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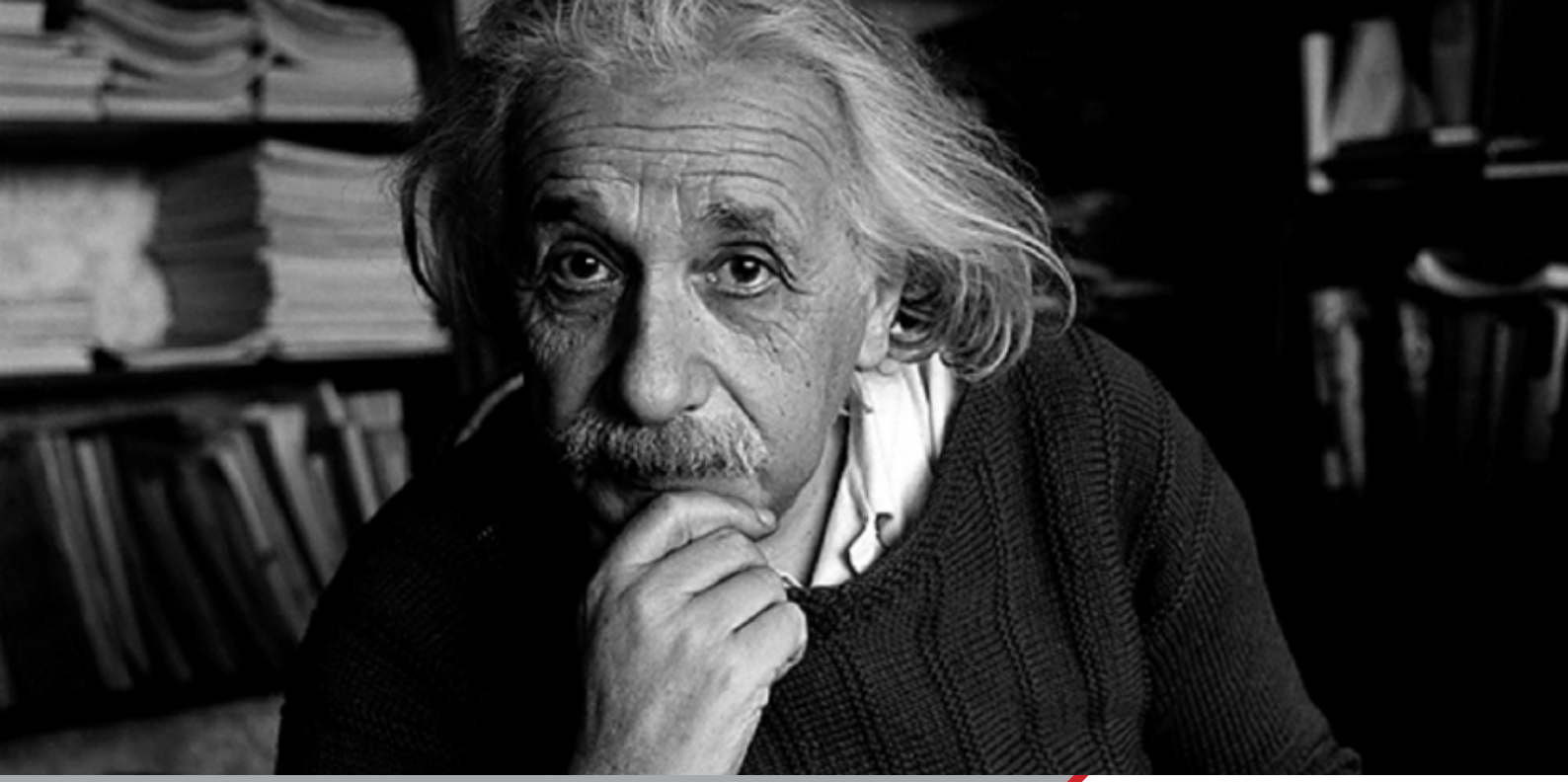

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ORIGINAL ARTICLE

Antimicrobial Effect of Different Inks on Microbes

Himanshu Jeetraj[1], Rajeev Kumar[2], Tripti Bhatnagar[3]

ABSTRACT

For ages, the main source of ink for writing documents and books has been natural dyes and vegetable inks. Biochemists have identified these natural inks and dyes that act as antibacterial and anti-fungal agent whereby they protect the historical writings and documents from bacterial or fungal infections. It also acts as moth deterrents. Natural colorants derived from plants have been used for at least 4,000 years as dyes and inks for writing because of their antimicrobial properties. Thus, documents and books written with these inks have survived moisture, humidity, and microbial degradation or deterioration. The type of ink used to write books or documents have forensic relevance. Therefore, the present study was undertaken to understand the antimicrobial properties of older dyes and inks compared to today's inks and their comparative antimicrobial activity. Fountain pen ink and gel pen pigments are water-based but even then their antimicrobial activity have been clearly seen against 5 different bacterial strains in the present study. Against all the bacterial strains, ball pen ink and gel ink demonstrated the greatest Inhibition region. It was also deduced from the results that of all the three, the black ink gave the maximum zone of Inhibition and toxicity to all the five strains of Gram -ve as well as Gram +ve bacterial strain. Thus the study proves that the ink pigments have high antibacterial effect and that both printed and written documents and manuscripts could be preserved without the fear of deterioration.

KEYWORDS | ink, documents, colourants, microbes, antimicrobial activity, bacterial strain

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INTRODUCTION

A majority of transactions in the world of trade and commerce takes place through written and printed documents. In today's culture, records play a critical role in about half of all cases involving disputed documents. Documents identify any matter conveyed or written or represented on any surface by letters, figures, or signs, or by more than one of these means, intended to be used, or that could be used, as proof of that matter.[1] As a result, determining what kind of ink was used in the writing of that document is critical to determining the age of the record. One of the earliest forms of forensic science has been the examination of the writing materials. The most difficult task accomplished through this study was determining of the age of the ink. There are a variety of approaches that can be used to determine the age of a newspaper. It could be calculated based on its own state, watermarks, makeup, and other factors.[2]

In certain unusual cases, using a pencil to determine the age of documents can be beneficial. Age of the ink may be used to determine the date of writing. Changes in the color of the ink caused by oxidation and chloride or sulfate diffusion may be

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one of the most effective methods for determining the age of writing was the antibacterial effect of the ink. Ink assessment is also critical for determining if a newspaper belongs to that time period or not. As some inks have characteristics that make them resistant to specific microbes such as antimicrobial markers used throughout the process, more inks have been developed with antimicrobial properties. Germs are the primary cause of the paper's rapid degradation and document damage.[4] One of the oldest branches of forensic science was the examination of writing materials, and ink analysis is an important field in forensic science. Inks of ballpoint pens are viscous liquids containing a combination of dyes and pigments, organic solvents, additives such as antioxidants, resins, preservatives, softeners, and trace elements.[5] Ink investigation is a crucial forensic technique that can provide answers to concerns about forged checks, legal contracts, wills, insurance claims, ransom letters and even suicide notes. The primary goal of ink analysis is to determine whether two pieces of written texts were produced using the same ink. To distinguish between different inks used in ballpoint pens, various analytical and spectroscopic techniques are used, and they're typically divided into two distinct categories: destructive/semi-destructive and non-destructive/non-invasive. A portion of the ink sample must be extracted before analysis using destructive/semi-destructive methods. Mass spectrometry combined with various ionization techniques are examples of destructive/semi-destructive procedures that have been used for ink analysis.[6,7] Capillary electrophoresis, Visible spectroscopy VIS, chromatography (thin layer, gas, higher output liquid), Fourier transform infrared spectroscopy, and other techniques for determining elemental composition are some of the techniques used. Non-destructive instruments, on the other hand, refer to ink monitoring on the document using methods that enable the conclusion of ink attributes without affecting the document, which include visual evaluation using a microscope, optical techniques using various lighting sources and filters, luminescence, reflectance IR, and Raman spectroscopy. [8,9]

METHODS

Sample Collection: Gel pen ink, Ball pen ink and printer inks were collected from different sources. Old typed and written documents were also collected for the extraction of inks from the documents.

Isolation of Soil and Water Bacterial Strains: To study the antimicrobial activity of different dyes and inks, bacterial cultures found in the soil, water and air were isolated. These bacterial cultures are the organisms which would easily grow on old books and documents and would degrade the written or typed inks as well as the cellulose of papers thus, resulting in degradation of important documents.

For isolation of bacterial strains inhabiting air, water and soil, serial dilutions of water and soil samples were made and spread on Nutrient Agar plates. The agar plates were left open in the air for an hour to isolate air microorganisms, and then all of the nutrient agar petri-plates were incubated at 37°C for 24 hours to obtain the relevant bacterial cultures.

Morphological & Biochemical Characterization of Bacteria: The isolated bacterial cultures were kept on Nutrient Agar plates and slants for further studies. The morphology of the bacterial cultures was studied by Gram Staining. Gram staining involves Crystal Violet purple staining of Gram +ve bacteria and pink counterstain by Safranin of Gram -ve bacteria. After gram stain the bacterial smears were observed under oil immersion 100X and the shape and color was identified and recorded.

Further characterization and partial identification of the bacterial cultures was done by biochemical tests, performed for each bacteria thus isolated. The biochemical tests performed were: Indole Test, methyl red test, Voges Proskauer test, citrate test, carbohydrates utilizing tests. (Glucose, Maltose, Mannose, Sucrose, Fructose etc.)

Preparation of Ink Solvent Mixture: Different types of ink samples were collected from the market to study their antimicrobial effect.

The Inks collected were as follows:

1. Fountain Pen Ink - Black and Blue color

2. Ball Pen Ink - Black and Blue color
3. Gel Pen Ink - Black and Blue color
4. Printer Ink - Black and Blue color

Then these ink samples were dissolved in different solvents like water, methanol, acetone etc., to analyze the extraction of pigments and their specific antimicrobial activity.

Study of antimicrobial activity of inks and dyes by Agar Well diffusion method: Under aseptic conditions, Nutrient Agar was prepared and plated. Using a 6mm diameter Agar medium well cutter, wells of similar length were created and sealed with melted agar. The test organism (isolated bacterial strains) were swabbed on the nutrient Agar plates. After which 80-100µl of ink or dye sample (different solvents) was added to different wells in case of antibacterial assay and related solvents were added to the other wells which are used as control. The swabbed plates with inks were incubated at 37°C for 24 hours. Ink and dye diffusion into the medium inhibits bacterial growth in the vicinity of the source, resulting in the creation of clear "zones" devoid of organism lawn.

Studying the Antimicrobial effect of ink and printer ink on old documents

Under aseptic conditions, nutrient Agar was prepared and plated. Small disks were cut from the old documents having pen ink or printer ink on them. The nutrient agar plates were swabbed with the five cultures and then the discs were carefully placed on the swabbed plate upside down to test the antimicrobial activity of the ink present on the old documents. Swabbed plates with inks were incubated for 24 hours at 37°.

RESULT

Five bacterial cultures were isolated from the surrounding environment using a serial dilution method and held in pure culture on Nutrient Agar and broth. To characterize and identify the bacterial cultures and understand which bacteria is more resistant, morphological analysis, Gram staining, biochemical tests were done (Table: 1).

Biochemical tests were performed for characterization of the bacterial isolates. The results are shown in Table: 2.

The carbohydrate test was performed by using different types of carbohydrates such as sucrose, fructose, lactose, maltose, mannose, etc.

S. No.	Strain Number	Gram Strain	Morphology Shape	Colony Color
1	Strain 1	Gram Negative	Rod	Pink
2	Strain 2	Gram Positive	Bacillus/rod shape	Purple
3	Strain 3	Gram Positive	Cocci	Purple
4	Strain 4	Gram Positive	Rod shape in chain	Purple
5	Strain 5	Gram Positive	Rod shape	Purple

Table 1 Table showing the shape, morphology and gram-staining of bacterial isolates

Sl. No	Tests	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
1	Glucose	Negative	Negative/ Positive	Negative	Negative	Negative
2	Fructose	Positive	Positive	Positive	Positive	Positive
3	Lactose	Positive	+/-	Negative	Negative	Negative
4	Glucose	Negative	+/-	Negative	Negative	Positive
5	Glucose	Negative	+/-	Negative	Positive	Positive
6	Glucose	Negative	+/-	Negative	Positive	Negative
7	Glucose	Negative	+/-	Negative	Positive	Positive
8	Glucose	Negative	+/-	Negative	Positive	Positive
9	Glucose	Negative	+/-	Negative	Positive	Positive

Table 2 Bio-chemical test result of isolated Isolates

The Gram stain and other biochemical and carbohydrate tests gave a clear identification of the type of genus of the bacterial isolates. On the basis of test results submitted to Bergey's online software, the isolates were identified as:

Strain 1 - Enterobacter sps. (85%)
Strain 2 - Lactobacillus sps. (87%)
Strain 3 - Staphylococcus aureus (93%)
Strain 4 - Actinomycetes sps. (89%)
Strain 5 - Bacillus subtilis (90%)

Once isolated, the bacterial strains were used for analyzing the antimicrobial effect of different inks which were diluted in 3 types of inks—printer ink, ball pen ink, gel pen ink and fountain pen ink—are tested against environmental bacterial cultures. The zone of Inhibition obtained in centimeter when tested against different inks and old documents are shown in Tables 3, and 4 and the comparative zone of Inhibition obtained against black, and blue printer ink, ball pen ink, fountain pen ink, gel pen ink is shown in Figures 1, and 2.

PRINTER INK	DILUTED IN	STRAIN1	STRAIN2	STRAIN3	STRAIN4	STRAIN5
		Zone of Inhibition (in centimeter)				
BLACK	Water	3.2	3.1	3.3	3.1	3.1
	Acetone	3.2	3.5	3.3	3.2	3.4
	Methanol	3.3	2.9	2.8	2.8	2.8
BLUE	Water	2.2	2.4	2.4	2.4	2.5
	Acetone	1.3	1.8	2.3	2.1	2.1
	Methanol	2.4	2.3	2.6	2.3	2.6
BALL PEN INK						
BLACK	Water	1.5	0.8	1.1	1.9	1.3
	Acetone	2	2.3	1.9	1.4	1.7
	Methanol	2.3	2.7	2.1	2.7	2.4
BLUE	Water	2.3	2	2	2.1	1.5
	Acetone	3	3.4	3.1	2.9	3
	Methanol	2.9	3.5	3.1	3	3
FOUNTAIN PEN INK						
BLACK	Water	0.9	1.5	1.4	1.2	0.9
	Acetone	0.9	1.4	-	0.9	0.9
	Methanol	1.2	1.2	1.1	1.1	0.8
BLUE	Water	1.2	-	1.2	1.2	1
	Acetone	-	2	1.5	1	0.9
	Methanol	-	-	1.5	0.8	0.9
GEL PEN INK						
BLACK	Water	2.4	2.1	2.4	1.9	-
	Acetone	-	-	-	-	-
	Methanol	2.5	2	2	2.1	1.9
BLUE	Water	2.1	2.1	2.2	2.2	2.3
	Acetone	-	1.9	1.9	1.7	1.9
	Methanol	2.2	2.2	2.4	1.9	2

Table 3 Zone of Inhibition obtained when Printer ink, Ball pen ink, Fountain pen ink, Gel pen ink (Blue & Black) are tested for antimicrobial effect

	STRAIN1	STRAIN2	STRAIN3	STRAIN4	STRAIN5
	Zone of Inhibition (in centimeter)				
BIO BOOK 1996	0.5	0.6	0.6	1.1	0.5
BILL BOOK CARBON INK	1	1.1	1.2	0.9	1.1
BILL BOOK PRINTED INK	1.1	0.8	1.2	1	0.8
	STRAIN1	**STRAIN2**	**STRAIN3**	**STRAIN4**	**STRAIN5**
	Zone of Inhibition (in centimeter)				
BOOK (FOUNTAIN INK BLACK)	0.7	1	0.7	-	0.8
BOOK (FOUNTAIN INK BLUE)	1	0.9	0.9	1.3	-
BOOK BALL PEN INK	0.9	-	0.9	0.9	0.8

Table 4 Zone of Inhibition obtained when written and typed text of very old Bill books and Text Books are tested for antimicrobial effect on Gel pen ink (Blue & Black) are tested for antimicrobial effect

- The printer ink when dissolved in three different solvents showed varying antimicrobial activity. The black Printer ink gave a better antimicrobial activity having zone of Inhibition from 2.8 cm to 3.5 cm. against different test bacterial isolates. Enterobactersps (gram -ve) gave the highest inhibition zone. The methanol dissolved blue ink gave the best results against all the test microorganisms.
- The ball pen ink when dissolved in three different solvents showed a higher

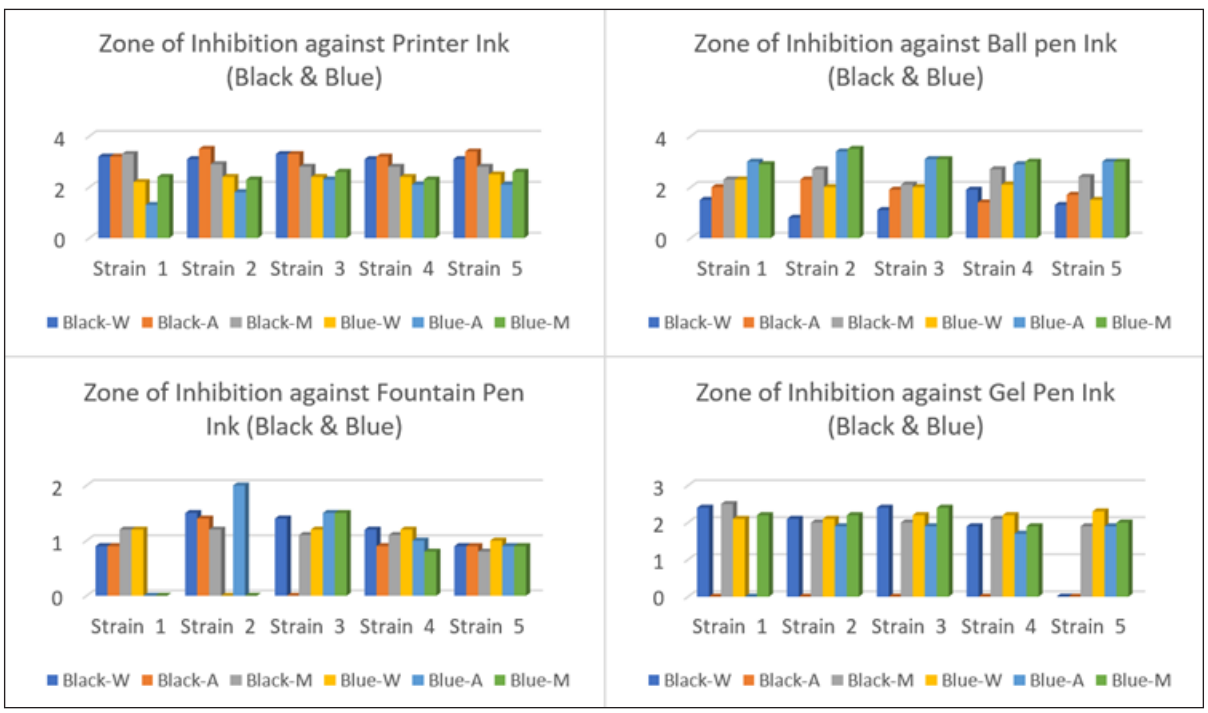

Figure 1 Comparitive zone of Inhibition obtained against black ink, blue ink, printer Ink, and Ball Pen ink

antimicrobial activity than printer ink. But the Black ball pen ink showed lesser antimicrobial activity as compared to the Blue ball pen ink. The three diluants of Black ball pen ink gave zone of inhibition from 1.5 cm to 2.7 cm against different test bacterial isolates. The least zone of inhibition was shown in case of Lactobacillus strain by black ink dissolved in water. The methanol and acetone dissolved blue ink gave the best results against all the test microorganisms.

- The Fountain Pen ink was observed to be very poor in its antimicrobial activity in both the Blue as well as Black Pigment. The strains Enterobactersps. And Lactobacillus sp. was resistant to both the colored fountain pen inks.
- The Gel Pen ink when dissolved in three different solvents did not show good antimicrobial activity. The three diluants of Blue ball pen ink gave zone of inhibition from 1.7 cm – 2.4 cm against different Test Bacterial isolates. All the bacterial strains were resistant to the Black gel pen ink dissolved in acetone. Thus, the blue ball pen ink pigment has more antimicrobial activity as compared to Black Ink pigment. The methanol and acetone dissolved blue ink gave the best results against all the test microorganisms.
- When the old books and documents were tested for antimicrobial activity by disc diffusion, it was found that all the inks showed antimicrobial activity against the five test bacterial cultures. The Control discs having only cellulose paper did not give any zone of inhibition. This gave proof that the ink present on the paper discs was actually showing the zone of Inhibition against the Gram +ve and Gram -ve bacterial strains.

Thus, it is clearly seen and observed through this study that inks and dyes of all kinds are antimicrobial to different extents depending on the dye pigments, which are its constituents. That is why documents and old books and copies are not easily degraded by bacterial cultures even if kept in moist condition for long duration. But, sometimes cellulose digesting fungi can cause degradation to the historical books and documents. The zone of Inhibition observed when discs were used was less as compared to well diffusion, because the concentration of inks on the paper discs was very less

DISCUSSION

The color of a pen, printer, or gel ink comes from either a water-soluble dye or a water-insoluble pigment. To make a specific ink, different pigments and dyes like eosin, carbon black, malachite gray, rhodamine, and others are combined with components like Titanium Oxide, Copper Zinc Alloy, or elements like bromine. However, the pigments in gel pen ink are usually copper phthalocyanine and iron oxides, with the gel consisting of water and biopolymers including xanthan gum and tragacanth gum.

Apart from the pigments in the ink, stabilizers, polar or non-polar solvents, and other additives such as Glycerides, triethanolamine, and other additives are all present in modern inks.

Ink pigments were derived from vegetables or fungal strains and made in aqueous solutions in ancient times, making the inks more vulnerable to microbe degradation when documents were stored for long periods of time. Inks are particularly toxic to bacteria since they are now made up of mostly organic and synthetic compounds. As a result, when these inks are used, they provide a higher

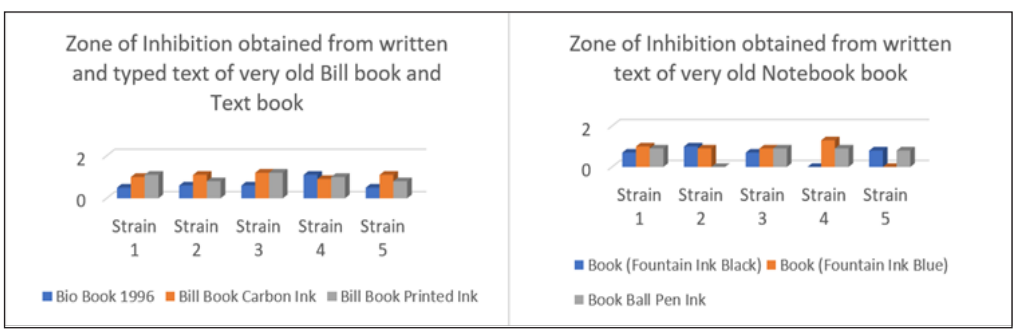

Figure 2 Comparitive Zone of Inhibition obtained against black and blue printer ink, Ball Pen

degree of Zone of Inhibition in almost all cases.

In the present study, different inks such as fountain pen ink, ball pen ink, gel pen ink and printer ink were used to study their effect on the bacterial strains commonly found in places where the books, documents or parchments are stored. The effect of inks and dyes on these bacterial strains are very important since they assess the maintenance and preservation of documents and parchments for longer period, without getting degraded or deteriorated. The antimicrobial activity of the dyes and inks prevents the degradation of documents and also prevent the growth of cellulose-eating or collagen-eating bacterial growth.

When it came to printer ink, the findings showed that black ink had a greater zone of inhibition against all of the bacterial test strains than blue ink. But in all other cases the Blue ink pigments fared better in acting as antimicrobial agent. In case of gel pen black ink, all the bacterial strains proved resistant to the dye as there was no zone of Inhibition. When old and new documents having ink or printer ink texts were used as antimicrobial discs, it was clearly seen that it was the ink which showed antimicrobial effect instead of the cellulose.

CONCLUSION

Documents play a very important role in our society. Roughly 50% of cases relate to the disputed documents. Documents denote any matter expressed or described upon any surface by means of letters, figures, or marks or by more than one of these means; intended to be used, or which may be used, as evidence of that matter. Hence, it is important to determine what kind of ink was used in writing that document and help in rough estimation of the age of that document. One of the oldest fields of forensic science was the examination of writing materials. Along with this aspect of inks and documents, the aspect of preserving papers, books, copies of documents etc., from bacterial deterioration has to be taken into the account. Thus, antimicrobial inks and dyes are becoming more common and are being manufactured for daily use as well as for use in official documents and papers. IJFMP

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ORIGINAL ARTICLE

Study on Expired and Unexpired Drugs on Different Pathogenic Bacteria

Divya Kommineni[1], Suneet Kumar[2]

ABSTRACT

There is a growing trend in the different antibiotics against microbes. However, studies were found that a microorganism interacts at molecular level in a unique way against a range of antibiotics. The present study was undertaken to analyze the effect of expired and unexpired drugs which are commonly used by Indians without doctor's prescription. Due to a change in chemical composition or a drop in potency, expired medical items may be less effective or dangerous. Expired drugs may harbor bacteria, and sub-potent medicines may even fail to treat infections, resulting in more serious diseases and antibiotic resistance. There is no assurance that the drug will be safe and effective after the expiry date. Sensitivity test and Spectrophotometry were performed for the selected expired and unexpired antibiotics and drugs - Ampicillin, Norflox-TZ, and Althorn. The growth pattern of microbes in antibiotics before and after their expiry was performed and then compared. It was evident that fresh forms of antibiotics have significantly inhibited the growth of microbes as compared to expired drugs. The expired forms of antibiotics lost their efficacy in expired drugs.

KEYWORDS | antibiotics, spectrophotometry, expired, unexpired drugs

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INTRODUCTION

Our health is regarded as the most valuable asset we own. Individuals, the government and commercial entities spend a lot of money on healthcare in today's medical system. So, it's critical to comprehend the economic concerns surrounding healthcare administration and medical therapy. Pharmaceutical drugs are chemicals that are used in medicines or drugs. Pharmaceutical drugs and medicines are available to all age groups via various channels (oral, topical, optic, ophthalmic) depending on the form of medicine (tablet, cream, ointment, ear drops, eye drops, etc.) for treatment of acute and serious illnesses caused by various microorganisms. They are used not only for illness treatment and diagnosis, but also for prevention of illnesses. However, even a small amount of contaminated medicines can be fatal to humans. The production of pharmaceutical drugs and medicines is a collaborative effort between pharmaceuticals and microbiologists. The process of drug development begins with the discovery of a drug molecule having therapeutic value in fighting, controlling, preventing, or curing illnesses. The creation and characterization of such molecules, known as "active pharmaceutical ingredients" (APIs), as well as their examination to provide preliminary safety and therapeutic

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effectiveness data, are prerequisites for drug development. Bacterial infection can cause degradation in a range of expired and unexpired drugs unfit for use. Pharmaceutical companies produce drugs and medicines to treat a variety of diseases, and t hey are labeled with an expiry date. The drugs are effective and safe for customers to ingest, but expired medicines are not. The expiry date printed on drugs and medicines indicates the day when the manufacturer guarantees full potency and safety of the drugs. Environmental conditions, microbial contamination, containers in which they are stored all contribute to the degradation of pharmaceuticals in three ways: physical, chemical, or microbial. Color and texture shift as a result of physical instability. Microbial proliferation leads to microbial deterioration, whereas chemical instability leads to oxidation, hydrolysis, and decarboxylation.

Expired Drugs: Medication lapse is the date after which a medication probably won't be effective or suitable for use by patient. Buyers can decide on the usability of a medicine by checking its expiry date printed on the bottle or packet. Medicines which are expired can be ineffective, inadequate or even dangerous.

Unexpired Drugs: Still valid or in use and effect but not terminated medicine that is not yet reached its expiration date. For the benefit of our immune system, sometimes we take antibiotic drug, which are chemicals, that go inside our body and attack the pathogenic bacteria so that it cannot live longer and multiply in our body. If the bacteria are susceptible to the antibiotic, then they stop growing and die simply.

Health: Wellbeing is seen as a source of happiness in our lives, or, to put it another way, health is regarded as a source of riches in our lives. Infections are treated and prevented by pharmaceutical products. These pharmaceutical items are designed to be safe during their development, storage, and use.

Pharmaceuticals are necessary for human health, but many of them include hazardous substances that can pollute the environment if they are not properly managed or disposed of. When pharmaceutical wastes are illegally disposed of, they can cause contamination in humans and animals, resulting in a wide range of toxicities. Many people keep unwanted, unused, or expired medications in their homes and then dispose them of into garbage cans or flush them down toilets.

Bacteria can infect a variety of expired and unexpired pharmaceutical items, resulting in corrosion of items that are not fit for use. Pharma companies produce medicines to treat a variety of diseases and label them with an expiry date. Due to a change in chemical composition or a drop in potency, expired medical items may be less effective or even dangerous. Expired drugs may harbor bacteria, and sub-potent medicines may even fail to treat infections, resulting in more serious diseases. There is no assurance that the drug will be safe and effective after the expiration date. As a result, the goal of this study was to assess the efficacy of expired medicines and determine how effective or ineffective they are in comparison to unexpired medicines. We employed mostly antibiotics in the experiment and conducted an antimicrobial investigation to determine the efficiency of widely used expired and unexpired medications against various harmful microorganisms.

OBJECTIVE

- Maintenance of Pathogenic Bacteria on nutrient agar media
- Morphological characterization of Bacterial strains.
- Study the effect of expired and unexpired drugs on bacterial growth by Agar Well Diffusion and Spectrophotometry.
- Study the effect of expired and unexpired drugs on bacterial growth by optical density and growth using Spectrophotometry.
- Potency is a measure of drug activity stated in terms of the amount necessary to generate an effect of a specific intensity in the discipline of pharmacology. At low doses, a very powerful medication elicits a bigger reaction, whereas a medication of lower strength elicits a tiny reaction. Affinity and effectiveness are proportionate. [Lipstich, 2012]
- A drug's shelf life is the period of time during which it keeps its true impact or effectiveness. It is the time span between the production date and the expiry date, starting from the day of manufacture.
- A drug's expiration date is the last day on which the manufacturer guarantees the drugs

complete potency and safety. Most drug labels, including prescription, over-the-counter (OTC), and dietary (herbal) supplements, include an expiration date. [Marshall et al 2009]

- For legal and liability concerns, many pharmaceutical manufacturers are required by law to provide expiration dates on prescription goods prior to marketing.
- Manufacturers will not offer recommendations on the stability of pharmaceuticals after the original expiration date.
- Drugs that have passed their expiration date may not be harmful, but their strength or efficacy may have deteriorated. Intake of such treatments may not be as effective as it should be, and infection-causing microorganisms may become resistant to the medicine. There will be little or no impact if the same medicine is given again in the future. The circumstances in which the medicine is stored are also a determining element in the drug's shelf life. The medication should be kept in a cool, dry location away from extremes of temperature, humidity, or light. Long-term exposure to light can cause chemical reactions, causing the drug's chemical properties to change and eventually degrade.
- Antimicrobial resistance can develop when a drug's potency is reduced. The latter is also the outcome of inappropriate medication administration. Antimicrobial resistance has now evolved into a major worldwide issue. To battle bacterial and fungal diseases, it's crucial to take the right antibiotic with the right potency at the right time.
- In many tropical nations, poor quality medications represent a critical yet underappreciated public health issue. In therapeutic research, policy choices and the implementation of high quality medications are critical. There are obstacles in the fight against low quality medications, particularly counterfeits.
- Third World countries have been increasingly threatened by counterfeit and substandard medications and pharmaceuticals that have lost their efficacy as a result of deteriorating economic situations and lax implementation of current pharmaceutical and customs rules. The distribution of low-quality products. Medicines are a critical clinical and public health problem in the poor nations.
- Problems include under or over concentration of ingredients, poor quality ingredients, poor stability, inadequate packaging and a decline in potency. [Alghasham *et al.,* 2018]
- Antimicrobial agents are most often tested against bacteria in the log phase of multiplication to produce the maximum bactericidal effect. In an infection, bacteria may multiply less optimally. Alekshun and Levy, 2007.

  The researchers studied the impact of a variety of antimicrobial drugs on gram-positive and gram-negative bacteria during non-growing and slow-growing stages. Only ciprofloxacin and ofloxacin were bactericidal (3-order-of-magnitude killing) against non-growing gram-negative bacteria, and no medications were bactericidal against Staphylococcus aureus. Gentamicin (an aminoglycoside), imipenem (a carbapenem), meropenem (a carbapenem), ciprofloxacin (a fluoroquinolone), and ofloxacin (a fluoroquinolone) showed up to 5.7 orders of magnitude greater killing than piperacillin or cefotaxime against the extremely slowly growing gram-negative bacteria examined. This is in contrast to ideally grown bacteria, which were killed by a wide range of antibiotic classes with a 99.9% success rate. Antibiotic king was highly dependent on the growth rate of the gram-positive and gram-negative bacteria we studied. Slow death by chemotherapeutic drugs has uncertain clinical implications for established bacterial infections and infections involving foreign substances.
- Barbara Tourette prosser *et al* (1987) Antibiotics are often ineffective against organisms in exopolysaccharide biofilms, according to research. The effect of antibiotics on bacteria in formed biofilms is studied using a simple approach. Escherichia coli ATCC 25922 cells were suspended in buffer and dispersed on 0.5-cm 2 catheter disks after being cultured overnight at 37°C on Mueller-Hinton agar. The disks were rinsed, moved to petri plates containing 20 ml of broth, and incubated for 20 to 22 hours at 37°C, during which time thick biofilms were formed. Disks were cleaned, put

in broth or antibiotic broth, and incubated for 4 hours at 37°C. With 400µl of amdinocillin or cefamandole per ml, viable bacterial counts fell from 103 to 104 CFU/cm2 in 24 hours. In 24 hours, a combination of 400 micrograms of each antibiotic per ml reduced viable counts to undetectable levels (100 CFU/cm2). This method was used to test a variety of drugs and microbes.

MATERIALS AND METHOD

Nutrient-Agar Media:

Requirement: Flask, Petriplates, cotton plugs, foil, peptone, beef extract, NaCl, agar, D/W, autoclave, laminar air flow chamber, weighing balance.

Composition of Nutrient Agar Media

Sodium Chloride =8gm		Sodium Chloride=0.8 gm	
Peptone	= 5 gm	Peptone	0.5 gm
Beef extract	= 3 gm	Beef Extract	0.3 gm
Agar	= 20 gm	Agar	2 gm
Distilled water	1000ml	Distilled water	100ml
pH	6.8	pH	6.8

Preparation of Nutrient Agar Slant/Petriplate

- Weigh each and every ingredients and transfer them to a conical flask containing D/W.
- After addition of every component, make up the final volume 1,000 ml by adding D/W and adjust the pH value. Heat it a little to dissolve the components.
- Plug the flask and cover its mouth with aluminum foil and autoclave it.
- Along with the media, autoclave the empty, wide-mouthed, cotton-plugged test tubes and petriplate at 121°C for 20 min at 15 partial pressure.
- After autoclaving transfer the material into laminar air flow cabinets and aseptically dispense 7-8ml of the medium into each sterilized test tube or 20-25 ml into the petriplates.
- Plug the tubes and also close the petriplates.
- For slant preparation, place a 10 ml pipette or any wooden stick on the bench top and lean the tubes of melted agar. Do this properly so that the slanted surface of the medium extends to the bottom of the tube.
- Leave the tubes and petriplates until it solidifies. Slant used for storage of pure culture and petriplates used for streaking and isolating colonies.

Culturing of Microorganism By Streaking Method:

Pathogenic microbes were taken from the lab and culture on nutrient agar plate by streaking method under sterile condition.

Morphological Characterization of Microbes by Gram Staining

Gram staining is a method of separating bacteria into two main groups via differential staining (gram positive and gram negative). The nucleic acids of bacteria and background tissues are stained with the cationic dye crystal violet. Iodine is used to launder the crystal violet staining, resulting in a purple complex. Due to the impermeability of their cell walls, certain bacteria resist differentiation. The tissue background and some kinds of bacteria lost their staining when an appropriate differentiator (eg., alcohol or acetone) was used, but they took up a cationic dye (saffranine) of contrasting hue (typically red/pink) that was afterwards applied. The purple staining microorganisms are termed "Gram positive", whereas the microorganisms that take up the counter stain (red/pink) are termed "Gram negative".

Materials Required: Grams iodine dye, saffranin dye, crystal violet, glass slides, inoculation loop, culture, distilled water, bacterial culture, burner, and microscope.

- Take clean slides and add a drop of water on it.
- Use the loop to pick a culture in the inoculation loop and make a smear on the slide.
- Heat fix the slide.
- Flood the slide with crystal violet for 1 minute and wash away the excess stain in water.
- Flood the slide with Grams Iodine for 1 minute and wash under water.
- Decolorize with acetone and immediately wash under water.
- Counter stain with saffranin for 45 secs.
- Air dry the slides and observe under microscope.

Collection of Different Drugs: Both expired and unexpired tablets of Ampicillin (500 mg), Norflox-TZ (1000 mg), Althrocin (250 mg) were collected from medical shops.

Preparation of Drugs: These capsules and tablets were dissolved in 10ml sterile water used for the experiments.

To Test the Effect of Expired and Non-Expired Drug On Bacteria By Agar Well-Diffusion Method:

The Agar Well-Diffusion test or the Kirby-Bauer Disk-Diffusion method is a means of determining the effect of an antimicrobial agent against different bacteria and fungus grown in culture.

Method:

- Nutrient agar media was prepared and plated under aseptic conditions.
- Wells were created at equal distance in the agar media by punching holes in the solidified agar with the help of a sterilized pipette tip.
- A drop of the melted agar was dropped into the well to seal the bottom of the well
- 200µl of the pathogenic bacteria inoculated in nutrient broth was spread nutrient agar plate
- 200µl of the expired drugs sample and 200µl of the non expired drugs sample was added in well 1 and 2 respectively.
- The plates were incubated at 37°C for 24 hours in incubator.

The antibiotics diffuses out from the wells into the agar in a gradient, so the agar closest to the well has the highest concentration and the concentration of the antibiotic decreases as antibiotic move further away from the well. The zone of inhibition was observed and further it was measured. This test was performed in order to test for the appropriate antibiotic concentration to be used. The four different antibiotics tested were Ampicillin, Combiflam, Norflox-TZ, Althrocin.

Effect of Drugs on Bacterial growth by Optical Density- Preparation of Nutrient Broth

Nutrient Broth is used to grow a wide variety of non-fastidious microorganisms. It is one of the non-selective medium used in ordinary microbial growth. Due to the presence of peptone and beef extract, this very basic formulation promotes the development of non-fastidious bacteria.

Materials Required:

Composition of Nutrient Agar Media

Sodium Chloride =8gm		Sodium Chloride=0.8 gm	
Peptone	= 5 gm	Peptone	0.5 gm
Beef extract	= 3 gm	Beef Extract	0.3 gm
Distilled water	1000ml	Distilled water	100ml
pH	7	pH	7

Method:

1. Weigh the chemical ingredients of the respective media and transfer to a beaker/ flask containing 500ml of distilled water.
2. Gently heat the contents with slight agitation to dissolve the ingredients properly.
3. Pour more distilled water to make the final volume to 1 liter.
4. Measure the pH of the medium and adjust it to the required pH using HCl or NaOH depending on the PH
5. Cap the mouths of the flask using cotton plug and tightly cover with aluminum foil.
6. Autoclave the flasks with the ingredients at 121°c for 30 minutes.
7. After autoclaving nutrient broth media was transferred in test tube all work was done under laminar air flow
8. All pathogenic bacteria taken from lab and maintained on nutrient agar plate was inoculated in nutrient broth media poured in test tube by using the inoculated loops in laminar air flow
9. And 200 µl of all selected antibiotics were added into the bacterial culture separately and incubated for 24 hrs and O.D. was taken at 0 hrs and then strains were kept in incubator at 37°C for 24hrs and again O.D. was observed on the next day at 600nm.

RESULTS AND DISCUSSIONS

In the present study microbes provided by the lab were inoculated on nutrient agar plate. For this purpose, Nutrient agar media was prepared.

Inoculation of Bacteria on Nutrient Agar Plates: Two Bacterial cultures were taken from the lab and the streaked onto nutrient agar plates. The plates were kept in an incubator at 37°C for 24 hrs. Growth was observed.

Morphological Identification of Bacteria

Gram Staining: It is done to distinguish between Gram positive and Gram negative bacteria.

Figure 1 Nutrient Agar Media

Figure 2 Strain 1

Figure 3 Strain 2

Bacteria	Gram +/-	Shape
E.coli (Strain1)	Negative	Rod
Bacillus cereus (strain2)	Positive	Rod

Table 1 Result of Gram Staining

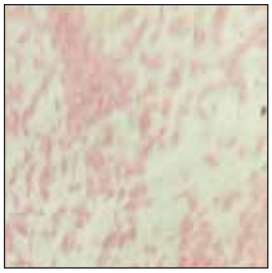

Figure 4 Microscopic result of E.coli and Bacillus subtilis

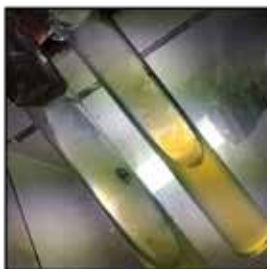

Figure 5 Maintained slants of both strains

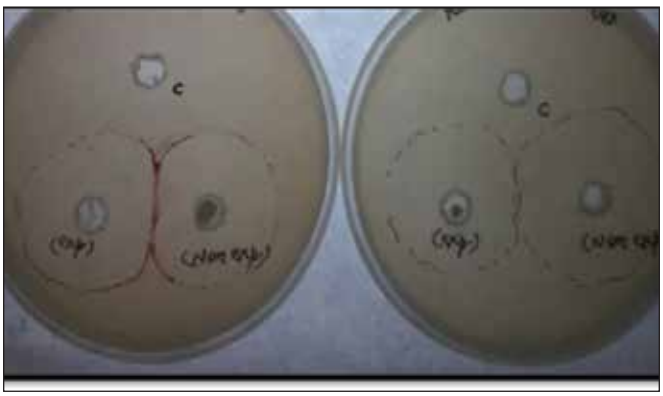

Figure 6 Well Diffusion result of expired and unexpired Ampicilin on E.coli and Bacillus cereus

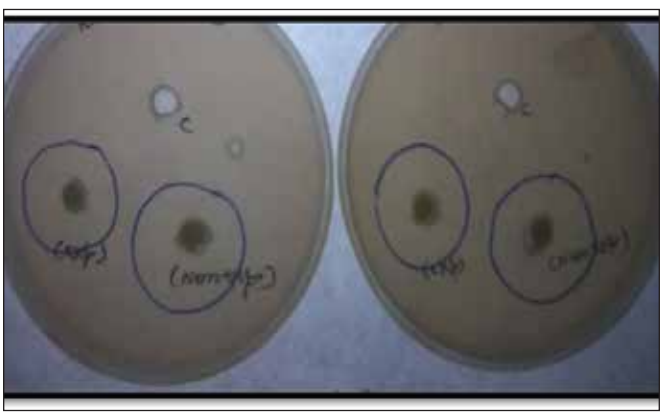

Figure 7 Well Diffusion result of expired and unexpired Norflox-TZ on E.coli and Bacillus cereu

Figure 8 Well diffusion result of expired and unexpired Althrocin on E.coli and Bacillus cereus

Micro organisms	**Expired Ampicillin** (Zone of Inhibition in cms)	**Unexpired Ampicillin** (Zone of Inhibition in cms)
E.coli	4.1	4.8
Bacillus cereus	3.5	3.7
	Expired Norflox-TZ (Zone of Inhibition in cms)	**Unexpired Norflox-TZ** (Zone of Inhibition in cms)
E.coli	4.3	4.7
Bacillus cereus	2.3	2.5
	Expired Althrocin (Zone of Inhibition in cms)	**Unexpired Althrocin** (Zone of Inhibition in cms)
Shigella dysenteriae E.coli	1.7	2.5
Bacillus cereus	1.0	1.4

Table 2 Antimicrobial Activity of Microorganisms against expired and unexpired drugs.

Micro organisms	**Expired Ampicillin** (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.076	0.025
Bacillus cereus	0.018	0.013
	Unexpired Ampicillin (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.212	0.258
Bacillus cereus	0.019	0.028
	Unexpired Norflox-TZ (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.533	0.528
Bacillus cereus	0.416	0.400
	Expired Norflox-TZ (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.388	0.475
Bacillus cereus	0.391	0.683
	Unexpired Althrocin (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.240	0.146
Bacillus cereus	0.161	0.145
	Expired Althrocin (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.155	0.159
Bacillus cereus	0.107	0.115

Table 3 Data represents OD of Microbial growth in antibiotics in 600 nm.

Well Diffusion Method:

Effect of expired and unexpired drugs on microorganisms was checked by Agar Well Diffusion method:

1 Effect of Ampicillin

2 Effect of Norflox-TZ

3 Effect of Althrocin

Effect of expired and unexpired drug Ampicillin, Norflox, Althrocin were studied against these two bacteriae - *E.coli* and *Bacillus cereus*, and zone of Inhibition was measured. It was clear that expired drug had less effective in comparison to unexpired drugs.

When expired and unexpired drug was added to check effect drug on bacterial growth, 24 hr grown culture of each strain was taken and growth was estimated by spectrophotometer, and used this O.D. as a control and drugs were added in each tube and incubated for 24 hr and again growth was checked and O.D. was compared from initial O.D. in both types of drugs. From O.D. it was clear that effect of expired drug showed lesser effect on bacteria so O.D. is higher in expired drugs, while O.D. was lesser in case of non-expired drugs.

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The authors declare that there is no commercial or financial links that could be construed as conflict of interests.
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CONCLUSIONS

- The study shows the broad spectrum of the Forensic pharmacy as the name states to examine the illegal drugs.
- Most commonly illegal scope in the pharmacy is the re-stamping the unexpired drugs. So the study made to the harmful effects of taking the expired drugs.
- Present study concluded that unexpired drugs showed maximum zone of inhibition as compared to expired drugs.
- Unexpired drugs have significantly inhibited the growth of microbes as compared to expired treatment.
- The expired forms of antibiotics lose their efficacy drastically.
- Thus, expired medical products are less effective or dangerous due to a change in chemical composition or a decrease in strength/ biological half-life.

The present study concludes that unexpired drugs showed maximum zone of inhibition as compared to expired drugs. Unexpired drugs have significantly inhibited the growth of microbes as compared to expired treatment.

The expired forms of antibiotics lose their efficacy drastically. Thus, expired medical products are less effective or dangerous due to a change in chemical composition or a decrease in strength. IJFMP

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ORIGINAL ARTICLE

Analysis and Protein Profiling of Toxic Plants and their Relevance in Forensics

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ABSTRACT

Plant toxins and poisons are deadly weapons which can kill humans without any bloodshed or violence. These deadly plant weapons in the form of extracts or plant tissue as whole are used by culprits for burglary, rape, and murder. They are also used in many suicide cases. In India, numerous murders, unsolved deaths and crimes have been related to the use of plant toxins. Criminals use them often as they are freely available at virtually no cost. The plant extracts or plant tissues can often be found on the crime site or even on the murderer or suspect. In this project, research has been initiated to study the polymorphism of proteins found in the different poisonous plants in India and identify its forensic importance. The profiling or polymorphism has been done using SDS-Page technique, which shows a series of Protein Bands present in the plant tissue used. This data shows the typical proteins present in the different plants and the polymorphism of plant proteins can be used as a Protein Fingerprinting to identify the toxic plant used to cause harm, death, murder on a crime site. The Protein fingerprint thus obtained can be used as an evidence to identify a particular death with the specific symptoms as well as the toxic plant used in the crime.

KEYWORDS | plant poison, SDS-PAGE, protein profiling, plant toxins, proteins

INTRODUCTION

A few plant species that are poisonous or harmful to humans are commonly found in our gardens or planted as roadside trees. Plant poisons are deadly weapons capable of taking down one's life in dark and mysterious ways. They have a unique appeal for criminal minds. Plant poison is used as a weapon or "Botanical Weapon" (BW) by the criminals in thefts, robbery, and murder.[4]

Poisoning can take many forms, including contact (skin irritation)[2], absorption (causing internal organ poisoning), retention (by the dermal upper layer), and aerosolization (by the respiratory system).[5,9] Castor bean seeds (Ricinus communis) and Jequiriti beans (abrusprecatorius), for example, have been known for their toxicity since antiquity. One of the poisonous plants is the oleander, which can cause poisonous effect while cooking of food.

Botanical toxins may be a product of toxic and poisonous plants, or they may be a harmful component of plants.

The most important poisonous concepts found in plants are alkaloids and other common components. Proanthocyanidins, diterpenes, flavonoids, tannins, cardiac and cyanogenic glycosides, cardiac and cyanogenic

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glycosides, phenyl propanoids, lignans, nitrogen-compounds, gums, oxalates, and certain amino acids or proteins. These cyanogenic glucosides are silent killers in a toxic-crime because they are easily available. They have played a very important role in crimes and romance. Research was initiated to study the polymorphism of proteins found in the different poisonous plants in India. This study would help in identifying the plant tissue extract found at the crime site where death occurs due to ingestion of poisonous plant.

MATERIALS AND METHODS

Plant Material collection: Leaves and seeds of different toxic plants commonly found on roadsides were collected for the study. The five plant samples were: *Dhatura stramonium, Parthenium hysterophorus, Riccinuscommunis, Calotropis* and *Nerium Oleander.*

Preparation of Extracts of wet and dry plant samples: The fresh and dry leaves of the plants were ground in a Protein Extraction Buffer (PEB) (10 mL: 0.625 mL of 1M Tris-HCl, pH6.8 + 2 mL 10% SDS (w/v) + 1 mL 1M DTT (Dithiothreitol) + 1 mL 100% Glycerol + 5.375 mL distilled water) and were incubated for 24 hours. These extracts were then centrifuged for 10 minutes at 10,000 rpm and the supernatant stored at 4°C for all experiments.

Quantitative Analysis of Protein in Plant samples by Biuret method:

Biuret or Alkaline Copper Reagent: 50 mL 2% Na_2CO_3 in 0.1% NaOH + 0.5 mL 2% sodium-potassium tartrate + 0.5 mL 1% copper sulphate.

Standard Protein Sample: Bovine serum albumin (BSA) solution- 5mg/mL.

Methodology: Distilled water was added to the unique algorithmic of standard protein solutions and unknown samples, resulting in a final volume of 1.0 mL. 5.0 mL. Biuret reagent was added to both tubes, blended thoroughly and kept for 10 minutes at room temperature. Each tube's optical density was measured at 520nm. The Calibration graph was created based on the readings.[3]

Isolation and Characterization of Proteins by SDS-PAGE

SDS-PAGE (Laemmli, 1970) designed a method for isolating and characterization of proteins. In an Eppendorf micro centrifuge tube, 20L of protein sample extracted from the plant samples (both fresh and dried samples) and 10L of (1X) loading dye were combined (1.5 mL). The sample was warmed at 100°C for 2-10 minutes before being centrifuged for 1 minute. Using a micro pipette, the sample was placed in the gel wells. The gel should be run at a constant voltage (100V) until the tracking color reaches the bottom. Using coomassie brilliant blue R-250, the gel was stained with staining pigment. Kept that gel in the staining for one night. After overnight staining, the solution was poured out and the gel rinsed several times with water. Approximately 50 ml of destain solution was added to the container and kept overnight. The gel was eventually exposed against a white background.

DISCUSSIONS AND RESULTS

The present study deals with the protein profiling or fingerprinting of the commonly available toxic plant leaves. Number of plants are highly toxic to humans and animals if ingested or rubbed on the skin. These plant extracts are used by criminals to cause death or harm to their victims. Since these plants are freely available and can be obtained in large quantity these are used by small-time or first time criminals.

The total protein once extracted was quantified by Biuret method. The estimation of total soluble protein in the plant samples was estimated by using the standard.

Sl.No	Samples	Protein Concentration (mg/ml)
1.	Oleander fresh	1.15
2.	Oleander dry	1.76
3.	Calotropis fresh	0.76
4.	Calotropis dry	1.0
5.	Riccinus fresh	1.23
6.	Riccinus dry	1.57
7.	Datura fresh	0.5
8.	Datura dry	1.96
9.	Parthenium fresh	1.08
10.	Parthenium dry	1.42

Table 3 The total soluble protein extracted is higher in dry samples, since the amount of fresh plant tissue being weighed would be lesser as compared to dry tissue.

Nerium Oleander Plant

The fresh and dry leaf extracts show a different protein banding pattern (Table 1).

The presence of lower M.wt protein bands in dry tissue could be due to denaturation & breakdown of proteins found in the fresh tissue.

Protein bands Obtained	Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
1	12.6	8.9
2	10.0	7.07

Table 1 Molecular Weights of Protein bands Obtained in Oleander leaves

Calotropis Plant

The fresh and dry leaf extracts show a different protein banding pattern (Table: 2). The pattern of peptide bands obtained in Calotropis leaves show many similar bands having the same Molecular Weight. Thus, it can be assumed that due to the condition of the leaf sample the proteins may vary in Molecular weight when further denatured using SDS- PAGE.

Riccinus Communis

The fresh and dry leaf extracts show a similar protein banding pattern (Table:3). In Riccinus also similar protein bands are obtained in fresh leaves and dry leaves. The Ricin protein toxin which is mostly produced in the seeds of Riccinus plant has a molecular wt. of 65 Kda. In the fresh leaf extract we find a band for 65 kDa which could be the Ricin protein. But in the dry leaf extract it has broken down into its subunits and peptides.

Protein bands Obtained	Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
	210	7943.2
	121	210
	125.9	100
	67	25
	25	20
	14	14
	7.07	7.07
	5.01	5.01

Table 2 Molecular Weights of Protein bands obtained in Colotropis leaves

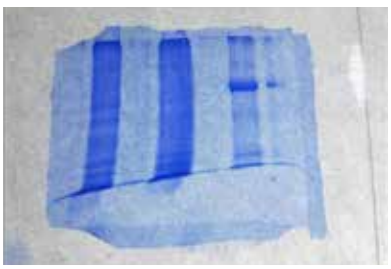

Figure 1 Calotropis protein bands On SDS PAGE gel.

Protein bands Obtained from Riccinus Communis

Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
7943	2511.8
2511.8	316
562	112
65	39.8
12.6	20
8.9	12
6.08	5.6
8.9	12
6.08	5.6
4.47	5.01
3.5	-

Table 3 Molecular Weights of Protein bands obtained in Riccinus Communis

Datura

The fresh and dry leaf extracts of Datura leaves show a very similar protein banding pattern (Table: 4). Here we observe that almost all the bands obtained in the both fresh and dry leaf extracts are of similar Molecular weights. Thus, here we can clearly develop a specific fingerprint pattern of SDS-PAGE separated protein bands in order to identify Datura plant extracts found in crime site.

Parthenium

The fresh and dry leaf extracts show a different protein banding pattern (Table: 5). The presence of lower M.wt protein bands in dry tissue could be due to denaturation and breakdown of proteins found in the fresh tissue.

This study would thus help in identifying the plant tissue extracts found at the crime site where poisoning and death has occurred due to ingestion of poisonous plants.

Protein bands Obtained from Datura

Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
15848	3981
210	121
121	100
100	50
50	48
48	11.2
11.6	9.5
9.5	8.9
8.9	5
6	3.7
3.9	–

Table 4 Molecular Weights of Protein bands obtained in Datura

Protein bands Obtained from Parthenium

Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
7943	3981
1584	210
210	100
100	67.6
67.6	20
10	10
5.6	3.16
3.9	–
3.16	–

Table 5 Molecular weights of Protein bands obtained from Parthenium

In the present study, we find that many of the protein bands in wet and dry tissue are same except for some bands which are absent. Thus, a protein SDS profile would help in identifying the plant species.

Pandey *et al.*,[8] (2017)used SDS-PAGE to extract proteins ranging in size from 45 kilo Dalton (kDa) to roughly 15 kDa for Ricinus communis and 45 kDa to roughly 10 kDa for cannabis sativa, respectively. They discovered that both of the methods used such as UV spectrophotometry and SDS-PAGE, are indispensable in analysing plant proteins, and that the methods can be of great assistance to scientists and other labs concerned with plant and proteomics management. IJFMP

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ORIGINAL ARTICLE

Study of a Two-Unit High-Performance Thin Layer Chromatography System with Partial Failure

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ABSTRACT

High-Performance Thin Layer Chromatography (HPTLC) is one of the revolutionary techniques that has the advantage of full optimization, minimum sample preparation, automation and hyphenation. Using HPTLC system is used frequently in forensic science for analysis of new drugs, poison, fingerprints, etc. In this paper, we study the major and minor faults of two units of HPTLC system by considering the fact that for minor and major faults, system goes to the partial failure and complete failure, respectively. On occurrence of a fault in a system, either the technician carries out the system to repair or if technician is busy the unit has to wait for repair. Systems parameters are derived by utilizing regenerative point graphical technique. Experimental results are performed to analyze system availability, server visit, busy period and profit. Graphical representations are shown for better understanding of the proposed technique.

Keywords: HPTLC, Regenerative Point Graphical Technique, RPGT

INTRODUCTION

HPTLC is a modern analytical method for analyzing chemical behavior of an object. It has wide applications in the field of forensic science due to its time saving nature and economic feasibility.[1-3] Some of its applications can be seen in the fields of poisoning, investigation, doping analysis, fingerprint analysis, analysis of drugs in blood, separating components of a mixture etc.[4-6] It is the fastest chromatography method since chromatography of the instances is performed in parallel. HPTLC technique is known for its purity, uniformity and best accuracy. Due to its importance in the field of forensic science, it has become an important tool for all researchers working in this area.[7-8]

HPTLC systems are inexpensive, easy and low-cost to install and has a very long service life. In this model, we discuss a two-unit HPTLC system taking both minor and major faults into account, considering online repairing of minor faults. After studying available data on a two-unit HPTLC system, it has been observed that the operative unit goes into failure state on occurrence of major fault. Due to this, sometimes the HPTLC standby unit usually does

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not come into operative state right after manual switching and the two-unit system reduces to single-unit system.

In reliability modeling literature, no researcher has ever discussed an HPTLC system with two units taking various major/minor fault into account. In order to fill this gap, we present a detailed analysis of a two-unit HPTLC system by considering both major as well as minor faults. When a major fault occurs, the unit goes into failure state resulting into non-function of standby unit after manual switching. Minor faults include motor overheating, overload, under or over voltage, abnormal sound, seal broken, etc. Such faults can be repaired online easily. Faults like bearings damage, motor damage, motor burn are examples of major faults, which should be repaired or replaced off-line.

Some assumptions are followed for the present system. First, it goes into partial failure state on occurrence of minor faults and into a state of complete failure, whenever a major fault disrupts the whole system. Secondly, in case of minor fault, technician should deflect the problem within a fraction of time and repair the system online. In case of major faults, the technician should inspect the system first for the faulty component and then carry out the offline repairing or replacing the faulty components, if required.

Gupta[9] constructed the single-unit shock model and analyzed its behavior by using RPGT. Goel *et al.*,[10] analyzed availability of banking server with warm standby unit by using RPGT. In order to analyze the nature of maintenance system, many researchers including[11-13] considered minor, neglected and stoppage on minor faults. Garg *et al.*,[14] measured various performance parameter of a stochastic model of a two-unit hardware software system. It is observed from the literature that researchers never discussed a two-unit HPTLC system by considering both major and minor faults simultaneously.

OTHER ASSUMPTIONS

1. Proposed System has two identical units.
2. Faults are self-announcing in the system.
3. Operative, partially failed and failed are the three only modes for each unit.
4. There is a single repair fault facility.
5. All the random variables are mutually independent.

Notations:

λ_1/λ_2	Minor/major failure rate
$h_1(t), h_2(t)$	Probability distribution function of repair rate
$H_1(t), H_2(t)$	Cumulative distribution function of repair rate
$O_r/O_w/O_{cs}$	Operative unit under repair/ waiting/Oil standby
F_r/F_w	Failed unit under repair/waiting

State Transformation Diagrams

In State transition diagram, all states are regenerative states while state 3 & 4 are failed states.

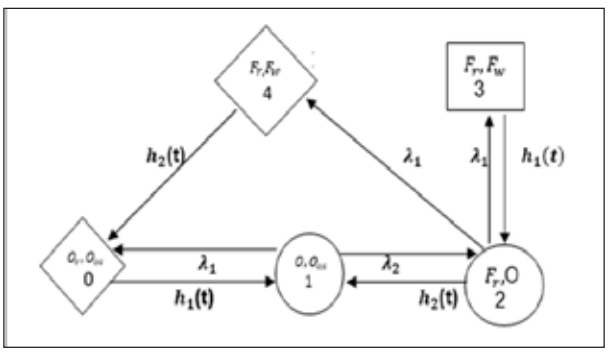

Figure 1 State Transmission Diagram

Transition Probability and Mean Sojourn Times:

The probability distribution function for each transition state is given below:

$$q_{01} = \lambda_1 e^{-(\lambda 1+\lambda 2)t}$$
$$q_{02} = \lambda_2 e^{-(\lambda 1+\lambda 2)t}$$
$$q_{20} = e^{-(\lambda 1+\lambda 2)t} G_2(t)$$
$$q_{23} = \lambda_1 e^{-(\lambda 1+\lambda 2)t} G_2(t)$$
$$q_{24} = \lambda_1 e^{-(\lambda 1+\lambda 2)t} G_2(t)$$
$$q_{32} = H_2(t)$$
$$q_{41} = H_2(t)$$

The Transition probabilities p_{ij} are obtained Laplace transform of q_{ij}:

$p_{01} = \lambda_1/\lambda_1+\lambda_2$
$p_{02} = \lambda_1/\lambda_1+\lambda_2$
$p_{23} = \lambda_1/\lambda_1+\lambda_2+\beta \rightarrow \lambda_1[1-h_2*(\lambda_1+\lambda_2)]/\lambda_1+\lambda_2 \ [h_1 = \alpha e^{-\alpha}$
$p_{24} = \lambda_1/\lambda_1+\lambda_2+\beta \rightarrow \lambda_1[1-h_2*(\lambda_1+\lambda_2)]/\lambda_1+\lambda_2 \ [h_2 = \beta e^{-\beta}$
$p_{20} = h_2*(\lambda_1+\lambda_2)$
$p_{32} = 1$
$p_{41} = 1$

Values of R*i*(t) Reliability:

$R_0 = e^{-(\lambda 1+\lambda 2)t}$
$R_1 = H_1'(t)$
$R_2 = e^{-(\lambda 1+\lambda 2)t} H_2(t)$
$R_3 = H_1'(t)$
$R_4 = H_2'(t)$

Values of $_{\mu i}$(t) (Mean Sojourn Times) and Probability factors Z (*i,j*):

$\mu_0 = 1/\lambda_1+\lambda_1$
$\mu_1 = 1/\alpha$
$\mu_2 = 1/(\lambda_1+\lambda_2+\beta)$
$\mu_3 = 1/\alpha$
$\mu_4 = 1/\beta$
$Z_{(0,0)} = 1$
$Z_{(0,1)} = p_{01}+p_{02}p_{24}p_{41}$
$Z_{(0,2)} = p_{02}$
$Z_{(0,3)} = p_{02}P_{23}$
$Z_{(0,4)} = p_{02}P_{04}/1-P_{23}P_{32}$

Measures of System Effectiveness: The MTSF and other parameters of the system are calculated by taking state 0 as a base state and using RPGT. Taking particular cases, $\lambda = \lambda_1 = \lambda_2 = \lambda_3$ & $W = \alpha = \beta$

Mean Time to System Failure: From Figure 1, states i=0, 1&3 are the operative states.

$T = Z_{(0,0)}\mu_0 + Z_{(0,1)}\mu + Z_{(0,2)}\mu_2 + Z_{(0,4)}\mu_4$

$T = ½ [(1/\lambda) + (1/W) + (\lambda/W)(2\lambda+W) + (\lambda/2\lambda) + W + \lambda(\lambda+W)]$

Availability: From Figure 1, all states are regenerative and states i = 0, 1 & 3 are only available states.

A = T/D

Where $T = Z_{(0,0)}\mu_0 + Z_{(0,1)}\mu + Z_{(0,2)}\mu_3 + Z_{(0,4)}\mu_4$

$D = Z_{(0,0)}\mu_0 + Z_{(0,1)}\mu_1 + Z_{(0,2)}\mu_2 + Z_{(0,2)}\mu_3 + Z_{(0,4)}$

Busy Period of the server: From figure 1, the server busy at states at j=1,2,3 & 4. For base state '0'.

$B = Z_{(0,1)}\mu_1 + Z_{(0,2)}\mu_2 + Z_{(0,2)}\mu_3 + Z_{(0,4)}\mu_4/D$

Table 1 MTSF for different value of repair rate.

λ	T (w = 0.6)	T (w = 0.8)	T (w = 0.9)	T (w=1)
0.01	50.86304	50.64489	50.57252	50.51475
0.02	25.89113	25.66398	25.58888	25.52903
0.03	17.58442	17.34898	17.27134	17.20953
0.04	13.44301	13.19995	13.11992	13.05627
0.05	10.96703	10.71691	10.63465	10.56926
0.06	9.323232	9.066587	8.98223	8.915207
0.07	8.154555	7.891864	7.805542	7.736971
0.08	7.282508	7.014205	6.926036	6.856003

Table 2 Availability for different value of repair rate

λ	A (w = 0.6)	A (w = 0.8)	A (w = 0.9)	A (w=1)
0.01	0.999736	0.99985	0.999881	0.999903
0.02	0.998995	0.99942	0.999538	0.999623
0.03	0.997851	0.998745	0.998996	0.999178
0.04	0.996367	0.997852	0.998275	0.998584
0.05	0.994602	0.996771	0.997395	0.997854
0.06	0.992607	0.995524	0.996375	0.997005
0.07	0.990426	0.994137	0.995232	0.996047
0.08	0.988098	0.992629	0.993983	0.994996

Table 3 Busy Period

λ	A (w = 0.6)	A (w = 0.8)	A (w = 0.9)	A (w=1)
0.01	0.017228	0.012882	0.011439	0.010286
0.02	0.035389	0.026437	0.023464	0.021092
0.03	0.054228	0.040535	0.035979	0.03234
0.04	0.073527	0.05506	0.048894	0.043961
0.05	0.093099	0.069909	0.062127	0.05589
0.06	0.112784	0.084988	0.075606	0.068067
0.07	0.132452	0.100215	0.089262	0.080438
0.08	0.151994	0.115519	0.103038	0.092952

Table 4 Server Visits

λ	T (w = 0.6)	T (w = 0.8)	T (w = 0.9)	T (w=1)
0.01	0.019814	0.019863	0.019879	0.019891
0.02	0.039187	0.039406	0.039477	0.039533
0.03	0.058036	0.058572	0.058745	0.058881
0.04	0.076298	0.077313	0.077641	0.077899
0.05	0.093929	0.095593	0.096132	0.096557
0.06	0.110902	0.113382	0.11419	0.114827
0.07	0.127201	0.13066	0.131794	0.132691
0.08	0.142822	0.147414	0.14893	0.150132

Table 5 Profit

λ	T (w = 0.6)	T (w = 0.8)	T (w = 0.9)	T (w=1)
0.01	79945.15	79958.65	79962.46	79965.26
0.02	79842.64	79889.43	79902.62	79912.27
0.03	79699.9	79795.65	79822.85	79842.79
0.04	79523.62	79680.4	79725.38	79758.48
0.05	79319.7	79546.5	79612.28	79660.93
0.06	79093.36	79396.56	79485.5	79551.59
0.07	78849.14	79232.94	79346.8	79431.84
0.08	78591	79057.76	79197.82	79302.95

Graphical Study

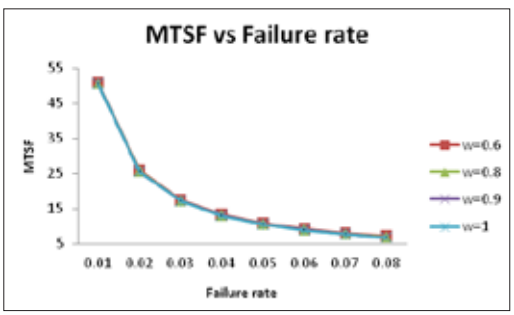

Figure 3 MTSF vs Failure Rate

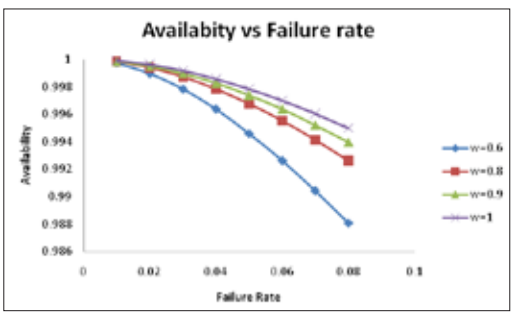

Figure 4 Availability vs Failure Rate

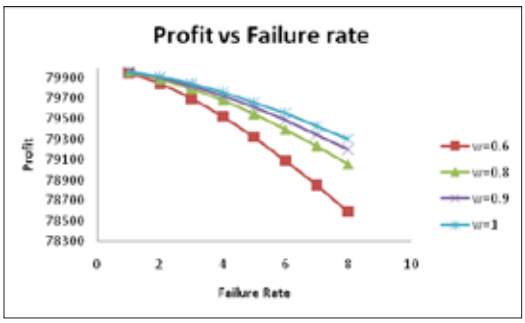

Figure 5 Profit vs Failure Rate

Expected visits by Server: From figure 1, the server visits anew for repair at j=1,2,3 & 4.

$E=Z_{(0,1)}+Z_{(0,2)}/D$

Profit: $P = C_1A-C_1B-C_1E-C_1$

Where,

C^0 = Revenue when the plant is in up-state per unit time

C^1 = Cost of repairmen engaging for repair

C^2 = Cost of repairmen visit

C^3= Loss during down-state of the plant

RESULT & DISCUSSION

Profit of the system model goes on decreasing with the increases of failure rate. Profit decreases if failed unit has more chances of repair. According to the analysis, there are several revenue cut-offs points in up-time per unit with full capacity to increase the system's profit.

CONCLUSION

A two-unit HPTLC system is considered having major and minor faults with single repairman. By using tables and graphs, we can easily conclude that availability of two-unit HPTLC system decreases with increasing failure rate. Moreover, profit also decreases with increase in failure rate. All performance measures decrease if unit has more chances of complete failure than partial failure. In future, we intend to focus upon the study of performance optimization of two-unit HPTLC system. IJFMP

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ORIGINAL ARTICLE

Lie Detector Test and Its Admissibility in the Court

Surabhi Verma[1], Selvaraj Premkumar Subashini[2]

ABSTRACT

Law enforcements round the world have always been inclined to the use of Lie Detectors. Lie Detector or the Polygraph test is one of the results of technological advancements. The Indian Law although doesn't allows the application of the Lie Detectors in the courts to be used as an evidence. This paper will give you a background check of lie detectors and despite of the fact that it isn't allowed, how effective has this been proved in cases. If we look at our ancient sciences also, the Ayurveda the technique to identify if a prisoner is at fault or not is the change in behavior, body language. We now call it in the modern language as psychology of an individual. This will also give a comparison of the admissibility rules of the same in different countries and how is it useful or not in their individual scenarios. Let us look at a brief history of how it was developed, the first application in a case and in the coming years do we see any changes in our Indian system of law for its application.

KEYWORDS | lie detector, forensic evidence, polygraph, narco test

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INTRODUCTION

The concept behind lie Detectors is that when a person is trying to deceive the investigator of his crime, his body tends to react under stress. The individual undergoes physiological and psychological changes such as happiness, sadness, anxiety etc. These different emotions produce different physiological and psychological responses. Talking about physiological responses, here we talk about the pulse rate, respiration rate, blood pressure, skin temperature, perspiration, etc. The physical responses indicate the body posture, the body movements, movements of limbs, facial expression, etc. This is what we put together in a test known as Polygraph Test. In a polygraph test when the accused is interrogated of his crimes, his body tends to produce a hyper arousal state/stress/fight and flight mode of his body will be turned on. This in turn will result into physiological and psychological changes. Hence, the intent of using this test is based on the fact that the suspect would show unusual psychological and physiological responses while the innocent will show none of these responses, which can be identified in a polygraph test.[1]

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HISTORY

The first polygraph is based on the works of an Italian Psychologist, Vitorio Benussi, whose seminal work on the respiratory system of the lie was published in 1914 following which, William M. Marston, a psychologist, lawyer and author of *Detection of Deception* using the concept of discontinuous systolic blood pressure invented the first prototype of Lie Detector. In 1921, the first polygraph was invented by John A Larson, a physiologist and a California-based policeman utilizing the above mentioned inventions. Device, which monitors ceaseless alterations in respiration rate, blood pressure and heart rate are an add-on in detection of deception. Larson's early research on polygraph became the basis of the Relevant/ Irrelevant Question Techniques of the polygraphy testing procedure by Leonarde Keeler.

Using the researches of Fordham University Graduate School Psychologist Reverend Walter G. Summers, Keeler created transportable polygraph and in 1938 seasoned the same with Galvanic Response channel(GSR).

The practice of new testing procedure and use of polygraphy did not seek a peer review of their published work, hence the practice was not in alignment to the academic advancements in the field. The limited researches in the field of polygraphy or deception detection in the later years became a basis for the academicians to question on techniques and principles for the same.[2]

INDIAN HISTORY

Going back to the Vedic times about 900 BC, vedic texts mention the identifiable behavior of prisoners that made them confess to their crime.[3] In ancient India, according to Vedas, we had a complete system of criminal investigation, the investigators, and jails. They even had to defend themselves in cases of self-defense, in defense of women or of weaker section who cannot take a stand for themselves.[4] Manusmriti, which is considered to be an ancient guide to legal laws and constitutions in the Dharamshastras of Hinduism, specifies the role and responsibilities of a judge to identify the accused and the witness by specifically analyzing their posture, mind, changes in tone of voice, movement of eyes. Chapter seven, twenty fifth paragraph of the Manusmriti states that, the judge should analyze the internal character of the person by examining his external signs for example, voice, their color, movements, aspects, movement of eyes, gestures, etc. Twenty sixth paragraph states that psychological aspect is apprehended by the aspect, motion, gait, speech, changes in the eyes and facial expressions. This ancient legal of law was one of its first to take into account the psychological aspect into judicial systems. Even flattening of voice, licking corner of lips, speaking in an unclear tone, paleness of faces, constant coughing are the manifestations of the guilty as per Mitaksara of the Yajanvalkya Smriti.[5]

INDIAN STATUS

Set of rules by the National Human Rights Commission, 12 November 1999, post collecting after receiving numerous grievances in opposition to the Polygraph Test, set of regulations, including non-compulsive and consented behaviors were constructed to deliver the test. The guidelines, weren't regulated by any law and could suggest to make a subject dragoon into becoming a witness against himself, as per The Indian Constitution Article 20 (3) "No person accused of any offence shall be compelled to be a witness against himself." Polygraph tests are still legal if the defendant requests one, which violates the constitutional immunity from testimonial compulsion. The Commission, after bestowing its careful consideration of this matter of great importance laid down, the following guidelines relating to the administration of Lie Detector Test:

- The Accused cannot be delivered without informed consent of the accused.
- If the accused volunteered, access to a lawyer to be given.
- The police and the lawyer should explain the physical, emotional and legal implication of the test.
- The procedure of consent towards the test be made in the presence of Magistrate, wherein the accused is represented by the lawyer.
- During the hearing it should also be explained to the accused that the statement made isn't "confessional" but a statement made to the police and not to the judge.

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- The Magistrate has to look into the points considering duration of detention and the type of interrogation
- The real-time procedure to be recorded in a separate unit like a hospital, in the presence of a lawyer
- An account of a complete medical and unprejudiced information must be recorded

The Commission disseminated the regulations to the Chief Secretaries and DGPs of States as well as Administrators and IGPs of UTs by a letter dated 11 January 2000.[6]

A woman was sentenced in 2008 based on the Brain Oscillation Signature Profiling as evidence in the court, it was a first of its kind of attempt.[7]

According to the Article 20(3) of the Indian Constitution : "No person accused of any offence shall be compelled to be a witness against himself." And Polygraph tests applicable on consideration of the requests of the defendant.[1]

The Criminal Procedure Code, Evidence Act and the Constitution nowhere accepts the results of polygraph as the only evidence in a court of law in India as the scientific basis is prone to error. Regardless of this, it is been immensely utilized as an abet to probing an accused.[1]

Again a bench, lead by the Chief Justice of India 2010, of the Hon'ble Supreme Court stated in a decree on polygraph and other new branches of forensic psychology. The salient features of this judgment, vis-à-vis polygraph interrogation are:

- Polygraph can only be disseminated to the accused on an informed consent in the presence of a magistrate
- Test cannot be enforced on the subject on compulsion
- The material and other evidence procured of the polygraph interrogation can be used as evidence under the Section 27 of the Indian Evidence Act
- Proof from a polygraphic examination cannot be individually admissible until supporting evidences are there
- Unconsented examinations results into third degree methods which aren't permitted by the court of Law
- The importance of undermining the accused cannot be done on the cost of witnessing against self
- The members of the parliament can consider formulating a law relating to the admissibility of the involuntary tests considering cases of public interest[1]

Effectiveness of Polygraph

Effectiveness of polygraph has contradictory statements by the various associations. The American Polygraph Association calls it "highly accurate" declaring the accuracy rate to be 90% considering the usage, methods and techniques to be correct. On the contrary, experts by their critical analysis say the accuracy rate is only 70%.[8] Prof. Don Grubin, a trainer of polygraph examiners, UK, states the same in agreement to the American Polygraph Association that a well-trained examiner, correct methodology, and quality controls contributes the test to be 80%-90% accurate which is more than the ability of even an experienced personnel to identify if someone is lying.[9] A contradictory work by Verschuere, B., Ben-Shakhar states that the polygraph are based on stress levels and thus point out that an individual can feel stressed even while he is stating truth during the process of interrogation during a Lie Detector test. They have alternatively proposed to rely on emotional responses during interrogation, called the Concealed Information Test.[10] In alignment to the above mentioned work, an article reviewing 16 empirical studies on the utilization of MRI to detect lie concludes that this technology is not scientifically reliable as it marks the absence of consistency amongst the findings and deprivation of.[11] In another book by Rutbeck-

Goldman, discusses the negative implications and civil rights concerns in the usage of polygraphs. [12]

The editor of the *Journal of Indian Academy of Forensic Medicine* points out that all the Deception Detection Tests as modern techniques in forensic medicine are fruitful in interrogating the criminals.

Instances of Use in India

A few of the high profile cases where polygraphy was applied:

The ₹3,000 crore stamp paper scam mastermind Abdul Karim Telgi was put through a truth serum or narco analysis test, the P-300 brain mapping test and a polygraph test by the Maharashtra police.

In the Aarushi-Hemraj murder case, the CBI had subjected her parents, Rajesh Talwar and Nupur Talwar, to a polygraph test in 2008. Three men, Krishna, Raj Kumar and Vijay Mandal – all servants from their neighborhood – were also put through the test.

In the Nithari Serial Murder case, in which skeletons of 17 missing children were recovered from inside a house in Noida, house owner Moninder Singh Pandher and his servant Surinder Kohli were arrested and were administered brain mapping and polygraph tests in 2007. [13]

Another case in the Kerala Court, Rojo George vs Deputy Superintendent for allowing the narco analysis test it was stated that traditional methods of interrogation could not give any result. So the Deception Detection Test are used in investigations. And tests conducted in supervisions guarantee the fundamental rights of the citizen of India, not violating them.

In a major judgment by Madras high court in Dinesh Dalmia vs State, the court stated that where the accused didn't confess to a crime the scientific tests are utilized by the agencies.

In case of Santokben Sharma bhai Jadeja vs State of Gujarat, the Gujarat High court held that "Narco analysis test is conducted under the supervision of doctors and proper care is taken and there is consent, with the observation of physical and mental state of the accused".

The Andhra Pradesh High Court in the State of Andhra Pradesh Vs. Inapuri Padma, relying on the previous decision by various courts on Deception Detection Tests, above decisions, another time upheld the constitutionality of Brain mapping and Polygraph tests, it was observed until the accused is apprehended by administering scientific methods, it gives them strength to accept that they cannot be taken down to make them answerable for the commission of the crimes. By administering Brain mapping, Narco analysis and Polygraph tests, it is not known what statement comes from the person who undergoes the test, implicating or not. Therefore, the protection given under Article 20(3) from compulsory testimony cannot be applied in respect of these tests.

In a recent judgment in July 2020, Haryana court in 2019 had allowed Rud and Baljinder to undergo Forensic Psychological test at DFSL Gujarat in Maninder Singh murder case. Maninder Singh, a resident of Radur village in Yamunanagar district, Haryana, was found missing after he stepped out with his friends - Rud and Baljinder. The two claimed that Maninder had drowned near Darbar Ghat. As Maninder was a swimmer, his family refused to accept the statement of his friends. The court admitted results of the forensic psychological test of BEOS (Brain Electrical Oscillation and Signature Profiling) and established the evidence that the friends had no participation in Maninder singh's drowning in the canal of Yamuna and it could be accidental. The forensic report validating no role of the duo in the drowning of Maninder was held valid in the court. It was revealed by the court that Maninder was in an intoxicated state when he jumped into heavy flow of water, which then led to the death of Maninder Singh. Thus, the court upheld the Forensic psychological test findings.[14]

DISCUSSION

The utilization of deception detection techniques has been a common practice. The utmost requirement is the growth of researches and hence developing of the best practices for this technique. And with the scientific basis of this technique, the acceptance will have solid grounds in the court of law. There will be more wide approval and fair usage of DDT all over the world.

CONCLUSION

Despite the fact that the Deception Detection techniques are extensively used by the police

interrogation, it still holds back its worth of being an individual evidence in the court of law. Over a century of utilization of these techniques, still the validity and extensibility remain questionable. An extensive research and objective evaluation of the new techniques should be available for the federal agencies. In the present era of developing new techniques, they will be developed and oversold by entrepreneurs. In addition to the above mentioned, the slow and progressive acceptance as evidenced by many cases in the court of Law states that there is still room for DDT being valid and accepted. Many journalists, officers accept the true utilization and acceptance of the same.

Though the use of lie detectors is pretty common, it remains the least researched area of forensic science. Considering this fact, it is to be stated that it still remains a vague area which need to be explored further. IJFMP

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ORIGINAL ARTICLE

Handwriting Accents to Reveal Cultural Identity of Author: An Indian Approach

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ABSTRACT

Handwriting is a neuro-muscular phenomenon, which is affected by the author's gender, age, ethnicity, the system of handwriting learned, content, purpose, writing protocol, whether written from memory, dictated, or copied out, writing instruments, changes in the handwriting of an individual over time, etc. It has been established by studies that handwriting is an acquired skill. Handwriting is also related to the cognitive aspects of the brain with the muscular movements. Therefore, it can be hypothesized that a certain degree of commonness exists in the handwriting features of the people of the same population group. Few researches had been conducted in this aspect to identify the ethnic origin of author through distinctive characteristics of their handwriting, which reflects their ethnic origin. Out of these works, a couple of studies took quantitative approach to statistically examine the characteristics of handwriting. This research will help in identifying the authors of anonymous writings. Also the work will help the anthropological researchers in identifying the ethnic origin of writer based on their ethno-linguistic handwriting features. We approach this problem using multilingual writers. We have focused on a very distinctive ethno-linguistic groups such as Telugu, Malayalam, Bengali and Hindi writers, to double check the results. Further comparison is done with the findings of the data obtained from control population. Each set of group contains 50 samples.

KEYWORDS | Forensic Linguistics, Handwriting, Author Identification

INTRODUCTION

India is home to a wide variety of ethno-linguistic groups, which can been witnessed in their different religious practices, cultural traditions and various languages spoken in the country (Pal, U., Jayadevan, R., and Sharma, N. 2012). Being an acquired skill perfected by an individual with practice over a long period of time, handwriting is categorized as the most complex perceptual-motor skill. It is a neuro-muscular phenomenon in which the image formed by brain is replicated on a writing surface via a writing instrument with the help of muscles of limb (Sharma, 2017). The chirography of an individual's writing is a highly individualistic characteristic which represents the form of writing. It includes the penmanship, use of abbreviations, different shape and size of letters. The chirography is affected by many factors which includes ethnicity, mother tongue, purpose of writing, writing surface, writing instrument, gender, age, changes in writing with time, and most importantly whether the writing is written from memory, dictation or copying (Osborn, 1929).

Any individual's handwriting development begins with mere scribbling on the surface and later these scribbles take the form of letters and figures of a particular language script. It has been observed that the handwriting practice

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which a child acquires generally is copied from the adults around him or her during their early cognitive development. It has been studied and widely accepted that for a 2-year-old child, it is acceptable that he/she start using straight strokes to imitate the orderly shapes given to him/her to copy. The usage of horizontal strokes is observed at the age of 2 years and 6 months and further 6 months later, that is, at the age of three years the children get familiar with the usage of round formations too. This habit of imitating shapes to get accustomed to the writing of script is being widely used across the world to ensure the learning of a particular script. It has been observed that at the age of 5 years 6 months the children are fully familiar with different types of strokes whether they are straight, horizontal, round, vertical, square or triangle, this implies that the child is ready for learning the formation of letter and figures.

The importance of which language is learnt as a first writing language cannot be more emphasized when viewed under the lens of study conducted by Feder in 1991. Feder studied the handwriting habits of children aging from age of 6 years up-to middle school. In this study it was concluded that children with age 6 years to 7 years the handwriting quality and penmanship grows quickly which reaches a stability by the age of 7 years to 8 years. The habit of chirography is seemed to be developed at the age of 8 years to 9 years. Another phase of development is seen in terms of involuntary action, organizations usage of vocabulary to characteristically arrange thoughts into words is observed at the stage of middle school.

Revealing the ethnicity of an individual by means of handwriting identification is termed as handwriting accents. (https://www.translatemedia.com/translation-blog/handwriting-accents-penmanship-can-betray-language-identity/). Handwriting recognition has been in practice since late 1800s. First case of handwriting recognition was reported in France in 1894 under the name *"Bordereau Story"* (http://www.lprinfo.com/history-handwriting-analysis.htm). The relevance of determining handwriting accents is not a new concept, it was first used in criminal investigation in 1932 in the case of kidnapping of Charles Augustus Lindbergh, Jr., in the state of New Jersey, USA (https://www.fbi.gov/history/famous-cases/lindbergh-kidnapping). Earlier the accent determination was based on the science of linguistic, now-a-days the researchers are basing this determination using the handwriting characteristics.

Related Works: The basis of handwriting recognition relies on the fact that every individual has his/her own personalized handwriting. The handwriting is an acquired skill, as an individual we start learning handwriting at primary school level where we learn writing by copying the pen-printed style of letter formation (Osborn, 1929). As the time progresses our handwriting becomes personalized with changes coming at regular intervals due to factors such as enhanced skill, more practice, increased speed of writing, increasing age, illness etc. (Hilton, 1963). These changes which occur in handwriting happens in the general handwriting characteristics whereas the basic letter formation i.e., the individual writing characteristics remains unchanged. This fact is the basis for the identification of the handwriting of an individual (Hilton, 1963).

As spoken language the handwriting of an individual is also affected by the fact of his/her own mother-tongue. As in spoken language the mother-tongue affects the accent of the any other language learnt to be spoken afterwards same accent can be detected in the secondary language learnt to write after the mother-tongue. This accent is referred to as the handwriting accent of an individual. (*https://www.translatemedia.com/translation-blog/handwriting-accents-penmanship-can-betray-language-identity/*). Many researchers have argued that determining the ethnicity or ethnic origin from the handwriting of an individual is difficult. But on the other hand, there are immense proof available in the literature where the ethnicity or the ethnic origin of an author can be determined from the way of writing the secondary language learnt (Kapoor & Saini, 2017; Hadhrami, 2017; Farooq, Lorigo & Govindaraju, 2006; Cheng 2005).

Determining the handwriting accent is extremely beneficial in the field of forensic science. With the results obtained one can establish the identity of the anonymous authors, threat, ransom

letters, which ultimately help the investigating agencies to narrow down the list of the suspects. Also, for this purpose the science of linguistic also plays a major role. The fact that the learning of a secondary language is greatly affected by the mother tongue is well established in the linguistic science (Shabani, *et al.* 2016; Denizer, 2017; Yigzaw, 2013; Mehrabi, 2014). The results of the studies indicate that when a secondary language is spoken its accent, grammar and pronunciation is greatly influenced by the mother tongue of the individual. When the same secondary language is written, the same influence is observed there also. This fact can be established by using the technique of text-mining of computing science (Ramaiah, *et al,* 2012). It has been observed that the characteristics influenced by the movement of the limbs are affected by the writing movements of mother tongue. Also, the basic letter formation, the movement of strokes is found to be influenced by the mother tongue. By determining such similarities one can establish the handwriting accent of the questioned author.

Objectives:

The objectives of the research work are summarized below.

1. To identify the individuals with bi-lingual writing characteristics in the target population.
2. Establish the similar letter formations in both first and second languages.
3. Identify the handwriting accent on the basis of regional ethnicity.

METHOD

For a study of handwriting pertaining to different individuals, the sample handwritings from various persons should be available. To collect such samples of handwritings the following protocol will be used:

The standard format to obtain Handwriting exemplar is known as 'London letter'. This letter comprises all the alphabets in lower case as well as the upper case of English script, and offers a good number of characters for thorough examination. The bi-lingual writers who can write in their mother tongue as well as in the English language easily will be asked to provide their handwriting samples in English language. The sample for each first language is 100 in the age group of 21 to 30 years. The analysis of the writing samples will be carried out by examining the handwriting habits (similarities and dissimilarities) along with the natural variations. For a thorough and scientific examination, lenses of various magnifications (2X approx.) were used along with stereomicroscope.

Examination is done to see whether the samples exhibit the characteristics of a freely and naturally prepared writing. Some of the characteristics of naturally prepared writing include consistent slant and size, thickening and thinning of the lines as the writing instrument changes direction, and tapered beginning and ending strokes that occur once the writing instrument comes into contact with or leaves the paper. Additionally, each body of writing is examined to assess internal consistency, comparability, and variation and to determine the presence or absence of individualizing characteristics. Statistical operations are to be utilized to test the validity of the hypothesis.

Sl. No.	General Characterists
1	Alignment
2	Skill
3	Slant
4	Speed
5	Spacing
6	Relative Size

Sl. No.	General Characterists
7	Proportion of Letters
8	Movement
9	Rhythm
10	Pen Pressures
11	Line Quality

Table 1 General Characteristics identified

General Characteristics	Sub-Category	Numerical Nomenclature
Alignment	Horizontal Ascending Descending	1 2 3
Skill	Inferior Medium Superior	1 2 3
Speed	Slow Medium Fast	1 2 3

Table 2 Sub-Categories and their Numerical Nomenclature

General Characteristics	Sub-Category	Numerical Nomenclature
Slant	Vertical Forward Backward	1 2 3
Relative Size	Small Medium Large	1 2 3
Line Quality	Smooth Consistent Normal	1 2 3
Proportion of Letters	Same Different	1 2
Movement	Finger Combined with Wrist	1
	Wrist Combined with Forearm	2
	Finger Movement	3
	Wrist Movement	4
	Forearm Movement	5
Spacing	Narrow Medium Wide	1 2 3
Pen Pressure	Light Medium Dark	1 2 3
Line Quality	Jerky Interrupted Smooth	1 2 3

Table 2 General Characteristics: Sub-Categories and their Numerical Nomenclature (continued...)

Type of Alphabet	Name of Alphabet
Letters above the Line	B, L
Letters below the Line	G, y
Letters on the line	a, R

Table 3 Individual Characteristics: Letter formation

Data Set	1st Language	2nd Language	Labeling
Set-I	Telegu	English	S1 to S50
Set-II	Malayalam	English	S51 to S100
Set-III	Bengali	English	S101 to S150
Set-IV	Hindi	English	C1 to C50

Table 4 Segregation of data based on first language

RESULT & DISCUSSION

For the purpose of analysis of the data the writers with Telugu as first language are treated as test samples, Set-I labeled as S1 to S50, Malayalam as first language are treated as test samples, Set-II labeled as S51 to S100, Bengali as first language are treated as test samples, Set-III labeled as S101 to S150 and the writers with Hindi as first language are treated as control samples, Set-IV labeled as C1 to C50 (refer Table 4). From the data analyzed for general and individual characteristics, it is found that the writers of set I exhibit similar general handwriting characteristics specially in terms of alignment, relative size and proportion of letters which is in accordance with that of their first language. The same results were observed for the writers of set II, III, IV.

For individual characteristics the following common observations were made for Set I:

- Spur commencement of 'g' with retrace at apex and rotundity of oval, formation of elongated loop with compression at base.
- Rotundity of oval of 'R', formation of retrace at buckle part as well as tapered curve finish and straight nature of staff of character.
- Inward hooked commencement of 'a', rotundity of oval, formation of a twist/ a retrace at staff and peculiar curve finish.
- Slanted nature of staff of 'B', rotundity of lobes, formation of leg at buckle part, direction of extent of character.
- Shape of body curves parts of 's', formation of cusp at apex and an impulse at base of these character as a variation.
- In 'i' dot, formation of circle and its relative location and size.

For individual characteristics the following common observations were made for Set II:

- tick commencement of 'g' with retrace at top and round circular formation, formation of elliptical loop with compression at base.
- Sphericalness of oval of 'R', formation of buckle at the mid body, with tick curve finish and round nature of staff of character.
- Outward hooked commencement of 'a', roundness of oval, formation of a twist/buckle at staff and distinct curve finish.
- Round/curved start of staff of 'B',

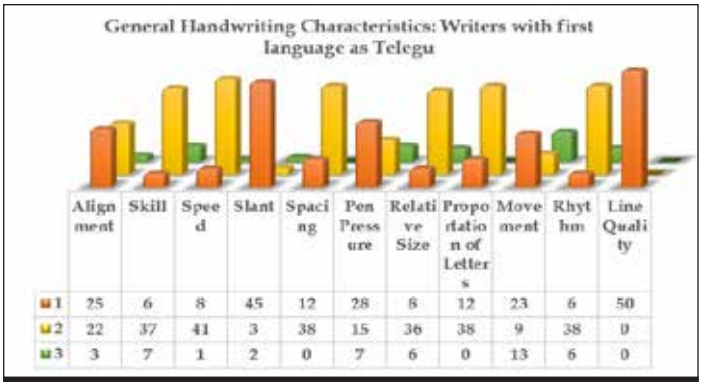

Figure 1 General Characteristics: Sub-Categories and their

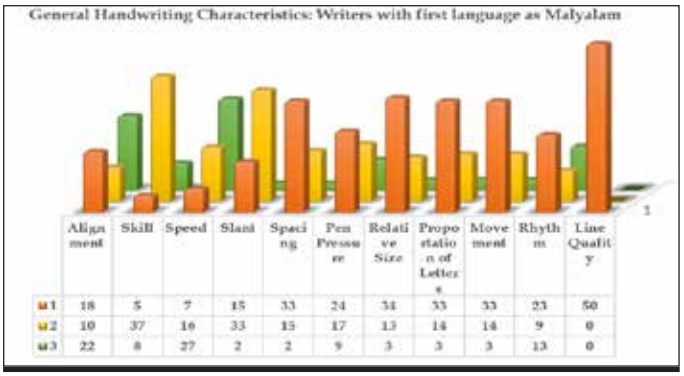

Figure 2 General Characteristics: Sub-Categories and their

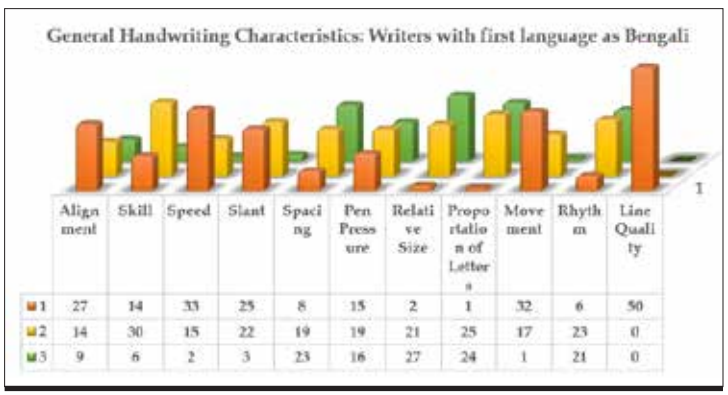

Figure 3 General Characteristics: Sub-Categories and their

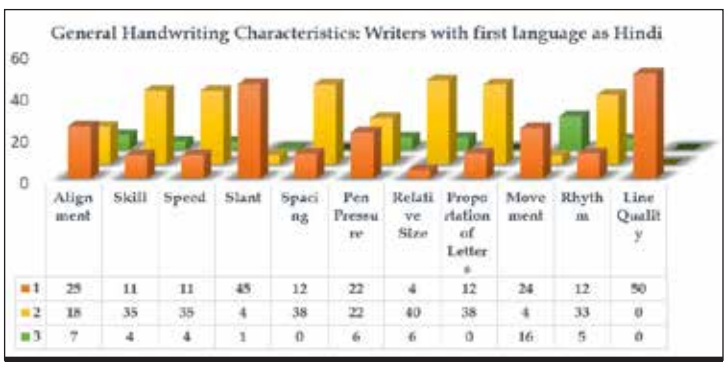

Figure 4 General Characteristics: Sub-Categories and their

sphericalness of lobes, formation of buckle at mid body, short and round appearance of character.

- Shape of body curves at start and end with small loops of 's', formation of point at apex and an impulse at base of these character as a variation.
- In 'i' dot, formation of circle and its relative location and size.

All these characteristics are found to be in accordance with the letter formation style of their mother tongue.

CONCLUSION

The examination of the writings of different individuals was undertaken. Their writing habits have been studied the both general and individual. The aspect of natural variation, with regard to its nature and extent, was also studied from these writings. Like-with-like comparison of the writings of each individual show that they show consistency. It was also observed that each individual has a unique set of writing habits. It is thus concluded that two individuals show similar set of writing habits.

Since the sets of handwritings show very surprising similarities of individual characteristics in letters of common words, which are greatly influenced by the script of the author's mother tongue, which explains why these characteristics are not found in concordance with the established principles of handwriting science.

With respect to the results obtained it can be concluded that the handwriting of an individual is greatly influenced by the mother tongue of the author, which in turn can determine the accent of handwriting. The results obtained here are in concordance with the hypothesis of the experimental work, which was spoken language affects the letter formation of the second written language learnt subsequently. IJFMP

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ORIGINAL ARTICLE

Bio-polymeric Nano-particles for Prospective Forensic Applications: A Futuristic Approach

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ABSTRACT

Despite its limited applications, nanotechnology has made significant contribution in a broad scientific arena. The technology is concerned with the development of new materials and reagents with conventional characteristics. Nano-particles are made from a variety of substances such as biopolymers, and synthetic polymers. The nano-particles synthesized from bio-polymers have benefits over synthetic polymeric nano-particles which include biocompatibility, non-toxicity, cost-efficiency and bio-degradability. The use of nano-particles have gained a lot of attention in recent years. The use of the bio-polymeric nanoparticles provides new opportunities in surface-based applications. The fingerprint detection is based on surface phenomena to which nanotechnology can be applied. This article reviews the forensic application of bio-polymeric nano-particles and their use in development and enhancement of latent fingerprints. The use of nano-particles has emerged as a powerful tool for improved forensic analysis. It also provides deep insight into the future possibilities of nanotechnology.

KEYWORDS | nano-particles, bio-polymer, synthetic polymer, latent fingerprint

INTRODUCTION

Nano-science deals with the nanotechnology which involve the use of nano-materials. Nano-materials are very compressed length scale structures ranging from 1-100nm. Their significant size have advantages over microspheres (>1μm) due to their large surface area. Nano-particles can be easily manipulated based on their size and surface characteristics. Efficient methodologies can be developed with minimized pollutant effect by understanding the formation and growth of nano-particles. Nano-particles provide surface for the covalent linkage via traditional coupling like carbodiimide-mediated amidation and esterification. Because of their efficient size, NPs are most preferably used in drug delivery system and can be easily absorbed by blood capillaries and cell barriers. Due to their multi-functional characteristics, NPs have varied range of applications in the field of agriculture, pharmaceutical, food packaging industries, medical sciences, and its blend with forensic techniques have improved effects in analysis tools.[1,2] Nono-particles can be synthesized using different materials, like metals, semiconductors, synthetic

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polymers which possess adverse effects like toxicity risks and are non-biodegradable in nature. To overcome the side effects of these nano-particles, biopolymeric nano-particles can be synthesized from proteins and polysaccharides. These bio-polymeric nano-particles revolutionized the world of bio-degradable, non-toxic and bio-compatible materials.[3,4] In addition to various applications, nanotechnology has its significant use in Forensic science.[7] Nanotechnology applications include pharmaceuticals, drug delivery systems and food packaging industries. Based on the characteristics like biosensing ability, nano-particle-protein linkage ability makes them suitable to be used as an aid to forensic analytical tools. The nano-particle-protein interactions can be used for multiple biological processes such as protein-protein interaction that can be used in fingerprinting analysis. The main goal of forensic analysis involve sensing of biological agents, diseases, and toxic materials. This paper gives an outline on the most available bio-polymers used for synthesis of nano-particles and their application in development and enhancement of latent fingerprints along with other future approach in forensic analysis.

Recently, there has been great interest in the use of nanotechnology in the design of new fingerprint detection systems. This is due to the fact that microparticles can provide improved latent fingerprint detection by using dye-functionalized microparticles (the dye or fluorophore may also be encapsulated within the microparticle) which can therefore provide an opportunity for improved visualization.

Bio-polymers used for preparation of nano-particles Chitosan based nano-particles

Chitosan is the most abundant natural occurring biopolymer having excellent physiological and biological properties. Though it is not soluble in aqueous solution but graft modifications due to its functional groups it can be chemically modified to improve its solubility and as consequence increases its applications. It is a linear homopolymer constituted of β-(1,4)-linked N-acetyle-glucosamine units derived from crustaceans and insect exoskeleton. They are the major sources of chitin from which partially decacetylated polymer chitosan is obtained. It has excellent biological properties like non-toxicity, biodegradability, mucoadhesive and antimicrobial properties which make it attractive even in biomedical field. Many of chitosan derivatives can also be obtained as it has active hydroxyl group and amino group that can undergo various chemical reactions such as hydroxylation, carboxylation, alkylation, acetylation, and esterification. These reactions help in acquiring the modified chitosan by introducing pendent group and destroying its crystal structure which consequently increases its solubility. These derivatives also increase its application except to be used in limited fields. Chitosan based nano-particles have the power to bind with the empty orbitals of some materials due to large number of lone pairs present in it. Mostly these are used in drug delivery, gene delivery, biosensors and in fractionated imaging. Chitosan nanoparticles can be prepared by ionic gelation method where the electrostatic interaction between the tripolyphosphate (TPP) and polyanion result in the formation of chitosan nanoparticles and hence the size of the chitosan nanoparticle can be controlled by controlling the concentration of chitosan and TPP under controlled pH conditions24. RecentlyChitosan based nanoparticles can be used for the enhancement of latent fingerprints due to its lipophilic nature. Chitosan aids in the attachment of nanoparticles to the frictional ridge and enhances contrast, making the fingerprint identification possible. The enhanced contrast evidently distinguishes the finger ridges and can be useful in the forensic identification. [25]

Dextran Based Nanoparticles

Dextran is a linear polysaccharide of glucose that is derived from bacteria growing in sucrose-containing media consisting of 1,6-D-glucopyranosyl linkages. It is clinically approved and biologically safe polymer. NPs of dextran can be formed due to the presence of aldehyde group in dextran chain after the oxidation. The units of glucose in dextran can self-assemble to form nanoparticles by forming bonds with molecules like fatty acids and amines. They are suitable to form bio-conjugates and nanogels because of their inimitable physiochemical properties. Acetal-modified dextran nanoparticles contribute to class of biocompatible and degradable materials. These are specifically used in drug delivery system and treatment

therapies. Its low cost and availability is another reason that broadens its applications. It has good water solubility and non-toxic properties. Such physiological properties of dextran and its derivatives can add an aid to different analytical tools. The application of dextran-based nanoparticles in the field of forensic science is not much explored even dextran powders showed promising result in the enhancement of latent fingers on different surfaces. The smaller dextran particles adhere to the sweat and lipid present in the fingerprint residues and result in developing latent finger impressions.[26]

METHOD & MATERIALS

Alignate-Based Nanoparticles Alginate is most of the widely investigated biopolymer in the area of nanoparticle preparation. It is naturally occurring anionic co-polymer of guluronic acid and mannuronic acid linked by a β-D-1-4 linkage, derived from cell of brown algae. It is a common pharmaceutical excipient and exhibit interesting biopharmaceutical properties, like pH sensitivity, low toxicity, biodegradability and biocompatibility. Alginate is a biodegradable, biocompatible polymer with very low toxicity. It has already claimed for the beneficial use due its properties in food and drug administration. Nanoparticulate systems made of biopolymers include promising properties as carriers and adjuvant for drug delivery as well use in the development of latent fingerprints. The nanoparticles of alginate can be prepared easily by number of methods like spray drying, ionic gelation, emulsification etc. Recent Studies reported that alginate can be used for the lifting and enhancement of footwear in blood which suggest they can also be used for the lifting of latent fingerprints on different surfaces. [23]

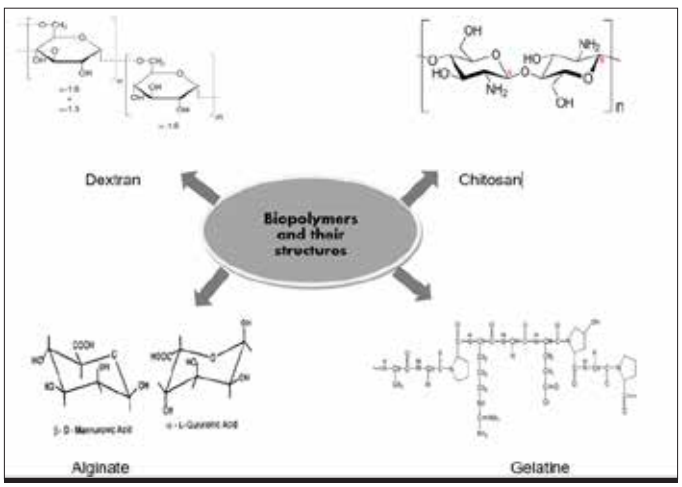

Figure 1 Biopolymers and their structures
Source: Author self.

Protine Nanoparticles

Protein-based nanoparticles are very promising as their properties related to biodegradability, less immunogenic, and non-toxicity. Protein nanoparticles can be prepared from proteins like fibroins, albumin, gelatin, gliadine, legumin, lipoprotein, and ferritin. They are relatively easy to monitor in size and easy to prepare. Due their defined primary structures, the nanoparticles offers possibilities of surface modification. Albumin is found in blood plasma and has remarkable molecular properties to its functions and applications. The particles formed are usable aid to the medication and treatment. The particles were mainly employed for treatment and diagnosis of tumor. The particles can be modified as the vehicle for the drug carrier. Collagen the most abundant mammalian protein,the structural building material of vertebrates. It has a unique structure, size and amino acid sequences that forms triple helix fibre. Nanoparticles fabricated from collagen can be modified for wide applications which include improvement and addition of other proteins, such as elastin, fibronectin and glycosaminoglycans. Collagen based nanoparticles are thermally stable and readily sterilized. Gelatin is a natural water soluble macromolecule, acquired from dissolution and partial hydrolysis of collagen. It of two types obtained from different processes. Type A-gelatin is obtained by acid treatment of collagen and Type B-gelatin is obtained from alkaline hydrolysis of collagen. It has large number of functional groups which aid to the chemical crosslinking and synthesis of nanoparticles. Keratins are cysteine rich structures. These proteins exhibit a high mechanical strength owning to a large number of disulfide bonds. Keratins are most frequently used in formation of nanosuspensions. It can provide inexpensive alternative to collagen or fibronectin. It is also applicable in tissue engineering. Gelatin lifters are used in lifting the fingerprints enhanced

using black powder. This is most effective method for lifting prints from the non-porous surface36. Nanocellulosic hydrogels are used in detection of latent fingerprints and the latent prints developed by nanocellulosic hydrogels can be easily read under the UV light. Use of luminescent hydrogels assure detection and analysis of latent prints with good visual effects.

Use of Nanoparticles for Fingerprint Enhancement

The major organic component of a fingerprint residue consists of lipids. The biopolymeric nanoparticles consists of long carbon chains which render them lipophillic.

Due to protonation of the amine group of the cationic chain of the nanoparticles formed from biopolymers creates electrostatic force on attraction towards any other ligand like gold, this stabilises negatively charged glutamic ions adsorbed in nanoparticles. This forms colloids. When any biopolymer is capped to other material like silver and gold, it makes it lipophillic. When latent fingerprints are exposed to these colloids, lipids within residues are attracted to colloid formed and improves their contrast. Their applications in fingerprint development and visualization is broad due to their small size and higher surface area of reactivity. The reaction of functional groups present in biopolymeric nanoparticles binds with the amine group in fingerprint residues. The small size of these nanomaterials allows them to bind efficiently to the minute ridges and minutae present on the fingerprint. This efficient binding results in the superior imaging of the minute details along with sweat pores. Quantum dots and rare earth fluorescent like YVO4, EU, and LaPo4, Ce, Tb significantly created more attention due to their small size, excellent fluorescent intensity, good chemical and photostability. C-dots' possess photoluminescent properties and are highly responsive to analysis such as illicit drugs, explosives, pesticides, and heavy metals. The powders enriched with C-dots significantly provide strong background surface for the development of latent fingerprints providing collaborative approach between scientist and practitioners among broad spectrum of science.

Fabrication techniques

There are three frequently used fabrication methods for the preparation of nanoparticles using polysaccharides and proteins. Emulsification, desolvation and coaservation are the three most followed techniques. Emulsification is based on spontaneous mixing of organic and an aqueous phase . The organic phase is formed of homogenous solution of oil, lipophilic surfactant and water-miscible solvent while the aqueous phase consists of hydrophilic surfactant and water. This method is called the dissolution of hydrophobic substances in an organic solvent which is emulsified at very high shear.[11] This process results in formation of very small droplets (50-100nm). Solid nanoparticles, after emulsification by removing organic solvent through evaporation. Nanoparticles were also fabricated by using desolvation process where, desolvation factors such as natural salts or alcohol are added to,protein solution. These desolvation factors changes the tertiary factors of protein. Due to crosslinking in reaching critical level with chemical substances it results in formation of nanoparticles. In two step desolvation, first step is to remove low molecule gelatin fractions present in supernatant by decanting and the second step is to redissolve the high molecule sedimented particle and desolvate again and again. Coacrvation is similar to that o desolvation where the aqueous solution is mixed with solvent like acetone or ethanol to yield coacervates. Agents like glutaraldehyde are used to limit the coacervates. The basic difference between coacervation and desolvation is the factors like pH, Protein concentration, temperature end cross linkers, affecting the properties of the desired substance to prepare nanoparticles.

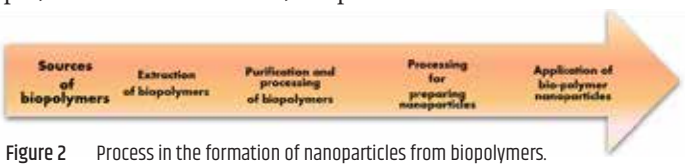

Figure 2 Process in the formation of nanoparticles from biopolymers. Source: Author self.

Application of biopolymeric nanoparticles in fingerprint analysis

The most common type of physical evidence is latent fingerprints. The visualization and enhancement techniques need to be modified with the advanced modus oprandi. Criminals

using defined methods to secure themselves from being suspected. Fingerprints are known for its uniqueness and individuality and is therefore the most trusted method to identify a suspect. There have been different chemical and physical methods used for development of latent fingerprints, which involve Ninhydrin, iodine fuming, but some of them have toxic and destructive chemical properties that unintentionally can affect the identity of the original marks obtained. Moreover, the chemical used for the latent print development are non-biodegradable. These properties of some latent print developing agents generated a need for preparation of materials that can be used without a doubt of their ill effects. There are some studies that involve the use of materials which can be used as effective alternates to the present chemical materials. These materials are obtained from the natural sources in the for of natural polymers and known as bio-polymers . These polymers are more reliable due to their biocompatible nature ,they are chemical less or non-toxic, they are biodegradable in nature and are cost effective. Their property of being easily modified they can be used for the fabrication of nanoparticles. These nanoparticles possess small and uniform size ,good adhesion properties to the sweat residues. The sweat residues obtained on the different types of surfaces like non-porous, semi-porous and porous surfaces can be tested using these prepared nanoparticles for the presence of latent fingerprints. Chitosan based micropowders and conjugates were prepared using precipitating method and further ionotropic crosslinking using sodium tripolyphosphate as the crosslinking agent. The experiment was done to test the functionality and possibilities of realistic use of the prepared powder. The powder was tested on the oily fingerprint samples on the different surfaces glass (non-porous), rubber (semi-porous) and paper (porous) surfaces. The samples were left to reduce the residues for 24 hours, till development of prints using developed powders. The smooth structure of the glass resulted in acquiring a satisfactory result as compared to rubber and paper surfaces. The papillary lines were not clearly visible on rubber surface but provided a limited result. The results on paper surface was totally unsatisfactory due to the yellowish color of the paper surface selected for the experiment22. Other experiment was performed to prepare four different dextran based powders using simple precipitation method and characterization was done to determine their potential application in latent fingerprint development. The initiating and crosslinking agents were used for obtaining aldehyde functionalities of the dextran chain and their crosslinking. This aimed for enhancement of fingerprints on interaction with residues. The small and uniform particles were allowed to bind with the fingerprint residues for their clear visualization21. These prepared formulation is less harmful and less costly. The result were not good on white paper. The dextran powders were easily handled and applicable. These bio-polymeric powders are non-destructive and irreversible loss of traces can be avoided.

Future Perspective

The researchers are being conducted on the expanded applications of biopolymeric nanoparticles based on the properties that allow them to be used as an alternative to existing chemicals in latent print development. Due to their

Figure 3: Fingerprint identification using nanopartticles.
Source: Author self.

excellent promising factors ,the bio-polymeric nanomaterials holds wide range of forensic applications. The previously developed functional nanomaterials has also been widespread in this field9.The bio-polymeric nanomaterials can be developed for the use in bio-analytical techniques for analysis of biological evidences like DNA analysis due to its non-destructive properties. Their non-toxicity and cost effectiveness can lift them as the substitutes for the current chemicals used in forensic analysis. Nanomaterials can be used in developing biosensing tools that can be used in biosensors. The products can be used as taggants for the purpose of counterfeiting. The use of such naturally developed nanomaterials can assist to minimize the environmental hazards due to their eco-friendly nature.

RESULT AND DISCUSSION

This review discusses the advantages of using the nanoparticles from natural polymers. The most frequently used bio-polymeric nanoparticles including chitosan-based micropowders and dexrtran-based powders are described with their applications in development and enhancement of latent fingerprints on different surfaces. Being eco-friendly nature, these nanomaterials can be used as tool for minimizing hazardous effects of chemical materials used as forensic analytical tools for ages. The smaller size of nanoparticles, good adhesion properties and higher surface reactivity makes it possible to develops latent prints and on reaction with silver and gold the electrostatic charge which makes it stable and helps in improvement in contrast of the developed prints. They support non-destructive and non-toxic behavior of the analytical methods used for sensitive forensic evidences and can be used as future toolkit in forensic applications. IJFMP

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REVIEW ARTICLE

Virtopsy in Covid-19 and Its Application in Forensic Science: A Review

Samiksha Chauhan[1], Sneha Yadav[2]

ABSTRACT

The outset of the year 2020 witnessed the outbreak of an epidemic known as CoVid-19 or novel coronavirus pandemic. Since then, the number of confirmed cases of this infection had increased rapidly all over the world. Autopsy provides relevant knowledge about the identification and determination of the cause of death in forensic medicine. Different non-invasive and minimally invasive approaches over the traditional autopsy are introduced into forensic science to deal with challenges presented by COVID-19 pandemic where performing invasive autopsy is not feasible. Virtopsy is a multi-disciplinary science. 'Virtopsy' or Virtual Autopsy aims at new imaging techniques in forensic pathology to facilitate the present postmortem examination. It offers advantages over invasive 'body opening' autopsy. Virtopsy consists of body volume documentation, optical scanning with imaging techniques such as Magnetic resonance imaging (MRI), computed tomography (CT) scan coupled with minimally invasive approaches such as postmortem biopsies. The importance of Virtopsy is noteworthy in post mortem examination of COVID-19 patients. Virtopsy has a broad spectrum of uses in forensic medicine, forensic odontology, firearm injury and road traffic injury etc. Hence, it can be used either independently or as an alternative to conventional autopsy. The present study highlights the importance of Virtopsy in postmortem examination and its contribution in forensic science. It also provides scope for future research in the subdisciplines of forensic science.

KEYWORDS | covid-19, virtopsy, minimal invasive autopsy, mri, ct scan

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INTRODUCTION

The severe acute respiratory syndrome SARS-2 (SARS-CoV-2) is a respiratory distress syndrome. It affects the functioning of other vital organs in cases. The first case of this epidemic was reported at Wuhan, the People's Republic of China, in the year 2019.[1–3] Older people with other diseases are more prone to this infection. Till 3rd April 2021, out of 129,902,402 confirmed cases worldwide, 12,392,260 cases with 164,110 deaths were reported in India only.[4] In Forensic medicine, postmortem examinations provide the required information regarding the exact cause and mechanism of death. An autopsy has been of great importance.[5] The word "autopsy" from the Greek words 'autos' and 'opsomei' meaning 'to see with one's eye'.[6] Under such circumstances, the contagious nature of COVID-19, forensic pathologists need to face numerous challenges of protecting themselves to analyzing the organs accurately while performing traditional body opening invasive autopsy.[7] Hence, there is a need to look for an alternative to conventional invasive autopsy to examine the dead accurately and effectively. Virtopsy is marked as one of the effective techniques to reduce the chance of infection to the practitioners. It also advances the radiological techniques

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in forensic science.[8] As the name suggests, Virtopsy is the combination of two words: virtual (obtained from the Latin language 'Virtus' meaning 'efficient, good') and autopsy eliminating the term 'autos' (self).[9] Virtopsy generally is composed of 3-D (three dimensional) body volume analysis by non-invasive techniques such as Multi-slice Computed Tomography (MSCT), Magnetic Resonance Imaging (MRI) and 3-D Photogrammetry and optical surface scanning strengthened by minimally invasive techniques.[10] Minimal invasive autopsy is the procedure that includes imaging techniques augmented with postmortem biopsies[11]. Virtobot is one of the newest robotics systems which are capable of performing body scanning and image-guided post mortem biopsies.[12] Virtopsy presents wide spectrum of applications in Forensic science. The imaging techniques help in postmortem examination in cases of hanging, drowning, shooting etc.[13] The present study illustrates the importance of imaging techniques such as CT, MRI and minimally invasive autopsy for future implications. But these imaging techniques are still not utilized today due to inadequate knowledge and expenses. The present study supports Virtopsy as an alternative to conventional invasive autopsy especially for COVID-19 patients and highlights its importance in Forensic Science.

METHODS

3-D Imaging Techniques:

X-ray is one of the oldest human imaging systems developed so far. Soon after its discovery in 1895, scientists have started using X-rays to produce postmortem images. Since then, various imaging modalities exist today.[14] Computed Tomography is a fast-imaging technique based on the attenuation of X-ray. It includes a rotating source of X-ray and detector that produce 3-D tomographic images using the algorithm. The cross-sectional images are detailed and sliced so that examiner can observe the internal organs thoroughly. It performed well in trauma cases.[15,16] It is one of the most widely used approaches in forensic radiology for post mortem examination.

Unlike CT, Magnetic Resonance Imaging (MRI) is a non-ionizing radiation imaging technique that creates images using a robust magnetic field and radio waves. The proton spins when the magnetic field is applied and the rate at which the spin return to its original normal alignment is different. Hence on calculating this, MRI forms an image.[17,18] MRI performed well in strangulation cases.

As compared to CT, the use of MRI is still underutilized, although it is gaining importance in forensic radiology. Both CT and MRI are non-invasive approaches, so they replace the surgical autopsy in many cases. Angiography coupled with CT and MR has also performed well in postmortem examination.[19]

3-D Photogrammetry & 3-D Body Surface Scans

Photogrammetry refers to the method of measurement of objects using photographs. It provides visual images using 2-D photographs taken from different angles. It works on the principle of triangulation. Forensic photogrammetry has been an essential approach in postmortem examination for body documentation. It usually worked well in traffic accidents cases.[20,21]

Surface scanning is another way of recording and documenting the object that provides images in 3-D view using optical scanners. The 3-D surface scan is often used non-invasive 3-D imaging technique in Forensic Science.[22,23] There are advanced surface scanners developed yet enhancing the use of 3-D scan in various field.

Forensic photogrammetry and 3-D Surface scanning are capable of providing 3-D representation. They are essential tools in 3-D reconstruction and providing 3-D optical models with their advantages and limitation.[24] Various software approaches are present along with photogrammetry, to construct 3-D model very efficiently.[25]

Minimally Invasive Autopsy

Minimal Invasive Autopsy, also known as Minimally Invasive Autopsy, is a systematic approach that involves the imaging procedures such as MRI and CT scan, as well as minor biopsies and needles. This autopsy procedure has been used for several years for its potential to provide outcomes with limited resources. It aims to target a variety of organs inside the human body. The main advantage is the less need for infrastructure and low-income settings. As it includes imaging technique, it provides a record of the whole body and lessens the risk of infection to practitioners.[26,27]

Virtual Autopsy in postmortem analysis:

Non-Invasive approach

Virtual Autopsy includes 3-D photogrammetry, optical scanning, computed tomography, Magnetic Resonance Imaging, Postmortem angiography, and postmortem biopsies. These techniques have the potential to work independently or augment with other. Photogrammetry-based approaches are easy and effective for 3-D body documentation in autopsy examination and provided high-quality models28. Photogrammetry is a reproducible and low-cost technique which doesn't require any professional training.[29] The Video recording is a faster method as compared to photo sessions for 3-D documentation.[30] Another approach for documentation is 3-D surface scanning by optical scanning, which is easy, time saving and efficient for dental identification.[31] In traffic accidents cases, external examination by 3-D surface scanning and internal by MSCT, MRI provide a better understanding in body analysis.[32] Even in the cases of late decomposition, CT scan and MRI successfully reconstructed the bullet trajectory through the skull.[33] The use of these imaging techniques is popularizing day by day in forensic medicine.

PMCT was able to detect major injuries in the body but couldn't completely surpass the conventional autopsy in the cases of traumatic deaths. It can be performed for all trauma cases. The performance of PMCT and PMMRI is comparable for determining the cause of death. Both the techniques have their advantages.[34] The studies show contrasting result on comparing these techniques. PMMRI detected extra cardiac and brain pathology efficiently in the cases of sudden death. Hence, it is a beneficial and informative approach when conventional autopsy is not possible.[35] Most of the comparative studies between Virtopsy and traditional autopsy promoted postmortem imaging techniques in autopsy examination. Virtopsy provides information about the cause of death but still can't completely replace the need of traditional autopsy. These imaging techniques are capable of coordinating with traditional autopsy. Virtopsy has the potential to be performed as an alternative to conventional autopsy. [36,37]

Virtopsy in postmortem analysis of COVID-19 patients:

Minimally Invasive Approach

The SARS-COV-2 infection present challenges in postmortem examination due to its contagious nature.[39] The minimal invasive approach has practiced for COVID-19 deaths to overcome the difficulties. Minimally Invasive autopsy includes use of the imaging techniques along with small organ biopsies. It enables the collection of tissue specimens for histologic examination.[40] This

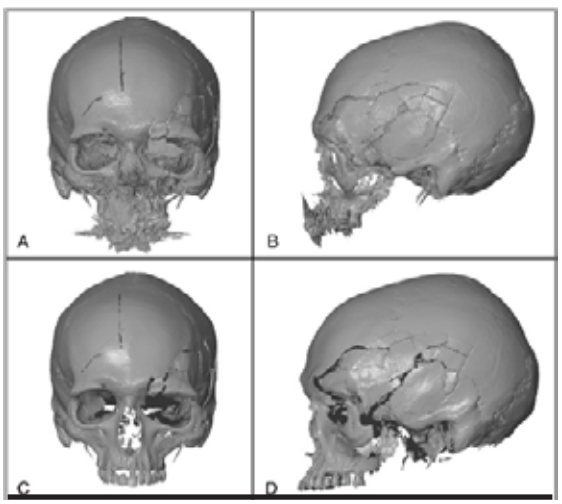

Figure 1 Comparative analysis of 3-D model of skull by CT scan A) Anterior view, B) Lateral view and 3-D surface scan C) Anterior view, D) Lateral view.

Source: *Scanning of a skull: first considerations regarding reproducibility issues . Forensic Sciences Research. 2017;2(2):93-99*

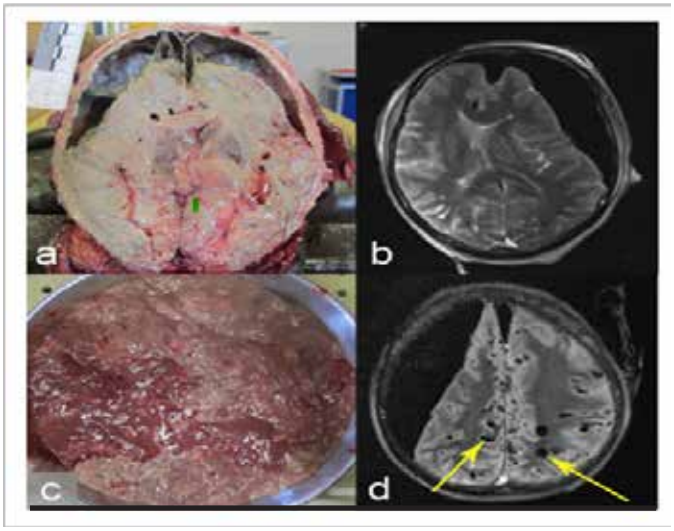

Figure 2 Shows Comparison representation between conventional autopsy in a) softened brain tissue, c) liquified brain at autopsy and PMMRI in b) softened brain tissue, d) liquified brain and yellow arrow indicating putrefied gas accumulation[38].

Source: *Tschui J, Jackowski C, Schwendener N, Schyma C, Zech WD. Post-mortem CT and MR brain imaging of putrefied corpses. International Journal of Legal Medicine. 2016;130(4):1061-1068*

method allows the collection of tissue samples, urine, bile, and blood samples for toxicologic and DNA investigations. In these circumstances, when the risk of transmission of the infection is high, minimally invasive autopsy reduce the risk of producing aerosols.[41] In terms of diagnostic performance, Minimal Invasive autopsy shows similar results as that of conventional autopsy in the diagnosing the cause of death.[42] Even when the consent of next-of-kin of dead is not present, it may work as an essential tool.[43] This approach is feasible for high infectious diseases for countries with middle and low income.[44] Ultrasound-guided minimally invasive autopsy performed well, providing 100% agreement conventional autopsy for the 2018 yellow fever epidemic in Sau Paulo.[45] MIA-US provided efficient in obtaining sufficient samples from multiple organs such as lungs, liver and spleen of COVID-19 patients.[46] MIA-US can help to determine the pathology of respiratory failure and systemic manifestation of COVID-19.[47] The main advantages offered by minimally invasive approach are its cost efficiency and low-invasiveness. It is a safer and quicker approach that provide accurate results during COVID-19. Hence, Minimal Invasive autopsy is an innovative approach in postmortem examination in the pandemic.[48]

Application of Virtopsy in Forensic Science

Age and Sex determination:

The CT scans have good reproducibility in estimating the age and gender of individuals. In a study on age estimation, CT scan well differentiated individual of age between 40-60 years from middle aged.[49] Through burnt and charred remains, Age can be easily estimated by CT scan using age estimation methods.[50] Sexually dimorphic bones are very often analyzed to determine individual's gender. A PMCT scan is also a simple and quick method to measure bone structures before an autopsy.[51]

Forensic Odontology & Personal identification

The comparison of antemortem and postmortem records and development of individual's details are usually done for dental identification.[52] Some of these antemortem radiographic reconstructions are possible using cranial CT records. Documentation and examination are possible in charred and decomposed bodies without surgical removal by Dent scan.[53] MSCT is a quick approach for gathering data and allowing comparison of dental radiological information for identification in mass disaster cases.[54]

Forensic Ballistics and Firearm Injuries

Entrance and Exit wounds are analyzed to locate the projectile inside the body using radiological techniques.[55] CT scan is capable of providing information of wound and cavity inside the body in clinical and forensic cases.[56] A doctor uses an X-ray to locate projectile to save the living person. MRI is capable of better visualizing soft tissues than the MSCT. It can be used as an alternative tool in cases where the bullet trajectory is not easily detectable.[57]

Forensic Reconstruction

Reconstruction of crime scene is a vital task in criminal investigation. In case of fracture, the impact of force and direction of force is analyzed.[58] 3-D photogrammetry, 3-D body surface scans and CT scans are also capable of Forensic reconstruction of body parts.[59]

Burn Injuries

For the identification of hidden signs of wounds, gas collections, and foreign bodies in burnt patients, a PMCT preliminary to autopsy is an addition to the postmortem forensic examination.[50]

Cause of Death

Virtopsy has great importance in diagnosing the cause of death in forensic pathology as it enables examination of vital organs for example heart, lungs etc.[60]

Mechanical asphyxia

In the case of death caused by obstruction created by a foreign particle, CT allows the preliminary screening to autopsy. CT is comparable to the traditional autopsy in detection of bone fracture and soft tissues analysis. MRI was able to detect microfractures in cases of manual strangulation.[61] The vital responses reveal the sequence of injuries and death. The determination of whether an accident occurred before or after death is a crucial forensic problem. MRI and CT can analyze signs of strangulation and collect internal neck detail.[62]

Cardiovascular system

The primary cause of natural death is cardiac insufficiency. Cardiac insufficiency may be caused by chronic heart disease or sudden ischemic events.[63] PMRI successfully detected ischemic lesions and myocardial infarction. It has the potential to perform in the absence of an autopsy.[64]

Respiratory system

In determining infections of lungs and natural causes of death, PMCT is as efficient as traditional autopsy. It is more capable of detecting vertebral fractures, which will rule out hanging and indicate spinal injuries.[65] In cases of non-traumatic death, PMCT of lungs was able to determine the cause of death.[66]

Central Nervous system

CT and MRI are the beneficial diagnostic tools in neuropathology and forensic science.[67] Even when the brain is liquefied, PMRI of putrefied brain accessed the multiple regions of the brain.[38]

Future Implementation of Virtopsy

With some advances in techniques used in Virtopsy, it can contribute more in forensic science. Virtopsy produces massive amounts of digital DICOM data. Digital format reduces the expense of films and video handling, making it more portable.[68] Virto Scan is capable of documenting the body. It is a multi-camera-based approach which saves money and time.[69] A new version of the robotic system named 'Virtibot' has been introduced to perform the task such as body surface scanning and incorporation of needles for sample collection. It has the potential to be combined with imaging techniques.[70] Recent developments in MR imaging have the potential to shorten MR imaging test times, which may take up to 3 hours per corpse.[71] The postmortem imaging techniques necessitates a significant investment of time and money, as well as professionals with the skills to obtain and analyze PM images. Imaging equipment needs to be maintained, modified, and replaced regularly. The PM imaging may not be developed properly without sufficient financial resources.[72]

DISCUSSION

This study shows how Virtopsy has widened the scope of postmortem examination in forensic science. A virtual autopsy can be used for postmortem examination in COVID-19 patients.[73] 'Virtopsy' is intended to implement radiological techniques in the field of Forensic Science.[74] It includes imaging techniques such as CT, MRI, scanning techniques such as 3-D photogrammetry, surface scans and postmortem biopsies. Postmortem radiology has developed incredibly since the decades. PMCT and PMMRI have used for examination of the vital organ after death. 3-D optical models and Forensic Reconstruction have been achieved by Photogrammetry and Surface scanners.[75] While there are substantial possible benefits of using postmortem MRI/CT imaging, it is doubtful that postmortem imaging would completely substitute conventional autopsy analysis shortly due to its inherent limitations. Besides that, for those cases where the next-of-kin refuse conventional autopsy, such "minimally invasive" autopsy examinations may provide at least some clinically valid details.[76] This approach has proved successful in postmortem examination in cases of highly infectious disease such as COVID-19.[77] The role of Virtopsy in forensic science includes detecting the sudden cause death due to failure of vital organs such as heart, lung, firearm injuries, burn injuries, traffic accident cases etc. It is also helpful in examining post mortem changes and in the cases of medical dispute. Even with so many advantages of Virtopsy, it is still not utilized to its complete extent.[78] Future researches are needed in this field to validate its use. However, depending on the situation, autopsy with histology or, in carefully chosen cases, non-invasive, minimally invasive or traditional autopsies are likely to be the most reliable method of determining the cause of death.[79]

CONCLUSION

Virtopsy is an essential diagnostic tool in forensic science. It can be used either independently or in combination with traditional autopsy, depending on the need of the hour. It has advantages in clinical and forensic fields. Minimal invasive autopsy is quick and safe. It can be an alternative to autopsy in the COVID-19 pandemic. Future research and funding can help in overcoming the limitations of the virtual autopsy. IJFMP

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REVIEW ARTICLE

Virtual Clinic: Best Practices for Patients and Doctors Under CoVid-19 Pandemic

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ABSTRACT

Virtual Clinic is a web based software application, where patients can consult with physicians, psychologists, therapists, and other health professionals. No physical presence of the patient is required in the virtual clinic. It is much better during the examination if the disease does not require it as the obligatory diagnostic prerequisite. The web-based VC has been designed for functional and nonfunctional purposes and specifications. These criteria have been met through interviews that have semi structured and open-ended questions on disease evaluation by medical practitioners. Finished questions concerning the evaluation by the experience of the disease. And it's a very open source application that any user can link a shared issue to a conference. This virtual clinic was used during lockdown due to Covid-19. There have been many lockdowns in countries all over the world. This was the only option during the pandemic. In developing the application, we were particularly concerned about the provision of services and the reliable, secure, and efficient storage of data. Here, the non-functional and functional requirements are proposed along with such clinic's design. Such requirements were collected depending on the input that medical practitioners have specified via interviews that had open ended and semi structured questions related to assessment of disease according to their experience.

KEYWORDS | Virtual Clinic, CoVid-19, Architecture, Design, Performance

INTRODUCTION

The worldwide outbreak of COVID-19 placed people under considerable strain. This new outbreak of 2019 coronavirus was first reported in China's Wuhan, as well as influenced the United Kingdom on 31 January 2020. The outbreak was reported. The WHO announced on 11 March 2020 that COVID-19 Social dissociation measures had been implemented in India in March. As an alternative to face to face consultations, they were presented at this time by 2020 and were virtually connected with the Internet (via telephone or video call). A virtual center is a way of service delivery that allows patients, physicians, and insurance companies to communicate and work together to help patients get a remote diagnosis and treatment.[1] It is particularly accurate in case the primary conditions are not severe as well as can be cured at home. It is normal when in cities there is availability of a doctor, for example. Although doctors are not readily available in developing world countries as well as in rural areas of the developed countries. Here, we propose the virtual clinic's conditions as well as design which allow patients, through text-based messaging and audio/video, to consult doctors or other healthcare professionals virtually anywhere in the world. The patients may be diagnosed and prescribed more easily at a time and place. The actual symptoms

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including temperature, blood pressure, and so on must be uploaded by patients for diagnosing. On the other hand, the doctors analyze the reports and the preliminary documents of the patient for writing as well as uploading the prescription. Also, patient records for future references are kept in a secure database by the virtual clinic. The proposed virtual clinic is developed from detailed interview data with doctors. The answers of doctors were utilized to address the virtual clinic's functional and non-functional needs. The quality improvement approach was followed through the Plan-Do-Study (PDSA) cycle by the Institute for Healthcare Improvements.[2] A process of activation and redesign The PDSA cycle was followed by an improvement approach for quality improvement. An improvement principles-based process of enabling, redesigning process, and support for delivery and evaluation were conducted.

An overview of certain potential reasons for patient satisfaction has been provided by open-ended qualitative data. Those were the following:

- The offer of an alternative use during the CoVid-19 pandemic to the virtual clinic.
- The journey times have been reduced.
- Reduced waiting times.
- Reduced effect on symptoms of travel.

This report shows that virtual consultation in response to CoVid-19 is fast and acceptable to a large extent. Additional measures to support CoVid-19 virtual consultations are also needed for clinically relevant and acceptable.[3]

LITEATURE REVIEW

A psychological software design / architecture may be analyzed from two different angles. Their virtual clinic offers mental health services, including prevention, early intervention, and recovery. We address first research initiatives focusing on the impact of other such practices on patients and the community in the following paragraphs. Then we explored the idea of the virtual clinic and its non-functional criteria in software design. Virtual clinic's all components that involve online personal advice, peer-to-peer support networks, referral to electronic self-help services, instruction, and confidential online screening. Have addressed the role played in e-health applications by the virtual clinic. They demonstrate that the use of such modern technologies in clinical practice has certain benefits and problems. This indicates that consultation process might be promoted by the connection

Among virtual and real worlds as well as affect treatment sessions positively, in particular for group. Also, these settings can also boost comfort levels among patients and therapists. Such benefits were noticed in comparison with traditional TV applications when the framework developed by authors (e.g., videoconferences, chat, and emails). Nevertheless, problems associated with the possible addiction to such virtual clinics and privacy and personal protection issues have not impeded patient entry.[4] Patients are enrolled in the online system as a first step. The virtual clinic allowed patients to speak to medical professionals, communicate and share information. The virtual clinic has found it to be effective in a variety of aspects of patient life and wellbeing in some areas. The alternative route of drug prescription and other related activities are discussed as a clinic word.[5] We found that a study for assessing loyalty of client to a content-dependent Web service, in particular, to a healthcare website, was performed on the Internet. We find that in customers there exists confidence issue in doing their medical for a virtual clinic. And they do not know about even about paying online. It concludes that patients' trust is motivated by infrastructure, protection, and accessibility on the website. A lot of papers speak about those points. Yet some of the study paper's miss points. Somewhere in the article, we add to it.

According to a report by Practo, an online platform, online consultation for virtual treatment increased by 500% between March 1 to May 31, 2020 during the pandemic. During this period Indian access to health services has added around 5 crore users with an average frequency of two online consultations per users per month, as approximately 80%, first-time virtual users, along with around 44% of users from non-Metros, were registered with reporting to healthcare professionals offering teleconsultation. 7.5% was linked to 19 symptoms of total online consultation. Eight minutes were the total time spent online with the doctor. In the overall consultation between the most discussed questions, a growth of 600% was observed. The virtual meeting during the

lockdown was time for an increase of 76% across several cities. Delhi, Pune, Mumbai, Hyderabad, Chennai, and soon.

3. Requirements of a Virtual Clinic

There are many features available in a virtual clinic. Including functional requirements, a system such as reliability, protection, and performance should also have functional requirement. Since a virtual clinic offers facility, the difference among death and life may be. The system's functional specifications are as defined in a patient of virtual clinic must have ability for registering as well as retaining his health and personal records.[6] The first time an expert should have the ability for sending a request to a patient/subscriber. The new medical examination reports should be submitted to your patient/subscriber. Only the physicians involved should have access to the medical records of a given patient. The first time, Health practitioners and organizations should be able to register and manage wiki pages as service providers. The patient should be properly advised and guided to a certain medical professional. Medics and organizations should be able to give notices to every subscriber (medical professionals or patient). To the patient subscriber, advisories must be sent through multiple channels, including SMS and e-mail.

A virtual medical center has been attempted in several ways. However, we also concentrate on the practical needs of such a device. The essence of the service (medical services are generally viewed as fundamental and fundamental human rights) rendered as well as the data value (information of patient health is confidential) treated through system makes these conditions especially relevant. Reliability is described as a device property which demands that in adverse circumstances it stays operational.[7]

A virtual clinic must be reliable as it can save a person's life by providing service. The intent of the service fails if the service is not available when most of it is required. Mechanisms to ensure the quality of operation were not part of a reliable system. One of the most significant criteria for such a system is protected because of the patient health information sensitivity.

A security requirement was defined as a security measure for preventing or removing vulnerabilities that may infringe the sensitive, integral, or accessible data. The security requirement is defined in this context. These constraints must be included in all phases of production. Not only should the details provided by the patient be kept confidential, but also in a way to prevent unauthorized persons from making changes (integrity).[8] If applicable, the data should also be available. For the system that provides medical care, a virtual clinic is appropriate. This especially applies to uploading or forwarding medical test results or medications quickly.

VARIABLE	COVID-19– POSITIVE (N=102)	COVID-19– NEGATIVE (N=3688)	p VALUE
Any VTE event	3 (2.9)	168 (4.6)	.43
PE	1 (1.0)	91 (2.5)	.34
LE-DVT	0 (0.0)	62 (1.7)	.19
UE-DVT	3 (2.9)	22 (0.6)	.004
Cerebral vein	0 (0.0)	6 (0.2)	.68

Table 1: COVID-19 positive and negative along with P value

Abbreviations: ICU - intensive care unit, PE - pulmonary embolism, UE-upper extremity, VTE-venous thromboembolism, DVT- deep venous thrombosis.

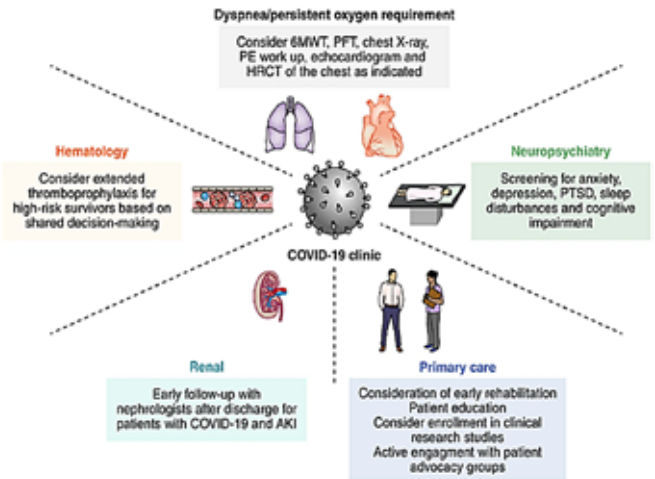

Figure 1: Multidisciplinary COVID-19 Clinic 5-tier views. **Source:** Author self.

RESULT AND DISCUSSION

During COVID-19, the results of the virtual clinic improved, as seen online. How patients in the covid-19 are virtually associated with physicians? A virtual clinic fills the gap between people, doctors, and health systems that allows all people, in particular patients with symptoms, to stay at home and to communicate across virtual networks with doctors. Science technologies are being used for the healthcare detection coronavirus, which includes case recognition, population monitoring,

ublic-health need	Digital tool or technology	Example of use	Refs.
igital epidemiological urveillance	Machine learning	Web-based epidemic intelligence tools and online syndromic surveillance	Web-based epidemic intelligence tools: 20–23,25 Based on social media or online search data: 30–33
	Survey apps and websites	Symptom reporting	32,38,48,49
	Data extraction and visualization	Data dashboard	39–45
apid case identification	Connected diagnostic device	Point-of-care diagnosis	58
	Sensors including wearables	Febrile symptoms checking	59–63
	Machine learning	Medical image analysis	65,66
nterruption of community ransmission	Smartphone app, low-power Bluetooth technology	Digital contact tracing	Paper: 71 Apps: 76–79 Frameworks: 80–83
	Mobile-phone-location data	Mobility-pattern analysis	Analysis: 84,87–89,93 Datasets: 86,90,91,92
ublic communication	Social-media platforms	Targeted communication	104,107
	Online search engine	Prioritized information	105
	Chat-bot	Personalized information	110
linical care	Tele-conferencing	Telemedicine, referral	58

Figure 2: shows that Virtual clinics seem to impact the general quality of life, happiness, or expectations that women have in their clinical episodes by using an online questionnaire framework (10-11). Source: Budd, J., Miller, B. S., Manning, E. M., Lampos, V., Zhuang, et.al (2020). Digital technologies in the public-health response to COVID-19. Nature Medicine, 26(8), 1183-1192. https://doi.org/10.1038/s41591-020-1011-4
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touch tracking, as well as assessment of behavior based on public communications like virtual or telecommunications clinics, and data on mobility.[9] There are huge millions of cellular telephones, large online datasets, connected Smart phones, and relatively cheap computer tools.

This result indicates the possibility of improved assistance for patients in expressing health problems and preventing embarrassment about medical issues of a delicate and personal nature as a useful element of adoption and use of a virtual clinic.[12]

CONCLUSION

Virtual visit to any doctor anywhere in the world can be made through video streaming and chat support via a proposed virtual clinic. The contact between patient and doctor is good and timely. It maintains a database of medical records and the qualifications, credentials, and timetables of patients registered at the virtual clinic. The patient can pick the time slot available and ask for doctor's appointment. All related reports can also be sent by the patient if required for medical check-up. The doctor is still available for these studies. The drug is available to physicians and patients at any time. The prescription is available once again. This project also discusses reliability, security, and performance specifications that are not functional. Such non-functional criteria are every program's required part which manages patients' medical details. Also, it demands reliability and efficiency as important part of the fundamental design of the service rendered by such applications. In this paper, we suggest a range of non-functions to improve reliability, protection, and performance. Initial results of the implementation indicate the minimal reliability, protection, and performance thresholds of the specified non-functional specifications, if implemented. IJFMP

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REVIEW ARTICLE

Detection of Mixed Profiling via Y-Filer Mode of Analyzation

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ABSTRACT

In this research study, we will look into the mixed profiling via Y marker analysis in a sexual assault case. Ascertaining the identification of the assailant or victim can be determined conclusively by DNA profiling of the samples found on the crime scene. There are two types of DNA's, nuclear DNA, and mitochondrial DNA. Generally, mitochondrial DNA is taken in cases where nuclear DNA cannot be extracted which are mostly present in hair and bone. Various types of DNA analysis, STR analysis, RFLP, mtDNA analysis, and Y-filer depending on the case involved. In the cases involving mixed profiling, it is Y-filer profiling which is used to find out the actual profile to give a report as conclusive evidence. In this research, we will look at the analysis of mixed profiling via Y-filer analysis in a sexual assault case. Problem in the cases involving mixed gene analyzer profiling that is two accused involved in a particular crime scene becomes a condition whose admissibility in the court comes under question. In such cases it is Y-chromosome present on "AMEL" that is the locus studying for the X- and Y-chromosome signifying the presence or absence of the criminal under question.

KEYWORDS | dna profiling, y-filer, mixed profiling, sexual assault

INTRODUCTION

DNA evidence can be used in various cases such as paternity testing, criminal identification, a study of evolution when considering the human population and inherited and autosomal disorder related study. In modern scenario, it has become a most common test in any criminal investigation, and is high in demand in the process of criminal examination. Due to the considerable authentic studies and challenges in courts, DNA is now regarded as 'gold standard' in forensic science.

The DNA test is just the first step while looking at DNA profiling. Matching DNA is the next step involving marker to analyze peaks, which is unique to each nucleotide when looking at an analysis, often known as the fingerprint of the individual. This leads to result in formation namely: inclusive, exclusive, or inconclusive in nature. In case that states the result to be inclusive involves the accused to be included in as a possible source of DNA considering it conclusive. In the result stating exclusion, the suspect's DNA is considered non-suspect and can be excluded from being a chance of a source of DNA origin. In the case that has the interpretation to be inconclusive, it is generally the result

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of DNA being degraded or in the case where the quantity is below 40 micro liters, which is the limit for analysis. The focus of this research is to detect individual DNA profiles in the cases involving mixed profiling via Y-filer mode in PCR.

For the purpose of this research, we have examined a collection of biological exhibits in the cases mostly involving rape preliminary cases of POCSO Act, Automation of exhibits in automator, PCR (Y-filer mode), Genetic Analyzer–ABI3130. The purpose is to study the analysis of mixed profiling with the use of Y marker analysis in sexual assault case.

THE PROCESS

After procuring the biological exhibits obtained at the crime scene, the exhibits were analyzed first to recognize the presence of semen, saliva, blood etc and, if present, on the sample using analytical techniques like chemicals (Sodium Acetate, Fast Blue B-salt, Glacial Acetic acid, Hydrogen peroxide solution, Sodium-1 Alpha naphthalene phosphate, Benzedrine Powder) and microscope. The second step involves the extraction of DNA using automater. The DNA thus extracted will be subjected to PCR loaded in a portable thermal cycler to magnify and amplify the targeted loci (sites). The amplified or magnified products are then filled within the capillaries of DNA Sequencing machine, which segregate and characterize the STR fragments.

MATERIALS & METHOD

Exhibits, chemicals (Sodium Acetate, Fast Blue B-salt, Glacial Acetic acid, Hydrogen peroxide solution, Sodium-1, Alpha Naphthalene Phosphate, Benzedrine powder), Distilled water Buffer-Prepfiler, DNA express, Forensic DNA Extraction kit, DDT, Master mix, Primer mix, Hydride, Allelic Ladder, Column, Pipette, Vortex, Sphinx, Automate Express, Genetic analyzer.

Instruments: Weighing Machine, Thermo mixture, Centrifuge Machine, Automate Express, PCR Machine, Genetic DNA Analyzer.

Preliminary and Confirmatory Test-Physical, Chemical and Microscopic Examination-

Biological examination for detection of Semen and Blood was done by preliminary test and confirmatory test and sent for DNA analysis and examination.

In the cases involving mixed gene analyzer profiling of the two accused involved in a particular crime scene becomes a condition whose admissibility in the court comes under question in such cases it is Y chromosome present on "AMEL" that is the locus studying for the X and Y chromosome signifying the presence or absence of the criminal under question.

This study was conducted in a case of mixed profiling in which a 12-year-old girl was raped by two men (suspects A and B) aged 22 and 47, respectively. Victim was found dead in B's house and was covered with a cloth. The victim had marks on her neck which seemed to be of a plastic rope which may be the cause of her death. Her hands and legs were tied with a plastic rope. Her mouth was taped shut with plastic tape. Both suspects were arrested by the police and their blood samples taken for examination. The exhibits received are listed below:

- A 5x11.5cm transparent tape stuck on the mouth of the deceased
- A mahanadi-colored underwear of the deceased (make-DIXCY 85cm).
- A white-colored banian of the accused A.
- A light blue underwear of the accused A.
- Pubic hair of the accused A.
- Nails of the accused A.
- Blood sample of the accused A in EDTA vial (reference DNA sample).
- Two vaginal slides of the deceased.
- Two cervix slides of the deceased.
- Cervix swab of the deceased.
- Nails of the deceased.
- Three vaginal swabs of the deceased.
- An anal swab of the deceased.
- Blood sample of the deceased in EDTA vial (reference DNA sample).
- A torn yellow and black colored full sleeves sweat shirt of the deceased.
- Blood sample of the accused B in EDTA vial (reference DNA sample).
- A dark brown-colored underwear of the accused B.
- 5 pieces of plastic rope tied around the legs of the deceased. (approximate sizes: 38.5cm, 49cm, 172cm, 31cm and 184cms).

Figure 1 Purple coloration signifies positive test

Figure 2 Green coloration signifies positive test

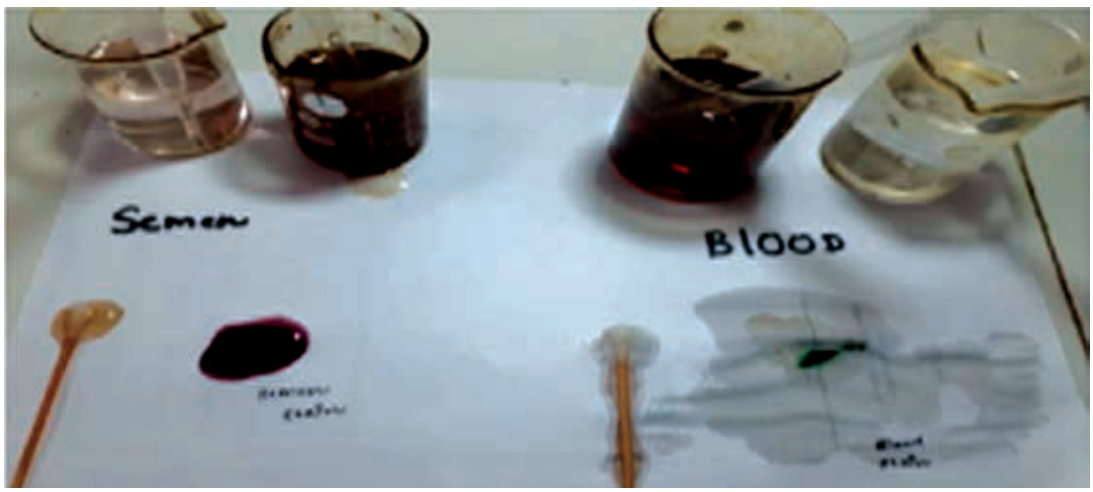


Figure 3 Purple coloration signifies positive test

Exhibit No.	Acid Phosphate Test	Barberious Crystal Test /Florence Crystal Test	Spermatozoa Detection
1	-ve	-ve	-ve
2	**Positive**	**Positive**	**Positive**
3	-ve	-ve	-ve
4	-ve	-ve	-ve
5	-ve	-ve	-ve
6	-ve	-ve	-ve
7	-ve	-ve	-ve
8	-ve	-ve	-ve
9	-ve	-ve	-ve
10	**Positive**	**Positive**	**Positive**
11	-ve	-ve	-ve
12	-ve	-ve	-ve
13	-ve	-ve	-ve
14	-ve	-ve	-ve
15	-ve	-ve	-ve
16	-ve	-ve	-ve
17	-ve	-ve	-ve
18	-ve	-ve	-ve
19	-ve	-ve	-ve
20	-ve	-ve	-ve
Control	**Positive**	**Positive**	**Positive**
U/S Control	-ve	-ve	-ve

Table 1 Semen Analysis

- 3 pieces of plastic rope tied around hands (approx sizes: 38cm, 37cm and 8cm) of the deceased.
- 5 pieces of plastic rope tied around neck (approx sizes: 48.5cm, 51.5cm, 29cm, 52cm and 39cm), of the deceased.

Material Method:

Biological examination for detection of semen and blood was done through preliminary test, and confirmatory test and then isolation from the exhibits was carried out by using Prep Filer Express™ DNA Extraction Kit. The quantified DNA was subjected to multiplex PCR reaction for fifteen Autosomal STR Loci and one amelogenin loci using commercially available AmpFISTR® Identifiler® plus kit and AmpFISTER® Y-Filer kit. The capillary electrophoresis of amplified products was done on automated DNA Sequencer and analysis was carried out using Gene Mapper IDX® software.

RESULTS

Result from examination of detection of semen and blood:

1. Semen was detected on exhibits of 2 and 10
2. Semen could not be detected on exhibits of 1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20
3. Human blood was detected on exhibits of 2, 4, 8, 9, 10, 12, 13 and 15
4. Human blood was not found on exhibits of 1, 3, 5, 6, 7, 11, 14, 16,17,18,19 and 20.

Results of DNA Examination

1. The DNA test was performed on exhibits 1-20
2. The alleles were amplified at each loci to obtain the DNA Profiles of the sources of the exhibits-1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19 and 20.
3. The DNA Profiles obtained from the exhibits-1, 4, 8, 9, 11, 12 and 13 (transparent tape, underwear of accused A, vaginal smear slide of deceased, cervix slide of deceased, nails of deceased, vaginal swab of deceased, and anal swab of deceased) belong to a single female human resource and matched with the DNA profile taken from exhibit-14(blood sample of deceased).
4. The mix DNA profiles collected from exhibits-2,10,18,19 and 20 (underwear of deceased, cervix swab of deceased, plastic rope tied around leg of deceased, plastic rope tied around hand of deceased and plastic rope tied around neck of deceased) were also matched with each of the DNA profiles taken from the

Exhibit No.	Benz./TMB Test	Takayama Test	Anti Human
1	-ve	-ve	-ve
2	Positive	Positive	Positive
3	-ve	-ve	-ve
4	Positive	Positive	Positive
5	-ve	-ve	-ve
6	-ve	-ve	-ve
7	-ve	-ve	-ve
8	Positive	Positive	Positive
9	Positive	Positive	Positive
10	Positive	Positive	Positive
11	-ve	-ve	-ve
12	Positive	Positive	Positive
13	Positive	Positive	Positive
14	-ve	-ve	-ve
15	Positive	Positive	Positive
16	-ve	-ve	-ve
17	-ve	-ve	-ve
18	-ve	-ve	-ve
19	-ve	-ve	-ve
20	-ve	-ve	-ve
Control	Positive	Positive	Positive
U/S Control	-ve	-ve	-ve

Table 2 Blood Analysis. **Method of Isolation:** Automate Express

Sample No.	Area/Volume of Sample used	Final Volume of Isolated DNA in L	Kit/Chemical Used
1	Tape	50µL	Prepfiler Express Forensic DNA Extraction Kit (500L Lysis Buffer + 5L DDT)
2	5*5mm^2	50µL	—Do—
4	5*5mm^2	50µL	—Do—
5	Pubic hair	50µL	—Do—
6	Nail	50µL	—Do—
7	40µL	40µL	—Do—
8	Slides	50µL	—Do—
9	Slides	50µL	—Do—
10	Swab	50µL	—Do—
11	Nails	50µL	—Do—
12	Swab	50µL	—Do—
13	Swab	50µL	—Do—
14	40µL	50µL	—Do—
15	5*5mm^2	50µL	—Do—
16	40µL	50µL	—Do—
17	5*5mm^2	50µL	—Do—
18	Rope	50µL	—Do—
19	Rope	50µL	—Do—
20	Rope	50µL	—Do—

Table 3 DNA Case Worksheet: DNA Isolation. **Method of Isolation:** Automate Express

exhibit-7 and 14 (blood sample of accused A and blood sample of deceased) and not matching with the DNA profile taken from the exhibit 16 (blood sample of accused B).

5. The DNA profiles obtained from exhibit- 6 (nails of accused A) is from a single male human source and matching with the DNA profile obtained from exhibit-7 (blood sample of accused A)
6. The mix DNA profiles collected from exhibits-15 (sweatshirt of the deceased) was matched with the DNA profiles taken from the exhibit-14 (blood sample of the deceased) but not matched with the DNA profile of the exhibit-7 and 16 (blood sample of accused A and blood sample of accused B.)

DISCUSSION

In a majority of the sexual assaults, blood and semen samples, which are left after the crime over the body of the victim, clothes as well as on the location of the crime generally plays a significant role during the investigation. A major challenge to the investigators is to properly extract the swab specimens from the crime scene, which are commonly found on those surfaces like the victim's body, the parts of the body of the victim with which the culprit came into contact. The priority was to collect the assailant's DNA as early as possible to ensure the chances of accurate recognition of the person who committed the crime.

Blood and semen can be exposed by preliminary or chemical test followed by confirmatory or microscopic test and DNA analysis. A DNA test will not set out to verify if the biological exhibit provided contains semen but rather its aim is to extract a DNA profile of the individual to whom the exhibit belongs using the semen and blood sample provided.

Microscopic tests of blood and semen will not help to recognize the criminal. In order to do this, examiners need a DNA profile and a finalized DNA profile can help precisely distinguish different people. No two individuals can have the same DNA profile (with the exception of monozygotic twins). Once the DNA profile has been made, it can be collated to the DNA profiles of any questionable person to look for a match. In cases where no match occurs, examiners and police might run a search in a DNA database. A DNA database will carry the DNA profiles of offender. However, a match between two DNA profiles does not surely confirm the person is culpable of the crime and is the true culprit. Other evidences will need to be provided in that case.

DNA is relatively long lasting. It is most likely that forensic samples assembled from a rape victim will yield results. However, time factors, chemical factors like washing using soaps and detergents, external factors such as temperature and humidity and internal factors like body fluids may damage the effectiveness of a sample. The earlier the samples are assembled and tested, the higher the chance of yielding solid, reliable results.

CONCLUSION

The DNA tests performed on the exhibits pro-

Exhibit No	Volume of DNA(L)	Primer Set(L)	PCR Reaction(L)	Additional Component	Total Volume(L)
1	10	10	5	– Nil –	25
2	10	10	5	– Nil –	25
3	10	10	5	– Nil –	25
4	10	10	5	– Nil –	25
5	10	10	5	– Nil –	25
6	10	10	5	– Nil –	25
7	10	10	5	– Nil –	25
8	10	10	5	– Nil –	25
9	10	10	5	– Nil –	25
10	10	10	5	– Nil –	25
11	10	10	5	– Nil –	25
12	10	10	5	– Nil –	25
13	10	10	5	– Nil –	25
14	10	10	5	– Nil –	25
15	10	10	5	– Nil –	25
16	10	10	5	– Nil –	25
17	10	10	5	– Nil –	25
18	10	10	5	– Nil –	25
19	10	10	5	– Nil –	25
20	10	10	5	– Nil –	25
Positive control	10	10	5	– Nil –	25
Negative control	10	10	5	– Nil –	25

Table 4: **DNA Case Worksheet:** DNA Amplification **Name of Kit:** ID

Notes: *Preheating: 95°C for 11 mins, Denaturation: 94°C for 20 mins. Annealing: 59°C for 3 mins, Extension: 60°C for 10 mins. Total Cycle: 29*

Exhibit No	Volume of DNA(L)	Primer Set(L)	PCR Reaction(L)	Additional Component	Total Volume(L)
2Y	10	0.5	9.5	0.5	25
7Y	10	0.5	9.5	0.5	25
10Y	10	0.5	9.5	0.5	25
15Y	10	0.5	9.5	0.5	25
16Y	10	0.5	9.5	0.5	25
17Y	10	0.5	9.5	0.5	25
18Y	10	0.5	9.5	0.5	25
19Y	10	0.5	9.5	0.5	25
20Y	10	0.5	9.5	0.5	25
Positive Control	10	0.5	9.5	0.5	25
Negative Control	10	0.5	9.5	0.5	25

Table 5: **DNA Case Worksheet:** DNA Amplification **Name of Kit: Y-Filer**

Notes: *Preheating: 95°C for 11 mins, Denaturation: 94°C for 01 min. Annealing: 61°C for 01 mins, Extension: 72°C for 01 mins. Total Cycle: 30*

Exhibit No	Amplified Product Allelic Ladder (L)	Size Standard (L)	Hi-Di Formamide (L)	Total Volume(L)
1	01	0.3	8.7	10
2	01	0.3	8.7	10
3	01	0.3	8.7	10
4	01	0.3	8.7	10
5	01	0.3	8.7	10
6	01	0.3	8.7	10
7	01	0.3	8.7	10
8	01	0.3	8.7	10
9	01	0.3	8.7	10
10	01	0.3	8.7	10
11	01	0.3	8.7	10
12	01	0.3	8.7	10
13	01	0.3	8.7	10
14	01	0.3	8.7	10
15	01	0.3	8.7	10
16	01	0.3	8.7	10
17	01	0.3	8.7	10
18	01	0.3	8.7	10
19	01	0.3	8.7	10
20	01	0.3	8.7	10
Negative Cuntrol	01	0.3	8.7	10
Positive Control	01	0.3	8.7	10
AL. ID	01	0.3	8.7	10

Table 6 **DNA Case Worksheet**: Genotyping. Sample Preparation and Electrophoresis

Notes: *Instrument: ABI 3130 Genetie Analyzer. Capillary Length: 36cms.*
Module: Genemapper 1D-X. Duration: 95°C for 3 mins. Snap Cooling in ice for 03 mins.

vided is sufficient to conclude that:

1. The DNA profiles obtained from the exhibits of transparent tape, underwear of the accused A, vaginal smear slide, cervix slide, nails, vaginal swab, and anal swab of the deceased belong to a single female human and matched with the DNA profile taken blood sample—exhibit-14—of the deceased).
2. The mix DNA profiles collected from the exhibits—underwear of the deceased, cervix swab, plastic rope tied around the leg of the deceased, plastic rope tied around hand of the deceased and plastic rope tied around neck of the deceased—were also matched with each of the DNA profiles taken from the exhibit-7 and 14 (blood sample of the accused A and blood sample of deceased) and not matching with the DNA profile taken from the exhibit 16 (blood sample of accused B).
3. The DNA profiles obtained from exhibit- 6 (nails of the accused A) is from a single male human source and matching with the DNA profile obtained from exhibit-7(blood sample of accused A).
4. The mix DNA profiles collected from exhibits-15 (sweatshirt of the deceased) was matched with the DNA profiles taken from the exhibit-14 (blood sample of deceased) but not

EXHIBIT 01

EXHIBIT 02

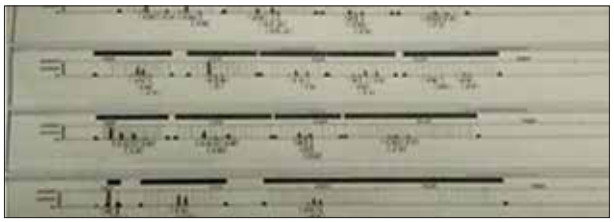
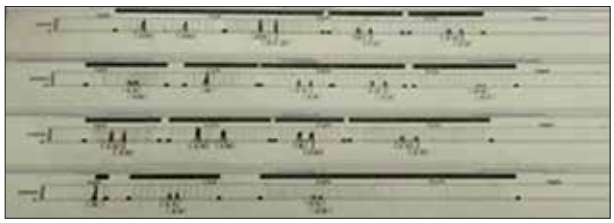


EXHIBIT 04



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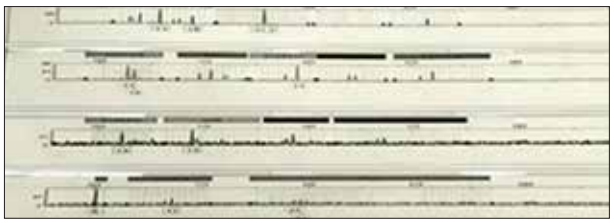

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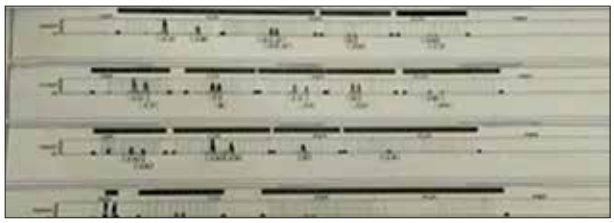

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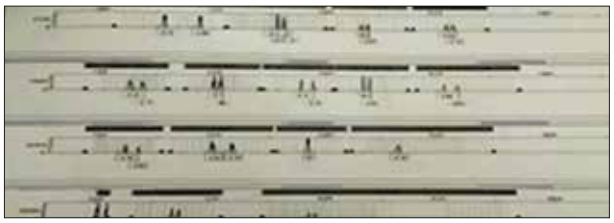

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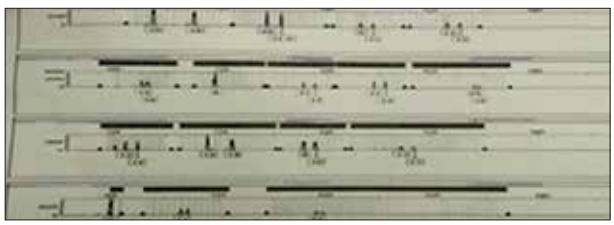

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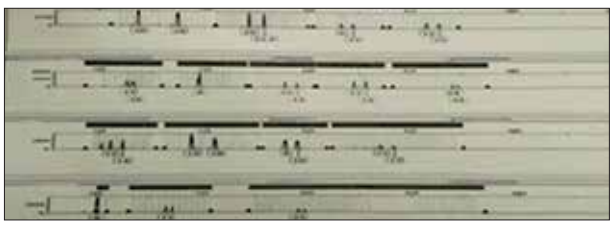

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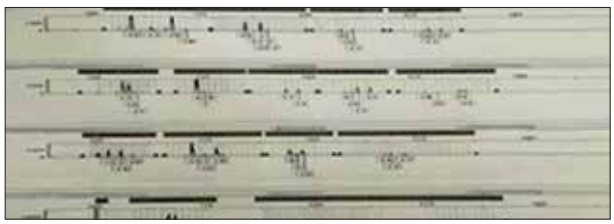

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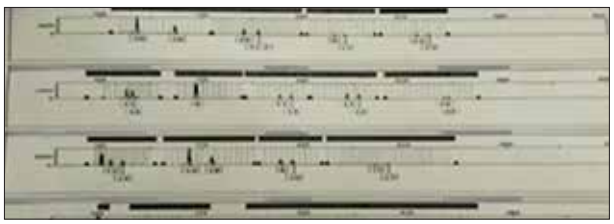

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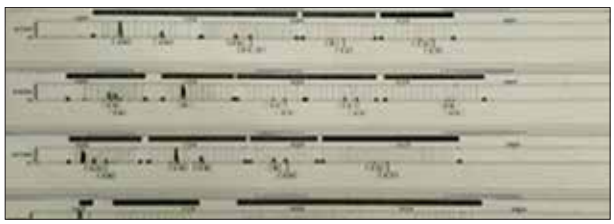

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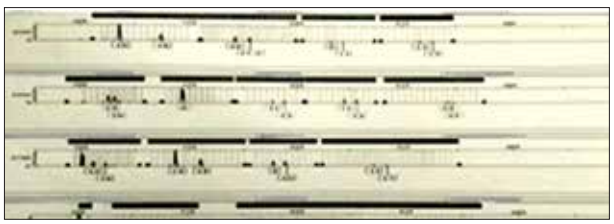


Table 7 DNA PEAKS. (Results: Identifiler Plus)

EXHIBIT 14

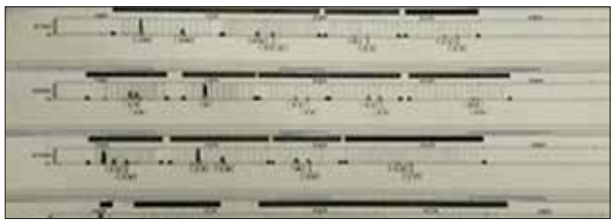
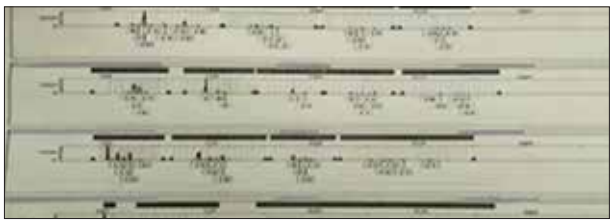


EXHIBIT 15



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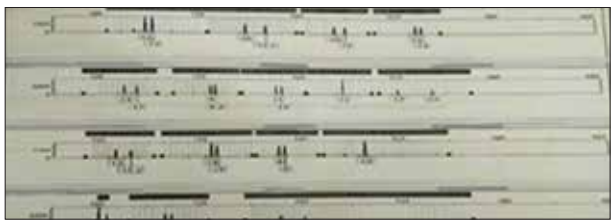

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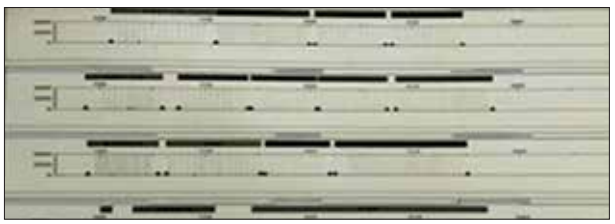

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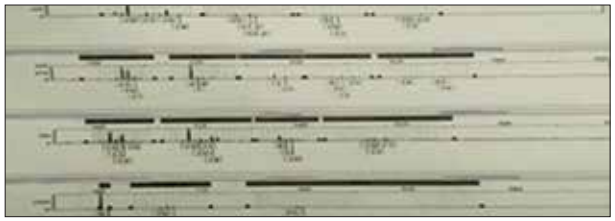

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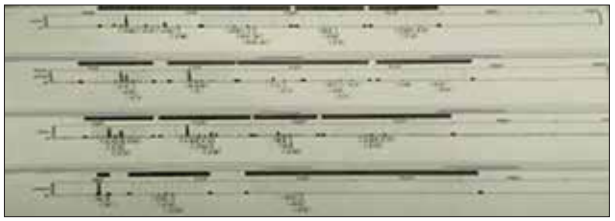

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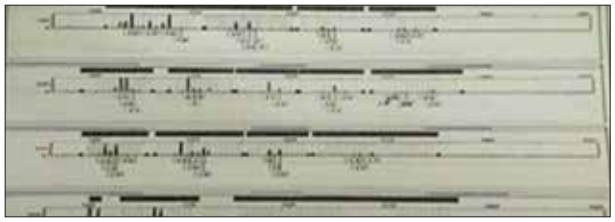
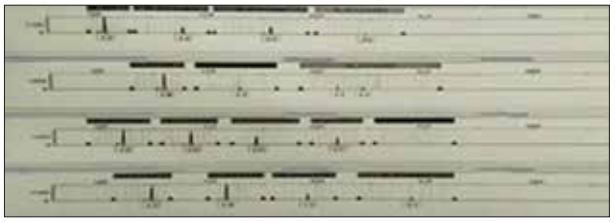


EXHIBIT 2Y



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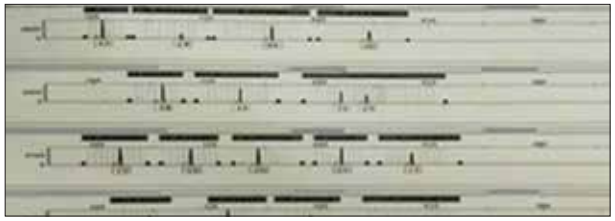

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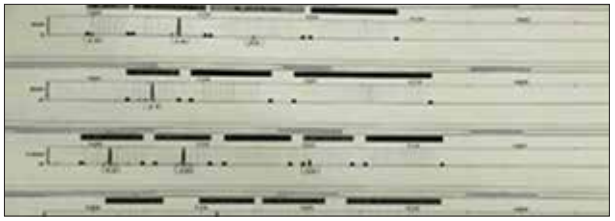

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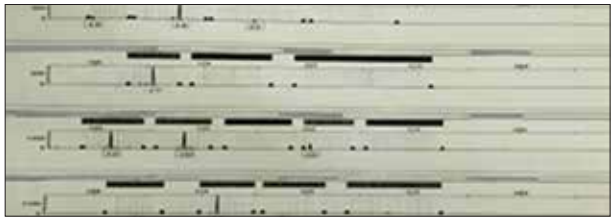

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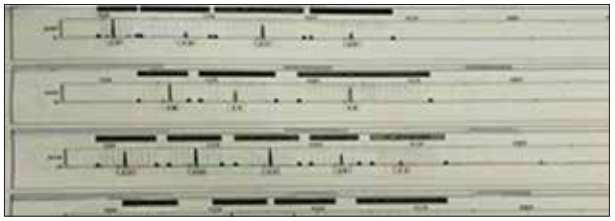

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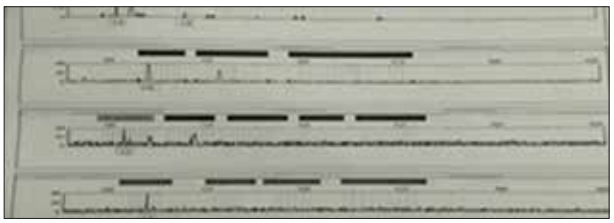

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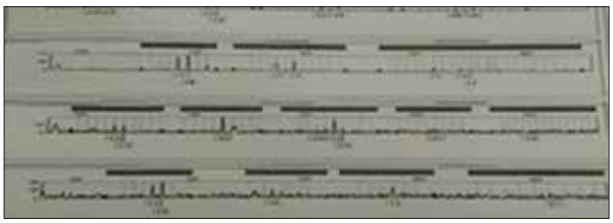


EXHIBIT 19Y

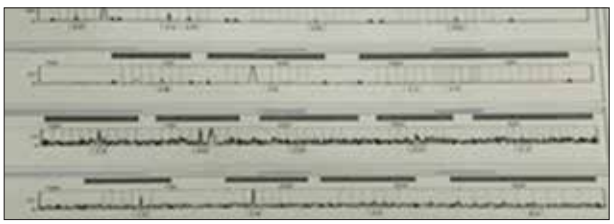
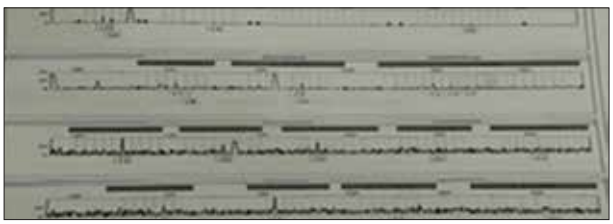


EXHIBIT 20Y



matched with the DNA profile of the exhibit- 7 and 16 (blood sample of accused A and blood sample of accused B.)

The DNA test performed on the exhibits provided is sufficient to conclude that the victim's DNA profile (of the deceased) matched with DNA profile of Suspect A and not with DNA profile of suspect B. So, this confirms that A is the accused.

Differentiation of Y gene via y-filer analysis in the case involving mixed profile. It would help solve the case, so as to match up to the profile of the accused. It would allow us to segregate from the mixed profile that occurs in the cases involving either sample degradation or the cases which involve two or more sample result crossover. IJFMP

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Conflict of Interest:

The author declares there is no conflict of interest in this project.

Source of Funding:

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ORIGINAL ARTICLE

The Forensic Investigation of Cloud Computing Using Different Techniques: Challenges, Issues & Security Risks

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ABSTRACT

Cloud computing is the most important and almost universally desired administration for all organisations because it allows organisations to exchange resources such as calculating, stocking, and programming units while also ensuring ease of use and management of virtualization technologies. Viva supports the internet. Comfort, mobility, stability, adaptability, speed, tremendous bandwidth limits, availability, and on-demand network access are just a few of the major benefits of cloud computing. The flexibility of options for cloud computing solutions has made it impossible for hackers to seek successful and pervasive future surveillance and security openings as emerging innovation. This paper examines cloud crime scene investigation research in order to include potential options, including questions and challenges, as well as several recommendations for overcoming these challenges.

KEYWORDS | cyber security, cloud computing, digital forensic, phishing

INTRODUCTION

In recent years, there has been a significant increase in the number of formal & informal online organizations, such as Facebook, Twitter, MySpace, and Friendster, which function with high levels of client personalization and client intercommunication. Online informal organizations, which can be described as cloud-based electronic administrations, which will allow each and every individual in a restricted context to create a public or semi-public identity, clarifying many clients they share with. Do, see, and overcome their relationships and the people they make. Some items inside the cloud-based structure.[1]

Sudden growth of the World Wide Web and disconnected clients with many gadgets are moving towards cloud computing for customer support. Along the main path in innovation there are potential threats to the client's data and if any attack is made by cyber criminals on the cloud, it calls for researching the cloud in light of an incredibly huge size. The most vexing difficulties to manage the diverse current liability on the classification of information received in different areas would be the issue of obtaining confirmation from the clouds and the capture of actual evidence for a coordinated effort of approval or proof of computerized crime. Does. Visual investigation in distributed computing starts from there. Cyber crime is a

fascinating field to examine non-ethical practices being used in the cloud. This happens with different types if examination and evaluation on a wide range of data on distributed computing. Completed in this area.[12]

Increasing cyberattacks and many offenses in the increasingly complex multi-legal and multi-local cloud world mean that the implementation of investigative processes in the cloud is urgently needed.[13]

METHOD & MATERIALS

The term "cloud" is being bandied around everywhere these days. This mysterious word seems to include almost every aspect of our lives. Although "the cloud" is merely a metaphor for the internet, cloud computing is a hot topic of conversation these days. It improves data collection, security, flexibility, and employee teamwork, as well as changing the workflow of small and large companies to help them make smarter choices while lowering costs.[14,15,16]

It is undeniable that cloud computing is becoming increasingly popular. The role and deployment of the cloud in companies like Alibaba, Amazon, Google, and Microsoft has already been predicted in our market intelligence trends report.

The cloud's importance is growing at an exponential rate. According to Gartner, the cloud computing industry grew to 17.3% in 2019 (US$206.2 billion), with 90% of the companies adopting cloud services by 2022.

Cloud Computing

The distribution of numerous hardware and software resources over the internet through a network of remote servers is referred to as cloud computing. These remote servers are collecting, handling, and processing information that allows users to extend or update their current networks.

The cloud's strengths and scope are immense. To further identify usage cases, the IT industry divided it into three groups.

a) Software as a Service (SaaS) – one or more vendors own, distribute, and operate software remotely. To begin, Software-as-a-Service, or SaaS, is a widely used method of obtaining and paying for software. Rather than downloading software on your own computers, SaaS companies allow you to rent software that is hosted, usually for a monthly or annual subscription fee. SaaS business intelligence and technology is being used by an increasing number of CRM, communications, and finance-related resources, and even Adobe's Creative Suite has followed the concept.

b) Infrastructure as a Service (IaaS) – operators own and host computing services, as well as storage and networking facilities, and make them accessible to consumers on request.

c) Platform as a Service (PaaS) is a term that refers to a group of providers that provide platform infrastructure (middleware). Application platform, automation, enterprise process management, and storage services are among these services.

Cloud Forensics

Cloud legal science or cloud forensics can be characterized as the use of advanced criminology in distributed computing for investigating crimes. Distributed computing is advantageous to the extent that it is making the legal local area so concerned about customer data. Adaptation of the cloud occurs at a certain point, information from different sources may include similar areas inside the capacity media that makes a commonality during e-disclosure, while an organization is being investigated; the expert unknowingly distributes the remaining information to another organization. The development of capacity limits in distributed computing is a hindrance to advanced criminology because more legal information and extra time will be used to examine the information, obviously, if nothing turns out badly. Traditional computerized criminology techniques (e.g., encoding) have different inadequacies and inefficiencies in distributed computing, in such a way that it is important to find out about advanced legal science information and set assessment in distributed computing to create fruitful pathways in distributed computing.

Towards declining cloud security opportunities

Distributed computing is another model and the computerized crime scene investigation local area is yet to investigate what inconvenience this new time innovation is. Countless creators of this sort expressed potential experienced troubles during the time spent maintaining a series of coherence.[5,6,7]

In any event, driving associations have not yet introduced a set of ideas or can be made to follow a definite arrangement of instructions when the most efficient method for moving the cloud across the associations is in the cloud or on the rules. A safety accident occurs inside. Many times, distributed computing and computing enabled to help network exams in their online exams for misdeeds. Lawbreakers can manipulate efficient free interchange frameworks, for example, Tor and Anonimizer which were initially shown to secure network client structure personalities criminals and delineations. In this manner, the law requires that many Amazon EC2 VMs be purchased, using the Tor network as a watchdog that can skip routes to the Tor circuit and leave the hub and attack inside the fitting. May examine sources [8]. Amazon Web Administration (AWS) is a more realistic model that can provide MD5 of every document that is on the framework, so when a bit of duplicate is being created, Microsoft continues to include the metadata record. Ease of use in the office, email store and careful reinforcement.

Related Work

Frank Y.W. Law et al.[4] has suggested a data-protection solution that is irrelevant with cryptographic aid to the investigative situation. The research begins with some insight into the situation, some keywords in the text base case may be. The suggested solution enables investigators to use approved keywords to scan for encrypted records. For an email server, the device is tested consisting containing 120 GB of 100,000 emails of which 600 words are used for all emails. Among all the addresses, 25% contain protection, and 25% contain both security and forensics, while 25% contain security. In order to create an index register, the system took 3536.984 seconds and calculated trapdoors took 0.25 seconds. To search through the whole emails and return the index file name which contains the text, protection or forensic, took 1705.672 seconds.

In the year of 2011, Ruan et al.[2] In the United Arab Emirates, a paper was distributed among advanced legal experts and experts around the world proposing another definition for the cloud exam in view of a review directive, facilitated by Zayed University. The review was planning to show a better understanding of some ideas, for example, cloud legal science definitions, difficult issues, most urgent investigative titles, and basic measures for cloud exam ability. In view of Ruan et al., By definition, cloud legal science is a cross layer between computerized distributed computing and advanced digital mis-examination. Additionally, it is a subset of the organization's wrongdoing experts that manage measurable examinations in any type of organizations, with current existing methods tailored for the distributed computing climate. Cloud Criminology can be extended into three components of special, authoritative and valid measurements.

Advantages	Disadvantages
Low or no cost infrastructure	Requires High speed internet connection.
Least administration cost	Downtime or stoppage time
No management hassles	Partial control of infrastructure
Very easy accessibility	Restricted or limited flexibility
Pay per use	Ongoing more costly
Better reliability	Security & security breaches issues
Data control	Vendor lock- in
Easier Data backup and recovery	Technical related Issues
Massive cloud storage available	Not available

Table 1 Advantages and Disadvantages of Cloud Computing

CONCLUSION

Advantages of Cloud Computing

1. *Low or no charge on infrastructure:* Distributed cloud-based computing that are separated into the three most popular significant classifications according to the administrations: Infrastructure as a Services (IaaS), Platform as a Services (PaaS) and Software as a Services (SaaS).

Each and every one of these classes, one thing that is normal that you don't have to put resources into equipment or any foundation. When all is said in done, each association needs to spend a ton on their IT foundation to set up and enlist a particular group.

Workers, network gadgets, ISP associations, stockpiling, and programming – these are the significant things on which you need to contribute on the off chance that we talk about broad IT framework. Yet, on the off chance

that you move to distributed computing administrations, you don't have to put resources into these. You just go to a cloud administrations supplier and purchase a cloud administration.[8]

2. *Minutest management and cost:* By selecting the cloud-based services, you must be saved the cost in multiple points of ways:
3. *Least administration cost:* You don't want to claim the foundation, you don't spend anything on its administration of the organization or staff to oversee it.

Cloud chips away at pay more only as costs arise model, so you spend just on assets of resources that you need. That's it!

At the point when you select the cloud-based services, the administration of its foundation is the sole obligation, commitment of the cloud service provider and none of the client or cloud-based customer.

4. *No managerial or management hassles:* At whatever point there is a buy or up-degree of equipment, a ton of time is squandered searching for best sellers, welcoming citations, arranging rates, taking endorsements, creating POs and sitting tight for conveyance and afterward in setting up the framework.

This entire interaction incorporates loads of authoritative/administrative errands that burn through a ton of time.

With cloud administrations, you simply need to look at the best cloud specialist co-ops and their arrangements and purchase from the one that coordinates with your necessities. Furthermore, this entire interaction doesn't take a lot of time and saves you a great deal of endeavors. Your framework support undertakings are additionally disposed of in the cloud.

5. *Easier Accessibility and pay-per-use:* Cloud assets are effectively open from around the globe – whenever, anyplace and from any gadget and you have total admittance to your assets.

This determines the charging as well - you only pay for what you need and how much you need. It seems like a phone bill or a fuel bill. However, like other IT foundations, the entire amount is spent all at once, and it is exceedingly rare if such funds are invested optimally, resulting in the investment being squandered.[17]

6. *Better Reliability:* Your foundation in the cloud expands the unwavering quality and accessibility of utilizations and administrations. Cloud administrations run on pooled and repetitive foundation which furnishes you with a higher accessibility of IT administrations.
7. *Data Control:* Another essential benefit of the cloud is that it concentrates all the information from different ventures and branch workplaces to a solitary area. You oversee the information without visiting singular spots for checking the data.
8. *Easier Data back-up & Recovery:* Loss of information can fundamentally affect your business. You may lose basic data which can cost you an immense amount of cash, burn through your important time and damage your coroprate image.

To forestall it, you can consequently reinforcement all the information to the cloud consistently. This assists you with recuperating any information in the event of unintentional erasure, misfortune in view of regular catastrophe or if the hard drive crashes.[18]

9. *Found Massive cloud storage:* Most cloud platforms give you a free, secure and enormous storage to store all your data.

Albeit most distributed storage administrations like OneDrive offer you a decent measure of free stockpiling, on the off chance that you use everything, you can generally go for purchasing safer capacity in the cloud.

10. *Automatic software updates for security purposes:* Refreshing a framework once in a while can be a disappointing undertaking for endeavors. The IT office needs to refresh the framework for each person which sits around idly as well as influences efficiency. Yet, in the event that you are utilizing cloud-based applications, they will get naturally refreshed, with no contribution from the clients.

Subsequent to examining the advantages of distributed computing, how about we currently examine a few burdens of distributed computing.[8]

Disadvantages of Cloud Computing

1. Required High-speed internet connection:

To get to your cloud administrations, you need to

have a decent web association consistently with great data transmission to transfer or download documents to/from the cloud

2. Downtime: Since the cloud requires high web speed and great transmission capacity, there is consistently a chance of administration blackout, which can bring about business vacation. Today, no business can bear the cost of income or business misfortune because of vacation or delayed down from a break in basic business measures.

3. Partial control of infrastructure: Since you are not the proprietor of the framework of the cloud, thus you don't have any control or have restricted admittance to the cloud infra.

4. Restricted or limited flexibility: The cloud gives an immense rundown of administrations, however devouring them accompanies a ton of limitations and restricted adaptability for your applications or advancements. Additionally, stage reliance or 'merchant lock-in' can now and then make it hard for you to relocate starting with one supplier then onto the next.

5. Ongoing more costly: Despite the fact that you save your expense of expenditure on entire framework and its administration, on the cloud, you need to continue to pay for administrations as long as you use them. In any case, in customary strategies, you just need to contribute once.

6. Security & security breaches issues: Everyone is concerned about information security. Since the public cloud is built on the internet, the data can become powerless.

Since it is a public cloud, it is up to the cloud provider to handle the data. As a result, before settling on cloud administrations, it is critical that you locate a provider who adheres to the most stringent information management strategies.

For full cloud protection, one must choose a much more expensive private cloud option or the crossover cloud solution, in which nonexclusive information can be stored on the public cloud and business-basic information is kept on the secret cloud.

7. Vendor Lock-in: It's better to sign a cloud computing contract than it is to break one. When switching suppliers is either prohibitively costly or impossible, "vendor lock-in" occurs. It's possible that the offering isn't standard or that there aren't any suitable vendor alternatives.[12]

It all boils down to prudence on the part of the consumer. Ensure that the services you use are standard and transferable to other suppliers, and that you are aware of the standards.[13]

While you are guaranteed to be able to turn to a specialized cooperative at some other point in time in cloud organizations, it is an extremely troubling interaction.

You may believe that moving all of the cloud administrations, beginning with one expert co-op and progressing to the next, is difficult. Similarity, interoperability, and backing issues can arise during the relocation process. To avoid these problems, often customers opt not to switch sellers.

8. Technical related issues: If you're a computer prodigy or not, specialized challenges will arise, and they can't all be resolved in-house. To avoid interfering with your work, you should seek assistance from your specialist co-op. In any case, only one out of every odd retailer provides day-to-day assistance to their clients, although emerging service providers are unable to do so.[8]

RESULT & DISCUSSION

In January 2018, RighScale published its annual report on emerging cloud dynamics. They polled 997 technology leaders from a wide variety of companies on their cloud computing practices. Their findings were eye-opening, especially in light of today's cloud computing challenges. To answer the main question on the challenges of cloud infrastructure, we've expanded on some of their findings and introduced additional cloud computing topics that businesses will need to address further down.

In terms of forensic science, we will not able to map entire events in traditional forensic cloud-based computing environments, any of which are mentioned below:

i) The majority of problems encountered during a criminological review of the cloud are a lack of customer data during the preparation period for a variety of reasons, such as worker closure, which can conflict with equivalent or random administrations.

ii) Not simple to get to all organize switches, network-based router, load balancers and other

systems administration gadgets.

iii) Access to a massive firewall is denied.

iv) Imaging challenges that bounce from one to the next but remain constant across cloud steering plans.

v) The issue was discovered through a log analysis of cloud apps.

vi) Log reinforcement and bendability.

vii) Log data access is limited.

viii) Assaulting Velocity is higher.

ix) At the time of the investigation, malicious hubs are present.

x) Clients will cancel or hide the majority of information.

xi) Hypervisor-level of research. Experts conducted a classification of research, for example, Lu *et al.,* to discuss the challenges of cloud-based distributed computing. In distributed computing, it was suggested that a stable installation be developed and managed to devise and monitor the history of knowledge properties. He stated that the secure starting point should address the two states' impossibility and protection issues, and then suggested a fair specification based on bilinear pairs after using the security technique to ensure their personal safety in the standard model.

Despite the suggested stable root plot computing being acknowledged as proof in the knowledge question, it was argued that the inquiry was conducted hypothetically and that there is no evidence that the administration and conscious models are distinct. Worked in conjunction with [6]

Digital Forensics Investigation Procedures

The event entails a series of tools and processes for remotely investigating the process of crimes in a cloud computing environment, with the following main aspects:

Figure 1 Digital forensic investigation: the process after the attack

Diagnosis Determine whether an unlawful act or indeed fraudulent activity is involved in IT based programs. These actions can include complaints submitted by an individual, irregularities noticed by IDS, tracking and profiling as a result of audit trail, and unusual events in a cloud. The use of deployment models (i.e., private, public, community and hybrid), the type of cloud resources (i.e., SaaS, Paas and IaaS) and the geographic region chosen for deployment.[10]

Clues Identification

The cloud as a concept has been shared as proof of finding possible validation wells, which is almost a concern. In this part, we'll look at the problems that the examinee can see at this point in more detail. The main problem in the Proof ID process is the log entry for validation. The testing framework is a piece of status and log record validation that isn't particularly noteworthy in SaaS and PaaS since the client's access is entirely restricted to the API or preplanned interface. Since it returns a virtual machine that behaves like a real machine, this is suitable for the IaaS cloud model.

Forensic Collection of Data

It is either the source-side or purpose-side to obtain, characterize, remove, and retrieve information about antiquities from other potential wells of information from the cloud. Due to the diverse cloud benefits and sent models, obligations begin with a help or conveyance model, then on the next in the cloud along these lines, requiring different tools and methods.

Preservation of Data

In a criminal investigation, proof is a confirmation of a crime, and no offence may use it to attach testimony. It is possible to assure the user that his or her recognition was taken and abused from someone in situations enabling evidence that the client is concerned in unethical activities. This is unlikely to refute the situation since the client will automatically and individually associate with the cloud.

Analysis of Data

The knowledge test is another crucial stage in a mediocre exam; in fact, in a PC science review, it necessitates a more sensitive assessment when the volume and quantity of objects to be tested is increased. It may also be referred to as a catastrophe

in distributed computing because the concept of distributed computing entails the utilisation of a vast number of properties with a reasonable chance of validation. This is a different viewpoint on cloud observable examination, owing to the difficulty of processing and distinguishing large amounts of data.

Reconstruction

The information recreation process of the science investigation yields a variety of dissect findings. As a characteristic of knowledge collection steps, computerised science practices are of concern for the era of brief inquiry to reliably replicate the non-moral demonstration. Because of the different ideas of distributed computation, which involve exploiting and distributing properties, any piece of misconduct could have arisen in a better position in distributed computing; this creates a problem that interferes with the era of the transitory test.

Reporting and Presentation

The final stage in scientific agents' trials entails selecting the appropriate court for the case's declaration. It is not difficult to select the court in a general PC criminal examination, and the matter will be taken to court in the country where the crime was wrongfully committed; however, it is totally muddled due to the virtue of distributed computing in accumulated networks, and especially in distributed computing.

It is not certain where the wrongdoing has been done and where the confirmation is actually located as cloud assets are usually split between different customers in many countries. This clearly enables the examinee to choose where and how the suit should be used in the overall set of laws.

CONCLUSION

Currently, there are no complete cloud-specific forensic solutions on the market. Despite this, forensic analysts continue to use existing technologies to collect data from the Cloud.

Stacks of wishes for organizations and organizations with issues of distributed computing and restricted processing assets with the beautiful proposition of PCs as administration. As the generality of cloud processing increases, digital faults associated with the cloud or direct tilt of the cloud are noted. In this paper, we examined and dissected the inconveniences and difficulties observed by computerized criminology experts when they were experiencing non-ethical practices related to the cloud and presented our ideas about cloud legal sciences, including most of the exam items, the discovery of the cycle and its requirements were included, and the final proposition 3 is the fundamental crime potential that agents must measure.

My future work will focus on developing the nonstop enhancement inspection methodology for cloud-related digital missteps, proposing a cloud legal science paradigm, and demonstrating policy and models using some of the cases that we've encountered. IJFMP

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CASE STUDY

Educational ERP Systems over Cloud Data Security, Threats & Risk Analysis

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ABSTRACT

Enterprise resource planning is an organizational management software for managing the data related to the functioning of an organization in a streamlined manner. ERP was initially designed for the business and industry sector, but with its wide applications in various sectors, it has been continuously been adopted by organizations of other domains too. A major domain that is quick to adopt the ERP system is the educational sector, wherein the institutions are adopting the ERP systems to manage their resources. ERP systems are majorly based on in house server and need huge investment. The educational institution has thus been found hesitating to implement the system. A new approach has been to use a Cloud-based ERP rather than use an in-house server to store data, as the cloud is being used for reducing the amount of investment needed for the set-up. This research studies data security, threats and risk analysis and concerns of the educational institutions while employing the Cloud based ERP systems. Also, the authors have suggested a mid-path solution to overcome these concerns and risk factors - A hybrid mode of ERP system combing both the traditional and cloud-based approach.

KEYWORDS | ERP, educational ERP, cloud-ERP, information-security

INTRODUCTION

The Enterprise resource planning (ERP) is a software that combines all business-related procedures and functions at a combined IT platform for easy management which helps the business to function efficiently and effectively.[1] ERP is an old term in the manufacturing and production industry that dates back to early of the 1960s when the ERP was in the form of an Inventory Control system where it acted as accounting software, later in the 1970s this inventory control system was modified into MRP - Material Requirements Planning which was a package that provided support to the planning and control unit of the business production houses. This system was replaced by a more advanced MRP II system in the 1980s. This new advanced system was aiming towards the integration of technology with the manufacturing to increase the manufacturing of products.[2]

In the new world keeping up with technology, the cloud ERP systems were introduced in the early 21st century with advanced technology. These technologies can be accessed from any kind of device such as mobile tablet and computer which has internet facility. These applications

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come with two major advantages: a) The one with cloud integration ERP shows all departments. b) The centralized DBMS help all business communications such as record, monitoring, and processing. [3,4,5]

In businesses, ERP systems keep a track of their resources such as raw materials, finance, production capacity, and the standing of business assurances like salaries, sale-purchase orders, etc. This system makes sure that the relevant data is shared with the associated departments of the business and links them together with the core data. In other words, ERP not only ensures the data flow amongst the various departments of the business but also manages the information sharing with the stakeholders of the said business.[6] It has been observed that the ERP modules may interface with the business or organization's own data management process resulting in an increased degree of effort to match the data.[7] But despite the shortcomings and the risks involved while employing the system, a huge rise has been observed in the last decade globally wherein the organizations have been employing the ERP systems to streamline their workings, especially in Higher Education.[8]

ERP in the educational sector is an application that joins all the modules and departments of an educational institution into a single system whose access is available with the fraternity members of the said institution and also with students, their parents, and other stakeholders.[9] Each individual who is part of the institution has his/her unique and distinct user id and password. All the activities can further be monitored by the said administration with the usage of master id and password access. The educational ERP structure is entirely different from that of the business sector.[10] It comprises programs, fees, library, events, hostel, faculty data, examination.

The main aim of the educational ERP system is to provide a platform that encompasses all the functionalities together at a user-friendly interface. The educational ERP system digitizes all the information and data of the institute which are updated by admin login only and grant access to all the students and faculties.[11] Educational ERP reduces the need of maintaining the data on paper and keeping a check on the store for ensuring data security. The digitized details once entered into the system are stored on the server which can be accessed only with valid login credentials.[13]

It has been observed by the authors that despite the rise in the implementation of ERP in the educational sector from the literature review, it can be deduced that 60 to 65 percent of ERP systems have a failure rate and 30 to 35 percent of ERP implementations are canceled for various reasons. In the area of the higher education system, the implementation and success of ERP is very critical, failure of ERP system is comparatively higher as well. [14,15,16,17] There can be numerous factors responsible for this rate of failure the end-user training, cost input, step-wise implementation rather than the big-bang approach, and lastly the technical training of the users.

Related Work

In an increasingly competitive world, organizations are forced to focus on the primary goal rather than on the support services that are leading to a rise in such services by a third party. Thus, making this one of the main reasons to seek the need for cloud-based ERP systems.[17] It has been observed that the organizations that have migrated from traditional ERP systems to cloud-based ERP systems have been able to manage their costs efficiently and effectively improve their functioning. This supports the argument that using an ERP system in an organization could support the growth of the organization by enhancing its resource and service management system.[18]

Gartner defines cloud-based services as capabilities of scalable systems that can be delivered to users using internet services [19]. Due to the increased demand for cloud-based ERP systems, a new market for the new subscription-based delivery model of ERP or SaaS ERP has emerged. This model of ERP is similar in function to the traditional ERP systems.[20] The SaaS ERP is accessed through the internet. The information and the system application is controlled using a cloud service by the third party that offers its services to the client at a monthly or annual subscription fee.[21] Figure 1 depicts a comparison of traditional ERP systems and cloud-based ERP systems in terms of costs, complexity, and implementation time.

In brief, the advantages of cloud-based ERP

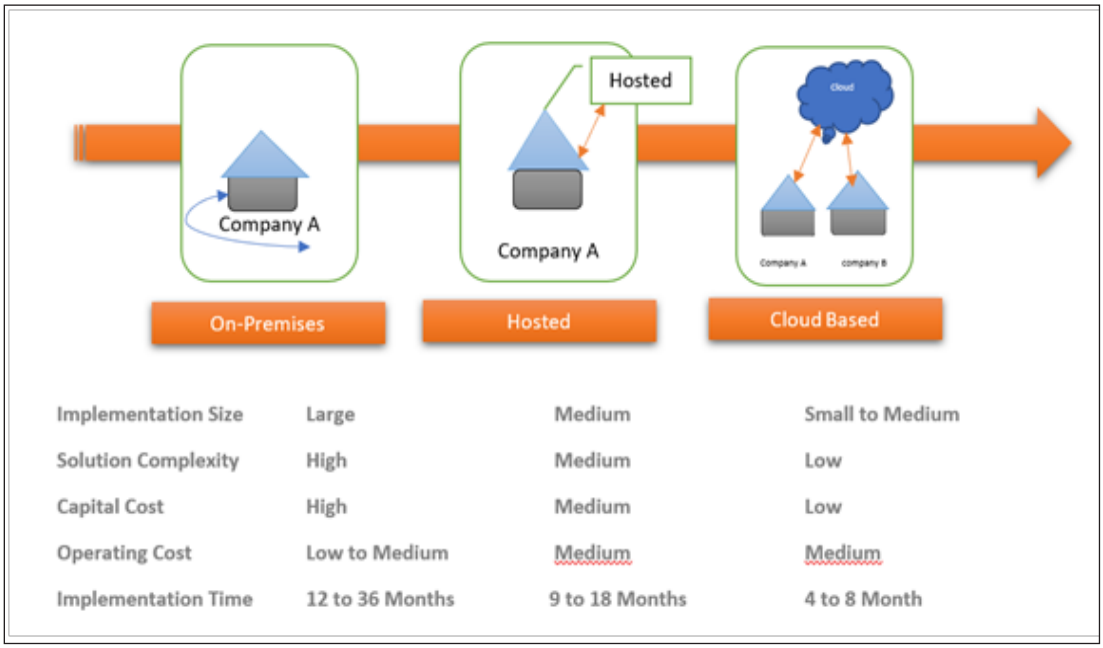

Figure 1: Comparison of Traditional ERP system and Cloud-based ERP system

over traditional ERP can be summarised as follows:[22]

- It permits small organizations to implement a complete ERP system without using much space.
- It saves on infrastructure costs, software maintenance and upgrade costs.
- Cloud-based ERP decreases the need for staff for ERP support and maintenance.
- Cloud-based ERP system is implemented faster, due to its agile approach.[23]
- It offers more scalability.
- It enables more mobility.

Despite having the aforementioned advantages, there are some concerning disadvantages of cloud-based ERP systems:

Issues in relation to Data Security

As ERP systems manage the essential data needed for the functioning of an organization, these organizations need to make sure that their data are secure in the cloud. As pointed out by Bishop that the security of any computer-based service depends on the integrity, confidentiality, and accessibility to the data stored cloud-based ERP systems are influenced directly by the security level ensured by the service provider.[24, 25]

Confidentiality

Weng and Hung (2014) discussed that when an organization implements a cloud-based ERP system, it must be ready to mitigate the risks surrounding the usage of cloud technologies and work on the tools to prevent unauthorized access of information stored.[26] Johansson (2015) disclosed that organizations are uncertain about storing their confidential data and information stored with the third-party service providers, who have not assured direct control over information. Another issue of concern is that the primary organization has no control over the staff of the cloud service provider, thus making it a worry over who could be accessing their data at the third-party vendor's end.[18]

Integrity

Another major concern experienced is to ensure the uniformity of the information stored. Puthal *et al.*, (2015), informed that the failures and errors that may occur from the cloud provider can easily affect the integrity of data. They also argued that the generally accepted method for data validation in the cloud is via public auditing, wherein the data validation can be done by a third party to check the integrity of the services.[27] A similar

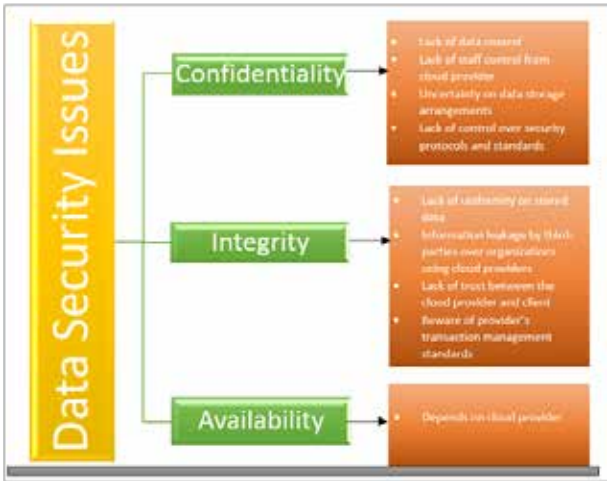

Figure 2: The major data security issues involved with Cloud-based ERP systems

concern was pointed out by Akande *et al.,* that the methods of verification of data and the levels of permission to manipulate the information are of utmost concern.[28] Figure 2 summarizes the major data security issues in cloud-based ERP systems.

Result and Discussion

Cloud-based ERP technology provides an attractive and cost-effective alternative to the traditional ERPs by offering a competitve edge in flexibility, scalability, ease of implementation, and cost savings.[29] Most major obstacles to the implementation of cloud-ERP are the risks surrounding information security and integrity of the data.

SaaS model of cloud-ERP is vastly growing in popularity, but the fears over information integrity and confidentiality need to be addressed before its implementation by any organization. Furthermore, the literature also highlights that the implementation rate for cloud-ERP is dependent on the organization type and functions.[30] As ERP system plays a very important role in the functioning of an organisation, the obstacles related to the implementation rate of SaaS-based ERP can be negatively impacted.

From the literature, it was showcased that the smaller organizations benefit from the cloud-ERP due to their low investment, and the issues about the risks in its implementation take a backseat.[31] Also, since smaller organizations are not able to employ proper IT experts and adapt apt security measures for traditional ERP systems, the cloud-ERP approach helps them to outsource these to third-party vendors thus saving money and efficiently utilizing their resources.

On the contrary, the larger organizations are more concerned about security issues of the cloud-ERP in relation to their sensitive and confidential data, which they have to share with the cloud service provider. They are more concerned about the security breach and its impact on their image amongst the stakeholders. As a result of these concerns the larger organizations are found to be a bit reluctant in adopting Cloud-ERP systems. A midway approach was suggested by Utzig *et al.*, wherein the approach was to approach cloud-ERP with 50 to 60 percent shift over 10 years with side-by-side existence of traditional ERP system.[29] Recently, a new approach has been gaining popularity amongst the larger organizations which is a 2-tier strategy for ERP also known as the hybrid cloud-based ERP. Ruivo *et al.,* (2015) maintained that approximately 77percent of organizations will employ the hybrid cloud-based ERP system, but currently, only 20 percent of such organizations have the plans and structure needed for this implementation.[32] This was evidently supported by Peng and Gala (2014) who also argued that the hybrid ERP system is an efficient solution for the organizations adopting the cloud approach as they can keep their core functioning modules and data on an in-house ERP server and rest they can share on the cloud-based server, before completely shifting to the full cloud-ERP system.[33]

Hybrid cloud-based ERP is, in a true sense, a mid-path between the traditional approach and the cloud approach wherein the best of the both approaches have been combined into one. This approach gives organization the freedom to choose which data they want to share on the cloud and which one they want to keep on an on-premise server. Thus, eliminating the risk of breach of confidential data via the cloud. Clarke *et al.,* (2014) shared that the biggest advantage of the hybrid approach is that it gives organizations the flexibility to reallocate their resources after moving the part of ERP and the associated services to the cloud enabling them to work more effectively. This approach also allows the organizations to enjoy the benefits of Cloud-ERP while minimising the

risks of storing of sensitive and confidential data on the cloud. [33]

CONCLUSIONS

Due to the great advantages of cloud-based ERP, educational institutions are considering using it for their institutions. Latest ERP platforms overcome a few of the weaknesses of older ERP systems. And due to the low cost investment and scalability of cloud-based ERP systems, even the smaller educational institutions are looking favorably at this.

However, cloud-based ERP systems are concerned about the integrity and security risks with regards to the data. Large institutions have been a bit wary of adopting this system, as storing sensitive data on third-party servers is a major concern. Another concern is the misuse of data and security breaches at the third-party servers, which make the switch rather difficult.

The takeaway of this work is to suggest a mid-path by combining both traditional and cloud-based ERP systems. This type of approach will allow the institutions to store their sensitive data by using an in-house server and less sensitive information on the cloud server of the ERP system. This hybrid approach will ensure the security and scalability of the data of the institution with ease-of-access to the data via the on-premise server and a wide range of access to ERP from different locations of end-users due to the cloud server usage. Also, the cloud-based ERP enables the administration to install different modules of ERP as per the need of the institution as it evolves along with the education policies of the government. The hybrid approach also enhances the mobility of the ERP system, as the cloud server provides high system performance and more customization of the system according to the needs of the institution. Hence, the hybrid traditional-cloud-based ERP systems are more suitable for educational institutions to ensure data security and ease of access. IJFMP

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ORIGINAL ARTICLE

A Cross-sectional Record-based Study on Homicidal Death Pattern in Bareilly Region, Uttar Pradesh

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ABSTRACT

Homicide is considered part of human behavior. Significant number of unnatural deaths have been reported in which tools like ax, hammer, kitchen knife, etc., were used, followed by other assault weapons. They were used by attackers due to their easy availability. Increasing rate of homicides worldwide reveals extreme violence, increased aggravation, lack of patience and mental disturbance in the culprits. The first step toward developing interventions to reduce the impact of homicidal crimes and violence is to study homicidal trends in civil society as it varies from region to region. It is a record based cross sectional study in which all the 137 homicidal deaths out of 151 cases of Bareilly region reported at Division of Forensic Serology, Forensic Science Laboratory, Moradabad, Uttar Pradesh from January 2019 - June 2019, were studied and analyzed. All the suicide cases with improper entry and those killed in police encounters were excluded. Various parameters like relationship of the victim to the accused, method of homicides, sex and gender of victims, category of lethal weapons, motive behind the homicide, place of incident etc., are considered. This research has been conducted with the objective to determine the most commonly used weapons for assault in Bareilly region. Moreover, parameters like determining the most vulnerable victims, age groups, place of incidence, gender, motive, accused-victim relationship, and cause of death behind the homicidal death were considered.

KEYWORDS | homicide, IPC 299, manslaughter, men's rea, actus reas

INTRODUCTION

Homicide basically is murder or manslaughter of one human being by Another, either intentionally or unintentionally.[1] Therefore, homicide is classified into two basic categories (i) Legal or lawful include excusable and justifiable homicide. (ii) Illegal or Unlawful homicide include felony murder or killing (Section 299 of IPC). Unlawful homicide basically include (i) Felony murder not amounting to intentional killing and it comes under the section 304 of Indian Penal code 9 (ii) Felony murder amounting to intentional killing and it comes under the section 300 of Indian Penal Code.

Ultimately, mens rea indicates guilty thinking or forethought, whereas actus reus implies planning with actual implementation which is major element for commitment of crime of murder. The major element of mens rea is not present in the culpable homicide offence not amounting to murder but during the conflicts, the offence of felony murder unknowingly committed out of human aggression or vengeance.[2]

The annual homicide rate prove to be a useful "gold standard" in evaluating the increasing heinous crimes because it is the best indication of social violence. Because homicide is such a serious crime,

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it attracts the attention of both the police and the general population. As a result, police agencies must be concerned about the availability and provision of fatal weapons, and strict preventive measures must be undertaken, such as changes in social, economic, moral, and academic status.

Homicide is becoming increasingly widespread all over the world, and the pattern is shifting dramatically due to rising population, changing lifestyles, modern man's requirements, and the ease with which it can be committed with the use of unconventional/improvised tools that are easily available in our surrounding.[3] According to the NCRB report for 2019, 51.56 lakh cognizable crimes were registered in 2019, including 32.25 lakh IPC crimes and 19.30 lakh Special & Local Laws (SLL) crimes, of which 10.5 lakh were crimes against the body (intentional and unintentional murder).[4]

The first step toward developing interventions to reduce the impact of homicidal crimes and violence is to study homicidal trends in any civil society. Also the availability of items that can be used as weapon, motive behind homicide, social, cultural and economical factors and the injury pattern influence the accountability of the assailant responsible for forming the particular trend of homicidal death in any particular region or geographical area. Therefore, to exclude and identify the mentally disable offender and habitual criminals data on the cause, age, gender involved, and weapon used, as well as other demographic studies, are required to achieve the objective of a peaceful society.

Previously no research has been conducted on the fatality profile of homicidal incidences from this specific region, the current study was conducted for the evaluation of homicidal trends regarding various parameters such as victim-accused relationship, method of homicide, sex and gender of victim, weapon used, motive for homicide, and location of homicide, cause of death etc. Moreover this study aimed at determining the most commonly used weapon for fatal/life threatening injury in the study of the geographical region.

METHODS

This study was conducted in the Division of Forensic Serology, Forensic Science Laboratory, Moradabad, Uttar Pradesh, over a period of 6 months, from January 1st - June 30th, 2019). A total of 151 incidents were reported for forensic examination by police stations from several districts of the Bareilly region with 137 of them satisfying the exclusion-inclusion criteria. The institutional ethical committee got ethical clearance prior to the conduct of study.

Source of Information

All descriptive data were collected from inquest and post-mortem reports. Firstly, basic information like victims' age, gender, location and time of occurrence of crime, motive behind homicide, and victim-offender relation were collected through inquest report. The data on the type of weapons from injury pattern were obtained from post-mortem report. All details were thoroughly analyzed, interpreted, and conclusions were obtained. The obtained observations and conclusions were compared to the previous study conducted by other authors and thus graphs and pie charts are used to show the behavior of data. Throughout the study, identity of the victims was kept secret for privacy.

Data analysis was done using data analysis software. An MS Excel spreadsheet was used to store the information and the statistics was obtained like percentage, frequency and proportion.

Inclusion Criteria

The investigating officer suspected and confirmed homicide instances of murder victims.

Post-mortem examinations have led to the suspicion of homicide. The study also included infanticide cases.

Exclusion Criteria

Suicide cases with improper entry or cases involving police encounters.

Deaths due to non-mechanical mode like poisoning, drowning, etc., were excluded.

RESULT

During the study period it was observed that out of 151 cases, 137 cases (90.72 %) were due to mechanical injuries by sharp and blunt weapons, as well as firearms. The current investigation reveals that male victims outnumber female victims in 97 cases (70.81 %). with the female victims 40 case (29.19%).

S.NO.	TIME OF INCIDENT	NO. OF CASES	PERCENTAGE (%)
1	Morning (6am - 12Noon)	9	6.57
2	Afternoon (12Noon – 6pm)	28	20.44
3	Evening (6pm - 12Midnight)	54	39.42
4	Late Night (12Midnight-6am)	30	21.90
5	Unknown	16	11.68
Total		137	100

Table 1 Arrangement of reported cases based on time at which homicide incidents took place

SL.NO.	METHOD	MALE	FEMALE	TOTAL	%
1	Hard and blunt tool	23	12	35	24.14
2	Sharp penetrating light tool	39	11	50	34.48
3	Heavy sharp pointed tool	11	5	16	11.03
4	Firearms	24	6	30	20.69
5	Strangulation	4	7	11	7.59
6	Throttling	2	1	3	2.07

Table 2 Arrangement of reported cases based on homicie mode versus gender of victim

SL.NO.	MOTIVE	TOTAL	PERCENTAGE
1	Revenge/Petty Quarrel	46	33.57
2	Argument/Personal dispute	22	16.06
3	Not known	18	13.14
4	Financial/Property conflict	14	10.22
5	Sudden provocation	8	5.84
6	Love affair	7	5.11
7	Mental illness	7	5.11
8	Dowry related	6	4.38
9	Family dispute	6	4.38
10	Rape	3	2.19
Total		137	100

Table 3 Arrangement of reported cases based on supposed intention behind homicide

SL.NO.	METHOD	MALE	FEMALE	TOTAL	%
1	Victim's home	18	23	41	29.93
2	Roadside/Riverside	22	6	28	20.44
3	Agriculture field	13	2	15	10.95
4	Temple/Masjid premises	8	0	8	5.84
5	Assailant's home	6	1	7	5.11
6	Forest	6	1	7	5.11
7	Street	5	2	7	5.11
8	Workplace/Market place	8	0	8	5.84
9	Open field/Plot/Garden	4	2	6	4.38
10	Peripheral Lonely Place	4	1	5	3.65
11	Marriage tent premises	1	0	1	0.73
12	Not Known	3	1	4	2.92
Total		96	41	137	100

Figure 4 Arrnagement of cases based on crime location as per inquest report

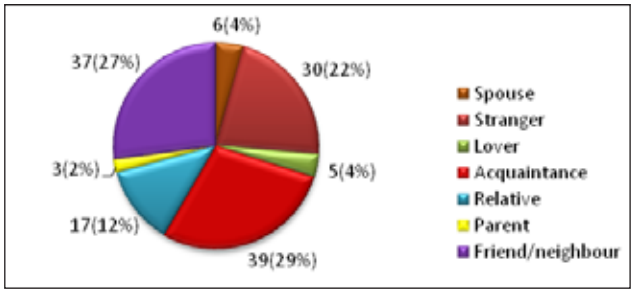

Figure 1 Time of Homicide Occurences

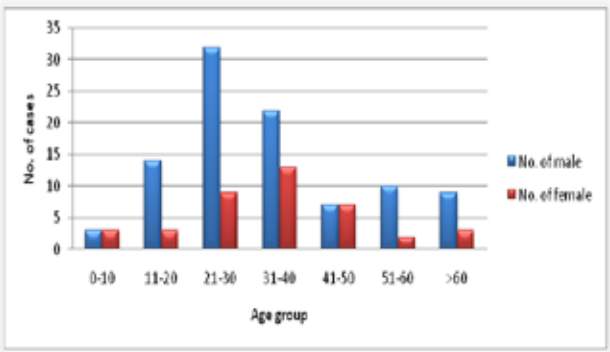

Figure 2 Time of Homicide Occurences

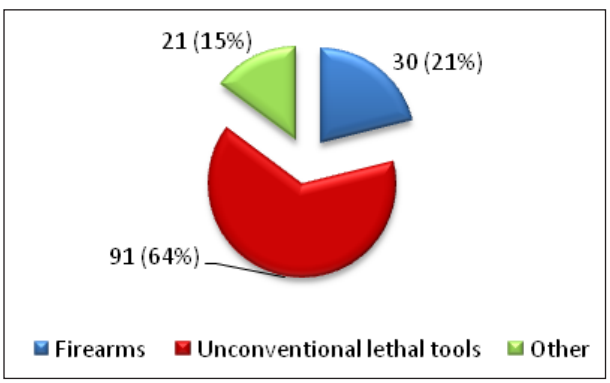

Figure 3 Time of Homicide Occurences

Time of Homicide Occurrence (Table 1)

The number of homicidal deaths was found to be highest in the evening (6pm-12midnight), with 54 cases (39.42 %), followed by late night (12midnight-6am), with 30 cases (21.90 percent). In 16 cases, the exact time of occurrence is uncertain/unknown (11.68 %). The morning had the fewest cases i.e. 9 (6.57 %), indicating that homicide was more likely throughout the night.

Homicide Distribution by Gender (Table 2)

Light and sharp piercing objects were found to be the most generally approached homicide mechanism in 50 cases (34.48%), followed by

hard and blunt objects in 35 cases (24.14 %). In 30 cases (20.69%), firearms were employed, heavy sharp pointed tools/items were used in 16 cases (11.03%), strangulation was used in 11 cases (7.59%), and throttling was used in one case (2.07 %). Light and sharp penetrating object was the most common means of homicide among males, with 39 cases (37.87%), followed by firearm in 24 cases (23.30%), and hard and blunt tools/items 23 cases (22.33 %). Hard and blunt object 12 cases (28.57 %) were the most common homicide mode observed in females, followed by light and sharp penetrating tools/items 11 cases (26.19 %), strangulation 7 cases (16.67 %), firearm 6 cases (14.29 percent), heavy sharp pointed tools/items 5 cases (11.90 %), and throttling 1 case (2.38 %).

Motive behind Homicide (Table 3)
The most common reason for homicidal death was vengeance/petty quarrel in 46 cases (33.57 %), followed by dispute in 22 cases (16.06 %), and financial/property conflict in 14 cases (10.22 %). In 18 cases, the intention was unknown (13.14). Love affairs and mental illness were the suspected motives with the same number of homicide cases (7.11%), followed by dowry-related and family disputes with each 6 cases i.e. (4.38 %). Rape was linked to the fewest number of homicide incidents 3 cases (2.19%). In 8 cases, homicidal death was caused by a sudden provocation (5.84%).

Location of Homicide (Table 4)
The highest occurrence (29.93%) occurred inside or near the Victim's home, followed by incidence along the roadside/riverside (outside), i.e. (20.44%). The majority of incidents involving female victims occurred in or around their residences (57%). Males had the highest occurrence (22.91 %) on the roadside/riverside (outdoor), followed by (13.13 %) on the inside or near the Victim's home. Because most interactions take place at home or outside, these were the primary venues of homicidal acts in our study. Due to cultural and religious beliefs, females are less involved in conflicts, according to this study

Age & Gender based Distribution of Cases (Figure 1)
According to the findings, the age range 21-30 has the highest number of victims (29.9%), along with the age group 31-40 with 35 cases (25.54 percent). This study includes victims who have been killed within less than a year (infanticide). 55.4 % homicides were occurred in age group 21-40 years, however least and the same number of victims age group were found in 51-60 and >61 years with each 12 case (8.75%) concluding that victims in this age group 20-30 are least tolerant which leads to arguments and scuffle and ultimately end to crime of homicide.

Offender-Victim Relation (Figure 2)
In the majority of cases, the accused was acquaintance 37 (28%) of the victim, followed by a friend/neighbor 37 (27%) and a stranger 30 (22%) of the victim. In the fewest situations, parents 3(2%) of victims were discovered. An acquaintance in this research is someone that one person recognizes but doesn't know personally. In this study, acquaintances include a shopkeeper, a peasant, an office worker, a criminal gang, a local eccentric person, and a factory owner, etc. This demonstrates that the attacker was well-known to the victim, and that the criminal took the victim's life out of vengeance.

It was discovered that in the majority of cases, unusual household/domestic instruments (91%) were used to commit homicide. In 30 cases (21%), firearms was used for causing grievous hurt. Least number of homicide cases, were observed with other category of weapons 21 case (15%). Unconventional tools like *hathoda* (ax), *hasiya* (sickle), *bhallam*, spade, *kodal*, kitchen knife, gas cylinder, sword, *gupti*, *vasula, nidaal*, scissor, *gandasa* (chopper), and other agriculture/domestic/household equipment were included in the study. Cement tiles, sugarcane, stone, bamboo stick, brick, iron rod, and other weapons fall under "Other" weapons group.

DISCUSSION

In the current research based study total 151 cases were reported out of which 137 were of homicides due to mechanical injuries. (i.e. by sharp, blunt weapons and firearms) accounting for 90.72% of total reported unnatural death cases over period of six month i.e. Jan 2019 to June 2019. The tendency of homicide in males was higher 70.80% as compared to female victims 29.19% which was 7:3. This tendency of homicide was less in female due to cultural and religious reasons.

Findings of our study shows that majority of the homicidal incidences took place during evening 54 cases (6pm-12midnight) and late night 30 cases (12midnight-6am). Reason may be that chances of assailant being recognized were less and the execution of crime and escape for the assailant become easy. Secondly, as the study shows the males outnumber the female victims so there may be chances that males usually consume alcohol in night or evening hours due to which they engage in arguments due to mental illness or disorder which

ultimately leads to homicide. Similar observations were made in studies conducted by other[3,5] in which frequency of homicidal incidence was maximum during the night and evening hours. Our study is in contrast to studies conducted by Vougiouklakis T *et al*.,[6] where in maximum (26.9%) of crimes occurred during noon. The frequency of homicide was higher during the day time (74.55%) in a study conducted by Vijayakumari N *et al.*[7] The present study found that the commonest type of weapon used was light sharp penetrating weapons in 50 cases (34.48%) followed by hard and blunt objects in 35 cases (24.14%). The observation goes consistent with the study of Dhaval J. *et al.*[8,3,9,10]

The use of only light and sharp penetrating objects points towards premeditated/planned crimes with motive of robbery, revenge, or property/ financial dispute. The findings were in contrast with study of Prashant Mada *et al.*,[11,12,13] where common weapon of choice was hard and blunt weapons. Exclusive use of hard and blunt weapons for homicide could be an unplanned/unpremeditated offensive/ confrontational reaction of a person to abrupt and dangerous provocation in dispute. The most common motive of homicides was petty quarrel/vengeance indicates that homicides were not predetermined and planned but due to less tolerance level and sudden provocation it ends up with homicide. Our observations and findings goes consistent with Shiva Kumar *et al.*,[10] (32.5%) and Dhaval8 (29.4%), B. C. whereas financial issues were found to be the major cause behind homicide by Sinha *et al.*[14]

As per our findings, the victim's home was the commonest place of homicide 41 cases (29.93%), as most of the interaction takes place at the home and secondly accused and victim are well acquainted with each other so they accounted as most frequent sites of murderous acts. Our findings are similar with that of Rodge.S *et.al.*,[22,3] whereas it differs with the study of Dhaval J. Patel *et al.*,[23,10,21,24] where outdoors was the primary site of homicidal act. Other location of crime recorded during study was street, forest, garden, agriculture field, open plot, marriage tent premises, temple/masjid premises etc. In our study, the injuries were most commonly seen over the head and face (30.4%), followed by chest (25.2%). The observation goes similar with the Gemechu T. *et al.*[25,16,19,21,26,14] The most used weapons were sharp and light penetrating weapons (34.48%).

Present study shows the predominance of victims of age group 21-40 which comprises of more than 55% of cases. Maximum involvement of 21-40years young people may be due to the fact that people in this age group are more aggressive, impatient, and less tolerant, which leads to arguments and scuffles, which eventually leads to homicide. The study shows overall predominance of male victims which is consistent with study of Shailesh Jhaveni *et al.*[31,32,11,8,15,17,18,3] In most of the homicides offenders were acquaintance 39 cases (29%) followed by friend/neighbor 37(27%). In all 17 relative homicides the offender was the male. Our finding were consistent with the observations made by Mohanty M.K.[5] 20(29%) of the perpetrators who were identifiable were usually an acquaintance of the victims and is in contrast to other[23,14] where in the stranger committed maximum number of homicides. This shows that the offenders have some personal issues and victim accused are closely related to each other due to which they take vengeance. In all the studies conducted worldwide by researchers[27,29,28,30,] the weapons were classified as sharp or blunt weapons but in this study we have focused on the various unconventional household/domestic tools which have been used in this area for homicide like ax, kitchen knife, sword, hammer, ice picks etc. These weapons had been the rising headlines of all the newspapers but no researchers had focused on main reason for the usage of these easily available weapons. It was found during the study that in majority of the cases 91 (64%) unconventional household/domestic weapons including domestic, agriculture, household, personal belongings tools or items etc. are used out of psychological mental disorder to fulfill the revenge to commit planned/ unplanned murder.

Limitations

This study was confined to specific region/area and the findings were based on documentary data (Inquest report and PM report) and information present in the respective reports submitted by the investigating officer during the autopsies of the dead bodies. Crime scene was not visited in any of the studied cases.

CONCLUSION

From the above findings, it can be concluded that social and demographic factors have an impact on homicide and homicide were the consequence of quarrels and scuffles between the parties involved. Nobody has the right to take the life of other. This youth victims are more aggressive and impatient therefore effective anger and stress management through mental psychotherapy may minimize homicide rates in this age group. Homicide reduction will be aided by socioeconomic well-being, poverty elimination, and more work possibilities. Children should be taught in schools the importance of ethical and moral ideals, which will aid in the formation of human interactions. Like other countries the strict provisions should be made in India for restricting the possession of lethal unconventional/ unusual tools at the public places or buildings. IJFMP

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REVIEW ARTICLE

Mathematical Models studying Crime Dynamics: A Review on Adoptd Approaches

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ABSTRACT

As crime is becoming a major issue over the last few decades in India, mathematical models came as a rescue to study and understand the dynamics of crime and to analyze the effect of control and preventive measures. This review aims at discussing several approaches to formulate such mathematical models, namely, economical approach, epidemic approach, predator-prey modeling approach and spatio-temporal approach. Strategies for combating financial crime are also examined. Through this review, it has been observed that study on the dynamics of crime against women is less considered. Such gap could be analyzed with the help of information obtained through the approaches discussed. The work of this paper could help crime analysts to predict the trends in the crime against women and prepare the optimal control and prevention policies.

KEYWORDS | mathematical modelling, crime against women, spatio-temporal

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INTRODUCTION

Crime is considered to be a wrong committed against the society as it shakes the social conscience and sense of societal stability and fearlessness. It is a law prohibited event, followed by prosecution of accused and later by punishment on conviction. Out of several criminal activities, some are listed as burglary, fraud, cybercrime, financial crime, domestic abuse, child abuse, robbery, rape and murder.

There is a social transmission view for the spread of crime. Crime can be considered a social epidemic process, which may infect people by means of behavior, messages, social structure and traditions or ideas. It is assumed to be spread by social contact which in turn depends on social environment including some factors like poverty, education level, religion, socio-cultural practices and inequality. Certain type of people can effectively broadcast their ideas and convince people to listen and follow suit, leading to the transmission of criminal activities.

The level and intensity of crime can vary from nation to nation. As per the statistics released by NCRB (National Crime Records Bureau), it was observed

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that crime in India is increasing at an alarming rate over recent years. In 2016, 48.3 lakh crime cases were recorded, which rose to 50.07 lakh in 2017 and further to 50.74 lakh in 2018.[1] Latest released report showed that crime increased by 1.6% in 2019, i.e., it reached to 51.56 lakh.[2] Report further showed that crime against women increased by 7.3%, making it to a total of 4,05,861 cases. Although rape cases which went up from 32,500 in 2017 to 33,356 in 2018, i.e., 91 rapes per day, lower down to 87 rapes per day in 2019. The average is still a matter of concern. The group that has been suffering the most is "Women". One in three women gets affected by such gender-based violence at least once in their lifetime. As per Indian census 2001-2011, women population comprises of approx. 48.04% of India's total population.[3] Such disturbing trends in crime shows that about half of the population is facing such gender-based crimes. Women, despite their age, religion or caste, social well-being, economic stature, education, are being victimized of sexual violence and offenses like rape, harassment at workplace, domestic violence, kidnapping, suicide, dowry-related deaths. These crimes are somehow hindering their empowerment and progress, which directly affects the societal and economic development. World Bank report of 2018 claimed that loss in productivity due to violence against women, costs an estimate of 3.7% of country's GDP.[4] Thus, addressing this issue is of utmost importance.

With crime becoming more sophisticated and organized, certain approaches were adopted to regulate it. Several studies were carried out based on data analysis such as survey, quantitative analysis, self-report and structured interview to get some insights into the nature of victimization. Appreciable review was presented by Ellsworth in 2019,[5] comprising of 33 such studies, where he discussed the effect of street crime victimization on homeless adults.

Another effective approach to study the dynamics of crime is by formulating a mathematical model. There can be a case where carrying out actual experiment to know the behavior of real-world system can be expensive, impractical or impossible. For example, to study the dynamics of crime against women, it is impractical to do experiments on women. In that case, analysing the model provides insight on the parameters involved and allows making predictions about systems behavior. Such qualities of mathematical models make them more appropriate than carrying out actual experiment. Several mathematical models have been introduced in the past to study the crime dynamics, using different approaches like economical approach, epidemic approach, predator-prey modeling approach and spatio-temporal approach. Some of those models are discussed in the following section.

METHODS

Economical Approach

The economical approach is adopted in mathematical modeling for observing, understanding and making predictions about economic issues and problems.

In 1996, US Presidents Commission on Law Enforcement and Administration of Justice presented a report on crime in America to address the causes of crime and gave recommendations to prevent the same.[6] They created a task force on Science and Technology whose aim was to introduce simulation modeling to demonstrate how system models can be used to evaluate cost associated to crime and analyze the effects on crime rates. In 1968, Becker,[7] initiated an economic approach in crime modeling to measure the social loss caused by offenses and to further calculate the resources and punishments expenditure that minimize this loss. He developed a model considering the behavioral relations behind the costs of crime (involving white-collar crimes), by placing these relations into five different categories. In order to minimize social loss, he discussed the optimal conditions for decision variables p and f, representing probability of conviction and punishments respectively. The effects on the optimal values of p and f due to changes in the basic behavioral relations like the damage, cost, and supply-of-offenses functions were also analyzed. Further, several interesting implications of optimality conditions were illustrated via examples and at last, the optimal control of crime was determined through statistical and economic analysis.

To get a more realistic and dynamical approach towards optimal control of crime, Zhao *et al.,* have

proposed a model consisting of ordinary differential equations, assuming constant population.[8] The population was divided into five sub-classes, namely the non-poor class, the poor class, the criminal class, the jailed class and the recovered class. Through stability analysis, they studied the dynamics of poverty and crime which further helped in identifying the cost-effective strategies to control such crime. Since people immigrate for employment and other purposes, their assumption of constant population was not always the case in real world scenario.

Further, in 1998, Chiu has proposed an economic model relating burglary and crime distribution.[9] Assuming fixed incarnation time for burglars, and the fact that burglars choose their target houses by looking at the quality of house, he investigated the relation between level of crime and distribution of income. Through a result introduced by authors, the model analyzed that more regressive tax results in more crime and rich neighborhoods may result in lower crime rate. Using Lorenz and 'relative differential' comparisons of income distributions, he showed burglary crime level may be increased by increase in income inequality. Later, after analysing that houses close to burgled house have high probability of crime than those which are far away, Curtis *et al.,* have formulated a model relating crime and security.[10] They analyzed the role of technology on crime statistics by showing how crime probability decreases upon imposing security. Their model was based on an assumption of constant population, which is again not always the case in real world scenario. To fill this gap, in 2013 Shukla *et al.,* have introduced a model considering dynamic population, i.e. both immigration and emigration of susceptible and criminals were taken into account.[11] To study the impact of technology in combating crime, they considered five interacting variables, — susceptible population density, criminal density, removed criminal's density, crime burden density (proportional to criminal density) and the technology level used to control crime. They used stability theory to analyze inverse relation between crime and level of technology. The model also concluded that crime burden may reduce preventing illegal immigration.

Another type of crime that exists is financial crime, a property crime where an entity illicitly transforms the property's ownership for their own use and benefit. Financial crimes involve fraud, money laundering, identity theft, bribery, forgery. Some causes of financial crime involve greed, unemployment, unsatisfactory salary, laziness, quest to get rich. Importance of such crimes should not be minimized as they can lead to violent crimes and terrorism. Keeping this in mind, Akhani *et al.,* have introduced a mathematical model to analyze the dynamics of financial crime under some optimal control strategies and preventive measure.[12] The model was examined through stability analysis and reproduction number. Suitable Lyapunov functional was constructed to obtain crime free equilibrium and unique crime-present equilibrium, which were globally asymptotically stable for reproduction number less than unity and greater than unity respectively. Several parameters were tested against sensitivity analysis to determine their importance in combating financial crime. Further, the significance of implementing time dependent optimal control measures including public enlightenment campaign was discussed. They also implemented cost effective analysis which showed that the optimal use of public enlightenment campaign is the most effective and least costly strategy in combating financial crime.

Through the study of these models, it was observed that economical approach helped in measuring the social loss caused by several crimes and identifying some strategies, which help in combating crime and hence minimising the loss. The strategies included imposing security, preventing illegal immigration, use of public enlightenment campaign etc.

Epidemic Approach

Here crime is considered to be a social epidemic process. The approach is similar to the one used in mathematical biology to describe the spread or containment of epidemic in a population.

Such methodological approach was adopted by Campbell *et al.*[13] after the evidences provided by Glaeser *et al.*[14] in 1996 on the importance of social interaction. They considered it an important feature in their model i.e. susceptible are likely to commit crime upon coming in contact with criminals. They split the population into three

groups, namely non susceptible, susceptibles and active criminals and studied the effect of social interaction between the agents. Moreover, they assumed that any agent can be influenced by social interaction, not just a proportion of population, as assumed by Glaeser *et al.* Using same approach Gonzlez-Parra *et al.,* have presented a mathematical model by taking into account behavioral changes of individuals.[15] The total population was divided into six categories, namely susceptible, free criminals, convicted criminals, criminals arrested in jail, judges and police officers. They analyzed the system of six nonlinear ordinary differential equations by examining the obtained equilibria. The method of next generation matrix was used to compute threshold parameter for the extinction of criminality, which helped in understanding best policies for controlling crime. Furthermore, sensitivity analysis investigated some important parameters related to transition of susceptible, police officers and judges to criminals, lower criminality. Lastly through elasticity analysis, where the impact of the parameters on criminality was compared, it was observed that honest judges might reduce criminality in the society.

There can be a case where youth having high social and financial needs gets involved in illegal drug trafficking groups, controlled by adults. The social disease caused by this criminal interaction among youth and adult will spread like an epidemic in highly dense and metropolitan areas. To study the above-mentioned scenario, in 2019, Rivera-Castro *et al.,* have presented a model based on SIR disease dynamics by dividing the heterogeneous population into two groups namely youth and adult. Interaction between the group was governed by three mixing patterns which were proportionate, preferred and like with like. Basic reproduction number was derived to analyze the model and stability analysis was carried out for homogeneous mixing. Further, sensitivity analysis showed some insights about various parameters involved. The model concluded that involvement of kids in drug trafficking can be decreased if they do not interact with adult gangs, provided they don't have any previous gang involvement. Also, the best ways to reduce spread of gangs as suggested by like with like mixing is prevention and indulging in activities like arts, music, recreation, sports etc.

A corruption control model, having similar behavior as epidemiological model, was proposed by Athithan *et al.*[17] They divided the population into three classes, namely corruption susceptible, corruption infected and recovered from corruption class. The model was analyzed through stability analysis and threshold quantity R_0. Stability analysis showed the presence of two non-negative equilibria, where the first one was corruption free equilibrium and other was endemic equilibrium, which indicates presence of corruption. It was observed that corruption free equilibrium always exists and is stable for $R_0<1$and unstable otherwise. Whereas, endemic equilibrium exists only when $R_0>1$. To analyze the findings, numerical simulations were performed. Furthermore, they showed that including optimal control parameter such as self-cure rate in the model could minimize the corruption and the cost on reducing the corruption. It was also observed that providing psychological pressure through media/advertisements increased the self-cure rate and hence reduced the corruption in the society.

Mohammad and Roslan have provided a dynamical 2-Dimensional model to analyze the spread of crime system.[18] They referred the model introduced by McMillon et *al.,* in 2014,[19] with basic components being criminally active, not active and imprisoned population. The main objective of the paper was to analyze the model via stability analysis, bifurcation and reproduction number. Numerical simulations were carried out, as a result of which two equilibria were found, namely crime free equilibrium and crime equilibrium. Eigen values were calculated using Jacobian Matrix, whose sign represent whether the equilibrium point is stable, unstable or saddle. The results showed that crime free equilibrium was asymptotically stable while the crime equilibrium was unstable. Further, bifurcation analysis revealed that increasing a contagion parameter regarding criminal behavior in the model increase the number of criminally active and incarcerated ones. The model also showed that increase in the above-mentioned parameter also increases $R_0>1$ and hence increases crime rate.

In 2019, Ugwuishiwu *et al.,* have presented a deterministic model to analyze the dynamics of interaction between crime, criminality and

victimization in the population.[20] Two forms of rehabilitation having substantial effect on crime when implemented effectively were considered in the model, namely, a reformation program for criminal individuals and psychotherapy for victims. The proposed compartmental model was studied through stability analysis and two equilibria were obtained, namely crime free and persistent crime equilibrium. Their stability was computed with the help of reproduction number, a factor responsible for persistence of criminality or victimization. The model analyzed that crime free equilibrium was globally-asymptotically stable when $R_0<1$, whereas unique endemic equilibrium was locally-asymptotically stable for $R_0>1$. Also, forward bifurcation was discussed using Centre Manifold theory. At last, the consequences of both effective and ineffective implementation on the rehabilitation forms were discussed. It was observed that non-criminalisation of victims is less effective in containing crime than the prevention of repeat victimization. A positive impact was observed from the removal of criminals either through quitting or death.

Summarizing the above models, it can be observed that the epidemic approach provided a better understanding on the influence of social interaction on spread of crime. The models also discussed several parameters to lower criminality, say honest judges, indulging susceptible in other activities like music and sports, reducing corruption by increasing self-cure rate, implementing rehabilitation forms, reducing criminal etc.

Predator-Prey Modeling Approach

Another approach to study the dynamics of crime is through Predator-Prey Modeling. Such approach helps in understanding the interaction of populations under natural environment.

Nuno *et al.,* consider such modeling[21] to discuss the interaction between three classes. Namely, owner, criminal and security guard. They considered owners to be the prey, criminals to be the predators of owners, and the security guards were predators of both owners and criminals. In this triangle model, all classes were competing for same resources. Later in 2016, Sooknanan *et al.,* then presented a modified predator-prey model in combination with an infectious disease (eco-epidemiological model), to examine the relationship between police (predator) and gang members (prey).[22] The model dealt with the corruption of police officers caused by gang members and other corrupted police officers. Further, the model analyzed various crime fighting strategies and policy changes. By assuming constant number of police officers, several behaviors of the model were interpreted through stability analysis. Response of gang members to various crime fighting strategies and policy changes were discussed. At last, they identified tripping points, which could lead to disappearance of such gangs and corrupted police officers from the population.

Motivated by both predator-prey and epidemic approach, Abbas *et al*., have introduced a model to examine the interaction between criminal and non-criminal population.[23] They changed the growth of non-criminal population from exponential to logistic. The logistic model showed that density of criminal population vanishes as carrying capacity goes below its threshold value (R_0). Whereas, it increases slowly when carrying capacity crosses its threshold value. In terms of financial conditions, this meant that rate of increase of crime can be controlled by improving the living standards. Logistic model was further modified to law enforcement model, which revealed that rate of crime decreases as enforcement parameter increases. Moreover, once the enforcement parameter reaches its threshold value, the population becomes crime free. Occurrence of saddle node bifurcations in law enforcement model was also discussed. However, their model did not incorporate the fact that under proper counseling criminals might recover and leave crime temporarily or permanently.

In 2019, Srivastav *et al.,* have provided an extended model by introducing a new class namely, recovered class.[24] They assumed that criminals might move to recovered class upon proper law enforcement and proper counseling. Also, they assumed that the negligible effects of counseling and unpredictable nature of human behavior could lead to a flow from recovered class to criminal and non-criminal class. The non-linear mathematical model was analyzed by computing basic reproduction number, to determine the persistence of crime. The existence and stability of different equilibria were discussed in detail. Further, the

authors discussed that improper law and orders can delay in flow of criminal to recovered class. Thus, causing delay in catching criminals. The fact was incorporated in the model to provide extended delay differential equation model. Stability analysis on the delay model revealed that large delays could cause periodic oscillations and hence further make it challenging to control the spread of crime. Lastly, the deterministic model was converted to stochastic model and their results were compared using numerical simulation, observing a lower level of criminal population in stochastic than in deterministic model.

The models based on predator-prey approach divide the population into two parts- predator and prey and study the relationship between them. Further, along with epidemic approach, the models showed that criminals could be recovered upon proper law enforcement and counseling.

Spatio-Temporal Approach

There is a line of research which talks about spatial and temporal dynamics of crime. In the last two decades, many researchers have focused on the spatio-temporal modeling of crime dynamics.[25-31] Empirical observation showed that crime is not uniformly distributed within time and space. Some areas are considerably safe whereas some may experience dense clusters of crime. Also, repeat victimization was observed over short intervals. Such spatial temporal clusters having high intensity crime are known as hotspots. Identification of such hotspots enables to analyze the conditions which leads to occurrence of crime. Several theories have been provided to understand the emergence of these hotspots, connection of their size and features with behavior of victims, offenders, law enforcements agents and local geography. It was observed that structure of urban environment involving features like population density, traffic volume, vacant property impacted crime patterns.[32-37]

Under certain conditions, crime hotspots may emerge, dissipate or diffuse. To examine the essential dynamics of these hotspots, in 2008, Short *et al.*, have introduced a quantitative 2-D model by focusing on simplest urban crime, i.e., residential burglary.[25] They started with a discrete system and derived a continuum model. Numerical simulations revealed that the two models were in good approximation under large criminal population. Lastly, stability analysis was done to determine the parameter values which lead to formation of stable hotspots. Further, in 2010, Short *et al.,* have included repeat victimization in their work and presented a model to study the repeat and near repeat burglary effects.[26] They concluded that such repeat and non-repeat victimization might lead to formation of crime hotspots and therefore might be analyzed for designing crime prevention strategies.

As a result of crime hotspot policing, it was observed that such hotspots may suppress, dissipate or displace. To study this scenario, in 2009, Short *et al.,* have presented reaction diffusion model.[27] Such models help in investigating the formation of crime patterns and the impact of alternative policing strategies on crime hotspot stability. They classified hotspots as supercritical and subcritical, where the former is a result of small spikes in crime which creates a local crime wave and latter arises due to large spike in crime. Their method showed that subcritical hotspots might be permanently destroyed whereas supercritical hotspots could only be displayed following a characteristic spatial pattern.

Thus, it can be concluded that spatio-temporal approach helps in identifying crime hotspots and further analysing such hotspots aids in designing crime prevention strategies.

DISCUSSION & CONCLUSION

Mathematical models can be used to guide decision-making, develop policies or to evaluate specific strategies aimed at reducing crime. In this review paper, we have discussed several approaches that can be adopted for formulation of crime models. One such approach was Economical approach, where the mathematical models were formulated to measure the social loss caused from offenses and to further identify the cost-effective strategies for the control of crime. As crime is considered a social epidemic, Epidemic approach was used, where crime was considered to spread in the population like an infectious disease. The authors assumed that susceptible were likely to commit crime upon coming in contact with criminals. Through such models, the impact of behavioral changes and policy implementations was discussed. Also, a deterministic model was

analyzed to identify the consequences of criminals and criminality on victimization. Further, we discussed about the models showing interactions among classes using predator-prey modeling approach, where one class was considered as prey and other as predator. This approach was combined with epidemic approach to formulate a model examining the relation between police and gang members. Such model helped in identifying the tripping points, which could lead to disappearance of gangs and corrupted police officers from the population. Interaction between criminal and non-criminal population was also studied through the combination of these two approaches. It was observed that criminals might move to recovered class upon proper law enforcement and proper counseling. Furthermore, empirical observations indicated that crime was not uniformly distributed but used to vary with time and location. Such spatio-temporal approach was adopted by several authors to identify the clusters having high intensity crime (known as *hotspots*).

In all the above reviewed models, we noted that mathematical models describing the dynamics of crime against women are given least importance. Several methods and approaches have been studied in this review, which are expected to give hints regarding the formation of models that would be helpful in controlling the spread of crime against women. This will open a window for counselors, bureau of police research and development and other law enforcement agencies to predict the trends in the crime against women and prepare the optimal control and prevention policies. IJFMP

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REVIEW ARTICLE

Fungal succession on carrion to determine the Post mortem interval: A Forensic Mycology

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ABSTRACT

Forensic mycology is a modern concept that has been used to explain the fungi succession and development of cadavers. It could lead to application in forensic medicine in determining presence of fungal strains which would, in turn, help in establishing the time of death. Since the corpse is an ample source of organic material, there has been an increase in research into the function of fungi in post-mortem decomposition. While some mentions of the participation of fungi in the post-mortem phase have been published in older literature and studies, they have rarely relied on the form or species of fungi that are present at each phase of decomposition. Apart from that, isolating fungal species in specified geographical areas will facilitate in the characterization and classification of region-specific microorganisms found on corpses under diverse growth condition. In this study, animal corpse and its specific organs were used to study the development of fungal strains developed in succession after specific intervals of time and stages of decomposition. The strains obtained were identified by lactophenol staining as *Rhizopus sps., Mucor, Aspergillus sps. and Alternaria sps.*

KEYWORDS | forensic mycology, fungi, postmortem, decomposition, staining

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INTRODUCTION

In medical terminology the word "thanatomicrobiome" refers to the array of germs present in different locations of decomposing bodies. An important outcome of these experiments is the potential to use forensic samples as microbial physical signs in medicolegal death investigations. Since the corpse is such a rich source of organic matter, there seems to be an uptick of study into the role of fungi in post-mortem decomposition, with an increasing number of laboratory accounts and case studies in forensic mycology. Any type of certain microorganisms have been established in these experiments, which offer useful hints for estimating the time of death.[1,2]

Thanatomicrobiome is a modern term for the study of microbes that colonize internal organs and orifices after death (thanatos, Greek for death). Recent discoveries in thanatomicrobiome have shown that obligate anaerobes, such as Clostridium spp., account for the overwhelming majority of microbes in the body and that the thanatomicrobiome inside internal organs grows across time. This knowledge can be used to predict the time of death when a human body decays.[3]

Many fungi, for example, are known

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to target insects and nematodes, and can play an important role in regulating their populations. Entomopathogens are fungi that infect flies, and they are classified as Ascomycota, Zygomycota, or Chytridiomycota. These fungi infect and ingest insects such as caterpillars and ants, and produce huge stromata that protrude significantly from the bodies of their victims. These fungi may also influence the insect's behavior. Insect brains are riddled with Brazilian "zombieant" fungi, which cause the victim to rise up plants and bite into plant tissue in a "death grip".[4]

Biological (bacteria, fungi, arthropods, nematodes, etc.) and abiotic (weather, atmosphere, temperature, humidity, etc.) factors affect the complex mechanism of decomposition of human or other mammalian cadavers. Without the presence of bacteria, chemical decomposition can occur at a glacial rate, resulting in the formation of biochemical waste pools. The necrobiome, which is derived from the microbial populations that inhabited the live host as well as the surroundings where the cadaver collapsed, includes these cadaver-associated microbes.[5,6,7]

Fungi can colonize decomposed corpses during the dry phase of decomposition, causing distinctive mildew stains and eventually transforming them into rotting corpses. On heavily decomposed cadavers, especially those that are highly mummified, visible fungal growth is normal. Artificial cultivation allowed the parasites that purge cadavers to be classified morphologically.[8,9]

The current research investigated the function of fungi during three stages of decomposition: bloated, putrefaction, and skeletonization, based on these results. When cadaverous material is destined for anatomy testing, it is often screened for these pathogens prior to delivery; however, this is not a standard practice in all research centers and universities.

Forensic importance and justification

The current research will focus on fungal species that begin colonizing cadavers. As a result, it will aid in detecting the various successions of fungal spores, and will aid in deciding the age of the cadaver and the time of death.

MATERIALS & METHODS

Preparation of Potato Dextrose Agar (PDA)

The microbiological medium potato dextrose agar, PDA, is made from potato infusion and dextrose (corn sugar). Potato dextrose agar is the most common medium for growing fungi and bacteria that attack live plants or decompose dead plant matter.

Fungus isolation from animal tissue

To obtain the growth of fungal strains, the tissues were split into smaller parts and held in different petriplates and incubated at different temperatures. Following that, the fungal growth was isolated on PDA and held for staining and detection.

Fungus identification

Lacto phenol cotton blue mounting of fungi is used for fungus detection.

Identification of yeast strains using biochemical tests.

To distinguish yeast strains, biochemical tests such as the IMViC test, carbohydrate test, and starch hydrolysis were conducted.

Preparation of potato dextrose broth

Potato Dextrose Broth is recommended for yeast and mold isolation and enumeration.

Setup for the experiment

- PDA plates were designed for carefully positioning the animal tissues, which were freshly sliced, at room temperature (370C) and at 400C for various times or days (0 day, 2 day, 7 day, 10 day, and 12 day).
- 1 gm tissue was measured and then placed on media plates aseptically.
- For proper fungal growth, these plates were incubated for 48 hours.
- After growth and sporulation have been detected on the plates, staining is used to distinguish the cultures.

RESULTS AND DISCUSSION

The aim of this study was to better understand and analyze the nature and growth of fungi on animal corpse tissue after various time intervals and storage temperatures. Different tissues from freshly cut mammalian tissues were gathered, cut into 1cm by 1cm sections,

DAY	TEMPERATURE	STRAIN	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	4°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Green	Dark colored hyphae, Elongated beak like conidia, segmented conidia	Alternaria alternata.
2	4°C	1	Cream	Yeast cells, unicellular, oval to elongated cells, single-celled, cream coloured colony	Candida sps.
		2	White to greyish colony	Yeast cells, unicellular, elongated cells, single celled, white coloured colony	Candida sps.
7	4°C	1	Green	greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
		2	Green	Eurotium herbariorum is formed when Aspergillus glaucus develops massive cleistothecia. The ascopsores released by the cleistothecia have a central groove that makes them look like hamburgers.	Aspergillus glaucus
		3	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillus niger
10	4°C	1	Green	greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
12	4°C	1	Green	Colorless conidiophore, green spores, Uni/Biserate conidial head, Round /radial head	Aspergillus flavus

Table 1 Detail and Identification of Fungal Strains in Liver Tissue.

DAY	TEMPERATURE	STRAIN NO.	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	37°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Black	Dark colored hyphae, Elongated beak like conidia, segmented conidia	Alternaria alternata.
2	37°C	1	No growth	--	--.
7	37°C	1	Green	Greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
		2	Light greenish to colorless	Colorless conidia and hyphae, Biserate conidiophore	Aspergillus nidulans
10	37°C	1	Black	Dark colored hyphae, elongated beak like conidia segmented conidia	Alternaria alternate
		2	Green	Umbrella like conidias with green hyphae	Rhizopus sps.
12	37°C	1	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillus niger

Table 2 Detail and Identification of Fungal Strains in Liver Tissue at 37° C.

DAY	TEMPERATURE	STRAIN NO.	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	4°C	1	Green	Coinidiaphore umbrella shaped	Rhizopus sps
2	4°C	1	Whitish	Single-celled structures	Candida sps
7	4°C	1	Green	Colorless conidiophore, green spores, Uni/Biserate conidial head	Aspergillus flavus
10	4°C	1	Light greenish to colorless	Colorless conidia and hyphae, Biserate conidiophore segmented conidia	Aspergillus nidulans
		2	Green	Colorless conidiophore, green spores, Uni/Biserate conidial head Round/radial head	Aspergillus flavus
12	4°C	1	Green	Dark colored hyphae, elongated conidia, segmented conidia	Alternaria sps.

Table 3: Details & identification of Fungal strains found to populate Muscle tissue after different intervals of time at 4°C

DAY	TEMPERATURE	STRAIN NO.	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	37°C	1	Brownish green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
2	37°C	1	Green	Single-celled oval to elongated cells, Whitish colony	Candida sps
		2	Green	Single celled oval to elongated cells, cream color	Candida sps.
7	37°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Black	Uniserate conidia, black spores in chains being released	Aspergillus terreus
		3	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillis niger
10	37°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Green	greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
		3	Green	Elongated club shaped conidiophore, uniserate	Aspergillus clavatus
12	370C	1	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillus niger
		2	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus

Table 4 Details & identification of Fungal strains found to populate Muscle tissue after different intervals of time at 37°C

S.NO.	TEST	Y1	Y2	Y3	Y4
1	Fructose	–	–	–	–
2	Dextrose	+	+	+	+
3	Maltose	+	–	–	+
4	Mannitol	+	+	+	+
5	Sorbitol	+	+	+	+
6	Xylose	+	+	+	–
7	Starch	+	+	+	+
8	Sucrose	+	+	+	+
9	Mannose	–	–	–	–
10	Lactose	+	+	+	+
11	Glycerol	+	+	+	+
12	Arabinose	+	+	+	+

Table 5 Test results of Carbohydrate for Yeast Characterization

and put in petriplates containing sterile potato dextrose agar for the sample.

All the experimentation was done in sterile condition.

The current study shows the presence of different fungal strains developing on different tissues at different times. The use of culture media, staining procedures, and other laboratory approaches to determine the existence of fungi is critical in forensic mycology science.

As is well established, (e.g. Aspergillus spp. most fungi reproduce asexually in nature, and many of their members are airborne strains that

can thrive on almost any substrate (1988, Sharma). Alternaria alternata was found in liver tissue on 0 Day, similar to Sharma's findings in 1988.

According to table 1, 2, 3, 4 and 5 on 0 day Liver and Muscle tissue, 7th day Muscle Tissue, 10th Day Muscle Tissue, and 12th Day Liver Tissue, Aspergillus flavus, a green filamentous fungus, was found. As a result, A. flavus appears to be a fungus that grows mostly on animal tissues. Aspergillus fumigatus was also observed to be prevalent in a 7-day-old liver tissue and a 10-day-old liver tissue preserved at both 40°C and 37°C. Aspergillus niger, a common black mold, was present in the liver and muscle tissue, but mainly in liver and muscle tissue stored 37°C.

However, very distinct and special filamentous fungi were found in the liver and muscle tissue. On the seventh day after being processed at 40°C, Aspergillus glaucus was discovered in liver tissue. On the 12th day of storage, Aspergillus clavatus with club-like conidiophore was found on Muscle tissue deposited at 37°C.

De Hoog *et al.,* discovered that there was a lot of Aspergillus spp. as well as Penicillium spp. Separation of standard soil plants, including hyphomycetes, was hampered under the conditions used, and hyphomycetes were not recovered even after the inhibitor cyclohexamide was added to the culture medium.

External collection sites were found to be better for fungal growth than internal collection sites for both airborne fungi and yeasts, particularly for Aspergillus and Candida, according to Collier (2005). Any of the observations on postmortem changes caused by fungi are similar to this one. On the scalp, yeasts sometimes grew faster than in the fur. This fact can be clarified by the after-death rupture of skin barriers and enhanced contact with mucous secretions, in addition to their potential inclusion in the skin microbiota.

Despite the recent discovery of fungi on the surface of bodies by forensic medical professionals, these species are not isolated on a routine basis.

Fungi need further analysis before they can be used as a forensic instrument since their description can also expose the site of death. (2006, Ishii).

The current study is just the beginning, and the findings are insufficient to show that fungi can be used as effective biological death markers. On the other hand, the isolation of fungi such *Aspergillus spp., Alternaria spp.,* and *Candida spp.* demonstrate that the fungi isolated during the corpse decomposition entomology process differ. These findings suggest that the existence of fungi on, in, and around cadavers can provide additional information that can help determine the exact time of death. As with forensic entomology, further study with larger samples and more detailed descriptions of conditions is required to validate the results reported here and to determine the true value of mycology as a forensic medicine tool.

RESULTS AND DISCUSSION

An investigation into the isolation and detection of fungal strains colonizing animal corpses and internal organs after death revealed a succession of *Aspergillus* strains. Various strains of *Aspergillus* seem to colonize dead organs in different ways depending on the temperature. *Aspergillus flavus* grows first in both liver and muscle tissue, followed by filamentous fungi such as *Aspergillus fumigatus, Aspergillus nidulans,* and *Aspergillus niger* in both tissues. Some filamentous *Aspergillus* fungi may only be found on liver or muscle tissue, which may be used as a biomarker in forensic mycological studies. At a two-day cycle, yeast-like fungi are often found on all tissues, which is unusual and can be used as a particular biomarker.As a result, the use of fungal succession on corpses for identification and tracking may be precise, leading to accurate time of death and other forensic parameters. IJFMP

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REVIEW ARTICLE

Forensic Nursing

Selvaraj Premkumar Subashini[1], Surabhi Verma[2], Simrat Kaur[3]

ABSTRACT

Forensic Nursing is the application of nursing process to public or legal proceedings in the healthcare with scientific investigation of trauma or death related to abuse, violence, criminal activity, liability and accidents. Nurse practices in all areas with specialized roles by taking care of the documentation which plays a major role for clarification. Forensic Nursing was recently recognized by American Nurses Association but it has its origin as early as 12th century from Italy Florence Nightingale (1820 – 1910), Angel of Crimea / Lady With A Lamp provided nursing care to war victims without any consideration whether of friend or enemy. In 1992 International Association of Forensic Nurses was found in the USA and other countries where the forensic nursing took its practice are Canada, South Africa, Japan, Hong Kong, Singapore, Sweden, Switzerland, and the Great Britain. In India, the journey of Forensic Nursing started in 2002 with the visit of Ms.Virginia A. Lynch to Punjab. In October 2015, Department of Health and family welfare, Government of India and Indian Nursing Council approved and started a one-year post-Basic diploma in Forensic Nursing at the Institute of Forensic Science, Gujarat Forensic Sciences University with fifteen students. Forensic nurses contribute to disaster planning and response. They are also consulted on legal cases, assisting attorneys to understand the medical terminology and how care is provided to the individual.

KEYWORDS | nursing, forensic nursing, forensic science

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INTRODUCTION

Nursing is a very important component of healthcare system to provide comprehensive medical services to the public. And forensic nursing is one of the specialties in Forensic discipline which, in addition to creating more satisfaction in nurses, is considered essential in holistic, quality, and safe care. It is one of the recent forms of discipline in forensic science, and it is the application of forensic science, combined with clinical nursing practice in the scientific investigation of death and injury resulting from criminal activity and accidents. The role of a nurse in medico-legal cases has been recognized since the mid-1970s when Dr. John Butt, the Chief Medical Examiner, Alberta, Canada, first established the role of a forensic nurse examiner in death investigations, representing the forensic pathologist at the scene of crime. Later, forensic nursing started as a specialty in the United States and then to various parts of the world like Sweden, South Africa, Japan, Singapore, and Malaysia. In this regard, Virginia A. Lynch, a forensic clinical nurse specialist, is recognized as the founder of forensic nursing as a formal discipline in the United States and

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all across the world. A forensic nurse is a nurse who provides specialized care for patients who are victims and/or perpetrators in medico-legal cases. Forensic nurses can play an important role in bridging the gap between the law and medicine. Nurses trained in this field can document injuries, collect biological fluids, and preserve clothing with evidence of assault. In medico-legal practice, it is not uncommon for some significant evidence in poisoning cases, namely, gastric aspirate, vomitus, urine sample, soiled cloth, etc., to be discarded in emergency departments. Trained forensic nurses are aware of the importance of these evidences, and they may help in collection, preservation, and maintenance of the chain of custody of these samples.[2]

A study aimed to describe the consequences of the presence of forensic nurses in the health system. This qualitative study was conducted between 2017 and 2018 in Iran. Semi-structured interviews were performed with 18 participant experts in the field of health and law. The interviews were analyzed using the inductive content analysis approach proposed by Graneheim and Lundman. The consequences of the presence of nurses in the health system were summarized into two categories: positive and negative consequences. The positive consequences consisted of improved performance, better legality of nurses, the calmness of nurses, prevention of patients' rights violations, advancement comparable with developed countries, reduced costs, increased accuracy and speed in dealing with forensic cases, improved performance of Iranian Legal Medicine Organization and increased employment. On the other hand, the negative consequences were role conflict with other involved professionals and nursing shortage. Participants included nurses (with different specialties), nurse lawyers, forensic medicines, forensic midwives, a judge, and a medical lawyer. If planners and policymakers have a positive attitude toward the presence of forensic nurses, we can anticipate better forensic services for clients through the development of systematic educational programs, the formation of forensic teams, and the expertise of this profession can provide many benefits.[1]

Sexual violence is a significant cause of physical and psychological harm and suffering for women and children. Although sexual violence mostly affects women and girls, boys are also subject to child sexual abuse. Nurse is the person who attends the victim first. In order to meet the rigid and ever-changing demands of providing care to the victim and complying with our confusing system of laws, the nursing should have been forced to expand into a Forensic nursing, specialty of its own. Nursing roles in the criminal justice service known by many names worldwide-Custody nursing, Prison/Correctional nursing, Immigration center nursing, Sexual Assault Nurse Examiner (SANE) or Sexual Assault Forensic Examiner (SAFE), SARTs (Sexual assault response team), SARCs (Sexual assault referral center) and FNDIs (Forensic nurse death investigator). The WHO and IAFN have urged inclusion of forensic content in both undergraduate and postgraduate nursing programs. Forensic Nurse Specialist can provide direct services to individual clients, consultation services to nursing, medical and law-related agencies, as well as providing expert court testimony in areas dealing with trauma and/or questioned death investigative processes, adequacy of services delivered, and specialized diagnoses of specific medical conditions. Research Findings on the Effectiveness of Sexual Assault Nurse Examiner (SANE) Programs suggests various improvements in each and every step in care of victim of sexual assault.[3]

ROLE AND RESPONSIBILITIES

Cases of sexual violence are on the rise, and a tremendous amount of physical and psychological trauma are suffered by the victims of such heinous crimes. A female forensic nurse can readily establish a rapport with these victims of sexual violence. At the same time, these nurses are qualified in providing sexual assault evaluations and victim management. They provide services to attending doctor, to individual clients, give counseling to victims and relatives, as well as provide expert court testimony.[3]

In the field of death investigation both at the crime scene and during postmortem examination, forensic nurses can also play an important role. In the mortuary, they may help in receiving dead bodies, police papers as well as in maintenance

of records and management of legal formalities, recording of the condition of the body, etc. A forensic nurse may also serve as a forensic pathology associate during dissection as well as in the collection of biological samples and trace evidences.[4] In present times, "death" has become a respectable field of inquiry, demanding answers to satisfy the public need, and demand to determine the cause and manner of death. Nurses can bring empathy and compassion as well as excellent observation, clinical, and communications skills to death investigation.[5]

On the other hand, forensic nurses may play a significant role in dealing with cases involving mental illness, especially when such people become involved in criminal cases or when criminals are feigning mental illness. They can provide a thorough forensic evaluation while observing for specific symptoms related to such a case. Moreover, crime victims face a higher risk of posttraumatic stress disorder, depression, suicide, and medical complications than other patients; forensic nurses improve both legal outcomes and quality of life for these patients.[6]

Interestingly, according to the American Forensic Association, the most important subspecialty of forensic nursing is sexual assault followed by other subspecialties such as death investigation, medicolegal consulting, and forensic psychiatric nursing.[7] Research findings on the effectiveness of Sexual Assault Nurse Examiner programs suggest various improvements in each and every step in care of victim of sexual assault. [3] Further, battered women, abused children, and the neglected elders will be more comfortable in explaining the circumstances of injury to female forensic nurses as compared to police personnel.[6]

CONCLUSION

In the present world, several new specialties are developing in various scientific fields. In nursing sciences, new areas of practice such as pediatric nursing, psychiatric nursing, and geriatric nursing have come up. The specialty of forensic nursing is a comparatively new entity in India. It was first introduced to a few select institutes and agencies in our country in the year 2003, and good response was emanated from this prolog in New Delhi, Punjab, and some other places in India.

To conclude, forensic nursing is an evolving specialty that has undergone substantive development in recent years. Unfortunately, in this part of India, most of the health-care professionals are not even aware of the existence of such a specialty. Even though efforts are still on, forensic nursing is yet to find a proper place in the nursing curriculum manuals in India.[2] IJFMP

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ORIGINAL ARTICLE

Forensic Study of Diatoms in Freshwater Sample Around Patna Region, Bihar, India.

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ABSTRACT

Diatoms are eukaryotic, unicellular algae that belong to a Bacillariophyceae family and it exists in water. Diatom is important in determining whether or not a person died as a result of drowning. When a corpse is retrieved from the water, it is generally believed that the death was caused by drowning, and that the body was drowned either before or after death. In such circumstances, the existence of diatoms in body tissues is a very useful evidence in determining the cause of death. Various types of diatoms found in dead body tissues are related to the types of diatoms present in bodies of water that are recovered from the body. The objective of this study was to find out which types of Diatom are present in different freshwater bodies around Patna district of Bihar, India. In this research, freshwater samples were collected by Random Sampling Technique from 17 different water habitats of Patna, during January to February, 2021. For diatom analysis, samples from ecological niches like river Ganga, ponds, and lakes were collected in clean water bottles with tight-fitting caps. Extraction of diatoms is done in two ways: The H_2O_2 method and the HNO3 method. The aim is to determine which extraction procedure — H_2O_2 or NHO_3 — is best for diatom extraction. After the extraction of diatoms, the examination and photography of diatoms species is performed by compound microscope (Leica DM750 with Leica ICC50 E camera). Different Diatoms species found in various water environments at different location were investigated in this research. Some of their names are Cymbella sp., Fragiliaria sp., Ehrenbergiulva granulosa, Coscinodiscus radiates, Cyclotella sp., Melosira sp., Actinoptychus sp., Triceratium sp., Nietzsche sp., Actinocyclus sp. The information from this experiment will help in forensic science laboratories to identify diatoms, criminal investigation and locating drowning sites or place of crime. In this analysis we found that nitric acid digestion method give better extraction as compare to hydrogen peroxide extraction method.

Keywords | Lie Detector, Forensic Evidence, Polygraph, Narco Test

INTRODUCTION

Diatoms are microscopic algae that are unicellular, photosynthetic organism with a worldwide distribution. They are the world's largest biomass producers, contributing 25% of the world's total oxygen production and being one of the most important contributor to the global carbon fixation.[1] Diatoms are highly effective species as assessed by their versatility, range, productivity and comparative antiquity. Diatoms have a siliceous cell wall made up of two intricately sculpted halves, which makes them exceptional for a wide variety of applications for understanding and analyzing varying degrees of species level complexity. The taxonomy of diatoms is

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based on morphological features such as frustule ornamentation shape, size, and patterns; the presence or absence of raphae; the number of striae, and so on. The diatoms are classified into two main orders based on the structure of the frustule: Centrales and Pennales. The Pennales have bilateral symmetry, while the Centrales are radially symmetric.[2]

Usually, similar types of diatoms occur in almost identical types of water bodies. It has been discovered that there is a significant shift in the diversity of diatoms with respect to seasonal changes. The quantitative and qualitative distribution of diatoms in water sources is strongly supported by climatic conditions.[3]

The significance of diatoms in drowning cases has always been recognized. When the cause of death cannot be determined by traditional postmortem examination, the presence of diatoms in the lungs and other body tissues can help determine whether or not the death was caused by drowning. Diatoms aren't found in the human body. When laboratory tests show diatoms in corpses that match those in the water where the corpses were discovered, drowning is indeed a strong possibility as a cause of death.[4]

Sample Collection and preservation

During the months of January and February 2021, water samples were collected from various locations in the Patna area in tidy and clean water bottles with tightly fitting caps to prevent contamination. 500ml of water samples were collected from each of the 17 water habitat from different location of Patna. The bottles were labeled properly with collection number, the location of sampling area along with date and month.

METHOD & MATERIALS

For sample preservation, we kept the samples in a refrigerator for a few days, at room temperature. When we needed to conduct the experiment, we took out the sample from the refrigerator and put it in a beaker with proper labeling. Then, into each beaker, we added 1% of Lugol's iodine solution and left it to rest overnight.[5] The next day we began the diatom extraction procedure.

In the study of diatoms, we used two extraction methods to see which one provided the better results: nitric acid digestion and hydrogen peroxide extraction.

Nitric Acid Digestion Method was used for the diatoms extraction from freshwater water samples. 10ml of water sample was taken and transferred into a separate 25ml acid washed test tube and the test tube were properly labeled. 2ml of concentrated nitric acid (HNO3) was added to the sample. Then the sample kept for hot water bath on hot plate at 40-45˚C for 30 minutes. After that the samples were kept undisturbed for 24 hours. Then the samples were moved to properly-labeled centrifuged tube and were centrifuged for 10 minutes at 2500rpm. The centrifugation was carried out repeatedly three times by taking 2ml of sample each time to increase the concentration. The supernatant was removed carefully with the help of a dropper. Again the pellets formed were suspended in the distilled and centrifuged at 2500rpm to remove the acid contents.[6]

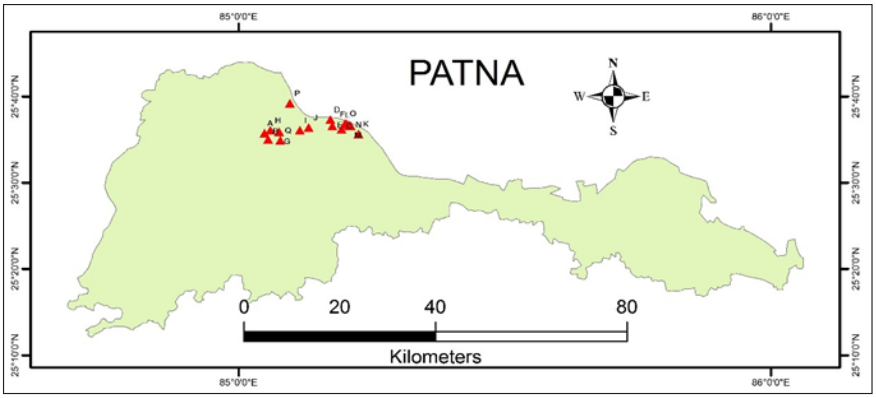
In the Hydrogen peroxide method, we took 10ml of water sample and transferred into a separate 25ml hydrogen peroxide washed test tube and the test tube were properly labeled. 10 ml of hydrogen peroxide (H2O2) were added to the sample. And then we follow the same procedure which we done in the case of Nitric Acid Digestion Method.[5]

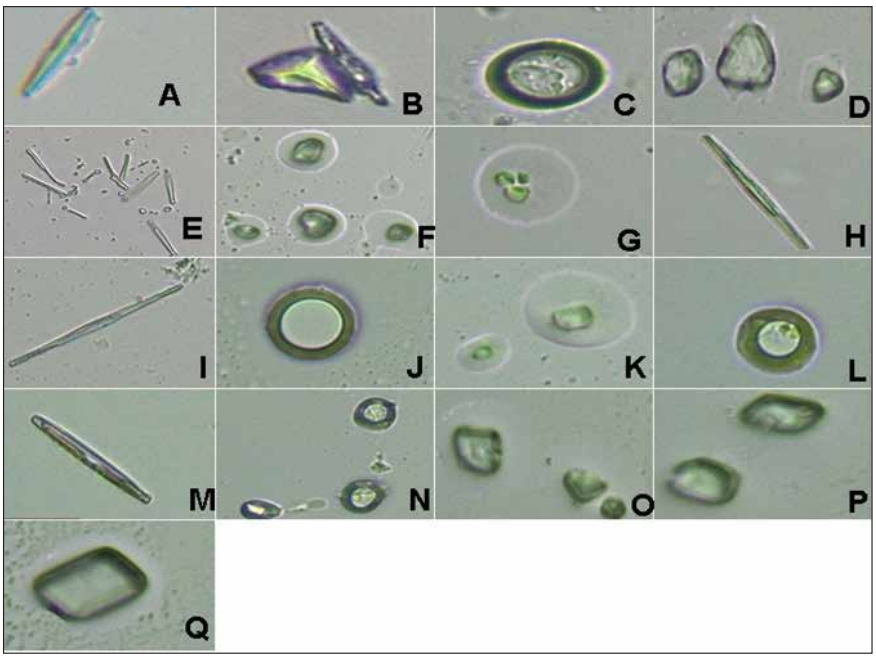
Mounting

After cleaning, the pellet is dropped onto the slide and covered with a cover slip. After that, the slides were dried on a heating plate at 30-40°C for 4-6 minutes. Then the slide was kept aside carefully for air dry, once the slide gets cool. It was examined under the compound microscope (Leica DM750) at magnification of 40X and digital images were taken with (Leica ICC50 E) camera connected to the compound microscope (Leica DM750). The diatom species were categorized based on the description provided by the various articles, site, book and reports.[7-11]

Map of the Sampling Areas

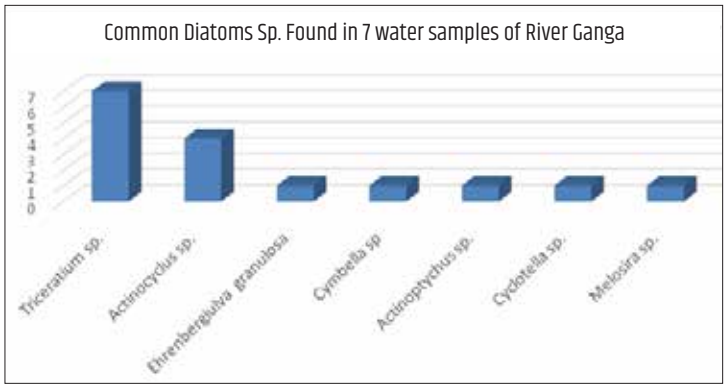
The following locations have been shown in the map. Namely, Eco Park lake, Adalat Ganj Talab, Mangal Talab, Kothwan pond, Digha Nahar, Adarshnagar Lake, B.M.P Mandir Pond, Bazar Samiti Talab, Danapur pond, Bajarang puri pond, Bhadra Ghat, Mahavir Ghat, Naujar Ghat, Hanuman Ghat, Gai Ghat, Gandhi Ghat, Digha–



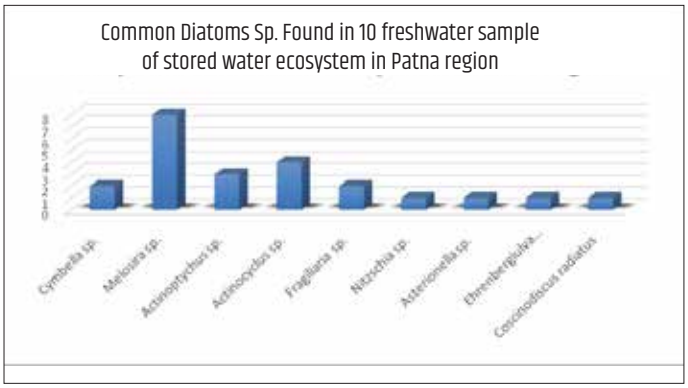


S.NO.	SAMPLING LOCATION	CO-ORDINATES	DIATOM SPECIES THAT FOUNDED
A	Kothwan pond	25.59588, 85.04866	Cymbella sp., Melosira sp., Actinoptychus sp.
B	Danapur pond	25.58417, 85.05563	Fragiliaria sp., Actinocyclus sp., Melosira sp.
C	Bajarang puri pond	25.60332, 85.19361	Actinoptychus sp., Actinocyclus sp.
D	Gandhi Ghat	25.62212, 85.17254	Triceratium sp., Actinocyclus sp.
E	Bazar Samiti Talab	25.60995, 85.17628	Nitzschia sp., Melosira sp.
F	Gai Ghat	25.61369, 85.20324	Triceratium sp., Actinocyclus sp.
G	B.M.P Mandir Pond	25.5986, 85.07604	Actinocyclus sp., Melosira sp.
H	Digha Nahar	25.60148, 85.05957	Cymbella sp., Actinocyclus sp., Melosira sp., Asterionella sp.
I	Eco Park lake	25.60179, 85.11492	fragilaria sp., Melosira sp.
J	Adalat Ganj Talab	25.60699, 85.13156	Ehrenbergiulva granulosa, Melosira sp.
K	Mangal Talab	25.59457, 85.22517	Coscinodiscus radiatus, Melosira sp.
L	Bhadra Ghat	25.61164, 85.20719	Ehrenbergiulva granulosa, Triceratium sp.
M	Mahavir Ghat	25.61074, 85.20891	Cymbella sp., Triceratium sp.,Actinoptychus sp.
N	Naujar Ghat	25.6099, 85.2108	Cyclotella sp., Triceratium sp., Melosira sp.
O	Hanuman ghat	25.61483, 85.20006	Triceratium sp., Actinocyclus sp.
P	Digha–Sonpur Rail Bridge	25.65375, 85.09635	Triceratium sp., Actinocyclus sp.
Q	Adarshnagar Lake	25.58215, 85.07821	Actinoptychus sp., Melosira sp.

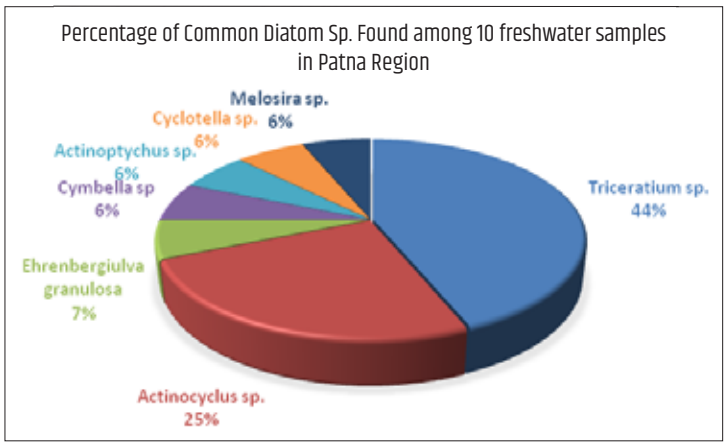
Table 1: Sample collection size 18-20km (during January to February, 2021)

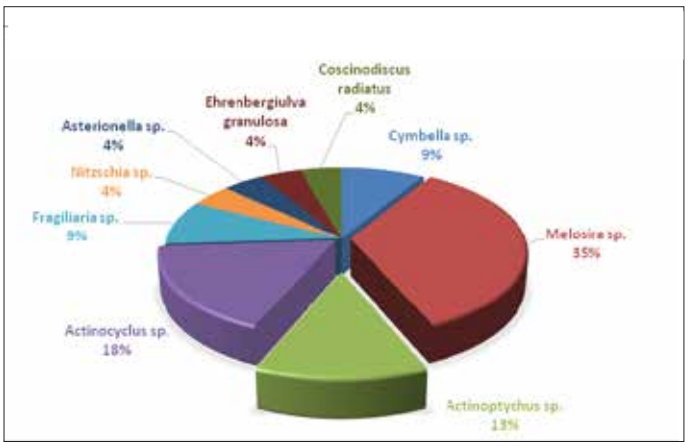


Graph 1: Some common diatoms found in the Patna water bodies



Graph 2: Some common diatoms found in the Patna water bodies





Sonpur Rail-Road Bridge (River Ganga).

Sample collected from River Ganga in different location are Bhadra Ghat, Hanuman Ghat, Gandhi Ghat, Mahavir Ghat, Naujar Ghat, Gai Ghat and Digha–Sonpur Rail-Road Bridge over Ganga River.

RESULT & DISCUSSION

In the drowning cases, diatoms play an important part. It is very helpful in deciding not just the cause of death, but also the site of drowning. The presence of a sufficient amount of diatoms in vital body organs (i.e. lungs and in between long bone) will confirm ante-mortem drowning to a certain degree. The scattering of diatoms in any body of water, and their interrelation with the diatom species retrieved from the drowned body, may be the method of choice for addressing the drowning site question. The presence of diatoms in water and biological samples can reveal a lot of information about the cause of mortality, season of death and the location.

In certain drowning cases, the outcome may be false positive, such as when a person drinks water from a pond, lake, or river on a frequent basis. As a consequence, when investigating drowning cases, authorities must understand this perspective.

Using the diatom test, we'll figure out whether the death was caused by drowning or not. Criminals frequently throw bodies into the river after committing a murder to it seem like the victim drowned.[12] The plume of froth on the

nose and mouth, respiratory emphysema, odema aquosum, froth in the trachea, Paltaufs spots, increased lung weights, and peripheral edema are the result in the change finding of autopsy in drowning deaths.[13] since diatoms can tolerate putrefaction, they are most useful in situations where decomposition has progressed and post-mortem drowning signs have faded. According to drowning death research, only the diatom test can detect ante mortem drowning in decomposed corpses and bodies' during late stages of decay. The diatom test identifies diatoms in organ samples and compares them to a control water sample.[14,15] From a scientific viewpoint, the study of drowning deaths allowed for a sensitive, accurate, and easily applicable examination. The diatoms test has emerged as the most effective test for detecting drowning deaths in forensic laboratories.

In this analytical study of diatoms we collect, preserves, extract and examine the diatom using a controlled water sample which is collected from various location of a Patna, Bihar region. Below are images of a few different diatom species. Few diatoms could be classified up to species level due to poor photographs. Since the samples were taken during the winter, the climatic conditions were not favourable to diatom formation, so there were few diatoms to be found. The growth of diatoms slows down because the climatic factors are not conducive for diatom growth in the winter, but only a few water samples had a stable diatom population.

We identified 11 diatom species in various water ecosystems in the Patna district region during this research, and their names are Cymbella sp., Fragiliaria sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Ehrenbergiulva granulosa, Cyclotella sp., Melosira sp., Actinoptychus sp., Triceratium sp., Nitzschia sp., Actinocyclus sp.

In most of Patna ponds, lakes and nahr, the diatoms species we identified are Cymbella sp., Melosira sp., Actinoptychus sp., Fragiliaria sp., Nitzschia sp. and Actinocyclus sp. In Ganga river of Patna, diatoms species we found are: Triceratium sp., Actinocyclus sp., Ehrenbergiulva granulosa, Cymbella sp., Actinocyclus sp., and Cyclotella sp.

Common diatoms Species were discovered in seven water samples taken from the River Ganga in Patna, Bihar are Actinocyclus sp., Triceratium sp., Cymbella sp., Actinocyclus sp., Ehrenbergiulva granulosa, Cyclotella sp. and Melosira sp.

Among the seven water samples of Ganga River 44% Triceratium sp. Presented, 25% Actinocyclus sp. Presented, 7%Ehrenbergiulva granulosa presented, and 6% each Cymbella sp., Cyclotella sp. and Melosira sp. Presented.

Common Diatoms sp. was discovered in ten freshwater samples from the Patna region's store water ecosystem are Cymbella sp., Fragiliaria sp., Actinoptychus sp., Nitzschia sp., Actinocyclus sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Cyclotella sp., Melosira sp.

Among the ten water sample of store water ecosystem, 35% Melosira sp. Presented, 18% Actinocyclus sp. Presented, 13%Actinoptychus sp. presented, 9% Cymbella sp., Fragiliaria sp. and 4% each Nitzschia sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Cyclotella sp., Melosira sp. Presented.

CONCLUSION

Diatoms can be studied qualitatively and quantitatively by detecting and counting the diatoms species within the samples. Diatom analysis on a large scale and by area can aid the forensic science laboratories in identifying and locating drowning sites.

In this analysis, we found 11 diatom species in various water habitats in the Patna district area, and their names are: Cymbella sp., Fragiliaria sp., Actinoptychus sp., Triceratium sp., Nitzschia sp., Actinocyclus sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Ehrenbergiulva granulosa, Cyclotella sp., and Melosira sp.

In most of the water ecosystem in Patna region, we identified the similar types of diatoms such as Cymbella sp., Melosira sp., Fragiliaria sp., Nitzschia sp., Actinocyclus sp., Ehrenbergiulva granulosa and Actinoptychus sp. In the waters of River Ganga, we found and identified mostly 7 different types of diatoms: Triceratium sp., Cymbella sp., Actinocyclus sp., Actinocyclus sp., Ehrenbergiulva granulosa and Cyclotella sp.

In this analysis of each 17 different slides of Diatoms which were extracted by nitric acid digestion method and hydrogen peroxide, we found that nitric acid digestion method gave

better extraction as compared to hydrogen peroxide extraction method. Using 'Hydrogen peroxide' to oxidise organic content is not adviced since washing off peroxide traces from samples is difficult due to the solution's bubbling, while nitric acid can be washed off quickly using the acid digestion process. As a practice for diatoms extractions, instead of hydrogen peroxide method, the use of nitric acid digestion method is advisable. IJFMP

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Conflict of Interest:

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ORIGINAL ARTICLE

Non-Equilibrium Multi-Ion Biosorption Isotherms for Removal of Heavy Metals from Drinking Water

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ABSTRACT

Biosorption isotherms define the relationship between biosorption capacity of the biosorbent and the equilibrium concentration of the ions in solution, at a constant temperature. Experiments are routinely performed under near-equilibrium because it is impossible to determine the exact time at which equilibrium was attained. A novel attempt to study multi-ion biosorption in non-equilibrium conditions has been made, based on the Probability Isotherm theory. *Materials and Methods:* Probability Isotherm theory was examined with cucumber and kiwifruit peel beads which are reported to be efficient biosorbents. The peels were incubated in a cocktail of seven ions (As, Cd, Cr, Cu, Hg, Pb and Ni) at the same initial concentration (0.1-15 mgL-1) and four different temperatures (25-55°C). Non-equilibrium biosorption data were modeled by Langmuir isotherm model. Data were analyzed using a one-way ANOVA coupled with a Bonferroni post-hoc test on GraphPad Prism 8 software. Cd and Ni ions showed the most well-defined trends with Langmuir isotherm model. The binding of ions was physico-chemical with simultaneously occurring physisorption and tchemisorption reactions. *Conclusions:* Probability Isotherm theory can be applied to multi-ion biosorption in non-equilibrium conditions. The behavior of each ion is unique and no two biosorption systems are alike.

KEYWORDS | kiwifruit peel, non-equilibrium isotherms, heavy metals

INTRODUCTION

The low cost biosorption methods for the decontamination of drinking water have been in research for decades. Peels from a range of fruits and vegetables have the ability to remove toxic ions from a cocktail solution. Some examples include peels from apple (AP)[1], cucumber (CP)[2], banana (BP)[3], orange (OP) 3, potato (PP)3 and kiwifruit (KP)[4] immobilised on sodium alginate (SA) that can remove As, Cd, Cr, Cu, Hg, Pb and Ni. Biosorption is a complex physical and/or chemical process.[5] A simplified theoretical description of the biosorption process is as follows: when a solid biosorbent with functional groups comes in contact with biosorbate ions in solution, the reaction tends to move towards a dynamic equilibrium where the rate of adsorption is balanced by the rate of desorption *(Equation: 1).*[6] Hence, biosorption equilibrium is not a steady state but a state of dynamic equilibrium defined by equal forward and backward rate constants ($k_f = k_b$) involving simultaneously occurring adsorption and desorption. Thus, the understanding of biosorption isotherms gives the overall direction of the reaction.

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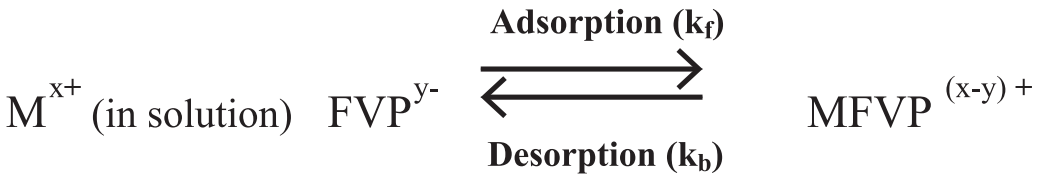

M^{X+} biosorbate ions; FVP^{Y-}=active sites on biosorbent surface; $MFVP(x-y)+ = MFVP^{(X-Y)+}$ = biosorbate ions bound to the active sites on biosorbent surfatce k_f = rate constant for forward reaction (adsorption); k_b = rate constant for backward reaction (desorption)6

Isotherms define the relationship between the biosorbent and the biosorbate ions when the system is at equilibrium[7]. The equilibrium time is usually determined by biosorption kinetic studies prior to arriving at biosorption isotherms. The concentration of the solution is analyzed at different time points and the point beyond which there is no significant decrease in solution concentration is the equilibrium time. However, such estimation may produce erroneous results since it is difficult to ascertain the exact point at which the reaction reaches equilibrium. Thus, when biosorption isotherm studies are performed, data are generated for biosorption system approaching equilibrium which in theoretical terms, is a non-equilibrium state. Additionally, various contact times ranging from 1 min to 75 days have been used by other researchers depending on the biosorption system and the time required to reach equilibrium.[8,9] However, in case of a multi-ion solution, the ions may or may not have similar equilibrium times. For example, in the studies on Cd and Cu ions, equilibrium was attained by 24 and 48 h, respectively.[1-4] Therefore, it is not possible to perform the experiment with the same equilibrium time for all ions as the ions physically bound to the biosorbent may desorb from the surface back into the solution upon continued agitation beyond equilibrium. Therefore, one or more ions may be in a non-equilibrium state in a multi-ion solution.

Chapman *et al.*,[10-12] have applied the Probability Isotherm theory, a probabilistic expression of the second law of thermodynamics, as a method for describing biochemical reactions that are valid both for equilibrium and non-equilibrium conditions. This approach is true for all isothermal conditions and is different from the typical kinetic expression that can be applied to equilibrium conditions only. They suggested that the equilibrium constant can be represented as a ratio of the overall probability of forward to backward reaction (*Equation - 2*), which is dependent on the concentration of the reactants and products but independent of the mechanism of reaction.[10-12] The modified form of the Van't Hoff equation equating the Gibbs free energy change (ΔG) with the equilibrium constant would then be valid for non-equilibrium conditions as well (*Equation - 3*).

$$K_{ne} = \frac{P_f}{P_b}$$

Equation 2:

$$G = -RT \ln \frac{P_f}{P_b}$$

Equation 3:

We have utilized this concept for multi-ion biosorption isotherms to analyze the uptake of heavy metal ions from water under non-equilibrium conditions. A comparative study of sodium alginate beads immobilised with various fruit and vegetable peels demonstrated that CP and KP beads had the highest biosorption capacities[1-4] and therefore these were selected for isotherm analysis. The aim of the present work was to use the Probability Isotherm theory to study the biosorption of heavy metals in non-equilibrium conditions. This novel concept will ease the strict requirements of fixed equilibrium time and allow further characterization of the non-equilibrium biosorption by CP and KP. This additional information will inform the potential practical use of these beads for treating drinking water contained with multiple ions.

MATERIALS AND METHODS

CP and KP Bead Preparation

CP and KP beads were prepared as described for other peels by Nathan et al.[1-4] Briefly, 6 cucumbers and 6 kiwi fruits were purchased from a supermarket in Dunedin, New Zealand. These

were pulverized and immobilised on sodium alginate (1%). Beads were formed by dropping them in 0.1 M CaCl2 and dried to increase their shelf life.

2.2 Batch biosorption experiments and ICP-MS analyses

Cocktail solutions were prepared by spiking deionized water with standard solutions of As (V), Cd (II), Cr (VI), Cu (II), Hg (II), Pb (II) and Ni (II) all at equivalent concentrations ranging between 0.1 and 15 mgL-1. The pH of the solution (25 ml) was adjusted to 7.0 ± 0.1 and the solutions were shaken with 4 beads of each type at 250 rpm for 10 h. The experiments were performed at four temperatures 25, 35, 45 and 55°C and all experiments were performed in triplicate. The results are expressed as the mean ± the standard error of the mean (SE).

All solutions before and after biosorption were analyzed by an Agilent 7900 quadrupole inductively coupled plasma detector coupled with mass spectrometry (ICP-MS) after appropriate dilutions with HNO3.

2.3 Statistical analysis

All analyses were performed using GraphPad Prism 8 software. Since metals were not compared with each other, all experiments contained only one factor. Thus, data were analyzed using a one-way ANOVA coupled with a Bonferroni post-hoc test. In all cases, $p < 0.05$ was the minimum requirement for a statistically significant difference.

RESULTS

Non-equilibrium Biosorption

To determine the equilibrium time for each of the ions a 15 mgL-1 cocktail solutions was incubated with four beads at 45°C. The results showed that the equilibrium time for each of the ions on both CP and KP beads was beyond 24 h and the difference between biosorption at 10 and 24 h was statistically significant (Figure 1). Therefore, 10 h was used as the non-equilibrium time in the remaining studies.

Effect of initial ion concentration and temperature

To determine how temperature and ion concentration affected the biosorption performance of CP and KP beads, experiments were performed at a range of ion concentrations and temperatures. The results indicated that biosorption capacity increased with an increase in ion concentration (Figure 2a, 3a). In contrast, biosorption percentage decreased with an increase in initial ion concentration (Figure 2b, 3b). Interestingly, the biosorption of Cd and Ni by CP beads increased with an increase in temperature at 1 mgL-1 and 6 mgL-1, respectively (Figure 2c,d). For KP bead, there was a significant increase in biosorption of ions (Figure 3c-e).

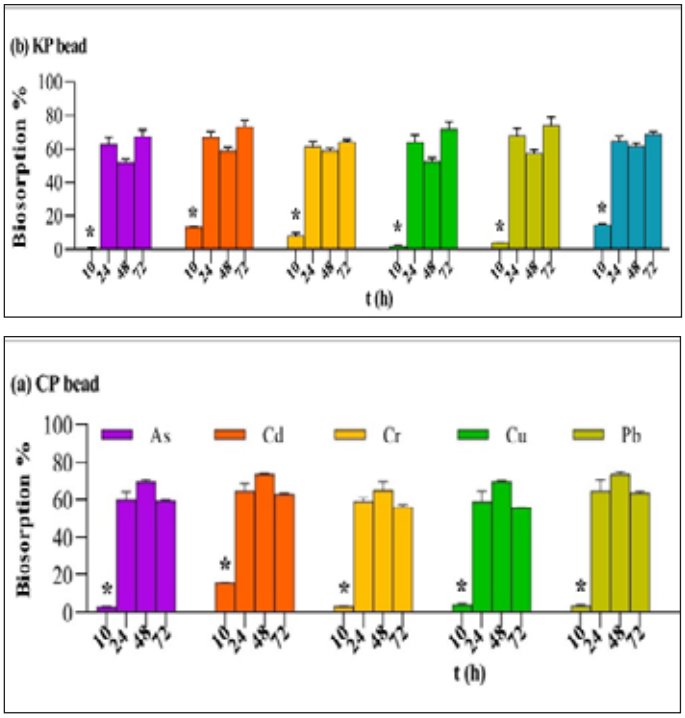

Figure 1: Equilibrium and non-equilibrium biosorption. Four beads of each type were incubated in a cocktail solution containing all seven ions each at 15 mgL-1, pH 7.0, 45°C with continuous shaking at 250 rpm for 10-72 h. The bars represent the mean ± SE for N=3. Data were analysed by a one-way ANOVA coupled with Bonferroni post-hoc test. *Significantly different to all other time points for each ion, p< 0.05. (a) CP bead, (b) KP bead.

Biosorption Isotherms

Biosorption isotherms were plotted with data collected at 10 h (non-equilibrium). Only Cd and Ni ions showed defined trends (R2 > 0.8) (Figure 4). Non-equilibrium data obtained for CP and KP beads at 10 h of contact time were then modeled using Langmuir isotherm model to describe the mechanism of biosorption.

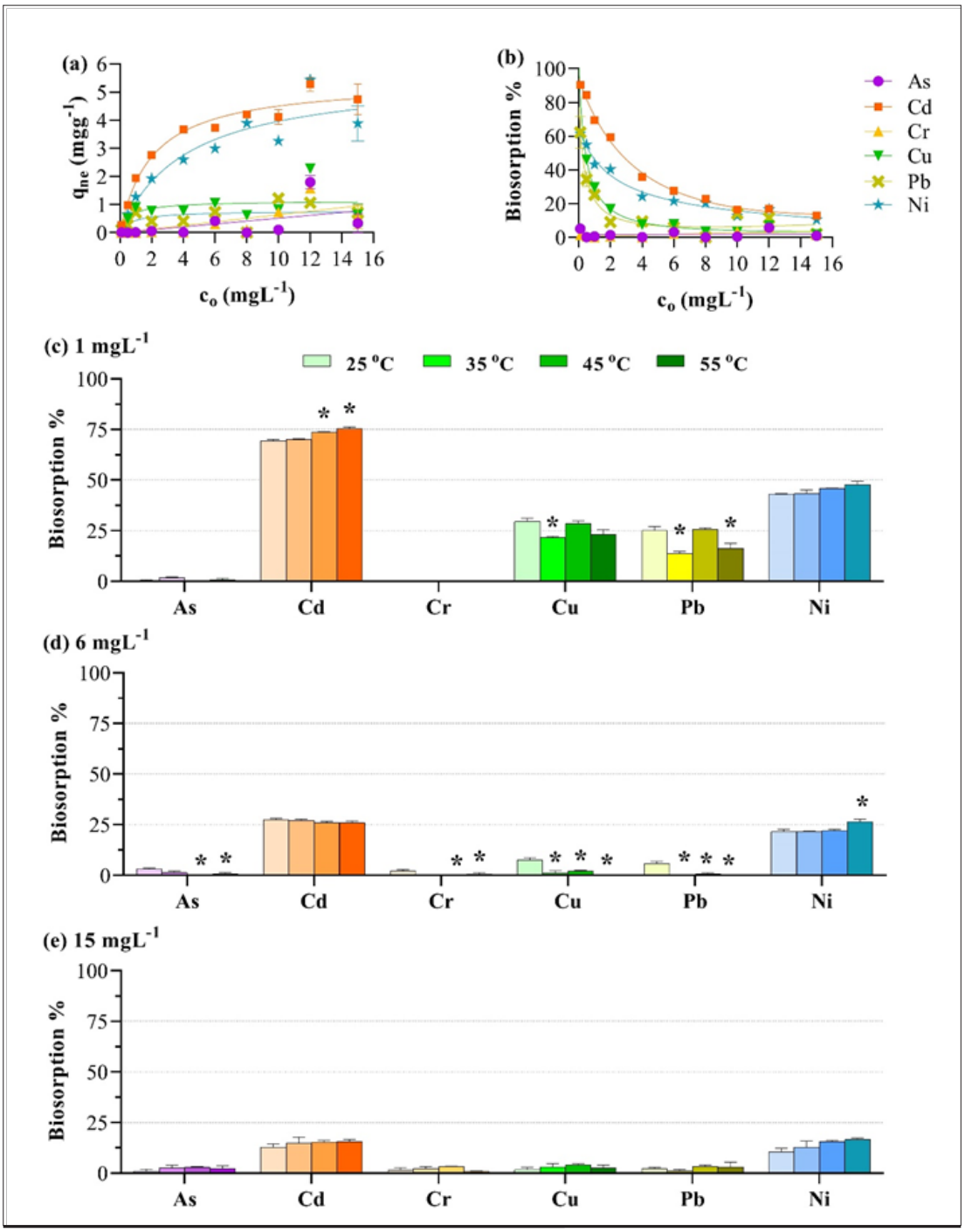

Figure 2: CP bead non-equilibrium biosorption. Four beads of each type were incubated in a cocktail solution containing all seven ions at pH 7.0 with continuous shaking at 250 rpm for 10 h. The points represent the mean ± SE for N=3. Data were analysed by a one-way ANOVA coupled with Bonferroni post-hoc test. *Significantly different from the corresponding value at 25°C, $p < 0.05$. (a) Biosorption capacity at 25^{0}C, (b) biosorption percentage at 25^{0}C, (c) biosorption percentage for ions at 1 mgL-1, (d) biosorption percentage for ions at 6 mgL-1, biosorption percentage for ions at 15 mgL-1.

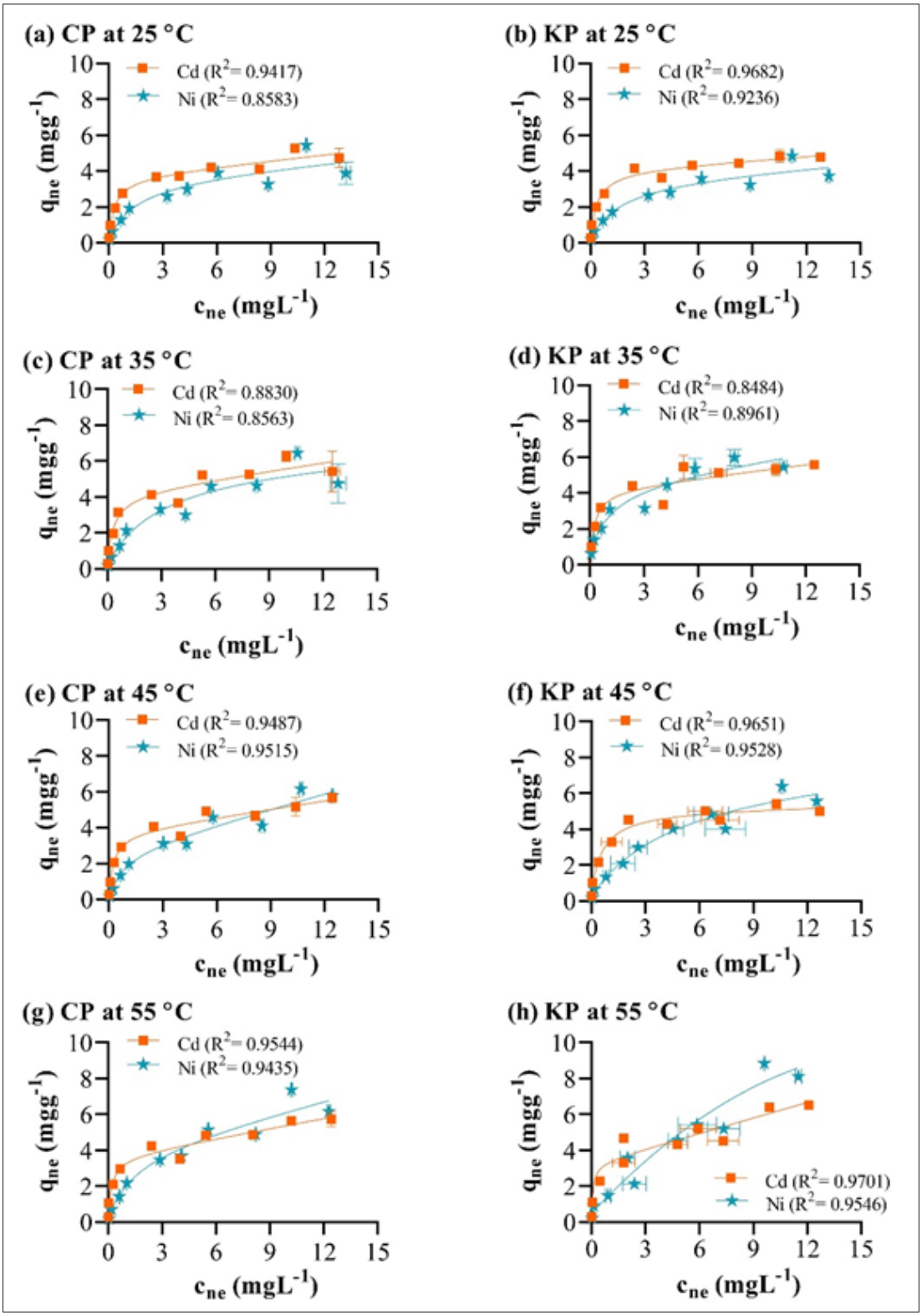

Figure 4: Non-equilibrium biosorption isotherms. Four beads of each type were incubated in a cocktail solution containing all seven ions each at 0.1-15 mgL-1, pH 7.0 with continuous shaking at 250 rpm for 10 h. The points represent the mean ± SE for N=3. (a) CP bead at 25°C, (b) KP bead at 25°C, (c) CP bead at 35°C, (d) KP bead at 35°C, (e) CP bead at 45°C, (f) KP bead at 45°C, (g) CP bead at 55°C, (h) KP bead at 55°C.

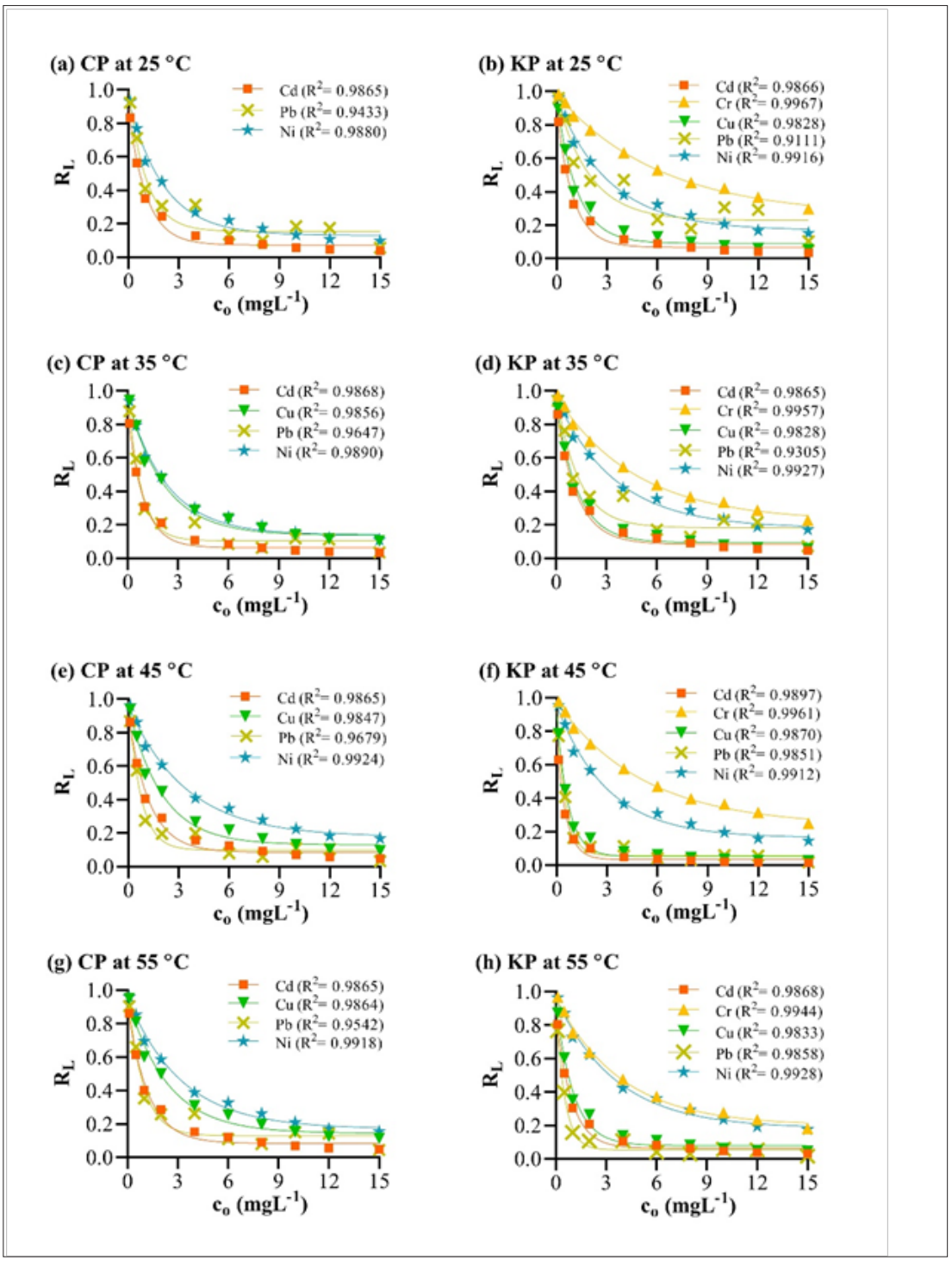

Figure 6: Non-equilibrium Langmuir separation factor. Four beads of each type were incubated in a cocktail solution containing all seven ions each at 0.1-15 mgL-1, pH 7.0 with continuous shaking at 250 rpm for 10 h. The points represent the mean ± SE for N=3. (a) CP bead at 25°C, (b) KP bead at 25°C, (c) CP bead at 35°C, (d) KP bead at 35°C, (e) CP bead at 45°C, (f) KP bead at 45°C, (g) CP bead at 55°C, (h) KP bead at 55°C.

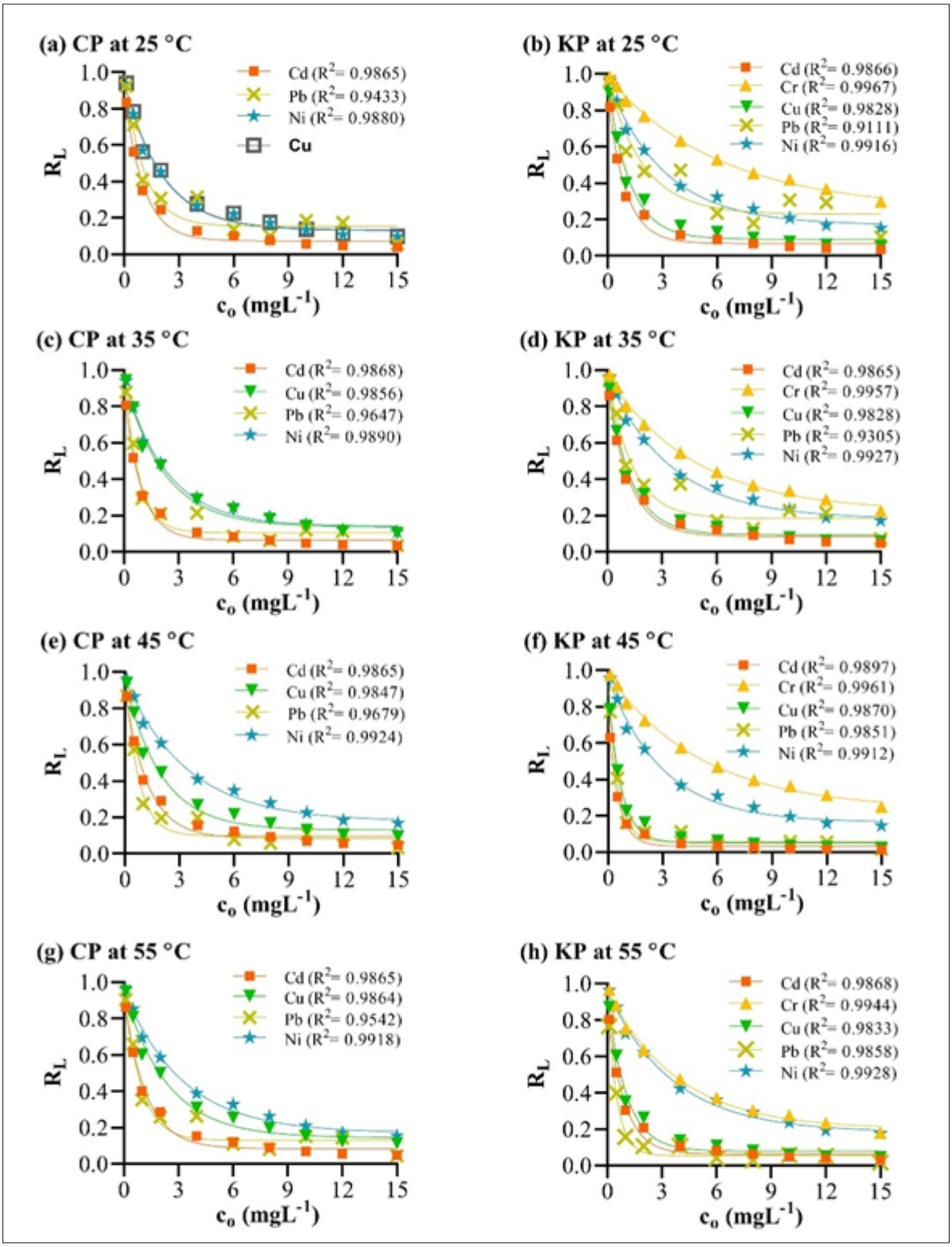

Figure 5: Non-equilibrium Langmuir isotherms. Four beads of each type were incubated in a cocktail solution containing all seven ions each at 0.1-15 mgL-1, pH 7.0 with continuous shaking at 250 rpm for 10 h. The points represent the mean ± SE for N=3. (a) CP bead at 25°C, (b) KP bead at 25°C, (c) CP bead at 35°C, (d) KP bead at 35°C, (e) CP bead at 45°C, (f) KP bead at 45°C, (g) CP bead at 55°C, (h) KP bead at 55°C.

Parameters	Cd	Cr	Cu	Pb	Ni
CP beads at 25°C					
Q_{max}	4.85	--	--	0.95	4.49
K_L	1.66	--	--	1.28	0.63
R_L	0.83-0.04	--	--	0.92-0.06	0.93-0.10
at 35°C					
Q_{max}	5.42	--	1.28	0.78	5.46
K_L	2.00	--	0.60	2.13	0.55
R_L	0.81-0.03	--	0.94-0.10	0.88-0.03	0.94-0.11
at 35°C					
Q_{max}	5.50	--	1.38	1.10	6.74
K_L	1.32	--	0.66	2.34	0.34
R_L at 55°C	0.86-0.05	--	0.94-0.09	0.87-0.03	0.96-0.17
Q_{max}	5.71	--	0.88	0.63	7.58
K_L	1.34	--	0.54	1.63	0.37
R_L	0.86-0.05	--	0.95-0.11	0.90-0.04	0.96-0.16
KP beads at 25°C					
Q_{max}	4.87	2.41	2.14	1.97	5.20
K_L	1.86	0.16	1.22	0.65	0.36
R_L	0.82-0.04	0.98-0.30	0.89-0.05	0.96-0.10	0.96-0.15
at 35°C					
Q_{max}	5.84	2.85	3.58	2.43	6.89
K_L	1.35	0.23	1.16	0.98	0.32
R_L	0.86-0.05	0.98-0.23	0.90-0.06	0.94-0.07	0.96-0.17
at 45°C					
Q_{max}	5.09	3.63	2.16	1.49	6.62
K_L	4.88	0.21	2.79	4.59	0.40
R_L	0.63-0.01	0.98-0.25	0.78-0.02	0.77-0.02	0.96-0.14
at 55°C					
Q_{max}	6.28	5.07	2.33	1.48	9.64
K_L	2.04	0.31	1.50	4.75	0.32
R_L	0.80-0.03	0.97-0.18	0.87-0.04	0.76-0.02	0.96-0.18

Table 1: Four beads of each type were incubated in a cocktail solution containing all seven ions each at 0.1-15 mgL-1, pH 7.0 with continuous shaking at 250 rpm for 10 h. qmax= maximum biosorption capacity (mgg-1); KL=Langmuir constant (Lmg-1); RL=Langmuir separation factor (dimensionless). -- Parameters could not be derived.

Non-equilibrium Biosorption Isotherms

In the present study, biosorption capacity increased with an increase in ion concentration (Figure 2a, 3a). This is likely due to the increase in the concentration gradient driving the reaction13. On the other hand, the decrease in biosorption percentage with an increase in initial ion concentration (Figure 2b, 3b) is because of the limited number of active sites on the surface for binding of ions at a fixed biosorbent concentration14. An interesting trend of increase with an increase in temperature for biosorption of Cd and Ni by CP beads (Figure 2c,d) and all ions by KP bead (Figure 3c-e) was observed. This may be due to the increase in the kinetic energy of the ions thus increasing the probability of collision with the biosorbent surface increasing their ability to diffuse into the pores of the bead. Additionally, there could be enlargement of pore sizes and/or an increase in the number of active binding sites due to the rupture of surface functional groups.[15] Similar results have been reported as biosorption increased with an increase in temperature for the uptake of As by date palm fibers and OP[16], Cd by bagasse[17], Cr by citrus peel18, Cu by OP carbon19, Hg by water hyacinth carbon20, Pb by modified walnut shell21 and Ni by banana leaf.[22] For KP bead, there was a significant increase in biosorption with increase in temperature thus indicating the more endothermic nature of the reaction (Figure 3c-e).

To further understand the biosorbent-biosorbate interactions, biosorption isotherms[23] were plotted in non-equilibrium conditions for both bead types and the data modeled using Langmuir isotherm model.

Langmuir Isotherm

The Langmuir isotherm describes the availability of a finite number of identical active sites that permit the formation of a monolayer (i.e. no stacking occurs) on a homogeneous biosorbent surface.[24,25] Thus, all binding sites have an equal affinity for biosorbate ions.[26, 27]

An increased rate of biosorption of these ions from solution at elevated temperature is determined by an increase in the qmax values with increase in temperature. This was observed in the uptake of Cd and Ni by CP and KP beads, and Cr by KP bead (Figure 5, Table 1). Similar observation was reported for the biosorption of Ni by activated carbon between 20 and 50°C from banana peel28 and Cu by peanut hulls between 25 and 65°C,[29] where the values of qmax increased with increase in temperature. On the other hand, qmax for Cu and Pb biosorption by both CP and KP beads first increased and then decreased with a further increase in temperature which was also reported for the biosorption of Ni by OP in the temperature range 25- 50°C.[30] This suggests that beyond a certain temperature, the ions may have started desorbing from the bead. This may have been due to the weakening of the binding forces.[31]

The dimensionless Langmuir separation factor (RL) (eq 5) indicates whether the biosorption is favorable or not; 0 < RL < 1 suggests a favorable reaction, RL > 1 indicates unfavorable biosorption, RL = 1 represents linear biosorption and RL = 0 shows that biosorption is irreversible.[32-34] All the RL values obtained were favorable for reaction, although for dilute solutions (closer to 0.1 mgL-1) the reaction was least favorable with a larger separation factor (RL ≤ 1) and for concentrated solutions (closer to 15 mgL-1) the reaction was more favorable and irreversible with a smaller separation factor (RL ≥ 0) (Figure 6, Table 1). The significant decrease in the affinity of the CP bead binding sites (KL) for Cu ions with an increase in temperature can be explained by the increase in the separation factor (RL) for moderately concentrated solutions. Similarly, the significant increase in KP bead KL values with respect to the uptake of Pb ions is confirmed by the corresponding decrease in RL values with increase

Nature of biosorption - physisorption and chemisorption

From the values of biosorption isotherm constants, the forces holding the biosorbate on the biosorbent surface can be determined. In physisorption, multilayer biosorption takes place.[35] While all multilayer adsorptions are physical in nature, monolayer adsorptions may occur via physisorption or chemisorption.[36-37] The results from Langmuir isotherm modeling suggest that the ion binding on CP and KP peel immobilised beads was physico-chemical in nature.

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CONCLUSIONS

We have shown that the Probability Isotherm theory given by Chapman *et al.,* for biochemical reactions can also be used to study multi-ion biosorption of heavy metal ions in non-equilibrium conditions. The uptake of Cd and Ni ions on CP and KP beads was most useful for calculating parameters in the Langmuir isotherm model as these ions gave the best fit among the seven ions in the cocktail solution. Langmuir was a suitable isotherm model with up to five ions showing intermediate to good fits (R2 > 0.8), and binding of the ions was physico-chemical in nature. This novel concept has opened new avenues for the study of biosorption in non-equilibrium conditions. IJFMP

LIST OF ABBREVIATIONS

AT	: Equilibrium binding constant corresponding to the maximum binding energy
cads	: Adsorbed biosorbate concentration
co	: Initial ion concentration
CP	: Cucumber peel
ICP-MS	: Inductively coupled plasma coupled with mass spectrometry
kb	: Rate constant for backward reaction
kf	: Rate constant for forward reaction
KL	: Langmuir constant
KP	: Kiwifruit peel
N	: Number of experiments/ Sample size
PFO	:Pseudo-first order
PSO	: Pseudo-second order
qe	: Biosorption capacity at equilibrium
qmax	: Maximum biosorption capacity
qne	: Biosorption capacity at non-equilibrium
R2	: Coefficient of determination
RL	: Langmuir separation factor
RSD	: Relative standard deviation
SE	: Standard error of the mean
Tb	: Terbium
V	: Volume of solution

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REVIEW ARTICLE

Driver Drowsiness Detection: A Review

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ABSTRACT

Safety of passengers has been a severe issue in all societies of any country in the world. Thousands of people lose their lives daily and many more lose their livelihood because of paralysis caused by accidents. Accidents not only cause physical injuries but also are responsible for high economic losses. According to various studies and investigations it is noticed that one of the major causes behind the road accidents is driver's drowsiness. This drowsiness can be the cause of many reasons. Fatigue or sleep deprivation are the major reasons. Thus, a countermeasure device is currently essential in many fields for sleepiness related accident prevention. Many researchers have been working on different aspects to deal with this drowsiness issue through various aspects like (1) Subjective Measures, (2) Physiological measures, (3) Vehicle-based measures, (4) Behavioural measures This paper proposes a comparative review on different methods used to detect drowsiness of drivers. It looks into the advantages and disadvantages of different methods used for the purpose and creates a detailed comparative analysis for a better future hybrid model to be taken into consideration. This would further help in enhancing the safety measures that should be taken for road safety.

KEYWORDS | drowsiness detection, road safety, ai measures, driver fatigue

INTRODUCTION

A survey published by the National Highway Transportation Administration states that 7.277 million traffic accidents occurred in the United States in 2016, causing 37,461 deaths and 3.144 million injuries, of which driver fatigue caused approximately 20–30%. Based on police investigation reports, it is estimated that every year a total of 100,000 vehicle crashes are due to driver drowsiness. These crashes were responsible for approximately 1,550 deaths, 71,000 injuries and $12.5 billion in financial losses. In the year 2009, the US National Sleep Foundation (NSF) reported that 54% of adult drivers has driven a vehicle while feeling sleepy and 28% of them actually fell asleep. The German Road Safety Council (DVR) claims that one in four highway traffic fatalities are a result of momentary driver drowsiness. These statistics suggest that driver drowsiness is one of the main causes of road accidents.

When a driver is under fatigue, the very next moment he would feel drowsy and this would affect the driver's mental faculty to process and respond on the road. As a result, the driver will lose control of the vehicle, leading to accident.

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There are many measures that are taken into consideration to monitor drowsiness and to avoid such accidents. Following four measures are used worldwide:-

1. **Subjective Measures:** The level of drowsiness is measured in terms of driver's personal fatigue estimation and using other tools the levels are translated into rating the drowsiness. Mostly theoretical approach.
2. **Vehicle-based Measures:** In some cases, a simulated environment is created where various sensors are placed inside the vehicle and the signals sent by these sensors are then analyzed to detect the level of drowsiness.
3. **Behavioral Measures:** This type of measure focuses on the driver's facial characteristics such as constant yawning, blinking of eyes, jaw drop etc to detect the drowsiness.
4. **Physiological Measures:** They help detect the physiological signals when the driver's head starts nodding. As a result, the vehicle veers off course and causes accidents.

All these measures are repeatedly studied and verified in detail and their advantages and disadvantages have been discussed.

METHOD & MATERIALS

The objective of the study is to verify the extent of the use of these measures to detect driver drowsiness. Some researchers created simulated environments to study drowsiness. Kokonozi et al., conducted an experiment to observe participants who were sleep-deprived prior to observation for 24 hours. Peter et al., took the same participants and studied them for four days at a stretch and concluded that even partially sleep-deprived participants tend to get sleepy after some time. Therefore, sleep quality plays a major role in influencing drowsiness.

According to past studies, the technologies that are used to detect the electrophysiological signals obtained when the driver was driving under fatigue, includes Electroencephalogram (EEG), Electrocardiogram (ECG), etc. In order to detect drowsiness, Khushaba et al., used a wavelet packet transform model to extract the information from EEG, EOG and ECG signals. Li et al., performed wavelet transform on the ECG signals to gain heart rate variability so as to measure the fatigue levels. These were the conventional methods.

Vehicle's turning angle, speed, deviation from centre line etc were measured via facial detection measures that closely focused on the facial characteristics of the driver. Wang et al., studied the relationship between vehicle steering wheel's lateral acceleration, longitudinal acceleration and steering angle with the level of fatigue in periodic time scales. The method has one drawback as it gets easily influenced by external factors such as the vehicle's condition, driving experience, etc.

With the advancement of technologies modern methods have been adapted by researchers to improve their study and get effective results. Facial features analyzing methods such as the PERCLOS (eyelid closure rate exceeds the pupil percentage per unit time), yawning rate, constant jaw dropping etc are acceptable as they do not interfere with the driver's potential to drive. Garcia et al., showed a 3-step method that first detects the traces and eye movements and then under different illumination analyze the performance of eyes. The system later uses PERCLOS measurement.

Another study by Jie et al., yawning detection was introduced in the field that extracted the appearance of eyes and mouth when they close.

Du et al., proposed an MFRNN model that measures the degree of mouth and eyes opening along with heart rate and comparing it with the driver's fatigue level.

Sun et al., proposed a two-level method which was based on MCSVM. Deng et al., explained a 3-level criteria that show the blinking frequency, closed time and yawning time of the driver's sleepiness.

Parkd et al., used IAA and FFA that accurately detects the level of driver's drowsiness.

Various studies have shown the use of these measures as successful methods to detect drowsiness by calculating the geometrical facial features and using those algorithms to improve the accuracy of the detectors. Many facial recognition models have come in handy for the purpose and gave precise results as and when needed.

Methods for Measuring Drowsiness

The section reviews the four most used drowsiness measuring methods, from which the first one is

purely questionnaire-survey based and the other three uses sensors or detectors for the study.

Subjective Measures

Subjective measures estimate the driver's level of sleepiness using some external tools (to create simulated tests). Karolinska Sleepiness Scale (KSS) is a nine-point scale that is mostly used to measure drowsiness. Hu et al., used KSS scale to measure the sleepiness of drivers at an interval of 5 minutes and collected EOG signals. Portouli et al., collected the EEG data of the drivers and verified the interpreted results through questionnaires and a medical practitioner. Ingre et al. established the relationship between the blinking of eyes and KSS data collected every 5 minutes during the driving.

Researchers concluded that lane-changing, blinking rate and drowsiness-related physiological signals give KSS rating between 5 and 9. Therefore, subjective measures output does not give conclusive results as compared to vehicle-based, behavioral and physiological measures.

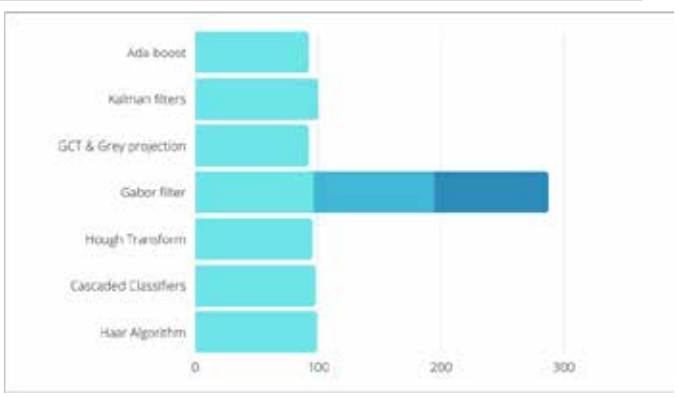
As the level of sleepiness changes even in time duration of as small as 5 minutes, subjective measures cannot detect that change. Also the subjective ratings perform poorly during self-introspection alerts that reduces the drowsiness of the driver in question. Therefore, subjective measures are only good when taken in a simulated environment and not in real conditions.

Vehicle-based Measures

Vehicle-based measurements include placing sensors in various parts of the vehicle including the steering wheel and the acceleration pedal. These sensors will send signals that will be analyzed and alert will be sent to the driver. Researchers found that sleep-deprivation can lead to large variation in driving speed. Given below are the two most commonly used vehicle based measures

Steering Wheel Movements: (SWMs) This method is widely used by the manufacturer like Renault and Nissan for detecting driver drowsiness. An angle sensor is placed on top of the steering column, when the driver is drowsy the micro-correction on the steering wheel reduces as compared to normal circumstances. Sleep deprivation leads the driver to make lesser steering wheel movements than in normal conditions (this characteristic was noted by Fairclough and Graham). Lane change is an important factor that is SWM, so to eliminate this effect, researchers considered only minimum steering wheel movements therefore between 0.5° and 5° which is needed to change lateral position within the lane. Although many companies have adopted SWMs but it comes with certain limitation and works only in very limited situations. This is because they are too dependent on the geometric characters of the road and very less dependent on the kinetic

SNO	DETECTION TECHNIQUES	EXTRACTED FEATURE	CLASSIFICATION	DETECTION RATE
1	Ada- boost	Texture detection and Red Eye Effect	Ratio of height and width of eye	92%
2	Kalman filters for pupil detection	Modified algebraic distance algorithm	Fuzzy classifier	Nearly 100%
3	Gravity center template and grey projection	Gabor wavelets	LDA	92%
4	Gabor filters	Wavelet decomposition	SVM	pp96%
5	Hough transform	Discrete wavelet transform	Neutral classifier	95%
6	Gabor filters	Local binary/condensation algorithm	Ada-boost/SVM	98.3%/93%
7	Cascaded Classifiers Algorithm detects face and Diamond searching algorithm to trace the face	Duration of eyelid closure, number of blinks, and its frequency	Region mark algorithm	98%
8	Haar algorithm to detect face	Unscented Kalman filter algorithm	SVM	99%



characteristic of the vehicle.

Standard deviation of lane position (SDLP)- this is also a useful technique to detect driver drowsiness. In case of a field experiment the position of lane is tracked using a field camera whereas in stimulated environment the software itself gives SDLP. In an experiment it was found that if the KSS rating increases, SDLP also increases. SDLP has a limitations; it is purely dependent on external factors like road markings, lighting conditions and climate. Moreover SDLP can be caused by impaired driving and driving under influence, when the driver is under drugs or alcohol.

Behavioural Measures

A lot of facial movement such as nodding of head, yawning and constant blinking is observed. These behavioural changes can be used to detect driver drowsiness. Following are the detection techniques using behavioural methods:

Physiological Measures

The measures discussed above have a limitation even though they are very precise and accurate. Behavioral measures are very accurate, but it can detect drowsiness of the driver only after the driver's head sways when they start getting sleepy. Same is the case of Vehicle based measures. Preventing accidents in this scenario becomes very difficult. But when we are talking about Physiological measures, they are very efficient in detecting drowsiness in the early stages and hence preventing accidents with these measures is an appropriate option. Physiological measures alert the driver timely and hence prevent any upcoming accidents.

The most common sensors that are being used to develop the physiological measures are:

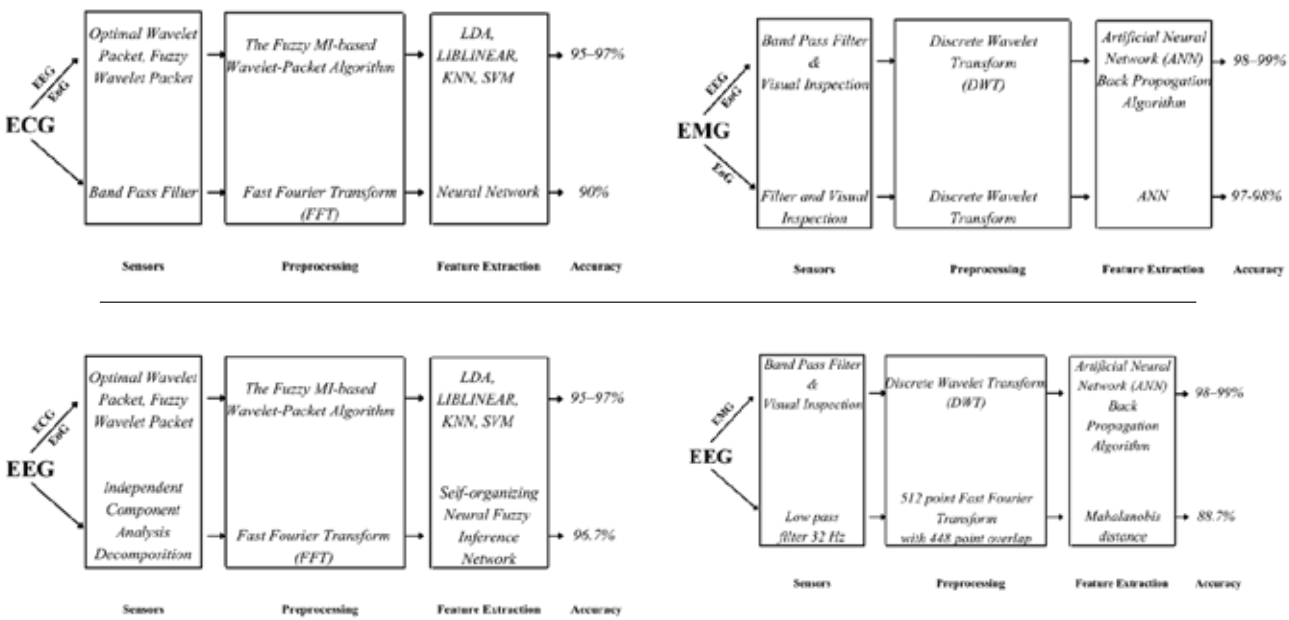
1) Electrocardiogram (ECG)
2) Electromyogram (EMG)
3) Electroencephalogram (EEG)
4)Electro-oculogram (EoG)

Electrocardiogram (ECG)

As the name suggests, ECG's role is to monitor cardiac activity. When a driver is drowsy, the heart rate is seen to be slower thcaan normal when the driver is sleepy. The heart rate drops from the driver being awake to being sleepy. ECG combinations for detection is shown below:

Electroencephalogram (EEG)

EEG's role is to monitor brain activity. Electroencephalography is an electrophysiological monitoring method to record electrical activity on the scalp that has been shown to represent the macroscopic activity of the surface layer of the brain underneath. It is typically non-invasive, with the electrodes placed on the scalp. EEG combinations for detection is shown below.



Electro-Oculogram (EoG)

Electro-oculogram (EoG) is used to detect eye movement of drivers such as Rapid Eye Movement (REM) and Slow Eye Movement (SEM). The REM and SEM is explained with a picture below. Electrooculography is a technique for measuring the corneo-retinal standing potential that exists between the front and the back of the human eye. The resulting signal is called the electrooculogram. EoG combinations for detection is shown below:
REM and SEM in sleep cycle:

Stage 1(SEM) It is the stage in which the person goes off to sleep slowly.
In this stage the heart rate, breathing and brain activity slows down. It lasts for several minutes
Stage 2 (SEM) In this stage the heart rate and breathing decreases more and the muscles starts relaxing. There is a drop in body temperature and eye movement stops.
Stage 3 (SEM) More decrease in heart rate and breathing. Muscles completely relax. Slow brain waves are observed.
Stage 4 (REM) Most dreams occur in this stage. It occurs 90 minutes after stage 1. The eye moves faster from one side to another and heart rate and blood pressure increases.

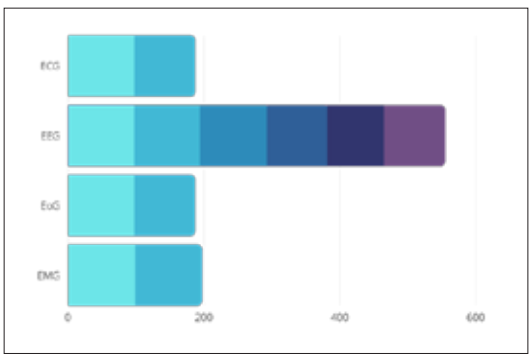
Below is a comparative chart of all physiological measures:

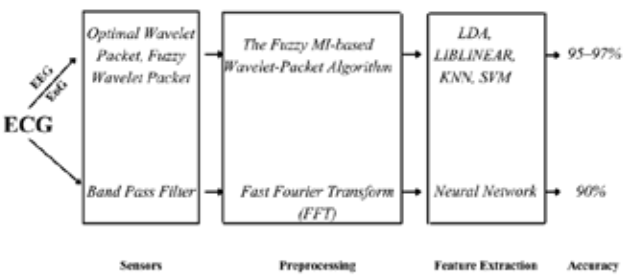
DISCUSSION

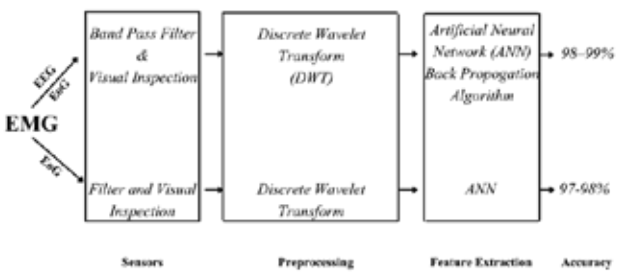
The review paper focused on various aspects of how drowsiness can be detected and measures can be improved to attain precise results in order to avoid devastating road accidents.

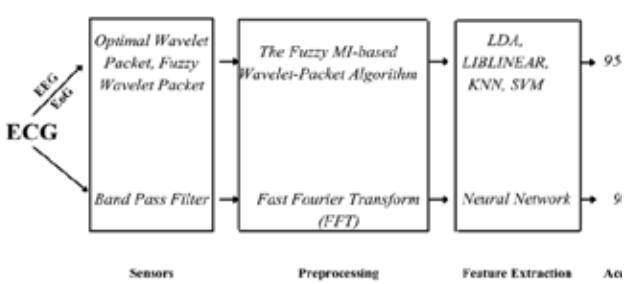
The detection of drowsiness depends on various factors for which four main types of measures came into existence.

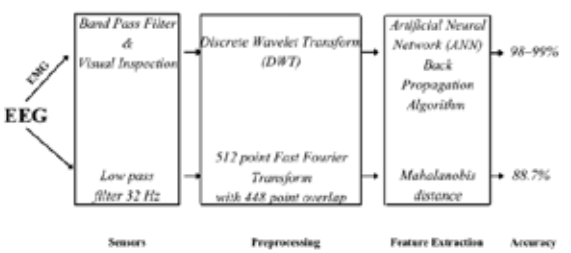
According to various researchers, experiments were conducted to draw a comparison between the simulated and real driving conditions and gave clear cut results that the simulated measures gave ratings that are not reliable when it comes to real driving experiences as the real conditions provide











more challenges that a controlled environment can not. And these significant differences prompted the researchers to search for better and reliable technologies that are advanced and can detect various aspects of drowsiness.

CONCLUSION

In this paper, we focused on a different approach to detect drowsiness in drivers and how these measures can be improved to get better results. The study focused on four measures: Subjective, vehicle-based, behavioral and physiological, to detect drowsiness. And their advantages and disadvantages were also mentioned. Out of all the measures, physiological measures showed the most accurate results but some more work needs to be done. Incorporating the vehicle-based approach along with the behavioral measures into the physiological measures can boost the precision to higher levels. Hence the detection can be implemented in real driving conditions. Fusing ECG results with other technologies can also help in providing optimal outcomes. IJFMP

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Conflict of Interest:

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REVIEW ARTICLE

Various Applications of Artificial Intelligence in Digital Forensic Investigation

Kiran Singh[1], T Poongodi[2] Shrddha Sagar[3]

ABSTRACT

Most data, including books, images, photographs, medical records, and even human genetic material, has moved to digital formats in recent years. Laptops, smartphones, and connected devices are the primary sources of this digital data transformation, and they are quickly becoming an integral part of our everyday lives. We are becoming a soft target for different forms of cybercrime as a result of this transition. Digital forensic investigation allows you to retrieve files from a suspect's laptop that have been accidentally removed or hidden. The available manpower and government resources, however, are insufficient to investigate cybercrime. Unfortunately, current automated forensic protocols and practices necessitate a lot of human contact, which slows down the process and slows down the rate at which digital crimes are committed. Over the last few years, the use of Artificial Intelligence (AI) in Digital Forensic Science (DFS) approaches to cybercrime investigations has gotten a lot of attention. Traditional DFS techniques are no longer applicable to Digital Forensic (DF) investigators, as they frequently require a DF investigator to manually sift through data in order to locate relevant evidence. In order for Digital Forensic Science to keep up with the demands, which are compounded by Big Data, more intelligent Digital Forensic investigation techniques are needed. The main focus of our paper is to conduct a thorough study of the various AI algorithms and their implementation specifically in Digital forensics.

KEYWORDS | AI, machine learning, digital forensics, cybercrime

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INTRODUCTION

The terms digital forensics (DF) is defined as "The application of scientifically proven and tested strategies in terms of preservation, compilation, authentication, identification, study, interpretation, recording, and presentation of digital evidence obtained from several digital sources in order to ease or further the reconstruction of criminal events, assisting in the detection of unethical practices that have been shown to be adverse to scheduled operations. Demand for DF are becoming increasingly significant in today's world. DF investigation protocols support in collecting critical data from a compromised computer system. And, businesses depend mostly on computers and mobile devices and the Internet nowadays. Moreover, it is essential to gather the necessary evidences from these devices. To promote or refuse any reasoning, an investigator might have information about the incident, where the digital evidences should be obtained from the connected systems. It's crucial to understand how to retrieve digital data that may be useful to investigators. Though, the existing human resources

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and other available resources are insufficient to adequately prosecute digital crimes committed on digital devices.

Furthermore, current digital forensic techniques and methods necessitate a high level of human involvement, which speeds down the process in comparison to the rate at which digital crimes are committed. The majority of data is now gathered using digital devices and websites confiscated from criminals, or accessible on the Internet or exchanged by telecommunication companies. Forensic investigators should gather and examine information to determine where the crime is committed, who the criminal is, whom the criminal is targeting, whether the criminal is committing the crime, and how the crime happens. Thus, DF is a branch of criminalistics concerned with identifying, acquiring, preserving, analyzing, and presenting the information content of computer systems, or digital devices in general.

It's important to understand how AI is used when investigating AI as a weapon of crime. Investigators can compile and evaluate the dataset, training model, learning model, inference model, and implementation of the AI method used to commit a crime. Investigators should be able to understand the purpose of using AI's function after examining it. It is essential for investigators to be able to distinguish between the developer's objective and the AI outcome. AI programs, unlike conventional programming, frequently have unintended consequences. Since several AI models exploit random weights in the learning process, AI parameters are often calculated with randomness. As a result, while using the same dataset and learning model, programs with different parameters and outputs can be developed. It would be difficult to prove if AI was used as a tool, how AI was used, and how much damage AI caused because investigators would be unable to investigate the case.

AN OVERVIEW

By 1970, digital forensics, also known as electronic forensics, had been introduced.[1] The financial crime is found in the first investigation using the criminal's computer. In 1996, the first electronic fraud was reported. In 1996, the initial computer crime was identified in Texas, which ended in a five-year sentence.[2] With the growing popularity of the Internet, computer-based digital crimes began to increase in 1990. At the end of 1990s and beginning of 2000s, computer forensics emerged as a separate discipline. According to CSI surveys, nearly 46% of respondents have been impacted by computer crimes in some way.[3] According to a Gallup poll from 2010, 11% of American adults have been victims of Internet/ Computer related crimes. And, the ratio is upto 6–8% high compared from the previous seven years. According to a study conducted by the "Australian Company Crime Survey," financial fraud and data breaches reached A$2,000,000 in 2006.[4] According to the Company Crime Survey, financial fraud and data breaches cost the company A$ 2,000,000 in lost revenue. With the emergence of various digital devices and their growing use for investigative purposes, the term "digital forensic" has become very common.

DF is a legal discipline that deals with the research, identification, conservation, security, extraction, recording, and other forms of digital data treatments. The aim is to transform the data to be assessed as part of a criminal or civil case after it has been processed into an information using appropriate types of reasoning. Consequently, the DF is a division of criminalistics whose practices are aimed at illustrating the presence of digital evidence related to the completion of a prosecution, or even prior to the investigative processes. It is a method of analyzing digital phenomena with the help of science and technology. Theories may also be used legally to address concerns about events that have occurred. DF has rapidly carved out a massive presence in business environments, with the aim of highlighting breaches of industrial secrets and/or security policies, as well as dealing with cyber incidents.

For the past two decades, global use of mobile smart devices has shot up dramatically, and they have become an integral part of our everyday lives. The word "smart computer" refers to a wide range of devices such as smartphones, laptops, GPS, tablets, and so on. Because of their processing power, large storage capacity, and low cost, these smart devices have become increasingly common. As a result, they can store a massive amount of commercial and personal data. These devices play

an indispensable role in our everyday lives because they store users' personal and vital information. As emerging technology such as digital devices and the internet become more predominant nowadays, so the number of digital crimes also increases. At last, we're becoming victims of a variety of cybercrime and cyber attacks.

CONCLUSION

In today's world, technology advances at a breakneck pace, and we are constantly exposed to new innovations. AI is one of the most interesting areas of computer science, with a bright future. AI has the ability to make a computer function like a person.

Software forensic tests require the ability to analyze vast volumes of data in a timely manner in order to find relevant evidence during criminal investigations. Time and resource constraints, both computational and human, have a negative effect on the outcomes. As a result, better utilization of available resources is needed, in addition to the capabilities of currently used forensic equipment. The use of Artificial Intelligence in computer forensics is described in this paper. This system is made up of specialized intelligent agents that operate based on technical domain experts' expertise. Their aim is to interpret and compare the data found in an investigation's evidences, then show the most interesting information to the human investigator based on their experience, minimizing the amount of data that must be individually examined. The correlation function aids in the discovery of ties between evidences that a human expert might overlook, particularly given the large amount of data involved. The preservation, collection, and analysis of evidence found in digital storage media are all steps in the forensic investigation of computer systems. In a criminal investigation, digital evidence may be crucial in cases involving child trafficking, document forgery, tax evasion, and even terrorism. The relentless growth in the ability of digital storage media, as well as their widespread presence in everyone's everyday lives, has resulted in an increase in demand for such tests, as well as the amount of data to be examined. Furthermore, the existing collection of forensic methods is insufficiently efficient when it comes to evaluating a large number of evidences and correlating the results. As a result, the work of computer forensic experts is extremely time consuming. Since most forensic instruments lack distributed processing capabilities, the computing resources needed to conduct such investigations are also a concern. To deal with the three issues listed above, a variety of approaches[5] have been proposed: (i) a reduction in the number of evidences to be investigated, (ii) proof correlation, and (iii) forensic examinations computational work distribution. As a branch of the forensic sciences, digital forensics is confronted with new challenges as potential digital evidence grows and expands.[6] Innovative methods for automated investigations are being developed at a rapid pace in the fields of computer science and information technology. Algorithms and techniques for machine learning are used in a wide range of applications. In order to work more effectively, machine learning engineers and forensic analysts must have a thorough understanding of the algorithms in use, how they work, and how they learn from raw data. Digital forensics[7] is becoming a more advanced topic, as well as an important field that often necessitates the analysis and extraction of a large amount of complex data from a crime scene. Examining digital information of the committed crime to be used as legal proof in a court of law is part of a digital forensic investigation. In the process of collecting and analyzing digital data, a variety of machine learning algorithms and techniques can be useful. Machine learning can improve this method by allowing it to deal with large amounts of data in a short period of time while maintaining a high degree of accuracy and producing high-quality results.

Algorithms of AI and ML in Digital Forensic

AI systems have made tremendous progress in solving increasingly complex computational tasks using machine learning as the core technology, making them crucial components of human society's future growth.[8] However, as ML algorithmic models chase prediction precision and become increasingly implicit, explainability becomes a challenge for black-box techniques like ensemble methods and deep neural networks.[9] An examination of current ideas about the application of artificial intelligence technology in forensic

science reveals that they are almost always linked to one of the following areas: Traditional peer research capacity building; algorithmizing of the crime investigation process; crime prevention (recognition of signs of imminent crime, etc.).

The described directions are focused on processing big data with machine learning elements that operate within a limited number of parameters to a greater extent. We are currently not considering the use of artificial intelligence in these areas in its entirety, since only a portion of the abilities inherent to the human intellect are used [10]. Simultaneously, technological advancements would inevitably lead to the creation of more advanced approaches that offer new artificial intelligence capabilities comparable to human intelligence.

Inductive reasoning and deductive reasoning are the two key methods used to characterize ML forensics:

i. Inductive Reasoning is based on a broad understanding of particular data. The knowledge gained is fresh and does not preserve the facts. This means that new information will invalidate previously acquired knowledge. There isn't a single well supported hypothesis.There are numerous objectives in this field, including the need to discover general concepts from a limited collection of examples. The examples are referred to as "experience." The foundation for this is to look for common characteristics in different instances. Inductive learning is the foundation of these approaches.
ii. Deductive Reasoning derives insight from well established logic techniques. Deductive reasoning uses well established techniques to derive information from experience. The information is not fresh. However, it is implied in the initial understanding. Established knowledge and its foundation in mathematical logic cannot be invalidated by new knowledge.

AI has been studied in a variety of academic settings, as mentioned in the introduction. From different viewpoints, this section discusses research on the AI security threat and AI-related crime. We also look at cybercrime as identified by the cybersecurity and digital forensics communities. Adopting online personae, known as socialbots, that act like humans is a prime example of malicious AI use.[11]

PRINCIPLE	TRADITIONAL FORENSICS	AI FORENSICS
Meaning	When gathered, the facts and context remain unchanged.	During the learning process, the interpretation of the AI system changes.
Errors	In forensic method, errors may be reported	
Transparency and trustworthiness	Several approaches have been tried and proven.	It's needed for the AI forensic process to be verified.
Reproducibility	It demonstrates a high degree of consistency in quality	Even using the same dataset and learning model, it would be impractical.
Experience	There is a wide range of research. and education available	It should be researched.

Table 1 describes the difference between the conventional forensics and digital forensic using AI techniques. Although the original goal of socialbot was to increase public awareness and collaboration, it has also been used for malicious purposes including phishing, fraud, and political manipulation of online social media campaigns.[12,13] Since it is based on a single user's past activities and public profiles, detecting the malicious socialbot has become a computer security problem. When malicious socialbots are programmed to carry out a political attack, according to social science, the technique has the potential to influence or inflame public opinion.

Some researchers claim that malicious hackers have already begun to use AI to improve their hacking skills and create new forms of cyber attacks. Financial frauds, cyber terrorism, and cyber extortion are all popular cybercrime techniques that have been enhanced with AI. When attempting voice phishing, for example, hackers can fool victims by convincingly imitating the voice of the victims' relatives or friends.[14]

In comparison to previous research, Brundage *et al.*,[15] concentrate on the potential problems that particular techniques can cause. They looked at three different shifts in the threat landscape: the proliferation of emerging threats, the rise of new threats, and a shift in the threat's traditional cost-cutting approaches (for example, mass spear phishing) allow attackers to invade more targets, resulting in the spread of established threats. The

cost of tasks that require human labor can be reduced because of the AI system's scalability. New threats can emerge in order to complete tasks that humans are incapable of completing (for example, imitating individual voices or controlling multiple drones).[16] The typical character of threats may change as highly successful AI attacks become more widespread.

King *et al.,*[17] offered a fresh take on AI defense. They are raising a threat by using the word 'AI crime.' AI crime includes trade, financial markets, and insolvency (e.g. market manipulation, price fixing, collusion), toxic or hazardous substances (e.g. drug distribution, selling, purchasing, possessing banned drugs), and crimes against individuals (e.g. harassment, torture), Theft and fraud, forgery and personation (e.g., spear phishing, credit card fraud). They asked that each of the offenses be labeled as having one or more threats associated with it. They focused on human nature when classifying AI security risks: emergence, transparency, surveillance, and control.The psychology danger, for example, means that AI can influence a person's mental state to the point that it promotes or triggers illegal behaviour.

Concerns about AI privacy resulting from the processing of personal data have been the subject of some study. AI applications in healthcare, banking, and education, according to Li and Zhang [18], can lead to privacy concerns. Developers want to obtain as much data as possible because the quantity and quality of training data has a huge effect on AI performance. According to Li *et al.,* the collection of detailed data entails inherent privacy risks.

The previous study has three implications for AI stakeholders. First and foremost, researchers and engineers should be aware that, due to AI's dual-use nature, the device could be used to conduct illegal acts, even though it is intended for legitimate purposes. Since artificial intelligence is a double-edged sword, anyone working in this area must adhere to strict professional ethics. Second, completely new forms of security threats that have never been considered before will arise.

Since AI may perform tasks that were previously thought to be difficult for humans or conventional systems to perform, the risks may be outside the reach of current threats. To avoid AI security threats and respond to AI crime, AI researchers should collaborate with experts from other fields. Finally, the cybersecurity sector's trials and tribulations could help the AI security field. Predictable AI crimes are inextricably related to cybercrime, as previously mentioned in previous studies. This object has two functions. The presence of ICT led to the emergence of cybercrime; the current state of AI security is similar to that of cybersecurity in its early stages.

Comparative study on various AI applications in Digital Forensics

The use of artificial intelligence (AI) improves the chances of detecting and investigating cybercrime. This enables forensic experts to get to the root of the problem rapidly and effectively.

AI aids in the quick resolution of a crime and saves police a lot of money. This will allow them to concentrate more on the areas where cybercrime is most likely to occur. By sifting through unstructured data collected by police, AI can identify suspicious and criminal activity.

Cognitive-Data Analytics is a form of AI that allows you to quickly digest and analyse data. It can also make it easier for investigators to search through criminal records and find possible suspects. [19]

AI will assist in the recognition of specific elements in images and videos that are being investigated. AI may also assist in identifying commonalities in contact, place, and time. This allows authorities to pinpoint the location of the next crime or incident.

Here are some AI techniques that have an effect on digital forensics:

Knowledge Representation

This has to do with what a computer program needs so that it intelligently executes tasks, and also how quantitative methods can feed this information to the program. It can be used to develop better strategies for defending against cyber attacks.

Expert Systems

This clarify why certain processes are carried out and the findings reached during the digital forensic' investigation. It enables an individual to examine and criticize the process and reasoning

employed. This can reveal shortcomings in the methods used to draw conclusions. They also make data processing faster.

c) Pattern Recognition

In an investigation, this distinguishes specific types of data clusters. It can be used to identify the contents of pictures, spam emails, and directories on hard drives that contain suspicious files. When used in conjunction with information exploration, it can help analysts spot trends in massive quantities of data.

Some Artificial Intelligence Techniques that Can Aid in Digital Forensics:

a) This recognizes, restricts, and removes threats using live forensics. It's also a good idea to scan criminal history to see who could be responsible.
b) Data recovery is the process of restoring data that has been lost or destroyed. In digital forensics, this is an important technique for retrieving possible evidence.
c) Password recovery is crucial during forensics when password-protected files are to be accessed. It gives you access to these files, which can be presented as evidence.
d) Investigators may use known file filtering to find files that are important to their investigation.
e) Investigators may use a timeline analysis to see the sequence of events that led up to the incident under investigation.

In view of the above, we can see how AI can help forensic analysts in a variety of ways. It removes the need for investigators to sift through various data sources, saving them precious time and effort.

CONCLUSION

After discussing the use of AI and its possible applications in forensic science and criminal investigation to support forensic scientists, police officers, and security personnel, it is obvious that Artificial Intelligence applications or software can assist investigators in greatly reducing the amount of time spent on various tasks at various stages of an analysis and investigation. Saving time will eventually lead to increased efficiencies in the disposition of cases, potentially contributing to the goal of reducing the number of cases that are pending due to sluggish and complicated investigation procedures. More precision, competence, and a lack of prejudice would ultimately lead to proper criminal justice. To make forensic investigation techniques more advanced, scientists and researchers have been designing more AI-based software programs and devices. Our forensic investigation and predictive policing systems as well as our security and defense systems will benefit from new advances in AI. IJFMP

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REVIEW ARTICLE

Heavy Metal Toxicity: Impact on Human Health: A Review

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ABSTRACT

The detrimental effects of heavy metals on human health are known as several health risks are associated with heavy metal toxicity. Rapid industrialization and enhanced use of metals in various industries resulted in the increased distribution of heavy metals in the environment. Industrial wastes in the form of liquid and gaseous effluents, as well as scrap in landfills are a major source of heavy metal pollution causing contamination of water bodies and the environment in general. These toxicants accumulate in the human body as individuals are exposed to them. The toxicity of heavy metals depends on various factors which include but not limited to amount of metal (dose), exposure duration, age, gender and health status of individuals. Heavy metals such as mercury, cadmium, arsenic, chromium, lead etc especially are of great concern for public health due to their acute toxicity. These metals are potent enough to induce multiple organ failure even at small concentrations. Studies have shown an association between heavy metals and carcinogenicity. In the present work, a comprehensive analysis of toxicity of eleven heavy metal namely, aluminum, antimony, arsenic, bismuth, cadmium, cobalt, iron, lead, mercury, tin and thallium has been reviewed along with their analytical aspects and management. Clinical features of metal toxicity with diagnosis techniques and treatment including hospitalization and post-hospitalization management are also elaborated.

KEYWORDS | heavy metals, metal toxicity, poisoning, analytical technique

INTRODUCTION

Heavy metals are naturally occurring components of the earth's crust. Degradation of heavy metals is not possible. With increasing pollution, the concentration of heavy metals is rapidly increasing in the environment. Consequently, humans are exposed to these metal toxins through food, air and water. Some metals are essential for the human body due to their role in metabolism but at higher concentration these are toxic for human health. In the present review, 11 heavy metals are discussed, namely, Aluminum, Antimony, Arsenic, Bismuth, Cadmium, Cobalt, Iron, Lead, Mercury, Thallium and Tin.

Aluminum:

Aluminum is denoted by the symbol '*Al*' and has atomic number 13. Aluminum is a highly conductive, corrosion resistant, silvery-white metal and derived from bauxite ore. Aluminum poisoning can be caused by three ways which includes ingestion, inhalation and dermal contact. The total body burden of aluminum (in healthy individuals) is estimated to be 30 to 50 mg.[1]

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Antimony

Antimony is denoted by the symbol *'Sb'* and has atomic number 51. Antimony, a hard and gray lustrous metalloid, is found in nature as the sulfide mineral, stibnite. Antimony trioxide exists in 4 valence states in which its trivalent state being most stable. Inorganic Antimony is more toxic than organic antimony. [2]

Arsenic

Arsenic is denoted by the symbol *'As'* and has atomic number 33. Arsenic is a toxic metalloid and found in different allotropic forms. There are three different metalloids of arsenic, each having different crystal structure. Arsenic is a natural component of the earth's crust and is widely distributed throughout the environment in the air, water and land. It is highly toxic in its inorganic form.[3]

Bismuth

Bismuth is denoted by the symbol '*Bi*' and has atomic number 83. Bismuth is a high-density, silvery, pink-tinged metal. Bismuth is brittle and due to its brittleness, it is usually mixed with other metals to make it useful. Its alloys with tin or cadmium have low melting points and are used in fire detectors, electric fuses, solders and extinguishers.[4]

Cadmium

Cadmium is denoted by *'Cd'* and has atomic number 48. It is soft, can be cut with a knife, malleable, ductile, bluish-white, divalent metal. Because of its unique physical, mechanical, and electrochemical properties, cadmium metal and other cadmium compounds such as cadmium sulphide, cadmium oxide and cadmium hydroxide are used in pigments, coatings, stabilizers, specialty alloys and electronic compounds, but it is mostly used in rechargeable nickel-cadmium batteries. Cadmium pigments are more stable than organic coloring agents at high temperatures and are not easily degradable by light.[5]

Cobalt

Cobalt is denoted by *'Co'* and has atomic number 27. Cobalt is a rare element of the earth's crust, which is essential to mammals in the form of cyanocobalamin.[6] Cobalt is a ferromagnetic metal. Cobalt is the active center of a group of coenzymes called cobalamins.[7]

Iron

Iron is denoted by the symbol *'Fe'* and has an atomic number 26. Iron plays an important role in the formation of different complexes with oxygen in hemoglobin and myoglobin (oxygen transporters). It is a very crucial component of different metalloproteins and plays a crucial role like oxygen sensing and transport, electron transfer and catalysis.[8]

Lead

Lead is denoted by the symbol *'Pb'* with the atomic number 204. Lead is bright and silvery when freshly cut, but rapidly fades in air and produces a dull luster. It is ductile, dense, very soft, and has poor electrical conductivity. [9,10,11]

Mercury

Mercury is denoted by the symbol '*Hg*' and has atomic number 80. Mercury is liquid at standard conditions of temperature and pressure. Mercury occurs in the form of elemental or metallic mercury, inorganic mercury and organic mercury.[12]

Tin

Tin is denoted by the symbol Sn, has an atomic number 50. Tin is a silvery metal that occurs in two stable oxidation states +2 and +4. Tin is used to preserve canned food and beverages. Common examples of organotin include agrochemicals, biocides, Polyvinyl chloride and some catalysts.[13]

Thallium

Thallium is denoted by Tl, has an atomic number 81. In its physical properties, it is a soft metal and resembles tin. It exhibits unipositive (Tl+1) and tripositive (Tl+3) valence states, of which the Tl+1 is more stable.1 The properties of Tl+1 resemble potassium and silver compounds, while Tl+3 resemble aluminum compounds.[14]

Thallium acetate and thallium sulphate are the chief salts of thallium having toxicological importance. Thallium salts being colorless, odorless and tasteless add to its potential to cause accidental ingestion, more so for homicidal intent.[15]

Sources of Heavy Metals

The Table 1 shown on page 47 gives a brief info on the most common sources of heavy metals and their usages.

Sl. No.	Name of the Metals	Source
1.	Aluminum	• Aerospace, Construction materials, Utensils manufacturer, Electrical Appliances, Packaging and automobiles. • Paints and Pigments. Fuel additives, propellants and explosives.
2.	Antimony	• Ores such as Valentinite (Sb203) and Stibnite (Sb2 S3) • Environment through waste incineration, metal processing mines and burning of coal.
3.	Arsenic	• Smelting industry (by-product of ores containing lead, gold, zinc, cobalt and nickel) • Pesticides • Paints and Dye industries
4.	Bismuth	• Sea Water (About 0.02 µg/litre Bi is present in Sea water). • Anthropogenic sources (copper, lead, silver and gold smelting, waste water and sewage sludge).
5.	Cadmium	• Environment, By-product of Zinc concentrates. • Batteries, Manures and Pesticides • Food such as liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed
6.	Cobalt	• Implants, Batteries (Lithium Cobalt Oxide), Coloring pigments and dyes. • Magnets
7.	Iron	• Supplementary Food and Drugs, Coloring pigments and dyes. • Toys and Sports Goods, and Cast Iron
8.	Lead	• Ores (Eg., Galena, Cerrussite and Anglesite • Batteries, metal products, Pipes and Solders and ammunition • Paints
9.	Mercury	• Fish and Shellfish (in the form of methylmercury which is highly toxic.a
10.	Thallium	• Glass, Photoelectric cells, Rodenticides and Fungicides
11.	Tin	• Canned Foods and Beverages, Tin industries, Mining, Soil (due to weathering process) • Biocides, Antifouling paints

Table 1 Sources of heavy metals

Human Exposure to Heavy Metals:

Sl. No.	Name of the Metals	Human Exposure
1.	Aluminum	• Environment Pollution, Inhalation and transdermal absorption through cosmetics containing aluminum. • Medicines (e.g., Antacids). • Workers in industries like refining, mining, smelting and those make tools by grinding and cutting using metal or compounds of aluminum. • Aluminum utensils for cooking purposes..
2.	Antimony	• Inhalation (exposure occurs during occupational activities while working with antimony compounds and inhaling antimony dust, or fumes)ds). • Ingestion (through contaminated vegetables) • Dermal exposure (occurs during working near antimony mines or antimony processing sites)
3.	Arsenic	• Smelting industry (by-product of ores containing lead, gold, zinc, cobalt and nickel) • Pesticides • Paints and Dye industries
4.	Bismuth	• Medicines • Cosmetics

Table 2 Source of heavy metal exposure (continued...)

Sl. No.	Name of the Metals	Human Exposure
5.	Cadminum	• Environment Pollution (contaminated food water) • Smoking • Occupational exposure (through inhalation of fine dust and fumes).
6.	Cobalt	• Environmental Pollution (Contaminated water, Air) • Prosthetics • Medicines (due to the overdose of vitamin B12 supplements). • Occupational exposure
7.	Iron	• Environmental Pollution (Contaminated water, Air). • Iron dust and fumes from welding, smelting, grinding • Medicines (excessive use of Iron supplements)
8.	Lead	• Environmental Pollution (Contaminated water, Air). • Occupational Exposure. • Paints, Ceramicwares, Cosmetics, Medicines, Pencils • Toys, Automobile Exhaust
9.	Mercury	• Environmental Pollution (Contaminated Food, water, Air). • Occupational exposure • Cosmetics
10.	Thallium	• Environmental Pollution (Contaminated Food as plants absorb thallium from thallium-treated soil, which further enters the food chain). • Inhalation of thallium oxides and salts • Absorption through skin.
11.	Tin	• Consumption of canned Food and beverages. • Inhalation (Landfills or industries dealing with the manufacturing of tin products).

Table 2: Source of heavy metal exposure

Pharmacokinetics of Heavy Metals

Aluminum

Absorption: About 0.1% to 0.6% of aluminum is absorbed through ingestion whereas absorption of less biovailable form such as aluminum hydroxide is as low as 0.1% and the rest is excreted in faeces.

After entering the bloodstream, it binds to various ligands and gets distributed in each organ system of the body with highest concentration in lungs and bones tissues.[18]

Distribution: In a healthy individual the total body burden of aluminum is approximately 30mg to 50mg. The level of aluminum in serum is 1 µg/l to 3µg/l. Half of the total body burden of aluminum is in bones, one fourth in lungs and rest in other tissues of the body. With the increase in age, the concentration of aluminum also increases in brain tissues and serum.[18]

Elimination: The unabsorbed aluminum gets eliminated from the body through excreta, whereas the absorbed aluminum gets eliminated through kidneys or urine. Extended exposure of Aluminum and its accumulation, the human body itself is not capable of eliminating aluminum and its compounds effectively from the body.[18]

Antimony

Absorption: Antimony compounds get absorbed through ingestion and inhalation. Gastrointestinal absorption being poor in man necessitates parenteral administration of pharmaceuticals of antimony2.

Distribution: Total body pool of antimony was estimated and observed that only 5 % of the ingested dose could be found in a patient who died of accidental antimony potassium tartrate ingestion, with high antimony concentrations found in the liver, gall bladder and gastrointestinal mucosa.[19]

Elimination: Reasand *et al*, 1980 demonstrated that about 80% to 90 % of the intramuscular dose of sodium stibogluconate is recovered in the urine within about 6 hour of administration. Kentner *et al,* 1995 estimated renal elimination half-life of about four days, upon occupational inhalation of antimony trioxide and stibine among[21] employees of a battery manufacturing plant.[20]

Arsenic

Absorption:

When ingested in dissolved form, inorganic arsenic is readily absorbed. About 80-90% of a single dose of arsenite As (III) or arsenate As(V) was absorbed from the gastrointestinal tract of humans and experimental animals.[21,22,23] A much lower degree of gastrointestinal absorption was reported for arsenic-contaminated soil[23], although the form of arsenic in the soil, as well as the type of soil, can be assumed to influence the degree of arsenic absorption. Also, arsenic compounds of low solubility (e.g., arsenic selenide)[24], arsenic trisulfide and lead arsenate[25] and gallium arsenide[26,27] are absorbed much less efficiently than is dissolved arsenic.

Distribution:

In the body, As (III) is mainly bound to SH groups. In particular, As (III) forms high-affinity bonds with vicinal thiols, as demonstrated with lipoic acid and DMSA.[28,29] A very stable complex appears to be formed between DMA and hemoglobin in the rat30. In vitro studies indicate the formation of mixed protein hemoglobin-GSH complex with As (III).[31]

Elimination

The major route of excretion of most arsenic compounds is via the urine. Following exposure to inorganic arsenic, the biological half-time is about 4 days. It is slightly shorter following exposure to As (V) than to As (III)32,33,21,34. In humans, about 78% of MMA and 75% of DMA were excreted in the urine within 4 days of ingestion of the dose34. Similar results were reported for mice in which the half-time of MMA and DMA was about 1 hr35. The 24-hr whole-body retention was about 2% of the dose.

With an average arsenic concentration in the skin of 0.18 mg/kg36 estimated that the daily loss of arsenic through desquamation was 0.1-0.2 µg in males with no known exposure to arsenic.

Bismuth

Absorption

About 0.2% of orally administered bismuth is absorbed systematically from the gastrointestinal tract.

Distribution

Bismuth accumulates in the kidney, bone (metaphysis), liver, spleen, heart and muscle. With a half-life of months to years, bismuth in bone is very slowly turned over.

Excretion

Bismuth is primarily excreted via the kidney as this organ contains the highest concentrations of bismuth.

Cadmium:

Absorption:

Cadmium has a long biological half-life from 17-30 years in man. After uptake from the lung or the gastrointestinal tract, cadmium is transported in blood plasma.

Distribution

Cadmium is widely distributed in the body, with the major portion of the body burden located in the liver and kidney.

Elimination

Most cadmium that is ingested or inhaled and transported to the gut via mucociliary clearance is excreted in the faeces.

Cobalt

Absorption

The cobalt enters into the body through food is absorbed at the small intestine, followed by absorption of metal into blood flow where it causes the binding with proteins and transport to various cells of the body resulting in accumulation in all organs mainly liver, pancreas, kidneys, heart and skeletal muscles[7].

Distribution

As cobalt is a major component of vitamin B12, it is found in most body tissues like bone, hair, lungs, muscle, lymph nodes, brain, pancreas, liver (largest amount), urinary bladder etc. reflecting the exposure from all sources and routes.

Elimination

Long term clearance is directly related to the solubility of cobalt compounds, e.g., higher (cobalt (II) oxide) the solubility faster the clearance from lungs than the less soluble ones (cobalt (III) oxide).

Iron

Absorption

Iron absorption is a complex process that occurs in the proximal small bowel and consists of a series of steps. These include binding of the iron molecule to the brush border, uptake of bound iron into the intestinal mucosal cell, intracellular handling of iron, transcellular transport and passage of the iron from the cell into the portal

circulation.

Distribution

Distribution of iron is very rapid. Entry of iron into tissues is an active process involving specific transferrin receptors and endocytosis.

Elimination

Excretion of iron after an overdose is insignificant as the body does not have any effective means of excreting it from the body8.

Lead

Absorption:

Lead is absorbed through mucous membranes in the mouth, nose, and eyes and through breaks in the skin. Tetra-ethyl lead passes through the skin37. Inorganic lead, found in food, paint and most lead-containing consumer products, are absorbed through inhalation and ingestion38.

Distribution:

The main body compartments that store lead are blood, soft tissues and bone; the half-life of lead is measured in weeks for blood, months for soft tissues and years for bone38.

Elimination:

Lead is excreted from the body very slowly, mainly through urine. Small amounts of lead are also eliminated through the faeces and very small amounts through hair, nails, and sweat.

Mercury

Absorption

Exposure to mercury occurs through ingestion, inhalation and occasionally by skin contacts.

Distribution

Non-occupational exposure occurs through air, food, drinking water and dental amalgams.

Elimination

Approximately 7-14 % of inhaled mercury vapour mercury is exhaled within a week after exposure. 80% excreted through faeces and urine.

Thallium

Absorption

Thallium oxides and salts are rapidly absorbed from mucous membranes of the respiratory tract, mouth and lungs as well as through skin40.

Distribution

After oral ingestion of a thallium salt, its peak blood level reaches within 2 hours and its occurrence in urine within 4 hours.

Elimination

The excretion of parenterally administered thallous ions continues for 3 months in urine and 35 days in faeces; however, the quantity of thallium decreases with time.[41]

Tin

Absorption

It is observed, with an increase in the dosage of tin in the body the gastrointestinal absorption decreases. Although there is very poor absorption of tin in the body, some compounds such as dibutyltin and trimethyltin were detected in post-mortem blood and liver suggesting their absorption in the body39.

Distribution

After absorption in the intestine, tin reaches various body parts via blood. Less than 17 mg of tin is found in the human body, and various experimental studies suggested the highest concentration of tin in kidneys and liver.

Elimination

Most of the ingested inorganic tin remains unabsorbed and is readily excreted in urine and faeces and a small amount in bile.

Sl. No.	Name of Metals	Matrix	Levels	
			Normal	Toxic
1	Aluminum	Blood Urine Serum	7 to 10 µg/l < 7µg/l 1 to 3 µg/l	> 60 µg/l 30 to 100 µg/l 50 to 100 µg/l
2	Antimony	Blood Urine Serum	0.7-2 µg/l 0.06-0.01 µg/l <0.066 µg/g	9mg/l 0.26-0.39 µg/l 0.088 µg/g
3	Arsenic	Blood Urine Serum	<1 µg/l <100 µg/l ≤1000 µg/l	>50 µg/l >5000 µg/day >1000 µg/l
4	Bismuth	Blood Urine Serum	<0.05µg/ml 0-20 mmol/l 1 to 3 µg/l	0.05-0.1µg/ml 400 mmol/l 50 to 100 µg/l
5	Cadmium	-	>1µg/l	>1µg/l
6	Cobalt	Blood Urine Serum	0.08 to 0.50 0.3 to 0.7 µg/L 60 µg/L	µg/L 5 µg/L 1- 5.1 µg/L >60 µg/L
7	Iron	Blood Urine	500-2000µg/l 65µg/g	>3500 µg/l >65 µg/g
8	Lead	Blood Urine 0.667µg/L	1.5µg/dl >25 µg/dl	>20µg/dl
9	Mercury	Blood-Plasma/Serum Urine < 10µg/L	1.5 - 2.0 µg/L < 20µg/L	50-200 µg/L
10	Thallium	Blood/Urine	<5 µg /mL	10-15mg/kg
11	Tin	Blood Urine	< 0.005 µg /mL 1 – 20 µg/L	> 0.009 µg/ml > 30 µg/ml

Table 3: Normal and toxic levels of heavy metal in biological samples

Diagnostic Investigation of Heavy Metals:

Aluminum

The diagnosis of aluminum toxicity depends upon the combination of both the clinical history of patient and the laboratory findings.

Antimony

The antimony concentration in blood is indicative of any recent exposure of it and is most useful in the diagnosis of acute antimony poisoning.

Arsenic

The urine test is the most reliable test for arsenic exposure. Tests on hair and fingernails can measure exposure to high levels of arsenic over the part 6 to 12 months.

Bismuth

Without a clear history of exposure of bismuth, it is very difficult to make diagnosis of bismuth toxicity.

Cadmium

In healthy, unexposed persons, β2-microglobulin levels average about 200μg/g creatinine. Excretion increases with age and cadmium exposure. In cadmium workers, urine levels greater than 300μg/g creatinine indicate possible early kidney disease.

Cobalt

The diagnosis relies upon the combination of both the clinical history of patient and the laboratory findings.

Iron

Testing for serum iron concentration is crucial for confirming iron toxicity. The serum iron concentration should be repeated after 4-6 hours after the initial determination. Abdominal radiographic examination can be useful to identify iron. Laboratory tests should include serum electrolytes, blood urea nitrogen (BUN), aniline and aspartate aminotransferases and bilirubin[8].

Lead

It has been suggested that all children should be screened for blood lead levels before the age of 1 year and if possible, at yearly intervals thereafter until they are 6 years old.

Mercury

Fecal metal tests helps determine if mercury eliminated is in normal range or not.

Thallium

Thallium is radio-opaque; therefore, an abdominal radiograph should be obtained especially in the cases of acute thallium poisoning by ingestion.

Tin

The physical examination is equally important; where forced vital capacity (FVC) posteroanterior chest roentgenogram, and forced expiratory volume per sec (FEV1) is performed.

G. Management of Heavy Metals Toxicity:

Aluminum

Hospital Management starts with complete and thorough examination of the patient which includes serum level of aluminum, hepatic function, whole blood test, renal function test and coagulation profile. In case of swelling and inflammation in lungs, blood, urine tests, ECG and X- rays are performed.

Antimony

Hospital management involves supportive and symptomatic measures as per the patient's conditions. Upon ingestion of an antimony compound, gastric lavage could be considered if presentation is within the first hour. The administration of 50 g activated charcoal within first hour of substantial ingestion could adsorb antimony.

Arsenic

In case of accidental arsenic ingestion, immediately 5 charcoal tablets should be given, and then 5 more every 15 minutes until reaching Health Care provider or emergency room of the nearest hospital.

Bismuth

Ingestion of single and small amount of bismuth are unlikely to cause systemic toxicity. Ingestion of acute overdose or large amount of bismuth should be evaluated in hospital.

Cadmium

When inhaled, take the person to fresh air, rest in a half upright position. If indicated provide artificial respiration and referred for medical attention.

Cobalt

Hospital Management starts with complete and thorough examination of the patient which includes serum level of cobalt, hepatic function, whole blood test, and renal function test and coagulation profile. In case of swelling and inflammation in lungs, blood, urine tests, ECG and X- rays are performed.

Iron

Intravenous access should be established and normal saline should be administered (0.9%) at an initial dose of 20 ml/kg followed by continuous infusion. Management includes thorough investigations such as serum iron levels, renal function test, electrolytes, complete hemogram, coagulation profile, liver function test and Arterial Blood Gas analysis of severely poisoned patients.

Lead

Garlic can be used for detoxification in cases of chronic lead poisoning. It also has prophylactic effect.

Mercury

In suspected mercury poisoning, remove mercury from body, intake of vitamin C foods, green leafy vegetables and cilantro should be increased. Cilantros is one of the best herbs to detox mercury.

Thallium

The decontamination should be done at the earliest by giving gastric lavage after the medical attendant wearing protective clothing.

Tin

Ingestion of a very small amount of tin or its compound is unlikely to cause any systemic toxicity, Intake of a large amounts of tin in any form should be evaluated in the hospital.

RESULTS AND CONCLUSION

Heavy metals show toxic effects in case of acute and chronic exposure in humans. Individuals working in industries dealing with heavy metals, mines, paints and other sources of toxins should be given special attention. Routine testing of blood, renal function test, urine albumin and other tests to monitor levels of heavy metals in the human body should be conducted to ensure early treatment. The use of canned food and beverages should be regulated. Any symptom, whether mild or severe, should not be neglected. Immediate medical help should be provided for treatment of exposed individuals. IJFMP

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REVIEW ARTICLE

Crocus Sativus: Comprehensive Pharmalogical Significance and Forensic Identification of Saffron in Illegal Trade

Tahir ul Gani Mir[1], Atif Khurshid Wani[2], Saurabh Shukla[3], Jaskaran Singh[4]

ABSTRACT

Saffron, a dried stigma derivative of *Crocus sativus*, is tremendously rich in phytochemicals like crocin and safranal. For a long time, the phytochemicals of saffron have remained obscure, and thereby its role in disease treatment remained subsidiary. Of late, researchers have gained momentum in studying its bioactivity because of its numerous beneficial health aspects. The pharmacological activities such as antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, and anti-atherosclerotic of crocin and safranal, have recently been a focus of many researchers. Considering market value and demand, saffron has become the center stage of adulteration and, subsequently, illegal trade. This has prompted researchers to develop advanced, cost-effective analytical tools for rapidly detecting possible adulteration common in illegal saffron trading. This review provides greater insight into methods and counter detection techniques in the adulteration of saffron worldwide. Moreover, this review gives a detailed account of the phytochemicals specific to saffron and their potential role in treatments of various diseases.

KEYWORDS | crocus sativa, saffron, phytochemistry, pharmacological activity

INTRODUCTION

Saffron known as red gold is one of the most expensive spices in the world. It is obtained from flowers (stigma) of the *Crocus sativa*.[1] Saffron is derived from the Arabic word, '*azaferan*' and blooms only in the autumn and is dormant in the summer. Crocus sativa belongs to the Asparagales family and is an angiosperm plant. The flower of Crocus is solitary and purple with six petals, three stamens, one style, and three red-orange stigmas. The Mediterranean Europe, and West Asia are the leading distributors of saffron. Iran supplies around 90% of total worldwide saffron output.[2] Saffron is highly expensive for its golden hue, flavor, and aromatic properties. It is recognized as a food spice and a powerful natural agent with a wide variety of health benefits. The ability of saffron and its key components to guard against natural and chemical toxins has increased the value of this spice. Since saffron is the most expensive spice and is in such high demand by the pharmaceutical industries, illicit trade and adulteration are rampant nowadays.[3,4]

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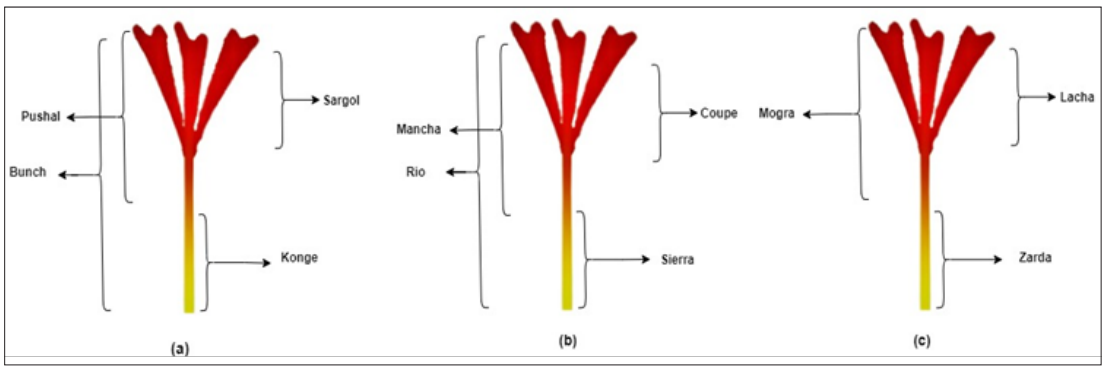


Figure 1: Grades of Saffron: a) Iranian Saffron b) Spanish Saffron c) Kashmiri Saffron. Source: Author self.

Grades of Saffron and ISO 3632

The quality and strength of saffron vary in terms of the amount of style attached to the red stigma. The age of the saffron is also an important factor. Since the color and flavor are concentrated in the red stigmas, the saffron is considered less efficient when more style is present. Saffron from Iran, Spain, and Kashmir are graded according to the proportions of red stigma and yellow styles present (Figure 1). The ISO 3632 certification assures consumers that the saffron they buy is genuine and safe to eat. Based on the following factors, Saffron is classified into grades I, II, and III by ISO 3632: 1. Moisture level (dried) 2. Crocin (color) 3. Picrocrocin (bitterness) 4. Safranal (aroma). The quantities of these key compounds are used to determine the saffron quality (Table 1). A higher concentration of these compounds implies greater quality of saffron. The highest quality saffron is graded in Group I by ISO 3632, which means direct readings of absorbance of E1% aqueous solution of saffron at 440 nm, 330 nm, and 257 nm for crocin, safranal, and picrocrocin are greater than 190, 20, and 70, respectively.[5]

Characteristic	Category I	Category II	Category III	Test Method
Crocin	>190	150-190	110-150	ISO3632-2, Clause 13
Safranal	20-50	20-50	20-50	ISO3632-2, Clause 13
Picrocrocin	>70	55-70	40-55	ISO3632-2, Clause 13
Moisture & Volatile matter	10	10-12	10-12	ISO3632-2, Clause 13

Table 1 ISO classification for various grades of saffron

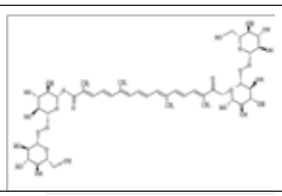
Phytochemistry of Saffron

The primary composition of saffron contains 14 to 16% water, 11 to 13% nitrogenous matter, 12–15% sugars, 41 to 44% soluble extract, 0.6 to 0.9% volatile oil, 4 to 5% fiber, and 4-6% overall ash. Two essential vitamins are found in Saffron: riboflavin and thiamin and a small amount of β-carotene. Saffron contains many volatile and non-volatile metabolites. Volatile elements constitute terpene alcohols, terpenes, and their esters. Non-volatile compounds of saffron include Crocin, picrocrocin, crocetin, and safranal (Figure 2).[6]

The orange-yellow color imparted to the saffron is due to the presence of α-crocin. The chemical nature of crocin is trans-crocetin di-β-D gentiobiosyl with an IUPAC name of 8,8

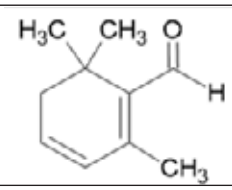
Crocin $C_{44}H_{64}O_{24}$ **Ref % 52**

Crocin is a carotenoid chemical compound primarily responsible for the characteristic colour of saffron



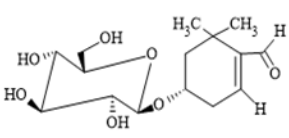
Safranal $C_{10}H_{14}O$ **Ref % 6**

Safranal is a chemical compound isolated from saffron responsible for the saffron aroma



Picrocrocin $C_{44}H_{64}O_{24}$ **Ref % 53**

Picrocrocin is responsible for the characteristic bitter taste of saffron. it is a monoterpene glycoside precursor of safranal



Crocetin $C_{20}H_{24}O_4$ **Ref % 52**

Crocetin is a natural apocarotenoid dicarboxylic acid present in the flower of crocus. The chemical composition of the crocetin forms the centre of crocin, the colouring agent for saffron.

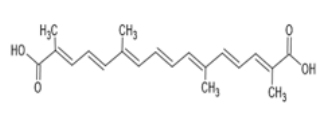


Figure 2: Chemical Structure, formula, properties of Saffron metabolites Source: Author self.

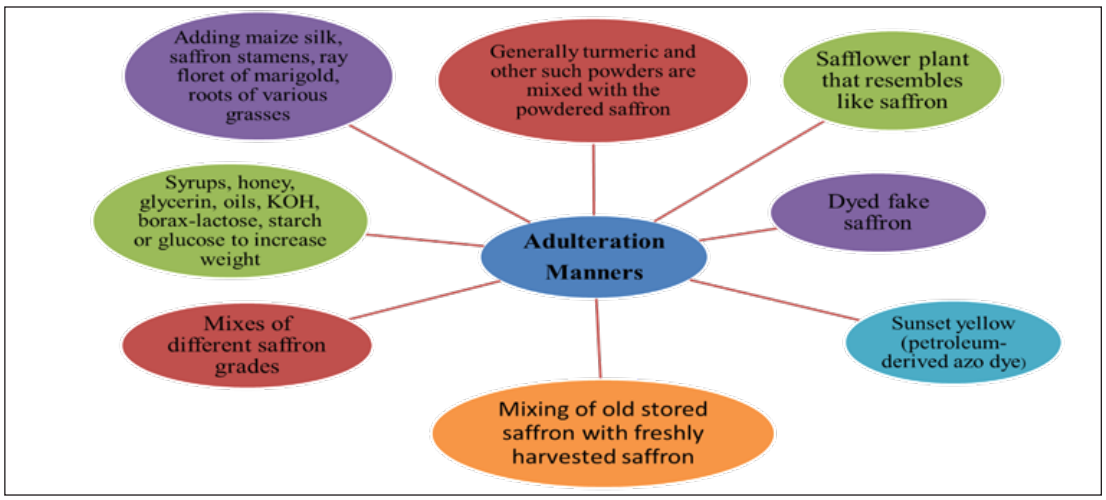


Figure 3: Various ways of Adulterating Saffron Source: Author self.

diapo-8,8 cartenoic acid. This is a testimony of the fact that crocin giving color to saffron is an ester of carotenoid crocetin (Digentiobiose)[7]. Crocins also belong to the hydrophilic series of carotenoids, either monoglycosyl polyene or diglycosyl polyene ester of the crocetin. Crocetin is a hydrophobic compound that is polyene conjugated dicarboxylic acid and also soluble in oil. The esterification of crocetin with two gentibioses that are water-soluble and yields a product that is water-soluble.[8] The carotenoid pigment, i.e., alpha crocin, makes 10 % mass of the dry saffron. Thus, the presence of esterified gentibioses enables crocin to be an ideal coloring agent in various foods and dishes. Crocin is a water-soluble carotenoid and is responsible for the characteristic golden-yellow color of saffron. 'Crocin' is the most significant carotenoid glycoside, giving distinctive color to saffron. In addition to these, it is a crocetin-digentiobiose ester (C20H24O4) which can hydrolyze with emulsion (β-glucosidase) with a beta-shaped glycosidic bond.

The pungent flavor of saffron is attributed to the presence of a bitter glucoside known as picrocrocin. The chemical formula of picrocrocin is C16H26O7 with a systematic name of 4-(β-D-glucopyranosyloxy)-2,6,6 trimethyl-cyclohexene carbaldehyde. Picrocrocin is a blend of carbohydrate and safranal (2,6,6 trimethylcyclohexaene carbaldehyde).[9] It is known to have pesticidal and insecticidal properties comprising 4% of saffron when dried. Picrocrocin is docked compound of zeaxanthin with a glycoside nature of aldehydic terpene safranal formed with oxidative cleavage. Post harvesting, when saffron is dried, the enzymatic action along with controlled heat breaks picrocrocin into glucose and safranal. Picrocrocin was discovered by Kajser and it is a glycoside that cracks into a glucose molecule and 4-hydroxy-b-cyclocitral (aglycon) because of acids and alkali. The aglycon loses a water molecule and becomes the safranal compound.

Safranal, a less bitter molecule, is a volatile oil that gives an aroma to the saffron.[10,11] The Safranal metabolite of saffron is a volatile oil and is mainly meant for the characteristic aroma of the spice. Safranal comprises up to 70% volatile fraction of saffron. The fresh stigma is not having any smell. After harvest, saffron undergoes processing and drying. The combined enzymatic action and the heat split picrocrocin to obtain D-glucose along with a free molecule of safranal in yield.[6] It has a distinctive fragrance that gives a characteristic aroma to the spice. Safranal is a cyclic terpenic aldehyde with brute formula C10H14O (m.w.= 150; e.p.70 OC/1 mm), IUPAC (2,6,6-trimethyl 1,3-cyclohexadiene-1-carboxaldehyde). The name derives from the first researchers Kuhn and Winterstein to obtain it through the hydrolyzing of picrocrocin.

Crocetin is hydrophobic in nature and therefore is oil soluble. It is a conjugated polyene dicarboxylic acid that is meant for the unique pleasant smell of the spice. Crocetin belongs to the broad natural dye family known as carotenoids, but the

provitamin function is lacking in this compound. Hydrocarbons with a general formulation of C40H56 or oxygenated derivatives are the main elements of this class.

Pharmaceutical significance of saffron derived phytochemicals

For the last couple of decades, phytochemicals of plant origin have been regarded as potential pharmaceutical agents with minimum side effects as compared to chemically formulated drugs.[12] Crocus sativus, as a popular spice agent, has also been found essential in treatment of various diseases including asthma[13], depression[14], menstruation disorders[15], cardiovascular disease[16], digestive ailments[17], cancer[18], insomnia[19], and many others. The therapeutic properties associated with saffron are attributed to its phytochemicals like crocetin, safranal, and crocins.[20] Even though the biomedical studies of the saffron-derived compounds have been experimented long ago, however, those early reports were more observational and the molecular relevance has remained in doubt for a long period.[21] One of the studies has shown saffron as a potent gastrointestinal modifier to prevent gastrointestinal atonia[22] and a significant therapeutic agent on female genitals.[23] Safranal is useful in treating respiratory ailments like chronic bronchitis. It acts on the alveoli through vagal nerves, thus sedating coughing.[24] Crocin, an analgesic, has been recommended for painful dysmenorrhea, thereby helping in reducing uterine contractions.[25] On the other hand, picrocrocin has been reported with tranquilizing properties, which induces a sedative effect on lumbar and spasm pains.[26]

Several in vitro and in vivo studies have been carried out to explore saffron as an excellent anti-cancer agent. Bathaie *et al.,* studied the importance of saffron extract (SE) in rats after being subjected to MNNG (1-methyl-3-nitro-1-nitrosoguanidine) gastric cancer induction. The administration of SE showed cancer progression inhibition and more than 15% of rats were found to be normal after treating them with higher SE doses.[27] Saffron has also been reported to cause the reduction of DEN (diethylnitrosamine) induced increase of hepatic dyschromatic nodules while carrying out studies on rats. It is known to counteract DEN-induced stress in rats as analyzed by catalase, superoxide mutase, and myeloperoxidase levels.[28]

The phosphorylation of acetyl-CoA carboxylase AMPK/ACC) and mitogenic activated protein kinases in saffron greatly enhance the peripheral insulin sensitivity; however, they could not enhance PI3-kinase/Akt. Crocetin therapies in diabetic rats have revealed an increase in insulin sensitivity by lowering adiponectin's protein and messenger RNA (mRNA) levels, tumor necrosis factor (TNF), and leptin in white adipocytes.[29] Crocetin also prevents insulin resistance induced by dexamethasone by reducing free fatty acids and triglycerides in plasma and downregulating TNF-α regulators.[30] Crocetin could also inhibit the expression of adiponectin contributing to improved sensitivity for insulin.[31] Examination of insulin susceptibility in HOMA-IR diabetic animals has shown that crocin significantly decreases glycosylated hemoglobin level and enhances insulin sensitivity by preventing oxidative stress and improving plasma lipid profile.[32] Safranal functions as a potent inducer of Insulin signal transduction (IST) by inhibiting protein tyrosine phosphatase 1B (PTP1B).[33] The crocin can improve insulin sensitivity by suppressing TNF-α and interleukin-1β (IL-1β) levels in plasma and the TNF-α and interferon-γ (IFN-γ) levels in pancreatic tissues.[34] It has been found that saffron can enhance the plasma glycemic profile by upregulation of the GLUT4/AMPK molecular pathway.[35] Thus, saffron's active metabolites display their hypoglycemic effects by enhancing IST and inducing insulin sensitivity.

Methods of Saffron adulteration and its detection:

Saffron is very costly and there is a great demand. Due to less production and high demand for this spice, it is highly prone to illicit trade and adulteration.[36] Adulteration of saffron is mainly done by adding safflower, maize silk, marigold floret, horsehair, grassroots, stamens of saffron, red dried silk fiber, etc. These kinds of adulterants resemble saffron in color and texture. Sugar, potassium hydroxide, borax lactose, glycerin, fats, glucose, starch, etc., are mainly added to increase weight. Adulteration is not only done by adding a foreign substance to the saffron but also mixing different grades is one of the common methods of adulteration. The powdered form of saffron is more likely to be adulterated. Usually, turmeric and other powders resembling saffron are blended and sold in the markets with powdered saffron (Figure 2).[37]

The ongoing adulteration of saffron has badly affected the production and economy of saffron cultivators. Various instrumentation methods are available to detect adulterations in saffron. Lozano *et al*., (1999) carried HPLC analysis on Mancha, Rio, and Sierra grades of Iranian saffron, and ten different metabolites were found in the saffron extract. Each chromatograph was depicted at three wavelengths (250, 310, 440), and it was concluded that Mancha showed the highest concentration for all sec-metabolites followed by Rio and Siera.[38] Sujata *et al.,* (1992) used TLC, HPLC, and Gas chromatography to check the authenticity of saffron. Crocin, picrocrocin, and crocetin were resolved using TLC and HPLC analysis. However, safranal was evaluated using Gas chromatography. In TLC analysis n-butanol, Acetic acid, and water (4:1:1) were used as mobile phase and Rf (Retention factor) was calculated as 0.63, 032, and 0.98 for crocin, picrocrocin, and crocetin, respectively. In HPLC analysis, the extract of pure saffron in 80% ethanol was passed through a cartridge eluted with 100% acetonitrile and Rt was obtained. It was found that Rt of crocin, picrocrocin, and crocetin was 13.5, 14-18, and 18 respectively. Gas chromatography was used to resolve safranal. Nitrogen at a flow rate of 30ml/min was used and it was found that safranal could be resolved into a sharp peak at a rate of 3.6 minutes.[39] Semiond *et al.,* (1996) studied the isotopic analysis and identification of saffron using Supercritical fluid extraction (SFE). Safranal metabolites obtained from saffron of different origins were analyzed and a difference was found between synthetic and natural safranal. Moreover, it was found that SFE allowed various volatile compounds to be selectively extracted from saffron under optical conditions. It is a quicker and safer way to extract volatile saffron compounds.[40] G. L. Alonso *et al.,* (1998) used the Thermal-desorption Gas Chromatography-Mass Spectrometry technique for analyzing the authenticity of Spanish saffron and found that major components of the aromatic composition are safranal. The fingerprint of the chromatograph was obtained for both genuine and fake saffron, which were different. In adulterated saffron, a chromatograph peak appeared, which was not produced by pure saffron fingerprint. Such fingerprint was similar to compound 2,6,6-trimethyl cyclohexane-carboxaldehyde, and it could be concluded that if beta-cyclocitral peak appears in chromatograph fingerprint, the saffron was adulterated.[41] Zalacian *et al.,* (2005) researched a testing tool for various colorant identification. This method was based on removing crocins from the sample by precipitation before the adsorption of colorants on a Polyamide Solid-phase Extraction (SPE) cartridge. Elution with methanol-ammonia solution was performed and after washing identification process was carried out with a spectrometer.[42] Anna Torelli *et al.,* (2004) used a system based on Sequence-Characterized Amplified Regions (SCARs) to detect adulteration with specific agents using various food products containing saffron. The use of SCAR markers proved to be an effective, quick, and low-cost screening method for authenticating saffron-containing food products.[43] The HPLC process for identifying, detecting and quantifying saffron metabolites was studied by Haghighi *et al.,* (2007) to detect adulterants like colored styles of Crocus sativa, safflower red beet, etc. The chromatograms were obtained at desired wavelengths, and then applying ANOVA to the chromatographic data obtained, the presence of adulterants in saffron were detected significantly.[44] In 2007, Gonzalo *et al.,* published a method for HPLC-DAD to simultaneously classify crocins and picrocrocin in aqueous saffron spice extracts. This method was able not only to determine crocins and picrocrocin but also to detect adulteration of saffron with water-soluble colorants.[45] Application of Proton Transfer Reaction-Mass Spectrometry (PTR-MS) in saffron quality control was studied by Nikolaos *et al* (2014). A minute quantity sample was used to capture volatile fingerprints. IPTR-MS/chemometry was examined to detect fresh addition of low-quality saffron to fine quality[46]. Kobra Heidarbeigi *et al.,* (2015) developed an electronic nose system combined with principal component analysis and an artificial neural network to detect adulteration in saffron. This was the first approach towards the detection of adulteration using sensors. The electronic nose could detect complex odors via an array of sensors. The specimen odors are drawn into the electronic nose chamber and then passed through the sensor array process, resulting in a reversible physical change or chemical alteration in the sensing material associated with electrical

properties like conductivity.[47] Ordoudi *et al.,* (2014) researched a multi-step method for detecting saffron fraud. HPLC, UV-Vis, FT-IR, and NMR were used to examine the saffron fraud. UV-Vis and FT-IR data were used to reveal the artificial colors used. NMR was used to identify others. The identification of authentic saffron by using a molecular genetic approach was investigated by Ma XQ *et al.* (2000). After analysis, they found that the nucleotide sequence of all the samples was distinct and different and served as a marker for authentic identification of pure saffron from counterfeit saffron.[48] N Javanmardi (2011) studied the petals of the sunflower plant that are mostly used as an adulterant of saffron. In their study, they employed the application DNA analysis (RAPD) to detect adulteration in saffron. DNA from safflower petals and saffron was extracted and after analysis, it was found two monomorphic bands (500 and 700 bp) present in safflower were found to be absent in saffron.[49] Luana magi *et al.,* (2011) studied the spectroscopic method to determine the safranal content in the saffron. To check the quality of saffron spice, this quantitative safranal study was based on non-polar solvent extraction followed by spectrometric analysis. Ultrasound-assisted safranal extraction was performed and UV-Vis spectrophotometric analysis showed satisfactory results in the performance of repeatability, linearity, and recovery.[50] Karimi *et al.,* (2016) used FTIR spectroscopy with pattern recognition to differentiate between true saffron and those samples adulterated with various food adulterants. The FTIR spectrum was obtained for all the samples and it was found that three region bands corresponding 1800-1830, 2600-2900 and 3700-3850 were responsible for recognizing true saffron from adulterated ones.[4] Soffritti G *et al.,* (2016) in their study, isolated DNA from the saffron sample, adulterated sample, and possible adulterants, then from isolated DNA new markers were developed to recognize genuine saffron from fake or adulterated saffron. This method could recognize saffron from a mixture with a low percentage of adulterants.[51]

CONCLUSION

Plants are tremendously rich in phytochemicals with a significant role in biological functions. Saffron has a substantial amount of crocin and safranal as phytochemical compounds that can treat diseases like diabetes, inflammation, neuro degenerative disorders besides helping in the fight against human pathogens. Although the bioactivity of saffron and its compounds is plenty, the mode of action is confined to *in-vitro* studies. As a result, the molecular mechanism of its action remains obscure and needs further study with regard to its therapeutic potential. Due to high demand and price, saffron is prone to adulteration. Lack of quality management is a threat to the saffron industry since a significant portion of the market share of saffron is exported through the selling of counterfeit saffron, necessitating the creation of a system for detecting adulterants on the spot. IJFMP

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REVIEW ARTICLE

Role of Nanotechnology in Techniques in Fingerprints Enhancement

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ABSTRACT

Fingerprints have been utilized in criminal and judicial investigations for establishing the identity of the accused/suspects for a long time. Fingerprints are an effective tool for determining who committed a crime. Nonetheless, easily detectable or visible marks are infrequently found at a crime scene; most of the time, they are latent, requiring the use of special methods to identify them. Fingerprint comparison should be cost effective and time bound to quickly obtain results with quality assurance. In this case, nanotechnology and its continued development have resulted in a range of applications in forensic science. Nano particles have been proposed as a solution for fingerprint detection and enhancement due to their versatility and smaller particle size. Because nanoparticles do not alter the composition of evidence, they can easily aid in the detection of high-resolution prints, and these evidences, unlike conventional techniques, can be re-used for any other chemical treatment. In most legal science research facilities, traditional methods such as black powder, fluorescent powder, and white powders are used. This review paper summarizes the various types of nanoparticle techniques that can be used for fingerprint identification and comparison, such as Camphor-based Nano carbon, Acetylated cashew gum-based silver nanoparticles and so on.

KEYWORDS | nanoparticles, fingerprint analysis, conventional techniques

INTRODUCTION

Fingerprints have been for ages the most important evidence collected from a crime scene. They are extremely useful in connecting the three dots of every crime: victim, author or individual, and object or location.[1] As LPs are constituents of natural secretions secreted from friction ridges and contaminants present on a surface, crime scene investigators regularly deal with the development and extraction of latent fingerprints from a crime scene.

Powder methods, silver nitrate methods, and iodine fuming are the general methods used in the enhancement of latent fingerprints. The method of choice is determined not only by the nature of the fingerprint but also by the substrate or supporting matrix on which the print is present.[22] The powder technique is the most widely used strategy in fingerprint development. Its mechanism is based on the sweat and oil components that have settled in the print's ridges. Powder is deposited in the ridges, and the print appears as a result of pressure and electrostatic forces between the powder and the oily components.[18] These methods, however, have less sensitivity, toxicity and, above all, the low contrast. Thus, nanoparticles are used to resolve this problem.[10]

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Ultrafine or minute particles between 1 to 100 nano meters in dimensions are defined as nanoparticles. These particles constitute nanotechnological components. Due to their smaller size, nanoparticles can be used in different industrial applications. In forensic science, their vital role and impact can be seen and this area is known as nano-forensics and is the most recent progress in forensic science. One of the major roles is seen in the development of fingerprints as they are very helpful in developing impressions of fingerprints regardless of their substrate.[26] Due to their smaller particles, they are incorporated into the ridge spacing of prints, and these particles are also non-reactive which is very useful in developing old-age finger marks. In recent years forensic researchers have developed different methods including nanoparticles, which have advantages such as high sensitivity, low toxicity, and high-contrast images, some of which are also cost-efficient.[5] As nanotechnology improves over time, new instruments have also been developed to keep pace with the progress. In the past years, nanotechnology has made a lot of advancements and these advancements need to be brought to notice so that forensic experts can use it to get results with more efficiency and accuracy. This review paper establishes a summary of different nanotechnology methods to develop and enhance fingerprints.

METHODS

As nanotechnology is still evolving, several techniques have been implemented to detect fingerprints. Each technique has distinct and significant advantages for detecting fingerprints, which are discussed below:

Acetylated cashew gum based silver nanoparticles

Cashew-gum is a gum secreted from the Anacardium occidentale L. tree, which is found in north eastern Brazil. This polymer provides steric hindrance to nanoparticles and occupies the surrounding surface due to charged particles that provide electrostatic repulsion. Cashew-gum separate is additionally blended in with silver nanoparticles to diminish the harmful and destructive nature of silver nanoparticles and further increasing the development power. Cashew gum diminishes the corrosive nature of AgNP (Silver Nanoparticles) as it synthetically comprises of β-D-galactose (72%), α-D-glucose (14%), arabinose (4.6%), rhamnose (3.2%), and glucuronic corrosive (4.7%).

A suspension of silver and cashew-gum was developed for the production of latent fingerprints, for which acylated cashew-gum was distilled and synthesized. It was further separated by different amounts (0.5, 1.0, 5.0 mg/mL) and blended separately for approximately 30 minutes with 1 mM AgNo3 solution. The solutions at low temperature were then mixed drop wise with a freshly prepared borohydride solution (NaBH4), a molar ratio of 1:10 silver. These were passed onto a falcon tube and left for 24 hours on the formulation of these solutions. They were centrifuged for 15 minutes at 3,600 R.P.M. Hence, forming acylated cashew gum silver nanoparticle suspension has been further analyzed with Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM).

For the enhancement of latent fingerprints from the surface of the alkaline paper, it is submerged in the suspension of varying focuses (0.5. 1.0, 5.0 mg/mL) trailed by withdrawal and prompt perceptions. The withdrawal and submersion were permitted to be saved for 3 distinctive time spans (1hr, 2hr, 24hr). This technique developed yellow color prints, therefore increasing the contrast and ridges were more defined and clearer as compared to traditional methods.

Hence, this technique is quick, easy, low-cost, and uses biodegradable chemicals, Due to its fast functioning and non-toxic nature it can also be used at crime scenes making fingerprint production fast and secure.[1,25]

Camphor bases enhancement

Impure camphor tablets ($C_{10}H_{16}O$) were used in this technique, which were burned into carbon soot and collected in a petri dish. Carbon sooth can be differentiated on the basis amount of carbon and size of the particles.

Camphor carbon powder was synthesized and used on absorbent and non-absorbent surfaces such as plastic files, book covers, paper, coffee cups, and so on. The prints were said to be provided by rubbing the donor's finger on the forehead in order for the sweat to collect. The powder dusting method was used to produce fingerprints

on different surfaces, and excess powder was extracted with the aid of a fiberglass brush. For the evaluation of developed fingerprints automated fingerprint identification system was used and for the evaluation of carbon soot particles SEM and EDX was used.

The outcomes were uncovered by AFIS (Automated fingerprint identification system) in view of different surfaces, for example, 27 minutiae were formed on metal and plastic surfaces. Further in the identical conditions the carbon soot showed more enhanced images of minutiae by showing 33 on plastic file surfaces and 36 on metal surface.

This method untangled the fact that the camphor-based powder produced better results than the conventional black powder. This powder is minimal expense and harmless to the ecosystem, and it tends to be utilized at a crime location. This method revealed characteristics of carbon soot such as water solubility, cytotoxicity, and biocompatibility.[2,15]

Red emitting CaTiO3: Pr3+ nanophosphors

In this technique Red emitting CaTiO3: Pr3+ nanoparticles with persistence timing of 20 minutes have been synthesized so as to produce or develop latent fingerprints. CaTiO3: Pr3+ was first synthesized further used in development of latent fingerprints through facile dusting powder method. These long after glow nanophosphors luminescence particles are great source of developing the prints as they have high quantum efficiency. Due to the purkinje effect and scotopic vision the human eyes are less sensitive to red color. Hence, making phosphorus strongly desirable in CaTIO3 compound. This compound reacts with the sweat present in the minutiae and provides high contrast red color latent fingerprints.

For the synthesis of CaTiO3, the sol-method was used, and Ca (NO3)2Pr(NO3)3.6H2O was homogeneously dissolved in iso-propyl alcohol for this experiment. The solution was vigorously stirred for 15 minutes before being combined with Titanium Tetra-iso-propoxide (Ti (OC3H7)4). A 1:10:4 ratio of Titanium isopropoxide: IPA: water was used to make the suspension. As a result of the exothermic reaction, a gel-like structure was formed, which was then placed on a hot plate to form powder. An additional annealing step is performed at temperatures ranging from 6000 to 10000 degrees Celsius.

SEM (Scanning electron microscope) was utilized to examine the surface geography, and surface highlights of CaTiO3 incorporated by sol-strategy. Since ordinary nano phosphor blend techniques produce huge molecule sizes going from 0.2 to 2 nm, sweat pores are filled and finger mark affectability is decreased. Tiny particle sizes formed finger marks with level-3 characteristics such as sweat pores and minutiae, according to Scanning electron microscope findings.

The test was also performed on fingerprints from a 28-year-old man who was asked to give fingerprints by rubbing his finger on his forehead and pressing it on a required substrate. CaTio3: Pr3+ was finely ground in a motor-pestle after being annealed at various temperatures. The excess powder was blown off after it was dusted over the finger marks with a soft squirrel brush. The finger marks were then excited for about 15 minutes in a UV chamber. The findings revealed that when the substrate is auto-reflective, the level-2 and level-3 characteristics of finger marks are not apparent. Otherwise, the finger marks were more noticeable and had a higher contrast, exposing the ridges, minutiae, and sweat pores.

The benefit of this approach is that by exciting the prints in a UV-Chamber, the finger marks can be seen at any time depending on the requirement. Phosphorus can also be preserved for longer periods of time due to its organic origin. Since they provide high contrast images with specified characteristics, these long after glow nanophosphors are the best alternative to conventional powder methods. 8,12,

Greener synthesis of Copper oxide nanoparticles

Copper oxide nanoparticles (CuO) is highly specific metal oxide that possess differment promoting features such as electrochemical activity, high specific area, redox potential and most importantly stability in the solutions. But, due to its corrosive nature it needed to be synthesized by sol method with an organic compound. So, in this experiment Green tea extract is used to coat the Cuo nanoparticles so, that it could form non-corrosive nature and eco-friendly.

In the initial phase Copper sulfate (CuSO4.5H2O) was used to form CuO without

purification and a green tea from the market. For the preparation of green tea. 1 gm of it is mixed in distilled water (50 mL) in an Erlenmeyer flask. A yellow color solution was obtained after 20 min of stirring at 700c to 800c. The extracted solution is then stored at 40c in a refrigerator. In an ultrasonic bath 40 ML of CuSO4.5H2O is mixed with 20 ml green tea extract. The greenish-yellow color of the extract gets converted to brownish-black. Integrated CuO arrangement was centrifuged at 10,000 rpm and the supernatant was eliminated. The brownish-black crystals obtained from centrifugation were removed from the flask. Further, these crystals were crushed and converted to powder form.

The latent prints were established on various non-permeable surfaces like glass, white paper, margarine paper, and steel utilizing by powder dusting strategy. Green tea goes about as a balancing out, covering specialist and a decreasing specialist for CuO. Hence, for the characterization FE-SEM (Field Emission Scanning Electron Microscope) was used and it revealed the magnitude of nanopartilces from 500 to 900 nm and spherical in shape. The results were easily visible by naked eyes and the black color given by the crystals was defining the ridges, minutiae, and sweat pores.

This method for development of latent fingerprints through CuO and green teas is eco-friendly, cost effective and majorily non- corrosive making it to use at crime scene. [4,11,17]

Tetraethoxysilane (TEOS) and Phenyltriethoxysilane (PTEOS) with Silica gel

In a rotator tube, 30 ml ethanol, 5 ml deionized water, 2.5 ml each of tetraethoxysilane (TEOS) and phenyltriethoxysilane (PTEOS), and 2.5 ml each of tetraethoxysilane (TEOS) and 2.5 ml phenyltriethoxysilane (PTEOS) were joined. 2 mL ammonium hydroxide arrangement was applied to this, and the arrangement was turned for the time being. The suspension was centrifuged during this period (3 min at 3000 rpm). The main cycle included centrifugation and extraction of the fluid/fluid stage from water to dichloromethane, joined by dissipation of the natural stage to dryness. Subsequently grouping of wash methodology and centrifugation, the item was disengaged in the second workup measure.

The connected color (25 mg) was broken down in the ethanol before the salinization reagents were added for the different color doped particles. Prior to applying the salinization reagents, 25 mg of titanium dioxide was added to the rotator tube. A 1:100 weakening of the provided carbon dark arrangement in water was applied to the antecedent answer for carbon dark particles. Particulate magnetite was set up as indicated by distributed techniques for TEOS: PTEOS-covered attractive particles, and 5 ml of the suspension in water was applied to the antecedent arrangement again for 5 ml of TEOS: PTEOS-covered attractive particles.

New prints (roughly 20 minutes before tidying), just as matured prints (different conditions itemized inside), were examined. A 21-year-old lady and a 33-year-old man both Caucasians stored their fingerprints on unused non-permeable glass magnifying lens slides (VWR Int, Leicester, UK), which were utilized precisely as taught. The prints were applied in one of two different ways: the fingerprints were scoured on brow prior to pushing on a superficial level, or the fingers connected with the surfaces after the latex glove was eliminated. A falling pipette was used to apply a small amount of suspension to the print (500 ml of 10% (w/v) in 97:3 (v/v) water/ethanol). The excess suspension was gently washed away after 2–3 minutes with surplus water.

The print was then permitted to dry normally. The slide could likewise be lowered in the suspension for 5–10 minutes. By gravity, the abundance engineer was taken out, and the surface had the option to air dry as in the past. As tidying specialists, various sieving divisions were utilized. The fluorescent sifter sizes of 45–63 mm and toned particles and 63–90 mm for sub-atom embedded particles were the most straightforward to use and gave the best portrayal prints. Instances of prints made on glass slides with the fluorescent color rhodamine.[6] The fluorescent and color doped particles would be advised to definitions when all is said in done, however the implanted particles actually furnished prints with great definitions. On a tempered steel sink top and a research center benchtop, great outcomes were acquired, and the idea was practically identical to that seen with fluorescent modern powders and enhanced were visible with aluminum powder.5,23,28

Fluorescent Starch-based Carbon Nanoparticles

Malic corrosive and ammonium oxalate were utilized as crude materials in a one-pot pyrolysis course to make N co-doped carbon nanoparticles. The carbon-nanoparticles of quantity wt1% were joined with characteristic starch to make a powder that fluorescent in dazzling blue in the dry strong state. These materials were joined with the uncommon benefits of the primary powder part's preeminent flowability and CNPs' remarkable photoluminescence.

The usage of nano-carbogenic powder as a novel UV fluorescent imprint for totally making whole fingerprints was the point of convergence of this research.

Conventional staining materials like iodine fume, industrially 502 cyanoacrylate stick fume, and TiO2 powder was utilized as controls to check the viability of fluorescent starch powder as fluorescence marks for the creation of inert fingerprints on the glass. Iodine fume, financially accessible 502 cyanoacrylate stick fume and TiO2 were discovered to be inadequate in creating dormant fingerprints on the glass side. The erosion edges of the fingerprints were hard to recognize from the foundation. fluorescent starch can be utilized as a successful powder for the advancement of inert fingerprints, with sharp edges, better goal, and less foundation obstruction, because of their improved solid blue iridescence. To analyze the affectability of the over four strategies, a progression of identifications on different substrates were performed. Iodine-fume, economically 502 cyanoacrylate stick fume, and Titanium oxide powder both have lower affectability than fluorescent starch powder.

The CNP-based nanocomposites had truly stable compound properties and tunable photoluminescent results, and were effectively utilized as a novel fluorescent name for the creation of idle fingerprints on different substrates, demonstrating moderately all around created qualities for finger edge data and great differentiation for improved location. The investigation presents a novel technique for producing whole inactive fingerprints utilizing CNPs in the legal sciences.[6,3]

Silicon Oxide Nanoparticles

Silicon Oxide Nanoparticles are one of a couple of nanoparticles with the entirety of the attributes for inert finger-mark location and improvement. The reversed micro-emulsion method can be used to make SiO2 NPs with a uniform size distribution. During the combination of SiO2 NPs, profoundly brilliant color atoms can likewise be typified in the center. It has been demonstrated in the literature that SiO2-based NPs can be used to detect latent finger marks.

In this method Triton X-100 (TX-100), 1-hexanol, ammonium hydroxide (30%), tetraethyl orthosilicate (TEOS), tris(2,20-bipyridyl) dichlororuthe- nium(II) hexahydrate (RuBpy) and sodium chloride (NaCl) were used . With the exception of the NP precipitation, silver oxide nanoparticles were synthesized using the reversed micro-emulsion technique stated by Moret et al.19. Here's a quick rundown of the synthetic technique. In a round bottom flask, 3.54 mL TX-100, 15 mL cyclohexane, and 3.6 mL 1-hexanol were inserted, followed by 960 mL RuBpy (16.6 mM), 200 mL TEOS, and 120 mL ammonium hydroxide (30 percent). For surface functionalization, 100 mL of TEOS and 100 mL of CES were mixed to the response combination following 24 hours of constant attractive blending.

For another 24 hours, the mixture was stirred. To start the precipitation of the NPs, the miniature emulsion blend was moved to a hawk axis cylinder and 20 mL of acetone was applied to it. The nanoparticles were secluded by centrifugation at 2500 RPM for 3 minutes, followed by decantation of the acetone. The isolated NPs were then treated with 15 mL of acetone. The nanoparticles were shaken in a falcon rotator tube with a vortex blender, and at that point centrifuged at 2500 RPM for 3 minutes until the acetone was tapped. Finally, RuBpy-doped CES-SiO2 NPs (0.1 g) were collected and dispersed in 20 mL RO/DI water.

Now the collected nanoparticles were used on the finger-marks obtained from different individuals. The finger-mark specimens were divided into two halves. All the finger-marks were treated with SiO2 and depending upon the age of finger marks (3 months old and 7 days old) were divided into different batches. Total 288 finger-marks were taken into account and analysed.

Though the bunch-to-group change from the examination study was noticed, assessment of the viability of the location of finger signs by changed identification boundaries would not be

influenced, as all fingerprints were handled and assessed in the significant half-finger marks. There were noticeable differences between the three donors from the results collected. However, there were improvements in the detection efficiency of three donors with regard to changed detection parameter. [17,3]

DISCUSSION

Technology in forensic science is improving day by day. And nanotechnology has played a major role in the field of fingerprints as they are enhancing the production. Some of the nanotechnology techniques are cost-effective as well as eco-friendly. For example, in red-emitting CaTiO3: Pr3+ nano phosphoric technique, the marks produced from this method can be maintained for longer periods of time. Because of their promising benefits and consistency of performance, nanoparticles have surpassed traditional methods.

CONCLUSION

Nanotechnology helps forensic science in two respects. Since it can identify and analyze samples at the nanoscale, vital information that could not previously be obtained and examined due to limitation of instruments can now be analyzed and used to support investigations. Due to its various advantages, nanotechnology has also became a branch of forensic science known as nanoforensic. This review summarizes how nanoparticles have created new advancements and branches in the field of forensic science. IJFMP

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CASE REPORT

Data Breaches in Academic Enterprise Resource Planning: The Rise of New White-Collar Crimes

Manjeet Kumar[1], Avadhesh Kumar [2], Adarsh Garg[3]

ABSTRACT

Enterprise Resource Planning (ERP) systems are widely used by the academia, and educational institutions. ERP are used by institutions for maintaining their records and sharing of data with the users and stakeholders. ERP modules have varying levels of end-user access restrictions depending on the type of module. Certain modules can be accessed from anywhere using an internet connection, whereas some are restricted to be accessed only through an admin account. These modules generally contain sensitive data stored in them. There are cases wherein the security breach has been reported for gaining access to the data stored and use them for perpetrating crimes, majorly financial crimes and identity thefts. The current paper discusses some of these breaches, along with the possible risks and corrective measures suggested to avoid such breaches.

KEYWORDS | erp, educational-erp, case study, data breach, hei, cyberattack

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INTRODUCTION

ERP is a computer software used to combine all business-related procedures and functions at a combined IT platform for easy management of businesses to work in an efficient way.

ERP is an old technology in manufacturing and production industry that dates back to the early 1960s when the ERP was in the form of Inventory Control system where it acted as accounting software. Later, in the 1970s, this was modified into MRP - Material Requirements Planning, a package that provided support to the planning and control unit of the business houses. This system was replaced by more advanced MRP II system in the 1980s. This new advanced system aims at integration of technology with the manufacturing to increase the manufacturing of products.[12]

In businesses, ERP systems keep track of their resources such as raw materials, finance, production capacity, and the standing of business assurances like salaries, sale-purchase orders, etc. This system makes sure that the relevant data is shared with the associated departments of the business and links them together with the core data. In other words, ERP not only ensures the data flow amongst the various departments of the business but also manages the information sharing with the stakeholders of the said business.[6]

ERP in the educational sector is an application that joins all the modules and departments of an educational institution into a single system whose access is available to the fraternity members of the institution and also to the students, their parents, and other stakeholders.[9] Each individual who is part of the institution has a unique user id and password. All the activities can further be monitored by the said administration with the usage of

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master id and password access. The educational ERP structure is entirely different from that of the business sector [10]. It comprises programs, fees, library, events, hostel, faculty data, examination, which is shown in Figure 1.

Main aim of the educational ERP system is to provide a platform that encompasses all the functionalities together at a user-friendly interface. The educational ERP system digitizes all the information and data of the institute which are updated by admin login only and grants access to all the students and faculties.[11] Educational ERP reduces the need for maintaining the data on paper and keeping a check on the store for ensuring data security. The digitized details once entered into the system are stored on the server which can be accessed only with valid login credentials.[13]

Figure 1: Modules in Academic ERP System

It has been observed by the authors that despite the rise in the implementation of ERP in the educational sector, it can be deduced that 60 to 65 percent of ERP systems have a failure rate and 30 to 35 percent of ERP implementations are canceled because of different factors. There can be numerous factors responsible for this rate of failure, the end-user training, cost input, data security, step-wise implementation rather than the big-bang approach, and lastly the technical training of the users.[14]

For any educational institution, the data of its students is the most valuable asset. With the rise in the technology, the academic institutions are making a change in how they store and process such data available to them, with majority of them shifting to the ERP system for the same. With this shift the concern regarding safeguarding this data becomes a top priority. The threat level has recently increased due to the rise in the cyberattacks, especially during the Covid-19 pandemic times. Another contributing factor in making the data vulnerable is the slow reaction of educational institutions towards cyber security.[15] It has been observed that the academic institutions invest less in cybersecurity making themselves prime targets of cyberattacks. The suggested approach is to make these organizations aware of the security-related issues and build necessary infrastructure.[16]

The survey gives a detailed view of the seriousness of the issue. The security breaches include unlawful disclosures, hacking attempts resulting in the breach of personnel data, ransomware attack, phishing attack, DOS attacks, and other cybersecurity threats, which results in disruptions of academic institutional activities resulting in unauthorized access or disclosure. In the year 2019, 348 incidents were reported related to cyberattacks associated with academic institutions, which is approximately thrice as much as in 2018 in the country of US alone. In 2020, this figure rose further to 377 and will continue to climb as establishments look to get cybersecurity under control. It has been observed that educational institutes are the no.2 target for ransomware attack. [17]

Another associated risk factor is 42% of educational organizations have students or staff as end-users who avoid cyber security protection. For academic organizations, it's compulsory to ensure that they are implementing the appropriate technology to protect themselves from cyberattacks, making sure that they are providing the necessary resources to their users and applying the needed restrictions using firewall to ensure unauthorised access to their network. Another study suggests that 41 percent of higher educational institutions cyber security incidents and breaches were results of social engineering attacks. It has been pointed out that 52% of cyberattack incidents resulting in the data breaches were caused by human error making it the top most cause of such attacks. The act of social engineering revolves around manipulation of its victims in sharing confidential personal information with another individual or third

party. To achieve this, the perpetrator most often impersonate as trusted friend or a colleague of an organization associated with the victim. Another approach employed in social engineering, is the usage of a phishing attack, that is done majorly via emails. It has been observed that an average of 30 percent of users in the academic sectors have been fallen prey of phishing emails received by them. In order to protect its users from such attacks, educational organizations should promote cyber security awareness trainings, ensuring to educate them on related topics of spotting a phishing attack, how to deal with such encounters appropriately when situation arises. [18]

Another survey indicates that 87 percent of educational institutions have encountered cyberattack at least once. These stats indicate that the majority of educational institutions have been the victims of cyberattacks, which is in consistence with the steep rise of attacks reported over the last couple of years. This should serve as a warning to educational administrators to ensure the updating of the cyber security protocols adopted by them. Amongst these organizations, 73 percent are found to be unprepared for cyberattacks, if encountered today.[19] This indicates that the educational institutes must employ the necessary means of technology needed to avoid any such future attacks before a breach occurs. Another study indicates that 85 percent of universities agreed that more investment should be made in order to ensure cybersecurity to protect critical research in IP. In the US alone, the academic sector in 2017, accounted for 13 percent of all data security breaches, which has resulted in the leak of approximate 32 million personnel records. These stats clearly indicate the need to understand the seriousness of cyber security in educational sector. Because of the education industry's approach to cyber security and the end users operating on campus, educational institutions are susceptible to cyberattacks. During the survey, it has been observed that, the educational sector as an industry is the least secured and most vulnerable industry amongst 17 industries studied when it comes to cyber security attacks, when the factors related to application security, endpoint security, and keeping software up to date on a regular basis is considered.[20]

Device standardization that is so common in business that it is much harder to achieve in an educational setting establishments should look to enforce their device management policies and authentication protocols for connected devices as strictly as possible. Awareness training should also be encouraged so that end users are prepared if and when they are targeted by a social engineering attack so they don't cause a breach that compromises the entire institution. According to BlueVoyant's Cyber security in Higher Education 2021 report, ransomware attacks on colleges increased 100% between 2019 and 2020. The report also found that two-thirds of assessed colleges lacked even basic email security measures and 86% of them demonstrated evidence of botnet targeting.

RESULT AND DISCUSSION

Majority of data breaches that struck the educational organizations involved the leaking of the personal information of the personnel and students, whereas about a quarter of the incident reported the exposure of their intellectual property and research work. In an attack on any educational institute the potential risks of data stealing can be related to:

- Complete access to the academic data of students viz, examination scores, overall results which can be changed or deleted.
- Administration can lose important information related to fees and the remaining balance which will result in huge monetary loss of the organization.
- Attackers may intrude in the institute management system and send spam emails or messages demanding confidential information and/or money.
- The biggest threat can be stealing and usage of sensitive personnel information viz, name, address, and age, for perpetuating heinous criminal activities such as blackmailing, loan applications amongst others financial scams. [21]

Any educational institute has numerous challenges which must be dealt to ensure protecting its information and data available with it. Some of such challenges includes:

- The educational sector completely relies on free-exchange of data and information

amongst the involved parties.

- Students and staff members are generally naïve about the technological development.
- Students who have superior technological skills can sometimes attempt cyberattacks out of curiosity.
- Users generally have been assigned more than one role within an organization which creates complications with the identity management software.
- The number of end-users is changed every year with the graduating students leaving and new enrollments coming in.
- In order to ensure smooth access by the end-users it is required to provide remote access of the system so that the parents and their wards can access the system from their personal computers and smartphones from varied locations.[22]

In a survey-based research conducted by Ed Guards Company, disclosed that the incidents related to data breach resulting from cybercrimes dates back to 2002 in US. According to this research in June of 2005, University of Hawaii, witnessed leak of personal data of approximately 150,000 students, staff and library when a former librarian compromised the integrity of the data in order to obtain fraudulent loans. A similar case was encountered at the University of Utah where the social-security number of approximately 100,000 employees was stolen from archived database. Another breach was reported in the year 2006 at UCLA, US, where the cyberattacks precisely aimed at gaining access to personal information, resulting in the leak of approximately 800,000 personnel information including student applicants, their parents, faculty and staff members.[23]

PeopleSoft a system developed by Oracle, which is most commonly adopted by the educational organizations in various countries. The first an foremost attack on this system occurred in the year 2007 wherein hackers by employing keylogger software on staff computers, had stolen the passwords and then used the same to log into ERP system of Florida, A&M University, with motive to change grades of students. Although, subsequently the information stolen was recovered later on, but troublemakers again attempted the similar attack and due to this the examination data of ninety students was modified. In 2008, another attempt was made to access the personal data. Nevertheless, the size of data breached increased from 70,000 to 700,000 stolen records.

In 2012, student of the University of Nebraska's notorious attempt compromised the database of the university which led to the leak of personal information of 654,000 students and employees. Along with the personal information the leaked data included the financial account details of 21000 people, too. Similar cyberattacks were reported at the Chadron State College, Peru State College and Wayne State College in the US.

During the years 2014 to 2016, cyber attacks on academic organizations not only increased in number but also became more advanced and aggressive in terms of data breaches. As per the details shared by the ADBI Report (annual data breach investigations) by Verizon, the occurrence of data breaches distressing the functioning of the educational institutions has grown approximately 10 times in US alone. It is also found that till 2017, the amount of reported cyberattacks was 393 which was only 5 in the year of 2012. In the month of march of 2018, more than 300 universities across the world were affected by a massive cyberattack orchestrated 9 hackers of Iranian origin. According to the information received form the investigating officials, 31TB of valued intellectual property and related data was leaked.[24,25,26]

All educational organizations store personal data of all the students and staff members of theirs. Making sure that this data stored is safe and not easily accessible by everyone is of prime importance to any such organization. The HEIs are rapidly experiencing the increased attacks on their databases by hackers on numerous counts. The data breach is attempted not only to gain access to the personal information of individuals but also include the research materials too.[27]

The most common assumption is that the fact data breach is generally associated with an attempted cybercrime, whereas a data breach can be accidental too, for example a member of staff

may lose his/her laptop or external storage device or the same may get stolen, resulting in loss of the data or confidential information. It may happen that the same can the downloaded on to a USB stick unofficially. Often it can be associated with carelessness which might result in accidental release of data by unintentional correspondence to the wrong person via email.[28]

The most common and effective solution to avoid data-breach is usage of encryption to ensure protection the information. By employing encryptions, academic organizations can be assured that only official users with whom encryption key is shared will be permitted to access and read the information/ data and for others it remains unreadable. This is supported by the study conducted by Ponemon Institute in the year 2017 which revealed that the with the usage of encryption the cost of data breached was reduced by US$16 per record. [29,30,31]

Another vital solution is to provide the necessary training to the IT professionals employed by the organization in case of attempted data breaches. Along with this the awareness training should be provided to the non-technical staff and student members in terms of the safe browsing of the data and safeguarding against the others means of the cyberattacks like social engineering and phishing scams. IJFMP

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REVIEW ARTICLE

Toxicological Aspects of Ayurvedic Medicines: A Review

Sneha Yadav[1], Monika Chauhan[2], Arvind Kumar Jain[3]

ABSTRACT

Ayurvedic medicine is considered to be one of the oldest medical systems in the world, and it is based on the ancient Indian medical philosophy i.e. depend on a "natural" and holistic mental and physical health approach and it remains one of the mainstream health care systems in India. Ayurvedic medicines are very popular in India and other Asian & Western countries as an alternative to chemical medicines because of their no / very few side effects on the human body. Ayurvedic medicines are known for the treatment of chronic diseases such as cancer, diabetes, arthritis, and asthma. This paper describes the possible causes for the various types of toxicity occurring/reported due to the intake of different types of Ayurvedic bhasma (nanoparticles) and herbal medicines, among them heavy metal toxicity (Lead, Mercury, Arsenic) is commonly seen. Many medicinal plant preparation could interfere with the proper functioning of certain medicines like Phenobarbital, Alprazolam, etc. The contamination of Herbal Medicines from heavy metals or microbial toxins can lead to nephrotoxicity, neurotoxicity, Hepatotoxicity, and severe damage to other body organs. Due to nonavailability of proper data on short, medium, and long term usage of Ayurvedic Bhasmas, heavy metal toxicity has been reported. Ayurvedic Bhasmas are safe though a lack of standard protocol or method of preparation.

KEYWORDS | heavy metals, toxicity, ayurvedic medicine, rasa shastra

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INTRODUCTION

Ayurvedic medical system is considered to be one of the oldest medicine systems in the world, originating in India during the 2000-1000BC. Ayurveda begant to spread to other countries such as China, Sri Lanka, and Mongolia during the 6th century BC through the Buddhist monks traveling around the world.[1,2] Ayurvedic medicines are prepared from herbs, minerals, and animal derivatives such horn, shells, feathers etc., both as single-ingredient drugs and as composite formulations. Typically, the formulations are divided into two groups, based on plants & minerals (Bhasmas), such as Shankh Bhasma, Hartal Bhasma, etc.[3] They are available in a variety of forms: tablets, pills, liquids, and semi-solid forms. Ayurvedic medicines extracted from plant extracts are usually considered safer, with no side-effects. There are various benefits of Ayurvedic medicines over allopathic medicines such as preserving the quality of life or developing a natural immune system. In today's COVID-19 pandemic times, most of the medicine systems have failed to provide a cure, whereas several formulations of Ayurvedic medicines, which are centuries old, have been popular wordwide have been helpful in boosting the immune system to fight against COVID pandemic.[4] But the view starts to change when Ayurveda, India's ancient system of medicine, is accused of toxicity in its medicines as many cases

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of severe toxicity due to intake of Ayurvedic medicines have started to surface.[5]

LITERATURE REVIEW

Several studies were performed on Ayurvedic medicines which were reported to have various amount of heavy metals in their formulations. A study was conducted in 2005 where 230 Ayurvedic medicines bought via the internet in the US and India, were randomly selected for analysis using an instrumental technique like X-ray fluorescence spectroscopy and it was recorded that nearly one-fifth of US and Indian-manufactured Ayurvedic medicines contain detectable lead, arsenic or mercury. All Ayurvedic medicines exceeded one or more standards for acceptable daily intake of toxic metals.[6] As per a media report in the USA, the New York City Health Department has asked the citizens to stop taking Ayurvedic medicines of Indian pharmacies as they were claimed to contain elevated levels of lead and mercury, which make it unsafe for human consumption.[7] The Houston Department of health services has detected lead and arsenic in seven South Asian traditional medicines and warned its residents against using these medicines.[8] In Another report of 2005, Health Canada has disapproved certain Ayurvedic products in the Canadian market due to detection of elevated levels of lead, mercury, and/or arsenic. It also warned consumers against buying these Ayurvedic products.[9] An incidence of death was reported when a person developed sub-acute toxicity after taking Dahana Bhasma prescribed for dyspepsia and appetite enhancement which ultimately led to his death. His death occurred after he took Ayurvedic Bhasma which had no labeling of ingredients. The said patient's case history also revealed that he had no habit of alcohol or any other recent medication taken apart from Vitamin B and Vitamin D. [10]

Kava, an herbal sedative prepared from plant (Piper methysticum), was found to have anti-anxiety or calming effects. It has been reported that the patient who was on a medication of Terazosin, Cimetidine, and Alprazolam, became disoriented and lethargic after ingesting Kava. A case has been reported in which severe hepatitis was seen in patients due to the use of Kava, as a result of which the patient eventually needed a liver transplant. Hence FDA issued an advisory and warned the public against the use of this medicine. [11-13]

Comfrey is a perennial herb that is used in the preparation of herbal medicines. Pyrrolizidine, an alkaloid present in its roots has shown hepatotoxic effects in animals and humans that can also induce tumors. Various studies show that Phenobarbital, a barbiturate, could induce the metabolism of pyrrolizidine alkaloids and convert it into a lethal metabolite. Some studies reveals that hepatic-veno-occlusive disease is related to the in-take of Comfrey. 14-16 The case study of a 70-year-old man who reported bleeding from the iris into the anterior chamber of the eye after one week of starting a self-prescribed regimen of medication of concentrated ginkgo biloba extract of 40 mg twice a day.[17] Hypertension was commonly seen, the adverse reaction was followed by a steep rise in heartbeat, tachycardia, strokes and even seizure. In some cases death has also been reported. [18,19] Various cases of unexpected lead poisoning due to the use of herbal medicines contaminated with lead have been reported. A case of acute porphyria was reported in a 23-year-old male. In his urine the porphyrin was seen positive indicating acute porphyria. Later on further examination lead (77ug/dL) was detected in his body. Another case of lead-poisoning occurs after taking Chinese medicine Cordyceps in which the lead content was found higher than 20000 ppm.[20, 21] Several case studies related to herbal medicine toxicity have been discussed herein which the drug or herbal medicine caused side effects after taking it for treating a particular disorder/disease. A report describes progressive renal failure in a forty-year African male after consuming a drug named Pausinystalia yohimbe which is an herb. [22] Chronic renal failure has been reported after taking Chinese herbal medicine Panax ginseng (Renshen Yangrong Tang), which is used for treating anorexia & hypoprotenimia. Cardiotoxicity and renal toxicity reported after consuming Tripterygium wilfordiian, an herb used for treating Arthritis.[23] Cases of neurotoxicity and liver toxicity were also reported after consuming an herbal medicine containing the herb Valeriana officinalis which is a popular sedative agent.[24]

It has also been seen that in a number of cases

interference in the functioning of an allopathic drug like Hyper perforatum interferes with Alprazolam[25], Ginkgo biloba interferes with sodium valproate[26], Panax ginseng interferes with Phenelizine[27], Cimicifuga interferes with Atoravastatin[28], Seutelluariae interferes with Losartan[29], Evolvulus alsinoides interferes with Phenytoin[30], Allium sativum interferes with Saquinavi.[31] This paper discusses the recent studies on Ayurvedic medicines and possible causes of their toxicity.

The Ayurvedic medicines are categorized into two types as rasa shastra, and non-*rasashastra*, both of which differ in their preparations.

Herbal Medicines

Herbal drug (Herbal medicine) is considered to be the entire or grained, desiccated part of a plant, algae, lichen, fungi, which is useful due to its medicinal properties. Both plant organs (flower, fruit, bark, root, seed, and leaf) and plant exudates like rubber, balsams, and resins can also be considered as Herbal medicine preparations. Herbal drugs are prepared from herbs, by following procedures of distillations, extraction and filtration, and a few more steps.[32] Various types of herbal medicines commonly available in the market are Echinacea, Ginseng, Ginkgo, biloba, Elderberry, Valerian, Camomile, Ashwagandha capsules, Cardimap, and many more. As per WHO, herbal drugs are classified into four different classes based on their origin, mode of current usage and evolution:

Indigenous Herbal Medicine: Used historically in a local community or region, it is very common among the local population for a very long time in terms of their composition, dosage, and treatment.

Herbal Medicine in System: These are in Ayurveda, Unani, and Siddha, some indigenous herbal medicines are now available in the market and are popular in the region or local community now, but it's important to fulfill the standards and requirements as per regulations for safety and efficacy of herbal medicine.

Modified Herbal Medicine: These are altered or modified in terms of compositions, Medical indications shape, methods of preparation, ingredients, dose, mode of administration, and dosage form also.

Imported product with an herbal medicine base: Include the raw material and products, the importing country and recipient country should ensure that the medicines meet the national regulatory requirements of exporting country as well as receiving country.

Major Causes of Toxicity

The toxicity caused by herbal medicines can be related to two major factors, direct (Intrinsic) and indirect (Extrinsic).

Intrinsic Factor: In orthodox medicine, the toxic effects are classified into four major categories in the same way toxic effects of the herbal medicines can be classified into four categories.

Type A (acute augmented) includes interaction with pharmaceuticals or overdose. It is connected with the integral pharmacological properties of herbal products; substance is poison or remedy depends on the dosage. Herbal medicines have been established to be an effective remedy for thousands of years, overdose or inappropriate or wrong consumption could lead to adverse drug effects. Different body organs and systems may get affected due to adverse drug effects.

Type B (bizarre/idiosyncratic) reactions are common adverse reactions triggered by herbal products which range from minor allergic reactions to severe anaphylactic shock. Its examples include Anaphylaxis and hives, acute asthma.

Type C (chronic/cumulative) reactions occur due to long-term therapy or use that are well known and anticipative or expected. Its examples include Hypokalemic paralysis that occurs after ingesting Licorice for a long period.

Type D (delayed) is not commonly reported due to the nonexistence of systemic assessment for herbal medicine, Delayed effects because of herbal medicines become more apparent in the future. Its examples include carcinogenesis which is associated with the Aristolochia species due to the presence of aristolochic acid. These herbal remedies are generally prescribed as a mixture of some medicinal plants and commonly taken as complementary medicine with conventional ones. The toxicity may occur when they are simultaneously present, which could occur differently like pharmacokinetic interaction which may change absorption, metabolism, distribution, and excretion of the drug and altering therapeutic/

beneficial properties also pharmacodynamics interaction affects the molecular target that intermediates various physiological responses.

Extrinsic Factor

The Extrinsic Toxic effects are related to contamination, misidentification, and adulteration of herbal medicines. It has been observed that lack of proper quality control of preparation & production of these herbal medicines' contamination with heavy metals and some toxic microbial substances has been reported worldwide.

Heavy metals: The commonly found heavy metals in herbal medicine are arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd). They are a serious public health concern because of their toxicity even at very low concentration. The contamination due to heavy metal in herbal medicines is a major concern in various Asian countries. The source of heavy metal contamination in herbal medicines could have come during the growth, development, processing of these herbal medicines. Medicinal plants could accumulate these heavy metals from agricultural soil, water, fertilizers and pesticides used. Also from air pollution. As a result of these, the WHO, which also regulates the permissible limits of toxic metals, has advised detecting the presence of these heavy metals in the raw materials that are used for the final product.

Microbial Toxins: The concern of microbial toxins in herbal products is also increasing worldwide even after so much research on bacterial, and fungal contamination on food is done. Mycotoxins (a type of microbial toxin), Aflatoxins, fumonisins, ochratoxin A, zearalenone, and deoxynivalenol have been reported in various medicinal plants globally.

Adulteration: The addition of some drugs or substances which are not labeled or due to improper preparation of herbal medicines requires heavy metals as an ingredient. As adulterants are not labeled on the ingredient listed it increases the chances of overdose and interactions, and serious outcomes.

Misidentification: Due to confusing nomenclature, similar appearance, and complication in processed products of herbal remedies and medicinal plants, a case has been reported in which a 23-year-old lady was hospitalized when she suffered a complete blockage of the heart after ingesting an herbal product, Chomper, due to the replacement of (Plantago major) Plantain with (Digitalis lanata) Woolly foxglove in the herbal product due to the similarity of a leaf. [33]

Ayurvedic Bhasma

On other hand, Ayurvedic Bhasma is considered to be ancient forms of nano medicine. Bhasmas are considered to be unique Ayurvedic metallic/mineral preparations that are treated with herbal juices or decoction and exposed to a certain degree of heat. Bhasmas are prepared by two methods putapaka and kupipakwa. The Bhasma is prepared by exposing metals or minerals to a three-step procedures that are (Shodhna, Bhavana, Marana).[34] This procedure is repeated continuously for a certain specific period of time. Finally, the prepared Bhasma is collected. The repetition of the process of incineration reduces particle size and it is observed that after passing all these stages, the Bhasma becomes biologically favorable for the body and thus it is seen that after the formation of Bhasma it passes through various parameters for quality of Bhasma. Rasa shasta method for preparing Ayurvedic Bhasma is considered for therapeutic use.[35] Rasa shastra claims that the inorganic form of mercury, which is toxic, can be converted into Rasa sindura, which is safe. Bhasma and various traditional medicines are forwarded to a process known as "alchemy" which alters the mineral form which is generally suitable for the medication (i.e. HgS, PbS, PbO, AsS4) which are different from the environmental metal contaminants like (NaAsO2, AsO4, HgCl2).[36] The particle size of these bhasmas are determined by various techniques such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Cryo TEM, Confocal laser scanning microscopy, Energy dispersive X ray (EDAX), X-ray induced photoelectron spectroscopy, and quantization of different heavy metals in Bhasma by Inductively Coupled plasma (ICP), Atomic absorption spectroscopy (AAS).[37-40] The metal poisoning cases especially of lead (Pb), Arsenic (As), Mercury (Hg) are on the rise because of inappropriate procedure followed during Bhasma preparation and inadequate testing. Therefore, pharmaco vigilance is required. The public health

influence of metals in Bhasma or rasa shastra in India is opaque and controversial, as Ayurveda claims that in India medicines produced by rasa-shastra process are safe and are in use for a long time, according to them the metal toxicity occurs due to inappropriate commercial manufacturing practices or else due to lack of proper monitoring by person skilled in rasa shastra. Ayurveda texts endorse proper detoxification processes in the preparation of Bhasma. The use of Bhasmas in such an era where enough studies on the short, intermediate and long-term use are not available, may pose a threat to the public. [41]

Possible Toxic Effects of Heavy Metals in the Human Body

Toxicity is a complex one with various influencing factors among them. Dosage is the most significant one. Toxicity is the outcome of various changes like macromolecular one, Biochemical, and Adverse cellular. Cell replacement which is seen in fibrosis, production of reactive chemicals, interruption of protein synthesis, Enzyme system damage, and even DNA damage are generally encountered in case of toxicity. [42]

Physiological Classification of Toxic Responses

The type of physiological effects they have on the human body so toxic substances can be classified as Teratogens (lead), Mutagen, Carcinogen, Reproductive toxins (lead), Pulmonary toxins (chromium), Hematopoietic Toxin, Neurotoxins (lead), Nephrotoxins (mercury), Hepatotoxin, Anesthetic, Asphyxias, Corrosive, Irritants.

Biochemical Effects of heavy metals

We will discuss the most toxic heavy metals and which are found in Ayurvedic preparations are arsenic, lead, mercury which can cause toxicity even in smaller doses.

Arsenic (As): This is considered the most toxic metal; exists in three valence states (0,+3,+5) called metalloid arsenic (with 0 oxidation state), arsenates (+5), arsenite (+3). The acute toxicity may result in fever, liver enlargement, anorexia, and even death. Various health effects of arsenic toxicity are due to chronic exposure that may produce poisoning of the nervous system, and severe liver damage, gangrene of the lower limb (Black Foot disease) which is a result of peripheral vascular disease. Major biochemical effects which lead to tissue respiration impaired that are:

Complexation with coenzymes/enzymes- Arsenic (+3) disrupts sulfhydryl containing enzymes by binding with (-SH) groups, which results in the inhibition of necessary metabolic activities of cellular respiration like stoppage of Tricarboxylic acid cycle (TCA), succinate, and pyruvate oxidation pathways which ultimately lead to no synthesis of ATP which is an important source of energy in the human body, this inhibitory action occurred due to inactivation of enzyme pyruvate dehydrogenase by As (+3) ion.

Coagulation of Proteins-Arsenic (+3) compounds attacks the sulfur bonds which are necessary for the primary & secondary structure of proteins.

Uncoupling of Phosphration: An alternate pathway is by substitution of as (+5) for phosphorus, due to its chemical similarity with phosphorus. The phosphorus anion in phosphate is a stable anion which is substituted by less stable as (+5) anion as a result of which there is speedy hydrolysis of high energy bonds in different compounds like ATP, as a result, loss of high energy phosphate bonds occurs and "uncouples" the process oxidative phosphorylation.

Mercury (Hg)- It exists in three forms metallic mercury (elemental mercury), inorganic & organic mercury. Various forms in which mercury occur naturally in the surroundings like metallic mercury, mercury sulfide (cinnabar), mercuric chloride, methyl mercury. Metallic mercury is not as toxic as methyl mercury due to slow absorption through the gastrointestinal tract various health effects of mercury toxicity depend upon its form which affects different parts of the body. Organic mercury (Methyl mercury) affects CNS and produces neurotoxic effects in adults and causes toxicity in the fetus of pregnant women, Inorganic mercury (mercuric salts) causes toxicity in the kidney it can also lead to abdominal cramps and bloody diarrhea.

Major biochemical effects of mercury are due to binding with sulfhydryl group or thio group which leads to interference in protein synthesis, and a strong affinity for sulfur which makes it easily bind to any compound containing sulfur or sulfur hydrogen combination

Elemental Hg *(mercury):* It is commonly found in liquid form, also is easily vaporized even at the room temperature and can be absorbed mostly

through inhalation, Mercury vapors inside cell converted into mercury (+2) which is more toxic form. This divalent form of mercury is responsible for causing erethism (mad hatter's disease).

Inorganic Hg *(mercury):* It is commonly found in mercuric salt form and is highly corrosive. It enters in body dermally or orally and gets stored in the kidney. In the GI tract, it causes sloughing away of the mucosa, even the pieces are found in stools.

Organic Hg *(mercury):* It has a high affinity to lipids, Alkyl organic mercury gets deposited into hair, nail, liver, kidney, brain. They also show teratogenic effects due to crossing blood placenta barriers, also crosses the blood-brain barrier, and also can easily penetrate erythrocytes' leading to neurological symptoms.

Lead (Pb) It is the most widely spread Toxic metal. Lead usually enters into human body from contamination like lead pipes or lead solder. Children are more vulnerable to lead poisoning because it absorbs more readily in growing bodies. It causes various abnormalities related to bone growth in children. It majorly affects three organ systems

Neurological System: In CNS it alters the functioning of cellular calcium leading to inhibition of the blood-brain barrier. This is followed by leakage of proteinaceous fluid and brain edema, which affects mainly the occipital lobe, cerebellum. This edema is manifested initially by headache, clumsiness, vertigo, ataxia leads to seizure, coma death or sometimes recovery is their permanent neurological loss.

Hematological System: It has a major effect on heme synthesis. Any compound having a sulfhydryl group is can be easily disrupted by lead. Lead crosses the blood-brain, placenta barrier and can easily be deposited into soft and hard tissue of the body.

Renal Effects: It causes irreversible nephropathy due to functional impairment of tubular regions characterized by glucosuria, Hyperphosphaturia. [42]

DISCUSSION

Natural medicine is safe but contrary to popular belief, herbal medicine can cause severe health problems and even death in some cases. Many medicinal plant preparations could interfere with the proper functioning of certain medicines like Phenobarbital, Alprazolam, etc. The contamination of herbal medicines from heavy metals or microbial toxins can lead to nephrotoxicity, neurotoxicity, hepatotoxicity, and cause severe damage to certain body organs.

CONCLUSION

Ayurvedic bhasmas are generally considered safe, as they are prepared in accordance with instructions mentioned in ancient Ayurvedic texts, but due to lack of standard protocol or method of preparation, these bhasmas are found to be a threat to public health due to the presence of heavy metal toxicity in them as there is a lack of proper data on short, medium and long-term usage of these bhasmas. IJFMP

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REVIEW ARTICLE

Voice Stress Analysis for Deception Detection

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ABSTRACT

The Air Force Research Laboratory (AFRL) has been asked by the National Institute of Justice to investigate voice stress analysis (VSA) technology and evaluate its effectiveness for both military and law enforcement applications. This technology has been marked as commercially available in computer based form, and marketed as being capable of measuring stress and, in some systems, deception. This technology is reported to be easier to use, less invasive and less constrained in its operation than standard polygraph technology. This study has found the VSA technology can identify stress better than chance with performance approaching that of current polygraph systems. However, it is not a technology that is mature enough to be used in a court of law. We also found that experience and training improve the accuracy than less trained individuals. Lastly, we explored how this technology may become an effective interrogation tool, when combined with polygraph technology. This article contains information and results of a primary work done to show how the stress changes for Mel Frequency Cepstral Coefficient features can be detected through FFT signal processing when a person is under psychological pressure. The principal purpose is to obtain a tool that could help the accused to prove their innocence in an offense or a crime.

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KEYWORDS | vsa, voice stress analysis, polygraph, deception detection

INTRODUCTION

Voice stress analysis (VSA) is accomplished by measuring fluctuations in the physiological micro tremor present in speech. A micro tremor is a low amplitude oscillation of the reflex mechanism controlling the length and tension of a stretched muscle caused by the finite transmission delay between neurons to and from the target muscle. Deception Detection refers to the investigative practices used to determine a person's truthfulness and credibility. This is largely determined through the consideration of certain behavioral and physiological cues as well as larger contextual and situational information.

The use of voice stress analysis for deception detection is arguable, since it's easy for an individual to change his/her feelings. On a voice decision, however, the recent advancements in computing have made it possible for humans to watch and analyze their inner feelings simply by analysing them. VSA is accomplished by activity fluctuations within the physiological small tremor gift in speech. A small tremor could be a low amplitude oscillation of the reflex mechanism dominant the length and tension of a stretched muscle caused by the finite transmission delay between neurons to and from the target muscle. Small tremors are felt in each muscle within the body as well as the vocal cords

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Objective

- The main idea behind the research was to understand the voice stress analysis for deception detection among criminals.
- To understand how stress in voice reflects the emotions of a criminal.
- Graphical representation of the emotions examined through the voice.
- To create a dataset of criminals so that it can be used in future to get the criminals behind the bars.

Review of Literature

Stress and its manifestation within the acoustic signal are the topic matter of the many studies in literature. Researchers have tried to see reliable indicators of stress by analyzing sure variable parameters of speech like first harmonic (pitch), amplitude, concentration of spectral energy, period and a number of other others. In literature, analysis of stress is performed through analysis of some parameters of stress like first harmonic, pitch, vowel period and formants in recorded emotional speech, namely, analyzing a speaker's speech once they are below stress, fatigue, significant work load, environmental noise, sleep loss or expressing some feeling like happiness, anger or sorrow.

Voice stress analysis originated from the idea that once an individual is below stress, micro-muscle tremors (MMT) occur within the muscles that compose the vocal tract that are transmitted through the speech. VSA literature points to a descriptor because the physiological basis for the MMT. This paper describes "a slight oscillation at or so ten cycles per second" (i.e., physiological tremors) throughout the conventional contraction of the skeletal muscle. All muscles within the body, as well as the vocal cords, vibrate within the eight to twelve cps vary. It's these MMTs that the VSA vendors claim to be the only real supply of detective work, if a person is lying.

In moments of stress, particularly if an individual is exposed to hazard, the body prepares for fight or flight by increasing the readiness of its muscles to spring into action. This successively causes the muscle vibrations to extend. In step with the Merck Manual, "enhanced physical tremors is also made by anxiety, stress, fatigue, or metabolic derangements or by sure medicine. VSA systems claim to live these tremors transmitted through the speech.

METHOD & MATERIALS

200 voice samples were collected and analysed for our research and a database was created (DB00002). The voice samples were then analysed on the basis of frequency. Change in frequency transfers from the muscles in the vocal tract to the voice produced.

To better perceive the aspects of stress speech during a human, the Air Force Research Lab (AFRL) worked with Dr. John Hansen of the University of Colorado, to see if it's possible to acknowledge and classify stress in a person's voice. Dr. Hansen is a world renowned professional within the space of voice stress. The report was enclosed during this report, associate degree is connected as an Appendix C. He states "it's not impossible that below extreme levels of stress, that muscle management throughout the speaker are going to be affected, as well as muscles related to speech production". During this study, he used the Speech Under Simulated and Actual Stress (SUSAS) info. This info includes stress speech like angry, loud, European (speaking below shouting conditions), and worry stress. In his report, he reviewed literature that mentioned past speech below stress studies. He analyzed stress in speech, within which he complete that voice stress is caused by factors that introduce variability into the vocalization method. These variabilities or options embody period, vocal organ supply factors, pitch distribution, spectral structure and intensity.

The workflow includes four areas:

1. Voice i.e., Overall word period,
2. Analysis: Individual speech category (vowel, consonant, semivowel, and diphthong) period,

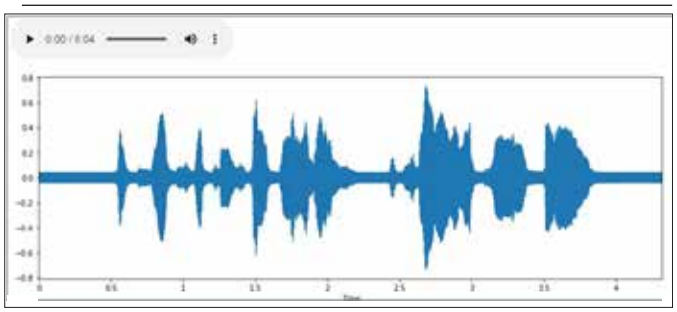
3. Data running and Period shifts between categories
4. Data acquisition and Speech category period ratios

Glottal supply factors measured the spectral slope of these vowels, that were longer than five frames or ninety-six-time unit. The primary and second formant's locations ar measured to see the spectral structure. Intensity could be a calculation of energy during a voice signal. These variabilities might even be speaker-dependent. By victimization these numerous linear and nonlinear options, and testing with the Bayesian hypothesis technique, it had been complete that differing kinds of emotional stress may well be classified. The Bayesian hypothesis technique could be a stress detection technique to see if a given piece of audio information is either neutral speech or an explicit classification of stress speech. From the results, it suggests that it's unlikely that one feature may well be wont to accurately sight deceptive stressed speech. There are lot of options that are united along, the strain kind recognition improves. It conjointly shows that some options, single two-handed, will sight a particular style of stress higher than alternative options. For associate degree example, the pitch feature might sight loud stress higher than angry and European stress.

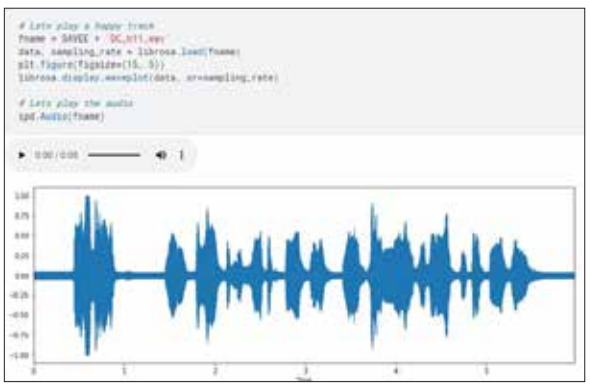
Whereas, the spectral structure feature may find angry stress higher. Classification of deceptive stress wasn't tested because of the inaccessibility of a deceptive information. the gathering of a deceptive information may be a recommendation of future work. The tests were performed, the information was documented, and therefore the results were compared. The Vericator and Diogenes lamp Systems were utilized during this analysis and their technology was tested. The first goal of this part of the VSA analysis was to work out if the microtremor claim is that the VSA's true theory of operation. For the needs of this check the character of the results, stress or no stress indicated, weren't taken into consideration. The results were found to be consistent across the board with very little variation within the leads to response to the adjustments/changes within the modulation or depth of modulation rates. for instance, the analysis of the 80Hz FM check wave, with a depth of modulation rate of one Hz and a modulation rate of one Hz, differed little or no from associate degree eighty Hz FM check wave with a depth of modulation of 4Hz and a modulation rate of twenty-five Hz.

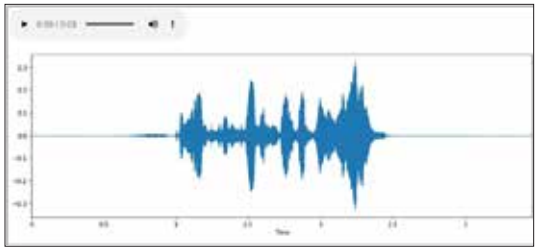
```
['JE_h09.wav', 'KL_f12.wav', 'DC_h03.wav', 'DC_d04.wav', 'KL_a14.wav']
```

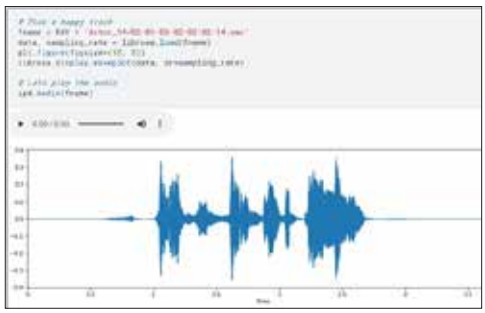
```
male_neutral     120
male_sad          60
male_happy        60
male_disgust      60
male_surprise     60
male_fear         60
male_angry        60
Name: labels, dtype: int64
```

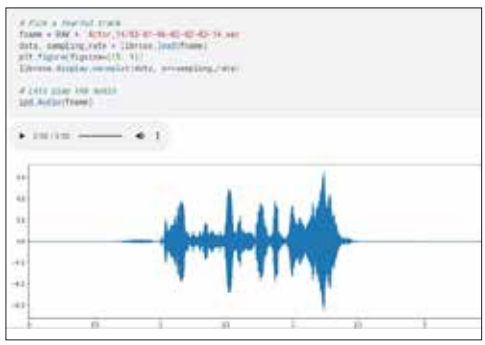


```
male_neutral       144
female_neutral     144
male_happy          96
female_fear         96
female_sad          96
male_disgust        96
female_disgust      96
male_fear           96
female_angry        96
female_happy        96
male_angry          96
male_sad            96
female_surprise     96
male_surprise       96
Name: labels, dtype: int64
```





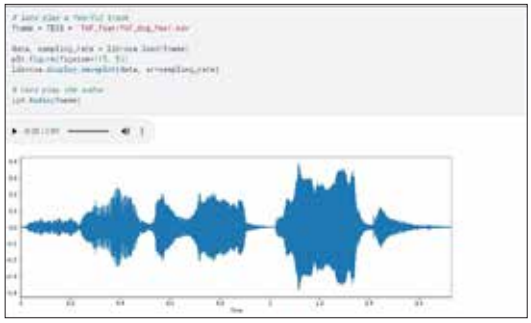




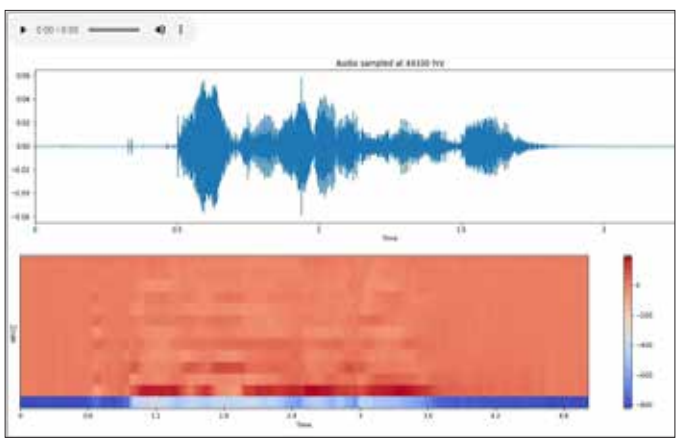
```
dir_list = os.listdir(TESS)
dir_list.sort()
dir_list
```

```
['OAF_Fear',
 'OAF_Pleasant_surprise',
 'OAF_Sad',
 'OAF_angry',
 'OAF_disgust',
 'OAF_happy',
 'OAF_neutral',
 'YAF_angry',
 'YAF_disgust',
 'YAF_fear',
 'YAF_happy',
 'YAF_neutral',
 'YAF_pleasant_surprised',
 'YAF_sad']
```

```
female_sad         400
female_disgust     400
female_happy       400
female_fear        400
female_neutral     400
female_angry       400
female_surprise    400
Name: labels, dtype: int64
```



Since there was no variation of indicated stress from totally different input signals, it may be assumed that the systems tested don't use microtremors as indicated in their claims. It was absolutely determined late within the testing part of this project, that the Diogenes lamp System live the energy amendment of the spectrum envelope between 20Hz and 40Hz. This can be what the Diogenes lamp System claims to be microtremors. It's the amendment of energy within the speech envelope. If a private is below stress, their vocal tract muscles square measure possible to constrain. Once the vocal tract muscles constrain the energy of the voice, signal becomes abrupt once the individual starts and finishes talking. Throughout the time a private talk, there's less variation of energy at intervals this the 20Hz band pass. Once a private isn't stressed, their voice energy slowly results in a peak after they begin to talk, then the energy varies till the individual stops speaking wherever the energy slowly trails off. This formula was coded within the laboratory with a similar audio signal input.

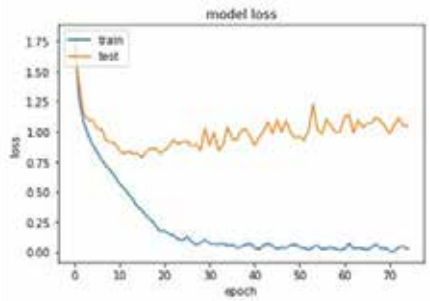


Source: All pictures are author self.

```
#concatinating the feature column into the complete dataframe
df = pd.concat([ref,pd.DataFrame(df['feature'].values.tolist())],axis=1)
df[:5]
```

	labels	source	path	0	1	2	3	4	5	6	...	206	207	208	209	210	211	212	213	214
0	male_happy	SAVEE	/kaggle/input/surrey-audiovisual-expressed-emo...	-25.742002	-26.182264	-25.466557	-25.196936	-25.429790	-23.347939	-16.800249	...	-3.119451	-4.260779	-5.274271	-5.775263	-7.272358	-7.340224	-7.019254	-8.643790	-15.420984
1	male_fear	SAVEE	/kaggle/input/surrey-audiovisual-expressed-emo...	-41.184326	-38.827896	-39.008781	-41.509396	-39.770164	-34.743958	-30.551401	...	-27.156342	-25.647068	-25.140005	-26.746456	-27.039560	-27.250130	-26.599070	-25.778673	-24.685337
2	male_happy	SAVEE	/kaggle/input/surrey-audiovisual-expressed-emo...	-25.528027	-23.866304	-22.509321	-22.320293	-17.513348	-12.073632	-9.537952	...	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
3	male_disgust	SAVEE	/kaggle/input/surrey-audiovisual-expressed-emo...	-24.961075	-23.702238	-24.368538	-23.824282	-20.085262	-20.491526	-21.797201	...	-11.261946	-12.151450	-13.157601	-13.586168	-14.427001	-15.093400	-13.683898	-13.934835	-9.288823
4	male_angry	SAVEE	/kaggle/input/surrey-audiovisual-expressed-emo...	-16.746674	-18.525646	-22.264179	-22.016502	-23.868864	-23.068270	-23.807617	...	-27.922167	-27.525471	-27.459106	-26.916111	-27.472073	-29.056606	-28.666271	-28.376913	-24.786333

```
plt.plot(model_history.history['loss'])
plt.plot(model_history.history['val_loss'])
plt.title('model loss')
plt.ylabel('loss')
plt.xlabel('epoch')
plt.legend(['train', 'test'], loc='upper left')
plt.show()
```

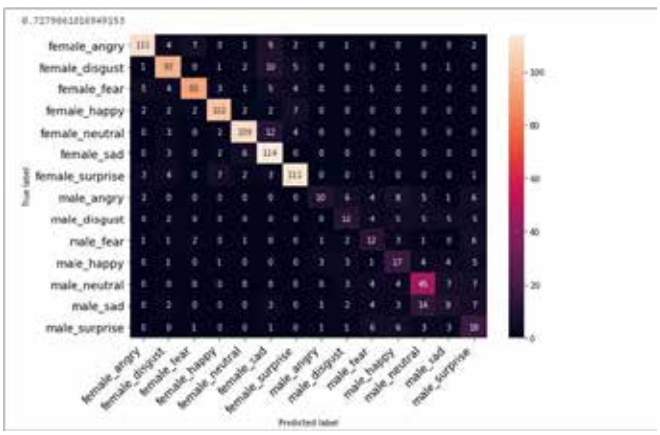



```
finaldf.to_csv('Predictions.csv', index=False)
finaldf.groupby('predictedvalues').count()
```

predictedvalues	actualvalues
female_angry	124
female_disgust	123
female_fear	105
female_happy	118
female_neutral	124
female_sad	159
female_surprise	133
male_angry	16
male_disgust	30
male_fear	37
male_happy	47
male_neutral	77
male_sad	30
male_surprise	57

```
classes = finaldf.actualvalues.unique()
classes.sort()
print(classification_report(finaldf.actualvalues,
```

	precision	recall	f1-score	support
female_angry	0.89	0.81	0.85	136
female_disgust	0.79	0.82	0.80	118
female_fear	0.89	0.80	0.84	116
female_happy	0.86	0.86	0.86	119
female_neutral	0.88	0.84	0.86	130
female_sad	0.72	0.91	0.80	125
female_surprise	0.83	0.84	0.84	132
male_angry	0.62	0.24	0.34	42
male_disgust	0.40	0.32	0.35	38
male_fear	0.32	0.40	0.36	30
male_happy	0.36	0.44	0.40	39
male_neutral	0.58	0.64	0.61	70
male_sad	0.30	0.20	0.24	45
male_surprise	0.32	0.45	0.37	40
accuracy			0.73	1180
macro avg	0.63	0.61	0.61	1180
weighted avg	0.73	0.73	0.72	1180



RESULT & DISCUSSION

The stress ground truth was obtained through the medical instrument examiner and court proceedings via the outcomes of every of the interviews each suspect confessed and were afterward guilty of murder. All of the relevant

stress sentences were verified. Every of the forty-eight utterances was analysed and compared to the bottom truth. Every system gave indications of high levels of stress wherever stress indicators were verified. The Vericator system scored 100 percent in its indication of some kind of stress, wherever because it displayed deceitful, high stress, or in all probability lying. The lamp system additionally scored 100 percent in its indication of stress through the wave shape analysis each system gave the examiner a conclusive indication of relevant stress.

Voiced analysis report table shows consistent results utilizing DAT and live voice. Every auditory communication was examined and located that everyone the waveforms and analysis was systematically identical. Once employing a recorder similar results were obtained as within the live information. Once recording with a electronic equipment, care has to be taken once adjusting the automated gain management (AGC). If the recording volume isn't set accurately, the input voice signal gets clipped. Thus once the output wave shape is processed, it gets distorted too. This might end in associate degree analysis that is totally different from the reality, so providing and incorrect result by the examiner. These discrepancies may be seen in figures, once exploitation the Diogenes lamp system. Once exploitation of the Vericator these discrepancies are evident. The Vericator results reacted otherwise every time a similar clipped information was inputted into the system. For instance, if a clipped audio phase was processed within the Vericator, the system might show truth, whereas once more that very same clipped information would cause the system to show untruth.

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CONCLUSION

The research was a success with an accuracy of 99.01%, we can say VSA can be done on an individual. For Deception Detection 200 samples were collected for this research and achieved successfully. Database can be created from Voice samples of Criminals which can be used in future for the purpose of identification of criminals.

It was not the target of this study to suggest one Psychophysiological Deception Detection technology was better than the other. Rather, it was our intent to produce unbiased analysis of VSA technology in conjunction with enough data to help them in creating selections on what kind of system to use. These instruments aren't "lie detectors". The choice on whether or not an issue is being truthful or lying ought to solely be created by a trained examiner. This call ought to be primarily based upon reviewing the information conferred by the instrument, the behavior of the topic, and different proof from the case. VSA systems square measure capable of providing associate degree examiner with a wave shape or different response which will be an affordable reflection of the strain level being toughened by the topic, in an exceedingly majority of the cases. The proper interpretation of this indicator is that the responsibility of the examiner. The goal in employing a VSA system or medical instrument ought to be to win over the topic that they cannot deceive the operator, which the instrument can find their deception and their best avenue is to confess to the crime. This study has shown that VSA systems can manufacture results that trained operators will use confidently to get confessions.

Finally, we tend to conclude that VSA technology will determine stress higher than medical instrument systems that have and coaching improves the accuracy of result. IJFMP

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REVIEW ARTICLE

Study on Disguised Voice Recording using Voice Changer Apps

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ABSTRACT

Speech science has a storied and illustrious past. Many studies are conducted for research on speech and voice acoustics, which focuses on speech. The human voice is our most basic and self contained instrument. When it comes to voice processing, the source filter paradigm may be used. It's common to think of the voice as having two distinct processes: one that produces the initial tone and another that produces the final tone. This is done in order to create a speaker's identity by providing an accurate phonetic profile of that individual. Other related fields include improving low quality audio files and verifying disputed recordings to see whether they have been manipulated, edited, or otherwise tampered with. In contrast, a digital recording samples the transduced waveform and transforms each sample into a binary number code similar to that used by computers. Speech analysis was also used in the investigation of recordings allegedly made by Osama bin Laden, the world's most wanted terrorist. This research will discuss if voice changer apps can be used to mask one's voice. There have been several instances where the perpetrator has used these apps to alter their speech. This research looks at how the characteristics of any voice can be altered or not by the use of these apps, how they function, and how they affect voice recognition. This paper also examines whether these apps really assist the perpetrator in eluding voice recognition experts.

Keywords | Forensic speech analysis, voice changer apps, Audio analysis

INTRODUCTION

Speech science has a long and glorious history. Many studies, including mine, which explores the speech, is conducting research on speech and voice acoustics.[1] Chatting, singing, laughing, moaning, crying, or shouting are examples of sounds produced by a person using the vocal tract. Human voice pitch is generated primarily by the vocal cords. (Other sound-generating mechanisms include unvoiced consonants, taps, whistling, and humming, which all come from the same general area of the body.) The human voice is our most basic and self-contained instrument.[2]

Indian languages represent the diverse layers of social order and caste. Individuals' speech repertoire includes a range of modes and dialects that are appropriate for various social situations. The better a speaker's rank is, the more speech forms he or she has at his or her fingertips.[3] To signify the speaker's social standing and the social context in which they speak, speech is altered in a number of ways.

When it comes to speech synthesis, the source filter paradigm may be used. It's common to consider the voice to have

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two distinct processes: one that produces the initial sound and another that modifies it. For example, the larynx (also known as the "speech box") emits a sound with a wide variety of frequencies. The root is the vibrating vocal folds in the larynx.[4] The filter is the vocal tract, which runs from the vocal folds to the lips or nostrils.

The larynx is a device located at the top of trachea. The vocal folds are two vibrating flaps of flesh in the voice. In a resonant system, standing waves can form. When the sound is up at the vocal folds and low at the mouth, standing vibrations, or resonances, occur in the vocal tract.[4]

Exhalation, phonation, and articulation are the three stages of speech development at the periphery level.

Formants are the more noticeable frequencies that differentiate distinct vowel sounds.

Voice Changer

A voice modulator (also called a voice changer) is a computer software programme that changes the human voice in real time. Not all voice changers are electronic devices of their own. Many of these voice changers are software programmes that can alter large swathes of vocabulary.[5]

Voice changers are also used as speech disguisers. When a voice changer is used, the accent varies so dramatically that even close friends and family members are unable to say who is speaking to, based solely on speech. They largely act by altering the user's voice pitch.[6]

A defendant was found guilty of delivering a bomb message over the internet in the case of United States v. Gilbert. According to the district court, the defendant purchased a toy voice changer on the day the phone bomb threat was made. To disguise the speaker's voice, this voice changer may alter the sound of the speaker's voice up or down.[7]

The defendant made some offensive calls by using the voice changer. Since adjusting the sound of the audio conversations so that the defendant's own voice could be heard, an investigator was able to prove that the defendant was the one making the calls. The majority of voice changers alter the tone of a person's natural voice.[8]

In Gilbert, the court dealt with the testimony of a voice changer by making an investigator testify to the defendant's name. Although an expert may be able to analyse metadata associated with a voice file, the required analysis, would be challenging.[9]

MATERIALS AND METHODS

For this research voice samples of 10 person (5 male & 5 female) with five filters including original in three voice changer apps has been calculated. The age group of the voice sample is 20-55 years.

Details of Different Apps

The transcript of the voice samples is in Devanagari script. The script is in Devanagari because Hindi is our mother tongue and it is easy to read by everyone and also easy to analyse. For analysis, different tools like Hash Cal, Gold Wave, Adobe Audition, Praat were used.

Hash Cal is used for Hash Calculation. This is essentially a set of numbers resulting from a complicated mathematical theorem. Gold wave is used for waveform Analysis. Adobe Audition is used for Spectral Analysis. Praat helps in Spectrographic Analysis.

RESULTS AND DISCUSSION

All the samples were properly arranged in a database with all the details including file name, file size, sampling rate, duration, format, mode of sample, details of recorder, hash value. Having a proper database concluded different variations in the samples.

Every filter is changing the file size, file duration, hash value of the original sample. The hash value is calculated by the software hash calculator.

In Hash cal the readings of SHA1 and MD5 is observed.

After having the details of each and every sample, it is observed that these apps are changing the audio properties of the original file.

When the details of each file are calculated, there is difference in every file. This shows that this app changes the details of original file. Here one original file's hash calculation and one modulated file's calculation is attached.

Then the file duration and sampling rate is notified from the Gold Wave app [10]. All conversions are performed by Gold Wave using Windows speech tools, so the consistency of the voice or the precision of the identification is solely

S.N	NAME	SAMPLE NAME	MALE/FEMALE	AGE
1.	Person 1	P1	Female	29
2.	Person 2	P2	Female	21
3.	Person 3	P3	Male	32
4.	Person 4	P4	Male	25
5.	Person 5	P5	Male	25
6.	Person 6	P6	Male	36
7.	Person 7	P7	Male	52
8.	Person 8	P8	Female	48
9.	Person 9	P9	Female	20
10.	Person 10	P10	Female	22

Table 1: Details of the persons

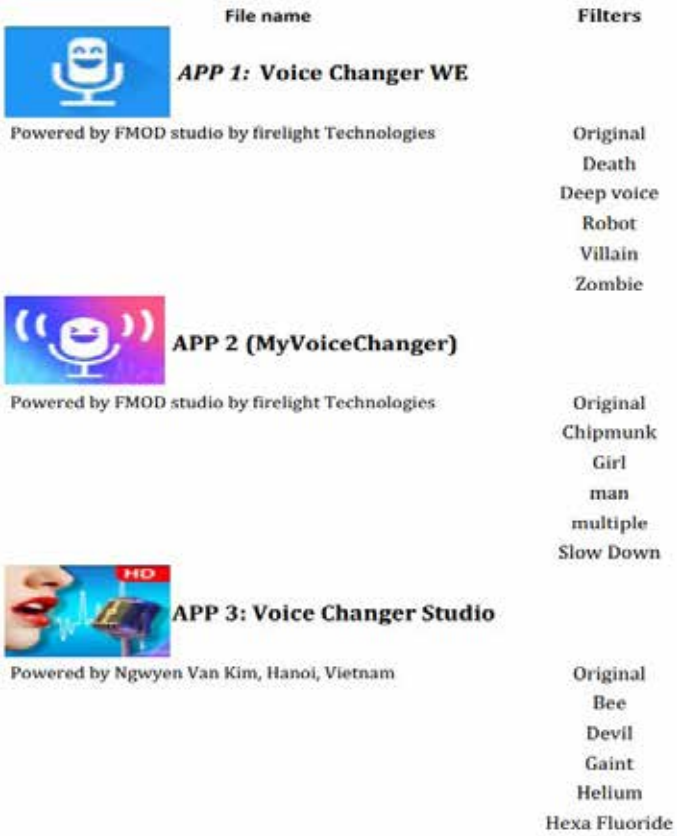

Table 2: Voice changer Apps details

FILE NAME	SAMPLE	FILTERS	SIZE	DURATION	LATENCY TIMES (ms)	
Person 6	S 31	Original	172kb	21.927	Start- 0.450	End- 0.146
	S 32	Death	234kb	29.887	Start- 0.874	End- 0.060
	S 33	Deep voice	214kb	27.363	Start- 0.759	End- 0.132
	S 34	Robot	150kb	19.119	Start- 0.390	End- 0.619
	S 35	Villain	228kb	29.127	Start- 0.579	End- 0.039
	S 36	Zombie	379kb	48.459	Start- 0.922	End- 0.084

Table 3: Difference in latency time

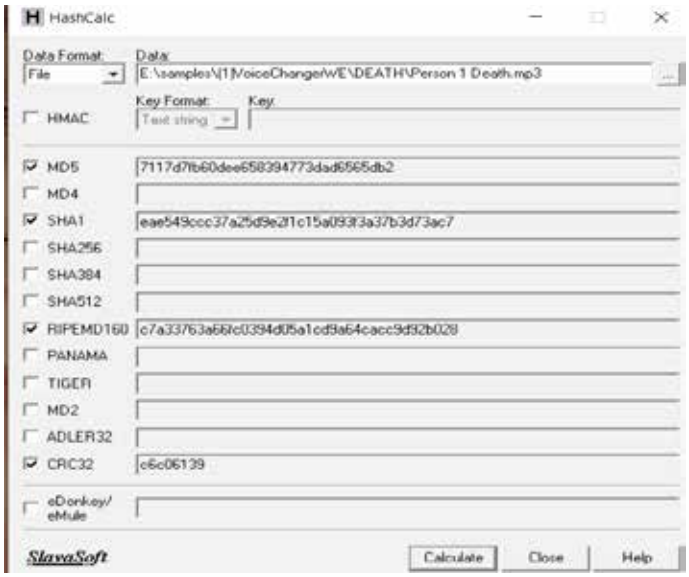

Figure 1: Representation of Hash calculations

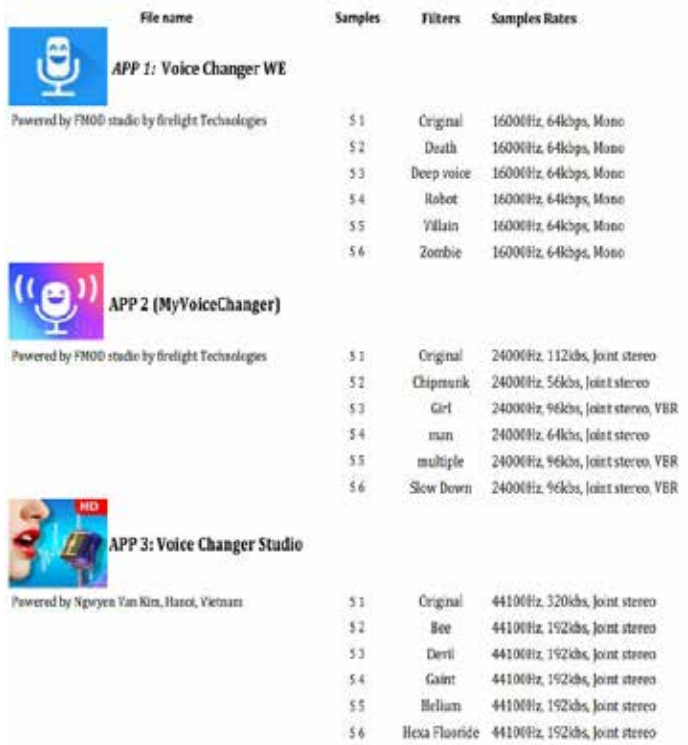

Figure 2: Difference in Sampling Rate

dependent on that software.

This result shows that there is no change in the sampling rate of the files of same voice changer app. There are changes in the sampling rate of different apps but this varies due to the app's coding.

Latency in Adobe Audition refers to the time between which an audio signal reaches a

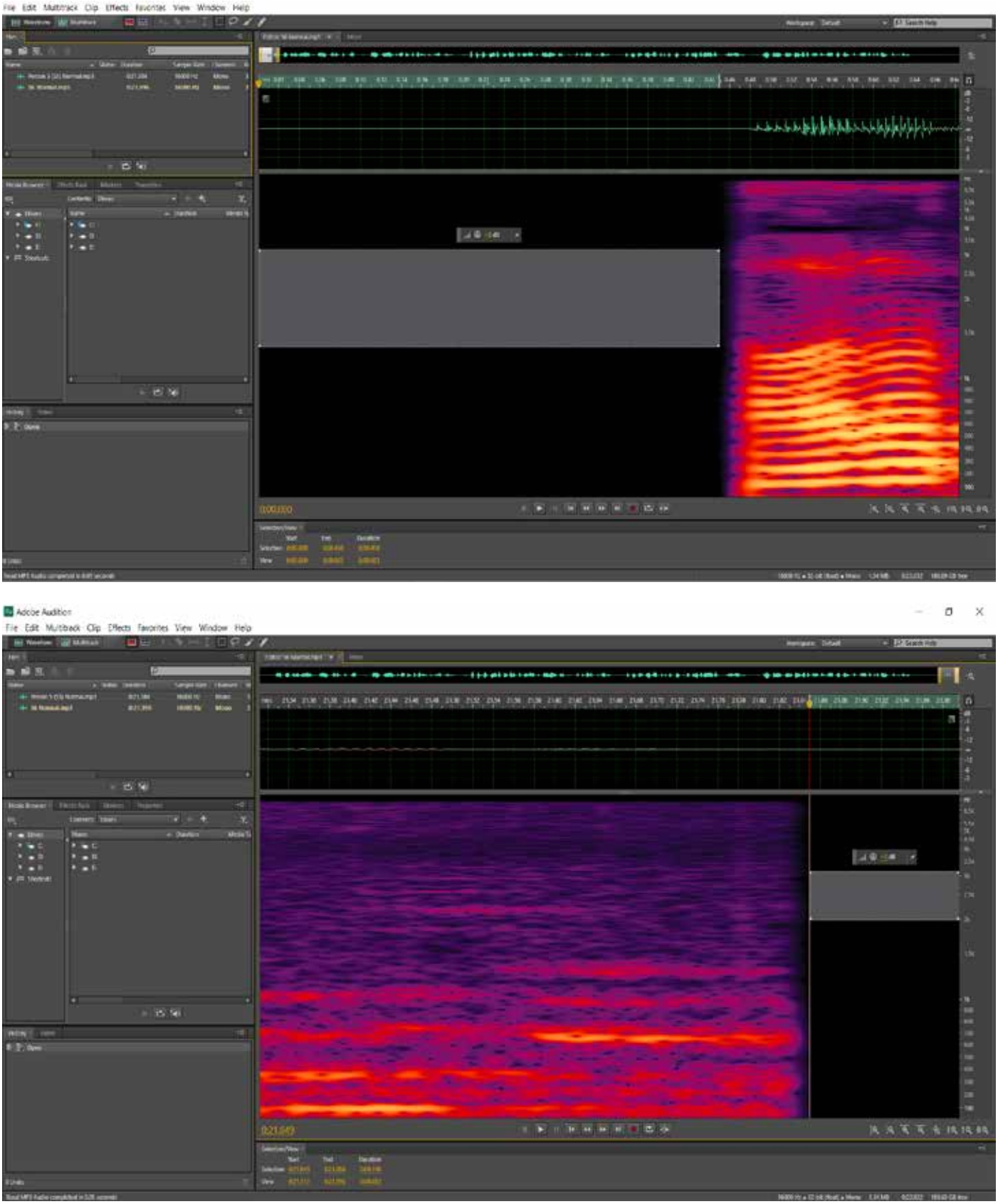

Figure 3: Calculation of Latency time.

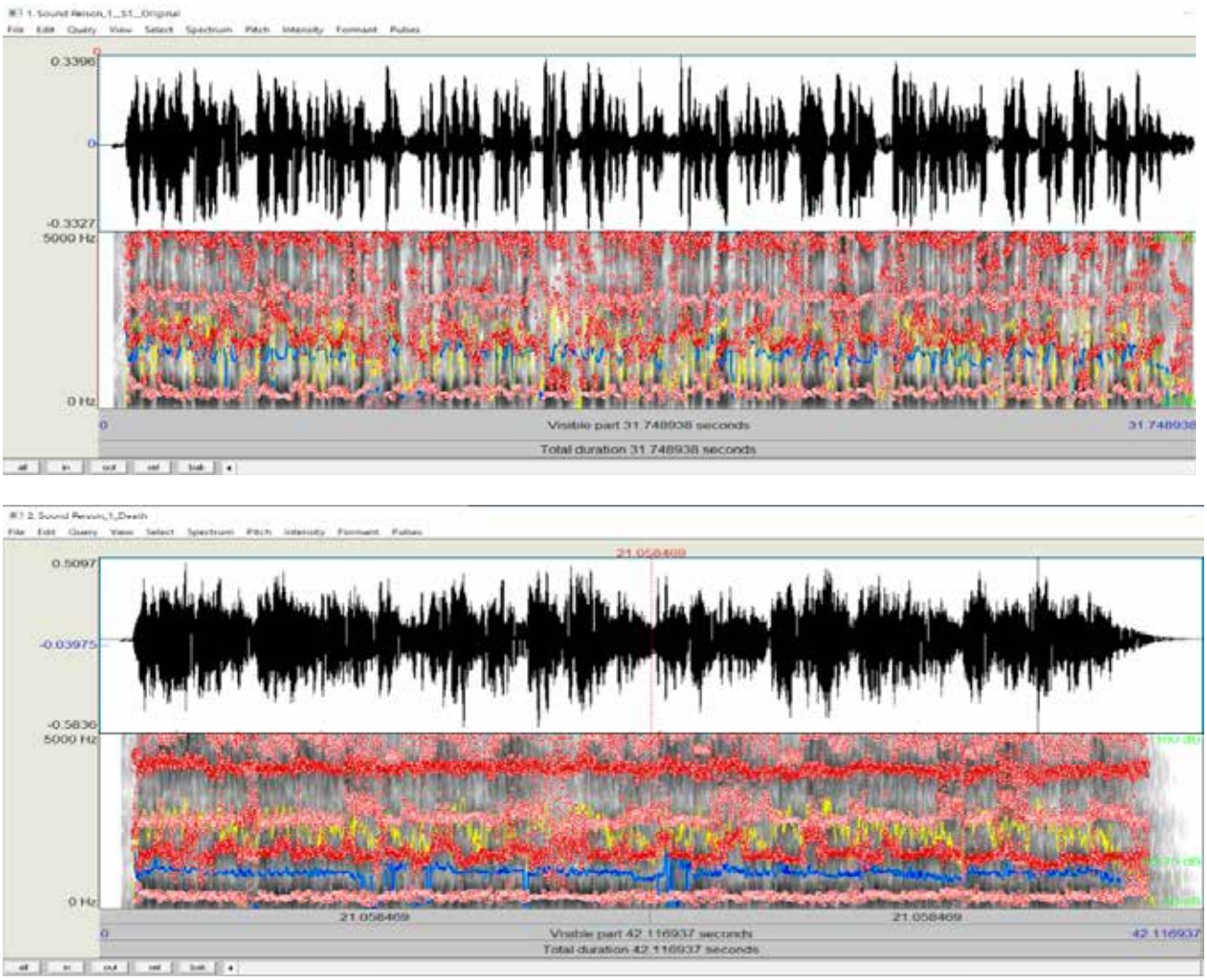

Figure 3: Spectral analysis of Samples.

device and when it returns (usually measured in milliseconds). Analog-to-digital switching, buffering, digital signal processing, transfer time, digital-to-analog conversion, and the speed of sound in the transmission medium are all potential contributors to delay in an audio system.[11,12] After analysis it is found that the latency of each sample differs from another sample. This shows that phonetic features changes.

Praat: Spectral analysis is the process of measuring or calculating the amount of acoustic energy present in a signal at various frequencies.[13,14] Note that this will change at any time, so specifying that spectral analysis applies to a certain point in time is normally necessary.

The spectral analysis shows the features of any individual's voice. The observation shows lack of differences in both of the file. This seems that identity cannot be concealed through these apps.

The results of the study are presented and discussed with reference to the aim of the study, which is to determine disguise on voice recording using voice changer app. The objectives of this paper is discussed above so here results are presented. The first objective of the research is about the features get changed through these apps. So, as per results of study, only phonetic features can be changed by the filters of this app. This means that the duration of the file, size of the file, sound effects is changed by these apps. Original

features of the person is not able to get changed. As the samples are observed under praat software, it is clearly visible, the original features is not disguised through these apps.

The second objective of paper is that it is possible for any perpetrator to hide his/her identity through these voice changer apps. Results of my study shows that it is not possible to hide one's identity through these apps. It is found from analysis that no original features of the voice is changed through these apps.

CONCLUSION

A voice changer is a software application that modifies human speech in real time. Many of these voice changers are computer software apps that can alter large chunks of language. Phone texts, voice mail, ransom demands, prank calls, and calls to emergency or police numbers are all examples of recorded fragments that may be investigated. Forensic experts can restore, recover, optimise, and interpret audio recordings using a variety of scientific instruments and procedures. For my study, recordings of the voices of ten people, using five filters, including the original, in three voice changer applications, have been collected. A variety of methods for analysis, including hash cal, goldwave, Adobe Audition, and Praat has been used.

According to the findings of this research, the filters in this app will only alter phonetic functions. These applications adjust the length of the file, the scale of the file, and the sound effects. When the samples are examined with Praat tools, it is apparent that the original features are not obscured by these applications. IJFMP

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REVIEW ARTICLE

Study on Variation in Speaker Identification under Different Conditions

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ABSTRACT

Voice is a fundamental way to communicate with people in a natural atmosphere where we come across many distortions. Speaker identification is a new boon in forensic science which is essential to identify a specific speaker and that a voice cannot be changed and it will prove that it belongs to a single individual. Some voices are naturally or accidentally distorted whereas some are intentionally distorted to disguise the identity of the speaker. The disguised or distorted voices give different values than the authentic ones. The voices can be accidentally disguised by natural environment, by being in a hot or cold atmosphere or deliberately by changing the accent, by keeping hand on mouth, by pulling cheeks, by creating nasal voice etc. The analysis of these voice samples is done by examining using software like Gold Wave, Praat and SSL (speech sound lab). The software help us to examine the voice samples right from extracting clue words to their spectral analysis which are known as spectrograms. Calculating the hash values of the samples provide another authentication to the original samples. Hash value is an alpha-numeric value which gives unique identity to the samples. Hash value has different algorithms but MD5(Message digest) and SHA1(Secure hash algorithm) are more reliable and secured, SHA1 being even more secured than MD5. The differences are made between the samples by looking at the pitch and intensity of the voice of the speakers. The pitch of the two voice samples of the same speaker can also be different because of the natural variation present in the speaker's voice.

KEYWORDS | gold wave, praat, spectrograms, hash value, MD5, SHA1

INTRODUCTION

VOICE THEORY: HUMANS communicate with each other through speech. Speech is produced by the movement of the lips and tongue. The air is pushed out of the lungs and the sound is made in the mouth or the throat. There are three main organs of speech in humans:

Respiratory: When we talk, air from the lungs goes up from the trachea or windpipe, then to the larynx. It has to pass two muscular folds known as vocal chords. If the vocal chords are separated, then the air from the lungs has a free passage into the pharynx and the mouth. But when these folds are not apart from each other, the air from the lungs make the folds vibrate.[2]

Phonatory: Phonation is defined as the vibration of vocal folds or vocal cords. When they vibrate, sound is produced and when they don't, sound is not produced. The vocal folds vibrate by the action of sub glottal air pressure and by Bernoulli's effect.[2]

Articulatory: Articulation occurs by

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the movement of the lips and tongue touching the roof of the mouth and the pharynx. The organs involved in speech production are known as articulators. There are two types of articulators:[2]

A. Active articulators: these are in motion helping in the process of articulation.

B. Passive articulators: these remain motionless and does not take part in articulation.

Sound waves: they are also known as speech sounds which may differ from each other on the basis of:

A. Pitch
B. Loudness
C. Quality

The vowels spoken may have the same pitch when they are said in the same note, they may also have the same loudness yet they differ when one vowel is said in a higher pitch or spoken more loudly than the other.[2]

When the sound waves reach the ear of the listener, they make the eardrums to vibrate.[2] This paper is based on voice recognition of different persons.

In this paper, voice samples of female speakers have been taken to analyze the difference between the various features of voice like their pitch, intensity, formant frequencies. This experiment is done with the help of Praat, SSL and GoldWave software in which different kinds of analysis have been done. The sampling is done in such a way that both original and disguised voice samples have been recorded in two ways: direct and telephonic. This research has been done to understand the difference between original and disguised voice recordings and how speaker identification in forensic science is important in solving different types of crimes happening now a days. As voice is also a biometric identifier because, like fingerprints, retina scan, palm prints etc, each voice is unique to a single individual. Therefore, an individual's voice known as voiceprint is always different comparing to the other person. And even if one tries to conceal it in some way, it is not possible for them to withhold the real qualities and characters of their voice.

Literature review

- Annu Choudhary *et al.,* (2013) proposed a programmed discourse acknowledgment framework for disengaged and associated expressions of Hindi language by utilizing Hidden Markov Model Toolkit (HTK). Hindi words are utilized for dataset separated by MFCC and the acknowledgment framework accomplished 95% exactness in secluded words and 90% in associated words.[7]
- Kersta L.G said that experimental data encourages me to believe in the fact that voiceprint could be used to make a unique identification of a particular individual. It is my opinion that perceptible uniqueness exists in each voice and that it cannot be changed by distorting or disguising and masking it will not escape identification if the speech is comprehensible.[4]
- Hirano *et al.,* did the acoustic analysis with respect to the perturbation of pitch, amplitude perturbation and energy related to noise. The evaluation efforts on the pitch, amplitude and noise energy. It emphasizes on the measurement of hoarse voices, vocal cord vibration, unsettled noise pathologies.[6]
- Bhuta *et al.,* (2004) determined the parameters related to noise of the Multi-Dimensional Voice Program (MDVP) in relation to the perceptual rating system. This algorithm or system is used to reduce the noisy background or the voice quality of the distressed voice signal. The resulting output produces the reliable, standard, consistent and valid measure against the voice pathology. The voice turbulence and soft phonation index and noise harmonic ratio for coarseness exposure and reduction of breath in the voice sample detection improvement tools are used.[5]

METHODS

The study includes samples collected from 6 female speakers and 6 voice samples from each speaker, comprising a total 36 voice recordings analyzed separately. The literature selected for sample collection is in Hindi language. The reason behind the collection of female voices is because the females have a very high pitch and it can go up to 3000-4000 Hz also. The samples were collected in two ways:

1. Direct recording
2. Telephonic recording

The samples collected are recorded under different conditions like:

- Normal voice
- Keeping cloth on phone
- Keeping hand on mouth
- Pulling cheeks
- Pinching nose making it a nasal voice

There are three types of voice samples collected for this study:

- Normal
- Disguise 1
- Disguise 2

The tools used in the study are:

1. Mobile recorder
2. High quality head phones
3. Hash calculator
4. Gold Wave software
5. Praat software
6. Speech Sound Lab

High quality headphones are used to hear the voices clearly with minimum background noise interference.

Gold Wave software has been used to extract the cue words from the voice recordings shown as spectrums. The original voice recordings were in .mp3 format which was then converted to .wav format. It was resampled from 48000Hz to 11025Hz and the bandpass was adjusted from 200-4000db. This is done because the normal hearing range of humans is 20-20,000 Hz.

It is used to show a difference between the normal and disguised voice and to find out if they are from the same speaker or not. Gold Wave has many features including:[3]

- Real time graphic visuals like bar, waveforms, spectrograms
- Basic and advanced filters like noise reduction, volume enhancer, effects like resampling, bandpass etc.
- It can support different file formats like .mp3, .wav, Ogg, FLAC, AIFF etc
- Supports large file editing

Praat is used for the spectrographic analysis of the voice samples. Praat provides different features including:

- Pitch
- Intensity
- Resonating frequency
- Formants
- Pulses etc

It has been used to study the differences between the pitch, intensity and the formants

SL. NO.	CUE WORDS	WAVE FILE: P1 S1(A).wav			WAVE FILE: P1 S1(C).wav			WAVE FILE: P1 S1(D).wav		
		FROM (SEC:MSEC)	TO (SEC:MSEC)	DURATION	FROM (SEC:MSEC)	TO (SEC:MSEC)	DURATION	FROM (SEC:MSEC)	TO (SEC:MSEC)	DURATION
1.	nahi	0.537	0.779	0.241	1.789	2.030	0.241	0.949	1.189	0.241
2.	haar	0.848	1.020	0.172	2.089	2.261	0.172	1.275	1.447	0.172
3.	rahi	1.054	1.248	0.194	2.296	2.491	0.194	1.486	1.680	0.194
4.	nirantar	2.279	2.697	0.418	4.098	4.517	0.418	3.100	3.519	0.418
5.	jaal	5.381	5.621	0.240	7.250	7.490	0.240	6.235	6.475	0.240
6	pura	5.684	5.945	0.261	6.965	7.226	0.261	6.569	6.829	0.261
7	liya	6.324	6.578	0.254	8.298	8.552	0.254	7.149	7.402	0.254
8	kar	7.397	7.544	0.147	9.618	9.765	0.147	8.181	8.329	0.147
9	raja	7.539	7.787	0.248	9.751	9.999	0.248	8.237	8.575	0.248
10	baar	9.836	10.057	0.221	11.857	12.077	0.221	10.257	10.487	0.221
11	yaad	14.905	15.115	0.209	16.362	16.572	0.209	14.957	15.166	0.209
12	Jo	15.896	16.077	0.181	17.384	17.565	0.181	15.794	15.976	0.181
13	usse	16.101	16.331	0.230	17.550	17.780	0.230	15.954	16.185	0.230
14	bada	17.085	17.278	0.193	18.410	18.603	0.193	17.036	17.229	0.193
15	gayi	18.043	18.224	0.181	19.242	19.424	0.181	17.950	18.162	0.181
16	jab	19.015	19.184	0.169	20.181	20.349	0.169	19.006	19.175	0.169
17	nah	20.607	20.706	0.099	21.296	21.395	0.099	20.197	20.296	0.099
18	tak	21.182	21.309	0.127	22.099	22.226	0.127	21.039	21.166	0.127
19	bina	22.094	22.295	0.201	22.906	23.107	0.201	21.848	22.048	0.201
20	lagataar	22.407	27.883	0.476	23.168	23.644	0.476	22.076	22.552	0.476

DIRECT

CUE WORD	FORMANT	Sample File (Normal)	Sample File (Disguise-1)	Sample File (Disguise-2)
nahi	F1	541	548	565
	F2	**1486**	**1529**	**1162**
	F3	2055	2281	2457
	F4	2866	3060	3048
haar	F1	882	883	741
	F2	**1496**	**1486**	**1482**
	F3	2091	2402	2090
	F4	2913	3059	2998
rahi	F1	454	540	451
	F2	**1893**	**1958**	**1678**
	F3	2232	2253	2188
	F4	2741	2805	2764
nirantar	F1	483	515	490
	F2	**1646**	**1848**	**1721**
	F3	2055	2281	2457
	F4	2866	3060	3048
jaal	F1	767	713	720
	F2	1431	1477	1771
	F3	2229	2844	1790
	F4	2969	3038	3061
poora	F1	591	545	593
	F2	1141	1211	1421
	F3	2253	2249	2593
	F4	2969	3038	3209
liya	F1	586	474	443
	F2	1573	1923	1429
	F3	2325	2306	2550
	F4	3005	3012	2887
kar	F1	585	668	692
	F2	1621	1827	1766
	F3	2488	1885	2310
	F4	2845	2848	2754
raja	F1	781	668	692
	F2	1490	1513	1626
	F3	2401	2810	1785
	F4	2942	3239	2906
baar	F1	808	794	775
	F2	1512	1426	1229
	F3	2718	2779	2422
	F4	3219	3490	2680
yaad	F1	859	729	850
	F2	1585	1585	1710
	F3	2199	2148	2357
	F4	2822	2892	2619
joh	F1	543	583	525
	F2	1747	1772	1718
	F3	2317	2848	2591
	F4	2726	2952	2978
usse	F1	541	617	559
	F2	1457	1419	1235
	F3	2517	2313	2275
	F4	3014	3032	3118
bada	F1	638	677	564
	F2	1714	1677	1546
	F3	2133	2295	2285
	F4	2958	3051	2718
gayi	F1	555	449	452
	F2	1856	1988	1640
	F3	2432	2355	2632
	F4	2806	3069	3117
jab	F1	700	660	586
	F2	1877	1918	1750
	F3	2690	2686	2059
	F4	2762	3172	2886
nah	F1	821	700	762
	F2	1752	1567	1258
	F3	1911	2415	1984
	F4	2926	3122	3075
tak	F1	601	810	765
	F2	1784	1651	1614
	F3	2549	2825	2301
	F4	2887	3042	2960
bina	F1	512	667	497
	F2	1952	1430	1425
	F3	2220	2223	2386
	F4	2395	3004	2892
lagatar	F1	677	671	571
	F2	1421	1494	1104
	F3	2645	2703	2387
	F4	3019	3397	3048

TELEPHONIC

CUE WORD	FORMANT	Sample File (Normal)	Sample File (Disguise-1)	Sample File (Disguise-2)
nahi	F1	509	601	500
	F2	**1661**	**1540**	**1611**
	F3	2756	1970	2431
	F4	3126	3220	3475
haar	F1	807	716	733
	F2	**1661**	**1540**	**1611**
	F3	2756	1970	2431
	F4	3126	3220	3475
rahi	F1	446	539	504
	F2	**1845**	**1762**	**1731**
	F3	2529	2408	2271
	F4	2928	2863	3227
nirantar	F1	605	553	680
	F2	**1750**	**1674**	**1453**
	F3	2485	1809	1936
	F4	3195	3237	2861
jaal	F1	788	836	768
	F2	1686	1606	1367
	F3	2085	2186	1723
	F4	3216	3130	3003
poora	F1	424	483	495
	F2	1503	1244	1130
	F3	2364	1752	1781
	F4	3136	3105	2936
liya	F1	425	463	611
	F2	1676	1198	1221
	F3	2558	1712	1912
	F4	3085	2887	3137
kar	F1	606	640	600
	F2	1583	1637	1415
	F3	2233	2505	1846
	F4	3031	2867	2639
raja	F1	779	805	788
	F2	1704	1539	1469
	F3	3213	2229	2228
	F4	3711	2885	2912
baar	F1	980	769	836
	F2	1726	1737	1640
	F3	3009	2218	2601
	F4	3766	2534	3133
yaad	F1	936	870	775
	F2	1825	1701	1769
	F3	2406	2679	2476
	F4	3240	2831	3953
joh	F1	515	559	541
	F2	1067	1423	1228
	F3	2963	2538	2310
	F4	3007	3546	3934
usse	F1	410	492	504
	F2	1543	1567	1538
	F3	2478	3117	2623
	F4	3334	3686	3322
bada	F1	713	625	650
	F2	1564	1480	1432
	F3	2488	2314	2305
	F4	2901	3218	2926
gayi	F1	534	459	522
	F2	1834	1884	1085
	F3	2591	2355	2632
	F4	2991	2970	2783
jab	F1	552	486	545
	F2	1785	1637	835
	F3	2529	2545	1634
	F4	3140	3085	2955
nah	F1	586	702	727
	F2	1711	1549	1409
	F3	2881	2886	2783
	F4	3140	3849	3576
tak	F1	556	672	570
	F2	1683	1701	1614
	F3	3007	2951	2672
	F4	3694	3848	3960
bina	F1	290	344	722
	F2	1761	1632	1804
	F3	2826	2782	3441
	F4	3113	3555	3617
lagatar	F1	443	700	529
	F2	1506	1446	1430
	F3	3102	2599	2669
	F4	4133	3988	3847

varying even in the voice samples of the same person speaking in a normal and disguised way. This study contains four different values of the formants (F1, F2, F3, F4). The value of F2 has been considered standard.

The formant frequencies of the speaker are mentioned in the above table. There is variation in the formant frequencies because of intraspeaker variation. The formants—F1, F2 and F3—are the intensification of the frequencies in the spectrum and indicates the resonance of the vocal tract. The first two formants are ample to label the said vowel.

The spectrograms taken using spectrographic tools have been examined and the values of pitch, intensity and all the formant frequencies have been noted down. The blue line represents pitch, yellow lines indicating intensity and the red dots are the formants. The black patches indicate the vowels. Each formant frequency is set to a similar value and then the desired formant value is remarked down. This procedure is reiterated for all the four formants. This spectral analysis adds another validation to the fact that each of the 6 voice samples taken from every speaker is same for each specific speaker. The values of F3 and F4 have been found to be very high. This has been seen mostly in the vowels I and E. The value for vowel A is comparatively lower than I and E. The formant frequency of the vowel U and O is low too.

DISCUSSION

The spectral analysis was done for all the speakers and their respective voice recordings. But only the result and data of a single speaker has been mentioned in this paper. The rest of the work was done in the same way.

The concept of spectrographs depends on the Fourier theorem. Putting into practice of the technique depends on the refined use of the electronic filtering or on the approach of complex computational algorithms. The Fourier theorem affirms that any periodic waveform can be analyzed into a series of sine waves with a number of frequencies, amplitudes and phase relationships.

The most conjoint method for spectrographic filtering of the speech signal are the bandpass filters that conduct frequencies within lower and higher range of frequencies passing. The lower and higher limits of the bandpass are demarcated in those frequencies where reduction is compared to the center of the band. These are known as the cut off frequencies of the filter. Filters with narrow bandpass are lethargic or inactive in their response whereas wide band filters respond in a very swift manner. Their time resolution is quite good except for their frequency resolution, which is very poor.[3]

The spectrum of an acoustic wave is basically the result of a Fourier analysis of the waves under examination, i.e., it is a proclamation of what frequencies are present and what their amplitudes are. Each frequency component (harmonic) of the wave is represented by a line sited approximately positioned on the frequency axis. The height of each harmonic line shows its amplitude in dB.[4]

The graph is not continuous and there are no points between the harmonics. The square waves are composed of discrete frequency components. The top of the harmonic lines cannot be joined together to form a continuous and a smooth curve. The blank spaces or the blank lines imply the absence of frequencies and not the absence of any data. This type of spectrum is known as line spectrum. [11,12]

The formant frequencies of the speaker are mentioned in the above table. There is variation in the formant frequencies because of intraspeaker variation.

These are the values of pitch and intensity of all the female speakers mentioned above in the table. The pitch of the female speakers is naturally very high. The average pitch and intensities have been noted down for each voice sample of each speaker individually to show that they vary each time even in the voice samples of the same speaker due to natural variation among them.

Cue words are similar-sounding words which have been selected from all the voice recordings (normal, disguise 1, disguise 2) for one speaker and this process is repeated for the rest of the speakers. This has been done for both ways of recordings (direct and telephonic). The clue words have been selected on the basis of CV (consonant-vowel), CVC (consonant-vowel-consonant), CVVC (consonant-vowel-vowel-consonant) format. The analysis is based on picking up the same words

available in the voice sample of a speaker. This is done for every possible word found from the samples. This authenticates that the particular voice sample belongs to a particular speaker.

Forensic speaker identification is given more importance nowadays than it was earlier. It is now argued that voice print identification is as valuable as fingerprint identification. By looking at different features of speech and by analyzing it on different software, it is concluded that voice print identification is a unique and helpful research tool in cases such as ransom calls, tapped phone conversations, etc. This experiment is based on mining of cue words of the speaker of all the 6 voice samples recorded directly and telephonically for each speaker. The analysis is based on selection of the similar sounding words available in the voice samples of the speaker. To substantiate the results found from gold wave, it then has been analyzed on SSL and Praat, to get a spectrographic analysis of the recorded samples. The spectrograms show pitch, intensity and the formants clearly as different colored lines. The varying values of the pitch, intensity and the formants have been determined and it shows that they vary even for the same specific speaker, too. This article concludes that all the 6 voice recordings of a specific person has been matched and they belong to the same person. This applies for the rest of the samples recorded by different speakers. The physical parameters mentioned above in the objectives responsible for distorted voice samples are:

- Bad throat condition
- Due to cold and cough
- Stammering in speech which may be original or fear-induced
- Some people tend to talk with a nasal tone

But these are the accidental distortions caused in a voice sample, as some people try to change their voice so as not to reveal their true identity by disguising it, but however hard one may try to hide one's real voice, one cannot succeed. With the help of above-mentioned tools, the individual features and the difference between original and disguised voice is acquired. IJFMP

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REVIEW ARTICLE

Offline and Online Handwriting Analysis: A Comparative Review

Vinny Sharma[1], AK Jain[2], Prashant Johri[3]

ABSTRACT

Handwriting analysis dates back to the late 1800s when the persons with knowledge and experience used to analyze the handwriting sample of the author. With the increase of knowledge of the subject and its need in the domain of justice system handwriting analysis, it grew from being an informal subject to an academic subject. Handwriting analysis has been readily used in forensic science investigations. It is one of the major branches of forensic science after fingerprint and DNA wherein maximum cases reports are sought after. The cases received for handwriting analysis are from both the domains of the legal system – civil and criminal. By realizing the importance of handwriting analysis and its further implications in the field, many researchers have tried to include the usage of computer technology for the purpose of analysis. This approach still has no approval in the legal system wherein the accepted method is the offline or manual mode of analysis. This current paper is an approach to first explain in detail both the modes of handwriting analysis – offline (manual) and online (computerized). For online handwriting analysis, different approaches are also reviewed. Along with this, a comparative analysis has been drawn between the two approaches to study the pros and cons of both.

KEYWORDS | forensic science, handwriting analysis, handwriting comparison

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INTRODUCTION

Handwriting is a skill which is a combination of perceptual-motor task, generally denoted as a neuromuscular activity. Accomplished handwriting movements are natural that one is persuaded to oversee their intricacy. Handwriting instigates in the brain where a mental image of figures and letters is formed. Then neural signal duplicates this mental image and sends it to the muscles of the arm and hand via the means nervous system. The resultant output is virtually never a precise match of the original mental image. (Will, 2015)

The chirography of an individual - the manner of writing - is specific and unique to an individual, which is in terms of the size, shape, and manner of execution of letters, style of penmanship, and other ornamentations which are specific to his or her writing chirography, which is uniquely distinctive of his or her writing from others. It has been a well-known fact that the handwriting of any individual begins at the age of two where the child scribbles using a writing instrument and establishes his or her handedness – left, right or ambidextrous. By the time the child learns to imitate different shapes by copying different strokes

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INTRODUCTION

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According to Hilton (1982) and Huber (1999), each individual's ability to replicate the letter formations varies from other individuals and this ability is significantly dependent upon the author's perception of the letter or figure image and his or her capacity to replicate the same via the usage of their muscular motion. In other words, each individual has his or her own cognitive capability which significantly affects their handwriting ability. Also handwriting is an art that is perfected through thorough practice and constant repetition. Once an individual has perfected the art of imitation, he/she starts deviating from the copy-book form and start embedding their own individual characteristics into their handwriting, making the art of handwriting a habit and an action of his/her subconscious brain, which repeats the letter formation every time in the same manner. (Mehta, 1970)

Since handwriting is highly individualistic, it has been used for the purpose of identification of individuals. Handwriting is the most common medium of communication among people and has slowly grown into human society as an easy means of identification. With the increased usage of handwriting as a means of authentication, the handwriting of forgery began at a faster rate for committing crimes. Thus, the need for establishing a scientific method for the analysis was needed which was proposed by A.S. Osborn, 1929 and was later modified by O. Hilton 1980, who gave the cardinal rules of handwriting identification and individualization for the purpose of handwriting analysis laying down the foundation of scientific methods for handwriting analysis.

These methods were solely based on the examination of the general and class characteristics of handwriting. It was propounded that the general handwriting features are the style characteristics, which are affected by the penmanship of the writer and individual characteristics are the features which are greatly affected by the chirography of an individual and are highly individualistic in nature which can be used for establishing the individualization of the author in the case of disputed or questioned identity (Sharma, 2018).

Although the popular practice for the analysis of handwriting is the usage of the manual or offline mode of examination based on the identification of similar or dissimilar general and individual handwriting characteristics, there is a copious research work available based on the online mode of handwriting examination, wherein different algorithms are devised to perform the handwriting identification. Along with this the methods of online examination of handwriting includes the methods of online character recognition, text-mining and, author attribution. (Dweik, 1986).

LITERATURE REVIEW

Manual or offline mode of handwriting recognition, examination, and analysis involves the evaluation of both general and individual characteristics which include slant, alignment, skill, speed, movement, proportion of letters spacing, rhythm, the proportion of letters, size of letters, amongst others. Individual characteristics include ornamentation, formation of letters,

t-cross bar (position, placement, and length), i-dot (placement, position, shape), use of pet phrases, punctuation marks, amongst others.

According to Harrison, Burkes and, Seiger in 2009, an author's identification can only be decided when both the components feature – general and individual – are equally used in combination to establish the authorship with no significant fundamental differences and verified natural variations.

In 2018, Sharma propounded that the analysis is carried out to establish the authors' identity by employing adequacy in characteristics analysis, authenticating natural variations and integrating both the general and individual characteristics together.

These observations are in accordance with the cardinal rules of handwriting identification viz., 1. Individualization of handwriting, and 2. Identification of handwriting, both sets of rules consist of 5 rules each. (Hilton, 1980)

Rules of Individualization of handwriting are stated as follows:

1. Each and every matured author has a handwriting, which is personal and individualistic to him or her alone.
2. Deterioration of a writer's writing which can be due to any cause will affect all of the handwriting characteristics and will not be limited to changes in one or two elements of writing.
3. Any author cannot exceed his or her maximum writing capability or skill without putting in tremendous effort and practice over a significant period of time.
4. Any type of attempted disguised or forgery will lead to a substandard quality of handwriting and never superior quality.
5. Rule of natural variation.

Rules of Identification of handwriting are stated as follows:

1. The uniqueness of handwriting is the basis of all identification.
2. Any handwriting is recognized by the amalgamation of all its characteristics and qualities, including both those derived from the writing movement – the general handwriting characteristics and those related to form – the individual handwriting characteristics.
3. Handwriting standard samples – admitted or specimen – are necessary to identify the writer's normal writing ways and also to establish the degree of natural variation of his or her handwriting.
4. To establish that any specimen sample of handwriting was written by an exact person the expert has to prove that all the identifying features are a part of his or her normal handwriting and additionally the variation within the specimen sample is covered in the range of natural variation of writing.
5. The rule for the fundamental difference.

From the foregoing observation , it can be justly assumed that a developed and matured writing is a product of a long period of variation and adaption in accordance with the wants and capabilities of the author which are highly specific to an individual. Due to the complex nature of the development of handwriting habits of an individual, the probability that two different individuals might have similar handwriting which when compared and analyzed with each other will not show the presence of dependable dissimilarities is extremely insignificant. (Ellen, 1997)

Although this is so, it does not mean that comparison and analysis of different samples of handwriting to establish the authorship is easy or forthright. The comparison and analysis of handwritings can never be skillfully achieved by placing the parts of jig-saw puzzle to solve it. The method involves thorough and repeated observations of natural variations and fundamental differences when the samples are compared keeping in mind the factors which affects the handwritings of any individual. (Harrison, 2012)

It has been widely advocated that the handwriting examination is at a cross-roads, due to the subjective nature of the analysis and examination methods and thus poses challenges to its scientific nature. Currently, a copious amount of research has been conducted where computer-based tools are being devised to form a scientific basis for handwriting identification.

These tools come in handy when the analysis is to done for comparison of handwriting and provide visual assistance to the handwriting expert

along with providing an automated response for the degree of match or variation between the suspected and questioned handwriting samples, which, in turn, verify the subjective analysis report or the report generated via manual examination by the expert. Along with this these tools help in creating a database of all the samples examined and analyzed which can be accessed when the authorship of unknown samples is to be determined. There are varied tools available which employ either the usage of image-based input method or real-time data collection method for the purpose of recognition of author (Leedham, 2003). Mentioned below are some of these tools:

1. **FISH** – Forensic Information System Handwriting: this system was made available to the experts in the 1990s, which was developed by the German law enforcement. This system was designed to effectively support the experts to retrieve the closest match of the handwriting under question from the copious database the system was supporting. The drawback of this system was it did not generate any scientific report of the analysis it made.
2. **WANDA Architecture:** FISH was succeeded by another system which was jointly developed by the researchers from Germany, Dutch, and the United States of America. This was an open-source generic architecture-based framework devised for handwriting examination and signature recognition and identification. This system was commonly known as WANDA Workbench. The framework of this system enabled the integration of different systems, like, English, German, Kanji, Arabic, etc.
3. **Cedar-Fox System:** This system was devised by US-based researchers for the purpose of handwriting examination and individualization. This system integrated the tools for handwriting examination and means for automated operations too. When it was made to function in automated mode, it was able to perform author verification, author identification, and signature matching. The identification was done using the available database of standard handwriting samples and a report was generated which was based on quantitative measures of similarity. (Leedham, 2003)
4. Louloudisa, Gatosb, Pratikakisb, and Halatsisa in 2008, proposed another method, the text line detection method, for handwritten documents. In this technique, they based the detection in three distinct steps viz., 1. Image binarization and enhancement, 2. Usage of block-based Hough transform for detecting the potential text lines and 3. To separate vertically connected characters and assign them to text lines. The performance evaluation of the proposed approach is based on a consistent and concrete evaluation methodology.
5. Verma and Sharma in 2017, proposed a zone identification algorithm and used it to perform online handwriting identification of Gurmukhi characters. Here, they grouped the strokes forming the characters into separate zones and each zone is identified using their allotted support-vector-machine model.
6. Raju, Moni, and Nair in 2014 advocated a novel approach wherein they used a combination of GBF–RLC (which is gradient-based features & run-length count). They used this approach on Malayalam script using a database of 19,800 handwritten characters. Their recognition accuracy was 99.78%. In their approach gradient of image was the intensity of each point and RLC was the count of contiguous group of 1's encountered when the image was scanned from top to bottom and left to right.
7. Lutf, You, and Li, in 2010, came up with another approach for the identification of authors of Arabic handwritings. Their study was conducted using 287 writing samples. They approached the identification problem by first separating the document into letters and diacritics. Then these diacritics were extracted and used to calculate the LBP histogram for each diacritic, which were then concatenated and used handwriting characteristics.
8. Vaidya and Bombade in 2013 presented another method wherein they have used the positional feature extraction method which was primarily based on the positional properties of each pixel present in the image of the character. Via this approach, they reported an accurate assessment of Devanagari and Kannada scripts up to 82.89%, and 85.62%, respectively.

RESULT & DISCUSSION

By nature, humans can never function with constant regularity and precision compared to what machines are for, and due to this, natural variation is part and parcel of each standard handwriting sample which will be provided for the purpose of examination and analysis. This is one of the major reasons that the handwriting sample of one single individual will not be the exact replica of his or her previous writings and will possess some degree of natural variation in them.

With this comes the expert who has to use his or her expertise to perform the assessment whether:

1. the differences observed in the handwriting are a natural variation or are fundamental differences making the authorship different, or
2. when the differences found are falling in the range of natural variation then advocating and provide apt reasons for why the differences observed are in actual the natural variation and the authorship belongs to one individual only. (Saudek 1978: 235)

In addition to the natural variation, there are other factors too that influence the changes in the letter formation of an individual's handwriting. These factors can be summarized as follows:

1. The type of writing instrument, writing material, and writing surface used: ink, pen, writing material, writing surface, etc.
2. The author's ability of realistic maturity.
3. The author's speed of handwriting.
4. The system of handwriting learnt by the author.
5. The author's nationality.
6. The author's degree of visual sensitivity and compliance.
7. The author's power of graphic appearance.
8. The author's chirography, vanity, artifice, and wish to copy others.
9. The author's familiarity with foreign script, special training, education, etc.
10. The author's physical and emotional condition.
11. Any chronic physical impairments the author may have. (Ron Morris, 2000)

Along with these the other factors such as age, ailment or wound; medicine, intoxication, alcohol, drug withdrawal, uncooperative writing position, temperature, weather conditions, fatigue, carelessness, content of the document, using of unaccustomed hand or attempted and/or disguise, need to be assessed while establishing an opinion as these factors greatly influence the natural handwriting of an individual. (Harrison, Burkes and Seiger, 2009)

Collectively, due to these reasons, the opinion of a handwriting expert is subjective in nature and is thus liable for human error. As a measure of judgment is called for, it follows that the comparison of handwriting must be, in a part, a subjective process and consequently liable to human error (NISTIR 8282, 2020).

Purohit *et al.,* in 2016, propounded that handwriting recognition is a growing topic of research in the arena of pattern recognition, and machine learning, as its applications are in varied fields. Both OCR (Optical Character Recognition) and HCR (Handwritten Character Recognition) have been gaining acceptance amongst the researchers. There are vivid techniques and algorithms which have been proposed and advocated for to be used as handwriting recognition systems. But the majority of studies are focused on how to convert the textual form present on a paper into computer-readable form. Table 1 summarizes

SYSTEM	ACCURACY	PURPOSE
Hand Printed Symbol (HPS) Recognition Method	97%	It extracts the geometric, topologic and local dimensions essential to recognize the letter
OCR for cursive script	88.8%	It implements S&R (segmentation & recognition) algorithms devised for cursive script.
Recognition of handwritten numeral based upon fuzzy model	95% for Hindi and 98.4% for English	The aim is to utilize the fuzzy technique to recognize handwritten figures for Hindi and English figures
HC (Hill Climbing) algorithm meant for handwritten character recognition	93% for upper-case letters	To implement hill climbing algorithm intended to select feature subset
Identification of Arabic alphabets and figures by using optimization feature selection	88% for figures & 70% for alphabets	To implement the method used for feature selection in an optimized manner.

Table 1 Comparison between different online handwritings

S.No.	Method	Advantages	Disadvantages
1	Convolutional-Neural-Network Method	• If Convolutional-Neural-Network is skilled, the accuracy of image recognition is high • This method has been employed to recognize different scripts.	• If Convolutional-Neural-Network is skilled, the accuracy of image recognition is high • This method has been employed to recognize different scripts.
2	Semi-incremental method	• The time taken for analysis is not visible • It studies both the latest & previous strokes	• Should be accompanied by other methods too • IT functioning is more complex than the incremental-method.
3	Incremental method	• The identification process is way simpler than the semi-incremental method	• Problem is encountered when the segmentation step is practised.
4	Line and Word Segmentation Method	• It is an effective method for printed forms of documents	• Cannot detect patterns
5	Part Based Method	• It is an effective method for handwriting recognition	• It can be made more accurate by increasing the sample size training period.
6	Slope-an-Slant-Correction Method	• Due to the simpler segmentation process the accuracy of this method	• It is not best approach for handwriting identification
7	Ensemble Method	• Highly accurate	• The step of Line segmentation of characters effect accuracy rate
8	Zone Method	• Highly accurate	• The image must have higher number of zones to get more accurate results.

Table 2: Pros and Cons of Different Methods

the comparison between different online handwriting recognition systems available on the basis of their accuracy and Table 2 summarizes the advantages and disadvantages of different techniques proposed by different researchers. (Rosyda, Shofia, Purboyo, and Waluyo, 2018).

CONCLUSION

Though there is huge evidence from the copious research which is in favor of the usage of handwriting recognition via online mode, there is a huge resistance from the handwriting experts in using this approach for the purposes of handwriting recognition. The major reasons cited for this resistance are from the cardinal rules of handwriting identification, which specifically mentions that the handwriting of any individual is affected by their age, the type of writing instrument used, the type of writing surface used, along with this the purpose for which the content is being written. It has also been promulgated that the handwriting is greatly influenced by the emotional condition of the author. The experts advocate that these factors will not be considered by an algorithm while performing the examination, making it a tool for performing preliminary analysis or for narrowing down the list of suspects. The usage of the online tools is also found to be limited to the general handwriting characteristics, whereas for forming a confirmatory opinion with regard to the authorship of any handwriting holds its ground on both general and individual characteristics.

The major issue being encountered in the online recognition system is how to recognize and classify the image of the handwritten matter for the purpose of analysis and comparison. Another area wherein the research needs to be done is how to make the computer think like the human brain and make it count the various factors which may affect the handwriting of an individual. This will make the recognition more effective as then the online system will be able to differentiate between natural variation and fundamental differences which is the cardinal rule for comparison of handwriting samples. In the near future, the online mode might serve as a key component of the handwriting comparison process as it will be providing a quantitative report. These systems will also be playing a key role in digitizing the existing paper documents. IJFMP

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REVIEW ARTICLE

Gender Identification Based on Handwriting Characteristics

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ABSTRACT

In the field of criminal justice, any testimony, no matter how minor or trivial, cannot be ignored, as supplementary evidence has always turned the case around. A common form of evidence is documentary proof. Document exhibits come in a number of formats and can be handwritten, digital, or printed. Handwriting may be used as a starting point for attempting to identify uniqueness. Different people have different writing patterns, which is a natural act. Even if the person deliberately wants to alter, certain characteristics are peculiar to a single person. On the basis of class and individual characters, an attempt was made to differentiate between male and female handwriting. In a case where the investigation involves handwriting, this can be a valuable piece of supplementary evidence. It can also be used to exclude possible suspects. With the writers using the same writing instrument and surface, over 200 samples were analyzed based on 6 parameters, and many of them yielded positive results in deciding whether the writers were male or female. The observation can be used as a first step in the field of Graphology in terms of gender identity.

KEYWORDS | handwriting, individual characters, class character

INTRODUCTION

Document is an object that has symbols, signs, and marks that conveys a message. Content of the documents is either partially visible or invisible. Questioned Document can be defined as a document whose authenticity or source is suspected or disputed. In 1910, Albert Osborn wrote a book on Questioned document in which he referred to the characteristics of handwriting that can be used for analysis. Any disputed document that is utilized in civil and criminal court is referred to as Questioned Document (Tripathi *et al.,* 2020).

The father of handwriting examination, Ordway Hilton, in 1956, wrote a book "*Scientific Examination of Questioned Document*", in which he mentioned some of the essential rules of handwriting examination and identification. Forty years later, William Harrison in 1996 showcased modern evolution in Questioned document and handwriting examination, used for verifying the writer of the document. Writing is an action in which the making of each word and letter is usually automatic, writers generally focus on the main matter instead of the process of writing. Writing is a combination of habitual patterns and innumerable

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subconscious [1]. Handwriting is a judicious procedure, which is obtained through comparing writing habits and handwriting characteristics.

Diverse elements that have an impact on handwriting are:

- Age
- Illness
- Physical or Mental disturbance
- Writing position
- Writing surface
- Writing instrument
- Lighting
- State of Drunkeness

Various Investigators:

Vikram Kamath *et al.,* in his investigation, discussed the conduct of an individual was anticipated through a mechanized handwriting examination framework. The handwriting was broken down through picture handling in MATLAB. Eight qualities highlights of penmanship were gotten to evaluate the character of personality, utilizing calculations and manual investigation. The framework created could be utilized for the location of Parkinson's infection and disease. It can likewise be utilized in record credibility and untruth recognition (Kamath *et al.,* 2011).

Nazeer and Shabaz, (2019) observed in their investigation chipped away at handwriting tests of 100 individuals having various considerations and differing states of mind. SSGBSAT calculation was utilized for the examination of seven assumptions (scorn, outrage, camouflage, bliss, tragic, tear, and shock). The investigation uncovered the pace of genuineness of seven assumptions present in an individual, while he/she was composing the content. The investigation could anticipate the adverse conduct of any individual so that advising could be given in the beginning phase.

Luria and Sara (2011) found in their investigation "A mechanized multidimensional estimation of mental responsibility through penmanship examination" discussed penmanship conduct which was utilized to decide mental responsibility in an individual. Three thematic movement of various troubles were composed by 56 members on a digitizer. Contrasts in worldly, spatial, and precise separating were seen to decide low and high responsibility in the individual. [7]

Gawda and Nikitha (2015) showed in their examination discussed the connection between clinical conclusion and graphological investigation to discover the connection between character qualities and the appearances in handwriting among kids by utilizing Children's Personality Questionnaire. The writer detailed that there were no solid contrasts between the psych diagnostics evaluation of character through Children's Personality Questionnaire and the handwriting examination.

Kenshin and Ohsawa, (2015) in their examination zeroed in on discovering the relationship between reasoning time and composing time in handwriting of 50 undergrad members, by utilizing computerized pen (Hitachi Maxell, DP-201). The writer noticed two sorts of gatherings in his analysis. The first took a long time in reasoning and more limited time recorded as a hard copy and the other one took more limited time in reasoning longer time recorded as a hard copy. The gathering taking longer time recorded as a hard copy showed 'back-following' which at last brought about the expanded nature of thoughts.

Dang and Kumar (2014) utilized different boundaries for the expectation of conduct of a person by utilizing PC. The boundaries utilized are pen pressure, letter development 'I' and 'f', letter incline which were contribution to the 'Counterfeit Neural Network' which helped in forecast of human conduct. The device utilized for the assessment of tests was 'MATLAB'.

Mavrogiorgou and Jucket (2011) observed in their examination they considered hand engine execution among 44 members of which 22 were patients with over-the-top enthusiastic issues (OCD) and 22 were well-being controls. The creator identified unobtrusive engine brokenness in OCD by utilizing digitizing realistic tablet and saw that the tedious engine design execution among patients with OCD was better in contrast with the sound controls.

Frank and Rejean (1989) in their examination they learned about handwriting investigation and closed the exploration on robotized composed language acknowledgment and investigation goes back quite a few years report with straightforward designs can be perceived dependably by off the

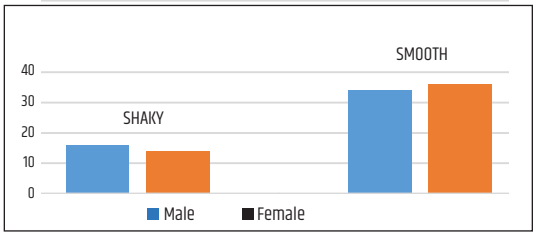

Figure 1: Graph showing line quality in handwriting

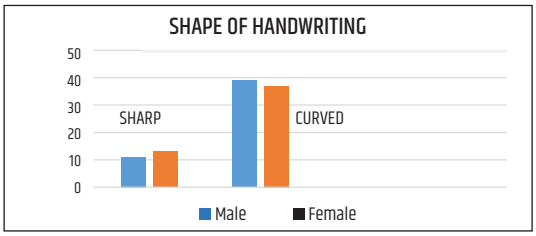

Figure 2: Graph showing shape of handwriting

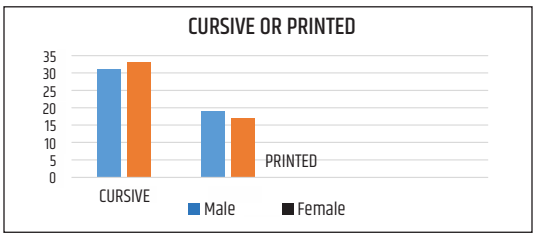

Figure 3: Graph showing style of writing

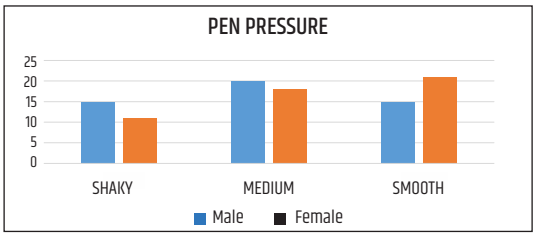

Figure 4: Graph showing pen pressure

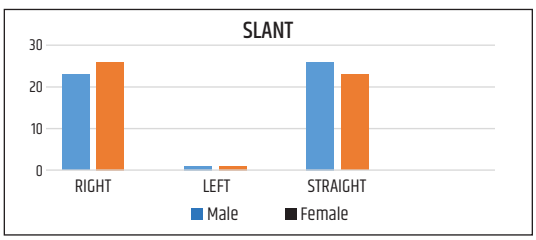

Figure 5: Graph showing Slant in handwriting

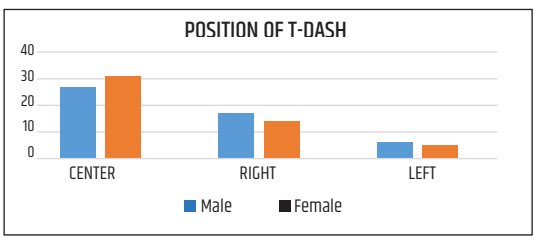

Figure 6: Graph showing T-dash position

rack or programming.

METHODOLOGY

For the comparison of samples, 100 samples of handwritings were taken which include 50 samples from males and 50 samples from females. The handwritings were taken from the age group 20-40 years. All samples are written using ball pens and the same surface of writing was provided.

Before the collection of samples, all the people were briefed about the collection process and their concerns were taken. Also, no samples are taken from people with any kind of mental disease.

Examination of samples:

Handwriting analysis involves a comprehensive comparative analysis of the writer. Specific habits, characteristics, and individualities of both the male and female samples are examined for similarities and differences.

- Analysis - The first step is to analyze the writing sample for distinctive characteristics.
- Comparison - The sample of males and females would be compared.
- Evaluation - The final step is to look at how male and female handwritings are different from each other.
- The sample card is given below. This card has London Letter. It is a paragraph that has all the letters from A- Z. the writers wrote on this sample card.
- The second page of this sample card has the 6 characteristics on which the sample has been evaluated.

The characteristics are as follows:

- Line quality - line quality describes if the writing is smooth, shaky or juvenile.
- Shape - handwriting shapes can be conical or curved as shown in the figure.
- Cursive and printed letter - the type of letter the person uses.
- Pen pressure - If there are no impressions on the backside of the paper.
  Medium – If there are light impressions on the

backside of the paper.

Heavy – If there are clear impressions on the back of the paper. This pen pressure can be judged on a few surfaces only such as paper, leaves, etc.

- Slant - The direction of the writing. It can be a right slant, left slant, or straight.
- Diactric Placement - It defines the position T-dash.

RESULTS AND DISUCSSION

Line Quality

According to figure 1, in the case of males out of 50 samples, 16 males have shaky handwriting and 34 have smooth handwriting.

In the case of females out of 50 samples, 14 have shaky handwriting and 36 have smooth handwriting.

If we look into this characteristic, we can see that comparatively, males have shaky handwriting and females have smooth handwriting as shown in Figure 1.

Shape of Handwriting

According to figure 2, in the case of males out of 50 samples, 11 males have sharp handwriting and 39 have curved handwriting.

In the case of females out of 50 samples, 13 have sharp handwriting and 37 have curved handwriting.

In this case, comparatively, females tend to have sharp handwriting and males have curved handwriting as shown in Fig. 2

Cursive or Printed Letter-

According to figure 3, in the case of males out of 50 samples, 31 males have cursive handwriting and 19 have printed handwriting.

In the case of females out of 50 samples, 33 have cursive handwriting and 17 have printed handwriting.

According to the data females use cursive letters more and males use printed letters more as shown in Figure 3.

Pen Pressure

According to figure 4, in the case of males 15 have light, 20 have medium and 15 have heavy pen pressure.

In the case of females 11 have light, 18 have medium and 21 have heavy pen pressure.

A significant difference can be seen in the case of pen pressure.

This data indicates that females apply more pen pressure than males as shown in Figure 4.

Slant

According to figure 5, in the case of males, 23 have a right slant, 26 have straight slant and 1 have a left slant.

In the case of females, 26 have right slant, 23 have straight slant and 1 has left slant. Comparatively very few people use left slant. According to the data, females use right slant more and males have straight slant as shown in Figure 5.

Diacritic Placement Position of T-Dash

According to figure 6, in the case of males out of 50 17 have T-dash at right, 27 have T-dash at center and 6 have T-dash at left.

In the case of females out of 50, 14 had T-dash at right, 31 had T-dash at the center and 5 had T-dash at left.

Not many had T-dashes at left. More females have T-dash position at the center and males have more T-dash position on the right as shown in Figure 6.

CONCLUSION

Document is an object that has symbols, signs and marks that convey a message. They are either visible, partially visible or invisible. Questioned Document can be defined as a document whose authenticity or source is suspect, questioned or disputed. In 1910, Albert Osborn wrote a book on Questioned document in which he referred to the characteristics of handwriting that can be used for analysis. Any disputed document that is utilized in civil and criminal court is referred to as Questioned document.

Documentary evidence is one of the most important supportive evidences. In the field of Forensic Science, every minute detail counts. Handwriting is the most common documentary evidence found. Handwriting can help us identify the individual and can also tell us about the writer's mental status. This work can help us identify the gender of the writer. As each individual carry both individual and class characters of handwriting, both genders have different characteristics of writing. The samples are collected and examined carefully on the basis of individual and class

characteristics of both male and female. The results have shown some positive results. The differences can be due to difference in their wrist and hand movement. Also studies prove that there are psychological factors which differentiate male and female handwriting. This study can be used for gender identification but one can't be 100% sure. Other supplementary evidence should also be looked at. This study shows that there is a significant difference between male and female handwriting. IJFMP

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REVIEW ARTICLE

Computational Examination of Signatures Using Digimizer

Geo Mariyam Joseph[1], Suneet Kumar[2]

ABSTRACT

Forensic Document Examination has become more diverse, which require authenticity or validation in determination of genuineness or non genuineness, to reveal forgery, alteration, addition, deletion, and personal identification. Signature identification is a most challenging mission in the field of forensic questioned document examination. Its aim is to determine forged signatures by matching the unknown signature with known signature. Signature is a handwritten deception of a person that engraves on document as a proof of uniqueness. A person's signature serves as a trademark. It is generally a person's most common writing act and such is largely habitual. Signature of a person may be constituted of only letters, or with letters as well as non-letter patterns, or may be constituted only with non-letter patterns. In this modern computational era, computational approach to handwritten signature is more relevant due to its accuracy, less time consumption. This paper efforts to give computational software used for identification of signature samples. The objective of the study was to identify the natural variation occur to signature with the help of image analyzing software "Digimizer". The efficiency of the proposed method is based on results of 100 writers with 4 signatures of each writer.

KEYWORDS | signature identification, characteristics, digimizer

INTRODUCTION

Document is anything that bears mark, sign, or symbols which have meanings or convey any message to someone. According to Section–3 of Indian Evidence Act, 1872 Document is defined as "any matter expressed or described upon any substance by means of letters, figures, or marks or by more than one of those means, intended to be used or which may be used for the purpose of recording that matter. Section-29 of Indian Penal Code defined Document as "any matter expressed or described upon any substance by means of letters, figures or marks or by more than one of those means, intended to be used or which may be used as evidence of that matter.

Questioned Document is any document whose authenticity is disputed or questioned; it is also referred as Forensic or Disputed Document. In 1910, Albert S Osborn who is known as the "Father of Questioned Document" authored a classic book "*Questioned Document*" in which he described the characteristics of handwriting which can be used for the examination.

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Handwriting is that the writing through with an editorial instrument like pen or pencil on any writing surfaces. Majority of people write with their hand;however, some are not able to use their hands in order that, they write with their foot and mouth etc. Handwriting is a neuromuscular activity in which, hand, foot, mouth etc. is merely a device with which instruction send to it by the brain are administrated. Writing includes each printing and running handstyles and is separate from proper calligraphy or typeface. Because to each adult's handwriting is unique and totally dissimilar, it will be want to verify author of document. Writing could be a deliberate action during which making of each letters and words are generally automatic, writer commonly focus on the matter instead of the process of writing.

A signature could be a written illustration of someone that engraves on documents as a symbol of individuality and intent.[13] A person's signature assists as a trademark. Signature is generally a person's most typical writing act and intrinsically is essentially habitual.[12] As handwriting signature is also a neuromuscular task and they each possess certain intrinsic characteristics that are unique for a person and therefore recognizable. The formation of signature will characterized as - initial in which a signature is created of letters only, second in which a signature is created of certain letters as well as certain non- letter patterns and third in which a signature is created of non- letter patterns only[14]

Writing variation in handwriting will be influenced by:

- Health of the author
- Psychological state of the author
- Declining health
- Advanced age, Illness
- Intoxication and drug use
- Writing surface and Writing instrument
- Writing position
- Nervousness

Handwriting identification is that the process of analysis, comparison and evaluation of questioned document with best known writing. Handwriting and signature examination is conducted on the premise of class and individual characteristics of writing.

Characteristics of Genuine Signature

No one ever signs identically constant from one time to another. There is some variation from signature to signature due to temporary effect of either intrinsic or extrinsic character, however, their overall construction remains same. This makes signature highly identifiable. We can identify genuine signature from fraudulent one by examination of some characteristics such as:

- Smoothness, and Fluidity
- Careless execution
- No Pen lifts and Pen Pause
- No tremors unless the writer is ill
- Variations

Class and Individual Characteristics of Handwritting

General or class characteristics of handwriting are those writing features which are common to a group. Class characteristics can be classified as – movement, pen pressure, pen presentation, speed, skill, slant/slope, shading, spacing, alignment, relative size, ratio, line quality.

Movement is that the motion of writing instrument with the action of hand on the writing surface. Author might use finger, wrist, elbow or shoulder and their combinations. Pen pressure is that the weight or pressure involuntarily applied to the writing instrument throughout the act of writing; writing created with a nib pen can clearly show the impact of applying different quantity of pressure; writer can be categorized as light, medium and heavy pen pressure. Pen presentation is the angle of the pen with the writing surface or with the line of writing; the best writing is produced when the angle is 45 degree; this angle can be measured by drawing tangent to the stroke and measuring its angle with baseline. Speed is the time it takes a writer to execute a handwriting; speed can be fast, medium or slow. Skill is the ability or quality learned through repetition till it become habit to do something, the act of writing could be a skill learned through repetition until it become habit; skill can be categorized as poor, medium or good. Slant/slope is that the leaning of axis of letters relative to perpendicular to baseline of writing; slant can be vertical, backward and forward. Shading is that the conscious or voluntary act of applying pressure to the pen whereas competitive sure strokes. Spacing is that

the amount of space between letters, words and line of writing. Alignment is that the arrangement of writing on the imaginary or actual line; some writing stay above the baseline, some stay below the baseline, some stay on the baseline. Relative size is the evaluation of size of letters. Ratio is the relation between height of tall and short letters; if tall letters are many times longer than the small letters the ratio is high, where difference between long and short letters is less the ratio is low. Line quality is defined as the smoothness, evenness, continuity, and directness of strokes.

Individual characteristics are writing features which are individual to each person. It happensonce a letter leaves from its normal copybook form. Individual characteristics are deviations from the structure of writing trained and later on are strong identifying characteristics when comparing handwriting samples. Individual characteristics are often considered as:

- Consciously acquired habits
- Subconscious acquisitions

Individual characteristics are classified as: I dot, J dot, loops and circle formation, formation of initial, terminal and medial stroke, formation of connectors, embellishments, arch, bow, cap, crossbar, cusp, eyelet, hiatus, hook, retrace, spur, shoulder, trough, ampersand, bar etc.

For the study of computational analysis of signature of different individuals, samples from various individuals should be available. These samples are collected from 100 individuals both male and female between the age of 18 – 45 years. Efforts were made to obtain a natural way of signature formation from individuals.

Collection of Sample

- After taking consent signature are collected from individuals
- Samples of signature are collected from 100 individuals (both male and female) between the age of 18 – 45 years old
- From each individual 4 signatures were collected

Examination of Sample

- Samples were scanned on a hi-res scanner at 300 dpi
- Examination of signature was carried out using the image analyzing software, "Digimizer"
- Scanned image is then subjected to extract significant features like: size of letter, slant, area, angle, alignment and cursiveness
- Result is calculated on the basis of observation made during examination

Instrumental Processing

Digimizer is auser-friendly and flexible image analysis software that permits exact manual measurements as well as automatic object detection with measurements of object characteristics. Images may be X-rays, micrographs, etc. Supported file formats are .jpg, .gif, .tiff, .bmp, .png, .wmf, .emf and .dicom files.

Experimental Setup for Examination

Each image of sample was gathered to make a data set of 100 signatures. The figures were transformed into an even format and saved in a single folder. For each signature their x, y coordinates were extracted and each coordinate were calculated to estimate the values for the parameters selected for this study. Formula for corresponding study as shown in Table 1 below:

Sr. no	Characteristics Observed	Formula for Calculation	Classified as
1	Slant	$\theta = \frac{(y1 - y2)}{(x1 - x2)}$	Right slant if, Θ> 90 Left slant if, Θ < 90 Vertical slant if, Θ = 90
2	Size of letters	$size \frac{y\sqrt{(x1-x2)2+(x1-y2)2}}{Average\ vertical\ distance\ of\ letter\ on\ x,\ y\ plane}$	Small Medium Large (based on unit in millimeter)
3	Alignment	$Tan\theta = \frac{Base}{\text{Hypotenuse}}$	Horizontal Uphill/Upward Downhill/Downward
4	Cursiveness	$Cursive = \frac{Horizontal\ Length}{No.of\ pen\ downs}$	Low Medium High
5	Angle of letters	Calculated by software	Acute Obtuse Right

Table 1 Formulae for Respective Study

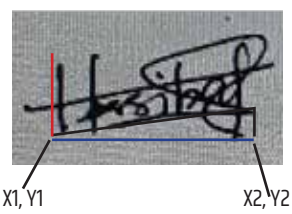

Figure 1: Coordinates of Signature at X and Y Plane

Related Studies

Vaibhav Saran in his study of "Computational Method for Forensic Verification of offline Signatures" analysed signatures through image processing in MatLab. Six characteristics features of signature were obtained to identify the genuineness of individual using pixel values based on coordinate geometry concept for forensic significance. The system could be used for classification of signature based on distance and angle.

Sargur N. Srihari "Computational Methods for Handwritten Questioned Document Examination" discussed about computational approach to handwriting Questioned document examination using algorithms. He analyzed nine characteristics features to access the uniqueness of writing in individuals.

Thameur Dhieb "Towards a novel biometric system for forensic document examination" discussed online handwriting examination through geometric characteristics. Segmented online handwriting into strokes and use uncertain perceptual elements to describe the writing of individuals. The system could be used as an authoritative instrument for online writer verification on Latin and Arabic scripts.

Antonio Parziale in "An Interactive Tool for Forensic Handwriting Examination" proposed a tool for quantitative examination of handwriting features for forensic Questioned documents. Four characteristics features of handwriting were examined through algorithms to recover the sequences of strokes, the tool automatically produces computed features value in both numerical and graphical form.

Ameur Bensefia "Handwritten Document Analysis for Automatic Writer

Recognition" in his study discussed writer identification and writer verification using PSI database and IAM database. He concluded nearly 96% correct verification of handwritten documents and writer verification.

Sargur Srihari Graham Leedham in "A Survey of Computer Methods in Forensic Handwritten Document Examination" proposed his study in the part of forensic handwriting examination with the help of software tools such as CEDAR-FOX system, FISH system, WANDA architecture.

Characteristics	Observed
Size of Letter	Small - 6% Medium - 4% Large - 90%
Alignment	Uphill - 73% Downhill - 16% Horizontal - 11%
Sland	Right - 6% Left - 4% Vertical - 90%
Angle	Acute - 6% Obtuse - 4% Right Angled - 90%
Cursiveness	Small - 57% Medium - 31% Large - 12%
Area	Small - 52% Medium - 46% Large - 2%

The system developed could be used to verify the degree of match between a questioned and non-document. The system can also be used in examination of white-collar crime such as check fraud by automatic and semiautomatic signature processing.

Raul Sanchez-Reillo "Forensic Validation of Biometrics using Dynamic Handwritten Signatures" studied the examination of signature created during the process of signing are seized via the dynamic signature biometric mode. The application is fully operational which decodes the information stored on the screen with chronological signals, linear and angular measurement can also be attained.

RESULTS

The study on signature analysis conducted between the 100 individuals from both male and female between the age of 18 – 45 years old to determine the class characteristics and natural variation of signature using computational examination.

After analysis of signature, nearly 90% of individuals have large size of letter, 6% of individuals have small size of letter and 4% have medium size of letter. On alignment, 73% of individuals have upward alignment, 16% downhill alignment 11% horizontal alignment. Moving on to slant, 54% had right slant, 41% had left slant and 5% had vertical slant. About angle of writing, 56% of individuals had acute angled signature formation, 43% have obtuse angled signature formation, only 1% of individuals shows right angled signature formation. Talking about the cursiveness of signature, 57% of individuals shows low cursiveness, 31% shows medium cursiveness and 12% shows high cursiveness. While analyzing area of the signature, 52% of individuals shows small area of signature formation, 46% shows medium area and only 2% shows large area of signature formation.

DISCUSSION

The study on signature analysis conducted between the 100 individuals from both male and female between the age of 18 – 45 years old to determine the class characteristics and natural variation of signature using computational examination.

After analysis of signature nearly 95% of individuals have large size of letter, 2% of individuals have small size of letter and 3% have medium size of letter. For alignment 73% of individuals have uphill or upward alignment, 16% downhill or downward alignment 11% horizontal or straight-line alignment.

Moving on to slant 54% of individuals have right or forward slant, 41% have left or backward slant and 5% have vertical or straight-line slant. About angle of writing, 56% of individuals have acute angled signature formation, 43% have obtuse angled signature formation, only 1% shows right angled signature formation.

About the cursiveness of signature, 57% of individuals shows low cursiveness, 31% shows medium cursiveness and 12% shows high cursiveness. While analyzing area of the signature 52% of individuals shows small area of signature, 46% shows medium area and only 2% shows large area of signature formation.

CONCLUSION

The signature samples were examined carefully with the help of Digimizer. Observation is based on the examination done by class characteristics and natural variation of signature by taking x and y coordinates. The class characteristics analyzed for examination are size of letter, slant, angle, alignment, cursiveness and area. The computational method is based on coordinate geometry concept help to verify natural variation in signature.

It concluded that most of the individuals have large size of letter formation, individuals with small and medium size of letter formation are almost similar in ratio. Most individuals form their signature in uphill or upward alignment, individuals with horizontal and downhill or downward alignment shows nearly similar ratio.

Most individuals having forward or right slant and backward or left slant, only few individuals show vertical or straight-line slant formation. Most individuals have acute and obtuse angled formation of signature, least number of individuals shows right angled signature formation.

While examining cursiveness of signature the greatest number of individuals shows low cursiveness, and least number of individuals shows high cursiveness, number of individuals shows high cursiveness lies in between the above two levels. The individuals showing medium and small area of signature is higher than that of individuals showing large area of signature.

In this study, we can say that the class characteristics and natural variation in signature can be examined with the help of coordinate geometry in computational analysis. IJFMP

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REVIEW ARTICLE

Latent Fingerprint Impressions Visualisation on Different Surfaces Using Burnt Paper Powder

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ABSTRACT

A fingerprint is a unique impression left by the friction ridges from fingers of our hand. The type of fingerprints at a crime scene is an important evidence in the field of forensic science. Many unique characteristics of the human body are like the fingerprints: our DNA, palm print, iris pattern, voice etc. So a fingerprint is a unique characteristic of the human body and every human has different fingerprint. This is true even in identical twins. Mayer, a German doctor and anatomist, was the first to write that friction ridge skin is unique. The fingerprint is the most important evidence at crime scenes for identification of the criminal. It offers clinching evidence in the criminal justice system. For latent/invisible fingerprint impressions recovery, we used mainly five types of Burnt Paper Powders to easily recover the impression by using this new method. Fine powder of burnt paper combined with fatty-acid and oil present in the sweat of a fingerprint even latent fingerprint patterns can be seen using burnt paper powder on the surface. Studies show that it delivers perfect results on various surfaces with clear fingerprint impression and ridges.

KEYWORDS | fingerprint, burnt paper, powder, human, surfaces

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INTRODUCTION

French scientist Edmond Locard gives us the "principle of exchange". Whenever two entities come in contact, mutual exchange of traces takes place. Based on this principle, finding invisible fingerprint impression is one of the most important hidden types of evidence at crime scenes. A fingerprint is an imprint left by the friction ridges of the human finger. Dermatoglyphics is a branch of Forensic science that deals with the analysis of skin ridge impressions.[1-3] The surface layer of human skin is protected by a variety of natural substances secreted by the eccrine, apocrine, and sebaceous glands. The main discharge from these glands is water. However, equally inorganic and organic substances are also secreted. We all know that the unique characteristics in our human body such as our DNA, fingerprint, voice, iris, etc. The most important thing about fingerprint is it is unique. In the criminal justice system, fingerprint evidence is primary evidence at the crime scene.[4-7] The fingerprint features like unique, permanent, universal, classifiable. Fingerprints of twins are also different. Fingerprint can be used in personal identification system. The idea was first proposed in 1858 by the District Magistrate of Hooghly District of Bengal, Sir William Herschel. Later, Dr Henry Fades found that criminals could be discovered from latent prints found at the crime scene and came to the conclusion that two fingerprints cannot be the same. Fingerprint examination has been the keystone of forensic examination for over 100 years. Three main types of fingerprint may be found at the crime scene:

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1) Visible or patent, 2) Invisible or latent, and 3) 3D fingerprint.[8,9]

The crime scene investigators often follow a 2-stage process when searching for fingerprints at the crime scene. In the first stage, they look for Visible or 3D prints. If they are not visible, a flashlight is often used for this. The second stage involves a blind search for Latent prints.[10-15] The Latent print impression recovery at crime scene is one of the most challenging parts of Forensic science because that invisible physical evidence can often help in the reconstruction of the scene. Nowadays, every forensic expert in India uses chemical, physical and instrumental method for the detection of Latent fingerprint impression. Basically, we invented a new method to detect latent fingerprint impression at the crime scene.[16-19]

METHOD & MATERIALS

In the present study, the latent or invisible fingerprint impression of nine samples were collected and developed to the various porous and non-porous surfaces i.e., plywood, paper, plastic box, door, marble tiles and on-porous plastic surface like non-porous wooden surface, iron, non-stick utensil, leather, mirror, stainless steel. The following surfaces developed by the burnt papers powder. We used four different types of burnt paper powders:

1. The one 200 × 210 mm of thick white paper and powder is formed light gray color.
2. The one 200 × 210 mm of thick white sheet and power is formed dark gray color.
3. The one 420 × 594 mm of thin printed newspaper and powder is formed dark black color.
4. The one 420 × 596 mm of thick printed newspaper and powder is formed ash color

This technique was used in the development of invisible or Latent fingerprint impression with the use of above-mentioned brunt paper powder. Then we used the most commonly applied technique for the secrets of latent prints which is "powder dusting method". The method is used on the suspected area and then the fingerprint powder is sprinkled over the suspected area and the powders attached to the oils and other constituents of the sweat left in a fingerprint. So based on the Locard principle, the burnt powders have very fine particles to put on the suspected area at crime scene. We used camel hair brush to develop the Latent fingerprint sample. After developing the fingerprint impression, Forensic photography was taken and a cellophane tape was put on the various contrast area and fingerprint pattern is clearly visible which type of print is there, it's clearly identified and preserved the using powder sample. The fine particles of the powder adhere to the print, hence the ridge characteristics are clearly visible.[20-25]

The results of the present study show nine samples of invisible fingerprints impression which

easily show the positive results developed in different porous or nonporous surfaces with the help of burnt paper powder. Due to very small and smooth fine particles of the burnt papers powder, pattern and ridge characteristics are clearly observed and identify the fingerprint impression pattern and their ridge features can be seen in figures.[1-7] The four burnt paper powder is used on the surfaces, for black surface we can use light color and for white surfaces we can use dark color, then the fingerprint impression is clearly visible. The fingerprint impression identified, and examination is positively done with the burnt paper powder. Related to these burnt paper powders of different parts, it states that it provides better results on opposite surfaces with testing the powder.

After developing the fingerprint impression by using burnt papers, the impression remains visible up to 72 hours. This research paper is based on the findings of Latent fingerprints which use a physical technique based on the behavior of fingerprint powder adjoining with fatty acid and oily component present on the sweat are deposited on the fingerprints and ridges. These results indicate a new way of identifying latent fingerprint impressions on different surfaces using burnt paper powder.

CONCLUSION

For the current research, any paper available at places like home, school, college, office, etc., can be used. So, we used burnt paper powder to develop latent fingerprints. In this process, nothing is high cost. In many cases, forensic experts do not have latent fingerprint development powder when they reached the crime scene. So, burnt paper powder can be used to develop latent fingerprint to solve major crimes. By using the burnt powder the result is developed on various surfaces during crime scene investigations. This type of research work has not been reported previously and can provide useful information to the investigators in case of unavailability of systematic traditional fingerprint development powder. This kind of powder can easily help visualize latent fingerprint impressions at the scene of crime. IJFMP




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REVIW ARTICLE

Role of Fluorescent Substances in Development of Latent Fingerprints: A Review

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ABSTRACT

Fingerprints are one of the crucial pieces of evidence in criminal investigations. As fingerprints are majorly found in latent form on the surface, it is necessary to develop those latent fingerprints appropriately without destroying them or leaving out any detail. There are plenty of methods for identifying hidden finger impressions. The powder dusting method, ninhydrin, iodine fuming, cyanoacrylate etc., are traditional methods. Though these procedures help to develop the latent fingerprints, they have some drawbacks such as sensitivity, low contrast, and selectivity as well as high toxicity. To overcome this inadequacy, advanced techniques have been developed using different chemicals and nanoparticles such as metal nanoparticles, fluorescent nanoparticles embedded with quantum dots, rare-earth upconversion nanoparticles etc. The fluorescent nanomaterials enhanced all three levels of fingerprints and provided the minute details of latent fingerprints. This review paper focuses on the role of fluorescence-based methods used for the enhancement of the latent fingerprint and their advantages over traditional methods. As compared to other approaches, fluorescent nanomaterials can obtain optimal contrast quality, enhancement, better sensitivity, and selectivity while exhibiting lower toxicity, less autofluorescence interference, and low background inference.

KEYWORDS | lie detector, forensic evidence, polygraph, narco test

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INTRODUCTION

Fingerprint impression is the most crucial evidence in criminal investigation. Fingerprints can be left at the crime scene when a person touches an object or surface. It helps in identifying an individual whether the suspect was present at the crime scene .[1] Sweat from the pores on the friction ridge skin of the hands leave finger impressions on objects and surfaces. Pores abound in the ridges of the fingers. Sweat from these pores is deposited in the form of outlines when the fingers touch any surface or object. It acts as a replica of the ridge patterns of the fingers.[2] Sweat is colorless; so when it is deposited on a surface it leaves behind colorless impressions known as latent fingerprints. Secretions from the eccrine (sweat), sebaceous, and apocrine glands on the hand, head, and nose make up latent fingerprint residues. Sweat consists of 0.5 percent minerals, approximately 0.5 % organic compounds, and 98 percent water. Sugars, urea, creatinine, Proteins, uric acid, amino acids, lactic acid, and choline make up eccrine sweat, while wax esters, glycerides, sterol esters, fatty acids, and squalene make up sebaceous sweat.[3]

Latent fingerprint impressions which are present at the crime scene, need to be developed using an appropriate technique. The powder method has been used most widely to develop fingerprint impressions at the crime scene. The powder method includes metallic and

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magnetic powders as well. These methods also possess certain disadvantages like high toxicity, background interference, contrast, selectivity, and less sensitivity with fewer development of all the ridges of finger impressio.[4] Apart from the traditional powder methods, there are chemical techniques that have been used like ninhydrin, iodine fuming but just like powder methods these techniques also have drawbacks.[5]

To overcome this inadequacy, researchers have been exploring many new techniques to enhance the quality of hidden finger impressions and make it cost effective, and providing better efficacy to assist in the investigation by identifying the suspect. The advanced techniques have been developed using different chemicals and nanoparticles such as metal nanoparticles, fluorescent nanoparticles embedded with quantum dots, rare earth upconversion, etc.[6]

Fluorescent Nanomaterials

The fluorescent nanomaterials enhanced all three levels of fingerprints and heightened the minute details of hidden finger impressions. As compared with other approaches, fluorescent nanomaterials can obtain optimal contrast quality, enhancement, better sensitivity, and selectivity while exhibiting lower toxicity, less autofluorescence interference, and low background inference. Oily substances are found in dormant finger impressions due to the normal human habits of touching the hair and face. As a result, if a fluorescent reagent would diffuse into the oily substances without rubbing out the hidden finger impressions with the substrates, it could be counted as an adaptable probe for latent finger impressions fluorescence imaging.[7] Strong fluorescence can be generated by fluorescent materials that have engrossed precise electromagnetic radiation such as ultraviolet, near-infrared, or visible light.[8] When working with multi-coloured or reflective surfaces, where contrast can be an issue with conventional fingerprint powders, the use of fluorescent methods to produce hidden finger impressions has an inexpensive benefit.[9]

Characteristics of Fluorescent Nanomaterials

Due to their peculiar ocular characteristics, the use of fluorescent nanomaterials like up conversion nanomaterials and quantum dots for the enhancement of latent finger impressions has gotten a lot of attention.[10] When fluorescent nanomaterials are utilized for hidden finger-marks growth, they have many benefits, including lox toxicity, high developing contrast and selectivity, and high sensitivity.[11,12]

Toxicity

Some components in quantum dots, like Cadmium, have the potential to be toxic. Furthermore, certain quantum dots have the potential to originate irritation and aversion.[13] Modification in the surface by adding a layer of silica dioxide was documented to reduce the toxicity of quantum dots to a large extent.[14] The upconversion nanomaterials modified at surface level contain low toxicity.[15] The near infrared light-based excitation of upconversion nanomaterials is less harmful to the DNA present in the remaining finger marks, hence, DNA analysis can be performed for the identification of an individual. The use of upconversion and quantum dots are suitable options as they opt for less toxicity or sometimes no toxicity.[9]

Contrast

The up-conversion nanomaterials and quantum dots can release visible fluorescence with high intensity under near-infrared and ultra-visible light. Strong fluorescent emissions can boost the emerging signal while reducing the distraction of the background color, resulting in high enhancing contrast.[11,16] Furthermore, since near-infrared lights emit low energy radiation, they do not cause background fluorescence to be emitted from substrates, eliminating the possibility of background color distraction and, as a result, allowing for high evolving contrast.[9] Because of their strong fluorescent properties, they can achieve a high emergent contrast by increasing the evolved signal and decreased background colour distraction when used for latent fingerprint growth.

Selectivity

Surface alteration is a versatile and efficient method for achieving high selectivity of finger impression. Surface modification can change the electric charge of the fluorescent nanomaterials, allowing them to bind to precise remains in finger impressions through electrostatic adsorption for high emerging selectivity.[17] Furthermore, these fluorescent nanomaterial surfaces can be

modified using the number of functional groups such as amino, carboxyl, and aldehyde. Chemical reactions allow them to selectively mark a particular component in finger marks, resulting in high selectivity.[18] By further addition of molecules like lysozyme, the binding aptamer can help in the conjugation with residues of finger impressions containing lysozymes leads to high selectivity.[19]

Sensitivity

Upconversion and quantum dots are usually small in size. In the case of upconversion fluorescent nanomaterial, the diameter is not more than 100 nm, and in the case of quantum dots, the diameter is not more than 10 nm. The ridge information, such as the fingerprint patterns, or individual characteristics like- termination points, bifurcation, dots, ridge ending, and the location of sweat pores, will not be heavily shielded when using these small-sized fluorescent nanomaterials for the enhancement of the hidden finger marks and gives high sensitivity.[11] Furthermore, these nanomaterials have a spherical shape that can be adjusted through the synthesis process. Surface modification may also be used to adjust the tackiness of the material. As a result, the adsorption on the finger mark remains can be modified, and the emerging sensitivity can be improved even more.[20,21]

METHOD & MATERIALS

Diatomaceous earth is made up of diatoms' remains and is found in the form of powdery silica. Diatoms vary in size from approximately 5 microns to 1000 microns. There are more than 200 genera of living diatoms which are estimated, and almost 100,000 species exist.[22] Diatomaceous earth's fine-grained, highly porous, and lightweight nature makes it suitable for use as an absorbent, insulation medium, mild abrasive, and agent in DNA purification and extraction.[23] Electrostatic force and hydrogen bonds can firmly attach the fluorophores of some cationic dyes present in this material.[25] Diatomaceous earth would be an excellent modulator for the generation of solid and photostable fluorescence because of the shielding effect of silica walls. Therefore, the selection of appropriate fluorescent dyes containing cationic fluorophores is critical. As a standard xanthene dye, rhodamine B is commonly used in solid-state lasers, optical filters, and other applications.[26] Even when encapsulated in a matrix material, it is well recognized for excellent spectroscopic features such as good fluorescence quantum yield and high photostability.[27] Rhodamine B's longer excitation wavelength allows it to be used as an excitation light source in the green region rather than the UV or violet-blue region, which is safer for humans and allows for better conservation of trace evidence such as DNA in fingerprint deposits. For the preparation of novel fluorescent fingerprint powders, diatomaceous earth and Rhodamine B may be excellent silica hosts and fluorescent hosts.

A series of Rhodamine B-diatomaceous earth composites have been successfully prepared as novel fluorescent fingerprint powders using a green, simple, and versatile preparation method based on physical adsorption between cationic fluorophores and porous silica. The fluorescence characteristics of the composite powders change with the amount of Rhodamine B present, due to the different accumulation phases of functional groups in the composite powders. The current research was the first to demonstrate a new mechanism for developing and improving latent fingerprints by using fluorescence properties of as-prepared fingerprint powders by qualitative and quantitative analyses. According to the mechanism, a photo recording and enhancement system with optical devices (532 nm alternate light source, cell phone camera, and 580 nm long-pass filter) and image analysis techniques (channel separation with PhotoShop and grey analysis with Image) were developed.[28]

Earth Upconversion Fluorescent Nanomaterials

Rare earth upconversion fluorescent nanomaterials (UCNMs) are materials that have been doped with rare earth elements. They emit a shorter wavelength light when excited by a longer wavelength light. When excited by NIR light, they produce visible light. UCNMs have a number of distinct attributes, including small emission spectra, low toxicity, and high intensity. They can also be chemically functionalized. Background fluorescent interferences are excluded since they can be excited using NIR light, resulting in increased evolving contrast. As a result, UCNMs show improvement for developing

latent fingerprints with high contrast, selectivity, and sensitivity. NaYF4 co-doped with Yb3–Er3 ions (NaYF4:Yb,Er) is the most widely used UC material today which is also capable of emitting the brightest UC fluorescence.[21]

Wang et al., also prepared the upconversion nanomaterials using NaYb0.98F4:Tm with the help of solvothermal technique. Generally, NaYF4:Yb, Er UCNM production was focused on a single near-infrared to visible method that allows finger impressions to be observed by sensing visible light under near infrared light excitation. Otherwise, it can be prepared by dual method that is near-infrared to near infrared and near-infrared to visible technique. The synthesized upconversion nanomaterials had a versatile fluorescent marker quality and used a dual-mode production on porous, non-porous semiporous surfaces. This method produced the enhanced fingerprints with better contrast, high selectivity, and sensitivity under both near-infrared to near infrared and near-infrared to visible methods.[29]

Bilayer systems based on conjugated polymers

Bersellini et al., suggested a new notion based on the electropolymerization of polypyrrole on surfaces such as gold, platinum, silver, and aluminum alloy (Ergal).[30] Hillman et al., used this technique to improve latent fingerprints on metallic surfaces like brass or stainless steel by electrodepositing conjugated polymers including Polypyrrole, Poly (3,4-ethylenedioxythiophene), and Polyaniline. The process in which the electrochromic material allows for the manipulation of optical properties (polymer color) by applying an electrical potential, has the efficacy to enhance the latent fingerprints. In spite of that, the visualisation of the fingerprint image under UV light is sometimes needed, particularly in case of dark or multicoloured backgrounds. Therefore, second layer of fluorescent conjugated polymer can be added to enhance the contrast.[31,32]

Conjugated polymer shows advantages over molecular fluorophores, including high emission, low-cost synthesis, and low toxicity. A series of fluorescent materials based on conjugated polymers for a variety of applications has been synthesised by C.V. Costa et al., The precursor monomers of those conjugated polymers were insoluble in water.[33]

The usage of an aqueous medium for electrodeposition of the polymer was one of the foundation approaches proposed by Hillman et al. This approach was based upon the assumption that the presence of fatty acids in the remains of the latent fingerprints serve as an insulating cover on which the polymer must not be deposited, resulting in a detailed finger impression imaging.[34]

C.V. Costa, et al., strategized a technique for the latent fingerprint development based upon the conjugated polymers bilayer, containing first layer of polypyrrole as electrodeposition on a stainless steel having a latent fingerprint. The second layer made up of fluorescent poly (2'2'-2"5'-terthiophene which was electrodeposited on the first layer. This technique showed the detailed structure of latent fingerprints containing ridge pattern, class and individual characteristics. The fluorescent bilayer system interacts with the unexposed area of the substrate which helped in the enhancement of the contrast between surface and fingerprint, has low toxicity as materials used for the synthesis are less toxic in nature, and easy development of the polymer film using Galvanostatically and Potentiostatically methods. This technique combines UV and visible light for better enhancement of fingerprints and can be used as suitable one on stainless steel surface.[33]

Quantom Dots

Cai et al., used an extremely fluorescent water-solvable cadmium-telluride quantum dots capped by mercaptosuccinic acid basic solution in 1–3 seconds to enhance hidden fingerprints on numerous nonporous exteriors. Mercaptosuccinic acid cadmium-telluride quantum dots showed the higher sensitivity and image excellence when developing dormant fingerprints.[35]

Chen et al., worked on dual visual senser based on polymer dots that helped in imaging of fingerprints. They embedded ninhydrin into the Polymer dot matrix after scheming and synthesising two forms of near-infrared fluorescent polymers. The characterization of nanoparticles has been done by transmission electron microscope and dynamic light scattering. The fluorescence remained measured using fluorometer under 450 nm excitation and absorption spectra under UV-Visible spectrophotometer. The colorimetric and fluorescent dual-readout capabilities of

the Polymer dot assay to spot dormant finger impressions on absorbent and non-absorbent exteriors were demonstrated. The chemical groups were also imbedded onto the surface of the nanoparticle to examine the process responsible in the growth of fingerprint. The assay has been used to image hidden fingerprints on checks and note paper. This technique helped in detecting hidden fingerprints on all smooth surfaces with low background interference, high resolution, and contrast. It showed all the particulars of fingerprints from level one to three.[36]

Conjugated polyelectrolyte dots have become a choice in bioimaging probes as they contain low cytotoxicity, strongly biocompatible, and have outstanding fluorescence brightness. Conjugated polyelectrolyte dots are made by dissolving water soluble conjugated polyelectrolytes in water to produce an aqueous colloidal solution. They are commonly found as particles like nano sized in water, despite their polar side chains increasing their water solubility. This is because the polymer chains appear to accumulate in aqueous environments due to the inherent hydrophobicity and structural rigidity of the main chains. The Conjugated polyelectrolyte dots amphiphilic features can be precisely balanced with the help of surfactants with sufficient hydrophile–lipophile-balance values and then the fluorescent nanoparticles can be efficiently transferred to the oily phase of a latent fingerprint while the latent fingerprint remains intact. Kim et al. worked on different variety of Conjugated polyelectrolyte dots to develop the hidden fingerprints using fluorescent imaging probes. The hidden finger impressions could not be stained by the aqueous Conjugated polyelectrolyte dots colloidal solutions alone. The Conjugated polyelectrolyte dots nanoparticles were transferred directly to the hidden finger impressions when an acceptable surfactant was applied to the aqueous mixture and then sprayed onto the hidden finger impressions. These showed the extremely distinct fluorescent images of latent finger impressions. Cationic surfactant also helped in enhanced the dormant finger impressions and made them brighter. This technique is convenient, simple, and universal which helps in the higher visualization of hidden finger impressions.[7]

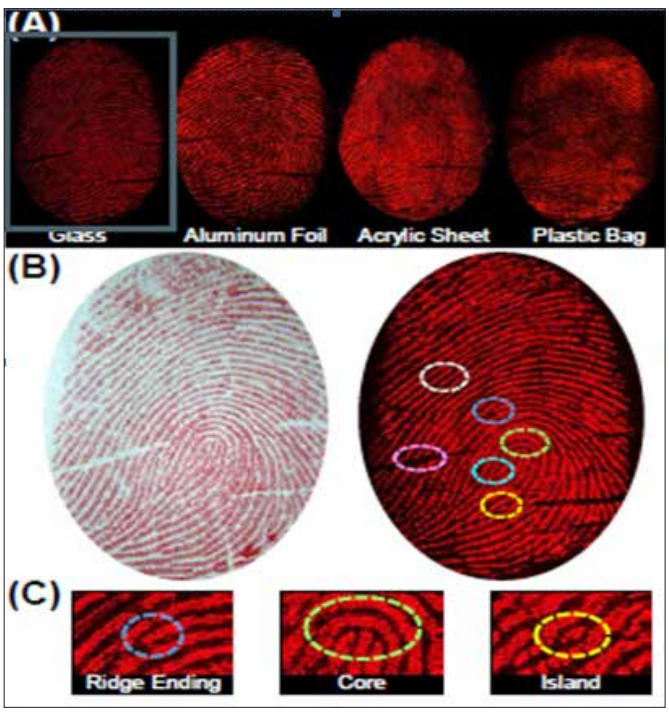

Figure 1: A) Hidden finger impression pictures enhanced using polymer dots on crystal, aluminum paper, acrylic piece, and plastic container. (B) finger impression assessment marked using a Polymer dots (right) and red inkpad (left). (C) Level one-three data, including centre, ridge ending, bifurcation, island, scar, and pore, are visible in high-resolution fluorescence images of latent fingerprints. A 450-nm LED light with an orangish filter was used to excite all fluorescence images. Copyright © 2016, American Chemical Society

Non-metals Nanoparticles with Fluorescence Characteristics

Rajan *et al.,* synthesized the silica nanoparticles using rice husk by thermochemical treatment which were then dyed with natural dyes. Fourier-transform infrared spectroscopy, Field emission scanning electron microscopy, X-ray diffraction analysis, and forensic alternate light source were used to record and characterise the powders' photoluminescence. By producing dormant fingerprints left on various multicoloured substrates, the efficacy of three fluorescent variants of silica nanoparticles powders and industrial fluorescent powder was studied. Rice husk was successfully used to make spherical fluorescent silica nanoparticles. Amorphous spherical silica nanoparticles with an average crystallite size of approximately 200 nm were discovered during characterization studies of coloured silica nanoparticles. Once heat was applied and aged at room temperature, silica

nanoparticles doped with curcumin pigment showed the best stability and greatest fluorescence. Electron microscopy and surface tests results on different surfaces showed that the dye doping method did not compromise the efficiency of the coloured silica nanoparticle. The results showed that it had strong photoluminescence, allowing for adequate contrast for fingerprint examination on problematic and challenging exteriors. The spherical mono-dispersed nanoparticles also improved the powder's transparency and selectivity.[37]

Nanofibres

Junior et al., prepared dansyl-based fluorescent nanofibers using Polycaprolactone and dansyl cadaverine or dansyl glycine derivative by electrospinning method. The characterization was done by thermal analysis, scanning electron microscope, FTIR, and fluorescence spectroscopy. With 2% fluorophore concentration in dansyl cadaverine or dansyl glycine, a working distance of 12 cm among the syringe tip and collector, and a voltage of 17 kV, the best experimental conditions for homogeneous, even, and bead-free fibre forming of all samples were discovered. Electrospun Polycaprolactone/ dansyl cadaverine and Polycaprolactone / dansyl glycine nanofibers emit large emission bands at 485 and 415 nm, respectively, and are fluorescent. This research provided a simple, low-cost method for producing electrospun fluorescent nanofiber materials for forensic applications such as the production of hidden fingerprints on metallic objects such as cartridge cases, knives etc. As compared to the majority of well-known traditional methods, like Powder dusting and cyanoacrylate fuming, the proposed technique has many benefits over traditional techniques including solvent and raw materials with low-toxicity, easy regulation of polymer film formation, and attainment of evidence containing hidden fingerprints on metallic objects of various sizes and shapes, liable on electrospinning collector settings. As a result, this novel approach enabled the high-definition visualisation of formed hidden fingerprint images, as well as the identification of class characteristics, ridge patterns, and singular points of fingerprints.[38]

Benzazole dyes have characteristics like high sensitivity, stable and intense photoluminescence which can enhance the latent fingerprints. Stefani et al. considered benzazole dye to prepare micro-structured fluorescent powder to develop the dormant fingerprints present on various surfaces having different color in background like multi-colored, white, and dark. The fluorescent powders were extremely selective and sensitive which only sticked to fingerprints rather than the whole treated area. Irrespective of the color of the surface, the high fluorescence produced by the powder provided a high disparity with the finger impressions, making them easy to recognize and capture. Silica was used as the matrix in the formulation of powder having a 1:100 and 1:300 mass ratio, having ethanol or aqueous solution underneath encompassing settings. Silica is non-toxic, biodegradable, and environmentally safe in nature. The excited-state intramolecular proton transfer mechanism and the high Stokes change detected for the integrated dyes were responsible for the proposed powders' high chemical and photophysical stability, resulting in a extended shelf lifespan. This too permits for the long-term storage of processed evidence without any deterioration of the exposed fingerprints. ultraviolet-visible absorption and fluorescence emission spectroscopy were used to determine the photophysical properties. Assessments were made with commercially obtainable fluorescent, white, and black powders for various types of surfaces to determine the efficacy of these industrialised residues. When exposed to long wavelengths of ultraviolet light at 365 nm, the created micro-structured powders showed intense visible light emission within the blue-green field, and a pointy distinction with the finger impression deposits was discovered, developed discrete ridge data on all studied exteriors. The method is cost- effective and also a simple technique to develop the hidden fingerprints on various kinds of surfaces with distinct levels of information. This fluorescent powder is versatile in nature and can be used in the forensic examination as a multipurpose choice.[39]

Numerous fluxes, such as NaCl, NH4F, and NaBr, were used by Dhanalaskhmi and her colleagues for their study to synthesise novel BaTiO3:Eu3+ (5 mol percent) nanophosphors through a solution combustion path. The effect of the fluxes on the prepared nanophosphors' physical,

structural, and photoluminescence possessions was thoroughly investigated. The cubic structure of the products is verified by powdered X-ray diffraction outcomes. The synthesized samples were sphere-shaped with accumulation, according to the morphological studies. The addition of fluxes results in a significant increase in the red emission intensity in the photoluminescence study. It was discovered that NH4F (3 weight%) was an effective flux for lowering the temperature formation, morphology improvement, and increasing the photoluminescence strength by two-fold. The samples' photometric properties were calculated and found to be very similar to commercial BaTiO3: Eu3+ phosphor. The fingerprints that have been visualised show that they were highly sensitive, have good contrast, and have no background interference. The results showed that the optimised sample opened up a new path for the easy visualisation of hidden finger impressions in anti-counterfeiting, forensic sciences applications.[40]

DISCUSSION AND CONCLUSION

Latent finger print development is cornerstone in any criminal investigation. Fingerprints are the crucial evidence for the identification of a suspect or culprit in any crime scene. It identifies the individual as fingerprints are unique; no two individuals share the same fingerprint. However, there is a profusion of fingerprint enhancement techniques for the visualization of hidden finger impressions. Powder method is one of the oldest techniques which are still used to develop the prints at the crime scene but it has its own drawbacks like less sensitivity, background disturbance, interference with cellular material visualization techniques, and might not enhance all the levels of finger impressions. Recently, the use of fluorescent nanomaterials like up conversion nanomaterials and quantum dots for the enhancement of latent finger impressions have attracted a lot of attention. Fluorescent nanomaterials for fingerprint development have shown many benefits like low toxicity, high developing contrast and selectivity, and high sensitivity. Rare earth upconversion fluorescent nanomaterials doped with rare earth elements can enhance the dormant finger impressions and emits the brightest upconversion fluorescence. On the other hand, a conjugated polymer based fluorescent material also develop a detailed image of finger impression that can be cost effective and having low toxicity with high emission of fluorescence. Similarly, quantum dots showed a higher sensitivity and image excellence when developing dormant fingerprints from level one to three. Therefore, fluorescence-based nanomaterials method developed here allows for successful imaging of hidden finger impressions on a variety of absorbent, non-absorbent, and semi-absorbent materials with high characterisation of first to third level detail, which is in line with the forensic science standard for finger impression identification. IJFMP

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REVIEW ARTICLE

Advancements in Potential Preservation and Decipherment Techniques of Charred Documents

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ABSTRACT

Documents have played a crucial part in our day-to-day life for ages. Documents contain vital information which can have evidentiary value that can link a criminal to a crime. Sometimes, such documents get destroyed either intentionally or accidentally by means of fire, water, moisture and humidity, dyes and paints, etc. Many a times, criminals try to destroy the valuable documents by setting them on fire in order to hide their criminal activity having wrong intention. Charred document is one such case where the documents under excessive heat become carbonized, brittle and fragile. The most perplexing problem faced in the area of arson investigation is the handling and preservation of charred documents which ultimately render the decipherment of contents written on it. This is due to the obvious reason, that is, its extreme fragile and brittle nature. Researches have also pointed towards this huge challenge for questioned document examiners to potentially preserve the charred documents for further investigation. Hence, the present paper aims to highlight the ancient and the recent advanced preservation and decipherment techniques of charred documents to summarize it with their pros and cons, so that the need to enhance and develop a better new technique of preservation can be looked into.

KEYWORDS | charred documents, decipherment, qd, polyvinyl acetate

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INTRODUCTION

Questioned document (QD) is a document whose legitimacy is questionable. Hence, the name Questioned document. Most often, valuable documents are ruined by fire and other means in order to cover up unlawful act. "Whether accidental or deliberate, fires have a devastating effect on paper or other writing materials."[1] Gases such as CO_2, CO, nitric oxide etc., and airborne particles like organic matter, soot come out of fire and causes damage to the material. Charred documents are one such class of QDs that become black and extremely fragile by the action of fire, extreme heat or smoke.

Charred documents are generally made up of paper (cellulosic fibers), obtained from wood or similar sources. In arson or other fire investigation, these documents are unlawfully linked to some or the other kind of crimes ranging from fraud and ransom, robbery to anonymous letters, extortion, phony, insurance and financial matters, even suicide and murder.[2,3] Written or printed documents will be severely damaged through fire, smoke or soot. The content of the document like, what was originally written, may provide some clue to who wrote it.

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Burning of document is one of the most common ways of destroying evidence, and in case of documents being deliberately burnt, many people think that as soon as the proof of their guilt has reduced to ashes, it cannot be retrieved. Although most of the paper that is set on fire, there are still chances Where a number of other factors like temperature, atmospheric condition, storage area, paper quality etc., come into play during the burning which may prevent the document from getting completely burnt to ashes. Thus, it is possible for paper to be not completely burnt into ashes, Rather it is burnt to a point of becoming black, making the writing illegible to someone looking at it with the unaided eye.[4]

Since ages in the field of QD and in cases of arson, one of the most confounding problems lies in the handling and preservation of charred documents, which ultimately render their decipherment process. Forensic document examiners apply range of techniques and make sufficient use of technology-based equipment to conduct various analysis on documents. However, its blackened, carbonised state renders ordinary restorative processes ineffective.[5] A completely new approach to a unique problem is thus needed. Therefore, this paper seeks to highlight and summarize the existing techniques and methods for handling, preservation and decipherment of charred documents, with their relative pros and cons, and to put forth the current advanced methods which gave satisfactory results in many cases, in order to guide QD examiner to benefit from the potential and appropriate technique of analysis.

Before getting into the techniques, let us look into certain important aspects with regard to this challenge, since the charring process is dependent on several factors and thus on the technique used for decipherment.[6] These factors are as follows:

Factors During Burning of Documents:

Place of Storage: Sometimes it is important to differentiate between slightly and completely charred documents. More often, slightly charred documents can be read with the naked eye. Moreover, charring that lead from burning in a closed storage area (e.g., in a box or metal cabinet) have been observed to create different effects than those produced under conditions of complete oxidation, i.e., in open air. Therefore, area in which document was placed and burnt affects the level of charring and its analysis.

Type of Flame and Temperature: The documents when burned in an open flame obeys different pace and unstructured path than those burned in a limited flame and temperature. This is due to the availability of oxygen at different portion of documents which makes each component of document to burn at its own pace. During charring, paper goes through various stages at different temperature turning the paper/document from light brown to black and finally gray ash to white ash.

Type and Quality of Paper and Ink: Another important factor which lacks attention, is the type of paper and ink used. Variety of papers and inks are available depending on the construction, loading and use. Those ink made up of mineral pigments do not get completely charred. On the contrary, printing ink (based on dye solutions made from non-volatile solvents) after the solvent evaporates, leave more dyes that are more susceptible to get charred than mineral pigment and can be completely destroyed. Writing inks usually contain iron, which is often left as an iron oxide deposit and is less visible. Therefore, the color reaction of the iron metal can be used to understand it.

Handling and Preservation of Charred Documents

Field Investigation: The fragile pieces of charred documents must be handled with utmost care. Some pieces of partly charred documents may be less vulnerable to get damaged, than others which have undergone extreme heat, may fall off even with the least interference. The fragility, sensitivity and pliable nature of charred documents merely depends upon the type of paper and composition. The solution to the problem of un-needed breakage can be by treating all the charred documents with some preservative solution. Most important documents can be found in a cupboard, in sturdy boxes, or in safe places, or that one document burned on the stove, or other open surface may need to be deciphered.

David A. Black (1948) safely delivered two boxes of charred documents from the Philippines, following this procedure by incorporating a pack

of cotton wool between and sides of the charred documents, thus creating a layer of protection against damage. Very small damage was observed in such a case.[7]

In case where much of the documents get burnt, then the best way is to take out the whole charred mass by gliding a thin metal wire or pie tin under the mass and transport them to the laboratory by carefully placing them in a container. If the charred bundles are tightly bound, each piled parcel should be taken out manually with the help of fingers and put separately. For single charred documents, a flat tweezers are used to carefully pick each document and placed in separate containers. All these processes must be carried out in an air-free condition to avoid the loss of any fragment.

Laboratory Treatment

In laboratory, the separation and segregation of the documents take place. It is of utmost importance that these charred documents are separated by causing least damage to each document; and this is not an easy part, because the documents are found in a folded or crumpled pieces of tightly adhered paper.[8] However, in cases where the documents get charred in a mass, it is possible to separate the paper individually without the use of solvents, or other chemical methods as was seen in John F. Tyrell's case using the manual method for separating the documents kept in a solid box.[9]

There are a number of other proposed theories for separating individual charred documents by several researchers:

- C. A. Mitchell (1922) attorneys immersing the mass of charred documents in hot water (40 0 C).[10]
- Water-bath with dilute glycerine was recommended which not only separates the documents, but also makes them softer to flatten.
- By keeping the charred documents in a damp/ humidified chamber for sometimes may aid to retain its moisture which can then be stabilized by some plasticizer.
- Immersion in alcohol, a process designed to break the bond between the pieces, thus allowing them to separate.[6]

Moreover, different authors also recommended several other tested solutions for this purpose, and the following were found useful by them:

- 5% aqueous solution of Sodium dodecyl sulphate (SDS) also known as Sodium lauryl sulphate (SLS), (NaC12H25SO4).
- Glycerine, distilled water, methanol (3:20:5 parts respectively) and 2% solution of sodium hydroxide, formalin and boric acid. The prepared solution is applied with pipette on the charred paper. Once the solution gets soaked before getting dried, the individual paper can be separated with a small spatula.[6]

Earlier, before the solution was tried upon. Black in Philippines handled the charred document by using standard 18x20 ferro-type plates to act as a supporting stage to congregate and safely place the charred document during the long decipherment process.[7] This was found to be time consuming and tedious process to follow.

Dr. Hans Gross (1906), also described a method for the purpose of charred document examination in a flat state.[11] His method suggested to firstly soften the fragments of charred documents in water, damp air or steam due to the fact that documents are hygroscopic in nature. Lastly, the drying process includes the usage of "gum Arabic" or "celluloid-acetone" type, while Dr. Gross principally used tracing paper, but a glass can provide a firmer and more transparent surface than tracing paper,[12] he concluded.

Stabilization Techniques of Charred Documents

NEATAN Treatment

Stabilization of charred document needs to be done in order to carefully handle the paper during decipherment since more than partly charred paper may be so fragile that it cannot be examined. When neaten new, developed by Merk, is applied over paper using eyedropper, it was possible to transfer to flexible sheets without much change in appearance and then the sheets could be handled relatively with ease.[8]

Lamination: Another way to preserve and make the charred paper stabilize is the lamination. This allows the papers to become flat, without breaking under the pressure of the heated rollers which makes it more readable. It also shields from any additional damage and makes handy to easily move and photograph. Moreover, it makes the

charred documents more impervious than the original paper.[8]

Plasticizers Treatment: Cellulose acetate solution in acetone or other like the 3% Polyvinyl acetate (PVA) solution in acetone (3 gms. PVA in 100 ml. acetone) are the plasticizer applied by spraying or glass rod. But there are various pros and cons of every plasticizer materials used. The cellulose acetate is a good plasticizer but it cannot be used as regular material here since it cannot be sprayed well which is one of the main characteristics of the stabilizing agents.

Before the application of polyvinyl acetate, the glass surface should be polished with 1% solution of silicon type water repellent substance in petroleum-ether. Due to this silicon type water repellent, the charred document does not get bound to the glass on application of the polyvinyl acetate. Thus, the document can be lifted with ease after being stabilized by stabilizing agent. The polyvinyl acetate solution is made in acetone gets evaporated after application.[13]

Gum acacia is another material which can be used for the purpose of stabilization of the charred document but it has a bad property of sticking to the glass on which the charred document is placed. Moreover, the spraying property of the gum acacia is also not good.

Methyl methacrylate (C5H8O2), commonly known as 40% Bed acryl, also serve this purpose and is applied by spraying.[14]

Decipherment Techniques of Charred Documents

The composition of writing inks, prior to the 20th century, had traces of metals such as iron and copper as a tagging material. Therefore, for deciphering the contents written with such inks, different researchers applied various techniques.[15] These are briefly summarized as follows:

- Blagden (1787), made use of 'potassium ferrocyanide' (K4Fe (CN)6), commonly known as Prussiate of Potash, to assess the nature of ink on ancient parchment.
- Davy (1821), discovered the 'Colour test method' wherein written content was deciphered by the use of potassium ferrocyanide.
- Davis (1922), developed the 'Photographic plate development method' to decipher content on the charred document.
- Mitchell (1925) used a 'Calcinated method' wherein the printed, typewritten contents and those written with pencil or some special inks, were deciphered by further burning the documents.
- Mitchell (1935), made use of 'IR light' incorporating filters and plates for making a contrast between the content and background, hence deciphering them.
- Radley and Grant (1940), used 'Fluorescent oil' and 'UV light' to decipher content of printed, photocopied, typescript as well as carbon-copied.
- Taylor and Walls (1941), advanced 'Chloral hydrate method' for printed and typescript contents.
- Gones (1941), used 'Photographic method' to improve the visibility of manually written content of charred document.
- Murray (1941), deciphered letter pressed printed documents as well as ink having metal content as a tagging material using 5% solution of 'Silver nitrate' (AgNO3).
- Black (1948), deciphered charred printed and typescripts using 'Alcohol-glycerine method'.

To categorize charred documents decipherment techniques, they have been classified in two broad categories: (I) Photographic, those processes that are completely based on photographic techniques, and (II) Visual, those processes which require the documents to at first visually examined, treated with some medium (if required) and then the contents are visually tabulated either under different lighting condition or through any specific instrument and finally photographically reproduced.

Photographic Techniques

Contact Process: This process was based on the fact that in the absence of light, some emitting gases and vapors would fog or make the emulsion of a photographic plate or film cloudy. Based on this principle, Davis (1922) of the "Bureau of Standards" conducted experiments16 wherein he was able to develop a latent image on photographic emulsion by making use of gases emitting from freshly burnt charred documents.[17]

Later, John F. Tyrrell (1939) by using the above principle and applying his own technique, was

able to decipher 85% of the charred documents burnt in a container producing a series of contact photographs. To obtain a more contrast of the image, a strong developer "Eastman Dl-type" was used, which produced the most satisfactory results.[9]

Furthermore, Tyrrell made little enhancement in his process. Prior to contact process, he exposed numerous pieces of charred document under UV radiation for long-term duration. The results obtained by this treatment was found to be far better than those obtained without it, particularly when the fragments were older and less effective to photographically. However, this whole process took larger care and time.

Filter Photography

Filter photography overcomes two of the cons of contact process, (i) time duration, and (ii) weaker photographic-activity of charred fragments i.e., the contrast. The filter photography make use of a "Wratten #48" deep blue filter in combination with commercial film. The function of the filter is unknown, but it seems to highlight the difference in the "actinic power" of the charred document compared to those areas of the paper on which printing ink was deposited, hence creating the difference in contrast with that of background.

Infrared (IR) Photography

Infrared photography is one of the highly utilized decipherment techniques. Though, it does not prove to provide much satisfactory results in many cases of charred documents decipherment. The development of an infrared viewer helped to provide better results, which resulted in probable success of IR photography.[18]

The technique of IR photography uses deep red 'Wratten #87' filter in conjunction with 'Eastman IR plates', and 'Eastman DK 50 is used as a developer, developed by Dr. Bendikson (1936). Incandescent light source can provide a good lighting for IR photography. The 'Wratten F' red filter was used to focus but later, 87-infrared filter took place of Wratten F filter. This method gave quite amazing outcome with inks of typescript, pencil, and dense iron-gall ink.[17]

Visual Decipherment Techniques

Reflectivity Technique: This is the one of the simplest yet most versatile decipherment procedures in which burnt documents are examined under controlled light source directed at various angles relative to the paper surface. This technique is applied by placing the charred documents between two suitable size glass plates and cautiously bind them together with tape after pressing plates from both sides. Setup is then analyzed under oblique lighting. "900 flare-type" lighting do not fulfill the required need, because of the reflective property of glass plates. For decipherment of content, a photographic record, only a single glass plate fulfils the setup by fixing the charred pieces over glass plate by means of some transparent adhesive20. This technique depends to a large extent on level of charring as well as the density of the ink or pencil made on the document.

Potassium Ferrocyanide Application: This method was developed by the fact and phenomena that even when the paper documents get charred, the residues of iron base ink retain in the paper fibers, thus can be made to react with come solution to produce a colour change. For this purpose, potassium ferrocyanide acidified with 2% solution of HCL (hydrochloric acid) was spread on the charred document with the help of a dropper or soft camel hair brush.

Davis (1922) reported an alternative to this procedure by using a portion of blotting paper immersed in potassium ferrocyanide16. However, later this technique with some charred documents gave disappointing results as the colour did not turn to blue rather it changed to deep pink and the cause of this was unknown whether the result did not appear because of non-presence of iron residue in the paper after getting charred or due to some other unknown factor.

Silver Nitrate Technique: The Superintendent Cherrill (1941) of Scotland Yard discovered this method. According to his procedure, "charred document is first allowed to rest on a glass plate in support of a standard pan placed at the bottom. Then a 5% aqueous solution of silver nitrate is poured on to it and at the top of it, a second plate is positioned. It takes 3 hours to the contents to get visible as a dark black writing against the lighter grey background. A lower concentration of this solution for longer duration is recommended in case when original writing appears to be faint. By wetting the pieces of charred document several

times in water and subsequently drying may result in obtaining a permanent image"20. For future record, image is instantly photographed. This technique was found fruitful for printed documents than for most ink and typescripts.

Chloral Hydrate Treatment: This method was developed by H. J. Walls and W. D. Taylor (1942) during World War II19. The active solution for this treatment is 25% chloral hydrate in alcohol. Applying this solution on both side of the charred sample using soft camel hair brush or immersing in the solution can serve best ways of application. The sample is then dried either in an oven at 600C or hot plate by placing it on heat resistant glass. After it gets dry, the process of immersion-drying continues several times and, on last immersion, a 10% glycerin solution is also added to prepared solution of chloral hydrate, then finally dried. The contents of charred documents become visible by the gradual accumulation of chloral hydrate crystals. Certain typewriting inks, iron gall inks, and printing ink show better legibility by undergo through this process, while washable aniline dye does not.

According to Grant's report (1941), this method gives more successful result with heavily loaded/higher grade paper than that of normal paper, thus concluded that test depends upon the incomplete combustion of the cellulose as in case of heavily loaded papers.[20]

4.2.5 Fluorescence in Ultraviolet Light: Another method of decipherment was developed by Julius Grant (1941). According to him, "the charred document when visualized under filtered UV light, before and after applying a solution of equivalent proportion of pale mineral oil and petroleum spirits, followed by drying, can observe a difference in florescence between written matter and paper background. The method depends on the composition and how old the ink is, paper type, and the level of charring. Quiet a number of samples were examined by this method, but in an instance, it failed to produce remarkable result in the case when the oil ink and paper absorbed oil equally, therefore not producing clear contrast"20. The technique gave positive result with printed, type written, even duplicate and carbon copy but they failed to give results with the ordinary pencil and old document.

Alcohol-Glycerin Immersion Technique: Black (1948) devised this simple, rapid and non-destructive method of decipherment by taking advantage of the phenomena that, "when the charred documents are immersed in certain liquids, a contrast between the writing and background at different portion of the charred document could be observed due to varied reflectivity. Therefore, when the American forces recaptured Manila, he devised a method for treating hundreds of valuable charred documents with alcohol-glycerin-water (5:3:2). About 90% of the contents of two boxes were possibly deciphered by this technique7. The readings were taken at varied stages of immersion in the solution. Each component used in this method has its own significance; alcohol act as wetting agent, whereas glycerin helps in partial drying of the burnt document by restoring some part of water before and after drying12. Moreover, this treatment with alcohol-glycerin-water does not permanently change the appearance of the charred fragment which give the additional success to this technique.

Ammonia Treatment

Tidke (2019) conducted an experiment, wherein the decipherment was carried out with alcohol treatment by first placing the charred documents in a fuming chamber containing 50ml ammonia for about 12 hours. This way, no writings could be deciphered. Thereafter, alternatively the ammonia solution was sprayed over the charred samples using spray pump. This way, the results were quite satisfactory and writings were clearly visible. As the ammonia solution readily evaporates, hence the deciphered contents were immediately photographed under visible light before the contents gets vanished.[15]

With growing advancement and development in technology, a wide range of optical instruments or systems are being developed to enhance the process of document analysis and decipherment. These are as follows:

Video Spectral Comparator (VSC): This instrument is the most commonly used, recent technology based optical instrument which utilizes a combination of Alternate Light Sources (ALS) under varied wavelength and high-quality camera to reveal the most desired features of document. Andrew (2016) conducted a study,

where the charred samples were analyzed under VSC using ALS and co-axial lighting at different wavelength of UV and IR radiations i.e., ranging from 300-900 nm.[4]

Another study by Moorthy (2016) revealed that "VSC 6000 could provide appreciable contrast between the background and writing, thus providing a safe and reliable alternative tool for deciphering the contents of charred documents when visualized under 'flood-light beam' and 'spot beam' setting of VSC". The samples were viewed at varied wavelengths, such as 645, 725 and 780 nm. Using a piece of equipment that is somewhat new to the field of forensics, the VSC, to show that ALS can be used for these purposes is good option to opt for minimal damage to the fragile documents.[3,18]

Projectina Inspec-8: This compact instrument, Inspec-8 was developed by Projectina for analyzing and detecting the alterations in documents and for verification of security documents such as driving license, bank notes, visa, passport, etc. It incorporates 8 integrated lighting sources: UV-IR for anti-stoke inks, blue-light 380-570 nm, LED (light emitting diode) for the OVD (optical variable device), Camera barrier filter, Excitation filter, Digital Video Interface (DVI) monitor to view live image, live camera port and USB remote control. In a study by Sharma et al. (2020), the security features of documents were analyzed by Projectina Inspec-8 under varied light sources before and after charring the documents. He analyzed those samples under UV and IR radiation at varied wavelengths for deciphering the charred contents21 and observed that some security features of the documents could be revealed by colour change while other does not. However, the stamp paper gave successful outcome as the details of it was visible under UV-IR light even after charring.

RESULT AND DISCUSSION

Based on the chronological study and development in the area of handling, preservation and decipherment techniques developed for the charred documents during the ancient times till today, it has been observed that not all techniques can be expected to be suitable for all types of charred documents because of several affecting factors as well as the type and composition of ink and paper. All the above techniques discussed provided successful results to a greater extent relating to the specific problem. However, some fails to do so because of one or the other factor. The most recent technology used today for decipherment such as, VSC and Projectina Inspec-8 are the potential techniques that allows a quick and thorough analysis of the documents, without causing any specific damage to the sample. However, the investigator and document examiner may realize the necessity to try several of the above techniques before choosing the one that will achieve a successful result.

CONCLUSION

By reviewing from most ancient to recent techniques followed in recent times for charred document examination, it can be concluded that there is a limitation of each technique when compared to the other. Therefore, with growing technology and development, it is the need of the hour to either differentiate the techniques based on the composition and type of inks, paper, documents, and other factors that render the process. The selective method of analysis should be fast, easy to use, cheap, handy, non-destructive, and provide result with higher accuracy. Hence, there is an urgent need to develop selective scientific techniques for the decipherment of destroyed documents which occupies the major areas in QD so that the investigators can reveal facts and findings more accurately and in a speedy manner with a less chances of error. IJFMP

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REVIEW ARTICLE

Persistence & Detection of Organic Gunshot Residue in a Forensic Investigation: A Review

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ABSTRACT

A method of identifying organic gunshot residue (GSR) is proposed as an alternative tool for unique identification in the forensic context. In recent trends of uses of non toxic/heavy metal free (HMF) ammunition, there must have been challenges in identification and detection of inorganic gunshot residue (GSR). The organic GSR preliminary emanate from the propellant of smokeless powder. Smokeless powder consists of either single base which is composed of nitrocellulose, double base which is composed of nitrocellulose and nitroglycerine or triple base which consists of nitrocellulose, nitroglycerine and nitro-guanidine. In order to preserve and lengthen the service life of smokeless powder different additives like stabilizer, plasticizer, flash inhibitors, coolant, moderate and surface lubricants etc were used.

Through this article review, we have summarized the study of the detection of diphenylamine (DPA), ethylcentralite (EC), methylcentralite (MC), dimethyl phthalate (DMP) and N-nitrosodiphenylamine (N-nDPA), which are generally found in the stabilizer, flash inhibitors, plasticizers and degradation products of the smokeless powder which could be separated and detected from the UPLC-MS (Ultra performance liquid chromatography – tandem mass spectrometer). This persistence and detection of organic GSR will be helpful for the forensic experts for testimony in a court of law.

KEYWORDS | organic gunshot residue particles, non-toxic ammunition

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INTRODUCTION

To overcome the challenges of frequent uses of non-toxic/heavy metal free (HMF) ammunition in the market space, the detection of organic GSR particles are very much useful for the forensic experts. Organic component of GSR generally comes from the propellant contained in ammunition and along with propellant to conserve and sustain the life time of the propellant some additive mentioned in Table 1, where added like stabilizer, plasticizer, flash inhibitors, coolant, moderator and surface lubricants etc,[2] which guides to enlighten organic GSR particle. However, the detection and analysis of GSR is foremost confirmation of evidence in the forensic ballistics fields and there are exigencies to develop more sensitive and selective technique that result in the more in number of cases. The gunshot residue particles are mainly referred to as inorganic particles deposited on the shooter's hands, surrounding areas of the firearm after discharge and travels through the discharged bullet with generally also lean at the target along with bullet residues.

The inorganic GSR particle originated from the primer contain in the percussion cap of a cartridge case. Customarily, the presence of GSR is confirmed by the

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distinctive constituent of Barium (Ba), lead (Pb), antimony (Sb) and bullet residues like copper (Cu), lead, zinc (Zn) nickel (Ni) etc., for supporting purposes.

The organic component in the GSR can be called as organic GSR originated from the propellant powder contain in the cartridge case which are composed of smokeless powder. Smokeless powders are categorized by their compositions single, double and triple base powder. Single base powder contained of nitrocellulose (NC), double Base consist of nitrocellulose (NC) nitroglycerine (NG) and triple base consist of nitrocellulose (NC) nitroglycerine (NG) and nitro-guanidine.[3]

Findings of inorganic elements in the GSR particle are facing challenges by the number of probability of run across casework including lead and heavy metal free (HMF) contained ammunition. So that organic component in GSR plays very important role to pinpoint the findings of GSR particles on the shooter's hands and at the target. In the present review we focused mainly on five compounds which are component of additives contained in gun propellant powder were inspected that are ethylcentralite (EC), methylcentralite (MC), diphenylamine (DPA) originated from stabilizer dimethyl phthalate (DMP) originated from plasticizer and N-nitrosodiphenylamine (N-nDPA) (as shown in Fig. 1) originated from the metabolites or degeneration product of the powder.[1,2]

Many other analytical techniques were employed for separation and detection of organic component of GSR mainly from them, gas chromatography (GC), Raman spectrometry, Micellar electro-kinetic capillary electrophoresis (MEKC), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and desorption electro-spray ionization-mass spectrometry (DESI-MS). Moreover, there are also other environmental presence and findings of OGSR additives such as EC, MC, DPA, DMP and N-nDPA which requires better understanding of forensic scientist about OGSR evidences in a context beyond the elementary questions of detection by analytical methods. Indeed progression of awareness about the persistence of such evidences is crucial when analyzing gun powder additive as a routine procedure for case work.[6] The technique of tandem mass spectrometry (MS) attached with chromatography has always been favorable due to the highly sensitivity and selectivity. The UPLC-MS was equipped with electro-spray ionization (ESI), adopting multiple reactive monitoring (MRM) modes to determine diphenylamine, ethylcentralite, methylcentralite, dimethyl phthalate, and N-nitro-sodiphenylamine, in the present article review. Ultra-performance liquid chromatography (UPLC) is one of the efficient liquid chromatography (LC) methods to deploy for the separation of different constituents in compound. It is additionally utilized for the recognition and quantitative analysis of compounds and has become trends over the world since many years. Moreover accomplish the considerable improve in speed, resolution and affectability in liquid chromatography, a critical progression in the column and instrumentation technique (column particle size and column measurement) were organized. To overcome the above goal, one of the analytical methods on the basis of small porous particles is Ultra-Performance Liquid Chromatography (UPLC).[26]

Techniques and their Analysis

As per Maitre[6] et al., the chromatographic separation of mixture was executed on a UPLC instrument. The mobile phases were used as a methanol having 1% formic acid and Milli-Q water, formic acid of 0.1% with the help of gradient method given in Table-II below, which comprise a 4.6% rise of methanol per minute. Thermostatically saturated column at 43°C and an injection volume of 2µL throughout were deployed.

Additionally evolution of LC-MS is concluded at UPLC with the optimized tandem mass spectroscopy method approach the identification of positive and negative ions which allows the detection of compound desired in even single run. There is an engagement of two ionization source called electrospray ionization (ESI) which is capable in both positive and negative mode and another one is atmospheric pressure chemical ionization (APCI) which is capable in negative mode and switching between them at high speed. This desires the detection of eighteen compounds in only 8 minutes running time taken.[7]

Other several forensic analysts have been separately examined organic constituent in gunshot residue particles by the technique of liquid chromatography assembled with mass spectrometry

(LC-MS) for segregation and identification. The exhibit evidence absorbed during examination is only disadvantage in the method of liquid – chromatography.

Maitre et al.,[6] ethylcentralite (EC), methylcentralite (MC), diphenylamine (DPA), dimethyl phthalate (DMP) and N-nitrosodiphenylamine (N-nDPA) (as shown in Fig. 1) were picked out as the compounds of interest deployed in the manuscript [15]. They may be the most important and typical compounds present in modern gun propellant and consequently the higher side probability of detection in OGSR samples.

Benito et al.[2] used liquid chromatography quadruple time of flight mass spectrometry (LC-QTOF-MS) to separate and detect 18 supplementary agents present in gunpowder (given in table – I) by using MS/MS mode.

Taudte et al.[15] derive and evaluate the development of an ultra high performance liquid chromatographic method (UHPLC) for less time dissociation of 32 compound inherently found in organic part of GSR (including EC, MC, DPA. DMP and N-nDPA). The total time taken for the analysis was 27 minute along with the limit of detection ranging from 0.03 to 0.21ng at 214nm.

As per studies undertaken Mucha et al.[9] the protocol of sampling of OGSR and technique of evaluation was carried for detection of following compounds: tri-nitroglycerine, nitroguanidine, 2,4-dinitrotoluene, stabilizers: diphenylamine and n-nitrosodiphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenyl stabilizer/plasticizer: ethylcentralite, methylcentralite, akardite-I, akardite-II, plasticizer: dibutylphthalate, dimethylphthalate, triethylcitrate and tributylcitrate.

As per Taudte et al.[10] a gradient UHPLC method was evolve for the quantitative analysis of approx. 32 organic components of gunshot Residue using artificial neural networks (ANNs) for rapid optimization. Identities of the compound detected by UHPLC analysis with UV detection where confirm by mass spectrometric detection and which is further associated to an atmospheric pressure chemical ionization (APCI) ion source. Multiple reaction monitoring (MRM) mode was engaged which yields high degree of authenticity in the identification of compound of interest i.e. DPA, N-nDPA, 2 naphthol 4-NDPA, DNG, 2-NDPA, EC and MC in APCI+ mode and NG, DNG in APCI- mode.[10]

Gassner et al.[11] experimented ultra high performance liquid chromatography (UHPLC) equipped with binary pump for maximum delivery separation was performed using kinetex core-shell column from phenomenex (2.6um, 2.1mm x 100mm) with a column C18 stationary phase. UHPLC system was bridged to a triple quadruple mass spectrometer. Electro-spray ionization was operated in positive mode. The [M+H] + of the

Explosives	Sensitizers	Stabilizers	Fresh Inhibitors	Plasticizers	Degradation Products
Nitrocellulose	Trinitrotoluene (TNT)	Diphenylamine	2, 4-dinitrotoluene	Diethyl Phthalate	2-Nitrodiphylamine (2-NDPA)
Nitroglycerine	Pentaerythritoltetranitrate	Methyl centralite	Nitroguanidine	Diethyl Phthalate	4-nitrodiphenylamine (4-NDPA)
Nitroguanidine	--	Ethylentralite	Nitroguanidine	--	2,4-Nitrodiphylamine (2,4-NDPA)
Cyclonite (RDX)	--	--	--	--	N-nitrosodiphenylamine (N-NDPA)
Octogen (HMX)	--	--	--	--	2-amine-4,6-dinitrotoluene (2-ADNT)
2,4-dinitroanisole (DNAN)	--	--	--	--	4-amine-2,6-dinitrotoluene (4-ADNT)

Table 1 Organic additives found in modern smokeless gun-propellant

Sl. No.	Curve	Rate of Flow (mL/min)	Mobile Phases (Water) (+0.1% v/v formic acid)	Mobile Phases (Methanol) (+0.1% v/v formic acid)	Run Time (in Mins.) (in Mins)
1	6	0.8	70%	30%	0.00
2	6	0.8	14.8%	85.2%	12.00
3	6	0.8	70%	30%	15.00
4	6	0.8	70%	30%	17.00

Table 2 UPLC Gradient Conditions [6]

target compound were explained as the precursor ions and quantification was obtained from SRM measurements MS/MS parameter and along with target compounds AKII, 1,3-DPU, MC, n-NDPA, EC, 2-NDPA, 4-NDPA, DPA were detected.

As per the studies by Leah ali[12] liquid chromatography coupled to tandem mass spectrometry (LC-MS /MS) has achieved a worldwide extensive method of separation and evaluation of gun-propellant GSR and explosive because of its higher level of sensibility and accuracy of Liquid Chromatography along with MS/MS mode from its potential to go through product and precursor ion analyze.

Laza *et al.,* engaged LC-MS /MS to analyze commonplace gun-propellant powder stabilizers (table –I) it was concluded that this technique is appropriate for the investigation of organic constituents of GSR. Although the samples had been lifted with the help of cotton swab and were detected for gunpowder.

Morelato *et al.,* observed that the primary and most effective group to investigate both primary contained particles and propellant GSR on aluminum adhesive stubs. The authors analyze the findings of organic GSR especially EC, MC and DPA first via the instrument desorption electro spray ionization mass spectrometry (DESI-MS). The existence of elements composition in percussion cap of cartridge case were detected through SEM/EDS. Gradient timetable was built to get complete segregation of desired compounds i.e. EC, AKII, DPA, DMP, N-nDPA, 2-nDPA and 4-nDPA.[28]

Optimization of tandem Mass Spectroscopy, conditioned with a multiple reaction monitoring (MRM) system was begun and optimized to abolish probable interference and enhance the detection sensitivity for the compound of interest included in Table-I below along with certified reference material. The method MRM scanning was chosen to enlighten the lower limit of detection by monitoring of particular ions continuously in shorter time.[12]

According to Leah Ali *et al.,*[12] with increasing uses of heavy metal or lead free ammunition, it is very much necessary to build up methods for the detection of propellant GSR besides the routine inorganic primer contained in GSR particle. Optimized LC parameter and optimized MRM scrutinizing method were evolved to determine the higher side sensitivity for compound present as an additive in organic component of GSR that is AKII, EC, DPA, DMP, N-nDPA, 4-nDPA, 2-nDPA.

Thomas *et al.,*[12] a reverse phase gradient UHPLC-MS/MS practice has been established for the identification and detection of additive in gun propellant. Approximately, 20 compound present as an additive in organic component of GSR which was segregated by UPLC as well as recognized by the mode of MS/MS in multiple reaction monitoring system. Particularly compounds of interest including diphenylamine, centralite, nitrotoluene, nitroglycerine and various compound of phthalate

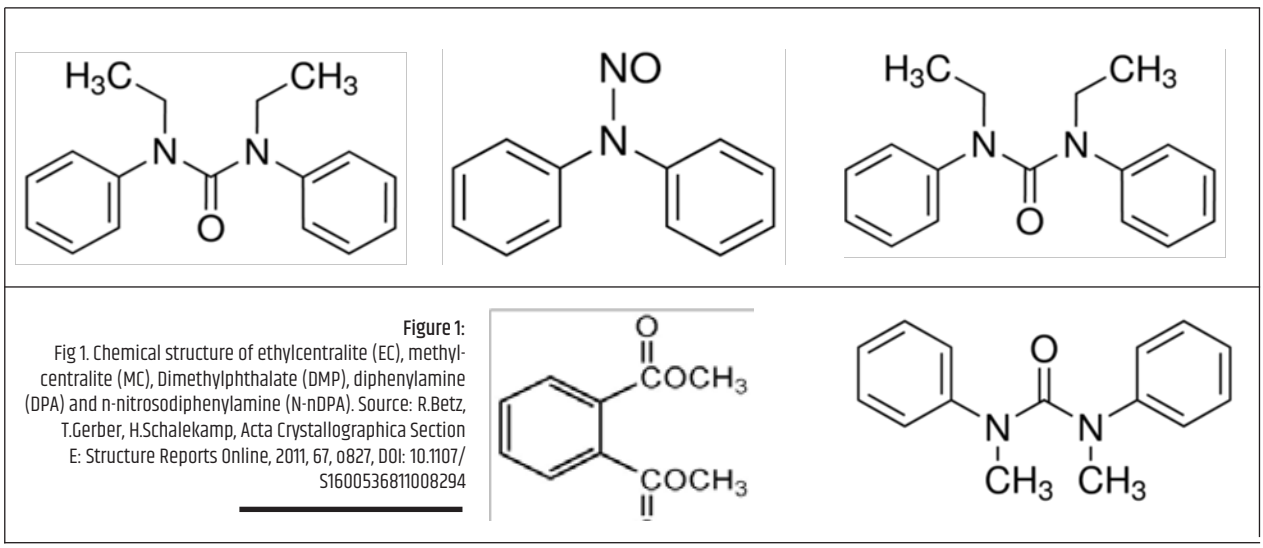

Figure 1:
Fig 1. Chemical structure of ethylcentralite (EC), methylcentralite (MC), Dimethylphthalate (DMP), diphenylamine (DPA) and n-nitrosodiphenylamine (N-nDPA). Source: R.Betz, T.Gerber, H.Schalekamp, Acta Crystallographica Section E: Structure Reports Online, 2011, 67, o827, DOI: 10.1107/S1600536811008294

group. Indeed electrospray ionization (ESI) with positive and negative modes were engaged along with negative atmospheric pressure chemical ionization (APCI) to evaluate all compounds in a single run time and analyzed within eight minute.

Wide spread smokeless powder propellant additives including both single and double base powder (Table-1) had been separated and detected to determine the relative compositions. Standard GSR mixture was spotted on C18 column and as well as detected in Ultra Violet light within approximately eight minute time taken.[13]

In accordance with capacity factor found by LC analyzer and their existence in two MRM indicators could be reliably recognized the various organic constituents present as additive in the smokeless gun-powder. Studies by Thomas et al. the technique of UPLC-MS/MS approx 18 compound could be separated via C18 column, the compounds of interest detected including EC, MC, DPA, DMP, n-NDPA and which might be individually distinguished by their capacity factor in LC with MS data and when detectable at low nano-gram level, this can be seen as an betterment over LC-MS/MS method published worldwide on account of the broad variety of analyst that can be separated and detected in a fast and effective mode. Moreover the working procedure is monitore with MRM transition for peak confirmation.[13]

Low limit of detection acquired with the UHPLC-MS/MS method should allow its applicability to follow up assays along with GSR samples lifted from the shooter's hands, which would be the one of the best subjects of future research.

RESULT & DISCUSSIONS

Many of the author's research work has focused on the detection of organic component of GSR via UHPLC-MS/MS along with MRM scanning method and compounds of interest mainly used in stabilizer, plasticizer, flash inhibitors and degradation products etc of gun powder.

The component DPA, EC, MC, DMP and N-nDPA (Fig.1) are basically present in the smokeless powder in modern ammunition which is mainly used as burning rate moderator and stabilizer for smokeless powder. Moreover, EC and MC can be considered as a signature compound for GSR. Separation and detection of these compounds by the method of UHPLC have made headway in the GSR detection.

Confirmation for the GSR would be after analysis of primer GSR as well as the majority of uses heavy metal free (HMF) ammunition, the detection of organic GSR increasingly should be taken on trends for analysis and also useful in evidence for the court of law by providing a trustworthy proof for existence of gunshot residue found in the sample for examination.

By the use of UHPLC-MS/MS method there is also less time consuming for separation and detection of each sample due to its feature of highly sensitivity, linearity, repeatability and authenticity for the validation of UHPLC-MS/MS method is more authentic and reliable. The proficiency to characterize various types of gun propellant additives, which are recognized on the basis of capacity factor and MRM chromatogram.

Maitre *et al.,* analyze all the target compound of interest whenever abundant was got the limit of detection (LOD). Whereas for N-nDPA - 5.64x10-3 ppm, for MC - 1.75x10-4 ppm, for DPA - 2.09x10-3 ppm and for EC-3.82x10-4 ppm are found their LODs.

Benito *et al.,* various organic supplement material were analyzed in GSR involve the plasticizer DEP (Diethyl phthalate) and DMP (Dimethyl phthalate), the stabilizer ethylcentralite (EC), methylcentralite (MC) and degrading product of diphenylamine (DPA). These findings are consonant with the usual organic component of GSR.

It is also known as DEP degradation product of DPA, 4-NDPA and centralite are some popular compounds in GSR. The types of phthalate ester (PAE) actually are diethyl phthalate (DEP) and their also uses in some industrial material in plastics, painting, pesticide, cosmetics and many more. Therefore the detection of DEP as additive in an organic gunshot residue is not sufficiently authentic for GSR as it could be originated from any product of materials made-up of plastic. The separation and detection of degradation product of DPA and centralite are evidential clue of having firearm discharged or having in the contiguity of a firearm discharged event. DPA is treated as stabilizer for propellant and explosive that extends the storage time. Diphenylamine reacts with nitric and nitrous acid leading to the post-combustion

oxidation of nitrocellulose and nitroglycerin.

Diphenylamine (DPA) is further transferred in mono-, di- and trinitro DPA imitative, which are individual character of gun-propellant. Centralites product are absolute to smokeless gunpowder reason behind their uses are restricted to the ammunition.[2,4,16,17] It is therefore investigation provides strong evidence in gunshot residue analysis.

With the aid of detection of these compounds as well as less time consuming we could overcome the challenges of non-toxic (NT)/heavy metal free (HMF) ammunition use in the current scenario. It is therefore suggestion that with the detection of EC, MC, DPA, DMP and N-nDPA etc. Forensic expert would secondary conformed about the Gunshot Residue (GSR) and firearm discharged event, which would be more beneficial for the forensic investigations.

However, UPLC is one of the most analytical method and primitive tools in the field of chemistry which increases the speed, resolution, as well as their sensitivity of the chromatographic technique and reduces the time of analysis, less consumption of solvent and economical. There is less noise and better signal to noise ratio for the peaks prevailed through UPLC. It yields very sharp and narrow peaks of interest in almost any desired compound.[26] IJFMP

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REVIEW ARTICLE

Comparative Study on Touch DNA Extraction Method

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ABSTRACT

Touch DNA viz. trace DNA is a perfect example of Locard's exchange principle, formulating the basis of forensic science. It basically states that any contact between two objects exchanges a trace amount of matter. Touch DNA is attained from the shed skin cells whenever a person comes in contact or touches any object. This kind of DNA can be found in every sample of fingerprint but its extraction is a cumbersome task in consideration of its trace amount. There is diversity in methods of its extraction but the main problem arises when task is to preserve fingerprint and extract DNA as well. Scientifically, the evidentiary value of fingerprints is 100% and that of DNA is 99.9%. It becomes crucial to preserve both of them to strengthen our case. In this era of vicious and cunning criminals where evidences are obscured. The extraction of touch DNA is practical but not practiced. This study aims at comparing various methods and finding best one out. The most appropriate method should be able to analyze the smallest possible amount of DNA in an economic way and of course leaving the fingerprint indifferent.

KEYWORDS | touch dna, fingerprint, trace evidence, extraction

INTRODUCTION

Recovering DNA from a crime scene is the most imperative task of a forensic analyst as it can directly link a suspect, a victim and the crime scene. DNA can be found on anything in any form. For example in sexual assaults, it can be found on condoms, bedsheets, clothes, in bite marks and in saliva etc.[5] Similarly, when a person handles or grasps any substrate from his hands a kind of DNA known as 'Trace DNA' or 'Touch DNA' is conveyed to the surface of substrate from the uppermost (epidermal) skin.[3] This DNA is found in the shed skin cells and can be majorly found in the fingerprints. The prerequisite of this type of DNA analysis is just 7-8 cells from the uppermost layer of a person's skin. Touch DNA can act as a ubiquitous tool in forensics by increasing the conviction rates in cases of robbery, sexual assaults as the DNA have a validity of 100%. The nature of Touch DNA deposition is majorly hooked on nature of surface, pressure applied (in case of fingerprints) and nature of contact. Majorly two questions always arise if we talk about touch DNA: 1) which is the best method for extraction of touch DNA? (2) What would be the amount of extracted DNA? According to published research papers, extraction of Touch DNA from various objects such as glass, fiber, clothes, metallic objects etc., is an unwieldly task.[4] But various methods such as Double swab, Hydrogel method, FTA paper, Mini tapes and FDF kit resolve all of these questions. Therefore, the aim of our study is to compare all of the above-mentioned methods and find

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METHODS & MATERIALS

Fingerprint DNA Finder Kit

In Fingerprint DNA finder kit (FDF), two surfaces were taken to gauge its proficiency.

Self-Adhesive Security seal sticker - On an intermediary polypropylene film layer a fingerprint was taken by pressing any of the thumb or index, which consequently is treated with adhesive acrylate polymer and along these lines disguised with upper film or cover. Around 146 samples were taken.

Latent Fingerprint - Four distinctive samples of Bersa 9 mm pistol, and one Smith&Wesson 357 magnum revolver were taken. For DNA isolation, 30µL of lysis cushion was pipetted on glue layer of seal having unique finger impression or either on a q-tip with wooden shaft to detach the DNA from the weapons having inactive finger impression. Each section of gun and seal was swabbed gently to isolate copious amount of nucleated cells or DNA. The cotton swab was further kept in a 1.5 mL plastic tube having 50µL of lysis buffer. Then incubation was executed for lysis at 60˚C (thermomixer) and centrifuged for 3 hours at 600 rpm. After incubation, cotton swab relocated to spin basket and for 1 min it was centrifuged at its maximum speed. The consequential extracted liquid from swab was added to left over liquid from incubation and the final volume was 55-60 µL. The solution was shifted to Nexttec clean column to centrifuge at 750×g for 1 min after incubation at room temperature for about 3 mins. The final supernatant contained purified DNA and other cellular debris DNA quantitative analysis was performed by Applied Biosystems Quantifile Human DNA Quantification kit.[5]

Double Swab Method

The twofold swab strategy comprises of scouring one pre-wet swab with 150 µL of sterile water followed by a drying swab for around 10 sec. This procedure was performed on both the tape and paper side of each unique finger impression "sandwich", bringing about four complete q-tips per test. All swabs were put away in singular swab encloses and dried for the time being a laminar stream hood before being joined and set in microcentrifuge tubes. All examples taken from the glue side were set in a 1.5 mL microcentrifuge tube, while tests taken from the paper side of a similar unique finger impression were put in another 1.5 mL microcentrifuge cylinder to ensure that all examples were totally lowered in the arrangement. Four individual chronicled inert fingerprints treated with every one of the three perception therapies from each of the 10 volunteers were acquired. A set from every perception treatment was prepared utilizing DNA extraction strategies—phenol-chloroform natural extraction. All DNA extricates were put away at 40C until measurement and fixation.

Lysis of natural material with 400 µL of strain extraction cushion with 15 µL of proteinase K was performed. At that point hatching at 56 ˚C on a shaking stage with turning in turn bins at 7500g for 5 mins was finished. 500 µL of phenol: chloroform: isoamyl liquor was added and turned at 18400 g for 5 min. The fluid layer was then moved to a microcentrifuge tube, 500 µL of chloroform isoamyl liquor, vortexed, and turned at 18400g for 5 mins. The watery layer is then moved to a pre-immersed cellulose film and turned at 350g for 13 min Filtrate was disposed of and TE support was added to each channel unit and turned at 350g for 18 min. Channels are presently rearranged into a clean miniature centrifugation tube and turned at 950g for 5 min. Test cuttings are then moved to miniature centrifugation tubes for decontamination.[2,6]

FTA Paper

In FTA Paper method, de-ionized (4 drops) water were smeared on a 3.2 cm of Whatman WB120205 formerly sample collection. Afterward, FTA Paper were dehydrated for 1 hr in drying box. After the FTA paper was dried, it was divided into small pieces and were placed in withdrawal tube. For the extraction of DNA QIAGEN® QIAamp DNA Kit was used. In the extraction tube 50 µL of nucleated free water was added to open DNA. After the DNA got eluted Real-Time qPCR was used for its quantitative analysis.[4]

Hydrogel Method

The solution of Dextran-methacrylate and LAP (Lithium phenyl-2,4,6 trimethyl benzoyl phosphonate) was newly arranged. Borosilicate magnifying lens cover slips were initiated before functionalization utilizing a Harrick's plasma cleaner for 240 seconds. They were moved to a

vacuum chamber containing 100 µL of 3-(trichloro silyl)- propyl-methacrylate and left under unique vacuum for 4 hours to activate the surface at room temperature.

A newly arranged arrangement of Dextran-methacrylate (10% w/v) and LAP (1% w/v) was applied on surface and functionalised cover slip was placed on it. Then, the sample was irradiated for 30 sec using 405 nm laser pen, after which the cover slip was removed leaving behind cross linked polymer known as hydrogel. Hydrogel was transferred into a beaker with MeOH-sonicated for 30 mins and IS solution (10 µL) was mixed to it. Extracted solution was inverted into polypropylene conical tube and solution was evaporated under Nitrogen. Material is dissolved in MeOH with 5% v/v Formic acid (50 µl) and relocated into injection vial after which quantification and analysis was performed.[9]

Mini-Tape Method

Smaller than normal tapes comprise acetic acid derivation strip with a part of twofold sided cement toward one side and which is secured by paper. The scaled down tapes are provided in clean individual plastic screwcap vials. For inspecting, the small tape was eliminated from vial, the defensive strip eliminated and the glue surface squeezed more than once over the outside of the item. The smaller than usual tapes were supplanted promptly in their vial and put away at room temperature. Little tapes were divided to little pieces and set in 1.5 ml miniature axis tube, at that point 180 ml of ATL cradle was mixed, the example tube was then vortexed and brooded at 85˚C for 10 min. 20 ml of Proteinase K was mixed, vortexed and hatched at 56 ˚C for 1 h. 200 ml of pre-warmed AL support was added, vortexed and hatched at 560c for 10 min centrifuged for 10 sec at 14,000 rpm. 200 ml of ethanol was mixed, vortexed and centrifuged at 14,000 rpm for 10 s. Tests were painstakingly added to the sections in the assortment tubes and centrifuged at 8000 rpm for 1 min. The section was taken out; 500 ml of each AW1 and AW2 support was added and centrifuged at 8000 rpm and 14000 rpm for 1 min and 3 mins individually and the segment was taken out. 65 ml of pre-warmed water was mixed, brooded at room temperature for 5 min from pre-centrifugation at 8000 rpm for 1 min. The concentrate (last volume 65 ml) was put away at 4˚C for the measurement investigation.[10]

Different techniques have different outcomes, depending upon various factors, such as surface (porous or non-porous), individual handler, activity before handling the surface, chemical composition of surface, time of recovery- sooner collection would prevent contamination of touch DNA but however, environmental factors the recovered quantity is independent of handling time.[8]

There were completely different results for swab method in two comparative study viz. FTA versus Double swab and mini tape versus double swab. When FTA Paper card was evaluated against double swab it gave significantly higher results as of chemical composition and better area of FTA Paper card. The chemical composition permits greater preservation and releases significant amount of DNA while on the opposite hand cotton swab traps DNA in its own fibres and this method is also time taking due to smaller area of cotton swabs. But the FTA papers didn't dry as fast because the cotton swab did, so there's requirement of drying procedure before the transportation and packaging. thanks to less sturdy matrix and rigorous applying of water on extraction from rough surfaces, there was loss of paper fibres on the surface itself.[4]

In tape lift method notably more DNA quantity was extracted on using scene safe fast mini tape on cloth material like cotton, but on flannelette material double swab method gave more prominent and better results. The potential reason of this outcome could be the presence of loose fibres on flannelette which either mask the cells underneath it or reduce adhesion of tape after contact. the main drawback of the tape lifting method might be when there's line increase in adhesion, the extraction of DNA could suffer in a huge amount. Another factor possibly implying on collection efficiency of the tape is pressure, however more research is required during this area for providing accurate results.[10,4]

The double swab method systemizes the methodology of cleaning in any case it's a troublesome errand to normalize the amount a swab should be soaked. Also, because it uses two

swabs at one surface it enhances extraction of DNA amount during a generous way. For poor absorbent surfaces, if we continue using one swab only it's going to result into a extended period of your time for drying off of the surface. this example is often avoided if we tend to use two swabs, the dry swab absorbs all the moisture left behind by the primary swab. Moreover, larger the world of interest or greater the absorbance of the surface, the need of additional swabs increases.[6]

This requirement is often fulfilled by using FDF kit which indeed may be a fast and one step protocol combining with the compelling expulsion of PCR inhibitors with suitable yield of DNA. LCN- Low Copy Number, is a section that consists 100 Pg of template DNA. Some samples fall under the category of LCN but most of them shouldn't be considered LCN because it may be an aftereffect of huge variety inside the measure of DNA in examples. With respect to utilize the DNA profiles acquired from the fingerprints kept on the Fingerprint Sticker, the outcomes exhibit the plausibility of utilizing this sort of tests as a DNA source to build data sets. The tactic facilitates various advantages like using of non-invasive sampling, no biological hazard, easy and enormous amount in single, transportation avoiding bacterial contamination as stored in dry condition in contradiction to the swab which require additional drying process etc. The upside of this framework is also that an identical weapon is frequently utilized both for finger impression and for DNA investigation, permitting the two kinds of proof to be acquired from a comparable firearm, yet these necessities cautious taking care of at the crime location and afterward.[5]

Now the comparison involves swab method and hydrogel which yielded 20%- 60% DNA of the amount extracted from swab method. Albeit this is regularly altogether not exactly the swab, still hydrogels go about as an expansion to the current work process, as DNA may be recuperated from fingerprints while enough material is abandoned as an expansion to the current work process, as DNA may be recuperated from fingerprints while enough material has been abandoned for unique mark representation. The greater assortment in DNA yielded by the hydrogels is probably on account of the extra strides inside the example planning, particularly the exchange of the lifted hydrogel to an aliquot, showing that further advancement of the example arrangement is significant. There is no single DNA extraction strategy that has been improved affected by representation treatment (shown in table.1). Hence, given the different testing ascribes identified with contact DNA and documented inert fingerprints explicitly, it is suggested that a post-extraction filtration/fixation step be thought of if tests are prepared utilizing protein-based freedom examines.[9,6]

RESULT & DISCUSSION

The success rate of touch DNA is limited even after so much advancements in technology. The potential reason tends to be the lack of research, knowledge and curiosity about it. The above-mentioned techniques throw a light on the legit techniques which can be further more improved and can deliver unbelievable results. The work here in reported should encourage laboratories and investigators to consider pursuing DNA analysis from archived latent fingerprints, particularly if they are the only potential source of physical or biological evidence available. It shows that DNA can be extracted from latent fingerprints for DNA

Method	Surface	DNA Extracted D(NG)	Mean Percentage Yielded	Fingerprint Extraction	Sample Size	Reference
Hydrogel	Glass	5.9	20%–60%	Possible	3	9
FDF	Gun	2.9	40%	Maybe possible	35	4
FTA	Steering wheel	1.89-7.89	91%	Not possible	3	9
Minitapes	Cotton cloth	0.69-7.8	0%–50%	Possible	28	10
Double Swab	Glass	0.65-5.2	75%	Not possible	100	2
	Steering wheel	0.62-5.33	25%–75%	Not possible	35	4
Hydrogel	Flannellette	0.8-1.8	20%–40%	Not Possible	28	10

Table 1 Organic Comparative study results of different methods that applied on different surfaces.

profiling although it should be considered that the work flow of this procedure might vary depending on the substrate on which the prints are present.

CONCLUSION

The various techniques are suitable for different type of substrate and unfortunately, there is not a single technique that can work as an all-rounder. So, the detailed knowledge of various substrate must be gained before touching any evidence. An important thing to be considered is that the fingerprint and DNA both are the confirmatory evidence and none of them deserves to be ignored in court of law, therefore a technique must be employed in which fingerprint as well as DNA both can be prevailed. However, in case of smudged prints, the highlight can be the Touch DNA. This leads us to the point where DNA extraction procedures must be devised for even treated fingerprints and even progress must be made in fingerprint visualisation techniques so that they do not interfere with the DNA profiling. The motive of this work is to motivate and enlighten forensic scientists and laboratories to treat Touch DNA just not as an option or choice but as a notion that has capability to change the vision. IJFMP

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REVIEW RATICLE

African Y-STR Haplotyping and Y-Chromosome Profiling: A Review

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ABSTRACT

Forensic genetics is an indispensable tool in forensic analysis that uses genetic evidence in crime investigation and/or identification of missing individuals or victims of mass disaster. It is highly reliable and can be used to substantiate evidence to prove the guilt or innocence of a suspect in question. There is paucity of data on African forensic DNA profiling. This is partly due to lack of funding and expertise. Moreover, there are very limited forensic genetic commercial kits that incorporate markers that are specific for African populations, markers that will provide highly specific information on the African Y-STR markers. Therefore, the purpose of this study is to consolidate the published Y-STR data of African population for forensic and population genetic reference. The review presents the Y-haplotype and genetic diversity of African male population. The review dissects the data into different regions of the African continent, viz., the Northern, Southern, Eastern, Western and Central African regions.

KEYWORDS | forensic genetics, dna profiling, y-str, y-haplotype, africa

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INTRODUCTION

The use of DNA profiling in criminal cases was first used a little above 30 years ago. The pioneer of this work was Professor Sir Alec Jeffreys, whose groundbreaking forensic work was able to link the assault and murder of two young girls to Colin Pitchfork in 1983 and 1986. The case served as the landmark criminal case that gave birth to the use of DNA fingerprinting in the criminal justice system.[1]

The Y-chromosome is male-specific in humans and follows a strict mode of paternal inheritance. It comprises of a major non-recombining region (NRY) that makes it suitable to providing one of the highest resolution tools for studying human population genetics.[2] This high resolution provides it with high discrimination power between individuals suitable for forensic investigations involving male victims or suspects.

The number of multiple alleles that are remarkably differentiated between individuals by the number of repeat units on Y-Chromosome is referred to as Y-Chromosomal Short Tandem Repeats (Y-STRs).[3] The forensic use of Y-STR genotyping has become instrumental in the identification of males involved in sexual assault, paternity and ancestral determination, missing and disaster victims investigations.[4] High mutation rates in Y-STR markers referred to as rapidly mutating Y-STRs or RM Y-STRs have been reported recently.[3] Some commercially available forensic analysis kits, for example, Y-Filer plus amplification kit of Thermo Fisher Scientific, USA, have introduced the RM Y-STRs with anticipation that they will assist in discriminating close male

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The African continent is inhabited by people with enormous linguistic, cultural and genetic diversity of more than 2,000 different languages and ethnic populations. The demographic timeline of the continent has recorded oscillations in population size, admixture, long and short-term migrations leading to rich and diverse variations and in modern populations. It can be said that the most genetically diverse region of the world is Africa.[5] Africa by size and population is the second-largest continent, comprising numerous countries and diverse populations. However, the information available on the Y-STR haplotype and allele frequencies in these populations is very little.[6]

There are several databases that the scientific community use to compare their data with already published data, forensic laboratories and other security agencies also use the databases, the most common databases include: YHRD and with Relia-Gene database and PowerPlex Y Haplotype Database.[7] The data provided in this review will help in consolidating the valuable information on the haplotype and allele frequencies of African Y-STR profile for population genetics and forensic reference.

Y-haplotype and Genetic Diversity in Central Africans

Studies on Central African population conducted by Arroyo-Pardo *et al.,* (2005) studied 16 Y-STR loci in 101 male samples of Equatorial Guinea origin who live in Madrid, Spain. Of the 101 studied individuals, 94 different haplotypes were obtained in the study. Another study conducted on 873 samples from Gabon and Cameroon from Central Africa by typing 18 Y-STRs found a total of 728 different haplotypes indicating high discrimination between the populations. They also observe high frequency of modal Bantu haplotype and its one-step neighbors as described by other literature in all the 24 Bantu populations in the study.[8] They also observed modal Bantu haplotype in two pygmy samples from Gabon and one of its one-step neighbors in one pygmy sample from Cameroon. Multi-Dimensional Scale Plot (MDS) showed all the Bantu population clustering together obviously separated from the Pygmies, indicating population homogeneity among the Bantus and some population admixture between the two populations.

Another study on Y STR among 165 Bantu population living on Bangui, Central African Republic, was conducted by Lecerf et al., (2007) who reported 88 different haplotypes, of which 83 were unique. The authors observed DYS385 and DYS392 to have the highest (0.9305) and lowest (0.1685) gene diversity (GD), respectively.

Data of Y-haplotype in Fang and Bubi populations from Bioko (Equatorial Guinea) were studied by Barrot et al., (2007), of the 133 samples studied in Bubi population, 102 and 87 different and unique haplotypes were reported respectively. Gene diversity in the study revealed DYS385 as the most polymorphic system.

The data generated by the researchers on the Y-haplotype diversity among Central Africans revealed that the populations are ideal for forensic casework due to their unique haplotype profile. It has also been established that DYS385 is the most polymorphic marker in the Central African population. It has also been revealed that the Central African population has intermediate allele 13.2 at DYS385 locus.

Y-Haplotype and Genetic Diversity in East Africans

Eighteen Y-STR profile of the population living in Maputo from Mozambique was characterized by Alves et al., 2003 who reported 101 defined haplotype out of a total of 112 studied samples, two individuals with seven shared haplotypes, while the most frequent was shared by five individuals. The most diverse and less polymorphic loci were DYS385 and DYS392 respectively.[11]

Twelve Y-STR of 40 Karimojong males from Karamoja, Uganda revealed 32 different haplotypes with high discrimination power. Comparison with the Y-STR data of Uganda, Mozambique, Cabinda and Equatorial Guinea revealed a large genetic distance between the populations.[12] Another study of 17 Y-STR haplotypes involving 118 males from the Nilotes population of Karamoja region in Uganda by Gomes et al., (2010) reported 94 different haplotypes, a total of 19 shared, 14 and 5 in two and three individuals respectively.[13]

Another study employing 27 Y-STR haplotypes among the Tigray populations of Northern Ethiopia by Haddish et al., (2019) revealed that the recent expansion of Yfiler to

study 27 loci produced a haplotype diversity and high discrimination capacity of 100%. Seventeen Y-chromosomal STR haplotypes in 69 Rwanda-Hutu unrelated male individuals from East Central Africa revealed a total of 62 unique haplotypes out of the 69 individuals.[6] The authors reported the lowest and highest gene diversity at locus DYS392 and DYS385 respectively.

Based on the data generated by the researchers, it is revealed that the highly discriminating or rather highly polymorphic allele in the East African population is DYS385 making it highly relevant in discriminating East African populations using the allele in forensic casework. It has also been established that DYS392 is at least a polymorphic marker in East African populations. The data presented by the researchers have demonstrated the uniqueness of the East African population with very few shared haplotypes among the populations which are of high forensic relevance.

Y-haplotype and Genetic Diversity in North Africans

A study on 185 individual Y-STR haplotype among four different populations: Southern Moroccan Berbers, Mozabites, Moroccan Arabs and Saharawis from North West Africa. The most informative markers in the populations were DYS390, DYS389II, DYS389I and DYS391 in the respective order of magnitude, the highest and lowest haplotype diversity were observed in Moroccan Berbers and Mozabites respectively.[14]

The first study on Y-STR of the Tunisian population was conducted by El Khil et al., (2001) who studied six Y-STR loci among 135 males from different ethnic groups: Berbers, Blacks of Jerba Island and Arabs. There was no significant difference between the Arabs and the Berbers except on locus DYS390. Contrastingly, a significant difference between Blacks and the other two islander groups was observed except for the locus DYS39I.[15]

In another study of 135 Jerban males, 67 different haplotypes were reported: 33 haplotypes out of 42 Jerban of Sub-Saharan Africa, 27 haplotypes out of 46 Arabs and 18 haplotypes out of 47 Berbers.[16] The study population had a haplotype diversity ranging from 0.987 to 0.827 where the Jerbans of Sub-saharan origin had the uppermost value, whereas the Berbers had the lowest value.

Another study on 13 Y-STR of 105 Southern Tunisian population by Ayadi et al., (2006) identified 81 different haplotypes, out of which 67 were unique, the most frequent haplotype was shared by five individuals in the study population.[17] The loci with the highest and lowest polymorphism are DYS385a/b and DYS436 respectively. In another study by Onofri et al., (2008) in Northern African populations: 52 Tunisian and 51 Moroccan samples, a lower haplotype diversity of 29 unique haplotypes out of 39 different haplotypes were observed in the Tunisian population, while higher haplotype diversity of 44 unique out of 47 different haplotypes in Moroccan population were reported.[16]

A study on a total of 267 Moroccan ethnic populations (Sahrawi, n=68, Berber-speaking, n=69 and Arab-speaking, n=130) revealed a total of 257 (96.25%) different haplotypes, out of which 10 alleles were found in two individuals each, 237 unique haplotypes were observed.[19] Highest Gene Diversity was recorded on alleles DYS385 (0.887) and DYS458 (0.820), the discrimination capacity (DC) and Haplotype Diversity (HD) were 0.963 and 0.9991 respectively.[19] Another study by Palet et al., (2010) on the Moroccan population from Figuig Oasis revealed 52 different haplotypes, and 36 unique in an overall total population of 96. The loci with the highest and lowest respective diversity were DSY458 and DYS392.

Another study on Berber and Arab-speaking populations in Morocco by[20] reported 74 different haplotypes out of the total 85 individuals. A non-significant difference in gene diversity between the Arab-speaking samples having higher (0.566) than the Berbers (0.472) was reported by the authors. The high polymorphic alleles in Arab-speaking and Berbers were DYS385 and DYS458 respectively, while the lowest polymorphic marker was DYS392 in both the populations. Two new alleles of DYS458 locus were observed in one Berber and one Arab.[20]

A study on 17 Y-STR of 208 individuals from South(Upper) Egypt reported 204 different haplotypes, of which 200 were unique and 4 alleles were found twice each.[21] The most polymorphic allele was DYS385a/b followed by DYS458. Another study on Y-STR of 238 Benghazi

population, East Libya revealed a total of 238 different haplotypes, out of which 214 were unique, and 24 shared haplotypes.[22] The most polymorphic loci were DYS385a/b and DYS458 with haplotype diversity of 0.82 and 0.73 respectively.[22] Another study on Libyan population by Triki-Fendri *et al.,* (2013) revealed 142 different haplotypes out which 124 were unique in a total population of 176 individuals.[23]

D'Atanasio *et al.,* (2019) studied the discrimination power of the Y-Filer Plus multiplex kit in 11 North African populations from Egypt, Libya, Algeria and Morocco. The authors observed null alleles at three different loci (FYF387S1, DYS448 and DYS389II). They determined the genetic diversity (GD) with the exclusion of the null alleles and observed DYS385 and DYS481 with the highest values of 0.86 and 0.85 respectively. DYF387S1 showed the fourth highest value comparable to the GD values of RM Y-STRs which the authors attributed to an observed low GD value in the Algerian population under study.[24]

The most polymorphic loci in North African populations are DYS385 and DYS458, while the least polymorphic locus in all the North African populations was DYS392. Some Tunisian populations revealed DYS710 as the most polymorphic marker. Intermediate alleles were observed in some of the North African populations. The North African populations also exhibited unique haplotype diversity even after search and comparison on Y-STR databases which makes them suitable for forensic casework.

Y-haplotype and Genetic Diversity in West Africans

A study on the population of Guinea Bissau was conducted by.[25] The authors studied Y-STR population data of 215 unrelated healthy males whose ancestors were known to have lived in Guinea Bissau for three generations. The authors observed that the range of the studied loci and the allele frequencies are similar to the ones observed in other Sub-saharan Africa. The authors noted high prevalence of alleles 11 for DYS392 (88%), 14 for DYS437 (72%), 11 for DYS438 (65%), 21 for DYS390 (67%), and 15 for DYS19 (42%). The highest genetic diversity was observed in DYS19 and DYS389II (0.7182 and 0.7239) respectively, while the highest haplotype diversity was observed in DYS385 (0.9031). One hundred and fifty-four distinct haplotypes were observed in 161 fully typed individuals.

Benin and Ivory Coast ethnic populations were studied by Fortes-Lima et al., (2015). The Authors studied 288 individuals. The data from the research showed 30 minimum haplotypes and a total of 45 Y-filer in Yoruba as well as 34 minimum haplotypes and 44 Y-filer in Bariba population. The Yoruba and the Bariba exhibited high genetic diversity values of 0.9937 and 0.9929 respectively.[26]

A study was conducted at the Institute of Legal Medicine, Cologne, Germany. Individuals from different countries of West Africa (Nigeria, Gambia, Niger, Senegal, Benin, Togo, Sierra Leone, Ghana, Ivory Coast and Liberia) were selected for the study.[5] High values of haplotype diversity (1.0000 ± 0.0018) were observed in 86 samples under study, the values obtained were similar to what was reported by other studies.[5]

Y-STR profile of 142 individuals from the three largest ethnic groups in Nigeria (Yoruba, Hausa and Igbo) was studied by Martinez et al., (2017). 140 different haplotypes were observed, comprising of two individuals with two shared haplotypes. The authors reported an increase in the number of shared haplotypes when Y-filer kit was used: four and one haplotypes shared by two and three individuals respectively.[27]

The data presented on the genetic profile of West African populations revealed low genetic diversity with very few shared haplotypes among the individuals which translates to population homogeneity. Upon comparison with other populations on YHRD, very few matches were obtained. DYS385 was found to be the most polymorphic allele in some West African populations.

Y-haplotype and Genetic Diversity in Southern Africans

A study was conducted by Sánchez-Diz P *et al.,* [28] on African population groups from Mozambique. The authors studied a sample of 308 unrelated healthy individuals from the following groups: Nguni, Rongas, Senas, Changanes, Nhungwes, Tswas, Macondes, Chopes, Yao, Bitongas, Chuabos, Shonas, Lomwe, Ndaus, Makuas and

Nyanjas. Lower gene diversity was observed on DYS391 and DYS392 in all the populations under study. Of the total 308 samples studied, only 126 different haplotypes were observed, with the most frequent haplotype present in 22 samples. The observed average haplotype diversity was 97%.

Study on individuals living in KwaZulu-Natal and Western Cape provinces in South Africa was conducted by Lea N *et al.*[29] Three subpopulations (88 Xhosa, 101 English Speaking Caucasian and 77 Asian Indian males) were recruited for the study. Of the total population, 77, 101 and 73 different haplotypes were observed in Xhosa, Caucasian and Asian Indian populations. The number of alleles ranges from three (DYS391 and DYS392) to 21 in DYS710, while the average gene diversity ranged from 0.32 in DYS391 to 0.89 in DYS711 loci. The authors reported DYS710, DYS711, DYS712, DYS713, DYS7114 as novel markers and among the most variable markers.[29]

In another study conducted by D'Amato et al., (2008) on 99 indigenous Xhosa, 100 Caucasian English, 86 Asian Indian, 114 mixed "colored" and 107 Caucasian Afrikaan populations. Of the 506 individuals, 394 different haplotypes were observed, shared haplotypes were observed in 33 individuals. The allele frequency and haplotype diversity in 54 Ovambo male population in Namibia were carried out.[30] The study was conducted on 28 Y-STRs, where a total of 51 different haplotypes and 48 unique haplotypes were observed. Three shared haplotypes were also observed in two individuals. DYS385 and DYS392 had the highest (0.9000) and lowest (0.036) respective diversity values.

Analysis of 17 Y-STR loci in 105 healthy, unrelated Muslim populations of Cape Town, South Africa was conducted.[31] Eighty-three, 102 and 89 Asian-Indian, European-English and native Xhosa respectively were used for the comparison. The most polymorphic (0.958) and least polymorphic (0.449) loci based on GD values reported were DYS385 and DYS391 respectively. Ninety-one unique haplotypes and DC values of 0.866 were observed when considering the nine minimal haplotype Y-STRs, while in the case of the remaining eight loci.

The Y-STR haplotypes in three ethnic groups of Angola were studied by Melo *et al.* (2011). The authors studied 11 Y-STR haplotypes from a total of 166 individuals of three main ethnolinguistic groups of Angola: 53 Ovimbundo, 57 Bakongo and 56 Kimbundo populations. The Ovimbundo ethno-linguistic group showed 39 and 46 different and unique haplotypes respectively with two shared haplotypes that appear twice and one shared haplotype that appeared three times. Fifty-three and 49 different and unique haplotypes were observed respectively in the Bakongo group, four shared haplotypes were observed twice in the Bakongo group. In the Kimbundo group, 53 and 50 different and unique haplotypes were reported, while three shared haplotypes were observed twice. The most polymorphic locus was DYS385. Of the total of 166 individuals, 138 and 120 different and unique haplotypes were observed respectively.

The first study on Y-STR in Botswana population was conducted by Tau *et al.,* (2015), the authors studied 17 Y-STR profiles of 252 individuals among Botswana population: The authors clustered the samples into two regions: Northern [North and North-Western (1 San, 1 Sobea, 1 Herero, 6 Yeyi, 3 Mbukushu) n=12] and Southern Botswana [South and South East(1 Pedi, 2 Ndebele, 5 Tswapong, 11 Birwa, 8 Kgalagadi, 24 Kalanga, and 189 Tswana) n= 240]. The authors observed Haplotype Diversity and Discrimination capacity of 0.9990 and 0.9444 respectively and 238 unique haplotypes. The most common haplotype was observed five times in the populations except in the Tswana and Mbukushu that had four and one most frequent haplotypes.

D'Amato & Kasu, (2017) designed a highly discriminating Y-STR kit to preferentially target and amplify African samples, this genetic tool has been developed to a commercial prototype called UniQTyper Y-10. It was made up of 10 Y-STR loci markers including four RM Y-STRs. The authors studied 957 individuals from native and immigrant South African ethnic populations: English, Afrikaan, Indian, Admixed and native Bantu groups such as Venda, Pedi, Xhosa and Zulu. Of the total 957 studied samples, 870 unique haplotypes were observed with an overall Discrimination Capacity of 0.909. Another study conducted by Lesaoana et al., (2019) also used UniQTyper Y-10 to type the Y-STR profile of 938

individuals in five Bantu ethnic groups living in 10 Lesotho districts composed of South, North and Central regions. The authors reported 698 and 588 different and unique haplotypes respectively. A total of 350 individuals shared 110 haplotypes, the most frequent haplotype was shared by 28 individuals. The same haplotype was also reported to have been observed in 17 unrelated samples in Northern South Africa.

Another study on 27 Y-STR profiles of 200 unrelated individuals of Shona ethnic group of Zimbabwe in Harare province was conducted by Shonhai et al., (2020a) using 5-dye SureID 27Y kit. The authors reported only 159 complete 27 loci profiles, in response to that, the authors downgraded the loci to 12 Y-STR of PowerPlex. A total of 154 unique haplotypes out of the 159 were observed, two and one haplotype appeared twice and four times respectively. With a high genetic diversity depicted by the haplotype diversity of 0.9994, the overall DC of the population was 0.9686 while haplotype match probability was computed as 0.0069. The authors performed a single locus analysis of the whole Y-STR profile where they reported several observations which included but were not limited to triallelic pattern for locus DYS387S1, microvariant allele markers at DYS387S1 and DYS385. The lowest GD values were observed at loci DYS392 (0.03748) and DYS437 (0.096702). Meanwhile, DYS449, DYS481, and DYS518 had the highest GD values of 0.867239, 0.85042, and 0.825179, respectively.

In another study by Shonhai et al., (2020b) on Zimbabwean Shona brother pairs using the same kit used by Shonhai et al., (2020a). Only four brother pairs out of the 18 pairs were distinguishable based on the variation of allele numbers on only one allele marker among the 27 loci studied in addition to RM Y-STR DYS518. The authors observed loci DYS481 and DYS518 to have the highest GD with values of 0.8252 and 0.8502 respectively. Although the authors described the loci DYS393 and DYS458 as mini Y-STRs, they could not clearly explain the reason why variation between the brothers was observed in the markers. It is clear that the kit is not suitable for discriminating between related individuals. However, it can be noted that the kit was discriminatory between unrelated male Shona population of South Africa.[36]

The studies conducted on the Southern African populations have revealed that the most polymorphic markers reported were DYS385 and DYS710 in some populations. DYS391 and DYS392 were reported with the lowest GD values in the South African populations. Analysis of molecular variance in the study population revealed variations within the study groups. Searches on YHRD and Applied Biosystem databases presented very few numbers of shared haplotypes with other African populations. Additionally, very few shared haplotypes were observed within the study samples. The high frequency of unique haplotypes in the populations has highlighted the suitability of Southern African populations for forensic casework.

CONCLUSION

It can be concluded based on the review that the African populations are unique populations with high discrimination haplotypes, thus making them unique for forensic reference. DYS385 is the most polymorphic allele, intermediate allele 13.2 at the same locus was observed in Central African populations. The most informative marker in East African is also DYS385, while DYS392 is the least polymorphic locus. The North African populations bear DYS385 as the most polymorphic marker in addition to DYS458, while DYS392 is also the least polymorphic marker. However, the Tunisian population has exhibited DYS710 as the most informative marker. DYS385 was also the most informative marker in West African populations. DYS385 is also the most informative marker in South African populations in addition to DYS710 in small populations as observed in Tunisian populations of Central Africa. DYS391 and DYS392 are as well the least polymorphic markers in the South African populations. However, based on the number of African populations and the number of countries where the studies were conducted, it can be said that the African genetic data for forensic reference is under-represented. There is also no dedicated African database for forensic reference.

It is recommended that African nations should embrace the use of DNA forensics to minimize the rate of crimes in their countries. The need for

African nations to embrace the use of Combined DNA Information System (CODIS) cannot be over-emphasized, as this will help in solving many mysterious criminal cases involving mass disaster victims, victims of rape, murder, and other criminal cases. Based on the data generated from the review, it is visible that there is a need to develop and validate Y-STR kits that will primarily target and amplify the unique African haplotypes.

RECOMMENDATIONS

It is recommended that Africans should embrace the DNA forensics to minimize the rate of crimes in Africa. The need for Africa to embrace the use of Combined DNA Information System (CODIS) cannot be over-emphasized, as this will help in solving many criminal cases involving mass disaster victims, victims of rape, murder, and other criminal cases. Based on the data generated from the review, it is visible that there is a need to develop and validate Y-STR kits that will primarily target the unique African haplotypes. IJFMP

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REVIEW ARTICLE

Emergence of Earthquake Resistant Buildings: Review of Earthquake Resistant Tall Buildings of India

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ABSTRACT

Earthquakes are a sign that the earth's internal structure is changing. Seismic activity is normal in most parts of the world, but the frequency with which it occurs is determined by the tectonic setup of the region. Past earthquakes have resulted in significant loss of life and property, impacting a country's social and economic conditions. Though an earthquake cannot be prevented, the least that can be done to minimize damage is to make buildings earthquake-resistant. Most countries have required the inclusion of seismic requirements in building design and architecture as our understanding of earthquakes has increased. This paper aims to create a review of some earthquake resistant tall building in various seismic zones in India. This study focuses on what are the different techniques adopted for these tall building to make it earth quake-resistant. This is a research with the approach consists of analysis from various case and literature studies and their comparison with regard to earthquake.

KEYWORDS | earthquake resistant building, techniques, seismic zones

INTRODUCTION

Earthquake is a tectonic or volcanic phenomena that depicts rock movement which causes the earth to shake or tremble. Earthquakes are one of the most terrifying natural occurrences. Due to its peculiar geophysical factors, India is extremely vulnerable to earthquakes of various magnitudes. Several million earthquakes occur each year all around the planet. Several devastating earthquakes have struck the country in the previous years, causing a considerable amount of fatalities and property damage.

Five earthquakes measuring M8 or more hit various areas of the globe during the last century:

- Uttarkashi (1991) M6.6
- Latur (1993) M6.4
- Jabalpur (1997) M6.0g
- Chamoli (1999) M6.8
- Bhuj (2001) M6.9.

Other earthquakes (Muzaffarabad in 2005 M7.6; Great Sumatra earthquake, 2004 M9.1) that occurred outside Indian territory had a significant impact on the country as well. Table 1 displays the recurrence of earthquakes by area over the last 110 years. (Safety_earthquake@Nidm.Gov.In, n.d.)

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METHOD & MATERIALS

Seismic Zones of India

According to the magnitude of the damage or the frequency at which earthquakes occur, the country has been divided into regions or zones. The seismic coefficient can be used to design buildings in various parts of the nation by referring zoning charts given by National Institute of Disaster Management.

Zone 5

This zone encompasses the areas that are most vulnerable to earthquakes of magnitude MSK IX or greater. The highest amount of seismicity is connected with Zone 5.

Very High Damage Risk Zone is what it's called. Kashmir, the western and central Himalayas, North and Middle Bihar, North-East India, and the Rann of Kutch, as well as the islands of the Andaman and Nicobar group.

Zone 4

Zone 4 is susceptible to MSK VI to MSK IX intensity. The High Damage Risk Zone is what it's called. Zone 4 includes the Indo-Gangetic basin, the country's capital (Delhi), and Jammu & Kashmir. The Patan area (Koyananager) in Maharashtra and the northern section of Bihar, such as Raksaul, near the India-Nepal border, are both in Zone 4.

Zone 3

This zone is vulnerable to MSK VI intensity. This zone includes the Andaman and Nicobar Islands, sections of Kashmir, and the Western Himalayas. The Moderate Damage Risk Zone is the name given to this area.

Zone 2

The Zone of Low Risk of Damage is defined as a zone that is vulnerable to MSK VI or less. The lowest level of seismicity is linked with this zone.

METHODOLOGY

This paper showcases idea of earthquake resistant tall buildings by studying some of the earthquake resistant tall buildings of the world. Since these buildings are located outside India; the data has been collected through online articles, journals and other internet sources. The main objective is to study their vertical shaping, plan shaping for wind, foundation treatment, materials used and may more. Study provides an understanding of advancements in the building industry which includes Foundation design, Innovative materials used, vertical shaping of the skyscraper. These tall buildings have incorporated such principles and withstand itself in high intensity earthquakes. This paper analyzes these innovative techniques and their capability to withstand earthquakes.

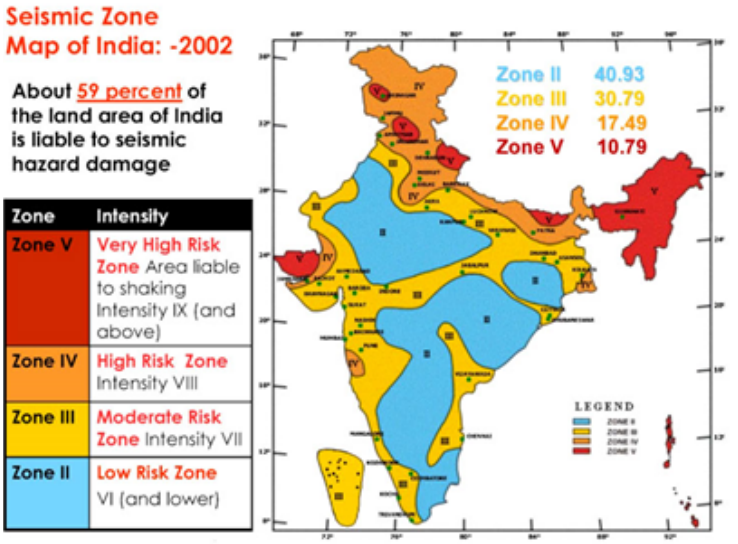

Figure 1 Seismic Zone Map of India - 2002 Source: The Hindu Times

SEISMIC REGION	NO.OF EARTHQUAKES OF MAGNITUDE				RETURN PERIOD
	5-0.5.9	6.0-6.9	7.0-7.9	8.0+	
Kashmir & Western Himalayas	25	7	2	1	2.5-3 yrs.
Central Himalayas	68	28	4	1	1yrs.
North East India	200	128	15	4	<4Months
Indo-Gangetic Basin and Rajasthan	14	6	-	-	5yrs
Cambay and Rann of Kutch	4	4	1	1	20 yrs.
Peninsular India	31	10	-	-	2.5-3 yrs.
Andaman & Nicobar	80	68	1	1	<8 months

Table 1 Regionwise major earthquakes in India

Source: Safety_earthquake @ Nidm.Gov.In, n.d.

Limitations

Some of the structures under investigation are approaching completion, so their earthquake resistance cannot be accurately assessed, but the features and techniques are useful for future research. As a result, only qualitative data is available for such structures.

Seismic Codes of India

India is among the nation's greatest catastrophe

countries, having been hit by practically every natural and man-made calamity.

Around 85% of the region is exposed to one or more disasters, and approximately 57 percent of the country, along with the capital, is positioned as a high earthquake zone. Technological intervention in buildings and infrastructure to makes them more resilient to natural disasters.

IS codes are intended to serve as a guideline for the construction and repair of buildings that are subject to seismic forces. (Safety_earthquake @ Nidm.Gov.In, n.d.).

Earthquake Resistance Construction

Earthquake-resistant construction refers to the design of a structure or building that can withstand the rapid ground shaking that happens during earthquakes, decreasing structural damage as well as human deaths and injuries. Appropriate construction procedures are required to ensure that correct design objectives for earthquake resistance are accomplished. Because construction methods vary so widely over the world.

The design of a building and the construction procedures utilised to construct that building are fundamentally different. Advanced earthquake-resistant designs can only be successful if suitable construction procedures are applied in the site selection, base, structural members, and link joints. Structures and structural elements designed to withstand earthquakes frequently have ductility (the ability to bend without breaking).

Behaviour of Tall Buildings to Ground Motion

During earthquakes, ground vibrations induce inertia forces at mass locations in the house. These forces pass to the base via the roof and walls. The main focus is on ensuring that these requirements are met. Without causing significant damage or failure, the powers hit the ground.

The roof, wall, and foundation are the three components of a masonry building. The walls are the most resistant to earthquake damage caused by horizontal forces. When pushed horizontally at the top in a direction perpendicular to its plane (the poor direction), a wall topples quickly, but when pushed along its length, it provides much more resistance (termed strong direction).

Source: architizer.com

CASE STUDIES

Spire Edge, Manesar

- Architects: T.R. Hamzah & Yeang Sdn Bhd
- Project: Spire Edge
- Location: Manesar, India
- Client: A.N Buildwell Pvt. Ltd
- Nos. of Floors: 20 floors + Roof Garden
- Site Area: 4,765 sq.m
- Proposed Development: Commercial and Offices
- Project Architect: Glen Pang
- Design Architect: Khairi Ismail

(Elements & Criteria, n.d.)

Winner of numerous awards Spire Edge is a Leed Platinum and Leed Gold Mainstream Green office complex designed to provide a financially stable, socially lively, and environmentally restorative work environment for IT/Ites organizations seeking development, expansion, or consolidation.

Design Strategies

The green Design Strategies of the building areas

Source: architizer.com

Source: www.designingbuildings.co.uk/

Source: http://hindupad.com

are as follows:

- Continuous Landscape Ramp
- Rainwater Harvesting/Recycling
- Roof Gardens
- Climate Responsive Façade
- Pocket Park
- Sun Shading Strategy
- Eco cell
- Earthquake resistant

Architectural features that make it earthquake Resistant

- Rubber shock absorbers to absorb earth tremors.
- Foundations sunk into bedrock avoiding clay.
- Computer controlled weights on roof to reduce movement.
- Automatic window shutters to prevent falling glass.
- Fire resistant building materials.
- Roads to provide quick access for emergency services.
- Steel frames that can sway during earth movements

Chandrodaya Mandir, Vrindavan, Mathura

The world's tallest temple skyscraper, Vrindavan Chandrodaya Mandir, is currently under construction (2016).

The Hindu temple will be built in the Mathura area of Uttar Pradesh, India, and would have a footprint of roughly 5 acres and a height of 213 meters (700 ft).

It is being erected at a cost of Rs. 300 crore (US$45 million) by ISKCON Bangalore, making it one of the most expensive temples ever built.

The building will be cutting-edge and earthquake-resistant.

It is being designed by Thornton Tomasetti, who has created some of the world's highest structures, including the Petronas and Shanghai Towers.

The project is projected to cost around Rs. 800 crore, with a total of 50 crore spent on piling work.

Vrindavan Chandrodaya Mandir, as the world's highest religious monument, demands a large number of construction materials.

It will have 32,516 m2 (350,000 sf) of glazing, 127,426 m3 (4.5 million c.f) of concrete, 17.2 million kg (19,000 tons) of reinforcing steel, and 5.8 million kg (6400 kg) of structural steel, and will be supported by more than 500 piles with a diameter of 1 m (3 ft) and a depth of 55 m (180 ft).

Under the Leadership in Energy and Environmental Design (LEED) standard, the project team is aiming for a Gold rating.

The project team is seeking a Gold rating under the Leadership in Energy and Environmental Design

(LEED) program.

The Indian Institute of Technology (IIT) Roorkee is also doing a seismic study of the site to guarantee that the building design is designed to withstand the projected amount of seismic activity. The temple is currently intended to withstand a seismic event nearly twice as powerful as the area's largest known earthquake, which struck in 1803 and measured 6.8 on the Richter scale.

RWDI, a Canadian consulting engineering firm, is one of the project's other consultants, having completed wind-tunnel design with a wind-load goal of 226 km/hr (140 mph),

Tata Consulting Engineers, based in India, built the queue systems to hold 35,000 people on a typical day. (These systems should be able to hold 250,000 people on a festival day.)

Aecom, an American corporation, will install and design a fire and life safety system, overseen by Sunil Shahani, who has worked on similar projects before.

Earthquake Resisting Techniques In High Rise Buildings

Building damage can be avoided or minimized by taking a few precautions during construction. Buildings' earthquake protection can be improved by good design and construction. Many high-rise buildings incorporate earthquake-resistant features such as shear walls, moment-resistant frames, or new strategies such as base isolation or energy dissipation systems to avoid or minimize damages and casualties.

Techniques Used

A. Shear walls

B. Base isolation

C. Energy dissipation methods

D. Addition of Composite with concrete

E. Addition of any above methods

A. Shear walls

Shear wall is a type of wall seen in structures that extends from the bottom to the top of the structure at various points in the plan, preferably along the periphery. Shear walls are employed in structures to resist seismic and wind forces acting in a lateral direction on the structure.

B. Base isolation

The devices that are positioned between the foundation and the superstructure are known as isolators. Base isolators function similarly to automobile suspensions in that they shield passengers from shock. One of the best tools for controlling vibration or shaking of buildings in an earthquake is base isolation.

As an effective technique, the base isolation system is employed to improve earthquake resistance. It is

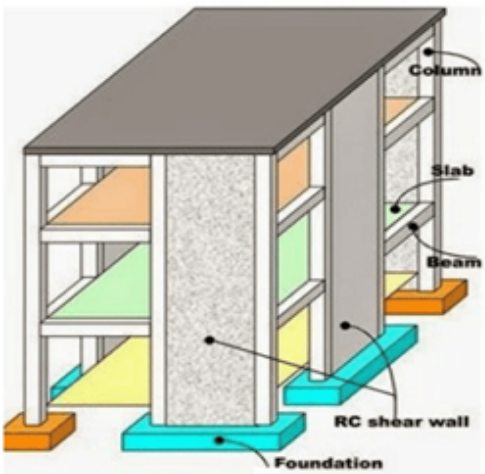

Fig. 6 Shear Wall Design to EC2 Source: civildigital.com

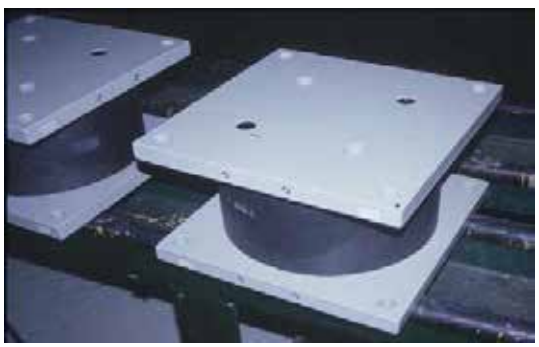

Figure 7: Elastomeric bearing (Mahadik & Bhagat, 2020)

Figure 8: Low friction bearing (Mahadik & Bhagat, 2020)

Source: civildigital.com

one of the most effective ways to reduce the rigidity of a structure so that it can withstand earthquake forces.

Bearings used in stiffness reduction

C. Energy Dissipation System

The use of energy dissipation devices known as dampers is another method of seismic strengthening. In this strategy, structures are outfitted with additional devices known as seismic dampers, which have a high dampening capacity and can significantly reduce seismic energy entering buildings while minimizing structural damage.

This system consists primary structure and secondary structure. Structure is pre strengthened with secondary structures called as seismic dissipaters.

D. Addition of Composite to Concrete

Another way for strengthening seismic resilience of structures is to use composite materials. These composite materials are used to improve the structural behaviour of concrete in structural members. Composites are made up of two or more components that are mixed together to create a new substance that is superior to the original.

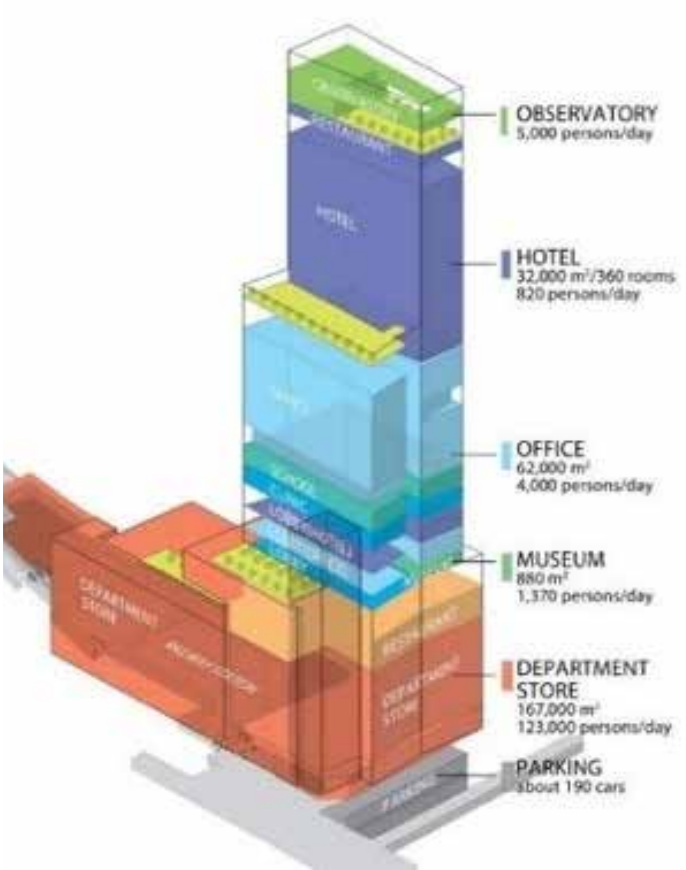

Figure 9: Vertical Zoning of Abeno Harukas, Japan.
(Mahadik & Bhagat, 2020)

Review of Tall Buildings Earthquake Techniques:

Earthquake-resistant or aseismic systems are designed to shield buildings from earthquakes to some degree or another. While no structure can be fully earthquake-proof, earthquake-resistant architecture aims to build structures that perform better than their traditional counterparts during seismic activity.

Building codes state that earthquake-resistant buildings must be able to withstand the largest earthquake with a reasonable chance of occurring at their site. This means that the loss of life should be reduced for rare earthquakes by avoiding building collapse, while the loss of functionality should be limited for more common ones. To avoid being destroyed by earthquakes, ancient architects had no choice but to design their landmark structures to last, often by making them overly rigid and solid.

Abeno Harukas, Japan

Abeno Harukas is a steel-structured multi-functional skyscraper tower stands on the west side of Osaka Abenobashi Station in Abeno ward.

The structure stands 300 meters tall, with 60 storeys above ground and 5 floors below. It is Japan's tallest skyscraper and the third tallest structure in the world, after Tokyo Sky tree and Tokyo Tower.

Takenaka Corporation, an architectural firm, designed the tower. The superstructure is made up of three blocks, each with a north side setback.

The bottom building has the Kintetsu Department Store, which has a platform on the second level for the Kintetsu Railway, the middle block houses offices and an Art Museum on the 16th floor, and the upper block houses a hotel with an observation deck on floors 58-60.

The building's foundation is a piled raft foundation, which comprises of a raft foundation with a bottom depth of 30.5 meters below ground level and cast-in-place concrete piles placed in a dense gravel layer below a depth of 70 meters.

Techniques To Achieve Seismic Design Concepts

High-capacity piled raft foundation with soil-

cement continuous wall construction

Because Harukas is a reconstructed structure over the city's central terminal station, it occupies nearly the entire site. Many constraints apply to the foundation structure, which necessitates excellent efficiency.As a result, for this building, a piled raft foundation consisting of piles and bottom plate, both of which bear the building weight, is employed, with an inverted placement method employing basement columns. As a result, the superstructure is built up to the 50th floor level before the bottom plate is installed. Accordingly, the piles bear approximately 90% of the column axial stress, while the bottom plate bears the remainder.

High-strength CFT columns

Because HARUKAS was built in such a small location on the site, it was essential to keep the column sections as small as possible. In order to assure the safety of the columns that bear significant axial forces, concrete filled steel tube ("CFT") columns consisting of high-strength concrete and high-strength steels were utilized in this structure.

New Joint System

To achieve the self-filling property of high-strength concrete, this structure uses a unique joint system consisting of an exterior diaphragm and aluminum spray jointing.

Rotational Friction Dampers

In the void of a low-rise department store, rotational friction dampers and oil dampers are installed to absorb the seismic energy that will be input into the building and help lessen seismic responses.

Corrugated steel plate walls

In the middle gap of the office area, corrugated steel plate walls are erected in the longitudinal direction of the building. A corrugated steel plate wall is an earthquake-resistant structure made up of a steel plate that is corrugated in the height direction and surrounded by flanged steel plates that are integrated with their frame.

Core Truss Dampers

The core truss dampers are positioned in the hotel's central void so as not to interfere with ventilation and to reduce the high-rise component's deformation.

Taipei 101 Taiwan

Taipei 101, located in Xinyi District, Taipei, Taiwan, is one of the world's tallest buildings. The 1,667-foot tower has a tuned mass damper to prevent damage in the event of a natural disaster such as a typhoon or earthquake. Near the top of the structure, steel cables suspend a massive mass that acts like a pendulum, swinging back and forth in the opposite direction of the building to dissipate steam. One of the world's largest tuned mass dampers is the gold ball, which weighs 730 tonnes and hangs from the Taipei 101.

Foundation

Soft soil in the form of clay and stiff colluvial soil is present just below the site of Taipei which has low load bearing capacity.

Soft rock in the form of sand stone is present beneath 40-60m; hence it is required matt foundation with bored piles. The tower required a 21 m deep basement

Water table at a site is 2m below ground which would create huge uplift forces on the foundation

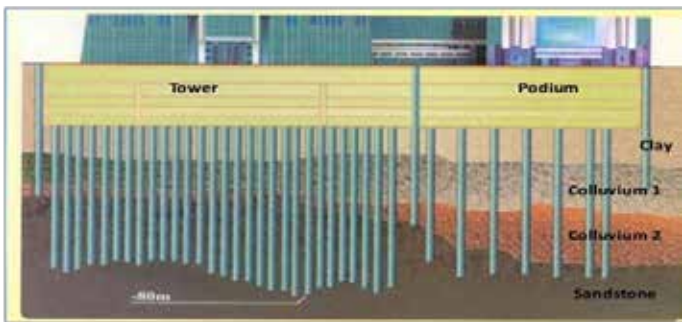

Figure 10: Spacing of Piles below Podium and Tower, Taipei 101

Source: structures-explained.com

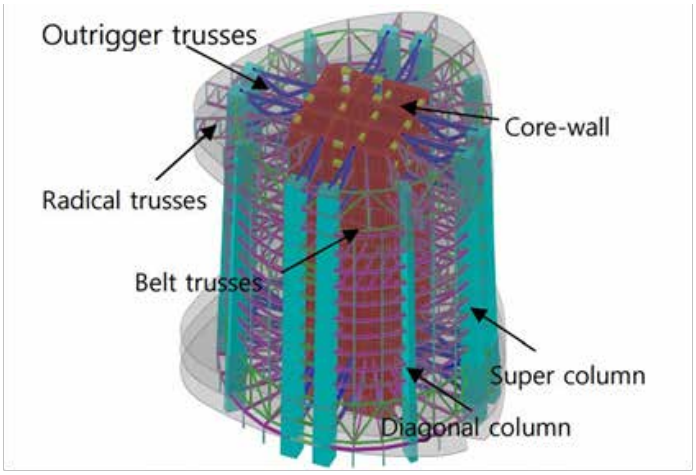

Figure 11:Outrigger trusses with central braced core, Taipei 101

Source: http://faculty.arch.tamu.edu/

of the building. hence slurry walls were constructed to lay the foundation below the tower.

Foundation Depth 80m.

These walls surround both the tower and the podium and are 1.2 m thick and up to 47m below the ground. Main foundation of the tower consists of 380 piles of 1.5 m dia. And 167 piles for podium area. They were spaced 4m apart in the staggered rows for tower portion. A concrete raft thickness 3-4.7 m capped the piles and transferred the load from columns and walls above. Use of steel in the superstructure minimized the building weight which reduce the cost of foundation.

Superstructure

The building is 508 m above ground and resembles ancient pagodas. it takes inspiration from bamboo which is flexible and light yet strong. The bamboo has joints at intermediate locations which are

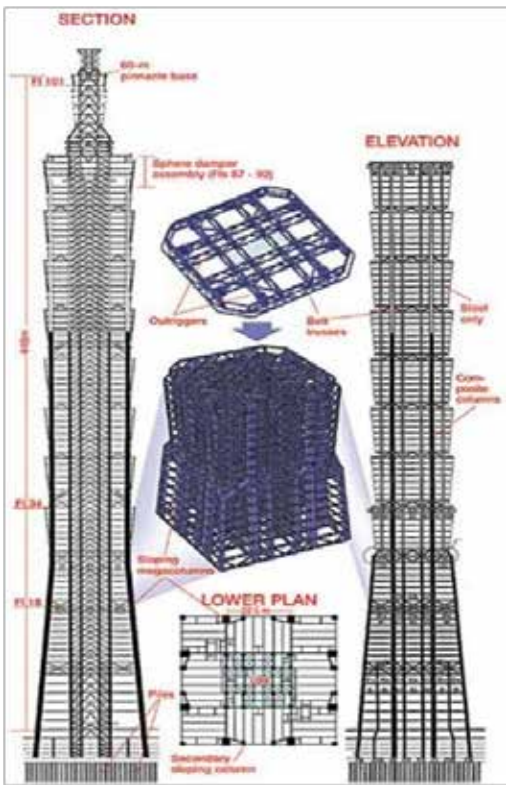

Figure 12: Seismic Design Components, Taipei 101, Taiwan
www.ctbuh.org/papers

mimicked by the building in the form of out riggers and belt trusses at every 8 floors. this separates the building into 8 identical modules.

At the top of 8th module sits on 9th module which has a smaller footprint. This module supports a spire and contains equipment and a observation deck. Below the 8th repetitive modules, a 25-story base shaped as a truncated pyramid is present. This base provides as an improved overturning resistance and lateral stiffness compared to a straight block. The story height of each floor is 4.2 m and retail floors below are 6.3 m. The floor is composite steel and concrete typically 135mm thick.

Tapai 101 is built in a high typhoon zone which experiences winds of 156km/hour with 100 year return period.

The building is impacted by alternating cross wind forces due to vortex shedding which means wind passing the building separated from the sites producing alternating whirlpools. These winds can also damage façade and partitions.

Sawtooth or double notch corners were provided which reduced the wind forces by up to 40%. The building has a square core made up og 16 box columns in four lines which are generally fully braced by moment frames between floors. The braced core is encased in concrete wall from foundation to the 8th level.

The core box columns were filled with concrete of strength 69Mpa till level 62, The building has 8 super columns or mega columns which are steel boxes filled with high strength concrete. they are present on the perimeter of the building to on each face. these super columns built up to the level 90 of tower. They were filled with concrete of Strength 69 Mpa from bottom of the basement till level 62,

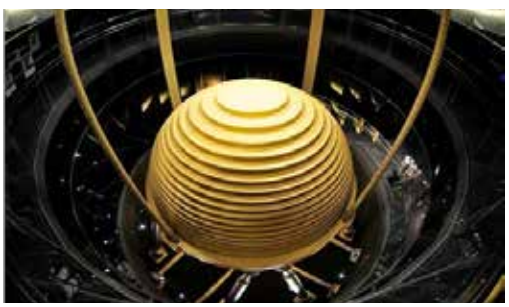

Figure 13 Mechanism of Damping, Taipei 101
Source: www.ctbuh.org/papers

these columns control drift as large portions of drift is created at lower stories due overturning rotations.

The building was design to be stiff for resisting

the wind forces first and then checked for seismic ductility and seismic strength.

The steel framing used in the building is a special moment resisting frame also known as SMRF Steel moment frames along sloping face of the building works in the braced core and out riggers to counter seismic forces.

MASS Dampers

At the top of the building between 86 and 92th floor is a huge pendulum which is called a tune massed damper or just TMD.

This pendulum counters the wind force and reduces sway of the building in typhoons. This damper uses building motion to push and pull giant shock absorbers to convert motion to heat by forcing fluid through small internal openings.

RESULT & DISCUSSION

The components that are placed in a structure to improve its seismic resistance are referred to as earthquake resisting elements, and the procedures used to apply these elements are referred to as techniques.

Even if structures are planned and detailed according to code, there is still the chance of damage or failure in the event of a significant earthquake. Building performance can be improved by incorporating earthquake-resistant materials and strategies.

As per analysis from the above case studies it is concluded that various techniques are used in Indian tall buildings as well as tall buildings in other countries, the major difference is the variation in seismic zones, accordingly, the design concepts were used and also techniques were used. So, the major thing is that there is a smaller number of a tall building are in India, so for future trend architects or engineers refers following techniques which were concluded from the above analysis that is Flexible foundation, Damping, Shield building from vibrations, Reinforce the structure by using shear walls, cross braces, diaphragms.

As a developing country, India has a limited number of tall buildings, according to the case study analysis. There is a lack of understanding about how to use earthquake-resistant materials and construction procedures.

As a result, it is critical to spread information on earthquake-resistant structures, technologies, and materials. To achieve this, we must introduce such basic programs at the elementary school level to raise awareness about earthquake-resistant structures, as well as higher-level awareness programs for people working in the construction industry such as architects, contractors, civil engineers, and structural engineers, to ensure that they will incorporate this knowledge into their upcoming projects.

CONCLUSION

In the past 50 years, India had faced six massive earthquakes which killed over 23,000 people and wreaked havoc. India has the world's second-largest population and, the second-largest number of buildings. However, few people realize that India also boasts of highest number of earthquake-prone structures. As everyone knows, earthquakes don't really kill people; nevertheless, falling buildings do, making India the world's most susceptible country. All structures in India are designed to the lowest seismic category-D, which means that after an earthquake, the building will be inoperable and will have to be abandoned. People who want better protection should have their structures designed in the same way that Japan does. Making India the world's most susceptible country. So, guidelines should be strictly followed in the construction. IJFMP

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REVIEW ARTICLE

Forensic Application of Non-destructive ATR-FTIR Spectroscopic Technique for Organophosporus Pesticides Analysis

Shivpoojan Kori

ABSTRACT

Over a long period organophosphorus pesticides (OPPs) such as Tusk, Dimex, Dysac, Sacban and Monovip contain Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos as active chemicals, respectively are used in agriculture to enhance the crops yield. However, these chemicals are extensively used in criminal cases such as suicidal, accidental, and homicidalthroughout the world. Such cases can conveniently be resolved with the help of spectral library as reference/ standard to compare with unknown or suspected samples recovered from scene of crime. The spectral library was generated using ATR-FTIR technique which reliable and non destructive in nature. This spectral library has been applied to identify the OPPs in different biological samples and investigate, characterize the OPPs. ATR-FTIR spectroscopic data are, therefore, a prerequisite for their identification. Finally, we believe that the use of ATR-FTIR should make it possible to identify all tested OPPs in one single analysis, even in the low ppm concentration range.

KEYWORDS | ATR-FTIR, organophosphorus, biological samples non-destructive, spectral library

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INTRODUCTION

Pesticides protect the crops from pests but their usage is harmful to humans and other animals due to potential toxicity. Only 1% of the total amount of pesticides (approx. 4 millions tones) annually applied reaches to target pests.[1] On the basis of targets, pesticides are divided into several types such as herbicides, insecticides, fungicides, rodenticides and others. Over long period, organophosphorus pesticides (OPPs) or organophosphates are being used for protecting the crops and also as warfare agents. Organophosphorus pesticides have a pentavalent phosphorous atoms attached to a sulfur or oxygen atoms by double covalent bond predicted by a number of chemicals e.g. Malathion, Dimethoate, Dichlorovos, Chlorpyrifos, Monochrotophos etc. (Fig. 1). OPPs are named on the basis of type of group attached to phosphorous such as phosphate, thiono, thiol and thithiol type. R1 and R2 predict different substituent groups (alkoxy, alkyl, amino, thioalkyl etc.) present at the skeletal base of OPPs.[2,3] R1 and R2 are replaced by propyl and ethyl groups to manage resistant pests respectively. Xrepresents the alkyl, alkoxy, aryl, heterocyclic, aryl oxy, arylthio and alkylthio groups. OPPs are structurally similar within a class and have a phosphorous atoms with characteristics phosphoryl bond (P=O) OR thiophosphoryl bond (P=S).[1,4] OPPs and their metabolites are used as a pesticide across the world population. The WHO (World Health Organization) was reported that every year 3 million peoples were affected

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by the acute poisoning of OPPs. OPPs are also caused neurotoxicity due to inhibition of acetyl cholinesterase (AChE) enzymes and butyryl cholinesterase (BuChE) enzymes, present in the synaptic membrane of CNS (central nervous system) and PNS (peripheral nervous system) of vertebrates. AChE and BuChE enzymes hydrolyze the neurotransmitter acetylcholine into acetyl and choline which regulates the signal transmission by sodium channel in the CNS and PNS system. OPPs block the active sites of AChE and BuChEand finally deactivate the acetylcholine (ACh), leads to inhibition cholinergic neurotransmission due to accumulation of the acetylcholine. These neurotoxic effects results in neuromuscular paralysis.[5,6] OPPs are also potent to causes immunotoxicity, genotoxicity and carcinogenicity, finally leading to death. Conclusively, OPPs are the most dangerous and determined organic pollutants chemicals due to their potential toxicity. Recently, most of the criminal cases are taking place due to pesticide toxicity. In forensic science set-ups, these types of cases are analyzed from blood, foodstuff, spits and the chemical bottles seized from crime scene to know the cause. Few OPPs such as Malathion, Dimethoate, dichlorovos, Chlorpyrifos and Monochrotophos are highly toxic in nature and have been used in criminal cases.[7] In Brazilian institute, research groups have been analyzed the acetyl cholinesterase enzymes inhibitors such as aldicarb, OPPs chemicals and confirming using FT-IR technique for forensic purpose.[8] In pesticide poisoning cases, OPPs are the most detectable in forensic intoxication viz. 63% and out of it, Quinalphos was the most common OPPs responsible for 28.8% of the total positive cases.[9] Suicidal cases are most common and approximate 60% cases of death due to pesticides poisoning have been reported in rural area of Asia.[10] WHO (1990) had published the data and found 3 millions/year detectable cases related to pesticides poisoning resulting in 220,000 deaths[11,12] OPPs categories of pesticides had been screened mostly in forensic science laboratory using thin layer chromatography (TLC). Criminal cases related to pesticides are occurring daily and are being forwarded to forensic science laboratory for pesticides analysis using thin layer chromatography and are further confirmed by using FT-IR and GC-MS techniques. GC-MS is destructive, costly and time consuming technique so cannot be used routinely in laboratory. However, FT-IR is non-destructive and reliable technique. Therefore, it has been planned to analyze the ATR-FTIR spectrum of commonly available OPPs, so as to obtain the standard or reference data and this spectral library has been applied in real spiked biological samples. The studies have been carried out using ATR-FTIR spectroscopic technique. It is a powerful technique for analysis of trace and bulk constituents of matrices sample. Infrared absorption technique has efficiently been used to detect the microscopic pesticide residue.[13] The technique is non-destructive which is makes it, the best suitable for forensic utility because, the re-analysis of sample can be carried for future purpose.

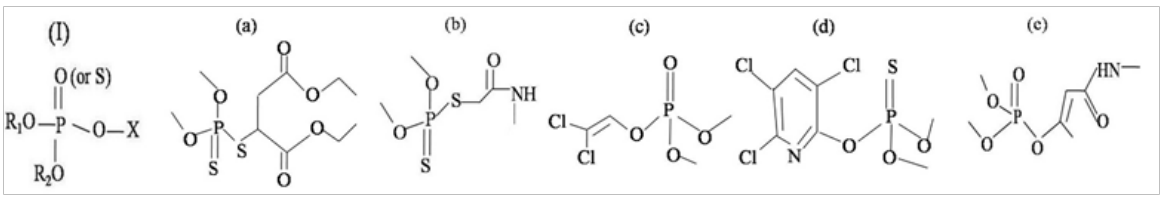


Figure 1: (i) General chemical structure of OPPs and (a) Malathion (b) Dimethoate (c) Dichlorovos (d) Chlorpyrifos (e) Monochrotophos.
Source: Author self.

METHOD

Five OPPs—Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos—were analyzed and characterized using ATR-FTIR spectroscopic technique.

Sample Preparation

Human biological control samples (free from milk, saliva and vomit materials) were obtained

from healthy volunteer and kept frozen at -20°C. 500µL volume of each biological samples were spiked three times (n=3) with each OPPs (Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos) up to 0.5ppm concentration in a scaled centrifugal vial. Samples were vortexed for FTIR analysis. Each sample was divided into four groups. First three groups were spiked with pesticides at the same concentration (0.5 ppm). However, pesticides were not spiked in the last group and served as a control.

Standard stock solution (2000ppm) was prepared for each OPPs separately. Tusk (50% EC), Dimex (30% EC), Dysac (76% EC), Sacban (20% EC) and Monovip (36% EC) contain Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos as active chemicals, respectively. OPPs pesticides were purchased from Shivalik agrochemicals at Chandigarh (India).

ATR-FTIR *Spectroscopy*

For ATR-FTIR spectra of each sample, four scans were recorded with a resolution of 4 cm-1, in the range from 4,000 to 700cm-1 using ATR-FTIR, (the Agilent Cary 630 FTIR Spectrometer). The spectral data was analyzed using Agilent Microlab FTIR software. 2.0µL samples were placed directly on the internal reflecting diamond crystal of FTIR with the help of micropipette.

RESULTS AND DISCUSSIONS

A number of methods had been used to analyse the organophosphorous pesticides (OPPs) residue in suspected samples of forensic interest. Use of infrared spectroscopy in forensic science is best technique because of sample is analysed without destruction& treatment. In spite of these efforts, an attempt in the present study has been equipped to obtain chemical fingerprinting of some commonly encountered OPPs using a non-destructive ATR-FTIR method. The mainmotiveis to generate ATR-FTIR spectra database of these OPPs due to makes it possible to positively identify the major OPPs found at crime scene. Finally, we believe that the use of ATR-FTIR should make it possible to identify all tested OPPs in one single analysis, even in the low ppm concentration range.

This study was to propose the spectral library of OPPs as a reference for any forensic chemist and toxicologist in field of forensic science. The generated ATR-FTIR spectral library at wavenumber (cm-1) was depicted in Table 1 and ATR-FTIR spectrum of spiked OPPs in biological samples was depicted in Table 3. The vibrational assignment of the signature peaks of each OPPs has been discussed in table 2. IUPAC name, Chemical structure and molecular formula of These reference OPPs compounds have been described which are used in this study such as (i) Tusk: The active component of Tusk is Malathion (C10H19O6PS2) with IUPAC name Diethyl 2-[(dimethoxyphosphorothioyl) sulfanyl] butanedioate. Chemical structure was depicted in Fig. 1(a). ATR-FTIR spectrum was shown in Fig. 2(a). The vibrational assignment of the principle peaks of ATR-FTIR spectrum has been depicted in Table 2.[14] (ii) Dimex: It contains Dimethoate (C2H12NO3PS2) which chemically is designated as O, O-dimethyl S-[2-(methyl amino)-2-oxoethyl] dithiophosphate. Structurally, Dimethoate was depicted in Fig. 1(b) and ATR-FTIR spectrum was shown in Fig. 2 (b). (iii) Dysac: Active component in Dysac is Dichlorovos (C4H7Cl2O4P) with specific chemical name as 2, 2-dichlorovinyl dimethyl phosphate. The chemical structure of Dysac was shown in Fig. 1(c). Fig. 2 (c)depicts the FT-IR spectrum.[14–16] (iv) Sacban: The commercial product contains Chlorpyrifos (C9H11Cl3NO3PS). It is chemically labeled as O,O-Dethyl O-3,5,6-trichloropyridin-2-ylphosphorothioate [Fig. 1(d)]. The FT-IR spectrum was shown in Fig. 2 (d). Table 2 shows the vibrational assignment of chlorpyrifos.[13,15,17,18] (v) Monovip: It contains Monochrotophos (C7H14NO5P) which chemically is represented as Dimethyl (E)-1-methyl-2-(methylcarbmoyl) vinyl phosphate (Fig. 1 (e)). FT-IR spectrum of product was shown in Fig. 2 (e). Table 2 shows the vibrational assignment of monochrotophos.[12,14,15,19,20] This OPPs spectral library has been employed to match the peaks of OPPs residue in spiked biological samples.

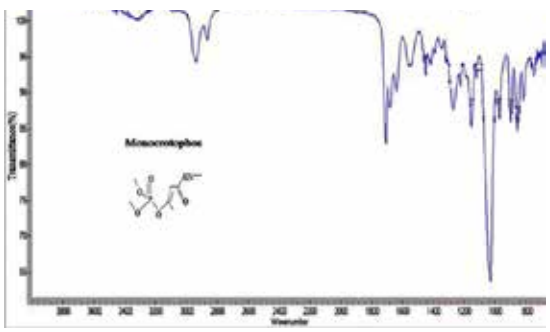
In this work, ATR-FTIR spectroscopy has been used to generate a spectral library of some organophosphorous pesticides of forensic interest. These types of pesticides can be seized as bulk market pesticides as well as traces found at the crime scene. There can be legal queries to identify OPPs to forensic expert has to answer. In these

cases, the standard reference database is required for comparison. The modern FTIR systems with diamond ATR proves to be a rapid, sensitive and non-destructive analysis of samples with very little effort. This spectral library can be used as a reference library when an unknown sample is suspected of being organophosphorous. Some of the closely related structures may have spectra very similar to that of particular pesticides, especially for organophosphorous. Thus, it is imperative that appropriate techniques should be used by the forensic chemist and toxicologist to ensure the optimal spectral data are obtained to get a feel for the discriminating features of a spectrum. Screening of pesticides using ATR-FTIR technique is particularly convenient because of both its speed and ability to use without sample preparation.

CONCLUSIONS

FT-IR is a very sensitive, rapid, easy, reliable, and non-destructive technique. Application of non-destructive analytical technique to analyze the forensic evidence as samples of forensic interest without destroying the sample. ATR-FTIR is a beneficial to forensic scientist/expert in field of forensic science. This generated spectral library of OPPs by ATR-FTIR can be used as reference/ standard for comparison of pesticides products, seized from crime scene.

In this work, ATR-FTIR spectral library was generated for some OPPs of forensic interests which had been frequently used in criminal cases and mostly recovered from crime scene. This



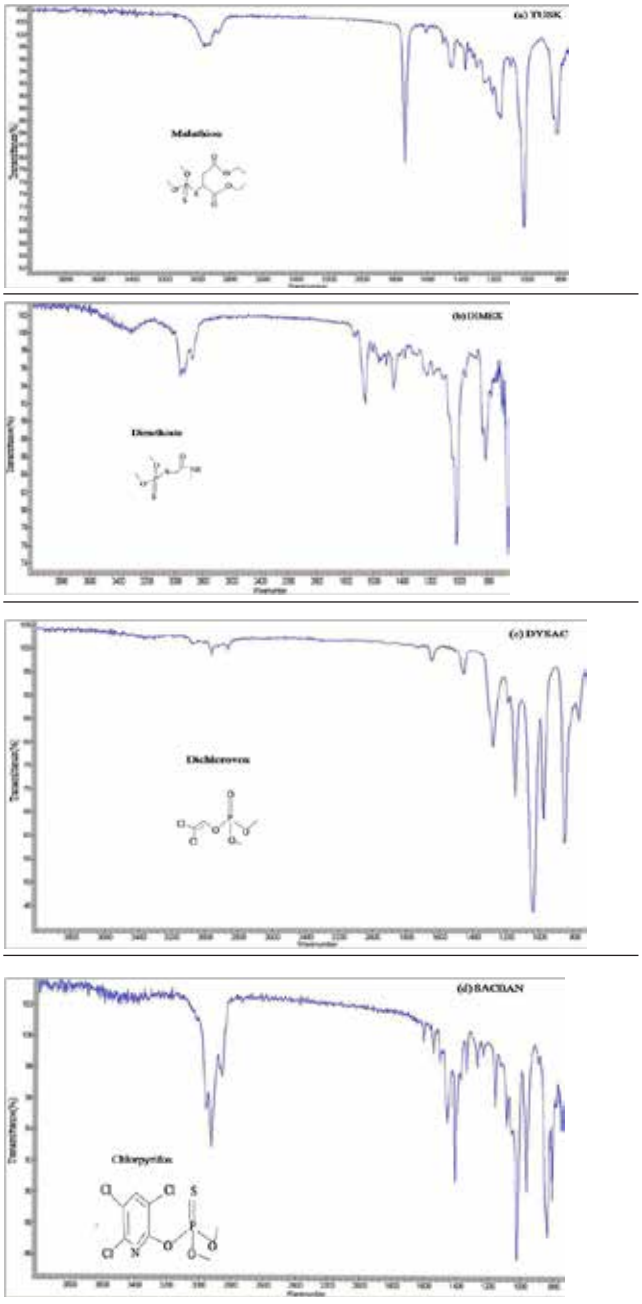


Figure 2(a): FTIR spectrum of Tusk OPPs product.
Figure 2(b): FTIR spectrum of Dimex OPPs product.
Figure 2(c): FTIR spectrum of Dysac OPPs product.
Figure 2(d): FTIR spectrum of Sacban OPPs product.
Figure 2(e): F TIR spectrum of Monovip OPPs product.
Figure 2(f): F TIR spectrum of Monovip OPPs product.
Source: Author Self

PURE OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1456(m), 1372(w), 1255(w), 1157(m), 1014(s), 817(m)
Dimex 30% EC (Dimethoate)	2948(m), 2874(m), 1661(s), 1550(w), 1457(m), 1296(w), 1221(w), 1176(w), 1016(s), 810(m)
Dysac 76% EC (Dichlorovos)	1644 (w), 1456(w), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1607(w), 1544(w), 1506(w), 1457(w), 1410(s), 1338(w), 1214(w), 1162(w), 1024(s), 984(m), 835(s), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1706(s), 1883(w), 1637(w), 1550(w), 1449(w), 1270(m), 1154(m), 1033(s), 970(w), 898(w), 855(w), 811(w)

Table 1: ATR-FTIR spectral peaks of OPPs of standard at transmittance (%) vs. wavenumber (cm-1) in OPPs products.

PURE OPPS	WAVENUMBERS (CM-1)	ASSIGNMENT
Tusk 20% EC (Malathion)	2869cm-1	aliphatic C-H stretching vibration
	1735cm-1	(C=O) carbonyl stretching vibration of ester group
	1456cm-1	C-H stretching vibrations of CH2
	1370cm-1	C-H Vibration of CH3 group
	1255cm-1	C-H rocking vibration of CH2
	1157cm-1	C-O vibration
	1014cm-1	P-O stretching of P-O-CH3 group
	817cm-1	P-O vibration of additional phosphates (PO43-) group
Dimex 30% EC (Dimethoate)	2948cm-1	aliphatic C-H asymmetric stretching vibration of CH3
	2874cm-1	aliphatic C-H symmetric stretching vibration of CH3
	1661cm-1	(C=O) carbonyl stretching vibration
	1550cm-1	P=S bending vibration
	1457cm-1	C-Hstretching vibrations of CH2
	1310cm-1	C-H Vibration of CH3 group
	810cm-1	P-O stretching of P-O-CH3 group, P-O vibration of additional phosphates (PO43-) group bands
		1296cm-1, 1221cm-1, 1176cm-1 C-N stretching vibration
Dysac 76% EC (Dichlorovos)	1644cm-1	C=C stretching in alkenes
	1456cm-1	C=H stretching vibrations of CH2
	1039cm-1	P-O stretching of P-O-CH3 group
	977cm-1	C-H bending vibration of CH2
	857cm-1	P-O vibration of additional phosphates (PO43-) group
	765 cm-1	C-Cl scissoring vibration
	1279cm-1, 1147cm-1	C-O bending vibration
Sacban 20% EC (Chlorpyrifos)	1607 to 984cm-1	C=N stretching, pyridine stretching, ring vibration, ring breathing, Cl-C stretching, trigonal ring breathing and P=S stretching
	2924cm-1	C-H asymmetric stretching vibration of CH3
	2857cm-1	C-H symmetric stretching vibration of CH3
	1607cm-1	C=C Vibration
	1544cm-1	P=S streching
	1410cm-1	CH2 bending
	1338cm-1	C-O stretching
	1024cm-1	CH2 in plane vibration
	984cm-1	CH3 wagging vibration, P-O stretching of P-O-CH3 and P-O vibration of additional phosphates (PO43-) group bands
	1506cm-1, 1457cm-1	vibration mode of CH2and CH3 group
	1214cm-1,1162cm-1	C-N stretching vibration
	835cm-1, 805cm-1	C-Cl rocking vibration
Monovip 36% EC (Monochrotophos)		2937cm-1 C-H asymmetric stretching vibration of CH2
	2862cm-1	C-H symmetric stretching vibration of CH2
	1706cm-1	(C=O) carbonyl stretching vibration
	1683cm-1	peaks of additional (C=O) carbonyl stretching vibration
	1637cm-1	C=C stretching in alkenes
	1550cm-1	N-H bending vibration
	1449cm-1	bending vibration of CH2
	1033cm-1	P-O stretching of P-O-CH3
	970cm-1	N-CO bending at out of plane
	898cm-1	out of plane bending CH in C-CN
	1270cm-1, 1154cm-1	C-N bending
	855cm-1, 811cm-1	mode of C-CH bending

Table 2: Vibrationl assignment of ATR-FTIR spectral peaks corresponding wavenumber (cm-1) of OPPs.

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1372(w), 1157(m), 1014(s),
Dimex 30% EC (Dimethoate)	2948(m), 1661(s), 1550(w), 1457(m), 1176(w), 1016(s), 810(m)
Dysac 76% EC (Dichlorovos)	1644 (w), 1279(m), 1039, 977(m), 857(m),
Sacban 20% EC (Chlorpyrifos)	2924(s), 1607(w), 1506(w), 1410(s), 1338(w), 1214(w), 984(m), 835(s),
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1883(w), 1637(w), 1449(w), 1154(m), 1033(s), 970(w), 855(w),

(A) ATR-FTIR spectral peaks of OPPs of spiked blood sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1456(m), 1372(w), 1255(w), 1014(s), 817(m)
Dimex 30% EC (Dimethoate)	2874(m), 1661(s), 1550(w), 1457(m), 1221(w), 1176(w), 1016(s),
Dysac 76% EC (Dichlorovos)	1644 (w), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 1544(w), 1506(w), 1457(w), 1338(w), 1214(w), 1162(w), 1024(s), 835(s), 805(s)
Monovip 36% EC (Monochrotophos)	2937(s), 2862(w), 1706(s), 1637(w), 1550(w), 1270(m), 1033(s), 970(w), 811(w)

(B) ATR-FTIR spectral peaks of OPPs of spiked urine sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(s), 1735(s), 1372(w), 1157(m), 817(m)
Dimex 30% EC (Dimethoate)	2948(m), 2874(m), 1550(w), 1457(m), 1296(w), 1176(w), 1016(s),
Dysac 76% EC (Dichlorovos)	1644 (s), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1544(w), 1457(w), 1410(s), 1214(w), 1162(w), 984(m), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1706(s), 1637(w), 1550(w), 1270(m), 1033(s), 970(w), 855(w), 811(w)

(C) ATR-FTIR spectral peaks of OPPs of spiked milk sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(s), 1735(s), 1372(w), 1157(m), 817(m)
Dimex 30% EC (Dimethoate)	2948(m), 2874(m), 1550(w), 1457(m), 1296(w), 1176(w), 1016(s),
Dysac 76% EC (Dichlorovos)	1644 (s), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1544(w), 1457(w), 1410(s), 1214(w), 1162(w), 984(m), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1706(s), 1637(w), 1550(w), 1270(m), 1033(s), 970(w), 855(w), 811(w)

(D) ATR-FTIR spectral peaks of OPPs of spiked saliva sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1456(m), 1255(w), 1157(m), 1014(s),
Dimex 30% EC (Dimethoate)	2948(m), 1550(w), 1457(m), 1296(w), 1221(w), 1016(s), 810(m)
Dysac 76% EC (Dichlorovos)	1644 (m), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1544(w), 1506(w), 1457(w), 1410(s), 1338(w), 1162(w), 1024(s), 984(m), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 1706(s), 1883(s), 1637(w), 1270(m), 1154(m), 1033(s), 855(w), 811(w)

(E) ATR-FTIR spectral peaks of OPPs of spiked vomit sample at transmittance (%) vs. wavenumber (cm-1) in OPs products.

(s): strong peak; (w): weak peak; (m): medium peak

generated ATR-FTIR spectral library has been employed in OPPs spiked biologicalsamples. This ATR-FTIR spectral library could be of great help to forensic scientist to characterize and screen the suspect OPPs for data assessment. It is a practicallypotential to screen out residual OPPs using ATR-FTIR directly. The present work can be scaled up to grant potential positive contribution to discriminate mysterious OPP. IJFMP

Recommendation

Present work leaves an off-shoot where, we recommend further, to generate ATR-FTIR spectral library of others standard pesticides and drugs as well as OPPs residue in real samples of forensic interest.

Abbreviations

OPPs: Organophosphorous pesticides, ATR-FTIR: Attenuated total reflection-fourier transforms infra red spectroscopy.

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REVIEW ARTICLE

Effect of Thermochromic Ink on Different Types of Papers

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ABSTRACT

Frixion Roller Thermochromic ink pens are incorporated with an erasure in each pen, which can be used to generate heat through friction which, in turn, decolourize the writings. Application of heat by other sources also have a similar effect. Thermal ink has a unique characteristic which makes it disappear, when heat is applied at a specific temperature. Due to this significant property of thermal ink, it is widely used for frauds. Easy availability and the unique characteristic of thermal inks attracts criminal minds and therefore increases criminal activities. The main purpose of this research paper is to describe different properties of thermal ink along with the derivation of an easy, inexpensive and non-destructive process to restore disappeared writings. Reaction of thermochromic ink with different varieties of papers has also been examined in this paper.

KEYWORDS | erasable ink, thermal ink, ink analysis, forensic science

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INTRODUCTION

Invisible ink is prepared by special chemical process specifically for some industrial purposes. Invisible ink pen looks just like any other pens available in the market but the ink is made of different chemical composition. It is commonly known as magic pen. Auto-vanishing fluid inks are easily available in the market which raise great deal of concern.[4,14,15] Two kinds of invisible inks are: disappearing ink and thermal ink. Disappearing ink is a mixture of different chemicals which causes the ink to become visible for a very short time duration after which it disappears.[17-18] It works on the principle of acid/base chemistry and is an irreversible reaction. This research paper is focussed on thermal ink only.

Thermal ink is a type of erasable ink which is removed easily by the friction produced by rubbers incorporated in each pen.[9-13] It has different colours like red, blue, green and black. Such inks can be removed from the paper surface mechanically through erasure or by exposure to heat and cooling simultaneously. It is a type of viscous ink that depends largely on the heat generated during erasure which affects the solvent of ink. For the ink to disappear or fade the ink requires an external heat such as friction through eraser or through direct exposure to temperature.[4,5]

These inks are used for committing various crimes. Criminals use thermal ink pen to erase the original writing and then rewrite with the intent to defraud. Such obliterations are not visible to the naked eyes and are difficult to detect.[19,21,28] Forensic document examiners are very much familiar with these type of thermal ink ball-pen manufactured by Paper Mate, they are sold in UK under the name "Replay". "Eraser Max" is a new brand

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name for Replay erasable pens in the UK. A latest collection of erasable pens are manufactured by Pilot under the name "Frixion" erasable roller ball pen.[3,6]

A small eraser is fitted at the tip or end of the erasable pen which helps remove the writing. While removing with rubber eraser small traces of writing remained visible to the eyes. Rubbing the rubber eraser on the paper generates heat by the action of friction and decolourize the writings but does not abrade it.[3,6]

Pigment-forming microcapsules are made up of mainly three substances: first, leuco dye which can switch between colored and colourless forms, second, a color developer which chemically bonds with the leuco dye to produce color, and third is a temperature regulator which changes color according to temperature. The leuco dye is the one which actually determines the color but it can produce color only when it is chemically bonded with the color developer. The bonding of leuco dye

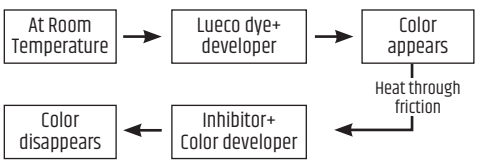

Figure 1: Reaction of Thermal Ink

and color developer is prevented by an inhibitor [color change temperature regulator] that inhibits bonding of the two above a particular temperature and makes the color disappear. There are several temperature regulators available that regulate colour change at different temperature.[7,22]

The solvent system used in thermal inks are leuco dye developer system. In the presence of a solvent an interaction between a colour former [leuco dye] and the developer results in the formation of the three components due to which colour change takes place.[20] Spirolactone molecule is commonly used as a colour former. One such possibility is CVL (CcrystalVvioletLlactone), which is colourless in grounded form. Opening of lactone ring gives colour to CVL, which results in increased conjugation due to the increased polarity or hydrogen bonding nature of developer. Phenols are generally used as developers. Some of the solvents which are commonly used in these inks are esters, acids having long chain aliphatic character, amides or alcohols.[2]

Properties of Thermal Ink

Erasable ink has acid-base sensitivity. It can be studied by adding 3M HCL or 3M H2SO4 to dry erasable ink at low temperature. This results in spreading of the colored form on the paper that helps to partially keep their colour at high temperatures. When acid is added to same dry ink after conversion at high temperature, it reverts back to coloured form.[1] However, addition of 3M NaOH or 3M NaCl showed negligible or little effect on the behavior of ink at high and low temperature.[8]

Examination through optical microscopy reveal the granular structure of ink which may be the result of micro-encapsulation of ink. Most of the granules are in the range of 1-2μm in size and some are upto 8μm in size. Most of the aqueous solutions does not affect the physical structure of ink granules, but some of the acids and bases can affect the granular structure by penetrating the granules and thus affects ink ability to change color.

Given enough time, the components of the ink reach their thermodynamically favoured colored form at low temperature and colorless at high temperature. Differential Scanning Calorimetry (DSC), when heated on a sample of black Frixion ink showed that the dominant endothermic transition takes place between 57°C to 60°C (without any exothermic transition in given range). When the ink was cooled, its dominant exothermic transition takes place at about −3°C to 0°C (without any endothermic transition in the given range). As observed it was found that these temperature ranges were consistent for dry ink, wet ink and aqueous solution inks like NH3, HClor NaOH added.[8]

The activation barrier that is responsible for the inter-conversion of different forms of ink components is high enough that at room temperature both the forms can coexist for a longer period of time. This is called as colour hysteresis which can be explained as the ink form at room temperature that depends on the way from which

that room temperature is reached or achieved.[8] It was found that α-anthracene terminated methoxy polyethylene glycol (An-PEG) aqueous solution can be used as a new type of ink to be written on conventional paper.[16,27]

METHOD & MATERIALS

Samples are made using Pilot Frixion Clicker Roller Pen (Blue) on three different types of papers. The papers used to prepare samples are White Copier paper (70gsm), Bond paper (90gsm) and Glossy paper (180gsm). There are total 90 samples recorded, 30 samples for each type of paper. In this process three ink removing methods are used i.e. erasure incorporated at the backside of each pen, hair dryer and domestic iron. A total of 10 samples with each method is prepared. Domestic freezer is used to restore all the writings. Observations were made by using a hand magnifying glass and unaided eye.

Observations

Frixion Roller pens are incorporated with an erasure at the tip of each pen which can be used

Figure 1: Pilot Frixion Clicker

to generate heat through friction which, in turn, decolourize the ink lines. This erasure can affect the physical properties of the paper depending upon the quality and type of the paper used.

We also used a domestic hair dryer to remove the writings made by thermal ink pen. It takes only 1 or 2 minutes to remove the writings from the paper. It was observed that the ink reappears in specific region of page with the movement of air stream.[6]

Domestic iron at a moderately hot, "two dot" setting was also used to remove the thermal ink writings. To protect the study paper, another sheet of paper is placed above the study paper before using the iron. The same effect was achieved when "one dot" setting of the iron was used to apply heat.[6]

All the samples with decolourized writings were placed in a domestic refrigerator (low temperature) to restore the writings. The following results were observed:

Sl.No.	Type of Paper	Method of Removal	Time taken to remove ink-lines	Reappearing Time at Low Temerature
1.	Copier Paper	Pen Erasure	1min.	45min
		Hair Dryer	1-2 min.	20min
		Domestic Iron	Few seconds	20min
2.	Bond Paper	Pen Erasure	1min.	1hour
		Hair Dryer	1-2 min.	20min
		Domestic Iron	Few seconds	20min
3	Glossy Paper	Pen Erasure	1min.	Doesn't reappear*
		Hair Dryer	1-2 min.	30min
		Domestic Iron	Few seconds	30min

Table 1: Pilot Frixion Clicker Roller [Blue]

It was observed that writings on the copier paper reappeared when removed with all the three methods i.e., by using erasure, hair dryer and domestic iron after putting it under the domestic refrigerator. Writings on the bond paper also shows similar results as shown by the copier paper.

Glossy paper reacts differently as compared to copier paper and bond paper. Glossy paper has a very smooth surface. When friction erasing

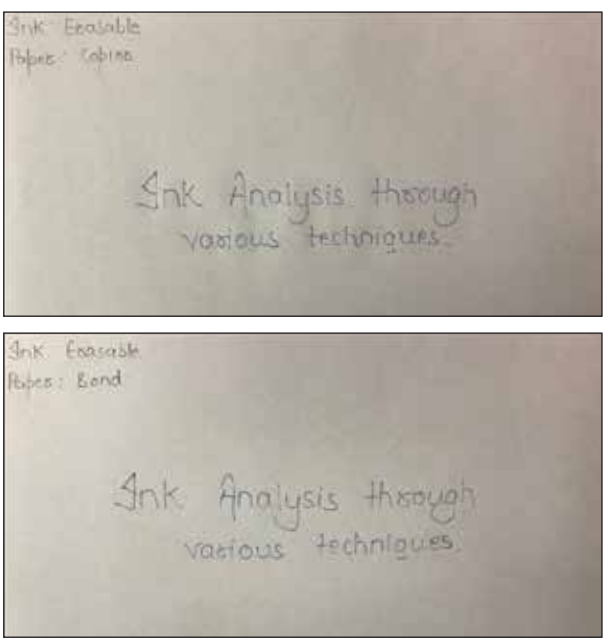

Figure 2 & 3: Reappeared writing on Copier, Bond papers respectively, by using domestic refrigerator (Blue)

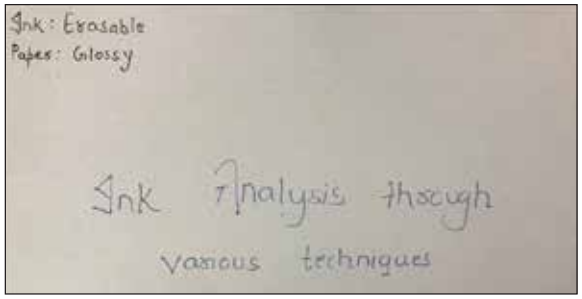

Figure 4: Reappeared writing on glossy paper using domestic refrigerator

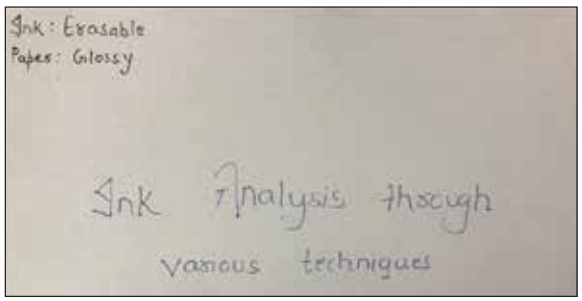

Figure 6: Reappeared writing on glossy paper using domestic refrigerator

method was used to remove ink lines from the glossy paper, it was observed that even after keeping it in a domestic refrigerator for sufficient time, the writings doesn't reappear. Only traces at the end of strokes reappeared. It was also observed that when ink lines were removed by using a hair dryer and a domestic iron then it reappears within 30 minutes when kept under the refrigerator.

CONCLUSION

It is difficult to detect disappeared thermal ink writings with naked eyes. Easy availability and unique quality of thermal inks attract criminal minds to indulge in increasing frauds. It was observed that applying heat by iron is the fastest method to remove thermal ink writings. This research paper provides information and alerts the forensic science community about the existence of thermal erasable roller ball-point pen and also describe an inexpensive, easy and non-destructive

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Figure 5: Few traces at the end of strokes reappeared when writings on the glossy paper is removed by using friction (rubber erasure)

method to decipher such writings. Some of the important features and properties of erasable ink are also elaborated in this paper. Restoration of disappeared writing by using an easily available domestic refrigerator provides a new avenue to detect these temperature dependent ink writings. Refrigeration method doesn't work on glossy paper when ink-lines are removed by pen erasure. In all other cases restoration by putting the samples in a refrigerator is possible. IJFMP

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REVIEW ARTICLE

Understanding the Psychology of Paraphilic & Violent Offenders

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ABSTRACT

Paraphilic and violent offenses have been committed for a very long time and have just gotten increasingly dangerous over time. Although the occurrences of such offenses have come down with the progression of the society, the need to study the underlying factors of these offenses is of vital importance. Acts of necrophilia have been reported from as early as the 15th century. Pedophilia, serial killing, and homicides, both catathymic and compulsive, are also discussed and reviewed. The role and use of psychology has proved extremely important in understanding, predicting, and potentially preventing such heinous acts from being committed. Criminal as well as forensic psychology are key to understanding the exact intricacies of the various crimes. The main aim of this review is to understand the underlying reason behind such heinous acts.

KEYWORDS | paraphilia, necrophilia, psychology, criminal psychology paedophilia, psychiatry, catathym

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INTRODUCTION

Criminal psychology is a study of the will, thoughts, intentions and acts of the criminals, and all that partakes in the behavior of criminals. This study goes deeply into what makes someone commit a crime. It also studies the reactions after the crime. Paraphilia is a psychiatric condition characterized by abnormal and often dangerous and deviant sexual desires and fantasies.[1] Conditions such as paedophilia, necrophilia, bestiality, voyeurism and sexual sadism and masochism among many others, are a few examples of paraphilia. This study is primarily focused on two paraphilia: paedophilia and necrophilia. Along with these, catathymic and compulsive homicides have also been taken up in the study.[2]

The aim of this study is to understand the rationale behind such offenses and preventing their occurrence. Two main questions arise in such cases, which generally hold the key to delve deep into the mind of the offender – 'why did the offender commit the crime' and 'why did he do that in that particular way'. The work and literature

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on the psychology behind these offenses is rather sparse. This, along with a profound interest in the subject, has served as a driving force behind this study.

The fact that the psychology of each and every person does differ and there is no method to exactly pinpoint the characteristics of an offender. Which makes the task of giving conclusive pointers about the characteristics of an offender even more challenging. A few behavioral attributes may be found to be overlapping in some of the offenses, but these only make up for the potential general characteristics of an offender and understanding or determining individual traits is a truly daunting task.

Necrophilia

A closed case file review of 211 sexual homicide cases revealed 16 cases of necrophilia.[3] The method used for the classification and characterization of these cases was simply just grouping and analyzing. Basically, all the offenders and victims in the cases were classified under various categories such as age, race/ethnicity, living conditions, marital status and childhood/background, and were characterized accordingly. Although inconclusive, there were certain points of interest that should be taken into consideration. All 16 offenders were male, with an average age of 26 (age range 14-38). Link between the crime and ethnicity of the offenders could not be established conclusively. Interestingly, 56% of the offenders suffered from anti-social personality disorder and more than 60% of the offenders were single. Almost 90% of the victims were Caucasian and fifteen victims out of sixteen were females.

The above mentioned review as well as the works of psychiatrist Richard Freiherr von Krafft-Ebing[4] and Rosman[5] suggested that necrophilia was rarely the main motive or driving force behind killing of a person. Rather, it was the fact that either the act of murder in itself wasn't satisfactory enough that an urge to further maim the victim arose, or the offender finally gained the ability to assert dominance over the deceased.

In a review of 122 cases that displayed acts of necrophilia conducted by Rosman and Resnick,[5] the cases were analyzed according to a number of variables. The data was accumulated from different decades, countries and ethnicities, to ensure a larger pool of data was taken into account as well as maintaining variance. The cases were divided into two broad classifications, genuine necrophilia (54 out of 122) and pseudo-necrophilia (33 out of 122) [data was insufficient to classify the remaining 35 cases].Genuine necrophilia is the persistent sexual attraction to corpses[6], whereas pseudo-necrophilia is a transient attraction towards corpses, while still preferring live partners for engaging in sexual activities. Contrary to belief, people who suffer from necrophilia do not have a subpar intelligence, as only 11 percent of the true necrophilia sample proved to be psychotic. Occupation was also an important factor as 57 percent of the perpetrators chose jobs that would allow frequent access to corpses.

Pedophilia

A pedophile, in clinical terms, is a person who exhibits a primary or exclusive sexual attraction or interest towards pre-pubescent children.[7] Seto conducted a study on pedophiles which aimed at the diagnosis, risk assessment and treatments of pedophilia offenders.[8] Being a pedophile is not a crime, but it certainly increases the risk that a pedophilic act might be committed. The diagnostic schemes used in this particular study for establishing pedophilia were DSM-IV-TR and ICD-109. Assessment methods used for the study were fairly simple to execute and understand. Self-report was the easiest assessment method which could be achieved by a simple clinical questionnaire or interview.

Albeit being the most direct method of assessing a pedophile's sexual urges and fantasies it had its advantages, while it also ran the risk of the subject concealing his pedophilic interests. Although a non-judgmental tone of the interviewer coupled with a well-structured and gradual line of questioning may have yielded better results. Since there were apparent loopholes in self-report method, researchers turned towards analyzing the sexual behavior history of the subjects. The Screening Scale for pedophilic Interests (SSPI) was developed by Seto and Lalumiere[10] to categorize and summarize an offender's potential pedophilic history, and also to serve as a proxy for psycho-

-physiological assessments of sexual arousal. The SSPI scores range from 0 to 5 that are positively unvarying and related to pedophilic responding in the laboratory.

Viewing child pornography is another major behavioral indicator. A large majority of pedophiles admitted to viewing child pornography on a regular basis. 61 out of a group of 100 pedophiles showed greater penile response to porn involving children than to adults. However, it should be noted that pornography was a clearer indicator of sexual interest rather than sexual contact or activity. 84% of the 43 child porn offenders admitted to masturbating while fantasizing sex with children.[11] Viewing time was also used as an indicator of degree of sexual arousal;[12] the more time he spent looking at kid porn, the more likely he was sexually aroused towards children and showing paedophilic behavior. The most important aspect of this method was that it must be carried out in an inconspicuous manner and the viewing time must be noted for each image the participant views. Since almost every paedophilic offender ever identified is male, phallometry was also used as method of assessment.[13] By measuring the penile response, sexual preferences were determined according to the stimuli. It was also used to establish the sexual orientation of the offenders.[14]

Generally, all the assessment methods were reported as relative sexual response of the offender towards a child and an adult. In cases of pedophilia, an accurate diagnosis and risk assessment is essential as the associated variables are very important for predicting a pattern as well as for preventing further recurrences. Furthermore, offenders with previous criminal record who view child pornography, were found more likely to commit a sexual act, or an offence of any kind.[15]

Catathymic and Compulsive Homicide

Sinnamon and Petherick in 2017 did a study on catathymic and compulsive homicides from a psychological perspective[16] which laid a foundation that the underlying reasons may range from episodic to pervasive characteristics that may give rise to antisocial or narcissistic behavior. When a catathymic reaction occurs, the control over impulses is compromised, which results inan outburst of uncontrollably excessive violence. General trend suggested that after committing the act, the offender felt a temporary relief from inner conflict and also a sense of normalcy.

Catathymic crisis may take either chronic or acute form. In chronic catathymic crisis (CCC), there were three stages, the incubation period, followed by the committing of violence, and finally the attainment of relief.[17] The incubation period may be as short as a few days or as long as several years. An offender may be fixated on a particular victim type and then, when they finally commit the act, the fixation helped them select a specific victim. In contrast to the gradual build-up of violence in the chronic case, there is a sudden outburst triggered often by an ordinary interaction between a casual acquaintance or a total stranger to the perpetrator in the case of acute catathymic crisis (ACC).[18] The reason for this sudden, explosive outburst was postulated to be a result of a comment or an incidence that triggered the underlying aggressive behavior. In most cases of acute catathymic violence, it was observed that the perpetrator had partial or no recollection of the aggressive event.

Compulsive homicide[19] is often confused with catathymic homicide but the difference lies in the fact that it is an act instigated by a deep-rooted, long-standing compulsion to kill.[20] The reason behind committing a homicide was not to achieve a feeling of inner relief from a prevalent turmoil, but to fulfill a deep rooted inherent fantasy. An act of unplanned compulsive homicide[21] (UCH) is different from acute catathymic homicide as there is no internal conflict acting as a driving force in the motivation of the homicidal act. Such an offender's desire to kill was found to be synergized with a combination of violence and sexual fantasies. There was only one similarity found between a planned compulsive homicide (PCH) and chronic catathymic crisis, and that was the obsession to kill over an extended time period.

DISCUSSION

The occurrences of the paraphilic and violent offenses mentioned above have certainly decreased in the last decade. With that being said, there has always been an omnipresent need to understand the thought process and psychology leading to

such crimes. The basic psychology of it remained more or less similar in all the offenses, but a few nuances were found in the background leading to various crimes. Childhood abuse and antisocial behavior (sometimes mixed with narcissism) was found in most offenders. The literature linking psychology and homicides due to necrophilia are sparse, and there are no scientific assessment methods as of yet to understand the intricate psychology of the offenders. The most common reason for necrophilia was found to be the presence of a dead victim.

Phallometric method in pedophilia assessment among men was found to be the most reliable indicator of a pedophilic preference. Using this method enabled the successful and consistent discrimination between pedophilic and non-paedophilic men and could also help in identifying sexual recidivism among offenders.

Cases of catathymia and compulsive homicide both produced very unique offender profiles. They fall in an unexplored category of seriously deviant behaviors, that involve a psychology so intricate that makes it very difficult to understand and hence prevent the crime.

DISCUSSION AND RESULTS

The above review concludes that although there may be a few nuances when it comes to the underlying factors of the offences committed and the psychology of the offenders, but a majority of the factors remained similar to a certain extent. It was also found that in certain cases, two or more factors led to a much more violent outcome. The most common explanation for necrophilia was found to be the need to assert sexual domination which would've been otherwise impossible, if the victim were alive.

According to Deriver, the motivation for committing necrophilia was to further destroy and degrade the already dead victim. In case of paedophilic offences, it was found that self-report analysis was less reliable than sexual behaviour history and phallometric analysis. SSPI proved a proper indicator of presence of pedophilia (phallometric response) and also for the prevention of repetition of the offence (for people with sexual behaviour history). The main driving factor that led to paedophilic offences was the fact that most of the offenders had been sexually violated in their own childhood. Also, the usually complying nature of children also acts a motivator for such offences. The motivation and psychological characteristics of homicides can vary, and since both catathymic and compulsive homicides are deviant behaviors, they are difficult to understand and predict and hence prevent.

The psychology of each crime varies from offender to offender and there needs to be varied strategy while trying to understand the psychology of every offender. Conducting interviews with a specifically constructed questionnaire for each type of crime is arguably the most efficient way of understanding the underlying factor of the crime. Since there are several ways to construct a 'productive' questionnaire, but the following points should be kept in mind:

- First of all, try to develop a relationship of empathy with the subject.
- More often than not, their reason for committing the offence is justified in their mind, so they will not crack down under conditions such as threats.
- People committing such heinous offenses generally possess a higher-than-usual threshold of emotional stimulation and a high ability to manipulate the opposite party (for example, Edmund Kemper).
- While framing the questions, it should be considered that such offenders do not get swayed by appeals of sympathy, remorse, regret or social obligation.
- They are highly egoistic and their confessions will be received only because their ego was satisfied and they finally get the spotlight (for example, Dennis Rader).
- The format of the questionnaire should be as much non-emotional as possible, with the interviewer being well-versed with the facts of the case(s).
- The interviewer, at any point during the interview, must not let the subject lead the conversation.
- Also there should be no diversion or deviation from the topic. IJFMP

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Preparation of the Manuscript

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, and Conclusion. References, tables, figures, legends, and Acknowledgment, Appendices and Abbreviations may be provided after the Abstract page.

Title Page

The title page should carry the following:

1. Type of manuscript (e.g. Original article, Review article, Case Report)
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The second page should carry the full title of the manuscript and an Abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. A minimum of 4 keywords and a maximum of 8 keywords may be provided by the author for indexing.

Introduction

State the background and purpose of the study and summarize the rationale for it. Describe in a few short sentences in simple English, the background and significance of your article in the following format.

Materials and Methods

The Meterials and Methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, study design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods.

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on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/l 7-c_e.html).

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Present your results in a logical sequence in the text, tables, and illustrations, describing the most important findings first. Do not repeat the data in the tables or illustrations; emphasize or summarize only important observations. Supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussions

Include a summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); strengths and limitations of the study (Study question, design, data collection, analysis and interpretation); interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now? What does this study add to the available evidence, effects on patient care and health policy, possible mechanisms). Controversies raised by this study; and future directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

References should be listed in alphabetical order and numbered accordingly. Each listed reference should be cited in the text. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

- Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.
- Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

- Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

- American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

- Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

- Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

- Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

No author Listed

- World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

- National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.
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- Tables should be self-explanatory and should not duplicate textual material.
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