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Effect of Anthropogenic Disturbances on the Distribution of Spider Species in Gopalpur and its Adjacent Villages of Ganjam District Odisha

Gitanjali Mishra[1], Somanath Sahoo[2]

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Author's Affiliations: [1]Professor, [2]Research scholar, PG Department of Zoology, Berhampur University, Odisha 760007, India.

Corresponding Author: Somanath Sahoo, Research scholar, PG Department of Zoology, Berhampur University, Odisha 760007, India.

E-mail: cool.somanath@gmail.com

Abstract

Anthropogenic disturbances cause many undesirable changes in our ecosystem that directly or indirectly affect the livelihood of people. In the present study, we investigated spider diversity and distribution in habitats with different degrees of anthropogenic disturbances in Gopalpur of Ganjam district, Odisha from November 2018 to January 2020. A total of 592 spider specimens belonging to 23 species were recorded. It was observed that the more the degree of anthropogenic disturbances, the lesser was the diversity of spider species. The spider population was negatively affected by the frequency of disturbances in their habitat. Ecological parameters such as temperature, humidity, wind speed and direction, and light intensity were changing at a higher rate in more disturbed areas, indicating differences, due to human impact. The study reveals that anthropogenic disturbances produce ecological changes that most of the spider species may not tolerate. Again, the distribution pattern of arboreal orb-web making spider species was greatly affected by the frequent disturbances whereas ground-dwelling species were least affected. To retain spider diversity in any locality, there needs a management plan for incorporating some undisturbed wild vegetation patches both in rural and urban areas. This could include rules on how to keep that vegetation patch undisturbed and not been influenced by any household waste materials or drained water from any industry, factory, or crop field.

Keywords: Spider; Vegetation; Diversity; Frequency.

Introduction

All living organisms including human depends on free services provided by natural ecosystems for their sustenance on earth. Although human can modify the natural ecosystems to grab most of the benefits for their own, anthropogenic disturbances cause many undesirable changes in the ecosystem that directly or indirectly affect the livelihood of people (Kremen*et al*., 2007; Winfree *et al*., 2009; Shrestha *et al*., 2013; Leal *et al*., 2014; Cambell*et al*., 2018).Spiders are the beneficial animal for agroecosystem as they control the pest population without degrading the quality of crop plants (Riechert, 1999; Maloney *et al*., 2003; Happe*et al*., 2019). The use of pesticides in crop fields can make the crops especially vegetables and fruits to be toxic for direct consumption (Fantke& Jolliet, 2016; Valcke, 2017; Carvalho, 2017). On the other hand, a low dose of pesticides in the field can induce resistant varieties of pests to be repopulated (Georghiou & Mellon, 1983; Guo *et al*., 1998). In this situation, integrated pest management can be helpful. Hereafter application of a low dose of pesticides in crop fields, spiders can be reintroduced to predating upon those resistant varieties. Therefore, in this context, it is very much important to know about the ecology of spider species to make them boons against pests in the modern system of integrated pest management. Anthropogenic disturbances influencing the diversity and distribution of most of the arthropod species.

In this present research work, it is hypothesized that the anthropogenic disturbances cause ecological changes that influence the distribution of spider species. This study aims to compare spider diversity and distribution at different grades of human-disturbed habitats in coastal areas of Gopalpur, Ganjam district, Odisha.

Materials and Methods

Study area: The study was conducted in a coastal belt of 5-kilometre width from the coastal line and20-kilometre length along the coastal line which includes Gopalpur

and its adjacent area in Ganjam district of Odisha. The whole study area was divided into three categorical areas such as human settled areas (HSA), agricultural crop fields (ACF), and wild vegetational areas (WVA). That division was done according to the degree of human interference in the habitat. In the human settled areas, the sampling was conducted inside the vegetation grown at the side of the road, factories, and dump yards. In the agricultural crop fields, sampling was conducted inside the rice and vegetable crop field before harvesting. Wild vegetational areas were existed far from both human settled areas and crop fields and were mostly contained varieties of shrubs with randomly grown trees. We have selected 5 sampling sites randomly from each categorical area. Thus, a whole total of 15 sampling sites were chosen for all types of data collection.

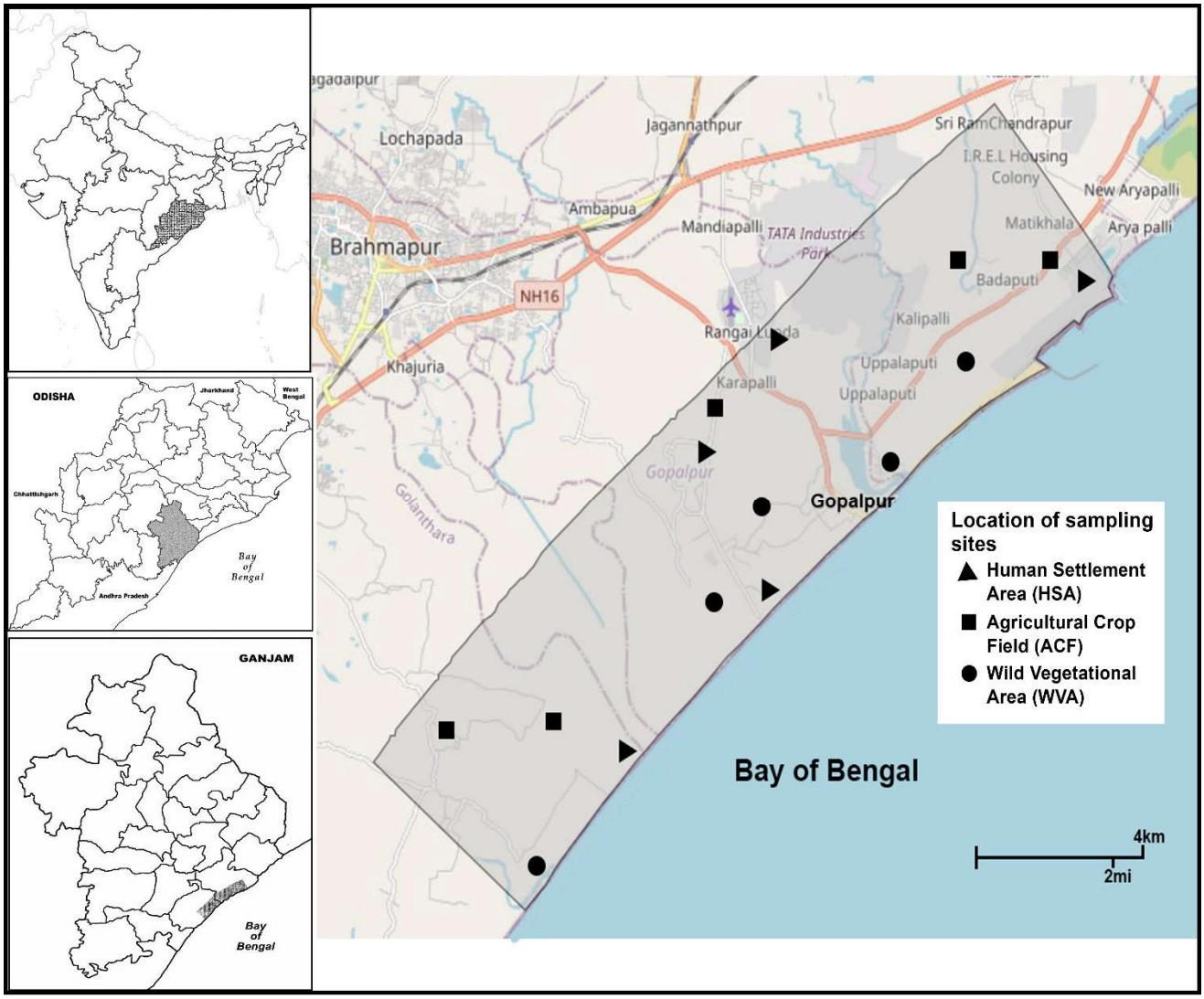

Fig. 1: Map showing study area and sampling sites. (Source: Google map)

Spider sampling: Spider sampling was done from the sampling sites of each category of the area twice a month for fifteen months from November 2018 to January 2020. Four methods were taken for spider samplings such as branch-beating and shaking technique for arboreal spiders, leaf litter sampling technique for ground-dwelling spiders, trapping technique (pit-fall trap for ground-dwelling spiders and hanging traps on shrubs and trees for arboreal spiders), and visual search technique. Some quadrates having the size of (1 X 1) m^2 were taken randomly from each categorical area for estimating spider density and distribution. During sampling, spiders were counted and identified in the field up to the family level by using standard field guides. Then collection and photography were done in the laboratory followed by preservation in 70% alcohol for later identification at least up to genus level.

Determination of ecological disturbances: Temperature, humidity, wind speed, wind direction, and light intensity was recorded near the vegetation at an interval of five minutes for one hour during mid-day time to find out the average change in the value of an ecological parameter per minute that represent the degree of anthropogenic disturbances in a particular habitat. Temperature, humidity, wind speed, and light intensity were measured by using portable standard digital instruments whereas wind direction was determined by the smoke direction

from a burning incense stick using a compass.

Data analysis: Spider abundance in each categorical area was calculated as the average density (number per meter square) of five sampling sites in that habitat. For measuring spider diversity, species richness (total number of species) and the Shannon-Weiner species diversity index were used. Mean species richness and mean diversity index per sampling site were considered for comparative analysis. Statistical calculations were done by using Microsoft Excel 2013.

Results

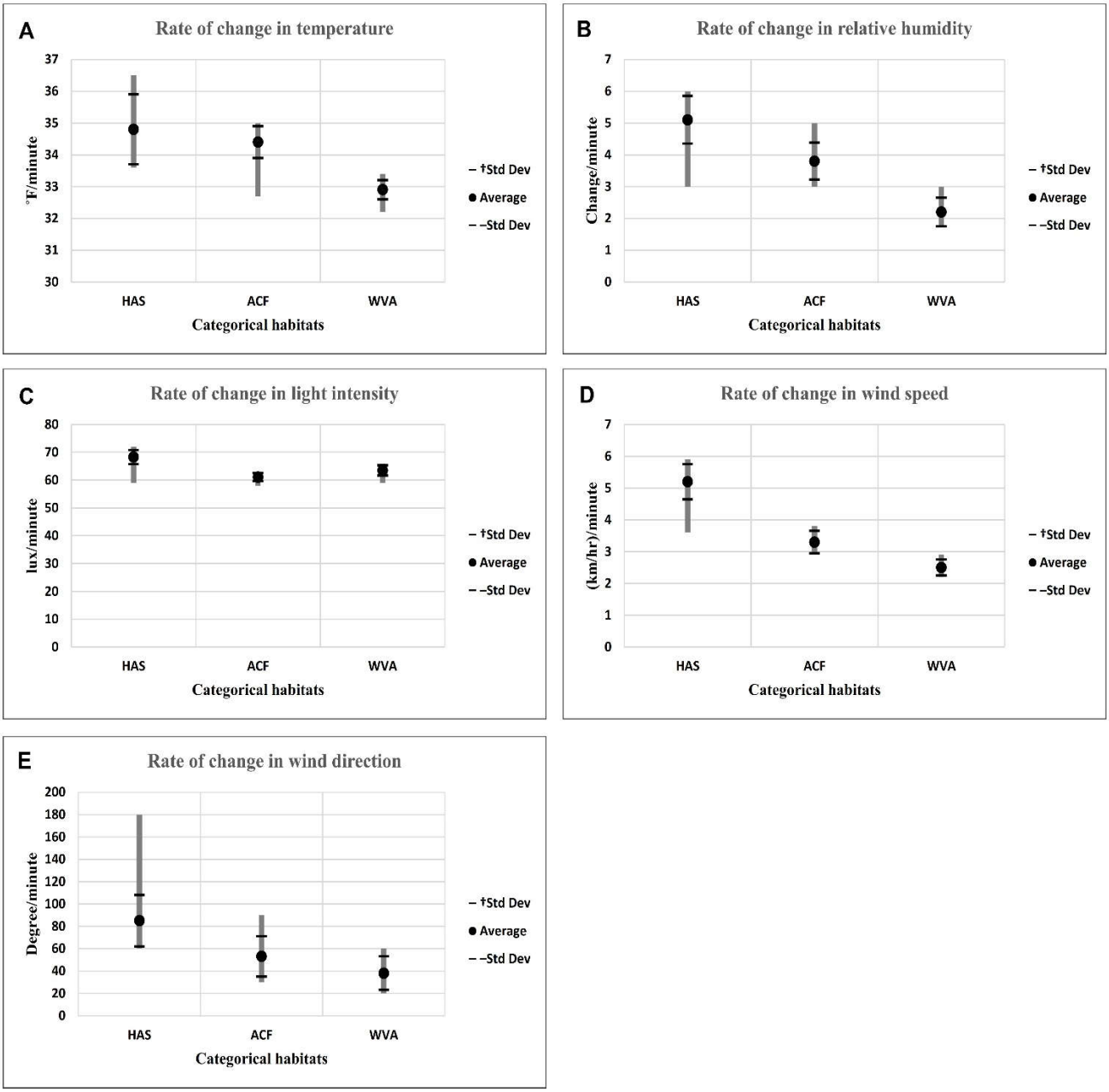

Fig. 2: Rate of change of ecological parameters at different categorical habitats.

Ecological parameters of each categorical habitat or areas: The average values of change in Fahrenheit temperature per minute in the five sampling sites of each categorical areas have been compared to found that, In the human settlement areas (HSA), there exhibits a large range of fluctuation in comparison to agricultural crop fields (ACF) and wild vegetational areas (WVA). There is a little significant difference between ACF and WVA. A similar result is found for change in the percentage of relative humidity per minute. Change in light intensity in lux per minute is slightly higher in HSA, but there was no significant difference between ACF and WVA. Therefore change in light intensity due to anthropogenic disturbances is insignificant to produce an effect on the ecological condition. Change in wind speed in (km/hour) per minute and change in wind direction in degree per minute has much significant difference among the three categorical habitats in the order of HSA>ACF>WVA.

Table 1: Diversity and distribution of spiders inside the whole studied area.

Family	Species	Habitat			Description
		HSA	ACF	WVA	
Araneidae	1. *Anepsion maritatum* (O.Pickard-Cambridge, 1877)	0	1	3	Arboreal, Orb-web builders
	2. *Cyclosa* sp.	0	1	3	Arboreal, Orb-web builders
	3. *Neoscona* sp.	0	0	1	Arboreal, Orb-web builders
	4. *Argiope pulchella* Thorell, 1881	1	2	3	Arboreal, Orb-web builders
	5. *Gasteracantha geminata* (Fabricius, 1798)	1	2	3	Arboreal, Orb-web builders
	6. *Araneus* sp.	0	0	1	Arboreal, Orb-web builders; Nocturnal
Ctenidae	7. *Ctenus* sp.	1	1	2	Ground dwelling; Found in leaf litter, crevices of the rock, sandy surface or on the muddy semi-dried soil; Nocturnal
Lycosidae	8. *Lycosa* sp.	2	3	2	Ground dwelling; Found in leaf litter, crevices of the rock, sandy surface or on the muddy semi-dried soil; Nocturnal
	9. *Hippasa* sp.	2	3	2	Ground dwelling; Tunnel sheet/funnel-web builder
	10 *Paradosa* sp.	1	3	2	Ground dwelling; Tunnel sheet/funnel-web builder
Oxyopidae	11. *Oxyopes birmanicus* Thorell, 1887	2	3	2	Arboreal, ambush hunter
	12. *Oxyopes sp.*	2	2	3	Ground dwelling on leaf litter or herbs, Diurnal
	13. *Peucetia viridana* (Stoliczka, 1869)	1	3	3	Arboreal, ambush hunter
Salticidae	14. *Telamonia dimidiata* (Simon, 1899)	1	2	2	Arboreal, ambush hunter
	15. *Hyllus semicupreus* (Simon, 1885)	1	2	2	Arboreal, ambush hunter
	16. *Phintella vittata* (C.L. Koch, 1846)	1	2	2	Arboreal, ambush hunter
	17. *Myrmaplata plataleoides* (O. Pickard-Cambridge, 1869)	0	2	2	Ant mimicking spider, Close proximity to ant
	18. *Myrmaplata* sp.	2	2	1	Ant mimicking spider, Close proximity to ant
	19. *Portia* sp.	0	0	2	Arboreal, hunt small spiders and termites.
Sparassidae	20. *Olios milleti* (Pocock, 1901)	0	1	3	Arboreal, ambush hunter
Tetragnathidae	21. *Tetragnatha javana* (Thorell, 1890)	1	1	2	Arboreal, horizontal orb-web builder
Theridiidae	22. *Argyrodes* sp.	0	1	2	Arboreal, Orb-web builder
Thomisidae	23. *Thomisus* sp.	1	3	3	Arboreal, Ambush hunters

0=Poor abundance, 1=Little abundance, 2=Moderate abundance, 3=High abundance.

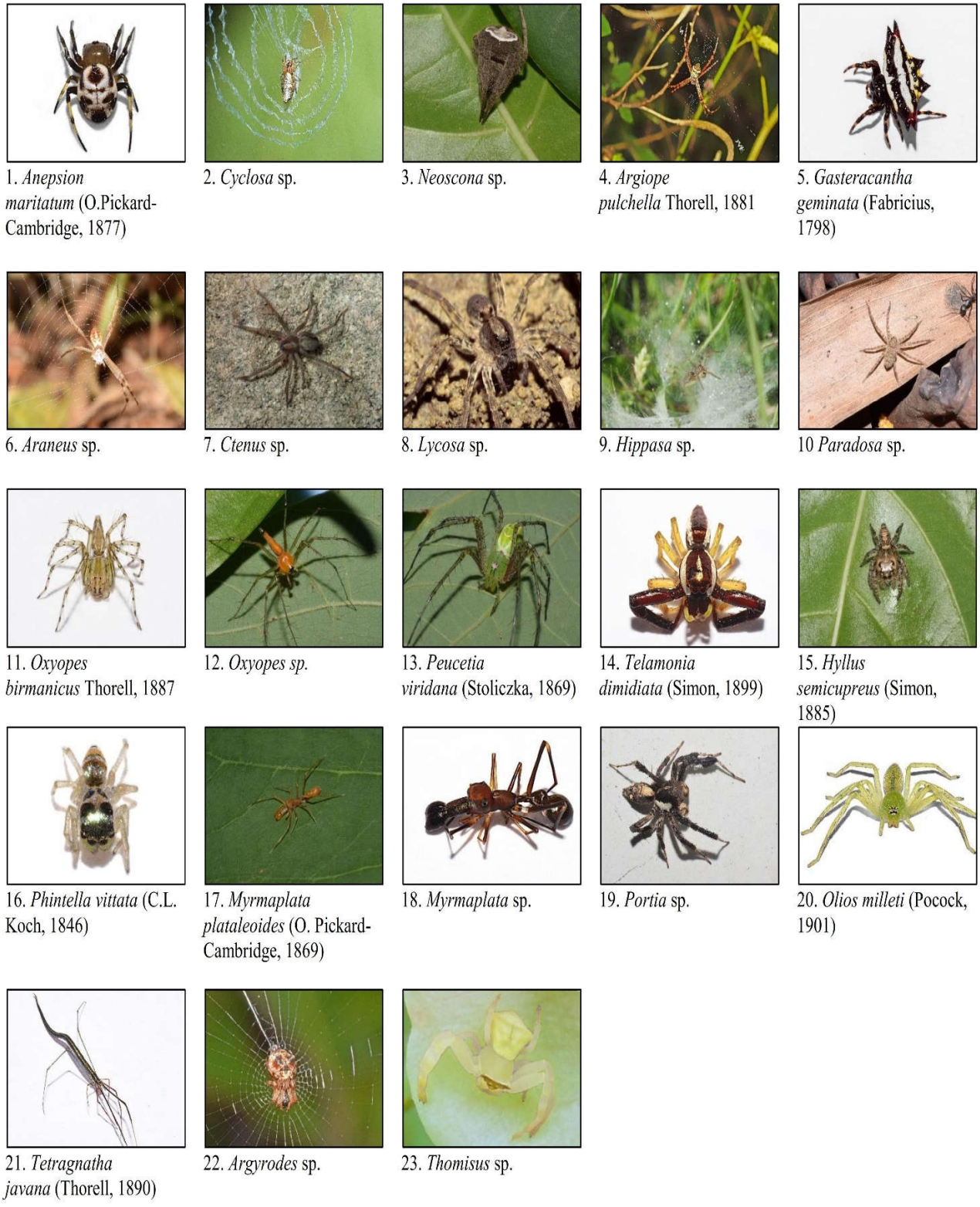

Fig. 3: Photographs of spiders from sampling.

Spider diversity and distribution: Overall 592 spider specimens were collected by all collecting methods from 15 sampling sites, representing 9 families and 23 species. From HSA and ACF, only 14 and 19 species were found respectively. All species were found to available in WVA. Spider abundance in number per square meter area is least in HSA and on the other hand, there is a little higher abundance in ACF in comparison to WVA. Mean species richness per sampling site and diversity indices per sampling site have values in the order of HSA<<ACF<WVA.

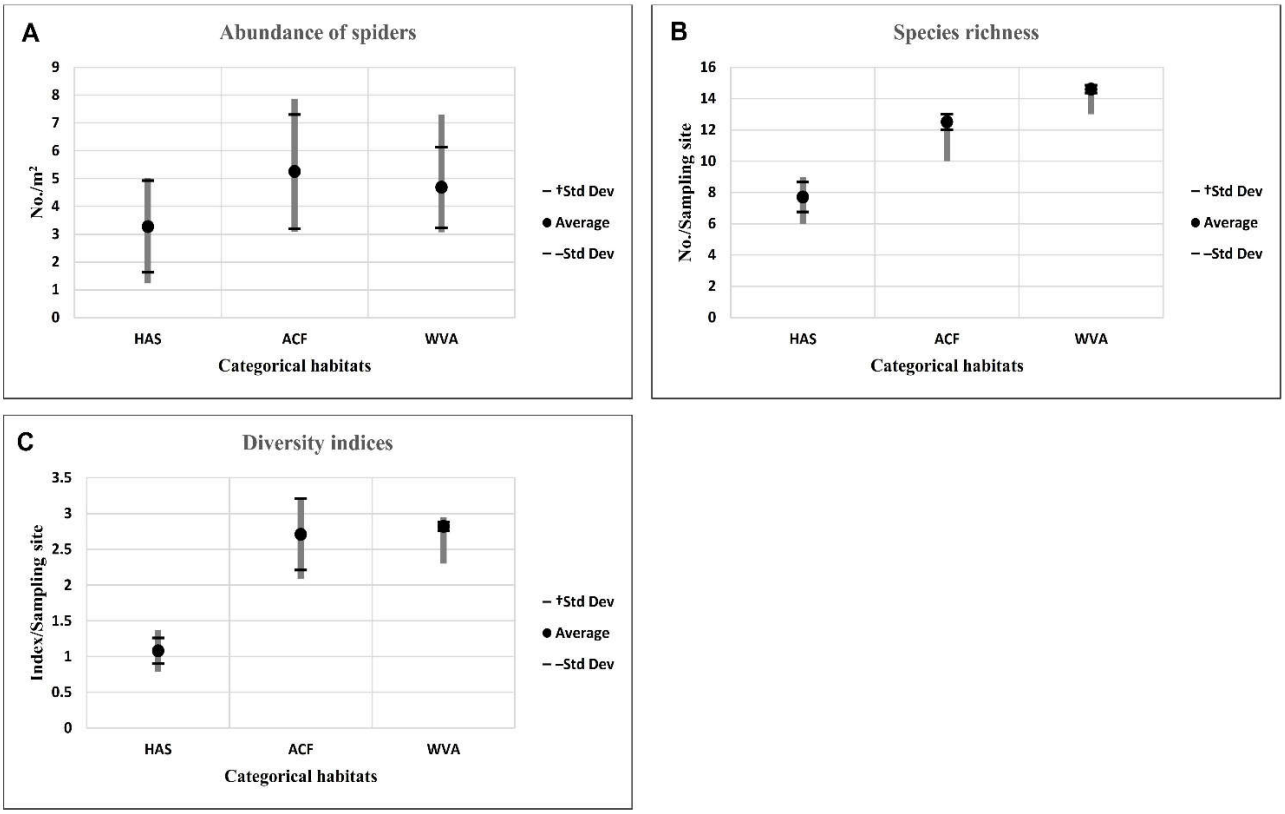

Fig.4: Spider abundance, species richness, and diversity indices at different categorical habitats.

Discussion

From the study, it is found that temperature and humidity in HSA are highly fluctuating, mostly due to burning action, the presence of concrete roads and buildings, smoke, and dust particles in the air. Wind speed and wind direction are not fluctuating to a greater extent at WVA due to the presence of trees and dense vegetation. In ACF, small height plants do not resist the change in wind speed and wind direction. Heavy vehicular movements is an important reason for the high rate of fluctuation of wind speed and wind direction at HAS. Anthropogenic disturbances make a high rate of change in the ecological factors that directly affect the population growth rate of different spider species in a different manner (Schweiger et. al., 2005; Lowe et. al., 2014; Picchi et. al., 2016). Changing and fluctuating ecological state reduces the predatory and mating behaviour, their fecundity rate, and developmental success by most of the wild spider species (Johonson et. al., 2019). Changing environmental conditions whether at a higher or lower degree, but in a constant rhythmic pattern (diurnal or seasonal changes), allows the growth of certain varieties of adaptable species to be populated and establish a good ecological community. On the other hand, habitats having frequently changing ecological factors which are mostly due to anthropogenic activities, resist the growth of most of the orb-web builder and other arboreal spider species. Only a few spider species such as ground-dwelling spiders and sheet web builder species can exploit the area to establish a poor diversified community structure (Shochat et. al., 2008). Ground dwelling spiders in the community can only participate in the ecological food chain at the ground part of the whole habitat, but not at the upper arboreal habitat. For establishing complete ecological balance, there needs the presence of spiders exhibiting all types of functional habitats or niches.

Conclusion

As spider diversity and distribution is considered as the indicator of sustainable ecological habitat (Maelfait & Hendrick, 1998; Langor & Spence, 2006), their conservation strategies can bring ecological balance which will be beneficial for the vegetational growth, both at human settlement areas and crop fields. Therefore, to retain spider diversity in any locality, there needs a management plan for incorporating some undisturbed wild vegetational patches both in rural and urban areas. This could include rules on how to keep that vegetational patch undisturbed and not been influenced by any household waste materials or drained water from any industry, factory, or crop field.

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Carbon dioxide and Suspended PM Variation in Barrackpore Cantonment Area during 2019-2020: Effect of Lockdown

Monojit Ray

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Author's Affiliations: Principal and Professor of Chemistry, Barrackpore Rastraguru Surendranath College, Barrackpore, Kolkata 700120, West Bengal, India.

Corresponding Author: Monojit Ray, Principal and Professor of Chemistry, Barrackpore Rastraguru Surendranath College, Barrackpore, Kolkata 700120, West Bengal, India.

E-mail: monojit1972@gmail.com

Abstract

Barrackpore cantonment, the oldest cantonment of India is very green indeed and have number of trees with low population density. In this study I had measured the amount of carbon dioxide and particulate matters namely PM 2.5 and PM 10 present in air throughout the years 2019 and 2020. From the experimental results it is evident that lockdown for pandemic and rainfall made a profound effect in air quality for at least seven months of 2020. Particulate matters are serious air pollutants and carcinogenic.

Keywords: Barrackpore Cantonment; Carbon dioxide; Lockdown.

Introduction

Barrackpore cantonment area (Location 22.78^{o} N, 88.48^{o} E) is situated within barrackpore subdivision, north 24 pargana district of West Bengal, India. Barrackpore cantonment is the oldest cantonment of India.[1] The cantonment area is surrounded by Barrackpore municipal area on the east and south, North Barrackpore municipal area on the north, Hooghly river on the west.[2,3] According to Census of India data 2011, the total population was 17,380 within barrackpore cantonment area. The only road connections are there and one army air base is situated within this area. Number of Schools and only one college having two campuses are situated within this area. The main source of air pollutions are human activity and automobile exhaust. According to Indian standard, moderate level of carbon dioxide, particulate matter 2.5 (PM 2.5) and particulate matter 10 (PM 10) in air are 900 ppm, 100µg/m^3, and 60 µg/m^3 respectively.

Sampling and Instrument

Sampling were done in different locations of barrackpore cantonment area at least twenty days in every month. The instrument used is a portable low volume sampler made by Airveda TM Model No PM2510CTH (India Made). The instrument measure PM by light scattering sensor and carbondioxide by nondispersive infrared sensor. The instrument was purchased by Barrackpore Rastraguru Surendranath College under UGC CPE fund.

Results

Table 1: Monthly average carbondioxide, PM 2.5, PM 10 and humidity data of barrackpore cantonment area during January 2019 to December 2019.

Month	Carbon Dioxide (ppm)	PM 2.5 (µg/m^3)	PM10 (µg/m^3)	Humidity (%)
January 2019	610.60	148.70	243.40	48.70
February 2019	582.00	113.50	210.16	51.50
March 2019	599.30	71.60	146.40	49.00
April 2019	594.57	50.14	95.57	56.00
May 2019	654.00	70.00	106.83	56.83
June 2019	745.00	47.40	85.80	57.20
July 2019	729.60	46.00	96.30	66.40
August 2019	688.80	27.50	50.50	65.30
September 2019	727.50	28.00	71.83	67.83
October 2019	823.00	41.50	69.00	64.50
November 2019	734.50	77.50	179.25	60.80
December 2019	653.60	129.30	225.60	51.30

Table 2: Monthly average carbondioxide, PM 2.5, PM 10 and humidity data of barrackpore cantonment area during January 2020 to December 2020.

Month	Carbon Dioxide (ppm)	PM 2.5 (µg/m³)	PM10 (µg/m³)	Humidity (%)
January 2020	738.25	138.00	216.00	55.50
February 2020	660.30	81.50	133.40	45.10
March 2020	678.75	80.50	133.50	61.50
April 2020	469.00	44.00	202.00	70.00
May 2020	656.00	38.75	62.00	74.00
June 2020	672.75	39.50	57.50	68.75
July 2020	672.80	32.00	42.80	72.00
August 2020	684.60	09.60	15.60	71.50
September 2020	725.60	32.50	40.00	72.25
October 2020	826.00	41.00	61.00	52.00
November 2020	768.00	22.00	61.00	46.00
December 2020	744.28	153.28	246.57	53.57

Table 3: Monthly average temperature and number of rain day data of barrackpore cantonment area.

Month	Temp High/Low(°C)	Rain
January	26°/13°	1 day
February	29°/16°	1 day
March	34°/21°	2 days
April	36°/24°	3 days
May	36°/25°	7 days
June	34°/26°	14 days
July	33°/26°	18 days
August	33°/26°	18 days
September	33°/26°	14 days
October	32°/24°	7 days
November	30°/19°	1 day
December	27°/14°	0 day

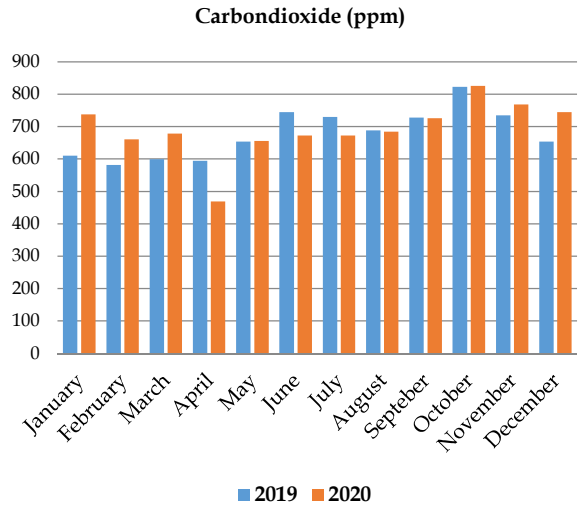

Fig. 1: Comparison of average Carbondioxide values.

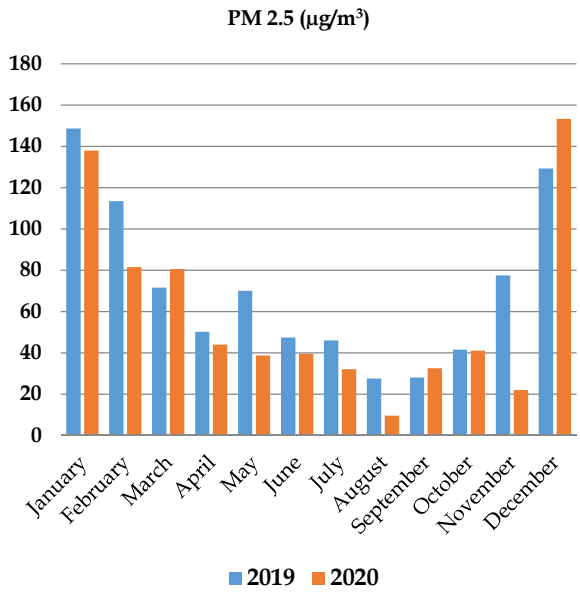

Fig. 2: Comparison of average PM 2.5 values.

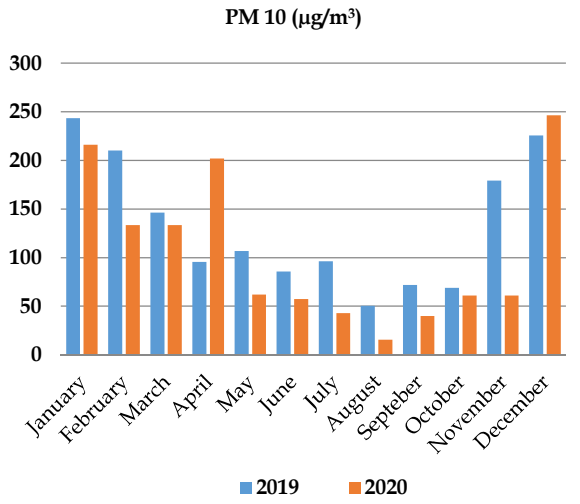

Fig. 3: Comparison of average PM 10 values.

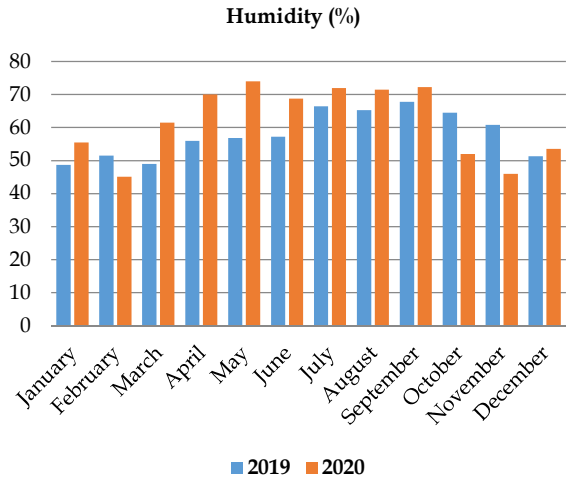

Fig. 4: Comparison of average humidity values.

Discussion

According to Indian standard air containing carbon dioxide above 1100 ppm is poor, around 900 ppm moderate and below 750 ppm is satisfactory. Carbon dioxide concentrations are comparable for the months august to November for both the year (Fig. 1). Carbon dioxide concentration majorly remain more than 600 ppm throughout the year with some exceptions (Fig. 1). The concentration of carbon dioxide is higher relatively during winter of 2019 (Table 1.) and also for 2020. Lowest carbon dioxide concentration is found during April 2020 (Table 2). According to Indian standard air containing PM 2.5 above 90 $\mu g/m^3$ is poor, around 60 $\mu g/m^3$ moderate and below 30 $\mu g/m^3$ is satisfactory. PM 2.5 remain higher than 80 $\mu g/m^3$ during winter (Fig. 2.) but gradually decreases with the number of rain days in a month. Rainfall removes particulates from air. The PM 2.5 value remain below 50 $\mu g/m^3$ during eight months (April 2020 to November 2020) (Table 2) whereas during 2019 for only five months (June 2019 to October 2019). The value of PM 2.5 stands below 100 $\mu g/m^3$ for nine months during 2019 and ten months during 2020. According to Indian standard air containing PM 10 above 250 $\mu g/m^3$ is poor, around 100 $\mu g/m^3$ moderate and below 50 $\mu g/m^3$ is satisfactory. Average PM 10 value were found higher than 100$\mu g/m^3$ during winter (November, December, January, February and March) but gradually decreases with the number of rain days in a month (Fig. 3). Rainfall removes PM 10 from air. The PM 10 value remain below 100 $\mu g/m^3$ during six months during 2019 (April, June, July, August, September and October 2019) whereas, during 2020 it remains below 100 $\mu g/m^3$ for seven months (May 2020 to November 2020) (Table 2). The minimum value of PM 10 was found $\mu g/m^3$ for 15.60 for August 2020. Humidity is maximum in rainy season and minimum during winter (Fugure 4).

Conclusion

Due to the effect of strict lockdown carbon dioxide concentration was low during April 2020 and the average value is lower than average April 2019 value by an amount of 21.12%. It may be concluded from data that with respect to carbon dioxide air quality of barrackpore cantonment area is "moderate". The air quality of barrackpore cantonment are poor during January, February, December 2019 and January & December 2020 with respect to PM 2.5. The barrackpore cantonment air quality with respect to PM 2.5 found "satisfactory" during August, September 2019 and July to November 2020. Within barrackpore cantonment area air quality is "satisfactory" with reference to PM 10 for august to October 2019 and May to November 2020. The extra four months PM 10 low data for the year 2020 can be attributed to lockdown due to pandemic. High PM 10 data are found for January, February, March, November, December 2019 and January, February, March, April & December 2020.

Particulate matter arises from soil dust, road dust, burning of fossil fuel in vehicles, industry exhausts, power plants etc. In barrackpore cantonment area particulate matter in air arises only from soil dust, road dust, burning of fossil fuel in vehicles and human activity only. Though there are no industry within barrackpore cantonment area still there are many industries within barrackpore subdivision. Particulate matter deposits on the leaf of plants, may clog the stomatal mouth and interfere with the photosynthesis activity of plant.[4] It is established fact that particulate matters are most powerful and harmful air pollutant as well as carcinogen. In the year 2016, throughout the world, 4.1 million deaths were reported on exposure to PM 2.5 through heart disease and stroke, lung cancer, chronic lung disease and lung disease.[5] Fine particulate matter PM 2.5 reach to alveolus of human lung. In present days due to COVID 19 pandemic most people are using face masks which prevent entry of PM 2.5 and PM 10 within human lung. Even when the pandemic will be over use of face mask is essential during winter (December, January and February) to keep safe from air pollutant particulates as during winter PM 2.5 and PM 10 remain higher than Indian standard and WHO standard. As a result of lockdown PM 10 values found lower than expected during the period May 2020 to November 2020 and it is evident from the comparison data of 2019 for the same period (May to November). It is clearly evident that air pollutants specially particulates found relatively low during 2020 for post lockdown period with an effect for seven months, but again became similar to earlier (before lockdown) during December 2020. Final conclusion can be made that May 2020 to November 2020 overall air quality of barrackpore cantonment area was highly satisfactory whereas in 2019 overall air quality was highly satisfactory for June to October only.

Acknowledgement

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RNA Profiling: More Help in Forensic Serology

Arushi Chawla[1], Chandrima Das[2]

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Author's Affiliations: [1]Professor, [2]BSc Student, Department of forensic science, Parul institute of applied sciences, Parul University, Vadodara, waghodia, Gujarat 391760, India.

Corresponding Author: Arushi Chawla, Professor, Department of forensic science, Parul institute of applied sciences, Parul University, Vadodara, waghodia, Gujarat 391760, India.

E-mail: arushi.chawla82130@paruluniversity.ac.in

Abstract

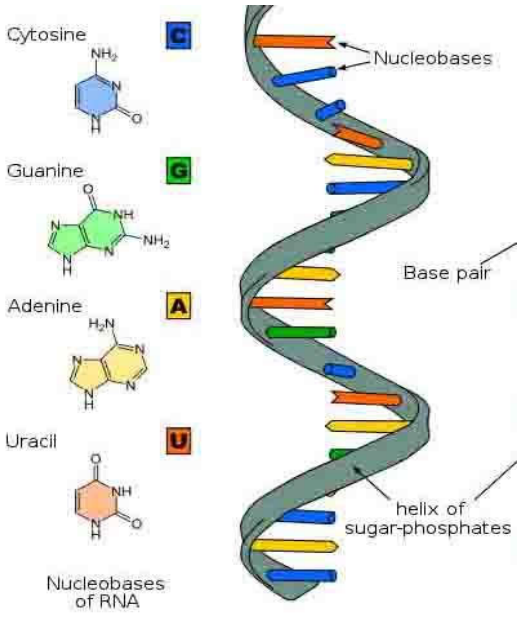
RNA profiling or more specifically messenger RNA (mRNA) profiling is a very useful method to study the body fluids. Various type of body fluids can be found from a crime scene. Because of the changes in the fluids over period of time, it requires more improvise identification method. To study the different biomarkers in different body fluids which is not distinguishable in general process or to analyse the body fluids present in back spatter of a gun, RNA typing or profiling is a safe process. The difference between menstrual blood and peripheral blood, nasal mucosa and vaginal mucosa can be very important in a case. Most of cases DNA profiling for those body fluids are sensitive than RNA profiling. Results of those process show false positive results more which affect the case progress. RNA profiling helps to establish the correlation between DNA profiling in those cases. Addition of many pre mRNAs form a circular RNA (cRNA) which plays the most important role in RNA profiling.

Keywords: Difference between Body Fluids; RNA Profiling; More Accurate Results; Microrna; Forensic Serology.

Introduction

RNA or ribonucleic acid, a single chain polyribonucleo -tide controlled every important function in the early life this fact is not very acceptable. More clearly RNA was first genetic material. It is also not much clear how it helped as first biocatalyst1. Though it is not so sure still there are some enzymes – ribozymes are made up of RNA. Through some medical changes it formed into DNA. Now RNA acts as carrier of coded genetic information from DNA to cytoplasm to help in protein and enzyme synthesis.

RNA is single stranded polynucleotides but some places it shows partially double stranded due to folding or coiling of the single strand. There are 70-12000 ribonucleotides joined from start to end2. The backbone of RNA is made up of alternate residue of phosphate and ribose sugar. These phosphate combines with 5′ of its sugar and 3′ of next sugar and form a DNA like structure. There are four types of nitrogen bases present in RNA 1) adenine 2) guanine 3) cytosine 4) uracil. Nitrogen bases arrange as a complementary to respective DNA template. There are 3 types of RNA mainly help in every major classes. It also acts as a genetic material in some viruses.



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Collected body fluids from the crime scene are major help to solve a particular crime. RNA was first mentioned in forensic literature by oehmichem et al in 1984 reporting on post mortal biosynthesis of DNA and RNA[3]. We can find RNA from dried blood stain, saliva mucosa, vaginal mucosa, buccal mucosa, semen. If there is very little amount of evidence, we still can apply PCR (polymerase chain reaction) to increase the amount of that specific biological fluid. To work with body fluids, we have to be cautious about degradation of its. We should consider sensitive and stable bio markers to prevent the effect of degradation, for that we can consider microRNAs which are less prone to degradation due to their small size and stable structure.[4] RNA profiling also helps to investigate postmortem time interval, age of the wound, identify the exact firearms used for murder from numerous numbers of fire arms in a gang fighting or robbery. But to get exact desirable results we should follow some precautions-1) how unbiasly we can execute the identification 2) distinguishable process adapt to extraction of RNA than DNA 3) consider physiological. Environmental factors.

Through this article I am giving the idea of more use of RNAs in forensic investigation as it gives more accurate result than DNA profiling for body fluids and reduces false positive results. With the guidance of proper procedures, we can solve more cold cases. We will be able to give those cases a proper breakthrough and it will be easier to narrow down the suspects.

Methods

Sample collection:

We can easily collect set of control samples of body fluids and tissues and through the process of evidence collection. This control set includes blood, salina, vaginal mucosa, menstrual secretion, semen and skin sampling from four individuals[5]. To called fresh nasal mucosa samples we have to consider both nostrils and for variation the more number will be beneficial.by using a single cotton swab per nostril the sample from 22 individuals were taken. From seven of these individuals were suffering from cold. A total of 11 nosebleed samples from six donors were collected on tissue paper[6]. From 10 donors the samples of sweat, tear, and urine has been collected on cotton swabs. We have to maintain the distance to avoid skin contact, semen samples are collected from four individuals. Two samples from fertile individuals and other two from vasectomised individuals. After collection we should keep those swabs were air dried and in room temperature in the dark until we used. The collection of 10 samples were collected and stored at -20c and waste materials from those specimens were also taken. Penile swabs were collected by 20 donors using 4N6FLOQ swabs™ with active drying system (Copan liagnostics)[7]. Before collecting any samples, consent has taken rom informed voluntary donors.

Marker name	Tissue	[primer] μM	Forward primer (5′–3′) Reversed primer (5′–3′)	Size (bp)	Dye	Reference
CD93	Blood	0.25	ACCAGTACAGTCCGACAC TTGCTAAGATTCCAGTCCAG	151	NED™	8
HBB	Blood	0.035	GCACGTGGATCCTGAGAAC ATGGGCCAGCACACAGAC	61	FAM™	8
HTN3	Saliva	0.2	GCAAAGAGACATCATGGGTA GCCAGTCAAACCTCCATAATC	134	VIC™	8
STATH	Saliva/nasal mucosa	0.3	TTTGCCTTCATCTTGGCTCT CCCATAACCGAATCTTCCAA	93	FAM™	8
SEMG1	Seminal fluid	0.8	GGAAGATGACAGTGATCGT CAACTGACACCTTGATATTGG	91	FAM™	8
PRM1	Spermatozoa	0.3	AGACAAAGAAGTCGCAGAC TACATCGCGGTCTGTACC	146	NED™	8
CYP2B7P1	Vaginal mucosa	0.8	AGTCTACCAGGGATATGGCATG CTATCAGACACTGAGCCTCGTCC	141	VIC®	9
MUC4	Vaginal mucosa	0.8	CTGCTACAATCAAGGCCA AAGGGAAGTTCTAGGTTGAC	88	FAM™	8
MMP7	Menstrual secretion	0.8	GAACAGGCTCAGGACTATCTC TTAACATTCCAGTTATAGGTAGGCC	107	VIC®	8
MMP10	Menstrual secretion	0.1	GCATCTTGCATTCCTTGTGCTGTTG GGTATTGCTGGGCAAGATCCTTGTT	76	VIC®	9
MMP11	Menstrual secretion	0.4	CAACCGACAGAAGAGGTTCG GAACCGAAGGATCCTGTAGG	71	NED™	9

Fig. 1: van den Berge & Sijen, 2017.

Presumptive tests

After collecting all the specimen presumptive tests for every specimen are required individually. For semen the rapid stain identification series (RSID) is used and to observe the presence of spermatozoa, microscopic analysis has been also conducted. For saliva sample RSID has been also performed. TB testing for blood was performed by transferring biological material to a with water moistened filter paper, to which on drop of tetra bass solution in 10% acidic acid was added10.one drop of barium per oxide solution was added next10. The colour formation will observe according to criteria.

RNA extraction

To extract RNA from biological sample we can proceed with commercial extraction kits.

1. Mirvana™mi RNA isolation kit.[11]

For RNA isolation we used the protocol described by lindenbergh et al.[12] DNase is used is to degrade. DNA in the process of RNA isolation. The RNA extracts were treated with DNase collected swabs were processed carefully. The swabs from excised from the nosebleed tissues were cut 1cm.[2] If the amount of extracted DNA was below 1ng then RNA extracts from ethanol precipitated was prioritize in the reverse transcription. To separate non sperm fraction and sperm fraction, the use of customized mild lysis is more effective. This mild lysis buffer is made up of phosphate buffered saline (PBS). which is composed with 1.6 mg proteinase k and 10µM ribonucleoside vanadyl complex, these two inhibits various ribonucleases, in 50µM of mild lysis buffer the swabs are incubated for 56 c for 20min. After using a QIA shredder column the lysate is separated from carrier material, during centrifugation at 11,000rpm. The non sperm fraction and sperm fraction got separated. Pellet of sperm fraction is washed using 400µL PBS buffer with 10 µM ribonucleoside vanadyl complex and after that it is centrifuged at 13.200rpm for 5min. Then the mirvana mi RNA is isolation kit is used for binding lysis, then the supernatant of non-sperm fraction after centrifugation is treated with lysis buffer and then addition of homogenate additive is placed.

RNA analysis

RNA analysis comprised of CDNA synthesis 19-plex reverse transcriptase PCR product purification and analyse using gene mapper. RNA marker signals help in identification of body fluids. There are several markers for each body fluids. Markers for blood (HBB, CD93, AMICA1) markers for saliva (STATH, HTN3, KRT13) and for mucosa, SPRR2A is used is general. Markers for semen (PRM1 and SEMG1) and for fertile men PRM1 is used. MUC4 for specific vaginal mucosa markers and some markers for menstrual secretion. Different types of marker help to differentiate between different body fluids. The marker for nasal mucosa did not show cross reaction with peripheral, BPIFA1 markers which can be used to differential between peripheral blood and nasal blood. Certain markers don't show the positive results for other body fluids.it discriminates between different type of body fluids.

Result

Through the process of RNA typing, it helps us to differentiate between body fluids. But in case of DNA profiling, it provides the accurate results of the source of the individuals. RNA profiling or typing concludes the cell types present in evidentiary body fluids. The process of RNA typing is followed by clear guidelines. In case of vaginal mucosa due to presence of microbes and bacteria DNA profiling shows false positive result. But in case of examination of skin cells DNA profiling shows more false positive results for whenever skin contact with body fluids, though for detailed cell type information RNA typing is more helpful.

Conclusion

RNA profiling is very recognized method for body fluid identification though it is used as a supplementary method. We can identify large number of body fluids through this process. It helps us to detect the type of body fluids through the RNA marker from a very trace amount. In some of the cases where DNA profiling is responsible as false positive results, there RNA typing comes as a blessing. It is helpful for very degraded body or evidence. In order to obtain accurate results of this method for RNA typing we require to maintain the chain of custody and also unbiased forensic interpretation. But the use of mRNA markers can cause of cross reaction. To identify a human body fluid accurately, we need to consider those cross reaction.

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Review Article

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Micropropagated Plants as Alternative Planting Material to Sugarcane Setts

Nand Lal

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Author's Affiliations: Professor, Department of Life Sciences, Chhatrapati Shahu Ji Maharaj University, Kanpur 208024, Uttar Pradesh, India.

Corresponding Author: Nand Lal, Professor, Department of Life Sciences, Chhatrapati Shahu Ji Maharaj University, Kanpur 208024, Uttar Pradesh, India.

E-mail: nl_pr@yahoo.co.in

Abstract

Sugarcane (Saccharum officinarum L., Family - Poaceae) is an economically important sugar and energy crop propagated conventionally by stem cuttings (setts). Low propagation rates, long time demand, huge land requirement and potential transmission of pathogens through seed cane from generation to generation are the major constraints of conventional propagation. In vitro propagation (Micropropagation) is the best alternative to overcome such limitations to produce disease-free genetic stock and sufficient amount of planting material in minimum possible time, however, it is a relatively sophisticated technique requiring aseptic conditions and technical skill at each stage viz. mother plant establishment, initiation and establishment of aseptic cultures, multiplication, rooting, and acclimatization. More knowledge is required regarding sensitivity of the micropropagation protocol for microbial contamination and the economical viability to support the conventional propagation. This review aims towards the scope of micropropagation for increasing yield by enhancing varietal life-span and allow the sugar industry to produce sufficient quality planting material within short period of time and cost effective manner.

Keywords: Cane Setts; Micropropagation; Seed Cane; Sachharum Spp; Sugarcane.

Introduction

Sugarcane (*Saccharum officinarum* L., Family-Poaceae), primarily used for sugar production, has a unique source-sink system. Its stem sinks store photosynthate as soluble disaccharide, sucrose, which can reach exceptionally high concentrations, up to 650 mM or 18% of stem fresh weight in commercial sugarcane varieties (Inman-Bamber *et al*., 2011). Lack of rapid multiplication procedures has long been a problem in sugarcane seed production, particularly in expansion of newly released varieties. Almost a decade is required to conduct various steps of sugarcane variety development i.e. hybridization, selection and different evaluation trials. Subsequently, it takes several years for seed multiplication before reaching to commercial plantations. The time spent for seed multiplication is considered a serious economic loss. The new varieties sometimes enter in degenerative phase due to continued contamination by systemic diseases in open fields. Sugarcane is propagated commercially by vegetative method, involving the planting of the stem cuttings of premature cane (about 8 to 12 months old) grown with special care (Sime, 2013). The seed cane that is used as planting material may be either whole stalks or stalk cut up in shorter segments called setts (Garside and Braunack, 2001). The growth of sugarcane has different stages: emergency, tillering, stalk growth, and maturation. The germination is a most critical and foundation event in the plant life to assure a good harvest. It is initially dependent on nutrients and water available within sett till developing its own root system for three weeks under proper conditions, though, the initial growth of sugarcane is influenced by several other internal and external factors such as sett age, cultivar, setts nutrients, temperature, soil aeration; setts position on the stalk and humidity (Sime, 2013).

A cane sett is the main conventionally propagation system for majority of the sugarcane growing countries in the world. In some instances, the buds scooped out of the cane using a bud-chipping machine or knives are used for raising the seed nursery. Higher seed rate of 75,000 three-bud setts per hectare is needed for raising breeder's seed to compensate for germination loss due to

heat therapy (Jalaja *et al.*, 2008). In general, non-availability of quality and true to type planting material of newly released varieties is a major bottleneck in their quick adoption for commercial use, and improving sugarcane productivity. Even the cultivation of well adopted commercial varieties requires availability of quality seed to ensure better cane yield, sugar yield, pathogens and pest-free crop (Flynn *et al.*, 2005). Further, traditional method of cultivation using three-budded setts requires large quantity of seed stalks, which is costly, time consuming and land demanding.

Micropropagation has emerged as most successful and commercially viable facet of plant biotechnology and is being routinely used for propagation of a range of species. The critical test for micropropagation is whether it can occur without introduction of genetic alternations. Although tissue culture work was initiated on sugarcane in 1962 in Hawaii, micropropagation studies remained incomplete for next 20 years. Earlier true shoot apex and axillary bud cultures in sugarcane were treated to be extremely difficult. A number of studies conducted between 1978-93 in France, Brazil and India proved that sugarcane can be propagated by induction of axillary shoot proliferation in cultured shoot meristems (Maretzki, 1987, Lal and Singh, 1994). Lee (1987) in Brazil made comparison of various methods of in vitro propagation in sugarcane and proposed that shoot apex culture was a better technique for propagation due to faster rate of multiplication and genetic stability of propagules.

Micropropagation in sugarcane results in production of planting material that enjoys a number of positive features such as:

- Faster rate of propagation.
- Availability of planting material throughout the year i.e. in all the seasons.
- Production of uniform plants of a selected genotype.
- Uniform cloning of highly heterozygous *Saccharum* complex.
- Elimination of systemic pathogens before planting.
- Preservation of breeding stocks as juvenile plants.
- International exchange of disease-free genotypes avoiding quarantine regulations.
- Transplanting to replace stalk piece (sett) planting.
- Filling of gaps in plant and ratoon crops.
- Requirement of small laboratory space in comparison to open fields.
- Cleaning of commercial varieties deteriorated due to diseases.

Micropropagation method for sugarcane involves following four stages for generating the planting material (Lal and Krishna, 1994):

- Culture establishment (Fig. 1A)
- Shoot (tiller) multiplication (Fig. 1B)
- Rooting of the tillers (Fig. 1C)
- Hardening of the propagules (Fig. 1D)

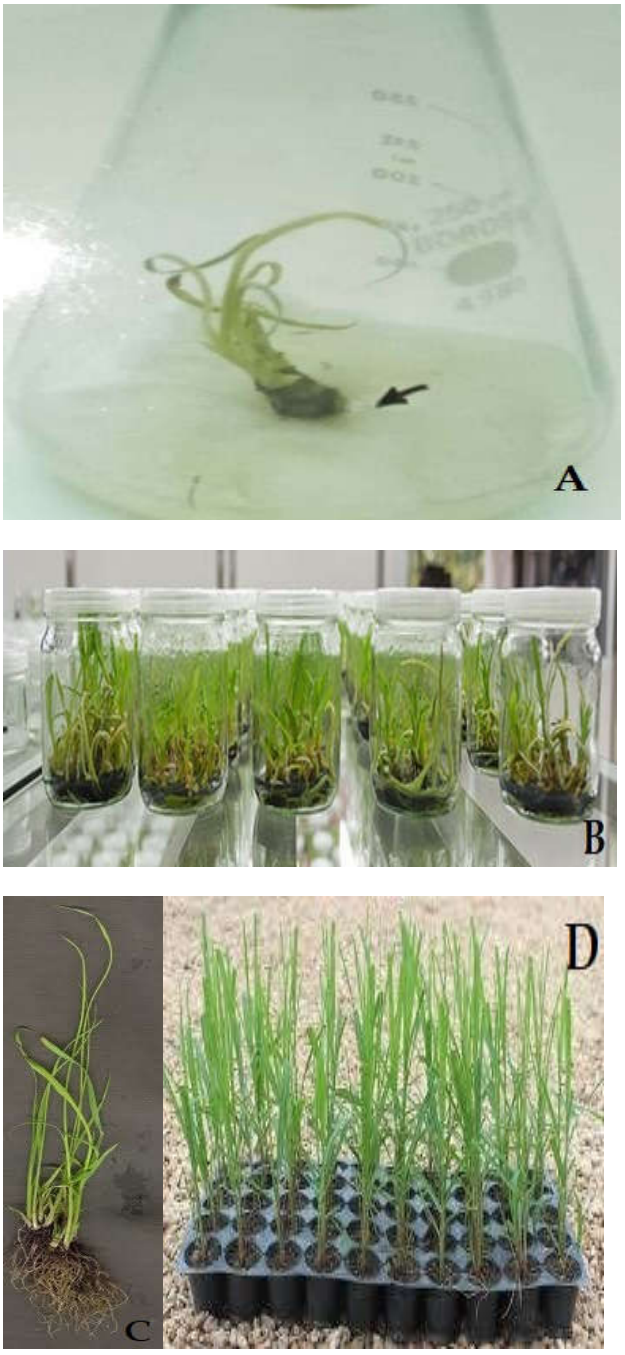

Fig. 1: Key steps/stages during Sugarcane micropropagation. **A.** Shoot culture establishment, **B.** Shoot multiplication, **C.** Rooting of tillers (plantlets), **D.** Hardening of plantlets.

Details of micropropagation protocol for sugarcane are well documented and being practiced in India, Brazil, Australia, Hawaii, French territories and African countries.

Use of micropropagated plants for raising seed cane crop is well studied in detail in several sugarcane growing countries. Generally, hardened plants of 30-45 days are used for transplantation in field. Hardening procedure for sugarcane propagules is simple and results in survival and establishment rates exceeding 90%. Transplanting of hardened plants in field in favourable

season shows almost 100% establishment. The hardened plants are transplanted in pits in levelled soil or furrows without disturbing the soil attached to roots. Trimming of leaves prior to transplantation has been found beneficial for establishment and early induction of tillers. A light irrigation is given to the field after transplantation. A single hardened plant is placed in one pit and appropriate amount of insecticide is also applied in pit. Generally the total fertilizer is applied as top dressing in 3 equal split doses however 1/5th of total fertilizers can be also given as basal dressing. Subsequent field care of micropropagated plants is done in same way as practiced for conventional seed nursery.

Studies conducted in various countries have revealed that micropropagated plants do not undergo genetic alterations and show improved tillering, millable stalks, yield and sugar recovery (Lal, 1996). Incidence of disease is also observed to a minimum level in nurseries raised from micropropagated plants (Flynn *et al.*, 2005).

Considering the potential of micropropagated plants in seed multiplication, it has necessitated the development of appropriate management practice. Lal (1997) observed that plants transplanted in deep pits i.e. pits in furrows show better establishment and vigourous growth. The most important consideration for planting is to decide optimum population and configuration to get economically viable seed nursery different inter- and intra-row spacing for transplanting. Comparison of spacing combinations with conventional sett planting system has shown that micropropagated plants transplanted at a low population i.e. 18,518/ha using inter- and intra-row spacings of 90 and 60 cm, respectively showed higher tillers and millable canes. There is only 20% extra cost required for managing seed cane nursery from micropropagated plants and this is credited to higher cost of the planting material (Jalaja *et al.*, 2008). Other inputs like fertilizers, agrochemicals, irrigation etc. are almost same for the seed nursery raised from either of the planting material. This area requires further attention since simplification of inputs for micropropagated plant based nursery can lower down the cost of cultivation. Another way is to further reduce the cost of planting material which is being attempted by integration of hydroponics at hardening stage and inducing one multiplication cycle outside the culture room. This attempt is likely to help in cost reduction of micropropagated plants. A marginal difference in benefit: cost ratio is observed in sett and micropropagated plant–derived crop and this is easily affordable for seed production purpose, particularly far newly released varieties. Development of appropriate agrotechnology can lead to substitution of setts with micropropagated plants for commercial plantations also and this goal seems not very far. Although higher inputs are required with micropropagated plants, net return/ha is almost same if compared with the sett-derived crop. Considering the net return factor, micropropagated plants can be well accepted by rich farmers.

The feasibility of micropropagation for seed multiplication in sugarcane has led to development of biotechnology units at a number of sugar factories and research institutes. A critical appraisal of the work done at various institutes to make micropropagation more economically viable has shown that tissue culture should be only initially used to produce a sizeable quantity of seed and subsequent multiplication should be done by STP technique (multiplication ration 1:30) and conventional methods (multiplication ratio 1:10).

To bring the feasibility from seed production to commercial plantations for micropropagated plants in sugarcane growing countries following areas require immediate attention:

- Reduction in cost of planting material.
- Development of technology for round the year planting particularly in India and Pakistan.
- Efficient use of inputs to minimize the cost of cultivation.

These challenges can be faced by a co-ordinated effort of tissue culturists, plant physiologists and agronomists. The micropropagated plants are being commonly used for plantations in Australia, Hawaii, Brazil and few Asian countries where the plants are required in low number due to mechanised farming and total cost involved in raising crop from setts and micropropagated plants are almost the same. This is not possible under Indian conditions where more planting material is required due to close inter-row spacings. There seems no scope to reduce the plant population beyond 18,518 per hectare at present. Solution to above mentioned problems can only bring the micropropagated plants within the reach of common farmers. If this happens to be true, micropropagated plants will substitute sett planting and become an ideal planting material for sugarcane cultivation.

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