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Original Articles

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Original Article

Isolation of Antibiotic and Heavy Metal Resistance Bacteria from Organs of Sewage Fed Farm Fish

Biplab Mandal*, Indranil Bhattacharjee, Sayantan Mukherjee**, Soroj Kumar Chatterjee***, Partha Sarathi Roy****

Abstract

Bacterial populations from organs (viz., liver, spleen, kidney and gill) of *Labeo rohita* of the sewage fed fish farm at Kalajharia, Asansol, West Bengal, India, were enumerated, followed by determination of resistance for antibiotics and heavy metals. The total viable counts of bacteria in these organs, observed, were 5.62×10^4, 4.12×10^4, 2.30×10^4 and 1.76×10^4 colony-forming units / mL, respectively. The random bacterial isolates from these fish organs showed resistance in decreasing order for ampicillin (95%), tetracylin (75%), amoxycillin (70%), amikacin (65%), chloramphenicol (50%), sparfloxacin (40%), gentamycin (30%), levofloxacin (25%), streptomycin (10%), and ciprofloxacin (05%). Most of the isolates exhibited an increasing order of tolerance for the metals (µg / mL) copper (200), cadmium (200), iron (400) and chromium (400), with minimum inhibitory concentration (MIC) ranging from <50 to 1600 µg / mL. A total of 40 bacteria have been successfully isolated from internal organs of *Labeo rohita* (8 isolates of *Aeromonas* spp., 21 of *Edwardsiella* spp., 6 of *Flavobacterium* spp. and 5 of *Vibrio* spp.). In terms of antibiotic susceptibility testing, each isolate was tested against 21 antibiotics, resulting in 482 (57.3%) cases of sensitivity and 61 (7.3%) cases of partial sensitivity. Meanwhile, 297 (35.4%) bacterial isolates were registered as resistant. The multiple antibiotic resistance (MAR) index of each bacterial species indicated that bacteria from raised bullfrogs have been exposed to tested antibiotics with results ranging from 0.27 to 0.39. These observations indicate that the significant occurrence of bacterial population in organs of fish with high incidence of resistance for antibiotics and heavy metals may pose risk to fish fauna and public health.

Keywords: *Labeo rohita*; Antibiogram; Heavy Metal Resistance; Multiple Antibiotic Resistance.

Introduction

Labeo rohita constitute a significant part of food for the global population and play an important role in the aquatic environment due to high value and huge demand from local and abroad. Heavy metals are ubiquitous and persist as environmental pollutants that are introduced into the environment through anthropogenic activities, such as mining and smelting, as well as through irrigation and other sources of industrial waste. However, untreated or partially treated wastewaters introduce a huge amount of contaminates particularly heavy metals into agricultural lands (Wang and Tao, 1998). The existence of heavy metals in the environment represents a significant and long-term environmental hazard since they are not biodegradable and tend to accumulate in living organisms (Kobya et al., 2005; Liao et al., 2008).

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Indiscriminate use of different antibiotics has caused development of resistance for various antimicrobials and chemotherapeutic agents among the gut flora of homeotherms. Use of antibiotics will exert more selective pressure and resistant pathogens will be encountered more frequently (MacMillan, 2001). Resistance to antibiotics and metals occurs simultaneously when the genes specifying resistant phenotypes are located together on the same genetic

element such as a plasmid, transposon, or integron (Chapman 2003; Frost et al., 2005; Venner et al., 2009).

Therefore, the present study was conducted to evaluate the antibiotic and heavy metal tolerance of these microorganisms obtained from internal organs of sewage fed farmed *Labeo rohita*.

Materials and Methods

Labeo rohita were collected from the sewage fed fish farms at Kalajharia Asansol, West Bengal, India, receiving municipal sewage and industrial effluent from Asansol town and its adjoining areas. Fishes were sacrificed by stunning to dissect out aseptically the liver, spleen, kidney and gills (Pathak and Gopal, 2005) by using sterile cotton bud; subsequently, spread-plate technique was employed with several selective agars such as cytophaga agar (CA), glutamate starch pseudomonas (GSP), thiosulfate citrate bile salt (TCBS) and xylose lysine deoxycholate agar (XLD) (Oxoid, England). Plates were incubated at room temperature (28-30°C)– since this the optimal growth temperature for mesophilic bacteria – for 24 to 48 hours. Colonies were then selected and kept in TSA deep tube for further studies.

Antibiotic susceptibility testing was conducted according to Kirby and Bauer disk diffusion method (Bauer et al., 1966) by employing Müeller-Hinton agar (Oxoid, England). 10 antibiotics namely 30 µg of amikacin, 25 µg of amoxicillin, 10 µg of ampicillin, 30 µg of chloramphenicol , 5 µg of ciprofloxacin, 10 µg of gentamycin, 5 µg of levofloxacin, 10 µg of sparfloxacin, 25 µg of streptomycin and 30 µg of tetracyline were used for studying antibiotic resistance (%) among bacterial isolates from fish organs.

Minimum inhibitory concentrations (MIC) of four heavy metals for the present bacterial isolates were determined by two-fold agar dilution method (Miranda and Castillo, 1998). $CdCl_2$, $K_2Cr_2O_7$, $CuSO_4.5H_2O$ and $FeCl_3$ were added to tryptic soy agar (TSA) (Merck, Germany). The MIC of these metals was observed after incubation overnight at 37±1°C.

The multiple antibiotic resistance (MAR) index of isolates were tested against 15 µg of erythromycin (E); 100 µg of spiramycin (SP); 30 µg of oxytetracycline (OT); 15 µg of furazolidone (FR); 30 µg of kanamycin (K); 30 µg of nalidixic acid (NA); 30 µg of chloramphenicol (C); 10 µg of ampicillin (AMP); 25 µg of sulfamethoxazole (RL); 25 µg of amoxicillin (AML); 25 µg of colistin sulfate (CT); 30 µg of doxycycline (DO); 30 µg of florfenicol (FFC); 30 µg of flumequine (UB); 50 µg of fosfomycin (FOS); 15 µg of lincomycin (MY); 50 µg of nitrofurantoin (F); 30 µg of novobiocin (NV); 15 µg of oleandomycin (OL); 2 µg of oxolinic acid (OA) and 100 µg of spiramycin (SP) antibiotics. The MAR index of isolates against tested antibiotics was calculated based on the following formula (Krumperman, 1983):

MAR index = X / (Y x Z)

X: total of bacteria resistant to antibiotics;

Y: total of antibiotics used in the study;

Z: total of isolates.

A MAR index value equal to or less than 0.2 indicated antibiotics that had been seldom or never used, in terms of treatment, whereas a value greater than 0.2 suggested that the animal had been highly exposed to those antibiotics.

Results and Discussion

Contamination of river water with municipal sewage and industrial effluent results in the occurrence of pathogenic microorganisms, particularly fecal bacteria and toxic metals, above their maximum permissible limits (Chatterjee et al., 2010). Fish in such water are exposed to these bacteria and metals, which bioconcentrate in different organs of fish. The bacterial load, i.e., total viable count, was found to be 5.62×10^4, 4.12×10^4, 2.30×10^4 and 1.76×10^4 c.f.u. / mL in liver, spleen, kidney and gill of the experimental fish, respectively. It has been observed to be maximum in liver and minimum in gills. Thus, it appears from these findings that soft tissues in massive organs are more prone to bioconcentration of bacteria, leading to incidence of infectious diseases among the aquatic fauna. This may be due to availability of more nutrients and lack of exposure to the surroundings. Bioconcentration of aquatic bacteria such as coliforms, streptococci, and aeromonads in gut, liver, and muscles of tilapia fish grown in a sewage-contaminated pond has also been noticed (Fattal et al., 1993).

The antibiotic resistance among random bacterial isolates from all four organs has shown a full range of resistance (0–100%) for ten common antibiotics of therapeutic and prophylactic use among human beings and in fish aquaculture. Resistance was found to be maximum among the isolates from spleen, kidney, and liver, while it was minimum among those from gill. Maximum average resistance was exhibited for ampicillin (95%) and tetracycline (75%) and minimum for ciprofloxacin (05%) (Table 1). The

resistance exhibited for ciprofloxacin, levofloxacin and gentamycin is a signal of the ineffectiveness of broad-spectrum antibiotics of the present generation. With these observations it appears that the source of the problem of antibiotic resistance in riverine ecosystems is fecally contaminated water and fish populations in them plays important role in creating resistance. Antibiotic resistance patterns in the bacterial population in an aquatic ecosystem have been found to be useful in identifying non point sources of fecal pollution (Wiggins et al., 1999). The occurrence of resistance for common antibiotics is, further, an indication of indiscriminate use of these antibiotics, leading to constraint in antimicro-bial therapy for infectious diseases. The loss of antibiotic susceptibility among the aquatic bacteria has been observed to be affected to a considerable extent by the physicochemical qualities of water and seasonal varia-tions (Pathak et al., 1993).

The maximum tolerance, in general, was observed for chromium and iron (400 µg/mL), while it was minimum for copper and cadmium (200 µg/mL). Likewise, the maximum MIC was observed for chromium and iron (400 µg/mL), while it was minimum for copper and cadmium (200 µg/mL) (Table 2). In addition to assessment of loss of antibiotic susceptibility, the test isolates were also found to be tolerant to different concentrations of various toxic heavy metals as evidenced by their MICs ranging from <50 to 1600 µg/mL (Table 3).Thus, the isolates from visceral organs, i.e., spleen, kidney, and liver, exhibited maximum resistance for ampicillin, tetracycline, and amoxicillin with highest tolerance for iron and chromium, while the isolates from gills showed minimum resistance for ampicillin and tetracycline with rather low tolerance for cadmium and copper (Tables 1 and 3). These observations indicate that visceral organs provide better conditions for bacterial growth and biological activity than the exposed organs such as gill. Therefore, this increase in the MIC of toxic metals as well as antibiotic resistance among aquatic bacterial populations is also an indication of risk to the safety of the aquatic ecosystem, fish fauna, and ultimately human health.

In the present study, a total of 40 bacterial isolates were successfully isolated comprising 8 isolates of *Aeromonas* spp., 21 of *Edwardsiella* spp., 6 of *Flavobacterium* spp. and 5 of *Vibrio* spp. A total of 482 cases (57.30%) were reported as sensitive, whereas 61 (7.30%) and 297 (35.40%) were, respectively, partially sensitive and resistant (Figure 1). MAR values were ranging from 0.27 to 0.39, *Vibrio* spp. presented the highest MAR value (0.39) while *Aeromonas* spp. revealed the lowest one (0.27) (Table 4). MAR indexes of the present work revealed that bacteria from locally raised fish may have been exposed to tested antibiotics. McPhearson et al., 1991 reported that the MAR index of bacteria from a catfish pond, near a river where antibiotic was commonly used as treatment, was as high as 0.76.

Table 1: Antibiotic resistance (%) among bacterial isolates from fish organs

Antibiotics (µg/mL)	Fish organs				Average
	Liver	Spleen	Kidney	Gill	resistance
Amikacin (30)	90	80	60	30	65
Amoxycillin (25)	90	80	70	40	70
Ampicillin (10)	100	100	90	90	95
Chloramphenicol (30)	80	50	50	20	50
Ciprofloxacin (5)	10	10	00	00	05
Gentamycin (10)	50	40	20	10	30
Levofloxacin (5)	50	40	10	00	25
Sparfloxacin (10)	60	50	30	20	40
Streptomycin (25)	20	10	10	00	10
Tetracyline (30)	100	80	70	50	75

Table 2: Heavy metal resistance (%) among bacterial isolates from fish organs

Heavy metals (µg/mL)	Fish organs				Average
	Liver	Spleen	Kidney	Gill	resistance
Copper (200)	10	00	00	00	2.5
Chromium(400)	100	100	80	70	87.5
Cadmium (200)	30	20	10	00	15
Iron (400)	90	100	80	50	80

Table 3: MIC values for different heavy metals among bacterial isolates from fish organs

Heavy metals	MIC values (µg/mL) for different Fish organs			
	Liver	Spleen	Kidney	Gill
Copper	< 50	-	-	-
Chromium	1600	1600	800	400
Cadmium	100	50	< 50	-
Iron	1600	800	800	400

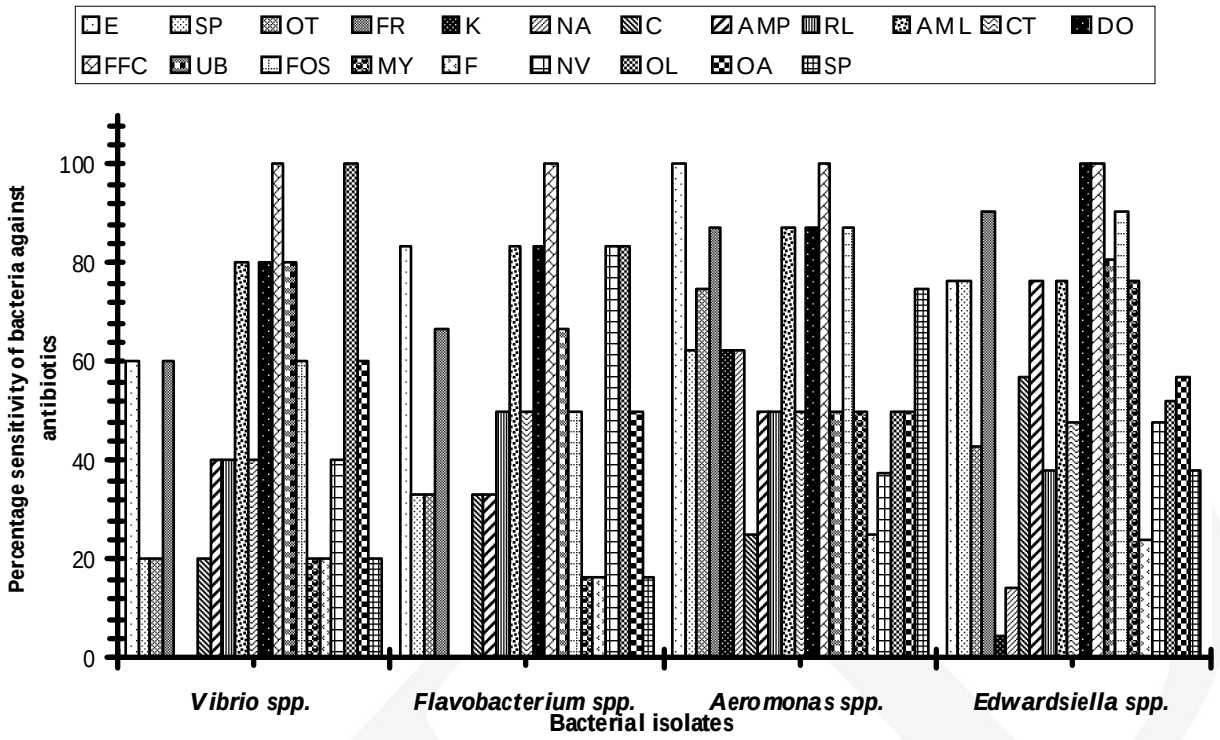

Fig. 1: Percentage sensitivity of *Vibrio* spp., *Flavobacterium* spp., *Aeromonas* spp. and *Edwardsiella* spp. against 21 antibiotics

In the current study, flumequine was found to be the most effective antibiotic for controlling bacterial disease in these fish farms. Other antibiotics that were also effective were oxolinic acid, florfenicol, nitrofurantoin, chloramphenicol and furazolidone. However, some of the antibiotics are banned for aquaculture use such oxolinic acid, nitrofurantoin, chloramphenicol and furazolidone. Therefore, based on published studies, farmers should use other drugs that are also effective for treatment and prophylactic purposes.

Currently, local fish farmers employ amoxicillin to treat fish diseases. The present results proven that amoxicillin is no longer effective, since not more than 27.5% of bacterial isolates were sensitive to it. These findings constitute an alert to antibiotic resistance of *Aeromonas* spp., *Edwardsiella* spp., *Flavobacterium* spp. and *Vibrio* spp.

It appears that the emergence of resistance is also influenced by the physicochemical characteristics of water and several environmental factors including hospital and aquaculture waste disposal (Rhodes et al., 2000) along with the form and bioavailability of metals in the ecosystem. Resistance may develop from a nonspecific mechanism with gene regulation of plasmids and chromosomes, which may be heritable or transfer-able due to the presence of a resistance factor (R-factor) among the aquatic bacterial population (Silver and Walderhaug, 1992). The infections caused by the pathogenic bacteria with R-plasmids may pose a risk of therapeutic problems to public health and fish population. Thus, the water bodies with antibiotic and metal resistant bacteria serve as an environmental reservoir and source for development of this trait among opportunistic pathogens and constitute a significant public concern. Therefore, such studies should be considered for selection of antibiotics in dealing with water-borne diseases, particularly among fishermen and fish consumers. These findings indicate that sewage and industrial pollution are responsible for the emergence of bacterial resistance and deterioration of water quality, along with risk to biodiversity of the hydrobionts and the human health.

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Original Article

The Influence of Seasonal Variation on the Testosterone Hormone of Black Bengal Bucks (*Capra aegagrus hircus)*

Mihir Bhatta*, Debasish Das*, Probal Ranjan Ghosh**

Abstract

The Black Bengal bucks of Indian sub-continent generally show a noted seasonal variation during in reproductive activity. India has a good number of Black Bengal goat populations, which has an important role in the lives of the goat rearers. The objective of thepresent study is to comprehendthe influence of seasonal variation on the testosterone hormone of Black Bengal bucks(*Capra aegagrus hircus*) in two different agro-climatic zones in India. The highest mean value oftemperature (42.6 ± 1.5 ºC) has been reported during the month of April or May in the season of pre-monsoon in Purulia. However, the lowest value of temperature (8.6 ± 0.9 ºC) has been reported during the month ofDecember or January in the season of post-monsoon again in Purulia.Serum testosterone was analyzed in blood samples collected once a week. It has been observed that, from January to April for both of the regions of Purulia and Nadia and the month May has the lowest serum testosterone level in Purulia (1.22 ± 0.18 ng / ml) and the serum testosterone levels stayed approximately the same from January to March in Purulia and more or less similar in Nadia.However, the serum testosterone level reached to its peak level in November in both the region Nadia (8.79 ± 1.3 ng / ml) and Purulia (6.59 ± 0.41ng / ml) respectively. It can also be presume thatthe early periods of the post-monsoon season can be taken as an alternate breeding seasons for Black Bengal breeds.

Keywords: Testosterone; Bucks; Purulia; Nadia; Pre-Monsoon; Post-Monsoon.

Introduction

Seasonality of reproduction mayhave a major role on the production rateof farm animal species such as goat, cattle and sheep. The levels of the different hormones which have major effects on reproductive system canundergo changes very much depending upon the photoperiodic variation[1]. The androgens have a significant effects on the reproductive capabilities in most of the male farm animals.The testosterone hormone in a male is much more effective as well as vital than other androgens found in an animal body. Excepttestosterone other androgen present are androstenedione and 5 alpha-dihydrotestosterone. They altogether induce a constructive effects on physiological development through the nitrogen build-up in cells [2]. Some goat breeds from temperate latitudes exhibita seasonal variation in reproductive activityduring the year[3]. Although, in Indian sub-continent it is known that monsoon is the ideal season for reproduction of most of the animals, especially farm animals[4]. So, in this present study it is an initialeffort to establish aperiod of time except the season of monsoon as an alternate period for reproduction of Black Bengal goats. Different studies have been revealed thatthere are other environmental factors, suchas availability of food and the presence of male and female [3 and 5] mayinfluence the season of reproduction.

The objective of the present study is tocomprehendthe influence of seasonal variation on the testosterone hormone of Black Bengal bucks (*Capra aegagrus hircus)[6]* in two different agro-climatic regions in West Bengal, India. Moreover, this will be a probable contribution to this field of research on Black Bengal goats where inadequate researches have been available and these results can be useful to the

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determine the best possibleseason of reproduction alternate to monsoon.

Materials and Methods

Animals

The animals have beentaken for these studies have been clinically healthy and sexually matureBlack Bengal bucksof 2 – 4 years of age and has an average body weight of 20Kg showing no parasitic infestation. The animals were taken from the local rearers of Jaharhatu (23°21´6´´N, 86°2´36´´E) village Purulia district and from the Mohanpur farm (22°56´ N, 88°31´ E) of Nadia district, both from the state of West Bengal but in two different agro-climatic regions, there are no feed restriction to the goats. Animals were maintained in its ambient condition for four weeks prior to blood sampling [7].

Study Areas

Planning Commission of India,during 2006has demarcated the geographical area of India into 15 agro-climatic regions. The present studies have been carried out into two agro-climatic zones of India. These are as follows:

Purulia, fall under Eastern Plateau and Hills region of India [8]. This agro-climatic zone is Located at the southern tip of Bihar. Thirty per cent of the area is classified as forests and only about a quarter of the area is cultivated. It receives about 1,200 mm of rainfall annually. The climate is moist sub-humid to sub-humid and the soil is red loamy, red and yellow. Average annual rainfall is varies from 1100 to 1500 mm. The humidity is high in monsoon season, from 75% to 85%. But in hot summer it goes down from 35% to 25%. Temperature varies over a wide range from 7°C in winter to 46.8°C in the summer(Table 1). Due to undulated topography just about fifty percent of the total rainfall flows away as run off [8]. The total goat population of Puruliahas been recorded as 813191 [9].

Nadia, fall under Lower Gangetic Plains region of India [10]. About 68% of the land is cultivated. The soil of this sub-zone is deltaic alluvial and the climate is per humid to humid. Annual rainfall ranging between 1,200 mm and 1,700 mm. The zone has a tropical climate with a short spell of winter season. The hot season lasts from mid-March to mid-June, with the day temperature ranging from 38°C to 45°C in different parts of this region. The monsoon arrives by the month of middle June. Winter extends about three months; the average minimum temperature not goes down below 10°C (Table 1). Average rainfall of this area is 1,435.8 mm [10]. The total goat population of Nadia has been recorded as 952143[9].

Climatological Measurement

The three year data on temperature of the study area has been collected from the state meteorological department and the mean of the three years with standard deviation was calculated (Table 1) using MS-Excel 2007 and shown here in a tabular form (Table 1) [11].

Table 1: Mean maximum and minimum temperature of last three years

			March	April	May	June	
Temperature	Purulia	Max (°C)	40.4 ± 2.3	42.6 ± 1.5	42.6 ± 1.5	38.6 ± 5.0	Pre - Monsoon
		Min (ºC)	21.6 ± 10.9	21 ± 0.7	22.4 ± 1.1	23.2 ± 0.8	
	Nadia	Max (ºC)	37 ± 2.45	38 ± 1	39.2 ± 1.5	36 ± 4.7	
		Min (ºC)	16 ± 3.9	19.4 ± 3.3	23.4 ± 1.5	23.6 ± 1.3	
			November	**December**	**January**	**February**	
	Purulia	Max (ºC)	32.0 ± 1.0	30.8 ± 2.2	30.0 ± 2.5	34.6 ± 2.5	Post - Monsoon
		Min (ºC)	13.6 ± 1.1	9.4 ± 1.7	8.6 ± 0.9	11 ± 2.5	
	Nadia	Max (ºC)	31.6 ± 1.2	28.75 ± 0.5	28.6 ± 1.5	32.2 ± 3.6	
		Min (ºC)	14.4 ± 2.8	11.5 ± 1.3	10.2 ± 1.6	12.6 ± 3	

Blood collection and Clinical Analysis

The blood have been collected from apparently healthy goats using purposive sampling technique [12]for the year and categorized into two seasons. The seasons include pre-monsoon and post-monsoon. About 4 ml of blood was collected by jugular venipuncture from each goat between 12 o'clock to 2 pm under the intense sun using disposable needles (SRS™ Sterivan) and vacutainertubes (Vacutech)[13]. Blood samples has been collected once in aweek. Blood samples has been collected always at the same time of the day to avoidcircadian variations[14].The collected blood has been dispensed into vials and labelled accordingly.

The Enzyme-Linked Immunosorbent Assay (ELISA)

The serum was separated by centrifugation at 4000 rpm for 15 min and stored at -20 °C until analysed.

Serum testosterone assayed using ELISA kits (abcam® ab108666). Serum testosterone (ng / ml) has been performed using ELISA technique [15].

Statistical Analysis

The statistical analysis of the data was performed using SPSS 21.01 [16]. A one-way analysis of variance (ANOVA) test has been used to determine the effects of seasons on the Black Bengal bucks serum testosterone parameters studied here [17]. Mean separation and standard error has been calculated using MS-Excel 2007.

Results

During in the month of March in the season of pre-monsoon (Table 2), the testosterone hormone level in the serum of Black Bengal bucks has been found lower in Purulia than the data obtained in Nadia, but the difference found has not been significant. Whereas the testosterone hormone level of the buck serum has been higher in Purulia than Nadia during in the month of April (Figure 1), here also the difference has not been significant. During in the month of May the testosterone hormone level in the serum of Black Bengal bucks in Puruliahave been reduced to 1.22 ± 0.18 ng / ml (Table 2), which is the lowest value of the testosterone hormone level obtained in the serum of Black Bengal bucks in the present study (Figure 1). The testosterone hormone level in the serum of Black Bengal bucks obtained during this time in Purulia has been significantly lower (P<0.01) than the value obtained (4.51 ± 0.63 ng / ml) in Nadia. Similar kind of results have been found during in the month of June, here also the testosterone hormone level in the serum of Black Bengal bucks in Purulia have been found significantly (P<0.01) lower than the testosterone hormone level found in the serum of Black Bengal bucks in Nadia (Table 2).

On the other hand, during in the season of post-monsoon (Table 3) the testosterone hormone level in the serum of Black Bengal bucks in Nadia during in the month of November have been found significantly (P<0.01) higher than that of Purulia. The value obtained in Nadia i.e. 8.79 ± 1.3 ng / ml, has been the highest value of the testosterone hormone in the serum obtained during the present study (Figure 1). The value of the testosterone hormone found in Purulia (6.59 ± 0.41 ng / ml), during in the month of November also the highest value among the months studied here in Purulia (Figure 1). More or less similar kind of result has been obtained during the month of December, where the testosterone hormone level in the serum of Black Bengal bucks in Nadia has been found significantly (P<0.05) higher than the testosterone level found in Purulia (Table 3). However the data collected on the testosterone hormone level in the serum of Black Bengal bucks obtained during the months of January and February are more or less similar in Purulia and Nadia and there are no significant difference have been observed between them (Table 3).

Table 2: The influence of seasonal variation on the testosterone hormone of Black Bengal Bucks (*Capra aegagrus hircus) during* in the season of pre-monsoon

Months	Purulia (ng/ml) Mean ±S.E.M	Nadia (ng/ml) Mean ±S.E.M	Overall (ng/ml) Mean ±S.E.M	P-value
March	2.7 ± 0.78	3.16 ± 0.59	2.44±0.59	0.203 NS
April	2.69 ± 0.52	2.39 ± 0.64	2.10±0.41	0.327 NS
May	1.22 ± 0.18	4.51 ± 0.63	2.35±1.00	0.000000001**
June	2.33 ± 0.48	6.09 ± 1.39	3.42±1.48	0.000004**

**: P < 0.01; NS: not significant

Table 3: The influence of seasonal variation on the testosterone hormone of Black Bengal Bucks (*Capra aegagrus hircus) during* in the season of post-monsoon

Months	Purulia (ng/ml) Mean ±S.E.M	Nadia (ng/ml) Mean ±S.E.M	Overall (ng/ml) Mean ±S.E.M	P-value
November	6.59 ± 0.41	8.79 ± 1.3	7.82±1.60	0.0004**
December	3.03 ± 0.65	3.96 ± 1.09	2.63±0.61	0.047*
January	2.86 ± 0.55	2.74 ± 1.19	2.11±0.73	0.784 NS
February	2.48 ± 0.64	2.34 ± 0.64	2.22±0.52	0.669 NS

*: P < 0.05; **: P < 0.01; NS: not significant

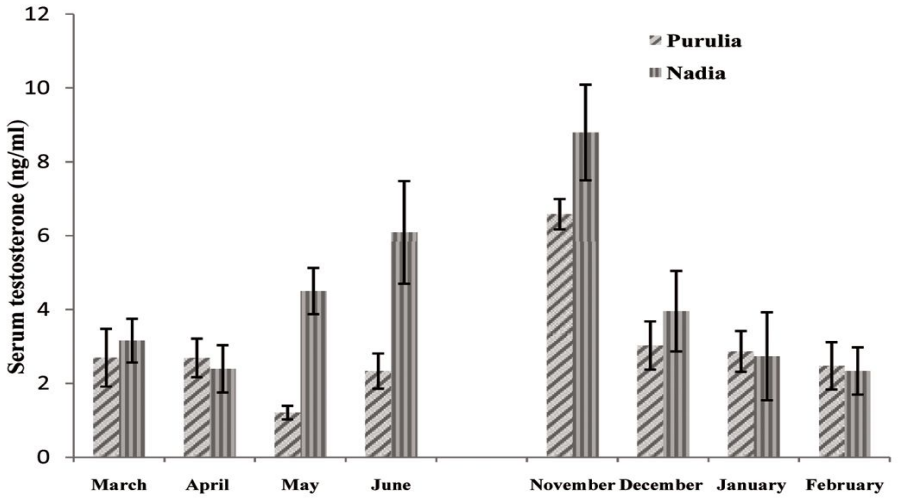

Fig. 1: The diagram itself depicted the influence of seasonal variation on the testosterone hormone of Black Bengal Bucks (*Capra aegagrus hircus) during* in the different months of Pre-monsoon and post-monsoon seasons.

Discussion

The present study demonstrated that the Black Bengal bucks maintained under two different agro-climatic region show noticeable seasonal variation of testosterone secretion. These variations have beenobserved despite of the animals have been feeding with a similar diet. The results indicate that season strongly influences testosterone secretion of these animals. Like sheep, photoperiod or the daylight duration is the principle factor forthe goats' reproduction [18]. The levels of the male or female hormones (hypothalamus to pituitary and subsequently to gonad axis) go through changes depending on the basis of photoperiod [19]. Like other mammals, the production of testosterone hormone in goats has beendirectly under the control of Gonadotropin releasinghormone (GnRH) and luteinizing hormone (LH). However the testosterone hormonelevel in blood generally increases during the breeding period of the bucks. In general all over the worlddoesshow reproductive activities mainly in the late monsoon while the sexual activities stop in pre-monsoon seasons[20].

During this present study the serum testosterone levels stayed approximately the same from January to March in Purulia and more or less similar in Nadia. However, the serum testosterone level reached to its peak level in November in both the region Nadia (8.79 ± 1.3 ng/ml) and Purulia (6.59 ± 0.41ng/ml) respectively. Delgadillo *et al*.[21] has been reported that the testosterone level in Creole bucks in Mexico,has been 0.1 ng/mL in January and February and 10 ng/mL in July and in August. Low level of testosterone hormone in serum of Black Bengal bucks may be due to the peak ambient temperature of the pre-monsoon seasons in the both of the region.Although it is known that, the reproductive functions of buckshave been generally least dependent on the seasons with respect to the does. So, that during in pre-monsoon season in May and June where the testosterone hormone level in the serum of Black Bengal bucks in Nadia has been increasing contrasting to the result observed in Purulia. It has been known that the seasonal productionand secretion models are generally parallel for most of the goatbreeds, changes in the quantity of the hormone productionhappensmostly due to the latitude and longitude and may be dueto other factors like genotype, feeding habit and feeding leveletc [22].

The seasonal influence on plasma testosterone and the traits of testosterone secretion model had been studied on Verata and Malaguena bucks and were showed an increase in plasma testosterone levels during pre-mosoon and late monsoon, during declining photoperiod[23], this result is however gone differ from our present findings.

Conclusions

During the present study it has been observed the lowest testosterone levels were observed from January to April for both of the regions of Purulia and Nadia and the month May has the lowest level of the testosterone hormone level in the serum of Black Bengalbucks in Purulia (1.22 ± 0.18 ng/ml). However, the serum testosterone level reached to its peak level in November in region Nadia (8.79 ± 1.3 ng/ml). In this breed i.e. Black Bengal goat, the higher serum

testosterone levelhas been reached during the decreasing photoperiod and it can be concluded that month of November and December i.e. early the periods of the post-monsoon season can be taken as an alternate breeding seasons for Black Bengal buck, still a lots of experiment has been needed to established this fact.

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Conflict of the Interest Statement

None of the authors has any financial or personal relationshipsthat could inappropriately influence or bias the content of the paper.

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Original Article

Oxidative Stress in Diabetic Patients

Diptikanta Acharya*, Sagarika Satapathy, Gitanjali Mishra****

Abstract

Oxidative stress plays a major role of free radicals generation disproportionately in diabetes mellitus by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, nitric oxide level and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Changes in oxidative stress biomarkers, including catalase, glutathione peroxidase, lipid peroxidation, nitrite concentration and their consequences, are discussed in this research article. Biochemical studies were carried out in 10 diabetes patient whose age range from 45-55 years. For control data, 10 individuals in the same group (45-55 years), socio-economic status and who were not suffering diabetes mellitus as a control groups.

Keywords: Oxidative Stress; MDA; GPx; SOD; Free Radicals.

Introduction

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy [1]. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, Diabetes, Heart Failure, Myocardial Infarction, Alzheimer's disease and chronic fatigue syndrome, but short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis. Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Reactive oxygen species are also used in cell signaling [2,3]. In modern medicine, regular physical exercise is an important tool in the prevention and treatment of diseases including diabetes. Although acute exhaustive exercise increases oxidative stress, exercise training has been shown to up regulate antioxidant protection. This highly reactive and short-living class of molecules is known as reactive oxygen species (ROS). ROS production is a side effect of the normal metabolism of the cell, which developed a series of enzymes able to disarm them. Superoxide dismutase, catalase, and glutathione peroxidase are powerful weapons that act together with antioxidant molecules introduced with diet to protect the organism. In healthy subjects there is a balance between ROS formation and elimination [4-5]. Every time this balance is lost due to an augmented production of reactive species or due to a reduction in antioxidant production or activity there is a condition of oxidative stress.

The foregoing research indicated the effect of free radicals generated in diabetic patients due to oxidative stress which involves the estimation of activity of antioxidant enzymes and the measurement of levels of nitric oxide and lipid peroxidation. The

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antioxidant enzymes includes Catalase and Glutathione peroxidase to be estimated in the blood of Diabetic patients and the level of nitric oxide and lipidperoxidation to be estimated by spectrophotometry analysis [6-7].

Materials and Methods

Biochemical studies were carried out in 10 diabetes patient whose age range from 45-55 years. For control data, 10 individuals in the same group (45-55 years), socio-economic status and who were not suffering diabetes mellitus as a control groups. 4ml of heparinized whole blood samples were collected from Gunupur hospital and clinics at Gunupur.

Nitrate and nitrate concentrations in plasma were determined by using Griess reaction in which NO_2 reacts with 3% sulfanilamide in 0.3% Napthalene-ethylene diamine dihydrochloride, forming chromophore [8-9].

The extent of lipid peroxidation in biological sample is estimated by the thiobarbutiric acid test (TBA test). In this the amount of malondialdehyde (MDA) formed in the samples is taken as the index for the extent of lipid peroxidation. MDA is a highly reactive three-carbon dialdehyde produced from lipid hydroperoxides. It is measured by the TBA test [10,11].

Catalase a 24.5 kDa molecular weight, antioxidant enzyme contains heme group bound to its active site. It catalyzes the conversion of high concentrations of hydrogen peroxides formed by the dismutation of SOD and oxygen. This activity measured spectrophotometrically at 240 nm. Azide or cyanide inhibits catalase [12].

Activity of Glutathione peroxidase was measured by the method of Paglia and Valentine [13]. In this method, reduced glutathione(GSH) is converted to oxidized glutathione (GSSG) by glutathione peroxidase which measured spectrophotometrically at 340nm.

Results

The result showed a significant increase in the level NO and lipid peroxidation and the activities of antioxidants includes GPx and Catalase were decreased significantly in diabetic patient's blood when compared to control groups.

Nitric Oxide Levels in Plasma

Measurement of nitrite in plasma is the indicative of the amount of nitric oxide. The nitrite levels were observed to be increased significantly ($P < 0.001$) in diabetes patient as compared to the values in control shown in Fig. 1 and Table 1.

Lipid Peroxides Levels

The lipid peroxidation products expressed as MDA equivalents observed significantly ($P < 0.001$) as compared to the values in control shown in Fig. 2 and Table 2. High levels of MDA in diabetic patient were indicative of increase oxidative stress.

Catalase Activity

Catalase activity decreased significantly ($P < 0.001$) in Diabetic patients when compare to control shown in Fig. 3 and Table 3.

Glutathione Peroxidase Activity

GPx activity decreased significantly ($P < 0.001$) as compared to the vales in control shown in Fig. 4 and Table 4.

Table 1: Nitric oxide (nitrite) levels in plasma values are expressed as mg of nitrite/ml of plasma

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.725 ± 0.06
P	< 0.001	
Diabetic	10	1.79 ± 0.08

Table 2: Lipidperoxidation (MDA) levels in plasma. Values are nanomoles of MDA/ml of plasma

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.116 ± 0.005
P	< 0.001	
Diabetic	10	1.036 ± 0.052

Table 3: Catalase Activity in Plasma values are expressed in mg/ml

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.264 ± 0.008
P	< 0.001	
Diabetic	10	0.086 ± 0.004

Table 4: GPx Activity in plasma values are u moles NADPH oxidized/min/mg protein

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.214 ± 0.004
P	< 0.001	
Diabetic	10	0.091 ± 0.003

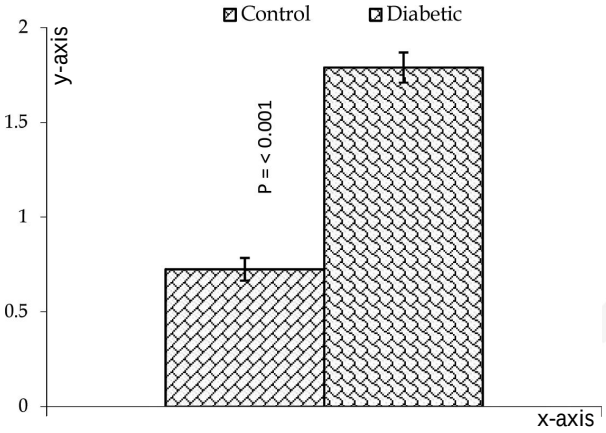

Fig. 1: Nitric oxide (nitrite) levels in plasma values are expressed as mg of nitrite/ml of plasma

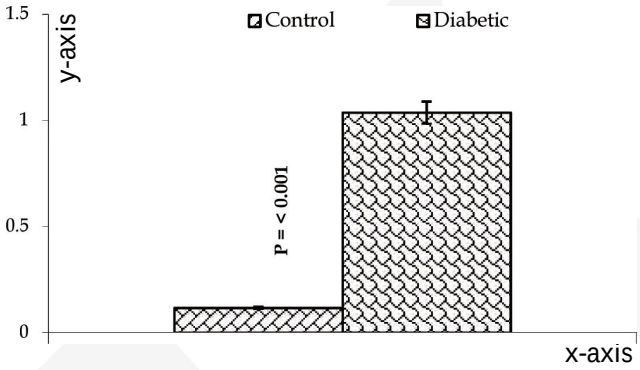

Fig. 2: Lipidperoxidation (MDA) levels in plasma values are nanomoles of MDA/ml of plasma

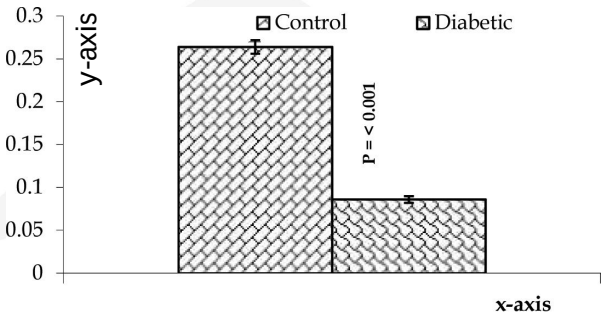

Fig. 3: Catalase Activity in Plasma. Values are expressed in mg/ml

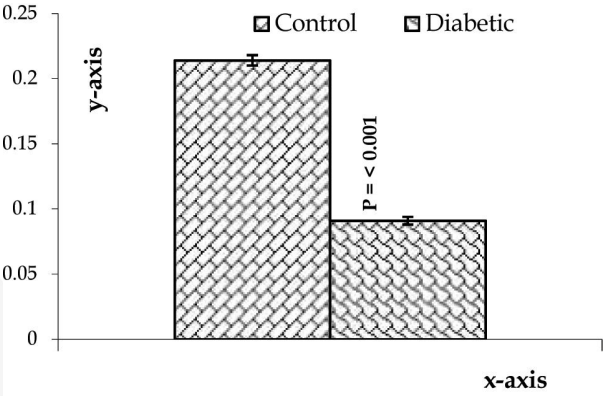

Fig. 4: GPx Activity in plasma values are u moles NADPH oxidized/min/mg protein

Discussion

In this study, we reported the frequency of catalase deficiency which observed among the patients with low activity and the blood catalase activity of randomly selected diabetic patients [14,15]. Increased in lipid peroxidation in diabetes mellitus is due to excess formation of free radicals. Glycosylated protein, auto oxidation, reduced superoxide dismutase enzyme and ascorbic acid and lack of reduced glutathione are other causes for oxidative stress. Here all groups of diabetes mellitus shows statistically significant increase in serum lipid Peroxide levels. In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipid peroxides and nitric oxide in plasma may be due to the abnormal lipid metabolism and by oxidation of amino acids. Maximum increase in lipid peroxide was found in group of diabetes mellitus with complication [8,16,17]. Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins [18]. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibits the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid per oxidation and tissue damage in diabetes [19]. The glycated protein might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid per oxidation in diabetes mellitus [12,20]. A deficiency of the antioxidant activity of superoxide dismutase and glutathione peroxidase has been related to higher concentration of peroxide. There may be imbalance between

production and scavenging of free radical produced due to the lack of antioxidant system [21,22]. Peroxidation of apolipoproteins may affect the lipoprotein metabolism. It is suggested that apo- A has an antioxidant effect, but due to the peroxidation the antioxidant property of apo-A is lost. Higher levels of lipid peroxides were observed in diabetic subject with vascular complication. This increase in lipid peroxide may be due to the increased activity of the free radical formation [23]. It has been suggested that the increase in triglyceride may be due to insulin deficiency which results faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by tissue resulting in hyperglycemia [24]. High level of cholesterol, triglyceride, LDL-cholesterol and low HDL-cholesterol may be due to the obesity, increase calorie intake and lack of muscular exercise in the patients of diabetes mellitus [25].

Conclusion

In diabetic patients, the persistence of hyperglycemia has been reported as a cause of increased production of oxygen-free radicals through glucose autoxidation and nonenzymatic glycation. The antioxidant capacity is always decreased in diabetic patients, but it seems necessary to measure all the components to ascertain the reasons. The activities of Catalase and GPx were significantly low in diabetic patients where as the level of nitric oxide and lipid peroxidation were significantly increases. The enzyme activities in diabetic patients are lower than that of control, but the differences are more significant. It seems that the reduction in levels of other antioxidant enzymes and substances are involved in the decreased antioxidant capacity in diabetic patients. In view of low activities of catalase and GPx in patient's supplementary trace elements such as Selenium, Copper, Zinc and Manganese, the essential components of the enzymes structures may be useful in prevention of oxidative stress.

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Original Article

Assessment of the Effect of Silver Nanoparticles on Haematological Profile and Total Protein Content in a Freshwater Fish, *Channa Punctatus* (Bloch)

Shraddha Basu*, Niladri Sekhar Bhunia*, Swarup Roy, Bibhas Guha***

Abstract

In the present experiment effects of silver nanoparticles (AgNPs) on various heamatological parameters and total protein content of some selected tissues (*i.e.,* muscle, liver, kidney and gill) of an Indian fresh water fish, *Channa punctatus* was evaluated. Two different concentrations of biologically synthesized AgNPs (100% and 50%) were intra-muscularly injected to the experimental fish is respect of distilled water treated control at different fixation intervals (*viz*., 6 hr, 24 hr, 72 hr, 96 hr, 7 day and 15 day). Treatment of AgNPs (100% and 50%) to the experimental fishes showed a significant decrease in number of the red blood cells and increase in number of white blood cells particularly at the longer fixation intervals (7 and 15 days) in respect of distilled water treated control. Total protein content of liver and kidney showed significant increase during 15 and 30 days of exposure compared to the control. In the contrary, total protein content of gill and muscle showed a significant decrease after exposure to AgNPs in respect of the control.

Keywords: Nanoparticles; RBC; WBC; Protein; Fish.

Introduction

As a result of the wide application of nanomaterials in industry, agriculture, business, medicine and public health; nanothechnology has gained a great deal of public interest (Ju-Nam and Lead, 2008). Uses of nanomaterials are likely to result in releases into aquatic systems and may pose a risk to aquatic ecosystems (Moore, 2006). Nanoparticles are part of our daily life in form of cosmetics (Perugini et al., 2002), drug delivery system (Jin and Ye, 2007), therapeutics (Czupryna and Tsourkas, 2006) and biosensors (Prow et al., 2006). Recently, significant concerns have been expressed about the potential risk of silver nanoparticles (AgNPs), due to the current and projected high exposure (Luoma, 2008) and their likely high hazard and toxicity in the environment (Klaine et al., 2008). Indeed, a number of ecotoxicology studies have been conducted to study the effect of AgNPs in algae, bacteria, invertebrates, fish and humans in both *in vivo* and *in vitro* studies (Obserdorster, 2004; Carlson et al., 2008; Gopinath et al., 2008; Govindasamy and Abdul Rahuman, 2011). In this context in-depth studies are indeed needed to evaluate the potential risks of AgNPs in the aquatic systems as well as its inhabitants like fish. The AgNPs synthesized biologically have been widely used in the field of medicinal industries (Asz et al., 2006) but its effects on fish had been hardly evaluated. In the present study an attempt has been made to evaluate the effect of biologically synthesized AgNPs (synthesis of AgNPs has been carried out by using the extracellular filtrate of the fungal strain, *Aspergillus foetidus* MTCC8876) on the haematological parameters and total protein content of some selected tissues of an important fresh water fish *Channa punctatus*. As the fishes are directly exposed to the aquatic environment, thus the assessment of its potential risks on the haematological parameter and the total protein content of selected tissues is of immense importance in scientific research.

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Materials and Methods

Test Fish

Live specimens of *Channa punctatus* belonging to the family Channidae, weighing between 15 and 18

gm and measuring from 11 to 14 cm in length, were procured from the local fish market and properly washed in tap water and treated with 0.02% $KMnO_4$ and 0.004% formalin solution to remove external infection of fungi, algae, etc. The fishes were acclimatized in a glass aquaria (20 L capacity) containing fresh tap water (stored from deep tube well) for seven consecutive days. During acclimatization the fish were fed with TOKYO (made in Japan) twice in a day. The experiment was conducted in 15 L glass aquaria each containing 10 L tap water (pH 8.57 ±0.14, temperature 28-30^{0}C) and five number of fish were used for both the control and experimental series.

Test Chemical

Biosynthesised silver nanoparticles (AgNPs) by fungi *Aspergillus foetidus* in crude condition (100%) was collected from the Biochemistry and Biophysics Laboratory of University of Kalyani, Kalyani, West Bengal, India (Roy and Das, 2014). 50% AgNPs solution was prepared through addition of required amounts of distilled water. Both these concentrations of AgNPs were injected to the test fishes intra-muscularly for experimentation. Distilled water treated fishes were served as control.

Experimental Design

The fishes were divided into three equal groups consisting of 10 fishes in each group and injected with test chemicals @ 1ml / 100 gm body weight- (i) group-I: distilled water treated control; (ii) group-II: 50% AgNPs; and (iii) group-III: injected with 100% AgNPs at six different fixation intervals (*viz.*, 6 hr, 24 hr, 72 hr, 96 hr, 7 days and 15days). During experiment, the waste products were removed by using good quality of aquaria water filter for keeping a good environmental condition within the aquaria.

Study of Haematological Profile and Total Protein Content

The blood from the caudal vein of control and treated fish was collected for heamatological investigation. RBC and WBC were examined following the procedures of Wintrobe (1957) and Sood (1996). For the study of total protein content in four selected tissues (*viz.,* liver, kidney, gill, muscle), the fishes were removed after 6 hr, 24 hr, 48 hr, 72 hr, 7 days and 15 days from each of the treated and control specimens separately and homogenized in 0.1% NaCl solution. After centrifugation at 4500g for 15 minutes, the supernatants were collected. Aliquotes containing known amount of protein for each tissue (5 to 10 micro gram) was used for estimating the quantity of protein as per the method of Bradford (Bradford, 1976). A standard curve was constructed from different known concentration of Bovine Serum Albumin (BSA) against their OD values. The amount of unknown protein (µg / gm) was calculated in the routine manner against the standard curve.

Statistical Analysis

The experiments were conducted in triplicates. Data of haematological and total protein contents have been presented as mean ± standard error (S.E.). Values of RBC and WBC of fish blood and total protein of tissues were compared statistically with control by using student's t test (2- tailed) with the help of SPSS 17. The level of significance was established at $p<0.05$.

Results

Total RBC Count

The erythrocyte count of healthy controls showed a mean value of 2.86×10^6 mm^{-3}. The fishes that were treated with AgNPs showed an alteration in mean value of RBC for both the treatment series. For 50% treated series the lowest RBC count was found at 7 days of fixation interval and the highest was at 6 hr, but the significant decrease of RBC count was found for 72 hr, 7 day and 15 day of fixation intervals ($p<0.05$) (Table-1). For 100% AgNPs treated series highest RBC count was found at 24 hr and the lowest at 96 hr interval, of which the data at 48 hr, 96 hr, 7 day and 15 day found significant ($p<0.05$) (Table-1).

Total WBC Count

The results of the total count of WBC revealed that the blood of the control fish showed a mean value of 57.84×10^3 mm^{-3}. The fishes treated with AgNPs reflect an alteration in mean value of WBC for both the treated series. For 50% treated series the lowest WBC count was found at 72 hr fixation interval and the highest was at 15 days, but the significance differences were found for 15 day of fixation interval ($p<0.05$) (Table-2). For 100% AgNPs treated series the result was significant for 7 day and 15 day of fixation intervals ($p<0.05$) (Table-2).

Total Protein Content of Selected Tissues

Total protein content of four selected tissues (*viz.*, liver, kidney, gill and muscle) of *C. punctatus* was

recorded against control for both the treatment series at different fixation intervals (Table 3 to 6). Highest value of total protein content in liver was 18.73 µg/mg for 50% and 26.88 µg/mg for 100% of AgNPs, of which the value of 48 hr and 96 hr (for 50%) and 24 hr and 15 days (for 100%) showed significant differences in respect of the controls ($p<0.05$) (Table 3). Highest value of total protein content in kidney was 12.46 µg/mg for 50% and 19.85µg/mg for 100% of AgNPs, of which the values at 6 hr (for 50%) and 24 hr and 15 days (for 100%) showed significant differences ($p<0.05$) (Table 4). The lowest value of total protein content in gill was 2.86 µg/mg for 50% and 1.76 µg/mg for 100% AgNPs, of which the result at 48 hr (for 50%) and 24 hr and 7 day (for 100%) showed significant differences ($p<0.05$) (Table 5). The lowest value of total protein content in muscle was 6.38 µg/mg for 50% and 5.43 µg/mg for 100% AgNPs, of which the result at 48 hr and 72 hr (for 50%) and 6 hr, 48 hr, 72 hr, 96 hr, 96 hr, 7days (for 100%) showed significant differences in respect of control ($p<0.05$) (Table 6).

Table 1: Effect of AgNPs on total RBC ($\times 10^6$ mm^{-3}) of *C. punctatus.* Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				2.86±0.06			
50% AgNPs	2.73± 0.002	2.06±0.001	2.26±0.001	1.49±0.005*	2.22±0.007	1.41±0.011*	1.85±0.004*
100% AgNPs	2.36±0.003	3.43±0.005	1.82±0.011*	2.63±0.009	1.14±0.008*	1.72±0.059*	1.88±0.007*

Table 2: Effect of AgNPs on t otal WBC ($\times 10^3$ mm^{-3}) of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				57.84±0.17			
50% AgNPs	58.73± 0.018	55.06±0.014	59.26±0.012	51.49±0.052	57.22±0.017	57.41±0.011	61.85±0.014*
100% AgNPs	52.86±0.032	53.43±0.053	59.82±0.11	52.63±0.019	56 .14±0.008	62.72±0.059*	64.88±0.047*

Table 3: Effect of AgNPs on the total protein content (µg/mg) of liver of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				13.93± 0.007			
50% AgNPs	18.73± 0.018	15.06±0.014	9.26± 0.012*	11.49±0.052	7.22±0.017*	17.41±0.011	15.85±0.014
100% AgNPs	12.86±0.032	33.43±0.053*	19.82±0.11	12.63±0.019	11.14±0.008	12.72±0.059	26.88±0.047*

Table 4: Effect of AgNPs on the total protein content (µg/mg) of kidney of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				6.31±0.028			
50% AgNPs	12.46±0.017*	2.69±0.009	2.53±0.009	2.3±0.006	3.13±0.002	5.78±0.012	2.78±0.004
100% AgNPs	4.04±0.008	19.85±0.001*	10.37±0.164	3.06±0.01	9.93±0.026	.72±0.008	19.85±0.001*

Table 5: Effect of AgNPs on the total protein content (µg/mg) of gill of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				7.19±0.004			
50% AgNPs	9.49±0.042	3.6±0.021	2.86±0.002*	3.29±0.011	8.96±0.027	8.22±0.026	10.75±0.034
100% AgNPs	7.75±0.008	14.04±0.013*	7.43±0.079	6.22±0.036	6.29±0.025	1.76±0.012*	7.77±0.003

Table 6: Effect of AgNPs on the total protein content (µg/mg) of muscle of *C. punctatus*. Data presented as mean ± SE, n= 5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				15.81±0.015			
50% AgNPs	15.57±0.015	10.17±0.003	6.38±0.003*	7.08±0.016*	9.47±0.011	20.8±0.026	14.53±0.044
100% AgNPs	5.43±0.011*	17.85±0.006	7.61±0.151*	4.25±0.043*	6.92±0.021*	7.10±0.027*	17.9±0.041

Discussion

The fate of nanoparticles in the aquatic environment, their interactions with biotic and abiotic components, and their potential to cause harm are still poorly understood, and these uncertainties are driving concerns on the risks they may pose to human and environmental health (Tessa et al., 2010). In the present experiment it was found that the total count of RBC in the experimental fishes for both the treatment series was lower than the control for all the fixation intervals (Table 1). Previously, Adakole (2012) showed that when *C. gariepinus* was exposed to metal finishing company effluents, RBC was initially increased and finally decreased after chronic exposure. Chandanshive et al. (2012) reported that decrease in RBC of fish *Labeo rohita* after exposure to mixture of heavy metals. In the present study, suppression of total RBC count as a result of AgNPs treatment may be due to destructive action of AgNPs on erythrocytes and the viability of the cells may be affected corroborated with the previous findings of Karuppasamy (2000). It was reported that multiple form of hemoglobin allows fish to adjust more efficiently to physiological stress such as varying water temperature and oxygen concentration (Hochachka and Somero, 1973). On the other hand, hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das et al., 1987). Thus, the present results of reduction in total RBC count in *C. punctatus* treated with AgNPs may be due to the deposition of nanoparticles within the cells.

Total count of WBC was found to be increased in the longer intervals (7 days and 15 days) for both the concentration of AgNPs in respect of control (table-2). Previously, progressive increased levels of total WBC count have been reported in *C. punctatus* exposed to lead (Hymavathi and Rao, 2000), *Clarias batrachus* exposed to mercuric chloride (Joshi et al., 2002) and *Clarias gariepinus* to metal finishing company effluents (Adakole, 2012). The increase in total WBC count in the present study was as a result of direct stimulation for its defense from diseases due to the presence of nanoparticles (Singh et al., 2008), or may be attributed to alteration in blood parameters and direct effects of AgNPs (Sinha et al., 2000).

Alteration of total protein content as a result of AgNPs injection to the experimental fish was found for all the tissues examined (*i.e.*, liver, kidney, gill and muscle) (Tables 3 to 6). The exact reason of this fluctuation of total protein content at different fixation intervals is not clearly understood. However, it can be said that the increase of total protein content may be due to increased protein synthesis in the particular tissue as an effect of AgNPs. The decreased levels of total protein in fish exposed to AgNPs suggest that the protein might be used as an alternative source of energy, due to high energy demand that induced by nanosiver intoxication (Hori et al., 2006).

Thus, the present results confirmed that stress due to AgNPs does create hematological disturbances, total protein alteration, affecting the immune system and making the fish vulnerable to diseases.

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Original Article

Dynamics of Diurnal Net Radiation Over Wheat (*Triticum Aestivum* L.) Intercropped with *Eucaluptus Tereticornis* Planted in Nelder Wheel Design

Ravi Kiran

Abstract

Plantation of trees in Nelder wheel design provides varied microclimatic conditions especially in terms of solar radiation penetration for understory crop in agroforestry system. The present investigation attempts to study the diurnal radiation over wheat as an understory crop below 8 year old *Eucaluptus tereticornis* planted in a Nelder wheel design planted in fifteen spokes of trees numbered serially starting from north direction as 0^0 . Wheat cv. PBW-226 was sown on 21st November, 1996 as an under story crop. Studies have been made on the diurnal variation of net radiation attributable to orientation of tree rows of the trees at four important phonological stage of the crop i.e. tillering, flag leaf emergence, flowering and maturing stage of the crop and compared with the control (sole crop) viz T1 (312^O-72^O), T2 (72^O-192^O) and T3 (192^O-312^O) in the Nelder wheel. Regression equations between net radiation in control and different treatments below trees were also developed.

Keywords: Diurnal Variation of Net Radiation; *Eucaluptus Tereticornis*; Wheat (*Triticum Aestivum* L.); Shallow Water Table.

Introduction

Tree crop integration has many effects on the components of the system. Trees act as wind-break and increase water use efficiency by retarding the water loss by evapotranspiration besides modifying radiation climate, crop energy balance temperature, photosynthesis and its rate and duration and plant growth (Messing and Noureddine 1991). Heat load reduction during reproductive phase can increase the production of wheat (Johnson *et al*., 1981). Excessive solar energy in sole cropped wheat fields at post anthesis period reduce wheat yield (Chinnoy, 1947; Wardlaw *et al*., 1989). Introduction of treed in the monoculture can be used to optimize the microclimate in field conditions for winter wheat in North India. Studies are scarse so far on tree-crop interactions of Wheat (*Triticum aestivum* L.) and *Eucaluptus tereticornis* planted in Nelder wheel design under shallow water table conditions. The present investigation attempts to quantify the diurnal variation of modified microclimate of wheat as an understory crop below *Eucaluptus tereticornis* planted in a Nelder wheel design.

Materials and Methods

The present field investigation was carried out at the Horticultural Research Center, Patharchatta, located in the campus of GB Pant University of Agriculture and Technology, Pantnagar, India (29° N, 79° 30 E, 243.83 m above mean sea level). Trees of *Eucaluptus tereticornis* planted in a Nelder wheel design in March 1989 (Nelder, J.A. 1962). It consisted of fifteen tree spokes of wheel each oriented at an angle of 24° from the adjacent tree spoke, arranged in ten concentric rings of trees of radii 2.0, 5.4, 13.5, 17.8, 21.5, 24.6, 27.4, 29.9, 32.2 and 34.4 m, respectively, enclosing a total number of 15 plots between the tree rows, numbered serially from 1 to 15 anticlockwise direction starting from a tree row oriented to 0° in north direction. each plot was divided into three sub plots of area 26.62 m^2 for the propose of the investigation. In each spoke the first, second and the tenth trees were considered as buffer trees to avoid border effect. Therefore, a constant tree stand of 333 trees per hectare was provided in the area between the third and the ninth trees of each spoke resulting

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each tree in the experimental area occupying an average area of 30 m^2. The climate of the region is humid subtropical, characterized by dry hot summers and cold winters having dry season from early October to mid June and a wet season from mid June to early October. Average annual total rainfall is 1434.4 mm (90% in mid June to end of September). The diurnal maximum air temperatures are highest in May–June and the diurnal minimum air temperatures are lowest in January (range 41.0- 3.0 °C) while relative humidity is highest in July–August and lowest in December–January; (range 80-35%) in April–May. The experimental area lies as a belt below and a few kilometers south of the foothills of the Himalayan mountains having gently slopped of less than 1% the soils are classified as Mollisols (Deshpande et al., 1971) along with characteristic fluctuating shallow water table ranging from surface to 1.8 m or so below the soil surface. The weather conditions during the experiment is depicted in Fig 1.

Field was harrowed four times with disc harrow, properly levelled for better germination and growth, before the sowing of experimental crop. Nitrogen, phosphorus and potassium were applied at the rate of 120kg, 80kg, and 60kg per hectare. Half of nitorgen and full doses of phosphorus and potassium were broadcasted during land preparation and mixed thoroughly by cross harrowing. The remaining half dose of nitrogen was top dressed at 25 DAS i.e. just after first irrigation. The wheat variety PBW-226 was sown below the tree canopies with the help of seed drill on 21st. November, 1996. The rate of seed was 100 kg / ha. The seed was sown in rows 23cm apart and at the depth of 5cm. The area not covered by seed drill was sown manually by hand hoe.Two hand weeding were given at 45 and 80 days after sowing. Only one irrigation was given at 20-25 days after sowing at crown root initiation (C.R.I.) Stage.

Trees of *Eucalyptus tereticornis* pruned appropriately to provide sufficient solar flecks penetration for growing of wheat as an understory crop. Radiation climate in the three microclimatic zones viz T1 (312^O-72^O), T2 (72^O-192^O) and T3 (192^O-312^O) in the Nelder wheel attributed to the trees row orientation were studied at four important stages of the wheat crop along with control.

Net radiation was measured with the help of portable Net Radiometer for each treatment at three points in the middle of the area for each subplot of each treatment along with control simultaneously at tillering, flag leaf emergence, flowering stage and maturing stage at half hour interval starting from morning to evening. Regression equations between net radiation in control and different treatments below trees were also developed.

Result and Discussion

Average increase in tree height, tree D.B.H. and tree horizontal area were 34 cm, 0.3 cm and 8.07 m^2, respectively.

Total diurnal net radiation in morning, noon and evening at tillering, flag leaf emergence, flowering stage and maturing stages of the wheat crop in various treatments is depicted in Fig 2-4. Diurnal net radiation availability among different treatments and all four stages of crop on half hourly basis under the agroforestry conditions was found highly dynamic. Net radiation was available more in South direction treatments than that of north direction treatments in Nelder design under tree canopies.

With the advancement of the stage of crop total diurnal net radiation in all the treatments showed increasing trend. During morning the net radiation was lowest at flag leaf emergencein T1 and highest at maturity stage in T3. During noon the net radiation was lowest at tillering in T3 and highest at same stage in T2. During evening the net radiation was lowest at flag leaf emergence in T1 and highest at flowering stage in T2 . Total diurnal net radiation at tillering flag leaf emergence, flowering stage and maturing stages ranged 31-55%,41-50%,49-63% and 46-61% of control, respectively. Net radiation availability was found more in T3 than that of other treatments under canopies. In Nelder design the radiation environment over inter crop is highly dynamic on time scale which can also be seen on short term (second) basis. The changes in light intensity has profound effect on photosynthetic responses of crop growing beneath trees (Knapp and Smith, 1990).

In general T2 and T3 had better net radiation availability at all stages of crop growth than T1. The foliated green tree canopy reduced radiation intensity along with the quality of light in PAR range (380–700 nm) (Zavitkovski 1982).

Relationship between net radiation in control and different treatments below trees in morning, noon and evening were developed separately for different stages of the crop. It was observed that there was a significant relationship between the net radiation in control and in understory crop in morning, noon and evening at four important stages which are presented in Fig 5.

In tarai region of Uttar Pradesh shallow water table can provide sufficient subirrigation to wheat crop intercropped with Eucalyptus tereticornis. Upward soil water ûux can contribute 36–73% of the total

water requirement of wheat in tarai region of foothills, under shallow water table conditions. (Saini and Ghildyal 1978). Water table depth in the study area ranged 68-120 cm during rabi season, 1996-97 under tree canopies. The depletion of water table occurred in reproductive phase of crop because of more water requirement of the wheat crop but have no significant effect on the plant water potential (Powell 1980).

Tree row orientation and distance affected considerably the different phase growth of the crop, but, reduced heat load from earhead developement to maturity and its more duration in an agroforestry system may possibly mitigate the effect of shading below trees as excessive solar radiation to sole

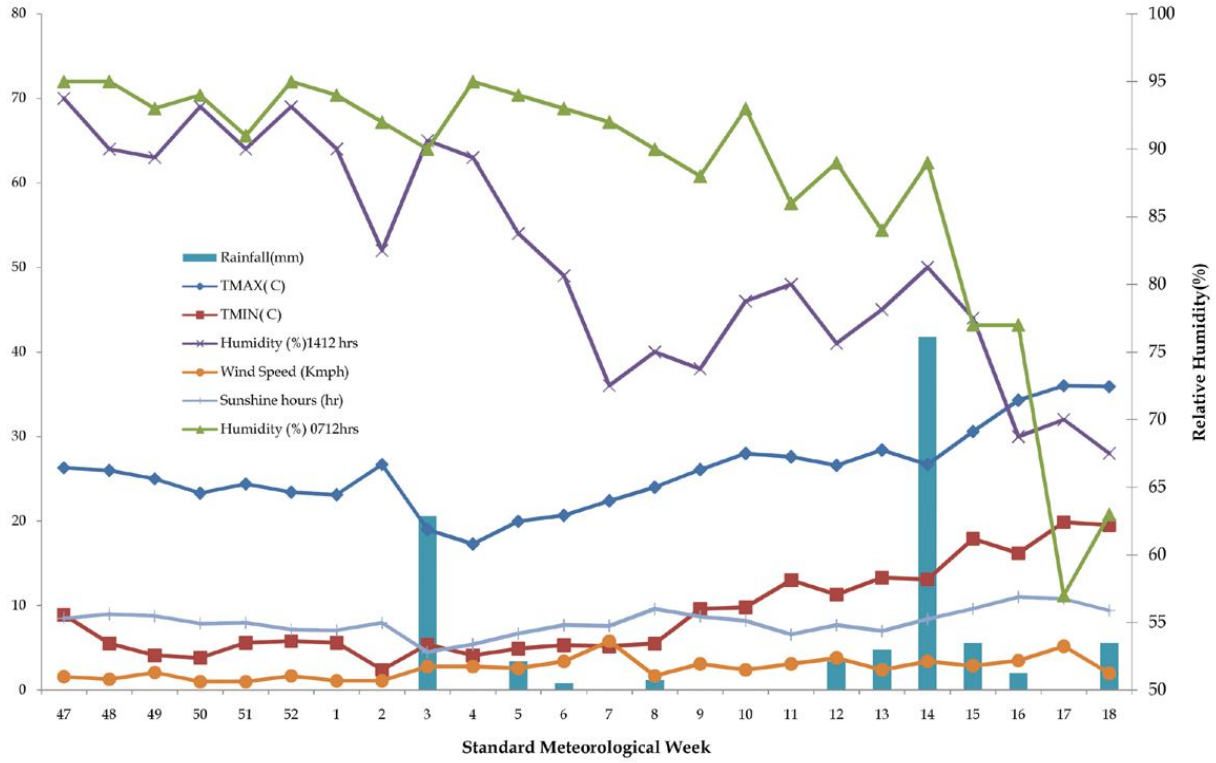

Fig. 1: Weekly average weather data for experimental period (November1996 - April1997)

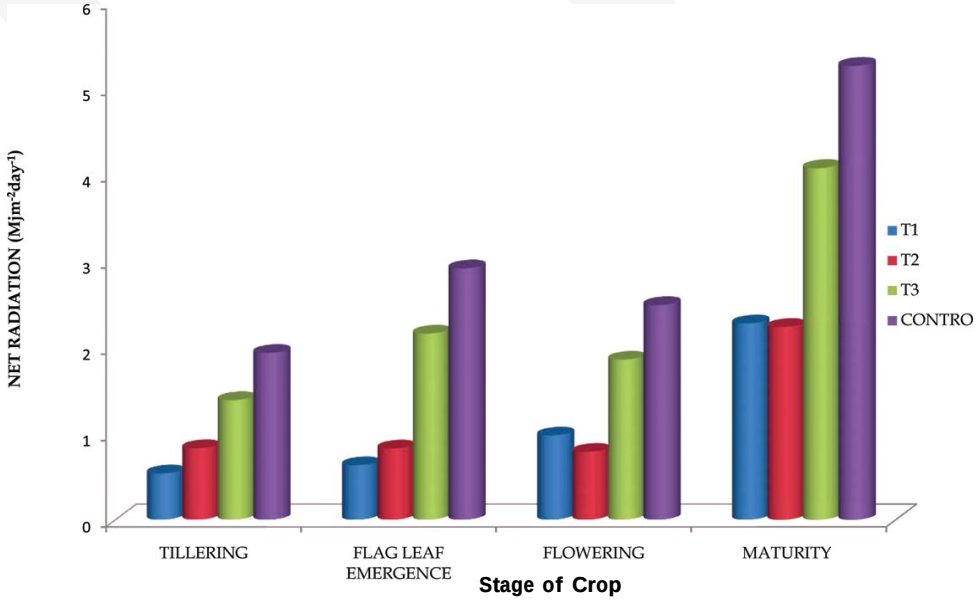

Fig. 2: Variation in net radiation in wheat intercropped with eucalyptus during morning hours

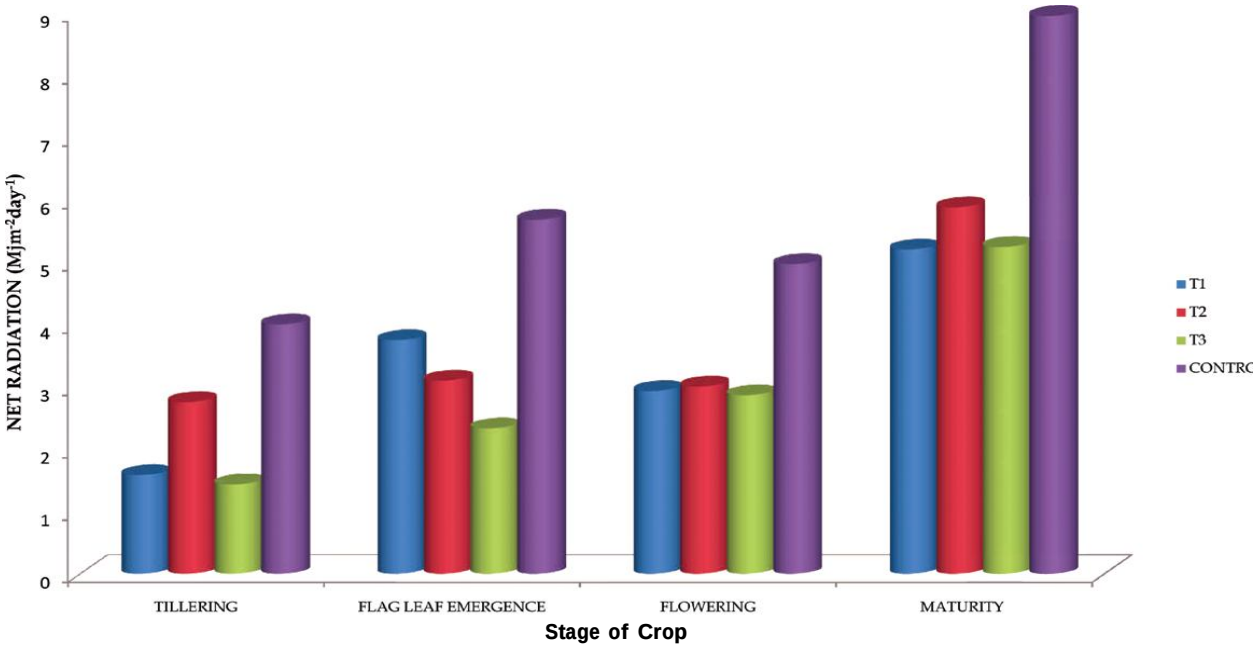

Fig. 3: Variation in net radiation in wheat intercropped with eucalyptus during noon hours

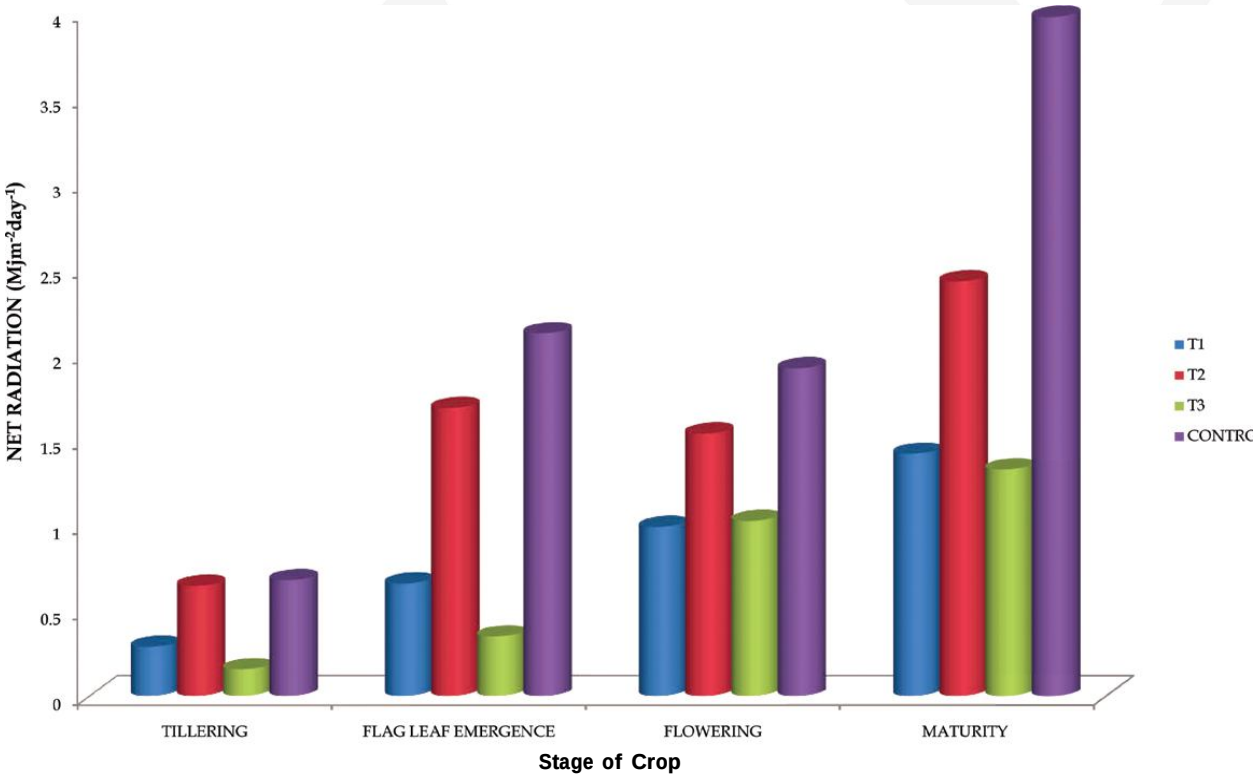

Fig. 4: Variation in net radiation in wheat intercropped with eucalyptus during evening hours

cropped wheat field at post anthesis period reduces wheat yield (Chinnoy J., 1947).

Agroforestry has vide scope to optimize the microclimatic conditions subjected to the proper tree spacing and their pruning for wheat in an agroforestry system.

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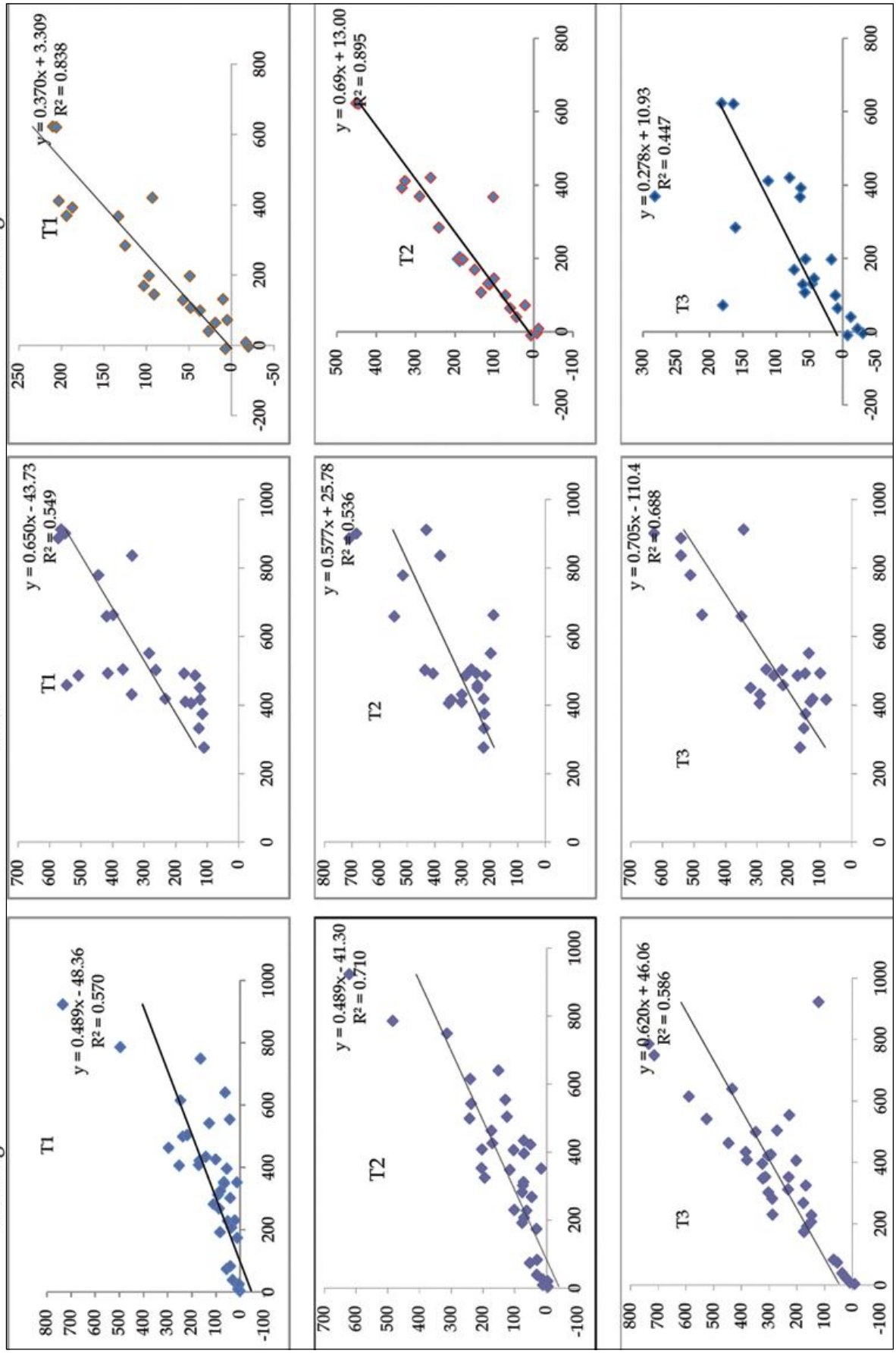

Fig. 5: Relationship between Net Radiation in different treatments and control during morning, noon and evening during crop growth period

during the course of investigation by All India Coordinated Research Project on Agroforestry (ICAR Project).

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Original Article

Studies on Antifertility Activity of Bark Aqueous Extracts of *Aegle marmelos* (L.) Corr. in male *Bandicota bengalensis* (Gray, 1835): In search for a potential Rodenticide

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Abstract

Rodents are considered as one of the major pests of agricultural crops and stored food grains. Rodents are highly adaptable and it is difficult to check their population effectively. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. There are several medicinal plants associated with reducing the male fertility potential in indigenous Indian medicine system. *Aegle marmelos* (L) Corr. commonly known as Bael, in Bengali, has various medicinal properties in the Ayurvedic system of medicine. Various parts of this plant (mainly the leaves, fruits, stem and roots) have been used in ethnomedicine for several medicinal properties. The present experiment is aimed at investigating the potential of bark extracts of *Aegle marmelos* (L) Corr. as an anti-fertility agent on male *Bandicota bengalensis* (Gray, 1835). Three various concentrations of aqueous extracts of barks were used for each group of male *Bandicota bengalensis* (Gray, 1835). The dose of 200mg / 100ml, 400mg / 100ml and 600mg / 100ml barks aqueous was administered orally for 60 days through food to male *Bandicota.* It was found that the extracts had a considerable effect on reducing male fertility and there is positive correlation between dose increase and anti-fertility. Significant decrease in the weight of testis, decrease in number of offspring were observed. Histo-pathological studies of testis revealed elongated spermatids, degeneration of Sertoli cells. Prominent spaces were detected within the germinal epithelium signifying testicular cytotoxicity and necrosis. Many tubules showed lumen with reduced spermatozoa and Leydig cell. Our studies suggest that *Aegle marmelos* (L) Corr. bark aqueous extract may be used as potential rodenticide for controlling *Bandicota bengalensis* (Gray, 1835) population.

Keywords: *Aegle Marmelos;* Anti-Fertility; *Bandicota Bengalensis;* Population Control; Rodenticide.

Introduction

Rodents are considered as one of the major pests of agricultural crops and stored food grains. Rodents are highly adaptable, prolific breeders and can cope up with new environments, new foods and adjust to new associates with a striking swiftness, and hence, it is difficult to check down their population for longer periods either by any cultural methods or natural enemies or synthetic chemical agents (Prakash and Mathur, 1987). The lesser bandicoot rat, *Bandicota bengalensis* (Gray, 1835) is a predominant rodent pest species in India (Jain and Tripathi, 1988). It has turned commensal and inhabits godowns and other premises in metropolitan cities (Chakraborty 1992). The effects of rodent's damage cause huge amount of crop losses and food shortages (Fayenuwo et al., 2007) in some parts of the world.

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Use of chemical rodenticides, mainly anticoagulants is the most common and dominant approach of rodent control in agriculture, rural and urban environment (Prasad, 1999). But application of these rodenticides is limited due to the reported intoxication of domesticated as well as wild animals through direct consumption of baits (primary hazard) and / or secondary hazard resulting from eating of poisoned rodents by non-target predators and scavengers (Valchev *et al*., 2008).

Natural products are an excellent alternative to synthetic pesticides (Isman and Machial, 2006) as a means to reduce negative impacts to human health and the environment. Plants are rich sources of ecologically developed secondary metabolites, which are potent remedies for different ailments (Ganesh *et al,* 2011). The plant *Aegle marmelos* (L.) Corr. is indigenous to India, and belongs to the family Rutaceae. It is known as Bael' in Hindi and Bengali; and 'Bilwa' or 'Sriphal' in Sanskrit. The Bael tree was originated in India and is presently growing in most of the countries of Southeast Asia. In India, it grows wild, especially in dry forest, outer Himalayas, Shivaliks, South Indian plateau with altitudes ranging from 250-1200 m and also cultivated throughout Indian sub continent for its fruits. It prefers dry and sunny or warm parts of the hill slopes with well-drained loamy soil. The bael tree has great mythological significance and abounds in the vicinity of temples. The leaves of the tree are traditionally used as sacred offering to Lord Shiva, the God of health. It is one of the most useful medicinal plants of India. Its medicinal properties have been described in the ancient medical treatise in Charaka Samhita.

Various parts of this plant (mainly the leaves, fruits, stem and roots) have been used in ethnomedicine for several medicinal properties: they are said to be astringent, anti-diarrhoeal, anti-dysenteric, demulcent, anti-pyretic, anti-scourbutic, haemostatic, aphrodisiac and an antidote to snake venom (Kirtikar & Basu 1993). *Aegle marmelos* (L) Corr. has been widely used in indigenous systems of Indian medicine due to its various medicinal properties (Sahare *et al* 2008; Dhankhar and Ruhil, 2011). Extensive investigations have been carried out on different parts of *Aegle marmelos* (L) Corr. and as a consequences, varied classes of compound viz, alkaloid (halfordin, ethylcinnamamide, marmeline), phynylpropenoids (hydroxylcoumarins, phenylpropenes, lignans), terpenoids (limonene, α-phellandrene) are found (Sharma *et al* , 2007).

There are several medicinal plants associated with male antifertility potential (Priya *et al* 2012). From Ayurvedic medicine, it has been claimed that the leaves of *Aegle marmelos* possess contraceptive efficacy (Bhattacharya 1982), and they are used for contraceptive purposes by men from different tribal areas of India. The ethanolic extract of Aegle marmelos leaf possesses antispermatogenic activity (Sur et al,. 1999) and aqueous extract of the leaf has anti-motility action on spermatozoa in rats (Sur et al., 2002). Sperm motility describes the ability of sperm to move properly towards an egg. This can also be thought of as the 'quality' of the sperm, which is a factor in successful pregnancies, as opposed to the 'quantity'. Sperm which do not properly 'swim' will not reach the egg in order to fertilize it. Sperm motility is an important factor in semen quality. Insufficient sperm motility is a common cause of sub fertility or infertility (Chauhan *et al*, 2007; 2009). Research on the contraceptive effects of *A. marmelos* (L) Corr. and its mode of action is scant (Remya *et al*, 2009). The present investigation has been carried out to determine the anti-fertility activity of *A. marmelos* (L) Corr. bark extract on male *Bandicota bengalensis* (Gray, 1835).

Materials and Methods

Bark of *Aegle marmelos* (L) Corr. were collected from local area. The bark was dried and pulverized in an electric grinder and bark powder was made and kept in refrigerator. The powder was dissolved in distilled water the following dose were prepared *viz.* 200mg / 100 ml (Dose I), 400mg / 100 ml (Dose II), and 600 mg / 100 ml (Dose III) and kept in separate container. 60 male *Bandicota bengalensis* (Gray, 1835) were taken and divided into 4 groups of 15 rodents each. The 3 groups were treated with 3 different doses (oral administration with food, three times a day). Another group was kept as control, where only food and distilled water was provided. After 60 days of the experiment, we observed the physical changes of those 4 groups. The testis were weighted and used for histological studies. For histology evaluation, testis was fixed in Bouin's fixative. Tissues were processed for wax embedding and embedded in paraffin, wax blocks were sectioned 7µm thick and stained with haematoxyline and eosin. Sperm morphology was observed under MSZ TR Stereo Zoom from the extract of epidedymis in the laboratory. 5 rodents from each group, the control as well as the treated, were kept with female *Bandicota bengalensis* (Gray, 1835) for the assessment of fertility

Results

Histopathology

In the control group there are no changes in sperm morphology and histology of testis as they are not treated with extract. The treated group shows following changes accordingly with doses of extracts. The changes are decreasing the weight of testis and depletion of the germinal layer. Prominent space detected within the germinal epithelium. There are reduction in the number of Sertoli cells were also observed. Histological examination of testes in control animal showed seminiferous tubules of the testes

possesses epithelia containing the sertoli cells and the germ cells at various stages, covering the complete process of spermatogenesis. Sertoli cells exhibited typical, irregular nuclei and well-defined cytoplasm, which appeared granular. The spermatogonia, oval in shape, were closely associated with the basal lamina. Spermatocytes showed various degrees of condensation of the nuclei and were closely associated with sertoli cell cytoplasm. The lumen contained mature spermatozoa, and the interstitium contained distinct Leydig cells (Figure 1). Group G I, G II and G III animals showed dose dependent defect on histopathology of testes. Figure 2 of Group G1 (Dose 1) shows mild to moderate effect. Large multinucleated cells were also present in a number of tubules in group G2 (Dose II) (Figure 3). In addition, prominent spaces detected within the germinal epithelium were consistent with structural disorganization in group G3 (Dose III) (Fig 4). The cytoplasm of all germ cells of group G1, G2 and G3 shows vacuoles and tubules suggesting degeneration of their germinal epithelium. Many tubules showed signs of restructuring lumen contained reduced spermatozoa, and the Leydig cells.

Effect on Reproduction

The testis weights were measured in the control and treated *Bandicota bengalensis*. The fertility of the male was also assessed from the pregnancy and birth

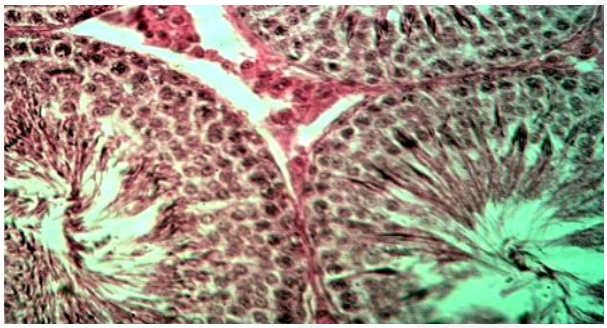

Fig. 1: T. S. of Testes of control shows normal normal Sertoli cells, germ cells and lumen contains mature spermatozoa

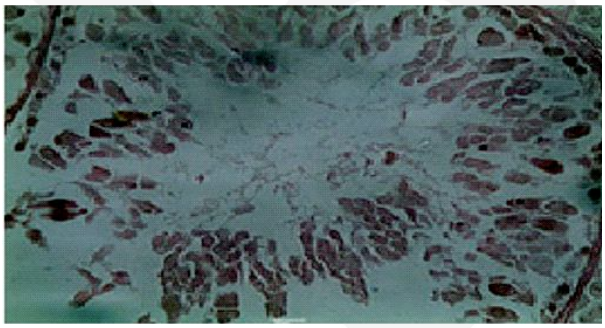

Fig. 2: T. S. of testis of Group 1 treated with 200mg/100ml (Dose 1) showing Sertoli cells started to vacuolize

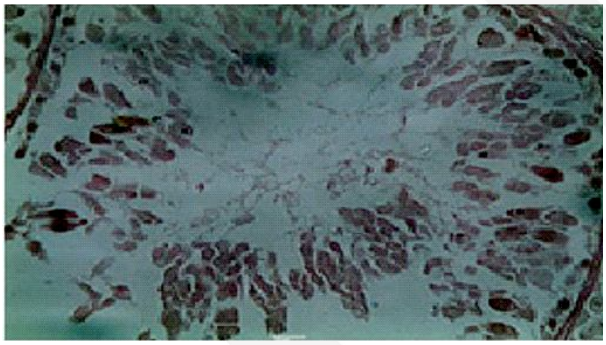

Fig. 3: T. S. of testis of Goup 2 Treated with 400mg/100ml (Dose II), quality of sperm is reduced, elongated spermatid

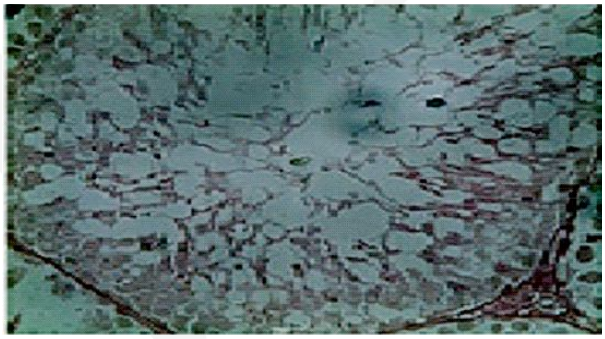

Fig. 4: T. S. of testis of Group 3 Treated with 600mg/100ml (Dose III) showing depletion in germinal layer resulting less production of spermatids

Table 1: Testes weight in different dose of mature *Bandicota bengalensis*

Control	1.82 ± 0.02 gm/100 gm body weight
Dose I (200mg/100 ml)	**0.72 ± 0.04 gm/100 gm body weight**
Dose II (400mg/100 ml)	0.51 ± 0.01 gm/100 gm body weight
Dose III600 mg/100 ml)	0.37 ± 0.12 gm/100 gm body weight

Table 2: Changes in fertility of *bandicota bengalensis* in treated and untreated

Treatment	Number of offspring produced/female/reproductive cycle
Control	7.4± 2.1
Dose I (200mg/100 ml)	2.1± 1.1
Dose II (400mg/100 ml)	00
Dose III (600 mg/100 ml)	00

rate of the partner female. The observations are shown in the table:

Discussion

Our research showed that oral administration of *Aegle marmelos* (L) Corr. barks aquous extract lead to dose dependent defects in the testicular spermatogenesis which leads to production of defective sperms. The results of the present study indicate that the bark extracts of *A. marmelos* (L) Corr. have a considerable effect on fertility. It has already been reported that aquous extracts of *A. marmelos* (L) Corr. decreases the motility of rat sperms in vitro (Agrawal *et al*, 2012). An earlier study on 50% ethanolic extracts of the leaves reported the potential of the same in suppressing the fertility of male albino rats (Sur *et al*, 1999). Phytochemical analysis of the bark extracts by TLC have revealed the presence of skimmaianine, fagarine, marmine (Uttam, K.D. *et al*, 2006). Studies have also indicated the presence of alkaloids, tannins, terpenoids, volatile oil, glycosides, phenolic group in aquous extracts of A. *marmelos* (L) Corr. This leads to the conclusion that the activity shown by the extracts could be because of the presence of the above mentioned secondary metabolites in them.

Conclusion

The findings of the present study clearly indicate that the bark extracts of *Aegle marmelos* (L) Corr. have a negative effect on the fertility of *Bandicota bengalensis* (Gray, 1835). The decrease the weight of testis and depletion of the germinal layer could be a possible explanation of reduced fertility. The future use of *A. marmelos* (L) Corr. as rodenticide on a commercial basis deserves further investigation.

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Original Article

Variation in Net Radiation Over Wheat (*Triticum Aestivum* L.) in Different Phenophase Intercropped with *Dalbergia Sissoo* in Nelder Wheel Design

Ravi Kiran

Abstract

The present investigation attempts to study the diurnal radiation over wheat as an understory crop below 8 year old *Dalbergia sissoo* planted in a Nelder wheel design planted in fifteen spokes of trees numbered serially starting from north direction as 0^0. Wheat cv. PBW-226 was sown on 21st November, 1996 as an under story crop. Tree spokes were pruned 0%, 30%, 45% and 75% starting from north direction respectively. Studies have been made on the diurnal variation of net radiation attributable to orientation of tree rows of the trees at four important phonological stage of the crop i.e. tillering, flag leaf emergence, flowering and maturing stage of the crop and compared with the control (sole crop) viz T1 (312^O-72^O), T2 (72^O-192^O) and T3 (192^O-312^O) in the Nelder wheel. Regression equations between net radiation in control and different treatments below trees were also developed.

Keywords: Diurnal Variation of Net radiation; *Dalbergia Sissoo;* Wheat (*Triticum Aestivum* L.).

Introduction

Forests and forest products are one of the basic needs, both for human beings and animals. Due to population increase in geometric progression, there is an increased demand of food, shelter, fuel, fodder, timber etc., resulting into the increased pressure on natural resources. The present day modern agricultural practices have increased the level of agricultural production considerably yet it is not sufficient. Agricultural production needs very specific soil, water and atmospheric conditions. The area under suitable soil and water condition was increased through intensive research, extension and management programmes. The atmospheric conditions could not be modified easily. However, efforts are now being made to improve the microclimatic conditions through agroforestry. Trees act as wind-break and increase water use efficiency by retarding the water loss by evapotranspiration besides modifying radiation climate, crop energy balance temperature, photosynthesis and its rate and duration and plant growth. (Messing and Noureddine 1991). Heat load reduction during reproductive phase can increase the production of wheat (Johnson *et al.*, 1981). Excessive solar energy in sole cropped wheat fields at post anthesis period reduce wheat yield (Chinnoy, 1947; Wardlaw *et al.*, 1989). Introduction of treed in the monoculture can be used to optimize the microclimate in field conditions for winter wheat in North India. studies are scarse so far on tree-crop interactions of Wheat (*Triticum aestivum* L.) and *Dalbergia sissoo* planted in Nelder wheel design under shallow water table conditions. The present investigation attempts to quantify the diurnal variation of modified microclimate of wheat as an understory crop below *Dalbergia sissoo* planted in a Nelder wheel design.

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Materials and Methods

The present field investigation was carried out at the Horticultural Research Center, Patharchatta, located in the campus of GB Pant University of Agriculture and Technology, Pantnagar, India (29° N, 79° 30 E, 243.83 m above mean sea level). Trees of *Dalbergia sissoo* planted in a Nelder wheel design in March 1989 (Nelder, J.A. 1962). It consisted of fifteen tree spokes of wheel each oriented at an angle of 24°

from the adjacent tree spoke, arranged in ten concentric rings of trees of radii 2.0, 5.4, 13.5, 17.8, 21.5, 24.6, 27.4, 29.9, 32.2 and 34.4 m, respectively, enclosing a total number of 15 plots between the tree rows, numbered serially from 1 to 15 anticlockwise direction starting from a tree row oriented to 0° in north direction. Tree spokes were pruned 0%, 30%, 45% and 75% starting from north direction respectively. Each plot was divided into three sub plots of area 26.62 m^2 for the propose of the investigation. In each spoke the first, second and the tenth trees were considered as buffer trees to avoid border effect. Therefore, a constant tree stand of 333 trees per hectare was provided in the area between the third and the ninth trees of each spoke resulting each tree in the experimental area occupying an average area of 30 m^2. The climate of the region is humid subtropical, characterized by dry hot summers and cold winters having dry season from early October to mid June and a wet season from mid June to early October. Average annual total rainfall is 1434.4 mm (90% in mid June to end of September). The diurnal maximum air temperatures are highest in May–June and the diurnal minimum air temperatures are lowest in January (range 41.0- 3.0 °C) while relative humidity is highest in July–August and lowest in December–January; (range 80-35%) in April–May. The experimental area lies as a belt below and a few kilometers south of the foothills of the Himalayan mountains having gently slopped of less than 1% the soils are classified as Mollisols (Deshpande et al., 1971) along with characteristic fluctuating shallow water table ranging from surface to 1.8 m or so below the soil surface. The weather conditions during the experiment is depicted in Fig 1.

Field was harrowed four times with disc harrow, properly levelled for better germination and growth, before the sowing of experimental crop. Nitrogen, phosphorus and potassium were applied at the rate of 120kg, 80kg, and 60kg per hectare. Half of nitorgen and full doses of phosphorus and potassium were broadcasted during land preparation and mixed thoroughly by cross harrowing. The remaining half dose of nitrogen was top dressed at 25 DAS i.e. just after first irrigation. The wheat variety PBW-226 was sown below the tree canopies with the help of seed drill on 21st. November, 1996. The rate of seed was 100 kg/ha. The seed was sown in rows 23cm apart and at the depth of 5cm. The area not covered by seed drill was sown manually by hand hoe. Two hand weeding were given at 45 and 80 days after sowing. Only one irrigation was given at 20-25 days after sowing at crown root initiation (C.R.I.) stage.

Trees of *Dalbergia sissoo* were pruned 0%, 30%, 45% and 75% starting from north direction respectively to provide sufficient solar flecks penetration for growing of wheat as an understory crop. Radiation climate in the three microclimatic zones viz T1 (312°-72°), T2 (72°-192°) and T3 (192°-312°) in the Nelder wheel attributed to the trees row orientation were studied at four important stages of the wheat crop along with control.

Net radiation was measured with the help of portable Net Radiometer for each treatment at three points in the middle of the area for each subplot of each treatment along with control simultaneously at tillering, flag leaf emergence, flowering stage and maturing stage at half hour interval starting from morning to evening. Regression equations between net radiation in control and different treatments below trees were also developed.

Result and Discussion

Total diurnal net radiation in morning, noon and evening at tillering, flag leaf emergence, flowering stage and maturing stages of the wheat crop in various treatments is depicted in Fig 2-4. On temporal scale diurnal net radiation availability among different treatments and all four stages of crop on half hourly basis under the agroforestry conditions was found highly dynamic. Net radiation was available more in South direction treatments than that of north direction treatments in Nelder design under tree canopies.

With the advancement of the stage of crop total diurnal net radiation in all the treatments showed increasing trend. During morning the net radiation was lowest at tillering in T1 and highest at flowering stage in T3. During noon the net radiation was lowest at tillering in T1 and highest at flowering stage in T2. During evening the net radiation was lowest at tillering in T1 and highest at flag leaf emergence stage in T2 . Total diurnal net radiation at tillering flag leaf emergence, flowering stage and maturing stages ranged 14-55%, 54-62%, 65-73% and 44-70% of control, respectively. Net radiation availability was found more in T3 than that of other treatments under canopies. The changes in light intensity has profound effect on photosynthetic responses of crop growing beneath trees (Knapp and Smith, 1990).

All treatments had sufficient net radiation availability at all stages of crop growth. The foliated green tree canopy reduced radiation intensity along with the quality of light in PAR range (380–700 nm) (Zavitkovski 1982).

Relationship between net radiation in control and different treatments below trees in morning, noon and evening were developed separately for different stages of the crop. It was observed that there was a significant relationship between the net radiation in control and in understory crop in morning, noon and evening at four important stages which are presented in Fig 5.

In tarai region of Uttar Pradesh shallow water table can provide sufficient subirrigation to wheat crop intercropped with trees. Upward soil water ûux can contribute 36–73% of the total water requirement of wheat in tarai region of foothills, under shallow water table conditions. (Saini and Ghildyal 1978). The depletion of water table occurred in reproductive phase of crop because of more water requirement of the wheat crop but have no significant effect on the plant water potential (Powell 1980).

Tree row orientation and distance affected considerably the net radiation over the crop, but, reduced heat load from earhead developement to maturity and its more duration in an agroforestry system especially the deciduous tree like *Dalbergia sissoo* may possibly mitigate the effect of shading below trees as excessive solar radiation to sole cropped wheat field at post anthesis period reduces wheat yield (Chinnoy J., 1947).

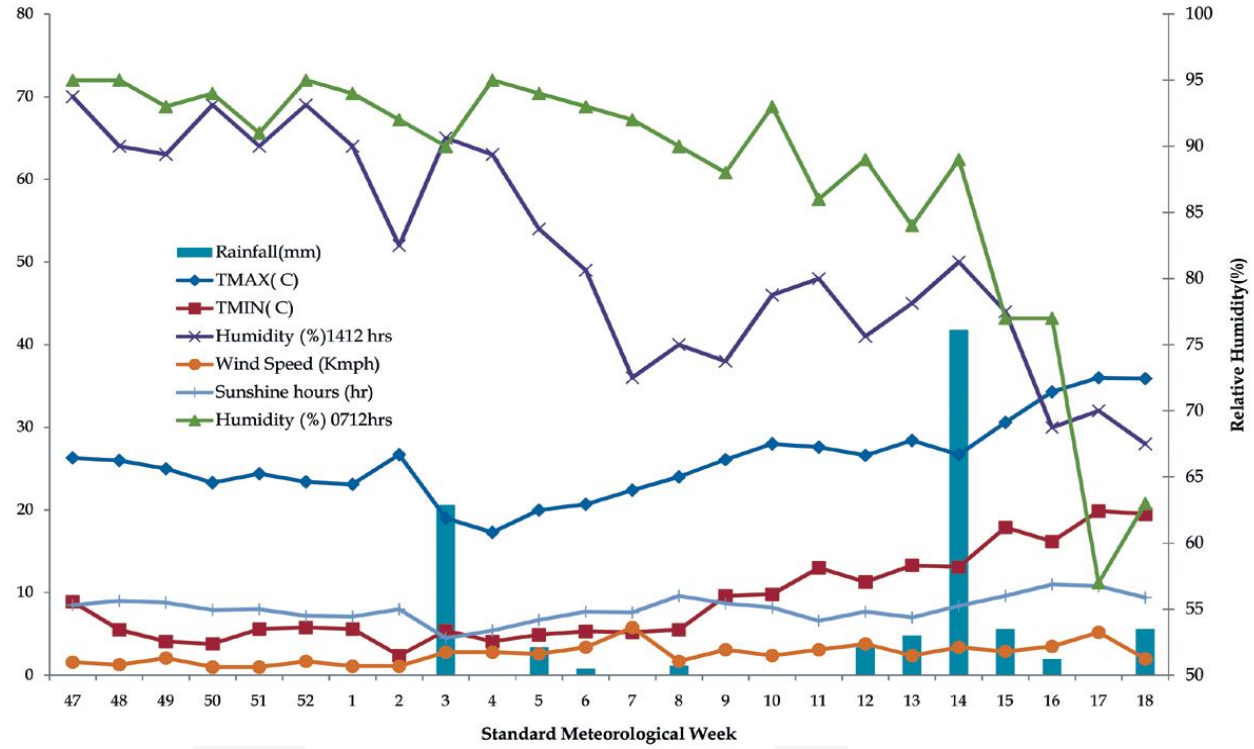

Fig. 1: Weekly average weather data for experimental period (November1996 - April1997)

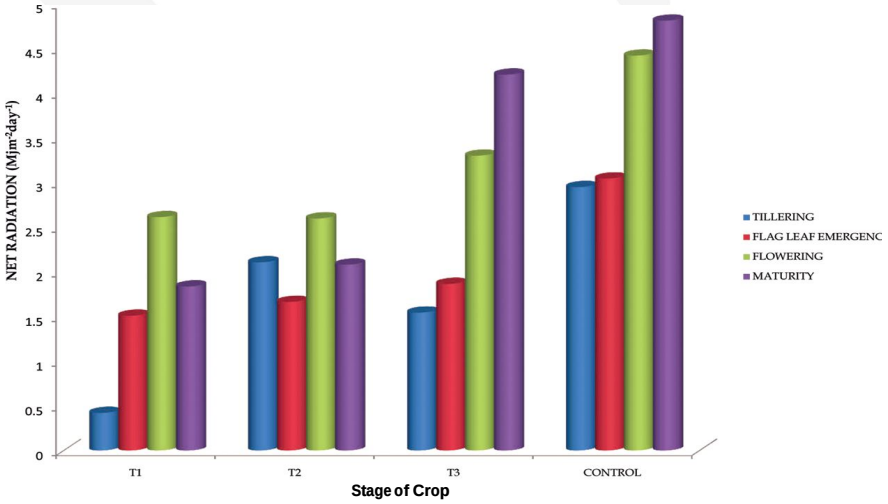

Fig. 2: Variation in net radiation in wheat intercropped with shisham during morning hours

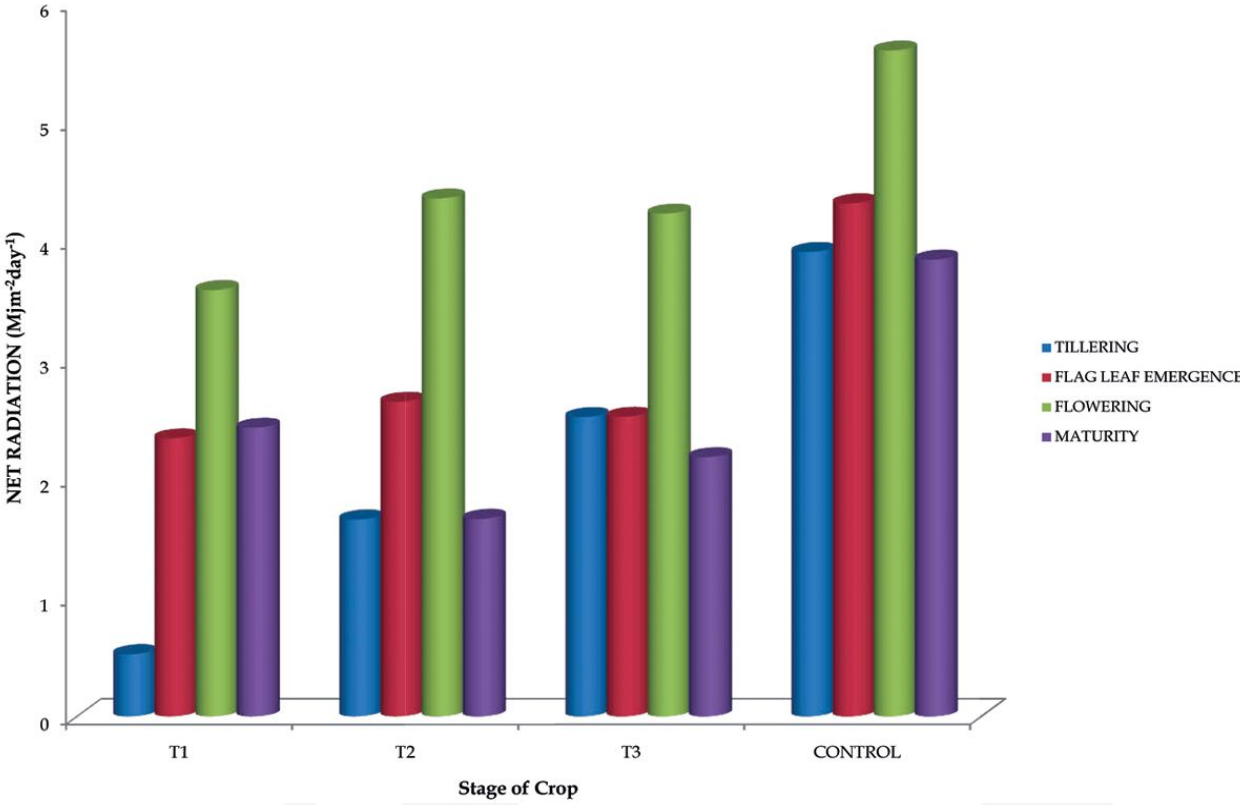

Fig. 3: Variation in net radiation in wheat intercropped with shisham during noon hours

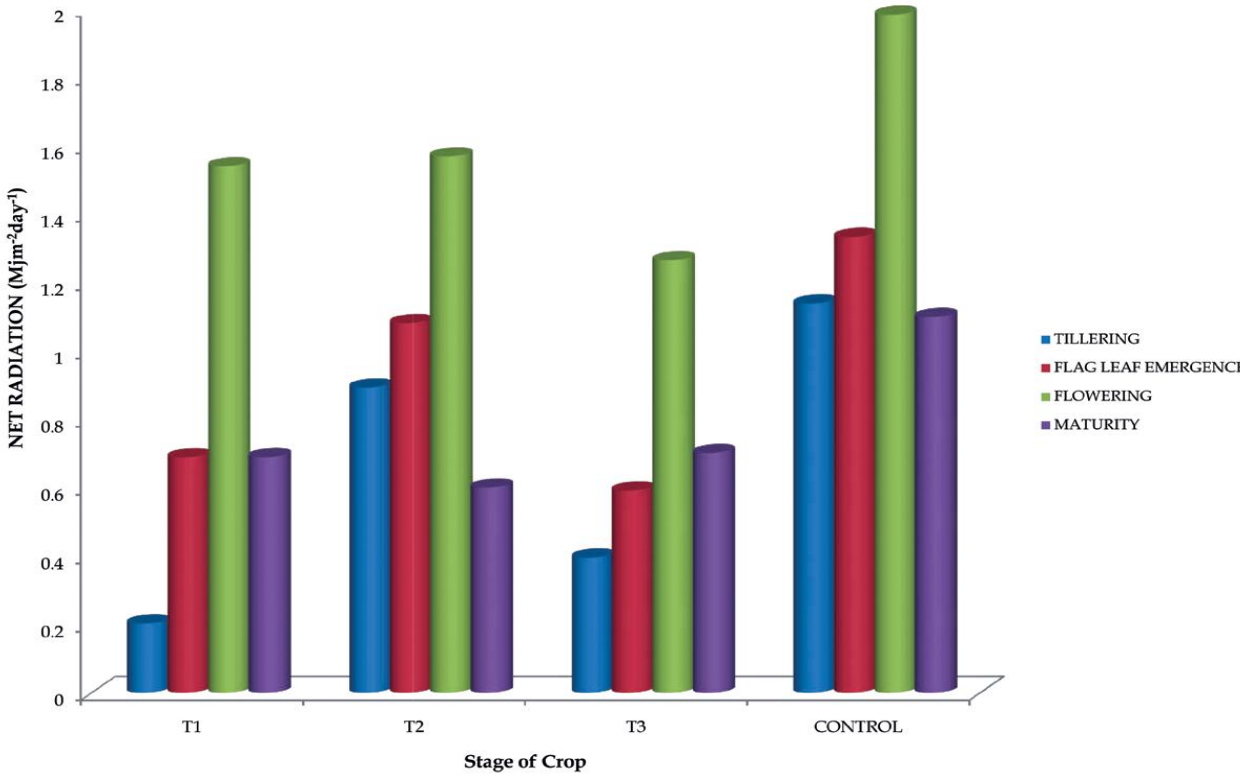

Fig. 4: Variation in net radiation in wheat intercropped with shisham during evening hours

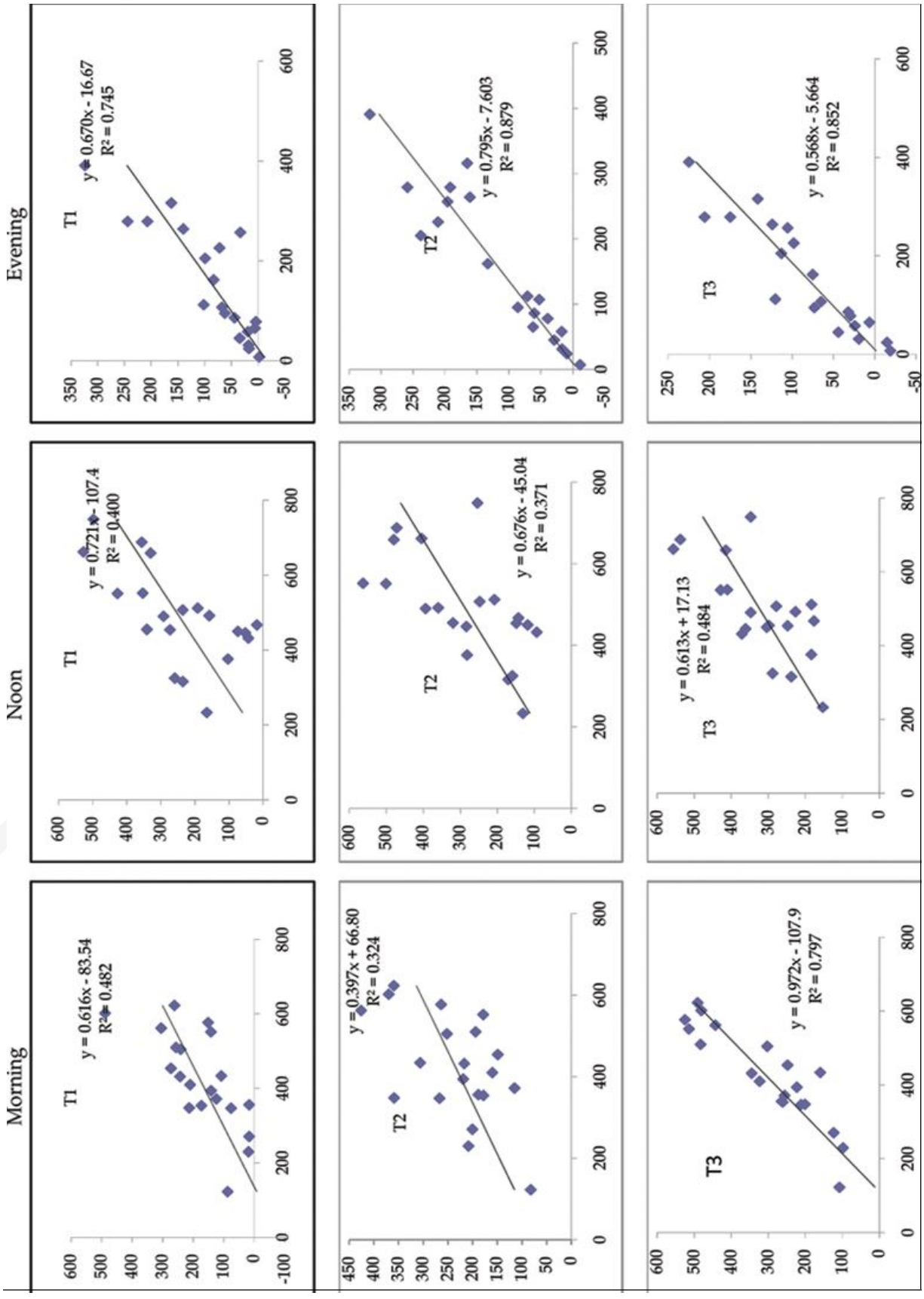

Fig. 5: Relationship between Net Radiation in different treatments and control during morning, noon and evening during crop growth period

Agroforestry has vide scope to modify the microclimatic conditions subjected to the proper tree spacing and their pruning for wheat.

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Original Article

Industrial Pollution Assessment of Tamla Rivulet in Industrial Town of Durgapur, West Bengal, India: Hazard Analysis Through GIS

K. Dasa*, S. Lahirib**

Abstract

The vehemence of industrial pollution has been studied along a stretch of effluent discharged canal, the Tamla Rivulet in the industry-intensive town of Durgapur, West Bengal, India. The present study monitors the surface water quality and relates it to the land use / land cover maps using Remote Sensing and Geographical Information System (GIS) techniques. Middle stretch (industry-rich) and lower stretch of Tamla (confluence area) manifested considerable pollution effects in surface water, ground water quality and agriculture, respectively compared to the upper stretch. The maximally affected lower Tamla region recorded high concentrations of phenolic compounds in ground water and concentrations of fluoride, lead, cadmium and chromium in the drinking water. The calculated industrial hazard index revealed that outfalls of industries in the middle and lower Tamla region exhibited very poor and poor water quality, respectively affecting the overall eco-biological health of the entire zone.

Keywords: Industrial Pollution; Surface Water; Ground Water; Industrial Hazard Index.

Introduction

Water is one of the most important constituents of life support system and a chemical medium with a unique property of dissolving and carrying in suspension a variety of chemicals and other materials (Kulshreshtha, 1998). Any material (or heat) harmful to humans, animals or desirable aquatic life when carried in excess amount causes water pollution (Katyal, 1989; Vorosmarty et al., 2000; Bhatia, 2003). It is a vehement global problem caused by various kinds of natural and man made activities such as agricultural, industrial, domestic and others affecting fresh water (lentic and lotic) bodies (Ramkrishna and Babu, 1999; World Bank, 1999; UNESCO, 2003; Puyate et al., 2007; Amaal et al., 2009), marine aquatic system (Edwards et al., 2001; Ylitalo, 2005; Verlecar, 2006; Saraswat et al., 2007) and ground water systems (Barber et al., 1998; Otto, 1999; Kinniburgh and Smedly, 2001; Chisala et al., 2004). Humans, especially young children and foetuses are very sensitive to heavy metal pollution of water (Jayprakash, 2005; Susheela et al., 2005; Pandey et al., 2010). Other severe cases of human health hazards (WHO, 2001; Chandrasekar, 2002; Clark et al., 2003; Rahman et al., 2005), aquatic food chain toxicity (Simon et al., 2005; Ekambaram et al., 2004; El. Shimy et al., 2007; Benson et al., 2007; Silva and Shimizu, 2004; Sciencedaily, 2008) etc have been reported from world wide.

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Durgapur city, the industrial metropolis of the state of West Bengal, India, is also one of the largest industrial cities of India. It covers an area of 154 sq km of which 13% is under industries and the rest is covered by settlements and cultural establishments. It serves as the home for a number of industrial units such as Durgapur Steel Plant, one of the integrated steel plants of Steel Authority of India Limited. Alloy Steels Plant of SAIL is also located here along with a number of power plants, chemical, engineering and metallurgical industries. Development of such an industry rich zone is due to its favourable position in respect to raw materials (good quality iron ore, coal and lime deposits), fresh water supply and easy transport for labour and market. As a result of this industrial activity, the basin draws about 329 MCM of water per year. At least 92.81 MCM of the water is

returned to the Damodar river (south of Durgapur) as an untreated effluent (CSME, 1998). The annual pollution load of the entire Damodar river (CSME, 1998) has indicated a high discharge (tones / year) of iron (16,800), copper (3,990), cadmium (470), chromium (1960) and phenolic compounds (1211).

The industrial effluents are discharged through the Tamla and Singaran Rivulets and hence the study has been done with specific reference to these discharge routes.

In this zone human populations and ecological receptors are in continuous contact with these industrial discharges because they are present in air, water, soil and food. These links manifest themselves through their effect on human health and ecology. Therefore, the link demands exact identification of the causes for formulation of sound environmental planning thereby necessitating studies and analysis of environmental hazards of the area. The environmental planning calls for scientific, eco-friendly and planned waste discharge methods, sites and treatment facilities in this area for most of the industries. To provide a comprehensive but easy to use tool in the assessment and evaluation of water quality in the industrially polluted area, the concept of water quality index (WQI) can be used (Lermontov, 2009) This index coupled with available pollution data after integration spatially in thematic maps prepared by interpretation of satellite imagery using a computer based analytical system of GIS (Asadi et al., 2007) will help in the environment planning and decision support system of the study area. The present study aims for development of the eco-health of the region through the seasonal and spatial analysis of effiuent load in outfalls of 5 major industries along the middle and lower stretch of Tamla and studying their effects on agricultural produce. Industrial Hazard Index (IHI) of the region has been generated for future risk analysis.

Materials and Methods

Study Area

Location

The study area is located between 23°20' to 23°30' N latitude and 87°15' to 87°30' E longitude along the left bank of river Damodar in the Durgapur city, Barddhaman district, West Bengal, India (Figure 1).

Climate

Extremely hot and humid summer persists from mid April to mid June. Rainy season starts from mid June to mid August with an annual precipitation of about 1424mm (Roy and Chakrabarty 2004). Winter is moderately cold and starts from November and lasts till February. Mean annual maximum and minimum temperatures are 42° C and 21.5° C respectively.

Soil and Drainage

Colour-Greyish brown, near neutral to slightly alkaline sandy clay loam in nature and medium to moderate sub-angular rocky in structure. The area is an interfluvial tract of the drainage system

i. two main rivers viz. Ajoy and Damodar flowing from west to east,
ii. two minor streams namely Tamla, rising just south of the village of Ukhra in Andal,
iii. rivulet Jor originating from north western direction of Banskopa village in Rajbandh.

Both the streams are joining the Damodar at Madhya Mana and Napara village respectively (Figure 2).

Experimental Sites

For the selection of suitable sites, the entire stretch of Tamla rivulet was categorized into three zones- upper Tamla (A), middle Tamla (B) and lower Tamla (C) based on the number of waste water outfalls into the rivulet from the different industries (Table 1). The outfalls of Alloy and Steel Plant (ASP) and Durgapur Steel Plant (DSP) are located in the middle segment whereas Durgapur Chemicals Limited (DCL) , East India Pharmaceuticals Works Limited (EIPWL) and Durgapur Projects Limited (DPL) near north-west of Ashishnagar area are located in lower stretch Five experimental sites, two located in the middle stretch and three in the lower stretch of Tamla rivulet, were selected altogether, each at the outfall point of DSP, ASP, DCL, EIPWL, DPL to study the surface water pollution. Map showing sampling points overlaid on satellite imagery is shown in Figure 3.

Further, three other places adjoining to Tamla rivulet corresponding to upper, middle and lower stretch (Table 2) were selected to study the pollution effect in groundwater, soil, and vegetations.

Sample Analysis

Collection of Samples

Samples of surface water, groundwater, soil and

vegetations were collected monthly over 4 years duration

Water

Samples of surface were from each site in 250 ml closed plastic container. Groundwater samples were taken from deep and shallow tubewells.

Soil

At each of the sites, one 100 x 100 m permanent plot was demarcated. Ten samples were collected randomly from the upper 10 cm layer (in 5x5x10 cm blocks) of each of the plots and the samples were divided into two parts, one part of which was sieved through a 2mm mesh screen. The screened samples were then mixed together and four subsamples from each site were drawn for further chemical analysis.

Vegetation

Vegetables grown in the experimental regions were also sampled in three replicates of 5 grams each for every vegetable of each site during winter seasons. The large pieces of plant materials were hand picked. Fine roots were carefully removed.

Testing of Samples

Surface and Groundwater

Samples were tested for magnesium, calcium, total hardness, alkalinity, chloride, total conductivity, pH, total dissolved solids, total suspended solids according to APHA (2005).

Soils

Mechanical analysis was conducted through pipette method ((Black et al. 1965) on the unsieved soil samples. pH was measured with pH analyzer. Materials prior to weighing were dried at 40°C. All the materials were grinded to pass through a 0.25 mm mesh. Iron, copper, zinc, chromium and magnesium were estimated through an atomic absorption spectrophotometer. Manganese and calcium were analysed by EDTA method (APHA 2005).

Total nitrogen was analysed according to macrokjeldahl analysis acid extraction method (Moore and Chapman 1986) and available N measurement as per Jackson (1958) and Wetzel and Likens (2000). Available phosphorus was measured through spectrophotometric method (TSBF, 1989). Organic matter was evaluated by multiplying 1.724 (Bemmelen factor) with the organic carbon content, the latter was measured by Walkley Black rapid titration method (Jackson, 1958). Atomic absorption spectrophotometer was used for heavy metal analysis of soil samples.

Vegetables

Three replicates of 5 mg of each vegetable from each site have been considered for analysis. Materials prior to weighing were dried at 40° C. All the materials were grinded to pass through a 0.15 mm mesh. Retained a representative sample of approximately 25 mg, eg coning and quartering, for analysis in 5 gm of each replicate. Iron, copper, zinc, chromium, cadmium and magnesium were estimated through Atomic Absorption Spectrophotometer (AAS). Magnesium and calcium were analysed EDTA method (Clesceri et al. 1998).

Spatial and Attribute Database Generation

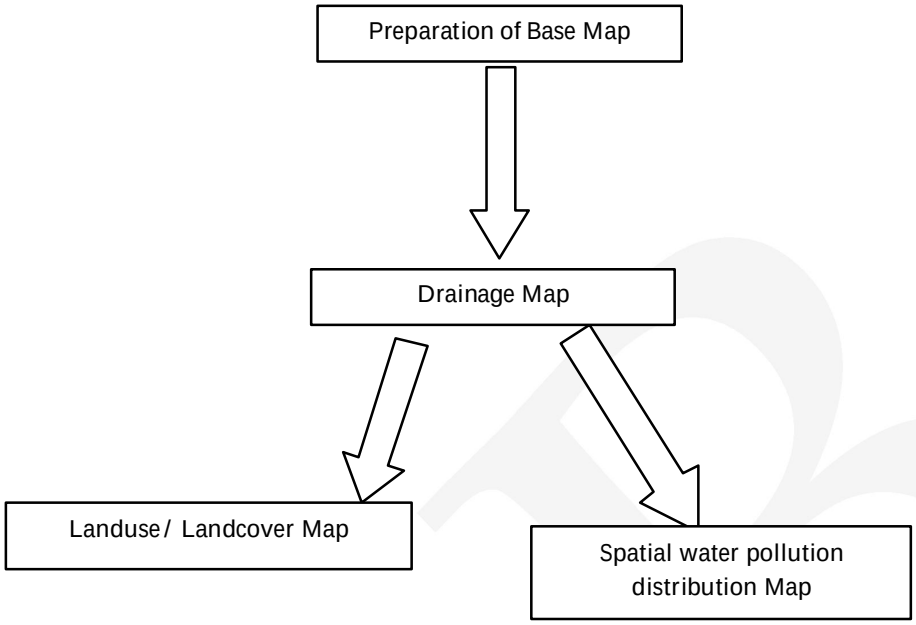
Preparation of Base Map

Thematic maps like base map and drainage maps (Figure.2) are prepared from the SOI toposheets (73 M / 2,73 M / 6 and 73 m / 7) on 1:50,000 scale. These Thematic raster maps are converted to vector format by scanning and digitized in Arc GIS 9.3 (Version) software to obtain a baseline data in digital mode.

A landuse / landcover map (at 1:162997 scale) was prepared using on screen digitization technique from IRS-ID,LISS-IV satellite imagery and SOI toposheet along with ground truth verification (Figure 4)

Integration of Spatial and Attribute Database

The spatial and the attribute database generated are integrated for the generation of spatial distribution maps of selected water quality parameters like pH, alkalinity, chlorides, sulphates, nitrates, TDS, total hardness, fluorides and Industrial Hazard Index (IHI) and overlaid on satellite imagery. The water quality data (attribute) is linked to the sampling location (spatial) in ARC / INFO and maps showing spatial distribution are prepared to easily identify the variation in concentrations of the above parameters in the ground water at various locations of the study area using curve fitting technique of ARC / VIEW GIS software.



Calculation of Industrial Hazard Index

$$\text{Industrial Hazard Index of surface water } (IHI_{sw}) = \frac{\text{Concentration of effluent at the outfall point}}{\text{Permissible limit of the effluent}} \times 100$$

All data were tested statistically for significance using LSD (Least Significance Difference) for relevant parameter.

Results and Discussion

Seasonal Variation of Surface Water Quality

The pre-monsoon period have a higher pollution load (Table 3) due to low discharge of water in the rivers and a low pollution load in the post-monsoon season owing to the effect of dilution brought about by higher discharge of water due to rains. But in the present case it is not always so. Further, because of the topography, there was a considerable amount of mixing of water of both the regions during the monsoon season consequently the difference in quality of water in up stream and down stream part decreased.

The industry-wise seasonal variation of different parameters is as follows :

pH

The values of pH at the effluent discharged points of Tamla rivulet for all the industries showed a fluctuating trend throughout the year. The value of pH ranged from 8.9 (EIPWL-winter) to 5.9 (DPL-February). The high pH value of water in the down stream region and its large fluctuation was related to the effluent discharged from DSP Steel Plant which varied from time to time depending upon the production schedule and quantity of municipal effluent entering to the river (Table 3).

TSS

The concentration of TSS remained low all throughout the year for ASP and DCL. The values remained comparatively higher during April to October than the remaining months although a fluctuating trend has been noticed all throughout. An unusual high value of 222.4 mg/l was observed at DPL outfall point in July (Table 3).

COD

The value of COD in the outfall point of EIPWL was markedly high (96 to 228 mg/l). This was followed by DPL (27 to 81.4 mg/l). The COD concentration ranged from 1.85 to 28.9 mg/l in the outfall points of ASP, DSP and DCL. Pre-monsoon and monsoon periods showed a comparatively lower COD concentrations in the outfall points of DSP, ASP, DCL and DPL than the other seasons. However, the result was reverse for EIPWL (Table 3).

BOD

The range of BOD concentration at the outfall point of EIPWL (4.8 to 48.6 mg/l) is remarkably higher all throughout the year than the effluent points of other

industries (0.8 to 13.12 mg/l). Higher values were recorded during May to July (Table 3).

Oil and Grease

At the effluent discharge point of EIPWL the concentration of O&G ranged from 1.36 mg/l to 4.7 mg/l which is 117 to 230 % higher than the concentrations at the other outfall points. Monsoon months from June to October recorded low values than the other months (Table 3).

Spatial Variation of Different Water Quality Parameters

Due to the continuous accumulation of discharge from various industries down the middle stretch of the Tamla rivulet, the concentration of total suspended solids increased by 33% towards lower stretch. Accordingly, the high density of industries discharging heavy effluent load towards the lower stretch of Tamla rivulet (Figure 5) resulted in higher concentration of COD and BOD in the outfalls of DCL, DPL and EIPWL compared to upper stretch. However, concentration of oil and grease in the surface water did not show much spatial variation among the outfalls of studied industries due to the quality variation of the effluents.

Groundwater Quality

The Table 4 shows that there is no significance difference ($P< 0.05$) between the three different sites in terms of electrical conductivity, iron, lead, copper, phosphorus, nitrate-N, fluoride, cadmium and chromium in the drinking water from shallow tubewells. Moreover in case of total alkalinity, total hardness, chloride, sulphate, sodium, potassium and phenolic compounds, tubewell water from DSP outfall point at Court More region and Madhya Mana region although did not differ significantly ($P> 0.05$), yet the same from the Tamla village differ significantly in terms of all these parameters from the water derived from other two experimental sites. TSS, TDS, calcium, magnesium and zinc contents however, differ significantly ($P<0.05$) in water from shallow tubewells of all the three experimental sites. pH value shows that in Tamla the water is near neutral alkaline whereas in other two sites it is significantly acidic.

If compared to the setup standards (WHO 1971) it can be inferred that in the water of the shallow tubewells from the three sites, only Tamla village water shows values of pH, total $CaCO_3$, Mg within acceptable limits (Table 4). However, TSS, Cl, S, Fluoride, NO_3 was acceptable in the water from all the three sites. On the other hand, concentration of phenolic compounds was 0.07 and 0.08 mg /l which was higher than the maximum limit of 0.001 mg/ltr in Bhiringi and Madhya Mana water.

Soil Quality

Testing of soil from the three experimental sites shows (Table 5) that the soil is very slightly acidic having no significant difference in pH values ($P>0.05$) between the sites. Organic contents were insignificantly ($P>0.05$) differing in all the three sites. In terms of available nitrogen Tamla village differs significantly ($P<0.05$) from Madhya Mana, however it does not differ significantly ($P>0.05$) from that of DSP outfall point at Court More region.

Available P was also higher (8 to 10 %) in Tamla village although the difference between sites was insignificant ($P>0.05$). Cadmium content was insignificantly differing in all the sites but the chromium and manganese content was much in samples from two sites namely DSP outfall point at Court More, Bhiringi and Madhya Mana than in the soil of Tamla village ($P<0.05$) whereas in case of manganese difference between Bhiringi and Madhya Mana was insignificant ($P>0.05$).

Iron content was less in case of Tamla Village soil, and highest in Madhya Mana soil ($P<0.05$). In case of lead, copper and zinc, the highest content was in Madhya Mana and lowest in Tamla village. Tamla village has sandy loam soil with about 56.4% sand content. Other two sites have loamy soil with about 50% sand content. Available nitrogen in Tamla is however higher than the other two sites probably because N-fertilizer is added to this soil from outside.

Accumulation in Vegetables

Among all elements iron accumulation was maximum in all vegetables (Table 6). Among vegetables Spinach in all the sites has maximum iron accumulation (456 mg/kg) at Madhya Mana whereas Bottle Gourd (*Lagenaria siceraria*) has lowest iron accumulation (17.2 mg/kg) at Tamla village. Copper accumulation was maximum in cabbage (*Brassica oleracea capitala*) 2.4 mg/kg followed by Cauliflower (*Brassica oleracea botrytis*) 25 mg/kg in Madhya Mana. It was lowest in Coriander leaf (*Coriandrum sativum*) at all the three experimental sites. Magnesium accumulation does not show any uniformity among vegetables and ranged from 3-6 mg/Kg. Zinc, chromium and cadmium were <5 mg/kg in all sites. The concentration of Manganese concentration increased from 11 mg/Kg in Tamla village to 17 mg/Kg at Madhyamana region.

It is apparent from the study that iron and copper accumulation was more than maximum permissible limit of 1.0 mg/kg and 1.0 mg/kg (WHO 1990) respectively in all vegetables from the two polluted sites of Bhiringi and Madhya Mana. Statistically differences are variable (LSD Test) in different vegetables.

Landuse and Landcover Distribution

An analysis of the nature and rate of land use change in the industrial area adjoining Tamla Rivulet and its associated impact on surface water and groundwater quality is essential for a proper understanding of the present environmental problems (Krishna et al. 2001). In the present study area, mixed built-up area comprises 56.40 km^2, industrial 20.46 km^2, cropped 24.65 km^2, forest5.08km^2, waterbodies 47.54km^2, vegetated areas 17.08 km^2 and others 2.53 km^2 out of the total area 154 km^2 respectively (Figure 6).

Industrial Hazard Index

The quality of the surface water at the outfall points is categorized into five types on the basis of the calculated Industrial Hazard Index These are Excellent (0-10), Good (10 to 40), Moderate (41-80) and Poor (81-120) and very poor (>120). Of the five industries DCL and DPL fall under the category of very poor quality, DSP in the poor category, ASP and EIPWL under the good category (Figure 7). It is observed that the outfalls of DCL and DPL rated as very poor are located in the middle stretch of Tamla river and is likely to affect the adjoining vegetated and double cropped area (Figure 4) since the farmers use this polluted water for irrigation purpose. However, the adjoining settlement region is less affected as they do not directly use the vegetables grown in the affected areas. The good hazard index rating of industries ASP and EIPWL reveal adequate treatment facilities of these two industries with a minimum environmental health impact of the fringing areas. Despite the variable industrial hazard ratings in the Tamla water, the pollutant load from the studied outfall points drains towards the lower stretch of the rivulet and deposits near the confluence point with the Damodar river. This pollutant deposition leaches to some extent through the soil and contaminates the groundwater and soil of the Madhyamana region which is severely affected as evident from the groundwater and soil quality (Table 4 and 5) and the accumulation of chemicals in the vegetables (Table 6). The industrial hazard index, therefore, clearly indicates that Tamla rivulet is under severe stress and use of water from this water body for any purpose is highly deleterious environmentally and biologically. The integration of attribute database comprising the quality of Tamla water, groundwater, soil and agriculture produce of Bhiringi and Madhya Mana region with the Remote sensing and GIS study proves to be an essential tool to evaluate the impacts of industrial hazard on land use / land cover of an industry rich zone of Durgapur city in West Bengal. Similar pollution study of ground water (Asadi et al., 2007) using Remote sensing and GIS techniques has been carried out for part of Hyderabad metropolis. Spatial distribution maps of pH, TSS, COD, BOD pollution parameters have been used to demarcate the locational distribution of water pollutants in a comprehensive manner and help in suggesting surface water pollution control and remedial measures by improving the industrial effluent treatment strategies in a holistic way.

It is inferred that highly polluted Tamla rivulet is contaminating its adjoining ecosystem vehemently and has created a highly profane environmental milieu that can no longer support a high biodiversity eco-system as well as healthy human society. This calls for an urgent planning strategies and intervention from pollution control/ regulatory authorities and civic administration along with NGO intervention.

Table 1: Outfalls of different industries of Durgapur city at Tamla nullah

Name of the industry	Number of outfalls	Waste water receiving body Tamla nullah			Others	Remarks
		Upper	Middle	Lower		
Durgapur Steel Plant (DSP)	6	-	3	-	3	
Alloy Steel Plant (ASP)	6	-	6	-	-	-
Durgapur Projects Limited (DPL)	1	-		1	-	-
East India Pharmaceuticals Works Limited (EIPWL)	1	-		1	-	-
Durgapur Chemicals Limited (DCL)	1	-		1		-

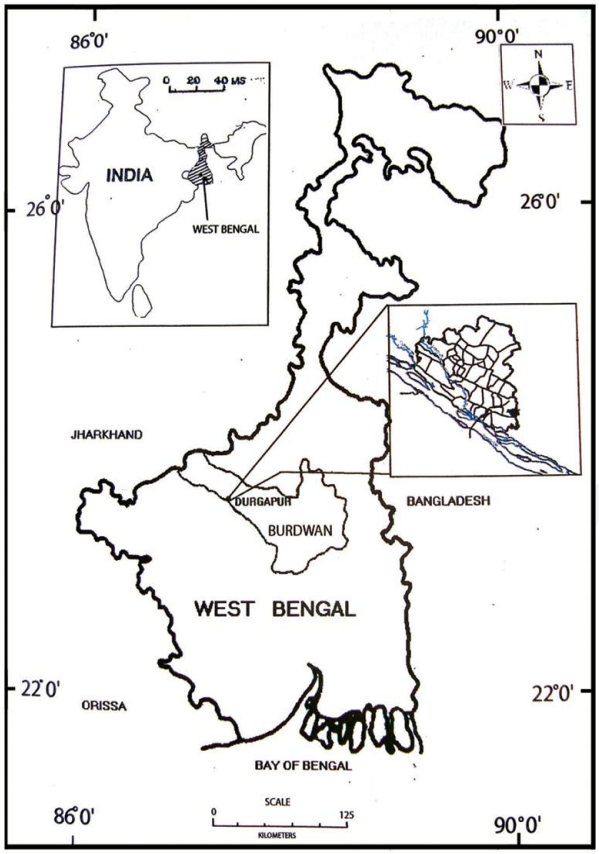


Fig. 1: Location map

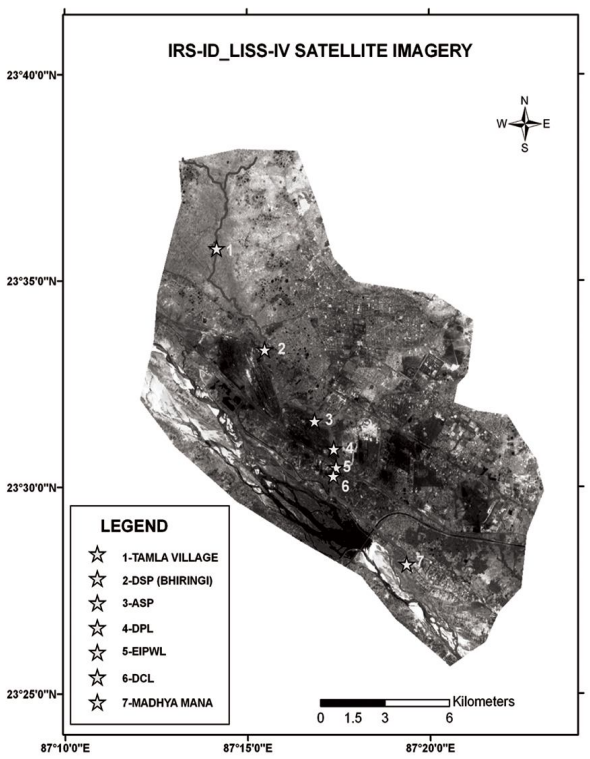


Fig. 3: Sampling sites overlaid on Satellite Imagery

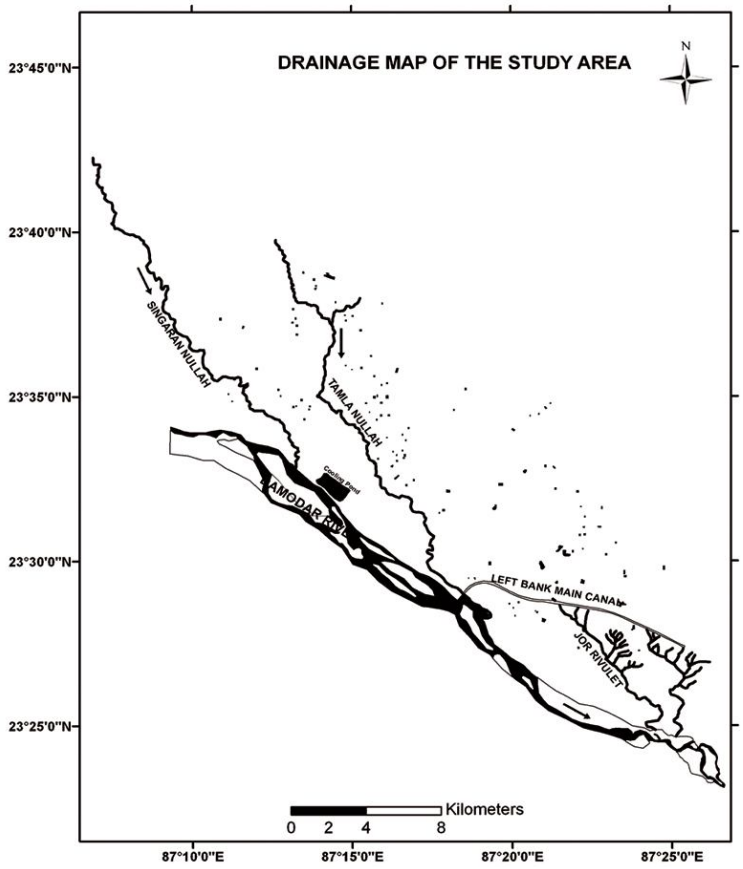


Fig. 2: Drainage Map of the study area

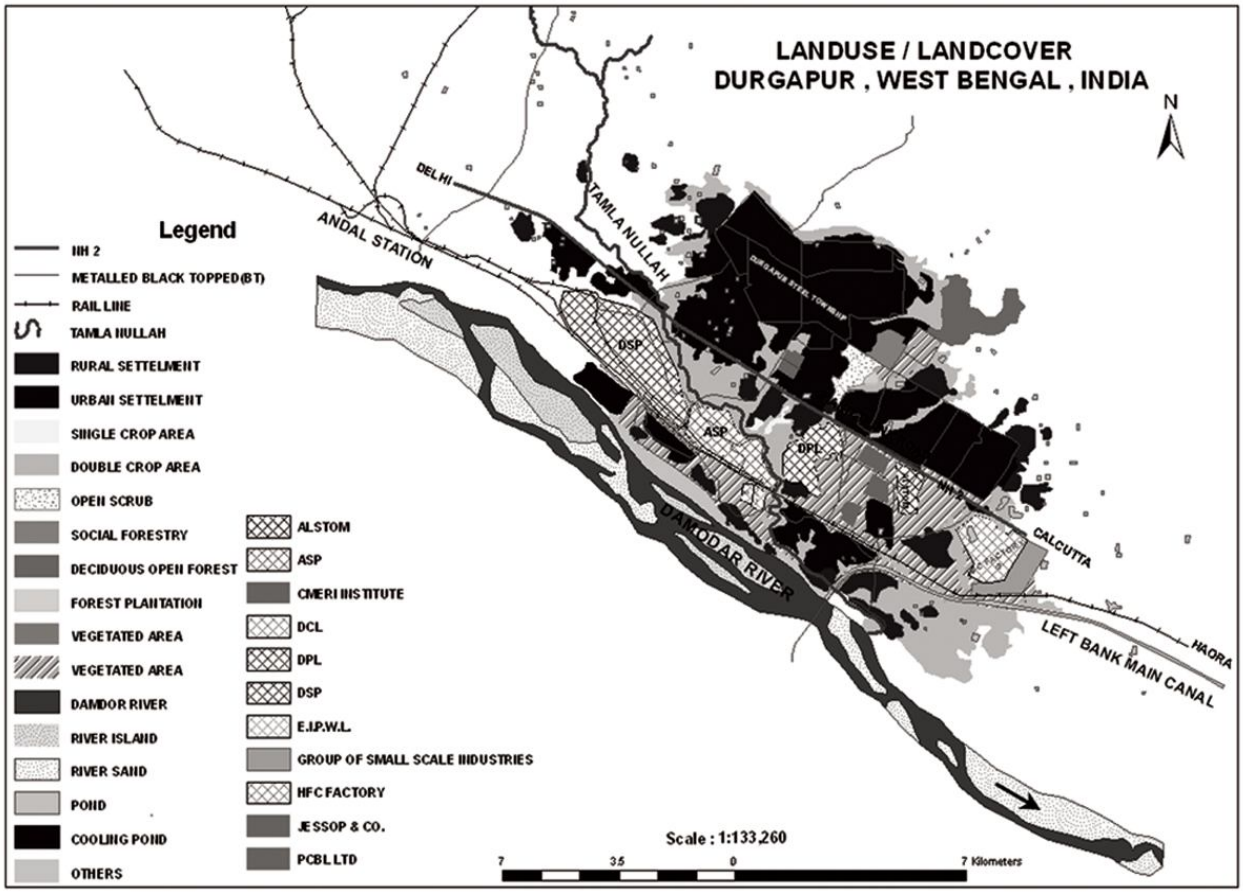


Fig. 4: Landuse/Landcover Map of the study area

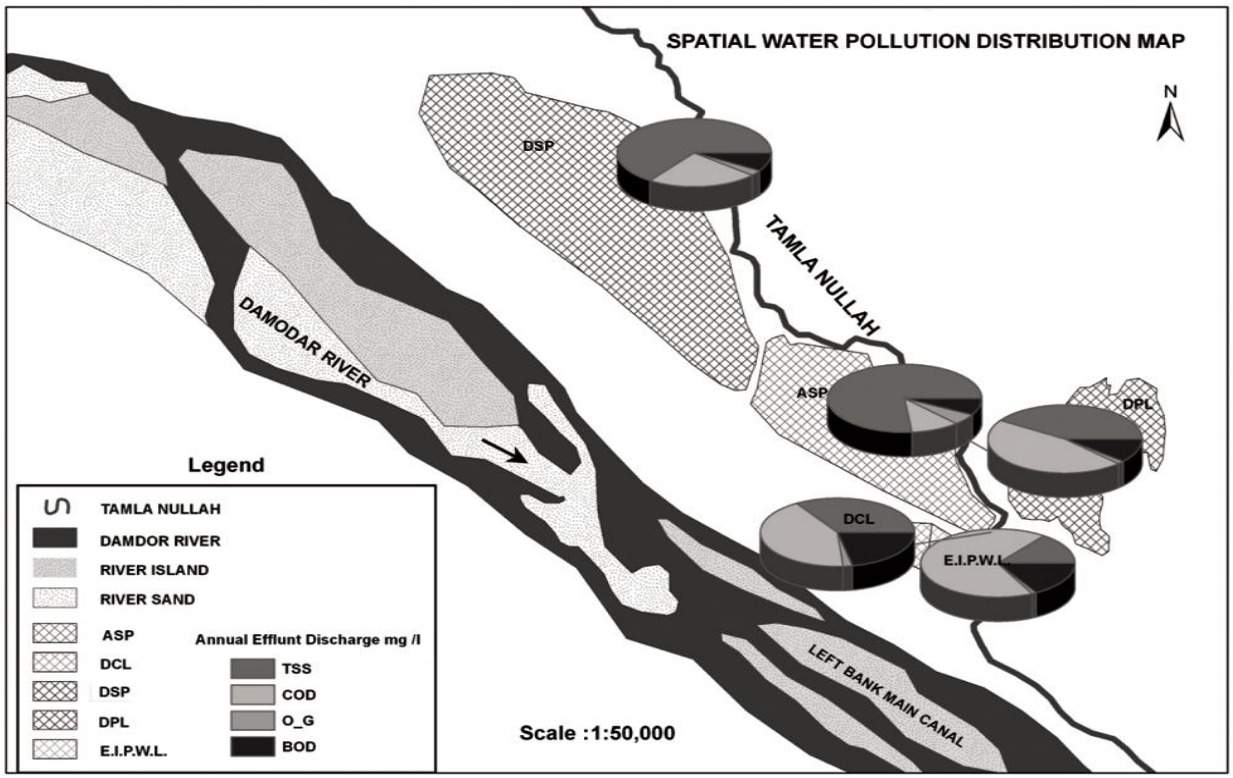


Fig. 5: Spatial and quantitative distriubution of annual effluent discharge in Tamla Nullah by the studied industries of Durgapur

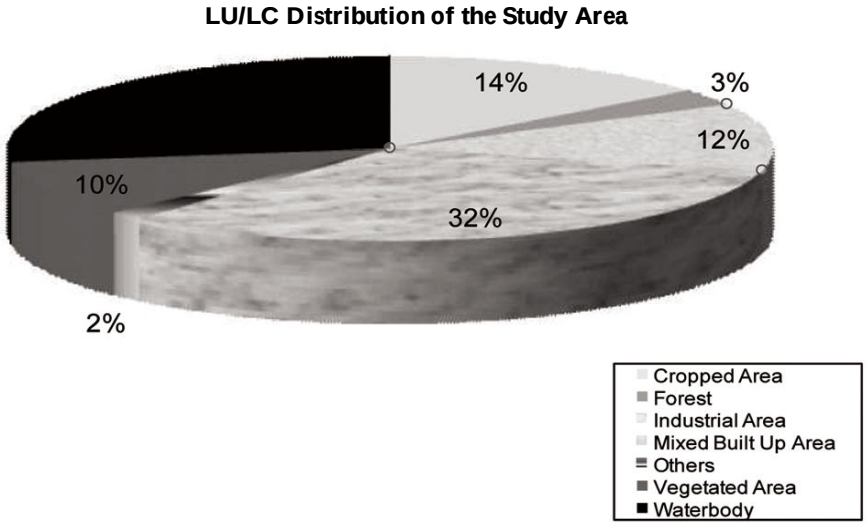


Fig. 6: Landuse / Landcover Distribution of the studied area

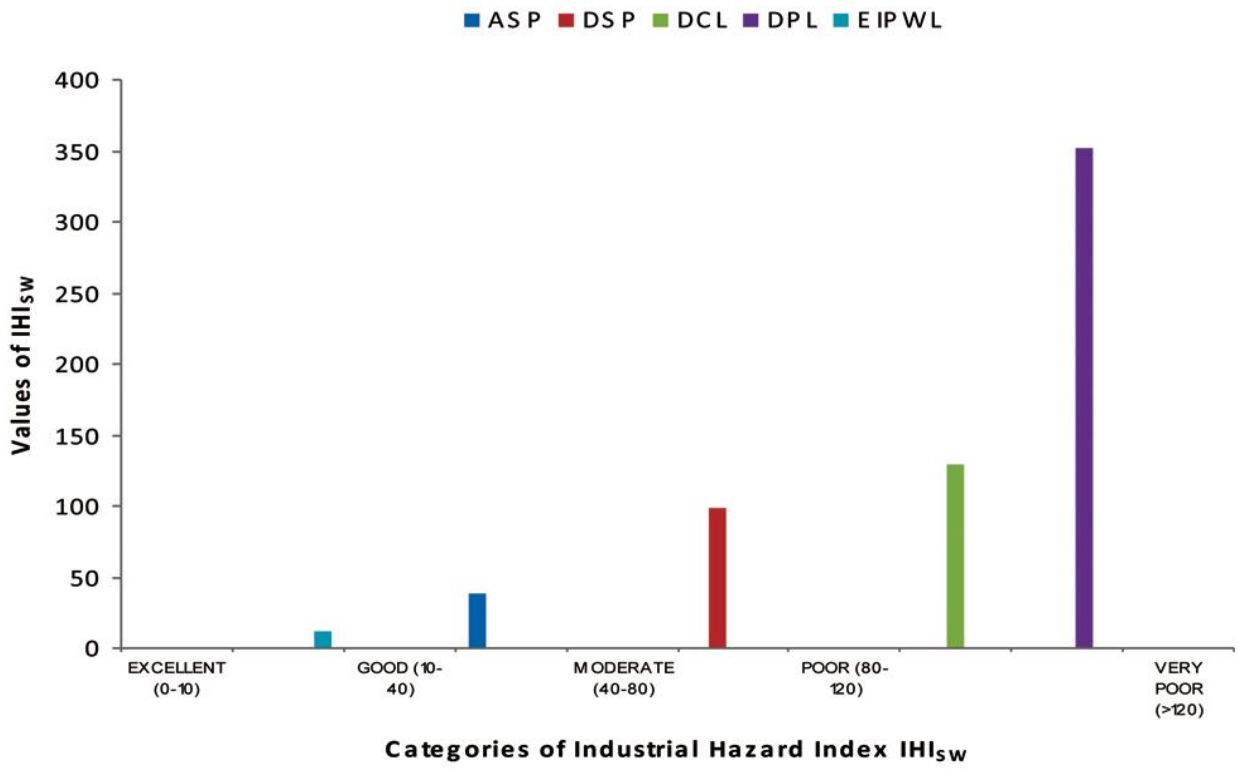


Fig. 7: Industrial hazard indices of the outfall points of the five industries studied

Table 2: Sampling sites for testing groundwater, soil, and vegetations

Segment of Tamla nullah	Names of the places	Location	Pollution status
Upper (A)	Tamla village	North-western part of the Durgapur city	No outfalls, Unpolluted
Middle (B)	Bhiringi Municipal area	Immediate vicinity of the Durgapur Steel Plant and other industries	Outfalls, Polluted with effluent discharge load
Lower (C)	Madhyamana	Eastern vicinity of Durgapur, confluence of Tamla nullah and river Damodar	No outfalls, Polluted with entire downstream effluent load

Table 3: Seasonal variation of surface water quality parameters in the selected outfalls of different industries

Selected outfalls of different industries	Parameters	Std. values	Mean Concentration mg/Ltrs. January	February	March	April	May	June	July	August	September	October	November	December
DSP	pH	7.0-8.5	8.0	7.50	7.80	7.60	7.40	7.8	7.40	8.0	7.15	7.94	7.0	6.38
	TSS	100	16.3	20.3	50.7	13.7	61.7	50.8	32.4	13.3	35.5	35.9	32	58.4
	COD	250	8.3	8.2	16.0	16.0	18.5	15.2	13.5	7.0	28.9	28	16.6	*16*
	BOD	30	3.8	4.7	2.6	1.8	1.5	1.23	3.0	5.74	8.5	*6.0*	2.15	3.4
	O&G	10	1.6	1.3	2.0	1.0	1.2	0.86	0.7	1.0	1.25	1.3	1.2	1.7
ASP	pH	7.0-8.5	7.57	7.20	7.30	6.04	6.5	7.54	7.0	7.00	7.04	7.35	7.6	7.45
	TSS	100	12.4	17.0	28.4	5.6	15.0	11.6	22.0	11.6	16.0	16.2	16.7	14.8
	COD	250	1.85	2.8	4.0	1.44	1.8	1.0	3.5	5.2	1.13	2.25	2.9	2.12
	BOD	30	0.8	1.36	1.78	0.76	1.0	0.98	1.11	2.36	0.8	1.2	1.13	1.06
	O&G	10	0.4	1.5	0.6	1.64	0.73	1.0	0.65	0.55	0.36	1.2	1.5	1.1
EIPWL	pH	7.0-8.5	8.9	8.9	7.8	7.5	8.6	8.1	7.6	7.8	8.1	8.6	8.65	8.8
	TSS	100	15.3	18	22	6.4	25.0	31.7	52.6	58	26.34	16	19.25	23.2
	COD	250	96.03	150.92	187	216.4	167.9	227.8	177.4	127.6	136	167.5	105.2	116.4
	BOD	30	21.2	47.5	27.3	18.34	48.6	61.6	39.2	25	23.5	4.8	19.2	30.2
	O&G	10	2.11	1.36	1.44	3.32	3.66	2	1.39	2.23	1.99	1.32	3.31	4.7
DCL	pH	7.0-8.5	8.2	7.36	7.8	7.9	7.1	8.3	8.1	8.1	7.43	8.21	7.67	8.7
	TSS	100	11.5	10.2	11.0	12.5	36	20.8	10.6	12	11.3	10.5	12.6	27.3
	COD	250	13.03	16.1	16.6	19.5	21.6	27.8	14.2	13.8	13.42	16.5	17.3	14.7
	BOD	30	7.2	10.4	11.3	12.4	9.5	15.9	11.3	9.2	6.4	5.8	2.2	3.6
	O&G	10	1.14	1.28	1.36	1.01	1.07	1.21	1.05	0.87	1.02	1.37	1.25	1.17
DPL	pH	7.0-8.5	6.8	5.9	7.2	7.4	7.6	8.1	7.5	6.5	7.4	8.1	8.9	7.2
	TSS	100	25.6	30.13	13.7	27.5	20.5	18.6	222.4	35.2	42.5	56.3	22.4	43
	COD	250	43.4	81.4	34.8	48.2	46	39.3	27	53.5	47.2	56.8	76.3	51.7
	BOD	30	12.5	13.12	3.71	5.0	10.4	13.39	3.9	6.8	9.2	12.5	11.5	11.7
	O&G	10	1.23	3.5	1.22	2.0	2.16	1.14	0.37	0.96	1.11	1.25	1.02	0.8

Table 4: Analysis of ground water of the three experimental sites (Data: Mean ± SE)

Sl. No.	Parameters	Tamla Village	DSP Out fall Point Court More, Bhiringi	Madhya Mana	LSD	ISI Standard
1	pH Value	<0.1± 0.3	6.24 ± 0.3	6.33 ± 1.4	0.7	6.5-9.2
2.	Electrical Conductivity (µs/cm)	289 ± 4.3	287 ± 7.4	290.0 ± 8.3	5.9	
3.	TSS (mg/L)	0.7± 0.3	15.0 ± 4.3	25.0 ± 3.2	3.7	
4.	TDS (mg/l)	16.0 ± 2.4	153.0 ± 18.9	186.0 ± 17.2	11.4	1500.0
5	Total Alkalinity (mg/L CaCO3)	32.0 ± 4.9	103.0 ± 6.4	115.0 ± 14 .7	17.3	
6	Total Hardness (mg/L CaCO3)	39.0 ± 3.1	124.3 ± 11.2	114.0 ± 17.9	14.7	
7	Chloride (Cl mg/L)	2.4±1.2	16.1 ± 2.4	20.0 ± 3.7	4.3	1000
8	Sulphate (SO 4mg/L)	4.2± 0.9	20.3 ± 3.2	24.8 ± 5.9	5.1	400
9	Calcium Ca mg/L)	6.1 ± 2.1	23.9 ± 3.2	32.1 ± 5. 8	5.2	200
10	Magnesium (Mg mg/L)	1.4 ± 1.0	10.2 ± 2.0	15.6 ± 3.1	2.7	100
11	Iron (Fe mg/L)	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	0.2	
12	Lead (Pb mg/L)	<0.01	<0.01	<0.01	-	
13	Copper (Cu Mg/L)	0.02 ± 0.0	0.03 ± 0.01	0.03 ± 0.01	0.01	
14	Zinc (Zn mg/L)	0.001 ± 0.0	0.82 ± 0.3	0.87 ± 0.02	0.02	
15	Sodium (Na mg/L)	0.9 ± 0.01	7.4 ± 1.0	9.0 ± 1.4	1.9	
16	Potassium (K mg/L)	0.03 ± 0.01	1.0 ± 0.01	1.0 ± 0.2	0.01	
17	Phosphorus (P mg/L)	<0.2	<0.2	<0.2	-	
18	Nitrate (N mg/L)	0.07 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.02	45
19	Flouride (F mg/L)	<0.1			-	1.5
20	Phenolic Compound (mg/l)	0.001 ± 0.0	0.08 ± 0.01	0.07 ± 0.02	0.01	
21	Cadmium (Cd mg/L)	<0.01	<0.01	<0.01	-	
22	Chromium (mg/L)	<0.01	<0.01	<0.01	-	

Table 5: Physico-chemical analysis of soil of the three experimental sites (Mean ± 1 SE)

Sl	Parameters	Tamla Village	DSP Out fall Point Court More, Bhiringi	Madhya Mana	LSD
1	pH Value (1 : 2.5)	6.9 ± 0.2	6.32 ± 0.4	6.47 ± 0.7	0.8
2	Particle S ize				-
	Clay %	20.2 ± 2.1	22.2 ± 3.1	20.2 ± 3.4	
	Slit %	23.4 ± 3.9	27.6 ± 2.1	28.5 ± 5.3	
	Sand%	56.4 ± 5.3	50.2 ± 4.4	49.5 ± 6.9	
3	Textural Class	Sandy Loam	Loam	Loam	-
4	Organic Carbon (%)	1.4 ± 0.2	1.3 ± 0.3	1.3 ± 0.2	0.3
5	Available Nitrogen (mg/kg)	68.7 ± 8.9	59.2 ± 9.4	57.75 ± 9.2	9.9
6	Available Phosphorus (mg/kg)	130.3 ± 12.8	118.0 ± 16.2	115.3 ± 18.7	12.3
7	Cadmium (Cd mg/kg)	<5.0	<5.0	<5.0	-
8	Chromium (Cr mg/kg)	4.6 ± 1.7	24.0 ± 6.2	32.0 ± 4.9	5.4
9	Manganese (Mn mg/kg)	17.4 ± 3.2	149.2 ± 2 1.0	174.0 ± 16.4	28.2
10	Iron (Fe mg/kg)	22.9 ± 4.2	27.2 ± 4.9	32.5 ± 4.3	6.9
11	Lead (Pb mg/kg)	3.1 ± 1.2	14.2 ± 4.9	16.0 ± 2.4	4.3
12	Copper (Cu mg/kg)	6.1 ± 1.2	29.0 ± 2.4	32.0 ± 3.2	4.3
13	Zinc (Zn mg/kg)	3.1 ± 1.2	15.2 ± 3.2	17.0 ± 3.8	5.4

Table 6: Mineral matter concentration in different vegetables at the three experimental sites (Data : Mean ± 1 SE)

Sl	Mineral Matters	Tamla Village					DSP Out fall Point Court More, Bhiringi					Madhya Mana				
		Brinjal	Palak	Cabbage	Chilli	Cauliflower	Brinjal	Palak	Cabbage	Chilli	Cauliflower	Brinjal	Palak	Cabbage	Chilli	Cauliflower
1	Iron (Fe mg/kg)	24.2 ± 3.4	170 ± 24.0	100.0 ± 18.0	80.6 ± 11.1	120.0 ± 11.0	31.4 ± 2.1	195.0 ± 20.0	118.0 ± 12.0	96.3 ± 12.1	125.0 ± 12.0	36.1 ± 9.2	456.0 ± 30.0	407.4 ± 22.0	113.4 ± 23.1	392.0 ± 18.7
2	Copper (Cu mg/kg)	1.1 ± 1.0	1.1 ± 0.4	1.0 ± 0.2	1.6 ± 0.3	1.0 ± 0.2	3.4 ± 1.0	9.3 ± 2.3	23.1 ± 3.6	9.3 ±2.4	20.0 ± 3.4	4.3 ± 0.1	16.3 ± 3.2	28.4 ± 9.2	13.2 ± 3.6	25.0 ± 3.8
3	Calcium Ca mg/kg)	5.0 ± 1.4	7.2 ± 1.9	17.0 ± 3.0	6.4 ± 2.3	15.0 ± 3.1	5.3 ± 1.4	10.4 ± 2.1	9.2 ± 2.3	7.9 ± 2.1	10.0 ± 2.1	6.2 ± 2.9	8.4 ± 2.7	13.3 ± 3.1	9.6 ± 2.1	20.0 ± 3.1
4	Magnesium (Mg mg/kg)	<5.0	7.9 ± 2.2	7.2 ± 1.2	4.0 ± 1.3	6.0 ± 1.4	<5.0	8.4 ± 2.4	3.4 ± 1.2	5.4 ± 2.1	3.0 ± 1.3	<5.0	3.7 ± 1.1	3.7 ± 1.3	12.5 ±3.1	4.0 ± 1.0
5	Zinc (Zn mg/kg)	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
6	Chromium (Cr mg/kg)	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
7	Cadmium (Cd mg/kg)1	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
8	Manganese (Mn mg/kg)	3.2 ± 1.0	5.9 ± 0.9	7.2 ± 1.3	<5.0	11.0 ± 3.1	5.2 ± 0.4	8.0 ± 1.0	12.0 ± 1.3	<5.0	14.0 ± 2.4	6.9 ± 2.1	15.0 ± 2.0	17.3 ± 3.4	<5.0	17.0 ± 4.9

Conclusion

In the study area human populations and ecological receptors are in continuous contact with industrial discharges because they are present in air, water, soil and food. These links manifest themselves through their effect on human health and ecology. Therefore, the link demands exact identification of the causes for formulation of sound environmental planning thereby necessitating studies and analysis of environmental hazards of the area. To provide a comprehensive but easy to use tool in the assessment and evaluation of water quality in this industrially polluted area, the concept of industrial hazard index (IHI) has been used. This index coupled with available pollution data after integration spatially in thematic maps prepared by interpretation of satellite imagery using a computer based analytical system of GIS will help in the environment planning and decision support system of the study area. The study results in Industrial hazard index mediated spatial demarcation of pollution stretch on GIS platform for stringent future environmental amendments.

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International Journal of Agricultural & Forest Meteorology	2	8000	800
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International Journal of History	2	6000	500
International Journal of Neurology and Neurosurgery	2	9000	276
International Journal of Political Science	2	5000	400
International Journal of Practical Nursing	3	3000	70
International Physiology	2	6500	240
Journal of Animal Feed Science and Technology	2	4000	280
Journal of Cardiovascular Medicine and Surgery	2	9000	238
Journal of Orthopaedic Education	2	4500	190
Journal of Pharmaceutical and Medicinal Chemistry	2	15000	350
Journal of Psychiatric Nursing	3	3000	70
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Original Article

Comparative Study of Electrophoretic Patterns of Proteins in the Parotoid Gland Secretion and its Extract of *Bufo melanostictus* (Schneider) through SDS-PAGE and Urea SDS-PAGE

Raju Neerati*, Thirupathi Koila*, Samatha Talari,Venkaiah Yanamala***

Abstract

The present study was under taken to analyze the qualitative analysis of the comparative study of eletrophoretic patterns of proteins in the parotoid gland secretion and its extract in terrestrial toad *Bufo melanostictus* (Schneider). The protein patterns indicated that the secretion has less number of protein bands compared to the gland extract. The patterns of protein bands were observed in the parotoid gland extraction of *B. melanostictus* through Sodium Dodecyl Sulphate and Poly Acrylamide Gel Eletrophoresis (SDS-PAGE) indicated a distinct of four protein bands and some additional bands with poor resolution and was compared with Urea SDS-PAGE. The protein bands indicated a distinct of six protein bands with some other additional bands in Urea SDS gels. In the parotoid gland secretion two protein bands in SDS-PAGE, where as four protein bands were observed in the parotoid gland secretion of *B. melanostictus* through Urea-SDS PAGE. The protein subunit patterns were identified by using standard marker protein and R_m values were calculated accordingly. The eletrophoretogram both the SDS-PAGE & Urea SDS-PAGE patterns of parotoid gland secretion and its extract showed homology in protein bands with minor variations.

Keywords: *Bufo Melanostictus*; Parotoid Gland; Protein Patterns; Urea SDS PAGE; Electrophoresis.

Introduction

Amphibians are treated as bio-indicators of aquatic and terrestrial ecosystem owing to their sensitivity to changes in the environment [1, 2, and 3]. Amphibians like toads are characterized by the presence of cutaneous glands spread over the skin. Basically two different types of glands developed in the amphibian skin i.e., I) Mucus secreting glands generally associated to maintain the humidity and cutaneous respiration and to protect the skin from mechanical damages and prevent microbial settlement on the skin; these glands secrete glycoprotein rich material which plays an important role in defense mechanism. II) Granular glands generally associated with chemical defense against predators and microbial infection [4-6]. The product secreted by such glands contain a wide variety of rich components like biogenic amines, bufo toxins, oligo peptides, proteins, guanidine derivatives, steroids and alkaloids in terms of pharmacological effects [4, 7-9]. The epidermal glands in amphibians are more evolved and are alveolar glandular cells and open on to the surface of the skin through ducts. In toads these glandular cells form the parotoid glands located between eyes and tympanum [8, 10]. The venomous secretions of the parotoid glands of the *Bufo* species are known to contain several bioactive compounds [11] and were used by Chinese, Indian traditional medicinal practice and Japanese physicians for centuries as folk medicines like “kyushin” and “Chan Su” [8,11]. The granular secretions are known to be secreting a variety of compounds which are species specific [12, 13].

The toad *Bufo melanostictus* (Schneider) is a very common amphibian in India So far, there are few reports on the protein patterns of *B. melanostictus* through Urea-SDS-PAGE. The present investigation has been undertaken for the comparative study of electrophoretic patterns of proteins in the parotoid gland secretion and it’s extract of *Bufo melanostictus* (Schneider) through Urea and SDS-PAGE in order to understand their possible defense role against

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microbial infections.

Material and Methods

Animal Materials Chosen for Study

The toads (7cm to 10cm in length, weighing about 45-70 grams) were collected from the vicinity of Kakatiya university hostel buildings, Warangal, Telangana, India.

Extraction and Collection of Samples

The parotoid glands were gently pressed to release the secretions. The secretions were collected in ice-jacketed containers. After collecting the secretions the gland was dissected out and were blotted free of blood clots and other adherent, tissues were weighed to the nearest milligram and gland as well as secretions were homogenized (10%) in 0.01M Tris-HCl buffer (pH 7.0) containing 0.1% Sodium Dodecyl Sulphate (SDS) and 0.9% NaCl the extracts were centrifuged at room temperature (30±2°C).

Experimental Procedure

The electroporetic patterns of proteins of parotoid gland secretion and its extract of *Bufo melanostictus*(scheinder) using SDS-PAGE and Urea-SDS-PAGE were performed through methods as described by Lammeli's [14] and Anderson et al.[15]. Thin layers (1.5mm thick) polyacrylamide slab gels were prepared by using the glass plates. The protein for electrophoretic studies was extracted by homogenizing the parotoid gland secretion and its extract (10%) in 0.01M Tris-HCl buffer pH (7.0) containing 0.1% sodium dodecyl sulphate (SDS) and 0.9% NaCl. The extracts were centrifuged at 2,000 rpm for 20 minutes in a clinical centrifuge at room temperature (30±2°C) and the supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, mercaptoethanol and bromophenol blue as the tracking dye; 0.1 ml (5 mg) of the parotoid gland secretion and its extract was loaded on to the separating gel directly. The electrode buffer, 0.025 M Tris and 0.192 M glycine, was used for Laemmli's method, whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with concentrated HCl as upper chamber buffer and 1M Tris, 0.2 % SDS adjusted to pH 7.8 with concentrated H_2SO_4 for the Urea SDS-PAGE. A constant current of 50 volts for the first 15 minutes followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8 cm from the origin. A solvent containing 0.25% Coomassive brilliant blue in methanol: water: acetic acid (5:5:1) was used for the staining proteins separated on gel by Laemmli's method. Silver nitrate [16] was used for staining proteins separated by the method of Andeson et al [15].

Standardization of Protein Bands

The molecular weight standards were used in comparing the variations noticed in the SDS-PAGE were the low molecular weight protein standards (14 to 66 KDa) from the SIGMA-Chemical company from USA and the Urea-SDS-PAGE were of molecular weight protein standards (14 to 200 KDa) from the Bio-Rad-Chemicals company from USA.

Results

The protein patterns of *Bufo melanostictus* observed in parotoid gland extract and its secretions and their relative mobility (Rm) are presented in Fig.1 and Table 1 respectively. The protein patterns observed on SDS-PAGE stained with Coomassie brilliant blue indicated distinct differences in the mobility of some bands of the parotoid gland extract and its secretion. Comparison of the protein bands of various regions with standard marker proteins revealed that the variation is higher in the regions of slow moving zones "A" (mol wt.66KDa) and those with fast moving zones "C" (mol.wt. 24KDa,14.2KDa). The pattern obtained in the middle region "B" (mol.wt. 45KDa, 36KDa) is more (or) less similar in secretion and gland extract.

The electrophoretogram obtained revealed that there is a decrease in the intensity of protein bands of parotoid gland secretion compared to protein bands of parotoid gland extraction. A protein band with Rm value 0.11 (nearer to molecular weight 66 KDa) showed decrease in the intensity in parotoid gland secretion whereas high intensity in parotoid gland extraction. The Rm values of protein bands 0.12, 0.21, 0.25 in between the molecular weight 66 KDa-45 KDa completely disappeared in parotoid gland secretion (Zone. A) in slow moving zone compared to parotoid gland extraction. The Rm value of protein bands 0.31, 0.50 and 0.52 in between the molecular weight of 45 KDa-35 KDa were completely absent in parotoid gland secretion (Zone. B) in the middle region compared to parotoid gland extraction. The protein bands of Rm value of 0.84 was absent in the parotoid gland secretion, whereas a protein band with Rm value 0.85 was absent in the parotoid gland extraction nearer to molecular weight 14.5 KDa in the fast

moving zone (Zone. C).

The patterns of proteins of parotoid gland extract and its secretion on Urea SDS-PAGE indicated a less number of protein bands in parotoid gland secretion with decrease in the intensity compared to parotoid gland extraction. In the slow moving zone "A" (mol.wt.200-97KDa) a protein band with Rm value 0.21 (molecular weight 116KDa) showed low intensity in parotoid gland extract and not observed in parotoid gland secretion. A protein band with Rm value 0.28 (molecular weight 97 KDa) was observed in both parotoid gland extract and its secretion. The Rm value 0.42 protein band was observed only in parotoid gland extract and the Rm value 0.45 protein band was observed only in parotoid gland secretion in between the molecular weight 66-45KDa.Protein bands with Rm values 0.54, 0.60 observed in parotoid gland extract and the Rm with 0.58 in parotoid gland secretion nearer to the molecular weight 45KDa in the middle region "B" (mol.wt. 66-45KDa). In the fast moving zone "C" (mol.wt. 31-14KDa) the Rm value with 0.68 (molecular weight 31KDa) was observed in the parotoid gland extract which was disappeared in parotoid gland secretion. The Rm value 0.78 (molecular weight 22KDa) was observed in parotoid gland secretion and disappeared in gland extraction. The Rm value 0.85 (molecular weight 14KDa) was observed in both parotoid gland secretion and its extract (Zone. C).

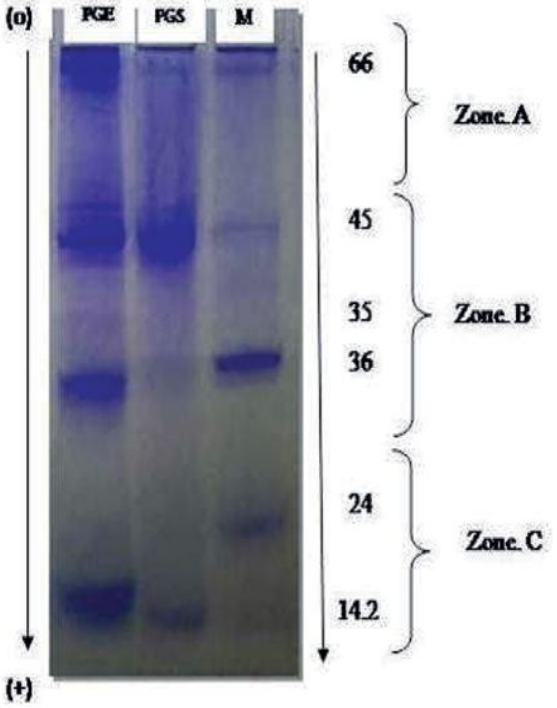

Figure-1 SDS-PAGE Electrophoretic Patterns of Proteins of *Bufo melanostictus*
Parotoid Gland Extract and Secretion stained with Coommassie brilliant blue.
Left lane indicates (M) mol. weight strands (66-14.2 KD.) 'A', 'B', 'C' zones.
PGE = Parotoid gland Extract.
PGS = Parotoid gland Secretion.
M = Molecular weight standards (14 to 66 KD).
Zone A = mol. wt. 66 KD.
Zone B = mol.wt.45 KD, 36 and 35 KD.
Zone C = mol.wt. 24 KD, 14.2 KD.
O = origin.
(+) = Anode.
↓ = Direction of run.

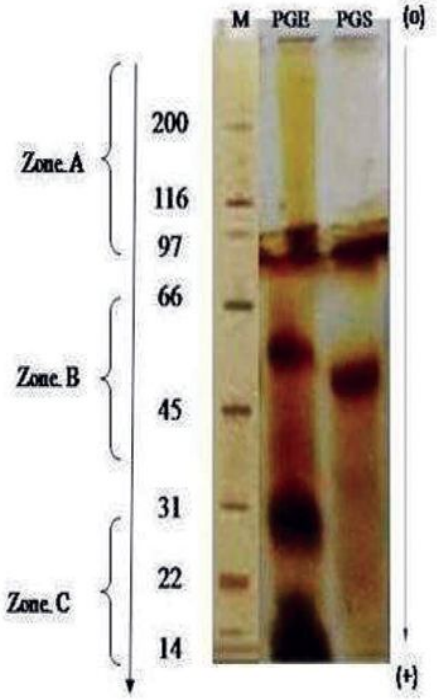

Figure -2 Urea-SDS-PAGE Electrophoretic Patterns of Proteins of *Bufo melanostictus*
Parotoid Gland Extract and its Secretion stained with Silver nitrate
Right lane indicates (M) mol. weight strands (200-14 KD.) 'A', 'B', 'C' zones,
PGE = Parotoid gland Extract,
PGS = Parotoid gland Secretion,
M = Molecular weight standards (14 to 200 KD),
Zone A = mol. wt. 200 KD, 116 KD and 97 KD
, Zone B = mol.wt.66 KD and 45 KD,
Zone C = mol.wt. 31KD, 22 KD and 14 KD;
O = Origin,
(+) = Anode,
↓ = Direction of run.

Table 1: Rm values of parotoid gland secretion and extract of Bufo melanostictus (Scheinder) on SDS-PAGE

Marker	Parotoid Gland Extract	Parotoid Gland Secretion
.07	0.07	0.07
-	0.11	-
-	0.12	-
-	0.21	-
-	0.25	-
0.28	0.28	0.28
-	0.31	-
0.50	0.50	-
0.52	0.52	-
0.64	-	-
-	0.84	-
0.85	-	0.85

Table 2: Rm values of parotoid gland secretion and extract of Bufo melanostictus (Scheinder) through Urea SDS-PAGE

Molecular Marker Standards	Parotoid Gland Extract	Parotoid Gland Secretion
0.14	-	-
0.21	0.21	-
0.28	0.28	0.28
0.35	-	-
-	0.42	-
-	-	0.45
0.50	-	-
-	0.54	-
-	-	0.58
-	0.60	-
0.68	0.68	-
0.78	-	0.78
0.85	0.85	0.85

Discussion

The patterns of protein bands observed in the parotoid gland extract and its secretion of the toad on SDS-gel indicated a distinct of four protein bands with several additional bands with poor resolution, exhibiting minor variations in the slow moving zone whereas a distinct of two protein bands were observed in the parotoid gland secretion. Therefore, the protein patterns observed in the parotoid gland extract and its secretion are more or less similar with minor variations.

The presence of protein bands with identical mobility in the secretions and gland extracts, indicate the similarity of proteins secreted probably by granular cells of epidermis. When the gland is pressed the secretion is released in the form of sticky fibrillar material [17]. Various authors have reported that the alkaloids and steroids present in *Bufo* as toxic and anti feeding agents, acting as a major chemical defense strategy against predators, and also act on the cardiovascular system by raising the blood pressure and / or increasing the contraction force of the heart [18-22]. The secretary proteins exist as coiled filaments within epidermal granular cells [23]. The presence of these arrays of proteins in Bufo parotoid gland secretions suggests a more complex role for these secretions than simply anti-predator defense. The peptides found in various species of toads and frogs which possess antimicrobial activities are of a much smaller molecular size range than encompassed by SDS-PAGE as used here. For instance, the magainins found in skin secretions of *Xenopus* are typically of 21-26 amino acid residues in length [24].

The electrophoretogram obtained from parotoid gland extract and its secretions of the protein patterns through Urea SDS-PAGE in *Bufo melanostictus* and their relative mobilities are presented in Figure 2 and Table 2, respectively. The protein patterns observed on Urea SDS-PAGE stained with Silver nitrate stain indicated distinct differences in the mobility of some bands of the parotoid gland extract and its secretion. The protein band comparison of various regions with standard marker proteins revealed that the variation is higher in the fast moving zone "C" (mol.wt. 31-14KDa) and those with middle region "B" (mol.wt. 66-45KDa). The pattern observed in the slow moving zone "A" (mol.wt. 200-97KDa) is more or less similar in the secretion and gland extract.

The pattern of proteins observed in the parotoid gland extract and its secretion of toad observed on Urea-SDS gel indicated a distinct of six protein bands and some additional bands with weak staining on the gel in fast moving zone, whereas a distinct of four protein bands were observed in the parotoid gland secretion and one additional band with weak staining on the gel in slow moving zone. Therefore, the protein patterns of parotoid gland extract as well as the gland secretions of the toad exhibited some regions of similarity (Fig 2).

Conclusion

The results of present investigation reveals that the analysis of protein patterns of *Bufo melanostictus* on SDS-PAGE and Urea-SDS gel, in spite of some minor differences in the total protein concentration and

relative concentration within the same sample, would lead to the conclusion that the secretions are very similar among themselves in *Bufo melanostictus.*

In view of the above results it can be concluded that the exudates of toad parotoid gland revealed that the presence of protein bands with identical mobility both in secretion and gland extract indicating homology of cell lines and its secretion in *B. melanostictus*.

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Original Article

Soil Composition and Nutrient Dynamics of Some Phytoplankton's of River Jalangi, Nadia, WB

Monojit Ray*, Koushik Sengupta**

Abstract

The river Jalangi was selected for the present studies not only to evaluate the increasing rate of pollution but also its effects on aquatic micro flora, because people incognito often use aquatic plants as vegetables, which are in turn the habitat of those microbes that may in turn cause severe human diseases. The river water contamination with hazardous waste and wastewater is becoming a common phenomenon. The water quality and human health are closely related. The domestic waste from each building along with the effluent of small scale industries is disposed off into the open drains and gutters which ultimately enter into the river. Thus, all these factors might have an effect on the soil composition and nutrient dynamics of phytoplankton. In our present study we tried our level best to sort out this fact by selecting the dominant phytoplankton of river Jalangi and their nutrient dynamics.

Keywords: Jalangi; Soil Composition; Nutrient Dynamics; Phytoplankton.

Introduction

The Jalangi, prime river of district Nadia is the main water source of the district along with its branch and ultimately merge into Bhagirathi at Nabadwip. The river contain plentiful algae, phytoplanktons and submerged aquatic herbs as, sufficient dissolved oxygen, carbondioxide and bicarbonate ions etc, present in the river water. This river is utilized variously by the people incognito. The pattern of algae, phytoplanktons and submerged aquatic herbs depends on majorly the bottom soil composition of a river. Moreover, it is a hard task to draw the interrelationship between the different biogeochemical cycles and that is why we shall treat only examples of the interrelationships between the cycles of phosphorus, nitrogen, carbon, sulphur. All of these are elements essential for the nutrition of plants. Algae phytoplanktons and submerged aquatic plants, the element carbon is derived from carbondioxide, carbonate ions, bicarbonate ions present in water. For algae phytoplanktons and submerged aquatic plants nitrogen is the most abundant element after carbon, oxygen and hydrogen. They derive the element from nitrate ion, nitrite ion, ammonium ions present in river water and bottom soil. Phosphorous occurs in river water as orthophosphate and in organic combinations. In fresh water sulphur is present as sulphate.

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Materials and Methods

Temporary slides were prepared for different algae like *Anabaena, Spirogyra* etc. with the help of lactophenol- cotton blue staining method. For aquatic macro flora morphological and chemical analysis was done. Elemental analysis (C, H, N and S) were done using Parkin Elmer CHN analyser machine at IACS, Jadavpur, Kolkata 700032. Analysis of Sodium, Potassium, Calcium, Total phosphorous, Total nitrogen , Boron, Iron and silica were done using standard methods using titrimetric methods, flame photometric methods, spectrophotometric methods etc. The analysis of the soil samples were carried out in the Department of Chemistry lab of Nabadwip Vidyasagar College. Some analysis were performed in outside laboratory (Scientific Research Laboratory, Santoshpur, Kolkata).

The Jalangi river bottom soil samples were collected from ten sampling sites, namely Taranipur, Chapra,

Haranagar, Anandanagar Ghurni, Krishnanagar kadamtala ghat, PWD more (Rail Bridge), Char-sambhunagar, Hulorghat(Mayapur). Soil samples were collected from different depths of sampling sites, by expert swimmers. Flora specimen collections were a continuous process and were done using hiring boat service. The study period is chosen as pre-monsoon due to stedy-equlibria among bottom soil-river water-ecosystem though river ecosystem is a lotic ecosystem.

Results

Table 1: Pre-monsoon data of some elements from soil of Janalgi and their relative percentage in different plant groups

Plant groups	C%	H%	N%	S%
Spirogyra	20.86	2.97	1.51	1.26
Anabaena	12.21	4.71	1.27	0.77
Alternanthera	43.88	5.89	3.21	0.94
Cyperus	34.40	4.81	1.94	1.09
Jussia	34.72	4.92	2.55	0.92
Saggitaria	42.26	5.89	3.52	1.07

Table 2: Average Pre-monsson data from both water and soil of Jalangi of available N,P,K and B

Jalangi water	(mg/Liter)	Jalangi soil	(mg/Kg)
Available nitrogen	≤ 0.1	Available nitrogen	161.5
Available phosphorus	0.054	Available potassium	179.6
Available potassium	4.32	Available phosphorus	49.5
Available nitrate	0.45	Available boron	18.4

Table 3: Average Dry Bottom Soil Composition of River Jalangi (g/Kg)

Available Nitrogen (as N)	**0.1615**
Available Potassium (as K)	0.1796
Available Phosphorous (as P)	0.0495
Silica(as SiO2)	664
Iron (as Fe)	37.815
Calcium (as Ca)	4.1014
Sodium (as Na)	0.476
Boron (as B)	0.0184
Carbon (as C)	9.7
Hydrogen (as H)	0.9
Sulphur (as S)	1.1

Discussion

The most abundant component of bottom soil is silica, i.e., 66.4% of the soil contain silica. Iron, carbon and calcium are also present in significant amount.

In our study a number of observations are noticed. Firstly, the effects of Phosphorus and other elements on biogeochemical cycle and growth of phytoplanktons and secondly, their effects on the metabolisms is well marked. When phosphorus 'input' is high with respect to nitrogen, the rate of growth or production of phytoplankton populations often becomes limited by nitrogen. When this happens, nitrogen-fixing Cyanophyceae usually out compete other forms so that atmospheric nitrogen contributes to the nitrogen requirements of the plankton. It is reported that, when several whole-lake experiments in the experimental lakes area of Northwestern Ontario were designed to yield information about the interplay between phosphorus and nitrogen. For example, Lake 227 (area 5.0 ha, mean depth 4.4 m), was fertilized for 6 years with an N:P ratio of 14:1 by weight. Algal standing crops were dominated by the green alga, *Scenedesmus* (Schindler *et al.,* 1973), and no nitrogen fIxation was detectable (Flett *et al.,* 1980).

In our present study percentages of different elements utilized by a number of plant groups exhibit differences (Table 1). It clearly indicates that different plant groups are in different stages of their respective growth cycle. It also found that carbon percentage is

quite high in two angiosperms as compared to the algal members and in dicots (*AIternanthera* and *Saggitaria)* the carbon percentage is more high than the rest monocot families. It might have happened due to variations in nutrient cycle. More over the availability of various elements in river Jalangi at pre monsoon stage also varies accordingly with its soil profile (Table 2 and Table 3).

This study presents a first attempt to quantify the biogeochemical transformations and fluxes of nutrients along the river Jalagi. The biogeochemical reaction-network is coupled to the hydrodynamic and transport processes of different nutrients, indicating the level of nutrient dynamics and also the biotic response thereof. In fact the residual transport field and in-situ turnover rates control the local nutrient availabilities and the emergence of distinct spatial patterns in ecosystem structure throughout the productive period.

On the other hand, nutrient enrichment by autochthonous recycling in fresh water ecosystems or through allochthonous input by feeder rivers have been considered a major stimulant for eutrophication and a threat to dwindling water resources. As a consequence of nutrient enrichment ecosystems experienced the events of toxic cyanobacterial bloom. Again a distinct effect of phosphorus on nitrogen cycle is also revealed. When phosphorus 'input' is high with respect to nitrogen, the rate of growth or production of phytoplankton populations often becomes limited by nitrogen. When this happens, nitrogen-fixing Cyanophyceae usually outcompete other forms so that atmospheric nitrogen contributes to the nitrogen requirements of the plankton. In a recent literature review of aquatic nitrogen flxation, Flett *et al.* (1980) found that fixation became important when the N:P ratio in nutrient loading fell below 10:1 by weight. While production and growth of nitrogen-fixing blue green algae are often lower than for other forms, their colonies are typically quite large, and therefore less susceptible to grazing or other mortality factors than smaller forms (Schindler and Comita, 1972). As a result, under steady-state conditions, the total algal standing crop is usually comparable to that which develops when the supply of ionic nitrogen is large. Thus, we think our present study will open a great scope regarding the study of correlation between soil composition and nutrient dynamics of some phytoplankton in the river Jalangi under a particular climate.

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Original Article

Effect of Male Hormone (17α-Methyl Testosterone) on the Histological Changes of Male Dwarf Gourami *Trichogaster Lalius* (Hamilton, 1822)

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Abstract

The present study was conducted to know about the effect of different concentrations of synthetic androgen 17α- Methyl testosterone (MT) on histological changes in gonads of male Dwarf Gourami, *Trichogaster lalius* (Hamilton, 1822). Fishes were fed with homogenous mixture of the hormone in ethyl alcohol in its feed for 90 days. On the basis of histological study, the hormonal actions on the testicular changes were distinguished on the basis of the spermatocytes present i.e. primary spermatocytes, secondary spermatocytes and spermatozoa. In lower doses (5 mg/Kg & 10 mg/Kg of feed), the concentrations of the gonadal materials were so closely placed that the identification of different stages was found very difficult up to 45 days. In the higher dose (15 mg/Kg of feed), the gonadal materials were found less concentrated which leads a negative impact on the gonads after 60 days of treatment.

Keywords: Histological Changes; 17α- Methyl Testosterone; Synthetic Androgen; *Trichogaster Lalius.*

Introduction

West Bengal is one of the states of India having a rich wealth of freshwater resources and fish germplasm diversity. It is also a pioneer state in ornamental fish production and export. Due to congenial climatic conditions, Kolkata and its surrounding districts have emerged as promising breeding centers for ornamental fish and a considerable number of small fish farmers and amateurs are engaged in this trade. It is found that 288 exotic varieties of ornamental fish populations are in West Bengal (Bhaskar *et al*., 1989) and 52 native ornamental fishes are available here (Ghosh *et. al*., 2003).

The Dwarf Gourami *(Trichogaster lalius)* is a peaceful freshwater fish, also known as the "Dwarf Gourami". Gourami is the name used for a big variety of perciform fish characterized by flat body and two elongated rays of pelvic fins used as sense of touch. Since they reach only 2 inches, they can be housed in small tanks and are a good fish' for beginners because of their low aggressiveness, easy care and nice look. Males can be easily distinguished from females for their colors. The male is a bit bigger than the female and has turquoise and orange-red iridescent vertical bands on the entire body and on fins; its color mutations with total orange-red body and turquoise dorsal fin, or total turquoise body with just some red at the edges of the fins. The dwarf gourami female is totally silver with pale turquoise vertical stripes (Patro *et al.,* 2015).

Nakorn and Sangsri (1995) reported that the testes of control Tawes (*Puntius gonionotus*) were relatively round with a single stalk attached to peritoneum. They comprised numbers of lobules in which various stages of spermatogenic cells, spermatocytes, spermatids and spermatozoa were found. In the groups of fish being treated for 30 days three types of gonads; testes, regressed ovaries and undifferentiated gonads were observed. The testes of genetic males represented the control fish.

Manosroi *et al*., (2004) reported that the presence of spermatogonia and ovocells in the gonads were used as an indicator of males and females respectively. Intersex gonads contained both oogenic and spermatogenic tissue. Sterile gonads contained large amounts of connective tissue with numerous vessels.

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The present study was conducted to investigate the effect of MT on histological changes of *T. lalius*, i.e., primary spermatocytes, secondary spermatocytes and spermatozoa.

Materials and Methods

The study was conducted during April 2009 to July 2009 in the laboratory of Department of Fisheries Resources Management, Faculty of Fishery Sciences, Chakgaria, Kolkata to understand the effect of different concentrations of dietary administration synthetic androgen 17α-Methyl testosterone on histological changes of male Dwarf Gourami, *Trichogaster lalius* (Hamilton, 1822). The samples were collected from Gullif Street, near to Syambazar, Kolkata, and West Bengal and acclimatized to the laboratory condition by feeding with the commercially available aquarium feed (i.e. Tokyo, Japan). The fishes were acclimatized for 15 days before starting of the experiment. During the experiment fishes were fed with the hormone incorporated feed upto 3 months.

Preparation of Hormone Incorporated Feed

An androgenic steroid hormone as 17α-methyltestosterone (MT) was used in the present study. It was obtained from the (Sigma chemicals Ltd). Three different kinds of feed were prepared by adding three doses of MT as 5 mg of MT per Kg of feed, 10 mg of MT / Kg of feed and 15 mg / Kg of feed. A hormone treated feed will be prepared as described by (Killian and Kohler, 1991).

Histological Study

For observing histological change fish were sacrificed in each 15 days and microscopic slides were prepared by the following procedure of Agarwal (1996). The development stages of germ cells in the testes were studies by the following methods.

Collection and Fixation of Tissue

For histological study, the middle parts of the gonadal tissues (testes) of *T. lalius* were selected. The tissues were put into Bouin's fluid for 24-48 hours as per size of tissue (Testes).

Post Fixation Treatment & Washing

The tissues (testis) were removed from the fixatives and subjected to overnight washing with flowing clear tap water till the noxious formaldehyde odour was not remain.

Dehydration

Then tissue was treated with graded alcohols (i.e. 30, 50, 70, 90 and 100 %) to dehydrate it.

Dealcoholization

Two changes of Xylene (1 hr each) were made to clean the tissues from alcohol. For better impregnation of wax into the tissue, the xylene penetration into the tissue is very important. After xylene treatment the tissue must be transparent and should come up to float on the top.

Infiltration

Paraffin wax (melting point 50-60^{0}C) was used for infiltration of tissue. Three changes of wax (45 min each) were made to make tissue xylene free.

Embedding and Block Preparation

For the preparation of blocks, pure paraffin wax melted in water bath in between 58-60^{0}C Metal 'L' moulds was adjusted according to the size of blocking materials. The melted paraffin was taken from water bath and the blocking disc was filled. After permitting a layer of wax to be solidifying on the bottom of the disc, the completely infiltrated tissue was carefully taken from the paraffin wax and put inside the blocking disc according to the size. Care must be taken, so that the wax on the top of the disc should not be solidified during keeping the material in the blocking disc. For this reason, a heated needle or forceps was put inside the wax of the disc. After the proper positioning of the tissues, the wax inside the disc was allowed to solidify. After few minutes, the 'L' moulds were removed from the wax block. The prepared blocks were kept separately inside the labeled polythene packets.

Trimming and Sectioning

The paraffin blocks were trimmed carefully to 6 to 7 mm^2 by sharp blades. The trimmed blocks were fixed to the wooden holder with the material facing away from it. Molted wax was poured on the holder and the block was kept on it. The block was padded with more wax at the base to make it strong. After being confirm, the blocks were firmly fixed with holder, the sectioning was done by using microtome (Spencer 820 Type). Each section was cut into 5µ

thickness. The ribbons containing tissue were collected on clear glass side with the help of fine brush.

Spreading and Fixing

Glass slides were cleaned properly by concentrated sulphuric acid, soap and finally with tap water. After cleaning, the slides were air dried and a thin layer of glycerin, egg albumin was rinsed over it. Then the ribbons with materials (about 10 to 12 sections depending on the size) were spread over the clean glass slides. The tissue were made wrinkle free allowed to fix on slides by keeping them on hot plates (30^0C) for 2-5 minutes.

Dewaxing and Staining of the Tissues

Tissues fixed on slides were dewaxed with descending order of graded alcohols (100%, 90%, 70%, 50% and 30%) and stained with Haematoxylin and Eosin by using standard techniques (Agarwal, 1996). After staining the slides were air dried.

Mounting

One or two drops of mountant (D.P.X) were put on the dried slide. Then a cover slip was put over it. During putting cover slip the slide was slowly lower when the mountant would flow ahead of the descending glass without trapping air bubble between the cover slip and slide. The excess of mountant on the slides was allowed for drying.

Labeling and Storing

Labeling was done on the slide by glass marking pens to avoid future confusion. The slides were stored inside box to protect them from dust and dirt.

Microscopic Observation

The prepared slides were thoroughly observed under Advanced Trinocular Research Microscope (Olympus Model 8x51) at different magnification. The development stages of germ cells inside the seminiferous tubules of the testis were noticed carefully. Coloured microphotographs of selected histological sections were taken as and when required.

Results and Discussion

In the present study from the GnSI value it is understood that the gonadal development in the treated fishes are found better than the control. Among the treated fishes the GnSI of 10 mg / Kg was

Table 1: Histological changes in testis of *Trichogaster laliaus* during May to July 2009

Months	Control	5 mg/Kg	10 mg/Kg	15 mg/Kg
Initial	ILS was more, LW was more prominent, and PS, SS and SZ were present.			
15thday	ILS was less, LW was less, PS, SS, and SZ was present. SZ was larger in shape.	ILS was more, ILW was packed with SZ. SZ was larger in shap e.	ILS was less, LW was less prominent. PS, SS and SZ were present and smaller in size.	LW was thin, ILS was packed with PS,SS and Sertoli cells were present
30thday	ILM was more, ILS was less, L W was more prominent, PS, SS, SZ was present. SZ was larger in shape.	ILS was more, ILW was thin, PS, SS were present and smaller in size.	ILM was packed with spermatogenic cells. SZ were larger in size, PS, SS was less and smaller in size.	ILS were more, LW were thin, PS, SS were pre sent and small in size.
45thday	LW was more prominent, ILS was more, more SZ, PS and SS was present.	ILW was thin, and ILW was packed with PS, SS and less number of SZ and small in size.	ILS was more, LW was thin, PS, SS was more, and SZ was smaller in size.	LW was prominent, ILS were more, PS, SS, SZ were present and small in size.
60thday	ILS was packed with SZ, ILS was less, and LW was prominent.	ILS was less, ILM were packed with PS, SS and SZ, spermatogenic cells were smaller in size.	ILS was less, LW was very thin, and LM was packed with SZ.	ILS was less, LW was thin, LM was packed with PS, SS and SZ.SZ was larger in size.
75thday	ILS were more, LW were thin, PS, SS were more and SZ present.	ILS were less, PS, SS and SZ were smaller in size.	ILS were more, PS, SS, SZ were sm aller in size.	LW were prominent but cells were not prominent tend to sterile conditions.
90thday	ILS was more, ILM contained more PS and SS, less SZ.	ILS were less, PS,SS, SZ were smaller in size,	ILS was more spermatogenic cells were more.	LW were pro minent, ILS were more, Sterile areas were more

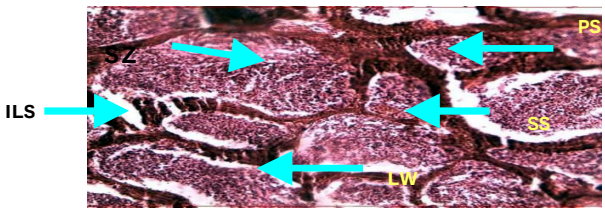
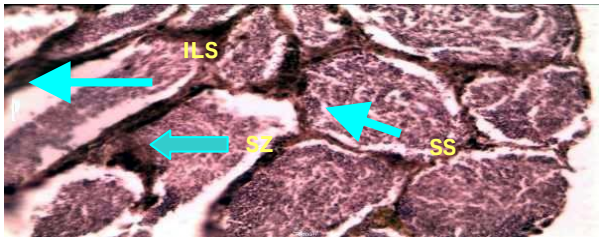


Plate-1



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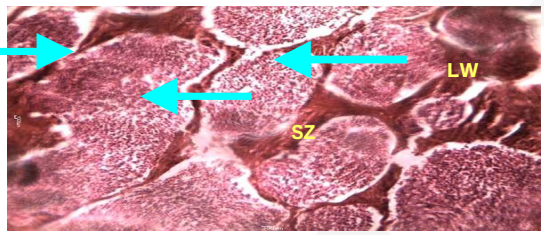

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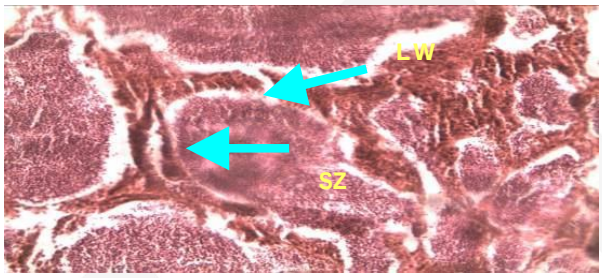

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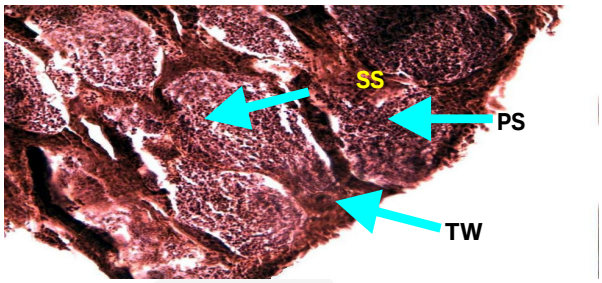

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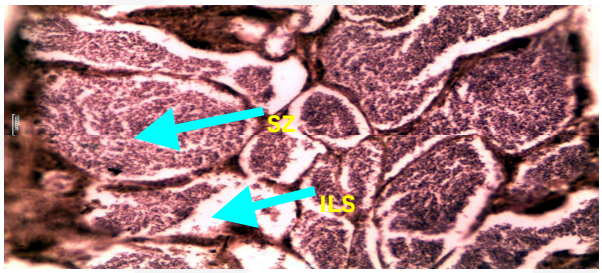

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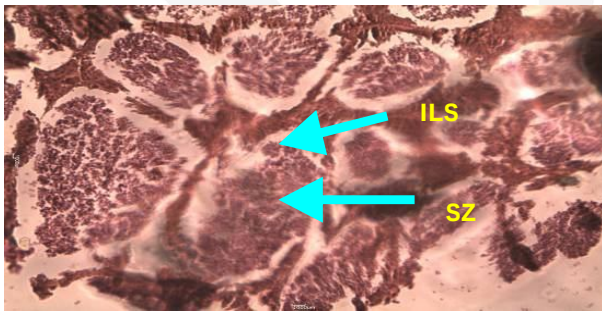


Plate-7

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing

Plate 1: Testis of control fish at initial period

Plate 2: Testis of control fish at 15th day)

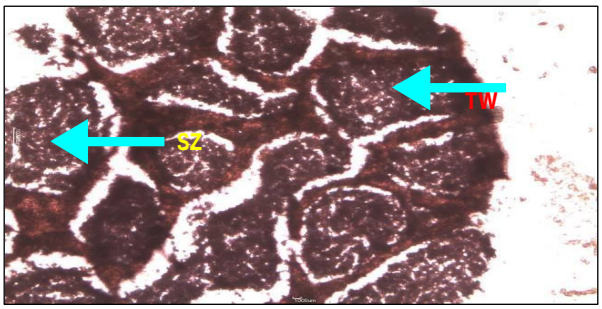
Plate 3: Testis of control fish at 30th day

Plate 4 : Testis of control fish at 45th day

Plate 5: Testis of control fish at 60th day

Plate 6: Testis of control fish at 75th day

Plate 7: Testis of control fish at 90th day



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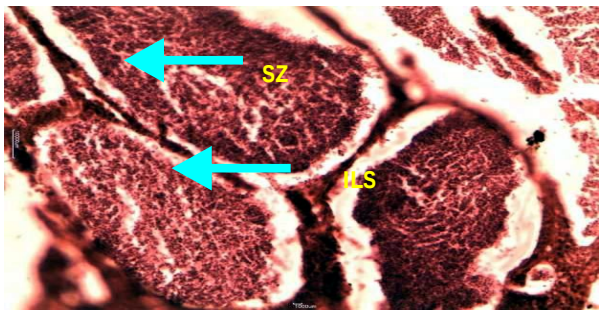

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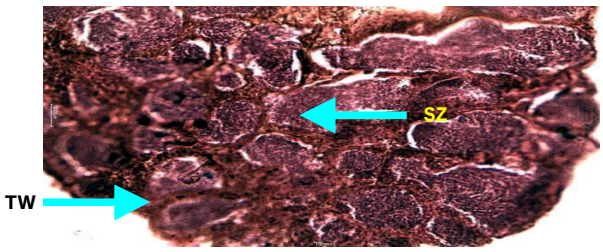

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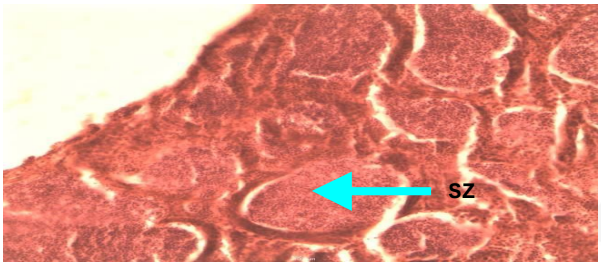
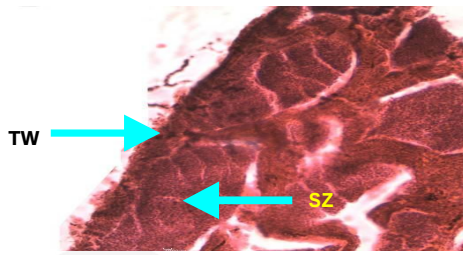


Plate-11



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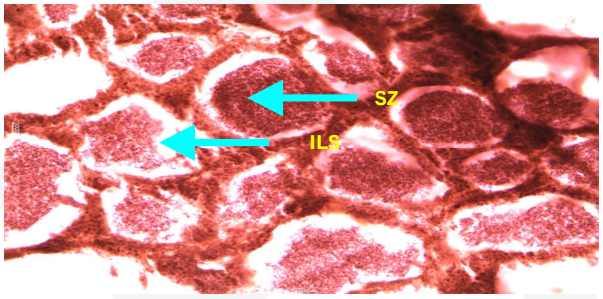
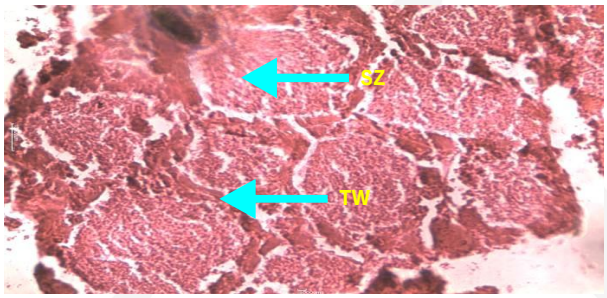


Plate-13

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing
Plate 8: Testis of 5 mg/Kg hormone treated fish at 15th day. **Plate 9:** Testis of 5 mg/Kg hormone treated fish at 30th day
Plate 10: Testis of 5 mg/Kg hormone treated fish at 45th day. **Plate 11:** Testis of 5 mg/Kg hormone treated fish at 60th day
Plate 12: Testis of 5 mg/Kg hormone treated fish at 75th day. **Plate 13:** Testis of 5 mg/Kg hormone treated fish at 90th day



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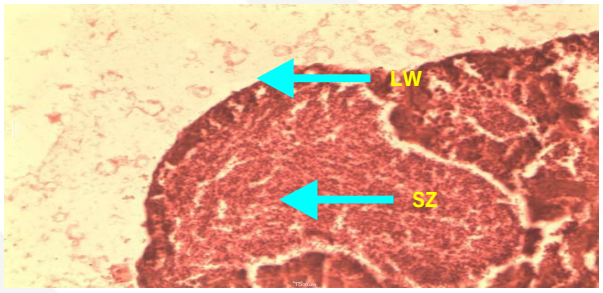

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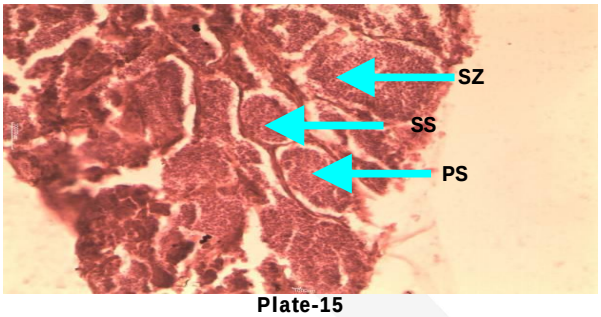

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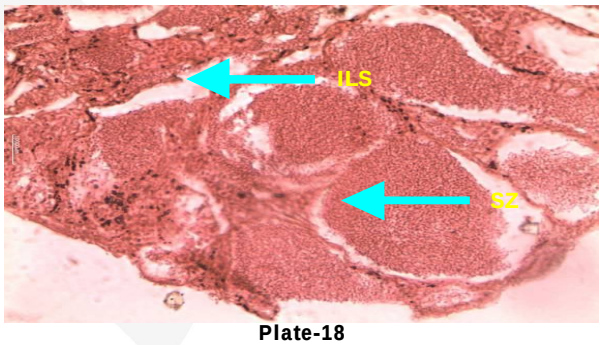

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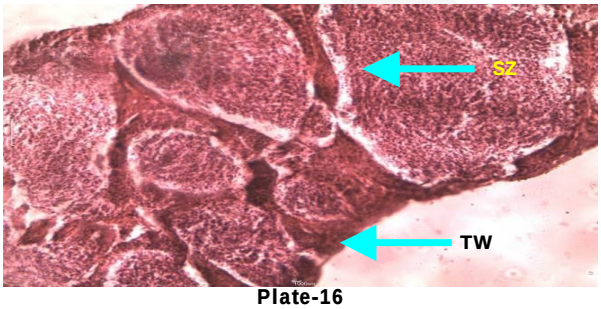

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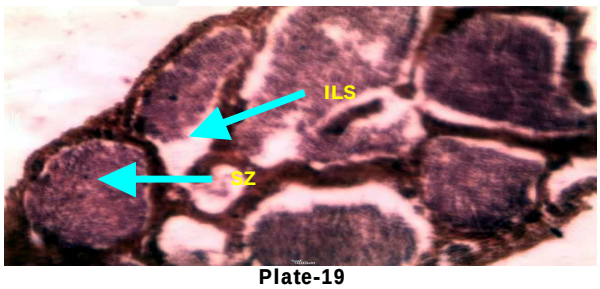
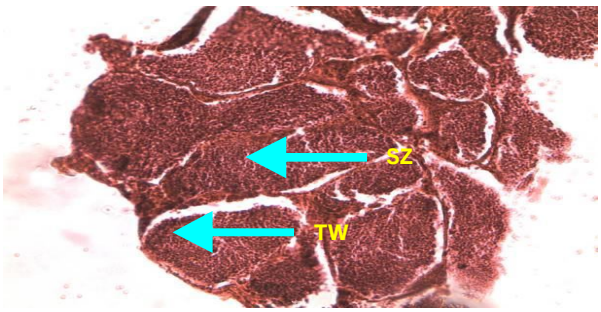


Plate-19

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing
Plate 14: Testis of 10 mg/Kg hormone treated fish at 15th day. **Plate 15:** Testis of 10 mg/Kg hormone treated fish at 30th day
Plate 16: Testis of 10 mg/Kg hormone treated fish at 45th day. **Plate 17:** Testis of 10 mg/Kg hormone treated fish at 60th day
Plate 18: Testis of 10 mg/Kg hormone treated fish at 75th day. **Plate 19:** Testis of 10 mg/Kg hormone treated fish at 90th day



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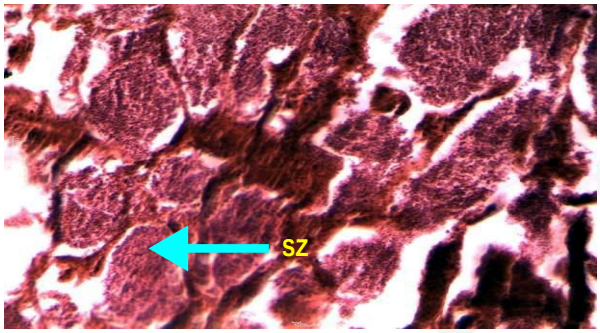
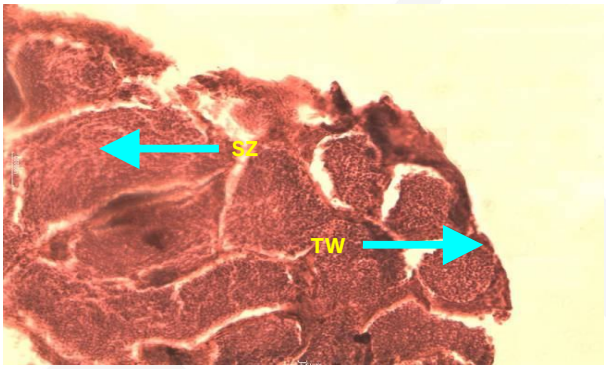


Plate-21



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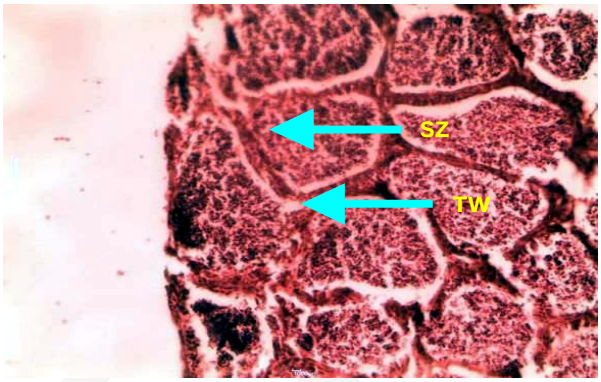

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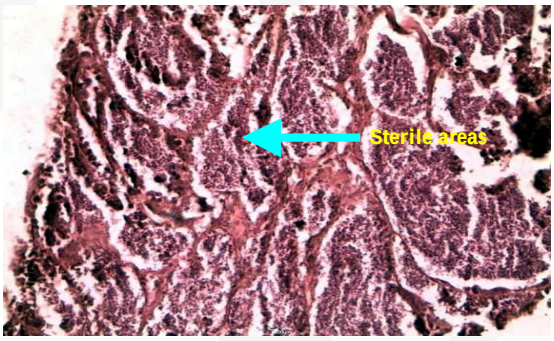

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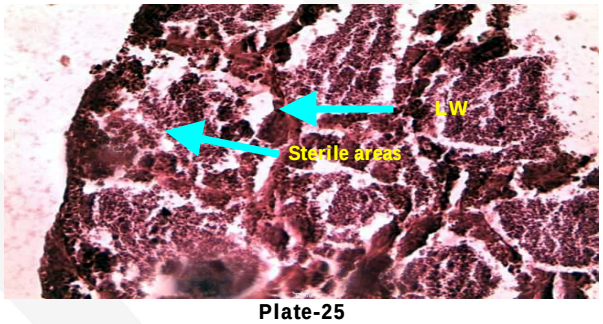

Plate-25

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing
Plate 20: Testis of 15 mg/Kg hormone treated fish at 15th day
Plate 21: Testis of 15 mg/Kg hormone treated fish at 30th day
Plate 22: Testis of 15 mg/Kg hormone treated fish at 45th day
Plate 23: Testis of 15 mg/Kg hormone treated fish at 60th day
Plate 24: Testis of 15 mg/Kg hormone treated fish at 75th day
Plate 25: Testis of 15 mg/Kg hormone treated fish at 90th day

found better than other two (5 and 15 mg/kg). The development of gonadal materials (Primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa i.e. sperms) insides the follicles of the male gonads was also seen accordingly in the different treatments. In the testis of fish, when undergoing reproductive activity (spermatogenesis), about six spermatogenic elements have been identified and described by Guraya (1986). The elements of spermatogenesis are produced from sperm mother cell of germinal epithelium and passes through different maturation stages as primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (sperms). On the basis of histological study, the testicular change of *T. lalius* due to hormone administration through diet was distinguished into primary spermatocytes, secondary spermatocytes and spermatozoa. This is because the concentrations of the gonadal material was so closely placed that the identification of different stages was found very difficult. In the present study sterile areas were also found in high dose of 15 mg/Kg hormone treated fish at the end of 60th day of treatment which is in agrrement of findings of the earlier workers (Simpson, 1976; Johnstone *et al*., 1978; Hurk and

Slof, 1981; Billard, 1992; Boris *et al*., 1994; Pandian and Sheela 1998).

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Original Article

Seasonal Variation of Different Biologically Significant ion Concentrations of the Jalangi River water

Monojit Ray

Abstract

Biodiversity of a river depends on the variation of nutrients, physico-chemical parameters and different biologically significant ion concentrations of the river water. Seasonal variation of nine biologically significant ion concentrations of Jalangi river water are reported in this article, month-wise, from April 2014 to March 2015. These ions, namely sodium, potassium, magnesium, calcium, carbonate, bicarbonate, nitrate, phosphate and chloride play vital role on the growth of aquatic flora and fauna present in the river. The average concentrations of arsenic(III), sulphate ion, fluoride ion, ferric ion and lead ions are also reported in this paper though are they are less significant. These data clearly helps to understand the background scenario of the river's ecosystem.

Keywords: Jalangi; Ion Concentrations; Biodiversity.

Introduction

River ecosystem is moving-water ecosystem. For such an ecosystem land-water interaction is very significant as water current and turbulence are present. These affect the biotic community of the river directly or indirectly. The flow of biologically significant ions within a river ecosystem greatly depends on the concentrations of these ions in the river water.

Sodium, potassium, magnesium and calcium are the most important metals for living systems. They are called bulk metals. Magnesium (II) ion is the absolute requirement for photosynthetic pigments. Calcium get precipitated as calcium carbonate within river. High temperature and high salinity of river water favors calcium carbonate precipitation as, the high evaporation rate and reduced quantity of free carbon dioxide in water can also be observed at high temperature only.

Some bacteria satisfy their nitrogen requirements through reduction of nitrate ions within river water. Carbonate and bicarbonate ions are responsible for effective buffering action of river water. For the aquatic flora and fauna, sufficient phosphates are required for phosphorylation and energy storage. Seasonal variation of these ions have profound influence on the seasonal abundance of different aquatic flora and fauna in the river water. The amount of sulphate, arsenite, fluoride, ferrous ion and lead(II) ions do not vary too much, with the change of seasons, round the year. Fluoride is a trace element. Sulphate is important as sulphur is a bulk element. Iron is also a trace metal and biologically significant. Ferrous ions play important role with respect to redox, catalysis, electron transfer, oxygen transfer and storage with in living systems.

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Materials and Methods

The Jalangi river water samples were collected from ten sampling sites, namely Jitpur, Taranipur, Chapra, Haranagar, Anandanagar, Ghurni, Krishnanagar kadamtala ghat, PWD more (Rail Bridge), Char-sambhunagar, Hulorghat(Mayapur). Water samples were collected from different depths of sampling sites, up to 8-10 ft depth of the river Jalangi by expert swimmers. The analysis of the water samples were

done using titrimetric methods, flame photometric methods, spectrophotometric methods etc. The analysis of the water samples were carried out in the Department of Chemistry lab of Nabadwip Vidyasagar College. Some analysis were performed in outside laboratory (Scientific Research Laboratory, Santoshpur, Kolkata). Water sample collections from sampling sites were a continuous process and done using hired boat services from the above mentioned sampling sites.

Analytical Results

Table 1: Variation of Biologically Significant Ion Concentrations of the river Jalangi

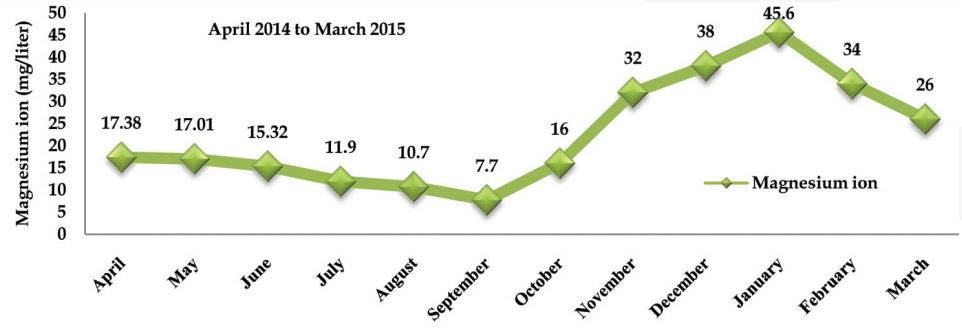


Fig. 2:

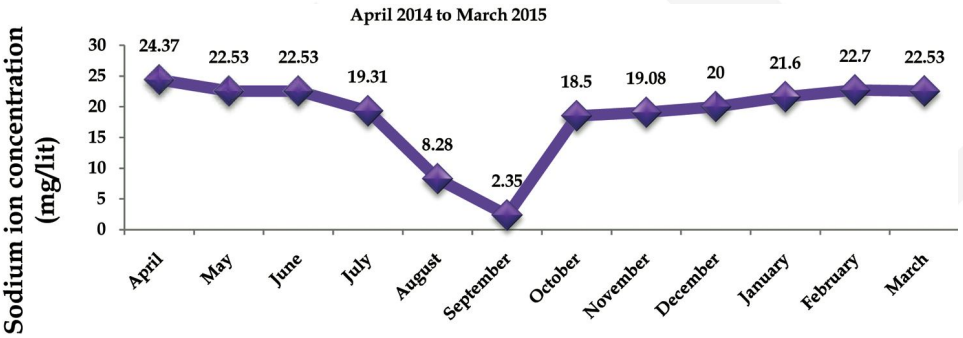
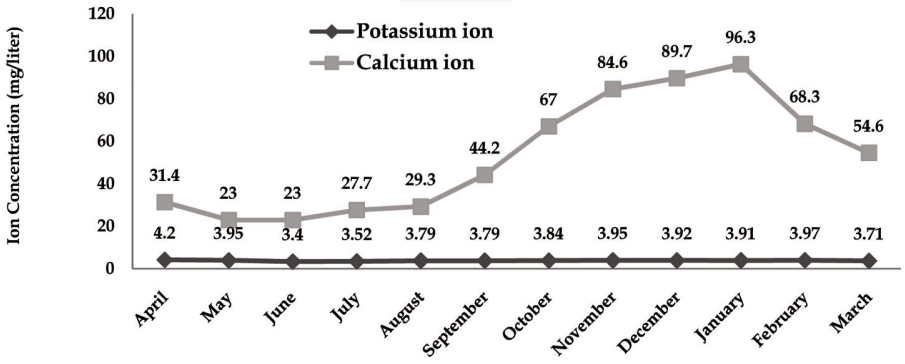


Fig. 3:



April 2014 to March 2-015

Fig. 4:

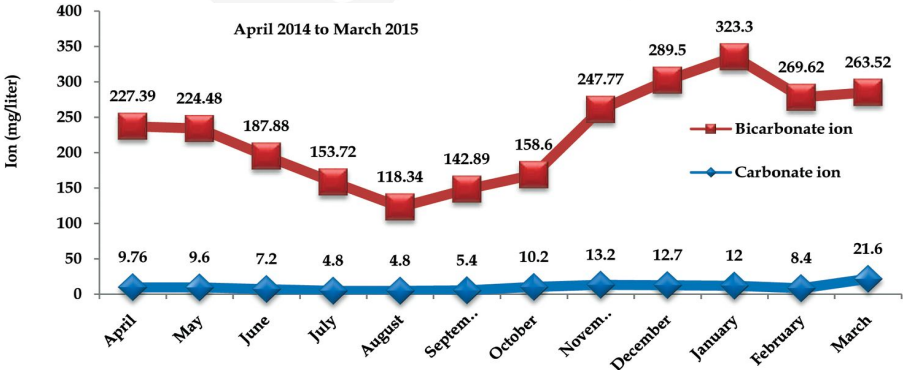


Fig. 5:

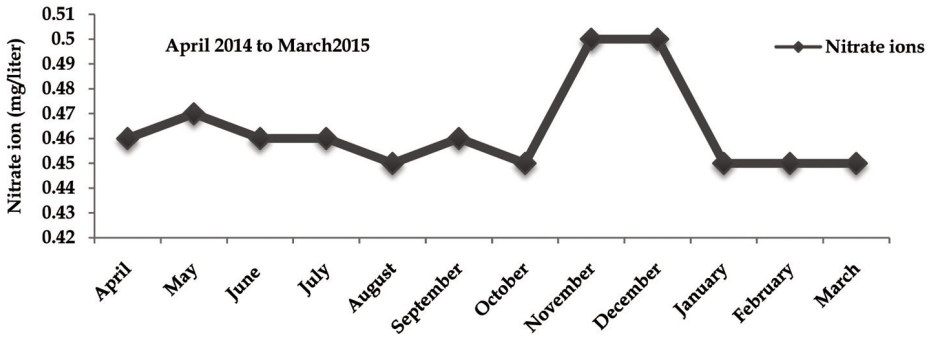


Fig. 6:

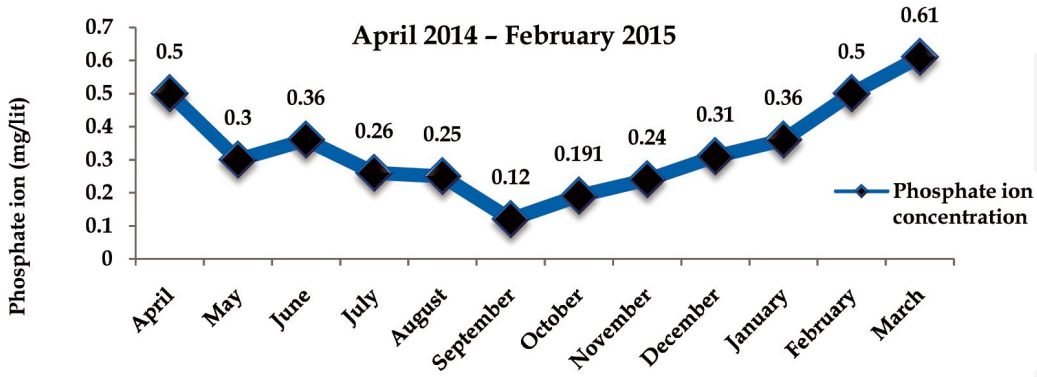


Fig. 7:

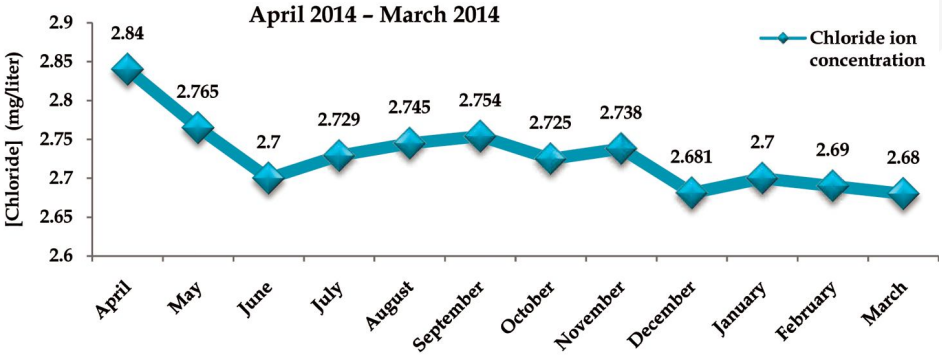


Table 2:

Ion	Average Concentration (mg/liter)
Sulphate, SO_4^{2-}	<2.5-6.3
ARSENIC (III)	0.032
FLUORIDE	0.17
LEAD(II)	<0.01
Fe(II)	0.0123
Total Cr	<0.05

Discussion

Phytoplankton and algal growth depends on the variation of nutrients, variation of physico-chemical parameters and the ion concentrations the of river water. High conductance value of river Jalangi indicates the presence of sufficient ions within river water. Sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), chloride (Cl^-), sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), carbonate (CO_3^{2-}), bicarbonate (HCO_3), nitrate (NO_3^-) etc. ions are responsible for the salinity of river water. Again, the water salinity acts as an important criteria for the distribution of flora and fauna.

Magnesium, sodium and potassium ion concentrations are maximum during maximum cold and minimum during late mansoon. The element magnesium is an absolute requirement for all algae, submerge aquatic plants and phytoplaktons as, it is

a constitute for chlorophyll. Magnesium is also essential for the formation of the enzyme catalase. Growth of blue green algae, like nostoc, anabaena show specific nutritional requirement of sodium ion. Sodium ion is also responsible for enzyme activation and water balance within living aquatic systems. The growth and photosynthesis of algae become low, when low potassium ion concentration is present. Potassium ions are also responsible for pH control, osmotic pressure regulations and stability of proteins within the cells of aquatic flora and fauna. Potassium ions maintain the electrochemical environment of the algal cells. Calcium concentration do not vary too much through-out the year. During winter conductance, salinity, total hardness and TDS values reaches maxima. In many algae like *chara sp*., *cladophora sp*., calcium remains deposited as calcite on or in the walls or in the mucilage. Calcium ion is responsible for enzyme activation, ion transport and specially skeletal structures of aquatic fauna within river. It should be noted that the breading season for fisher and residential-birds are monsoon and the processes required enhanced bio-mineralisation rate i.e., formation of calcium carbonate, calcium phosphate etc. from the river water source. Bio-mineralisation is very significant as huge amount of freshwater mussels, fishes, *Pilla sp*., *Turritella sp.* along with Clam and Oyster are present within river Jalangi. This also must be noted that, large cladocerans such as Daphnia require higher specific quantity of calcium ion. High river water flow rate along with enhanced bio-mineralisation rate during monsoon is significant and the conductance, total dissolved solid, salinity, total alkalinity and hardness reaches minimum value.

Bicarbonate and carbonate concentrations are also maximum during winter; minimum during August-September. Aquatic flora, derive the element carbon, from bicarbonate and carbonate ions, apart from carbondioxide and organic compounds. Through-out the year, the change of nitrate concentration is insignificant and lies between 0.44 – 0.48 ml/liter. Some algae utilize nitrate ions as a source of nitrogen. Phosphate concentration is maximum during spring and minimum during autumn. Algal cells etc. need phosphorous for the formation of phospholipids, nucleic acids and various ester phosphates such as NADP, phosphorylated sugars and ATP. Chloride concentration is maximum during summer and minimum during winter. The element chlorine is significant and essential for algae with respect to Photosystem-II.

Iron is the most important trace metal for the phytoplanktons, aquatic mammals. Iron is an essential element for algae as it is a constituent of cytochromes. In the river Jalangi, average ferrous concentration is 0.0123 mg/liter. Arsenic is a trace element. Sulphur is present in small amount in all aquatic plant and animal cells. Sulphur is present as sulphate ions within river water. Lead and chromium are toxic metals. High fluoride concentration have adverse effect on flora and fauna, however the Jalangi river water contain very low lead, chromium, arsenic and fluoride ions. During winter, Jalangi river contain huge red water blooms of *Azolla pinnata* which can remove chromium, nickel, copper, zinc and lead from effluent. It can also remove lead from solutions containing 1-1000 ppm.

Acknowledgement

Monojit Ray, Associate Professor, Department of Chemistry, Nabadwip Vidyasagar College, thanks University Grant Commission, India for financial assistance.

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Original Article

A Study on Diversity Ofherpetofauna in Sanghagara Forest Ecosystem

Sureshbala Nayak*, Prafulla Kumar Mohanty**

Abstract

The present paper focuses on diversity of serpentofauna in Sanghagara forest ecosystem on the basis of observational method. This forest is located in between $21^0$1'-$22^0$10' N latitude and $85^0$11'-$86^0$22' E longitude. Sanghagara, one of the most significant and alluring natural scenic spots of Odisha, deserves its weightage, validity and importance because of assemblage of both floral and faunal diversity. The field study was undertaken twice a month and 27 herpetofaunal species were recorded during the survey. Among these, 5 families, 11 genera and 9 species of lizards; 5 families, 14 genera and 16 species of snakes are reported. Recorded lizard diversity is dominated by members of family Geckonidae, Lacertidae followed by Scincidae. Recorded serpentofaunal diversity is dominated by members of the family Elapidae, Colubridae followed by Viperidae. Among these, *Ptyas mucosa* (Indian rat snake) and *Amphiesma stolatum* (Buff striped keelback) were most frequently observed followed by *Bungarus caerulus* (krait) and *Bungarus fasciatus* (Banded krait). Findings include both poisonous and non-poisonous species of snakes. The body of snakes is different in different habitats. The diversity of herpetofauna has been observed to be decreased with increase in altitude of the forest area.

Keywords: Sanghagara; Reptiles; Lizards; Snakes; Diversity.

Introduction

Reptiles are the first successful terrestrial tetrapods which are evolved from Labyrinthodont amphibians 300 million years ago (Romer, 1949). The development of internal fertilization enabled reptiles to be the first vertebrates which radiated out across the landscape, diversified quickly and become the dominant life form on the planet during the Triassic, Jurassic and Cretaceous period of Mesozoic era (245 million to 65 million years ago) of geological time scale. Healthy biodiversity is a healthy indicator of an ecosystem. Today, a drastic decline in biodiversity has been observed in different parts of the world in an alarming rate. The destruction in different forms such as degradation, fragmentation or outright loss prompted mainly by several factors such as poverty, demographic factors, inadequate polices and economic incentives, anthropogenic activities such as overgrazing, deforestation, bushflies, shifting cultivation, developmental activities like mining, urbanization and road construction inside the protected areas are found to be the major causes of loss in biodiversity. Documentation, conservation and finding enhancement strategies of biodiversity is considered to be one of the important challenges in present day conservation, biological research and policy making process (Scott Mills,2013). In view of the above background, several studies on species diversity have been undertaken (Romer, 1949; Selvanayagam et al., 1995; Shrestha, 1996; Urfi, 1997). Since investigation on herpetofauna in Keonjhar district is inadequate, the present study was undertaken to enlist and evaluate the status of Lizards and snakes in hill forest, Sanaghagara natural ecosystem, Keonjhar, Odisha.

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Methodology

For this study, field visits were carried out twice a month at the study site to survey the reptilian diversity

from 2008 to 2013 in entire terrestrial site. The study site is situated between 2101'-220 10'N latitude and 850 11'-860 22'E longitude. It is bounded by Mayurbhanja district and Bhadrak district to the east, Dhenkanal and Sundergarh district to the west, Jajpur district to the south and Singhbhum of the State Jharkhand to the north. Individuals of a particular species were recorded by visual encounter surveys (Rodel and Ernst, 2000). However, active search involving turning rocks and logs, excavating burrows and termite mounds by the help of snake catcher also provided good results. During day time, attempt was made for heliothermic (basking) reptiles along forest trails, forest edges and stream sides. Data were collected for each individual species encountered during field work. The various aspects such as locality, date, time, weather condition, habitat, sex of each individual (when possible), co-existing species if any and behaviour were noted in a field data sheet. Taxonomic characters of captured species were noted during field work. Photographs of different species and habitat were collected by digital camera from vario positions and angle of the species. Species were identified using the described keys (Smith, 1929; David and Vogel, 1996; Schleich and Kastle, 2002). Common English name of herpetofaunal species is followed after Captain and Bhatt (2000) and Jude Sekar (2012). The status of reptilian species is enlisted by using the keys of Anderson (1871); Smith (1950); Bellairs and Underwood (1957); Caroll (1969); Das, (1994), Bhupathy (1995); Hermann (1997) and Venkateswarulu et al. (1995). Endangered species are checklisted by using Biswas et al. (1976); Grombridge (1981); Narayan and Rosland (1990); Murthy (1995); Aengels (1995); Klemens and Thorbjarnarson (1995); and Allen (1996). Data were collected from the secondary information sources like hunters and local inhabitants through interaction and discussion.

Results and Discussion

Twenty Seven reptilian species were recorded during the survey. Among these, 5 families, 11 genera and 9 species of lizards (Table 1); 5 families 14 genera and 16 species of snakes (Table 2) were recorded. Among lizards, the family Geckonidae is the dominant family with 4 species followed by family Lacertidae (3 species) and Scincidae (2 species). Similarly, the recorded serpentofaunal diversity is dominated by members of the family Colubridae followed by Elapidae and Viperidae.

The Recorded Reptiles are As Follows.

Lizards-1:

Eutropis multifasciate (Fig 1) It is commonly called as "many lined grass skink". It is diurnal; basking in the sun, along forest tracks or on the trees.Skin is smooth and scaled with small legs. Its body colour is olive brown.2. *Eutropis macularia* (Fig 2). It is commonly called as "bronze grass skink". The body is brown or olive in colour. Tail is 1.25-1.75 times the length of head. It has short snout. 3. *Calotes emma* (Fig 3). It is commonly called as "Gray's forest lizard". Its dorsal crest is well developed on the neck and on the anterior part of the trunk, gradually disappearing behind. Tail is compressed. 4. *Calotes versicolor* (Figs 4 and 5). It is commonly called as "Indian garden lizard". Its colour ranges from brownish-buff to greyish. It has a short crest above the neck. Spines are present above the tympanum. 5. *Japalura planidorsata*. (Fig.6). It is commonly called as "flat backed japalura". Dorsal side is golden yellow in colour and ventral side is grey in colour. Black lines are present on its dorsal side. 6. *Gecko gecko* (Fig.7). It is commonly called as "tokay gecko". Its body is grey in colour having large eyes. Foot pads are well developed. 7. *Cosymbotus platyurus* (Fig8). Body colour is brown. Its size is small. 8. *Hemidactylus frenatus* (Fig. 9). It is commonly called as "house gecko". This species is largely recorded from human habitations and buildings at various localities of study area. Body colour is dark brown. Length is about 4-6 inches. 9. *Varanus benghalensis* (Fig. 10). It is commonly called as "Bengal monitor". It is dark grey or brownish in colour. It is a tree climber or inhabited in dryer land and road sides of forest.

Plate 1: Lizards of sanaghagara forest

Fig. 1: Eutropis multifasciate

Fig. 2: E. acularia

Fig. 3: Calotes emma

Fig. 4: C.versicolor

Fig. 5: C. versicolor

Fig. 6: Japalura planidorsata

Fig. 7: Gecko gecko

Fig. 8: Cosymbotus platyurus

Fig. 9: Hemidactylus frenatus

Fig. 10: Varanus bengalensis

Table 1: List of lizards from Sanghagara forest

Family	Sl. No.	Common Name	Scientific name	Date of Encounter	Time	Habitat
Scincidae	01	Many lined grass skink	*Eutropis multifasciata*	O7.03.2008	11:20am	Secondary and degraded forest areas, road sides
	02	Bronze grass skink	*Eutropis macularia*	12.06.2008	4:40pm	Scrub forest,near human habitation
Lacertidae	03	Gray's forest lizard	*Calot esemma*	27.07.2008	10:20am	Stream side, scrub forest

	04	Indian garden lizard	*Calotes versicolor*	02.05.2008	05:30	Scrub forest, mesic forest, near human habitation
	05	Flat backedJapalura	*Japalura planidorsata*	10.04.2008	11:25am	Rocky dry stream bed,shrub forest
Geckonidae	06	Tokay Gecko	*Gecko gecko*	27.07.2008	8:30am	Shrub forest, road side shrubs along forest trails
	07	Flat tailed Gecko	*Cosymbotus platyurus*	12.06.2008	09.50am	Secondary forest,on rocks trees
	08	Asian house Gecko	*Hemidactylus frenatus*	10.04.2008	4:30pm	Human habitation, ruined gardens, scrub and mesic forest
	09	Bengal monitor	*Varanus benghalensis*	08 02 2009	09.40am	Peripheral region of forest

Snakes-1.*Typhlops diardii* (Blind snake) (Fig. 11). Its body is elongated, cylindrical, and wormlike with blunt snout. Body scales are small. 2. *Python molurus* (Fig.12). Body is greyish-brown with black and red spots. Head is distinct from the neck. Tail is short and prehensile. 3.*Dendrelaphis pictus* (Bronze back tree snake) (Fig. 13). It is a long, slender snake with a pointed head and a bronze coloured line running down its back . This harmless snake prefers the tree tops to life on the ground. This active snake was restless and quick, both on the ground as well as in the trees. 4. *Boiga cynea* (Green cat snake) (Fig. 14) it was tree dweller and green in colour. 5 .*Lycodon aulicus* (Common wolf snake) (Fig.15). It was reported near human habitat at study area. 6. *Ptyas mucosa*. (Figs.16 and 17) It was commonly called as "Indian rat snake". Snout was obtuse which was slightly projecting with large eyes. Dorsal side was brown above, frequently with more or less distinct black cross bands on the posterior part of the body and on the tail. 7. *Amphiesmaa stolatum*(Fig.18). It was commonly known as "buff striped keelback". It was a small, slender snake, generally olive-brown to gray in colour. The head and the body are of the same colour. The body of the buff striped keelback was short, and it has a long slender tail. Two yellow stripes along the length and to the sides of the spine are the distinctive feature of this snake. *8. Trimeresurus erythrurus*. (Spot tailed pit viper) (Fig.19). It was long and tree dweller. 9. *Echis carinata*(Fig.20). It was commonly called as "saw scaled viper". Size ranges between 38 and 80 cm (15-31.5 inches) in total length (body + tail). Head was distinct from neck and snout was very short. 10.*Daboi russelii* (Fig. 21). This snake can grow to a maximum total length (body + tail) of 166 cm (5.5 ft). The head was flattened, triangular and distinct from the neck. The snout was blunt, rounded and raised. 11. *Bungarus fasciatus* (Fig. 22). It is commonly called as "banded krait". Body is elongated and slender, measuring about one metre in length. The colour of the body is steel-blue with narrow cross bars or white specks dorsally and the underparts are uniformly white. Head with normal shields and is not differentiated from neck. Eyes are of moderate size. 12. *Bungarus caerulus*. (chiti) (Fig.23). It is brown in colour and small in size. 13. *Bungarus niger* (Fig.24) Lenth is about 4.5 ft . Color is black. Pointed tail and obtuse head was present. 14. *Naja naja* (Cobra). (Fig.25). Body is elongated, measuring one and half to two metres in length. The colour of the body is brown. Head is differentiated from the neck. Neck is dilatable. 15. *Naja kaouthia*(Monocled cobra)

Plate 2: Snakes of sanghagara forest

Fig. 11: Typhlops diardii

Fig. 12: Python molurus

Fig. 13: Dendrelaphis pictus

Fig. 14: Boiga cynea

Fig. 15: Lycodon aulicus

Fig. 16: Ptyas mucosa

Fig. 17: Ptyas mucosa

Fig. 18: Amphiesma stolatum

Fig. 19: Trimeresurus erythrurus

Fig. 20: Echis carinata

Fig. 21: Daboia russelii

Fig. 22: Bungarus fasciatus

Fig. 23: Bungarus caerulus

Fig. 24: Bungarus niger

Fig. 25: Naja naja

Fig. 26: Naja kaouthia

Fig. 27: Ophiophagus hannah

Fig. 28: Ophiophagus hannah

Table 2: List of snakes from Sanghagara forest

Family	Sl No	Common Name	Scientific Name	Habitat
Typhlopidae	01	Blind snake	*Typhlops diardii*	Near human habitation,shrub forest below rock boulder
Boidae	02	Burmese rock python	*Python molurus*	Trees, forest in mesic forest
Colubridae	03	Painted bronze back tree snake	*Dendrelaphis pictus*	On trees, near human habitation,scrub forest
	04	Green cat snake	*Boiga cynea*	Branches of trees
	05	Common wolf snake	*Lycodo naulicus*	Forest floor,leaflitter,below rock boulder,scrub forest
	06	Indian rat snake	*Ptyas mucosa*	Forest floor, termitemound,below rock boulder
	07	Buff striped keelback	*Amphiesma stolatum*	Grassland, near human habitat
Viperidae	08	Spot tailed pit viper	*Trimeresurus erythrurus*	Grass land ,peripheral region of forest
	09	Saw-scaled viper	*Echis carinata*	Schrubforest,near human inhabitants
	10	Daboia	*Daboia ruselii*	Scrub forest, near human habitats
Elapidae	11	Banded krait	*Bungarus fasciatus*	Scrub forest, near human habitat
	12	Krait	*Bungarus cærulus*	Near human habitat
	13	Black krait	*Bungarus niger*	Scrub and mesic forest, road side
	14	Cobra	*Naja naja*	Scrub forest, near human habitat, forest floor, degraded termite mound
	15	Monocled cobra	*Naja kaouthia*	At degraded forest edge, scrub forest
	16	King cobra	*Ophiophagus hannah*	Tree, schrub forest.

(Fig.26).The monocled cobra has an O-shaped, or monocellate hood pattern, the dorsal surface may be yellow, brown, gray, or blackish, with defined cross bands. It has a black spot on the lower surface of the hood on either side, and one or two black cross-bars on the belly behind it. 16. *Ophiophagus hannah* (Figs. 27 and 28). It is commonly called as King cobra. Length is about 3-5 metre. Skin is either olive-green or black and it has faint, pale yellow cross bands down the length of the body. It has an expandable hood.

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