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Original Article

Effect of Sublethal Concentration of Zinc Sulphate on the Serum Biochemical Parameters of Freshwater Cat Fish, *Clarias Batrachus* (LINN)

Navneet Kunwer Srivastava[1], Sadguru Prakash[2]

Abstract

Zinc is a necessary trace element that contributes cumulative polluting effect to aquatic organism. The effect of sublethal concentration of zinc sulphate was investigated on the serum biochemical parameters of *Clarias batrachus.* This study was carried out to evaluate the sub chronic toxicity of zinc at three levels (10mg/L, 20mg/L and 30mg/L) for 15, 30 and 45 days. The biochemical changes in plasma showed significant increased in glucose, lipids (Triglycerides and cholesterol), serum phosphatases (acid and alkaline phosphatases) and serum transaminases (SGOT & SGPT) where as decreased in bilirubin and protein level. These results indicate that both concentration and period of exposure of zinc sulphate can alter the plasma's biochemical contents of *Clarias batrachus.* Thus it can be concluded that fishes can effectively used as monitors of water quality with respect to heavy metal, zinc as well as toxicant.

Keywords: Zinc; Chronic Toxicity; Biochemical Changes; *Clarias Batrachus*.

Introduction

The rapid development of industry and especially chemical industry has created serious problems of water pollution. Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface water. These elements are generally released in small amounts into the environment by processes like weathering of rocks, volcanic eruption etc. and their intake/exposure is necessary in trace amounts for good health. But, presently, due to anthropogenic activities there is a steady increase in their concentration in all the habitats due to discharge of these metals into natural aquatic ecosystems. Sometimes, aquatic organisms are exposed to unnaturally high levels of these metals. Heavy metals easily get absorbed in the body and get transported to various organs through blood. When in circulation, these metals not only affect the blood components but also elicit marked alterations in the histology as well as physiology of the target organs. Fish are relatively sensitive to changes in their surroundings environment. Fish health may therefore reflect and give a good indication of the health status of a specific aquatic ecosystem.

Heavy metal, Zinc is essential for proper functioning of the body. Zinc is a necessary trace element that contributes to the structure of more than 300 proteins which play a role in the growth, reproduction, development and immune system catalysts in fish (Watanabe et al., 1997). It is used in various industrial operations forms and excessive zinc finds its way into reservoirs, lakes and rivers. Excessive zinc enters the environment as a result of human activities such as mining, purification of zinc, lead and cadmium ores, burning of coal and burning

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of waste. Although, small quantities of zinc are required for normal development and metabolism of organism, but if levels exceed the normal physiological requirements, it can act as a toxicant. Exposure to excess zinc has been reported to bring about haematological as well as biochemical changes in various organs of fishes. This results in general enfeeblement, retardation of growth and may bring about metabolic and pathological changes in various organs in fish.

In recent years, biochemical variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances. Hadi *et al.*, (2009) suggested that biochemical changes of fish be used in determining the toxicity of pollutants. Therefore, estimation of serum biochemical parameters thus, proves to be a diagnostic tool in toxicology to access the general health status of an organism and their impact on target organs.

Clarias batrachus is widely distributed fish in Asia. In these areas, it is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high fish market price. It inhibits tropical swamps, lakes and rivers. These are generally strong fishes and possess accessory respiratory organ for air breathing that enable them to tolerate adverse aquatic conditions where other cultivated fish species cannot survive. Not much work has been carried out on the effect of zinc on the biochemistry of freshwater cat fish, *Clarias batrachus*. Therefore, the present study was undertaken to evaluate some serum biochemical effects resulting from the exposure of the freshwater cat fish, *Clarias batrachus* to sublethal concentrations of zinc sulphate in the water.

Materials and Methods

Healthy specimens of Indian fresh water catfish, *Clarias batrachus* were collected from local fish farm at Balrampur, U.P. and were transported in containers to the laboratory .In the laboratory, the fishes were carefully examined for any injury and then kept in 1% solution of $KMnO_4$ for few hours to get rid off dermal infection, finally they were kept in large plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they were fed on boiled egg yolk and commercial fish food . The fish specimen weighing 50±5 gram and measuring 15±5 cm selected for experiments. The fishes were inspected for disease conditions and general fitness. Water was changed every other day. Feeding was stopped 48 hours prior to the toxicity test, to minimize the contamination of metabolic wastes.

Srivastava and Prakash (2018) reported that the LC_{50} of zinc sulphate was 37.22mg/L at 96hours for *Clarias batrachus*. The selected sublethal concentrations zinc sulphate were 10.0mg/L, 15.0mg/L and 20.0 mg/L for 15,30 and 45 days, respectively . After 15, 30 and 45 days exposure, blood samples were collected from both the control and experimental fishes by puncturing the caudal vein to assess the effect of zinc sulphate on the serum biochemical parameters. The collected blood was allowed to centrifugal tubes, stored in a slanting position to clot for 3 minutes. It was then centrifuged at 3000 rpm for 15 minutes. The supernatant serum was separated by a fine rubber bulb pipette in separate tube. The serum samples were used for the estimation of biochemical parameters i.e. bilirubin, serum glucose, total proteins, cholesterol and enzyme activity of transaminases (SGOT and SGPT), acid and alkaline phosphatase. The serum glucose, total protein, triglycerides, cholesterol, transaminases, phosphatases and bilirubin were estimated by following standard methods. The mean values of the various biochemical parameters for the control and experimental fish were analyzed for statistical significance using the student's t-test. The calculations of statistical significance by the student's t- test at 0.01 and 0.05 levels were made using Microsoft Excel 2003.

Results and Discussion

Heavy metals are known for their strong action on biological tissues. Metal ions once absorbed into body are capable of reacting with a variety of active binding sites and thus can disturb the normal physiology of an organism. In the present study, an attempt was made to examine the sub-lethal toxic effect of zinc sulphate on the serum biochemical parameters of C*larias batrachus.* Changes in the blood biochemical values often reflect alteration of physiological state of fish. Although no mortality was observed in the present study, we found physiological effects in the fish after the exposure to zinc sulphate. Result of the quantitative estimation of serum bilirubin, glucose, cholesterol, triglycerides, acid and alkaline phosphatases, SGOT and SGPT in Clarias batrachus exposed to zinc are presented in Table 1-6.

Serum bilirubin levels were significantly decreased in *Clarias batrachus* exposed to zinc sulphate as compared to control groups (Table 1) may be hepato-dysfunction. Similar report of decreased in bilirubin level was previously recorded by Srivastava *et al.* (2007) in *Clarias batrachus* exposed to distillery effluent and also by Srivastava *et al.* (2012) in *Heteropneustes fossilis* exposed to sodium fluoride.

The serum glucose levels were significantly ($p<0.05$ & $p<0.01$) higher in zinc exposed groups of fishes as compared to control groups (Table 2). Serum glucose levels increased progressively with the increasing concentrations of metals and exposure periods under studies. Similar hyperglycemic response has reported by Hadi *et al.*, (2009)in *Tilapia zillii* exposed to aluminium and by Canli (1995) in *Cyprinus carpio* exposed to Hg, Cr and Ni. The hyperglycemic condition in metal treated fish in present study may be an effort to provide additional energy required during times of high metabolic activities such as 'fight or flight 'response in order to counter the metal toxicity (Goss and Wood,1988). Another plausible reason for elevated glucose levels presently seems to be the incomplete metabolism of

Table 1: Effect of Zinc sulphate on the serum Bilirubin (mg/ml) level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↓ %)	30 Days Mean±SD (↓ %)	45 Days Mean±SD (↓ %)
Control	0.58±0.32	0.60±0.28	0.61±0.23
10 mg/L	0.48±0.16 (20.83)	0.46±0.33 (23.33)	0.48±0.31 (21.31)
20mg/L	0.45±0.38 (22.41)	0.43±0.21* (28.33)	0.39±0.18** (36.06)
30mg/l	0.41±0.33* (29.31)	0.39±0.28** (35.00)	0.31±0.41** (49.18)

*Significant at $p< 0.05$; ** significant at $p< 0.01$.

Table 2: Effect of Zinc sulphate on the serum glucose (mg/dl) level in *C.batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
Control	11.10±0.20	11.20±0.30	11.30±0.33
10 mg/L	13.30±0.10 (19.81)	13.45±0.18 (20.18)	13.85±0.19 (22.56)
20mg/L	15.45±0.15* (39.19)	15.90±0.19* (41.96)	16.15±0.20* (42.92)
30mg/l	17.18±0.18** (57.77)	17.78±0.22** (58.75)	17.98±0.20** (59.11)

*Significant at $p< 0.05$; ** significant at $p< 0.01$.

Table 3: Effect of Zinc sulphate on the serum protein (g/dl) level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↓ %)	30 Days Mean±SD (↓ %)	45 Days Mean±SD (↓ %)
Control	3.98±0.51	3.78±0.48	3.65±0.50
10 mg/L	2.90±0.52 (27.13)	2.79±0.18 (26.19)	2.75±0.38* (24.65)
20mg/L	2.60±0.53* (34.67)	2.38±0.23** (37.03)	2.75±0.38* (32.05)
30mg/l	2.45±0.18** (38.44)	2.30±0.20** (39.15)	2.10±0.38** (42.46)

*Significant at $p< 0.05$; ** significant at $p< 0.01$.

blood sugars (Zikic *et al*., 2001). Thus it can be calculated that zinc affects glucose dynamics in *Clarias batrachus* in order to obtain more energy to withstand and overcome the existing stress condition.

Proteins are highly sensitive to heavy metals and happen to be one of the earliest indicators of heavy metal poisoning. In the present study zinc induced gradual and significant decline in the serum proteins contents in all zinc exposure fishes groups (Table 3). This hypoproteinemia in the present study can be attributed to the enhanced proteolysis. Proteolysis seems to offer a physiological mechanism in a bid to provide energy to cope up with the stressful situation caused by metal toxicity. Depletion in protein level in metal exposed fish thus might be due to its enhanced use to build up new cells or enzymes to reduce the stress. Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Shalaby *et al*., 2006). Thus, it can be calculated that decline in serum protein content can be attributed to the increased cost of homeostasis, tissue repair and detoxification during stress. Therefore, the influence of metal on total protein concentration which act as potential metabolic biomarker of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy.

Triglycerides represent the major energy reserve in the fish. Serum triglycerides levels are usually used to evaluate the metabolic status of an organism. In the present study it is clear from table that triglycerides undergo significant ($p<0.05$ & $P<0.01$) increase in all the groups of zinc exposed fishes when compared to control (Table 4) and hence lead to hypertriglyceridemic conditions may be due to impairment in liver function as also pointed by Hadi *et al.*, (2009). As such, various lipolytic enzymes which function locally and convert triglycerides into fatty acids and glycerol probably get released in blood due to the degeneration of liver cells leaving triglycerides unprocessed. Thus, it seems that reduced rate of lypolysis ultimately results in the elevated serum triglycerides levels.

Alteration in the cholesterol level of blood is the indication of liver dysfunction. In the present study significant increase in cholesterol values have been observed in all the zinc exposed groups of fishes as compared to controls (Table 4). This hypercholesterolemia in metal treated fishes m ight be due to the degenerative changes in the liver tissue. Since homeostasis of lipids is one of the principal functions of liver, any change in serum cholesterol concentration may thus be a clear indicative of liver dysfunction. Moreover, it seems that the rise in cholesterol levels (which are potential energy reserves) in response to

Table 4: Effect of Zinc sulphate on the serum Total Lipid level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
Triglyceride(mg/dl)			
Control	49.80±0.31	53.62±0.33	52.85±0.28
10 mg/L	52.32±0.35 (5.06)	56.28±0.32 (4.96)	56.30±0.24 (9.57)
20mg/L	54.58±0.41 (9.60)	59.32±0.42 (10.63)	60.42±0.41* (14.32)
30mg/l	56.18±0.18* (12.81)	63.28±0.31* (18.01)	64.18±0.34** (21.43)
Cholesterol (mg/dl)			
Control	72.20±0.22	78.38±0.33	78.65±0.53
10 mg/L	80.32±0.18* (11.24)	83.15±0.32 (6.08)	86.18±0.31 (9.57)
20mg/L	83.18±0.42* (15.21)	86.18±0.18 (9.95)	89.14±0.18* (13.34)
30mg/l	85.72±0.26** (18.72)	90.25±0.34* (15.14)	91.21±0.42* (15.97)

*Significant at $p< 0.05$; ** significant at $p< 0.01$.

unfavourable condition possibly may be one of the way these intoxicated fishes employ to strengthen the excess energy reserves which are required by them to mitigate the effects of stress. Liver releases various lipolytic enzymes in the blood which convert cholesterol into bile. Impairment in the liver tissue under the stress of zinc exposure, by inhibiting the release of such enzymes which catabolize cholesterol may be another probable causative of increment in the serum cholesterol content in *Clarias batrachus.*

Thus triglycerides and cholesterol are known to participate in the rise of total lipid. The rise of these energy reserves in response to pollution could be due to the fact that excess energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress (Lee

Table 5: Effect of Zinc sulphate on the serum phosphatases level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
Acid phosphatase (U/L)			
Control	1.34±0.78	1.36±0.72	1.38±0.48
10 mg/L	1.38±0.32 (2.99)	1.40±0.18 (2.94)	1.44±0.32 (4.34)
20mg/L	1.42±0.48 (5.97)	1.48±0.12 (8.82)	1.52±0.28 (10.14)
30mg/l	1.65±0.28* (23.13)	1.68±0.18* (23.52)	1.72±0.33* (23.91)
Alkaline phosphatases(U/L)			
Control	3.28±0.56	3.30±0.45	3.34±0.26
10 mg/L	4.22±0.18 (28.65)	4.43±0.28* (34.24)	4.02±0.26 (20.36)
20mg/L	4.28±0.30* (30.48)	4.78±0.31** (44.84)	4.92±0.28** (47.31)
30mg/l	4.52±0.18* (37.80)	4.98±0.32** (50.90)	5.22±0.34** (56.28)

*Significant at p< 0.05 ; ** significant at p< 0.01.

Table 6: Effect of Zinc sulphate on the serum Transaminases level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
SGOT(U/L)			
Control	65.05±0.08	66.10±0.20	66.70±0.32
10 mg/L	69.42±0.43 (6.72)	70.81±0.30 (7.12)	71.31±0.52 (6.91)
20mg/L	71.38±0.42 (9.73)	72.18±0.18 (10.11)	73.72±0.16 (10.52)
30mg/l	74.42±0.43* (14.40)	74.38±0.28* (12.52)	76.82±0.42* (15.17)
SGPT(U/L)			
Control	32.35±0.41	32.83±0.32	33.05±0.18
10 mg/L	38.18±0.33 (18.02)	46.42±0.12* (4139)	49.18±0.32* (48.35)
20mg/L	45.18±0.28* (39.65)	58.18±0.14** (77.21)	55.10±0.35* (66.21)
30mg/l	50.45±0.23* (55.95)	62.18±0.28** (89.39)	65.25±0.38** (96.83)

*Significant at p< 0.05; ** significant at p< 0.01.

et al.,1983). Since homeostasis of lipids is one of the principal liver functions, any change in serum triglyceride concentration is used as an indicator of liver dysfunction (Kaplan *et al.*,1988). In addition, the abnormal accumulation of fats (both cholesterol as well as triglycerides) in experimental animals could be due to induced imbalance between fat production and utilization (Moore *et al.*,1988).

Serum enzymes such as acid and alkaline phosphatases are membrane bound lysosomal enzymes and the sensitive biomarkers in ecotoxicology as they provide an early warning of potentially hazardous alterations in contaminated aquatic organisms. These enzymes synthesized in liver catalyze the hydrolysis of monophosphate esters and their activities usually find relation to cellular damage. Alkaline phosphatase is a polyfunctional enzyme acts as transphosphorylase at alkaline p^H and plays an important role in mineralization of the skeleton of aquatic animals and in membrane transport activities (Bernt *et al.*, 2001 and Lan *et al.*, 1995). Acid phosphatase is a hydrolyzing enzyme and act as a good indicator of stress conditions in biological systems (Verma et al., 1980). In the present study significantly increased in serum phosphatases activity was found in zinc exposed fishes compared to control (Table 5). Hadi *et al.* (2009) also advocated increase in enzymatic activities of fish under stress of xenobiotics. Jiraungkoorskul *et al.* (2003) and Thangamalathi *et al.* (2016) showed that the change in ALP activity was a result of physiological and functional alteration in metal exposed fish.

Liver is an important organ metabolism and detoxification of xenobiotic as well as biocides in the fish (Sharma *et al.*, 2007).The serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxalate transaminase (SGOT) produced in liver, on the other hand, play an important role in protein and amino acid metabolism. In liver of healthy fishes, transaminases are located in mitochondria. Any variation in the concentration of these enzymes thus very clearly reflects the status of hepatic condition of the fish.

Thus SGOT and SGPT are markers of liver function. The elevated levels of SGOT and SGPT in zinc induced *Clarias batrachus* indicate the liver damage (Table 6). Similar report of increased in the level of transaminases was shown in fluoride induced fish (Srivastava *et al.*, 2012). Therefore, elevated level of these enzymes in serum of zinc induced *Clarias batrachus* indicated liver damage and disruption of normal liver function.

Thus metal intoxication appears possibly results in liberation of these enzymes (phosphatases and transaminases) into circulation which might have been due to the damage of the hepatic tissue under the state of stress created by metal toxicity. Due to the impaired cellular architecture, these enzymes it appears may get released from the hepatic cells which have become necrotic with degenerated cell membranes. Once released from the liver cells, these enzymes enter the general circulation and thus may lead to increase in their concentration in the blood.

On the basis of present results and discussion, it can be concluded that heavy metal, zinc induced marked serum biochemical alterations in *Clarias batrachus*. Examination of biochemical parameters thus can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicant.

Metal concentrations in the aquatic environment is seen as potential threat for aquatic organisms that are exposed to significant amount of heavy metals as a consequence of industrial, agricultural and anthropogenic activities. From the observations of present data it can be concluded that, although heavy metal, zinc is an essential trace element for various physiological processes but at high concentration it poses toxic metabolic stress hence altered the serum biochemistry of fishes thereby affecting human population. Also, it can be concluded that the fishes can effectively used as monitors or water quality.

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Original Article

Langerhans Cells Distribution in Oral Mucosa of Immunosuppressive Mice Associated with and without Oral Candidiasis: A Comparative Study

Khulud Mohammed Alshehri

Abstract

Context: Langerhans cells (LCs) are dendritic cells residents in epithelial tissue, which motivate the immune system against to pathogen entry, self-antigen, commensal microbes and protect the mucosa. Langerhans cells in oral mucosal are responsive to wide microbes such as oral Candida species that play immunological activity in immunosuppressed cases. *Aims*: The study was aimed at compering the distribution of LCs in in immunodeficiency mice associated with/without oral candidiasis. *Settings and Design:* Thirty female mice were distributed in three groups; control group (C group), immunosuppressed group without candida infection (IM group) and immunosuppressed group with candida infection (IM+*C. albicans* group). *Methods and Material:* The dorsal tongue surface stained with CD1a marker and the number of LCs was calculated. *Statistical analysis used:* was performed using analysis to determine the difference in the number of LCs between groups by analysis of variance or Student t test with unequal variances. A P value of <0.05 was regarded to be statistically significant. *Results:* The statistically examination of lingual sections showed high significantly different in LCs cells number between each group ($p< 0.05$). The greatest number of LCs was observed in (IM+ *C. albicans* group) with the Mean±SD: 102±31.30, and the lowest number was observed in (C group) with the Mean±SD: 17±14.29, while (IM group) recorded Mean±SD: 44.2±9.95. *Conclusions:* The increase in (IM+*C. albicans* group) might return to the role of antigenic exposure that leading to cell mediated immunity in oral candidiasis.

Keywords: Langerhans Cells (LCs); CD1a; Oral Mucosa; Immunosuppressed; *Candida Albicans*.

Introduction

Stratified squamous epithelium of oral mucosa contain Langerhans' cells (LC) which are dendritic, antigen-presenting cells. Langerhans' cells (LC) have a role function the immune systems the peripheral arm [1]. LCs is driving immune response against antigenic pathogens in the epithelial surface for secure of body's integrity and homeostasis , most of them peripheral organ, As sentinels of immune system [2]. The mucosal LCs is imperative mediators of mucosal immunity, it have immune reaction to antigen of microbial and tumor, yet additionally of resilience to self-antigen and commensal microbes. oral mucosal LCs have been discovered receptive to several other diseases such as nickel in patients with nickel allergies, oral Candida species, oral lichen planus, immunodeficiency virus (HIV) infection and oral squamous cell carcinoma [3]. Oropharyngeal candidiasis and gastrointestinal *C. albicans* infection are more wide demonstrated in, immunocompromised patient populations (e.g. AIDS and transplant patients, and patients undergoing steroid therapy) [4]. The defenses mechanisms of innate and adaptive immunity in host against *C. albicans* incorporate mechanical barriers to fungal entrance epithelial surfaces and soluble antimicrobial factors [5].

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Cytokines and chemokines are regulating responses proteins to infection secreted by epithelial cells in response to *C. albicans* hypha attack and activate immune cells. The innate and adaptive immune response mechanisms ultimately clear the fungus or reduce fungal burdens below the activation threshold, there by re-establishing the commensal phenotype [5]. The adaptive arm of the anti-*C. albicans* response is initiated through recruitment of dendritic cells (or langerhans cells) to the site of mucosal infection, Dendritic cells will recognise *C. albicans* through established pattern recognition receptors and traffic to the local lymph node where processed fungal antigen will be presented to T cells to initiate adaptive immunity [4] the current study goals are to quantitatively the number of oral mucosal LCs in immunodeficiency mice associated with/without oral candidiasis with a view to elucidate their role in pathogenesis. Subjects and Methods:

Animal Model

Reduce immunity thirty female mice, 6 week old, weighing 25–30 gm each, were gotten from the Animal House, KFMR and kept under controlled temperature (22±2°C), humidity (55±10%), and 12/12 hours cycle of light and dark with an access to nourishment and drinking water ad libitum. The animals were immunosuppressed and treated with Takakura et al., 2004 method . [6] and arranged into 3 groups; first group was control group (C group) which was resaved physiological solution. Second group was immunosuppressed group with no candaidal infection (IM group). Third group was immunosuppressed group infected with Candida albicans ATCC66027 was obtained from Microbiology laboratory, KAU Hospital (IM+*C. albicans* group).

Immunohistochemistry

The tongue were fixed in 10% formalin, after embedding in paraffin were sectioned of 4µm thickness Narayanan and Narasimhan 2015 method using CD1a expression [7].

Evaluation

The stained slides were viewed and analyzed under light microscope. Under the microscope brown surface stained cell was taken as positive for CD1a as CD1a is a surface marker. The quantity of LCs per high power field (400x) were calculated from 6 fields in the varying layers of epithelium and the average LCs in a high power field was calculated for each of the sections.

Statistical Analysis

Statistical analysis was performed using analysis to determine the difference in the number of LCs between groups by analysis of variance or Student t test with unequal variances. A P value of <0.05 was regarded to be statistically significant. Computations were done by SPSS software [8].

Results

Immunohistochemistry of lingual sections stained with CD1a revealed amount of LCs population with distributed length of oral basement membrane in each group study and morphological appearance of LCs cells was shown with highly or fewer dendrites.

Histologically examination of lingual sections showed the normal view of tongue in the control group with stratified squamous keratinized epithelium with a few numbers of LCs in basement membrane figure 1, while the IM group specimens

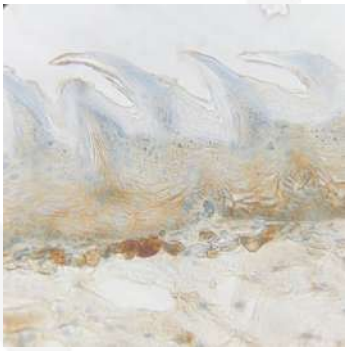

Fig. 1: Control group with a few numbers of LCs in basement membrane CD1a magnification × 400

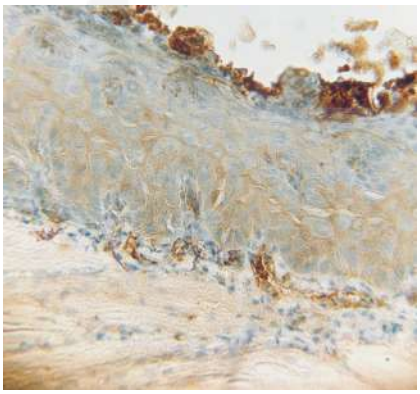

Fig. 2: Immunosuppressive with oral candidiasis showed a large distribution of LCsCD1a magnification × 400

Table 1: Statistical comparison of Langerhans cell counts in control C ,immunosuppressed with no *candida* infection IM, immunosuppressed with *candida* infection IM+*C. albicans* (unpaired student's t test)

	Mean ±SD	C	IM	IM+*C.albicans*
C	5.8±23		*p*= 0.0001	*p*= 0.0001
IM+*C.albicans*	102±31.30	*p*= 0.0001		*p*= 0.0001
IM	45.2±9.07	*p*= 0.0001	*p*= 0.0001	

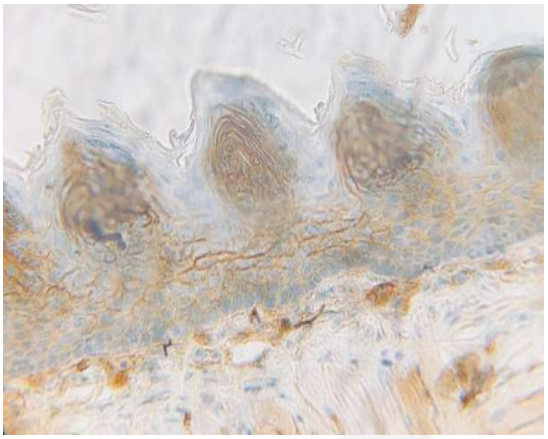

Fig. 3: Immunosuppressive without oral candidiasis showed numbers of LCs in basement membrane CD1amagnification × 400

showed a slight change in filiform papillae distributed and largest number of LCs in basement membrane figure 2, on the other side highly Candidal colonization showed in IM+ *C. albicans* group specimens lead to candida pseudohyphae penetrating the superficial keratinous epithelial layers and number of LCs in basement , connective tissue and few number of LCs cells reach to keratinized layer figure 3.

The Mean number of LCs per unit area in groups is presented in table 1. The greatest number of LCs per unit area was observed in (IM+*C. albicans* group) with the Mean±SD: 102±31.30, and the lowest number of LCs per unit area was observed in (C group) with the Mean ±SD: 17±14.29, while (IM group) recorded Mean ±SD: 44.2±9.95.

The Statistically compared of Langerhans cell counts in control, immunosuppressed, candidal infected immunosuppressed groups table1, showed highly significantly different between C and IM+ C.albicans group (p<05) as was different between IM and C+IM group (p<05). The LCs cells counts for C and IM+ *C. albicans* groups was also significantly different (p< 0.05).

Discussion

This study has shown the normal distribution of LCs cells in the basement membrane of control specimens C, while LCs cells reach to connective tissue in IM group specimens, and the largest distribution of LCs cells appeared in IM+C.albicans group specimens. The authors viewed lowest quantities of LCs in the floor of mouth and highest quantities in the buccal mucosa and dorsum of the tongue.

LCs cells arranged as a line along the length of epithelium and were found in basal and supra-basal layers of lateral tongue border and floor of mouth [9]. The recent result recorded two types of LCs have been defined based on their dendritic to highly dendritic LCs and fewer dendritic LCs in common with Breathnach 1977 the electron microscopic appearance of LCs noted two types: Type 1: described by highly dendritic with an electronlucent cytoplasm, numerous granules and is usually found in the suprabasal layers; Type 2: described by fewer dendrites, a more electron-dense cytoplasm with fewer Birbeck granules and is usually located in the basal layer [10].

The result shown significantly different between C and IM+ *C. albicans* group (p<05), many studies have highlighted LCs are capable of engaging with a wide kind of pathogens, and, upon endocytosis, they can process antigens, prime naive T-cells, and initiate adaptive immune responses. Also an increase of LCs number associated with the development of the oral micro flora has been observed [11].

LCs mobilize and mature in response to inflammatory cytokines and pathogen associated molecular patterns from oral mucosal Pathogens. Oral mucosal LCs appear to be oriented in a manner to efficiently sample the oral fluids and bacteria, with their dendrites extending toward the surface, and often represent a hetrogenous population [12]. The LCs counts in immunosuppressed with no candida infection group IM and, immunosuppressed with candida infection group IM+ *C. albicans* was p<05 that result reflect to LCs response for candida

infection. Newman and Holl report the LCs have defense role against candidiasis with phagocytic capacity of candidal yeasts and hyphae as well as processing their antigens [13].

Our histological recorded data similar to Romagnoli et al., 1997 in heavily candida-infected sections the CD1a positive LCs were particularly intense and the localization was quite variable. The dynamic nature of LCs and its involvement in local disease mechanisms such as chronic hyperplastic candidiasis lead to highly LCs variation in its number or localization. Furthermore, LCs were significantly numerous and richer in dendrites and Birbeck granules in erythematous areas than in areas of pseudomembranous candidiasis. [14] This finding is consistent with the prevailing view that a high density of Langerhans cells CD1a in the epithelium of Oral lichen planus has an important role in the antigen presentation and immunity cells activation [15].

Some reports, however, Confirmed LCs in oral mucosa have been response to some oral disease; Candida species, oral lichen planus (OLP), rhomboid median glossitis, human immunodeficiency virus (HIV) infection, hairy leukoplakia of the tongue, oral squamous cell carcinoma, and several other diseases [3-16-17].

This study shown highly significant between LCs cells counting in IM and IM+ C.albicans groups with $p< 0.05$ this difference is refers to candida in IM+ *C. albicans* sections. This reason agrees with Romagnoli et al., 1997 reported this difference is appears because the clinical appearance of a reaction to Candida antigens as a result of defense mechanism activation in HIV seropositive subjects where oral tissues influenced by candidiasis showed decrease of LCs number, than oral tissues without candidiasis; this reduction being more significant in oral pseudomembranous than erythematous candidiasis [14].

Conclusion

From the above results it was obvious Langerhans cells (LCs) played an important role in the immune system against pathogens attack especially in immunosuppressed cases, finally, it recommended that studies investigating the factors effected the numbers and activation of oral LCs found in immunosuppressed candidiasis cases, and immune interaction between epithelial host and pathogens.

Key Messages

The quantities of Langerhans' cells (LC) in oral mucosa are clear evidence on the efficacy of the peripheral immune system. The number of Langerhans' cells (LC) in oral mucosa influenced in immunosuppressed cases and increased in microbial infections such as white *Candida albicans* attacked.

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Original Article

Qualitative and Quantative Analysis of Macrozoobenthos of Baghel Taal, A Wetland of U.P.

Ashok Kumar Verma[1], Sadguru Prakash[2]

Abstract

The present study was conducted on Baghel Taal of Baharaich district of U.P., dealing with the qualitative and qualitative analysis of macrozoobenthic diversity. During the present investigation 36 genera were recorded which belonged to phyla Annelida, Arthropoda and Mollusca. During the present study molluscs show rich diversity contributing about 42%, to the total benthic population as shown in table, arthropods also shoes good diversity contributing about 30% while as annelids contributing only 28% of the total population. The overall benthic population was estimated to be 1712 nos/m². Highest diversity (15 genera) and population density (752 nos/m²) was contributed by molluscs followed by arthropods (11 genera and 472 nos/m²) and annelids (10genera and 488 nos/m²).

Keywords: Macrozoobenthos; Baghel Taal; Wetland.

Introduction

Wetlands are considered to be one of the richest sources of biological diversity. Due to urbanization and anthropogenic pressure most of the wetlands are succumbed to greater degree of biologically active nutrient accumulation.

Benthic invertebrates occupy the bottom of the water body. The functional role of benthic communities in the trophic dynamics of aquatic ecosystem is well acknowledged. The composition, distribution of benthic organisms over a period of time provide index of the ecosystem. In recent years, there is greater emphasis world over for better understanding of benthic environment.

The present study was conducted on Baghel Taal of Bahraich, U.P., with special reference to macrozoobenthic diversity. The benthic communities composed of a wide range of flora, fauna and bacteria from all levels of food web and inhibit different types of habitat such as mud, sand attached to rocks, stones, macrophytes and other solid organic matter. As we know that each species is important component of food chains and food webs which helps in transfer of energy to trophic levels and cycling of nutrients in any ecosystem. Macrozoobenthic organisms act as food for many aquatic birds and fishes also benthic organisms are used as potent pollution indicators, so it is utmost important to document the benthic diversity. On these aspects, Benthic diversity of lentic waterbodies were studied by many ecologists in India (Gupta, 1976; Dutta *et al.*,1887; Shrivastava, 1997; Pani and Misra, 2000; Kumar, 2001; Sisodia, 2001; Pani and Misra, 2005; Srinivasan and Hamlatha, 2006; Bhat and Pandit, 2009; Vyas and Bhat, 2010) but no such information is available in fresh water body of North-Tarai region of U.P. Keeping this mind an attempt has been made to document macrozoobenthic diversiy of Bhagel Taal and their composition as well as to know the variation of the macrozoobenthic diversity with depth.

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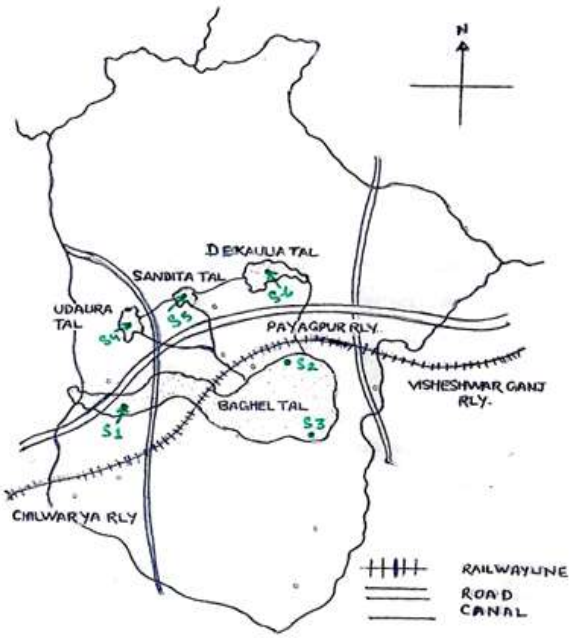
Study Area

Baghel Tal is a large shallow perennial lentic waterbody with irregular margins and dense growth of macrophytes. It is situated in village Baghel, Payagpur block of district Bahraich at a distance of about 1.60 km. to the south-east of Payagpur Railway station. It is about 31 km. away from Gonda, 39km. from Bahraich and 45 km. from Balrampur. It is half oval in shape with maximum diameter of 3800m and connected with three small waterbodies namely Udavra Tal, Sandita Taal and Dekaulia Taal. It receives water from three main streams, Babia Nallah from north-west side, Jamvar Nallah from north and Sakarpatti Nallah from north-east side during rainy season. It is also a Bird sanctuary extending around 32 km. with total catchment area of reservoir 441.5575 acre. Out of this only 121.22 acre is water body. In rainy season but in summer its area becomes limited to 438 ha. Its maximum depth in summer season was found 3.6m. It is habitat of rich micro- and macro living organisms including *Nymphaea, Nelumbo*, Narkul, Tinna Rice, vegetation as well as snails, fishes and frogs. This abundant food attracts hundreds of resident and migratory birds including Siberian crane during winter season. Five stations were selected throughout the water body on the basis of habitat, nutrient type and supply which are as S_1, S_2, S_3, S_4 , S_5 and S_6. Sites S_1 , S_2 and S_3 are located in inshore region of Baghel Taal receiving organic nutrients which act as food for growth of benthos. Sites S_4 , S_5 and S_6 are located in deepest region of Udavra Taal, Sandita Tal and Dekaulia Taal respectively.

Materials and Methods

The sediment sample from the bottom at all station were collected during morning time by using Peterson Grabe mud sampler, collected samples were sieved through 0.5 mm sieve (Ankar and Elmgreen, 1976) the material which retained on sieve were collected and from it benthic organisms were stored out with the help of forceps and brush and were collected in narrow mouthed plastic bottle, containing 4% formalin and 70% alcohol as preservative depending upon the type of organisms to be preserved. The soft-bodied organisms were preserved in 70% alcohol while the shelled organisms like mollusks in 4% formalin (Borror *et al.,* 1976) . All macro fauna of bottle were identified with the help of available key and manuals Neetham and Needham (1962), Borror *et al*. (1976) and Pennak (1989) under the light microscope. The population of organisms was counted and number of individuals of a species per sample and was expressed as number/m² .



Location of Baghel Tal

Results and Discussion

In normal condition the distribution of macro benthos fauna has been reported to be dependent on the availability and distribution of preferably food items. In fact, their capacity to exploit areas with optimum food supply might be explained by their abundance (Zahoor *et al.*, 2010). The benthic population of the water body was estimated to be 1712 nos/m² during twelve month study period in bimonthly sampling. Vyas and Bhat (2010) and Shrivastava (1997) reported 1782 nos/m² 845nos/ m² intropical water body and Ravishankar reservoir, respectively. Benthic diversity of all the stations is given in the table; during the present investigation 36 genera were identified throughout the study period. Out of 36, 10 species belonged to annelids, 11 belonged to arthropods and 15 belonged to molluscs. Among the macrobenthos, *Tubifex* sp., *Aumbriculus* sp., *Lumbriculus* sp. and *Nais* sp. of annelid; *Chironomus* sp, *Spaniotoma* sp. and *Cyclops* sp. of arthropods where as *Bellamya* sp, *Vivipara* sp, *Pila* sp, and *Pissidium* sp. of molluscs were most dominant forms being present in all the six stations of Baghel Tal.

During the present study molluscs show rich diversity contributing about 42%, to the total benthic population as shown in table, arthropods also shoes good diversity contributing about 30% while as annelids contributing only 28% of the total population. Molluscs performs key role in functioning the aquatic ecosystems. In the present study it was observed that diversity as well as density of macrobenthos was maximum in spring months followed by summer months due to maximum decomposition of macrophytes leaf litters on the bottom of waterbody. These leaf litter decomposed by decomposers because the increased water temperature, activating the process of decomposition of these organic sediments.

Stations S_1 (33 genera & 448nos/m²), S_2 (31 genera & 445 nos/m²), and S_3 (29 genera & 433 nos/m²)are rich in diversity and population density as compared to stations S_4 (19 genera & 140 nos/m²),S_5 (18 genera & 133 nos/m²), and S_6 (16 genera & 113 nos/m²) supporting less benthic diversity and population density due to inadequate nutrients supply because S_1, S_2 and S_3 are inshore stations of main waterbody where as S_4 , S_5 and S_6 are deepest

Table 1: Macrozoobenthos diversity of Baghel Taal during 2016 – 2017

Phylum/Genera	Number of Macrozoobenthos (number/m²)					
	S_1	S_2	S_3	S_4	S_5	S_5
Annelida						
Tubifex sp.	32	34	23	5	9	6
Aumbriculus sp.	19	25	12	10	4	5
Lumbriculus sp.	34	4	9	16	12	15
Poecilobdella sp.	6	-	8	12	8	-
Glassiphonia sp.	7	4	8	-	-	-
Batracobdella sp.	12	6	1	1	-	-
Branchiura sp.	6	8	2	-	4	3
Limnodrillus sp.	11	14	9	2	-	-
Nais sp.	26	15	19	3	7	2
Hemiclepsis sp.	5	6	-	-	-	-
Diversity / Density	10/158	9/116	8/90	7/49	6/44	5/31
Arthropoda						
Chironomus sp.	37	32	42	12	11	16
Spaniotoma sp.	18	13	15	8	1	9
Polycetropus sp.	6	3	11	-	-	-
Philopotamus sp.	8	15	10	-	-	-
Tinodes sp.	6	6	11	-	-	-
Hydroptila sp.	2	-	-	-	-	-
Psphenus sp.	33	10	35	-	-	-
Caenidae sp.	-	-	-	6	8	-
Gammarus sp.	-	12	-	-	-	-
Cyclops sp.	12	11	19	2	8	4
Atydae sp.	3	-	9	-	-	8
Diversity / Density	9/125	8/102	8/152	4/28	4/28	4/37
Mollusca						
Lymnaea sp.	12	-	10	7	-	-
Bellamya sp.	32	25	26	11	15	14
Vivipara sp.	4	6	9	2	5	2
Gyraulus sp.	3	9	-	-	-	-
Thiara sp.	29	24	27	-	-	-
Pila sp.	24	29	38	11	12	18
Unio sp.	11	14	12	-	8	2
Planorbis sp.	2	31	14	-	-	-
Gibbia sp.	7	14	4	8	-	-
Corbicula sp.	12	14	11	7	9	4
Lymnaea sp.	11	15	15	-	5	2
Perreysia sp.	4	12	7	4	3	-
Pissidium sp.	11	15	14	13	2	3
Melanoides sp.	3	8	4	-	-	-
Planorbis sp.	-	11	-	-	2	-
Diversity / Density	14/165	14/227	13/191	8/58	9/66	7/45
Total	33/448	31/445	29/433	19/140	19/133	16/113

stations of small waterbodies connected with Baghel Taal. Due to low depth, transparency increases which helps in penetration of sunlight to the bottom layer by which process of decomposition get accelerated resulting increase in benthic diversity.

The findings of the present study agreed with the findings of Efitre *et al.* (2001), Pani and Misra (2005), Srinivasan and Hamlatha (2006) and Vyas and Bhat (2010). Thus it can be concluded that shallow inshore area of water bodies are suitable for growth of benthic organisms because these zones are rich in macrophytes and solid organic wastes.

Conclusion

Macrozoobenthos function in different ways that are important to maintaining ecosystem functions such as energy flow in food webs. In the process of maintaining energy flow, these benthic species simultaneously provide essential ecosystem services, such as nutrient cycling and aeration of sediments. Different species comprise distinct functional groups that provide ecological integrity . The present study shows that benthic organisms grow easily in shallow zones. Thus the present water body is cradle for benthic organisms especially shallower regions were macrophytes are abundant from diversity point of view habitat i.e. bottom of the body showed posses mud, sand, rocks, stones, macrophytes and solid organic wastes to which benthic organisms get attached and act as on organic debris.

Acknowledgements

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Original Article

Initiation and Establishment of Agrometeorological Obesrvatory and Quantification of Weather at VCSG College of Horticulture, Bharsar

Ravi Kiran

Abstract

V.C.S.G. College of Horticulture, Bharsar, Pauri Garhwal, Uttarakhand,was established in 2001 under Govind Ballabh Pant University of Agriculture & Technology, Pantnagar till 2011 as 11th College of the university. V.C.S.G. College of Horticulture, Bharsar. The Latitude of bharsar (pauri garhwal) is 30.060 N. The Longitude of bharsar (pauri garhwal) is 78.990 E and height is 2000 m MSL altitude. An agrometeorological Observatory was established by the sole author of this research paper by the sole author of this paper, Dr. Ravi Kiran who was appointed as Assistant Professor (Agrometeorology) in January 2006 by the Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, during 2007-2008 and daily data recording of different weather parameters has been started by the author of this paper since May 2008 onwards.

Keywords: Agro Meteorological Observatory; Weather Data; Maximum Temperature; Minimum Temperature; Rainfall; Snowfall; V.C.S.G. College of Horticulture; Bharsar.

Introduction

Crop yield is influenced by several factors like weather, soil type and its nutrient status, management practices and other inputs available. Weather is the only environmental factor which influences the growth and final yield of the crop cycle. It governs the crop phenological development and the efficient conversion of biomass into economic yield. Weather assumes significance in nearly every aspect of agricultural activity, storage and plant protection measures, hence success or failure of crop is intimately related to the prevailing weather conditions. Therefore records of weather are a must for future planning.

Pauri Garhwal is located between 29^0 20'-29^0 75' N latitude and 78^0 10'-78^0 80' E longitude, which covers around 5540 km^2 area. The district is the most fascinating segments of Himalaya, stretches from the Ram Ganga river that separates Pauri-Kumaon border in the East, and to the Ganga depicting the Western border. Almora, Nainital (East), Chamoli, Tehri and Dehradun (North-West) and adjacent plains of Bijnor, Hardwar (South) districts, surround this district. Bharsar is about 60 km from the district head quarter (Pauri) in the East-South direction on the road side area of Pauri-Thalisain-Ram Nagar National Highway. Bharsar meaning in local dialect is 'flourished with natural wealth'. It contains the temperate evergreen forest towards North-East (Budha Bharsar), North-West (Chauri Khal), East-South direction have terracing crop fields and village namely Dhulet, Sakniyana, Buransi, Nauntha, Sainji.

The climate of the Bharsar is represented by the mild summer, higher precipitation and colder or severe cold prolonged winter. Major output of precipitation is in the form of rain fall, alongwith occasional occurrence of due, hailstorm, fog, frost, snow fall. The South-West monsoon starts by the end of June while the western disturbances cause occasional winter

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showers during November-February. During winter, snow fall is common in this region. During summer months, the valley has warm climate prevailing for few hours in a day, but the nights are cool. Climate change is expected to have serious environmental, economic, and social impacts in mountainous regions worldwide.

This region is situated in mid hills of garhwal region representing temperate climate.

Major farming systems/enterprises (based on the analysis made by the KVK's) are:

Farming System / Enterprise

1. *Bhabar Irrigated*: Rice, Wheat, Sugarcane, Rapeseed, Mustard, Potato, Lentil, Mango, Guava and Litchi
2. *Irrigated Lower Hills*: Rice, Wheat, Onion, Chilies, Peas, Potato, Radish, Cauliflower, Soybean, Mango, Guava, Plums and Peaches
3. *Rainfed Lower Hills*: Finger Millet, Maize, Rice, Wheat, Pulses, Mango, Plums, Guava, Peaches
4. *Mid Hills*: Barnyard Millet, Ffinger Millet, Rice, Wheat, Soy Bean, Potato, Tomato, Peas, Cole Crops, Pulses, Citrus, Plums and Peaches
5. *High Hills*: Barnyard Millet, Amaranth, Finger Millet, Cole Crops, Potato, Peas, Peaches, Plums, Pear, citrus, stone fruits.

Description of Agro-climatic Zone & major agro ecological situations (based on soil and topography)

S. N.	Agro - Climatic Zone	Characteristics
1	Zone A (Up to 1000m)	Alluvial mixed with boulders and shingles, Alluvial sandy soil, residual sandy loam.
2	Zone B (1000-1500 m)	Sandy loam.
3	Zone C (1500-2400 m)	Red to Dark.

Source: http://paurigarhwal.kvk4.in/district-profile.html)

Having a wide variation in climate and weather the area had an urgent requirement of recording of weather data on regular basis in an agrometeorological observatory.

Materials and Methods

V.C.S.G. College of Horticulture, Bharsar, Pauri Garhwal, Uttarakhand, was established on June 12, 2001 under Govind Ballabh Pant University Of Agriculture & Technology, Pantnagar. There was no agrometeorological observatory till 2006. The imitative was taken to establish an agrometeorological observatory for regular records of various weather data on daily basis, by the sole author of this paper, Dr. Ravi Kiran who was appointed as Assistant Professor (Agrometeorology) in January 2006 by the Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, to establish an agrometeorological observatory for regular records of various weather data on daily basis So, in the campus out of 3 proposed sites for establishment of agrometeorological observatory, one at the heart of campus was selected by the sole author of this paper. Site finally selected was situated at the area adjacent the road near Dean's office / girls' hostel adjacent to floriculture block and medicimnal and aromatic block in the campus. Hill top of the selected site was levelled before installation of the instruments. Required masonry work was done for installation of the instruments. Following instruments were installed in agrometeorological observatory (plate 1).

1. Maximum and minimum thermometers.
2. Wet and dry bulb thermometers.
3. Soil thermometers (5, 10, 20 cm).
5. Rain gauge (ordinary).
6. Wind vane and anemometer.
7. U.S.W.B. open pan evaporimeter.
8. Sunshine recorder.
9. Snow gauge

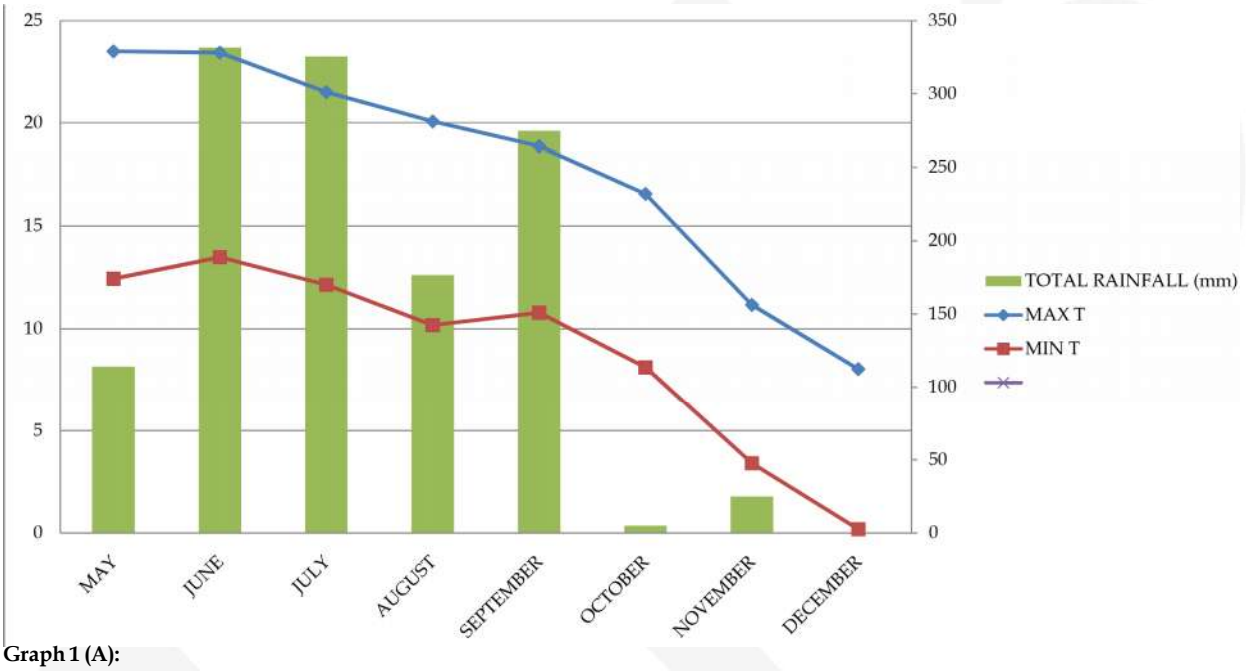
A temporary fencing of the agrometeorological observatory area was made to watch and ward off the wild animals. The recording of weather data was started by May, 2008.

Result and Discussion

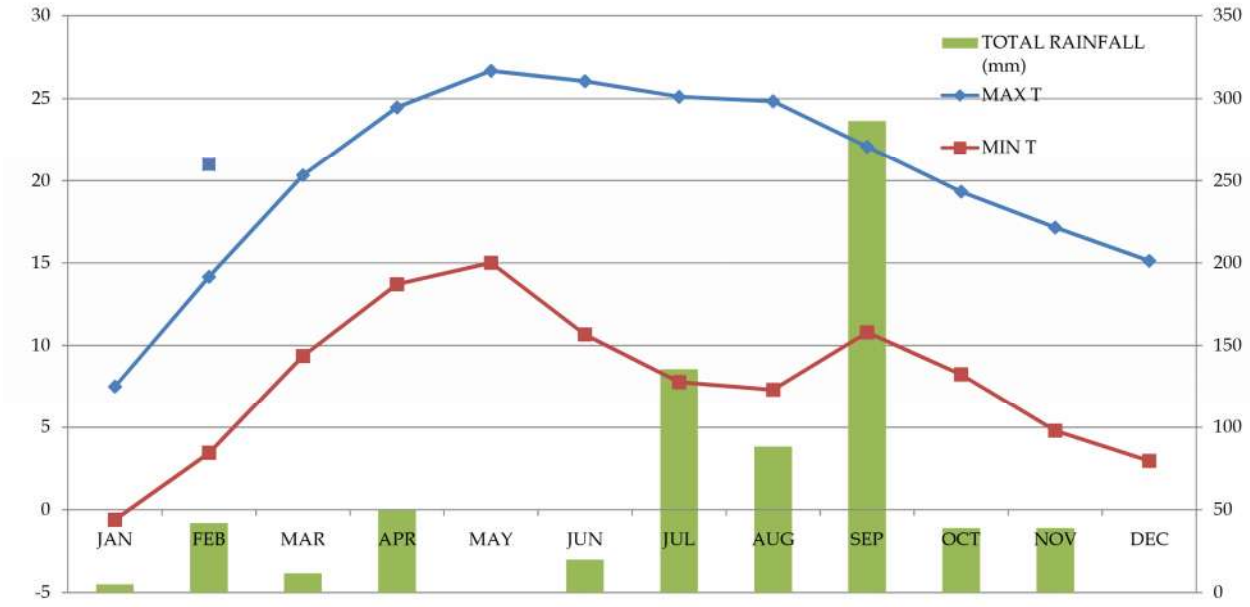
The climate of the Bharsar comprises of the mild summer, higher precipitation and cold prolonged winter. The weather parameters like. precipitation, temperature, relative humidity and wind, along with elevation (valleys or mountain range from temperate zone), proximity to Great Himalaya, slope aspects, drainage, vegetation etc are responsible for highly variable the micro-climate of this area. Precipitation comes in the form of rainfall, besides occasional hailstorm, fog, frost, snow fall etc. The South-East monsoon commences towards the end of June while the western disturbances cause occasional winter showers

during November-February. During winter, snow fall is common in this region. During summer months, the valley has hot climate prevailing for noon hours in a day, the maximum temperature during May-June is recorded between 30^{0}C-35^{0}C however, and nights are cool. December and January are the coldest months, the minimum temperature reaches to 1^{0}C to -4^{0}C. The average monthly maximum temperature was 20.24, 16.25 and 15.45^{0}C having highest and lowest monthly values 26.68, 19.86, 20.38^{0}C and 7.5, 9.0, 7.3^{0}C, respectively during 2009, 2010 and 2011.The average monthly minimum temperature was 6.98, 7.0 and 5.3^{0}C having highest and lowest monthly values 10.8, 11.57, 10.75^{0}C and 2.9, -0.9, -2.5 ^{0}C, respectively during 2009, 2010 and 2011. The annual rainfall was 667.6, 1684.4 and 970.3 respectively during 2009, 2010 and 2011.

The rainfall during sowth west monsoon was 530.4, 1411.8 and 877.3 respectively during 2009, 2010 and 2011.The rainfall during October-December was 39.0, 13.8 and 2.4 respectively during 2009, 2010 and 2011 (Graph 1 A-E).



Graph 1 (A):



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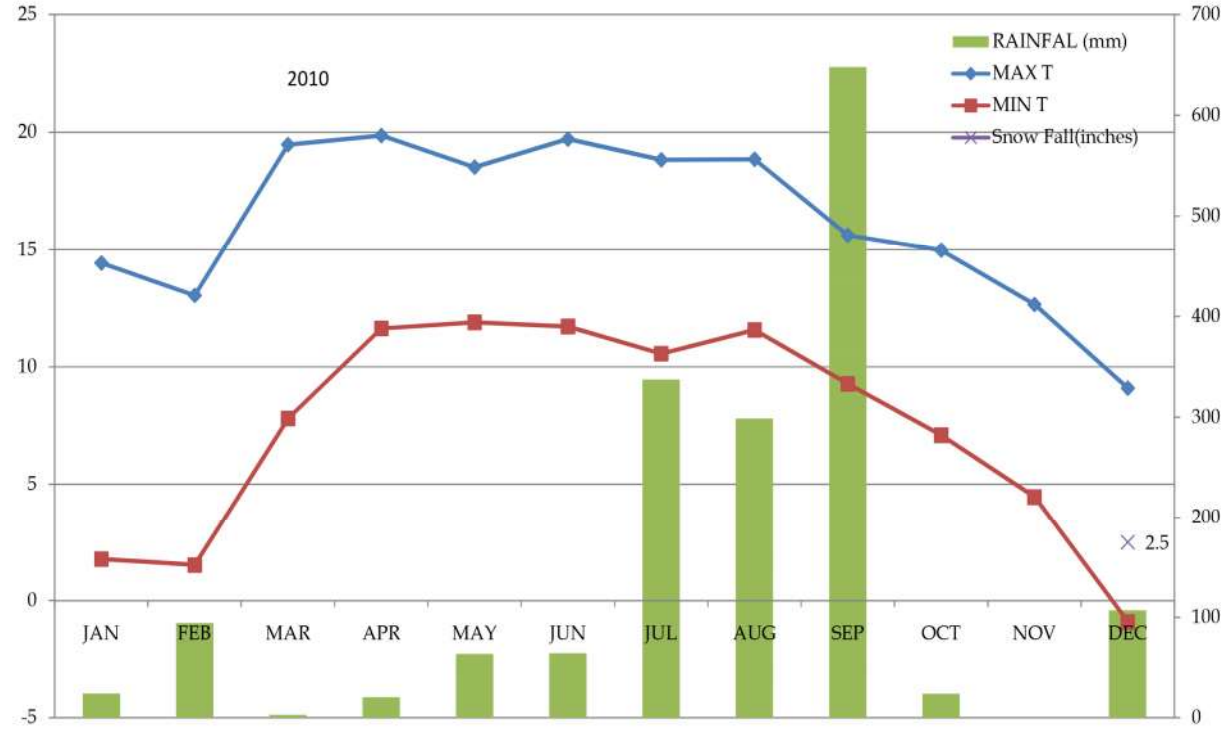

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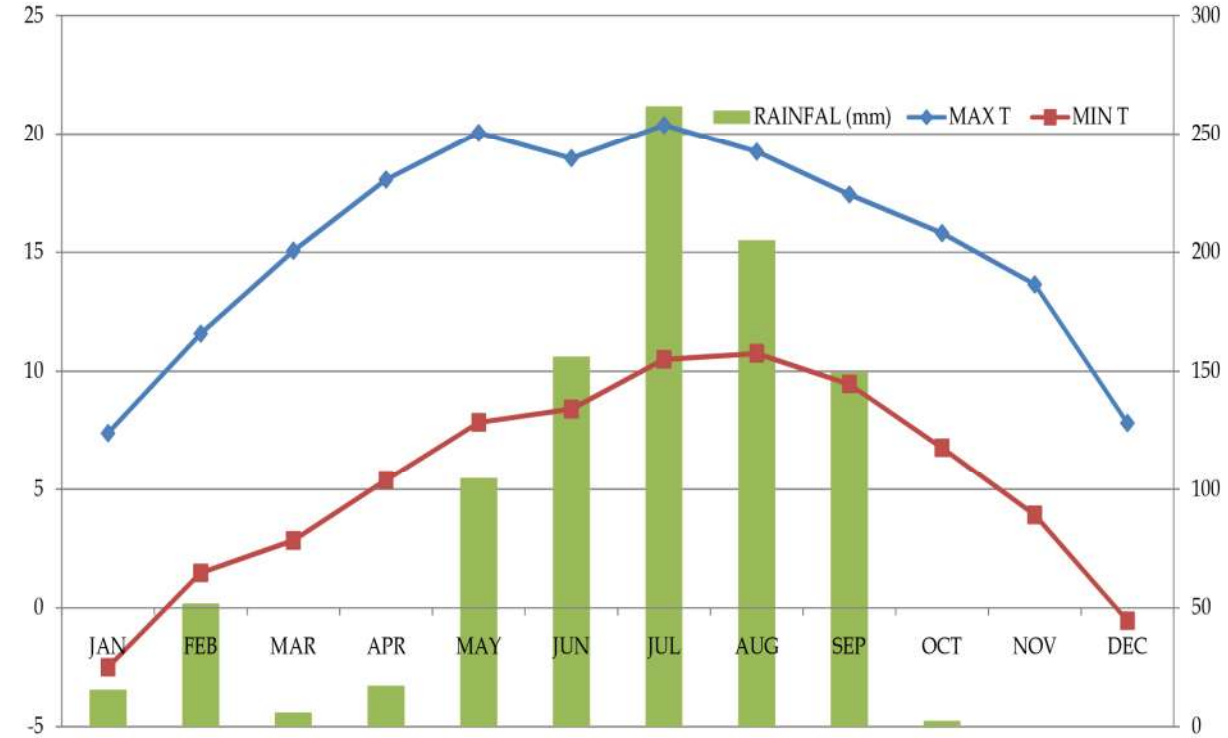

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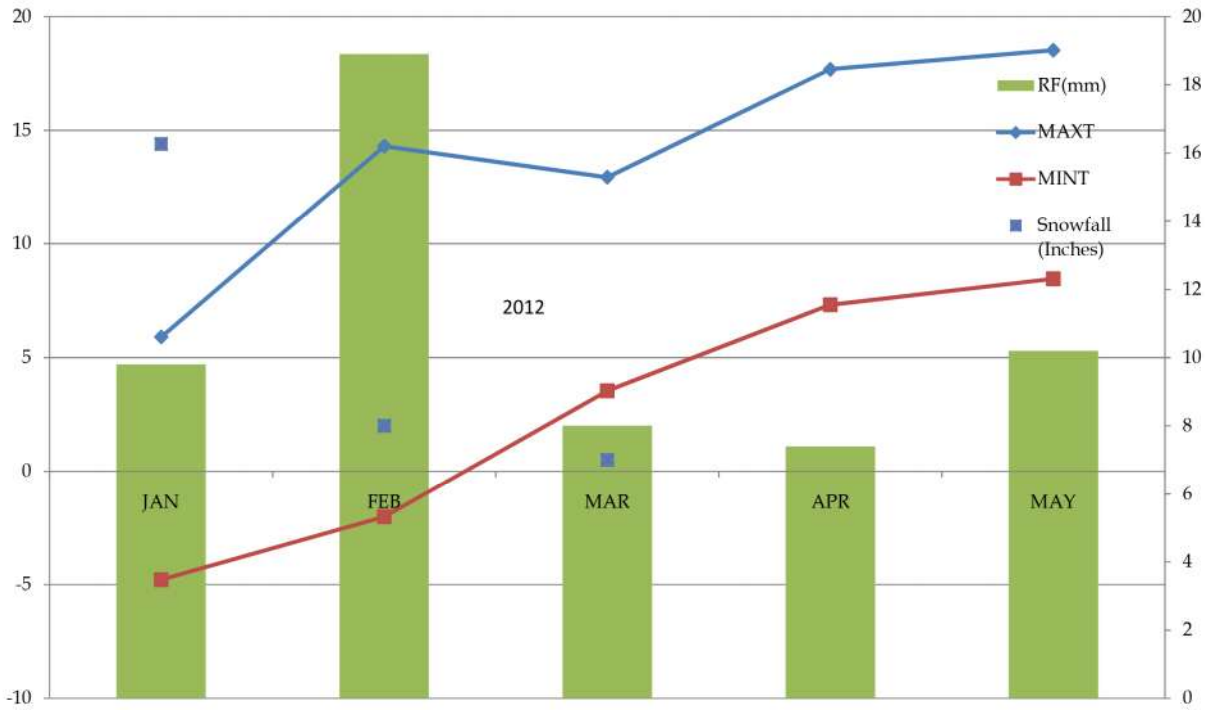

Graph 1 (E):

Graph 1 (A, B, C, D, E): Variations in Maximum (^{O}C), minimum temperature (^{O}C) , snowfall (inches) on primary Y axis and rainfall (mm) on secondary Y axis recorded during 2008- 2012 at VCSGC, Bharsar

Plate 1 (A):

Plate 1 (B):

Plate 1 (C):

Plate 1 (D):

Plate 1 (E:

Plate 1 (F):

Plate 1 (G):

Plate 1 (H):

Plate 1 (I):

Plate 1 (J):

Plate 1 (K):

Plate 1 (L):

Plate 1 (A, B, C, D, E, F, G, H, I, J, K, L)**:** Preparation and installation of agrometeorological Observatory at VCSG College of Horticulture, Bharsar.

Conclusion

On the basis of data it may be concluded that there is wide variation in case of Maximum, minimum temperature, rainfall in the region. There is wide variation in the temperature range also. The average monthly maximum temperature range was 19.18, 10.77 and 12.99^{0}C respectively during 2009, 2010 and 2011. The average monthly minimum temperature range was 7.84, 12.47 and 13.25^{0}C respectively during 2009, 2010 and 2011. Therefore, a thorough analysis is highly needed for proper planning of agriculture under the climatic conditions of Bharsar.

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Original Article

Studies on Weed Diversity of Coastal Rice (*Oryza sativa* L.) Fields in Lucknow Region

Swati Verma

Abstract

A survey was conducted at fifty different rice fields in coastal areas of Lucknow region to identify most common and prevalent weeds associated with rice. Fields surveyed were done according to the quantitative survey method by using 0.5m× 0.5m size quadrate with twenty samples from each field. Total thirty weed species were collected which related to fifteen families and twenty six generas from the rice field in the Lucknow region. The weed survey of this region was made during August to November 2013. Maximum number of species (eight) belongs to family Euphorbiaceae, followed by Family Asteraceae (three species), Amaranthaceae and Cyperaceae (three each species). Three species were found to be Poaceae, two Solanaceae and Molluginaceae. One species were found to be remaining families. In floristic survey, two weeds were found to be climber, six were prostrate and remaiing was erect. Thirteen weeds were found to be common, eleven were occasional and six were rare in kharif season rice fields.

Keywords: Weed Diversersity; Rice Field; Lucknow Region; Weed Survey.

Introduction

Weeds are notorious pest of rice causing serious yield reduction in rice worldwide. Rice (*Oryza Sativa*) belongs to family 'Graminae'. Among the food crops, Rice is a staple food and one of the most important cereals of the world. It is grown approximately one-third of the total world population (Johnson, 1984). The average per hectare yield of rice in India of 2.71 tons per hectare (Anonymous, 1997). Annual worldwide rice yield loss by weeds is 15-21% (Karim et al., 2004). Losses caused by weeds very from one location to another, depending on the predominant weed flora and on the control methods practiced by farmers. A crop loss due to weed competition varies with the duration of weed infestation of the crop. The crop is likely to experience yield reduction, unless field is kept weed free during a part of its growing period (Azmi et al., 2007). The main factors for which crops and weeds compete are light, water and nutrients. Weeds commonly absorb added nutrients as much or more rapidly than crops (Moody, 1990). Salinity is another dramatic factor causing yield reduction in rice. Weeds also are adversely affected by a combination of increased salinity and high temperatures (Greenwood and Mac Farlane, 2006). The composition of weed flora is also expected to differ depending on location (Uddin et al., 2010). Salinity could also cause changes in weed in coastal areas. The distribution and nature of weeds in coastal areas would be different from non-saline areas.

The present study was therefore undertaken to investigate the distribution and severity of weed flora prevailing in major coastal rice growing areas of Lucknow region. The Lucknow zone of Province Uttar Pradesh is situated at Latitude and Longitude 26.8470^0N and 80.9470^0E respectively. The aim of

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the study is the diversity of weeds in the rice fields and find out the common and dominant, occasional and rare weeds in rice crop fields of Lucknow region.

Materials and Methods

The survey were conducted in some selected coastal rice field areas in Lucknow region to identify and evaluate the major weed species in rice fields during August to November 2013. The age of rice plants was around 60 days at the time of survey, and fields were flooded with 2-4 cm of water. The soil salinity was measured using a conductivity meter (Model: ECTestr, Spectrum Technologies, Inc). The quantitative field survey was performed according to the method described by (Thomas, 1985). An inverted "W" pattern was used to systematically walk through each sample field. Fifty rice fields were sampled with a total of 20 quadrats per field. Rice field size ranged from 1.1 to 2.8 acre. A quadrat of 0.5×0.5 m size was used. The distance between quadrats was fixed based on the size and shape of the field. All weeds in each quadrat were identified, counted, and recorded. Species that could not be identified in the field were tagged and transported for later identification (Chancellor and Froud-Williams, 1982; 1984). Probable anomalies in sampling areas, such as shoulder and foot slopes, potholes, ditches, bluffs, power lines, and paths were avoided. The identified weeds were categorized as herbs and climbers as per the methods described by (Bisht, 2004) and also separated into common, occasional and rare weeds described methods (Siddique, 2005): (Dalvi, 2010).

Results and Discussion

India has a characteristic geographic location at the junction of the three major biogeographic realms, namely, the Indo-Malayan, the Eurasian and the Afro-tropical region. It is considered to be one of the twelve centres of origin and diversity of several plant species in the world. Phytogeographically the Indian mainland may be divided into three distinet regions: the Himalaya, the Indo-Gangetic plain, and the Peninsular India. The Indo- Gangetic plain comprises allulvial low lands Gangetic plain to the south of the Himalaya. This region is agricultrually more productive. India is

Table 1: Studies on weed diversity in the field of Rice crop

S. No.	Name of the weeds	Common Name	Family	Habit	Category
1.	*Ageratum conyzoids* L.	Billygoat weed	Asteraceae	Erect	Rare
2.	*Alternanthera sessilis* (L.) R.Br. ex DC	Sessile joyweed	Amaranthaceae	Prostrate	Occasional
3.	*Amaranthus Polygamus* L.	Pigweed	Amaranthaceae	Prostrate	Common
4.	*Calotropis gigantea* (L.) R.Br.	Milkweed	Asclepiadaceae	Erect	Rare
5.	*Cardiospermum helicacabum* L.	Balloon vine	Sapindaceae	Climber	Common
6.	*Cassia tora* L.	Sickle pod	Caesalpiniaeae	Erect	Occasional
7.	*Chrozophora rottleri* (Geis.) Juss.ex	Suryavarti	Euphobiaceae	Erect	Rare
8.	*Cynodon dactylon* (L.) Pers.	Bermuda grass	Poaceae	Erect	Common
9.	*Cyperus rotundus* L.	Nut-grass	Cyperaceae	Erect	Common
10.	*Cyperus triceps* Endl.	White water sedge	Cyperaceae	Erect	Rare
11.	*Digera muricata* (L.) Mart.	Kanejaro	Amaranthaceae	Erect	Common
12.	*Eragrotis tenella* Roem. & Schult.	Lovegrass	Poaceae	Erect	Common
13.	*Euphorbia heterophylla* L.	Mole plant	Euphobiaceae	Erect	Common
14.	*Euphorbia hirta* L.	Asthma plant	Euphobiaceae	Erect	Common
15.	*Euphorbia prostrata* Ait.	Prostrate sandmat	Euphobiaceae	Prostrate	Occasional
16.	*Fembristylis aestivalis* Retz.	Hurricanegrass	Cyperaceae	Erect	Occasional
17.	*Grangea maderaspatana* (L.) Poir.	Madras carpet	Asteraceae	Prostrate	Rare
18.	*Mollugo disticha* L.	Green carpetweed	Molluginaceae	Erect	Occasional
19.	*Mollugo nudicaulis* Lamk.	Daisy leaved chickweed	Molluginaceae	Erect	Occasional
20.	*Oryza sativa complex* (weedy rice)	Weedy rice	Poaceae	Erect	Common
21.	*Parthenium hysterophorus* L.	Whitetop weed	Asteraceae	Erect	Common
22.	*Phyllanthus amerus* Schumach & Thonn.	Hurricane weed	Euphobiaceae	Erect	Common
23.	*Phyllanthus madewraspatensis* L.	Velvet bushwillow	Euphobiaceae	Erect	Occasional
24.	*Phyllanthus niruri* Linn.	Pick-a-back	Euphobiaceae	Erect	Occasional
25.	*Portulaca oleracea* L.	Hogweed	Portulaceae	Prostrate	Common
26.	*Solanum nigrum* auct.	Black nightshade	Solanaceae	Erect	Occasional
27.	*Solanum xanthocarpum* Schrad.	Yellow-fruit nightshade	Solanaceae	Erect	Rare
28.	*Tragia plukenetii* A.R. Sm	Cannabis leaf nettle	Euphobiaceae	Climber	Occasional
29.	*Tribulus terrestris* L.	Tackweed	Zygophyllaceae	Prostrate	Occasional
30.	*Vicoa indica* (L.) DC.	Sonkadi	Asteraceae	Erect	Common

mainly a tropical country but due to great altitudinal variations, almost all climatic condition from hot deserts to cold deserts exists. Of the fifty fields surveyed, a total thirty weeds were collected which belong to fifteen families and twenty six genera from the kharif season of rice fields. Two weeds were found to be climber, six were prostrate and remaining was erect. Thirteen weeds were found to be common and dominant, eleven were occasional and six were rare in kharif season rice fields.

Conclusion

Weeds compete for light, nutrients, moisture and space with the crop and thus cause severe losses to yield. As mentioned in the title of the present proposal was therefore undertaken to investigate the distribution and severity of weed flora prevailing in major coastal rice growing areas of Lucknow region. This study is based on diversity of weeds of kharif rice crop fields, which provides a preliminary data of the different categories of weeds in rice crop fields. It will be helpful to farmers, students and researchers related to this field for identification of weeds and their weed specificity. Further study is required for distribution and quantification of weeds for ecological management.

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Original Article

Degradation of Water Quality by Mining Effluents: Its Impact on *'Puntius Narayani'*

Nandini Vaz Fernandes

Abstract

Unregulated mining causes pollution of air, land and water. Mining effluents are the main source of degradation of water bodies in their vicinity. *Aim:* The present study was designed to determine the change in the water quality on account of mining activity and its effect on the growth and development of the fishes. *Settings and Design:* Water bodies in four mining areas were selected for the study. Upstream site of the river was taken as a measure of uncontaminated water as compared to down stream site which had influx of mining effluents from the surrounding mines. *Methods and Material:* Catch sampling method was used to compare the parameters in the upstream and downstream sites of eight study sites. Water samples were evaluated for physicochemical parameter and heavy metals. Fishes from these sites were examined for body length, width and weight. Gills, liver and muscle tissues of these fishes were examined for heavy metals. *Statistical analysis used:* The statistical significance of associations between various qualitative parameters was evaluated through Fisher's exact test. *Results:* The study showed degradation of water quality because of the influx of mining effluents in the downstream sites. Physicochemical parameters significantly altered were turbidity, total suspended solids and sulphates in the downstream sites. The metals detected were iron, copper, zinc, manganese, cadmium and nickel. Examination of the fishes revealed decrease in the body weight of the fishes in the downstream site as compared to the upstream site. *Conclusions:* We attribute the impaired growth of fishes to the synergistic effect of change in water quality and increase in heavy metal concentration in the downstream region on account of mining effluents.

Keywards: Mining Effluents; Heavy Metals; Impaired Growth in Fishes.

Introduction

Mining is the major economic activity of Goa. The mining belt of Goa covers an area of approximately 700 sq.kms. Mining has been a very important element in the economic history of modern Goa and a significant foreign exchange earner for the state. It has provided the trigger to boost the economy of the mining talukas. However, unregulated mining of both small and large-scale mining operations are inherently disruptive to the environment, producing enormous quantities of waste that can have deleterious impacts for decades. The mine wastes constitute a potential source of contamination to the environment, as heavy metals and acid are released in large amounts (Ledin M et al., 1996). Aquatic pollutants produce multiple consequences at the organism, population, and community and ecosystem level, affecting organ function, reproductive status, population size, species survival and thus biodiversity. Besides altering the water quality, mining activity is also contributing release of heavy

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metals. Therefore assessment of water quality around mining areas is essential as all aquatic organisms are directly or indirectly affected by the physical characteristics of their environment, especially the chemical composition of the water. The high concentration levels, damage tissues and interfere with the normal growth, proliferation, and reproduction (Gillis et al., 2008). The data available on the degradation of water bodies on account of mining in Goa is restricted. Therefore the present study was undertaken to determine the change in water quality on account of mining activity, by comparing the physicochemical parameters of water samples collected from upstream and downstream sites and analyzing the effect of the change in water quality on the growth of fish Puntius narayani.

Materials and Methods

Four study areas of Kalay, Ponda, Codli and Rivona in south Goa, were identified using GPS / Google maps. From each area, we identified two sampling sites. The upstream and the downstream location of the river from each area were considered for sample collection. Selection of sampling site was based on the location of the influx of effluents from the nearby prominent mines. Sampling point which was located four kms upstream of the mining area was meant to establish the water quality before the influence of mining activities. The 'upstream' region was thus considered to be a reflection of water condition devoid of mining effluents. Downstream site of the river was considered as the second site for sample collection as it had visible sources of influx of mining effluents. Catch sampling method was used to compare the parameters in the upstream and downstream site. Water samples and live fishes were collected from the upstream and downstream sites of the four sampling areas. The study involved analysis of all physicochemical parameters of water in upstream and downstream regions of Kalay, Ponda, Codli and Rivona. The water samples were also subjected to heavy metal analysis. The heavy metal analysis was done by atomic absorption spectrophotometer. Heavy metals tested included iron, manganese, arsenic, lead, zinc, mercury, copper and hexavalent chromium. Physicochemical parameters analyzed were pH, turbidity, temperature, conductivity, biochemical oxygen demand, carbonates and bicarbonates, dissolved oxygen, phosphates, nitrates, sulphates and free carbon dioxide. Fishes collected from the upstream and downstream site were analyzed for body size, body length, and weight. The gills, liver, and muscles were also evaluated for detection of heavy metals. The tissue samples obtained from Puntius narayani, dried, powdered, digested according to standard procedure of Sreedevi et al. (1992) and analyzed using Atomic Absorption Spectrophotometer. The standardized procedures used for heavy metal detection in the tissues was as per IS 3025 Part 53: 2003, SOP/ITG/AAS/INST-01. Values of heavy metals were recorded in mg/ltr. The data was tabulated analyzed statistically. The statistical significance of associations between various qualitative parameters was evaluated through Fisher's exact test (two tail). Online calculators of statistic were used for standard deviation at www.easycalculation.com and fisher's test at www.graphpad.com.

Results

The main ores extracted in these regions include iron, basalt, and manganese. The selection of the sampling areas was based on the presence or absence of effluents released by the mines into the water body.

Analysis of Water

The water samples of the upstream and downstream of the 04 study areas were analyzed for physicochemical parameters and heavy metals.

Physicochemical Parameters

The physicochemical parameters that the water samples were tested for include the Ph, Colour, Odour, Turbidity, Total Hardness as $CaCO_3$, Total Dissolved Solids, Sulphates as SO_4, Nitrates as NO_3, Total Alkalinity as $CaCO_3$, Conductivity, Total Suspended Solids, Bio-Chemical Oxygen Demand, Chemical Oxygen Demand, Residual Chlorine, Calcium as Ca and Fluorides as F. In the present study the physicochemical parameters which were significantly altered in the downstream sample were turbidity, total suspended solids and sulphates (Table 1). Others factors affected were lowered pH, conductivity, temperature, hardness, carbon dioxide, salinity, nitrates and BOD. However, the increases or decrease was statistically insignificant.

Heavy Metals in Water

The metals detected in the water samples of eight sites were Iron, Copper, Zinc, Manganese, Cadmium, and Nickel. Test for Lead and Boron were negative.

Table 1: Physicochemical parameters in the upstream and downstream sites of the 04 study areas

Sr. No	Parameter	Unit	Kalay	Ponda	Rivona	Kodli	Remark
1.	pH		U-6.98 D-6.87	U- 7.48 D- 7.48	U- 7.15 D- 7.33	U- 7.85 D- 7.27	Decreased pH in the downstream sites *(p=1.000).*
2.	Color	Hazen	U-5 D-5	U- 5 D- 5	U- 5 D- 5	U- 5 D- 5	reddish tinge in the downstream
3.	*Turbidity	NTU	U-1.4 D-13	U- 5.17 D- 20.3	U- 0.97 D- 3.15	U- 2.98 D- 3.49	increased significantly *(p=0.001)* in the downstream
4.	Total Hardness as CaCO3	mg/ltr	U-14 D-13	U- 23.09 D- 23.33	U- 21.23 D- 25.29	U- 40.27 D- 12.25	decreased level of conductivity in the downstream *(p=1.00)*
5.	Total Dissolved Solids	mg/ltr	U-47 D-50	U- 50 D- 45	U- 51 D- 52	U- 52 D- 40	Higher in upstream
6.	Chlorides as Cl	mg/ltr	U-11.7 D-12.9	U- 10.65 D- 10.65	U- 14.2 D- 14.2	U- 21.3 D-17.75	--
7.	Calcium as Ca	mg/ltr	U-8.12 D-11.1	U- 5.61 D-6.81	U- 7.21 D- 9.22	U- 12.42 D- 13.21	---
8.	Magnesium as Mg	mg/ltr	U-1.83 D-1.5	U- 2.19 D-1.46	U- 0.73 D- 0.49	U- 2.19 D- 1.7	--
9.	Sulphates as SO4	mg/ltr	U-1.93 D-2.9	U- 1.23 D- 1.75	U- 2.98 D- 3.33	U- 0.88 D-1.58	Higher concentration in downstream *(p=0.4065).*
10.	Nitrates as NO3	mg/ltr	U-0.18 D-0.24	U- 0.18 D-0.23	U- 0.08 D-0.13	U- 0.09 D-0.1	Higher concentration in the downstream *(p=0.5795).*
11.	Total Alkalinity	mg/ltr	U-19 D-19	U- 17 D-17	U- 17 D-18	U- 35 D-29	--
12.	Conductivity	µs/cm	U-52.14 D- 54.98	U- 69.52 D-67.73	U- 60.25 D-63.75	U- 103.6 D-73.84	Decreased conductivity in the downstream
13.	*Total suspended solids	mg/ltr	U-ND D-2	U- 1 D-7	U- ND D-1	U- ND D-7	Significantly higher in the downstream *(p= 0.0001).*
14.	BOD	mg/ltr	U-0.8 D-0.6	U- 0.8 D-0.8	U- 0.9 D-0.9	U- 1 D-1	--
15.	COD	mg/ltr	U- 4 D- 3	U- 3.96 D-3.96	U- 4 D-4	U- 3.95 D-3.95	--

Abbreviations: U- upstream; D-downstream; ND- not detected; BDL - below detectable level

Table 2: Showing fish body length, width and weight in the 08 sites

Study Area	Study Site	Body Length (Cms)	Body Width (Cms)	Weight (Gms)	Metals Detected In Water
Kalay	SITE 1 (U)	4.1 ±0.29	1.18 ±0.15	0.29 ±0.07	Fe
	SITE 2 (D)	3.12 ±0.43	1 ± 0.16	0.21 ±0.03	Fe, Cu, Zn, Mn
Usgao	SITE 3 (U)	4.18 ±0.78	1.25 ±0.89	0.63 ±0.42	Fe, Cu, Zn, Mn, Cd
	SITE 4 (D)	4.07 ±0.43	1.13 ±0.24	0.59 ±0.19	Fe, Cu, Zn, Mn
Rivona	SITE 5 (U)	4.25 ±0.60	1.25 ±0.23	0.63 ±0.40	Fe, Mn
	SITE 6 (D)	3.96 ±0.13	1.24 ±0.09	0.55 ±0.13	Fe, Mn
Kodli	SITE 7 (U)	3.77 ±1.21	1.42 ±0.55	0.89 ±0.90	Fe, Cd, Ni
	SITE 8 (D)	3.23 ±0.97	1.17 ±0.40	0.48 ±0.24	Fe, Mn

*U- Upstream sites, D- Downstream sites

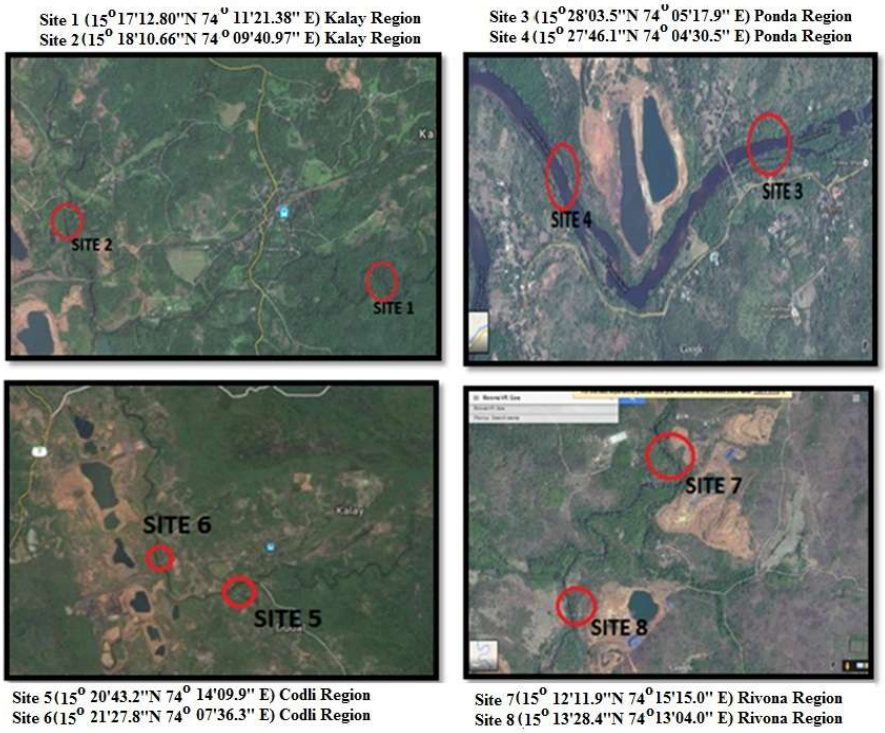

Fig. 1: Eight study of the four sampling areas in Kalay, Ponda, Codli and Rivona

Table 3: Bioaccumulation factor of the different metals in the tissues of *Puntius narayani*

Metal	Bioaccumulation Factor (BAF) in Tissues				
Iron	Gill (15.3)	>	Muscle (1.5)	>	Liver (0.86)
Copper	Gill (79.8)	>	Liver (8.2)	=	Muscle (6.4)
Zinc	Liver (47.3)	>	Gill (41.6)	>	Muscle (24.3)
Manganese	Liver (373)	>	Gill (100.3)	>	Muscle (23.5)
Cadmium	Gill (10.8)	=	Muscle (0.71)	>	Liver (0.35)
Nickle	Muscle (4.0)	=	Liver (3.7)	>	Gill (0.01)

Iron, zinc, and manganese were the most common metal pollutants detected in higher concentrations in the water bodies at the mining sites. The types of metals and their concentration varied at different sites (Fig. 1). There was a significant increase in the concentration of Copper, Iron and Zinc in the downstream samples in all the four study areas.

Analysis of Fish – Puntius narayani

The fishes collected from the upstream and downstream sites of the four sampling areas were studied.

Body Length and Body Weight

The average body length, width, and weight of the fishes in the downstream site were decreased as compared to the fishes in the upstream sites (Table 2). Significant reduction in body weight was observed in the fishes of the downstream region ($p = 0.02$).

Tissue Wise Distribution of Metals in Puntius Narayani

The Gill, Liver, and Muscle tissues of the fish Puntius narayani were studied for the deposition of the metals. The tissues were evaluated for various metals. We found deposition of iron, copper, zinc, manganese, cadmium, and nickel in the tissues. The study showed interesting findings of tissue preference of these metals (Table 3).

Gills: We observed that gill tissue showed higher metal deposition as compared to the liver and muscles. Analysis of the gills revealed presence of all metals studied. We observed high concentrations of Zinc, Manganese, copper and iron

in the gill tissues. The Tissue preference was Zinc ≥ Manganese ≥ copper > iron. Assessment of bioaccumulation factor (BAF) showed many folds increase of Iron, copper and cadmium in the gills.

Liver: Liver tissue revealed presence of all metals studied. We observed high concentrations of Manganese, Zinc, copper and iron in the liver tissues. The Tissue preference was Manganese ≥ Zinc ≥ copper > iron. Assessment of bioaccumulation factor (BAF) showed many folds increase of manganese and zinc in the Liver.

Muscles: Compared to the gill and the liver, the muscles showed less concentration of most of the metals. Assessment of bioaccumulation factor (BAF) showed that muscles showed a tendency to accumulate nickel.

Discussion

The present study shows that the mining effluents degrade the water quality by altering the physicochemical parameters. The heavy metals released are a major concern for the humans as there is deposition of metals in the tissues of the fishes. The concentration of total suspended solids (TSS) in the water sample from the downstream sample was significantly higher as compared to the upstream sample. TSS can increase because of organic particles from decomposing organic material and chemical precipitates. Since the BOD was not altered significantly we attribute the increased TSS, to the chemical precipitates. Studies of Berli et al., 2014, shows that increases TSS can have a negative effect across multiple scales of fish communities, from individual level to the system-level.

The turbidity of the downstream sample was found to be significantly higher as compared to the upstream sample. Turbidity increases in river and lake water mainly due to floating algae, soil washing from banks and industrial activities such as mining. Turbidity in the present sites was due to the mining as other causative factors were not observed. The turbidity seen may be due to the increased TSS and also the constant disturbance of water with the mining effluents. Large amounts of suspended solids and clay materials from mining effluents contribute to the turbidity which can clog the gills of fish species (Hickin, 1995). It is also hazardous to the fish population as it can smother fish eggs and suffocate benthic organisms.

The present study showed increased concentrations of nitrates and sulphates in the water samples of the downstream site though it was found to be statistically insignificant. Nitrates provide nutrition for algae leading to an increase in their growth and demand for dissolved oxygen. This decreases the amount of dissolved oxygen present for other aquatic organisms. Nitrites are intermediates in the oxidation of ammonia to nitrates. It is a well-known toxicant for fish as well as a disruptor of multiple physiological functions including iron regulatory, respiratory, cardio-vascular, endocrine and excretory processes. Nitrite accumulation causes oxidation of haemoglobin to methaemoglobin compromising blood oxygen transport (Kroupova et al., 2005).

Heavy metals released in the water bodies through the mining effluents may be at levels below their toxic thresholds. Though the metals present in the water were in small quantities, the consequence of it on the aquatic organisms is of importance because of bioaccumulation. Metals enters into the organs through the respiratory and the integumentary system. Due to their non-degradable nature, such sub-lethal concentrations may still pose the risks of damage via uptake and subsequent bioaccumulation. Examination of the fishes from the upstream and downstream region revealed a decrease in the body weight of the fishes in the downstream site as compared to the upstream site. The reduction in body size and weight may be due to environmental stress experienced by the fish on account of metals such as iron and zinc as well as some physicochemical parameters of the water such as turbidity and suspended solids. The change in the water quality can be attributed to the mining effluents. Studies of Schoenfuss et al., 2008; Giesy and Dobson, 2000, Fernandes NV, 2015) also revealed that exposure to mining effluents can lead to abnormal physiological responses and cause adverse effects on the development, growth, behaviour, and reproduction of fish. The present study showed interesting findings on the risk posed to humans on account of metal deposition in the tissues of the fishes. The study showed interesting findings of tissue preference of these metals. Gill tissue showed higher metal deposition as compared to the liver and muscles. Metal deposition in the muscles was the least. These differences result from different uptake, deposition and excretion rates. The gills are considered the main site of entry for the dissolved metals. Thus they represent the target for the toxic action of some metals. Another explanation for finding high concentration in gill is mucous excretion by this organ. The excreted mucous has affinity to be bound with metal ions. We conclude

that Zinc, manganese, copper and Iron have tendency to be deposited in the gills because the entry of these metals may be more through the surface exposure. The present study also showed that Liver had greater affinity to concentrate manganese and zinc. The liver is considered the main detoxifying organ. Manganese accumulation in the liver tissue may be due to biogenic materials especially phytoplankton's readily incorporating the metals in them and transferring to the fishes through the various trophic levels. Deposition of these metals may be attributed to the cumulative effect of the quantity of these metals ingested by the fishes through the food chain, and the ability of the fishes to digest these metals or transport it to the liver for detoxification. Constant exposure of the fishes to the metals on account of the continuous leaching of the metals from mining effluents will lead to significantly high levels of these metals which can apparently have chronic effects on fishes. Since fish is one of the major components of the human diet, the deposition of these metals may lead to bioaccumulation and is, therefore, a matter of grave concern. In humans, the presence of even trace heavy metals in diets can cause serious health problems ranging from neuro-, nephro-, carcino, to immunological disorders, if ingested over a long period of time (Tanee, et al., 2013; Uwem et al., 2013). Therefore, though the metals present in the water were in small quantities, the consequence of it on the aquatic organisms is of importance because of bioaccumulation.

Conclusion

Synergistic effect of the change in water quality and an increase in heavy metal concentration in the downstream region on account of mining may be the possible cause of impaired growth of fishes indicated by the reduction in body length and weight of the fishes and genetic damage. Though the increase in each of the physicochemical factor is low, the interplay of all factors in totality can impose severe stress on the body metabolism of the fishes, thereby acting as an impediment to body growth. The present study is a small reflection of the consequences of environmental degradation on account of mining effluents. The larger picture encompasses the damage to air, water, and land on account of unregulated mining. Therefore it is recommended that mining effluents should be treated specially to remove metals before releasing into the water bodies and that bioaccumulation studies should be component of environment impact assessment for renewal of mining leases.

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Conflicting Interest: None.

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Key Messages

We recommend that mining effluents should be treated especially to remove metals even if they are in traces, before releasing into the water bodies as it may lead to health hazards on account of bioaccumulation and biomagnifications. Environmental impact assessment should include bioaccumulation studies for renewal of mining licences.

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Original Article

Sustainable Conservation and Management of Indian Sarus crane (*Grus antigone antigone*) in and around Alwara Lake of District Kaushambi (U.P.), India

Ashok Kumar Verma[1], Shri Prakash[2]

Abstract

The Indian Sarus crane, *Grus antigone antigone* is the only resident breeding crane of Indian subcontinent that has been declared as 'State Bird' by the Government of Uttar Pradesh. This is one of the most graceful, monogamous, non-migratory and tallest flying bird of the world that pair for lifelong and famous for marital fidelity. Population of this graceful bird now come in vulnerable situation due to the shrinking of wetlands at an alarming speed in the country. Present survey is aimed to study the population of sarus crane in the year 2017 in and around the Alwara Lake of district Kaushambi (Uttar Pradesh) India and their comparison to sarus crane population recorded from 2012 to 2016 in the same study area. This comparison reflects an increasing population trend of the said bird in the area studied. It has been observed that the prevailing ecological conditions of the lake, crane friendly behaviour of the local residents and awareness efforts of the authors have positive correlation in the sustainable conservation and increasing population trends of this vulnerable bird.

Keywords: Alwara Lake; Conservation; Population Census; Sarus Crane; Increasing Trend.

Introduction

The Indian Sarus Crane, *Grus antigone antigone* (Linnaeus, 1758) prefers to inhabit close to human habitation. It belongs to phylum: Chordata, class: Aves, order: Gruiformes and family: Gruidae. Its population density is inseparably associated with wetland habitats. There are three subspecies of sarus crane *namely* the Indian sarus crane *Grus antigone antigone*, Eastern sarus crane *Grus antigone sharpii* and the Australian sarus crane *Grus antigone gillae*. Sundar *et al*., (2003) gave the literature review of sarus crane in detail while Archibald *et al*., (2003) gave the first comparative review of these three subspecies.

Due to widespread reductions in the extent and quality of their wetland habitats, exploitation and the effects of pollutants, unsustainable agriculture, unplanned irrigation and non-adoption of wild life rules and regulations as well, the number of sarus cranes is gradually decreasing at global level. Due to its declining number, Indian sarus crane has been now listed as globally threatened i.e. vulnerable avian species (The IUCN Red List 2017 and Bird Life International, 2017).

Only a few researchers have tried to study the demography, ecology and status of Indian sarus crane on large scale in Uttar Pradesh. As far as the study of this sarus crane from demographic and conservation point of view, in and around the Alwara lake is concerned, it is done only by few Zoologists like Prakash *et al*., (2014, 2016a) and Verma *et al*., (2015, 2016a, 2016b, 2017 and 2018). Prakash *et al*., (2016b) and Verma *et al*., (2016c) worked a little on the nesting materials, their medicinal values and suitable selection of nesting sites of this crane.

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Present exploration is aimed to study the population of sarus crane in the year 2017 in and around the Alwara Lake of district Kaushambi (Uttar Pradesh), India and their comparison to sarus crane population recorded from 2012 to 2016 in the same study area.

Material and Method

The Alwara lake (Fig. 1, Google map) is a natural lake (Fig. 2) and a part of perennial wetland and is situated between the latitude 25^{0}24'05.84"S – 25^{0}25'10.63"N and longitude 81^{0}11'39.49"E-81^{0}12'57.95"W with altitude MSL – 81.08 meter. It is surrounded by agricultural fields and connected to the river Yamuna and covers more than 1750 hectares. It is located in Sarsawan block of Manjhanpur tahsil of Kaushambi district of Uttar Pradesh. The lake is skirted by villages like; Ranipur, Dundi, Hatwa and Bhawansuri in east, Paur Kashi Rampur, Alwara and Gaura in the north, Shahpur, Umrawan in the south and Mawai, Tikra and Dalelaganj in the west.

Fig. 1: Study area in Kaushambi district of U.P. (India).

Authors used binocular, camera, motorbike, chappu boat, field stick etc. for various purposes. Since sarus crane is a huge bird and visible from a distance hence sarus count was easy. The study area was visited and examined regularly but the counting of sarus crane was done during first and third Sunday of every month in the year 2017. This counting was accomplished on a single day to avoid the possible double counting due to local movements of the birds to neighbouring habitat. Authors recorded cranes in maximum number during third Sunday of June 2017 as they remain confined around the wetlands in search of water. Besides actual sightings, inquiries from local people were also made to ensure the estimate of existing population and their perceptions about the existence of the crane. All the observations were made while moving through the chappu boat and walking along the croplands, mud lands, natural areas using binoculars (7x35 and 8x40-BEZIF BM-9) and canon cameras.

Counting procedure, identification and other demographic parameters were aided by using standard guides such as Ali (1941), Wild Life Institute of India Wetland Research Methodology (1999) and methods adopted by Ali *et al.,* (1980), Aryal *et al.,* (2009) and Jha *et al.*, (2014).

Fig. 2: A view of Alwara Lake with a pair of sarus crane

Result and Discussion

Sarus crane, the monogamous bird occurs mostly in pair (Fig. 3) or in pair with one juvenile (Fig. 4) or in pair with two juveniles (Fig. 5) and rarely in solo condition. During non-breeding season, cranes are seen in congregation mostly in evening for mate finding or pair formation activities. Prakash *et al.*, (2016a) reported a congregation of 155 cranes in 2014.

Prakash *et al.*, (2014) counted a population of 335 cranes in 2012 in three different transects of Alwara lake; Verma *et al.,* (2016a) counted their population as 425 in 2013; Verma *et al.,* (2016b) reported 510 cranes in 2014; Verma *et al.,* (2017) recorded 537 cranes in total in 2015 and Verma *et al.,* (2018) recorded 575 cranes in and around Alwara lake in 2016. In the latest survey, authors collected a data of 605 cranes in the same study area in 2017. The result is shown in table and pie diagram (Fig. 6).

Table 1: Year wise population of sarus crane from 2012 to 2017

No. of cranes in 2012	No. of cranes in 2013	No. of cranes in 2014	No. of cranes in 2015	No. of cranes in 2016	No. of cranes in 2017
335	425	510	537	575	605

During demographic survey in and around Alwara Lake, authors realized that the presence of abundant paddy fields, land under irrigation, vegetation at the edge of the crop field, type of crop grown, wetland and the openness of habitat are the major factor for the existence and survival of sarus crane. Verma (2018) observed a positive correlation between the crane numbers and the area of agricultural land. Authors also observed that openness of habitat is a requirement for the existence and growth of the crane.

Fig. 3: Paired sarus crane in study area around Alwara Lake

Fig. 4: Sarus crane pair with one juvenile in mustard field around Alwara Lake

Fig. 5: Sarus crane pair with two juveniles in agro field around Alwara Lake

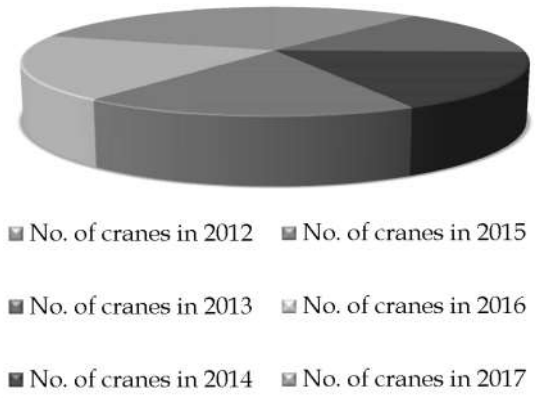

Fig. 6: Pie diagram to show year wise population of sarus cranes from 2012 to 2017

The authors and their team visited the villages concerned a number of times especially on first and third Sunday of every month, contacted the people and told as well as convinced them not to kill or hunt the sarus cranes, their eggs and juveniles. The authors organized awareness programme regularly with group of local people and continued it even when 1 or 2 villagers were there. They were trained about the safety of this sarus crane and its legal aspect was also explained. Importance of its protection, conservation and maintenance of its natural habitat were also emphasized (Prakash *et al.*, 2016c).

It is not only a favourable site for sarus crane distribution but also support a wide variety of other fauna and flora. Globally the sarus crane is threatened i.e. vulnerable avian species but in the area studied, its increasing trend is observed from 2012 to 2017, as clearly shown by table and pie diagram. All these positive efforts and proper management finally led such a sustainable state of conservation of this vulnerable bird.

Conclusion

In the present survey, a continuous gradual increase is clearly observed, as indicated in the table and pie diagram. This increasing population trend is an important aspect of sustainable conservation and management of the sarus crane in the area studied. Prakash *et al.*, (2014) and Verma *et al.*, (2015, 2016a, 2016b, 2017 and 2018) strongly argued that this is happening because of awareness of local people, sustainable conservation and management and quite supportive nature of ecological and environmental conditions in and around the Alwara Lake. A positive correlation was observed between the crane numbers and the wetland. This

conservation model can therefore be applied elsewhere for the conservation of other such species. The authors strongly recommend continuous population census of this bird and declaration of the entire Alwara Lake as *Sarus Sanctuary* to make it safe zone for the conservation of Sarus crane.

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International Journal of Practical Nursing	Triannual	5500	5000	430	391
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RFP Indian Journal of Medical Psychiatry	Semiannual	8000	7500	625	586
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Original Article

Cytomorphometrical Analyses of Blood of Black rat *Rattus rattus* (Linnaeus, 1758)

Aryadhara Das[1], Prafulla Kumar Mohanty[2]

Abstract

Quantitative microscopy has strengthened conventional diagnostic scheme through better understanding of microscopic features from clinical perspective. Towards this, analysis of pathological image analysis havegained immense significance among medical fraternity through visualization and quantitative evaluation of clinical features. Till date, pathological inspection of rodents' blood is solely dependent on subjective assessment which usually leads to significant inter-observer variation in grading and subsequently resulting in late diagnosis of certain diseases. This paper aims at a systematic approach to morphologically characterize of five types of white blood cells, and its nuclei from light microscopic image of blood samples. Hence, cellular and nuclei based geometric features are computed and statistically analyzed with t- test to show the discriminating potentiality of the species.

Keywords: *Rattus Rattus;* Blood Cells; Differential Leucocyte Count; Morphometry.

Introduction

Cytomorphometry is a quantitative description of geometrical structures in all dimensions (Baak 1985; Vandiest et al. 1991). Morphometry is the simplest form of image cytometry and refers to the evaluation of cells or tissues by measurement of various cellular features in a two-dimensional view. Abnormal and insufficient white blood cell function is most often reflected in modified cell morphology, and mathematical analysis of morphometrical cell characteristics is very useful for its estimation (Bins 1985). Furthermore, changes in morphometrical erythrocyte indicators have been detected in certain humans(Alexandratou et al. 1999; Manjunatha and Singh 2000) and dog ailments (Berezina et al. 2001). A complete blood count is an ideal indicator of general health, as stress and numerous illnesses can modify haematological parameters, especially with regard to erythrocyte and lymphocyte count (Hinton et al. 1982). A complete blood count is undisputedly the most important diagnostic method available to veterinarians, along with proper anamnesis and physical examination of the animal. The modern computerized geometric, and morphometric methods have been established as efficient tools to quantify differences in the cell shape or morphological structures in particular and can provide a better characterization in describing the complexity of anatomical structures (Grizzi and Chiriva- Internati 2005; Russ 2007; Rosioru et al. 2012).

Morphometry is used as the prognosis and diagnosis of diseases of animals.It enables analysis of changes in to the entire cell, changes in cytoplasm and changes in the nucleus and structures of nucleus (Dalton 1992; Russack 1994). Since thehaemocytomorphometrical data on rodents are inadequate, the particular study onthe blood cells of two species of rats are analyzed, and interpreted.

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Materialsand Methods

The investigation was conducted on wild black rats *R. rattus* (five from each sex) which were caught by wooden and wire net trapping in evening time from the backyard of the residential complex of the Utkal University campus, Bhubaneswar, Odisha. These were allowed to acclimatize to captive condition prior to experimentation and were carefully handled to minimize the stress. Trapped rats were kept at room temperature and were fed with paddy, cereals, and grains. After a week, the rats were anaesthetized by using chloroform in a jar and two milliliter of blood was drawn from the jugular vein with the help of a disposable syringe. Thin blood smears of peripheral blood were prepared from a small drop of fresh blood directly from the needle and were made for each sample to determine the differential blood count and morphometrical analyses of leucocytes and erythrocytes. The morphometry like cellular and nuclear length and breadth of erythrocytes, and leucocytes (monocytes, lymphocytes, neutrophils, eosinophils, and basophils) was undertaken. Cytomorphometry of cells and nuclei of blood of both the species with sexual dimorphism was carried out with the help of Microscope Eyepiece Digital Camera [CatCam130–1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra, India] attached to Hund Wetzlar Microscope [MICROSCOPE H 600 WILOZYT PLAN, Serial No. 1024980, Helmut Hund GmbH, Wetzlar-Nauborn, Germany] and computer.

Results

In this investigation, the differential leucocyte count and cytomorphometrical analyses are taken into account. The findings of this study reveal the effect of sex on blood parameters. The results of differential leucocyte count are tabulated in detail (Table 1). The number of neutrophils is found to be 36.8±1.48 in males and 32±0.59 in females. The number of monocytes is 12.6±0.61 in males and 12.8±0.44 in females. The concentration of lymphocytes is 48.5±1.21 in males and 50.6±0.85 in females. The number of eosinophils is 3±0.33 in males and 3±0.33 in females. The number of basophils is 0.7±0.15 in males and 0.6±0.16 in females.

The morphometrical parameters like length and breadth of both RBC and five categories of WBC such as monocyte, lymphocyte, neutrophil, eosinophil and basophil are measured and expressed in micron metre (µm) (Table 2). The cell

Table 1: Differential leukocyte count of *R. rattus* (per sex n= 05) in number

Sl. No.	Parameters	*Rattusrattus*	
		Male	Female
1	Neutrophil	36.8±1.48	32±0.59
2	Monocyte	12.6±0.61	12.8±0.44
3	Lymphocyte	48.5±1.21	50.6±0.85
4	Eosinophil	3±0.33	3±0.33
5	Basophil	0.7±0.15	0.6±0.16

Table 2: Morphometry of blood cells of *R. rattus* (per sex n=05) in micron

Sl. No	Types of cell	Cell/ Nucleus	Parameters	*Rattusrattus*	
				Male	Female
1	Erythrocyte	Cell	Length	19.8±3.12	6.57±0.11
			Breadth	10.80±0.32	5.91±0.10
2	Monocyte	Cell	Length	4.47±0.36	6.34±0.27
			Breadth	3.90±0.34	5.91±0.24
3	Lymphocyte	Cell	Length	8.14±0.80	5.72±0.29
			Breadth	8.29±0.83	5.22±0.30
		Nucleus	Length	6.34±0.76	6.30±0.73
			Breadth	5.36±0.68	5.31±0.63
4	Neutrophil	Cell	Length	10.59±0.94	5.88±0.39
			Breadth	9.59±0.91	5.19±0.39
5	Eosinophil	Cell	Length	7.93±0.70	4.5±0.32
			Breadth	7.78±0.65	4.08±0.27
6	Basophil	Cell	Length	7.45±0.86	4.39±0.37
			Breadth	6.59±0.86	6.14±2.05

length of erythrocyte is 19.8±3.12, and6.57±0.11 in males and females, respectively. The cell breadth of erythrocytes is 10.80±0.32 in males, and 5.91±0.10 in females. The length of monocytes is 4.47±0.36, and 6.34±0.27 in males and females, respectively. The breadth of monocytes is 3.90±0.34 in males and 5.91±0.24 in females. The cell length of lymphocyte is 8.14±0.80, 5.72±0.29 in males and females, respectively. The cell breadth of lymphocytes is 8.29±0.83, and 5.22±0.30 in males and females, respectively. The nucleus length of lymphocytes is 6.34±0.76 in males and 6.30±0.73 in females. The nucleus breadth of lymphocytes is 5.36±0.68, 5.31±0.63 in males and females, respectively. The cell length of neutrophils is 10.59±0.94, 5.88±0.39 in males and females, respectively. The cell breadth of neutrophils is 9.59±0.91 in males and 5.19±0.39 in females, respectively. The cell length of eosinophil is 7.93±0.70, and 4.5±0.32 in males and females, respectively. The breadth of eosinophils is 7.78±0.65 in males and 4.08±0.27 in females. The cellular length of basophils is 7.45±0.86, and 4.39±0.37 in males and females, respectively. The breadth of basophils is 6.59±0.86 in males and 6.14±2.05 in females.

Fig. 1: Black rat in wire net cage

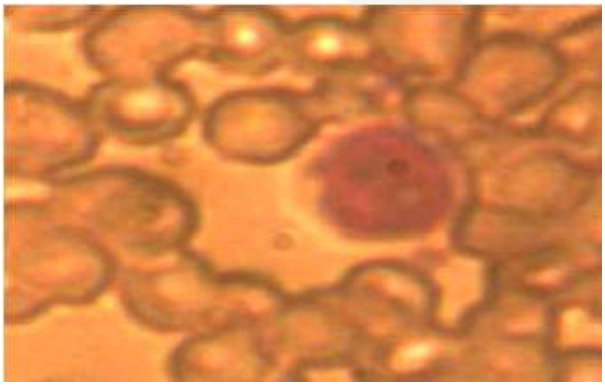

Fig. 2: Lymphocyte

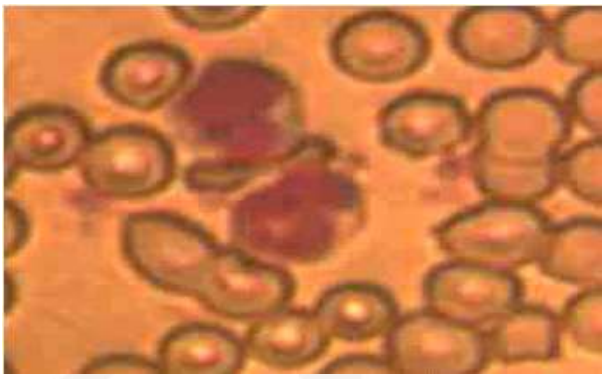

Fig. 3: Neutrophil

Fig. 4: Eosinophil

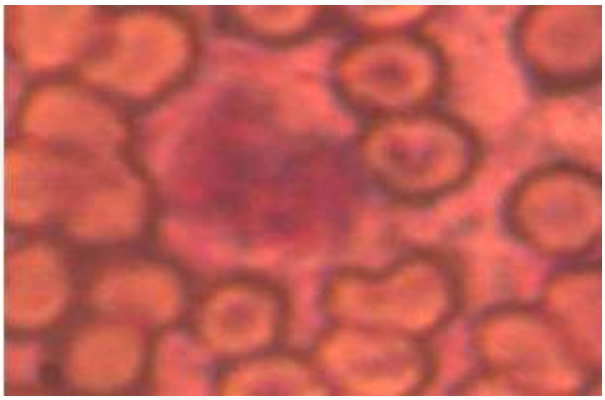

Fig. 5: Basophil

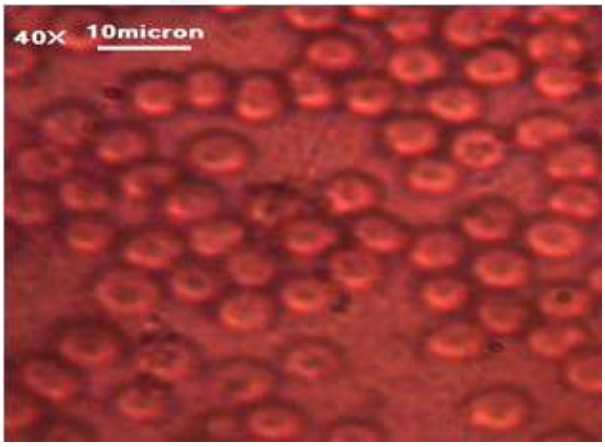

Fig. 6: Erythrocyte

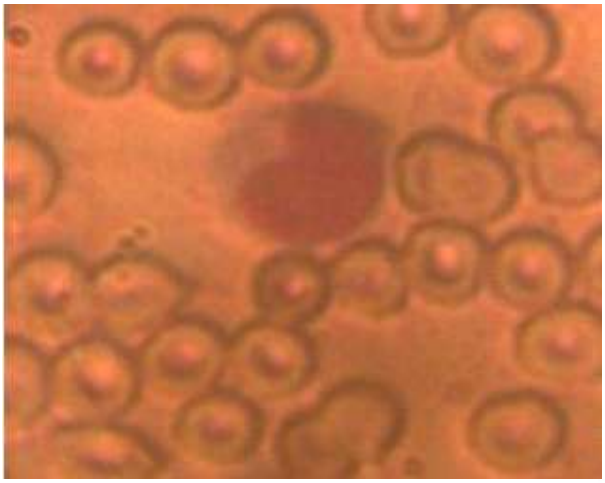

Fig. 7: Monocyte

Discussion

The findings of this study reflect the effect of sex on the size of erythrocytes and leucocytes (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) of *R. rattus*. In this investigation, the cytomorphometrical parameters of blood of healthy black rats have been observed. The findings of cytomorphometrical analyses of *R. rattus* can be helpful in clinical investigation and interpretation. In differential leucocyte count, the highest number of lymphocytes is found in males than females and shows significant difference at $p < 0.01$ which is due to variation in species. It is observed that the percent of monocytes increases in females and males which is due to variation in sexual dimorphism. The neutrophils are found to be more in females in comparison to males which is possibly due to some pathogenic infections. The number of eosinophils is same for both males and females of *R. rattus*. The basophils are observed to be more in males than females which may due to inflammatory responses such as certain types of skin inflammation, asthma or parasite infections, settings in which basophils may appear in the affected tissues.The parameters like monocytes, neutrophils, eosinophils, and basophils do not show any significant difference.

The length and breadth of leukocytes do not vary much due to their round shape. Among agranulocytes (lymphocytes and monocytes), lymphocytes are larger than monocytes.The cellular length and breadth of erythrocytes are noted to be more in males than females which deviates significantly sex wise at $p<0.001$. The cell length and breadth along with nucleus of lymphocytes have more value in males whereas less infemales. The parameters show sex wise significant difference at $p<0.01$. The morphometric parameters of neutrophils are found to be higher value inmalesand lower in females and deviate significantly sex wise at $p<0.001$. The morphometric parameters of eosinophils are observed to be higher in males than females anddeviate with respect to sex which is significant at $p<0.01$, and basophil cell breadthdoes not show significant difference.

Conclusion

The study reveals that as a whole, effect of sex on morphometry of blood cells of black rat but,certain genetic and non-genetic factors such as, onset of maturity, sexual dimorphism, breeding, and environment are believed to affect the shape and size of blood cells. So, it is equally important to consider these factors and detailed investigation as to the stated aspects is suggested to arrive at accurate clinical and physiological interpretations.

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Review Article

An Overview on Parthenium as a New Menace for Indian Agro-Ecosystems and its Management

Shiv Prasad Vishwakarma[1], Sheetla Prasad Verma[2]

Abstract

Parthenium hysterophorus L. commonly known as Congress grass is an aggressive and noxious weed among top worst weeds in the world. Now, it is widely distributed in all the crops of almost all the states of the India threatening natural agro-ecosystems and biodiversity. It a great menace due to causing skin allergy, asthma in human being and animals too. *Parthenium* has got major weed status in India. This weed alone may lowered the average yield of cropup to 40% and forage production about 90%. Sustainable crop production of many crops, grasslands and orchard ecosystems are being greatly affected by invasion of this noxious weed in the country. Various approaches viz., physical, mechanical, agronomical, chemical and biological have been employed for *Parthenium* management but most of them are not so effective due to invading characteristics of this weed as well as other limitations. Integrated weed management practices have been found effective to minimize this noxious weed. An attempt has been made to review its impact on various crops production, human and animal health and its effective management.

Keywords: Noxious; Agro-Ecosystem; Forage.

Introduction

Parthenium hysterophorus L., popularly known as carrot weed or gajar grass because of its appearance like carrot plants, white top orcongress grass in India. It belongs to family Asteraceae or Compositae.This weedwas introduced to India in seed form as a contaminant of food grains imported from Mexico. First time *Parthenium* reported as waste land weed in Pune (Maharashtra) during 1956. It is now widely distributed in all the crops of almost all the states of the country threatening natural agro-ecosystems and biodiversity. It has also been considered as a one of the most noxious and problematic weed due to poisonous, pernicious, causing skin allergy, asthma in human being and animals. It has great potential of invasiveness, for quick spread and environmental impacts. In Australia, this weed has been considered as one of the greatest source of dermatitis, asthma, nasal-dermal and naso-bronchial types of diseases. Besides, it also reduced the yield and quality of field crops and animal products (Aneja *et al.*, 1991).

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Parthenium has occupied almost all the states and areas of our country in arable lands, crops, orchards, pastures and waste lands like railwaystracts, canals, roadsides, industrial areas, forest areas and even national parks and residential colonies. Within few decades it widely spread in the plains of India and became the country's "worst weed".

It is bushy and leafy herb and attains an average height of 1 to 1.30 m. The stem becomes comparatively hardy at maturity stage. *Parthenium* bearshead of capitulum type flowers surrounded by bracts around flower and appears a flower cluster (Warshaw and Zug, 1996). It has capability

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to produce millions of pollen grains per plant and easily be transportedand spread new areas by wind and has a great seed production potential which can produce average 10,000 to 25,000 seeds per plant. Due to its photo-thermo insensitivity and wide adaptability of soils it grows round the year except in severe winters. However, it grows in almost all types of ecosystems except where soil is not saline because salinity is harmfulforitsflowering (Chembolli and Srinivas, 2007).

Harmful effects of *Parthenium hysterophorus* L. on Agriculture and Ecosystems

Parthenium weed cancomplete its vegetative and reproductive phases only in four weeks. This weed has invaded about 35 million hectares of land in our country today. Its infestation in crop area in recent past years is alarming. (Sushilkumar, 2009). In India, it reduces about 40% of crop yield (Khosla and Sobti, 1981). *Parthenium* plant contains harmful biochemicals, like parthenin, hymenin, hysterin and ambrosin etc.The weed exerts strong allelopathic effects on different crops by releasing these exudates in rhizosphere (Gunaseelan, 1998). *Parthenium* badly affects root nodulation and activities of rhizobium in leguminous crops. It also affects the free living microorganisms viz. *Actinomycetes, Azotobacter,* and *Azospirillum* in the soil.Itis prolific in nature and produce a huge number of pollens i.e. on an average 624 million/ plant, which are transported for shorter distance and in clusters of 600-800 grains. These pollen grains settles on the vegetative and floral parts inhibits fruit setting in some crops like maize, brinjal, capsicum, tomato, and sunflower.

Parthenium has the potential to damage the natural ecosystems. This menace totally changed Australian grasslands, open woodlands and flood plains river banks, (Chembolli and Srinivas, 2007). This weed has great capability to invade new surroundings rapidly and can dominate the native indigenous species and pose a serious threat to local biodiversity (Seema Patel, 2011). It colonizes in roadsides, railway tracts, water resources and crop lands. Reports disclosedthat this aggressive weedoccupied about 14.25 million hectares of cultivated land during 2001-2007 compared to 2 million hectares in 1991-2000 (Javaid and Adrees, 2009). A survey conducted in Bangladesh on various invasive alien species and indicates that *Parthenium* can easily flourished on different types ofland viz., fallow land, road sides, waste land, low land, railway tracks and even in colonies. Reports further said that *Parthenium* easily established in new habitats and replace the number of local indigenous species (Akter and Zuberi, 2009). It is also well known fact that *Parthenium* primarilyexerts severe detrimental effect on human being and livestock by causing many skin and respiratory problems. The dermatitis caused by this weed affects the face, neck, eyelids, chest and other exposed organs (Warshaw and Zug, 1996). Italso affects other exposed organs shows as scattered scaly papules over the mainly forehead, ears, cheeks, nape of neck, forearms, hands and under surface of chin etc. through chronic actinic dermatitis reported by Kaur *et al.,* 2014. In *Parthenium* dermatitis extracts of *Parthenium* responded with positive skin reaction to mAb2 and also involvesTH type cytokines (Akhtar *et al.,* 2010).

The *Parthenium* also acts as a host for various pests and pathogens which caused diseases in crops. This weed also caused severe dermatitis problems in livestock including horses and cattle and scattered lesions appeared on their skin. Grazing animals, fed on grass mixed with this weed produce bitter milk and low quality of meat from goats, cows and buffaloes (Aneja, 1991). If it is eaten in small amount, it is responsible for mouth ulcers with excessive salivation. However, significant amount (10 to 50%) of *Parthenium* weed in the diet can kill cattle (Narasimhan *et al.,* 1977). Besides, it causes itching, eye irritation, anorexia, diarrhea, pruritus, alopecia, in dogs.

Management of *Parthenium hysterophorus* L.

For the management of *Parthenium v*arious methods viz., preventive, physical, mechanical, agronomical, chemical, biological, legal and integrated approaches are in practice. Singh *et al.,* 2004 reported that use of biological tools like using insects, microorganisms and competitive plants have been found the most economic ways of managing this problematic weed.The use of botanical extracts as germination and growth inhibitors is a new emerging concept for effective management of *P. hysterophorus*. Several studies on plants extract andtheir active ingredientshave also been attempted to control this weed. (Sushilkumar and Saraswat, 2001).

Preventive measures for management

Sunilkumar *et al.*, 2017advocated that no any weed control programme is successful if adequate preventive measures are not taken to reduce weed infestation.Prevention comprised of all possible measures taken to prevent the introduction and or establishment and distribution of weeds in new local, regional or national level. It includeduse of *Parthenium* free clean and certified seeds, creation of weed free good tilth, clean cultivation, feeding

of *Parthenium* seed free material to the farm animals, incorporation of well rotten organic manures, cleaning of farm machinery and irrigation channels before use to avoid adding the *Parthenium* are some effective measures.Successful management of *Parthenium* is based on integration of all the available techniques and their implementation round the years are as under:

1. Physical management

Hand pulling and burning are most effective methods to reduce the weed seed bank.Hand pulling should be done during rainy season or in moist soil. Before flowering is the best time to uproot the plants regarding its management because it is less effective after flowering. In other seasons especially in summer, it is difficult to uproot the plant and if tried, plants are broken, from which further regeneration takes place. Burning is more effective in wastelands and isolated areas.In small areas and isolated pockets such as flower beds, lawns, kitchen garden and in intensively cultivated agricultural fields, hand weeding may be really effective and should be preferred. Persons should wear hand gloves and full sleeve shirts, mask and goggle to avoid direct body contact with the weed during uprooting programmes. According to researchers about 4% human population is sensitive to *Parthenium.* If any symptoms like itching, swelling of skin etc. reflected such person should not be employed for *Parthenium* removal programme.

2. Mechanical management

Mechanical deep ploughing before flowering is good and economical incrop fields where *Parthenium* germinate profusely.The weed is incorporated into the soil as green manure. Precaution should be taken to give spot chemical treatment over those plants which remain on surface as such plants not buried completely may rejuvenate. Weeding with small tools in early stage is also effective in newly introduced cropped area.

3. Agronomical/Cultural management

Selection of competitive crop and adoption of suitable crop rotation may be a good tool to manage *Parthenium* in crop fields.Farmersare advised for quick growing crop like sorghum and *Sesbania* (daincha) to suppress the growth of *Parthenium* in their crop field, particularly when fields are supposed to keep as fallow. This method may be employed in colonies and gardens. Several cultural practices like thorough land preparation, growing of suitable varieties which have smothering effect, maintaining optimum plant population, mulching, soil solarisation, stale seed bed technique, balance fertilizer application, proper fertilizer placement and water management are some other important factors in controlling this weed (Ray and Gour, 2012 and Javaid, 2007).

4. Legal management

The government of India and state governmentsshould declare *Parthenium* as noxious weed and also a weed of national significance and constitute and implement law to held responsible the owner of a vacant field or land. Municipalities in towns or cities, ministry of transport on road side, railway ministry on railway tracks side; irrigation departments on the bunds of irrigation canals should take appropriate step to control the weed by available methods.

5. By use of chemicals

Hand pulling of *Parthenium* is labour and cost intensive while manual control method become ineffective in some cases or conditions. Khan *et al.*, 2012 reported that itcan be controlled by the use of chemicals like use of Glyphosate (2.5kg a.i./ha) or Paraquat (0.51 kg a.i./ha), Metribuzin (0.3 to 0.5%) or 2,4-D (2-2.6 kg a.i./ha) or Hexazinone (3.5 kg a.i./ha). The similar results were obtained by Ramamoorthy*et al.,* 2004; Reddy *et al.,* 2007; Singh *et al.,* 2003; Mishra and Bhan, 1994).

The use of herbicides for different crops should be done only after consulting weed scientists because chemicals are highly specific and alteration in time, dose or method may cause severe injury if applied in crop or efficacy may be lower.Alachlor (2.0 kg a.i/ha) can be applied as pre-emergence to control *Parthenium* in soybean, rajmash, banana and tomato crop. Metribuzin (0.50 to 0.75 a.i/ha) can be used as pre-emergence just after sowing to control *Parthenium* in potato, tomato and soybean crop. Atrazin is effective to control *Parthenium* in maize. There are some limitations using chemicals like herbicidal effect of chemicals which persist for a longer time. Sometimes the plants so suppressed by chemicals have regenerated after remaining dormant for a shorter time. Also chemical treatment repeatedly can kill the existing plants but cannot prevent the entry of seeds getting deposited from outside. The remaining seeds as well as newly deposited seeds are always ready for germination with a slight moisture available to them in the soil. Because of

continuous seed production without any interval in the entire calendar year, reinvasion of the areas can hardly be avoided unless the seed source itself is checked (Mahadevappa, 1999).

6. By use of biological control agent

• *Maintenance of natural biodiversity*

Biological control is an effective measure by intentional manipulation of natural enemies for the purpose of controlling harmful weeds. Biological control is less expensive andnot poses any threat to non-target organisms, environment and biodiversity if systematically applied. Different types of bio-control agents like competitive plants, insects, fungi, nematodes, viruses etc. are used to manage the weed (Shushilkumar, 2009; Singh *et al.,* 2004). Insects have received maximum attention in biological control of *Parthenium* followed by competitive plants and pathogens. It is self-perpetuating and can spread on its own while other control measures require inputs periodically. It is easy to integrate with other control measures (Jayaramaiah *et al*., 2017).

A botanical survey in relation to *Parthenium* control across the country has revealed an interesting factor that the *Parthenium* cannot penetrate into areas where the natural flora have not been disturbed. Wherever there is indiscriminate destruction of naturally existing plant species, the chances of *Parthenium* proliferation are more. In Maharashtra, *Stylosanthesscabra* has been found to compete with *Parthenium* though allelopathic effect. This has been confirmed by the field observation made by the officers of the Department of Forests, Karnataka. Several other plant species were also identified as having similar impact but with varying degrees. The strongest species effective for *Parthenium* control listed in the order are

i. *Cassia sericea*
ii. *Tephrosiapurpurea*
iii. *Stylosanthesscabra*
iv. *Crotonsparsifiorus*
v. *Hyptis spp*.
vi. *Cassia tora*
vii. *Amaranthusspinosus.*

Further, *Parthenium* growth is very intensive in places where new constructions like extensions in cities and towns are going on. All these observations lead to the conclusion that maintenance of biodiversity, that is natural flora wherever possible, is important to check *Parthenium* entry or invasion and its growth as weed (Mahadevappa, 1999).

• *Use of Insects*

A good number of insect and non-insect pests may be used to control *Parthenium* under bio-control approach. In India, use of more than 50 insects have been reported against *P. hysterophorus* but none of the insect has been found to be host specific yet. The classical approach was started by Jayanth in 1987 from Mexico with the introduction of host specific leaf feeding *Zygogramm abicolorata pallister* (Coleoptera: Chrysomelidae) and the stem galling moth *Epiblema strenuana.* These two insects have showed good potential to manage this weed (Jayanth, 1987). Both adult and larvae of *Zygogramm abicolorata* feed on leaves. In early stage, larvae feed on the auxillary and on the terminal buds and move on to the leaf blades as they grow and the fully-grown larvae enter the soil and pupate. The density of insect's one adult per plant caused skeletonization of leaves within 4-8 weeks. But findings of other scientists disclosed that little successful was made due to very high germination of weed and moreover the insect is not a host specific and found that this insect can attack to other crops like sunflower in India (Dhileepan, 2001; Jayaramaiah *et al.*, 2017). In our country, insect species have been reported on *Parthenium,* but none of the indigenous insects was found host-specific yet. Based on well documented success by Mexican beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), in other countries where they were introduced, beetle were imported from Mexico to India. After in intensive laboratory and field studies, it was found host specific, which can eat only *Parthenium*, hence, its use was permitted by Government of India. Therefore, Mexican beetles may be multiplied and released anywhere in India for *Parthenium* suppression.

• *Use of Viruses*

A joint view of several scientists is that few viruses can be used for biological control of *Parthenium*. *Parthenium* phyllody disease is very common on *Parthenium* weed in India. The incidence varies from 10% in February-June to 100% in August-December. The leafhopper (*Orosius albicinctus*) population in the field is positively correlated with the incidence of this disease. The phyllody disease of *Parthenium* is transmitted by *O. albicinctus* and the active transmission is found to be 55%. The minimum acquisition access period is found to be 20 minutes and the inoculation access period is 15 minutes. Incubation

period in the vector varies from 15 to 20 days. The pathogen persists throughout the life of the *O. albicinctus*. *Parthenium* phyllody was transmitted by *O. albicinctus* to cowpea, urdbean, moongbean, horsegram, pigeonpea, sunhemp, fieldbean and soybean. Scientists researching on this area feel that there is no hope of using this as a bio-control method in the near future, but their efforts continue work with the hope of positive results in future.

7. Other methods

Growing competitive crops is also not practicable because *Parthenium* is not such a big problem in cultivated lands where regular weeding operation is done. Further, cropping pattern has to be decided based on the suitability, cropping sequence, crop compatibility and changing the crop for the sake of *Parthenium* control is not going to become practicable. One possibility of using a plant enemy in intensively cultivated land to control *Parthenium* is only growing of *Stylosanthus scabra* and *Tephrosea spp.* in lands exclusively devoted to forage production. An experiment conducted by National Research Centre for Weed Science, Jabalpur, India and found that marigold can easily suppress the *Parthenium* weedby both shade and also by allelopathic effects. Some information of relevance to this aspect may also be found relating to biological control of *Parthenium* through plants (Mahadevappan, 1999).

8. Awareness programmes

Since *Parthenium* is a noxious weed of national significance having characteristics of high allergy, disturbing indigenous flora and endangering biodiversity. Therefore, a campaign of awareness programmes should be organized for the public welfare. Besides, "*Parthenim* Day" should also be celebrated especially in affected areas.

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