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# Environment Conservation Journal

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## Stress induced alterations in the outer membrane of *Escherichia coli* K-12 strain

Bindu Arora

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### Abstract

In the present study, gram-negative (*Escherichia coli* K-12) bacterial biomembrane involvement was studied in the presence of modulating factors such as EDTA,  $Mg^{+2}$  ions and EDTA and  $Mg^{+2}$  ions in combination. The release of proteins and their involvement during the transport of  $\beta$ -Lactams namely Ceftriaxone and Cefazolin were also studied. The broader applications of Ceftriaxone for pharmaceutical implications offer greater advantage as compared to pre-existing  $\beta$ -Lactams. Due to the availability of more signal molecules in the membranes there is enhanced toxicity at 5 mM EDTA concentration, and easy entrapment of antibiotics, thus enhanced sensitivity levels. A concentration of 15 mM  $Mg^{+2}$  ions was found to be toxic for *E.coli* whereas it exhibited luxuriant growth with decreasing  $Mg^{+2}$  ion concentration under antibiotic stress. On the contrary, when 5 mM EDTA is treated in combination with  $Mg^{+2}$ , it attributed reduced signals available on the membrane surface therefore, reduced drug sensitivity. To identify the involvement of specific proteins and to know the site of proteins released which are directly or indirectly involved in transport of antibiotics across the biological membrane, the protein release was monitored from intact cells, as well as, membrane vesicles derived from *E.coli* cells and studied upto a level of molecular weight determination and measured by using a high-pressure liquid chromatography (HPLC). The study confirms the induction of certain stress signal proteins from the outer membrane, thereby rendering the bacteria more susceptible to therapy.

**Keywords:-** HPLC- High pressure liquid chromatography, CEF- Ceftriaxone Sodium, CEZ- Cefazolin Sodium, Outer Membrane Proteins- OMPs

### Introduction

Outer membrane protein A (OmpA) is located in the membrane of *Escherichia coli* and other gram-negative bacteria and plays a multifunctional role in bacterial physiology and pathogenesis. (Alfredo *et al.*, 2006). Also, many new outer membrane proteins have recently been identified by proteomics techniques in *Escherichia coli* (Marani *et al.*, 2006). In the outer membrane of gram-negative bacteria, the porins, are present in large amounts and form water-filled channels that permit the diffusion of small hydrophilic solutes across the membrane (Nikaido, 2003; Nikaido, 1994; Nikaido, 1985; Nikaido and Vaara, 1985) including bacterial nutrients and

antimicrobials. The permeability of outer membranes of gram-negative bacteria to hydrophilic compounds is mostly due to presence of porin channels (Dela Vega and Delcour, 1995). Since  $\beta$ -Lactam antibiotics penetrate the outer membrane of gram-negative bacteria, resistance could also be caused by loss or deficiency of specific porins that reduce the outer membrane permeability to  $\beta$ -Lactam antibiotic. This might be an important factor in mediating  $\beta$ -Lactam resistance in multidrug *E.coli* (Nikaido, 2003). The Cephalosporins have been known to induce stress signal proteins, from the outer membrane, inhibiting cross-linking step of peptidoglycan biosynthesis in the cell wall of *E.coli* thereby rendering the bacteria more susceptible to therapy (Russel and Chopra, 1990).  $\beta$ -Lactams

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binding to these PBP's may be eliminated by the action of 3 enzymes, viz.,  $\beta$ -Lactamases, the acylases and the esterases. Bacteria may become more resistant to these  $\beta$ -Lactams by producing altered transpeptidases (PBP's) with greatly reduced affinity for the binding of these antibiotics (Nikaido *et al.*, 1990).

## Materials and Method

*Escherichia coli* K-12, wild type strain was procured from IMTECH, Chandigarh. Cultures were grown on Nutrient Agar at  $37^\circ\text{C} \pm 1$  and stored at  $4^\circ\text{C}$ . Ceftriaxone sodium (CEF) and Cefazolin Sodium (CEZ) was purchased from Aristo pharmaceutical India Ltd. (M.P.).

*E. coli* was grown for growth studies on Spencer and Guest (1973) media, pH 6.9. Incubation was carried out at  $37^\circ\text{C} \pm 1$  in a thermostatically controlled orbital shaker (Labline model No. 3521) under aerobic conditions with a platform rotation of  $180 \text{ rev min}^{-1}$ .

Growth was monitored under various conditions spectrophotometrically (Spectronics-20 Bausch and Lomb) at  $550 \text{ nm}$ .

For transport and protein release studies, membrane vesicles were prepared by Koning and Kaback (1973) with some modifications. Membrane protein analysis was carried out by Warburg and Christian (1941) method. The qualitative study of protein release was done by HPLC (Waters, USA) at 4,000 psi pressure, at absorbance of  $280 \text{ nm}$ . The solid phase was Protein-PAK column (Waters, USA).

## Results and Discussion

A highly significant and negative correlation between concentration of CEF and CEZ with percent survival was found to be  $r = -0.646$  ( $p < 0.02$ ) and  $r = -0.757$  ( $p < 0.02$ ) respectively. *E. coli* K-12 wild type strain was found to be ten times sensitive to CEF ( $\text{LD}_{50}$  of  $0.005 \text{ ppm}$ ), than with CEZ ( $\text{LD}_{50}$  of  $0.05 \text{ ppm}$ ) (Fig. 1). This high sensitivity of *E. coli* to CEF as compared to CEZ was attributed to the high  $\text{Na}^+$  ion concentration outside *E. coli* ( $\text{Na}^+$  ion

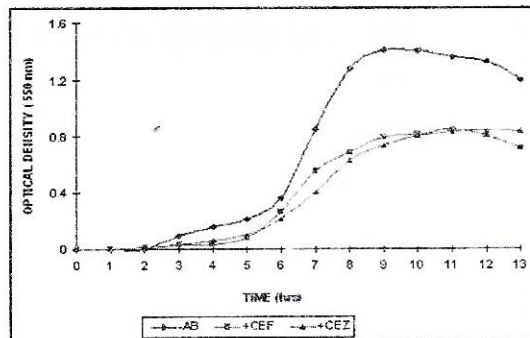


Fig. 1: Effect of  $\text{LD}_{50}$  concentrations of CEF and CEZ on the growth of *E. coli*

content of  $\text{CEF-Na}^+$  ion,  $83 \text{ mg/gm}$ ) cell wall as compared to its interior, leading to  $\text{Na}^+\text{-H}^+$  ion antiporter activity and thus dissipation of pH gradient, ultimately interrupting the pmf and increase in the CEF uptake through its porins. Loss of porin-mediated resistance mechanism against cephalosporin has been observed among the multidrug resistant *E. coli* (Ananthan and Subha, 2005). In relation to sensitivity of *E. coli* cells to  $\beta$ -Lactams in the presence of varying  $\text{H}^+$  ion concentrations, the protein thus, obtained in suspension medium shows variations with respect to time of incubation. The protein content present in this suspension medium (Tris-HCl Buffer), in the presence of  $\beta$ -Lactams as well as the release of proteins under antibiotic stress shows variations with respect to time of treatment (Fig. 2a, 2b).

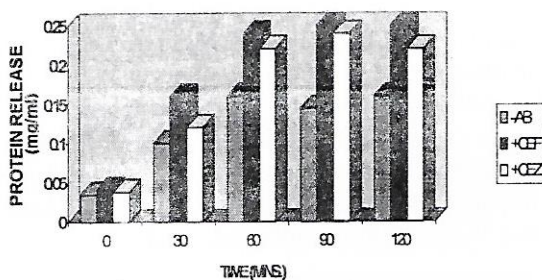
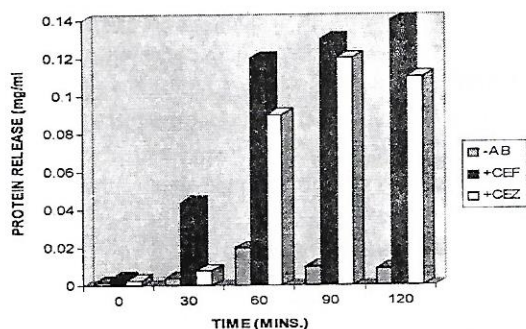


Fig. 2a: Protein release from intact cells of *E. coli* at varying time intervals

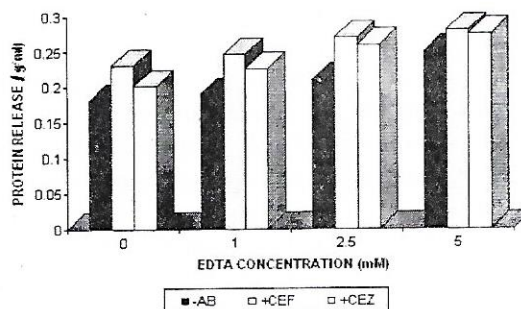




**Fig. 2b: Protein release from membrane vesicles of *E. coli* at varying time intervals**

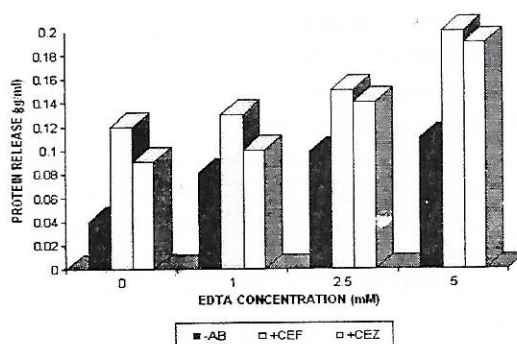
Bacterial adaptation to various environmental stresses has been extensively investigated (Yohannes *et al.*, 2004; Yohannes *et al.*, 2005). Chelation property of EDTA, thus, facilitates antibiotic transport, thereby resulting in antibiotic toxicity (Yamada *et al.*, 1978). It has also been demonstrated that the effect of  $Mg^{+2}$  ions at high concentrations include reduction in the growth rate, extension of lag phase and perturbation in morphology and physiology (Hughes and Poole, 1989). The cell extract of *E. coli* exhibited a significant degradation of EDTA only in the presence of  $Fe^{+3}$  ion. There have been some reports regarding the interaction of non-toxic metals with toxic metals or metal chelators diminishing or enhancing the toxicity of the latter by the former. The ion antagonism has been studied but not sufficiently. Leive (1968) found that the concentration of  $Na^{+}$  ion and  $K^{+}$  ion do not affect EDTA action but the divalent cation like  $Mg^{+2}$  ion prevent its action. Attempts to revive EDTA-treated cells with cation such as  $Mg^{+2}$  ion have met with slight success.

It was observed that with varying concentrations of EDTA (1.00, 2.50 and 5.00 mM), antibiotic-treated cells were inhibited more than normal cells. The protein release in CEF-treated cells along with 1.00, 2.50 and 5.00 mM EDTA concentrations was 0.245, 0.270 and 0.280 µg/ml; whereas with CEZ-treated cells was observed as 0.225, 0.260 and 0.275 µg/ml respectively, after 60 min of incubation, as illustrated in Fig. 3a.



**Fig. 3a: Protein release from intact cells of *E. coli* in the presence of varying conc. of EDTA**

These observations were found consistent with the existing evidence (Matzushita *et al.*, 1978). The enhanced protein release with higher concentration of EDTA explained the pronounced activity of antibiotics against the EDTA treated cells at higher concentration. The effect of varying concentrations of EDTA on the membrane vesicles of *E. coli* is illustrated in Fig. 3b.



**Fig. 3b: Protein release from membrane vesicles of *E. coli* in the presence of varying conc. of EDTA**

In the presence of 1.00 mM EDTA, the release of protein was quite less *i.e.*, 0.13 and 0.10 µg/ml with CEF and CEZ, respectively in comparison to 2.50 mM and 5.00 mM EDTA (in combination with antibiotics) releasing 0.15 and 0.20 µg/ml in the presence of CEF; whereas, 0.14 and 0.19 µg/ml in the presence of CEZ respectively, in the medium after 60 mins of incubation. In contrast to that, the



inhibition of 5.00 mM EDTA was more pronounced than 1.00 mM EDTA in membrane vesicles of *E. coli*. The less quantity of protein released with 1.00 mM EDTA supports the earlier observations that in growing cultures of *E. coli*, 5.00 mM EDTA along with cephalosporins showed maximum inhibition of growth.

In case of intact cells, the amount of protein released in the presence of 5.00 mM EDTA was four-fold greater than the protein from membrane vesicles derived from gram-negative bacteria. It is very obvious that outer membrane comprises of low protein content in contrast to intact cells which has all the cellular proteins in combination with structural component, as well as, functional components such as enzymes. But with intact cells, as well as, membrane vesicles the speciation of proteins differs with respect to their molecular size as apparent from the Fig. 6 (a), (b). As far as molecular size is concerned, EDTA with cephalosporin resulted in release of similar group of proteins which had molecular size more than 67.00 kD. In case of EDTA also, peak C at 5 min was very prominent and differs in concentration reflecting high peak in case of EDTA and CEF, in contrast to EDTA and CEZ; after 60 min of incubation. In all the cases, peak height of the proteins were greater in the presence of CEF and CEZ. It is evident from growth, as well as, enzyme studies, that gram-negative cells were more sensitive to CEF treatment in contrast to CEZ as a counterpart. EDTA although potentiated the cephalosporin toxicity by many folds in case of CEF than CEZ. It is clear from the figure that the protein leaked out at 10 min run was at much higher conc. in case of CEF than CEZ. The protein release was found to be directly dependent upon the increasing concentration of EDTA from the cell. The amount of cellular protein, thus calculated by carrying a difference between protein release from intact cells and membrane vesicles shows a constant trend with a similar concentration at 1.00, 2.50 and 5.00 mM concentration, in case of both the cephalosporins. However, the difference

in toxicity can be then considered as involvement of OM proteins exclusively in conferring sensitivity towards antibiotics.

In *E. coli*, when intact cells were treated with varying concentrations of  $Mg^{+2}$  ions, it was observed that the extent of protein release was quite higher as the concentration of  $Mg^{+2}$  ions was increased. The protein release from the cell wall of *E. coli* when treated with 1.00 mM, 5.00 mM, 10.00 mM and 15.00 mM  $Mg^{+2}$  ion was observed as 0.160, 0.175, 0.225 and 0.247  $\mu\text{g/ml}$ ; in the absence of antibiotics after 60 min of incubation. Thus, increasing concentration of  $Mg^{+2}$  ions had released more proteins. The inhibitory effects induced by high  $Mg^{+2}$  ion concentration which release more protein were explained in terms of stress-induced injury of OM. On the other hand in the presence of antibiotics, 15.00 mM  $Mg^{+2}$  ions drastically inhibits the transport of antibiotics, therefore resulting in fewer protein release. The protein release at 1.00, 5.00, 10.00 and 15.00 mM  $Mg^{+2}$  ions in the presence of CEF was found to be 0.229, 0.225, 0.215 and 0.190  $\mu\text{g/ml}$ ; whereas, with CEZ was observed as 0.202, 0.206, 0.20 and 0.185  $\mu\text{g/ml}$  respectively after 60 min of incubation (Fig. 4a). However, the protein release from the membrane vesicles (OM) of *E. coli* was observed as 0.042, 0.05, 0.072 and 0.097  $\mu\text{g/ml}$  when the cells were suspended in the presence of 1, 5, 10 and 15.00 mM  $Mg^{+2}$  ion concentration respectively;

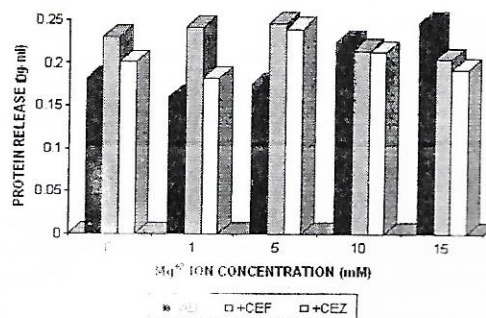
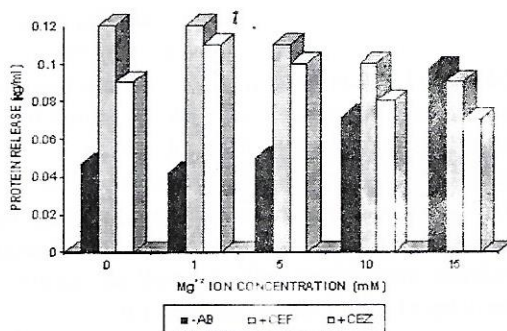


Fig. 4a: Protein release from intact cells of *E. coli* with and without antibiotics in presence of varying concentrations of  $Mg^{+2}$  ions





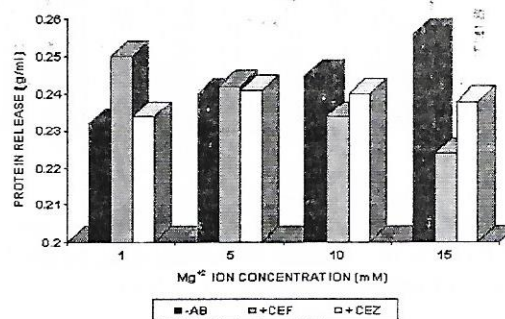
**Fig. 4b: Protein release from membrane vesicles of *E. coli* in presence of varying conc. of Mg<sup>2+</sup>** after incubation period of 60 mins (Fig. 4b). The variation in protein release at varying concentrations of Mg<sup>2+</sup> ions could be attributed to the differential behaviour of Mg<sup>2+</sup> ions at different concentrations i.e., at lower concentration Mg<sup>2+</sup> ions acted as a potentiator and a stabilizing agent of the membrane while at higher concentrations they are inhibitory. With  $\beta$ -Lactams, the contrast behaviour of Mg<sup>2+</sup> ions has been observed in relation to protein release.

At MIC<sub>50</sub> conc. of CEF and CEZ, the protein released in the medium when the cells were suspended with 1.00, 5.00, 10.00 and 15.00 mM Mg<sup>2+</sup> ion concentrations was observed as 0.12, 0.11, 0.10 and 0.09  $\mu$ g/ml with CEF, whereas with CEZ, the protein release was 0.11, 0.10, 0.08 and 0.07  $\mu$ g/ml, when the aliquots were drawn after 60 min of incubation. This might be due to the fact that the antibiotic accessibility to the inside of the cell is reduced at higher concentration of Mg<sup>2+</sup> ions which releases fewer proteins at high concentrations (15.00 mM Mg<sup>2+</sup> ions).

The effect of  $\beta$ -Lactams on the protein release of *E. coli* in the presence of EDTA as well as, Mg<sup>2+</sup> ions have been studied. The extent of protein release was less when intact cells were suspended in 15 mM Mg<sup>2+</sup> (in combination with 5 mM EDTA), than in 1.00 mM (in combination with 5.00 mM EDTA), after 60 min of incubation. The protein released in the medium when cells were suspended in 1.00,

5.00, 10.00 and 15.00 mM Mg<sup>2+</sup> ion concentration (each in combination with 5.00 mM EDTA) was observed as 0.250, 0.242, 0.234 and 0.224  $\mu$ g/ml respectively, in the presence of CEF, whereas protein release was 0.234, 0.241, 0.240 and 0.238  $\mu$ g/ml respectively, in the presence of CEZ; after 60 min of incubation (Fig. 5a).

This must be attributed again to the differential behaviour of different concentrations of Mg<sup>2+</sup> ions in relation to release of proteins. The enhanced protein release by antibiotics and EDTA had been limited by high concentrations of Mg<sup>2+</sup> ions i.e., 15.00 mM to a great extent. The protein release was observed as 0.19, 0.18, 0.154 and 0.13  $\mu$ g/ml in the presence of CEF; and in the case of CEZ, the protein



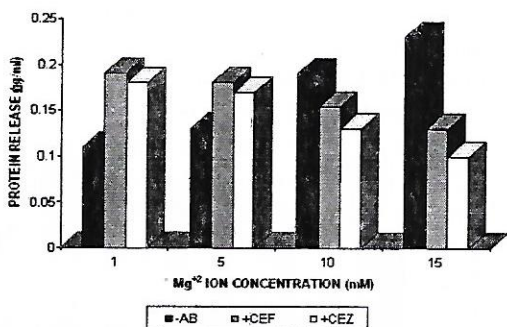
**Fig. 5a: Protein release from intact cells of *E. coli* in the presence of varying conc. of Mg<sup>2+</sup> each followed by the addition of 5mM EDTA**

release from the membrane vesicles of *E. coli* was observed as 0.18, 0.17, 0.13 and 0.10  $\mu$ g/ml, when the cells were suspended in 1.00 mM, 5.00 mM, 10.00 mM and 15.00 mM Mg<sup>2+</sup> ion concentration (each supplemented with 5 mM EDTA) respectively, after 60 min of incubation (Fig. 5b). The variation in protein release might be attributed to the fact that the inhibitory effect of antibiotics was potentiated with the help of EDTA, which has resulted in more protein release, however, Mg<sup>2+</sup> ions would have retained the stability of OM thus protecting the synergistic behaviour of EDTA in combination with  $\beta$ -Lactam. Contrary to that Mg<sup>2+</sup> ions at higher conc. when supplemented would have saturated the



molecules of EDTA present into the medium thereby, protecting possible chelation of OM ions by EDTA; thus, maintaining integrity of cell wall.

As far as, speciation of protein leakage is concerned magnesium in combination with EDTA and cephalosporins resulted in release of possibly similar group of proteins that were greater than 67 kD which appeared after 11 min of run. In the absence of EDTA,  $Mg^{+2}$  ion causes effectively release of protein A and B in case of CEF; whereas with CEZ peak B was omitted. (Fig 6c). A remarkable phenomenon observed with CEF when treated with



**Fig. 5b: Protein release from membrane vesicles of *E.coli* in the presence of varying conc. of  $Mg^{+2}$  each in combination with 5 mM EDTA**

EDTA and  $Mg^{+2}$  ion the potentiality of third generation cephalosporin can be possibly related by the appearance of peak G after 20 min of run resembling exactly with cytochrome c with a molecular weight of 12.5 kD (Fig. 6d). The appearance of cytochrome "c" peak into the medium under the stress of CEF might have resulted in making gram-negative cells sensitive towards CEF by reducing cellular energy level required to carry out possible cellular metabolism. Omp's located in the outer membrane of *E.coli* plays a multifunctional role in bacterial physiology and pathogenesis (Alfredo *et al.*, 2006). Recently it has been identified by proteomics techniques that there are many new *E.coli* outer membrane proteins, out of eight predicted outer membrane proteins, the outer membrane localization for five- YfiM, YaiO, YfaZ,

CsgF, and YliI- are confirmed and also the sixth one- YfaL- may be an outer membrane autotransporter. Since  $\beta$ -Lactam antibiotics penetrate the outer membrane, resistance could also be caused by loss or deficiency of specific porins that reduce the outer membrane permeability to  $\beta$ -Lactam antibiotic. Also, the cause for antibacterial drug resistance has been known to be the active efflux. The study of protein release from intact cells, as well as, membrane vesicles of *E.coli* shows the direct involvement of outer membrane components in developing sensitivity towards  $\beta$ -Lactams. The observations, thus, confirms the greater effectiveness of CEF as compared to CEZ towards *E.coli*.

The extent of EDTA- induced outer membrane losses from cells of wild- type *Escherichia coli* K-12 were concentration dependent. An additional  $Mg^{+2}$  ions immediately following the EDTA treatment decreased the release of outer membrane proteins and reduced the leakage of periplasmic proteins, suggesting that the temporary increase in outer membrane "permeability" caused by EDTA treatment was rapidly reversed by the redistribution of outer membrane components, a process which is favored by a low  $Mg^{+2}$  ion concentration. The envelope alterations caused by EDTA reveals that the biochemical, as well as, cellular disturbances were found to be more easily related to the toxic action of  $\beta$ -Lactams, CEF and CEZ. The susceptibility of bacterial culture for specific  $\beta$ -Lactams can be assessed by gradual exploration of membrane/cellular proteins responsible for making possible transport of antibiotics, across the cellular membrane against concentration gradient.

The study thus reveals that some of the membrane proteins as, well as some of the intracellular proteins get affected by the  $\beta$ -Lactam transport, revealing the mediation of direct antibiotic-protein interaction. The study confirms the orientation of the outer membrane and the energy status of the cell, determining the therapeutic value of cephalosporins.





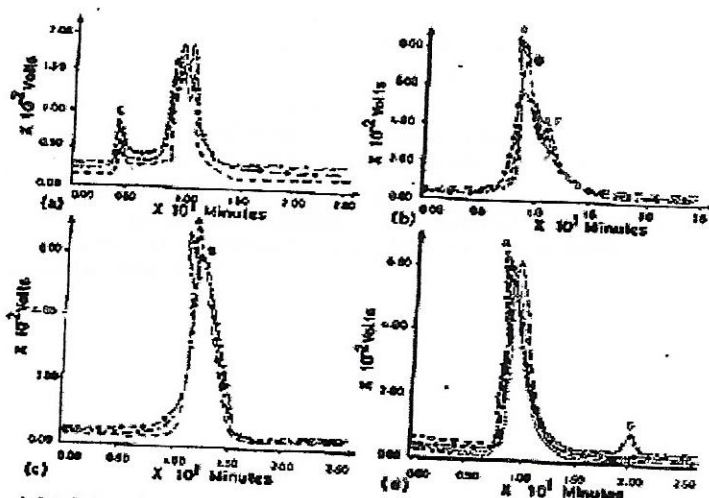


Fig. 6: Molecular weight determination of protein released from *E. coli* cells in the presence of antibiotic stress as monitored by HPLC, in the presence of 5mM EDTA, 5mM  $Mg^{+2}$  ions and 5 mM EDTA in combination with 5 mM  $Mg^{+2}$  ions, separately after 60 mins of incubation. (a) Membrane vesicles- EDTA (5 mM) (b) Membrane vesicles-  $Mg^{+2}$  ions (5 mM) (c) Intact cells - EDTA (5 mM) (d) Intact cells - EDTA (5 mM) +  $Mg^{+2}$  ions (5mM). Here — indicates -AB; -x-x indicates +CEF; -o-o- indicates + CEZ; ..... indicates control conditions

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## Zooplankton diversity in relation to certain physico-chemical parameters of swamp of Kishanganj District, Bihar

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### Abstract

The zooplankton fauna of Kishanganj swamps in relation to certain physico-chemical parameter was studied. Out of 28 species recorded, 18 belong to *Rotifera*, 6 to *Cladocera* and 4 to *Copepoda*. The total zooplankton density was more in summer and least in rainy season. The quantitative relationship amongst the group of zooplankton was *Rotifera* > *Cladocera* > *Copepoda*. Zooplankton also comprised of some pollution tolerant species like *Brachionus*, *Keratella* and *Mesocyclops* etc. The Rotifers showed negative correlation with pH, sulphate and dissolved oxygen Cladocerans showed negative correlation with pH, sulphate and phosphate while Copepods revealed negative correlation with pH, sulphate and water temperature.

**Keywords:-** Zooplankton, *Rotifera*, *Cladocera*, *Copepoda*, Physico-chemical

### Introduction

Zooplankton are the microscopic free swimming animals. They are found almost universally in all the aquatic environment comprising the first trophic level of heterotrophic food chains and form a link between phytoplankton and aquatic animals. They provide main food to fishes and can be used as indicators of trophic level of water body. Zooplankton play an integrated role in transferring energy to consumer, hence they make a higher trophic level in energy flow after phytoplankton. They respond to change in water quality (Holland *et al.*, 1983). Zooplankton play an important role in the trophic dynamic of aquatic ecosystems (Venkataraman, 1981). The occurrence and diversity of planktonic organisms are almost universal in all aquatic habitats and the greatest concentration of zooplankton occurs in upper layers of water (Cable, 1966). The extent of degradation of water bodies can be reliably evaluated with plankton (Vareethiah and Haniffa, 1998).

Investigation of biological diversity and primary productivity rates are of great importance to determine the potential for organic production and subsequent exploitation. The need for knowledge of zooplankton diversity is often stressed as a reliable indicator for the integrity of aquatic ecosystem (Barbosa *et al.*, 1995). The occurrence and physiological condition of zooplankton can be an indicator of environmental conditions. Hence in the present paper an attempt has been made to study the population density of zooplankton of Kishanganj swamps in relation to certain physico-chemical factors.

### Materials and Method

The monthly observation of physico-chemical factors and zooplankton population was made. Surface water and zooplankton were collected on every 15<sup>th</sup> day of the month at a fixed time. Water temperature was recorded with ordinary thermometer and free CO<sub>2</sub> was measured on the spot. The pH, dissolved oxygen, carbonate, bicarbonate, chloride, nitrate, sulphate and phosphate were analyzed according to methods of APHA (1975) and Trivedy and Goel (1984).

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Zooplankton were collected by filtering 50.00 liters of water through a plankton net made up of bolting silk (No. 21) and preserved in 5% formalin. Quantitative and qualitative analysis of zooplankton were made by Lackey (1938), as modified by Edmondson (1974). Correlation between zooplankton and certain physico-chemical parameters were computed.

## Results and Discussion

The physico-chemical characteristics of the swamps water and zooplankton populations are shown in the Table. 1 and 2 respectively. The

zooplankton taxa collected from the swamps water belong to three dominant groups viz. *Rotifera*, *Cladocera* and *Copepoda*. There were 28 zooplankton species identified from which 18 belong to *Rotifera*, 6 belong to *Cladocera* and 4 belong to *Copepoda*. Rotifers were the most dominant and abundant group showing highest percentage (56.85%) composition and diversity followed by *Cladocera* (27.47%) and *Copepoda* (15.68%).

Following zooplankton were found in the swamps: *Rotifera* : *Branchionus angularis*, *B. rubens*, *B. caudatum*, *B. calyciflorus*, *B. forticula*, *B. falcatus*, *B. bicenata*, *Keratella tropica*, *K.*

Table 1: Physico-chemical characteristics of swamps water

Month	Temp.		pH	DO	Free CO <sub>2</sub>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>2-</sup>	Cl	SO <sub>4</sub> <sup>2-</sup>
	Atm.	Water									
January	20.90	19.10	7.50	8.20	8.50	0.00	125.00	0.36	0.50	14.90	49.60
February	23.80	21.10	7.40	7.80	6.70	0.00	122.00	0.37	0.52	16.50	60.40
March	25.50	22.40	7.20	7.30	9.80	0.00	126.00	0.38	0.60	20.40	72.20
April	30.10	26.80	7.10	7.10	9.60	0.00	130.00	0.42	0.84	22.20	75.60
May	33.90	31.70	7.90	6.80	8.80	0.00	135.00	0.49	0.94	28.70	71.80
June	32.80	31.90	6.80	6.10	7.20	0.00	118.00	0.52	0.72	26.20	70.90
July	33.50	30.80	6.60	5.80	9.90	0.00	105.00	0.58	0.74	19.90	78.50
August	30.80	29.40	6.70	5.10	12.80	0.00	98.60	0.62	0.72	16.40	73.20
September	29.20	28.60	6.40	4.80	11.40	0.00	106.70	0.56	0.70	12.40	80.50
October	29.30	26.20	6.90	5.20	10.50	0.00	110.20	0.52	0.73	15.10	63.80
November	26.80	25.40	7.10	5.90	9.60	0.00	112.40	0.49	0.71	16.20	57.40
December	21.40	22.10	7.30	7.80	8.10	0.00	114.20	0.41	0.68	17.10	60.50

*vulga*, *Filinia longiseta*, *F. terminalis*, *Monostyla lunaris*, *M. bulla*, *Hordelia sp.*, *Lepadella sp.*, *B. plicatilis*, *Rotaria sp.*, *Polyarthra sp.*

*Cladocera* : *Moina micrura*, *M. dubia*, *Alonella sp.*, *Daphnia sp.*, *Ceriodaphnia, sp.*, *Chdorus gibba*.

*Copepoda* : *Cyclops sp.*, *Nauplius sp.*, *Diaptomus, Cyclops sp.*

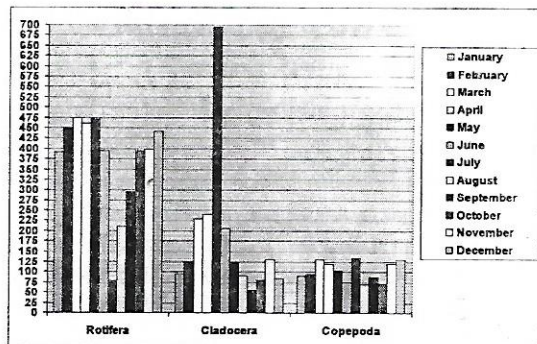
The quantitative zooplankton analysis shows that total plankton density is more in summer and least in rainy (Table 2; Fig. 1 and 2). The quantitative relationship amongst the groups of zooplankton is



**Table 2: Monthly variation of zooplankton groups in swamps water (Unit/l)**

Month	Rotifera	Cladocera	Copepoda
January	390.00	95.00	90.00
February	450.00	125.00	95.00
March	475.00	230.00	130.00
April	460.00	240.00	120.00
May	470.00	695.00	105.00
June	395.00	205.00	75.00
July	78.00	125.00	135.00
August	210.00	90.00	70.00
September	295.00	55.00	88.00
October	395.00	80.00	72.00
November	398.00	130.00	120.00
December	442.00	85.00	130.00

*Rotifera* > *Cladocera* > *Copepoda*. Rotifers showed superiority over other groups both in terms of number of species, genera and population density. Dominance of *Rotifera* in seasonal data of zooplankton in the present study is in accordance with the earlier finding of Ferneska and Lewkowicz, 1966; Schindler and Noven, 1971; Pandey *et al.*, 1994, 2004. Dominance of Rotifers over other groups is an indication of congenial conditions of the system (Arora, 1966). Daggett and Davis (1974) suggested that a rich Rotifer community requires a stable medium as they depend upon certain species of phytoplankton, whereas cladocerans and copepods due to their vast adaptability can withstand a wide range of environmental stress and utilize generally the phytoplanktonic cells as their natural food items. The rotifers communities have some association with water quality and any variation in the dissolved oxygen, organic matter, suspended solids would immediately affect their distribution (Holland *et al.*, 1983). The observed rotifers- *Branchionus calyciflorus*, *B. rubens*, *B. forticula*, *B. falcatus* can be considered to indicate the eutrophicated nature of the water body under

**Fig 1: Showing month-wise distribution of zooplankton groups in swamp water**

the present investigation as they are most abundant in the present study. George (1966) and Bansei (1976) have reported summer peak of rotifers while Nasar (1977), Baker (1979) and Edmondson (1996) have shown winter peak of rotifers. But in the present investigation peak of rotifers was observed in summer season. The abundance of rotifers in any ecosystem as compared to other groups is an indication of eutrophication (George, 1966). Laal and Karthikeyan (1993) have reported the maximum rotifers at polluted zone of different rivers. Biswas and Konar (2000) have reported more rotifers at station of mixing zone of waters in Damodar river water. However, in the present investigation no such correlation was observed.

The seasonal occurrence and abundance of different species of rotifers showed that *B. angularis* was dominant in number over other species. Such result has been reported by Pandey *et al.* (1992). *B. calyciflorus* and *Keratella tropica* were observed throughout the year while other species showed fluctuations, *Keratella sp.* was regarded as an indicator of eutrophication. *Polyarthra* was abundant during winter indicating its preference for clear water. Seasonality was not shown by any other species.

The cladoceran population was less scanty in comparison to the rotifers. The cladoceran peak was observed during winter followed by summer





and rainy seasons. Prasad (1977) has reported the abundance of cladocerans in the month of May. Copepods were abundant during rainy season. Rainy peak of copepods has been also reported by Maruthanyagam *et al.* (2003) and Pandey *et al.* (2004). *Mesocyclops* were mainly represented by *Mesocyclops* sp. They indicate presence of particular matter in water and form primary food of planktivorous fish (Ivlev, 1961). Presence of copepods indicate rich trophic status of the water body (Pejler, 1983).

Presence of maximum zooplankton population in summer (Fig. 2) might be due to the presence of higher population of bacteria. The lowest number recorded during rainy season may be related to the flood and fast currents. This finding support to Bilgrami and Datta Munshi (1985) and Yousuf (1989). Seasonal fluctuation was observed in zooplanktonic community during the course of present investigation. Patra and Dutta (2004) have noted the seasonal fluctuation of zooplankton which is governed by abiotic and biotic factors. To assess the importance of abiotic interaction an

attempt was made to analyze the data statistically (Table 3). Rotifers showed positive correlation with all the studied parameters except pH sulphate and dissolved oxygen, cladocerans showed negative correlation with pH and phosphate while copepods revealed negative correlation with water temperature, pH and sulphate.

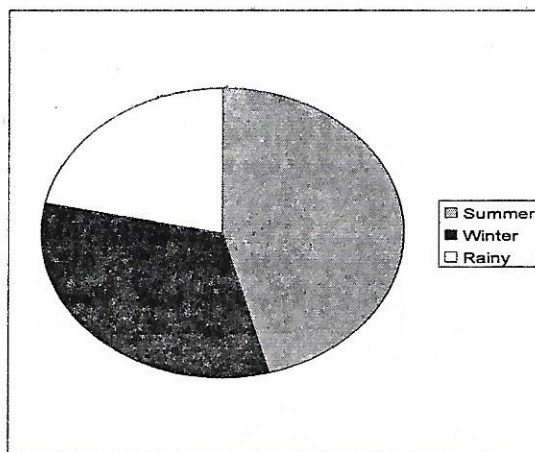


Fig. 2 : Seasonal variation of zooplankton

Table 3: Karl Pearson's correlation coefficient (r)

Variables	Rotifera		Cladocera		Copepoda	
	r	r <sup>2</sup>	r	r <sup>2</sup>	r	r <sup>2</sup>
Water Temp. (°C)	0.22	0.494	0.053	0.0028	-0.094	0.0089
pH	-0.075	0.0057	-0.323	0.1045	-0.079	0.00062
DO (mg/l)	-0.349	0.0057	0.149	0.0223	0.527	0.2774
Free CO <sub>2</sub> (mg/l)	0.327	0.1070	0.620	0.3846	0.495	0.2455
Bicarbonate (mg/l)	0.124	0.248	0.434	0.1887	0.223	0.0499
NO <sub>3</sub> <sup>-</sup> (mg/l)	0.183	0.336	0.519	0.2693	0.432	0.1866
PO <sub>4</sub> <sup>3-</sup> (mg/l)	0.198	0.0393	-0.009	0.001	0.079	0.0063
Cl <sup>-</sup> (mg/l)	0.078	0.006	0.454	0.206	0.530	0.2806
SO <sub>4</sub> <sup>2-</sup> (mg/l)	-0.233	0.0543	0.278	0.00775	-0.287	0.0824



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# Dietary effect of water soluble gummy fibre and water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds on blood glucose, glucose tolerance and some intestinal enzymes in normal and diabetic male albino rats

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## Abstract

In the present study, the effect of feeding of diets containing 12% and 18% water soluble gummy fibre and 5% and 10% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds were carried out in normal and alloxan treated diabetic rats for 21 days. The results obtained from present study indicates that incorporation of 12% and 18% water soluble gummy fibre significantly reduces blood glucose, notably improved glucose tolerance and decreased activity of intestinal enzymes *i.e.* amylase and invertase while 5% and 10 % water insoluble neutral detergent fibre (NDF) did not exhibit any hypoglycaemic effect in both normal and treated diabetic rats.

**Keywords:-** *Syzygium cumini* seed fibre, Neutral detergent fibre (NDF), Blood glucose, Glucose tolerance, Intestinal enzymes

## Introduction

Hypoglycaemic effect of *Syzygium cumini* seeds are mainly due to water soluble gummy fibre has been studied earlier (Pandey and Khan, 2002). In earlier study, feeding of diet containing 6% water soluble gummy fibre isolated from *Syzygium cumini* seeds by solvent fractionation method for 21 days significantly reduces the blood glucose levels, notably improve the oral glucose tolerance (Pandey and Khan, 2002) and decreases the activity of intestinal enzymes *i.e.* amylase and invertase in both normal and diabetic treated rats whereas feeding of diets containing 2.25 % water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds did not show any hypoglycaemic effect (Pandey and Khan, 2002). Keeping in view the hypoglycaemic effect of 6% water soluble gummy fibre, the present study was conducted to

investigate whether the increase in the amount of fibre *i.e.* 12% and 18% water soluble gummy fibre and 5% and 10% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds had same or any enhancing hypoglycaemic effect in both normal and alloxan treated diabetic rats for 21 days.

## Materials and Method

### Chemicals

Casein (Vitamin free), soluble starch, glucose oxidase, peroxidase, O-dianisidine were purchased from Sigma Chemicals, St. Louis, Co, USA. Benzoic acid, Sodium hydroxide, Zinc Sulphate, Sodium dihydrogen phosphate and D-glucose were procured from Qualigen's fine chemicals, Co. Bombay. Rest of the chemicals used were of analytical grade.

### Animal and diet

The male albino rats of Wistar strain weighing between 150-170 gm were divided into five groups each of normal (designated as Group I N, Group II N, Group III N, Group IV N, Group V N and diabetic

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(designated as Group I D, Group II D, Group III D, Group IV D and Group V D) rats. The animals were made diabetic by the method of Lazaro and Pally (Pandey and Khan, 2002). Group I from normal (*i.e.*, Group I N) and diabetic rats (*i.e.*, Group I D) served as control and were fed the control diets for 21 days whereas remaining groups were fed the experimental diets containing different amounts of water soluble gummy fibres and water insoluble detergent fibre (NDF) isolated from *Syzygium cumini* seeds as mentioned below:

#### Groups Diets

Group II N and Group II D diets containing 12% water soluble gummy fibre. Group III N and Group III D diets containing 18% water soluble gummy fibre. Group IV N and Group IV D diets containing 5% water insoluble neutral detergent fibre. Group V N and Group V D diets containing 10% water insoluble neutral detergent fibre. The numbers of animals in each group are mentioned in the table in parenthesis. The 20.00 gm of all the diets were fed daily for 21 days. The composition of control and experimental diets are given in (Table 1). All the animals were allowed free access to deionized distilled water. Body weights were examined at weekly intervals before feeding till the termination

of the experiment. At the end of 21 days, the rats of all the groups were fasted for 18 hours. After fasting, half of the animals from each group were sacrificed by decapitation, small intestine were removed and used for the determination of activity of intestinal enzymes, amylase and invertase (Williams and James, 1979). After fasting for 18 hours followed by feeding of 2400 mg and 3600 mg which corresponds to 12% and 18% of water soluble gummy fibre and 1000 mg and 2000 mg which corresponds to water insoluble neutral detergent fibre (NDF) to the animals of respective groups, blood samples were collected from the tail vein of the remaining animals and blood glucose was estimated by glucose oxidase method by (Pandey and Khan, 2002). In order to determine the effect of feeding of diets containing 12% and 18% water soluble gummy fibre and 5% and 10% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds on oral glucose tolerance of the rats, the same pattern of grouping of animals, feeding of diets to the respective group and fasting was followed as described for above parameters.

The glucose tolerance was performed by administering glucose orally (10 mg/Kg body

Table 1: Composition of controlled and experimental diet

Ingredients	Control diet	Experimental diets containing water soluble gummy fibre		Experimental diet containing water insoluble neutral detergent fibre	
		12%	18%	5%	10%
Casein	20.00	20.00	20.00	20.00	20.00
Water soluble gummy fibre isolated from <i>Syzygium cumini</i> seeds	-	12.00	18.00	-	-
Water insoluble neutral detergent fibre (NDF) isolated from <i>Syzygium cumini</i> seeds	-	-	-	5.00	10.00
Hydrogenated oil	10.00	10.00	10.00	10.00	10.00
Starch	65.00	53.00	47.00	60.00	55.00
Hawk's Oser salt mixture*	4.00	4.00	4.00	4.00	4.00
Vitamin mixture*	1.00	1.00	1.00	1.00	1.00

Each diet contains (%w/w) salt mixture \*4 g ; Vitamin mixture 1g.





**Table 2: Effect of feeding of diets containing water soluble gummy fibre and water insoluble neutral detergent fiber isolated from *Syzygium cumini* seeds for 21 days on blood glucose and glucose tolerance in normal and alloxan treated diabetic male albino rats**

Group	Blood glucose (mg/100 ml)	Glucose tolerance test				
		0 hr	1/2 hr	1 hr	11/2 hr	2 hr
Normal						
Group I N (6) Control diet fed	97.56±8.09	96.11±7.79	138.00±9.61	163.00±10.00	177.00±8.99	95.25±7.01
Group II N (6) diets containing 12% water soluble gummy fibre fed	72.43**±5.98	71.00**±6.00	92.00**±7.43	94.00**±7.67	84.00**±6.59	65.00**±6.03
Group III N (6) diets containing 18% water soluble gummy fibre fed	67.25***±5.75	72.06***±5.95	85.13***±6.60	91.00***±6.86	80.00***±6.00	63.00***±5.81
Group IV N (6) diets containing 5% waer insoluble neutral detergentfibre fed	62.33 <sup>NS</sup> ±5.53	62.13 <sup>NS</sup> ±5.49	82.00 <sup>NS</sup> ±6.64	84.00 <sup>NS</sup> ±6.86	70.00 <sup>NS</sup> ±4.84	60.36 <sup>NS</sup> ±4.07
Group V N (6) diets containing 10% water insoluble neutral detergent fibre fed	96.22 <sup>NS</sup> ±7.94	91.05 <sup>NS</sup> ±7.06	132.00 <sup>NS</sup> ±8.60	158.00 <sup>NS</sup> ±9.14	112.31 <sup>NS</sup> ±7.02	89.00 <sup>NS</sup> ±6.91
Diabetic						
Group I D (6) Control diet fed	285.10±14.63	281.03±15.07	357.00±18.25	442.19±21.20	316.16±16.40	309.00± 16.11
Group II D (6) diets containing 12% water soluble gummy fiber fed	132.00**±8.04	130.00**±7.33	243.00**±9.80	256.00**±14.00	165.00**±9.92	139.00**±9.90
Group III D (6) diets containing 18% water soluble gummy fiber fed	125.00***±7.00	126.00***±6.92	230.00***±9.00	240.00 ***±12.52	163.00***±9.65	132.00**±7.61
Group IV D (6) diets containing 5% water insoluble neutral detergent fiber fed	280.25**±15.16	277.00 <sup>NS</sup> ±12.76	356.00 <sup>NS</sup> ±17.49	438.00 <sup>NS</sup> ±19.91	313.00 <sup>NS</sup> ±16.00	305.00 <sup>NS</sup> ±15.89
Group V D (6) diets containing 10% water insoluble neutral detergent fibre fed	280.00 <sup>NS</sup> ±15.01	277.05 <sup>NS</sup> ±12.80	355.00 <sup>NS</sup> ±17.00	438.00 <sup>NS</sup> ±19.90	308.00 <sup>NS</sup> ±15.95	298.00 <sup>NS</sup> ±15.04

The results presented in table are means ± SD of 6 rats each. Number of animals used are given in parenthesis. The data were analyzed statistically by student t-test. The blood glucose levels of normal and diabetic treated rats at 0, 1/2, 1, 1 1/2, 2 hrs. compared with corresponding glucose levels at 0, 1/2, 1, 1 1/2, and 2 hrs of normal and diabetic control rats after glucose loading for statistical analysis. p\*\*< 0.01 . \*\*\*p< 0.001. NS Not Significant



weight) to the animals of all groups and blood samples were collected for glucose estimation at 0, ½, 1, 1 ½ and 2 hours after the administration of glucose by glucose oxidase method (Pandey and Khan, 2002). The experiments were conducted in accordance with the internationally accepted principles for laboratory animals.

## Results and Discussion

In previous study, feeding of diets containing 6% water soluble gummy fibre isolated from *Syzygium cumini* seeds significantly reduced the blood glucose, improved the glucose tolerance (Pandey and Khan, 2002) and significantly decreased the activity of intestinal enzymes *i.e.* amylase and invertase in both diabetic and normal rats when

compared with their respective controls while feeding of diets containing 2.25% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seed diets did not show any hypoglycaemic effect (Pandey and Khan, 2002). Feeding of diets containing 12% and 18% water soluble gummy fibre isolated from *Syzygium cumini* seed resulted in more decrease in blood glucose level and more improvement in glucose tolerance than the feeding of diet containing 6% water soluble gummy fibre isolated from *Syzygium cumini* seeds is observed by the result of our study (Table 2). A significant decrease in the activity of intestinal enzymes *i.e.* amylase and invertase was observed in the normal and diabetic rats fed the diets containing 12% and 18% water soluble gummy fibre

**Table 3: Effect of feeding of diet containing water soluble gummy fibre and water insoluble neutral detergent fibre isolated from *Syzygium cumini* for 21 days on intestinal enzymes in normal and alloxan treated diabetic male albino rats**

Group	$\alpha$ -Amylase (U/l)	Total invertase (U/mg protein)
<b>Normal</b>		
Group I N (6) control diet fed	5.449 $\pm$ 0.486	0.135 $\pm$ 0.051
Group II N (6) diets containing 12% water soluble gummy fibre fed	3.505*** $\pm$ 0.367	0.086*** $\pm$ 0.032
Group III N (6) diets containing 18% water soluble gummy fibre fed	2.338*** $\pm$ 0.247	0.056*** $\pm$ 0.021
Group IV N (6) diets containing 5% water insoluble neutral detergent fibre fed	4.885 <sup>ns</sup> $\pm$ 0.430	0.140 <sup>ns</sup> $\pm$ 0.056
Group V N (6) diets containing 10% water insoluble neutral detergent fibre fed	3.982 <sup>ns</sup> $\pm$ 0.383	0.148 <sup>ns</sup> $\pm$ 0.056
<b>Diabetic</b>		
Group I D (6) control diet fed	5.943 $\pm$ 0.507	0.149 $\pm$ 0.020
Group II D (6) diets containing 12% water soluble gummy fibre fed	3.602** $\pm$ 0.377	0.079** $\pm$ 0.010
Group III D (6) diets containing 18% water soluble gummy fibre fed	2.891*** $\pm$ 0.305	0.039*** $\pm$ 0.005
Group IV D (6) diet containing 5% water insoluble neutral detergent fibre fed	4.874 <sup>ns</sup> $\pm$ 0.429	0.144 <sup>ns</sup> $\pm$ 0.019
Group V D (6) diets containing 10% water insoluble neutral detergent fibre fed	4.117 <sup>ns</sup> $\pm$ 0.395	0.150 <sup>ns</sup> $\pm$ 0.020

The results presented in table are means  $\pm$  SD of 6 rats each. Numbers of animals used are given in parenthesis. The data were analyzed statistically, by student's 't' test. The activity of intestinal enzymes,  $\alpha$ -amylase and total invertase of normal and diabetic treated rats compared with the corresponding activities of intestinal enzymes,  $\alpha$ -amylase and total invertase of normal and diabetic control rats for statistical analysis. \*\*P<0.01, \*\*\*P<0.001





isolated from *Syzygium cumini* seeds where as 5% and 10% water insoluble neutral detergent fibre (NDF) did not exhibit any hypoglycaemic effect shown in (Table 3).

The amount of water soluble gummy fibre is very high i.e. (40 g %) in *Syzygium cumini* seeds and viscosity of water soluble gummy fibre i.e. (14 centipoises) is very nearer to that of guar gum i.e. (16 to 20 centipoises) which has been shown to be gummy fibre (Jenkins *et al.*, 1977). Our observation confirmed by guar gum studies. The guar gum has been reported to reduce serum levels of glucose of both in normal and diabetic animals and human beings (Anderson and Chen, 1979 and Anderson and Clark, 1986). It has been demonstrated that viscous guar gum and water soluble gummy fibre isolated from *Syzygium cumini* seed caused more impairment in glucose diffusion than the less viscous gum Arabica.

Our observation that increase in the amount of water soluble gummy fibre caused enhancing hypoglycaemic effect is mainly due to factors like impaired gastric emptying or decreased intestinal transit time which may be described to physical property of the water soluble gummy fibre i.e. viscosity. It was confirmed by in vitro dialysis bag experiment that increase in the amount of guar gum and water soluble gummy fibre from *Syzygium cumini* seeds caused more impairment of glucose diffusion due to increased viscosity. In vitro dialysis bag experiment, impairment in glucose, diffusion in presence of water soluble fibre isolated from *Syzygium cumini* seeds has been correlated to its viscosity (Morgan, 1979). Viscosity has a considerable on absorption and transit time in that guar gummy fibre, and the most viscous substance was more effective in decreasing post prandial glucose and insulin concentration. This was confirmed by the fact that the destruction of the viscous character of guar by hydrolysis prevented these actions. The two fold actions of viscous agents may depends on both delayed gastric emptying and delayed absorption of glucose from the small intestinal lumen (Nuttall, 1993). The increase in the

sugar at 2 hrs and its correlation with viscosity may be another factor of slower absorption. In other words, the enhanced flattening of oral glucose tolerance curve observed in the rats fed diets containing 12% and 18% in both normal and diabetic treated rats in our study may be considered an another factor of slower glucose absorption caused by viscosity of fibre. Other factors such as a changed hormonal background and the gut endocrine system play an important role in reducing blood glucose concentration. GIP and GLI, i.e. gastric inhibitory polypeptide and gut glucagon like immuno reactivity, these two gastric hormones markedly stimulate the release of insulin in the presence of glucose, were not carried out in our study but their corelationship with gummy fibre in the animals fed gummy fibre has been established as also reported by Ribes *et al.*, 1984 and Vohouny and Washington, 1981. They are released from the gut in response to glucose and may be regulated by the rate of absorption of glucose (Williams and James, 1979).

Reduced level of blood glucose observed in our study in treated rats may partly be attributed to the decreased activity of intestinal enzymes i.e. amylase and invertase, which are responsible for digestion of carbohydrates and are trapped in the gummy gel which reduces their accessibility for the substrates resulting in the slowing down of the absorption of end product i.e. glucose.

In conclusion, the present study clearly indicates the enhancing hypoglycaemic effect of the water soluble gummy fibre isolated from *Syzygium Cumini* seeds and not due to water insoluble neutral detergent gummy fibre [NDF] or other constituents isolated from *Syzygium cumini* seeds.

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## Occurrence of small free-living amoebae from natural water resources

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### Abstract

Small free-living amoebae are also called as amphizoic amoebae, because of their capability to exist both as free-living as well as opportunistically pathogenic. Some of the species of *Naegleria* and *Acanthamoeba* are known to cause fatal disease (Primary Amoebic Meningoencephalitis= PAM and Granulomatous Amoebic Encephalitis= GAE) of central nervous system affecting brain and amoebic keratitis affecting eyes. The purpose of our study was to find out whether small free-living amoebae were also found in natural resource of water such as river and pond from Lucknow region. A total 68 samples of water were collected from six different sites of Gomti River and four different ponds from Lucknow city. A total 38 samples were found to be positive for small free-living amoebae. The most common amoebae present were species of *Naegleria*, *Schizopyrenus*, *Acanthamoeba*, *Hartmannella* and *Vannella*. Out of total 47 water samples from Gomti River, 27 samples were positive for amoebae and out of 21 samples of ponds examined 11 were found to be positive. The occurrence of these amoebae in natural water sources such as river and pond in Lucknow region poses a threat to human being for meddling with water without taking adequate care. Since the pathogenic strains of amoebae are known to infect human being via nasal route during swimming and/or via cut in the skin/ body via haematogenous route. Exposure of eyes with contaminated water may cause amoebic keratitis in human being. A great awareness among masses is essential to educate about these new amoebic disease, their possible preventive measures and occurrence of these pathogenic amoebae from natural water resources.

**Keywords:-** Free-living, Amphizoic, *Naegleria*, *Acanthamoeba*, PAM, GAE

### Introduction

Small free-living amoebae are widely distributed in the human environment (soil, water and air). These amoebae have been isolated from various natural water sources such as rivers, pond, lake etc. (Kasprzak and Mazur, 1974 and Jonckheere, 1981), from thermal acidic stream (Sheehan *et al.*, 2003), from artificial well (Shenoy *et al.*, 2002) and from domestic tap water (Kilvington *et al.*, 2004, Pandey and Sharma, 2006).

These amoebae are called "amphizoic" because of their capability to exist both as free-living as well as opportunistically pathogenic (Page, 1974). People probably have more frequent contact with these organisms particularly with their resistant cysts and

temporary amoeboid-flagellate stage (Martinez and Visvesvara, 1997). The species of *Naegleria*, *Acanthamoeba* and *Balamuthia* have been identified as opportunistic pathogens of human beings and other domestic animals and are known to cause a spectrum of infections in immunocompromised individuals including persons with AIDS (Denny *et al.*, 1997 and Sison *et al.*, 1995).

Occurrence of Primary Amoebic Meningoencephalitis (PAM) has also been reported from India. Pan and Ghosh (1971) from Calcutta, first reported the occurrence of PAM in two children. Third case was reported by Bedi *et al.* (1972) from Udaipur, Rajasthan. One more case has been reported, in an infant of five months old from Manglore, south India (Shenoy *et al.*, 2002).

Recently, Jain *et al.* (2002) from Chandigarh also reported survival of a patient after the infection of

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*Naegleria*. Recently, Kaushal *et al.* (2008) reported one more case of primary amoebic meningoencephalitis from Ludhiana, who had the history of taking bath in the village pond. Till date a total of nine cases have been reported between 1971- 2008 from India. More than 200 cases of PAM have been reported so far abroad and most of them are from America. This may be because of the greater awareness about these diseases rather than the incidence. The aim of our investigation was to detect these amphizoid amoebae from aquatic environment used by human being and to know the distribution of the pathogenic and non-pathogenic free-living amoebae in natural water resources.

### Materials and Method

The sampling sites included Gomti River at different point and pond of different localities in ten parts of Lucknow city. Using sterile 500 ml screw-cap bottle, 200 ml surface water samples were collected at proposed collecting sites. Water temperature and pH were recorded at the sampling sites. The water samples were filtered through a sterile filter paper using sterile conical funnel. The residue was collected in the inner side of the cone of filter paper and the 1.00 cm cone of this filter paper was cut by sterilized scissors and was placed in the center of non-nutrient agar petri-dish plates already pre-seeded with *Escherichia coli* (as food for amoebae) keeping the inner side of paper downward. These plates were incubated at 37.00 °C for 8-10 days or longer for the growth of amoebae for their detailed study and biological characterization.

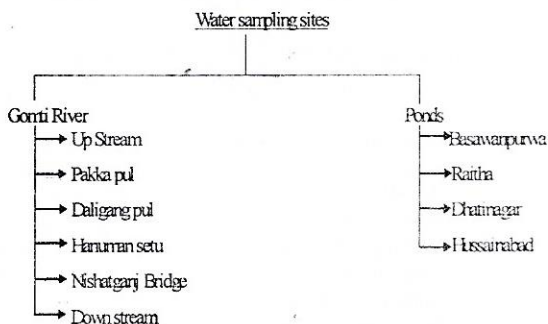
#### Amoeba Identification

Amoebae were identified on the morphological basis of trophozoites, cysts and flagellates following Singh (1985) and the latest classification of protozoa (Levine *et al.*, 1980).

### Results and Discussion

All the samples were collected throughout the year. Total 68 samples were collected from different points of Gomti River and various ponds of

### The Experimental design of water sampling sites is giving below



All samples were collected throughout the year

Lucknow city and surrounding areas. The pond water samples were collected from the villages of Bakshi-Ka-Talab area of Lucknow. Out of total 68 water samples, 38 samples were found to be positive for small free-living amoebae (57%). Out of total 47 samples from river, 27 were positive (57%) and out of 21 samples of pond examined, 11 samples were found to be positive for amoebae (52%) (Fig. 1 and 2). The water temperature recorded was in the range of 29.00 °C-37.00 °C and pH from 5.00 to 7.00. *Acanthamoeba* strains and other free-living amoebae could be recovered from pH range of 5.00 to 7.00 and temperature range (27.00 °C to 35.00 °C) of water, where as *Naegleria* strains were found in the water temperature from 28.00 °C to 35.00 °C and at the pH range of 6.50-7.00.

In our investigation, the strains of free-living amoebae isolated from different water samples were identified as different strains and species of *Naegleria*, *Acanthamoeba*, *Schizopyrenus* and *Hartmannella* (Plate-1 and 2).

The identification of the amoebic isolates was done following their detailed biological characterization (studying the morphology of cysts and trophozoite, locomotion, nuclear division and pathogenicity) using the method of Singh, 1985 (Table-1 and Plate-





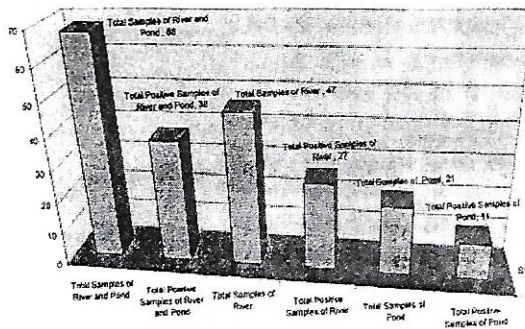


Fig. 1: Number of positive sample from river and ponds in ten different site of Lucknow

1 and 2). In pathogenicity test using *Albino* mice (weight approximately 12 gm) the strain (R-4) of *Naegleria fowleri* was found to be highly pathogenic. The occurrence of small free-living amoebae from water sources has also been reported from different parts of India and abroad (Singh and

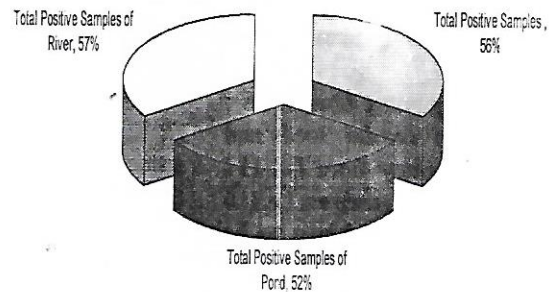


Fig. 2: Percentage of positive water samples for free-living amoebae from river and pond of Lucknow

Das, 1972a; Gogate *et al.*, 1984; Pandey and Sharma, 2006). Human infection by *Naegleria* and *Acanthamoeba* has been reported world wide (Nacapunchai *et al.*, 1999). The environmental sources of amoebic isolates range from air to water and to soil (John and Howard, 1995).

Table-1: Isolation of amphizoic amoebae from water resources (River and pond) in Lucknow

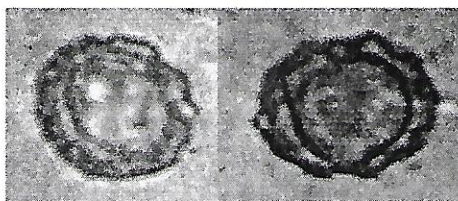
Site name	Site code	Water sample		A m o e b a
		Total sample	Positive sample	
River and Pond				
Up-stream	R - 1	9	6	A , N , H
Pakka pul	R - 2	5	3	A , N , V n
Daligang pul	R - 3	6	3	A , V , H
Hanuman setu	R - 4	12	7	A , N , S c z
Nishat ganj Bridge	R - 5	9	5	A , N , H , V n
Down -stream	R - 6	6	3	N , A
Basawan Purwa pond	P - 1	5	3	A , N , H
Raith pond	P - 2	7	4	A , N , V n
Dhatingara pond	P - 3	5	2	A , N , S c z
Hussainabad pond	P - 4	4	2	A , N , S c z
Total		68	38	A , N , S c z , H , V n

Note: N = *Naegleria*, Scz = *Schizopyrenus*, A = *Acanthamoeba*, Vn = *Vannella*, H = *Hartmannella*



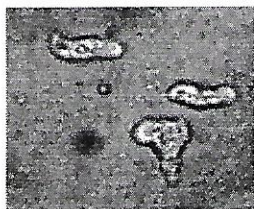


**Cyst**

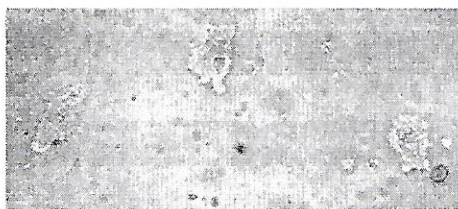


**Trophozoites**

*Naegleria spp.*



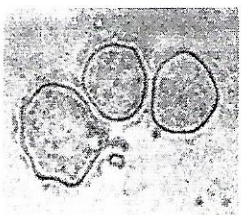
**Cyst**



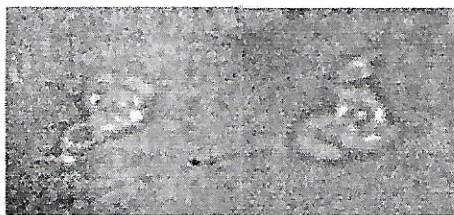
**Trophozoites**

*Acanthamoeba spp.*

**Plate no. 1**

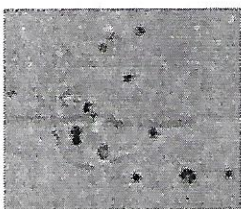


**Cyst**

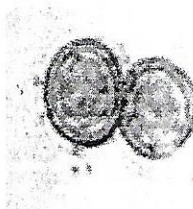


**Trophozoites**

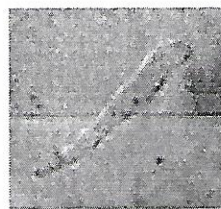
*Schizopyrenus spp.*



**Trophozoites**  
*Vanella*



**Cyst**



**Trophozoites**  
*Hartmannella spp.*

**Plate no. 2**

Our results are inconformity with that of specific characteristic of amoebae isolates by other workers (Pandey and Sharma, 2006; Singh and Das, 1972a, Martinez *et al.*, 1997 and Rohr *et al.*, 1998).

## Conclusion

These finding serve as additional evidence for the presence of either pathogenic or non-pathogenic free-living amoebae responsible for human diseases under natural conditions, therefore, these results may have profound implication with regard surveillance of water system for amoebae and especially for the prevention of diseases, either directly or indirectly. A greater awareness among masses is essential to educate about the new amoebic diseases and occurrence of these pathogenic amoebae in natural water sources like rivers, ponds, lake etc.

## Acknowledgement

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## Some additional records of follicolous fungi from North Central Tarai Forests of U.P.

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### Abstract

The follicolous fungi was collected from Feb. 03-05, 2008 from Sirsa forest range of Shrawasti District. The authors collected twenty plant species, representing fourteen families, parasitized by sixteen fungi species. *Leptoxylum buteae* was found on *Butea frondosa* (Fabaceae), where as *Pseudocercospora nigricans* on *Cassia occidentalis* (Fabaceae); *Pseudocercospora* sp. on *Hetrofragma* sp (Boraginaceae) *Alternaria* on *Achyranthes aspera* (Amaranthaceae), *Syzygium* sp. (Myrtaceae) and *Corchorus olitoris* (Tiliaceae); *Corynespora* on *Lantana* in (Verbenaceae), *Croton roxburghii* (Euphorbiaceae), *Clerodendron* sp. (Verbenaceae); *Sirosporium lantanae* on *Lantana camera* (Verbenaceae); *Sirosporium* sp. on *Carica papaya* (Caricaceae); *Stenella tectonic* on *Tectona grandis* (Verbeaceae); *Stenella* sp. on *Eucalyptus lanculatus* (Myrtaceae); *Stenella grewiae* on *Grewia elastica* (Tiliaceae); *Cercospora* sp. on *Galycosmis pentaphyla* (Rutaceae), *Corchorus olitorius* (Tiliaceae); *Meliola* sp. on *Streblus asper* (Moraceae); *Astrostromella* on *Litsea chinensis* (Lauraceae); *Acrodytis* sp. on *Tinspora malaverica* (Menispermaceae); *Passalora* sp on *Eopatarium cannabinum* (Asteraceae); *Oidium* sp on *Syzygium* sp. (Myrtaceae) and *Coccinia indica* (Cucurbitaceae).

**Keywords:-** Follicolous fungi, Ethnomedicine

### Introduction

The leaves provide a very suitable habitat for the growth and development of fungal pathogens by providing ample surface area and nutrient supply. Such leaf inhabiting fungi are known as follicolous fungi and invaded area of the leaf appear as leaf spot or leaf lesions. Taxonomic studies of such fungal forms have been generally considered as only of academic interest, taxonomic treatment of a fungal organism is the first requirement for any studies concerning its biology. Correct identification of a fungus absolutely free from ambiguities is vital for its employment in applied disciplines. Infact without being equipped for ascertaining the correct identity of a fungul pathogen all studies concerning its phytopathological aspects would be misleading. The weed and forest plants serve

as reservoirs of leaf spot pathogens which on getting opportunity may spread to agriculture and horticulture plants keeping this in view the author surveyed the Sirsia forest range of Shrawasti District on February, 03-05, 2008.

### Materials and Method

During collection, infected leaf samples were taken in separate polythene bags. Suitable amounts of suface scrapping and free hand cut sections were prepared from infected portions of the leaf samples. Slides were prepared in cotton-blue lactophenol mixture, slides were examined and *Camera lucida* drawings were made which seems to be new. Morphotaxonomic determinations of taxa were done with the help of current literature and resident expertise available. All the fungal taxon were identified after making microscopic preparations and later confirmed by Prof. Kamal, Emeritus Scientist (DST), DDU Gorakhpur University, Gorakhpur.

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## Results and Discussion

The author surveyed the Sirsia forest range of Shrawasti District on Feb. 03-05, 2008 so as to collect the follicolous fungi. The authors collected twenty plant species, representing fourteen families, parasitized by sixteen fungi species. *Leptoxylum buteae* was found on *Butea frondosa* (Fabaceae), where as *Pseudocercospora nigricans* on *Cassia occidentalis* (Fabaceae); *Pseudocercospora* sp. on *Hetrofragma* sp (Boraginaceae) *Alternaria* on *Achyranthes aspera* (Amranthaceae), *Syzygium* sp. (Myrtaceae) and *Corchorus olitoris* (Tiliaceae); *Corynespora* on *Lantana* in (Verbenaceae), *Croton roxburghii* (Euphorbiaceae), *Clerodendron* sp. (Verbenaceae); *Sirosporium lantanae* on *Lantana camera* (Verbenaceae); *Sirosporium* sp. on *Carica papaya* (Caricaceae); *Stenella tectonic* on *Tectona grandis* (Verbeaceae); *Stenella* sp. on *Eucalyptus lanculatus* (Myrtaceae); *Stenella grewiae* on *Grewia elastica* (Tiliaceae); *Cercospora* sp. on *Glycosmis pentaphylla* (Rutaceae), *Corchorus olitorius* (Tiliaceae); *Meliola* sp. on *Streblus asper* (Moraceae); *Astrostromella* on *Litsea chinensis* (Lauraceae); *Acrodytis* sp. on *Tinospora malaverica* (Menispermaceae); *Passalora* sp. on *Eopatarium cannabinum* (Asteraceae); *Oidium* sp. on *Syzygium* sp. (Myrtaceae) and *Coccinia indica* (Cucurbitaceae).

The literature (Bilgrami *et al.*, 1981; 1991; Goos and Hosagoudar, 1998; Hosagoudar and Goos, 1990; Hosagoudar 1996; Hosagoudar *et al.*, 1997; Hosagoudar and Abraham 1998; Hosagoudar *et al.*, 2007; Jamaluddin *et al.*, 2004; Jana *et al.*, 2005; and Singh and Mall, 2007) reveals that all fungal taxon has not been reported from north central tarai forest of Uttar Pradesh.

The follicolous fungal pathogens interfere with the manufacturing rate of food and other valuable substances by damaging the photosynthetic elements of living leaves, bringing about qualitative and quantitative damage in the living tissue of the host in various ways. Several leaf spot pathogens

are known to produce toxins of the various kinds. When the leaf spots are numerous or large, there is a considerable reduction in the photosynthetic area of the leaf. Some times rapid defoliation occurs due to such infections. The productivity of the host plants is reduced. The weeds and forest plants serve as reservoir of leaf spot pathogens which may also spread to agriculture and horticultural plants. The destruction caused by these enemies of leaves is serious problem because they also cause degradation of quality of ethnomedicine present therein.

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## Effect of Bio-Pesticide (Agroneem) on the biochemistry of kidney of *Clarias batrachus*

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### Abstract

In the present study authors studied the effect of bio-pesticide (Agroneem) on the biochemistry of kidney of *Clarias batrachus*. The biochemical parameters studied were RNA, total protein, acid phosphatase, alkaline phosphatase, GOT and GPT. These parameters in the experimental animal were found decline. The order of toxicity of Agroneem to biochemical parameters from higher to lower were- acid phosphatase (-78.623%)< total protein (-78.078%)< alkaline phosphatase (-70.594%)< RNA (-44.049%)< GPT (-39.618%)< GOT (-29.948%). Thus on basis of obtained results in the present investigation it can be concluded that 96 hrs. exposure of 30 ppm of Agroneem aqueous solution has toxic effect and alter the biochemistry of kidney. Therefore, it is recommended to the user of this bio-pesticide that they should be careful about the dose they are using.

**Keywords:-** Bio-pesticide, Biochemistry, Toxicity, *Clarius batrachus*

### Introduction

Biological pesticides vary in their toxicity and in their potential ecological impact. They are relatively non-toxic to people with few side effects. Agroneem (Azadiractin) is a Neem seed based bio-pesticide. It is very complex tetranotriterpenoid obtained from the seed. It is considered relatively non-toxic and it is not likely to accumulate or cause long-term effects (Miller and Uetz, 1998).

Very few workers have studies toxicity of neem products to fish species. Attri and Prasad (1980) have reported toxic concentration of neem extract to fish and frog tadpoles. Deshmukh and Periyal (1992) observed acute toxicity of Neemark to fish *Tilapia mossembica*. Wan *et al.* (1996) have described 96 hrs.  $LC_{50}$  value of Azadiractin to juvenile salmon. Consulted literature showed that Azadiractin (Agroneem) effects the biochemistry of various organs of fishes, were not extensively investigated. Therefore, present study was under taken to investigate the Azadiractin induced change in the biochemistry of kidney of *Clarias batrachus*.

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### Materials and Method

#### Experimental animal

Healthy *Clarias batrachus* were purchased from local fish market and acclimatized to the laboratory conditions for one week, during which they were regularly fed with Prawn powder and Soya meal.

#### Experimental design

In the present investigation experimental fishes were divided into two groups.

- (1) Control group: - In this group 15 fishes were kept and exposed to normal distilled water.
- (2) Experimental group: - In this group 15 fishes were exposed to 30.00 ppm Agroneem solution.

**Experimental duration:** In both control and experiment group fishes were exposed to maximum 96 hrs.

**Autopsy:** Fishes of control and experimental groups were scarified at 0 hrs., 12 hrs., 24 hrs., 48 hrs., 72 hrs., and 96 hrs. The kidney were removed, blotted, weighed and then processed for various biochemical assay.

**Biochemical analysis:** Following standard biochemical methods were used, which are described in the laboratory manual in biochemistry (Jayaraman, 2000).



- (1) Extraction and estimation of RNA.
- (2) Estimation of total protein (TP) by Biuret method.
- (3) Determination of alkaline phosphatase (ALP) by King and King method.
- (4) Determination of acid phosphatase (ACP) by King and King method.
- (5) Estimation of activity of glutamate oxaloacetate transaminase enzyme (GOT).
- (6) Estimation of activity of glutamate pyruvate transaminase enzyme (GPT).

### Results and Discussion

**RNA content:** The total content of RNA in the kidney of *Clarias batrachus* was found to be

**Table 1: RNA changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**

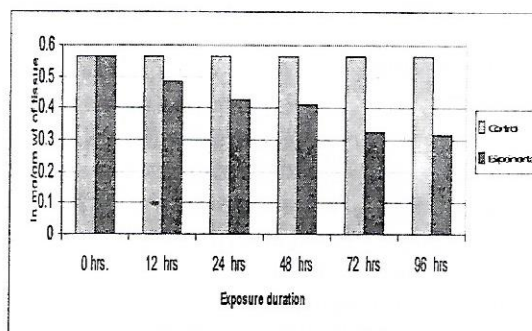
Exposure duration in hrs.	RNA content in mg/gm wt. of tissue		Difference	% alter
	Control	Experimental		
0	0.563	0.563	0.00	0.00
12	0.563	0.418	0.082	14.065
24	0.563	0.427	0.136	24.156
48	0.563	0.414	0.149	26.465
72	0.563	0.326	0.177	31.438
96	0.563	0.315	0.248	44.049

respectively. The kidney of experiment animals showed decrease in TP activity, which was gradual and exposure duration dependent. The total protein content decrease 78.078 per cent after 96 hrs.

**Alkaline Phosphatase (ALP):** The content of ALP in the kidney of *Clarias batrachus* was also observed decreased gradually after the exposure of 30 ppm Agroneem. The normal value of ALP content in control animal was observed 7.230 KA units/100 ml of kidney, which after 12, 24, 48, 72 and 96 hours of exposure reduced to 7.142, 5.763, 4.213, 2.376 and 2.126 KA units/100 ml respectively. This experiment showed the inhibition ALP upto 70.564

decreased gradually after the exposure of 30 ppm Agroneem. The normal value of RNA content in animal was observed 0.563 mg/gm wt. of kidney which after Agroneem exposure of 12, 24, 48, 72 and 96 hours reduced to 0.481, 0.427, 0.414, 0.386 and 0.315 mg/gm of tissue wt. respectively. In the experiment total 44.049 per cent inhibition in RNA activity was observed after 96 hrs.

**Total protein (TP):** The total protein in the kidney of *Clarias batrachus* was found decreased gradually in experiment group. The normal value of total protein content in control animal was observed 4.726 mg/gm wt. of kidney which after 12, 24, 48, 72 and 96 hours exposure of biopesticide reduced to 4.519, 2.187, 2.147, 1.122 and 1.036 mg/gm wt. of tissue



**Fig. 1: RNA changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**

per cent in just 96 hrs of duration. The inhibition in the enzyme activity was gradual and exposure duration dependent.

**(4) Acid phosphatase (ACP):** The content of Acid phosphatase in the kidney of *Clarias batrachus* was found decreased gradually upto 96 hrs after the exposure of 30 ppm Agroneem. The control value of Acid phosphatase was observed 6.437 KA units/100 ml in kidney, which after 12, 24, 48, 72 and 96 hours exposure of test solution, reduced to 6.125, 5.629, 4.326, 2.479 and 1.376 KA units/100 ml respectively. The inhibition of Acid phosphatase activity in 96 hrs was 78.623 per cent.



**Table 2: Total protein change in kidney *C. batrachus* exposed to 30 ppm Agroneem**

Exposure duration in hrs.	Total protein in mg/gm wt. of tissue		Difference	% alter
	Control	Experimental		
0	4.726	4.726	0.00	0.00
12	4.726	4.519	0.207	4.380
24	4.726	2.187	2.539	53.724
48	4.726	2.147	2.577	54.528
72	4.726	1.122	3.604	76.258
96	4.726	1.036	3.690	78.078

**Table 3: Alkaline  $PO_4^{2-}$  change in kidney *C. batrachus* exposed to 30 ppm Agroneem**

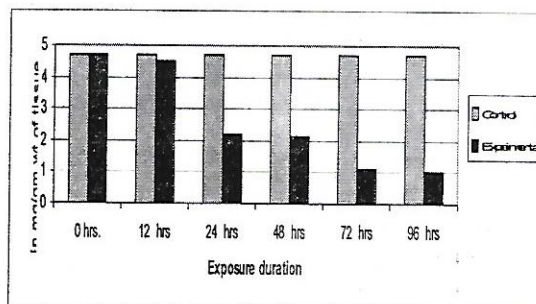
Exposure duration in hrs.	Alkaline phosphatase content in KA unit/100 ml		Difference	% alter
	Control	Experimental		
0	7.230	7.230	0.00	0.00
12	7.230	7.142	0.088	1.217
24	7.230	5.763	1.467	20.290
48	7.230	4.213	3.017	41.728
72	7.230	2.376	4.854	67.136
96	7.230	2.126	5.104	70.564

**Table 4: Acid  $PO_4^{2-}$  changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**

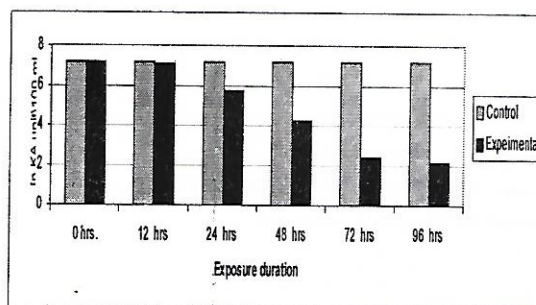
Exposure duration in hrs.	RNA content in mg/gm wt. of tissue		Difference	% alter
	Control	Experimental		
0	6.437	6.437	0.00	0.00
12	6.437	6.126	0.312	4.847
24	6.437	5.629	0.808	12.552
48	6.437	4.326	2.111	37.794
72	6.437	2.479	3.958	61.488
96	6.437	1.376	5.061	78.623

**GOT:** The content of GOT in the kidney of *Clarias batrachus* was decreased very slowly and gradually after the exposure of 30 ppm Agroneem. The control value of GOT was observed 0.975 mg/gm in the kidney of experimental fish, which after 12, 24, 48, 72 and 96 hours of exposure decreased to 0.927,

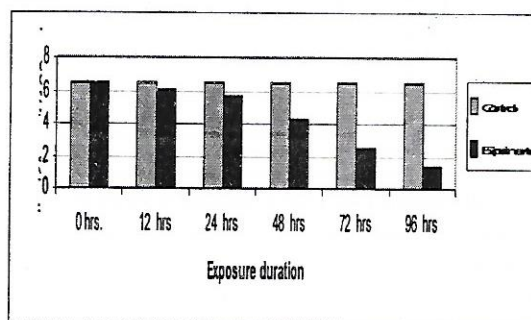
0.856, 0.804, 0.742 and 0.683 mg/gm respectively. This experiment showed that inhibition of GOT was very low in comparison to RNA, Protein, Acid and Alkaline phosphatase as the value was found decrease only 29.948% in 96 hrs of exposure of studied biopesticide chemical.



**Fig. 2: Total protein changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**



**Fig. 3: Alkaline  $PO_4^{2-}$  changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**



**Fig. 4: Acid  $PO_4^{2-}$  changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**



**GPT :** The content of GPT in the kidney of *Clarias batrachus* was also decreased gradually after the exposure of 30 ppm Agroneem. The control value of GPT content was observed 0.313 KA units/100 ml of kidney, which after 12, 24, 48, 72 and 96 hours exposure reduced to 0.287, 0.284, 0.239, 0.211 and 0.189 KA units/100 ml respectively. GPT also showed very less reduction in 96 hrs in term of per cent i.e. 39.616%. This finding was closer to GOT finding in contrast to RNA, Protein, Acid and Alkaline phosphatase.

**Table 5: GOT changes in kidney *C. batrachus* exposed to 30 ppm Agroneem**

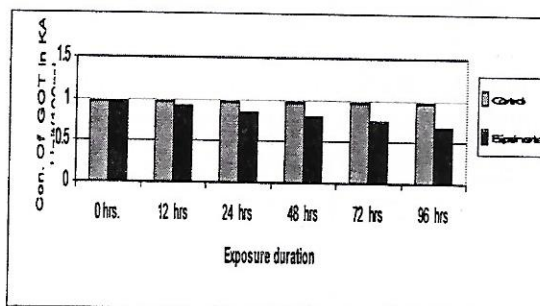
Exposure duration in hrs.	Total protein in mg/gm wt. of tissue		Difference	% alter
	Control	Experimental		
0	0.975	0.975	0.00	0.00
12	0.975	0.927	0.048	4.923
24	0.975	0.856	0.119	12.205
48	0.975	0.804	0.171	17.538
72	0.975	0.742	0.233	23.897
96	0.975	0.683	0.292	29.948

**Table 6: GPT change in kidney *C. batrachus* exposed to 30 ppm Agroneem**

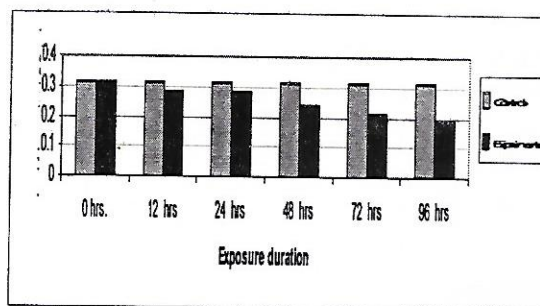
Exposure duration in hrs.	Alkaline phosphatase content in KA unit/100 ml		Difference	% alter
	Control	Experimental		
0	0.313	0.313	0.00	0.00
12	0.313	0.287	0.026	8.306
24	0.313	0.284	0.029	9.265
48	0.313	0.239	0.074	23.642
72	0.313	0.211	0.102	32.587
96	0.313	0.189	0.124	39.616

Attri and Prasad (1980) have reported toxicity of neem extracts to fish and frog tadpoles. Deshmukh and Periyal (1992) have observed acute toxicity of neem to fish, *Tilapia mossambica*. Wan *et al.* (1996) have studied 96 hrs LC<sub>50</sub> values of Azadirachtin to juvenile of *Salmon*. Farah *et al.* (2006) described that neem extract exhibit

strong antimutagenic activity in fish, *Channa punctatus*. Gandhi *et al.* (1988) documented acute toxicity of neem oil in rats and rabbits and described dose related pharmacotoxic symptoms with a number of changes in the biochemical and histopathological indices of toxicity. Kasutri *et al.* (1997) reported that oral administration of 20, 40, 60 mg of dry *Azadirachta indica* leaf powder for 24 days resulted in decrease in the weight of seminal vesicle, nuclear diameter and the secretory material in the lumen. Biochemically they observed decrease in total



**Fig. 5: GOT changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**



**Fig. 6: GPT changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem**

protein, acid phosphatase activities. Kanungo (1996) also observed neem extract as hepato-toxic and nephrotoxic to poultry birds. Rahman *et al.* (2001) also reported that a neem based pesticide (Vepacide) altered the biochemical profile of serum, kidney and lungs of Albino Wistar rats. In the present investigation Agroneem (30 ppm) exposure



for 96 hrs to *Clarias batrachus* was found toxic as it altered rather decreased all the studied biochemical parameters (RNA, TP, ALP, ACP, GOT and GPT) of kidney and thus support the observation of previous authors. Therefore it is recommended to the user of this bio-pesticide that they should be careful about the dose they are using.

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## Environmental ethics: An essence for environmental protection

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### Abstract

With the rapid and unplanned industrialization the human society is facing different environmental problems due to variety of destructing activities. There is an urgent need to stop further degradation of nature or at least keep it under control. We have to decide and choose the right code of conduct which calls the human conscience and environmental ethics. Environmental ethics are our beliefs about our social, moral and scientific behavior towards the nature concerning what is right or what is wrong. There are several approaches to environmental ethics. Present paper deals with certain principles which are recognized as environmental ethics. Some ethical justifications are discussed with reference to moral sense, spiritual thinking and practical utility on the grounds of present need and the future of our next generation.

**Keywords:-** *Environmental degradation, Environmental ethics, Ethical theories, Global problems*

### Introduction

As the human society is entering the third millennium, new reports are pointing us regarding our fragile environment, vanishing forests, depleting resources, dwindling floral and faunal diversity and endangered state of the planet earth. In last five billion years of existence of our green planet earth, it has witnessed bombardments by meteors, sudden changes in its magnetic fields, drifting of landmasses, abrupt changes in the continents, reshaping of ice caps and glaciers and other variety of changes. The life appeared three and a half billion years ago on the earth has proved flexible. Many individual species appeared and vanished but the life has persisted without much interruption, against many powerful physical and chemical forces and interactions (Silver and Defries, 1991).

The activities of human being are exploiting the nature and disrupting the natural rhythms of earth's ecosystem. Though these destructing activities cannot completely suppress the earth's natural system, certainly can affect it significantly through various activities like energy use, release of

pollutants, exploitation and overexploitation of natural resources and other industrial activities. Due to drastic and dramatic increase in various such regular and repeated polluting and destructing activities, many local, regional and global environmental problems are experienced. The consequences of such activities are expanding to a global scale in the form of global environmental problems like ozone depletion, loss of biodiversity, acidification of rain, global warming and green house effect. The cumulative effect of all these problems is catastrophic for certain species and making the life of mankind miserable on earth (Cunningham and Cunningham, 2003).

There is an urgent need to control the consequences of all these adverse activities. We need to stop degradation of nature or at least keep under control. But the question is how to bell the cat? Who is to bell the cat? Certainly, if do not do it today, we will have to suffer. The question is whether to flourish the nature or perish the nature. Anyway, we have to control the degradation by limiting our activities and damaging trends of development (Agarwal and Dubey, 2002). We have to decide and choose the right code of conduct

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and regulate human activities and behaviors towards the nature so that the quality of life will not deteriorate further. The future generations will live without compromised lifestyle. The only way is to call the human conscience and the device and follow environmental ethics.

### Concept of Ethics

A set of guiding principles which appeal human conscience about our ethical convictions about good and bad, the right and wrong is often and effective way to control unmanageable environmental problems. According to a naturalist Aldo Leopold any decision of a conscious human mind is right when it tends to preserve the integrity, stability and beauty of the nature preferring the biotic community and any decision of a conscious human mind is wrong when it tends contradictory to it, even after understanding it. We care the nature for better human health, welfare and our concern about the environment. The environmental ethical principles are practiced with self imposed restraints by human being. These are therefore the morally acceptable behaviors or mannerisms. Environmental ethics are our beliefs about our social, moral and scientific behaviors towards the nature concerning what is right or what is wrong. These are acceptable codes of conduct. Such moral reflects feelings and ethics reflects actual activity.

Environmental ethics is the branch of knowledge concerned with moral principle. It is a branch of philosophy dealing with right and wrong with nature regardless to cultural attitudes. It is the philosophical concern about the nature with moral and values and the relationship between human being and world around it. According to Krishnamoorthy (2005), environmental ethics is a subject without definition and without consensus.

### Some facts concerning Environmental Ethics

Human being is one of the moral agents to judge the situation as he is capable of acting morally or immorally. The capabilities of human being to form

moral judgments include deliberation, which are to carry out the decisions and responsibilities to answers rights things and actions. It emphasizes that the other species of plants, animals and microorganisms have certain rights associated with environment (Chavan, 2008). There are certain facts concerning the environmental ethics. These are related to distribution of resources of the world. These facts give equal opportunities to compete for the comfort and richness of the world for all life forms including human being. These are:

Earth has adequate resources to support unlimited economic growth.

Nature is production unit and store house for all human needs.

Human progress is the satisfaction of his real needs and availability.

Technological progress helps to sustain human needs and comfort of life.

Human being has a strong sense of judging the need and responsibilities to act as a caretaker or custodian of resources.

### Classification of ethical issues

There is definite relationship between the ethical feelings and environmental issues providing the scope for worth of actions. Based on such actions, such issues can be classified into following three classes (Khan, 2006).

**A. Progressive ethics:** These are the issues of developmental activities governing the actions. It believes that the nature is for the pleasure of human being. But such pleasure should be without disturbing the natural rhythms.

**B. Preservative ethics:** These are the issues related to recreational, real, aesthetic feelings for nature preservation. These involve reverence for the nature and oppose overexploitation of nature and natural components. It desires the care for nature. Ultimately, if we care for environment, it will care us.

**C. Conservation ethics:** These are the issues related to maintenance and preservation of environment. These tresses on the expectation of unpolluted,





clean and pious environment with balance of resources. Conservation ethics recognizes the desirability of decent living standard with justified, proper and most reasonable use of natural resources (Rao, 2001). Exploitation beyond certain limits is not permitted for insuring the natural balance harmony of human society with the nature. It suggests for the suitable balance between development and preservation of natural resources.

### Historical Development of Environmental Ethics

The history of environmental concern dates as long as our primitive ancestors (Carson, 1960). The Book Sand County Almanac-'The Land Ethic' wrote in 1949 by Aldo Leopold, claimed that roots of ecological crisis were philosophical. But the consciousness in developed world was invoked by Lynn White in March, 1967 through his paper 'The historical roots of our ecologic crisis', and another paper 'The tragedy of the commons' by Garrett Hardin published in Dec. 1968. Those were published in *Science*.

The first philosophical conference was organized by William Blackstone in 1972 at the University of Georgia. "Philosophy and Environmental crisis", a paper by Pete Gunter in the proceedings of conference held at Georgia in 1974 caught the attention. Another book 'Is it too late? A theory of ecology' written by John B. Cobb on theology and religion based on philosophical roots of ethics. An Australian philosopher Richard Routley-Sylvan contributed his thoughts in a paper 'There is a need for a new environmental ethic' presented at 15<sup>th</sup> Congress of philosophy in 1973. In 1979 Eugene C. Hargrove started special journal 'Environmental Ethics'. These are a few milestones in the development of Environmental Ethics. Thereafter, many philosophers, environmentalists contributed the subject making it more popular and triggered public thinking.

### Ethical Theories

To support certain facts on moral basis of nature protection the ethical thoughts in the form of ethical

theories are formed. These are being shaped with the contributions from philosophers, environmental scientists and naturalists. Following are the major theories on environmental ethics.

**Consequential Theories:** The consequential theories supports that, a right action is one that overall has good consequences, a wrong action is one that in general has bad consequences. These theories are useful to evaluate human acts, policies, practices and traditions.

**Deontological Theories:** Deontological theories suggest that certain actions are right or wrong regardless of their consequences, like the universal truth. Criminal deserves to suffer solely as broken the law. These are the truths which are always required.

**Other Theories:** There are other few ethical theories which make the attempts to specify the matters related to environment. Following are a few of these.

1. Situation ethics: It is based on ethical responsibility. It follows that any action is right or wrong depends on situation.

2. Virtue ethics: Virtue ethics emphasizes that bringing about good consequences does not matter, development of a particular character matters.

3. Feminist ethics: Feminist ethics is based on emotional and spiritual living of women with nature. It assumes that women are inherently co-operative and caring rather than aggressive and competitive than men.

4. Existentialists: Existentialism is based on the responsibility of individual to make his own choices with his unique choice in environmental matters. Willing acceptance of pollution in return of consumer product as cost and benefit is best example of this kind.

### Spiritual approaches to environmental ethics

There are several spiritual approaches to environmental ethics. This list includes transcendentalism, deep ecology, eco feminism, Judeo-Christian, Islam and Asian religions. Most of these spiritual approaches are ultimately offered



an opportunity to construct useful and satisfying environmental ethics. Transcendentalists learn the truth from the facts in Universe. According to their thoughts, nature was there before human being started spoiling it and it will be there but we have to find spiritually where we could find ourselves in it. Self realization and bio-centric equality evolved the sense of deep ecology. Recognition of one-self not just an individuals identity or as a member of limited human society but as a member of Universe. Deep ecology says that human beings must eat and use other creatures may include non-vegetarian too for his survival but should not exceed the limits of vital needs. Judeo-Christian tradition considers that god created nature for human life and comfort. Nature is creation of god. Saint Francis of Assisi says that the nature is important to god and human being should take care of god's creation those also exist in the form of creatures. Buddhism expresses the compassion for all forms of life. Central theme of Hinduism is on the care and compassion for all life forms in the nature. Hinduism has elevated many plants and animals to god and goddess. The prophet Mohammed was very sensitive to the sufferings of living creatures.

#### **People and Environmental Ethics**

Environmental ethics are based on knowledge, belief and faith. Environment is more important than you, me and all of us. Reality status of all environmental components and their status is concern of matter for the future. Destruction of nature results in spread of diseases, pollution, soil erosion, desertification, acid rain, loss of biodiversity and number of problems which are difficult to tackle and control. If the nature is healthy, pure, clean and without much deteriorated form then human existence is comfortable for many years.

#### **Attitude governing relationship to Environment**

Human concern for the environment is of the obligatory nature. Human being are not to destruct the nature but to preserve it for the future generations with fulfillment of present needs.

Attitude of killing, slow killing negligence towards the living creatures needed to be controlled and nurturing of nature should be promoted. Attitude of saving eco-centrism and consciousness should be given priority than the anthropocentrism of human race. Human centric thinking (anthropocentrism) is to be controlled. If it is continued without control, it may lead to slow killing of the world. Environmental responsibility and animal rights and obligation towards nature should be recognized. Because, the direct responsibility of nature protection (eco-centrism) lies on the head of human being more than any other organisms on the planet earth.

#### **Environmental ethics and population Control**

We know that the world comfortable for the life-forms is finite, space as habitat is finite, many natural resources are finite hence needs must be finite. The needs can be finite only if growth is controlled and stopped. Therefore, human population should be kept finites by adopting different means and ways to limit the needs and restrict the excess exploitation and overexploitation. To insure such ecological stability in future, present population growth rate must tend to zero.

#### **Candidates for Environmental Ethics**

The concept of sustainable development is a representing core of new environmental ethics. Some ethical justifications are challenged by new moral sense, spiritual thinking and practical utility on the grounds of present need and the future of our next generation. But the thinking convergence emphasizes that we have to stop the damaging trend and prefer the path of sustainable development. We should not infringe the rights of future generations. It is necessary for healthy sustenance that the needs should be fulfilled without any major conflicts due to the activities of the present generation and short supply of needful resources. There are some suggested candidates of environmental ethics to act in this direction. These are as follows.





1. Integrity for the infusion of ecological percepts and ethos in lifestyle.
2. Humanity for sharing of resources on the basis of equality for today and tomorrow.
3. Determination to arrest pollution, stop degradation and promote quality of life.
4. Promotion of green consumerism in a controlled manner.
5. Judgment for environmental components with humility for nature to promote trusteeship.
6. Nourishment of nature by taking care for present and future generations.

#### Conclusion

The comfortable world for the existence of life is finite in space as habitat and natural resources. We must keep our needs finite not to infringe the rights of future generations for their healthy sustenance. We must regulate our conducts by adopting certain ethical principles for healthy environment with zero population growth rates.

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# Effect of herbicide (sodium penta chloro phanate) on pollen germination and tube length of stored pollen of Apocynaceae: Further evidence of a criticism of Banerji and Gangulee (1937), Brewbaker and Kwack (1963), Sudhakaran (1967- Ph.D.Thesis), Dharurkar (1971- Ph.D. Thesis), Nair, Nambudiri and Thomas (1973), Berg (1973), Brandt (1974), Vick and Bevan (1976), Rasmussen (1977), Navara, Horvath and Kaleta (1978), Mhatre (1980- Ph.D. Thesis), Mhatre, Chaphekar, Ramani Rao, Patil and Haldar (1980), Shetye (1982- Ph.D. Thesis) and Giridhar (1984- Ph.D. Thesis)– A critical review

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## Abstract

Even the lowest concentration ( $10^{-17}$ mg/ml) of sodium penta chloro phanate tried suppressed the germination of pollen of F and F-24 series of red-flowered cultivar of *Nerium odorum* and F-48 and F-72 series of pink-flowered cultivar of *Catharanthus roseus*. The herbicide stimulated the germination of pollen of successive flowers of all the cultivars of the Apocynaceae throughout the experiment. However, it stimulated the tube growth of only 3 out of 10 series.

**Keywords:-** Palynology, Toxicology, Environmental Sciences, Herbicides

## Introduction

Herbicides drastically reduced pollen germination as well as tube growth. It was therefore important to study the effect of such chemicals on germination as well as tube growth since inhibitory effects of these chemicals eventually reduce fruit and seed-set.

## Materials and Method

Pollen of successive flowers (viz. F, F-24, F-48, F-72 series i.e. open flowers and the flower buds which require 24, 48, 72 hours to open respectively) of 5 cultivars of Apocynaceae e.g. red-, pink- and white-flowered cultivars of *Nerium odorum* Soland. and pink- and white-flowered cultivars of *Catharanthus roseus* (L.) G. Don. were collected soon after the

dehiscence of anthers in the open flowers and stored at room temperature (22-31.8°C) having RH 57% and in diffuse laboratory light at the Department of Botany, Govt. Institute of Science, Mumbai. Germination of stored pollen grains of successive flowers was made soon after the dehiscence of anthers and with 2 hours intervals for the first 10 hours in the optimum concentrations of sucrose (acts as control) as well as in the optimum concentrations of sucrose supplemented with the optimum concentrations of sodium penta chloro phanate (Table 1). Observations were recorded 24 hours after incubation. For each experiment a random count of 200 grains was made to determine the percentage of pollen germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a magnification of 100x.

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**Table 1: Effect of sodium penta chloro phenate on stored pollen on their germination and tube length of five cultivars of Apocynaceae**

					PG & TLSADA				TRADAFMPGTAL							
					%PG		TL in mm		C				T			
Cultivars	SF	PV	SC	HC	C	T	C	T	H	PG	H	TL	H	PG	H	TL
<i>N. odorum</i> pink-flowered	F	80	50	10 <sup>-17</sup>	35	37	1485	745	8	52	0	1485	4	45	4	836
<i>N. odorum</i> red-flowered	F	74	20	Ng	20	Ng	1250	Ng	6	36	0	1250	Ng	Ng	Ng	Ng
<i>N. odorum</i> white-flowered	F	62	50	10 <sup>-17</sup>	20	40	675	148	4	24	0	728	2	45	4	210
<i>C. roseus</i> pink-flowered	F	90	20	10 <sup>-17</sup>	60	74	1575	1800	6	72	0	1575	0	74	0	1800
<i>C. roseus</i> white-flowered	F	88	20	10 <sup>-17</sup>	40	64	1256	1220	8	75	0	1256	4	75	0	1220
<i>N. odorum</i> red-flowered	F-24	74	20	Ng	06	Ng	485	Ng	6	8	0	485	Ng	Ng	Ng	Ng
<i>C. roseus</i> pink-flowered	F-24	90	50	10 <sup>-17</sup>	28	29	240	425	6	36	4	300	0	29	0	425
<i>C. roseus</i> white-flowered	F-24	88	50	10 <sup>-17</sup>	16	56	248	786	6	50	6	264	2	60	0	789
<i>C. roseus</i> pink-flowered	F-48	90	50	Ng	14	Ng	95	Ng	4	16	0	95	Ng	Ng	Ng	Ng
<i>C. roseus</i> pink-flowered	F-72	90	80	Ng	10	Ng	65	Ng	0	10	0	65	Ng	Ng	Ng	Ng

C, in control; HC, herbicide concentrations in mg/ml; Ng, no germination of pollen even 24 hours of their sowing; PG, pollen germination; PG&TLSADA, Pollen germination & tube length in the sets which were set soon after dehiscence of anthers; PV, pollen viability in %; SC, sucrose concentrations in %; SF, successive flowers; TL, tube length; TRADAFMPGTAL, Time required after dehiscence of anthers for maximum pollen germination & tube length.

## Results and Discussion

Pollen viability is a subject that has a great deal of practical as well as theoretical interest. In the present investigation even the different cultivars of the same species showed the variations in the percentage of their pollen viability (Table 1). Reduced pollen viability has been interpreted as an indication of suspected hybridity in wild populations. Nevertheless, variations in pollen viability may affect the breeding systems of the species concerned, and if the pollen viability can be altered by the environment, then the breeding system itself may be under some degree of environmental control.

Potentiality of pollen germinability was recorded in F series of all the 5 cultivars of the Apocynaceae studied. It was the pollen of F-24 series of red-flowered cultivar of *Nerium odorum* and both the cultivars of *Catharanthus roseus* found germinated in the optimum concentrations of sucrose. It should be pointed out that the pollen of F-48 and F-72 series of pink-flowered cultivar of *C. roseus* showed their germination in the optimum concentrations of

sucrose. Thus the potentiality of pollen germinability in Apocynaceae was observed in 10 out of 20 series investigated (Table 1).

As a rule the percentage of pollen germination is always less than the pollen viability (Table 1). However, Banerji and Gangulee (1937) and Dharurkar (1971) reported higher percentage of pollen germination than the pollen viability in *Eichhornia crassipes*. The claim of Banerji and Gangulee (1937) and Dharurkar (1971) is challenged by Salgare (1986c, 1995, 2000b, 2006a, 2006j, 2006m, 2006q, 2006s, 2007b-2007c, 2007e, 2007f, 2007g, 2007i and 2007j) who stated that the observations of Banerji and Gangulee (1937) and Dharurkar (1971) are exaggerating.

Germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus* in vitro culture of sucrose was noted in the present investigation. However, Palathingal (1990) failed to germinate the pollen of F-72 series of pink-flowered cultivar of *C. roseus* in Brewbaker and Kwack's (1963) culture medium. This proves that the culture medium is also having the bearing on the germination of pollen.





This also confirms that Brewbaker and Kwack's (1963) culture medium is not ideal for pollen cultures. This was also pointed out earlier by Salgare (2006a, 2006j, 2006q, 2006s, 2006u, 2007e, 2007f and 2007h). Even the lowest concentration ( $10^{-17}$  mg/ml) of sodium penta chloro phanate tried suppressed the germination of pollen of F and F-24 series of red-flowered cultivar of *Nerium odorum* and F-48 and F-72 series of pink-flowered cultivar of *Catharanthus roseus* (Table 1). Sharma (1984) stated that even the lowest concentration ( $10^{-17}$  mg/ml) of sodium penta chloro phanate tried prevented the germination of pollen of F series of white cascade, sonata, F-24 series of white cascade, duet, sonata and F-48 series of red as well as white cascade. All these are the cultivars of *Petunia grandiflora*. This proves that the pollen of the said series are highly sensitive and acts as an ideal indicators of pollution. Thus it is confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant (Berg, 1973; Brandt, 1974; Vick and Bevan, 1976; Rasmussen, 1977; Navara *et al.* 1978; Mhatre, 1980; Mhatre *et al.* 1980; Shetye, 1982 and Giridhar, 1984) as an indicator of pollution is a very crude method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. This is confirmed in the present critical review (Table 1) as well as by the previous extensive work of Salgare (1983, 1984, 1985a, 1985b, 1985c, 1986a, 1986e, 1986f, 2000a, 2001a, 2001b, 2005b, 2005d, 2005e, 2006a, 2006f, 2006j, 2006l, 2006o, 2006q, 2006u, 2007b, 2007c, 2007d, 2007e, 2007f, 2007g, 2007h, 2007i, 2007j, 2007k, 2007l), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Singh (2002, 2006a, 2006b), Salgare and Pathak (2005) and Sharma (1984).

Sodium penta chloro phanate stimulated the germination of pollen of successive flowers of all the cultivars of the apocynaceae throughout the

experiment. However, it stimulated the tube growth of only 3 out of 10 series (Table 1). Maximum pollen germination was noted in all the 10 series except for F-72 series of pink-flowered cultivar of *Catharanthus roseus* with the stored pollen *in vitro* culture of sucrose. Pollen of F-72 series of pink-flowered cultivar of *C. roseus* showed an equal percentage of germination of pollen with that stored pollen. The time interval of the period of storage ranges right from 4 to 8 hours. However, the pollen of F-72 series of pink-flowered cultivar of *C. roseus* showed the highest germination in the sets which were set soon after the dehiscence of the anthers (Table 1). The herbicide stimulated the germination of stored pollen in 4 out of 10 series, while the tube growth was stimulated in 2 out of 10 series of the Apocynaceae. *In vitro* culture of sucrose the longest pollen tubes were noted in 5 out of 10 series in the sets which were set soon after the dehiscence of the anthers. *In vitro* culture of sucrose supplemented with the herbicide the maximum germination of pollen was noted in 6 out of 7 series in the sets which were set soon after the dehiscence of the anthers. However, the longest pollen tubes were noted in 3 out of 7 series in the sets set soon after the dehiscence of anther (Table 1). Thus it is confirmed that the pollen germination and tube elongation are two distinct processes. However, Nair *et al.* (1973) stated that the pollen germination and tube elongation is one and the same process. Present work (Table 1) as well as previous extensive work of Salgare (1979, 1983, 1986d, 2004, 05a, 2005c, 2006e, 2006j, 2006k, 2001, 2006s, 2007i, 2007j), Salgare and Bindu (2002, 2005) and Salgare and Tessy Mol Antony (2005a, 2005b) it could be concluded that the observations of Nair *et al.* (1973) are superficial and misleading.

In this connection it should be pointed out that Sudhakaran (1967) stated that in *Vinca rosea* L. {*Catharanthus roseus* (L.) G. Don.} besides pollen grains which produced single pollen tube, it has also been noticed that tetraploid grains frequently produce more than one pollen tube. Pollen tubes





are branched quite frequently. Aberrations of this type in the pollen tube development are not observed in diploid pollen tubes, but quite frequently met with the pollen grains of irradiated plants. Salgare (1983) made it very clear that Sudhakaran (1967) had failed to trace out the branched pollen tubes and polysiphonous condition which is fairly common even in diploid pollen grains. Apart from this Sudhakaran (1967) was not able to report the various types of pollen tube deformities either with diploid or tetraploid grains. Present investigation as well as the extensive work of Salgare (1983, 1986b, 2006b, 2006c, 2006d, 2006h, 2006j, 2006n, 2006p, 2006q, 2006s, 2006t, 2007a, 2007b, 2007c, 2007e, 2007f, 2007h, 2007i) also proved that Sudhakaran's (1967) observations are superficial and misleading.

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## Effect of *Eclipta alba* on loss of fecundity and phagodeterancy of *Callosobruchus maculatus* (FAB.)

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### Abstract

*Eclipta alba*, which is herbaceous plant, has shown phagodeterant activity, equally good as insect sterilitant. Three different concentrations of the plant extract in petroleum ether and acetone showed loss in fecundity and fertility in *Callosobruchus maculatus*, a serious stored pest of green gram. The detail phytochemistry of chemosterilant is still in progress.

**Keywords:-** *Callosobruchus maculatus*, *Eclipta alba*, Fecundity, Fertility, Plant extract

### Introduction

Bruchus are the most important stored grain pest of pulses which causes loss to the national economy, also impaired the quality of the seeds. Several workers have reported phagodeterant, repellent and insecticidal activity of plant extract. Prominent among them are, Rathore and Sharma (2002), Jha (2008) Manohar and Yadav (1990), Baby (1994) and Dwivedi and Bhati (2006). Pulses, which are essential constituents of human diet, suffered severely from the stored grain pest *Callosobruchus maculatus*. The present paper reports the loss of fecundity and fertility caused by the extract of *Eclipta alba* to the *Callosobruchus maculatus*.

### Materials and Method

**Rearing of bruchus:-** The initial culture of experimental insect *Callosobruchus maculatus* was obtained from laboratory stock. The insect were reared in the pre-sterilized jars containing disinfected green gram seeds in a glass jar (15.5 x 10.5 cm) under controlled conditions (Temp.  $26 \pm 1^\circ\text{C}$  and Rh60  $\pm$  5%) in the insectary at pest control research laboratory, Vidisha.

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**Experimental design:-** For this experiment, the newly emerged adult female beetles were separated and 1  $\mu$ l dose of the extract was applied topically to them. They were released in the petri dishes containing 20 gm. treated seeds. Untreated males of the same age in 1:1 ratio were introduced into the same petridishes for observing mating behavior. The oviposition, rate of egg laying (Fecundity) and percent of hatching (Fertility) rates were observed. Parallel controls were maintained throughout the course of experiment. Visual observations were noticed on the behavioral responses of bruchus due to the effect of plant extracts as well as laboratory cultured normal ones. All the experiments were conducted in the insectary maintained at ad libitum.

**Plant material:-** *Eclipta alba*, which is commonly known as 'Bhangra' grows plentifully in the moist places throughout India. This is medicinal plant, which is used as purgative, hepatoprotective and against liver and spleen enlargement. The plant was collected locally; shed dried and powdered material was Soxhlet extracted in petroleum ether and acetone. Purification and characterization of the crude extract was done using column chromatography and TLC techniques. Three different concentration of the plant extract were used against 10 pairs of adult bruchus in glass jar tied with muslin cloth.

## Results and Discussion

The present study recorded the effect of petroleum ether and acetone extract of *Eclipta alba* on the fecundity and fertility of *Callosobruchus maculatus* for this experiment newly emerged adult beetles were separated and 1µl of each concentration is applied topically and then they were released in the glass jars containing 20 gm. seeds of green gram, i.e. *Phaseolus mungo*. Untreated males of the same age in 1:1 ratio were introduced in the same jars. Oviposition, egg laying (fecundity and percentage of hatching (fertility)) was observed and the results are shown in Table 1. From the results, it is quite clear that 1.5% concentration caused complete loss in fecundity and fertility to beetle extract of *Eclipta alba* against *Callosobruchus maculatus*. In other two concentrations, a dose dependent loss in fecundity and fertility was noticed that egg laid by the females succeeded in hatching out in adult stage. However, effect on subsequent metamorphosis was not observed.

Plant extract have shown promising results on variety of insects. The plant extracts species specific may act as insecticidal, growth inhibitor, anti-ovipositional and phagodeterrent. The present paper reports, the loss in fecundity and fertility of *Callosobruchus maculatus* adult beetle when treated with three different doses of petroleum ether and acetone of *Eclipta alba* the plant material was focus quite effectively at five concentrations. The finding of present study is quite comparable with that of Sangappa(1977) reported, the protection of red gram seed against *Callosobruchus chinensis* by several vegetable oils. Saxena and Yadav (1983) have studied the effect of extract of the lowers of *Delonix regia* on the reproduction of *Triboleum castaneum* and concluded that the suppresses fecundity and fertility of *Triboleum castaneum*. This is agreement with results of *S. indicus* extract when tested against LC<sub>50</sub> concentration resulted in complete inhibition in fecundity and fertility of

*Callosobruchus chinensis* and *Callosobruchus maculatus*.

**Table 1: Effect of *Eclipta alba* extracts on fecundity and fertility of *Callosobruchus maculatus***

Concentration %	Name of extract					
	Petroleum ether			Acetone		
	Average no. of egg laid	% of hatching	GI	Average no. of egg laid	% of hatching	GI
5	21	82.53	3.05	18	82.14	3.04
1	9	71.42	2.64	9	77.77	2.88
1.5	-	-	-	-	-	-
Control	98	98.71	3.65	98	98.71	3.65

Note: 10 paired of adult beetles were taken in each treated group. Results are the average of three replicates.

Similarly, Chander and Ahmed (1986), studied the efficacy of oils from medicinal plants as protectants of green gram against pulse beetles *Callosobruchus chinensis* and opined that oils of *Acorus calamus*, *Curcuma amada* at 0.25 and 0.5 ml/Kg. Significantly, reduced the expected adult emergence. Oils of *Carum copticum*, *Nigetta sativa* and *Bassia longifolia* had no effect on the adult emergence. Chellayan and Karnavar(1990), observed the influence of neem kernel extract against *Trogoderma gramurum* and stated that the extract caused reduction in fecundity and fertility at 10-100µg concentration. Similarly, Dixit and Saxena(1990a,b) observed that plant extract of *Adhatoda vasica* and *Azadirachta indica* inhibited the fecundity and fertility of *Callosobruchus maculatus*.

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## Studies on antibacterial activity of extracts from *Tinospora cordifolia* (Giloy) against *Staphylococcus aureus*

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### Abstract

The active components of stem bark of *Tinospora cordifolia* were extracted using cold water and organic solvents (methanol, diethyl ether and acetone) and were tested against *Staphylococcus aureus* using the agar disc diffusion method. All the four extracts inhibited the growth of *S. aureus*, with methanol extract exerting the highest activity whereas water extract was least active. The results were compared with the reference antibiotic ciprofloxacin.

**Keywords:-** Antibacterial, *Tinospora cordifolia*, *S. aureus*, Stem extract, Methanol, Diethyl ether, Acetone.

### Introduction

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Nascimento *et al.*, 2000). According to WHO medicinal plants would be the best source to obtain a variety of drugs (Santos *et al.*, 1995). Therefore such plants should be investigated to better understand their properties, safety and efficacy (Ellof, 1998). Antimicrobial activity of 120 plant species from 28 different families was carried out (Santos *et al.*, 1990). Antifungal activity of leaf extracts of medicinal plants used by Himalayan people was investigated against *Alternaria alternate* and *Curvuleria lunata* (Guleria and Kumar, 2006). 18 plants belonging to zingiberaceae family was evaluated for their antioxidant and antimicrobial activity (Chen *et al.*, 2008). *T. cordifolia* (Giloy) is a large glabrous and climbing succulent shrub with rocky bark. It is found throughout the tropical India ascending up to an altitude of 300m

and has been used from ancient times to cure different types of ailments like general debility, dyspepsia, fever and urinary diseases (Negi and Pant, 1994).

### Materials and Method

The matured leaves of *T. cordifolia* were collected from Hardwar and the bacterial strain of *S. aureus* (MTCC-737) was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. For the preparation of plant extract the powdered stem bark of *T. cordifolia* were extracted with methanol, diethyl ether and acetone for 24 hrs using Soxhlet apparatus and aqueous extract. Three different dilutions of plant extracts i.e. 800, 400 and 200 mg/ml DMSO were used for primary screening which was carried out through agar disc diffusion method (Bauer *et al.*, 1966). DMSO served as negative control and standard antibiotic ciprofloxacin (500 ppm) as positive control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones in mm.

### Results and Discussion

Preliminary evaluation of antibacterial activity clearly indicates that all the stem bark extracts prepared in four solvents exhibited activity against

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*S. aureus* but the 800 mg/ml concentration of methanol extract was found to possess higher level of antibacterial activity (14.3 mm) in comparison to diethyl ether, acetone and aqueous extract which was comparable to that of antibiotic ciprofloxacin (15 mm). Hence, it can be concluded that keeping in mind the side effects of allopathic medicines and the drug resistance in microbes it will be of great

interest to use plant based medicines to combat against diseases. The discovery of a potent remedy from plant origin will be of great advancement in bacterial infection therapies.

**Table 1: Antibacterial activity of stem bark extract of *T.cordifolia* and that of reference antibiotic on *S.aureus***

Solvents	*Effective zones of inhibition				
	Concentration of sample(mg/ml)			Antibiotic control(500ppm)	DMSO control
	800	400	200	Ciprofloxacin	
Methanol	14.3	12.3	10.3	15	0
D.E.E	14.0	10.3	7.6	15	0
Acetone	11.6	9.6	7.3	15	0
Aqueous	11.3	9.3	6.6	15	0

\*Effective zone of inhibition = Total zone of inhibition – Diameter of the disc (5mm)

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## Water quality status of Dharampuri ward lake, Aheri, Distt. Gadchiroli (M.S.)

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### Abstract

The Dharampuri Ward lake is the oldest lake of Aheri, build up by the Gond Maharaja for domestic purposes. The lake is now used for domestic, irrigation and pisciculture on lease basis. Monthly variations of different physico-chemical characteristics were studied for period of one year from July-06 to June-07 on three sites to enhance the water quality status. Various physico-chemical parameters reveals that the fluctuation in water temperature, pH, DO and sulphate are within desirable limits, slight increase in the values of Hardness, Phosphate and Nitrate indicates the mesoeutrophic nature of the lake at site S<sub>2</sub>.

**Keywords:-** *Physico-chemical, Dharampuri Lake, Water quality*

### Introduction

Water resources are of critical importance to both natural ecosystem and human development. The study of inland water bodies has gained immense importance in recent years because of their multiple uses for the human consumption, agriculture and industry. The men's influence on lotic and lentic ecosystems caused increase in silt and nutrient load, pushing lakes towards eutrophied at a very early stage and lead to changes in their trophic status and render them unsuitable for aquaculture and domestic purpose.

Dharampuri Ward lake situated in eastern region of Aheri and extensively used for domestic purpose. The total area of lake is 7.12 hectare. In India and abroad large number of studies on lentic water bodies have been carried out among those are Welch

(1952), Munnawar (1970). Study of different water parameters is very important for understanding metabolic events in aquatic ecosystem. The parameters influences each other and also sediment parameters, as well as they govern the abundance and distribution of flora and fauna, hence the regular monitoring of physico-chemical parameters is essential to determine the status of water body.

### Materials and Method

Dharampuri Ward lake is located in Aheri city of Gadchiroli district, Maharashtra State having depth of 3.4 meters. Physico-chemical characteristics of water were studied monthly during the period of July-2006 to June-2007. For the sampling, three sites were selected viz. S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub>. Samples were collected in 5.00 liters dried plastic cans during 9.00 A.M. to 10.30 A.M., brought to laboratory and analyzed for alkalinity, hardness, free CO<sub>2</sub>, TS, TDS, chloride, sulphate, phosphate and nitrate. For determination of dissolved oxygen, samples were fixed in field and brought to laboratory in an ice box for further process, the parameters like temperature, pH, conductivity, and transparency were analyzed on the spot. The parameters analyzed with the help

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of standard methods APHA (1986) and Khanna (1993).

## Results and Discussion

Temperature is one of the most important physical parameter for the water body since the rise in temperature speed up the bio-chemical reactions, reduces the solubility of gases and affects population fluctuation. Air temperature of the area ranged from 25 °C to 44 °C. High atmospheric temperature was recorded during summer, moderate in monsoon and slightly lower in winter season. Similar pattern of temperature fluctuation was noted by Swarnalata and Rao (1991) from Saroomagar lake, Hyderabad.

**Water temperature:** Water temperature of Dharampuri lake ranged from 23.5 °C to 35 °C. The water temperature varied between 23.8 °C to 34.8 °C, 23.6 °C to 34.9 °C and 23.89 to 34.9 °C at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. It was highest in the summer months (May) and relatively low in monsoon and winter months. The minimum water temperature was recorded at S<sub>2</sub> and Maximum at S<sub>3</sub>. Water temperature consistently lower than atmospheric temperature of the lake, to summaries, air and water temperature followed a common pattern. It was higher in summer and relatively lower in monsoon and winter. A similar pattern with regard to temperature variation is in conformity with Singh and Swarup (1979), Vyas and Jain (1979) and Swarnalatha (1994) in Saroomagar lake, Hyderabad.

**pH:** pH is the value expressed as the negative logarithm of hydrogen ion concentration. The pH varied between 7.14 to 8.1, 7.56 to 7.99 and 7.56 to 8.02 at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. Water which has a pH value more than 9.6 or less than 2.5 becomes unsuitable for most life forms. In present investigation, pH was observed to be alkaline, ranged from (7.54 to 8.01) throughout the period of investigation with no definite seasonal variation. Generally, slightly alkaline conditions are favourable for growth of algal species in lotic systems (Welch,

1952) while acidic conditions are detrimental. The similar studies were observed by, Vyas and Jain (1979), Sreenivasan (1974) and Bohra (1976).

**Conductivity:** Conductivity ranged between 0.89 to 0.199 µmhos/cm. The conductivity varied between 0.89 to 0.171 µmhos/cm., 0.101 to 0.175 µmhos/cm. and 0.91 to 0.177 µmhos/cm. at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. It was high in summer months (May) and relatively low in winter months (December). The minimum conductivity was recorded at site S<sub>1</sub> and maximum at site S<sub>3</sub>. In present study, maximum conductivity was observed in summer may be due to evaporation of water and minimum during winter season due to dilution from large volume of rain water.

**Transparency:** The extent to which light can penetrate into the water column depends on the transparency of standing water column. Further, transparency of water is inversely proportional to turbidity created by suspended inorganic and organic matter (Saxena and Baskaran, 1981).

Transparency ranged between 34.6 cm to 76.5 cm. It varied between 35.3 cm to 76.5 cm, 33.5 to 76.00 cm. and 35.00 to 77.00 cm at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. The maximum transparency observed during summer season and minimum during monsoon and winter. The low transparency during late summer is attributed to decline in water level due to evaporation and plankton growth while its lower values in monsoon is due to addition of silt and clay particles along with runoff water from the catchment area.

**Alkalinity:** Alkalinity is the capacity of water or sediments to neutralize strong acid and is characterized by presence of hydroxyl ions capable of combining with hydrogen ions. In natural water, carbonates and bicarbonates mainly contribute to alkalinity. In general, alkaline water is more productive and supports the diversity of aquatic life. Alkalinity ranged between 54.00 mg/l to 186 mg/l It varied between 56 mg/l to 183 mg/l, 54 mg/l to 186 mg/l, and 56 mg/l to 180 mg/l, at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>





respectively. The maximum alkalinity was during summer months and the minimum during winter season. The acceptable limit for alkalinity is 200 mg/l, observed values are not more than acceptable limit.

**Hardness:** Alkaline earth metals mainly Ca and Mg present in it impart hardness of water. The total hardness of water recorded between 76 mg/l to 227 mg/l. The hardness varied between 76.00 mg/l, to 227 mg/l, 81.00 mg/l to 239 mg/l and 77.00 mg/l to 232 mg/l, at site  $S_1$ ,  $S_2$  and  $S_3$  respectively. Total hardness may be correlated with alkalinity was found to be maximum during summer months while minimum during winter. These observations are similar to that of Vyas and Jain (1979) in Gordhan Vilas lake near Udaipur, India.

**Total Solids:** All matter in water except water is called as solids. The TS is the matter remains after the temperature of water at 103 °C to 105 °C. During the present study, total solids ranged between 254 mg/l to 615 mg/l, 270 mg/l to 702 mg/l and 255 mg/l to 622 mg/l, at site  $S_1$ ,  $S_2$  and  $S_3$  respectively. The maximum values recorded during monsoon and minimum during winter months. These findings were in accordance with Tripathi and Pandey (1990). The total solids recorded low during winter may be due to sedimentation and high during monsoon due to high surface run-off, silt- sewage from catchment area.

**Total Dissolved Solids:** Total dissolved solids include salts and variety of organic substances. TDS level plays an important role in community structure due to limiting impact on primary production of trophodynamics. In present study TDS values ranged between 57 mg/l to 134 mg/l, 58 mg/l to 130 mg/l, and 59 mg/l to 130 mg/l at site  $S_1$ ,  $S_2$  and  $S_3$  respectively. The low values recorded in winter months and high during summer. This may be due to low water level and similar in accordance with Chandrashekhar (1996).

**Dissolved Oxygen:** Dissolved oxygen is one of the most important parameter of water quality affecting

survival and distribution of flora and fauna and reflects the physical and biological processing prevailing in water (Trivedy and Goel 1984). In present investigation DO values ranged between 5.1 mg/l to 10.2 mg/l, 5.7 to 9.7 mg/l and 4.7 to 11.2 mg/l at  $S_1$ ,  $S_2$  and  $S_3$  respectively. The minimum values recorded during summer months, Pahwa and Mehrotra (1966) have reported a minimum DO in summer and maximum in winter. Normally high DO is encountered in unpolluted while lower levels of the same in polluted areas of an aquatic ecosystem. It is a well known fact that the oxygen balance of lake is tagged with the photosynthetic and respiratory activities of biota and chemical oxidization on one hand and prevailing physico-chemical condition on the other, High winter values of DO could be attributed to relatively stable abiotic conditions and higher algal-biomass stimulating rate of photosynthesis. Intermediate values in monsoon could be attributed to dilution factor that adversely affects algal biomass and photosynthetic replenishment of oxygen, Kulshrestha *et al.* (1989) recorded DO from 6.9 mg/l to 12.9 mg/l in lower lake and from 4.8 mg/l to 9.9 mg/l in Chunnabhatti lake.

**Free CO<sub>2</sub>:** Free CO<sub>2</sub> is directly proportional to the bi-carbonates and indirectly to carbonates and is highly soluble in water. The respiratory activity of aquatic organisms and decomposition of organic matter are important sources of carbon dioxide in fresh water bodies. The free CO<sub>2</sub> is released during the decomposition of certain substances and metabolic activities of the living organism since; higher temperature accelerates the decomposition of organic substances as well as the respiratory activity of the biota. A direct relationship was established between water temperature and free CO<sub>2</sub>. In present study, CO<sub>2</sub> ranged between 2.8 mg/l to 6.7 mg/l, 3.02 mg/l to 6.4 mg/l and 3.25 mg/l to 6.9 mg/l at site  $S_1$ ,  $S_2$  and  $S_3$  respectively. Minimum values recorded during winter and maximum during summer. The high utilization by more algal blooms in winter causes depletion of CO<sub>2</sub> in winter.





**Biological Oxygen Demand:-** Biochemical oxygen demand (BOD) can be defined as the quantity of dissolved oxygen in mg/l required under the condition for complete oxidation of the organic matter in sample. In present study, the BOD values recorded 1.7 mg/l to 3.9 mg/l, 1.5 to 4.2 mg/l and 1.6 mg/l to 3.7 mg/l at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. The high BOD values recorded during summer months and low during winter. Biochemical Oxygen Demand (BOD) is an important parameter that indicates the magnitude of water pollution by oxidizable organic matter. The main sources of organic pollution include untreated domestic sewage; agricultural run off containing residual fertilizers and certain industrial effluents.

**Chemical Oxygen Demand:-** In present study, COD ranged between 11.2 mg/l to 19.5 mg/l, 11.3 mg/l to 19.9 mg/l and 11.2 mg/l to 19.6 mg/l at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. The maximum seasonal value was recorded during winter and minimum during summer.

**Sulphate:-** Sulphates are produced by biological oxidation of sulphur containing organic matter and weathering of rocks. Sulphate exists in number of oxidation state from the most oxidized sulphate to the most reduced sulphide. The biological reduction of sulphur can take place in both aerobic and anaerobic condition.

The sulphate values at three sampling stations S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> in the present investigation were recorded

**Table 1- Average value of monthly variation in physico-chemical parameters of Dharampuri ward lake, Aheri**

Parameter	July	August	September	October	November	December	January	February	March	April	May	June
Temp. (°C)	30.6 ± 0.09	29.3 ± 0.08	28.7 ± 0.09	28.6 ± 0.04	26.6 ± 0.14	24.4 ± 0.14	23.7 ± 0.09	44.0 ± 23.4	29.9 ± 0.09	32.6 ± 0.09	34.8 ± 0.04	33.6 ± 0.12
pH	7.9 ± 0.00	7.7 ± 0.00	7.6 ± 0.01	7.5 ± 0.00	7.5 ± 0.00	7.8 ± 0.00	7.9 ± 0.03	7.9 ± 0.01	7.8 ± 0.00	7.8 ± 0.01	7.9 ± 0.03	7.8 ± 0.24
Cond. (micro/cm)	0.152 ± 0.0	0.127 ± 0.0	0.121 ± 0.0	0.122 ± 0.0	0.446 ± 0.46	0.093 ± 0.3	0.096 ± 0.4	0.110 ± 0.0	0.145 ± 0.0	0.168 ± 0.0	0.173 ± 0.2	0.167 ± 0.0
Trans. (cm)	55.5 ± 0.81	34.6 ± 0.84	38.9 ± 0.29	45.5 ± 0.40	46.8 ± 0.47	54.1 ± 2.01	66.0 ± 1.08	73.8 ± 2.32	75.3 ± 1.64	76.3 ± 0.23	66.3 ± 1.24	62.8 ± 1.02
Albca. (mg/l)	137 ± 1.63	105 ± 0.84	84.3 ± 1.88	79.3 ± 1.24	55.3 ± 0.94	88.6 ± 0.47	92.0 ± 0.84	111 ± 1.24	153 ± 49.8	139 ± 9.89	158 ± 0.81	183 ± 2.44
Hard. (mg/l)	155 ± 2.62	152 ± 2.62	90.6 ± 1.69	78.3 ± 2.62	94.0 ± 1.63	103 ± 1.69	71.8 ± 49.7	147 ± 1.69	189 ± 2.16	220 ± 1.63	227 ± 6.64	227 ± 6.64
TS (mg/l)	646 ± 39.4	619 ± 46.67	452 ± 72.6	415 ± 47.7	415 ± 47.7	324 ± 16.9	188 ± 114.3	259 ± 7.31	279 ± 3.85	298 ± 4.02	386 ± 17.72	450 ± 1.69
TDS (mg/l)	129 ± 2.16	118 ± 1.24	74.3 ± 51.8	71.3 ± 20.7	76.8 ± 0.6	61.8 ± 0.62	58.0 ± 0.81	75.3 ± 0.94	105 ± 0.94	112 ± 1.24	130 ± 2.49	123 ± 2.05
DO (mg/l)	6.03 ± 0.17	7.13 ± 0.17	7.37 ± 0.20	7.37 ± 0.17	7.8 ± 0.04	8.30 ± 0.16	9.07 ± 0.26	10.3 ± 0.66	9.27 ± 0.68	6.67 ± 1.15	5.57 ± 0.89	5.17 ± 0.41
CO <sub>2</sub> (mg/l)	5.60 ± 0.21	5.30 ± 0.21	4.37 ± 0.30	4.33 ± 0.12	3.97 ± 0.17	3.23 ± 0.41	2.87 ± 0.24	5.33 ± 0.30	5.53 ± 0.09	5.97 ± 0.17	6.27 ± 0.47	6.70 ± 0.16
BOD (mg/l)	3.50 ± 0.28	2.67 ± 0.25	2.30 ± 0.21	2.03 ± 0.09	1.80 ± 0.81	1.60 ± 0.28	1.47 ± 0.18	1.63 ± 0.09	2.27 ± 0.12	2.7 ± 0.21	3.67 ± 0.20	3.93 ± 0.20
COD (mg/l)	14.4 ± 0.04	14.6 ± 0.04	12.8 ± 0.45	18.53 ± 0.3	18.8 ± 0.04	19.6 ± 0.08	19.7 ± 0.14	16.6 ± 0.14	17.5 ± 0.16	14.1 ± 0.49	11.2 ± 0.04	11.3 ± 0.04
Sulph. (mg/l)	21.7 ± 0.35	20.0 ± 0.83	15.4 ± 1.32	13.3 ± 0.1	11.5 ± 0.26	12.3 ± 0.08	11.1 ± 0.56	15.7 ± 1.39	27.5 ± 0.47	30.3 ± 0.89	36.6 ± 1.48	38.3 ± 0.63
Phos. (mg/l)	0.36 ± 0.03	0.28 ± 0.02	0.23 ± 0.01	0.19 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.29 ± 0.00	0.36 ± 0.02	0.40 ± 0.00	0.43 ± 0.00	0.52 ± 0.92	0.60 ± 0.02
Nitrate (mg/l)	0.32 ± 0.00	0.39 ± 0.06	0.20 ± 0.00	0.19 ± 0.00	0.40 ± 0.41	0.36 ± 0.37	0.56 ± 0.35	0.36 ± 0.36	0.16 ± 0.01	0.22 ± 0.00	0.60 ± 0.46	0.24 ± 0.04



between 10.30 mg/l to 37.9 mg/l, 11.2 mg/l to 39.2 mg/l and 10.7 mg/l to 37.8 mg/l. The maximum value recorded in the summer months and minimum during winter. Higher concentration of sulphate in summer may be due to activity of bio degradation, Munnawar (1970).

**Phosphate:-** In natural water, phosphates are present in small quantities. Generally aquatic ecosystems receive excess of this nutrient through untreated domestic sewage and agriculture run off. Normally phosphate acts as a limiting nutrient in the process of eutrophication and lakes can be aesthetically classified into good, fair, bad, very bad, and lawful on the basis of % phosphates loading. Like nitrate, phosphate plays an important role in determining trophic status of water body. In the present study the phosphate ranged between 0.169 mg/l to 0.632 mg/l. The phosphate values varied between 0.169 mg/l to 0.589 mg/l, 0.170 mg/l to 0.632 mg/l and 0.169 mg/l to 0.579 mg/l at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. The maximum value of phosphate noted down during summer months due to less volume of water and minimum during winter.

**Nitrate:** The determination of nitrate is very important as it helps in measuring the pollution status and gives relative picture of availability of decomposable organic matter. Aquatic ecosystems in urban environment receives excess of nitrates through untreated domestic sewage and along with phosphates, are responsible for the process of lake degradation called eutrophication. The determination of nitrate is very important as it helps in measuring the pollution status. In the present investigation, nitrate ranged between 0.077 mg/l to 0.342 mg/l, 0.92 mg/l to 0.348 mg/l and 0.69 to 0.339 mg/l at site S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. The minimum value of nitrates was recorded in summer and maximum was in monsoon months. Nitrate peaks followed by rain in the catchment area have also been reported by various workers (Venkateshwarlu, 1969 and Adwant 1981). In the

present investigation, the Dharmapuri Ward lake show physico-chemical parameters within a permissible limit at site S<sub>1</sub> and S<sub>3</sub> however slight increase in values of hardness, phosphate and nitrate indicating the lake is towards mesoeutrophic status of lake at site S<sub>2</sub>.

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## Physico-chemical status of soil between Lowang to Gatti, Sewa catchment, Kathua District, J&K

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### Abstract

Four stations were selected in Sewa river catchment, district Kathua, J&K for soil studies for 2 years on monthly basis. Geologically and from pH studies have revealed acidic character of soils. The soils are mostly clay-loam in texture. Most of its parts is barren and soil moisture is accounted from persistent snow cover on high peaks and frequent rain for 8 months during the year. Moisture ranges between 8.73% to 12.90% in all profiles and results in high ingress of water. The loss of nutrition among  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{TiO}_2$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{Na}_2\text{O}$  and  $\text{K}_2\text{O}$  and  $\text{MnO}$  by chemical analysis, revealed that  $\text{TiO}_2$ ,  $\text{Fe}_2\text{O}_3$ , Na and K get depleted from composite samples and as such need compensation.  $\text{Al}_2\text{O}_3$  and  $\text{MnO}$  get added to the composite soils, because of excessive erosion usually common most of the Himalayan catchments.  $\text{SiO}_2$ ,  $\text{CaO}$  and  $\text{MgO}$  do not show any impoverishment in composite samples and remain almost same in both *in-situ* and composite samples. Organic carbon too, in all the four profiles indicates uniform distribution. The present studies shows soil of catchment area of River Sewa moderately to highly degraded and prone to soil erosion, landslides and rock falls—leading ultimately to loss of nutrients essential for maintaining the fertility of the soil. The catchment areas should properly be forested, planted with long rooted grass and vetiver grass to control erosion. Various land management practices, such as mixed cropping, intercropping, strip cropping, rotational cropping, mulching, application of organic manures will go a long way to reduce soil erosion and conserve sub-soil moisture effectively.

**Keywords:-** Soil, Sewa river, Catchment, Kathua district, Erosion

### Introduction

River Sewa, a snowfed river that originates from the Siwalik range, is one of the major tributaries of River Ravi. It traverses through the Bani and Basohli blocks of District Kathua, which is situated in the south-east of Jammu and Kashmir State. The northern part of District Kathua is situated in the foothills of Himalaya which includes the Siwalik range. The southern part consists of alluvial plain. Ravi river, which possesses rich power and irrigation potential, flows in the eastern part of the District. Sewa river originates from Sarthal hills, at an altitude of 4200 m above mean sea level on the south-western slopes of the Chattar watershed and after traversing through Bani and Basohli covers a distance of 53 km, then merges with River Ravi at Mashka at an altitude of 578 m.

Sewa river catchment is 481 sq km and the topography is diverse on account of altitudinal gradient from 578 to 4200 m above mean sea level. This catchment has a large number of small and big glaciers. There is a steep gradient at some places along the smaller stretches of River Sewa.

The soil studies in a catchment area of any River is very important dynamic component of ecosystem and is one of the natural resources required for the growth of land plants, the essential supply of water and the nutrients for the plants are available from it. The physical and chemical characteristics of the soil determine the type of vegetation which can be supported by it. The top layer of the soil is a vital component, since all the nutrients required by the plants are present in this layer. In fact, it is the source of 13 elements out of 16 elements essential for the plant growth. The state of Jammu and Kashmir has in all seven types of soils namely Udalfs, Orthents,

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Ochrepts, Ochrepts-Orthents, Ochepts-Orthents-Udalfs, Udolls and soils under glaciers and snow caps. The Sewa river catchment has the ochrepts type of soil (Fotedar, 2006).

These soils are found in lower plains of Kathua District and are mostly alluvial and loamy consisting of little clay contents. The knowledge of physico-chemical composition of the soil and its variation during the different parts of the year is pre-requisite for the proper utilization and management of soil. Since no study has been carried out in respect of soils of Sewa catchment so far, it is thus important to collect the primary data about soils in different profiles. The data needs to be in respect of texture, chemical composition, soil moisture, pH, high and low altitude variation because of erosion and loss of nutrition etc., all these have been studied in detail in the present paper. The four stations in the Sewa catchment have been included in the present study, namely Lowang, Bani, Sarthali and Gatti, all situated in Kathua District, J&K state.

## Materials and Method

Soil samples were collected from four different sampling stations of Sewa watershed representing the higher elevations (*in-situ* i.e. higher elevation in the watershed area) and the stations below the drainage lines near the river banks at correspondingly lower elevations on monthly seasonal basis (Fig. 1). Samples collected from higher elevations were named as higher elevation (*in-situ*) soil samples, and the one which were collected from the stations near the River banks at correspondingly lower elevations were named as composite soil samples. For collecting the soils at the higher elevation i.e. the *in-situ* soil samples, first the overburdened accumulated soils consisting of humus, litter, lots and pots of forest residues, were removed by scaling and digging upto 0-15 cm from the surface and then around 100 gm of soil samples were collected in the polythene bags. Similarly,

composite soil samples were collected at the termination of gullies, near the banks of Sewa river. The sampling of soil was done in this way in order to assess the loss of nutrients.

Soil texture of the samples was determined according to structural triangle method of "US Department of Agricultural Hand Book No. 18" after asserting the percentage of various soil components viz., coarse sand, fine sand, silt, clay and loam by sieving the sample through electrically operated sieve set having sieve mesh numbers 25, 53, 72, 120, 200 and bottom pan. Soil moisture was determined by gravimetric method. Soil sample of known weight was dried in oven at 105 °C for 24 hours. The percentage moisture was calculated by the loss of weight of the sample. Soil pH meter "HANNA" make was used for recording the pH of the soil samples. For this, 1:2 soil water suspensions were prepared and then the pH was recorded. pH was further confirmed in the laboratory by Phillip pH meter.

Seven elemental oxides were determined by the following methods from 'A' solution and 'B' solution according to Shapiro and Brunnock Method (1956).  $Al_2O_3$  was analysed volumetrically using EDTA and Zinc Sulphate (Shapiro and Brunnock, 1956).  $TiO_2$  was determined calorimetrically using Ferric Ammonium Sulphate,  $KMnO_4$  and Ammonium Thiocyanate (Shapiro and Brunnock, 1956). Total iron was determined calorimetrically using Tartaric Acid, P-Nitrophenol, 1-10 Phenanthroline and Hydroxylamine Hydrochloride (Shapiro and Brunnock, 1956).

CaO and MgO was determined volumetrically by EDTA Method (Vogel, 1962 and APHA, 1998).  $Na_2O$  and  $K_2O$  was determined on Flame Photometer (EEL-Model). MnO was determined by using Potassium Periodate (Vogel, 1962). Organic carbon was estimated according to Walkley and Black's rapid titration method.

## Geology in brief

The area from north to south falls in pre-cambrian terrain. In the extreme south, tertiary rocks bind the



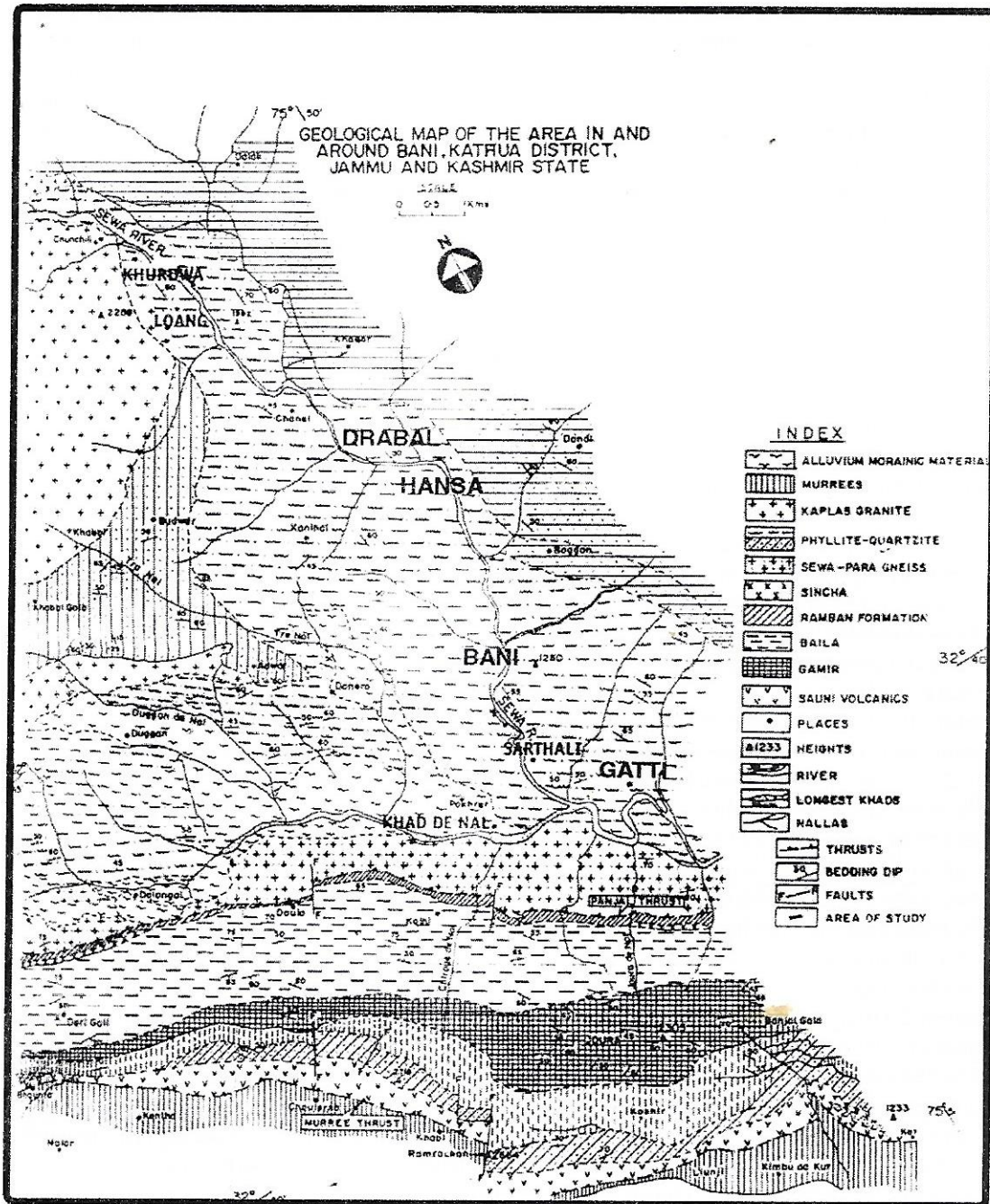


Fig. 1: Map showing sampling site



area. The area is highly folded, faulted and metamorphosed due to two granitic intrusions, one in north-west as Kaplas granite of Bhaderwah and second in the south-west as Dalhousie granite. Besides, two important thrusts, namely Murree Thrust in the south and Panjal Thrust in the north are responsible for formation of anticlines and synclines causing plications in the rocks. Bhaderwah-Chamba is an example of such a syncline and the rocks have been referred to as Bhaderwah Slates panjal Thrust passes east-west above Ramban and Sincha formations to the south of Gatti area. Sewa para gneiss is overlain conformably by phyllite-slate sequence of late-pre-cambrian Salkhala group (Fig. 1). The present area of study falls strictly in late-pre-cambrian Bhaderwah Formation of Salkhala group of rocks. These rocks are acidic in character and constitute mostly granitic and reworked metamorphosed sediments slightly containing more ferromagnesian minerals soils (Dhar *et al.*, 1996).

### **Physico-chemical characteristics of higher elevation and composite soil samples**

#### **Physical parameters**

At station 1 (Lowang), coarse sand varied from a minimum value of 9.7% to 16.0% and the average being 12.6%. Fine sand fluctuated between 8.2% to 21.8% with an average value of 14.0%. Silt concentration varied from 21.7% to 39.4% with an average of 33.5%. Clay concentration ranged between 1.4% to 23.7% with an average value of 18.4%. Loam concentration fluctuated from 11.7% to 30.7% with an average value of 21.5%. The texture class is clay-loam.

At station 2 (Bani), coarse sand varied from a minimum of 9.3% to 16.0% with an average value of 12.35%. Fine sand fluctuated between 9.8% to 17.0% with an average value of 13.17%. Silt varied between 3.8% to 43.9% with an average value of 36.06%. Clay ranged between 11.3% to 27.0% with an average value of 18.20%. Loam ranged between 11.7% to 33.5% with an average value of 20.2%. The texture class is clay-loam.

At station 3 (Sarhali), coarse sand varied between 9.3% to 16.7% with an average value of 13.5%. Fine sand fluctuated between 10.4% to 23.2% with an average value of 14.2%. Silt concentration varied between 19.8% to 45.8% with an average value of 34.0%. Clay fluctuates between 2% to 24.1% with an average value of 19.1%. Loam ranged between 12.9% to 34.6% with an average value of 19.2%. The texture class is clay-loam.

At station 4 (Gatti), coarse sand ranged between 8.3% to 14.2% with an average value of 11.9%. Fine sand ranged between 9.4% to 18.4% with an average value of 13.8%. Silt fluctuates between 25.8% to 45.9% with an average value of 35.7%. Clay fluctuates from 15.6% to 22.1% with average value of 18.4%. Lastly, loam ranged between 14.0% to 33.5% with an average value of 20.2%. Texture class is clay-loam.

#### **Soil moisture:**

Station 1 (Lowang)- Soil moisture varied from 5.4% to 19.2% in case of higher elevation (*in-situ*) soils, whereas in composite soils, moisture varies from 5.4% to 15.0%. The average concentration of moisture does not show much variations.

Station 2 (Bani)- Soil moisture varied from 5.2% to 20.6% in case of *in-situ* soils, whereas in composite soils, it ranges between 5.2% to 17.3%. In higher and lower elevation soils, the moisture does not show much variation.

Station 3 (Sarhali)- Soil moisture varied between 4.5% to 23.8% in higher elevation soils, whereas in lower elevated soils (composite samples), it ranged between 4.5% to 21.8%. Concentration of moisture does not show much variation in this profile.

Station 4 (Gatti)- In higher elevation soils, the moisture fluctuates between 4.7% to 18.2%, whereas in composite soils, it ranges between 4.7% to 17.8%. The moisture condition in this profile for two sites does not show much fluctuation. The moisture condition in higher and lower elevation soils is same because of nearness of Panjal Thrust. The area is highly unstable and due to thrusting, uniform



Table 1: Mean monthly percentage values of soil texture from January 2002 to December 2004

Texture separates	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
Coarse sand	11.4	14.9	11.8	13.3	16.0	15.0	12.0	13.3	9.7	10.1	12.5	11.4	12.6
Fine sand	16.3	10.8	11.0	8.2	9.8	1.6	11.8	12.9	15.2	21.8	16.2	1.5	14.0
Silt	33.8	36.8	38.4	39.4	43.9	39.2	33.2	24.2	27.0	33.2	21.7	31.4	33.5
Clay	19.5	11.3	18.7	17.6	16.3	16.5	18.1	21.4	1.4	18.9	20.9	23.7	18.4
Loam	19.0	26.2	20.1	21.5	14.0	11.7	24.9	28.2	30.7	16.0	28.7	17.0	21.5
Texture class	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay	Clay	Clay	Clay-loam	Clay	Clay	Clay-loam
Coarse sand	9.3	16.0	10.0	13.4	15.0	12.0	14.9	11.8	12.5	11.2	12.0	10.1	12.35
Fine sand	15.8	9.8	11.3	15.9	17.6	11.8	10.8	11.0	15.2	13.9	12.5	12.5	13.17
Silt	34.8	43.9	36.8	31.0	39.2	33.2	3.8	38.4	27.2	25.8	41.1	42.6	36.06
Clay	19.9	16.3	27.0	19.1	16.5	18.1	11.3	18.7	17.1	15.6	19.8	19.1	18.20
Loam	20.2	14.0	12.9	20.6	11.7	24.9	26.2	20.1	28.0	33.5	14.6	15.7	20.2
Texture class	Clay-loam	Clay-loam	Clay-loam	Clay	Clay-loam	Clay	Clay-loam	Clay-loam	Clay	Clay	Clay-loam	Clay-loam	Clay-loam
Coarse sand	13.7	9.3	16.7	16.2	13.0	10.6	13.4	15.8	12.8	11.2	15.2	15.1	13.5
Fine sand	20.6	15.6	11.9	10.4	12.0	11.3	15.9	16.1	19.0	20.0	23.2	17.9	14.2
Silt	29.9	34.8	35.2	35.4	45.8	38.8	31.0	26.5	19.8	29.8	21.9	29.4	34.0
Clay	17.8	19.4	18.5	20.5	15.2	2.0	19.1	17.1	13.6	17.9	18.6	24.1	19.1
Loam	18.0	21.2	17.7	17.5	14.0	12.9	20.6	24.5	34.6	21.1	21.1	13.5	19.2
Texture class	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay	Clay-loam	Clay	Clay-loam	Clay-loam	Clay-loam	Clay-loam
Coarse sand	12.8	12.9	12.2	13.5	12.5	9.6	13.5	12.5	11.2	8.3	9.0	14.2	11.9
Fine sand	17.5	9.9	11.4	9.4	12.7	10.9	16.2	15.2	13.9	12.8	18.4	16.8	13.8
Silt	35.0	36.6	39.3	41.5	41.2	45.9	35.8	27.2	25.8	32.0	37.9	30.7	35.7
Clay	15.8	19.4	22.1	18.0	18.1	16.0	20.5	17.1	15.6	21.4	19.0	18.3	18.4
Loam	18.9	21.2	15.0	17.5	15.5	17.6	14.0	28.0	33.5	25.5	15.7	20.0	20.2
Texture class	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay-loam	Clay	Clay	Clay	Clay-loam	Clay-loam	Clay-loam

Note:- All the readings represent the average of 2 years





**Table 2: Mean monthly values of physico-chemical parameters of soil collected from higher elevated (*in-situ*) and composite samples of Station 1 (Lowang) from January 2002 to December 2004**

Month	Soil moisture (%)	Soil pH	SiO <sub>2</sub> (%)	Al <sub>2</sub> O <sub>3</sub> (%)	ThO <sub>2</sub> (%)	Fe <sub>2</sub> O <sub>3</sub> (%)	CaO (%)	MgO (%)	Na <sub>2</sub> O (%)	K <sub>2</sub> O (%)	MnO (%)	Organic carbon (%)
<b>Higher elevated (<i>in-situ</i>) soil samples</b>												
Jan	14.8	7.1	75.47	12.10	0.19	4.60	2.12	1.82	2.90	3.34	0.03	0.19
Feb	15.0	7.0	75.48	12.12	0.21	1.62	2.14	1.84	2.91	3.35	0.03	0.22
Mar	10.0	7.0	74.43	13.13	0.20	2.61	2.10	1.82	2.91	2.45	0.09	0.59
Apr	6.5	6.6	74.44	13.14	0.22	3.62	2.12	1.84	2.92	2.46	0.09	0.20
May	5.9	5.9	74.45	13.15	0.21	1.63	2.14	1.86	2.93	2.47	0.07	0.30
Jun	5.4	5.7	75.49	12.99	0.20	2.40	2.38	1.82	2.30	2.22	0.03	0.30
Jul	5.6	6.7	75.50	13.00	0.22	2.42	2.40	1.83	2.32	2.27	0.03	0.24
Aug	7.8	6.6	75.51	13.01	0.24	3.44	2.42	1.84	2.34	2.32	0.03	0.22
Sep	13.6	7.0	75.40	11.96	0.14	1.57	1.08	1.48	2.87	3.50	0.03	0.45
Oct	11.9	6.3	75.45	11.97	0.19	1.59	1.10	1.50	2.89	3.53	0.04	0.35
Nov	10.1	6.5	75.50	11.98	0.24	4.61	1.12	1.52	2.91	3.56	0.05	0.14
Dec	19.2	5.8	75.46	12.80	0.20	4.58	2.10	1.80	2.89	3.33	0.03	0.28
Average	10.31	6.5	75.21	12.61	0.20	2.89	1.93	1.74	2.75	2.90	0.04	0.29
<b>Composite soil sample</b>												
Jan	10.8	6.0	62.10	23.10	0.10	3.00	2.10	2.20	1.28	3.00	0.06	0.19
Feb	15.0	6.4	61.90	23.11	0.10	2.10	2.11	2.21	1.29	3.10	0.07	0.18
Mar	9.0	6.0	62.00	23.30	0.11	2.01	1.85	2.20	1.27	2.0	0.06	0.38
Apr	6.5	6.9	62.10	23.10	0.12	2.02	1.90	2.21	1.30	2.00	0.06	0.17
May	5.9	7.0	62.20	22.90	0.13	1.03	1.70	2.10	1.22	2.40	0.06	0.30
Jun	5.4	7.0	62.11	22.09	0.09	1.11	1.79	2.24	1.10	2.29	0.07	0.23
Jul	5.6	5.9	62.12	22.08	0.10	1.12	1.80	2.23	1.08	2.24	0.08	0.24
Aug	7.8	6.6	62.13	22.07	0.11	2.13	1.81	2.22	1.22	2.00	0.08	0.22
Sep	13.6	6.8	60.92	24.15	0.10	2.03	1.90	2.16	1.20	2.16	0.07	0.38
Oct	7.9	6.9	60.93	24.10	0.10	2.04	2.00	2.10	1.24	3.10	0.06	0.35
Nov	8.1	6.6	60.94	23.95	0.10	1.05	2.10	2.24	1.10	3.00	0.06	0.14
Dec	11.2	5.9	62.00	23.09	0.10	1.90	2.09	1.27	1.00	2.00	0.07	0.19
Average	8.73	6.5	61.78	23.08	0.10	1.79	1.95	2.21	1.19	2.55	0.06	0.24

Note: All the readings represent the average of 2 years



**Table 3: Mean monthly values of physico-chemical parameters of soil collected from higher elevated (*in-situ*) and composite samples of Station 2 (Bani) from January 2002 to December 2004**

Month	Soil moisture (%)	Soil pH	SiO <sub>2</sub> (%)	Al <sub>2</sub> O <sub>3</sub> (%)	TiO <sub>2</sub> (%)	Fe <sub>2</sub> O <sub>3</sub> (%)	CaO (%)	MgO (%)	Na <sub>2</sub> O (%)	K <sub>2</sub> O (%)	MnO (%)	Organic carbon (%)
<b>Higher elevated (<i>in-situ</i>) soil samples</b>												
Jan	18.0	7.0	66.27	18.40	0.33	5.00	1.50	1.28	2.40	3.80	0.03	0.24
Feb	17.3	5.8	66.29	18.41	0.36	5.02	1.52	1.30	2.44	3.82	0.02	0.28
Mar	20.6	5.6	66.23	20.02	0.22	4.10	1.56	0.98	1.19	3.90	0.01	0.37
Apr	8.5	7.0	66.24	20.04	0.21	4.12	1.58	0.99	1.20	3.88	0.01	0.24
May	8.2	7.0	66.25	20.06	0.20	4.08	1.57	1.00	1.21	3.92	0.01	0.27
Jun	6.7	6.7	64.13	22.00	0.20	3.96	1.48	0.94	2.08	3.48	0.01	0.16
Jul	9.9	5.9	64.14	22.10	0.21	3.95	1.50	0.96	2.09	3.50	0.01	0.30
Aug	15.2	7.0	64.15	22.20	0.19	3.97	1.52	0.98	2.10	3.52	0.01	0.25
Sep	9.8	6.8	65.42	19.10	0.40	5.10	1.62	1.16	1.65	3.63	0.01	0.48
Oct	10.5	5.7	65.40	19.11	0.38	5.18	1.64	1.18	1.67	3.65	0.01	0.35
Nov	18.5	7.0	65.38	19.09	0.42	5.26	1.66	1.20	1.69	3.67	0.01	0.24
Dec	14.6	7.1	66.25	18.39	0.30	4.98	1.48	1.26	2.36	3.78	0.04	0.37
Average	12.9	6.5	65.51	19.91	0.28	4.56	1.55	1.10	1.84	3.71	0.02	0.29
<b>Composite soil sample</b>												
Jan	12.0	6.6	64.10	20.22	0.20	4.98	1.48	0.90	2.00	3.70	0.03	0.24
Feb	17.3	7.0	64.11	20.23	0.21	4.99	1.49	0.91	2.01	3.71	0.04	0.28
Mar	10.6	7.1	62.90	22.31	0.28	3.98	1.50	0.96	1.09	3.85	0.02	0.37
Apr	8.5	7.0	63.00	22.32	0.32	4.00	1.52	0.98	1.10	3.86	0.02	0.15
May	5.2	6.8	63.10	22.33	0.36	4.02	1.54	1.00	1.11	3.87	0.04	0.27
Jun	6.7	6.3	64.08	21.09	0.19	3.97	1.46	0.95	1.98	3.47	0.02	0.14
Jul	9.9	7.0	64.10	22.00	0.20	3.98	1.48	0.96	1.99	3.00	0.02	0.19
Aug	12.2	7.1	64.12	22.01	0.21	3.99	1.50	0.97	2.00	2.30	0.05	0.20
Sep	9.8	6.8	64.51	23.09	0.36	3.40	1.60	1.05	1.64	2.10	0.06	0.28
Oct	10.5	6.4	64.52	23.11	0.37	3.00	1.62	1.07	1.68	2.00	0.07	0.35
Nov	13.5	7.0	64.53	23.13	0.38	3.10	1.64	1.09	1.72	2.09	0.08	0.24
Dec	10.6	6.2	64.09	20.21	0.19	2.90	1.47	0.89	1.99	2.05	0.09	0.37
Average	10.31	6.7	63.93	21.83	0.27	3.50	1.52	0.97	1.69	2.97	0.05	0.25

Note:- All the readings represent the average of 2 years





**Table 4: Mean monthly values of physico-chemical parameters of soil collected from higher elevated (*in-situ*) and composite samples of Station 3 (Sarhali) from January 2002 to December 2004**

Month	Soil moisture (%)	Soil pH	SiO <sub>2</sub> (%)	Al <sub>2</sub> O <sub>3</sub> (%)	TiO <sub>2</sub> (%)	Fe <sub>2</sub> O <sub>3</sub> (%)	CaO (%)	MgO (%)	Na <sub>2</sub> O (%)	K <sub>2</sub> O (%)	MnO (%)	Organic carbon (%)
<b>Higher elevated (<i>in-situ</i>) soil samples</b>												
Jan	23.8	6.5	65.08	22.35	0.21	3.08	1.08	0.50	2.70	4.30	0.04	0.21
Feb	15.0	5.6	64.90	22.37	0.20	3.04	1.01	0.51	2.78	4.31	0.03	0.32
Mar	9.0	5.6	64.61	23.00	0.18	3.95	1.28	0.52	2.83	3.97	0.02	0.43
Apr	6.5	7.0	60.62	23.01	0.17	3.96	1.30	0.51	2.84	3.98	0.03	0.28
May	6.9	7.0	60.63	23.02	0.19	3.97	1.32	0.53	2.85	3.99	0.01	0.30
Jun	5.4	6.7	61.00	22.20	0.22	4.97	1.11	0.53	2.87	4.00	0.02	0.38
Jul	6.6	5.9	64.10	22.00	0.23	4.98	1.10	0.54	2.86	4.11	0.03	0.24
Aug	7.8	7.0	63.20	21.80	0.21	3.99	1.09	0.55	2.85	4.22	0.01	0.26
Sep	9.6	6.4	66.17	22.20	0.22	3.50	0.72	0.52	2.81	4.56	0.05	0.47
Oct	11.9	5.7	63.18	22.21	0.21	3.52	0.70	0.50	2.82	4.55	0.03	0.39
Nov	10.1	6.8	60.19	22.22	0.20	3.54	0.68	0.48	2.80	4.57	0.03	0.24
Dec	16.2	7.0	64.00	22.36	0.19	3.96	1.03	0.49	2.80	4.29	0.03	0.28
Average	10.73	6.4	63.13	22.39	0.20	3.87	1.03	0.51	2.82	4.23	0.03	0.313
<b>Composite soil sample</b>												
Jan	21.8	6.2	63.68	23.70	0.19	1.98	0.98	0.47	2.78	4.12	0.03	0.21
Feb	16.0	5.0	63.69	23.71	0.20	1.99	0.99	0.48	2.79	4.13	0.05	0.21
Mar	8.0	5.6	64.64	23.65	0.16	1.94	1.00	0.39	2.80	3.98	0.03	0.24
Apr	4.5	7.0	64.60	23.66	0.17	1.96	1.02	0.40	2.83	3.99	0.05	0.28
May	4.9	7.4	64.56	23.67	0.18	1.98	1.04	0.41	2.86	4.00	0.07	0.30
Jun	5.4	6.7	64.90	23.64	0.16	1.90	1.00	0.40	2.80	4.08	0.08	0.29
Jul	6.6	5.9	65.00	23.67	0.18	1.97	1.04	0.41	2.00	2.04	0.08	0.20
Aug	7.8	7.0	65.10	23.70	0.20	2.04	1.08	0.42	2.00	2.01	0.05	0.23
Sep	9.6	6.4	62.27	24.66	0.15	2.48	0.66	0.47	1.80	2.00	0.05	0.29
Oct	10.9	5.7	62.28	24.68	0.20	2.50	0.68	0.49	1.80	1.90	0.04	0.34
Nov	8.1	6.3	62.29	24.70	0.25	2.52	0.70	0.51	1.70	1.70	0.08	0.24
Dec	7.2	7.0	63.67	23.69	0.18	1.97	0.97	0.44	1.00	1.03	0.07	0.28
Average	9.23	6.3	63.89	23.92	0.19	2.10	0.93	0.44	1.42	2.08	0.05	0.25

Note:- All the readings represent the average of 2 years



**Table 5: Mean monthly values of physico-chemical parameters of soil collected from higher elevated (*in-situ*) and composite samples of Station 4 (Gatti) from January 2002 to December 2004**

Month	Soil moisture (%)	Soil pH	SiO <sub>2</sub> (%)	Al <sub>2</sub> O <sub>3</sub> (%)	TiO <sub>2</sub> (%)	Fe <sub>2</sub> O <sub>3</sub> (%)	CaO (%)	MgO (%)	Na <sub>2</sub> O (%)	K <sub>2</sub> O (%)	MnO (%)	Organic carbon (%)
<b>Higher elevated (<i>in-situ</i>) soil samples</b>												
Jan	12.0	6.6	67.13	18.20	0.20	2.85	1.50	0.44	2.84	4.15	0.02	0.14
Feb	10.3	5.8	67.15	18.18	0.22	2.86	1.51	0.43	2.85	4.16	0.02	0.24
Mar	8.6	5.6	67.29	19.20	0.12	1.84	1.54	0.36	2.80	4.15	0.03	0.28
Apr	5.4	7.0	67.28	19.21	0.16	1.86	1.56	0.38	2.78	4.16	0.04	0.18
May	5.2	7.0	67.30	19.22	0.20	1.88	1.58	0.40	2.76	4.17	0.05	0.30
Jun	4.7	6.7	69.11	16.76	0.13	2.83	1.56	0.40	2.12	4.10	0.05	0.19
Jul	8.9	5.9	69.12	16.78	0.12	2.85	1.57	0.41	2.14	4.09	0.06	0.18
Aug	18.2	6.4	69.13	16.80	0.14	2.87	1.58	0.39	2.16	4.08	0.07	0.25
Sep	17.8	6.8	66.67	18.36	0.26	2.88	1.48	0.38	2.76	4.28	0.03	0.40
Oct	10.5	5.7	66.68	18.37	0.24	2.90	1.52	0.41	2.78	4.24	0.04	0.35
Nov	17.5	6.9	66.66	18.35	0.22	2.92	1.56	0.44	2.80	4.20	0.05	0.44
Dec	9.6	5.6	67.14	18.22	0.18	2.84	1.49	0.45	2.88	4.17	0.02	0.38
Average	10.72	6.3	67.55	18.13	0.18	2.61	1.53	0.40	2.63	4.16	0.04	0.27
<b>Composite soil sample</b>												
Jan	9.00	6.7	63.38	23.78	0.19	1.84	1.52	0.39	2.80	4.14	0.02	0.11
Feb	10.30	5.7	63.39	23.80	0.20	1.86	1.54	0.40	2.82	4.16	0.04	0.24
Mar	8.60	6.0	66.00	23.00	0.14	1.80	1.50	0.37	2.75	4.12	0.06	0.20
Apr	5.40	6.9	66.01	23.03	0.15	1.85	1.51	0.38	2.77	4.08	0.07	0.18
May	5.20	7.0	66.02	23.06	0.16	1.90	1.52	0.39	2.79	4.10	0.08	0.24
Jun	4.70	7.0	65.10	24.25	0.09	1.80	0.46	0.34	2.02	3.99	0.08	0.19
Jul	8.90	6.9	65.12	24.30	0.10	1.83	0.48	0.36	2.04	4.00	0.09	0.18
Aug	9.20	6.6	65.14	24.35	0.11	1.86	0.50	0.38	2.06	4.01	0.08	0.25
Sep	17.80	6.8	63.20	24.67	0.18	1.88	1.48	0.36	2.05	3.00	0.07	0.32
Oct	10.50	6.9	63.28	24.68	0.20	1.86	1.50	0.40	2.00	1.80	0.08	0.35
Nov	8.50	6.6	63.36	24.9	0.22	1.84	1.52	0.44	2.00	1.78	0.10	0.24
Dec	9.60	5.9	63.37	23.76	0.18	1.82	1.50	0.38	2.00	1.78	0.05	0.38
Average	8.97	6.5	64.44	23.94	0.16	1.84	1.25	0.38	2.40	3.13	0.06	0.24

Note:- All the readings represent the average of 2 years





drainage flows occur from top to bottom of the rock sequences.

#### Soil pH:

Station 1 (Lowang)- At this station, soil pH fluctuated between 5.6 to 7.1 for higher elevation and 5.9 to 7.0 in composite soils.

Station 2 (Bani)- Values ranged between 5.0 to 7.1 for higher elevation and 6.2 to 7.1 for composite soils.

Station 3 (Sarhali)- At this station, soil pH ranged between 5.6 to 7.0 in higher elevation (*in-situ*) samples, while as for composite samples, it ranged between 5.0 to 7.4.

Station 4 (Gatti)- At this station, the soil pH ranged between 5.6 to 7.0 for *in-situ* soil samples while as for composite soils it ranged between 5.7 to 7.0.

## Results and Discussion

### Physical parameters

As far as textural classification of soils of Sewa catchment is concerned, the soils belong to clayey-loam in texture for all the four stations. From pH measurement data for all the four profiles, acidic nature of the soils is revealed. This is consistent with the acidic character of the rocks (Dhar *et al.*, 1996). The soil pH reflects both, the chemical properties of the mineral soils as well as of the organic residue deposited as litter (Wazir, 1984). However, along the longitudinal profiles for Station 1.0 to 4.0, pH has shown fluctuation from slightly acidic and slightly alkaline. This range of pH favours the breaking of silicate chain aiding the denudation (Wedepohl, 1978). Also, burial metamorphism in Salkhala group of rocks, occurring in Sewa catchment is an additional factor causing erosion. Soil moisture appears to be uniformly distributed in forest soils and soils in the lower elevations. The ability of forest soils to retain water depends upon the amount of silt and clay present. The amount of silt and clay exists almost uniformly distributed (50% or nearly so) and as such moisture does not show much variation. The frequent rains in the upper

reaches and presence of glaciers keeps the moisture conditions almost uniform.

### Chemical parameters

The concentration of silica in composite soil samples in all the four stations is less at almost all stations as compared to higher elevation soil samples, which shows that the area of study has suffers from extensive erosion. Silica is rich in acidic soils as has been reported by Nesbitt and Young (1982), so under favourable pH and metamorphism, the acidic rocks correspond to high values of silica in the soils of Sewa catchment. Along the longitudinal profiles from Station 1 (Lowang) to Station 4 (Gatti), silica recorded high values at Lowang as compared to rest of the stations. The reason for this is that Lowang is surrounded by granitic rocks which are acidic in nature and silica is rich in acidic rocks.

Aluminium oxide in the present study has been found higher in case of composite soils than *in-situ* soils. Aluminium contents get added up in the composite soils, where intensity of erosion is more. The soils have got weathered to a large extent and this has resulted in more clayey type of composite soil rich in aluminium content. This observation is supported by studies of Wedepohl (1978) in the present case. More aluminium in soil samples give indirectly an idea of severe erosion having undergone by the rocks of Sewa catchment. According to longitudinal profile,  $\text{TiO}_2$  in soils of Sewa catchment is lower in composite soils as compared to *in-situ* higher elevation soils. This indicates more of erosion suffered by the rocks of Sewa catchment. This influence is supported by the study of Nesbitt and Young (1982) as  $\text{TiO}_2$  is considered one of the best indicators to assess the rate of weathering. In the present study, ilmenite has been best carrier of  $\text{TiO}_2$  which has impoverished in composite soils with prolonged course of weathering.

CaO percentage in Lowang (Station 1), is the highest points in the area of study. In higher elevation (*in-situ*) soils, the average came to 1.93%, while as in



composite soils it was found as 1.95. In Bani (Station 2), CaO percentage average was found as 1.55 in higher elevation soils, while as in composite soils it came to 1.52%, loss of nutrition being merely 3.0%. In Sarthali profile (Station 3), CaO percentage was 1.03 in *in-situ* soils and 0.93% in composite soils, loss of nutrition being merely 0.1%. In Gatti (Station 4), CaO percentage registered a loss from *in-situ* to composite soils as 0.28%. Albrecht and Smith (1952) considered that calcium-deficiency is prominent feature of the adverse effects of soil acidity upon plant growth. An insufficiency of calcium permits the accumulation of undesirable ions in plants. In all profiles of Sewa catchment, the loss of nutrition that has occurred is not much and cannot be considered undesirable.

Combined alkalis ( $\text{Na}_2\text{O} + \text{K}_2\text{O}$ ) in Lowang were found to be 5.65% in higher elevation soils, while as it was found as 3.74% in case of composite soils. At Bani, the percentage fluctuated from 2.94% to 2.66%. At Sarthali, the percentage dropped from 7.05% to 3.50% and at Gatti, the percentage dropped from 6.79% to 5.53%. The nutrition loss in case of total alkalis, thus, came to be 0.91%, 0.28%, 3.55%, 1.16%, respectively in all the four profiles. Sarthali registers maximum loss of alkalis, because the whole catchment in this profile is barren for the most part.

Mg is very important component of chlorophyll pigment and its deficiency in general can cause chlorosis in plants. In the present study, MgO has been found to lie between 1.74% in *in-situ* soils of Lowang to 2.21% to composite soils; at Bani station, MgO has been found to lie between 1.10% to 0.97% for *in-situ* and composite soils, respectively. At Sarthali, MgO percentage for higher elevation soils was found to be 0.51% and for composite soils as 0.44%. At Gatti, MgO was found to be 0.40% in *in-situ* soils and 0.38% in composite soils.

The present values in all the profiles are in accordance with the values got by Wazir (1984) in the Bhaderwah soils existing in the north of the

present area of study. Overall, MgO percentage in all the profiles of Sewa catchment does not show much impoverishment when we examine values passing from *in-situ* to composite soil samples.

Mg in forest soils is usually from 1/5th to 1/3rd of Ca (Wilde, 1958). In the present case, Mg has been recorded higher than this ratio. Mg in the composite samples on an average is lesser than *in-situ* samples by a margin of 0.15%. The Mg ions in excess produce harmful effects in isolation, but in forest soils, these effects are neutralized by the amount of Ca present in the soils (Puri and Gupta, 1951; Ray and Datta, 1964; Champion and Seth, 1968; Wazir, 1984).

MnO percentage in all the profiles shows an increase from *in-situ* soils to composite soils. Close examination of Tables 2, 3, 4 and 5 reveals that in the longitudinal profile from Lowang to Gatti, Mn gets considerably added up. This is consistent with extensive studies done by Horowitz (1974) in down water soil profiles, which he ascribes to most of Mn getting concentrated by the mechanism of adsorption on fine grained sediments because of large surface area. Similar adsorption and concentration increasing in low profiles has been noticed in Jajjar Nalla, Udhampur, J&K by Fotedar and Loan (2004) and Tikoo (2004) in a tributary of Chenab in J&K.

$\text{Fe}_2\text{O}_3$  found in higher elevation soils of Lowang was 2.89% and in composite soils as 1.79%. At Bani,  $\text{Fe}_2\text{O}_3$  was found as 4.6% in *in-situ* samples and as 3.50% in composite samples. At Sarthali, in high elevation soils (*in-situ*),  $\text{Fe}_2\text{O}_3$  was found as 3.87% going down to 2.10% in composite soils. At Gatti,  $\text{Fe}_2\text{O}_3$  in high elevation soils (*in-situ*) came to be as 2.61% and 1.84% in composite soils. The decrease in concentration observed in four profiles came to be 1.10%, 1.06%, 1.77%, 0.77% in Lowang, Bani, Sarthali and Gatti, respectively.

Organic carbon in Lowang for *in-situ* soils and composite soils is 0.29% and 0.24%, respectively. For Bani *in-situ* samples, it is 0.29% and 0.25%,





respectively. In Sarthali higher elevation, organic carbon is 0.32% for *in-situ* soils and for composite soils only 0.25%. For Gatti, organic carbon is 0.27% for *in-situ* samples and 0.24% for composite samples. In all the four profiles from Lowang to Gatti, there is not much variation as far as organic carbon is concerned.

In conclusion, loss of nutrition of four element oxides has taken place in Sewa catchment, namely  $\text{TiO}_2$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{Na}_2\text{O}$  and  $\text{K}_2\text{O}$ , which can be mitigated by improving the soil by modern silvicultural methods using adequate organic manures.

### Suggestions for control

1. The enthusiastic endeavours by the Forest Department, J&K Government alongwith the local communities, will surely pave a way in checking enormous erosion occurring in the whole area.
2. Diversion of land suitable for sustainable farming to non-farming uses should be checked by legislation.
3. The stoniness in all the transects persists due to high intensity of erosion in the area of study. The stones can be removed manually as there exists manpower and this can make the soil worth fruit cultivation. There exists a huge promise for planting fruit trees in the whole area of study. The climate and soil are both congenial for starting fruit industry.
4. Modernized farming and use of manures is necessary to make soils completely fertile for three crops (rice, wheat and maize) that are mainly cultivated on repose slopes, on both sides of river Sewa.
5. Long rooted grass and vetiver grass should be used as a soil builder in the watershed areas. In many Himalayan terrains, vetiver grass is being used successfully for checking erosion (Rao, 2002; Lavania, 2004).
6. Afforestation in the catchment area is needed on a largescale so as to combat erosion. Forests in barren areas should be started on regeneration principle. It will check erosion and besides this, it

will screen all the contaminants from entering into the solution of the Sewa river, thus protecting riverine ecology.

7. It is necessary to construct a series of staggered contour trenches on slopes, series of stone check dams in gullies and plantation of ecologically suited soil builder species.

8. Besides the above, various land management practices such as mixed cropping, intercropping, strip cropping, rotational cropping, mulching, application of organic manures, appropriate residue management, go a long way to reduce soil erosion and to conserve sub-soil moisture effectively.

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## Phytodiversity of Katarniaghat Wildlife Sanctuary

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### Abstract

The floral diversity in the Wild life Sanctuary is immense. The present documentation indicates the presence of 95 tree species, 57 shrubs, 28 climbers and 23 species of grasses. The main tree species are Sal (*Shorea robusta*), Asna (*Terminalia alata*), Sheesham (*Dalbergia sissoo*), Bel (*Aegle marmelos*), Kusum (*Schieuchera oleosa*), Ficus spp and semal (*Bombax ceiba*), etc. The main grass species occurring in the area are Kaans (*Saccharum spontaneum*) and Moonj (*Saccharum munja*). Calamus tern.

**Keywords:-** Katarniaghat wildlife sanctuary, Bahraich, Flora, Phytodiversity

### Introduction

The Katarniaghat wildlife sanctuary is located in the Nanpara Tehsil of District Bahraich. The Indo-Nepal border constitutes the northern boundary of the WLS. The entire area, totaling 40009.35 ha. is situated between 28° 06' N and 28° 24' N latitude and 81° 02' E and 81° 19' longitude. The sanctuary, together with the adjoining 15002.75 ha. of reserve forest, which serve as buffer, constitutes one ecological unit. It is one of the few remnants of the rich and diverse tarai ecosystems. Katarniaghat Wildlife Sanctuary is one of the most significant representative of highly rich, diverse and fragile tarai ecosystems, presently under threat if not zealously guarded against anthropogenic pressures. The rich soils of tarai coupled with heavy monsoon downpour results in immense floral diversity, which rise to a mosaic of diverse habitats. The whole of the area is subjected to the climate variation typical of the plains of northern India with their extremes of heat and cold. The winter nights are very cold and foggy and heavy dews fall regular, with the result that the vegetation remains damp

for most of the day. The days at this time of year are cool and bright. Frost occur generally in January. The nights remain cool and dew falls until late in spring, the hot weather commencing in April and lasting until the rains break towards the end of June. Heavy monsoon rains fall from then onwards until October and give with the winter rains, an average annual fall of about 1300 mm. The prevailing winds are from the east, but during the hot weather there are often strong west winds and mild hurricanes from the north and west accompanied by showers. Since no study was conducted earlier to catalogue the range of biodiversity available in the same sanctuary and to conserve the known range of biodiversity with emphasis on endangered threatened and rare elementary floras the present work was undertaken to study the flora.

### Materials and Method

The regular survey of the forest area under study was made and sample was collected in separate polythene bags so as to identify the same at rest home or head quarters with the help of available floras Hookers, 1972-1897, Duthie, 1994; Cooke, 1998. The herbarium were prepared as recommended by Jain and Rao, 1967; Jain, 1989; and Rao, 1989.

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## Results and Discussion

The floral diversity in the wild life sanctuary is immense. The present documentation indicates the presence of 95 tree species, 57 shrubs, 28 climbers and 23 species of grasses. The most common plants found in the sanctuary are enumerated as below with their local names.

### Trees

*Mangifera indica*, Linn. (Am), *Cassia fistula*, Linn. (Amaltas), *Emblica officinalis*, Goertn. Syn. *Phyllanthas embilica*, Linn. (Aonla), *Lagerstroemia parviflora*, Roxb. (Asidh, Dhauri), *Terminalia tomentosa*, W. and A. (Asan, Sian), *Acacia arabica*, Willd. (Babul), *Terminalia bellerica*, Roxb. (Bahera), *Crataeva unilocularis*, (Biabarna), *Ficus benghalensis*, Linn. (Bargad), *Aegle marmelos*, Correa. (Bel), *Salix tetrasperma*, Roxb. (Bhainsh), *Semecarpus anacardium*, Linn. f. (Bhilawa), *Hymenodictyon excelsum*, Wall. (Bhurkul, Baurang), *Pterocarpus marsupium*, Roxb. (Biiaisal), *Casearia tometosa*, Roxb. (Chilla), *Butea monosperma* (Lamk) Taub, Syn. *B. frondosa*, Roxb. (Dhak), *Grewia vestita*, Wall. (Dhaman), *Anogeissus latifolia*, Wall. (Dhau 'Bakli'), same as *Asidh* (*L. parviflora* Roxb.) (Dhauri), *Ficus rumphii*, Bl. (Gaihar), *Ficus glomerata*, Roxb. (Gular), *Trewia nudiflora*, Linn. (Gutal), *Adina cordifolia*, Hook. f. (Haldu), *Terminalia chebula*, Retz. (Harra), *Tamarindus indica*, linn. (Imli), *Syzygium cumini*, Linn. Skeels. Syn. *Eugenia jambolana*, Lamk. (Jamun), *Lannea corromandelica*, Houtt. Merr. Syn. *L. Grandis*, Engl. (Jigna jhingan'), *Bavhinia variegata*, Linn. (Kachnar), *Anthocephalus cadamba*, Miq. (Kadam), *Mitragyna pervifolia*, Roxb. Korth, *Stephygyne parvifolia*, Korth. (Kaim 'Phaldu'), *Saccopetelum tomentosum*, HK, F. and T. (Kairauta), *Garuga pinnata*, Roxb. (Kakar 'Kaikar'), *Celtis tetrandra*, Roxb. (Kakai), *Gmelina arborea*, Linn. (Kambhar), *Holoptelea integrifolia*, Planch. (Konju), *Artocarpus heterophyllus*, Lamk. Syn. *A. Integra*, Thunb, Me-rr (Kathal' Jack fruit'), *Acacia catechu*, Willd. (Khair), *Bridelia retusa*,

Spreng. (Khaia), *Phoenix sylvestris*, Roxb. (Khajur) *Careya arborea*, Roxb. (Kumbhi), *Schleichera oleosa*, Lour, Oken. Syn. *S. Trijuga* Willd. (Kusum), *Coridia dichotoma*, Forst. F. Syn. *C. Myxa*, Auct. Plur. Non Linn. (Lisora), *Madhuca indica*, Gmel. Syn. *M. Latifolia*, Roxb. Macbride. (Mahua), *Azadirachta indica*, Juss, Syn. *Melia azadirachata*, Linn. (Neem), *Stereospermum suaveolens*, DC. (Padal), *Ougenia dalbergioides*, Benth. (Panan 'Sandan'), *Putranjiva roxburghii*, Wall. (Patju), Same as *Kaim* (Phaldu), *Buchanania lanzan*, spreng, Syn. *B. latifolia*, Roxb. (Pial), *Eugenia operculata*, Roxb. (piaman), *Ficus religiosa*, Linn. (Papal), *Kydia calycina*, Roxb. (Pula), *Tectona grandis*, Linn. f. (Sagon 'Teak'), *Morus australis*, Poir. Syn. *Morus acidosa*, Griff. (Sahtut), *Shorea robusta*, Goertn. (Sal), Same as *Panan* (Sandan), *Salmalia malabarica*, DC. Schott. and Endl. Syn. *Bombax malabaricum*, DC. (Semal), *Dalbergia Sissoo*, Roxb. (Shisham), *Streblus Asper*, Lowr. (Sihor), *Albizia species*. (Sir-is), *Diospyros tomentosa*, Roxb. (Tendu), *Cedrela toona*, Roxb. (Tun), *Sterculia villosa*, Roxb. (Udala).

### Shrubs

*Dillenia pentagyna*, Roxb. (Agai), *Adhatoda vasica*, Nees. (Arusa), *Pogostemon plectranthoides*, Desf. (Bantulsi), *Zizyphus mauratiana*, Lamk. Syn. *Z. Jujuba*, Lamk. *Ardisia solanaces* (poir) Roxb. *Ardisia humilis*. (Bhakmal 'Majrawa'), *Clerodendrum viscosum*, Vent, Syn. *Clerodendron infortunatum*, Auct. Non Linn. (Bhant), *Ehretia leavis*, Roxb. (Chamror), *Gardenia turgida*, Roxb. (Churga), *Zizyphus xylopyrus*, Willd. (Chittaina), *Glycosmis pentaphylla*, Correa. (Guturu), *Nyctanthes arbortristis*, Linn. (Harsinger), *Barritonia acutangula*, Goertn. (injur), *Tamarix dioica*, Roxb. (Jhau), *Carissa opaca*, Staph. (Karaunra), *Miliusa velutina*, HK. f. and Thom. (Kari 'Dom-Sal'), *Moghania brevipes* Syn. *Flemingia chappar* (Kasraut), *Murraya koenigii*, spreng (Mitha neem), *Holarrhena antidysenterica*, R. Br. (Kura), *Jatropha gossypifolia*, Linn. (Lal arand),





*Calotropis procera*, Br. (Madar), *Randia dumetorum*, Linn. (Maniphal), *Helictres isora*, Linn. (Marorphal), *Colebrookia oppositifolia*, Sm. (Pichera), *Clausena pentaphylla*, DC. (Ratanjot), *Mallotus philippensis*, Syn. *M. philippinensis*, Muell. Arg. (Rohini), *Piliostigma malabaricum*, Syn. *Bauhinia malabarica*, (Sihuli).

#### Scandent shrubs and Climbers

*Acacia pennata*, Willd. (Alis), *Dendrophthoe falcata*, Linn. F. Ettin. Sny. *Loranthus longiflorus*, Derr. (banda), *Calamus tenuis*, Linn. Roxb. (Bent), *Ichnocarpus frutescens*, Br. (Dudhi- bel), *Milletia auricullata*, W & A. Baker. (Gauj), *Tinospora codifolia*, Miers. (Gulod), *Tiliacora aouminata*, Lamk. Miers. Sny. *T. Racemosa*, Colebr. (Karwanth), *Zizyphus oenoplia*, Juss. Mill. (Makoi), *Banhinia vablii*, Linn. W & A. (Maurain), *Vitis latifolia*, Linn./ Roxb. (pani-bel), *Piper longum*, Linn. (Pipal), *Smilx nolifera*, Roxb. (Ram Dataum).

#### Grasses

*Eulaopsis binata*, Retz. C. E. Hubb. Syn. *Ischaemum angustifolium*. Hack. (Baib), *Dendrocalamus strictus*, Nees. (Bens), *Imperata cylindrica*, Linn. Beae. Syn. *Imperata arundinacea*, Cyrill. (Charni 'Puhs'), *Cynodon dactylon*, Pers. (Dub), *Saccharum spontaneum*, Linn. (Kans), *Bambusa bamos*, Syn. B. *Rundinacea*, Willd. (Kanta bans), *Vetiveria zizanioides*, Staph. (Khas), *Arundo donax*. (Kilak), *Erianthus munja* (Muni),

*Pharagmites Karka*, Trin. (Narkul), *Heteropogon contorus*, Roem. (Parua 'Paura'), *Typha elephantina*, L, Roxb. (Pater), *Sclerostachya fusca*, Roxb. A. Camus (Retwa), *Bothriochloa intermedia*, A. Camus, Syn. *Andropogon intermedius*. (Sandhar), *Themeda arundinacea*, Roxb. A. Camus, Syn. *Anthistiria Arundinacea*, Roxb. (Ullah).

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## Occurrence of zooplankton in a perennial freshwater reservoir of Wadgaon Dam during monsoon season at Nagpur District

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### Abstract

The zooplankton occupy a central position between the autotrophs and heterotrophs and form an important link in the food chain of a freshwater ecosystem. The occurrence and abundance of zooplankton is always influenced by physico-chemical factors and the level of nutrients present in the water body. In this context a perennial freshwater reservoir Wadgaon dam is studied with respect to zooplankton population to assess the types of zooplankton present. Qualitative studies on the zooplankton in the Wadgaon dam reservoir situated in the Nagpur district were undertaken in monsoon months to assess the extent of forms present. The reservoir water is uncontaminated and clean with no influence of man made activities in the vicinity. The zooplankton population of the reservoir water is found to be represented by five different groups viz. *Protozoa*, *Rotifera*, *Cladocera*, *Copepoda* and *Ostracoda* represented by about 21 different forms. The present study indicate the uncontaminated nature of reservoir water due to absence of pollution indicator species in the reservoir water.

**Keywords :-** Zooplankton, Autotroph, Heterotroph, Wadgaon dam, Physico-chemical

### Introduction

The inland water bodies are closed ecosystem in which zooplankton hold a key position in the trophic level, food chain and energy flow of the ecosystem. As producers and consumers, plankton play an important role in the transformation of energy from one trophic level to the next higher trophic level ultimately leading to fish production which is the final product of aquatic environment.

The occurrence and abundance of zooplankton in freshwater ecosystems depends on its productivity, which in turn is governed by the physico-chemical parameters and level of nutrients available in the ecosystem. A large amount of work has been done on plankton world-wide by various researcher on various water bodies like Reddy (2001), Kodarkar (1994), Malin (1984), Pai and Berde (2005), Pawar

and Pule (2005), Schindler and Noven (1971). But still there are many reservoirs and water bodies worldwide where there is no work reported till date. So keeping this in view present work on zooplankton was undertaken on a water body situated in Nagpur district of Maharashtra.

### Materials and Method

The Wadgaon dam is a big reservoir situated near butibori on state highway connecting Nagpur to Chandrapur. This beautiful water body has abundant water available through out all the seasons and is a oligo-trophic type of water body with clear water. The zooplankton samples were collected from the littoral zone at two sites by filtering 50 liters of water through plankton hand net of bolting nylon cloth (mesh size 45 µm in early morning hours between 9 to 11 A.M. twice a month. The procedure for collection storage and analysis of samples were followed as per Standard Methods (APHA, 1989). The zooplankton samples were preserved in 4% formalin. The samples were

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identified using Standard literature (Battish, 1992; Edmondson, 1992; Dhanpathi, 2000 and Michel and Sharma, 1988).

**Table 1: List of zooplankton Species in Wadgaon reservoir during monsoon months**

Type of species present in the reservoir water	Month	
	July	August
<b>Protozoa</b>		
<i>Diffugia</i> sp.	+	+
<b>Rotifera</b>		
<i>Asplanchna</i> sp.	-	+
<i>Anuropsis fissa</i>	-	+
<i>Brachionus diversicornis</i>	-	+
<i>Brachionus calyciflorus</i>	+	+
<i>Brachionus felcatus</i>	+	+
<i>Epiphanes senta</i>	+	+
<i>Filinia</i> sp.	-	+
<i>Keratella tropica</i>	-	+
<i>Tesudinea</i> sp.	+	+
<b>Cladocera</b>		
<i>Bosmina</i>	+	+
<i>Chydorus</i>	+	+
<i>Macnethrix</i> sp.	+	+
<i>Simocephalus</i>	+	+
<i>Sida crystallina</i>	-	+
<i>Alona costata</i>	+	+
<b>Copepoda</b>		
<i>Cyclops</i> sp.	+	+
<i>Diaptomus</i>	+	+
<i>Copepod nauplius</i>	+	+
Total forms present in the reservoir water	13	19

## Results and Discussion

The zooplankton in Wadgaon dam reservoir is composed of five distinct groups viz. protozoa, rotifera, cladocera, copeopda and ostracoda represented by about 21 different species. In July month five different groups of zooplankton are

present represented by 15 different forms while in August month 5 different groups of zooplankton are represented by about 21 forms. The protozoa is represented by only one form, rotifera by 9 different forms, cladocera by 6 different forms, copepoda by 3 forms and ostracoda by 2 forms. The ostracoda thrives on fine detritus and as the detritus is available in monsoon in abundance the ostracods occur in large number.

The rotifers play an important role as grazers and suspension feeders within the zooplankton community. The difference in periodicity and population density of different rotifer species can be analyzed by considering the nutritional ecology and biotic interactions. The rotifer species exhibit marked differences in their tolerance and adaptability to changes in physico-chemical and biological parameters. Chandrasekhar (1996) observed that in summer and monsoon the factors like water temp, turbidity, transparency and dissolved oxygen play an important role in controlling the diversity and density of rotifers. In the present study 9 different kinds of rotifers are present in the water body.

The water of the lake was clear with very less turbidity observed in monsoon months i.e. July and August from the sampling points. The present studies confirm that the lake is uncontaminated and free from human interference as no indicator species is found in the water body.

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# Monitoring of heavy metal (zinc sulphate) toxicity by using pollen as Indicators - Pollen of *Vigna unguiculata*: Further Evidence of a Criticism of Banerji and Gangulee (1937), Dharurkar (1971 - Ph.D. Thesis), Nair, Nambudiri and Thomas (1973), Berg (1973), Brandt (1974), Vick and Bevan (1976), Rasmussen (1977), Navara, Horvath and Kaleta (1978), Mhatre (1980 - Ph.D. Thesis), Mhatre, Chaphekar, Ramani Rao, Patil, Haldar (1980), Shetye (1982 - Ph.D. Thesis) and Giridhar (1984 - Ph.D. Thesis) - A Critical Review

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## Abstract

Zinc sulphate affected the germination of seed of *Vigna unguiculata* (L.) Walp. The treatment of 0.1 mg/ml of zinc sulphate showed the adverse effect on the phenology. It caused decrease in the fertility of pollen with an increase in the concentrations. This proves the gametocidal behaviour of the heavy metal. Though it affected the fertility of pollen, however, none of the concentration could bring down the fertility to zero percent. Potentiality of the germinability of pollen was noted in all the 4 series i.e. F, F-24, F-48, F-72 investigated. The heavy metal stimulated the germination of pollen of F, F-24 and F-48 series. All the concentrations of zinc sulphate inhibited the germination of pollen of F-72 series as well as the pollen tube growth of all the 4 series. The present investigation also shows that pollen germination and tube elongation are two different processes differing in their sensitivity to different concentrations of the heavy metal. It is also confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant as an indicator of pollution is a very crude method and rather a wrong choice.

**Keywords :-** Genetics and Plant Breeding, Palynology, Crop Physiology, Heavy Metals

## Introduction

Zinc increases the fibre strength of cotton. It has been also seen that if cotton seeds are soaked in a zinc before sowing or if zinc applied to the roots of cotton seedlings, the zinc is translocated to actively growing parts of the plant. However, an excess of zinc in soil suppresses phosphorus uptake by plants and can cause leaf chlorosis (Bertrand and Wolf, 1961).

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## Materials and Method

Certified seeds of *Vigna unguiculata* (L.) Walp. Var Pusabarsati (cowpea) of Delhi were obtained from the authorized dealers from which healthy seeds were selected. To study the effect of zinc sulphate, 20 seeds of *V. unguiculata* were sown in white-transparent polythene bags (35x25 cm) containing garden soil and each bag was treated with a 500 ml of different concentrations (0.001, 0.01, 0.1, 1, 10, 100, 1000 mg/ml) zinc sulphate immediately after sowing the seeds. The treatment was given on every alternate day till the life cycle of the crop.

A set of control plants was also grown simultaneously with only water in the same quantity as the treated sets. Excess plants were removed after 15 days of sowing leaving the identical and healthy 5 plants in each bag. There were 10 replicates of each treatment. The observations regarding mortality, morphology, anatomy, phenology *etc.* were recorded on every alternate day. After 4 weeks of an uniform flowering, successive flowers (*viz.* F, F-24, F-48, F-72 series *i.e.* open flowers and the flower buds which require 24, 48, 72 hours to open respectively) were plucked at the same time after the dehiscence of anthers (in open flowers). Pollen viability was tested by using 2,3,5-Triphenyl tetrazolium chloride (Hauser and Morrison, 1964). To find out the germination potential of pollen in the bud stage of floral development, the flower buds of various sizes marking the various stages of development and the open flowers were plucked at the same time, after the dehiscence of the anthers (in open flowers). Germination of pollen grains of successive flowers was studied by standing-drop technique in an optimum concentrations of sucrose as: 10% sucrose for F-24 and F-48 series, 20% sucrose for F-72 series and 50% sucrose for F series. The cultures were then transferred to a moist filtered chamber, stored at room temperature (27-31°C) having RH of 53% and in diffuse laboratory light. The experience were run in triplicate and average results were recorded. Observation were made by 24 hours after incubation. For each experiment a random count of 100 grains was made (from different fields of the slide) to determine the pollen viability and germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a magnification of 100x. The data obtained was statically analyzed applying 't' test.

## Results and Discussion

Zinc sulphate affected the germination of seed of *Vigna unguiculata*. The treatment of the lowest

concentration of the heavy metal showed 38.00% seed germination against 50.00% in control, while only 3.50% was noted with the treatment of 1000 mg/ml. Zinc sulphate could not cause cent percent mortality. The treatment of 1 mg/ml of zinc sulphate showed the adverse effect on the phenology, as the result of which F-72 series stopped further flowering after 29 days of their initiation. The treatment of 100 mg/ml inhibited an initiation of flowering of all the series except for F series after 31 days of their initiation, while further an initiation of all the series had been completely suppressed after 32 days of their onset.

Zinc sulphate caused decrease in the fertility of pollen of *Vigna unguiculata* with an increase in the concentrations (Table 1). This proves the gametocidal behaviour of the heavy metal. Salgare and Suwarna Gawde (1991) recorded the gametocidal effect of the heavy metal on the leguminous crop. It should be noted that though zinc sulphate affected the fertility of pollen of *V. unguiculata*, however, it could not bring down to zero percent (Table 1).

As a rule the percentage of pollen germination is always less than the pollen viability (Table 1). However, Banerji and Gangulee (1937) and Dharurkar (1971) reported higher percentage of pollen germination than the pollen viability in *Eichhornia crassipes*. The claim of Banerji and Gangulee (1937) and Dharurkar (1971) is challenged by Salgare (1986a, 95, 2000b, 2006a, 2006i, 2006j, 2006k, 2006m, 2007a, 2007b, 2007d, 2007e, 2007f, 2007h, 2007i) who stated that the observations of Banerji and Gangulee (1937) and Dharurkar (1971) are exaggerating.

Potentiality of the germinability of pollen was noted in all the 4 series *i.e.* F, F-24, F-48, F-72 investigated (Tables 2 and 3). The treatment of 0.001, 0.01, 0.1 mg/ml concentrations of the heavy metal stimulated the germination of pollen of F and F-24 series. The treatment of all the concentrations of zinc sulphate inhibited the germination of pollen of F-48 and F-72





**Table 1: Effect of zinc sulphate (supplied through water) on the fertility of pollen of successive flowers of *Vigna unguiculata***

Conc.	Successive flowers							
	F		F-24		F-48		F-72	
	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%
0.001	66.20±1.98	-14.03	63.20±3.05	-17.92	57.00±3.47	-25.97	53.20±3.02	-30.91
0.01	63.80±2.19	-17.14	61.60±1.39	-20.00	53.20±5.98	930.91	50.60±3.42	-34.29
0.1	61.20±2.79	-20.26	56.80±2.17	-26.23	49.20±2.17	-36.10	44.00±3.08	-42.86
1.0	60.20±4.55	-21.82	56.20±2.08	-27.01	48.40±2.37	-37.14	Nf	Nf
10.0	57.80±2.39	-24.94	55.00±4.50	-28.57	Nf	Nf	Nf	Nf
100.0	56.00±2.55	-27.27	Nf	Nf	Nf	Nf	Nf	Nf
1000.0	APD	APD	APD	APD	APD	APD	APD	APD

Note: APD, all plants died; Conc., Concentrations of heavy metal in mg/ml; DFC, difference from control; Nf, no flowering; T, pollen viability in treated sets. Values given are mean ± SE of 500 (Tested 4 weeks after initiation of flowering)

**Table 2: Effect of zinc sulphate (supplied through water) on the germination of pollen of successive flowers of *Vigna unguiculata***

Conc.	Successive flowers							
	F		F-24		F-48		F-72	
	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%
0.001	24.00±2.82	+60.00	9.40±1.86	+56.67	5.00±1.09	-13.79	3.20±0.86	-33.33
0.01	20.80±1.85	+38.67	8.00±1.89	+33.33	4.20±0.86	-27.59	2.00±0.55	-558.33
0.1	17.80±2.19	+18.67	7.00±2.02	+16.67	3.40±0.68	-41.38	Ng	Ng
1.0	7.80±1.28	-48.00	3.00±0.89	-50.00	2.00±0.45	-65.52	Nf	Nf
10.0	4.20±1.32	-72.00	1.00±0.32	-83.33	Nf	Nf	Nf	Nf
100.0	2.00±0.71	-86.67	Nf	Nf	Nf	Nf	Nf	Nf
1000.0	APD	APD	APD	APD	APD	APD	APD	APD

Note: APD, all plants died; Conc., Concentrations of heavy metal in mg/ml; DFC, difference from control; Ng, no pollen germination; Nf, no flowering; T, pollen viability in treated sets. Values given are mean ± SE of 500 (Tested 4 weeks after initiation of flowering)

series (Table 2). The treatment of all the concentrations of the heavy metal inhibited the pollen tube growth of all the 4 series of *V. unguiculata* (Table 3).

The present investigation also shows that pollen

germination and tube elongation are two different processes differing in their sensitivity to different concentrations of the chemical (Tables 2 and 3). This was also pointed out earlier by the author (1986, 2006c). However, Nair, Nambudiri and Thomas



**Table 3: Effect of zinc sulphate (supplied through water) on the pollen tube growth of successive flowers of *Vigna unguiculata*.**

Successive flowers								
Conc.	F		F-24		F-48		F-72	
	Tin (mm)	DFC%	Tin (mm)	DFC%	Tin (mm)	DFC%	Tin (mm)	DFC%
0.001	158.00±15.91	-21.78	194.00±22.89	-19.17	110.00±16.99	-26.67	32.00±3.74	-36.00
0.01	146.00±15.97	-27.72	184.00±26.52	-23.33	96.00±18.02	-36.00	30.00±7.06	-40.00
0.1	136.00±34.38	-32.67	170.00±18.13	-29.17	68.00±10.66	-54.67	Ng	Ng
1.0	128.00±15.27	-36.63	160.00±14.12	-33.33	40.00±10.47	-73.33	Nf	Nf
10.0	122.00±19.81	-39.60	150.00±18.68	-37.50	Nf	Nf	Nf	Nf
100.0	70.00±7.06	-65.35	Nf	Nf	Nf	Nf	Nf	Nf
1000.0	APD	APD	APD	APD	APD	APD	APD	APD

Notes: APD, all plants died; Conc., Concentrations of heavy metal in mg/ml; DFC, difference from control; Ng, no pollen germination; Nf, no flowering; Tinn, pollen tube length in mm in treated sets  
Values given are mean ± SE of 50 (Tested 4 weeks after initiation of flowering)

(1973) stated that it has been significant that the optimum percentage of germination and tube length were attained in the same growth medium. Present work (Tables 2 and 3) as well as previous extensive work of Salgare (1979, 1983, 1986b, 2004, 2005a, 2005c, 2006b, 2006g, 2006m, 2007h, 2007i), Salgare and Bindu (2002, 2005), Salgare and Tessy Mol Antony (2005a, 2005b) and Salgare and Joshi (2007) it could be concluded that the observations of Nair, Nambudiri and Thomas (1973) are superficial and misleading.

Thus it is confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant (Berg, 1973; Brandt, 1974; Vick and Bevan, 1976; Rasmussen, 1977; Navara, Horvath and Kaleta, 1978; Mhatre, 1980-Ph.D. Thesis; Mhatre, Chaphekar, Ramani Rao, Patil, Haldar, 1980; Shetye, 1982-Ph.D. Thesis and Giridhar, 1984-Ph.D. Thesis) as an indicator of pollution is a very crude method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. This is confirmed in the present critical review (Table. 1) as well as by

the previous extensive work of Salgare (1984, 85a-c, 86c-d, 2000a, 01a-b, 05b, d-e, 06a, c-f, h, j-o, 07a-k), Salgare and Theresa Sebastian (1986, 2006), Salgare and Phunguskar (2000), Salgare and Sanju Singh (2002, 06a-b) and Salgare and Sanchita Pathak (2005).

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## A survey of plants used in homoeopathic pharmacy from Sirwel hills of Madhya Pradesh, India

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### Abstract

The present paper deals with a preliminary survey of plants used in homoeopathic pharmacy from Sirwel hills situated in Khargone district of Madhya Pradesh. In all 35 angiospermic taxa belonging to 33 genera and 24 families reported, which are used in this therapy.

**Keywords:-** *Vegetation, Flora of Khargone, Therapy, Medicinal plants*

### Introduction

Homoeopathic method is based on the principle of "Similia Similibus" which means let likes be treated as likes. According to homoeopathic principles, the system favours "The law of the single remedy". It was first adopted by Heinemann in 1805 who was earlier an allopathic physician. This method treats a particular disease by prescribing minute doses of drugs, which in maximum dose would produce symptom of the disease. The unique feature of this therapy is its small dose. It seems that by a special method of dilution used in homoeopathy, all the energy of the drug is liberated and transferred to the medium of sugar or alcohol. In comparison to the pharmacy of the other systems of medicine, this therapy is much simpler and less complicated. The main preparation under this system is the mother tincture, which is prepared either from fresh drugs by maceration or from dry drugs by percolation processes. These methods more or less follow the same line as done in the allopathic pharmacy.

Sirwel hills (21°59'N Lat., 75°50'E Long. and 666.46 m above msl), where the present survey was done, are a part of Satpura ranges. Biogeographically, it is a part of Central India. Sirwel proper is situated in South-West region of Madhya Pradesh. It is about 60 km away from Khargone bus stand towards South. The average annual rainfall is 770 mm while minimum and maximum temperatures are 11.2°C (in January) and 42.7°C (in May) respectively. The area is rich in bioresources and its detailed survey is yet to be done. Earlier works on homoeopathic pharmacy indicate that only few workers have done research work in this direction (Sanyal, 1982; Chandrakala, 2000). Hence the present investigation was undertaken, which deals with such plants that are being used in homoeopathic therapy to cure various human ailments.

### Materials and Method

The present survey was done during the year 2007-08. For plant collection, various villages, which were visited around Sirwel were Khaparjamali, Raisagar, Palaspur, Kumarbari, Kumi and Salpali. These places are 6, 10, 10, 5, 4 and 6 km away from Sirwel respectively. After plant collection with the help of three homoeopathic practitioners, the uses of such plants were noted that are used in homoeopathic therapy. Herbarium sheet of these plants were prepared after proper drying of

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specimens. Standard literature was consulted for identification of the plants (Cooke, 1957; Shastri, 1977; Solanki, 1984 and Mahajan, 1987). The herbarium sheets

are deposited in the Botany Department of Govt. P.G. College, Khargone for future record. The results are shown in Tables 1 – 2 and Fig. 1.

Table 1. List of medicinal plants used in the formulated Homoeopathy drugs

Plant Name	Family	Phytogeography	Habit	Plant parts
<i>Abus precatorius</i> L.	Fabaceae	Pantropical	Climber	Seeds
<i>Achyranthes aspera</i> L.	Amaranthaceae	Central Asian	Herb	Entire plant
<i>Aegle marmelos</i> Cor.	Rutaceae	Central Asian	Tree	Aerial parts
<i>Allium cepa</i> L.	Liliaceae	Central Asian	Herb	Mature fruits
<i>Allium sativum</i> L.	Liliaceae	Central Asian	Herb	Mature bulb
<i>Andrographis paniculata</i> (Burm. f.) Nees.	Acanthaceae	Tropical Asian	Herb	Entire plant
<i>Azadirachta indica</i> A. Juss.	Melastomaceae	Indo-Malayan	Tree	Aerial parts
<i>Bryonia laciniosa</i> L.	Cucurbitaceae	Europe	Climber	Root
<i>Boerhaavia diffusa</i> L.	Nyctaginaceae	Central Asian	Climber	Entire plant
<i>Calotropis gigantea</i> Br.	Asclepiadaceae	Pantropical	Shrub	Entire plant
<i>Capsicum annum</i> L.	Solanaceae	Tropical America	Herb	Ripe fruits
<i>Carica papaya</i> L.	Caricaceae	Tropical America	Tree	Unripe fruits without seeds
<i>Chenopodium album</i> L.	Chenopodiaceae	Central Asian	Climber	Seeds
<i>Citrullus colocynthis</i> (L.) Schrader	Cucurbitaceae	Mediterranean	Climber	Fruit pulp
<i>Cynodon dactylon</i> Pers.	Poaceae	Africa	Herb	Entire plant
<i>Datura metel</i> L.	Solanaceae	North America	Herb	Entire plant
<i>Eucalyptus globulus</i> Labill.	Myrtaceae	Australian	Tree	Leaves
<i>Euphorbia tirucalli</i> L.	Euphorbiaceae	African	Tree	Leaves
<i>Ficus religiosa</i> L.	Moraceae	Indo-Malayan	Tree	Leaves
<i>Holarrhena antidysenterica</i> Wall.	Apocynaceae	Central Asian	Tree	Leaves
<i>Leucas aspera</i> Spr.	Lamiaceae	Central Asian	Herb	Leaves
<i>Mangifera indica</i> L.	Anacardiaceae	Indo-Malayan	Tree	Fruits
<i>Melilotus indica</i> All.	Fabaceae	Euroasian	Herb	Inflorescence
<i>Ocimum gratissimum</i> L.	Lamiaceae	Central Asian	Herb	Entire plant
<i>Psoralea corylifolia</i> L.	Fabaceae	Central Asian	Herb	Seeds
<i>Rauvolfia serpentina</i> Bth.	Apocynaceae	Indo-Malayan	Shrub	Root
<i>Saraca asoca</i> (Royle) Wille.	Caesalpiniaceae	Indo-Malayan	Tree	Bark
<i>Solanum xanthocarpum</i> Schd.	Solanaceae	Central Asian	Climber	Entire plant
<i>Syzygium cumini</i> (L.) Skeeh.	Rutaceae	Indo-Malayan	Tree	Fruits
<i>Terminalia arjuna</i> (DC) W. and A.	Combretaceae	Indian	Tree	Bark
<i>Tinospora cordifolia</i> Miers.	Menispermaceae	Central Asian	Climber	Stem
<i>Tribulus terrestris</i> L.	Zygophyllaceae	Paleotropical	Herb	Entire plant
<i>Withania somnifera</i> (L.) Dunal.	Solanaceae	Afro-Indian	Shrub	Root
<i>Zingiber officinale</i> Roscoe.	Zingiberaceae	Indian	Herb	Dried rhizome



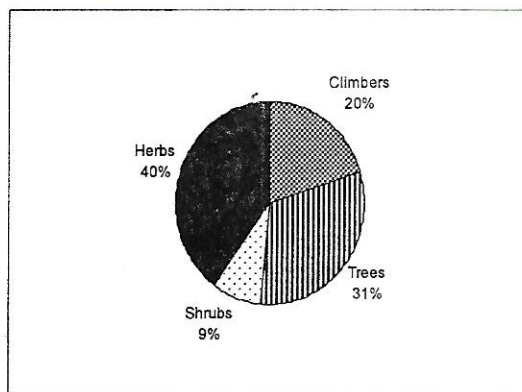


**Table 2. Plant parts used in the formulated Homoeopathic drugs**

Plant parts	No. of species
Entire plant	10
Aerial parts	2
Stem	1
Leaves	5
Rhizome/root	4
Mature bulb	1
Bark	2
Fresh inflorescence	1
Fruits	6

## Results and Discussion

From Table 1 it is revealed that in all 35 plants belonging to 33 genera and 24 angiospermic families are used in the treatment of various human ailments. Out of 35 species, 14 species (40%) exhibit herbaceous, 11 woody (31%), 7 climbing (20%) and 3 shrubby (9%) habitat. The dominance of herbs is possibly due to predominant weed flora occurring in tropical climate. The dominant families are Solanaceae (4 spp.), Fabaceae (3 spp) and Lamiaceae (3sps) while 2 species each belong to Myrtaceae, Apocynaceae, Cucurbitaceae and Liliaceae families. From Table. 2 it emerges that majority of homoeopathic drugs are prepared from the entire plant followed by fruits, leaves and rhizome or roots. It has also been found that 29 taxa out of 35 are indigenous and the remaining 6 taxa are non-indigenous or introduced. Important taxa are *Achyranthes aspera*, *Andrographis paniculata*, *Boerhaavia diffusa*, *Calotropis gigantea*, *Carica papaya*, *Cynodon dactylon*, *Holarrhena antidysenterica*, *Nyctanthes arbor-tristis*, *Ocimum sanctum*, *O.gratissimum*, *Psoralea corylifolia*, *Rawoulfia serpentina* and *Tinospora cordifolia* Sanyal(1982) has mentioned that 67 angiospermic taxa are extensively used by the homoeopathic practitioners in India. According to Chandrakala *et al.* (2004), about

**Fig. 1. Classification of medicinal plants used in formulated homoeopathic drugs based on habit**

127 homoeopathic medicinal plants are used in Tamil Nadu in various formulated preparations. Of these 34 plants sources are considerably used as ingredients in many formulated drugs sold in commercial outlets. In order to make policies for sustainable supply and health care, a detailed study needs to be done on various herbal products.

## Acknowledgement

The author is thankful to all the homoeopathic practitioners who have extended their cooperation to supply useful informations regarding the uses of the collected plants. Thanks are also due to Dr. N.K.Soni, Principal, Govt.P.G.College, Khargone, M.P. for facilities and encouragement.

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## Effect of textile effluent on chemical characteristics of Wunna river of Wardha District (M.S.)

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### Abstract

The paper describes the chemical characteristics of Wunna River at Hinganghat, Wardha district of Maharashtra State. Various chemical characteristics such as BOD, COD, total alkalinity, total hardness, total dissolved solids, chloride, sodium, nitrate, chromium and iron were studied at 3 different station of Wunna River viz. upstream region ( $S_1$ ), effluent mixed region ( $S_2$ ) and 700 m away from effluent mixed region ( $S_3$ ). Marginal variations in chemical characteristics were noticed at station 1 and 3. However, higher values were recorded at station 2 i.e. effluent mixed region. Data analysis indicates heavy pollution of eco system of the river at the site of release of textile industry waste water i.e.  $S_2$ .

**Keywords:-** Analysis, BOD, COD, Chemical characteristics, Eco-system, Textile industry Waste water, Wunna river

### Introduction

Water is one of the most precious gifts of nature. It occupies over 71% of the earth surface of which about 97% is saline in nature while 2.14% water is trapped in the giant glaciers and polar ice caps. This means that not even 1% quantity of water is available for drinking, agriculture, domestic and industrial consumption.

There are 14 major rivers in India that share 83% of the total drainage basin and contribute 85% of the total surface flow (Chaudhari, 1982). Water used by the consumers must be free from microbial contamination, toxic substance and excessive amount of minerals and organic matter (Godi *et al.*, 2003). Anthropogenic activities like rapid industrialization, urbanization and improper waste management techniques lead to heavy pollution of water.

The effluents of one of the textile industries involved in fabric dyeing are being released in Wunna River. Hence, the present investigation was undertaken to assess the changes in chemical characteristics of the river due to release of industrial effluents.

### Materials and Method

#### Wunna River

It originates from Pilkapar rows of Mahargarh valley in Tahsil Katol, District Nagpur of Maharashtra State. The latitude of the river is  $20^{\circ}32'58''$ , the longitude is  $78^{\circ}49'00''$ , and altitude is 214.2 metre. It is the main tributary of river Wardha that joins the Pranhita river which ultimately flows into the Godavari river. People residing in the vicinity of Wunna river heavily depend on it for drinking and other domestic purposes.

#### Study stations

Water samples from three stations of the Wunna river were collected monthly from Station-1 (upstream region), Station-2 (effluent mixed region) and Station-3 (700m away from effluent mixed region) during June 2007 to March 2008 between 10

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A.M. to 11 A.M. The samples were collected 2 meter away from the bank and 0.3 meter below surface to prevent the surface micro layer.

#### Sample collection and analysis

The samples were collected in pre-cleaned plastic

bottles. The samples were brought to the laboratory and stored at 4 °C till the analytical work was carried out. The chemical analysis of samples were undertaken as per American Public Health Association (APHA, 1989) and Bureau of Indian Standards (IS-3025).

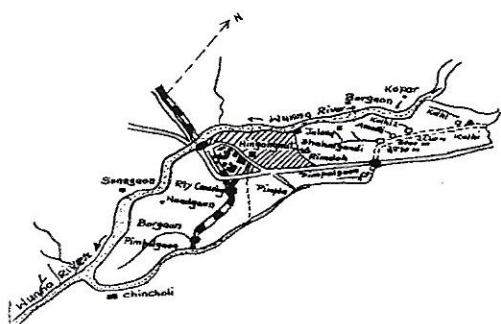


Fig. 1: Location Map of River Wunna at Hinganghat

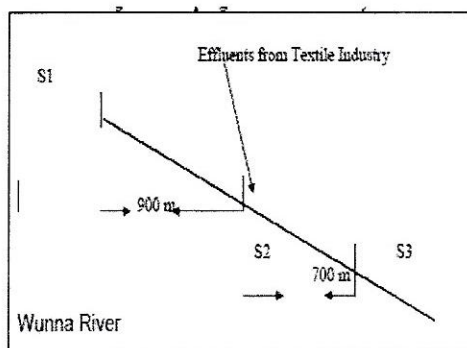


Fig. 2: Sampling locations in the study area

## Results and Discussion

The chemical analysis of samples of three different locations distribution of these parameters for the three stations of the river was analyzed using descriptive statistics and the results are given in the Table. 1 and 2.

**Alkalinity:** The estimated levels of alkalinity at different stations along the course of the river were presented in table 1-2. At station 1, maximum value of alkalinity recorded was 303 mg/l, while minimum value was found to be 105 mg/l. For station 2, the maximum value was 696.6 mg/l and minimum value was found to be 309 mg/l. For station 3, the maximum value was 397 mg/l and minimum value was found to be 132 mg/l.

**Hardness:** At station 1, maximum value of hardness was found to be 332.8 mg/l, while minimum value was 109 mg/l. For station 2, the maximum value was found to be 450 mg/l. For station 3, the maximum value was found to be 327 mg/l and minimum value 128 mg/l. (Table 1-2).

**BOD :** In the present study, the estimated levels of BOD at station 1, maximum value of BOD recorded was 4.9 mg/l, while minimum value observed was 2 mg/l. For station 2, maximum value was 112 mg/l in October 2007 which indicated heavy organic load. While minimum value was 69 mg/l. For station 3, maximum value of BOD recorded was 19 mg/l and minimum value was 13 mg/l (Table 1-2).

**Chemical Oxygen Demand (COD):** The estimated levels of COD at station I had ranged from 23-54mg/l (Table 1). Station II were recorded with minimum level of 240mg/l and the maximum levels of COD recorded were 289 mg/l. At station 3 recorded levels ranged from 55-119 mg/l (Table 2). Variations in the estimated levels of COD at all the stations studied during the period of investigation, station II shows estimated COD levels more than 10 folds increased than the reference site (Station I) due to the influx of industrial waste water (Table 1 and 2).

**Total Dissolved Solids:** At station I TDS levels ranged form 180-290 mg/l during the period of study;



the minimum levels were recorded during the November 2007 and March 2008; the maximum level during the month of January 2008 (Table 1). A station II values ranged from 1600-2050 mg/l (Table 2). And 410-650mg/l respectively at station III (Table 2) Among the stations studied station II was recorded with highest levels of TDS during the period (June 07-March08) of study.

**Table 1: The ranges of values obtained for different parameters**

Parameter	WHO/Indian Standards	Ranges of values		
		Station 1	Station 2	Station 3
Alkalinity(mg/L)	200	105-303	399-696.6	132-397
Hardness(mg/L)	200-300	109-332.8	266.24-450	128-327
BOD (mg/L)	1-2	2-4.9	69-112	13-19
COD (mg/L)	0.5	23-54	240-289	55-119
Total Dissolved solids (mg/L)	500	180-290	1600-2050	410-650
Chloride (mg/L)	200-300	11.1-19.7	750.9-905.1	30-48
Sodium (mg/L)	-	11-19	611-938	29-58
Nitrate (mg/L)	45	1.1-3.1	550-620	40-80
Chromium (mg/L)	0.05	0.02-0.05	0.08-0.10	0.04-0.07
Iron (mg/L)	0.3	0.1-0.2	0.15-0.29	0.12-0.27

January and March 2008 and minimum was 30 mg/l recorded in August 2007. (Table 1-2).

**Sodium:** Sodium was recorded maximum value of 19 mg/l in August 2007 and February 2008 and minimum was 11 mg/l in November 2007. For station 2, maximum value was 938 mg/l in October 2007 and minimum 611 mg/l in February 2008. At station 3, maximum value was 58 mg/l in December 2007 and minimum 29 mg/l in July 2007. (Table 1 and 2).

**Nitrates:** Table 1-2 provided the data on variations in the nitrate levels at different stations along the course of Wunna river. At station 1, maximum value was found to be 301 mg/l in July 2007 and minimum was 1.1 mg/l in February 2008. For station 2, maximum value was 620 mg/l in December 2007 and minimum value was 540 mg/l in November 2007. For station 3, maximum

**Chloride:** Station I were recorded levels of chloride ranging from 11.1 to 19.7 mg/l (Table1) For station 1, maximum value was 19.7 mg/l in October 2007 and minimum 11.1 mg/l in July 2007. For station 2, maximum value observed was 905.1 mg/l in January 2008 and minimum was 750.9 mg/l in July 2007. For station 3, maximum value was 30 mg/l recorded in

**Table 2: The mean and SD values obtained for different parameters**

Parameter	Mean and SD values		
	Station 1	Station 2	Station 3
Alkalinity(mg/L)	213.45±74.39	564.06±93.27	270.88±93.34
Hardness(mg/L)	140.88±67.83	350.02±54.27	164.10±58.78
BOD (mg/L)	3.53±0.79	83.40±12.55	16.40±2.01
COD (mg/L)	32.80±9.08	265±13.39	69.50±18.76
Total Dissolved solids (mg/L)	220.00±3.97	1779.0±13.67	516.0±7.66
Chloride (mg/L)	14.59±2.36	814.34±44.13	38.59±5.52
Sodium (mg/L)	15.90±2.60	776.50±106.27	43.90±10.39
Nitrate (mg/L)	2.00±0.56	588.10±29.74	56.70±12.75
Chromium (mg/L)	0.04±0.01	0.09±0.01	0.06±0.01
Iron (mg/L)	0.14±0.03	0.22±0.05	0.19±0.05

value recorded was 80 mg/l in September 2007 and minimum value was 40 mg/l in the month of March 2008.

**Chromium:** At station 1, maximum value recorded was 0.05 mg/l in the month of October 2007 and February 2008 and minimum was 0.02 mg/l recorded in June 2007. For station 2, minimum value was 0.1 mg/l in the month of August 2007, while minimum value was 0.08 mg/l in December 2007. For station 3, maximum value was 0.07 mg/l in July 2007, while minimum value was 0.04 mg/l in June 2007 (Table 1 and 2).

**Iron:** The estimated levels of iron content at different stations during the period of study were presented in the tables 1-2, maximum value observed was 0.20 mg/l in March 2008, while minimum value was 0.10



mg/l in August 2007. Station 2, maximum value was 0.29 mg/l in March 2008 while minimum value was 0.15 mg/l in January and February 2008. For station 3, maximum value was 0.27 mg/l in January 2008 while minimum value was 0.12 mg/l in August 2007.

### Conclusion

From the exceptionally higher values of BOD, COD, TDS, Hardness, Alkalinity, Sodium, Chloride and Nitrates at station 2 *i.e.* effluent mixed region, it may be concluded that the water of station 2 is unsuitable for drinking and other domestic purposes.

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## Antifungal activity of *Lantana camara* L. and *Syzygium aromaticum* L. against *Candida albicans*

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### Abstract

*Candida* is a commensal organism found in 40-80% of normal humans. Candidiasis is observed in immunocompromised individuals such as HIV-positive patients, it may also occur in blood and genital tract. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients. The *in vitro* antimicrobial activity was performed by Agar disc diffusion and Agar well diffusion method. The crude aqueous fractions and Methanolic extracts of leaves of *Lantana camara* and buds of *Syzygium aromaticum* were investigated for their antifungal activity against yeast *Candida albicans*. Methanolic extract of *Syzygium* was found to be effective against *Candida* while aqueous extract showed no activity. Both aqueous and methanolic extract of *Lantana camara* showed no antifungal activity. These observations could be the basis for the usefulness of the buds of *Syzygium* in treatment remedies for candidiasis or microbial infections.

**Keywords:-** *Candida albicans*, *Syzygium aromaticum*, *Lantana camara*, Antifungal activity

### Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people everyday. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Pidcock and Wise, 1989; Singh *et al.*, 1992; Mulligen *et al.*, 1993; Davis, 1994; Robin *et al.*, 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients (Rinaldi, 1991; Diamond, 1993). In the present scenario, emergence of multiple drug resistance to human pathogenic organisms, has necessitated a search for new antimicrobial

substances from other sources including plants. The increasing resistance to antifungal compounds and the reduced number of available drugs led us to search therapeutic alternatives among aromatic plants and their essential oils, empirically used by antifungal properties (Fontenelle *et al.*, 2007). The use of traditional medicine for the treatment of various diseases has been practiced for generation and a large number of populations in the country use traditional medicines for day to day healthcare needs. (Moshi *et al.*, 2006; Hamza *et al.*, 2006). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microbes, plants and animals. (Janovska *et al.*, 2003). Fungi are opportunistic organisms, which are ubiquitous in nature. Prolonged antibiotic therapy, invasive therapeutic procedures, immunosuppressive therapies and AIDS have all contributed to the rise in systemic fungal infections. (Advani *et al.*, 1992). Ayurveda is the system of traditional medicine prevalent in India since 2000 B.C. Herbal plants from nature

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provide rational means for the treatment of many diseases, which are considered to be obstinate and incurable in other systems of medicine. It is a form of treatment by natural remedies, which makes use of the power of nature to restore human beings to a state of balance. Genuine and pure powdered medicinal herbs of Indian origin are used in herbal medicines, herbal extracts, herbal cosmetics and nutrition foods and other alternative medicines and herbal remedies for natural healings (Heinerman, 1996).

Plants that are traditionally used in treatment of bacterial and fungal infections or related ailments could be a good source for new, safe and biodegradable antimicrobial drugs (Hamza *et al.*, 2006) and could offer potential lead towards

development of novel compounds that are active against pathogenic microbes (Runyoro *et al.*, 2006). In the present communication, an attempt has been made to explore antimycotic principles, which involves an investigation on the efficacy of essential oil against *Candida*. Ethnobotanical data is given in Table 1.

## Materials and Method

### Test Organisms

The strain of *Candida albicans* ATCC 0231 was obtained from American type culture Collection from IMTECH, Chandigarh and was biochemically and serologically characterized by standard methods. The culture was maintained at  $4 \pm 1^\circ\text{C}$  on slants, plates of *Candida* Agar and corn meal agar (HiMedia) and overnight grown culture in sterile glycerol (15%).

**Table 1: Ethnobotanical data of the plants used in the present study**

Botanical name	Family	Common name	Part used	Traditional uses (Chopra <i>et al.</i> , 1992)
<i>Lantana camara</i> L.	Verbenaceae	Ghaneri	Leaves	Decoction used for sore throat
<i>Syzygium aromaticum</i> L.	Myrtaceae	Laung	Bud	Stimulant, carminative used in dyspepsy

### Plant Materials

Leaves of *Lantana camara* were collected from Garhwal region and bud of *Syzygium aromaticum* was procured from local market of Dehradun.

### Preparation of Plant Extracts

Plant extracts were prepared by the methods of Alade and Irobi (1993) with minor modification. Both aqueous as well as alcoholic extracts were used to check the antifungal activity. The leaves of *L. camara* and bud of *Syzygium aromaticum* were air dried, ground and soaked in water for aqueous extract and in methanol for alcoholic extract for 72 hrs with continuous shaking and homogenized using high speed homogeniser (REMI RQ 127 A/D). It was filtered (Whatman No. 1) and extracts were obtained using

soxhlet assembly. The extracts were concentrated in check at  $30^\circ\text{C}$  and stored at  $4^\circ\text{C}$  for further use.

### Inoculum preparation

For the preparation of inoculum, the tested fungus was cultured in mycological broth (HiMedia) at  $37^\circ\text{C}$  for 24hrs and standardized for the same absorbency, number 0.5 of the McFarland Standard, which corresponds to the order of  $10^8$  CFU/ml (Barry and Thornsberry, 1985).

### Antifungal susceptibility testing

The antifungal assay was performed by two methods viz. agar disc diffusion method (Bauer *et al.*, 1986) for aqueous extract and agar well diffusion method (Perez *et al.*, 1990) for solvent extract. The





molten PDA (at 45°C) was inoculated with 100 µl of the inoculum ( $10^8$ cfu/ml) and poured into petri dishes. The plates were allowed to gel for an hour. 50 mg of both extracts were dissolved in 100 µl of sterile ethanol (15%) separately and sterilized by passing through 0.45 µm millipore syringe filter. For agar disc diffusion method, the sterile disc (0.7 cm) Hi-Media, was saturated with 100 µl of both extract, allowed to dry and was introduced on the upper layer of the seeded agar plates separately. Sterile paper disc treated with 15% of sterile ethanol was used as control. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85cm). 100 µl of the both extracts was introduced into the well in different plates. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition (ZOI). Pure solvents instead of extract were used as Control. The result was obtained by measuring zone diameter. The experiment was done three times and the mean values were taken.

#### Minimum inhibitory concentration

MIC method using broth dilution method was applied on extracts that proved their high efficacy against test microorganism by disc diffusion method and agar well diffusion method. The dried plant extract was dissolved in 15% ethanol and serially diluted to fungal broth in order to observe their activities at low concentration. *Syzygium* extract was evaluated against *Candida* by dilution of stock to various concentrations from 100 mg/ml to 6.25 mg/ml. Equal volume of stock and fungal broth was mixed in a test tube. Specifically 0.1ml of standardized inoculum ( $10^8$  CFU/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18-24 h. Two control tubes were maintained for each batch as antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest

dilution) of the extract that produced no visible fungal growth (no turbidity) when compared with the control tubes were regarded as MIC. However, MFC was determined by sub-culturing the test dilution on to a fresh drug-free medium and incubated further for 18-24 h. It is defined as the highest dilution that yielded no single fungal colony.

#### Results and Discussion

The antifungal activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter respectively as shown in Table 2. Alcoholic extract was found to be a better solvent for extraction of antimicrobial active substances compared to water and hexane as also shown by Ahmad *et al.* (1998). The antifungal activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter, respectively as shown in Table 3. The results showed that the growth of *C. albicans* was inhibited by *S. aromaticum*. Methanolic extract was found effective against *Candida* and displayed potent antifungal activity but *Candida* was not inhibited by aqueous extract (Fig. 1 and 2). Zone of inhibition of *Syzygium* against *Candida* was 26 mm obtained after 24 hrs of incubation. MIC was observed at 6.25 mg/ml and MFC was observed at 12.50 mg/ml (Table 4). *Syzygium* extract evaluated against *Candida* by serial two fold dilution of stock concentration (100mg/ml) showed inhibition upto 6.25 mg/ml (Fig. 3). *Syzygium* was more potent and maximum zone of inhibition was observed. Aqueous and methanolic extract of *L. camara* showed no activity, confirming that *Candida* is resistant to this extract. A drug resistant strain of *C. albicans* was found to be sensitive to the tested plant extract. This indicated that antibiotic resistance does not interfere with antifungal action of plant extract. Similar reports on anticandidal activities of these



**Table 2- Antifungal Activity of *Lantana camara* and *Syzygium aromaticum* Zone of Inhibition (mm)**

	<i>Lantana camara</i>		<i>Syzygium aromaticum</i>	
	Aqueous	Methanolic	Aqueous	Methanolic
<i>C. albicans</i>	NI	NI	NI	25

NI: No Inhibition

**Table 3: Antifungal Activity of *Syzygium aromaticum* Zone of Inhibition (mm)**

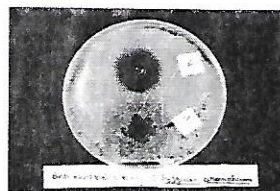
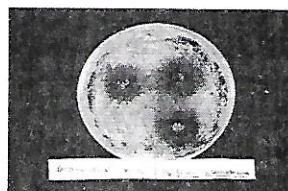
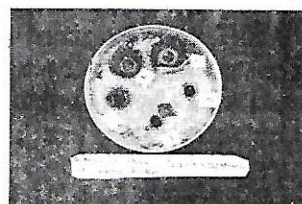
Stock (100 mg/ml)	50.00 mg/ml	25.00 mg/ml	12.50 mg/ml	6.25 mg/ml
25.00	19.00	13.00	10.00	9.00

Note: ZOI in mm; &lt; 10mm- Low activity; 10-20mm- Moderate activity; &gt;20mm- High activity

**Table 4: Minimum Inhibitory concentration (MIC) and Minimum Fungicidal Concentration (MFC) (*Syzygium aromaticum*)**

Organism	MIC (mg/ml)	MFC (mg/ml)
<i>C. albicans</i>	6.25	12.50

plants were also reported by Ahmad *et al.*, 2001 with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction (Nimri *et al.*, 1999). Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but our studies showed that

**Fig.1: Antifungal activity of *Syzygium aromaticum* against *C. albicans***  
A: Methanolic extract B: Aqueous extract**Fig.2: Antifungal activity of *Syzygium aromaticum* (methanolic) against *C. albicans* showing zone of inhibition****Fig.3: Antifungal activity of *Syzygium aromaticum* in various dilutions against *C. albicans***

Well A: 100mg/ml; Well B: 50mg/ml; Well C: 25mg/ml; Well D: 12.5mg/ml; Well E: 6.25mg/ml

methanolic extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in the organic solvent (De Boer *et al.*, 2005). These observations can be rationalized in terms of polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. (Lin *et al.*, 1999).

The results of the present study supports the traditional usage of the studied plants and suggests that some of the plant extracts possess compounds with antifungal properties that can be used as antifungal agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.



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## Bioevaluation of antibacterial potential of Sarpagandha (*Rauwolfia serpentina*)

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### Abstract

The in vitro antibacterial activity of *Rauwolfia serpentina* plant extract has been investigated against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* using the disc diffusion method. The alcoholic extract was found effective against *Staphylococcus aureus* only. The antibacterial activity is attributed to the presence of alkaloids, which was confirmed by gas liquid chromatography and positive alkaloid test. The minimum inhibitory concentration (MIC) was determined by paper disc diffusion method. The results were compared with reference antibiotic tetracycline (one unit solution).

**Keywords:-** Antibacterial, *Rauwolfia serpentina*, Plant extract, Disc diffusion method, MIC

### Introduction

The evaluation of plant extract for their antibacterial activity has been known for more than seventy years (Machat and Kankel, 1920). Various medicinal plants have been used for years in daily life to treat diseases all over the world. The medicinal herbs represent a rich source of antibacterial activity. Herbal medicines are still the mainstay of about 70% of world population for health care (Kaushik and Dhiman, 1999). Evaluation of plant extract for their antimicrobial activity has been done by several workers. Ansari (1995) studied effect of plant extract against the pathogen of leaf sheath blight of rice. Chakroborty and Brantner (1999) studied antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. Earlier literature on antibacterial potential of plants has been reviewed by Kaushik and Dhiman (1999). Antibacterial activity of various plant parts of *Aerva persica* has been tested against human pathogenic bacterial strains and pathogenic fungal species (Gehlot and Bohra, 1998).

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### Materials and method

The plant material of *Rauwolfia serpentina* was collected from state Ayurvedic college, Gurukul, Haridwar, and bacterial strains viz. *Escherichia coli* (MTCC-739), *Staphylococcus aureus* (MTCC-537), *Salmonella typhi* (MTCC-531) and *Klebsiella pneumoniae* (MTCC-432) were self purchased from IMTECH, Chandigarh. For the preparation of plant extract, plant material were first washed 2-3 times with tap water and then again with sterilized double distilled water. Finally the surface sterilization was done with 90 % ethyl alcohol. 100 grams of plant material were crushed in ware blender resulting in the formation of a paste which was mixed in 250 ml of absolute ethyl alcohol. Alcoholic extract so prepared was allowed to evaporate at room temperature until 80 ml of this was left. This extract was squeezed through double layer muslin cloth and filtered through Whatman's filter paper no-42 and was centrifuged at 5000 r.p.m. for 20 minutes and was then sterilized by passing through 0.2 micron disposable filters for primary screening of antibacterial testing procedure, 100%, 50% and 20% dilution of extract were taken.

For antibacterial screening and minimum inhibitory concentration (MIC), agar and disc diffusion



method was used (Bauer *et al.*, 1966). In this method nutrient agar medium was prepared and autoclave and than cooled up to 42- 45 °C. To each 100 ml nutrient agar medium 1.0 ml of 24 hrs old bacterial cultures was added from nutrient broth and then shaken properly to ensure complete distribution of microorganisms in the medium.

The culture medium which was already inoculated with bacterial suspension, was poured in Petri dishes when it was in solid phase, Whatman's filter paper no-42 discs, which were already dipped in different dilutions of the plant extract were placed on nutrient agar surface. D.W. and absolute alcohol served as negative control and the standard antibiotic tetracycline (one unit solution) as positive control. After inoculation plates were kept at 30° C for 24 hrs in incubator. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone in mm. The alcoholic extract was tested for presence of alkaloid by Dragondraff's

test also followed by Stahi (1969) and Gas liquid chromatography (GLC) which was carried under University Science instrumentation centre, Indian institute of Technology (IIT) Roorkee. In GLC HP-5 column and FID detector 250 °C were used for analysis. This column detected alkaloids from extract and solvent was ethyl alcohol.

### Results and Discussion

Results of present investigations clearly indicate that the alcoholic extract of *Rauwolfia serpentina* is effective against *S.aureus* only, and was found non effective against *E.coli*, *S.typhi* and *K. pneumoniae*. The effective zone of inhibition was 24.5 mm against 100 % concentration, 16 mm against 50 % concentration and 8.5 mm against 20 % concentration (Table – 1). The MIC was reported at 14 % extract concentration against *S.aureus*. The results indicated that the undiluted alcoholic extract more effective in comparison to the antibiotic.

**Table 1: The antibacterial effect of *Rauwolfia serpentina* plant extract**

Test organism	Inhibition zones in mm								
	Antibiotic zone in mm	Extract zone (A)			Control alcohol zone (B) in mm	D.W. zone (C)	Effective zone of inhibition (A-B)		
		100%	50%	20%			100%	50%	20%
<i>Staphylococcus aureus</i>	18	29.5	21	13.5	5	Nil	24.5	16	8.5
<i>Salmonella typhi</i>	19	-	-	-	5	Nil	-	-	-
<i>Escherichia coli</i>	17	-	-	-	5	Nil	-	-	-
<i>Klebsiella pneumoniae</i>	18	-	-	-	5	Nil	-	-	-

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## Impact of fabric dyeing effluents on the physical parameters of Wunna river in Wardha district of Maharashtra

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### Abstract

The physical parameters like temperature, odor, electrical conductivity, turbidity and pH of Wunna River in Maharashtra (India) at three different stations (upstream region- S<sub>1</sub>, effluent mixed region- S<sub>2</sub> and 700 m away from effluent mixed region-S<sub>3</sub>) were studied during the pre, mid and post-monsoon season. Marginal variations were recorded in temperature and turbidity while rigorous effect of effluent discharge on odor, electrical conductivity and pH was observed at the effluent mixed region as compared to the other stations. The flow of water in the river in different seasons was found to be an important factor responsible for variable impact of pollution. The data analysis confirms a great extent of pollution of the riverine eco-system at the site of release of textile industry effluents.

**Keywords:-** Analysis, Eco-system, Pollution, Textile industry effluent, Wunna river

### Introduction

Rivers are the lifeline of living beings as a source of drinking water and fish culture. There are 14 major rivers in India that share 83% of the total drainage basin and contribute 85% of the total surface flow (Chaudhari, 1982). Water used by the consumers must be free from microbial contamination, toxic substance and excessive amount of minerals and organic matter (Godi *et al.*, 2003). The pollution of water may be attributed to anthropogenic activities like rapid industrialization, urbanization and improper waste management techniques (Rao and Rao, 1995; Todd, 1995, Ranga Raj *et al.*, 1996).

The effluents of one of the textile industries involved in fabric dyeing are being released in Wunna river. At the moment, very scanty information on study of physical parameters of Wunna river is available. Hence to know the

changes in physical parameters of the river due to release of industrial effluents, the present investigation was undertaken.

### Materials and Method

#### Wunna river

It originates from Pilkapar rows of Mahargarh valley in Tahsil Katol, District Nagpur of Maharashtra State. The latitude of the river is 20°32'58", the longitude is 78°49'00" and altitude is 214.2 metre. It is the main tributary of river Wardha that joins the Pranhita river which ultimately flows into the Godavari river. People residing in the vicinity of Wunna river heavily depend on it for drinking and other domestic purposes.

#### Study stations

Water samples from three stations of the Wunna River were collected monthly from Station-1 (upstream region), Station-2 (effluent mixed region) and Station-3 (700 m away from effluent mixed region) during June 2007 to March 2008 between 10 A.M. to 11 A.M. The samples were collected 2 meter away from the bank and 0.3 meter below surface to prevent the surface micro layer.

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### Sample collection and analysis

The samples were collected in pre-cleaned plastic bottles. The spot tests for temperature and pH were done instantly after collection of samples at the sampling sites. The samples were brought to the laboratory and stored at 4 °C till the analytical work was carried out. The physical analysis of samples were undertaken as per American Public Health Association (APHA, 1989) and Bureau of Indian Standards (IS-3025).

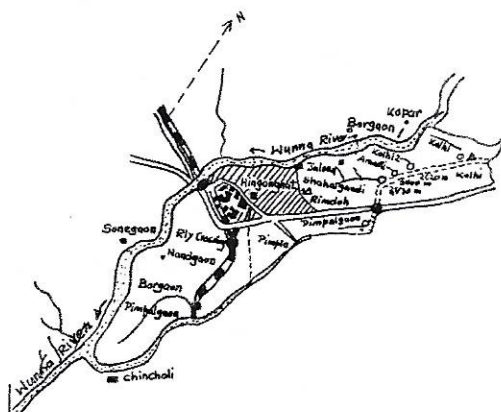


Fig. 1: Location Map of River Wunna at Hinganghat

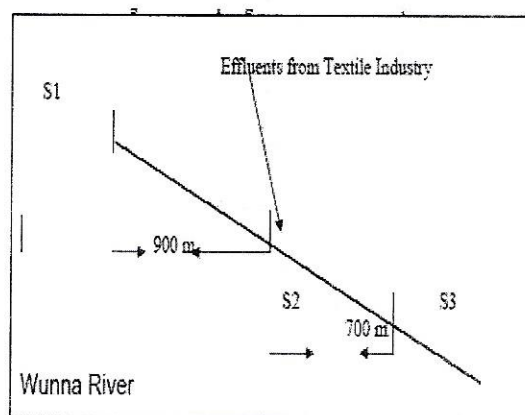


Fig. 2: Sampling locations in the study area

### Results and Discussion

The physical analysis of samples of three different locations distribution of these parameters for the three stations of the river was analyzed using descriptive statistics and the results are given in the following table.

Table 1: The mean and SD values obtained for different parameters

Parameter	WHO standards	Mean and SD value		
		Station-1	Station-2	Station-3
Temperature(°C)	--	25.05±3.45	25.67±3.82	25.24±3.50
Electrical conductivity (µS/cm)	750-2000	426.80±91.94	2739.40±827.02	499.70±97.12
Turbidity(N.T.U)	5-10	50.70±4.52	46.10±2.38	51.70±3.16
pH	6.5 -8.5	7.79±0.25	7.95±0.37	7.85±0.15
Odor	UO	UO	O	PO

The present study was undertaken to evaluate changes in the river water temperature along the course of Wunna River, the changes in the recorded levels of temperature are presented in Table 1.

**Temperature:** The maximum temperature recorded at station 1 was 30 °C in the month of June 2007, while minimum temperature 20 °C recorded in the month of December 2007. At station 2, the maximum temperature recorded was 31.4 °C in June and July 2007 while minimum temperature was 21.1 °C in the month of December 2007. For station 3, the maximum temperature recorded was 30.4 °C in the month of June 2007 while the minimum temperature recorded was 20.2 °C in the month of December 2007 (Fig.-3). The rise in temperature at effluent mixed region may be attributed to the heat generated by the effluents.

**Electrical Conductivity:** Table 1 provides variations in EC at all the stations study. At station 1, maximum conductivity recorded was 545 µS/cm in the month of October 2007, while minimum value recorded was 308 µS/cm in the month of June 2007. Station 2, maximum value recorded was 4580 µS/cm in the



month of August 2007, while minimum value recorded was 1814  $\mu\text{S}/\text{cm}$  in June 2007. At station 3, maximum value recorded was 609  $\mu\text{S}/\text{cm}$  in the month of December 2007 and minimum value recorded was 354  $\mu\text{S}/\text{cm}$  in June 2007 (Fig. 4). The maximum value recorded at station-2 is well above the maximum permissible limit stated by WHO.

**Turbidity:** The variations in the turbidity (NTU) levels estimated at all stations was presented in table 1. At station 1, the maximum value of turbidity recorded was 58 N.T.U. in July 2007, while minimum value recorded was 43 N.T.U. in February 2008. For station 2, maximum turbidity observed was 50 N.T.U. in June and July 2007, while minimum turbidity observed was 43 N.T.U. in June 2007. For station 3, the maximum value of turbidity recorded was 53

N.T.U. in the month of August 2007 and December 2007 while minimum turbidity observed was 46 N.T.U. in February 2008 (Fig. 5).

**pH:** At station 1, the maximum pH value recorded was 8.4 recorded in the month of June 2007. While minimum value recorded were 7.5 in the month of January 2008. For station 2, the maximum pH value recorded was 8.68 in July 2007, while minimum value recorded was 7.46 in the month of March 2008. For station 3, the maximum value of pH recorded was 8.2 in the month of June 2007, while minimum recorded value of pH was 7.71 in the month of July 2007 (Fig. 6). The values of pH at the study stations clearly indicate the alkaline nature of water. Maximum pH value recorded at station -2 exceeds WHO permissible limits.

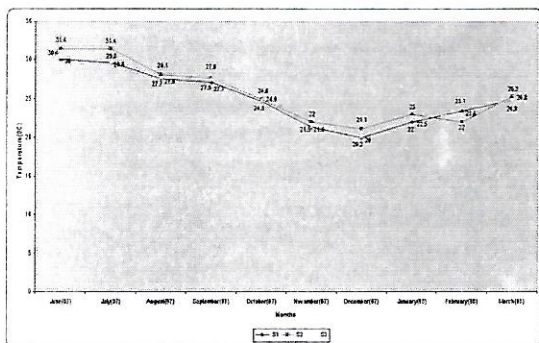


Fig. 3: Temperature recorded at different stations

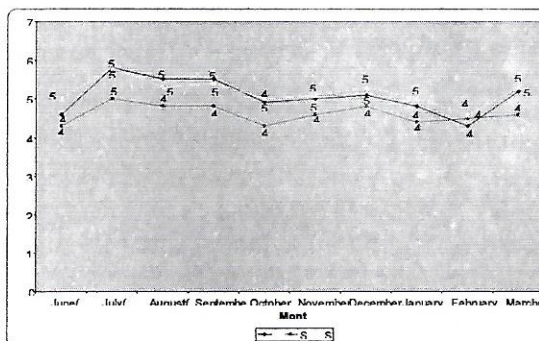


Fig. 5: Turbidity (NTU) recorded at different station

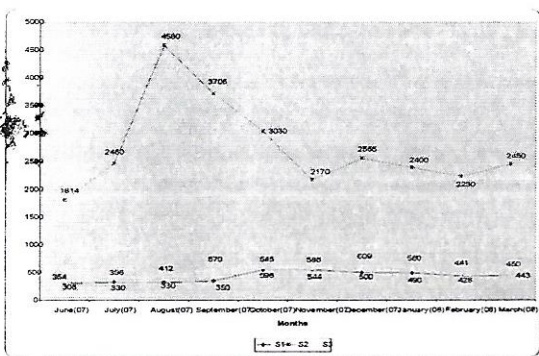


Fig. 4: Fluctuation in electrical conductivity ( $\mu\text{S}/\text{cm}$ ) recorded at different stations

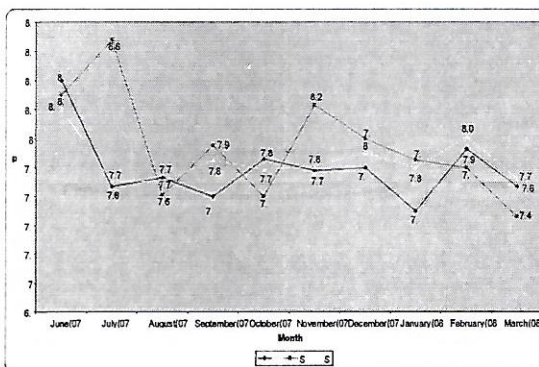


Fig. 6: pH recorded at different stations

## Conclusion

The discharge of textile industrial effluents into the Wunna river is known to alter the hydrographical parameters at different levels in the stations studied. Textile industrial effluents influx in to the river may be considered as pollutant added to the river water thereby imparting bad odour along the course of the Wunna river at site-2 of the study area. As a result of this water of station-2 (S-2) has become unsuitable for drinking. These tremendous changes in the hydrography of the Wunna river could be expected to exert deleterious effects on the river ecosystem along the course of the down stream.

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## Antimicrobial activity of *Vitex negundo* leaf extracts

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### Abstract

Methanol, diethyl ether and acetone extracts of leaf of *Vitex negundo* were tested for their antibacterial activity against two human pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and one fungus *Aspergillus niger* using the disc diffusion method. It was found that all the extracts produced inhibitory effect but the methanol extract of leaves exhibited a superior level of antimicrobial activity. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was determined by broth dilution method. The results were compared with the reference antibiotics.

**Keywords:-** Antibacterial activity, *Vitex negundo*, leaf extract, MIC

### Introduction

Infectious diseases are a serious problem worldwide and account for high proportion of health problems in the developing countries (Sashikumar *et al.*, 2003). On the other hand some of the drugs currently in use result in adverse side effects (Covington, 1988). Therefore the search for new antimicrobial substances exhibiting minimal side effects is warranted (Kandil *et al.*, 1994). One of the most promising area in the search for new biologically active compounds are the plants used in traditional medicine (Alonso *et al.*, 1995).

*Vitex negundo* (verbenaceae), a large aromatic shrub upto 4-5 meters in height is found throughout the greater part of India growing upto an altitude of 1500m in the outer Himalayas. In addition to India, it is also found in Sri Lanka, Burma, China, Pakistan, Afganistan, Malaysia, tropical Africa and the Philippines. It can be propagated readily by vegetative cutting. The leaves of *Vitex negundo* are reported to possess

pesticidal, antifungal and antibacterial properties. The plant is also used as a commercial drug in the indigenous system of medicine (Anon, 1976).

### Materials and Method

The present investigation was conducted to evaluate the antimicrobial potential of leaf extracts of *Vitex negundo* against three pathogenic microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Aspergillus niger* which was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Local isolates were obtained from B.H.E.L Hospital, Haridwar.

The matured leaves of *Vitex negundo* were collected from Haridwar and its adjoining areas, dried in shade and crushed in mortar. The crushed leaves were extracted with methanol, diethyl ether and acetone for 24h using Soxhlet apparatus. The solvent was removed in rotary evaporator and the crude extract was used. Three different dilutions of the extracts will be prepared in dimethyl sulphoxide (DMSO). The working concentrations of the extracts were 200, 400 and 800 mg mL<sup>-1</sup> respectively.

The antibacterial activity was tested *in vitro* by disc diffusion assay (Bauer *et al.*, 1966) on nutrient agar medium (NAM) and Sabouraud's dextrose agar medium (SDA) by taking Whattmann no 42 filter

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paper discs which was saturated with 10 $\mu$ l of different dilutions of different extracts. DMSO as negative control and ciprofloxacin, gentamycin and clotrimaxazole (500ppm) were used as positive control for *S. aureus*, *P. aeruginosa* and *A. niger* respectively. The plates were incubated at 37°C for

**Table 1: Inhibition zones of the leaf extracts of *Vitex negundo* against *Staphylococcus aureus***

Extract	Concentration (mg/ml)	*Effective zone of inhibition	
		<i>S. aureus</i> (MTCC)	<i>S. aureus</i> (local)
Methanol	800	17.6** $\pm$ 0.57	13.6** $\pm$ 0.57
	400	14.6** $\pm$ 0.57	12.0** $\pm$ 0
	200	11.3** $\pm$ 0.57	9.6** $\pm$ 0.57
Diethyl ether	800	17.3** $\pm$ 0.57	13.3** $\pm$ 0.57
	400	12.0** $\pm$ 1.0	11.3** $\pm$ 0.57
	200	9.6** $\pm$ 0.57	9.3** $\pm$ 0.57
Acetone	800	17.0** $\pm$ 0	12.0** $\pm$ 1.0
	400	14.3** $\pm$ 0.57	10.6** $\pm$ 0.57
	200	10.0** $\pm$ 0	9.3** $\pm$ 0.57
Ciprofloxacin	500ppm	15** $\pm$ 1.0	12** $\pm$ 1.0
DMSO	100%	-	-

Note: Values are the average of 3 replicates  $\pm$  SD, \*Effective zone of inhibition=Total zone of inhibition-diameter of the disc(5mm), \*\*Significant at 0.05 %level

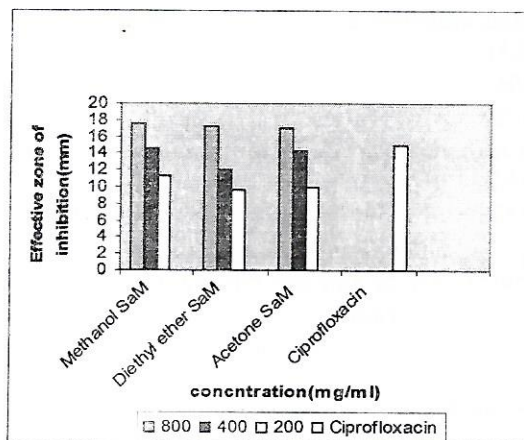
24 h in case of bacteria and at 28 °C for 5 to 7 days in case of fungi. The activity was measured in terms of diameter of inhibition zone appearing around the filter paper discs saturated with leaf extracts. The tests were made in triplicates.

The minimum inhibitory concentration (MIC) was determined in sterile microtiter plates each having 24 wells (Forbes et al., 1998). 1 $\mu$ l of methanol extract was taken from the stock solution having a concentration of 800mg mL<sup>-1</sup> and serially diluted using nutrient broth. 0.1  $\mu$ l of the bacterial and

**Table 2: Inhibition zones of the leaf extracts of *Vitex negundo* against *P. aeruginosa***

Extract	Concentration (mg/ml)	*Effective zone of inhibition	
		<i>P. aeruginosa</i> (MTCC)	<i>P. aeruginosa</i> (local)
Methanol	800	12.3** $\pm$ 0.57	10.6** $\pm$ 0.57
	400	10.3** $\pm$ 0.57	8.0** $\pm$ 1.0
	200	8.3** $\pm$ 0.57	5.3** $\pm$ 0.57
Diethyl ether	800	11.6** $\pm$ 0.57	9.0** $\pm$ 1.0
	400	9.3** $\pm$ 0.57	6.3** $\pm$ 0.57
	200	6.6** $\pm$ 1.15	3.3** $\pm$ 0.57
Acetone	800	10.3** $\pm$ 0.57	9.6** $\pm$ 0.57
	400	8.3** $\pm$ 0.57	7.6** $\pm$ 0.57
	200	5.6** $\pm$ 0.57	6.0** $\pm$ 0
Gentamycin	500ppm	11** $\pm$ 1.0	9** $\pm$ 1.0
DMSO	100%	-	-

Note: Values are the average of 3 replicates  $\pm$  SD, \*Effective zone of inhibition=Total zone of inhibition-diameter of the disc(5mm), \*\*Significant at 0.05%level



**Fig. 1. Comparative analysis of the activity of leaf extract of *V. negundo* and that of antibiotic control against *S. aureus***





**Table 3: Inhibition zones of the leaf extracts of *Vitex negundo* against *A. niger***

Extract	Concentration (mg/ml)	*Effective zone of inhibition	
		<i>A. niger</i> (MTCC)	<i>A. niger</i> (local)
Methanol	800	10.3**±0.57	7.6**±0.57
	400	7.6**±0.57	5.3**±0.57
	200	5.3**±0.57	3.6**±0.57
Acetone	800	9.3**±0.57	7.0**±1.0
	400	5.6**±0.57	5.3**±0.57
	200	4.0**±1.0	3.6**±1.15
Diethyl ether	800	6.6**±0.57	5.0**±1.0
	400	5.0**±0	3.6**±0.57
	200	3.6**±0.57	2.0**±0
Clotrimazole	500ppm	10**±1.0	14±0.47
DMSO	100%	-	-

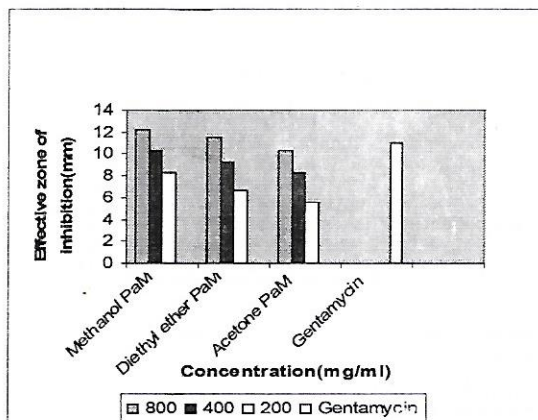
Note: Values are the average of 3 replicates ± SD, \*Effective zone of inhibition=Total zone of inhibition-diameter of the disc(5mm), \*\*Significant at 0.05% level

**Table 4: MIC (Minimum inhibitory concentration) of methanol extract of *Vitex negundo* against *S. aureus***

Replicates	Minimum inhibitory concentration (MIC in mg/ml)	
	<i>S. aureus</i> (MTCC)	<i>S. aureus</i> (local)
1	12.5	25
2	12.5	25
3	6.25	12.5
Mean	10.4	20.8
SD	±2.9	±5.8

**Table 5: MIC (Minimum inhibitory concentration) of methanol extract of *Vitex negundo* against *P. aeruginosa***

Replicates	Minimum inhibitory concentration (MIC in mg/ml)	
	<i>P. aeruginosa</i> (MTCC)	<i>P. aeruginosa</i> (local)
1	25	50
2	25	50
3	12.5	25
Mean	20.8	41.6
SD	±5.8	±7.2



**Fig. 2. Comparative analysis of the activity of leaf extract of *V. negundo* and that of antibiotic control against *P. aeruginosa***

fungal inoculum was added to each well and incubated. The tests were made in triplicates. The lowest concentration (highest dilution) of the plant extract preventing the turbidity is considered to be the MIC.

## Results and Discussion

All the three dilutions of the leaf extract showed antimicrobial activity and the 800 mg/ml concentration was most effective and was

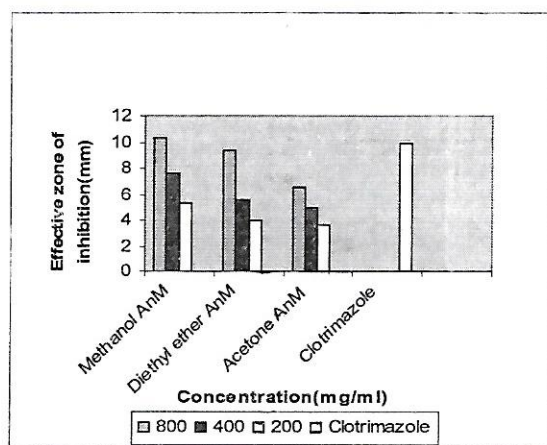


Fig. 3. Comparative analysis of the activity of leaf extract of *V. negundo* and that of antibiotic control against *A. niger*

Table 6: MIC (Minimum inhibitory concentration) of methanol extract of *Vitex negundo* against *A. niger*

Replicates	Minimum fungicidal concentration (MFC in mg/ml)	
	<i>A. niger</i> (M TCC)	<i>A. niger</i> (local)
1	200	200
2	200	400
3	400	400
Mean	41.6	333.3
SD	± 94.2	± 94.2

comparable to the one unit solution of antibiotic (Table 1, 2 and 3 and Fig. 1, 2 and 3). The minimum inhibitory concentration (MIC) of the methanol extract of *V. negundo* against *S. aureus*, *P. aeruginosa* and *A. niger* was found to be 10.4, 20.8 and 41.6 mg/ml respectively and the minimum bactericidal concentration (MBC) and

minimum fungicidal concentration (MFC) was found to be 41.6, 83.3 and 800 mg/ml respectively (Table 4, 5 and 6). The extract was found to be cidal in nature as there is no visible growth after subculturing the sample from the wells. Thus the results confirm the presence of antimicrobial components in the leaves of *Vitex negundo*.

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## Abiotic factors of Yashwant Nagar Taalab, Mhow (M.P.)

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### Abstract

In the present paper the status of water quality of Yashwant Nagar Taalab was determined. The different parameters which were analyzed during the study are temperature, conductivity, pH, total suspended solid, DO, BOD, silicates, chloride, nitrate, phosphate, sulphate. During course of study minor difference were observed in parameter studied.

**Keywords:-** Abiotic factor, Yashwant Nagar

### Introduction

Aquaculture demands productive status of water but productivity of water body is determined by its abiotic and biotic factors. Thus for the aquaculture point of view both the factors of urban water body in have sufficient role in limnological study. A lot of work has been done on abiotic factors of urban water body in comparison to rural water body.

### Materials and Method

For the present study a perennial lentic water body- Yashwant Nagar Taalab was selected, situated on Mhow- Manpur, AB Road at a distance of about 23 km from Mhow. Geographically it is located 22.37° 30" F 75.37° 00" W at 579.2 m above the mean sea level. Yashwant Nagar Talab is a multipurpose water body, used to irrigate the adjacent agriculture lands to provide drinking water *etc.* to near village population. Water samples were collected monthly in sterilized glass bottles throughout the period of investigation from surface. Collection time was between 8 A.M. to 11 A.M. collected samples were kept in an ice-box and brought to the laboratory for further analysis. Analysis of various parameters were done according to the standard method of APHA (1989).

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### Results and Discussion

The mean values of physico-chemical parameters of water present water are described in the Table 1 and 2, the physico-chemical character of water plays an important role in maintaining pond eco-system. Which directly have relationship with biological characters of pond.

The temperature is the key parameters governing metabolic activity of aquatic organisms. Shaikh (1993) observed surface water temperature range 16.5 to 30.5 °C at Hathikheda reservoir. Prakash (1994) recorded the temperature variation between 17.1 °C to 39.5 °C at Mehta pond Jhabua. Shukla (1995) observed water temperature range in between 17.20 to 34.20 at Gandhi Sagar reservoir. Dave *et al.* (1999) reported surface water temperature range from 16.2 to 30.4 °C (1998-90) and 14.0 to 30.2 °C (1990 -91) in Kaila pond, Dhar. Ansari and Prakash (2000) reported water temperature range from 12.0 to 33.0 °C with maximum and minimum value in summer and winter months in Motisagar Tal (U.P.) The range of surface water temperature temperature of Yashwant Sagar, Indore was from 13.0 to 31.0 (Shrivastava, 2002). Pathak (2004) observed surface water temperature range in between 19.2 to 34.2 at Virla resvoir Khargone (M.P.). Garg *et al.* (2005) described that water temperature ranged 19.15 to 29.52 °C in Harshi reservoir Gwalior (M.P.). In the present investigation surface water temperature ranged from 14.44 °C (Dec.) to 32.22 °C (May) during

Table 1: Mean value of physico-chemical parameters of Yashwant Nagar Taalab, Mhow 2005-2006

S.N.	Parameter	Unit	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
1	Air temp	$^{\circ}\text{C}$	29.11	33.54	30.11	25.22	23.54	22.6	20.2	18.16	18.67	16.4	21.1	26.03
2	Water temp	$^{\circ}\text{C}$	28.9	32.22	29.09	24.11	22.37	20.2	18.1	15.33	14.44	15.4	20.1	25.77
3	Conductivity	$\mu\text{moh/m}$	45	41	21	19	14	18	24	28	32	35	38	42
4	pH		8.5	8.2	8.1	8	7.8	7.4	7.3	7.2	7.8	8.1	8.2	8.3
5	TDS	$\text{mg/l}$	345	395	449	585	605	580	525	435	400	305	285	325
6	SS	$\text{mg/l}$	34	34	38	43	50	54	50	48	45	40	38	36
7	$\text{CO}_2$	$\text{mg/l}$	0	0	0	0	2.61	2	2.51	0	0	0	0	0
8	Silicate	$\text{mg/l}$	2.18	2.2	2.28	1.22	1.21	1.03	1.06	1.21	1.26	1.23	1.2	1.26
9	DO	$\text{mg/l}$	6.9	6.4	3.6	3.4	3.1	3	7.6	7.7	7.6	7.7	7.9	6.4
10	BOD	$\text{mg/l}$	3.3	3.5	4.1	3.9	3.1	2.8	2.5	2.6	2.8	3.2	3	3.1
11	COD	$\text{mg/l}$	30	30	30	29	28	26	25	22	24	26	28	26
12	Chloride	$\text{mg/l}$	33	33	29	26	24	22	24	26	28	30	28	32
13	Sulphate	$\text{mg/l}$	4.2	4.7	5.1	5.6	5.7	6.1	6.3	6.9	6.9	5.1	4.6	4.5
14	Nitrate	$\text{mg/l}$	0.18	0.2	0.34	0.26	0.36	0.32	0.34	0.32	0.26	0.2	0.13	0.11
15	Phosphate	$\text{mg/l}$	0.34	0.62	0.36	0.38	0.36	0.3	0.31	0.39	0.3	0.46	0.42	0.45

Table 2: Mean value of physico-chemical parameters of Yashwant Nagar Taalab, Mhow 2006-2007

S.N.	Parameter	Unit	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
1	Air temp	$^{\circ}\text{C}$	28.1	33.07	30.22	24.11	22.7	20.3	18.4	18.43	16.88	16.3	16.4	20.11
2	Water temp	$^{\circ}\text{C}$	27.09	31.33	27.23	21.98	19.76	19.1	17.8	15.98	14.33	15.6	14.3	19.09
3	Conductivity	$\mu\text{moh/m}$	42	39	17	15	13	15	22	26	33	34	36	39
4	pH		8.4	8.4	8.6	8.4	8	7.8	7.6	7.2	7.6	7.9	7.8	8
5	TDS	$\text{mg/l}$	335	389	439	580	598	560	524	455	390	398	290	320
6	TSS	$\text{mg/l}$	31	32	36	40	45	48	46	44	43	38	36	32
7	$\text{CO}_2$	$\text{mg/l}$	0	0	0	0	2.32	1.86	2.33	0	0	0	0	0
8	Silicate	$\text{mg/l}$	2.2	2.2	1.86	1.67	1.08	1.1	1.15	1.18	1.06	1.08	1.04	1.98
9	DO	$\text{mg/l}$	7.8	7.4	4.4	4.2	4	3.6	6.4	6.3	6.2	6.8	6.6	6.9
10	BOD	$\text{mg/l}$	2.1	2.4	3.3	2.5	2	1.9	1.6	1.9	2	2.1	2.2	2.3
11	COD	$\text{mg/l}$	26.3	27.5	28.5	25.7	26.6	24.5	23.5	21.88	22.3	24.8	25.1	24.6
12	Chloride	$\text{mg/l}$	29.2	26.6	25.3	24.4	23.5	20.7	21.4	23.8	24.98	26.9	25.3	29.54
13	Sulphate	$\text{mg/l}$	3.8	3.6	4.44	4.5	4.77	5.08	5.1	5.66	4.9	4.6	3.8	3.7
14	Nitrate	$\text{mg/l}$	0.16	0.98	0.54	0.44	0.33	0.36	0.34	0.32	0.23	0.26	0.21	0.18
15	Phosphate	$\text{mg/l}$	0.24	0.56	0.26	0.28	0.27	0.28	0.27	0.29	0.28	0.35	0.36	0.28





2005-06 and from 14.33 (Dec.) to 31.33 °C (May) during 2006-07. These values are closely similar to findings of Shrivastava, (2002) and Pathak (2004) and very much suitable for fish culture.

According to Dhawan (1970) the occurrence of minimum and maximum temperature coincide with winter and summer respectively. In the present study minimum maximum water temperature was in the winter and summer as reported by earlier scholars. The air temperature of water body ranged from 16.4 °C (Jan.) to 33.54 °C (May) during 2005-06 and 16.3 °C (Jan.) to 33.22 °C (May) during 2006-07. These reading suggested that warming and cooling of the surface water of Yashwant nagar Talaab was influenced considerably by meteorological factors such as air temperature and wind as described by Hutchinson (1957).

Conductivity (specific conductance) is the numerical expression of the water's ability to conduct an electric current and depends on the total concentration, mobility and the temperature of the solution of ions. In the present investigation conductivity ranged from 14.0 moh/m to 45.0 moh/m lowest and highest in August and April during 2005-06. In the second consecutive year (2006-07) of study the minimum value (13.0 moh/m) was observed again in the month of August and maximum value (42.0 moh/m) was noted in month of April. The highest values of conductivity was in summer, mostly due to the increased concentration of salts because of evaporation, where as lowest values of conductivity in rainy months due to dilution. In the present investigation pH range was found between 7.2 to 8.5 during 2005-06 and 7.2 to 8.6 during 2006-07. These were matched with findings of Hutchinson (1957) who reported pH range between 7.5 and 9.0. In monsoon months, hydrogen-ion concentration of water at Yashwant Nagar Talaab was low may be due to mixing of rain water. Sreenivasan (1969) stated that the range from 5.0 to 6.6 and 9.1 to 11.0 give low productivity results. Banerjee (1967) described pH range 6.5 to 7.5 as most favorable for production in ponds and

7.5 to 8.5 just favorable for an average production. In the present investigation, water of the Yashwant Nagar Talaab was noted on an alkaline range mean value varying from 7.3 to 8.68, which is considered as productive moreover the water of this talaab is also suitable for potable purpose as pH is well within permissible limits as described by Kadam *et al.* (2008).

Total solids is the term applied to the materials residue left in the vessel after evaporation of the sample and its subsequent drying in an oven at a temperature of 103-105 °C. total solids (TS) include total suspended solids (TSS) and Total dissolved solids (TDS). In the present investigation TS (during 2005-06) ranged from 305 mg/l to 605 mg/l with lowest and highest value in the months of February. Regulates biological process among the aquatic communities and can form many compounds (Agarkar *et al.*, 1994). Unni (1992) emphasized that the rate of changes in the free carbon-dioxide concentration is considerable due to decomposition of organic matter at the bottom. In the present investigation carbon dioxide during 2005-06 ranged between 2.00 mg/l to 2.61 mg/l with the lowest in September and highest in August. During 2006-07 Carbon dioxide range was in between 1.86 mg/l to 2.33 mg/l with lowest September and maximum in October. Except Aug to Oct in the rest of the months CO<sub>2</sub> was absent. Kumar *et al.* (2008) also reported absence of CO<sub>2</sub> in his observations. The presence and absence of the free carbon-dioxide in the surface water is mostly governed by the utilization by algae during photosynthesis at Yashwant Talaab and also through its diffusion from air (Sreenivasan, 1974).

Dissolved Oxygen is an important limnological indicator of water quality and organic production in the lake Wetzel *et al.* (2006). Rawson (1939) stated variation in dissolved is related with utilization of Oxygen during chemical oxidation and respiration of animals, plants and bacteria present in the water. In the present investigation dissolved oxygen ranged between 4.3 to 7.9 mg/l (2005) and 4.7 to 7.8





mg/l (2006-07) with maximum value in April and minimum in September. These findings corroborate with the observations made by Agarwal (1978), Nair *et al.* (1988), Prakash (1994), Kumar and Singh (2002), Shrivastava (2002). Silicates in 2005-06 ranged from 1.03 mg/l to 2.28 mg/l. minimum value were 1.03 mg/l in the month of September. Maximum value 2.28 mg/l was in the month of June. While in 2006-07 it ranged from 1.04 mg/l to 2.20 mg/l. minimum 1.04 mg/l in the month of February and maximum (2.20 mg/l) in the month of April and May.

Biological Oxygen demand and chemical oxygen demand are important parameters to estimate the pollution level and water quality of a particular water body. In the present investigation BOD value ranges between 2.6 to 4.1 mg/l with maximum value was in June and minimum in October. Similar trends were obtained by Belsare (1984), Badge and Verma (1985), Pant *et al.* (1985) and Khanna and chugh (2004). In the present investigation higher value of BOD was observed in summer month may be due to high temperature, rich of eutrophication and decreased water level as described by Kumber *et al.* (2008).

Chemical oxygen demand is a measure of oxygen required for complete oxidation organic matter by a strong oxidant. In the present investigation COD in 2005-06 ranged from 22 mg/l to 30 mg/l. minimum (22 mg/l) value was in the month of November and maximum (30mg/l) value was in the month of April, May and June. COD in 2006-07 ranged from 21.88 mg/l to 27.5 mg/l with minimum (21.88) in the month of November and maximum (27.5mg/l) in the month of May. Higher value of COD in Summer was also reported by Manimepalai *et al.* (2008).

The chloride in the present study varied in between 22.0 to 33.0 mg/l during 2005-06 and 20.66 to 29.54 mg/l during 2006-07. the minimum value was recorded in the month of September and maximum in April and May. Gonzalves and Joshi (1946); Singh (1960); Ganpathi (1962); Zafar (1964); Singh (1986); Nair *et al.* (1998); Dave *et al.* (1999); Shrivastava (2002) and Pathak (2004) had also found higher

chloride in summer due to increased rate of evaporation of water.

The rate of Nitrate in present investigation between 0.11 to 0.36 mg/l during 2005-06 and 0.16 to 0.58 mg/l during 2006-07. Shrivatava (2002) observed nitrate range between 0.13 to 0.73 mg/l at Yashwant Sagar, Indore. Garg *et al.* (2005) described its range from 0.010 to 0.028 mg/l at Harshi reservoir, Gwalior. In the present study maximum nitrate was observed in summer (May) and minimum in December and April.

In aquatic ecosystem Phosphorous occurs both in organic and inorganic forms. The main source was detergent and sewage containing high contiontration of Phosphorus. In the present investigation Phosphorus content varied from 0.03 to 0.62 mg/l (2005-06) and 0.24 to 0.56 mg/l (2006-07). Maximum value was recorded in May and minimum in September may be related with concentration and dilution of water body. On the basis of the observations made on physico chemical features of water body it is regard as of great economic, ecological and biological importance. The present water body would prove ideal, specially for the purpose of fish culture.

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## Fish and fisheries of Virla Reservoir of West Nimar, District M.P., India

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### Abstract

During course of study on virla reservoir West Nimar (Kargone) M.P., India, total 29 species of fish biodiversity were recorded these belong 6 orders, 10 families and 16 genera were observed. On the basis of economic importance fishes were divided into three major groups viz. catfish (*Mystus seenghala*, *Mystus tengara*, *Wallago attu* and *Ompok bimaculatus*), major carp (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) and miscellaneous (*Channa* spp., *Mastacembelus* spp. and *Notopterus notopterus*). The water body was divided into three zones viz. upstream zone, reservoir zone and downstream zone for fish catch. The total annual fish catch computed in this water body was 2465.40 kg. and 2576.40 kg. during the 2001-02 and 2002-03 respectively. The minimum catch were observed in August while maximum in May. The percentage composition of major carp shows decline trend and catfishes shows increasing trend during two years study time. Catch per unit effort (CPUE) ranged between 0.015 to 0.191 kg/ hrs. The water level fluctuations and fish production show inverse relationship.

**Keywords:-** Fisheries, Virla reservoir, Economical, Fish production

### Introduction

India ranked third in inland fish production in the world. The total fish production is 47.89 lakh tones contributed 20.97 lakh tones from inland and 26.92 lakh tones from marine sector respectively in the year 1994-95 (Pandy and Unyal 1907-1998). The contribution of inland sector is was nearly one third of the total fish production with 57% of total domestic consumption supply. The major portion of protein rich food of domestic consumption come from inland fish production. Madhya Pradesh occupy 2.75 lakh hectares area with 60 reservoir and got second position in India. The total fish catch is 2619 is about tones per year from reservoirs of M.P. the state fishery department governs the development of reservoir fisheries since 1962 and has only 40 % stocking facility of total production at major reservoirs. The management of reservoir fisheries in India is not well developed when we compare with other countries. So many impending

factors i.e. regular monitoring of reservoir, excessive growth of aquatic vegetation, introduction of commercial fish seed, use of fishing gears, untrained staff, transportation and marketing facilities are responsible for development of reservoir fisheries in the country. A numbers of national and foreigner researchers (scientists) have contributed our knowledge in the fisheries aspects. Review of literature indicates that not much work has been done on the reservoir of M.P. and no attempt has been made on Virla reservoir in this aspect. The first limnological study on this reservoir has been conducted and details of fish biodiversity and potential structure were observed, which is helpful for the management and development of fishery in the reservoir.

### Materials and Method

The present study was done for a period of two years from July 2001 to June 2003. Four collection centers was selected in the studied water body viz. one at upstream site, two at reservoir site and one

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at downstream site. Besides this, fish collection and marketing survey were done in one week of every month during the study period. The main fishing gears operated in the reservoir were gillnets of mesh ranging from 23- 180 mm bar. Further the fish caught by small meshed gill net, cast net and traps. The fishes soon after collection were cleaned and preserved in 5 % formaline. The fishes were identified to genus/ species with the help of keys provided by Day (1958) and Jhingram (1992). Catch per unit effort (CPUE) is calculated based (by) on the mean monthly fluctuation of fish landings.

## Results and Discussion

The reservoir receives rainwater through local nalla started from Jalabad of Satpura ranges. The west of the reservoir is covered by the agriculture fields and remaining three by hill and village of Virla. The reservoir is mainly used for culturing of fishes. The near by villagers also use water for irrigation, bathing, washing and drinking for domesticated animals. The dam is constructed by cement concrete and is provided with one small siphon to let out the excess water from the reservoir particularly during rains. The length of dam is 270 m and height is 19.29m. The above details of morphometry and I Morphometric and hydrological Characteristics Particular of the Dam

**Water elevation:** Maximum water level-154.40 m  
Full reservoir level- 153.20 m river bed level- 36.91 m.

**Capacity:** Gross storage- 4.47 M.CuM. Live storage- 4.29 M.CuM.

**State:** M.P.

**District:** Khargone

**Tehsil:** Segon

**River:** Local nala

**Location:** Near village Virla

**Total length:** 270 m Maximum height – 19.29

**Area:** Gross storage level- 525 Hectares, full reservoir level- 445 Hectares, average level- 495 Hectares

**Depth:** Maximum at FSL- 16.29 Maximum at DSL- 9.69.

hydrology of reservoir denote the static value. The water volume and water level changed seasonally and depend upon rainfall, water inflow and erosion inputs from catchment area.

## II Fish diversity

The fish diversity is mainly depend on the type of habitat and its abiotic and biotic factors. During the study at Virla reservoir of west Nimar (Khargone) 29 species of fishes were noted which belong to 6 orders, 10 families and 17 genera (Table 1). Choudhary (1977) observed 39 species after the impoundment of Gandhi Sagar reservoir. Kartha (1987) reported 41 species of fish from Gandhi Sagar reservoir. Singh (1993) reported 84 species from Sardar Sarover dam of Narmada River. Saxena (1997) reported 42 species from upstream region and 35 species from down stream region in river Satluj. While comparing this Virla reservoir consist very less quantity of fish diversity. As there is no previous data available on Virla reservoir therefore it is not possible to evaluate the quantity of fish depletion in this reservoir.

## Commercial fisheries

Due to lack of knowledge of fisheries management techniques in Virla reservoir the commercial fishery is unorganized. The history of Virla reservoir represents the natural stock. The commercial fishes of Virla reservoir were dominated against non commercial fishes. In the present study fishes were classified as major carps, cat fishes and a group of miscellaneous fishes. The dominated fisheries include *Catla catla*, *Labeo rohita*, *Mystus seenghala*, *Cirrhinus mrigala*, *Wallago attu*, *Channa* sps. *Notopterus notopterus* and *Mastacembelus* sps.

The commercially important species of fishes of virla reservoir were grouped according to their economic importance into three major categories viz. major carp (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) cat fish (*Wallago attu*, *Mystus tengara*, *Mystus seenghala*, *Ompok bimaculatus*).





**Table 1 : List of fishes recorded in Virla Reservoir during July 2001 to June 2003**

Order	Family	Genera
Cupriniformes	Cyprinidae	<i>Catla catla</i> (Ham)
		<i>Cirrhinus Mrigala</i> (Ham)
		<i>Cirrhinus reba</i> (Ham)
		<i>Labeo rohita</i> (Ham)
		<i>Labeo calbasu</i> (Ham)
		<i>Labeo bata</i> (Ham)
		<i>Puntius ticto</i> (Ham)
		<i>Puntius sophore</i> (Ham)
		<i>Rasbora daniconius</i> (Ham)
		<i>Nemacheilus botia</i> (Ham)
		<i>Nemacheilus beavani</i> (Ham)
		<i>Nemacheilus auratus</i> (Ham)
	Siluridae	<i>Ompok bimaculatus</i> (Bloch)
		<i>Wallago attu</i> (Schm)
	Bagridae	<i>Mystus seenghala</i> (Skyles)
		<i>Mystus tengara</i> (Ham)
		<i>Mystus aor</i> (Ham)
	Schilbeidae	<i>Eutropichthys vacha</i> (Ham)
	Saccobranchidae	<i>Heeropneustes fossilis</i> (Bloch)
Clupeiformes	Notopteroidae	<i>Notopterus notopterus</i> (pallus)
Belontiiformes	Belontiidae	<i>Xenentodon cancila</i> (Ham)

Ophiocephaleiformes	Ophiocephalidae	<i>Channa marulius</i> (Ham)
		<i>Channa gachua</i> (Ham)
		<i>Channa striatus</i>
		<i>Channa punctatus</i>
Perciformes	Cenropomidae	<i>Ambassis ranga</i> (Ham)
		<i>Ambassis nama</i> (Ham)
Mastacembeliformes	Mastacembelidae	<i>Mastacembelus armatus</i>
		<i>Mastacembelus pancalus</i> (Ham)

and miscellaneous (*Notopterus notopterus*, *Channa* species and *Mastacembelus*). Dubey and Mehra (1959) reported 70 species from Chambal river in which 46 species were identified as commercially important fishes. Rao *et al.* (1988) noted 9 commercially important fishes from Gandhi sagar reservoir. Kartha (1987) reported 77% growth rate of *Catla catla* during the period of 1980-81 to 1985-86 in Gandhi sagar reservoir. Gandhi Sagar Reservoir is known as Catla sagar because of increased landing of *Catla catla* in this reservoir. In the present study of Virla reservoir the percentage contribution of commercially important fishes like *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* shows 7.57%, 6.93% and 3.79% of the total catch respectively. There are no storage facilities available for caught netted fishes from the reservoir. Hence, after catching they have to sold out at any rate. The seed of major carps should be introduced for the enhancement of further production of commercially important fishes in the virla reservoir. Kartha (1987) in Gandhi Sagar and Pandey (1998) made similar observations in Gambhir dam.

#### Annual fish yield

For the fisheries output, hydrological characteristics of the collection centers play an important role to a greater extent. In the present



study of Virla reservoir four sampling station were identified according to their morphometry, water flow and hydrobiological characteristics. The sampling station I exhibits lotic condition to some extent. This centers contributed 26.85% of total fish catch of Virla reservoir. The sampling station II and III represents lentic habitat and contributed total fish catch 28.33% and 34.27% respectively. The sampling station IV (downstream) exhibits rapid water current very similar to river during rainy season. It contributes 10.55% to the total fish catch. The sampling station wise data of annual catch showed minimum 251.87 kg/year at collection station IV and maximum 873.58 kg/year at collection station III (Table 2). In the present study fish catch includes 4.69 and 4.90 kg/ha/yr. landings were estimated in 2001-02 and 2002-03 respectively. The present results indicate that the total fish yield/ha/yr. shows increasing trend. Similarly the total fish yield at Virla reservoir also showed increasing trend i.e. 2465.40 kg/yr. in 2001-02 and 2576.24 kg/yr. in 2002-03. Kartha (1987) reported the total fish landings at Gandhi Sagar reservoir 2437.0 tons/yr. and 13420 tons/yr. in 1984-85 and 1985-86 respectively. According to all India coordinator reservoir research project the annual fish production from important reservoir during 1983-84 were 1.4,

4.1, 41.2, 1.1, 25.3, 4.3 and 2.8 kg/ha/yr. from Ukai, Nagarjunsagar, Getal Sud, Kangabati reservoir respectively (Saxena, 1990). Kumar (1990) reported 78.4 kg/ha/yr. annual landing from Govind Sagar reservoir during 1988-89.

#### Species wise percentage composition

In the present study composition of *Catla catla* was 48.88% and 49.36% to the total fish landings during 2001-02 and 2002-03 respectively. *Labeo rohita* was recorded 20.44% in 2001-02 but in 2002-03 it was decreased upto 19.80%. *Cirrhinus mrigala* contributed 7.38% during 2001-02 and it increased upto 7.39 during 2002-03. Among the catfishes *Mystus seenghala* was 8.05% in 2001-02 and this increased upto 8.16% in 2002-03 the presentage contribution was *Mystus tengara* 6.78% and 6.73% in 2001-02 and 2002-03 respectively. *Wallago attu* contributed 2.975 in 2001-02 and 3.00 in 2002-03. *Ompok bimaculatus* contributed 1.82% in 2001-02, which further increased upto 1.90% in 2002-03. Among the miscellaneous group the *Channa* sps. contributed 0.77% and 1.03%. *Mastacembelus* sps., 1.46% and 1.36% and *Notopterus* sps. 0.62% and 0.61% during 2001-02 and 2002-03 respectively. Other small fishes shared 0.83% in 2001-02 and 0.66% in 2002-03 (Table 3).

Kartha (1987) reported maximum contribution of *Calla catla* (58.67%), *Cirrhinus mrigala* (22.18) and *Labeo rohita* (17.14) in Gandhi Sagar reservoir. Singh (1993) reported the maximum Catch of *Tor tor* (23.84 to 26.66%), *Labeo fimbriatus* (18.1 to 18.8%), *Labeo calbasu* (5.2 to 5.66%), *Cirrhinus mrigala* (2.26 to 3.06%), *Mystus seenghala* (7.7 to 8.6%), *Mystus tengara* (0.26 to 0.4%), *Wallago attu* (7.3 to 8.14% and Miscellaneous species (5.1%) in Sarover of Narmada river. Pandey (1998) reported maximum contribution of *Mystus seenghala* (49.35 to 49.64%), *Mystus tengara* (19.45 to 20.12), *Wallago attu* (8.80 to 10.35%), *Catla catla* (6.78 to 7.45%), *Labeo rohita* (4.33 to 4.72%), *Cirrhinus mrigala* (3.65 to 3.77%), *Ompok bimaculatus* (1.58 to 1.66%), *Channa* species (0.99 to 1.185), *Mastacembelus* sps.

**Table 2: Annual fish yield in Virla reservoir Zone and Site wise during July 2001 to June 2003**

Name of the zone	Name of the site	2001-02		2002-03	
		Yield	%	Yield	%
Up stream	Site No. 1	654.39	26.54	699.73	27.16
Reservoir zone	Site No. 2	705.93	28.64	722.26	28.03
Reservoir zone	Site No. 3	853.21	34.62	873.58	33.92
Down stream	Site No. 4	251.87	10.21	280.67	10.89
Total		2465.40	100	2576.24	100





**Table 3: Percentage composition of fishes at Virla reservoir: Group wise during July 2001 to June 2003**

Group of fishes	2001-02		2002-03	
	Catch (Kg)	% Composition	Catch (Kg)	% Composition
Major carps	1891.04	76.70	1971.85	76.55
Cat fishes	483.70	19.62	509.90	19.79
Miscellaneous	70.26	2.85	77.41	3.00
Other small fishes	20.40	0.83	17.08	0.66
Total	2465.40	100	2576.24	100

(0.69 to 1.46%) and *Notopterus notopterus* (1 to 1.21%) and other small fishes (0.77 to 0.98%), in Ghambir dam. In Virla reservoir the major carps were the most dominant followed by catfish and miscellaneous group. The percentage contribution of major carps showed increasing trend except *Labeo rohita*. Similarly, among catfishes except *Mystus tengara*. Other fishes showed decreasing trend. It is therefore, suggested that for further increase in the production of fishes appropriate fish seed stocking is required. Special attention is needed for *Labeo rohita* and *Mystus tengara*.

#### Water level variation and fish catch

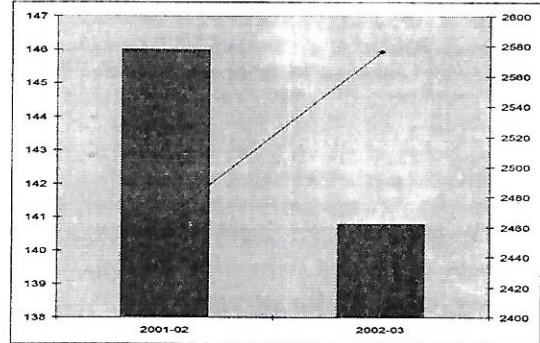
The impact of water level variation on total fish landings were observed at Virla reservoir (Table 4 Fig. 1). The value of average water level and total fish landings were recorded 145.59 m. and 2465.40 kg. during 2001-02 respectively and in 2002-03 the value of average water and total fish landing were 140.80 m. and 2576.24 kg. respectively. The impact of water level variation on total fish landings is inversely proportional.

#### Catchper Unit Effort (CPUE)

On the basis of monthly mean value of fish catch the catch per unit effort has been calculated. The abiotic biotic factors, volume of water and velocity

**Table 4: Water level variation and fish catch at Virla Reservoir During the year 2001-02 and 2002-03**

Water level in meter	Year	
	2001-02	2002-03
Maximum	149.30	145.20
Minimum	142.68	136.40
Average	145.99	140.80
Fish catch (kg)	2465.40	2576.24

**Fig. 1: Average water and fish landings in Virla reservoir during July 2001 to June 2003**

of current also affect the CPUE. In the present study CPUE was estimated in four study sites of Virla reservoir. The CPUE values ranged 0.015 to 0.191 kg/h the highest value of CVPUE in the month of May at site III and lowest in August at site IV (Table 5, Fig.2). the value of CPUE ranged 21.57 to 31 kg/hr. at Gandhisagar reservoir (Karthi, 1987). Singh (1993) observed CPUE from 3.3 to 3.41 kg./hr. at Sardar sarover dam. Shyam Sundar *et al.* (1995) recorded CPUE in between 0.35 to 639 gms/h at river Guala. Pandey (1998) reported 0.375 to 1.137 kg/hr. at Gambhir dam. The Value of CPUE is very less in Virla reservoir when compared with above findings. Probable reason of less CPUE value might be due to non-availability of Stocking facility in this reservoir.

Table 5: Catch Per Unit Effort (CPUE) in Kg. at four sites of Virla Reservoir During July 02 to June 03

Month	Site-1		Site-2		Site-3		Site-4	
	Production	CPUE	Production	CPUE	Production	CPUE	Production	CPUE
July 02	30.10	0.040	28.50	0.038	30.60	0.041	14.35	0.019
Aug.	27.68	0.037	15.90	0.021	27.00	0.036	11.22	0.015
Sept.	32.26	0.044	22.45	0.031	35.60	0.049	15.30	0.021
Oct.	36.41	0.048	30.24	0.040	42.50	0.057	18.40	0.024
Nov.	45.70	0.063	48.62	0.067	64.63	0.089	21.20	0.029
Dec.	60.35	0.0081	52.30	0.070	75.25	0.101	24.50	0.032
Jan.	67.40	0.090	62.44	0.083	88.20	0.118	26.65	0.035
Feb.	73.00	0.108	82.60	0.122	99.35	0.147	28.15	0.041
March	82.21	0.110	107.35	0.144	108.50	0.145	30.75	0.041
April	86.50	0.120	118.60	0.164	120.40	0.167	35.20	0.048
May	89.95	0.121	134.15	0.180	142.75	0.191	42.85	0.057
June	38.27	0.053	19.11	0.026	38.80	0.053	12.10	0.016

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## Snowtrout fishery in Garhwal Himalaya: Causes of depletion and strategy for propagation

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### Abstract

Snowtrout (*Schizothorax* sp.) is an important group of fishes in Indian uplands and is prone to decline due to several anthropogenic activities and natural disasters. However, the natural behaviour of the group is also one of the important constraints for its self-propagation in the nature. It requires attention for the conservation and propagation through *in-situ* as well as *ex-situ* measures. The *in-situ* refers to - aquaculture under controlled conditions for propagation of the group by developing artificial breeding programmes for high seed requirement, better management of incubation patterns and rational management of water bodies including development of sanctuaries, where the fishing would be banned. The artificial breeding and snowtrout seed production technique in flow through hatchery can be adopted at commercial level for mass scale snowtrout seed production. Sometimes unavailability of either sex of the mature brooder at the time of artificial fertilization in hatchery is a major constraint in the artificial breeding programmes. Cryopreservation of gametes has emerged as a promising and a very useful technique to facilitate artificial breeding in several fishes. The cryopreservation of milt of snowtrout finds its role right here. Initial attempts for developing cryopreservation protocol for snowtrout milt are very much promising. More concerted efforts for commercializing the reproductive techniques including cryopreservation of gametes will certainly be helpful for the strategic propagation of snowtrout in cold-water bodies.

**Keywords:-** Snowtrout, Habitat destruction, Breeding, In-situ conservation, Ex-situ conservation, Cryopreservation

### Introduction

India is blessed with the vast and varied fish germplasm resource distributed widely in various aquatic bodies. With an estimate, the coldwater fish germplasm resource contributes around 3.32% to the total fish germplasm resource of the country (Das and Pandey, 1999). More than 725 freshwater fish species have been reported to occur in India and 68 species dwell in the Garhwal Himalayas. Among these fishes, some species are on decline. Likewise, the schizothoracids, the dominant group of fishes in Garhwal Himalayas, are rapidly decreasing since past 3-4 decades due to various

factors including over exploitation, habitat destruction besides having its own specific genetic reasons. It requires urgent consideration for its conservation and propagation by making scientific strategies and sustainable exploitation.

In view of the global slogan of "seed, feed and breed" for intensification of fish farming and organized pisciculture, fish sperm bank has gained current importance (Gjedrem, 1981). Therefore, the preservation of gametes has emerged as a promising and a very useful technique to facilitate artificial breeding. In order to meet high fish seed requirements, pressure on harvesting of brooders from wild stocks, in the absence of availability of the farm-raised bloodstock, is on the increase. At the same time, fish farming is in a state of expansion. This situation further emphasizes the need for the

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cryopreservation of gametes for propagation of fishery in captivity as well as in the wild.

## Materials and Method

The snowtrout were surveyed for their occurrence in river Ganga from Chilla-barrage to Rishikesh, in river Bhagirathi from Tehri to Harsil, in river Bhilangana from Tehri to Ghansali, in river Alaknanda from Devprayag to Chamoli, in river Mandakini from Rudrapur to Agastmuni. The fishermen and the local inhabitants were interviewed for their past knowledge regarding the fish catch experiences. The breeding and feeding ground of the fishes were identified on the basis of presence of eggs, fry and brooders.

## Results and Discussion

The fishery in Garhwal Himalaya is observed mainly in the nature of capture fishery. The survey conducted during the present study has noticed that snowtrout fishery contributes a large (nearly eighty percent of total fish catch), for subsisting fish food resources to the local denizens of the villages settled nearby to rivers and streams. The old aged experienced fisherman largely reported that now they have to make more efforts to catch fish as their nets often found without fish when they do fishing. It is not like as such those of 30-40 years back when they fished out their nets from rivers contained number of fish. They remember it as a past story. The fish catch per unit effort is declining year after year resulting in to overall decline in capture fishery of the region. This has also well documented in several previous reports by number of workers (Singh *et al.*, 1987; Agarwal *et al.*, 2005)

### Factors affecting snowtrout population

With the rapid ever-increasing demand of fish as food, the aquatic ecosystems are under constant pressure of man-induced stresses to the detriment of the aquatic flora and fauna (Jhingran, 1991). Though the decline of snowtrout species in the Garhwal Himalayan region is very often related to

more than one proximate factor, the various causes of decline in the population of snowtrout has been observed and grouped as –Anthropogenic or man induced factors and Natural or Genetic factors of the population.

### *Anthropogenic or man induced factors:*

The anthropogenic or man induced factors responsible for the decline of the snowtrout population are-

#### *Habitat Destruction*

The feeding and breeding grounds of snowtrout including many others were found adversely affected by heavy silt load in the river Bhagirathi and Bhilangana due to developmental activities such as road construction etc. from the catchment areas. Collection of stones and boulders from riverbanks as building material resulted into the destruction of breeding grounds of snowtrout and mahseer. Recent emergence of reservoirs due to hydroelectric projects (Maneri Bhali and Tehri Project) has dramatically changed the breeding and feeding habitats of the fish. Singh *et al.* (1992), Kirchhoffer and Hefti (1996) and Shrestha (1997) have also reported that siltation in the feeding and breeding grounds and damming of rivers for power generation are the reasons of habitat degeneration for local fish communities. The migration routes of the important native fishes such as mahseer (*Tor putitora* and *T. tor*) and snowtrouts (*Schizothoracichthys progastus*) have also been blocked by the construction of dam infrastructures at Tehri and Maneri in Bhagirathi River and Veer Bhadra Barrage at Rishikesh in River Ganga.

The recent emergence of Tehri dam reservoir has resulted into the large destruction of natural habitat of snowtrout species (*viz.* *Schizothorax richardsonii*, *Schizothorax plagiostomus*, *Schizothorax curvifrons*) that are basically bottom feeder and lithophil spawner, thrive in the snowfed river habitat of clear, shallow water of stony substratum with a average depth from 1 to 3 meters, and river flow not less than 0.5 meter per sec (Singh



*et al.*, 1985; Singh and Agarwal, 1986; Agarwal, 1996, Agarwal, 2001). The impoundments of the river Bhagirathi and Bhilangana for Tehri dam reservoir has caused a loss of riverine habitat of 44 km stretch between Tehri and Chinyalisaur and 25 km stretch between Tehri and Ghansali. The impoundments of these two rivers for Tehri Hydro-Electric project has affected the snowtrout population in this area up to the extent that snowtrout population has totally disappeared from the reservoir and shifted upstream to Chinyalisaur and Ghansali in the river Bhagirathi and Bhilangana respectively due to its highly sensitive nature to river bed, being bottom feeder and lithophil spawner. (High water column and steep banks of the reservoir do not support feeding and breeding behaviour of the snowtrout)

#### *Over-exploitation*

Increasing dependence of human population for nutrition on fishes has caused diminishing of the resources in Uttaranchal. The fish resource has also been found over-exploited for extraordinary economic benefits and has caused increasing vulnerability of the snowtrout population as evidenced by decline in catch of snowtrout in per unit effort in the Bhagirathi, Bhilangana and Alaknanda Valleys.

#### *Mass-destruction*

Use of dynamites and electric shocks by the persons involved in road construction & hydroelectric projects are very often for catching fishes. Some people also used ichthyotoxic plants (viz. *Xenthoxyllum armatum*, common name Timuru) and chemical poisons (viz. bleaching powder) to catch fish with fewer efforts in the shallow portion of the river. These methods not only kill adults but juveniles also and cause severe damage to the micro habitat by affecting the survival of micro-organisms including periphyton and benthic invertebrates that serve as food of the fish. Thus, cumulative effect of it leads to decline in the fish population. Dehadrai *et al.* (1994), Shrestha (1997) and Ponniah *et al.* (1998) also opined that the unscientific fishing

techniques causes mass-destruction of brooders, small sized fishes, fingerlings and fry, which have distinct impact on capture fisheries in subsequent years.

#### *Uncontrolled Introduction of Exotics*

Many exotic fish species have been introduced and well established in Indian waters due to their fast growing capability. However, these species have initiated competition with indigenous fish species at different levels and are becoming dominant in Indian waters (Pullin, 1994; Singh and Pandey, 1995). Common carp introduced in the Kashmir has almost exterminated the indigenous schizothoracids of the Valley. Though in the Garhwal Himalayan rivers, common carps were not introduced till the year 2006. But recent survey to Tehri dam reservoir has shown the presence of substantial number of common carp in the catch along with mahseer population. Although snowtrout from the reservoir has shifted itself to upstream due to its highly sensitive nature with the river bed but there is a further threat to riverine population of snowtrout from the exotic common carp. If common carp established to themselves in the reservoir and somehow migrate to the downstream (passive migration with downstream water into the river after dam) or upstream (where river entering into the reservoir at Chinyalisaur and Ghansali), may give tough competition to the indigenous local population of snowtrout and may dominate over them in coming years.

Another exotic fish species brown trout and rainbow trout have been introduced in the coldwater of Garhwal Himalaya. The seeds of trout are being raised in Varagana, Talwari and Gangori hatcheries and stocked in the adjacent high altitude streams by state fishery department. These exotic trout being carnivore in nature may cause danger to the survival of native herbivore snowtrout by feeding upon their hatchlings and small fry. Therefore one must be aware of the potential hazards of the introduction of the exotics and this should be done under careful monitoring.





### **Genetic Factors**

In addition to above-mentioned factors, snowtrouts have some own genetic constraints for its vast and speedy propagation in the nature. Important ones are-

#### ***Asynchronisation of Gonadal Maturation***

It is one of the major constraints that impede breeding in schizothoracids. Males often show testicular recrudescence earlier during the season, therefore causing earlier ripeness of the males than females when they are not yet mature and ready for spawning (Agarwal, 1996, 2001). Similarly, during the later periods of the breeding season, the males become spent while the females are still capable of producing eggs. This asynchronisation in the maturation of the sexes causes lesser chances of fertilization thereby less production.

#### ***Low Egg Production Potential***

The egg production potential (fecundity) of snowtrouts is comparatively very less than other plain counterparts as well as other coldwater fishes (Agarwal *et al.*, 1988, Agarwal, 1996; Thapliyal, 2002). However this may be due to the struggle for existence in adverse coldwater environment, but it has also caused low production of the group.

### **Strategies for Snowtrout Protection and Propagation**

The further decline of the fish germplasm resource may be prevented by devising all the possible measures of conservation and rehabilitation (Pavolov, 1993; Penczak, 1996; Das and Pandey, 1999). The irreparable harm caused to the snowtrouts and its habitat need to be compensated through strict implementation of Indian Fisheries Act, 1897 (modified, 1956) along with adoption of the following strategies. Legal measures for protecting and propagating fish germplasm resources cannot be implemented without taking the ground socio-economic realities in the consideration.

### ***In situ Protection & Propagation***

*In situ* protection of fish is useful where genetic diversity exists and where wild forms are present. This can be done through their maintenance within natural or man-made ecosystems in which they occur. Rishikesh, Har-ki-Pauri (Haridwar) and Baijnath (Almorah) are some examples of religiously protected areas in Uttaranchal where fishing is totally banned and protected by the community. Such type of protected areas can be developed with the support of community participation. The successful artificial breeding and larval rearing of the snowtrout, *Schizothorax richardsonii* (Agarwal *et al.*, 2002, 2007; Thapliyal, 2002) has opened up the new avenue of river ranching in depleted areas for stock replenishment and conservation. The stocking-restocking pattern by establishing flow through hatcheries may be one of the strategies for the development of snowtrout fishery in rivers and reservoirs. But the natural breeding grounds of the snowtrout should also be protected and developed all along the riverside and near the headwater of reservoir.

### ***Ex situ Conservation***

In this measure, the targeted species are conserved outside their natural habitats. The two main pillars of *ex-situ* conservation programmes are live gene bank and Gamete/Embryo banks. In a live gene Bank, the endangered species are reared in captivity, bred therein and genetically managed avoiding inbreeding depression, domestication and unintended selection (Minckley and Deacon, 1991). In Gamete/Embryo Banks, adequate samples representative of the natural genetic variations of the threatened species are kept in liquid nitrogen (LN<sub>2</sub>) at extreme low temperatures (-196° C). Establishment of Sperm Bank by cryopreserved milt assures further availability of genetic materials of desired species for intensive breeding programmes. Cryopreservation of snowtrout milt (Semen) may



play an important role in the protection and propagation of species through intensive seed production in the hatcheries.

### **Cryopreservation of Snowtrout milt**

Cryopreservation of fish milt (sperm) without loss of viability is of considerable value in propagation of snowtrout fishery. It helps in the availability of male gametes round the year for seasonal breeders, and in organizing easy transport of germplasm, genetic selection and hybridization programmes. The cryopreservation of snowtrout milt overcome the problem of asynchronization of maturation of males and females by making available cryopreserved milt round the year for fertilizing eggs in hatcheries. This will not only improve the snowtrout seed production efficiency of the hatchery but also greatly minimize the cost of maintenance of male brooders. By raising snowtrout seed in the hatchery, river ranching programme can be initiated for stocking the rivers and streams to replenish the snowtrout in the wild. The research group has developed short term and long term preservation techniques for snowtrout milt. The milt of *Schizothorax richardsonii* and *Schizothoracichthys progastus* can be stored in refrigerator (0-4 °C) upto 5 days with 50% motile sperm in Mounib's and KCl extender respectively (Agarwal 2005). The protocol for the cryopreservation of snowtrout (*S. richardsonii* and *S. progastus*) milt in Liquid nitrogen (-196 °C) has been developed (Agarwal *et al.*, 2009). The initial success has been achieved in fertilizing the eggs with 375 days old cryopreserved milt and developing the snowtrout seed. Commercialization of the cryopreservation technique for snowtrout milt at the level of field trial is now awaited. This will certainly open up an avenue for the development of cold-water fishery through protection and propagation of snowtrout in cold-water bodies of Indian uplands.

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## *Nyctanthes arbor-tristis* Linn. (Harsinghar): A potential medicine

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### Abstract

*Nyctanthes arbor-tristis* Linn. is widely used in the traditional medicinal systems of India. It possesses hepatoprotective, antileishmonial and antiallergic, antiviral and antifungal activities. The petroleum ether, ethanol and water extract of stem, root, flowers, seeds and leaves of the plant were screened for the antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* by using well diffusion method. The results were compared with reference antibiotic ampicillin. The water extract of root shows minimum inhibition zone (10 mm) against *S. aureus* and *B. subtilis* and petroleum ether extract of leaves against *E. coli* and ethanolic extract showed the maximum activity against *S. aureus* and *B. subtilis*.

**Keywords:-** *Nyctanthes arbor-tristis*, Antibacterial activity, Pathogens

### Introduction

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents (Prabhat and Navneet, 2007). The Gram-positive bacteria such as *S. aureus* are mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning. The Gram-negative bacteria such as *E. coli* are present in human intestine and causes lower urinary tract infection, coenocytes or septicemia. Several plants are indicated in folk and other traditional systems of medicine that act as aseptic agents. *Nyctanthes arbor-tristis* commonly known as Harsinghar belongs to the family Oleaceae (Chitravanshi *et al.*, 1992). The tree is small in size and found in abundance in the forests of central India and sub-Himalayan region. Traditionally in India the plant is used in snake bite, animals, bites cancer, sores, ulcers dysentery, menorrhagia (Badam *et al.*, 1987) and obstinate sciatica. The leaf of *Nyctanthes arbor-tristis* is very active from the immunologic point of view. It strongly stimulates

antigen specific and non-specific immunity as shown by increase in humoral and delayed type hypersensitivity response to sheep erythrocytes and in macrophage migration index (Puri *et al.*, 1994). In the present study the antibacterial activity of different parts i.e. seeds, root, stem, leaves and flower against *E. coli*, *B. subtilis*, *S. aureus* and *K. pneumoniae* were carried out.

### Materials and Method

The plant material of *Nyctanthes arbor-tristis* was collected from Hardwar and the plant was identified by the Botanical Survey of India, Dehradun (Uttarakhand). The each part of the plant was dried separately in shade and powdered by using grinder. The 100 gm of powdered plant material was loaded in soxhlet assembly extracted successively with petroleum ether, ethanol and water (400ml of each solvent). By removing the solvents with vacuum evaporator crude extract was used for antibacterial activities. The Mueller Hinton Agar media (Hi media No. M 173) was poured (25ml.) into sterilized Petri plate and left for solidification at room temperature and used to test the antibacterial activity of the extracts prepared from the plant against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* by well

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diffusion or cup plate method (Ahmad *et al.*, 1998 and Prabhat *et al.*, 2005a,b). 8mm diameter wells were punched in the agar media by borer and filled with extracts and antibiotic ampicillin (100 mg/ml) was used as positive control and solvent as negative control. The plates were incubated at 37°C for 24 hours to obtain inhibition zones.

## Results and Discussion

Table 1 enumerates the effect of the different solvents and aqueous extract of the plant against 4 pathogens. All plant extracts showed significant activity against the bacteria. Similar results of biological activity of plant against fungi and bacteria were reported by Ahmad and Beg (2001).

The methanolic extracts showed the strong activity against both gram positive and gram negative bacteria. In general gram +ve organisms were more sensitive than gram-ve, similar differences in the sensitivity were also observed by Suresh and Chauhan (1992). The extracts are tested for their antibacterial activity against the Gram-positive and Gram-negative bacteria. The activity was compared with the antibiotic ampicillin. All the extracts show activity against *E.coli*, *K.pneumoniae*, *S.aureus* and *B.subtilis*. The inhibition zones of all parts of the plant extracts are found to be less as compared to ampicillin. The results indicate that the petroleum ether extracts exhibit the lower degree of antibacterial activity as compared to aqueous and ethanol. The

**Table 1: The antibacterial activity of *Nyctanthes arbor-tristis* extracts in mm**

Pathogen	Root			Leaves			Seeds			Flowers			Stem			Ampicillin
	P.E.	EOH.	W	P.E.	EOH.	W	P.E.	EOH.	W	P.E.	EOH.	W	P.E.	EOH.	W	
E.C.	12	17	16	10	18	14	13	20	20	16	22	20	14	20	21	23
K.P.	14	16	15	11	20	20	14	20	21	17	20	19	15	19	20	21
S.A.	13	15	10	12	23	20	14	21	20	18	21	20	14	22	20	24
B.S.	12	14	10	14	23	21	12	20	20	17	20	21	11	19	19	25

E.C.=*E.coli*, K.P.=*K. pneumoniae*, S.A.=*S. aureus*, B.S.=*B. subtilis*. \*The solvents did not showed any zone of inhibition. P.E.=Petroleum ether\*, EOH=Ethanol\*, W= Water\*

maximum inhibition zone measured for antibiotic control is (25 mm) against *B. subtilis*. The minimum inhibition zone is (10 mm) of water extract of root and petroleum extract of leaves for *S.aureus*, *B.subtilis* and *E.coli* respectively. The ethanolic extract of leaves show maximum inhibition zone is (23 mm) against *S.aureus* and *B.subtilis*.

The inhibition zone by flowers extracts in ethanol and stems extract in water against *E. coli* was very effective nearly exhibition with ampicillin. Similarly water extracts of seeds, leaves, flowers and a stem inhibition is almost resembles inhibition in ampicillin against *K. pneumoniae*. The leaves extract with ethanol shows inhibition against *S.aureus* was found to be the same with ampicillin. The use of leaves, seeds, and stem

can be used in place of ampicillin antibiotic against some diseases caused by these bacteria.

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## Bacteriological characteristics of raw water of the river Tawi near Sitlee water treatment plant, Jammu

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### Abstract

Bacteriological analysis of raw water of the river Tawi, near Sitlee water treatment plant, has shown the seasonal presence of *Klebsiella*, *Citrobacter*, *Escherichia* and *Pseudomonas*. Total bacterial count (MPN) recorded summer (April, May), monsoon and post-monsoon (June- October) increase and winter (November, December, February) decrease. It varied between 92 and >180 during both the years of study. MPN index above 10 /100 ml. indicates that raw water is not suitable for drinking purposes and comes under the category of unsatisfactory. It needs proper treatment before supplied to the consumers.

**Keywords:-** River Tawi water, Bacteriological, MPN

### Introduction

Growing population, increasing living standards, rapid industrialization and wide sphere of human activities, have resulted in steady increase in the demand for water resources. This requires regular water monitoring to ensure supply of clean water to meet the consumers' demands. According to WHO, about 600 million cases of diarrhea and 46,00,000 childhood deaths are reported per year because of contaminated water and lack of sanitation. Although some work on physico-chemical characteristics of water of the river Tawi has been attempted by Zutshi (1992), but there is no report on bacteriological characteristics of water of the river. The river Tawi, an important tributary of the river Chenab, having its origin in the middle Himalayas, below Seoj Dhar peak at Kalikund near Bhaderwah, is a major source of potable water supply prior to the inhabitants of Jammu city since 1916 when Dhaonthly was the only supply point. At present, water supply from this river for Jammu city is from Sitlee, Dhaonthly and Gorkha Nagar treatment plants. In order to assess the water quality

of the river Tawi, two years study was undertaken and has been described.

### Materials and Method

**Presumptive Coliform Count (MPN):** MPN count of coliform organisms was done by Multiple Tube method (Senior, 1989; APHA, 1998).

**Confirmed phase:** Submitted all presumptive tubes showing growth, any amount of gas, or acidic reaction within 24 hrs. of incubation to the confirmed phase. Calculated the MPN value from the number of positive BGLB tubes (Senior, 1989; APHA, 1998).

**Completed phase:** Using aseptic technique (Senior, 1989; APHA 1998), streaked one each MacConkey agar plate, from each tube of positive BGLB showing gas.

**Faecal Coliform Count (MPN):** MPN count of faecal coliform was done by multiple tube method.

**Biochemical Tests:** To confirm the presence of various coliform bacteria, the following biochemical tests (IMViC) (Senior, 1989; APHA, 1998) were conducted (Table. 1):

### Results and Discussion

The results of various bacteriological characteristics of water have been tabulated in Table 2 and 3 and depicted in Fig. 1.

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**Qualitative Composition:**

In the raw water of the river Tawi, near Sitlee water treatment plant, *Klebsiella*, *Citrobacter*, *Escherichia* and *Pseudomonas* showed their seasonal qualitative presence, during both the years. During the first year, *Klebsiella* was observed only once (June), *Pseudomonas* four times (September - November and July), *Escherichia* ten times (August, October - May and July) and *Citrobacter* eleven times (August - June). During the second year, *Citrobacter* showed its presence five times (November, December, April, May and July); *Klebsiella* six times (August, September, January and February, May and June); *Escherichia* seven times (August - October, December, March, April and July) and *Pseudomonas* eight times (October,

November, January - March and May - July) (Table 1). Qualitative seasonal presence of these microbes in lotic waters has also been reported by Gupta and Gupta (1999), Gaur *et al.* (2000), Koshy and Nayar (2000), Fokmare and Musaddiq (2001), Singh *et al.* (2001), Srivastava (2002), Bhadra *et al.* (2003), Tavior (2003) and Sharma (2004). Entry of these microbes in river Tawi may result from inflow of sewage and human and animal excreta in the upstream region (Taylor, 2003) and surface runoff during rains (Taylor, 2003). Re-growth of some microbes present in sediments of river, derived from faecal pollution and enriched organic matter, may also account for the presence of these microbes in the river Tawi (Taylor, 2003).

**Table 1: Biochemical tests (IMViC) to confirm the presence of various coliform bacteria**

Organism	Indole	Motility	TSI	MR	Urease	Citrate	PPA	H <sub>2</sub> S	Dry Filter Paper/ Oxidase
<i>Escherichia</i>	+	+	+	+	-	-	-	-	-
<i>Klebsiella</i>	-	-	+	-	+	+	-	-	-
<i>Citrobacter</i>	+	+	+	+	-	+	-	+	-
<i>Proteus</i>	+	+	+	-	+	+	+	+	-
<i>Pseudomonas</i>	-	-	-	-	-	-	-	-	+

**Table 2: Monthly variations in qualitative composition of microbes in the river Tawi near Sitlee water treatment complex, Jammu**

Month	2000	2001
August	<i>Escherichia</i> , <i>Citrobacter</i>	<i>Escherichia</i> , <i>Klebsiella</i>
September	<i>Citrobacter</i> , <i>Pseudomonas</i>	<i>Escherichia</i> , <i>Klebsiella</i>
October	<i>Citrobacter</i> , <i>Pseudomonas</i> , <i>Escherichia</i>	<i>Pseudomonas</i> , <i>Escherichia</i>
November	<i>Citrobacter</i> , <i>Pseudomonas</i> , <i>Escherichia</i>	<i>Pseudomonas</i> , <i>Citrobacter</i>
December	<i>Citrobacter</i> , <i>Escherichia</i>	<i>Citrobacter</i> , <i>Escherichia</i>
January	<i>Citrobacter</i> , <i>Escherichia</i>	<i>Pseudomonas</i> , <i>Klebsiella</i>
February	<i>Citrobacter</i> , <i>Escherichia</i>	<i>Pseudomonas</i> , <i>Klebsiella</i>
March	<i>Citrobacter</i> , <i>Escherichia</i>	<i>Pseudomonas</i> , <i>Escherichia</i>
April	<i>Citrobacter</i> , <i>Escherichia</i>	<i>Citrobacter</i> , <i>Escherichia</i> , <i>Klebsiella</i>
May	<i>Citrobacter</i> , <i>Escherichia</i>	<i>Pseudomonas</i> , <i>Klebsiella</i> , <i>Citrobacter</i>
June	<i>Citrobacter</i> , <i>Klebsiella</i>	<i>Pseudomonas</i> , <i>Klebsiella</i>
July	<i>Escherichia</i> , <i>Pseudomonas</i>	<i>Citrobacter</i> , <i>Escherichia</i> , <i>Pseudomonas</i>





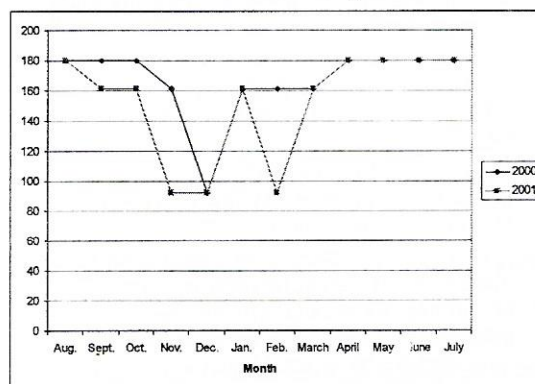
**Quantitative Analysis:**

In the Tawi water, MPN index per 100 ml., during the first year, ranged between 92 (December) to >180 (August - October, April - July). During the second year, MPN range was between 92 (November, December, February) to >180 (August, April - July). A very high bacterial count, seen in the river Tawi raw water, has also been reported from lotic waters by Bhosle and Rao (2001), Fokmare and Musaddiq (2001), Singh *et al.* (2001), Begum *et al.* (2003), Rajurkar *et al.* (2003), Thakur *et al.* (2003), Bankar and Deshmukh (2004) and Sharma (2004). Microbial enrichment in lotic waters is caused by discharge of sewage and entry of human (open defecation)

**Table 3: Monthly variations of microbes (MPN/100 ml) in the river Tawi, near Sitlee water treatment complex, Jammu**

Month	2000	2001
August	>180	>180
September	>180	161
October	>180	161
November	161	92
December	92	92
January	161	161
February	161	92
March	161	161
April	>180	>180
May	>180	>180
June	>180	>180
July	>180	>180

and animal excreta and other decomposing organic matters (animal slaughtering and washing of stomach and other internal parts) at various places (Begum *et al.*, 2003). This indicates that bacteria, always and under all conditions, remain in water body and this signifies the organic pollution. Summer (April, May) microbial highest count may be attributed to bacterial survival and increased production under warm conditions and is in conformity with the findings of Gaur *et al.* (2000), Singh *et al.* (2001) and Bhadra *et al.* (2003). High temperature and increased surface runoff in catchment areas may account for monsoon (June - September) increase in MPN count in the river Tawi (Table 3). Comparison of MPN per 100ml with National and International Standards (BIS, 1991; WHO, 1992) reveals that the water quality exceeds the allowable limits of drinking water standards.



**Fig. 1 : Monthly quantitative variations of microbes (MPN/100 ml) in the river Tawi, near Sitlee water treatment complex, Jammu**

**Table 4: Comparison of water quality of the river Tawi, near Sitlee water treatment complex, Jammu with National and International standards**

Parameter	PCC/100 ml	WHO	Ac	AI	BIS	Ac	AI
	1st Year	2nd Year	0	10		0	10
River Tawi	92->180	92->180					
British Ministry of Health	Excellent 0/100		Satisfactory	1-3/100			
	Suspicious 4-10/100		Unsatisfactory	>10/100			

PCC: Presumptive Coliform Count Ac: Acceptable AI: Allowable



The water quality remained unsatisfactory during both the years. as per British Ministry of Health, 1957 classification (Table 4).

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## Spiders fauna from G.V.I.S.H. Campus, Amravati ( M.S.)

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### Abstract

The spiders from G.V.I.S.H. Campus, Amravati (M.S.) were collected from first week of August to last week of November 2008, using insect nets, tapping sticks, umbrella, pit fall trap etc. During the study 470 specimens were collected from different area of campus. 35 species belonging to 12 families were identified. Family Araneidae represented 7 species, Salticidae represented 6 species, Oxyopidae and Eracidae represented 4 species, Therididae and Thomisidae represented 3 species each, families Lycosidae and Pholcidae represented 2 species, families Clubionidae, Philodromidae, Tetragnathidae, Uloboridae, represent single species. The population of spiders was abundant. Species richness and diversity was high during the month of September and October, the population of species *Stegodyphus* was observed largely during the month of November.

**Keywords:-** *Spiders, Araneae, Species diversity, Insect*

### Introduction

Order Araneae is a large group of animals, which is commonly called as spiders. They are one of the most diverse animals group in the world. They are widespread and found in all types of habitats. Spiders are carnivorous creatures, feeding on insects and small Arachnids, which is one of most abundant predatory groups. More than 37777 species of spiders belonging to 3496 genera, under 109 families are known from all over the world. From the Indian subcontinent, 1035 species of spiders belonging to 240 genera under 46 families are known. Spiders inhabit diverse habitats, they may be found on or near water, in or on the ground, from underground caves to the top of mountains, on or under the bark of trees, found on tall grasses, on bushes, inside human habitations etc.

The spider varies greatly in their size and shape. They also show great variations in colours, mostly mimics their surroundings in body form and colours. Some spiders resembles other animals, some time ant-like spiders, beetle-like spiders confuses with insects, but can be distinguished from insects by

having four pairs of legs, one pair of pedipalp, six or eight pairs of simple eyes etc. All spiders possess spinnerets and produce silk, which is used in many ways. Hunting spiders do not built any web, but the silk produced is having specific use for them. Web spiders use its silk to ensnare the trapped prey. Poison glands are found in most of the spiders, which open by a pore near the tip of each cheliceral fang. Spiders use their venom to kill or subdue the prey and as mean of defense. Most of the spiders are not dangerous to human being. The spiders are present everywhere and are exclusively carnivorous, prey mainly on insects. They are one of the most important biological agents in nature, which help in keeping the insect population in control. A large number of spiders are found in cultivated fields and must be preying on a large number of insect pests of crops. Spiders may be a significant enemy of insect pests. The main aim of present study was to investigate spiders fauna of G.V.I.S.H. campus, Amravati (M.S.)

### Materials and Method

To observe and investigate the spiders fauna of G.V.I.S.H. Campus, Amravati (M.S.), spiders specimen were collected during the first week of

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September to last week of November 2008. Spiders were collected from different areas of campus, such as Botanical garden, Apsara Uddyan, Bohsale Auditorium, social forest, Boys hostel, fencing etc. For collection of spiders insect nets, pitfall trap, stroking sticks, umbrellas were used. The specimens were put in to 70 % alcohol, labeled and identified according to Tikader (1962, 1973, 1982). Before preservation the photographs were taken in different views, to get the clear eye position, pattern and shades of cephalothorax and abdomen, hair and spines pattern etc.

## Results and Discussion

During this study 470 specimens were collected from campus of G.V.I.S.H. Amravati (M.S.). 35 species belonging to 12 families were observed and identified (Table 1). Among the specimens most of the individuals were adult and only few males were observed. The most abundant species were observed from families Araneidae and Salticidae. Species of *Stegodypus* belonging to family Eracidae was abundant during early November and their webs were observed having more than 300-400 eggs, all over the fencings and trees like *Tamarindus*, *Zizipus*, *Bougainvillia* etc.

**Table 1: Spiders recorded from G.V.I.S.H. Campus Amravati (M.S.)**

- |   |  |
|---|--|
| <p>i) Family:-Araneidae (Orb-web spiders)</p> <ol style="list-style-type: none"> <li>1) <i>Araneus</i> sp. (Female)</li> <li>2) <i>Araneus mitifica</i> (Female)</li> <li>3) <i>Araneus shillongensis</i> (Female)</li> <li>4) <i>Argiope</i> sp. (Female)</li> <li>5) <i>Cyclosa</i> sp. (Female)</li> <li>6) <i>Neoscona theis</i> (Female)</li> <li>7) <i>Neoscona</i> sp. (Female)</li> </ol> <p>ii) Family:- Clubionidae</p> <ol style="list-style-type: none"> <li>8) <i>Clubiona</i> sp. (Female)</li> </ol> <p>iii) Family:- Erasidae</p> <ol style="list-style-type: none"> <li>9) <i>Stegodypus</i> sp. (Female)</li> </ol> | <ol style="list-style-type: none"> <li>10) <i>Stegodypus</i> sp. (Female) NEW</li> <li>11) <i>Stegodypus prakashii</i>. (Male)</li> <li>12) <i>Stegodypus sarasinorum</i> (Female)</li> </ol> <p>iv) Family:- Lycosidae (Wolf spiders)</p> <ol style="list-style-type: none"> <li>13) <i>Hyppasa</i> sp. (Female)</li> <li>14) <i>Lycosa</i> sp. (Female)</li> </ol> <p>v) Family:- Oxyopidae (lynx spiders)</p> <ol style="list-style-type: none"> <li>15) <i>Oxyopus Chittrae</i>. (Female)</li> <li>16) <i>Oxyopus pankaji</i>. (Female)</li> <li>17) <i>Oxyopus pawani</i>. (Female)</li> <li>18) <i>Oxyopus</i> sp. (Female)</li> </ol> <p>vi) Family:- Philodromidae</p> <ol style="list-style-type: none"> <li>19) <i>Philodromous</i> sp. (Female)</li> </ol> <p>vii) Family:- Pholcidae</p> <ol style="list-style-type: none"> <li>20) <i>Pholcus</i> sp. (Female)</li> <li>21) <i>Pholcus</i> sp. (Female)</li> </ol> <p>viii) Family:- Salticidae</p> <ol style="list-style-type: none"> <li>22) <i>Euophrys</i> sp. (Female)</li> <li>23) <i>Marpissa</i> sp (Female)</li> <li>24) <i>Phidippus</i> sp. (Female)</li> <li>25) <i>Phidippus</i> sp. (Male)</li> <li>26) <i>Plexipus</i> sp. (Female)</li> <li>27) <i>Telamonia dimidiata</i> (Female)</li> </ol> <p>ix) Family:- Tetragnathidae (Long-)</p> <ol style="list-style-type: none"> <li>28) <i>Tetragnatha mandibulata</i> (Female)</li> </ol> <p>x) Family:- Therididae</p> <ol style="list-style-type: none"> <li>29) <i>Leucauge decorata</i> (Female)</li> <li>30) <i>Leucauge</i> sp. (Female)</li> <li>31) <i>Theridion</i> sp. (Female)</li> </ol> <p>xi) Family:- Thomisidae (Crab spiders)</p> <ol style="list-style-type: none"> <li>32) <i>Thomisus</i> sp. (Female)</li> <li>33) <i>Thomisus</i> sp (Male)</li> <li>34) <i>Xisticus</i> sp. (Female)</li> </ol> <p>xii) Family:- Uloborodae</p> <ol style="list-style-type: none"> <li>35) <i>Uloborus</i> sp. (Female)</li> </ol> |
|---|--|

## Conclusion

The spiders were found to be living in different types of habitat. The spiders belonging to families





Thomisidae, Salticidae, Araneidae, Tetragnathidae, Oxyopidae and Errasidae were mainly found in campus vegetation. Spiders living in residential places included those belonging to families Pholcidae, Uloboridae and Salticidae. Most spiders were found living on the ground, under the stones or in vegetation exhibiting some kind of colouration for camouflage. No exceptionally poisonous spiders was found among the species recorded in the campus. The spiders are exclusively carnivorous and hence help naturally to control insect pests.

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## Water quality of natural springs in Garhwal Himalayas

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### Abstract

Instead of having plenty of water in the Garhwal Himalayas in the form of Glaciers and snow fed rivers, a large population is facing the problem of easy availability of freshwater. Natural springs This paper deals with the study of natural water springs in Garhwal region. The parameters studied were alkalinity, acidity, DO, BOD, free CO<sub>2</sub>, nitrate, H<sub>2</sub>S, chlorides, hardness, inorganic phosphates, temperature, pH and coliform number. The study elucidates that the water quality of selected natural water springs is suitable for drinking purpose.

**Keywords:** - *Water quality, Natural springs, Garhwal, Physico-chemical, Biological*

### Introduction

Natural springs are the major source of potable water in most of villages in Garhwal Himalaya and are naturally emerging from earth. In hills, middle and upper dense vegetation of broad leaved trees viz. *Quercus leucotrichophora* (Banj Oak), *Quercus floribunda* (Moru Oak), *Rhododendron arboretum* (Buarns) etc., absorbs rain water during monsoon. This water is slowly released over the year by these broad leaved plants. Released water percolates in land and forms numerous channels which flows over the year and called as natural springs. Water is the most essential commodity for the entire living system on the earth (Shivashankara and Sharmila, 2004). Till now a very less amount of studies has been carried out on the springs of Garhwal region. Rare are the studies that have been conducted on the riverine ecology in the torrential reaches of the Indian uplands, to which the major rivers, the Ganga and Brahmaputra owe the existence (Nautiyal, 1986). Khanna and

Bhutiani (2004) worked on fish and their ecology of river Ganga at Gohri Ghat, Garhwal. The main objective of the present study, which is the first of its kind in this region, was to assess the current situation and state of spring in Garhwal region. The present study was conducted in order to obtain an overall picture of the prevailing ecological conditions and thus the water quality, in the spring fed Alkananda.

### Materials and Method

The physico-chemical and biological parameters of the water were recorded monthly at different stations followed APHA (1998) and Khanna and Bhutiani (2008). Total four sites were selected for the study as followings:

**Site-1 (Kothar dhara)** - Water emerges after passing through dense vegetation at this sampling site.

**Site-2 (Sweeth Bridge)** This spring lies adjacent to Government Medical College, emerges directly from earth and is surrounded by cemented pool which remains covered by slabs.

**Site-3 (Bhola Mahadev)** - This spring is situated at Srikot. Spring is surrounded by cemented pool.

**Site-4 (Barkot Spring)** - The sampling site lies 3.0 km away from Chauras campus in north. Water was flowing in cemented channels.

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## Results and Discussion

Table 1 represents results of various physico-chemical and biological parameters and is graphically presented in Fig. 1 and 2 at different sampling sites in natural springs.

The alkalinity of water samples was recorded as  $180.31 \pm 4.10$ ,  $178.12 \pm 7.43$ ,  $170.25 \pm 5.50$  and  $230.06 \pm 7.23$  at site 1, 2, 3 and 4, respectively, whereas the permissible limit as 200-600 mg/l. D.O. was recorded as  $2.30 \pm 0.66$ ,  $2.9 \pm 0.37$ ,  $1.3 \pm 0.28$  and  $6.4 \pm 0.36$  mg/l at site 1, 2, 3 and 4, respectively. The acceptable limit varies from 4-6 mg/l. BOD of water sample was recorded as  $0.83 \pm 0.16$ ,  $1.6 \pm 0.16$  and  $0.60 \pm 0.12$  and  $1.0 \pm 0.26$  mg/l at site 1, 2, 3 and 4, respectively. The acceptable limits of BOD are 2-3 mg/l, above this limit water is considered to be not fit for drinking purpose. Nitrate of water sample was recorded as  $0.58 \pm 0.08$ ,  $0.38 \pm 0.06$  and  $0.32$  mg/l at site 1, 2 and 4, respectively.  $H_2S$  was reported as

$5.90 \pm 0.40$ ,  $5.30 \pm 0.35$ ,  $5.30 \pm 1.08$  and  $3.76 \pm 0.63$  at site 1, 2, 3 and 4, respectively. Chlorides of water samples were recorded as  $34.17 \pm 2.11$ ,  $15.12 \pm 1.14$  and  $35.36 \pm 1.89$  and  $11.79 \pm 1.04$  mg/l at site 1, 2, 3 and 4, respectively. Free  $CO_2$  recorded as  $46.1 \pm 2.33$ ,  $34.7 \pm 2.13$  and  $72.6 \pm 3.83$  and  $37.31 \pm 2.21$  mg/l at site 1, 2, 3 and 4, respectively. Hardness of water samples were recorded as  $176 \pm 3.71$ ,  $141 \pm 10.12$ ,  $247 \pm 11.89$  and  $263 \pm 5.92$  mg/l at site 1, 2, 3 and 4. Inorganic phosphate in given water sample recorded as  $0.06 \pm 0.22$ ,  $0.06 \pm 0.01$  and  $0.09 \pm 0.01$  mg/l at site 1, 2 and 4, respectively. pH was recorded as  $6.90 \pm 0.65$ ,  $7.30 \pm 0.41$ ,  $7.26 \pm 0.12$  and  $7.29 \pm 0.14$  at site 1, 2, 3 and 4, respectively. Temperature was recorded as  $23.8 \pm 0.39$ ,  $23.7 \pm 0.86$ ,  $23.9 \pm 2.69$  and  $24.9 \pm 0.79$  at site 1, 2, 3 and 4, respectively. Coliform in given water samples was recorded as 0.50-22.0, 7.0- 63.0, 0.5-10.0 and 32.00 to 450/100 ml at site 1, 2, 3 and 4, respectively. This test is indicator of fecal

**Table 1: The value of physico-chemical and biological parameters observed in natural springs of Garhwal region**

Sampling sites Parameters	Kothar-dhara	Sweeth Bridge	Bhola Mahadev	Barkot	Permissible limit (PL)
Alkalinity (mg/l)	$180.31 \pm 4.10$	$178.12 \pm 7.43$	$170.25 \pm 5.51$	$230.06 \pm 7.23$	200-600*
Acidity (mg/ L)	$81 \pm 1.84$	$63 \pm 1.79$	$121 \pm 3.74$	$76 \pm 2.75$	N.R.
D.O. (mg/ L)	$2.3 \pm 0.66$	$2.9 \pm 0.37$	$1.3 \pm 0.28$	$6.4 \pm 0.36$	4.0-6.0*
B.O.D. (mg/ L)	$0.83 \pm 0.16$	$1.6 \pm 0.16$	$0.6 \pm 0.12$	$1.0 \pm 0.26$	2.0-3.0*
Nitrate (mg/ L)	$0.58 \pm 0.08$	$0.38 \pm 0.06$	NT	$0.32 \pm 0.04$	45-100*
$H_2S$ (mg/ L)	$5.9 \pm 0.40$	$5.3 \pm 0.35$	$5.3 \pm 1.08$	$3.76 \pm 0.63$	N.R.
Chlorides (mg/l)	$34.17 \pm 2.11$	$15.12 \pm 1.14$	$35.36 \pm 1.89$	$11.79 \pm 1.04$	250-1000*
Free $CO_2$ (mg/ L)	$46.1 \pm 2.33$	$34.7 \pm 2.13$	$72.6 \pm 3.83$	$37.3 \pm 2.21$	N.R.
Hardness (mg/ L)	$176 \pm 3.71$	$141 \pm 10.12$	$247 \pm 11.89$	$263 \pm 5.92$	300-600*
Inorganic Phosphate (mg/ L)	$0.06 \pm 0.22$	$0.06 \pm 0.01$	NT	$0.09 \pm 0.01$	0.046-0.068*
Temperature (°C)	$23.8 \pm 0.39$	$23.7 \pm 0.86$	$23.9 \pm 2.69$	$24.9 \pm 0.79$	N.R.
pH	$6.90 \pm 0.65$	$7.30 \pm 0.41$	$7.26 \pm 0.12$	$7.29 \pm 0.14$	6.5-7.5*
Coliform no. (Per 100 ml.)	L = 0.5 U = 22	L = 7.0 U = 63	L = 0.5 U = 10.0	L = 32.0 U = 450	50-500*

\* Public Health Engineering Department, Govt. of West Bengal

NT=Not Traceable; L=Lower Limit; U=Upper Limit



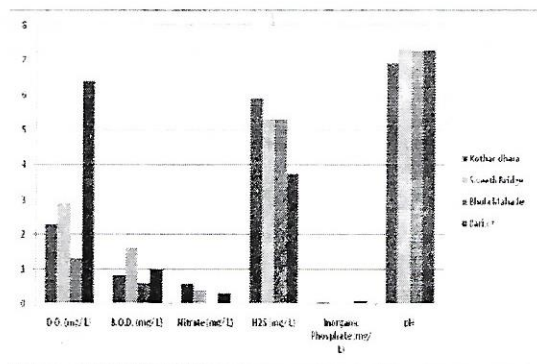


Fig. 1: The graphical representation of of physico-chemical and biological parameters observed in natural springs of Garhwal region

contamination. The permissible value of coliform in the sample is very much disputed. WHO recommends the coliform count 0/100 ml for drinking water while the Indian standards are very much flexible and permissible value of coliform is 50-500/100 ml.

Groundwater is rich in carbonic acid and dissolved oxygen usually possesses a high solubilizing potential towards soil or rocks that contain appreciable amount of mineral calcite, gypsum and dolomite and consequently hardness level may increased. That's why the values of conductivity, TDS and DO were observed beyond the limit of drinking purpose (Singh *et al.*, 2007). Negi *et al.*, (2008) have reported water temperature to range between 14.62 to 20.25 °C at Ganga River. It is a well established fact that the dissolved oxygen budget of a river is a direct indicator of its biological state, as was also suggested by Lamb (1985). The temperature may not be considered as most important factor in case of pure water due to the eurythermic nature of aquatic biota, but in polluted waters, the temperature has serious impact on dissolved oxygen and BOD (Palharya and Malviya, 1988). Phosphate-phosphorus is one of the most important limiting nutrients of primary concern to aquatic ecology (Datta *et al.*, 1988). The world

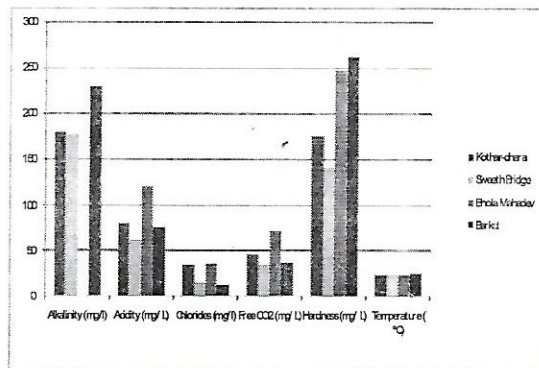


Fig. 2: The graphical representation of of physico-chemical and biological parameters observed in natural springs of Garhwal region

average for nitrate in unpolluted freshwaters as reported by Reid (1961) is 0.30 mg/l.

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## Studies on antibacterial activity of extracts from *Tinospora cordifolia* (Giloy) against *Staphylococcus aureus*

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### Abstract

The active components of stem bark of *Tinospora cordifolia* were extracted using cold water and organic solvents (methanol, diethyl ether and acetone) and were tested against *Staphylococcus aureus* using the agar disc diffusion method. All the four extracts inhibited the growth of *S. aureus*, with methanol extract exerting the highest activity whereas water extract was least active. The results were compared with the reference antibiotic ciprofloxacin.

**Keywords:-** Antibacterial, *Tinospora cordifolia*, *S. aureus*, Stem extract, Methanol, Diethyl ether, Acetone.

### Introduction

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Nascimento *et al.*, 2000). According to WHO medicinal plants would be the best source to obtain a variety of drugs (Santos *et al.*, 1995). Therefore such plants should be investigated to better understand their properties, safety and efficacy (Ellof, 1998). Antimicrobial activity of 120 plant species from 28 different families was carried out (Santos *et al.*, 1990). Antifungal activity of leaf extracts of medicinal plants used by Himalayan people was investigated against *Alternaria alternate* and *Curvuleria lunata* (Guleria and Kumar, 2006). 18 plants belonging to zingiberaceae family was evaluated for their antioxidant and antimicrobial activity (Chen *et al.*, 2008). *T. cordifolia* (Giloy) is a large glabrous and climbing succulent shrub with rocky bark. It is found throughout the tropical India ascending up to an altitude of 300m

and has been used from ancient times to cure different types of ailments like general debility, dyspepsia, fever and urinary diseases (Negi and Pant, 1994).

### Materials and Method

The matured leaves of *T. cordifolia* were collected from Hardwar and the bacterial strain of *S. aureus* (MTCC-737) was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. For the preparation of plant extract the powdered stem bark of *T. cordifolia* were extracted with methanol, diethyl ether and acetone for 24 hrs using Soxhlet apparatus and aqueous extract. Three different dilutions of plant extracts i.e. 800, 400 and 200 mg/ml DMSO were used for primary screening which was carried out through agar disc diffusion method (Bauer *et al.*, 1966). DMSO served as negative control and standard antibiotic ciprofloxacin (500 ppm) as positive control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones in mm.

### Results and Discussion

Preliminary evaluation of antibacterial activity clearly indicates that all the stem bark extracts prepared in four solvents exhibited activity against

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*S. aureus* but the 800 mg/ml concentration of methanol extract was found to possess higher level of antibacterial activity (14.3 mm) in comparison to diethyl ether, acetone and aqueous extract which was comparable to that of antibiotic ciprofloxacin (15 mm). Hence, it can be concluded that keeping in mind the side effects of allopathic medicines and the drug resistance in microbes it will be of great

interest to use plant based medicines to combat against diseases. The discovery of a potent remedy from plant origin will be of great advancement in bacterial infection therapies.

**Table 1: Antibacterial activity of stem bark extract of *T.cordifolia* and that of reference antibiotic on *S.aureus***

Solvents	*Effective zones of inhibition				
	Concentration of sample(mg/ml)			Antibiotic control(500ppm)	DMSO control
	800	400	200	Ciprofloxacin	
Methanol	14.3	12.3	10.3	15	0
D.E.E	14.0	10.3	7.6	15	0
Acetone	11.6	9.6	7.3	15	0
Aqueous	11.3	9.3	6.6	15	0

\*Effective zone of inhibition = Total zone of inhibition – Diameter of the disc (5mm)

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## Water quality analysis of River Panv Dhoi in reference to its physico-chemical parameters and heavy metals

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### Abstract

This paper deals with the analysis of different water parameters of River Panv Dhoi which flows through Saharanpur district. It is a streamfed river and a tributary of Hindon. The sample collection was usually completed during morning hrs. between 8:00 AM to 10:00 AM. The parameters like Temperature, Turbidity, Conductivity, Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), pH, Dissolved Oxygen (DO), Free Carbon dioxide (CO<sub>2</sub>), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Alkalinity, Total Hardness, Chloride (Cl<sup>-</sup>) and heavy metals like Lead, Zinc, Mercury and copper were analyzed.

**Keywords:-** Water, River, Panv Dhoi, Saharanpur, Heavy metals

### Introduction

Water is the most precious commodity of life. It is not only the basic need for sustaining human life but also vital to all the segments of economic development. Water is being adversely affected, qualitatively and quantitatively by all kinds of human activities on land, in air and/or in water. River Panv Dhoi flows through Saharanpur district. It is a streamfed river and a tributary of Hindon. This river originates near Shanklapuri Shiv Mandir of Panwarka, than it goes to Saharanpur. It is about 15 km in length and then it mixes with river Dhamola (Fig. 1). Metallic scraps, metallic dust, oil, paper and wooden products, besides domestic garbage appear to be the main items of the sewage. Main drain carries away some effluent from the factories and also from the residential colonies. This drain carries domestic sewage, which is poured into river Panv Dhoi. Therefore, it can be well considered that it carries a variety of pollutants of equally different in physico-chemical nature. A lot of work

has been done to evaluate the impact of human interferences on different water bodies by Khanna *et al.* (1997), Khanna *et al.* (2000), Khanna and Bhutiani (2003) and Khanna and Chugh (2004) and many more. It is very much essential to monitor the water quality of different water bodies present to assess their suitability for different purposes. Therefore the physico-chemical parameters of river Panv Dhoi were analysed for different seasons during 2002-2003.

### Materials and Method

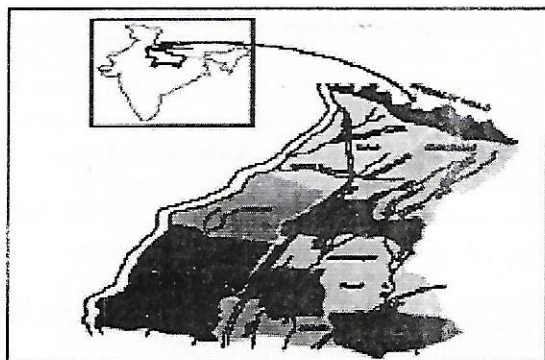
The samples for different parameters were analysed with the help of the procedure described by Welch (1948), APHA (1980), Mathur (1982), Ross (1983), Trivedi and Goel (1984) and Khanna (1993). The water samples were collected from five different sampling sites Shanklapuri Shiv Mandir (A), Makhraj Ka Pul (B), Laldas Ka Baada (C), Jogyan Pul (D) and Near Dhamola (E). The sample collection was usually completed during morning hrs. between 8:00 A.M. to 10:00 A.M. The parameters like Temperature, Turbidity, Conductivity, Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), pH, Dissolved Oxygen

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**Fig. 1: Map of District Saharanpur showing the situation of Panv Dhoi River**

(DO), Free Carbon dioxide ( $\text{CO}_2$ ), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Alkalinity, Total Hardness, Chloride ( $\text{Cl}^-$ ) and heavy metals like Lead, Zinc, Mercury and copper were analyzed.

## Results and Discussion

In an aquatic ecosystem the physico-chemical parameters are the main important factors responsible for the biotic healthiness of the system. They show their effect individually and also collectively. The values of different physico-chemical parameters in different seasons were tabulated in the Table 1-10 and are graphically represented in Fig. 2 to 11.

The temperature is one of the most important parameter in an aquatic environment. In the present study, a difference in the fluctuation of water temperature was observed  $10.4 \pm 1.5$  (minimum) in the winter season and  $28.0 \pm 0.5$  (maximum) in monsoon season. Annual average values of temperature varied between  $15.6^\circ\text{C} \pm 4.5$  to  $25.0^\circ\text{C} \pm 4.4$ . Minimum annual average value of temperature was observed at sampling station A and maximum was observed at sampling Station E. The water temperature showed an upward trend from winter season to summer season followed by a downward trend from monsoon season onwards. A more or less similar trend has been observed in the river Yamuna by Chakrabarty *et al.* (1959) and in the Kallayi (John, 1976). Badola and Singh (1981) also reported similar trend in

the river Alaknanda. Same trend of temperature was observed by Khanna *et al.* (2001) in river Ganga at Haridwar.

The present study showed conductivity fluctuation of  $65.10 \text{ mmhos/cm}^2 \pm 2.98$  in winter season to  $336.78 \text{ mmhos/cm}^2 \pm 0.58$  in monsoon season. Annual average values of conductivity varied between  $142.07 \text{ mmhos/cm}^2 \pm 104.39$  to  $191.44 \text{ mmhos/cm}^2 \pm 127.32$ . Minimum annual average value of conductivity was observed at sampling station E and maximum was observed at Sampling Station A. Identical results were observed by Raina *et al.* (1982) and Mittal and Sengar (1990) from various Indian rivers. Khanna *et al.* (2003) also observed similar trend of conductivity in Ganga river at Bulandshahar.

The water of the river Panv Dhoi becomes start turbid from summer season onward and in rainy season the water was highly turbid. The lowest turbidity was recorded ( $19.78 \text{ JTU} \pm 1.00$ ) in winter season and highest ( $1000.00 \text{ JTU} \pm 150.21$ ) noted in monsoon season. Annual average value of turbidity varied between  $49.58 \text{ JTU} \pm 40.44$  to  $908.46 \text{ JTU} \pm 101.05$  in which maximum average value observed at sampling station E and minimum at sampling station B. Similar pattern was also reported by Badola and Singh (1981), Dobriyal *et al.* (1983) in the hill streams of the Garhwal Himalaya, Khanna *et al.* (1997) in river Ganga, and Seth *et al.* (2000) in the river Ganga.

In the present investigation it was noted that the total solids were maximum in monsoon season ( $2400.00 \text{ mg/l} \pm 226.63$ ), which were responsible for the turbidity in the river. The total solids were recorded minimum ( $68.44 \text{ mg/l} \pm 1.36$ ) in winter season due to gradual sedimentation of the filterable residue. Annual average values of total solids varied between  $179.53 \text{ mg/l} \pm 168.53$  to  $1726.94 \text{ mg/l} \pm 651.22$ . Minimum average value of total solids was observed at sampling station A and maximum at sampling station E. The highest value of total solids range between  $1873 \text{ mg/l}$  and  $3573 \text{ mg/l}$  reported by Kumar and Sharma (2002) in the river Krishna. Total solids cause ecological imbalance in the aquatic ecosystem by mechanical abrasive action. Similar trends were shown by Khanna and Chugh (2004) during study





of water quality of River Ganga at Haridwar. But Khanna and Singh (2000) found that total solids were maximum in summer in the water of Suswa River.

Minimum values of total dissolved solids were obtained during winter season ( $48.33 \text{ mg/l} \pm 1.57$ ) and maximum values during monsoon season ( $1100.00 \text{ mg/l} \pm 55.00$ ). Annual average value of total dissolved solids varied between  $105.24 \text{ mg/l} \pm 13.10$  to  $766.66 \text{ mg/l} \pm 351.18$ , in which minimum average value was obtained from sampling station C and maximum from sampling station E. Same study was also done by Khanna and Chugh (2004) in the river Ganga at Haridwar.

Minimum values of total suspended solids were obtained during winter season ( $20.11 \text{ mg/l} \pm 0.11$ ) and maximum values during monsoon season ( $1300.00 \text{ mg/l} \pm 120.04$ ). Annual average value of total suspended solids varied between  $57.85 \text{ mg/l} \pm 54.94$  to  $960.26 \text{ mg/l} \pm 307.78$ , in which minimum average value was obtained from sampling station A and maximum from sampling station E. Same study was also done by Khanna and Chugh (2004) in the river Ganga at Haridwar.

The Panv Dhoi river at Saharanpur showed high pH value ( $8.88 \pm 0.19$ ) in monsoon season which might be due to increase chemical load in the river. The minimum pH values ( $7.07 \pm 0.26$ ) were observed in winter season. The annual average values of pH varied between  $7.32 \pm 0.25$  to  $8.65 \pm 0.37$ . Highest annual average value was recorded at sampling station E and minimum at station A. It was recorded during this study that pH of the river Panv Dhoi is slightly alkaline in nature. Identical results were reported by Sangu and Sharma (1985) in the river Yamuna and Khanna *et al.* (1999) in Ganga river. Khanna *et al.* (2001) in the river Ganga at Haridwar and Khanna *et al.* (2003) in the river Ganga at Bulandshahar have also shown the alkaline nature of river.

The bio-chemical oxygen demand was observed maximum ( $530 \text{ mg/l} \pm 15.98$ ) in monsoon season and minimum ( $1.39 \text{ mg/l} \pm 0.17$ ) in winter season. The annual average value of biochemical oxygen demand ranged between  $2.26 \text{ mg/l} \pm 0.82$  to  $468.33 \text{ mg/l} \pm 80.98$ . The minimum average value was found at sampling station

A and maximum at sampling station E. Highest annual average value of bio-chemical oxygen demand at sampling station E may be due to drainage of several small sewage drains into the river and runoff of sludgy, silted sewage during months of rainy season. Khanna & Singh (2000) noticed peak values during summer in Suswa river and Khanna *et al.* (1997) observed peak values in monsoon season in river Ganga.

Chemical Oxygen Demand (COD) represents chemically oxidizable load of organic matter in water. It was noted highest ( $1240.00 \text{ mg/l} \pm 145.26$ ) in monsoon season and minimum ( $4.69 \text{ mg/l} \pm 0.18$ ) in winter season. The annual average value of COD ranged between  $5.62 \text{ mg/l} \pm 0.69$  to  $1075.00 \text{ mg/l} \pm 220.17$  whereas least average value was found at sampling station B and maximum at sampling station E. Similar trends of COD have shown by Khanna *et al.* (2002, 2003) in the river Ganga and Khanna & Singh (2000) in Suswa River at Raiwala.

Maximum dissolved oxygen was recorded ( $11.78 \text{ mg/l} \pm 0.25$ ) in the winter season. The minimum value of dissolved oxygen ( $0.07 \text{ mg/l} \pm 0.01$ ) was observed in monsoon season. The annual average value of dissolved oxygen ranged between  $1.68 \text{ mg/l} \pm 1.47$  to  $9.61 \text{ mg/l} \pm 1.41$ , whereas the minimum annual average value of DO was observed at sampling station E and maximum was observed at sampling station B. The dissolved oxygen reduced gradually from summer onward due to turbidity which retarded the photosynthetic activity of aquatic flora. The temperature showed an inverse relationship with the DO almost throughout the study. The cause of maximum dissolved oxygen in winter is due to reduced rate of decomposition by decreased microbial activity at low temperature (Strommer and Smock, 1989). Chopra *et al.* (1990), Gopal and Sah (1993) and Sharma (1999) also have got the same result and have opined that low temperature in winter increases the oxygen retaining capacity of water and solubility of  $\text{O}_2$  in water. This trend was also observed by Badola and Singh (1981) in the river Alaknanda. Khanna (1993, 2001) and Khanna and Chugh (2004) has also reported the same trends in the river Ganga at Haridwar. Free carbon dioxide was observed maximum ( $6.24 \text{ mg/l} \pm 0.96$ ) in monsoon season due to





higher turbidity and water temperature, but was recorded minimum ( $0.07 \text{ mg/l} \pm 0.01$ ) in winter season. Annual average value of free carbon dioxide varied between  $2.02 \text{ mg/l} \pm 1.95$  to  $4.74 \text{ mg/l} \pm 1.56$  in which maximum average value obtained from sampling point E and minimum observed at sampling point A. Pahwa and Mehrotra (1966) and Ray *et al.* (1966) have reported that the Ganga river contains maximum free carbon dioxide in rainy season at Allahabad. Khanna *et al.* (1997) and Seth *et al.* (2000) have also reported the same trends in the river Ganga at Haridwar but Khanna and Singh (2000) observed maximum free carbon dioxide during summer in Suswa River at Raiwala, Dehradun.

Alkalinity of water is a measure of weak acid present in it and of the cations balanced against them (Sverdrup *et al.*, 1942). The highest concentration ( $820.00 \text{ mg/l} \pm 55.27$ ) was observed in summer season and lowest ( $35.00 \text{ mg/l} \pm 1.38$ ) in winter season. The annual average value of alkalinity varied between  $50.02 \text{ mg/l} \pm 15.76$  to  $781.66 \text{ mg/l} \pm 62.11$  in which maximum average value was obtained from sampling station E and minimum from sampling station A. Similar observation was also obtained by Khanna and Chugh (2004), Holden and Green (1960), Tallying and Rzoska (1967) and Abidin (1948).

Maximum values of total hardness were recorded in monsoon season ( $390.82 \text{ mg/l} \pm 26.71$ ) and minimum was recorded in summer season ( $83.78 \text{ mg/l} \pm 0.56$ ). The annual average values of total hardness ranged from  $95.66 \text{ mg/l} \pm 11.71$  to  $279.74 \text{ mg/l} \pm 17.27$  in which maximum average value is recorded from sampling station E and minimum at sampling station A. Khanna *et al.* (1993, 2003) and Mishra (2003) observed hardness in river Ganga at Haridwar and found more or less similar trends in their study.

The highest and lowest values of chloride were found in monsoon and winter season,  $68.32 \text{ mg/l} \pm 4.56$  and  $1.97 \text{ mg/l} \pm 1.66$  respectively. The annual average value of chloride varied between  $5.51 \text{ mg/l} \pm 5.29$  to  $59.67 \text{ mg/l} \pm 3.16$  in which maximum average value was recorded from the station E and minimum was at sampling station A. Chlorides are present in

sewage, sewage effluents and farm drainage. Significant levels of chloride were shown by many rivers like Yamuna (Sengar *et al.*, 1985); Tungbhadra (Reddy and Venkateswarlu, 1987); Jhelum (Raina *et al.*, 1984) and Kshipra (Mishra and Saxena, 1984). CPCB (2003) reported the value of chloride in between 14 to 51 mg/l during Ganga monitoring from Bithur, Kanpur to Sangam, Allahabad.

The minimum value  $0.0638 \text{ mg/l} \pm 0.0072$  of lead was found from sampling station A and maximum  $5.3975 \text{ mg/l} \pm 0.7123$  from sampling station E. The annual average of lead (Pb) ranged between  $0.0702 \text{ mg/l} \pm 0.0076$  to  $4.8452 \text{ mg/l} \pm 0.6109$ . The minimum value  $0.0214 \text{ mg/l} \pm 0.0022$  of copper was found from sampling station A and maximum  $1.9774 \text{ mg/l} \pm 0.3996$  from sampling station E. The annual average value of copper varied between  $0.0550 \text{ mg/l} \pm 0.0292$  to  $1.5945 \text{ mg/l} \pm 0.3729$  in which minimum average value of copper was obtained at sampling station A and maximum average value at sampling station E.

Hg is not required even in small amount by any organisms. Virtually all metals, including the essential metal micronutrients, are toxic if exposure levels are sufficient high. The increased circulation of toxic metals in recent times resulted in the unavoidable build up of such toxic substances in the human food chain. The minimum value  $3.1224 \text{ mg/l} \pm 0.0467$  of zinc was found from sampling station A and maximum  $5.0012 \text{ mg/l} \pm 0.1814$  from sampling station E. The annual average value of Zinc ranged between  $3.1651 \text{ mg/l} \pm 0.0528$  to  $4.8695 \text{ mg/l} \pm 0.1763$  in which minimum average value of Zinc was obtained from sampling station A and maximum was recorded at sampling station E. The annual average value of Mercury varied between  $0.0000 \pm 0.0000$  to  $0.0005 \text{ mg/l} \pm 0.0006$ . The minimum was found as nil at sampling station A, B, C and maximum ( $0.0009 \text{ mg/l} \pm 0.0007$ ) at sampling station E in. The concentration of these metals at sampling station C, D and E give a highly misleading picture of the degree of metal pollution. Khanna *et al.* (2003) also reported heavy metals in water of Ganga river at Bulandshahar.



Table 1. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station A (2002-2003)

Physico-Chemical Parameters	2002-2003			
	Summer	Monsoon	Winter	Average
Temperature(°C)	18.0 ± 1.3	18.4 ± 1.3	10.4 ± 1.5	15.6 ± 4.5
Conductivity (µmhos/cm <sup>2</sup> )	138.56 ± 0.40	336.78 ± 0.58	99.00 ± 0.56	191.44 ± 127.32
Turbidity (J.T.U.)	33.22 ± 0.36	120.28 ± 0.37	19.83 ± 0.45	57.78 ± 54.54
Total Solids (mg/L)	96.70 ± 1.35	373.45 ± 1.26	68.44 ± 1.36	179.53 ± 168.53
T.D.S. (mg/L)	64.13 ± 1.41	252.56 ± 1.48	48.33 ± 1.57	121.67 ± 113.62
T.S.S. (mg/L)	32.57 ± 0.04	120.89 ± 0.08	20.11 ± 0.11	57.85 ± 54.94
pH	7.30 ± 0.31	7.57 ± 0.29	7.07 ± 0.26	7.32 ± 0.25
BOD (mg/L)	2.30 ± 0.27	3.08 ± 0.35	1.39 ± 0.17	2.26 ± 0.82
COD (mg/L)	5.02 ± 0.29	7.36 ± 1.09	4.69 ± 0.18	5.69 ± 1.45
DO(mg/L)	9.52 ± 0.34	7.34 ± 1.11	11.78 ± 0.25	9.55 ± 2.18
Free CO <sub>2</sub> (mg/L)	2.02 ± 0.29	3.98 ± 0.19	0.07 ± 0.01	2.02 ± 1.95
Alkalinity (mg/L)	66.67 ± 1.34	48.39 ± 1.22	35.00 ± 1.38	50.02 ± 15.76
Total Hardness (mg/L)	83.78 ± 0.56	107.20 ± 0.71	96.00 ± 0.85	95.66 ± 11.71
Chloride (mg/L)	2.96 ± 1.57	11.58 ± 1.90	1.97 ± 1.66	5.51 ± 5.29

Table 2. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station B (2002-2003)

Physico-Chemical Parameters	2002-2003			
	Summer	Monsoon	Winter	Average
Temperature(°C)	19.8 ± 1.6	20.2 ± 1.2	12.6 ± 0.7	17.6 ± 4.2
Conductivity (µmhos/cm <sup>2</sup> )	138.15 ± 1.00	336.61 ± 31.12	99.58 ± 7.04	191.44 ± 127.18
Turbidity (J.T.U.)	33.34 ± 1.92	95.62 ± 0.63	19.76 ± 1.00	49.58 ± 40.44
Total Solids (mg/L)	97.75 ± 1.82	377.55 ± 14.51	70.47 ± 1.21	181.92 ± 169.98
T.D.S. (mg/L)	65.17 ± 1.74	254.57 ± 10.79	49.35 ± 4.98	123.03 ± 114.19
T.S.S. (mg/L)	32.58 ± 1.98	122.98 ± 2.03	21.12 ± 1.23	58.88 ± 55.79
pH	7.35 ± 0.93	7.59 ± 0.28	7.09 ± 0.05	7.34 ± 0.25
BOD (mg/L)	2.88 ± 0.62	3.18 ± 0.58	1.57 ± 0.35	2.54 ± 0.86
COD (mg/L)	5.52 ± 0.98	6.36 ± 1.67	4.98 ± 0.79	5.62 ± 0.69
DO(mg/L)	9.65 ± 0.72	8.18 ± 1.02	11.01 ± 0.58	9.61 ± 1.41
Free CO <sub>2</sub> (mg/L)	2.08 ± 0.06	4.20 ± 0.12	1.24 ± 0.97	2.56 ± 1.52
Alkalinity (mg/L)	67.67 ± 1.72	49.08 ± 1.21	35.94 ± 0.78	50.89 ± 15.94
Total Hardness (mg/L)	84.01 ± 1.67	108.33 ± 2.61	96.94 ± 0.76	96.44 ± 12.19
Chlorides (mg/L)	3.15 ± 0.92	12.05 ± 0.99	2.37 ± 1.22	5.85 ± 5.37





Table 3. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station C (2002-2003)

Physico-Chemical Parameters	2002-2003			
	Summer	Monsoon	Winter	Average
Temperature(°C)	22.1 ± 0.7	24.2 ± 0.9	14.2 ± 0.6	20.2 ± 5.2
Conductivity (µmhos/cm <sup>2</sup> )	111.26 ± 6.27	300.24 ± 15.25	85.20 ± 1.79	165.56 ± 117.35
Turbidity (J.T.U.)	250.00 ± 1.34	270.00 ± 9.50	210.00 ± 4.56	243.33 ± 30.55
Total Solids (mg/L)	176.22 ± 13.57	213.32 ± 9.28	170.26 ± 2.67	186.60 ± 23.33
T.D.S. (mg/L)	99.20 ± 5.42	120.28 ± 14.26	96.26 ± 6.21	105.24 ± 13.10
T.S.S. (mg/L)	77.02 ± 4.31	93.04 ± 2.74	74.00 ± 1.28	81.53 ± 10.23
pH	7.48 ± 0.18	7.62 ± 0.20	7.40 ± 0.10	7.50 ± 0.09
BOD (mg/L)	3.44 ± 0.63	11.16 ± 0.29	5.67 ± 0.12	8.42 ± 2.74
COD (mg/L)	26.30 ± 1.26	35.40 ± 1.04	22.65 ± 0.44	29.11 ± 6.56
DO(mg/L)	7.85 ± 0.27	6.56 ± 0.45	8.27 ± 0.89	7.56 ± 0.89
Free CO <sub>2</sub> (mg/L)	2.21 ± 0.27	4.12 ± 0.39	1.75 ± 0.34	2.69 ± 1.25
Alkalinity (mg/L)	272.98 ± 9.02	261.55 ± 2.54	249.00 ± 1.04	261.17 ± 11.99
Total Hardness (mg/L)	221.00 ± 1.39	229.32 ± 4.58	215.51 ± 7.21	221.94 ± 6.96
Chloride (mg/L)	32.72 ± 1.85	39.39 ± 1.09	31.26 ± 0.77	34.45 ± 4.33

Table 4. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station D (2002-2003)

Physico-Chemical Parameters	2002-2003			
	Summer	Monsoon	Winter	Average
Temperature(°C)	26.0 ± 0.6	26.9 ± 0.8	17.0 ± 0.7	23.4 ± 5.5
Conductivity (µmhos/cm <sup>2</sup> )	101.90 ± 1.02	274.78 ± 6.84	70.20 ± 3.22	148.96 ± 110.11
Turbidity (J.T.U.)	550.80 ± 9.95	650.70 ± 17.12	400.24 ± 6.27	533.93 ± 126.08
Total Solids (mg/L)	1536.42 ± 41.23	2315.28 ± 112.21	1030.50 ± 79.62	1627.40 ± 647.20
T.D.S. (mg/L)	755.62 ± 22.32	1015.28 ± 14.21	390.50 ± 7.96	720.46 ± 313.86
T.S.S. (mg/L)	780.80 ± 65.83	1300.00 ± 120.04	640.00 ± 29.32	906.93 ± 347.60
pH	8.80 ± 0.24	8.40 ± 0.15	8.00 ± 0.10	8.42 ± 0.40
BOD (mg/L)	315.00 ± 8.94	325.00 ± 10.06	205.00 ± 14.28	281.66 ± 68.58
COD (mg/L)	750.00 ± 45.21	950.00 ± 40.75	605.00 ± 20.29	768.33 ± 173.22
DO(mg/L)	4.90 ± 0.98	4.00 ± 0.34	5.10 ± 0.45	4.66 ± 0.58
Free CO <sub>2</sub> (mg/L)	3.74 ± 1.22	5.36 ± 0.46	2.67 ± 0.94	3.93 ± 1.36
Alkalinity (mg/L)	715.00 ± 47.65	710.25 ± 22.98	708.00 ± 38.12	711.08 ± 3.57
Total Hardness (mg/L)	272.50 ± 6.74	294.00 ± 2.34	261.00 ± 41.08	275.83 ± 16.75
Chloride (mg/L)	58.37 ± 14.78	60.00 ± 8.96	53.12 ± 21.49	57.16 ± 3.59



Table 5. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station E (2002-2003)

Physico-Chemical Parameters	2002-2003			
	Summer	Monsoon	Winter	Average
Temperature (°C)	27.0 ± 0.7	28.0 ± 0.5	26.0 ± 0.6	25.0 ± 4.4
Conductivity (µmhos/cm <sup>2</sup> )	100.20 ± 4.27	260.90 ± 8.42	85.10 ± 2.88	142.07 ± 104.39
Turbidity (J.T.U.)	925.23 ± 69.32	1000.00 ± 150.21	800.00 ± 22.34	908.46 ± 101.05
Total Solids (mg/L)	1680.82 ± 167.34	2400.00 ± 226.63	1100.00 ± 175.00	1726.94 ± 651.02
T.D.S. (mg/L)	800.00 ± 28.82	1100.00 ± 55.00	400.00 ± 12.05	766.66 ± 351.16
T.S.S. (mg/L)	880.82 ± 14.99	1300.00 ± 87.73	700.00 ± 63.29	960.26 ± 307.76
pH	8.85 ± 0.18	8.88 ± 0.19	8.22 ± 0.10	8.65 ± 0.37
BOD (mg/L)	500.00 ± 35.20	530.00 ± 15.98	375.00 ± 14.71	468.33 ± 80.98
COD (mg/L)	1160.00 ± 0.99	1240.00 ± 145.26	825.00 ± 88.74	1075.00 ± 220.17
DO(mg/L)	2.02 ± 0.24	0.07 ± 0.01	2.96 ± 0.19	1.68 ± 1.47
Free CO <sub>2</sub> (mg/L)	4.88 ± 0.64	5.24 ± 0.96	3.12 ± 0.72	4.74 ± 1.56
Alkalinity (mg/L)	820.00 ± 55.27	815.00 ± 45.72	710.00 ± 41.38	781.66 ± 62.11
Total Hardness (mg/L)	276.40 ± 18.45	390.82 ± 26.71	262.00 ± 22.94	379.74 ± 17.27
Chloride (mg/L)	60.54 ± 2.50	68.32 ± 4.56	50.17 ± 5.04	59.67 ± 3.16

Table 6. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station A (2002-2003)

Heavy Metals	2002-2003			
	Summer	Monsoon	Winter	Average
Lead (Pb) (mg/L)	0.0880 ± 0.0072	0.0798 ± 0.0070	0.0636 ± 0.0072	0.0762 ± 0.0076
Copper (Cu) (mg/L)	0.0695 ± 0.0067	0.0740 ± 0.0059	0.0214 ± 0.0022	0.0550 ± 0.0292
Zinc (Zn) (mg/L)	3.1224 ± 0.0467	3.1488 ± 0.0513	3.2242 ± 0.0538	3.1651 ± 0.0528
Mercury (Hg) (mg/L)	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 7. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station B (2002-2003)

Heavy Metals	2002-2003			
	Summer	Monsoon	Winter	Average
Lead (Pb) (mg/L)	0.0697 ± 0.0077	0.0760 ± 0.0072	0.0647 ± 0.0055	0.0711 ± 0.0073
Copper (Cu) (mg/L)	0.0739 ± 0.0284	0.0814 ± 0.0301	0.0324 ± 0.0324	0.0626 ± 0.0253
Zinc (Zn) (mg/L)	3.2258 ± 0.0265	3.2260 ± 0.0251	3.1810 ± 0.0231	3.2109 ± 0.0259
Mercury (Hg) (mg/L)	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000





Table 8. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station C (2002-2003)

Heavy Metals	2002-2003			
	Summer	Monsoon	Winter	Average
Lead (Pb) (mg/L)	1.645 ± 0.3930	1.992 ± 0.4136	1.224 ± 0.2184	1.6170 ± 0.3797
Copper (Cu) (mg/L)	0.0872 ± 0.0181	0.0935 ± 0.0186	0.0649 ± 0.0161	0.0835 ± 0.017
Zinc (Zn) (mg/L)	3.6527 ± 0.2056	3.8928 ± 0.2132	3.4912 ± 0.2001	3.6755 ± 0.2067
Mercury (Hg) (mg/L)	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 9. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station D (2002-2003)

Heavy Metals	2002-2003			
	Summer	Monsoon	Winter	Average
Lead (Pb) (mg/L)	3.2755 ± 0.0796	3.3972 ± 0.08120	3.2527 ± 0.0612	3.3084 ± 0.0776
Copper (Cu) (mg/L)	0.8475 ± 0.1761	0.9976 ± 0.1810	0.6474 ± 0.161	0.8308 ± 0.1756
Zinc (Zn) (mg/L)	4.2186 ± 0.01230	4.3270 ± 0.0112	4.1874 ± 0.0149	4.2443 ± 0.0732
Mercury (Hg) (mg/L)	0.0001 ± 0.00000	0.0002 ± 0.00000	0.0000 ± 0.0000	0.0001 ± 0.0000

Table 10. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station E (2002-2003)

Heavy Metals	2002-2003			
	Summer	Monsoon	Winter	Average
Lead (Pb) (mg/L)	4.9492 ± 0.6260	5.3975 ± 0.7123	4.1889 ± 0.5600	4.8452 ± 0.6109
Copper (Cu) (mg/L)	1.5737 ± 0.3631	1.9774 ± 0.3996	1.2324 ± 0.2998	1.5945 ± 0.3729
Zinc (Zn) (mg/L)	4.9384 ± 0.1796	5.0012 ± 0.1814	4.8692 ± 0.1816	4.8695 ± 0.1763
Mercury (Hg) (mg/L)	0.0002 ± 0.0004	0.0009 ± 0.0007	0.0004 ± 0.0005	0.0005 ± 0.0006

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Fig. 2. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station A (2002-2003)

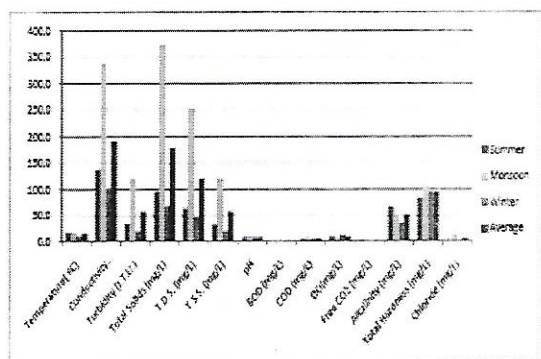


Fig. 3. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station B (2002-2003)

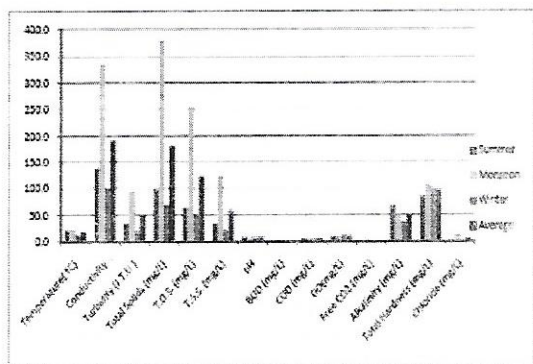


Fig. 4. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station C (2002-2003)

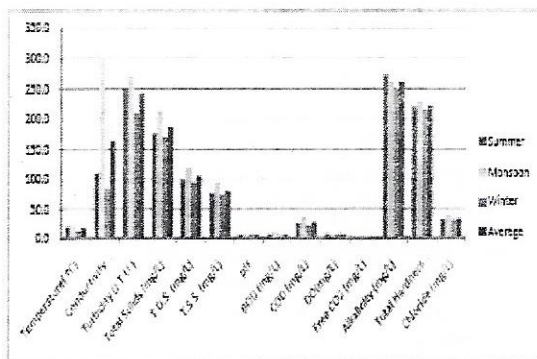


Fig. 5. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station D (2002-2003)

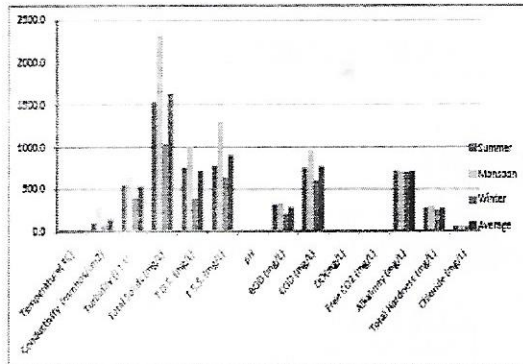


Fig. 6. Seasonal variation in physico-chemical parameters of River Panv Dhoi at sampling station E (2002-2003)

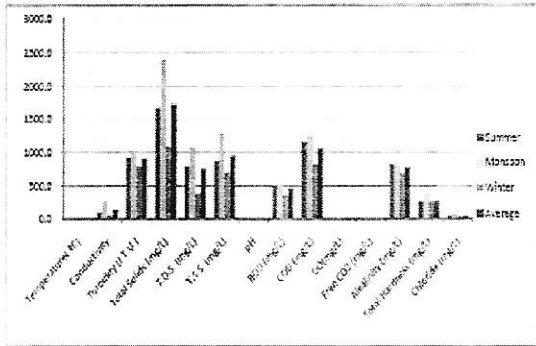


Fig. 7. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station A (2002-2003)

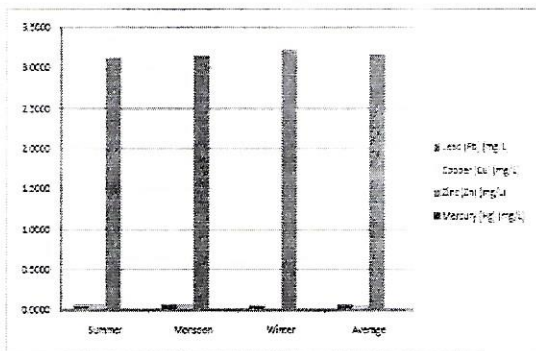


Fig. 8. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station B (2002-2003)

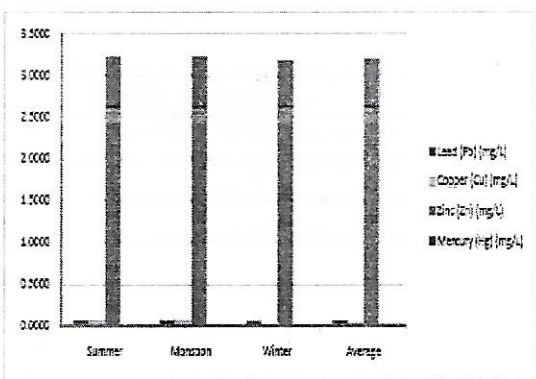


Fig. 9. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station C (2002-2003)

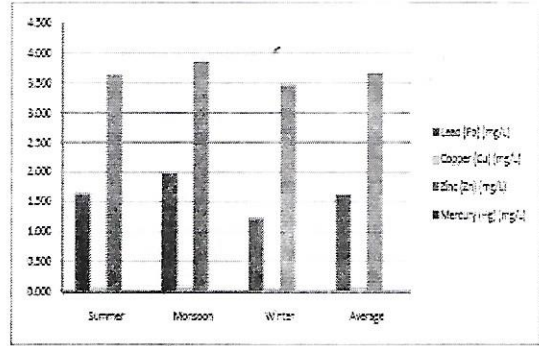


Fig. 10. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station D (2002-2003)

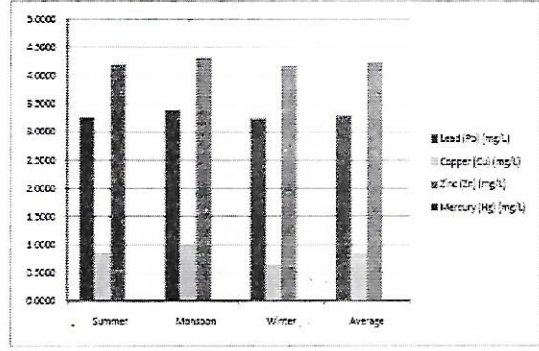
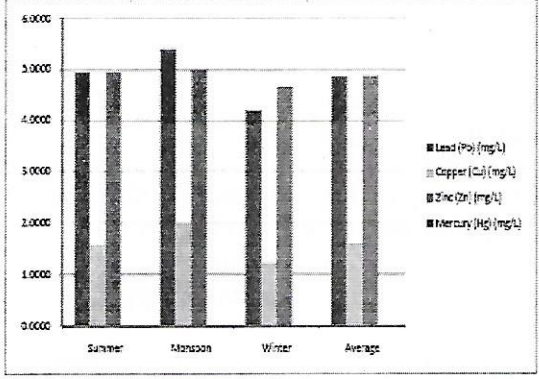


Fig. 11. Seasonal variation in Heavy Metals of river Panv Dhoi at sampling station E (2002-2003)







## Riffles inhabiting benthic macroinvertebrate communities in a spring-fed tributary of River Alaknanda (Garhwal Himalaya)

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### Abstract

Bachchan Gad is a spring-fed tributary of River Alaknanda in the Garhwal region of Uttarakhand. Benthic macroinvertebrates in the riffles of Bachchan Gad was investigated during March 2008 to February 2009 at four sampling sites between 590 m to 1600 m above sea level. Preliminary observations revealed that riffle inhabiting benthic macroinvertebrates communities in the Bachchan Gad comprised of 36 insect genera belonging to 8 orders and 29 families along with 1 genus each of crustacean and annelida. Dominated by *Ephemeropteran*, *Trichopteran* and *Coleopteran* genera the density was highest ( $1300 \pm 142$  Individual  $m^{-2}$ ) during winter at confluence, the downstream site, it was recorded lowest ( $80 \pm 15.2$  Individual  $m^{-2}$ ) during monsoon at Pata, the headwater site.

**Keywords:-** Benthic macroinvertebrate, Spring fed, River Alaknanda

### Introduction

Fast or swiftly flowing stream is usually dominated by riffles or fast moving water habitats. These are the habitats preferred by many bottom dwelling macroinvertebrates as the water current is relatively faster, the depth vary from moderate to shallow and the substrate is coarser. These areas generally alternate with deeper pools with finer deposits and slower water (Moss, 1998).

Located almost centrally in the long Himalayan sweep the Garhwal Himalaya is endowed with rich water resources in the form of rivers, streams, rivulets and lakes. The major rivers namely Bhagirathi, Alaknanda, Yamuna and some of their tributaries originate in the greater Himalayas, there by owing their origin to the glaciers. In addition, a large number of streams originate as spring in the lesser Himalaya. These small spring-fed fluvial systems are locally known as Gad. A number of invertebrate organism associated with the solid-liquid interface inhabit these water bodies these

are referred as zoobenthos, benthic invertebrates and macroinvertebrate benthos. The macroinvertebrates of freshwater are dominated by insects and their diversity is greatest in running water.

Aquatic insects play potentially major qualitative role in the processing and turnover of nutrients in freshwater ecosystems (Merritt *et al.*, 1984). Being involved in the recycling of organic detritus and serving as the main food source for stream fishes, estimates of the benthic production are of considerable importance for understanding the stream fish ecology. The benthic macroinvertebrate have been frequently studied to evaluate streams conditions (Hynes, 1970) and *in situ* assessment of environmental stress in aquatic communities has been increasingly utilized in biomonitoring programmes and toxicity test procedure (Vuori, 1995). Macroinvertebrate responses have increasingly received attention as a part of evaluation programmes of restoration works (Friberg *et al.*, 1998; Lassonen *et al.*, 1998). Benthic macroinvertebrates have been the subject of investigations in many parts of the world since the

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beginning was made by Needham and Christenson (1927), Shelford and Eddy (1929) and Hynes (1984). However such studies in Garhwal Himalaya have been fewer and fragmentary. Some noteworthy work in this regard are by Badola and Singh (1981), Dobriyal (1985), Nautiyal (1986), Sharma (1986), Negi (1989), Negi and Singh (1990), Kumar (1991), Gusain, (1994a), Gusain, (1994b), Singh *et al.*, (1994), Kumar *et al.*, (1999), Kishor *et al.*, (1998), Singh (2002), Sharma *et al.*, (2004) and Gusain and Gusain (2005). Understandably, there is relatively little or no information available on macroinvertebrates in the remote tributary habitats of the river systems in Garhwal Himalaya. Data collected during the present study gave an insight into the riffle inhabiting benthic macroinvertebrates in Bachchan Gad, a spring-fed stream in Garhwal Himalaya.

## Materials and Method

### Study Area

Bachchan Gad a spring-fed tributary of River Alaknanda in the Rudraprayag district of Uttarakhand originates upstream to Pata at an altitude of 1600 m above sea level (Fig. 1). This approximately 12 km long perennial stream receive water from several streams, underground seepages and surface runoff before meeting the River Alaknanda, about 21 km (590 m above sea level) upstream to Srinagar near the NH 58 on route to the shrine resort of Badrinath. The investigated area is located between latitude 30°11'45.08" to 30°15'32.77" N and 78°54' 58.87" to 78°59'38.64" E.

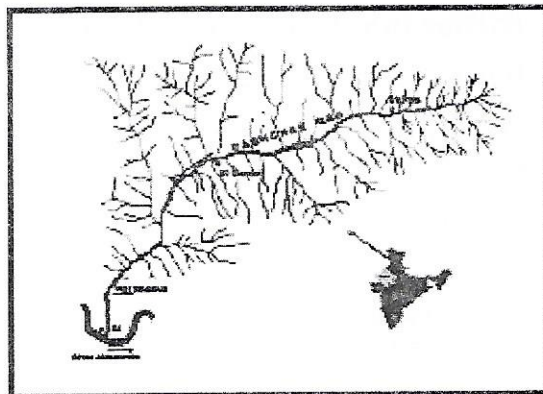


Fig. 1: Location map of study area Bachchan Gad

After a preliminary survey, depending on the accessibility, four sampling sites namely Pata ( $S_1$ ), Kandai ( $S_2$ ), Khankra ( $S_3$ ) and Confluence ( $S_4$ ) were selected (Table. 1). The vegetation type influenced by altitudinal limits, in the Bachchan Gad catchment constituted of sub montane (Tropical Forest) and montane (Temperate Forests) (Meher-Homji, 1978). Terraced agriculture land along with open mixed forest is abundant in the riparian zone of the catchment.

Monthly sampling for analyzing the selected physicochemical parameters and benthic macroinvertebrates was conducted during March 2008 to February 2009. Air and water temperature were recorded with the help of a centigrade thermometer. The mean water velocity was measured using float drift method. pH of water was estimated by the Control Dynamic pH meter.

Table 1: Sampling sites on Bachchan Gad

Sampling sites	Altitude (m above sea level)	Substratum Characteristics
Pata	1600	Pebbles, cobbles (dominant) and boulders; Cascade and riffles.
Kandai	820	Pebbles, cobbles (dominant) and boulders; Cascade, riffles and pools.
Khankra	610	Small pebbles (dominant), cobbles and boulders; Riffles and pools.
Confluence	590	Large pebbles (dominant), cobbles and boulders; Riffles and pools



Turbidity was measured with the help of Nephelometer. Dissolved oxygen, free  $\text{CO}_2$ , total alkalinity, total solid and total dissolved solid, were estimated by following standard methods outlined in Trivedi and Goel (1984) and APHA (1995). The 'MAC' conductivity meter was used for measuring the conductivity of water.

Benthic macroinvertebrates from the riffles were collected from one square feet area by lifting stones and sieving substratum using modified Surber's square foot sampler (Welch, 1952). Samples were preserved in 4% formalin. Identification was carried out to the lowest recognizable level usually genera, in the laboratory with the help of keys by Usinger (1950), Ward and Whipple (1959), Needham and Needham (1962), Macan (1979), Tonapi (1980) and Edington and Hildrew (1995). The data collected were analyzed seasonally as the study area experiences five seasons in a year namely Spring (March-April), Summer (May-June), Monsoon (July-August), Autumn (September-October) and Winter (November-February).

## Results and Discussion

Bachchan Gad has more or less, typical longitudinal profile, with the headwater reaches characterized by a steep gradient, swift current and large boulder bed. In the middle reaches, the stream has intermittent pools and rapids sequences. Thereafter, it usually tends to widen near the mouth, depositing the finer sediment on its banks. Such streams provide ample opportunities to a large number of bottom dwelling faunal elements especially the macroinvertebrates. According to Moss (1998) stones, the pockets between them and the interstices of gravel and sand provide a complex architecture on stream bottom for habitation by the benthic organisms.

Several factors are known to influence the distribution of benthic invertebrates, but the most important factors which affect the density and diversity of benthic macroinvertebrates in aquatic ecosystem are water temperature, water velocity,

substrate composition, hydromedian depth and turbidity. However, the spring-fed streams often vary in physico-chemical and biological characteristics from other types of streams (Covich, 1988). Most importantly, they tend to maintain a more constant aquatic environment than the streams supplied by runoff. In the present study similar environmental regime was evident all along the stream from headwater to the downstream region. Seasonal variation in the physicochemical parameters in the riffles of Bachchan Gad is depicted in Table 2. A total of 36 insects genera belonging to 29 families and 8 orders along with one genus each of annelida and crustacean were recorded from the riffles of Bachchan Gad (Table. 3). Out of the 36 insect genera recorded 11 belonged to trichoptera, 9 to ephemeroptera, 7 to diptera, 3 to odonata 2 to plecoptera and coleoptera each and 1 genus each to hemiptera and neuroptera.

The density of benthic macroinvertebrates in the riffles of Bachchan Gad showed an increase in the downstream region (Table 4). The density was found to be maximum ( $1300 \pm 142$  Individual  $\text{m}^{-2}$ ) during winter at confluence, the downstream region and it was recorded minimum ( $80 \pm 15.2$  Individual  $\text{m}^{-2}$ ) during monsoon at Pata, the headwater region.

The abundance of macroinvertebrates during the winter may be explained as due to the relatively low velocity of water current, high dissolved oxygen and low turbidity of stream water. The macroinvertebrates density declined in the month of summer and was recorded lowest during monsoon. The reason for such decline during monsoon may be attributed to the disturbance in substratum, increased water level, depth of stream, current velocity and turbidity (Crayton and Sommerfeld, 1979). During rainy season the spate causes disturbance in the stream by overturning the stones, underneath which the insects live. Also, the organisms living on the upper surface of stones are seriously affected due to scouring and stress (Malmqvist and Otto, 1987). Some reduction in number can also be attributed to the difficulties in



Table 2:. Seasonal variation in the physicochemical parameters recorded in the riffles of Bachchan Gad

Parameters	Seasons				
	Spring	Summer	Monsoon	Autumn	Winter
Air Temperature(°C)	27.5±4.93	30±3.35	28.7±1.76	24±2.0 <sup>r</sup>	13.5±2.19
Water Temperature(°C)	17.6±1.92	20.8±1.96	20.2±1.45	17.5±0.91	10±0.81
Mean Depth(m)	0.18±0.04	0.23±0.06	0.31±0.09	0.24±0.09	0.20±0.05
Mean Width(m)	0.63±0.14	2.06±1.20	2.55±1.47	1.76±1.22	0.76±0.23
Mean Velocity(ms <sup>-1</sup> )	0.59±0.16	0.76±0.07	0.80±0.08	0.55±0.13	0.44±0.13
Turbidity(NTU)	4.66±3.08	2.6±0.38	6.5±2.12	1.25±0.35	1.9±0.141
Conductivity(Scm <sup>-1</sup> )	140±12.83	153±17.77	131±21.05	115±15.29	93.25±3.12
Dissolved Oxygen(mgl <sup>-1</sup> )	9.4±1.22	7.16±0.06	8.05±0.46	7.5±0.27	8.9±0.63
Free CO <sub>2</sub> (mgl <sup>-1</sup> )	1.50±0.20	0.92±0.41	0.99±0.66	1.37±0.36	1.81±0.23
Alkalinity (a) P.A.(mgl <sup>-1</sup> )	0	0	0	0	0
(b) T.A.(mgl <sup>-1</sup> )	77.5±5.36	71±5.24	70±5.77	70±4.59	69±12.12
pH	7.85±0.14	8.8±0.23	9.5±0.26	8.37±0.28	7.87±0.29
Total Solids (gl <sup>-1</sup> )	0.24±0.05	0.16±0.01	0.14±0.02	0.145±0.03	0.105±0.02
Total Dissolved Solids(gl <sup>-1</sup> )	0.14±0.01	0.11±0.018	0.10±0.008	0.11±0.02	0.07±0.01

sampling during high water but much was due to losses caused by wash out (Hynes, 1970). Ward (1976) stated that sedimentation decrease substrate heterogeneity fills interstices with silt, may severely reduce algal population which directly affects the zoobenthos.

Site wise distribution revealed that 30 genera were recorded from middle region (S<sub>2</sub>), while the downstream region (S<sub>3</sub> and S<sub>4</sub>) recorded 25 genera each (Fig. 2 to 5). Among the benthic macroinvertebrates inhabiting the riffles of Bachchan Gad, 18 genera were recorded at all the sampling sites, whereas 6 genera were absent at only one site, 4 were absent at 2 sites and 10 genera were recorded only at one site. Among the genera *Epeorus*, *Leptophlebia*, *Psephenus*, *Brachycentrus*, *Corydalis* and Leech were found at three sites. *Ephemera*, *Ophiogampus* and *Perla* were absent at S<sub>3</sub> and S<sub>4</sub> in the downstream region, while *Plea* was absent at S<sub>2</sub> and S<sub>4</sub>. *Phacopteryx*, *Phrygenia*, *Antocha* and *Elliptera* were found only at S<sub>2</sub>, and *Tabanus*, *Lestes*, *Polycentropus* and Crab were recorded only at S<sub>1</sub> i.e., the headwater region.

Also, *Agapetus* and *Cinygma* were found only at S<sub>4</sub>, the downstream region.

Ephemeroptera was the dominant macroinvertebrate order in the riffles of Bachchan Gad followed by Trichoptera and Diptera. Similar composition of benthic invertebrates has been observed by Suren (1994), while investigating the invertebrate communities of the streams of Western Nepal. The taxonomic composition of the fauna was similar to that reported by Rundle *et al.* (1993) and Ormerod *et al.* (1994) in Central and Northeast Nepal.

The present study though preliminary still revealed that the riffle inhabiting benthic macroinvertebrates in spring-fed stream are diverse and varied seasonally along an altitudinal gradient.

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Table 3: Check list of benthic macroinvertebrates inhabiting the riffles of Bachchan Gad

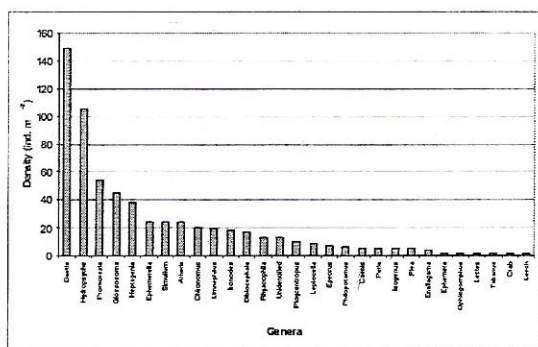
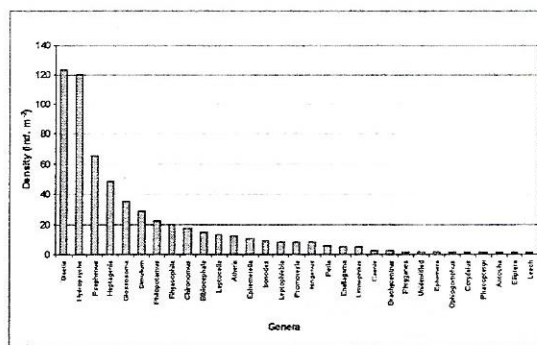
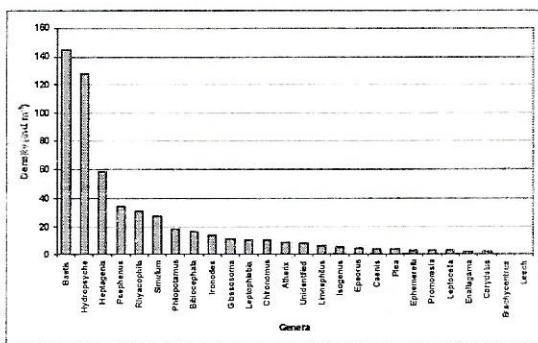
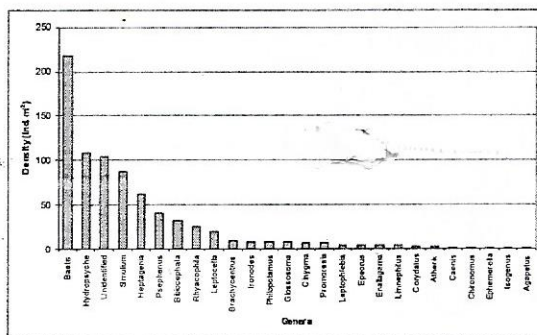
Phylum	Order	Family	Genera
Arthropoda			
Class-Insecta			
	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>
			<i>Epeorus</i>
			<i>Ironodes</i>
			<i>Cinygma</i>
		Baetidae	<i>Baetis</i>
		Leptophlebiidae	<i>Leptophlebia</i>
		Ephemerellidae	<i>Ephemerella</i>
		Caenidae	<i>Caenis</i>
	Ephemeridae	<i>Ephemera</i>	
	Odonata	Agrionidae	<i>Enallagama</i>
		Lestidae	<i>Lestes</i>
		Gomphidae	<i>Ophiogomphus</i>
	Plecoptera	Perlidae	<i>Perla</i>
			<i>Isogenus</i>
	Hemiptera	Pleadae	<i>Plea</i>
	Neuroptera	Corydalidae	<i>Corydalus</i>
	Coleoptera	Psephenidae	<i>Psephenus</i>
		Elmidae	<i>Promoresia</i>
	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>
		Philopotamidae	<i>Philopotamus</i>
		Rhyacophilidae	<i>Rhyacophila</i>
		Glossosomatidae	<i>Glossosoma</i>
			<i>Agapetus</i>
		Brachycentridae	<i>Brachycentrus</i>
		Limnephilidae	<i>Limnephilus</i>
			<i>Phacopteryx</i>
		Leptoceridae	<i>Leptocella</i>
		Polycentropodidae	<i>Polycentropus</i>
		Phryganeidae	<i>Phryganea</i>
	Diptera	Blepharoceridae	<i>Bibiocephala</i>
		Simuliidae	<i>Simulium</i>
		Athericidae	<i>Atherix</i>
		Chironomidae	<i>Chironomus</i>
		Tabanidae	<i>Tabanus</i>
		Tipulidae	<i>Antocha</i>
Class- Crustacea			Crab
Annelida			Leech



Table 4. Density (Individual  $m^{-2}$ ) of benthic macroinvertebrates inhabiting the riffles of Bachchan Gad

Sampling sites	Density (Individual $m^{-2}$ )	
	Minimum	Maximum
Pata	80±15.2 (MO)	765±44.63 (WI)
Kandai	490±58.88 (SU)	825±67.9 (AU)
Khankra	394±27.2 (SP)	990±136.0 (SU)
Confluence	320±24.7 (MO)	1300±142.0 (WI)

SP-Spring, SU-Summer, MO-Monsoon, AU-Autumn, WI-Winter

Fig. 2: Density (Individual  $m^{-2}$ ) of benthic macroinvertebrates in the riffles of Bachchan Gad at  $S_1$ Fig. 3: Density (Individual  $m^{-2}$ ) of benthic macroinvertebrates in the riffles of Bachchan Gad at  $S_2$ Fig. 4: Density (Individual  $m^{-2}$ ) of benthic macroinvertebrates in the riffles of Bachchan Gad at  $S_3$ Fig. 5: Density (Individual  $m^{-2}$ ) of benthic macroinvertebrates in the riffles of Bachchan Gad at  $S_4$



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