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Studies on toxicity of endosulfan on edible fresh water fish *Nemacheilus botia*

Anita P. Patil, Sunil D. Patil ✉[1] and Kailas H. Kapadnis

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Abstract

The present paper deals with acute toxicity test carried out for pesticide endosulfan. The LC_{50} value, standard error (for accuracy of variance) and maximum and minimum fiducial limit were calculated. The safe concentration for endosulfan was found 2.038 ml µg/lit. The biochemical component glycogen was studied in the control and polluted water.The glycogen content of liver decreased after acute treatment by endosulfan at 2.024 and 3.039 milli µg/lit conc.

Keywords: *Nemacheilus botia, LC_{50}, Glycogen, Liver, Endosulfan*

Introduction

The marked increase in total pesticides usage and rapid proliferation of synthetic organic compounds has deteriorated the water quality which enters through the agriculture discharge, chiefly the pesticides may reach fresh water bodies by runoff or by accident and ultimately enters the fauna residing there. Today over 1000 chemicals are used against about 2000 pest species.

Fishes are the most widely used organisms to determine the toxicity of water and other pollutant. The wide use of fishes is probably due to their adaptability to laboratory conditions as well as their availability and their varying degree of sensitivity to the toxic substance (Verma *et al.* 1980). Undesirable effects caused by pesticide to the aquatic organisms and their hazard are elegantly reviewed by many workers (Sanger, 1964). Brown (1976) listed 12 basic types of investigations of toxicity; these are for preliminary screening of chemicals, for monitoring influence to determine the extent of risk to aquatic organisms and to determine the component causing death. Determination of acute toxicity is essential for determining the sensitivity of the animals to the toxicants and is also useful for evaluating the degree of damage to the target organs and consequent physiological and behavioral disorders. Toxicity tests are useful for suitability of environmental conditions for aquatic life, favorable and unfavorable environmental factors, such as DO, pH, temperature, salinity or turbidity and effect of environmental factors on waste toxicity. The present study was carried on *Nemacheilus botia* which is a fresh water fish. An attempt has been made to study the changes in biochemical composition in tissues of organs like liver of fish, when it is exposed to different concentrations of endosulfan. Glycogen and proteins have main role in the energy metabolism. Therefore the change in glycogen level was studied in the present investigation.

Materials and Method

Nemacheilus botia were collected regularly in live conditions from River Godavari at the place Nandur Madhmeshwar, a famous bird sanctuary in Nashik district. These fishes were kept in the aquarium containing tap water. Fishes are acclimatized to laboratory conditions. The important parameters like pH, temperature, dissolved oxygen, total hardness were determined for the water as per standard methods (APHA, 1981). Endosulfan is a broad spectrum, extremely toxic organochlorine pesticide that is widely used in India. The effect of 35% endosulfan on biochemical constituents of *Nemacheilus botia*,

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were evaluated by exposing fishes to 1/2, 1/3 … $1/10^{th}$ to sublethal concentrations of endosulfan at 24, 48, 72 and 96 hours. The controlled and treated fishes were used for analysis of biochemical constituent glycogen in liver. The fresh isolated tissues were used for estimation of glycogen according to (Dezwann and Zandee, 1972) and protein according to (Lowry *et al.* , 1951).

The results were statistically analyzed by applying standard deviation and chi-square test. Control animals were treated exactly in the same way as the experimental animals but without toxicants.

Results and Discussion

Pesticides have enormous value in agricultural field to control agricultural pest and diseases. The use of chemicals in the field increases along with overgrowing populations because of which pesticides have become environmental contaminants. The toxicity of particular pollutant depends upon many factors such as animal weight, time of exposure, temperature, pH and hardness of water. The evaluation of LC_{50} concentration of pollutant is an important step, before carrying further studies on physiological changes in animals. In present investigation endosulfan showed LC_{50} gradually reducing from 5.76 to 3.35 milli µg/liter as time duration increased from 24 to 96 hrs, whereas safe concentration was found to be 2.038 milli µg/liter. During the course of study it was noticed that the toxicity increases as the exposure time as well as concentration of pollutant increased. The pesticide endosulfan might affect the nervous tissues by blocking the passage of impure across the synaptic junctions and inhibition of acetyl cholinesterase activity because the mode of action of organochlorine pesticide is indicated through the impairment of nerve tissue (Murthy and Devi, 1982).

Table 1 : Relative toxicity of pesticide Endosulfan when fish *N. botia* were exposed to 24 hrs. to 96 hrs.

Hrs. of Exposure	Regression Equation	LC_{50} ± SE	Variance	Fiducial Limit		Lethal dose ml µg/Lit	Safe Concentration
				M_1	M_2		
24	$Y = 1.14 + 4.9\ \bar{}$	5.76 ±1.32	1.767	-1.840	3.360	114.240	2.038 milli µg/Lit
48	$Y = 0.71 + 6.09\ \bar{}$	4.88 ±0.98	0.967	-1.240	2.618	234.330	
72	$Y = 2.31 + 4.4\ \bar{}$	4.47 ± 0.90	0.898	-1.140	1.590	322.488	
96	$Y = 2.62 + 4.5\ \bar{}$	3.35 ± 1.10	1.212	-1.390	2.790	430.080	

The LC_{50} value of various fish species varies with pesticide to pesticide which is highly useful in the final evaluation of extent of pollution of aquatic environment by agricultural chemicals. Vasait and Patil (2005) investigated the LC_{50} values of organochlorine pesticide and calculated its effect for 7 and 14 days exposure period. The result indicates decrease in LC_{50} concentration with increase in concentration and duration of exposure. Similar results was shown by Joshi (2001) Many chemicals induce similar precipitation of mucous which fills the space between filaments and gill lamellae ultimately affecting the gaseous exchange leading to stasis of blood and death of the fishes. Many researchers suggested that cytological damage to gills, rather than mucous accumulation results in death by asphyxia. Chindah *et al.* (2001) noted that aquatic organisms (shell and for fishes) in direct contact with the medium in addition to breathing and feeding is vulnerable to respiratory tract damage and other organs of the body.

The acute toxicity effects are generally evolved due to action of the pesticides on the target organs.

The results of the acute toxicity test was observed to be for 24, 48, 72 and 96 hours. The LC_{50} values were calculated for 24, 48, 72 and 96 hours by method described by Finney (1971).

The obtained regression equation to pesticide endosulfan for 24, 48, 72 and 96 hours are listed in Table-1. LC_{50} values for 24, 48, 72 and 96 hours exposures to mutation were found as 5.76, 4.88, 4.47 and 3.35 milli μg/lit respectively. The calculated accuracy for log LC_{50} values are summarized in under column variance, which are 1.767, 0.967, 0.898, 1.212 for 24, 42, 72, 96 hours respectively. The standard error for (accuracy of variance) 24, 48, 72 and 96 hours are 1.32, 0.98, 0.90, and 1.10 respectively. The fiducial limits for log LC_{50} value are summarized in Table 1 under the column fiducial limit M_1 and M_2. The 95% confidence of LC_{50} values are (fiducial limit) to pesticide are M_1 (Minimum limit) and M_2 (Maximum limit). The maximum and minimum fiducial limit for 24, 48, 72, and 96 hours log LC_{50} value of endosulfan are -1.840 to 3.360, -1.240 to 2.618, -1.140 to 1.590, and -1.390 to 2.790 respectively.

Table-2: Glycogen content in milli μg/gm of wet liver tissue of *N. botia* in control and endosulfan exposed

Concentration of Endosulfan	24 hours	48 hours	72 hours	96 hours
Control	7.000(± 0.121)	7.000(± 0.121)	6.833(± 0.171)	6.666(± 0.0906)
Endosulfan Conc. 2.024 milli μg/lit.	6.666(± 0.182)	6.331(± 0.207)	6.166(± 0.1314)	5.833(± 0.1052)
Endosulfan Conc. 3.039 milli μg/lit.	6.333(± 0.186)	6.166(± 0.122)	5.833(± 0.171)	5.666(± 0.0192) 3.833

The standard error for (accuracy of variance) 24, 48, 72 and 96 hours are 1.32, 0.98, 0.90 and 1.10 respectively. The fiducial limits for log LC_{50} value are summarized in Table 1 under the column fiducial limit M_1 and M_2. The 95% confidence of LC_{50} values are (fiducial limit) to pesticide are M1 (Minimum limit) and M_2 (Maximum limit). The maximum and minimum fiducial limit for 24, 48, 72 and 96 hours log LC_{50} value of endosulfan are -1.840 to 3.360, -1.240 to 2.618, -1.140 to 1.590, and -1.390 to 2.790 respectively.The safe concentration for endosulfan is 2.038 ml μg/lit. Lethal dose for pesticide are entered in column 'lethal dose', for immediate 100% mortality of fish, the lethal dose was calculated. The lethal doses for pesticide endosulfan at 24, 48, 72, 96 hours exposure are 114.240, 234.330, 322.488 and 430.080 ml μg/lit. respectively. For 100% immediate mortality the fish require highest lethal dose of pesticide endosulfan. The glycogen content of liver decreased after acute treatment by endosulfan at 2.024 and 3.039 milli μg/lit conc.

The glycogen content in liver decreased from 7.000 to 6.666, 7.000 to 6.330, 6.830 to 6.166, 6.666 to 5.833 mg/gm of wet tissue in 24, 48, 72 and 96 hours respectively at 2.024 milli μg/lit. From the present investigation it is quite clear that glycogen content of fish *N. botia* after endosulfan treatment was altered indicating the effect of tested endosulfan. The average glycogen content after acute treatments were decreased. Whatever may be reasons, the decrease in the level of glycogen contents will adversely affect the growth, development and reproduction of organisms, which in turn disrupt the effectiveness of aquatic organisms in biological control programmes.

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Microbiological examination of macronutrients (C & N) for production of Antibiotics produced by Actinomycetes

Aishwarya Tandon✉ and Laxmi Chandra

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Abstract

The present study indicates that C and N sources are very important for antibiotic production. Streptomyces isolates DC-25 and DC-30 are specific to utilize C and N source for their growth. The compounds which have generally amino group is easily utilized by streptomyces and promote its growth for production of antibiotics by adding various amino acids like glycine, alanine, phenyl alanine, leucine and thyroxin. It was noted during course of study that various carbon sources like xylose, maltose, lactose *etc*. were utilized by actinomycetes but cellulose was poorly utilized.

Keywords: *Streptomyces isolate, DC-25, DC-30, Carbon and Nitrogen source*

Introduction

The presence of different carbon and nitrogen sources is very important for the growth of actinomycetes and production of antibiotics. Sometimes it was found that presence of carbon sources like glucose, maltose, starch *etc.* causes lower production for streptomycin (Dulany, 1948). But actinomycetes species like *S.gresius* can be easily grown on xylose , glucose, galactose, while it doesn't shows any growth on arbinose, lactose, inocitol and ducitol.Similarly few of nitrogen sources like protein, peptone and amino acid supported the growth of actinomycetes and nitrate and urea followed the production of antibiotics.

Materials and Method

The DC-25 and DC-30 isolates of streptomyces were cultured on different broths in 250 ml flask which were sterilized at 15 lbs for 30 minute.

Now 1ml of spore suspension was inoculated on the DC-25 and DC-30 and incubated for 15 days at 28 . Different carbon sources including sugar, alcohol and nitrogen sources were then examined for the measurement of growth for DC-25 and DC-30. The pH was maintained at 6.6 and autocleved

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Similarly, nitrogen compounds were incorporated on basal medium and pH was maintained to 6.6. After that the streptomyces was inoculated and incubated at 30 for 15 days.

Media Used

Sucrose Nitrate

Sucrose	3.0 gm
$NaNO_3$	0.2 gm
$FeSO_4$	0.001 gm
$MgSO_4$	0.05 gm
Pot. Dihydrogen Phosphate	0.1 gm

Glucose asparagin

Glucose	1.0 gm
$MgSO_4$	0.025 gm
Asparagines	0.5 gm
Dipotassium hydrogen phosphate	0.5 gm
Distilled water	1.0 liter

Gelatin Broth

Peptone	0.5 gm
Beef extract	0.3 gm
Gelatin	0.4 gm

Nutrient Broth

Peptone	0.5 gm
Beef extract	0.5 gm
NaCl	0.5 gm

Results and Discussion

The results of the present study are tabulated in Table. 1 to 4. Table 1 shows the effect of different carbon sources on the growth and productionof antibiotic by actinomycetes (Isolate DC-25). The maximum value was observed in Xylose i.e. 16.8 after 20 days of inhibition while lowest value was observed in Sucrose i.e. 9.4. Table 2 shows the effect of carbon sources in production of antibiotic substances by actinomycetes (Isolate DC 30). In DC-30 the maximum inhibition in incubation after 20 days was observed in Xylose i.e 17.0 mm while lowest was observed in starch i.e. 12.2 mm. Table 3 shows the effect of nitrogen sources in production of antibiotic substances by actinomycetes (Isolate DC 25). The maximum value was observed in Arginine i.e. 16.2 mm while lowest was observed in Glycine i.e. 13.2 mm. Table 4 shows the effect of nitrogen sources in production of antibiotic substances by actinomycetes (Isolate DC 30). The highest value was observed in Glycine i.e. 17 mm while lowest was observed in Arginine i.e. 12.4 mm. It is evident from various culture mediums that the maximum yield of antibiotic occurred in sucrose nitrate medium in comparison to other medium. Glucose asparagines medium showed less yield of antibiotic. Gupta and Tandon (1977) have found that chemically defined media is able to enhance antibiotic production.

During our study of different culture medium the best yield occured in sucrose nitrate medium in comparison to glucose asparagines which showed mild yield whereas the gelatin broth and nutrient broth media showed very poor growth in case of *S. ganmycicus* it showed maximum antibiotic yield but showed poor mycelial growth. It showed less antibiotic production with decreased medium. Basuchaudhary (1961), Baldacci (1961), Chestster and Rollinson (1955) and Clutterbuck et *al.* (1932) found similar results.

Table.1: Effect of different Carbon sources in the growth and production of antibiotics by actinomycetes (Isolate DC-25)

S.No	Carbon Source	Inhibition in incubation (mm) after 20 days of *A. alternata.*
1	Xylose	16.8
2	Sucrose	9.4
3	Lactose	15.2
4	Starch	16.4
5	Maltose	16.1

Table. 2: Effect of different Carbon sources in production of antibiotic substance by actinomycetes (Isolate DC--30)

S.No	Carbon Source	Inhibition in incubation (mm) after 20 days of *A. alternata.*
1	Sucrose	16.0
2	Xylose	17.0
3	Maltose	15.3
4	Lactose	14.0
5	Starch	12.2

Table.3: Effect of different Nitrogen sources in production of antibiotic substance by actinomycetes (DC-25)

S.No	N Source	Inhibition in incubation (mm) after 20 days of *A.* alternata.
1	Glycine	13.2
2	Alanine	14.8
3	Glutamic Acid	14.3
4	Arginine	16.2
5	Aspartic Acid	12.2

Table.4 : Effect of different Nitrogen sources in production of antibiotic by actinomycetes (DC-30)

S.No	N Source	Inhibition in incubation (mm) after 20 days of *A. alternata.*
1	Glycine	17
2	Alanine	14
3	Glutamic Acid	15.2
4	Arginine	12.4
5	Aspartic Acid	15.3

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A study on pollution status and its impact on water quality of River Ganga at Haridwar

D.R. Khanna, R. Bhutiani ✉ and Dipali Bhaskar Kulkarni

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Abstract

The present study deals with the study on pollution status and its impact on water quality of River Ganga at Haridwar. For the present study four sampling stations viz. Saptrishi ghat, Har-ki-pauri, Chandi ghat and Pul Jatwara were selected and various physico-chemical parameters i.e. temperature, conductivity, total solids, pH, velocity,turbidity, BOD, COD, DO, free CO_2, acidity, alkalinity,total hardness, chlorides, calcium, magnesium, phosphates were analyzed. Minor fluctuations in physico-chemical parameters were observed during course of study at all the sampling stations. Correlation coefficients between different parameters were also calculated during present study.

Keywords: *Physico-chemical, Pollution, Impact, Effluent, Water quality*

Introduction

River Ganga, the holiest river of all rivers and lifeline of the north India originates from Gangotri glacier. It emerges from the confluence of two important rivers of hills, River Bhagirathi and River Alaknanda at Devprayag. Beside this, Ganga's head streams Mandakini, Nandakini, Bhilangana, Dhauliganga and Pinder, all originates from northern Himalayas. After descending 2827 meters at Hardwar, the River Ganga cuts across the Shivalik hills and for the first time it enters the great plain of the Uttrakhand state in India. Therefore, Hardwar is known as the Gate Way of God. From Hardwar it flows down towards south and then south- east touching many important cities and towns like Garh Mukteshwar, Anupshahar and Narora in Bulandshahar, metro towns like Kanpur, Allahabad,Varanasi and lastly terminates in the Bay of Bengal covering about 2,506 km in India. Due to rapid industrialization, urbanization and increasing population day by day, water consumption rate has increased and major causes of water pollution are extended as a result of treated and untreated sewage and industrial effluents discharging into water sources. Global water consumption rose six folds between 1900-1995. According to the text, the United Nations (New York) has determined that one third of the world's people live in countries dealing with "moderate to high" water resources strains and warns that the situation will worsen in absence of major changes in the way water is distributed and used. Better management of water resources is the key to mitigating water scarcities in the future and avoiding further damage to aquatic ecosystems. On the banks of River Ganga several towns/major cities and industries are situated which have no proper management of sewage drains and effluent treatment plant for controlling industrial pollution. Consequently sewage, treated and untreated industrial effluents are being discharged directly or indirectly into the river and deteriorating the water quality of River Ganga day by day.

It is well known that Ganga is one of the most important rivers of India and has served as cradle for Indian civilization. Although the Ganga river serves as source of water supply to several large cities located on its banks over the years the river has been indiscriminately polluted and misused.

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Despite its extra ordinary resilience and recuperative capacity the river is severely polluted. Due to increase in population and industrialization, the water quality of River Ganga has deteriorate due to inflow of domestic sewage and industrial effluents, containing large number of chemicals and heavy metals. Waste materials react with each other and as a result, the water is polluted and may become toxic which would ultimately make the water unpotable and severely affect the bio productivity of the aquatic system.

The present study was conducted on the water quality of river Ganga in a long stretch of approximately 25 km. in Haridwar.

Sampling stations of the study area

The study was conducted over a period of two years *i.e* 2007-2009. Four sampling stations were selected with in the study area comprising of long stretch of about 25 km. Sampling station-A (Saptrishi ghat), Sampling station-B (Har-ki-pauri), Sampling station-C (Chandi ghat) and Sampling station-D (Pul Jatwara).

Materials and Method

Materials used for sample collection and analysis work were used as per standard method (APHA-1998), ISI- Methods (1982), Trivedi and Goel (1984) and Khanna and Bhutiani (2008).The water samples of River Ganga were collected in a neat and clean two liter capacity white plastic Jericanes for general parameters. Collected samples were preserved in ice box and refrigerated till analysis.The water samples for DO were collected in a neat and clean 300 ml capacity borosil glass stoppered bottles by dipping the DO bottles in water below water surface. When bottles DO fully filled with water then bottles were stoppered below water surface. Immediately at the sampling point DO was fixed by adding 2 ml of each manganous sulphate ($MnSO_4$) and alkaline KI azide solutions on site.

Results and Discussion

The results of various physico-chemical parameters (Mean value) observed during study period are tabulated in Table-1. While the correlation coefficient between different parameters are given in Table-2. Minimum water temperature (21.17 ± 2.57 ^oC) of River Ganga was recorded in 2007-08 at Sampling station-B while maximum (23.80 ± 2.19 ^0C) was recorded in 2008-09 at Sampling station-C. The average value for the study period (2007-09) was observed as 22.61± 0.84 ^oC. The trend of water temperature was found to be upward from winter season to summer season followed by downward from monsoon season onwards. A more or less similar status of temperature was reported by Badola and Singh (1981) in River Alaknanda and in River Kallayi by John (1976). Similar trends were also observed by Singh *et al.* (1988; 1989a,b) in River Ganga, Yamuna and Sangam. Same study was made by Gautam *et al.* (2000) in Ganga River at Rishikesh. Minimum value of Conductivity (0.01 ± 0.003 Siemens/cm) in river Ganga was recorded in 2007-08 at Sampling station-B while maximum (0.31 ± 0.002 Siemens/cm) was recorded in 2007-08 at Sampling station-A. The average value of conductivity for the study period (2007-09) was observed as 0.08 ± 0.11 Siemens/cm. Similar trends were observed by Singh *et al*. (1989; a, b) in River Ganga. CPCB (2003) also reported conductivity from 0.517 to 0.641 μmhos in the stretch of Bithur, Kanpur to Sangam Allahabad. As compared to results of CPCB water quality of river Ganga is better in respect of conductivity.

Minimum turbidity (7.21 ± 14.43 J.T.U) in river Ganga was recorded in 2008-09 at Sampling station-D while maximum (12.02 ± 20.20 J.T.U) was recorded in 2008-09 at Sampling station-C. The average value of turbidity for the study period (2007-09) was observed as 9.77± 2.00 J.T.U. The turbidity and total solids were closely interrelated with one another and cause common effect upon the river and aquatic life as also stated by Verma and Shukla (1969). Bhatt *et al.* (1984) attributed that during monsoon months, the river water contained large amount of silt, fine sand particles, organic matter and clay. Bilgrami and Duttamunshi (1985) observed minimum values of turbidity in winter and summer seasons while maximum in monsoon period. Minimum total solids (740.00 ± 2.40 mg/l) in River Ganga was recorded in 2008-09 at Sampling station-A while maximum (1100.00 ± 375 mg/l) was recorded in 2008-09 at Sampling station-C. The average value of total solids for the study period (2007-09) was observed as 907.65 ± 134.43 mg/l. Higher values of total solids may cause a significant role in deterioration of the surviving conditions of aquatic organisms by

mechanical abrasive action and enhance the turbidity of the river. Total solids can be in the form of settleable, coarser, fine or colloidal particles. It is interested to note that, total solids were recorded minimum during winter season due to gradual sedimentation of settleable particles at the bottom of the river and also due to lower velocity of the river which favour effective sedimentation.

Table- 1: Physico-chemical characteristics of Ganga river at different sampling stations during 2007-09

Physico-Chemical Parameter	Sampling station A		Sampling station B		Sampling station C		Sampling station D		Average
	2007-2008	2008-2009	2007-2008	2008-2009	2007-2008	2008-2009	2007-2008	2008-2009	2007-2009
Temp. (^{0}C)	22.50 ± 2.02	22.12 ± 2.72	21.17 ± 2.57	22.44 ± 2.93	23.67±3.32	23.80±2.19	22.41±3.27	22.82±3.57	22.61±0.84.
Cond. (S/cm)	0.31 ± 0.002	0.05 ± 0.03	0.01 ± 0.003	0.20 ± 0.005	0.02±0.005	0.02±0.006	0.02±0.007	0.03±0.004	0.08±0.11
Turbidity (JTU)	7.33 ± 14.43	9.50 ± 17.32	8.11 ± 14.43	11.33 ± 20.20	11.65±23.09	12.02±20.20	11.12±23.09	7.21±14.43	9.77±2.00
T. Solids (mg/I)	779.58 ± 204.83	740.00 ± 2.40	858.3 ± 72.16	891.66±260.20	1050.00±.125	1100.0±375.0	816.66±260.20	1025±433.01	907.65 ±134.43
Velocity (m/s)	1.25 ± 0.06	0.76 ± 0.03	1.31 ± 0.02	1.11± 0.02	2.30.±0.04.	1.95±0.04	2.39±0.08	2.20±0.07	1.56±0.61
pH	7.45 ± 0.21	7.42 ± 0.10	7.35 ± 0.12	7.30± 0.06	7.37±0.15	7.41±0.03	7.35±0.14	7.48±0.05	7.39±0.05
BOD (mg/I)	1.64 ± 0.10	1.50 ± 0.12	2.72 ± 0.02	1.95±0.17	2.25±0.21	2.10±0.35	1.76±0.30	1.79±0.14	1.96±0.38
COD (mg/I)	5.35 ± 0.65	3.45 ± 0.61	6.06 ± 0.16	3.00±0.78	5.30±0.30	3.14±1.46	3.08±1.33	3.20±0.22	4.07±1.26
DO (mg/I)	10.22 ± 0.13	11.07 ± 0.47	7.43 ± 0.61	9.04±0.86	7.14±0.27	7.96±0.98	9.75±0.21	10.70±0.20	9.16±1.51
Free CO_2 (mg/I)	3.11±1.05	2.20±0.74	3.08±1.91	2.99±1.87	2.68±2.08	2.98±2.15	3.26±1.97	2.67±2.10	2.87±0.33
Acidity (mg/I)	63.90 ± 3.18	53.46 ± 10.52	51.94 ± 7.18	49.57±5.00	55.58±4.67	58.87±9.38	56.89±8.54	63.44±9.74	56.70±5.17
Alkalinity (mg/I)	265.55 ± 5.91	295.16 ± 28.24	244.29 ± 13.34	265.16±23.92	257.21±21.22	287.09±21.24	254.27±11.97	275.92±20.26	268.08±17.09
T. hardness (mg/I)	227.43 ± 11.33	232.46 ± 3.33	218.80 ± 8.88	236.52±3.69	249.91±8.94	230.26±8.67	246.41±3.00	253.50±9.30	236.91±12.05
Chlorides (mg/I)	16.68 ± 1.49	19.60 ± 2.17	20.28 ± 2.22	22.21±4.53	17.82±0.64	22.87±2.27	24.09±3.22	19.98±3.44	20.44±2.51
Magnesium (mg/I)	52.20 ± 3.17	39.58 ± 4.83	44.79 ± 3.06	51.34±0.74	41.33±3.59	43.51±6.65	46.40±5.07	39.77±6.37	44.86±4.87
Phosphates (mg/I)	0.09 ± 0.04	0.08 ± 0.07	0.06 ± 0.04	0.16±0.02	0.08±0.04	0.07±0.05	0.09±0.04	0.05±0.02	0.08±0.03
Calcium (mg/I)	69.42 ± 3.26	75.97 ± 6.09	59.24 ± 1.62	48.72±6.53	62.50±2.79	55.70±4.36	54.19±4.52	54.63±11.26	60.04±8.93

All values are mean values, ± = standard deviation

Maximum total solids were recorded in monsoon season which may be due to more turbulence of high velocity of river water and waste water run off from sewage drains and other drains and surface water run off from agricultural land. Similar trends were shown by Chugh (2000) in his thesis during the study of water quality of River Ganga at Hardwar. Similar conditions were also recorded by David (1956) in River Bhadra, Mysore and Verma and Shukla (1969) in their studies. Kudesia and Verma (1985) and Reddy and Venkateshwarlu (1987) reported that most of the Indian rivers show similar tendency with respect to fluctuations of total solids. Minimum velocity (0.76 ± 0.03 m/s) in River Ganga was recorded in 2008-09 at Sampling station-A while maximum (2.39 ± 0.08 m/s) was recorded in 2007-08 at Sampling station-D. The average value of velocity for the study period (2007-09) was observed as 1.56± 0.61 m/s. In the present study it has been observed that the velocity and the total solids show positive relationship. Total solids may be in the form of coarse, floating, settleable, fine or colloidal particles as a floating film. Most of Indian rivers showed a similar tendency with respect to fluctuations of total solids (Kudesia and Verma, 1985; and Reddy and Venkateshwarlu, 1987). Minimum pH (7.30 ± 0.06) in River Ganga was recorded in 2008-09 at Sampling station-B while maximum (7.48 ± 0.05) was recorded in 2008-09 at Sampling station-D. The average value of pH for the study period (2007-09) was observed as 7.39± 0.05. Minimum values of pH were obtained mostly in winter season and maximum during rainy season. It may be due to

draining of several small sewage drains into the river and high value obtained during rainy season may also be due to rainy water run off of sewage drains. Besides this higher values of pH may be due to increase in bathing/ washing activities during summer period. However annual average values of pH are with in the limits prescribed for pH (6.5 to 8.5). Hence water quality of river Ganga is slightly alkaline. CPCB (2003) found the pH of Ganga river water from 7.46 to 8.18 in their study at different sampling points in Kanpur and Kannauj. CPCB (2003) also reported the pH values 8.1 to 8.6 in River Ganga from Bithur, Kanpur to Sangam, Allahabad. Similar trends of pH was also reported by Singh *et al.* (1988) in Ganga, Yamuna and Sangam at Allahabad. Minimum BOD (1.50 ± 0.12 mg/l) in River Ganga was recorded in 2008-09 at Sampling station-A while maximum (2.72 ± 0.02 mg/l) was recorded in 2007-08 at Sampling station-B. The average value of BOD for the study period (2007-09) was observed as 1.96± 0.38 mg/l.Singh *et al.* (1988) reported biochemical oxygen demand values (1.5 to 2.6 mg/l) of river Ganga in its upstream of Sangam at Allahabad. Similar trends of biochemical oxygen demand was also observed by Singh *et al.* (1988) in River Yamuna and Khanna and Bhutiani (2003) in River Ganga and Khanna *et al.* (2006) in River Suswa. Minimum COD (3.00 ± 0.78 mg/l) of River Ganga was recorded in 2008-09 at Sampling station-B while maximum (6.06 ± 0.16 mg/l) was recorded in 2007-08 at Sampling station-B. The average value of COD for the study period (2007-09) was observed as 4.07± 1.26 mg/l. Annual average values of chemical oxygen demand may be higher due to running off of rainy water characterizing chemically oxidizable load of organic matter (Chugh 2000). CPCB (2003) reported chemical oxygen demand values of River Ganga in between 26.0 mg/l to 44.0 mg/l during river Ganga monitoring from Bithur, Kanpur to Sangam, Allahabad. However chemical oxygen demand values observed in our study area are very low as compared to chemical oxygen demand values of CPCB. It indicates that no more contamination of industrial effluent are being discharged into river Ganga within the study area as well as in its upstream. Similar trends of chemical oxygen demand were shown by CPCB (1990-91).Minimum DO (7.14 ± 0.27 mg/l) of river Ganga was recorded in 2007-08 at Sampling station-C while maximum (11.07 ± 0.47 mg/l) was recorded in 2008-09 at Sampling station-A. The average value for the study period (2007-09) was observed as 9.16± 1.51 mg/l. Mostly dissolved oxygen was recorded minimum during monsoon at all sampling points and the maximum was found in winter season at all sampling stations. Similar trends were observed by CPCB (1990-91) and annual mean values of dissolved oxygen reported between 6.0 to 8.0 mg/l in the stretch of Rishikesh to Kanpur D/S and Behrampur. Singh *et al*. (1988, 1989 a, b) also found similar trends of dissolved oxygen in River Ganga, Yamuna and at Sangam Allahabad. Chugh (2000) has also reported the same trends in his thesis. Gautam *et al.* (2000) also reported dissolved oxygen from 8.0 to 10.0 mg/l at Rishikesh. Hence water quality of River Ganga with respect to dissolved oxygen may be good for drinking/bathing purposes within the study area. Minimum free CO_2 (2.20 ± 0.74 mg/l) in River Ganga was recorded in 2008-09 at Sampling station-A while maximum (3.26 ± 1.97 mg/l) was recorded in 2008-09 at Sampling station-A. The average value for the study period (2007-09) was observed as 2.87 ± 0.33 mg/l. Pahwa and Mehrotra (1966) have reported that the Ganga river contains maximum free carbon dioxide in monsoon season at Allahabad. Chakrabarty *et al*. (1959) also recorded the maximum free CO_2 in Jamuna during monsoon at Allahabad. Free Carbon dioxide 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. Minimum acidity (49.57 ± 5.00 mg/l) in river Ganga was recorded in 2008-09 at Sampling station-B while maximum (63.90 ± 3.18 mg/l) was recorded in 2007-08 at Sampling station-A. The average value of acidity for the study period (2007-09) was observed as 56.70 ± 5.17 mg/l. Alkalinity is the measure of weak acid present in water and of the cations balanced against them. Minimum alkalinity (244.29 ± 13.34 mg/l) in River Ganga was recorded in 2008-09 at Sampling station-B while maximum (295.16 ± 28.24 mg/l) was recorded in 2008-09 at Sampling station-A. The average value of alkalinity for the study period (2007-09) was observed as 268.08 ± 17.09 mg/l. Similar trend was also obtained by Chugh (2000), Holden and Green

(1960), Talling and Rzoska (1967) , Abdin (1948), Sverdrup *et al*. (1942), Khanna *et al.* (2010) and Khanna *et al.* (2009). Factors such as mixing of ashes, waste water from sewage drains into the river may also be responsible for its fluctuation. The decomposition of organic matter leads to high alkalinity of water as per Hay and Anthony (1958) and Venkateshwarlu and Jayanti (1968).The presence of total hardness is governed by the contents of calcium and magnesium salts, largely combined with bicarbonate, carbonate sulphate and chloride.According to Barrett (1953), hard water is more productive than soft water. Minimum hardness(218.80 ± 8.88 mg/l) in River Ganga was recorded in 2007-08 at Sampling station-B while maximum (253.50 ± 9.30 mg/l) was recorded in 2008-09 at Sampling station-D. The average value for the study period (2007-09) was observed as 236.91 ± 12.05 mg/l. Chopra and Patrick (1994) observed positive relationship between chloride and hardness in River Ganga at Rishikesh. Hardness showed a positive relationship with alkalinity while Chopra and Patrick (1994) observed negative relationship in River Ganga at Rishikesh.

Table 2: Correlation between physico-chemical parameters of Ganga River during 2007– 2009

Parameters	Temp.	Conductivity	Turbidity	Total Solids	Velocity	pH	BOD	COD	DO	Free CO_2	Acidity	Alkalinity	Hardness	Chloride	Mg	Phosphates
Temperature (˚C)																
Conductivity (Siemens/cm)	-0.09															
Turbidity (J.T.U.)	0.53	-0.28														
Total Solids (mg/l)	0.74	-0.41	0.36													
Velocity (m/s)	0.55	-0.44	0.14	0.55												
pH	0.10	0.06	-0.60	0.10	0.17											
BOD (mg/l)	-0.15	-0.39	0.11	0.40	0.04	-0.44										
COD (mg/l)	-0.30	0.13	-0.38	-0.13	-0.35	-0.01	0.56									
DO (mg/l)	-0.21	0.31	-0.47	-0.53	-0.07	0.52	-0.88	-0.47								
Free CO_2 (mg/l)	-0.10	0.25	0.07	0.01	0.45	-0.37	0.33	0.15	-0.34							
Acidity (mg/l)	0.38	0.20	-0.46	0.20	0.49	0.85	-0.39	-0.001	0.37	0.09						
Alkalinity (mg/l)	0.36	-0.009	0.07	0.08	-0.19	0.52	-0.61	-0.58	0.51	-0.66	0.21					
T. Hardness (mg/l)	0.51	-0.27	0.19	0.39	0.63	0.15	-0.32	-0.43	0.20	-0.20	0.27	0.04				
Chloride (mg/l)	0.08	-0.40	0.53	0.13	0.52	-0.46	0.05	-0.68	-0.06	0.35	-0.33	0.02	0.08			
Magnesium (mg/l)	-0.20	0.81	-0.01	-0.36	-0.18	-0.38	-0.05	0.12	-0.02	0.70	-0.05	-0.38	-0.37	0.02		
Phosphates (mg/l)	0.68	0.56	0.42	-0.25	-0.37	-0.65	-0.19	-0.29	0.02	0.20	-0.51	-0.08	-0.03	0.22	0.68	
Calcium (mg/l)	-0.15	0.15	-0.34	-0.50	-0.57	0.45	-0.35	0.38	0.33	-0.57	0.15	0.36	-0.29	-0.66	-0.23	-0.31

Minimum chloride (16.68 ± 1.49 mg/l) in River Ganga was recorded in 2007-08 at Sampling station-A while maximum (24.09 ± 3.22 mg/l) was recorded in 2007-08 at Sampling station-D. The average value for the study period (2007-09) was observed as (20.44 ± 2.51 mg/l).Similar trends were obtained by Chugh (2000) in the River Ganga at Hardwar. CPCB (2003) reported the value of chloride in between 14 to 51 mg/l during Ganga monitoring from Bithur, Kanpur to Sangam Allahabad. CPCB (1990-91) also studied the chloride from Rishikesh to Uluberia and showed a significant increasing trend on chloride at all monitoring stations in west Bengal stretches. Minimum magnesium (39.58 ± 4.83 mg/l) in River Ganga was recorded in 2008-09 at Sampling station-A, while maximum (52.20 ± 3.17 mg/l) was recorded in 2007-08 at Sampling station-A. The

average value for the study period (2007-09) was observed as 44.86 ± 4.87 mg/l. Singhai (1986) reported a positive correlation between magnesium and total hardness as also observed in present study. The magnesium hardness was always observed lower than calcium hardness. Minimum phosphates (0.05± 0.02 mg/l) of River Ganga was recorded in 2008-09 at Sampling station-D while maximum (0.16 ± 0.02 mg/l) was recorded in 2008-09 at Sampling station-B. The average value for the study period (2007-09) was observed as 0.08 ± 0.03 mg/l. Minimum calcium (48.72 ± 6.53 mg/l) in river Ganga was recorded in 2008-09 at Sampling station-B while maximum (75.97 ± 6.09 mg/l) was recorded in 2008-09 at Sampling station-A. The average value for the study period (2007-09) was observed as 60.04 ± 8.93mg/l. Calcium is one of the most abundant substance of natural waters. Being present in high quantities in the rocks, it is leached from there to contaminate the water. Calcium is essential for metabolic processes in all-living organisms. Lund (1965) suggested calcium, main effect on phytoplankton by buffering pH of water. Atkin and Harris (1924) and Mohanty (1981) reported negative relationship between pH and calcium in dried water ponds in some water bodies of Bhubaneswar.

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Heavy metal contamination in seafood of two suburban areas of Mumbai (West Coast) of India

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Abstract

Seafood is a major source of food for large number of people residing in the coastal areas of Maharashtra. Fish samples namely *Escuolosa thoracata, Carcharhinus limbatus, Ilisha filigera, Johnius sina* and *Sardinella longiceps* (Goregaon market) and *Megalaspis cordyla, Ilisha filigera, Harpadon nehereus, Coilia dussumieri* and *Lepturacanthus lepturus* (Borovali market) were collected directly from the two suburban markets (Goregaon and Borivali markets) of Mumbai coast. In the present study, the level of Zn in fishes from Goregaon and Borivali market was found above the tolerable limits, while the concentration of Fe in different species of fishes was found quite high as also reported in earlier literature. Iron was found tobe the dominant metal measured during the study period.The level of Pb was found within the tolerable limits. The concentration of Cd in marketed fishes was far lower than the consumption safety tolerance in fishes. Hg level in the samples of the fishes was found below the tolerable limits. The study concludes that the value of Fe represents severe contamination in the seafood and necessary steps are required to minimize heavy metal contamination.

Keywords: *Heavy metals, Fish, Contamination, Spectroscopy, Tolerance limit*

Introduction

Increased industrialization, urbanization, population growth and overall man's greed to overexploit mother nature has created a serious threat to all kind of life in the form of pollution which has now become a global problem. Massive amounts of domestic wastewater and industrial effluents are transported by rivers and finally discharge to the sea, containing rivers and coastal waters. Such anthropogenic pollutants are the main sources of heavy metal contaminants in the ocean. These contaminants entering the aquatic ecosystem may not directly damage organisms; however, that can be deposited into aquatic organisms through the effects of bio-concentration, bio-accumulation and passes into the food chain process and eventually threaten the health of humans by seafood consumption. Metals may occur in the environment as hydrated ionic species or they may form a variety of complexes with inorganic and organic ligands (VanLoon, 1977).

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The global heavy metal pollution of water is a major environmental problem with the advent of agricultural and industrial revolution by which most of the water resources are becoming contaminated (Khare and Singh, 2002). Industrial discharges containing toxic and hazardous substances, including heavy metals (Gbem *et al*., 2001; Woodling *et al.*, 2001) contribute tremendously to the pollution of aquatic ecosystem causing cytotoxic, mutagenic and carcinogenic effects in animals (More *et al*., 2003). Fish are often at the top of aquatic food chain and may concentrate large amounts of metals from the water (Mansour and Sidky, 2002). Metal bioaccumulation is largely attributed to different fish species (Tiwari-Fufeyn and Ekaye, 2007). Multiple factors including season, physiological and chemical properties of water (Kirgin, 1996) can play a significant role in metal accumulation in different fish tissues. The natural concentrations of these metals in sea water are very low and hence the risk of contamination in living tissues is high. Industrial effluent is one of the prime sources of metal contamination in coastal waters and the Bay of Bengal and Arabian Sea is no exception (Mitra and Choudhury, 1993).

The contaminants contributed in water, sediments and tissues of several marine organisms have also been reported along with toxicity tests. The pollution of the aquatic environment with heavy metals has become a world wide problem during recent years because they are indestructible and most of them have toxic effects on organisms (MacFarlane and Burchett, 2000). Among environmental pollutants, metals are of particular concern due to their potential toxic effect and availability of bioaccumulation in aquatic ecosystems (Censi *et al*., 2006).

At present the population of Mumbai is severely suffering from lots of disorder particularly respiratory and digestive, due to air and drinking waters. Most of these causes have been identified and remedial measures have been taken up. However, toxic effect due to contamination of sea food, which is a main diet of majority of the population of Mumbai is not primarily addressed and completely neglected. In fact the relevant toxic effect may be already prevalent in the society and most probably they may become severe in due course of time. Hence, the stage has already reached to address the problem in detail and to dig the thought under the problem.

However, in India, the contamination of sea food studies have not been seriously attended so far.The present study has been undertaken with an aim to determine the current status of heavy metal contamination in seafood and to highlight the information regarding sources of pollution and measures to mitigate it.

Materials and Method

a) Collection of fish Samples

Fish samples namely *Escuolosa thoracata*; *Carcharhinus limbatus, Ilisha filigera, Johnius sina* and *Sardinella longiceps* (Goregaon market) and *Megalaspis cordyla, Ilisha filigera, Harpadon nehereus, Coilia dussumieri* and *Lepturacanthus lepturus* (Borivali market) were collected directly from suburban markets of Goregaon and Borivali respectively. The samples were identified in the Department of Zoology S.S & L.S. Patkar College Goregaon (West), Mumbai. These samples were brought to the laboratory and washed in sea and dried in oven at 80 ^{0}C. The dried fishes were crushed into a fine powder by mortal and pestle and pass through a 2 mm sieve and stored in amber colored bottles in vacuum dessicators.The samples were then analysed following the standards methods of APHA (1998).

Results and Discussion

The range of heavy metals in seafood collected from Goregaon and Borivalli markets are given in Table-1.

Zinc (Zn)

The mean concentration of zinc was found to be highest in *Harpadon nehereus* (55.358 ppm) collected from Borivali market, whereas the lowest mean concentration of Zn was found in *Carcharhinus limbatus* (2.45 ppm) collected from Goregaon market. It was found that Zn is above the tolerable limits in *Escuolosa thoracata* (10.04 ppm), *Ilisha filigera* (10.321 ppm) and *Sardinella longiceps* (10.449 ppm) collected from Goregaon market and *Megalaspis cordyla* (9.357 ppm), *Ilisha filigera* (16.969 ppm) and *Lepturacanthus lepturus* (12.111 ppm) collected from Borivali market. The level of zinc was found below the tolerable limit in *Johnius sina* (6.346 ppm) collected from Goregaon market and in *Coilia dussumieri* (6.128 ppm) collected from Borivali market. Denton and Burdon (1986) have reported higher mean value of Zn (1.9 to 35.0 ppm) in *Thalassorna* sp., in *A. saxatils* the highest concentration was found in the liver of these fishes (30.0 ppm- 44.9 ppm). Similar range of concentration (4.3 ppm-41.8 ppm) was found by them in the muscles of fish species from the Great Barrier Reef. They have also reported relatively high concentrations of Zn in the liver of these fishes. In comparison to this Hanna (1989) found much higher and wider concentrations of Zn in the muscles (8.4-195.0 µg g^{-1}), livers (43-620 µg g^{-1}), and gonads (72-259 µg g^{-1}) of fishes from the Red Sea. The present study shows that Zn levels in the fishes collected from Goregaon and Borivali markets are within the levels reported from the Red Sea and other regions of the world. During the study the level of Zn was found above the tolerable limits.

Manganese (Mn)

Manganese is an essential element and is subject to some internal regulation in human body. Although this element is of low toxicity, it has a considerable biological significance and seems to accumulate in certain fish species (Eustace, 1974; Uthe and Bligh, 1971). The highest mean concentration of Mn was

recorded in the fish *Escuolosa thoracata* (0.849 ppm*)* collected from Goregaon market, while the lowest mean concentration was recorded in the fish *Lepturacanthus lepturus* (0.216 ppm) collected from Borivali market. It is evident that the level of Mn was found above the tolerable limits in *Ilisha filigera* (0.783ppm), *Johnius sina* (0.523 ppm) *Harpadon nehereus* (0.282 ppm), *Ilisha filigera* (0.249 ppm), *Megalaspis cordyla* (0.28 ppm), *Sardinella longiceps* (0.299 ppm) and *Carcharhinus limbatus* (0.315 ppm) collected from Goregaon and Borivali markets respectively. Cross *et al.* (1973) reported lower Mn concentrations (0.20-0.28 µg g-1 wet weight) in the muscle of the blue fish *P. saltatrix*. Eustace (1974) found that 39 species of marine fish from Derwent Estuary, Tasmania contained up to 0.6- 4.4 µg g^{-1} wet weight Mn when homogenized whole. By comparison, Wahbeh and Mahasneh (1987) reported higher mean concentration (5.6-26.8 ppm) in various organs of fish they examined from the same study area within the Gulf of Aqaba. Our data is generally within the tolerable limits and does not indicate any particular contamination issue as reported in abovesaid literature.

Table-1: Range of heavy metals in seafood collected from Goregaon and Borivali markets

GOREGAON MARKET							
SAMPLE		**Zn (ppm)**	**Mn (ppm)**	**Fe (ppm)**	**Pb (ppm)**	**Cd (ppm)**	**Hg (ppm)**
1	*Escuolosa thoracata*	10.04	0.849	16.82	0.085	0.015	0.08
2	*Carcharhinus limbatus*	2.45	0.315	8.46	0.331	0.078	0.038
3	*Ilisha filigera*	10.321	0.783	26.167	0.861	0.037	0.164
4	*Johnius sina*	6.346	0.523	58.425	0.22	0.036	0.074
5	*Sardinella longiceps*	10.499	0.299	11.365	0.21	ND	0.042
BORIVALI MARKET							
SAMPLE		**Zn (ppm)**	**Mn (ppm)**	**Fe (ppm)**	**Pb (ppm)**	**Cd (ppm)**	**Hg (ppm)**
1	*Megalaspis cordyla*	9.357	0.280	13.254	0.204	0.011	0.084
2	*Ilisha filigera*	16.969	0.249	12.197	0.198	0.011	0.037
3	*Harpadon nehereus*	55.358	0.282	67.279	0.113	0.006	0.029
4	*Coilia dussumieri*	6.128	0.301	7.394	0.100	0.008	0.037
5	*Lepturacanthus lepturus*	12.111	0.216	11.067	0.441	0.017	0.02

N = 3 (Average of three readings) ND = Not detected or less than 0.0001ppm

Iron (Fe)

In the present study, it was found that Fe was dominantly present in the samples collected from Goregaon and Borivali markets. Our observations are similar to the observations of other workers (Okoye *et al*., 2002; Asuquo *et al*, 1999). It has also been observed that iron is the dominant metal in the muscle of *C. gariepinus* (Adeyeye *et al*., 1996). There is wide variation in mean concentrations of Fe among different species of fishes. The mean concentration of Fe was recorded highest in the fish *Johnius sina* (58.425 ppm) and *Harpadon nehereus*(67.279 ppm) collected from Goregaon and Borivali market whereas the concentration of Fe was found lowest in *Coilia dussumieri* (7.394 ppm) and *Carcharhinus limbatus* (8.46 ppm) from Borivali and Goregaon markets respectively. The mean concentration of Fe was recorded above the tolerable limits in *Escuolosa thoracata* (16.82 ppm), *Ilisha filigera* (26.167 ppm) and *Sardinella longiceps* (11.365 ppm) collected from Goregaon

and *Megalaspis cordyla* (13.254 ppm) and *Lepturacanthus lepturus* (11.067 ppm) collected from Borivali market. The mean concentration of Fe was recorded below the tolerable limits in *Carcharhinus limbatus* (8.46 ppm) collected from Goregaon market and *Coilia dussumieri* (7.394 ppm) collected from Borivali market. Similar variations were also found by Wahbeh and Mahasneh (1987) for fish species from the Gulf of Aqaba. Cross *et al*. (1973) reported lower mean levels of Fe in the muscles of the blue fish, *Pomatomus saltatrix* (4.5-5.0 µg g^{-1} wet weight). During our study it was found that the concentration of Fe in different species of fishes collected from Goregaon and Borivali markets correlate with the earlier data.

Lead (Pb)

Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults (Commission of the European Communities, 2001). FAO (1983) of the United Nations and WHO (1990) have established a provisional tolerable weekly intake (PTWI) of lead as 25 µg/kg body weight for humans, equaling 1,500 µg/g lead/week for a 60-kg person.

The maximum lead level permitted for canned fishes is 0.2 ppm according to the European communities (Commission of the European Communities, 2001). In the present study, the mean lowest and highest levels of lead in fish samples ranged from 0.085 ppm to 0.861 ppm collected from Goregaon market, whereas the level of Pb in fishes ranged from 0.1ppm to 0.441ppm collected from Borivali market. The fact that toxic metals are present in high concentrations in fishes is of particular importance in relation to the FAO/WHO (1972) standards for lead as a toxic metal. The maximum permissible dose for an adult is 3 mg lead per week, but the recommended sources are only one-fifth of those quantity. Lead is a neurotoxin that causes behavioural deficits in vertebrates (Weber and Dingel, 1997) and can cause decreases in survival, growth rates, learning, and metabolism (Eisler, 1988; Burger and Gochfeld, 2000). Levels of 50 ppm of lead in the diet can cause reproductive effects in some predators, and dietary levels as low as 0.1–0.5 ppm are associated with learning deficits in some vertebrates (Eisler, 1988). In our study, the levels of lead are within the tolerable limits.

Cadmium (Cd)

In the present study the concentrations of cadmium in market fishes were found to be far lower than the consumption safety tolerance in fishes set by countries worldwide. The contamination of Cd in fishes ranged from 0.015ppm to 0.078 ppm in fishes collected from Goregaon market and from 0.006 ppm to 0.017 ppm in fishes collected from Borivali market. These values are below the range reported by (Hanna, 1989). Cadmium is accumulated primarily in major organ tissues of fish rather than in muscles (Moore and Ramamurthy, 1984). In contrast, Cd levels in muscles of fish *Mullus barbatus* and *Sardinella aurita* from the Great Barrier Reef were consistently lower than 0.1ppm (Denton and Burdon Jones, 1986), while in liver of *Mullus barbatus and Sardinella aurita*, Cd concentrations varied from less than (0.6 ppm to 0.7 ppm) Roth and Hornung (1977). In general, it can be stated that the concentrations of Cd found in the present study was below the tolerable level as compared to those of uncontaminated fish (< 1.5) reported by Moore and Ramamurthy (1984).

Mercury (Hg)

According to the results obtained, the mercury levels in the samples of the fishes collected from the Goregaon and Borivali markets were found below the tolerable limits than the permissible level, *i.e*., 1 ppm. (WHO, 1994). The Food and Drug Administration (FDA) has set a maximum permissible level of one part of methylmercury in a million parts of seafood (1 ppm). The higher level of mercury can be attributed to the sewage–sludge outfall present along this western coast. This sewage outfall consists of treated industrial effluents from industries and other biochemical manufacturing units situated in that part of Mumbai. It is possible that though the sewage–sludge was treated, traces of heavy metals might have leached into the sea. Fish analyzed from Goregaon and Borivali markets showed normal mean levels of Hg which were in the range of 0.02 ppm to 0.164 ppm. In the case of Goregaon market, mercury levels range from 0.038 ppm to 0.164ppm and in Borivali market, the mercury levels were found to be in the range of 0.02 to 0.084 ppm which

suggests that the fish brought to the market was relatively less contaminated with mercury. The PTWI (permissible tolerable weekly intake) of mercury has been set at 5 µg/kg body weight (FAO-WHO 1972), equaling 300 µg mercury/week for a 60-kg person. Mercury is known to be a latent neurotoxin compared to other metals like lead, cadmium, copper and arsenic. A high dietary intake of mercury (organic) from consumption of fish has been hypothesized to increase the risk of coronary heart disease (Salonen *et al*., 1995). When deposited in biota, mercury undergoes biotransformation, in which inorganic mercury may convert to organic mercury (methyl mercury). Microbes subsequently concentrate mercury through the food chain in the tissue of fish and marine animals (Altindag and Yigit, 2005).

Conclusion

From the above results, Zn, Fe, and Pb was found to be high in fish samples collected from Goregaon and Borivali markets. It can be assumed that the sea from where the fishes were collected might be receiving outfalls from industrial waste and sewage from the city as it faces the open Arabian Sea. The levels of heavy metals such as Mn, Cd, and Hg in fish samples collected from Goregaon and Borivali markets were within permissible limits. These elemental toxicants may be transferred to man on consumption of fish obtained from the market. These heavy metals transferred to man through the consumption of fish pose health hazards because of their cumulative effect in the body. Therefore, it was concluded that the fishes are not heavily burdened with metals, but a danger must be considered depending on the agricultural and industrial developments in this region. The fish from Arabian Sea should be monitored periodically to avoid excessive intake of trace metals by human and to monitor the pollution of aquatic environment. In view of these findings strict method of waste disposal control should be adopted to ensure the safety of the environment and safeguard our aquatic life.

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Morphological and biochemical studies of the milt (Spermatozoa) of the snow-trout fish S*chizothorax richardsonii* (Gray)

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Abstract

The present study was undertaken to evaluate the biochemical and morphological nature of the milt (=spermatozoa) of the snow-trout fish *Schizothorax richardsonii.* The study was conducted to find out the possibilities of evolving cryopreservation, artificial propagation for better fishery development. Scanning Electron Microscopy was employed to find out the structural details of the sperm cell. Biometric and mobility studies were also done on the sperm cells to obtain their sizes and movements. The present findings showed that *S. richardsonii* has high protein and lipid content in gonads. The spermatozonic characteristics of these fishes can help in maintaining stocks of their populations in natural aquaculture resources.

Keywords: *SEM*, *Milt*, *Biometric studies*, *Motility*

Introduction

Garhwal Himalayas in India is home to various fish fauna of which *Schizothorax* spp. holds a very significant position. They are key group in the snowfed water of Himalayan belt. In Garhwal Himalayas, *Schizothorax* has three species namely *Schizothorax richardsonii* (Gray), *Schizothorax plagiostomus* (Heckel) and *Schizothorax sinatus* (Heckel). The family Cyprinidae to which the *Schizothorax richardsonii* belong is the richest and most important family of fish and its members are distributed throughout the world comprising 220 genera and 2420 species (Nelson, 2006). Their spawning seasons depend upon various interceptive factors such as photoperiod, temperature, pH, flood, turbidity *etc* (Sunder, 1986). These fishes are widely distributed along the Himalayan region of India, Pakistan, Bhutan, Bangladesh and Indonesia (Menon,1992). The physical appearance of *S. richardsonii* is highly modified accordingly to the fast flowing waters of Garhwal hills. They are bottom-feeders and are well adapted to live in rocky and stony bed with icy cold super oxygenated and fast flowing waters of the Himalayan region. They are mainly found in upper stretches of Alaknanda and Bhagirathi rivers of this region.

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In the present study, *Schizothorax richardsonii* was chosen for the determination of its spermatozonic characteristics. The study was also significant from the fact that it was closely associated with the development of cryopreservation. Therefore, establishing the spermatozonic characteristics is an urgent prerequisite for launching conservation policies. Moreover the study of sperm morphology provides fair amount of idea about the modifications in fishes and their utilities (Billard and Cosson, 1992). Spermatozoa of fishes are characterized by wide divergences in their structural organization which has become field of interest from taxonomical point of view (Jamieson, 1991). Sperm morphology also reflects the mode of fertilization. The biochemical characteristics of the milt (=spermatozoa) is highly correlated with gonadal maturation and development. The possibilities of sperm preservation and their motility studies can help in their proper preservation by providing them suitable conditions.

Materials and Method

Experiments were carried out on various samples of *S. richardsonii* at Premature, Mature and Post Mature Stage to evaluate the protein and lipid content along with pH and temperature estimation. The ecomorphological adaptations in the fish sperm cells were studied using Scanning Electron Microscopy.

Specimen Collection

The samples were collected from River Alaknanda (Lat 30º 13'North, Long 78º 47'East) at an altitude of 1780-2500 feet during various seasons. The live fishes were transported to the laboratory and were kept in a well-aerated hatchery at 15-22 ºC before analysis to get acclimatized to the existing conditions. After correct identification and taking morphometric data at species level, the specimens were properly cleaned.

Semen collection

For semen collection, specimens of *S. richardsonii* at various stages were administered with intraperitoneal hormone Ovaprim (Ovaprim Syndel Laboratories, Vancouver Canada) at the rate of 0.2 ml/kg body weight. Semen samples were collected in ice cooled sterilized test tubes after 5 h of hormone administration. During semen collection, attention was paid to prevent contamination by faecal matter, urine *etc*. The tubes were stored at 4 ºC for further analysis.

Lipid estimation

The lipid estimation was done by conventional Bligh and Dyer method (1959) in which the weight of the empty vial was first taken in which the lipid is to be weighed. Then chloroform and methanol in equal volume were added; centrifuged at 3300 rpm at 5 ^{o}C for 10 minutes; decant and chloroform layer was retained. The layer was then passed through 2.5 cm anhydrous sodium sulfate layer using Whatman filter paper 1. The solvent was then removed using rotary evaporator under vacuum at 40ºC.Now the weight of the extracted lipid plus the weight is taken. The lipid weight is taken according to the formula.

Weight of lipid = (weight of container + extracted lipid) − (weight of empty container)

Protein estimation

The protein estimation was done according to Lowry's *et al*. (1951) with modifications from Hartree (1972). This modification makes the assay linear over a larger range than the original assay. Gonad fragments were first homogenized. The homogenate of fresh sample was prepared in 20% trichloroacetic acid. It was then centrifuged at 200 rpm for 10 minutes. The supernatant was then used to estimate soluble sample protein and residues to evaluate insoluble protein. Bovine Serum Albumin (BSA 1mg/ml) was used as standard. The absorbance was determined at 660 nm using the calibration curve.

Electron Microscopy

Gonad fragments of *Schizothorax richardsonii* were fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M Phosphate buffer (pH 7.4) for 7 h at 4 ºC. The material was post-fixed for 2h in 1% osmium teraoxide at 4 ºC. The sample was then critical point dried and electromicrographs were obtained using Leo-435 VP scanning electron microscope at 20 KV.

Statistical Analysis

Statistical evaluation for the semen parameters was performed by Duncan's multiple range test (DMRT). A P value of $P<0.05$ was considered as statistically significant.

Biometric Studies

Biometric measurements were recorded with the help of ocular and stage micrometer on the slides prepared for morphological studies. All lengths were reported in micrometers.

Motility determination

The collected milt of the fishes were evaluated for motility, pH and temperature estimation. Spermatozoa motility assessment was carried out by diluting milt with sterile water (1: 50) at room temperature (31°C) on glass slide, observed immediately under an inverted microscope (200 X) (Zeiss, Germany) with a CCD camera attachment. Estimation of spermatozoa motility was started immediately (approximately 15 s) after dilution and the movement was observed till 2 min. The motility was recorded in a computer by using computer aided motility software (Biovis motility software, Expert Vision Pvt. Ltd, India) and computer assisted sperm analyzer (CASA).The assessment was done at two different temperatures (at 4ºC and at 28 ºC) in order to evaluate the effect of temperature on sperm motility. The percentage of total number of observed (immotile and poorly motile) spermatozoa were counted.

Results and Discussion

The milt collected from different samples was taken in measuring vials and the biochemical characteristics were analyzed. The pH value was recorded more towards alkaline region in mature stage than the immature and post mature stage. The temperature of the collected milt was also comparatively higher than in immature fry and post mature (spent) fishes. The pH, temperature, protein and lipid content at premature, mature and post mature stage are summarized in Table-1 to 3. The

biometric parameters are shown in Table-4. The spermatozoa of teleost fishes are characterized by wide divergences and structural organization which is also significant from taxonomical point of view (Jamieson, 1991). Generally spermatozoa of externally fertilizing teleost fishes are differentiated in a head, a small mid-piece and a tail region, but no acrosome. Evidences are mounting to suggest that the amount of milt produced by a fish is of vast significance in fertilization process as large amount of spermatozoa in these externally fertilizing fishes gets wasted due to short mobility and hostile external environment. Hence study of their sperm characteristics is necessary for the development of breeding programmes and conservation policies (Routray *et al.*, 2007).

Table-1: Biochemical study of milt in *Schizothorax richardsonii* (Premature stage). (May 15-20/2009)

S. No.	Wt. of fish (gm)	Length of Fish (mm)	No. of Samples	pH	Temp. (^{0}C)	Protein (mg/gm)	Lipid (mg/gm)
1.	160	350	5	7.2	15	91.00±14.5	43.8±4.70
2.	165	400		7.3	16	95.80±15.1	45.8±3.99
3.	170	410		7.2	16	94.60±13.9	44.5±3.68
4.	200	300		7.3	16	98.90±14.8	34.0±3.98
5.	166	380		7.2	16	95.90±14.2	45.8±3.68
Mean value	**172.2**	**368**	**5**	**7.24**	**15.6**	**95.24±2.85**	**42.78±4.12**

Data is expressed as mean± SEM

Table-2: Biochemical study of milt in *Schizothorax richardsonii* (Mature stage). (September 15-20/2009).

S. No.	Wt. of fish (gm)	Length of Fish (mm)	No. of Samples	pH	Temp.(^{0}C)	Protein (mg/gm)	Lipid (mg/gm)
1.	218	210	5	7.40	18.0	189.80±12.2	91.50±4.70
2.	280	250		7.40	18.0	190.00±14.5	91.20±3.99
3.	300	280		7.50	18.5	191.50±13.2	90.00±4.24
4.	420	350		7.60	19.0	190.00±15.2	88.50±3.88
5.	450	410		7.90	20.0	192.50±16.2	90.50±4.20
Mean value	**333.6**	**300**	**5**	**7.56**	**18.7**	**190.76±14.26**	**90.34±4.19**

Data is expressed as mean± SEM

The ultrastructure of the spermatozoa reveals that the flagellum is fastened to the sperm cell by a centriolar complex located in an invagination of the nucleus and the flagellum is separated from the mitochondria by a cytoplasmic channel (Koch and Lambert, 1990). Ovaprim is quite useful for the better production of semen from the fishes. The enhanced mobility of spermatozoa at 4 ºC was observed (> 90%) and mobility occurs for 90 to 97 seconds. The motility and viability of sperm is directly related to the metabolism (Lahnsteiner, 1999). The duration of spermatozoa motility in cyprinids is reported to be till 120 seconds (Suzuki, 1959). The mobility of the spermatozoa is an important indicator of the fertility and a necessary parameter to evaluate the sperm value. Besides this, the mobility of fish spermatozoa is an important factor for its ability to enter into the egg. The results also illustrate that the percentage of motile spermatozoa at low temperature in incubated environment is higher than the non-incubated sperms. Since these fishes inhibit cold waters, the spermatozoa demonstrate better motility at lower temperature. There is no prominent acrosome in *Schizothorax richardsonii* which is typical of all teleosts. However an acrosome like structure was observed on the sperm head of *S. richardsonii*, (Figure -1).High magnification shows that the head

of *Schizothorax richardsonii* is ovoid in shape. The head possess a rough surface throughout (Figure-3).The insertion of the flagellum is central into the head of the sperm cell (Figure-2). The lower temperature of the milt was according to the environment in which the fish lives. The pH value of the milt was more towards alkaline region in mature male gonads but near to neutral in immature and spawned gonads of all fishes examined. The biochemical components *viz.* protein, lipid and water content in *Schizothorax richardsonii* was higher during mature stage. The protein content is reported to be higher in milt than any other tissue *i.e.* intestine, muscles and kidneys in some fishes (Geetha *et al*., 1990). Lipids are quantified more in mature *S. richardsonii* specimens.

Table-3: Biochemical study of milt in *Schizothorax richardsonii* (Post mature stage). (December 15-20/2009).

S. No.	Wt. of fish (gm)	Length of Fish (mm)	No. of Samples	pH	Temp. (^{0}C)	Protein (mg/gm)	Lipid (mg/gm)
1.	260	496	5	7.8	14	110.5±14.2	53.2±3.42
2.	210	310		7.5	14	109.6±13.5	54.0±4.20
3.	206	300		7.5	14	112.0±14.5	53.5±3.99
4.	209	310		7.5	13	112.0±12.5	54.4±4.20
5.	160	250		7.4	13	115.0±12.2	55.0±3.42
Mean value	**209**	**333.2**	**5**	**7.54**	**13.6**	**111.82±13.38**	**54.02±3.84**

Data is expressed as mean± SEM

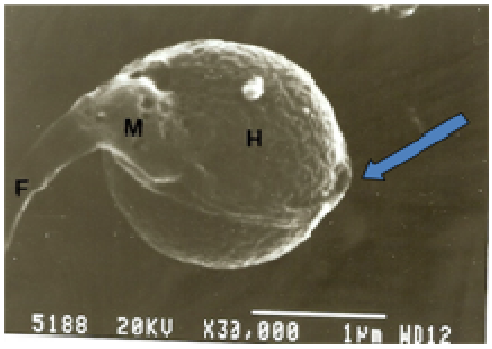

Figure-1. Sperm head of *Schizothorax richardsonii* showing the ovoid head and acrosome like structure which is unusual of teleosts (x 30, 000). Scale bar= 0.1µm.

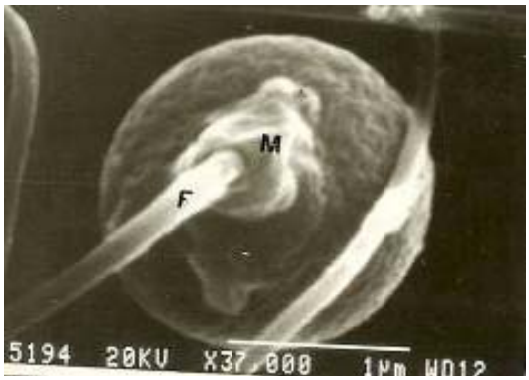

Figure-2. Shows the central insertion of flagella in the sperm head (x 37, 000). Scale Bar= 1 µm

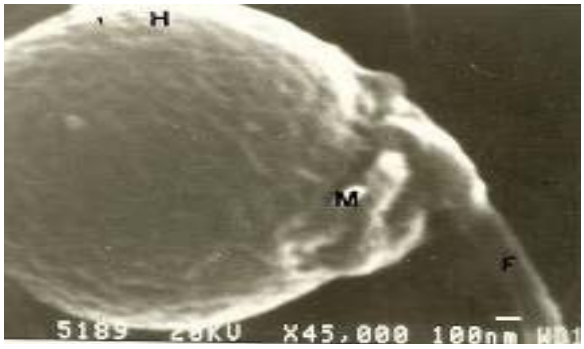

Figure-3. Shows the rough surface of the sperm cell with no acrosome like structure (x 45,000), Scale bar=100 nm

This implies the corresponding requirements of more lipids for males as they utilize a lot of their energy in moving around the spawning areas and breeding grounds. The higher length of flagella in these fishes increases the efficiency of undulating movement of sperm in high velocity water current (Stoss, 1983). Scanning Electron Microscopy (SEM) is a powerful tool to describe the morphology of these hill-stream fishes and various modifications which have occurred in them. The fine structure of spermatozoa in fishes is also being studied previously using electron microscopy (Iwamatsu and Ohta, 1981; Lahnsteiner and Patzner 1990; Di Lauro *et al*., 1999; Burns *et al.*, 2002).

However reports on hill-stream cold water fishes is not being done earlier. SEM studies showed the variations between the morphology of these fishes. These divergences in sperm morphology are mainly phylogenetic and do not truly represents the mode of reproduction (Mattei, 1991). Acrosome is completely absent in both the fishes which is typical of all the teleost sperm organization. In the absence of true acrosome, the sperm cells reach the egg plasma membrane through a narrow micropyle. However an acrosome like structutre was observed on the sperm head *of S. richardsonii* which is a

Table-4: Showing the percentage Mobile and Non-Mobile sperms at two different temperatures

S. No.	No. of oozing for each sample	1 hrs experiment in incubated (1-4 ºC milt 50 times dilution)		1 hrs in non-incubated (20-25 ºC) milt with 50 times dilution	
		Average percentage of mobile spermatozoa	Average percentage of non mobile spermatozoa	Average percentage of mobile sperms	Average percentage of non-mobile sperms
1	6	92.5±1.50	7.5	87.6± 1.2	12.4
2	5	94.0± 1.75	6.0	88.5± 1.5	11.5
3	9	93.0± 1.50	7.0	90.0±2.0	10.0
4	8	89.5± 1.50	10.5	86.5± 1.80	13.5
5	7	90.5± 1.50	9.5	85.5± 1.50	14.5
Mean	**7**	**91.5± 1.55**	**8.1**	**87.62±1.60**	**12.38**

Table-5: Biometric parameters of *Schizothorax richardsonii*

Sperm	***Schizothorax richardsonii***
HEAD (i) Length (µm)	Ovoid 5.07± 1.49 Ellipsoidal
FLAGELLUM (i) Insertion (ii) Length (µm) (iii) Total Length of spermatozoa(µm) Total No. of Cells counted	 Central 78.00 ± 3.50 83.07 ± 4. 56 48

Data expressed as Mean±SEM

peculiar modification to which the fish has undergone that somewhat differs from other teleosts. A variety of acrosomal structures are known to occur in other fish spermatozoa (Stanley, 1971; Mattei, 1970). The rough surface of *S.richardsonii* may be an ecological adaptation against the fast water current in which the fish dwells. The rough surface helps them to bind to the eggs effectively in the pacedwater currents of the streams and helps the sperm cell to perform the processes of capaciation and egg penetration. The establishment of spermatozoon characteristics can be of immense help to differentiate between the species when morphometric and meristic characters do not resolve its proper species identification. Thus morphologically similar *Schizothorax* spp. (Bahuguna and Bisht, 2005) could be distinguished using spermatozoan characters. These spermato-zonic characterizations of *S.richardsonii* may also help in determining taxonomic ambiguities for future breeding and maintaining stocks of their populations in natural aquaculture resources.

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Dynamics of zooplankton diversity in relation to water quality of Heggere tank, Kanale Sagara Karnataka, India

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Abstract

The present study reveals that the diversity of zooplankton communities in Heggere tank, Kanale varies with the physico-chemical parameters of water. The presence of different zooplankton community indicates the nutrient status of water body. The zooplankton communities were recorded more during the post monsoon and pre monsoon seasons. However, the variation of physico-chemical parameters of water in relation to zooplankton population has been discussed in detail in this paper. The trend of monthly occurrence of zooplankton was found as cladocerans>copepods>rotifers>protozoans.

Keywords: *Cladoceran population, Nutrient composition, Physico-chemical, Zooplankton diversity*

Introduction

Zooplankton is ecologically and economically important heterogenous group of tiny aquatic organisms that can move at the mercy of water currents, as they have weak power of locomotion. Their ecology is closely related to fishery limnology, oceanography and meteorology. Also temporal and spatial change in zooplankton abundance and composition reflected the dynamic nature of both physical and biological factors of freshwater resources. Zooplankton are either herbivorous, feeding on phytoplankton or carnivorous, feeding on other zooplankton. They themselves fed upon by fish and are thus the vital transition between primary production (phytoplankton) and fish. Without these primary consumers, herbivorous and other levels of food chain would collapse.

Study Area

Heggere tank (Kanale) is a perennial fresh water body situated at about 14 km away towards North of Sagara town. It lies between 14° 12' to 14° 17' North latitude and 74° 54' and 74° 59' East longitude. This is a medium sized tank. The total water spread area of the tank is 22.1 hectare. Rain water is main source of water for the tank. The river basin of the tank is Krishna. The catchment area of this tank is 1.30 sq. km and is covered by Natural vegetation *i.e.* Areca and Acasia plantation. The water of this tank is used for agricultural and aquaculture practices and domestic activities.

Materials and Method

Surface water samples were collected at an interval of 30 days from January, 2004 to December, 2004 for physico-chemical analysis. Water samples were collected in black colored carboys of 2 liter capacity. Factors like pH, air and water temperature were recorded on the spot. For dissolved oxygen (DO) the samples were fixed on the spot using Wrinkler's reagents. Later the samples were brought to the laboratory for estimation of other chemical parameters. The remaining parameters were analyzed as per the standard methods (APHA, 1998).

Microscopic studies (Zooplankton)

For the qualitative and quantitative analysis of plankton, two liters of composite water samples at the surface level were collected at an interval of 30 days. One liter of sample was fixed with 20 ml of 1% lugol solution. After sedimentation 100 ml of sample is subjected to centrifugation at 1500 rpm

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for 20 min for further microscopic investigation. The filtered plankton were collected in separate bottles and preserved using 10% formalin. The identification of plankton up to the level of species was done by standard manual and monographs. Quantitative estimation of zooplankton was done using Sedgwick rafter counting cell.

Results and Discussion

The results of all the four categories of the zooplankton encountered during course of study *viz*., Cladocera, copepoda, rotifers and protozoan are given in Table-1 to 6. While the seasonal fluctuation of physico-chemical parameters are given in Table-7.

The study of physico-chemical parameters and their effects on the aquatic biota are important in understanding the trophic state of a water body. Each factor plays its role in regulating the ecosystem of the waterbody. The concentration of the various constituents along with factors such as rainfall, agricultural runoff is also of equal importance. The changes in one factor are directly or indirectly related to the other factors.

Zooplankton plays an important role in the aquatic food chain and also contributes significantly to secondary productivity and energy flow in fresh water ecosystem. This is due to their rapid turnover rates, metabolism and capacity to build up populations in short duration. They serve as food for both fry and adult fish and hence is cultured as supplementary food in aqua cultures.

In the present study Cladocera invariably constitute a dominant component of freshwater (Table-1). Temperature, pH, alkalinity, calcium and phosphate were the factors found to influence the cladoceran population. Datta *et al.* (1986) have considered the cladoceran abundance to lower temperature, phosphate and salinity. High densities of cladoceran population during rainy seasons may be due to availability of certain nutrients entering from the agricultural runoff. Cladocerans are known to be abundant in water with good littoral vegetation, while ponds and lakes without vegetation have fewer cladoceran species (Idris and Fernando, 1981). Decay of this vegetation during summer may serve as food, thus maximum during that season. Low densities during the other season may be due to predation by copepoda (Hessen, 2003). Another reason may be the positive phototactic swarming from littoral areas to pelagic zone (Kairesalo and Penttila, 1990). These observations are in conformity with the findings of present investigation. If monthly density is considered, cladocerans recorded a minimum of 312 O/l in the month of July 2004 and maximum of 361 O/l in the month of April 2004 (Table-1).

Table-1: Monthly occurrence of different groups of zooplankton density in Heggere tank, Kanale

Months	Cladocera (org./l)	Copepoda (org./l)	Rotifers (org./l)	Protozoans (org./l)
January	321	258	210	10
February	343	265	218	12
March	352	245	214	13
April	361	267	203	08
May	342	241	222	12
June	357	236	198	09
July	312	229	202	08
August	324	245	210	12
September	321	261	214	15
October	317	241	221	16
November	328	242	214	12
December	325	251	215	15

Seasonwise, cladocerans were found to be more during pre monsoon with 349 O/l and low during post monsoon season with 322 O/l (Table 2). A total of six species were found during the course of the study *i.e. Alona pulchella*, *Daphnia carinata*, *Diaphanosoma sarsi*, *Macrothrix goeldi*, *Macrothrix laticornis* and *Moina carinata* (Table-3).

Copepods are aquatic crustaceans, smaller relatives of the crabs and lobsters, in terms of their size, abundance and diversity of way of life. Calanoids copepods are small crustaceans, 1-5 mm in length, commonly found as part of the free living zooplankton in freshwater lakes and ponds (Williamson, 1991). In shallow waters, no thermal stratification is observed and distribution of

zooplankton is highly variable. Well developed aquatic macrophytes, copepods are more abundant in littoral than pelagic areas.Large species of copepods find shelter in temporary and weedy ponds and can be found among macrophytes (Arcifa, 1984).The present study witnessed with these reports (Paterson, 1993; Lauridsen and Buenk, 1996).During the present study, copepoda species were found to be in higher densities during pre monsoon season and low densities during monsoon season (Table- 2). Copepods species are regarded as pollution sensitive zooplankton as they disappear from polluted water (Verma *et al*., 1984.).Contrary to this observation is the findings that *Cyclops* sp. are pollution tolerant, found abundantly nutrient rich environment and thus can be considered as eutrophication indicators (Adholia and Vyas, 1992). However, in the present study, copepods were not found in high numbers along with frequent absence of *Cyclops* species.

Table-2: Seasonal variation of zooplankton density in Heggere tank, Kanale (O/l)

Sl. No.	Zooplankton	January, 2004 – December, 2004		
		Pre Monsoon	Monsoon	Post Monsoon
1.	Cladocera	349	328	322
2.	Copepoda	254	242	248
3.	Rotifera	214	206	215
4.	Protozoans	11	11	13

Thus, it can be concluded that, the waterbody showing low nutrient composition and free from pollution except agriculture runoff . With regard to their periodicity, they reached their peak of 267 O/l in the month of April and the minimum population density of 229 O/l in the month of September (Table -1).Seasonally, they were more during pre monsoon season *i.e.* 254 O/l and less during monsoon season with 242 O/l (Table-2).A total of eight species of copepods were found *i.e. Heliodiaptomus vidus, Heliodiaptomus* sp, *Mesocyclops hyalinus, Mesocyclops leuckarti, Naupliar larve, Neodiaptomus stregilipes, Paracyclops fimbriatus and Tropocyclops prasinus* (Table-4).

Table-3: Occurrence of Cladocera in Heggere tank

S.No.	Organisms	Heggere
1.	*Alona pulchella*	+
2.	*Daphnia carinata*	+
3.	*Diaphanosoma sarsi*	+
4.	*Macrothrix goeldi*	+
5.	*Macrothrix laticornis*	+
6.	*Moina carinata*	+

Rotifers are the smallest animals and occur worldwide in primarily freshwater habitats. They are important in freshwater ecosystem as they occur in all biotypes. About 95% of the rotifers are encountered in fresh waters, while 5% are from brackish or marine waters and most are free living. Like the other zooplankton, rotifers also form a link in the aquatic food chain. They have a rapid turnover and high metabolic rates and feed on detritus. These organisms serve as bioindicators to depict water quality and are extensively cultured for use as fish feed.

Table-4: Occurrence of Copepoda in Heggere tank

Sl. No.	Organisms	Heggere
1	*Heliodiaptomus vidus*	+
2	*Heliodiaptomus sp*	-
3	*Mesocyclops hyalinus*	+
4	*Mesocyclops leuckarti*	-
5	*Naupliar larve*	+
6	*Neodiaptomus stregilipes*	+
7	*Paracyclops fimbriatus*	-
8	*Tropocyclops prasinus*	-

Table-5: Occurrence of Rotifers in Heggere tank

Sl. No.	Organisms	Heggere
1.	*Brachionus calyciflorus*	+
2.	*Brachionus caudatus*	+
3.	*Brachionus falcatus*	+
4.	*Rotatoria neptunia*	+

Table-6: Occurrence of Protozoan in Heggere tank

Sl. No.	Organisms	Heggere
1.	*Difflugia* sp.	+
2.	*Vorticella* sp.	+

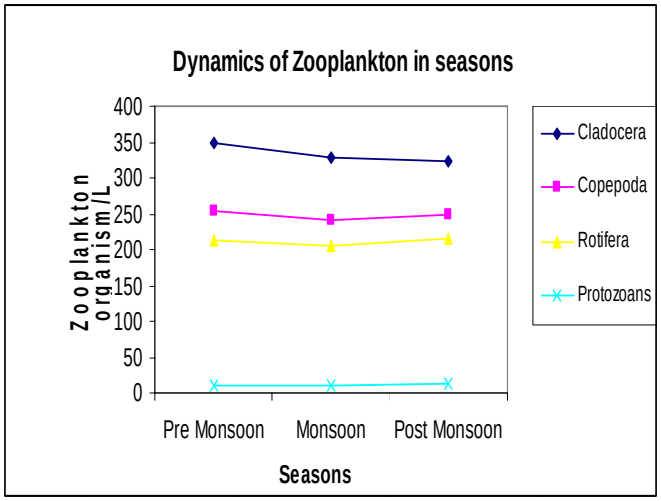
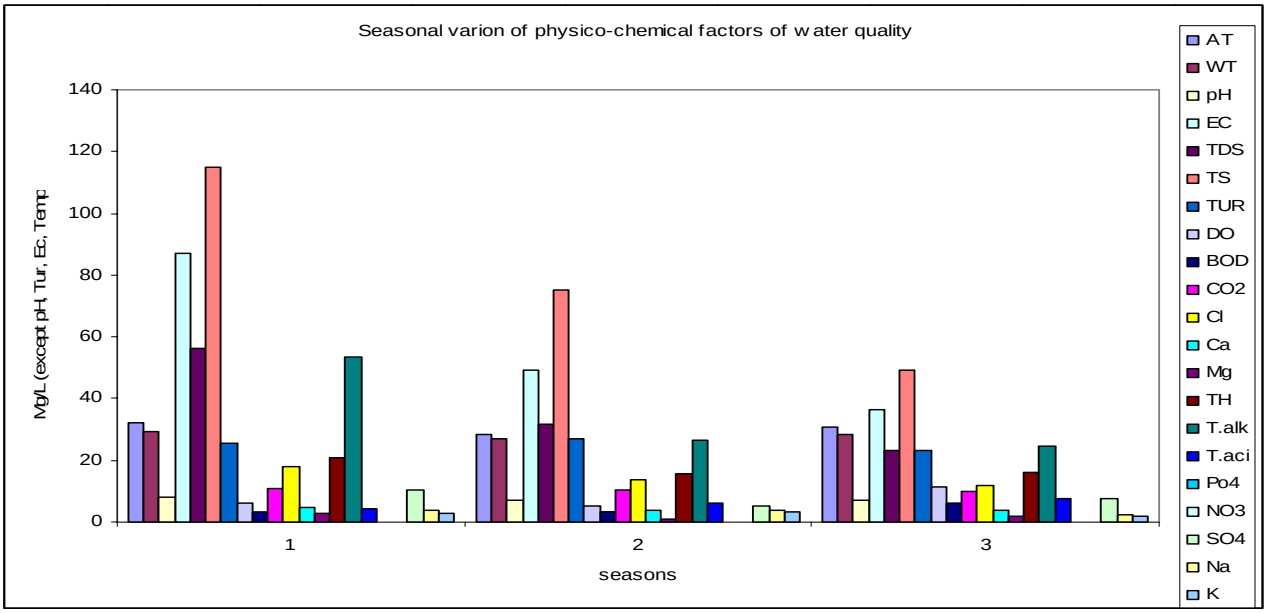


Table-7: Seasonal variation in physico-chemical parameters of Heggere tank, Kanale

S. No	Parameters	Pre-monsoon	Monsoon	Post-monsoon
1	Atmospheric temperature	32.37	28.37	30.87
2	Water temperature	29.12	27.12	28.37
3	pH	7.87	7.12	7.25
4	Electrical conductivity	87.00	49.25	36.25
5	Total dissolved solids	56.35	31.52	23.17
6	Total solids	115.12	75.4	49.10
7	Turbidity	25.65	26.97	23.25
8	Dissolved oxygen	6.14	5.13	11.20
9	Biological oxygen demand	3.15	3.46	5.94
10	Free carbon dioxide	11.00	10.45	9.9
11	Chloride	18.07	13.82	12.05
12	Calcium	4.62	3.84	3.57
13	Magnesium	2.64	0.93	1.74
14	Total hardness	20.81	15.66	16
15	Total alkalinity	53.25	26.5	24.5
16	Total acidity	4.37	6.25	7.5
17	Phosphate	0.15	0.085	0.05
18	Nitrate	0.14	0.19	0.16
19	Sulphate	10.49	5.22	7.79
20	Sodium	3.8	3.95	2.47
21	Potassium	2.85	3.22	1.95

Note: - All the parameters are in mg/l except pH, Electrical conductivity (µmhos/cm) and temperature °C.



Where, high densities were detected from post monsoon season. However, rotifers persisted during all the months. Similar results of bimodal pattern were reported by Pandey *et al* ., (1994) and Goswami (1997) during their limnological studies. Previous observation shows that lower temperature and availability of nutrients favour the rotifers population. Whereas, in the present study, temperature ranges from 24 ºC to 30 ºC and availability of nutrient is also very less. Hence, our observations are in agreement with above researchers.

The temperature, turbidity, transparency, dissolved oxygen were important factors controlling diversity and density of rotifers. In the present study, low rotifers density was found during monsoon season this may be due to unavailability of nutrients. A total of four species *i.e. Brachionus calyciflorus, Brachionus caudatus, Brachionus falcatus* and *Rotatoria neptunia* were found during the study period (Table-5). A number of studies have shown macrophytes to provide protection from planktivorous fish as well as food on decaying (Junk, 1977). Thus in general, it was observed that water bodies rich in macrophyte growth are rich in rotifer fauna (Narayana, 1994). Similar, observations are noticed in the present study.

Protozoan showed minimum population density in the present study. A total of two species *i.e. Difflugia* sp. and *Vorticella* sp. were recorded during the study period in the waterbody. Zooplankton species were fluctuated seasonally and no single species showed dominant throughout the study period.

Conclusion

The present investigation of physio-chemical parameters and zooplankton population indicates that the tank waterbody contains lower nutrients. The zooplankton communities were more during postmonsoon and premonsoon, as no dilution takes place in the waterbody during that seasons which automatically increases nutrients through anthropogenic and some climatic process.

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Analysis of solid waste generation in hospitals of Kathua Town (J&K), India

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Abstract

The present paper deals with the seasonal variations in the generation of solid waste in hospitals of Kathua Town. The dangerous waste generated by hospitals has become a serious hazard which threatens public life, so variation in the qualitative and quantitative composition of waste was worked out seasonally. The study also includes the observations on the separation of recyclable solid waste at source so as to evaluate net solid waste generation per day that needs disposal. In the last some recommendations are given in this paper.

Keywords: *Infectious waste, Radioactive waste, Biodegradable waste, Non biodegradable waste, Recyclable waste, Biomedical waste*

Introduction

Hospital waste is broadly defined as any solid or liquid waste that is generated during diagnosis, treatment or immunization of human beings or animals or in research activities pertaining there to, or in the production or testing of biological samples or material. In India, the waste generated during the process of patient care is also referred to as biomedical waste. The discharge of hundreds of tones everyday of such an unregulated and untreated toxic waste into the environment creates an imbalance in the composition of the environment. This highly infectious waste can create serious pollution problems and may prove to be a source of varying type of health hazards. The waste generated by the hospitals includes wide variety of hazardous substances like solvents, chemotherapy waste, anesthesia gases, radioactive waste, intravenous drips, used bandages, cotton plasters, stools and urine collection bags, nasal gastric tubes, syringes, needles, scalpels, blades, rubber catheters, suction catheters, urinary catheters, gloves *etc*. Careless dumping of infectious waste like living or non living pathogens, human body tissues, solid cotton, dressing linen, blood soaked bandages, laboratory culture stocks, waste of experimental animals used in research, waste generated by veterinary hospitals, colleges, animal houses and livestock and other medical waste outside hospitals and nursing homes were checked under a legal provision. Many times used syringes, blood bags, gloves and other items which should ideally be disposed off after they are chemically disinfected are sold back into the market. Even used cotton after being washed and fluffed is stuffed in quilts and sold. Rag pickers gather unseggregated medical waste and sell it in the market.Reports pertaining to recycling of disposable medical items have been published from different parts of the country from time to time. Human lives are put at risk, when it involves recycling of dangerous and toxic medical waste products (Agarwal, 2000). Many hospitals and nursing homes dump untreated infectious medical waste in municipal dumping grounds. Infected medical waste can cause fatal diseases like AIDS, meningitis, hepatitis B and C, liver failure, tuberculosis and brain fever. Most of them even throw them out on the road sides. Heavy metals like mercury and cadmium are present in significant quantities in these hospital (medical) wastes which are extremely toxic even in small quantities. In view of its hazardous nature and serious environment threats, biomedical waste requires special handling, especially in view of the available new technologies for its disposal in a scientific

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manner. Hospital waste, which constitutes relatively a small fraction of urban municipal waste (1.5 to 2.0 percent), is proving to be a big menace. Due to increase in medical facilities in recent years, the medical waste also increases many folds and the subject is of great concern for the Public and the Government.

Study area

The sites of study area were hospitals of Kathua Town. Geographically Kathua district lies in South-East of the state. It is situated 32.17' to 32.55' North latitude and 75.32' to 75.76' East longitude, spread over an area of 2651 sq. kms constituting 1.9 % of the total area of the state. Town has population of over 40000 as per 2001 estimates.

Kathua, the site of present study is about 85 kms form Jammu city on Jammu-Pathankot National Highway. The two main rivers of the district are Ravi and Ujh which are two major contributors to the prestigious Ravi-Tawi Irrigation –complex. The significance of the study area, Kathua is important because it is situated near Lakhanpur, which is the Gateway of India to Jammu Kashmir state.

Materials and Method

The study was conducted in the hospitals of Kathua Town during two years *i.e.* 2007-2008. The average solid waste generation per capita per day along with standard deviation was calculated by taking 08 samples from the study area per three months period for two years. During each sampling the total solid waste generated by the patient and his attendants during the period of 24 hours was collected in a polythene bag of 10 kg capacity and weighed. The qualitative and quantitative composition of biodegradable and non biodegradable waste per capita per day was calculated. The quantity of various recyclable/reused like plastic ware (glucose bottles, spirit bottles, H_2O_2 bottles *etc*.), glassware (savlon bottles, betadine bottles *etc.*) which were separated by sweepers to be sold to Itinerant Waste Buyers (IWB) or Small Enterprises Middle man (SEM) at weekly or monthly intervals were recorded to calculate average separation and net generation of solid waste. The per capita per day values of solid waste separation and net generation in hospitals during eight seasons of the study period were compiled to find the average per capita per day separation and net generation at hospitals. There were about 250 medical beds in Kathua town and finally per capita was multiplied with total number of medical beds.

Results and Discussion

The analysis of solid waste generation in two years revealed that average solid waste generation/capita/day decreased from January-March to April-June, increased from April-June to July-September and it again decreased from July-September to October-December, during first year as well as in second year of study period. The biodegradable solid waste generation also exhibited the same trend, but non-biodegradable wastes did not followed a specific trend during course of study. The average qualitative composition of various biodegradable and non-biodegradable solid wastes in different seasons of two years study period has been tabulated in Table-1a- 1d.

The data generated over two years study revealed that average/capita/day solid waste generation ranged from 0.510-5.919 kg with an average value of 2.217±0.344 kg. The average separation of recyclable wastes to be sold to waste buyers at source ranged from 0.022-0.350 kg with an average value of 0.087±0.031 kg/capita/day. The net average/capita/day solid waste generation in the study area was observed to be ranged from 0.415-5.773 kg with an average value of 2.130±0.342 kg/capita'day (Table-2). As per record of BMO office Kathua, there are 250 medical beds. Taking this value same at present, the total solid waste generation/day in the hospitals in the Kathua Town comes to be 0.55 tones/day, of which 0.02 tones/day is separated at source to be sold to waste buyers by hospital's authority themselves without the involvement of municipality and net solid waste generation/day in the study area was calculated to be 0.53 tones/day (Table-2), of this 0.32 tones/day was the biodegradable, 0.16 tones/day non-biodegradable and 0.05 tones/day inert material. The generation of average potential recyclable solid waste/capita/day was to be 0.555 kg (0.14 tones/day *i.e.*, 25.54 % of gross average value of 0.55 tones/day). This included average recyclable non-biodegradable solid waste/capita/day was to be 0.555 kg (0.14 tones/day *i.e*. 25.54 % of 0.55 tones/day) which included plastic ware 0.380±0.157 kg, glassware 0.175±0.048 kg (Table-3). Presently on an average 0.040±0.037

kg/capita/day of plasticware and 0.047±0.017 kg/capita/day of glass ware out of 0.380±0.157 kg/capita/day of plastic ware and out of 0.175±0.048 kg/capita/day glassware respectively were actually collected at source to be sold to waste buyers. Thus the recyclable waste was observed to be separated from the generated potential recyclable waste on a total 3.64 % *i.e*. 0.02 tones/day @ 0.087 kg/capita/day was observed to be separated at source. Net recyclable material /day was observed to be 84.14 % *i.e*. 0.12 tones @ 0.467 kg/capita/day (Table-3). Banerjee and Bagchi, (1999), Basu,(1998) and Goudar and Subramanyam, (1996) also suggested various methods of solid waste management.

Thus the awareness of hospital's authorities regarding the sale of various reuses, recyclable waste can decrease the waste load to maximum of 25.54 % (0.14 tones/day @ 0.555 kg/capita/day) without the involvement of the municipality services. In the last but not the least I would like to say that we all must follow the triple 'R' policy *i.e*., REUSE, REDUCE and RECYCLE policy in order to overcome this problem, as this problem will become more acute with better medical facilities in coming years.

Table-1a: Seasonal variations in average solid waste generation (kg/capita/day) in Hospitals of Kathua Town

Wastes	(January-March, 2007) Average solid waste (kg/capita/day)			(April-June, 2007) Average solid waste (kg/capita/day)		
	Gross solid waste (G)	Recyclable solid waste (r)	Net solid waste (G-r)	Gross solid waste (G)	Recyclable solid waste (r)	Net solid waste (G-r)
Biodegradable	1.214±0.599 [0.7912.618] (48.46)	-	1.214±0.599 [0.791-2.618] (48.46)	0.861±0.293 [0.220-1.454] (43.55)	-	0.861±0.293 [0.220-1.454] (43.55)
Paper	0.098 (3.91)	-	0.098 (3.91)	0.144 (7.30)	-	0.144 (7.30)
Cloth ware	0.491 (19.61)	-	0.491 (19.61)	0.419 (21.18)	-	0.419 (21.18)
Cotton	0.113 (4.53)	-	0.113 (4.53)	0.104 (5.25)	-	0.104 (5.25)
Wood	-	-	-	0.022 (1.10)	-	0.022 (1.10)
Food/garbage	0.511 (20.41)	-	0.511 (20.41)	0.172 (8.72)	-	0.172 (8.72)
Non-biodegradable	0.975±0.560 [0.461-1.400] (38.92)	0.070±0.06 [0.051-.099] (2.79)	0.905±0.496 [0.410-1.300] (36.13)	0.880±0.410 [0.267-1.000] (44.51)	0.053±0.041 [0.022-0.096] (2.68)	0.827±0.386 [0.246-0.904] (41.83)
Plastic ware	0.644 (25.71)	0.040 (1.58)	0.604 (24.13)	0.515 (26.06)	0.017 (0.85)	0.498 (25.21)
Metallic ware	0.108 (4.32)		0.108 (4.32)	0.172 (8.71)	-	0.172 (8.71)
Glass ware	0.168 (6.70)	0.030 (1.21)	0.138 (5.49)	0.147 (7.43)	0.036 (1.83)	0.111 (5.60)
Rubber	0.031 (1.23)	-	0.031 (1.23)	0.032 (1.61)	-	0.032 (1.61)
Egg shell/bones	0.024 (0.96)	-	0.024 (0.96)	0.014 (0.70)	-	0.014 (0.70)
Inert material	0.316±0.209 [0.110-0.991] (12.61)	-	0.316±0.209 [0.110-0.991] (12.61)	0.236±0.085 [0.143-0.419] (11.94)	-	0.236±0.085 [0.143-0.419] (11.94)
Total	2.505±1.100 [1.415-4.761] (100)	0.070±0.064 [0.051-0.099] (2.79)	2.435±1.090 [1.091-4.714] (97.21)	1.977±0.621 [0.630-2.451] (100)	0.053±0.041 [0.022-0.096] (2.68)	1.924±0.615 [0.601-2.376] (97.32)

Table-1b: Seasonal variations in average solid waste generation (kg/capita/day) in Hospitals of Kathua Town

Wastes	(July-September, 2007) Average solid waste (kg/capita/day)			(October-December, 2007) Average solid waste (kg/capita/day)		
	Gross solid waste (G)	Recyclable solid waste (r)	Net solid waste (G-r)	Gross solid waste (G)	Recyclable solid waste (r)	Net solid waste (G-r)
Biodegradable	1.544±0.476 [0.281-3.180] (63.80)	-	1.544±0.476 [0.281-3.180] (63.80)	1.433±0.516 [0.619-3.390] (60.57)	-	1.433±0.516 [0.619-3.390] (60.57)
Paper	0.220 (9.10)	-	0.220 (9.10)	0.159 (6.73)	-	0.159 (6.73)
Cloth ware	0.532 (22.00)	-	0.532 (22.00)	0.594 (25.10)	-	0.594 (25.10)
Cotton	0.151 (6.26)	-	0.151 (6.26)	0.134 (5.65)	-	0.134 (5.65)
Wood	0.010 (0.40)	-	0.010 (0.40)	-	-	-
Food/garbage	0.630 (26.04)	-	0.630 (26.04)	0.546 (23.09)	-	0.546 (23.09)
Non-biodegradable	0.716±0.259 [0.272-1.110] (29.59)	0.089±0.030 [0.041-0.146] (3.68)	0.627±0.233 [0.255-0.970] (25.91)	0.740±0.284 [0.215-1.319] (31.28)	0.150±0.044 [0.080-0.350] (6.34)	0.590±0.247 [0.143-0.999] (24.94)
Plastic ware	0.314 (12.97)	0.039 (1.62)	0.275 (11.35)	0.291 (12.32)	0.123 (5.21)	0.168 (7.11)
Metallic ware	0.140 (5.79)	-	0.140 (5.79)	0.201 (8.50)	-	0.201 (8.50)
Glass ware	0.210 (8.66)	0.050 (2.06)	0.160 (6.60)	0.188 (7.95)	0.027 (1.13)	0.161 (6.82)
Rubber	0.032 (1.34)	-	0.032 (1.34)	0.050 (2.10)	-	0.050 (2.10)
Egg shell/bones	0.020 (0.83)	-	0.020 (0.83)	0.010 (0.41)	-	0.010 (0.41)
Inert material	0.160±0.053 [0.044-0.219] (6.61)	-	0.160±0.053 [0.044-0.219] (6.61)	0.193±0.074 [0.056-0.283] (8.16)	-	0.193±0.074 [0.056-0.283] (8.16)
Total	**2.420±0.711 [0.610-4.440] (100)**	**0.089±0.030 [0.041-0.146] (3.68)**	**2.331±0.681 [0.574-4.363] (96.32)**	**2.366±0.708 [0.510-3.910] (100)**	**0.150±0.044 [0.080-0.350] (6.34)**	**2.216±0.669 [0.415-3.590] (93.66)**

Table-1c: Seasonal variations in average solid waste generation (kg/capita/day) in Hospitals of Kathua Town

Wastes	(January-March, 2008) Average solid waste (kg/capita/day)			(April-June, 2008) Average solid waste (kg/capita/day)		
	Gross solid waste (G)	**Recyclable solid waste (r)**	**Net solid waste (G-r)**	**Gross solid waste (G)**	**Recyclable solid waste (r)**	**Net solid waste (G-r)**
Biodegradable	1.933±0.613 [0.860-4.490] (69.81)	-	1.933±0.613 [0.860-4.490] (69.81)	0.965±0.277 [0.342-1.671] (53.31)	-	0.965±0.277 [0.342-1.671] (53.31)
Paper	0.317 (11.46)	-	0.317 (11.46)	0.129 (7.10)	-	0.129 (7.10)
Cloth ware	0.773 (27.91)	-	0.773 (27.91)	0.384 (21.19)	-	0.384 (21.19)
Cotton	0.210 (7.60)	-	0.210 (7.60)	0.094 (5.19)	-	0.094 (5.19)
Wood	0.058 (2.10)	-	0.058 (2.10)	0.040 (2.22)	-	0.040 (2.22)
Food/garbage	0.574 (20.74)		0.574 (20.74)	0.319 (17.61)	-	0.319 (17.61)
Non-biodegradable	0.620±0.237 [0.285-1.415] (22.39)	0.073±0.032 [0.036-0.155] (2.64)	0.547±0.212 [0.233-1.269] (19.75)	0.590±0.117 [0.159-0.826] (32.60)	0.064±0.036 [0.022-0.109] (3.54)	0.526±0.090 [0.131-0.723] (29.06)
Plastic ware	0.122 (4.40)	-	0.122 (4.40)	0.328 (18.10)	0.026 (1.42)	0.302 (16.68)
Metallic ware	0.150 (5.40)	-	0.150 (5.40)	0.089 (4.90)	-	0.089 (4.90)
Glass ware	0.274 (9.90)	0.073 (2.64)	0.201 (7.26)	0.140 (7.76)	0.038 (2.12)	0.102 (5.64)
Rubber	0.051 (1.83)	-	0.051 (1.83)	0.026 (1.42)	-	0.026 (1.42)
Egg shell/bones	0.024 (0.86)	-	0.024 (0.86)	0.008 (0.42)	-	0.008 (0.42)
Inert material	0.216±0.064 [0.096-0.386] (7.80)	-	0.216±0.064 [0.096-0.386] (7.80)	0.255±0.071 [0.060-0.622] (14.09)	-	0.255±0.071 [0.060-0.622] (14.09)
Total	**2.769±0.776 [1.644-5.919] (100)**	**0.073±0.032 [0.036-0.155] (2.64)**	**2.696±0.759 [1.613-5.773] (97.36)**	**1.810±0.381 [0.599-3.132] (100)**	**0.064±0.036 [0.022-0.109] (3.54)**	**1.746±0.359 [0.561-3.054] (96.46)**

Table-1d: Seasonal variations in average solid waste generation (kg/capita/day) in Hospitals of Kathua Town

Wastes	(July-September, 2008) Average solid waste (kg/capita/day)			(October-December, 2008) Average solid waste (kg/capita/day)		
	Gross solid waste (G)	**Recyclable solid waste (r)**	**Net solid waste (G-r)**	**Gross solid waste (G)**	**Recyclable solid waste (r)**	**Net solid waste (G-r)**
Biodegradable	1.109±0.339 [0.519-2.010] (55.39)	-	1.109±0.339 [0.519-2.010] (55.39)	1.055±0.360 [0.460-2.218] (55.85)	-	1.055±0.360 [0.460-2.218] (55.85)
Paper	0.138 (6.90)	-	0.138 (6.90)	0.153 (8.11)	-	0.153 (8.11)
Cloth ware	0.325 (16.24)	-	0.325 (16.24)	0.330 (17.46)	-	0.330 (17.46)
Cotton	0.090 (4.51)	-	0.090 (4.51)	0.096 (5.06)	-	0.096 (5.06)
Wood	-	-	-	-	-	-
Food/garbage	0.555 (27.74)	-	0.555 (27.74)	0.476 (25.22)	-	0.476 (25.22)
Non-biodegradable	0.718±0.349 [0.215-1.610] (35.86)	0.110±0.083 [0.035-0.240] (5.49)	0.608±0.278 [0.173-1.393] (30.37)	0.673±0.241 [0.219-0.918] (35.63)	0.090±0.031 [0.029-0.150] (4.76)	0.583±0.233 [0.180-0.768] (30.87)
Plastic ware	0.440 (22.00)	0.048 (2.38)	0.392 (19.62)	0.383 (20.30)	0.030 (1.57)	0.353 (18.73)
Metallic ware	0.110 (5.50)	-	0.110 (5.50)	0.111 (5.90)	-	0.111 (5.90)
Glass ware	0.139 (6.93)	0.062 (3.11)	0.077 (3.82)	0.135 (7.16)	0.060 (3.19)	0.075 (3.97)
Rubber	0.022 (1.11)	-	0.022 (1.11)	0.024 (1.25)	-	0.024 (1.25)
Egg shell/bones	0.006 (0.32)	-	0.006 (0.32)	0.019 (1.42)	-	0.019 (1.42)
Inert material	0.175±0.11 [0.043-0.223] (8.74)	-	0.175±0.110 [0.043-0.223] (8.74)	0.161±0.096 [0.046-0.190] (8.52)	-	0.161±0.096 [0.046-0.190] (8.52)
Total	**2.002±0.798 [0.796-3.919] (100)**	**0.110±0.083 [0.035-0.240] (5.49)**	**1.892±0.715 [0.761-3.679] (94.51)**	**1.889±0.641 [0.593-3.562] (100)**	**0.090±0.031 [0.029-0.150] (4.76)**	**1.799±0.623 [0.543-3.510] (95.24)**

Table – 2: Average solid waste generation and separation at Kathua Town

Average solid waste	Biodegradable (B)	Non-biodegradable (NB)	Inert Material (IM)	Total = (B+NB+IM)
Gross average/capita/day at source (G) kg/day	1.264±0.354 (0.220-4.490)	0.739±0.129 (0.159-1.610)	0.214±0.054 (0.043-0.991)	2.217±0.344 (0.510-5.919)
Average/capita/day separated at source (r) kg/day	-	0.087±0.031 (0.022-0.350)	-	0.087±0.031 (0.022-0.350)
Net average/capita/day generated (G-r) kg/day	1.264±0.354 (0.220-4.490)	0.652±0.138 (0.131-1.393)	0.214±0.054 (0.043-0.991)	2.130±0.342 (0.415-5.773)
Gross average/day at source G x 250* (tones/day)	0.32	0.18	0.05	0.55
Average/day separated at source r x 250* (tones/day)	-	0.02	-	0.02
Net average/day generated (G-r) x 250* (tones/day)	0.32	0.16	0.05	0.53

*** 250 medical beds as per record of BMO office Kathua**

Table – 3: Average generation and separation of recyclable solid waste at source in Kathua Town

Average solid waste (kg/capita/day)	Non-Biodegradable (NB) kg.	Non-Biodegradable (NB) kg.	Total Non-Biodegradable (NB) kg/capita/day
	Plastic ware	Metallic ware	Total (kg) NB= PI+MW
Average/capita/day generated at source (R)	0.380± 0.157 (17.73)	0.175±0.048 (7.81)	0.555 (25.54)
Average/capita/day separated at source (r)	0.040±0.037 (10.52)	0.047±0.017 (26.85)	0.087 (15.67)
Net average/capita/day generated at source (R-r)	0.339±0.160 (89.21)	0.128±0.045 (73.14)	0.467 (84.14)

Figures in ( ) is showing percentage by weight (Table-1a-1d) **Figures in [ ] showing ranged values (Table-1a-1d)**

* Metallic ware – tin boxes, scrap.

* Plastic ware – plastic bottles, buckets, scraps, plastic woven sack.

Avg. Recyclable Non-Biodegradable solid waste (kg/day) generated at source = 0.555 × 250 = 0.14 tones/day.
Avg. Total Recyclable solid waste (kg/day) at source = 0.555 × 250 = 0.14 tones/day.
Avg. Recyclable Non-biodegradable solid waste (kg /day) separated at source = 0.087 × 250 = 0.02 tones/day.
Avg. Total Recyclable solid waste (kg /day) separated at source = 0.087 × 250 = 0.02 tones /day.
Net avg. Recyclable Non-biodegradable solid waste (kg /day) = 0.467 × 250 = 0.11 tones /day.
Net avg. Total Recyclable solid waste (kg /day) = 0.467 × 250 = 0.011 tones/day.

Recommendations

Ideally, medical wastes must be segregated category wise and rendered harmless through physical separation and disinfection and disposed off in secured landfills or incinerated.

The authority must require those persons deployed for handling medical wastes must have the basic knowledge and technical skills for this specialized task.

A well directed public awareness campaign.

A strong monitoring system, which determines accountability of the polluter and of the handler of the hazardous wastes.

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Resource utilization and anthropogenic pressure in a part of Submontane forest of outer Himalaya, Uttarakhand

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Abstract

Forests and wild life are essential for ecological balance of an area. Forests are important components of our environment and economy. Present study was conducted in a part of submontane forest of Kumaun, Outer Himalaya adjacent to Kashipur, at (29º 14-43.6)–(29º 19-50.5) N latitude and (79º 03-22.6)–(79º 04-23.2) E longitude at an elevation of 253.4–265.5 meter above the sea level, within the districts of Nainital and Udham Singh Nagar to check the various resources and effect of anthropogenic pressure in forest ecosystem.

Keywords: *Submontane forest, Anthropogenic pressure, Ecosystem, Chopping, Lopping, Grazing, Forestfire*

Introduction

Forests are an important renewable natural resource dominated by trees. Forests are linked with our culture and civilization. The chief product that forests supply is wood, fuel, raw material for paper industries, timber for furniture *etc.* however, canes, gums, resins, dyes, tannins, lac, fibers, flocs, medicines, katha *etc.* are minor products supplied by forests. Besides, this they are the major factor of environmental concern by providing protection to wild life, help in balancing the gaseous cycles of atmosphere, tend to increase local rainfall and water holding capacity of soil, maintain the soil fertility and regulate the earth's temperature regimes, check soil erosion and landslides.

The forest cover of India is 63.7 million hectare and it is one of the richest areas for biodiversity in the world (Anon, 1999). According to the latest report of the Forest Survey of India, forest covers 19.39 % of India's geographical area. Only 11.48 % are well stocked dense forests (canopy density > 40%) (Bahuguna and Upadhyay, 2002). The Uttarakhand state has 64.79 % of its total geographical area declared as forest area with forest against all India state only 45.65%, forest area is legally under forest department. The per capita forest area of Uttarakhand is 0.41 hectare (Verma, 2009). Various informations are available on impact of anthropogenic pressure on Himalayan forests given by Shah (1982), Pandey and Singh (1984), Khoshoo (1987), Singh *et al.* (1988), Singh (1989), Yadav *et al.* (1993), Sundriyal and Sharma (1996), Singh *et al.* (1997), Metz (1997), Samant *et al.* (1997), Silori (2001) and Chhetri *et al.* (2006).

The Shivalik foothills are one of the world's most spectacular landscapes, encompassing the tall grasslands and the *Shorea robusta* (Sal) forests. Construction of roads, urban expansion, settled agriculture, industrialization and deforestation cause massive forest destruction in this area. There are also many abiotic factors like forest fires, weather *etc.* however many biotic factors like human influence in terms of competition between species, insects, diseases, grazing, chopping and lopping of trees, alcohol formation that can cause changes in forests over time. Thus, this study highlights the effect of biotic and abiotic factors in a submontane forest of outer Himalaya of Uttarakhand.

Geographical Location

This study area is located in submontane forest of Kumaun, outer Himalaya adjacent to Kashipur, at (29^0 14-43.6)–(29^0 19-50.5) N latitude and (79^0 03-22.6)–(79^0 04-23.2) E longitude at an elevation of 253.4–265.5 msl, within the districts of Nainital and Udham Singh Nagar and occupies the middle

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reaches of the River Kosi and Dabka. The area of study site was 674.61 ha (Source: Office of Tarai West Forest Division, Kumaun, Ramnagar, (Uttarakhand). The study was conducted from April, 2007 to March, 2008.

Materials and Method

There was no standard method available for studying anthropogenic pressure and resource utilization in forests. In present study, anthropogenic pressure and resource utilization sites was studied by frequent field visits, from experience of personals of forest department and the local natives.

Results and Discussion

Biotic factors play an important role in resource-limited habitats due to plant competition (Chapin and Shaver, 1985; Tilman, 1988). However, abiotic factors become important in the nutrient poor habitats (Campbell *et al.*, 1991; Grime, 1977 and Keddy, 1989). Forests are important components of our environment and economy. Various types of resources are present in forests of present study area. So due to utilization of these resources, forest are directly or indirectly affected.

(A) Timber Resources

Various species of trees are present in forest area. In Tarai and Bhabhar, wood have high commercial uses. These include *Tectona grandis* L.f., *Shorea robusta*, *Dalbergia sisso* Roxb., *Eucalyptus hybrid* L. Herit., *Haplophagma adenophyllum* Wall., *Cedrela toona* Roxb. and *Adina cordifolia* (Roxb.) Benth & Hook, f. for timber. Collection and utilization of timbers from these forests is prohibited. However, villagers surrounding to forest areas collect timber by lopping (Fig.3).

Fig. 1: Preparation of alcohol in forest

Fig. 2: Utilization of wood in alcohol formation

Fig. 3: Collection of fuel wood from forest

Fig. 4: Collection of vegetation after chopping in forest

Liquor preparation is a common practice in the outlying villages for which extra fuel wood is consumed, but it is prohibited in forests (Fig.1&2). Due to this activity they utilize wood and forest land and waste material after alcohol preparation contaminate water of river site in forests. The nearby villages had extra consumption of fuel wood to protect their crops during nights from animals of nearby forest area.

(B) Chopping

Local native and Van Gujjars collect fleshy leaves and small twigs for forage to their domestic animals by chopping (Fig.4). They cut down small twigs and some time whole plants for firewood. Forests are good source of food, fodder and forage. Local natives surrounding to forests collect forage for their domestic animals from forests. For this purpose, they cut down small leafy twigs of trees having up to 22cm diameter. This process is called chopping. *Acacia catechu* Willd., *Bauhinia malabarica* Roxb., *Broussonetia papyrifera* Vent., *Ficus racemosa* L., *Ficus religiosa* L., *Terminalia bellerica* Roxb. and *Trewia nudiflora* L. are mostly affected by chopping. *Dalbergia sisso* Roxb., *Eucalyptus hybrid* L.Herit., *Eugenia jambolana* Lam., *Mallotus philippenensis* Muell. Arg. and *Tectona grandis* L.f. were not affected by chopping, because cattle do not like leaves of these plants.

(C) Grazing

In Tarai and Bhabhar regions of Kumaun Uttarakhand local native and Van Gujjars directly use forest as grazing land (Fig.5).

Fig. 5: Movement of buffaloes in forest for grazing

Fig. 6: Extraction of minerals from River Kosi

(D) Minerals, Stones and Sand

River Kosi flows in the center of the forest. It provides huge amount of minerals, stones and sand (Fig. 6). Minerals, stones and sand are good source of money for forest department.

(E) Medicinal Plants

There are various medicinal plants present in the form of trees, shrubs and trees at forest sites, which have high medicinal value for human being and were used by local natives for treatment and remedy of disease. Collection, utilization and trading of medicinal plants from these forests are prohibited. *Acacia catechu* Willd., *Biophytum sensitivum* Zucc., *Centella asiatica* (L.) Urb., *Holarrhena antidysenterica* Wall., *Piper nepalense* Miq. (E.) and *Zingiber capitatum* Roxb. are mostly used medicinally.

(F) Other Resources

In rainy season *Saccharum spontaneum*, L. (Kans) mostly grows in forest area. Van Gujjars and local villagers use this plant in making of cottage roof and domestic animals use it as forage. Wax and honey are also good resources of forest. Honey contains carbohydrate, minerals and vitamins. Local villagers collect them illegally from forest, utilize it and sell at very high rates.

In winter and early spring, fruits of *Zizyphus xylopyra* Willd. get matured, local villagers collect it and sell it on their own prices. In rainy and winter seasons *Themeda arundinacea* (Roxb.) Ridley (Sarkanda grass) grows in forest. This grass is good raw material for paper industry. So it can be good economic source for forest department if they make arrangement to sell *Themeda arundinacea* (Roxb.)

Various biotic and abiotic factors are also harmful for forests, because they reduce forest vegetation silently. These are fungi (*Fomis badius* - *Acacia catechu* Willd., *Gonoderma* sp. - *Dalbergia sissoo* Roxb., *Ciliandro caladium* – *Eucalyptus hybrid* L.Herit.), shrubs (*Lantana camara* L. and *Lantana indica* Roxb.), climbers (*Bauhinia vahlii* W. and A., *Ichinocarpus fructescens* R.Br. and *Tinospora cordifolia* Miers.), floods and forest fire.

Forest fire is a major problem during spring and summer season in forests (Fig.8). Fire affects flora and fauna of forest directly and indirectly. Removal of ground vegetation during forest fire is major problem. Fire plays a key role in ecosystem process and can change the vegetation composition (Timoney and Wein, 1981). Fire also stimulates flowering, fruiting and vegetative reproduction of many herbaceous species as the overstorey is reduced (Pyne, 1991). Fire also temporarily reduces competition for moisture, nutrients and light thereby selectively favoring some species. In present study, forests of Tarai and Bhabhar also suffer from forest fire during late spring and summer season.

Fig. 7: Flood cause forest land degradation

Fig. 8: Forest fire

Based on above investigation it is concluded that regular monitoring of forest is necessary and excess influence of local villagers in forests should be prohibited, because their excess involvement in forest caused massive forest destructions in terms of grazing, chopping, lopping, collection of wood for fuel and other resources.

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A study on planktonic components of River Yamuna

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Abstract

The present study deals with the plankton component in Yamuna river basin. During the study period (2006-07) total number of plankton comprises of zooplankton, Bacillariophyceae, Chlorophycea, Desmideaceae and Myxophyceae and range from 90 unit/liter (August) to 531 unit/liter (February) for Kuthnor, 96 unit/liter (August) to 557 unit/liter (February) for Naugaon and 105 unit/liter (August) to 569 unit/liter (February) for Haripur. Bacillariophyceae was found as dominating group followed by Chlorophyceae, Desmideaceae and Myxophyceae.

Keywords: *Plankton*, *River*, *Chlorophyceae*, *Desmideaceae*, *Myxophyceae*

Introduction

In India, few detailed studies are available on the ecobiological characteristics of main stream of Rivers Bhagirathi and Ganga for its total length, mainly on account of Ganga Action Plan. On the other hand no such comprehensive systematic study is available on River Yamuna mainly in its Himalayan regime. Besides the tributaries of these two major rivers *viz.*, the Bhagirathi – Ganga and the Yamuna within their Garhwal Himalayan catchment areas. Except for a project based study by Joshi and Singh (1997) for river Ganga and its two minor tributaries in between Dev prayag and Rishikesh. The present study is mainly centered to assess the plankton components of River Yamuna. Plankton are the heterogeneous assemblage of minute organisms which occur in the natural water and float by the wave action and movement of water. The quantitative and qualitative changes in the planktonic constituents of the river system under this study was observed with special attention of their contribution to evaluate in the form of primary and secondary productivity of the system.

Study site

The Yamuna river originates from the Yamunotri glacier near Bander Punch district of Uttarkashi in Uttarakhand state. Three sites were selected along the Yamuna stretch to monitor the plankton components within Uttarakhand Himalaya Site-I (Yamuna River near Barkot at Kuthnor).Site - II (Yamuna River at Naugaon) and Site–III (Haripur near Kalsi)

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Materials and Method

Planktonic samples were collected in 2006 and 2007 with the help of plankton net. The samples were collected by filtering a known volume of water through the plankton net. For preservation of plankton 4% formalin was used (prepared from 40% formaldehyde solution). The sample was concentrated by sedimentation method, removing the supernatant by decanting and the desired final volume was obtained. For counting, 1ml of concentrated sample was taken and placed Sedgwick rafter counting cell following the Standard methods of APHA (1995).

Results and Discussion

The result of present study is shown in Table-1 & 2 and in Fig. 1 & 2. The plankton component comprises of zooplankton, Bacillariophyceae, Chlorophyceae, Desmideaceae and Myxophyceae. During the present study total number of plankton ranged from 90 unit/liter (August) to 531 unit/liter (February) for Kuthnor, 96 unit/liter (August) to 557 unit/liter (February) for Naugaon and 105 unit/liter (August) to 569 unit/liter (February) for Haripur. Whereas in second year of study the total number of plankton varied from 90 unit/liter (August) to 527 unit/liter (February) for Kuthnor,84unit/l (Sep.) to 547 unit/l (Feb.) for Naugaon and 92 unit/liter Sep) to 565 unit/l (Feb.) for Haripur.

Table-1: Monthly mean values of plankton component (unit /lit) of River Yamuna at three sites for year 2006

Month	Kuthnor (Site I)						Naugaon (Site II)						Haripur (Site III)					
	Zoo	Bac	Chl	Des	Myx	Total	Zoo	Bac	Chl	Des	Myx	Total	Zoo	Bac	Chl	Des	Myx	Total
Jan	5	399	83	33	0	**520**	8	404	90	35	0	**537**	9	410	90	41	0	**550**
Feb	11	415	89	13	3	**531**	15	422	95	20	5	**557**	17	429	93	23	7	**569**
Mar	22	357	67	29	7	**482**	25	342	72	35	9	**483**	29	341	75	31	11	**487**
Apr	25	147	44	21	9	**246**	25	148	45	25	10	**253**	28	149	49	29	10	**265**
May	31	118	39	15	9	**212**	30	120	40	16	14	**220**	31	125	45	17	14	**232**
June	27	32	33	17	15	**124**	25	36	38	18	17	**134**	27	44	40	17	17	**145**
July	34	14	21	11	13	**93**	35	15	25	12	18	**105**	33	17	39	15	19	**123**
Aug	31	15	24	9	11	**90**	32	16	26	11	11	**96**	33	17	30	13	12	**105**
Sep	18	29	39	12	9	**107**	20	30	40	12	12	**114**	21	31	41	19	15	**127**
Oct	17	51	41	34	0	**143**	16	55	46	35	3	**155**	17	57	40	41	2	**157**
Nov	15	114	69	37	0	**235**	14	115	75	38	0	**242**	15	115	83	43	0	**256**
Dec	3	239	101	33	0	**376**	5	250	105	40	0	**400**	6	251	111	45	0	**413**
Mean	**19.2**	**160.8**	**54.17**	**22.0**	**6.3**	**263.3**	**20.8**	**162.8**	**58.1**	**24.8**	**8.25**	**274.7**	**22.2**	**165.5**	**61.3**	**27.8**	**8.9**	**285.8**

Zoo = Zooplankton, **Bac** = Bacillariophyceae, **Chl** = Chlorophyceae, **Des** = Desmideaceae, **Myx** = Myxophyceae

Almost similar results were recorded for various other aquatic systems by Pahwa and Mehrotra (1966) in the Ganga River. Das and Upadhyaya (1979) observed maximum phytoplanktonic concentration during March and April in lakes of Kashmir and Nainital. Badola and Singh (1981) reported high values of plankton during January to March. Joshi *et al.* (1996) opined that plankton production was mainly influenced by temperature, while Bhatt *et al.* (1984) stated that temperature is lesser effective for the abundance of biotic population as observed in the Kosi river. In present study Bacillareophyceae was the dominating group followed by Chlorophyceae, Desmideaceae and Myxophyceae. Joshi and Singh (1997) reported higher planktonic population during winter months in Ganga at Hardwar and also observed Bacillareophyceae as the dominating group among phytoplankton.

Conclusion

The present study concludes that the Yamuna river ecosystem support a rich and colourful planktonic diversity as compared to some other riverine ecosystems of Garhwal Himalayas. Though quite few factors seems to play significant role in the build up of planktonic population. The hilly basin of the stream has shallow water in comparison to their counterparts of plain segment. From the present observation it can be concluded that Bacillareophyceae was the dominating group followed by Chlorophyceae, Desmideaceae and Myxophyceae.

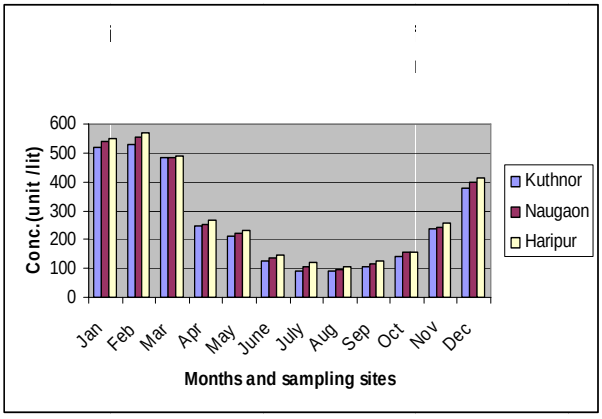

Fig. 1 Monthly mean values of plankton component (unit/l) for the second year

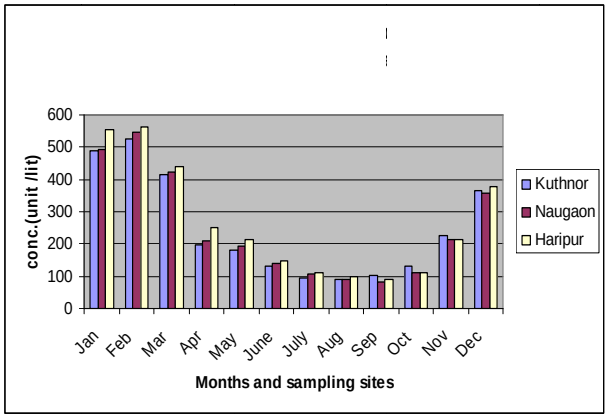

Fig. 2 Monthly mean values of plankton component (unit/l) for the second year

Table -2: Monthly mean values of plankton component (unit /lit) of river Yamuna at three sites

Month	Kuthnor (Site I)						Naugaon (Site II)						Haripur (Site III)					
	Zoo	Bac	Chl	Des	Myx	Total	Zoo	Bac	Chl	Des	Myx	Total	Zoo	Bac	Chl	Des	Myx	Total
Jan	8	365	90	24	0	**487**	7	368	92	26	0	**493**	9	414	101	29	0	**553**
Feb	13	401	98	15	0	**527**	13	417	98	17	2	**547**	12	419	111	21	2	**565**
Mar	21	295	62	27	9	**414**	22	297	67	29	7	**422**	22	303	69	35	12	**441**
Apr	18	118	35	12	14	**197**	19	121	37	14	19	**210**	26	135	47	21	21	**250**
May	47	85	29	9	11	**181**	49	90	28	11	17	**195**	33	105	41	17	19	**215**
June	28	42	31	18	12	**131**	30	45	35	17	11	**138**	34	44	36	18	15	**147**
July	34	18	22	10	11	**95**	36	19	29	15	8	**107**	37	17	30	13	12	**109**
Aug	30	17	24	9	10	**90**	32	17	25	10	8	**92**	33	15	28	12	10	**98**
Sep	22	28	35	11	8	**104**	23	27	26	8	0	**84**	25	26	31	8	2	**92**
Oct	18	45	38	30	0	**131**	17	47	35	12	0	**111**	19	45	34	11	0	**109**
Nov	17	110	68	32	0	**227**	16	101	65	31	0	**213**	18	97	65	33	0	**213**
Dec	3	222	105	37	0	**367**	6	214	103	34	0	**357**	7	219	114	39	0	**379**
Mean	**22.0**	**146.0**	**53.0**	**20.0**	**6.0**	**245.9**	**23.0**	**147.0**	**53.0**	**19.0**	**6.0**	**247.0**	**23.0**	**153.0**	**59.0**	**21.0**	**8.0**	**264.0**

Zoo = Zooplankton, **Bac** = Bacillariophyceae, **Chl** = Chlorophyceae, **Des** = Desmideaceae, **Myx** = Myxophyceae

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Localization of dye degrading enzymes in *Xanthomonas campestris* MTCC 10, 108

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Abstract

Direct Red 28 is a carcinogenic direct diazo dye used for the coloration of paper products. It is recalcitrant and is mostly found in effluents of paper factories. Bacteria in consortia and monocultures those capable of decolorizing Direct Red 28 were isolated previously. The culture *Xanthomonas campestris* MTCC10, 108 was found able to decolorize dye consortia of Direct Red 28, Amido Black, Reactive Black, Reactive Blue, Reactive Red concentration of 20 mg/l each, thus making final concentration approximately to 100 mg/l. It was observed that the rate of decolorization by *Xanthomonas campestris* MTCC10, was varied when incubated under optimum environmental conditions. Dye degradation occurred in the supernatant of sonicated cells, indicating that the dye degrading enzyme was located intracellularly. In present study the active component responsible for decolorization. Direct Red 28 was found as azoreductase rather than laccase and peroxidases enzymes. The optimum concentration of NADH was 0.10 mM and 250 µg of enzyme resulted reduction of 100 µg/ml (highest) Direct Red 28. Based on these results, the optimal enzyme assay conditions were 100µg/ml Direct Red 28, 0.1mM NADH and 250 µg/ml enzyme in 1 ml assay mixture.

Keywords: *Azoreductase, Decolorization, Direct Red 28, Laccase, NADH, Xanthomonas campestris*

Introduction

Azo dyes are characterized by the presence of one or more R_1-N=N-R_2 bonds and is widely used in the paper, textile, plastic, pharmaceutical, food, cosmetic, enamels and drug industries (Collier *et al*., 1993; Dillon *et al*., 1994; Levine, 1991). The ability of micro-organisms to degrade textile azo dyes has been studied extensively in both aerobic and anaerobic processes (Banat *et al*., 1996; Pearce *et al*., 2003). More and more recalcitrant dyes are manufactured with the hope of improving the delivery of color onto fabric, at the expense of becoming increasingly difficult to bioremediate. This has created a need to investigate and understand the actual mechanisms behind the biodegradation of textile waste water. Several enzymes from fungi and bacteria have been identified and used in the breakdown of azo dyes.

Azo dye metabolites are produced after being reductively cleaved at the –N=N– position and are considered toxic aromatic amines. For example, the metabolism of the azo dye Direct Red 28 yields benzidine derivatives, a potential carcinogen (Cerniglia *et al*.,1982). In addition, *in vivo* and *in vitro* experiments supported the toxicity of these metabolites (Morgan *et al*., 1984; Chung, 1983*)*.

Sulphonated azo dyes are the largest and most versatile class of dyes. During textile processing, large amounts of dyestuff directly lost to the wastewater which ultimately finds its way into the environment. The discharge of such effluents from textile industries can result in serious environmental damages. Bioremediation is seen to be an attractive method for the treatment of textile effluent due to its low cost and environmental friendly nature (Banat *et al*., 1996*)*. The sulphonic acid groups that are introduced to increase the water solubility of the dye and azo group confer resistance to microbial attack and make them recalcitrant to oxidative decolorization (Nachiyar and Rajkumar, 2003). Microorganisms are efficiently used for the bioremediation due to their natural catalytic activities. Enzymatic treatments have less impact on the ecosystem as they present no risk of biological contamination. The efficiency of enzymatic reactions in textile processing has been recognized for many years and increasingly

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gained importance as biocatalysts in textile wet processing. Number of different redox enzymes has been noted for their stability to transform a wide variety of toxic pollutants. Redox enzymes may encounter field of application not only in bioremediation of polluted environments, but also in the development of novel clean technologies to avoid or diminish the environmental contamination. The textile dyes are able to undergo extensive oxidation coupling reactions mediated by the naturally occurring biocatalysts such as peroxidases, laccases and azoreductase (Huang *et al.*, 2002).

Laccase, Lignin peroxidase has ability to oxidize large number of aromatic compounds including highly polluting and recalcitrant compounds such as azo dyes. The enzyme produced by bacteria that is involved in reducing azo dyes is called azoreductase and the properties of the azoreductase vary from species to species.

Numerous enzymes for the pharmaceuticals and cosmetic industries are currently isolated by multi stage processes such as precipitation, dialysis, followed by several column chromatographic steps. In the present study, enzyme responsible for Direct Red 28 decolorization and location of active component (azoreductase) in *Xanthomonas campestris* cells was determined and optimization of enzyme assay conditions.

Materials and Method

Test for the Location of Azo Dye Degrading Enzyme

Extracellular Location

The decolorized culture broth of monocultures was centrifuged at 3,500 rpm for 10 minutes at 4°C. The supernatant was collected and sterilized using 2 µm sterile syringe filter. The sterile supernatant was incubated at 30 °C with 100 µg/ml Direct Red 28 and was observed for decolorization.

Intracellular Location

Cells from 2 l cultures were harvested by centrifugation at 3,500 rpm for 10 minutes, It was then washed three times with 50 mM sodium phosphate buffer (pH 6.0) and then suspended in 100 ml of the same buffer. Lysozyme and DNase I were added at final concentrations of 1 mgml $^{-1}$ and 10 µgml^{-1} respectively and the sample was incubated at 30 °C for 20 minutes (Punj and John, 2008). The sample was cooled by sonication (30 s, 70% output, 16×) using a Bandelin Sonopuls sonifier. Cellular debris and unbroken cells were removed by centrifugation at 8,000 rpm for 45 min at 4 °C. The supernatant thus obtained constitutes the crude bacterial extract (soluble protein fraction). Protein was determined by the method of Bradford using bovine serum albumin as a standard.

Enzyme screening assays

Laccase assay

Laccase enzymes have been widely shown to catalyze the degradation of azo dyes through a one step electron oxidation using molecular oxygen as a terminal electron acceptor (Stolz, 2001). In light of this, it became necessary to investigate the potential of terminal electron acceptor to replace the oxygen under anaerobic conditions. Laccase activity was determined using a modified protocol from Zarvazina *et al.* (2004), which used 2, 2´- azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) as a substrate. The reaction mixture contained ABTS (2 ml) dissolved in 50mM sodium phosphate buffer, (pH 6.0). The reaction was started by adding 1 ml of sample and was monitored at 30 °C for 3 minutes. The change in absorbance was monitored spectrophotometrically at 436 nm. One unit of activity was regarded as the amount of enzyme capable of converting 1µmole ABTS per min per ml.

Lignin peroxidase assay

Lignin peroxidase activity was measured by recording the increase in absorbance at 310 nm at 30°C due to the oxidation of veratryl alcohol (VA) to veratraldehyde (VAD) (Have *et al.*, 1997). The total volume of reaction mixture was 2 ml that contained 1 ml of sample and 1 ml of VA (2 mM) dissolved in 50 mM sodium phosphate buffer (pH 6.0). The reaction was started by adding 100 µl of 0.5 mM H_2O_2 and was monitored for over 10 minutes. One unit of activity was regarded as the amount of enzyme capable of converting 1µmole VA per min per ml.

Azoreductase assay

Azoreductase activity was assayed by the method of Zimmermann *et al.* (1982) using Direct Red 28 as dye substrate. The activity of azoreductase was determined spectrophotometrically at room temperature, using a UV/Visible spectrophotometer. In general, enzyme preparation was added to 50 mM sodium phosphate buffer (pH 6.0) containing 100 µM NADH (Sigma), 5 µM Direct Red 28 and 100 µl enzyme solutions to the total volume of the reaction mixture was 1.0 ml.

The reaction mixture was pre-incubated for 5 minutes followed by the addition of NADH and was observed the decrease in absorbance at 497 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the oxidation of 1µmol of dye/min. All experiments and assays were carried out in triplicate. Protein concentration was measured as per standard methods of (Bradford, 1976) using bovine serum albumin (BSA) as a standard.

Optimization of Enzyme assays

All enzyme assays were carried out in a quartz cuvette with a total reaction volume of 1ml. The activity of the enzyme was assayed by measuring the decrease in the optical density for the azo dye at 497 nm for Direct Red 28 with a UV-visible spectrophotometer. The 1ml reaction mixture contained 50mM sodium phosphate buffer (pH 6.0), different concentrations of the azo dye Direct red 28 (60 to 140 µg/ml), different concentrations of NADH (0.05 mM, 0.1 mM, 0.15 mM) and at three different concentrations of the enzyme (100 µg, 250 µg , 500 µg) were used for the assays. Enzyme denatured by boiling and the addition of few drops of HCl was used as a control for all enzyme assay experiments and in second part of experiment under varying enzyme concentrations with optimum concentrations of NADH and dye was conducted to optimize the concentration of enzyme (Macwana, 2007). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the decolorization of 1 µM of azo dye per minute. Enzyme reactions were carried out under static conditions at room temperature and the reactions were initiated with the addition of NADH. All reactions were done in triplicates. A time course experiment was carried out for 2 minutes and readings were acquired every second.

Results and Discussion

Location of Azo Dye Degrading Enzyme

In this study, it was observed that dye degradation occurred in supernatant of the sonicated cells of the culture incubated with Direct Red 28. In the case of the filter sterilized culture broth with Direct Red 28, no decolorization occurred even after 10 days of incubation, indicating that the enzyme responsible for the reduction of Direct Red 28 is located intracellular (Stolz, 2001). Enzyme activity was expressed as a relative percentage in order to allow comparison of the azoreductase activity of the different cell fractions. The sonicated cell supernatant fraction was considered as 100% because it exhibited the highest activity of the enzyme. The disruption of the cells by sonication resulted in approximately 3-fold increase in azoreductase activity of the cell free extract. This indicates that there was release of intra-cellular azoreductase that did not have access to the substrate when the cell was intact, and this together with the periplasmic azoreductase gave the activity.

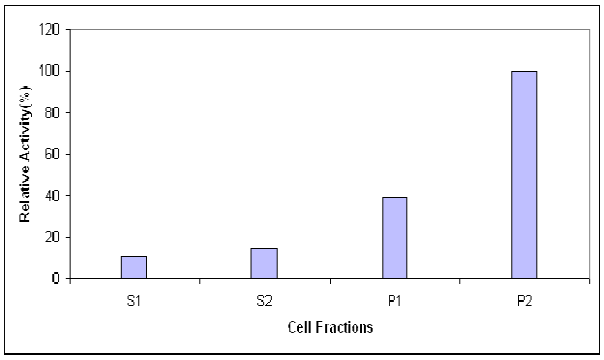

Figure 1: Relative Activity of azoreductase in different cell fractions before and after cell disruption by sonication, S_1=Supernatant before sonication, S_2 = Supernatant after sonication, P_1 = Pellet before sonication, P_2 = Pellet after sonication

Under anaerobic conditions, it has been reported that azoreductase require reducing equivalents to provide the electrons for the reductive cleavage of the dyes which act as the terminal electron acceptors. In our current experiment, NADH was used as a redox mediator since previous work indicated that azoreductase enzymes strictly require NADPH to provide hydrogen and electrons required for reductive cleavage of the azo bond (Zimmermann *et al.*; 1982, Stolz, 2001).

Screening for dye degrading enzymes

Once the ability of *Xanthomonas campestris* MTCC 10,108 to grow in the presence of a Direct Red 28 azo dye had been demonstrated, the next step was to identify the enzymes responsible for the reductive cleavage of the azo bond. Lignin modifying enzymes (laccase, lignin and manganese peroxidase) were investigated in this experiment with the aim of finding a substitute enzyme that would operate under partial anaerobic conditions. Unfortunately, but expectedly the activities for the laccase and the peroxidases were 0.171 and 0.147 Uml^{-1} respectively which were significantly lower than that for azoreductase activity as shown in Fig.2

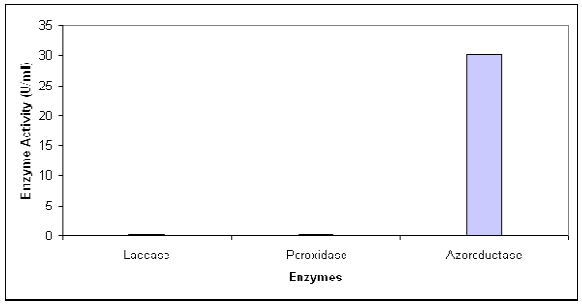

Figure 2: Identification of enzymes that can degrade azo dyes produced in *Xanthomonas campestris* cells

Azoreductase assays resulted in an activity of 30.20 Uml^{-1} which was significantly high in comparison to that of Laccase and Peroxidase activity. These enzymes have been successfully isolated and purified under aerobic conditions using *Pseudomonas* strains K22 and KF46 (Zimmermann *et al.*, 1982)

Optimum Enzyme Assay Conditions

To test azoreductase activity, three experimental approaches were used and the result obtained was at the concentration of 1.435 mM Direct Red 28 the percent relative activity was high. The significant activity was observed at 0.1 mM NADH and 250g enzyme concentration as shown in Figure 3, 4, 5. The result was almost similar at the enzyme concentration 250 µg and 500 µg. Hence, 250 µg was the optimum enzyme concentration at which further assay were conducted where as in case of NADH there was increase in enzyme activity to certain extent and afterwards there was no significant change observed in enzyme activity so the optimum concentration of NADH was 0.1mM To determine if the enzyme was responsible for the decrease in dye concentration, a few drops of HCl (2 µl, 36 %) was added, followed by boiling for 30 minutes. When the reaction was carried out in the presence of denatured enzyme, there was no reduction of Direct Red 28 demonstrating azoreductase activity of the enzyme. In addition different dye and cofactor NADH concentrations were tested with the same enzyme concentrations and a similar conclusion resulted. Interestingly, the different dye and NADH concentrations caused some change in the reduction of the concentration of Direct Red 28, suggesting some enzyme conditions were not optimal. Based on these results, the optimal enzyme condition was 150 µM dye and 0.15 mM NADH.

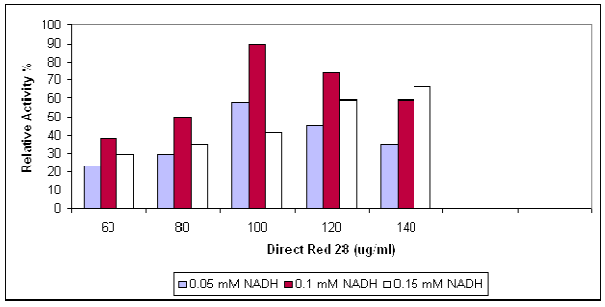

Figure 3: Rate of Decolorization at 100 µg enzyme concentration and different concentration of NADH and Direct Red 28

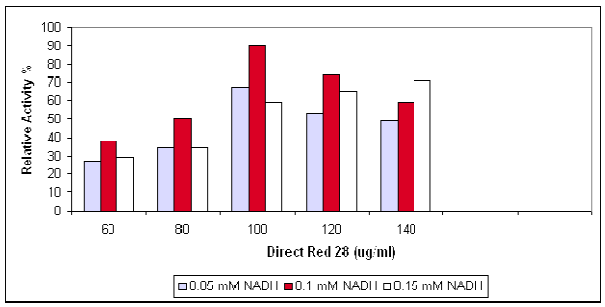

Figure 4: Rate of Decolorization at 250 µg enzyme concentration and different concentration of NADH and Direct Red 28

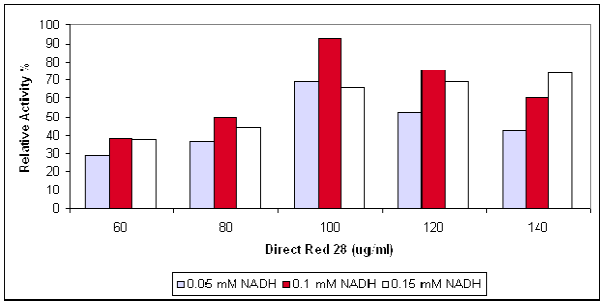

Figure 5: Rate of decolorization at 500 µg enzyme concentration and different concentration of NADH and Direct Red 28

Reduction of Direct Red 28 under optimal conditions

Based on the pH and temperature experiments, a time point measurement of the complete reduction of Direct Red 28 was taken. Using three different concentrations (100 µg, 250 µg and 500 µg) of enzyme, 0.1 mM NADH and 100 µg/ml of Direct Red 28 incubated at 30 ºC, it took approximately 14 minutes to reduce most of the dye (Table-1 and 2). The varied and incomplete reduction of the dye with three different enzyme concentrations suggested that Direct Red 28 concentration is limiting or inhibitory factor.

Table-1: Mean residual dye reduction of the concentration of the azo dye Direct Red 28(100 µg/ml) with three concentrations of the enzyme and differentconcentrations of NADH (0.01-0.2mM)

Enzyme Conc.	Direct Red 28 (100 µg/ml)				
	NADH				
	0.01 (mM)	0.05 (mM)	0.1 (mM)	0.15 (mM)	0.20 (mM)
100 (µg)	60.76±0.053	56.48±0.020	34.57±0.011	50.31±0.043	47.65±0.022
250 (µg)	46.76±0.040	32.17±0.047	11.60±0.079	38.9± 0.039	40.14±0.032
500 (µg)	41.62±0.020	26.35±0.022	9.68±0.007	15.32±0.056	19.19±0.020

Table 2: Mean residual dye reduction of the concentration of the azo dye Direct Red 28(120 µg/ml) with three concentrations of the enzyme and different concentrations of NADH (0.01-0.2 mM).

Enzyme Conc.	Direct Red 28 (100 µg/ml)				
	NADH				
	0.01 (mM)	0.05 (mM)	0.1 (mM)	0.15 (mM)	0.2 (mM)
100 (µg)	88.18±0.053	79.48±0.020	54.47±0.043	68.21±0.043	71.92±0.011
250 (µg)	49.76±0.002	34.67±0.016	23.41±0.005	12.70±0.003	19.46±0.011
500 (µg)	55.83±0.040	51.28±0.047	17.71±0.079	26.32±0.039	28.74±0.031

Under optimum conditions Direct Red 28 (100µg/ml), 0.1 mM NADH and 250 µg enzyme concentration. The reduction in the concentration of dye was observed as shown in Figure 6 after 14 minutes of reaction. Azo dyes are used extensively in many industries. These azo dyes have been shown to be reductively cleaved by a wide range of microorganisms. Bacteria, both aerobic and anaerobic from different environment possess the ability to reduce azo dyes.

The study concluded that *Xanthomonas campestris* MTCC 10, 108 possessed the ability to reduce mono and diazo sulfonated dyes Direct Red 28 with no inhibitory effect on the growth of the bacteria. This indicates that the enzyme azoreductase is functionally expressed in *Xanthomonas campestris* MTCC 10, 108. When the supernatants were tested for activity, no activity was observed which showed that the enzyme is not extracellular but an intracellular protein and azoreductase from *Xanthomonas campestris* cells was released by sonication.

In conclusion, we have shown that the *Xanthomonas campestris* possess azo reductase enzyme rather than Laccase and Peroxidase enzymes.The enzyme azoreductase was capable of reducing the water soluble diazo dye Direct red 28

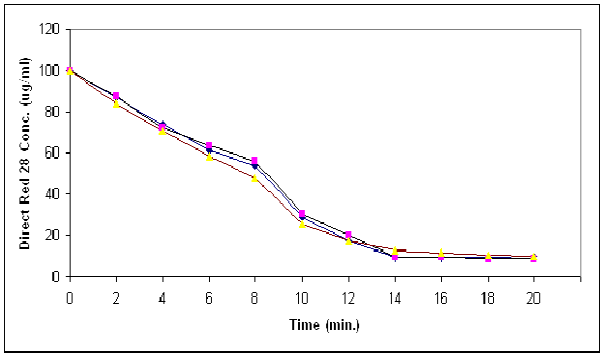

Figure 6: Time course reduction of Direct Red 28 (100 µg/ml) using optimal conditions for the enzyme. The optimal conditions were 0.1mM NADH, pH 6, 30°C (Experiments were done in triplicates).

Direct in the presence of the cofactor NADH. The reduction of the dye by the enzyme was not linear. This may include several factors. These factors may include the NADH and enzyme concentration which may affect enzyme activity. The optimal conditions for enzyme activity were found to be 30 °C, pH of 6.0, 0.1 mM NADH and 250 µg enzyme concentration.

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Vesicular arbuscular mycorrhiza (VAM) mediated solubilization of phosphorus in clayey soil

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Abstract

One of the major essential macronutrient for plant is phosphorous and is applied to soil in the form of chemical phosphatic fertilizers which is immobilized rapidly and becomes unavailable to plants. Microorganisms are involved in the transformation of soil P and is thus an integral part of the soil P cycle. In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization. P-solubilization ability of the microorganisms is considered to be one of the most important properties. The adverse impact of chemical fertilizers on the environment and the less cost effectiveness stimulates the exploration of Phosphate solubilisers. 2/3rd of phosphate fertilizer is unavailable within a very short period of its application due to fixation in the soil complex. To overcome the problem of phosphorus solubilisation and to raise its concentration in soil, the present work was undertaken which deals with the isolation and inoculation of VAM spores from four sets of soil sample mainly clayey textured soil as classified on the basis of its morphological characteristics done through particle size analysis. The result of the present study showed that AM symbiosis associated with plant roots and soil aggregates optimizes the phosphorus solubilization and it is confirmed by the physico-chemical and biochemical estimations along with the mineralogical studies, where the results are within expectations.

Keywords: *Clayey soil, VAM spores, Solubilisation, Phosphorus, Biochemical*

Introduction

The solubilization of mineral phosphates to low molecular weight organic acids contributes in phosphorous solubilisation (Halder *et al.*, 1990). The presence of hydroxyl and carboxyl groups in these organic acids results in chelating the cations bound to phosphate, thereby converting it into soluble forms. Microbial solubilization of inorganic and organic phosphatic compounds has been extensively studied under Indian conditions. Therefore, one of the approaches would be to increase the number and activity of efficient Phosphate Solubilizing Microorganisms (PSM) in the root zone of plants by use of microbial inoculants for increasing phosphorus availability to the plants from the soil as well as added phosphate. It is estimated that India alone has about 140 million tones of rock phosphate deposits, most of which are low grade and contain impurities. Only high grade rock phosphates, free from impurities are utilized for the manufacture of phosphatic fertilizers. Direct use of even low-grade rock phosphate as fertilizer is feasible in neutral to alkaline soil if PSM are used as inoculants. Mycorrhizal symbiosis play an important role in nutrient cycling in agricultural and natural ecosystems. VAM fungi colonize the root cortical region cells of plants and develop an extrametrical hyphae network that could absorb nutrients from the soil. In ecosystem they play a vital role and their association improves the capacity and longevity of root uptake of growth nutrients, enhanced the absorption of remotely available nutritional elements especially phosphorus and reduces the susceptibility of plants to soil-borne pathogens (Rokni *et al*., 2010). Phosphorus is an important plant nutrient classed as a major plant nutrient element. It is associated with several vital functions and is responsible for several characteristics of plant growth such as utilization of sugar and starch, photosynthesis, nucleus formation and cell division, fat and albumin formation, cell organization and the transfer of heredity (Saha and Biswas, 2009). As reported by Khiari and Parent (2005), VAM plays a major role in converting the complex organic phosphorus into inorganic

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solubilized form through phosphatase enzyme which varies with pH. Its mechanism at initial level of its presence in complex form gradually converted into the soluble form but for a short period and automatically get converted into more stable form and in a fraction of time become unavailable to the soil system for uptake by plants (Khan *et al.*, 2009).

Materials and Method

Sample collection: Freshly soil samples were collected from four different locations within the Bhopal city, India from depth of 5-10 cms by adopting conventional coning and quartering method for property evaluation. The soil samples were named as SS1, SS2, SS3 and SS4 under two categories *i.e.*, VAM (+) and VAM (-). The soil samples were brought to the laboratory in polybags and were kept under ice cold conditions for initial level studies. These soil samples were then transferred in to earthen pots for further experimental analysis.

Physico-chemical and biological properties: The samples were tested in terms of physical properties like pH, conductivity, water holding capacity, density, porosity and texture, adopting the conventional experimental details of Page *et al.*, (1982), mineralogical analysis was done by X- ray diffract meter (PW-1710), the technique applied to identify different phases present in initial soil and in treated with VAM inoculants soil after 90 days; available phosphorous was estimated by Bray and Kurtz method (1945); Phosphatase enzyme activity was estimated by Tabatabai and Bremner (1969).

Isolation of Spores (**Gerdemann and Nicolson, 1963):** The top soil was sieved through a series of sieves of maximum size 500 microns and minimum of 50 microns. VAM spores were collected on the 50 microns sieve. Generally the soil in the top sieve is washed 3 times.

Proliferation and inoculation of VAM Spores in Test Soil: Starter inoculums (spores) of VAM fungi were isolated from soil by Wet sieving and decantation technique. Spores were transferred to the sterile soil (in duplicate) and mixed well. Now, seeds of *Zea mays* and VAM spores were added in sterilized soil kept at 37 ºC for incubation.

Isolation and Screening of Phosphate Solubilizing Bacteria: Soil samples were collected from the rhizosphere of different soils. A total of 9-10 composite soil samples (pH 6.0-9.0), were used for the isolation of phosphate solubilizing bacteria. These bacteria were isolated from soil sample by serial dilution technique (Sharma, 2005) on Pikovskaya agar medium plates. Pikovskaya medium was used for the isolation, cultivation and maintenance of phosphate solubilizing bacteria (Gaur, 1990). All the flasks were maintained at 30˚C for 14 days with intermittent shaking twice a day. Un-inoculated medium served as control and each experiment was done in triplicate set.

Identification of Phosphate solubilising bacteria (Mac Faddin, 1980): Pure cultures were identified on the basis of their morphological, cultural and biochemical reactions. Isolates were spot inoculated on Pikovskaya medium (Pikovskaya, 1948) for detection of their phosphate solubilizing ability and incubated at 37 ºC for 48 hours of their phosphate solubilizing ability and incubated at 37 ºC for 48 hours. Halo surrounding the colonies were measured and the solubilizing efficiency (SE) was calculated by the following formula:

$$SE = \frac{\text{Solubilization diameter X 100}}{\text{Growth diameter}}$$

Results and Discussion

Spores from four sets of soil sample are isolated and identified as *Glomus aggregatum.* This genus could grow/multiply in a wide range of pH tolerance (pH 6.0-9.0). Most spores are isolated from the top soil (5-20 cm). Phosphorus is one of the major elements utilized by the plant largely used in membrane, cell division, nucleic acid and high energy compound. It is considered as important plant growth limiting factor because of many abiotic and biotic properties which restricts its mobility in soils. Table–1 incorporates the results of physico-chemical and biological properties at initial and final level of soil genesis for 90 days. The texture of the soil is clayey. The results showed view of various parameters which played an important role in enhancing soil properties. The pH is found to decrease with number of sampling at 14 days interval for a total period of 90 days, the water holding capacity of clayey soils is noted to be increase around 10-18% with raised conductance from 350 to 455 µmhos/cm, the porosity value ranges from 34 % at

initial level to 48% in the final soil. An increase in the available phosphorus concentration is noted in all the samples upto third sampling. Fig.1 shows the pattern of pH noted during the study of 90 days, where the initial level of pH begins at 8.2 and reduced to 5.8 *via* 6.2. Fig.2 indicates available phosphorous in ppm in test and control soil against number of samplings. An initial increase in the concentration with gradual decrease at later stages is observed. The possible explanation for such pattern is the production of organic acids in soil which solubilises the phosphorous into available form but due to its unstable nature, if not utilized by the plants immediately again revert to its unavailable form. In sandy soil an easy source for the uptake of phosphate by ions specifically Ca^{2+} and Mg^{2+} in alkaline pH range and Al^{3+} and Fe^{3+} in acidic range, is reported by Harley and Smith (1983), the same pattern along with noted depletion of available phosphorus in the test soils is measured and this observation confirmed the solubilization of the non labile to labile form of phosphorus. Arbuscular mycorrhizal produce a range of phosphatase enzyme and through these enzymes the phosphatase are taken up into cells and incorporate into nucleic acid, phospholipids which are stored as polyphosphates in vacuoles.

Table -1: Physico-chemical and biological properties of treated and untreated soil

Soils	pH	Conductivity (µmhos/cm)	WHC (%)	Porosity (%)	Av. PO_4 (ppm)	Phosphatase enzyme (µmoles/ml)
SS -1	8.2	340	38	22	0.424	0.600
SS 1+	6.4	445	44	36	1.042	0.297
SS -2	8.2	340	38	22	0.423	0.533
SS 2+	6.4	445	44	36	1.042	0.299
SS -3	8.2	340	38	22	0.439	0.625
SS 3+	6.4	445	44	36	1.042	0.319
SS -4	8.2	340	38	22	0.400	0.609
SS 4+	6.4	445	44	36	1.042	0.297

SS- : Soil Sample minus VAM spores
SS+: Soil Sample plus VAM spores

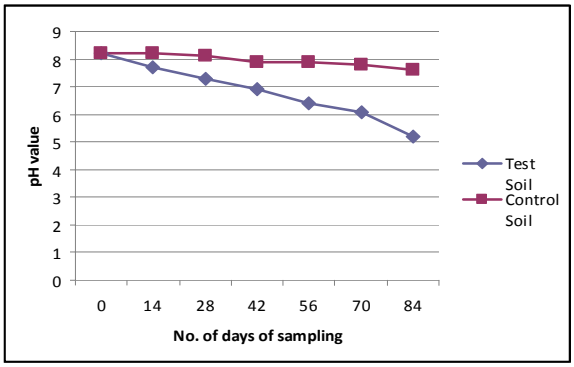

Fig.- 1: pH range in control soil (-VAM) and test soil (+VAM)

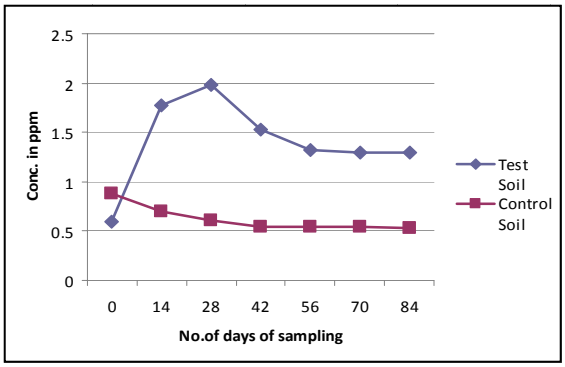

Fig.- 2: Available phosphorus range in control soil (-VAM) and test soil (+VAM)

The phosphatase enzyme activity in test soils is measured at 14 days interval after each spore inoculation in soil Fig. 3, where the enzyme activity is increased gradually and contributed in the formation of organic acid which is an indication of the formation of inorganic (soluble) phosphorus with phosphatase enzyme activity. The state has reached to saturation due to which no further increase in the concentration of phosphatase activity is observed. After sixth sampling, the

amount of available phosphorous as well as phosphatase enzyme activity is noted to be constant. The constant value of available phosphorus and phosphatase enzyme activity in soil confer to the presence of channel of phosphorus in soluble form transported through hyphae and the transfer of phosphorus across the host-fungus interface. Along with these observations, during the process of solubilization of phosphorus in soil the pH of the soil more importantly is taken in to account. The pH changes from 5.8 to 8.2 in the entire sampling of test soils. It is imperative from the observation that all these parameters are interdependent. The results of pH shows a decline from initial to the final level while in control where no spores are inoculated the pH is slightly fluctuated. Fankem *et al.,* 2006, reported the same experimental results with pH and could strengthen our studies.

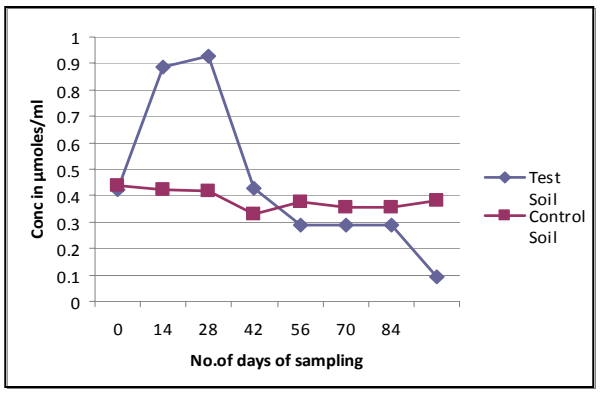

Fig.-3: Phosphatase enzyme activity in control (-VAM) and test soil (+VAM)

Phosphate dissolving microbial consortium are developed in Pikovskaya liquid medium from the soil. On screening the consortium, numerous colonies are noticed on the plates, which gave a zone of clearing. Two different bacterial colonies and a fungus are picked up from the plates of Pikovskaya agar showing the maximum zone of the clearing around these colonies. These two bacterial colonies and a fungus are purified and identified as *Pseudomonas striata*, *Bacillus* sps, through Probablistic identification of bacteria (PIB) (Bryant, 2003) and *Aspergillus niger* through its lawn and spore morphology microscopically. Table – 2 shows different mineral phases present in initial and treated soil detected by X ray diffractometer. It indicates that most of the elements were present in their oxide and silicate form. VAM spores inoculation is favored by certain factor which leads to release of phosphorus as elements in the ionic state after undergoing various chemical reactions as discussed here. The phosphate mineral reported in the table was lazulite along with different other minerals.

Table -2: Mineralogical Phases of Soils identified by XRD

Minerals	SS1	SS2	SS3	SS4
Quartz	Present	Present	Present	Present
Albite	Present	Present	Present	Present
Augite	Present	Present	Present	Present
Mizzonite	Present	Present	Present	Present
Lazulite	Present	Present	Present	Present
Meta- Present **aluminite**	-	Present	Present	
Magnetite	Present	Present	-	Present
Tenorite	Present	Present	Present	-

SS- : Soil Sample minus VAM spores
SS+: Soil Sample plus VAM spores
SS1, SS2, SS3, SS4 are soil samples in four replications

Conclusion

The overall results showed that the mycorrhizal inoculation could help in effective utilization of rock phosphate by changing it into available form, which is later taken up by the plants for their better growth and development.

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Traditional use of some leguminous plants in Tarai and Bhawar regions of Kumaun Himalaya, Uttarakhand

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Abstract

The medicinal properties of leguminous plants were analyzed in Tarai and Bhawar regions of Kumaun adjacent to Kashipur at 29º 14-43.6 to 29º 19-50.5 N latitude and 79º 03-22.6 to 79º 04-23.2 E longitude at an elevation of 253.4–265.5 meter above the sea level, within the districts of Nainital and Udham Singh Nagar. The present study documents the ethnomedicinal uses of 25 leguminous plants, which are prevalent in study area along with botanical name, family, vernacular name, habit, plant parts used and mode of ethnomedicinal use.

Keywords: *Ethnomedicinal plants, Leguminous plants, Tarai, Bhawar*

Introduction

Indian traditional medicines are based on different systems such as Ayurveda, Siddha and Unani used by various tribal communities (Gadgil, 1996). 1748 species of medicinal plants have been reported from Indian Himalayan Region (IHR), of these 701 species occur in Uttarakhand state (West Himalaya). In the region most medicinal plants are being extracted for drug and pharmaceutical industries from the wild (Mehta, 2001). People living in the developing countries rely quite effectively on traditional medicine for primary health care (Sullivan and Shealy, 1997; Singh, 2002). Kumaun Himalaya especially Tarai and Bhawar region have high floristic diversity. The present study has been designed to report the medicinal uses of leguminous plants for curing and healing of common diseases on the basis of field surveys and taxonomic identification of plants. The objective of this study is utilization, cultivation and preservation of traditional plants.

Study Area

The study site is situated in Tarai and Bhawar region of Kumaun adjacent to Kashipur at 29º 14-43.6 to 29º 19-50.5 N latitude and 79º 03-22.6 to 79º 04-23.2 E longitude at an elevation of 253.4–265.5 meter above sea level, within the districts of Nainital and Udham Singh Nagar.

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Materials and Method

The present study is the outcome of the two years of critical field survey in the different parts of Tarai and Bhawar region of Himalaya in every season. Medicinal uses was gathered by taking interviews of the local and tribal people. The plants were identified with the help of a plant taxonomist and from the forest flora of Kumaun (Osmoston, 1926), Flora Simlensis (Collet, 1971), Flora Nainitalensis (Gupta, 1968) and Flora of Mussoorie (Raizada, 1978).

Results and Discussion

All known 25 species of leguminous plants were encountered in Tarai and Bhawar region of Kumaun. Botanical names, family, vernacular name, habit, mode of use and plant parts used are given below:

(1) *Acacia auriculaeformis* Cunn. ex Benth.

Family: Mimosaceae; Habit: Tree

Use: It contains tannin useful in animal hide tanning. In India, its wood and charcoal are widely used for fuel. Gum from the tree is sold commercially, but it is said not to be as useful as gum arabic. The tree is used to make an analgesic by indigenous Australians.

(2) *Acacia catechu* Willd.

Family: Mimosaceae; Vernacular Name: Khair, Kattha; Habit: Tree

Use: The bark of this plant is used as an astringent in the treatment of cough.

(3) *Acacia farnesiana* Willd.
Family: Mimosaceae; Vernacular Name: Gand babul; Habit: Tree
Use: The bark of this plant is used as an astringent agent and demulcent.
(4) *Acacia nilotica* (L.) Willd.
Family: Mimosaceae; Vernacular Name: Babul; Habit: Shrub
Use: The bark is used as a tan while gum obtained from stem is used in dysentery. Tender leaves are used as blood purifier while young twigs are used as "Datoon" for cleaning teeth.
(5) *Albizzia chinensis* (Oseck) Merr.
Family: Mimosaceae; Vernacular Name: Kala Siris; Habit: Tree
Use: The infusion of bark is used as lotion for cuts, scabies and skin diseases. Leaf is useful in curing fish poisoning.
(6) *Albizzia procera* Benth.
Family: Mimosaceae; Vernacular Name: Safed siris; Habit: Tree
Use: Leaves are used as insecticides and poultice applied to ulcer.
(7) *Alysicarpus vaginalis* DC.
Family: Fabaceae; Habit: Herb
Use: Decoction of roots are used in treatment of cough.
(8) *Bauhinia malabarica* Roxb.
Family: Caesalpiniaceae; Vernacular Name: Kachnar; Habit: Tree
Use: Decoction of leaves are used in headache and malaria. Bark is used in treatment of diarrhoea and dysentery.
(9) *Bauhinia vahlii* W. & A.
Family: Caesalpiniaceae; Vernacular Name: Maljan; Habit: Climber
Use: Seeds are used as an aphrodisiaic agent. Leaves as demulcent agent.
(10) *Butea monosperma* (Lamk).Thub.
Family: Fabaceae; Vernacular Name: Dhak; Habit: Tree
Use: Seeds are used as an anthelmintic agent. Gum is astringent used in treatment of diarrhoea and dysentery. Flowers are astringent, diuretic, depurative and aphrodisiac. The seeds and bark of this plant are used in curing snake bite.
(11) *Cassia fistula* L.
Family: Caesalpiniaceae; Vernacular Name: Amaltas; Habit: Tree
Use: The pulp from the pods is of great therapeutic value. It is a mild pleasant and safe purgative, even for children and expectant mothers. Its confection is given in diabetes. The leaves are emollient; their juice makes a useful dressing for ringworm and chilblains. The root is a tonic febrifuge and a strong purgative.
(12) *Cassia mimosoides* L.
Family: Caesalpiniaceae; Vernacular Name: Patwa Ghas; Habit: Herb
Use: The roots are used for treating spasm of stomach.
(13) *Cassia obtusifolia* L.
Family: Caesalpiniaceae; Vernacular Name: Chakunda; Habit: Herb
Use: Decoction of leaves are used as laxative. Leaves and seeds are used in skin diseases such as ringworm and itch. Roots are used in treatment of snake bite.
(14)*Cassia occidentalis* Vahl
Family: Caesalpiniaceae; Vernacular Name: Kasondi; Habit: Herb
Use: Whole plant is used as a febrifuge, purgative, diuretic and bitter tonic. Seeds and leaves are used externally in skin diseases, antiperiodic. Leaves, seeds and roots are used as purgative, however roots are also used in treatment of snake bite.
(15)*Crotalaria mucronata* Desv.
Family: Fabaceae; Vernacular Name: Sen; Habit: Herb
Use: The seeds are used as a substitute of coffee.
(16)*Crotalaria spectobilis* Roth
Family: Fabaceae; Vernacular Name: Jhunjhunia; Habit: Herb
Use: Plant used in scabies. Seed, leaves and stem used in treatment of hypertension.
(17)*Dalbergia sisso* Roxb.
Family: Fabaceae; Vernacular Name: Shisam; Habit: Tree
Use: The decoction of leaves is useful in treatment of gonorrhea. Roots are used as an astringent agent.
(18)*Delonix regia* Raf.
Family: Caesalpiniaceae; Vernacular name: Gulmohar; Habit: Tree
Use: Leaves are used in treatment of rheumatism.
(19)*Desmodium gangiticum* (L.) DC.
Family: Fabaceae; Vernacular Name: Sarivan; Habit: Herb
Use: Roots are used as an astringent in the treatment of diarrhoea, diuretic, chronic fever, biliousness, cough, vomiting, asthma, snake bite and scorpion sting.

(20)*Desmodium pulchellum* Benth. ex Baker

Family: Fabaceae; Vernacular Name: Ladrom; Habit: Herb

Use: Decoction of the bark is used in hemorrhage, diarrhoea, poisoning and eye diseases. Flowers are used in biliousness.

(21)*Dolichos biflorus* L.

Family: Fabaceae; Vernacular Name: Kulthi; Habit: Herb

Use: Seeds used as an astringent, diuretic. Decoction of plant used in leucorrhoea and menstrual disorders.

(22) *Indigofera tinctoria* L.

Family: Fabaceae; Vernacular Name: Mehandi; Habit: Herb

Use: Extract of plant is used in bronchitis and hepatitis. Juice of leaves are used as prophylactic against hydrophobia. Extract of plant is given in epilepsy and nervous disorders, in bronchitis and as ointment in sores, old ulcers and hemorrhoids. Root are used in hepatitis and scorpion sting treatment.

(23)*Melilotus indica* All.

Family: Fabaceae; Vernacular Name: Vanmethi; Habit: Herb

Use: Seeds are used in bowel complaints, infantile diarrhoea and given in gruel. Whole plant is used as a emollient, externally as a fomentation and plaster or poultice for swellings.

(24)*Mimosa pudica* L.

Family: Mimosaceae; Vernacular Name: Lajwanti, Chuimui; Habit: Shrub

Use: Decoction of root is useful in gravellish complaints. Leaves and root are used in piles, scorpion sting and fistula. Leaves rubbed into a paste is applied to hydrocele.

(25) *Tephrosia purpurea* Pers.

Family: Fabaceae; Vernacular Name: Sarphonka; Habit: Herb

Use: The pills made from fresh root bark with a little black pepper is given in case of obstinate colic. Whole plant is used as an anthelmintic for children and is used internally as a blood purifier.

Conclusion

The leguminous plants are widely used traditionally by the local people in Tarai and Bhawar of Kumaun region. The study documented 25 leguminous plants which are used in curing and healing of different diseases. This data could be useful for phytochemists and pharmacologists to determine their true therapeutic compounds. It may bring light to new sources of drugs of herbal origin because many medicinal plants are reported to be threatened to extinction.

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Ecological characteristics of Sahastradhara stream at Dehradun (Uttarakhand)

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Abstract

Sahastradhara sulphur stream is a natural perennial hill stream which originates from the upper mountainous terrains of Mussoorie in Garhwal region. The climate of Garhwal region depends on the temperature variability. on monthly and seasonally changing patterns. Sahastradhara stream had a cool and pleasant climate but at current stage it has changed at a great extent due to natural and anthropogenic factors. In the present study, the changes were recorded as annual average temperature (3.4 °C – 38.3 °C), wind velocity (0.9 km - 2.5 km), rainfall (225 mm – 371 mm), precipitation and sedimentation rate just double from two decay periods. Twenty tree genera of macrobenthic organisms in sediments and other existed native species of macrophytic vegetation in littoral zones of stream. The physico-chemical characteristics of Sahastradhara hill-stream showed seasonal variations and influenced the distributional patterns of macrobenthic communities. Presently, eco-biological characteristics of Sahastradhara stream exhibited continuous degradation nature in and around stream ecosystem in terms of biological productivity and macro-benthic diversity.

Keywords: *Stream variables, Sulphur spring, Macro-benthic diversity*

Introduction

Hill streams are generally the important source of clear crystal water on earth. The process of economic growth & development, virtually have inverse relationships with hill stream resources and quality of aquatic environment. Hill streams are unique habitats, which sustain substantial biodiversity and provide many tangible and intangible benefits on a sustainable basis, not only to a local society but also to the associated dependent ecosystems. Sahastradhara hill stream situated in foothills of Dehradun in Garhwal Himalayas is a major tributary of River Song which flows downwards through Dehradun Valley. Sahastradhara stream is situated on the globe at 29°57'-31°20' N Latitude and 77°35'-79°20' E Longitude in Garhwal region.Macro-benthic diversity and water quality are interrelated to each other, as they are potential indicators of water quality of any aquatic system or a body. Benthic macro-invertebrates are one of the most common group of organisms used to assess the health of aquatic ecosystem (Rosenberg and Resh, 1993). Benthic aquatic organisms are sensitive indicators to environmental changes in streams because they express long term changes in water and habitat quality rather than instantaneous conditions (Armitage *et al.*, 1983). The presence and absence of such macro-invertebrates indicates the degree of pollution, though specific causative physico-chemical pollutant may be identified by physico-chemical methods. Due to anthropogenic activities and heavy soil erosion from surrounding fragile hill terrains, could add nutrient load in aquatic ecosystem of the stream which would result in eutrophication. The main focus of the study is to describe the degradation of water quality in Shastradhara stream.

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Materials and Method

The sampling sites were selected in Sahastradhara valley at a distance of 15 km far away from Dehradun. The five sampling stations were selected for ecological study. The water samples were collected monthly from different sampling stations during May, 2009 to April, 2010 in morning period (9:00 AM). The samples were examined on site for selected parameters and brought to the laboratory for remaining physical and chemical analysis. The selected variables of the stream were analyzed with the help of the procedure described by APHA

(1995), Trivedi and Goel (1984). The sample of benthic organisms were collected between 8:00 to 10:30 AM on seasonal basis with an Ekman's Dredge Sampler and sieve having size US No. 60 cms and preserved in 4.0 % formalin. In laboratory, the benthic organisms were sorted out and identified to genus/species level with the help of identification keys (Edmondson, 1992).

Results and Discussion

Ecological study of hill stream significantly contributed in assessment of existed nutrient load and their impacts on distribution and abundance of aquatic organism in aquatic ecosystem. The physico-chemical observations of Sahastradhara stream in different seasons were observed at different sites S_1, S_2, S_3, S_4 and S_5 during the year 2009 – 2010 (Table-1). In the present study, the maximum water temperature (23.5 °C) was recorded during summer at site S_5 and minimum (15.6 °C) at site S_1 during winter. Lower temperature was recorded during winter and higher during summer may be due to extreme cold and extreme sunshine period. The flow of stream is directly related to the amount of water flowing off watershed into the stream channel. The maximum velocity (0.74 m/s) was recorded at site S_1 during monsoon and minimum (0.32 m/s) recorded at site S_4 during summer season due to magnitudes of stream slope gradients.

The pH was maximum (8.5) during monsoon at site $S_1$and minimum (7.8) during winter at site S_3 indicates that water was alkaline. Similar observation was observed by Sharma (1986 and Joshi (1996)) in the Bhagirathi river and other hills rivers in Garhwal Himalaya.

Table 1: Physico-chemical characteristics of Sahastradhara Stream at different sampling stations during 2009 – 2010

Parameters	Karligarh Upstream (S_1)		Main tourist spot (S_2)		Kalirov Downstream (S_3)		Kalagaon Downstream (S_4)		Bajhat Confluence point (S_5)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Temp. ˚C	15.6 - 21.9	19.1	15.7 - 21.8	18.7	15.9 - 22.1	19.2	16.2 - 22.6	19.3	16.2 - 23.5	19.9
Velocity m/s	0.37 - 0.74	0.59	0.39 - 0.71	0.58	0.35 - 0.62	0.53	0.32 - 0.61	0.49	0.37 - 0.69	0.54
pH	8.5 - 7.9	8.1	8.3 - 7.9	7.9	7.8 - 7.9	7.8	7.7 - 7.9	7.8	8.1 - 8.2	8.1
Alk. (mg/l)	39.4 - 54.4	37.3	40.4 -51.2	45.07	40.1 - 46.3	43.7	41.5 - 52.1	45.9	43.1 - 62.1	51.2
Free CO_2 (mg/l)	1.1 - 1.9	1.7	0.87 - 1.12	0.94	0.91 - 1.67	1.2	2.13 - 2.27	2.25	1.86 - 2.43	2.18
DO (mg/l)	7.28 - 10.87	8.64	7.29 - 9.13	8.08	7.26 - 8.64	7.81	7.24 - 8.37	7.68	7.71 - 8.45	8.13
BOD (mg/l)	0.84 - 2.39	1.55	0.95 -2.42	1.61	1.05 - 2.33	1.62	1.13 - 2.45	1.71	1.45 - 3.12	1.89
Calcium (mg/l)	83.62 - 85.15	84.42	83.8 - 85.18	84.55	83.92 - 85.11	84.4	84.79 - 87.16	84.64	84.07 -88.64	84.74
Mag. (mg/l)	50.51 - 53.97	51.78	50.7 - 54.3	52.14	49.61 - 55.12	51.24	50.45 - 53.57	51.64	50.92 -53.81	52.08
Sod. (mg/l)	14.92 - 16.97	15.85	15.21 - 17.31	16.24	15.66 - 17.77	16.49	16.06 - 18.77	17.22	15.16 - 17.7	16.34
Pot. (mg/l)	7.66 - 10.97	9.09	7.9 - 11.3	9.39	8.62 - 11.6	9.85	9.7 - 12.73	10.9	8.81 - 11.53	9.9
Chl. (mg/l)	14.43 - 16.42	15.33	14.6 - 16.5	15.49	14.76 - 16.67	15.56	14.99 - 16.86	15.87	15.76 - 6.23	15.93

Alkalinity of water was strongly correlated with pH value, which was recorded maximum (62.1 mg/l) at site S_5 during mansoon, due to increase in the concentration of bicarbonates by runoff and domestic waste discharge near by the village of stream and minimum (39.4 mg/l) at site S_1 during winter season. Streams with limestone soil characteristics have high alkalinity and good

buffering capacity along with domestic sewage effluents drained directly into stream contributed the high alkalinity. The free CO_2 ranged from 0.87-2.96 mg/l during the study period. Higher free CO_2 in water samples in monsoon season was due to discharge of domestic waters, inflow of sewage and mostly due to decomposition of organic wastes on the site by enormous tourism activities. A similar trend of free CO_2 was reported by Khannna *et al.* (2006) in Suswa and Khanna *et al.* (2008) in Nalhota stream at Dehradun. Dissolved oxygen is an important factor to assess the biological productivity and ecological health status. Maximum DO (10.87 mg/l) was recorded during winter at site S_1 due to continue flow of stream with low temperature and minimum (7.24 mg/l) during monsoon at site S_4 due to increase in the temperature after the rainfall and decaying of macro-vegetations in the water. Mishra and Yadav (1978) reported same seasonal fluctuation of DO in river and lake water in Central India. Biochemical oxygen demand has contributed in estimating the pollution level and water quality of a particular water body. In the present investigation, BOD value ranged between 0.84 mg/l to 3.12 mg/l. The fluctuation in the value may be due to accumulation of maximum load of organic substances with microbial reactions at the littoral zone and bottom of stream. Similar trend of microbial degradation and increasing trends of BOD was obtained by William *et al.* (1993) The maximum value (88.64 mg/l) of calcium was reported at site S_5 during monsoon due to runoff water from rocks and constriction hotels and shops near the stream and minimum (83.62 mg/l) was recorded at site S_1 during winter. Similarly, Khanna and Singh (2000) reported the fluctuation in calcium and magnesium ion in Suswa river at Dehradun. Magnesium is also an essential and beneficial element but it is toxic at higher concentration. The maximum concentration (55.12 mg/l) of magnesium was recorded at site S_3 during monsoon and minimum (50.45 mg/l) recorded at site S_4 during summer. Jenkins *et al.* (1995) recorded similar findings in the streams of middle hills and high mountains of the Himalaya. Sodium is one of the most common cations has no adverse effect on human health at low concentration. The maximum concentration (18.77 mg/l) of sodium was observed at site S_4 during monsoon due to runoff in downstream and minimum (14.92 mg/l) was recorded at site S_1 during winter. Pande and Mishra (2000) observed similar trend of potassium deposition in Sahastradhara at Dehradun. Potassium is naturally occurring element, released by clay minerals; weathering and leaching from growing vegetation and decomposition of organic matter (Berndtsson, 1990). The potassium was recorded maximum (18.77 mg/l) at site S_5 during monsoon and minimum (7.66 mg/l) at site S_1 during winter. Bond (1979) observed similar nutrients concentration pattern in a stream ecosystem in Utah. Miller *et al.*, (1997) in Potomac river and Cameron (1996) reported sodium accumulation in aquatic system in their study. Chloride generally occurs in the form of chloride ion and is major inorganic anion present in natural water. The chloride was recorded maximum (16.86 mg/l) at site S_4 due to the runoff during monsoon and minimum (14.43 mg/l) at site S_1 during winter. Khanna and Singh (2000) found similar trend in River Suswa at Raiwala. Chopra and Patric (1994) reported the similar observation in the Ganga river at Rishikesh. Water temperature showed negative correlation with DO (-0.76) and Magnesium (-0.113), Free CO_2 and Chloride showed negative correlation with DO (-0.89). However, a positive correlation was found between Free CO_2 and water temperature (0.39) (Table-3).

Total 23 genera of macro-invertebrates were encountered in the stream (Table-2). The macro-invertebrates were represented by different groups *e.g. Oligochaeta*, *Plecoptera*, *Trichoptera*, *Diptera*, *Ephemeroptera*, *Odonata* and *Hemiptera*. Maximum contribution to total macro-invertebrates were observed by *Ephemeroptera* (23.94 %), followed by *Oligochaeta* (21.15 %), *Hemiptera* (14.89 %), *Odonata* (12.48 %), *Diptera* (10.13 %) *Plecoptera* (10.85 %) and *Trichoptera* (6.56 %). During study period high abundance of aquatic organisms were reported during winter season it may be due to low velocity of water, high dissolved oxygen, low hydro-median depth and low turbidity. However, the minimum abundance of aquatic organisms were observed in Sahastradhara stream during monsoon season which may be due to increased water velocity, high turbidity, low DO and low primary productivity. A significant difference in the density of macro-invertebrate was recorded between the sampling sites S_2 and S_3 which may be attributed to the anthropogenic disturbance by mass bathing activities, tourist movement, drainage of waste water from the near

by the hotels and restaurants into stream water at site S_2 and S_3. Such types of observation was also observed by Mitra (1999) and Sharma and Rawat (2009) with respect to the dragonflies (Odonata) of Asan wetland in the Central Himalayas. The macrophytes vegetation were observed near the stream from upstream to downstream were *Slix tetraspherma, Arundodonex, Epomoea carnea,*

Table-2: The distributional pattern of benthic organisms (ind./m^2) in Sahastradhara stream at Dehradun.

OLIGOCHAETA	S_1	S_2	S_3	S_4	S_5	Total %
Tubifex sp.	++	+++	++	+	+	21.15
Branchiora sowerbyii	++	++	+	++	++	
Limnodrillus hoffmeisteri	+	+++	+++	++	++	
PLECOPTERA						
Pteronarcys	+	-	++	++	++	10.85
Acroneuria	++	-	+++	++	+	
Isoperia	++	-	++	++	+	
TRICHOPTERA						
Hydrosyche	+	+++	++	++	++	6.56
Leptocella	++	+++	++	+	+	
Ochrotrichia	++	++	++	+	++	
DIPTERA						
Chironomous plumosus	++	+++	+++	++	++	10.13
Tendipestentans	+	++	++	++	+	
Culicoides	++	+++	++	++	+	
Alabesmyia	+	++	++	++	+	
EPHEMEROPTERA						
Adult Mayfly	+	+++	++	++	++	23.94
Stenononema	++	++	++	++	++	
Leptophlebia	+	++	++	++	+	
Ephemeralla indica	+	+++	+++	++	+++	
Cinygma	++	++	++	++	++	
ODONATA						
Epicordulia (Dragonfly)	+	+++	++	++	-	12.48
Macromia	++	++	+++	++	+	
HEMIPTERA						
Aquarius remigis (Water striders)	+	+++	+++	++	++	14.89
Sigara mckinstryi (Water boatmen)	+	++	+++	+	-	
Notonecta unifasciata (Backswimmers)	+	++	++	++	+	

+++: Abundant; ++: Common; +: Rare, -: Absent

Vitex negundo, Leucaena leucaena, Lannea grandis, Erythrina-suberosa, Bouhinia retusa, Giant napier, Eulaliopsis binata. Among all the species *Slix tetraspherm, Leucaena leucaera* and *Giant napier* were abundant and common near and n littoral zone of Sahastradhara stream.The proper monitoring of the stream is essential to know the current status of stream water quality for sustainable, holistic solid waste management and to treat the untreated domestic sewage wastes, which is directly drain into the stream water which is degrading the quality of the Sahastradhara stream water.

Table- 3: Correlation between physico-chemical parameters of Sahastradhara stream during 2009 – 2010

Parameters	Temp. ˚C	WV m/s	pH	Alk	CO_2	DO	BOD	Ca	Mg	Na	K	Cl
Temperature (˚C)	1											
Water Velocity (m/s)	-0.69	1										
pH	0.04	0.69	1									
Alkalinity (mg/l)	0.08	0.67	0.99	1								
Free CO_2 (mg/l)	0.39	0.41	0.94	0.95	1							
DO (mg/l)	-0.76	0.049	-0.68	-0.71	-0.89	1						
BOD (mg/l)	0.46	0.33	0.91	0.92	0.99	-0.92	1					
Calcium (mg/l)	0.27	0.51	0.97	0.98	0.99	-0.83	0.98	1				
Magnesium (mg/l)	-0.113	0.79	0.99	0.98	0.87	-0.56	0.83	0.93	1			
Sodium (mg/l)	0.37	0.42	0.94	0.95	0.99	-0.89	0.99	0.99	0.88	1		
Potassium (mg/l)	0.29	0.49	0.97	0.98	0.99	-0.84	0.98	0.99	0.92	0.99	1	
Chloride (mg/l)	0.39	0.39	0.93	0.95	0.99	-0.89	0.99	0.99	0.87	0.99	0.99	1

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Isolation and determination of biochemical nature of water soluble anticoagulant from earthworm

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Abstract

Earthworms are commonly known as farmer's friend. Various previous studies have confirmed the anti-inflammatory, analgesic, antipyretic and anticancer effects of earthworm extract. In the present study selected species of earthworm was used to study the anticoagulative activity of earthworm extract by APTT test. The study involves the extraction and isolation of anticoagulant from earthworm which was found to be in the form of DNA. In order to uncover the biochemical nature of this molecule, the anticoagulant was processed with various hydrolases such as Proteinase-K, Dnase, Rnase and lysozyme. Simultaneously APTT test and agarose gel electrophoresis were performed to confirm the results. Standard herring sperm DNA and yeast RNA were also used to compare the anticoagulative activities with that of anticoagulant purified from earthworm. Individual components of nucleotide were also checked which might be responsible for the anticoagulative capability.

Keywords: *Anticoagulant activity, APTT, DNA, Eudrilus eugeniae, Hydrolases*

Introduction

Earthworm has been recognized as an anti-inflammatory, analgesic and antipyretic agent (Noda *et al*., 1996). It shows anticancerous effect by preventing excess glucose uptake (Nagasawa *et al*., 1991). It is also implicated in hemostasis by acting either as a fibrinolytic or anticoagulatory agent which results in the facilitation of blood circulation (Wang *et al*., 1989). Anticoagulation activity was reported by Woo (1996) on an earthworm species *Lumbricus rubellus*. The earthworm has been suspected to contain proteases which specifically dissolve the fibrin clots or anticoagulant(s) which selectively interfere with the intrinsic pathway of the blood coagulation cascade (Mann *et al.*, 1990; Davie *et al.*, 1991; Leipner *et al.*, 1993; Kim *et al.*, 1995; Woo, 1996). Antimicrobial and Anti-inflammatory activities of earthworm, *Eudrilus eugeniae* were investigated by Mathur *et al*. (2010 a and b) Pharmaceutical significance of earthworm *Eudrilus eugeniae* was also reported by him. *Eudrilus eugeniae* is an African earthworm specie and is having good reproduction capability. As no work like the present investigation is reported till yet thus we have emphasized our study on this specie. The aim of our study was to investigate the anticoagulant activity from earthworm specie, *Eudrilus eugeniae* and to determine the biochemical nature of the anticoagulant.

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Materials and Method

Collection of material

Adult earthworms were provided by Jai Bharat Nursery, Rani Pokhari, Rishikesh (U.K), India. The worms were washed in order to remove the sand debris and were kept in N-saline for washing the gut. The step was repeated several times until the gut gets thoroughly cleared.

Purification of Anticoagulant

A detailed purification procedure (Woo, 1996) was adopted. The earthworms were homogenized in distilled water in the ratio 1:1 (w/v) followed by heat extraction at 100 ^{0}C for 30 minutes after centrifugation, the supernatant was subjected to ammonium sulphate fractionation at final concentration of 80 %. The precipitate was suspended in a minimum volume of 50 mM Tris

HCl (pH 8.0) and the anticoagulatory activities were measured by Activated partial thromboplastin time (APTT) test.

Activated Partial Thromboplastin time (APTT) test

An *in vitro* coagulation test of APTT was performed according to the manufacturer's instruction. The data was analyzed in percent coagulation time.

Agarose gel electrophoresis

1% agarose gel was prepared in 1X TAE buffer according to the standard protocol and the sample was loaded along with marker in the wells along with gel loading buffer. The observation of the bands was done under UV-transilluminator.

Treatment of anticoagulant with various hydrolases

The concentrated sample of 50 µl (0.92 mg/ml) was separately incubated with Proteinase-K (2 µg), DNase (5 µg) in the presence of 2mM $MgCl_2$, Rnase (5 µg) and lysozyme (20 µg) at 37^{o}C overnight in a total volume of 150 µl adjusted with 50mM Tris HCl (pH 8.0). Later on APTT test and agarose gel electrophoresis of the hydrolases digested samples were carried out.

APTT of Standard DNA, RNA and individual components of nucleotide

APTT test was performed according to the manufacturer's instruction available in the kit. Calf thymus DNA and Yeast RNA were used as positive standards. Pentose sugars (Deoxyribose and Ribose sugars), phosphoric acid nitrogenous bases (purines and pyrimidines) were used for the determination of APTT in order to assess the individual component responsible in anticoagulant activity of DNA.

Results and Discussion

From the present investigation it was revealed that the anticoagulant purified from earthworm extract was in the form of DNA which produced reddish-orange colored bands with ethidium bromide under UV transilluminator. When APTT test was performed of this extracted anticoagulant it showed 76.66% coagulation time (Table-1) with respect to Standard Diagnos Thrombo reagent (available in the kit).

To confirm our studies, when the anticoagulant was treated with various hydrolytic enzymes at 37^{0}C overnight, a diffused band of DNA was observed on treatment with Dnases under UV light, which confirmed our studies that the anticoagulant is in the form of DNA (Fig.1). When such Dnase treated sample was subjected for APTT the anticoagulant activity gets reduced. This decrease in value of APTT might be due to the digestion of anticoagulant (DNA) by Dnases (Table-2).

Table-1: Activated prothrombin thromboplastin time (APTT) of purified anticoagulant from earthworm extract

Reagent\Earthworm extract	Mean values of APTT (seconds)	% Coagulation time
Diagnos Thrombo Reagent	120	130.43
Earthworm extract	92	76.66

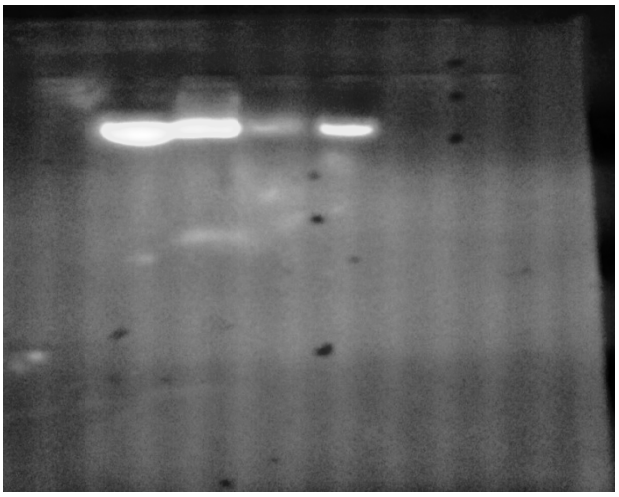

Fig.1: Bands of digested DNA after the treatment of hydrolases. (1, proteinase-k treated; 2, lysozyme treated; 3, Dnase treated; 4, Rnase treated)

Table-2: Activated prothrombin thromboplastin time (APTT) of hydrolases treated purified anticoagulant

Reagent\ Earthworm extract	Mean values of APTT(seconds) Hydrolases treated Dnase	Lysozyme	Proteinase -k	Rnase
Diagnos Thrombo Reagent	62	56	66	62
Earthworm extract	136	51	70	154

Our next question was whether this effect of the DNA was unique for the earthworm, *E.eugeniae*. When herring sperm DNA was used to measure its effect on the coagulation, the APTT value gets reduced *viz.* 45 seconds (Table-3) which confirmed our findings that anticoagulant (DNA) purified from earthworm, *E. eugeniae* is a potent anticoagulant. When the results of APTT of standard yeast RNA were compared with that of anticoagulant (DNA) purified from earthworm and herring sperm DNA, the coagulum appeared in no time in the plasma treated with RNA sample. Simultaneously no values of APTT were observed in pentose treated plasma sample (Table-3).

It may be due to the fact that single stranded RNA could be more compact than double stranded DNA. We assumed therefore that the effect of anticoagulation was due to negatively charged matrix provided by the extended DNA. If this assumption is valid, the DNA could be compared with heparin in terms of their anticoagulatory mechanisms. It has been already shown that thrombin inhibition by AT-III was accelerated in the presence of heparin since it provides a negatively charged matrix as a template (Ehrlich *et al.*, 1991; Nesheim, 1983).

Table-3: Activated prothrombin thromboplastin time of Standard DNA, RNA and components of nucleotide

Samples	Mean values of APTT (seconds)
Herring sperm DNA	45
Yeast RNA	NA
Nitrogenous bases	90
Phosphoric acid	36
Pentose sugar	NA

NA= No activity

We further confirmed that nitrogenous bases present in the DNA are the active components responsible for anticoagulant activity. The mean value of APTT was found to be 90 seconds much higher than that of phosphoric acid *viz.* 36 seconds (Table-3) while pentose sugar showed no values of APTT. Further studies are needed to refine the technique. Thus the present investigation revealed the fact that DNA could be considered as alternative thrombotic agent to heparin and various other anticoagulants used routinely in pathological labs.

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Mini Forest - An approach to evaluate the adaptability of Western Ghats species for afforestation

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Abstract

Saplings of forty nine species of trees from Western Ghats forests were planted on a 1.5 hectare tract of Deccan plateau (in the campus of Indian Institute of Science, Bangalore) and their performance was monitored for 23 years. The objective was to evaluate their adaptability to a habitat and conditions apparently alien to these species. The study was also meant to understand the linkages of these trees with the surrounding environment. Contrary to the belief that tree species are very sensitive to change of location and conditions, the introduced trees have grown as good as they would do in their native habitat and maintained their phenology. Further, they have grown in perfect harmony with trees native to the location. The results showed that the introduced species are opportunistic and readily acclimatized and grew well overcoming the need for the edaphic and other factors that are believed to be responsible for their endemicity. Besides *ex situ* conservation, the creation of miniforest has other accrued ecosystem benefits. For instance, the ground water level has risen and the ambient temperature has come down by two degrees.

Keywords: *Western Ghats, Ecological services, Mini forest*

Introduction

It is general belief that tree species are adapted to such specialized natural conditions that they are unsuitable for translocation, particularly to planting in urban environs. Contrary to this opinion, it has been observed in the present study that trees have a remarkable ability to adapt to change in locations which are totally alien, a fact that was demonstrated by scores of exotic species naturalised and flourishing in parts of the world other than the region of their origin or nativity (Sankara Rao, 2008, 2009, Hanumaiah *et al*., 1967). There has been an almost continuous process of introduction of alien trees into Karnataka state, especially to Bangalore (Hayavadana Rao, 1930). Within a short time, species such as Paper mulberry (*Broussonetia papyrifera* Vent.), Tabebuias (*T. aurea*, *T. chrysotricha*, *T. impetiginosa*, *T. pallida*, *T. rosea*), Leucaena (*Leucaena latisiliqua* L. Gillis) and some Australian Acacias (*Acacia auriculiformis* Cunn. ex Benth.) have come to dominate Bangalore's tree flora and become the principal cause for a number of native species in the city edging towards local extinction. There is a growing concern that we should be helping to maintain our native woodland land and in cities which are suffering from a continuous process of attrition, particularly in the urban spaces in the face of modern developments.

Flora of India belongs to diverse vegetation types. Virtually it supports every kind of vegetation, tree species, small and big, deciduous and those that remain leafy most part of the year. The species diversity is enormous and as such, there is no dearth for selection of species among these native trees for afforestation and urban greening. There is also the impending danger of climate change, which is likely to affect some of our native tree species and their phenology and thereby effecting further regeneration and continuity of the species, which would result in loss of diversity. It might therefore become necessary to bring different wild indigenous species to other locations and also into city confines where they might have better opportunity to thrive under a watchful eye.

With this conservation strategy in mind, creation of miniforest was mooted three decades ago at the Centre for Ecological Sciences (CES), Indian Institute of Science (IISc), Bangalore and tree species of Western Ghats forests were sought to be evaluated for their performance in the Deccan plateau region of which Bangalore is a part. A small vacant space (about 1.5 hectare) that was beset with scrub vegetation opposite to the CES in the campus of Indian Institute of Science was

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chosen for planting tree saplings from the forests of the Western Ghats that came to be known as the miniforest. Saplings (480 no's.) belonging to forty nine species (Table-1) which were raised at the CES Field Station Nursery at Sirsi, Uttara Kannada district were obtained and planted along with few species already existing on the plot with a spacing of 3 x 3 m.

Figure 1: Picture showing the type of terrain on which the miniforest was raised

Table 1: List of tree species in the miniforest

S No.	Tree Species
1	*Adenanthera pavonina* L.
2	*Adina cordifolia* (Roxb.) Hook.f. ex Brandis
3	*Ailanthus triphysa* (Dennst.) Alston
4	*Albizia amara* (Roxb.) Boiv.
5	*Alstonia scholaris* (L.) R. Br.
6	*Areca catechu* L.
7	*Artocarpus heterophyllus* Lam.
8	*Artocarpus hirsutus* Lam.
9	*Artocarpus lacucha* Roxb. ex Buch.-Ham.
10	*Bambusa arundinacea* (Retz.) Willd.
11	*Bombax malabaricum* DC.
12	*Broussonetia luzonica* Bureau
13	*Butea monosperma* (Lam.) Taub.
14	*Calamus prasinus* Lak. & Renuka
15	*Calophyllum apetalum* Willd.
16	*Calophyllum inophyllum* L.
17	*Cananga odorata* (Lam.) Hook. f. & Thoms.
18	*Canarium strictum* Roxb.
19	*Ceiba pentandra* (L.) Gaertn.
20	*Chukrasia tabularis* A. Juss.
21	*Commiphora wightii* (Arn.) Bhand.
22	*Duabanga grandiflora* (Roxb. ex DC.) Walp.
23	*Elaeocarpus serratus* L.
24	*Elaeocarpus tuberculatus* Roxb.
25	*Entada rheedei* Spreng.
26	*Ficus benghalensis* L.
27	*Ficus racemosa* L.
28	*Garcinia indica* (Thouars) Choisy
29	*Holigarna grahamii* (Wight) Kurz
30	*Holigarna arnottiana* Hook. f.
31	*Hopea ponga* (Dennst.) Mabb.
32	*Lagerstroemia lanceolata* Wall. ex C. B. Clarke
33	*Lophopetalum wightianum* Arn.
34	*Madhuca longifolia* (Koenig) Macbr.
35	*Mallotus philippensis* (Lam.) Muell.-Arg.
36	*Mangifera indica* L.
37	*Memecylon umbellatum* Burm. f.
38	*Mimusops elengi* L.
39	*Mitragyna parvifolia* (Roxb.) Korth.
40	*Pajanelia longifolia* (Willd.) K. Schum.
41	*Sterculia guttata* Roxb. ex DC.
42	*Syzygium cumini* (L.) Skeels
43	*Syzygium laetum* (Buch.-Ham.) Gandhi
44	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.
45	*Terminalia crenulata* Roth
46	*Vateria indica* L.
47	*Vitex altissima* L.f.
48	*Xylia xylocarpa* (Roxb.) Taub.
49	*Ziziphus rugosa* Lam.

The area encompassing Western Ghats is recognised as one of the most eco-sensitive regions of the world and is one among the 34 biodiversity hotspots on the basis of its species richness (Myers *et al.*, 2000). Western Ghats run along the West

coast of India from the Vindhya-Satpura ranges in the North to the southern tip of the peninsula to a stretch of 6000 km, covering an area of nearly 1, 59,000 sq. km and consist of mountains ranging from 50 m to 2695 m in height. Western Ghats receive an average of 6000 mm of rainfall every year. The vegetation is quite diverse, broadly having evergreen, semi-evergreen, deciduous, scrub forests, sholas, grasslands and bamboo clumps. Factors including sunlight, rainfall, humidity, altitude, topography and location contribute to the uniqueness of this habitat, its animal and plant diversity. Plants such as *Holigarna grahamii* (Wight) Kurz, *Garcinia* sp., *Mitragyna parvifolia* (Roxb.) Korth., *Lophopetalum wightianum* Arn., *Syzygium leatum* (Buch.-Ham.) Gandhi, *Entada rheedei* Spreng., *Calamus prasinus* Lak. & Renuka and the like represent evergreen, semi evergreen and moist deciduous species of the Western Ghats (Pascal and Ramesh, 1987; Pascal, 1988). Outside the habitats in Western Ghats and the unique climatic and edaphic factors therein, these species are not generally found thriving in other plateau regions.

It is observed that in less than 25 years, the experimental plot, now termed 'Miniforest' on account of the limited area, is transformed into a lush green forest on a terrain that was originally a scrub vegetation of the Deccan plateau type with apparently conditions alien to most of the species that have been introduced. The miniforest, in this respect, presented an opportunity to study the adaptations and succession of the Western Ghats forest species (Table-1) in comparison with native species existing in the area. The species composition that emerged in the experimental plot is quite interesting. Majority of them are the Western Ghats species whereas the others, the native to scrub vegetation, both found growing in perfect harmony, in spite of the difference in rainfall (850 mm), humidity, temperature and soil conditions for the former species (Fig.-2). The miniforest trees exhibited normal robust growth, flowered and set fruit as they would do in their native habitat. Some of the trees, for example *Mitragyna parvifolia* (Roxb.) Korth., *Chukrasia tabularis* A. Juss., *Duabanga grandiflora* (Roxb. ex DC.) Walp., *Garcinia indica* (Thouars) Choisy, *Holigarna grahamii* (Wight) Kurz, *Lophopetalum wightianum* Arn. and *Syzygium laetum* (Buch.-Ham.) Gandhi (Fig.-3) have grown as well as they would do in the evergreen forests.

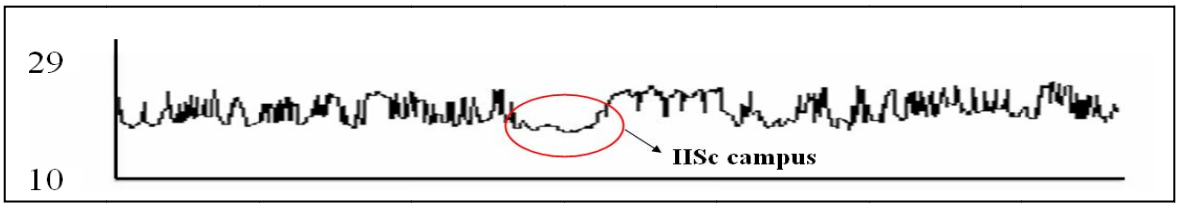
Figure 2: A view of Miniforest

A gigantic liana *Entada rheedei* Spreng., that was not known to grow outside the moist forests has thrived very well and spread prolifically to nearby areas (Maheshwari *et al.*, 2009) and flowered since 2001 (Fig.-3). *Calamus prasinus* Lak. & Renuka, being a rattan, which is rarely reported to survive in drier tracts, has also grown considerably well exhibiting normal flowering (Bhat, 2003). These observations provide evidence that most of the trees of the Western Ghats forests are opportunistic and grow under factors largely different from those believed to be responsible for their endemicity. A microclimate prevails in the plot, the miniforest. There is a slight dip in temperature, an increase in humidity and humus enrichment on account of the survival of many moist evergreen species and their good canopy cover. The miniforest plot is kept undisturbed. Progressively, the area developed rich micro- and macro-fauna, from insects, frogs, snakes to birds and smaller mammals like the most elusive Slender Loris. Smaller plants such as mosses, algae, fungi, ferns, herbaceous plants and climbers have grown well adapting to the change. The entire plot is amazingly transformed into the type of a habitat that prevails in the moist forests of Western Ghats. Other ecological benefits have resulted from creating the miniforest. Temperature profile analysis through the computation of Land Surface Temperature (LST) using LANDSAT ETM thermal regions (Fig. 4). The water table at this location was

Figure 3: A gigantic liana *Entada rheedei* Spreng. (with fruits)

in the range of 60-70 m depth before creating the miniforest. Present monitoring of water table has showed that the level of water is at about 3 to 3.5 m below the ground. This indicates that land cover dynamics play a decisive role in recharging the groundwater sources. Four families of Slender Loris (*Loris tardigradus*) inhabiting here is an indication of total wilderness prevailing in the miniforest, further confirming the ecological richness of the habitat.

Figure 4: Temperature profile of IISc campus (Transect passing through miniforest)



***Syzygium laetum* (Buch.-Ham.) Gandhi**

***Lophopetalum wightianum* Arn.**

***Holigarna arnottiana* Hook. f. (Fruiting)**

***Garcinia indica* (Thouars) Choisy (Fruiting)**

Figure 5: Evergreen species of miniforest

The results have further showed that the experiment of the miniforest can be replicated to create such green pockets in and around other urban spaces. This kind of green patch not only can be an arboretum for evergreen tree species but also serves as a home for several refuge fauna and adaptable

species. The patch will also serve as an efficient carbon sink, trapping free carbon in the atmosphere, bringing the temperature to less than a degree, thus helping in mitigating climate change issues. Similar experiments also can be valuable in establishing germplasm banks to offset any loss of species in the wild due to climate change and other factors.

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Environmental assessment of Tapti river water quality in Betul district, M.P. India

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Abstract

Tapti river water is the main source for drinking, irrigation, fish culture and other important activities in Central India. Hence the present investigation has been done to observe the chemical and physical constituents of Tapti river water flow. The quality of water with the view to being out a transparence image of the water pollution and its effect and bringing forth suggestions for improvement. Samples were collected from different monitoring points of the Tapti water flow on bimonthly basis.The sample collection, preservation and pre-treatment has been done according to standard methods. Prior to this a survey was conducted to know about probable pollution sources and other relevant features.

Keywords: *Dissolve oxygen, BOD, COD, Turbidity, Total hardness*

Introduction

The Tapti river originates in the Betul district from place called Multai. It is one of the major rivers of peninsular India with a length of around 724 km. It is one of the three river, others being Narmada and Mahi river that runs from east to west.

The Tapti river basin extends over an area of 65, 145 km^2 which is nearly two percent of the total area of India. The basin lies in the states of Maharashtra (51,504 km^2), Madhya Pradesh (9,804 km^2) and Gujarat (3,837 km^2). It covers Betul, Burhanpur district of M. P., rest of Maharashtra and Gujarat.

Materials and Method

The Tapti river has been surveyed throughout the year, covering a distance of about 20 km. Three sampling sites were selected. Site I^{st} is situated at Multai where river originates, here people take bath and wash their clothes daily. Site II^{nd} is situated at Kerpani and Site III^{rd} Kolgoan where continuous discharge of chemicals and sewage take place. Physical and biological parameters were studied as per method suggested by APHA, (1995) and Trivedi and Goel (1986), Manivaskam (2002) and Khanna and Bhutiani (2004). pH and dissolved oxygen were recorded immediately after collection of samples on the site.

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Results and Discussion

The survey of the river water resources includes the identification and characterization of three sites (Table–1).Colour of sewage at Kolgaon site is brown and all the sites have fairly bad smell. Values of pH (7.3 – 8.4) have been observed at the polluted site throughout the year. Low dissolved oxygen values (1.33) was recorded at Multai site. The value of D.O. ranged from 13.3 to 18.90. The low D.O value may be due to oxygen demand of microorganism. The values of chloride is more in pre monsoon (173.48 -253.51) compared to post monsoon (110.0-143) the value of C.O.D (53.83-790.67) and the values of BOD ranged from 2.27-29.40 recorded at all the sites. High values (56 ppm) of free CO_2 were recorded at Kolgaon site. High values (308.67) of bicarbonate alkalinity were observed at Multai but low value (37.33) of carbonate alkalinity was observed at polluted site in monsoon season.The chemical analysis showed that polluted site II^{nd} and III^{rd} containes high value of chloride, total hardness and bicarbonate alkalinity and exhibited a high biochemical oxygen demand, but dissolved oxygen was recorded in low range, which indicated a high pollution load.The high COD show the presence of accumulated organic matter, which reflects its incomplete oxidation. The cause of high BOD may be due to excessive growth (eutrophication) of aquatic fauna (Hasan and Pande, 1983). Total viabal counts, *E-coli* counts were highest in post monsoon season.

Table 1: Mean value of physio-chemical parameters sampled at different sites of River Tapti, Multai, Distt. Betul

S. No.	Parameters	Pre Monsoon			Monsoon			Post Monsoon		
		Multai	Kerpani	Kolgaon	Multai	Kerpani	Kolgaon	Multai	Kerpani	Kolgaon
1	TDS	533.33	366.67	366.67	580.00	533.33	583.33	266.67	366.67	323.33
2	pH	7.17	7.83	8.40	6.63	7.33	7.40	6.70	6.80	7.30
2	EC	0.62	0.63	0.50	0.51	0.33	0.26	0.79	0.77	0.60
4	Turbidity	0.00	10.33	3.33	6.67	5.00	7.33	0.00	0.00	0.00
5	Ca hardness	186.67	165.33	93.33	392.00	360.00	354.67	600.00	480.00	197.33
6	Mg hardness	229.33	173.33	234.67	162.67	130.67	157.33	269.33	293.33	160.00
7	Total hardness	426.67	366.67	328.00	458.67	389.33	432.00	357.33	293.33	176.00
8	Carbonate alkalinity	1.33	4.00	9.33	0.00	1.33	34.67	5.33	7.33	0.00
9	Bi-carbonate alkalinity	308.67	289.33	264.67	49.33	37.33	166.67	188.00	110.00	74.67
10	Total alkalinity	310.00	293.33	271.33	134.67	246.67	233.33	245.33	325.33	114.67
11	D.O.	11.37	15.60	16.27	18.00	18.90	7.20	1.33	9.00	7.83
12	BOD	2.70	9.60	7.60	14.80	15.93	29.40	2.27	2.40	0.20
13	COD	210.67	88.00	53.33	456.00	356.00	504.00	762.67	790.67	766.67
14	Free CO_2	16.00	17.33	10.67	41.33	28.00	56.00	2.00	4.33	0.00
15	Chloride	173.48	210.17	253.51	104.00	164.67	147.33	146.67	133.33	110.00
16	Residual chloride	0.00	0.00	0.00	2.12	2.60	1.95	2.99	2.88	2.18
17	Acidity	356.67	290.00	136.67	30.00	2.67	4.00	0.00	0.00	0.00
18	Ammonium nitrate	0.90	1.87	0.70	0.53	1.33	1.03	1.33	2.67	1.33
19	Total iron	1.17	1.47	1.53	0.08	0.08	0.08	1.50	1.40	1.37
20	Phosphate	0.10	0.13	0.17	0.50	0.60	0.73	0.50	0.20	0.33
21	Sulphide	0.00	0.00	0.00	44.80	38.40	36.67	18.67	32.00	22.40
22	Zinc	0.18	0.04	0.06	0.10	0.12	0.13	0.13	0.12	0.10
23	Copper	0.13	0.05	0.04	0.07	0.06	0.37	0.30	0.05	0.06
24	Magnesium	0.13	0.07	0.08	0.07	0.11	0.10	0.24	0.20	0.09
25	*E. coli*	60000	128000	96667	97000	20833	53467	720000	171333	46667

All data are in mg/l except pH and *E. coli*= unit/l

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Liquid bio-medical waste management strategy

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Abstract

Bio-medical waste has become a major concern in the world over as it poses serious environmental hazard. The collection and disposal of bio-hazardous liquid can pose a significant risk and occupational challenge to hospital staff as microbial content in this waste may contain significant pathogens. Occupational risks associated with working in Health Care Establishments (HCE's), need to take proper precautions in handling any material from these centers.The scope of this study is limited to bio-medical liquid waste management as per Bio-medical waste (Management & Handling) Rules, 1998 prescribed by CPCB. The objective of this study was to assess the waste handling and treatment system of hospital biomedical liquid waste & its mandatory compliance with Regulatory Notification of Bio-medical waste (Management & Handling) Rules, 1998, under Environmental Protection Act-1986, Ministry of Environment and Forestry, Government of India. In accordance with rules, every hospital generating liquid BMW needs to set up requisite treatment facilities of BMW in site. Here we have carried out detailed field study for liquid bio-medical waste in selected HCE's for quantification and characterization of liquid medical waste streams from the different facilities *i.e.* operation theatre laboratories *etc*. Also study the existing wastewater management system of these selected HCE's. To assess the feasibility of discharging the liquid bio-medical waste into sewer, with or without treatment and if treatment is required then use the techno-viable treatment schemes for HCE's.

Keywords*: Biomedical waste, Health care establishment, Effluent treatent plant*

Introduction

Bio-medical waste has become a major concern world over as it poses serious environmental hazards. The collection and disposal of bio hazardous liquid can pose a significant risk and occupational challenge to hospital staff as microbial content in this waste may contain significant pathogens. Occupational risks associated with working in Health Care Establishments (HCE's), need to take proper precautions in handling any material from these centers. In India, very little has been done in the area of BMW management so far Notification (1998). Regulations for management of BMW are different all over the world but the risk of exposure is almost same for health care workers.

The comprehensive interim policy has been developed to provide standards and guidelines for collection, storage, handling, treatment and disposal of BMW along with safety and precautionary measures for all HCE's in western countries.

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However in Indian legislative framework there is no comprehensive guideline for occupation health and safety aspect.The scope of study is limited to Bio-medical liquid waste management as per Biomedical Waste (Management and Handling) Rules, 1998 prescribed by CPCB. The present study was undertaken with following objectives:-

To identify common type of HCE's existing and clubs them into broad categories.

To carry out detailed field study for liquid bio-medical waste in selected HCE's for quantification and characterization of liquid medical waste streams from the different facilities *i.e.* operation theatre, laboratories *etc*.

To study the existing wastewater management system of selected HCE's and also to analyze the efficacy of the ETP's, if existing.

To assess the feasibility of discharging the liquid bio-medical waste into sewer, with or without treatment.

To come out with the techno-viable treatment schemes for HCE's, if the treatment is required.

To recommend comprehensive wastewater management system for HCE's.

Quantification and Characterization of Liquid bio-medical waste-detailed field study.

The detailed field studies of selected HCE's were conducted to generate base line data on water use pattern, wastewater generation and characterization of wastewater streams from different streams/sections of the establishment and the existing liquid BMW management system.

Quantity of wastewater generated was assessed by interacting with concerned medical staff and on site assessment by knowing actual washing duration and pattern. An assessment was made regarding the feasibility of discharging of liquid waste to the sewer with or without treatment so that it meets the prescribed discharge standards.

Materials and Method

Main Sources of Liquid Bio-Medical Waste

It is the waste which is generated from the different sources from the HCE's as given in Table 1

Table-1: Sources of Bio-Medical Liquid Waste Generation

S.No.	Section	Source
1.	Microbiology lab	Spent savalon solution used for soaking vials Wastewater during vials washing Wastewater during bottle washing Wastewater during dish washing
2.	Operation theatre	Wastewater during scrubbing and instrument washing Body fluids generated during operations Wastewater from bleach wash of clothes used in operation theatre
3.	Bio-chemistry lab	Wastewater generated during the testing Wastewater generated during instrument washing
4.	Blood bank	Hypo solution used in disinfecting of used syringes
5.	Histo-pathology lab	Spent formaldehyde solution Spent alcohol solution Spent acetone solution Spent xylene solution Spent dye solution

Hazards from Liquid Waste

Contaminated liquid is produced by wards treating patients with enteric diseases and is a particular problem during outbreak of diarrhoea disease. Radioactive isotopes from oncology department could cause risk to human health, ehen found its way to sewer etc, but can be minimized by suitable measures. The toxic effect of any chemical pollutant in liquid waste can cause retardation of the active bacteria in sewage purification process which may give rise to additional hazards.

Legislative Framework for Liquid BMW

As per the BMW Rules, 1998 and amendment there of all liquid medical waste must be disinfected by chemical treatment using at least 1% hypo-chlorite solution or any other equivalent chemical reagent before discharge in to drains, CPCB (2000). The liquid chemical waste should be neutralized before discharged into drains. According to schedule-III of above rules, standards for effluent generated from hospital are as below:-

Table-2: Standards for effluent generated from hospital

Parameters	Permissible Limit
pH	6.5-9.0
Suspended solid	< 100 mg/l
Oil & grease	< 10 mg/l
BOD	< 30 mg/l
COD	< 250 mg/l
Bioassay test	90% survival of fish after 96 hrs

These limits are applicable to those hospitals, which are either connected to sewers without terminal sewage treatment plant or not connected to public sewers U.S. Environment protection agency (1986).

Treatment of Wastewater

In modern society proper management of wastewater is a necessity, not an option. Wastewaters are usually classified as industrial wastewater or municipal wastewater. Many industrial wastewaters require pre-treatment to remove non-compatible substances prior to discharge into the municipal system. Characteristics of industrial wastewater vary greatly from industry to industry and consequently, treatment processes for industrial wastewater also vary. A wastewater treatment system is composed of combination of unit operations and unit processes designed to reduce certain constituents of wastewater to an acceptable limit. The treatment systems are often divided into primary, secondary and tertiary systems.

Pre-Treatment of Wastewater

This stage is the key to the whole management process, because at this stage wastes are segregated into infectious and non-infectious, thus minimizing the risks to staff and public as well as resources used for the treatment purpose. Segregation of waste allows special attention to be given to the relatively small quantities of wastes.

Segregation starts mainly with doctors and nurses, and therefore they should be made aware of the important responsibilities that lies upon them.

The containers for storing segregated waste should be clearly identifiable. The best system is to use colored plastic bags/containers. The color coding and types of containers shall be followed as per the schedule II of Bio-medical Waste (Management & Handling) Rules, 1998, MoEF, (1998).Sharps need special attention while segregating and storing because needles can act as reservoirs of pathogens in which the pathogens may survive for a long time because of the presence of blood, and also that the sharps can provide a direct route into the bloodstream by puncturing the skin. Syringe and needles should be damaged before putting into the containers, so that rag pickers cannot collect them for the purpose of resale, which may get recycled at some later stage. Sharps must always be kept in puncture-proof containers to avoid injuries and infection to those handling them.Plastic bags for storing the waste may be suspended inside a frame or be placed inside a study container. A lid should be provided to cover the opening of the bag at the top. Every room such as ward, laboratory, operation theatre, etc. should have containers/bags for the types of wastes that are generated in that room. In all rooms except isolation wards there should be a container for general waste. All wastes from isolation wards should be regarded as infectious waste. Each container may be clearly labeled to show the ward or room where it is kept. The reason for this labeling is that it may be necessary to be able to trace the waste back to its source. For example, if a porter is injured by a syringe or blade that has been put into a bag rather than into the correct sharps container, it is possible to determine the origin of that waste and identify the member of staff who was responsible for that ward. It may also help in knowing the type of infection that may have been transmitted.

Primary Treatment of Wastewater

The purpose of primary treatment is to remove solid materials from the incoming wastewater. Large debris may be removed by screen or may be reduced in size by grinding devices. A typical primary system should remove approximately one-half of the suspended solids in the incoming wastewater. Wastewater contains a wide variety of solids of various shapes, sizes and densities. Effective removal of these solids may require a combination of unit operations such as screening, grinding and settling.

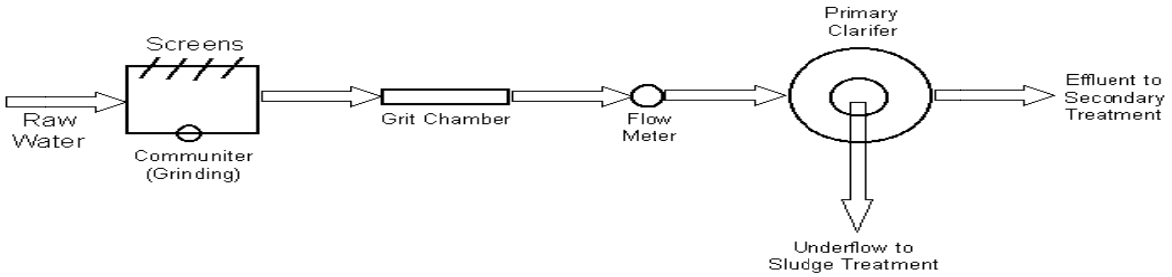


Fig. 1: Primary Treatment of Waste Water

A) Screening Screening devices are used to remove coarse solids from wastewater. A coarse solid consists of sticks, rags, boards and other large objects. Wastewater screens are classified as fine or coarse, depending on their construction. Coarse screens usually consists of vertical bars spaced 1 or more cm apart and inclined away from the incoming flow. Fine screens usually consist of woven-wire cloth or perforated plates mounted on a rotating disc or drum partially submerged in the flow, or on a traveling belt. Screening devices are contained in rectangular channels that receive the flow from the collection system. Proper ventilation must be provided to prevent accumulation of explosive gases. Hydraulically, flow velocity should not exceed 1m/s (3.3ft/s) in the channel. Clean bars and screens result in a head loss of less than 0.1m. The quantity of solids removed by screening depends primarily on screen opening size. Screened solids are coated with organic material of a very objectionable nature and should be promptly disposed of to prevent a health hazard Gayatri *et al.* (2004).

B) Grit Removal Wastewater consists a wide assortment of inorganic solids such as pebbles, sand, silt, eggshells, glass and metal fragments. Most of the substances in grit are abrasive in nature and will cause accelerated wear on pumps and sludge-handling equipment with which it comes in contact. Grit deposits in areas of low hydraulic shear in pipes, sumps and clarifiers may absorb grease and solidify. Grit removal facilities basically consist of an enlarged channel area where reduced flow velocities allow grit to settle out. The deposited grit is removed by mechanical scrapers. Hydraulically, grit chambers are designed to remove, by type-1 settling, discrete particles with diameters of 0.2 mm and specific gravity of 2.65. Since a wide variation in flow rates may be encountered, the horizontal velocity must be artificially controlled. In larger treatment plants, the trend is toward aerated grit chambers. Turbulence created by the injection of compressed air keeps lighter organic material in suspension while the heavier grit falls to the bottom. Adjustment of air quantities provides settling control. If the sewage is anaerobic when it arrives at the plant, aeration serves to strip noxious gases from the liquid and to restore it immediately to an aerobic condition, which allows for better treatment. Grit, particularly from channel-type grit chambers, may contain a sizeable fraction of biodegradable organics that must be removed by washing.

Secondary Treatment of Wastewater

The effluent from primary treatment still contains 40 to 50% of the original suspended solids and virtually all the original dissolved organics and inorganic. To meet the minimum EPA standards for discharge, the organic fraction, suspended and dissolved, must be significantly reduced. This organic removal, referred to as secondary treatment, may consist of chemical-physical processes or biological processes. Combinations of chemical-physical operations such as coagulation, micro screening, filtration, chemical oxidation, carbon adsorption, and other processes can be used to remove the solids and reduce the BOD to acceptable limits.

In biological treatment, microorganisms use the organics in the wastewater as a food supply and convert into biological cells, or biomass. Because

wastewater contains a wide variety of organics, a wide variety of organisms, or a mixed culture, is required for complete treatment. Most mixed cultures also contain grazers, or organisms that prey on their species. The microorganisms involved in wastewater treatment are essentially the same as those that degrade organic material in natural freshwater systems Singh and Sharma (1996).

Activated Sludge Process – The process derives its name from the fact that setteled containing living or active, microorganisms is returned to the reactor to increase the available biomass and speed up the reactions. The process is aerobic, with oxygen being supplied by dissolution from entrained air.
The rate at which oxygen is consumed by the microorganism in the biological reactor is called the oxygen utilization rate. For the activated sludge process, the oxygen utilization rate will always exceed rate of natural replenishment, thus some artificial means of adding oxygen must be used. With the exception of the pure oxygen system, oxygen is supplied by aerating the mixed liquor in the biological reactor. Aeration techniques consist of using air diffusers to inject compressed air into the biological reactor and/or using mechanical mixers to stir the contents violently enough to entrain and distribute air through the liquid.

Tertiary Treatment of Wastewater

The secondary effluent will probably contain at least 20mg/l suspended organic matter, which is to high for efficient disinfection. It should therefore be subjected to tertiary treatment, such as lagooning, if no space is available for creating a lagoon, rapid sand filtration may be substituted to produce a tertiary effluent with a much reduced content of suspended organic matter (< 10 mg/l).

A) Disinfection The disinfection of wastewater is usually required where portions of the effluent may come in contact with humans. Chemical oxidants are generally considered the most effective disinfectants, with required dosages being much higher than those used for cleaner water. Chlorine is the most common disinfectant in use.

B) Chlorine Disinfection To achieve pathogen concentration comparable to those found in natural waters, the tertiary effluents would be subjected to the breakpoint. This may be done with chlorine dioxide, sodium hypochlorite or chlorine gas. Another option is UV light disinfection.

C) Lagooning In a region or a regular health care establishment that cannot afford sewage treatment plants, a lagooning system is the minimal requirement for treatment of wastewater. The system should comprise two successive lagoons to achieve an acceptable level of purification of bio-medical sewage. Lagooning may be followed by infiltration of the effluent into the land, benefiting from the filtering capacity of the soil.

General Preservation Schemes

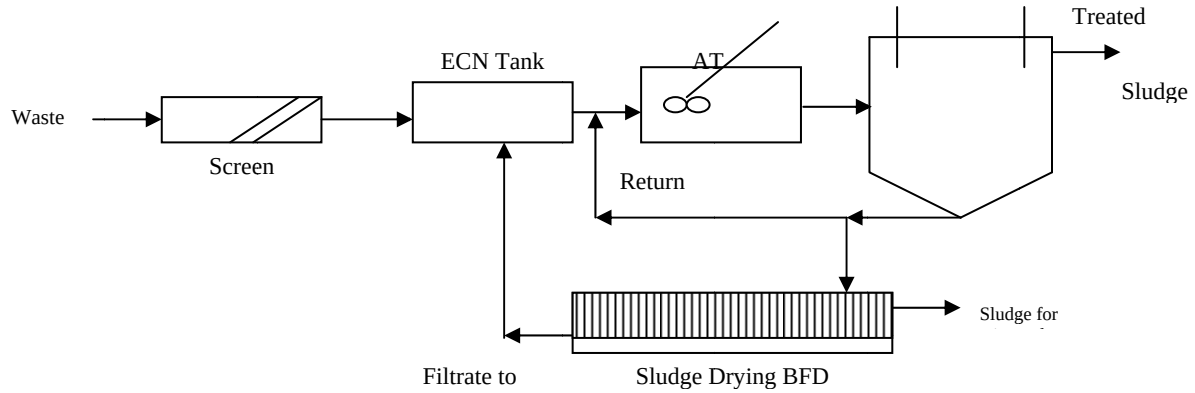
To minimize the potential for volatilization or biodegradation between sampling and analysis, keep samples as cool as possible without freezing. Avoid using dry ice because it will freeze samples and may cause glass containers to break. Keep composite samples cool with ice or a refrigeration system set at 4˚C during composting. Analyze samples as quickly as possible on arrival at the laboratory. Use chemical preservations only when they are shown not to interfere with the analysis being made. When they are used, add them to the sample bottle initially so that all sample portions are preserved as soon as collected. Because a preservation method for one determination may interfere with another one, samples for multiple determinations may need to be split and preserved separately.
Methods of preservation are relatively limited and are intended generally to retard biological action, retard hydrolysis of chemical compounds and complexes and reduced volatility of constituents Acharya and Singh (2000). Preservation methods are limited to pH control, chemical addition, and the use of amber and opaque bottles, refrigeration, filtration and freezing.

Activity Description

The study was undertaken with the objective to quantify and characterize liquid medical wastewater from different facilities so as to assess the wastewater treatment requirement.

Results and Discussion

The body suction fluid should be disinfected before being discharged to the drain.



It is recommended to put more bleach during the bleach wash of clothes in operation theatre. It will not only reduce the MPN, COD and BOD values of the wastewater but also improve the bleaching of the clothes.The wastewater from the bio-chemistry lab should be disinfected before being discharged to the drain.

It is recommended to avoid the discharge of spent formaldehyde, alcohol, acetone, xylene and dye solutions to the drain as these solutions will render very high COD and BOD values to the wastewater. Since the quality of solution is very less, these solutions may be incinerated at scientifically designed incinerator Baveja *et al*. (2000).

Effort may be made to reduce the quality of wastewater generation in the laundry section. From the table given above, it can be seen that the final outlet wastewater is already complying with the applicable sewer discharge standards having terminal ETP, thus the wastewater can be discharged to municipal sewers without any further treatment. However, since the MPN value for the wastewater is quite high it is recommended to have an arrangement for disinfecting the wastewater before being finally discharged into sewer.

However, in case the sewer is not connected with the terminal ETP, the wastewater doesn't comply with the standard. Therefore the total wastewater from the entire hospital, including general wastewater (kitchen, toilets etc), is required to be treated to meet surface water discharge standards. The proposed treatment scheme for the total wastewater is as given below.

A) Screening – Wastewater should be passed through screens from different sources to remove particles larger than the screen size. The coarse screen should be placed nearer to the source of generation and the fine screen should be placed in the combined drain of all sources.

B) Equalization Tank – The wastewater from different sources should be passed into an equalization tank to homogenize the waster characteristics. Residence time of one day needs to be provided for homogenizing characteristics of the wastewater.

C) Activated Sludge Process (extended aeration system) Aeration tank – The wastewater from equalization tank has to be pumped into an aeration tank.

The wastewater needs to be aerated using surface/diffused aerators to supply oxygen for the respiration and growth of bacteria. The aeration should ensure dissolved oxygen content between 1-2mg/l to enable growth of bacteria and help flocculate and settlement of sludge. Residence time of 4hrs should be provided for the wastewater. MLSS in the range of 3500 mg/l has to be maintained in the tank.

Settling tank/clari-flocculator – The biodegraded wastewater from aeration tank has to be passed into a settling tank or clari-floccculator. The supernatant from settling tank will overflow as treated wastewater. The bottom sludge will be pumped out. Part of the sludge pumped will be recycled for maintaining MLSS in aeration tank. When the MLSS exceeds the design limit the excess sludge should be disposed of into sludge drying bed.

Sludge drying bed – The excess sludge should be disposed into a sludge drying bed for solar drying. Once the sludge is dried it has to be removed and the bed prepared for disposing another batch of sludge. Typical drying times for sludge may vary between 4-7 days at accordingly number of beds should be provided.

Conclusion

At present in most HCE's the wastewater generated from different sources is directly (after disinfections) discharged into municipal sewer without segregation at source.Waste water characteristics shows two broad trends:

High volume and low concentration

Low volume and high concentration

The glassware and other sample testing apparatus are disinfected with hypo cleaned with (germicide) washed with soap solution before being reused.

This washing activity leads to generation of high amount of wastewater.

The wastewater from X-rays ultrasound laboratories arises from developed and fixer spent solution .The fixer spent solution is sold out for extraction of silver but the developer spent solution is discharged in drain, which is low in volume and high in strength.

In OPD the wastewater arise from needles; gloves washing etc. in tap water and some components are dipped in 1% hypochlorite solution for 3-4 hrs before washing in tap water.

The wastewater from the laboratories of manufacturing drugs, vaccines etc have high pollution load.

There is lack of awareness regarding proper waste management among health care professional.

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Analysis of cyanophycean biodiversity in Munshi Hussain tank, Bhopal

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Abstract

The present study focused on cyanophitic diversity of Munshi Hussain Tank. In this study an attempt has been made to identify the taxa of Cyanophyta in Premonsoon, monsoon, summer and Post monsoon seasons *i.e.* from July 2007 to June 2009. A total of 61 species of cyanophyta were observed during the course of study. Oscillatoria, Microcystis and chlorococcus were found as dominant genera.

Keywords: *Cyanophyta*, *Bhopal*, *Munshi Hussain tank*, *Biodiversity*

Introduction

Plankton are primary producers responsible for a large part of the Earth's global primary photosynthetic production. These organisms are thus the objects of intensive multidisciplinary studies at different levels of organization, from molecular genetics and physiology to population dynamics and community ecology. The success of these photosynthetic organisms lies in their ability to use solar energy and nutrients and to cope with a fluctuating environment. Thus, light, nutrients, and water mixing plays a key role in the evolution of their life history traits, their physiology and their ecology. Moreover, in recent decades, ecologists have considered to an increasing extent their interactions with other biological communities, as herbivores or decomposers.

Cyanophyta is a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in freshwater, marine and terrestrial habitats. Cyanophyta have been identified as one of the most promising group of prokaryotes from which various biologically active natural products were isolated. Cyanophyta from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms. Recently compounds from cyanophyta have been isolated which display inhibitory effects on bacterial growth, on mycobacterium species on fungal growth, on cancer cells and against viruses and enzymes.

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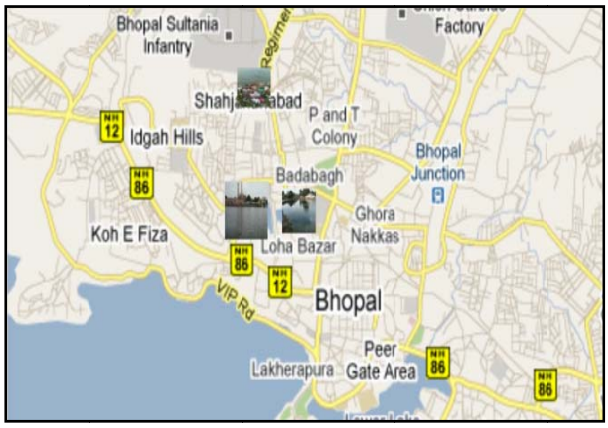
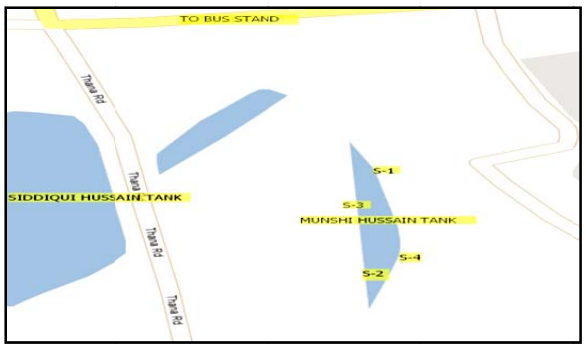
Munshi Hussain Tank is situated amidst the old city of Bhopal near Taj-ul-Masjid on the northwest of the Bhopal City. This Tank is one of the important aquatic reservoirs. It is situated at longitude 77°23'45''E, latitude 23.15°55" N having the catchment's area 2 ha., Submergence area 1.1 ha., Gross storage 1.4 m cum, live storage 4.11 meters, Maximum depth 3.23 meters, Minimum depth 2.03meter, Lowest still level 0.46 ha., Full reservoir level 7.13 m, Water spread at FRL Approx. 0.58 ha. It is a part of the exemplary water management system constructed by Muslim rulers, which resembles the water management system of Islam Nagar fort.

On earlier days, the rainwater flowing down the Idgah Hills was collected at a point for supply to the Benazir palace. The wastewater from the palace used to join the Motia Tank, which subsequently trickled down to Munshi Hussain Khan Tank. Thus a level was maintained in the Tank.





Materials and Method

Fortnightly collection of water samples was done from all the stations *i.e.* four sampling station S1, S2, S3 and S4 of Munshi Hussain Tank. Physico-chemical parameters were analysed using standard methods of APHA (1998) and Khanna and Bhutiani (2008).The algal sample collection was carried out with the help of truncated cone shape plankton net. The sample was concentrated by sedimentation method, removing the supernatant by decanting and the desired final volume was obtained. For counting, 1ml of concentrated sample was taken and placed Sedgwick Rafter Counting Cell following the Standard methods of APHA (1998), Trivedi and Goel (1986), Hutchinson (1967) and Khanna and Bhutiani (2008). The concentrate was preserved in 4% formalin for study (Welch, 1952).

Given formula is used to calculate percentage:-

$$Percent = \frac{\text{No. of Taxa}}{\text{Total No. of taxa}} x100$$

Results and Discussion

The results of percent composition of various genera of cyanophyta in Munshi hussain tank is given in Table-1 and Fig. 1. During course of study a total of 61 species of Cyanophyta were found *i.e. Microcystis aeruginosa*, *M. elongata*, *M. flos-aquae*, *M. protocystis*, *M. pseudofilamentosa*, *Chlrococcus limneticus*, *C. micrococcus*, *C. minor*, *C. minutus*, *C. turgidus*, *Gloeothece rupestris*, *G. samoensis*, *Aphanocapsa koordersi*, *A. biformis*, *A. pulchra*, *Aphanothece nidulans*, *A. pallida*, *Dactylococcopsis fascicularis*, *D. raphidiodes*, *Gomposphaeria aponica*, *G. lacustris*, *Merismopedia elegans*, *M. glauca*, *M. punctata*, *M. tenuissima*, *Oscillatoria acuta*, *O. amphibian*, *O. amphigranulata*, *O. chalybea*, *O. foreaui*, *O. jasorevensis*, *O. laete-virens*, *O. princeps*, *O. salina*, *O. sancta*, *O. subbrevis*, *Phormidium calcicola*, *Lyngbya magnifica*, *L. majuscule*, *L. spirulinoides*, *Anabaenopsis arnoldii*, *Cylindrospermum indicum*, *C. sphaerica*, *Nostoc commune*, *N. sphericum*, *Anabaena ambigua*, *A. aphanizominoides*, *A. flos-auae*, *Raphidiopsis indica*, *R. mediterranea*, *Aulosira fritschii*, *Scytonema coactile*, *S. pascheri*, *Tolypothrix nodosa*, *Calothrix castellii*, *Rivularia aquatica*, *R. bacearianа*, *R. dura*, *Gloeotrichia kurziana* and *G. raciborskii*.

Table-1: Percentage composition of various genera of Cyanophyta in Munshi Hussain Tank

No.	Genera	No. of taxa	Percentage
1	*Microcystis*	5	8.19
2	*Chlorococcus*	5	8.19
3	*Gloeothece*	2	3.27
4	*Aphanocapsa*	3	4.91
5	*Aphanothece*	2	3.27
6	*Dactylococcopsis*	2	3.27
7	*Gomphosphaeria*	2	3.27
8	*Merismopedia*	4	6.55
9	*Oscillatoria*	11	18.03
10	*Phormidium*	1	1.63
11	*Porphyrosiphon*	1	1.63
12	*Lyngbya*	3	4.91
13	*Anabaenopsis*	1	1.63
14	*Cylindrospermum*	2	3.27
15	*Nostoc*	2	3.27
16	*Anabaena*	3	4.91
17	*Raphidiopsis*	2	3.27
18	*Aulosira*	1	1.63
19	*Scytonema*	2	3.27
20	*Tolypothrix*	1	1.63
21	*Calothrix*	1	1.63
22	*Rivularia*	3	4.91
23	*Gloeotrichia*	2	3.27
	Total	**61**	

In the Munshi Hussain Tank different genera in order of frequency of occurrence were Oscillatoria, Microcystis and Chlorcoccus. These were dominant out of total 61 genera and by predominance species of Microcystis, Chlorococcus and Merismopedia in Munshi Hussain Tank. Several workers such as Agarkar (1975), Anand (1988), Hammer (1964), Narayan *et al*. (2006), Oommachan (1981) found similar frequency of algae during their study.

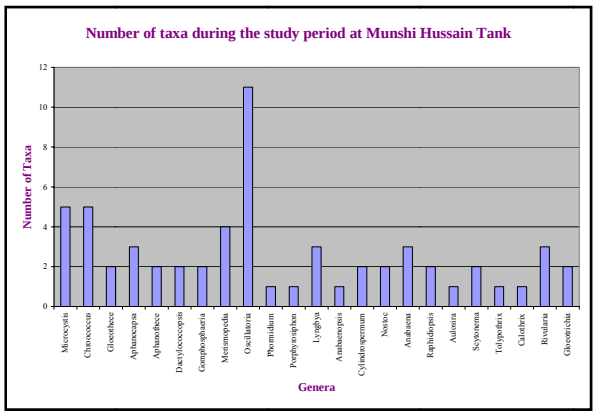


Fig. 1: Number of taxa at Munshi Hussain tank

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Shoot induction and multiplication of an endangered medicinal plant *Rauvolfia serpentina*

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Abstract

***Rauvolfia serpentina* is an endangered medicinal plant which is used to treat various diseases. Shoot induction and multiplication was achieved from nodal, apical and leaf explants. MS media supplemented with IAA and BAP was found suitable for shoot induction and multiplication. The apical portions of shoot segment gave good results in multiplication. The regenerated shoot when subcultured to same medium shown better proliferation.**

Keywords: *Endangered*, *Medicinal plant*, *Micropropagation*, *Subculture*

Introduction

Rauvolfia serpentina belongs to family Apocynaceae represented by 200 genera representing 2000 species. Its common name is Sarpagandha. It is small undershrub generally about 45cm height (George and Sherrington,1984). It is widely distributed within tropical Himalaya and plains near the foothills from Sirkind, edge worth, Muradabad to Sikkim. Rarely found in forest of Bastar, Raipur and Amarkantak.

Family: Apocynaceae
Genus: *Rauvolfia*
Species: *serpentina*

Active compounds, ajmaline, ajmalinine azamalicine, serpentine, serpentinine, reserpine, raupine, sarpagine, reserpinine can be extracted from roots of the plant (Bhojwani, 1990).Yield of alkaloid. 0.8- 2.29 % (standard as per international pharmaceutical codex). Leaves stem and seeds also contain alkaloid. IUCN (International Union for Conservation of Nature and Natural Resources) kept *Rauvolfia serpentina* in endangered species. Besides this it has great importance in treating high blood pressure, hypertension, neuropsychiatric condition, gynological disorder and insomnia Because of the above reasons and it's high medicinal values it is propagated by both conventional and tissue culture method.

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Materials and Method

Glasswares like test tubes, bottles, petriplates, conical flasks, pipettes, beakers were washed with chromic acid or Labolene (Neutral liquid detergent). Washed glassware were sterilized in vertical autoclave at 121˚C and 15 pressure for 30 minutes then transferred to hot air oven for drying at 60˚C for 15-20 minutes. Along with glassware, equipment like scalpels, forceps, scissors, distilled water were also sterilized.Explant like apical portion, nodal portion and leaf were collected from nursery grown plant of *Rauvolfia serpentina*. Explants were washed with DDW (Double distilled water) for 4-5 times and then the explants were treated with 5% solution of Extran and 1% Bavistin. In LAF (Laminar Air Flow) the explant were washed by presterilized DDW, then surface sterilization was done with 0.1% solution of $HgCl_2$. In the present study, Murashige and Skoog's media referred as MS media was used. Different plant growth regulators like, Auxin-IAA, IBA and Cytokinin-BAP were supplemented in low concentration (0.5-5.0mg/l.). pH was adjusted to 5.7 to 5.8 with 1N HCl and 1NaOH. The semi solid growth medium was prepared with the addition of 0.8 % agar-agar powder in the basal media. All stock solutions were prepared in double distilled water and were stored in refrigerator. The cultures were maintained in culture room at 25 ± 2˚C for less than 16 hours photo period in presence of florescent light (1000 lux). Relative humidity *i.e.* 70-80% was also maintained.

Results and Discussion

Growth media were standardized for micropropagation by adding different concentration and combination of plant growth regulators. **Multiplication**: Incorporation of BAP with IAA showed good morphogenetic response for shoot multiplication when the level of IAA (1.0 mg/l) and BAP (2.0 mg/l) was low, better shoot proliferation occurred with 1mg/l IAA and 3.0 mg/l BAP and 2.0 mg/l IAA and 3.0 mg/l BAP also gave good result in case of shoot multiplication (Table-2 and Fig. 2). Shoot length increase when MS media supplemented with 0.5 mg/l of IAA and 2.0 mg/l of BAP excellent growth were observed (Table-1 and Fig. 1). In just 3-weeks shoot get elongated to 3.0-3.5 cm in length.

Interaction of Cytokinin and Auxin on *R. Serpentina* for shoot Induction: High multiplication rate was observed when medium was supplemented with 1.0 mg/l + 2.0 mg/l of BAP and optimal growth was observed in medium containing 0.5 mg/l IAA and 2.0 mg/l BAP. Whereas no morphogenetic response was observed in control medium. Similar observations has been recorded by Kataria *et al*. (2005), Sarkar *et al*. (1996), Bhuya *et al*. (2000) and Kirillova and Komov (2002).

On the basis of this work following PGR combinations and concentrations were recommended for –

Shoot proliferation

IAA 1.0 mg/lit + BAP 2.0 mg/lit

Shoot elongation

(a) IAA 0.5 mg/lit + BAP 2.0 mg/lit

(b) IAA 2.0 mg/lit + BAP 3.0 mg/lit

Table- 1: Morphogenetic resposnse for growth in *Rauvolfia serpentina*

Media composition (in mg/l)	Morphogenetic response				Remark
		After 10 days	After 20 days	After 30 days	
IAA:BAP	Growth				
1:2		+	++	+++	
1:3		-	++	++	
1:4		+	++	+++	
2:3		++	+++	+++	Swelling observed at base after 18-20 days (Fig 1)
2:4		-	++	+++	
5:2		+	+++	+++	
Control		_	+	+	

Table-2: Morphogenetic resposnse for Shoot multiplication in *Rauvolfia serpentine*

Media composition (In mg/l)	MORPHOGENETIC RESPONSE				REMARK
		After 10 days	After 20 days	After 30 days	
IAA:BAP	Shoot multiplication				
1:2		+	++	+++	
1:3		-	+++	+++	
1:4		-	+	+	
2:3		-	+	+++	
2:4		++	+++	+++	
5:2		+	+++	+++	Elongation in internode was observed after subculturing (Fig. 2)
Control		_	+	+	

Now from the above result it is concluded that plant tissue culture of *R. Seprentina* can be done under aseptic condition and according to the need one can obtain the shoot height and more number of plant by applying different plant growth regulators.

Fig. 1: Growth response

Fig. 2: Shoot multiplication

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Pollution studies of River Bhadra at Industrial town Bhadravathi, Karnataka, India

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Abstract

Bhadra river supplies water for irrigation, drinking and industrial zone of Bhadravathi town. Water samples were collected from two different sites along the river. Physical and chemical parameters were studied during January to December 2010. The main aim of the study was to determine the pollution status of Bhadra river and the suitability of water for domestic and other purposes. The study revealed that there is indication of pollution at station-B and the river water at the station-A is fairly good and is free from pollution. There is an urgent need of action plan for the conservation of the river at station-B.

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Keywords: *Bhadra river, Biodegradation, Physico-chemical parameters, Pollution, Sewage load, Western ghats*

Introduction

Water is precious for every living being on this planet. In India 80% of the surface water is vulnerable to pollution as more than 95% of the sewage in the country is not treated (Manjappa *et al.*, 2008). Pollution is as old as man himself. Rivers are considered to be lifeline for most of the developing countries as they meet drinking water needs. Most of the perennial rivers and their tributaries are being used as a site for disposal of domestic and industrial waste in India which impairs their water quality (Chandanshive *et al*., 2008). Studies on physico-chemical dynamics of lotic water bodies were reported by (Nataraja *et al.*, 2009; Patil *et al*., 2009; Sayeswara *et al*., 2010). Lotic water bodies like rivers and streams play very important role in maintaining the biodiversity and over all ecological balance in nature. However, the water quality of fluvial systems is deteriorating due to increase in the amount of raw sewage entering the rivers. The increase of pollution is caused by population growth and increasing urbanization. Indiscriminate use of fertilizers and pesticides in the irrigated lands has significantly contributed to the non point sources of pollution (Prakash *et al.*, 2005). Now-a-days, increasing effects of pollution have become a serious threat. Thus, periodic monitoring of river water quality is necessary to access its suitability for drinking and other purpose. Bhadravathi is a growing town of Karnataka. The position of town on the globe is on latitude 13^{o} 50' N and longitude 75^{o} 40' E. With rapid growth of the Bhadravathi town both in urban and industrial areas, the pollution load in the river Bhadra has increased. The Bhadra river rises from Varsha hills at a place called Ganga moola in the Western ghats about 24 km west of kalasa in Chikamangalore district. After flowing for about 190 kms, it joins the River Tunga at Kudli, 14 kms east of Shivamogga city and becomes Tungabhadra river which is a major tributary of Krishna river. The Bhadra basin gets rain both from the South-West monsoon (June-September) and North-East monsoon (October-December).

The Bhadra river can be considered as lifeline of this area, which fulfills the needs of hundreds of villages, situated along the banks of the river. Due to anthropogenic activities, rapid industrial growth, domestic and agricultural activities of the region, the river water is being polluted, which is the case

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with almost all major rivers of the country. The present investigation highlights the effects of pollution on the physico-chemical aspects of water of the Bhadra river at Bhadravathi town in different months at two different sampling stations.

Materials and Method

In the present investigation, we have selected two stations based on the pollution sources.

Station-A: This is located near Sunnadahalli, 6.2 km away from Bhadravathi town. It is without human disturbances.

Station-B: This is located near down stream of New Bridge near Bus stand which is at the distance of 8.3 km away from station-A. It is partially fed by municipal sewage water from the adjacent areas. The human disturbances include disposal of garbage and organic wastes.

The study was carried out during January, 2010 to December, 2010. The water samples were collected once a month by immersing a wide mouth bottle at the subsurface level during the morning hours between 7:00 to 9:00 A.M. Water temperature was recorded on the spot. The samples for dissolved oxygen fixed immediately on the field itself. The remaining parameters were analyzed as per the standard methods (APHA, 1998).

Results and Discussion

The values of various physico-chemical characteristics of station-A and station-B of Bhadra river at Bhadravathi town have been tabulated in Table-1 and Table-2 and depicted in Fig. 1 (A-H). The water temperature depends on the season, solar radiations and other climatic conditions. The temperature directly influences the changes in dissolved oxygen, alkalinity, salinity and the taste of water (Hosetti and Venkateshwarlu, 1991). Values of water temperature ranged from 22.1 to 27.3 ^{o}C at Station-A and 21.9 to 28.1 ^{o}C at Station-B. The temperature difference might be either due to difference between the collection times or due to the geographical difference in the locations (Pejaver and Gurav, 2008).

Table-1. Physico-chemcial characteristics of Bhadra River water at Station-A (Unpolluted station)

Parameters	Months-2010											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature	22.3	23.4	26.1	26.8	27.3	25.2	23.7	25.4	23.3	24.1	23.8	22.1
pH	7.3	7.1	7.1	7.0	6.8	7.3	7.4	7.2	6.9	7.2	7.0	7.1
DO	6.9	7.2	7.3	6.3	6.2	6.5	6.9	7.1	7.8	7.3	7.1	7.2
BOD	1.9	2.8	2.9	2.7	2.9	3.1	2.6	2.7	2.3	2.1	2.4	2.9
CO_2	1.2	1.3	1.3	1.1	1.8	2.6	2.3	2.4	2.1	2.3	2.3	2.4
TDS	33.7	32.1	36.4	40.2	49.3	44.1	42.7	39.8	36.4	37.3	53.7	34.7
Alkalinity	82	76	53	59	60	70	72	61	68	65	73	71
Sulphate	4.1	4.3	4.9	5.2	5.1	4.8	5.1	5.3	5.4	5.1	5.2	5.7
Phosphate	0.063	0.071	0.068	0.073	0.080	0.079	0.093	0.091	0.089	0.083	0.068	0.071
Nitrate	1.1	0.82	1.1	0.93	0.69	1.2	1.3	1.1	0.99	1.2	1.3	0.97
Chloride	23.1	26.3	28.1	22.2	22.3	18.2	16.1	17.3	19.9	22.1	22.4	23.6

All values are expressed in mg/l except Temperature (^{o}C) and pH

pH refers to a scale of intensity of acidity or alkalinity. This is regarded as a measure of concentration of H^+ ions in water. The pH values ranged between 6.8 and 7.4 in Station-A and 7.0 and 7.9 in Station-B. pH values are slightly acidic to slightly alkaline and found within permissible limit of 6.5 to 8.5 as per the Bureau of Indian Standards (BIS). The pH is an important parameter in a water body since aquatic organisms are well adapted to specific pH range and do not withstand abrupt changes in it (George, 1997). Dissolved oxygen is another vital parameter regulating survival of aquatic life. The permissible standard of DO is above 5 mg/l (Perk and Park, 1980). Values of DO ranged form 6.2 to 7.8 mg/l at Station-A and 2.1 to 2.7 mg/l at Station-B. The sampling Station-B falls under polluted zone because in this zone there is entry of Bhadravathi town sewage rich in bacteria. So the bacteria utilize the dissolved oxygen in the process of decomposition. Due to the process of biodegradation, the DO has reach lowest level at Station-B. The variation of DO depends on

the primary production and respiration of aquatic organisms.

BOD is the measure of degradable organic matter present in water. BOD and other microbial activities generally increase by the introduction of sewage (Hynes, 1972). The BOD values ranged between 1.9 to 3.1 mg/l at Station-A and 6.6 to 9.9 mg/l at station-B. Higher values of BOD in Station-B indicate the higher consumption of oxygen and higher population load in river water. Higher values of BOD at Station-B during summer could be a result of reduced rate of water flow, degradation of organic matter and accumulation of wastes due to anthropogenic activities, while, low BOD values during monsoon could be attribute to the dilution of river water (Upadhyaya and Rana, 1991).

Carbon dioxide is added to aquatic system by directly being mixed from atmosphere. Carbon dioxide in water bodies is also contributed by therespiratory activity of organisms. CO_2 content was minimum in Station-A (1.1 to 2.6 mg/l) and maximum in Station-B (12.1 to 16.7 mg/l). Free CO_2 helps in buffering the aquatic environment against rapid fluctuations in the acidity or alkalinity and also regulates biological process of aquatic communities (Prassanakumari *et al.*, 2003).

Dissolved solids of the water are termed as Total dissolved solids (TDS).

Dissolved materials result from the solvent action of water on solids, liquids and gases. A large number of salts found dissolved in natural water. TDS values ranged form 32.1 to 53.7 mg/l in Station-A and 114.3 to 190.3 mg/l in Station-B. The values of both stations are within

Table- 2: Physico-chemcial characteristics of Bhadra river water at Station-B (Polluted station)

Parameters	Months, 2010											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Temperature	23.2	22.7	25.9	26.7	28.1	26.0	24.1	25.3	24.2	23.9	23.2	21.9
pH	7.2	7.4	7.1	7.2	7.1	7.4	7.8	7.0	7.7	7.9	7.4	7.5
DO	2.1	2.3	2.6	2.6	2.2	2.7	2.2	2.3	2.2	2.6	2.2	2.3
BOD	7.9	7.7	8.7	9.3	9.9	6.6	6.9	7.3	7.2	7.8	7.7	7.4
CO_2	14.3	15.4	16.7	16.3	17.4	11.3	12.1	13.4	14.3	13.7	12.9	13.7
TDS	114.3	121.6	132.1	190.3	181.4	179.2	162.4	168.3	149.1	156.3	162.1	167.7
Alkalinity	73	86	94	79	66	77	74	62	65	56	63	77
Sulphate	14.1	12.9	12.3	11.9	13.4	12.8	13.1	12.8	12.6	13.1	13.6	12.8
Phosphate	1.6	1.2	1.2	1.6	1.7	1.4	1.3	1.3	1.5	1.6	1.1	1.3
Nitrate	5.3	4.3	5.2	4.9	5.3	5.4	5.9	6.2	5.3	4.8	5.7	5.3
Chloride	163.1	150.3	161.1	147.2	149.1	136.3	132.7	135.2	141.2	138.3	143.1	144.7

All values are expressed in mg/l except Temperature (^oC) and pH

permissible limits of 1500 mg/l (BIS, 1982). High values of TDS and sulphates in drinking water are generally not harmful to human beings but high concentration of these may affect persons, who suffering from kidney and heart diseases (Guptha *et al*., 1980). Alkalinity in the water samples is primarily a function of carbonate, bicarbonate and hydroxide content. Alkalinity ranged from 53 to 82 mg/l at Station-A and 56 to 94 mg/l at Station-B. It is within permissible limit of 600 mg/l (WHO, 1991). Surface alkalinity may result from the discharge domestic wastes.

Sulphate is naturally occurring anion found in almost all kinds of water bodies. The sulphates are derived from the discharge of domestic sewage, surface and agricultural runoff (Trivedi and Goel,1995). Sulphate values fluctuated between 4.1 to 5.7 mg/l in water samples collected from Station-A and 11.9 to 14.1 mg/l in water samples collected from Station-B. Phosphorus occurs in natural water as various types of phosphates.

The most important sources of phosphates are the discharge of domestic sewage, detergents and agricultural runoff. Values of phosphate ranged form 0.063 to 0.093 mg/l in Station-A and 1.1 to 1.7 mg/l in Station-B. Phosphate concentration increases in water bodies that receive domestic waste (Nirmalkumari, 1984). Nitrate is a critical nutrient for the growth of algae in the aquatic realm. Nitrate level was maximum at Station-B (4.3 to 6.2 mg/l) and minimum at Station-A (0.69 to 1.3 mg/l). The increase of nitrate in Station-B indicates

the river receives very large amount of organic matter. Chloride is an important parameter in assessing the water quality. Chloride values fluctuated between 16.1 to 28.1 mg/l in water samples collected from Station-A and 132.7 to 163.1 mg/l in water samples collected from Station-B. The most important sources of chlorides in the fresh water are the discharge of domestic and industrial sewage. The concentration of chlorides is thus the indicator of water pollution. High chloride content indicates deterioration of water quality usually linked with sewage load (Mini *et al*., 2003).

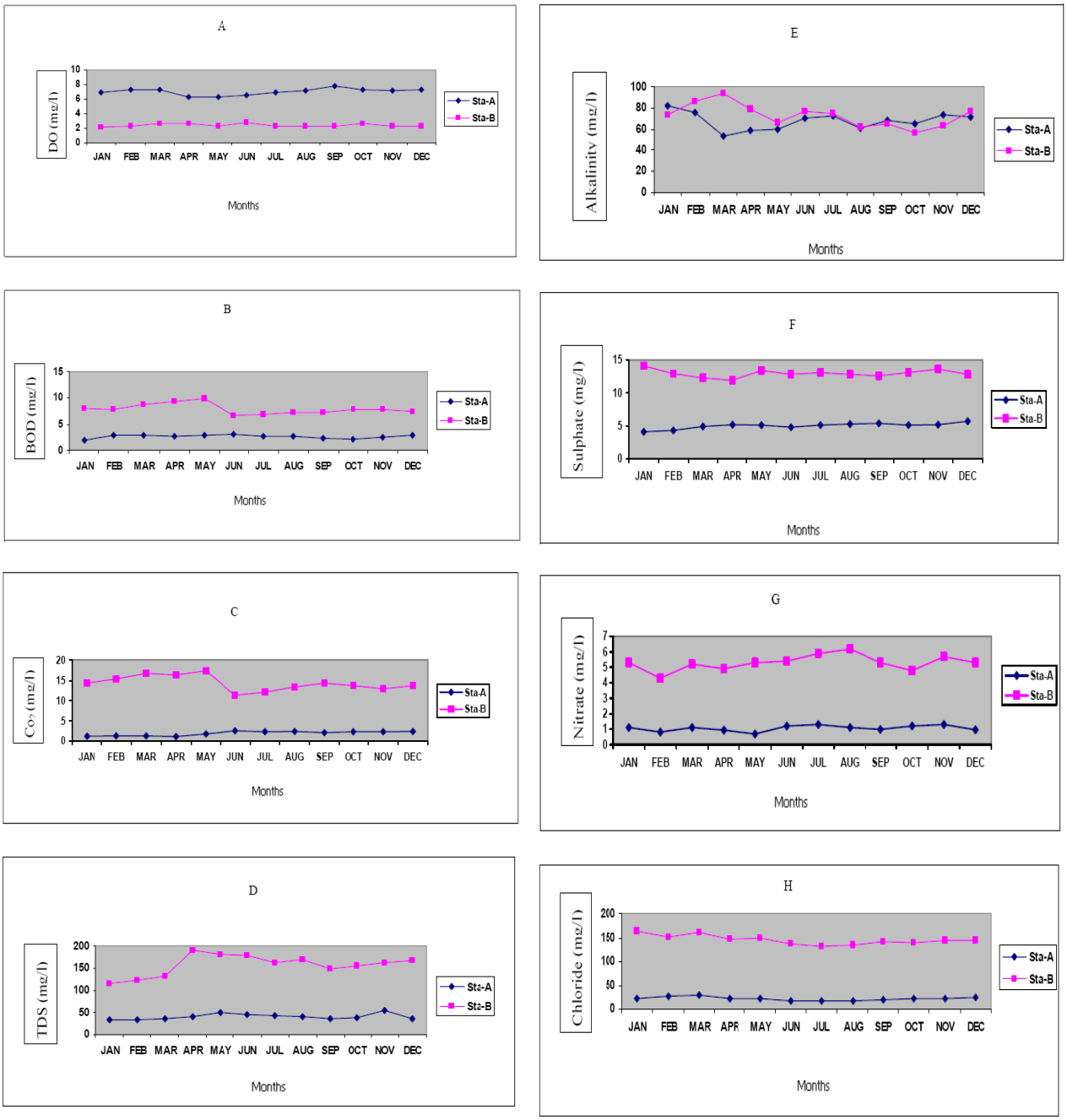

Fig.1 (A-H): Monthly variations in DO, BOD, CO_2, TDS, alkalinity, sulphate, nitrate and chloride at Stations A and B of Bhadra river.

Conclusion

The results of the physico-chemical analysis have revealed that the Station-B of Bhadra river is contaminated due to human disturbances. In the present investigation, most of the values of some physico-chemical parameters exceed the desirable limit according to BIS specifications at Station-B. It is advocated to take urgent steps by governmental and non governmental organizations to protect the river at Station-B. The river water quality at Station-A is fairly good and the data reveals that river at Station-A is free from pollution. This water can be used for the human consumption after proper treatment.

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Molecular characterization of the keratinophilic fungi isolated from high altitude regions of Kashmir

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Abstract

Keratinophilic fungi is an ecologically important group that cycle one of the most abundant and highly stable animal protein on the earth 'Keratin'. The keratin degrading ability of these fungi has been attributed to the production of the extracellular proteases known as keratinases. They have great potential in wool and silk cleaning, leather industry, developing cost effective feather by-products, valorization of the keratin containing wastes, bioremediation and curing skin diseases. In addition, prospective application in prion degradation can revolutionize the protease world in the near future. In the present study, we focussed on the isolation of keratinophilic fungi from the soils of high altitude areas of Kashmir. The sites selected were Khanyar (5173 ft) and Tangmarg (8900 ft). Nineteen isolates of keratinophilic fungi were isolated from these soils by keratin bait technique. These were purified and identified by studying the micro and morphological characters by using relevant literature. Molecular characterization offers more discrimination in fingerprinting an organism and studying its lineage, we thereby relied on PCR based RAPD technique. It is a sensitive and rapid molecular tool for species identification as many fungi do not produce characteristic spores. For molecular characterization, genomic DNA from fungal isolates were isolated and purified. These were then amplified using twenty-one RAPD primers for detecting the polymorphism. PCR products were then separated on the agarose gel. The data was analysed using RAPD-PLOT, PHYLIP and TREE VIEW softwares. Dendrogram generated divided the isolated keratinophiles into three main groups. This data supported the morphological analysis to a noticeable extent.

Keywords: *Dendrogram*, *Keratinophilic*, *Polymorphism*, *Phylogeny*, *RAPD*, *Fungi*

Introduction

Keratin is a rigid scleroprotein, fibrous in nature and insoluble in water. Its main sources are feathers, hair, wool, nails, horns, hooves *etc*. Its structure comprises of α and β chains with molecular weight around 20,000-25,000 Da. Cysteine is a major constituent of this protein (18-24%) (Powell *et al*., 1995). Keratin is used as a source of energy by insects, bacteria, actinomycetes and fungi. Amongst them the largest group of organisms that utilize keratin as the sole source of carbon is the keratinophilic fungi. The term '*keratinophilic*' is derived from a greek word meaning 'keratin loving' but may not hold true always. It refers to the specialized group of fungi, for which keratinized substrates are the natural habitats. They have been reported to be both saprophytic as well as pathogenic (Aho, 1988). Another class of fungi that occur commonly in the soil as kertain decomposers is called 'Keratinolytic fungi' and generally belong to the groups Dueteromycetes and the Ascomycetes (Kaul and Sumbali, 1997; Marchisio *et al*., 1994). Some potential pathogens with keratinolytic activity include dermatophytes belonging to the genera *Trichophyton*, *Microsporum* and *Epidermatophyton* (Buchta and Hejtmanek, 1985; Aho, 1988). The keratin degrading ability of these fungi is attributed to their potential of producing an extracellular protease known as 'keratinases'. These keratinases are of high commercial importance due to its numerous applications (Bockle *et al*., 1995; Lin *et al*., 1995; Gupta and Ramnani, 2006). They are being exploited in the detergent and leather industries, wool and silk cleaning industries, personal care products and more recently in prion degradation (Okoroma *et al*., 2009).

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Keeping the above in mind, in the present study we aimed at the isolation and characterization of such fungi from high altitude regions which may have native enzymatic properties. PCR based characterization is rapid, sensitive, specific and hence highly promising. DNA polymorphism is a common trait of many fungal species and can be studied using specific molecular markers. We used the RAPD (Random Amplified Polymorphic DNA) approach which has proved to be potent and useful technique in the taxonomical classification of the fungi which are morphologically indistinguishable. Another prominent reason for the application of these molecular tools is for the species identification as many fungi do not produce characteristic spores which are key to fungal species identification.

Materials and Method

Sample collection and fungus isolation- The soil samples were taken from Khanyar and Tangmarg regions of the Kashmir in the pre-sterilized polythene bags. It was sieved and pH was checked by pH meter. For the isolation of the keratinophilic fungi, the 'Tokawa' hair baiting method was used with slight modifications (Benedek, 1962). Briefly, 10-20 grams of the soil samples were taken in autoclaved petridishes and tyndallized baits (hair, chicken feathers) were used for isolation (Table 1). The samples were incubated and observed for the growth of fungal mycelia.

Morphological characterization-After pure culturing, the slides were made using methylene blue stain to observe the spore shape, hyphal bearing and mycelium type. Further spore size was calculated using ocular micrometery.

Soil sample	Set	Subset	Bait	Incubation temperature
A	IA	AHI	Hair	37 °C
	IIA	AH2	Hair	18 °C
		AF1	Feather	37 °C
		AF2	Feather	18 °C
B	IB	BH1	Hair	37 °C
	IIB	BH2	Hair	18 °C
		BF1	Feather	37 °C
		BF2	Feather	18 °C

Molecular characterization- Total genomic DNA was isolated following the protocol of Maroof *et al*. (1984). The DNA hence obtained was purified by treatment with 1µl of RNAse per sample for 2 hours at 37 °C. DNA quantification was done on 0.7% agarose gel by comparing with λ DNA marker (300 µg/µl). The DNA was diluted to a uniform concentration of 40 ng/µl for the PCR amplification.

PCR amplification- The DNA was PCR amplified using the ingredients as shown in Table- 2. The sequences of the RAPD primers are shown in Table 3. The PCR was carried out in Eppendorf master cycler gradient thremalcycler. The final optimized condition for amplification was initial denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 1 minute, annealing temperature at 45 °C for 2 minutes and extension at 72 °C for 1 minute. The program was repeated for 40 cycles and final extension was done at 72 °C for 10 minutes. The PCR products were electrophoresed on a 2 % agarose gel and visualized under UV trans illuminator.

S. No	Ingredients	Reaction mixture	Stock concentration	Working concentration
1	Buffer	2.0 µl	10X	1X
2	dNTPS	2.5 µl	1mM	-
3	$MgCl_2$	3.0 µl	25 mM	2 mM
4	Taq pol	0.4 µl	6 U/µl	1.8 U/µl
5	Primers	2.0 µl	0.5 M	0.4 M
6	DNA	3.0 µl	-	40 ng/µl
7	MilliQ	7.1 µl	-	-
	Total	20.0 µl		-

Table-2: Optimised PCR assay

S. No.	Primer code	Primer sequence
1	OPK3	CCAGCTTAGG
2	OPK4	CCGCCCAAAC
3	OPK5	TCTGTCGAGG
4	OPM14	AGGGTCGTTC
5	OPA3	AGTCAGCCAC

Table-3: Sequences of the RAPD primers

Phylogenesis- For the analysis of the phylogenetic relatedness amongst the fungal isolates, a dendrogram was generated using the RAPD-PLOT, PHYLIP and TREE VIEW softwares.

Results and Discussion

A noteworthy number of keratinophilic fungal species were found to be present in the collected soil samples. Although some of them showed similar macroscopic characteristics, a significant polymorphism was revealed in the molecular analysis. After sieving soil samples, the pH of the soil sample 'A' was found to be 8 while that of sample 'B' was 7.5 indicating a slightly high alkaline nature of soil sample 'A'. Nineteen pure cultures of the keratinophilic fungi were isolated using the keratin bait technique (Figure-1).

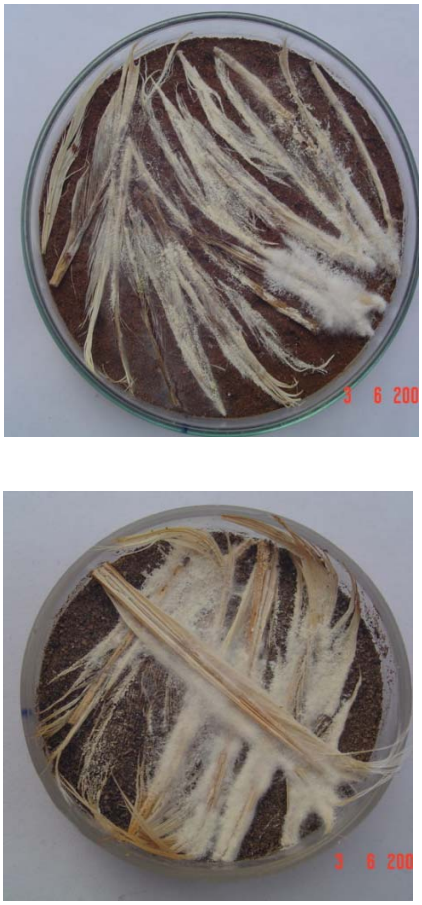

Fig. 1: Plate showing culture

These were purely cultured on the SDA medium and then subcultured (Figure-1). Nine cultures out of the total isolates were selected for the further work. These fungal cultures were all white in colour, but showed variations in the texture and reverse colony characteristics. Ac1 culture had a velvety texture with small oval shaped spores of size (7-4) × (2-4) µm, while Ac2 had a puffy growth and thin walled oval spores of size (7-5) × (2-4) µm. Ac3 showed the presence of pyriform shaped arthospores of (9-14) × (7-9) µm, showing a powdery white texture on the plates. Ac4 showed absence of sporulation, while Ac5 too had rough walled pyriform spores of size (7-10) × (4-8) µm on a racquet hyphae. Ac3 and 5 were identified as members of *Chrysosporium* species. The spores of Ac6 were thin walled and elongated doughnut shape of size (9-14) × (5-7) µm. The Ac7 had thin walled oval spores of size (9-10) × (2-3) µm. Ac8 had (5-7) × (3-4) µm sized oval spores, while Ac9

had pyriform spores of size (10-11) × (2-3) µm and velvetty white texture.

Molecular analysis: About twenty one RAPD primers were used for PCR amplification, of which five primers showed proper polymorphism amongst the selected fungal samples (Figure 2 and 3). The PCR amplified products were directly scored from the electrophoresis gel for the presence and absence of the bands. Each band was treated as RAPD marker. The presence of the band was scored as 1 while absence of the band was scored as 0.

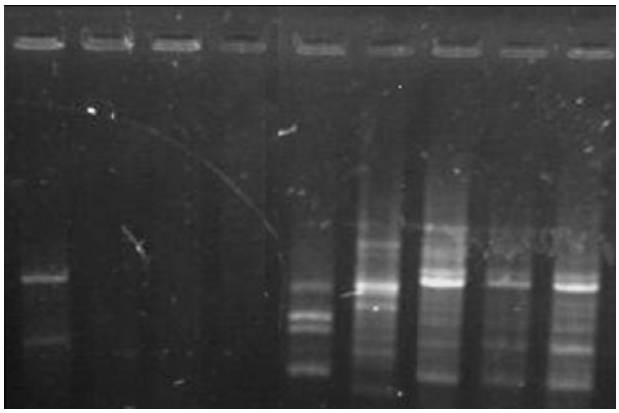

Fig. 2: RAPD polymorphism using primer pair 1-2

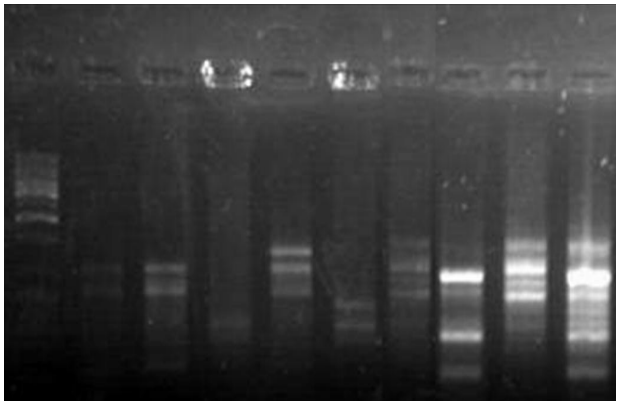

Fig. 3: RAPD polymorphism using primer pair 3-4 (wells 1-4) and 4-5 (wells 5-9)

The generated dendrogram divided the fungal isolates into three main groups (Figure-4). Acession numbers 3, 4, 5, 9, 6, 7 were placed in one group, wherein Ac6 and 7 were more closely related. Ac9 was closer to this subgroup followed by Ac5. Ac4 was close to Ac3 which was distant from other members. Ac1 and Ac2 formed another cluster present near Ac6 and Ac7. Ac8 formed a separate branch distant from the other two clusters.

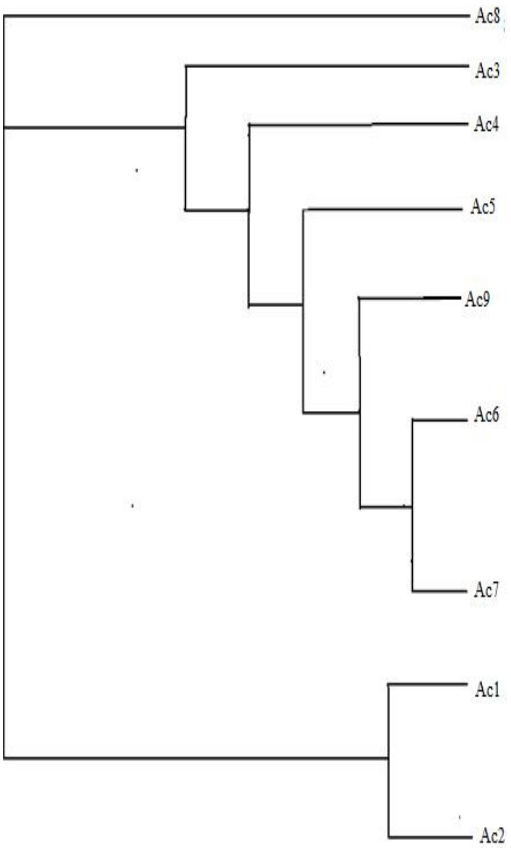

Fig. 4- Dendrogram generated using RAPD PLOT and PHYLIP

Till date, fungal systematics is chiefly based on the morphological criteria and identified basically by their phenotypes. Numerous alternative approaches have been developed like nutritional and physiological studies, secondary metabolites, serological testing, molecular markers *etc*, but they are used as complimentary tools of morphological data. A number of molecular targets like mitochondrial cytochrome B gene (Yokoyama *et al*., 2001), aflR gene (Chang, 2001), TOP2 gene (Kanbe *et al*., 2002), β tubulin gene and rRNA genes (Iwen *et al*., 2002) *etc* for identification of various fungi have been developed. Kaul and Sumbali (1997) have made a special study on the keratinolytic fungi in the Jammu region and have added new fungi like *M. chrysosporodia* and *M. flava* to the list. Prevalence of the keratinophilic fungi in the alkaline soils has been reported and alkaline pH of our soil samples was an early indication of the presence of the keratinophilic fungi (Kaul and Sumbali, 1999; Mercantini *et al.*, 1980). Research on the soil samples collected from

the glacier banks of Gulmarg, Khilanmarg, Sonamarg and Tangmarg regions of the Kashmir valley revealed the prevalence of the keratinophilic fungi and related dermatophytes (Deshmukh, 2002). *Chrysosporium keratinophilum* (3.7 %), *Chrysosporium tropicum* (5.6 %), *Ctenomyces serratus* (11.2 %), *Geomyces pannnorum* (2.8 %), *Microsporum nanum* (1.9 %), *Trichophyton ajelloi* (15 %). There was a significant difference in the microscopic characteristics of the fungal isolates as discussed above. These differences were confirmed by the molecular characterization where marked polymorphism was observed among the isolates. A study reported the intraspecific variation in the *Metarhizium anisopliae* populations by RAPD and ITS primer based approach (Velasquez *et al*., 2007). Moreover, RAPD and RFLP assays were performed for the characterization of the obligate biotroph *Spongospora subterranean* (Qu, 2006).The phylogenetic data supported the morphological analysis to a noticeable extent. Ac6 and Ac7 were the fungal isolates from the soil sample A, isolated using feathers as bait. Both of them were white in colour and brown reverse colony characteristics, but Ac6 had a puffy texture while Ac7 had velvety texture. Ac6 had elongated doughnut shaped, thin walled spores with size (9-14) x (5-7) µm, while Ac7 had oval spores of size (9-10) x (2-3) µm. This indicates that these may be different species of a same genus. Ac3, 5 and 9 were isolated from the soil sample 'B' using feather baits. They had white colonies with cottony texture and reverse colony characteristics were yellowish brown. Ac5 had pyriform rough walled spores, racquet hyphae and abundant arthrospores were also present. The spore size was calculated as (7-10) x (4-8) µm. It was closely related to Ac4 which was isolated from the soil sample A using feather baits. It too had pyriform spores and spore size was (9-14) x (7-9) µm. Ac8 emerges as an outgroup and remained aloof from the other isolates as revealed from the dendrogram. It was isolated from soil sample A using hair bait. It was white in colour and had cottony mycelia. Spore size could not be calculated due to absence of sporulation.

The other phylogentic cluster contained Ac1 and Ac2 indicates their close relatedness and identical origin. These were isolated from the soil sample B using hair bait. The morphological colony characteristics were similar with slight differences in the textures. Ac1 had a velvety texture while Ac 2 was cottony in appearance. The spore shape was oval in both of them but spore sizes differed to a small extent.

Conclusion

With the inventions on the newer techniques of isolation, the studies on the keratinophilic fungi are in progression. Soil is the main reservoir of this group of fungi, where they are involved in the degradation of the keratinous substances like hair, nails, feathers *etc*. A marked effect of the habitat on the biological properties of the microbes is well known. We isolated these fungi from high altitude regions. Morphologically all the selected fungal isolates had white colonies, so their differentiation was difficult. But at the molecular levels the variations were revealed using RAPD based approach. Furthermore, their phylogenetic relatedness was a better indicator for the identification of these fungal isolates. Though RAPD has some limitations like non specificity, presence of repetitive sequences but still it serves as an informative molecular tool. Further biochemical screening and protein characterization of the isolated fungal isolates may offer them as a boon for industries.

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A study to access heavy metal concentration in Paniyala Fish Pond near Roorkee (Haridwar)

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Abstract

Paniyala fish Pond is a multipurpose pond with emphasis on fisheries, irrigation and washing. The present study was conducted to find out the heavy metal concentrations in the fish pond. Water samples were collected on monthly basis from January, 2008 to December, 2008. Concentration of heavy metals (Copper, Nickel, Iron, Lead, Zinc, Aluminium and Arsenic) was analyzed in the pond water by Atomic Absorption Spectrophotometer. Significant variations were found between winters (December, January) and wet summer period (July, August, September) for the studied metals. The relative variability followed the order Fe >Zn>Pb>Cu>Ni>Al>As.

Keywords: *Heavy metals*, *Fish Pond*, *Heavy metal concentration*.

Introduction

Lakes and ponds are habitats of great human importance as they provide water for domestic, industrial and agricultural use as well as providing food. In spite of their fundamental importance to humans, freshwater systems have been severely affected by a multitude of anthropogenic disturbances, which have led to serious negative effects on the structure and function of these ecosystems. The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years because they are indestructible and most of them have toxic effects on organisms (MacFarlane and Burchett, 2000). Heavy metals are introduced to the environment through a variety of sources such as combustion, extraction, agricultural runoff, transportation *etc* (Lars, 2003). Besides, the dangers involved from the presence of metals in the environment derive not only from their persistence and toxicity, but also from the remarkable degree of bioaccumulation they undergo through the trophic chain, thus becoming serious danger to man (Bishop, 2000). Heavy metal contamination in aquatic environment exerts an extra stress on fish which tend to accumulate the heavy metals in metabolically active tissues and organs (Langston, 1989).

The problem of chemical contamination in water bodies like nitrate, sulphate, iron, manganese, zinc and copper may cause several health problems to human beings. Their compounds are destroyed in the water body, that is how heavy metals are referred to conservation substances toxic for hydrobionts and man (Natalia *et al*., 1997).

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. Since heavy metals are rapidly absorbed to particulate materials (*e.g.* detritus, plankton, suspended sediments) and assimilated by living organisms. Heavy metals, especially copper, nickel, lead and zinc, have adverse effects on terrestrial and in aquatic environments. However, their impact can vary depending on the nature of organisms (Clark, 1997; Seidl *et al.* 1998). Although heavy

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metal concentration remains within the permissible limit but regular immersion activity may increase the concentration of heavy metallic ions in the pond water, which may ultimately cause serious health hazards in human beings when get accumulated through food chain.

Materials and Method

Detection of heavy metals in pond water was done following the standard methods of APHA (1998). The surface water samples were collected at four sampling sites for one year period in the Paniyala State Fish Pond. The determination of heavy metals in the water samples was done by the Atomic Absorption Spectrophotometer (AAS). Using the appropriate element, hollow-cathode lamp, monochrometer was set at the selected wavelength. Standard solutions of the different elements of interest were prepared separately. The instrument was zeroed with distilled deionized water. The water samples for this analysis were subjected to acid digestion and subsequently different mineral elements were determined using appropriate methods.

Results and Discussion

Although, these trace metals differ widely in their chemical properties, their relative concentrations and discharges and hence, their bioavailability are very important to terrestrial, aquatic and marine organisms in terms of toxicity (Alloway and Ayres, 1997). The main health risks due to Arsenic are considered to be severe poisoning and carcinogenicity, specially cancer of respiratory system and gastrointestinal tract. During the study time in the water sample of Paniyala pond, Copper, Nickel, Iron, Lead, Zinc and Aluminium were detected while Arsenic was found below detection limit (Table- 2 and Fig. 1-6).

Copper is malleable, ductile metal, and is an excellent conductor of heat and electricity. Adraino (2001) reported that copper toxicity in humans is rare, aquatic organisms are potentially at risk from Cu exposure. During the study Copper concentration was found maximum 0.0058 mg/l in November and minimum value 0.0022 mg/l was found in January. The range obtained was under the WHO permissible limit which is 0.05 mg/l. Zinc has been known for a very long time; it was used in alloys since the 7th century in India and in the 11th century in China. Zn is an essential macronutrient for plants but is phototoxic when in excess (Muvanga and Barifaijo, 2006). Zn was maximum 0.0386 mg/l in September and minimum 0.0270 mg/l was present in June and July, and the observed values were under the WHO permissible limit (5.00mg/l).

Metal	Drinking Water (mg/l)
Aluminium	0.2
Arsenic	0.05
Copper	0.05
Iron	0.30
Lead	0.05
Nickel	-
Zinc	5.00

Table-1: Maximum Permissible limit for Heavy Metals (WHO,2006)

Cronstedt discovered nickel in 1751; its name is derived from the Swedish *kopparnickel* (Goblin Copper) Nickel is a hard, malleable, ductile metal, crystallizing in the face-centred cubic system. The metal is produced by roasting the sulphide ores and reducing the oxide with carbon; it is purified by electrolysis (Adriano, 1986). Nickel salts significantly increase the level of lipid peroxidation and simultaneously decrease glutathione level and glutathione peroxidase activity in the liver (Das *et al.*, 2001). Nickel concentration was maximum 0.0036 mg/l in June and July and minimum 0.0017 mg/l in the month of December. It is estimated that 8% of nickel is used for household appliances (IPCS, 1991). Aluminium was observed maximum 0.0027 mg/l and minimum 0.0010 mg/l in the month of June and January respectively during the study period. For Aluminum the permissible limit of WHO is 0.2 mg/l. The main effects of aluminum exposure in fishes are respiratory and ion regulatory disturbances (Neville, 1985; Gensemer and Playle, 1999). Lead has been known since ancient times. Often, it is one of the most widely used metals in industry: in piping, conducting materials, accumulators, lead chambers, printing characters, soldering, anti-knock substances and coloured pigments. Bowen (1966) explained that lead is not essential as a trace metal to nutrition in animals, but is a cumulative poison. In study period maximum concentration of Lead was found in September 0.0075 mg/l and minimum 0.0016 mg/l in March. The observed values were under the permissible limit WHO which is 0.05 mg/l.

Table-2: Monthly average concentration of Heavy Metals of the water of Paniyala Fish Pond

Month	Copper (mg/l)	Nickel (mg/l)	Iron (mg/l)	Lead (mg/l)	Zinc (mg/l)	Aluminium (mg/l)	Arsenic (mg/l)
January	0.0022	0.0018	5.2621	0.0026	0.0340	0.0010	BDL
February	0.0024	0.0021	5.1797	0.0019	0.0340	0.0013	BDL
March	0.0025	0.0023	5.2769	0.0016	0.0314	0.0013	BDL
April	0.0027	0.0030	5.3267	0.0019	0.0336	0.0021	BDL
May	0.0027	0.0033	5.2959	0.0021	0.0336	0.0025	BDL
June	0.0028	0.0036	5.3202	0.0033	0.0270	0.0027	BDL
July	0.0035	0.0036	5.6055	0.0041	0.0270	0.0020	BDL
August	0.0035	0.0027	5.6410	0.0061	0.0323	0.0015	BDL
September	0.0054	0.0025	5.6309	0.0075	0.0386	0.0014	BDL
October	0.0053	0.0022	5.6219	0.0067	0.0351	0.0016	BDL
November	0.0058	0.0019	5.6128	0.0056	0.0353	0.0013	BDL
December	0.0043	0.0017	5.2570	0.0052	0.0327	0.0012	BDL
Average±SD	0.0036 ±0.0013	0.0026 ±0.0006	5.4192 ±0.1832	0.0040 ±0.0021	0.0329 ±0.0032	0.0016 ±0.0005	

±SD- Standard Deviation; BDL-(Below Detection Limit)

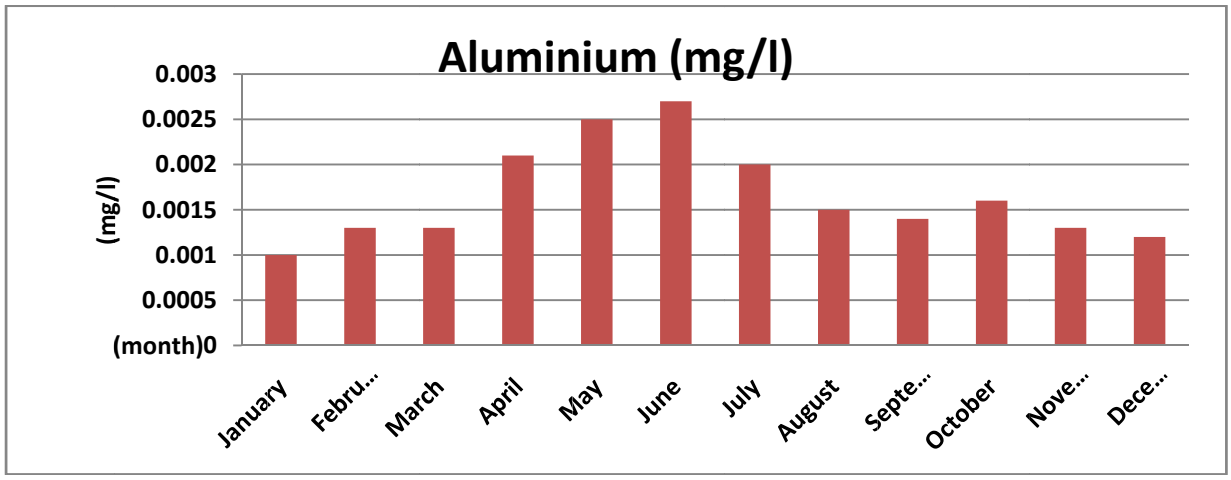

Fig.1:Showing monthly fluctuation of Aluminum in Paniyala Fish Pond in 2008.

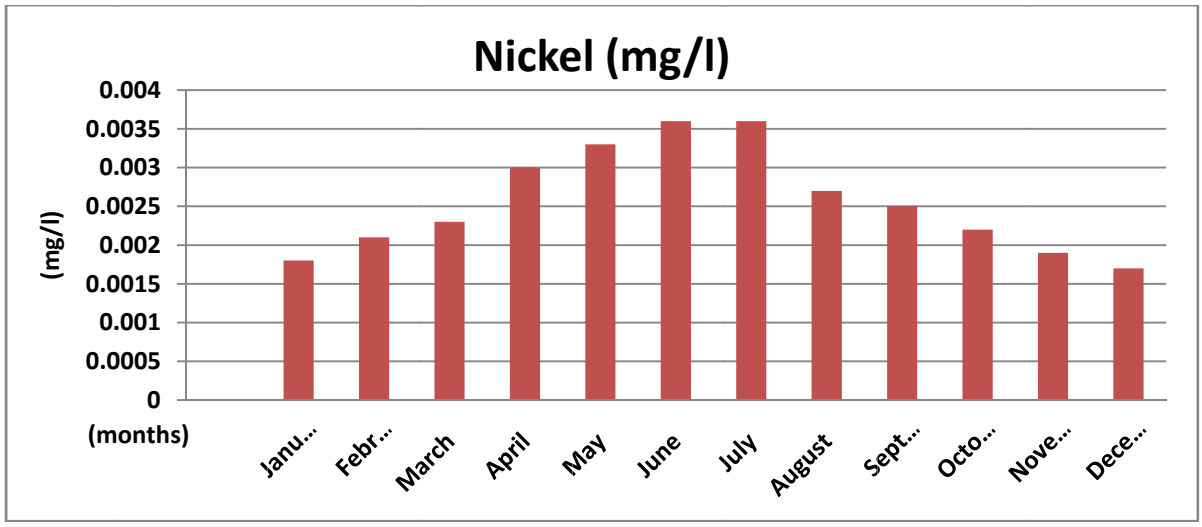

Fig. 2: Showing monthly fluctuation of Nickel in Paniyala Fish Pond in 2008.

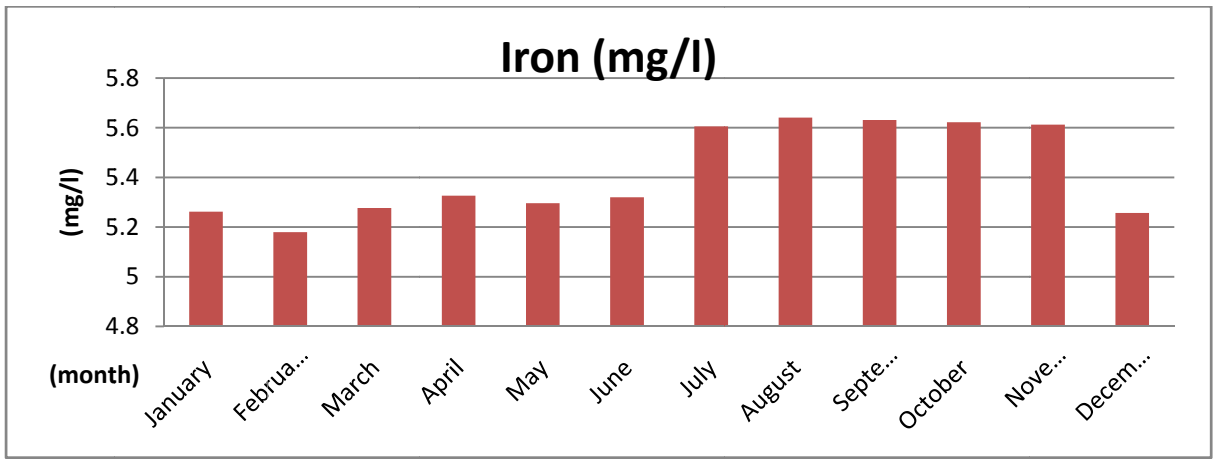

Fig. 3:Showing monthly fluctuation of Iron in Paniyala Fish Pond in 2008.

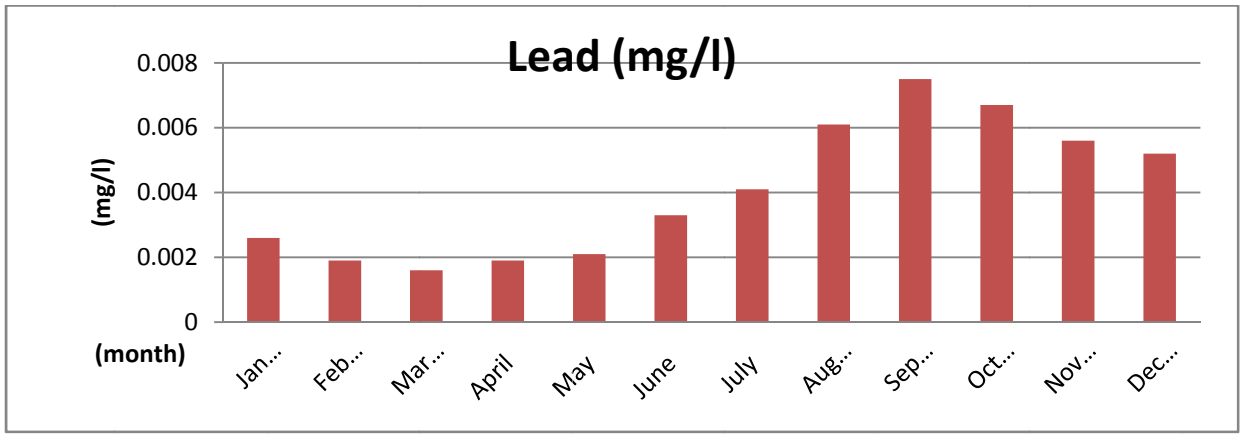

Fig. 4: Showing monthly fluctuation of Lead in Paniyala Fish Pond in 2008.

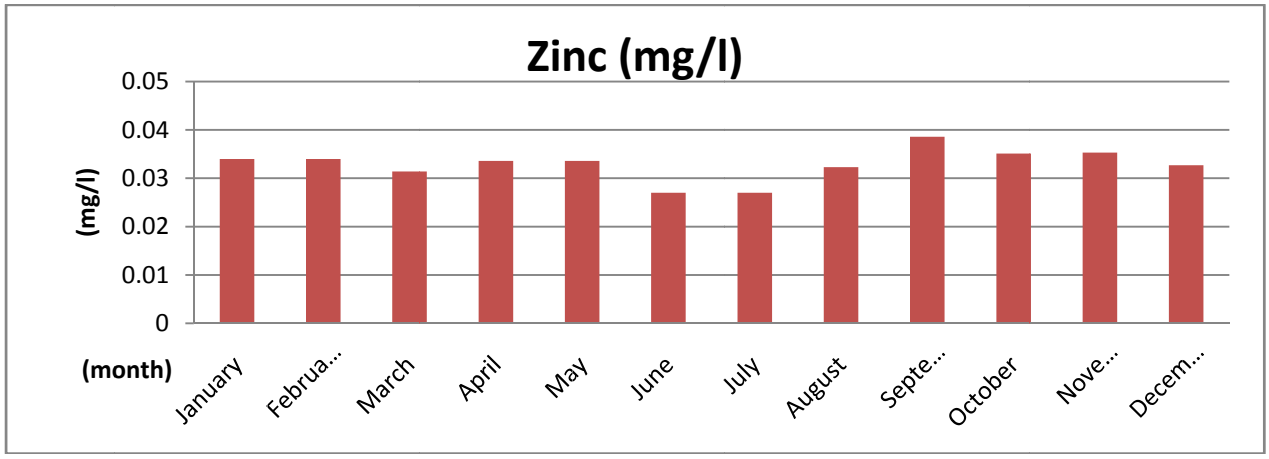

Fig. 5: Showing monthly fluctuation of Zinc in Paniyala Fish Pond in 2008.

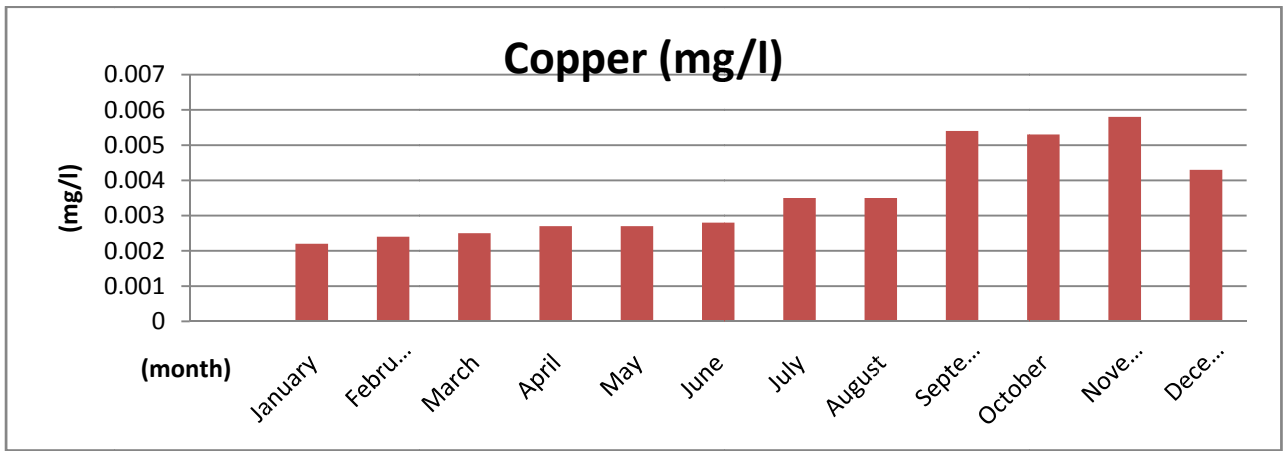

Fig. 6 : Showing monthly fluctuation of Copper in Paniyala Fish Pond in 2008.

Water containing iron does not show deleterious effect on human health, its presence in drinking water is objectionable for various reasons. Iron is moderately toxic to many species of aquatic plant, above permissible limit. Excessive iron content makes the water turbid, discoloured and imparts an astringent taste to water. As per the standards set by WHO, the permissible level of iron is 0.3 mg/l. Iron concentration was maximum 5.6410 mg/l in August month and minimum 5.1797 mg/l was present in February month. This observation is similar to Adefemi *et al.*, (2008) who studied heavy metal concentration in Ureje dam in south-western Nigeria.

The maximum values in summer months was may be due to the discharge of huge amount of domestic sewage and agricultural runoff from surroundings in to the pond. The solubility of trace metals in surface water is predominantly controlled by the water temperature (Iwashita and Shimamura, 2003). At a higher temperature, plants grow and die faster, leaving behind matter that requires oxygen for decomposition. Trace elements where are accumulated to phytoplankton may become soluble during the decay of plants (Pendias and Pendias, 1992). Except for iron and zinc, the concentrations of the other heavy metals were relatively low. The result shows that only Iron in pond water exceed the WHO permissible level and Copper, Nickel, Lead, Zinc and Aluminium does not exceed the WHO permissible limits, while Arsenic was found below detectable limit.

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Geothermal spring sites as excellent reservoir of novel microorganisms

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Abstract

Geothermal sites at the earth's surface are known to be present both on land and deep inside the sea. Thermophilic microorganisms are the only life forms that can survive under such harsh conditions. These extremophiles can resist the high temperature prevalent at a geothermal site for their capability to produce a variety of thermotolerant enzymes. Many of these enzymes are known to have applicability in a variety of industrial processes. The last three decades have witnessed the spectacular growth of industries producing thermostable enzymes either employing the pure strain or a recombinant one. In addition to that thermophilic microorganisms have been a source of other important metabolites. The evaluation of microbial wealth of every such site is therefore, a need of the hour.

Keywords: *Geothermal sites, Microorganisms, Enzymes*

Introduction

Greek words *geo* (earth) and *therme* (heat) defines the hot environment formed due to heat within the earth as 'geothermal' sites. Geothermal springs are biotopes with controlled environmental conditions round the year offering excellent conditions for the growth of thermophilic microorganisms. The diversity of such tiny life forms is a thrust area which is drawing attention of the researchers worldwide. A considerable temperature gradient exists inside the earth which is found to be quite high in certain geographical locations. The rainwater and snowmelt continue to seep underground owing to cracks and deep subterranean faults. As this water is heated by the hot rock a geothermal reservoir is formed beneath the earth. This hot water, through the openings at the earth's surface in the nearby region, forms geothermal springs, geysers or fumaroles (Bhardwaj and Tiwari, 2009).

A variety of flora and fauna is known to exist at the high temperature sites (Table-1). However, it is interesting to note that except prokaryotic organisms none of them is adapted for optimum growth at elevated temperature. Thermophilic microorganisms are capable to do so because of their ability to produce biocatalysts active at high temperature. Generally enzymes, being proteins, begin to denature at a temperature above 40 ^{o}C, and are completely inactive beyond 50-60 ^{o}C. However, thermotolerant enzymes have specific protein motiffs rendering the overall structure of its proteinaceous part unaffected at higher temperature. Some thermophilic enzymes are known to maintain at least half of their specific activities at temperature as high as 90 ^{o}C or above or in rare cases even higher. In other cases, enzymes might partially denature at high temperatures but have adaptive systems that allow them to renature into a functional form once removed from such extreme conditions.

Microbial wealth of thermal springs

Microorganisms that can tolerate high temperatures are commonly found in hot springs or deep-sea thermal vents and are typically part of the group of Archaea. Although the Archaea might not grow fast enough or produce enough enzyme under "normal" conditions to make their harvest practical, their enzymes do have many potential applications in a wide variety of industrial processes where extreme conditions are required. Therefore, once the gene

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for potentially useful thermophilic enzyme has been identified, cloning techniques can be applied to express the enzyme under the control of a strong promoter, in a fast-growing organism that has been proven to show robust growth in large-scale fermentation systems. The heat stable enzymes from thermophilic and hyperthermophilic microorganisms have unlimited potential in biotechnological applications. The heat stable enzymes from the extremely thermophilic and hyperthermophilic microorganisms, virtually due to their unlimited potential in biotechnological applications are expected to fill the gap between biological and chemical processes (Leuschner and Antranikian, 1995). The extremophiles thus hold the promise of providing such enzymes which could be effective at high temperatures in industrial processes. Proteases, lipases, amylases and other hydrolases which are active at high temperature would be boon in food processing as fats could be hydrolysed, proteins digested and fibres modified enzymatically to make food more palatable and healthful (Sony and Sandhu, 1999).

Some of the previous work related to the isolation and characterization of microbial wealth of thermal springs across the globe is summarized in Table-2. The production of hydrolytic industrial enzymes (*e.g*. Protease, lipase, amylase, pullulanase, xylanase, pectinase, cellulase, lactase *etc*.) for the manufacturing of various valuable products has shown a spectacular rise during the last three decades. In 1983, the estimated sale of all industrial enzymes worldwide was estimated as US $ 1 billion. In 2009, the whole market for industrial enzymes has gone to be in the range of US $ 2.4 billion.

Table-1: Temperature range for the growth of various groups of organisms (Brock, 1978)

Organisms	Upper temperature limit
Animals	
Fish and other aquatic vertebrate	38 ^{0}C
Insects	45-50 ^{0}C
Crustaceans	49-50 ^{0}C
Plants	
Vascular plants	45 ^{0}C
Mosses	50 ^{0}C
Eukaryotic microorganisms	
Protozoa	56 ^{0}C
Algae	55-60 ^{0}C
Fungi	60-62 ^{0}C
Bacteria	
Cyanobacteria	70-73 ^{0}C
Photosynthetic bacteria	70-73 ^{0}C
Chemilthotrophic bacteria	90 ^{0}C
Heterotrophic bacteria	90 ^{0}C

Future Prospectus

There are some definite advantages in screening for wild type microbial genes among nature's own diversity. The gene expressed in nature represents proteins which presumably, through the evolutionary process has been undergoing hard and long selection pressure. The pressure thus exerted drives the gene evolution towards enzyme production which is most fit for solving interaction with the substrate needed for a given organism in an ecological niche in which it is adapted to inhabit. Careful selection of the taxonomic group and the ecological niche to screen, as compared to the industrial process conditions under which these metabolites should work gives optimal chances for discovery of novel microorganisms capable of producing them. Hot springs provide such opportunity in terms of wealth of biotechnologically important microflora.

Table -2: Microorganisms isolated from thermal springs having industrial utility

Thermal spring location	Microorganism	Usefulness/properties	References
Assam, India	*Brevibacillus laterosporus* BPM3	Biocontrol agent maximum growth and antagonistic activity at 30 °C, pH 8.5	----------
Siberian hot spring	*Crenarchaeota* sp.	Ammonia oxidizing, highly active at 0.14 and 0.79 mM ammonium	Hatzenpichler *et al*. (2008)
Iceland	*Thermoanaerobacter thermohydrosulfuricus*	Hydrogen and ethanol production Active at 50-78 ^{0}C	Koskinen *et al*. (2008)
Jae Sawn hot spring, Thailand	Recombinant *E.coli*	Lipase active 50-70 ^{0}C and pH (7-10)	Tieawongsaroj *et al*. (2008)
China	*Silanimonas lenta* and *Schlegelella aquatica*	Lipase producer, active at high temperature and alkaline conditions	Lee *et al*. (2005) and Chou *et al*. (2006)
Himachal Pradesh, India	*Bacillus* sp. J33	Thermostable lipase, heat stability increased after immobilization	Nawani *et al*. (2006)
Atri, Taptapani and Deuljhari, Orissa	*Bacillus* sp. *Pseudomonas* sp.	Heat stable lipase activity at 60^{0}C	Rath (1999)
Egyptian thermal spring	*Bacteria*	Thermostable cellulose production	Ibrahim and EI-diwany (2007)
Russia	*Caldicellulosiruptor kronotskyensis* and *Caldicellulosiruptor hydrothermalis*	NA	Miroshnichenko *et al*. (2008)
Korea	*Chlamydomonas taiwanensis*	Thermostable Amylase production	Chen *et al*. (2005)
Manikaran, Himachal	*Bacillus* sp.APR-4	Thermostable protease	Kumar *et al.* (2002)

Exploration of areas with extreme environments and the isolation and investigations on the associated microbial wealth for biotechnological applications are of great significance, both for basic and applied research within the country. In the whole world several attempts have been made by scientists in this direction but in India still a lot more attention has to be paid. The thermal springs of Uttarakhand are almost untouched for any such exploration.

Therefore, it seems to be of utmost importance to study the microbial diversity of these geothermal sites for their functional attributes. Further, novel techniques of metagenomic are expected to bring forth the hidden gold in the form of novel genes producing important metabolites from the uncultivated microbial world of such geographically distinct region.

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Survey and conservation of some useful aquatic insects of Betul District of Madhya Pradesh, India

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Abstract

In India, freshwater ecosystems are most threatened by the man made reservoirs, loss of seasonally flooded forests, polluted wetlands and deforestation of surrounding watersheds. Specific actions are needed for conservation of the water valuable insects through detailed scientific studies. A study has been carried out in the Sapna Dam of Betul District, Madhya Pradesh. Field surveys have been carried out to prepare geographical coordinates. Water depth, water quality and biotic characteristics at different locations were measured with the help of limnological equipments. Results of these studies are presented in this paper.

Keywords: *Conservation, Reservoir, Aquatic pollution, Odonata, Aquatic insects*

Introduction

There are many wetlands available in different parts of the country. The wetlands are highly productive areas with rich biodiversity. They serve as a spawning and nursery ground for fishes, birds *etc*. and hence can be used as a excellent area for conservation of rare and endangered species (Rao, 2002).

In Madhya Pradesh, there are many freshwater wetland areas in the form of lakes and man-made reservoirs. The reservoirs are constructed primarily for flood control, conservation of rainwater, irrigation, power generation and water supply to cities and industries. Fishing development in these water bodies is considered as a secondary activity. Our present knowledge on various aspects of reservoirs in central Madhya Pradesh is inadequate. Few studies on Tighra reservoir have been conducted (Sharma, 1991; Singh, 2003).

Wetlands are used for extensive aquaculture operations (Sugunan, 1995). Wetlands play a role in wastewater treatment and function as natural filter systems (Anon, 1989). Development of water resources has affected fish and wildlife resources in many wetlands (Rao, 2002).

Many wetlands have been constantly used for dumping of garbage, sewage disposals, tanneries disposal *etc*. An increased level of water quality deterioration has been observed year by year. Some species in the water bodies are likely to become extinct in the near future (Anon, 1989). The changes in the characteristics of the wetlands in the form of water quality pollution and water development projects also have greatly altered habitat conditions for aquatic animals. The habitat loss has caused concern for the welfare of the aquatic animals that live in different water bodies. As it is clear that some aquatic insects are very much useful for the fishes growth, eradication of harmful mosquitoes larvae and aquatic plant growth. Present paper deals with the conservation of these useful aquatic insects for maintaining the ecosystem and aquatic environment.

Materials and Method

The study was carried out of Sapna reservoir. It is located approx. 12 kms south of Betul city of Madhya Pradesh. It is a large man-made reservoir. The water from the reservoir is being supplied to the city of Betul, Betul -bazar and surrounding places in addition to irrigation and fisheries use. Sampling in the reservoir at different points was made by moving on a hired boat. Insects werecollected regularly from the reservoir and the collected insects were brought to the laboratory and sorted out seperately in glass aquarium. Different nymphal instars were maintained regularly for the duration of research. The insect of following

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families were identified and maintained for the study. Identification of insects was done by using different entomological reference books. Physico chemical analyses were done by using standard method APHA (1998).

Results and Discussion

A total of twenty-one species belonging to four different families were identified. *Anisogomphus occipitalis*,*Burmogomphus sivalikensis*, *Mesogomphus lineatus*, *Macromia moorei*, *Orthetrum taeniolatum*, *Trithemis aurora*, *Trithemis festiva* and *Tholymis tillarga* were found abundantly. *Anisopteran nymphs* were found in shallow running water having a considerably sandy bottom and an abundance of vegetation they were studied in relation to certain ecological conditions *Brachythemis contaminata*, *Macromia* sp. and *Orthetrum* sp. were observed in water body at lower altitude where *Zyxomma petiolatum* found which preferred decaying plant debris. These nymphal communities were found in a great variety of habitats and in association with an abundance of algae and macrophytes. *Orthetrum taeniolatum*, *Tholymis tillarga* are found in shallow water and *Orthetrum* sp., *Macromia* sp., *Brachythemis contaminata* found in deep water. *Macromia moorei*, however preferred sandy bottom. *Anax guttatus*, *Potomarcha* sp. were found in organically polluted water bodies where effluents mixed with reservoir. *Orthetrum taeniolatum* and *Tholymis tillarga* are indicators of highly alkaline water where industrial effluents occur. Odonata, dragonflies and damsel flies larve were also found. These insects constitute a small, well known order of insects that are widely distributed all over the world (Tillyard, 1917). They are denizens of many aquatic ecosystems and their distribution covers a great deal of continum from temporary to permanent water bodies (Corbet,1999; Johansson and Suhling, 2004). Earlier 54 species of Odonata: Anisoptera (33) and Zygoptera (21) inhabitating temporary water bodies were recorded from different parts of India (Fraser, 1933, 1934, 1936; Kumar, 1973 a,b; Singh and Prasad, 1976). Odonata were collected from water body of Sapna reservoir which were present during all the season. Only adult Odonata was collected with the help of a sweep net (35 cm dia. and 70 cm ht.) by slowly walking around the water bodies. Anisoptera and Zygoptera were found in equal proportion), both were represented by two families each *viz*., Gomphidae, Libellulidae (Anisoptera) and Coenagrionidae and Lestidae (Zygoptera). Less abundance of damselflies were found, it is probably due to their limited dispersal ability, absence of shade over the habitat from the trees present around the water bodies and due to the absence of aquatic vegetation. This is in confirmation with the findings of Fraser (1933) and Subramanian (2005) who revealed that shade and aquatic vegetation could favour Zygoptera more than Anisoptera. The size of the water body determines the species richness and diversity of Odonata (Lounibos *et al*. 1990; Clark and Samways, 1996; Stewart and Samways 1998; Schindler *et al*. 2003; Kadoya *et al*., 2004; Carchini *et al*. 2005; Suh and Samways, 2005). The maximum Odonata diversity in the dam was due to their larger size. Factors affecting Odonata species assemblage were due to human disturbances (modification of habitat structure) (Moore, 1982; Brown, 1991; Stewart and Samways, 1998; Norma-Rashid *et al*., 2001; Timm *et al*., 2001; Clausnitzer 2003; Oppel, 2005a, b), contamination of water bodies (Watson *et al*., 1982) and the presence of predators (Williams, 1987; Blaustein, 1992). Minimum diversity of species were found due to the discharge of sewage water into the reservoir and presence of insectivorous fish. The abundance of Libellulidae (Anisoptera) and Coenagrionidae (Zygoptera) in the present study might be due to their shorter life cycle and widespread distribution (Norma-Rashid *et al*., 2001) and tolerant to wide range of habitats (Gentry *et al*., 1975; Samways, 1989).

Recommendations

Trees present around the water bodies provide shade over the habitat. Aquatic vegetations need microclimate for their proliferation, so small trees should be planted near the reservoir. Due to mixing of pesticides through water run off from the agriculture fields to reservoir, infected the fishes as well as aquatic insects, it should be checked. Overexploitation, conversion of habitats, destructive land-use practices and pollution are greatest threats for normal animal and plant life, therefore, specific actions are needed for conservation of Sapna reservoir in Betul district, Madhya Pradesh, India.

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Importance and role of Green Productivity in Industries: A Review

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Abstract

The economic development policies of most developing countries have lead to industrialization and urbanization. This results in major environmental problems, such as forest degradation, air pollution and soil degradation. Thus, it is necessary to provide and compile like of base and principle of green business, green productivity and green government in order to economize in limited resources rationally and to retain resources for next generations (Pineda, 2004). It is the combined application of appropriate productivity and environmental management tools, techniques and technologies that reduces the environmental impact of an organization's activities, products and services while enhancing profitability and competitive advantage. Using the GP approach, companies can put in place waste minimization programmes first and thereafter build a formal management system to support those programmes. By implementing GP, companies can enjoy many cost savings.

Keywords: *Green productivity, Sustainable Development, waste minimization, Eco friendly, EMS, ISO 14000*

Introduction

The economic development policies of most developing countries have lead to industrialization and urbanization. These results in major environmental problems, such as forest degradation, air pollution and soil degradation Improvement in the quality of life is often associated with an increase in demand for goods and services. Production of goods and services often has two negative aspects on our environment. The demand for energy, initially through the burning of wood and charcoal and later by consumption of coal, oil, natural gas has resulted in depletion of natural resources and has produced adverse effects (Nguyen and Nguyen, 2001). Production of goods and services involves processes, which either use and/or discharge toxic and hazardous substances thus posing great risks to the environment and health. Such techniques may sometimes be economically attractive but are not sustainable because of their potential threats to society. Economic policies emphasizing productivity and economic growth alone may lead to an adverse irreversible environment (MOEA, 2002).

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Access to the goals of sustainable development would emphasize the necessity of carefulness in consumption of natural resources. Thus it is necessary to provide and compile of bases and principles of green business, green productivity and green government in order to economize in limited resources rationally and to retain resources for next generations (Pineda, 2004).

Green productivity has attained importance due to the following reasons (Avishek *et al*., 2008):

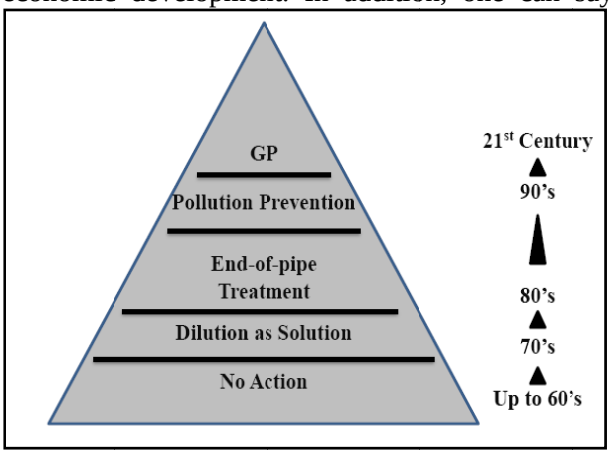
- Deteriorating Global Environment
- International Environment Treaties
- Environment & Trade
- Consumer Demand
- Need of Eco labeling
- Resource scarcity
- Economic Competitiveness
- Eco efficiency
- Occupational and Health Hazards
- Industrial Policies

Green Productivity Concept

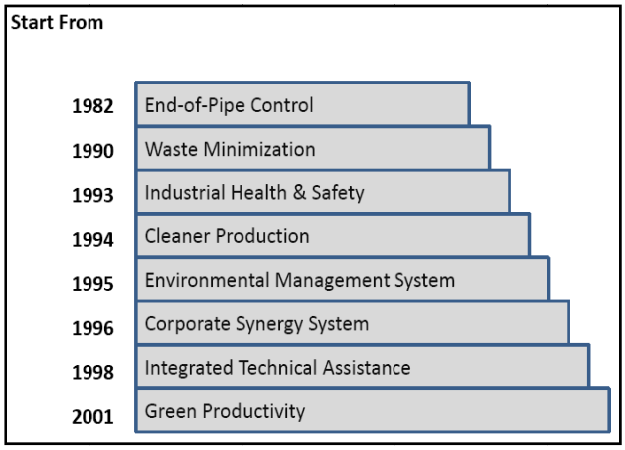
Green Productivity (GP) was launched in 1994 in line with the 1992 Earth Summit. It laid stress on economic development and environmental protection to be the key elements of sustainable development. It was initiated in Japan as APO

(Asian Productivity Organization) with an objective to enhance productivity and simultaneously reduce the negative impacts on the environment. The concept of Green Productivity is drawn from the integration of two important development strategies *via* productivity improvement and environment protection.

Productivity provides the framework for continued improvement while environmental protection provides the foundation for sustainable development. Therefore, green productivity is a strategy for enhancing productivity and environmental performance for overall social-economic development. In addition, one can say that there are essentially two reasons for the importance of Green Productivity: firstly, innovation is a primary driver of economic growth. Green productivity enhances the process of innovation, under the umbrella of Green Productivity. Innovation, a key engine of economic growth, because part of holistic strategy to move towards a sustainable future. Secondly, productivity is essentially a marathon without a finishing line. Just as productivity was the essential strategy that enabled such country like Japan to rebuild after the second war, other Asian nations are being attracted to the lure of their success (Ahmed, 2009).



(Source- MOEA, 2002)



Green productivity aims at attaining quality, productivity & environmental sustainability.

1. Quality improvement
2. Productivity improvement
3. Environmental protection
4. Sustainable development

Its quiet obvious from the above aims that green productivity involves a linkage between man, his environment and occupation

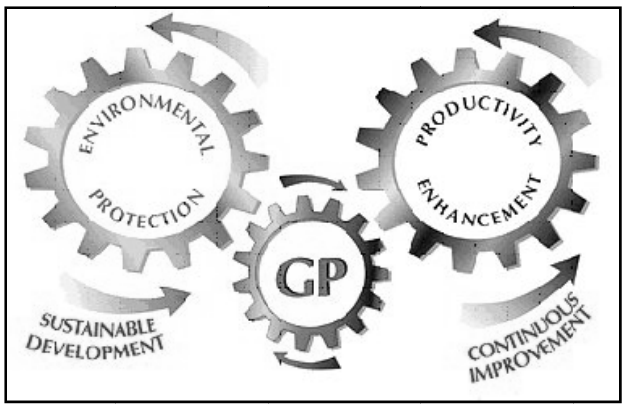
The **ecological principles** which guide green productivity are given below.

Sustainable use of Natural resources: As such earth caters to our daily needs through the vast expanse of natural resources. An optimal use of these resources will lead to sustainable development of mankind and its environment.

Protecting Ecological Biodiversity

Biodiversity plays an important role in achieving sustainability. There is a linkage between different food chains and hence a loss of one will affect the other. Hence for achieving green productivity we need tools that are self sufficient and fast in analyzing at larger scales. According to the Asian productivity Organization (APO), Japan the Tools to obtain Green productivity are:

- Cost benefit analysis
- Flow charts & Process Diagrams
- Bench marking
- Environmental Impact Assessment & Audit
- Eco Mapping
- Risk Assessment
- Life Cycle Assessment
- Energy Conservation
- Waste Reduction, Recycling, Reuse, Recovery
- Good House Keeping



(Source- MOEA, 2002)

Materials and Method

GP recommends use of various Environmental Management tools such as Environmental Management System (EMS), Design for environment (DfE), Life Cycle Assessment (LCA), Environmental Performance Evaluation (EPE) and Corporate Environmental Reporting (CER) as required in the international environmental standards under the ISO 14000 series (MOEA, 2002).The implementation and establishment of environmental management system (EMS) was first introduced following Rio De Janeiro Environment and Development Conference that resulted implementation of Agenda 21 (ANSI, 1999). International Standard Organization (ISO) developed the environmental management standard series (ISO 14000) which expanded worldwide (ISO, 1996).

Green productivity is the combined application of appropriate productivity and environmental management tools, techniques and technologies that reduce the environmental impact of an organization's activities, products and service while enhancing profitability and competitive advantage. Traditional methods of increasing productivity were not eco-friendly and the pollution control measures were not optimal for sustainable environmental protection. Green productivity programme for any unit should focus on – Increased Profitability and quality production, Environment Protection, Health & Safety, Ensure regulatory compliance and lead to Sustainable Development. Green productivity uses a set of tools & techniques that focus on eco-friendly options & alternatives in production units that can provide an overall healthy quality of life along with increased productivity (Avishek *et al*., 2008)

During the implementation of GP, proven productivity and management tools such as the cause –and effect analysis (also known as Ishikawa diagrams or "Fish Bone Diagram"), run charts for waste generation, Pareto diagrams, quality circle activities and the Japanese 5-S method for improving shop floor productivity are employed. GP is therefore not a new set of skills to be learned rather it is the application off well known tool and skills to a new set of priorities (Guan, 1999).

Results

The GP approach is an effective resource management tool. It can be used in the framework of an environment management system (*e.g.* ISO 14000) by delivering the continual improvement as required by the standard. GP is also applicable for companies working towards ISO 14000 certification. Using the GP approach, companies can put in place waste minimization programmes first and thereafter build a formal management system to support those programmes. By implementing GP, companies can enjoy many costs saving (Guan, 1999).

Other benefits include:

1. A better working environment
2. Better employ participation and team work
3. Greater job satisfaction
4. Improved corporate image & responsible citizenship of organization
5. Save money
6. Increase competitive advantages
7. Reduced environmental damage
8. Good reputation
9. Comply to regulation reduce the need to regulate the targeted sectors for environmental performance

Benefits to Business

1. Provide business with a competitive advantage
2. Increase productivity growth rates
3. Market share and profitability increase
4. Less operational and environmental compliance costs
5. Less generation of waste

6. Efficient resource utilization

GP- Steps, Tasks and Tool		
Steps	**Tasks**	**Tools**
Step I: Getting Started	▪ Form a GP team ▪ Walk through survey and information collection	▪ Brain storming ▪ Attribute analysis ▪ Needs analysis ▪ Responsibility matrix ▪ Checklists, tally charts ▪ Flow charts and process flow diagram ▪ Material balance ▪ Benchmarking
Step II: Planning	▪ Identification of problems and causes ▪ Setting objectives and targets	▪ Brainstorming ▪ Causes and effect ▪ Analysis (Ishikawa) ▪ Critical Path analysis ▪ Eco-Mapping ▪ Gantt chart ▪ Force field analysis
Step III: Generation and evaluation of GP options	▪ Generation of GP option ▪ Screening and evaluation of GP options ▪ Preparation of Implementation plan	▪ Brainstorming ▪ Cost benefit analysis ▪ Eco-mapping ▪ Failure mode and effect analysis ▪ Pareto charts ▪ Program Evaluation Review Techniques (PERT)
Step IV: Implementation of GP Option	▪ Implementation of selected options ▪ Training, awareness, building and developing competence	▪ Training need analysis ▪ Team briefing ▪ Responsibility matrix ▪ Critical path analysis ▪ Gantt chart ▪ Spider web diagrams
Step V: Monitoring and Review	▪ Monitoring and evaluation of results ▪ Management review	▪ Solution effect analysis ▪ Eco-mapping ▪ Failure mode and effect and analysis ▪ Charts (control, tally, *etc*.)/spider-web diagram
Step VI: Sustaining GP	▪ Incorporate changes ▪ Identify new/additional problem areas for continuous improvement	▪ The tools are repeated here, since the activities are looped back to the previous steps

Source: APO (2004)

Benefits to Environment

1. Economic development while maintaining productivity
2. More value to society
3. Less damage to the environment
4. Steer production and consumption patterns
5. Recycle and reuse materials
6. Conserve energy and water

Benefits to Society

1. Cleaner environment
2. Better quality of life
3. Supply of goods and services in a sustainable manner
4. Shapes society's demand

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