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Floristic composition, life form classification and biological spectrum of the catchment of Ratle H.E. project, District Kishtwar-J&K (India)

Anil K. Raina✉ and Ravinder Kumar

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Abstract

Based on floral inventorization of the vegetation of Ratle catchment area, biological spectrum on life form was prepared and compared with Raunkiaer's normal biological spectrum as well as the spectra of the adjoining areas prepared by other workers. The floristic list of the Ratle catchment consists of 384 species belonging to 96 families and 242 genera. Asteraceae has been recorded as the largest family (31 genera/52 species) followed by Fabaceae (12 genera /23 species), Lamiaceae (14 genera/ 23 species), Rosaceae (10 genera/ 17 species) etc. Thirty nine families show monotypic representation. The ratio of family to genera was calculated as 1:2.52; family to species as 1:4 and genera to species as 1:1.59. According to the Raunkiaerian life form classification (1934), Therophytes (33.85%) and Hemicryptophytes (18.75%) were found to be dominant thus indicating thero-hemicryptophytic type of phytoclimate in the study area.

Keywords: *Biological spectrum, life forms, phytoclimate, Ratle H.E. Project*

Introduction

Study of floristic composition and phytoclimate of an area is important as the change in structure and compositions of vegetation are sensitive indicators of whole environment. It reflects the adaptation of plants to climate and ecological conditions of an area. The life form of a plant is the physiognomic form produced as a result of all life processes after interaction with the environment. The relative proportion of different life forms for a given region or an area is called its biospectrum. Biological spectra are useful in comparing geographically widely separated plant communities and are also regarded as indicators of biotic interaction, climate and habitat deterioration. Plants can be grouped in life form classes based on their similarities in structure and function (Mueller-Dombois and Ellenberg; 1974). According to Cain (1950) life form study is an important part of vegetation description, ranking next to floristic composition.

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Different life form classifications and modification have been proposed by various workers including Raunkiaer (1905, 1918, 1934), Braun-Blanquet (1932) and Cain (1950) from time to time. However, the Raunkiaer's (1934) classification is most convincing and has been widely accepted. Raunkiaerin approach explains and helps in understanding the flora and structure of vegetation in relation to prevailing eco-biological conditions. It reflects the impact of current biotic factors, like overgrazing, over harvesting, deforestation on the overall vegetation structure and composition. It also influences the economic value of plants in various ways. The approach is useful in developing management plan for the sustainable harvest of plant resources. The Raunkiaer's Normal Spectrum prepared for the phanerogamic flora of the whole world is still widely used for comparing biological spectra of different regions. In Jammu and Kashmir, several workers have studied the floristic composition and biological spectra of different areas. This includes the works of Sapru (1975), Kaul and Sarin (1976), Kapur (1982), Dhar and Kaul (1986), Kumar (1987),

Singh and Kachroo (1994), Kumar (1997), Kour (2001), Singh (2002), Kesar (2002), Sharma (2003), Jhangir (2004), Dutt (2005), Rai (2007) and others. However, the work on this aspect in district Kishtwar has not been done so far. Therefore, in the present study, biological spectrum of the study area has been prepared by following Raunkiaer (1934) life form classification after enumeration of the floristic composition.

Study area

The study area includes the total catchment of Ratle Hydro-electric project that is a run-off the river scheme on the River Chenab with its proposed dam site at village Drabshalla in district Kishtwar, Jammu & Kashmir. The dam site is located at Latitude 32°06' N to 34°12.5'N and longitude 75°23'E to 77°48'E. The catchment area of River Chenab up to dam site of the project is estimated to be 14965 km^2. The project area falls mostly in between sub-tropical to temperate zone. In the project area, average maximum and minimum temperature during winter and summer are reported to be 25° C & -0.5° C and 43° C & 15° C, respectively (at Ratle dam site). Average annual rainfall in the project area is 843 mm. The Ratle catchment area lies in inner lesser Himalayas under Kishtwar group of rocks. All the project components lie mainly in gneiss/schist rock of Salkhalas formation.

Materials and Method

Plant diversity and floristic composition of the area was studied by making field trips from October 2007 to March 2010. The area was surveyed during all the four seasons of the year and care was taken to cover all the possible watersheds, habitats and vegetation types. Plant species were photographed and accordingly identified using local herbaria, flora and relevant literature available for the region. Taxonomic identification was done by using different floras and by consulting taxonomic experts. Utmost care was taken during survey and enumeration to avoid disturbance to flora and fauna. The plant species so inventorised have been classified into various life form classes as proposed by Raunkiaer (1934) classification.Biological spectrum of the area was prepared and was compared with the Raunkiaer's normal Spectrum as well as the spectra of the adjoining areas prepared by other workers.

Results and Discussion

The study area represents sub-tropical to temperate vegetation. The lower altitude belt near the proposed dam site is represented by sub-tropical vegetation with dominance of *Alnus nitida, Pinus roxburghii, Populus ciliata, Pyrus pashia, Robinia pseudoacacia, Dalbergia sissoo, Berberis lycium, Justicia adhatoda, Vitex negundo* etc. The temperate vegetation is prominently represented by *Quercus semicarpifolia, Pinus wallichiana, Cedrus deodara, Quercus baloot* etc.

Among the climbers, *Hedera nepalensis* is one liana that climbs mostly on *Cedrus deodara.* The other lianas of this area are *Tylophora hirsuta, Convolvulus arvensis* and *Cissampelos pareira. Cuscuta reflexa* is one of the parasites found in the region.

During the field inventorization, a total of 384 plant species belonging to 242 genera and 96 families have been recorded. The highest species representation has been observed in the family Asteraceae (52) which is followed by Fabaceae (23), Lamiaceae (23), Rosaceae (17) and others. Asteraceae has also been reported to be dominant family in the adjoining areas like Bhaderwah (Kumar, 1987); Patnitop Hills (Kumar, 1997); Trikuta Hills (Kour, 2001) and Neeru Watershed, Bhaderwah (Dutt, 2005). The comparison of dominant families recorded in the study area with that of adjoining areas have been presented in Table-1. The analysis of data reveals the presence of 363 angiosperms (330 dicots and 33 monocots), 16 pteridophytes and 5 gymnosperms in the study area. 39 families have been recorded to be monotypic in this area represented by single species. The genus with maximum number of species in the study area are *Anaphalis* (7), *Geranium* (6), *Ipomoea* (6), *Cyperus* (5), *Euphorbia* (5) and *Leucas* (5) etc. The analysis of the data further reveals that the ratio of family to genera is 1:2.52; family to species is 1:4 and genera to species is 1:1.59. The ratio of genera to species which reflects the floristic pattern in given time and space is lower than that derived for British India – 1:7 (Hooker, 1872-97); India alone – 1: 6 (Chatterjee, 1939); Himachal Pradesh – 1: 2.93 Bashar Himalayas – 1: 2.29 (Aswal and Mehrotra, 1994): Shimla – 1:20 (Collet, 1902); Great Himalayan National Park – 1:1.94 (Singh and Rawat, 2000);

Kullu – 1: 1.84 (Dhaliwal and Sharma, 1999); Kangra – 1:1.72 (Kapur, 1985); Mussourie – 1: 1.87 (Raizada and Saxena, 1978). The ratio tends to match with the Valley of flowers – 1:43 (Kala and Rawat, 2004); Sirmour – 1: 1.65 (Kaur and Sharma, 2004): Trilkuta Hills – 1:42 (Kapur and Sarin, 1990), Patnitop and adjoining areas – 1:1.44 (Kumar, 1997) and Trikuta Hills – 1:1.42 (Kour, 2001). As per the available records, the percentage of dicots and monocots species in the world flora

Table-1: Comparison of dominant families of study area with adjoining areas

S.No	Study area	Patnitop Hills (Kumar, 1997)	Bhaderwah (Kumar, 1987)	Neeru Watershed Bhaderwah (Dutt, 2005)	Trikuta Hills (Kour, 2001)
1.	Asteraceae	Asteraceae	Asteraceae	Asteraceae	Asteraceae
2.	Fabaceae	Labiatae	Poaceae	Labiatae	Fabaceae
3.	Lamiaceae	Poaceae	Apiaceae	Apiaceae	Poaceae
4.	Rosaceae	Fabaceae	Labiatae	Ranunculaceae	Labiatae
5.	Poaceae	Roasaceae	Ranunculaceae	Roasaceae	Euphorbiaceae
6.	Amaranthaceae	Ranunculaceae	Cruciferae	Cruciferae	Scrophulariaceae

is 81.3% and 18.7%, respectively. Different studies carried out in different parts of Jammu by Kumar (1987), Kumar (1997), Kour (2001) and Singh (2002) have reported higher percentage of dicots.

The present study also revealed the higher percentage of dicots (90.9%) from the study area. The ratio of the monocot to dicot families have been recorded as 1:5.3, of genera 1:8.1 and of species 1:10 (Table-2).

Table-2: Percentage and ratios of families, genera and species of dicots and monocots (excluding gymnosperms and pteridophytes)

Taxa	Dicots		Monocots		Total	Ratio	
	Total Number	Percentage (%)	Total Number	Percentage (%)		Monocots	Dicots
Families	74	84.1	14	15.9	88	1	5.3
Genera	202	89.0	25	11.0	227	1	8.1
Species	330	90.9	33	9.1	363	1	10.0

In the present study, the representative flora constituting the class therophyte is mainly characterized by species like *Chenopodium album, Euphorbia indica, Impatiens balsaminea, Malvastrum coromandelianum, Ranunculus arvensis, Solanum erianthum, Sonchus asper, Taraxacum officinale, Tridax procumbens* etc. Class hemicryptophyte is represented by *Androsace rotundifolia, Asplenium oxyphyllum, Cyperus rotundus, Gentiana kurroo, Lathyrus humilis, Leucas capitata, Plantago ovata, Saussurea costus* etc. Class chamaephyte includes species like *Achyranthes aspera, Artemisia brevifolia, Barleria cristata, Cannabis sativa, Lotus corniculatus, Rubus ellipticus, Rumex hastatus etc.* Class macrophanerophyte is represented by *Aesculus indica, Ailanthus excelsa, Albizia chinensis, Alnus nitida, Toona ciliata, Cedrus deodara, Dalbergia sissoo, Ficus palmata, Grewia optiva, Juglans regia, Pinus roxburghii, P. wallichiana, Quercus baloot, Q. floribunda, Q. glauca, Q. leucotricophora, Q. semecarpifolia, Robinia pseudoacacia* etc. whereas class nanophanerophyte is mainly constituted by *Berberis lyceum, Dendrocalamus strictus, Euphorbia royleana, Ipomoea carnea, Justicia adhatoda, Prinsepia utilis, Vitex negundo* etc.The biological spectrum of study area reveals the

dominance of Therophytes (33.85%) followed by Hemicryptophytes (18.75%), Chamaephytes (14.32%), Macrophanerophytes (11.72%), Nanophanerophytes (10.94%), Geophytes (4.95%), Lianas (3.91%), Hydrophytes (1.04%) and Epiphytes (0.52%). The total species count and percent values of life form classes found in study area has been summarised in Table-3. From this, it can be derived that the phytoclimate of the study area is of Thero-Hemicryptophytic type. The comparison of the biological spectrum of the study area with Raunkiaer's normal biological spectrum is presented in the Table-4.

The thero-hemicryptophytic climate of the area is attributed to various factors like prevalent microclimate of the region coupled with anthropogenic activities like grazing, developmental activities and longitudinal and latitudinal difference, as has also been advocated by other workers (Kumar, 1997; Kour, 2001; Kesar, 2002; and Singh 2003).The predominance of therophytes indicates a disturbed environmental condition where phanerophytes cannot establish themselves. Anthropogenic activities including overgrazing, overharvesting and developmental activities reduce the macro element of the vegetation. This facilitates the dominance of other life form classes. Sher *et al*. (2004a) have also reported that extensive biotic influences increased short lived annuals. Ansari & Singh (1979) and Sharma & Dhakre (1993) also attributed the biotic interference, overgrazing etc. for the dominance of therophytes in their respective study areas.

The comparison of life forms of study area with adjoining areas having similar climatic conditions in Northwestern Himalayas is represented in the Table 5. Perusal of the table reveals that all these regions show different type of phytoclimate despite being similar to one another. This may be because of the varied amount of disturbances and longitudinal and latitudinal difference in these areas. Among these areas, the phytoclimate of study area resembles to that of Patnitop (Kumar, 1997) and Trikuta hills (Kour, 2001).

Table-3: Total number of species and percentage of different life form classes

Lifeform class	No. of species	Percentage (%)
Therophytes (TH)	130	33.85
Hemicryptophytes (H)	72	18.75
Chamaephyte (CH)	55	14.32
Macrophanerophytes (M)	45	11.72
Nanophanerophytes (N)	42	10.94
Geophytes (G)	19	4.95
Lianas(L)	15	3.91
Hydrophytes and Helophytes (HH)	4	1.04
Epiphytes (E)	2	0.52
Total	384	100

Table-4: Comparison of biological spectrum of study area with Raunkiaer's (1934) Normal Biological Spectrum

Life Form	TH	HH	G	H	CH	N	M	L	E
Percentage lifeform (present study)	33.85	1.04	4.95	18.75	14.32	10.94	11.72	3.91	0.52
Percentage lifeform in normal spectrum	13	2.0	4.0	26.0	9.0	15.0	28.0	–	3.0
Percentage deviation	+20.85	-0.96	+0.95	-7.25	+5.32	-4.06	-16.28	+3.91	-2.48

Table-5: Comparison of life forms of study area with adjoining areas having similar climatic conditions in Northwestern Himalayas

Life forms		TH	HH	G	H	CH	N	M	L	E
Study Area	Author	33.85	1.04	4.95	18.75	14.32	10.94	11.72	3.91	0.52
Bhaderwah	Kumar, 1987	29.30	3.27	4.50	37.09	11.47	6.14	6.35	1.43	0.40
Patnitop	Kumar, 1997	29.8	3.2	3.5	26.4	15.2	8.5	10.4	2.6	0.2
Trikuta Hills	Kour, 2001	27.31	3.09	7.73	26.00	16.49	15.0	28.0	1.03	3.0
Kalakote Forest Range	Singh, 2002	30.89	1.40	3.65	12.64	16.85	12.35	16.57	5.61	-
District Jammu	Sharma, 2003	35.45	1.78	2.02	9.11	13.93	11.64	16.70	8.87	0.50
District Kathua	Jhangir, 2004	32.89	1.27	2.55	15.77	12.36	12.79	16.63	5.11	0.63
Mansar-Surinsar Wildlife Sanctuary	Rai, 2007	34.70	4.46	2.06	7.56	15.46	10.65	15.80	8.59	0.68

Conclusion

The forests of the study area represent sub-tropical to temperate vegetation. During the floristic survey Asteraceae, Fabaceae, Lamiaceae and Rosaceae have been found to be dominant families. Phytoclimate of the study area has been worked out to be of Thero-Hemicryptophytic type. The therophytes, chamaephytes and geophytes constitute the higher percentage than the normal spectrum. It clearly indicates that some anthropogenic (overgrazing and developmental activities) are operating together and favouring the chances of growth of short lived annuals.

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In vitro anti-malarial activity of aerial part of *Boenninghausenia albiflora*

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Abstract

Boenninghausenia albiflora **belonging to the family Rutaceae is well known for its medicinal properties in traditional system of medicine. The crude ethanolic extracts of aerial part were tested for *in-vitro* anti-plasmodial activity against two strains of *Plasmodium falciparum*: MRC-pf-20 (chloroquine resistant strain) and MRC-pf-303 (chloroquine sensitive strain), using the parasite lactate dehydrogenase (pLDH) assay. Crude ethanolic extracts showed good anti-plasmodial activity *in vitro* ($IC_{50} \leq 50$ µgml^{-1}). It showed promising anti-plasmodial activity with an IC_{50} = 22.18µgml^{-1} on MRC-pf-20 strain and IC_{50} = 27.43µgml^{-1} on MRC-pf-303 strain. Crude ethanolic extracts were be further fractionated by partitioning in water and dichloromethane. The dichloromethane fraction revealed stronger anti-plasmodial activity than crude ethanolic extracts. The dichloromethane extract of *B. albiflora* showed promising anti-plasmodial activity with an IC_{50} = 12.32 µgml^{-1} on MRC-pf-20 strain and IC_{50} = 17.41 µgml^{-1} on MRC-pf-303 strain. Therefore, there is need to continue the extensive study including isolation and identification of the active compound and their *invitro* and *invivo* activity.**

Keywords: *Boenninghausenia albiflora, Plasmodium falciparum, Chloroquine, Anti-malarial activity*

Introduction

Malaria is still the most destructive and dangerous parasitic infection in many tropical and subtropical countries. The burden of this disease is getting worse; mainly due to the increasing resistance of *Plasmodium falciparum* against the widely available anti-malarial drugs. The use of chloroquine to prevent and treat falciparum malaria has lead to the wide spread appearance of chloroquine-resistance strain of *P. falciparum* throughout the affected regions. The resistance has at the same time increasingly extended to other available antimalarial drugs (Peters, 1982).

Approximately 80% of the world's population still depend on traditional medicine as a source of the treatment of disease (Phillipson and Wright, 1991). Local medicinal plants continue to be used in the treatment of malaria and update the evaluation of antimalarial activity of medicinal plants against *P. falciparum has been*s extensively studied (O'Neill *et al*., 1986; Chen *et al*., 1994; Basco *et al*., 1994; Gakunju *et al*., 1995; Connely *et al*., 1996).

History reveals that plants have always been considered as an important source of medicine against malaria both quinine and artemisinin have been derived from traditional medicine and plant extracts. Artemisinin derivatives are now recommended by the World Health Organization worldwide (WHO, Mutbingawa, 2005), in combination with other drugs, such as lumefantrine, amodiaquine, mefloquine, sulphadoxine-pyrimethamine (SP), as the first-line treatment of malaria.

This fact has encouraged the continuing search for new natural product-derived anti-malarial drugs. In malaria-endemic countries, several plants are utilized in traditional medicine for the treatment of malaria and fever. Furthermore, several studies have been undertaken to evaluate not only the inhibitory effects of various plant extracts on *P. falciparum* (Tran *et al*., 2003, Wanoiyke, 2004) using *in vitro* culture, but also *in vivo* anti-malarial properties on *P. berghei*-infected mice (Andarade, 2003, Sudhanshu, 2004). There is an urgent need for new, more affordable and accessible antimalarial agents possessing original modes of action. Natural products have played a dominant

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role in the discovery of leads for the development of drugs to treat human diseases, and this fact anticipates that new antimalarial leads may certainly emerge from tropical plant sources.

Therefore based on ethnical knowledge the plant *Boeninghausenia albiflora* was selected for the antimalarial activity from Himalayan region of India. *B. albiflora* is a slender, erect, glaucescent, gland dotted, glabrous to somewhat pubescent, perennial herb, 15-60 cm in height. *B. albiflora* belonging to the family Rutaceae and is well known for its medicinal properties in traditional system of medicine. In ethnobotanical literature, the aerial as well as the root part has been described as an antiseptic. Gaur (1999) mentioned that leaf part has been used to apply on cuts and wounds whereas root powder is being used as antiseptic. Sometimesits juice is also being given in vomiting and dysen tery. Some workers also reported this plant to have flea repellent as well as calcium blocking activity (Yamaha *et al*., 1987). It is distributed from Kashmir to Arunachal Pradesh, Meghalaya and Mizoram, India between 660-2,640 m height having good medicinal value (Fig.1).

The present study was undertaken to investigate the antimalarial activity of *B. albiflora* (aerial part) due to good medicinal value and there has been no report on its activity against malaria parasite. The aim of this study was to discover novel, effective plant based medicines for the treatment of malaria. In light of this *in vitro* screening, the anti-plasmodial properties of this plant species were determined.

Material and Methods

Selection and collection of plant materials was carried out based on literature, ethnical knowledge, plants representing Rutaceae families, that used against fever, inflammation and microbial infection have been selected. The plants were collected from Chakrauta, Garhwal region of North West Himalayas India (Fig. 1).

The plant was identified by Dr. Sumer Chand, Botanist, Forest Research Institute Dehradun India. A voucher specimen was preserved at herbarium of Forest Research Institute of Dehradun, India. After identification, aerial parts of this herb were air-dried in shadow at room temperature. The plants were crushed into fine powder using anelectric grinder. The powdered samples were stored inappropriate containers and were kept at a cold room (4°C).

Fig.1. Aerial part of *B. albiflora.*

Extraction and Isolation

100 g of powdered materials was extracted by percolation three times using 80% ethanol at room temperature. The ethanol extracts (named BA1) were filtered, pooled and dried at 40°C or below using a rotary evaporator. Further BA1 extract was kept in airtight containers and were stored at 4°C for use in anti-plasmodial bioassay. Crude ethanolic extracts that showed good anti-plasmodial activity against *P. falciparum* strains, were further fractionated by partitioning in water (name BA2) and dichloromethane (BA3). The organic and aqueous phases were concentrated and dried by rotary evaporator and were dissolved in di-methylsulphoxide (DMSO) and double distilled water, respectively, and then stored at -20°C for use of anti-plasmodial assay.

P. falciparum strains and *in vitro* culture

Two strain laboratory-adapted *P. falciparum* MRC-pf-20 (chloroquine sensitive) and MRC-pf-303

(chloroquine resistant) were taken from parasite bank of National Institute of Malaria Research, New Delhi India and maintained to continue on human erythrocytes. The cultures have been maintained in the laboratory using the Candle Jar method of Trager and Jensen (1976) in human red blood cells (blood type A+) using RPMI 1640 medium supplemented with ABRh +ve human serum (10%), sodium bicarbonate (0.2%), HEPES buffer (25mM) and gentamycin (50μgml^{-1}). The cultures were incubated at 37^0C in an atmosphere of 93%N_2, 4%CO_2 and 3%O_2.

In vitro anti-plasmodial assay

The parasite cultures, prior to experimentation, were synchronized by treatment with 5% D-sorbitol (Lambros C. and Vanderberg, 1979). Synchronise cultures containing ring staged parasites were suspended in equal volume of human serum. Plant extracts were assessed for anti-plasmodial activity *in vitro* using modified parasite lactate dehydrogenase (pLDH) method as described previously (Makler and Hinrichs ,1993 and Makler , *et. al.*,1993).

Crude plant extracts were first dissolved in DMSO at concentration of 50 $mgml^{-1}$, sonicated for 10 min and then diluted in malaria culture medium to prepare a 2 $mgml^{-1}$ solution. The highest concentration of solvent that the parasites were exposed to was < 1%, which was shown to have no measurable effect on parasite viability. Microtitration techniques were used to measure the activity of samples over a wide range of concentrations (ranging from 200-1.56 μgml^{-1}). Chloroquine diphosphate and artemisinin (both from Sigma Chemical, USA) were dissolved in double distilled water (1 $mgml^{-1}$) and DMSO (1 $mgml^{-1}$), respectively and served as controls in all experiments. All tests were performed in triplicate. Synchronous cultures with parasitaemia of 1% and a final haematocrit of 2% were aliquoted into the plates and incubated at 37°C for 72 h. After incubation period, the plates were frozen at -20°C overnight, followed by thawing at room temperature to haemolyze the red blood cells. Parasite growth was determined spectrophotometrically at 650 nm, by measuring the activity of the pLDH in control and drug treated cultures, using a microplate reader. At the end of incubation, the cultures were re-suspended, and aliquots of 20 µl were removed and added to 100 µl of the Malstat reagent in a 96- well microtiter plate. The spectrophotometric assessment of pLDH activity was obtained by adding 25 µl of a solution of 1.9 µM NBT (Nitro Blue Tetrazolium) and 0.24 µM PES (PhenazineEthosulphate) to the Malstat reagent. The anti-malarial activity of the test compound was expressed as Inhibitory Concentration IC_{50} (mean ± S.D.) of the least three separate experiments performed in triplicate). The OD values from control wells devoid of plant extracts or drug were referred to as having 100% pLDH activity. The inhibition of each extract or drug concentration was calculated as compared to the untreated control to obtain the IC_{50} values. The percentage of inhibition was calculated as (1-Number of schizonts in test well/Number of schizonts per control well) x 100. The 50% inhibitory concentration (IC_{50}) of plant extracts were estimated from the graph drawn on the %inhibition data (Table.1).

Results and Discussion

In vitro activity of ethanolic extract of aerial part of *B. albiflora* were carried out against Chloroquine sensitive (MRC-pf-20) and chloroquine resistant (MRC-pf-303) results showed in (Table.1). The ethanolic extract (BA1) of *B. albiflora* was further fractionated in water and dichloromethane mixture solution. Further anti-plasmodial activity of organic and aqueous phases was evaluated against CQ-sensitive and CQ-resistant *.P. falciparum* strains. The dichloromethane fraction showed stronger anti-plasmodial activity than the ethanol and water extracts. The inhibitory concentration values which kill 50% and 90% of the parasites (IC_{50} and IC_{90}) were calculated for anti plasmodial activity (Table 2). Results revealed that IC_{50} value with standard deviation of dichloromethane is 12.33 ± 0.33μgml^{-1} which, is the more effective than other fractions. Recently Ali Ramzani *et al.*, 2010 reported invitro and invivo anti malarial activity of ten plants from Iran showed only two plants (*Boerhavia elgans* and *Prosopis juliflora*) out of ten are effective with IC_{50} value is 15.33 ± 0.07μgml^{-1} and14.78 ± 0.08μgml^{-1} respectively. While the remaining eight plants showed the IC_{50} value > 200μgml^{-1} which is more in comparison to *B. albiflora* fraction. Thus, there are so many work has been carried out earlier in this field but result is not effective. Overall, increasing the global spread of multi drug resistant malaria parasite showed that

Table:1.Percent(%) Inhibition of Schizonts by Ethanolic extracts of *B. albiflora* aerial part against CQ-sensitive and CQ-resistant strain.

Plant extracts	Concentration of drug (µg/ml)	Chloroquine sensitive (MRC-pf-20) (%± S.D)	Chloroquine resistant (MRC-pf-303) (%± S.D)
Ethanol(BA1)	2.0	8.33 ± 1.30	7.40 ± 1.17
	4.0	13.67 ± 1.02	12.00 ± 1.58
	8.0	22.37 ± 1.06	19.60 ± 0.7
	16.0	33.17 ± 1.55	29.50 ± 0.98
	32. 0	61.47 ± 0.81	59.47 ± 1.20
	64.0	84.43 ± 1.35	82.43 ± 0.91
	128	97.33 ± 1.22	94.66 ± 0.94
	256	100	100
	512	100	100
Control	0	0	0
Dicholoromethan (BA3)	2.0	10.33 ± 1.30	8.83 ± 1.30
	4.0	22.43 ± 1.35	17.67 ± 1.02
	8.0	41.77 ± 1. 05	35.60 ± 1.13
	16.0	65.50 ± 1.08	59.50 ± 1.08
	32. 0	86.90 ± 1.05	80.33 ± 1.15
	64.0	97.43 ± 1.35	94.43 ± 1.35
	128	100	100
	256	100	100
	512	100	100
Control	0	0	0

CQ = Chloroquine, n = 3

Table.:2 In-vitro anti plasmodial activity(IC_{50}) ofaerial part of *B. albiflora*(BA)against both strain (CQ-Sensitive and CQ-Resistance).

Plant extracts	Chloroquine sensitive (MRC-pf-20) µgml^{-1}		Chloroquine resistant (MRC-pf-303) µgml^{-1}	
	IC_{50} ± S.D	**IC_{90} ± S.D**	**IC_{50} ± S.D**	**IC_{90} ± S.D**
Ethanol (BA1)	22.18 ± 0.22	100.35 ± 0.17	27.43 ± 0.25	112.44 ± 0.30
Water (BA2)	>200	Not Calculated	>200	Not calculated
Dichloromethane (BA3)	12.33 ± 0.33	60.89 ± 0.20	17.41 ± 0.42	62.45 ± 0.31

N = 3 , S.D = standard deviation

there is a need for new drug to combat malaria. In this study, the main aim to search for new antimalarial drug, it was found for the first time *in-vitro* good anti plasmodial activities of the *B. albiflora*. This study provides the important information to the area of malaria research where always is in need of alternative anti malarial drug. The present finding is only preliminary, but the next step will be to isolate and identify the active compound of *B. albiflora*. There is need of further investigation of anti-malarial activity at compound level and the cyto-toxicity as well.

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Determination of aflatoxin level in peanut using immunoaffinity column combined with ELISA

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Abstract

Peanut (*Arachis hypogaea* L.) is a largest source of edible oil in India, extensively consumed in the central and western parts of the country. The variability in the total aflatoxin and aflatoxin B_1 levels in the different peanut samples collected was investigated. Quantitative analysis of total aflatoxin and aflatoxin B_1 (AFB_1) content was performed by competitive ELISA micro plate reader using total aflatoxin and aflatoxin B_1 test kit. All the seed samples investigated were found positive for aflatoxin. The total aflatoxin content ranged from 24.53 to 250.34 ppb, whereas the concentration of AFB_1 was in the range of 18.55 to 234.50 ppb. More than 86% of samples showed aflatoxin content above regulatory limits. 40% of the samples showed high levels (.>100 ppb) indicating high health risk of exposure to aflatoxin. Aflatoxin contamination of peanut seeds and oil is therefore an important public health concern. More precaution should be taken for proper storage of peanut seeds in order to prevent microbiological and chemical hazards.

Keywords: Peanut, *Arachis hypogaea*, aflatoxins, aflatoxin B_1, ELISA

Introduction

The problem of food and feed contamination with toxigenic moulds especially *Aspergillus* species has received a great deal of attention during the last three decades (Ardic *et al*., 2008; Rustom, 1997). These fungi are capable of growing on a great variety of food commodities and animal feed materials when the conditions of temperature, relative humidity and moisture are favorable (Iqbal *et al*., 2006; Rosi *et al*., 2007). Mycotoxins are highly toxic, mutagenic and carcinogenic compounds contaminating a wide variety of agricultural commodities (Bilotti *et al*., 2000; Abdulkadar et al., 2000; Shenasi *et al*., 2002; Arrus *et al*., 2005). Aflatoxins are a group of extremely toxic metabolites produced by some *Aspergillus* species namely *Aspergillus flavus*, *Aspergillus parasiticus* and the rare *A. nomius*, during the growth on food and feeds. *A. flavus* produce only aflatoxin B, while other species produce both B and G aflatoxins (Sweeney and Dobson, 1998; Creppy, 2002). There are four major aflatoxins referred to as B_1, B_2, G_1 and G_2, which are often found in tropical and subtropical climates (Shenasi *et al*., 2002). Aflatoxin B_1 (AFB_1) is the most potent of these naturally occurring aflatoxins (Leontopoulos et al., 2003). The peanut (*Arachis hypogaea* L.) which is also popularly known as groundnut is one of the world's most popular and universal crops, cultivated in more than 100 countries of six continents (Patil *et al*., 2009). Aflatoxin contamination in peanut has been reported in Nigeria (Thomas *et al*., 2005) and India (Bhat *et al*., 1996). Earlier workers (Blesa *et al*. 2003; Yentur *et al*., 2006) have reported a high incidence of occurrence of aflatoxins in peanuts and peanut products such as peanut butter.

Due to their frequent occurrence and toxicity, regulatory agencies are imposing uniformly rigorous standards on the level of acceptance in imported commodities. Acceptable levels regulatory limit of aflatoxins in different countries is shown in Fig. 1 (Anonymous, 2009). Despite lots of studies on aflatoxin in agricultural products, only a few are concerned with peanut that is more

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commonly used in cooking and play an important role in the economy of the country. The result of this study can contribute to the evaluation of peanut consumed by a lot of people in different states of India, from the point of view of food safety. Our aim was to determine the presence and levels of aflatoxins in peanut seed samples and to evaluate whether aflatoxin levels were within the Indian regulatory values or not.

Materials and Methods

Collection of Samples and Fungal counts

Peanut samples (5 kg each lot) were purchased from store merchants and open market vendors of Lucknow. Fifty grams seeds from each lot were drawn in triplicate and dried in an oven at 35 ^{o}C till complete removal of moisture content. Dried seeds were ground to fine powder using laboratory grinder and analysed for their aflatoxin content. Fungal count (C.F.U. g^{-1}) from seed samples were made by dilution method on potato dextrose agar using serial dilution following the method of Sidhu *et al*. (2009).

Estimation of aflatoxin

Five grams ground powder of peanut kernel was extracted with 25 mL of 70% aqueous methanol using a laboratory homogenizer and filtered through Whatman #1 filter paper. One hundred microliters of each filtrate was diluted with 600 µL of dilution buffer and 50 µL of diluted sample employed to immunoaffinity column for cleaning the samples. Aflatoxin fraction was finally eluted with 0.5 mL of HPLC grade methanol and total aflatoxin content and aflatoxin B_1 were determined using aflatoxin detection kit obtained from R-Biophram AG, Darmstadt, Germany. Fifty microliters of standard solution of aflatoxin and eluted samples (in duplicate) were added to the wells of microtiter plate. Further, 50 µL of peroxidase enzyme conjugate and 50 µL of mouse monoclonal anti-aflatoxin antibodies were added to each well. The plates were incubated at room temperature in the dark for 30 min. After washing thoroughly with 250 µL distilled water three times, 50 µL of urea peroxidase (substrate) and 50 µL of tetramethylbenzidine (chromogen) were added to each well, mixed thoroughly and incubated for 30 min at room temperature in the dark. Reaction was stopped by adding 100 µL 1 M sulphuric acid (stop reagent) and the absorbance was measured at 450 nm using Bio-Rad ELISA microplate reader Model 680. There were three replicates for each seed lot investigated in the present study. Kernels of peanut were extracted by cold press expeller (Komet, IB6 Monforts, Germany) to determine the total aflatoxin and aflatoxin B_1 contamination in peanut kernels, cake and oil samples.

Results and Discussion

Quantitation of aflatoxins

Quantitative analysis of total aflatoxin and aflatoxin B_1 (AFB_1) was performed by competitive ELISA Microplate reader using total aflatoxin and aflatoxin B_1 test kit (RIDASCREEN, Dermstadt, Germany). Aflatoxins were separated and purified by immunoaffinity columns and purified fractions were analysed for total aflatoxin content and AFB1 by antigen-antibody reactions using ELISA. A calibration curve was drawn using a wide range of total aflatoxin standards with concentration of 0–4050 ppt and for aflatoxin B_1 (AFB_1) with concentration of 250–4000 ppt. A plot in between the percentage absorbance and concentration of both the total aflatoxin and AFB_1 for a set of standard indicated a linear relationship (Sidhu *et al*., 2009). Several methods have been reported for the determination of aflatoxins in a number of food commodities (Gilbert and Anklam, 2002). Methods based on thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assays (ELISA) are mainly used in routine analysis. Nowadays, these methods involve immunoaffinity column cleanup procedures, which offer the extraction of aflatoxins from most food matrices with simple aqueous solvent mixtures (Stroka *et al*., 2000).

Occurrence of aflatoxin in peanut seeds

Peanut samples were purchased from local markets of Lucknow in triplicate and analyzed for variability in their fungal counts, total aflatoxin and aflatoxin B_1 content. Fungal count, total aflatoxin and aflatoxin B_1 content of the investigated samples are presented in Table 1. Fungal counts ranged from 2.9×10^3 to 2.5×10^6 C.F.U. g^{-1} (Table 1). All the seed samples investigated were found positive

for aflatoxin. Out of fifteen seed lots screened for their aflatoxin content, six were found to be contaminated with >100 ppb of total aflatoxin and aflatoxin B_1 content. Total aflatoxin content ranged from 24.53 to 250.34 ppb, the lowest being in Rajajipuram and the highest in Daliganj. The concentration of aflatoxin B_1 was in the range of 18.55 ppb in Rajajipuram to 234.50 ppb in Daliganj (Table 1). Peanut oil was extracted using cold press expeller and the aflatoxin content was determined in peanut oil and cake samples. The concentration of total aflatoxin content was 250.34 ppb in kernels, 205.6 ppb in oil and 40.77 ppb in cake whereas aflatoxin B_1 content was 207.55, 175.6 and 25.88 ppb in kernel, oil and cake, respectively (Fig. 2). by Loosmore *et al*. (1964) and Wagon (1965) have reported aflatoxins contamination in peanut products, cotton seed cake and other nuts and oilseeds samples. A number of survey and monitoring programs have been carried out in several countries for aflatoxin contamination in food products (Yndestad and Underdal, 1975; Girgis *et al*., 1977; Tabata and Kamimura, 1988).

India has been reported to be a World leader in peanut farming (FAO, 2004). Peanut is the single largest source of edible oils in India and constitutes roughly about fifty percent of the total oilseeds production and extensively used in cooking in India. Whole kernels are used for table purpose by frying, soaking, roasting and boiling and in different types of *namkeens*. Roasted peanut is the most popular way of eating (Patil *et al*., 2009). Aflatoxin contamination of market peanut, therefore, is an important public health concern.

Table 1 : Fungal count, total aflatoxin, aflatoxin B_1 and total aflatoxin/aflatoxin B_1 ratio in peanut grain samples collected from different markets of Lucknow, India

S.N.	Site of Collection	Fungal count (C.F.U. g^{-1})	Total Aflatoxin (ppb)	Aflatoxin B_1 (ppb)	Total/AFB_1 Ratio
1	Rajajipuram	2.9×10^3	24.53 ± 0.76	18.55 ± 0.22	1.32
2	Chattarmanzil	2.8×10^4	34.40 ± 1.15	28.65 ± 0.26	1.20
3	Rajajipuram	3.4×10^5	34.83 ± 0.52	26.65 ± 0.48	1.30
4	Talkatora	6.0×10^4	40.26 ± 0.55	32.60 ± 0.65	1.23
5	Hussainganj-I	3.2×10^4	45.73 ± 1.73	32.48 ± 0.45	1.40
6	Sikandarbag	3.1×10^6	62.30 ± 1.02	54.19 ± 1.05	1.15
7	Rakabganj	2.8×10^4	72.40 ± 0.97	66.50 ±1.10	1.09
8	Hazartganj-I	3.2×10^3	80.64 ± 1.02	64.29 ± 0.78	1.25
9	Hussainganj-II	5.0×10^5	83.27 ± 1.95	68.56 ± 1.08	1.21
10	Banthra-II	4.2×10^4	126.35 ± 1.11	118.62 ± 1.03	1.06
11	Nishatganj-I	4.2×10^5	126.62 ± 2.62	115.65 ± 1.02	1.09
12	Banthra-I	2.5×10^6	144.27 ± 1.62	126.58 ± 1.64	1.14
13	Nishatganj-II	5.7×10^5	162.18 ± 2.03	144.74 ± 1.07	1.12
14	Gomti Nagar	3.6×10^6	164.10 ± 2.04	141.35 ± 1.95	1.16
15	Daliganj	2.2×10^6	250.34 ± 2.50	234.50 ± 2.35	1.06

± = standard error

All the samples investigated had aflatoxin levels greater than the Indian regulatory standard of 30 ppb. Forty percent samples had exceedingly high levels (>100 ppb) indicating consumers risk for exposure to high levels of aflatoxin. Bhat *et al*. (1996) reported exceeded permissible Indian regulatory limit of 30 µg/kg aflatoxin in peanut samples. Peanut is prone to attack by various pests and diseases (Ghewande *et al*., 1987; Subrahmanyam and Ravindranath, 1988). In the assembling markets, decorticating factories and oil mills, the produce is generally stored in the form of nuts, either loose or in gunny bags in India. The period of storage may be varying from short or it may be stored for several months in anticipation of better prices. Aflatoxin contamination in peanut seed samples screened in the present study may be due to long time under improper storage conditions. High rate of fungal infection in maize (65%), peanut (70%) and soybean (66%) during long time

storage (8-9 months) have been reported by Bhattacharya and Raha (2002). Iqbal *et al*. (2006) have investigated aflatoxin contents of stored cereals and nuts and reported that *Aspergillus* growth and toxin production are directly correlated with increase in humidity. More than 40 ppb of total aflatoxin content in the cake sample indicates a risk for animal health concern using as feed. The regulatory level of aflatoxin B_1 has been set at 5 µg kg^{-1} for complete feedstuffs for dairy animals by European Union (Delmulle *et al*., 2005).

Fig. 1. Regulatory limits of aflatoxins.

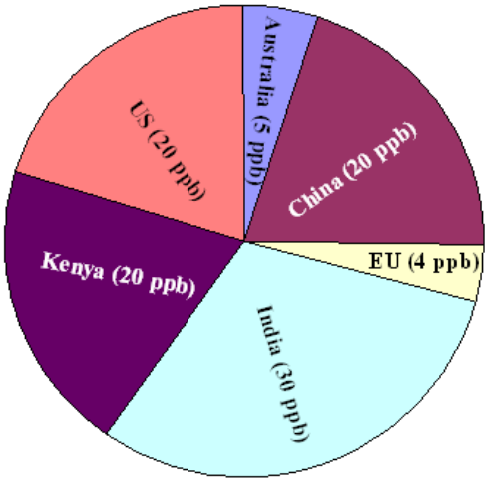
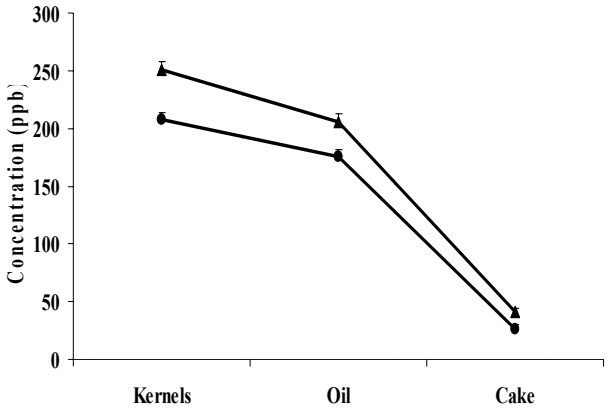


Fig. 2. Total aflatoxin (▲) and aflatoxin B1 (●) in peanut seeds, oil and cake samples.



Conclusion

All the seed samples investigated were found positive for aflatoxin. More than 86% samples showed aflatoxin content above the regulatory limits used in the European Union and in India. Forty percent samples had exceedingly high levels (> 100 ppb) indicating consumers risk for exposure to high levels of aflatoxin. Aflatoxin contamination of market peanut, therefore, is an important public health concern. Precautions should be taken for proper storage of groundnut seeds in order to prevent microbiological and chemical hazards.

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A study of geomorphologic factors restricting the expansion of Ardebil city using analytical hierarchy process (AHP)

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Abstract

Cities occupy vast and expansive terrain. Land is composed of several topographic and geomorphological units. As cities expand and develop, they encounter with topographic and geomorphologic units and issues pertaining to them also increase. Ardebil with its increasing population and urban sprawl is a city that is not an exception to this rule and its expansion has been influenced by the dynamics of the natural environment. In this paper, the hindrances to the expansion of this city, often due to geomorphologic factors, are discussed. To this end, topographic maps of 1:5000 and geological maps of 1:100000 were prepared followed by a field study that yielded images of geomorphologic phenomena in the region. After matching the maps with the geomorphologic phenomena in the region, these maps were scanned in Arc view GIS, digitalized and theme maps of altitude, slope, slope direction, lithology were prepared. The values of each of these factors were determined using the AHP, and faults, networks of watersheds, slopes, slope direction, thickness of matrices etc were plotted on lithology maps in GIS environment. Finally, after overlaying the maps, sensitive and susceptible points of the region were identified and zonation maps of unstable and sensitive points were prepared in GIS environment.

Keywords: *Expansion obstacles, morphological factors, unstable points, Ardebil, GIS, AHP*

Introduction

The city of Ardebil is the capital of the Ardebil province and is located between the latitudes 37˚ 45’ and 39^0 42’ north and longitudes 47˚ 25’ and 47^0 30’east and covers an area of about 4072 Km ^{2}. Several studies have been carried out on this issue in the world as well as in Iran which includes, the work of Teimoori (2004), Ghaffari (2001),Fathi (2006), Basirat (2003), Eqbal (2004), Shafi’ee (1994), Esfandiary (1998; 2008), Ostad (2000), Bird & Boomer (2004), Boomer & Rodriquez (2002), Dai *et al*., (2002),Koho, (2009), Katz (2007).

Methods and Materials

The present study probed into eight processes restricting urban development and the relative importance of each was explored. This was accomplished by using the paired comparison method (hierarchical). In the initial stage, by taking into account their quantitative and qualitative values, the factors were rated from 1 to 8 (from least to most important). Next, to determine relative weights of the main parameters, mean geometrical matrices were constructed for each and the relative weightage of each was computed.

Processes restricting city expansion in the study area are as follows:

1. Land settlement
2. Slope
3. Earthquake
4. Climatic factors
5. High levels of underground water
6. Pollution of surface water
7. Lack of adequate sewage disposal system in the city
8. Existence of adequate agricultural land around the city

The following calculations were performed:

W1= 0.31 coefficient of significance of elevated levels of underground water

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W2= 0.22 coefficient of significance of ground subsidence
W3= 0.15 coefficient of significance of the existence of land suitable for agriculture in the periphery of the city
W4= 0.11 coefficient of significance of slopes
W5= 0.07 coefficient of significance of the lack of appropriate sewerage system in the city
W6= 0.05 coefficient of significance of climatic factors
W7= 0.03 coefficient of significance of earthquakes
W8= 0.02 coefficient of significance of pollution of surface water

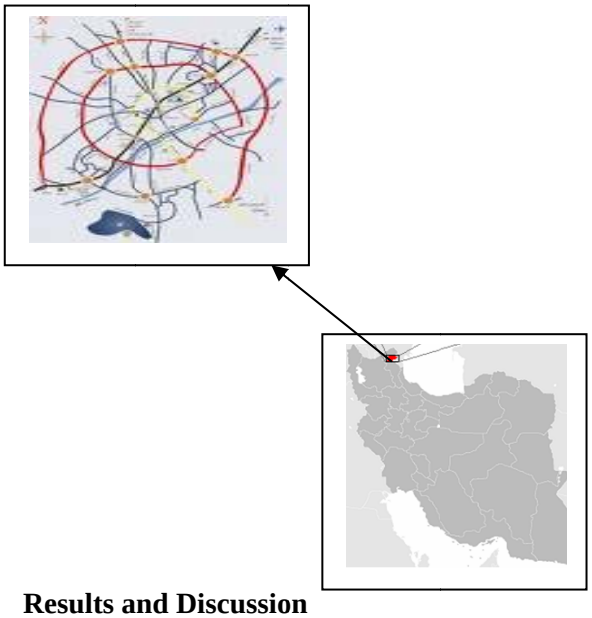
$$\lambda = \frac{85.32}{n(8)} = 9.48$$

$$CI = \frac{\lambda - n}{n-1} = \frac{0.48}{7} = 0.06$$

$$CR = \frac{CI}{RI} = \frac{0.06}{1.45} = 0.04$$

Given that CR (relative stability) in the above computation is less than 0.1, acceptable stability is implied in the paired comparisons.

Figure1: Geographical position of the study area



Results and Discussion

The zones in the north, west and north western parts of the city, on account of having sufficient water, appropriate slope, and fertile soil, possess adequate capability for cultivation (figure 5); on the other hand, the southern and western parts of the city possess less potential for agriculture and cultivation.

Other likely restricting factors are the Ardebil airport, located in the north-west of the city, the industrial town of Niyaar to the east of the city and the expansion of irrigation and drainage networks of the Ardebil dam to the west and north of the city. On the whole, the existence of areas suitable for agriculture in the periphery of the city is considered to be the primary factor limiting future expansion of the city. From a topographical view, a negative slope of greater than 9 degrees is not suitable for city expansion.

In terms of base rock characteristics, the existence of layers of clay beneath the base rock, schist, and sand hills, flood plains, and visible and invisible faults restrict the expansion of the city. From the point of view of pedology, shallow soil with a sand texture, heavy or partially heavy clay soil, or hydromorph soil with indequate conditions for drainage and soil with very fine grains and particles have been estimated as conditions detrimental to city expansion.

Dry river beds, flood-prone areas and passages of natural waterways are not considered to be conditions favorable to urban expansion. Paths of tornados and heavy seasonal winds and the areas with where the speed of permanent winds exceeds 50 km per hour have been evaluated as unfavorable to city expansion. Forest areas (density of tree coverage of greater than 60% and grass coverage of more than 50%) and irrigated land are considered to be unfavorable for city expansion.On the whole, it can be concluded that the expansion of the city in the southern, south-western and north-western areas appear to be reasonable. Among these areas, considering the conditions of the land and scatter of villages, the southern zones are suggested as the most favorable zones for future expansion of Ardebil. However, expansion in other directions is possible provided certain conditions are met. Figure 6 illustrates the mapping of the most unfavorable points in the city in view of surface water pollution, sewage disposal into the agricultural land of Sina town (figures 2 & 3) and obstruction of roads (figure 4). It is evident this map can provide guidance to environmental planners in tackling the obstacles to the expansion of the city of Ardebil.

Figure 2: Sewage disposal into agricultural land in Sina town

Figure 3: Sewage disposal into agricultural land in Sina town

Figure 4: Insufficient dimensions of most streams and canals along roads in the city, leading to less than optimal capacity for maximum surface water drainage

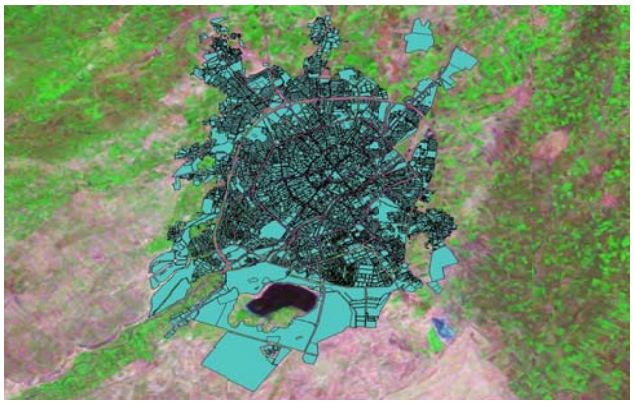


Figure 5: Agricultural land in the periphery of the city as a hindrance to city expansion

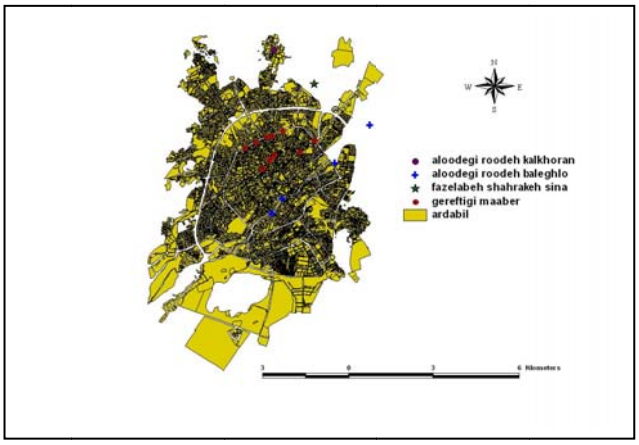


Figure 6: Zonation of unfavorable points in terms of city expansion

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Responsiveness to photostimulation in two passeriform birds

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Abstract

Two studies were performed to analyze the photoperiodic interaction of testicular growth in brahminy myna and weaver bird. In the first study, birds were exposed to stimulatory long day lengths (15L:9D) and natural day length (NDL) for 60 days. The second study investigated the interpretation of a light pulse as 'morning (entraining)' or 'evening (inducing)' depends on the time during night at which they fall. Five groups (6L:6D:1L:11D, 6L:13D:1L:4D, 11L:13D, 13L:11D and NDL respectively) of birds were exposed under skeleton and complete photoperiods for two months. Body mass and testicular volume was measured on monthly intervals. In the first study, testicular volume among both groups (15L:9D) and (NDL) gradually increased, but more inductive effect was found in 15L:9D. In the second study more induction occurred in testicular volume of groups 6L: 6D: 1L: 11D and 13L: 11D with different magnitude as if it was exposed to long days. Taken together, results demonstrate that birds were sensitive to the stimulatory photoperiod and strongly show that brahminy myna and weaver bird at 29^0N, 77^0 45'E latituderesponded similar to the populations living at higher latitudes and these species use the photoperiodic cues from the environment to regulate their reproductive cycles.

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Keywords:*Body mass, testes, circadian rhythm, complete and skeleton photoperiod*

Introduction

Day length regulates seasonal responses in many vertebrates, including several songbird species. The cycle of growth–regression–refractoriness can be reproduced under laboratory conditions by exposing birds to long days. Few studies suggest the role of light intensity and photoperiod in initiation of gonadal growth and development (Rani *et al*. 2009; Rani *et al*. 2007; Budki*et al*., 2009; Dixit and Singh, 2011). Long day lengths (15L:9D) induce gonadal growth and development, while short day lengths (photoperiods less than 9h per day) are ineffective or inhibitory in many series of investigation in Indian species; Indian weaver bird (Singh and Chandola, 1981; Rani, *et al*., 2007; 2009), redheaded bunting (Tewary and Tripathi, 1983; Budki*et al*., 2009), blackheaded bunting (Tewary and Kumar, 1982; Misra*et al*., 2004). In this study, we investigated the comparison between NDL (~14L to ~15L) and LDL (15L:9D) in two subtropical Indian species on reproductive cycle. So it was interesting that when we compare the two resident Indian species in breeding season in NDL and LDL (artificial photoperiod) and both species breeding cycle are relatively close and faces the same environmental condition for survival. And also we have aimed to address this question that do different photoperiodic species have 'different reaction norms' to stimulatory day lengths?

Skeleton photoperiods or Scotophase experiments shows the effects of a light pulse which would vary depending where it falls in a 24 h day. This is best illustrated by the ability of two short light pulses introduced at a fixed hour in the circadian rhythm of photoperiodic photosensitivity (CRPP) to induce the metabolic and gonadal functions (Kumar and Follett, 1993; Tewary and Kumar, 1983). It is suggested that in a two pulse light:dark (LD) cycle paradigm (two light pulse at fixed intervals in a 24hr LD cycle), that constitutes a 'skeleton' photoperiod (SKP), the first (usually

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longer) light pulse (main photoperiod) entrain CRPP and the second (usually shorter) light pulse falling in the night (Φi) induces photoperiodic responses (Bhardwaj,*et al*., 2006). Thus, two light periods tend to simulate the effects of corresponding long light period. In our study, the first light period (called the entraining light pulse, E-pulse) given early in the subjective day 'entrains' the CRPP, and photoinducible phase (Φi) begins some 'fixed' hours later in day. Thus, the E-pulse entrains photoperiodic response curve and decides the timing of the Φi. Light coinciding with the Φi initiates photoperiodic reaction. Later, these experiments were applied on a number of species including *Passer domesticus* (Kumar *et al*., 2004, Anushi and Bhardwaj, 2006, 2010), *Coturnixcoturnix japonica* (Follett and Sharp, 1969; Wada, 1981), *Passer montanus* (Lofts and Lam, 1973), *Ploceusphilippinus* (Singh and Chandola, 1981), *Carpodacuserythrinus* (Kumar and Tewary, 1982), *Emberizamelanocephala* (Tewary and Kumar, 1984) and *Sturnuspagodarum* (Kumar and Kumar, 1993). Singh *et al.* (2002), also found the effects of duration and the timing of the E-pulse on photoperiodic induction in the blackheaded bunting.So, in this investigation we answered that 'Is the interpretation of a light pulse as 'morning (entraining)' or 'evening (inducing)' depends on the time during night at which they fall'. In other words, does position of Φi determine the effects of light pulse? We introduced 1 h light pulse at the beginning of night or after midnight and measure the photoinduction to determine the entrainment/re-entrainment pattern in weaver birds.

Materials and Method

Birds were procured from local animal catchers (Meerut 29^0N) in the month of early May 2008 and were acclimatized to captive conditions under natural day lengths (NDL) for a period of two weeks after they were exposed to experimental conditions. First study was performed on adult male brahminy myna (*Sturnuspagodarum*) and baya weaver (*Ploceusphilippinus*) which were divided into four groups (n=6) and were exposed to 15L:9D and NDL.

The second study was performed in February 2008 on adult male brahminy myna which were grouped into five (n=6) and were exposed to 6L:6D:1L:11D (group 1), 6L:13D:1L:4D (group 2), 11L:13D (group 3), 13L:11D (group 4) and NDL (group 5). Day and night situation was provided by artificial illumination of CFL lamps (Phillips) providing cold white light at an intensity of ~500 lux at perch level by switching 'on' and switching 'off' by automatic time switches (Muller clock). Food and water were provided *ad libitum* condition to all groups in both studies.

Body mass was recorded using top pan balance on an accuracy of 0.1g.The dimensions of the left testis were recorded, and testis volume was calculated from formula $4/3\pi ab^2$ where a and b denote the half of the long (length) and short (width) axis, respectively. The data from the experiments was analyzed by one-way repeated measure analysis of variance (one-way RM ANOVA). Newman-Keuls Multiple Comparison Tests compared different means if ANOVA indicated the significance of difference between mean values. Significance was taken at $P < 0.05$.

Results and Discussion

Results of first study are shown in figure 1. There was no significant change in body mass of brahminy myna exposed to 15L:9D, but significant change in weaver birds exposed to 15L:9D and NDL. (fig. 1a and c) [$F_{2,8}$=0.6314, P=0.6540, (15L:9D) brahminy myna, $F_{2,8}$=4.470, P=0.0344, (15L:9D) baya weaver and $F_{2,8}$ =0.6914, P=0.0344, (NDL) baya weaver]. There was significant change in NDL birds of brahminy myna [$F_{2,8}$=17.27, P=0.0005] (fig.1 a).Mean testicular volume in the group exposed to 15L:9D gradually increased throughout the experiment in both species (fig. 1b and d). There was significant increase in testis volume of brahminy myna and baya weaver, subjected to 15L:9D and NDL [$F_{2,8}$=142.6, P<0.0001, (15L:9D) brahminy myna $F_{2,8}$=16.36, P=0.0006, (NDL) brahminy myna; $F_{2,8}$=40.25, P<0.0001, (15L:9D) baya weaver and $F_{2,8}$=18.14, P=0.0004, (NDL) baya weaver; One-way RM ANOVA]. Results of second study are shown in figure 2. Mean body mass among all groups gradually increase throughout the study with different magnitude (fig. 2a and c). The mean body mass among the five groups had significantly different [group 1: $F_{2,8}$=21.24, P=0.0006 (6L:6D:1L: 11D); group 2: $F_{2,6}$=14.39, P=0.0051 (6L:13D:1L:4D); group 3: $F_{2,6}$=4.21, P=0.0053

(11L:13D); group 4: $F_{2,6}$=20.06, P=0.0022 (13L:11D); group 5: $F_{2,6}$=40.01, P=0.0003 (NDL), One-way RM ANOVA]. Figure (2b) shows that mean testicular volume in group 1 and 4 gradually increased by day 30 and then significantly increased on 60 days [$F_{2,8}$=671.2, P<0.0001, (6L:6D:1L:1D) group 1 and $F_{2,6}$=46.62, P=0.0002, (13L:11D) group 4]. In group 2 (6L:13D:1L:4D), marginal response occurred on day 30 and in group 3 (11L:13D) on day 60, [fig. 2d ($F_{2,6}$=3.479, P= 0.0993, (6L:13D:1L:4D) group 2 and $F_{2,6}$ = 5.537, P = 0.0434, (11L:13D) group 3; One-way RM ANOVA]. There was no photoinduction occurred in the group exposed to NDL throughout the experiment in comparison to group 1 and group 4. More induction occurred in the groups exposed to 6L:6D:1L:11D and 13L:11D with different magnitude.

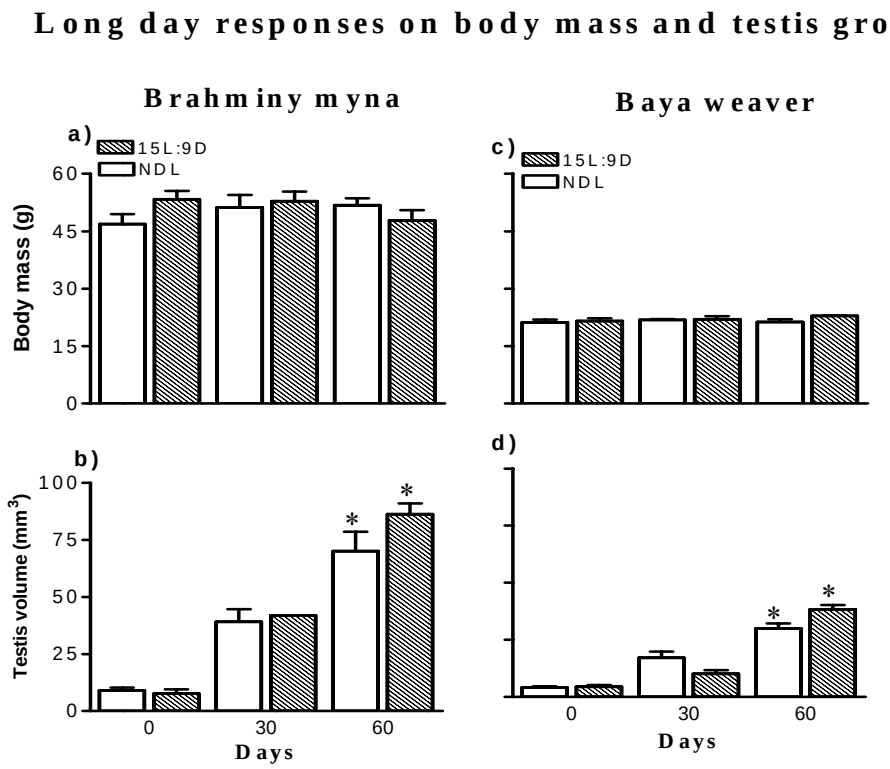

Fig. 1: Results of body mass and testicular volume of brahminy myna (fig. a and b) and baya weaver (fig. c and d) subjected to long day lengths (15L:9D) and natural day lengths (NDL). Asterisk indicates the significance of difference at P<0.05.

In the first study we investigated that the photo-responsiveness of myna and baya weaver under long day lengths to test the photoresponsiveness of neuroendocrine system that regulates reproductive response under long day lengths. Myna and baya are long day breeders and observe the changes in the photoperiod occurring in the nature. There was gain in body mass up to 60 days and testis attaining a peak value in both brahminy myna and baya weaver subjected 15L:9D and NDL groups. The annual cycles of gain in body mass and testes in brahminy myna correspond to increasing day lengths of spring and summer, similar to a number of temperate and tropical/subtropical species (Kumar and Tewary,1983; Dittami and Gwinner, 1985; Kumar and Kumar, 1991, 1993; Deviche and Small, 2001). Male brahminy myna exhibited a seasonal change in its responsiveness to long day lengths, which is comparable to that reported in the Indian weaver bird (Singh and Chandola, 1981). This suggests that under long day lengths induction of a photoperiodic response was faster. A similar photoperiodic response to such photoperiods (9L, 12L and 15L) is reported in another species, the Indian weaver bird, at 25^0N, 83^0E that often shares habitat with the house sparrow. A long day species usually do not show gonadal response under short photoperiods (light

below critical day length) and this indicates the importance of photoperiodic cues over an endogenous circannual rhythm in control of reproductive cycle of tree sparrows. On the other hand, gonadal recrudescence under short day length in a long day breeder may be the consequence of seasonal rhythm rather than of the photoperiod. The present results of study 1 shows that both species under 15L:9D and NDL remained stimulated during the entire duration of experiment and thus it indicates that they were sensitive to the stimulatory effects of these photoperiods. Male brahminy myna and baya weaver exhibits similar reaction norms to long days. The results from both the species also suggest that there is no difference in the responsiveness of two photoperiodic species and survive better in natural environment.

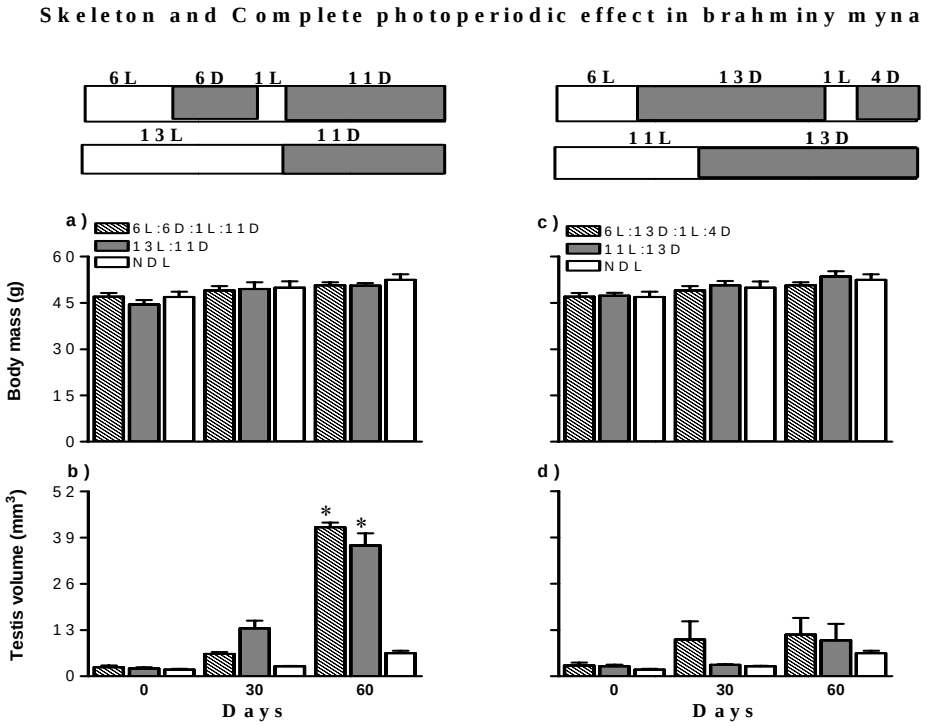

Fig. 2: Results of body mass and testicular responses in brahminy myna subjected to skeleton and complete photoperiods 6L:6D:1L:11D and 13L:11D (fig. a and b) and 6L:13D:1L:4D and 11L:13D (fig. c and d). Asterisk indicates the significance of difference at $P<0.05$.

In second study a long day response is believed to result from the interaction of long light pulse (LLP) simultaneously with two different phases of the circadian rhythm of photoperiodic photosensitivity (CRPP). This is best illustrated by the ability of two short light pulses introduced at fixed hour in the CRPP to induce the metabolic and gonadal functions (Follett, 1984; Kumar and Follett, 1993; Tewary and Kumar, 1983, 1984; Kumar, 1986, 1988). It is suggested that in a two-pulse light: dark (LD) cycle paradigm (two light pulse at fixed intervals) in a 24 h (LD) cycle, that constitutes a 'skeleton' photoperiod (SKP), the first (usually longer) light pulse (main photoperiod) entrain CRPP and the second (usually shorter) light pulse falling in the night induces photoperiodic responses. Considered together, this means that a SKP will be as effective as a single complete photoperiod (CP, a single continuous light period in a 24 h LD cycle) in influencing the photo-neuroendocrine system in a photoperiodic species.The traditional view of how skeleton photoperiods can stimulate long days is based upon concept of how circadian rhythms might be used as a day length-measuring device. Normally, this coincidence occurs only at one season of the year when the day lengths are of sufficient length to engage the photosensitive phase, but certain skeleton photoperiods can mimic this situation, one of the light

pulsecoinciding with the period of peak photosensitivity. The present results on the effect of complete photoperiods (CP) and skeleton photoperiods (SKP) in altering the timing of spontaneous regression. The present data supporting that birds were the photosensitive and when exposed to complete and skeleton photoperiods, their exposure to 6L:6D:1L:11D and 13L:11D did evoke a long day response in brahminy myna (fig 2b) the reason of this behind, because the dark pulse was short duration (6D) so the birds read 6L:6D:1L:11D photoperiod as 13L photoperiod. There was no photoinduction occurred in the group exposed to NDL because the study period was non-breeding phase for brahminy myna. More induction occurred in the groups exposed to 6L: 6D: 1L: 11D and 13L: 11D with different magnitude (fig. 2b) and marginal response occurred in the group exposed to 6L: 13D:1L:4D and 11L:13D (fig. 2d) and mean body mass among all groups gradually increased throughout the experiment (fig. 2a and c). In skeleton photoperiod (6L:6D:1L:11D) the dark pulse was short duration (6D) so the birds entrained according to 13L but in skeleton photoperiod (6L: 13D: 1L: 4D) the dark pulse was longer duration (13D) so the birds reads its, short day length. Result of this study shown that the gonadal response occurred in 13L:11D photoperiod. So, 13L:11D is stimulatory and 11L:13D is non-stimulatory in brahminy myna. Considering together, studies suggest that both birds are photosensitive for long day lengths.

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Diversity indices of phytoplankton at Munj sagar talab (Dhar, Madhya pradesh, India).

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Abstract

Phytoplankton is the significant formal natural occupier of all water bodies. They play an important role in the biosynthesis of organic material. Being an index of trophic status phytoplankton reflects the overall environmental condition of the system and its potentiality. Present investigation was carried out for a period of twenty four month to study the diversity indices. Simpson's Index of Dominance ranged from 0.0441 to 0.0952. Shannon-Wiener Diversity (H) index ranged from 2.6298 to 3.2112.273. Evenness Index ranged from 0.5593 to 0.9808 during the study period. Keywords: Diversity index, Evenness Index (J), Shannon and Weaver diversity index (H), Simpson's Index of Dominance (D) and phytoplankton,

Keywords: *Diversity, phytoplankton, dominance, Simpson's index*

Introduction

Plankton are minute organism and are effective tools in environmental bio monitoring of aquatic system. They are essential link in food chain. Phytoplanktons are the significant formal natural occupier of all water bodies. They play an important role in the biosynthesis of organic material. Being an index of trophic status phytoplankton reflects the overall environmental condition of the system and its potentiality. Plankton population is directly or indirectly governs by the interaction of the number of physical, chemical and biological factors of the water body (Reid and Wood, 1976). Using the biological approaches to determine the ecological effects of pollution has been preferred widely for decades. These approaches have more advantages than determining the pollution with only using physico-chemical methods, because physicochemical variables give information about only the situation of water at the time of measuring (Rosenberg and Resh, 1993). Diversity index is a statistical method which is planned to evaluate the variety of a data group consisting of different types of components. Features of a population such as number of existing species (Richness), distribution of individuals equally (Evenness) and total number of existing individuals underlie the basis of diversity indices (Wilhm and Dorris, 1968, Allan, 1975). Thus, any changes in any of these three features will affect the whole population, so that the diversity indices depending upon these features are used effectively to determine the changes in a population (Mandaville, 2002, Dügel, 1995). The diversity index is a measure of the relationship between the number of species collected and the evenness of their distribution. Diversity index based on the Shannon-Wiener function of information theory, and describes the uncertainty of predicting the species of a randomly chosen individual from the community (Heister, 1972).There are three different levels of biological diversity ,first one is spices diversity which embraces the variety of living organism on earth, second is genetic diversity which is concerned with variation in genes with in a particular species and third one is ecological diversity which is related to variety habitat,(Salam and Rizvi,1999;Salam *et al*.,2000;Ali *et al*.,2005).

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Material and Method

Dhar is located in the Malwa region of Western Madhya Pradesh. The climate of Dhar is tropical and the month of November announces the winter and it continues until February. The summer season starts in mid March and continues through out the June. Munj Sagar is located in the district Dhar. It covers an area of about 49.596 h .The altitude of Munj Sagar Talab is 554m.In Year 2005 it was deepen by removing the bottom soil. This water body was basically constructed for drinking water purpose but now-days its water is mainly utilize for irrigation and fish culture purpose.

Munj Sagar talab has mainly three Pakka Ghats. These ghats were chosen as sampling station. First one is Ganesh Ghat (S1) geographically located at $22^0$36'13.55" North latitude and $75^0$17'54.25" East latitude. Second is Shankar Ghat (S2) geographically located at $22^0$36'05.28" North latitude and $75^0$17'59.66" East latitude and third is Chatri Ghat (S3) geographically located at $22^0$35'58.34" North latitude and $75^0$17'45.82" East latitude .These sampling stations named as S1, S2 and S3 during the course of study .

The samples were collected in the first week of every month from November 2006 to October 2008 between 7 to 9 a.m. Plankton sample were collected by filtering the 50 liters of water through a plankton net (No. 20).The counting of plankton was done with the help of a Sedgwick Rafter cell count of 1 ml capacity. All planktons were counted according to the procedure given by Welch (1952). The identification of phytoplankton was done by taking the help of standard books and publications. Phytoplanktons were identified up to genus in most cases by key given by Turner (1982), Smith (1950), Prescott (1962), Ward and Whipple (1959) and Ruttner-Kolisko (1974). The Shannon and weaver diversity index (H), Simpson's Index of Dominance (D) and Evenness Index (J).were calculated by diversity calculator software.

Result and Discussion

29 species of phytoplankton have been identified in Munj Sagar Talab. Phytoplankton group/class consists of Chlorophyceae (12), Bacillariophyceae (9), Cynophyceae (6), and Euglenophyceae (2).The Shannon-Weaver Index (H) for phytoplankton was always found above one at all the station during the study period of 24 months. The maximum observed value of H (3.2111) was at station S3 in the month of June 2008. While the minimum observed value of H (2.6298) was at Station S1 in the month of November 2006. The Simpson's diversity Index (D) for phytoplankton was always less than one. In the present study the maximum value of D (0.0952) was obtained at Station S1 in the month of August '07. The minimum value of D (0.0441) was obtained in month of May'07 at station S2 and April'08 at Station S1. The Evenness Index (J) for phytoplankton was always less than one. In the present study the maximum value of J (0.9808) was obtained in month of April'08 at station S2. The minimum value of J (0.8893) was obtained in the month of August'07 at station S2

The diversity index is a good tool for measuring the health of an ecosystem. It measures the stability of an ecosystem, which increases with its diversity. Shannon-Wiener Diversity index (H) greater than 3 indicates clean water, values in the range of 1-3 are characteristics of moderately polluted condition and values less than one characterize heavily polluted condition(Mason, 1981) Almost in all cases an increase in spices diversity is considered as an increase in ecological quality (Magurran,1996).

Staub *et al.* (1970) suggested another scale for categorizing the status for water body , if Shannon-Wiener Diversity index(H)ranging between 3.5 to 4.5 indicates slightly polluted water , values in the range of 2-3 are characteristics of light polluted condition, values in the range of 1-2 are characteristics of moderate polluted condition and values less than one characterize heavily polluted condition

In the present study the Shannon-Wiener Diversity index (H) for phytoplankton ranged from 2.629to 3.208 during the study period 2006 and 2007 and during the study period 2007-08 the diversity index ranged between 2.880 to 3.268.

Shannon-Wiener Diversity index (H) almost near to 3 for phytoplankton suggests that water is good for growth of phytoplankton in water body (Ali *et al.*, 2005). Theses value of Shannon-Wiener Diversity index (H) also indicates presence of longer food chain (Margalef, 1968) present study also indicates the same. In present study the Simpson Diversity index (D) for phytoplankton ranged from 0.0441to 0.0952 during the study period 2006 and 2007. During the study period 2007-08 the diversity ranged in-between 0.0403 to 0.0604.

Table-1: Monthly variations in diversity indices of phytoplankton at Munj sagar talab(2006-07)

MONTHS	STATION 1			STATION 2			STATION 3		
	Simpson Diversity Index(D)	Shannon-Wiener Diversity Index (H)	Evenness (J)	Simpson Diversity Index(D)	Shannon-Wiener Diversity Index (H)	Evenness (J)	Simpson Diversity Index(D)	Shannon-Wiener Diversity Index (H)	Evenness (J)
Nov 06	0.0502	3.0849	0.9707	0.0489	3.0967	0.9744	0.0578	2.9340	0.9637
Dec 06	0.0614	2.9583	0.9309	0.0592	2.9843	0.9390	0.0635	2.8814	0.9464
Jan 07	0.0803	2.7868	0.9016	0.0764	2.8003	0.9059	0.0549	3.0479	0.9469
Feb 07	0.0651	2.9082	0.9408	0.0593	2.9411	0.9515	0.0543	3.0759	0.9333
Mar 07	0.0513	3.1202	0.9577	0.0614	3.0060	0.9226	0.0497	3.0987	0.9627
Apr 07	0.0453	3.2086	0.9629	0.0493	3.1386	0.9523	0.0566	3.0260	0.9401
May 07	0.0449	3.2059	0.9621	0.0441	3.2142	0.9646	0.0453	3.1860	0.9667
Jun 07	0.0533	3.0928	0.9384	0.0619	2.9206	0.9449	0.0474	3.2037	0.9514
Jul 07	0.0618	2.9764	0.9247	0.0714	2.8869	0.9207	0.0461	3.1978	0.9597
Aug 07	0.0952	2.6298	0.8932	0.0887	2.7076	0.8893	0.0534	3.0155	0.9617
Sep 07	0.0593	2.8979	0.9674	0.0629	2.8726	0.9589	0.0529	3.1108	0.9439
Oct 07	0.0524	3.0300	0.9664	0.0621	2.9021	0.9532	0.0518	3.1090	0.9433

TABLE- 2: MONTHLY VARIATIONS IN DIVERSITY INDICES OF PHYTOPLANKTON AT MUNJ SAGAR TALAB(2007-08)

MONTH	STATION 1			STATION 2			STATION 3		
	Simpson Diversity Index(D)	Shannon-Wiener Diversity Index (H)	Evenness (J)	Simpson Diversity Index(D)	Shannon-Wiener Diversity Index (H)	Evenness (J)	Simpson Diversity Index(D)	Shannon-Wiener Diversity Index (H)	Evenness (J)
Nov 07	0.0609	2.9046	0.9540	0.0617	2.8957	0.9511	0.0597	2.9292	0.9621
Dec 07	0.0634	2.8800	0.9460	0.0546	2.8344	0.9461	0.0604	2.8948	0.9508
Jan 08	0.0627	2.9863	0.9166	0.0651	2.9906	0.9291	0.0628	3.1166	0.9456
Feb 08	0.0565	3.0624	0.9292	0.0601	3.0498	0.9475	0.0508	3.0188	0.9265
Mar 08	0.0548	3.0681	0.9417	0.0548	3.1063	0.9650	0.0457	3.1228	0.9585
Apr 08	0.0441	3.1121	0.9442	0.0484	3.2682	0.9808	0.0495	3.1661	0.9606
May 08	0.0513	3.2082	0.9527	0.0403	3.0715	0.9427	0.0474	3.1796	0.9647
Jun 08	0.0463	3.2058	0.9727	0.0446	3.2290	0.9589	0.0459	3.2111	0.9536
Jul 08	0.0489	3.1511	0.9457	0.0459	3.1966	0.9593	0.0481	3.1726	0.9521
Aug 08	0.0664	2.9203	0.9189	0.0575	2.9639	0.9589	0.0705	2.8930	0.9103
Sep 08	0.0581	3.0470	0.9245	0.0550	3.0670	0.9413	0.0593	3.0335	0.9204
Oct 08	0.0555	3.0763	0.9334	0.0529	3.0565	0.9617	0.0554	3.0697	0.9422

Theoretically Simpson Diversity index (D) varies between 0 to 1.0 and value more than 0.5 considered as a higher value. According to Dash (2003) mature and stable communities in general have high diversity value (0.6 to 0.9) and rare communities or unstable communities and communities under stress exhibiting low diversity, usually shows nearer to zero value. In light of these facts it can be concluded that the low value of Simpson Diversity index (D) in present study shows that species studied are under stress condition. According to Whittaker (1693) the value

of Simpson Diversity index (D) is always higher where the community is dominated by fewer numbers of spices and when the dominance is shared large number of species. This supports the results of present study.

In present investigation, during 2006-07 maximum and minimum degree of evenness (J) were (0.974) and (0.889) respectively for phytoplankton, while during 2007-08 these values were 0.980 (maximum) and 0.910 (minimum). Balloch (1976) and Suresh *et al*. (2009) reported high association of Shannon Wiener diversity index with high evenness index, reflecting the multi dominance pattern in cluster. Present study also shows same pattern in these two indices for phytoplankton. Present study indicates that, whenever diversity index (D) was higher the evenness index was lower and vice versa this finding is in agreement with the finding of Walting *et al.* (1979).

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Impact of municipal solid waste (MSW) dumping on ground water quality at Muthi Jammu -A Case Study

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Abstract

The present paper deals with the physico -chemical analysis of ground water quality near a municipal solid waste dumping site at Muthi, Jammu. The impact of MSW dumping on ground water quality varies in different seasons and at different distances from the waste dumping sites .The depth of ground water also indicates the level of ground water contamination. Ground water analysis was carried for a period of seven months during (June to Dec. 2007) and thirteen parameters viz., pH, Turbidity, EC, TDS, DO, Free CO_2 , HCO_3, Total hardness,Ca^{++}, Mg^{2+} , NO_3^-, SO_4^{2-}, Cl^- were analysed for present studies. Results revealed that in some samples pH, turbidity, DO , HCO_3, total hardness, Ca^{++}, Mg^{2+}, NO_3^- were not within the standards prescribed by WHO, 1993 and BIS, 1992.

Keywords: *Physico-chemical, dumping Site, contamination, groundwater, quality*

Introduction

Water, the priceless gift of nature, vital for the existence of all life forms and hence the life line on planet Earth. Ground water accounts for more than 90% of water supply resources for many developing countries (Kolaja *et al.* 1986) and this is true for India .Rapid growth of urban areas has affected the ground water quality due to over exploitation and improper waste disposal. According to WHO, about 80% water pollution in developing countries, like India is caused by domestic waste. Ranga Raj (1996) and Indra Raj *et al.* (2000) have indicated that the ground water crosses the limits of health criteria due to anthropogenic factors like disposal of domestic waste, sewage, industrial waste and septic tanks. Auragabadkar (2000) carried out ground water quality monitoring around a municipal waste dumping site at Chennai and reported that concentration of iron and manganese were exceeding the permissible limits for drinking water. The surface runoff samples collected around the dumpsite show high organic and inorganic pollution. Lechate of soil samples also showed higher concentration of chromium, zinc, lead, iron and manganese.

Chandrasekar and Ayyappan (2006) studied impact of municipal solid waste dumping on ground water quality and results shows higher amount of contamination in the water samples which are taken within 500 meters from the dumping site which is not suitable for drinking purpose and the parameters are within the permissible limits for the remaining water samples which are suitable for drinking purposes. Ravinder *et al.* (2005) studied the impact on ground water quality due to leachate caused by solid waste.

The solid waste in Muthi is dumped in open, one of the dumping site is adjacent to a community hand pump. To study the effect of this solid waste dumping on the ground water quality, present study has been envisaged.

Materials and Methods

The contamination level of ground water has been evaluated by collecting the water samples from hand pumps located at various distances from the waste dump site. One situated near the

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MSW dumping site station(a) and the other 2.5km away station (b). The physico-chemical parameters were studied in monsoon and post monsoon season. Nearly 14 representative water samples were analysed for 13 parameters pH, Turbidity, EC, TDS, DO, Free CO_2 , HCO_3, Total hardness, Ca^{++}, Mg^{2+} , NO_3^-, SO_4^{2-} and Cl^- were selected to asses the ground water quality and samples were analysed as per standard methods of APHA, 1998.TDS was determined from electrical conductivity using the equation TDS (ppm) = 0.64 × EC (David, 1980).

Results and discussion

Results of various physico-chemical parameters of groundwater have been depicted in table–1 and figure-1.

pH

At station (a), the ground water near the waste dumping site recorded maximum pH of 9.4 in December followed by November which recorded a pH of 9.2 while the minimum pH of 5.9 was observed in October followed by 7.9 in September. The pH values of six study water samples are not within limits of standard values prescribed by WHO, 1993(6.5 to 8.5); Bureau of Indian Standards 1992 (7.0 to 8.3). Analysis of water samples have revealed that five water samples have values higher than the prescribed limits and one sample have value lower than prescribed values. A lower pH value below 4 will produce sour taste and high value above 8.5 gives bitter taste thus rendering it unsuitable for human consumption. Higher values induces the formation of trihalomethanes, which may induce cancer in human beings (Shivakumar *et al*., 2004).

At station (b), the ground water 2.5 km away from the waste dumping site recorded maximum pH of 8.8 in December followed by November which recorded pH of 8.7 while the minimum pH of 7 was observed in October followed by 7.4 in September. All water Samples are well within the limits prescribed by WHO, 1993(6.5 to 8.5); Bureau of Indian Standards, 1992 (7.0 to 8.3). pH of water samples indicates the neutral to alkaline nature which may be due to the presence of bicarbonates which undergoes hydrolysis in solution (Bindiya Langer *et al*., 2003).

Turbidity

At station (a), the ground water near the waste dump site recorded maximum turbidity of 20 NTU in June. The month of July recorded turbidity of 18 NTU while the minimum turbidity of 5.7 NTU was observed in December followed by 5.8 NTU in November. The turbidity values of all water samples are observed to be higher than the limits of International standard prescribed by WHO, 1993(5NTU); Bureau of Indian Standards, 1992 (5 NTU).

At station (b), the ground water 2.5km away from the waste dump site recorded maximum turbidity of 6 NTU in June. The month of July recorded turbidity of 5NTU while the minimum turbidity of 1.7NTU was observed in December followed by 2NTU in November. Turbidity values of all water samples are within the limits of standard prescribed by WHO, 1993 (5 NTU); Bureau of Indian Standards, 1992 (5 NTU).

Electrical Conductivity (EC)

At station (a), the ground water near the waste dumping site recorded maximum electrical conductivity of 73µsiemens/cm in October. The month of November recorded electrical conductivity of 50µsiemens/cm while a minimum of 8µsiemens/cm electrical conductivity was observed in August followed 9µsiemens/cm in July. The EC values of all water samples studied during the present investigations are within the limits of standard prescribed by WHO, 1993 (600µmhos/cm).

At station (b), the ground water 2.5km away from the waste dumping site recorded maximum electrical conductivity of 69µsiemens/cm in October, the month of November recorded electrical conductivity of 47µsiemens/cm while the minimum electrical conductivity of 4µsiemens/cm was observed in September followed by 5µsiemens/cm in July. The electrical conductivity values of all samples of present studies are within the limits of standard prescribed by WHO, 1993 (600µmhos/cm).

The EC decreased with the increase in distance of water sample from the waste dumping site, which indicates leaching effect in ground water adjacent to the waste dumping site as addition of contaminants increase EC.

Total dissolved solids (TDS)

At station (a), he ground water near the waste dumping site recorded maximum TDS of 46.72 mg/l in October, the month of December recorded TDS of 33.92 mg/l while the minimum TDS of 5.12mg/l was observed in August followed by 5.76mg/l in July. Concentration of TDS in all water samples studied during present investigations were within the limits prescribed by WHO, 1993(75mg/l); Bureau of Indian Standards, 1992 (75mg/l).

At station (b), the ground water 2.5km away from the waste dumping site recorded maximum TDS of 44mg/l in October, the month of December recorded TDS 31.36mg/l while the minimum TDS 2.56 mg/l was observed in September followed by 4.48mg/l in August. Concentration of TDS in all water samples studied during present investigations were within the limits prescribed by WHO, 1993 (75mg/l); Bureau of Indian Standards, 1992 (75mg/l).

Total hardness (TH)

At station (a), the ground water near the waste dumping site recorded maximum total hardness of 627.9mg/l in October the month of July recorded total hardness of 508.87mg/l while the minimum total hardness of 442mg/l was observed in July followed by 462mg/l in November. The total hardness values of all water samples of present studies exceeded the limits prescribed by WHO, 1993 (300mg/l); Bureau of Indian Standards, 1992 (200mg/l). Hardness of water mainly depends upon the amounts of calcium or magnesium salts or both.

Hardness is an important property of ground water from its utility point of view. The water containing excess hardness is not desirable for potable purpose. It forms scales on water heaters and utensils when used for cooking, and consumes more soap during washing of cloth. Higher values of TH may be due to leaching from solid waste dumping site.

At station (b), the ground water 2.5km away from the waste dumping site recorded maximum total hardness of 502.89mg/l in October, the month of September recorded total hardness of 496.23mg/l while the minimum total hardness of 404.786mg/l was observed in June followed by433.52mg/l in November.

Though the value for total hardness in all two stations is not within the limits prescribed by WHO, 1993 (300mg/l); Bureau of Indian Standards, 1992 (200mg/l) but comparative study reveals higher values of total hardness at station (a) located near the waste dumping site than station (b) located 2.5km from the waste dumping site.

Bicarbonate (HCO_3)

At station (a), the ground water near the waste dumping site recorded maximum bicarbonate content of 674.9mg/l in December, the month of November recorded bicarbonate content of 670.89mg/l while the minimum bicarbonate content of 320.24mg/l was observed in October followed by 386.33mg/l in July. Four water samples of present studies were found to posses' values higher than limits prescribed by WHO, 1993(500mg/l). High concentration of bicarbonate is due to the presence of humic acid which comes from decaying of organic substances present in soil and solid waste. The leachate generated from the waste dumping site might have percolated through the soil and combined with ground water.

At station (b), the ground water samples 2.5km away from the waste dump site recorded maximum bicarbonate content of 570mg/l in December. The month of November recorded Bicarbonate content of 559.16mg/l while the minimum bicarbonate content of 279.58mg/l was observed in October followed by 318.5mg/l in July. Three samples of present studies were found to posse's values higher than limits prescribed by WHO, 1993 (500mg/l)

Bicarbonate alkalinity showed a direct positive correlation with pH as they mostly tend to rise and fall together. This observation is similar to that of Zafar (1964), Sinha (1969), Singh and Sahai (1979) and Prakash (1996) who reported a direct positive correlation between Bicarbonate alkalinity and pH.

The concentration of bicarbonate decreases as we move away from the waste dumping site as the leaching effect decreases with increasing distance from the waste dumping site.

Free CO_2

At station (a), the ground water near the waste dumping site recorded maximum CO_2 content of

37.64mg/l in June, the month of July recorded CO_2 content of 32.52mg/l while the minimum CO_2 content of 8.6mg/l was observed in December followed by 9.1mg/l in November. Ground water is extra rich in CO_2 because precipitated water percolates through the soil and dissolves CO_2 from soil air and carries it into ground water, moreover decomposition of organic waste is an important source of CO_2 in water.

At station (b), the ground water samples 2.5km away from the waste dump site recorded maximum CO_2 content of 28.09mg/l in June, the month of July recorded CO_2 content of 26.47 mg/l while the minimum CO_2 content of 7.92mg/l was observed in December followed by 8.4mg/l in November.

Ground water contains considerable amount of carbon-di-oxide. The concentration of carbon-di-oxide in the water samples near waste dumpsite was found to be higher compared to water samples away from waste dumping site.

Dissolved Oxygen (DO)

At station (a), the ground water near the waste dumping site recorded maximum DO content of 5.6mg/l in December, the month of November recorded DO content of 4.9mg/l while the minimum DO content of 2.02mg/l was observed in June followed by 2.7mg/l in October. Concentration of DO in all water samples was much below the standard prescribed by WHO, 1993 (6 mg/l). Concentration of DO in four water samples was below the standard prescribed by WHO, 1993(6 mg/l).

At station (b), the ground water 2.5km away from the waste dump site recorded maximum DO content of 6.4 mg/l in December, the month of November recorded DO content of 6 mg/l while the minimum DO content of 3.7mg/l was observed in October followed by 3.9mg/l in September.

Calcium (Ca^{++})

At station (a), the ground water near the waste dump site recorded maximum calcium content of 168.46mg/l in August. The month of September recorded calcium content of 159.72mg/l while the minimum calcium content of 135.24mg/l was observed in July followed by 140.23mg/l in June. Concentration of calcium in all water samples exceeded the limits prescribed by WHO, 1993 (75mg/l); Bureau of Indian Standards, 1992 (75mg/l).

At station (b), the ground water 2.5km away from the waste dump site recorded calcium content of 133.28mg/l in October. The month of August recorded calcium content of 116mg/l while the minimum calcium content of 104.40mg/l was observed in July followed by 105mg/l in September. Concentration of calcium in all water samples exceeded the limits prescribed by WHO, 1993 (75mg/l); Bureau of Indian Standards, 1992 (75mg/l).

While comparing the concentration of calcium at all three sites, it was found that though most samples exceeded the prescribed limit but the calcium concentration was found to be much higher at site (a) located near the waste dumping site.

Minimum calcium concentration was found during monsoon season and maximum concentration was found during post monsoon season. Generally groundwater contains Ca^{2+} contents less than 100ppm and Mg^{2+} contents 50ppm (Todd and Keith, 1995). Calcium and magnesium ions in greater quantities may be present in groundwater either by leaching of soil deposits (lime stone, dolomite, gypsum, granite, siliceous, sand, serpentine etc.) or through seepage of ions from domestic waste water. The hard water causes ill effects on digestive system and moreover, the possibilities of forming calcium oxalate crystals (leading to stone formations) in the urinary tracts.

Magnesium (Mg^{2+})

At station (a), the ground water near the waste dump site recorded maximum magnesium content of 84.5 mg/l in September, the month of October recorded magnesium content of 71mg/l while the minimum magnesium content of 19.7 mg/l in December followed by 20.36mg/l in November . Concentration of magnesium in all water samples exceeded the limits prescribed by WHO, 1993 (75mg/l); Bureau of Indian Standards, 1992 (75mg/l). At station (b), the ground water 2.5km away from the waste dump site recorded maximum magnesium content of 37.9mg/l in September, the month of October recorded magnesium content of 30.90mg/l while the minimum magnesium content of 7.37mg/l was observed in November followed by 8.6mg/l in December. Concentration of magnesium in all water samples were well within the limits

prescribed by WHO, 1993 (75mg/l); Bureau of Indian Standards, 1992 (75mg/l).

Nitrate (NO_3^-)

At station (a), the ground water near the waste dumping site recorded maximum nitrate concentration of 23mg/l in July, the month of June recorded nitrate concentration of 20mg/l while the minimum nitrate concentration of 1.5mg/l was observed in October followed by 2.5mg/l in September. The concentration of nitrate in the three water samples of present studies exceeded the limits prescribed by WHO, 1993 (10mg/l).At station (b), the ground water sample 2.5km away from the waste dump site recorded maximum nitrate concentration of 4mg/l in July. The month of June recorded nitrate concentration of 3.5mg/l while the minimum nitrate concentration of 1.2mg/l was observed in October followed by 1.4mg/l in August. The concentration of nitrate in all water samples presently studied were within the limits prescribed by WHO, 1993 (10mg/l) ;Bureau of Indian Standards ,1992 (45 mg/l) .Nitrate is a poisonous component of groundwater which is natural as well as of anthropogenic origin. This is the highest oxidized form of nitrogen. Biological oxidation of nitrogenous substances from sewage is the main source of nitrate (Suresh *et al.* 1993). Shrivastva *et al.* (1998) and Olaniya and Saxena (1997) has reported the leaching of nitrate ions from soil to ground water. Excessive nitrate concentrations in drinking water pose an immediate and serious health threat to infants under three months of age the nitrate ions react with blood hemoglobin, reducing bloods capacity to carry oxygen. This produces a disease called blue baby or methemoglobinemia.

Sulphate (SO_4^{2-})

At station (a), the ground water near the waste dumping site recorded maximum sulphate concentration of 45mg/l in July. The month of June recorded sulphate concentration of 41.5mg/l while the minimum sulphate concentration of 21.5mg/l was observed in December followed by 24mg/l in November. The concentration of sulphate in all water samples were within the limits prescribed by WHO, 1993 (150mg/l); Bureau of Indian Standards 1992, (200 mg/l).At station (b), the ground water 2.5km away from the waste dumping site recorded maximum sulphate concentration of 32mg/l in July.The month of June recorded sulphate concentration of 30mg/l while the minimum sulphate concentration of 3.4mg/l was observed in December followed by 4 mg/l in November. The concentration of sulphate in all water samples were within the limits prescribed by WHO, 1993 (150mg/l); Bureau of Indian Standards 1992, (200 mg/l).However it was higher at station (a) in comparison to station (b). Sulphate is a naturally occurring anion found almost in all types of water. Sulphate salts are mostly soluble in water and impart hardness. The variation of sulphate content mainly depends on the decomposition of organic matter present in the solid waste. In anaerobic decomposition of waste, Sulphates are reduced to hydrogen sulphide causing obnoxious odours and promote corrosion. Waters with about 500mg/l sulphate have a bitter taste and those with 1000mg/l or more sulphate may cause intestinal disorders.

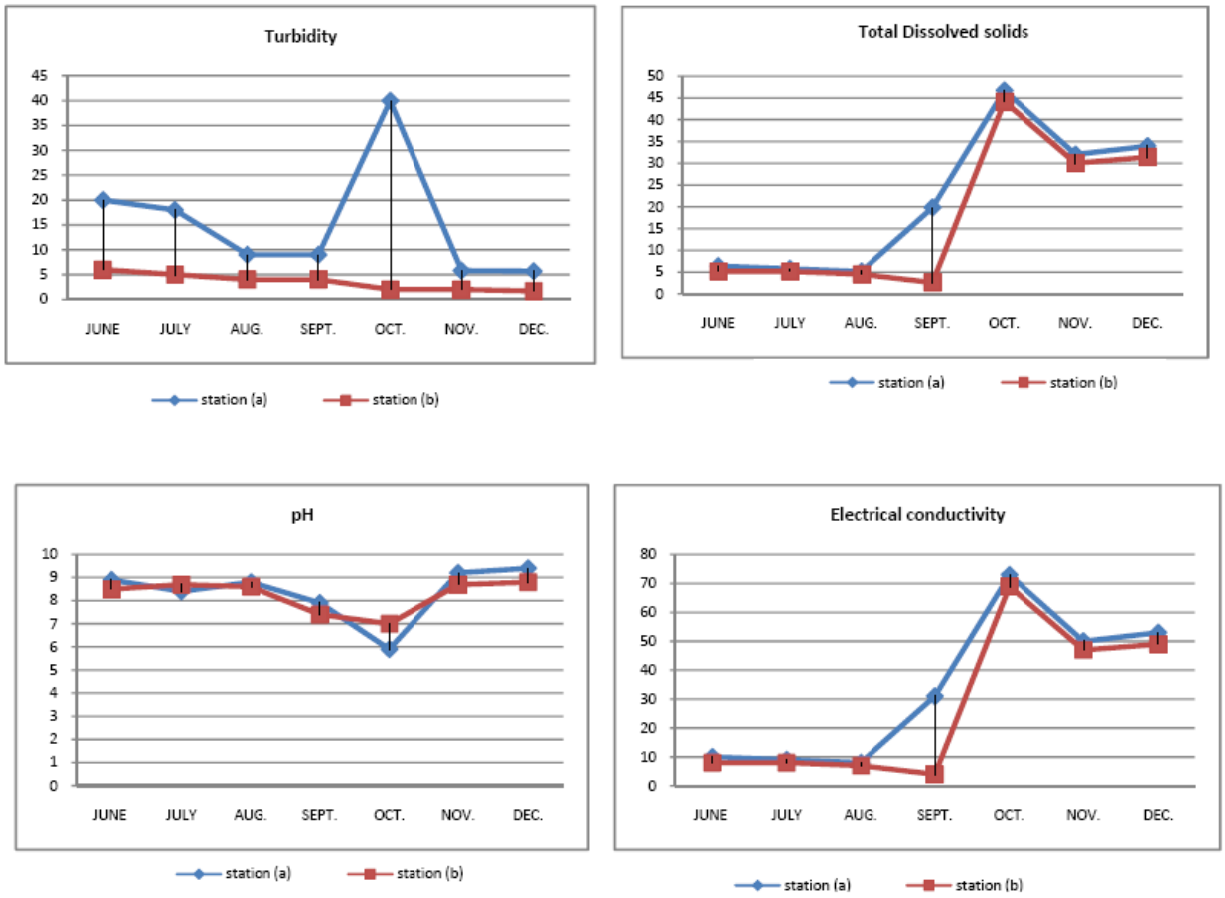
Chloride (Cl^-)

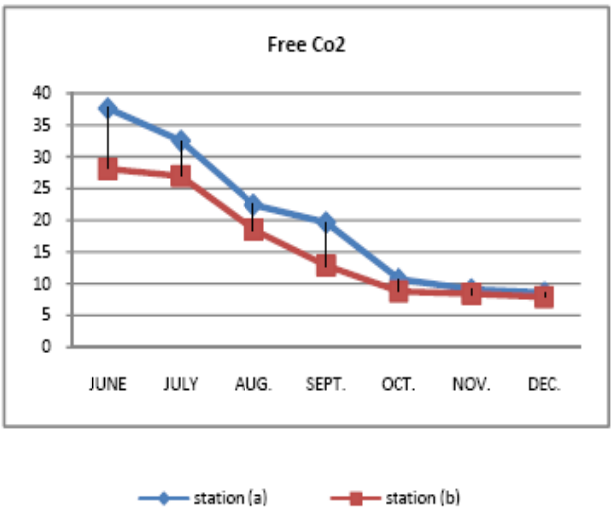
At station (a), the ground water near the waste dumping site recorded maximum chloride concentration of 103.74mg/l in October, the month of November recorded chloride concentration of 101.4mg/l while the minimum chloride concentration of 41.2mg/l was observed in July followed by 40mg/l in August. The concentration of chloride in all water samples studied during present investigations were within the limits prescribed by WHO, 1993 (200mg/l); Bureau of Indian Standards, 1992 (250 mg/l).High content of Cl^- in natural waters is regarded as pollutant from organic waste of animal origin. At station (b), the ground water 2.5km away from the waste dump site recorded maximum chloride concentration of 81.12mg/l in December, the month of November recorded chloride concentration of 76.94mg/l while the minimum chloride concentration of 18.2mg/l was observed in August followed by 26.09mg/l in July. The concentration of chloride in all samples studied were within the limits prescribed by WHO, 1993 (200mg/l); Bureau of Indian Standards, 1992 (250mg/l). However chloride concentration was higher at station (a) than station (b).Chloride is a very important parameter of detecting the contamination of groundwater by waste water. Chloride imparts bad taste to water when present at higher level than prescribed limit of 250mg/l.

Table 1: Showing physico-chemical parameters of ground water at station (a) and (b) near the municipal solid waste dumping sites

Parameters	Station (a)	Station (b)	BIS (1992)	WHO(1993)
pH	5.9-9.4	7-8.8	7.0-8.3	6.5-8.5
Turbidity	5.7-2 0	1.7-6	5.0	5.0
Electrical conductivity	8-73	4-69	-	600
Total dissolved solids	5.12-46.72	2.5-44	500	500
Total hardness	442-627.9	404.78-502	200	150
Bicabonate	320-674.9	279-570	-	500
Free CO_2	8.6-37.6	7.92-28.09	-	-
Dissolved O_2	2.02-5.6	3.7-6.4	---	6
Calcium	135.4-168.46	104.40-135.28	75	75
Magnesium	20.36-84.5	7.37-37	-	30
Nitrate	1.5-23	1.2-4	45	10
Sulphate	3-45	1-32	200	150
Chloride	35.43-103.74	26.09-81.12	250	200

Fig. 1: Showing physico-chemical parameters of ground water at station (a) and (b) near the municipal solid waste dumping sites




Free Co2
40
35
30
25
20
15
10
5
0
JUNE
JULY
AUG.
SEPT.
OCT.
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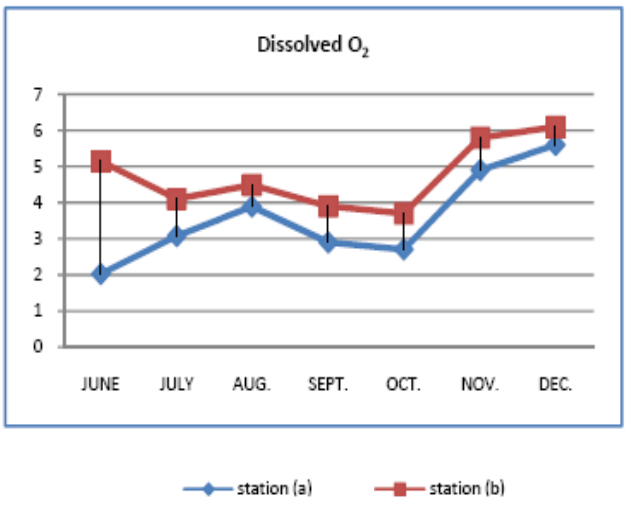
DEC.
station (a)
station (b)


Dissolved O_2
7
6
5
4
3
2
1
0
JUNE
JULY
AUG.
SEPT.
OCT.
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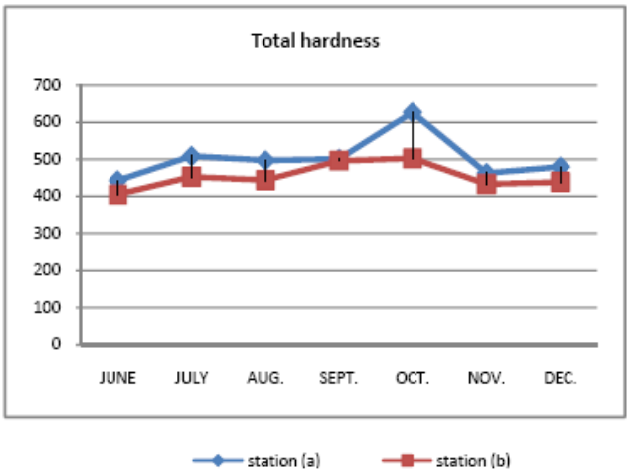
DEC.
station (a)
station (b)


Total hardness
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600
500
400
300
200
100
0
JUNE
JULY
AUG.
SEPT.
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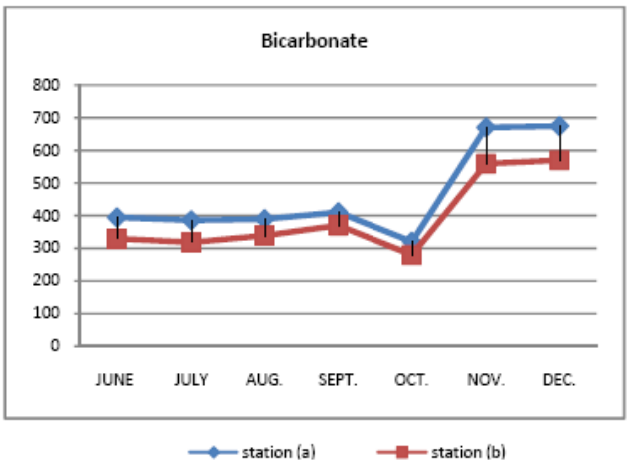
station (a)
station (b)


Bicarbonate
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700
600
500
400
300
200
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0
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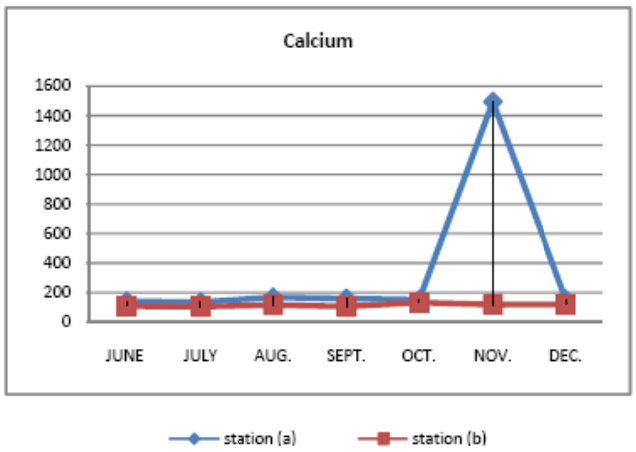
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
station (a)
station (b)


Calcium
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1400
1200
1000
800
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400
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AUG.
SEPT.
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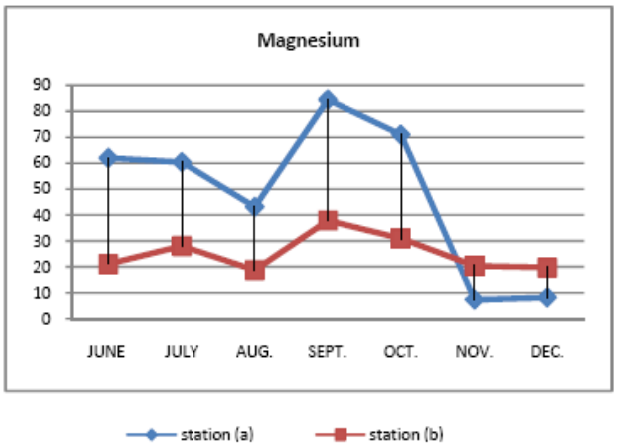
DEC.
station (a)
station (b)


Magnesium
90
80
70
60
50
40
30
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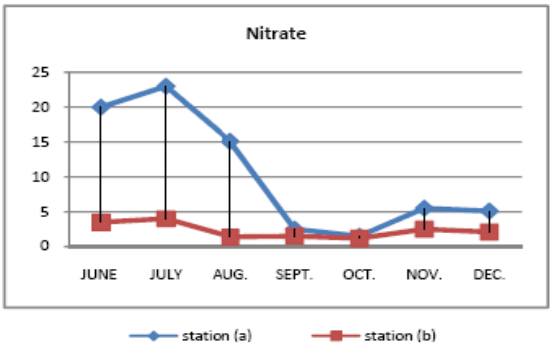

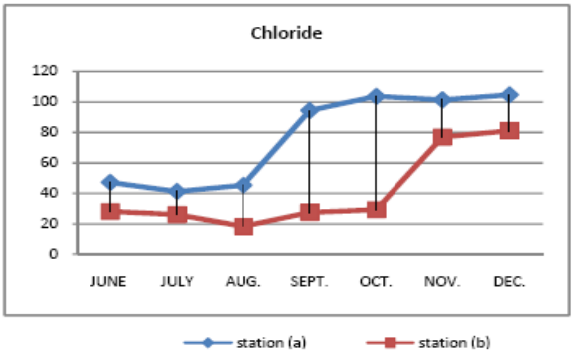

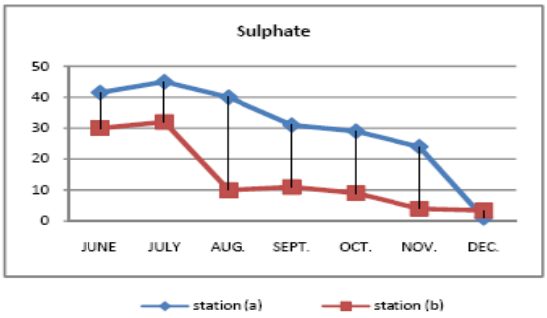

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Microbiological Screening of river Ganga before and after Shivratri

Nisha Sharma✉ and Padma Singh

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Abstract

Rivers in India have been regarded from time immemorial as sacred water bodies. The holy Ganges flowing in the most populated northern India is also declared unfit for even bathing. The present investigation has been carried out to monitor the water quality of river Ganga. Water samples were taken from five different sites and were checked out for microbiological study by evaluating SPC. Later on enumeration, isolation and identification of bacteria was done. Sensitivity tests were also carried out. The results obtained after performing the experiments indicates that the water in the tested stretch is unfit for both bathing and drinking purposes.

Keywords: *Ganga, microbiological, monitor, river, sacred, SPC*

Introduction

Water is a prime natural resource and is a basic need of life. The water is available abundantly in nature. The water covering about 75% of earth's crust. Water is found in everything in different forms. It is an essential component of all cellular organism.

The main problem now a days before the world is of safe drinking water which is affected due to various pollutants in different proportion, alteration in physical, chemical and biological characteristics of fresh water due to human activities which ultimately cause harmful effects on human beings and aquatic biota. The underlying assumption in traditional water resource planning process still continues. Fresh water is a gift of god which could continue to be available in perpetuity and in abundance. This is not valid as both quantity and quality of water pose serious problems (Kaul *et al*., 1999).

The river Ganga occupies unique position in the cultural ethos of India. Even today, people carry treasured Ganga water all over India and abroad because it is "holy water" and known for its "curative" properties (Sharma, 1997). The holy Ganges flowing in the most populated northern India is also now declared unfit even for bathing (Pandey *et al*., 2005).

During recent years due to the urbanization and industrial growth, the quality of Ganga water has deteriorated considerably. Due to increasing pollution, chemical contents of the water have gone over eight to nine units beyond the permissible limit of seven units. Besides this the water is becoming saline and flow of Ganga is slowing down gradually. According to scientists of the Pollution control research institute (PCRI) in their study warned that unless steps are taken in time, Ganga will become unfit for use (Agarwal, 1992). At Haridwar thousand of pilgrims washing away their sins in the Ganga everyday, considerable deterioration has been witnessed in the quality of water during peak bathing hours. Ganga is not only polluted by bathing but also by garbage. At Haridwar Central Pollution Control Board puts the figure at 17.5 million litre of waste dumping per day in Ganga (Banerjee, 1987) several pathogenic microorganisms enters into the water body which cause harmful effects and chronic diseases in man and animals. The bacteria, viruses and protozoa may begin to grow on sewage under anaerobic conditions. This may cause the spread water borne disease like viral hepatitis, polio, cholera, dysentery, typhoid, amoebiasis, giardiasis etc. such activities are increased many folds on the occasion

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of festivals. Due to mass bathing Ganga water becomes highly polluted and many water borne disseases spread rapidly.

Ganga water has significance due to its self purification capacity. So Ganga water considered as "Holy" water due to its purification property. Ganga water has self purification property as the bacteria degrade or decompose everything in Ganga ecosystem. Velz (1947) explained the process of recovery and self purification capacity of river.

Material and Methods

The study was conducted on Ganga water at five different sites in Haridwar before and after Shivratri festival. The five different sites selected for collection of water were:-

Site I -Har Ki Pauri, which is the main place of 'Ganga Snan" during the festival.

Site II -Prem Nagar Ghat, which is 4.5 km from Site I

Site III -Daksh Mandir, which is 7 km from Site I

Site IV -Singh Dwar Ghat, which is 5 km from Site I

Site V -Jatwara Pul Ganga Canal, which is 8 km from Site I

Sampling Procedure

The sterilized sampling bottle were held at its base by hand and dipped in to the water with its neck downwards upto 6 inches below the water surface and then its mouth was opened inside the water and removing its stopper against its flow. The bottle was filled completely and about 10% of the volume of bottle was left unfilled, the mouth of bottle was closed inside the water.

Analysis of water

Water was analysed for Microbiological study (SPC). Bacteria were isolated and identified and then sensitivity tests were carried out against multidisc antibiotics.

Isolation and identification

Isolation of bacteria was carried out by serial dilution method. The bacteria were identified with the help of Gram staining and biochemical tests as described in Bergey's manual of Systematic Bacteriology (Holt *et al*., 1994).

Antibacterial sensitivity test

The susceptibility test was carried out by Disc diffusion method (Bauer *et al*., 1966)

Results and Discussion

The numbers of bacterial colonies obtained in Ganga water before and after festival are tabulated in table 1 and 2. The number of bacterial colonies obtained in Har Ki Pauri were 145±0.67 CFU before shivratri and 172±0.54 CFU after Shivratri. In Prem Nagar Ghat the number of colonies were 132±0.14 CFU before Shivratri and 154±0.10 CFU after Shivratri. In Daksh Mandir the number of colonies were 148±1.65 CFU before Shivratri and 161± 0.73 CFU after Shivratri. In Singh Dwar Ghat the number of colonies were 141±0.54 CFU before Shivratri and 157±1.32 CFU after Shivratri. In Jatwara Pul Ganga Canal the number of bacterial colonies were 137±0.47 CFU before Shivratri and 146±0.02 CFU after Shivratri (Table-1).

Bacteria were identified on the basis of Gram's staining, their morphology, cultural characteristics and biochemical characterisation. There were large number of bacterial colonies obtained like *Bacillus* sp*, E.coli, Micrococcus* sp., *S. aureus, Serratia* sp. etc. some of the bacteria were pathogenic while others were found as non-pathogenic.

The most abundant bacterial colonies after shivratri festival was *Micrococcus* sp. It was observed by the data that non-pathogenic bacteria was generally present in the Ganga water but after festival usually pathogenic bacteria such as *S. aureus* and *Bacillus* sp. were increased in the water due to arrival of large number of pilgrims who took bath in the holy river Ganga and washing away their sins in the Ganga (Table-2).

During the study activity of different antibiotics and chemotherapeutic drugs on *Bacillus* sp., *E.coli, Micrococcus* sp., *S. aureus, Serratia* sp. following results are obtained –

The zone of clearance of *Bacillus* sp. to various antibiotics and chemotherapeutic agents are as follows –

Ofloxacin > Ceprofloxacin > Tetracycline > Roxythromycin > Pefloxacin > Gentamycin > Cephalexin > Cefotaxime. The isolate was completely resistant to Co-trimaxazole, Amphicilin, Cloxacillin and Lincomycin.

The zone of inhibition of *E. coli* to various Antibiotics and chemotherapeutic agents are as follows –

Ciprofloxacin > Amphicillin > Tetracycline = Chloramphenical > Gatifloxacin > Cefotaxime > Amikacin = Ofloxacin. The isolate was completely

resistant to Pipercillin, Co – trimaxazole, There is no zone of clearance in *Micrococcus* sp. therefore it shows resistance against all the antibiotics and chemotherapeutic agents.The zone of inhibiton of *S. aureus* to various antibiotics is as follows: -

Gentamycin > Pefloxacin > Ofloxacin > Ceprofloxacin > Roxythromycin > Cefotaxime = Lincomycin > Tetracycline > Cloxacillin > Cephalexin.

ceftizoxime and Gentamycin.

The isolate was completely resistant to Ampicillin and Co-trimaxazole.

The zone of inhibiton of *Serretia* sp. to various antibiotics is as follows:

Amphicillin > Ciprofloxacin = Getifloxacin > Tetracycline > Cefotaxime = Ofloxacin > Chloramaphenical > Co-trimaxazole = Amikacin.

The isolate was completely resistant to Pipercillin, Ceftizoxime, Gentamycin. (Fig 1-4, Plate-1)

Table-1: Bacterial Population in Ganga Water Before & After Shiv Ratri Festival

S. NO	SITE	COLONY FORMING UNIT (CFU) ±	
		Before Festival	After Festival
1.	HAR-KI-PAURI	145±0.67	172±0.54
2.	PREM NAGAR GHAT	132±0.14	154±0.10
3.	DAKSH MANDIR	148±0.65	161±0.73
4.	SINGH DWAR GHAT	141±0.54	157±1.32
5.	JATWARA PUL GANGA CANAL	137±0.47	146±0.02

± = Standard error

Table-2: Bacterial Population(CFU) of Individual Bacteria Before & After Shiv Ratri Festival

S.NO.	Isolated Bacteria	Har Ki Pauri		Premnagar Ghat		Daksh Mandir		Singh Dwar Ghat		Jatwara Pul Ganga Canal	
		BS	AS	BS	AS	BS	AS	BS	AS	BS	AS
1.	*Bacillus sp.*	28	35	28	24	33	27	-	24	-	28
2.	*E.coli*	37	41	35	40	37	38	42	40	33	38
3.	*Micrococcus sp.*	56	68	55	63	56	61	58	64	57	62
4.	*S.aureus*	11	14	-	15	12	24	20	17	31	-
5.	*Serretia sp.*	8	12	11	12	-	7	14	12	14	10
6.	**Unidentified**	5	2	3	-	10	4	7	-	2	8

BS = Before Shiv Ratri
AS= After Shiv Ratri

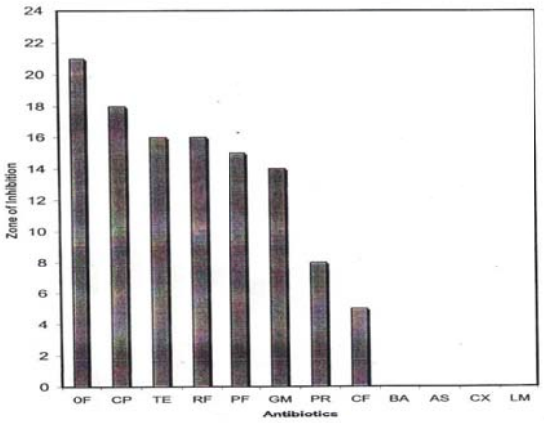

Fig. 1. Showing sensitivity pattern of *Bacillus* spp. against different antibiotics.

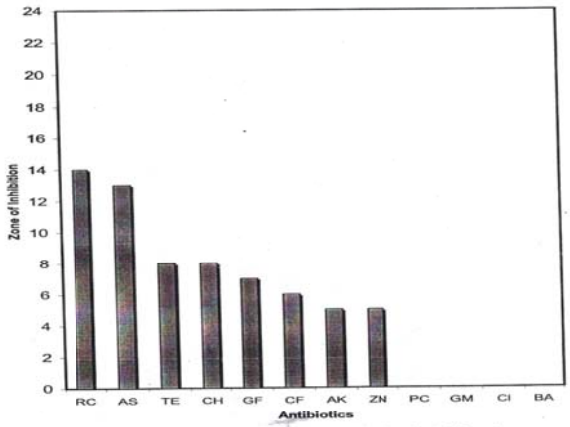

Fig. 2. Showing sensitivity pattern of *E.Coli* against different antibiotics

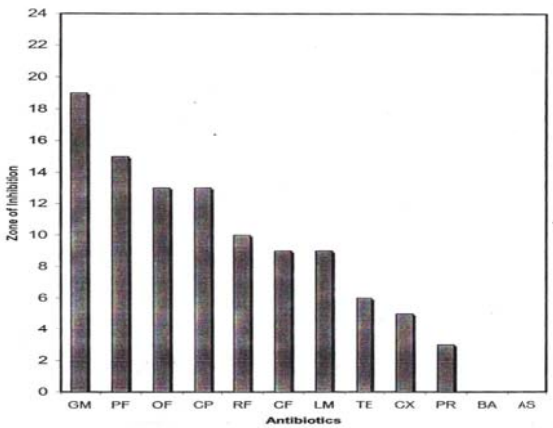

Fig. 3. Showing sensitivity pattern of *S. aureus* against different antibiotics

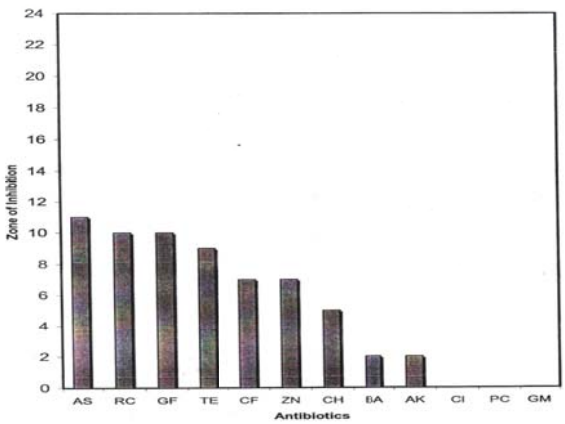

Fig. 4. Showing sensitivity pattern of *Serretia* Spp. against different antibiotics

Standard plate count (SPC) technique enumerate the total population of bacteria present in water. The total bacterial population may include many pathogenic and non-pathogenic bacteria in addition to the coliform group. Total bacterial count of 100 colonies / ml. of water is acceptable but the test sample showed the striking higher population after festival between 146-172 colonies/ml. It indicates towards the height of microbial population in the holy river due to large number of pilgrims who took bath in Ganga during festivals and due to domestic and industrial disposal.

A total of 43 different bacterial species were obtained in Ganga water, which were distributed in 24 genera. Of these 31 species were isolated from Alaknanda, 25 from Bhagirathi and 28 from Lower Ganga in Uttarakhand (Sood *et al.,* 2010).

Many pathogenic bacteria are found in Ganga water which cause serious disease in all living beings e.g. *Bacillus* sp. *E.coli, S.aureus* and some non-pathogenic bacteria like *Micrococcus* sp. and *Serretia* sp. are also found.

***Bacillus* sp**. is a Gram +ve bacteria. It can cause food borne gastroenteritis. Some species may be responsible for opportunistic infection. It is found at all the sites but its population is increasing after shivratri mainly at Har Ki Pauri and Daksh Mandir during peak bathing hours.

E. coli is Gram –ve bacteria. It causes urinary tract infection, diarrhoea, pyrogenic infection and septicemia etc.

***Micrococcus* sp**. is found at all the five sites of Ganga water before and after Shivratri almost in equal amount, is a Gram Positive non-pathogenic bacteria.

S. aureus is also a Gram positive bacteria and is responsible for skin allery and throat infection. Highest population of *S. aureus* is found after Shivratri at Daksh Mandir.

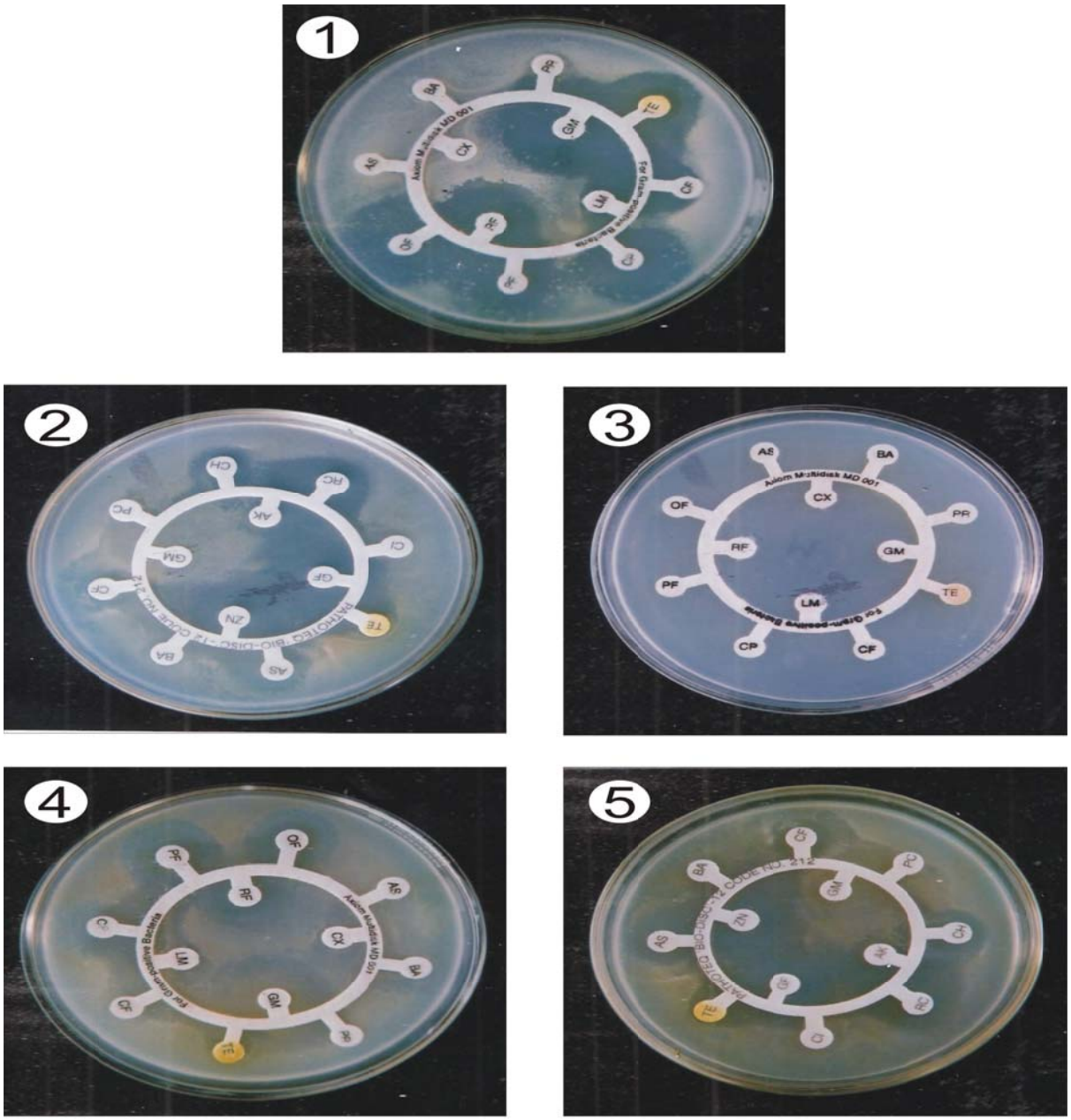

Plate 1 – Zone of inhibition exhibited by different antibiotics :
1. *Bacillus* sp., 2. *E.coli*, 3. *Micrococcus* sp.,
4. *S.aureus*, 5. *Serretia* sp.

***Serratia* sp**. is found in Ganga water at very lowest amount. It is a Gram –ve and non-pathogenic bacteria.

The study of different antibiotics and chemotherapeutic drugs were tested against isolated bacteria. ***Bacillus* sp.** showed maximum zone of inhibition was about 21mm against the antibiotic Ofloxacin.

E. coli showed maximum zone of inhibition was about 14mm against Ciprofloxacin.

In Case of ***S. aureus*** the some of inhibition was maximum about 19mm against Gentamycin.

Amphicillin gave best results in case of ***Serretia sp.,*** the zone of inhibition was maximum about 11.

Conclusion

From the present study it can be concluded that large number of bacteria are present in Ganga water and showed resistance against some antibiotics and chemotherapeutic agents. The main source of pollution includes urban liquid waste discharge, Industrial liquid waste discharge and large scale bathing of local pilgrims, cattle, throwing of dead bodies in the river, mass bathing during festival and many others.

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Status of brown trout (*Salmo trutta fario* L.) in Garhwal Himalaya with a note on it morphometric characteristics

M.S. Rawat✉[1], Babita Bantwan[1], Dhyal Singh[1] and O.P. Gusain[2]

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Abstract

The history of introduction of brown trout in Garhwal Himalaya is 100 years. However, the scientific information on brown trout is grossly lacking. The present study is a part of investigation on various aspects of brown trout inhabiting the River Asiganga in Uttarkashi district of Uttarakhand. The status of brown trout was ascertained in River Asiganga and other reports from elsewhere in the region. The morphometric study was based on 253 fish specimens collected from River Asiganga. In addition to the 12 body measurements of the fish, red/orange and brown spots on body were also studied.

Keywords: *River Asiganga, brown trout, body spots, teeth*

Introduction

Brown trout (*Salmo trutta fario* L.) belonging to family salmonidae are indigenous to Europe, North America, Africa, Australia, New Zealand, Papua New Guinea (Moyle 2002), while, native western Asian countries are Armenia, Afghanistan and Turkey. In Asia, the fish has been introduced in India, Sri Lanka and Nepal. With large variability the existing populations of brown trout significantly differ among them either by geographic isolation of specific conditions from each specific spreading area (Bud *et al*. 2009). It was introduced in India in the early 19th century for food and sports, and in spite of being ecologically, economically and scientifically a valuable species, brown trout is too less studied in India. The details of introduction of trout in India has been well described by Molesworth and Bryant (1912), Howell (1916), Mitchell (1918), Mackey (1945) and Jones and Sarojini (1952). Also, the length weight relationship and food and feeding habit of brown trout in Himachal Pradesh and Jammu and Kashmir has also been studied (Khan and Tandon, 1941; Shah, 1975; Kumar *et al*., 1979; Sehgal *et al*., 1984 and Sehgal, 1992). As such, various aspects of brown trout have been extensively dealt with in different parts of the world by Frost (1939), Ball (1961), Michael (1970), Elliott (1972,1976), Edwards *et al*. (1979), Lobon-Cervia *et al*. (1986), Belaud (2002), Alp *et al*. (2003, 2005), Arslan *et al*. (2004), Maric *et al*. (2004), Oscoz *et al*. (2005), Montori *et al*. (2006), Power *et al*. (2007), Fochetti *et al*. (2008), Hao and Chen (2009), Demir (2010), Kara *et al*. (2011), Sanchez-Harnandez (2011).

The history of the introduction of brown trout in the Garhwal Himalaya region of the state Uttarakhand (the hill districts of erstwhile Uttar Pradesh) dates back to 1910 when, the then Tehri State Ruler stocked the eyed ova of brown trout, carried from Kashmir, into Kaldyani (Uttarkashi) and Talwari (Chamoli) hatcheries (Singh *et al*., 1983). From these hatcheries the seeds were introduced into different rivers and lakes in the region. Surprisingly, in spite of hundred years of introduction in Garhwal region, scientific study on brown trout is grossly lacking.

As of now, this exotic fish has established itself successfully in some of the Garhwal Himalayan water bodies viz., Lake Dodi Tal, River Asiganga, Balkhila Gad, Rupin-Supin, Madhu Ganga etc. The present study is the first attempt to collect information on the biology and ecology of brown

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trout inhabiting the river Asiganga-a tributary of River Bhagirathi in the Garhwal Himalaya region (Fig. 1). The paper gives an account of the status of brown trout in aquatic environment of Garhwal Himalaya along with its morphometric characteristics.

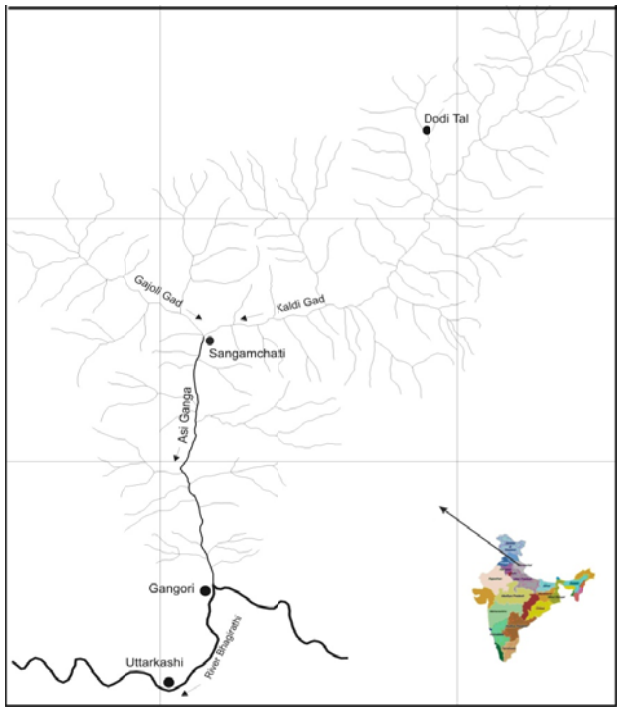

Fig 1. Location map of river Asiganga

Materials and Method

Kaldi Gad and Gajoli Gad originated from 4521 m asl and 3836 m asl respectively and joins at Samgamchati (1505 m asl) to form River Asiganga. River Asiganga (Latitude $30^0$48'N and Longitude $78^0$27'E) traverses a distance of 15 km before meeting River Bhagirathi at 1160 m asl at Gangori 5 km upstream to Uttatkashi township. Specimens of brown trout were collected during August 2007 to July 2009 from local fishermen at the river. In all, 253 specimens were examined fresh in the laboratory. The brown trout specimen ranged from 12.8 to 48.0 cm in length, while the weight was measured in between 20.61 to 1280 gm. The morphometric measurements were taken on the left side of body as described by Lowe-McConnel (1971). The morphological features measured were: total length (TL), standard length (SL), fork length (FL), head length (HL), snout length (SL), eye diameter (ED), caudal length (CL), anal length (AL), pelvic length (PL), pre pectoral length (PPL), dorsal length (DL), post orbital length (POL), maximum body depth (MBD). The measurements were further subjected to range difference and correlation analysis. All the statistical analysis were made using MS Excel. Also, the number of red/orange and brown spots on the body and fins were counted.

Morphometric characteristics of brown trout

The body of brown trout is cylindrical with shinning silvery grey colour and a squarish tail having red/orange and brownish spots on its body (Fig. 2). The colouration of body changes somewhat according to age; brown trout with the age group of 5^+ years is less shinning, dark brown in colour from dorsal side and slightly from lateral side above the lateral line. The brownish colour originating from head region decreases towards dorsal fin and finally finishes at adipose fin. The ventral side of the fish is creamish-white in colour. According to Kottelat and Whitten (1996) and Crivelli *et al*. (2000) the physical-chemical features of aquatic basins are significant conditions of the brown trout body colour. The body of brown trout has red/orange to dark brown colour spots. The reddish/orange spots mostly observed near and below the lateral line, are less as compared to the dark brownish spots. With the increase in age the reddish/orange spots get converted into dark brownish spots. These spots were also present on dorsal and adipose fin, which ranges from 2-98 in number while in pelvic, pectoral, anal and caudal fins no such spots were observed. The approximate number of spots on whole body ranged between 86-312 (Table 1).

The jaws of the brown trout are dome shaped, equal in size and contained sharp teeth. In the upper jaw the number of teeth ranged from 36 to 38, while the lower jaw contained 22-24 teeth. The ventral side of upper jaw was folded and also contained teeth. The tongue has 4 folds and each fold contained teeth (Fig. 3a & b).

Fig. 2. *Salmo trutta fario* L. from River Asiganga, Uttarkashi

Table 1. Variability of spots on the various regions of body of brown trout.

Specification	Head	Lateral Line	Above L. L	Below L.L.	Dorsal Fin	Adipose Fin	Total
Range	2-14	12-46	10-180	8-100	26-98	0-6	86-312
Average	7.57	24.94	62.03	29.03	53.33	4.00	186.67
SD	2.88	8.76	43.31	20.14	28.47	1.73	75.64

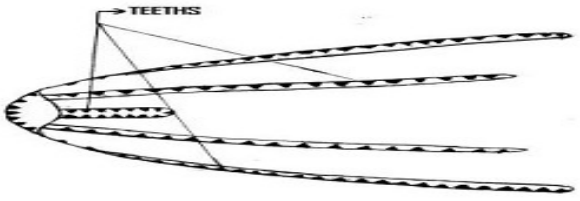

Fig 3a. vertical portion of upper jaw containing teeths

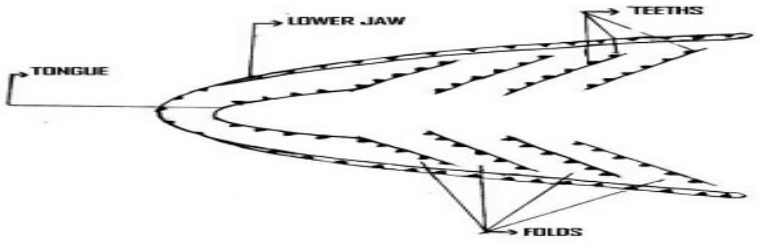

Fig 3b. Structure of lower jaw and tongue showing folds and teeths

The pectoral, pelvic and anal fins are paired while dorsal and caudal fin are unpaired. In addition to these fins an adipose fin is also present in between caudal and dorsal fin. The various body parameters of brown trout (*Salmo trutta fario* L.) showed highly positive correlation in relation to total length and head length and presented in Table 2.

Table 2. Mean standard deviation, (SD), range difference, correlation coefficient (*r*), of different morphometric characters of brown trout in relation to total length (TL) and head length (HL) in River Asiganga.

S. N.	Parameters in relation to total length (cm)	Mean	SD	Range of %	Range Difference	*R*
1	Standard length	24.9146	6.9465	20.4841-19.3612	27.8	0.9977
2	Caudal length	4.2906	1.2820	3.3519-3.5928	5.4	0.9329
3	Pre Pectoral Length	5.4866	1.6946	3.9106-4.9401	7.8	0.9462
4	Pelvic Length	13.5333	3.8522	11.1731-0.4935	16.1	0.9860
5	Anal Length	19.2720	5.4787	15.4562-0.4962	22.7	0.9961
6	Fork Length	28.9040	8.2501	22.7188-0.4850	33.7	0.9987
7	Head Length	6.6973	2.0545	4.8417-0.4850	9.3	0.9721
8	Dorsal Length	11.9973	3.4507	9.3109-0.4903	14.7	0.9826
9	Max body depth	8.4440	2.6088	6.1452-0.4787	10.6	0.9594
10	In relation to Head length (cm) Snout length	2.0880	0.7839	26.9230-8.18	3.8	0.9816
11	Eye diameter	0.9720	0.2010	19.2307-7.5466	0.8	0.9056
12	Postorbital Length	8.6373	1.1095	53.8491-8.0833	5.2	0.9940

Habitat characteristics

The brown trout inhabiting rivers and lakes requires cold water, high dissolved oxygen and fast water current in the river to maintain dissolved oxygen level**.** Trout streams in the mountains are typically fast flowing soft-water streams with rocky and stony riverbeds, clear water and usually with no macrophytes growing in the streams. Brown trout reportedly prefer water temperature ranging from 12–19^{0}C / 20^{0}C (Bud *et al*., 2009; Raleigh *et al*., 1986). Similarly, in the present study the brown trout was recorded inhabiting the high-oxygenated (7.3 to 14.3 mg l^{-1}) and cold water (5.0-21.0^{0}C) of River Asiganga. The catchment area of Asiganga is predominantly mixed forest of Oak, Pine, Deodar, Rhododendron, Walnut *etc*. The river substratum is constituted of rocks, boulders of various size, pebbles, cobbles along with gravel and sand in the lower section. The gradient is very high in the upper reaches which decreased below 1450m asl. The water velocity varied between 0.8 to 1.96 m s^{-1}. Brown trout inhabiting Asiganga is extremely carnivorous and feed on aquatic insects of order ephemeroptera, plecoptera, diptera, coleoptera, trichoptera *etc*. Terrestrial insects like ants, grasshoppers and spiders were other common food present in the gut. Food items, like small fishes, and earth worms were also consumed by brown trout.

Status in Garhwal Himalaya

Keeping in view the prospect of brown trout propagation in Garhwal, the then Tehri Garhwal Ruler, in the year 1910 developed two hatcheries at Kaldyani (Uttarkashi) and Talwari (Chamoli). Later, during 1992-94 Fishery Department, Govt. of Uttar Pradesh (U.P.) constructed one more hatchery at Barangana (Chamoli). However, Talwari hatchery is not functional since long.

Kaldyani hatchery is located about 15 km. from Uttarkashi along River Asiganga at an altitude of 1404 m asl. Setup in year 1910, fish seeds collected from Kashmir was introduced in 4 tanks. In 1947-48 this hatchery was handed over to Fishery department, U.P., Lucknow. At present, with a total area of about 1.10 ha, the hatchery has 21 ponds which include nursery, rearing and stocking ponds. The hatchery was functional up to 2001; however, the inlet channel and some ponds of the hatchery were destroyed in the flood of 2002. Later in 2009 the renovation work was completed and now the hatchery is in the functional state.

Barangana hatchery constructed by Fishery department, Govt. of U.P. spread over an area of 2.4 ha, on the bank of snow-fed Balkhila Gad (a tributary of Alaknanda River), is situated about 14 km from Gopeshwar (Chamoli). This hatchery is producing seed of Rainbow trout (*Oncorhynchus mykiss*), Brown trout (*Salmo trutta fario*) and Common carp (*Cyprinus carpio*).

Fisheries department, Govt. of U.P. had also introduced brown trout seeds in different fluvial systems of Garhwal like Balkhila Gad, Birahi Gad, Nandakini and Pinder in Chamoli district, Madhuganga in Rudraprayag, Kaldi Gad, Asiganga and Rupin-Supin in Uttarkashi (Fisheries in Uttaranchal, 2005) (Fig. 4).

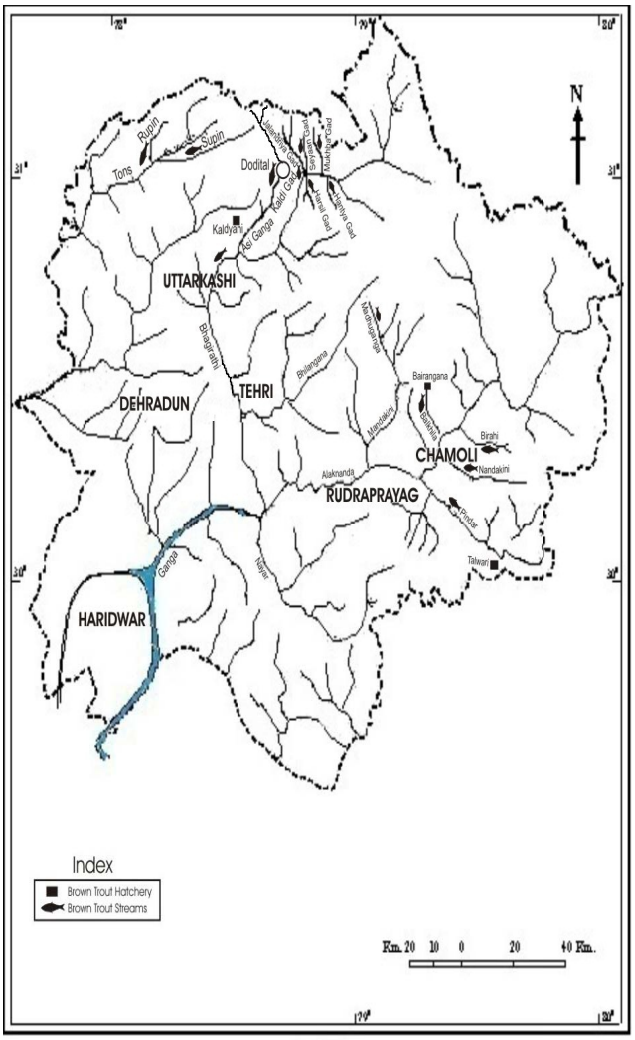

Fig 4. Brown trout in water bodies of Garhwal Himalaya

It is also observed that the fish have reached the tributaries of these rivers. During the present survey, brown trout was also reported from the upper reaches of tributaries of River Bhagirathi (between 2550 and 2685 m asl). These tributaries are Jalandriya Gad, Seyaan Gad, Harsil Gad, Mukhba Gad and Hantya Gad. These are perennial, snow-fed, clear and fast flowing water bodies with highly oxygenated water. Notably, brown trout has established it self in the Bhalkhila Gad and River Asiganga along with other indigenous fish species like *Schizothorax, Barilus, Noemachelus etc.*, in the Bhalkhila Gad mainly due to the fact that seeds are introduced every year from the nearby Barangana hatchery. Also River Asiganga is stocked with seeds from Kaldyani hatchery and Lake Dodi Tal is yet another permanent source of brown trout as during monsoon the fry and fingerlings reach it through Kaldi Gad.

Acknowledgement

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Impact of plantomycin spray on soil algae of paddy fields in Maouda, Nagpur district, Maharashtra

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Abstract

Several antibiotics are used to protect the crop plants from bacterial diseases by spraying it on them. Plantomycin and Paushamycin are the two antibiotics commonly used in Vidarbha region of Maharashtra state. These antibiotics create an impact on the soil micro flora when it falls on the soil surface during spraying. The soil micro flora performs conditioning of the soil for better crop production. In present study we observed the impact of Plantomycin on the soil algae of paddy fields in Maouda of Nagpur district, Maharashtra. Total 52 algal taxa could be indentified from the experiment field of which 31 belongs to Cyanophyta, 13 to Chlorophyta and 8 to Bacillariophyta. The different paddy field soil algal taxa show variable resistance to Plantomycin. The blue green algae show more resistance to the antibiotic than the green algae. Higher concentration of antibiotic used in this experiment gave 100% algicidal value in both Cyanophyta and Bacillariophyta, but Chlorophyta could not respond so, due to the two species of the genera, Chlorococcum. *C. humicolo* and *C. vitiosum* were highly resistant to antibiotic used. It may be due to their sheath or cell wall organization.

Keywords: *Soil algae, impact, plantomycin, pady fields*

Introduction

The paddy fields usually show abundance of algal flora due to the varying conditions available during the paddy crop. Nagpur district paddy fields show dominance of Cyanophyta over other groups of algae (Cherian, 2010). The uses of antibiotics to control plant bacterial diseases is an important advancement in the science of plant protection .In modern agricultural practices, the crop fields are sprayed with antibiotics. During this operation some of these antibiotics spray does fall on the soil and may exert a harmful effect on the soil algae. Addition of gypsum, sulphur, ground nut oil cakes increase the number of rhizosphere flora (Sunar & Chohan 1971). The Phorate granules, a systemic insecticide affects the rhizosphere micro flora of various plants (Tiwari 1972, Visalakshi & Nair 1979). Introduction of any chemicals organic or inorganic will definitely create an impact on the soil micro flora. Usually it is negative impact rather than positive (Cherian, 2010).

The author had worked out the impact of Paushamycin, another antibiotic commonly used in paddy field.

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The present investigation is aimed to find out the effect of Plantomycin on the growth of soil algae. The soil algae play an important role in soil conditioning by increasing the humus and other nutrients available to crop plants. It also prevents the erosion of soil. Thus any negative impact on these important soil organisms will adversely affect the crop production.

Several phycologists used different antibiotics to obtain pure cultures of algae (Provasoli *et al*. 1951, Shelubsky 1951, Pappus & Hoffman 1952, Barkley 1956, Zender & Hughes 1958). The inhibitory effects of antibiotics on some groups of algae were reported by the observations made by Hunter & Veigh (1961), Kumar (1964a), Taylor (1965), Tarar & Kelkar (1979) and Cherian (2010). The potential values of antibiotics in relation to their action on algal forms have been investigated by Foiter et. al. (1953), Hunter & Veigh (1961), Lampmen & Arnow (1961), Perlman (1964) and Zender & Hughes (1958). They opined that algae are less sensitive towards antibiotics than bacteria and fungi.

Some contents in algae also show antibiotic properties. Hornsey and Hide (1976) examined 151 marine algae for antimicrobial substances. They

studied the seasonal variations in antibiotic production. The fresh water algal extracts showed the least antimicrobial activity against the bacteria, *E.coli* and *Staphylococcus aureus* than marine algae (Mesmar. & Abussaud, 1991). The chlorellin from Chlorella, pendorine from Pandorina, phormidine from Phorphidium etc. shows antimicrobial properties. Many saline extracts of algae are active against selective Vibrios. The Lectins extracted from red algae *Eucheuma serra* and *Pterocladia capillaceae* is active against *V. pelagiushe* a fish pathogen (Liao *et al*, 2003). Some sugars and glycoprotein inhibits the antibiotic activities of Lectins.

Different types of substances are produced in different microorganisms for different utility in their life stages. The properties of some of them were studied and reported by different workers. The Lectins play an important role in recognition and adherence of gametes during sexual reproduction. The sulphated polysaccharide from cell wall of Porphyridium species display pronounced antiviral activity (Huleihel, *et al*.2001). The antibiotic characteristic is depending on type, season and growth conditions of the alga (Centeno and Ballantine,1999). The carrageenan oligosaccharides mediate the association of red alga and its green algal pathogen (Bourrab *et al.*, 2001). The experiments were conducted in paddy fields in Maouda of Nagpur district, which is about25 km. away from Nagpur city. It receives approximately 75-85 cm of rain fall and the temperature varies from 7.5 c. in January to 46 c in May. The soil is of clay type and it is light brown in color.

Materials and Method

1. **Plantomycin:**It is a broad spectrum antibiotics for agricultural use.

Composition : Each 40 gm contains Streptomycin sulphate 36 gm tetracycline hydro chloride 0.4 gm With vitamin B_{12} along with safe and effective melting agent and acidifying agent in an inert diluents.To study the algicidal potential of Plantomycin on algae, different concentrations were prepared and treated with the culture of algae.

A. **Algal flora of the experiment Field :** The algal flora of the experiment field were identified and listed in Table II. The lists were made complete by algal collections as well as soil culture studies. Total 52 algae were identified of which 31 belongs

Five concentrations were used as 0.2%, 0.4%, 0.6%, 0.8% and 1.0%. These concentrations of the antibiotic solution are prepared by dissolving the required quantities of antibiotic in distilled water. Plantomycin do not dissolve completely in water and hence its suspension is used as stock solution.

Algal cultures:

Multiple sets of cultures were made in De's modified Beneck's media, Allen & Arnon's media and Chu 10 media for each percentage treatment. The inoculated culture media were kept in racks with illumination for 12 hrs. per day.

Treatment:

The desired concentrations i.e. 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of Paushamycin were prepared by dilution of the stock solution with sterilized distilled water. 7.5 ml of solution of 5 different concentrations were added to separate flasks containing 5 gm of soil sample and 75 ml of nutritive media. After inoculation of the antibiotic all the culture samples were agitated to ensure a uniform distribution. A control sample is also kept with each set without treatment of Plantomycin.

Algal culture is made from soil with each type of media and another set of the same treatments were done with fresh culture obtained previously. Each flask with required media and Plantomycin is inoculated with 5ml of well stirred algal cultures. The treated and control culture flasks were kept in ideal condition of light and temperature for 45 days for the growth of algae.

Results and Discussion

Impact of selected antibiotic on soil algae:

Plantomycin, a commonly used antibiotic on crop plants in Vidarbha region is selected for the experiment. The experiments were conducted in paddy field soils of Maouda region in Nagpur district. The selected fields are located about 25 km. away from Nagpur city. The area is 289.28 meters above M.S.L. and receives approximately 75-85 cm of rain fall. The climate is extreme with cold winter and hot summer. The minimum temperature is 7. 5°C in January and maximum 46°C in May. The soil is of clay type and it is light brownish in colour.

to Cyanophyta,13 to Chlorophyta and 8 to Bacillariophyta. The Cyanophycean members consist of 8 genus. Anabaena is represented by a single species while *Lyngbya* is represented by 2 species. The rest genus were represented as follows; *Chroococcus* with 4 species, *Gloeothece* with 3 species, *Aphanocapsa* with 4 species, *Oscillatoria* with 9 species, *Phormidium* with 5 species and *Nostoc* with 3 species. The nitrogen fixing blue greens were represented by Nostoc (3 taxa) and Anabaena (1 taxa).The 13 Chlorophycean members were represented by *Chlorococcum* (2 taxa) *Scenedesmus* (3 taxa), *Ulothrix* (3 taxa), *Geminella* (2 taxa) and *Closterium* (3 taxa). The 8 members of bacillariophyta were represented by *Fragillaria* (1 taxa) *Synedra* (1 taxa) *Achnanthes* (1 taxa) *Navicula* (2 taxa) *Cymbella* (1 taxa) and *Nitzchia* (2 taxa). The observations on the algal flora of the experiment field shows that it is dominated by *Cyanophyta.*

Table – I: Total Number of viable forms, their survival, algicidal percentage in various concentrations of Plantomycin.

Antibiotics gm/100ml	Cyanophyceae			Chlorophyceae			Bacillariophyceae		
	No. of Survival forms.	Survival %	Algicidal Value %	No. of Survival forms.	Survival %	Algicidal Value %	No. of Survival forms.	Survival %	Algicidal Value %
0.2	31	100.0	0.0	8	61.53	38.47	8	100.0	0.0
0.4	31	100.0	0.0	6	46.15	53.85	8	100.0	0.0
0.6	23	74.19	25.81	4	30.76	69.24	2	25.0	75.0
0.8	10	32.25	67.75	2	15.37	84.63	0	0.0	100.0
1.0	0	0.0	100.0	2	15.37	84.63	0	0.0	100.0

B. Effect of Plantomycin on survival of algae :

The occurrence of algal species of experiment field in different concentration of plantomycin treatment is given in Table No.I. All the 31 Cyanophycean member were found resistant to 0.2% and 0.4% plantomycin treatment. *Chroococcus spelaeus* is found more sensitive than the other three species of the genus as it made its occurrence only upto 0.4% treatment. *Gloeothece samoensis* was more resistant and occurred up to 0.6% treatment whereas the other two species of the genus i.e G. membranceae and G. palea could occur only up to 0.4% treatment. All the 4 species of *Aphanocapsa* were resistant in 0.6% treatment. In 0.8% treatment were tolerated by *Aphanocaps nivalis* and *Aphanocapsa pulchra* whereas the other two species of the genus could resist only up to 0.6% treatment. Majority of the *Oscillatoria* species were resistant up to 0.6% treatment except *Oscillatoria grunowiana* and *Oscillatoria jenensis* which comparatively much sensitive as it could withstand only up to 0.4% treatment. *Oscillatoria decolorata*, *Oscillatoria subbrevis* and *Oscillatoria terebriformis* were most resistant as they survived up to 0.8% treatment whereas the rest could resist up to 0.6% treatment.The genus Phormidium was found to be most resistant as its species could occur upto 0.8% treatment except for *Phormidium foveolarum* which resisted upto 0.6% treatment only. Both the species of Lyngbya was very sensitive as it could resist only upto 0.4% treatment. All the nitrogen fixing members of Cyanophyceae was found sensitive, above 0.6% treatment except for two species of Anabaena which resisted upto 0.8% treatment. The Chlorophycean members showed varying sensitivity to Plantomycin treatment as some members are highly sensitive and could not resist even the lowest concentration treatment whereas others could resist even the highest concentration used. Both species of *Chlorococcum* resisted even in the 1% treatment. Species of *Scenedesmus* resisted upto 0.6% treatment except *Scenedesmus bijugatus* var. *bicellularis* which could resist only upto 0.4% treatment.

Table –II: Algae observed in different types of treatment of antibiotics

S. N	Name of Algae	Plantomycin % of concentration				
		0.2	0.4	0.6	0.8	1.0
I.	**CYANOPHYCEAE**					
1.	*Chroococcus macrococcus*	p	p	p	-	-
2.	*Chroococcus schizodermaticus*	p	p	p	-	-
3.	*Chroococcus spelaeus*	p	p	-	-	-
4.	*Chroococcus turgidus Var. fuscescens*	p	p	p	-	-
5.	*Gloeothece membranacea*	p	p	-	-	-
6.	*Gloeothece palea*	p	p	-	-	-
7.	*Gloeothece samoensis*	p	p	p	-	-
8.	*Aphanocapsa biformis*	p	p	p	-	-
9.	*Aphanocapsa grevillei*	p	p	p	-	-
10.	*Aphanocapsa nivalis*	p	p	p	p	-
11.	*Aphanocapsa pulchra*	p	p	p	p	-
12.	*Oscillatoria animalis*	p	p	p	p	-
13.	*Oscillatoria curviceps Var. anqusta*	p	p	p	-	-
14.	*Oscillatoria decolorata*	p	p	p	p	-
15.	*Oscillatoria grunowiana*	p	p	-	-	-
16.	*Oscillatoria ienensis*	p	p	-	-	-
17.	*Oscillatoria limosa Var. disperse-granulata*	p	p	p	-	-
18.	*Oscillatoria princeps*	p	p	p	-	-
19.	*Oscillatoria subbrevis*	p	p	p	p	-
20.	*Oscillatoria terebriformis*	p	p	p	p	-
21.	*Phormidium africanum*	p	p	p	p	-
22.	*Phormidium ceylanicum*	p	p	p	p	-
23.	*Phormidium foveolarum*	p	p	p	-	-
24.	*Phormidium jenkelianum*	p	p	p	p	-
25.	*Phormidium uncinatum*	p	p	p	p	-
26.	*Lyngbya aerugineo-coerulea*	p	p	-	-	-
27.	*Lyngbya lachneri*	p	p	-	-	-
28.	*Nostoc microscopicum*	p	p	p	-	-
29.	*Nostoc muscorum*	p	p	p	-	-
30.	*Nostoc piscinale*	p	p	p	-	-
31.	*Anabanena laxa*	p	p	p	p	-
II.	**CHLOROPHYCEAE**					
1.	*Chlorococcum humicolo*	p	p	p	p	p
2.	*Chlorococcum vitiosum*	p	p	p	p	p
3.	*Scenedesmus bijugatus Var. bicellularis*	p	p	-	-	-
4.	*Scenedesmus arcuatus*	p	p	p	-	-
5.	*Scenedesmus dimorphus*	p	p	p	-	-
6.	*Ulothrix oscillarina*	p	p	-	-	-
7.	*Ulothrix subtillisima*	p	-	-	-	-
8.	*Ulothrix tenuissima*	p	-	-	-	-
9.	*Geminella minor*	-	-	-	-	-
10.	*Geminella protogenita*	-	-	-	-	-
11.	*Closterium acerosum*	-	-	-	-	-
12.	*Closterium acutum*	-	-	-	-	-
13.	*Closterium parvulum*	-	-	-	-	-
III.	**BACILLARIOPHYCEAE**					
1.	Fragillaria brevistriata f. elongata	p	p	p	-	-
2.	Synedra affinis	p	p	p	-	-
3.	Achnanthes delicatula	p	p	-	-	-
4.	Navicula clavata	p	p	-	-	-
5.	Navicula grivilleri	p	p	-	-	-
6.	Cymbella austriaca	p	p	-	-	-
7.	Nitzschis dissipata	p	p	-	-	-
8.	Nitzschia gracilis	p	p	-	-	-
	(P) = Present Total (-) = Absent	**46**	**44**	**29**	**12**	**2**

Species of *Scenedesmus* resisted upto 0.6% treatment except *Scenedesmus bijugatus* var. *bicellularis* which could resist only upto 0.4% treatment. *Ulothrix subtilissima* and *Ulothrix tenuissima* were much sensitive and occurred only upto 0.2% treatment. Comparatively Ulothrix ocillarina was more resistant as it made its appearance up to 0.4% treatment. Both the species of Geminella were very sensitive to the Plantomycin since it could not withstand even 0.2% treatment. Similar effects were observed in the

species of *Closterium* too. Thus Plantomycin has 100% algicidal value on Geminella and Closterium.All the members of the Bacillariophyta from the experiment field resisted plantanycin upto 0.4% treatment. *Fragillaria brevistrata f. elongata* and *Synedra* affinis could resist upto 0.6% treatment where as other forms were found sensitive above 0.4% plantomycin treatment.The lower % of Plantomycin treatment on soil showed 100% survival value by the Cyanophycean algal forms i.e. up to 0.4%. The 0.6 and 0.8% treatment with Plantomycin showed 74.19% and 32.25% of survival value. All the members of Cyanophyceae showed 100% algicidal value in 1.0% antibiotic treatment.The Chlorophycean members are more sensitive than other class as its treatments gave 61.53%, 46.15%, 30.76%, 15.37% and 15.37% with concentration as 0.2%, 0.4%, 0.6% 0.8% and 1.0% respectively. The 15.37% of survival value in 0.8% and 1.0% concentrated antibiotic treatment were due to the resistances of specific species of Chlorococcum.Lower concentration treatment of Plantomycin showed 100% survival value that is in 0.2% and 0.4% treatments by the Bacillariophycean members. The 0.6% antibiotic treatment showed only 25.0% survival value by Bacillariophyta. The higher concentration treatments showed 100% Algicidal value by all the 8 Bacillariophycean members of the experiment field.

Conclusion:

The paddy field shows more variety as well as abundance in soil algae due the environmental condition during the crop cultivation as compared to other crop field. The Blue green alga dominates the other groups of alga. The different paddy field soil algal taxa show variable resistance to Plantomycin. The blue green algae show more resistance to the antibiotic than the green algae. Higher concentration of antibiotic used in this experiment gave 100% algicidal value in both Cyanophyta and Bacillariophyta, but Chlorophyta could not respond so, due to the two species of the genera, Chlorococcum. *C. humicol*o and *C. vitiosum* which were highly resistant to antibiotic used. It may be due to their sheath or cell wall organization.

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Coccinella sptumpumctata (Coccinellidae: Coleoptera) as a predator of cecidozoan, hymenopteran and dipteran in Garhwal Himalaya, India.

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Abstract

The gall forming insects (cecidozoan), *Allirhytis semicarpifoliae, Rhopalomyia baijali Labopteromyia bivalve and Amaradiplosis amaermyia, Andricus sp.* are widely distributed in Garhwal Himalaya causing galls on different economically important plants. In this regard, out of theses Coccinellids oftenly crawl on gall bearing portions in search of their prey, but it is frequently seen in case of grubs. Maximum numbers of grubs prefer to feed upon the larvae of *R. baijali.* The abundance of predators synchronized with peak population of prey insects. The relative abundance of predators always higher in tropical region than those of other geographical zones. The grubs of Coccinellids find their way by cutting the gallicolous tissues with help of their serrated mandibles so as to secure their prey inside the gall. Experiments on feeding propensity of grubs of *Coccinella saptempunctata* show that this insect devour 15.2 to 22.8 larvae of *R. baijali* per 24 hrs. in laboratory condition *(Table-1).* It was also noticed that the predatory activity was recorded high in the lower altitude in comparision to the higher one.

Keywords: *Galls, cecidozoan, predator, larva*

Introduction

In the Garhwal Himalayan region (situated between north latitude 29° 26' 15" and 31° 5' 31" and between east longitudes 70° 18' 45" and 80° 8' 0") due to high and low mountain ranges and valleys climatic conditions vary from one place to another. This region is very rich for florastic and faunastic population. The population of galls is also very high in comparision to the other parts of the country. In the natural condition variation enemies of gall and gall insects

(i. e. parasites and predators) are found. They not only feed upon the gall producing insects, but also play a significant role in controlling the population of these cancer causing agents of the plnats. The predatory nature of Coccinelids on various groups of insects, (aphids and other soft body insects) has been recognized for a long time (Atwal and Sethi 1963), but studies on feeding upon gall insect is magre and far from complete. Very few published work on predatory related study is available e.g., Barnes 1929, 1930 and 1933). Harris, (1967) recorded cecidomyiid (Diptera) as predators upon some coccids. Yukawa (1983) observed that the worker ant *Monomorium nipponenset* to feed upon gall insect Pseudasphondylia neolitseae (Diptera : Cecidomyiidae) from Kagoshima., Japan. Smith (1939), Atwal and Sethi (1963) and Kaczmerek (1973) done work on predatory nature of Coccinellids upon aphids. But published work on Coccinelidsas the predators of the gall insects exists. Therefore, the present study on the predatory insects was undertaken in the Garhwal Himalayas during 1988-1990.

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Materials and Method

To study feeding nature of grubs and adults of Coccinelids in natural conditons the daily visits were conducted during seasons of abundance of galls and predators. Some galls were also carefully cut, opened and calculated for damage done by the predators.

Feeding experiments were done upon the 2nd instar larvae of R. Baijali, for that ten sets of experiments were kept. In each set of experiment one grub and 100 larvae were kept on tender bud of *A. vulgaris*

inside the glass chimeny or beaker and covered with thin cloth net (for proper ventilation and prevent to escape the predator and prey from the beaker). Some experiments of the same type also conducted inside the insect raring cage. After every 24 hours of intervals remaining larvae inside the beaker were counted. This experiment was continued for 10 days. The time of complete exploitation of larvae is also calculated during different field visits of the sites.

Results and Disscussion

However, the predatory nature of Coccinella is well known Kaczmareh (1973) used nine species of Coccinella as a predator of aphids. In majority of cases (e.g. grubs and adults of *Coccinella septumpunctata)* was observed that maximum abundance of these predatory insects coincides with the most favorable period (higher population) of the gallicolous insects such as *Andricus sp., Amaradiplosis amraemyi.,* We observed as many as seven species of Coccinella crawling on the gall bearing portion of the host plant in many localities of Garhwal Himalaya. Out of the seven species of this predatory insects (immature stage and adults), the most common and widely disturbed species was *Coccinella septumpunctata*. The grubs of this species were feed upon by the larvae of *Rhopalomyia baijali* under laboratory conditions (temp. 9.0°C to 28.2°C) Maximum 39 larvae were ate by *Coccinella septumpunctata* in 24 hours *(Table.1),* Sethi and Atwal (1963) observed in their experiments that the Coccinella can eat on an average 62 aphids of cabbage plant.

Table-1: Feeding propensity experiments on grubs of *Coccinella septempunctata*. On mature 2nd larval stages of *R. baijali* in laboratory conditions (for 24 hours).

Days	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
1.	13	25	14	13	16	35	14	23	14	16
2.	15	18	23	17	24	15	17	19	16	25
3.	23	24	17	15	18	17	34	18	23	16
4.	14	17	15	13	25	26	19	23	35	18
5.	19	18	19	18	26	27	27	15	24	27
6.	15	17	23	died	20	25	18	10	19	19
7.	8	17	11	-	27	38	26	9	27	38
8.	27	10	18	-	28	21	20	22	24	17
9.	12	19	17	-	25	12	29	11	29	18
10.	19	10	39	-	11	12	17	10	8	16
Mean	16.50	17.50	19.60	7.60	22.00	22.80	22.10	16.00	21.50	21.00
S.D.	±2.85	±2.67	±2.11	±1.43	±2.63	±2.64	±2.91	±2.85	±2.56	±2.69

When we correlate the distribution and rate of feeding of predatory Coccinelids on the basis of abiotic factors, we found that the predators were much active and higher in number in the low altitudinal zones (up to 1800 meters), where the temperature was high and humidity was low as compared to the higher altitudes (more than 1800 meter), where the average annual temperature was low and humidity was high. In the present study, out of a total of 11 prey for these coccinelids, 7 were gall producing insects from lower altitudes and 4 species were from the higher altitudes Coccinelids *(Table-2).*Although the grubs and adults of this species were observed frequently on the gall bearing areas of many host plants in the field *(Table-2),* but occasionally the adults were noticed to utilize the gallicolous insects. On the contrary grubs were frequently noticed to prey upon the cecidozan insects. This was more so in case of larvae of R. baijali (on *Artemisis vulgaris* as their food, but frequently of abundance of grubs on these galls radically differs (i.e. a few in *Andricus sp*. and *Callirhytis semicarpifoliae* both on *(Q. incana).* In general it is also noticed that the relative abundance of the predators remain higher in lower altitudes than in higher altitudes mostly during the month from February to May *(Table-2).* The grubs of Coccinella find their way by cutting the gallicolous tissues with the help of their sharp mandibles, so as to secure their prey inside the gall. The anterior half body portion of grubs was inserted the gall cavity by making peristetitic movements. The grubs captures its prey with the help of its serrated mandibles which act like a forecep.

Table-2: Showing the food spectrum of predatory insects with their distribution at various localities in Garhwal.

SN	Predator	Food spectrum (Cecidozoan)	Host plant	Abundance of predator on a gall twigs	Period of maximum occurrence of the predators	Localities of occurence	Altitudinal distribution (m. asl)
1.	*Coccinella septumpuntata* (grubs)	*Andricus sp.* (larvae)	*Quercus incana*	++	May-June	CHM,BTH,CHO, RAN,PAU,ADW, KAN,CBU,KAD	1400-2610
		Callirhytis semicarpifoliae (larvae and adult)	*Quercus incana*	++	May-June	CBU,CHM,BTH, KAD	1650-2610
		Rhopalomyia baijali	*Artemisia vulgaris*	+++	Jan.-Feb.	KTW,MAT,TIP, KAN	350-1800
2.	*Coccinella Septumpunctata* (adult)	*Andricus* (larvae and adults)	*Quercus incana*	++	Feb.-May	CHM,BTH,CBU, KNK	1400-2610
		Labopteromyia bivalvae(larvae)	*Acacia catechu*	++	Feb.-May	GHN,GAD,UPH, SIM	550-1500
		Amaradiplosis amraemyia (larvae)	*Magnifera indica*	+	March-April	SRI,THE,DEB,K TW,SAT	350-1500
		Rhopalomyia baijali (larvae and adults)	*Artemisia vulgaris*	++	Jan.-April	PAU,DHU,KNK, UK,KAN, KTW	350-1800
3.	*Coccinella sp.* (Adult and grub)	*Andricus sp.* (larva)	*Quercus incana*	+++	Feb.April	CHM,BTH,CHO, RAN,PAU,ADW, KAN	1400-2610
		Rhopalomyia baijali	*Artemisia vulgaris*	++	Jan.-April	PAU,DHU,KNK, UK,KAN, KTW	350-1800
		Labopteromyia bivalvae (larvae)	*Acacia catechu*	++	Feb.-April	GHN,GAD,UPH, SIM,TAK	550-1500
		Amaradiplosis Amraemyia (larvae and adult)	*Magnifera indica*	++	Feb.-April	SRI,DEB,KTW,S IR,TEH	350-1500

Fore-legs also assist the larve during feeding. The duration taken by this predatory insects from the time of captivity of an insect (such as *Rhopalomyia baijali)* upto the complete exploitation of its body contents was observed to be 7 to 12 minutes (average 9.1 minutes) under laboratory experiments. As feeding is over the larvae was pushed back and out by making peristelitic movement of its body. The effect of predation by the above mentioned insect is quite embracing. The death of cecidozoan causes early drying of gall.Occasionally adults of *Coccinella septumpunctata* and Coccinella sp. were found predatory on larvae of *Andricus sp*. *Labopteromyia bivalvae, Amaradiplosis amraemyia* and *Rhopalomyia baijali* on their host plants *Quercus incana, Acacia catechu, Mangifera indica* and *Artemisis vulgaris* respectively. These predotory insects are noticed to cut the interlocular tissue of the oak galls so as to expose the larval chambers to secure the prey.The considerable frequency of predation by the predotors on a gall insect (larvae/adults) thus definitely seem to play a prime role in decreasing the biotic potential of gall insect in Garhwal Himalayas.

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A preliminary study on sewage quality improvement through water hyacinth (*Eichhornia crassipes*)

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Abstract

In the present study, the role of water hyacinth *(Eichhornia crassipes*) in reducing nutrient concentration from municipal wastewater treatment plant effluent by phytoremediation methods was evaluated. The paper is the outcome of in-situ experiments conducted on samples collected from Singh Dwar sewage pumping station Hardwar. Results indicates that water hyacinth is capable in improving water quality by reducing nutrient concentration

Keywords: *Eichhornia crassipes, phytoremediation, macrophyte*

Introduction

Increasing urbanization, industrialization and over population are the main factors responsible for increasing pollution. Water bodies are the main receiving end for capturing these pollutants. They receive industrial wastewater, residential wastewater, surface runoff etc (Dhote and Dixit 2009). According to Central Board for Prevention and Control of Water Pollution about 90-94% (by volume) wastewater are domestic sewage where as industries produce only 6-10% wastewater (Sharma, 2007). It means water get more polluted by domestic sewage than industrial effluents.

Various conventional methods are in practice for purification of water and removing these contaminants. Most of the conventional methods in practice are costly and non eco-friendly. Green plants are not only the lungs of nature with an ability to uptake, tolerate and even hyper accumulate heavy metals and other toxic substances from soil and water through their roots and concentrate them in roots, stems and leaves. These include some aquatic weeds, such as Salvinia, Lemna, Azolla, Hydrilla and Eichhornia sedges like *Typha latifolia* and some herbaceous as well as woody plants.

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Phytoremediation is an alternative or complimentary technology that can be used along with or in some cases in place of mechanical conventional cleanup technologies that often require high capital inputs and are labour and energy intensive. Phytoremediation is an in-situ remediation technology that utilizes inherent abilities of living plants. It is also an eco-friendly, solar energy driven cleanup technology, based on the concept of using nature to clean.In respect to the phytoremediation aquatic macrophytes plays an important role in wastewater treatment. Each species contribute its special function and co-operate in the purification process. Some nutrients (pollutants) were absorbed by the macrophytes and were removed from the effluents. The macrophytes used for BOD removal seemed to function as fixed film reactors with the submerged plant structure of the macrophytes which can transport atmospheric oxygen from foliage into the roots.Oxygen not required for root respiration may diffuse into the wastewater and utilized by bacteria for the oxidation of BOD.Floating aquatic macrophytes are also capable of assimilating large quantities of trace elements, some of which are essential for plant growth (Reddy and Suton, 1984).Aquatic macrophytes particularly floating species, such as the water hyacinth and pennywort

are exhibiting very high rate of growth e.g.: 10gm/sqm/day such growth rates are associated with high level of nutrient uptake and demand particularly for nitrogen and phosphorous. It has been estimated that water hyacinth are capable of removing nitrogen from the water by direct uptake 5850kgN/ha/year and of storing within their biomass between 300 kg N/ha and 900 kg N/ha. Significant quantities of phosphorus can also be removed 350-1125 kg P/ha/year and accumulation (20-57 kg P/ha).

Materials and Method

Collection of samples: Grab samples were collected from the Singh Dwar sewage pumping station.

Collection of hydrophytes: Young and healthy macrophyte were collected from pond situated at Bhadarabad.

Preparation of aquarium: Two concentration were taken for study i.e. (50%
and 100%).

Set-1- 50% wastewater + 50% tap water

Set-2- 100% wastewater

The experiment was performed in the Department of Environmental Sciences, KGM, Hardwar (U.K.). All the parameters were analysed every week during the study schedule.

Results and Discussion

Phytoremediation refers to the natural ability of certain plants hyper accumulators to bio-accumulate, degrade or render harmless contaminants in water, air or soil. Contaminants such as metals, pesticides, solvents, crude oil and its derivatives have been mitigated in phytoremediation projects worldwide. It is considered a clean, cost-effective and eco-friendly technology, as mechanical cleanup methods, such as pumping polluted ground water or soil excavation. A new technology of purification of sewage by water hyacinth (*Eichhornia crassipes*) is a possible solution (Alade and Ojoawa, 2009). In the present study the parameter considered for the study were turbidity, pH, TDS, hardness, DO, BOD, COD, total kjeldhal nitrogen, chlorophyll-a and chlorophyll-b. From table. 1 and 2 it is evident that turbidity was decreased from 35 NTU to 9.6 NTU in 50% concentration and 21.6 NTU to 15.9 NTU in 100% concentration by using water hyacinth.

The reduction in turbidity is due to the reduction in total dissolved solids (Dhote and Dixit, 2007). Reduction in turbidity is due to the roots hairs which have electrical charges that attract opposite charges of colloidal particales such as suspended solids and cause them to them to adhere on the roots where they are slowly digested and assimilated by the plants and micro-organism.Total Dissolved Solids were decreased from 1200 mg/l to 665 mg/l in 50% concentration and 1800 mg/l to 790 mg/l in 100% concentration (table 1 and 2).

Besides enabling growth of microbial colonies root system is also good medium for filteration and adsorption of suspended materials, nutrients and heavy metals.

Table1: Effect of *Eichhornia crassipes* on some parameters in 50% concentration

S.N	Parameters	0 day	7th day	14th day	21st day	28th day	35th day	42nd day
1	Turbidity (NTU)	35±0.23	20±0.46	18±0.87	15±0.37	13±0.84	10.5±0.45	9.6±0.82
2	TDS (mg/l)	1200±1.27	1050±1.48	970±1.38	810±1.64	776±0.92	6.86±1.19	6.65±0.83
3	pH	6.82±0.48	7.9±0.57	7.8±0.83	7.65±0.93	7.56±0.18	7.46±0.28	7.3±0.38
4	Hardness (mg/l)	330±0.98	300±0.27	275±0.86	235±0.72	198±0.38	159±0.72	148±0.94
5	DO (mg/l)	3.4±1.72	3.9±1.28	4.3±1.19	4.8±1.38	5.1±1.47	5.3±1.76	5.5±1.84
6	BOD (mg/l)	43±1.83	54.8±0.98	48.3±0.87	41.5±1.68	37.5±0.92	32.2±1.15	28±0.93
7	COD (mg/l)	170±0.00	123±0.00	110±0.00	98±0.00	85±0.00	71±0.00	52±0.00
8	TKN (mg/l)	3.5±0.00	3.0±0.00	2.8±0.00	2.5±0.00	2.2±0.00	1.8±0.00	1.5±0.00
9	Chl.a	9.91±0.36	9.71±0.83	9.45±0.75	8.20±0.47	6.39±0.41	6.15±0.18	4.32±0.14
10	Chl.-b	4.11±0.47	4.09±0.16	3.58±0.71	31.6±0.58	2.65±0.19	2.05±0.38	1.85±0.17

Table2: Effect of *Eichhornia crassipes* on some parameters in 100% concentration

S.N.	Parameters	0 day	7th day	14th day	21st day	28th day	35th day	42nd day
1	Turbidity (NTU)	21.6±0.16	19.5±0.39	18.3±0.28	17.2±0.16	16.8±0.42	16.2±0.38	15.9±0.47
2	TDS (mg/l)	1800±0.23	1600±0.25	1100±0.48	1050±0.25	950±0.31	870±0.29	790±0.25
3	pH	6.54±0.58	8.12±0.36	7.96±0.43	7.78±1.28	7.65±1.24	7.45±0.97	7.22±0.93
4	Hardness (mg/l)	386±0.72	342±0.46	270±0.82	220±0.42	190±0.73	175±0.25	168±0.29
5	DO (mg/l)	1.21±1.82	2.02±0.95	2.03±0.97	3.64±0.15	4.12±0.82	4.45±0.47	5.2±0.71
6	BOD (mg/l)	75±1.43	72.26±1.83	65±1.28	59.28±1.49	54.8±1.48	41.2±0.98	35.2±1.11
7	COD (mg/l)	285±0.0	198±0.00	158±0.00	13.3±0.00	128±0.00	113±0.00	85±0.00
8	TKN (mg/l)	5.6±0.00	5.0±0.00	4.7±0.00	4.3±0.00	3.9±0.00	3.3±0.00	2.8±0.00
9	Chl.-a	9.91±0.32	9.65±0.58	9.22±0.63	8.06±0.29	6.69±0.73	5.18±0.43	3.31±0.14
10	Chl.-b	4.21±0.54	4.05±0.81	3.27±0.49	2.96±0.73	2.11±0.69	1.72±0.81	1.13±0.87

pH was decreased from 7.9 to 7.3 in 50% concentration and 8.12 to 7.22 in 100% concentration. Dhote *et al*. (2009) also reported same decreasing trend in pH in lake water by using water hyacinth (*Eichhornia crassipes)* and hydrilla (*Hydrilla verticillata*).Hardness decreased from 330 mg/l to 148 mg/l in 50% concentration and 386 mg/l to 168 mg/l in 100% concentration (table 1 and 2) by using water hyacinth. Dar *et al*. (2011) also reported same decrease in trend of hardness. They reported 54% reduction in 50% concentration and 45% reduction in 100% concentration in sewage treatment potential of water hyacinth.

The level of DO in any water shows the condition of pollution level. In the present study, water hyacinth increased the DO level in both the sewage concentration of sewage. DO was increased from 3.14 mg/l to 5.5 mg/l in 50% concentration and 1.21 mg/l to 5.2 mg/l in 100% concentration (table 1 and 2) by using water hyacinth.BOD is the worst problem of sewage. BOD was decreased from 43 mg/l to 28 mg/l in 50% concentration and 75 mg/l to 35.2 mg/l in 100% concentration (table 1 and 2) by using water hyacinth. Dhote *et al.* (2007) reported the reduction of 75% in BOD in lake water. COD also reduced from 170 mg/l to 52 mg/l in 50% concentration and 285 mg/l to 80 mg/l in 100% concentration.Total nitrogen was decreased from 3.5 mg/l to 1.5 mg/l in 50% concentration and 5.6 mg/l to 2.8 mg/l in 100% concentration (table 1 and 2). Water hyacinth is capable in assimilating both ammonium and nitrate. The impact of sewage on the plant is observed by plant chlorophyll estimation. At a very first day the chlorophyll-a was 9.9156 mg/dry wt. and chlorophyll-b was 4.1188 mg/dry wt. in 50% concentration, while in 100% concentration chlorophyll-a was 9.915 mg/dry wt. and chlorophyll-b 4.2136 mg/dry wt. But after 7 weeks chlorophyll-a was 4.3265 mg/dry wt. and chlorophyll-b was 1.8589 mg/dry wt. in 50% concentration where as in 100% concentration chlorophyll-a was 3.31 mg/dry wt. and chlorophyll-b was 1.1372 mg/dry wt. so the result shows that the sewage affects the plant severely.

Conclusion

Water has been polluted and is suffering from ongoing chronic pollution. This has become one of the most pervasive environmental problems throughout the world. High cost technologies though effective cannot be employed by most developing countries including India. So, we have to find alternative cost effective methods; one such system could be phytoremediation. Phytoremediation is a potential remediation strategy that can be used to treat water contaminated with pollutants.

In the present study, various physical, chemical and biological parameters were studied and it was

observed, that water hyacinth is a potential tool for treating municipal wastewater. The plant performed well in 100% wastewater. It may be used for at least primary and secondary treatment. This plant might be utilized as an efficient, economical and ecological alternative to accelerate the removal and degradation of agro-industrial wastewater pollutants.

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A reliable rapid protocol for characterization of *in vitro* totipotency in *Spilanthes oleracea*

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Abstract

***Spilanthes oleracea* is an important medicinal herb and is also called 'Toothache Plant' or 'Eye Ball Plant'. It is used to prepare herbal formulation to cure many diseases. Its property to heal the dental wounds can be exploited as an alternative to synthetic medicines used presently. An efficient protocol for *in vitro* shoot multiplication of *Spilanthes oleracea* has been developed from axillary bud explants. Nodal segments, from young plants, were taken as explants; shoot multiplication was induced on slightly modified Murashige and Skoog's (MS) medium supplemented with 6- Benzyl amino purine, (BAP, 0.5 ppm) and Naphthalene Acetic Acid, (NAA, 0.1 ppm) and BAP (0.5 ppm) + Indole 3-Acetic Acid, (IAA, 0.3 ppm). Shoot proliferation could be induced using different combinations of BAP, IAA and NAA. Shoots were further multiplied through continued subculture of nodal segments with sprouted shoots. Micro-shoots were rooted in the basal medium supplemented with NAA (1.71 µM) alone and BAP (0.44 µM) + NAA (1.0 µM) concentration.) Survival of *in vitro* grown plantlets 2 months after transplantation in the pots, containing equal parts of sand and top soil, was found to be 97 per cent.**

Keywords: *Axillary buds, Conservation, Micropropagation, Multiple shoots, Plant growth substances Spilanthes oleracea*

Introduction

Spilanthes oleracea Linn (Asteraceae) is a herbaceous, tropical/tender perennial with a growing height of 12-18 in. (30-45 cm); its bloom color is red, bright yellow and blooming time is mid summer/late summer, aromatic and blooms repeatedly (Akah and Ekekwe, 1995). *Spilanthes oleracea* is very beautiful, and can be grown as an annual in most climates. It has striking cone-like flowers, much smaller than *Echinacea*. There are no flower petals, but golden buds with a rust-red center, which look like an eyeball (Raju and Raju, 1996).

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This plant is called Toothache plant because we can chew on the fresh or dried flower, or take the extract to help deaden pain from a tooth until we can visit the dentist. It is not only topically anesthetic for gums and teeth, but it is also bacteriostatic, helping to fight tooth decay. Plant parts used to cure ailments like throat pain, constipation and other diseases (Holetz, *et al*., 2002; Kala, 2005). Leaves are pretty potent and cause tingling or numbing of the gums when chewed, which depends on the age of plant. It is supposed that leaves are strongest near the time of flowering.

Tissue culture is now being commonly used for clonal propagation of a large number of horticulture plants, medicinal plants and also for forest trees (Murashige, 1974). Rare and endangered plants as well as medicinally important plants are being conserved and exploited by using *in vitro* techniques all over the world (Ang and Chan, 2003; Baskaran and Jayabalan, 2005). Here, authors have tried to standardize the protocol for optimum

production of this medicinal herb in *in vitro* conditions.

Materials and Methods:

Young plants of *Spilanthes oleracea* were collected from villages of Azamgarh Dstrict, Uttar Pradesh, India and were grown in the green house of Society of Pollution and Environment Conservation Scientists (SPECS). Nodal explants with axillary buds were taken from young & healthy plants for culture initiation (Goel *et al*., 2009).

Explants were first washed in running tap water. Afterwards the explants were placed in a beaker containing water; 3-4 drops of Tween-20 were added and the beaker was shaken lightly for 3-4 minutes, followed by thorough washing with tap water (x 4) and double distilled water (x 4). Surface disinfection was carried out with 0.1% $HgCl_2$ (w/v) for 4 minutes followed by several washings with sterilized distilled water (Gamborg and Phillips, 1995, Goswami *et al*., 1999, Singh *et al*., 2010). The explants were allowed to dry in laminar hood for 20 minutes to remove surface water, and the nodal segments were then inoculated under aseptic conditions on agar solidified Murashige and Skoog's (MS) medium (Murashige and Skoog, 1962; Murashige, 1974) with slight modifications (Manganese sulphate dihydrate was used in place of Manganese sulphate tetrahydrate) and concentration of $Na_2EDTA.2H_2O$ was 30.5mg/l in place of 37.25 mg/l.). The medium was supplemented with usual salts and vitamins and 3.0% sucrose (w/v; Hi- Media), 100mg/litre myo-inositol (E. Merck) and 0.8% agar (w/v; Difco-Bacto, Becton Dickinson U.S.A.).

Media were supplemented with various concentrations of BAP (6-benzylamino purine) alone and in combinations with NAA (α-naphthalene acetic acid) and IAA (Indole-3- Acetic acid). The pH of the media was adjusted to 5.8 before the addition of agar and autoclaved at 121^0C with 1.5 kg lb/cm^2 for 20 minutes. The cultures were kept at 25$\pm$ 2^0C under illumination with white fluorescent tubes (50 μM $m^{-2}s^{-1}$) at 78% relative humidity. They were maintained under light for 14 hours followed by 10 hours dark period. Each treatment had 5 replicates and the experiments were repeated 3 times. Sprouting of axillary buds was seen on nodal segments after 15-25 days of culture (Figure 3 A). These buds, with part of the growing nodal segments, were subcultured on modified medium supplemented with BAP (1.30-4.40 μM) + IAA (1.40-2.30 μM) or NAA (0.44-1.33μM) for further shoot multiplication. Nodal explants (0.7-0.9 cm) from the axenic shoots were recultured on agar solidified medium containing different concentrations of BAP, NAA and IAA. Gibberellic Acid, (GA_3, 0.15-3.10 μM) for elongation of shoots (Fig 4 & 5); the shoots attained height of 2.3$\pm$0.10 cm. Roots were then induced in these shoots measuring 2-3 cm long by transferring to MS medium supplemented with different combinations of IAA, and NAA. The roots were initiated in rooting medium as well as in basal medium. The roots produced in basal medium were thin and short.

Eight weeks old plantlets were transferred to pots containing sterilized soil and sand (1:1), covered with polythene bags with perforations, for 10 days and the pots were kept below 25$\pm$2^{o}C, for acclimatization. These were then transferred to green house, after removing polythene covers, for hardening (Saritha *et al*., 2003).

Results and Discussion

Best induction of multiple shoot formation from nodal explants occurred on medium containing BAP (4.84 μM) and IAA (2.60 μM) - Figure 3 A. After that the cultures were transferred to the medium that favored multiple shoot regeneration. Amongst different combinations of plant growth substances used, maximum shoot regeneration per explant was found to take place with BAP (4.00 μM) and NAA (2.80 μM) - (Figure 1 & Figure 3 B) with about 25 shoots in 40 days. Use of BAP (2.22 μM) with IAA (1.50 μM) also gave multiple shoots (15 shoots per explant after 40 days). In contrast, the number of shoots formed in control cultures was only 2$\pm$ 0.5; shoot induction was late as well as shoots formed were less viable (survival only 10%). Further it was found that the slight increase in sucrose concentration was able to increase the shot multiplication rate (Figure 1) while higher concentrations of BAP inhibited the shoot multiplication rate as well as induction of shoots (Figure 2). Root initiation was tried with combinations of BAP, IAA and NAA, but best root growth was promoted by BAP (0.40 μM) used with IAA (1.75 μM; Figure 3 C) and with IAA alone (1.75 μM). Plants transferred after acclimatization in green house (Figure 3 D), showed 93% survival capacity.

Table 1. Effect of Plant Growth Substances on shoot multiplication from cultured nodal explants in *Spilanthes oleracea* (Values are means ± SE of five replicates per treatment)

S.No.	Plant Growth Substances (conc. in µM)	25 Days		45 Days	
		No. of shoots	Average length of shoots (mm)	No. of shoots	Average length of shoots (mm)
1.	Control	0	-	0	-
2.	BAP (2.20)	4	1.5 ±0.3	7	16 ±0.9
3.	BAP(2.20)+NAA (2.20)	9	1.7 ±0.4	15	12 ±0.8
4.	BAP (2.20)+ IAA (1.80)	10	3.0 ±0.7	18	20 ±1.3
5.	BAP (3.40)+ NAA (2.80)	11	3.5 ±0.9	20	25±1.8
6.	BAP (3.30)+ NAA (2.40) + GA_3 (0.28)	12	4.9 ±1.2	22	35 ±1.4
7.	BAP (4.50)+ NAA (2.80)	5	1.8 ±0.2	9	22 ±0.9

*Only those combinations are shown that produced optimum results.

Table 2. Effect of Plant Growth Substances on rooting of *in vitro* raised microshoots (Values are means ± SE of five replicates per treatment)

S.No.	Plant Growth Substances (conc. in µM)	25 Days		45 Days	
		No. of roots	Average length of roots (mm)	No. of roots	Average length of roots (mm)
1.	Control	0	-	0	-
2.	IAA 1.70	3	1.7 +0.8	5	6 +0.8
3.	NAA 1.70	5	1.8+0.4	5	13 +1.3
4.	BAP (0.40)+ NAA(1.70)	9	3.9+0.8	16	20+2.4
5.	BAP (0.80)+ NAA(1.70)	4	2.5+0.3	9	9 +0.6
6.	BAP (0.40)+ IAA(0.80)	7	1.8+0.2	11	10 +0.9

*Only those combinations are shown that produced optimum results.

Table 3: Survival of plantlets under *ex vitro* conditions (Values are means ± SE of five replicates repeated thrice)

Group No.	No. of plantlets produced & transferred to pots	No. of surviving plants after 60 days	Survival percent (%)
1	45	42	93.33
2	37	33	89.12
3	42	41	97.62
4	53	49	92.45
5	35	33	94.29
		Average survival % 93.362	

It was observed in the present investigations that multiple plant regeneration from nodal explants of *Spilanthes oleracea* could be induced on slightly modified MS medium. Plant multiplication rate was dependent on appropriate combinations of plant growth substances (PGSs). Higher concentrations of PGSs, especially BAP was found to inhibit shoot multiplication. The current work provides preliminary information and methodology for rapid propagation of this valuable plant from nodal explants that might help in the improvement of conservation methods.

Conclusion

Spilanthes oleracea is widely used in many traditional medicines prescribed under different systems of medicine. *Spilanthes* species have long been used as traditional medicine for local anesthetic, antibacterial (Sabitha Rani and Suryanarayana Murty, 2005), antiviral, antihypertensive, larvicidal (Pandey *et al*., 2007) and diuretic actions. The whole plant leaves and roots are used for a variety of purposes in many herbal medicines (Ley *et al*., 2006). For example, the leaves are used to cure throat infections (Chandra Prakash Kala, 2005) and for the treatment

of ulcers (Chauhan *et al.*, 2003). It is shown that the plant is being used traditionally in treatment of several respiratory diseases. It is, therefore, important to maintain a balance between its use and conservation status. Many researchers have paid attention in this direction.

Propagation and conservation of some pharmaceutically important *Spilanthes species* was attempted using tissue culture technique (Ang and Chan, 2003). Suspension cultures widely used for the *in vitro* production of secondary metabolites using large and small scale fermenters, proved the importance of tissue culture technology (Curtin, 1983). In the present study, direct shoot multiplication was preferred for generating true-to-type plants than callus regeneration. This study supported the rapid multiplication of this useful medicinal plant by *in vitro* conditions.

In vitro mass propagation of *Spilanthes oleracea* reported here may provide some help in this direction. The protocol developed is easy and reproducible through which its mass multiplication can be attempted at commercial level.

It is less time taking and the survival rate of *in vitro* grown plants was also found to be more than 97%, a considerable improvement over earlier studies (Karthikeyan, *et al*., 2007; Babeet *et al*., 2010).

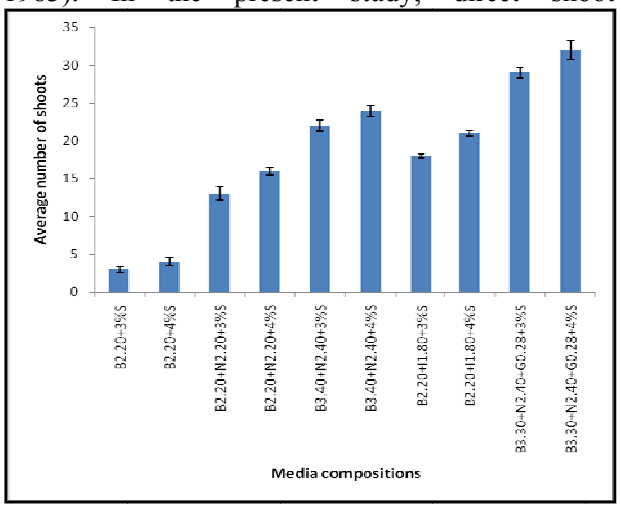

Figure 1. Frequency of multiple shoot regeneration with varying concentrations of sucrose.
B= BAP, N= NAA, I= IAA, G= GA_3 and S = Sucrose; Error bar indicates standard deviations.

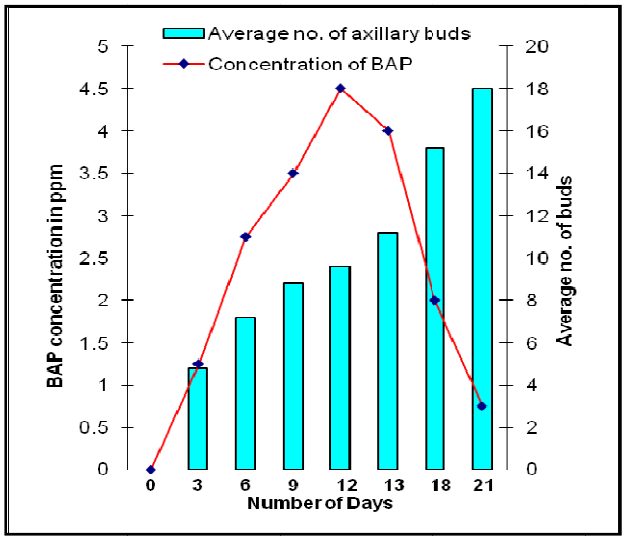

Figure 2. Effect of increasing BAP concentration on axillary bud proliferation in *Spilanthes oleracea*

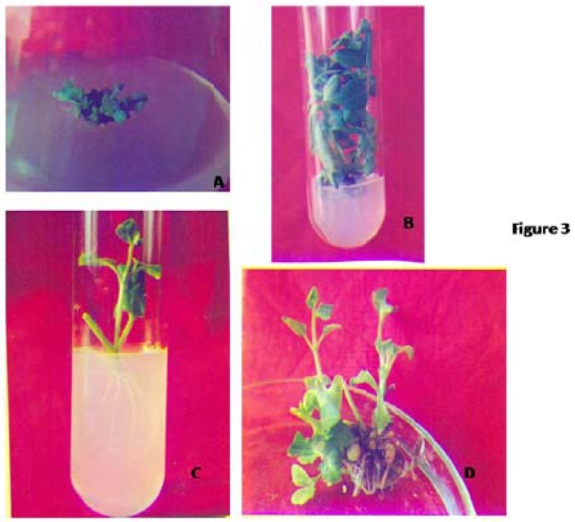

Figure 3: *In vitro* propagation of *Spilanthes oleracea*.
(A) Origin of multiple shoots from nodes, after 3 weeks on MS medium supplemented with BAP (4.84 µM) and IAA (2.60 µM).
(B) Mass multiplication of shoots after 4 weeks on the MS medium supplemented with BAP (4.00 µM) and NAA (2.80 µM).
(C) Development of healthy and viable roots on medium supplemented with BAP (0.40 µM) used with IAA (1.75 µM after 18 days.
(D) A 6 week old plant ready to be planted in pots; the survival of such plantlets, 4 weeks after plantation, was found to be more than 93%.

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Phytosociological characters of forest vegetation in Tarai of KumaunHimalaya, Uttarakhand

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Abstract

The present work aimed to study the phytosociological characters of forest vegetation in mixed deciduous forest of Tarai of Kumaun Himalaya near Kashipur. Phytosociological characters of vegetation were calculated for all forest layers i.e. trees, shrubs and herbs. In this review, we are discussing different phytosociological characters and compare it with various forest types of Himalaya.

Keywords:*Forest vegetation, herb, Kumaunhimalaya, phytosociological characters, sapling, seedling, shrub, tarai, tree.*

Introduction

Vegetation of an area varies from place to place according to habitat heterogeneity of the area itself. It is also a key factor, which determines the structure of an ecosystem and ecological parameters within a plant community such as microclimate, energy budget, photosynthesis, water regimes, surface runoff and soil temperature (Tappeiner and Cernusca, 1996). The description and classification of the plant community in an ecosystem is known as phytosociology (Braun-Blanquet, 1932; Odum, 1971). Himalaya, the youngest mountain system of the world, constitutes an important link between the vegetation of the southern peninsular India on the one hand, the eastern Malaysian, the northeastern Sino-Japanese and the northern Tibetan areas on the other (Puri*et al.*, 1983). The various changes in the Himalayan forests are appearing in their structure, density and composition due to global warming (Gaur, 1982), uncontrolled lopping and utilization of trees for fuel wood, fodder and grazing (Kumar *et al.*, 2004).

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There is little information available for vegetational analysis in Tarai and Bhawar area of Kumaun by Pant (1976), Jain and Sastry (1983) and Pant *et al.*, (1981). Therefore, the aim of this paper is to incorporate the seasonal variation in phytosociological characters of the submontane forest vegetation at Tarai of Kumaun Himalaya.

Geographical Location and Climate

For the present study, the forest of Tarai area near Kashipur of Kumaun Himalaya was selected having 558.38 hectare forest area (Source: Office of Tarai West Forest Division, Kumaun, Ramnagar). This study was conducted from April 2007 to March 2008. The study site was situated in the foothills of Shivalik Mountain of the Outer Himalaya and southeast to Corbett National Park at an elevation of 253.4 m above msl, within the district of Udham Singh Nagar. The climate is monsoonic with 1414.70±175.46 mm year^{-1}annual rainfall. The average monthly maximum temperature ranged from 16.7±2.26°C to 38.0±0.70°C and minimum temperature was in the range of 8.2±1.20 to 23.4±0.98°C.

Material and Methods

Phytosociological Analysis of Vegetation

Herbs

The phytosociological analysis of forest floor vegetation was conducted by using 200 quadrats of 1x1meter with in seasonal intervals. The quadrats were laid randomly, covering all area and directions. The quadrats size was determined by the species area curve following Misra (1968).

Shrubs

The phytosociological analysis of shrubs in study sites were conducted by using 100 quadrats of 10x10 meter with in seasonal intervals. The quadrats were laid randomly, covering all area and directions.

Trees

The phytosociological analysis of trees in study sites were conducted seasonally by using 25 quadrats of 10x10meter. The size was determined following Saxena and Singh (1982). The quadrats were laid randomly, covering all area and directions.The data so obtained was calculated on seasonal basis i.e. summer (April and May), Rainy (June, July, August and September), winter (October, November, December and January) and spring (February and March). The phytosociological characteristics were quantitatively analyzed following methods described by Curtis and Mc. Intosch (1950), Curtis (1959), Phillips (1959) and Misra (1968). A/F ratio was calculated by Whitford (1949) method. Species diversity (H) and Concentration of Dominance (Cd) for all the tree layers at each site was calculated by using Shannon–Wiener Information Index (Shannon and Wiener, 1963) and Simpson's index (Simpson, 1949) respectively.

Results and discussion

In present study density for trees, saplings, seedling, shrubs and herbs were reported in a range of 664-808 ind ha^{-1}, 36-336 ind ha^{-1}, 344-596 ind ha^{-1}, 1096-1776 ind ha^{-1} and 345-481 ind m^{-2}. These results were similar as reported by Devi and Yadava (2006) in a tropical semi evergreen forest of Manipur as density for trees, saplings, seedling, shrubs and herbs amounting as 685-820 ind ha^{-1}, 95-795 ind ha^{-1}, 15500-17504 ind ha^{-1}, 2340-3060 ind ha^{-1} and 27.3-42.65 ind m^{-2}. In addition, they reported Shannon-Weiner Index for trees, saplings, seedling, shrubs and herbs as 0.1094-1.1782, 0.6285-0.7595, 1.3180-1.3323, 1.6432-2.4544 and 2.4985-2.2944 respectively. Simpson Index for trees, saplings, seedling, shrubs and herbs as 0.5554-0.9712, 0.7106-0.7340, 0.4486-0.4581, 0.2574-0.3467 and 0.2259-0.2304 respectively. Total tree density for temperate forests of Kumaun Himalaya was ranged from 420-1640 trees/ha (Saxena and Singh, 1982).Gairola*et al.,* (2008) reported the tree density 243-843 ind ha^{-1}, sapling density 2200-8333 ind ha^{-1}, seedling density 1867-10135 ind ha^{-1}, shrub density 813-4357 ind ha^{-1} and herb density 5.51-21.35 ind m^{-2} in Garhwal and Kumaun region of West Himalaya.They also reported basal area of trees as 8.94-69.84 m^2 ha^{-1}. Kumar *et al.,* (2004) estimated the density of trees in sub tropical forest of Garhwal Himalaya amounting 656 to 888 ind ha^{-1}. Kumar and Bhatt (2006) observed Shannon-Weiner Index (H) for forest vegetation amounting 4.580-4.643 for trees, 4.695-5.021 for shrubs and 4.962-4.986 forherbs

Table 1.0: Phytosociological characters of herbs

S. No	Season	No. of Plant Species	D	A/F Ratio	TBA	IVI	H	Cd
1	Summer	47	345±13.00	0.01-4.08	26.28±0.81	0.46-57.96	1.333±0.03	0.0866±0.009
2	Rainy	70	430±14.30	0.01-3.60	101.53±5.40	0.33-142.51	1.361±0.025	0.0906±0.008
3	Winter	58	403±12.47	0.01-3.34	55.39±2.17	0.35-52.42	1.446±0.025	0.0718±0.005
4	Spring	54	481±12.54	0.02-3.64	38.01±1.05	0.46-34.40	1.368±0.027	0.0545±0.003

Abbreviation: D (Density [plant m^{-2}]), TBA (Total basal area [cm^2 m^{-2}]), IVI (Important Value Index), H (Shannon-Weiner Index), Cd (Simpson Index)

Table 2.0: Phytosociological characters of shrubs in summer, rainy, winter and spring seasons

S. No.	Season	No. of Plant Species	D	A/F Ratio	H	Cd
1	Summer	09	12.92±1.75	0.21-8.00	0.676±0.06	0.2588±0.046
2	Rainy	14	10.96±1.21	0.22-12.00	0.771±0.054	0.2287±0.039
3	Winter	13	17.76±1.60	0.18-8.00	0.843±0.055	0.1743±0.022
4	Spring	11	15.48±1.47	0.40-4.00	0.821±0.054	0.1817±0.025

Abbreviation: D (Density [plant 100m^{-2}]), A/F (Abundance / Frequency), H (Shannon-Weiner Index), Cd (Simpson Index)

Table 3.0: Phytosociological characters of trees

S. No.	Season	No. of Plant Species	D	A/F Ratio	TBA	IVI	H	Cd
1	Summer	17	7.64±0.81	0.06-1.25	5414.74±538.27	2.38-103.14	0.814±0.044	0.2439±0.045
2	Rainy	18	7.12±0.59	1.00-7.14	5354.04	2.94-86.14	0.95±0.04	0.1757±0.025
3	Winter	17	8.08	0.06-0.50	5761.83±641.96	2.40-107.20	0.774±0.045	0.2650±0.047
4	Spring	19	6.64±0.63	0.05-0.50	5289.43±466.79	2.39-99.29	0.892±0.040	0.2200±0.039

Abbreviation: D (Density [plant 100m^{-2}]), A/F (Abundance / Frequency), TBA (Total basal area [cm^2 100m^{-2}]), IVI (Important value index), H (Shannon-Weiner Index), Cd (Simpson Index)

Table 4.0: Phytosociological characters of sapling

S. No	Season	No. of Plant Species	D	A/F Ratio	TBA	IVI	H	Cd
1	Summer	04	0.36±0.035	0.04-0.12	16.92±1.82	42.90-2.36	0.569±0.023	0.2838±0.045
2	Rainy	08	3.36±0.86	0.07-0.50	69.84±14.38	8.32-72.78	0.412±0.044	0.6168±0.203
3	Winter	07	2.16±0.44	0.17-0.75	51.10±8.15	10.86-122.74	0.496±0.053	0.4122±0.112
4	Spring	06	1.12±0.16	0.11-0.75	38.63±5.26	14.12-87.44	0.632±0.044	0.2805±0.067

Abbreviation: D (Density [plant 100m^{-2}]), A/F (Abundance / Frequency), TBA (Total basal area [cm^2 100m^{-2}]), IVI (Important value index), H (Shannon-Weiner Index), Cd (*Simpson Index*)

Table 5.0: Phytosociological characters of seedling

S. No.	Season	No. of Plant Species	D	A/F Ratio	H	Cd
1	Summer	5	3.44±0.69	0.12-0.81	0.485±0.059	0.3818±0.109
2	Rainy	10	5.96±0.85	0.08-1.25	0.661±0.054	0.2942±0.063
3	Winter	9	4.96±0.86	0.07-2.00	0.571±0.054	0.3698±0.080
4	Spring	7	3.72±0.95	0.11-1.91	0.408±0.034	0.5698±0.199

Abbreviation: D (Density [plant 100m^{-2}]), A/F (Abundance / Frequency), H (Shannon-Weiner Index), Cd (Simpson Index)

Table 6.0: List of Plants reported during phytosociological analysis of forest vegetation

S. No.	Name of Plant Species	Habitat	S. No.	Name of Plant Species	Habitat
1	*Acacia catechu* Willd.	Herb	**74**	*Helminthostachyszeylanica*L.	Fern
2	*Achyranthesaspera*L.	Herb	**75**	*Hemigraphisrupestris*(Heyne ex T. Andr.)	Herb
3	*Adenostemmalavenia*L.	Herb	**76**	*Holarrhenaantidysenterica*Wall.	Tree
4	*Adiantumcapillus-veneris*L.	Fern	**77**	*Hydrocotyljavanica*Thunb.	Herb
5	*Adiantumincisum*Forssk.	Fern	**78**	*Ipomoea eriocarpa*R.Br.	Herb
6	*Aervascandens*Wall.	Herb	**79**	*Justiciaprocumbens*L.	Herb
7	*Ageratum conyzoides*L.	Herb	**80**	*Lactuca sativa* L.	Herb
8	*Ajugabracteosa*Wall. exBenth.	Herb	**81**	*Lanneacoromandelica*Hontt.	Tree
9	*Albizziaprocera*Benth.	Tree	**82**	*Lantana camara*L.	Shrub
10	*Alternantherasessilis*R.Br.	Herb	**83**	*Lepidagathispurpuricaulis*Nees	Herb
11	*Amaranthusviridis*L.	Herb	**84**	*Leucascephalotes*(Roth) Spreng.	Herb
12	*Anagallisarvensis*L.	Herb	**85**	*Limnophilarugosa*Roth.	Herb
13	*Antirrhinum orontium*L.	Herb	**86**	*Linderniaanagallis*(Burm.f.)	Herb
15	*Arthraxonlancifolius* (Trin.) Hochst	Herb	**87**	*Ludwigiaoctovalvis* (Jacq.) Raven.	Herb
16	*Bidensbiternatea*Merr. &Sherf	Herb	**88**	*Ludwigiaprostrata*Roxb.	Herb
17	*Biophytumsensitivum*Zucc.	Herb	**89**	*Lygodiumflexuosum* (L.) Sw.	Fern
18	*Blumeamollis*D.Don	Herb	**90**	*Mallotusphilippenensis*Muell. Arg.	Tree
19	*Blumeaoxyodenta* DC.	Herb	**91**	*Malvastrumcoromandelianum*Garcke.	Herb
20	*Boehmariascabra*Gaud.	Herb	**92**	*Mazusjaponicus*Thunb.	Herb
21	*Boerhaaviadiffusa*L.	Herb	**93**	*Meliaazedarach*L.	Tree
22	*Bombaxceiba*L.	Herb	**94**	*Melochiacorcorifolia*L.	Herb
23	*Bothriospermumtenellum*Hornem.	Herb	**95**	*Murrayakoenigii*Spreng.	Shrub
24	*Buteamonosperma*(Lamk).Thub.	Tree	**96**	*Nicotianaplumbaginifolia*Viv.	Herb
25	*Callicarpamacrophylla*Vahl	Shrub	**97**	*Ophioglossumreticulatum*L.	Fern
26	*Calotropisprocera*R.Br.	Shrub	**98**	*Oxalis acetosella*L.	Herb
27	*Cedrelatoona*Roxb.	Tree	**99**	*Oxalis corniculata*L.	Herb
28	*Centellaasiatica*(L.) Urb.	Herb	**100**	*Oxalis dehradunensis*Raizada	Herb
29	*Ceratopteristhalictroides*Brong.	Fern	**101**	*Partheniumhysterophorus* L.	Herb
30	*Chlorisdolichostachya* Lag.	Herb	**102**	*Paspalidiumflavidum* Retz.	Herb
31	*Cissampelospareira*L.	Climber	**103**	*Peristrophebicalyculata*Retz.	Herb
32	*Clerodendrumviscosum*Vert.	Shrub	**104**	*Peucedanumdhana*Ham.	Herb
33	*Cocciniacordifolia*Cogn.	Climber	**105**	*Phalaris minor* Retz.	Herb
14	*Argemonemexicana*L.	Herb	**106**	*Phyla nodiflora*(L.) Greene	Herb
34	*Colebrookiaoppositifolia*Smith	Shrub	**107**	*Phyllanthusdebilis*	Herb
35	*Commelinapadulosa*Blume	Herb	**108**	*Phyllanthusfraternus* Webster.	Herb
36	*Conyzastricta*Willd.	Herb	**109**	*Phyllanthusniruri*L.	Herb
37	*Corchorusaestuans*L.	Herb	**110**	*Physalis minima* L.	Herb
38	*Cordiamyxa*L.	Tree	**111**	*Plectranthusjaponicus*Burm.f.	Herb
39	*Crotalaria medicaginea*Lamk.	Herb	**112**	*Pogostemonebenghalense*(Burm.f.) Kuntz	Shrub
40	*Croton sparsiflorum*Morong.	Herb	**113**	*Polygonumbarbatum*L.	Herb
41	*Cynodondactylon*(D) Pers.	Herb	**114**	*Polygonumhydropiper*L.	Herb
42	*Cynoglossumlanceolatum*Forssk.	Herb	**115**	*Polygonumplebejum*R.Br.	Herb
43	*Cyperusbrevifolius* (Rottf.) Hassk.	Herb	**116**	*Pouzolziaindica*Gaud.	Herb
44	*Cyperusiria*L.	Herb	**117**	*Pterisvittata* L.	Fern
45	*Cyperuspaniceus* (Rottb.) Boeck.	Herb	**118**	*Ranunculus scleratus*L.	Herb
46	*Cyperuspumilus* L.	Herb	**119**	*Rumexdentatus*L.	Herb
47	*Cyperusrotundus*L.	Herb	**120**	*Rungiapectinata*L.	Herb
48	*Dalbergiasisso*Roxb.	Tree	**121**	*Salvia plebeiaR.Br.*	Herb
49	*Desmodiumconcinnum* DC.	Herb	**122**	*Saussureaheteromalla*D.Don	Herb
50	*Desmostachyabipinnate*Stapf	Herb	**123**	*Scopariadulcis*L.	Herb
51	*Diclipteraroxburghiana*Nees	Herb	**124**	*Setariaglauca*Beauv.	Herb
52	*Digitariacruciata*Nees	Herb	**125**	*Sidaacuta*Burm.	Herb
53	*Digitariastricta* Roth. ex R. & S. Syst.	Herb	**126**	*Siegesbeckiaorientalis*L.	Herb
54	*Diplaziumesculentum*(Retz.) Sac.	Fern	**127**	*Solanumincanum*L.	Shrub
56	*Ecliptaprostrata*Roxb.	Herb	**128**	*Solanumnigrum*L.	Herb
57	*Elephantopusscaber*L.	Herb	**129**	*Solanumverbascifolium*L.	Shrub
58	*Eleusineindica*Gaertn.	Herb	**130**	*Sonchusoleraceous*L.	Herb
59	*Emilia sonchifolia*DC.	Herb	**131**	*Sporobolusdiander*Beauv.	Herb
60	*Equisetum diffusum*D.Don	Fern	**132**	*Stellaria media* L.	Herb
61	*Erigeron bonariensis*L.	Herb	**133**	*Tectonagrandis*L.f.	Tree
62	*Eucalyptus hybrid* L.Herit.	Tree	**134**	*Tephrosiapurpurea*Pers.	Herb
63	*Eugenia jambolana*Lam.	Tree	**135**	*Thelypterisprolifera* Retz.	Fern
64	*Euphorbia heliosocopia*L.	Herb	**136**	*Toreniacordifolia*Roxb.	Herb
65	*Euphorbia hirta*L.	Herb	**137**	*Trewianudiflora*L.	Tree
66	*Ficuspalmata*Forssk.	Tree	**138**	*Tridaxprocumbens*L.	Herb
67	*Ficusracemosa*L.	Tree	**139**	*Vernoniacinerea*Less.	Herb
68	*Fimbristylisdichotoma*(L.) Vahl	Herb	**140**	*Vernonicaanagallis-aquatica*L.	Herb
69	*Fumariaindica*Haussk.	Herb	**141**	*Vetiveriazizaniodes*Nash.	Herb
70	*Galiumvestitum*D.Don	Herb	**142**	*Vicia sativa* L.	Herb
71	*Glycosmispentaphylla*Corrca.	Tree	**143**	*Youngia japonica* DC.	Herb
72	*Gnaphaliumluteoalbum*L.	Herb	**144**	*Zingibercapitatum*Roxb.	Herb
73	*Haplophagmaadenophyllum*Wall.	Tree	**145**	*Zizyphusjujuba*Lamk.	Shrub

andSimpson Index value for trees was 0.053-0.114, 0.040-0.049 for shrubs and 0.039-0.039 for herbs in forests at foot hills of Garhwal Himalaya.

The analysis of distribution pattern of herbs, shrubs and trees shows contagious distribution. Kumar and Bhatt (2006) also reported the contagious distribution pattern in forests of Garhwal Himalaya. Kharkwal and Rawat (2010) reported the total abundance-frequency A/F ratio of tree, shrub and herb species in different sampling sites ranged from 0.23 to 1.25, 0.25 to 1.79 and 3.4 to 27.3, respectively.

According to Odum (1971), contagious distribution is the commonest pattern in nature and random distribution is found in uniform environments.

Several workers (Kershaw, 1973; Singh and Yadava, 1974) have reported the contagious distribution in natural vegetation. Based on IVI values, the name of herbaceous communities is *Cynodondactylon-Saussureaheteromalla* in summer season, *Ageratum conyzoides-Rungiapactinata* in rainy season, *Ageratum conyzoides-Stellaria media* in winter season and *Cynodondactylon-Saussureaheteromalla* in spring season. However based on IVI values, the tree communities can be named as *Eucalyptus hybrid–Tectonagrandis* (Summer Season), *Tectonagrandis- Eucalyptus hybrid* (Rainy Season), *Eucalyptus hybrid–Tectonagrandis*(Winter Season) and *Eucalyptus hybrid–Tectonagrandis*(Spring Season).

Conclusion

The study points out phytosociological characters of forest vegetation in Tarai region of Kumaun Himalaya. The density of forest vegetation (herb, shrub, trees, sapling and seedling) is higher as compare to temperate forest of Garhwal and Kumaun (Central) Himalaya. Therefore, there is an urgent need for the conservation of biodiversity in Tarai forests of Kumaun Himalaya. Thus, this study will helpful for researcher for better understanding about structure of forest vegetation in Tarai of Kumaun Himalaya.

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Vermicomposting of the sugarcane trash using local earthworm species of Jammu

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Abstract

In the present investigation, an attempt has been made to vermicompost i)the sugarcane trash only and ii) sugarcane trash and cow-dung in ratio of (1:1) using local earthworm species viz. *Metaphire posthuma* (Vaillant), *Metaphire houlleti* (Perrier) and *Amynthas morrisi* (Beddard). Overall from study it is concluded that local species of earthworm particularly *M.posthuma* can be used to vermicompost sugarcane trash in combination with cow-dung in ratio of (1:1) so that the nuisance and environmental pollution due to unmanaged disposal of sugarcane trash can be reduced.

Keywords: *Vermicompost, sugarcane trash, cow-dung, local earthworm species*

Introduction

Vermitechnology is the process by which biological degradation of organic wastes takes place in controlled conditions due to earthworms feeding on the materials. There are mainly two approaches of vermitechnology: one is the process of vermicomposting resulting in the production of organic manure and aiding in waste management and the other is its application in the conservation processes of land or reclamation of waste lands (especially organic farming) (Abbasi and Ramasamy, 2001). Vermicomposting – is the bioconversion of organic waste materials through earthworm consumption (Gupta and Dwivedi, 2001).Sugarcane trash is the waste generated after extracting juice from sugarcane. Sugarcane juice is taken as a soft drink particularly in summer. In Jammu large number of sellers are involved in sugarcane juice selling and thus producing a lot of sugarcane trash which is either burnt or thrown into rivers or becomes breeding ground for flies, mites and microbes.

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If this is used as a raw material for production of vermicompost, it not only abates pollution load of this waste but also leads to conservation of resources. In present investigation, an attempt has been made to vermicompost i) the sugarcane trash only and ii) sugarcane trash and cow-dung in ratio of (1:1) using local earthworm species from Jammu.

Material and Methods

From the different parts of Jammu, three epigeic species of earthworm i.e. *Metaphire posthuma* (Vaillant), *Metaphire houlleti* (Perrier) and *Amynthas morrisi* (Beddard) were collected and got identified by Dr. J. M. Julka, former Jt. Director and Emeritus Scientist Zoological Survey of India, presently working as Director (planning) at Shoolini Institute of Life Sciences and Business Management, Solan (HP).The twenty four vermibed were prepared in wooden boxes of size 35 cm × 20 cm × 17 cm. At the bottom saw dust and paddy straw was placed and filled up to 3-4 cm, followed by 3 cm layer of fine sand and then 3cm layer of garden soil. Finally, 2-3 inches of a week old cattle dung was spread over the surface of soil as a bait to acclimatize earthworms. Three vermibeds were used as replicas for each species

of earthworm and thus a total of nine vermibeds (i.e. three sets for inoculation with specific earthworm species) for vermicomposting of sugarcane trash only and nine vermibed (i.e. three sets for inoculation with specific earthworm species) for vermicomposting of sugarcane trash and cow-dung (1:1) were used. Each vermibed was inoculated with approx. 100 gms. of specific earthworms species and the remaining six vermibeds with no inoculation of earthworms were set as control sets i.e. three vermibed with Sugarcane trash only and three vermibed with Sugarcane trash and Cow-dung (1:1).Sugarcane trash was chopped into smaller pieces dried and soaked at the rate of 100 gms / vermibed into water for 24 hours to be used as follow:

i) 100 gms (on dry wt. basis) Sugarcane trash only per vermibed for 12 vermibeds (i.e. nine experimental sets and three for control set).

ii) Sugarcane trash and Cow-dung in ratio of (1:1) i.e. 100 gms (on dry wt. basis) Sugarcane trash and 100 gms of dry cow-dung per vermibed for 12 vermibeds (i.e. nine experimental sets and three for control set).

After complete composting, the loose layer of soil along with decomposed organic material and worm casts was collected, dried in oven at 100^0C crushed and sieved. This was termed as vermicompost. After harvesting, fresh organic waste was again added twice to respective vermibeds to get IInd and IIIrd harvestings respectively. Number of days for completion of vermicomposting during each harvesting was also recorded. (Tables I and II).

Results and Discussion

The analysis of the data regarding Vermicompost production potential of *M.posthuma, M.houlleti* and *A.morrisi* on 100 % sugarcane trash revealed that *M.houlleti* vermicomposted on an average 21.7±0.9 % of Sugarcane trash in 52 days during 1st harvesting, 18.7±2.4 % of Sugarcane trash in 51 days during 2nd harvesting and 20.3±1.4 % of sugarcane trash in a period of 49 days during 3rd harvesting thereby exhibiting average vermicompost production potential of 20.23 % per harvesting in average number of 50.6 days per harvesting. The vermicompost production potential of *A.morrisi* during 1st, 2nd and 3rd harvesting exhibited values of 19.3±2.4 %, 21.3±1.4 % and 19.9±1.1 % respectively in a period of 54 days, 53 days and 51 days respectively with average vermicompost production potential of 20.17 % per harvesting in average number of 52.6 days per harvesting.*M.posthuma* vermicomposted on an average 19.0±1.8 % of sugarcane trash in 50 days during 1st harvesting, 16.2±2.1 % of sugarcane trash in 49 days during 2nd harvesting and 17.6±1% of Sugarcane trash in a period of 47 days during 3rd harvesting thereby exhibiting average vermicompost production potential of 17.6 % per harvesting in average number of 48.6 days per harvesting.The analysis of data regarding vermicompost production potential of *M.posthuma, M.houlleti* and *A.morrisi* on sugarcane trash and Cow-dung (1:1) revealed that the vermicompost production potential of *M.posthuma* during 1st, 2nd and 3rd harvesting exhibited values of 61.9±3.6 %, 67.6±4.2 % and 64.0±1.2 % respectively in a period of 45 days, 43 days and 42 days respectively with average vermicompost production potential of 64.5 % per harvesting in average number of 43.3 days per harvesting. *A.morrisi* vermicomposted on an average 61.3±4.8 % of Sugarcane trash and cow-dung (1:1) in 55 days during 1st harvesting, 56.5±1.8 % of sugarcane trash and cow-dung (1:1) in 53 days during 2nd harvesting and 59.3±2.6 % of Sugarcane trash and cow-dung (1:1) in a period of 50 days during 3rd harvesting thereby exhibiting average vermicompost production potential of 59 % per harvesting in average number of 52.7 days per harvesting. The vermicompost production potential of *M. houlleti* during 1st, 2nd and 3rd harvesting exhibited values of 57.4±2.8 %, 62.5±4.8 % and 59.52±2.8 % respectively in a period of 49 days, 48 days and 47 days respectively with average vermicompost production potential of 59.8 % per harvesting in average number of 48 days per harvesting.

From above analysis it is concluded that all the three local species of earthworm were observed to be inefficient for vermicomposting of exclusive sugarcane trash but all the three species were observed to be efficient for vermicomposting of sugarcane trash along with cow-dung in the ratio of (1:1). Muthukumaraswamy *et al.* (1997**)** composted sugar industry by products such as

Table 1: Vermicomposting potential of local earthworm species on 100% Sugarcane Trash

S. No	Name of species	Average % age of Sugarcane Trash vermicomposted per vermibed during					
		Ist harvesting	No. of days	2nd harvesting	No. of days	3rd harvesting	No. of days
1	*Metaphire posthuma*	19.0±1.8 (17.36-20.92)	50	16.2±2.1 (13.80-17.15)	49	17.6±0.99 (16.48-18.36)	47
2	*Metaphire houlleti*	21.7±0.9 (20.87-22.71)	52	18.7±2.4 (16.50-21.30)	51	20.3±1.4 (18.68-21.48)	49
3	*Amynthas morrisi*	19.3±2.4 (16.56-20.96)	54	21.3±1.4 (19.78-22.50)	53	19.9±1.1 (19.02-21.11)	51

Table 2: Vermicomposting potential of local earthworms' species on Sugarcane Trash and Cow-dung (1:1)

S. No	Name of species	Average % age of Sugarcane Trash and Cow-dung (1:1) vermicomposted per vermibed during					
		Ist harvesting	No. of days	2nd harvesting	No. of days	3rd harvesting	No. of days
1	*Metaphire posthuma*	61.9±3.6 (58.16-65.38)	45	67.6±4.2 (64.80-72.40)	43	64.0±1.2 (62.84-65.18)	42
2	*Metaphire houlleti*	57.4±2.8 (55.0-60.44)	49	62.5±4.8 (57.4-67.0)	48	59.52±2.8 (57.04-62.63)	47
3	*Amynthas morrisi*	61.3±4.8 (58.08-59.00)	55	56.5±1.8 (54.58-58.26)	53	59.30±2.6 (56.54-61.78)	50

press-mud and surplus bagasse through vermicomposting technique into a valuable organic rich vermicompost. They found this ecofriendly vermicompost superior to lignite in physical and chemical properties, higher density, porosity and water holding capacity and also in maintenance of bacterial population. Nogales *et al*. (1999) also observed that dairy biosolids were more effective in supporting earthworm growth and reproduction as compared to sheep manure. Sinha and Sinha (2000) also vermicomposted kitchen waste and garden waste alongwith cattle dung using *Eudrilus eugeniae, Eisenia foetida* and *Perionyx excavatus* and observed that the worms acted more faster during the summer days (June-August) than in winter days (January-March). *E.eugeniae* was found to have higher feeding growth and biodegradation capacity as compared to *E.foetida* and *P.excavatus*. Of all the three species *M.posthuma* was observed to be most efficient i.e. it vermicomposted 64.5 % of Sugarcane trash in 43.3 days in the mixture of Sugarcane trash and cow-dung (1:1), this was followed by *M.houlleti* which vermicomposted 59.8 % of Sugarcane Trash in 48 days and *Amynthas morrisi* which vermicomposted 59 % of Sugarcane trash in 52.7 days in the mixture of Sugarcane trash and cow-dung (1:1).Overall from above study it is concluded that local species of earthworm particularly *M.posthuma* can be used to vermicompost sugarcane trash in combination with cow-dung in ratio of (1:1) so that the nuisance and environmental pollution due to unmanaged disposal of sugarcane trash can be reduced or abated in an ecofriendly manner along with generation of vermicompost which can be used in agricultural fields or Kitchen gardens which would further cut down the use of chemical fertilizers.

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High intensity ultraviolet radiation induced changes in aquatic arthropod with retene and riboflavin

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Abstract

Ozone depletion is resulting into increase in ultraviolet radiation level in the world. Exposure to UV radiation has been found to have negative effects on aquatic and terrestrial organisms. Adverse effect of natural solar and artificial ultraviolet-B and UV-A radiations was observed in crustacean species *Daphnia magna* in presence of retene and riboflavin. *Daphnia magna* exposed to artificial ultraviolet-B with retene causes maximum physiological changes and mortality, indicating that enhanced solar UV-B exposure could be lethal to aquatic fauna. Artificial UV-B had a stronger damaging effect than solar radiation and become highly toxic in presence of retene. Riboflavin is slightly phototoxic in presence of solar and artificial UV radiation. Results on mortality rate indicated highest mortality in retene + ultraviolet-B exposed group followed by riboflavin + artificial ultraviolet - B radiation. A dose and intensity dependent change in mortality rate was observed. Retene and riboflavin photoproducts with ultraviolet radiation generate reactive oxygen species leading to cell injury and mortality thus are threat to aquatic biodiversity.

Keywords: *Aquatic biodiversity, Ozone depletion, phototoxicity, retene, riboflavin, ultraviolet radiation.*

Introduction

The role of stratospheric ozone layer in absorbing biologically harmful UV radiation is well known. Ultraviolet radiation is the most photochemically reactive wavelength of solar energy reaching the earth surface and has a broad range of effects on aquatic and terrestrial ecosystem (Williamson 1996). The intensity of solar (UV- A and UV-B) is increasing due to ozone depletion (Mckemzie *et. al.* 2007). Life of aquatic environments experiences extreme conditions with respect to temperature, food availability and radiation. UV radiation especially UV-B (280-320 nm) is found to be harmful for the aquatic organisms. Aquatic organisms of shallow arctic water must reproduce successfully within a very short breeding season under these extreme environmental conditions.

Daphnia magna occur circumpolar and play an important role in food web. The knowledge of the effect of solar ultraviolet radiation on *Daphnia magna* is of great interest. Most of the irradiation experiments on *Daphnia* and other zooplankton have been conducted under standardization conditions in the laboratory using artificial light sources for irradiation (Borgeraas and Hassen, 2008). Some authors demonstrated that aquatic organisms of shallow habitats such as ponds, with high UV radiation doses or intensities, may be harmed, even killed due to natural irradiation. The effect of solar (UV-A and UV-B) on some sensitive model species i.e. *Tubifex* and *amphibians* has also been studied by (Formicki et. al. 2003). Still there is a lack of information on phototoxicity of endogenous and exogenous chemicals present in our body and in environment. The effects of (UV-A and UV- B) on aquatic organisms depend on the dose of the harmful radiation to which an individual organism is exposed. Ultraviolet radiation penetration in aquatic habitats is modulated by some factors as dissolved organic carbon, suspended particles, phytoplankton and reflection (Daiz *et al.,* 2000, Hargreaves, 2003). Certain chemicals become phototoxic in presence of solar and ultraviolet radiation. Natural photosensiizers are present in many organisms including bacteria, protozoa, plants, invertebrate and vertebrates (Al-Akhras *et al*. 2007, Kumar et. al. 2010). Retene is found naturally in resinous plants and riboflavin is vitamin B-2 commonly present in our body. This study was performed to investigate the adverse

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effect of natural and artificial (UV-A and UV-B) on crustacean species *Daphnia magna* in presence of retene and riboflavin.

Materials and Method

Solar terrestrial UV-A and UV- B was recorded by Cole-Parmer radiometer (USA) having, Vilber Laurmat France calibrated UV-A and UV- B sensors with spectral sensitivity 365 and 312 nm. *Daphnia magna* were collected from Doon valley Uttarakhand and cultured in the laboratory by the method of Songlake and Tisher (2001). During the experiment water depth was about 6cm and water transparency was very high. Transmission was measured with a Spectrophotometer.

Daphnia placed in Petri dish with pond water. Twenty five adult *Daphnia,* egg carrying size were selected for each group. Experimental protocol with two replicates was designed and *Daphnia magna* were divided into twelve groups. Group one was control. Group two was exposed with retene, group three was exposed with riboflavin and group four was treated with low intensity of natural solar radiation. Group five was exposed with low intensity of artificial UV-A radiation and group six was exposed with low intensity of artificial UV-B radiation, group seven was exposed with solar radiation + retene, group eight was exposed with solar radiation + riboflavin. Group nine was exposed with artificial UV-A radiation + retene and group ten was exposed with artificial UV-A radiation + riboflavin, group eleven was exposed with UV-B radiation + retene and group twelve was exposed with UV-B radiation + riboflavin. Another experiment was setup with high intensity of solar and artificial UV-A and UV-B radiation having twelve groups. Dose used for retene and riboflavin was 25mg/liter. Low intensity of UV- A and UV-B radiation used was 0.600 mw/ cm^2 and higher intensity was 0.900 mw/ cm^2. Exposure time was 1 hrs and 2hrs through Philips UV-A and UV- B lamps emitting 365 and 312 nm wavelength radiation. During the experiment morphological, physiological and reproductive changes were observed in *Daphnia*. Results were statistically analyzed using students "t" test (Fisher, 1963).

Results and Discussion

Results on mortality rate of *Daphnia magna* indicates that retene and riboflavin is not harmful when given separately but become photo-toxic with solar light and artificial UV-A and UV-B. Non significant changes in mortality were observed with low intensity of ultraviolet radiation exposure. 10% mortality was found with high intensity of natural solar light. Mortality rate in *Daphnia* was found higher i.e. 23% with high intensity of artificial UV-B exposure (Table-1 & 2). Maximum mortality 42% was found in artificial ultraviolet- B radiation with retene. UV-B radiation and retene show maximum changes in physiological activity, growth, movement, mortality and reproduction. UV- A and solar radiation is less toxic in comparison to artificial UV-B (Table-2). Significant increase in mortality rate was observed after high intensity of solar radiation and artificial UV-B exposure. Co-treatment of retene and riboflavin with solar, artificial UV-A and UV-B exposure further increase the mortality showing phototoxic effect. Artificial UV-B was found more toxic than solar radiation and UV-A (Table- 1 & 2). Retene was found more phototoxic than riboflavin. A dose wavelength and intensity dependent change in mortality rate was observed (Fig-1).Retene (7- isopropyl- 1-methylanthrene) is a compound formed from resin by anaerobic microbes. In natural waters, retene is mainly formed anaerobically from resin acids, oleoresinous constituents of coniferous trees. It has been found in sediment particles contaminated by treated pulp and paper mill effluents as well as in the sediments surface in lake areas contaminated by the industry (Leppanen *et. al.* 2000). Riboflavin is vita-B_2 present in the body. Results on mortality rate and behavior of *Daphnia magna* in presence of solar light UV-A, UV- B individually and with retene and riboflavin indicates that retene is not harmful when given separately but, become phototoxic in presence of solar light, artificial UV-A and UV- B. Difference in phototoxicity of retene, riboflavin with UV-A and UV-B was observed through growth, movement and behavioral change. Reversible effect of UV radiation which recovered within 3-4 hr. of withdrawal of exposure was observed (McKim *et. al*. 2001). Results on dose and intensity dependent increase in phototoxicity are supported by our studies on photohemolysis of erythrocytes (Kumar *et. al*. 2009). Lake having high content of dissolved organic material shields and protect the organism from UV radiation. It act as natural sunscreen as it influence water transparency, therefore determine the light penetration. Low vegetation at alpine areas lakes offer less protection from UV to the organism.

Transparency of water maximizes the penetration and effect of UV radiation however; organisms develop adaptation towards increase radiation (Carbol *et. al*. 2004, Rautio and Tartarotti 2010). Shallow Water Lake is more sensitive to UV radiation and could be harmful to flora, fauna including mammals (Laura *et. al*. 2010). Results on crustacean species are supported by the studies on *Metaphire* (Kumar et. al. 2010).

Table - 1: **Effect of low intensity 0.600mw/ cm^2 of natural and artificial UV radiation on mortality rate in *Daphnia magna*.**

Group	Treatment	Mortality %	
		1 Hour	2 Hour
1.	Control	2 ± 0.4	2 ± 0.4
2.	Retene	4 ± 0.4 ^{NS}	4 ± 0.3 ^{NS}
3.	Riboflavin	3 ± 0.5 ^{NS}	3 ± 0.6 ^{NS}
4.	Solar radiation	6 ± 0.5 ^{NS}	8 ± 1.4 ^{NS}
5.	Artificial Ultraviolet-A	6 ± 0.7 ^{NS}	8 ± 1.06 *
6.	Artificial Ultraviolet-B	12 ± 1.12*	14 ± 1.71*
7.	Solar radiation + retene	13 ± 0.8*	18 ± 0.9*
8.	Solar radiation + riboflavin	12 ± 0.7*	16 ± 0.8*
9.	Artificial Ultraviolet-A + retene	18 ± 1.08*	22 ± 3.05**
10.	Artificial Ultraviolet-A + riboflavin	15 ± 1.45*	19 ± 1.92*
11.	Artificial Ultraviolet-B + retene	28 ± 1.2**	32 ± 1.1**
12.	Artificial Ultraviolet-B + riboflavin	26 ± 1.4**	30 ± 0.9**

Results are mean ± S.E. of 5 observations in each group. P value * 0.05, *0.01, NS not significant

Table - 2: **Effect of high intensity 0.900 mw/ cm^2 of natural and artificial UV radiation on mortality rate in *Daphnia magna*.**

Group	Treatment	Mortality %	
		1 Hour	2 Hour
1.	Control	2 ± 0.4	2 ± 0.4
2.	Retene	4 ± 0.5 ^{NS}	4 ± 0.3 ^{NS}
3.	Riboflavin	3 ± 0.6 ^{NS}	3 ± 0.4 ^{NS}
4.	Solar radiation	7 ± 0.6 ^{NS}	10 ± 1.05 *
5.	Artificial Ultraviolet- A	9 ± 1.65*	10 ± 2.24*
6.	Artificial Ultraviolet-B	20 ± 1.27*	23 ± 1.06*
7.	Solar radiation + retene	24 ± 1.28*	28 ± 2.09*
8.	Solar radiation + riboflavin	22 ± 1.18*	26 ± 3.17*
9.	Artificial Ultraviolet-A + retene	25 ± 1.43**	28 ± 2.16**
10.	Artificial Ultraviolet-A + riboflavin	23 ± 1.43*	25 ± 3.12**
11.	Artificial Ultraviolet-B + retene	38 ± 3.31**	42 ± 2.42**
12.	Artificial Ultraviolet-B + riboflavin	32 ± 2.24**	36 ± 3.19**

Results are mean ± S.E. of 5 observations in each group. P value * 0.05, *0.01, NS not significant

Riboflavin and retene are important chromophores for photoinduced lethality in *Daphnia*. Retene and riboflavin photoproducts generate reactive oxygen species with UV radiation leading to cell injury and mortality. Those species whose early life stage occur near the surface, there may be circumstances- such as a cloudless sky, lack of wind, calm seas, low nutrient loading- under which the contribution of UV-A and UV- B radiation to the productivity and mortality of a population could be far more significant. Reproductive parameters seem to be very sensitive in determining UV radiation induced damaged. Further increase in solar UV due to stratospheric ozone depletion may leads to significant change in the zooplankton communities and aquatic ecosystem.

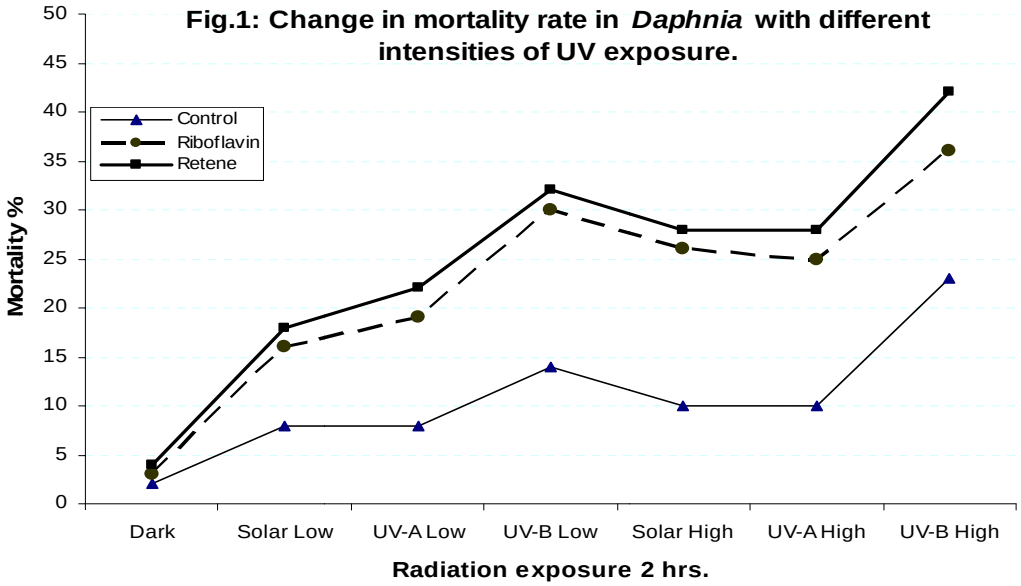

Fig.1: Change in mortality rate in *Daphnia* with different intensities of UV exposure.

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Chronic effect due to changes in the contents of urea & creatinine in edible cat fish *Channa punctatus* (Bloch), under the stress of sub lethal concentration of methyl parathion – a pesticide

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Abstract

The chronic effect of methyl parathion on cat fish *Channa punctatus* was observed by comparing the amount of urea & creatinine in the bio chemical blood plasma profile of a control group & a group exposed to methyl parathion pesticide, in sub lethal concentration. Lc50 value of fish was found 35µg/l. During the study it was found that the amount of urea in the experimental fish was increased upto alarming level, although the creatinine concentration increased gradually. As such fish food is consumed by the non target organisms & more specifically by the human being, its consumption usually results in the development of such symptoms, which are not mentioned in the routine literature.

Keywords: *Methyl parathion, Channa punctatus, Urea, Creatinine, Human being*

Introduction

For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors (Prakasham, *et al*., 2001). Among these pesticides, organophosphorus compounds (OPs) are commonly used as insecticide. Organo phosphorus (OPs) pesticides have long been of serious environmental concern. They form the largest group of chemicals used in the control of pests, including invertebrates, vertebrates and, to a lesser extent, plants. There are some 200 OPs pesticides available in this class, which have been formulated into literally thousands of different products (Hill, 2003). Methyl parathion is a non synthetic, wide spectrum organophosphorous pesticide. It was one of the earliest organophosphorous pesticides developed (Introduced in 1950). It was used for agricultural and non-agricultural purposes. Once methyl parathion is introduced into the environment, it may cause serious trouble to aquatic organisms and is notorious for causing several metabolic disturbances in non-target species, like fish and fresh-water mussels etc. (Anonymous,2005).

Organophosphorous pesticides have replaced the persistent chlorinated pesticides in the 1970's and in the beginning of 1980's, it is completely replaced. The main advantage of the organophosphorous pesticides was their low cumulative ability and short-term persistence in the environment (Svoboda, *et. al.*, 2001). Methyl parathion is a contact organophosphorous pesticide and extensively used, both in agriculture and households to control insects in soil, plants, fruit and vegetable crops. After its application on crops and plants, methyl parathion is easily washed into surface waters and enters the ground water. Eventually, it enters the aquatic environment in large quantities (Kuivila and Foe, 1995). Methyl parathion degrades rapidly, but under conditions of low temperature, low moisture, high alkalinity, and lack of suitable microbiological degraders, it may remain biologically active in soil for six months or longer. Because of its aquatic distribution, methyl

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parathion affects a wide range of non-target organisms, like inverterbrates, fishes, birds and mammals, especially those inhabiting aquatic environment (Burkepile, *et. al.,* 2000) Due to its chemical properties, widespread use and application, methyl parathion is frequently found in point sources (wastewater treatment plant effluent) and non-point sources (storm water runoff) in urban and agricultural areas. Methyl parathion is known to be extremely toxic to birds and aquatic life (USEPA, 2005). Methyl parathion is transported into rivers largely via storm water runoff and also with rain, events producing pesticide pulses in rivers and streams (Ferrari, *et. al.,* 1997).

Teleost fish may be good indicator of contamination because their biochemical responses are quite similar to those found in mammals. The response of some aquatic organisms to pollutants has been studied through the measurement of hematological and physiological parameters (Begum, 2004). The major reason for carrying out toxicity tests with fish and other aquatic organism is to determine which concentrations of pesticide are harmful to the organisms and which have no apparent effect. A second objective toxicity tests is to monitor the toxicity of effluents or evaluate the quality of surface water. The edible fish was taken into considerate. To asses the quality of water with meaningful procedure, especially if many waste substances are present or if it is not known exactly what is presents.

The purpose of the present investigation is to evaluate the effect of altered amount of urea & creatinine in the body of *Channa punctatus,* exposed to methyl parathion sub lethal concentration and to establish the fish as bio-marker. In food chain it is also used by the several organism including human being.

Materials and Methods

Channa punctatus, (Bloch) is regionally called **SOLI,** belongs to the family ophiocephalidae is collected from the local fish ponds & reservoirs. Specimens of more or less same sized and weight were selected for the present investigation. The average size of fish was 16cm.-19cm. & weight was 60gm.-70gm. During acclamatization commercial fish food was given to them and on alternate day water was changed. Physiochemical nature of water was determined by using the APHA techniques (APHA 1998). With this technique water temperature was recorded $28^{o}c \pm 1^{o}c$ & pH recorded was 7.0±1.5. BOD was normal as standard value.

Effects were made to maintain the temperature & pH of water containing experimental & control fish. Fish were kept in six groups, each one had ten specimens. Fish of four groups were treated experimentally and the remaining two as control. Sampling was done on 3, 7, 15, and 30^{th} day and every time feeding was stopped at least 24hrs. , before taking blood samples.

After anaesthetization with MS222 blood was taken out from caudal vein with the help of heparinized syringe. Blood in sterilized tube was kept in centrifuge for 20 minutes at a speed of 3500 rpm.

After separating, plasma analysis was done with the help of Semi-Autoanalyser using test reagents manufactured by Span Diagnostic Ltd., Sachin, Surat (India).

Results and Discussion

Fish were treated with sub lethal doses of methyl parathion to determine the concentration of urea and creatinine in the blood plasma, causing the level damage in kidney and other body organs. Efforts have also been made to observe similar changes in other organisms including human being in the food chain.

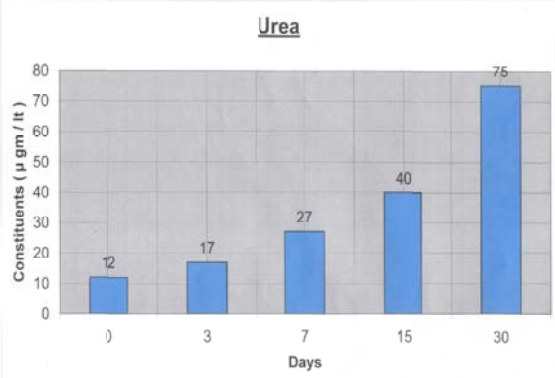
Both the groups of fish (experimental and control) were kept under observation to find out the changes in urea and creatinine. The amount of urea in control fish was observed 12 µg/l ± 5 µg/l and in experimental fish, the amount of urea increased upto 75 µg/l. Similarly the concentration of creatinine in blood plasma was observed about 0.40 µg/l ± 0.35 µg/l in control group, and in experimental group the value increased upto 2.1 µg/l.

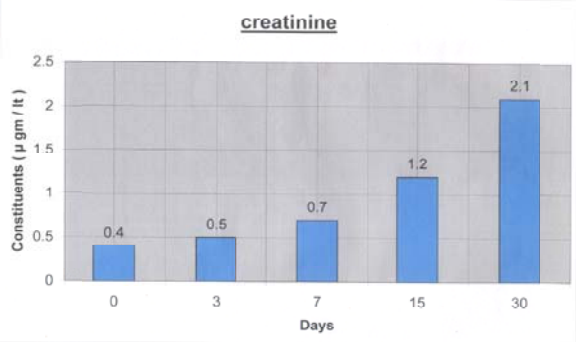
	Control	Experimental			
Urea	12±5	17	27	40	75
Creatinine	0.40±0.35	0.50	0.70	1.2	2.1

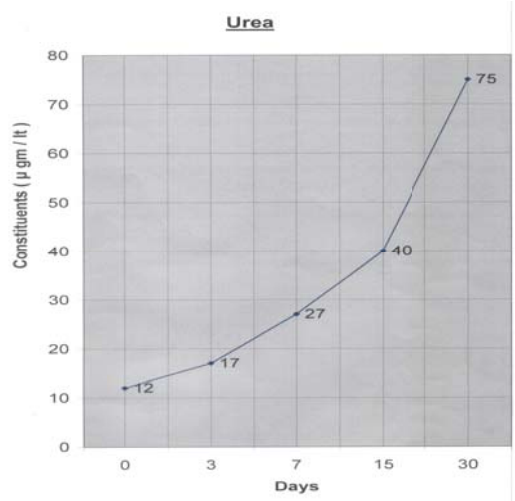
Days

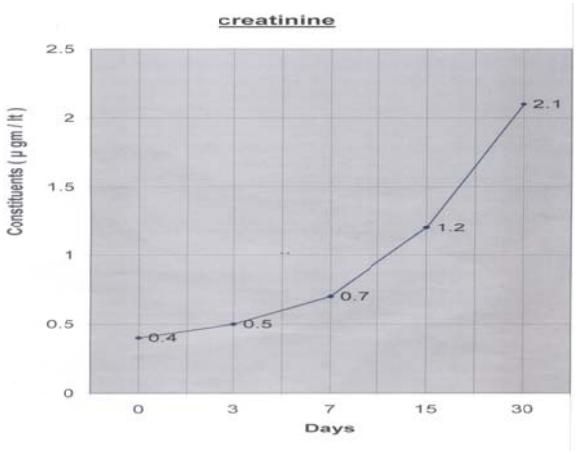
0 3 7 15 30

The extra cellular fluid constitutes the internal environment of the cells of the body. The cells carry out their vital activities in this medium. This fluid should be maintained relatively constant in composition for the normal functioning of the cells. Cytoplasm is the intracellular fluid and is affected functionally by the extracellular fluid. filtration of plasma by glomeruli (2) selective absorption (3) secretion and (4) disorder caused by them. Due to the disturbance in the above mentioned processes and mainly due to decreased glomerular filtration, waste products particularly nitrogenous substances such as urea, creatinine etc. increased in blood.









Any biochemical change in extracellular fluid or tissue fluid affects the biochemistry of cells. Lungs and Kidney are the primary organs maintaining the internal environment.

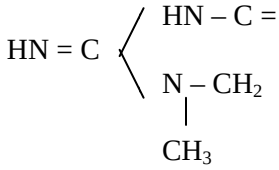
This internal environment is regulated mainly by two pairs of organs. The lungs control the concentration of oxygen and CO_2 and the kidney maintain optimal chemical composition of the body fluids by acidification of urine and also by removing metabolic wastes such as urea, creatinine. The regulation of the internal environment by kidney is a composite of four processes (1)

The term blood non protein nitrogen comprises urea and creatinine etc. Estimation of total blood nonprotein nitrogen (NPN) is commonly undertaken as a measure of protein catabolism of renal function.

Accumulated amount of the methyl parathion in fish may develop disorder and when taken by human being as a final consumer, various abnormalities may be developed. In this manner, the Azotemia like disorder appears showing high concentration of plasma non protein nitrogen causing following abnormal situations:

- Higher tissue protein catabolism.
- Excess break down of blood protein, and is taken up by human being may develop diabetes mellitus, dehydration, cardiac failure and high fever. (Balis, 1976 and Baron *et. al.* 1955)

Creatinine is end product of creatine metabolism. It is an anhydride of creatine. The chemical formula of this creatinine is as follow



Creatinine is mainly found in muscle by the irreversible and non-enzymatic removal of water from creatine phosphate. Formation of creatinine is a preliminary step required for the excretion of most of the creatine. Three amino acids glycine, arginine and methionine are directly involved in the synthesis of creatine. (Meister, 1965)

When the concentration of creatinine becomes alarming in human being, due to consumption of such effected fish, it may cause renal failure, heart failure / shock and obstruction in urinary track. (Bonsnes and Taussing, 1945 and Brod and Sirota 1948).

Conclusion

Exposure of fish *Channa punctatus* to the methyl parathion - a pesticide in sub lethal concentration for about 30 days, with few intervals, directly indicate that the exposure of this pesticide not only affect the health of fish but also the human being and develop the various alarming symptoms which may be proved fatal.

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A checklist of benthic macroinvertebrates of River Manuni, Himachal Pradesh

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Abstract

Himachal Pradesh is drained by five main rivers and their tributaries. River Manuni originates from the southern slopes of the Dhauladhar range and joins River Baner on the rear side of the Kangra fort to finally meet the River Beas near Haripur in district Kangra of Himachal Pradesh. The present study was undertaken to investigate the benthic macroinvertebrate communities of River Manuni during March 2009 to February 2011. Monthly samples of benthic macroinvertebrates were collected from the three designated sampling sites within an altitudinal range of 610m to 1240m asl. A total of 67 taxa were identified in River Manuni during the study period. Out of which 44 insects were identified up to generic level which belonged to 8 orders and, 12 up to family level belonging to 5 orders. Among other benthic macroinvertebrates, 8 were identified up to generic level belonging to 5 orders. In addition, earthworm, leech and crab were also recorded in Manuni water. Of the taxa recorded, 44 were common in all reaches i.e. higher (1240m), middle (770m) and lower reach (610m) of the River Manuni, whereas some taxa were restricted to specific reach only i.e., 10 taxa were limited to higher reach, 03 taxa to middle reach and 01 taxon was present only in lower reach of River Manuni.

Keywords: *Benthic macroinvertebrates, insects, crustacean, annelids, molluscs, River manuni.*

Introduction

A fundamental characteristic of river ecosystems is the unidirectional movement of water, nutrients, inorganic materials and organic matter down altitudinal gradients from headwater mountain streams to lowland streams (Suren, 1994). The stream located in the hills is called as hill stream and support wide range of flora and fauna and these in turn determines the health of major rivers systems (Johal and Rawal, 2005). Among the freshwater biota, benthos (benthonic or benthic organisms) is a collective designation for all the bottom dwelling aquatic organisms that live on or within the sediments at the bottom of water body. Among these the zoobenthos are the animals inhabiting the sediment, or living on or in other available bottom substrates. They mainly dwell at the bottom but may occasionally travel upward. Further, macroinvertebrates are defined broadly as animals with body length $\geq$ 0.5 mm which could be retained by a net of 200 µm mesh size (Dudgeon, 1999).

Stream macroinvertebrates are typical objects of community ecology and environmental monitoring studies (Rosenberg and Resh, 1993; Allan, 1995). The sensitive species inhabiting these habitats because of adverseness of environmental conditions are gradually eliminated and tolerant species establish their colonies and grow in abundance (Hellawell, 1986; Rosenberg and Resh, 1993). Most aquatic habitats with acceptable water quality and substrate conditions support diverse macroinvertebrate communities. Usually, in the hill streams, insects of principal groups Ephemeroptera, Odonata, Plecoptera, Hemiptera, Tricoptera, Coeleoptera and Diptera are among the most visible benthic animals.As such, the macroinvertebrates present several advantages compared to other groups of organisms: they are ubiquitous and diverse, exhibit different feeding habits, are sedentary and have life cycles ranging from few weeks to a few years and are of convenient size for field examination, storage and transport (Chessman, 1995; Miserendino and Pizzolon, 1999). Understandably, the macroinvertebrate

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communities have been the most commonly used tool for making an integrated assessment of water quality in rivers (Karr, 1991; Palmer et al., 1996). The Himalayan region remains comparatively lesser studied as regards to benthic macroinvertebrates. Moreover in Himachal Pradesh such study has been fewer and fragmentary, though some notable contributions have been made by Prasad (1918), Hora (1930), Dutta (1992), Farmahan (1994), Joshi (1994), Julka *et al*.(1999) and Sharma *et al*. (2006). Therefore, considering the number of rivers and streams draining Himachal Pradesh and its obvious importance, the proposed study envisaged inventorying, benthic macroinvertebrates of River Manuni, a tributary of River Beas.

Study area

Himachal Pradesh is located between $30^0$22' -$33^0$12' North Latitude and $75^0$45' -$79^0$04' East Longitude. to East, it forms India's border with Tibet, to the North lies the state of Jammu and Kashmir, Uttarakhand in the Southeast, Haryana in South and Punjab in West. There is a general increase in elevation from West to East and from South to North. The entire territory of Himachal Pradesh is mountainous with altitude varying from 350m to 7000m asl.River Manuni originates from the southern slopes of Dhauladhar range in district Kangra. Steep slopes form the upper catchment of River Manuni. It is covered by small glaciers in upper and dense forest in lower ridges. The upper catchment lies in heavy rainfall region where substantial precipitation occurs during monsoon and winter months. Manuni joins Baner (tributary of Beas) on the rear side of the historical Kangra fort at Sangam to finally meet River Beas near Haripur in district Kangra of Himachal Pradesh. The present study was undertaken during March 2009 to February 2011. For the purpose three sampling sites were selected in River Manuni within an altitude range of 610-1240m asl. (Fig.1).The sites were:

S1 (Khaniyara 1240 m asl): Located 10 km (approx.) from district headquarter, Dharamshala at an altitude of 1240m asl. The area is famous for Himachal slate mines, broken pieces of which can be seen scattered in the river. Upstream to S1 the river descends through thick forest having natural vegetation, where numerous streamlets emerge from the Dhauladhar range to form the mainstream Manuni. Khaniyara locality falls in the northern region of the study area. The litho-units exposed in the area include slates, shales, sandstones and occasional basalts. Two important thrust systems viz.; Main Central Thrust and the Main Boundary Thrust traverse this area (Dhar, 2004). Scarification and land degradation due to the haphazard mining activity of slate is conspicuously seen in this region. This area shows the presence of glacio-fluvial deposits in the upper regions. Upward to S1 i.e. Khaniyara there is sudden rise in the height of Dhauladhar having difficult terrain hence was not included in the study. Some of the important vegetational elements of the higher reaches of Dhauladhar in the Manuni watershed are *Acer caesium* (Mandar), *Aconitum heterophyllum* (Patis), *Cotoneaster microphylla* (Res), *Diplazium frondosum* (Lugru)**,** *Fagopyrum tataricum* (Fafru), *Gentiana kurroo* (Karu), *Jurinea dolomiaea* (Dhoop), *Pieris ovalifolia* (Ailan), *Quercus leucotrichophora* (Ban) and *Rhododendron arboreum* (Barah). *Cedrus deodara* is generally absent in the watershed, although few planted trees are present as low as 1250m in Khaniyara.

S2 (Bhadwal 770m asl): Located 8 km (approx.) downstream to S1 at an altitude of 770m asl, this site is surrounded by extensive agriculture fields and stream water has been directed for irrigating the crop fields. This locality falls south to Khaniyara and comprises the terrace deposits of the Manauni stream. The upper Shiwalik conglomerates and minor sandstones are encountered in this area hidden under the thick veneer of terrace deposits. Drini thrust passes in close vicinity to this area (Dhar, 2004). However the area shows gentler slope and occurrence of terrace deposits more significant owing to lesser intensity of erosional activity. Much of the natural vegetation at lower altitude (downstream Khaniyara) has been replaced by irrigated terraces locally known as 'khet'. The important crops grown in the area are wheat and rice in rotation along with maize, potatoes and pulses.

S3 (Purana Kangra 610m asl): Located 7.0 km (approx.) downstream to S2 at an altitude of 610m asl, The river further travels 1.0 km (approx.) through deep gorge to meet Baner a tributary of River Beas at Sangam. This locality as the name

signifies is the erstwhile town of Kangra. The area dominantly comprises of upper Siwalik conglomerate with intercalations of loose sand stones (Dhar, 2004). Owing to its lithological characters the area shows scanty forest cover and the signature of high erosional intensity is visible here.

Methodology

Regular monthly samples of benthic macroinvertebrates were collected following stratified random sampling (Cummins, 1962) along transects using modified Surber's square foot sampler (Welch, 1952). Benthic macroinvertebrate samples were collected during March 2009 to February 2011 at three selected sampling sites. Benthic macroinvertebrate visible to naked eye were collected. They were then transferred to small plastic bottles containing 4-5% formalin solution and taken to laboratory for analysis. Identification was carried to lowest recognizable level as far as possible with the help of keys by Burks (1953), Usinger (1956), Needham and Needham (1962), Hynes (1977), Macan (1979), Edington and Hildrew (1981), Elliott et al. (1988), Wallace et al. (1990), Dudgeon (1999) and Jessup et al. (2003).

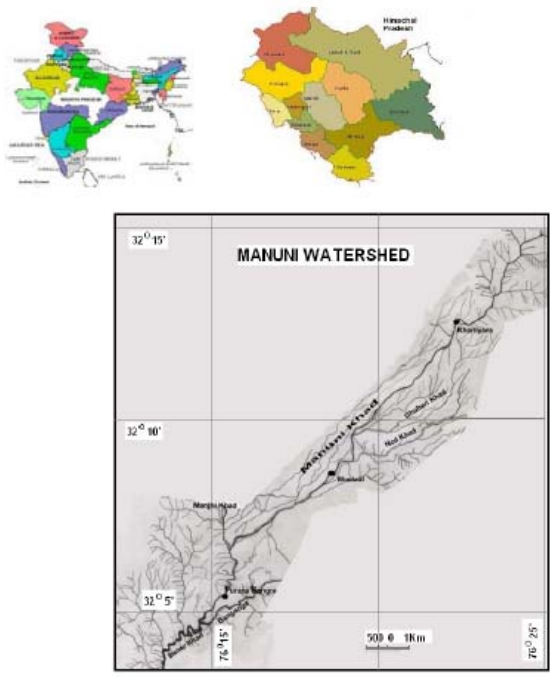

Fig.1. Location Map of Manuni Watershed

Results and Discussion

A total of 67 benthic macroinvertebrates taxa were identified in River Manuni during present study (Table 1). Of these, 44 insects were identified up to generic level which belonged to 8 orders, and 12 up to family level belonging to 5 orders. Among other benthic macroinvertebrates, 8 taxa belonging to 5 orders were identified up to generic level. These included 01genus of Platyhelminthes, 01 crustacean and 06 molluscs. Besides, crabss, earthworm and leeches were also recorded in Manuni water. Of the taxa recorded, 44 were common in all reaches i.e. higher (1240m), middle (770m) and lower reach (610m) of River Manuni.

During the present study, 60 taxa of benthic macroinvertebrates were identified from S1 followed by 56 from S2 and 48 from S3. The high number of taxa in the upper zone of study area may be due to the presence of thick riparian forest in the headwater region as the riparian zone provides food and shelter for aquatic biota **(**Bretschko and Moser, 1993**).** The middle zone (S2) is surrounded by agriculture fields, whereas the forest cover is very scanty in lower zone (S3) of Manuni. The low diversity in streams with human modified riparian land use type is attributed to change in habitat brought out by decreased detritus input, increased sedimentation and runoff (Hershey and Lamberti, 1998).Some taxa of benthic macroinvertebrates also showed restricted distribution. 10 taxa were limited to higher reach, 03 taxa were restricted to middle and 1 taxon was present only in lower reach of River Manuni. Taxa recorded only at higher reach (S1) were *Nemoura*, *Leuctra*, *Himalopsyche*, *Rhyacophila*, *Stenopsyche*, *Brachycentrus*, *Limnephilus*, *Dicranota*, *Blepharicera*, and *Gammarus*. Whereas, Crab, *Melanoides* and *Indoplanorbis* were restricted to mid reach (S2) and *Rhinocypha* to the lower reach (S3) of study area. Similar distribution of benthic macroinvertebrates has also been reported by other workers. Nemourid larvae can be quite abundant in collections from small streams draining forested catchment (Dudgeon, 1999). A thick riparian forest is present in the upper reach of Manuni, thus providing suitable habitat. *Leuctra* though basically Palaearctic genus, is also found in India and Nepal (Harper, 1977; Sivec, 1981). Similarly, *Himalopsyche* seems to be restricted to habitats where torrential flows predominate, especially at

Table1. Check list of benthic macroinvertebrates of River Manuni, Himachal Pradesh.

Phylum	Class	Order	Family	Genera
Arthropoda	Insecta	Ephemeroptera	Baetidae	*Baetiella*
				Baetis
				Platybaetis
			Heptageniidae	*Ecdyonurus*
				Epeorus
				Iron
			Leptophlebiidae	*Leptophlebia*
				Paraleptophlebia
			Ephemeridae	*Ephemera*
			Ephemerellidae	*Ephemerella*
			Caenidae	*Caenis*
		Odonata	Calopterygidae	-
			Chlorocyphidae	*Rhinocypha*
			Euphaeidae	*Euphaea*
			Coenagrionidae	*Enallagma*
			Gomphidae	-
			Macromiidae	-
			Libellulidae	-
		Plecoptera	Nemouridae	*Nemoura*
			Leuctridae	*Leuctra*
			Perlidae	-
		Hemiptera	Nepidae	*Nepa*
			Naucoridae	*Naucoris*
			Corixidae	*Micronecta*
		Megaloptera	Corydalidae	*Corydalus*
		Trichoptera	Rhyacophilidae	*Himalopsyche*
				Rhyacophila
			Glossosomatidae	*Agapetus*
			Hydroptilidae	*Hydroptila*
			Philopotamidae	*Chimarra*
			Stenopsychidae	*Stenopsyche*
			Polycentropodidae	*Polycentropus*
			Hydropsychidae	*Hydropsyche*
			Brachycentridae	*Brachycentrus*
			Limnephilidae	*Limnephilus*
			Leptoceridae	*Leptocella*
		Lepidoptera	Pyralidae	-
		Coleoptera	Dytiscidae	-
			Hydrophilidae	-
			Scirtidae	*Hydrocyphon*
			Psephenidae	*Psephenoides*
			Elmidae	-
		Diptera	Tipulidae	*Tipula*
				Antocha
				Dicranota
				Hexatoma
				Limnophila
			Blephariceridae	*Blepharicera*
			Psychodidae	*Psychoda*
			Dixidae	*Dixa*
			Simuliidae	*Simulium*
			Ceratopogonidae	-
			Chironomidae	-
			Tabanidae	*Tabanus*
			Athericidae	*Atherix*
			Dolichopodidae	-

	Crustacea	Decapoda (Crab)		-
		Amphipoda	Gammaridae	*Gammarus*
Platyhelminthes	Turbellaria	Tricladida	Planariidae	*Planaria*
Annelida	Oligochaeta (Earthworm)			-
	Hirudinea (Leech)			-
Mollusca	Gastropoda	Mesogastropoda	Thiaridae	*Melanoides*
		Basommatophora	Lymnaeidae	*Lymnaea*
			Physidae	*Physa*
			Planorbidae	*Gyraulus*
				Indoplanorbis
	Pelecypoda	Heterodonta	Sphaeriidae	*Pisidium*

high altitude, like the upstream region of the study area. The taxon has also been reported in two high mountain streams at about 1500m asl. in Nagano, Central Japan (Tsuruishi, 2006). *Rhyacophila* is wide spread and extremely speciose especially in north India (Kimmins, 1953). *Rhyacophila* larvae are probably the most restricted to conditions of high current speed (Scott, 1958). Indian *Stenopsyche* species appear to be confined to altitudes north of Tropic of Cancer (Higler, 1992). Also, *Brachycentus* has been reported from high altitude of the Himalaya (Mani, 1968) and Limnephilidae are characteristics of high altitude streams (Suren, 1994). Similarly, *Dicranota* also was reported in streams of western Nepal ranging in altitude from 850-4250m (Suren, 1994). Blephariceridsl larvae (mountain midges) have specialized morphology that is associated with living on rocks surface in swift currents at high altitude (Dudgeon, 1999). They may be found crawling on rocks in rushing water of mountain or hill streams, living directly in the water or in the perpetual spray of waterfalls (Usinger, 1956). Sehgal (1991) recorded *Gammarus* in two out of the eleven tributaries of Indus and Jehlum in northwest Himalaya (North of 32^0N). Also, it has been recorded to be confined to rather higher altitudes (Sehgal, 1983; Melkania, 1991). Ng (1988) says that crabs individual species tend to be confined to particular altitudes and hence shows longitudinal zonation along the river. The middle stretch of Manuni is surrounded by extensive agriculture fields and crabs inhabit the marginal waters. Pulmonates like planorbidae in Asian streams are less widespread and diverse than prosobranchs and tend to be most abundant in slow flowing streams (Dudgeon, 1983). *Melanoides* have been introduced widely to the regions of Tropics beyond their natural ranges (Dudgeon, 1999). Bath et al. (1999) found higher abundance of molluscs with increased water temperature and decomposed organic matter. The mean annual water temperature (20.44 ^{0}C) at S2 also seems to favour molluscs in the river. In the lower reach of Manuni the river widens and the substratum is dominated by pebbles and cobbles with isolated boulders, thus providing suitable habitat to the Chlorocyphid larvae (*Rhinocypha*) which are mainly confined to stony streams where they hide under stones or submerged wood (Dudgeon, 1999) The benthic macroinvertebrate community of River Manuni was dominated by Ephemeroptera, Trichoptera and Diptera. This observation corresponds to the study of Farmahan (1994) in Barog hill stream of Himachal Pradesh. Similar composition of benthic macroinvertebrates has also been observed by Suren (1994) and Brewin *et al*. (2000) in streams of Nepal.

The physicochemical parameters of River Manuni also varied during its course. Thus increase in mean annual- water temperature (14.34-20.50^{0}C), total dissolved solids (0.04-0.14 gl^{-1}), nitrate (0.023-0.214 mgl^{-1}) and phosphate (0.012-0.112 mgl^{-1}) in the downstream and velocity (0.64-1.12 ms^{-1}) in the upstream could also be important contributing factors along with riparian vegetation in determining diversity of benthic macroinvertebrate communities among the different reaches of river Manuni. Similarly Subramanian et al., (2005) found that, the streams with natural riparian vegetation had higher insect richness than the ones with human modified ones in Kudremukh National Park Karnataka state, India.

To conclude the macroinvertebrate communities of River Manuni showed variation in distribution from headwater to downstream region. The upstream region supported a diverse benthic fauna which decreased in the downstream with the change in riparian land use by man.

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Present status of Icthyofaunal diversity of Garhwal Himalayan river Bhilangna and its tributaries with reference to changing environment

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Abstract

Fish as a group, from biodiversity view point has the highest species diversity among all vertebrate taxa. Present communication deals with the reassessment of ichthyofaunal diversity of the river Bhilangna and its two sub tributaries, the Balganga and the Nailchami of Bhagirathi river system in Garhwal Himalaya. The observation made during study showed the occurrence of 22 fish species belonging to 2 orders, 3 families and 9 genera from varying habitat of falls, cascades, rapids, riffles and pools in various sections of river Bhilangna and its tributaries. *Schizothorax richardsonii, S. plagiostomus* are dominate species in the riverine segment of river Bhilangna while *Cyprinus carpio* (common carp) is the dominate species in impoundment segment of river Bhilangana (reservoir area). The comparison of results of present study with earlier reports revealed that fish fauna has decreased with passage of time in the Bhilangana river system which may be due to degradation and fragmentation of riverine habitat caused by various developmental activities, changes in the natural flow pattern of river, indiscriminate fishing by the use of destructive and unscientific fishing methods, and other natural calamities.

***Keywords*:** *Fish diversity, Bhilangana river, habitat degradation, River fragementation*

Introduction

Biodiversity is essential for stabilization of ecosystem, protection of overall environment quality and for understanding the intrinsic worth of all species on the earth (Gadgill and Kar, 2000). The fish faunal diversity has its own importance like other aquatic and terrestrial animals. Fishes occupy all the possible habitat of aquatic ecosystem. Some of them are commercially important species with good economic value as food while other small sized species has own ecological importance being of important tropic link in water bodies. India has very rich fish diversity of approximately 2500 fish species, of which 930 are fresh water inhabitants (Jayaram, 1999, 2010). Several attempts have been carried out to document the fish diversity of different parts of India. Ponniah and Gopalakrishnan (2000) have documented the fish diversity of Western Ghats in form of a compendium entitled 'Endemic Fish Diversity of Western Ghats'. Lakra and Sarkar (2007) have edited a book entitled 'Fresh water fish diversity of Central India' based on the papers presented in a national workshop on 'Conservation Assessment of Fresh water Fish Diversity for central India' organized by NBFGR at Bhopal. Fish Biodiversity of North east India has also been reported in the proceeding of the workshop on 'North East Fish Germplasm Inventory and Conservation' edited by Ponniah and Sarkar (2000). Coldwater of Indian uplands also have rich diversified fish fauna. A total of 258 fish species, both indigenous and exotic ones, has been reported from Indian upland by Sunder *et al*. (1999). While Garhwal Himalayan water bodies encountered 64 species (Singh *et al.*1987). Pristine water resources with tremendous range of thermal regime support this diversity. Although several studies have been carried out on Ichthyo-faunal diversity of Garhwal Himalayan rivers and streams in past (Badola and Singh, 1980; Bahuguna and Singh, 1981; Singh and

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Bahuguna, 1983; Lakra *et al* 1987; Tilak and Baloni, 1983; Khanna and Badola 1991; Nautiyal and Lal, 1994; Agarwal *et al.*, 2005; Uniyal and Kumar 2006; Bisht *et al.*, 2009) but there is a need to revalidate the diversity and distribution of fish species over time and space as since past 3-4 decades the rich cold water fish biodiversity of Garhwal Himalayan water bodies is under intense pressure from a wide range of anthropogenic disturbances (Agarwal and Singh, 2009). Important among them are –altered land and water use, changes in river flow and habitat, over exploitation of natural stocks, and invasion of exotic species. Considering this fact, present study is made to re-evaluate the status of fish diversity of Bhilangna river –a major tributary of river Bhagirathi of Garhwal Himalaya from viewpoint of degradation and fragmentation of river habitat due to dam construction and various other developmental activities carried out in recent past. The tributaries of river Bhilangana -the Balganga and the Nailchami streams are also explored for stream habitat and Ichthyofaunal diversity in the present study.

Study area and physiography of the river Bhilangana and its tributaries

The Garhwal Himalaya situated between the latitude 29^0 26' to 31^0 28'N and longitudes 77^0 49' to 86^0 06'E is blessed with large number of river system. The river Bhilangna is a major tributary of river Ganga in Garhwal Himalaya. It originates from the Khatling glacier approximately 50 km south of the ice cave at Gaumukh at an elevation of 3717 masl. Earlier, the river Bhilangana flowed to a length of 95 Km from its origin to join river Bhagirathi at old Tehri. But after its impoundment for the Tehri dam reservoir, 25 km river length has inundated to lacustrine habitat thus shrinking riverine habitat to 70 Km length. Further, 45 km from the origin of the river Bhilangana, a small Bhilangana hydropower project of 22.5 MW has been constructed at Ghuttu village obstructing the natural flow of river while two small hydro power projects on this river are under planning stage (Fig 1).

The River Bhilangana has its own two sub-tributaries, namely the Balganga- right bank tributary and the Nailchami - left bank tributary (Fig 1). The Balganga is also facing the fragmentation of riverine habitat due to the construction of a small hydro-power project at 25 km upstream to Ghansali near Budakedar. The stream Nailchami- left bank tributary of river Bhilangna is a life line for people settled in the small hamlets along its banks. Water from the streams is abstracted for irrigation and drinking. The villagers do fishing in the stream for their own consumption and recreation. In summer and winter, river has very less water discharge due to abstraction of water to irrigate agricultural fields.

The change in river habitat, flow pattern and land and water uses have affected the density and diversity of fish fauna in the river Bhilangana and its tributaries. Considering this aspect, the river Bhilangana and its tributaries- the Balganga and Nailchami were thoroughly surveyed for the occurrence of fish fauna. Four sampling sites viz. S1, S2, S3, S4 were selected for the study. Sampling site S1 is located at Ghansali in lotic water and S2 at Pilkhi in lentic habitat of river Bhilangana. S3 site is located in Balganga stream at Chamyala and S4 is located on river Nailchami, 5 km upstream to Ghansali (Fig. 1).

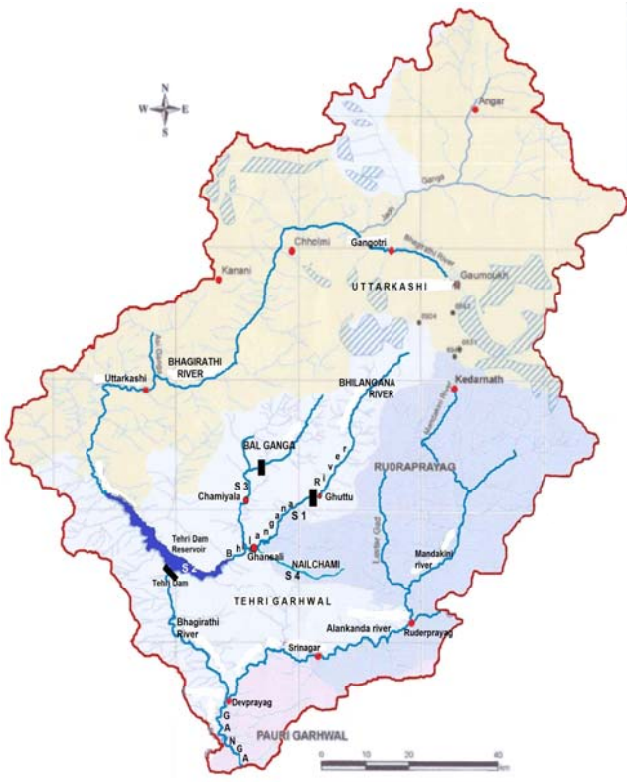

Fig. 1: Physiographic map of river Bhilangana and its tributaries.

Material and Methods

Regular sampling was conducted for collection of fishes from the river Bhilangana, the Balganga and the Nailchami during the year 2009-2011. Besides personal fish collection, fishes were also procured from local fishermen fishing at different sites using indigenous fishing methods. The common fishing methods namely -Gill net, Cast net, Hook and lines, Baur (Phans), Goda were used for fish collection. Colored images of fresh collected specimen were taken prior to their preservation. Fish specimens were preserved in 10% formaldehyde solution at the sampling sites. Large sized specimens were also injected with formalin for their better preservation. Extensive care was paid for proper maintaining of specimen during their transport from sampling sites to laboratory. The identification of fishes was done on the basis of various morphometric and meristric characters. Standard keys, literature and work of Day (1878), Srivastava (1968, 1980), Menon (1974), Tilak, (1987) Talwar and Jhingran (1991) Jayaram (1999), Badola (2009) was consulted for identification.

Results and Discussion

In our present study, 22 fish species belonging to 2 orders, 3 families and 9 genera have been encountered from varying habitat of falls, cascades, rapids, riffles and pools in various sections of river Bhilangna and its tributaries (Table 1). *Cyprinus carpio* (Common carp) is the new record of fish species reported from the lentic habitat of this river. Studies revealed that there is heterogeneity in habitat and ecological characteristics of both the river Bhilangna and its tributaries. In the upper reaches from its origin to Ghansali, river Bhilangana (S1) flows torrentially due to high gradient. River has rocky substratum with big and small sized boulders in this section. The torrentially flowing river has become almost stand still, downstream to Pilkhi as it is impounded for Tehri dam reservoir. River has deep water and sandy substratum for 25 km stretch of impounded area (S2). The pattern of species distribution and abundance highly varied in these two different habitat (lotic and lentic).

From river Bhilangna upstream to Ghansali (S1), 21 species belonging to 2 order, 3 families and 9 genera have been observed during study period (Table 1). Family Cyprinidae (representing 13 species) was found dominated over Cobitidae family (representing 6 species) and Sisoiridae family (representing 2 species). The *Schizothorax richardsonii* was recorded most abundant fish species throughout all seasons. Species of genus *Tor* and *Pseudochenus* were also reported along with other *Schizothorax* species but in limited number. Further *Noemacheilus* and *Barilius* spp. were also reported in some catches during study period but species of genus *Garra*, *Botia* and *Glyptothorax* were found rarely only in few catches. However, earlier reports of Badola (1979) showed the occurrence of 43 species and Singh *et at.* (1983) reported 37 species from same river Bhilangna (Table 2). The observation of present study when compared with the work on fish faunal diversity reported during 1980's, it appears that there is continuous decrease in fish fauna with passage of time. The factors responsible for this decline are the unscientific and unsustainable fishing methods (Dynamiting Poisoning, Electrocuting, and Channel diversion), various developmental activities such as Dam, and road construction, and other natural calamities like adverse weather conditions especially flood in monsoon season which flows away all the eggs and small fishes. *Schizothorax richardsonii*, *S. plagiostomus* were dominant species in past and these are still dominant at present but the total fish catch has drastically decreased with the passage of time.

The fish catch of impounded area of river Bhilangna (S2) (lentic habitat) is found dominated by *Cyprinus carpio* followed by *Tor putitora* with occasional occurrence of *Tor tor* and Snow trout *Schizothoraichthys progastus*. No other species was observed from this stretch. *Tor putitora*, *Tor tor* and *Schizothoraichthys progastus* are column feeder native fish species. These were also present in the Bhilangana river before the reservoir came into existence. These can very well withstand in the impounded water/lentic habitat due to column feeding habit. But introduction of exotic carp *Cyprinus carpio* has proved detrimental to these native fish species.

From the Balganga stream (S3), 20 species belonging to 2 orders, 3 families and 7 genera have been reported. Species of genus *Schizothorax* were observed abundantly. *Tor* and *Pseudecheneis* spp. were procured in limited number.

Table1. Status of Ichthyofaunal diversity of river Bhilangna and its tributaries.

Name of species	Local name	Sampling sites			
		Balganga	Nailchami	Bhilangana	Bhilangana river impoundment
Order Cypriniformes					
1. Family Cyprinidae					
Tor putitora	Khasra	c	c	c	c
Tor tor	Khasra	r	n	r	r
T. chilinoides	Mahaser	c	r	a	n
Schizothorax richardsonii	Maseen	a	c	a	n
S. plagiostomus	Asela	c	c	a	n
S. sinuatus	Maseen	r	r	r	n
Schizothoraichthys progastus	Chongu	r	n	r	r
S. curvifrons	Chongu	r	n	c	n
Barilius bendelisis	Fulra	c	c	c	n
B.barna	Fulra	r	c	c	n
B.bola	Dhaur	r	r	n	n
B.vagra	Fulra	c	c	r	n
Garra gotyla gotyla	Gunthala	r	n	r	n
Cyprinus carpio	Carp	n	n	r	a
2. Family Cobitidae					
Noemacheilus rupicola	Gadiyal	c	c	c	n
N. montanus	Gadiyal	c	c	c	n
N. bevani	Gadiyal	c	c	c	n
N. savona	Gadiyal	r	r	r	n
N. multifasciatus	Gadiyal	c	c	c	n
Botia Dario	Gadiyal	n	n	r	n
Order Siluriformes					
1. Family Sisoiridae					
Glyptothorax pectinopterus	Kathrua	c	r	c	n
Pseudecheineis sulcatus	Kathrua	c	r	c	n
Total number of texa reported = 22		**20**	**16**	**21**	**4**

a=Abundant; c=Common; r=Rare; n=Nil

From the stream Nailchami (S4), 16 species belonging to 2 orders, 3 families and 6 genera were recorded. Species of genus *Schizothorax* were also found dominated over other species in this stream but average size of them are less in comparison to the fishes procured from the rivers Bhilangana and Balganga. Both the tributaries of river Bhilangana – the Balganga and the Nailchami were unexplored from the view point of their fish diversity. The result of present study has filled this lacuna and is an attempt to develop base line data of icthyofaunal diversity of these streams. In River Balganga and Nailchami, maximum fish diversity was observed during monsoon and summer months in contrast to winter months.This may be related with the

migratory behavior of some fishes which migrate upstream when river is flooded. Substratum of river largely influences distribution of fish fauna. The substratum of Bhilangna River in the lentic habitat (S2) is sandy while upstream to Pilkhi in the lotic habitat of river, the substratum is stony.Due to such type of sandy substratum, only four species of column feeding habit has been recorded from S2.

Table 2. Fish fauna of river Bhilangana in relation to previous reports

Name of species	Badola (1979)	Singh *et al.* (1983)	Present study
Schizothorax richardsonii	P	c	a
S. plagiostomus	p	c	a
S. sinuatus	p	c	r
Schizothoraichthys progastus	p	r	r
S. esocinus	p	r	n
S.micropogon	p	c	n
S. longipinnis	p	n	n
S. curvifrons	p	c	c
S. niger	p	c	n
S. planifrons	p	n	n
S. intermedius	ab	c	n
Barilius bendelisis	p	c	c
B. shacra	p	n	n
B. barna	p	c	c
B. barila	p	c	n
B. vagra	p	c	r
B. bola	ab	r	r
Labeo dyocheilus	p	c	n
L. dero	p	c	n
Tor tor	p	r	r
T. putitora	p	r	c
T. chilinoides	p	c	a
T. hexastichus	p	n	n
Noemacheilus rupicola	p	a	c
N. montanus	p	a	c
N. bevani	p	a	c
N. savona	p	a	r
N. denisonii	p	n	n
N. zonatus	p	n	n
N. multifasciatus	p	c	c
Garra gotyla gotyla	p	c	r
G. lamta	p	r	n
Garra prashadi	ab	c	n
Glyptothorax madraspatanum	p	n	n
G. pectinopterus	p	c	c
G. telchitta	p	n	n
G. conirostris	p	c	n
G. cavia	p	c	n
G. trilineatus	p	n	n
G. kashmirensis	p	n	n
G. brevipinnis	p	c	n
Pseudecheneis sulcatus	p	c	c
Crossocheilus latius latius	p	c	n
Clupisoma garua	p	r	n
Euchiloganis hodgarti	p	n	n
Chagunius chagunio	p	n	n
Botia dario	ab	r	r
Balitora brucei	ab	r	n
Mastacembelus armatus	ab	c	n
Cyprinus carpio	ab	n	r*
Total number of texa reported	**43**	**37**	**22**

P=present,ab=absent,a=Abundant;c=Common;r=Rare;n=Nil,r*= rare in river and abundant in impoundment

However, twenty one species are found in the upstream as fast flowing meandering river having rocky bed with stones and pebbles supports the survival of various life stages of fish. A striking observation during the collection of fish fauna is- the high dominance of *Cyprinus carpio* (introduced exotic carp) in the fish catch from impounded water of river Bhilangana. The change in river habitat (from riverine to lacustrine) has lead to the drastic changes in fish species composition and distribution. Prior to impoundment, fishes of snowtrout group namely *Schizothorax richardsonii, S. plagiostomus, S. curvifrons,* and *Schizothoraichthys progastus* were dominated in fish catch. *Tor putitora, S. sinuatus, Pseudecheneis sulcatus. Glyptothorax pectinopterus* were also found regularly in the fish catch. The *Schizothorax richardsonii, S. plagiostomus, S. curvifrons, Garra gotyla gotyla* are bottom feeder, herbivorous fish species highly adapted for scrapping periphyton (algae, diatoms) from submerged stones and boulders in the river (Fig. 2). The *Pseudecheneis* and *Glyptothorax* spp. are also bottom feeder, feeds on stony substratum and highly adapted to withstand in fast water current of torrential hill streams. Thus, these species were surviving very well in fast flowing environment of river Bhilangana due to presence of good feeding and breeding ground on its shallow banks with stony and bouldary substratum. As the river has been impounded, this peculiar hill stream river habitat is now replaced with the stagnant/slow moving deep water, steep banks and sandy substratum. The peculiar feeding and breeding ground characteristics of fish have disappeared. This has forced the bottom feeder hill stream fish species to migrate upstream in fast flowing shallow areas having stony substratum. Thus, the impounded area of Bhilangana river (25 km stretch) is now totally devoid of above bottom feeder resident species, restricting these species to riverine reaches only. The native fish species -*Tor putitora, Tor tor* (Mahseer) and *Schizothoraichthys progastus* are of column feeder habit thrive in deep water, hence have remained in impounded segment of river. These are now struggling for their existence in changed habitat with the introduced exotic carp *Cyprinus carpio*- giving tough competition due to its fast growth and prolific breeding habit. Thus, fragmentation of river habitat and change in river flow has affected species distribution. At present, impounded section of river has only four species in comparison of 21 species (unpublished observation) observed earlier, before the reservoir came into existence.

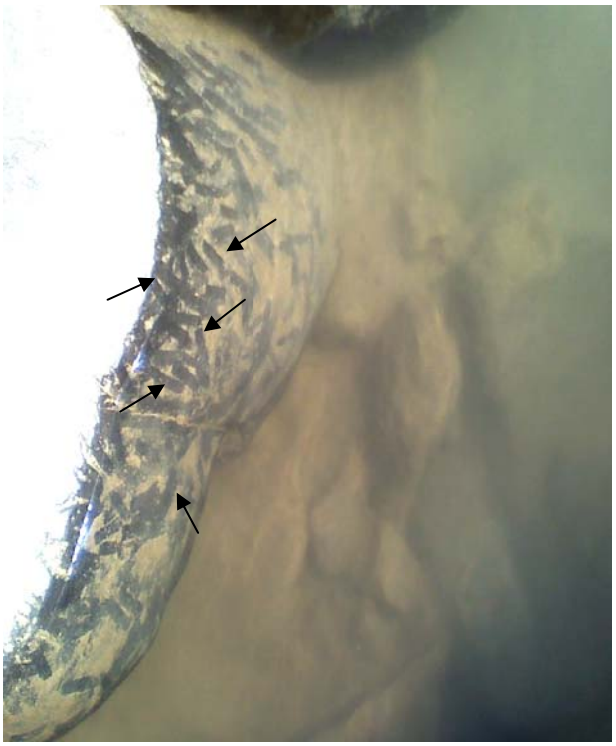

Fig.2: Feeding marks of bottom feeder snowtrout species on a boulder. Fish scrapes algae and periphyton from stones and boulders by hard cartilaginous lower labial fold on ventrally situated mouth.

Total water discharge of river is directly proportional to distribution of fish fauna. High discharge of water will have large size fishes. The Nailchami stream having less water discharge has small sized fishes as compare to river Bhilangna and Balganga stream. The water of Nailchami stream is abstracted for agricultural fields located on both sides along its length thus, further reducing the water discharge in summer and winter months restricting the occurrence of small size fishes with low faunal diversity. Stream also has high gradient with cascade type habitat providing frequent falls and shallow pools among big boulders in upper reaches. Though, in lower reaches, stream has shallow runs with stony, pebbly substratum- ideal for small sized *Barilius* sp. and loaches. Stream is highly disturbed due to anthropogenic activities. Use of bleaching powder, icthyotoxic plants, and

channel diversion for fishing has drastically affected the fish population in the stream.

Gradient is inversely proportional to distribution of fish fauna. River Bhilangna and its tributaries-Balganga and Nailchami have high gradient towards their sources, thus density and diversity of fish is low in the upper stretches of these streams.

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Conservation of alpine pasture in Himachal Pradesh, India

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Abstract

Himalayan Alpine pasture occurs at high altitude mountains in between the tree line and perpetual snow line. These constitute one of the important land cover. These pastures contain very good grasses and medicinal plants thus traditionally tribal people depend on these pastures for grazing their animals during summer. 75% of the total alpine pastures in Himalayan region are situated in Himachal Pradesh.The alpine pasture of Himachal Pradesh provides a matchless wealth of highly priced medicinal, aromatic plants and known as a natural reservoir of these herbs. In alpine pasture and meadows due to continuous loss of forest land, uncontrolled grazing and irregular exploitation of medicinal herbs by commercial enterprises have resulted in depletion of valuable medicinal plants used since ancient times. There are many medicinal plants which have become rare in several tracts while a few others have fallen in the list of endangered species. Therefore it has been felt that there is an urgent need for ex-situ and in-situ conservation of these valuable and threatened species.

Keywords: *Medicinal plant, grazing, ecosystem, trible people*

Introduction

75% of the total alpine pastures in Himalayan region are situated in Himachal Pradesh. 16-19%of the total geographical area of the state constitute the alpine pasture. The alpine pasture occupies 827.31 sq.km.area which account for 12.84% of the chamba district. In the chamba district the alpine pasture girdles around three prominent ranges .starting from south they are:-

1. Dhaulandar range (303.03 sq.km.)
2. Pir Panjal or Pangi range (445.58 Sq.km.)
3. Zanskar range (78.70 sq.km.) (a\c to data provided by Remote sensing office, Shimla)

The use of medicinal plants for curing diseases in human society is almost as old as man himself. The earliest mentioned use of medicinal plants is found in Rigveda.After the Vedas there is no information on the development of this science in India for a period of about 1000 years. The study of Indian indigenous drugs first started in the early part of the first century and it was then confined to the collection of available information with regard to various medicinal plants growing in different parts of the country .Extensive studies has been carried out in exploring the medicinal plants specially of alpine pasture from different region:

Chawdhery and Wadhwa (1984) studied the Alpine flora of Himachal Pradesh, Chopra, and Chopra (1955) reviewed work on medicinal plants, Dey. (1996) studied indigenous drugs of India, Gammie (1898) studied botanical tour to Chamba and Kangra. Jain and Sartry (1979) studied threatened plants of India; Joshi (1962) worked on preliminary study of the Alpine Flora of Rudranath Bugyal of Distt. Chamoli (North Garhwal). Samant and Palni (2001) reported diversity, distribution and indigenous uses of essential oil yielding plants of Indian Himalayan region. Sharma and Singh (1990) reported phytogeographical observations on the flora of Chamba district, Himachal Pradesh.

The demand of these high qualities of medicinal herbs from alpine region of Himachal Pradesh is increasing day by day within and outside the

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country .Time has come when serious and effective measures are required to meet the challenge. Therefore this is a time to think and formulate strategy for the conservation for supply of raw materials to industries, users and above all to maintain ecological balance of these areas.

Materials and Method

The medicinal plants were collected from time to time during flowering and fruiting season. The plants collected from study area were consisted of almost all parts so that they can be easily recognized and able to provide maximum information. Herbarium of plants was also prepared.

Beside this information related to collected plants was also gathered by discussing with people inhabiting the area. Study material from various reference books was also referred.

Results and Discussion

The following alpine medicinal plants of Himachal Pradesh have been collected likely to be endangered (rare) groups therefore there is an urgent need for ex-situ and in-situ conservation.

1. *Aconitum benthamii*
2. *Artemisia brevifolia*
3. *Atropa acuminata*
4. *Angelica glauca*
5. *Colchicum luteum*
6. *Corydalis govaniana.*
7. *Dactylorhiza hatagirea*
8. *Delphinium denudatum*
9. *Delphinium brunonianum*
10. *Ephedra gerardiana*
11. *Fritillaria roylei*
12. *Gentiana kurroo*
13. *Hedychium spicatum*
14. *Hyoscyamus niger*
15. *Hyoscyamus niger*
16. *Jurinea dolomiaca*
17. *Meconopsis aculeate*
18. *Nardostachys grandiflora*
19. *Picrorhiza kurroa*
20. *Picrasma quassioides*
21. *Podophyllum hexandrum*
22. *Rheum australe*
23. *Saussurea costus*
24 *Saussurea hypoleuca*

The species mentioned above are mostly from alpine and sub-alpine regions of Himachal Pradesh, which have limited scope for their sustenance in wild imperative need to conserve these species of rhizomes and roots, which remain dormant more than two to six months under heavy snow. So there is an urgent need to save these plants for their multiplication by growing them on mass scale in temperate regions of Himachal Pradesh , for internal consumption of pharmaceutical enterprises, such as Ayurvedic, Unani, Homeopathic, Sidha and also in Allopathic system of medicines

Socio-economic and ecological importance of Alpine Pasture-

Grass lands are both a component and product of ecosystem. As a product of ecosystem they provide extractable products which have been utilized for food, essential oil, paper making, ornamental and medicinal purposes etc.

As a component of ecosystem they play an important role in protection of water shed

And soil conservation, nutrient cycling and biological diversity.

In Himachal Pradesh particularly in Chamba and Kinaur district grazing pressure is tremendous. The region supports 2,47,117 cattle,34,718 buffaloes , 2,69,923 sheep and 1, 73,169 goats. (a\c Data provided by censes department).

From available information from the forest department of chamba district, some of live stock from adjoining states of Haryana and Punjab are brought for grazing during summer in this alpine pasture. Due to this pressure of grazing, these alpine pastures are being degraded continuously.

Environmental impact of Grazing -

The environmental impact of grazing has to be evaluated in terms of grazing practices.Most of the damage to grass land is done along the migratory routes.Grazing by sheep and goat harm younger shoot tips, effecting shoot growth and causing soil compaction and thus reducing soil fertility. The other impact of over grazing is loss of vegetation cover which accelerates, soil erosion, land slides and subsequently results in salutation of rivers and dams. Beside this, overgrazing causes species destruction by trampling of reproductive plant points. Continuous grazing at the same place over a period causes loss of palatable species and increase

in the number of unpalatable species and ultimately effects ecological succession.

Consideration Points

The following facts need consideration while formulating action plan.

Alpine pasture constitute unique ecosystem having economical, social and ecological importance.

The tribal population is closely linked with this ecosystem and subsides on it for their livelihood.

Apart from good grasses this zone is a repository of very valuable herbs having medicinal value.

Proximity of Chamba district to Punjab plains and as plains depends on the hill for various kinds of raw material.

Considering the above facts it goes without saying alpine pastues need systemic study and scientific management.

Management Needs -

The following measures may be taken up for sustained productivity of the pasture lands.

Constituting an alpine pasture development society with the involvement of users especially 'Gaddies and Gujjars' (Tribal Communities) .It could be termed under the gaddies development board and gujjars development boards.

Alpine pasture should be divided into different sectors based on the migratory routes and each sector should be opened for grazing on rotational basis.

A mission should be launched with the help of beneficiaries to eradicate the undesirable growth of weeds and undesirable grass species.

Aerial broadcasting of suitable seeds should be taken up before the snowfall season preferably in the month of Oct. and early Nov.

The tribal people should be trained in the scientific collection of herbs and medicinal plants.

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Identification of xylanase producing *Bacillus licheniformis* strain C1 and properties of crude xylanase

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Abstract

In the present investigation xylanase producing bacteria was isolated from compost. A total of 95 xylanolytic bacteria were isolated on oat spelt xylan agar medium and screened by the xylanolysis method. Out of these 95 isolates, only one bacterial isolates, strain C1 was selected for further study on the basis of zone of hydrolysis on xylan-congo red agar plate. This strain was identified by 16S rDNA analysis. The phylogenetic analysis using 16S rDNA sequence data showed that isolate C1 showed highest nucleotide identity of 98% with *Bacillus licheniformis* strain CICC 10181 (GenBank accession no. GQ375235) and identified as *Bacillus licheniformis* strain C1. *Bacillus licheniformis* strain C1 was gram positive and rod shaped. Morphology of *Bacillus licheniformis* strain C1 showed- smooth texture, medium size, opaque transparency, creamish-white colour and serrated margin. Maximal xylanase production for *Bacillus licheniformis* strain C1 was achieved at the incubation period of 48 h. Xylanase and cellulase activities were determined as 20.0 U/ml and 1.3 U/ml, respectively. The optimum pH and optimum temperature for xylanase activity was found to be 7.0 and 60°C, respectively. Xylanase was found to be thermostable at 60°C for 1h and retained 90% of its activity upto 6 h at this temperature. Approximately, 74% and 70% of its activity was retained at 70°C and 80°C respectively, after 6 h of incubation. All of these properties of the *Bacillus licheniformis* strain C1 xylanase make the suitability of this enzyme for its use in feed and baking industry.

Keywords: *Feed and baking industry; phylogenetic analysis; 16S rDNA analysis; xylanase.*

Introduction

Xylans are linear polysaccharides formed from β-1,4 -linked d-xylopyranoses. In cereals, xylans frequently contain side chains of α-1,2, α -1,3, or α -1,2 and α -1,3 linked L-arabinofuranoside. This substituted xylan is commonly referred to as arabinoxylans. Xylanases (endo-1,4- β -xylanase,E.C. 3.2.2.8) hydrolyze internal β -1,4-xylosidic linkages in xylan to produce smaller molecular weight xylo-oligomers.Xylanase can be used in animal feed, in baking and in brewing which are rich in arabinoxylans. The addition of xylanase to feeds (e.g. for monogastric animals, including poultry or swine) which contain cereals (e.g. barley, wheat, maize, rye, triticale or oats) or cereal by-products, improves the break-down of plant cell walls which leads to better utilization of the plant nutrients by the animal. This leads to improved growth rate and feed conversion, also reduced the viscosity of the feeds containing xylan. The xylanase may be used as a supplement in animal feed by itself or in combination with vitamins, minerals, and other feed enzymes, agricultural co-products (e.g., wheat middlings or corn gluten meal). The xylanase may be used in monogastrics as well as in polygastrics. Diets supplemented with xylanase improve feed conversion ratio. Xylanases have also been used in bread making industry as bread improvers (Javier *et al*., 2007), where it improves dough and bread quality leading to improved dough flexibility, machinability, stability, loaf volume and crumb structure (Polizeli *et al*., 2005). Xylanases directly or indirectly improve the strength of the gluten network and therefore, improve the quality of bread (Baillet *et al.,* 2003). Xylans have an important role in bread quality due to their water absorption capability and interaction with gluten (Guy and

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Sarabjit, 2003). The hydrolysis of xylan using xylanase improves the dough properties, leading to a greater uniformity in quality characteristics (Gray and BeMiller, 2003). Xylanases make the dough soft, *i.e.* reduce the sheeting work requirements and significantly increase the volume of the baked bread (Dervilly *et al*., 2002, Harbak and Thygesen, 2002). It significantly improves manufacturing conditions: made dough more 'machine-friendly' and it does not stick to the machinery parts (Nuyens *et al*., 2001, Rouau *et al*., 1994). Xylanases have gained much importance owing to their application in feed, food and fermentation industries (Rouau, 1993). Most commercial xylanases designed for feed applications are not very thermotolerant, especially when neutral or alkaline pH conditions are used. These xylanases are generally inefficient or inactive at temperatures higher than 60° C and often work under acidic and neutral conditions. The aim of present study was to isolate and identify new xylanase producing bacteria which secrete thermostable xylanase active under neutral and acidic condition and to make the applicability of this xylanase for feed and baking industry.

Material and Methods

A thermophilic bacteria was isolated from compost (MRDC, Pantnagar). This strain produced extracellular, thermostable xylanase on oat spelt xylan agar medium and screened by the xylanolysis method.

Isolation and screening

Sample suspensions in sterilized water were poured and spread onto nutrient agar plates. These plates were incubated at 60°C for 2 days and constantly observed for the appearance of bacterial colonies. Colonies found on these plates were transferred onto oat spelt xylan agar plates and incubated at 60°C for 2 days. This thermophilic bacterial strain was tested for their capabilities to produce xylanase by growing them on xylan-congo red agar medium. Bacteria was further screened by growing them in liquid medium and assayed for xylanase activity in cell-free culture supernatant fluid and cell pellet.

Culture media

The thermophilic bacteria were screened into nutrient agar media having pH 7.0. The screening of xylanase producing bacteria were done by growing them into following media: oat spelt xylan, 1%; yeast extract, 0.2 %; peptone, 0.5%; $MgSO_4$, 0.05%; NaCl, 0.05 %; $CaCl_2$, 0.015 % and agar, 2% at pH 7.0 (Cordeiro *et al*., 2002). Qualitative analysis of xylanase positive isolates was done on xylan-congo red media consisting: K_2HPO_4, 0.05%; $MgSO_4$, 0.025%; congo red, 0.02%; oat spelt xylan, 0.5%; agar, 0.5% and gelatin, 0.2% having pH 7.0 (Hendrickset, 1995).

PCR amplification of the 16S rDNA and sequence determination

For the 16S rDNA sequence analysis, bacterial genomic DNA was extracted, than 16S rDNA gene was amplified by PCR using 5' AGAGTTTGATCCTGGCTCAG-3' and 5' AAGGAGGTGATCCAGCCGCA-3' as the forward and reverse primers, respectively (Edwards *et al*., 1989). The amplification was carried out in 25 µl of reaction mixture containing 4.0 µl of DNA template, 2.5 µl of PCR buffer (10x) (Bangalore, Genei), 1.5 µl of dNTP (10 mM) (Bangalore, Genei), 2.0 µl of the primers FP (40 ng) and RP (40 ng) respectively, 1.5 µl of Taq polymerase (5 U/µl) (Bangalore, Genei) and 11.5 µl of autoclaved Milli-Q water (Millipore). The PCR program was run for 35 cycles in thermal cycler (Eppendorf, Germany). The following thermal profile was used for PCR: denaturation at 94°C for 1 min, annealing at 64°C for 1 min, extension at 72°C for 1min 30sec. Final cycle included extension for 10 min at 72°C to ensure full extension of product. The amplified PCR products were analyzed in a 1.0 % (w/v) agarose gel, excised and purified from the gel (by Spin gel extraction kit, Genei). The sequencing of purified PCR product was done by the Bangalore, Genei (India) using automated ABI 3100 Genetic Analyzer with fluorescent labeled dye terminators. The ABI's AmpliTaq FS Dye terminator cycle sequencing is based on Sanger's sequencing method. Databases (GeneBank) were searched for sequences similarity analysis of the 16S rDNA sequence obtained. The 16S rDNA gene sequence of the isolate was aligned with reference 16S rDNA sequences of the European Microbiological Laboratory (EMBL), GenBank (gb, Germany) using the BLAST algorithm (Altschulet, 1997) available in NCBI (National Centre for Biotechnology information) in internet.

Enzyme production

Growth media used for enzyme production contained: oat spelt xylan (OSX) 1%, yeast extract 0.2%, peptone 0.5%, $MgSO_4$ 0.05%, NaCl 0.05%, $CaCl_2$ 0.015%, pH 7.0. Media was inoculated with 10% of overnight culture and incubated at 60°C

with aeration in shaker at 200 r.p.m. for 2 days. Before assay the cells were separated by centrifugation at 10,000g and clear supernatant used as crude enzyme.

Enzyme assay

Xylanase activity was assayed by measuring the release of reducing sugar from oat spelt xylan. Reaction mixture consisted of 1% xylan in 0.1 M Tris-HCl buffer (pH, 7.0) and enzyme to give a final volume of 1.0 ml. After incubating for 10 min at 60^0C, the release of reducing sugar was determined by Nelson-Somogyi method (Nelson, 1994 and Somogyi, 1952). The cellulase activity was measured under the same condition as described above using carboxymethyl cellulose as a substrate.

One unit of xylanase and cellulase is defined as the amount of enzyme required to release 1µmol of reducing sugar, xylose or glucose, per min under above assay condition.

Protein estimation

The protein content was determined by Lowry method using bovine serum albumin as standard (Lowry, 1951).

Incubation time on xylanase production

Bacillus licheniformis strain C1 was tested for xylanase producing ability in production media containing xylan. The active culture was inoculated into production medium and incubated under shaking at 60□C for 96 h. The enzyme activity and growth (at 600nm) were determined periodically after time intervals of 12h.

Effect of pH on activity of xylanase

The activities of xylanase at various pH values were measured by using oat spelt xylan (OSX) as substrate. The reaction pH was adjusted from 5.0 - 10.0 with various buffers by incubating xylanase with suitable amount of buffer solution containing xylan as substrate. The buffers used were 0.1M citrate-phosphate buffer for pH 5.0-6.0, 0.1M Tris-HCl buffer for pH 7.0-8.0 and 0.1M Glycine-NaOH buffer for pH 9.0-10.0.

Effect of temperature on activity and stability of xylanase

The effect of temperature on the enzyme activity was determined by assaying xylanase within temperature range of 40°C -80°C at pH 7.0 (0.1mM Tris-HCl buffer). Thermostability was determined by incubating crude xylanase at temperature ranging from 40°C-80°C for 1-6 hrs and residual enzyme activity was determined. The residual activity was quantified, at optimum temperature i.e. on 60°C, using Nelson-Somogyi method at their optimum pH (7.0).

Results and Discussion

Screening of bacterial isolates

In the present study a total of 95 isolates were screened. Among these isolated strains good xylanase producers were screened by growing into xylan-congo red agar media. The large zone of hydrolysis was produced by *Bacillus licheniformis* strain C1 on xylan-congo red agar plates (Fig: 1). This strain was further tested for xylanase production by growing into oat spelt xylan agar medium.

Characteristics of bacterial isolate

Gram staining

Bacteria were identified by Gram stain reaction (Gerhardt *et al.,* 1994). Isolate *Bacillus licheniformis* strain C1 was rod shaped and gram positive (Fig: 2).

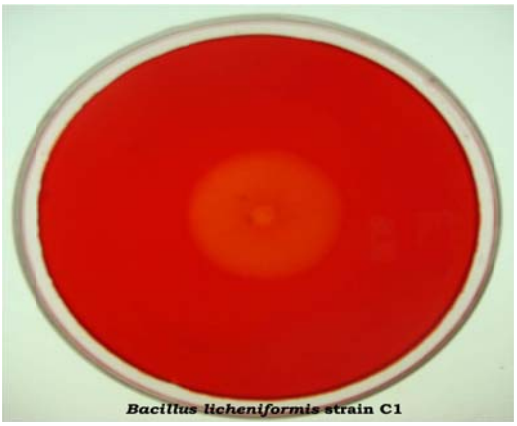

Fig 1: Zone of hydrolysis on xylan-congo red agar plate for *Bacillus licheniformis* strain C1

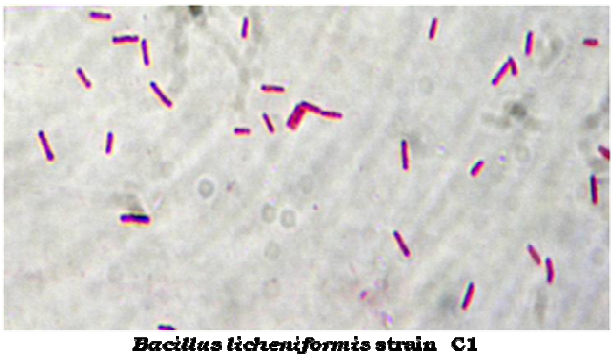

Fig 2: Gram staining of *Bacillus licheniformis* strain C1

Colony morphology of bacterial isolate

Bacillus licheniformis strain C1 was studied for their colony morphology-texture, size, transparency, colour and margin as shown in Table: 1.

Strain C1 identification by 16S rDNA sequence

In order to confirm the identification of strain C1, the 16S rDNA was amplified after PCR amplification of genomic DNA. The amplified product was analyzed in 1.0% agarose gel (Fig: 3). Sequencing result shows that amplified 16S rDNA sequence was 1502 bp (Fig: 4). The16S rDNA analysis of isolate C1 shows its close phylogenetics to the genus *Bacillus* rRNA group. Isolate C1 showed highest nucleotide identity of 98% with *Bacillus licheniformis* strain CICC 10181 (GenBank accession no. GQ375235). Therefore phylogenetic analysis confirmed that isolate C1 was one strain of *Bacillus licheniformis* and identified as *Bacillus licheniformis* strain C1 (Fig: 5).

Bacterial growth and xylanase production

Bacterial isolate was evaluated for their growth in terms of O.D. at 600nm, enzyme activity U/ml, enzyme activity U/mg and protein concentration after 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h and 96 h of growth. The highest xylanase activity for *Bacillus licheniformis* strain C1 was achieved after 48 h of incubation. Further incubation after this time cause decreased in xylanase production (Fig: 6).

Table 1: Colony morphology of bacterial isolate

S. N.	Isolates	Texture	Size	Transparency	Colour	Margin
1.	*Bacillus licheniformis* strain C1	Smooth	Medium	Opaque	Creamish White	Serrated

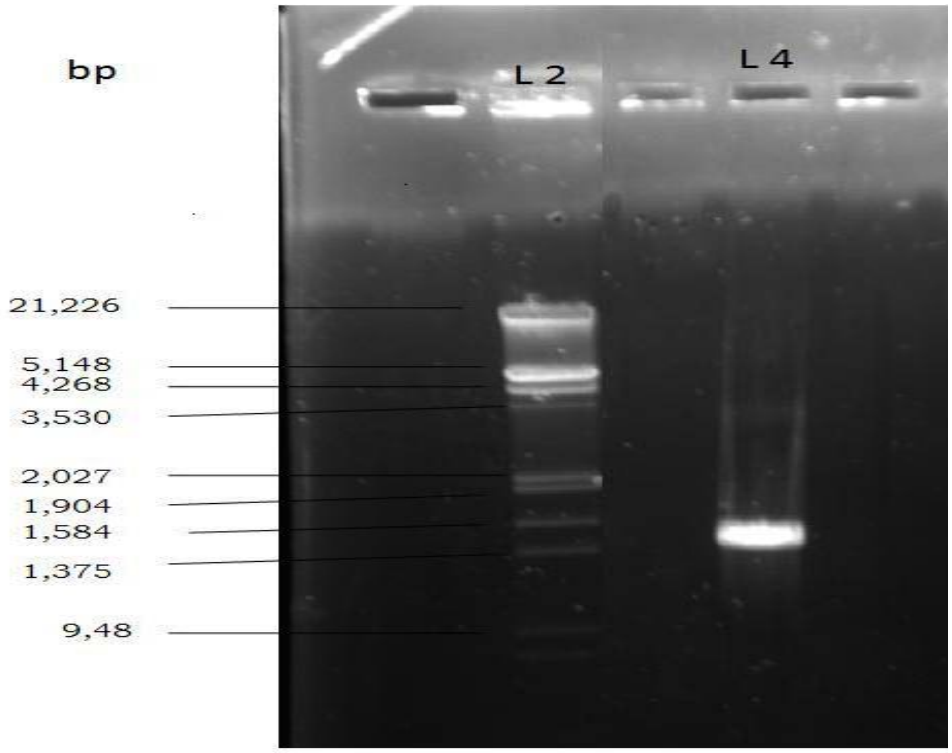

Fig 3: Agarose gel electrophoresis of amplified 16S rDNA product of *Bacillus licheniformis* strain C1. (1) Marker (Lambda DNA/Eco RI/ Hind III double digest, at lane 2) (2) amplified PCR product (16S rDNA, at lane 4).

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   1 TGTTTTTTTG TTAAGAGCGA GTCCTATAAT GCAGTCGAGC GGACAGATGG GAGCTTGCTC
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 121 AACTCCGGGA AACCGGGGCT AATACCGGAT GCTTGATTGA ACCGCATGGT TCAATTATAA
 181 AAGGTGGCTT TTACCTACCA CTTACAGATG GACCCGCGGC GCATTAGCTA GTTGGTGAGG
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 301 ACTGAAACAC GGCCCAAACT CCTACGGGAG GCAGCAGTAG GGAATCTTCC GCAATGGACG
 361 AAAGTCTGAC GGAGCAACGC CGCGTGAGTG ATGAAGGTTT TCGGATCGTA AAACTCTGTT
 421 GTTAGGGAAA AACAAGTACC GTTCGAATAG GGCGGTACCT TGACGGTACC TAACCAGAAA
 481 GCCACGGCTA ACTACGTGCC ACCAGCCGCG GTAATACGTA GGTGGCAAGC GTTGTCCGGA
 541 ATTATTGGGC GTAAAGCGCG CGCAGGCGGT TTCTTAAGTC TGATGTGAAA GCCCCCGGCT
 601 CAACCGGGGA GGTTCATTGG AAAGCTGGGG AACTTGAGTG CAGAAGAGGA GAGTGGATTT
 661 CCGCGTGTAG CGGTGAAGTG AGTAGAGATG TGGGAGGAAC ATCAGTGGAG AAGGCGTCTC
 721 TTTGCTGTGT AACTGACGCT GAGGCGCGAA AGCGTGGGGA GCGAACAGGA TTAGATACCG
 781 TGGTAGTCCA CGCCGTAAAC GATGAGTGTT AAGTGTTAGA GGTTTTCCGC CCTTTAGTGC
 841 TGCAGCAAAC GCATTAAGCA CTCCGCCTGG GGAGTACGGT CGCAAGACTG AAACTCAAAG
 901 GAATTGACGG GGGCCCGCAC AAGCGGTGGA GCATGTGGTT TAATTCGAAG CAACGCGAAG
 961 AACCTTACCA GGTCTTGACA TCCTCTGACA ACCCTAGAGA TAGGGCTTCC CCTTCGGGGG
1021 CAGAGTGACA GGTGGTGCAT GGTTGTCGTC AGCTCGTGTC GTGAGATGTT GGGTTAAGTC
1081 CCGCAACGAG CGCAACCCTT GATCTTAGTT GCCAGCATTC AGTTGGGCAC TCTAAGGTGA
1141 CTGCCGGTGA CAAACCGGAG GAAGGTGGGG ATGACGTCAA ATCATCATGC CCCTTATGAC
1201 CTGGGCTACA CACGTGCTAC AATGGGCAGA ACAAAGGGCA GCGAAGCCGC GAGGCTAAGC
1261 CAATCCCACA AATCTGTTCT CAGTTCGGAT CGCAGTCTGC AACTCGACTG CGTGAAGCTG
1321 GAATCGCTAG TAATCGCGGA TCAGCATGCC GCGGTGAATA CGTTCCCGGG CCTTGTACAC
1381 ACCGCCCGTC ACACCACGAG AGTTTGTAAC ACCCGAAGTC GGTGAGGTAA CCTTTTGGAG
1441 CCAGCCGCCG AAGGTGGGAC AGATGATTGG GGGAAGTCGA TCAAGAGCTG GAAAAAAAAT
1501 AT
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Fig 4: *Bacillus licheniformis* strain C1, 16S rDNA partial sequence (Length=1502)

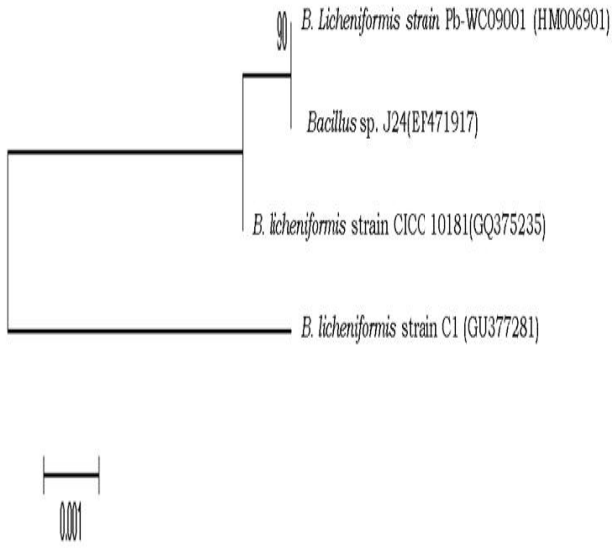

Fig 5: Phylogenetic analysis of partial 16S rDNA gene sequence of *Bacillus licheniformis* strain C1 and related microorganism, accession no. are given after species names. Bootstrap values (1,000 replicate runs, shown as percent)

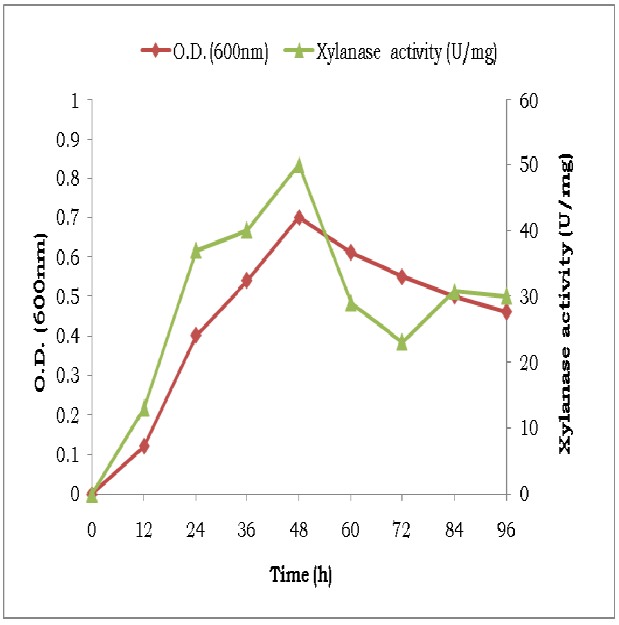

Fig 6: Effect of time of incubation on growth and xylanase activity for *Bacillus licheniformis* strain C1

Xylanase activity

The xylanase activity for *Bacillus licheniformis* strain C1 was found to be 50.0 U/mg or 20.0 U/ml. The xylanase activity of *Bacillus licheniformis* strain C1 may be compared with previously reported *Bacillus substilis* strain (3.2 U/ml) (Khanongnuch *et al*., 1998); *Bacillus subtilis* (12 U/ml) (Pereira *et al*., 2002); *Bacillus* sp. AA3 (4.2 U/ml), *Cellulomonas* sp. CX 38 (5.1 U/ml) (Ten *et al*., 2006) and *Bacillus* NT-9 (10.5 U/ml) (Fang *et al*., 2004).

Cellulase activity

Cellulolytic activity of *Bacillus licheniformis* strain C1 crude enzyme preparations was obtained at pH 7.0 and 60^{0}C. The cellulolytic activity was found to be 1.3 U/ml. Xylanases along with cellulase activity improve the properties of wheat bread and reduce staling during storage (Haros, 2002). Microbial xylanolytic enzymes in mixture with cellulase can be used for modification of baking products and for improvement of poultry diets. Xylanases and cellulase directly or indirectly improve the strength of the gluten network, this results in increased loaf volume, bread score and produce softer crumb and therefore, improve the quality of bread (Baillet *et al.,* 2003). Our results indicate that, xylanase produced by *Bacillus licheniformis* strain C1 could meet the requirement of baking industry.

Effect of pH on xylanase activity

Optimum pH of *Bacillus licheniformis* strain C1 xylanase was found to be 7.0 (Fig: 7). Xylanase from *Bacillus licheniformis* strain C1 active at pH 7.0 make it applicable for feed industry. Similar, optimum pH of 7.0 was reported by Choudhary *et.al*. (2006) for *Bacillus coagulans* xylanase and Kitamoto *et al.,* (1999) for *Aspergillus terreus xylanase.* Chadha *et al.,* (2004) isolated *Streptomyces* sp. having optimum xylanase activity at pH 6.0-8.0.

Effect of temperature on xylanase activity

Temperature optima for *Bacillus licheniformis* strain C1 xylanase was 60°C (Fig: 8), enzyme also showed good activity at 70-80°C. This optimum temperature value for *Bacillus licheniformis* strain C1 xylanase is similar or even somewhat higher than the optimal temperature reported by Durate *et al*., (2000), Dhillon *et al*., (2000), Morales *et al*., (1993) Muthezhilan *et al*., (2007) and Grabski and Jeffries (1991).

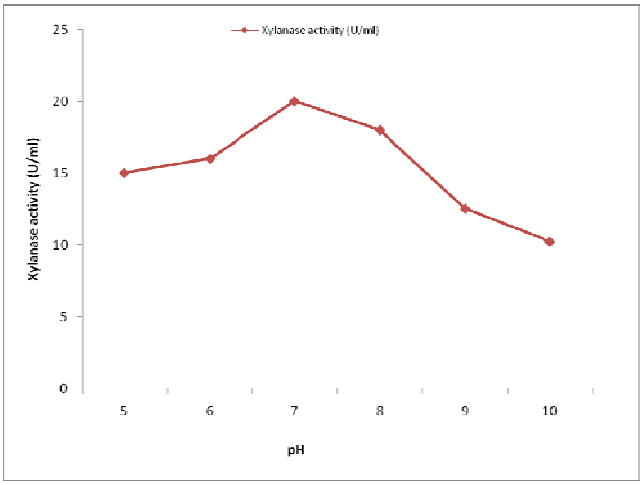

Fig 7: Effect of pH on xylanase activity.

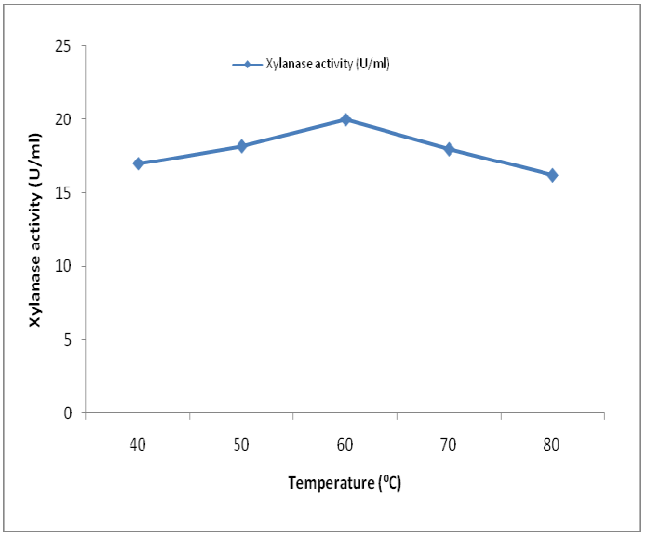

Fig 8: Effect of temperature on xylanase activity.

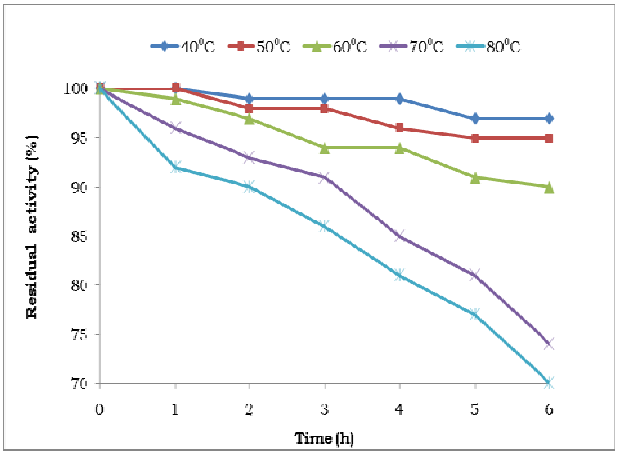

Fig 9: Thermostability profile of *Bacillus licheniformis* strain C1 xylanase

Thermostability of promising bacterial isolate

Xylanase thermostability was estimated at different temperature ranging from 40^0C to 80^oC for period of 1 to 6 h. Xylanase showed good thermostability at 60^0C for 6h, it retained 99% of its activity after 1 h and 10% activity was lost after 6h of incubation at this temperature. Approximately, 74% and 70% of its activity was retained at 70°C and 80°C respectively, when incubated for 6 h (Fig: 9). Comparable to our results, Durate *et al.*, (2000) showed that xylanase from *Bacillus pumilus* 5_2 retained around 40% of their original activity, while 5_{14}, 4_a and 13_a retained 60% of its activity after 2 h of incubation. However, activity decreased gradually over time, with 30% of the activity remaining for strain 5_2, 50% for strain 5_{14} and 40% for strains 13_a and 4_a after 6 h.

Thermal stability of xylanase is important property due to its potential applications in baking industry. Strains isolated by us could be a good source for its biotechnological applications.

Conclusion

In the present investigation we have isolated a bacterial source *Bacillus licheniformis* strain C1 that produced thermostable xylanase. Isolated xylanase is not only active at neutral pH but also exhibit broad range of thermostability at 60□-70□C for upto 6 h. *Bacillus licheniformis* strain C1 xylanase also showed good cellulase activity of 1.3 U/ml. Xylanases along with cellulase activity improve the properties of wheat bread (Haros, 2002) and can be used for modification of baking products and for improvement of poultry diets. Enzymatic hydrolysis of highly viscous arabinoxylan in diets based on cereals results in improved poultry growth and increased feed efficiency. *Bacillus licheniformis* strain C1 xylanase active at neutral pH, temperature optima 60□C, thermostability at 70-80□C with 1.3 U/ml of cellulase activity fulfills the criteria of using this enzyme in feed and baking industry.

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Comparative study of paired fin epidermis of hill-stream fishes: A scanning electron micrioscopic investigation

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Abstract

The adhesive nature of pectoral and pelvic fins of hill-stream fishes *G. gotyla, G. pectinopterus and P. sulcatus* as examined by scanning electron microscopic investigation is an attempt to understand the structural and functional modifications in epithelial cells in relation to life in torrential streams. The outer rays of these fins are modifies into structures that bear prominent transverse ridges and grooves in *G. pectinopterus* and *P. sulcatus*, where as the rough epidermis covered the ventral surface of entire length of first anterior ray of both the fins and also the proximal part of third and fourth rays of pectoral fin only in *G. gotyla*, the rough epidermis provided with horny projections. The outer epidermal cells of ridges are thrown into elongated spines. Mucous pore (opening to mucous glands) are frequently present in the epidermis of ridges. These spines are absent in the cells that line by the groove regions. Presence of these grooves and ridges could be interpreted as the means of adhesion, affected by suction pressure generated by the musculature attached to the grooves and ridges and mucus and spines aid in this process.

Keywords: *Fin epidermis, Kumaun himalaya, hill-stream fish, SEM*

Introduction

The aim of present the investigation is to study surface morphology of adhesive structures located in paired fins of mountain stream fishes, *G. gotyla, G. pectinopterus* and *P. sulcatus.* The paired fins of the hill stream fishes *G. gotyla*, *G. pectinopterus* and *P. sulcatus*, in the alternate to understand the structure and the functional modifications in epithelia, in relation to life in torrential streams. The glacier-fed mountain-streams of the Himalayas are perennial shallow-water bodies, characterized by low temperature, high turbulent currentand sandy-rocky substratum (Nag & Bhattacharjee 2002). To thrive successfully against the action of strong water currents, many Himalayan fishes demonstrateseveral unique adaptive modifications. One notable feature is the possession of a ventral adhesivedisc, surrounding the mouth in the cyprinids and at the thoracic region in the mountain-stream catfishes of the family Sisoridae (Hora 1922, 1930; Saxena 1966; Sinha et al. 1990; Singh & Agarwal 1991, 1993). In the latter, a rather unusual form of the adhesive.

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organs is present in the pectoral and pelvic fins. In these fishes, the skin of the pectoral and pelvic fins, instead of being plain, is thrown into a series of alternate grooves and ridges, especially at the outer rays (Hora 1922, 1930). Observations on the adhesive nature of the pectoral and pelvic fins of the mountain-stream catfishes are limited to gross morphology only (Hora 1922, 1930). The aim of the present investigation is to study the detailed general organization of the epidermis of pectoral fin and pelvic fin of *G. gotyla, G. pectinopterus* and *P. sulcatus*, adapted to life in torrential streams.

Material and Methods

Live adult specimens of *G. gotyla* (7-9 cm long) were collected from Kosi River at Kakrighat, Distt Nainital, *G. pectinopterus* (5-7 cm long) from west Ramganga River at Chaukhutiya, Distt. Almora, and *P. sulcatus* (6-7 cm long) from east Ramganga River at Thal, Distt. Pithoragarh respectively water current was very fast having velocity 0.5 to 2.0 m/sec. in Kosi, 1.5 to 2.5 m/sec. in west Ramganga and 2.0 to 3.0 m/sec. in east Ramganga (Bhatt & Pathak, 1991). Specimens were maintained in laboratory at 25 ± 2^0C. The fish were cold anesthetized, following Mittal & Whitear (1978), for SEM preparation of

paired fins. Tissue were excised and rinsed in 70 % ethanol and one change saline solution to remove debris and fixed on 3% Glutaraldehyde in 0.1M phosphate buffer, at p^H 7.4 for one night at 4^oc at Refrigerator. The tissue were washed in 2-3 changes in phosphate buffer and dehydrated in the graded series of ice cold Acetone (30%, 50%, 70%, 90%, and 100% approximate 20-30 min.) and critical point dried, using Critical Point Dryer (BIO-RAD England) with liquid carbon dioxide as the transitional fluid. Tissues were glued to stubs, using Conductive Silver Preparation (Eltecks, Corporation, India) Coated with gold using a sputter Coater (AGAR, B 1340, England) and examined in a Scanning Electron Microscope (Leo, 435, VP, England). The results were recorded using Kodak T-MAX 100 professional film (Kodak Ltd., England).

Results and Discussion

The paired fins of *G. gotyla, G. pectinopterus and P. sulcatus* are large, expanded and fan-shaped in appearance they are pushes outward and placed horizontally on the side of the body. The epidermis covering of paired fin of all three fishes is rough and keratinized (Fig. 1, 2, 3,).

In *G. gotyla, G. pectinopterus and P. sulcatus,* the epidermis of ventral surface is much thick at ray than that of the interrays region in between the first four anterior rays of pectoral and two rays of pelvic fins of all the three fishes.

In *G. gotyla,* the epidermis on ventral surface covering the entire length of first two anterior rays of pectoral and pelvic fins shows remarkable modification from the epidermis of rest of the parts of fins. The above epidermis is non-glandular and provided with a large number of horny projections (unculi) (Fig.4) at the surface and so is designated as uncular epidermis where as epidermis of rest of the parts of fins is devoid of such projections, hence is designated as smooth epidermis which is further differentiated into glandular and non-glandular epidermis on the bases of presence and absence of gland cells.

In *G. pectinopterus*, the epidermis on ventral surface covering the entire length of first anterior ray of both the fins shows significant modification from the epidermis of rest of the parts of fins, epidermis is here non-glandular and provided with unculi at surface (Fig. 5). It is, however, glandular in remaining parts of the fins both at rays and interrays region, except that at interrays region between the first and second rays at parts of rays having poorly developed elevations where, it is non-glandular (Fig. 5).

In *P. sulcatus,* also the epidermis is differentiated into rough and smooth. The epidermis on ventral surface along the entire length of first ray of pectoral fin and pelvic fin and proximal part (approximate $^1/_{10}{}^{th}$ part) of second ray of pectoral fin only, is rough and is provided with a large number of irregularly arranged transverse ridges separated by superficial grooves. The epidermis of rest of the parts of fins is smooth at surface and is devoid of unculi (Fig. 6, 7).

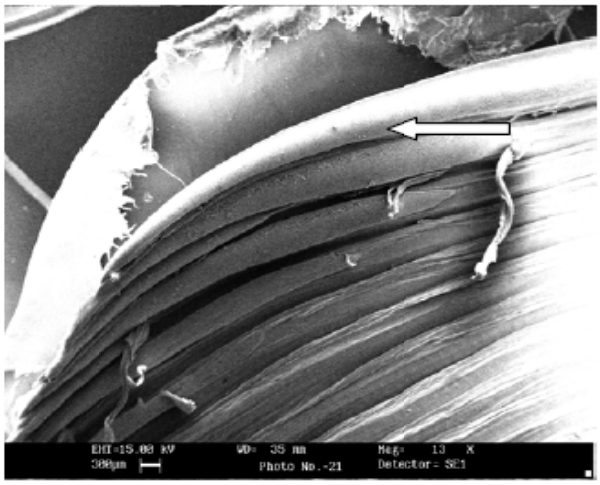
In *G. gotyla,* in the smooth epidermis, epithelial cells are characterized by microridges. The microridges are compactly arranged, branched as like microridges of general body epidermis (Fig. 8). In *G. pectinopterus,* and *p. sulcatus,* microridges are compactly arranged, numerous and filamentous (Fig. 9 and 10). Interspersed between the epithelial cells mucous cells apparatus are distinguished. The mucus cells appear with developed mucous cell opening in all of three fishes in glandular epidermis. In *G. pectinopterus,* the unculi are tall and conical with broad base projection. Each unculi are separated by groove (Fig. 10, 11). In *P. sulcatus*, these unculi are blunt with separated by groove (Fig. 12). These unculi are of uniform size shape and remain projected at the free surface. Each unculus represents a modified surface relief of compactly layer of epithelial cells all three fishes (Fig.13, 14 and 15). Some developed taste buds are described in the smooth epidermis of *P. sulcatus* (Fig. 16).

The epithelial cells are keratinized in the both pectoral and pelivic fins in *G. gotyla, G. pectinopterus* and *P. sulcatus* as revealed by a SEM techniques.

The adhesive nature of the pectoral and pelvic fins is examined by scanning electron microscope. The outer rays of these fins are ventrally and dorsally modified into structures that bear prominent ridges and grooves in *G. pectinopterus* and *P. sulcatus*. Such structures regressively developed on dorsal side of paired fin.

In *G. pectinopterus and P. sulcatus,* a rather usually form of the adhesive apparatus is present in the pectoral and pelvic fin. In these fishes, the skin of the pectoral and pelvic fins, instead of being plain, is thrown into a series of alternate groove and ridges, especially at the outer rays (Hora 1922, 1930).

In the flying fish, *Exocoetus volitans,* the modified pectoral fins are used for gliding for a considerable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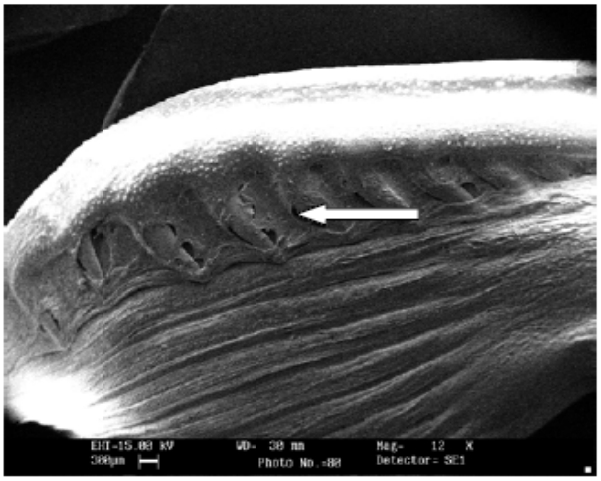
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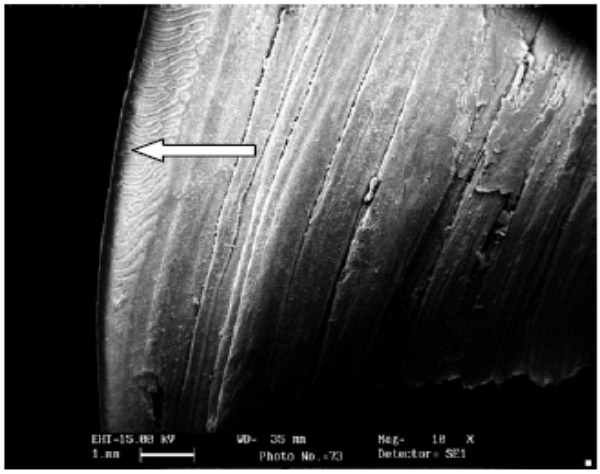
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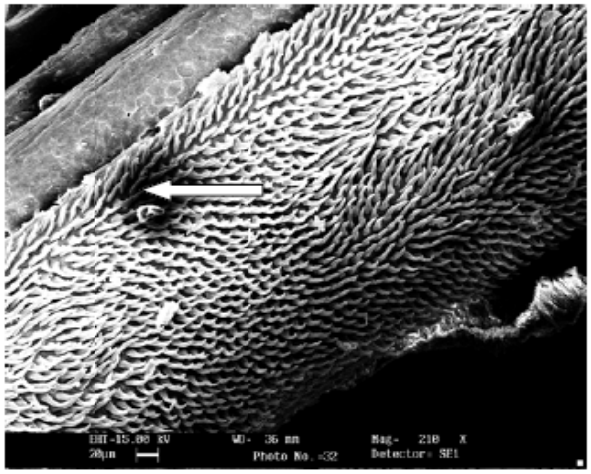
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20µm
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Detector= SE1

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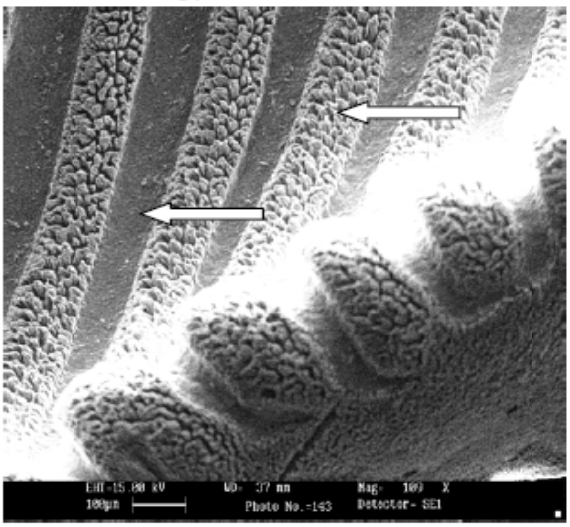
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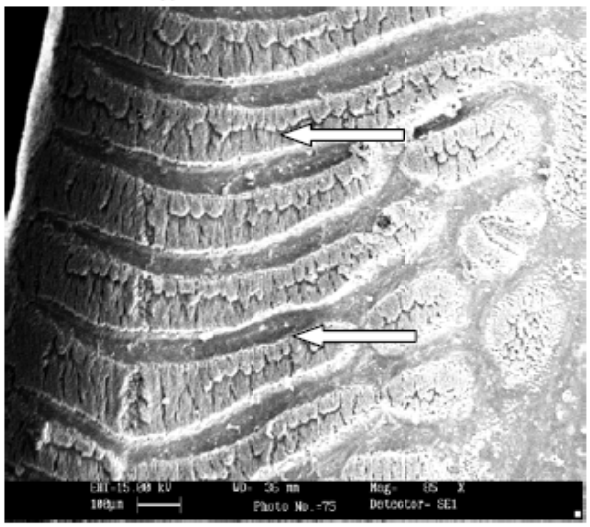
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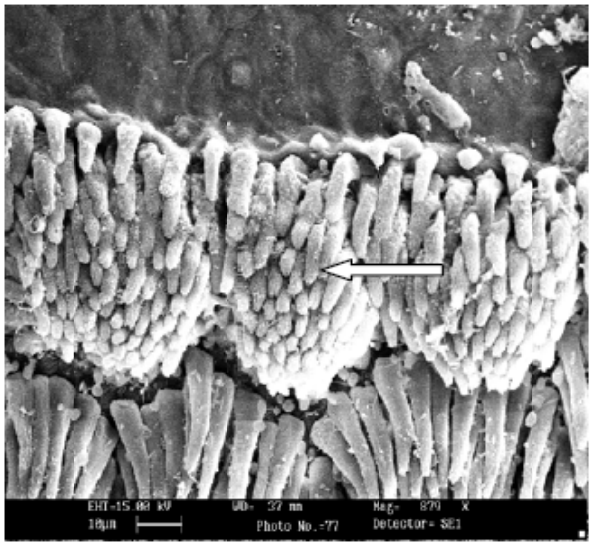
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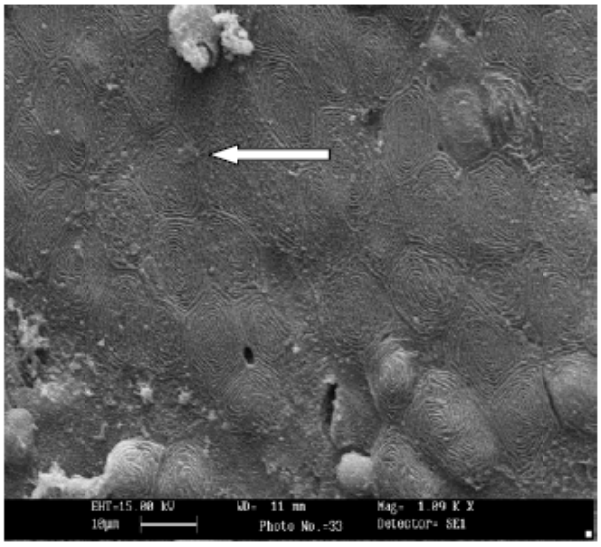
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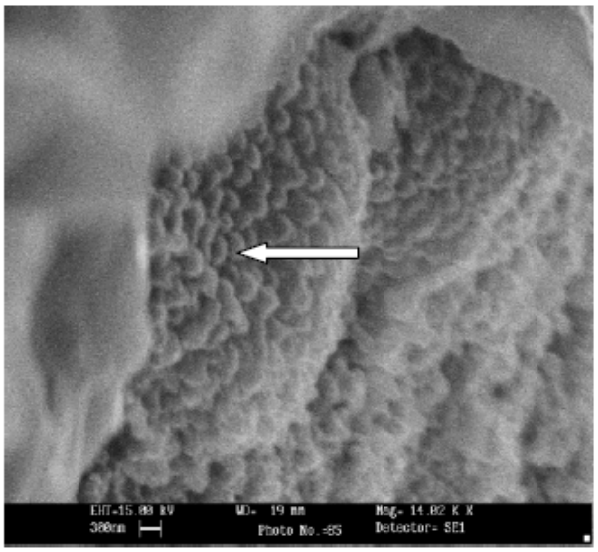
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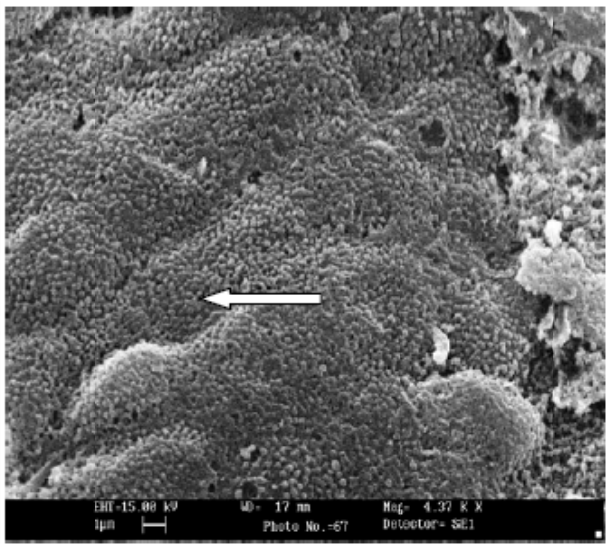
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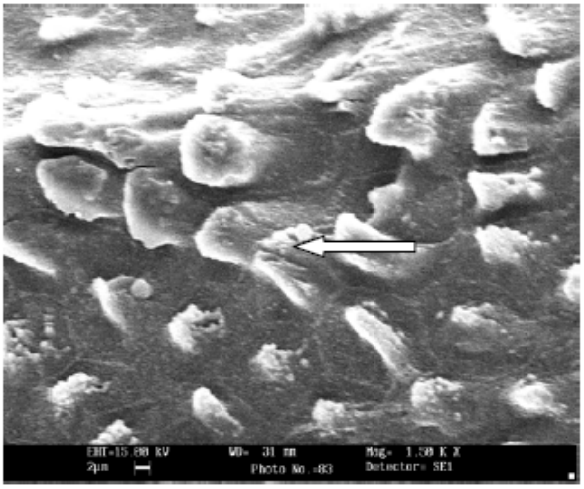
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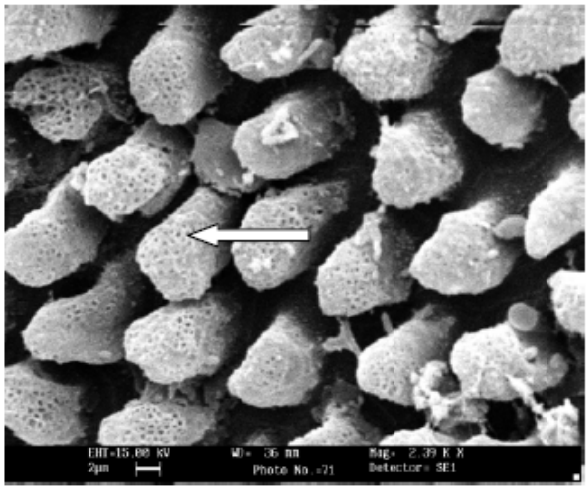
11.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2µm
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Detector= SE1

12

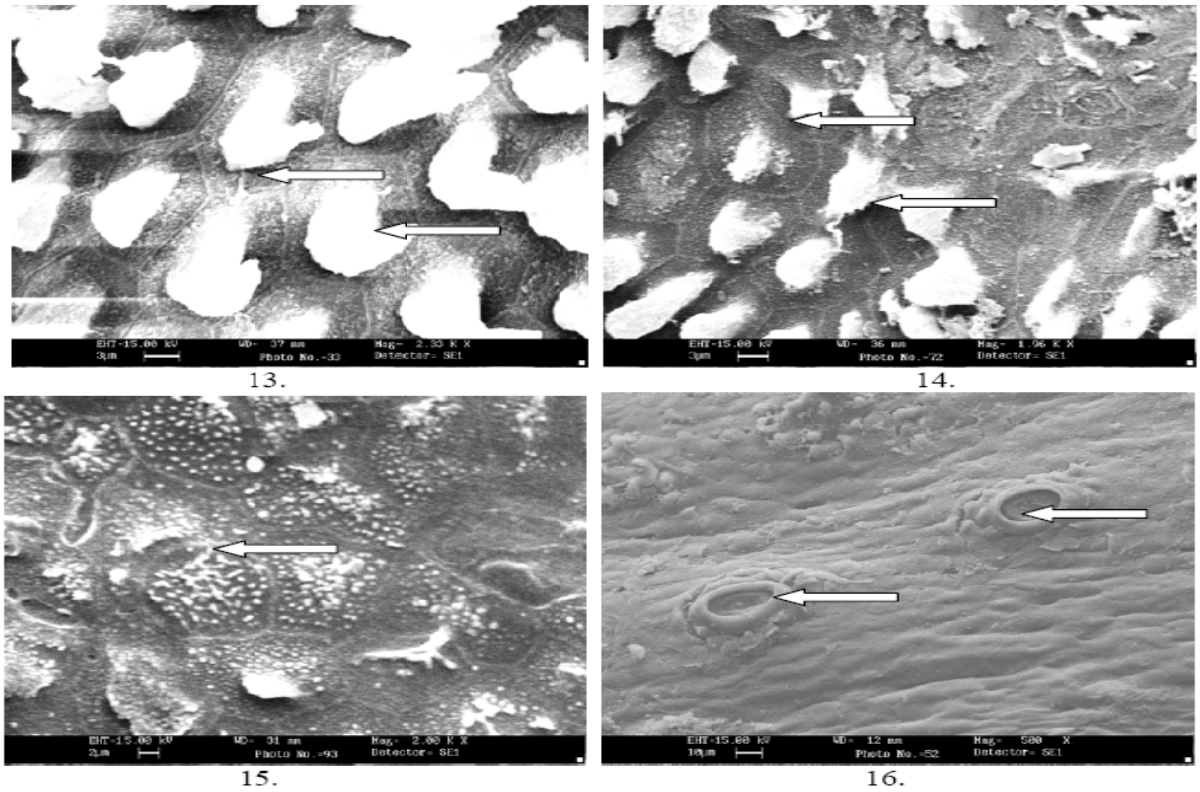

Fig 1: Surface electron microphotograph (SEMPH) of the paired fin (Pectoral and pelivic) epidermis of *G. gotyla* showing well developed fin rays (marked by arrow) (Scale bar - 300 µm and 1 µm).

Fig 2: Surface electron microphotograph (SEMPH) of the paired fin (Pectoral and pelivic) epidermis of *G. pectinopterus* showing well developed fin rays (marked by arrow) (Scale bar - 300 µm and 1 µm).

Fig 3: Surface electron microphotograph (SEMPH) of the paired fin (pectoral and pelvic) epidermis of *P. sulcatus* showing well developed fin rays (marked by arrow) (Scale bar - 1 µm and 200 µm).

Fig 4: SEMPH of paired fin (pectoral and pelvic) epidermis of *G. gotyla* showing the unculi (Marked by arrows) at the surface of epithelia of first fin ray (Scale bar- 20 µm and 20 µm and 2 µm).

Fig. 5: SEMPH of the paired fin (pectoral and pelvic) epidermis of *G. pectinopterus* showing well developed longitudinally ridges separated by groove (marked by arrow) (Scale bar 1 µm and 10µm).

Fig. 6 & 7: SEMPH of the paired fin epidermis of *P. sulcatus* showing well developed longitudinally ridges separated by groove (marked by arrow) (Scale bar 100 µm).

Fig. 8: SEMPH of the paired fin epidermis of *G. gotyla* showing microridges (marked by arrows). (Scale bar- 10 µm and 1 µm).

Fig. 9: SEMPH of the paired fin epidermis of *G. pectinopterus* showing the filamentous microridges (marked by arrows) at the surface of epithelia of groove region. (Scale bar- 300 µm).

Fig. 10: SEMPH of the paired fin epidermis of *P. sulcatus* showing the numerous filamentous microridges (marked by arrows) at the surface of epithelia of groove region. (Scale bar- 300 µm).

Fig 11: Surface electron microphotograph (SEMPH) of the paired fin epidermis of *G. pectinopterus* showing well developed cluster of unculi (marked by arrow) (Scale bar – 10 µm).

Fig 12: SEMPH of the paired fin epidermis of *P. sulcatus* showing blunt type of unculi on the surface of ridges (marked by arrows) (Scale bar –2 µm).

Fig 13: SEMPH of f the paired fin epidermis of *G. gotyla* showing hexagonal epithelial cells (marked by arrows) at the base of each unculi (marked by arrows head) (Scale bar – 3 µm).

Fig 14: SEMPH of the paired fin epidermis of *G. pectinopterus* showing hexagonal epithelial cells (marked by arrows) at the base of each unculi (marked by arrows head) (Scale bar – 2 µm).

Fig 15: SEMPH of the paired fin (pectoral and pelvic) epidermis of *P. sulcatus* showing hexagonal epithelial cells (marked by arrows) at the base of each unculi (marked by arrows head) (Scale bar – 3 µm).

Fig 16: SEMPH of the paired fin (pectoral and pelvic) epidermis of *P. sulcatus* showing well developed taste bud (Scale bar – 30 µm and 10 µm).

distance above the water surface, and in the sucking fish *Remora remora,* the first dorsal fin is utilized for adhesion (Migdalski & Fichter 1983). In several genera of the mountain-stream fishes (eg. *Pseudecheneis sp.*, *Glyptothorax sp.*, *Balitora sp.*), the expanded pectoral and pelvic fins are used for swimming against the strong water current. At rest, however, these fins are involves in adhesion (Hora 1930). The outer rays of these paired fins are generally employed for this purpose. This change seems to have been brought about for two reasons. First, it allows the ventral surface of the body to be firmly applied to rocks and second, to enable the fins to act as organs of attachment. The epidermis covering of the outer rays of these fins is an extension of the abdominal skin, and in order to achieve the function of adhesion, the epidermis has undergone remarkable modifications. The epidermis covering the ridges of the outer rays is characterized by the presence of spines, whereas the part that lines the grooves between the ridges is devoid of spines. These ultrastructural features allow us to speculate about the possible mechanism operative in the process of adhesion by the pectoral and pelvic fins in these teleost. It is likely that the outer rays of these fins work on the principle of suction for adhesion. When the fins are pressed against the substratum, a reduced pressure is created by the musculature attached to the ridges and grooves. The spiny projections might then assist in organic growth on the submerges rocks. The mucus secretion from the mucous glands causes a weak adhesion and prepares the sub-stratum for subsequent action of the spines. In addition, the mucus seems to afford protection to the spines from abrasion during adhesion. The apparent lack of spines in some of the epidermal cells (located near the base of the ridges) indicate that these structures are often damaged and then possibly lost, to generate new spines. The factors causing damage to the spines could be the constant mechanical abrasion or reduced mucus secretion from the surrounding mucous glands in altered physiological states.Hora (1930) considered that the non unculiferus groove as sulci on the thoracic adhesive apparatus and paried fins of sisoridae served as channels for the exit of water from beneath the surface, so that the unculiferus laminae can be brought into more intimate contact with the substrate and ‘seizing’ can take place. Hora (1930) also suggested a seizing function of papillated structures including the lips of hill-stream fishes, although he was unaware that such papillae frequently are unculiferous.The well-developed unculiferous pads on the ventral surface of paired fins, in addition to providing adhesion, may help achieve the seal for the suction device; possibly the seal enhanced by suction or seizing due to exit of water from the interradial grooves, between unculiferous pads.

The presence of unculi and differences in these structures of different fishes could be considered as adaptive modifications that reflect varied functional demands.A complementary relationship between development of multicellular tubercles on the dorsal surface of paried fins and unculiferus unculi on the ventral surface in many species of *Paraerossocheilus, Homolaptera* and *Gastromyzon*, multicellular tubercles are well as in adults and are equally well developed in sexually mature individuals as both sexes. Roberts (1982) described some multicultural tubercles are well developed on the dorsal surface of the paired fin in sexually mature males and poorly developed or absents in female, the unculiferous pads appear to be equally well developed in both sexes.In *G. gotyla*, *G. pectinopterus* and *P. sulcatus* the currents investigation shows the presence of characteristic microridges on the surface of epithelial cells on the interpapillary region.A dense network of microridges could be interpreted as a means to retain more and more mucus at the surface of epithelial cells possessing few mucus cell in this region as suggested by Hughes and Wrignt (1970); Ojha & Hughes (1988); Fishelsones (1984).

The mucus secretion from the mucous cells caused a weak adhesion and prepares the substratum for subsequent action of the spine. In addition the mucus seems to afford protection to the spines from abrasions during adhesion.

The apparent lack of spines in some of the epidermal cells indicates that these structures are after damages and then possibly lost to generate new spines. The factors causing damage to the spines could be the constant mechanical abrasion to reduced mucus secretion from the surrounding mucus glands in altered physiological states (Das & nag 2004 and 2006).Mucous cells occupy much higher area in epidermis of *G. gotyla* and secrete profuse amount of mucus on surface than those in epidermis of *G. pectinopterus* and *P. sulcatus*, that has to withstand a high strength of current of water, and may protect the fish from frictional stress by lubricating the surface.

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Ethno-botanical study of some medicinal plants used for treatment of Cancer in Narendra nagar block, District Tehri Garhwal (Uttarakhand) India

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Abstract

Ethno-botanical survey of some medicinal plants used in the treatment of cancer was carried out in the Narendra Nagar block, district Tehri Garhwal (Uttarakhand), India. Herbalists, herb sellers and traditionalists living within the study area were interviewed by the administration of questionnaires. Twenty six plant species of Angiosperm were found to be used in the treatment of cancer. Prominent species among these are the members of family Liliaceae and Rutaceae which were found to be very important and useful in the treatment of the disease based on their frequency of occurrence in the recipes. Several plants parts which were said to be useful were indicated in the recipes.

Keywords: *Cancer, ethnobotany, medicinal plants, traditional healers*

Introduction

Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. This is particularly true in developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations (WHO, 1998). Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals and healthcare (Koduru *et al.,* 2007). In addition, herbal medicines are more acceptable in these countries from their cultural and spiritual points of view. Use of plants for medicinal remedies is an integral part of the Uttarakhand cultural life, and this is unlikely to change in the years to come .Several studies employing methodologies of modern medicine have been conducted on a multitude of herbs of ethno-botanical importance (Dahanukar *et al*., 2000; Duke and Ayensu, 1985). Ayurveda, the Traditional Indian System (TIS) of medicine, has been successful from ancient times in using natural drugs, mostly herbal preparations, in preventing or suppressing various diseases using several lines of treatment.

Among the human diseases treatment of cancer with medicinal plants, which is probably the most important genetic disease as well as other factors. Cancer has been defined as a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells (Rang *et al.,* 2001). All cancer types arise through a series of steps characterized by progressive loss of normal growth control. There are proteins in the cells that ensure this continuity (Brooks and La Thanque, 1999). Death from cancer often comes not from the primary site but from metastages. Cancer may affect people at all ages even foetus but the risk for most varieties increases with age. Thousands of herbal and traditional compounds are being screened

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worldwide to validate their use as anticancerous drugs (Diwanay *et al.,* 2004; Liu *et al.,* 1998.

The disease causes about 13% of all death. Reports have shown that during 2007, about 7.6 million people died from cancer in the world. All cancers are caused by abnormalities in the genetic material of the transformed cells and these abnormalities may be due to the effect of carcinogens such as tobacco, smoke, radiation, chemicals and infectious agents.

Every year, millions of people are diagnosed with cancer, leading to death in a majority of the cases. According to the American Cancer Society (ACS) 2006, deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide. In India, cancer rates are increasing every year, breast cancer being the most common form of cancer in women worldwide and the second most common cancer amongst Indian women (Mouli *et al.,* 2009). Current statistics indicates that across all ethnic groups, one in every 31women in this country is likely to develop breast cancer. Many traditional healers and herbalists have been treating cancer patients for many years using various medicinal plant species. Despite the long history of cancer treatment using herbal remedies in the study area, the knowledge and experience of these herbalists have not been scientifically documented. Information on traditional herbal practice is passed from one generation to the other through oral tradition. Considering the rapid rate of deforestation and loss of biodiversity, there is a need for accurate scientific documentation of the knowledge and experience of these herbalists. In this paper, we report the informations on plants gathered from traditional and elder rural dwellers, used in the study area for the treatment of cancer.

Methodology

The study area falls within the block Narendra Nagar, district Tehri Garhwal, Uttarakhand, India. It lies in between 30^0 10'-30^0 17' N latitude and 78^0 18'-78^0 30'E longitude and has an average elevation upto 1700m a.s.l. and covering an area of 6,8,123 hectors. The head quarter of the block is away from Rishikesh (16km) on the route of Yamunotri and Gangotri, offering a splendid view of the snow-capped Himalaya. It streches from Dhalwala Than, Amsera, Jaikot, Gaja to Marora, Nigyer, Shivpuri, Kaudiyala, Byasi etc. The information was collected from herbalists, traditional healers and rural dwellers in the study area and was compiled through scientifically guided questionnaires, interviews and general conversations. Although informants were not scientifically literate, they were born in the region and had lived there for most of their lives. Healings homes were not left out in this exercise. Relevant information regarding the plant species, recipes, their local names, modes of administration and dosage pattern were also collected to enhance permanent record (Table 1). The plants were initially identified by their vernacular names through consultations with the local people. Standard method of collection, preservation and maintenance of specimens in the herbarium were followed by Jain and Rao, (1977), Singh and Subramanyam, (2008). The plant specimens were properly identified with the help of available taxonomic literature and monographs (Hooker, 1872-1897; Osmaston, 1927; Polunin and Stainton, 1984) etc. The collected plant specimens were deposited in the Herbarium of H.N.B. Garhwal Central University, S.R.T. Campus, Botany Department Badshahi Thaul, Tehri Garhwal.

Enumeration of recipes

Recipe:-1.

Botanical Name	**Vernacular Name**	**Plant part used**
Calotropis procera (Aiton) Dryander	Aak	Leaves
Kigelia africana (Lam.) Benth.	Kanguli	Leaves and bark
Diospyros malabarica (Desrousseaux) Kosteletsky	Gab	Bark and fruit

Preparation: - Leaves bark and fruit should be rinsed and boiled in 4 liters of fermented corn water for 6 hours.

Application:- It is taken as a tea thrice a day with a cup.

Recipe:-2

Botanical Name	Vernacular Name	Plant part used
Mangifera indica L.	Aam	Bark
Citrus medica L.	Nimbu	Fruit juice
Allium cepa L.	Pyaz	Leaves
Bryophyllum pinnatum (Lam.) Oken	Bish-Kapru	Root

Preparation: - The Root, bark and leaves as indicated above should be rinsed and boiled in the water for 40 minutes. *Citrus medica* fruit juice is added when cooled.
Application: - Cup full 3 times daily upto 2 months .

Recipe: - 3

Botanical Name	Vernacular Name	Plant part used
Citrus medica L.	Nimbu	Fresh juice
Citrus aurantifolia (Christmann) Swingle	Kagjinimbu	Fresh juice
Plumbago zeylanica L.	Chitrak	Root

Preparation: - It should be ground together smoothly and mixed with black soap and gum powder.
Application: - Use the preparation to wash all the part of the body, once in a week.

Recipe: - 4.

Botanical Name	Vernacular Name	Plant part used
Zingiber officinale Roscoe	Adrak	Rhizome, Seed/fruit
Curcuma domestica Valeton	Haldi	Whole plant
Allium sativum L.	Lahsun	Bulbs
Allium cepa L.	Pyaz	Leaves

Preparation: All the plants should be ground together when dried and taken with honey.
Application: - Take one full cup as tea 3 times daily after meal.

Recipe:-5

Botanical Name	Vernacular Name	Plant part used
Chenopodium ambrosioides L.	Bethuali	Twigs and roots
Citrus aurantifolia (Christmann) Swingle	Kagjinimbu	Fruit Juice
Allium sativum L.	Lahsun	Leaves
*Oroxylum indicum (*L.) Ventenat	Tantia	Fruit, bark and leaves
Potash		

Preparation: - Soak all the above with lime and dry gin with gun powder for 30 days**.**
Application: - Two teaspoonful morning and evening after meal.

Recipe: - 6.

Botanical Name	Vernacular Name	Plant part used
Cannabis sativa L.	Bhangulu	Leaves
Solanum nigrum L.	Makoi	Leaves, root, bark and fruit.

Preparation: Whole plants are boiled until they burst into pieces. It is filtered and the decoction is made.
Application: - One teaspoonful is taken once a day till recovery.

Recipe: - 7.

Botanical Name	Vernacular Name	Plant part used
Celtis eriocarpa Decne.	Kharik	Bark and roots
Citrus aurantifolia (Christmann) Swingle	Kagjinimbu	Fruit juice

Preparation:- Bark and root dried in sun light and made a powdered and infused in lemon juice or milk.
Application: Taken orally every day till signs of relief are obvious.

Recipe: - 8

Botanical Name	Vernacular Name	Plant part used
Solanum nigrum L.	Makoi	Fruit extract
Catharanthus roseus (L.) G. Don.	Sadhabahar	Leaves
Butea monosperma (Lam.) Kuntze	Dhak	Leaves, flowers and seeds,
Triticum aestivum L.	Gehun	Seed

Preparation: **-** All the plants should be ground together when dried and taken with honey.

Application: Take one full glass cup as tea 3 times daily.

Recipe: - 9

Botanical Name	Vernacular Name	Plant part used
Azadirachta indica A.H.L. Juss.	Neem	Leaves and flower
Plantago depressa Willd.	Luhurya	Leaves and seeds
Artemisia nilagirica (C.B Clarke)	Kunjaa	Leaves and flowers

Preparation: - Leaves, flowers and seeds are stamped and boiled in water to make a decoction. It is administered orally till signs of relief are obvious.

Application: Drink when hot with a glass cup twice daily.

Recipe:-10.

Botanical name	Vernacular Name	Plant part used
Aloe vera (L.) Burm.	Patanguar	Leaves
Alstonia scholaris (L.) R.B.	Satni	Whole plant
Allium sativum L.	Lahsun	Bulbs

Preparation: - Plant should be ground together when dried and taken with honey or milk

Application: - One teaspoonful is taken 3 times daily.

Recipe: - 11.

Botanical Name	Vernacular Name	Plant Part Used
Saccharum officinarum L.	Ganna	Crushed stem (Juice)
Citrus aurantifolia (Christmann) Swingle	Kagajiinimbu	Fruit juice

Preparation:- Crushed stem and fruit juice should be rinsed and boiled in one liter of Palm oil for two hours.

Application: - Two tea spoonful morning and evening before meal.

Results and Discussion

It is revealed that several ethno-medicinal plant species parts such as leaves, roots, barks and seeds have been found efficient in the treatment of cancer. However, the prominent plant species in the recipes are *Solanum nigrum, Catharanthus rosesus, Butea monosperma, Triticum aestivum, Diospyrus malabarica, Kigelia africana, Citrus medica, Citrus aurantifolia, Allium cepa* and *Allium sativum* which are indicative of their importance in the treatment of cancer disease. Similarly, Fabaceae and Liliaceae families occurred more frequently in the list of plants identified but the occurrence of other families also suggested the importance of all those families as repository of useful chemical compounds which may be explored for drugs in the treatment of cancer (Madhuri and Pandey, 2009).

In orthodox medicine cancer can be treated with drugs and radiotherapy if detected early. Otherwise surgical operation is used at some stage after which it can become very difficult and hopeless. However, nature has some remedy for cancer patients. Some substances have been found to be anti-carcinogenic, *i.e.* they fight cancer forming cells and help to eliminate them from the body, for example cumaric acid and lycopen which are found naturally in tomatoes fruits (*Lycopersicum esculentum* L.) and the leaves of bitter leaf (*Vernonia amygdalina* Del.).

Also, a lot of research has been and is still being done on the effectiveness of *Aloe vera* (L.) Burm.f. *Azadirachta indica* A.H.L. Juss., *Catharanthus rosesus* (L.) G. Don., *Butea monosperma* (Lam.) Kuntze for treating cancer. Literature has revealed that most of the synthetic drugs that have been used in the past have negative effects that were of grave consequence in some cases, especially when taken by patients on self prescription after an initial visit to the physician (Olapade, 2002).

For this reason, it is imperative for ethno-botanists and pharmacognosists to do more analysis on the 26 wonderful plants mentioned in this paper (table 1).

Our medical health practitioners should also focus attention on more intense research on ethno-medicinal plants which can save the life of peoples without side effects.

Table 1: Medicinal Plants Used by Treatment of Cancer in the Narendra Nagar block, district Tehri Garhwal (Uttarakhand), India

S.No	Botanical Name	Family	Vernacular Name	Plant parts used
1.	*Allium cepa* L.	Liliaceae	Pyaz	Leaves
2.	*Allium sativum* L.	Liliaceae	Lahsun	Bulbs
3.	*Aloe vera* L.	Liliaceae	Patanguar	Leaves
4.	*Alstonia scholaris* (L.) R.B.	Apocynaceae	Satni	Bark and leaves
5.	*Artemisia nilagirica* (C.B Clarke)	Asteraceae	Kunjaa	Leaves and flowers
6.	*Azadirachta indica* A.H.L. Juss.	Meliaceae	Neem	Leaves, bark and flowers
7.	*Bryophyllum pinnatum* (Lam.) Oken	Crassulaceae	Bish-kapru	Root
8.	*Butea monosperma* (Lam.) Kuntze	Fabaceae	Dhak	Leaves flowers and seeds
9.	*Calotropis procera* R.B	Asclepiadaceae	Aak	Leaves
10.	*Cannabis sativa* L.	Cannabaceae	Bhanglu	Leaves
11.	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Sadabahar	Leaves
12.	*Celtis eriocarpa* Decne.	Ulmaceae	Kharik	Bark and roots
13.	*Chenopodium ambrosioides* L.	Chenopodiaceae	Bethuli	Twigs and roots
14.	*Citrus aurantifolia* (Christmann) Swingle	Rutaceae	Kagjinimbu	Fruit juice
15.	*Citrus medica* L.	Rutaceae	Nimbu	Fruit juice
16.	*Curcuma domestica* Valeton	Zingiberaceae	Haldi	Whole plant
17.	*Diospyros malabarica* (Desrousseaux) Kosteletsky	Ebenaceae	Gab	Bark and fruit
18.	*Kigelia africana* (Lam.) Benth.	Bignoniaceae	Kanguali	Leaves and bark
19.	*Mangifera indica* L.	Anacardiaceae	Aam	Bark
20.	*Oroxylum indicum* (L.) Ventenat	Bignoniaceae	Tantia	Fruit bark and seeds
21.	*Plantago depressa* Willd.	Plantaginaceae	Lahurya	Leaves and seeds
22.	*Plumbago zeylanica* L.	Plumbaginaceae	Chitrak	Root
23.	*Saccharum officinarum* L.	Poaceae	Ganna	Crushed stem (Juice)
24.	*Solanum nigrum* L.	Solanaceae	Makoi	Leaves, root, bark and fruit
25.	*Triticum aestivum* L.	Poaceae	Gehun	Seed
26.	*Zingiber officinale* Roscoe	Zingiberaceae	Adrak	Rhizome, Seed/ fruit

Formulation of the dosages of extracts from the recipes must be strictly adhered for maximum efficacy and also the avoidance of over dosage which may lead to other complications in patients. One major advantage of traditional medicine is that, it is cheaper than orthodox medicine. While drugs alone are not the only means of providing health care, they do play an important role in protecting, maintaining, and restoring the health of people. Total information gathered from the herbalist's shows that increasing number of people is turning to the use of anti-cancer which shows that they are effective and efficient in the treatment of cancer. According to Olapade (1995), traditional medicine has higher benefits than any other health care system as it is cheaper, readily available and could cure permanently. Apart from this, it has no side effect and is capable of saving for the nation, huge foreign exchange which can be used for other development programme. The vulnerability of medicinal plants to over exploitation and extinction needs to be dealt with seriously. Issues relating to the conservation of these medicinal plants should be addressed by the Government and Non-governmental Organizations. Conservation methods such as *in-situ* and e*x-situ* should also be adopted to protect our natural biodiversity (Soladoye *et al.,* 2006).

A need for further scientific research based on the findings of this survey is indeed very necessary and recommended so that adequate records of indigenous methods for the management of cancer can be kept for posterity especially in the study area. A need for analytical work on the plants identified as useful for the management of cancer is also necessary in order to determine the actual dosage applicable so that the medicinal value of these plants could be made available to humanity and hence reduce pain, cost and sudden death of the peoples.

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Utilization of waste heat generated from thermal power plants

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Abstract

The present paper describes the main features of waste heat recovery. Waste heat is heat, which is generated in a process by way of fuel combustion or chemical reaction, and then "dumped" into the environment even though it could still be reused for some useful and economic purpose. The energy lost in waste gases cannot be fully recovered. However, much of the heat could be recovered and loss minimized by adopting various measures as outlined in this paper.

Keywords: *Duct, Dumped, Slagging*

Introduction

The present communication deals with the utilization of waste heat generated from thermal power plants. Waste heat is the heat that is generated in a process by way of fuel combustion or chemical reaction (Gordon, 2001) which is then dumped in to the environment and not reused for useful and economic purpose. Boilers, Klins, oven and furnace are such examples which generate large quantity of hot flue gases. The energy lost in waste gases can not be fully recovered. Hence in the present investigation and attempt has been made to describe some of the methods which are useful to utilize waste heat generated from various sources. Some of the commercial equipmentswhich can be used to recover waste heat for others applications are Recuperator, Regenerator, Economizer, Heat wheels, Heat pipe and Waste heat recovery boiler (Nag.,1987 and Kumar, 2008).In order to evaluate the potential for waste heat recovery determination of heat quality and heat quantity are necessary. In this connection waste heat can be recovered by the mechanical equipment for different industrial processes.

Methodology for Waste Heat Recovery

This section describes the various commercial equipments that can be used to recover waste heat for other application.

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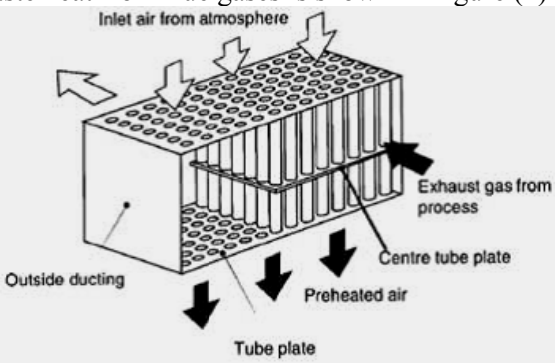
Recuperator

In a recuperator, heat exchange takes place between the flue gases and the air through metallic or ceramic walls. Duct or tubes carry the air for combustion to be pre-heated, the other side contains the waste heat stream. A recuperator for recovering waste heat from flue gases is shown in Figure (1)



Waste heat recovery using Recuperator

Regenerator (Fig. 2)

The Regeneration which is preferable for large capacities has been very widely used in glass and steel melting furnaces. Important relations exist between the size of the regenerator, time between reversals, thickness of brick, conductivity of brick and heat storage ratio of the brick.

In a regenerator, the time between the reversals is an important aspect. Long periods would mean

higher thermal storage and hence higher cost. Also long periods of reversal result in lower average temperature of preheat and consequently reduce fuel economy.

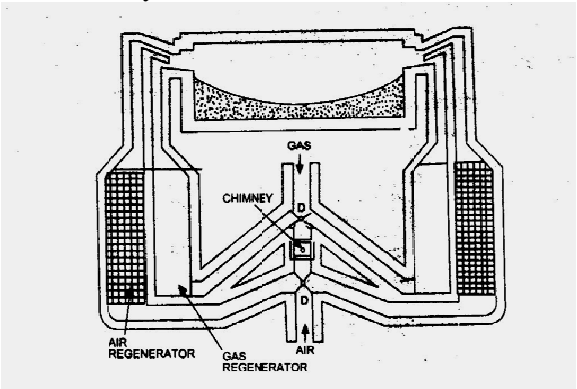


Fig. 2. Regenerator

Accumulation of dust and slagging on the surfaces reduce efficiency of the heat transfer as the furnace becomes old. Heat losses from the walls of the regenerator and air in leaks during the gas period and out-leaks during air period also reduces the heat transfer.

Heat Pipe (Fig. 3)

A heat pipe can transfer up to 100 times more thermal energy than copper, the best known conductor (Kumar, 1985). In other words, heat pipe is a thermal energy absorbing and transferring system and have no moving parts and hence require minimum maintenance.

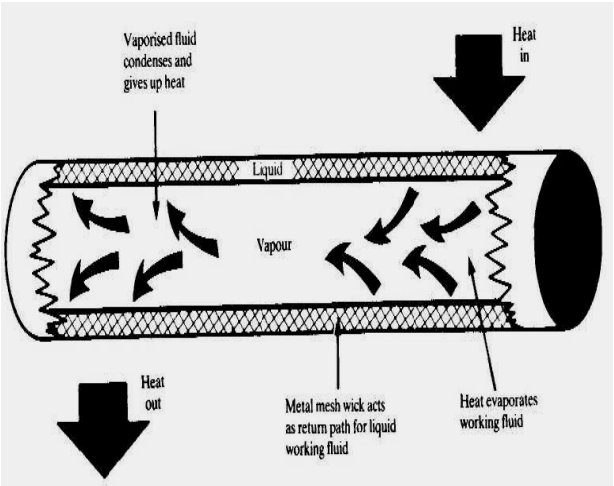


Fig. 3 : Heat Pipe

The Heat Pipe comprises of three elements – a sealed container, a capillary wick structure and a working fluid. The capillary wick structure is integrally fabricated into the interior surface of the container tube and sealed under vacuum. Thermal energy applied to the external surface of the heat pipe is in equilibrium with its own vapor as the container tube is sealed under vacuum. Thermal energy applied to the external surface of the heat pipe causes the working fluid near the surface to evaporate instantaneously. Vapor thus formed absorbs the latent heat of vaporization and this part of the heat pipe becomes an evaporator region. The vapor then travels to the other end of the pipe where the thermal energy is removed causing the vapor to condense into liquid again, thereby giving up the latent heat of the condensation. This part of the heat pipe works as the condenser region. The condensed liquid then flows back to the evaporated region.

Waste Heat Boilers (Fig. 4)

Waste heat boilers are ordinarily water tube boilers (Yadav ,2003) in which the hot exhaust gases from gas turbines, incinerators, etc., pass over a number of parallel tubes containing water. The water is vaporized in the tubes and collected in a steam drum from which it is drawn off for use as heating or processing steam.

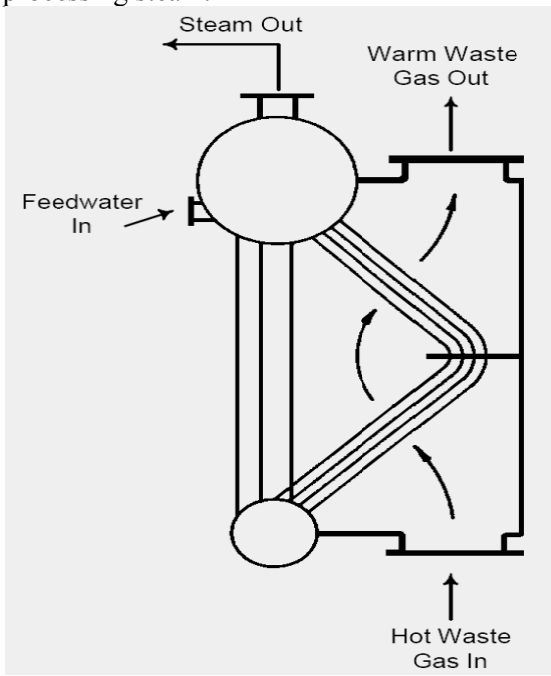


Fig. 4: Two-Pass Water Tube Waste Heat Recovery Boiler

Because the exhaust gases are usually in the medium temperature range and in order to conserve

space, a more compact boiler can be produced if the water tubes are finned in order to increase the effective heat transfer (Kumar, 1985) area on the gas side. The pressure at which the steam is generated and the rate of steam production depends on the temperature of waste heat. The pressure of a pure vapor in the presence of its liquid is a function of the temperature of the liquid from which it is evaporated. The steam tables tabulate this relationship between saturation pressure and temperature. If the waste heat in the exhaust gases is insufficient for generating the required amount of process steam, auxiliary burners which burn fuel in the waste heat boiler or an after-burner in the exhaust gases flue are added. Waste heat boilers are built in capacities from 25 m^3 almost 30,000 m^3 / min. of exhaust gas.

Results and Discussion

This section explains how to evaluate the potential for waste heat recovery along with examples.

Determining the waste heat quality

When recovering the waste heat, the quality of waste heat must be considered first.

Depending upon the type of process, waste heat can be rejected at virtually any temperature from that of chilled cooling water to high temperature waste gases from an industrial furnace or kiln. Usually higher the temperature, higher the quality and more cost effective is the heat recovery. In any study of waste heat recovery, it is absolutely necessary that there should be some use for the recovered heat. Typical examples of use would be preheating of combustion air, space heating, or pre-heating boiler feed water or process water. With high temperature heat recovery, a cascade system of waste heat recovery may be practiced to ensure that the maximum amount of heat is recovered at the highest potential. An example of this technique of waste heat recovery would be where the high temperature stage was used for air pre-heating and the low temperature stage used for process feed water heating or steam generation.

Quality, potential and their uses

In considering the potential for heat recovery, it is useful to note all the possibilities, and grade the waste heat in terms of potential value as shown in the Table 1.

Table 1: Waste source and quality

S.No.	Source	Quality
1.	Heat in flue gases.	The higher the temperature, the greater the potential value for heat recovery
2.	Heat in vapour streams.	As above but when condensed, latent heat also recoverable.
3	Convective and radiant heat lost from exterior of equipment	Low grade – if collected may be used for space heating or air preheats.
4.	Heat losses in cooling water.	Low grade – useful gains if heat is exchanged with incoming fresh water.
5.	Heat losses in providing chilled water or in the disposal of chilled water.	a) High grade if it can be utilized to reduce demand for refrigeration. b) Low grade if refrigeration unit used as a form of heat pump.
6.	Heat stored in products leaving the process	Quality depends upon temperature.
7.	Heat in gaseous and liquid effluents leaving process.	Poor if heavily contaminated and thus requiring alloy heat exchanger.

Recovery potential for different industrial processes

Waste heat can be recovered from various industrial processes. A distinction is made between high, medium and low temperatures of waste heat.

Table 2 gives the temperatures of waste gases from industrial process equipment in high temperature range. All these results from direct fuel fired processes.

Table 2 Typical waste heat temperature at high temperature range from various sources

Types of Device	Temperature, °C
Nickel refining furnace	1370 –1650
Aluminium refining furnace	650-760
Zinc refining furnace	760-1100
Copper refining furnace	760- 815
Steel heating furnaces	925-1050
Copper reverberatory furnace	900-1100
Open hearth furnace	650-700
Cement kiln (Dry process)	620- 730
Glass melting furnace	1000-1550
Hydrogen plants	650-1000
Solid waste incinerators	650-1000
Fume incinerators	650-1450

Table 3 gives the temperatures of waste gases from industrial process equipment in the medium temperature range. Most of waste heat in this temperature range comes from the exhaust of directly fired process units.

Table 3 Typical waste heat temperature at medium temperature range from various sources

Type of Device	Temperature, °C
Steam boiler exhausts	230-480
Gas turbine exhausts	370-540
Reciprocating engine exhausts	315-600
Reciprocating engine exhausts (turbo charged)	230- 370
Heat treating furnaces	425 - 650
Drying and baking ovens	230 - 600
Catalytic crackers	425 - 650
Annealing furnace cooling systems	425 - 650

Table 4 lists some heat sources in the low temperature range. In this range it is usually not practical to extract work from the source, though steam production may not be completely excluded if there is a need for low-pressure steam. Low temperature waste heat may be useful in a supplementary way for preheating purposes.

Table 4 Typical waste heat temperature at low temperature range from various sources

Source	Temperature, °C
Process steam condensate	55-88
Cooling water from:	
Furnace doors	32-55
Bearings	32-88
Welding machines	32-88
Injection molding machines	32-88
Annealing furnaces	66-230
Forming dies	27-88
Air compressors	27-50
Pumps	27-88
Internal combustion engines	66-120
Air conditioning and refrigeration condensers	32-43
Liquid still condensers	32-88
Drying, baking and curing ovens	93-230
Hot processed liquids	32-232
Hot processed solids	93-232

Determining the Waste Heat Quantity

In any heat recovery situation it is essential to know the amount of heat recoverable and also its usage.

The total heat that could be recovered can be calculated using this formula:

$$Q = V \times \rho \times C_p \times \Delta T$$

Where,

Q is the heat content in kcal.

V is the flow rate of the substance in m^3/hr.

ρ is the density of the floe gas in kg/m^3.

Cp is the specific heat of the substance in kCal/kg^{o}C.

ΔT is temperature difference in ^{o}C

Conclusion

Heat recovery technology is an excellent tool to conserve energy. The waste heat recovery brings in related economic benefits for the local community and would lead to sustainable economy and industrial growth in the region. The waste heat recovery activity would be able to replace electricity generated by grid-connected power plant thus saving further exploitation and depletion of natural resources – coal, or else increasing its availability to other important process.

The electricity generated from the waste heat recovery would help to reduce carbon dioxide emission and other associated pollution at thermal power plants. By placing waste heat recovery power plants in heavy industries which are generating huge amount of waste heat, we can reduce 10-12 % of Global Warming effect. This will helpful for Nation too. This project can be treated as the efficient method of utilization of waste gases for production of electrical energy and one can hope that the "Waste Heat Recovery" will play even great roll in industrial development of twenty first century.

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Status of noise pollution in households of Kathua city (J&K)

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Abstract

The present study has been carried out to assess the seasonal variations of L_{eq} ,NC and L_{NP} noise levels in households located at different areas of Kathua city. Indoor as well as outdoor L_{eq} in the households located in the study area revealed lower values during winter season as compared to summer season of first year as well as second year study period. The indoor L_{eq} in Households exhibited higher values as compared to that of outdoor but both values exceeded the prescribed limits of noise level in the residential and commercial area but within the limits in industrial area. Households located in the residential area and near the institutes exhibited statistically significant ($p<0.05$) lower values of L_{eq} than the households located in the commercial area and industrial area.

Keywords: *Noise pollution, L_{eq}, NC, L_{NP}*

Introduction

There is growing evidence that noise pollution is not merely an annoyance like other forms of pollution, it has wide-ranging adverse health, social and economic effects. It is more severe and widespread than ever before and it will continue to increase in magnitude and severity because of population growth, urbanization and the associated growth in the use of increasingly powerful, varied, and highly mobile sources of noise.

Noise produces direct and cumulative adverse effects that impair health and that degrade residential, social, working and learning environments. It interferes with sleep, concentration, communication and recreation.

One challenge for researchers today is to increase our understanding of the possible health impacts of being exposed to noise pollution for longer period of time. With research indicating that a very large number of people spend more than 90% of their time indoor, the indoor noise pollution could also be great risk to the health. In many countries of the world steps are being taken to stop the damage to our environment from noise pollution i.e. scientific groups study the ill effects of noise on living organisms, Legislative bodies write laws to control noise pollution and educators in schools and universities teach students about effects of noise pollution.

The first step for solving noise pollution is assessment. In present study an attempt has been made to assess the status of noise pollution in households located at different areas of Kathua city.

Materials and Method

The study area was divided into 4 zones viz. Residential, Commercial, Institutional and Industrial to cover all sites having potential sources of noise pollution.

Sampling of noise level at each site of the study area was done with the help of Digital Sound Level Meter, Model-8928 at 'A' weight age. For collection of noise level data, each household was further divided into subsites viz. drawing room, bedroom, kitchen and outside. Three sampling of noise levels were recorded at each sub-site of Households during Morning hours (0800-1000 hours), Afternoon hours (1200-0200 hours) and Evening hours (0600-0800 hours).

The sampling of noise level was done twice during each of summer season and winter season in the two years study period i.e. sampling was done once during April to June and once during July to September of the summer season of first year as

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well as second year study period. Similarly sampling was done once during October to December and once during January to March of the winter season of first as well as second year study period.

Monitoring was carried out at a height of 1.5m, away from the chest. During each sampling of noise, 20 readings of SPL were recorded at an interval of 30 seconds in a period of 10 minutes. The minimum and maximum SPL were also recorded. From the observed readings of SPL obtained for each time-interval, following Noise Indices were calculated:

Equivalent Noise Level $\mathbf{L_{eq}}$:-

$$L_{eq}(A) = 10 \log \left(\sum_{i=1}^{n} fi\ 10^{Li/10}\right) dB$$

where,

fi=fraction of time for which the sound level persists.

i=time interval.

n=number of observations.

Li=sound intensity.

- Combined $\mathbf{L_{eq}}$

$$L_{eq}(A) = 10 \log \left(\sum_{i=1}^{n} 10^{Li/10}\right) dB$$

where,

i=time interval.

n=number of observations.

Li=L_{eq} of particular room.

- $\mathbf{L_{10}}$=the noise level exceeding 10% of the time.
- $\mathbf{L_{90}}$=the noise level exceeding 90% of the time.
- Noise Climate **NC** is the range over which the sound levels are fluctuating in an interval of time and is given by the following relation:
  - $\mathbf{NC=L_{10}-L_{90}}$
- Noise Pollution Level $\mathbf{L_{NP}}$:
  - $\mathbf{L_{NP}= L_{eq}+ (L_{10}-L_{90})}$

Equivalent Noise Level L_{eq} was calculated for each indoor subsite i.e. drawing room, bedroom and kitchen and combined L_{eq} of the particular household was calculated.

Results and Discussion

The analysis of the data of indoor as well as outdoor L_{eq} in the households located in the study area revealed lower values during winter season as compared to summer season of first year as well as second year study period (Table I).Overall analysis of the L_{eq} values of the different sites (i.e. households located at different sites) revealed that indoor L_{eq} during all the seasons of two year study period exhibited higher values than outdoor L_{eq}. A significant positive correlation was observed between outdoor L_{eq} and indoor L_{eq} in households at all sites during summer season of first year as well as second year study period i.e. residential (+0.3), institutional (+0.1), commercial (+0.4), industrial (+0.6) during summer season of first year study period and r value of +0.2 (residential), +0.7 (institutional), +0.1 (commercial) and +0.5(industrial) during summer season of second year study period. During winter season of first year as well as second year study period no correlation (r) was observed in households (Table II).The positive correlation between outdoor and indoor noise levels in households at all the sites during summer season clearly indicated that outdoor noise from various sources penetrated households through open doors, windows but no specific correlation between outdoor and indoor noise levels during winter season indicated that due to closed doors and windows penetration of outdoor noise was reduced so that indoor and outdoor noise levels acted as independent variants.The compiled indoor as well as outdoor L_{eq} values in the average household located in the study area during second year of study period were observed to be higher as compared with that of first year study period but at the same time the overall survey of the compiled noise level data revealed that average household in the study area exhibited higher value of indoor Noise Pollution Level (L_{NP}) and both indoor as well as outdoor Noise Climate (NC) during winter season of first year as well as second year study period as compared with that of summer season. But the computed outdoor Noise Pollution Level (L_{NP}) value exhibited higher value during winter season of first year study period and summer season of second year study period (Table I).

Table I: Average Outdoor and Indoor Noise Levels in the Households located in Kathua city.

Area	Noise Level Indices	Site	Noise Levels dB(A) during			
			First Year Study Period		Second Year Study Period	
			Summer Season	Winter Season	Summer Season	Winter Season
Residential Area	L_{eq}	Indoor	68.6±5.2 *(+13.6) (58.4-78.5)	64.4±6.3 *(+9.6) (56.2-79.3) #	68.7±7.5 *(+13.7) (53.7-78.1)	65.7±4.3 *(+10.7) (56.8-72.4)
		Outdoor	59.4±6.7 *(+4.4) (50.8-72.3)	58.2±5.1 *(+3.2) (49.2-66.0)	60.2±4.9 *(+5.2) (53.2-68.5)	57.1±5.1 *(+2.1) (50.1-67.5)
	NC	Indoor	12.0±4.0 (2.7-20.0)	15.9±4.9 # (6.5-25.1)	11.5±6.6 (2.9-29.4)	15.7±5.4 # (5.0-29.0)
		Outdoor	13.1±5.8 (3.8-24.7)	15.3±4.6 (3.8-21.7)	14.5±7.3 (3.0-29.3)	13.6±5.6 (4.6-21.7)
	L_{NP}	Indoor	80.6±5.8 (69.8-91.6)	80.2±8.6 (66.6-97.2)	80.1±9.2 (65.1-96.2)	81.4±6.3 (67.8-89.9)
		Outdoor	72.5±10.8 (54.8-93.9)	73.5±8.2 (60.0-87.6)	74.8±11.6 (57.4-94.7)	70.7±8.7 (57.3-86.6)
Near Institutes	L_{eq}	Indoor	68.7±3.6 (61.3-73.2)	65.7±4.0 # (60.5-73.5)	68.7±5.2 (56.5-76.0)	66.1±4.7 (58.1-75.9)
		Outdoor	62.1±5.3 (53.1-70.3)	61.2±4.1 (55.1-72.6)	62.5±4.9 (54.3-72.5)	61.5±4.9 (54.5-69.6)
	NC	Indoor	12.2±4.7 (5.2-26.8)	14.4±4.0 # (6.2-22.5)	12.6±2.8 (3.9-19.9)	15.6±4.1 # (9.7-30.0)
		Outdoor	13.9±3.6 (6.8-20.1)	13.9±3.4 (8.9-19.9)	14.2±3.3 (9.0-20.4)	14.3±3.4 (8.9-20.1)
	L_{NP}	Indoor	80.9±4.7 (72.4-89.2)	80.1±6.2 (72.0-94.0)	81.3±6.5 (66.3-91.9)	81.7±7.3 (69.7-100.0)
		Outdoor	76.1±8.7 (59.9-90.3)	75.1±6.9 (64.4-92.5)	76.7±8.1 (63.7-92.9)	76.0±7.8 (64.8-88.2)
Commercial Area	L_{eq}	Indoor	69.6±3.3 *(+4.6) (64.1-74.3)	68.2±3.7 *(+3.2) (61.3-74.0)	70.0±3.3 *(+5.0) (62.8-74.6)	68.0±4.5 *(+3.0) (57.2-73.3)
		Outdoor	65.5±6.0 *(+0.5) (53.4-75.2)	61.8±6.6 *(-3.2) (46.5-70.8)	65.9±5.7 *(+0.9) (54.5-74.8)	63.3±6.3 *(-1.7) (50.3-71.9)
	NC	Indoor	12.9±4.1 (6.5-26.2)	15.3±5.2 # (4.0-29.3)	13.4±3.4 (6.8-22.2)	15.3±4.0 # (6.7-26.7)
		Outdoor	11.9±4.1 (4.4-21.6)	14.8±4.0 # (8.7-23.0)	13.4±3.3 (7.5-18.2)	15.1±3.2 (10.5-21.2)
	L_{NP}	Indoor	82.5±4.5 (76.4-91.9)	83.5±5.7 (73.9-93.6)	83.4±4.0 (75.9-90.9)	83.3±5.5 (69.9-90.0)
		Outdoor	77.4±9.3 (57.8-96.8)	76.6±9.4 (57.7-91.0)	79.3±8.2 (62.0-92.0)	78.4±8.2 (61.1-91.5)
Industrial Area	L_{eq}	Indoor	70.4±6.2 *(-4.6) (54.6-78.3)	68.1±4.3 *(-6.9) (58.7-75.7)	71.0±4.5 *(-4.0) (61.0-77.8)	68.6±4.0 *(6.4) (60.3-75.5)
		Outdoor	67.7±4.3 *(-7.3) (56.7-72.3)	66.5±3.9 *(-8.5) (57.9-73.8)	68.4±3.5 *(-6.6) (60.5-72.7)	67.2±4.5 *(-7.8) (58.5-73.1)
	NC	Indoor	10.5±3.6 (4.0-19.6)	15.9±5.1 # (6.7-33.0)	12.2±2.7 (7.3-23.6)	16.0±4.3 # (8.6-26.7)
		Outdoor	12.5±3.5 (8.0-20.3)	14.7±2.6 # (11.5-21.1)	12.3±2.5 (6.9-16.3)	13.9±3.2 (9.9-20.0)
	L_{NP}	Indoor	80.9±7.9 (62.6-89.4)	84.0±8.1 (69.0-101.4)	83.2±5.5 (73.4-93.7)	84.6±6.8 (72.7-96.7)
		Outdoor	80.1±7.2 (64.7-90.4)	81.3±5.9 (69.4-93.3)	80.7±5.8 (67.4-89.0)	81.1±7.1 (69.6-91.1)
Study Area	L_{eq}	Indoor	69.3±4.7 (54.6-78.5)	66.6±4.9 # (56.2-79.3)	69.6±5.3 (53.7-78.1)	67.1±4.5 # (56.8-75.9)
		Outdoor	63.7±6.4 (50.8-75.2)	61.9±5.8 (46.5-73.8)	64.3±5.7 (53.2-74.8)	62.3±6.3 (50.1-73.1)
	NC	Indoor	11.9±4.2 (2.7-26.8)	15.4±4.8 # (4.0-33.0)	12.4±4.2 (2.9-29.4)	15.6±4.4 # (5.0-30.0)
		Outdoor	12.8±4.3 (3.8-24.7)	14.7±3.7 # (3.8-23.0)	13.6±4.5 (3.0-29.3)	14.2±4.0 (4.6-21.7)
	L_{NP}	Indoor	81.2±5.8 (62.6-91.9)	82.0±7.3 (66.6-101.4)	82.0±6.6 (65.1-96.2)	82.8±6.5 (67.8-100.0)
		Outdoor	76.5±9.3 (54.8-96.8)	76.6±8.1 (57.7-93.3)	77.9±8.8 (57.4-94.7)	76.5±8.7 (57.3-91.5)

L_{eq}: Equivalent Noise Level

NC: Noise Climate

L_{NP}: Noise Pollution Level

* values in paranthesis indicate deviation from CPCB Prescribed noise levels.

\# Seasonal difference statistically significant ($p<0.05$) during particular year of study period.

Table II: Correlation coefficient (r) of Outdoor and Indoor Leq at Households in different areas of Kathua city.

Outdoor Leq of Households	Indoor Leq during			
	First Year Study Period		Second Year Study Period	
	Summer Season	Winter Season	Summer Season	Winter Season
at Residential Area	+0.3	0.0	+0.2	0.0
near Institutes	+0.1	0.0	+0.7	0.0
at Commercial Area	+0.4	0.0	+0.1	0.0
at Industrial Area.	+0.6	0.0	+0.5	0.0

From above analysis, it can be concluded that various external sources of noise like traffic, public noise, noise from industries were responsible for increase in indoor noise level along with various indoor sources of noise like domestic appliances, fans, exhaust fan, desert coolers, television, grinder, whistling of cooker, washing machine etc.

The indoor L_{eq} in Households exhibited higher values as compared to that of outdoor but both values exceeded the prescribed limits of noise level in the residential and commercial area but within the limits in industrial area.Srivastava and Dhabal (1998) also reported the penetration of traffic noise and noise from other sources to increase indoor noise level in the residential buildings in Delhi and Kolkatta. Wilson (1963) in London, Ali (1988) in Rourkela, Dhillon *et al.* (1990) in Ludhiana, Singh and Mahajan (1990) in Kolkatta, Rao and Rao (1990) in Vishakhapatnam, Pandya and Verma (1997) in Nagpur, Koijam *et al.* (1998) in Imphal, Rampal (2005) Jammu city, Patel *et al.* (2006) in Jarsuguda and Rampal and Pathania (2008) in Bishnah also observed higher values of noise levels in residential area as compared to noise level prescribed by Central Pollution Control Board. They also observed that increasing values of noise was the most disturbing factor for residents of the area.The overall average values of L_{eq} and NC showed statistically significant ($p<0.05$) difference in the winter season as compared to the summer season of both years of study period at households located in the study area.Households located in the residential area and near the institutes exhibited statistically significant ($p<0.05$) lower values of L_{eq} than the households located in the commercial area and industrial area.

This indicated that higher outdoor noise levels in the commercial and industrial area raised the indoor noise levels in the households.

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Effect of pressmud incorporation on physiology of *Cicer arietinum*

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Abstract

Pressmud also known as filter cake or filter mud produced as a by product by sugar mills has been used as a fertilizers and ameliorant in sodic and saline sodic soils. In the present study, different concentrations of sulphitation pressmud with soil were taken i.e., 20%, 40%, 60% and 80%. Equal number of seeds were sown in each pot and irrigated daily. Effect of different concentrations of pressmud was observed on the physiology of *Cicer arietinum* after 21 days of sowing. The percentage of seed germination, vigour index, root length, shoot length, root:shoot ratio, chlorophyll a, chlorophyll b, total chlorophyll, ascorbic acid, fresh biomass and dry biomass were found to be maximum in 20% concentrations of pressmud as compared to control, though high concentration of pressmud result in reduction of growth.

Keywords: *Pressmud, Cicer arietinum, filter cake, biomass*

Introduction

Sugarcane is grown in different parts of the world since middle of the 19th century, primarily for the production of sugar. It was only after the global energy crisis of 1973, that the scientists and technologists realized the value of sugarcane, its by products and co-products. The main by products of sugar industry which have greater economic value:

i. Baggasse
ii. Molasses
iii. Pressmud or Filter Press Cake

The sugar industry by-products are vast potential reserves for human and animal consumption as well as capable of providing energy as renewable source. Bagasse is the fibrous residue left over after sugarcane is crushed. Molasses is a byproduct in the manufacture of sugar. Commercial products made by fermentation of molasses are ethyl alcohol, CO_2, Citric acid, Baker's yeast, butyl alcohol, etc. a mixture of bagasse and molasses is burnt and the ash as used as fertilizers. Pressmud is the residue obtained from sedimentation of the suspended materials such as fiber, sugar, wax, ash, soil and other particles from the cane juice. For every tonne of sugarcane crushed 30-40 Kg pressmud is produced. It is reported that 9 million tones of pressmud is generated in India (Bakthavatsalam, 1999).

Pressmud is produced at the rate of around 3% of weight of cane in sulphitation factories and 7% in carbonated factories. It contains sugar, fiber, coagulated colloids including cane wax, albuminoids, inorganic salts and soil particles. On an average, each ton of sulphitation pressmud contains 17, 36,14, 23 Kg of nitrogen, phosphorus, potassium and sulphur respectively. One of the best and cheapest alternative for chemical fertilizers is organic manure. Pressmud like other organic manures has great potential to supply nutrients in addition to its favourable effects on physico-chemical and biological properties of soil. The organic matter is highly soluble and readily available to the microbial activity and so to the soil for plants (Gaikwad, 1996; Rangaraj et. al., 2007). Filter cake when applied to the land, increase soil fertility by providing nitrogen and phosphorus for crops or ground cover growth (Ossom et. at. 2009). It improves soil nutrient availability and uptake by plants. It is a useful fertilizer, especially when applied to phosphate deficient soil and to fields in which the top soil has

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been removed or re-distributed for any reason. It is useful as a conserver of moisture and as a soil conditioner. Pressmud is also hotbed of microbial population. About 14 sp. of bacteria, 13 sp. of fungi, 5 types of yeast, 5 species of actinomycetes and a few protozoans were identified in pressmud (The Hindu, 2000). Thus, keeping all the above points in view, "Effects of Pressmud Incorporation on Physiology of *Cicer arietinum*" has been studied.

Materials and Methods

The materials and methods include the following aspects:

1. Description of sampling sites
2. Collection and storage of samples
3. Preparation of pots for experiment
4. Physico-chemical parameter of pressmud and soil
5. Mycological parameters
6. Plant parameters

1. Description of sampling sites

Sampling sites for pressmud and soil wereb Sugar factory, Saraswa, Saharanpur and backyard of Kanya Gurukul Mahavidyalaya, Haridwar respectively.

2. Collection and storage of samples

Sampling was carried out for 3 months i.e, February to April, 2010. Soil trower was used for soil sampling. For the present study, five randomly distributed sites over the field was selected, for each composite samples. Samples of soil were thoroughly mixed and for analysis purpose, soil and pressmud was air dried or oven dried and then stored in clean polythene bags.

3. Preparation of pots for experiment

Different concentrations of suphitation pressmud and soil were taken, i.e, 20% (200 gm pressmud+800 gm soil), 40% (400 gm pressmud+600 gm soil), 60% (600 gm pressmud+400 gm soil), 80% (800 gm pressmud+200 gm soil). Ten seeds of the *Cicer arietinum* were sown in each pot and irrigated daily. Effect of different concentrations of pressmud was observed on the physiology of *Cicer arietinum*. After 21 days of sowing i.e., percentage of seed germination, root-length, shoot-length, vigour index, root: shoot ratio, chlorophyll a, chlorophyll b, total chlorophyll, fresh biomass and dry biomass.

4. Physico-chemical parameter of pressmud and soil

Analysis of pressmud and soil were done according to the method as prescribed by Trivedy and Goyal (1998). Physico-chemical parameters analyzed were Moisture content, Porosity, Water holding capacity, pH, EC, Organic carbon, Organic matter, Total Nitrogen, Nitrate Nitrogen, Sulphate, Ferrous iron, Phosphate, Calcium, Sodium, Potassium and Carbon:Nitrogen ratio.

5. Mycological parameters

Five different dilutions were made and numbers of colonies and percentage occurrence of fungal proportions were observed in each dilutions (Aneja, 1993).

6. Plant parameters

Various plant parameters were studies on the *Cicer arietinum* after 21 days of sowing in pots i.e., percentage of seed germination, vigour index, chlorophyll a, chlorophyll b, total chlorophyll, ascorbic acid, biomass estimation by harvest method.

Results and Discussion

Results are presented in tables given below. Table-1 contains results of physico-chemical parameters of sulphitation pressmud and soil. Table-2 shows, total number of colonies in different dilutions. Table-3 shows, percentage occurance of fungal proportions in different dilutions and table-4 contains results of physiological parameters of *Cicer arietinum*(Black Gram).From table-1, it is evident that pressmud has high value of moisture content, porosity and water holding capacity i.e., 39.56±0.41, 0.36±0.01, 78.56±0.06 respectively. High moisture content of pressmud is due to water absorbing capacity of pressmud. 10-30% moisture is favourable for crops. Yaduvanshi and Yadav (1990) reported that 10t/ha sulphitation presssmud having 40% moisture was applied with 75 kgN/ha produced cane yield equal to that by 150 kgN/ha. Water holding capacity of soil is governed by porosity or soil structure.

Table-1: Values of some selected physico-chemical parameters of sulphitation pressmud. (Values are mean±S.E. of 10 observations each).

S. No.	Parameters	Sulphitation pressmud	Soil
1	Moisture content (%)	39.56±0.41	9.66±0.53
2	Porosity(Sec^{-1})	0.36±0.01	0.24±0.01
3	Water Holding capacity(%)	78.75±0.06	43.0±0.91
4	pH	6.8±0.08	7.1±0.03
5	Conductivity(S/cm)	1.65±0.092	0.053±0.007
6	%Carbon	24.3±0.11	0.166±0.09
7	Organic matter (%)	41.89±0.19	0.287±0.18
8	Total Nitrogen (%)	1.72±0.16	0.052±0.06
9	Nitrate Nitrogen (mg/l)	33.3±1.36	26.9±2.11
10	Sulphate (mg/l)	79.75±0.21	41.25±2.16
11	Ferrous Iron (mg/l)	2.89±0.27	0.18±0.03
12	Phosphate (%)	1.45±0.05	0.53±0.23
13	Calcium (%)	0.504±0.015	0.035±0.004
14	Sodium (ppm)	20.00±0.001	14.25±0.002
15	Potassium (ppm)	21.40±0.003	5.00±0.020
16	C:N ratio	14.12	3.19

Gaikwad (1996) reported that addition of pressmud increases the water holding capacity of soil that certainly promotes the growth of plants. pH of sulphitation pressmud and soil were 6.8±0.08 and 7.1±0.03 respectively. According to Muhammad and Khattak (2009), the mean value of pH decreased with increasing levels of presumed. Patel and Singh (1993)reported that application of pressmud reduced the soil pH. The decrease in soil pH with pressmud was possibly due to replacement of exchangeable Na during Na-Ca exchange (Kumar and Abrol, 1984) and subsequent leaching of biocarbonates or the effect of salts on pH (Khattak and Jarrell, 1988). Electrical conductivity of pressmud and soil were 1.65±0.092 and 0.053±0.007 respectively. According to Omar Hattab (1998), pressmud increases the EC because decomposition process of organic matter favours the accumulation of CO_2 and release of large amounts of salts in solution which result in high Electrical Conductivity. Mathakiya and Meiseri (2003) also reported that application of pressmud increases the EC of soil. Organic matter and organic carbon of pressmud were 41.89±0.19 and 24.3±0.11 which is very high as compared to that of soil i.e., 0.287±0.18 and 0.166±0.09 respectively. The amount of organic carbon and available N, P, K increases with increasing with increasing rate of application of pressmud. Gaikwad (1996) reported that addition of pressmud increases the soil organic matter of soil that certainly promotes growth of plants. Nitrogen and Nitrate nitrogen in pressmud accounts for 1.72±0.16 and 33.3±1.36 while in soil it is 0.052±0.06 and 26.9±2.11 respectively.

The increase in available soil nitrogen on account of the pressmud application indicate that nitrogen present in the pressmud was immediately available for crop nutrition (Indiraraj and Raj, 1979). Pressmud also show high value of Sulphur 79.75±0.21, Phosphate 1.45±0.05, Iron 2.89±0.27, Calcium 0.504±0.015, Sodium 20.00±0.001 and Potassium 21.40±0.003 as compared to that of soil. According to Partha and Sivasubramanian (2006), pressmud contains about 1.15 to 3.0% nitrogen, 0.6 to 3.50% phosphorus and 0.30 to 1.80% potassium.

Table-2: Number of colonies present in different dilutions.

S.No.	Dilution	Number of colonies
1	10^{-1}	45.5±0.35
2	10^{-2}	29.5±0.35
3	10^{-3}	21.5±0.53
4	10^{-4}	6.75±0.17
5	10^{-5}	2.75±0.17

(Values are mean±S.E. of 2 observations each).

Table-3: Percentage occurrence of fungal proportions in different dilution.

S.No.	Name of fungi sp. occurrence	Dilutions				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1	*Aspergillus* sps.	23.63±0.57	27.12±0.32	32.89±0.84	29.66±0.77	45.00±3.53
2	*Penicillium* sps.	18.67±0.41	19.48±0.36	18.76±1.19	25.82±1.94	39.66±3.3
3	*Mucor* sps.	15.93±0.72	16.95±0.21	15.35±1.21	18.40±1.15	-
4	*Rhizopus* sps.	13.18±0.14	12.69±0.44	11.77±0.41	10.98±0.98	-
5	*Alternaria* sps.	8.77±0.70	6.77±0.07	4.70±0.11	-	-
6	*Fusarium* sps.	8.78±0.01	5.08±0.05	4.70±0.11	-	-
7	*Cladosporium* sps.	11.53±0.29	11.86±0.14	10.64±1.09	15.10±1.01	18.33±1.18

(Values are mean±S.E. of 2 observations each).

Table-4: Physiological parameters of *Cicer arietinum* after 21 days in different concentrations.

S.No.	Physiological parameters	Concentrations used				
		Control	20%	40%	60%	80%
1	Seed germination (%)	80	90	90	90	90
2	Root length (cm)	5.3±0.49	8.45±0.03	6.6±0.63	7.2±0.21	7.5±0.11
3	Shoot length (cm)	13.6±0.56	18.36±0.39	15.4±0.35	16.1±0.26	17.9±0.14
4	Vigour index	1504	2407.5	1980	2097	2286
5	Root:Shoot ratio	0.38	2.71	0.42	2.23	2.38
6	Chlorophyll a (mg/g) Fresh weight	4.17±0.07	6.69±0.05	4.86±0.06	5.96±0.05	6.49±0.02
7	Chlorophyll b (mg/g) Fresh weight	1.70±0.01	2.44±0.08	1.59±0.04	2.08±0.09	2.05±0.07
8	Total Chlorophyll (mg/g) Fresh weight	5.87±0.08	9.13±0.13	6.45±0.10	8.05±0.14	8.54±0.09
9	Ascorbic Acid (mg/g) Fresh weight	1.29±0.03	1.56±0.10	1.36±0.08	1.46±0.19	1.53±0.11
10	Fresh Biomass (gm/m^2)	119.4	230.5	138.8	170.0	184.3
11	Dry Biomass (gm/m^2)	16.9	26.4	17.1	20.0	21.3

(Values are mean±S.E. of 2 observations each).

Table 2-3 shows the mycological study of sulphitation pressmud. From table-2, it is evident that the total colony counted in different dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were 45.50±0.35, 29.5±0.35, 21.25±0.53, 6.75±0.17 and 2.75±0.17 respectively. 10^{-1} dilution showed high concentration of fungal colony while 10^{-5} showed low concentration of fungal colony, hence the occurrence of fungal colony is inversely proportional to concentration of dilutions.

From table-3, it is evident that percentage occurrence of *Aspergillus* species and *Pencillium* species were found abundantly in all dilutions because these are fast growing species. These species achieve good growth when moisture content is more than 14%. Pressmud has high moisture content, appropriate pH range and high organic matter with N, P, and K favourable for growth of fungi (Barnett and Hunter, 1999) .Table-4, assess the effect of sulphitation pressmud on *Cicer arietinum* after 21 days of sowing. Percentage of seed germination was observed same in all different concentrations of pressmud i.e., 90% as compared to control i.e., 80%. Raman and Sundram (1996) found that the application of pressmud compost in the soil has significantly increased the germination of tomato seedlings. The maximum root length and shoot length were observed in 20% pressmud i.e., 8.45±0.03 cm and 18.3±0.39 cm and minimum was observed in control i.e., 5.3±0.49 cm and 13.6±0.5 cm respectively. According to the Arvind and Pushpalata (2006), there is an increase in root growth and shoot growth of the *Cicer arietinum* plant by addition of rhizobium and pressmud in the soil. The maximum total chlorophyll content was calculated in 20% pressmud i.e., 9.13±0.13 mg/gm and minimum was in control i.e., 5.87±0.08 mg/gm respectively. An increasing trend of chl.a, chl.b and total chlorophyll was reported in farmyard manure incorporated treatment followed by pressmud and inorganic fertilizer treated plots alone (Bokhtiar and Sakurai, 2005). Ascorbic acid in the present study was highest in 80% pressmud i.e., 1.53±0.11 mg/g and lowest in control i.e., 1.29±0.03 mg/g. Ascorbic acid content increases with increase in level of pressmud. The fresh and dry biomass of *Cicer arietinum* was highest in 20% pressmud i.e., 230.5 g/m^2 and 26.4 g/m^2 respectively against control i.e., 119.4 g/m^2 and 16.9 g/m^2 respectively. All growth parameters showed maximum results in 20% pressmud as compared to control which indicated that this concentration is favourable for plant growth.

Conclusion

Present study showed that application of pressmud improves the soil structure and provide better aeration for exchange of gases. It also increases the soil organic matter and water holding capacity of soil that promotes the growth of plant. Pressmud has great potential of increasing nutrient content, thereby improving the yield of crop upto significant extent. The values of root length, shoot length, vigour index, root:shoot ratio, chl.a, chl.b, total chlorophyll, fresh biomass and dry biomass were maximum in 20% pressmud which may be due to its high organic matter, adequate nitrogen and phosphorus content. This indicates that 20% pressmud is most favourable for plant growth.

Hence, the physico-chemical and mycological study of pressmud suggests that pressmud is a useful organic fertilizer which helps to reduce the use of chemical fertilizer and its application improves the soil structure that promotes the growth of plant.

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Generation, composition and management of solid waste at Muthi, Jammu

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Abstract

The paper deals with the generation, composition and management of solid waste at Muthi, Jammu. Study on Generation and Composition of solid waste was carried during January to December, 2007. In this paper characteristics of solid waste and its probable impacts on the environment in general and population in particular have been discussed. Recommendations regarding solid waste management have also been given. Findings revealed various short comings in the present disposal system which affected the quality of environment in the study area.

Keywords: *Solid waste, composition, characteristics, management*

Introduction

The western model of development by means of aggressive industrialization and insane consumerism has invaded urban India and converted innocent elegant spaces into technologically driven factories and slews of concrete skyscrapers. All over the urban areas, we find waste, garbage, factory refuse, medical leftovers, plastics and municipal filth. Much of it is miasmatic, foul and toxic. A recent report on ambient air quality around Chennai's Kodungaiyar waste dump site has sent out a strong health warning to over 100,000 residents living in its vicinity. Every day the municipal corporation of Chennai dumps around 3,200 tonns of waste either at Kodungaiyar or the near by Perungudi dumpyard. Released by a Chennai based NGO Community Environmental Monitoring (CEM) in December 2006, the report notes the presence of chemicals in the ambient air of Kodungayar. Analysis showed five of the chemicals exceeded permissible levels of USEPA out of which 1, 2 dichlorobenzene, benzene and chloromethane are known carcinogens. The data also indicated that unsegregated waste, including medical waste and plastics is being openly burnt at Kodungaiyar (Nidhi Jamwal, Down To Earth Jan., 2007).

Currently the city of Kochi is a victim of garbage macro crisis. It faces the imminent peril of fatal fevers. There is even the potential for a plague outbreak**.** Avijit Ghosh (2007) highlighted the role of waste pickers in municipal solid waste management and reported that every day waste picker picks 50-60 kg of waste which means he helps recycle between 15000-18000kg waste every month. NGOs estimate that their efforts save the MCD about Rs 6 lakh every day. Waste pickers often pick up diseases such as TB, anemia, gastrointestinal ailment and eye infection diseases related to lungs and skin are common. Choudhary and Malik (2007) explained various kinds of health hazards related to solid waste. Except for some preliminary investigations made by Rampal *et al.* (2002) Sambyal (2006), Manhas (2007) who have tried to gather information on this aspect of environmental concern, very less is known about the impact of unscientific and improper waste disposal on the environment in general and population in particular from J&K state in general and Jammu in particular.

Methodology

The study area Muthi lies between 32°27'16.16"N latitudes and 75°21'47.08" E longitudes in the North of Jammu city within the municipal limits.

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In order to analyze the composition of solid waste at Muthi, the area was divided into four study sites which include **Site I: Primary health centre, Site II: School, Site III: Main bazaar and Site IV: Minibus stand.** Sampling of solid waste was carried out thrice a month for a period ranging from January 2007 to December 2007. During each sampling, solid waste generated for 24 hours was collected and segregated into various categories viz., biodegradable material (vegetative/putrescible matter, paper and rags), non-biodegradable material (plastic, metal, glass) and inert and miscellaneous waste (sand, pebbles, stones, chalk pieces, construction & demolition waste etc.). Each component of the waste was then weighed and the average weight percentage, standard deviation was computed for a period of one year.

During present investigations, for analyzing household waste, the residential area of Muthi was divided into four zones. From each zone five houses were selected and the average waste generation/capita/day was studied. Samples were collected thrice a month from each house over a period of twelve month (January,2007-December,2007).During each sampling, the waste generated during period of twenty-four hours in a household was collected in a polythene bag and weighed, with the help of spring balance of different scales viz; 200gm, 1kg, 10kg, 25kg. The total number of residents of the house during the sampling period was also recorded to calculate per capita per day values. Per capita per day waste generation values were also calculated for 12 months.

Results and Discussion

Site: (I): Primary health centre: The critical examination of the table: 1 reveals that the total average solid waste (gm/month) ranges from 16014.6 to 22505.07gm with an average value of 20040.69gm comprising 5787.52±2388.24gm of biodegradable10210.13±2230.81gm of non-biodegradable and 4043.9±786.23gm of inert & miscellaneous waste.

Site: (II): School: A close examination of the table: 1 reveals that the total average solid waste (gm/month) ranges from 21313.72-28140gm with an average value of 24847.32gm and comprised 15792.47±1669.61gm of biodegradable waste, 4563.12± 449.85gm of non-biodegradable and 4491.78±993.99 gm of inert & miscellaneous waste.

Site: (III); Main bazaar: An observation of the table: 1 reveals that the total average solid waste (gm/month) ranges from 1160101.9-17332227gm with an average value of 14287718gm comprised 11485467.5±2259815.16gm of biodegradable, 1835142.56±1105039.96gm of non-biodegradable and 990361.7±412908gm of inert& miscellaneous waste.

Site: (IV): Minibus Stand: The close examination of the table: 1 reveals that the total average solid waste (gm/month) ranges from 71025-249996.43gm with an average value of 106626.6gm and comprised 50078.16±46401.22gm of biodegradable, 28685.33± 5601.25gm of non- biodegradable and 27863.12±8271.5 gm of inert& miscellaneous waste.

Household waste generation at Muthi: The results of twelve months data have revealed that the average solid waste/capita/day generation was observed to be 935.35gm.The total domestic waste generated per day at residential area was recorded to be 7950475.2gm (7950.47kg).

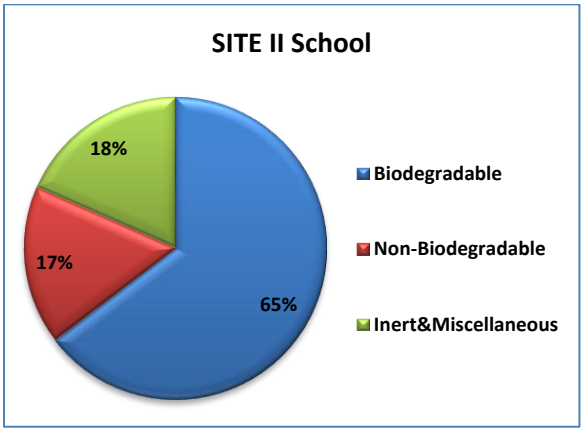
When a comparative study of solid waste generation at different sites was made it was observed that the average solid waste gm/month was maximum at Site: III; Main bazaar (14287718gm) followed by household waste (7950475.2 gm), Site: IV; Minibus stand (106626.6gm), Site: II; School (24847.32gm), Site: I; Primary health centre (20040.69gm).

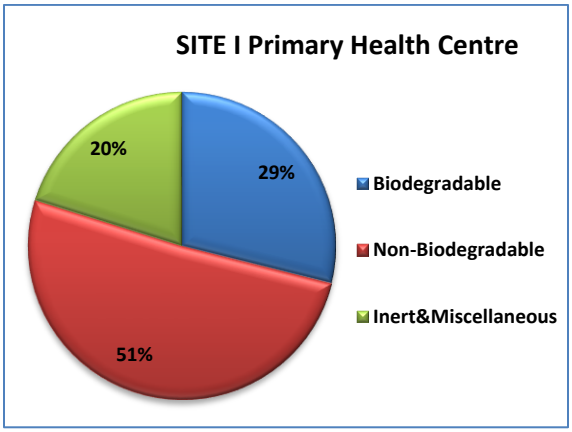
The present findings reveals that the biodegradable waste was found to exhibit the maximum percentage at all sites except the primary health centre where non-biodegradable waste showed dominance the reason being vaccination programmes carried out their, lots of medicines, glucose bottles are wasted as they exceed the expiry date, as very few people visit their for the purpose of treatment and mostly its visited for purpose of vaccination and minor injuries & illness. The waste collected from the

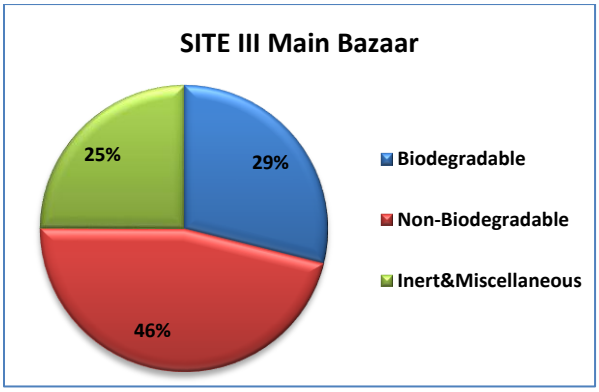
Table 1: Showing average solid waste generation (gm/day) generation & composition at different Sites (I, II, III, IV) in the study area, Muthi.

Categories	Average	Standard deviation	Range
SITE I Primary health centre			
Biodegradable	5787.52	2388.24	3209.54 -9771.2
Non-biodegradable	10210.13	2230.81	6947.1-15370.4
Inert & miscellaneous	4043.9	786.23	3000-5626.2
Total	20040.69	2206.15	16014.6-22505.07
SITE II School			
Biodegradable	15792.48	1669.56	13300-18290
Non-biodegradable	4563.12	449.85	3042.0-5042.0
Inert & miscellaneous	4491.78	994.05	2948.4-6045
Total	24847.32	2010.50	21313.72-28140
SITE III Main bazaar			
Biodegradable	11485467.5	2259815.16	8970016.8-15960000
Non-biodegradable	1835142.56	1105039.96	651027.5-1372006.6
Inert & miscellaneous	990361.7	412908	382209.2-1798003.4
Total	14287718	2084339	11160101.9-17332227
SITE IV Minibus stand			
Biodegradable	50078.16	46401.22	26123.7-195350.53
Non-biodegradable	28685.33	5601.24	20399.1-41863.02
Inert & miscellaneous	27863.12	8271.5	18298.06-45024.0
Total	106626.6	48039.56	71025-249996.43

municipal limits is commonly disposed off by open dump method. The temporary dumping site are near the pond in Muthi village, vacant plots, streets adjacent to roads, at the bank of canal, in Muthi nullah and near a community handpump in Muthi camp from the temporary dumping sites only a fraction of waste is being collected every week on Wednesday, rest remains unattended. The final disposal site is the bank of river Tawi. This presents a clear picture of ill management of waste generated in Muthi.







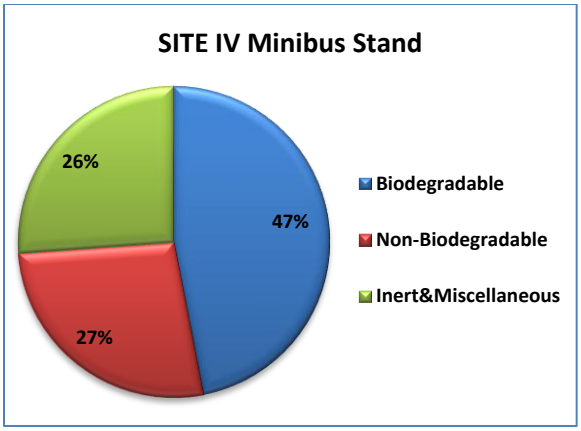


Fig.1. Pie diagrams showing percentage of different components of solid waste at different study sites

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Isolation, characterization and identification of bacteria by FAME based analysis for herbicide degradation

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Abstract

Fatty acid profile using Sherlock system is a technique in which unique fatty acid is matched with Sherlock micro-organism library. In the current investigation, the bacterial species degrading 2, 4-Dichlorophenoxy acetic acid (2, 4-D) were isolated from soil and monitored for their ability to degrade herbicide. These species were cultivated on Bushnell Hass Agar (BHA) and Bushnell Hass Broth (BHB) with increasing concentration of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) as a sole carbon source. The growth of organisms and percentage degradation of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) was studied by turbidometric method. In the given study, by using FAME based analysis system, two species were obtained which degrade herbicides and they were confirmed on the basis of fatty acid profile as *Escherichia coli* and *Citrobacter koseri*. Hence from the current investigation one may conclude that, these two species can be used in the field for purpose of bioremediation in near future.

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Keywords: *2,4-Dichloroacetic acid (2,4-D), FAME based analysis, fatty acid profile, herbicides, Sherlock microorganism library, turbidometric method.*

Introduction

Herbicides are the agents, usually chemicals used for killing or inhibiting growth of unwanted plants or weeds. They compete with important crop plant for water, light, nutrients, space and carbon dioxide. Other than they reduce crop quality by contaminating the commodity, interfere with harvesting, serving as hosts for crop diseases or providing shelter for insects to overcome winter and also have some effects on human and animal health. Herbicides provide a convenient, economical, and effective way to manage weeds (Dwight ,1998). An herbicide's mode of action is the biochemical or physical mechanism by which it kills plants. Most herbicides kill plants by disrupting or altering one or more of their metabolic processes. Some disrupt the cellular membranes of plants, allowing cellular content to leak out, but do not directly disrupt other metabolic processes. (Tu, 2001).

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In the current study, we have taken 2, 4-dichlorophenoxy acetic acid (2, 4-D).2, 4-dichlorophenoxy acetic acid (2, 4-D)2, 4-dichlorophenoxy acetic acid, commonly abbreviated as 2, 4 D is one of the most widely applied phenoxy herbicides in many parts of the world. (Wilson *et. al* 1997; Botre *et al*, 2000). 2, 4-D products of can be used at very low application rates as growth regulators by application of aqueous foliar spray 20-40mg 2,4-D/liter on apple trees to reduce premature fruit drop, on potato plants to increase the proportion of medium size tubers or to intensify the tuber skin colour of the red varieties (Bristol *et.al.*, 1982). At low doses, these herbicides act as plant growth regulators and stimulate plant cell growth. However, at high doses, they induce phytotoxic effects. (Naylor, 2002; Vencill, 2001)

Mode of action

2, 4-D is generally applied to the foliage of broadleaf plants or directly to the soil as either a liquid or granular products. Plants absorb 2, 4-D through their roots and leaves within 4-6 rain free

hours after application (Munro *et al*.1992); if rain occurs it will dissolve in the rain water and runoff of the plants and soil before sufficient amount are absorbed by the plants. Following foliar absorption 2, 4-D progress through the plant in the phloem most likely moving with the food materials. If absorbed by roots it moves upward in the transpiration stream . It mimics the effect of auxins or other plant growth regulating hormones and stimulates growth rejuvents old cells. (Mullison, 1987).

Health effect

Acute and chronic exposure to 2, 4-D causes inhalation, ingestion, eye or skin contact and absorption through the skin, low blood pressure, etc. exposing to large amount of 2,4-D develop extreme stiffness of the extremities, lethargy,stupor, coma, severe dilation. It is also an excitant and a depressant of the central nervous system. (Hathway *et al.* 1991)

Biodegradation of 2, 4- D

Microbial degradation of 2, 4- D has been subject for the extensive studies and more recent studies has elucidated the kinetic of degradation of 2,4 D (Igbinosa, *et al*. 2007). It is considered to be the major route in the breakdown of 2, 4-D in the soil. Some microorganisms are capable of using 2, 4-D as their sole carbon source (IPCS,1989). Hemmett and Faust (1969) conclude that the size of the microbial population, the concentration of 2, 4- D and the ratio of the two factors determine 2,4-D degradation rates. Soil conditions that enhance microbial population that is warm and moist facilitate 2, 4-D degradation.(Foster & McKercher 1973). Six species of microorganisms were isolated from the soil previously treated with herbicides. These were *Flavobacterium peregrinum, Pseudomonas fluorescence, Arthrobacter globiformis, Brevibacterium* species, *Streptomyces viridochromogenes*, and an unidentified *streptomyces* species. *Flavobacterium* was the most active organism in the degrading the 2, 4,-D.

The present paper highlights on the isolation and screening of bacteria which can degrade herbicide from the soil as well as its identification by FAME based analysis. For more than 15 years a substantial portion of the pharmaceutical industry has relied on the MIDI Sherlock Microbial Identification System for the identification in their microbial testing laboratories as well as it is used in many clinical, environmental and biodefence laboratory. The Sherlock System identifies microorganisms based on fully automated gas chromatographic (GC) analysis of extracted microbial fatty acid methyl esters (FAME). More than 300 fatty acid and related compounds are found in bacteria. This system show both qualitative differences (genus level) and quantitative differences (species level).(Craig kunitsky., 2007)

Materials and method

1] Isolation and Screening

Soil sample were collected from College of Agriculture Maharajbagh, Nagpur and serially dilution from 10^{-1} to 10^{-6} was performed. For isolation of hydrocarbon degrading bacteria 0.1ml of soil sample from each dilution was inoculated to the Bushnell Hass Agar ($MgSO_4$ 0.02%, $CaCl_2$0.0002%, K_2HPO_4 0.1%, KH_2PO_4 0.1%, NH_4NO_3 0.15% ,$FeCl_3$ 0.0005%, Agar 2% pH 7.0-7.2) by spread plate techniques. Similarly for isolating Pseudomonas species process was repeated in Pseudomonas isolation agar. Incubate all the plates at 37^0c for 24 hrs; and that pure culture slants were prepared for each bacterial isolates. Now, isolated bacteria were inoculated to the Bushnell Hass agar (BHA) with 5ul of herbicide (2, 4-D) by centre streaking and incubate the plates at 37^0c for 24 hours to 48 hours. Similar process was repeated in the presence of 10ul of 2, 4-D. That bacteria were able to grow even in the presence of 10µl of 2, 4- D were selected for further studies.

2] Characterization

Gram staining, capsule staining, endospore staining methods and biochemical tests like catalase production test, urease production, gelatinase production, hydrogen sulphide production, fermentation of carbohydrates, and IMViC test were performed according to standard procedure to categorize all the bacteria isolates depending on their morphological and biochemical characteristics.

3] Study of growth of bacterial isolates in different amounts of 2, 4-D

Broth cultures were prepared by inoculating the bacteria in 5ml of Nutrient Broth and incubate at 37^0c for 24 hours. Then 5ml Bushnell Hass Broth (BHB) was taken in 18 test tubes in three sets. 2,4-D was added to each set with increasing amount

starting from 0.5µl, 0.6µl, 0.7µl, 0.8µl, 0.9µl to 1.0µl and add 0.1ml broth culture to each tube expect for one set consider as a control. Incubate all the tubes at 37^0c for 24 hours. Take optical density of broth of each tube with respect to their corresponding controls at 610 nm using UV/Vis spectrophotometer.

Graph was plotted of optical density against amount of 2, 4-D added for all bacterial isolates.

4] Comparison of growth activities of bacterial isolates in the presence and absence of 2, 4 D

Take 50 ml of BHB in 3 conical flasks and sterilized. Add 1ml broth culture in each flask except the control. Take optical density with respective to control at 610 nm using spectrophotometer starting from the time of inoculation and continuing with the interval of 1 hour. About 7 readings were taken in all. Similar procedure was followed in the presence of 5ul and 10ul of 2, 4-D in each conical flask and reading was recorded. The growth activity was compared by plotting graph of optical density against time in hours for each bacterial isolates in the absence as well as in the presence of lowest and highest amount of 2,4-D.

5] Determination of presence of 2, 4-D biodegradation

Take 50ml BHB in 5 conical flasks with one blank, two as control (I0 and I24) and other two as experimental and sterilized. Add 5ul 2, 4-D in each conical flask except blank and 1 ml culture broth was inoculated in only two experimental conical flask and incubate it at 37^oC at 24 hours. Take O.D just after inoculation of 5µl of 2, 4-D for control I0 at 284nm using spectrophotometer with respect to blank containing only BHB, after filtering and centrifuging them at 10,000 for 10 min and take reading. After incubation, the remaining flask were filtered and centrifuged and optical density was taken at 284 nm with blank. Percentage of biodegradation was determined through calculation. Similar procedure was repeated for 10µl of 2, 4-D.

6] Bacterial identification by fame based analysis.

For identification of aerobic bacteria isolated from environmental sample, bacterial cultures were inoculated by four ways streaking on Trypticase Soy Broth Agar (TSBA) (Casein peptone 1.5%, soya peptone 0.5%, Sodium chloride 0.5%, Agar 1.5%) and incubated at 28^0C for 24 hours.

6.1 **Sample processing** – The five steps to prepare GC ready extracts are as follows-

Harvesting- A 4mm loop is used to harvest about 40mg of bacterial cells from the third quadrant (second or first quadrant if slow growing) of the quadrant streaked plate. The cells are placed in a clean culture tube.

Saponification-1.0ml of Reagent 1(45g sodium hydroxide,150 ml methanol, 150 distilled water) is added to each tube containing cells. The tubes are securely sealed with Teflon lined caps, vortexed briefly and heated in a water bath at 100^0 c for 5 minute; the tubes are vigorously vortexed for 5-10 seconds and returned to the water bath for further 25 minute heating.

Methylation-The cooled tubes are uncapped, 2.0ml of reagent 2 (325ml certified 6N hydrochloric acid, 275ml methyl alcohol) is added. The tubes are capped are briefly vortexes. After vortexing, the tubes are heated for 10 minute at 80^0 c (This step is critical in time and temperature.)

Extraction-Addition of 1.25ml of reagent 3 (200ml hexane, 200 ml methyl tertiary butyl ether) to the cooled tubes is followed by recapping and gentle tumbling on a clinical rotator for about 10 minutes at 6 rpm. The tubes are uncapped and aqueous (lower) phase is repeated out and discarded.

Base Wash-About 3.0ml of reagent 4 (10.8g sodium hydroxide, 900ml distilled water) is added to the organic phase remaining in the tubes. The tubes are recapped and tumbled for 5 minutes at 6 rpm. Following uncapping, About 2/3 of the organic phase is pipette into a GC vial which is capped and ready for analysis.

6.2 Run the calibration mixed and samples into the Sherlock MIDI by rapid method.

Results and discussion

1) Isolation & Screening:-

Bacterial cultures were obtained after incubation of Pseudomonas isolation agar and Bushnell Haas agar (BHA) containing plates inoculate with serially diluted soil sample. After incubation number of colonies appeared on pseudomonas isolation agar but all were single type. Out of them 1 colony was chosen from 10^{-5} dilution plate for preparing pure culture of *Pseudomonas* species, has been represented by culture A. Similarly on

Bushnell Haas Agar (BHA) three different type's of hydrocarbon degrading bacterial colonies were found to be appeared. Two were grown on 10^{-5} dilution plate and the third one from 10^{-3} dilution plate. Then pure cultures were prepared which are represented as culture B, culture C, and culture D respectively. On inoculation of these isolated bacteria in Bushnell Haas agar (BHA) with 5µl and 10 µl of 2, 4-D similar growth pattern was obtained after incubation period of 24 hours and 48 hours.

The results obtained in the presences of 10 µl of 2, 4-D has been represented in Table 1. It has been found that culture A was unable to growth in presence of 2,4-D even after 48 hours were as out of other three, Culture B and C was showing good growth just after 24 hours also their growth was fairly good after 48 hours while Culture D was good only growth after 48 hours. Therefore, only culture B and C was selected study and the growth of culture B & C in the presence of 10 µl of 2, 4-D is represented in Table 2 and 3.

Bacterial isolates	Bacterial growth after 24 hours	Bacterial growth after 48 hours
Culture A	-	-
Culture B	++	+++
Culture C	++	+++
Culture D	+	++

Table 1:- Growth of isolated bacteria in BHA with 10 µl of 2, 4-D

No growth -
Poor growth +
Good growth ++
Very good growth +++

Sr. No	Staining techniques	Culture B	Culture C
1	Gram staining	Gram negative	Gram negative
2	Capsule staining	Non capsulated	Non capsulated
3	Endospore staining	Non endosporic	Non endosporic
4	Motality	Motile	motile

Table 2:- Staining techniques for culture B and culture C

Sr.	Biochemical Test	Culture B	Culture C
1	Catalase	+	+
2	Urease	-	-
5	Gelatinase	-	-
6	Hydrogen sulphide production	-	-
7	Fermentation of carbohydrates Glucose Lactose Sucrose	 A+G A+G A+G	 A+G - A+G
8	IMViC Indole test Methyl Red test Voges proskauer test Citrate test	 + + - -	 - + - +

Table 3:- Biochemical tests for culture B and culture C

A=Acid
G=Gas
+ =Producing
- =Not producing

2) Characterization:

The morphology & biochemical character of the two bacterial isolate were identified by performing various staining techniques and biochemical test. The result obtain are represented in Table 2 and 3.

3) Study of growth activity of bacteria of the bacterial isolated in different amount of 2, 4-d:-

The growth of both culture B and C in different amounts of 2, 4-D has been observed by the help of turbidometric method. The optical density obtained at 610 nm of the culture B and C with increasing amount of 2,4-D in BHB after incubation is represented in Table 4 and 5 respectively. From figure 1 it has been found that growth of culture B initially increases with increasing amount of 2,4-D but after 0.9µl it has decreased. Therefore 0.9 is the most optimum amount of 2,4-D in 5ml BHB which show the maximum growth.

From figure 2 it has been found that growth of culture C initially increases upto 0.8ul but after it start decreasing. Therefore 0.8µl of 2, 4-D in BHB media is the amount at which it show maximum growth. Thus their growth is concentration

dependant and at optimum concentration they show maximum growth.

4) Comparison of growth activities of isolated bacteria in absence and presence of 2, 4-d

This study was also done by the turbidometric method. Table 6 and 7 represents O.D. of Culture B and C in the absence of 2, 4-D and in the presence of 5µl and 10 µl of 2,4-D in BHB every hour. The optical density obtained verses time were plotted for culture B and culture C and are represented in figure 3, 4 and 5 respectively and shown in table 8,9,10 and 11. It was found that the bacterial growth increases on adding 2, 4-D and also growth is more in the presence of higher amount of 2,4-D. presence of 10µl of 2,4-D in 50 ml of BHB is represented in figure 6 From this graph it has been found that culture C grows faster as compared to culture B.

Sr. No.	Amount of 2,4-D added (µl)	Optical density
1	0.5	0.109
2	0.6	0.182
3	0.7	0.199
4	0.8	0.381
5	0.9	0.604
6	1.0	0.383

Table 4:- O.D. obtained at different amount of 2, 4-D for culture B

Sr. No.	Amount of 2,4-D added (µl)	Optical density
1	0.5	0.120
2	0.6	0.209
3	0.7	0.219
4	0.8	0.356
5	0.9	0.162
6	1.0	0.177

Table 5:-O.D.obtained at different amount of 2, 4-D culture C

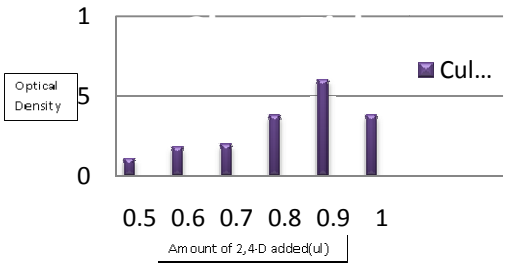

Figure 1:- Graph of growth activity of Culture B in increasing amount 2,4-D

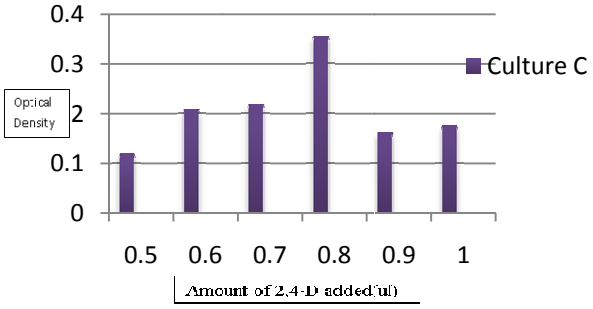

Figure 2:- Graph of growth activity of Culture C in increasing amount 2,4-D

Sr No	Time (hr)	Optical density		
		0 µl	5 µl	10 µl
1	0	0.049	0.057	0.064
2	1	0.054	0.051	0.076
3	2	0.055	0.080	0.089
4	3	0.058	0.068	0.090
5	4	0.058	0.075	0.104
6	5	0.056	0.065	0.110
7	6	0.061	0.079	0.122

Table 6:- O.D. obtained every hour for culture B in 0 µl and 5 µl and 10 µl of 2, 4-D.

Sr. No.	Time (hr)	Optical density		
		0 µl	5 µl	10 µl
1	0	0.059	0.055	0.065
2	1	0.052	0.063	0.075
3	2	0.077	0.056	0.079
4	3	0.070	0.095	0.096
5	4	0.061	0.096	0.128
6	5	0.060	0.090	0.122
7	6	0.065	0.112	0.141

Table: 7:-O.D. obtained every hour for culture C in 0 µl and 5 µl and 10 µl of 2, 4-D.

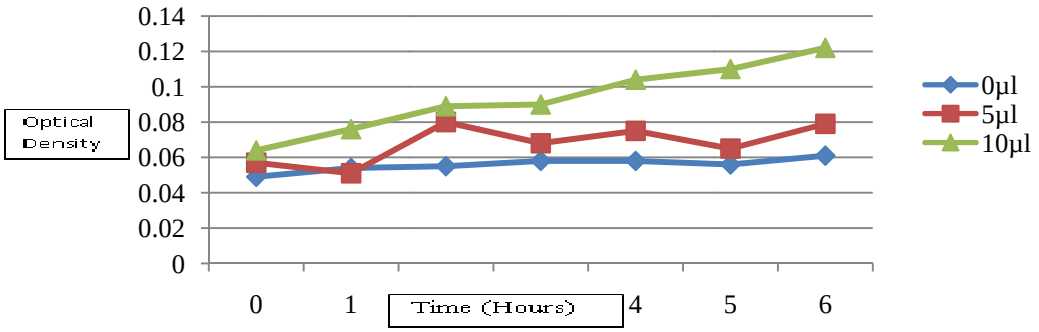

Figure 3:- graph of growth activity culture B in 0 µl, 5 µl and 10 µl of 2,4-D

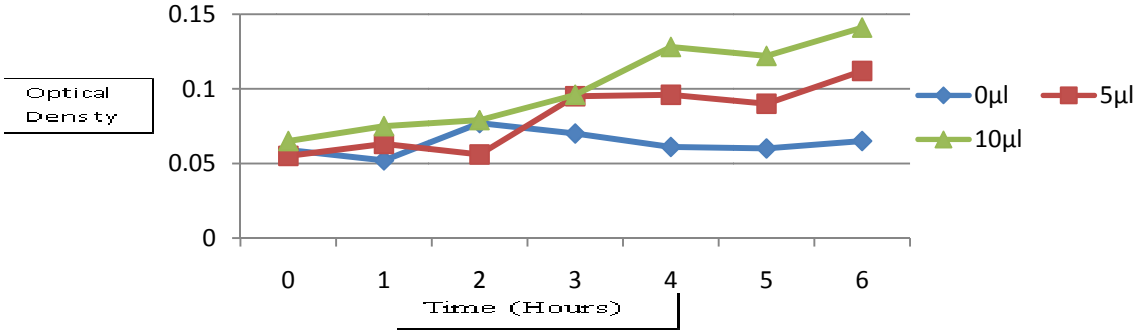

Figure 4:- graph of growth activity culture C in 0 µl 5 µl and 10 µl of 2,4-D.

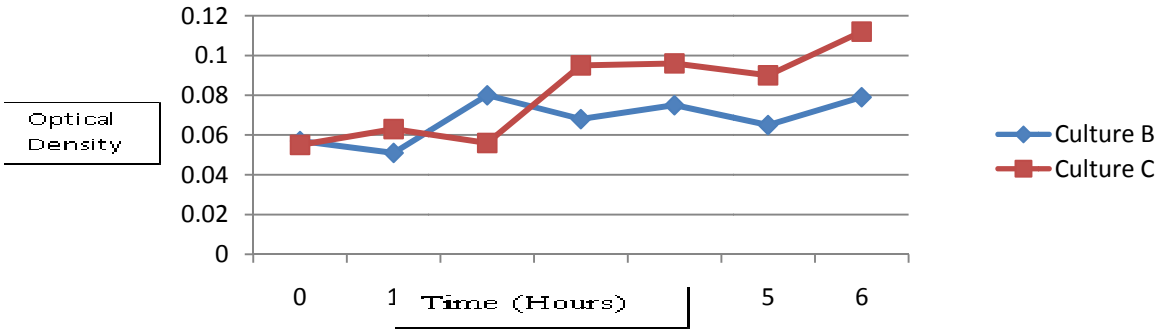

Figure 5:- Graph comparing growth acitivities of culture B and C in 5µl of 2, 4-D

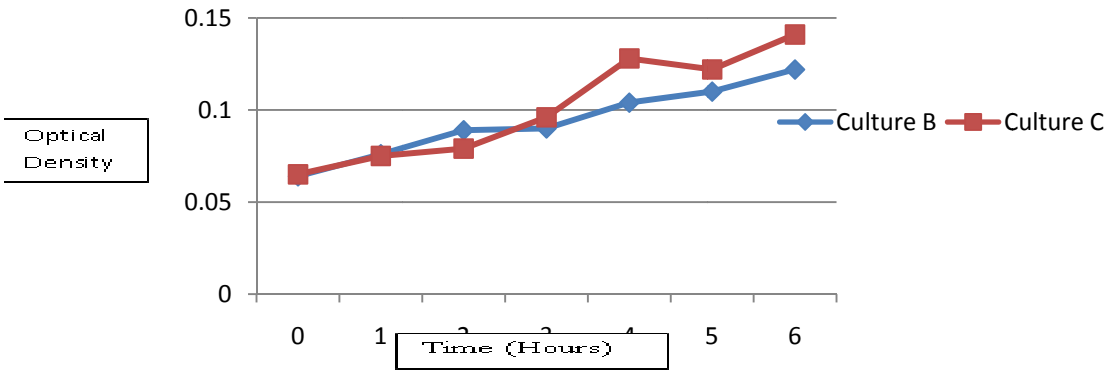

Figure 6:- Graph comparing growth acitivities of culture B and C in 10µl of 2, 4-D

Table 8:-O.D. obtained for control and cultures in presence of 5 µl of 2, 4-D

Sr. No.	Sample	Incubation period	Optical density
1	Control I_0	0 hour	0.291
2	Control I_{24}	24 hour	0.223
3	Culture B	24 hour	0.198
4	Culture C	24 hour	0.179

Table 9:- Percentage of degradation 5 µl of 2, 4 -D

Sr.No.	Sample	Amount of 2,4-D remaining (ppm)	Aount of 2,4-D lost(ppm)	Percentage of degradation (%)
1	Control I_{24}	76.63	-	-
2	Culture B	68.04	8.59	11.21%
3	Culture C	61.51	15.12	19.73%

Table 10:- O.D. obtained for control and culture in presence of 10 µl of 2,4-D

Sr.No.	Sample	Incubation period	Optical density
1	Control I_0	0 hour	0.309
2	Control I_{24}	24 hour	0.224
3	Culture B	24 hour	0.178
4	Culture C	24 hour	0.158

Table 11:- Percentage of degradation 10 µl of 2,4-D

Sr.No.	Sample	Amount of 2,4-D remaining (ppm)	Aount of 2,4-D lost(ppm)	Percentage of degradation (%)
1	Control I_{24}	72.49	-	-
2	Culture B	57.60	14.89	20.54%
3	Culture C	51.13	21.36	29.47%

5) Determination of percentage of 2,4-dbiodegradation:

The percentage of 2, 4-D biodegradation by Culture B and Culture C was measured by the help of turbidometric method.

CALCULATION:

The biodegradation percentage of 2, 4-Dis calculated by following way.

For 5µl 2, 4-D

The O.D. of control I_0 at 0 hour obtained is 0.291 considering it has 100 ppm then control I_{24} with O.D of 0.223 contains = 0.223 x 100/0.291 =76.63 ppm of 2,4-D

Culture B after 24 hours with O.D. of 0.198 contains =0.198 x 76.63/0.223 =68.04 ppm of 2, 4-D

Culture C after 24 hours with O.D. 0.179 contains=0.179 x 76.63/0.223 =61.51 ppm of 2, 4-D

The amount of 2,4-D degraded by culture B =76.63-68.04=8.59ppm

The amount of 2,4-D degraded by culture C =76.63-61.51=15.12ppm

The biodegradation % of 2,4-D by culture B=8.59x 100/76.63 =11.21 %

The biodegradation % of 2,4-D by culture C =15.12x 100/76.63=19.73%

For 10µl 2, 4-D

The O.D. of control I_0 at 0 hour obtained is 0.309 considering it has 100 ppm then Control I_{24} after with O.D of 0.224 contains =0.224x 100/0.309 = 72.49ppm of 2,4-D

Culture B after 24 hour with O.D. of 0.178 contains=0.178x72.49/0.224 =56.60ppm of 2, 4-D

Culture C after 24 hours with O.D. 0.158 contains=0.158x72.49/0.224 = 51.13 ppm OD 2,4D

The amount of 2, 4-D degraded by culture B =72.49-57.60=14.89pp

The amount of 2, 4-D degraded by culture C =72.49-51.13=21.26ppm

The biodegradation % of 2, 4-D by culture B=14.89x100/72.49=20.24%

The biodegradation % of 2, 4-D by culture C=21.36x 100/72.49=29.47%

From the calculation it has been found that Culture B degrade 11.21% 2,4-D in the presence of 5ul and 20.54% in the presence of 10ul of 2,4-D in BHB media. Whereas Culture C degrade 19.73% 2,4-D in the presence of 5ul and 29.47% in the presence 10ul of 2,4-D in BHB media. Thus the percentage of biodegradation is more at higher amount of 2,4-D in case of both Culture B and Culture C. also the percentage of biodegradation of 2,4-D by culture c is more compared to culture B.

6) Bacterial identification by fame based analysis:

Two herbicides 2, 4-D degrading bacteria that are culture B and culture C were identified by FAME based analysis using gas chromatography (GC). The extract was prepared according to the procedure and then was run in GC. The growth of culture B and C obtained on Trypticase Soya Broth agar (TSBA). The technique is used by Sherlock system to present result is based on similarities index (SI). The SI numerical value which express the fatty acid composition of unknown compare with mean fatty acids composition of the strain used to create library entry listed as if match. The SI is not "probably" or percentage but an expression of the relative distance of the unknown sample from the population mean. An exact match between fatty acid profile of the unknown and the mean of the library entry will result in SI of 1.0000. As each fatty acid varies from the mean percentage, the SI will decrease in proportion to the cumulative variances between the composition of the unknown and the library entry. Sample with a SI of 0.500 or higher and with a separation of 0.100 between the first and second choice are considered good library comparisons. If the SI is between 0.300 and 0.500 and well separated from the second choice (>0.100 separation), it may be a good match, but an atypical strain (it would fall still very far away from the mean on the normal distribution curve).Values lower than 0.300 suggest that the organism is not a species in the library, but the software will still indicate the most closely related species, which can be useful when a new species is encountered.

The FAME based analysis of bacterial isolates were showed that culture B is *Escherichia coli* with SIM index 0.825 and culture C is *Citrobacter koseri* with SIM index 0.813.

Conclusion

Bacterial species were isolated and monitored for their ability of herbicide (2,4-D)degradation, isolated from land of college of Agriculture Maharajbagh, Nagpur. All bacterial isolates were cultivated in solid media (Bushnell Haas agar) and in liquid media (Bushnell Haas Broth) with 2,4-D as a sole source of carbon. 2 out of 4 bacteria were found to have the ability to utilize 2, 4-D rapidly. Bacterial species capable of degrading 2, 4-D were characterized for their morphological and biochemical feature to the first step of identification. They were further identified as *Escherichia coli* and *Citrobacter koseri* by their fatty acid profile using Sherlock (software use for FAME analysis) in which their unique fatty acid profile was match with the Sherlock microorganisms' library. Increasing amount of 2, 4-D and percentage of 2, 4-D degradation in bacterial growth was studied by turbidomatric method and also compare the growth activities in absence and presence of 2, 4-D by the same method.

The aim of current work is to isolate bacteria that are able to degrade herbicide (2,4-D) has been successfully achieve. Thus it can be concluded that *Escherichia coli* and *Citrobacter koseri* are able to degrade 2, 4-D. their growth concentration dependent and at optimum concentration they show maximum activity. Also *Citrobacter koseri* is better than *Escherichia coli* in herbicide degradation and its growth activity. Thus they can be used in the field for bioremediation. However, in order to increase the feasibility the bacterial isolates as commercial strains there is need of further studies

on the biodegradation pathway and the byproduct produce after it. The byproduct should be less toxic otherwise it can cause more damage to environment as compare to earlier. The oxygen, nutrient, optimum temperature range, salinity and physical state of the soil and other inhibitory factors that can affect their growth in the presence of 2,4-D should also be studied. It is essential to identify the enzyme that is responsible for biodegrading ability and the gene responsible for secreting that enzyme. Other factors such as mutation can also affect their biodegradation capability.

If *Escherichia coli* and *Citrobacter koseri* meets every required standards then they can be applied in bioremediation processes on an industrial scale.

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Physico-chemical property of River Ganga at foot hills of Garhwal Himalayas

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Abstract

To assess the water quality of river Ganga at foot hills of Garhwal Himalaya five sampling station have been selected in a long stretch of 125 Km from Devprayag to Roorkee. The investigation was carried out for a one year (2010-2011). In the present study of river Ganga its physio-chemical characteristics*viz* temperature, turbidity, conductivity, total solid, BOD, COD, DO, Alkalinity, Acidity, Hardness, Chloride were done. A minor difference in all the physico-chemical parameters were observed in all the sampling station studied during the course of study.

Keywords:*BOD,Conductivity,Physico-chemical*

Introduction

The River Ganga (2,525 km long) is the largest river basin in India, covering 26.2 percent of India's total geographical area. The Bhagirathi River emerges at Gaumukh (30°92” N, 79°08” E, elevation 4100 m) from Gangotri glacier in Western Himalaya. Alaknanda River emerges from the Saptonath-Kharak group of glaciers, 8 km away from Badrinath(30°44" N, 79°32" E, elevation 3123 m). The stream formed after their merger at Devprayag is formally known as Ganga River.The country is blessed with so many river systems that have a history of sustaining civilizations as old as Harappa and Indus Valley civilizations. That is why rivers are held in awe and revered in our country. But we have taken an unfair advantage of these lifelines of our country by polluting them. Water resources are national assets and holds key of the economy of the country. This vital resource is becoming a scarce commodity and as such required to be planned, developed and managed with most care.

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But due to growing population and industrialization, maintains and safe guarding of this precious resources is however, neglected (Anchal*et al*., 2007).The maintenance of healthy aquatic ecosystem is depended on the physico-chemical properties and biological diversity. A regular monitoring of water bodies with required number of parameters with reference to the quality of water not only prevents the outbreak of diseases and occurrence of hazards but checks the water from further deterioration. A number of investigations have been conducted to study the physicochemical properties of water in different Rivers. The River Ganga is a part and parcel of everyday life in the city and thousands of people bath daily in the River Ganga. Pressure on the river increasing is enormously due to ever increasing population, industrial and urban growth in the river basins. The Ganga has been worshipped by Indians from time immemorial and the practice still continues. The water of the Ganga was considered to be holy, having powers to rid us from all our sins. But now the water has become contaminated to such an extent that it has the potential to cause many life threatening diseases. At Haridwar domestic sewage and untreated industrial effluents along with the excreta of various warm blooded animals are directly or indirectly discharged into

river Ganga which had adversely affected physicochemical property of River Ganga. In the present paper an attempt has been made to assess the impact of changes on the physiochemical properties of water of river Ganga at foot hills of Garhwal Himalaya.

Materials and method

The investigation was performed at five different sampling sites for a period of one year i.e. March 2010-March 2011in a long stretch of about 125 km. Sampling station A (Devprayag), Sampling station B (Rishikesh), Sampling station C (Haridwar) Sampling station D (PulJatwara) and Sampling station E (Roork**e**e).

Water samples were collected at monthly interval for a period of one year between 8.00AM to 10.00 AM in clean borosil glass bottles of 300 ml capacity and plastic containers from five selected sampling sites. Standard method for the examination of water and waste water was used APHA–AWWA–WPCF (1998), Trivedy and Goel (1986) and Khanna and Bhutiani (2008) for analysis work.The temperature was recorded at the sites with the help of mercury thermometer. DO in water samples were fixed with the help of mangnoussulphate and alkali-iodide-azide solution (2ml each) at the sites and analyzed in the laboratory using wrinkler´smodified iodide- azide method.

Results and discussion

On the basis of analysis result of various physico-chemical parameter are given in table 1 while correlation coefficient between different parameter are given in table 2. Temperature is the important factor which influences the chemical, biochemical and biological characteristic of the aquatic system. In the present investigation minimum water temperature was recorded 15.54±3.32 at sampling site A while maximum was recorded 17.04±4.54 at sampling site E. The average value of temperature was observed as 16.47±0.61 during the study period.The water temperature showed an upward trend from January to April followed by a downward from May onwards. A more or less similar trend has been observed in the River Yamuna by Chakrabarty*et al*. (1959). Badola and Singh (1981) also reported similar trend in the river Alaknanda. Same study was made by Khanna*et al.* (2011) in river Ganga at Haridwar, and Kulshrestha and Sharma (2006) in Ganga river at Haridwar. Same trend of temperature was observed by A. Mohan*et al*. (2007) in river Ganga at Moradabad. The electrical conductivity has always been used as a valuable method to estimate the degree of salt contents and total dissolved solids in water.Minimum Conductivity was recorded 124.23±21.75 at sampling site A while maximum was recorded 152.02±49.18 at sampling site E. The average value of conductivity was observed as 16.47±0.61 during the study period. It can be said that the present higher conductivity values in July to October month due to the input of large amount of salts and silt carried by the river.EC showed significant positive correlation with TS, chlorides, alkalinity, free CO_2, Acidity, DO and hardness and had negative correlation with Turbidity, BOD and COD. Identical results were observed byAbida and Harkrishna(2008)Dobriyal, *et al*., (1983) and Kudesiaand Verma (1985) from various Indian rivers. Singh, *et al*. (2006) also observed similar trend of conductivity in Ganga river at Bulandshahar.The Turbidity of any water sample is the reduction of transparency due to the presence of particulate matter such as lay or slit, finely divided organic matter, plankton and other microscopic organisms. The water of the river Ganga becomes start turbid from June month onward and in July to September the water was highly turbid. Minimum turbidity was recorded 111.42±132.63at sampling site B while maximum was recorded 182.32±192.32at sampling site A.The average value of turbidity was observed as 131.47±29.69 during the study period. Significant positive correlation was found withBOD, COD and free CO_2 and had negative correlation with DO, TS, acidity, alkalinity, hardness and chloride. Similar pattern was also reported by Badola and Singh (1981), Khanna*et al*. (2010) in the hill streams of the Garhwal Himalaya, Malik*et al*. (1995) in river Ganga, and Ray*et al*. (1966) in the river Yamuna and Ganga. In the present investigation it was noted that the minimum total solid was recorded 336.02±317.89 at sampling site C while maximum was recorded 627.77±753.39 at sampling site E.The average value of Total Solid was observed as 434.57±115.87during the study period. Total solids cause ecological imbalance in the aquatic ecosystem by mechanical abrasive action.

Table 1: Physico-chemical characteristics of Ganga River at different sampling stations during 2010-11

Parameter	Sampling site A	Sampling site B	Sampling site C	Sampling site D	Sampling site E	Average
Temp.(°C)	15.54±3.32	16.79±3.40	16.16±3.82	16.83±3.73	17.04±4.54	16.47±0.61
Cond. (µmhos/Cm^2)	124.23±21.75	128.69±20.74	135.81±23.76	140.35±19.18	152.02±49.18	136.22±10.80
Turbidity (JTU)	182.32±192.32	111.42±132.63	115.24±123.05	114.99±116.87	133.39±147.11	131.47±29.69
Total Solid (mg/l)	365.91±411.18	393.11±420.35	336.02±317.89	627.77±753.39	450.02±585.91	434.57±115.87
BOD (mg/l)	2.72±0.67	1.78±0.54	0.89±0.79	0.95±0.48	1.85±0.46	1.63±0.18
COD (mg/l)	8.86±2.30	8.89±2.06	6.88±1.33	6.22±0.86	9.00±1.34	7.97±1.31
DO (mg/l)	6.84±2.62	6.98±2.20	9.26±1.18	9.09±1.31	6.47±1.16	7.73±1.33
Free CO_2 (mg/l)	1.42±0.70	0.98±0.31	1.08±0.20	1.14±0.21	1.85±0.57	1.29±0.35
Acidity (mg/l)	52.19±11.60	50.00±11.91	48.56±19.67	49.55±11.84	51.25±10.68	50.30±1.98
Alkalinity (mg/l)	36.87±7.74	43.28±9.83	43.42±9.91	73.39±16.31	74.60±17.78	54.31±18.16
Hardness (mg/l)	54.65±9.44	52.88±9.76	56.65±10.58	60.98±9.74	74.02±14.78	59.84±8.48
Chloride (mg/l)	4.65±0.57	4.67±1.12	4.98±0.91	5.40±0.69	4.79±1.82	4.90±0.30

All values are mean values ,± = Standard Deviation

Higher values of total solids may cause deterioration of the surviving condition of aquatic organisms by mechanical abrasive action and enhance the turbidity of the river. Same conditions were shown by Khanna (1993), Kudesia&Verma(1985) in their studies. Sah*et al*. (2000), and Shraddha*et al*. (2011) studied that most of the Indian rivers show similar tendency with respect to fluctuations of total solids. Khanna*et al*. (2001) and Dwivedi*et al*. (2002) also made out the same study. Similar trends were shown by Bilgramiand Duttamunshi (1985).Biological oxygen demand has been used as a measure of the amount of organic material in an aquatic solution which supports the growth of microorganism. Minimum biological oxygen demand was recorded 2.39±0.46 at sampling site E while maximum was recorded 2.83±0.79 at sampling site E. The average value of biological oxygen demand was observed as 2.61±0.18 during the study period. Highest annual average value of bio-chemical oxygen demand at sampling station E may be due to drainage of several small sewage drains into the river and runoff of sludgy, silted sewage during months of rainy season. A negative relationship has been observed between BOD and COD contents. A similar pattern has been reported by Khanna&Chugh (2004) and Singh, (1999). BOD determination is a most useful technique to assess the level of organic pollution in river system. BOD levels were probably influenced by heavy metals with regard to seasonal fluctuation. Meenakshi*et al*. (2002) reported similar trends in river Yamuna and Singh, (1999) for the river Ganga. Singh*et al*. (2009) noticed peak values during summer in Irilriver and Singh &Rai (1999) observed peak values in monsoon season in river Ganga.COD determines the amount of oxygen required for chemical oxidation of organic matter using a strong chemical oxidant such as potassium dichromate under reflux conditions. Minimum Chemical oxygen demand temperature was recorded 6.22±0.86 at sampling site Dwhile maximum was recorded 9.00±1.34 at sampling site E. The average value of COD was observed as 7.97±1.31 during the study period. Similar trends of COD have shown by Khanna&Bhutiani (2003) in the river Ganga and Khanna&Chugh (2004) inGangaRiver.Temperature plays an important role in determining DO in an aquatic body. Dissolved

oxygen data are valuable in determining the water quality criteria of an aquatic system. In the system where rate of respiration and organic decomposition are high,the DO values remain lower than those of system where the rate of photosynthesis is high.A high pollution load may also decrease the DO values to considerable level.Minimum Dissolved oxygen was recorded 6.47±1.16 at sampling site E while maximum was recorded 9.26±1.18 at sampling site D. The average value of dissolved oxygen was observed as 7.73±1.33 during the study period. The cause of maximum dissolved oxygen in November to February is due to reduced rate of decomposition by decreased microbial activity at low temperature Swarnali*etal*. (2009). Meenakshi*etal*. (2002),Abowei, (2010)and Singh and Rai(1999) also have got the same result and have opined that low temperature in November to February increases the oxygen retaining capacity of water and solubility of O_2 in water. This trend was also observed by Badola and Singh (1981) in the river Alaknanda. Khanna (1993) has also reported the same trends in the river Ganga at Haridwar. Same study is also made by Joshi*et al*. (2009a) in Ganga canal at Hardwar. Concentration of dissolved oxygen is one of the most important parameter to indicate water purityand to determine thedistribution and abundance of various algal groups. It has been recommended that a minimum of 4 mg/l of dissolved oxygen should be maintained in water for healthy growth of fish and other microbial population. Different workers have pointed out various influencing factors on oxygen level that include discharges from industries, water current, velocity and biota.Minimum Free CO_2 was recorded 0.98±0.31 at sampling site B while maximum was recorded 1.85±0.57 at sampling site E. The average value of Free CO_2 was observed as 1.29±0.35during the study period.Singh*et al*. (2006) and Seth *et al*. (2000) have also reported the same trends in the river Ganga.A direct relationship was established between the water temperature and free carbon dioxide.The dissolved oxygen and free carbon dioxide usually inversely related to one another because of photosynthetic and respiratory activity of the biota (Hynes, 1970). The fluctuations in the dissolved oxygen content were mainly influenced by the factors like TOC, plankton and microbial activity.

Minimum Acidity was recorded52.19±11.60 at sampling site A while maximum was recorded 56.81833±19.67 at sampling site C. The average value of acidity was observed as 53.88±1.98 during the study period. Similar trend was found by Khanna and Bhutiani (2003).

Table 2: Correlation between physico-chemical parameter of Ganga River at different sampling stations during 2010-11

Parameter	Temp.	Cond.	Turbidity	TS	BOD	COD	DO	CO_2	Acidity	Alkalinity	Hardness	Chloride
Temp.												
Cond.	0.72											
Turbidity	-0.73	-0.38										
Total Solid	0.55	0.44	-0.30									
BOD	-0.72	-0.74	0.14	-0.77								
COD	-0.07	-0.14	0.48	-0.51	-0.01							
DO	-0.03	0.59	-0.50	0.29	-0.84	-0.96						
Free CO_2	0.12	0.59	0.47	0.02	-0.56	0.48	-0.59					
Acidity	0.10	0.48	-0.37	-0.35	0.21	-0.25	0.36	0.13				
Alkalinity	0.77	0.88	-0.35	0.79	-0.92	-0.27	0.07	0.46	-0.05			
Hardness	0.57	0.93	-0.06	0.35	-0.78	0.11	-0.25	0.83	0.34	0.82		
Chloride	0.32	0.36	-0.47	0.79	-0.36	-0.92	0.80	-0.26	0.09	0.58	0.15	

Alkalinity constitutes an important parameter in determining the quality of water. Minimum Alkalinity was recorded 36.87±7.74 at sampling site A while maximum was recorded 74.60±17.78 at sampling site E. The average value of alkalinity was observed as 54.31±18.1 during the study period.This result was supported by the finding of Kumar *et al.*(2010) Galy and Lanord (1999). The decomposition of the organic matter leads to the high alkalinity of the waters. (Maiti, 2004).Minimum Hardness was recorded 52.88±9.76 at sampling site B while maximum was recorded 74.02±14.78 at sampling site E. The average value of hardness was observed as 59.84±8.48 during the study period. Calcium and Magnesium are the two component of Total hardness. It is present in form of carbonate and bicarbonate. Calcium In is one of the most abundant substances 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. Khanna*et al*. (2001) and Joshi *et al.* (2009a) observed hardness in river Ganga at Haridwar and found more or less similar trends in their study.Minimum Chloride was recorded 4.65±0.57 at sampling site A while maximum was recorded 5.40±0.69 at sampling site D. The average value of Chloride was observed as 4.90±0.30 during the study period. Chlorides are present in sewage, sewage effluents and farm drainage. Chloride showed positive correlation with temperature, EC, TS, DO, acidity and alkalinity and had significant negative correlation with turbidity, BOD,COD and free CO_2.Significant levels of chloride were shown by many rivers like Yamuna (Meenakshi*et al*., 2002), Harshley*et al*.(1982). Khanna and Chugh (2004)also reported chloride in Ganga river at Hardwar.

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Beak abnormality in *Coturnixcoturnix japonica*

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Abstract

A reporting of an abnormality in the beak in a male Japanese quail, *Coturnixcoturnix japonica*, has been discussed in this paper. During the behavioral study of Japanese quails, a male differed markedly from the others due to its comparatively longer beak. The maxilla was abnormally elongated and curved over the mandible, which made it difficult to feed and drink.Beak deformities has been previously reported in the Antarctic Cormorant,*Phalacrocoraxbransfieldensis*chick; White winged Becard,*Pachyramphuspolychopterus*; Passerines (Craves); Brown headed Cowbird; Black Wheatears, *Oenantheleucura*; Southern Giant Petrel, *Macronectesgiganteus* chick. But no such record of beak deformity in japanese quail has been studied. The reason behind this anomaly can be a subject of research.

Keywords:*Beak abnormality, Japanese quail, Coturnixcoturnix japonica*.

Introduction

Craves (1994) considered abnormal bills to be 'noticeably different from the normal'. Normally, the maxilla (upper jaw) and the mandible (lower jaw) of the bird's beak have a bony base with a horny keratin covering at the tip, which grows continuously, and is called the rhamphotheca. The constant contact between the maxilla and the mandible mutually inhibit the growth of rhamphotheca (Rintoul, 2005). Indeed deformities like overgrowth in either mandible or maxilla or both, crossed mandible and maxilla, curvature in the maxilla on either side of the mandible, torsion in the maxilla or mandible have been reported because of injury, poor nutrition, genetic or developmental diseases and chemical pollutants (Vasconcelos and Rodrigues, 2006), or parasites (Marti *et al* 2008).

Materials and method

Here we report a male Japanese quail (*Coturnixcoturnix japonica*) with a deformed bill for the first time, during their behavioral study, at Nagpur (21.07°N, 79.27°E), central India. Out of a total 100 birds one male was strikingly different than the rest in the structure of the beak. The maxilla was elongated and curved downwards overlapping the mandible.

Results and Discussion

The measurements of the deformed bird were compared with that of other normal males without deformity (Table 1).

The deformed bird fed by tilting the head on its side. But still, morphologically the bird appeared to be normal.

Also, the comparison between the data does not shows any significant difference between the body mass, body length, tarsus length, mandibular length and bill width and bill depth of both deformed and the non deformed birds.

Only the beak length differed due to the extension of the maxilla by 1.28 times the normal length (mean=18.44). This shows that, inspite of the abnormality in the beak, the bird behaved normally.

The present study concludes that in nature beak deformities do occur and they are very rarely noticed and reported.

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Fig1. Male Japanese quail (*Coturnixcoturnix japonica*) with a deformed beak (on the left), and other male with a normal beak.

Table1. A comparison between the male Japanese quail (*Coturnix coturnix japonica*) with beak deformity and the other males without beak deformity. For comparison, the averages are taken from 20 males without deformity, represented as 'Mean+SD' with their ranges Units are presented as mass in gm; lengths in mm.

S.N.	Trait	Male with bill deformity	Males without bill deformity (n=20)
1.	Beak length	23.66	18.447+0.96 (16.94-20.04)
2.	Maxillary length	23.66	18.447+0.96 (16.94-20.04)
3.	Mandibular length	17.75	18.447+0.96 (16.94-20.04)
4.	Bill width	5.76	6.256+0.48 (5.34-6.83)
5.	Bill depth	6.79	7.875+0.41 (7.11-8.47)
6.	Body mass	210	193.66+31.32 (166.8-250.4)
7.	Body length	215	211.8+17.49 (185-250)
8.	Tarsus length	39	39+1.76 (35-40)

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