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## Sediment characterization of a sub-tropical pond of Birpur, Jammu (J&K) in relation to its macrobenthic and bacterial fauna

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### Abstract

Macrobenthic and bacterial fauna play an important role in maintaining the food chain and for exchange of nutrients in the aquatic ecosystem. The presently investigated subtropical perennial pond of Jammu situated near Birpur area. The study was carried to analyse water quality of pond in relation to its physico-chemical status and biological characteristics. Bottom sediment sample from which bacterial colonies and macrobenthic invertebrates were isolated had also been analysed for various physico-chemical parameters. Macrobenthic invertebrates were represented by *Chironomus chironomus*, *Chironomus pupae*, *Pentaneura* sp, *Melanoides tuberculata*, *Physa* sp, *Tubifex tubifex*, *Branchiura* sp. and *Dero digitata*. From the bacterial study, bacterial colonies identified were Gram +ve Bacilli, Gram -ve Bacilli, Gram +ve Cocci and Gram -ve Cocci. Comparative analysis between Bacterial colonies inhabiting sediments and water the results revealed fewer bacteria in Pond water as compared to sediment sample. Presence of few pollution indicator organisms infers that the water is getting polluted and approaching towards eutrophication.

**Keywords:** Eutrophication, Macrobenthic, Bacterial, Perennial and Physico-chemical.

### 1. Introduction

Role of various water bodies in nature is unique as it sustains life on earth and entire fabric of life is woven around water. Thus conservation of the natural environment, particularly of water requires monitoring of physical, chemical and microbiological characteristics, also are important all sediment characteristics of aquatic ecosystem which influence the biotic components as well. Sediments act as substratum for benthic flora and fauna and also act as rich source of nutrients for different inhabiting bacteria because most of the organic nutrients are released into the bottom sediments before mixing into water. Bacteria are unicellular organisms differently inhabiting different types of water and soil. Bacteria act as decomposers and consume simple sugar and simple carbon compounds, such as root exudates and fresh plant litter. Bacteria can act as pathogens and there are some species which are commonly used as indicator of faecal and sewage pollution. They are *Streptococcus* sp, *Staphylococcus* sp, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aeromonas* sp, *Enterobacter* sp, *Shigella* sp. and other coliforms. Macrobenthic communities are extremely diverse and are represented by all phyla from protozoans, through large macro invertebrates to vertebrates [1]. These ubiquitous benthic organisms react strongly and often predictably to human influences in aquatic ecosystem [2] thereby also contributing a lot to the food chain. They play an important role in transitional ecosystem, by filtering phytoplankton and then acting as food source for larger organisms such as fish, thereby linking primary production with higher trophic levels. The abundance of benthic fauna mainly depends on physical and chemical properties of their habitat as they respond more quickly if any changes in water quality occur [3]. The present study was aimed to know the sediment characteristics along with physico-chemical and biological characteristic of the perennial pond of Jammu and Kashmir, as this pond has lot of religious and biological importance.

### 2. Material and methods

The sub-tropical pond is located at 32°39'50" N and 74°57'10" E just 17 kms from Jammu city in Samba district of J&K state of India.

Important element in HR functions, because it has close relationship and connection with the organization's development. Selection is collecting the information about the applicants to decide who is fit and should be employed each work position. For studying physico-chemical and biological parameters (Macrobenthic invertebrates and bacteria) of pond, sampling was done using following methods:

### 2.1 Analysis of Physico-Chemical Parameters of sediments

Random sediment sample was collected from the bottom of the pond using Ekman's dredge. This sample was brought to the laboratory in polythene bags and then air dried. Bottom sediments were analysed for its chemical and biological parameters. Methodology adopted for sediment analysis is as under:

- **pH:** 20 gm of dried sediment sample was taken in a beaker and a suspension was made in 50 ml of distilled water. After shaking it well, pH was recorded by using a standard digital pH meter (Hanna).
- **Carbonates and Bicarbonates:** Carbonates and bicarbonates in the sediment sample were estimated following A.P.H.A. [4].
- **Chlorides:** Argentometric method using Potassium chromate as indicator was used for the determination of chlorides in sediments [5].
- **Calcium and Magnesium:** The estimation of calcium and magnesium in the sediment was done by the EDTA-titrimetric method [5].
- **Organic Carbon:** Organic carbon was calculated by Walkley and Black's rapid titration method [6].
- **Organic Matter:** Organic matter was calculated by multiplying the value of organic carbon with a factor 1.724.
- **Total Nitrogen:** Total nitrogen was calculated by the following formula:

Total nitrogen = 5% of Organic matter

### 2.2 Analysis of Bacteria

For bacterial analysis of water and sediments, water sample was collected in autoclaved glass bottles and sediment sample was collected in petriplate with the help of sieve.

The pure cultures were isolated using spread plate and rapid qualitative streak plate method. In order to identify bacteria, different methods which were used are Cultural characteristics, Morphology and Staining reaction, Biochemical reaction. The cultural characteristics such as colour and surface of colonies were observed along with any other peculiar feature of culture growth on agar plates. Morphology of these sixty seven cultures was studied by gram staining technique. Bacterial colonies were isolated on the basis of different biochemical test viz, Starch hydrolysis, Lipid hydrolysis, Casein hydrolysis, Gelatin hydrolysis, Triple sugar-iron agar, H<sub>2</sub>S production, Citrate utilization test, Catalase test, Urease test, Indole Production test. Morphological and biochemical tests were done for the identification of particular bacteria as per methods given by Cappuccino and Sherman and Bergey's Manual 9th edition [7, 8].

### 2.3 Analysis of Macrobenthic Invertebrates

The samples were collected with the use of a sampler and the collected samples were washed through sieve no 40 (256 meshes/cm<sup>2</sup>) and macrobenthic invertebrates were transferred to vials containing 5% formalin for further identification. The organisms were segregated and their abundance was calculated as no. per square meter according to Welch [9]. Preserved samples of macro benthic invertebrates were identified according to Ward and Whipple, Tonapi, Adoni and Pennak [10, 11, 12, 13]. However, for quantitative analysis, species-wise individual counting was done in the whole sample or sub sample. The number of benthos per unit area was calculated as follows:

$$\text{Benthos No. /m}^2 = \frac{N}{A \times S} \times 10,000$$

Where,

N = Number of organism collected per sample

A = Area of sampler (cm<sup>2</sup>)

S = Number of samples taken

## 3. Results

### 3.1 Bacterial Analysis

During the study period, a total of 67 isolates were obtained for pure culture and Gram's staining results revealed that, out of 49 isolates of sediment 23 were Gram-positive cocci and 18 were Gram-negative cocci while 5 were Gram-positive rods and three were Gram-negative rods. Similarly, out of 18 isolates of water, three were Gram-positive cocci and 10 were Gram-negative cocci while two were Gram-positive rods and three were Gram-negative rods (Table 1). Similar studies by using Gram's staining technique were used by various workers [14, 15, 16] (Ibe and Okpelye, 2005; Saha *et al.* 2009 and Saha *et al.* 2012) for studying different Gram-positive and Gram-negative bacteria.

10 isolates were selected for biochemical analysis from sediments and out of ten Gram-positive rods were identified as *Bacillus* sp. whereas Gram-positive cocci were identified as *Staphylococcus* sp. and *Streptococcus* sp. Among gram-negative isolates, Gram-negative cocci were identified as *Shigella* sp. and rest five were unidentified coccus species. Gram-negative rods were identified as *Escherichia* sp, *Enterobacter* sp, *Salmonella* sp, *Pseudomonas* sp. and *Bacillus* sp. [17, 18, 19, 20]. The microbial analysis results are presented and enlisted in Table 1.

During the study period, bacterial count in dilution 10<sup>-1</sup> being 1.35 x10<sup>4</sup> CFU/ml in water sample and too numerous to count (TNTC) in sediment sample, the number decreases further with dilution 10<sup>-2</sup> to 10<sup>-4</sup> (Table 2). The results revealed that the sediment is much more dynamic source of microorganisms and contains higher quantity of bacteria than pond water. The present observation is in complete conformity with the findings of Jun *et al.* and Burh [21, 22] who concluded that the soil is a much more productive source of microorganisms than is water.

### 3.2 Macrobenthic invertebrate Analysis

Macrobenthic invertebrates analysis showed the presence of three species of phylum Annelida belonging to the class Oligochaeta (*Tubifex tubifex*, *Branchiura* sp, *Dero digitata*), six species of phylum Arthropoda belonging to two orders, Diptera (*Chironomus chironomus*, Chironomous pupae,

*Pentaneura* sp, *Culicodes* sp, *Tabanus* sp.) and Coleoptera (*Berosus* sp, *Paracymus* sp, *Hydroglyphus* sp. and *Canthydrus* sp.) and three species of phylum Mollusca belonging to three families Thiaridae (*Melanoides tuberculata*), Physidae (*Physa* sp.) and Planorbidae (*Gyrulus* sp.) (Table 3).

In the present study presence of some macrobenthic invertebrates such as *Chironomus chironomus*, *Tubifex tubifex* etc. infers that the water is getting polluted and this pond is in the initial stages of eutrophication. Similar work on macrobenthic invertebrate fauna was done by various workers in different water bodies [23, 24, 25, 2, 26, 27, 28].

### 3.3 Physico-chemical Analysis of Sediments

Bottom sediments were analysed for different physico-chemical parameters viz. pH, organic carbon, organic matter, total nitrogen, bicarbonates, chlorides, calcium and magnesium. Values of different parameters were recorded as pH, 8.1 ( Slightly alkaline); organic carbon, 1.62%; organic matter, 2.79%; total nitrogen, 0.140%; bicarbonates, 85.4 mg/l; calcium, 28.87 mg/l; magnesium, 25.27 mg/l and chlorides, 7.98 mg/l. Sediment of the pond was recorded muddy with high organic matter (2.79%). (Table 4)

### 4. Discussion

Bottom sediments are dynamic part of any aquatic ecosystem and contains large number of heterotrophic bacteria because process of mineralization of organic matter takes place most intensively. The upper layer of sediments, a few centimeters thick is particularly abundant in bacterial cells [29].

#### Colour and Texture

Dark brownish colour of bottom sediments reveals high organic matter. Texture of the sediment was observed to be muddy. Muddy sediments, being richer in organic matter usually contain considerably more bacteria as compared to sandy sediments [30]. This type of sediments also helpful in the flourishing of rich macrobenthic biodiversity.

#### pH

pH of the soil reflects the conditions associated with the availability of nutrients, physical conditions of soil having a pronounced affect on the sediment biota including macrobenthos and microbial fauna [31,32]. Most of the bacterial growth occurs at pH 4 to 9. The results revealed that the sediment of pond showed pH value of 8.1, which was slightly alkaline and good for bacterial and macrobenthic growth.

#### Organic Carbon

Decomposition of plants and animal matter by the action of microorganisms acts as a primary source of organic carbon which on mineralization results in the evolution of water and carbon dioxide [33]. Organic carbon present in the pond sediment was 1.62% which is indicative of its mineral nature with low to moderate organic matter content [34].

#### Organic Matter

Organic matter act a reservoir of nutrients and it is indicated by plants, microbes and other organisms inhabiting water body. Organic matter is also known to affect other properties of soil [35]. The organic matter content of sediment sample was 2.79% which is enough for the growth of pollution indicator bacteria.

#### Total Nitrogen

Total nitrogen content represents an important source of ammonium and a sink for nitrate [36].

In the present study of pond, total nitrogen was 0.140%. In aerobic conditions, ammonium (produced as a by product of microbial degradation of organic matter and metabolism of benthic organisms) can undergo nitrification and form nitrate. Nitrates thus released are assimilated by bacteria and macrobenthic organisms and later on released into bottom sediments by decay [37].

#### Bicarbonates

Bacterial degradation of organic matter results into release of bicarbonates thereby increasing the alkalinity and stimulate the decay rate by enhancing buffer capacity and pH of the sediments [38]. In the present study, value of bicarbonates was recorded to be 85.4 mg/l.

#### Calcium and Magnesium

Calcium and magnesium is important exchangeable base in terms of physical, chemical and biological action in soil. Calcium amendment stimulates microbial life too [39]. Values of calcium and magnesium were 28.87 mg/l and 25.27 mg/l. Low values of calcium and magnesium are due to lack of sources.

#### Chloride

Chloride is an important indicator of pollution. Its higher concentration in aquatic ecosystem is very harmful for aquatic biota [40]. During the study of chloride level in sediment sample, was having low value of 7.98 mg/l revealing low rate of mineralization of accumulated organic matter at the bottom and thus releasing less of chloride ions. Sediment characteristics play a significant role in determining microbial features of water body. In the present investigative study of subtropical pond all physico-chemical parameters of sediments showed significant relationship with bacterial count. Dark brown colour of sediments, organic carbon (1.62%) and high organic matter (2.79%) showed high content of organic waste in the bottom sediments. Such characteristics of bottom sediments reveals organic pollution and help in the growth of pollution indicator, bacteria such as *Escherichia coli*, *Pseudomonas*, *Enterobacter* etc. and macrobenthic invertebrates such as *Chironomus chironomus*, *Chironomus pupae*, *Pentaneura* sp, *Culicodes* sp, *Tabanus* sp, *Berosus* sp, *Paracymus* sp, *Hydroglyphus* sp. and *Canthydrus* sp. etc. pH of the sediment sample showed alkaline nature (8.1) and most of these isolated bacteria grow at slightly alkaline pH with high organic content.

## 5. Tables

**Table 1:** Inventory of bacteria isolated during the sampling period.

				Hydrolysis Reaction											
1	S1	- ve	Cocci	-	+	-	-	+or -	+	-	-	No fermentation	-	-	<i>Shigella sp.</i>
2	S2	+ ve	Rod	-	-	+	+	-	+	-	-	Lactose and/or Sucrose	-	-	Unidentified <i>Bacillus sp.</i>
3	S3	- ve	Rod	+	-	-	-	-	+	-	+	Lactose and/or Sucrose	-	-	<i>Enterobacter sp.</i>
4	S4	- ve	Cocci	-	+	+	-	-	+	-	+	Glucose	+	+	Unidentified <i>Coccus sp.</i>
5	S5	- ve	Rod	+	-	-	-	-	+	-	+	Glucose	+	+	<i>Salmonella sp.</i>
6	S6	- ve	Cocci	+	-	+	+	-	+	-	+	Lactose and/or Sucrose	-	-	Unidentified <i>Coccus sp.</i>
7	S7	+ ve	Cocci	+	-	+	+	-	+	-	-	Glucose	-	-	<i>Staphylococcus sp.</i>
8	S8	+ ve	Rod	+	-	+	+	-	+	-	-	Glucose	-	-	<i>Bacillus sp.</i>
9	S9	+ ve	Cocci	-	-	-	-	-	-	-	-	-	-	-	<i>Streptococcus sp.</i>
10	S12	- ve	Rod	+	+	-	+	-	+	-	+	No fermentation	-	-	<i>Pseudomonas sp.</i>

**Table 2:** Bacterial count for water and sediment sample

S. No.	Dilutions	Dilution factor	Number of colonies		Inoculum size (ml)	CFU/ml	
			Water	Sediments		Water	Sediments
1	10 <sup>-1</sup>	10	135	TNTC	0.1	1.35 x 10 <sup>4</sup>	-
2	10 <sup>-2</sup>	100 (10 <sup>2</sup> )	79	200	0.1	7.9 x 10 <sup>4</sup>	2.0 x 10 <sup>5</sup>
3	10 <sup>-3</sup>	1000 (10 <sup>3</sup> )	64	150	0.1	6.4 x 10 <sup>5</sup>	1.5 x 10 <sup>6</sup>
4	10 <sup>-4</sup>	10000 (10 <sup>4</sup> )	60	76	0.1	6.0 x 10 <sup>6</sup>	7.6 x 10 <sup>6</sup>

**Table 3:** Inventory of macrobenthic invertebrates reported during the sampling period.

Phylum	Class	Order	Family	Organisms
Annelida	Oligochaeta		Tubificidae	<i>Tubifex tubifex</i>
				<i>Branchiura sp.</i>
			Naididae	<i>Dero digitata</i>
Arthropoda	Insecta	Diptera	Chironomidae	<i>Chironomus chironomus</i>
				Chironomus pupae
				<i>Pentaneura sp.</i>
			Ceratopogonidae	<i>Culicodes sp.</i>
			Tabanidae	<i>Tabanus sp.</i>
		Coleoptera	Hydrophilidae	<i>Berosus sp.</i>
				<i>Paracymus sp.</i>
			Dytiscidae	<i>Hydroglyphus sp.</i>
Noteridae	<i>Canthydrus sp.</i>			
Mollusca	Gastropoda	Mesogastropoda	Thiaridae	<i>Melanoides tuberculata</i>
			Physidae	<i>Physa sp.</i>
		Bsommatoophora	Planorbidae	<i>Gyalus sp.</i>
<b>Total</b>				14

**Table 4:** Physico-chemical parameters of water sediments of the pond.

S. No.	Physico-chemical parameters of sediment	Results
1	Colour of the sediment	Dark brown
2	Hydrogen ion concentration (pH)	8.1 (Slightly alkaline)
3	Percentage of organic carbon	1.62
4	Percentage of organic matter	2.79
5	Percentage of total nitrogen	0.140
6	Bicarbonates (mg/l)	85.4
7	Chlorides (mg/l)	7.98
8	Calcium (mg/l)	28.87
9	Magnesium (mg/l)	25.27

## 6. Conclusion

This study indicates the rich diversity of macrobenthos and bacteria in this water body and present species were favoured due to the conducive physico-chemical status recorded presently. An attempt was done to record the pollution status of the pond and to find the diversity of bacteria and macrobenthic invertebrates. Most of the identified bacteria (*Shigella* sp, *Escherichia* sp, *Enterobacter* sp, *Salmonella* sp, *Pseudomonas* sp. etc) and macrobenthic invertebrates (*Chironomus chironomous*, *Tubifex tubifex*) are pollution indicators and cause various health hazards.

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