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USE OF CILIATES IN POLLUTION MONITORING AND BIOREMEDIATION IN VEMBANAD LAKE, INDIA

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ABSTRACT

Vembanad Lake, an important Ramsar site has been subjected to a number of serious stresses from anthropogenic influences. Pollution stress in an aquatic environment is often observed by abrupt changes associated with discharges from industries and sewage. This lake thus requires an active bioremediation programme using a fast, efficient and cost effective biotic system. Ciliates are excellent water qualifiers as they call attention to a system, overload, indicate cause and gravity of the situation in their own way. With this in view a study was conducted in Karaparambil site located in the southern part of Vembanad Lake. The diversity of the free-living ciliates in the site was observed for a period of three months. The frequently noticed ciliate species in the study area belonged to 3 genera namely Euplotes, Tachysoma and Coleps. Interestingly they were pollution indicators possessing the property of heavy metal uptake (Rehman et al. 2006, 2008). These ciliates survive in the polluted waters and accumulate the heavy metals in their body. The water quality analysis and heavy metal analysis also proved the waters of the lake is polluted with heavy metal concentrations. Thus the present study points out the possibility of using these dominant ciliate species for bioremediation in this lake. The above ciliate species can be cultured in the laboratory and released into the lake for controlling heavy metal pollution in the lake.

KEYWORDS: Bioindicators, Biomonitoring, Free Living Protozoans, Heavy Metal Uptake

INTRODUCTION

Vembanad Lake has attracted most of the naturalists, limnologists and scientists due to its unique ecological significance. Consequently the lake has been subjected to intense biological research in the last few decades. This wetland system has been facing many threats among which pollution is the most serious one arising out of agricultural run-off, domestic sewage and discharge of untreated and partly treated effluents from the industries. Sixteen major industries around Cochin discharge nearly 0.104 M m^3/d of waste containing organic load into the lake (Balachandran *et al*., 2002). The effluents contain a large dose of heavy metals (Ouseph, 1987). Geochemical fractions such as Fe, Co, Cr, Cu, Mn, Ni, Pd and Zn were detected in the coastal sediments of central south-west coast of India, in and around Cochin (Balachandran *et al.,* 2003). More over 40% of its area has also been lost due to human invasion. As the economic consequence of this area is great with returns to the tune of Rs.300 crores yr-1, there is an immediate need to protect the system in view of its importance. While bioindicators can be used for monitoring the pollution, suitable organisms have to be found out for carrying out bioremediation.

Importance of protozoa as bioindicators for pollution and environmental bio-monitoring has been recognised since long particularly in water purification plants and in activated sludge processes (Kelkwitz and Marsson, 1908). The spectacular protozoan abundance that develops wherever organic matter accumulates and decomposes can be effectively used for bioremediation. Protozoa also plays an additional role by grazing bacteria. Several field and experimental studies carried out showed that protozoa may be conveniently used for environmental bio-monitoring of water quality (Liebmann, 1962; Bick ,1972; Curds,1973; Madoni and Ghetti 1981; Salanki,1986; Ricci,1995). Their role in water purification systems is very significant. Several major taxonomic groups of protozoa, *viz.*, flagellates, naked and testate amoebae, actinopods and ciliates occur in biological sewage treatment plants. Amongst these ciliated protozoa is the most significant component. The free swimming forms, crawling/creeping forms, sessile attached forms which stick to the substrate by a contractile stalk are 3 kinds of ciliate forms that are used for the regulation of the entire complex of purification systems. In this backdrop, information on the diversity of protozoans and their bioremediating capacity is becoming highly important. As no information is available on the above aspects, the present study has been undertaken to find out the diversity of protozoans in the Vembanad Lake.

METHODS

Study Area

Vembanad Lake, an important Ramsar site, lies 0.6-2.2m below mean sea level (MSL) along the south-west coast of India ($9^0 35' N$ $76^0 25' E$ of the Arabian Sea) and has a permanent connection with the Arabian Sea at bar mouth. The lake has a freshwater dominant southern zone and a salt-water dominant northern zone, both separated by a bund at Thanneermukkom where the lake has its minimum width. Since the entire Cochin estuary is a part of the Vembanad-Kol wetland system, the pollutants from the industrial area of Cochin are transferring towards the fresh water region of the Vembanad Lake (Harikumar *et al.*, 2007). The collection of the free living protozoan's was from Karaparambil site (figure 1) located in the southern region of Vembanad Lake. The collection was done periodically during June, July and August 2012.

Collection of the Free Living Protozoans

The brackish water samples were collected during the early morning hours (between 6.00am to 8.00am) using 63µm mesh sized plankton net. Water samples were brought to the laboratory in wide mouthed plastic bottles, their lids were removed and they were kept open in a place where adequate light is available which promotes the increase in the number of protozoans occurring in those samples. Rice bran was given as feed for these protozoans. Then the samples were thoroughly examined under the microscope from time to time. The free living ciliates were observed in both 10x and 40x magnification. Then different species were observed and photographs were taken and identified.

Water Quality Assessment

The major portion of the lake being highly eutrophicated along the southern sector reducing it to a virtually biological desert with poor water quality, its water quality was monitored to make an assessment of the status of pollution. Physical, chemical and biological parameters were analysed. Nutrients like nitrate, nitrite, sulphate, phosphate were analyzed in the water samples. These parameters were analysed by adopting relevant methods from APHA (1998). The air and water temperature readings were taken using an accurate centigrade thermometer. Dissolved oxygen content was analyzed according to classic Winkler's method. One more water sample taken in 300ml BOD bottle at the same time as the sample was kept in the incubator for five days and the similar procedure was followed to obtain the value of BOD. Total hardness was measured adopting APHA (1998). The calcium and magnesium hardness were also found out. Total alkalinity of water sample was also determined by titrating hydrochloric acid and with phenolphthalein and methyl orange indicators. Then the nutrients like nitrate, phosphate, nitrite, sulphate and chloride were analysed and their concentrations in the sample were determined using the method of APHA (1998).

Determination of the Ciliate Communities

Identification of the ciliates was done by observing their body shape, other morphological features, movements and behaviour (Bick 1972, Curds, 1982; Curds *et al*., 1983).

Heavy Metal Analysis

The digestion of water samples were done according to the EPA vigorous digestion method described by Gregg (1989) and after digestion using conc. HNO_3, HCl and NaOH, the water samples were subjected to heavy metal analysis. A total of six metals were determined in the pre-treated water samples using Atomic absorption spectrophotometry as described by Gregg (1989). These include Cadmium, Chromium, Zinc, Lead, Nickel and Manganese.

RESULTS

Observed Water Quality Parameters

The temperature fluctuation (table 1) in different locations of the site during the period of study was recorded and the air temperature ranged from 29^{o}C to 32^{o}C. The minimum water temperature recorded was 27^{o}C during the last week of July. The mean temperature of the site during the study period of June- August was 31^{o}C. Dissolved oxygen concentration during the period of study ranged from the 3.2mg/L to 5mg/L (table 2). The Mean value from the study was found to be 4.06mg/L. The total alkalinity ranged from 105mg/L to 150mg/L in the study. The mean alkalinity of the water samples from the study area was found to be 125mg/L. The acidity ranged from 20mg/L to 50mg/L and the mean acidity of water samples from the study site was found to be 25 mg/L. The total hardness ranged from 40mg/L to 100 mg/L during the study period. The mean of the total hardness of various locations of the study area was found to be 44mg/L. The mean calcium hardness was found to be 8.016 mg/L and that of the mean Mg hardness was found to be 5.847m/L.

Nutrient Concentrations

The nutrients that include phosphate, Sulphate, Nitrate, Nitrite and Chloride were analysed (table 2). The Phosphate concentration ranged from 0.1mg/L to 0.3mg/L. The average phosphate level found during the study was 0.271. The sulphate content ranged from 5mg/ L to 7mg/L. The mean sulphate content was found to be 5.22mg/L. The Nitrite concentration ranged from 0.1mg/L to 0.5mg/L during the study period and the mean nitrite conc. was found to be 0.160mg/L. The nitrate concentration ranged from 0.01 mg/L to 0.04 mg/L. The mean Nitrate concentration was 0.0315mg/L. The chloride conc. in the study area ranged from 120mg/L to 132 mg/L to 132mg/L. The average chloride in the study site was found to be 127.8mg/L. The salinity was also determined, it was found to be 0.260 gm/L.

Ciliate Diversity

Ciliate diversity was tremendous in the study area. Ciliates which were found included *Tachysoma* sp., *Euplotes* sp., *Coleps* sp., *Paramaecium amoebae*, *Tetrahymena* sp. *and Vorticella* sp. But the most important ciliate communities that dominated the study area were that of *Tachysoma*, *Euplotes* and *Coleps* communities. *Tachysoma* (figure 2) and *Euplotes* spp. (figure 3) were frequently observed throughout the study period.

Heavy Metal Concentration

The heavy metals (table 3) analysed showed concentration above the maximum admissible concentrations suggested by the World Health Organization, this indicates that the water in the study area is polluted with the heavy metals.

DISCUSSIONS

From the present study 3 genera of ciliates were frequently observed, they include *Euplotes, Tachysoma and Coleps*. Ciliates are usually found in the polluted waters containing less than 10 µgm/mL concentrations of toxic metal ions (Shakoori *et al.* 2004). Ciliates have a most significant role in water purification system. They regulate the bacterial population and control the BOD levels (Curds, 1973), control pathogenic and faecal bacteria, release mucous substances to facilitate flake formation and successive sedimentation.

From the analysis of water quality parameters the BOD and DO levels significantly indicated the water from the study area to be polluted, the phosphate level was found to be 0.271 mg/L which were above the permissible limit of 0.1mg/L, similarly the Nitrite level were above 0.02 mg/L (ISI standard), the Nitrite was 0.160 suggesting the pollution in waters. But the sulphate, nitrate and chloride were within the permissible limits. Water pollution from agro-chemical residues, municipal sewage, effluents from the motor boats, and coir retting has deteriorated the quality of the water and most striking indicator of eutrophication is the spread of invasive plant, water hyacinth (*Eichornia crassipes*).

The total hardness of sample analysed was 44 mg/L which indicates that the water is soft water based on the classification (Sawyer *et al*., 1994) in waters with low hardness, fish and other aquatic organisms become more sensitive to heavy metals uptake because the metals are more soluble. This is how heavy metals become incorporated into the food chain. Turbidity is a measure of the ability of light to pass through water, that is, a measure of the Water's murkiness. Measuring murkiness gives an estimate of suspended solids in the water. It is measured in Nephelometric Turbidity Units (NTU's). High turbidity also has the capacity to significantly increase water temperature. Water temperature needs to remain fairly constant so aquatic fauna can survive. Water sample analysed had turbidity above 30 NTU's. Alkalinity is not a specific pollution substance as it represents the buffering capacity of the water. Natural alkalinity of the surface water is frequently increased by the addition of municipal and industrial wastewater effluent. Decreased alkalinity levels on aquatic organisms are increase of toxicity of heavy metals.

Effect of Alkalinity on aquatic organisms depends on pH of water. The ciliates identified from the study site in Vembanad Lake strongly suggest the present polluted environmental condition. Also it has been confirmed after analyzing the results of the heavy metals in sample. Based on the earlier works it is quite evident that *Euplotes* and *Tachysoma* spp. reported are possessing heavy metal uptake properties and can be used in bioremediation of industrial waste water. *Euplotes mutabilis*, a ciliated protozoan isolated from heavy metals laden industrial waste water has been shown to tolerate multiple heavy metals, thus suggesting its significance in bioremediation of industrial effluents. The metal removal efficiency of *Euplotes mutabilis* is greater than 80% in metal contaminated wastewaters (Rehman *et al*. 2006., 2008) and these ciliates are excellent and convenient bioindicators for evaluating the toxicity of waste waters polluted by heavy metals (Madoni and Romeo, 2006).

Shakoori *et al*. (2004) have reported 99% and 48% reduction of Zn^{2+} and Cr^{6+} by *Vorticella microstoma*, these microorganisms actively contribute to the amelioration of the effluent quality, since the majority of them feed upon dispersed bacteria (Madoni, 2000).

In a previous work by Rehman *et al*. (2006), *Tachysoma pellionella* species has been highlighted in the heavy metal absorption from industrial effluents and details on its role in bioremediation has been given. *Tachysoma* was found to be resistant to Cr^{6+} at a concentration of 40µg/mL. The ciliate was also found to tolerate Cu^{2+}, Pb^{2+} and Cd^{2+} at concentrations of 20µg/mL, 40µg/mL and 23µg/mL respectively. There was apparently no reduction in size of *T. pellionella* cells. In the present study the heavy metals concentration show a trend of Pb> Cr>Cd>Mn>Ni>Zn, this strongly suggests that lead concentrations are high and the high level of Pb disposal in the effluents in the study areas is indicated, this could exert toxic effects on human beings if consumed from the water or irrigated agricultural products from the sites. This is where the role of ciliates as effective agents in bioremediation is to be highlighted. These organisms can take these metals in their body.

Heavy metal uptake processes by biological cells are known under the general term of bio absorption. The phenomena include both passive adsorption of heavy metals to the cell walls and metabolically mediated uptake by the cells (Gadd, 1990). Excessive industrialization and unplanned disposal of industrial effluents have led to increase the emission of pollutants into ecosystems (Diagomanolin *et al*., 2004).

Accumulation of toxic metals like Hg, Cu, Cd, Cr and Zn in humans have several consequences such as growth and developmental abnormalities, neuromuscular control defects , mental retardation , renal malfunction and wide range of other illness(Thiele ,1995). Cr is embryotoxic, carcinogenic and teratogenic, this metal was present above the maximum admissible concentration suggested by WHO in the present study. Initially from the work of Rehman and Shakoori (2006) it is evident that ciliate like *Tachysoma* sp. is able to accumulate this metal.

So microbial metal removal has received much attention in the past years due to potential use of micro organisms for cleaning metal polluted water (Ledin, 2000). The long-term survival of protozoa in media containing relatively high concentration of heavy metal ions show that these organisms have evolved strategies to tolerate, resist or detoxicate organic substances and heavy metals (Haq *et al*., 2000).

FIGURES AND TABLES

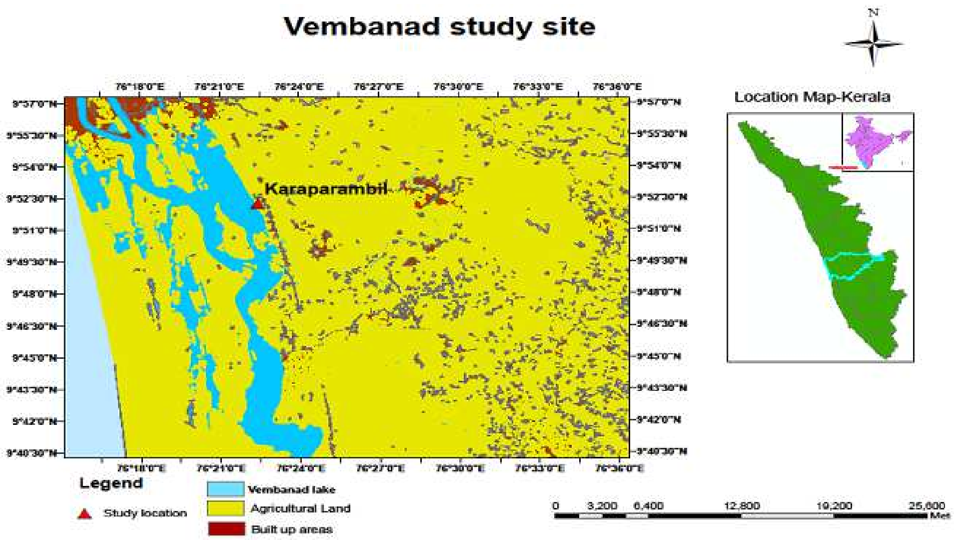
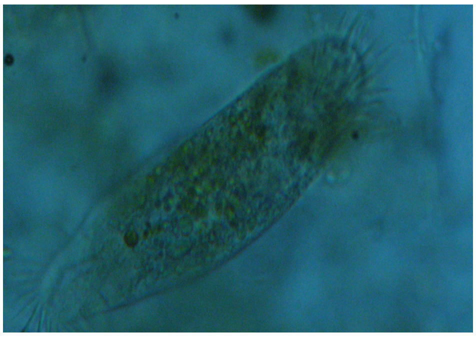


Figure 1: Map Showing the Study Area Karaparambil Site in Vembanad Lake



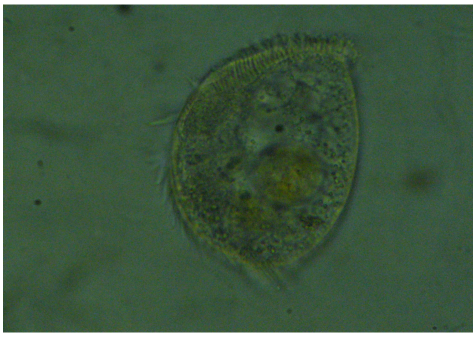


Figure 2: *Tachysoma* spp **Figure 3: *Euplotes* spp**

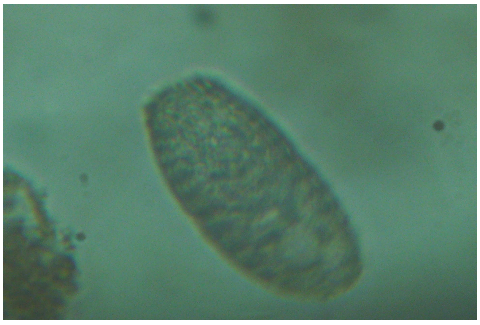


Figure 4: ***Coleps*** **spp**

Table 1: On Site Parameters

Sl.No	Parameters	Mean Value
1.	TEMPERATURE(^{o}C)	
	AIR	32
	WATER	29
2.	COLOUR	COLOURLESS
3.	ODOUR	UNOBJECTIONABLE
4.	TASTE	TASTELESS
5.	FOAM/FROATH	NIL

Table 2: Mean Value of Water Quality Parameters

Sl. No.	Parameters	Mean Value(Mg/L)	Standard Deviation
1	DO	4.06	±1.427
2	BOD	1.5	±0.022
3	ALKALINITY	125	±1.63
4	ACIDITY	25	±1.63
5	TOTAL HARDNESS	44	±1.87
6	Ca^{2+} HARDNESS	8.016	±0.2
7	Mg^{2+}HARDNESS	5.847	±0.083
8	CHLORIDE	127.8	±0.081
	NUTRIENTS		
9	NITRATE	0.0315	±0.1
10	SULPHATE	5.22	±0.026
11	NITRITE	0.160	±0.01
12	PHOSPHATE	0.271	±0.01

Table 3: Heavy Metal Concentrations

Heavy Metals	Concentration
Cadmium	1.012
Chromium	1.032
Lead	1.54
Nickel	0.42
Zinc	0.260
Manganese	0.739

CONCLUSIONS

The presence of *Euplotes* and *Tachysoma* species in the study area strongly indicates that the heavy metal contamination could be tackled and the possible bioremediation of the water of Vembanad Lake by applying these indicator species in pollution control is possible in the near future.

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