USE OF PROTOZOA AS BIOLOGICAL INDICATORS OF WATER QUALITY AND POLLUTION

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ABSTRACT

Protozoa have many advantages for assessing water quality due to their short life generations and more rapid response to environmental stress. This approach has been used for the investigations of the aquatic biota which take into account the general ecology of an area and the classification of water quality with respect to organic pollution ([1]Antipa, 1977 and [2]Wilber, 1969). The Jammu and Kashmir which was once bestowed with a large number of fresh water lakes is losing sheen due to over exploitation and negligence in conservation strategies. Thus, attention has been focused on bio-assessment using protozoa as bio-indicators for evaluating water quality and pollution. The protozoa as biological indicators have been used extensively in assessment of water quality throughout the world. However, very little investigations of this nature have been carried out in the lake under study. The water quality status of Anchar Lake has been seriously influenced by human activities. It is in this backdrop that the study on "Use of Protozoa as Biological Indicators of Water Quality and Pollution" was carried out in the Anchar Lake of Kashmir under different environmental conditions for a period of two years in which 30 protozoan taxa were recorded. The present investigation also aims to assess the impact of agriculture and sewage disposal on its water quality and protozoa by using different statistical tools.

Key words: Anchar Lake, environment stress, fresh water, protozoa, urban wetland, water quality

I INTRODUCTION

The lakes of Kashmir are socio-economically important aquatic ecosystems harboring a rich and diverse gene pool. Increasing population has been responsible for introducing many undesirable changes to aquatic environment. In recent years some of these lakes have shown increased nutrient concentration due to human activities ([3]Zutshi *et al.,* 1980). Open water areas of the lakes have been converted into floating gardens to enhance agricultural production while lake peripheries have been encroached upon to construct residential houses etc. Enhanced population explosion and rapid rate of encroachments and increased utilisation of lake waters for disposal and dilution of sewage have not only deteriorated the water quality of the lakes but have also affected the biotic flora. Most of the liminoloigical investigations in this region have centered on phytoplankton

([4]Khan and Bhat, 2000 and [5]Jeelani *et al.*, 2005), zooplankton ([6]Yousuf, 1988 and [5]Jeelani *et al.*, 2005). The lake environment has greatly eroded during the last three decades. A large number of illegal houses have encroached within the lake areas. House hold wastes and sewage from these human habitats are drained into the lakes indiscriminately at several sites. Agricultural run-off from the surrounding fields, especially the fertilizer and pesticide wastes also find their way into the lake waters. The Anchar Lake under study, too, is contaminated by ample amount of sewage coming from the bordering human settlements and agricultural run-off added from the surrounding fields.

With this background, it becomes imperative to study the impact of various anthropogenic activities on the Anchar Lake by "Use of Protozoa as Biological Indicators of Water Quality and Pollution". However, the studies on protozoa have not received much attention from limnologists. In view of this, detailed investigations of the Anchar Lake with respect to protozoa were undertaken for two years to provide base line data on the water quality and protozoan fauna and to understand the magnitude of threat imposed by discharges from urban human settlements to the ecology of the lake so that possible conservative measures could be undertaken to restore the natural lake ecology. The present study would prove useful in understanding the conservative planning and management of polluting factors.

II MATERIAL & METHODS

(2.1) Selection of sites**:** A two year study with three sampling sites were selected in the lake that represents different environmental features. The Site-I is located at the point where a channel from the Sindh river enters the Anchar Lake. It is characterized by flowing water. Site-II is located in the centre of the lake where water is standing while Site-III is located close to the point at the back of the hospital. Several sewerage outlets from the catchment area also discharge their contents into water at this site.

(2.2) Collection of samples: The sampling was carried out for a period of two years once in a month in one litre polyethylene bottles. The pH and conductivity values were determined electrometrically. The detailed chemical analysis of water samples was carried out according to the standard methods of [7]Golterman and Clymo (1969) and [8]APHA (1989). Collection of samples for biological study was done using plankton net. Five litres of water was sieved through from each sampling site on each occasion. The samples were preserved in 5% formalin and concentrated through centrifugation. Samples were studied under inverted microscope (Nikon) in a Sedgwick-Rafter cell. Identification was done by using standard monographs of [9]Ward & Whipple (1959), [10]Mellanby (1963), [11]Pennak (1978) and [12]Tonapi (1980). The statistical analysis was done using Shannon-Wiener (Species diversity, H'), Pielou evenness (J') and Margalef richness(D) indices were computed: where P_i=proportion of the total count arising from the i^{th} species; S=total number of species; and N=total number of individuals.

III RESULTS AND DISCUSSION

The protozoa found to be distributed in the Anchar lake are given

(Table 2). In all, 30 protozoan taxa have been recorded, which include *Arcella mitrata, A. discoides, A. vulgaris, A. megastoma, Bullinaria indica, Centropyxis stellata, C. ecornis, C. constricta, C. aerophila, Diffulgia lebes, D. oblonga, D. rubescens, D. acuminatA, D. lobostoma, D. aurela, D. corona, D. tuberculata, Euglypha ciliate,* *E. tuberculata, E. laevis, Lesquereusia modesta, L. spiralis, Nebula dentistoma, Paraeuglypha reticulate, Paraquadrula irregularis, Paraeuglypha sp., Pyxidicula scutella, Paramecium sp., Trinema sp. and Wailesella sp.* Out of these, 20 taxa were recorded at Site-I; 23 each at Site-II and 23 Site-III.

At Site-I, the maximum density of protozoan was observed during summer followed by spring. The lowest density was recorded during winter. [13]Yousuf and Qadri (1985) recorded lowest population density during winter and a rise during summer. [14]Baruah and Das (2001) recorded protozoan density to increase in post monsoon period and decrease in winter. At Site -II, a maximum density of protozoan was observed during summer and the lowest during autumn. At Site-III, a similar trend with respect to density of protozoa was recorded. In overall terms, a single peak depicting the maximum density of protozoan was recorded during summer at all the sites (fig.1). [15]Ramakrishnaiah and Sarkar (1982) and [16]Kaur *et al.* (1997) also observed population density of protozoans to be highest during summer. They opined that during summer, organic matter accumulates which triggers the growth of zooplankton including protozoans.

The taxa which were recorded at Site-I are *Arcella mitrata, A. discoides, A. vulgaris, A. megastoma, Bullinaria indica, Centropyxis stellata, C. ecornis, C.constricta, C. aerophila, Difflugia lebes, D. corona, D. aerula, D. accuminata, D. lobostoma, D. rubescens, Euglypha ciliate, Lesqueresia modesta, Nebula dentistoma and Paraeuglypha reticulate and Paraquadrula irregularis.* This site lie where the river Sind feeds the Anchar lake and water here is flowing. The macrophytic vegetation is scarce. However, the littorals at this site are with willow plantation. This site is almost free from organic pollution source. Species diversity here was observed to be lowest of all the sites. The population density was also observed to be lowest at this site. [17]Sajeev (1999) in Ropar Headworks waters observed protozoan species diversity and population density very low at the site which is full of aquatic plants and where water flow is swift. Similar suggestions were made by [15]Ramakrishnaih and Sarkar (1982) on Konar Reservoir of Bihar and by [18]Bath (1996) in Harike Reservoir who opined that protozoa thrive well in relatively calm and undisturbed water, harboured with lot of aquatic plants.

The protozoan taxa which were recorded at Site-II are *Arcella disciodes, A. vulgaris, A.megastoma, Bullinaria indica, Centropyxis stellata, C. aerophila, C. ecornis, Difflugia lebes, D. lobostoma, D. rubescens, D. acuminate,*

D. corona, Euglypha laevis, E.tuberculata, E. ciliate, Lesquereusia modesta, Paraeuglypha reticulata, P. araeuglypha sp., Paramecium sp., Paraquadrula irregularis, Pyxidicula scutella and *Wailesella sp.,* respectively. This site is located in the centre of the lake and is the deepest of all the sites. The site harbours rich growth of macrophytes. The site itself does not receive any sewage or agricultural run-off, however, the water coming from Site-III mixes at this site. The population density is highest of all the sites. [17]Sajeev (1999) in Ropar headworks waters observed species diversity and population density very high at the site which is characterized by relatively less disturbed and calm waters and harbouring a rich growth of plants. Similar reports have also been given by [19]Munawar (1970b), [15]Ramakrishnaiah and Sarkar (1982) and [20]Kaur *et al.* (2003). Our findings are in agreement with all these authors.

The species which were recorded at Site-III are *Arcella discoides, A. vulgaris, A. megastoma, Centrophyxis constricta, C. stellata, Difflugia lebes, D. aurela, D. tuberculata, D. acuminata, D. oblonga, Euglypha ciliata, E. tuberculata, Lesquereusia modesta, L. spiralis, Nebula dentistoma, Paraeuglypha sp., Pyxidicula scutella,* *Paraquadrula irregularis*, *Paramecium sp.*, *Trinema sp.* and *Wailesella sp.*. This site is located at the littorals of the lake. Here water is shallow and highly turbid. This site recieves effluents from the adjoining agricultural fields, sewage from local catchment area. The site is highly enriched with nutrients and harbours a rich macrophytic vegetation. The present study reveals high species diversity and population density of protozoans here. [21]Hellawell (1986) opined that organic matter accelerates the growth of aquatic plants which in turn support zooplanktons. [26]Kaur *et al.* (2003) observed high population density of protozoan at site characterised by rich growth of aquatic vegetation and nutrient rich waters. Similar observations were made by [19]Munawar (1970b).

The taxa which were encountered at all the study sites of the lake included *Arcella discoides, A. vulgaris, A. megastoma, Centropyxis stellata, Diffulgia lebes, D. acuminata, Euglypha ciliata, Lesquereusia modesta* and *Paraquadrula irregularis*. [22]Syal (1996) found *Arcella sp*. in both clean and polluted waters while [23]Sukumaran *et al.* (1984) found it only in clean waters. [24]Algarsamy *et al.* (1967) and [25]Verma *et al.* (1978) discovered it in sewage contaminated waters. *Centropyxis* sp. was found in clean waters by [26]Bilgrami and Datta Munshi (1979) while [22]Syal (1996) found it in waters contaminated with sewage. [27]David and Ray (1966) found it in both clean as well as polluted waters. *Difflugia sp*. was found to be present both in polluted and clean waters by [27]David and Ray (1966), [28]Verma and Dalela (1975) and [29]Sexena and Mishra (1990). [24]Algarsamy *et al.* (1967), [26]Bilgrami and Datta Munshi (1979) and [16]Kaur *et al.* (1997) found it in waters contaminated with sewage. [22]Syal (1996) found it in waters contaminated by industrial wastes. However, [23]Sukumaran *et al.* (1984) found it only in clean waters. *Euglypha ciliata* was recorded at the sites which receive sewage and fertilizer factory waste by [22]Syal (1996) in river Satluj. [27]David and Ray (1966), however, reported its occurence in both clean as well as polluted waters.

Out of total of 20 protozoan taxa, 2 taxa were found exclusively at Site-I. These are *Arcella mitrata* and *Centropyxis ecornis*. [23]Sukumaran *et al.* (1984) also found *Arcella sp.* in clean waters only. [27]David and Ray (1966) and [18]Bath (1996) found *Arcella sp*. in clean waters as well as in waters receiving effluence. [24]Algarsamy *et al.* (1967) and [25]Verma *et al.* (1978) discovered it in sewage waters. *Centropyxis sp.* was discovered both in clean waters and sewage receiving waters by [27]David and Ray (1966), [18]Bath (1996), and [22]Syal (1996). [24]Algarsamy *et al.* (1967) and [26]Bilgrami and Datta Munshi (1979) on the other hand found it in waters contaminated with sewage.None of the protozoan taxon was found to be exclusively at Site-II of the lake.

The taxa which were exclusively found at Site-III during the study period are *Difflugia tuberculata*, *Difflugia oblonga*, *Lesquereusia spiralis* and *Trinema sp*. The presence of these protozoan taxa at this site suggests that these are tolerant towards pollution by sewage, agricultural run-off and other wastes. Among the above mentioned protozoan taxa, *Difflugia* sp. was found to be ubiquitous in nature by [27]David and Ray (1966) and [22]Syal (1996), who found it in both clean and polluted waters. [24]Algarsamy *et al.* (1967) and [26]Bilgrami and Dutta Munshi (1979) found it in waters contaminated by sewage. [28]Verma and Dalela (1975) and [29]Sexena and Mishra (1990) found it in waters contaminated by industrial wastes. On the other hand, [23]Sukumaran *et al.* (1984) found it in clean waters. Presencce of *Trinema sp.* and *Lesquereusia spiralis*, indicates that these protozoans can thrive well in polluted waters. [18]Bath (1996) also found *Trinema sp.* in the Satluj waters at the site which is polluted by industrial and domestic effluents.

The relationships between the protozoan communities and environmental conditions at the sampling sites were shown in (Table-3) (p<0.05). The analysis demonstrated a significant correlation between protozoan communities and changes in environmental variables. The analysis showed that the changes in water temperature, DO, NH_4-N, NO_3-N, TP brings changes in protozoan communities best (ρ=0.701, p<0.05). The values of species number, richness, evenness and diversity represented a significant or a strong significant correlation with nutrients (NO_2-N, TP or TN or NH_4-N). In this study, the statistical analysis showed a significant correlation between the protozoan taxa and the variations in environmental variables. The protozoan taxa (Table-4) are significantly correlated with nutrients. The findings imply that protozoa may be used as a feasible tool for assessing the water quality. The species number, individual abundance, species diversity, evenness and richness indices have widely been utilized as a useful indicator to evaluate water quality in many aquatic environments. [30]Connell (1978) suggested that with small environmental stress raise, the diversity will be increased because of the decreasing competition, while if the environmental stress increases up to a higher point, the diversity starts to decrease then. Thus, increased eutrophication could lead to either an increase or a decrease in diversity for a certain community. In our study, the three diversity indices of protozoan communities were lower at Site-I (located at the point where a channel from the Sindh river enters the Anchar Lake) and at Site-II (located in the centre of the lake where water is standing) as compared to Site-III (close to the point where water receives multiple types of organic wastes.

IV CONCLUSION

All the water quality parameters of lake fall in the category of eutrophication where the lake receives organic wastes except at Site-I. Eutrophication has promoted excessive plant growth and decay, favouring certain weed species over others, and is likely to cause severe reductions in water quality. DO levels declined to hypoxic levels (Table-1). Finally this ecosystem experienced an increase in nutrients. Hence lake water cannot be much fit for drinking, irrigation and domestic use. The alkalinity values of the lake water have exceeded. It is recommended that lakes water analysis should be carried out from time to time to monitor the rate and kind of contamination. A regular environmental monitoring programme must be conducted in the Anchar Lake for pollution abatement needs to be initiated. The unsafe water in the lake has been implicated as one of the major causes of the diseases for the populations living around. As one of the urban lakes, as well as a potential lake for the future generations to come, Anchar Lake water should be preserved for the protection of natural environment. A massive cleanup of lake is expected so that the lake will be restored to a condition comparable to what existed at Site-I, thus biotic pressure on the lake's water quality can be eliminated or reduced. Proper regulation regarding the dumping of wastes should be taken. As far as the problem of sewage introduction is concerned, it should be properly treated and strict enforcement of the laws would be more adequate. However, little information on community-based bio-assessment, using protozoa documented in the present study, should be further investigated to understand the lake system further.

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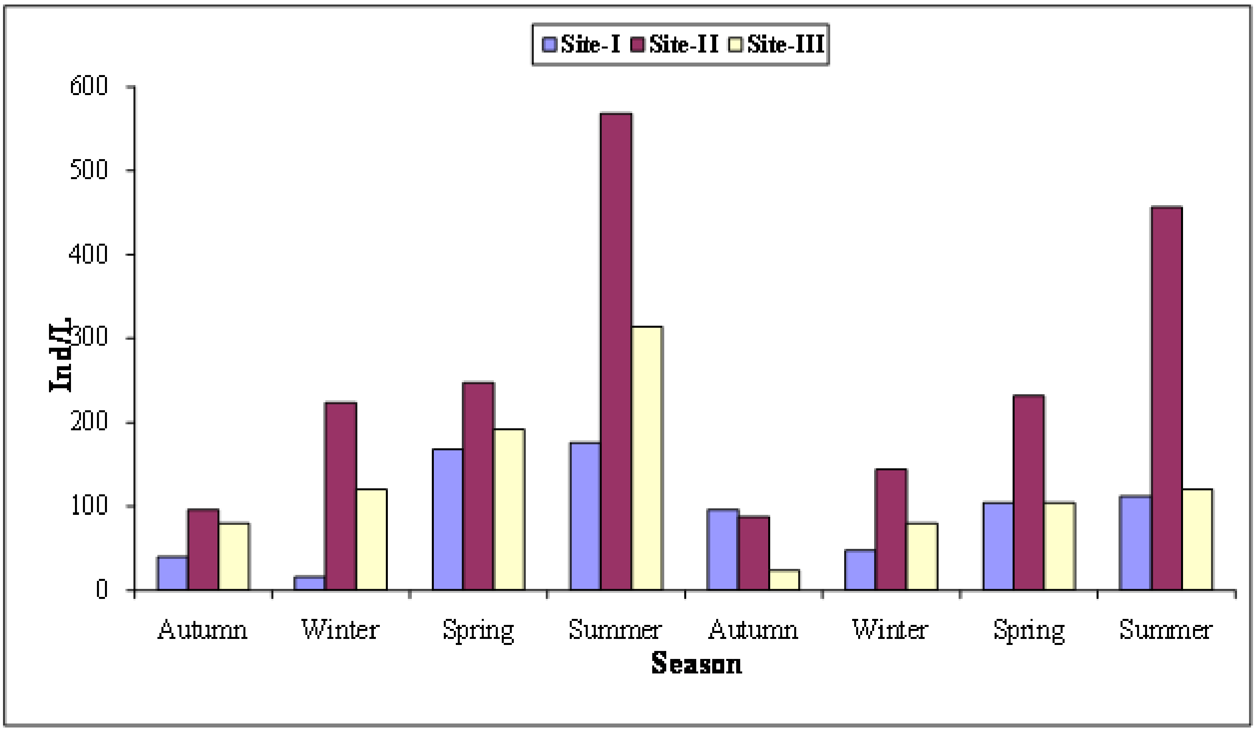

Figure 1: Seasonal variations in the population density (Ind/L) of the protozoa at the investigated sites of the Anchar Lake

Table 1. Range in physico-chemical characteristics in Anchar lake.

Parameter	**Unit**	**Range**	**Desirability**
Temperature	°C	3.0-28	---
pH	-----	6.9-8.6	Alkaline
Conductivity Dissolved oxygen Calcium	µS/cm mg/L mg/L	205 - 580 0.4 - 9.5 9.0 - 43.0	Not desirable
Magnesium Tota1 Alkalinity	mg/L mg/L	4.3 - 28.5 59 - 315	Not desirable
Chloride Nitrate nitrogen Total phosphorus	mg/L µg/L µg/L	9.0 - 95.0 245-611 200-505	Not desirable

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Table 2: List of protozoan taxa recorded at the investigated sites of the Anchar Lake

S. No.	Protozoa Taxa	Site-I	Site-II	Site-III
1	*Arcella discoides* Ehrenberg	+	+	+
2	*A. megastoma* Penard	+	+	+
3	*A. mitrata* Leidy	+	-	-
4	*A. vulgaris* Ehrenberg	+	+	+
5	*Bullinaria indica* Penard	+	+	-
6	*Centropyxis ecornis* Ehrenberg	+	+	-
7	*Centropyxis stellata* Wailes	+	+	+
8	*C. aerophila* Penard	+	+	-
9	*C. constricta* Ehrenberg	+	-	+
10	*Difflugia acuminata* Penard	+	+	+
11	*D. oblonga* Ehrenberg	-	-	+
12	*D. tuberculata* Wallich	-	-	+
13	*D.aurela* Ehrenberg	+	-	+
14	*D. corona* Ehrenberg	+	+	-
15	*D. lebes* Penard	+	+	+
16	*D. lobostoma* Leidy	+	+	-
17	*D. rubescens* Penard	+	+	-
18	*Euglypha laevis* Ehrenberg	-	+	-
19	*E. ciliata* Ehrenberg	+	+	+
20	*E. tuberculata* Dujardin	-	+	+
21	*Lesquereusia spiralis* Ehrenberg	-	-	+
22	*L. modesta* Ehrenberg	+	+	+
23	*Nebula dentistoma* Penard	+	-	+
24	*Paraeuglypha reticulata* Penard	+	+	-
25	*Paraeuglypha sp.*	-	+	+
26	*Paramecium sp.*	-	+	+
27	*Paraquadrula irregularis* Archer	+	+	+
28	*Pyxidicula scutella* Playfair	-	+	+
29	*Trinema sp.*	-	-	+
30	*Wailesella sp*	-	+	+

Table 3: Correlations between average values of the species number (*S*), abundance (*N*), richness(*D*), diversity (*H'*) and Pielou's evenness (*J'*) and environmental variables.

Species	Water temperature	Dissolved Oxygen ($D.O_2$)	pH	NH_4-N	NO_3-N	Total (P-PO_4)
Arcella discoides Ehrenberg	0.690	0.736	0.597	-0.513	-0.951	-0.499
Centropyxis stellata Wailes	0.322	0.747	0.711	-0.083	-0.865	-0.301
Difflugia acuminata Penard	-0.799	-0.321	-0.301	0.851	0.531	0.705
D. lebes Penard	-0.802	0.001	0.156	0.899	0.645	0.928
Euglypha ciliata Ehrenberg	0.801	0.649	0.579	-0.649	-0.697	-0.561
E. tuberculata Dujardin	0.347	-0.498	-0.599	-0.443	-0.152	-0.678
Paraeuglypha sp.	0.059	-0.669	-0.669	-0.271	0.301	-0.189
Paramecium sp.	0.279	0.079	-0.219	-0.443	-0.510	-0.601
Paraquadrula irregularis Archer	-0.111	0.111	-0.151	0.051	-0.220	-0.102

Table 4 : **Environmental variables with protozoan abundances at three sampling sites of Anchar Lake**

Environmental variables	ρ value
Temperature (T), DO, NH_4-N, NO_3-N, TP	0.701
DO, NH_4-N, NO_3-N, TP	0.598
DO, pH, NH_4-N, NO_3-N, TP	0.598
DO, NH_4-N, NO_3-N, TP	0.598
DO, NH_4-N, NO_3-N	0.682
T, DO, TP	0.682
T, DO, pH, NH_4-N, NO_3-N	0.682
T, DO, pH, TP	0.682
T, DO, NH_4-N, NO_3-N,	0.682
DO, pH, NH_4-N, NO_3-N	0.679