ECOLOGY AND SEASONAL VARIATION OF MICROALGAL COMMUNITY IN AN OIL REFINERY EFFLUENT HOLDING POND: MONITORING AND ASSESSMENT

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Abstract. The microalgal community as primary producers has to play a significant role in the biotic and abitoic interactions of any aquatic ecosystem. Whenever a community is exposed to a pollutant, responses can occur because individuals acclimate to pollutant caused changes and selection can occur favouring resistant genotypes within a population and selection among species can result in changes in community structure. The microalgal community of industrial effluent treatment systems are continuously exposed to pollutants and there is little data available on the structure and seasonal variation of microalgal community of industrial effluent holding ponds, especially of a complex effluent like that of refinery. The aim of the present study was to investigate the annual variation in the ecology, biomass, productivity and community structure of the algal community of a refinery effluent holding pond. The results of the study showed the pond to be a eutrophic system with a resistant microalgal community with distinct seasonal variation in species composition.

Keywords: chlorophyll, community respiration, gross primary productivity, microalgal community, refinery effluent holding pond

1. Introduction

The microalgal community as primary producers has to play a significant role in the biotic and abitoic interactions of any aquatic ecosystem. Any change in water quality due to industrial, agricultural or municipal wastewater can alter the normal algal function, thus inducing major changes in community structure. Whenever a community is exposed to a pollutant, responses can occur because individuals acclimate to pollutant caused changes, and selection can occur favouring resistant genotypes within a population, and selection among species can result in changes in community structure (Forbes and Forbes, 1994). Oxidation ponds are usually used in industrial effluent treatment as a major treatment process, or as a polishing process after other treatments. The performance of oxidation ponds is dependent on the biotic community present (Wong *et al.*, 1995), and the biotic community of treatment ponds is continuously exposed to pollutants. The diversity at the level of producers and decomposers, in particular may influence the ecosystem productivity (Morin, 2000). In this context the phytoplankton of wastewater lagoons are quite

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significant (Soler *et al.*, 1991). There is little data available on the structure and seasonal variation of micro-algal community of industrial effluent holding ponds, especially of a complex effluent like that of refinery.

Palmer (1969) negatively correlated the species diversity of the algae in waste stabilisation ponds to the organic loading. The seasonal succession of algal flora in waste stabilisation ponds treating sewage has been studied by Govindan (1990). The biological features of waste stabilisation ponds treating specific industrial effluents have also been investigated (Copeland and Dorris, 1962; Thripathi and Pandey, 1990). Provided sufficient solar radiation is available, non-toxic wastewaters of various origins and nature can support good algal growth. It is reported that oil pollution generally reduces the growth of phytoplankton and the number of algal species (El Sheikh *et al.*, 2000). Low molecular weight hydrocarbons were found to stimulate or inhibit phytoplankton growth, depending upon the species (Dunstan *et al.*, 1975). Recently, biodegradative capacity is reported in many blue green algae (Subramanian and Uma, 1997), and algal species were found to be tolerant to phenol and phenolic effluents (Shashirekha *et al.*, 1997; Joseph and Joseph, 1999, 2001a and b). Therefore, the community composition in an effluent holding pond depends upon the adaptability of the species to organically rich and polluted waters.

The aim of the present study was to investigate the annual variation in the ecology, biomass, gross primary productivity, community respiration and community structure of the algal community of a refinery effluent holding pond.

2. Materials and Methods

2.1. SAMPLING PROCEDURE

The effluent treatment system of the refinery investigated incorporates API oil separators for oil removal, and trickling filters followed by two fire ponds. The fire ponds are natural ponds meant to polish the effluent. The effluent after filtration through the trickling filters flows into the fire pond No. 1 and from there to the fire pond No. 2. The retention time of the effluent in each pond is four days. The site of study was fire pond No. 2. The pond has a mean depth of 2 m and holding capacity of 60,00 m^3. Effluent samples were collected from four locations in the pond at random and combined to get a composite sample. The surface and bottom samples were collected separately using a Van Dorn sampler. Sampling was done in fifteen days interval during February 1994 to February 1995. The transparency of the pond was measured each time using a secchi disc. The water samples for the measurement of primary productivity, chlorophyll and phytoplankton were collected from the euphotic depth. Effluent samples for estimation of dissolved oxygen were fixed immediately after collection using manganous sulphate and alkali-iodide-azide reagents. Temperature was recorded while sampling.

2.2. ANALYTICAL METHODS

The pH was determined using a pH meter. The dissolved oxygen, biochemical oxygen demand, primary productivity, phosphate, nitrate, primary productivity and community respiration were analyzed as per standard methods (Lenor *et al.*, 1992). The chlorophyll was determined by dimethyl sulfoxide method (Burnison, 1980). The identification of algae was done following monographs on algae (Desikachary, 1959; Prescott, 1954; Philipose, 1967).

2.3. DATA ANALYSES

The monthly averages of the data were integrated to obtain the water column mean values and, the mean annual variation in different parameters is represented in figures. The data on the composition of phytoplankton community was analyzed to define the dominant forms. The species, which formed more than 75% of the population, were grouped in the category dominant. The species that formed 40–75% of the population were ranked common and those less than 40% in the category present. The values for similarity index were calculated for different months based on the equation:

$$C = \frac{2C \times 100}{a + b} ,$$

where, C is the number of species common to both months; a, the number of species present during one month and b, the number of species present during the other month (Sorensen, 1948).

3. Results and Discussion

The mean depth of the sampling pond was 2 m and the euphotic zone was quite above the pond bottom in the range 0.82 to 1.59 m. There was significant difference in light penetration into the pond at various periods of the year (Figure 1a). The highest amount of chlorophyll *a* and the lowest depth of penetration of light were recorded in February 1994 (Figure 3a). The monthly values of temperature, pH, dissolved oxygen, biochemical oxygen demand, phosphate, ammonia and nitrate in the surface and bottom of the pond is given in Figures 1a through 2d. There were quite significant variations in the nutrient concentrations. The premonsoon phosphate concentrations were higher than the rest of the year. Nitrogen was present more as ammonia and the highest levels were recorded in the post monsoon months. Nitrate was lower in premonsoon months and high during July to September.

The depth integrated values of algal biomass in terms of chlorophyll *a* and *b*, and that of gross primary productivity varied significantly from month to month (Figures 3a through 3c). The algal productivity was low during the monsoon months

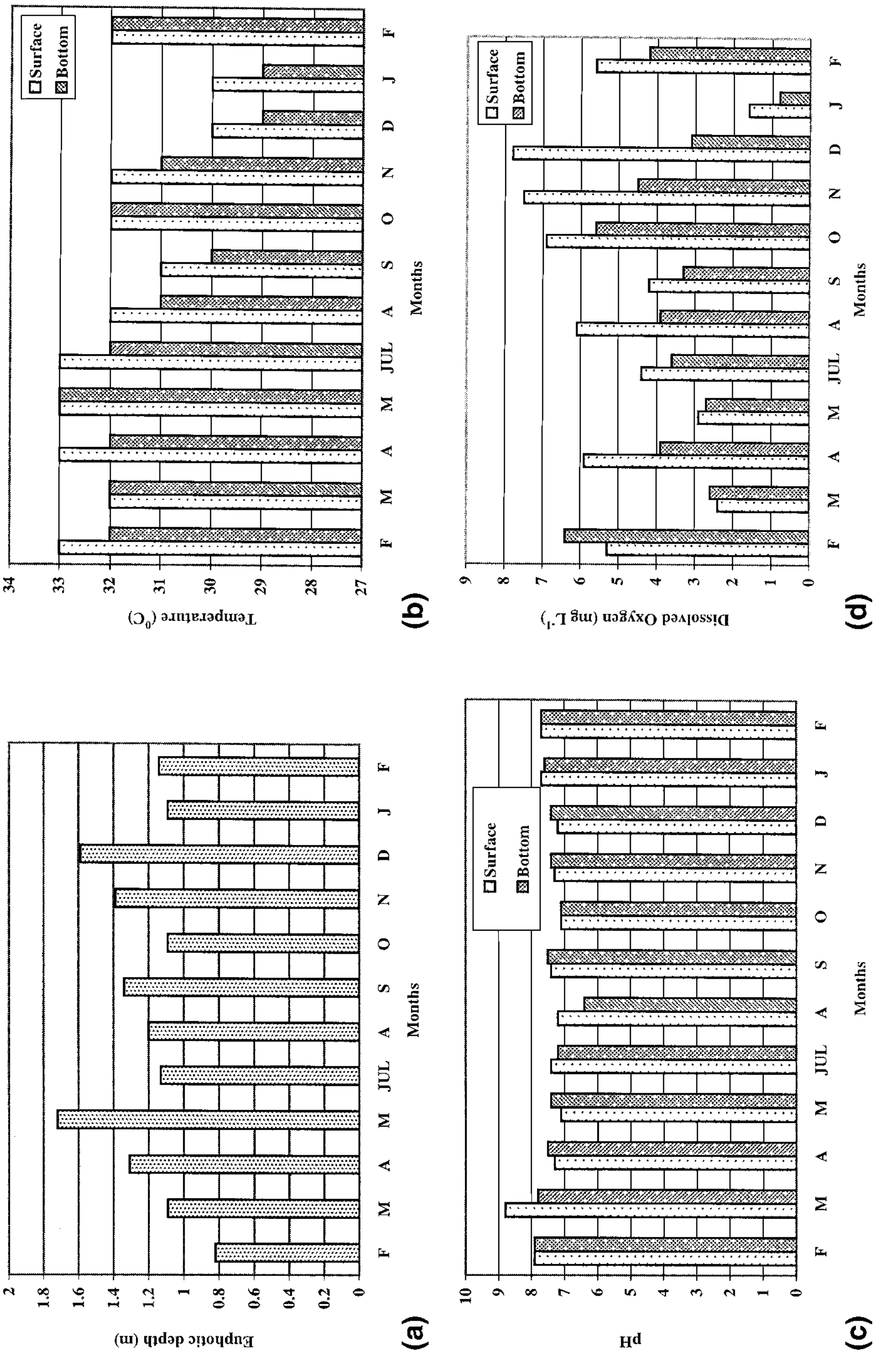


Figure 1. Mean annual variations in euphotic depth, temperature, pH and dissolved oxygen in the refinery pond from February 1994 to February 1995.

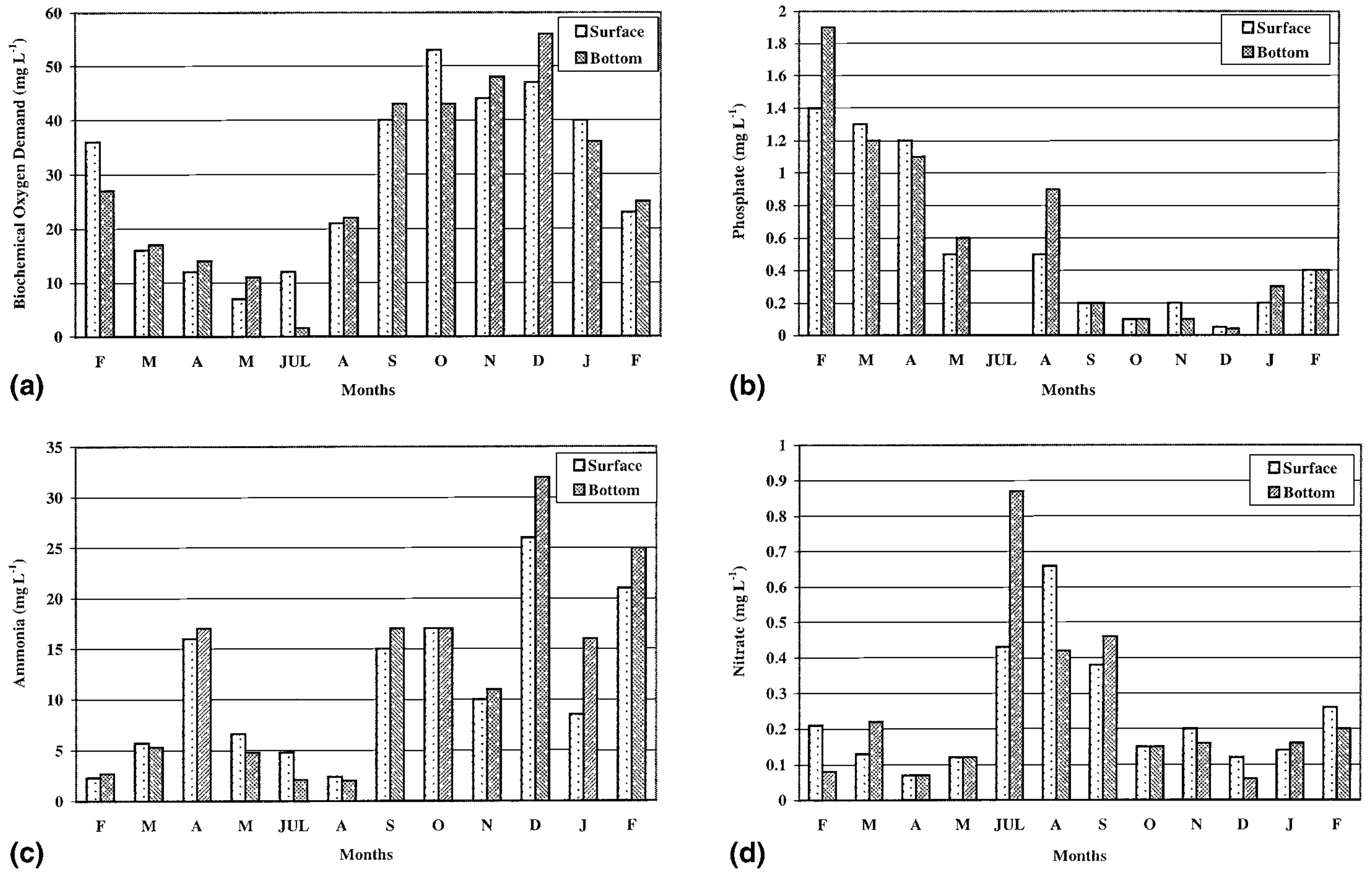


Figure 2. Mean annual variations in biochemical oxygen demand, phosphate, nitrate and ammonia in the refinery pond from February 1994 to February 1995.

compared to the pre and post monsoon. The community respiration was high during the post monsoon period (Figure 3d).

The phytoplankton species composition of the pond during the sampling period is given in Table I. The algal community composed of members of Chlorophyta, Cyanophyta, Euglenophyta and Bacillariphyceae. *Chlorella pyrenoidosa* was present during 11 months out of the 12 months of sampling. *Scenedesmus* had the highest species richness, being represented by six species.

In February 1994, when the sampling was started there was an algal bloom represented by Chlorophycean and Cyanophycean members. *Volvox aureus*, *Pandorina morum* and *Oscillatoria curviceps* were the dominant species. *Oscillatoria quadripunctulata* and *Chlorella pyrenoidosa* were common. Chlorophycean and cyanophycean algae continued to be present till May with *Chlorella pyrenoidosa* dominating in April–May.

The diatoms, *Nitzschia palea* and *Navicula* appeared in July and were present till September. From August to December there was a shift in dominance from *Chlorella-Scenedesmus-Volvox-Pandorina-Eudorina* cluster to that of *Chlamydomonas-Euglena-Phacus*. The waxing and waning of green algal community was reflected in the variation in chlorophyll *b* content as well.

The Chlorophycean members gained dominance from December till February 1995, *Pandorina morum* being the common species present. *Nitzschia palea*, *Oscillatoria quadripunctulata* and *O. subbrevis* were also present.

The similarity index for algal population between different periods of sample collection is represented in a Trellis diagram (Figure 4). The value of similarity index varied between 0 to 87. The maximum similarity was observed between the months of February and March 1994 and April and May 1994 and the minimum similarity between July and October 1994.

Quantitative evaluation of the gross primary productivity revealed significant correlation to organic loading measured as BOD; but no correlation with ammonia, nitrate, and phosphate concentration in the pond water (Joseph and Joseph, 2001b). However, it was observed that Chlorophycean and Cyanophycean algae are present predominantly in premonsoon period, from February to May when phosphate is high and nitrate and ammonia are comparatively low. During monsoon months of July to September Euglenophytes and Diatoms appear and during this period phosphate concentration is low and nitrogen high.

Seasonal variation in the primary productivity of the waters in the nearby estuarine system of the refinery is well documented. Generally, there is a decrease in primary production during monsoon months (Nair *et al.*, 1988; Ramachandran Nair *et al.*, 1975). A similar trend was observed in the refinery pond also. The decrease in the primary productivity during the monsoon season may be due to the dilution of the water and the dispersion of algal cells along the water column.

As in natural freshwater systems, there was a tendency for the seasonal fluctuation in algal flora of the pond. El Sheik *et al.* (2000) also reported changes in community composition of an algal flora with respect to oil pollution in the river

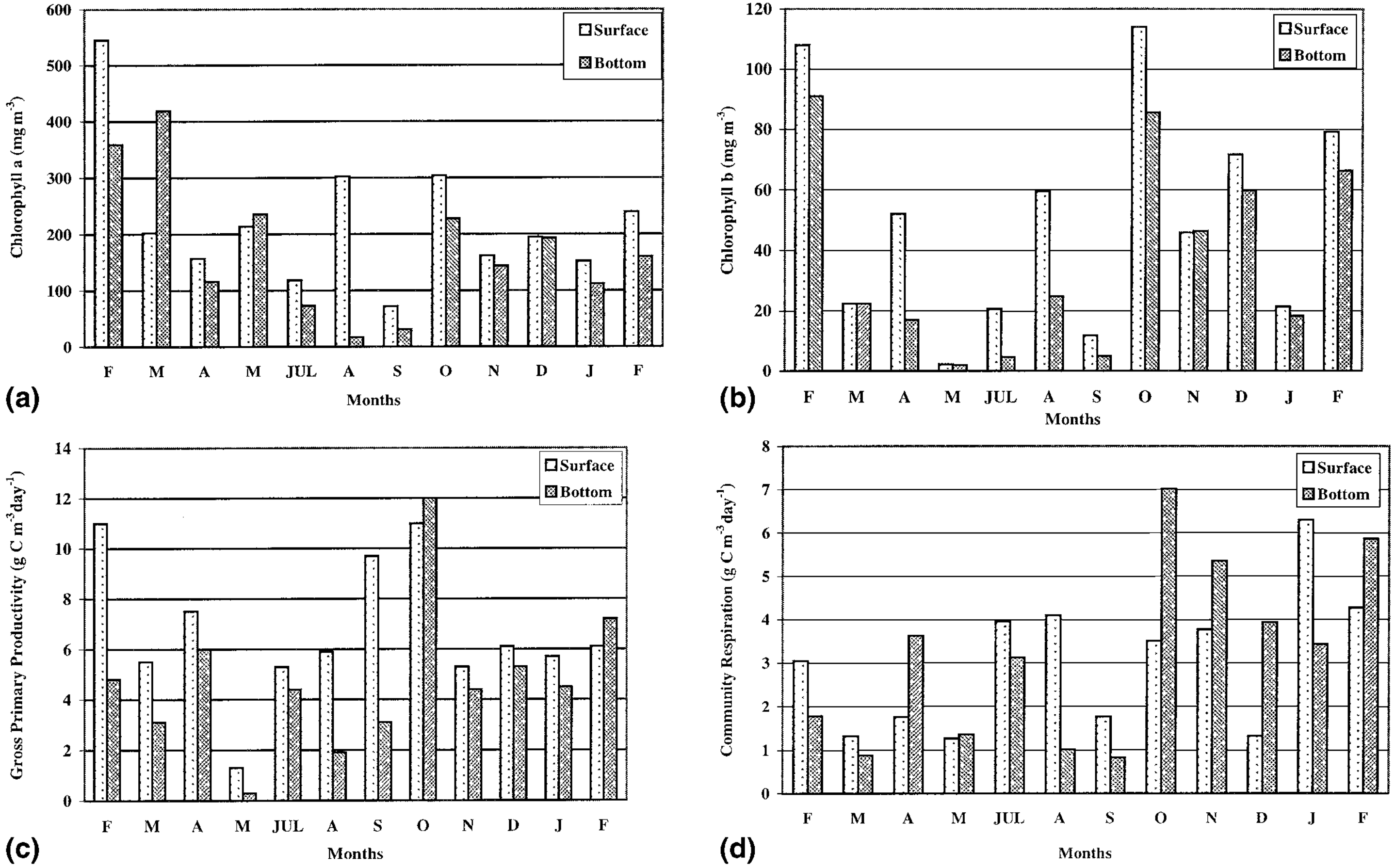


Figure 3. Mean annual variations in chlorophyll *a*, chlorophyll *b*, gross primary productivity and community respiration in the refinery pond from February 1994 to February 1995.

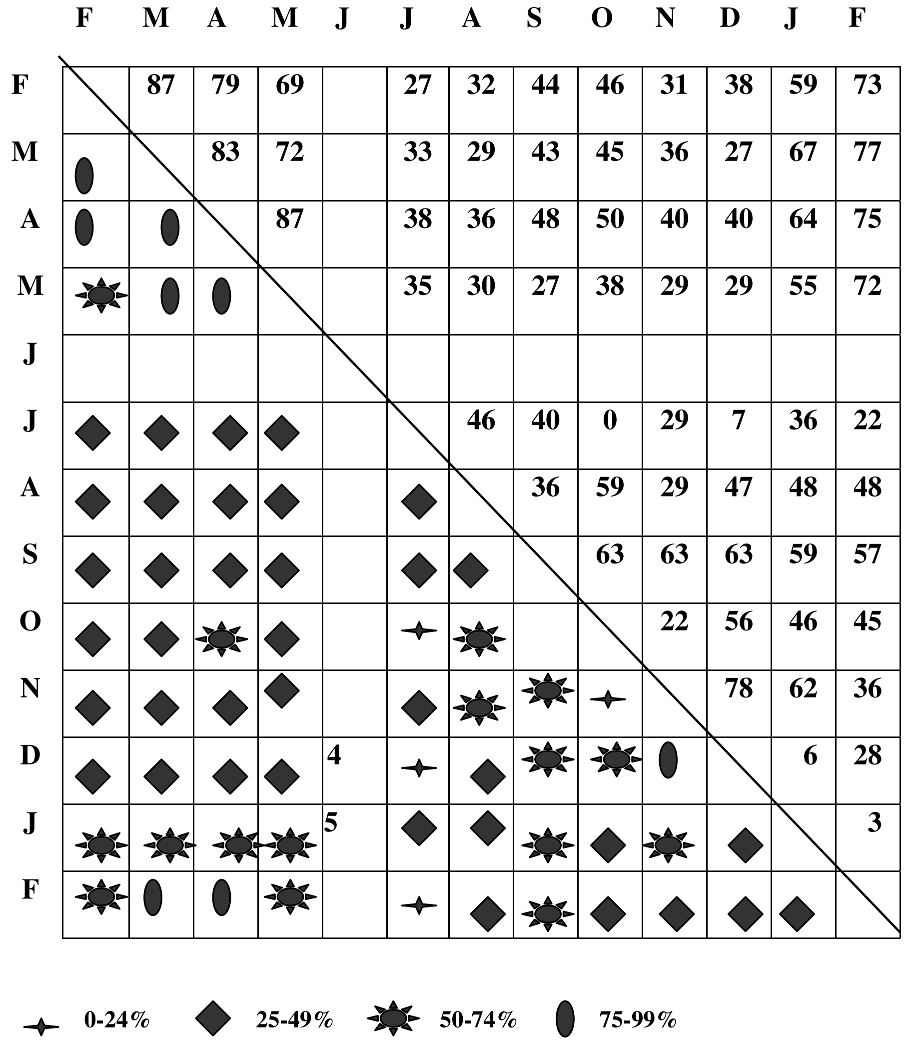
TABLE I

Microalgal species composition and seasonal variation in the refinery effluent holding pond from February 1994 to February 1995

Algal species	Months												
	F	M	A	M	J[a]	J	A	S	O	N	D	J	F
Cyanophyta													
Arthrospira sp.	+	+	–	–		–	–	–	–	–	–	–	–
Nostoc sp.	+	–	–	–		–	–	–	–	–	–	–	–
Oscillatoria curviceps	+	+	–	+		+	–	+	–	–	–	+	+
O. quadripunctulata	++	++	+	+		–	–	–	–	+	+	+	–
Phormidium inundatum	+++	+	++	+		–	–	+	+	–	–	+	+
Synechococcus sp.	+	–	–	–		–	–	–	–	–	–	–	–
Chlorophyta													
Actinastrum hantzschii	+	–	–	–		–	–	++	+	–	+	–	–
Ankistrodesmus falcatus	+	++	–	–		–	–	–	–	–	–	+	+
Chlamydomonas sp.	–	–	–	–		–	+++	+++	+++	+++	++	+	+
Chlorella pyrenoidosa	++	++	+++	++		+++	+	+	–	+	+++	+	+
Chlorococcum infusionum	+	++	++	+		–	+	+	+	–	–	–	+
Closteriopsis longissima	–	–	–	+		–	–	–	–	–	–	–	–
Eudorina sp.	+	+	+	+		–	–	–	–	–	+	+	+
Oocystis pusilla	+	+	+	+		+	–	–	–	–	–	+	+
Pandorina morum	+++	+++	+	+		–	–	–	–	+	–	++	–
Scenedesms bijugatus	+	–	+	–		–	–	–	–	–	+	+	–
S. carinatus	–	–	–	+		–	–	–	–	–	–	–	–
S. dimorphus	+	–	–	–		–	–	–	–	–	–	–	–
S. quadricauda var. longispina	+	+	+	–		–	–	+	+	+	+	+	–
S. q. var. bicaudatus	–	+	–	–		–	–	–	–	–	–	–	+
S. smithii	–	–	–	–		–	–	–	–	+	+	–	–
Volvox aureus	+++	+++	+	++		–	+	–	+	–	–	+	+
Euglenophyta													
Euglena oxyuris	–	–	–	–		–	++	++	+	+	+	+	–
Phacus longicauda	–	–	–	–		–	++	++	+	+	+	+	+
Trachelomonas similis	–	–	–	–		–	–	–	–	–	–	+	–
Bacillariophyceae													
Navicula cuspidata	–	–	–	–		+	+	+	–	–	–	+	–
Nitzschia palea	–	–	–	–		+	+	+	–	–	–	+	–

+++ = Dominant; ++ = common; + = present; – = absent.

[a] In June 1994, sampling could not be done due to heavy rains.



	F	M	A	M	J	J	A	S	O	N	D	J	F
F		87	79	69		27	32	44	46	31	38	59	73
M	⬮		83	72		33	29	43	45	36	27	67	77
A	⬮	⬮		87		38	36	48	50	40	40	64	75
M	✹	⬮	⬮			35	30	27	38	29	29	55	72
J													
J	◆	◆	◆	◆			46	40	0	29	7	36	22
A	◆	◆	◆	◆		◆		36	59	29	47	48	48
S	◆	◆	◆	◆		◆	◆		63	63	63	59	57
O	◆	◆	✹	◆		✦	✹			22	56	46	45
N	◆	◆	◆	◆		◆	✹	✹	✦		78	62	36
D	◆	◆	◆	◆	4	✦	◆	✹	✹	⬮		6	28
J	✹	✹	✹	✹	5	◆	◆	✹	◆	✹	◆		3
F	✹	⬮	⬮	✹		✦	◆	✹	◆	◆	◆	◆	

✦ **0-24%** ◆ **25-49%** ✹ **50-74%** ⬮ **75-99%**

Figure 4. Trellis diagram showing algal similarity index between different sampling periods from February 1994 to February 1995.

Nile. It has been established that toxic chemical stress causes large changes in community structure (Howarth, 1991). In aquatic ecosystems new populations can grow very quickly to replace those, which are damaged by pollution. The relationship between the nutrients and the algal community in these systems is not well understood. Sometimes changes in nutrient levels do not alter algal species composition and sometimes it do (Borchardt, 1996). The response of the same species to seemingly similar nutrient enrichment conditions differed among studies, and it is suggested that parameters other than nutrients are more important in determining species composition. It has been found that high algal density results in efficient nutrient removal from primary settled wastewater. But it would lead to self-shading, an accumulation of auto-inhibitors and reduction in photosynthetic efficiency (Lau *et al.*, 1995). The bioactivity of a complex waste is probably related to interactions among components with no substance having dominant effect (Walsh *et al.*, 1980).

4. Conclusions

The results of the study concluded that the effluent holding pond is of eutrophic nature. In a eutrophic state ecosystems become structurally simpler, more dominated by opportunistic species, and more resistant to toxic chemical stress. The mechanisms determining the phytoplankton abundance viz. bottom-up control and top-down control has not been considered in the present study. Further, it was found that in an aquatic ecosystem producer-decomposer co-dependency influences producer biodiversity effects (Naeem *et al.*, 2000). The factors such as species interaction, competition between species, and interrelationships between other organisms and the production of antibiotics and phenolics by freshwater algae, should be taken into consideration from the ecological point of view (El Sheik *et al.*, 2000). Naeem *et al.* (2000) reported in a mesocosm study that the ability of bacteria to use a variety of carbon sources did not depend solely on the bacterial abundance, but also increased with the increasing algal diversity and the relationship between the two is not straightforward. Integration of all these factors is essential to explain the complexity of community changes in a system exposed to effluent like that of a refinery.

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