Turkish Journal of Biology

Volume 27 | Number 3 Article 7

1-1-2003

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Turk J Biol
27 (2003) 163-170

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Received: 24.12.2002

Abstract: The present study describes a multispecies toxicity test that uses community level responses of plankton samples from a natural lake using polyurythane foam (PF) as artificial substrate. These substrates were suspended and left for a period of 14 days. Colonized substrates were then placed in a series of microcosms dosed with a gradient of tannery effluent from 1 to 15% concentration. The species composition and abundance of the planktonic communities in PF substrate with the exposure of 4 different concentration of tannery effluent (1%, 3%, 5% and 15%) were assessed on days 1, 3, 7, 14, 21 and 28. Species diversity and abundance in colonized PF substrate showed a significant difference in different effluent treatments. Similarly, an inverse relationship was noted between effluent dosage and species surviving in source PF substrate at the end of the experiment period.

Key Words: Microcosm, toxicity, colonization rate, sensitivity, endpoints

Introduction

The direct discharge of effluents from industries and municipal wastewater treatment plants into bodies of water has become a growing environmental problem. Most of these wastewaters are extremely complex mixtures containing inorganic and organic compounds (1). The best way to analyse risk assessment has been to develop biological test systems, which, combined with chemical analysis, can be useful for evaluating aquatic assessments and establishing relevant water quality criteria (2).

In order to assess the toxicity of anthropogenic chemicals, single species toxicity tests often conducted. These tests are much easier to perform and often their results are easier to interpret (3). In contrast, multispecies microcosm toxicity tests have the advantages of providing the effects of toxicants and their interaction between species and their environment (4). In the natural environment it is difficult to handle such a study because of the complexity and dimensions of water bodies. A microcosm study with a laboratory environment is more effective and useful. The information from research with microcosm s relatively rich. The influence of pH variations on the periphyton was investigated by Muller (5) and on cladoceran population dynamics by Havens and De Costa (6). In addition, several microcosm experiments have been carried out on the distribution and fate of trace metals and their effects on phyto- and zooplankton communities (7,8). Pratt et al. (9) studied the prediction of permissible concentrations of copper on naturally derived protozoan communities. The effects of total phosphorus reductions in a lake plankton community structure were investigated in a microcosm study by Holz and Hoagland (10).

This approach may help to identify endpoints that consistently respond to different kinds of chemical stress, making it possible to focus monitoring resources in expensive mesocosm or whole ecosystem tests. The ultimate purpose of this investigation was to examine the effects of tannery effluent (TE) on the colonization rate of plankters in laboratory established systems.

Materials and Methods

Polyurethane foam (PF) units are used as an artificial substrate for the colonization of microorganisms because of their three-dimensional character and their Mowing the ready colonization by a wide variety of microorganisms. Free swimming forms easily invade the interstices of the foam, while sessile forms attach to the solid pillars (11).

PF units measuring 6 x 5 x 3.75 cm were cut and used for the colonization experiment. These units were rinsed with 10% hydrochloric acid, distilled water and

50% ethanol and thoroughly rinsed with distilled water. The units were placed in a net bag and suspended at a 1-m depth in lake Porur, a 3.20 km^2 man-made impoundment in the western suburban area of the city of Chennai. The PF units were colonized for 14 days and then retrieved and transported to the laboratory in a glass jar containing enough water so that it was immersed completely. These substrates act as an epicenter for the laboratory -based microcosm study.

Microcosm Test Design

The test containers were rectangular glass tanks (35.5 x 15 cm) containing 4 l of filtered lake water. TE was used as a toxicant in this experiment. The typical effluent characteristics from tanneries are given in Table 1. The TE was diluted with source water (lake water), which act as treatment/experiment with 1%, 3%, 5% and 15% concentrations and a control was kept without adding the effluent. Duplicate samples were maintained simultaneously. Total volume was kept at 4 l throughout the experimental period.

The PF substrate obtained from the sampling location (acting as an epicenter) after 14 days of colonization served as the source. A single colonized substrate (epicenter) was suspended in the center of each tank and surrounded by six barren PF units (6 x 5 x 7.5 cm). A single unit was randomly chosen from each microcosm tank and harvested after 1, 3, 7, 14, 21 and 28 days of colonization.

Table 1. Typical effluent characteristics from tanneries (mg/l).

Parameter	Quality of Effluent	
	Chrome tanning	Vegetable tanning
Total suspended solids	2004	11,184
Total dissolved solids	15,152	17,254
Total volatile solids	1660	-
Chloride	2470	9335
Sulfate	339	1605
Oil and Grease	11	12
Biochemical oxygen demand	930	1,262
Chemical oxygen demand	8000	3800
Ammonia - Nitrogen	-	70
Sulfide	228	-
% sodium	98	73.65
Chromium	11.2	-

- Not given
Sources: Tamil Nadu Environmental Monitoring and Pollution Control, Final Report, Vol.2, June 1994.

Sample Collection, Counting and Enumeration

Physicochemical parameters like temperature, pH, alkalinity, hardness, chloride, phosphate and nitrite were estimated by following the methods given in APHA (12). Plankton samples were collected by squeezing the foam (PF) until dry in a borosil beaker. The sample volume recorded from these three -dimensional substrates were £ 100 ml. The samples were fixed in 5% formalin for further analysis. The plankton were identified by following Edmondson (13), Sharma (14), Michael and Sharma (15), Battish (16) and Anand (17) and counted under an inverted microscope (NIKON TMS-4) at 100x magnification. A Sedgwick-Rafter cell was used for the counting process (18).

All values of the physicochemical parameters are expressed as means. The significance of the difference between the mean values of the control and experimental microcosms were analysed using one-way ANOVA (19). Statistical significance was inferred at $P \leq 0.05$.

Results

System Parameters

Two replicates of four concentrations and a control were tested. There was significant variation noted in the physicochemical features of both the control (Figures 1-3) and treated microcosms. However, there was no noticeable increase in the surface water temperature during the study period (Figure 1). The maximum pH (7.5) was noted on day 28 in the 5% TE treated microcosm and on days 14, 21, and 28 in the 15% TE treated microcosms (Figure 1). Further, the lowest alkalinity value (74.75 mg/l) was recorded in the control microcosm on day 1 and the maximum value (172.5 mg/l) was recorded in 15% TE on day 14 (Figure 2). The hypothesis that this was due to the alkaline nature of the effluent was supported statistically. The alkalinity values showed significant variations between the control and experiment microcosms on days 1 ($P < 0.005$), 3 ($P < 0.025$), 7 ($P < 0.025$) and 21 ($P < 0.025$).

Hardness showed significant variations between the control and experiment microcosms on day 1 ($P < 0.005$) (Figure 2). Nutrient concentrations (nitrite and

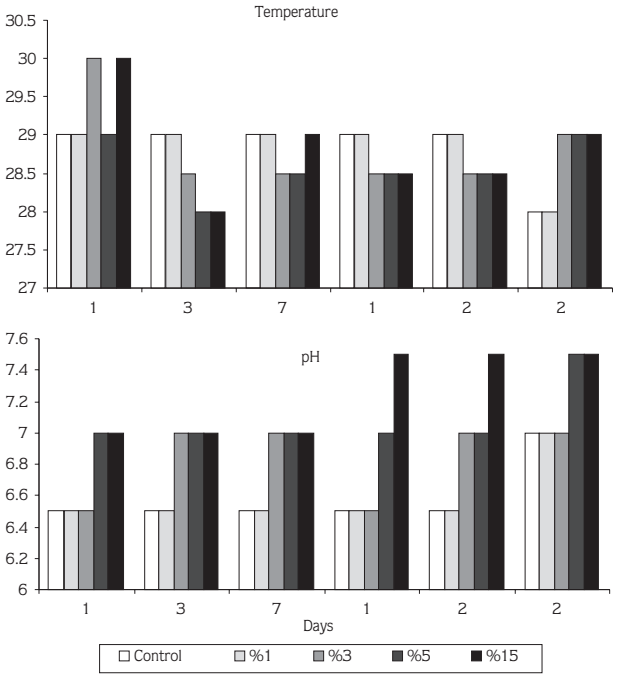

Figure 1. Changes in temperature and pH in the microcosm tanks during the experiment.

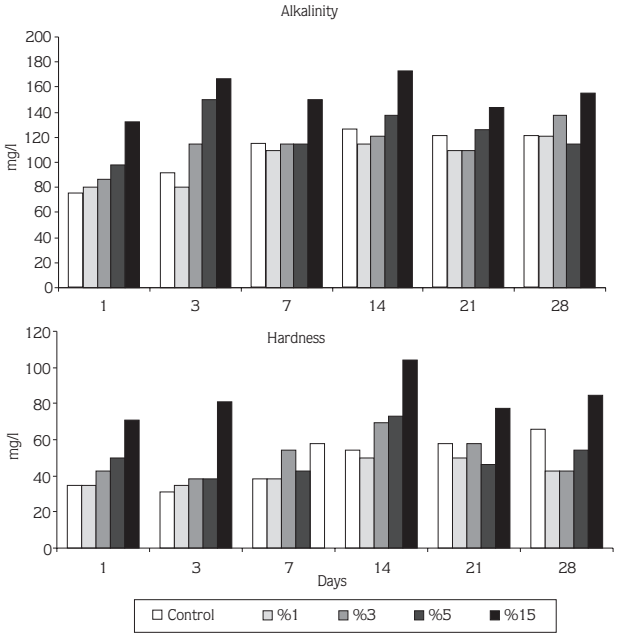

Figure 2. Changes in alkalinity and hardness concentration in the microcosm tanks during the experiment.

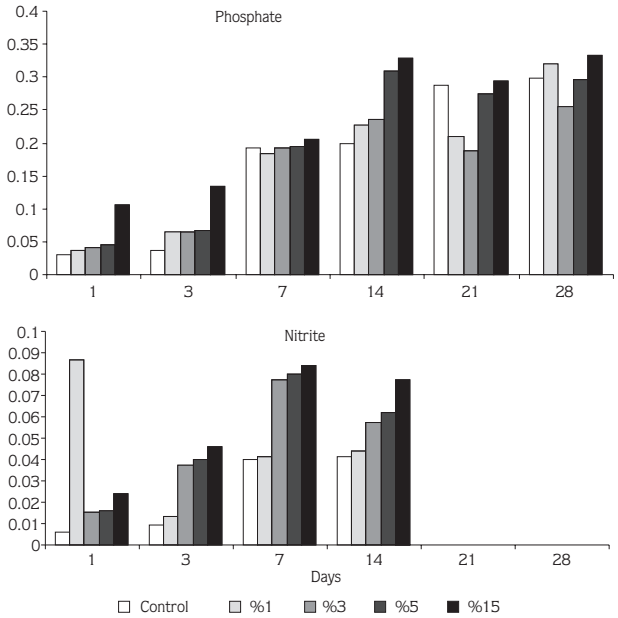

Figure 3. Changes in phosphate and nitrite concentration in the microcosm tanks during the experiment.

phosphate) also deviated between different treatments after the addition of effluent (Figure 3) and the concentration increased with dosage levels. The maximum value of phosphate (0.334 mg/l) was noted on day 28 in the 15% TE treated microcosm and the minimum value (0.03 mg/l) on day 1 in the control microcosm (Figure 3).

Colonization rate

A total of 43 phytoplankton taxa were identified during the experiment: 12 of green algae, 27 of diatoms and 4 of blue green algae. Among the zooplankton, Rotifera were the dominant group, comprising 14 taxa, whereas Copepoda and Cladocera were represented by 4 and 2 taxa respectively (Table 2). The majority of species were found only in a few samples during the experiments.

On day 1 of colonization, phytoplankton were relatively greater in number than zooplankton and they comprised 13 taxa of diatoms, 8 taxa of green algae and 3 taxa of blue green algae. Among the zooplankton, Rotifera were the dominant group, represented by 7 taxa (Table 2). Copepoda were represented by *Halycyclops* sp. and *Microcyclops* sp. only. The total mean abundance of diatoms and green algae were lower in the treatment microcosms than in the control microcosm (Figure 4). There was a significant change in total mean phytoplankton counts on day 1 ($P < 0.005$) and zooplankton did not show any significant variation between the control and treatment microcosms.

Table 2. List of planktons identified during the microcosm study.

S. No.	PHYTOPLANKTONS	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Epicenter
I.	**Chlorophyceae (Green algae)**							
1.	*Chlorella* sp.	+	+	–	+	–	–	+
2.	*Closterium* sp.	–	–	+	–	–	–	–
3.	*C. ocutum*	+	–	+	+	–	–	–
4.	*C. dubium*	+	–	+	–	–	–	–
5.	*Closteriopsis* sp.	+	+	+	+	+	+	+
6.	*C. longissima*	+	+	+	+	+	+	+
7.	*Coelastrum chodati*	–	+	–	–	–	–	–
8.	*Microspora willeana*	+	–	–	–	+	–	+
9.	*Pediastrum duplux*	+	+	+	+	+	+	+
10.	*P. simplex*	+	–	+	+	+	+	+
11.	*Scenedesmus quadricauda*	–	+	+	–	+	+	+
12.	*S. dimorphus*	–	–	–	–	–	–	+
II.	**Bacillariophyceae (Diatoms)**							
1.	*Achnanthes inflata*	–	+	–	–	+	–	+
2.	*Amphora ovalis*	+	+	+	+	+	+	+
3.	*Anomoneneis ineronises*	–	–	–	–	–	–	+
4.	*A. sphaerophora*	–	–	–	–	–	–	+
5.	*Cosinodiscus* sp.	–	–	–	–	+	–	+
6.	*Cymbella cistula*	+	–	+	–	–	–	–
7.	*C. tumida*	+	+	+	+	+	+	+
8.	*C. cymbiformis*	+	+	–	–	+	+	+
9.	*Diatoma* sp.	+	+	+	+	–	+	+
10.	*Diploneis robustus*	–	–	–	–	–	–	+
11.	*Meridion* sp.	+	–	–	–	–	–	–
12.	*Mastogloia* sp.	–	–	+	–	–	–	–
13.	*Navicula* sp.	–	+	+	+	–	–	–
14.	*Navicula radiosa*	+	+	+	–	–	+	+
15.	*N. closterium*	+	–	–	–	–	–	–
16.	*N. cuspidata*	+	+	+	+	+	+	+
17.	*N. productum*	–	+	+	–	–	–	+
18.	*N. cryptotenella*	–	–	+	+	+	+	–
19.	*Neidium affinie*	+	+	–	–	+	–	+
20.	*N. productum*	–	+	–	–	+	+	+
21.	*Nitzschia* sp.	+	–	–	–	–	–	–
22.	*Nitszchia obtusa*	+	+	+	+	–	–	+
23.	*N. ublicata*	+	–	–	–	–	–	+
24.	*N. palea*	–	+	+	+	+	+	+
25.	*N. vitrea*	–	–	–	–	–	–	+
26.	*Pleurosigma* sp.	–	+	–	+	–	–	+
27.	*Tabullaria* sp.	–	–	+	–	–	–	–
III.	**Cyanophyceae (Blue green algae)**							
1.	Anabena variabilis	+	+	+	+	–	+	+
2.	Gloeotheca rupestris	+	+	–	–	–	–	–
3.	Microcystis sp.	–	–	–	–	+	–	–
4.	Spirulina sp.	+	+	+	+	+	+	+

Table 2. (Contunued).

S. No.	ZOOPLANKTON	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Epicenter
I.	**Rotifera**							
1.	*Brachinous sp.*	–	–	–	–	–	–	–
2.	*B. plicatilis*	–	–	–	–	–	–	–
3.	*B. cadatus*	+	–	–	–	–	–	–
4.	*Colurella obtusa*	–	+	+	+	+	–	+
5.	*Eothinia sp.*	–	–	–	–	–	–	–
6.	*Lecane bulla*	+	–	+	–	+	+	–
7.	*L.quadridentata*	–	+	–	–	–	–	–
8.	*Lepadella ovalis*	–	+	+	–	–	–	–
9.	*Monostyla sp.*	+	–	+	+	+	+	+
10.	*Notholca sp.*	+	+	–	–	+	–	+
11.	*Polyathra vulgaris*	+	–	–	–	–	–	–
12.	*Testudonella sp.*	+	–	+	+	+	+	+
13.	*Trichocera longiseta*	+	–	+	+	+	+	+
14.	*T. pussilla*	–	+	–	–	–	–	–
II.	**Copepoda**							
1.	*Cyclops sp.*	–	+	–	–	–	+	+
2.	*Halycyclops sp.*	+	–	–	–	–	–	–
3.	*Microcylops sp.*	+	–	+	–	–	–	–
4.	*M. diversus*	–	+	+	–	–	–	+
III.	**Cladocera**							
1.	*Alona sp.*	–	+	+	+	+	–	+
2.	*Moina sp.*	–	+	–	–	–	–	–

+ → Present

- → Absent

The species composition of phytoplankton showed a slight decrease during day 3 of colonization. Similar to on day 1, diatoms were the dominant group, represented by 14 taxa, and the colonization rate of zooplankton was relatively increased from day 1. Cladocera were a pioneer group that appeared on day 3 (Table 2). Rotifera continued to be the dominant group among the zooplankton. Rotiferan species such as *Coluralla obtusa, Lecane quadridentata, Lepadella ovalis* and *Trichocera pussilla* (Table 2) were found on day 3. The total mean abundance of green algae was at its maximum in the 1% TE treated microcosm (63 ind/l) (Figure 4). No statistically significant changes were observed in the total mean abundance of phytoplankton between the control and treatments, whereas zooplankton showed significant changes ($P < 0.025$).

A total of 23 phytoplankton species were identified on day 7. A single species of green algae (*Closterium* sp.) was found on the day 7 of colonization (Table 2). Out of 23 species, 8 were green algae, 13 were diatoms and 2 were blue green algae. Among the zooplankton, Rotifera were represented by 6 taxa (*Colurella obtusa, Lecane bulla, Lepadella ovalis, Monostyla* sp., *Testudinella* sp., and *Trichocera longiseta*). The abundance of phytoplankton showed a decreasing trend from the control to the 15% effluent treated microcosm, except for in 1% and 3% treatments. Diatoms showed a very marked decrease in abundance from the control to the 15% treatment microcosm. Statistically there was no significant variation in phyto- or zooplankton abundance.

Seventeen phytoplankton taxa and 6 zooplankton taxa were identified on day 14 out of 43 phytoplankton taxa

and 20 zooplankton taxa respectively. Diatoms were the dominant group (7 taxa) among the phytoplankton and Rotifera were the dominant group (4 taxa) among the zooplankton. Green algae showed a maximum abundance in the control microcosm (73 ind/l) (Figure 4). Phytoplankton and zooplankton showed a steady decrease between the control and 15% TE treated microcosms (Figure 5). A total of 18 phytoplankton and 7 zooplankton were identified on day 21. Blue green algae was represented by 2 species (*Microcystis* sp. and *Spirulina* sp.). The abundance of phytoplankton did not show any significant variation, but zooplankton showed a significant variation between the control and effluent treated microcosms ($P < 0.05$).

On day 28, the phytoplankton were represented by 16 taxa and the zooplankton by 5. Among the phytoplankton, diatoms were the dominant group (9 taxa). The zooplankton were represented by Rotifera and

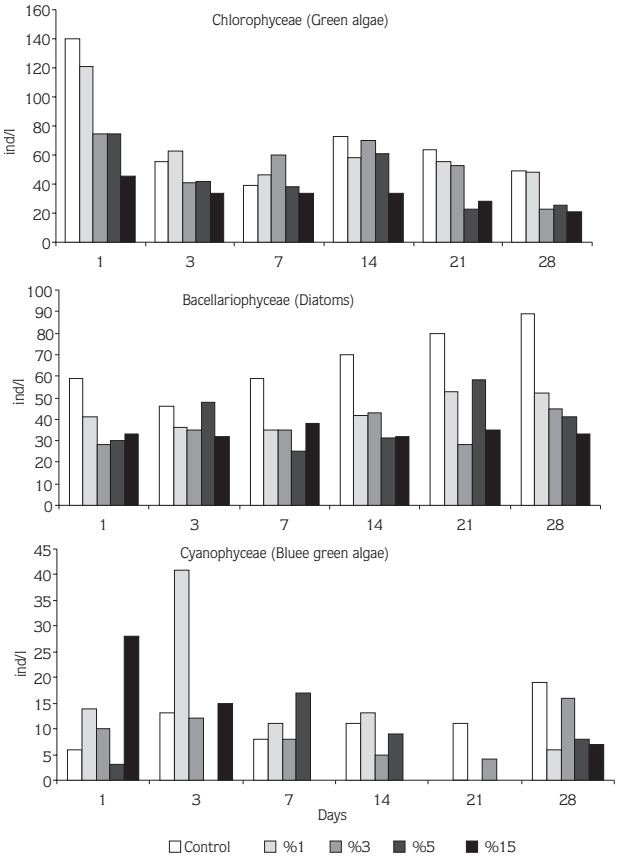

Figure 4. Changes in phytoplankton (total mean abundance) colonization rate in the microcosm tanks during the experiment.

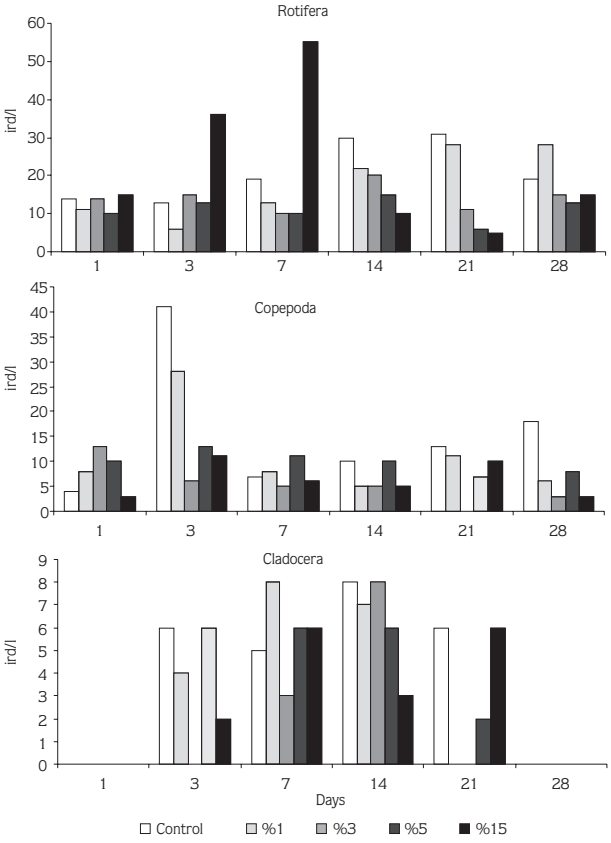

Figure 5. Changes in zooplankton (total mean abundance) colonization rate in the microcosm tanks during the experiment.

Copepoda. Cladocera were completely absent on day 28. The phytoplankton abundance showed a decreasing trend between the control and the 15% TE treated microcosms (Figure 4). A highly significant variation in total mean phytoplankton and zooplankton count was noted both in the control and effluent treated microcosms ($P < 0.001$ and $P < 0.025$ respectively).

The epicenter (source) was analyzed after 28 days of exposure to various concentrations of TE. The phytoplankton were represented by 29 taxa and the zooplankton by 8. Diatoms were the dominant group with 19 taxa; green algae and blue green algae were represented by 8 and 2 taxa respectively. Among the zooplankton, Rotifera were the dominant group with 5 taxa, followed by Copepoda (2 taxa) and Cladocera (1 taxon). The taxonomic richness of the epicenter substrate was not adversely affected at the concentration tested, but a significant variation in the total mean abundance of phytoplankton was observed ($P < 0.025$).

Discussion

The colonization dynamics of artificial substrates are an essential prerequisite for their use in biomonitoring studies (20). Much of the effects on the community structure in ecotoxicology must be estimated through the use of microcosms/mesocosms due to the need for experimental control not attainable in the field or practical necessity due to the highly hazardous nature of some contaminants. The response of taxonomic richness as the end point of microcosm toxicity was reasonably sensitive for many types of toxicants (21). Relatively few species were identified in these experiments. This suggests that the early community development was dominated by pioneer species capable of surviving minimal conditions i.e. the early stage of organic accumulation on the substrate (22). The number of migrant species from these communities that reached barren PF substrate between days 1 and 7 was greater. Migrant species from more mature communities (14 to 28 days) that have reached species equilibrium represent a consistent proportion of the source community (Figure 6). As the day proceeds a smaller proportion of the community migrates to barren islands, but the absolute number of migrant species is relatively similar.

TE addition was an effective means of increasing nutrients from days 1 to 28. Further increases in the phytoplankton community were shown to be correlated with the nutrient increase (10,23). Schinder (24) stated that phosphate is well recognized as the primary factor controlling freshwater production and is frequently limiting. Among the phytoplankton, diatoms were the dominant group sampled from PF substrates but they did not show increasing species numbers over time. This suggests that the colonization of diatoms in PF substrates occurs very rapidly. This agrees with Stewart (25), who examined the colonization process for diatoms and protozoans in Michigan lakes.

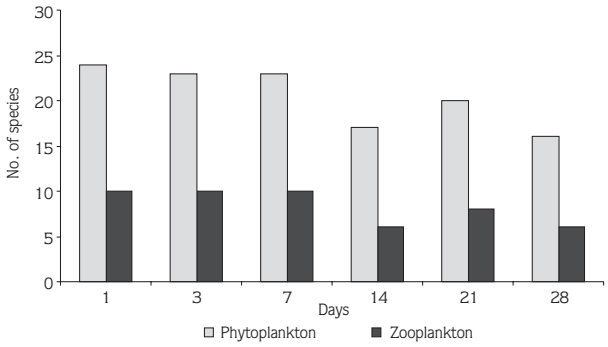

Figure 6. Number of species on barrel PF substrates during the experimental periods.

Species richness has a consistent response to different effluent treatments. The response of taxonomic richness is likely to be useful for many types of toxicants (9). Chemical flocculation also initially effected phytoplankton as well as zooplankton counts because of physical entrapment, but species richness and composition increased following floc settling and few species were lost from the species pool in the treatment tanks. A significantly lower total zooplankton count in the high TE treated microcosm tanks and changes in zooplankton composition at all TE treatment levels may suggest that the toxicity of the effluent and also the food base reduction affected the zooplankton community (26). Flemer et al.'s (27) laboratory colonized experiments also indicated that the total number of organisms experienced a significant decrease in response to the addition of pesticide.

The taxonomic richness of the epicenter substrate was not adversely affected at the concentration tested. Using changes in taxonomic richness at the end point of the microcosm toxicity test was reasonably sensitive and is intuitively appealing for several reasons. It is an empirical determination of toxicant effluent on a parameter. A microcosm toxicity test with substrate communities to determine the concentration of a toxicant affecting 5% of taxa is similar in expense to a conventional 21-day chronic test with *Daphnia* (28). In general, changes in species composition and abundance were more consistent with and sensitive to the end point tested. Decreases in species richness were also a consistent and sensitive response.

Acknowledgment

The first author (YNK) is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, Government of India, for financial assistance (SRF).

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References

1. Fu LJ, Stares RE and Stahy Jr. RG. Assessing acute toxicities of pre and post treatment industrial wastewaters with *Hydra attenata*: a comparative study of acute toxicity with the fathead minnows, *Phimephales*, Promelas. Environ Toxicol Chem 13: 563-569, 1994.
2. Nielsen MH and Rank J. Screening of toxicity and genotoxicity in wastewater by the use of *Allium* test. Hereditas 121: 249-254, 1994.
3. Streit B, Community ecology and population interactions in freshwater systems. Ed. Genit Scrllrmann and Bernd Markets. Ecotoxicology 12: 133-139, 1998.
4. Cairns Jr. J, Niederlehner BR and Eric P.Smith. Microscale testing in aquatic toxicology: Advances, Techniques and Practice. Wells PG, Lee K, Blaise C and Ganthier J eds. CRC Press, Boca Raton, FL, USA, 1998.
5. Muller P. Effects of artificial acidification on the growth of periphyton. Can J Fish Aquat Sci 17: 355-363, 1980.
6. Havens K and De Costa J. The effect of acidification in enclosures on the biomass and population size structure of *Bosmina longirostris*. Hydrobiologia 122: 153-158, 1985.
7. Blinn DW, Tompkins T and Zaleski L. Mercury inhibition on primary productivity using large volume plastic chambers *in situ*. J Phycol 13: 58-61, 1977.
8. Niederlehner BR, Pratt JR, Buikema AL, et al. Laboratory tests evaluating the effects of cadmium on freshwater protozoan communities. Environ Contam Toxicol 4: 155-165, 1985.
9. Pratt, JR, Niederlehner, BR, Nancy Bowers, et al. Prediction of permissible concentrations of copper from microcosm toxicity tests. Toxicity Assessment: An International Quarterly 2: 417-436, 1987.
10. Holz JC and Kyle D. Hoagland, Experimental microcosm study of the effects of phosphorus reduction on plankton community structure. Can J Fish Aquat Sci 53: 1754-1764, 1996.
11. Paul Jr. RW, Kuhn DL, Plafkin JL et al. Evaluation of natural and artificial substrate colonization by scanning electron microscopy. Trans Am Microsc Soc 96: 506-519, 1977.
12. APHA (American Public Health Association, American Water Works Association and Water Pollution Control Federation), Standard Methods for the examination of water and wastewater, 18th ed., Washington, D.C., 1992.
13. Edmondson WT, Freshwater Biology, II nd edition, John Wiley and Sons Inc., New York, USA, 1959.
14. Sharma BK. The Indian species of the genus Brachionus (Eurotatoria: Monogononta: Brachionidae). Hydrobiologia 104: 31-39, 1983.
15. Michael R and Sharma BK. Fauna of India and adjacent countries, Indian Cladocera (Crustacea: Branchiopoda: Cladocera). Zoological Survey of India, Calcutta, India, 1988.
16. Battish SK. Freshwater zooplankton of India. Oxford and IBH Publishing Co. Pvt., Ltd., New Delhi, 1992.
17. Anand N. Indian freshwater microalgae, Shiva Offset Press, Dehradun, India, 1998.
18. Woelkerling WT, Robert R. Kowal and Stephan B. Gouch, Sedgwick- Rafter cell counts: a procedural analysis. Hydrobiologia 48: 95-107, 1976.
19. Zar JH. Biostatistical analysis, Printice-Hall International, Inc., 1987.
20. Ramanibai R and Elayaraja P. The effect of toxicant on the colonization rate of plankters - A microcosm study. Indian J. Environ & Ecoplan 5: 417-421, 2001.
21. Nederlehner BR and John Cairns Jr. Consistency and sensitivity of community level endpoints in microcosm test. J. Aquatic. Ecosystem Health 3: 93-99, 1994.
22. Pratt JR, Cairns Jr. J and Meier K. Migration of species during early colonization. The American Midland Naturalist 113: 92-101, 1985.
23. Flemer DA and Robert J. Livingston, Seasonal growth stimulation of sub-temperate estuarine phytoplankton to nitrogen and phosphorus: An outdoor microcosm experiment. Estuaries 21: 145-159, 1998.
24. Schindler DW. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol Oceanogr 23: 478-486, 1978.
25. Stewart PM, James R. Pratt, John Cairns Jr. and et al. Diatoms and protozoan species accrual on artificial substrates in lentic habitats. Trans. Am Microsco Soc 104: 369-377, 1985.
26. Nederlehner B, Pratt J, Buikema Jr. A et al. Laboratory tests evaluating the effects of Cadmium on freshwater Protozoan communities. Environ Toxicol Chem 3: 155-165, 1985.
27. Flemer DA, Roman S Stanley, Barbara F Ruth et al. Recolonization of estuarine organisms: effects of microcosm size and pesticides. Hydrobiologia 304: 85-101, 1995.
28. Niederlehner BR, Pratt J, Bukema Jr. A, et al. Comparison of estimates of hazard derived at three levels of complexicity. Community toxicity testing, American Society for Testing and Materials, Philadelphia, pp.30-48, 1986.