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Evaluation of Toxicity of Tannery Effluent on Plankton Community Structure: A Multispecies Microcosm Study II

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Abstract: Laboratory experiments were conducted to evaluate the dose-dependent response of tannery effluents on plankton community structure using polyurethane foam (PF) as artificial substrate. Naturally derived planktonic communities on PF provided colonists for the development of new communities under stress conditions in the laboratory. Predictions of colonization rate, taxonomic richness and relative abundance in a series of microcosms dosed with a gradient of tannery effluent from 20% to 35% concentration were assessed after the initial dosage study (1% to 15%). Changes in taxonomic composition occur at high levels of stress. End points responding at increasing levels of stress are declines in species numbers relative to expected numbers followed by an increase in nutrient concentration from control to tannery effluent treated microcosms.

Key Words: Multispecies, plankton community structure, tannery effluent, taxonomic richness

Introduction

Many questions about the response of complex systems to toxicity are difficult to address by describing different natural environments (1). In order to explain the relationships between changes in communities and chemical stress, experiments with similar artificial ecosystems have been useful. Single species toxicity tests have served as efficient ranking tools to monitor the effect of existing discharges on aquatic organisms (2). But multispecies microcosm toxicity tests permit the determination of the effects of direct toxicant discharge on characteristics of complex communities, such as diversity, and by testing many species simultaneously they can efficiently establish the range of biological sensitivity to a toxicant (3).

Many human activities cause pollution of the aquatic environment, modification of the environmental conditions and thereby changes in the aquatic communities (4). The best way to analyze 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 (5). The information from research with multispecies microcosms is relatively rich. Pratt et al. (6) studied the prediction of permissible concentrations of copper on naturally derived protozoan communities. Niederlehner and Cairns (1) reviewed the microcosm toxicity tests with 12 chemical stressors and found that the relative sensitivity of certain end points is consistent over toxicant type. The effects of total phosphorus reductions in a lake plankton community structure were investigated in a microcosm study by Holz and Hoagland (7). Nutrient limitation on phytoplankton biomass production with emphasis on nitrate - nitrogen and orthophosphate - phosphorus was conducted by Flemer and Livingston (8), on a mixture of metals and 3-4 dichloroaniline by Jack et al. (9,10) and on thermal effluent by Ramanibai and Elayaraja (11). Recently Sandesan et al. (12) determined the lowest effect concentration for freshwater zooplankton exposed to perflurooctanoic acid (PFOA), and the effects of aluminum and copper on aquatic microbial communities were studied by Fuma et al. (13). The preliminary investigation of toxicity of tannery effluent (TE) (1% to 15%) on colonization rate of plankton was given by Koteswari and Ramanibai (14), where the plankton community structure was relatively less affected. This approach may help us to identify end points that constantly respond to different kinds of chemical stress. The purpose of this study was to examine the colonization

process of naturally derived planktonic communities and to estimate the toxicity of TE on taxonomic richness as the end point.

Materials and Methods

A standard procedure for the artificial substrate microcosm (AS-M) is described by Pratt and Bowers (15). A general description of the AS-M is as follows. Polyurethane foam (PF) units are used as an artificial substrate for the colonization of microorganisms because the 3-dimensional characteristics of the foam permit ready colonization by a wide variety of microorganisms. Free swimming forms easily invade the intersects of the foam, while sessile forms attach to the solid pillars (16).

PF substrates measuring 6 x 5 x 3.75 cm were cut and used for the colonization experiments. These units were rinsed first with 10% hydrochloric acid, and then with distilled water and 50% ethanol and thoroughly rinsed with distilled water. The substrates were placed in a net bag and suspended at 1 m depth with cotton string in lake Porur, a 3.20 km^2 man-made impoundment in the western suburban area of Chennai metropolitan city. The PF substrates were colonized for 14 days and then immediately transported to the laboratory in a glass jar containing enough water so that they were immersed completely. These substrates were used as the epicenter for the laboratory-based microcosm study.

Microcosm Test Design, Sample Collection and Enumeration Procedures

Test vessels were rectangular glass tanks (35.5 x 15 x 10 cm) containing 4 l of filtered lake water. A single colonized substrate (epicenter) was suspended from a hook, in the bottom of the container at the center of each tank and surrounded by 6 initially barren substrates (6 x 5 x 3.75 cm) (islands). Tests were conducted at ambient room temperature and under 12:12 h light:dark photoperiod conditions. TE was used as a toxicant in this experiment. Typical effluent characteristics of tanneries are given in our previous study (14). The effect of initial concentration of TE (1%, 3%, 5% and 15%) on plankton colonization was tested initially (14). The TE was diluted 20%, 25% and 35% with filtered lake water for the treatment procedure and a control was kept without adding the effluents. Triplicate 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 island from each tank was randomly chosen and sampled after 1, 3, 7, 14, 21 and 28 days of colonization.

Physicochemical variables like temperature, pH, alkalinity, hardness, and phosphate and nitrate concentrations were estimated by following methods given in APHA (17). The material from the interstices of the foam substrate was collected by decanting the excess liquid, and then squeezing each substrate to dryness in a borosilicate beaker. Sample volumes recovered from these 3-dimensional substrates are 75% ± 3% (N = 3) of the total displacement volume of the substrates. The plankton samples were fixed in 4% formalin for further analysis. The plankton were identifed by following taxonomic keys (18-22) and counted under an inverted microscope (Nikon TMS-4) at 100 x magnification. A Sedgwick-Refter cell was used for the counting process (23).

All values of the physicochemical variables are expressed as means. The significance of the difference between the mean values of the control and experimental microcosms was analyzed using one-way ANOVA (24) and statistical significance was inferred at $P \leq 0.05$.

Results

Results of the physicochemical variables of the effluent toxicity test are given in Figure 1. A triplicate of 3 concentrations and a control were tested. Significant variation was determined in the physicochemical features between the control and TE treated microcosms (Figure 1). However, there was no noticeable increase in surface water temperature during the study period, except for a slight increase (0.5 ºC) during day 14 in the 35% TE treated microcosm. The pH was between 6.5 and 8.0 and the maximum value (8.0) was noted in the 35% TE treated microcosm during the entire study period (Figure 1). Further, the lowest alkalinity value (86 ± 24.39 mg/l) was recorded in the control microcosm on days 3 and 7 and the maximum value (264 ± 16.26 mg/l) was detected in the 35% TE treated microcosm on day 3 (Figure 1). The alkalinity values showed significant differences between the control and treatment tests on day 1 ($P < 0.001$), day 3 ($P < 0.005$), day 14 ($P < 0.05$) and day 21 ($P < 0.025$).

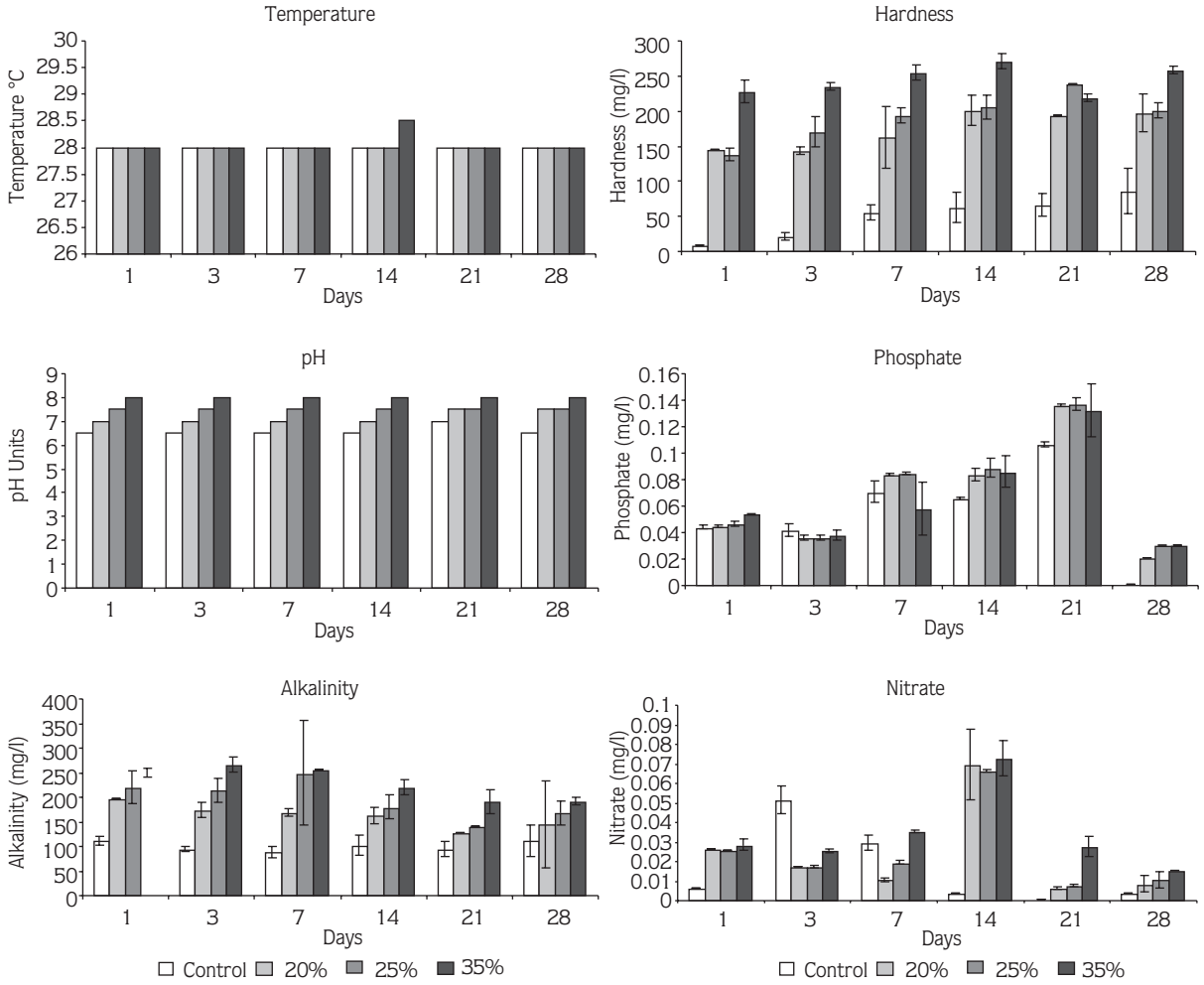

Figure 1. Mean response of physical and chemical variables to tannery effluent additions (± SD) in the microcosm tanks during the experiment.

A wide variation in hardness values was determined between the control and experimental microcosms, ranging from 7.7 ± 0.00 to 269 ± 10.89 mg/l. Hardness showed significant variations between the control and experimental microcosms on day 1 ($P < 0.005$), day 3 ($P < 0.0005$), day 7 ($P < 0.005$), day 14 ($P < 0.0025$) and day 21 ($P < 0.0005$) but not on day 28 (Figure 1).

Nutrient concentrations (phosphate and nitrate) also deviated between different treatments after the addition of effluent (Figure 1) and the concentration increased with dosage. The maximum concentration of phosphate (0.136 ± 0.005 mg/l) was found on day 21 in the 25% TE treated microcosm and the minimum concentration (0.02 ± 0.00 mg/l) on day 28 in the 20% TE microcosm (Figure 1). Nitrate showed significant differences between the control and experiment microcosm on day 1 ($P < 0.001$), day 3 ($P < 0.0025$), day 7 ($P < 0.0025$), day 14 ($P < 0.025$), and day 28 ($P < 0.0025$) but not on day 21.

Colonization Rate

A total of 29 phytoplankton and 14 zooplankton taxa were identified during the experiment. Phytoplankton was represented by 7 taxa of Chlorophyceae (green algae), 21 taxa of Bacillariophyceae (diatoms) and 2 taxa of Cyanophyceae (blue green algae). Among the zooplankton, rotiferans were the dominant group, consisting of 10 taxa, whereas Copepoda and Cladocera were represented by 3 and 1 taxa, respectively (Table 1). The majority of the species were found in only a few samples during the experimental period.

Table 1. List of planktons identified during the microcosm study.

S. No.	PHYTOPLANKTON	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Epicenter
I	CHLOROPHYCEAE							
1	*Closteriopsis* sp.	+	+	+	+	+	+	+
2	*Closteriopsis longisemma*	+	+	+	+	+	+	+
3	*Microspora williana*		+				+	+
4	*Pediastrum duplex*	+						+
5	*P. simplex*	+	+	+	+	+	+	+
6	*S. dimorphus*							+
7	*Scenedesmus quadricauda*	+	+	+	+	+	+	+
II	BACILLARIOPHYCEAE							
8	*Achnanathus inflate*	+	+	+	+	+	+	+
9	*A. elata*		+		+	+	+	
10	*Amphora* sp.	+						
11	*A. ovalis*	+		+				+
12	*Anomoneis ineronises*							+
13	*Cymbella cymbiformis*	+	+					+
14	*C. tumida*	+	+	+	+	+		+
15	*C. cistula*							+
16	*Diatoma* sp.		+	+	+	+	+	+
17	*Fragillaria* sp.			+	+	+		
18	*Navicula closterium*	+						+
19	*N. cuspidate*	+	+	+	+	+	+	+
20	*N. radiosa*	+	+	+		+		+
21	*N. crytotinella*			+	+	+	+	
22	*N. productum*		+	+				+
23	*Nitzshia palea*		+	+	+	+	+	+
24	*N. vitria*							+
25	*N. umblicata*	+						+
26	*N. obtuse*	+	+	+	+			+
27	*Pinnularia interrupta*							+
III	CYANOPHYCEAE							
28	*Anabaena variabilis*	+			+			
29	*Spirulina* sp.	+	+	+	+	+	+	+

S. No	ZOOPLANKTON	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Epicenter
I	ROTIFERA							
1	*Brachionus quadridentatus*			+				
2	*Collurella obtuse*	+		+	+	+		+
3	*Eiothena* sp.							+
4	*Lecane inopioneta*		+				+	+
5	*L. ploenensis*		+					
6	*Lecane* sp.	+						
7	*Lepidella ovaslis*	+		+		+		+
8	*Monostyla bulla*			+	+	+	+	+
9	*M. decipiensis*		+	+	+	+		
10	*Monostyla* sp.	+	+	+			+	+
II	COPEPODA							
11	*Cyclops* sp.	+		+				
12	*Halicyclops* sp.		+	+	+	+	+	+
13	*Microcyclops diversis*					+		+
III	CLADOCERA							
14	*Alona* sp.	+	+	+	+	+		+

+ Presence

A total of 16 phytoplankton species were identified on day 1. Bacillariophyceae was the dominant group, represented by 10 taxa, followed by Chlorophyceae with 4 taxa and Cyanophyceae with 2 taxa. Among the zooplankton, rotiferans were the dominant group, represented by 4 taxa (Table 1). Copepoda and Cladocera were represented by single species of *Cyclops* and *Alona,* respectively. The total mean abundance of Chlorophyceae, Bacillariophyceae and Cyanophyceae was lower in the treatment microcosms than in the control microcosm (Figure 2). There was a significant change in total mean phytoplankton counts on day 1 ($P < 0.001$) but zooplankton did not show any significant differences between the control and treatment microcosms.

The phytoplankton was represented by 16 taxa on day 3, but a slight increase in the zooplankton taxa was noted (7 taxa) (Table 1). Out of 16 taxa, Chlorophyceae was represented by 5 taxa *Closteriopsis* sp., *Scenedesmus quadricauda, Pediastrum simplex, Closterium longisemma* and *Microspora williana.* Similar to day 1, Bacillariphyceae was the dominant group, represented by 10 taxa, and the colonization rate of zooplankton was relatively increased from day 1. Rotifera continued to be the dominant group among the zooplankton (Table 1). *Lecane inopionata*,

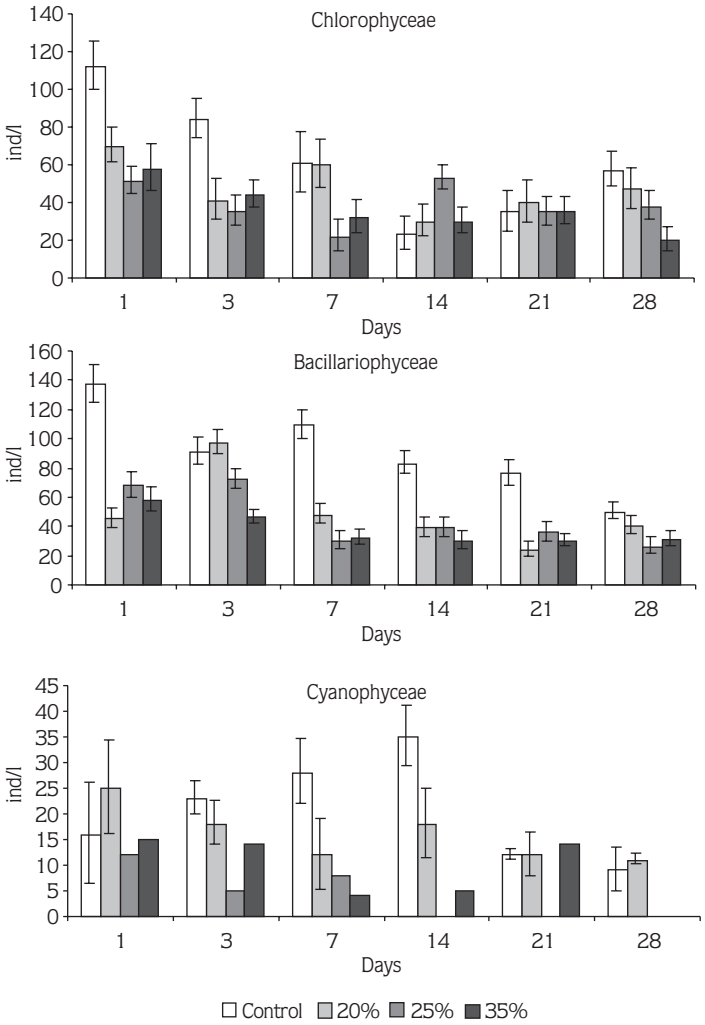

Figure 2. Colonization rates of phytoplankton groups exposed under different gradients of tannery effluent. Shown are the mean ± standard deviation based on 3 replicates.

Lecane pleonensis and *Monostyla decipenes* were the pioneer species found on day 3. The total mean abundance of Chlorophyceae and Cyanophyceae was maximum in the control microcosm (84 ind/l and 23 ind/l) (Figure 2), but Bacillariophyceae was relatively increased from the control to the 20% TE treated microcosm (Figure 2). Statistically significant changes were observed in total mean abundance of phytoplankton and zooplankton between the control and treatments ($P < 0.025$ and $P < 0.025$).

Sixteen phytoplankton taxa and 11 zooplankton taxa were identified on day 7, out of 30 phytoplankton taxa and 17 zooplankton taxa. Diatoms were the dominant group (11 taxa) among the phytoplankton and Rotifera were the dominant group (8 taxa) among the zooplankton. Cyanophyceae was represented by a single *Spirulina* species. Bacillariophyceae showed a maximum abundance in the control microcosm (109 ind/l) (Figure 2). Phytoplankton and zooplankton showed a steady decrease between the control and 35% TE treated microcosms (Figure 2 and 3). A significant interaction in the total mean abundance was found between the control and effluent treated microcosms ($P < 0.005$ and $P < 0.05$).

The species composition of phytoplankton and zooplankton showed a slight decrease during day 14 of colonization. Similar to on days 1, 3 and 7, Bacillariophyceae were the dominant group (9 taxa), followed by Chlorophyceae (4 taxa) and Cyanophyceae (1 taxon). Rotifera continued to be the dominant group among the zooplankton. Rotiferan species such as *Colurella obtuse, Monostyla decipienes* and *Monostyla bulla* (Table 1) were found on day 14. The highest total mean abundance of Bacillariophyceae (83 ind/l) and Cyanophyceae (35 ind/l) was detected in the ontrol microcosm (Figure 2) and a steady significant decrease in abundance was observed between the control and effluent treated microcosms ($P < 0.025$). Copepoda showed a very marked decrease in abundance from the control to the 35% TE treated microcosm (Figure 3). There were no significant differences in zooplankton abundance in the experiment.

A total of 13 phytoplankton taxa and 7 zooplankton taxa were identified on day 21. Bacillariophyceae was the dominant group (8 taxa) followed by Chlorophyceae (4 taxa) and Cyanophyceae 1 taxon) (Table 1). The colonization rate of zooplankton was relatively increased from day 14. Cyanophyceae was completely absent in the 25% TE treated microcosm (Figure 2). Among the zooplankton, Cladocera was represented only in the 35% TE treated microcosm. The total abundance of Rotifera and Copepoda showed a significant steady decrease from the control and effluent treated microcosms ($P < 0.025$) (Figure 3).

On day 28, the phytoplankton was represented by 13 taxa and the zooplankton by 4 taxa. Bacillariophyceae was the dominant group (7 taxa). The zooplankton was represented by Rotifera and Copepoda. Cladocerans were completely absent on day 28. Phytoplankton and zooplankton abundance showed a decreasing trend from the control to effluent treated microcosms (Figure 2 and 3). A significant variation in total mean phyto- and zooplankton count was observed between the control and effluent treated microcosms ($P < 0.025$ and $P < 0.05$).

The epicenter (source) was analyzed after 28 days of exposure to various concentrations of TE. A total of 23 phytoplankton taxa and 9 zooplankton taxa were identified. Bacillariophyceae was the dominant group (15 taxa), followed by Chlorophyceae (7 taxa) and Cyanophyceae (1 taxon) (Table 1). Among the zooplankton Rotifera were the dominant group, with 6 taxa. Copepoda species such as *Halicyclops* sp. and *Microcyclops diversus* were also found. The taxonomic richness of the epicenter substrate was not adversely affected at the concentrations tested but a significant variation in the total mean abundance of phytoplankton was determined ($P < 0.05$).

Discussion

It is necessary to establish a reasonable method to evaluate the ecological effects of toxicants on microbial communities in the aquatic environment, since these microorganisms play a vital role in the food web (25). Plankton colonizes on PF substrates, rather the reverse of the single species tests frequently used in ecotoxicology studies. Changes in the taxonomic composition and abundance of communities were observed in response to different concentrations of effluent. The response of taxonomic composition as the end point of microcosm toxicity was reasonably sensitive to many types of toxicants (26).

The colonization dynamics of artificial substrates are an essential prerequisite for their use in biomonitoring

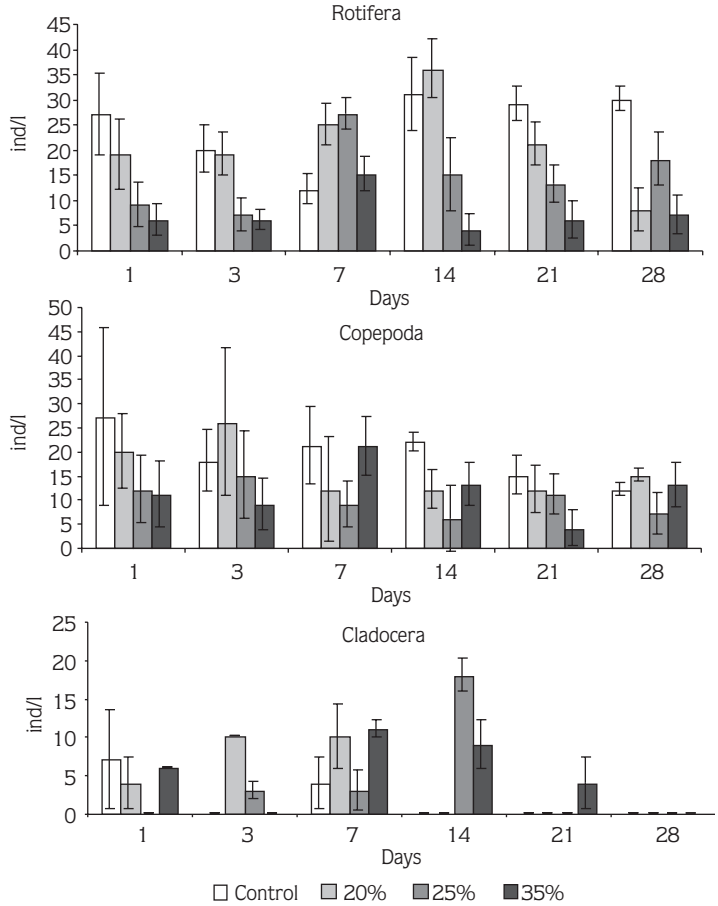

Figure 3. Colonization rate of zooplankton groups exposed to different gradients of tannery effluent. Shown are the mean ± standard deviation based on 3 replicates.

studies (11). Ecological perturbation in an aquatic environment generally produces certain predictable changes in the community structure (27). Species with low tolerances are eliminated, while those species best suited for survival in enriched habitats become extensively dominant. Relatively few species were identified in our experiments. This suggests that the early community development was dominated by pioneer species capable of surviving minimal organic accumulation on the substrates (28).

The diversity and number of taxa colonizing the substrates stabilized on day 7 (Figure 4). The colonization rate increased from day 1 to day 7; migrant species from more mature communities (14 to 28 days) that have reached species equilibrium represent a consistent proportion of the source community. As the day proceeds a smaller portion of the community migrates to barren islands, but the absolute number of migrating species is similar in terms of phytoplankton count, whereas zooplankton showed a decreasing count (Figure 4).

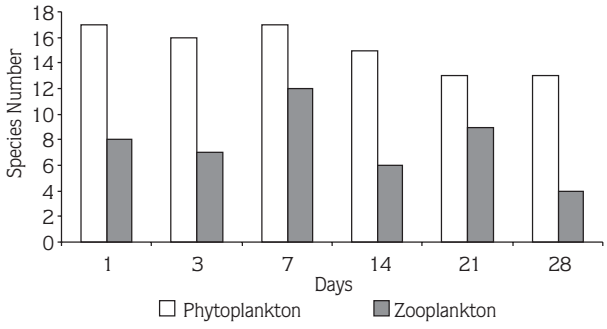

Figure 4. Number of species on barren PF substrates during the experimental period.

Effluent addition was an effective means of increasing nutrients from the control to the 35% TE treated microcosms. A further decrease in the phosphate concentration on day 1 to day 28 in the control microcosm was shown to be correlated with an increase in plankton productivity (7,29). Diatoms showed strong substratum affinity and colonized in greater numbers in the control microcosms (Figure 2). This suggests that the colonization of diatoms in PF substrates occurs very rapidly. This does not imply that changes in species composition and relative abundance in the later colonization or arrival of new species do not occur. These observations suggest that PF substrates do not behave as islands for diatoms in lake plankton since they appear to be distributed throughout the water column (30).

The tolerance of a community of interacting organisms can change through additional mechanisms that are unique to communities (31). Taxonomic richness and relative abundance cause a consistent response to different effluent treatments. Changes in the plankton community can be explained by the reduction in the water column by the addition of different concentrations of effluents. lower abundance of phytoplankton and subsequently zooplankton in the high TE treated microcosm and changes in zooplankton taxonomic composition at all TE treatment levels suggest that food base reductions affected the zooplankton community. Comparisons among effluent treatments suggest that there is a significantly different phyto- and zooplankton community in the microcosms with high levels of TE, as indicated by the lower total phyto- and zooplankton biovolumes. In addition, the magnitude of changes in the relative abundance of diatoms and rotiferans was much greater than that of the other groups such as green algae, blue green algae, copepods and cladocerans. Furthermore, the relative abundances of rotiferans and copepods were higher only in the control microcosms (Figure 3). This result suggests that the plankton community's response to a toxicant may be nonlinear with relative abundance and taxonomic composition changes occurring in high concentrations of effluent.

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