

## Chapter 3

### Diatoms in the microfilm

### 3.1. INTRODUCTION

Marine and freshwater biofouling is one of the major unsolved problems currently affecting the shipping industry and industrial aquatic processes. In the aquatic environment, any submerged solid surface gets coated by a complex layer, initially consisting of an organic conditioning film. Formation of this film is immediately followed by an accumulation of microorganisms and the secretion at their cell surface of extracellular polymeric substances (EPS) during attachment, colonization, and population growth. This process is known as microfouling and the resulting layer termed as microfilm/biofilm. The microfilm subsequently induces settlement of larvae of higher organisms such as barnacles, mussels, tubeworms etc., thus leading to macrofouling. These fouling organisms cause serious technical problems by settling on shiphulls, power plant cooling systems, aquaculture systems, fishing nets, pipelines and other marine infrastructure. Ships suffer increased drag and surface corrosion leading to lower speeds, thereby causing higher fuel consumption and maintenance costs.

Fouling is a dynamic process. The specific organism that develops in a fouling community depends on the substratum, geographical location, the season and factors such as competition and predation. In the entire process of biofouling, diatoms are amongst the earliest eukaryotic colonizers of submerged artificial surfaces and one of the most conspicuous organisms in biofilms (Evans 1988). However, till date, few studies have focused on the community structure of fouling diatoms in the marine environment (Cooksey et al. 1984; Edyvean et al. 1985; Karande 1987; Bhosle et al. 1989; Kelkar 1989; Shantakumaran 1989; Rao 1990; Raveendran et al. 1991; Bhosle 1993; Pangu 1993; Venugopalan 1994; Prabhadevi 1995a; 1995b; Redekar 1997; 2000b). Another aspect, which has been neglected in this area, is the approach towards the quantitative study of fouling diatoms. Scraping has been used as a traditional method for enumerating

the fouling diatom community from various types of substrata. However, there is no comparative and standardized method as to check the amount of cell loss that occurs through the use of traditional methods.

In this investigation, efforts were made to find an appropriate technique for the quantitative study of fouling diatoms by comparing the use of different tools for removal of microfilms. Also, the volume of the microfilm sample to be taken for observation was standardized. Subsequent to this study, taking the appropriate method into account, an evaluation of the temporal variation (both qualitative and quantitative) in the fouling diatom community was undertaken. This study included the evaluation of exposure period and types of substrata.

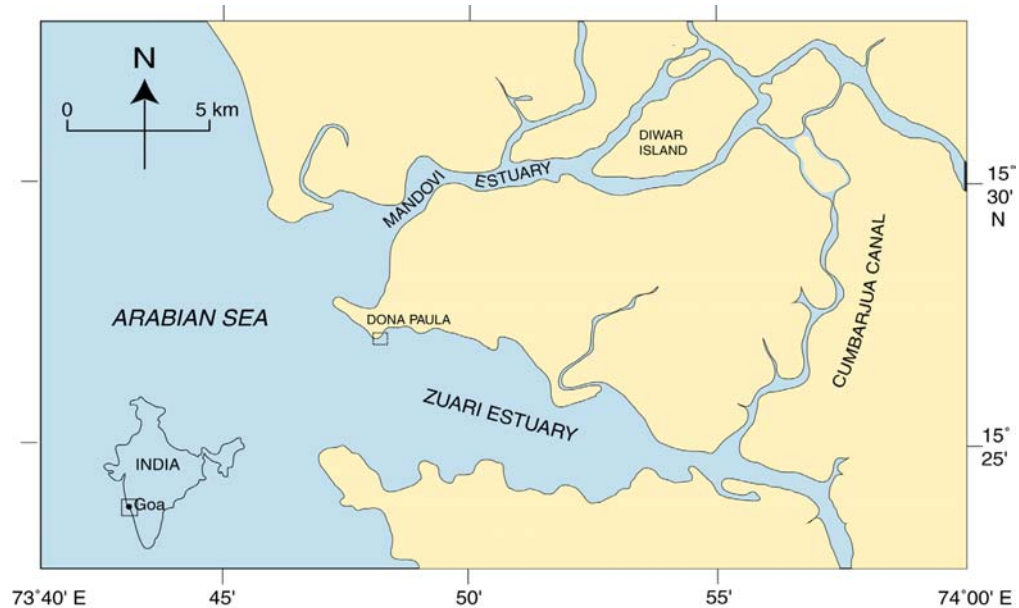
## **3.2. MATERIALS AND METHODS**

### ***3.2.1. Study area***

All the investigations were carried out at Dona Paula Bay located at the mouth of the Zuari estuary, Goa, ( $15^{\circ} 27.5' N$ ,  $73^{\circ} 48' E$ ), on the west coast of India (Fig. 3.1). This estuary is classified as a tide-dominated, coastal, plain estuary. The river Zuari has its source in the Western Ghats, and it extends up to 70 km before meeting the Arabian Sea. This river is influenced by seawater inflow up to a considerable distance inland, and it receives a large quantity of fresh water during the southwest monsoon season. Based on this, a year has been classified into three seasons viz. monsoon season (June–September), followed by a recovery period during post–monsoon (October–January) and thereafter a stable pre-monsoon period (February–May).

### ***3.2.2. Experiment 1.***

A comparative evaluation of various tools employed for the removal of microfouling material and estimation of diatom population has been carried out. The aim of the study



**Fig. 3.1.** Geographical location of study area.

is to develop a very simple and reliable method for the estimation of microfilm diatom population. Evaluation of the diatom population from the microfilm was done by using different techniques such as brushing, scraping and direct observation.

For this study, 40 cleaned fiberglass panels measuring 15 x 10 x 0.2 cms bolted to fiberglass flats with PVC nuts and bolts and 60 glass-slides measuring 2.5 x 7.5 cms fixed to a wooden frame were deployed at subsurface level at Dona-Paula Bay, Goa, India. Ten fiberglass panels and 15 glass slides were retrieved every alternate day for a period of four days and were transported to the laboratory in an insulated cool-box containing seawater.

For the study, two different types of tools (nylon brush and ceramic scraper) were used for removing the microfilm from the test substrata. Out of the 10 fiberglass panels, microfilms from 5 panels were removed with a nylon brush whereas a ceramic scraper was used for the remaining 5 panels. The removal of microfilm from individual panels

was done separately in a dried glass tray with 100 ml of 0.45  $\mu\text{m}$  membrane filtered seawater. The scraped material, in replicates of five for each tool, was fixed with 4% Lugol's iodine for enumeration of diatom population.

Similar tools and methods were employed for removing microfilm from glass-slides. Out of 15 glass slides, 3 glass slides were used for direct observation of diatom population. Considering 2 glass slides as a single unit, microfilm from 3 units were removed with a nylon brush and the remaining 3 units with a ceramic scraper. The scraped material was fixed with 4% Lugol's iodine for enumeration of diatom flora.

The microfilm samples obtained were allowed to stand for  $\sim 2$  days for sedimentation in a 100 ml measuring cylinder. The top 90 ml of the sample was siphoned out. The remaining 10 ml of the sample was mixed thoroughly and sub-samples (2 ml, 3 ml and 5 ml representing 20%, 30% and 50% of the samples) were taken for enumeration of diatom population. The results are presented in terms of diatom abundance per  $\text{dm}^2$ .

### *3.2.2.1. Data analysis*

The results were further subjected to various statistical analyses to evaluate the efficiency of the tools used for the removal of microfilm.

Univariate measures included the calculation of species count (S), Shannon-Wiener's diversity index ( $H'$ ), species richness (d) and evenness ( $J'$ ) of the diatom community, for the different volumes of the microfilm samples obtained from glass and fiberglass substrata using both tools for microfilm removal (Pielou 1984). Similar analyses were also performed on the data obtained from the direct observation of the microfilm developed over glass slides. This analysis was performed using a statistical software PRIMER version 5.

Linear regression analysis on diatom abundance (after transforming to %) for the different volumes of the microfilm samples for all days was performed to compare the different volumes.

Two-way analysis of variance (ANOVA) with equal replications was performed on the fourth root transformed diatom abundance data to evaluate the sub-volume variation in the univariate measure of the microfilm sample obtained from fiberglass substrata and also to compare between the tools (nylon brush and ceramic scraper) used for removing the microfilm with respect to values of univariate measures.

### ***3.2.3. Experiment 2.***

#### ***3.2.3.1. Environmental parameters***

Surface water samples (in triplicate) were collected every month for 5 consecutive days from the study site, during the period of panel submersion. Analysis of temperature, salinity (Mohr-Knudsen titration method) (Strickland and Parsons 1965), dissolved oxygen and nutrients (NO<sub>2</sub>-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P and SiO<sub>3</sub>) were carried out by standard procedures (Parsons et al. 1984). A known volume of the water sample was preserved with 4% Lugol's iodine solution for estimating the diatom population by the sedimentation method (Hasle 1978).

#### ***3.2.3.2. Type of substrata***

Fiberglass, glass, copper and cupro-nickel (70:30) panels admeasuring 15 × 10 × 0.2 cm were used in this study.

#### ***3.2.3.3. Substrata submersion***

The panels, with 8 mm holes drilled at one end, were bolted to fiberglass flats with PVC nuts and bolts and suspended at the sub-surface level (~ 1m below lowest low tide level).

Earlier reports (Smita et al. 1997; Smita and Anil 2000) and personal observations of the present study area have revealed the dominance of microorganisms on substrates for one to four days, followed by the arrival of macro organisms. So the submersions were carried out for four consecutive days, once every month in order to obtain 1, 2, 3 and 4 day old microfilm over a period of 14 months (November '98 to January 2000).

#### *3.2.3.4. Assessment of settlement*

Panels (in replicates of 5) were removed every consecutive day till the fourth day. They were then scraped with a nylon brush (Sharma et al. 1990) into known quantities (~ 100 ml) of 0.45  $\mu\text{m}$  membrane filtered seawater. The scraped material was then fixed with 4 % Lugol's iodine. This preserved material was subjected to sedimentation in a settling chamber (Hasle 1978) and the diatom cells enumerated both qualitatively and quantitatively using a compound microscope. Diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyam (1946), Desikachary (1987), Round et al. (1990) and Tomas (1997). The results are presented in terms of diatom abundance per  $\text{dm}^2$ . Simultaneously, another set of scraped material from the panels was used for chlorophyll *a* analysis (Parsons et al. 1984).

#### *3.2.3.5. Data analysis*

The water column and fouling diatom community were subjected to univariate and multivariate analyses.

*Univariate analysis:* Univariate techniques included the calculation of species count (S), Shannon-Wiener's diversity index ( $H'$ ), species richness (d) and evenness ( $J'$ ) of the diatom community, in both, the water column and the fouling film (Pielou 1984).

*Multivariate analysis:* The fourth root-transformed data on the fouling diatom abundance was subjected to Multiple Analysis of Variance (MANOVA) (Sokal and Rohlf 1981) to evaluate temporal (days and months) and substratum variance.

The water column and fouling film diatom community (which averaged more than 5% of the total diatom community) were subjected to cluster analysis with respect to species for 1, 2, 3 and 4 days of submersion. Clustering was performed through Bray Curtis similarity and group average method (Pielou 1984). Data was subjected to fourth root transformation prior to analysis. Univariate and cluster analysis were performed using PRIMER software version 5.

Linear regression analysis was performed on the log-transformed values of diatom abundance and chlorophyll *a* concentration of the fouling diatom community.

### ***3.2.4. Experiment 3.***

Copper and cupro-nickel (70:30) panels admeasuring  $15 \times 10 \times 0.2$  cm were used in this study. The panel submersion and assessment of diatom settlement was similar to experiment 2. The only difference is that submersion was carried out for four consecutive weeks, once every season in order to obtain 1, 2, 3 and 4 week old microfilm over a period of one year (1999). The results are presented in terms of diatom abundance per  $\text{dm}^2$ .

#### ***3.2.4.1. Data analysis***

The analysis includes the calculation of univariate and multivariate measures (clustering and MANOVA). These analyses were performed as explained in experiment 2.



### 3.3. RESULTS

#### 3.3.1 Experiment 1.

##### 3.3.1.a. To standardize the volume required for the enumeration of diatoms obtained from microfilm

The microfilm samples obtained by employing different removal techniques from fiberglass and glass slides were used for the standardization of volume required for the enumeration of diatom population. The results obtained were similar for both glass slides and fiberglass i.e. the diatom counts followed a similar trend between the sub-volumes (Tables 3.1 and 3.2). The regression analysis of diatom abundance showed a

**Table 3.1a.** Values of the univariate measures of different volumes of microfilm (1 to 4 day-old) samples obtained from glass using nylon brush. (Note: Species count-S, Abundance -N, Species richness-d, Evenness-J' and Diversity-H').

Days	S			N (cells dm <sup>-2</sup> )			d			J'			H'		
	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%
1	23	21	28	464	627	923	3.53	3.16	3.92	0.71	0.65	0.67	3.19	2.88	3.21
2	24	27	30	862	1204	1881	3.45	3.67	3.82	0.56	0.59	0.57	2.59	2.81	2.78
3	30	33	34	1776	2491	3668	3.83	4.05	4.02	0.62	0.61	0.56	3.05	3.06	2.87
4	34	37	37	4205	6192	9252	3.92	4.10	3.93	0.63	0.60	0.58	3.21	3.13	3.03

**Table 3.1b.** Values of the univariate measures of different volumes of microfilm (1 to 4 day-old) samples obtained from glass using ceramic scraper. (Note: Species count-S, Abundance -N, Species richness -d, Evenness-J' and Diversity-H').

Days	S			N (cells dm <sup>-2</sup> )			d			J'			H'		
	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%
1	22	26	28	498	771	1031	3.43	3.77	3.87	0.77	0.76	0.73	3.41	3.56	3.50
2	25	27	29	725	1146	1597	3.72	3.78	3.86	0.65	0.65	0.64	3.02	3.08	3.10
3	29	28	32	1443	1987	2810	3.81	3.56	3.95	0.60	0.60	0.58	2.88	2.87	2.90
4	33	37	36	3985	5786	8529	3.83	4.13	3.89	0.64	0.62	0.60	3.23	3.20	3.08

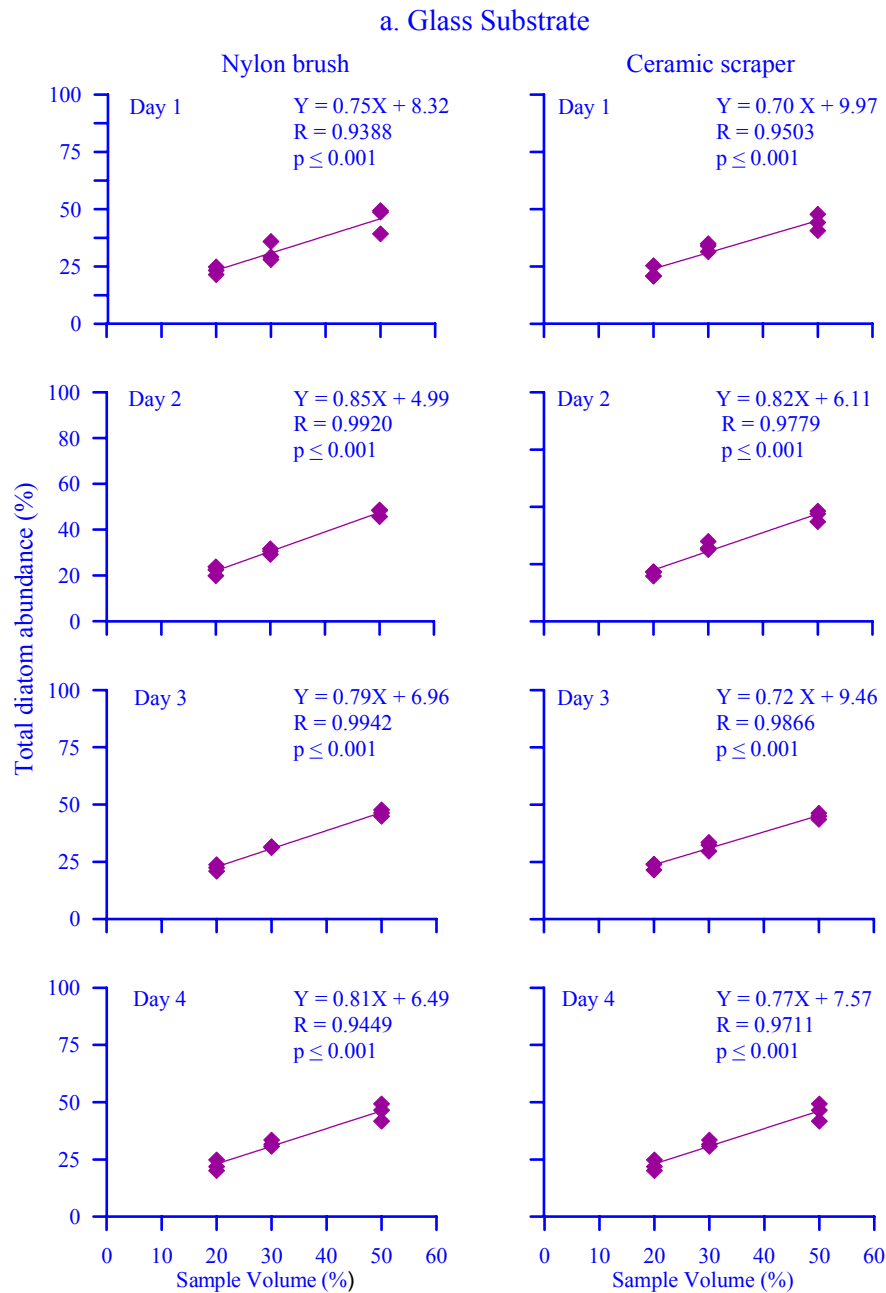
**Table 3.2a.** Values of the univariate measures of different volumes of microfilm (1 to 4 day-old) samples obtained from fiberglass using nylon brush. (Note: Species count-S, Abundance -N, Species richness -d, Evenness-J' and Diversity-H').

Days	S			N (cells dm <sup>-2</sup> )			d			J'			H'		
	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%
1	19	23	25	78	131	201	4.35	4.64	4.49	0.77	0.75	0.76	3.25	3.41	3.50
2	17	20	21	58	105	155	4.09	4.15	4.16	0.64	0.66	0.63	2.60	2.81	2.74
3	20	22	23	102	155	244	4.09	4.17	4.04	0.61	0.59	0.55	2.61	2.60	2.47
4	27	26	27	517	1150	1555	4.24	3.77	3.68	0.59	0.59	0.59	2.80	2.75	2.79

**Table 3.2b.** Values of the univariate measures of different volumes of microfilm (1 to 4 day-old) samples obtained from fiberglass using ceramic scraper. (Note: Species count-S, Abundance -N, Species richness-d, Evenness-J' and Diversity-H').

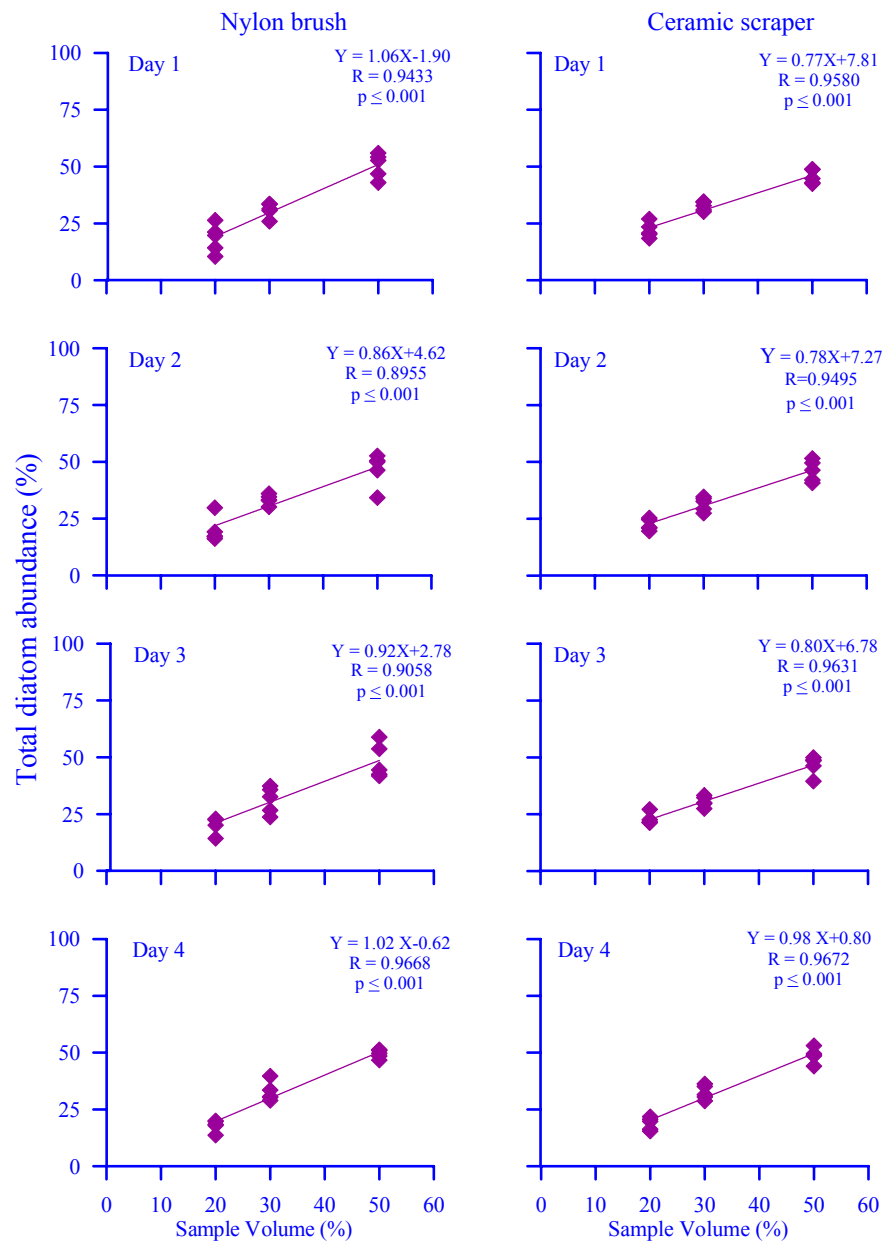
Days	S			N (cells dm <sup>-2</sup> )			d			J'			H'		
	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%	20%	30%	50%
1	21	22	24	79	116	162	4.63	4.43	4.58	0.70	0.74	0.73	3.07	3.27	3.37
2	18	21	21	87	116	163	4.07	4.54	4.03	0.67	0.65	0.64	2.80	2.87	2.81
3	24	24	26	207	270	427	4.30	4.22	4.23	0.54	0.54	0.54	2.49	2.48	2.53
4	25	24	25	304	543	815	4.13	3.64	3.53	0.56	0.57	0.56	2.58	2.60	2.57

significant linear relationship between the sub-volumes i.e. 20, 30 and 50 % of the scraped material for all experimental days and also between the tools used for removing microfilm (Fig. 3.2a and b). The result of the univariate measures obtained for different volumes of samples analyzed is given in tables 3.1 (a and b) for glass and 3.2 (a and b) for fiberglass. The univariate measures did not differ much for different volumes of the



**Fig. 3.2a.** Comparison of the sub volumes (2ml, 3ml and 5ml representing 20%, 30% and 50% of the  $1/10^{\text{th}}$  concentrated sample) with respect to diatom abundance (%) obtained from the microfilm developed over glass slide substrata.

## b. Fiberglass Substrate



**Fig. 3.2b.** Comparison of the sub volumes (2ml, 3ml and 5ml representing 20%, 30% and 50% of the 1/10<sup>th</sup> concentrated sample) with respect to diatom abundance (%) obtained from the microfilm developed over fiberglass substrata.

sample. ANOVA of univariate measures showed insignificant variation between the different sub-volumes of scraped samples obtained from fiberglass (Table 3.3) and glass (Table 3.4). These results indicate that 20% of the 1/10<sup>th</sup> concentrated sample is sufficient for enumeration of diatom population from the microfilm developed.

**Table 3.3.** Two-way ANOVA to evaluate the sub-volume variation in the univariate measures of the microfilm sample obtained from fiberglass substrata. (\*\*\*\*\*  $\leq 0.001$ , \*\*\*\*  $\leq 0.005$ , \*\*\*  $\leq 0.01$ , \*\*  $\leq 0.025$ , \*  $\leq 0.05$ , ns = not significant).

a. Species count													
	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>
A(Tools)	1	0.00	0.00	0 <sup>ns</sup>	2.70	2.70	0.16 <sup>ns</sup>	73.63	73.6	7.21**	38.53	38.53	35.6*****
B(Sub-volumes)	2	84.87	42.43	4.15*	67.40	33.70	1.94 <sup>ns</sup>	39.27	19.6	1.92 <sup>ns</sup>	4.87	2.43	2.2 <sup>ns</sup>
A*B (interaction)	2	13.40	6.70	0.66 <sup>ns</sup>	4.20	2.10	0.12 <sup>ns</sup>	4.07	2.0	0.20 <sup>ns</sup>	0.07	0.03	0.03 <sup>ns</sup>
within subgroup error	24	245.2	10.22		416.0	17.33		245.2	10.2		26.00	1.08	
Total	29	343.5			490.3			362.2			69.47		

b. Diversity													
	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>
A(Tools)	1	0.17	0.17	6.65**	0.09	0.09	0.70 <sup>ns</sup>	0.03	0.03	0.27 <sup>ns</sup>	0.30	0.30	9.13***
B(Sub-volumes)	2	0.38	0.19	7.38*****	0.10	0.05	0.42 <sup>ns</sup>	0.02	0.01	0.07 <sup>ns</sup>	0.00	0.00	0.03 <sup>ns</sup>
A*B (interaction)	2	0.00	0.00	0.05 <sup>ns</sup>	0.03	0.02	0.12 <sup>ns</sup>	0.06	0.03	0.26 <sup>ns</sup>	0.01	0.00	0.11 <sup>ns</sup>
within subgroup error	24	0.61	0.03		3.03	0.13		2.62	0.11		0.79	0.03	
Total	29	1.16			3.25			2.73			1.11		

c. Evenness													
	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>
A(Tools)	1	0.01	0.01	9.59*****	0.00	0.00	0.90 <sup>ns</sup>	0.01	0.01	3.11 <sup>ns</sup>	0.01	0.01	4.13 <sup>ns</sup>
B(Sub-volumes)	2	0.00	0.00	0.43 <sup>ns</sup>	0.00	0.00	0.56 <sup>ns</sup>	0.01	0.00	0.83 <sup>ns</sup>	0.00	0.00	0.06 <sup>ns</sup>
A*B (interaction)	2	0.00	0.00	1.50 <sup>ns</sup>	0.00	0.00	0.48 <sup>ns</sup>	0.00	0.00	0.52 <sup>ns</sup>	0.00	0.00	0.12 <sup>ns</sup>
within subgroup error	24	0.03	0.00		0.06	0.00		0.09	0.00		0.03	0.00	
Total	29	0.04			0.07			0.11			0.04		

d. Species richness													
	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>	SS	MS	F <sub>s</sub>
A(Tools)	1	0.02	0.02	0.08 <sup>ns</sup>	0.05	0.05	0.14 <sup>ns</sup>	0.17	0.17	0.45 <sup>ns</sup>	0.12	0.12	1.80 <sup>ns</sup>
B(Sub-volumes)	2	0.01	0.01	0.02 <sup>ns</sup>	0.45	0.23	0.62 <sup>ns</sup>	0.03	0.01	0.03 <sup>ns</sup>	1.93	0.96	14.4*****
A*B (interaction)	2	0.31	0.15	0.56 <sup>ns</sup>	0.38	0.19	0.52 <sup>ns</sup>	0.03	0.02	0.05 <sup>ns</sup>	0.00	0.00	0.01 <sup>ns</sup>
within subgroup error	24	6.57	0.27		8.79	0.37		9.11	0.38		1.61	0.07	
Total	29	6.91			9.68			9.34			3.66		

### 3.3.1.b. To compare the efficiency of nylon brush and ceramic scraper as tools for removal of diatoms from microfilms

The diatom population obtained from the microfilm developed over fiberglass and glass by using two different tools for microfilm removal revealed that the nylon brush is more efficient than the ceramic scraper in removing diatoms from microfilms (Figs. 3.3 and 3.4).

**Table 3.4.** Two-way ANOVA to evaluate the sub-volume variation in the univariate measures of the microfilm sample obtained from glass substrata. (\*\*\*\*\*  $\leq 0.001$ , \*\*\*\*  $\leq 0.005$ , \*\*\*  $\leq 0.01$ , \*\*  $\leq 0.025$ , \*  $\leq 0.05$ , ns = not significant).

a. Species count

	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$
A(Tools)	1	9.39	9.39	0.44 <sup>ns</sup>	0.50	0.50	0.07 <sup>ns</sup>	26.89	26.89	2.91 <sup>ns</sup>	1.39	1.39	0.30 <sup>ns</sup>
B(Sub-volumes)	2	88.11	44.06	2.09 <sup>ns</sup>	65.33	32.67	4.29*	50.78	25.39	2.75 <sup>ns</sup>	44.78	22.39	4.86**
A*B (interaction)	2	23.44	11.72	0.56 <sup>ns</sup>	1.33	0.67	0.09 <sup>ns</sup>	11.44	5.72	0.62 <sup>ns</sup>	0.78	0.39	0.08 <sup>ns</sup>
within subgroup error	12	253.33	21.11		91.33	7.61		110.67	9.22		55.33	4.61	
Total	17	374.28			158.50			199.78			102.28		

b. Diversity

	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$
A(Tools)	1	0.72	0.72	14.3*****	0.52	0.52	17.3*****	0.06	0.06	1.84 <sup>ns</sup>	0.01	0.01	4.53*
B(Sub-volumes)	2	0.06	0.03	0.56 <sup>ns</sup>	0.07	0.04	1.22 <sup>ns</sup>	0.03	0.01	0.43 <sup>ns</sup>	0.09	0.04	17.6*****
A*B (interaction)	2	0.19	0.09	1.86 <sup>ns</sup>	0.02	0.01	0.32 <sup>ns</sup>	0.04	0.02	0.72 <sup>ns</sup>	0.00	0.00	0.40 <sup>ns</sup>
within subgroup error	12	0.61	0.05		0.37	0.03		0.36	0.03		0.03	0.00	
Total	17	1.57			0.98			0.49			0.13		

c. Evenness

	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$
A(Tools)	1	0.03	0.03	58.6*****	0.02	0.02	16.6*****	0.00	0.00	0.24 <sup>ns</sup>	0.00	0.00	6.60**
B(Sub-volumes)	2	0.00	0.00	5.13**	0.00	0.00	0.30 <sup>ns</sup>	0.01	0.00	1.99 <sup>ns</sup>	0.01	0.00	33.4*****
A*B (interaction)	2	0.00	0.00	2.70 <sup>ns</sup>	0.00	0.00	0.26 <sup>ns</sup>	0.00	0.00	0.48 <sup>ns</sup>	0.00	0.00	0.06ns
within subgroup error	12	0.01	0.00		0.02	0.00		0.02	0.00		0.00	0.00	
Total	17	0.04			0.04			0.02			0.01		

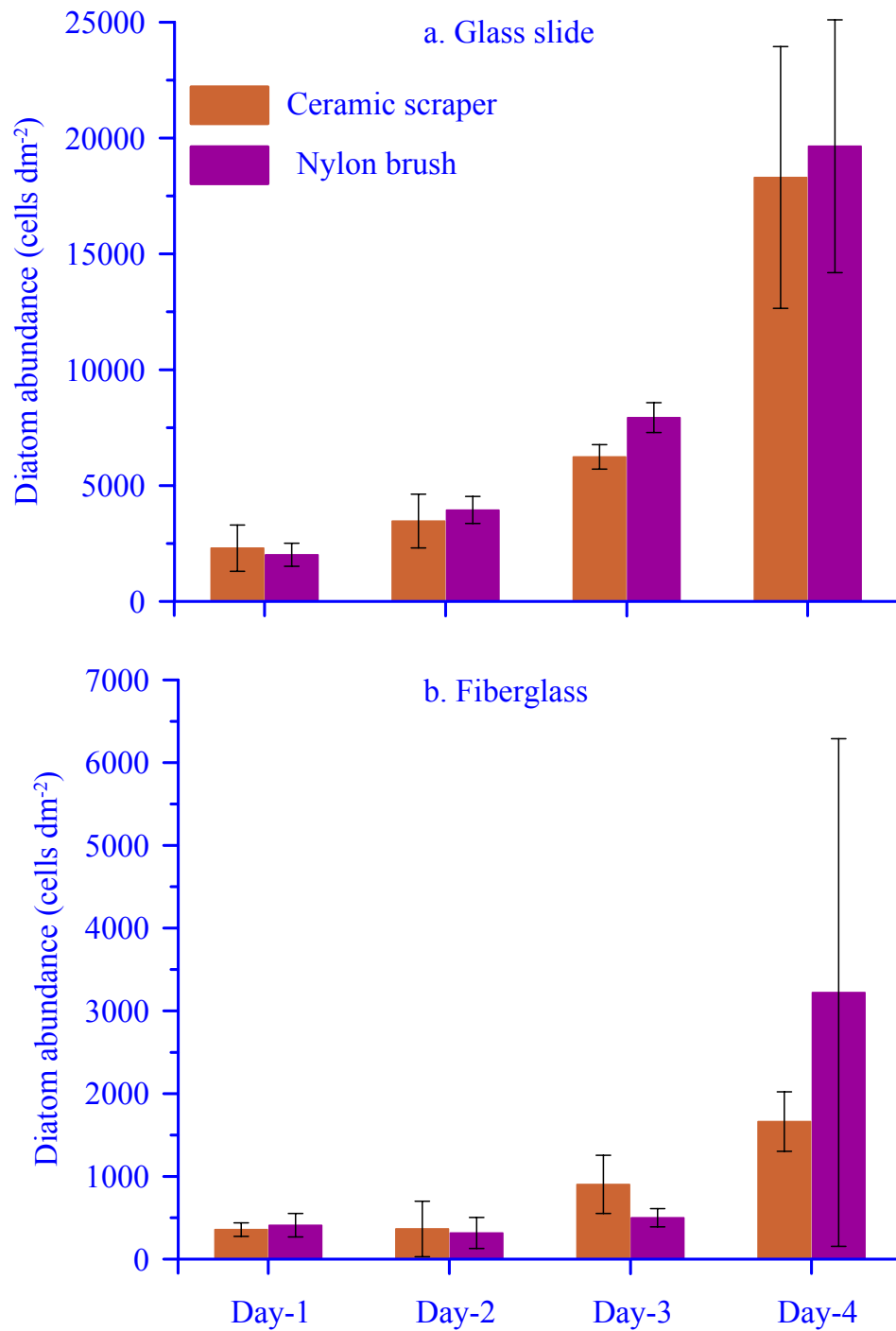
d. Species richness

	df	Day-1			Day-2			Day-3			Day-4		
		SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$	SS	MS	$F_S$
A(Tools)	1	0.11	0.11	0.40 <sup>ns</sup>	0.08	0.08	0.57 <sup>ns</sup>	0.18	0.18	1.04 <sup>ns</sup>	0.00	0.00	0.06 <sup>ns</sup>
B(Sub-volumes)	2	0.71	0.36	1.31 <sup>ns</sup>	0.19	0.09	0.63 <sup>ns</sup>	0.12	0.06	0.36 <sup>ns</sup>	0.20	0.10	1.40 <sup>ns</sup>
A*B (interaction)	2	0.47	0.24	0.87 <sup>ns</sup>	0.04	0.02	0.14 <sup>ns</sup>	0.20	0.10	0.59 <sup>ns</sup>	0.01	0.01	0.09 <sup>ns</sup>
within subgroup error	12	3.28	0.27		1.79	0.15		2.02	0.17		0.86	0.07	
Total	17	4.58			2.11			2.52			1.07		

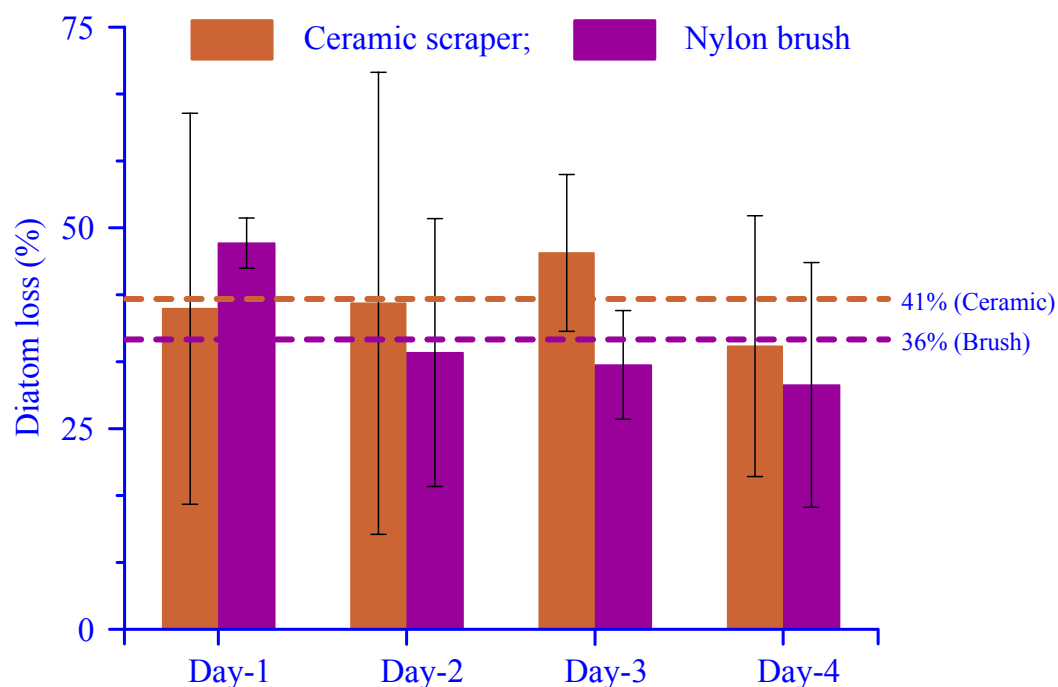
**3.3.1.c. To estimate the loss of diatoms (%) encountered during removal of microfilm**

Only glass slides were used for estimation of diatom (%) loss during removal of microfilm. Glass slides were used because of their smaller dimensions, transparency and ease for carrying out direct observations under the microscope. The results revealed that the maximum loss was encountered with ceramic scraping (41%) than nylon brushing (36%) (Fig. 3.4). In case of nylon brush, the percentage loss decreased with the increase in diatom abundance in the microfilm i.e. increase in exposure period (Fig. 3.4). The results of the univariate measures, presented in table 3.5, revealed that the number of

species decreased during scraping. However the less abundant species were lost during removal of microfilm.



**Fig. 3.3.** Comparison of the efficiency of tools (nylon brush and ceramic scraper) employed for the removal of microfilm diatoms from (a) glass slide and (b) fiberglass.



**Fig. 3.4.** Diatom loss (%) encountered during removing the microfilm developed over glass slide by using different tools (nylon brush and ceramic scraper)

**Table 3.5.** Comparison between the tools (nylon brush-Br and ceramic scraper-Ce) used for removing the microfilm and direct observation of the glass slide (Gl-Sl) with respect to values of univariate measures (species count-S, Species richness -d, evenness-J' and diversity-H').

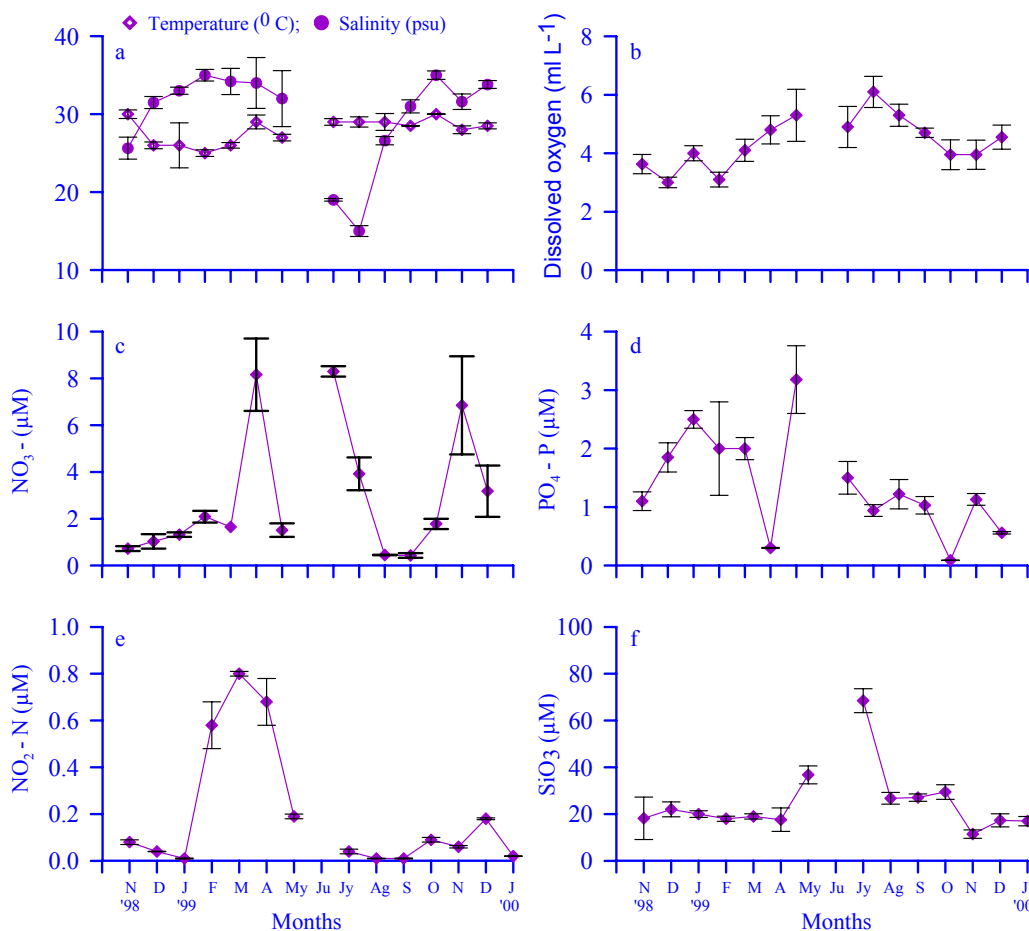
Days	S			d			J'			H'		
	Gl-Sl	Ce	Br	Gl-Sl	Ce	Br	Gl-Sl	Ce	Br	Gl-Sl	Ce	Br
1	41	36	32	4.85	4.57	4.08	0.67	0.70	0.64	3.60	3.63	3.20
2	41	37	33	4.60	4.44	3.87	0.59	0.61	0.56	3.13	3.16	2.81
3	43	38	40	4.45	4.27	4.38	0.61	0.56	0.57	3.32	2.92	3.01
4	46	40	40	4.39	4.02	3.99	0.62	0.60	0.58	3.39	3.17	3.12

### 3.3.2. Experiment 2: Diatoms in water column and 1 to 4 day old microfilms developed over fibreglass, glass, copper and cupro-nickel substrata.

#### 3.3.2.a. Environmental parameters of the surrounding water column

Temporal variation in environmental parameters such as water temperature, salinity, dissolved oxygen and nutrients is shown in Fig. 3.5. Temperature ranged from 25 °C (February) to 30 °C (November) and salinity ranged from 15 psu to 35 psu. Dissolved oxygen ranged from 3.0 to 6.1 ml L<sup>-1</sup>. Nitrate (NO<sub>3</sub>-N) peaks were observed in April, July and December. Nitrite (NO<sub>2</sub>-N) peaked in March and Phosphate (PO<sub>4</sub>-P) in May.

Silicate ( $\text{SiO}_3$ ) peaked in July (Fig. 3.5). The reasons for such variations have been provided in chapter 2 (2.3 Results section).



**Fig. 3.5.** Temporal variation in temperature, salinity, dissolved oxygen and nutrient concentrations of the ambient waters.

### 3.3.2.b. Diatoms in the water column

In the present study, 51 species (28 centrics and 23 pennates) belonging to 34 genera (21 centrics, 15 pennates) have been recorded from the water column (Tables 3.6a and b). The diatom cell numbers ranged from a low of 1 to a high of  $33 \times 10^3$  cells L<sup>-1</sup> (Fig. 3.6a). In September, blooms of *Skeletonema costatum*, *Fragilariopsis oceanica* and *Thalassionema nitzschioides* were observed. Blooms of *Leptocylindrus danicus* and *Cerataulina* sp. were encountered in the post-monsoon (November 1999) period. The



**Table 3.6a.** List of pennate diatom species recorded from the surrounding water column (WC) and microfilm (1 to 4 day-old) developed over different substrata (fiberglass-Fg, glass-Gl, copper-Cu and cupro-nickel-CN during the study.

Sl. No.	Taxon	Abbrev.	WC	Fg	Gl	Cu	CN
1	<i>Achnanthes brevipes</i> Agardh	Ac. br		+	+	+	+
2	<i>A. longipes</i> Agardh	Ah. lo		+	+	+	+
3	<i>A. subsessilis</i> Kützing	Ac. su		+	+		
4	<i>Amphiprora gigantea</i>	Am. gi		+	+		
5	<i>Amphora coffeaeformis</i> (Ag.) Kützing	A. co	+	+	+	+	+
6	<i>A. hyalina</i> Kützing	A. hy		+	+	+	+
7	<i>A. ovalis</i> (Kützing) Kützing	A. ov		+	+		+
8	<i>A. rostrata</i> Wm. Smith	A. ro		+	+	+	+
9	<i>A. turgida</i> Gregory	A. tu		+	+	+	+
10	<i>Asterionellopsis glacialis</i> (Castracane)	As. gl	+				
11	<i>Bacillaria paxillifera</i> (O. F. Müller)	Ba. pa		+		+	+
12	<i>Cocconeis scutellum</i> Ehrenberg	Co. sc	+	+	+	+	+
13	<i>Cocconeis</i> sp.	Co. sp		+	+	+	+
14	<i>Cylindrotheca closterium</i> (Ehrenberg) Smith	Ci. cl	+	+	+	+	+
15	<i>Cymbella gastroides</i> Kützing	Cy. ga	+	+	+	+	+
16	<i>Diploneis smithii</i> (de Brebisson) Wm. Smith	D. sp		+	+	+	+
17	<i>Fragilariopsis oceanica</i> (Cleve) Hasle	F. oc	+	+	+	+	+
18	<i>Grammatophora marina</i> (Lyngbye) Kützing	Gr. ma	+	+	+	+	+
19	<i>G. serpentina</i> Ehrenberg	Gr. se		+	+	+	+
20	<i>Gyrosigma</i> sp.	Gy. sp		+		+	
21	<i>Hantzschia</i> sp.	Hn. sp		+	+	+	+
22	<i>Licmophora flabellata</i> (Greville)Agardh	L. fl		+	+	+	
23	<i>L. gracilis</i> (Ehrenberg) Grunow	L. gr		+	+	+	+
24	<i>L. juergensii</i> Agardh	L. je		+	+	+	
25	<i>L. paradoxa</i> (Lyngbye) Agardh	L. pa	+	+	+	+	+
26	<i>Meuniera membranacea</i> (Cleve) P. C. Silva comb. nov	Me. me		+	+	+	
27	<i>Navicula clavicularis</i>	N. cl		+	+		+
28	<i>N. crucicula</i> (Wm. Smith) Donkin	N. cr		+	+	+	+
29	<i>N. granulata</i>	N. gr		+	+	+	+
30	<i>N. subinflata</i> Grunow	N. su	+	+	+	+	+
31	<i>N. transitans</i> var. <i>derasa</i> f. <i>delicatula</i> Heimdal	N. de	+	+	+	+	+
32	<i>Navicula</i> sp. 1	N. sp1	+	+	+	+	+
33	<i>Navicula</i> sp. 2	N. sp2		+	+	+	
34	<i>Navicula</i> sp. 3	N. sp3		+	+	+	+
35	<i>Navicula</i> sp. 4	N. sp4		+	+	+	+
36	<i>Nitzschia acicularis</i>	Ni. ac		+	+		
37	<i>N. angularis</i> Wm. Smith	Ni. an	+	+	+	+	+
38	<i>N. bilobata</i> Wm. Smith	Ni. bi	+	+	+	+	+
39	<i>N. longissima</i> Ralfs in Pritchard.	Ni. lo	+	+	+	+	+
40	<i>N. panduriformis</i> Gregory	Ni. pa	+	+	+	+	+
41	<i>N. sigma</i> (Kützing)Wm.smith	Ni. si	+	+	+	+	+
42	<i>Pinnularia rectangulata</i> (Gregory) Rabenhorst	Pi. sp		+	+		
43	<i>Pleurosigma angulatum</i> Sensu W. Smith	Pl. an	+	+	+	+	+
44	<i>P. elongatum</i> WM Smith	Pl. el	+	+	+	+	+
45	<i>Pleurosigma</i> sp.	Pl. sp		+		+	
46	<i>Pseudo-nitzschia seriata</i> (Cleve) H. Pergallo	Pn. se	+	+	+	+	+
47	<i>Pseudo-nitzschia</i> sp.	Pn. sp		+	+		
48	<i>Rhabdonema</i> sp.	Rd. sp		+			
49	<i>Stauroneis</i> sp.	St. sp		+			
50	<i>Surirella ovalis</i>	Su. ov	+	+	+	+	
51	<i>Surirella</i> sp.	Su. sp	+	+	+		
52	<i>Synedra affinis</i> Kützing	Sy. af		+	+	+	+
53	<i>S. gallioni</i> (Bory) Ehrenberg	Sy. ga	+	+	+	+	+
54	<i>Synedra</i> sp.	Sy. sp		+	+		
55	<i>Tabellaria</i> sp.	Tb. sp		+			
56	<i>Thalassionema frauenfeldii</i> (Grunow) Hallegraeff	Th. fr		+	+	+	+
57	<i>T. nitzschiioides</i> (Grunow) Mereschowsky	Th. nit	+	+	+	+	+
58	<i>Thalassiothrix longissima</i> Cleve and Grunow	Th. lo		+	+	+	+

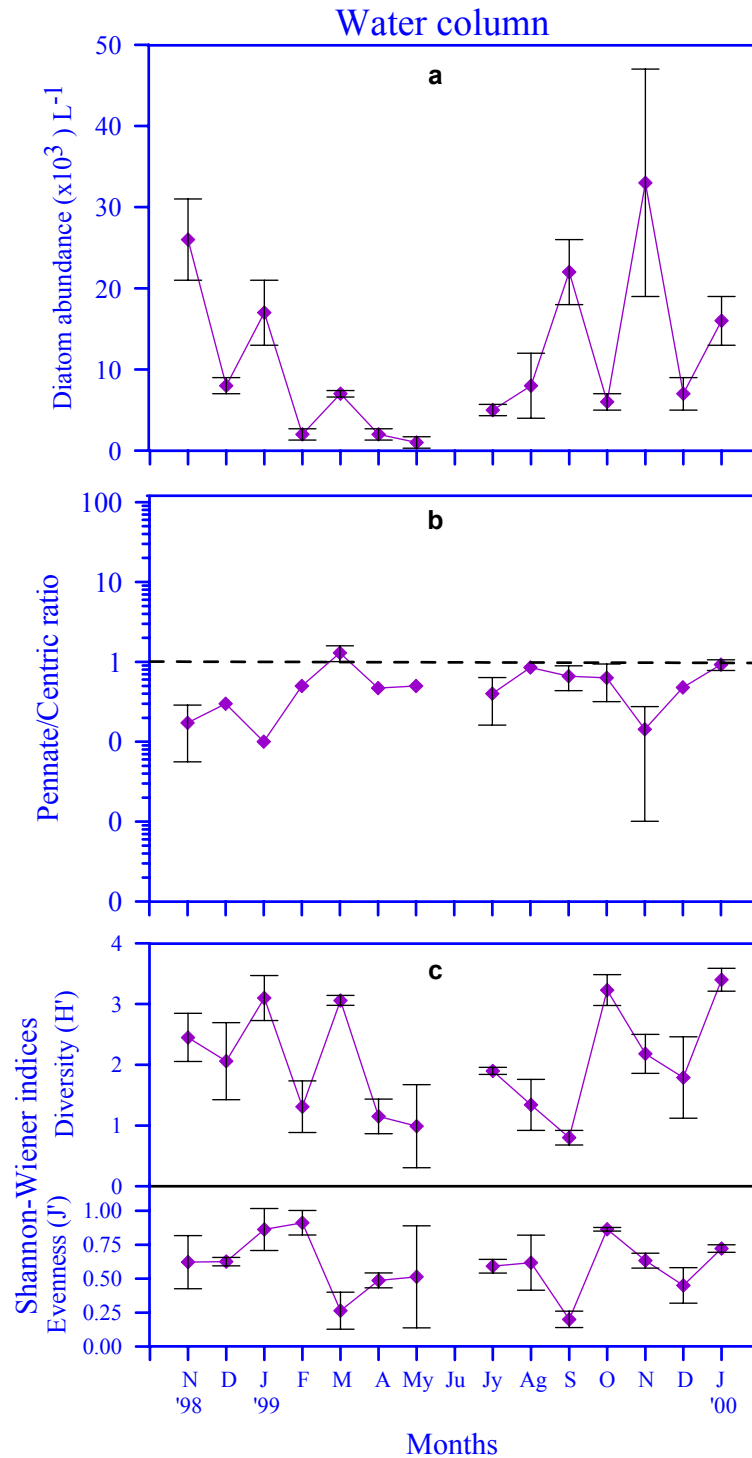
**Table 3.6b.** List of centric diatom species recorded from the surrounding water column (WC) and microfilm (1 to 4 day-old) developed over different substrata (fiberglass-Fg, glass-Gl, copper-Cu and cupro-nickel-CN during the study.

Sl. No.	Taxon	Abbrev.	WC	Fg	Gl	Cu	CN
1	<i>Asteromphalus</i> sp.	Ast. ma		+			
2	<i>Bacteriastrum hyalinum</i> Lauder	Ba. hy	+				
3	<i>Biddulphia pulchella</i> Gray	Bd. pu		+	+	+	
4	<i>B. rhombus</i> (Ehrenberg) Smith, W.	Bd. rh		+	+	+	+
5	<i>Cerataulina pelagica</i> (Cleve) Hendy	Ce. pe	+				
6	<i>Chaetoceros curvisetus</i> Cleve	Ch. cu	+	+	+	+	+
7	<i>C. diversus</i> Ehrenberg	Ch. dv	+				
8	<i>C. lorenzianus</i> Grunow	Ch. lr	+				
9	<i>Climacodium frauenfeldianum</i> Grunow	Cl. fr	+				
10	<i>Corethron criophilum</i> Castracane	Cr. cr	+				
11	<i>Coscinodiscus marginatus</i> Ehrenberg	C. ma	+	+	+	+	+
12	<i>C. radiatus</i> Ehrenberg	C. rad		+	+	+	+
13	<i>Coscinodiscus</i> sp.	C. sp	+				
14	<i>Cyclotella</i> sp.	Cyc. sp		+	+	+	+
15	<i>Ditylum brightwellii</i> (West) Grunow	Di. br	+				
16	<i>Eucampia zodiacus</i> Ehrenberg	Eu. zo	+				
17	<i>Guinardia flaccida</i> (Castracane) Pergallo	Gu. fl	+				
18	<i>G. striata</i> (Stolterfoth) Hasle com. Nov	Gu. st	+				
19	<i>Helicotheca tamesis</i> (Shrubsole) Ricard	He. ta	+				
20	<i>Hemiaulus sinensis</i> Greville	He. si	+				
21	<i>Lauderia annulata</i> Cleve	La. an	+				
22	<i>Leptocylindrus danicus</i> Cleve	Lp. da	+	+	+		
23	<i>Melosira nummuloides</i> C.A. Agardh	M. nu	+	+	+	+	+
24	<i>Odontella regia</i> (M. Schultze) Ostenfeld	Od. re	+				
25	<i>O. sinensis</i> (Greville) Grunow	Od. si	+				
26	<i>Paralia sulcata</i> (Ehrenberg) Cleve	Pa. su		+	+		
27	<i>Planktoniella sol</i> (Wallich) Schutt	Pk. so	+	+	+		
28	<i>Proboscia alata</i> (Brightwell) Sundstrom	Pr. al	+	+			
29	<i>Rhizosolenia stolterfothii</i> H. Peragallo	Ri. st	+	+			
30	<i>Skeletonema costatum</i> Greville (Cleve)	Sk. co	+			+	+
31	<i>Striatella</i> sp.	St. sp	+				
32	<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve	Th. ec	+	+	+	+	+
33	<i>T. subtilis</i> (Ostenfeld) Gran	Th. sb	+				
34	<i>Thalassiosira</i> sp.	Th. sp	+				

percentage ratio of pennate to centric diatoms remained below one during most of the months, indicating the dominance of centric diatoms (Fig. 3.6b).

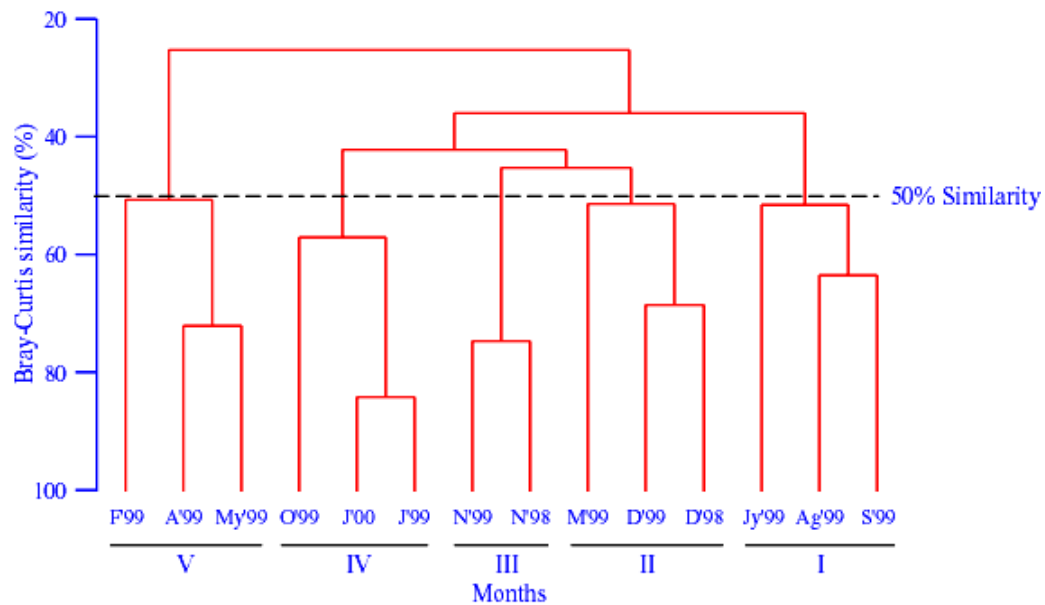
Diatom species diversity was comparatively higher during the post monsoon period. The lowest species diversity was encountered in September (0.8), which was due to blooms of *S. costatum*, *F. oceanica* and *T. nitzschioides* (Fig. 3.6c).

Month-wise clustering of the diatom population at 50% Bray–Curtis similarity level revealed five groups (Fig. 3.7a). Group I represented the monsoon months (July–September). These months are characterized by the presence of *S. costatum*, *F. oceanica* and *T. nitzschioides* in high numbers. The groups II to IV represented the post-monsoon season (October–January) except March. An interesting feature is that all the post-



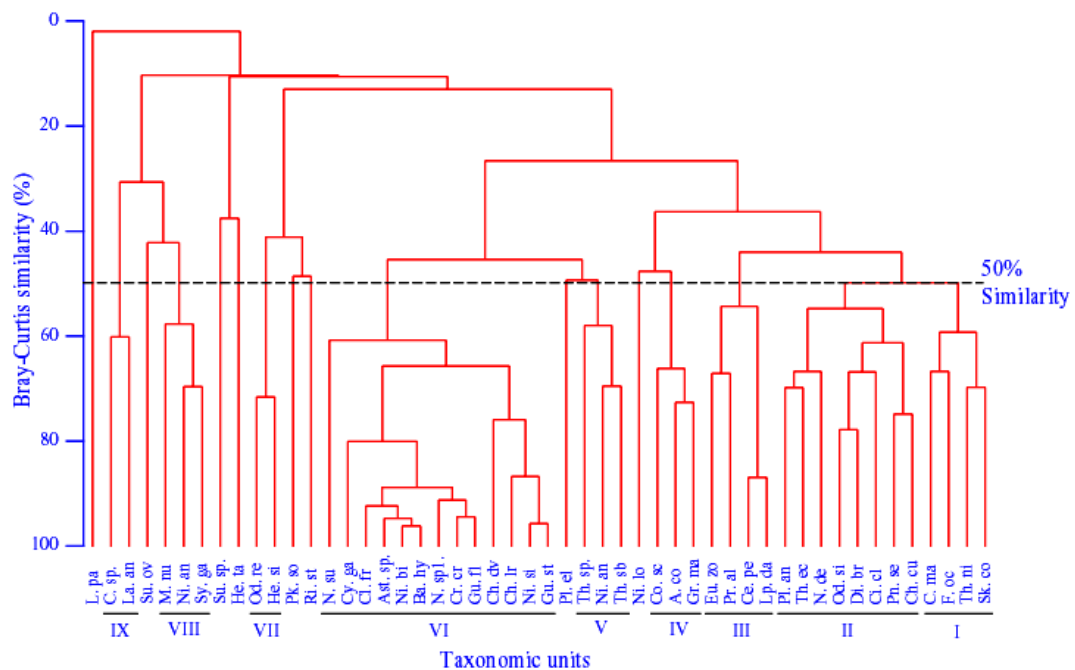
**Fig. 3.6.** Temporal variation in (a) diatom abundance; (b) ratio of pennate /centric diatoms; (c) diatom diversity and evenness in the ambient waters.

monsoon months except October grouped together, indicating repetitive events. Group V comprised of pre-monsoon months (February-May) except March, which are characterized by low diatom population.



**Fig. 3.7a.** Dendrogram of the diatom community from the ambient waters with respect to months.

Cluster analysis at 50% similarity level divided 50 species into 9 clusters with 8 ungrouped individuals (Fig. 3.7b). Among them, 16 species belonging to clusters I to III were dominant. Clusters I to III comprised of 4, 8 and 4 species respectively. Clusters I to IV comprised *S. costatum*, *T. nitzschioides*, *Cerataulina* sp., *L. danicus*, *F. oceanica*,



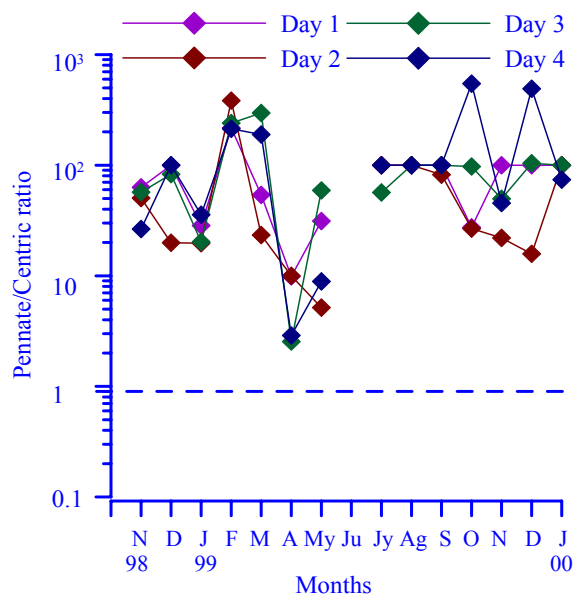
**Fig. 3.7b.** Dendrogram of the diatom community from the ambient waters with respect to species.

*Pseudo-nitzschia seriata*, *Cylindrotheca closterium* and *Chaetoceros curvisetus*, which resulted in occasional blooms in the surrounding water column. *N. transitans* var. *derasa* f. *delicatula* belonging to cluster II was found round the year in the water column and the same was also reflected in the microfilm diatom community. Rest of the species, depending upon their abundance and frequency of occurrence, clustered together and are considered as minor components.

### 3.3.2.c. Diatoms in 1 to 4 day old microfilm

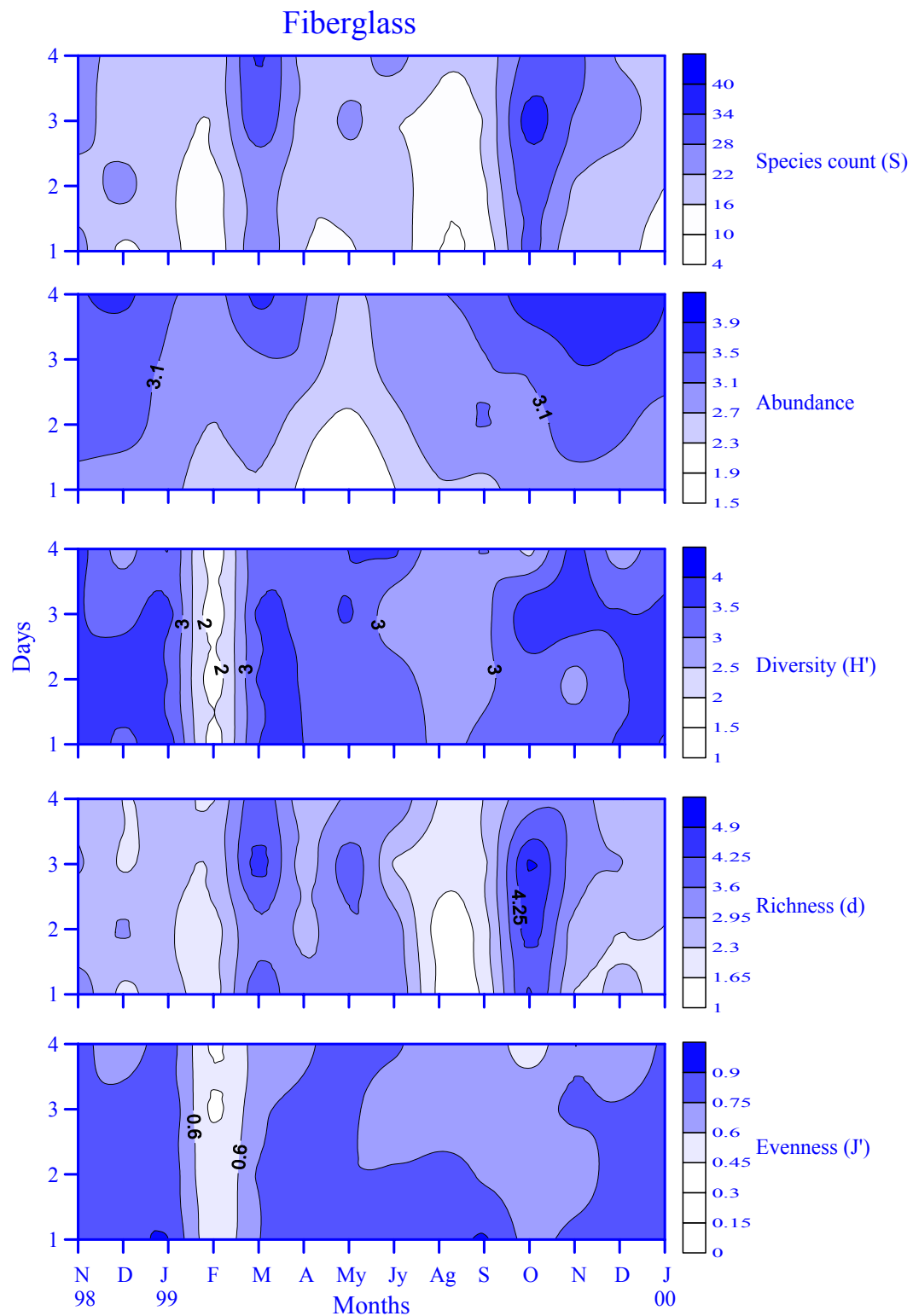
#### 3.3.2.c.1. Fiberglass:

Diatoms encountered on fiberglass substrata from day 1 to 4 are presented in Table 3.6a and b. The fouling population included 71 diatom species (57 pennates and 14 centrics) belonging to 38 genera (26 pennates and 12 centrics). The pennate/centric diatom percentage ratio on this substrata remained above 1 on all occasions indicating the dominance of pennate diatoms (Fig. 3.8).



**Fig. 3.8.** The ratio of pennate /centric diatoms in the microfilm developed over fiberglass substrata.

The temporal variation in the fouling diatom abundance and univariate measures from fiberglass is shown in Fig. 3.9. The diatom abundance on fiberglass increased with

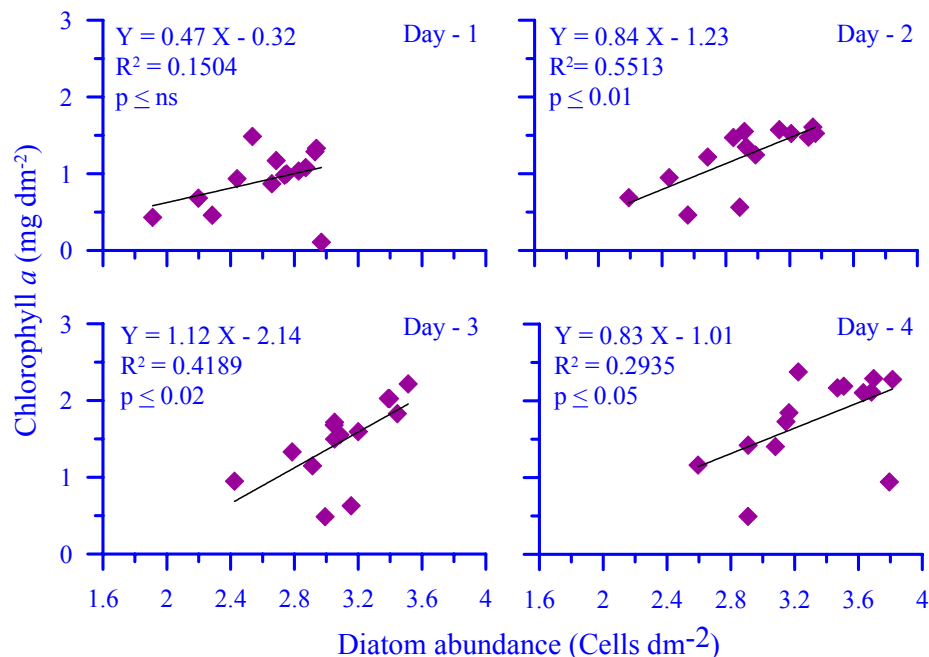


**Fig. 3.9.** Temporal variation in the univariate measures of the fouling diatom population on fiberglass substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

exposure period. On day 4 diatom abundance was maximum during December 98, Mar 99, Oct 99 to Jan 00. Maximum diatom abundance was observed in December during

both the years (98 and 99). Minimum diatom abundance was observed during late pre-monsoon (April 99) and early monsoon (July 99) season. Diatom abundance was least during May 99 from day 1 to 4. The species count increased with exposure period during March, October and November 1999 indicating new recruitment. During the rest of the period, the species count remained almost constant indicating no new recruits. Diversity and species richness was minimum during February 99 for all experimental days followed by late monsoon (August and September 1999). During October and December (98 and 99), diversity decreased on day 4 indicating dominance of *N. transitans* var. *derasa* f. *delicatula*.

Linear regression analysis between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship for 2 day ( $P \leq 0.01$ ), 3 day ( $P \leq 0.02$ ) and 4 day ( $P \leq 0.05$ ) old diatom communities (Fig. 3.10).



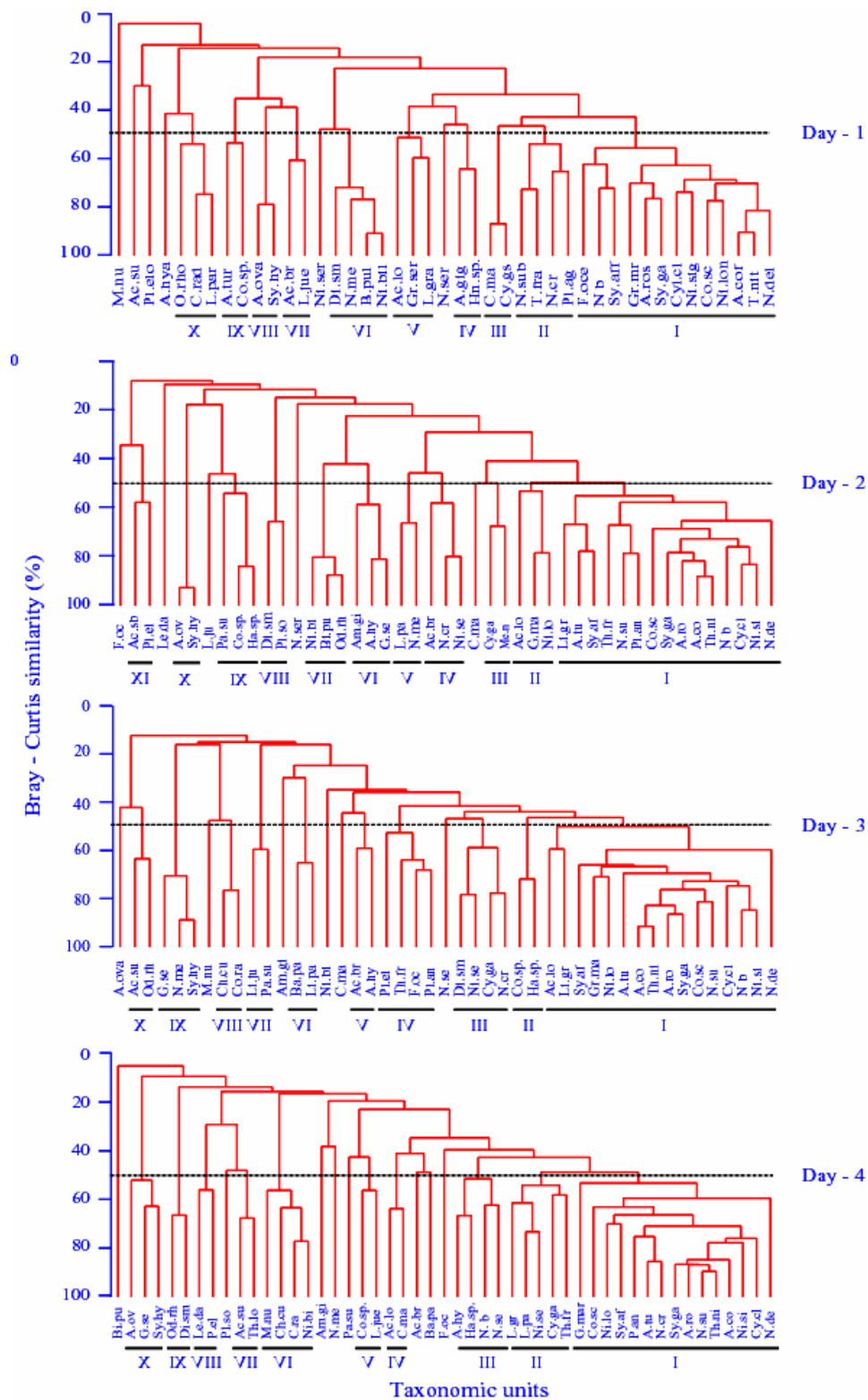
**Fig. 3.10.** Linear regression analysis between diatom abundance and chlorophyll *a* concentration on fiberglass substratum for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

Cluster analysis at 50% similarity level divided 43 species into 10 clusters with 6 ungrouped individuals (day 1), 45 species into 11 clusters with 5 ungrouped individuals (day 2), 45 species into 10 clusters with 6 ungrouped individuals (day 3) and the 49 species into 10 clusters with 8 ungrouped individuals (day 4) (Fig. 3.11). This indicates that the number of species (>5%) increased with exposure period. For all days, species belonging to cluster I (13 species–day 1, 15 species–day 2, 14 species–day 3 and 15 species–day 4) dominated the respective day old microfilm community. Among the species belonging to cluster I for all days, *N. transitans* var. *derasa* f. *delicatula* was the most dominant followed by *A. coffeaeformis*, *T. nitzschioides*, *C. closterium* and *N. sigma*. The remaining species of the cluster formed the next dominant forms. Among the ungrouped individuals, *F. oceanica* and *M. nummuloides* contributed significantly to the microfilm community. *F. oceanica*, the bloom-forming species in the present study site, contributed significantly to the microfilm during August and November (Table 3.7 a-d), while *M. nummuloides* contributed significantly only during April 99 to 3 and 4 days-old microfilm (Table 3.7a and b) and thus altered the pennate/centric ratio (Fig. 3.8). The rest of the species belonging to other clusters or individual units for all exposure days formed the minor component depending upon their abundance and frequency of occurrence.

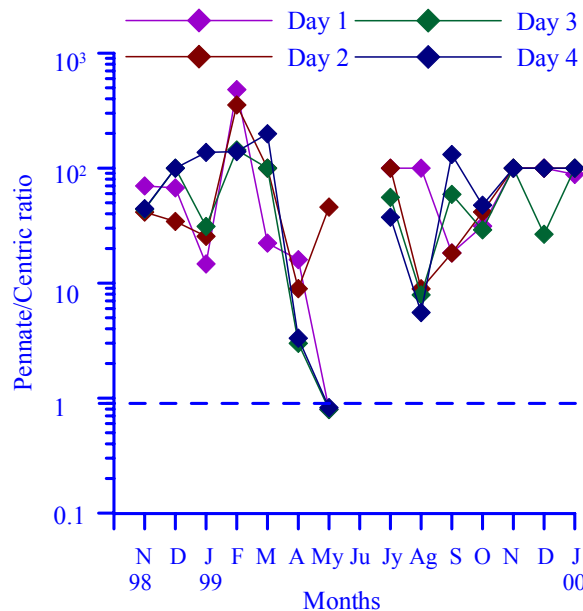
### 3.3.2.c.2. Glass

Diatoms encountered on glass substrata from day 1 to 4 are shown in Table 3.6a and b. The fouling population included 62 diatoms (51 pennates and 11 centrics) belonging to 30 genera (21 pennates and 9 centrics). The pennate/centric diatom percentage ratio, on this substratum, remained above 1 on all occasions, except during May, indicating the dominance of pennate diatoms (Fig. 3.12).





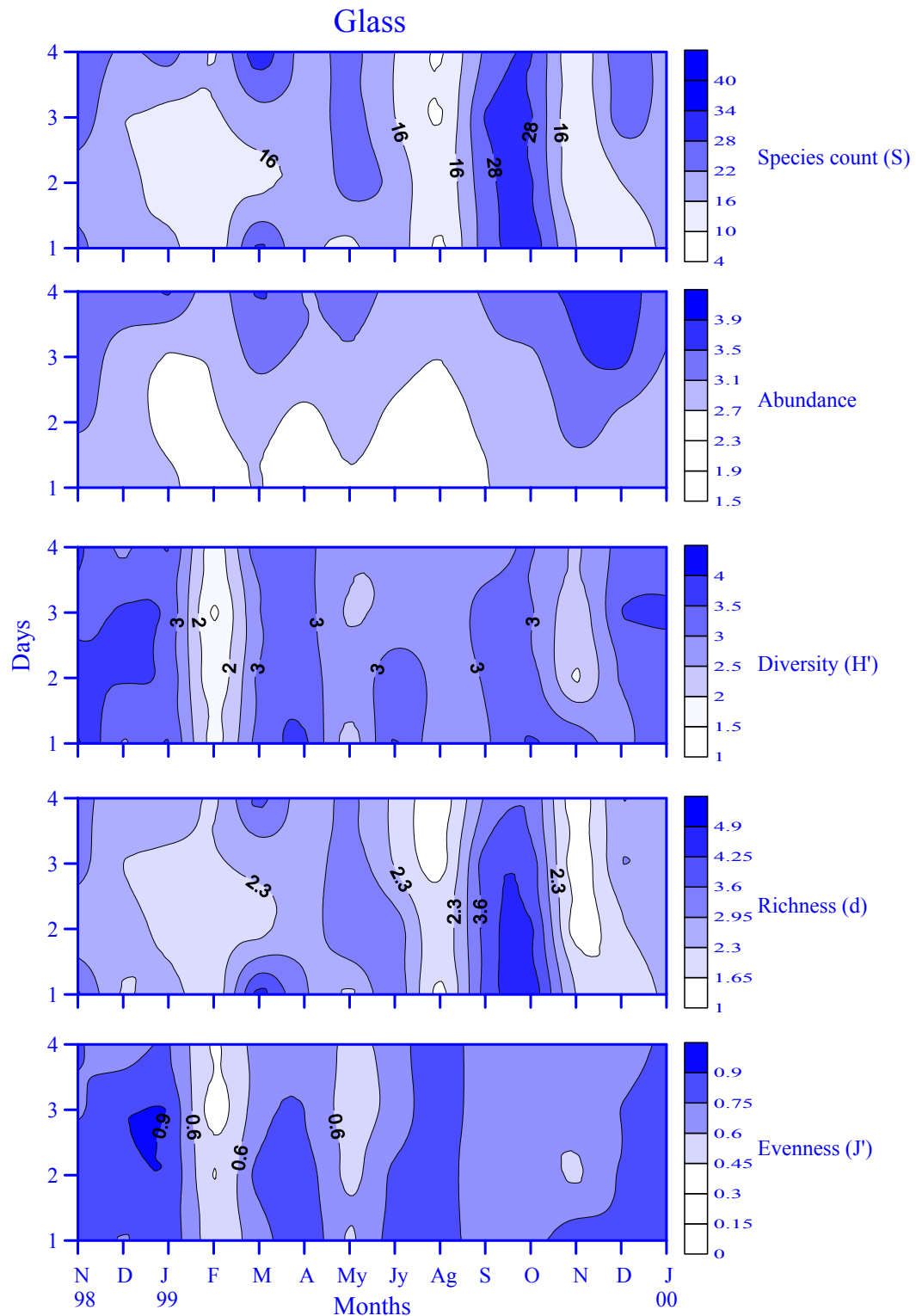
**Fig 3.11.** Dendrograms of the fouling diatom community developed on fiberglass with respect to species for different submersion periods (Day 1, Day 2, Day 3, and Day 4).



**Fig. 3.12.** The ratio of pennate / centric diatoms in the microfilm developed over glass substrata.

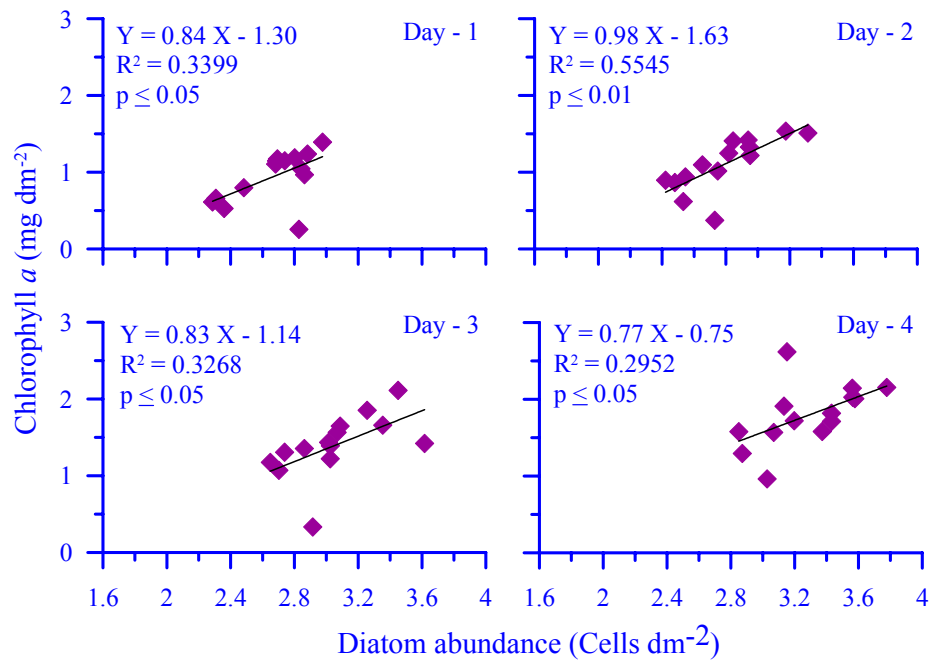
The temporal variation in the fouling diatom abundance and univariate measures from glass is shown in Fig. 3.13. Diatom abundance showed increasing trend with exposure period (Fig. 3.13b). Diatom abundance was minimal during February 99, April 99 and the entire monsoon season. Maximum diatom abundance was observed during March and December 99. Maximum species count was observed during October 99; from day 1 to 4, it remained constant indicating no new recruitment (Fig. 3.13a). Species count was minimal during August. Diversity remained almost constant with exposure period. Minimum diversity, richness and evenness were observed during February 99 (from day 1 to 4). Diatom richness was maximum on day 1 and decreased with exposure period during March, September and October 99.

Linear regression analysis between the total diatom abundance and chlorophyll *a* concentration showed a significant relationship in case of glass for 1 day ( $P \leq 0.05$ ), 2 day ( $P \leq 0.01$ ), 3 day ( $P \leq 0.05$ ) and 4 day ( $P \leq 0.05$ ) old diatom communities (Fig. 3.14).



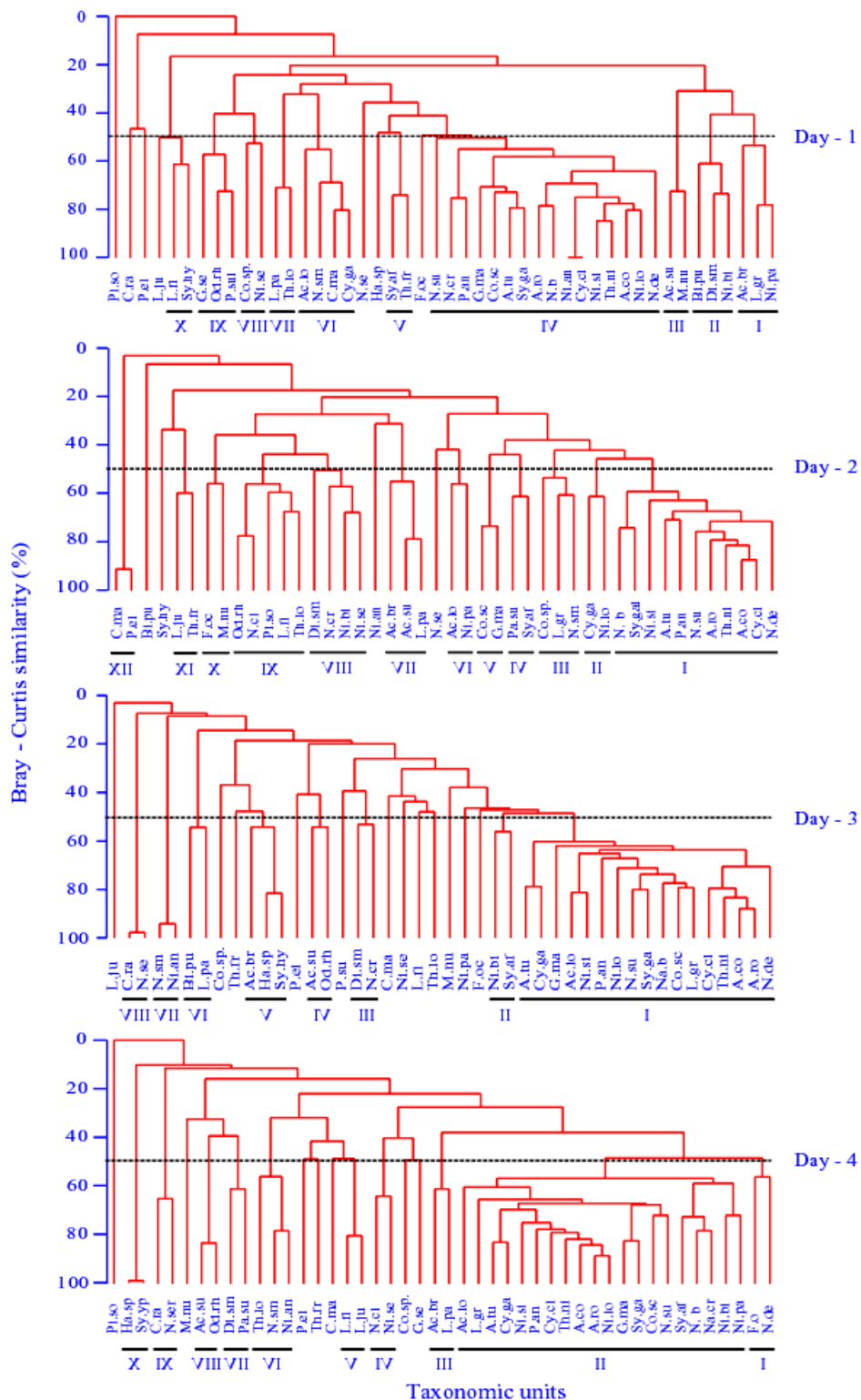
**Fig. 3.13.** Temporal variation in the univariate measures of the fouling diatom population on glass substrata for different submersion periods (Day 1; Day 2; Day 3 and (Day 4)

Cluster analysis at 50% similarity level divided the 46 species into 10 clusters with 7 ungrouped individuals (day 1), 44 species into 12 clusters with 4 ungrouped individuals



**Fig. 3.14.** Linear regression analysis between diatom abundance and chlorophyll *a* concentration on glass substratum for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

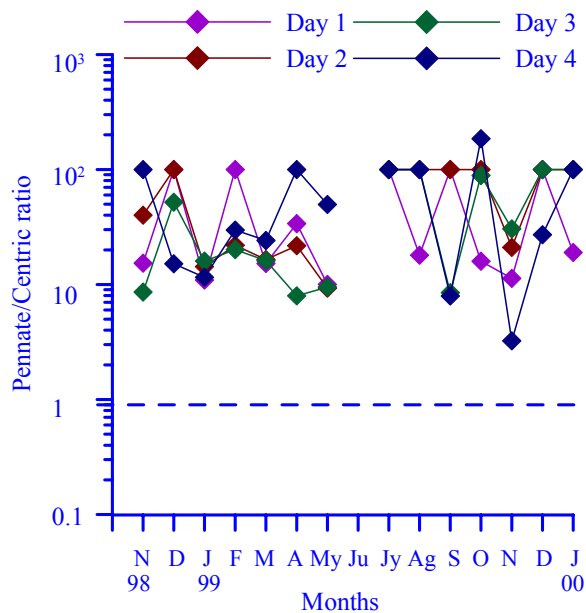
(day 2), 44 species into 8 clusters with 12 ungrouped individuals (day 3) and the 46 species into 10 clusters with 7 ungrouped individuals (day 4) (Fig. 3.15). This also indicates that the number of species (>5%) increased with exposure period. For all days, species belonging to cluster III for day I (16 species) cluster I of other days (15 species-day 2, 17 species-day 3 and 2 species-day 4) and cluster II of day 4 (20 species) dominated the respective day old microfilm community. Among the species belonging to these clusters, similar to fiberglass panels, *N. transitans* var. *derasa* f. *delicatula* was the most dominant followed by *A. coffeaeformis*, *A. rostrata*, *T. nitzschioides*, *C. closterium* and *N. sigma*. The remaining species of the cluster formed the next dominant forms. Among the ungrouped individuals, *F. oceanica* and *M. nummuloides* contributed significantly to the microfilm community. *F. oceanica* contributed significantly to the microfilm during August and November (Table 3.7a-d), while *M. nummuloides* contributed significantly during May 99 on all days (Table 3.7a-d) and thus altered the pennate/centric ratio to a centric dominant one (Fig. 3.12). The rest of the clusters or individual units for all exposure days formed the minor component.



**Fig 3.15.** Dendrograms of the fouling diatom community developed on glass with respect to species for different submersion periods (Day 1, Day 2, Day 3, and Day 4).

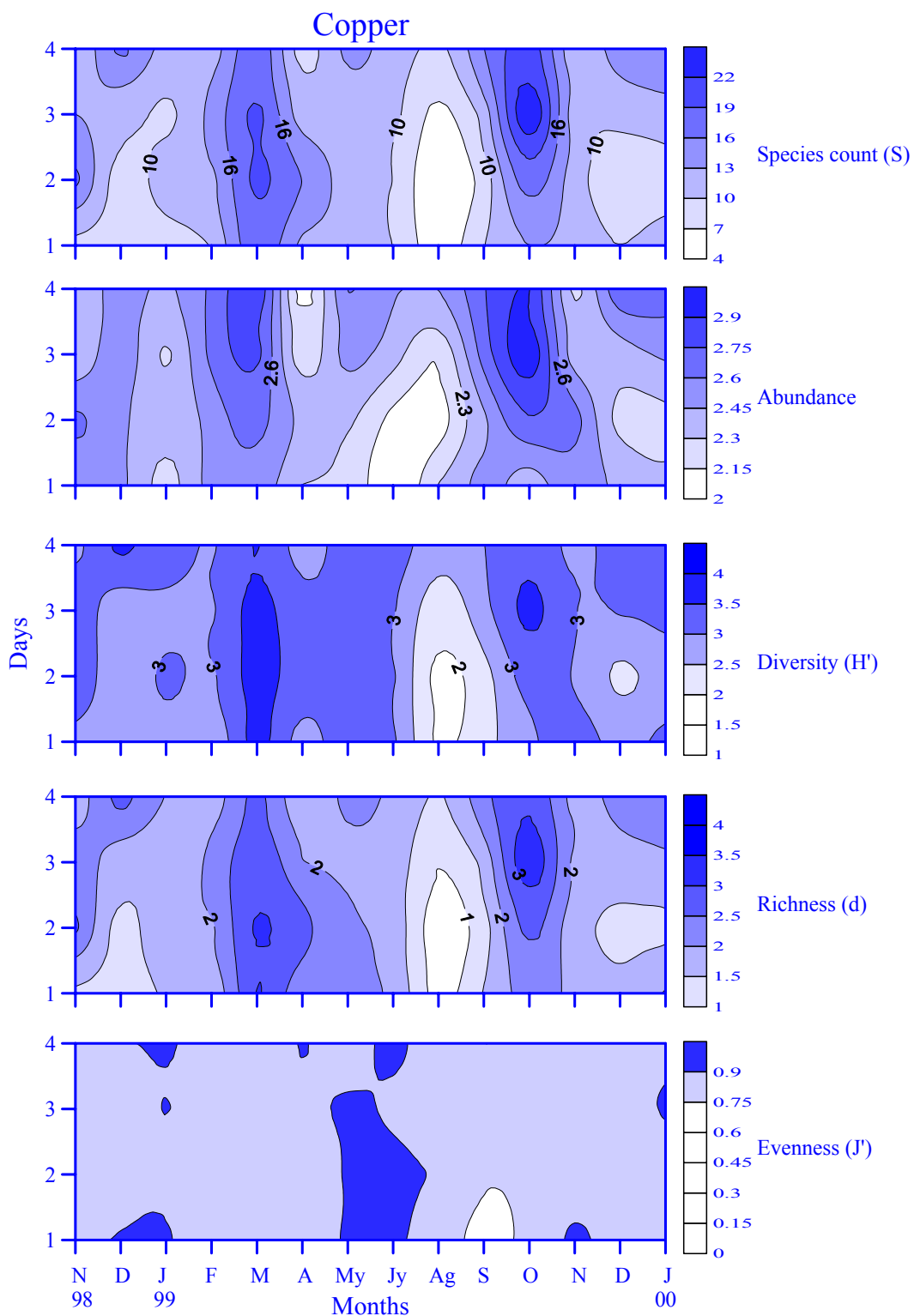
### 3.3.2.c.3. Copper

Diatoms encountered on copper substrata from day 1 to 4 are presented in Table 3.6a and b. The fouling population included 54 diatom species (45 pennates and 9 centrics) belonging to 28 genera (21 pennates and 7 centrics). The pennate/centric diatom percentage ratio on this substratum remained above 1 on all occasions indicating the dominance of pennate diatoms (Fig. 3.16).



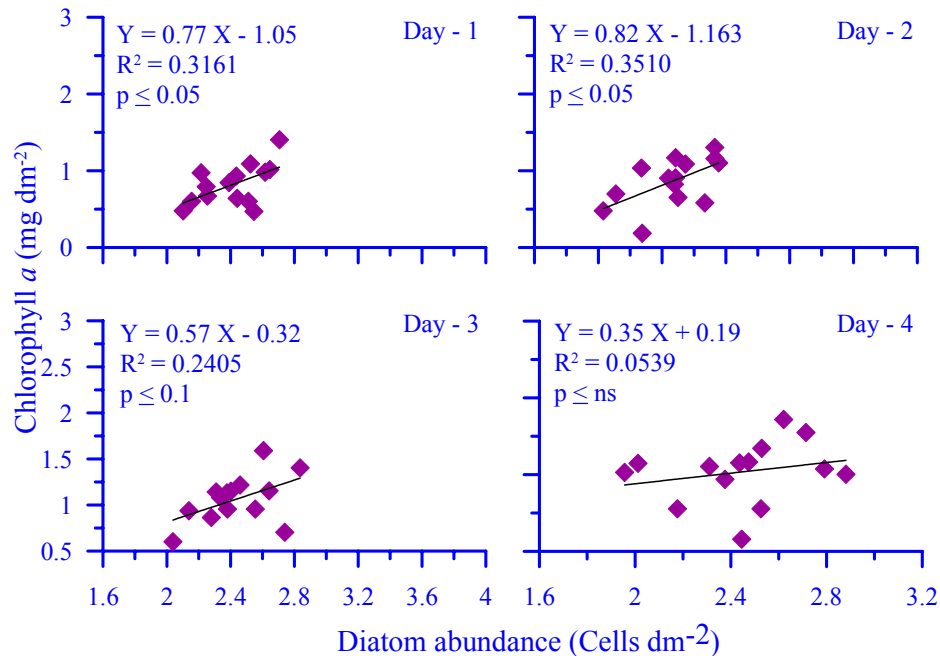
**Fig. 3.16.** The ratio of pennate/centric diatoms in the microfilm developed over copper substrata.

The temporal variation in the fouling diatom abundance and univariate measures for copper is shown in Fig. 3.17. Diatom abundance and species count were minimum during the monsoon season (July and August 99) whereas the maximum values were observed during March and October 99. During these periods, diatom density increased with exposure while, during the rest of the period, the abundance decreased or remained constant with exposure. Diversity and richness also followed a similar trend. The diatom population was evenly distributed during the study period.



**Fig. 3.17.** Temporal variation in the univariate measures of the fouling diatom population on copper substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

Linear regression analysis between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship in case of the 2 days-old diatom communities (Fig. 3.18).

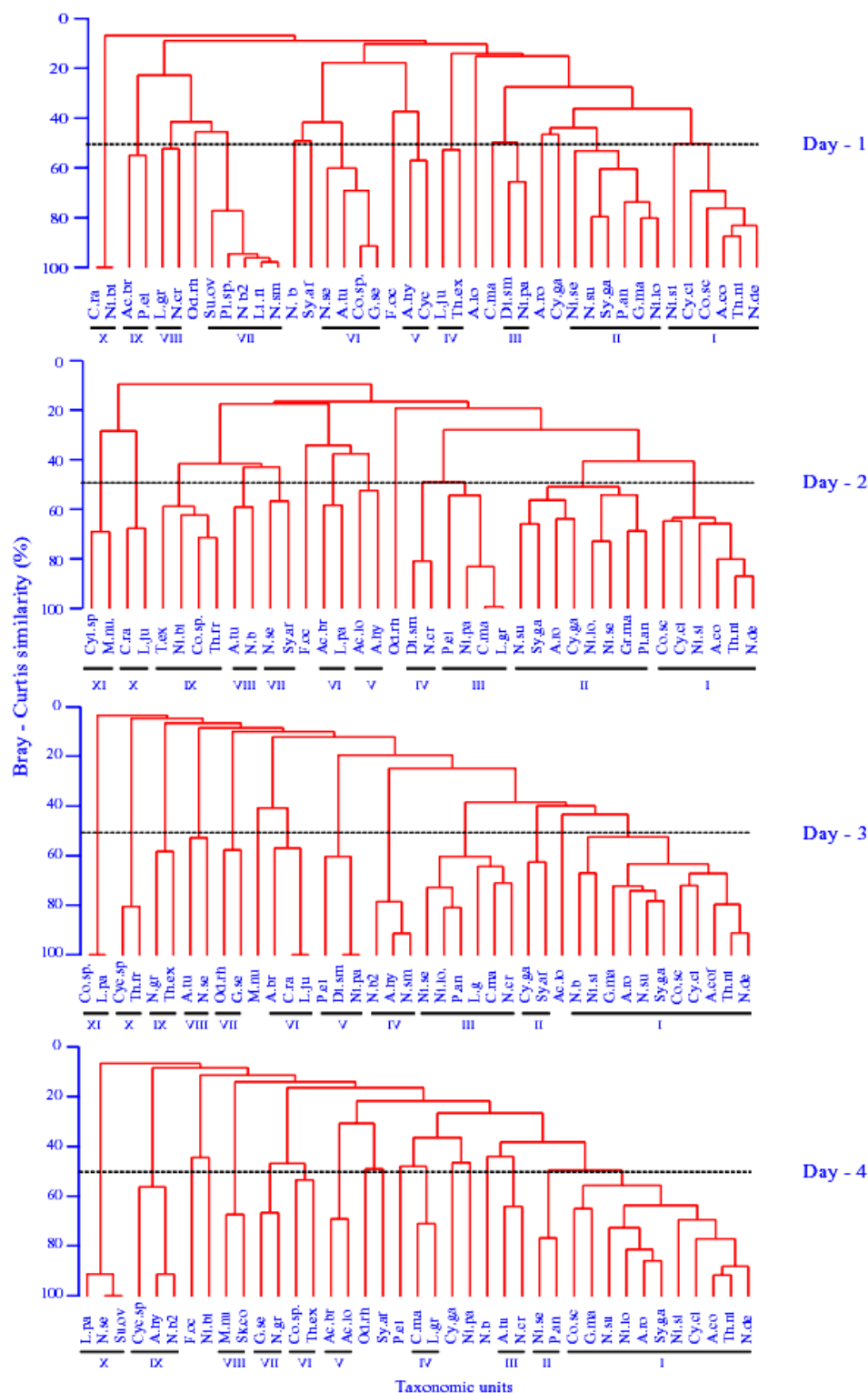


**Fig. 3.18.** Linear regression analysis between diatom abundance and chlorophyll *a* concentration on copper substratum for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

Cluster analysis at 50% similarity level divided the 41 species into 10 clusters with 8 ungrouped individuals (day 1), 38 species into 11 clusters with 2 ungrouped individuals (day 2), 40 species into 11 clusters with 2 ungrouped individuals (day 3) and the 39 species into 10 clusters with 8 ungrouped individuals (day 4) respectively (Fig. 3.19). This indicated that the number of species (>5%) remained constant with exposure period. For all days, species belonging to cluster I (6 species - day 1 and 2, 11 species - day 3 and 4) dominated the respective day old microfilm community. Among the species belonging to these clusters, *N. transitans* var. *derasa* f. *delicatula* was the most dominant followed by *A. coffeaeformis*, *A. rostrata*, *T. nitzschioides*, *Cylindrotheca closterium*, *Cocconeis scutellum*, *G. marina* and *N. sigma*. The remaining species of the cluster



formed the next dominant forms. Among the ungrouped individuals, *F. oceanica* contributed significantly to the microfilm community [about 20% during monsoon for

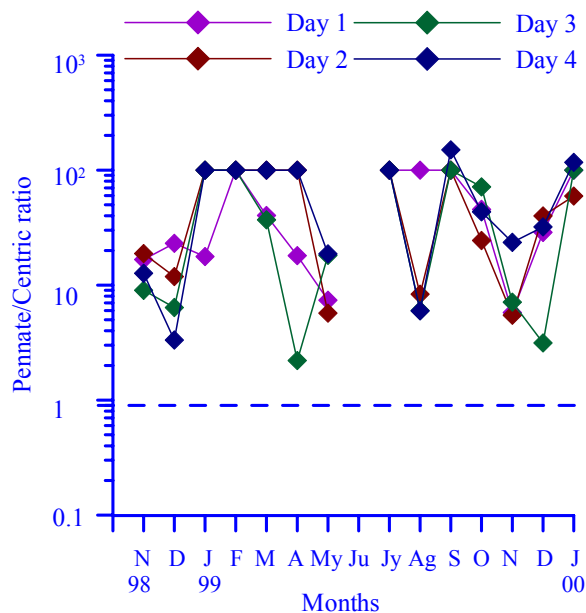


**Fig 3.19.** Dendrograms of the fouling diatom community developed on copper with respect to species for different submersion periods (Day 1, Day 2, Day 3, and Day 4).

day1 and 2 (Table 3.7a-b)] whereas the rest of the clusters or individual units for all exposure days formed the minor component.

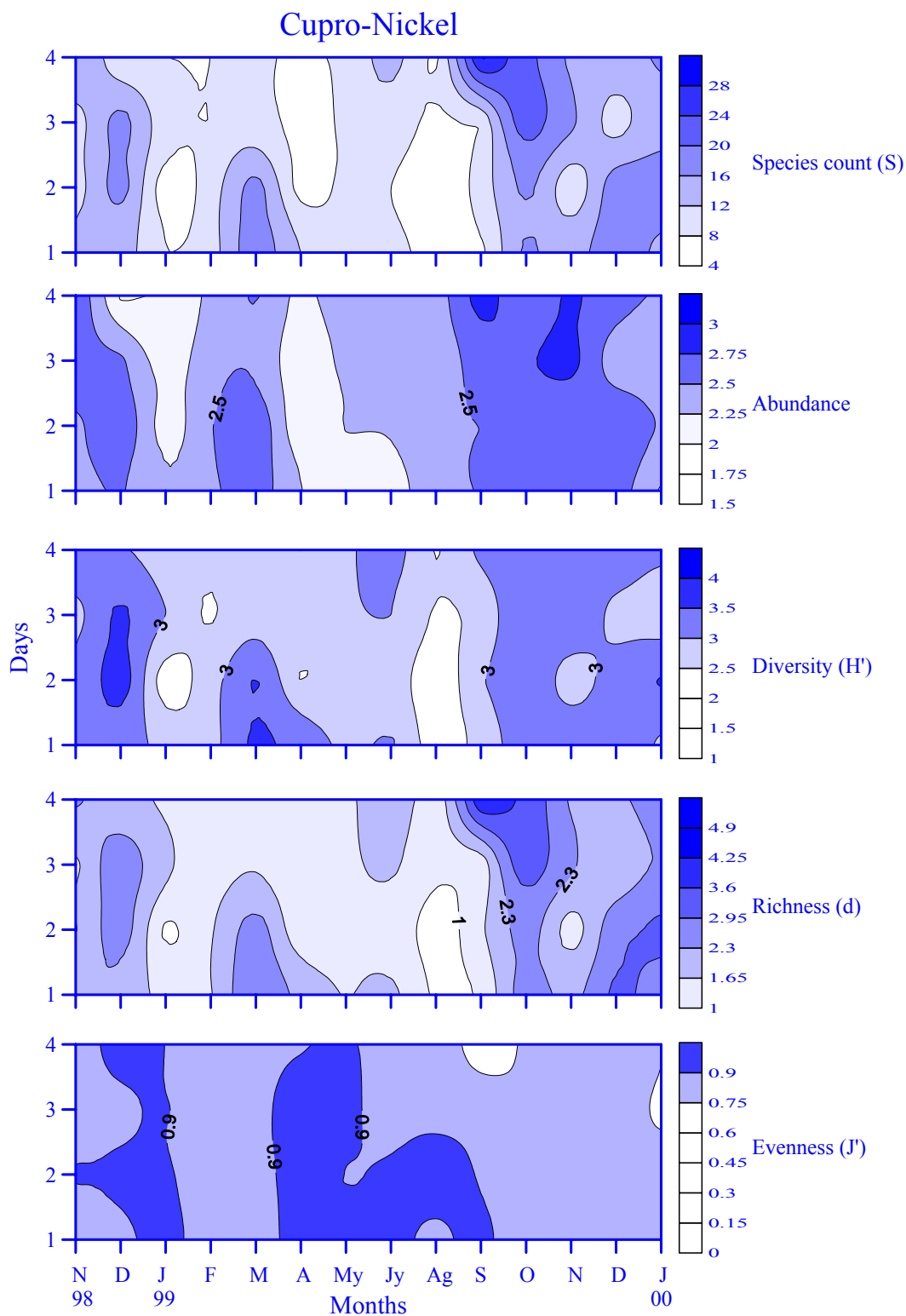
### 3.3.2.c.4. Cupro-nickel

Diatoms encountered on cupro-nickel substrata from day 1 to 4 are presented in Table 3.6a and b. The fouling population included 48 diatom species (40 pennates and 8 centrics) belonging to 25 genera (18 pennates and 7 centrics). The pennate/centric diatom percentage ratio on this substrata remained above 1 on all occasions indicating the dominance of pennate diatoms (Fig. 3.20).



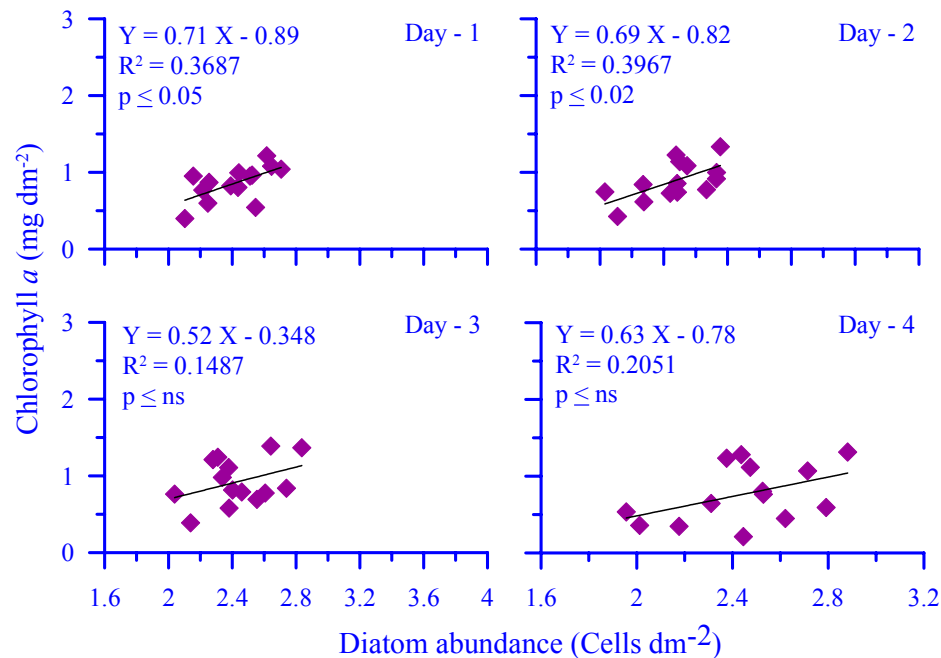
**Fig. 3.20.** The ratio of pennate/centric diatoms in the microfilm developed over cupro-nickel substrata

The temporal variation in the fouling diatom abundance and univariate measures from cupro-nickel is shown in Fig. 3.21. Minimum diatom abundance was observed during May and early monsoon. Diatom abundance almost remained constant with exposure period. Diversity and richness followed a similar pattern. Species count increased with exposure during September and October 99 while, during the rest of the period, species count decreased with increase in exposure. Diatom evenness remained constant with exposure period indicating that the diatoms are evenly distributed.



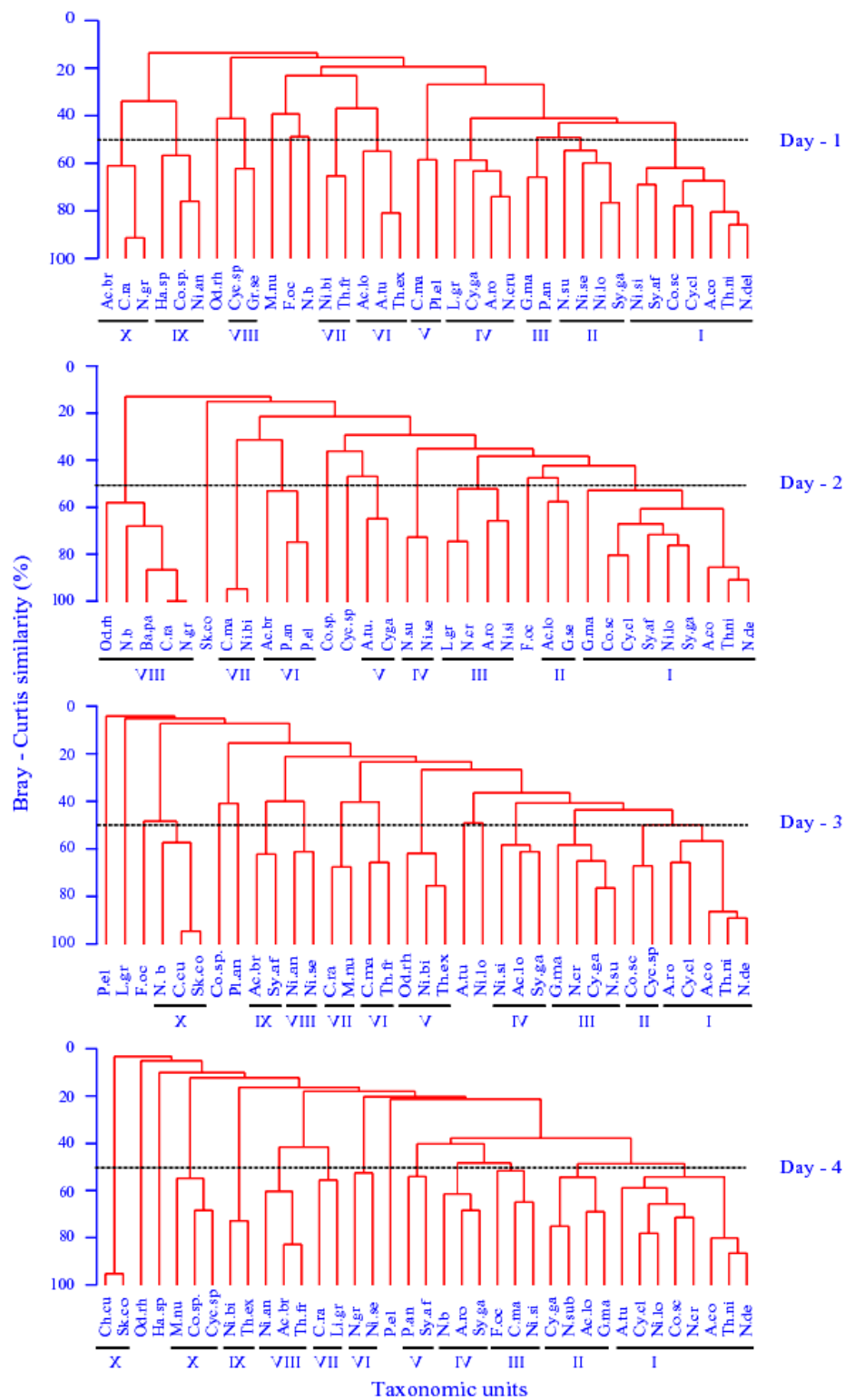
**Fig. 3.21.** Temporal variation in the univariate measures of the fouling diatom population on cupro-nickel substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

Linear regression analysis between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship only for 1 and 2 days-old diatom communities (Fig. 3.22).



**Fig. 3.22** Linear regression analysis between diatom abundance and chlorophyll *a* concentration on cupro-nickel substratum for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

Cluster analysis at 50% similarity level divided the 36 species into 10 clusters with 4 ungrouped individuals (day 1), 33 species into 8 clusters with 4 ungrouped individuals (day 2), 35 species into 10 clusters with 7 ungrouped individuals (day 3) and the 37 species into 10 clusters with 3 ungrouped individuals (day 4) (Fig. 3.23). This also indicates that the number of species (>5%) decreased with exposure period. For all days, species belonging to cluster I (7 species - day 1, 9 species - day 2, 5 species - day 3 and 8 species - day 4) dominated the respective day old microfilm community. Among the species belonging to these clusters, similar to that of copper substratum, *N. transitans* var. *derasa* f. *delicatula* was the most dominant followed by *A. coffeaeformis*, *A. rostrata*, *T. nitzschioides*, *Cylindrotheca closterium*, *Cocconeis scutellum*, *G. marina* and

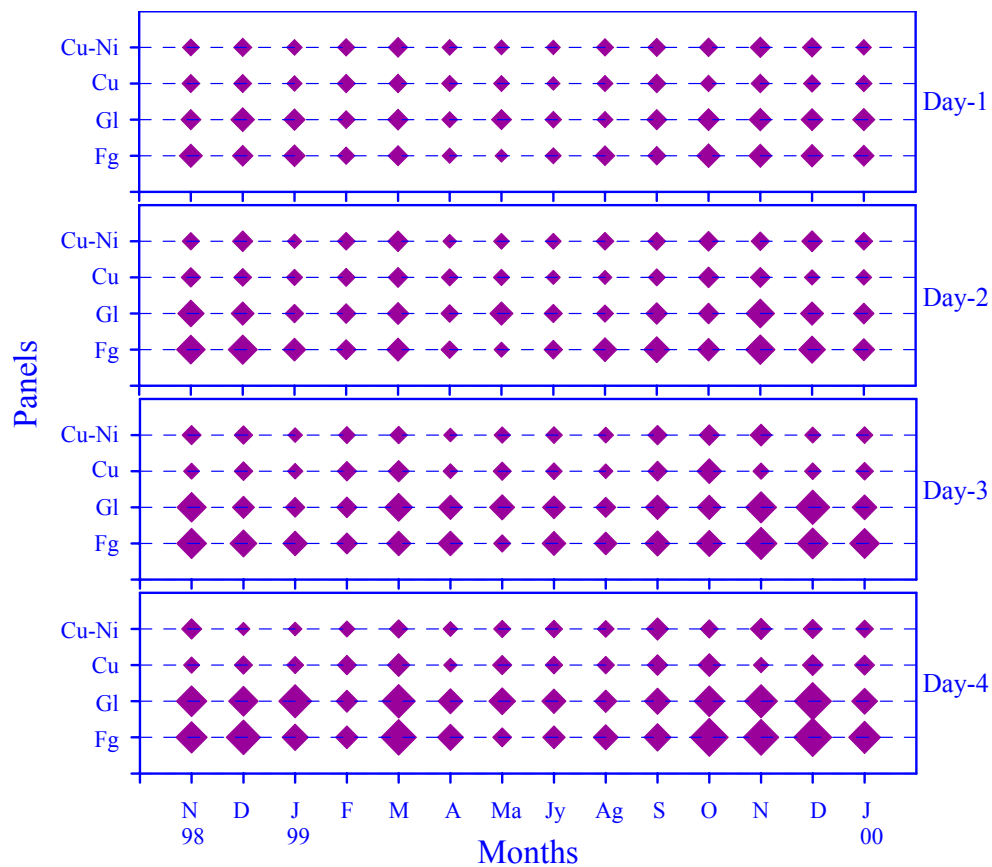


**Fig 3.23.** Dendrograms of the fouling diatom community developed on cupro-nickel with respect to species for different submersion periods (Day 1, Day 2, Day 3, and Day 4).

*N. sigma*. The remaining species of the cluster formed the next dominant forms. Among the ungrouped individuals, *F. oceanica* contributed significantly to the microfilm community [about >30% during monsoon (Table 3.7a-d)] for all exposure days. The rest of the species belonging to other clusters or individual units for all exposure days formed the minor component.

### 3.3.2.c.5. Comparison of microfilm diatom community from fiberglass, glass, copper and cupro-nickel exposed for short period i.e. (1 to 4 days)

Generally, the total number of diatom species (Table 3.6 a and b) as well as total diatom abundance was higher on non-toxic (fiberglass and glass) than toxic (copper and cupro-nickel) substrata (Fig. 3.24). Diatom distribution encountered on the test substrata is given in Table 3.7a-d. *N. transitans* var. *derasa* f. *delicatula* was the most dominant



**Fig. 3.24.** Temporal and substratum variation in the abundance of microfilm diatoms from fiberglass (Fg), glass slide (Gl), copper (Cu) and cupro-nickel (Cu-Ni) at different exposure periods. Note: fourth root transformed raw abundance data is used and the range of symbol height is 0 to 0.32 inch at data value 0 and 9

**Table 3.7a.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from one day old microfilm developed over different substrata (fiberglass-Fg, glass-Gl, copper-Cu and cupro-nickel-CN) during the study.

Mon	Sub	Species																
		<i>Ac</i>	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Gr</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
N-98	Fg	0	40	53	27	13	13	212	106	93	13	53	146	27	0	13	0	0
	Gl	0	27	27	20	7	7	107	75	88	7	34	74	14	0	7	0	0
	Cu	0	51	76	32	0	0	76	0	28	0	0	19	9	0	0	0	19
	CN	7	14	28	0	0	0	87	7	54	0	0	28	7	0	0	0	14
D-98	Fg	0	64	48	80	16	0	160	49	49	0	33	48	0	0	0	0	0
	Gl	0	101	27	0	0	0	449	42	227	14	21	54	0	0	0	0	0
	Cu	16	66	16	0	0	0	89	0	57	0	0	64	0	0	0	0	0
	CN	0	80	35	0	7	0	88	35	28	0	14	28	0	7	0	0	7
J-99	Fg	89	100	23	0	0	56	122	11	78	0	68	66	23	11	11	0	0
	Gl	0	61	51	0	20	0	210	40	131	0	40	40	0	20	0	20	0
	Cu	0	21	28	0	0	0	34	0	34	0	21	14	0	7	0	0	7
	CN	0	9	0	0	0	0	47	32	41	0	19	19	0	0	0	0	9
F-99	Fg	3	14	1	0	9	1	183	0	37	25	0	1	0	0	1	1	0
	Gl	1	12	2	0	4	0	205	0	59	18	0	3	0	0	1	0	0
	Cu	21	21	14	0	40	0	157	0	41	21	0	7	0	0	0	0	0
	CN	35	35	14	0	54	0	93	0	14	21	0	7	0	0	0	0	0
M-99	Fg	0	35	32	0	4	18	157	22	74	11	4	96	9	0	4	0	0
	Gl	8	44	3	3	2	2	181	44	66	5	9	143	8	0	6	16	0
	Cu	0	54	22	0	11	21	80	11	56	6	6	32	21	0	21	0	0
	CN	0	75	16	0	32	21	127	22	51	11	22	32	21	0	11	0	0
A-99	Fg	0	15	0	0	0	0	39	14	60	5	0	5	6	12	0	0	0
	Gl	0	20	0	0	0	0	42	14	74	8	0	13	9	9	0	0	0
	Cu	0	55	6	0	0	0	86	11	6	0	17	11	0	0	0	0	0
	CN	9	47	9	0	0	9	38	19	9	0	19	9	0	9	0	0	0
My-99	Fg	1	15	0	0	2	0	33	5	3	7	2	9	1	3	0	0	0
	Gl	34	10	5	0	25	3	96	0	10	3	0	0	27	0	0	268	0
	Cu	6	33	0	0	16	0	43	17	11	6	11	22	6	17	0	0	0
	CN	6	22	0	0	16	0	43	11	0	0	11	11	6	17	0	0	0
Jy-99	Fg	1	15	6	0	3	5	57	33	28	17	16	7	0	0	0	0	0
	Gl	0	15	8	0	1	11	63	35	40	23	21	8	0	0	0	0	0
	Cu	0	11	0	0	11	0	27	6	23	6	6	11	0	0	0	0	0
	CN	0	16	0	0	16	0	32	6	28	6	6	16	0	0	0	0	0
Ag-99	Fg	6	15	0	116	0	0	105	45	0	115	0	52	0	0	0	0	0
	Gl	0	8	0	64	0	0	25	8	49	0	0	48	0	0	0	0	0
	Cu	0	0	0	49	0	0	90	0	48	0	0	6	0	0	11	0	0
	CN	0	0	0	97	0	0	65	0	73	0	8	33	0	0	0	0	0
S-99	Fg	0	40	0	80	13	0	66	27	40	0	53	27	0	0	0	0	0
	Gl	0	27	0	32	5	15	246	18	56	5	5	49	4	1	0	24	0
	Cu	0	27	7	27	0	0	110	0	14	0	14	140	0	0	0	0	0
	CN	0	34	0	54	0	0	61	21	94	0	21	40	0	0	0	0	0
O-99	Fg	39	129	32	28	12	5	273	42	61	3	18	239	6	3	11	0	0
	Gl	39	122	35	20	5	3	221	6	76	1	11	83	4	1	8	0	0
	Cu	0	0	0	0	0	63	5	27	0	20	0	0	67	8	0	0	0
	CN	15	19	15	0	15	0	61	15	64	0	15	125	0	0	0	0	8
N-99	Fg	0	145	63	0	16	0	302	16	145	0	48	81	33	0	0	0	0
	Gl	0	121	76	0	19	0	247	11	150	0	42	58	21	0	0	0	0
	Cu	0	38	38	0	19	0	57	38	38	0	19	66	0	0	0	0	28
	CN	19	38	38	28	0	0	131	28	66	0	47	38	0	0	0	56	19
D-99	Fg	0	146	40	53	40	13	199	13	172	13	13	40	0	0	0	0	0
	Gl	0	68	0	0	0	14	312	40	146	0	27	73	0	0	0	0	0
	Cu	0	9	19	0	0	0	75	19	57	0	19	66	0	0	0	0	0
	CN	7	28	41	0	0	7	131	14	56	7	14	67	14	0	14	0	0
J-00	Fg	48	63	16	0	80	16	159	49	16	0	33	33	49	0	0	0	0
	Gl	0	90	49	96	8	0	183	25	112	8	32	96	24	0	8	0	0
	Cu	0	21	11	0	11	11	64	21	11	11	0	32	0	0	0	0	11
	CN	8	16	8	0	3	0	76	13	16	2	10	8	5	0	0	0	0

**Table 3.7b.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from two day old microfilm developed over different substrata (fiberglass-Fg, glass-Gl, copper-Cu and cupro-nickel-CN) during the study.

Mon	Sub	Species																
		<i>Ac</i>	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Gr</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
N-98	Fg	40	359	80	0	0	0	557	180	260	20	160	359	0	0	0	0	0
	Gl	38	278	57	0	0	0	416	106	189	18	152	208	0	0	0	0	0
	Cu	11	54	11	0	11	0	140	43	54	21	32	43	0	0	0	0	11
	CN	0	28	28	0	0	0	81	0	42	0	42	42	0	0	0	0	14
D-98	Fg	53	477	40	0	53	13	491	159	331	0	133	292	13	106	0	0	0
	Gl	0	121	25	0	0	25	253	105	130	16	16	138	16	25	0	0	0
	Cu	0	35	0	28	0	0	107	28	0	0	35	74	0	0	0	0	0
	CN	28	14	47	28	41	0	73	28	81	0	35	80	40	14	14	0	14
J-99	Fg	0	199	13	0	13	80	172	13	80	0	93	80	27	0	0	0	40
	Gl	0	20	47	0	14	20	100	20	54	0	27	34	0	0	0	13	0
	Cu	0	35	28	0	0	0	54	7	34	0	21	14	7	7	0	0	7
	CN	0	21	21	0	0	0	54	0	11	0	0	21	0	0	0	0	0
F-99	Fg	5	27	5	0	39	1	335	4	55	12	0	0	0	1	0	0	0
	Gl	4	23	5	0	32	1	319	5	49	9	0	0	0	0	1	0	0
	Cu	21	21	14	0	40	0	119	0	48	28	0	7	0	0	0	7	0
	CN	27	40	14	0	54	0	107	0	21	28	0	14	0	0	0	0	0
M-99	Fg	9	116	31	0	0	63	289	42	57	8	16	85	81	0	26	0	9
	Gl	20	144	0	0	0	0	90	222	91	0	0	102	20	0	0	0	0
	Cu	0	74	27	0	14	28	107	14	69	14	7	48	28	0	27	0	0
	CN	0	95	21	0	40	28	154	28	63	14	28	40	28	0	0	0	0
A-99	Fg	0	25	0	0	0	0	71	20	106	10	0	10	10	20	0	0	0
	Gl	0	35	0	0	0	0	76	5	116	13	0	15	9	24	0	0	0
	Cu	0	60	11	0	0	0	96	28	11	0	17	11	0	0	0	0	0
	CN	0	32	0	0	0	0	21	0	21	0	11	21	0	0	0	0	0
My-99	Fg	1	30	0	0	0	0	57	6	13	3	3	12	3	15	0	11	0
	Gl	117	30	2	6	0	443	0	196	20	0	4	0	44	8	0	2	2
	Cu	7	42	0	0	21	0	54	14	7	7	14	21	7	21	0	0	0
	CN	7	28	0	0	28	0	54	7	0	0	7	14	14	28	0	0	0
Jy-99	Fg	2	33	4	0	3	13	114	58	28	32	5	63	0	0	0	0	0
	Gl	2	29	4	0	3	28	88	67	33	25	4	54	0	0	0	0	0
	Cu	0	14	0	0	14	0	35	7	28	7	7	14	0	0	0	0	0
	CN	0	25	0	0	16	0	65	8	49	0	0	25	0	0	0	0	0
Ag-99	Fg	11	23	0	288	0	0	203	110	0	244	0	88	0	0	0	0	0
	Gl	1	30	0	64	0	0	33	6	64	5	0	33	0	0	0	25	1
	Cu	0	8	0	0	0	0	32	0	49	0	0	25	0	0	0	0	0
	CN	0	0	0	107	0	0	54	0	43	0	0	64	0	0	0	0	32
S-99	Fg	16	256	0	0	0	16	605	176	129	0	65	81	0	0	0	0	0
	Gl	3	51	0	16	0	15	0	341	73	0	6	4	95	3	0	27	5
	Cu	8	25	0	0	0	0	81	16	25	0	0	105	0	0	0	0	0
	CN	8	55	0	33	0	15	68	7	52	0	23	56	0	0	0	0	0
O-99	Fg	4	92	3	34	2	8	273	32	61	8	38	174	8	0	2	9	0
	Gl	3	77	3	33	1	4	246	20	54	5	24	38	8	0	0	8	0
	Cu	10	58	0	118	0	0	135	20	102	0	0	80	20	0	0	0	0
	CN	9	55	0	0	8	0	130	15	120	0	24	54	24	0	0	0	18
N-99	Fg	0	260	20	1075	0	20	498	81	61	40	60	61	20	0	0	80	0
	Gl	0	139	0	1102	0	0	644	0	85	20	0	40	30	0	0	0	0
	Cu	0	75	32	0	0	0	150	11	129	0	0	54	0	0	0	11	11
	CN	0	54	0	0	0	0	120	0	40	0	14	54	14	0	0	0	54
D-99	Fg	32	208	49	0	64	0	383	98	448	16	49	160	16	0	16	32	48
	Gl	0	136	16	0	0	0	382	128	96	23	40	56	0	0	0	0	0
	Cu	7	7	21	0	0	0	42	0	81	0	7	7	0	0	0	0	0
	CN	21	42	28	53	7	0	146	0	42	0	28	167	14	0	0	0	14
J-00	Fg	13	93	40	0	93	13	146	0	199	0	27	40	27	0	0	0	0
	Gl	9	113	19	0	9	9	214	76	73	9	19	9	0	0	0	0	0
	Cu	0	9	19	0	0	0	66	9	9	19	9	38	0	0	0	0	0
	CN	13	23	10	0	15	10	97	15	45	8	19	40	0	0	5	0	0



**Table 3.7c.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from three day old microfilm developed over different substrata (fiberglass-Fg, glass-Gl, copper-Cu and cupro-nickel-CN) during the study.

Mon	Sub	Species																
		<i>Ac</i>	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Gr</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
N-98	Fg	43	426	149	85	0	21	786	86	129	21	43	595	43	0	0	0	0
	Gl	35	293	111	61	0	21	808	74	133	19	57	540	35	0	0	0	0
	Cu	0	42	14	0	7	0	54	14	14	0	7	21	0	0	0	0	21
	CN	27	40	14	27	0	0	160	28	0	0	40	28	0	0	0	0	40
D-98	Fg	0	281	130	0	38	0	557	57	318	0	37	94	56	0	19	0	0
	Gl	0	107	47	0	19	19	193	75	99	28	28	66	47	0	0	0	0
	Cu	14	21	7	0	7	0	105	14	53	0	14	119	7	7	0	0	0
	CN	0	56	27	0	7	0	71	14	40	0	0	74	7	0	28	7	14
J-99	Fg	0	212	40	0	40	119	225	40	93	0	146	93	40	0	0	0	53
	Gl	0	37	64	0	28	27	121	26	61	0	34	34	0	0	0	14	0
	Cu	0	0	11	0	21	0	32	21	32	0	0	54	0	0	0	0	11
	CN	0	21	21	0	14	0	40	14	0	7	0	14	7	0	0	0	0
F-99	Fg	12	23	4	0	29	1	435	0	78	22	1	1	0	0	3	0	0
	Gl	6	24	2	0	25	1	431	0	38	8	3	4	0	0	4	0	0
	Cu	35	35	14	0	54	0	181	0	53	28	0	14	0	0	7	7	0
	CN	14	41	0	0	28	0	114	0	21	14	0	21	0	0	0	0	0
M-99	Fg	12	101	13	0	0	117	398	33	225	18	32	68	59	0	0	4	0
	Gl	39	126	0	0	11	42	720	260	335	0	11	192	43	0	0	0	0
	Cu	0	103	37	0	19	38	150	19	95	19	9	66	38	0	37	0	0
	CN	0	117	19	0	0	0	87	8	27	0	0	23	0	0	0	8	0
A-99	Fg	0	81	21	0	25	0	342	74	99	0	84	76	0	0	32	253	32
	Gl	0	87	14	0	22	0	366	82	86	0	66	51	0	0	38	202	0
	Cu	0	25	0	0	0	0	49	8	16	0	16	8	8	0	0	16	0
	CN	0	21	0	0	0	0	11	0	0	21	0	21	0	0	23	11	0
My-99	Fg	11	47	8	0	3	0	90	35	29	15	0	13	8	4	0	0	0
	Gl	174	23	0	0	6	4	234	6	42	0	9	30	11	9	0	670	0
	Cu	11	68	0	0	32	0	87	21	11	11	21	32	11	32	0	0	0
	CN	11	27	0	0	32	0	64	16	0	0	11	27	17	11	0	0	0
Jy-99	Fg	11	27	0	269	12	0	426	55	0	103	9	53	0	9	3	6	0
	Gl	15	17	0	234	14	0	328	42	0	97	8	52	0	8	3	4	0
	Cu	0	33	0	0	33	0	65	8	33	8	16	41	0	0	0	0	0
	CN	7	21	0	0	21	0	81	21	56	0	0	27	7	0	0	0	0
Ag-99	Fg	3	15	1	254	0	0	264	81	3	155	0	31	8	0	0	0	0
	Gl	28	0	0	47	0	0	112	0	84	0	9	167	0	0	19	38	0
	Cu	0	17	0	0	0	0	59	6	38	0	0	22	0	0	0	0	0
	CN	0	0	0	64	0	0	33	6	16	0	0	44	0	0	0	0	27
S-99	Fg	13	186	0	0	66	40	597	13	93	27	93	53	40	0	0	0	0
	Gl	9	153	3	25	39	44	491	37	69	25	16	93	20	9	0	9	0
	Cu	0	16	0	0	0	0	144	49	49	33	0	128	0	0	33	16	0
	CN	20	81	0	0	0	38	98	0	15	0	45	140	0	0	0	0	0
O-99	Fg	35	164	16	33	30	11	429	125	196	25	93	201	42	0	12	0	0
	Gl	13	140	13	51	6	11	543	34	90	9	27	51	15	0	0	29	0
	Cu	18	73	4	0	12	12	273	73	318	9	44	186	24	0	13	0	0
	CN	15	95	0	0	0	0	159	28	134	5	20	71	4	0	8	0	0
N-99	Fg	0	532	86	171	0	21	998	405	319	21	129	320	86	21	0	0	0
	Gl	0	348	0	1237	0	0	947	0	125	32	0	57	69	0	0	0	0
	Cu	7	21	14	0	0	0	54	7	7	0	21	81	0	0	0	0	7
	CN	25	109	27	0	0	0	218	54	81	0	14	64	0	27	0	0	58
D-99	Fg	53	504	119	106	27	13	876	172	504	40	80	172	27	0	13	0	0
	Gl	134	896	33	33	0	116	1349	100	878	83	133	66	126	0	83	0	0
	Cu	0	42	7	0	0	0	48	14	55	0	20	35	0	0	0	0	0
	CN	0	16	8	0	0	0	57	0	16	0	8	49	0	0	8	33	8
J-00	Fg	97	304	113	0	33	0	684	320	527	0	160	144	32	0	0	0	0
	Gl	25	196	63	0	22	19	391	189	167	19	41	35	0	0	0	0	0
	Cu	11	43	21	0	21	0	75	32	21	0	21	54	0	0	0	0	0
	CN	6	8	9	0	10	0	104	3	33	1	15	48	0	0	0	0	0

**Table 3.7d.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from four day old microfilm developed over different substrata (fiberglass-Fg, glass-Gl, copper-Cu and cupro-nickel-CN) during the study.

Mon	Sub	Species																
		<i>Ac</i>	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Gr</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
N-98	Fg	43	405	21	0	0	43	913	299	320	43	170	278	299	0	21	0	0
	Gl	44	322	124	61	0	40	884	88	246	38	95	629	35	0	0	0	0
	Cu	0	34	0	47	0	0	54	7	28	0	7	28	7	0	0	0	0
	CN	19	131	9	0	28	0	141	47	57	0	19	28	0	0	0	28	9
D-98	Fg	0	495	0	175	0	16	1656	159	1371	33	64	255	0	0	0	0	0
	Gl	0	582	24	25	0	8	1122	57	354	57	0	80	16	0	0	0	0
	Cu	0	55	21	0	7	0	74	14	56	0	42	27	0	0	0	0	7
	CN	0	14	0	0	0	7	21	7	7	0	0	14	0	0	14	7	0
J-99	Fg	0	212	66	0	40	133	398	40	133	0	186	133	53	0	0	0	40
	Gl	53	585	80	0	319	133	1167	27	480	54	186	477	27	0	0	0	0
	Cu	0	21	14	0	28	0	54	42	28	0	0	47	0	0	0	0	21
	CN	0	21	14	0	14	0	33	7	0	0	0	7	7	0	0	0	0
F-99	Fg	13	74	11	0	54	1	593	0	30	16	0	11	0	0	4	0	0
	Gl	9	44	4	0	30	5	518	0	59	10	9	8	0	0	5	0	0
	Cu	28	47	14	0	67	0	146	0	47	35	0	28	0	0	0	14	0
	CN	14	41	0	0	28	0	80	0	21	7	0	14	0	0	0	0	0
M-99	Fg	87	144	8	0	4	367	1434	380	1337	53	270	473	100	0	9	8	8
	Gl	15	152	15	0	19	377	1174	338	1024	42	159	375	48	0	19	0	8
	Cu	0	138	33	0	49	33	239	33	106	16	25	73	33	0	32	0	0
	CN	0	136	20	0	0	0	114	8	30	0	0	30	0	0	0	0	0
A-99	Fg	0	117	21	0	27	0	530	74	106	0	86	76	0	0	32	297	32
	Gl	0	102	20	0	22	0	436	88	86	0	88	57	0	0	38	215	0
	Cu	0	33	0	0	0	0	33	8	16	0	0	8	8	0	0	0	0
	CN	0	43	0	0	0	0	54	11	0	11	11	21	0	0	0	0	0
My-99	Fg	44	107	0	0	8	0	71	63	8	4	26	0	21	22	0	0	18
	Gl	210	32	0	0	8	8	274	9	59	0	34	54	17	11	0	852	0
	Cu	16	66	8	0	49	0	137	25	16	16	25	33	16	8	0	0	0
	CN	14	35	0	0	40	0	81	21	0	0	14	34	21	14	0	0	0
Jy-99	Fg	3	132	25	3	8	62	273	100	45	40	31	74	0	0	0	0	0
	Gl	19	24	0	309	16	0	402	44	0	122	11	88	0	13	5	10	0
	Cu	0	47	0	0	40	0	53	21	56	14	21	40	0	0	0	0	0
	CN	7	22	16	0	16	6	90	28	54	0	0	27	11	0	0	0	0
Ag-99	Fg	3	27	3	368	0	0	318	119	6	243	3	84	17	0	0	0	0
	Gl	38	0	0	104	0	0	158	0	114	0	28	189	0	0	38	76	0
	Cu	0	49	0	22	11	0	105	11	59	0	0	27	0	0	0	0	0
	CN	0	0	0	80	0	0	41	7	21	0	0	54	0	0	0	0	34
S-99	Fg	27	239	0	0	119	40	756	80	119	27	133	80	53	0	0	0	0
	Gl	13	174	0	90	5	24	720	32	151	32	13	144	6	11	0	0	0
	Cu	0	33	0	0	0	0	228	35	69	14	14	93	0	0	14	21	27
	CN	0	48	5	87	3	0	330	28	90	13	27	95	8	0	3	0	0
O-99	Fg	37	917	0	125	6	23	3973	299	513	54	61	141	50	0	11	0	0
	Gl	57	364	25	271	13	19	1271	52	364	30	45	66	32	0	0	43	0
	Cu	101	64	0	15	0	0	264	20	174	43	28	90	8	0	0	0	4
	CN	19	23	0	0	0	8	131	19	53	8	0	42	4	0	8	0	0
N-99	Fg	0	703	150	319	0	43	1591	553	468	21	213	532	150	21	0	0	0
	Gl	0	571	0	1263	0	0	1307	0	242	81	0	87	91	0	0	0	0
	Cu	0	14	0	0	0	0	54	21	0	0	0	35	0	0	0	0	40
	CN	0	145	13	0	0	0	241	25	109	0	13	49	0	0	13	0	13
D-99	Fg	27	1167	66	0	0	0	3237	398	1207	13	80	212	80	0	13	0	0
	Gl	17	1394	33	133	17	0	2653	399	700	100	234	117	100	0	0	0	17
	Cu	0	122	27	0	0	0	107	28	100	0	42	107	0	0	0	0	20
	CN	0	25	37	24	13	13	108	13	63	0	25	73	13	0	13	0	0
J-00	Fg	85	554	128	0	213	0	1146	191	427	0	128	170	43	21	0	0	0
	Gl	25	183	57	0	25	25	562	164	189	25	38	63	0	0	0	0	0
	Cu	19	47	38	0	38	0	122	28	47	0	28	75	9	0	0	0	0
	CN	6	15	9	0	10	5	111	15	42	4	15	58	0	0	3	0	0

diatom on all the substrata followed by species belonging to *Amphora*, *Nitzschia* and *Thalassionema* and was present round the year. However, the percentage composition and abundance of dominant diatoms varied depending on the nature of substratum, season and distribution of the other co-existing species. In May, *M. nummuloides* (all days) and during August and November, *F. oceanica* were abundant on fiberglass and glass substrata but were absent on copper and cupro-nickel substrata indicating substratum preferences.

Diatom diversity increased with exposure period on toxic substrata (copper and cupro-nickel) whereas, in case of non-toxic substrata (fiberglass and glass substrata), the diversity either decreased or remained constant (Figs. 3.9, 3.13, 3.17 and 3.21). MANOVA of total diatom abundance, species diversity, species richness and evenness for the four substrata is shown in Table 3.8. A significant variation between the four substrata was observed with respect to months. With respect to days, the total diatom abundance and evenness revealed significant variation.

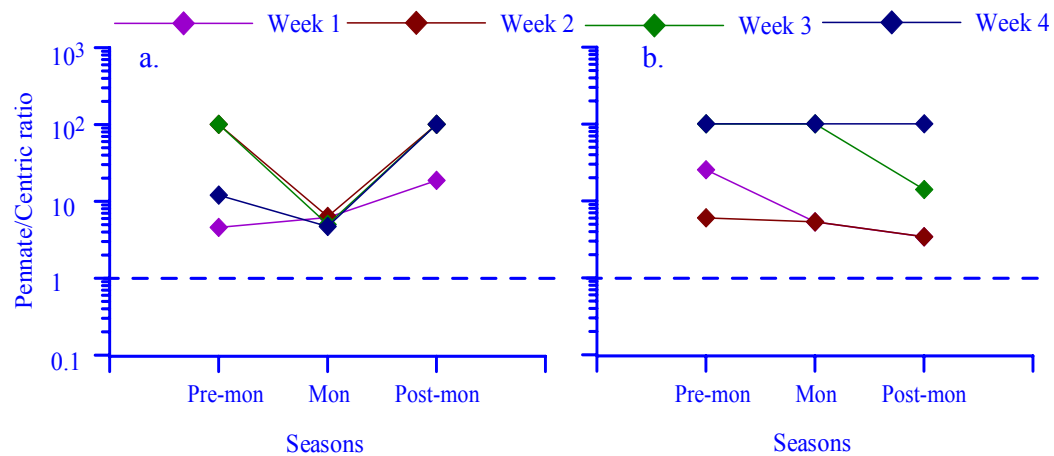
**Table 3.8.** MANOVA to evaluate the temporal variation in the diatom species count, abundance, diversity, species richness and evenness on fiberglass, glass, copper and cupro-nickel with respect to exposure (short term). (\*\*  $\leq 0.001$ , \*  $\leq 0.05$ , ns = not significant).

Source of variation	df	Species count			Abundance			Diversity			Species richness			Evenness		
		SS	MS	$F_s$	SS	MS	$F_s$	SS	MS	$F_s$	SS	MS	$F_s$	SS	MS	$F_s$
A: Substrata	3	3154.0	1051.3		140.6	46.9		1.8	0.6		32.4	10.8		0.85	0.28	
B: Months	14	3754.6	268.2		60.6	4.3		26.7	1.9		70.8	5.1		0.90	0.06	
C: Days	3	345.0	115.0		49.3	16.4		0.1	0.0		0.8	0.3		0.10	0.03	
A*B	42	1590.1	37.9	2.76**	36.5	0.9	3.86**	16.3	0.4	4.19**	36.8	0.9	3.27**	0.76	0.02	5.95**
A*C	9	177.9	19.8	1.44 <sup>ns</sup>	30.8	3.41	5.21**	1.4	0.2	1.70 <sup>ns</sup>	2.5	0.3	1.03 <sup>ns</sup>	0.07	0.01	2.52*
B*C	42	430.0	10.2	0.75 <sup>ns</sup>	10.5	0.3	1.12 <sup>ns</sup>	4.4	0.1	1.14 <sup>ns</sup>	10.8	0.3	0.96 <sup>ns</sup>	0.12	0.00	0.94 <sup>ns</sup>
A*B*C	126	1730.1	13.7		28.3	0.2		11.7	0.1		33.7	0.3		0.38	0.00	
Total	239	11181.6			356.7			62.3			187.8			3.17		

### 3.3.3. Experiment 3: Diatoms in 1 to 4 week old microfilms developed over copper and cupro-nickel substrata.

#### 3.3.3.a. Copper

Diatoms encountered on copper substrata from week 1 to 4 are presented in Table 3.9a and b. The fouling population included 28 diatom species (21 pennates and 7 centrics) belonging to 16 genera (10 pennates and 6 centrics). The pennate/centric diatom percentage ratio on this substratum remained above 1 on all occasions indicating the dominance of pennate diatoms (Fig. 3.25a).



**Fig. 3.25.** The ratio of pennate/centric diatoms in the microfilm developed over a. copper and b. cupro-nickel substrata exposed for different submersion periods (Week 1; Week 2; Week 3 and Week 4).

The temporal variation in the fouling diatom abundance and univariate measures for copper is shown in Fig. 3.26a. Diatom abundance decreased initially and increased thereafter with exposure period. Diatom abundance was minimal during the monsoon period. Species count and diversity decreased with exposure period. Maximum diversity was observed during post - monsoon followed by pre-monsoon and monsoon period. Species richness also followed a similar pattern. During the pre-monsoon period, the diatom population was evenly distributed up to two weeks and, thereafter, the

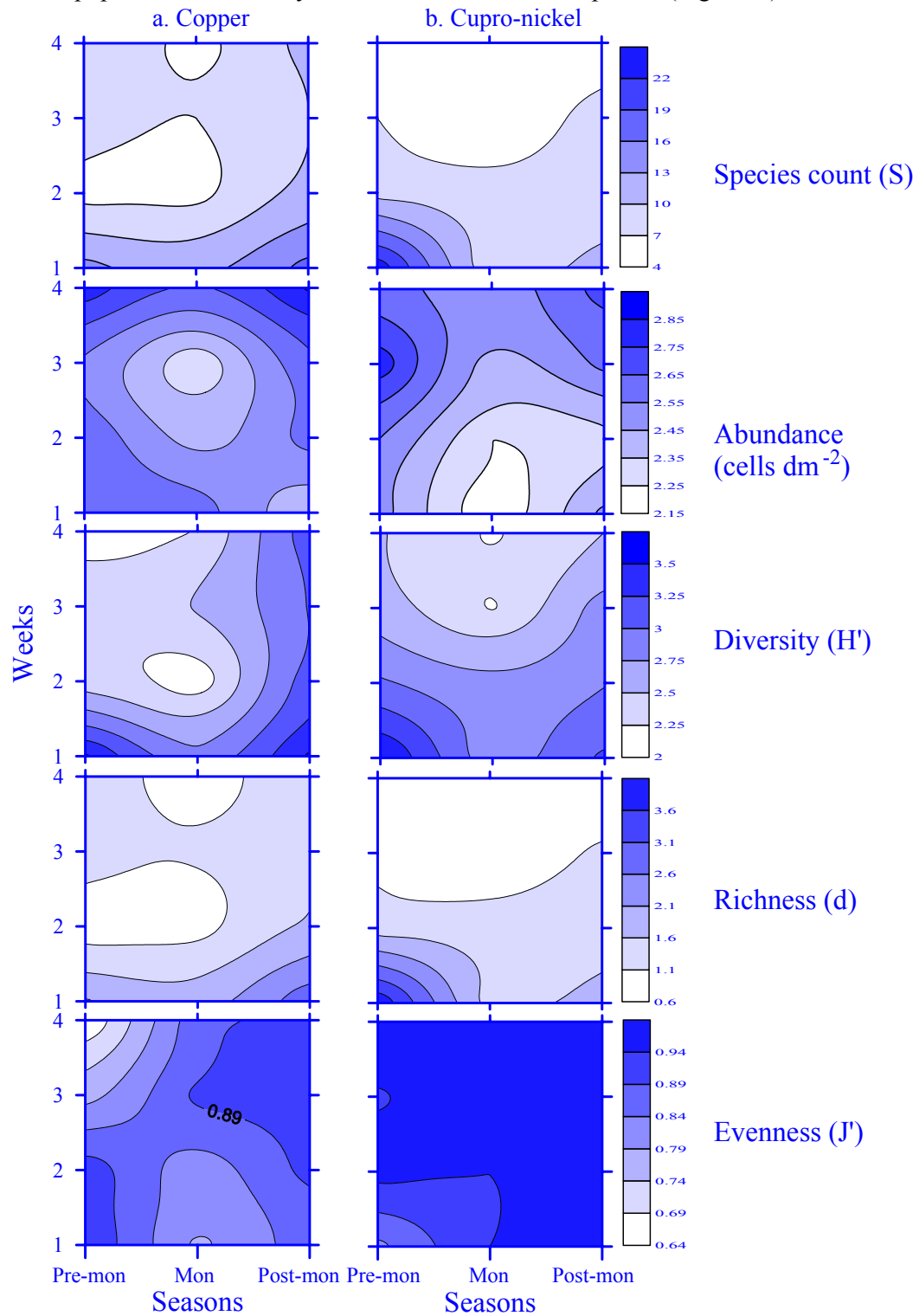
**Table 3.9a.** List of pennate diatom species recorded from the microfilm (1 to 4 week old) developed over copper (Cu) and cupro-nickel (CN) during the study. (Abbrev-abbreviation of the diatom species used in the cluster analysis)

Sl. No.	Taxon	Abbrev.	Cu	CN
1	<i>Amphora coffeaeformis</i> (Ag.) Kützing	A.co	+	+
2	<i>A. hyalina</i> Kützing	A.hy	+	+
3	<i>A. rostrata</i> Wm. Smith	A.ro	+	+
4	<i>Cocconeis scutellum</i> Ehrenberg	Co.scu	+	+
5	<i>Cocconeis</i> sp.	Co.sp	+	
6	<i>Cylindrotheca closterium</i> (Ehrenberg) Smith	Ci.cl	+	+
7	<i>Cymbella gastroides</i> Kützing	Cy.ga		+
8	<i>Fragilariopsis oceanica</i> (Cleve) Hasle	F.oc		+
9	<i>Grammatophora mariana</i> (Lyngbye) Kützing	Gr.ma		+
10	<i>G. serpentina</i> Ehrenberg	Gr.se	+	
11	<i>Hantzschia</i> sp.	Hn.sp		+
12	<i>Licmophora flabellata</i> (Greville)Agardh	L.fl		+
13	<i>L. gracilis</i> (Ehrenberg) Grunow	L.gr		+
14	<i>L. juergensii</i> Agardh	L.je		+
15	<i>L. paradoxa</i> (Lyngbye) Agardh	L.pa		+
16	<i>Navicula crucicula</i> (Wm. Smith) Donkin	N.cr	+	
17	<i>N. granulata</i>	N.gr	+	
18	<i>N. subinflata</i> Grunow	N.su	+	+
19	<i>N. transitans</i> var. <i>derasa</i> f. <i>delicatula</i> Heimdal	N.de	+	+
20	<i>Navicula</i> sp. 1	N.sp1	+	
21	<i>Navicula</i> sp. 2	N.sp3		+
22	<i>Navicula</i> sp. 3	N.sp4		+
23	<i>Nitzschia bilobata</i> Wm. Smith	Ni.bi	+	+
24	<i>N. longissima</i> Ralfs in Pritchard.	Ni.lo	+	+
25	<i>N. panduriformis</i> Gregory	Ni.pa		+
26	<i>N. sigma</i> (Kützing)Wm.smith	Ni.si	+	+
27	<i>Pinnularia rectangulata</i> (Gregory) Rabenhorst	Pi.sp	+	
28	<i>Pleurosigma angulatum</i> Sensu W. Smith	Pl.an	+	+
29	<i>Pseudo-nitzschia seriata</i> (Cleve) H. Pergallo	Ni.se	+	
30	<i>Synedra affinis</i> Kützing	Sy.af	+	+
31	<i>S. gallioni</i> (Bory) Ehrenberg	Sy.ga	+	+
32	<i>Thalassionema frauenfeldii</i> (Grunow) Hallegraeff	Th.fr		+
33	<i>T. nitzschoides</i> (Grunow) Mereschkowsky	Th.nit	+	+

**Table 3.9b.** List of centric diatom species recorded from the microfilm (1 to 4 week old) developed over copper (Cu) and cupro-nickel (CN) during the study. (Abbrev-abbreviation of the diatom species used in the cluster analysis).

Sl. No.	Taxon	Abbrev.	Cu	CN
1	<i>B. rhombus</i> (Ehrenberg) Smith, W.	Bd.rh		+
2	<i>Chaetoceros curvisetus</i> Cleve	Ch.cu	+	
3	<i>Coscinodiscus marginatus</i> Ehrenberg	C.ma	+	
4	<i>C. radiatus</i> Ehrenberg	C.rad	+	+
5	<i>Cyclotella</i> sp.	Cyc.sp	+	
6	<i>Melosira nummuloides</i> C.A. Agardh	M.nu	+	+
7	<i>Proboscia alata</i> (Brightwell)Sundstrom	Ri.al	+	
8	<i>Skeletonema costatum</i> Greville (Cleve)	Sk.co		+
9	<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve	Th.ec	+	+

distribution was uneven with exposure period. During monsoon and post-monsoon, the diatom population was evenly distributed with time of exposure (Fig. 3.26).

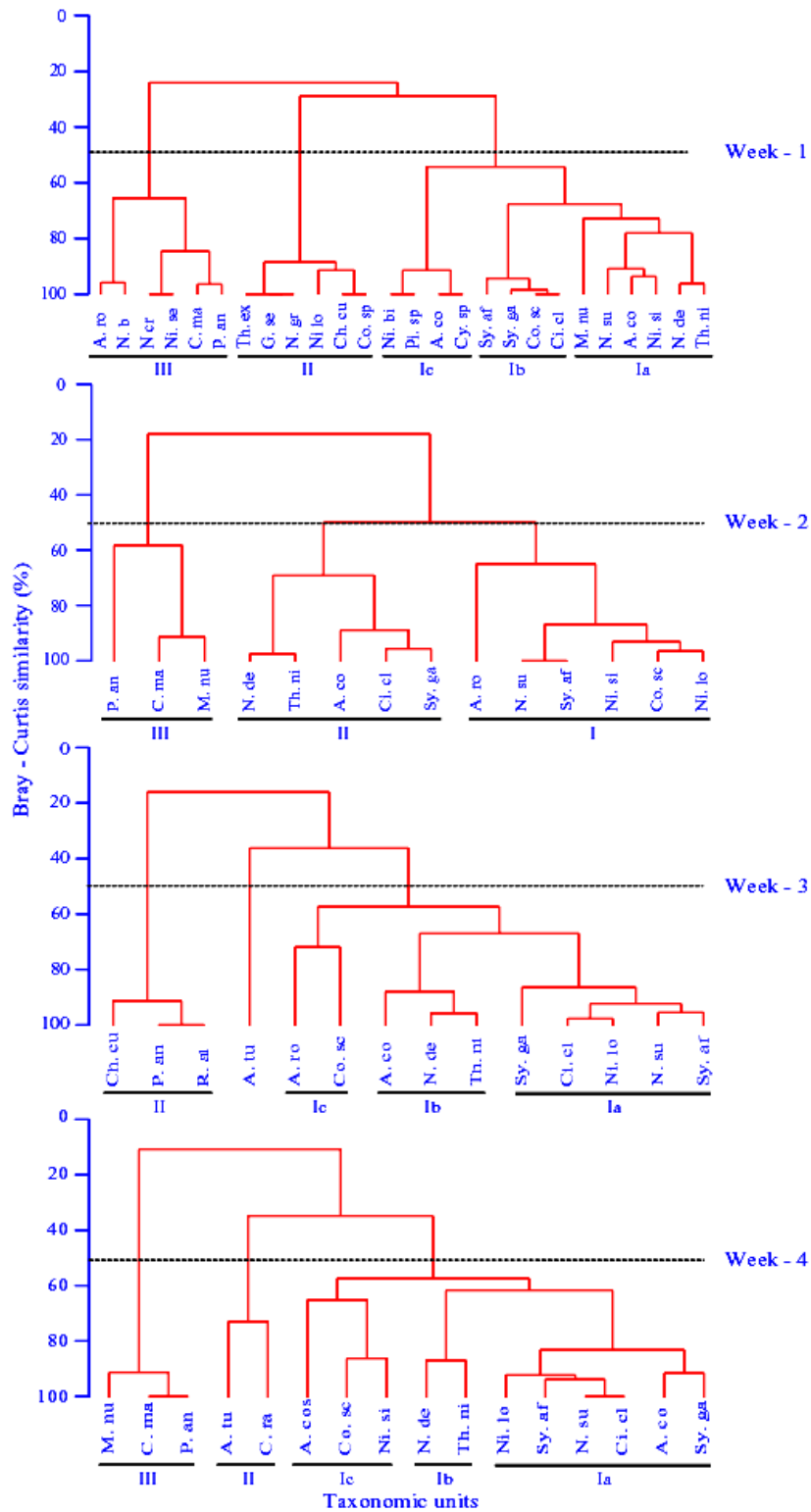


**Fig. 3.26.** Seasonal variation in the univariate measures of the fouling diatom population on copper and cupro-nickel substrata for different submersion periods (Week 1; Week 2; Week 3 and Week 4).

Cluster analysis at 50% similarity level divided 26 species into 3 clusters (week 1), 14 species into 3 clusters (week 2) and 2 clusters and 1 ungrouped individual (week 3) and 16 species into 3 clusters (week 4) (Fig. 3.27). This indicates that the number of species decreased with exposure period. For 1 week exposure, cluster 1 comprised of 3 subgroups i.e. 1a, b and c (Fig. 3.27). Subgroup 1a consists of 6 species (*N. transitans* var. *derasa* f. *delicatula*, *T. nitzschioides*, *A. coffeaeformis*, *N. sigma*, *N. subinflata* and *M. nummuloides*) that were dominant and present in all the seasons whereas the species belonging to others subgroups and clusters formed the minor components. Among them, *N. transitans* var. *derasa* f. *delicatula* and *T. nitzschioides* were the most dominant forms forming about 31% (pre-monsoon), 61% (monsoon) and 42% (post-monsoon) of the community. In case of week 2, cluster 2 comprised of 5 species which contributed >70% of the community. Among them, *N. transitans* var. *derasa* f. *delicatula* and *T. nitzschioides* were the most dominant forms whereas the species belonging to other clusters formed the minor components. In week 3, cluster 1 comprised of 10 species and formed the major component of the community while the species belonging to other clusters occurred at few occasions were considered as minor components. This cluster is further divided into 3 subgroups i.e. 1 a, b and c. Subgroup 1b comprised of *N. transitans* var. *derasa* f. *delicatula*, *T. nitzschioides* and *A. coffeaeformis*, the most dominant species that were present during all the periods. In week 4, cluster 1 is further divided into subgroups i.e. 1a, b and c. Among these subgroups, subgroup 1b consisted of *N. transitans* var. *derasa* f. *delicatula* and *T. nitzschioides* that were dominant in all the seasons.

### 3.3.3.b. Cupro-nickel

Diatoms encountered on cupro-nickel substrata from day 1 to 4 are presented in Table 3.9a and b. The fouling population included 31 diatom species (26 pennates and 5



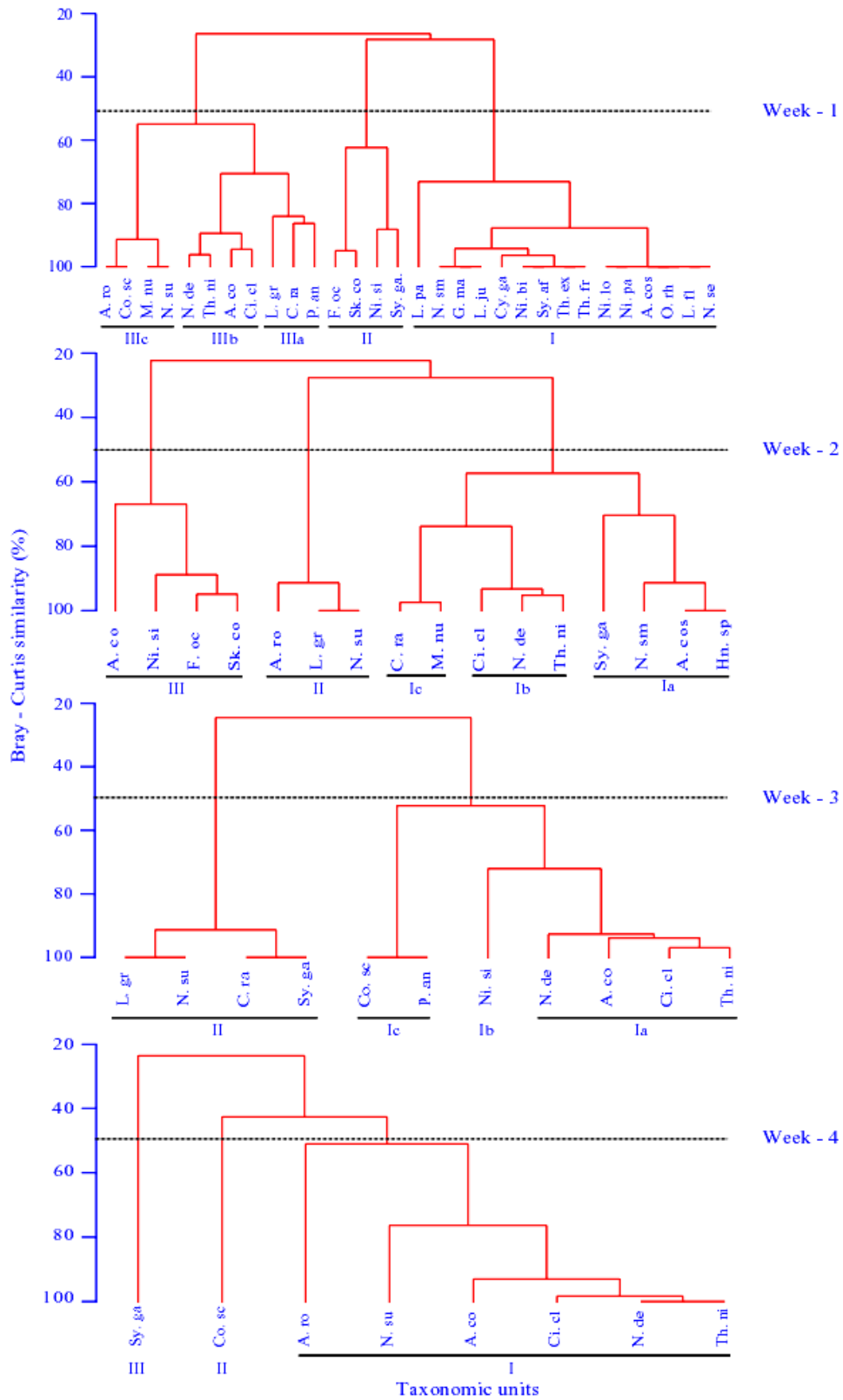
**Fig 3.27.** Dendrograms of the fouling diatom community developed on copper with respect to species for different submersion periods (Week 1, Week 2, Week 3, and Week 4).



centrics) belonging to 18 genera (13 pennates and 5 centrics). The pennate/centric diatom percentage ratio on this substrata remained above 1 on all occasions indicating the dominance of pennate diatoms (Fig. 3.25b).

The temporal variation in the fouling diatom abundance and univariate measures for cupro-nickel is shown in Fig. 3.26b. Diatom population increased with exposure period except during the pre-monsoon period. During pre-monsoon, the diatom abundance decreased during the fourth week. Diatom abundance was minimal during the monsoon period. Species count, diversity and richness decreased with the exposure period with minimum values observed during the monsoon. The diatom population was evenly distributed throughout the study period.

Cluster analysis at 50% similarity level divided the 30 species into 3 clusters (week 1), 16 species into 3 clusters (week 2), 11 species into 2 clusters (week 3), and the 8 species into one cluster with 2 ungrouped individuals (week 4) (Fig. 3.28). This indicates that the number of species decreased with exposure period. For 1-week exposure, cluster I comprised of 15 species (each contributing <3%); these were observed only during the pre-monsoon period. Cluster II comprised of 4 species, of which *S. costatum* and *F. oceanica* are found only during the monsoon season and contribute about 27% of the community whereas the *N. sigma* and *S. gallioni* are found only during the pre-monsoon and monsoon periods. Cluster III is again divided into 3 sub-groups; subgroup IIIa consists of 3 species present during the post-monsoon period, each species contributing between 5–10%. Subgroup IIIb consists of 4 species, *N. transitans* var. *derasa* f. *delicatula*, *T. nitzschoides*, *A. coffeaeformis* and *C. closterium* which formed the most dominant group, comprising about 50% during all the seasons. Subgroup IIIc consists of 4 species and they contributed significantly as the dominant forms in the community



**Fig 3.28.** Dendrograms of the fouling diatom community developed on cupro-nickel with respect to species for different submersion periods (Week 1, Week 2, Week 3, and Week 4).

only during the pre- and post-monsoon season. For 2 weeks exposure, cluster I is further divided into 3 subgroups (Fig. 3.28). Subgroup Ia, Ib and Ic consisted of 4, 3 and 2 species respectively (Fig. 3.28). Species belonging to subgroup 1b, *N. transitans* var. *derasa* f. *delicatula*, *T. nitzschioides* and *C. closterium* formed the most dominant group, each contributing 49%, 53% and 50% during pre-monsoon, monsoon and post-monsoon season. The species belonging to subgroups Ia (pre-monsoon) and c (pre- and post-monsoon) were not present during all periods. Cluster II consisted of three species: *A. rostrata*, *L. gracilis* and *N. subinflata* and contributed 23% during the post-monsoon season; cluster III consisted of 4 species, *F. oceanica* and *S. costatum*, that were present only during the monsoon, forming 27% of the community. For week 3, cluster I is divided into 3 subgroups i.e. Ia, b and c (Fig. 3.28). Subgroup Ia consisted of 4 dominant species (*N. transitans* var. *derasa* f. *delicatula*, *T. nitzschioides*, *A. coffeaeformis* and *C. closterium*) that were present throughout the investigation period. Species belonging to other subgroups and clusters formed the minor components and were grouped/clustered according to their occurrence. For week 4, cluster I consisted of 6 species of which 4 species (*N. transitans* var. *derasa* f. *delicatula*, *T. nitzschioides*, *A. coffeaeformis* and *C. closterium*) were dominant and were present throughout the investigation period while the rest formed minor components.

### **3.3.3.c. Comparison of microfilm diatom community from copper and cupro-nickel panels exposed for long period (i.e. 1 to 4 weeks)**

The long-term exposure of copper and cupro-nickel panels revealed that the diatom diversity decreased with exposure period. The diatom distributions encountered on the test substrata are given in Table 3.10a-d. *N. transitans* var. *derasa* f. *delicatula* was the most dominant diatom on all the substrata followed by species belonging to *Amphora*, *Nitzschia*, and *Thalassionema*; all these were present round the year. However, the

**Table 3.10a.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from the one-week-old microfilm developed over copper (Cu) and cupro-nickel (CN) during the study.

Seasons	Sub	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
Pre-mon	Cu	43	33	0	0	87	11	65	0	54	43	11	0	0	54	22
	CN	16	0	0	82	47	7	40	7	23	61	14	2	2	0	7
Mon	Cu	19	0	0	0	112	28	28	28	0	130	0	0	37	19	0
	CN	16	0	16	0	39	0	23	0	8	23	0	0	0	0	23
Post-mon	Cu	27	19	0	0	50	12	35	0	23	47	4	0	0	0	12
	CN	27	14	0	27	54	27	27	14	0	41	0	0	41	27	0

**Table 3.10b.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from the two-week-old microfilm developed over copper (Cu) and cupro-nickel (CN) during the study.

Seasons	Sub	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
Pre-mon	Cu	109	0	0	0	109	0	27	54	27	109	0	0	0	0	0
	CN	25	0	0	0	50	50	50	0	25	76	25	0	25	25	0
Mon	Cu	12	0	0	0	82	0	0	23	0	105	0	0	23	12	0
	CN	19	0	19	0	47	0	28	0	9	28	0	0	0	0	28
Post-mon	Cu	58	29	0	0	97	10	78	0	49	68	0	0	0	0	0
	CN	19	0	0	19	47	19	19	0	0	37	0	0	28	19	0

**Table 3.10c.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from the three-week-old microfilm developed over copper (Cu) and cupro-nickel (CN) during the study.

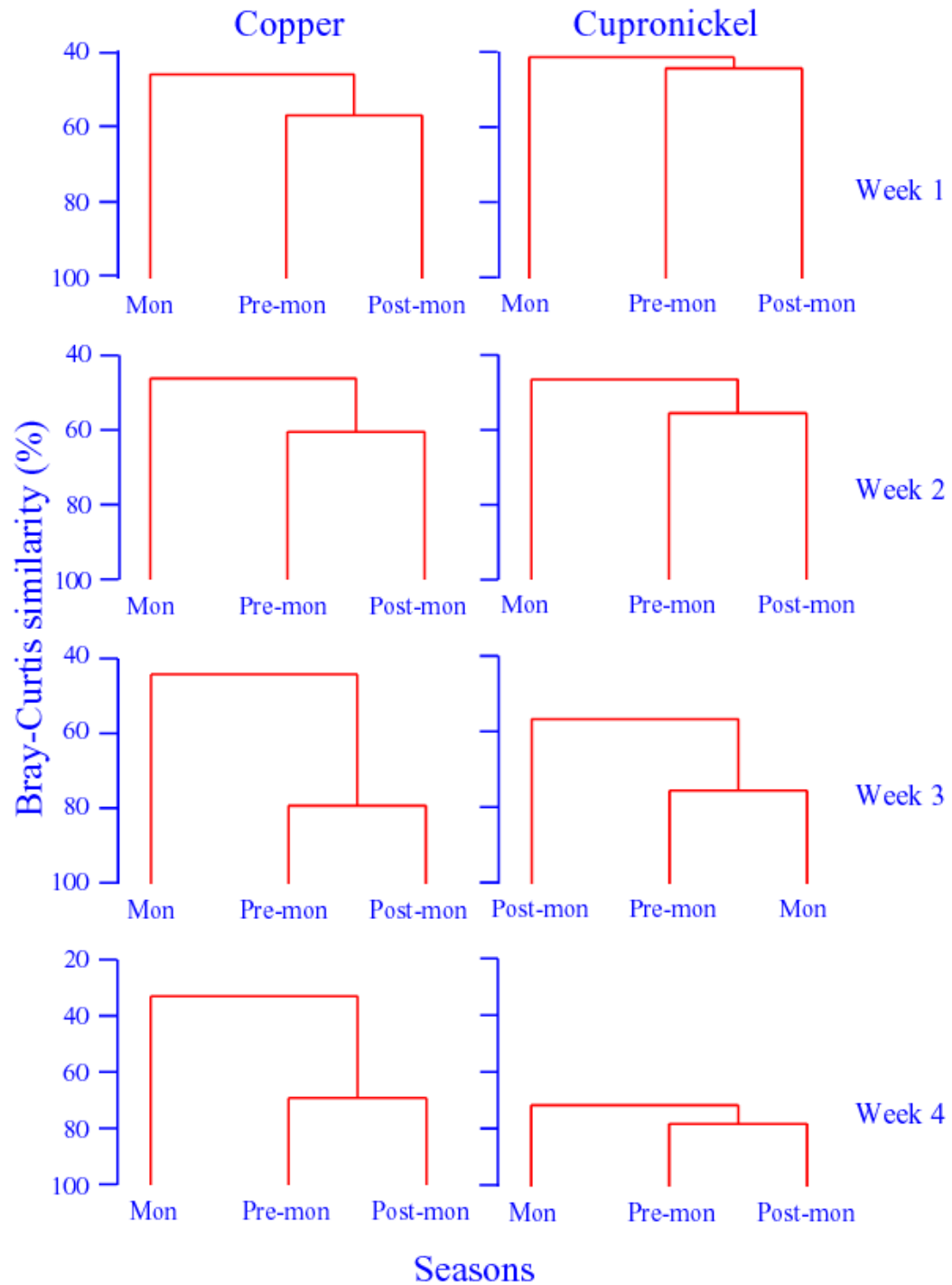
Seasons	Sub	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
Pre-mon	Cu	33	0	0	0	77	17	28	0	38	143	0	0	0	0	0
	CN	109	54	0	0	54	0	217	54	0	163	0	0	0	0	0
Mon	Cu	30	0	0	0	40	0	0	20	0	61	0	0	0	0	30
	CN	47	0	0	0	70	0	70	0	0	70	0	0	0	0	0
Post-mon	Cu	68	27	0	0	95	14	54	0	68	95	0	0	0	0	0
	CN	25	0	0	50	76	50	50	0	25	76	0	0	25	0	0

**Table 3.10d.** Values of diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from the four-week-old microfilm developed over copper (Cu) and cupro-nickel (CN) during the study.

Seasons	Sub.	<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
Pre-mon	Cu	106	0	0	0	22	17	28	0	62	364	0	0	50	0	0
	CN	109	54	0	0	54	54	54	0	0	54	0	0	0	0	0
Mon	Cu	25	0	0	0	126	0	0	50	0	151	0	0	50	25	0
	CN	54	0	0	0	82	0	54	0	27	82	0	0	0	0	0
Post-mon	Cu	151	76	0	0	177	25	101	0	76	126	0	0	0	0	0
	CN	109	0	0	0	109	54	109	0	0	109	0	0	0	0	0

percentage composition of the dominant groups varied depending on the season and distribution of other co-existing species. The persistence of these diatoms throughout the investigation period further reveals their tolerance towards copper and cupro-nickel panels.

Cluster analysis of the sampling periods based on diatom abundance at 40% similarity level revealed one group with one ungrouped individual sampling period for both copper and cupro-nickel substrata (all weeks) (Fig. 3.29). Cluster I comprised of 2 sampling



**Fig 3.29.** Dendrograms of the fouling diatom community developed on copper and cupro-nickel substrata with respect to season for different submersion periods (Week 1, Week 2, Week 3, and Week 4).

seasons (pre- and post-monsoon) whereas monsoon formed a separate individual unit. The ungrouped sampling period is characterized by low diatom population compared to other sampling periods.

MANOVA of total diatom abundance, species diversity, species richness and evenness for copper and cupro-nickel substrata is shown in Table 3.11. A significant variation between the four substrata was observed with respect to months. With respect to days, the total diatom abundance and evenness also revealed significant variation.

**Table 3.11.** MANOVA to evaluate the temporal variations in the diatom species count, abundance, diversity, species richness and evenness on copper and cupro-nickel with respect to exposure (long term). (\*\*\*\*\*  $\leq 0.001$ , \*\*\*\*  $\leq 0.005$ , \*\*\*  $\leq 0.01$ , \*\*  $\leq 0.025$ , \*  $\leq 0.05$ , ns = not significant).

Source of variation	df	Species count			Abundance			Diversity			Species richness			Evenness		
		SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>
A: Substrata	1	1.5	1.5		0.1	0.1		0.0	0.01		0.0	0.02		0.0	0.01	
B: Seasons	2	1718.4	859.2		352.4	176.2		147.2	73.6		40.6	20.31		15.2	7.60	
C: Weeks	3	42.6	14.2		0.27	0.09		0.5	0.17		1.45	0.48		0.00	0.00	
A*B	2	35.9	17.9	1.53 <sup>ns</sup>	0.33	0.16	0.88 <sup>ns</sup>	0.5	0.23	3.59*	0.98	0.49	1.25 <sup>ns</sup>	0.04	0.02	2.47 <sup>ns</sup>
A*C	3	3.7	1.2	0.10 <sup>ns</sup>	0.14	0.05	0.25 <sup>ns</sup>	0.1	0.02	0.29 <sup>ns</sup>	0.12	0.04	0.11 <sup>ns</sup>	0.00	0.00	0.09 <sup>ns</sup>
B*C	6	206.5	34.4	2.93*	1.36	0.23	1.22 <sup>ns</sup>	2.3	0.39	6.12**	6.8	1.13	2.91*	0.02	0.00	0.46 <sup>ns</sup>
A*B*C	6	70.5	11.7		1.11	0.18		0.4	0.06		2.3	0.39		0.05	0.01	
Total	23	2079.0			355.6			151.0			52.3			15.3		

### 3.4. DISCUSSION

Diatoms occupy two principal habitats in nature i.e. moist habitats or submerged surfaces (benthic) and open waters (planktonic). Even though benthic diatoms are more diverse than their planktonic counterparts, their ecology has been less understood. This is because benthic diatoms are far more difficult to sample and quantify than planktonic forms. Removal of diatoms from microfilms is perhaps the most critical step in evaluating the diatom community structure from biofilms developed on solid substratum immersed in aquatic environments. Until recently, several methods have been employed to assess the microfouling biomass (Jeffrey and Paul 1986, Afring and Taylor 1979, Burton and Margray 1979, Mayack et al. 1984, Sharma et al. 1990). However,

efficiency of these methods is based on the estimation of biomass by chemical methods. In this study, efficiency of the tools employed for removal of microfilm and the loss encountered during removal are discussed.

The microfilm sample volume used for diatom analysis did not influence total diatom count (Tables 3.1a, b, 3.2a and b). The fact that 20%, 30% and 50% of the 1/10<sup>th</sup> concentrated sample volume did not show any significant difference in the total diatom counts (Fig. 3.2a and b) and diversity (Tables 3.3 and 3.4) reveals that sample volume did not play an important role in diatom enumeration. However, the differences in abundance through the use of tools such as nylon brush and ceramic scraper makes it evident that the tools employed for microfilm removal are critical. Nylon brush was found to be a better tool than ceramic scraper (Fig. 3.3 and 3.4) as the percentage loss of diatom cells was comparatively higher in the latter (Fig. 3.4). Also, there were insignificant differences in the diversity between the tools. However, both the tools resulted in the loss of minor species contributing to the microfilm community. This loss is unavoidable due to the very low numbers of such species, which can go unnoticed even during direct observation. The loss of minor species is evident after comparison of results obtained through direct observations (complete slide) with that of scraped material (Table 3.5). However, such direct observations, involving the complete substratum are very time-consuming and laborious and cannot be employed for opaque materials. Taking the above observations into consideration, the nylon brush was employed for removal of microfilms developed over different substrata from Dona Paula Bay.

Dona Paula Bay exhibits strong seasonal gradients in both environmental variables as well as planktonic assemblages. Of these, physical environmental variability, driven

primarily by external forcing events like meteorology and climatology, is fundamental. Wind- and weather-driven events cause physical changes and disruptions. These events lead to variability in temperature, irradiance, precipitation, run-off, nutrient loading, etc. in coastal environments. Such changes are instrumental in the complete transformation of the phytoplankton community structure and production (Devassy and Goes 1988, Bhattathiri et al. 1976). The results revealed that the diatom community structure in the water column differed considerably from that of the microfilm. Obviously, such differences arise depending upon the mode of life (free living/attached) of diatoms. Earlier literature (Characklis and Cooksey 1983; Cooksey et al. 1984) as well as the present study suggests that pennate diatoms often dominate the diatom fouling community irrespective of the nature of substratum and exposure period (Figs. 3.8, 3.12, 3.16, 3.20 and 3.25) except during May, in case of glass substrata, due to the dominance of a centric diatom, *M. nummuloides*. The reverse was observed in case of the water column (Fig. 3.6b). The dominance of pennate diatoms has also been reported in biofouling on test panels exposed in the Arabian Sea (Bhosle et al. 1989; Kelkar 1989; Bhosle 1993; Raveendran et al. 1991; Pangu 1993; Venugopalan 1994; Prabhadevi 1995a; 1995b; Redekar 1997; 2000a; 2000b) and elsewhere (Aleem 1957, Reisen and Spencer 1970, Patrick and Roberts 1979, Stevenson 1981, Summer and McIntire 1982, Hudon and Bourget 1983, Edyvean and Moss 1986, Wetherbee et al. 1998). Among the pennates, *N. transitans* var. *derasa* f. *delicatula* was the most dominant followed by *A. coffeaeformis*, *Amphora* spp., *Navicula* spp., *Thalassionema* spp., *Fragilariopsis* spp., *C. closterium*, *Nitzschia sigma*, *Pleurosigma angulatum*, *Cocconeis* spp. and *Grammatophora marina*; followed by the centric diatoms (*Melosira nummuloides*, *Odontella* spp. and *Coscinodiscus* sp.) (Table 3.7a-d). These diatoms are primarily natives of the surrounding intertidal sediment flat (Mitbavkar and Anil 2002). Among



them, *N. transitans* var. *derasa* f. *delicatula* (an epipellic diatom) and *A. coffeaeformis* (an epipsammic diatom) are the most dominant in the microphytobenthic community of this study area (Mitabavkar and Anil 2002). The microphytobenthic diatoms get frequently resuspended into the surface waters through physical forcing factors like waves, tides, currents, etc. (de Jonge and van Beusekom 1992, Tomas 1997) and thereby influence the water column primary productivity (de Jonge and van Beusekom 1995). In the present study, dominance of these microphytobenthic diatoms in the water column was also reflected in the microfilm (Tables 3.7a-d and 3.10a-d). This suggests that hydrodynamic conditions might play an important role in the microfilm diatom community structure. Breznak et al. (1985) reported that fouling diatoms reach surfaces purely by hydrodynamic means. Hence it might be possible that once resuspended, benthic diatoms come under the influence of water medium for their survival until they settle to the bottom sediment or find a suitable substratum in the euphotic zone. If these diatoms colonize any substratum, then their growth and community structure is further dependent upon the physico-chemical and biological nature of the: 1) surrounding/ambient waters and 2) substratum.

#### **3.4.1. Surrounding/ambient waters**

This region is highly dynamic in terms of physico-chemical and biological properties. In general, waters are highly disturbed during monsoon and calm during pre- and post-monsoon periods. This study reveals that the maximum diatom population in the microfilm was found during post-monsoon followed by pre-monsoon and monsoon (Table 3.7a-d and Figs. 3.9, 3.13, 3.17 and 3.21) periods. However, the species composition and their contribution (%) to the microfilm diatom community varied with time and exposure period (Table 3.7a-d). *N. transitans* var. *derasa* f. *delicatula*, the most dominant species, occurred round the year both in the water column as well as in the 1 to

4 day-old microfilm (Table 3.7a-d). Maximum density was observed throughout the post-monsoon period followed by pre-monsoon (March). This species usually forms a mat (similar to bloom) during the post-monsoon period in the surrounding intertidal sand flat of this region (Mitbavkar and Anil 2002, personal observation). *Amphora* spp. was the next abundant during the post-monsoon followed by *Nitzschia* spp. during pre-monsoon (Table 3.7a-d). The timing of diatom peaks both in the microfilm and water column coincided (Figs. 3.6a, 3.9, 3.13). Hence it might be possible that the physico-chemical factors of the surrounding environment, not only influence planktonic diatoms but also the microfilm diatoms. However, these aspects required a detailed investigation. In the present investigation, two peaks of diatoms were observed as a recurrent feature of the annual diatom cycle. The first peak was observed during monsoon possibly when there is a break in rainfall or after the end of southwest monsoon (November 1999 and October 2000) and the second small peak (autumn bloom) was observed during the pre-monsoon period (March 2000) (Fig. 3.6a). The first bloom was dominated by planktonic diatoms like *Chaetoceros curvisetus*, *Leptocylindrus danicus*, *Cerataulina* sp., *S. costatum*, *Helicotheca tamesis* and pennate diatoms like *N. transitans* var. *derasa* f. *delicatula*, *F. oceanica*, *Thalassionema nitzschioides* and *Pseudo-nitzschia seriata* and the autumn bloom (March) was dominated by *N. transitans* var. *derasa* f. *delicatula*. Among them, only pennate diatoms dominated the microfilm irrespective of the nature of substratum (Figs. 3.8, 3.12, 3.16, 3.20 and 3.25). These observations indicate that the dominance of particular species in the microfilm can be attributed to their dominance in the surrounding waters resulting in a higher substrate-encountering probability.

With the onset of the monsoon, the study site experiences a high freshwater influx (Shetye et al. 1995) at a flow rate of  $>10,000$  ML day<sup>-1</sup>, when most of the annual precipitation occurs over the catchment area (Unnikrishnan et al. 1997). Hence, during

this period, stratification develops (Chapter 2-Fig. 2.2), with a warmer, lower salinity layer arising from Zuari river and local precipitation in the surface and the cold, saline and oxygen-depleted, upwelled water at the bottom (Naqvi et al. 2000). These processes have a pronounced influence on the native phytoplankton community in this estuary. The increased flow rate caused by riverine discharge can physically influence the diatom community in the surface waters. During monsoon, low diatom abundance in the microfilm was observed (Figs. 3.9, 3.13, 3.17 and 3.21) even though the nutrient levels were high (Fig. 3.5). This can be attributed to physical disturbances like high wave action, low light (cloud and high turbidity) and salinity levels (Chapter 2). Earlier literature suggests that such high physical disturbance reduces the rate and type of diatom colonization (Young 1945, Cattaneo and Kalff 1978, Hudon and Bourget 1981 and 1983). During this investigation, it was observed that the salinity drops to 15 psu. The peak in nitrate and silicate concentration in July followed by a decline during August and September (Fig. 3.5) coincided with blooms of centric (*S. costatum*) and pennate (*F. oceanica* and *T. nitzschioides*) diatoms in the ambient waters suggesting nutrient uptake by these species. During August and September, these species, except *S. costatum*, dominated the microfouling community developed over fiberglass and glass substrata, as they turned out to be better competitors than the others suggesting the influence of blooms on the microfilm community developed over fiberglass and glass (Table 3.7a-d).

### **3.4.2. Substratum**

The diatoms, which have an attached mode of life, once dislodged, attach to any suitable substratum. In the present study, the results of the population dynamics of such diatoms with respect to their choice of substratum are discussed. The highest diatom abundance and species were encountered on fiberglass followed by glass as compared to that on

copper and cupro-nickel substrata (Tables 3.6a and b; Fig. 3.24). This can be attributed to their toxic and nontoxic surface properties respectively. The influence of surface properties was also evident with increase in exposure time wherein; abundance and diversity increased on non-toxic surfaces (Figs. 3.9 and 3.13) and decreased or remained constant on toxic surfaces (Figs. 3.17 and 3.21). The long-term exposure of copper and cupro-nickel substrata also did not show any considerable increase in diatom abundance with exposure period; however, the species diversity decreased as compared to that during short-term exposure (Figs. 3.26). These substratum preferences indicate either an active choice by the diatoms, or that the physical and chemical conditions of the substrata encourage or discourage settlement or entrapment of some species. While selective community development on toxic surfaces is well known (Daniel and Chamberlain 1981), any differences in communities developing on non-toxic surfaces are due to more subtle environmental influences, for example topography, surface charge, surface tension or nutrient availability (Fletcher 1980, Escher and Characklis 1980, Fattom and Shilo 1984, Becker and Wahl 1991, Sekar et al. 2003). Fiberglass and glass are comparatively hydrophobic and hydrophilic in nature respectively (Becker and Wahl 1991, Sekar et al. 2003, unpublished data). Earlier literature suggests that the rate of microfouling is quicker on hydrophobic than on hydrophilic surfaces (Characklis and Cooksey 1983, Wahl 1989, Becker and Wahl 1991, Castlow and Fletcher 1994). This could be one of the reasons for the difference in abundance of diatoms on both these substrata (Figs. 3.9 and 3.13). Although the diatom communities are similar, most of the diatom species showed significant substratum preferences (Table 3.7a-d). These substratum variations, which resulted in higher diatom recruitment on non-toxic substrata was also responsible for the significant relationship in diatom abundance and chlorophyll *a* concentration for fiberglass (Figs. 3.10) and glass (3. 14), while it was restricted up to

2 days-old community on toxic substrata (Figs. 3. 18 and 3. 22), indicating that diatoms are the major contributors among the autotrophs in the microfilm. Similar findings have also been reported elsewhere, both for natural (Scheer 1945, Santelices et al. 1981), and artificial substrata (Neushul et al. 1976, Edyvean and Moss 1986). The diatoms are not only dominant among micro-algal groups but also to other unicellular eukaryotes like yeasts, protozoans etc. (Marshall et al. 1971, Corpe 1972, Cuba and Blake 1983). These diatoms attach to the substratum by mucus secretion (Cooksey et al. 1984, Ferreira and Seeliger 1985), may densely cover wide substratum areas, and contribute significantly to the chlorophyll pool of the microfilm developed over non-toxic substratum i.e. fiberglass and glass; however, in the case of toxic panels i.e. copper and cupro-nickel, the non-significant relation between diatom abundance and chlorophyll *a* on day 3 and 4 (Fig. 3.18 and 3.22) can be attributed to the toxic effect on microfilm diatoms. Literature also revealed that the copper exposure caused stronger effects on algal (periphyton) biomass and community structure (Guasch et al. 2002).

Maximum diatom recruitment was observed on non-toxic substrata (fiberglass and glass) compared to toxic substrata (copper and cupro-nickel) (Table 3.7a-d). It was further evident that dominant diatoms like *N. transitans* var. *derasa* f. *delicatula* (2- to 15-fold), *Amphora* spp. (2- to 10-fold), *Nitzschia* spp. (upto 25-fold), *Thalassionema* spp. (upto 4-fold) showed many-fold increase on day 4 while the others showed up to 3-fold increase or remained constant whereas some species disappeared from the non-toxic panels (3.7a-d). Among the dominant diatoms, recruitment of *N. transitans* var. *derasa* f. *delicatula* was higher than that of the other species (Table 3.7a). On certain occasions, it was found that the initial recruitment of *N. transitans* var. *derasa* f. *delicatula* was almost the same as that of other species like *Amphora* spp., *Nitzschia* spp. and *T. nitzschioides* (Table 3.7a) but on day 4, *N. transitans* var. *derasa* f. *delicatula* turned out to be the best

competitor (Table 3.7d). Probably, the dominance of *N. transitans* var. *derasa* f. *delicatula* might have suppressed the development of other species. However, this aspect requires a detailed investigation. Such a trend was not observed on the toxic panels (for both short term and long term exposure) (Tables 3.7a-d and 3.10a-d) indicating that the dissolution of the panels by bacteria (Sreekumari et al. 2003) and the toxic nature of the substratum (Chamberlain and Garner 1988, French and Evans 1988) might have influenced the development of diatom community. However, the presence of diatoms such as *N. transitans* var. *derasa* f. *delicatula*, *Amphora* spp. *Thalassionema* spp. and *Nitzschia* spp. throughout the study period (Table 3.10a-d) further reveals their tolerance to the toxicity of the substrata.

In the pre-monsoon period (April-May), the comparatively higher turbulence caused by the water movement, resulted in the dislodgement from bottom substrates of a centric diatom, *M. nummuloides* into the surface waters. *M. nummuloides* is known to lead an attached mode of life (Aleem 1973; Rendall and Wilkinson 1983; Ferreira 1985; Raveendran et al. 1991). When brought into the water column, such tychopelagic forms, which require a space for attachment, will tend to settle on a suitable substratum. In this study, *M. nummuloides* was found to dominate on the glass substrata in May thereby changing the pennate/centric ratio to  $< 1$ . This species was also encountered on fiberglass substrata, but only in 1 and 2 day-old microfilm community and were absent on toxic substrata. It was also evident that the recruitment of *F. oceanica* during the monsoon was negligible on copper and cupro-nickel substratum. These observations indicated species-specific substratum preferences.

In conclusion, the results of the present investigation revealed a significant difference in the abundance and diversity of diatoms encountered on different substrata i.e. toxic and

non-toxic but the species composition remained almost constant (Figs. 3.9, 3.13, 3.17, 3.21 and 3.26). Earlier literature has also reported similar observations (Wetzel and Westlake 1969, Neushul et al. 1976). The only indications of different microfilm diatom community structures are between the period (months/seasons) i.e. temporal variations and the implication of substratum specificity (Tables 3.7 and 3.10), which can be attributed to physico-chemical and biological changes of the ambient waters and the substratum. Temporal variation in the tychopelagic diatom community structure (temporarily dislodged benthic diatom community) of the surrounding water column was observed to exert its influence on the microfilm community. Such differences in the diatom community structure in the microfilm might offer differing signals to settling larvae of sedentary organisms, which are critical in determining the functional ecosystem of the benthic environment.