

## Abundance of Bacterial and Diatom Fouling on Various Surfaces

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Abundance of bacterial and diatom fouling on aluminium, fibreglass and stainless steel were studied from Dona Paula waters of the Zuari estuary. Both these forms were reversibly attached in large numbers to surfaces during the initial 24 hr of exposure and thereafter an irreversible attachment was observed until the end of sampling (144 hr). *Nitzschia* sp was found to be most abundant on surfaces as well as in the subsurface waters. The other forms present included *Navicula* sp, *Pleurosigma* sp, *Grammatophora* sp, *Coscinodiscus* sp, *Fragilaria* sp, *Licmophora* sp, *Chaetoceros* sp and *Rhizosolenia* sp. Pennate forms were more dominant as compared to the centric forms. The mucilaginous fibrillar material entrapping bacteria and diatoms formed a two-tiered layer on study surfaces. Significant correlation existed between the two forms on all the surfaces for the study area.

**Key Words:** Microfouling, Diatoms, Bacteria, Various substrata

### Introduction

Studies on the short term effect of microbial fouling, caused mainly by diatoms and bacteria, are scanty. An attempt is made here to quantify the primary settlers like bacteria and diatoms on different surfaces (Characklis 1973, Characklis & Cooksey 1983) and study their interrelationship. Visual morphology of the surfaces of coupons (small panels of size 5 × 5 cm) with special reference to diatoms is also presented.

### Materials and Methods

**Study area:** The site chosen for the present study was situated at the mouth of the Zuari estuary (15.31° N, 73.59° E), located on the west coast of India, along the Arabian Sea. It receives a large quantity of fresh water (150-400 m<sup>3</sup>. sec<sup>-1</sup>) during the south-west

monsoon season, while, the discharge for the pre- and post-monsoon season is reported to be insignificant (10 m<sup>3</sup>. sec<sup>-1</sup>), (Shetye & Murty 1987). In this area the waters were well mixed for most part of the year.

**Study surfaces:** Three types of surfaces viz. aluminium, fibreglass and stainless steel, measuring 15 × 10 cm (as test panels) and small sized 5 × 5 cm (test coupons) for SEM studies were used. Test coupons were thoroughly polished using emery paper of gradient sizes and treated with xylene and ethanol. Test panels were thoroughly washed as described by Bhosle et al. (1990), dried and arranged on fibreglass frames using PVC nuts and bolts. The coupons were tied using nylon strings onto a frame of fibreglass. Both the frames were immersed in the surface waters (~ 1m) of Dona Paula.

They were retrieved at daily intervals (24 hr) over a period of 6 days (144 hr), during the first week of April '89, May, August, September, December and January '90. After retrieval, test panels were scrapped using a nylon brush as suggested by Sharma et al. (1990) and used for experimental purposes. Simultaneously subsurface water samples were collected using Niskin water sampler (5L) to determine the microbial count in the waters of the study area.

#### *Diatom Count*

Samples of microfouling as well as water samples (known volume) were fixed with Lugol's iodine solution. Dilutions were made and replicates (10) of known quantity (0.01 ml) were taken on slides to determine the diatom numbers using a microscope (Olympus, BH-2) as suggested by Hitchcock (1982).

#### *Bacterial Count*

Scrapped material was diluted while water samples (known volume) was added to a test tube containing 0.1% acridine orange, filtered through 0.2  $\mu\text{m}$  nucleopore filter (previously stained with amido black) and the filter paper containing the filtered material was fixed in a drop of immersion oil and mounted by adding another drop of oil on top of the coverslip. The bacteria which fluoresce green against a black background were counted in 10 random fields for each sample using epifluorescence microscope (Nikon) as suggested by Parsons et al. (1984).

A number of precautions were taken to obtain consistent results. First the acridine orange solution as well as water used for dilutions of samples were filtered through 0.2  $\mu\text{m}$  (pore size) nucleopore filters each day before the start of counting. Blanks of acridine orange and dilution water was run each day. At least 2 ml of the fluid was made to pass through the filter to give a random

distribution of cells. A 2% solution of formaldehyde was used to fix samples after collections.

#### *Scanning Electron Microscopic Study*

Test coupons were rinsed with filtered seawater and placed in 5% (V/V) solution of glutaraldehyde, then washed in a series of increasing concentrations of acetone solution and freeze dried (Marszalek & Small 1969), in order to minimize the shrinkage and distortion of artifacts caused by air drying. Dried specimens were then fixed onto SEM stubs using epoxy resin and sputter coated with gold for 2 min. at 1.3 KV ( $\sim 600^\circ\text{A}$ ) which provides a conducting material so as to eliminate or reduce electric charge which builds up rapidly in a nonconducting specimen when scanned by a beam of high energy electron. Scanning electron microscope (Cambax EPMA) was used to examine specimens.

## **Results**

#### *Temporal Variations of Diatoms*

During the first sampling all the surfaces showed high diatom numbers. Subsequently, for the next sampling (48 hr), there was a drop in the diatom count for all the surfaces. Thereafter, the number showed an increase over the period of immersion.

Variation was also evident with respect to the nature of surface used for the study. Initially (24-48 hr), higher values were observed on fibreglass test panels during all the sampling months. With the increase in exposure time aluminium showed higher diatom abundances as compared to fibreglass and stainless steel. Stainless steel on the other hand, showed lower diatom numbers over the period of sampling.

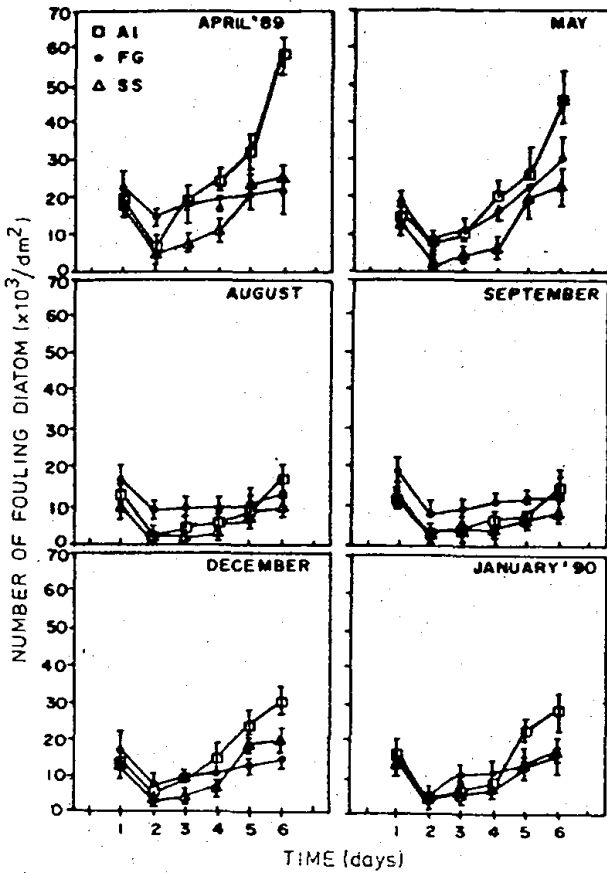
Diatom settlement on test panels also showed seasonal fluctuations, highest during April and May (pre-monsoon

season) and the least during August and September (monsoon season), while intermediate during December and January (post-monsoon season).

The average count of diatoms from the subsurface waters of Dona Paula showed a high number ( $23 \times 10^2$  cells/l) during April and May (pre-monsoon season), intermediate ( $9 \times 10^2$  cells/l) during December-January (post-monsoon season) and minimum ( $3 \times 10^2$  cells/l) during August-September (monsoon season).

*Temporal Variation of Bacteria (figure 2)*

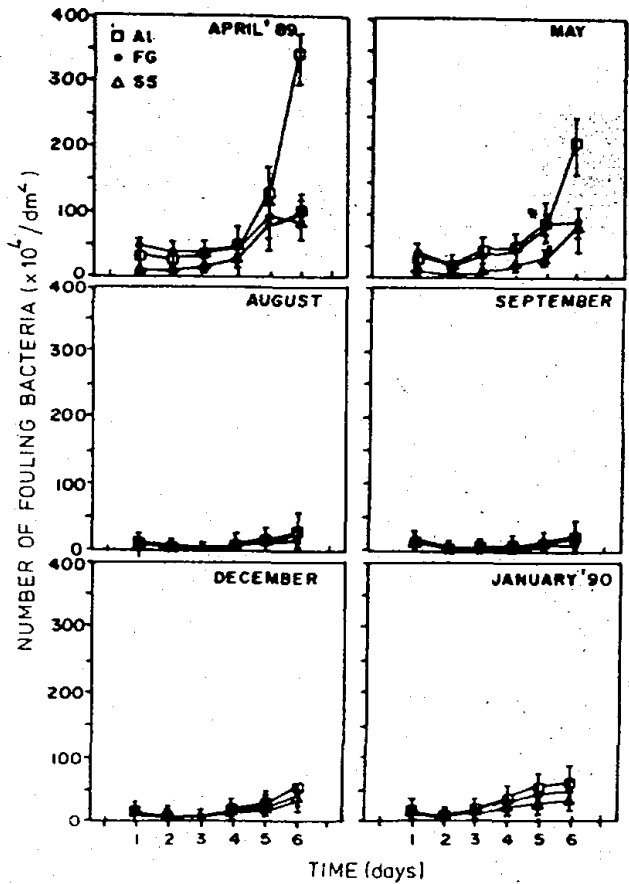
Bacterial counts, in general were higher than the diatoms. Bacterial densities were high initially (24 hr) which dropped during the second sampling (48 hr) followed by a steady increase until a maximum was reached on the last sampling day (144 hr).



*Surface preference:* Initially a very high bacterial count was seen on fibreglass test panels. However, with the increase in time aluminium showed maximum number. Stainless steel on the other hand showed minimum number for all the months sampled.

*Seasonal trend:* The bacterial numbers was highest for the pre-monsoon (April-May) intermediate during the post-monsoon (December-January) and minimum during monsoon season (August-September).

The average bacterial count for the subsurface water was highest ( $17 \times 10^3$  cells/l) during the pre-monsoon, followed by post-monsoon ( $9 \times 10^3$  cells/l) and monsoon season ( $2 \times 10^3$  cells/l).



**Figure 1** Abundance of fouling diatom developed on aluminium, fibreglass and stainless steel surfaces during different months

**Figure 2** Abundance of fouling bacteria developed on aluminium, fibreglass and stainless steel surfaces during different months

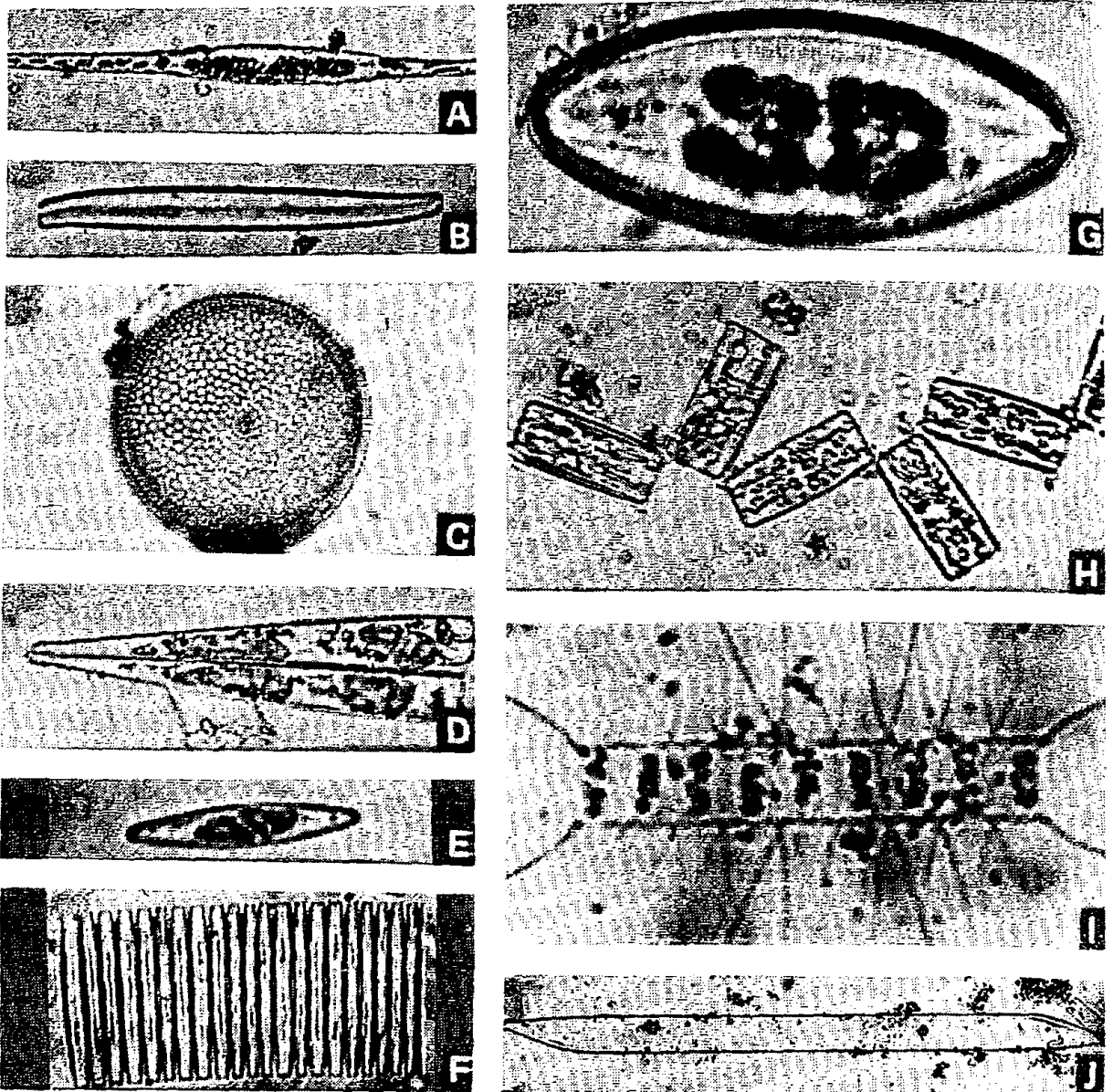


Figure 3A-J Graphs showing fouling diatoms: A, *Nitzschia* sp; B, *Pleurosigma* sp; C, *Coscinodiscus* sp; D, *Licmophora* sp; E, *Navicula* sp i; F, *Fragilaria* sp; G, *Navicula* sp ii; H, *Grammatophora* sp; I, *Chaetoceros* sp; J, *Rhizosolenia* sp from the various study surfaces

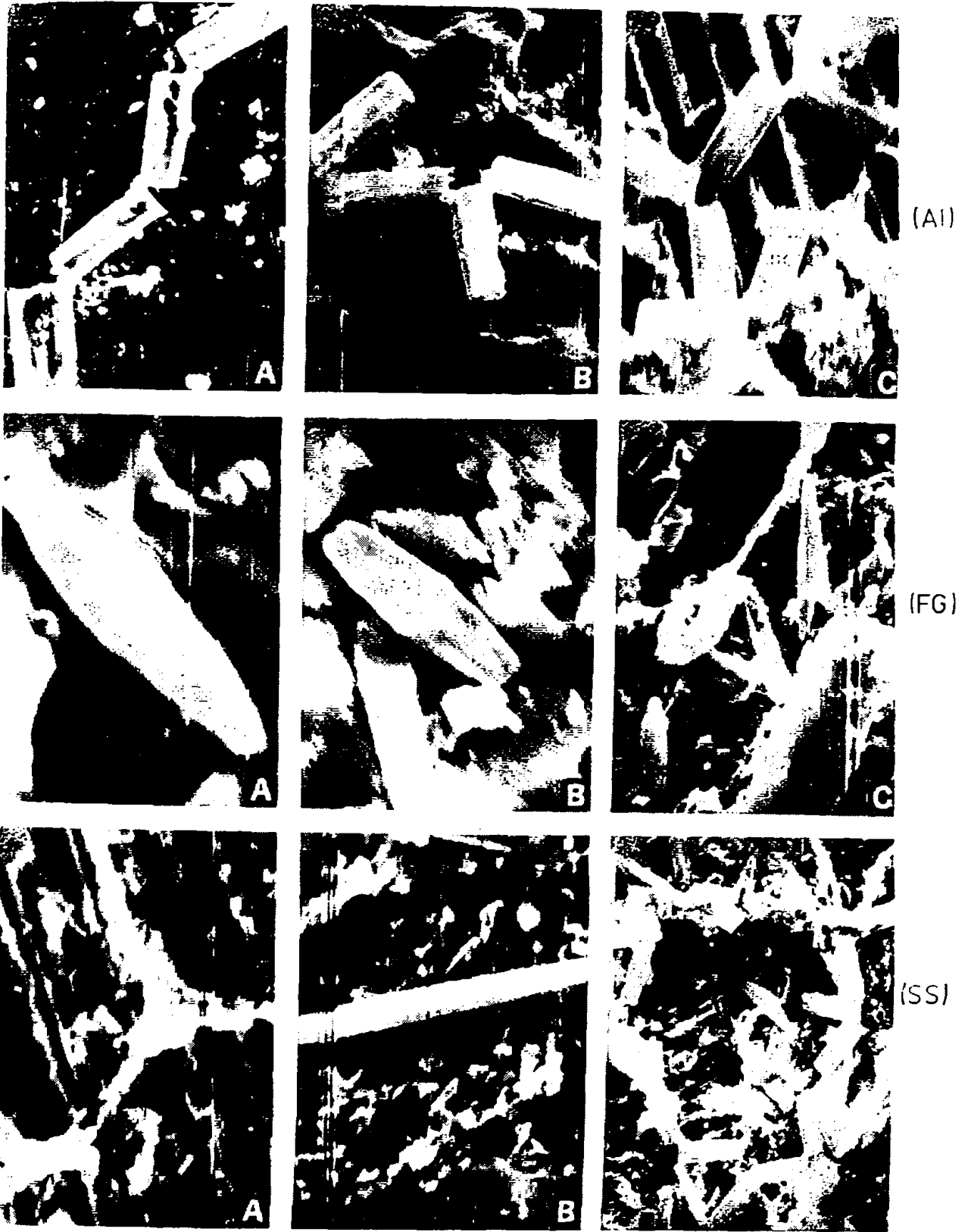


Figure 4 SEMs of aluminium, fibreglass and stainless steel coupons exposed for 24, 96 and 144 hr ( $\times 2000$ )

### Microscopic Photographs/Scanning Electron Micrographs

The diatoms commonly encountered from the microfouling material were: *Navicula*, *Nitzschia*, *Pleurosigma*, *Coscinodiscus*, *Fragilaria*, *Licmophora*, *Chaetoceros*, *Rhizosolenia*, *Grammatophora* (figure 3A-J). SEMs of aluminium, fibreglass and stainless steel coupons exposed for 48, 96 and 144 hr (figure 4 A-C) showed an abundance of *Grammatophora* sp on aluminium test coupons exposed for 48 hr, whereas, *Navicula* sp was more abundant on fibreglass and stainless steel test coupons. Coccoid bacterial cells were most commonly observed on all the surfaces. Coupons which were exposed for 96 hr showed mucilaginous fibrillar material entrapping several microbial cells. Towards the end of sampling (144 hr) the slime film on the surface enclosed clusters of microbial cells to form a two-tiered layer giving rise to a complex microsystem on surfaces (figure 4C).

Interrelationships between the two forms of fouling organisms were attempted and significant correlations were obtained for aluminium ( $r=0.86$ ,  $p < 0.001$ ,  $n=36$ ), fibreglass ( $r=0.83$ ,  $p < 0.001$ ,  $n=36$ ) and stainless steel ( $r=0.80$ ,  $p < 0.001$ ,  $n=36$ ).

### Discussion

Any clean surface when immersed in seawater becomes a site for the attachment of microorganisms. These microorganisms produce a mucilaginous material which entraps detrital matter to constitute a slime film (Loeb & Neihoff 1977). Such film is usually the first form of fouling to appear on submerged surfaces. Earlier investigators have reported bacteria to be the first organisms to attach onto surfaces (Zobell & Allen 1935, Corpe 1970). According to these investigators bacterial population along with mucilaginous slime may act as a trap for other organisms. Miyachi et al. (1989),

reported that biofilm consisting of bacterial strains had a promoting effect on diatom settlement. On the other hand, Kawamura et al. (1988), was of the opinion that bacterial biofilm had no significant effect on the attachment of diatoms. Another school of thought opined that surfaces which are under water and are well illuminated support algal population and in many cases these algae are diatoms. According to them, these diatoms colonize prior to bacteria (Skerrmann 1956, O'Neil & Wilcox 1971). There are others (Paul et al. 1977) who are of the opinion that the diatoms, fungi and cyanophytes can occur at any stage before or after bacterial proliferation.

Thus, we may suggest that the microbial community formed on test surfaces generally showed bacteria and diatom to be most abundant (Zobell & Allen 1935, Callow et al. 1976). The abundance of these organisms in the primary film may influence the physical and chemical conditions of the surface (Terry & Edyvean 1984, Edyvean 1984) as well as subsequent colonization by macrofouling larvae (Crisp & Ryland 1960, Barnes 1970).

The irregular growth pattern of diatom and bacteria especially in the earlier stages of microfouling (figure 1 & 2) seems to be a regular phenomena on various surfaces immersed in marine waters (Yanshun et al. 1984). This was probably due to reversible attachment of microorganisms to surfaces initially, followed by an irreversible attachment towards the end of sampling.

The differences shown by the settlement of microfoulers on various surfaces was because of the differences in the nature of the surfaces used. Fibreglass being hydrophobic accumulated higher microbial number initially. A similar case was also reported by Fletcher (1988) and Pedersen (1990). On the other hand aluminium being hydrophilic accumulated lower microbial number

initially. However, the microbial count increased drastically with time, until the end of sampling. Stainless steel being an alloy had a more electropolished surface and is known to be more resistant to microbial attack (Zoltai et al. 1981, Dunsmore et al. 1981).

As in the present study, predominance of *Navicula* from the microfouling material has also been reported from tropical and temperate waters (Cooksey et al. 1984, Bhosle et al. 1989). Of the eight different forms of diatom species reported from Dona Paula waters, five belonged to the pennate forms and three to the centrales. From this it is evident that pennate forms dominated in the present study area. Such forms are known to foul man-made structures in temperate and tropical waters too (Characklis & Cooksey 1983, Cooksey et al. 1984, Bhosle et al. 1989, Raveendran et al. 1991). Similar dominance of pennate forms has been reported from microfouling material developed on test surfaces exposed to the offshore waters of the Arabian Sea (Bhosle et al. 1989, Kelker 1989).

Several workers suggest that bacteria were the first organisms to appear on surfaces placed in seawater (Mitchell & Krichman 1984, White & Bensen 1984, Yanshun et al. 1984). However, our observations showed that in addition to bacteria, diatoms also existed in large numbers during the initial 24 hr. This probably showed that both diatom as well as bacteria co-existed on surfaces.

Although bacterial number was several times higher than the diatom number in the microfouling film, for all the seasons, seasonal pattern was identical for both forms of organisms. The pre-monsoon season contributed to the peak numbers of both forms for all the surfaces. This was probably due to the high number of cells in the subsurface water during the study period as compared to the post-monsoon and monsoon season when the count was minimum.

Furthermore, it was interesting to note higher microbial numbers on surfaces for all the seasons as compared to the number in the ambient surface seawater. This could probably be due to selective increase in survival, rather than to an increase in growth, because the surface matrix offers protection against lethal agents unlike in the bulk phase. It could also be due to mutualism between community members or facilitation of extracellular enzyme activity (Fletcher 1984).

Significant correlations between diatom numbers and bacterial count for all the surfaces could mainly be due to the fact that bacteria grow by consuming the organic material produced by algae. The algal community to some extent influences the composition of bacteria (Riquelme et al. 1987). Biofilm containing both green algae and bacteria gave rise to a condition wherein the heterotrophs utilized extracellular products from the phototrophs which in turn depends on bacterial products such as vitamins (Furuki et al. 1985), siderophores (Murphy et al. 1976) and other trace elements. Thus, the dependence between the two different groups of microorganisms within a biofilm allowed growth of both these forms on various surfaces.

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