BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE MARINE MICROFOULING MATERIAL DEVELOPED ON VARIOUS SUBSTRATA

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BY

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UNDER

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Dedicated to

My

Parents

&

Mrs. Parukutty Panikkar

INTRODUCTION

Due to the considerable potential of resources from the seas, man is dependent on them for food, energy, raw materials, transportation and also recreation. The increasing gap between the demand and supply of the world's resources emphasizes the urgency to exploit the marine resources. One of the major problems facing anthropogenic marine activities in exploitation of these resources, is the biodeterioration of various materials of the systems deployed for these activities. They comprise of sea-going vessels, harbour and offshore structures, OTEC plants, marine engineering and scientific equipments, etc.

Biodeterioration may be defined as the destruction of desirable materials in the sea through the action of marine organisms (Eggins & Oxley, 1980). Traditionally, the phenomenon of biodeterioration has been divided into three categories viz., biocorrosion, wood-boring and biofouling.

The process of marine biofouling which has been accepted since centuries as a natural phenomenon, is caused due to the settlement of organic, inorganic and biotic

STATEMENT

As required under the Ordinance No. 15.3, I state that the present thesis entitled " Biochemical and molecular characterization of the marine microfouling material developed on various substrata", is my original contribution and that the same has not been submitted for any degree of this or any other university on any previous occasion, to the best of my knowledge.

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Chapter 1

Introduction

matter on solid substratum. This settlement is called biofouling when it causes impediment to the proper functioning of these materials. Biofouling is further classified into microfouling and macrofouling.

Microfouling

The living cells which attach, grow, reproduce and produce exopolymers on surfaces, extend to form a fibrilar matrix. This matrix which encloses dead and living cells and other debris is termed as biofilm. Such a film which is responsible for deterioration of materials of human interest is called microfouling.

A simple modified diagramatic representation of the marine fouling cycle is given in Fig 1.1 (Zahuranec, 1988). The highlighted points are the main area of research today. The initial processes occurring with the introduction of materials into the sea are so complex, that a thorough study of each process is needed. One such process is microfouling and hence these studies have been undertaken.

1. CONDITIONING FILM

A clean surface of any type of material, when introduced in seawater gets conditioned by a layer of organic macromolecules and other low-molecular weight species (Neihof & Loeb, 1972; Baier, 1975; Loeb & Neihof, 1977; Characklis & Escher, 1988; Zutic & Tomaic, 1988). Low molecular weight species include, sugars, amino acids and biogenic salts such as NH_4^+, PO_4^{-3}, etc., as well as inorganic ions like Ca^{+2}, Na^+, H^+, etc., (Fletcher, 1984). The presence of these compounds alter the surface charge and free energy (measured in terms of wettability) of the surface (Zobel, 1943; Smith, 1961; Chave, 1965; Baier, 1973; Dexter et al, 1975; Baier, 1980; Walch, 1986; Marshall, 1992). The formation of a conditioning film is the most rapid process and takes place within minutes of the surface being exposed to sea water (Neihof & Loeb, 1972; Baier, 1975). It is a relatively selective process and not all organic species in the water column are adsorbed onto these surfaces. (Corpe et al, 1976). Marshall (1979) has described this conditioned surface as a relatively nutrient-rich heaven in an otherwise nutrient deficient environment. Thus, the conditioned layer is important in establishing a base for subsequent build-up of

the microlayer. The molecular surface that a microorganism comes into contact with, is not the original surface, but is the modified one formed by the conditioned film. The chemical composition of the conditioning film is reported to consist mainly of polysaccharide and sugars (Stotzky, 1985). In addition, glycoproteins (Humphrey et al, 1979), proteins and nucleic acids (Nishikawa and Kuriyama, 1968) are also reported to be present.

Earlier, chemical characterization of this layer was studied by several research scientists like Baier, (1973); Loeb & Neihof, (1975); Baier, (1984); Little & Zsolnay, (1985). These studies were difficult due to the chemical complexity and minute quantity of the material involved. However, with sophisticated instrumentation and advanced techniques, it is now possible to determine nanogram quantities of substance present in the microfouling material.

2. BIOFILM OR PRIMARY FILM

It is very difficult to make a clear demarcation between a conditioned film and a biofilm. However, a

conditioned film with microorganisms embedded within the matrix can be labeled as a biofilm. The various stages in biofilm development consist of initial colonisation of the conditioned surface. This is followed by growth and further adhesion of microorganisms to form a multilayer in a polymer matrix, which finally leads to the formation of a biofilm (Marshall, 1992). Such biofilms are reported to render even toxic surfaces like copper, relatively non-toxic and thus permit a variety of bacteria to settle on them (Bitton & Freihofer, 1978). Much confusion exists over the terminology for the extracellular material intimately related to biofilms. Glycocalyx, slime, capsule or sheath, have all been used in referring to extracellular polymeric substance/s (EPS) associated with individual cells, cell aggregates or biofilms (Bowler & Marsh, 1982). Therefore, unless extensive identification has been done, the organic matrix will be referred to as extracellular polymeric substances (EPS). The slime film was first studied by Bray (1923), with reference to the control of fouling. A number of workers have observed that bacteria are the first to colonize on conditioned metal and non-metal substrata in marine environment (Marshall et al, 1971b; Corpe, 1977; Marszalek et al, 1979; Sieburth, 1979). However, this is true in the case of surfaces which are not

illuminated. On surfaces which are wet and exposed to sufficient sunlight, in addition to bacteria, algal populations occur in large numbers (Daniel & Chamberlain, 1981; Terry & Edyvean, 1981; Escher & Characklis, 1982; Characklis & Cooksey, 1983; Edyvean & Terry, 1983). Hence, initially, the surface is reportedly covered with a layer of bacteria, fungi and non-motile small diatoms. Later, motile diatoms, microalgae, filamentous fungi, debris, flagellates and other protozoa attach onto this layer to complete the formation of the primary film (Marszalek et al, 1979).

2.1 **Adhesion of cells to surfaces**

Two distinct phases have been proposed to explain the initial attachment of microorganisms to solid substrata. (Zobel, 1943; Marshall et al, 1971; Marshall, 1976; Brusscher & Weerkamp, 1987).

The microorganisms are initially held weakly to the surface. This is termed as "reversible sorption" It is so called due to the ease with which the microbes can be dislodged from the surface. Motile organisms are attracted

to the nutrient rich conditioned surface and they actively swim towards it (Young & Mitchell, 1973). On the other hand non-motile organisms depend on currents, wave motion and capillary flow.

Once the cells are temporarily attached to the surface, they begin to secrete extracellular polymeric substances or proteinaceous appendages (Corpe, 1970b; Fletcher & Floodgate, 1973; Sutherland, 1982; Jones & Isaacson, 1983). Extracellular polymeric substances help the cells to anchor firmly onto the substratum by polymer bridging, using various combinations of chemical bonding (electrostatic, co-valent, hydrogen), dipole interactions (dipole-dipole, dipole-induced dipole, ion-dipole) and hydrophobic interactions (Marshall, 1992). Marshall et al, (1971a) suggest that polymer bridging has been responsible for firm anchoring of bacteria to the surface. Fig 1.2 (Jones & Isaacson, 1983) gives a diagramatic representation of bacterial adhesion.

Three distinct interaction regions are defined by the separation distance between the bacterium and substratum. At separation distances > 50nm, only Van der Waals forces operate. This stage is reversible. At separation distances

between 20 and 10nm, both Van der Waals forces and electrostatic repulsions are active. Adhesion during this stage is initially reversible but may change with time to an essentially irreversible stage. At separation distance of < 10nm, Van der Waals forces, electrostatic and specific interactions, such as the production of exopolysaccharides, lead to irreversible bonding (Marshall et al, 1971a; Marshall, 1976; Dempsey, 1981; Kelly, 1981).

2.2 **Factors affecting biofilm formation**

Physical, chemical and biological properties of a biofilm are dependent on the immediate environment to which the surface is exposed (Characklis & Cooksey, 1983).

The major factors which affect microfouling settlement are:

i. Microbial cell concentration in the bulk medium (Fletcher, 1977; Bryers & Characklis, 1981)

ii. Temperature (Kinne, 1970; Costlow & Bookhout, 1971; Characklis, 1980)

iii. Salinity (Kinne, 1963; 1970)

iv. Nutrient concentration (Trulear & Characklis, 1982)

v. Fluid-shear stress at the liquid-solid interface (Bryers & Characklis, 1981; Trulear & Characklis, 1982) and

vi. Characteristics of substratum surface (Dexter, 1975; Loeb & Neihof, 1975; Fletcher & Loeb, 1979).

Influence of microfouling on macrofouling settlement

The question whether macrofouling is dependent on microfouling is still a subject of considerable debate. The opinion that microfouling is essential for macrofoulers (Barnes, 1970) was prevalent till quite recently and this fact was proved in the laboratory (Miller et al, 1948; Knight & Jones, 1951; Meadows & Williams, 1963; Muller, 1973; Kitamura & Hirayama, 1986) and in the field (Zobel & Allen, 1935; Wood, 1950; Daniel, 1955; O'Neill & Wilcox, 1971; Mitchell et al, 1977). However, the precise role played by the primary film to induce settlement remains unclear. Microfouling organisms trap nutrients from the bulk phase and this activity leads to maturation of the film into ecologically more complex system capable of supporting a diverse array of species (Kitamura & Hirayama, 1986; Blenkinsopp & Costerton, 1991). The nutrients

present in the biofilm could be as a result of cell death or cell metabolism. Metabolite of one species may be nutrient for another. The substratum thus acts as a stimulus for attracting larvae of macrofoulers (Crisp 1974; Scheltema 1974; Fletcher, 1976; Burke 1983; Zaidi et al, 1984; Hadfield 1986). The biofilm is known to act as a buffer which is important, especially to hard shelled animals, as deposition of $CaCo_3$ is pH dependent (WHO1, 1952). Microfouling layer also acts as a holdfast for larvae of macrofoulers. Inspite of all these studies there are researchers today who insist that the primary film is not a must for secondary development of fouling (Crisp, 1984; Rittschof et al, 1984; Maki et al, 1988). Although it is not a prerequisite, it may hasten the settlement of macrofoulers on surfaces.

Implications of microfouling

Merits

Microfouling may prove to be beneficial in natural waters as well as in some modulated or engineered biological systems, by controlling water quality and by

influencing dissolved oxygen levels. According to Characklis & Escher, (1988), they are also responsible for the removal of soluble and particulate contaminants from natural streams and from waste water treatment plants. Glycocalyx which is the extracellular polymeric material intimately related to the biofilm has a tendency to attract heavy metal ions by forming chelates, thus helping in removal of heavy metal contaminants (Blenkinsopp & Costerton, 1991).

Demerits

i) Increased frictional resistance
ii) Loss of heat efficiency
iii) Microbial corrosion

The development of microfouling on under-water marine structures like buoys (Anon, 1952) and sea-going vessels have long been of concern. Fouling increases a ship's frictional resistance (Christie, 1973), leading to increased fuel consumption, loss of speed and thus causing additional engine stress. This necessitates expensive

periodic scraping to remove the growth and also the application of antifouling paints.

In heat transfer equipments, microfouling impedes the flow of heat across the interface (Aftring & Taylor, 1979), and in water distribution & waste water transport system, causes closing of valves and fitters (Picologlou et al, 1980).

Another major disadvantage of microfouling is the problem related to microbial corrosion. Miller & King (1975), have discussed several aspects of this topic in detail. Microbial metal corrosion takes place due to

a) Uptake of nutrients including oxygen by microbial growth attached to metal surfaces (Fig 1.3)

Mechanism of corrosion occurring due to uptake of nutrients in the presence of microbes is explained by Miller & King (1975), showing the setting up of a differential aeration cell as shown in Fig 1.3. The corrosion mechanism is simply the formation of concentration cells by the uptake of nutrients, including oxygen, during growth. Once established, the concentration cell maintains itself even when nutrient uptake by organisms has ceased.

b) Liberation of corrosive metabolites or end products of fermentative growth such as organic acids,

c) Production of sulphuric acid by sulphate reducing bacteria (-SRB-) such as Thiobacillus sp., and

d) Interference with the cathodic process in oxygen-free conditions by SRB at the biofilm-metal interface by consuming hydrogen and causing cathodic depolarization (Filip & Hattori, 1984; Marshall, 1992).

Thus, the role of microorganisms is either to assist in the establishment of the electrolytic cell (indirect) or to stimulate the anodic or cathodic reactions (direct). According to Freeman (1978), such deteriorations are more severe in the tropical waters.

Thus, it can be seen that the disadvantages outweigh the advantages. Hence, growing awareness of the problems posed by microfouling has led to a more detailed study of this problem.

Aim of present study

From the foregoing account, it is evident that microfouling plays a central role in the overall process of material deterioration. The review of literature shows

that there has been very little work done on this topic in tropical waters and hence these studies were undertaken. The present study deals with the biological, biochemical and molecular characterization of microfouling material developed on various surfaces, when immersed in the marine environment. It deals with the monitoring of various environmental parameters, such as, temperature, salinity, dissolved oxygen, nutrients like nitrate, phosphate and silicate. The suspended load was also monitored as suspended particulate matter (SPM), particulate organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC), particulate carbohydrate (PCHO), particulate organic nitrogen (PON) as well as chlorophyll of the sub-surface waters of the Zuari estuary at Dona Paula point.

Simple regression analysis using Lotus 1-2-3 software package was used to study the interrelationship between various environmental parameters and microfouling.

In addition, an assessment of microfouling on various substrata such as aluminium (metal), fibreglass (non-metal) and stainless steel (alloy) for different durations, has been carried out.

Evaluation of various techniques to estimate microfouling biomass in terms of total dry-weight (DW), fouling organic matter (F-OM), fouling organic carbon (F-OC), fouling organic nitrogen (F-ON) and fouling chlorophyll^{a} (F-CHL^{a}) was done. The carbohydrate composition as well as fouling inorganic matter (F-IM) of the microfouling biomass was also studied.

Furthermore, an attempt has been made to estimate the abundance of bacteria and diatoms which were the two most abundant microfoulers on surfaces studied. In addition to these studies, morphology of the surface coupons (small size test panels) was also evaluated using scanning electron microscope.

Molecular characterization of amino acids to assess the application of ratios of individual amino acids, to determine the source and abundance of microfouling, has been done.

Thus, it may be said that these studies would contribute to our knowledge of microfouling in tropical estuarine waters. This in turn may help in our understanding of the nature of settlement on various materials when exposed to marine environment.

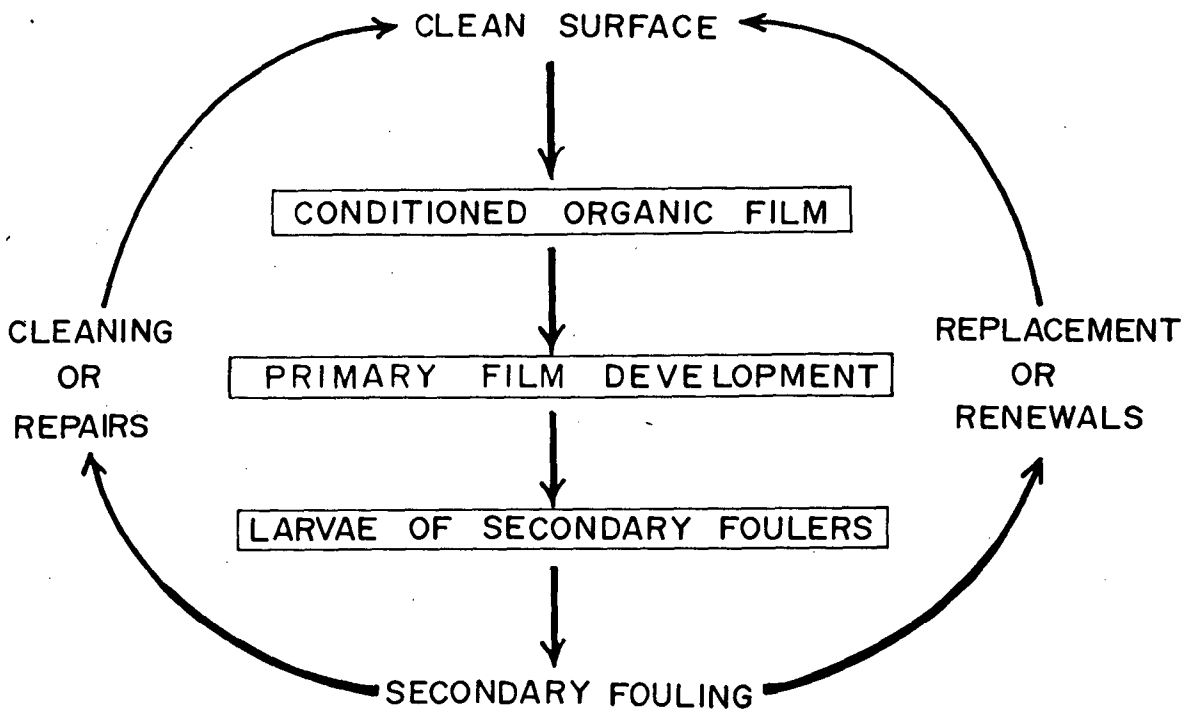


Fig.1.1 DIAGRAMATIC REPRESENTATION OF THE MARINE FOULING CYCLE (MODIFIED FROM ZAHURANEC, 1988).

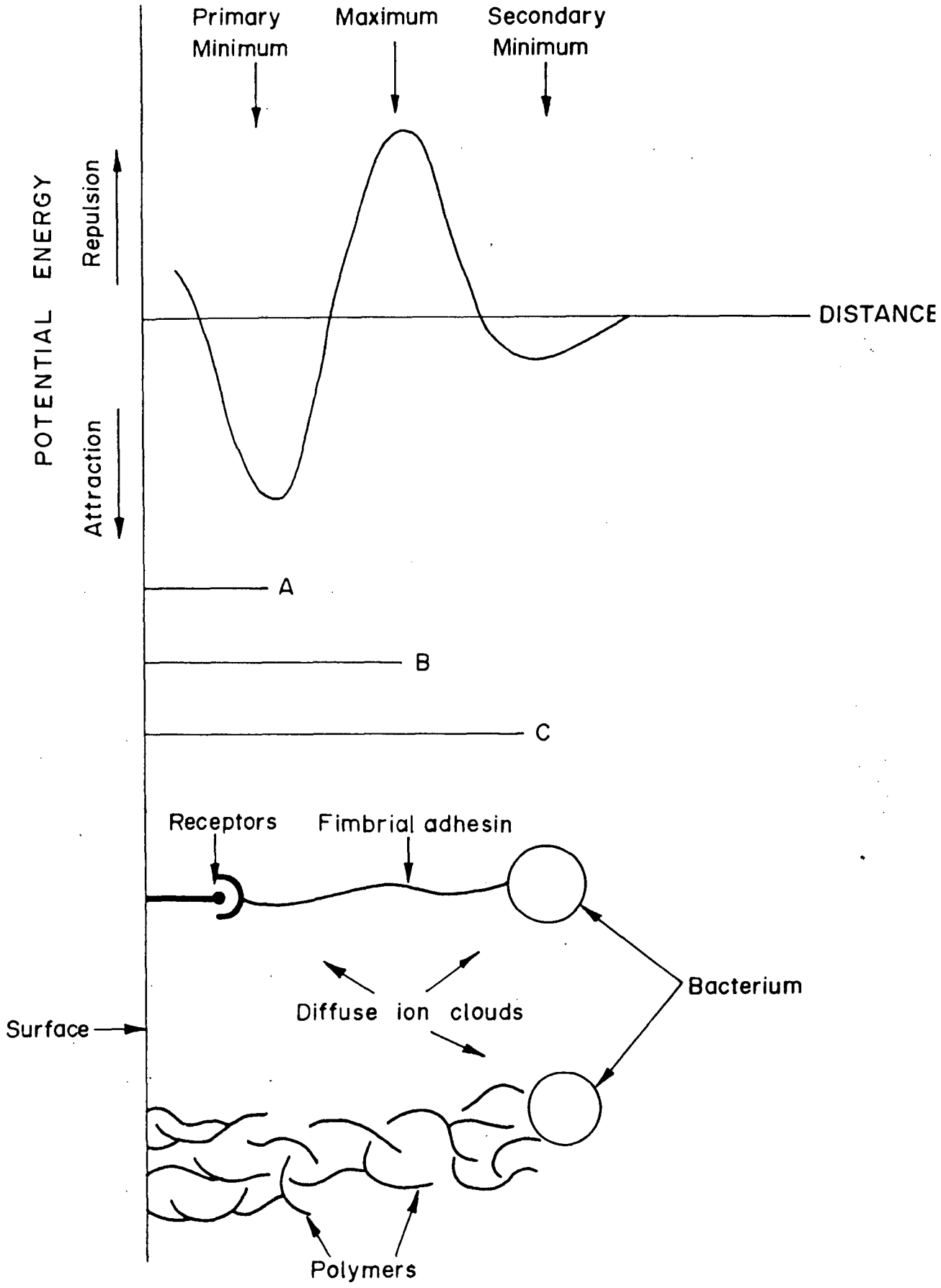


Fig.1.2 DIAGRAMATIC REPRESENTATION SHOWING BACTERIAL ADHESION TO SURFACES (JONES & ISAACSON, 1983)

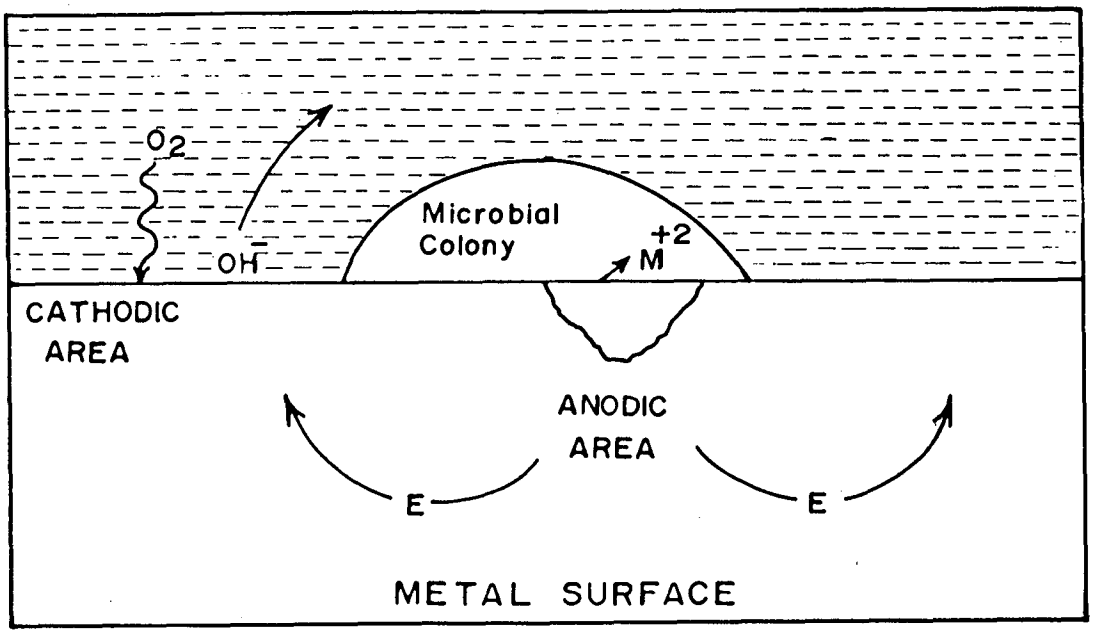


Fig.1.3 MICROBIAL METAL CORROSION TAKING PLACE ON SURFACES DUE TO UPTAKE OF NUTRIENTS INCLUDING OXYGEN BY MICROBIAL GROWTH. (MILLER & KING 1975)

Chapter 2

HYDROGRAPHIC PARAMETERS OF THE STUDY AREA

HYDROGRAPHIC PARAMETERS OF

THE STUDY AREA

1. INTRODUCTION

Any study on microfouling on material surface is considered incomplete without a thorough knowledge of the surrounding environmental parameters to which the material is exposed. The development of microfouling is the result of dynamic, complex phenomena wherein several environmental parameters are intimately related to one another through various processes.

Although a number of physico-chemical and biological parameters influence fouling settlement temperature, salinity, dissolved oxygen and nutrients are some of the important factors which need to be properly studied. Ahmed et al, (1984) and Guenzennec, (1986), have reported the influence of some of these parameters on individual organisms. Alabiso et al, (1984), have highlighted the effect of temperature on microfouling in OTEC pipes. Nevertheless, no consolidated effort seems to have been made to assess the implications of the above mentioned parameters on the microfouling development on

substrata. Moreover, statistical approach to evaluate the influence of these parameters does not seem to have received adequate attention from the earlier researchers. Hence, an elaborate study consisting of short-term daily sampling and long-term weekly sampling, was undertaken, to provide a valuable insight into the microfouling succession. Therefore, a detailed monitoring of the various environmental parameters and their seasonal variations in the study area, has been carried out and is presented in this chapter.

1.1 **Description of the study area** (Fig 2.1)

The Zuari estuary (15.31^{o} N, 73.59^{o} E) is located on the west coast of India, in the Arabian Sea. It is influenced by inflow of sea water and receives a large quantity of fresh water (~150-400$m^3.sec^{-1}$ of rainfall) during the south-west monsoon season (Shetye & Murty, 1987). The Zuari river has its source in the Western Ghats, and extends upto 70kms before meeting the Arabian Sea. The present study was carried out at **"Dona Paula"** point which is situated at the mouth of this estuary. The physical, chemical and biological parameters of the waters of Dona Paula are reported to explain the seasonal cycle. Heavy precipitation coupled with land runoff, occurring

during the south-west monsoons (June-September), reportedly reduce the salinity of the waters at Dona Paula point, considerably. This in turn causes changes in temperature, dissolved oxygen, nutrients and suspended load. The monsoon season is followed by the post-monsoon season (October-January) and finally by the pre-monsoon season (February-May). During the pre-monsoon season, waters of Dona Paula exhibit marine conditions (Qasim & Sen Gupta, 1981). For most part of the year the water at the study area is well mixed.

2. MATERIAL AND METHODS

2.1 Sample collection

Sub-surface seawater (~ 1m) samples were collected using a Niskin water sampler (5L). Collections were made at daily intervals (24 h) for a period of 6 days, called short-term daily sampling. Such samplings were made during April 1989, May 1989, August 1989, September 1989, December 1989, January 1990, April 1990 and May 1990. Simultaneously, long term weekly sampling were also carried out at weekly intervals, for a period of four weeks. Collections for weekly sampling were made during April-May 1990, August-September 1990 and December-January 1991.

These collections were done simultaneously along with studies on microfouling on surfaces immersed in the waters at Dona Paula.

2.2 Sample analysis

Immediately after collection of samples, various parameters like temperature, dissolved oxygen, salinity and nutrients such as nitrite, nitrate, phosphate and silicate were determined as described below.

2.3 Temperature

Temperature measurements of the sub-surface waters were made immediately after collection, using a graduated centigrade mercury thermometer which was calibrated upto 50°C.

2.4 Dissolved Oxygen

Dissolved oxygen content of the water samples collected was analysed following the standard Winklers method (Parsons et al, 1984)

2.5 Salinity

Salinity values were determined by the Mohr-Knudsen titration method, wherein first the chlorosity was obtained. From chlorosity, salinity was determined from the Knudsen hydrographic table (Strickland & Parsons, 1965).

Nutrients

2.6 Nitrite-nitrogen

Nitrite-nitrogen present in seawater was determined by using the method suggested by Parsons et al, (1984).

2.7 Nitrate-nitrogen

Nitrate-nitrogen from sub-surface waters was estimated by reducing them quantitatively to nitrite by passing through a nitrate glass column containing amalgamated cadmium filings (Parsons et al, 1984).

2.8 **Phosphate**

A known volume of seawater sample was allowed to react with a mixed reagent containing molybdic acid, ascorbic acid and trivalent antimony. The resulting blue-coloured phosphomolybdate complex was measured at 885nm spectrophotometrically (Parsons et al, 1984).

2.9 **Silicate**

Seawater sample of known volume was allowed to react with molybdate under conditions which resulted in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate complex. A reducing solution of metol and oxalic acid was then added which reduces the silicomolybdate complex to a blue colour and decomposes phosphomolybdate and arsenomolybdate complexes, if formed. Concentration of silicate present in the sample was measured spectrophotometrically at 810nm (Parsons et al, 1984).

3. RESULTS

A) Daily variations

3.1 Temperature (Fig 2.2)

The distribution of temperature values during the period of study is shown in Fig 2.2. The temperature values of the sub-surface water remained high during the pre-monsoon (29-32°C) period as compared to the monsoon (27-28°C) and post-monsoon (28-29°C) periods. Although there was no wide variation in the values during the six day study period for April 1989, the end of the pre-monsoon period (May 1989) showed relatively higher values ranging from 31-32°C than in April 1989 (29-31°C). Temperature values did not show any major change for the monsoon season where they ranged from 27-28°C in August 1989 and from 27.5 to 28°C in the month of September 1989. Temperature values during the post-monsoon period remained intermediate as compared to the pre-monsoon and monsoon period with values ranging from 28-29°C in December 1989 and January 1990. Sampling for the pre-monsoon season of 1990 once again showed a rise in temperature which was in agreement with the data for 1989.

3.2 **Salinity** (Fig 2.2)

The salinity values for April and May 1989 as well as for December 1989 and January 1990, showed very small variations. On the other hand in August 1989, there was a considerable variation, with a low value of 11.98%. on the 1st day which increased to 19.99‰ on the 2nd day. It decreased for the next two days, the values being 16.33 and 12.79‰ Thereafter, it increased to 18.29 and 29.65‰ for the 5th and 6th day respectively. In September too, for the first three days salinity values were almost constant, being 20.47‰ for the first two days and 21.55‰ for the 3rd day. It then slowly increased to 27.50‰, on the 4th, 5th and 6th days of sampling. During the pre-monsoon season of 1990, the salinity showed a maximum of 35.54‰ and a minimum of 25.88‰. In April 1990, there were not much variations (34.47-35.54‰). However, during May 1990, there was an increase on the 3rd day (33.42‰) and decrease on the 5th day (29.13‰) of sampling. On the other days the values were between 28.59 & 29.66‰.

3.3 **Dissolved oxygen** (Fig 2.2)

In April 1989, as seen in Fig 2.1, the initial higher value of dissolved oxygen (3.89ml/l) dropped to 2.16ml/l on

the 2nd day and again increased to 3.67 and 3.99ml/l for the 3rd and 4th days respectively. The value of this parameter for the 5th and 6th days were found to be 3.99 & 3.07ml/l. In May 1989, there was no considerable change in the values (2.99-3.32ml/l) except for an increase on the 4th day (3.88ml/l).

During the month of August, there was significant increase in the dissolved oxygen concentration, with a decrease on the 2nd day (4.7ml/l) and on the 5th day (4.32ml/l). For all the other days, the values of dissolved oxygen were found to be more or less stable ranging between 4.86 & 5.24ml/l. During September 1989, all six days showed values ranging from 5.17 to 5.61ml/l.

There appeared to be a decrease in the dissolved oxygen concentrations to 3.12ml/l on the 2nd day from 5.87ml/l on the 1st day in December. The value increased to 4.37ml/l on the 3rd day and remained steady (3.25ml/l) on the 4th and 5th day respectively, which again increased to 4.5ml/l on the 6th day of sampling. For the month of January 1990, low values were exhibited on the first two days (3.33 & 3.18ml/l) whereas, for the remaining four days the oxygen content remained quite stable with only slight variation (3.89-4.01ml/l). The observations for the pre-monsoon season (1990) were identical with the pre-monsoon

of 1989. In April 1990, on the 1st day the value recorded was 3.87ml/l. It decreased to 3.25ml/l on the 2nd and 3rd day. The concentration of dissolved oxygen remained steady on the 4th day (3.62ml/l) which further decreased to 2.5ml/l on the 5th day and on the 6th day it was 2.88ml/l. Similarly in May 1990, dissolved oxygen concentration for the first two days was more or less stable (2.87 & 3.12ml/l) which dropped to 2.5 ml/l on the 3rd day. The value increased to 3.62ml/l on the 4th day and later dropped on the 5th (3.24ml/l) and 6th day (2.5ml/l).

Thus, it can be said that the values of dissolved oxygen for the Dona Paula waters showed a considerable variation for all the three seasons of the year. It was lowest during the pre-monsoon months (2.99-3.99ml/l), intermediate during the post-monsoon months (3.12-4.5ml/l) and the highest during the monsoon months (4.32-5.61ml/l).

NUTRIENTS

3.4 **Nitrite-nitrogen** (Fig 2.3)

The value of nitrite-nitrogen was lower than nitrate-nitrogen for all the three seasons of the year, as presented in Fig 2.3. For the pre-monsoon month of April 1989, there was no significant variation in the concentration of nitrite. The 1st day showed a value of 0.46ug-at/l. There was a decrease in the concentration for the 2nd day (0.36ug-at/l) as well as for the 3rd day (0.29ug-at/l). Once again a very meagre increase was evident for the 4th day (0.44ug-at/l) which again decreased to 0.37ug-at/l on the 5th day and 0.35ug-at/l on the 6th day, respectively. During May 1989, the value of nitrite observed on the 1st day was relatively low (0.26ug-at/l), which increased to 0.42ug-at/l on the 2nd day. A drop in the value of nitrite was evident on the 3rd day (0.32ug-at/l) but once again increased on the 4th day to 0.39ug-at/l. The 5th and the 6th day showed a value of 0.24ug-at/l and 0.32ug-at/l for the same month. Thus, their concentration in the sub-surface water was quite steady, without any drastic changes, for all the six days of both the sampling months.

During the monsoon month of August 1989, the 1st day showed a value of 0.19ug-at/l which increased to 0.25ug-at/l on the 2nd day. Once again a decrease in the nitrite concentration was observed for two consecutive days i.e. 3rd day (0.17ug-at/l) and 4th day (0.11ug-at/l). For the 5th and the 6th day an increase in the concentration from 0.22 to 0.26ug-at/l was evident. Nitrite concentration during the 1st day of September showed a value of 0.2ug-at/l, which then decreased on the 2nd day (0.12ug-at/l) and so also on the 3rd day (0.13ug-at/l). A sudden increase was observed on the 4th day to 0.26ug-at/l, however, the increasing trend did not continue to increase but dropped to 0.23ug-at/l on the 5th day and to 0.14ug-at/l on the 6th day of sampling.

The nitrite concentration for the post-monsoon month of December 1989, showed a low value of 0.46ug-at/l on the 1st day which was near in comparison to the concentration on the 3rd day (0.42ug-at/l). The 2nd day, however, showed a comparatively high value of 0.57ug-at/l. The increasing trend continued for all the other days as seen for the 4th day (0.60ug-at/l), 5th day (0.66ug-at/l) and the 6th day (0.77ug-at/l) of sampling. During January 1990, the 1st day showed a value of 0.57ug-at/l, while a decrease was evident for the 2nd day (0.49ug-at/l). There was an

increase in the nitrite concentration to 0.63ug-at/l for the 3rd day. The month's maximum was recorded on the 4th day (0.77ug-at/l), which was very close to the concentration on the last day (0.71ug-at/l) of sampling. The 5th day showed a lower value of nitrite concentration (0.65ug-at/l), as compared to the 4th day.

Sampling for the pre-monsoon months of 1990, showed values which were comparable with those of 1989. The month's maximum for April, 1989, occurred on the 1st day (0.44ug- at/l). There was a subsequent decrease in the nitrite concentration for the 2nd day (0.36ug-at/l) and 3rd day (0.27ug-at/l) respectively. A sudden increase (0.41ug-at/l) was evident on the 4th day. Thereafter, a decrease was observed from 0.39ug-at/l on the 5th day to 0.34ug-at/l on the 6th day, for the same month. May, 1990, showed identical values of nitrite for both the 1st and 2nd days (0.32ug-at/l) of sampling. The month's maximum was evident on the 3rd day (0.42ug-at/l), which decreased to 0.39ug-at/l on the 4th day and to 0.26ug-at/l on the 5th day. The 6th day however, showed a value of 3ug-at/l, which was only marginally higher than the concentration on the 4th and 5th days.

3.5 **Nitrate-nitrogen** (Fig 2.3)

The changes observed for this nutrient parameter are presented in Fig 2.3. During April 1989, the 1st day of sampling exhibited a high value of 2.94ug-at/l, which dropped marginally till the 4th day (1.45ug-at/l) and again increased on the 5th day (2.05ug-at/l) and on the 6th day (2.62ug-at/l). During May 1989, the values remained more or less steady on the first two days (1.75, 1.81ug-at/l) and increased marginally on the 3rd and 4th days (2.29 & 2.62ug-at/l) respectively. It decreased on the 5th day (1.77ug-at/l) and finally increased on the 6th day (2.85ug-at/l).

During August, the values showed a drop from the 1st day (0.71ug-at/l) to the 3rd day (0.49ug-at/l) and increased to 1.01ug-at/l on the 4th day. A decrease in the values of nitrate concentration to 0.82 and 0.91ug-at/l was observed on the 5th and 6th day of August 1989, sampling. During September 1989, on the other hand, steady value for nitrate concentration for the 1st (0.56ug-at/l), 2nd (0.68ug-at/l) and 3rd (0.42ug-at/l) days were observed, which later increased to 0.73ug-at/l on the 4th day. There was a drop on the 5th day (0.53ug-at/l), which later increased to 0.84ug-at/l, on the last sampling day of the month.

In December, 1989, the values of nitrate-nitrogen were more or less the same, with slight variations. It was 1.75ug-at/l for the 1st day. For the 2nd day (2.09ug-at/l), 4th day (2.87ug-at/l), 5th day (2.08ug-at/l) and 6th day (2.67ug-at/l) the change was marginal except for an increase on the 3rd day, to 3.15ug-at/l. In case of the 2nd month for the same season (January 1990), on the 1st day a low value of 2.66ug-at/l was recorded, which increased on the 2nd (3.01ug-at/l) and the 3rd (4.00ug-at/l) days. Thereafter, the values dropped to 2.09ug-at/l on the 4th day which later increased and remained steady for the 5th (3.99ug- at/l) and 6th (3.98ug-at/l) days. In April, 1990, intermediate values of nitrate concentration have been observed on the 1st day (2.62ug- at/l), which reduced to 2.05ug-at/l and 2.09ug-at/l on the 2nd and 3rd days respectively. However, the value increased to 2.45ug-at/l on the 4th day, to again exhibit a lower concentration of 2.16ug-at/l on the 5th day. Finally, on the last day (6th), the value was the month's maximum, it being 2.99ug-at/l. In May 1990, high concentration was observed on the 1st (4.77ug-at/l) and 3rd (4.15ug-at/l) days, while on other days the values recorded were comparatively low ranging from 3.06 to 3.78ug-at/l.

Thus, it may be said that the values for nitrate-nitrogen during the pre-monsoon 1989, monsoon 1989 and post-monsoon 1989-90 ranged from 1.45-2.94ug-at/l, 0.42-1.01ug-at/l and 1.75-3.98ug-at/l respectively. During the pre-monsoon season of 1990, values for nitrate-nitrogen ranged from 1.77-2.99ug-at/l.

3.6 **Phosphate-phosphorus** (Fig 2.3)

In April, 1989, wide fluctuations in the phosphate concentration were observed. To begin with a low value of 1.12 and 1.06ug-at/l were observed on the first two days of sampling, which increased to 1.83 and 2.09ug-at/l on the 3rd and 4th day respectively. It decreased once again on the 5th (1.67ug-at/l) and 6th (1.97ug-at/l) days. In May 1989, the values for this nutrient showed a steady decrease from the 1st day (2.97ug-at/l) till the 3rd day (0.91ug-at/l). Later there was a steady increase in the concentration on the 4th (1.44ug-at/l), 5th (1.64ug-at/l) and 6th (1.93ug-at/l) days. The values of phosphate concentration in August 1989, were quite high on the 1st (3.03ug-at/l) and 2nd (3.72ug-at/l) day, whereas, on other days the concentration decreased and remained more or less steady (2.04 to 2.67ug-at/l). During September 1989, month of the same season, the 1st day (2.23ug-at/l) as well as

the 2nd day (2.08ug- at/l) did not show much variation in the concentration of this parameter. The 3rd day of sampling showed higher value of 3.53ug-at/l. It increased further on the 4th day to 4.55ug-at/l. There was a sudden drop on the 5th day to 2.15ug-at/l, the value finally rising to 3.91ug-at/l on the 6th day of sampling.

In December 1989, low values of phosphate concentration were exhibited on all six days with the months lowest value being 0.32ug-at/l which was recorded on the 1st day. It increased to 0.79ug-at/l on the 2nd, to 0.64ug-at/l on the 3rd day, 0.76ug-at/l on the 4th day and 0.66ug-at/l on the 6th day. The 5th day of sampling exhibited the maximum value for the month, the value being 0.82ug-at/l. In January 1990, a more or less uniform concentration was observed as shown in Fig 2.3, with the months maximum value observed on the 2nd day of (0.79ug-at/l) and the months minimum on the 4th day (0.32ug-at/l). On the other days, the values were found to be stable with phosphate concentration ranging from 0.44 to 0.52ug-at/l.

During April 1990, the 1st day showed a concentration of 1.52ug-at/l. Minimum concentration was observed on the 2nd day (1.36ug-at/l). The other days showed an increase without much variation in the phosphate values from the 3rd to the 5th (2.3 to 2.96ug-at/l) day. The phosphate

concentration during May 1990, was found to be almost the same on the 1st (1.42ug-at/l), 5th (1.43ug-at/l) and 6th (1.22ug-at/l) days. On other days the values were found to be quite high, they being 2.15ug-at/l on the 2nd day and 2.03ug-at/l on the 3rd day. The concentration for the 4th day was 1.68ug-at/l.

The values of phosphate concentration during the pre-monsoon season ranged from 0.91 to 2.97ug-at/l, in the monsoon season, from 2.04 to 4.55ug-at/l, and in the post-monsoon season from 0.32 to 0.82ug-at/l. Thus in the monsoon season highest values as compared to pre-monsoon and post-monsoon season were recorded. Pre-monsoon of 1990, showed a range from 1.36 to 2.96ug-at/l.

3.7 **Silicate** (Fig 2.4)

During April, 1989, sampling showed the silicate concentration in the sub-surface water for the first two days as well as the 5th day to be 10.12, 10.18 & 10.53ug-at/l. Slightly lower concentration was observed on the 4th day (9.74ug-at/l). Lower values for this season were observed on the 3rd (5.11ug-at/l) and the 6th (5.43ug-at/l) days. In May, 1989, on the 1st day the silicate concentration was found to be 7.48ug-at/l. It was

5.04ug-at/l on the 2nd day with an increase on the 3rd (6.5ug-at/l) and 4th (9.7ug-at/l) days. The concentration of silicate decreased and then remained almost the same on the 5th (7.8ug-at/l) and 6th (7.9ug-at/l) days of sampling.

The samples in August 1989, showed high values on the 1st (18.4ug-at/l) and 6th (16.92ug-at/l) days. The concentration of silicate was found to be more or less the same on the 2nd (13.36ug-at/l), 4th (13.77ug-at/l) and 5th (12.24ug-at/l) days of sampling. Still lower value (11.34ug-at/l) was observed on the 2nd day. The concentration of silicate observed for September 1989, was similar to that of August 1989, with respect to its wide fluctuation. Maximum concentration of silicate was evident on the 2nd day (19.94ug-at/l), followed by the 3rd day (18.36ug-at/l) and then the 1st day (17.92ug-at/l). The 4th day showed a low value of 12.93ug-at/l, which decreased still further to 10.64ug-at/l on the 5th day and 10.86ug-at/l on the 6th day.

In December 1989, the silicate concentration remained almost the same on all days, the values ranging between 4.04 and 5.99ug-at/l. However, it was quite low (3.18ug-at/l) on the 5th day. In January 1990, the concentration was similar to that in December 1989, with noticeably low

concentration ranging from 3.32 to 4.22ug-at/l, except for a slightly higher value on the 5th day (5.32ug-at/l).

April 1990, sampling showed a very marginal change from the 1st to the 3rd day, the values being 6.56ug-at/l on the 1st day, 6.22ug-at/l on the 2nd day and 6.49ug-at/l on the 3rd day. There was increase in the silicate concentration from the 4th day (8.28ug-at/l), to the 5th (10.21ug-at/l) and 6th (10.8ug-at/l) days respectively. In May 1990, the first four days exhibited a more or less steady concentration for this parameter, ranging between 6.32 to 7.96ug-at/l. Comparatively higher concentration was observed on the 5th (6.73ug-at/l) and 6th (9.7ug-at/l) days.

Silicate concentration during the pre-monsoon, monsoon and post-monsoon months showed values ranging from 5.11 to 10.53ug-at/l, 10.64 to 19.94ug-at/l and 3.18 to 5.99ug-at/l, respectively. Sampling in the pre-monsoon months during 1990, showed values ranging from 12.74 to 18.88ug-at/l.

B) **Weekly variations**

3.8 **Temperature** (Fig 2.5)

Temperature variation for the weekly sampling did not show wide fluctuations with 30°C being recorded for week I. During weeks II and III the values remained at 29.5°C and the week IV once again showed a temperature of 30°C. During August-September, 1990, the value was much lower, with week I showing 27°C on the mercury thermometer. This was followed by a linear drop in the values thereafter, for weeks II (26.5), III (26.5°C) and finally (26°) in week IV of the sampling period. In December-January, 1991, the values were only marginally higher when compared to those of August-September, with week I showing a value of 27.5°C. In the weeks II and III the temperature was observed to be 28°C. In week IV the value was once again almost similar (28.5°C) to the week III.

Thus, it can be seen that in the pre-monsoon season the temperature varied from 29.5° to 30°C, for monsoons it was lower and ranged between 26°-27°C, whereas post-monsoon season exhibited temperature ranging from 27.5° to 28.5°C.

3.9 **Salinity** (Fig 2.5)

The same figure as above also represents the variation in salinity for the sub-surface waters observed during weekly sampling. In April-May all the four weeks showed salinity values ranging between 33.41 to 34.47‰ However, low values between 18 to 19.05‰ were observed during August-September and between 29.25 to 29.80‰ during December-January. Thus, the pre-monsoon season showed maximum values of salinity for the study period, when compared with the post-monsoon and monsoon seasons. Minimum values were observed during the monsoon season.

3.10 **Dissolved oxygen** (Fig 2.5)

In April-May, the value of dissolved oxygen for week I was as low as 4.42ml/l which remained the same upto week III and then increased marginally (4.46ml/l) for week IV.

In August-September, week I showed DO content to be 4.99ml/l which increased significantly to 6.62ml/l in week II. The value then showed a steady decline for week III (5.87ml/l) and week IV (4.62ml/l) respectively.

The concentration of dissolved oxygen in the sub-surface waters in December-January was recorded as 4.99ml/l for week I which increased to 5.35ml/l during week II. DO values were 4.7ml/l and 5.12ml/l for weeks III and IV respectively.

Thus, during the pre-monsoon season, values for dissolved oxygen ranged between 4.42 to 4.46ml/l. Monsoon season showed higher values ranging between 4.62 to 6.62ml/l and finally values observed during the post-monsoon season ranged between 4.79 to 5.35ml/l).

Nutrients (Fig 2.6)

The values for both nitrite-nitrogen and nitrate-nitrogen were comparatively low in the surface waters of Dona Paula (Fig 2.6). Nitrite-nitrogen was lower than nitrate-nitrogen for the study period.

3.11 **Nitrite-nitrogen** (Fig 2.6)

In April-May, week I showed a value of 0.6ug-at/l which dropped to 0.48ug-at/l for week II and then increased to 0.64ug-at/l for week III. Once again the value dropped to 0.44ug-at/l which occurred during week IV.

In August-September very low values occurred for week I (0.08ug-at/l), week II (0.063ug-at/l), week III (0.04ug-at/l) and week IV (0.04ug-at/l).

The sampling during December-January showed similar values during weeks I and II (0.24ug-at/l), which increased to 0.46ug-at/l for week III and dropped to 0.36ug-at/l during the last week of observation.

3.12 **Nitrate-nitrogen** (Fig 2.6)

The condition for nitrate-nitrogen was identical with weeks I and II of April-May showing concentrations of 2.16ug-at/l and 2.59ug-at/l respectively. A decrease in its concentration occurred during week III (1.92ug-at/l) and week IV (1.69ug-at/l).

During August-September, low values were observed (Fig. 2.6) showing 1.24ug-at/l for week I and 1.17ug-at/l for week II which did not change significantly for week III (1.14ug-at/l). There was a decrease to 1.04ug-at/l in the nitrate concentration for the last week of sampling.

In December-January, 1991, the nitrate-nitrogen concentration values were quite high when compared to August-September, with week I showing a value of 1.54ug-at/l, which dropped to 1.43ug-at/l for week II. An increase in concentration was observed for week III (1.68ug-at/l) and week IV (1.66ug-at/l) respectively.

Thus, the pre-monsoon season exhibited values ranging between 0.44 to 0.64ug-at/l for nitrite and between 1.69 to 2.59ug-at/l for nitrate. Minimum values for both these nutrients were observed during the monsoon season (0.04 to 0.08 & 1.04 to 1.24ug-at/l). Intermediate values prevailed during the post-monsoon season for nitrite (0.24 to 0.46ug-at/l) and nitrate (1.43 to 1.66ug-at/l), respectively.

3.13 **Phosphate-phosphorus** (Fig 2.6)

During April-May, there was no significant change observed in the phosphate concentrations for week I (0.23ug-at/l), week II (0.22ug-at/l) and week IV (0.21ug-at/l). Week III, however, showed a value of 0.17ug-at/l which was comparatively low.

Phosphate-phosphorus during August-September, showed consistent values for all the four weeks ranging between 2.01 to 2.07ug-at/l.

During December-January, phosphate concentration for week I & II showed similar values (1.16ug-at/l). This is also evident from Fig 2.6. There was a sudden increase in its value during week III (1.61ug-at/l) which once again decreased in week IV (1.19ug-at/l).

Thus, this was the pattern for the pre-monsoon, monsoon and post-monsoon seasons for phosphate. These values showed a pattern which was dissimilar to the other nutrients such as nitrite and nitrate.

3.14 **Silicate** (Fig 2.6)

During April-May,90the values for the above nutrient did not change to a significant extent with 3.24, 3.58, 3.16 and 3.38ug-at/l being recorded during week I, II III & IV week respectively.

During August-September, the values of silicate concentration were comparatively higher for all the observations made. Week I showed a value of 7.84ug-at/l. Week II and III, did not show any significant change (7.3 & 7.26ug-at/l). The last week showed a much higher value of 8.28ug-at/l.

Silicate concentration for the sub-surface waters of Dona Paula, showed 6.83ug-at/l for week I during December-January. There was a sudden decrease in its concentration amounting to 4.59ug-at/l for week II. Week III did not show much of a change (4.7ug-at/l) as compared to week II. The last week once again showed an increase in the concentration of silicate, the value being (6.4ug-at/l).

The value for this parameter, like the phosphate concentration, was at its peak during the monsoon season. Intermediate values were observed during the post-monsoon season and low values during the pre-monsoon season.

Linear relationship studies between the various hydrographic parameters for the daily as well as weekly data was computed. Significant positive correlation was obtained when temperature verses salinity were correlated. On the other hand, temperature verses DO and salinity verses DO exhibited inverse relationships (Fig 2.7). Similarly, nutrients correlated with salinity as shown in the Fig 2.8.

4 DISCUSSION

Variation in the environmental parameters of the waters of Dona Paula for both short term as well as long term is important in understanding the changes occurring in the system. Fresh water discharge into the system causes changes in the environmental parameters. Variation in temperature and salinity for both daily as well as for weekly sampling showed one clear indication that with high temperature (29 to 31.5°C), high salinity (34.47 to 37.89‰) was noticeable during the pre-monsoon season. On the other hand, the monsoon season showed lower temperature (27 to 28°C) and low values of salinity (11.98 to 29.65‰). Intermediate values of temperature and salinity were exhibited for the post-monsoon season. Lower salinity values during the monsoons could be due to the

fresh water influence (rainfall, river runoff), which may alter the concentration of nutrients. Low values of salinity for the monsoons (10.5%.) were also reported by Qasim & Sen Gupta (1981) for the surface waters of the Zuari estuary. Thus, clearly indicating that salinity values changed with the salt intrusion into the system, that varied seasonally in relation to the amount of fresh water discharge causing dilution during the monsoons. Shetye & Murty, (1987), reported discharge of fresh water amounts to ~150-400m^3/sec during the monsoon period. During the pre-monsoon and post-monsoon seasons when the fresh water addition is reported to be insignificant (~10m^3/sec), the salinity values remained high (29.25 to 34.47‰).

A significant co-relation between temperature and salinity values have been observed for both daily ($r=0.61$, $p<0.001$, $n=48$) as well as for weekly ($r=0.64$, $p<0.001$, $n=12$) sampling periods (Fig 2.7). Similar observations were reported by Chandran & Ramamoorthy (1984), for the Goutami-Godavari, Ganapati & Ramasarma (1965), for the Vellar estuary and De Souza et al, (1981), for the Zuari and Mandovi estuary.

Dissolved oxygen of the subsurface waters of Dona Paula showed wide fluctuations during the study period.

Peak concentration of dissolved oxygen was a feature of the monsoon season. Intermediate DO content was evident during the post-monsoon season. Minimum concentration occurred during the pre-monsoon season. This could probably be related to high temperature and salinity existing during the pre-monsoon season, as was also reported for the Vellar estuary and for the Mahanadi Estuary (Chandran & Ramamoorthy, 1984; Sai Sastry & Chandramohan, 1990). The influence of temperature and salinity on dissolved oxygen has been assessed using simple linear correlation studies. Significant negative correlation between temperature and dissolved oxygen for daily ($r=-0.70$, $p<0.001$, $n=48$) as well as weekly sampling ($r=-0.66$, $p<0.001$, $n=12$) was observed. Negative correlation was observed for salinity and dissolved oxygen which was quite pronounced for the daily sampling ($r=0.66$, $p<0.01$) as compared to weekly samplings ($r=0.46$, $p<0.1$, $n=12$). These may probably be explained to be due to the fact that high salinity decreases the solubility of gases including oxygen and high temperature causes high salinity conditions (Head, 1985).

Nutrients showed a great deal of variation throughout the year. The input of nutrients could mainly be by river runoff, effluent discharge into seas and from insitu input from the system itself.

In the present study nitrogen in the form of nitrite as well as nitrate were lower than phosphates and silicates during the monsoon season. In other seasons, however, silicate concentration was most abundant followed by the phosphate values. Nitrite-nitrogen values were lower in comparison with the nitrate-nitrogen at this station. It has been established that nitrite-nitrogen is the intermediate oxidation state between ammonium and nitrate-nitrogen, and as such it can appear as a transient in both the oxidation of ammonium nitrogen and reduction of nitrate-nitrogen (Spencer, 1975). It could also be present as an excretion product of phytoplankton (Head, 1985). All these processes are mainly activated in the marine environment by biological agents (Spencer, 1975).

Nitrate is mainly added to the system by river run-off, land drainage and precipitation. During both daily and weekly sampling nitrite and nitrate-nitrogen were the highest in the pre-monsoon season. Low to intermediate concentrations during the monsoon and post-monsoon seasons, were observed. This could be due to the dilution of Dona Paula waters when the river discharge is maximum during the monsoon season. Nitrate depletion at this station could also be due to its utilization for biological productivity by primary producers and

denitrification (Boynton et al, 1982; Dehadrai, 1970). However, since the waters in this area seem to be well aerated as indicated by high dissolved oxygen concentration, especially during the monsoon season, the loss due to denitrification could be ruled out.

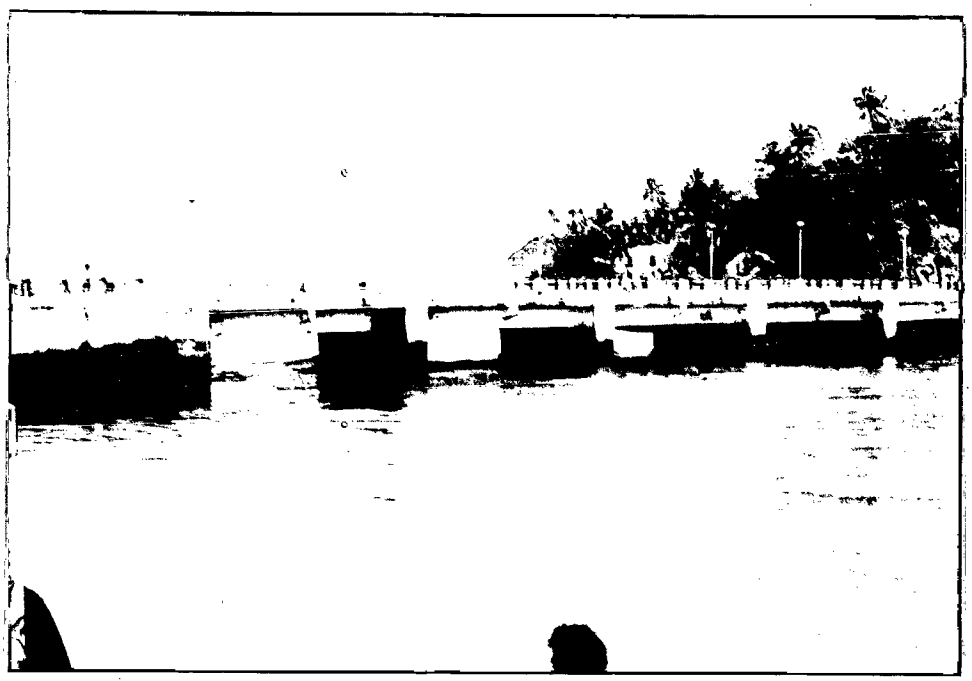
Nutrients like phosphate and silicate for both daily as well as weekly sampling showed high concentrations in the monsoon season which could be attributed to the seawater dilution during this period. Phosphate concentration during this season could also be due to the considerable waste material brought in by the rivers (Simpson et al, 1977). Similar results were also observed by Sarla Devi (1991) for the Periyar river estuary. Among all the nutrients analysed, silicate was found to be the most abundant in the waters at Dona Paula. Silica is the structural material of several types of biota including diatoms, radiolarians, siliceous sponges and silicoflagelates (Reimann et al, 1969). Hence, after death of these biota, their skeleton settle in the water column (De Master, 1979).

Phosphate and silicate concentrations were low for the pre-monsoon and intermediate for the post-monsoon seasons, indicating their utilization or removal by plankton, which were high during the same period and also

due to non-biological removal by adsorption onto suspended particulates (Liss & Spencer, 1970). High temperature, high salinity values and well aerated waters also favour the removal of phosphates (Jitts, 1959; Pomeroy, 1975). Low to intermediate concentration of nitrite and nitrate during the monsoon and high concentration of silicate and phosphate for the same season probably indicate low concentration of these two nutrients in the river itself and subsequent high concentration of phosphate and silicate in the river waters (DeSouza et al, 1981). This fact shows that the river could be the main source for these inorganic nutrients for this season (Liss & Spencer, 1970; Bien et al, 1958).

A study of the correlation between nutrients and salinity was important to predict the possible source of nutrients in the Bay. As shown in Fig 2.8, Nitrite/Salinity showed $r=0.59$, $p<0.001$, $n=48$ for daily sampling and $r=0.92$, $p<0.001$, $n=12$ for weekly sampling. Correlation between Nitrate/Salinity showed a linear positive relationship for daily ($r=0.70$, $p<0.001$) and for weekly sampling ($r=0.85$, $p<0.001$). This indicates that both the above mentioned nutrients do not seem to be of riverine origin. It could be the insitu degradation of marine organic matter as indicated by De Souza, (1983). In

contrast to this, phosphate/salinity values showed an inverse relationship for daily ($r=-0.52$, $p<0.001$, $n=48$) and for weekly sampling ($r=-0.92$, $p<0.001$, $n=12$). Silicate/Salinity also showed a similar relation for daily ($r=-0.73$, $p<0.001$, $n=48$) and for weekly ($r=-0.91$, $p<0.001$, $n=12$) sampling. Such an inverse linear relationship implies that both these nutrients could have been brought in by the river runoff. Thus it may be said that these observations could help in identifying the source of the nutrients in the study area.



STUDY SITE

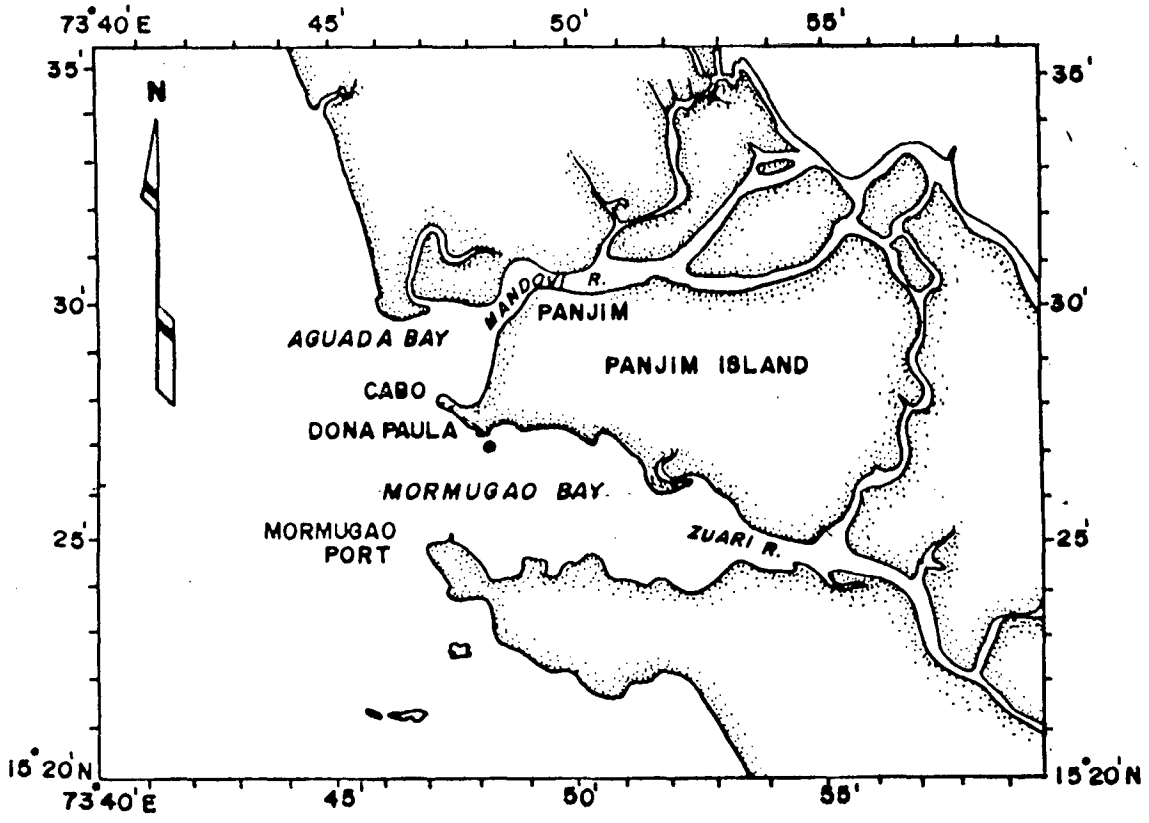


Fig. 2.1 Map showing the study area.

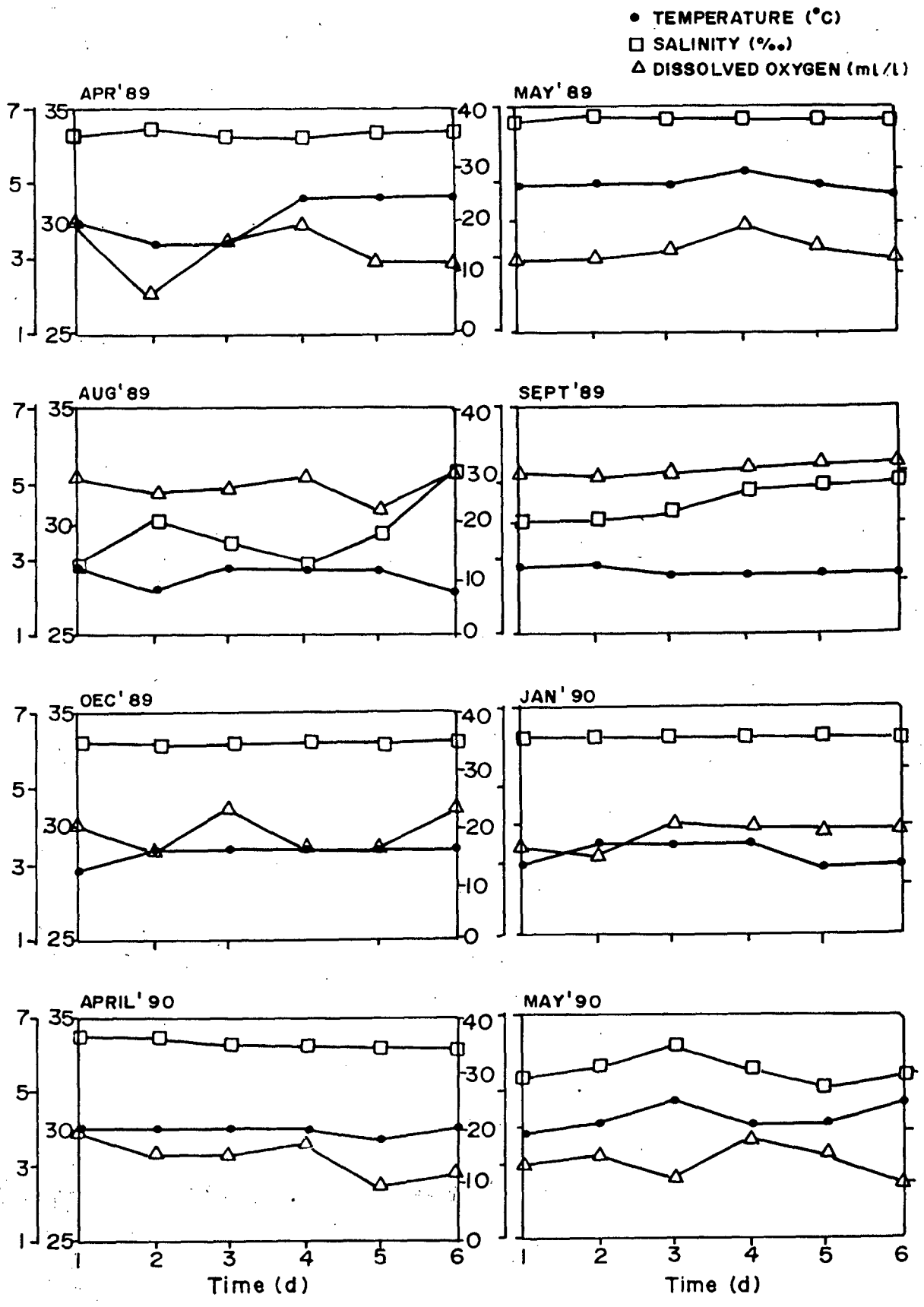


Fig.2.2 DAILY VARIATION IN TEMPERATURE, SALINITY & DISSOLVED OXYGEN OF THE SUB-SURFACE WATERS OF THE STUDY AREA FOR VARIOUS MONTHS.

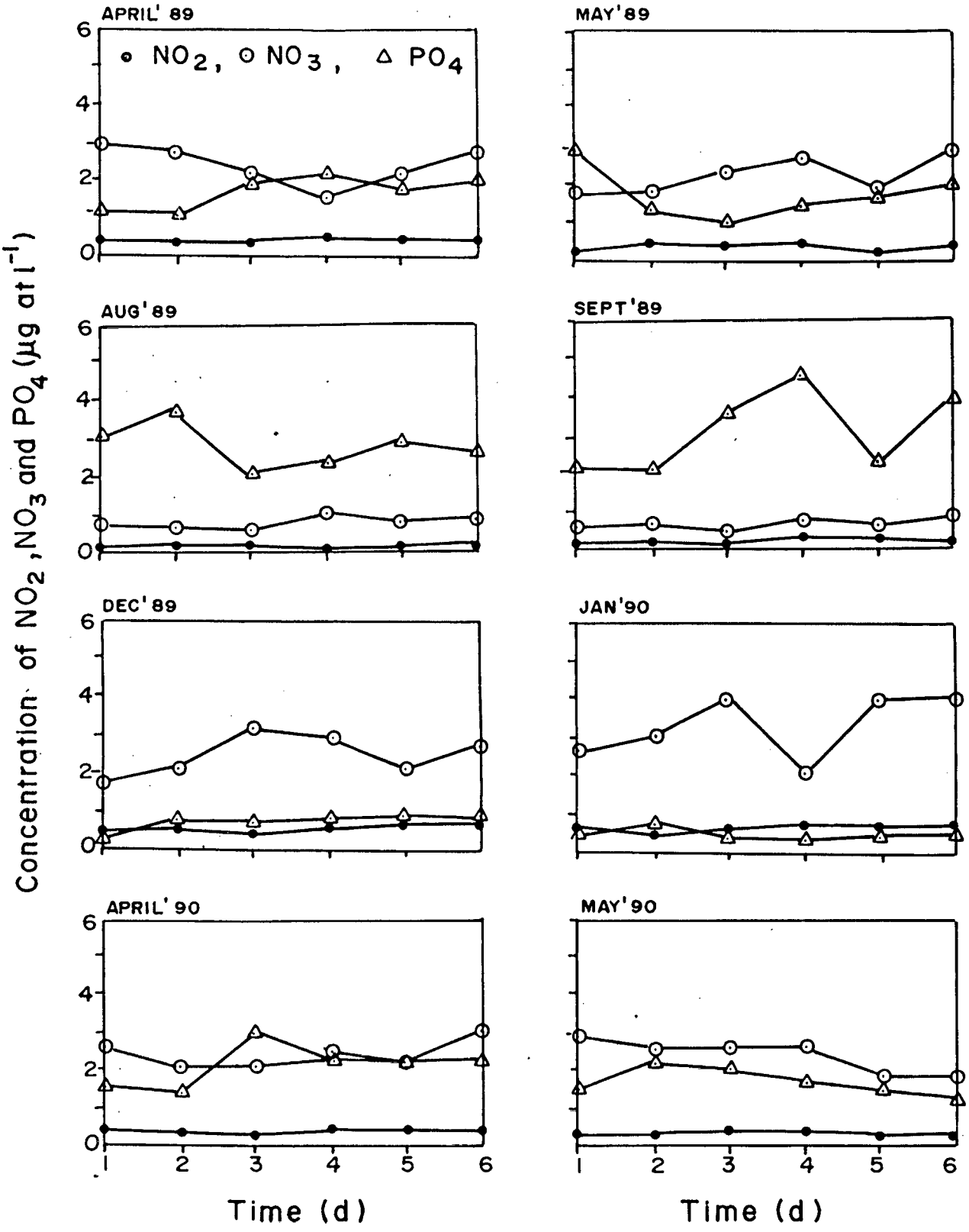


Fig. 2.3 DAILY VARIATION IN NITRITE, NITRATE & PHOSPHATE OF THE SUB-SURFACE WATERS OF THE STUDY AREA FOR VARIOUS MONTHS.

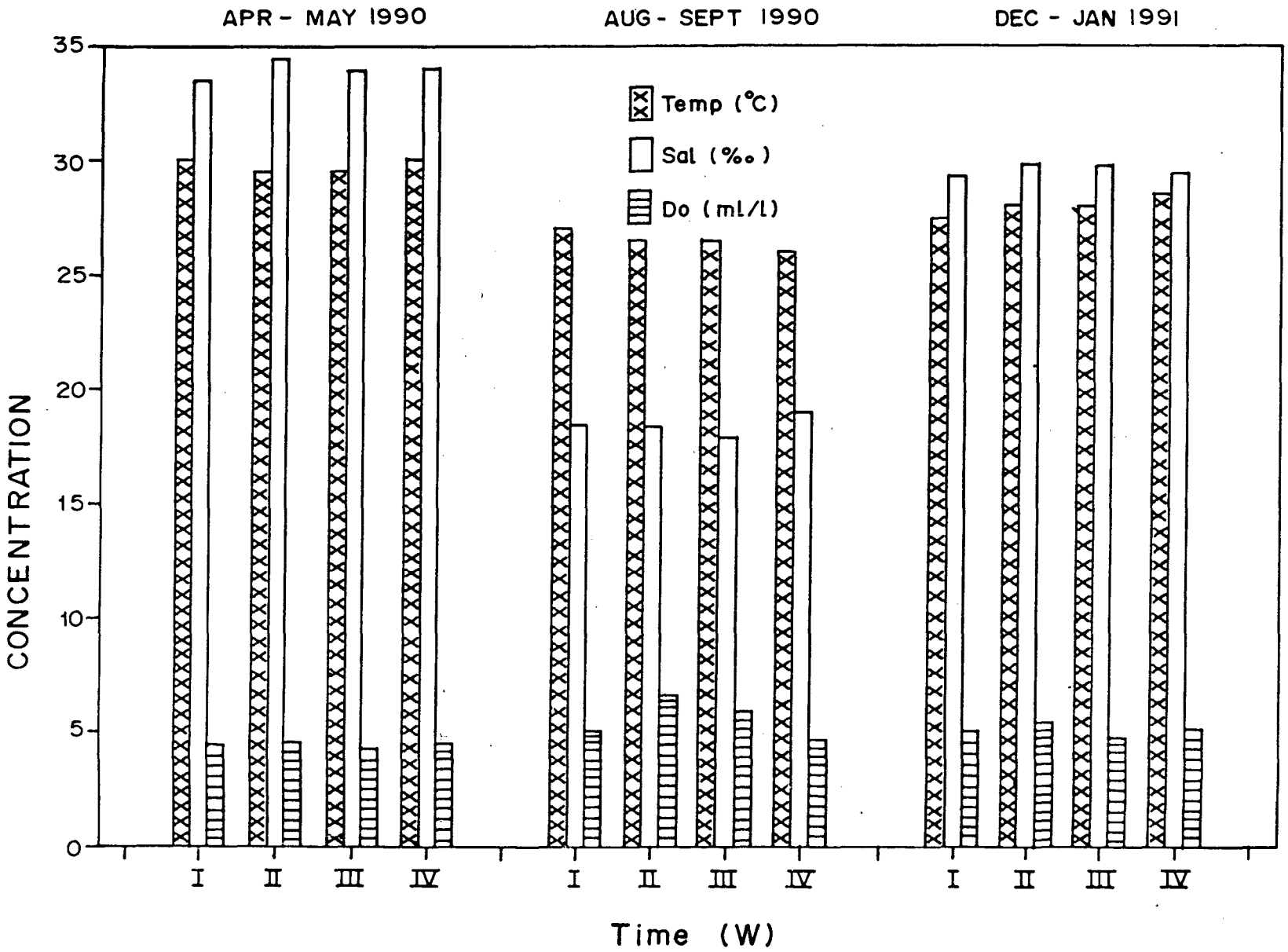


Fig.2.5 WEEKLY VARATION IN TEMPERATURE, SALINITY & DISSOLVED OXYGEN OF THE SUB-SURFACE WATERS OF THE STUDY AREA.

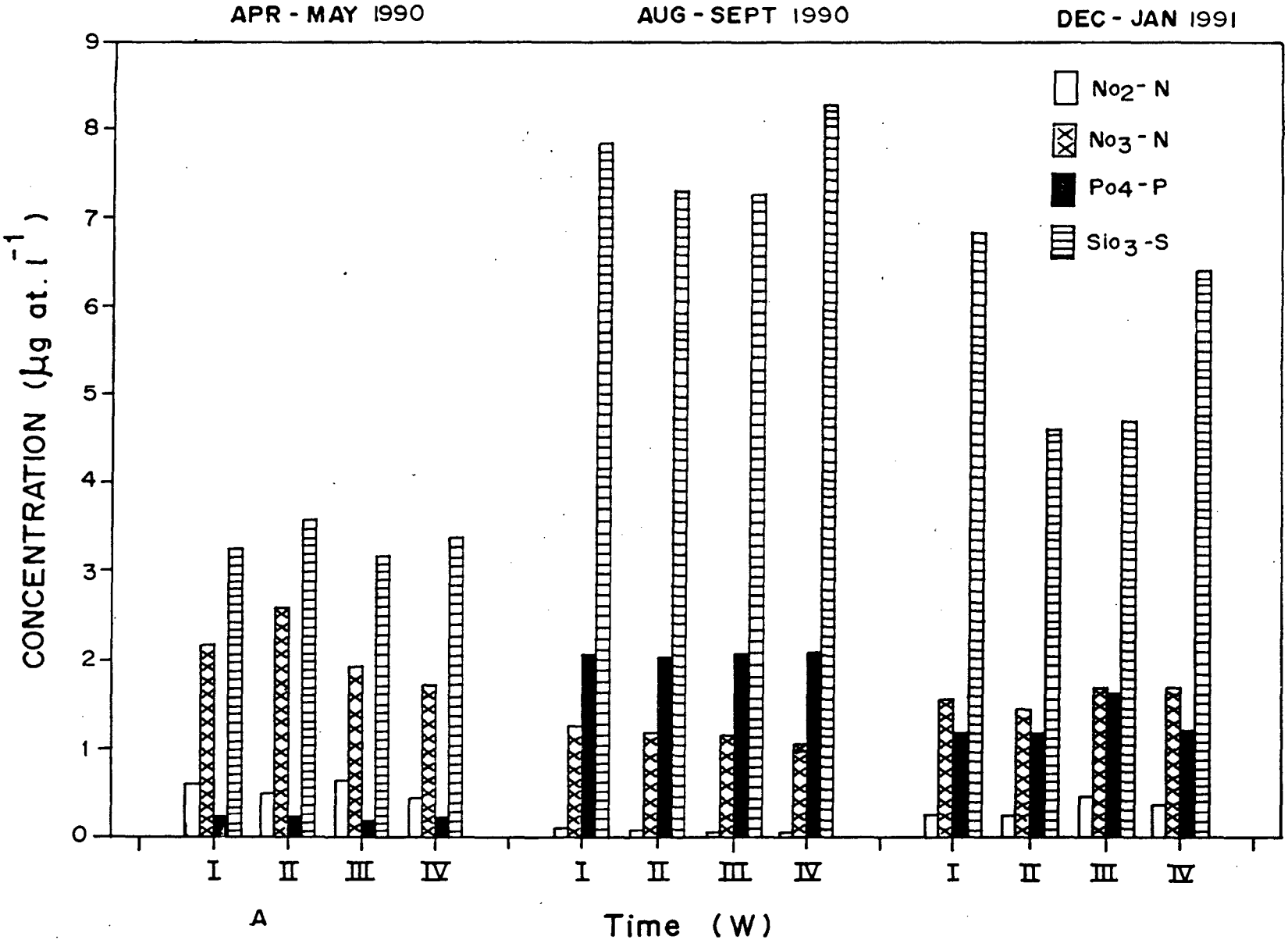


Fig.2.6 WEEKLY VARIATION IN NITRITE, NITRATE, PHOSPHATE & SILICATE OF THE SUB-SURFACE WATERS OF THE STUDY AREA.

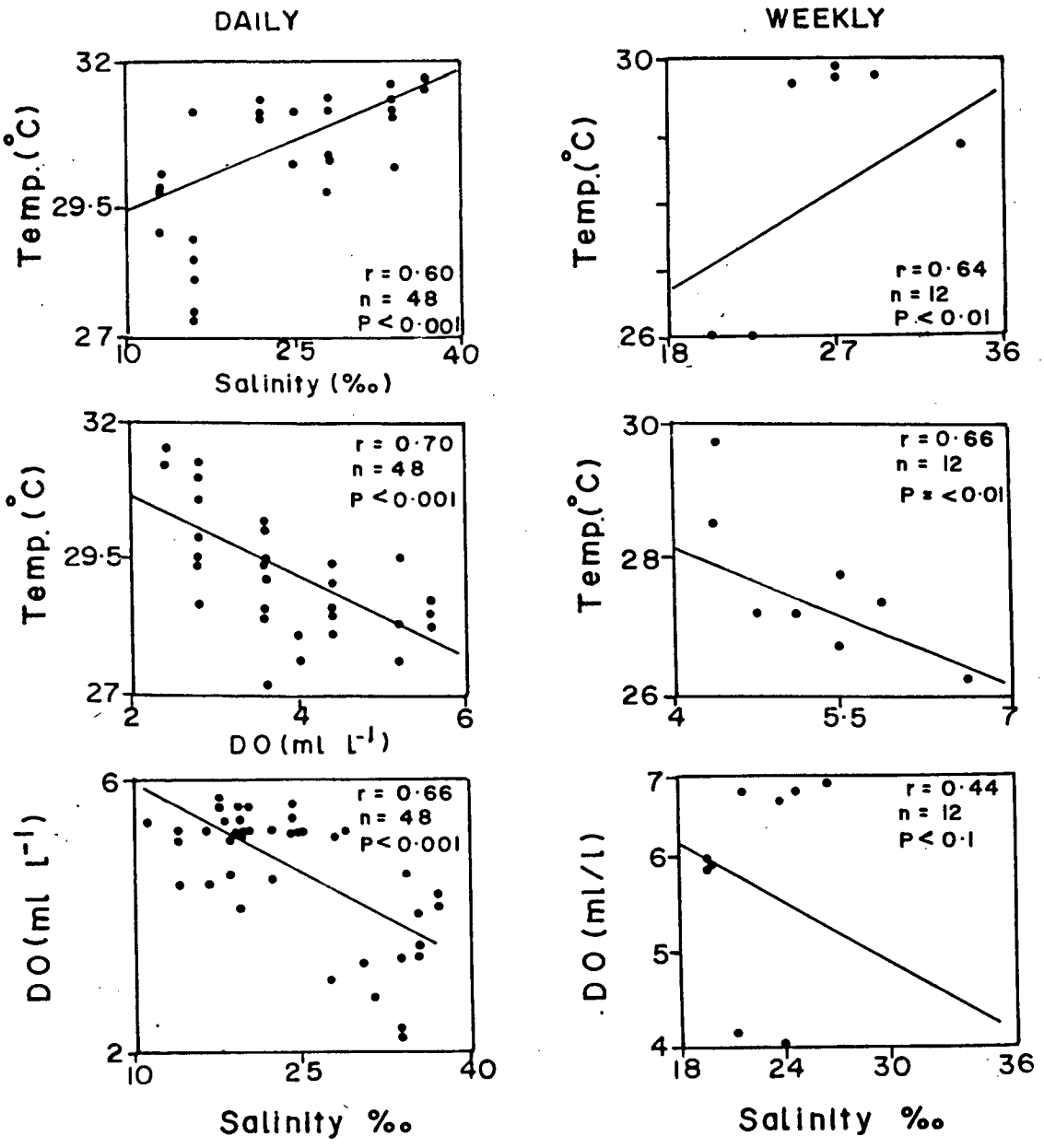


Fig.2.7 RELATIONSHIP BETWEEN TEMPERATURE, SALINITY AND DISSOLVED OXYGEN OF THE SUB-SURFACE WATERS OF THE STUDY AREA.

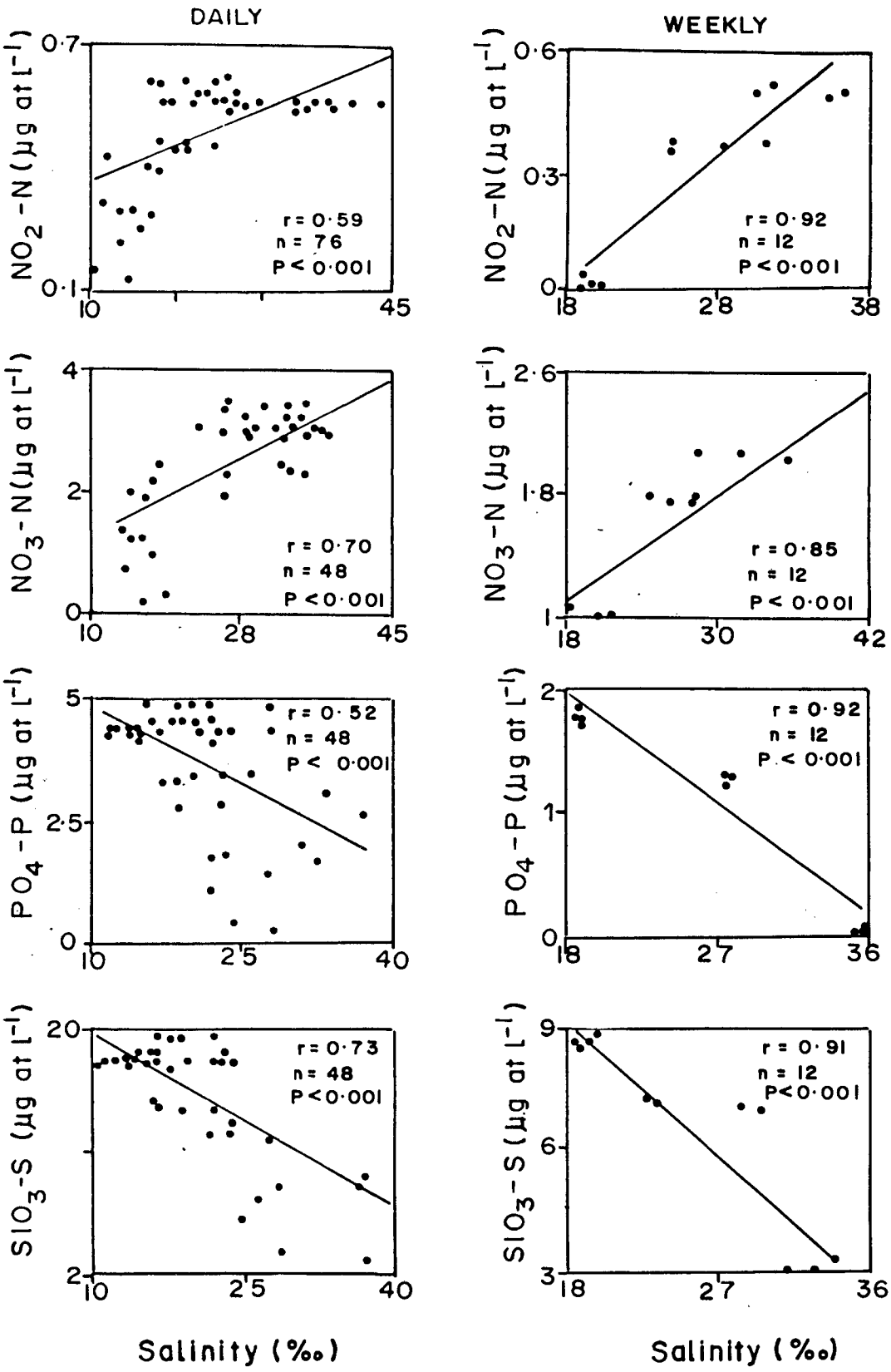


Fig. 2.8 RELATIONSHIP BETWEEN NITRITE, NITRATE, PHOSPHATE & SILICATE WITH SALINITY OF THE SUB-SURFACE WATERS OF THE STUDY AREA.

Chapter 3

NATURE AND DISTRIBUTION OF SUSPENDED PARTICULATE MATTER

NATURE AND DISTRIBUTION OF

SUSPENDED PARTICULATE MATTER

1. INTRODUCTION

Suspended particulate matter (SPM) in seawater originates either from autochthonous (biological life) or allochthonous (derived from terrestrial matter) sources as suggested by Degens & Ittekkot, (1985). It mainly comprise of inorganic and organic components. The inorganic fraction forms the most abundant constituent of SPM. However, since less energy is obtained from this fraction of SPM, it is not a good food source for marine organisms (Lanne, 1982). It is the organic content of the SPM which is more important and is subjected to variations in aquatic environment (Bordovskiv, 1965). The major producers of organic fraction of SPM are the phytoplankton. Under favourable conditions, they multiply extremely rapidly resulting in blooms. Such biogenic particulate material is a useful source of food for micro and macroplanktonic and benthic organisms (Dhargalkar & Verlencar, 1992). Thus, the abundance of SPM may influence the development and growth of microfouling organisms. Hence, monitoring of SPM and its constituent fraction such as particulate

organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC), particulate carbohydrate (PCHO), particulate organic nitrogen (PON), and chlorophyll a (Chl^{a}) may provide useful information on the implication of SPM on the development of microfouling organisms.

Several workers have reported the distribution of suspended particulate matter (SPM) from oceanic waters (Menzel, 1964; Gordon, 1970; Tanoue & Handa, 1979; Deuser, 1986; Cadee, 1988). However, very little work appears to have been done on this aspect in the Indian waters and especially in waters around Goa. Therefore, this work has been undertaken.

2. MATERIAL AND METHODS

Water samples were collected using Niskin water sampler (5L) approximately 1m below the surface as described in chapter II. The sample collections were done on daily and weekly basis. For daily sampling, water was collected after every 24 hours for a period of 6 days and for weekly sampling after every one week for a period of 4 weeks, for the three seasons of the year, namely, pre-monsoon (April/May, 1989), monsoon (August/September, 1989)

and post-monsoon (December/January, 1990) seasons. Known volumes of the water samples thus collected were filtered through ashed (450°C, 3h) GF/C glass fibre filters (47mm dia. & 1.2um pore size) in replicates of three. These filter papers containing the particulate material were digested in 90% acetone in dark vials for the estimation of chlorophyll^{a}. While, for the other parameters namely, total particulate matter (SPM), particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate carbohydrates (PCHO), the filter papers were dried at 40°C for 24h and stored in a desiccator until analysed.

2.1 Chlorophyll^{a}

The acetone extract of each sample was centrifuged, filtered and measured spectrophotometrically for chlorophyll^{a} (chl^{a}), as suggested by Parsons et al, (1984).

2.2 Suspended Particulate Material (SPM)

Filter papers containing the filtered material were dried in an oven at 60°C and reweighed to calculate suspended particulate matter (SPM).

2.3 Particulate Organic Carbon (POC)

POC was analyzed spectrophotometrically at 440nm after wet oxidation of carbon by chromic acid. The measurement of carbon was made by the standard procedure of Parsons et al, (1984).

Particulate organic matter (POM) was calculated by multiplying POC by a factor 1.724 (Bhosle & Dhople, 1988). Particulate inorganic matter (PIM) was then calculated using the relation PIM = SPM-POM

2.4 Particulate Carbohydrate (PCHO)

PCHO was analyzed by the phenol-sulphuric acid method (Dubois et al, 1956), and extinction was measured spectrophotometrically at 490nm. Glucose was used for the standard calibration.

2.5 Particulate Organic Nitrogen (PON)

Filter papers containing the filtered material were treated with an oxidizing solution ($K_2S_2O_8$-Potassium peroxodisulphate), digested under 15 lb pressure at 120°C for 30 minutes, cooled and acidified. PON was obtained by

following the method of Grasshoff, (1964).

Interrelationship studies were carried out using a software package Lotus 1-2-3.

3. RESULTS

A) DAILY OBSERVATIONS

3.1 Suspended Particulate Matter (SPM) (Table 3.1)

The values of suspended particulate matter (SPM) for the pre-monsoon season ranged from 9.29 to 17.85mg/l.

In April, 1989, the SPM for the 1st day showed a high value of 15.31mg/l, which decreased on the 2nd day to 9.29mg/l. An increase was observed on the 3rd day (17.85mg/l), which successively decreased for the 4th (16.12mg/l), 5th (11.18mg/l) and 6th (11.02mg/l) days for the same month.

In May, 1989, the 1st day represented the minimum value for the month (10.1mg/l), which increased for the 2nd day to 12.14mg/l. There was a decrease on the 3rd day to 11.73mg/l. For the 4th and 5th day the values increased and remained almost steady (16.12 & 16.88mg/l) and finally

dropped again for the 6th day to 14.27mg/l.

As seen in Table 3.1, the values for August, 1989, were comparatively higher for all the 6 days (34.35 to 38.97mg/l), when compared to those of April, 1989. The same fact was also observed during September, 1989, wherein the 1st day showed a value of 33.53mg/l, which increased to 41.88mg/l for the 2nd sampling day. The 3rd day exhibited a value of 34.2mg/l, which decreased on the 4th day (30.11mg/l). This value increased marginally to 33.25mg/l on the 5th day and once again increased on the 6th day (39.51mg/l).

The concentration during December, 1989, was shown to exhibit low values ranging between 11.8 to 17.34mg/l for all the 6 days sampled. In January, 1990, there was once again no significant variation, with values ranging between 15.08 to 18.76mg/l.

Just as in the case of April, 1989 and May, 1989, the same months of 1990 showed values of 10.11mg/l for the 1st day of April, which increased to 15.03mg/l on the 2nd day. A sudden decrease was observed (13.64mg/l) on the 3rd day. The maximum for the month was 18.58mg/l on the 4th day, which reduced marginally for the 5th day (17.16mg/l) and 6th day (13.44mg/l), respectively.

In May'90, the values observed ranged between 8.17 and 15.2mg/l, which were lower than those observed during May, 1989.

3.2 **Particulate Organic Matter (POM)** (Table 3.1)

As seen in the same Table 3.1, the concentration of particulate organic matter (POM) in April, 1989, ranged between 2.15 and 3.05mg/l. In the case of sampling during May, 1989, there was no remarkable change with values ranging between 2.05 and 3.25mg/l for the study period.

In the month of August, 1989, minimum value were observed (5.41mg/l) on the 6th day, followed by the 3rd day (5.6mg/l). The other days for the same month, did not show any significant change (7.53 to 8.39mg/l).

In September, 1989, minimum value of 4.5mg/l was observed on the 1st day of sampling. For all the other days, the values of POM ranged from 6.84 to 8.31mg/l. Sampling during December, 1989 and January, 1990, showed lower values when compared with August, 1989 and September, 1989. The values for both these months ranged between 2.9 to 4.66mg/l. April, 1990, showed very low values with 2.45mg/l on the 1st day, which remained almost steady on the 2nd day (3.17mg/l), 3rd day (3.33mg/l) and 4th day

(3.59mg/l). The 5th day showed value of 4.31mg/l which dropped remarkably on the 6th day to 2.54mg/l.

In May, 1990, the minimum value for the month was observed on the 5th day (1.99mg/l) and all the other days showed values between 2.58 and 3.31mg/l.

3.3 **Particulate Inorganic Matter (PIM)** (Table 3.1)

When PIM was measured for the study period it was observed that the 1st day of April, 1989, showed a value of 12.82mg/l. This value dropped to 6.62mg/l on the 2nd day. The months maximum of 14.8mg/l was observed on the 3rd day which decreased successively on the 4th day (13.1mg/l), 5th day (8.56mg/l) and 6th day (8.87mg/l), respectively.

In May, 1989, PIM concentration showed a minimum value of 6.85mg/l on the 1st day. The 2nd (9.38mg/l) and 3rd days (8.58mg/l) did not vary significantly. There was a sudden increase for the 4th day, the value being 14.07mg/l which decreased to 13.85mg/l for the 5th day and 11.29mg/l for the 6th day respectively.

In August, 1989, the values were comparatively high with the 1st day showing a value of 29.26mg/l, which dropped on the 2nd day (28.31mg/l) as well as on the 3rd

day (26.74mg/l). The maximum concentration for the month was observed on the 4th day (30.76mg/l), while the 5th day (27.6mg/l) and 6th day (28.94mg/l) did not show any significant change for the study period.

During September, 1989, the concentrations were quite high like those of August, 1989, with values ranging between 23.23 and 35.04mg/l.

December, 1989, however showed values which were very much lower (8.52 to 13.08mg/l) than those observed during August and September.

January, 1990 however, showed very minor changes with a minimum and maximum of 11.34 and 15.4mg/l during the sampling period.

In April, 1990, the month's minimum was observed on the 1st day (7.66mg/l) while the other days did not show significant changes (10.31 to 14.99mg/l).

During May, 1990, once again the minimum for the month was 5.39mg/l which was evident on the 1st day and maximum value of 12.91mg/l was observed on the 5th day. The other days showed values between 6.93 and 9.91mg/l.

3.4 Particulate Organic Carbon (POC) (Table 3.1)

The concentration of POC was found to be comparatively low during April, 1989, with insignificant changes in the concentration ranging between 1.25 and 1.77mg/l. An identical condition existed during May, 1989 (1.6 to 1.89mg/l).

During August, 1989, and September, 1989, higher values were observed which ranged between 3.14 and 4.87mg/l, respectively. Once again during December, 1989 the values dropped and varied from 1.81 to 2.65mg/l. A similar feature was also evident during January, 1990 (0.60 to 2.17mg/l).

In April, 1990, the minimum concentration for the month was observed during the 1st day (1.42mg/l) of sampling. There was marginal increase on the 2nd day (1.84mg/l), 3rd day (1.93mg/l), 4th day (2.08mg/l) and 5th day (2.5mg/l). However, the last day (6th day) showed a drop in POC to 1.47mg/l.

During May, 1990, minimum value was observed to occur on the 1st day (1.5mg/l), which increased till the 3rd day (1.92mg/l) and then dropped to 1.16mg/l on the 5th day. The 6th sampling day showed a value of 1.68mg/l.

3.5 Particulate Carbohydrate (PCHO) (Table 3.1)

Total carbohydrate concentration of the particulate material for April, 1989, showed a range between 0.179 and 0.273mg/l.

In May, 1989, the 1st day showed a value of 0.239mg/l. This value decreased to 0.128mg/l on the 2nd day. There was a still further decrease in the concentration from 0.157 on the 3rd day to 0.114mg/l on the 4th day. Maximum for the month was observed on the 5th day (0.292mg/l) which once again dropped to 0.141mg/l on the last sampling day for the same month.

During August, 1989, the values ranged between 0.258 and 0.425mg/l and during September from 0.324 to 0.428mg/l. Unlike such high values during August, 1989 and September, 1989, December, 1989, and January, 1990, showed lower values ranging between 0.123 and 0.250mg/l and between 0.111 and 0.294mg/l.

During the sampling of April, 1990, the 1st day showed a value of 0.173mg/l, which decreased on the 2nd day to 0.153mg/l. There was a further drop to 0.147mg/l on the 3rd day, which decreased still further (0.096mg/l) on the 4th day. The PCHO concentration on the 5th day (0.28mg/l)

represented the month's maximum and on the 6th day the value was observed to be 0.125mg/l.

In May, 1990, the 1st day showed a value of 0.127mg/l which increased to 0.245mg/l on the 2nd day. From the 3rd day onwards (0.182mg/l) there was a regular decrease till the last sampling day (0.094mg/l) for the study period.

3.6 **Chlorophyll^{a} (Chl^{a}) (Table 3.1)**

Chlorophyll^{a} concentration during April, 1989, showed very high values ranging from 0.617mg/l on the 1st day and 2nd day, and 0.986mg/l on the 5th days. The last day showed an intermediate value of 0.748mg/l. Even during May, 1989, the values remained high ranging between 0.569 and 0.858mg/l.

In August, 1989, there was a decrease in the concentration of chl^{a}, with the 1st day exhibiting a value of 0.194mg/l. This value increased to 0.21mg/l on the 2nd day, which remained almost similar even on the 3rd day (0.201mg/l). The maximum concentration of chl^{a} was observed on the 4th day (0.240mg/l), which decreased from 0.230mg/l on the 5th day to 0.179mg/l on the 6th day.

During September, 1989, like in the case of August, 1989, the chl^{a} concentration varied between 0.109 and 0.254mg/l.

A higher range when compared to August, 1989, and September, 1989, but lower than that of April, 1989 and May, 1989, was observed during December, 1989, and January, 1990. During December, 1989, chl^{a} values exhibited a minimum of 0.312mg/l and maximum of 0.696mg/l. On the other hand minimum and maximum value in January, 1990 was 0.225 and 0.392mg/l, respectively.

In April, 1990, high concentration of chl^{a} was observed, just as in the case of April, 1989. A value of 0.747mg/l was observed on the 1st day. There was a decrease for the 2nd day (0.627mg/l) and 3rd day (0.518mg/l) of sampling. A sudden increase in the chl^{a} concentration was observed on the 4th day (0.844mg/l), which once again dropped on the 5th day (0.742mg/l) and finally showed the month's maximum on the 6th day (0.956mg/l).

In May, 1990, the highest value for the month was observed on the 1st sampling day (0.936mg/l) and minimum value was evident on the 2nd day (0.549mg/l). All the other days showed values ranging between 0.799 and

0.888mg/l.

4 **Seasonal variation for daily sampling (Table 3.2)**

Concentration of particulate chl^a unlike the particulate parameters (SPM, POM, PIM, POC & PCHO) showed maximum concentration during the pre-monsoon season. Minimum values were evident for the monsoon season. Intermediate values occurred during the post-monsoon season.

4.1 **Suspended particulate matter (SPM) (Table 3.2)**

The values of SPM for pre-monsoon, monsoon and post-monsoon season of 1989, were 13.50, 35.63 and 16.01mg/l, whereas, for the pre-monsoon season of 1990, SPM concentration was seen to be 13.39mg/l.

4.2 **Particulate organic matter (POM) (Table 3.2)**

POM concentrations for the pre-monsoon, monsoon and post-monsoon seasons, showed values of 2.76, 7.09 and 3.61mg/l for the year 1989. Sampling for the pre-monsoon season of 1990, showed values of 2.98mg/l.

4.3 Particulate inorganic matter (PIM) (Table 3.2)

PIM concentration for the pre-monsoon season was lowest for both 1989, (10.73mg/l) and 1990, (10.41mg/l). Highest concentration was observed for the monsoon season (28.54mg/l) and intermediate values were recorded for the post-monsoon season (12.40mg/l)

4.4 Particulate organic carbon (POC) (Table 3.2)

POC content for the pre-monsoon 1989, monsoon and the post monsoon season showed values of 1.61, 4.16 and 1.95mg/l, while the pre-monsoon season of 1990, showed a value of 1.73mg/l.

4.5 Particulate carbohydrate (PCHO) (Table 3.2)

For the pre-monsoon season of 1989 and 1990, the PCHO concentration was 0.19mg/l & 0.16mg/l, respectively. Intermediate values were observed for the post-monsoon season (0.19mg/l) and peak value observed during the monsoon season (0.35mg/l).

4.6 **Chlorophyll^{a} (Chl^{a}) (Table 3.2)**

Chl^{a} exhibited peak values for the pre-monsoon seasons of 1989 & 1990, (0.751 & 0.776mg/l). Low values for the monsoon (0.200mg/l) and intermediate values were observed for the post-monsoon season (0.418mg/l).

5 **Percentage composition of SPM** (Table 3.3)

Daily variation in the percentage composition of SPM showed that POM did not vary significantly. Minimum percentage of POM recorded for the study period was 12.72% and maximum was 32.32%. On the other hand the minimum and maximum of PIM was 66.67% and 87.28% of the SPM.

The percentage contribution of POC to the SPM was also not significant and the percentage distribution varied between 3.93 and 19.05%.

The PCHO percentage of SPM for the study period varied between 0.52 and 2.29%.

Results of similar studies to understand the percentage contribution of PCHO and POC of POM as well as the contribution of carbohydrate carbon to POC are also presented in Table 3.4. The contribution of PCHO to POM

varied between 2.98 and 9.64%, while PCHO carbon's contribution to POC was between 1.85 and 12.87%. .

B) **WEEKLY OBSERVATIONS**

6.1 **Suspended particulate matter (SPM) (Table 3.5)**

As evident from Table 3.5, in April-May, 1990, week I showed SPM concentration of 13.88mg/l. There was no significant change in the concentration of SPM for week II (13.54mg/l) and week III (13.16mg/l). The maximum concentration for this period was observed during the sampling for week IV (14.64mg/l).

In August-September, 1990, high value of 36.40mg/l was observed for week I, which decreased successively for week II (29.01mg/l) and also for week III (28.67mg/l). A sudden increase in the concentration of SPM was observed for week IV (33.40mg/l).

During December-January, 1991, the values of SPM ranged between 14.70 and 21.20mg/l. Week I represented a value of 17.40mg/l, which increased to 19.75mg/l for week II. Minimum concentration was observed during week III (14.70mg/l), while the maximum concentration occurred in week IV (21.20mg/l).

6.2 Particulate organic matter (POM) (Table 3.5)

POM during April-May, 1990, showed a steady concentration for weeks I to III (2.41 to 2.83mg/l). Week IV showed a minimum value of 1.78mg/l during the study period.

In August-September, 1990, weekly samples showed comparatively higher values ranging between 5.31mg/l (week III) and 8.50mg/l (week II). The values which were observed for week I and IV were 7.72 and 8.48mg/l respectively.

In December-January, 1991, POM concentration was higher than in April-May, 1990 but lower than that observed for August-September, 1990. The value observed during week I of December-January, 1991, was 6.52mg/l. This value decreased subsequently for week II (4.64mg/l) and week III (4.30mg/l). There was an increase in the POM concentration to 5.44mg/l for week IV of sampling.

6.3 Particulate Inorganic Matter (PIM) (Table 3.5)

PIM concentration, like POM and SPM, during April-May, 1990, showed comparatively low values. Week I, for the

month showed a value of 11.34mg/l. Week II as well as week III did not show any significant change in the PIM concentration (10.71 & 10.74mg/l). The maximum concentration for the month was observed during week IV (12.86mg/l) of the study period.

In August-September, 1990, higher values of PIM were observed when compared to those of April-May, 1990, with a minimum value of 20.50mg/l (week II) and maximum of 28.68mg/l (week I) for the study period. Weeks III & IV showed values of 23.35 and 24.92mg/l respectively.

During the period of study, for December-January, 1991, intermediate values of PIM concentration were observed. Week III represented the month's minimum concentration of PIM (10.40mg/l), as compared to the value observed for week I (10.87mg/l). Week II as well as week IV showed PIM concentrations of 15.11 & 15.76mg/l respectively.

6.4 Particulate Organic Carbon/Nitrogen (POC/PON) (Table 3.5)

Concentration of POC and PON showed a similar trend with maximum concentration observed during August-September, 1990, (3.08 to 4.93mg/l and 1.14 to 2.11mg/l).

The period of April-May, 1990, represented minimum values (1.03 to 1.64 & 0.08 to 0.09mg/l). Intermediate concentrations were present in the December-January, 1991, period (0.08 to 0.18 and 2.04 to 3.36mg/l).

6.5 Chlorophyll^{a} (Chl^{a}) (Table 3.5)

Chlorophyll^{a} concentration for weekly sampling was maximum during April-May, 1990, (0.455 to 0.778mg/l), minimum in August-September, 1990, (0.148 to 0.398mg/l) and intermediate in December-January, 1991, (0.204 to 0.336mg/l).

7 Percentage composition for weekly sampling (Table 3.6)

The percentage composition of the SPM for weekly sampling is represented in Table 3.6. It was evident that the concentration of POM for the study period during weekly sampling showed a minimum of 12.17% and a maximum of 37.5%. The PIM percentage of the SPM was much higher than POM for the weekly sampling. This was also the case for the daily sampling. The minimum percentage for this parameter was 62.47% and the maximum was 87.83% for the study period. The percentage composition of POC for weekly sampling showed a minimum of 7.06% and a maximum of 17.00%.

However, no particular trend was evident for the different weekly samples.

The percentage of PON was low during the pre-monsoon season (0.61 to 0.75%) and maximum during the monsoon season (3.42 to 7.37%). Intermediate percentage contribution of PON was evident during the post-monsoon season (0.41 to 1.06%) of the SPM.

The POC:PON ratio was also studied for weekly samples. It was observed that this ratio mostly showed a decrease from week I to week IV (17.33 to 11.24%) during the April-May, 1990. The August-September, 1990, samples showed a decrease from week I (3.21) to week III (1.46%). However, week IV showed a sudden increase to 4.30%. This was also the case with December-January, 1991, for week I to III (20.57 to 16.22%). Week IV once again showed a discrepancy with a high value of 36.26%.

Seasonal distribution for the composition of the SPM is represented in Fig 3.1.

8 DISCUSSION

Suspended particulate matter (SPM) for the sub-surface waters of Dona Paula, for both daily (30.11-

39.51mg/l) and weekly (28.67-36.48mg/l) sampling showed a peak during the monsoon season. This was followed by the post-monsoon and finally the lowest concentration of SPM was observed during the pre-monsoon season. According to Shètye & Murty (1987), the mean fresh water runoff during the monsoon season was 150-400m^3/sec, whereas for the other seasons it was 10m^3/sec which is about <0.5%. Despite such wide seasonal fluctuations, differences in the trend of the various parameters of the particulate material distribution were found to be pronounced. Ward & Twilley (1986), have reported the concentrations of SPM to be 3.3 to 98.6mg/l for the coastal plain estuary. Such high values of SPM in the coastal estuary as in the sub-surface waters of the study area, indicated the extent of turbidity of these waters. SPM can broadly be divided into particulate inorganic matter (PIM) and particulate organic matter (POM). The PIM contributed 62 to 87% and formed the major portion of the SPM throughout the period of study. This was in agreement with the findings of Cadee (1982). PIM concentration was the highest during the monsoon season and consisted largely of terrestrial material. Similar finding were also reported by Whitehouse, (1985) and Gardner et al, (1989).

Simple regression analysis between PIM and salinity showed significant inverse relations ($r=-0.79$, $p<0.001$ & $n=12$). High content of PIM was associated with low chl^a values suggesting that inorganic matter may not directly influence the primary productivity of Dona Paula waters (Ward & Twilley, 1986). Low to intermediate concentrations of PIM with high to intermediate chl^a values once again confirms the assumption. Particulate organic matter fraction contributed 17% to 28% to the total SPM of subsurface seawater i.e. 21.4%, 20% and 23 % for the daily sampling and 17.5%, 23.6% & 28.9% for the weekly sampling in the order of pre-monsoon, monsoon and post-monsoon seasons respectively (Fig 3.1).

The distribution of particulate organic matter is mostly influenced by hydrographic conditions (Faganeli, 1989). The concentration of organic fraction showed a wide variation with time which could be due to its greater susceptibility to degradation. A greater portion of the decomposition products are recycled. In addition to these, primary producers also contribute strongly in direct or indirect ways, leading to the formation of organic particulates in the surface waters.

Particulate organic carbon (POC) showed a pronounced seasonal variation with peak values during the monsoon and

the lowest to intermediate values were observed for the pre-monsoon and post-monsoon seasons respectively. Such an increase in the POC concentration during the monsoons could be due to the increased water flow bringing in more amount of resuspended particulate material rich in organic carbon. Such a phenomenon was also observed by Cadee, (1982) and Faganeli, (1989). High concentration of POC in estuarine and coastal waters, as compared with ocean waters was reported by several workers (Wallace & Duce, 1975; Faganeli & Malej, 1981). In the eastern Harbour of Alexandria, absolute POC values were higher, with values ranging between 1.0 and 6.0mg/l. Sreepada et al, (1993) also showed high values of POC for coastal waters (0.380mgC/l) as compared to Oceanic waters (0.331mg.C.l). The present study showed that Dona Paula waters are characterized by high POC carbon with values ranging between 0.6-4.93mg/l. The percentage of organic carbon to the SPM accounted for approximately 12.2% for daily sampling and 13.5% for weekly sampling (Fig 3.1). These values were found to be comparatively higher than those reported for coastal waters. For example Biggs (1970), reported POC values of 7.8% of the SPM, whereas, Hobson & Menzel (1969) observed 5-7% POC in coastal waters. Still lower values were observed by Bhosle et al (1985) for the Mahi estuary and Lodar & Hood (1972) for Alaskan estuary.

An inverse relationship was observed between POC and salinity ($r=-0.59$, $p<0.01$ & $n=12$), indicating that a major part of the POC is transported to the study area through sewage discharge or any other allochthonous source. Gardner et al, (1989), for the coastal waters of South California (Georgetown, USA) and Sreepada et al, (1993), for the coastal waters along the east coast of India, also reported that POC in waters of these areas were mainly of allochthonous origin. For the present observation the POC percentage of POM ranged between 50-71% for daily sampling and between 57-58% for weekly sampling. Thus, more than 50% of the organic matter in this area seems to be composed of organic carbon. Organic nitrogen contributed 1-39% of the particulate organic matter. Such knowledge on the distribution of POC is essential to understand the carbon cycle and its balance in the waters of the area under investigation. Such data is of importance in understanding the process of microfouling settlement. Moreover, it was observed that the POC percentage of SPM during the post-monsoon season was higher for both daily (12%) as well as weekly sampling (16.8%) as compared to the other seasons of the year, indicating the possibility of a phytoplankton bloom during this season. Although the concentration of organic matter is high during the monsoon season, chl^a concentration was low indicating allochthonous

origin of organic matter. Thus, in addition to terrestrial input during the monsoon season, POC was also introduced into the system through primary production by plankton, both in the pre-monsoon and post-monsoon seasons.

Particulate organic nitrogen (PON), concentration was found to be the highest during the monsoon season and its percentage contribution to the SPM ranged between 0.7 to 4.70% for the weekly sampling. PON concentration was influenced by the vascular plant debris brought by river runoff during the monsoon season and probably by plankton biomass during the pre-monsoon and post-monsoons seasons. This observation was further confirmed by studying the ratios of POC/PON variation seasonally (Table 3.6). High concentration of SPM, POM, POC and PON in the monsoon season and low to intermediate concentrations during the other seasons indicate a common source for these compounds. POC:PON ratios were found to be >13 indicating significant contribution from plant detritus (Parsons et al, 1961).

Highly significant but inverse correlation between PON and salinity ($r=-0.90$, $p<0.001$ & $n=12$) indicates riverine origin of the particulate organic nitrogen (PON). Negative correlation of PON was also observed with chl^a indicating poor contribution of organic nitrogen by

phytoplankton.

Particulate carbohydrate (PCHO) was lowest for the pre-monsoon season and highest for the monsoon season. As shown in Table 3.3, PCHO contributed 1.00 to 1.50% to the SPM. On the other hand its contribution to POM was from 2.98 to 9.64% (Table 3.4). The carbohydrate carbon contribution to the POC was from 1.85 to 12.87%. Carbohydrate chemistry has a complex hydrology and is complicated further by a multiplicity of sources of organic material, sewage, industrial effluents, rivers runoff, planktonic and benthic processes as well as mixing processes with the sea. All these may introduce PCHO into the system. The fresh water discharge during monsoon probably explains such high concentration of carbohydrate during this period. Such a trend of high concentration during monsoon was earlier reported by Kamat,(1976),for the Dona Paula waters for the year 1974-75 (0.00-9.00ug/l).

Once again an inverse linear relationship with salinity explains the probable allochthonous detrigenous sources. During the monsoon season when minimum salinity was recorded, the production was found to be maximum as recorded by Qasim et al, (1969). The fall in salinity in the monsoons and thereafter its gradual rise in the pre-monsoon and post-monsoon seasons, may induce several

complex changes in the system. Low values during post-monsoon could be due to the active utilization by the filter feeding organisms and a part of it by bacteria (Bordovsky, 1965). In the pre-monsoon season low to intermediate values were recorded for the present study. Biggs & Wetzell (1968) have reported similar behaviour of PCHO for the Chesapeake Bay waters. Marshall & Orr (1964) studied PCHO in Ioch Strive waters and observed that increase in carbohydrate concentration was accompanied by increase in diatom number during monsoons or early post-monsoon. This fact was contradictory with our results which indicated a low diatom count during this season as compared to the pre and post-monsoon seasons. Laane (1982) observed 0.1-103ug/l of carbohydrates in the EMS Dollart-Estuary and 0-35.8ug/l was reported by Burney & Sieburth (1977) for coastal waters. Bhosle et al (1985), observed 4.37-15ug/l for the surface waters of Mahi estuary.

Although it is difficult to establish what portions of the carbohydrate are living or non-living, a general idea can be obtained from the chlorophyll^{a} values, assuming that 45% of the phytoplankton carbon is carbohydrate carbon (Parsons et al, 1977). PCHO:CHL ratio indicates that only a small fraction of the living source (1-38%) contributes to the PCHO concentration. These rough estimates only give

a general picture of the proportion of living and non-living carbohydrates. A better estimation as was done by Lancelot van Beveren, (1980), for the Southern Bight of the North Sea was not possible, because there was no positive relation between chl^a and PCHO. This fact also indicates that most of the carbohydrates could be of non-living origin.

Chl^a values were maximum during the premonsoon season followed by the post monsoon season. During the pre-monsoon season the less turbid waters allowed greater light penetration. This relation between high phytoplankton and greater light penetration during the pre-monsoon season was earlier also reported by Murugan & Ayyakannu, (1993). The percentage of phytoplankton carbon in these waters is comparable with the values given for coastal waters (3.27%) by Hung. et al, (1982) and (5.30%) Cadee, (1984). Finally, it may be inferred that during the pre-monsoon season the waters of the study area experiences no increase in SPM. This could be due to the two transport processes namely runoff induced advection of material out of the system and tidal induced diffusion into the estuary is minimum (Shetye & Murty, 1987). On the other hand during the monsoon season the runoff into the system increases greatly due to the wet spell of the south-

west monsoons, thereby, increasing the suspended load in these waters.

Table 3.1

Daily variation in the concentration of suspended particulate matter (SPM), particulate organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC), particulate carbohydrate (PCHO) and chlorophyll[a] of the sub-surface waters of Dona Paula

Time (d)		A (mg/l)	B (mg/l)	C (mg/l)	D (mg/l)	E (mg/l)	F (mg/l)
Apr'89	1	15.31	2.49	12.82	1.45	0.1790	0.617
	2	9.29	2.67	6.62	1.77	0.2130	0.617
	3	17.85	3.05	14.80	1.55	0.1910	0.929
	4	16.12	3.02	13.10	1.76	0.2730	0.986
	5	11.18	2.62	8.56	1.52	0.2070	0.986
	6	11.02	2.15	8.87	1.25	0.1970	0.748
May'89	1	10.10	3.25	6.85	1.89	0.2390	0.602
	2	12.14	2.76	9.38	1.60	0.1280	0.625
	3	11.73	3.18	8.55	1.80	0.1570	0.569
	4	16.12	2.05	14.07	1.19	0.1140	0.803
	5	16.88	3.03	13.85	1.76	0.2920	0.668
	6	14.27	2.80	11.29	1.73	0.1410	0.858
Aug'89	1	36.80	7.53	29.26	4.37	0.3044	0.194
	2	36.67	8.36	28.31	4.85	0.3172	0.210
	3	32.34	5.60	26.74	3.25	0.3079	0.201
	4	38.97	8.21	30.76	4.76	0.2944	0.240
	5	35.99	8.39	27.60	4.87	0.4254	0.230
	6	34.35	5.41	28.94	3.14	0.2581	0.179
Sept	1	33.53	4.50	29.03	3.20	0.4110	0.219
	2	41.88	6.84	35.04	3.97	0.3510	0.254
	3	34.20	7.94	26.26	4.61	0.3240	0.240
	4	30.11	6.88	23.23	3.99	0.4280	0.112
	5	33.25	8.31	24.94	4.82	0.3790	0.109
	6	39.51	7.09	32.42	4.11	0.3410	0.217

(contd..)

Table 3.1 contd..

	Time (d)	A (mg/l)	B (mg/l)	C (mg/l)	D (mg/l)	E (mg/l)	F (mg/l)
Dec	1	15.72	3.13	12.59	1.81	0.1370	0.435
	2	14.40	3.60	10.80	2.09	0.1230	0.457
	3	17.34	4.26	13.08	2.47	0.1270	0.312
	4	13.98	4.66	9.32	2.65	0.2500	0.696
	5	11.80	3.28	8.52	1.90	0.1560	0.634
	6	17.20	3.45	13.75	1.99	0.1440	0.529
Jan'90	1	18.31	3.75	14.56	1.95	0.2830	0.386
	2	18.76	3.36	15.40	2.17	0.1940	0.277
	3	16.26	3.44	12.82	1.99	0.2950	0.392
	4	18.09	2.90	15.10	1.74	0.1110	0.313
	5	15.28	3.94	11.34	0.60	0.1930	0.225
	6	15.08	3.57	11.49	2.07	0.2940	0.373
Apr'90	1	10.11	2.45	7.66	1.42	0.1730	0.747
	2	15.03	3.17	11.86	1.84	0.1530	0.627
	3	13.64	3.33	10.31	1.93	0.1470	0.518
	4	18.58	3.59	14.99	2.08	0.0961	0.844
	5	17.16	4.31	12.85	2.50	0.2810	0.742
	6	13.44	2.54	10.90	1.47	0.1250	0.956
May'90	1	8.17	2.58	5.59	1.50	0.1270	0.936
	2	15.20	2.70	12.50	1.57	0.2450	0.549
	3	10.24	3.31	6.93	1.92	0.1820	0.876
	4	11.40	2.88	8.52	1.67	0.1710	0.841
	5	14.90	1.99	12.91	1.16	0.1030	0.799
	6	12.80	2.89	9.91	1.68	0.0940	0.888

A= SPM, B= POM, C= PIM, D= POC, E=PCHO, F=CHL^{a}

Table 3.2

Seasonal variation in the concentration of suspended particulate matter (SPM), particulate organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC) particulate carbohydrates (PCHO) and chlorophyll^{a} (CHL^{a}) of the sub-surface waters of Dona Paula.

Time (s)	A (mg/l)	B (mg/l)	C (mg/l)	D (mg/l)	E (mg/l)	F (mg/l)
Pre-mon'89	13.50	2.76	10.73	1.61	0.19	0.751
Mon'89	35.63	7.09	28.54	4.16	0.35	0.200
Post-mon'89	16.02	3.61	12.40	1.95	0.19	0.419
Pre-mon'90	13.39	2.98	10.41	1.73	0.16	0.777

A= SPM, B= POM, C= PIM, D= POC, E= PCHO, F= Chl^{a}

Table 3.3

Daily variation in the percentage of particulate organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC) and particulate carbohydrates (PCHO) present in the suspended particulate material from the sub-surface waters of Dona Paula.

Time (d)		%POM	%PIM	%POC	%PCHO
Apr'89	1	16.26	83.74	9.47	1.17
	2	28.74	71.26	19.05	2.29
	3	17.09	82.91	8.68	1.07
	4	18.73	81.27	10.92	1.69
	5	23.43	76.57	13.60	1.85
	6	19.51	80.49	11.34	1.79
May'89	1	32.18	67.82	18.71	2.37
	2	22.73	77.27	13.18	1.05
	3	27.11	72.89	15.35	1.34
	4	12.72	87.28	7.38	0.71
	5	17.95	82.05	10.43	1.73
	6	19.62	79.12	12.12	0.99
Aug'89	1	20.46	79.51	11.88	0.83
	2	22.80	77.20	13.23	0.87
	3	17.32	82.68	10.05	0.95
	4	21.07	78.93	12.21	0.76
	5	23.31	76.69	13.53	1.18
	6	15.75	84.25	9.14	0.75
Sep'89	1	13.42	86.58	9.54	1.23
	2	16.33	83.67	9.48	0.84
	3	23.22	76.78	13.48	0.95
	4	22.85	77.15	13.25	1.42
	5	24.99	75.01	14.50	1.14
	6	17.94	82.06	10.40	0.86

(contd..)

Table 3.3 (contd..)

	Time (d)	%POM	%PIM	%POC	%PCHO
Dec'89	1	19.91	80.09	11.51	0.87
	2	25.00	75.00	14.51	0.85
	3	24.57	75.43	14.24	0.73
	4	33.33	66.67	18.96	1.79
	5	27.80	72.20	16.10	1.32
	6	20.06	79.94	11.57	0.84
Jan'90	1	20.48	79.52	10.65	1.55
	2	17.91	82.09	11.57	1.03
	3	21.16	78.84	12.24	1.81
	4	16.03	83.47	9.62	0.61
	5	25.79	74.21	3.93	1.26
	6	23.67	76.19	13.73	1.95
Apr'90	1	24.23	75.77	14.05	1.71
	2	21.09	78.91	12.24	1.02
	3	24.41	75.59	14.15	1.08
	4	19.32	80.68	11.19	0.52
	5	25.12	74.88	14.57	1.64
	6	18.90	81.10	10.94	0.93
May'90	1	31.58	68.42	18.36	1.55
	2	17.76	82.24	10.33	1.61
	3	32.32	67.68	18.75	1.78
	4	25.26	74.74	14.65	1.50
	5	13.36	86.64	7.79	0.69
	6	22.58	77.42	13.12	0.73

Table 3.4

Daily variation in the percentage of PCHO & POC present in the particulate organic matter (POM), and carbohydrate carbon as percentage of particulate organic carbon (POC) of the study area.

Time (d)		%PCHO	%POC	C %POC
Apr'89	1	7.19	12.34	4.94
	2	7.98	12.03	4.81
	3	6.26	12.32	4.93
	4	9.04	15.51	6.20
	5	7.90	13.62	5.45
	6	9.16	15.76	6.30
May'89	1	7.35	12.65	5.06
	2	4.64	8.00	3.20
	3	4.94	8.72	3.49
	4	5.56	9.58	3.83
	5	9.64	16.59	6.64
	6	5.04	8.15	3.26
Aug'89	1	4.04	6.97	2.79
	2	3.79	6.54	2.62
	3	5.50	9.47	3.79
	4	3.59	6.18	2.47
	5	5.07	8.74	3.49
	6	4.77	8.22	3.29
Sep,89	1	9.13	12.84	5.14
	2	5.13	8.84	3.54
	3	4.08	7.03	2.81
	4	6.22	10.73	4.29
	5	4.56	7.86	3.15
	6	4.81	8.30	3.32
Dec'89	1	4.38	7.57	3.03
	2	3.42	5.89	2.35
	3	2.98	5.14	2.06
	4	5.36	9.43	3.77
	5	4.76	8.21	3.28
	6	4.17	7.24	2.89

Table 3.4 (contd..)

Time (d)		%PCHO	%POC	C %POC
Jan'90	1	7.55	14.51	5.81
	2	5.77	8.94	3.58
	3	8.58	14.82	5.93
	4	3.83	6.38	2.55
	5	4.90	32.17	12.87
	6	8.24	14.20	5.68
Apr'90	1	7.06	12.18	4.87
	2	4.83	8.32	3.33
	3	4.41	7.62	3.05
	4	2.68	4.62	1.85
	5	6.52	11.24	4.50
	6	4.92	8.50	3.40
May'90	1	4.92	8.47	3.39
	2	9.07	15.61	6.24
	3	5.50	9.48	3.79
	4	5.94	10.24	4.10
	5	5.18	8.88	3.55
	6	3.25	5.60	2.24

Table 3.5

Weekly variation in the concentration of suspended particulate matter (SPM), particulate organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC), particulate organic nitrogen (PON) and chlorophyll[a] (CHL[a]).

Time (w)	A (mg/l)	B (mg/l)	C (mg/l)	D (mg/l)	E (mg/l)	F (mg/l)
Apr-May 1990 I	13.88	2.539	11.34	1.4730	0.085	0.778
II	13.54	2.830	10.71	1.6400	0.093	0.657
III	13.16	2.417	10.74	1.4020	0.099	0.444
IV	14.64	1.782	12.86	1.0340	0.092	0.596
Aug-Sep 1990 I	36.40	7.726	28.68	4.4810	1.396	0.148
II	29.01	8.505	20.50	4.9330	1.252	0.228
III	28.67	5.315	23.35	3.0830	2.112	0.398
IV	33.40	8.480	24.92	4.9180	1.143	0.290
Dec-Jan 1990-91 I	17.40	6.525	10.87	3.7850	0.184	0.336
II	19.75	4.640	15.11	2.6922	0.174	0.288
III	14.70	4.306	10.40	2.4979	0.154	0.288
IV	21.20	5.440	15.76	3.1550	0.087	0.204

A= SPM, B= POM, C= PIM, D= POC, E= PON, F= CHL[a]

Table 3.6

Weekly variation in the percentage of particulate organic matter (POM), particulate inorganic matter (PIM), particulate organic carbon (POC), particulate organic nitrogen (PON) of the suspended particulate matter and the ratios of POC:PON of the sub-surface waters of Dona Paula.

Time (d)		%POM	%PIM	%POC	%PON	POC:PON
Apr-May 1990	I	18.29	81.70	10.61	0.61	17.33
	II	20.90	79.10	12.11	0.69	17.63
	III	18.37	81.61	10.65	0.75	14.16
	IV	12.17	87.83	7.06	0.63	11.24
Aug-Sep 1990	I	21.23	78.79	12.31	3.84	3.21
	II	29.32	70.67	17.00	4.32	3.94
	III	18.54	81.44	10.75	7.37	1.46
	IV	25.39	74.61	14.72	3.42	4.30
Dec-Jan 1990-91	I	37.50	62.47	21.75	1.06	20.57
	II	23.49	76.51	13.63	0.88	15.47
	III	29.29	70.75	16.99	1.05	16.22
	IV	25.66	74.34	14.88	0.41	36.26

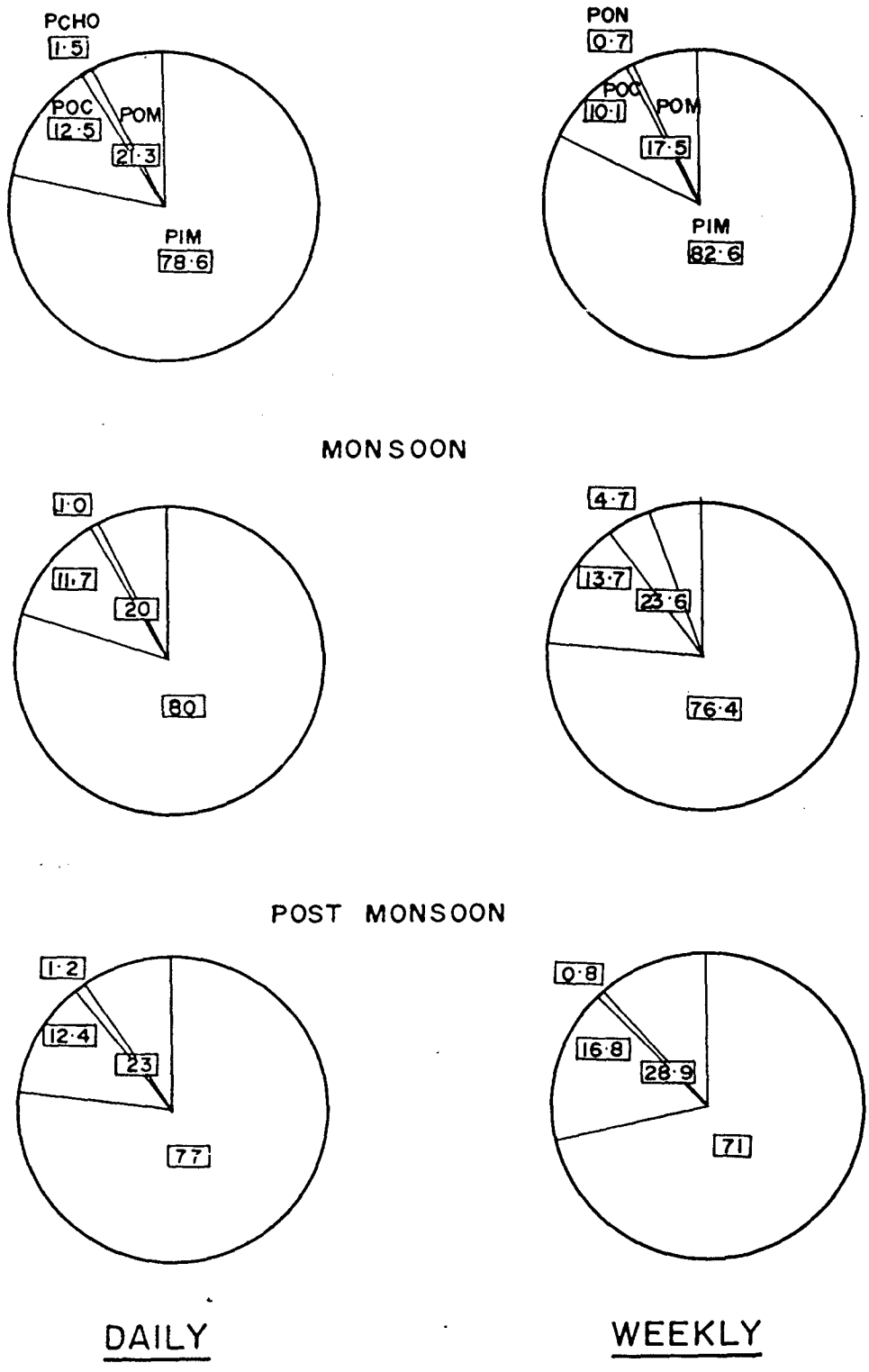


Fig 3.1 SEASONAL VARIATION IN THE PERCENTAGE COMPOSITION OF THE SUSPENDED PARTICULATE MATTER (SPM) FOR BOTH DAILY AND WEEKLY SAMPLING.

Chapter 4

ABUNDANCE OF DIATOM AND BACTERIAL FOULING ON VARIOUS SURFACES

ABUNDANCES OF DIATOM AND BACTERIAL

FOULING ON VARIOUS SURFACES

1. INTRODUCTION

It is apparent from the multitude of world wide studies that any object when introduced into seawater accumulates, with respect to its wettability, organic and inorganic detritus along with microorganisms and their extracellular products (Henchel & Cook, 1990). This accumulation may take place within hours or may take days together, depending upon the environmental conditions and the nature of the substrata.

The slime film was first studied by Bray, (1923), with reference to the control of fouling. Many researchers have reported bacteria to be the first organisms to get attached to surfaces (Corpe, 1970; Sieburth, 1979; Bhosle et al, 1989; Sharma & Wagh, 1990), while others are of the opinion that diatoms colonize prior to bacteria (Skerman, 1956; O'Neil & Wilcox 1971. There are still others (O'Neil & Wilcox, 1971; Paul et al, 1977) who are of the opinion that diatoms, fungi and cynophytes can occur at any stage before or after bacterial proliferation. Miyachi et al, (1989),

have reported that biofilm consisting of bacterial strains had a promoting effect on the diatom settlement. On the other hand, Kawamura et al, (1988), are of the opinion that bacterial biofilm had no significant effect on the attachment of diatoms. Although the presence of bacterial film may facilitate the attachment of diatoms, it is not a prerequisite (Horbund & Freiberger, 1970; Cooksey & Cooksey, 1980).

Such accumulation of microorganisms and their byproducts may lead to tremendous losses in the performance of handmade structures, seagoing vessels and other marine equipment (Characklis, 1973). Several high molecular weight polymeric compounds appear on the surface even before and after the accumulation of microorganisms. These polymeric materials have to be decomposed by extracellular enzymes which are secreted from living cells or liberated through lysis (Meyer-Reil, 1987). The enzymatic hydrolysis of polymers on surfaces is generally considered the rate limiting step in the biofilm formation. Such hydrolysis produces oligomers or monomers which are easily taken up by microbial cells to meet their energy demand and build up biomass on the substrata. Such work, involving carbon flow has been undertaken in considerable detail for marine sediments by Christensen &

Blackburn, (1982); Novitsky, (1983). However, they have not looked into detail as far as microfouling is concerned. Despite the fact that most of the studies of microbial fouling showed the presence of diatom on fouled surfaces, little is known about the contribution of these organisms to the biofilm. Many researchers have highlighted that bacteria represent the major fouling problem, which could be true for surfaces that are dark. However, surfaces, which are underwater and receive sufficient light will support the growth of algal population, and in many cases these algae are diatoms.

Hence, in the present study, an attempt has been made to quantify the primary settlers namely bacteria and diatoms on test panels, since these bacteria and diatoms have been recognized to be the primary biological colonizers on surfaces placed in aquatic environment (Characklis, 1973; Corpe, 1980; Marshall, 1981; Cooksey, 1981). In addition to this, visual morphology of the surface coupons with special reference to diatoms using Scanning Electron Microscopy (SEM) is also presented. In the present study, we investigated the seasonal changes in the communities of algae and bacteria in the waters of **Dona Paula** as well as on surfaces exposed to the subsurface waters of the same area, and attempted to examine their

interrelations in an annual cycle. An attempt is also made to study the preferential attachment of these particular groups to various types of material and to determine which material initiates settlement in preference to the other, over the period of immersion. Such studies evaluating or describing how different types of materials differ in their biofilm development, give valuable information on the ecology of attached microbial communities and hence this aspect has been studied.

2. MATERIAL AND METHODS

2.1 Test surfaces

Three types of panels, viz., aluminium, fibreglass and stainless steel of size 10 x 15 cm. and test coupons of size 5 x 5 mm were cleaned thoroughly with hydrochloric and chromic acid, washed with tap water and then rinsed with distilled water. The test panels were arranged on the fibreglass frames using PVC nuts and bolts. Test coupons were thoroughly polished using emery paper of gradient sizes and treated with xylene and ethanol. The coupons were then tied using fine nylon strings onto a frame of fibreglass. Both the frames were immersed in the sub-surface waters of the Bay (~ 1m). They were retrieved at

every 24 hourly intervals over a period of one week. Test panels were scraped with a nylon brush using filtered sea water as suggested by Sharma et al, (1990).

2.2 Diatom Count

A known volume of scraped material was fixed with lugol iodine solution. Dilutions were made and a known quantity (0.01 ml) was taken on the slides to count the total diatom number using a microscope (OLYMPUS, BH-2), in replicates of 10 for each sample. The total number was then calculated on the panel exposed, by estimating the area of the test panels used (Hitchcock, 1982).

2.3 Bacterial Count

Scraped material was diluted, and filtered and added to a test tube containing 0.1% acridine orange. It was then filtered through 0.2um nucleopore filter which was previously stained black using amido black stain. The filter was then placed on a microscopic slide containing a drop of immersion oil. A coverslip was placed over the filter and another drop of oil was placed on top of the cover slip. The bacteria which fluoresce green against a black background were counted in ten random fields for each

sample. Counting was done using an epifluorescence microscope (Nikon) as suggested by Parsons et al, (1984).

A number of precautions were taken to obtain good results. First the acridine orange solution as well as water used for dilution of samples were filtered through 0.2um nucleopore filters each day before the start of counting. Blanks of acridine orange and dilution water were run each day. Atleast 2ml of the fluid was made to pass through the filter to give a random distribution of cells. Bacterial numbers can change soon after the samples are collected, so preservation with an aldehyde may be necessary. We used 2% formaldehyde for the present study.

2.4 **Scanning Electron Microscopic study (SEM)**

Test coupons were rinsed with filtered sea water and placed in 5% (V/V) solution of gluteraldehyde. They were then washed in a series of increasing concentration of acetone solution. The coupons were then freeze dried (Marszalek & Small, 1969) in order to minimize the shrinkage and distortion of artifats caused by air drying. Dried specimens were then fixed onto SEM stubs using epoxy resin and sputter coated with gold for two minutes at 1.3 kV (~600°A) which provides a conducting material so as to

eliminate or reduce electric charge which builds up rapidly in a non conducting specimen when scanned by a beam of high energy electron. Scanning Electron Microscope, Cambax EPMA, was used for the present study.

3. RESULTS

3.1 Variation of diatom number with time (Fig 4.1)

Aluminium test panels during the pre-monsoon season showed relatively high values. For example the first 24h during April, 1989, showed a value of 19 x $10^3/dm^2$. Subsequently, the diatom number dropped to 7 x 10^3 $/dm^2$ during the next 48 hour period of exposure. Thereafter, the number showed an increasing trend for the entire period of study and reached a peak value of 59 x $10^3/dm^2$ after 144 hours of immersion. More or less similar observations were made when the study was carried out during the month of May, 1989. A high value of 15 x 10^3/dm2 was observed for the first 24h. This value dropped drastically to 9 x 10^3/dm2 for the next 24h and then increased for 72 (11 x 10^3/dm2), 96 (21 x 10^3/dm2), 120 (27 x 10^3/dm2) & finally for 144h (47 x 10^3/dm2) for the said period. The months of August and september, 1989, also showed a similar trend. Both the months showed a high value (13 & 12 x 10^3/dm2) for

the first 24h. This was followed by a decrease to 2 & 3 x 10^3/dm2 for the 48h sampling. Thereafter, a gradual increase in diatom numbers was evident (17 & 14 x 10^3/dm2) until the end of sampling (144h) for both the months.

The month of December and January, 1990, were quite similar in comparison to the above months, as far as the pattern of diatom numbers was concerned. The first 24h showed a high value of 14 & 17 x 10^3/dm2 respectively. This was followed by a drop to 5 & 4 x 10^3/dm2 for December and January, 1990. Thereafter, an increase was evident till a maximum number was attained, the values being 31 x 10^3/dm2 for December and 29 x 10^3/dm2 for January, 1990.

As observed in the case of aluminium, fibreglass test panels for April and May, 1989,also showed a very high number of diatoms during the first 24 hours (23 & 19 x 10^3/dm2) which gradually decreased during the next 24 hours (15 & 7 x 10^3/dm2). Thereafter, the diatom numbers showed a non-linear increase in diatom abundance on this substratum. However, the increase which was observed from 72 to 144 hour samples of fibreglass panels, was very marginal during the above months. During the months of August and September, 1989 the, initial 24 h sampled, showed a high value of 17 & 19 x 10^3/dm2 which dropped to 9 & 8 x 10^3/dm2 for the next 24h for fibreglass panels. Later for

72 and 96h panels showed a slow increase for both the months from 10 & 11 x $10^3/dm^2$ to 11 x 10^3/dm2 for both the months. The 144h exposed panels showed a steady increase to 14 & 13 x 10^3/dm2 respectively. Finally, the months of December & January, 1990, showed a value of 17 & 15 x 10^3/dm2 for 24h exposure period. The next 24h immersion period showed a drop in diatom numbers to 8 & 5 x 10^3/dm2 for both the months, which again increased marginally to 15 & 17 x 10^3/dm2 for the sampling time of 144 hours for fibreglass panels.

The test panels of stainless steel for the month of April, 1989, showed a high value (18 x 10^3/dm2) for the initial 24 hours exposure. It dropped to 5 x 10^3/dm2 for the next 24h sampling. Thereafter, a steady increase over the period of immersion was observed with highest count of 26 x 10^3/dm2. This was also the case during May, 1989, wherein a high value of 13 x 10^3/dm2 dropped to 2 x 10^3/dm2 during 48h sampling. There was a further increase until a value of 24 x 10^3/dm2 was attained for 144h sampling period. During the months of August and September, 1989, the first 24h recorded a value of 10 & 11 x 10^3/dm2 which decreased for the next 24h to 1 & 3 x $10^3/dm^2$. This was followed by a steady increase to reach a value of 11 & 9 x 10^3/dm2 for 144h sampling time. The month of December and

January, 1990, showed a value of 13 & 14 x 10^3/dm2 for the first 24h sampling. The number decreased drastically to 2 & 3 x 10^3/dm2 for 48h sampling. From the 72h sampling till 144h there was a steady increase in diatom numbers.

3.2 **Variation with Season**

The average count of diatoms for the sub-surface waters showed high values for the pre-monsoon season of 23 x 10^2 cells/l, intermediate values for the post-monsoon season (9 x 10^2 cells/l) and the lowest value in the monsoon season (3 x 10^2 cells/l).

Average diatom settlement on test panels also showed the highest value for the pre-monsoon season with a minimum of 5 x 10^3/dm2 and maximum of 59 x 10^3/dm2. During the monsoon season, settlement was the lowest with a minimum and maximum of 1x10^3/dm2 and 17 x 10^3/dm2, respectively. Post monsoon season showed intermediate values for diatom numbers on all the test panels with 2 x 10^3/dm2 and 31 x 10^3/dm2, representing the minimum and maximum values respectively, for the said season (Fig 4.2).

3.3 **Variation of bacterial count with time** (Fig 4.3)

The test panels of aluminium for the month of April and May ,1989, showed a total bacterial count of 32 & $37x10^4$/dm2 for the 24h exposure period. This value decreased to 27 & 24 x 10^4/dm2 for 48 hours sampling. There was an increase to 342 & 209 x 10^4/dm2 for the test panels, towards the end of sampling period. For the month of August and September, 1989 also, aluminium showed a higher value on the first day (9 & 11 x 10^4/dm2) which dropped to 2 & 4 x 10^4/dm2 for the next 24 hours of sampling. The sampling for 72h showed an increase to 4 & 7 x 10^4/dm2, until it reached a figure of 29 & 24 x 10^4/dm2 for the last sampling. The month of December and January, 1990, showed a similar trend, as the monsoon season, with a high initial value of 14 & 12 x 10^4/dm2 which sharply decreased to 5 & 6 x 10^4/dm2 and then increased gradually to 57 & 62 x 10^4/dm2 for 144 hours exposure for both the months.

The fibreglass panels during April and May,1989, showed an increase in total bacterial count over the period of 48 to 144 hours of immersion, with values ranging from 38 x 10^4/dm2 to 95 x 10^4/dm2 and 23 to 92 x 10^4/dm^2. The first 24h, however showed a high value of 49 & 42 x 10^4/dm2 respectively. In the month of August and september, 1989, also, the panels exposed for 24 hours showed a high bacterial count of 14 & 17 x 10^4/dm2 which dropped to 6 & 7

x 10^4/dm2 and then increased steadily until the last day of sampling (27 & 21 x 10^4/dm2). In December and January, 1990, a similar pattern with a linear increase (7 to 43 x 10^4/dm2 and 9 to 50 x 10^4/dm2) from 48 to 144 hours of exposure was evident. However, the first 24h showed a value of 17 x 10^3/dm2 for December, 1989 and 19 x 10^4/dm2 for January, 1990.

The stainless steel panels for the month of April and May, 1989, showed an initial high value of 11 & 7 x 10^4/dm2 which decreased to 3 & 2 x 10^4/dm2 for the next 24h. Thereafter, an increase was observed until a value of 103 & 281 x 10^4/dm2 was evident during the 144h sampling. During August and September, 1989, as well as December and January, 1990, also a similar trend was observed. It comprised of high value on the first day of 7 x 10^4/dm2 (August), 9 x 10^4/dm2 (September), 11 x 10^4/dm2 (December) and 17 x 10^4/dm2 (January). The values then decreased to 1 x 10^4/dm2 during August, 3 x 10^4/dm2 during September, December & January. Towards the end of sampling, maximum values were observed to be present. The values being 17 x 10^4/dm2 during August, 15 x 10^4/dm2 during September, 38 x 10^4/dm2 during December, 1989 and 35 x 10^4/dm2 during January, 1990, respectively.

3.4 Variation with respect to season

The average bacterial count in the subsurface water, was found to be 17 x 10^3cells/l for the pre-monsoon 2 x 10^3cells/l for the monsoon and 9 x 10^3cells/l for the post-monsoon seasons.

The settlement on the panels for the pre-monsoon season showed highest values ranging from 3 to 342 x 10^4/dm2, whereas, during the monsoon, it ranged from 1 & 29 x 10^4/dm2 and between 3 & 42 x 10^4/dm2, for the post monsoon season (Fig 4.2).

3.5 Microscopic photographs/scanning electron micrographs

PLATE 4.1
(~~Fig 4.4~~ a-j)

Among the commonly occurring diatom from the microfouling material, Navicula, Nitzschia, Pleurosigma, Coscinodiscus, Fragilaria, Licmophora, Chaetoceros, Rhizosolenia, Gramatophora, are reported from the study area and some of them are shown in the PLATE 4.1 ~~Fig 4.4~~ a-j. Scanning Electron Micrographs of Aluminium, fibreglass and stainless steel coupons exposed for 48, 96 and 144 hours are shown in PLATE 4.2 ~~Fig 4.5~~ A-C. Aluminium test coupons showed abundance of Gramatophora species. Fibreglass and stainless steel showed Navicula sp. in abundance. Coccoid

bacterial cells were most commonly observed on all the surfaces for the study period. Coupons which were exposed for 96 hours showed mucilagenous fibrillar material entrapping several microbial cells. Towards the end of sampling, the slime film on the surface was shown to enclose clusters of microbial cells to form a two-tiered layer to form a complex microsystem (PLATE 4.2 ~~Fig 4.5C~~)

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 ($r=0.80$, $p<0.001$, $n=36$).

4. DISCUSSIONS

Among the microbial community formed on test surfaces, bacteria usually comprise the greatest percentage of microbial colonizers (Zobell & Allen, 1935; Corpe, 1973). Diatoms also contribute significantly to the microfouling community (Callow et al, 1976; Rao, 1989). These microorganisms may develop within a few days on test surfaces (Marshall et al, 1971a). The abundance of these organisms in the primary film may influence the physical and chemical conditions at the surface (Terry & Edyvean,

1984; Edyvean, 1984) as well as subsequent colonization by macrofouling larvae (Crisp & Ryland, 1960; Barnes, 1970). Due to these reasons, abundances of the above mentioned organisms was evaluated with special interest. Further, continuous monitoring of the microfouling material developed on various substrata during the different exposure periods, also provides useful data to explain the dynamics of primary film formation and organism succession.

A very high abundance of diatom and bacteria during the first 24 hour period of exposure in the sub-surface seawater was evident (Fig 4.1 & 4.3) Their numbers reduced drastically for the subsequent 24 hours. Such an irregular pattern of diatom and bacterial numbers, especially in the earlier stages of microfouling, seems to be a regular phenomenon on various surfaces immersed in marine waters as also reported by Corpe, (1972) and Yanshun et al, (1984). This phenomenon observed on all test surfaces was probably due to reversible attachment of microorganisms to surfaces. According to Marshall et al, (1971b), mere attraction to surfaces does not guarantee that the cells will adhere irreversibly. The reversibly adhered organisms are removed by small changes in water velocity or other forms of disturbances. Alternatively, the irregular pattern of microfouling bacteria and diatoms observed in

the present study could also be ascribed to grazing by zooplanktons, fishes or crabs (Marshall et al, 1971; Marshall, 1976; Characklis et al, 1984). After the initial high value during the first 24h sampling, a decrease in numbers of both the forms were observed. Thereafter, a linear increase was observed until the end of sampling period (Fig 4.1 & 4.3). This showed that irreversible attachment was obtained by the end of 48 hours of immersion. Such an irreversible attachment is time dependent and may involve extracellular materials excreted by the microorganisms (Marshall, 1976)

Of the various materials used, fibreglass showed the highest number of diatom as well as bacterial cells, as compared to aluminium and stainless steel, especially during the initial 24 hours immersion period. Fibreglass is a non-metal and being hydrophobic it accumulates maximum microbial numbers initially (Fletcher, 1988; Pedersen, 1990). The slime film which develops on any solid surface along with microorganisms attach to substrata and exude high molecular weight polysaccharides. These polysaccharides adsorb water and have a tendency to maintain a water layer even on objects which are initially non-wetting, such as fibreglass (Kenis et al, 1974). However, with increase in immersion time from 48 to 144h

only a marginal increase was observed for this surface.

On the other hand aluminium being a metal was hydrophilic and unlike fibreglass, showed a different behaviour with respect to settlement. It accumulated lower microbial number initially in comparison to fibreglass, which increased drastically with increase in time. Stainless steel, being an alloy was also hydrophilic and showed low microbial number initially. This is because metals and alloys are known to be chemically active and each provides a unique physical and chemical environment to the colonizing microorganisms and behave quite similarly at least during the initial immersion period. However, the figure remained low on stainless steel when compared with those of aluminium and fibreglass till the end of sampling. This may be because stainless steel had a more electropolished surface and is known to be more resistant to microbial attack than are those with higher surface roughness such as aluminium (Dunsmore et al, 1981; Zoltai et al, 1981). This consequently implies that in addition to the hydrophobic or hydrophillic nature of the surfaces, the microstructure, its chemical composition and their interaction with the environment might play an important role in the development of microfouling settlement (Characklis & Escher, 1988; McEldowney & Fletcher, 1988).

Reports on fibre glass accumulating higher microbial biomass for short term study period have also been documented by Bhosle et al, (1989) and Raveendran et al, (1991). Similarly, Shrivastava et al (1990) have observed that metals accumulated higher biomass over a longer study period for Bombay Coastal waters.

Amongst the different algae, Nitzschia sp was found to be the most abundant form in the water column and on surfaces during sampling. This was followed by Navicula sp and Pleurosigma sp on test substrata. Aluminium panels also showed the presence of Grammatophora sp to be present in large numbers, especially during the initial 24 hours. Predominance of Navicula and Licmophora from the microfouling material has been reported from tropical and temperate waters (Cooksey et al, 1984; Bhosle et al, 1989). Of the 8 different forms of diatom species reported from the Dona Paula waters, 5 belonged to the pennate forms and 3 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 (Characklis & Cooksey, 1983; Cooksey et al, 1984; Bhosle et al, 1989; Raveendran et al, 1991). Similar dominance of pennate diatoms have also been reported from microfouling material developed on test surfaces exposed in

the open ocean waters of the Arabian Sea (Bhosle et al, 1989; Kelkar, 1989). They are known to secrete extracellular polysaccharides and mucilage (Haug & Myklestad, 1976) which could probably help in its adhesion to the substrata (Edgar & Pickett-Heaps, 1983; Webster et al, 1985)

The surface also showed the presence of non-living material which could help in the development of a conditioning film (Dempsey, 1981). Fibre glass test coupons for the same period showed pennate forms on the surface along with coccoid bacteria. The latter was also observed on stainless steel coupons along with mucopolysaccharide secretions from the initial 24h period of immersion. In a number of cases bacteria and the mucopolysaccharide secretions were so closely associated with the microorganisms that it was difficult to distinguish between the two, even under highest magnification (Perkins & Kaplan, 1978; Hamilton & Duthie, 1984). Mucopolysaccharides probably help in binding the organisms to the surface by bridging the gap between the cell and surface by adhesive polymers (Fletcher & Floodgate, 1973). Hence microorganisms attach immediately after maturation of the conditioning film to develop into a rich microbial community indicating that polymer bridging is important even at the earliest stages (Zachary et al,

1980, Dempsey, 1981). It was also observed that substrata preference shown by certain species initially, disappeared with longer immersion time.

Several workers (Mitchell & Krichman, (1984), White & Bensen, (1984) and Yanshun et al, (1984) suggest that bacteria were the first organisms to appear on surfaces placed in seawater. The present observation on the microfouling material developed on aluminium, fibreglass and stainless steel however, showed that a large number of diatoms were also present during the initial 24 hours of sampling. Thus, both diatoms and bacteria co-existed on surfaces simultaneously. Significant correlation existed between diatom and bacterial numbers for surfaces.

Although it was observed that bacterial count was several times higher than the diatom count in the microfouling material during all the seasons, the pattern for each season was identical for both types of organisms. The pre-monsoon season contributed to the peak counts for all the surfaces. In this season, the microbial count in the bulk medium was also high as compared to the post-monsoon and monsoon seasons. This was followed by the post-monsoon season which showed intermediate values for microbial settlement. In the monsoon season however, minimum count was observed as compared to the other two

seasons of the year. It was also supported by recording of a low count in the ambient surface seawater of the study area.

Further, it was interesting to note that higher microbial numbers were observed on surfaces as compared to the ambient seawater. This could probably be due to a selective increase in survival, rather than due to an increase in growth, because the intertidal 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 as mentioned by Fletcher, (1984).

Significant correlations between diatom and bacterial counts for all the surfaces, could mainly be due to the fact that bacteria grow by consuming the organic material produced by algae. The algal communities to some extent influence 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 inturn depend on bacterial products such as vitamins (Furuki et al, 1985), siderophores (Murphy et al, 1976) and other trace elements. Thus, cross feeding between two different microorganisms within a biofilm allowed a growth of

copiotrophs under oligotrophic nutrient conditions that were restricted to the overlying water column.

In addition to the count method, the surface morphology was further studied using Scanning Electron Microscope, wherein the micrographs help in the visual comparison of various surfaces. Such a qualitative assessment of test coupons made the initial microfouling studies easier since a large diverse biological and non-living community on the surface could be compared. Definite evidence for the involvement of polysaccharides bridging in microbial adhesion was obtained by Scanning electron microscopic examination (Fletcher & Floodgate, 1973; Read & Costerton, 1987).

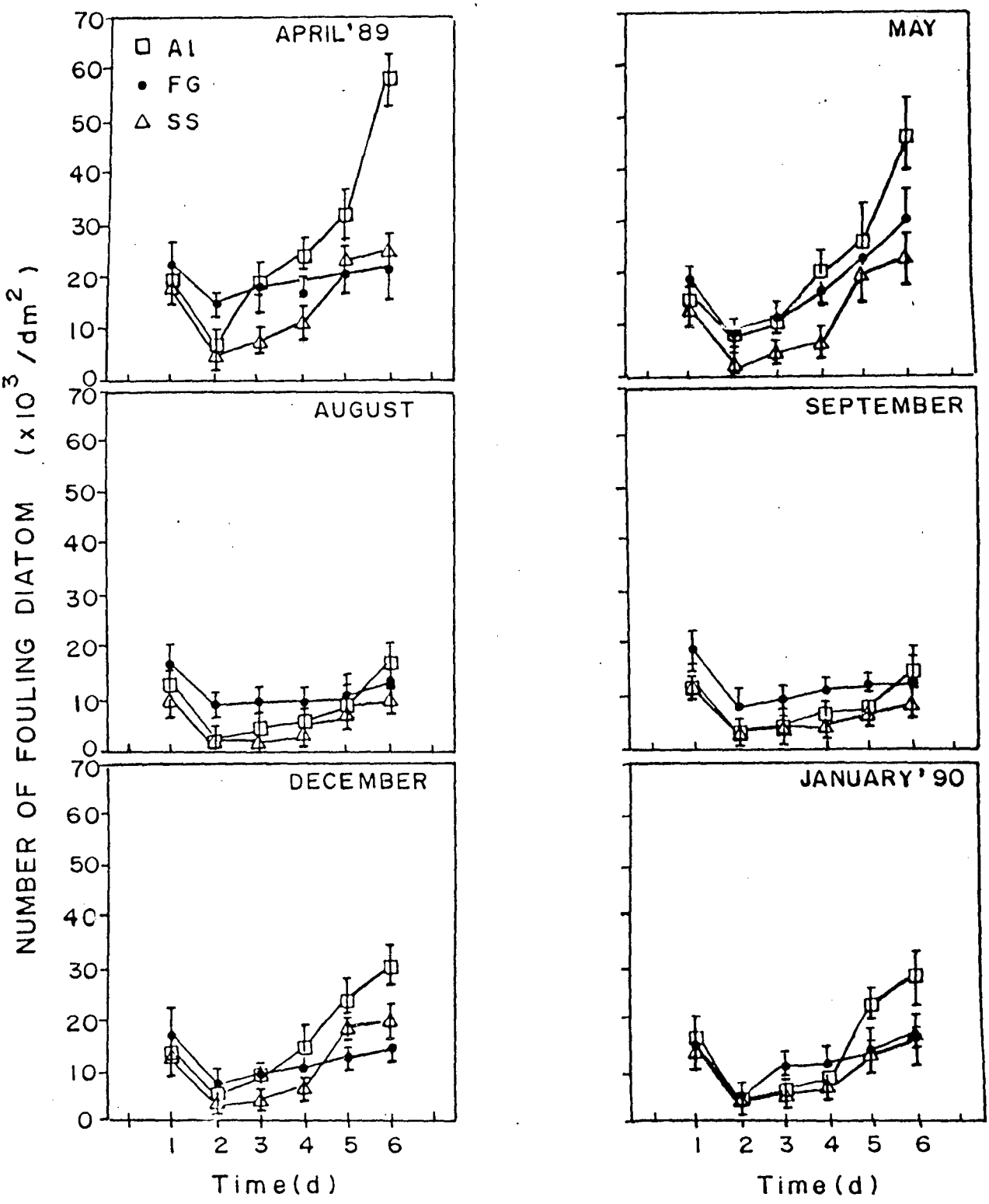


Fig.4.1 NUMBER OF DIATOMS SETTLED ON DIFFERENT SURFACES WHEN EXPOSED TO THE STUDY AREA.

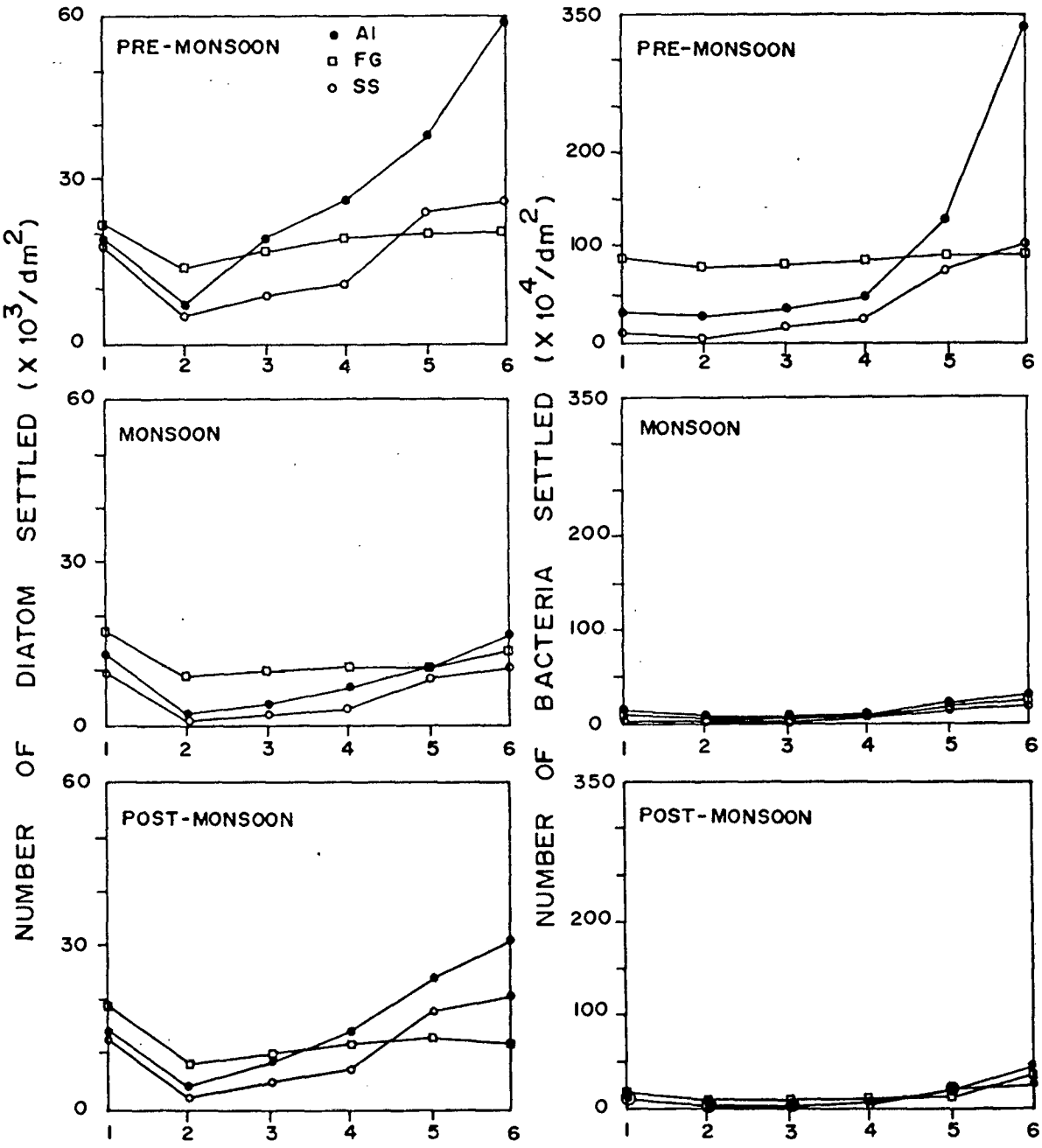


Fig.4·2 NUMBER OF DIATOMS AND BACTERIA SETTLED ON DIFFERENT SURFACES WHEN EXPOSED TO THE STUDY AREA(SEASONAL)

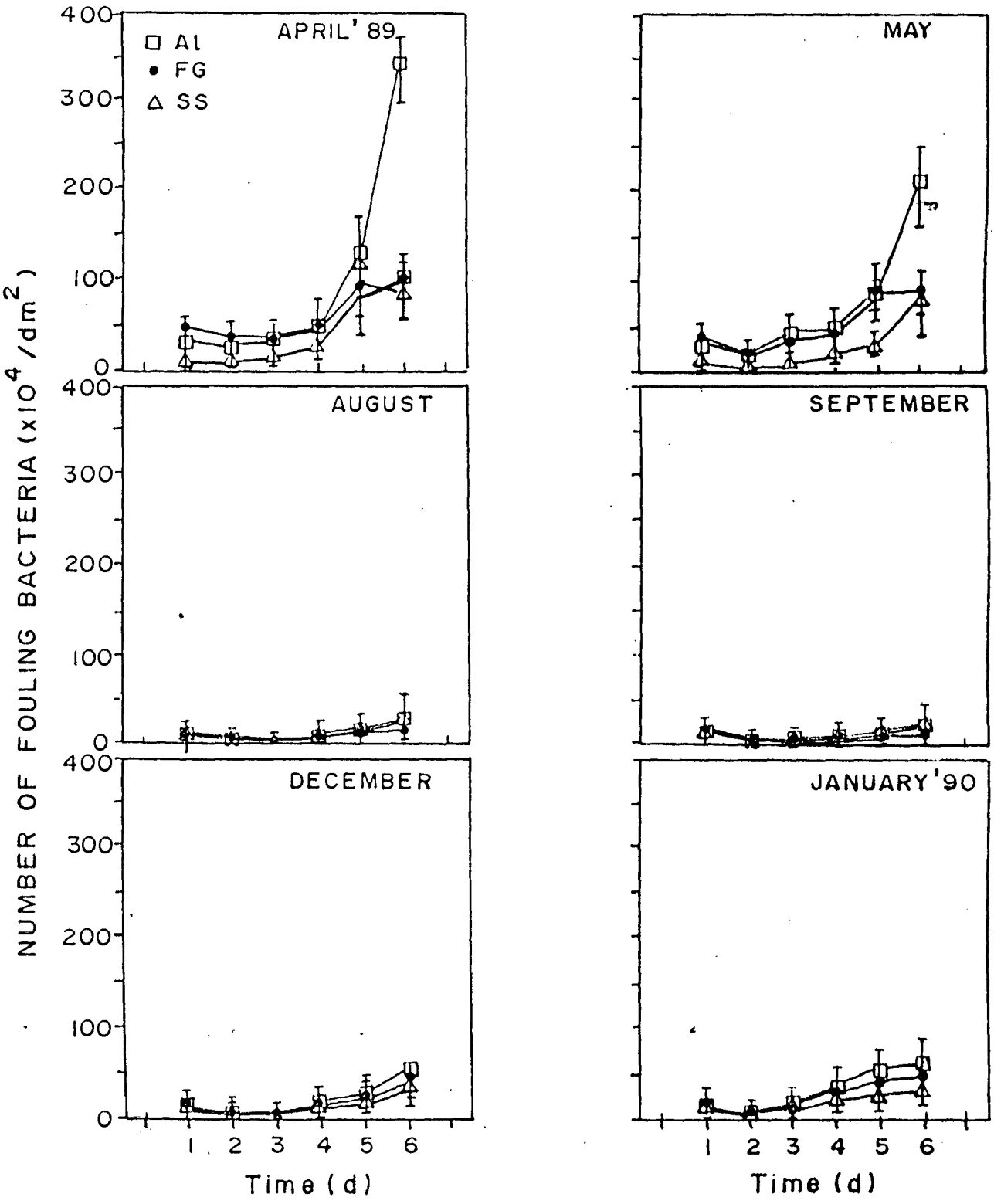
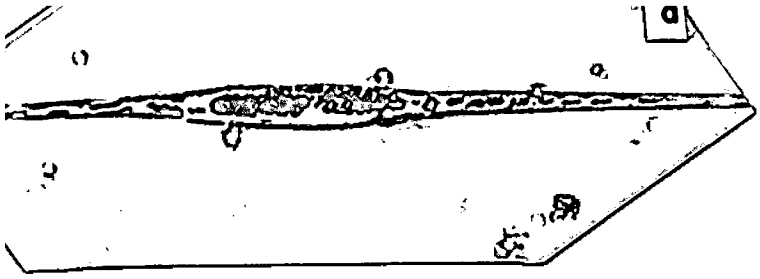


Fig. 4.3 NUMBER OF BACTERIA SETTLED ON DIFFERENT SURFACES WHEN EXPOSED TO THE STUDY AREA.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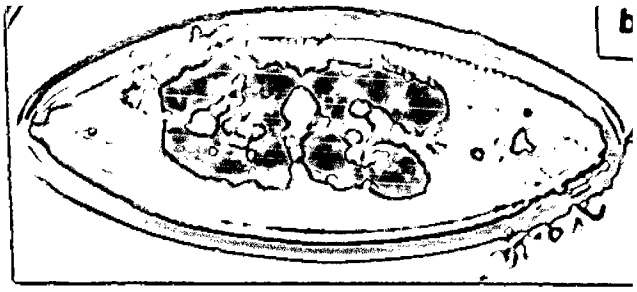
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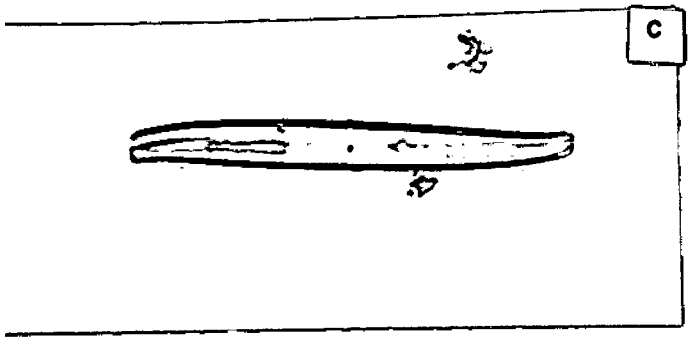
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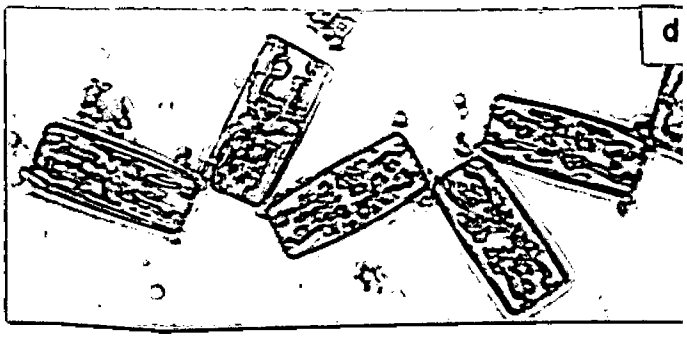
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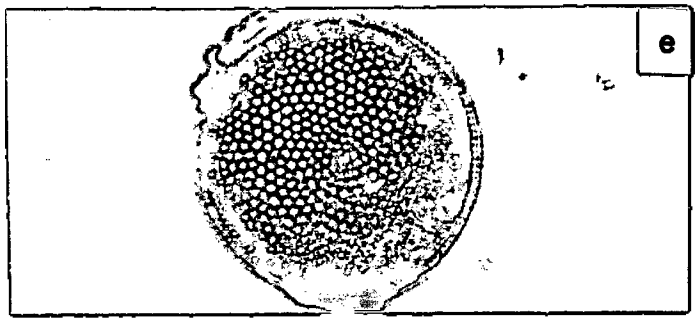
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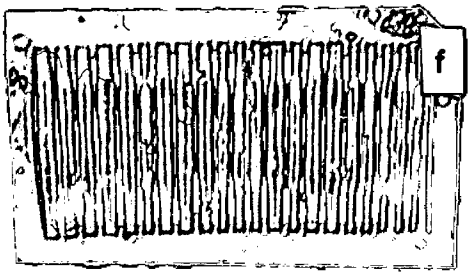
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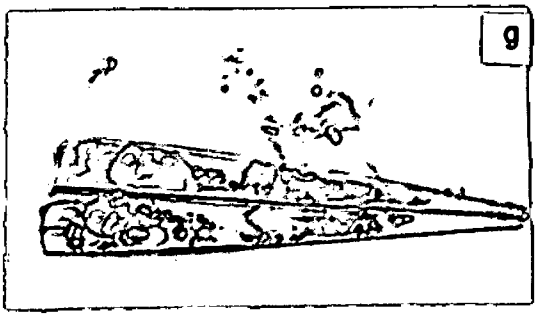
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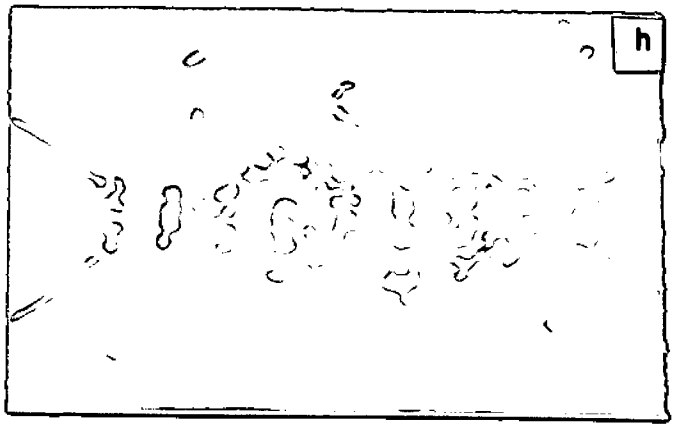
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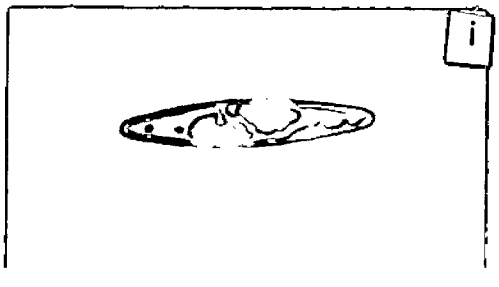
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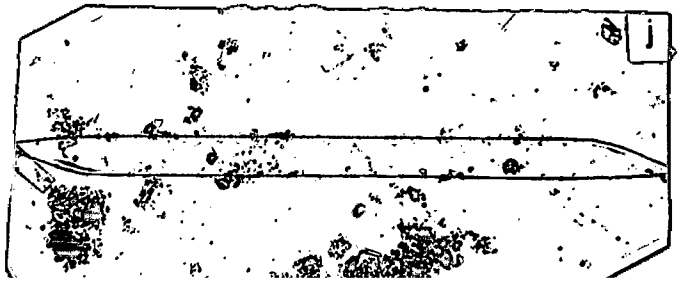
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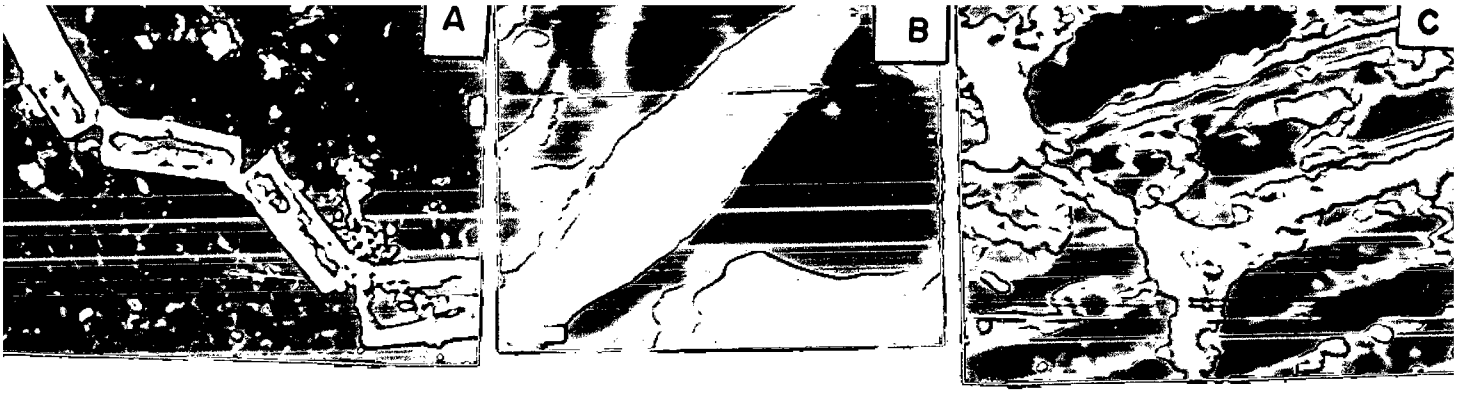
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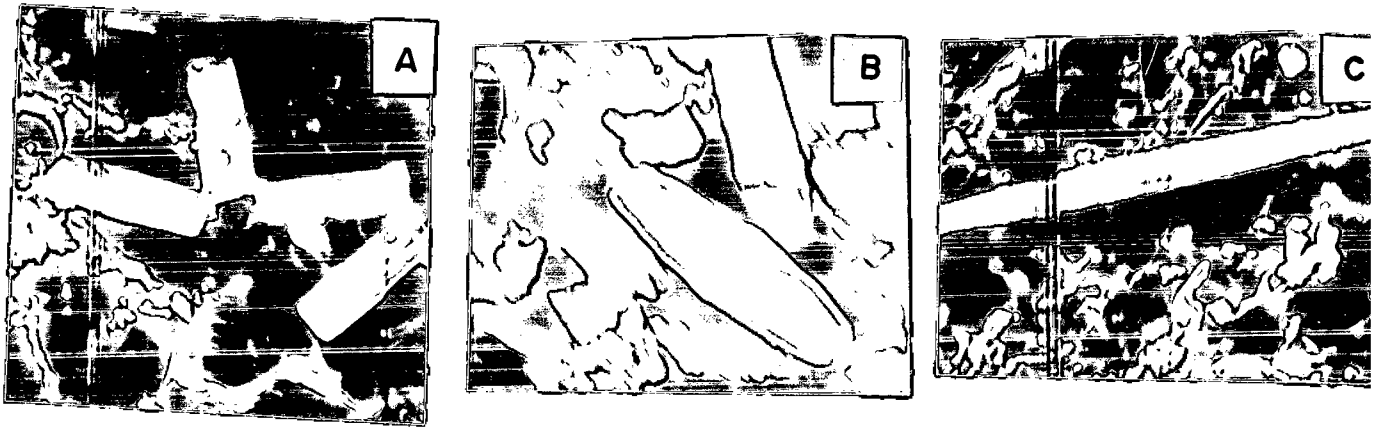
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Chapter 5

OBSERVATIONS ON THE MICROFOULING BIOMASS ON VARIOUS SURFACES

OBSERVATIONS ON THE MICROFOULING BIOMASS ON VARIOUS SURFACES

1. INTRODUCTION

Before the advent of the visible organisms on the surface, the conditioning film is the primary step in toning the surface to microfouling (Loeb & Neihof, 1977; Characklis & Escher, 1988; Zutic & Tomaic, 1988) and subsequently macrofouling (Miller et al, 1948; Crisp & Ryland, 1960; Mihm & Banta, 1981; Little, 1984; Mitchell & Kirchman, 1984). The conditioning film involves macromolecular agents such as glycoproteins, (Baier, 1980), humic material, (Loeb & Neihof, 1975) or unspecified macromolecules, (Zaidi et al, 1984). This phenomena of microfouling seems to significantly influence the larval settlement of many, if not all macrofoulers. Hence detailed observations of the particulate material developed on various surfaces over the short term period ranging from 1 to 6 days and I to IV weeks were made to assess fouling biomass.

It is known that larvae of macrofoulers require specific stimuli to induce settlement and metamorphosis and it has been suggested that such stimuli may be partially derived from the substratum (Crisp, 1974; Scheltema, 1974; Burke, 1983; Hadfield, 1986). Hence the organic and inorganic nature of the particulate matter which forms this substratum needs to be analysed in detail. Such an analysis could help in determining the role of these particulates in microfouling settlement, which inturn is believed to be responsible for inducing settlement of macrofoulers. Several reports are available on the biological and chemical characterization of the microfilm (Paul & Loeb, 1983; White & Benson, 1984; Paul et al, 1985; Karande, 1987; Bhosle et al, 1989, Sharma & Wagh, 1990; Bhosle et al, 1990; Srivastava et al, 1990; Raveendran et al, 1991). However no particular attempt is made to determine the percentage of organic and inorganic components from the total particulate matter of various substrata, for the different seasons of the year. Therefore, in the present chapter an attempt is made to study the major fractions of the particulate material developed on aluminium, fibreglass and stainless steel immersed in the Dona Paula waters.

Further, the formation and succession of microfouling organisms in a community, are affected by a variety of environmental factors such as temperature, salinity, light, organic and inorganic materials (Characklis et al, 1984; Yanshun et al, 1984; Fletcher, 1988; McEldowney & Fletcher, 1988). Interrelationship studies were made between the microfouling biomass measured as total dry weight (DW), organic matter (F-OM) and inorganic matter (F-IM) with various hydrographic parameters and biological components such as bacteria and diatoms and suspended load of the sub-surface waters.

2 MATERIAL AND METHODS

2.1 Preparation of test panels, deployment and retrieval

Aluminium, fibreglass and stainless steel test panels (10 x 15cm) were deployed in Dona Paula waters to study microfouling at daily and weekly intervals. The method used for cleaning the panels and preparation of the frame is described in detail, in chapter IV.

Sampling was done after every 24 hours for a period of 6 days, during April 1989, May 1989, August 1989, September 1989, December 1989, January 1990, April 1990 and

May 1990. Sampling was also done at weekly intervals for a period of 4 weeks during April-May 1990, August-September 1990 and December-January 1991. Five replicate panels were used for the microfouling studies.

2.2 Removal of microfouling material

After retrieval, the panels were gently rinsed with filtered seawater, then scraped using a nylon brush as suggested by Sharma et al, (1990), and made to a known volume (100ml) using filtered seawater. Aliquots (10ml) from the above were filtered using ashed (450° C, 4h), and preweighed glass fibre (GF/C) filters (4.7mm dia. & 1.0um pore size). The filter papers were dried in an oven at 40° C for 24h before analysis of microfouling dry weight (DW), organic matter (F-OM) and inorganic matter (F-IM).

2.3 Chemical analysis

Dry weight (DW)

The weight difference of the dry filter papers before and after filtration of the microfouling material gave the dry weight of the microfouling material formed on various study surfaces.

Organic matter (F-OM)

Fouling biomass estimated as organic matter was obtained from the organic carbon values by multiplying the latter by a factor (1.724). Organic carbon was estimated as suggested by Parsons et al, 1984.

Inorganic matter (F-IM)

The difference in the values between dry weight (DW) and organic matter (F-OM) resulted in the inorganic content of the microfouling material.

3 RESULTS

The change in the physical appearance of the panels with the increasing period of exposure was very much evident. Aluminium test panels changed from a silvery white colour to a pale black colour, whereas fibreglass and stainless steel panels appeared slimy. The observed change in colour on these surfaces could be due to the corrosion products in the case of metals and/or due to microbial attachment, in the case of both metals and non-metals.

3.1 SHORT TERM VARIATION

The changes in biomass values varied with the type of material exposed. Variations in biomass values are described for aluminium, fibreglass and stainless steel. The total-dry weight (DW) of the microfouling material for all the surfaces, fluctuated between 21.15 to 236.33mg.dm-2.

3.1a Aluminium surface

Dry weight (DW) (Fig 5.1)

The microfouling biomass expressed as DW developed on aluminium test surface showed values between 31.62 and 236.33 mg.dm-2 (Fig 5.1). During April, 1989, as well as in May, 1989, all the 6 days showed a similar trend with a low value of 131.22 & 119.54mg.dm-2, for the initial exposure period of 24h. This was followed by a sharp increase in the dry weight concentration on the 2nd day, for both the months of April, 1989, (197.65mg.dm-2) and May, 1989, (180.55mg.dm-2). The 3rd day showed a decrease in the value to 182.25 and 170.25mg.dm-2, which finally increased to 236.63mg.dm-2 and 214.15mg.dm-2 for both the months, during the end of the sampling period (6th day).

During August, 1989, as shown in Fig 5.1, low value of 32.19mg.dm-2 was observed on the 1st day which decreased only marginally on the 2nd day (31.62mg.dm-2). Thereafter, a steady increase was observed till the 6th day, which recorded a value of 50.92mg.dm-2. During the month of September, 1989, the 1st day exhibited 37.55mg.dm-2 of DW. A steady increase was observed with increase in the immersion time, till the 5th day (58.76mg.dm-2). On the last day of sampling (6th day), a sudden decrease to 54.32mg.dm-2 was observed.

During the month of December, 1989, the values varied from a minimum on the 1st day (39.66mg.dm-2) to a maximum on the 6th day (77.02mg.dm-2), with all other days showing a steady increase till the last day. Similar observations were also recorded during January, 1990, when the minimum value of 49.32mg.dm-2 was observed on the 1st day and maximum of 93.62mg.dm-2 was seen on the 6th day. Sampling conducted during April and May, 1990, are also presented in the Fig 5.1. In April, 1990, the trend was identical to that prevailing during December and January, 1989, with a minimum of 72.33mg.dm-2 and increasing till it reached a maximum of 107.22mg.dm-2 on the 6th day. An identical pattern was observed for the month of May, 1990, (63.32 to 97.65mg.dm-2).

Organic matter (F-OM) (Fig 5.2)

When microfouling biomass was studied as organic matter, on aluminium test surface, it was observed that the values ranged between 3.29 and 53.80mg.dm-2 for the entire study period (Fig 5.2).

During April, 1989, the lowest value of 21.17 was observed on the 1st day, which increased significantly to 37.27mg.dm-2 on the 2nd day. From then on there was an increase till the 6th day (53.80mg.dm-2). When sampling was done in May, 1989, a minimum value was once again observed on the 1st day (19.77mg.dm-2), which increased on the 2nd day (21.65mg.dm-2) and 3rd day (29.55mg.dm-2). However, there was a decrease in the concentration of organic matter (27.21mg.dm-2) on the 4th day, which increased therefrom till the end of sampling period (38.96mg.dm-2).

When the samples of August, 1989, was analysed, it was observed that (Fig 5.2) a low value of 3.29mg.dm-2 was seen on the 1st day which increased to 11.01mg.dm-2 on the 6th day. Increase from the 1st till the 3rd day was marginal. On the 4th day however, a sudden steep increase (10.32mg.dm-2) was noted which once again remained more or less stable (10.98mg.dm-2) for the 5th day and increased

only marginally (11.01mg.dm-2) for the last day.

During September, 1989, the values were somewhat erratic. This was because on the 1st day a value of 7.77mg.dm-2 was observed which increased to almost two times (13.77mg.dm-2) on the 2nd day. There was a decrease on the 3rd day to 12.33mg.dm-2, and a further decrease marginally on the 4th day (12.00mg.dm-2) and 5th day (11.42mg.dm-2). There was a very slight increase on the 6th day to 11.67mg.dm.-2

The results obtained during December, 1989, revealed a value of 4.31mg.dm-2 on the 1st day, which showed a linear increase with time, till the end of the study period (10.99mg.dm-2). However, during January, 1990, on the 1st day a value of 8.77mg.dm-2 was observed which increased to 9.67mg.dm.-2 on the following day. There was a drop to 8.58mg.dm-2 on the 3rd day increasing thereafter, till the last day, the value being 15.53mg.dm-2.

The biomass during April, 1990, showed an increase in the concentration from the beginning (11.77mg.dm-2), till the end of sampling (19.82mg.dm-2). During May, 1990, the lowest value was observed on the 2nd day (7.05mg.dm-2). All other days showed a steady increase, as can be seen in Fig 5.2.

Inorganic matter (F-IM) (Fig 5.3)

Inorganic component of the microfouling material (F-IM) on aluminium panels showed fluctuations for the entire study period. It exhibited a maximum value of 182.53mg.dm-2 and a minimum of 25.65mg.dm-2 during the study period.

As evident from Fig 5.3, the change in the F-IM concentration showed a similar trend as that of DW. During the months of April and May, 1989, lowest values viz., 110.05 and 99.77mg.dm-2 were observed on the 1st day of these months. This was followed by a steep increase to 160.38mg.dm-2 and 158.90mg.dm-2 on the 2nd day for both the months. The 3rd day showed a decrease in the concentration of F-IM for both the months (142.08 & 140.70mg.dm-2). Values thereafter, increased till maximum were obtained during April and May, 1989, with values being 182.53mg.dm-2 and 175.19mg.dm-2, respectively.

During August, 1989, the value recorded was 28.9mg.dm-2 on the 1st day as could be seen from the Fig 5.3, which decreased to 26.87mg.dm-2 for the 2nd day. The values till the 6th day showed an increase with time. A similar pattern was observed during September, 1989, with the value being 29.78mg.dm-2 on the 1st day, which decreased to 25.65mg.dm-2 on the 2nd day. The

concentration thereafter, increased till the 5th day (47.34mg.dm-2). On the 6th day, however, there was a decrease to 42.65mg.dm-2 in the concentration of F-IM.

The sampling during December, 1989 and January, 1990, showed a linear increase with time, showing values to range from 35.35 to 66.03mg.dm-2 in December, 1989, and 40.55 to 78.09mg.dm-2 in January, 1990.

Similar observations were also made during April, 1990, with values ranging from 60.56 to 87.40mg.dm-2 and from 55.79 to 85.89mg.dm-2 during May, 1990.

3.1b **Fibreglass surface**

It was found that the DW developed on fibreglass surface varied from 30.35 to 266.45mg.dm-2 during the study period.

As can be seen from Fig 5.4, short term variation during April, 1989, showed wide ranging changes in the DW content. The 1st day exhibited a value of 143.62mg.dm-2, which increased to 152.52mg.dm-2 on the 2nd day. The 3rd day showed a considerable increase in the DW content amounting to 215.16mg.dm.-2 The biomass values however, decreased to 192.77mg.dm-2, on the 4th day. It once again

increased on the 5th day to 266.45mg.dm.-2 The last day (6th day), showed a sudden decrease to 233.42mg.dm-2. Hence, the variation in DW for this month was highly erratic with time, on fibreglass test surface. However, during May, 1989, on the 1st day a concentration of 128.42mg.dm-2 was observed. An unexpected high value of 180.44mg.dm-2 was observed on the 2nd day. There was a sudden drop in the DW concentration for the 3rd day (119.96mg.dm-2). On all the other days, a marginal increase in the values from 119.96mg.dm-2 on the 3rd day, to 157.06mg.dm-2 on the 6th day, has been observed.

The value observed during August, 1989, was 30.34mg.dm-2 on the 1st day and 49.51mg.dm-2 on the 6th day. In September, 1989, values ranged between 38.04 and 46.25mg.dm-2. While during December, 1989, biomass values varied between 39.77 and 62.77mg.dm-2. Similarly, in April & May, 1990, a somewhat identical trend was observed, the values ranging between 80.37 & 98.66mg.dm-2 and 77.65 & 93.21mg.dm-2, respectively. Sampling for the month of January, 1990, unlike for the other months also showed such a trend from the 1st day (52.85mg.dm-2), till the 3rd day (78.78mg.dm-2). Thereafter, a decrease in the value was observed on the 4th day of sampling, amounting to 64.60mg.dm-2. Subsequently, the increasing trend was quite evident as shown in Fig 5.4.

Organic matter (F-OM) (Fig 5.5)

The organic matter content of the microfouling material developed on fibreglass test surface showed remarkable variations in April, 1989, as shown in Fig 5.5. On the 1st day a value of 24.22mg.dm-2 was observed. There was a sudden increase in value on the 2nd day to 37.23mg.dm-2. However, the value on the 3rd day was low (31.09mg.dm-2), and decreased even further on the 4th day to 30.28mg.dm-2. Later on, for the 5th and 6th day there was a marginal increase in the values (40.29 & 40.32mg.dm-2 respectively). During the month of May, 1989, of the same season, there was a linear increase with time from 20.40mg.dm-2 on the 1st day till it reached 35.88mg.dm-2 on the 6th day. Most of the other months such as August, 1989 (4.21 to 9.96mg.dm-2), December, 1989 (6.32 to 10.89mg.dm-2), April, 1990 (11.16 to 15.39mg.dm-2) as well as May, 1990 (9.32 to 12.17mg.dm-2) showed a linear increase in the organic matter content with increase in the duration of exposure.

In September, 1989 and January, 1990, the values were low, they being 9.05mg.dm-2 and 10.74mg.dm-2 on the 1st day. The values increased during September, 1989, on the 2nd day to 12.34mg.dm-2 and then dropped on the 3rd day to

10.58mg.dm-2 and on the 4th day to 9.95mg.dm-2. However, on the 5th day there was an increase to 11.73mg.dm-2 and the value was almost the same on the 6th day (11.80mg.dm-2). On the other hand, during January, 1990, there was a drop in the value from 10.74mg.dm-2 on the 1st day to 9.77mg.dm-2 on the 4th day. From the 5th day, a regular increase was observed till the 6th day (11.93mg.dm-2).

Inorganic matter (F-IM) (Fig 5.6)

This component on fibreglass showed wide fluctuations as illustrated in Fig 5.6, with a minimum of 21.29mg.dm-2 and a maximum of 226.16mg.dm-2 registered over the study period. During April, 1989, it showed considerable fluctuation exhibiting a value of 119.40mg.dm-2 on the 1st day which recorded a decrease to 115.29mg.dm-2 on the 2nd day. The 3rd day showed a major increase to 184.07 and 226.16mg.dm-2 on the 5th day, with a drop in between on the 4th day to 162.49mg.dm-2 and 193.10mg.dm-2 on the 6th day. Such a loss in the inorganic matter content during April, 1989, was very much significant for fibreglass, as compared to any other surface. Such a variation, though, to a lesser extent, was also observed during May, 1989, when the 1st day recorded a value of 108.02mg.dm-2 (Fig 5.6). It increased to

157.79mg.dm-2 on the 2nd day. The month's minimum was observed on the 3rd day which was 90.24mg.dm-2 and from then on there was an increase till the 6th day of sampling (the value being 121.18mg.dm-2).

The changes observed in the F-IM content during August, 1989, were less irregular as compared to the former months. Minimum value registered on the 1st day was 33.83mg.dm,-2 which increased to 35.84mg.dm-2 on the 3rd day. A decrease was observed in the inorganic matter content on the 4th day (34.94mg.dm-2). It increased to 36.29mg.dm-2 for the 6th day.

The sampling conducted during September, 1989, showed a linear increase in the inorganic matter content of the microfouling material developed on fibreglass from 21.29 on the 1st day till 37.71mg.dm,-2 on the 6th day of the study period. Similar was also the case during April, 1990, with values ranging from 69.21 to 83.27mg.dm-2.

In the month of December, 1989, the values showed a very marginal change for the first three days (33.45, 33.20 & 33.96mg.dm-2). An increasing trend was evident from the 4th day onwards (40.09mg.dm-2) till the 6th day (51.88mg.dm-2). The results obtained for January, 1990, showed an irregular pattern with the 1st day showing a

value of 42.11mg.dm-2 which increased to 50.17mg.dm-2 on the 2nd day. The maximum for the month was observed on the 3rd day (68.97mg.dm-2). There was a sudden drop in the concentration for the 4th day (54.63mg.dm-2), which later showed a linear increase with time till the 6th day (65.46mg.dm-2).

In the month of April, 1990, a low value of 69.21mg.dm-2 was observed for the 1st day. Thereafter, a gradual increase was evident with time till the 6th day (83.27mg.dm-2). During May, 1990, the inorganic matter content of the microfouling material on fibreglass was 68.33mg.dm-2 on the 1st day, which dropped to 67.44mg.dm-2 on the 2nd day. Thereafter, a linear increase till the end (81.04mg.dm-2) was observed with increase in exposure time.

3.1c Stainless steel

Dry weight (DW) (Fig 5.7)

The biomass as dry-weight developed on stainless steel showed a trend similar to that observed for aluminium and fibreglass. The values for the study period fluctuated between 21.15 and 106.39mg.dm-2.

During April, 1989, (Fig 5.7), the value of 79.51 was

observed on the 1st day which increased to 106.39mg.dm-2 towards the end of sampling. During May, 1989, a low value of 63.27 increased to 71.25 on the following day. A decrease was observed (69.05mg.dm-2), on the 3rd day. Thereafter, there was a gradual increase till the 6th day (96.01mg.dm2). Results obtained during August, 1989, showed values ranging between 21.15 and 35.64mg.dm-2. Sampling during December, 1989, showed a minimum value of 21.16mg.dm-2 and a maximum of 60.34mg.dm-2. Values during January, 1990, April and May, 1990, showed fluctuation between 42.42 & 68.78mg.dm-2, 51.34 & 73.50mg.dm-2 and 45.17 & 68.67mg.dm-2, respectively.

Biomass during September, 1989, on the 1st day was 26.41mg.dm-2, which decreased to 25.14mg.dm-2 representing the months minimum value. The concentration then increased to 36.19mg.dm-2 on the 3rd day and further increased thereafter, till the 5th day of sampling (46.70mg.dm-2). However, a drop in the dry weight content was observed on the 6th day (41.64mg.dm-2) of sampling during September, 1989.

Organic matter (F-OM) (Fig 5.8)

Most of the time, microfouling biomass as organic matter developed on stainless steel, showed a regular

trend (Fig 5.8) i.e., linear increase in concentration with increase in time for most of the months. An exception occurred during August, 1989, and December, 1989, sampling, where a sudden drop in the concentration was observed, especially on the 5th day of August, 1989, and on the 3rd day of December, 1990.

Inorganic matter (F-IM) (Fig 5.9)

When compared to the organic matter content the inorganic matter also appeared to have followed the same pattern. However, variation observed for this biomass developed on stainless steel was found to be more. The values fluctuated between a minimum of 16.02mg.dm-2 and a maximum of 71.45mg.dm-2, for the study period.

The observations during April, 1989 as shown in Fig 5.9, revealed a concentration of 55.77mg.dm-2 on the 1st day which increased till the 3rd day (60.76mg.dm-2). There was a sudden decrease on the 4th day to 56.89mg.dm-2 which again increased for the 5th (70.27mg.dm-2) and 6th (71.45mg.dm-2) days. During May, 1989, however, a monthly minimum was observed on the 1st day (50.22mg.dm-2), which increased to 53.34mg.dm-2 on the 2nd day. There was a drop in the concentration of F-IM on the 3rd day (51.40mg.dm-2),

observed on the 1st day which increased to 106.39mg.dm-2 towards the end of sampling. During May, 1989, a low value of 63.27 increased to 71.25 on the following day. A decrease was observed (69.05mg.dm-2), on the 3rd day. Thereafter, there was a gradual increase till the 6th day (96.01mg.dm2). Results obtained during August, 1989, showed values ranging between 21.15 and 35.64mg.dm-2. Sampling during December, 1989, showed a minimum value of 21.16mg.dm-2 and a maximum of 60.34mg.dm-2. Values during January, 1990, April and May, 1990 showed fluctuation between 42.42 & 68.78mg.dm-2, 51.34 & 73.50mg.dm-2 and 45.17 & 68.67mg.dm-2, respectively.

Biomass during September, 1989, on the 1st day was 26.41mg.dm-2, which decreased to 25.14mg.dm-2 representing the months minimum value. The concentration then increased to 36.19mg.dm-2 on the 3rd day and further increased thereafter, till the 5th day of sampling (46.70mg.dm-2). However, a drop in the dry weight content was observed on the 6th day (41.64mg.dm-2) of sampling during September, 1989.

Organic matter (F-OM) (Fig 5.8)

Most of the time, microfouling biomass as organic matter developed on stainless steel, showed a regular

trend (Fig 5.8) i.e., linear increase in concentration with increase in time for most of the months. An exception occurred during August, 1989, and December, 1989, sampling, where a sudden drop in the concentration was observed, especially on the 5th day of August, 1989, and on the 3rd day of December, 1989.

Inorganic matter (F-IM) (Fig 5.9)

When compared to the organic matter content the inorganic matter also appeared to have followed the same pattern. However, variation observed for this biomass developed on stainless steel was found to be more. The values fluctuated between a minimum of 16.02mg.dm-2 and a maximum of 71.45mg.dm-2, for the study period.

The observations during April, 1989 as shown in Fig 5.9, revealed a concentration of 55.77mg.dm-2 on the 1st day which increased till the 3rd day (60.76mg.dm-2). There was a sudden decrease on the 4th day to 56.89mg.dm-2 which again increased for the 5th (70.27mg.dm-2) and 6th (71.45mg.dm-2) days. During May, 1989, however, a monthly minimum was observed on the 1st day (50.22mg.dm-2), which increased to 53.34mg.dm-2 on the 2nd day. There was a drop in the concentration of F-IM on the 3rd day (51.40mg.dm-2),

which increased thereafter, till the last day (66.51mg.dm-2).

The amount of F-IM during August, 1989, showed an increase from 18.10mg.dm-2 on the 1st day to 28.20mg.dm-2 on the 4th day. The 5th day showed a value of 23.89mg.dm-2 while the 6th day showed a value of 27.84mg.dm-2. During September, 1989, although sampling showed increasing values from 18.70mg.dm-2 on the 1st day to 37.81 on the 5th day, there was a sudden decrease to 16.02mg.dm-2 on the 2nd day. Similarly, a sudden decrease was observed on the 6th day of sampling (30.84mg.dm-2) of this month. All the other remaining months sampled showed an increase from 17.30 to 52.38mg.dm-2 in December, 1989, 34.30 to 59.70mg.dm-2 in January , 1990, 46.48mg.dm-2 to 65.23mg.dm-2 in April, 1990 and 41.61mg.dm-2 to 62.44mg.dm-2 in May, 1990.

3.2 SEASONAL VARIATION

The variations in microfouling biomass were also studied with respect to seasonal changes. Irrespective of the type of material used, biomass as dry weight ranged from 32.56 to 185.68mg.dm-2 for the study period. The subsequent account presents the details for each surface viz., aluminium, fibreglass and stainless steel.

3.2a **Aluminium**

Dry-weight (DW) (Fig 5.10)

Fig 5.10 shows biomass as DW, developed on aluminium surface. Maximum value prevailed during the pre-monsoon season of 1989, (185.68mg.dm-2) and minimum values of 45.43mg.dm-2 occurred during the monsoon season. This decrease observed during the monsoon was four times lower than that observed during the pre-monsoon season. Intermediate value of 66.70mg.dm-2 was observed during the post monsoon season. Once again, when pre-monsoon sampling was done during 1990, the value was high (83.48mg.dm-2), but lower when compared with that of pre-monsoon, 1989.

Organic matter (F-OM) (Fig 5.10)

Biomass as organic matter is also shown in the same figure as above. Here, the trend is the same, with the pre-monsoon season of 1990, showing maximum values (34.99mg.dm-2) and minimum values observed during both monsoon (9.62mg.dm-2) and post-monsoon (9.6mg.dm-2) seasons of the same year. However, pre-monsoon season of 1990, showed higher values when compared to the monsoon and post-

monsoon seasons. On the other hand, when the pre-monsoon season of 1989 and 1990 were compared, it was observed that lower values (12.64mg.dm-2) occurred during pre-monsoon, 1990.

Inorganic Matter (F-IM) (Fig 5.10)

Seasonal distribution shown by this parameter was similar to that exhibited by dry-weight parameter. Pre-monsoon seasons of 1989 and 1990 showed a value of 150.69mg.dm-2 and 70.84mg.dm-2, respectively. Minimum value (35.8mg.dm-2) as usual, was seen during the monsoon season and intermediate value (57.10mg.dm-2) during the post- monsoon season.

3.2b **Fibreglass**

The biomass parameters when analysed for this surface showed the following changes.

Dry-weight (DW) (Fig 5.11)

Biomass as dry-weight on fibreglass test surface showed values ranging between 40.84 & 173.74mg.dm-2. Maximum values were exhibited during the pre-monsoon

seasons and minimum during the monsoon season. Intermediate values were observed during the post-monsoon season (58.59mg.dm-2) as shown in Fig 5.11

Organic matter (F-OM) (Fig 5.11)

As also shown in Fig 5.11, variation in the distribution of biomass as organic matter was maximum during the pre-monsoon season of 1989, (31.57mg.dm-2) and 1990, (11.88mg.dm-2). Both the monsoon season (8.93mg.dm-2) and post-monsoon season (9.8mg.dm-2), did not show significant changes in the concentration.

Inorganic matter (F-IM) (Fig 5.11)

The distribution of inorganic matter varied in a similar manner as that of DW with 142.16mg.dm-2 occurring during pre-monsoon, 31.9mg.dm-2, during monsoon and 48.79mg.dm-2 during the post-monsoon seasons of 1989.

3.2c **Stainless steel**

This alloy was also analysed for the various parameters and the results obtained for the various seasons are presented below, are also shown in Fig 5.12

Dry weight (DW) (Fig 5.12)

The biomass as DW showed seasonal changes with a maximum value observed during the pre-monsoon season of both 1989, (85.32mg.dm-2) and 1990, (59.12mg.dm-2). A low value of 32.56mg.dm-2 which was nearly three times lower than that of the pre-monsoon season was evident during the monsoon season. Intermediate value of 52.82mg.dm-2 was observed during the post-monsoon season.

Organic matter (F-OM) (Fig 5.12)

Biomass as organic matter on stainless steel showed a similar trend of maximum value (25.42mg.dm-2) during the pre-monsoon season. Minimum value of 6.91mg.dm-2 was observed during the post-monsoon season, in contrast to other cases where a minimum was exhibited during the monsoon season. The value during the monsoon season (7.55mg.dm-2) was closely related with the value of the post-monsoon season but, was marginally higher in comparison. Once again a very low value of 5.82mg.dm-2 was observed for the pre-monsoon season of 1990. This was an abnormal phenomenon observed for this biomass parameter.

Inorganic matter (F-IM) (Fig 5.12)

The inorganic matter showed a pattern identical with that of DW, with a maximum value exhibited during the pre-monsoon season of 1989, (59.89mg.dm-2) and 1990 (53.30mg.dm-2). A minimum value of 25mg.dm-2 was observed for the monsoon season and an intermediate value of 45.9mg.dm-2, for the post-monsoon season.

3.3 WEEKLY VARIATION

Microfouling biomass was also measured during weekly intervals as dry-weight (DW) and organic matter (F-OM). In addition to these parameters, the inorganic matter (F-IM) of the microfouling material was also studied for various surfaces.

3.3a Aluminium

Dry-Weight (DW) (Fig 5.13)

As seen in Fig 5.13, biomass as dry-weight for April-May, 1990, showed a low value of 166.99mg.dm-2 for week I. This value increased sharply for week II, (215.08mg.dm-2), week III (250.99mg.dm-2) and week IV (258.76mg.dm-2). The

increase from week III to week IV was less significant.

During August-September, 1990, weekly sampling, biomass as DW showed a rise with increase in exposure time, from 63.81mg.dm-2 for week I to 88.11mg.dm-2 for week IV. This increase was considerably less than that observed during April-May, 1990. Fig 5.13 also shows the weekly changes observed during December-January, 1991. During this period the minimum value recorded was 87.10mg.dm-2 during week I, which increased, although marginally, till week III (106.52mg.dm-2). However, from week III to week IV the increase was significant.

Organic Matter (F-OM) (Fig 5.14)

Biomass as organic matter for week I during April-May, 1990, was 21.21mg.dm-2. This value almost doubled during week II and remained steady for week III (45.86mg.dm-2). Thereafter, it increased to 56.28mg.dm-2 for week IV.

On the other hand, during August-September, 1990, week I showed a very low value of biomass amounting to 16.62mg.dm-2 which increased very marginally till the end of sampling, the final value being 19.15mg.dm-2. Results obtained during December-January, 1991, showed a value of

18.47mg.dm-2 during week I. It showed a significant increase thereafter, till week IV (33.24mg.dm-2). These results have been presented in Fig 5.14.

Inorganic matter (F-IM) (Fig 5.15)

Variations in the inorganic component are presented in Fig 5.15. During April-May, 1990, the inorganic matter content of the microfouling material showed a minimum and maximum value of 145.78mg.dm-2 and 202.48mg.dm-2 respectively. A very low value of 47.19mg.dm-2 was observed on the panels for week I during August-September, 1990, which increased till week IV (68.96mg.dm-2). However, during December-January, 1991, there was only a marginal increase from 68.63mg.dm-2 during week I to 76.57mg.dm-2 for week III. Week IV however, showed a sharp increase to 136.53mg.dm-2.

3.3b **Fibreglass**

Dry-Weight (DW) (Fig 5.16)

As can be seen in Fig 5.16, the fibreglass surface, like aluminium test surface, showed an increase from 161.67 to 231.15mg.dm-2 during April-May, 1990, from 55.62 to

71.55mg.dm-2 during August-September, 1990, and from 73.26 to 90.64mg.dm-2 during December-January, 1991.

Organic Matter (F-OM) (Fig 5.17)

Fig 5.17 shows the changes in organic matter concentration on fibreglass during weekly sampling. During April-May, 1990, the biomass showed a sharp increase from 20.16 to 41.16mg.dm-2 for the sampling days. However, during August-September, 1990, a value of 14.38mg.dm-2 was seen in week I, with a very marginal increase till week IV (18.97mg.dm-2). Like April-May, 1990 samples, those of December-January, 1991, also showed considerable increase in biomass from 15.39 during week I to 29.24mg.dm-2 in week IV.

Inorganic matter (F-IM) (Fig 5.18)

The inorganic matter content of the microfouling material developed on fibreglass during April-May, 1990, showed a high value of 141.51mg.dm-2 for week I panels, which increased considerably till 189.99mg.dm-2 during week IV. During August-September, 1990, the rate of increase remained low, although the increase, with time, was quite evident as shown in Fig 5.18. Even during

December-January, 1991, changes were observed. It was found to be 57.87mg.dm-2 in week I, which decreased abnormally to 55.93mg.dm-2 during week II. The value once again increased to 57.95mg.dm-2 in week III and then increased again for week IV amounting to 61.4mg.dm-2.

3.3c **Stainless steel**

Dry weight (DW) (Fig 5.19)

The biomass as DW on stainless steel test panels is presented in Fig 5.19. As in the case of aluminium and fibreglass, stainless steel also showed increase in values with the increase in exposure time. During April-May, 1990, August-September, 1990 and December-January, 1991, the values showed a minimum and maximum of 145.71 & 181.20, 38.00 & 51.79 and 59.41 & 81.88mg.dm-2, respectively.

Organic matter (F-OM) (Fig 5.20)

The biomass as organic matter showed a very sharp increase as evident from Fig 5.20. This was especially the case during April-May, 1990, (12.18 to 34.44mg.dm-2) and December-January, 1991, (13.08 to 24.55mg.dm-2). During

August-September, 1990, the values (11.11 to 17.73mg.dm-2), showed a lower range.

Inorganic matter (F-IM) (Fig 5.21)

Fig 5.21 shows the weekly changes represented by the inorganic matter component of the microfouling material on stainless steel test panels. This parameter showed very little changes with values ranging between 133.53 & 146.76mg.dm-2 during April-May, 1990. The values observed during August-September, 1990, were almost steady for weeks I, II & III, being 26.89, 26.09 & 26.17mg.dm-2. The last week (IV) showed a sudden increase to 34.06mg.dm-2. December-January, 1991, was no exception with values ranging between 46.33 & 57.33mg.dm-2 for the study period.

3.4 SEASONAL VARIATION

The samples of April-May, 1990, August-September, 1990, and December-January, 1991, were grouped into pre-monsoon, monsoon and post-monsoon seasons to obtain seasonal data.

3.4a **Aluminium**

Dry weight (DW) (Fig 5.22)

Fig 5.22 shows the seasonal fluctuations of biomass on aluminium panels. Pre-monsoon season showed a maximum values of 222.95mg.dm-2 followed by the post-monsoon season (115.19mg.dm-2). Minimum value of biomass was observed during the monsoon season (75.41mg.dm-2). The value observed for the monsoon season was considerably lower than that of the pre-monsoon season and only a little higher than the one recorded for the post-monsoon season.

Organic matter (F-OM) (Fig 5.22)

Biomass as organic matter showed a similar trend with a maximum concentration exhibited during the pre-monsoon season (41.26mg.dm-2). It was followed by the post-monsoon season (17.89mg.dm-2). Minimum concentration for this parameter was evident during the monsoon season. However, the difference in the range was very marginal.

Inorganic matter (F-IM) (Fig 5.22)

Seasonal variation in the inorganic matter followed an identical pattern, (Fig 5.22) with a maximum (181.68mg.dm-2), intermediate (88.43mg.dm-2) and minimum value (57.52mg.dm-2), shown during the pre-monsoon, post-monsoon and monsoon season respectively.

3.4b **Fibreglass**

Seasonal fluctuation for the fibreglass test panels also showed a maximum concentration during the pre-monsoon season for all the parameters.

Dry Weight (DW) (Fig 5.23)

The fluctuation of this biomass parameter is shown in Fig 5.23. The highest concentration of 202.73mg.dm-2 was evident during the pre-monsoon season. Minimum concentration was recorded during the monsoon season (63.04mg.dm-2) and intermediate value was observed during the post-monsoon season (81.47mg.dm-2).

Organic matter (F-OM) (Fig 5.23)

Biomass as organic matter, as seen in Fig 5.23, did not show very distinct changes. The values during pre-monsoon and post-monsoon seasons were quite close (29.90 & 23.18mg.dm-2 respectively). Even the monsoon season value which was low (16.93mg.dm-2) remained close to the value of the post-monsoon season.

Inorganic matter (F-IM) (Fig 5.23)

Unlike the organic component of the microfouling material, seasonal variations of inorganic matter also showed maximum concentration during the pre-monsoon season (172.82mg.dm-2). Post-monsoon and monsoon seasons showed values of 58.28 and 46.11mg.dm-2. For this parameter, the value for the monsoon season was more than three and half times lower as compared to the pre-monsoon season. The post- monsoon season, however, showed values which were three times lower than the pre-monsoon season.

3.4c **Stainless steel**

Stainless steel also showed a distinct seasonal fluctuation in biomass values as evident from Fig 5.24.

Dry-Weight (DW) (Fig 5.24)

The dry-weight during the pre-monsoon season was maximum with a value of 164.71mg.dm-2. This was followed by the post-monsoon season (69.69mg.dm-2) which was a little less than two times the former. The monsoon season showed a biomass value of 42.73mg.dm-2 which was minimum as compared to the other two seasons.

Organic Matter (F-OM) (Fig 5.24)

Biomass as organic matter showed values of 23.71mg.dm-2, 14.43mg.dm-2 and 18.96mg.dm-2 during the pre-monsoon, monsoon and post-monsoon seasons.

Inorganic Matter (F-IM) (Fig 5.24)

Inorganic matter of the microfouling material showed the highest value during the pre-monsoon season (141.00mg.dm-2), which dropped to more than two and half times during the post-monsoon season (50.72mg.dm-2) and to almost five times during the monsoon season (28.30mg.dm-2). A wide difference was thus observed on this surface, as compared to either aluminium or fibreglass.

Relative comparisons between surfaces

Fibreglass test panels for the initial sampling period (24 to 48h) showed higher biomass values as compared to aluminium or stainless steel. However, as the immersion period increased as observed in the case of the weekly sampling, values on aluminium were seen to be higher in comparison to the other two surfaces, till a seasonal maximum was attained towards the end of sampling time. Stainless steel when compared to aluminium or fibreglass, showed low biomass for all the three seasons of the year.

Relative comparison between the seasons

The pre-monsoon season was observed to exhibit maximum microfouling biomass as compared to the monsoon or post-monsoon seasons. This fact was evident for both short term daily sampling as well as for weekly sampling. This was once again confirmed by sampling done for the pre-monsoon season of the next year. The monsoon season showed low values for all the parameters. Intermediate values were observed during the post-monsoon season. Although such differentiation was made between the seasons, some of the parameters showed more or less closely related values.

The regression analysis of the various biomass parameters with each other has been presented in Table 5.1 & 5.2. All the fouling parameters were very well correlated, indicating their interdependence on each other. Biomass parameters were also correlated with various hydrographic parameters, and are presented in Table 5.3 & 5.4.

The various percentage contribution by organic and inorganic matter of the total DW has been shown in Tables 5.5a,b & 5.6. Irrespective of the surface used, the percentage values of inorganic matter exceeded organic matter throughout the study period. The percentage concentration of organic matter was found to range between 8.72% and 31.34% for daily sampling. To be specific, for each surface, viz., aluminium, fibreglass and stainless steel, the respective values of percentage contribution by organic matter were 12.15 to 26.07%; 12.96 to 22.79% and 8.72 to 31.84% (Table 5.5a).

On the other hand, the percentage of inorganic matter for the study period ranged from 68.66 to 90.20%. The percentage of inorganic matter varied from 73.93 to 87.85% for aluminium, 61.98 to 87.04% for fibreglass and 68.16 to 90.73% for stainless steel (Table 5.5b).

As shown in Table 5.6, for weekly sampling, organic matter ranged between 8.36 & 37.00%, and inorganic matter from 63.00 & 91.64%. It was observed that both organic matter as well as inorganic matter for aluminium (12.70 to 28.12% & 71.88 to 87.30%), fibreglass (12.37 to 33.21% & 66.79 to 87.63%) and stainless steel (8.36 to 37.00% & 63.00 to 91.64%) also showed variation. The general trend observed for both daily as well as weekly sampling was the increase in the concentration of organic matter with time, while the F-IM decreased with time, irrespective of the type of material used for the study.

4 DISCUSSION

The scraping of the microfouling material from the various test surfaces was one of the important factors for determining microfouling biomass. From the work carried out by Sharma et al, 1990, it was evident that the use of nylon brush was the most appropriate method for the removal of the microfouling material.

Secondly, various parameters which were formerly used to quantify the extent of biofouling were carbon, nitrogen, carbonate content, ATP, dry weight etc, (Aftring & Taylor, 1979; Bhosle et al, 1989). In the present chapter,

microfouling biomass was estimated as dry weight and total organic matter. The IM of the microfouling material was also studied. These methods were used because they were relatively simple, reproducible and gave reliable results.

The chemical analysis of the biofilm developed on various types of substrata revealed that 68.66 to 90.20% comprised of inorganic material, (Tables 5.5 & 5.6). Such high concentration of inorganic material as compared to the organic material has been earlier reported by Berger & Little (1980). When the organic and inorganic load from the water column was 17 to 28% and 76 to 82%, respectively, the settlement on panels was found to vary from 8.72 to 31.34% and 68.66 to 90.20% for daily sampling, (Table 5.5a & b). For the weekly sampling, the organic and inorganic content of the water column ranged from 20 to 23% and 77 to 80% respectively and for settlement on test panels, these values were found to vary from 13.5 to 35.4% and 64.6 to 86.5% (Table 5.6). The change observed was found to be irrespective of the type of material used for the study.

High concentration of inorganic matter coincided with subsequent large number of fouling diatoms as well as bacteria found on the test panels. Diatoms have the ability to form an external skeleton largely of silica called frustules (Kelker, 1989). Moreover, diatoms as well

as bacteria which occurred in large numbers on test surfaces are reported to produce polysaccharides thereby forming a gelatinous film (Fletcher & Floodgate, 1973; Hoagland, 1993). Such production of polysaccharides may also increase the inorganic matter content by adsorbing cations due to their acidic nature (Ford et al, 1987). This appears to be the case because many functional groups such as carboxyl and hydroxyl of the polysaccharides are known to play an important role in binding of metal ions and inorganic nutrients (White & Benson, 1984; Ford et al, 1987). Organic matter gets adsorbed onto surfaces at rates proportional to their relative electronegativities. Thus, they actively adsorb onto non-metal surfaces and passively onto metal surfaces, having the uncharged polymers (Sechler & Gundersen, 1972). Alternately, high inorganic content could also be ascribed to the presence of detrital particles containing higher abundance of inorganic components.

Increase in the biomass values with increase in the duration of exposure was evident in the present study. This corroborates with the earlier reports by several workers, who also suggested a linear increase in build-up with corresponding increase in exposure time (Bhosle et al, 1990; Raveendran et al, 1991). This was, however, in contrast with other studies suggesting a non-linear

increase in microfouling biomass with immersion period (Martinez et al, 1984; White & Benson, 1984; Yanshun et al, 1984; Chamberlain & Garner, 1988).

It appears that the increase in the microfouling biomass observed for the present study was due to irreversible adsorption of microorganisms onto surfaces, their growth and increase in the production rates. Occasional low values recorded during certain sampling days for some of the surfaces could perhaps be due to grazing by aquatic organisms (Hardings et al, 1987). Alternately this could also be due to shearing and/or sloughing of microfouling material because of the physical forces occurring in the water column (Marshall et al, 1971; Marshall, 1976; Characklis, et al 1984).

Fibreglass test surfaces accumulated higher biomass as DW for the first 74h, as compared to aluminium and stainless steel. This could be due to the fact that fibreglass being hydrophobic and having a low energy surface could have accumulated higher biomass initially (Fletcher, 1988; Pedersen, 1990). Similar behaviour of fibreglass was reported by Bhosle, et al, (1989); Srivastava et al, 1990; Raveendran et al, (1991) for short term study period. Nevertheless, as the immersion period

increased, aluminium panels showed higher biomass of microfouling (DW) and for all the months reached a seasonal peak towards the end of sampling time. This could be explained to be due to the hydrophilic nature of aluminium panels, due to which metals accumulate higher microfouling biomass. In addition, the rough surface texture of aluminium as compared to fibreglass and stainless steel, facilitated adhesion and subsequent growth. This also suggests that the hydrophobicity observed in the case of fibreglass is important only initially, however, with increase in time, such forces do not seem to play a significant role. The most probable reason might be that the biofilm process of exponential growth, product formation and debris entrapment dominated (Characklis et al, 1984), over the initial, surface energy dependent processes (Fletcher & Marshall, 1982).

Stainless steel showed lowest values for all the biomass parameters. This may be because stainless steel has a highly electropolished surface, due to the presence of a passivating film of mixed iron and chromium oxides (Chamberlain & Surrinderjit, 1987; Dunsmore et al, 1981; Zoltai et al, 1981). Such a polished surface does not initially form a suitable substratum for microfoulers.

Biomass also varied with the season as illustrated in the results for both daily as well as weekly observations. It was observed that during the monsoon season, the fresh water input into the study area was 150 to 400m^3/sec (Shetye & Murty, 1987). This causes the temperature and salinity to decrease, whereas, dissolved oxygen and suspended load of the water column increased. The increase in the suspended load of the water column lowers the light penetration in the said area. This was less evident during the post-monsoon season and to a still lesser extent during the pre-monsoon season. Such seasonal variation mainly causes a wide variation in the settlement behaviour on test surfaces.

Despite the fact that several researchers have suggested that various hydrographic factors may influence the development of microfouling settlement (Martinez et al, 1984; Yanshun et al, 1984; Characklis & Escher, 1988; McEldowney & Fletcher, 1988; Bhosle et al, 1989b), only a few studies have been carried out to verify the actual statistical analysis of the data on the development of microfouling (Bhosle et al, 1990). As there is a paucity of data a detailed statistical analysis was carried out to evaluate which of the above mentioned factors could significantly influence microfouling settlement.

Biomass parameters for all the substrata viz., aluminium, fibreglass and stainless steel were well correlated indicating interdependence of these parameters on each other. This was further confirmed by the fact that the percentage composition of organic matter increased with time, while the IM decreased with time. Therefore, both these fractions were dependent on each other and the concentration of one affected the concentration of the other.

The effect of nutrients, temperature, salinity, dissolved oxygen, suspended load etc. with microfouling biomass of the sub-surface waters was correlated (Tables 5.3 & 5.4). In the present study it was observed that temperature and salinity of the water column showed highly significant correlation with microfouling biomass. Although this was suggested earlier by Yanshun et al, (1984); Karande, (1987); Characklis & Escher, (1988), only a few have attempted to verify the statistical significance of the data (Bhosle et al, 1990). Temperature affects the metabolic activities, physiological tolerance and phytoplankton productivity (Levinton, 1982). Moreover there is a complex relationship between salinity and temperature whereby, changes in salinity can modify the effects of temperature and vice versa (Kinne, 1963). Their

variations may affect the metabolic processes like diffusion, osmosis, as well as water density, gas solubility and viscosity (Guillard, 1962; Chlebowics, 1988).

Dissolved oxygen also showed a significant but inverse relation with biomass. This could probably be due to the formation of oxidised radicals of water like .OH and $.OH_2$ in the presence of oxygen, which subsequently formed H_2O_2, all of which tend to have a lethal action on living cells (Norris & Ribbons, 1969).

It was quite interesting to note that nutrients like nitrate, phosphate and silicate of the sub-surface waters also showed poor relationship with settlement on test surfaces. The probable reason could be due to the dependence of microfoulers on both the dissolved and particulates from the substrata and the ambient waters. When the nutrient supply is scarce in the immediate environment the surface of test panels may provide an advantage by assisting the capture and/or uptake of scarce nutrients (Marshall, 1976; Ladd et al, 1979). According to Kjelleberg (1986) even low nutrient conditions gave higher adhesion value for various surfaces. Hence high concentration of organic compounds were observed in natural waters poor in nutrients (Vaccaro et al, 1968). Thus, DO

and nutrients from the sub-surface waters do not seem to directly influence the microfouling settlement, at least in the present study area.

Another point worth mentioning in this study area was the significant but inverse relationship observed between the biomass from surfaces and suspended load of the sub-surface waters. This was contradictory with the earlier studies by Bhosle et al, (1990) wherein, they have reported a highly significant correlation between biomass from surfaces and particulate matter of the water column from the offshore environment. However, it may not be advisable to compare our results with those of Bhosle et al, (1990), because their samples were obtained from the water column, from oceanic environments and the study was restricted to one particular season of the year. The present study on the other hand was carried out in the sub-surface waters of the nearshore environment, for three cumulative seasons of the year.

Highly significant correlation was observed between the microfouling biomass (DW, F-OM) and the diatom and bacterial number formed on aluminium, fibreglass and stainless steel. Thus, indicating that these forms largely contributed to the formation of the microfouling material either directly or indirectly.

Table 5.1

Statistical correlation between microfouling biomass developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) for daily sampling (n=48)

		F-DW		F-OM		F-IM	
		r	p	r	p	r	p
F-DW	AL			0.96	<0.001	0.74	<0.001
	FG			0.94	<0.001	0.97	<0.001
	SS			0.86	<0.001	0.98	<0.001
F-OM	AL					0.59	<0.01
	FG					0.90	<0.001
	SS					0.84	<0.001
F-IM							

r= correlation coefficient, p= level of significance, F-DW= fouling dry weight, F-OM= fouling organic matter, F-IM= Fouling inorganic matter.

Table 5.2

Statistical correlation analysis between microfouling biomass developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) for weekly sampling (n=12)

	F-DW			F-OM			F-IM	
	r	p	S	r	p		r	p
FDW			AL	0.93	<0.001		0.99	<0.001
			FG	0.89	<0.001		0.99	<0.001
			SS	0.67	<0.001		0.99	<0.001
FOM						AL	0.88	<0.001
						FG	0.70	<0.001
						SS	0.59	<0.01
FIM								

r= correlation coefficient, p= level of significance, S= surface, F-DW= fouling dry weight, F-OM= fouling organic matter, F-IM= fouling inorganic matter.

Table 5.3

Statistical correlation between physico-chemical parameters (W) and microfouling biomass (F) developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) test panels for the daily sampling (n=48).

		F-DW		F-OM		F-IM	
	S	r	p	r	p	r	p
Temperature	AL	0.92	<0.001	0.86	<0.001	0.63	<0.01
	FG	0.91	<0.001	0.90	<0.001	0.90	<0.001
	SS	0.86	<0.001	0.88	<0.001	0.84	<0.001
Salinity	AL	0.65	<0.01	0.53	<0.02	0.50	<0.05
	FG	0.67	<0.001	0.59	<0.01	0.69	<0.001
	SS	0.68	<0.001	0.54	<0.02	0.69	<0.001
Dissolved oxygen	AL	-0.68	<0.001	-0.55	<0.02	-0.64	<0.01
	FG	-0.74	<0.001	-0.63	<0.01	-0.74	<0.001
	SS	-0.74	<0.001	-0.57	<0.02	-0.75	<0.001
Nitrate	AL	0.37	<0.1	0.22	<0.1	0.27	<0.1
	FG	0.37	<0.1	0.26	>0.1	0.44	<0.05
	SS	0.41	<0.1	0.21	>0.1	0.46	<0.05
Phosphate	AL	0.26	>0.1	0.01	NS	0.18	<0.1
	FG	0.18	>0.1	0.06	NS	0.25	<0.1
	SS	0.24	>0.1	0.02	NS	0.26	<0.1
Silicate	AL	0.30	>0.1	0.18	NS	0.23	<0.1
	FG	0.30	>0.1	0.21	>0.1	0.33	<0.1
	SS	0.38	<0.1	0.15	NS	0.42	<0.1
SPM	AL	-0.56	<0.02	-0.40	<0.02	-0.60	<0.01
	FG	-0.58	<0.02	-0.44	<0.01	-0.60	<0.01
	SS	-0.76	<0.001	-0.28	NS	-0.92	<0.001
F-DIATOM	AL	0.69	<0.001	0.74	<0.001	0.22	<0.1
	FG	0.63	<0.001	0.67	<0.001	0.58	<0.01
	SS	0.40	<0.1	0.53	<0.02	0.41	<0.05
F-BACTERIA	AL	0.67	<0.001	0.75	<0.001	0.30	<0.1
	FG	0.93	<0.001	0.95	<0.001	0.89	<0.001
	SS	0.42	>0.05	0.72	<0.001	0.45	<0.05

S= surface, r= correlation coefficient, p= level of significance, SPM= suspended particulate matter, F-OM= fouling organic matter, F-IM= fouling inorganic matter, F-DW= fouling dry weight, NS= not significant

Table 5.4

Statistical correlation between water parameters (W) and microfouling biomass (F) developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) test panels for weekly sampling (n=12).

		F-DW		F-OM		F-IM	
	S	r	p	r	p	r	p
Temperature	AL	0.78	<0.001	0.84	<0.001	0.72	<0.001
	FG	0.78	<0.001	0.78	<0.001	0.76	<0.001
	SS	0.83	<0.001	0.76	<0.001	0.78	<0.001
Salinity	AL	0.37	NS	0.45	NS	0.32	NS
	FG	0.34	NS	0.22	NS	0.38	NS
	SS	0.48	<0.1	0.25	NS	0.39	NS
Dissolved oxygen	AL	-0.59	<0.05	-0.64	<0.02	-0.55	<0.05
	FG	-0.58	<0.05	-0.60	<0.02	-0.56	<0.05
	SS	-0.61	<0.02	-0.58	<0.05	-0.54	<0.05
Nitrate	AL	0.10	NS	0.14	NS	0.10	NS
	FG	0.08	NS	0.08	NS	0.14	NS
	SS	0.18	NS	0.04	NS	0.35	NS
Phosphate	AL	0.05	NS	0.10	NS	0.03	NS
	FG	0.02	NS	0.10	NS	0.06	NS
	SS	0.20	NS	0.06	NS	0.50	<0.1
Silicate	AL	0.19	NS	0.27	NS	0.15	NS
	FG	0.14	NS	0.08	NS	0.22	NS
	SS	0.29	NS	0.03	NS	0.54	<0. 05
SPM	AL	-0.85	<0.001	-0.92	<0.001	-0.99	<0.001
	FG	-0.58	<0.01	-0.76	<0.001	-0.99	<0.001
	SS	-0.45	<0.1	-0.67	<0.01	-0.99	<0.001

S= surface, r= correlation coefficient, p= level of significance, SPM= suspended particulate matter, F-DW= fouling dry-weight, F-OM= Fouling organic matter, F-IM= Fouling inorganic matter, NS= Not significant

Table 5.5a

Percentage of organic matter present in the microfouling material developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) test panels (daily).

		Percentage of organic matter		
Season	Days	AL	FG	SS
Apr-May 1989	1	16.33	16.40	25.77
	2	15.58	17.98	29.57
	3	19.78	18.15	29.09
	4	19.04	18.31	31.34
	5	20.47	18.14	30.05
	6	20.59	19.51	31.84
Aug-Sept	1	15.86	19.39	22.62
	2	26.07	22.44	26.84
	3	20.45	20.61	22.17
	4	21.71	22.79	23.19
	5	21.19	22.71	21.30
	6	21.55	22.72	24.07
Dec-Jan	1	14.70	18.42	18.84
	2	15.65	17.20	13.71
	3	12.15	15.60	12.29
	4	14.50	17.26	10.85
	5	13.98	16.28	12.44
	6	15.54	16.28	13.20
Apr-May 1990	1	14.37	12.96	8.72
	2	13.70	13.69	9.27
	3	14.97	13.27	9.80
	4	15.97	13.17	10.40
	5	16.06	14.33	10.27
	6	15.41	14.36	10.20

Table 5.5b

Percentage of inorganic matter present in the microfouling material developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) test panels. (daily)

	Percentage of inorganic matter			
Season	Days	AL	FG	SS
Apr-May 1989	1	83.67	83.60	74.23
	2	84.42	82.02	70.43
	3	80.22	81.85	70.91
	4	80.96	81.69	68.66
	5	79.53	81.86	69.95
	6	79.41	80.49	68.16
Aug-Sept	1	84.14	74.76	77.38
	2	73.93	61.98	73.16
	3	79.55	72.66	77.83
	4	78.29	75.73	76.81
	5	78.81	72.10	78.70
	6	78.45	76.60	75.93
Dec-Jan	1	85.30	81.58	81.16
	2	84.35	82.80	86.29
	3	87.85	84.40	87.71
	4	85.50	82.74	89.15
	5	86.02	83.72	87.56
	6	84.46	83.72	86.80
Apr-May 1990	1	85.63	87.04	91.28
	2	86.30	86.31	90.73
	3	85.03	86.73	90.20
	4	84.03	86.83	89.60
	5	83.94	85.67	89.73
	6	84.59	85.64	89.80

Table 5.6

Percentage of organic and inorganic matter developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) test panels for weekly sampling.

Months	Weeks	organic			inorganic		
		AL	FG	SS	AL	FG	SS
Apr-May	I	12.70	12.47	8.36	87.30	87.53	91.64
1990	II	19.40	12.37	12.59	80.60	87.63	87.41
	III	18.27	15.32	16.36	81.73	84.68	83.64
	IV	21.75	17.81	19.01	78.25	82.19	80.99
Aug-Sept	I	26.05	25.85	29.24	73.95	70.66	70.76
1990	II	23.74	28.56	34.15	76.26	70.67	65.85
	III	24.05	25.66	37.00	75.95	74.34	63.00
	IV	21.73	26.51	34.23	78.27	73.49	65.77
Dec-Jan	I	21.21	21.01	22.02	78.79	78.99	77.98
1991	II	26.09	25.65	26.97	73.91	74.35	73.03
	III	28.12	33.21	28.50	71.88	66.79	71.50
	IV	19.58	32.26	29.98	80.42	67.74	70.02

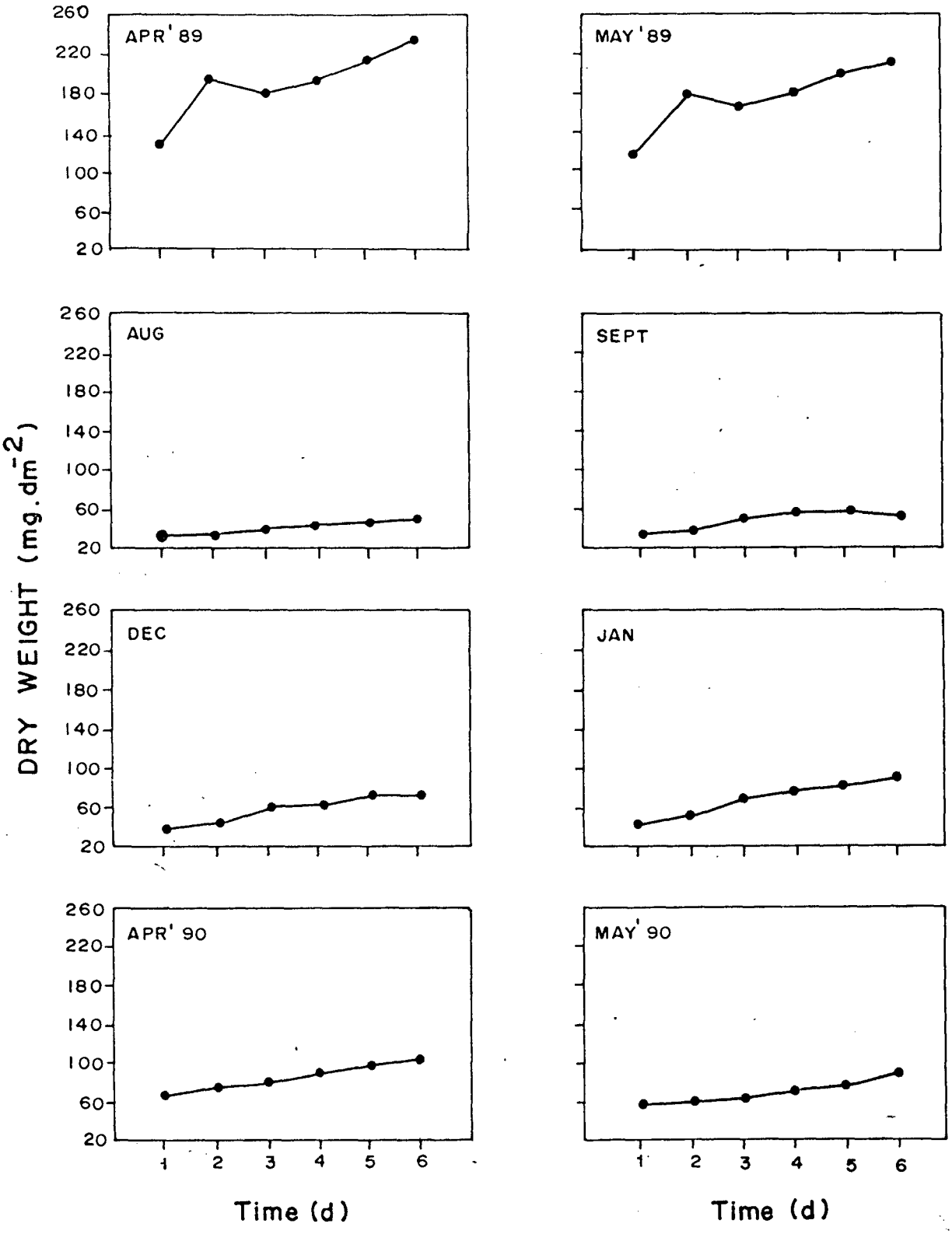

Fig.5.1 DAILY VARIATION IN MICROFOULING BIOMASS (DRY WEIGHT) DEVELOPED ON ALUMINIUM TEST PANELS FOR DIFFERENT MONTHS FROM THE STUDY AREA

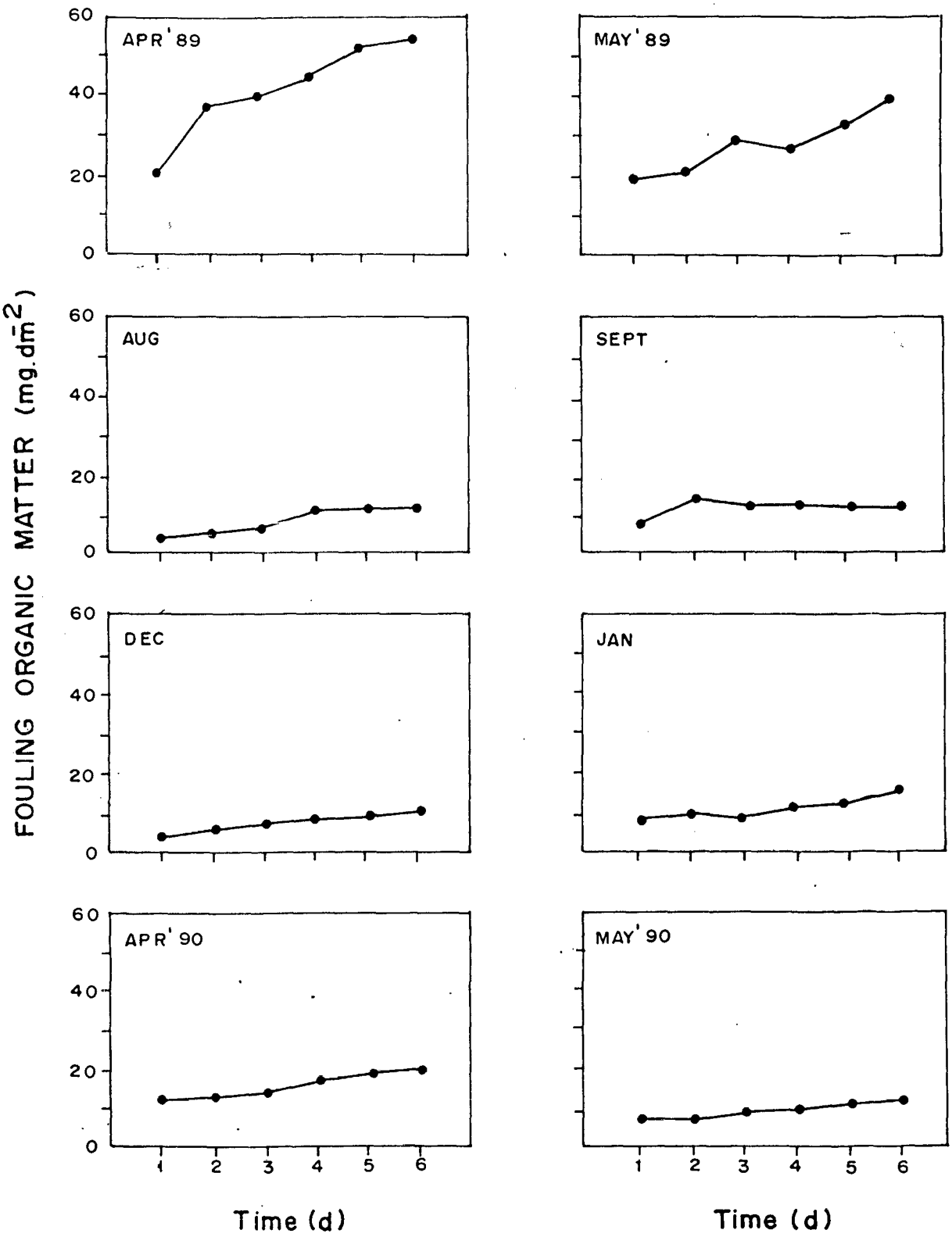

Fig.5.2 DAILY VARIATION IN THE FOULING ORGANIC MATTER FROM ALUMINIUM SURFACE FOR THE SUB-SURFACE WATER OF DONA PAULA.

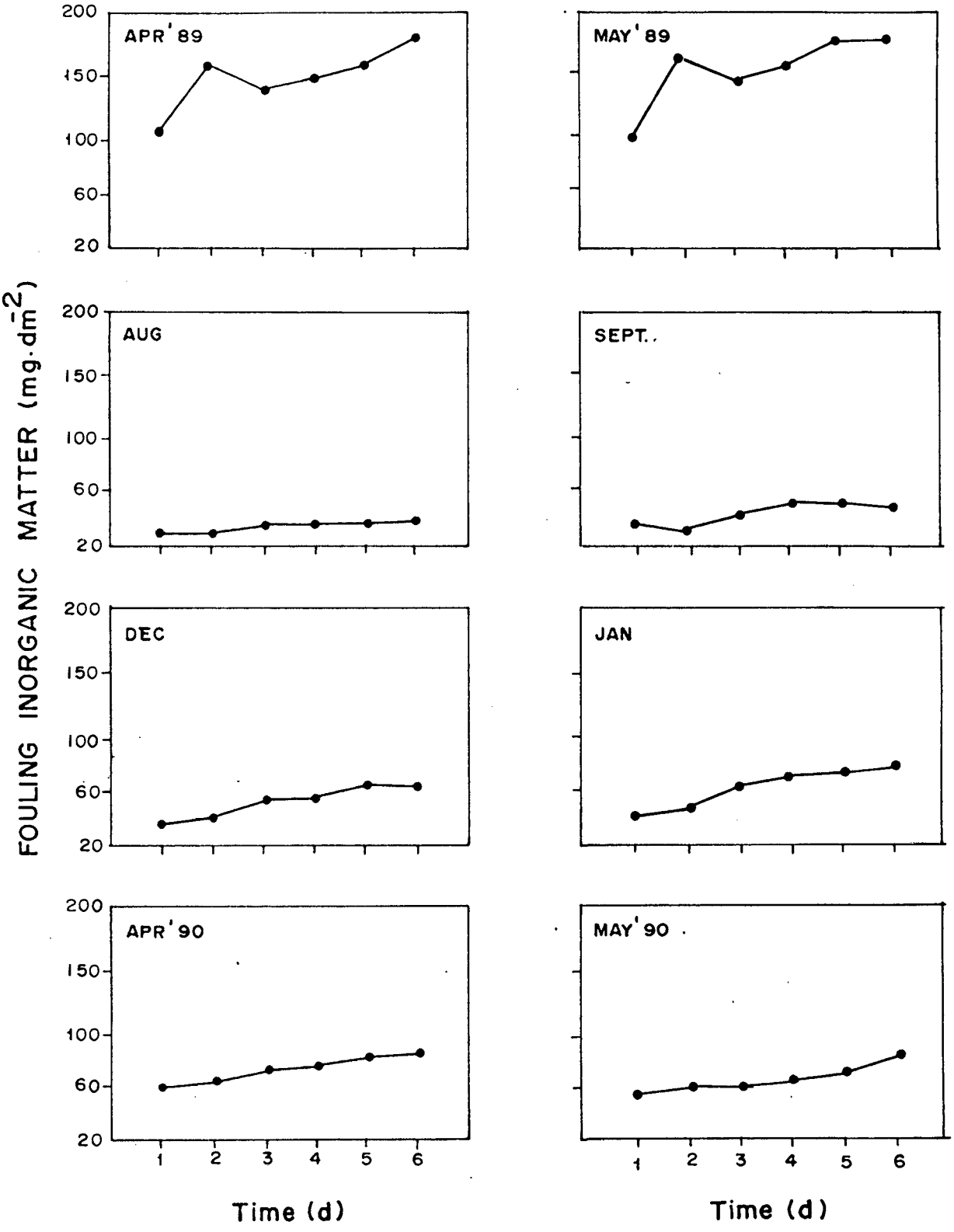

Fig.5.3 DAILY VARIATION IN FOULING INORGANIC MATTER DEVELOPED ON ALUMINIUM FOR VARIOUS MONTHS FROM THE STUDY AREA

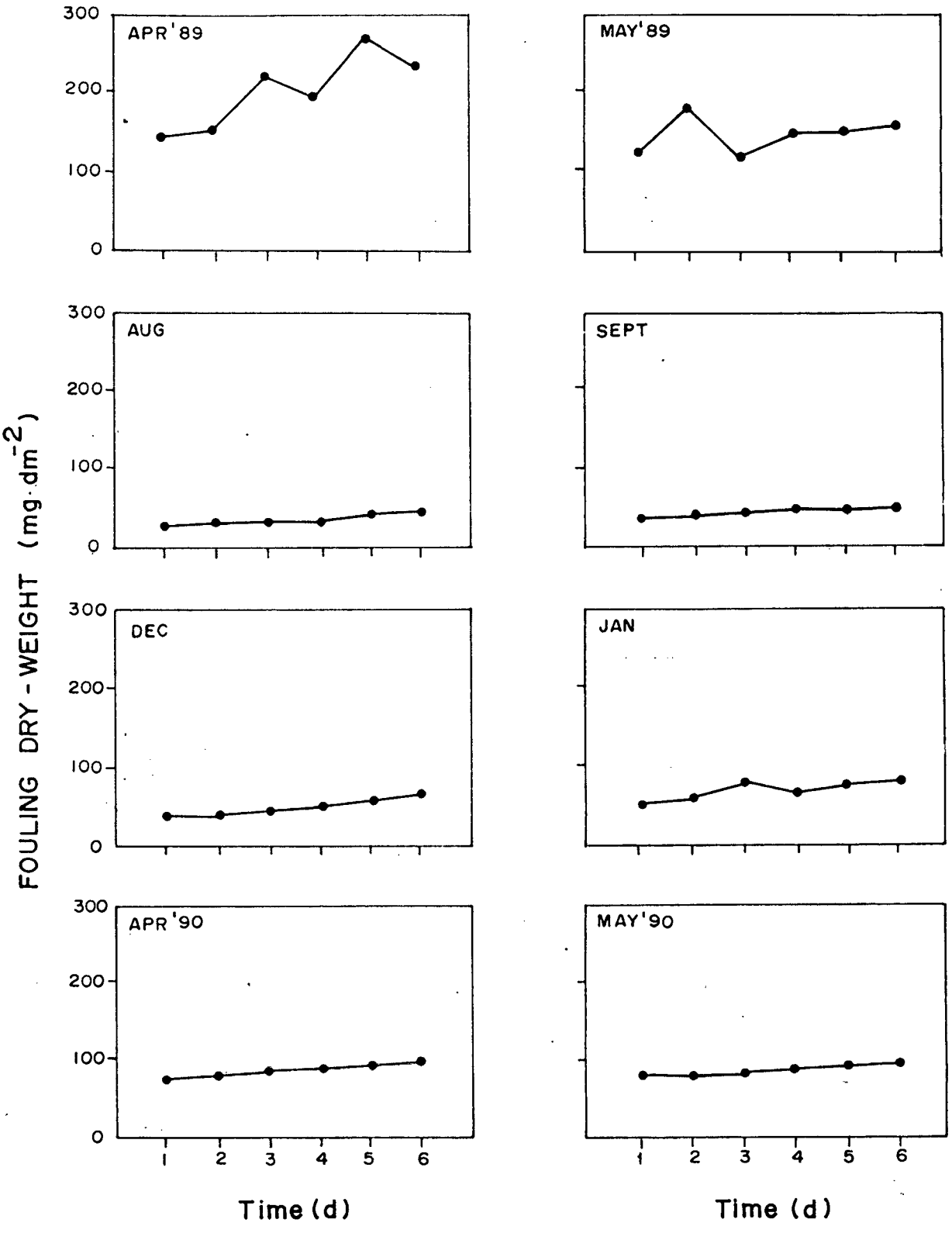

Fig.5.4 DAILY VARIATION IN MICROFOULING BIOMASS (DRY WEIGHT) DEVELOPED ON FIBREGLASS TEST PANELS FOR DIFFERENT MONTHS FROM THE STUDY AREA

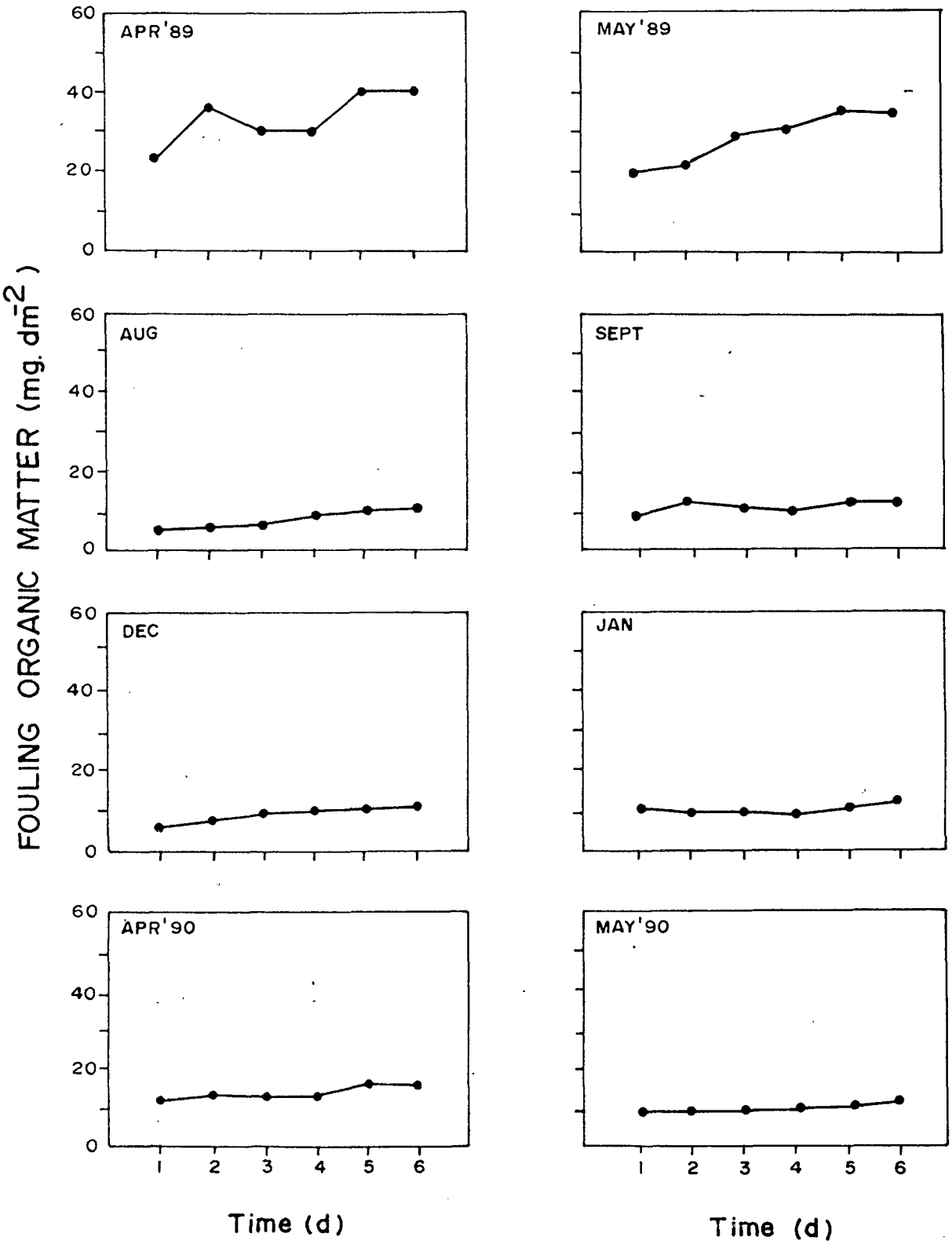

Fig.5.5 DAILY VARIATION IN THE FOULING ORGANIC MATTER FROM FIBREGLASS SURFACE FOR THE SUB-SURFACE WATERS OF DONA PAULA

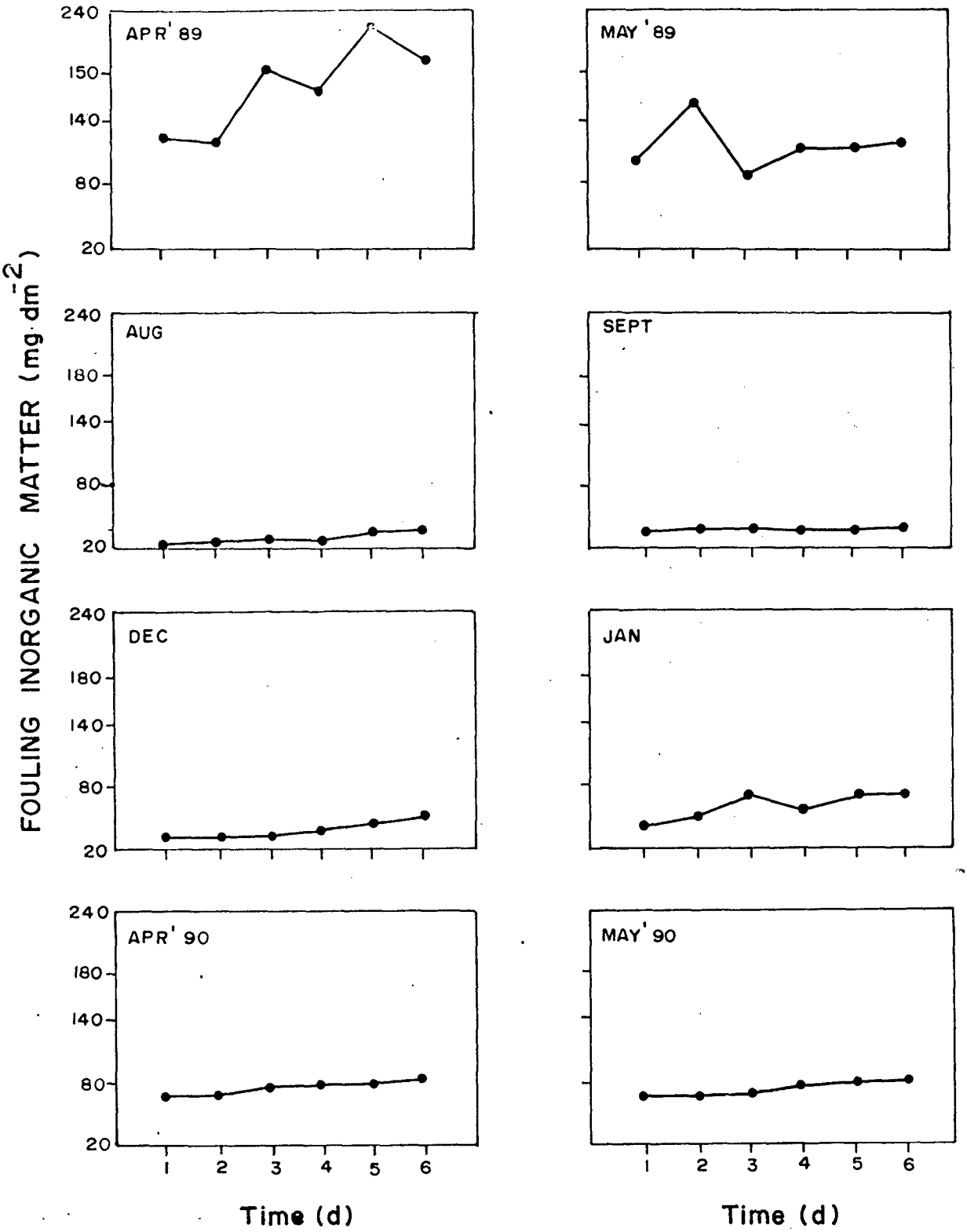

Fig.5.6 DAILY VARIATION IN FOULING INORGANIC MATTER DEVELOPED ON FIBREGLASS FOR VARIOUS MONTHS FROM THE STUDY AREA

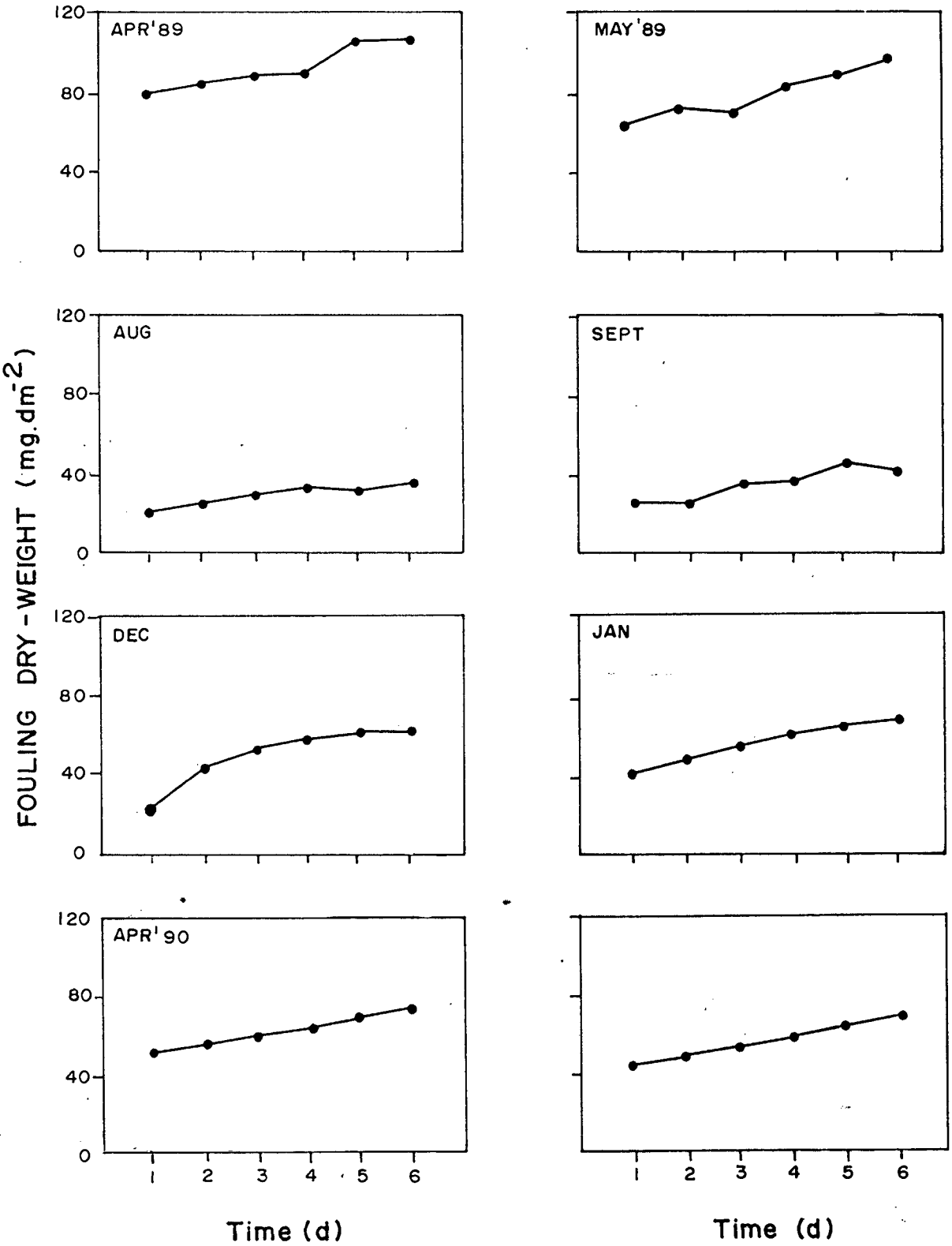

Fig.5.7 DAILY VARIATION IN MICROFOULING BIOMASS (DRY WEIGHT) DEVELOPED ON STAINLESS STEEL TEST PANELS FOR DIFFERENT MONTHS FROM THE STUDY AREA

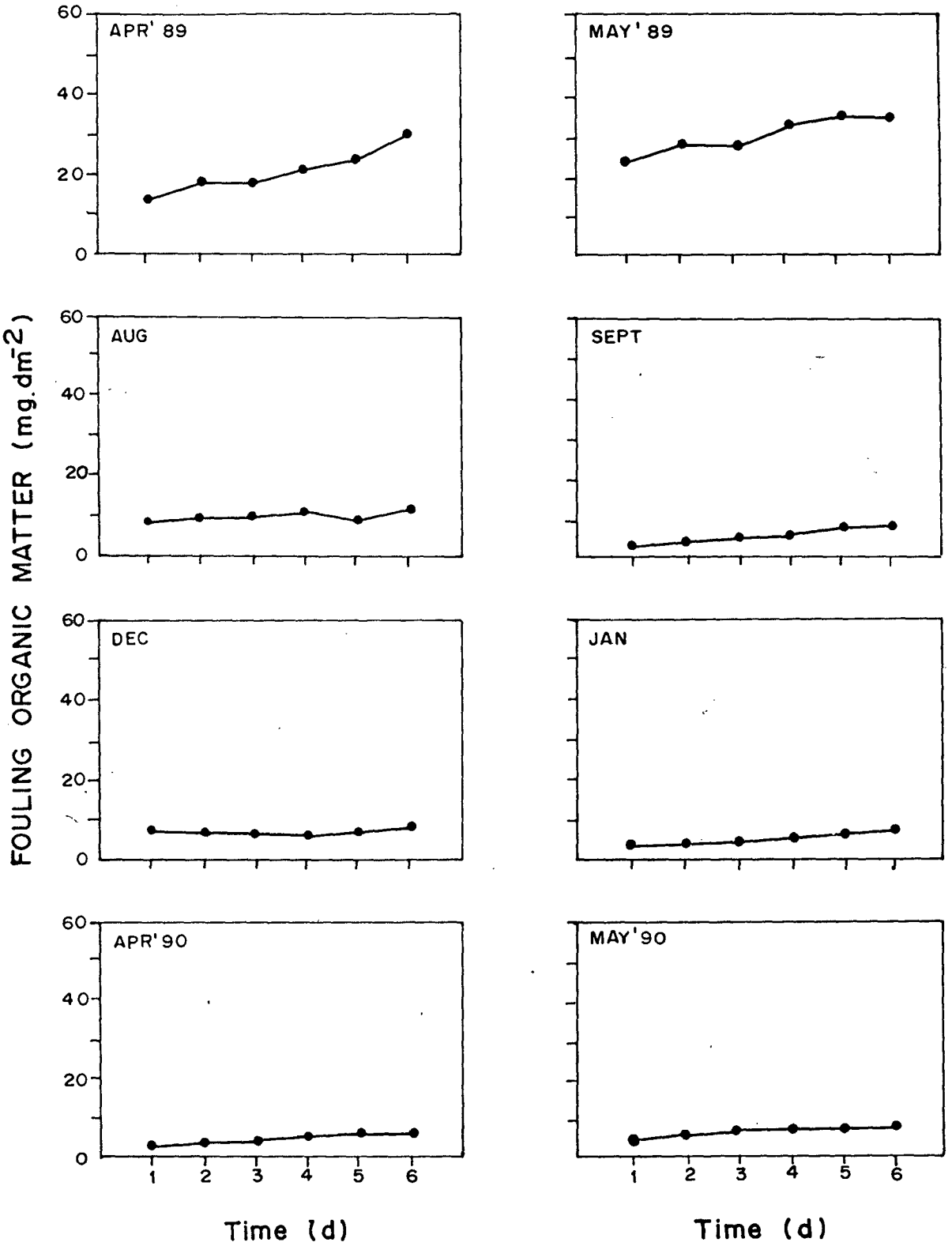

Fig.5.8 DAILY VARIATION IN THE FOULING ORGANIC MATTER FROM STAINLESS STEEL SURFACE FOR THE SUB-SURFACE WATERS OF DONA PAULA.

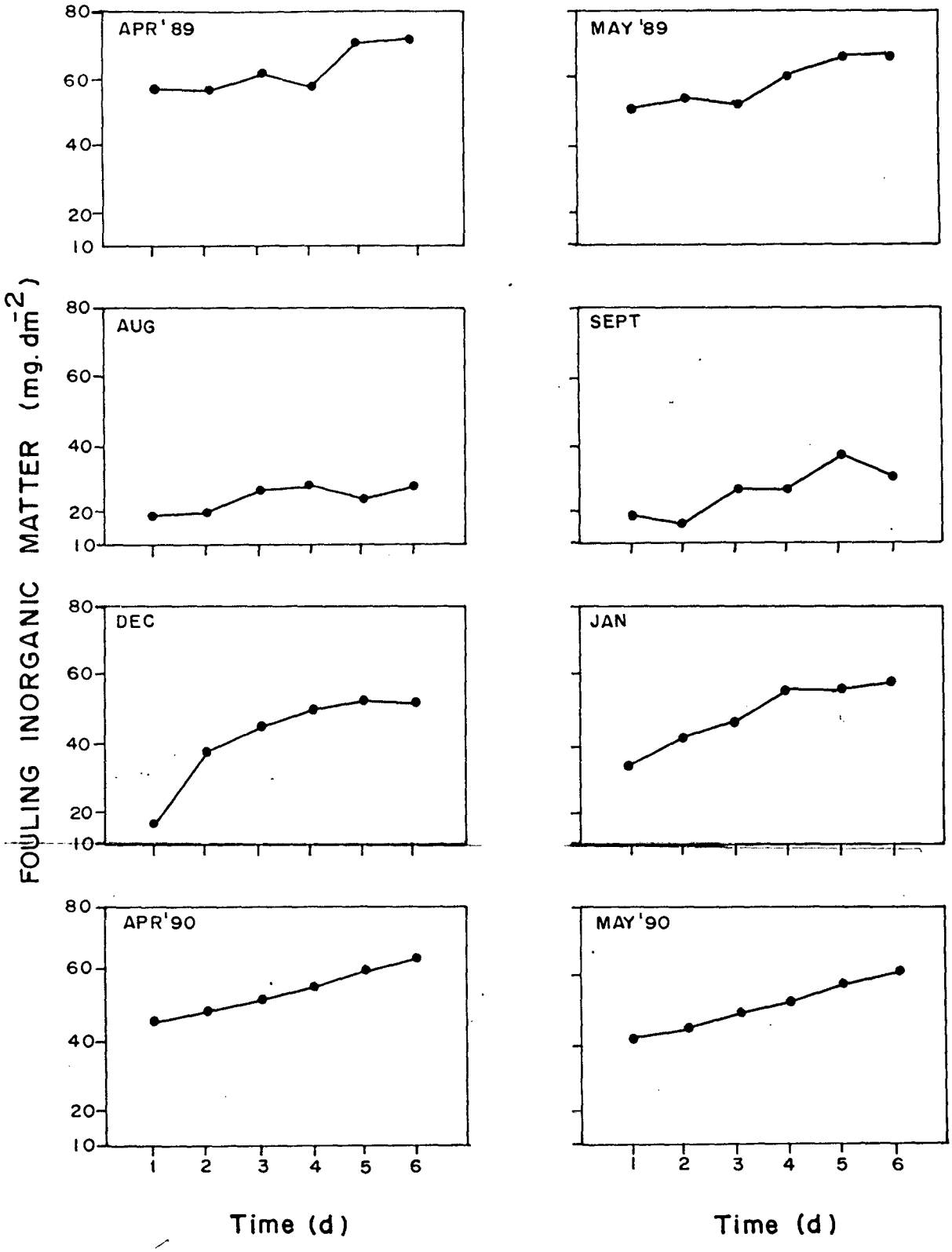

Fig.5.9 DAILY VARIATION IN FOULING INORGANIC MATTER DEVELOPED ON STAINLESS STEEL FOR VARIOUS MONTHS FROM THE STUDY AREA

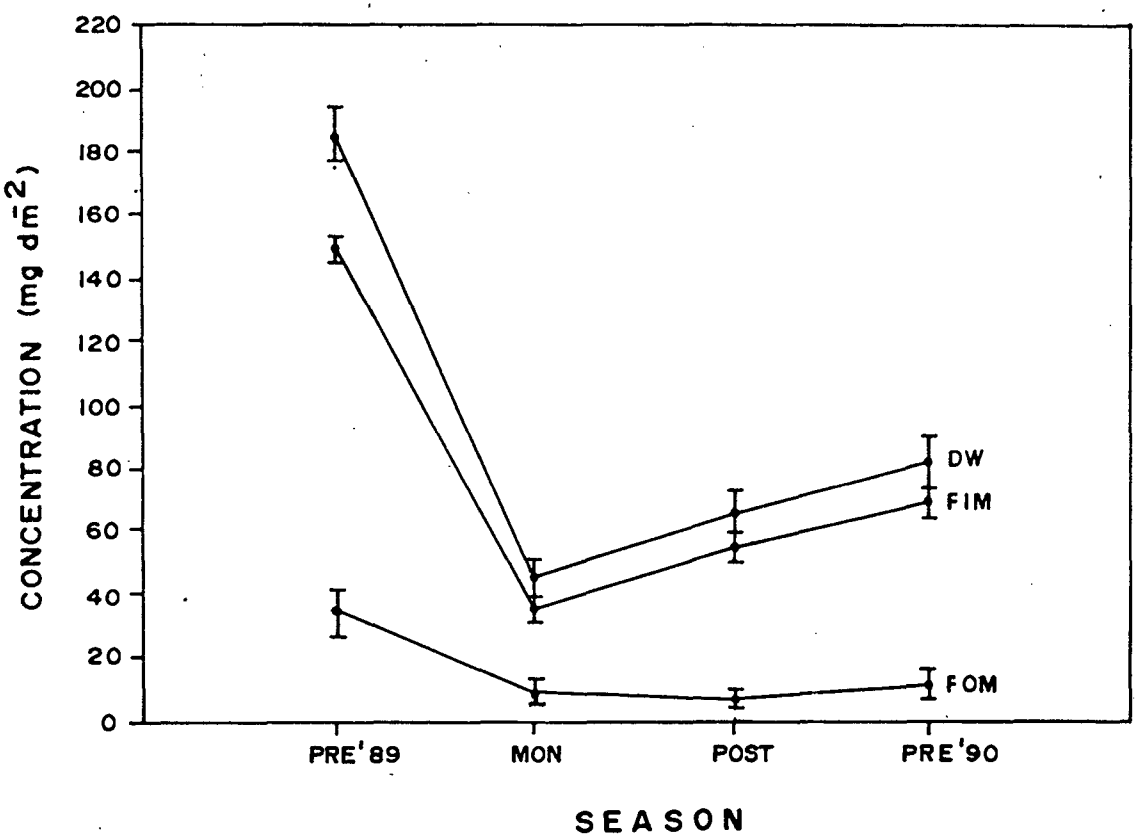

Fig. 5.10 SEASONAL VARIATION IN MICROFOULING MEASURED AS DRY-WEIGHT, ORGANIC & INORGANIC MATTER DEVELOPED ON ALUMINIUM TEST PANELS IMMEPSED IN THE SUB-SURFACE WATERS OF THE STUDY AREA

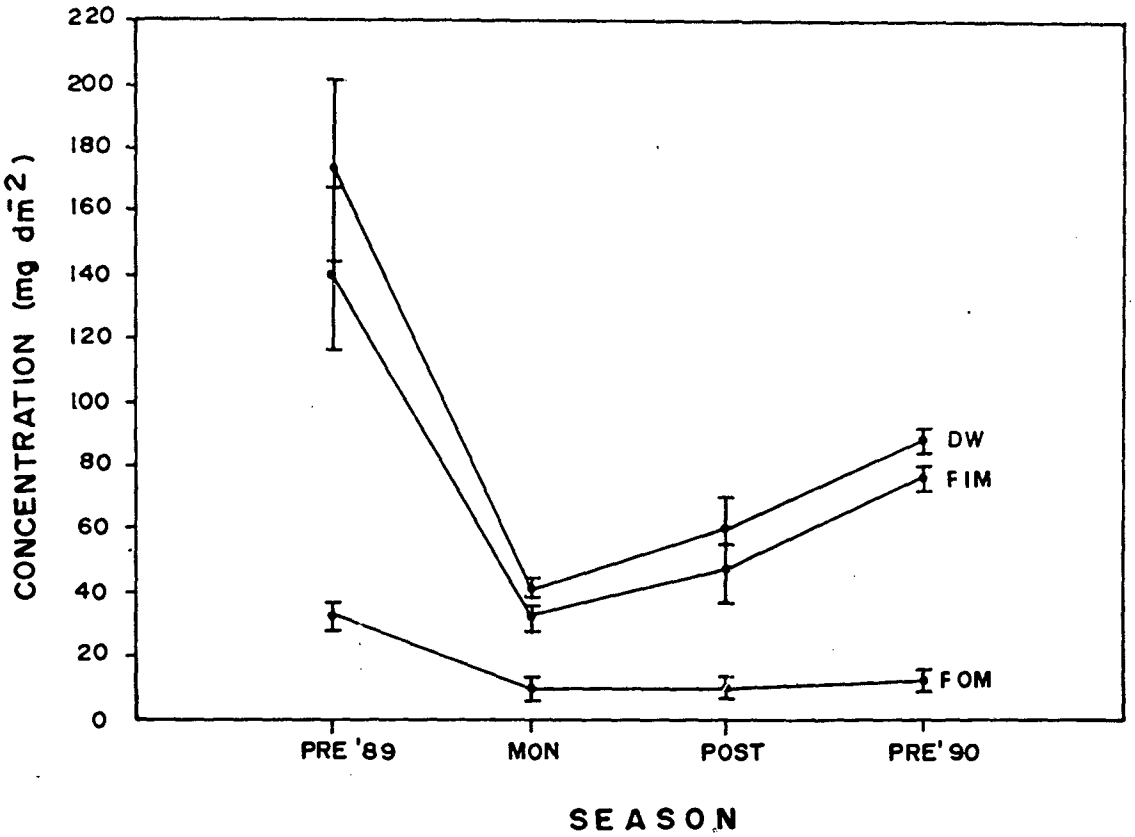

Fig.5.11 SEASONAL VARIATION IN MICROFOULING MEASURED AS DRY-WEIGHT, ORGANIC & INORGANIC MATTER DEVELOPED ON FIBREGLASS TEST PANELS IMMEPSED IN THE SUB-SURFACE WATERS OF THE STUDY AREA.

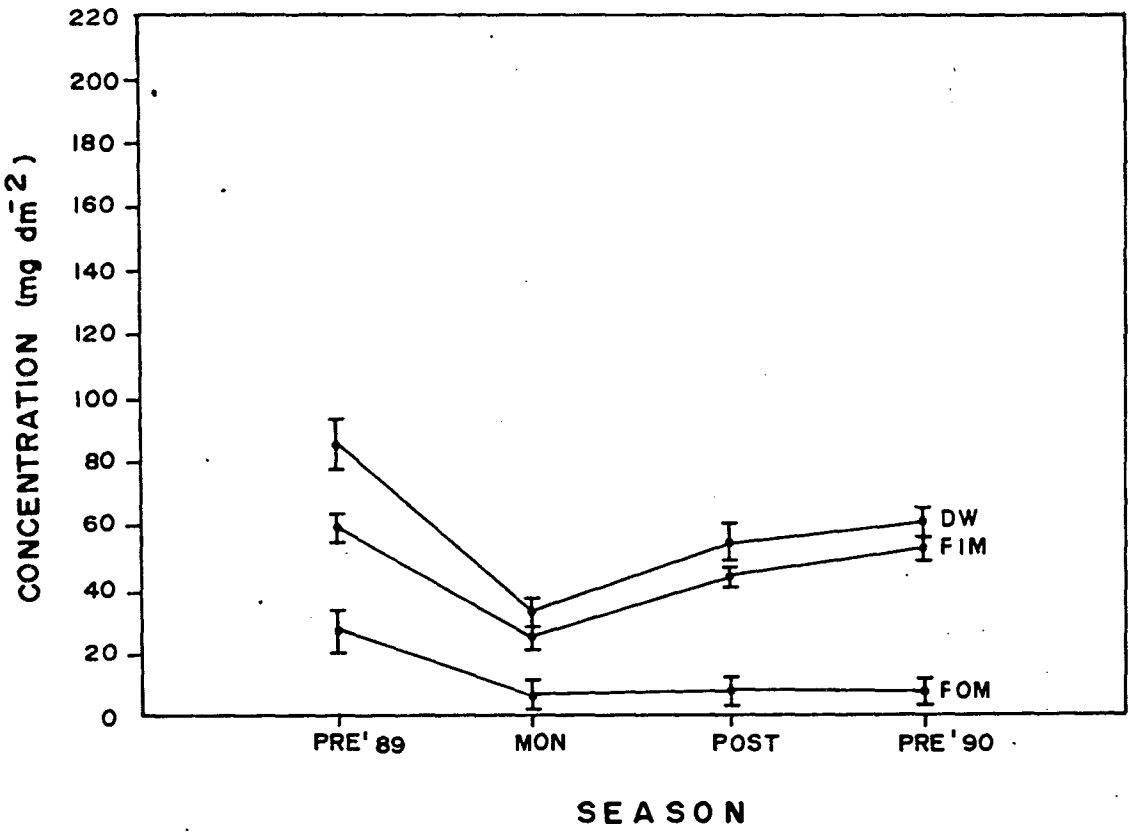

Fig. 5.12 SEASONAL VARIATION IN MICROFOULING MEASURED AS DRY-WEIGHT, ORGANIC & INORGANIC MATTER DEVELOPED ON STAINLESS STEEL TEST PANELS IMMERSED IN THE SUB-SURFACE WATERS OF THE STUDY AREA.

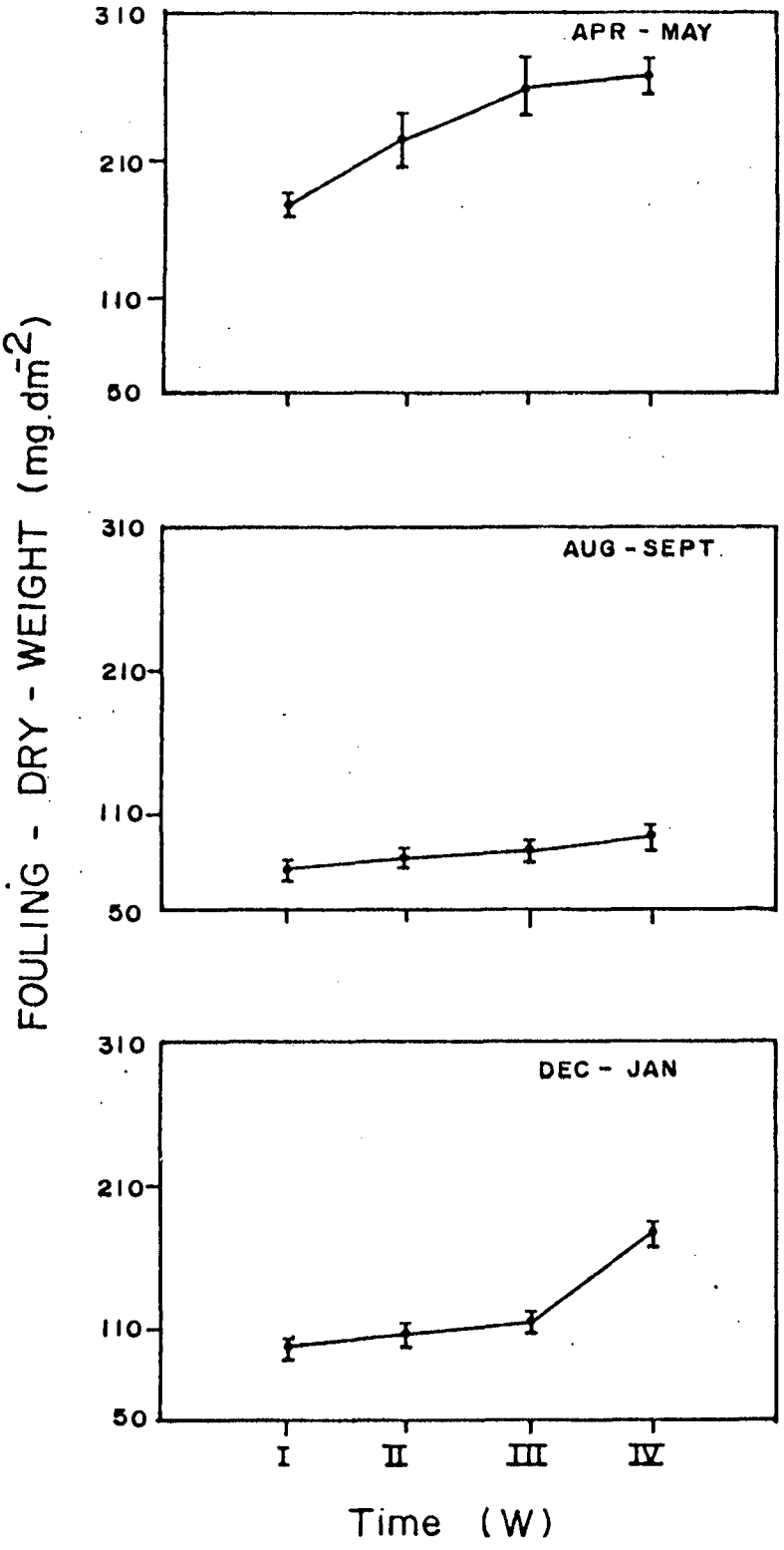

Fig.5.13 WEEKLY VARIATION IN MICROFOULING AS DRY WEIGHT ON ALUMINIUM TEST PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA

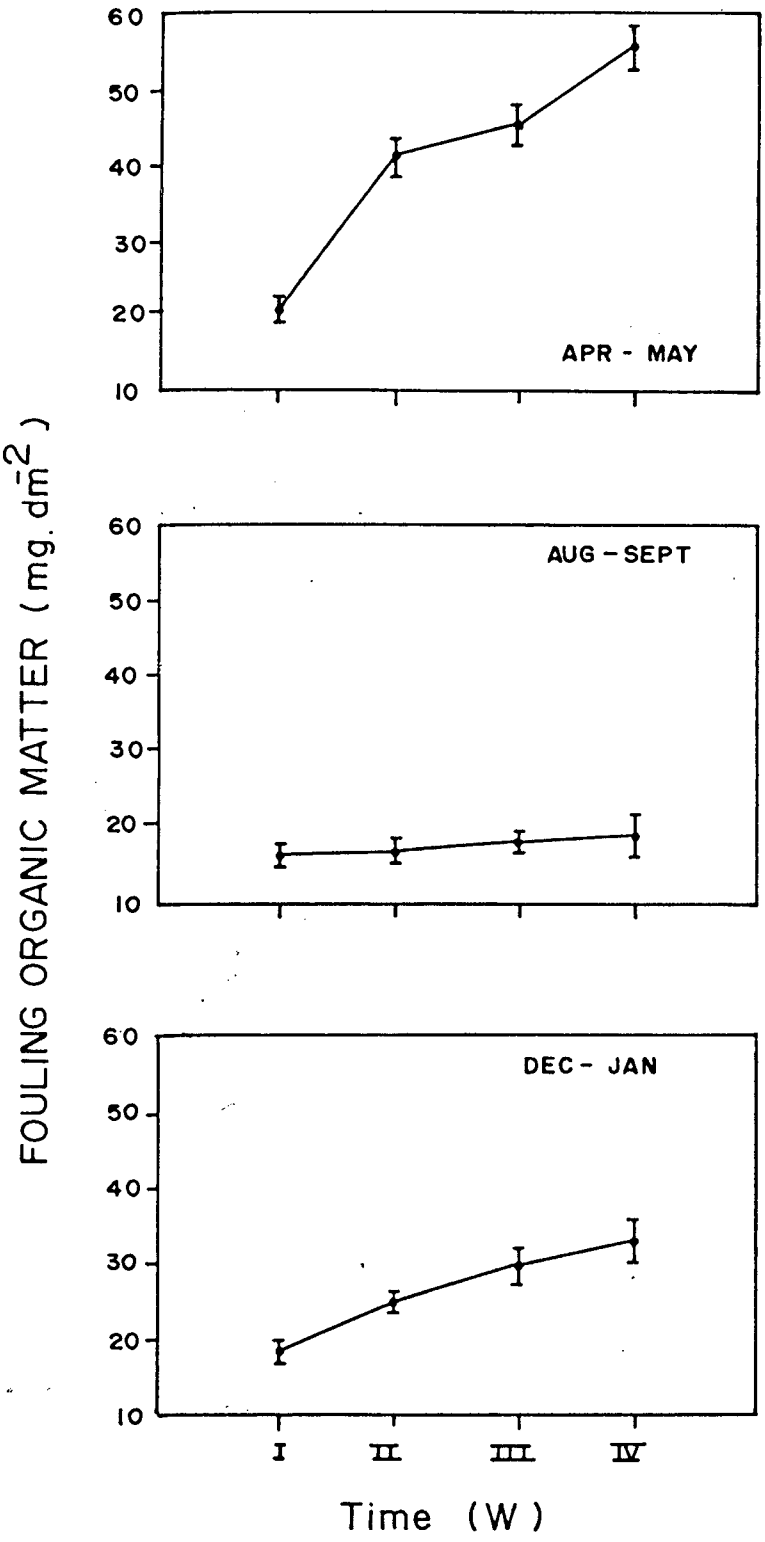

Fig.5.14 WEEKLY VARIATION IN MICROFOULING AS ORGANIC MATTER DEVELOPED ON ALUMINIUM TEST PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

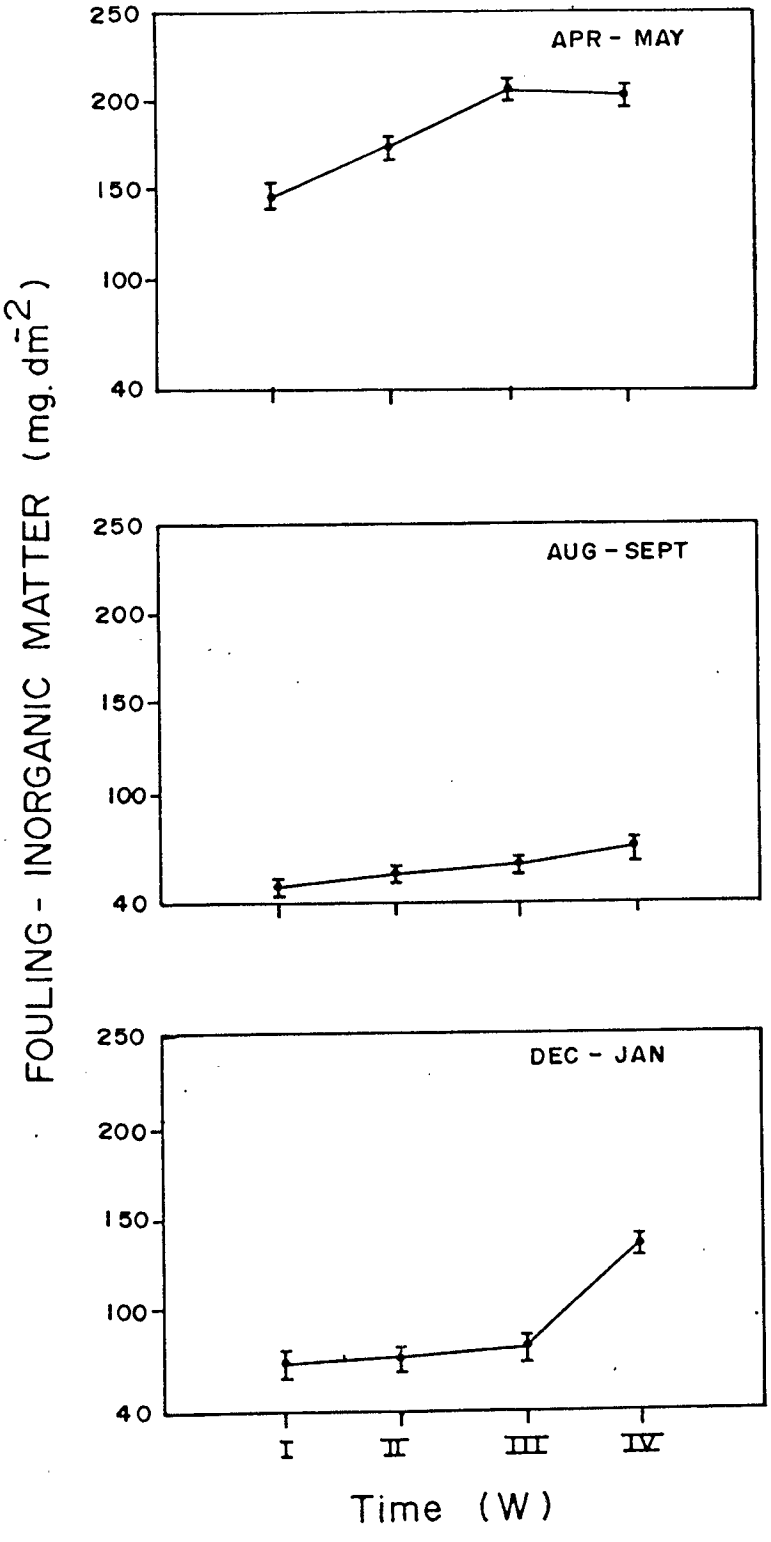

Fig.5.15 WEEKLY VARIATION IN MICROFOULING AS INORGANIC MATTER DEVELOPED ON ALUMINIUM TEST PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

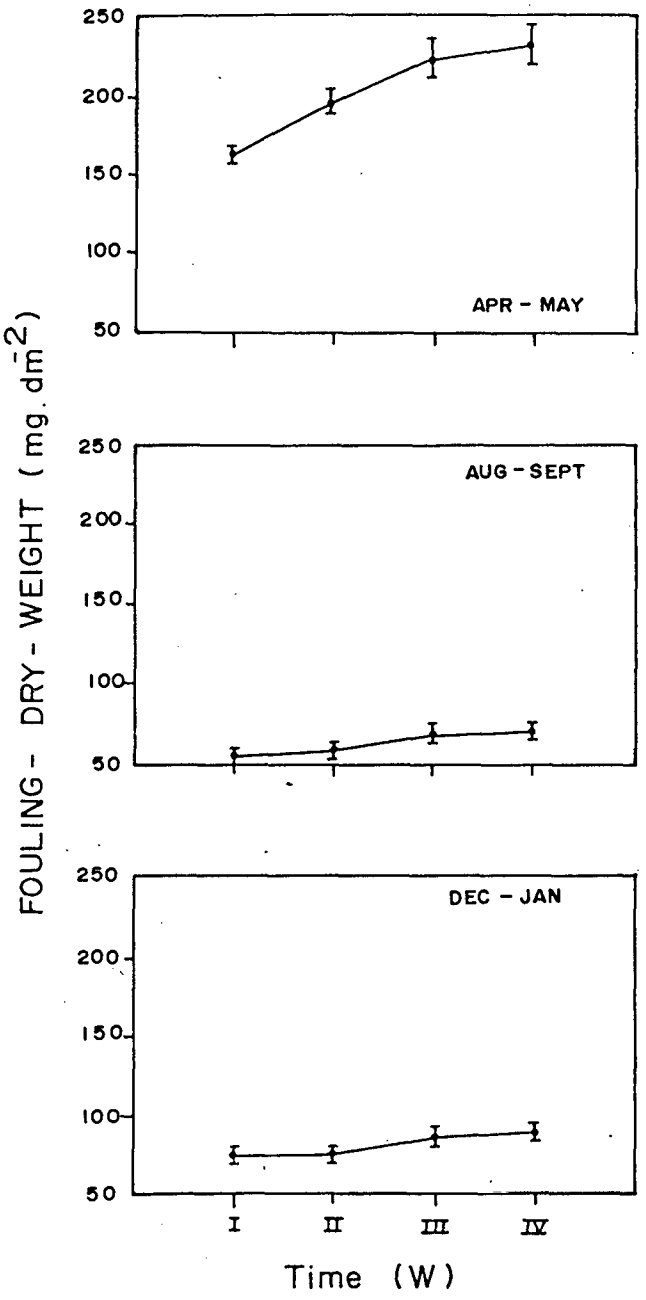

Fig.5.16 WEEKLY VARIATION IN MICROFOULING AS DRY WEIGHT DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

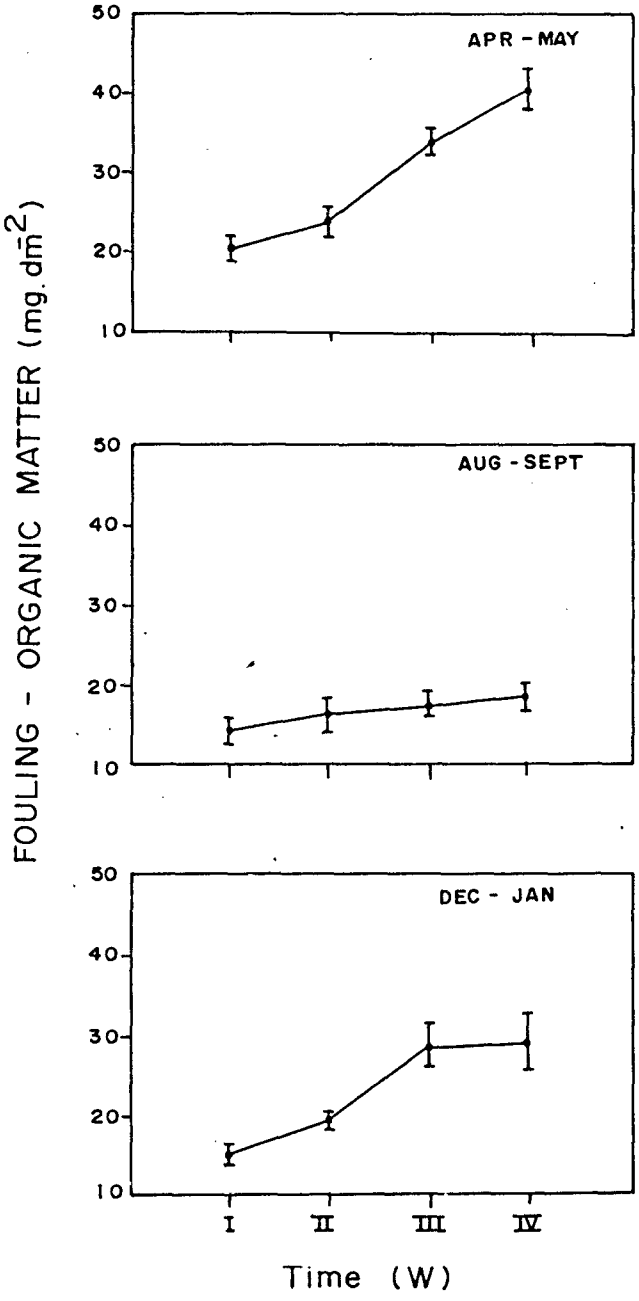

Fig.5.17 WEEKLY VARIATION IN MICROFOULING AS ORGANIC MATTER DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

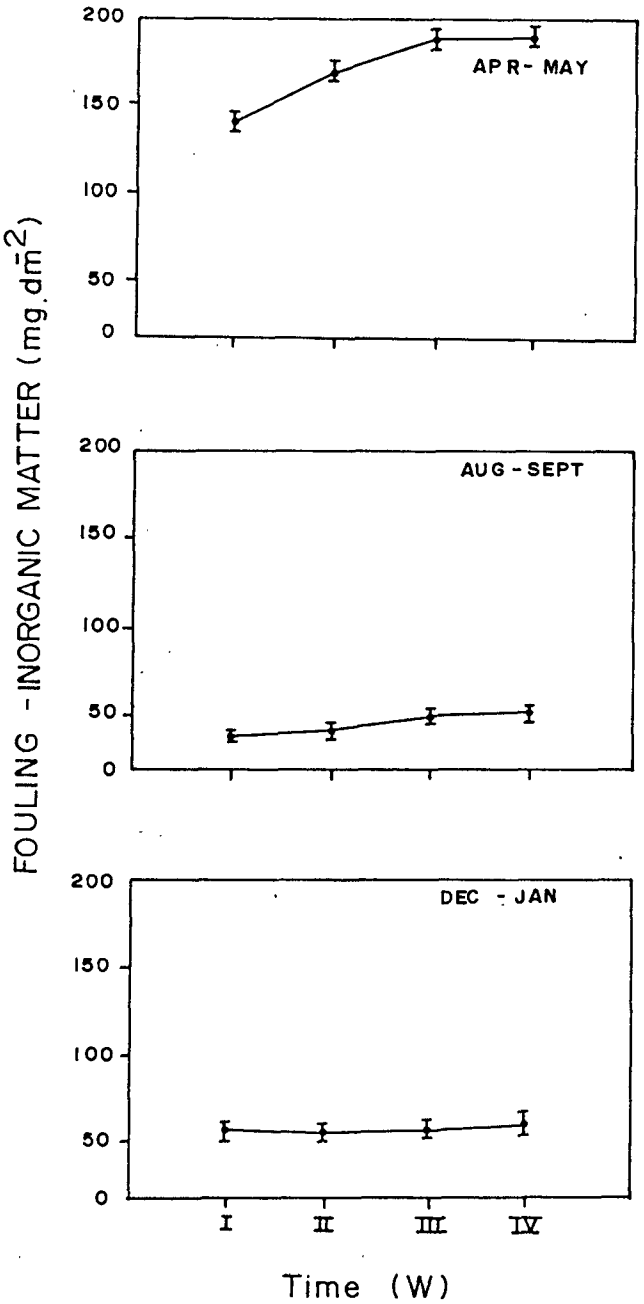

Fig.5.18 WEEKLY VARIATION IN MICROFOULING AS INORGANIC MATTER DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

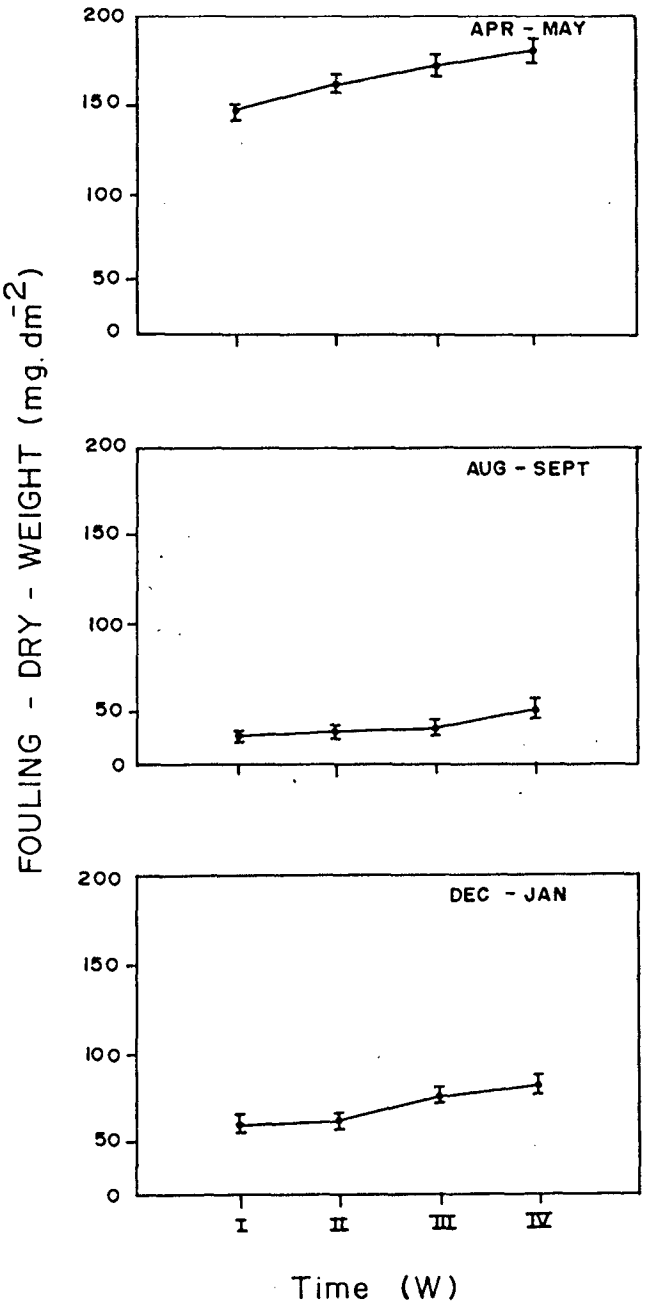

Fig.5.19 WEEKLY VARIATION IN MICROFOULING AS DRY WEIGHT DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

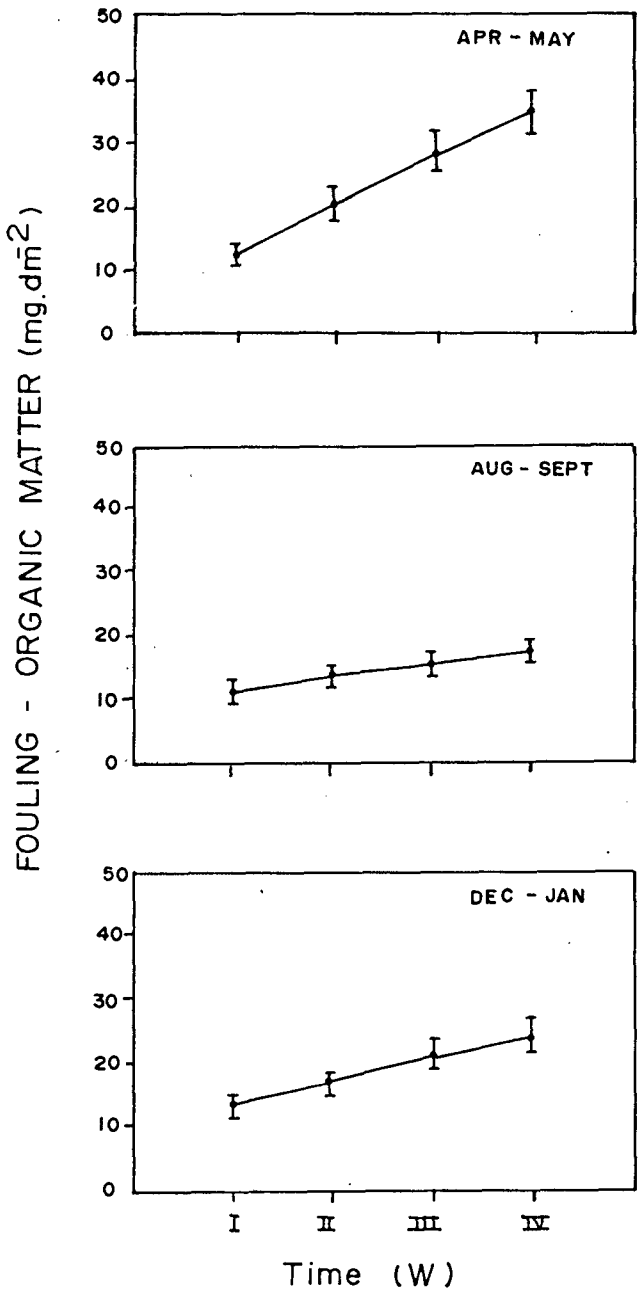

Fig.5.20 WEEKLY VARIATION IN MICROFOULING AS ORGANIC MATTER DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

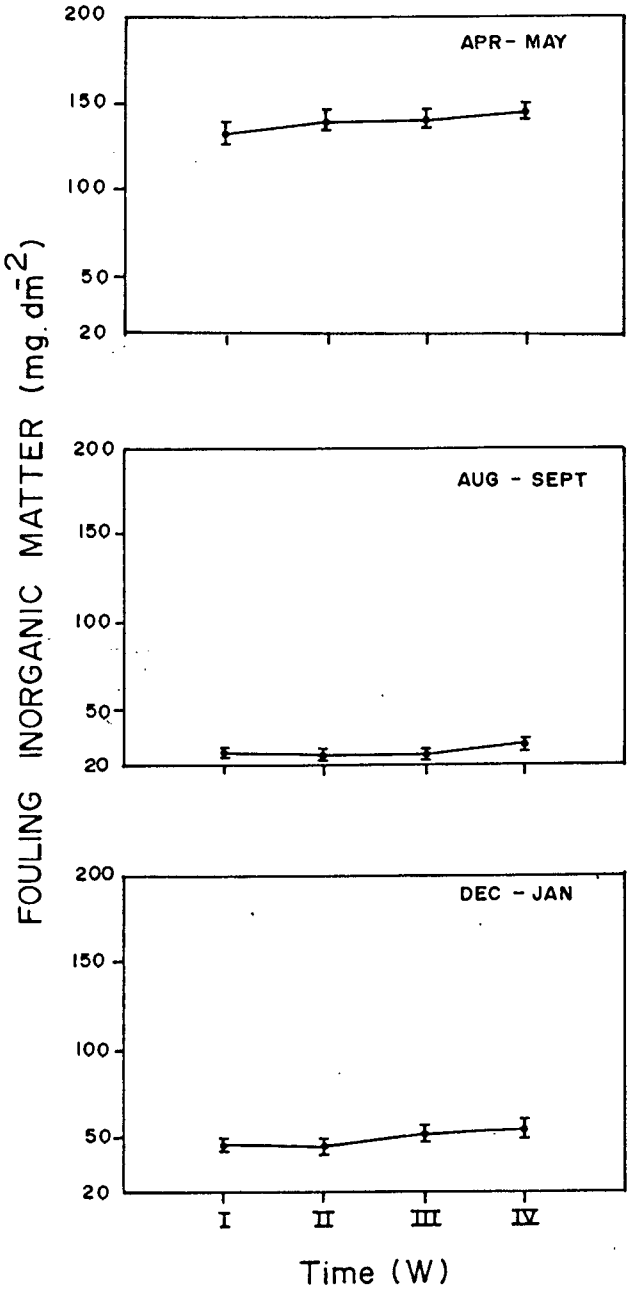

Fig.5.21 WEEKLY VARIATION IN MICROFOULING AS INORGANIC MATTER DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

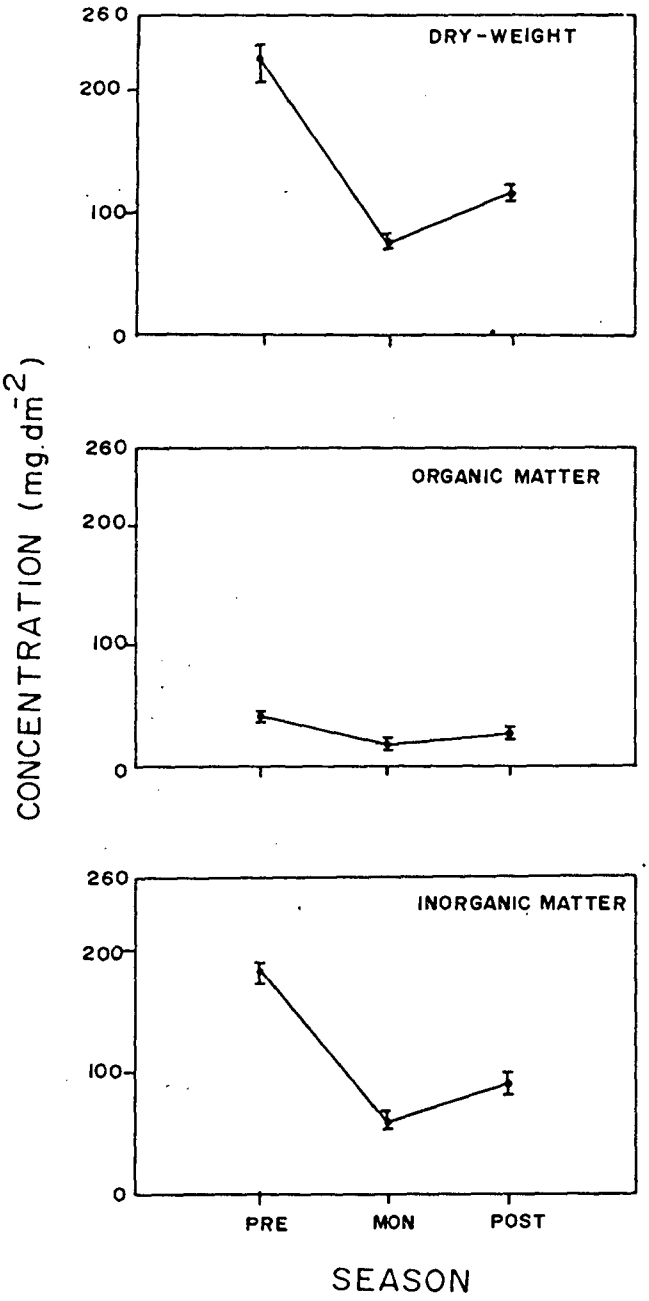

Fig 5.22 SEASONAL VARIATION IN MICROFOULING AS DRY-WEIGHT, ORGANIC AND INORGANIC MATTER DEVELOPED ON ALUMINIUM PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA.

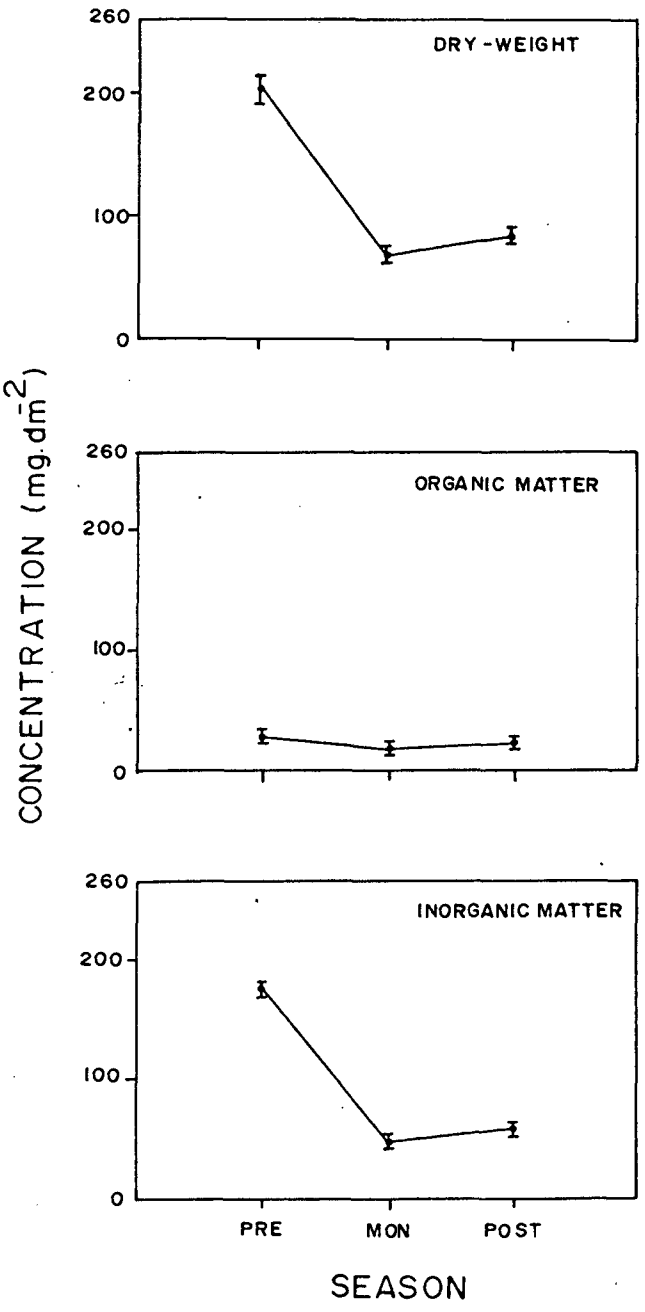

Fig 5.23 SEASONAL VARIATION IN MICROFOULING AS DRY-WEIGHT, ORGANIC AND INORGANIC MATTER DEVELOPED ON FIBRE GLASS PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA.

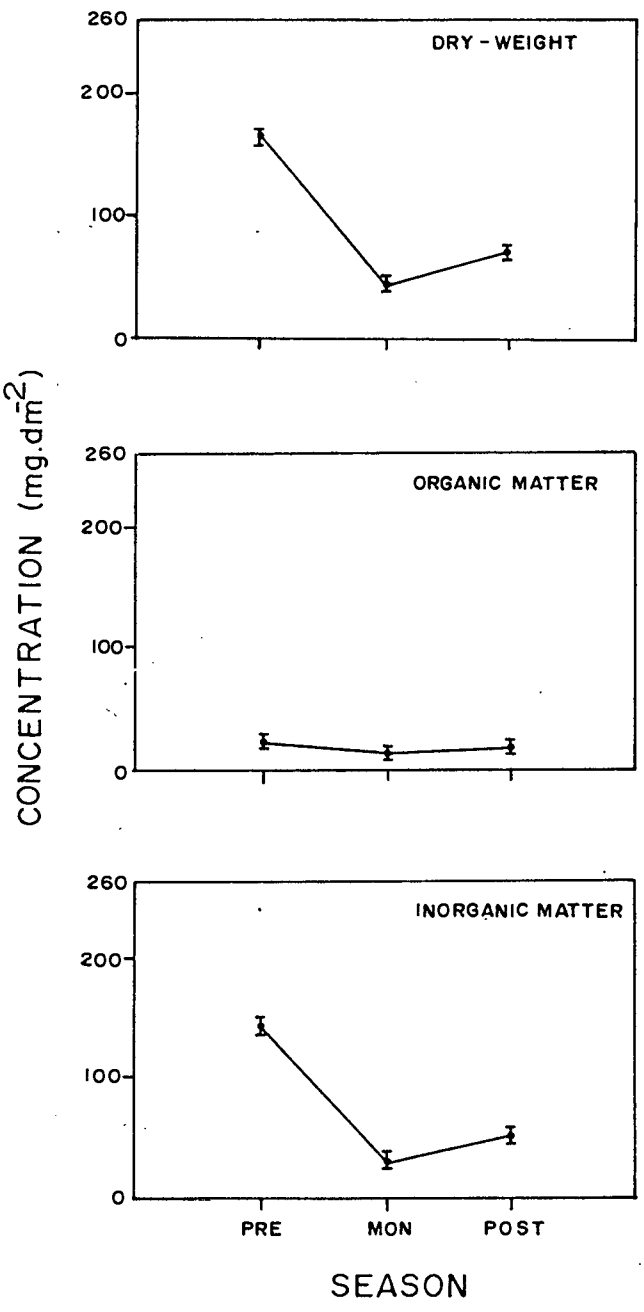

Fig.5.24SEASONAL VARIATION IN MICROFOULING AS DRY-WEIGHT, ORGANIC AND INORGANIC MATTER DEVELOPED ON STAINLESS STEEL WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA.

Chapter 6

NATURE OF PARTICULATE ORGANIC MATTER SETTLED ON SOLID SUBSTRATA

NATURE OF PARTICULATE ORGANIC MATTER SETTLED ON SOLID SUBSTRATA

1 INTRODUCTION

Conditioning of solid surfaces by the adsorption of organic matter is a commonly observed phenomenon in an aquatic environment (Loeb & Neihof, 1977; Characklis & Escher, 1988; Zutic & Tomaic, 1988).

The percentage of inorganic matter in the conditioning film is much higher as compared to the organic matter (Berger & Little, 1980; Srivastava et al, 1990). However, the latter is of greater significance due to its importance as a source of food for various fouling organisms and hence considered to be of higher energy value (Laane, 1982).

Organic compounds are one of the main constituents of the microfouling material developed on solid substrata (Bhosle et al, 1989; Raveendran, et al, 1991). Many such organic compounds are secreted by microorganisms, during their adhesion to solid surfaces (Alison & Sutherland, 1984). Diatom mucilage containing polysaccharides appears to be similar with marine bacterial exopolymers (Fletcher &

Floodgate, 1973). However, relative composition of these compounds varies with the nature of the surface, period of exposure and with season. Decomposition of such organic matter is mainly performed by bacteria (Williams & Yentsch, 1976). Hence, in the present study microfouling biomass was studied as organic carbon (F-OC), organic nitrogen (F-ON) and chlorophyll *a* (Chl *a*). In addition to this, the carbohydrate composition of the microfouling material was also studied. A thorough knowledge of the composition and source of organic matter on substrata is of prime interest for understanding the biology and chemistry of microfouling material developed on test surfaces. Such studies serve as a baseline data for any future study of microfouling settlement in the Dona Paula waters.

In addition, since the physico-chemical parameters also played an important role in influencing settlement and development of microfouling (Characklis *et al*, 1984; Yanshun *et al*, 1984; Fletcher, 1988), simple linear relationship of microfouling biomass with environmental parameters are discussed.

2 MATERIAL AND METHODS

2.1 Removal of microfouling material

The study area, the method of deployment of panels, as well as interval for retrieval is explained in the earlier chapters. After retrieval panels were gently rinsed using filtered sea water, scraped using a nylon brush, as suggested by Sharma et al, (1990), and made to a known volume (100 ml) using filtered sea water. Aliquots (10 ml) from the above, were filtered through ashed (450° C, 4 h) and preweighed GF/C glass fibre filter papers (1.0um pore size, 47 mm. dia). These papers were used for the analysis of organic carbon (F-OC), organic nitrogen (F-ON), chlorophyll *a*, (Chl *a*) and carbohydrate composition (F-CHO) of the microfouling material. Filter papers for all the parameters except chlorophyll *a* were dried in an oven at 40° C for 24h.

2.2 Chemical analysis

Chlorophyll *a*

For the analysis of chlorophyll *a* (Chl *a*), triplicate samples were extracted by homogenization of the filter

papers along with the filtered material in 90% acetone using dark vials. After 24h, the concentration was determined spectrophotometrically, after correcting for pigments, as described by Parsons et al, (1984).

Organic carbon (F-OC)

The organic carbon content of the microfouling material was analyzed following the method of Strickland & Parsons, (1965). This method involves the wet oxidation of carbon by acid dichromate. The decrease in the extinction of the yellow dichromate solution is taken as the measure of carbon. Extinction was measured at 410nm using a spectrophotometer. Phytoplankton carbon was calculated from the chlorophyll a values by multiplying them with a factor 40 (F-OC:F-Chl a wt/wt). Bacterial carbon was calculated after multiplying the bacterial number by 20 assuming the average cell volume to be 0.073, as suggested by Lee & Furmann, (1987).

Organic nitrogen (F-ON)

Organic nitrogen was analyzed by the method suggested by Grasshoff, (1964), wherein the filter papers containing the microfouling material was oxidised using $K_2S_2O_8$

(potassium peroxodisulphate) and digested at 120° C and 15 lb/cm2 pressure for 30 minutes. This technique converts all nitrogen compound to nitrate. The nitrate was then reduced to nitrite by using a cadmium column and nitrite was estimated as suggested by Parsons et al, (1984).

Total carbohydrates (F-CHO)

Total carbohydrates of the microfouling material was analysed by the phenol-sulphuric acid method (Dubois et al, 1956). This method was modified by Handa, (1966), to include hydrazine sulphate. The carbohydrate carbon was calculated by multiplying F-CHO values with 0.40 (Bhosle & Dhople, 1988).

3 RESULTS OF SHORT TERM VARIATIONS

3.1a ALUMINIUM

Organic carbon (F-OC) (Fig 6.1)

Microfouling biomass as organic carbon developed on aluminium test panels showed that the values for the entire study period ranged between 1.91 and 31.21mg.dm-2.

As shown in Fig 6.1, during April, 1989, short term variation in biomass exhibited a value of 12.28mg.dm-2 for samples on the 1st day, which increased significantly on the 2nd day (21.62mg.dm-2) and then marginally till the 6th day (31.21mg.dm-2). During May, 1989, the trend was as follows, samples on the 1st day exhibited a value of 11.47mg.dm-2, which increased upto 17.14mg.dm-2 on the 3rd day. For the 4th day the value was lower than that observed on the 3rd day (15.78mg.dm-2) samples. On subsequent days the increase was steady till the 6th day amounting to 22.60mg.dm-2.

During August, 1989, as can be seen from Fig 6.1, the value was very low for samples on the 1st day (1.91mg.dm-2). The 2nd and 3rd day showed marginal increase, the values being 2.76mg.dm-2 and 3.69mg.dm-2, respectively. Whereas, on the 4th day, the increase was more significant (5.99mg.dm-2). It was almost steady for samples on the 5th day (6.37mg.dm-2) as well as for the 6th day (6.39mg.dm-2).

The pattern obtained during September, 1989, was altogether different. The biomass value for samples on the 1st day was 4.51mg.dm-2, which increased to 7.99mg.dm-2 on the 2nd day. The value then decreased successively till it reached 6.77mg.dm-2 for samples on the 6th day.

Biomass values observed during December, 1989, showed a clear distinct increase from 2.50mg.dm-2 on the 1st day till the end of sampling on the last day (6.37mg.dm-2). Low value for samples on the 1st day of January, 1990, amounting to 5.09mg.dm-2 was higher than that observed during December, 1989. It remained steady during the 2nd (5.61mg.dm-2) and 3rd days (4.98mg.dm-2). The samples on the 4th day showed a steady increase to 6.89mg.dm-2, which continued in this fashion till the 6th day (9.01mg.dm-2).

During April as well as May, 1990, an increase in concentration was observed from 6.83 to 11.50 and 4.39 to 6.82mg.dm-2, respectively.

Chlorophyll *a* (F-Chl *a*) (Fig 6.2)

The biomass as Chl *a* on aluminium surface ranged from 49 to 265ug.dm-2, for the whole of the study period as shown in Fig 6.2. During April, 1989, the samples on the 1st day showed a value of 250ug.dm-2, which decreased on the 2nd day to 245ug.dm-2 and 244ug.dm-2 on the 3rd day. For samples on the 4th day, a relatively higher value of 265ug.dm-2 was recorded. Thereafter, it showed a decrease till the last day of sampling to 257ug.dm-2. During May, 1989, of the same season, lowest value was observed for

samples on the 1st day (190ug.dm-2), which increased till the 3rd day (220ug.dm-2). The value of chl$_a$ then dropped for samples on the 4th day to 207ug.dm-2 and once again showed an increase till the 6th day (223ug.dm-2).

During the months of August, 1989, as well as September, 1989, there was a significant resemblance in the biomass values. For example, low values of 59 and 117ug.dm-2 were observed for samples on the 1st day. These values showed a regular increase till the 3rd day for both August (93ug.dm-2) as well as September (173ug.dm-2). The minimum for both the months were exhibited by samples on the 4th day (49 and 73ug.dm-2 respectively). The value kept on increasing till 75 ug.dm-2 was reached for samples on the 6th day during August, 1989. In September, 1989, the value increased till the 5th day to 86ug.dm-2 and decreased to 83ug.dm-2 on the 6th day. The results obtained for December, 1989 (62 to 108ug.dm-2), January, 1990 (68 to 158ug.dm-2), April, 1990 (101 to 206ug.dm-2) and May, 1990 (90 to 183ug.dm-2), showed a somewhat similar pattern. There was a linear increase in the values of chl$_a$, with increase in the period of exposure.

Total carbohydrate (F-CHO) (Fig 6.3)

Total carbohydrate concentration of the microfouling

material developed on aluminium test panels showed a similar increase in the concentration with corresponding increase in the immersion time. During the entire period of study a minimum value of 0.061mg.dm-2 and a maximum value of 1.606mg.dm-2, was observed in Fig 6.3.

During the month of April, 1989, as well as in May, 1989, the microfouling material showed values which were comparatively high. The values ranged between 1.023 and 1.606mg.dm-2 and 0.932 to 1.266mg.dm-2 for samples on the 1st and 6th days, respectively. Samples for all the days during December, 1989, (0.131 to 0.539mg.dm-2), January, 1990 (0.240 to 0.746mg.dm-2), April, 1990 (0.532 to 0.957mg.dm-2) and May, 1990, (0.456 to 0.856mg.dm-2), showed a similar pattern. However, during August as well as in September, 1989, the values were low (0.061 and 0.114mg.dm-2) on the 1st day which increased till the 4th day (0.216 and 0.283mg.dm-2). The 5th day showed a decrease in carbohydrate concentration to 0.117 and 0.220mg.dm-2, which once again showed an increase to 0.237 and 0.268mg.dm-2 for samples obtained during August & September, 1989, respectively.

A similar set of observation was carried out for fibreglass panels, in order to assess the change in the biomass values as well as the carbohydrate content of the

microfouling material.

3.1b **FIBREGLASS**

Organic Carbon (F-OC) (Fig 6.4)

Microfouling biomass when estimated as organic carbon for the study period, showed values ranging between 2.44 and 23.39mg.dm-2. During April, 1989, a value of 14.05mg.dm-2 was observed on the 1st day, which increased to 21.60mg.dm-2 on the following day. Thereafter, there was a decrease in the biomass for the samples on the 3rd day (18.03mg.dm-2) as well as on the 4th day (17.56mg.dm-2). On the 5th day, however, an increase in the value was observed, recording 23.37mg.dm-2, which remained more or less steady until the 6th day (23.39mg.dm-2).

During May, 1989, as shown in Fig 6.4, biomass values varied linearly (11.83 to 20.81mg.dm-2), with corresponding increase in time. Such a trend was also observed during August, 1989 (2.44 to 5.78mg.dm-2), December , 1989 (3.67 to 6.32mg.dm-2) April, 1990 (6.47 to 9.04mg.dm-2) and May, 1990 (5.41 to 7.06mg.dm-2).

During September, 1989, biomass showed a low value initially (5.25mg.dm-2), which, increased for samples on

the 2nd day to 7.16mg.dm-2. This was followed by a decrease to 6.14mg.dm-2 on the 3rd day. Thereafter, a marginally increasing trend was seen as shown in Fig 6.4, till the end of sampling time. The values ranged from 5.77 to 6.84mg.dm-2.

During the month of January, 1990, the 1st day samples showed a value of 6.23mg.dm-2. On the subsequent day it decreased to 5.67mg.dm-2, which was the minimum value observed for this month. The value remained somewhat steady for samples on the 3rd day, being, 5.69mg.dm-2. Thereafter, an increase was observed from the 4th day (5.78mg.dm-2) till the 6th day (6.92mg.dm-2).

Chlorophyll a (F-Chl a) (Fig 6.5)

The microfouling biomass expressed as chl a developed on fibreglass fluctuated between 67 and 271ug.dm-2 during the study period.

For microfouling samples on the 1st day in April, 1989, the value exhibited by fibreglass test surface as presented in Fig 6.5, was 255ug.dm-2. The 2nd day showed an increase to 271ug.dm-2. Thereafter, a steady drop was observed in the biomass values till the 5th day (250ug.dm- 2). Samples on the 6th day showed a slight increase (252ug.dm-2)

as compared to the previous day. Sampling during May, 1989, showed a linear increase in biomass with increase in exposure period from 205 to 236ug.dm-2. Such was also the case during September, 1989, (109 to 159ug.dm-2), December, 1989, (96 to 147ug.dm-2), April, 1990 (115 to 217ug.dm-2) and May, 1990, (99 to 167ug.dm-2). During August, 1989, as well as in January, 1990, the samples on the 1st day showed a low value of 67 and 120ug.dm-2 which increased on the 2nd day to 91 and 120ug.dm-2, respectively. However, during the 3rd day a decrease for both the months to 78 and 139ug.dm-2, was evident. The value then increased for all the remaining days till the end of sampling (136 & 163ug.dm-2).

Total carbohydrate (F-CHO) (Fig 6.6)

Carbohydrate concentration of the microfouling material developed on fibreglass fluctuated from 0.106 to 1.766mg.dm-2. There was a significant increase in the concentration of carbohydrates with increase in the study period during April, 1989 (1.242 to 1.766 mg.dm-2), May, 1989 (0.986 to 1.236mg.dm-2), August, 1989, (0.106 to 0.378mg.dm-2), September, 1989, (0.163 to 0.486mg.dm-2), December, 1989 (0.275 to 0.498mg.dm-2), January, 1990 (0.435 to 0.549mg.dm-2), April, 1990 (0.623 to 0.788mg.dm-2)

and May, 1990 (0.513 to 0.732mg.dm-2) respectively.

3.1c STAINLESS STEEL

Organic carbon (F-OC) (Fig 6.7)

Microfouling biomass as organic carbon developed on stainless steel was observed to vary between 1.77 and 20.27mg.dm-2 for the sampling period. All the months sampled showed a similar linear increasing trend with time as was evident from Fig 6.7, without any abnormality.

Chlorophyll $\underline{a}$ (F-Chl $\underline{a}$) (Fig 6.8)

Biomass as chl $\underline{a}$ showed fluctuations ranging between 49 and 265ug.dm-2 for the study period.

During April, 1989, a value of 198ug.dm-2 for samples on the 1st day increased to 265ug.dm-2 on the 3rd day. The 4th day showed a decrease to 247ug.dm-2 which increased on the 5th day to 254ug.dm-2. The value decreased once again for samples on the 6th day (240ug.dm-2). An irregular change with time was also evident during May, 1989, (Fig 6.8), when the samples on the 1st day showed a value of 142ug.dm-2, which decreased to represent a minimum value of

107ug.dm-2 for samples on the 2nd day. From the 3rd day onwards there was an increase in the biomass (111ug.dm-2) till the 6th day (198ug.dm-2). Linear increase was observed for August, 1989, (49 to 91ug.dm-2), September, 1989 (83 to 109ug.dm-2), December, 1989 (42 to 108ug.dm-2), January, 1990 (54 to 136ug.dm-2), April, 1990 (67 to 146ug.dm-2) and May , 1990 (55 to 131ug.dm-2).

Total carbohydrate (F-CHO) (Fig 6.9)

Carbohydrate, which is one of the important abundant constituents of the microfouling material, was also studied for stainless steel test surfaces. As illustrated in Fig 6.9, an increase in the concentration was observed with time. The minimum and maximum values recorded were 52.7ug.dm-2 and 1636.6ug.dm-2 for the study period.

3.2 SEASONAL VARIATION

3.2a ALUMINIUM

Organic Carbon (F-OC) (Fig 6.10)

Microfouling biomass as organic carbon for aluminium surface for the three seasons showed values differing

significantly for the pre-monsoon, 1989 (23.16mg.dm-2), monsoon, 1989 (5.58mg.dm-2), post-monsoon, (5.57mg.dm-2) and pre-monsoon season of 1990, (7.33mg.dm-2).

Chlorophyll a (Chl a) (Fig 6.10)

Biomass as chlorophyll a was very much similar to organic carbon and organic matter as shown in the same figure. Samples for the pre-monsoon seasons of 1989 & 1990, showed maximum concentration for chl a (0.232 & 0.136mg.dm-2). Minimum value of 0.090mg.dm-2 was observed for samples during the monsoon season of 1989. This value was almost three times lower than that observed during pre-monsoon, 1989. Although intermediate values were observed for the post-monsoon season (0.095 mg.dm-2), they were more closely related to the values of the monsoon season.

Carbohydrates (CHO) (Fig 6.10)

The same figure also showed the variation of total carbohydrates in the microfouling material observed for the three seasons of the study period. This parameter showed an extremely high value of 2.379mg.dm,-2 during the pre-monsoon season of 1989. This was closely followed by the

samples for the same season of 1990 (1.370mg.dm-2). The value dropped to almost four times during the monsoon season (0.399mg.dm-2) and to a little less than three times during the post-monsoon season (0.812mg.dm-2). Carbohydrate concentration showed highest value for samples during the pre-monsoon season for both the years of 1989 & 1990.

3.2b **FIBREGLASS**

Organic carbon (F-OC) (Fig 6.11)

Organic carbon concentration for fibreglass did not really show significant variations. The minimum and maximum for the study period was 5.18 and 18.31mg.dm-2, respectively.

Chlorophyll-a (chl a) (Fig 6.11)

Chl a concentration ranged between 0.117 and 0.238mg.dm-2 for the study period. As seen in Fig 6.11, there was very little difference between the concentration for samples during the monsoon (0.117mg.dm-2) and post-monsoon (0.130mg.dm-2) seasons.

Total carbohydrate (F-CHO) (Fig 6.11)

Results obtained for total carbohydrates of the microfouling material developed on fibreglass showed maximum value during the pre-monsoon season (2.599mg.dm-2). The value of F-CHO during the monsoon season was minimum (0.546mg.dm-2) and was more than four times lower than that observed for the pre-monsoon season. During the post-monsoon season the value (0.907mg.dm-2) for samples was higher than that of the monsoon season, but more than two times lower than the value for pre-monsoon season of 1989. The same season of 1990, showed a value of 1.305mg.dm-2, which was comparatively lower than that of 1989.

3.2c **STAINLESS STEEL**

Organic carbon (F-OC) (Fig 6.12)

As seen in Fig 6.12, the variation in F-OC was maximum for the samples in the pre-monsoon season of 1989 (16.74mg.dm-2), minimum for the monsoon season (4.37mg.dm-2) and intermediate for the post-monsoon season (4.05mg.dm-2) samples. A low value during the pre-monsoon season, 1990, as compared to that of 1989, was once again evident for this parameter.

Chlorophyll^{a} (Chl^{a}) (Fig 6.12)

Seasonal variation in Chl$_{a}$ concentration for stainless steel is shown in Fig 6.12. It was clearly evident that both the pre-monsoon sampling of 1989 as well as 1990, showed high values of 0.190 and 0.109mg.dm-2, respectively. Concentration for samples during the monsoon (0.082mg.dm-2) and post-monsoon (0.089mg.dm-2) seasons, did not vary significantly.

Total carbohydrate (F-CHO) (Fig 6.12)

Microfouling material when analysed for total F-CHO concentration, showed results as presented in the above figure. F-CHO did not show any significant variation, for samples, with the maximum value of 1.408mg.dm-2 obtained during the pre-monsoon season of 1989, while the same season of 1990 showed a value of 0.995mg.dm-2. Minimum value of 0.287mg.dm-2, was observed for the monsoon season and intermediate value of 0.397mg.dm-2 occurred during the post-monsoon season.

3.3 **Algal contribution to total carbon**

Algal contribution to the total microfouling carbon

was obtained from the Chl a values. Values on aluminium are presented in Table 6.1a. On an average, for aluminium surface, algae contributed to more than 63% of the total carbon. Fibreglass surface showed algal contribution to be 79% (Table 6.1b) and for stainless steel it was 62% (Table 6.1c).

Bacterial contribution to the total carbon accounted for <1%, for all the surfaces. Thus, living carbon present on surfaces was obtained as a sum of algal and bacterial carbon. Living carbon contribution to the total carbon was found to vary from 45 to 94% on aluminium (Table 6.1a), from 44 to 98% on fibreglass (Table 6.1b), and from 46 to 98% on stainless steel (Table 6.1c).

The other major source could be contribution by detrital or non-living material. Detrital/non-living carbon was obtained from the difference in the total and living carbon. The non-living carbon contribution to the total carbon was between 1 and 64%. On an average, on aluminium surface, non-living carbon contribution was around 38%, followed by contribution on stainless steel (27%) surface. On fibreglass surface, contribution by non-living carbon to total carbon was approximately 20%. As seen in Fig 6.13, the contribution by non-living carbon was less when compared with living carbon for the three

surfaces, during the monsoon and post-monsoon seasons. However, in the case of the pre-monsoon season there was not much of a difference between the nonliving and living carbon contribution, for all the surfaces.

The amount of carbohydrate carbon present for every 100mg of total carbon on various surfaces, was determined. An average value for the different months is presented in (Table 6.2). This gave a general idea about the probable source of carbon in the microfouling material. For example value below 20 indicated biogenic origin of the microfouling material which was also the case for the present study.

3.4 Studies on the various ratios of the microfouling material

Various ratios like F-CHO/F-OC (wt/wt), F-OC/Chl$\underline{a}$ (wt/wt) and F-CHO/Chl$\underline{a}$ (wt/wt) for daily sampling were plotted with time as shown in Fig 6.14.

Ratio of F-CHO/F-OC (wt/wt) on aluminium and fibreglass decreased with time for the pre-monsoon season. On the other hand, stainless steel surfaces for the same season showed a somewhat irregular pattern. During the

monsoon season, an increase in ratios was evident with time. This pattern was more pronounced on fibreglass and to some extent on stainless steel, as compared to aluminium. A distinct increase, for all the surfaces, was seen for the post-monsoon season. More than 90% of the ratio occurred between 0.3 to 0.8% for all surfaces, for the three seasons. Low ratio was observed to exist during the monsoon season as compared to the post-monsoon and pre-monsoon seasons.

Ratios of F-OC/Chl$_a$ (wt/wt), when plotted with time, showed a rise with increase in the immersion time, for the pre-monsoon season. However, for the monsoon season, aluminium, fibreglass and stainless steel did not show any significant changes. Just like the monsoon season, the post-monsoon season also showed the ratio to remain somewhat stationary, with increase in the duration of study period. These values were found to lie between 40 and 80. This value was more or less similar for the monsoon and post-monsoon seasons, whereas, for the pre-monsoon season they were comparatively higher.

F-CHO/Chl$_a$ ratios when plotted against time, showed an increase which was remarkably distinct for the pre-monsoon season, as compared to the monsoon and post-monsoon seasons. The F-CHO values showed a wide fluctuation,

ranging from 10 to 60 as shown in Fig 6.14. Ratios were minimum for the monsoons, followed by an increase for the post-monsoons, and a still further increase for the pre-monsoon season.

3.5 Interrelation between various biomass parameters & carbohydrates

Simple linear correlation studies between biomass parameters and F-CHO is shown in Fig 6.15. It was observed that carbohydrate was well correlated with both organic carbon (F-OC) and chlorophyll *a* (Chl *a*). Similarly F-OC was also significantly correlated with F-Chl *a*, to give a level of significance greater than 98% for all the surfaces.

Cell density (diatom & bacteria) verses biomass

Diatom and bacterial abundances from surfaces also showed significant positive correlation with both carbohydrates as well as F-OC & F-Chl *a* (Table 6.3).

Hydrographic parameters of sub-surface waters

The details of the hydrographic parameters are described in chapter II. Using these data, statistical

correlation analysis of the microfouling biomass with the hydrographic parameters was done, as shown in Table 6.3, for the daily sampling. Significant correlations were observed with temperature and salinity for all the surfaces. On the other hand, microfouling biomass was inversely related with dissolved oxygen. Poor correlation was observed with nutrients of the subsurface waters.

3.5 WEEKLY VARIATIONS

For weekly sampling, microfouling biomass was estimated as organic carbon (F-OC), organic nitrogen (F-ON) and chlorophyll$_a$ (F-Chl$_a$). All these parameters showed an increase in values with increase in immersion time.

3.5a ALUMINIUM

Organic carbon (F-OC) (Fig 6.16)

Biomass, as organic carbon, showed that during April-May, 1990, a value of 12.30mg.dm-2 was observed. The concentration then increased two times for samples on week II (24.50mg.dm-2) and almost three times for samples on week IV (32.65mg.dm-2). During August-September, 1990,

samples on weeks I and II did not show any significant differences in the biomass values (9.64 & 9.95mg.dm-2). Marginal increase was observed for week III (10.81mg.dm-2) and week IV (11.11mg.dm-2). During December-January, 1991, the variation was more evident than during August-September, 1990. Week I showed a value of 10.71mg.dm-2 which increased till week IV (19.28mg.dm-2)

Organic nitrogen (F-ON) (Fig 6.17)

Fluctuations in biomass as organic nitrogen, are presented in Fig 6.17. During April-May, 1990, there was a progressive increase from samples on week I (4.09mg.dm-2) until week IV (6.21mg.dm-2). Even during August-September, 1990, as observed in the same figure, although the margin was low increase was evident from week I (0.78mg.dm-2), until the last sampling week (1.26mg.dm-2). Similar was the case with December-January, 1991, showing values between 2.65 and 3.81mg.dm-2, for the four sampling days.

Chlorophyll *a* (Chl *a*) (Fig 6.18)

Weekly sampling for biomass as chl *a* is shown in Fig 6.18. Results obtained during April-May, 1990, showed significant increase from 0.291mg.dm-2, for samples on week

I to 0.721mg.dm-2 in week IV. During August-September, 1990, the values were initially low (0.130mg.dm-2), and increased steadily till the end of study period (0.246mg.dm-2). Quite similar was the case in December-January, 1991, (0.162 to 0.378mg.dm-2)

3.5b **FIBREGLASS**

Organic carbon (F-OC) (Fig 6.19)

Biomass as organic carbon, for fibreglass during April-May, 1990, showed values between 11.69 & 23.87mg.dm-2, during August-September, 1990, between 8.34 & 11mg.dm-2 and during December-January, 1991, between 8.93 & 16.96mg.dm-2 respectively.

Organic nitrogen (F-ON) (Fig 6.20)

Biomass as fouling organic nitrogen during April-May, 1990, was 3.45mg.dm-2 for samples on week I. There was marginal increase during week II (3.69mg.dm-2), week III (4.22mg.dm-2) and week IV (4.87mg.dm-2) samples. Even during August-September, 1990, the variation was only marginal, ranging from 0.70 to 1.08mg.dm-2 for the microfouling samples of the four weeks. Minimum and

maximum values observed for organic nitrogen during December-January, 1991, were 1.82 & 2.62mg.dm-2 for weeks I and IV respectively. Week II and III showed values of 2.05 and 2.49mg.dm-2 respectively.

Chlorophyll a (Chl a) (Fig 6.21)

During April-May, 1990, biomass as chl a showed a value of 0.293mg.dm-2 for samples during week I, which increased for week II (0.319mg.dm-2), week III (0.436mg.dm-2) and finally for week IV (0.527mg.dm-2), as evident from the Fig 6.21. During August-September 1990 also, there was an increasing trend for samples from week I (0.104mg.dm-2), till week IV (0.240mg.dm-2). This was also the case with December-January, 1991 (0.139 to 0.366mg.dm-2).

3.5c **STAINLESS STEEL**

Organic carbon (F-OC) (Fig 6.22)

Just as in the case of aluminium and fibreglass surfaces, biomass as organic carbon for stainless steel also showed a distinct increase in the values (Fig 6.22). A value of 7.06mg.dm-2 was observed for samples on week I which increased to 11.77, 16.20 & 19.98mg.dm-2 for samples

on weeks II, III & IV respectively. During August-September, 1990, a minimum of 6.44mg.dm-2 for week I and a maximum of 10.28mg.dm-2 for samples on week IV were evident. During December-January, 1991, the values for samples ranged from 7.59 to 14.24mg.dm-2 for the period of study.

Organic nitrogen (F-ON) (Fig 6.23)

Biomass on stainless steel was low for samples on week I for all the months. To begin with, during April-May, 1990, the minimum and maximum values were 1.81 & 3.28mg.dm-2. During August-September, 1990 and December-January, 1991, the values were 0.36 & 1.25mg.dm-2 and 1.66 & 2.19mg.dm-2 respectively.

Chlorophyll $\underline{a}$ (Chl $\underline{a}$) (Fig 6.24)

As observed in Fig 6.24 biomass values during April-May, 1990, fluctuated between 0.127 & 0.432mg.dm-2. During August-September, 1990 and December-January, 1991, the fluctuations were between 0.09 & 0.223mg.dm-2 and 0.106 & 0.335mg.dm-2, respectively.

3.6 SEASONAL VARIATION

Seasonal variations were obtained for the three surfaces. There was a somewhat similar trend for the study period.

3.6a ALUMINIUM

Organic carbon (F-OC) (Fig 6.25)

For this surface, the biomass as organic carbon as seen in Fig 6.25, showed a maximum concentration during the pre-monsoon season (23.94mg.dm-2), followed by the post-monsoon season (15.53mg.dm-2). This inturn was closely followed by the monsoon season (10.38mg.dm-2). Concentration during the monsoon season was two times lower than the concentration observed for the pre-monsoon season.

Organic nitrogen (F-ON) (Fig 6.25)

As presented in the same figure, this biomass also showed a similar trend. It was maximum during the pre-monsoon season (5.30mg.dm-2). This was followed by the post-monsoon season (3.22mg.dm-2), which was almost half of that of the former season. Low biomass value was evident

during the monsoon season (1.05mg.dm-2), which was almost five times lower than the value observed for samples during the pre-monsoon season and three times lower than the value for the post-monsoon season.

Chlorophyll_a (Chl_a) (Fig 6.25)

Biomass as chl_a was also identical in the seasonal changes like the above two parameters. The pre-monsoon season showed a value of 0.517mg.dm-2, followed by the post-monsoon season (0.270mg.dm-2). Minimum concentration was reported during the monsoon season (0.195mg.dm-2).

3.6b **FIBREGLASS**

Organic carbon (F-OC) (Fig 6.26)

As shown in Fig 6.26, the trend shown by fibreglass was similar to the aluminium test surface. However, the values were much lower during the pre-monsoon (17.35mg.dm-2), post-monsoon (13.45mg.dm-2) and monsoon (9.82mg.dm-2) seasons.

Organic nitrogen (F-ON) (Fig 6.26)

Biomass as organic nitrogen on fibreglass showed a maximum value of 4.06mg.dm-2 (pre-monsoon) and minimum value of 0.89mg.dm-2 (monsoon season), whereas, intermediate value of 2.25mg.dm-2 was observed for samples during the post-monsoon season.

Chlorophyll$\underline{a}$ (Chl$\underline{a}$) (Fig 6.26)

This biomass parameter also exhibited a similar trend during the pre-monsoon (0.393mg.dm-2); monsoon (0.176mg.dm-2) and post-monsoon (0.231mg.dm-2) seasons.

3.6c **STAINLESS STEEL**

This surface also showed biomass as organic carbon, organic nitrogen and chlorophyll$\underline{a}$ values to be maximum during the pre-monsoon season (13.75mg.dm-2, 2.78mg.dm-2 & 0.271mg.dm-2) as evident in Fig 6.27. Minimum values were observed during the monsoon season (8.37mg.dm-2, 0.72mg.dm-2 & 0.147mg.dm-2 respectively). Intermediate values were evident during the post-monsoon seasons (11.00mg.dm-2, 2.03mg.dm-2 & 0.209mg.dm-2 respectively).

3.7 Studies on various ratios (Fig 6.28)

The various ratios like F-ON/Chl*a* (wt/wt), F-OC/Chl*a* (wt/wt) and F-OC/F-ON (wt/wt) were plotted against time.

F-ON/Chl*a* (wt/wt) ratios for aluminium, fibreglass and stainless steel, in general showed a decrease for all the seasons. The decrease was more evident during the pre and post-monsoon season for all the surfaces. On the other hand, during the monsoon season, the change was insignificant. Such a general decrease in the ratio of F-ON/Chl*a* showed that the contribution of organic nitrogen by algae increased with the increase in exposure time.

F-OC/Chl*a* ratio decreased with time for all the three surfaces. This was most evident during the monsoon and post-monsoon season. The decrease during the pre-monsoon season was not linear, with some values in between increasing as seen in the case of aluminium and stainless steel surfaces. Values on fibreglass surface remained more or less steady with a gentle decreasing curve.

F-OC/F-ON like the other ratios also showed remarkable variations with respect to different surfaces and with different seasons. For the pre-monsoon and post-monsoon seasons, the ratios of F-OC/F-ON for aluminium, fibreglass and stainless steel showed a gradual increase

with time. However, during the monsoon season, all the surfaces showed a decrease with increase in exposure time. The decrease was very significant in the case of aluminium and fibreglass as compared to stainless steel.

3.8 Interrelationship between biomass parameters

Linear regression analysis for the various parameters (F-ON, F-OC, Chl$_a$) for weekly sampling also showed highly significant correlation with each other (Fig 6.29).

Interrelationship between biomass and hydrographic parameters

Correlation studies were carried out between hydrographic parameters (given in chapter II) and microfouling biomass (Table 6.4) Just as in the case of daily sampling, temperature and salinity were found to be significantly correlated with biomass. Whereas, dissolved oxygen showed inverse relations. Nutrients of the water column like nitrates, phosphates and silicates showed poor correlation with microfouling biomass.

4 DISCUSSION

In order to better understand the nature of particulate organic matter settled on surfaces when exposed to the marine environment and chemical changes taking places on surfaces, short term studies were undertaken. In addition to this, the tremendous degree of short term changes occurring in our tropical climate, lays further emphasis on such short term studies. Thus, biochemical composition of the initially formed microfouling material becomes almost a necessity. The studies were, therefore carried out daily and at weekly intervals.

Recently, various parameters such as dry weight, total carbon, total nitrogen, protein, ATP, chlorophyll a and bacterial density, have been employed to quantify the extent of microfouling (Aftring & Taylor, 1979; Mayack et al, 1984; Bhosle et al, 1989; 1990). In the present study, carbon, nitrogen and Chlorophyll content, were used to estimate microfouling biomass because these methods were comparatively simple, sensitive, gave reproducible results and were less time consuming.

Microfouling biomass, irrespective of the method used, exhibited an increasing trend over the period of exposure. Such a linear increase with time has been

reported from many environments. For example Bhosle et al, (1990), while working in the Bombay High area of the Arabian Sea, have reported a linear increase in the microfouling biomass. Studies carried out in Bombay coastal waters (Srivastava et al, 1990) and Lakshadweep waters (Raveendran et al, 1991), also showed similar trends. In contrast, several others have reported a non-linear increase in microfouling biomass with immersion period (Martinez et al, 1984; White & Bensen, 1984; Yanshun et al, 1984; Chamberlain & Garner, 1988). It appears that the increase in the microfouling biomass observed for the present study was due to irreversible adsorption of organisms onto surfaces, their growth and increase in production rates. Occasionally however, low values were recorded during certain sampling days for some of the surfaces. The observed decrease was perhaps, due to grazing by aquatic organisms (Hardings et al, 1987). Alternatively, this could also be due to shearing and/or sloughing of microfouling material because of the physical forces occurring in the water column (Marshall et al, 1971: Marshall et al, 1976; Characklis et al, 1984).

Microfouling biomass as organic carbon ranged between 1.77 and 32.65mg.dm-2, during the study period, irrespective of substrata. The values of carbon were comparable with those reported for surfaces exposed to

Bombay coastal waters by Srivastava et al, (1990). However, these values were relatively higher than those reported by Bhosle et al, (1990), for aluminium panels exposed in the open waters of the Arabian Sea. Thus, from this it is obvious that, for coastal waters, microfouling showed higher biomass as organic carbon as compared to oceanic waters. This was probably due to higher productivity of the coastal waters as compared to oceanic waters.

Nitrogen from the microfouling material showed values between 0.36 and 6.21mg.dm-2 for the study period. These values of nitrogen were comparable with those reported by Sharma & Wagh, (1990), for the panels exposed to estuarine waters.

chlorophyll a has been routinely used to assess the phytoplankton biomass. High Chl a concentration indicates high phytoplankton biomass (Hitchcock, 1977; Ittekkot, 1982). Steele & Baird, (1961) had shown that labeled carbon (C^{14}) uptake at a fixed light intensity was linearly related to the Chl a with no indication of dead cells. Thus, it is natural to interpret chl a values as indicators of living cells. When the values of chlorophyll a was multiplied with a factor of 40 (obtained from the F-OC:Chl a ratios), resulted in the phytoplankton carbon contribution

of the microfouling material (60-71%). Finally from chl*a* distribution and bacterial abundance data, it was possible to establish the living carbon contribution to the total carbon of the microfouling material.

As already mentioned earlier, (chapter IV), bacteria were more abundant than diatoms. Bacterial carbon was obtained by multiplying the total number of cells by 20fg for an average cell size of 0.073um. The total carbon contributed by bacteria were highly insignificant (~0.003). Similar reports of bacterial contribution to the total F-OC being insignificant was reported by Berger & Little, (1980); Srivastava *et al*, (1990). One point worth mentioning for the present study was the possibility of underestimating bacterial numbers due to their nature of agglomeration with particulate material to form clumps. Hence, it was rather difficult to count each and every bacterial cell present on surfaces.

A sum of the above two carbon sources gave an account of the living carbon contribution to the total carbon budget. On various substrata, it was found that, the living forms contributed significantly to the microfouling community.

Nature of the substrata may influence microfouling. Hence, it was not surprising to note the variation in microfouling biomass on these surfaces. Among the various surfaces used, fibreglass, a hydrophobic surface, accumulated higher biomass initially as evidenced from the daily sampling. Similar observations were made by others (Fletcher, 1988; Pedersen, 1990). The reason being the surface alteration as a result of adsorption causing hydrophobic nature of the surfaces to become hydrophilic. Organic molecules which form a slime film, have a tendency to maintain a water layer (Kenis et al, 1974).

On the other hand, aluminium being a hydrophilic surface exhibited enhanced electrochemical activity and it acquire adsorbed materials. Aluminium accumulated higher biomass throughout the period of study, irrespective of the season. This was clearly evident from the weekly sampling wherein aluminium superseded all other surfaces with respect to biomass development. Similar observations were also made by Bhosle et al, 1989; Srivastava et al, 1990; Raveendran et al, 1991).

Stainless steel, which is an alloy, supported poor microfouling biomass, which was considerably lower than that on aluminium and fibreglass surfaces. This could be ascribed to its more electropolished surface, which is

known to be more resistant to microfouling than materials with higher surface roughness (Dunsmore et al, 1981; Zoltai et al, 1981).

The highly significant correlation between microfouling biomass such as F-OC, F-ON & F-Chl*a* indicates a common origin of these compounds on the various surfaces. Such significant correlation also suggests that these methods are good parameters for biomass estimation. In addition, significant relationships between F-Chl*a* & F-OC as well as F-Chl*a* & F-ON indicate that phytoplankton contributed a fairly constant percentage to the total microfouling carbon and nitrogen. All these parameters were well correlated with both diatom as well as bacterial abundance (Table 6.3). Thus, these organisms may be strongly associated with the microfouling material.

When the percentage of CHO present in 100mg of F-CHO was studied, it was observed that values below 20 were obtained (Table 6.2). This was another clear indication to prove the biogenic source of origin of microfouling material.

Based on the interrelation study we have valuable information regarding the source of carbon in the microfouling material. In order to better understand their

contribution, we studied the various ratios.

The C:N ratio was used as it serves as a biomarker to detect the source of nitrogen in the microfouling material, present during the time of sampling. It is also used as a good indicator of the food value on surfaces. In the present study, the C:N (wt/wt) ratios is relatively low (3 to 4.5) and comparable with that for phytoplankton (Moal et al, 1977; Checkley, 1980; Abdel-Moati, 1990). In general, the C:N ratios of naturally occurring particles, including most of the living and non living materials available in the sea, vary from 2 to 35 (Man, 1972; Eppley et al, 1977; Le Masson et al, 1977).

C:N ratio was found to decrease with time (Fig 6.28), suggesting increasing abundance of living cells on their surfaces. Alternately higher amount of nitrogen was fixed by these organisms as compared to carbon. There was an increase in the C:N ratios for fibreglass during the post-monsoon season and stainless steel during the pre-monsoon season. This probably indicates the more rapid degradation and/or removal of nitrogen compounds like proteins, than the carbon containing compounds for these periods or rather it reflects the beginning of nitrogen limitation.

F-OC:Chl*a* (wt/wt) ratio was used to calculate the factor for determining the phytoplankton carbon from the F-

Chl· values. The values obtained for our study were between 40 to 88%. They were comparatively higher than the ratio normally used for phytoplankton i.e. 35. This increase in F-OC:Chl*a* indicates efficient photosynthetic activity by healthy living cells inhabiting this layer (Subramanian & Venugopalan, 1980). A plot of F-OC:Chl*a* with time, indicated a more or less regular phytoplankton contribution except for an increase during the pre-monsoon season.

Carbohydrates are the main constituents of the microfouling material developed on solid substrata (Bhosle *et al*, 1989; 1990). These compounds are produced by microorganisms during as well as after their adhesion to solid surfaces (Fletcher & Floodgate, 1973). They also form an important constituent of the terrestrial matter that settle on substrata. Hence, in view of their importance and abundance in biofouling, their concentration in the microfouling material was estimated for the entire study period. Carbohydrate was found to vary between 0.052 & 1.766mg.dm-2. These values were higher than those reported by Raveendran *et al*, (1991), for test panels exposed in Agatti waters of the Lakshwadeep Island and for those observed for coastal waters of Bombay (Srivastava *et al*, 1990). However, a similar comparison cannot be made

with the carbohydrate data reported by Bhosle et al, (1990), due to the differences in the analytical methods used. Carbohydrates showed highly significant correlation with the abundance of diatom as well as bacteria for all the surfaces used. Thus, the most likely source of carbohydrates developed on substrata could be from living organisms (diatom, fungi, protozoans and larvae of macrofoulers). However, microscopic observations did not reveal the presence of protozoans, fungi and larvae of macrofoulers. Hence, carbohydrates of the microfouling material could have been derived from diatoms, bacteria and to a certain extent from terrestrial material.

Similarly, Chl$_a$ was also well correlated with F-CHO indicating that on surfaces with high living algal population there is proportionately high concentration of carbohydrate. This suggests that living algal biomass was associated with the production of carbohydrate (Zsolnay, 1973).

F-CHO:F-OC (wt/wt) ratios were used to understand the contribution of carbohydrate carbon to the total carbon of the microfouling material. For the present study, it was found to decrease for all surfaces with time during the pre-monsoon season, indicating less production of these compounds with time. It also suggests nutrient abundance

during this sampling season. However, for the monsoon and post-monsoon seasons, an increase in the F-CHO:F-OC (wt/wt) ratios was observed with time. This trend indicates a high production of carbohydrates, which is essential to maintain adhesion by microorganisms. It also indicates a nutrient deficient condition on surfaces, which enhances carbohydrate production as reported by Ittekkot et al, (1981); Vaccaro et al, (1968) in natural waters.

Microfouling is a very complex process, in which interaction of hydrodynamic and biochemical factors occur. It was suggested by several workers that environmental factors greatly influenced microfouling settlement (Martinez et al, 1984; Yanshun et al, 1984; Characklis & Escher, 1988; Bhosle et al, 1989). It is rather difficult to assess the actual influence of hydrodynamic conditions due to the complexity of the surrounding factors. Simple linear correlation coefficients of fouling biomass with hydrodynamic factors showed that temperature and salinity of the water column actually influenced settlement, irrespective of the type of material. Dissolved oxygen showed an inverse relation. Nutrients showed an unexpected poor correlation with the fouling settlement. This was reported for the first time in the present chapter for such a kind of study. The probable reasons could be due to the dependence of the microfoulers on both the

particulate from the substrata and the ambient waters (Marshall, 1976; Ladd et al, 1979). Till date, it was generally speculated that nutrients of the water column greatly enhanced microfouling settlement, which now could not be a totally accepted factor. From the present studies, however, this could not appear to be the case, since nutrient deficiency could also increase the carbohydrate production on surfaces (Kjelleberg, 1986)

Thus, we may conclude, by summarising that microfouling biomass can be studied using simple methods like carbon, nitrogen & Chl*a*.

Microfouling showed a linear increase with time and varied with respect to surfaces and seasons.

Among surfaces, although non-metals showed higher microfouling biomass initially (24-48h), however, with the lapse of time, metals were observed to show higher biomass values.

Seasonal variations showed minimum biomass build-up for the monsoon season and maximum for the pre-monsoon season. Post-monsoon season formed an intermediate stage when compared to the other two seasons.

Interrelation studies showed all parameters to be well correlated, which explains the common origin of these compounds. Further, correlation with algae and bacteria, showed living source to be the major contributor to the microfouling material. Among the living sources, diatom contribution accounted for 60%.

Ratios were studied to confirm the contributions of living source to the microfouling material.

Biomass parameters were significantly correlated with temperature and salinity and inversely correlated with dissolved oxygen. Nutrients however, were poorly correlated with microfouling biomass.

Table 6.1a

Contribution of algal-carbon (A), viable bacterial-carbon (B)), living carbon (A+B=C), detrital or non-living carbon (D) of the microfouling material developed on aluminium panels immersed in the sub-surface waters of Dona Paula

Time (d)	A (%)	B (%)	C (%)	D (%)
Apr-May'89				
1	74.11	0.00005	74.11	25.89
2	52.19	0.00003	52.19	47.81
3	45.90	0.00003	45.90	54.10
4	45.16	0.00005	45.16	54.84
5	38.88	0.00010	38.88	61.12
6	35.68	0.00025	35.68	64.32
Aug-Sept				
1	94.49	0.00005	94.49	05.51
2	77.40	0.00001	77.40	22.60
3	98.98	0.00001	98.98	01.02
4	37.68	0.00003	37.68	62.32
5	46.81	0.00006	46.81	53.19
6	48.02	0.00009	48.02	51.98
Dec-Jan				
1	68.51	0.00007	68.51	31.49
2	62.34	0.00002	62.34	37.66
3	75.13	0.00003	75.13	24.87
4	62.44	0.00003	62.44	37.56
5	74.52	0.00004	74.52	25.48
6	69.18	0.00011	69.18	30.82
Apr-May'90				
1	70.71	0.00028	70.71	29.29
2	76.32	0.00032	76.32	23.68
3	70.92	0.00034	70.92	29.08
4	65.12	0.00420	65.12	34.88
5	61.99	0.00045	61.99	38.01
6	69.53	0.00460	69.53	30.47

Table 6.1b

Contribution of algal-carbon (A), viable bacterial-carbon (B)), living carbon (A+B=C), detrital or non-living carbon (D) of the microfouling material developed on fibreglass panels immersed in the sub-surface waters of Dona Paula

Time (d)	A (%)	B (%)	C (%)	D (%)
Apr-May'89				
1	71.09	0.00014	71.09	28.91
2	55.50	0.00009	55.50	44.50
3	53.53	0.00009	53.53	46.47
4	53.24	0.00010	53.24	46.76
5	44.02	0.00008	44.02	55.98
6	44.16	0.00009	44.16	55.84
Aug-Sept				
1	91.55	0.00007	91.55	08.45
2	83.00	0.00002	83.00	17.00
3	92.77	0.00003	92.77	07.23
4	92.67	0.00003	92.67	07.33
5	90.82	0.00005	90.82	09.18
6	93.15	0.00009	93.15	06.85
Dec-Jan				
1	87.28	0.00007	87.28	12.72
2	98.74	0.00003	98.74	01.26
3	88.49	0.00003	88.49	11.51
4	93.54	0.00003	93.54	06.46
5	90.87	0.00004	90.87	09.13
6	93.66	0.00010	93.66	06.34
Apr-May'90				
1	72.05	0.00011	72.05	27.95
2	81.83	0.00021	81.83	18.17
3	76.92	0.00026	76.92	23.08
4	94.31	0.00028	94.31	05.69
5	82.44	0.00032	82.44	17.56
6	76.84	0.00034	76.84	23.16

Table 6.1c

Contribution of algal-carbon (A), viable bacterial-carbon (B)), living carbon (A+B=C), detrital or non-living carbon (D) of the microfouling material developed on stainless steel panels immersed in the sub-surface waters of Dona Paula

Time (d)	A (%)	B (%)	C (%)	D (%)
Apr-May'89				
1	63.73	0.00002	63.73	36.27
2	50.76	0.00000	50.76	49.24
3	56.34	0.00003	56.34	43.66
4	49.59	0.00003	49.59	50.41
5	48.50	0.00009	48.50	51.50
6	46.87	0.00011	46.87	53.13
Aug-Sept				
1	52.55	0.00004	52.55	47.45
2	74.64	0.00001	74.64	25.36
3	73.52	0.00001	73.52	26.48
4	70.45	0.00003	70.45	29.55
5	76.81	0.00005	76.81	23.19
6	74.15	0.00006	74.15	25.85
Dec-Jan				
1	55.26	0.00006	55.26	44.74
2	85.38	0.00002	85.38	14.62
3	98.82	0.00002	98.82	01.18
4	97.91	0.00004	97.91	02.09
5	93.71	0.00008	93.71	06.29
6	98.75	0.00009	98.75	01.25
Apr-May'90				
1	62.21	0.00028	62.21	37.79
2	46.07	0.00032	46.07	53.93
3	45.45	0.00034	45.45	54.55
4	42.67	0.00420	42.67	57.33
5	42.21	0.00045	42.21	57.79
6	38.24	0.00460	38.24	61.76

Table 6.2

Amount of carbohydrate present for every 100mg of carbon on aluminium (AL), fibreglass (FG) and stainless steel (SS) surfaces expressed in mg.dm-2

Time(M)	AL	FG	SS
April'89	5.37	7.61	7.24
May'89	6.69	6.51	1.11
Aug	3.86	5.42	3.42
Sept	3.39	5.18	3.21
Dec	6.59	7.35	5.28
Jan	7.80	8.53	4.74
April'90	8.00	9.04	12.89
May'90	11.63	10.02	17.33

Table 6.3

Statistical correlation between hydrographic parameters and microfouling biomass developed on aluminium (AL), fibreglass (FG) and stainless steel (SS) test panels for daily sampling (n=48)

		A		B		C	
	s	r	p	r	p	r	p
Temp	AL	0.86	<0.001	0.75	<0.001	0.74	<0.001
	FG	0.90	<0.001	0.64	<0.01	0.57	<0.01
	SS	0.88	<0.001	0.72	<0.001	0.84	<0.001
Sal	AL	0.53	<0.02	0.57	<0.01	0.38	<0.1
	FG	0.59	<0.01	0.55	<0.02	0.40	<0.1
	SS	0.53	<0.02	0.53	<0.02	0.59	<0.01
DO	AL	-0.57	<0.01	-0.51	<0.02	-0.34	NS
	FG	-0.63	<0.01	-0.51	<0.02	-0.20	NS
	SS	-0.57	<0.01	-0.50	<0.02	-0.55	<0.02
No3	AL	0.22	NS	0.43	<0.1	0.10	NS
	FG	0.26	NS	0.48	<0.05	0.09	NS
	SS	0.20	NS	0.36	<0.1	0.34	NS
Po4	AL	0.02	NS	0.17	NS	0.07	NS
	FG	0.06	NS	0.22	NS	0.14	NS
	SS	0.02	NS	0.10	NS	0.14	NS
Sio3	AL	0.17	NS	0.34	NS	0.04	NS
	FG	0.20	NS	0.40	NS	0.06	NS
	SS	0.14	NS	0.10	NS	0.26	NS
Diatom	AL	0.72	<0.001	0.67	<0.01	0.72	<0.001
	FG	0.67	<0.01	0.96	<0.001	0.69	<0.001
	SS	0.53	<0.02	0.74	<0.001	0.60	<0.01
Bacteria	AL	0.73	<0.001	NC		0.55	<0.02
	FG	0.95	<0.001	NC		0.96	<0.001
	SS	0.72	<0.001	NC		0.67	<0.01

s= surfaces, r= correlation, p=level of significance, A= fouling organic carbon, B= fouling chlorophyll$_a$, C= fouling carbohydrate, NS= no significance, NC= not correlated.

Table 6.4

Statistical correlation between hydrographic parameters and microfouling biomass developed on aluminium (AL), fibre-glass (FG) and stainless steel (SS) test panels for the weekly sampling (n=12)

		A		B		C	
	s	r	p	r	p	r	p
Temp	AL	0.84	<0.001	0.83	<0.001	0.76	<0.001
	FG	0.78	<0.001	0.84	<0.001	0.77	<0.001
	SS	0.76	<0.001	0.78	<0.001	0.45	<0.1
Sal	AL	0.46	<0.1	0.43	NS	0.58	<0.05
	FG	0.22	NS	0.26	NS	0.52	<0.1
	SS	0.24	NS	0.28	NS	0.44	NS
DO	AL	-0.59	<0.05	-0.60	<0.02	-0.60	<0.05
	FG	-0.60	<0.05	-0.63	<0.02	-0.65	<0.02
	SS	-0.58	<0.05	-0.67	<0.02	-0.44	NS
No3	AL	0.17	NS	0.20	NS	0.24	NS
	FG	0.03	NS	0.03	NS	0.20	NS
	SS	0.04	NS	0.03	NS	0.16	NS
Po4	AL	0.15	NS	0.13	NS	0.34	NS
	FG	0.10	NS	0.05	NS	0.24	NS
	SS	0.06	NS	0.04	NS	0.33	NS
Sio3	AL	0.28	NS	0.04	NS	0.38	NS
	FG	0.02	NS	0.04	NS	0.31	NS
	SS	0.03	NS	0.06	NS	0.33	NS

s= surfaces, r= correlation, p=level of significance, A= fouling organic carbon, B= fouling nitrogen, C= fouling chlorophyll$_a$, NS= no significance, NC= not correlated.

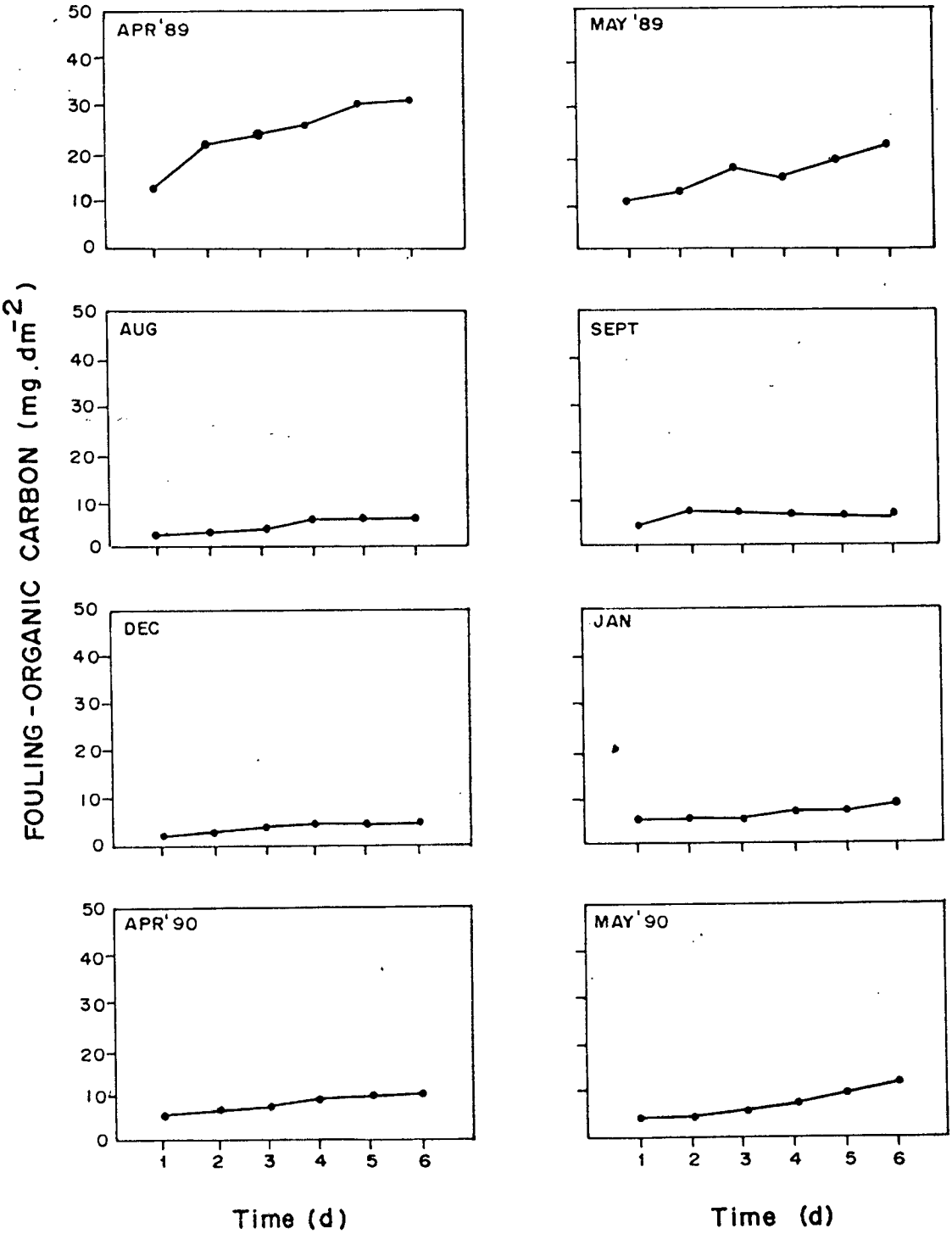

Fig.6.1 DAILY VARIATION IN ORGANIC CARBON DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA

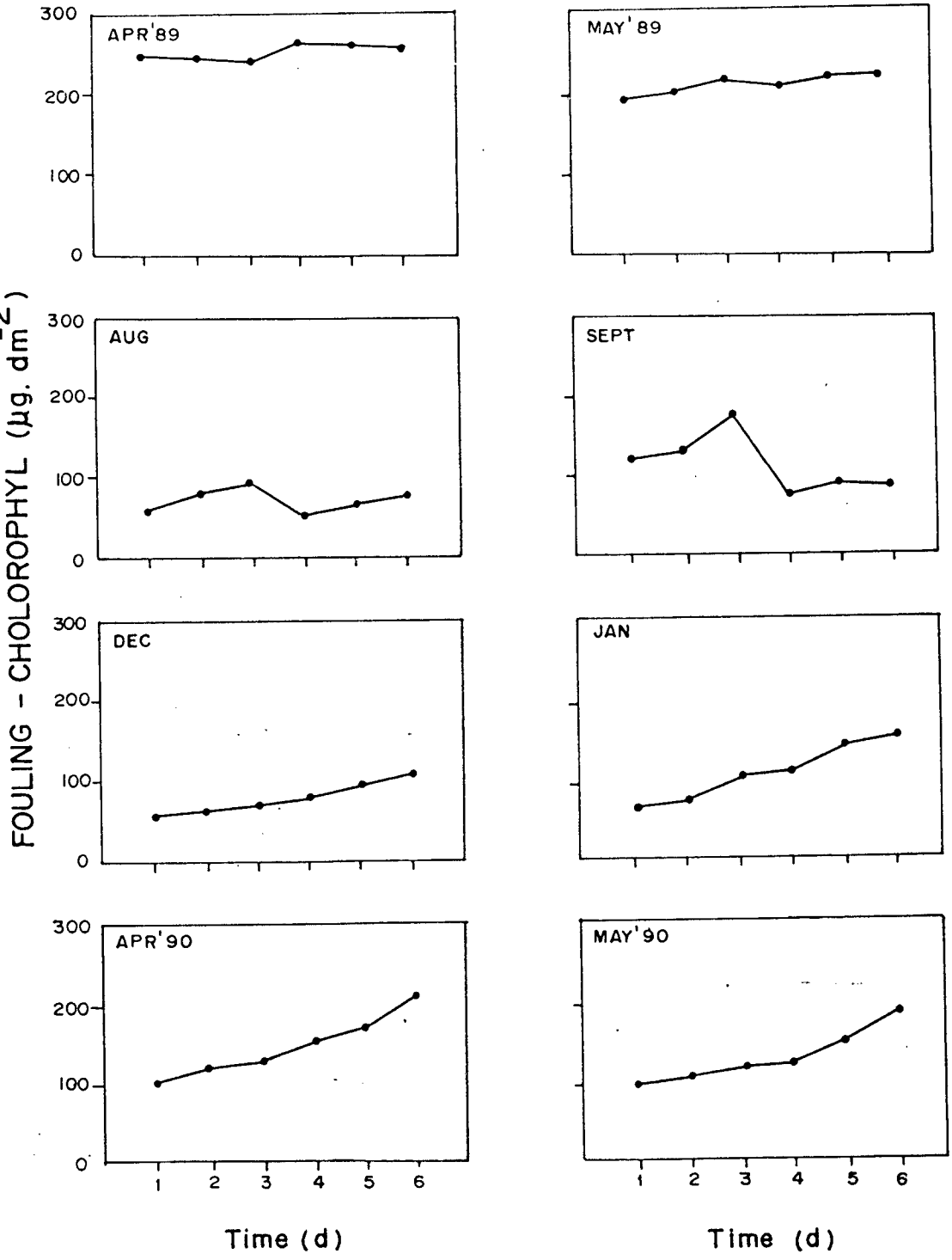

Fig.6.2 DAILY VARIATION IN CHLOROPHYLL DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

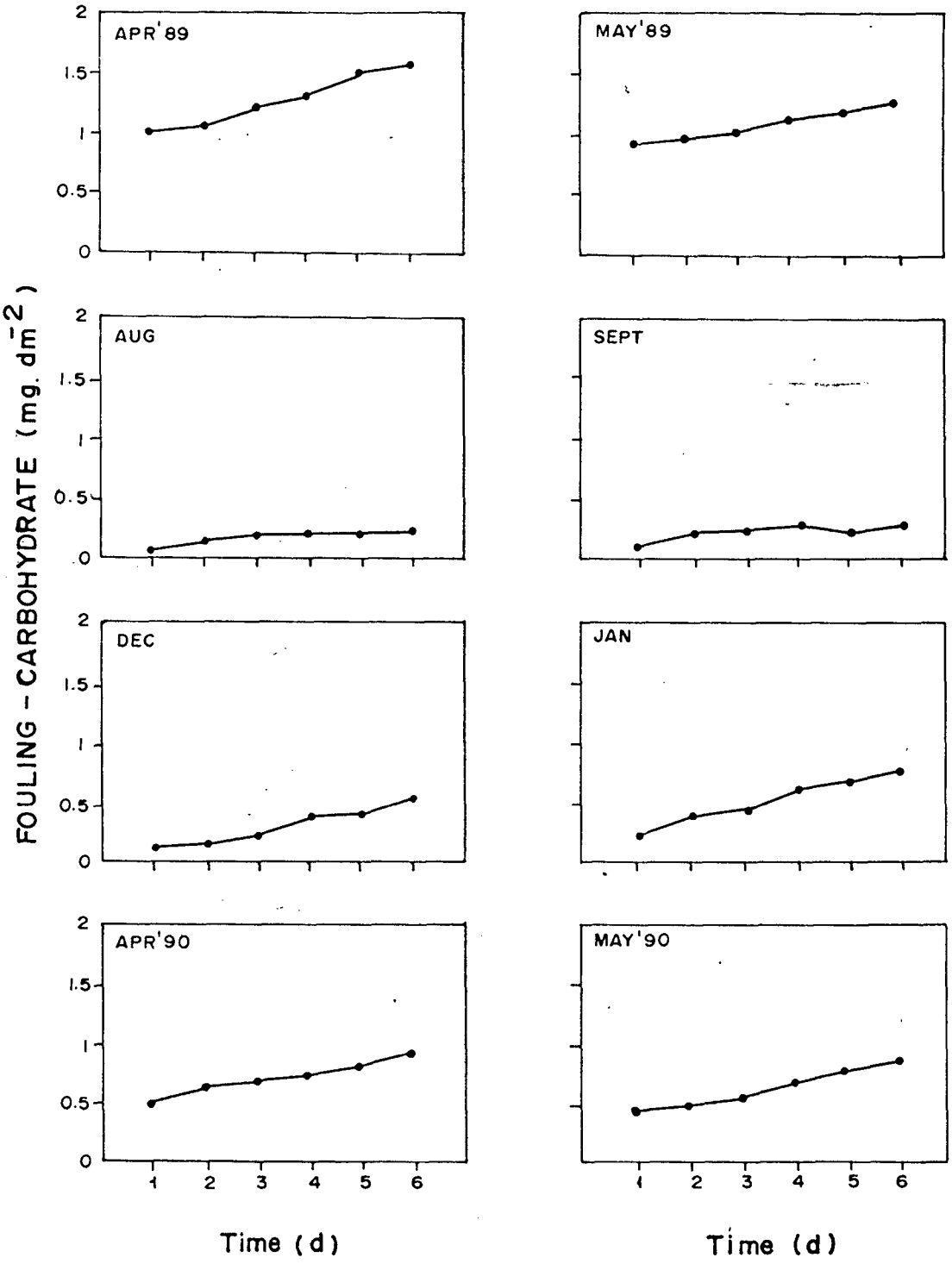

Fig.6.3 DAILY VARIATION IN CARBOHYDRATES DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

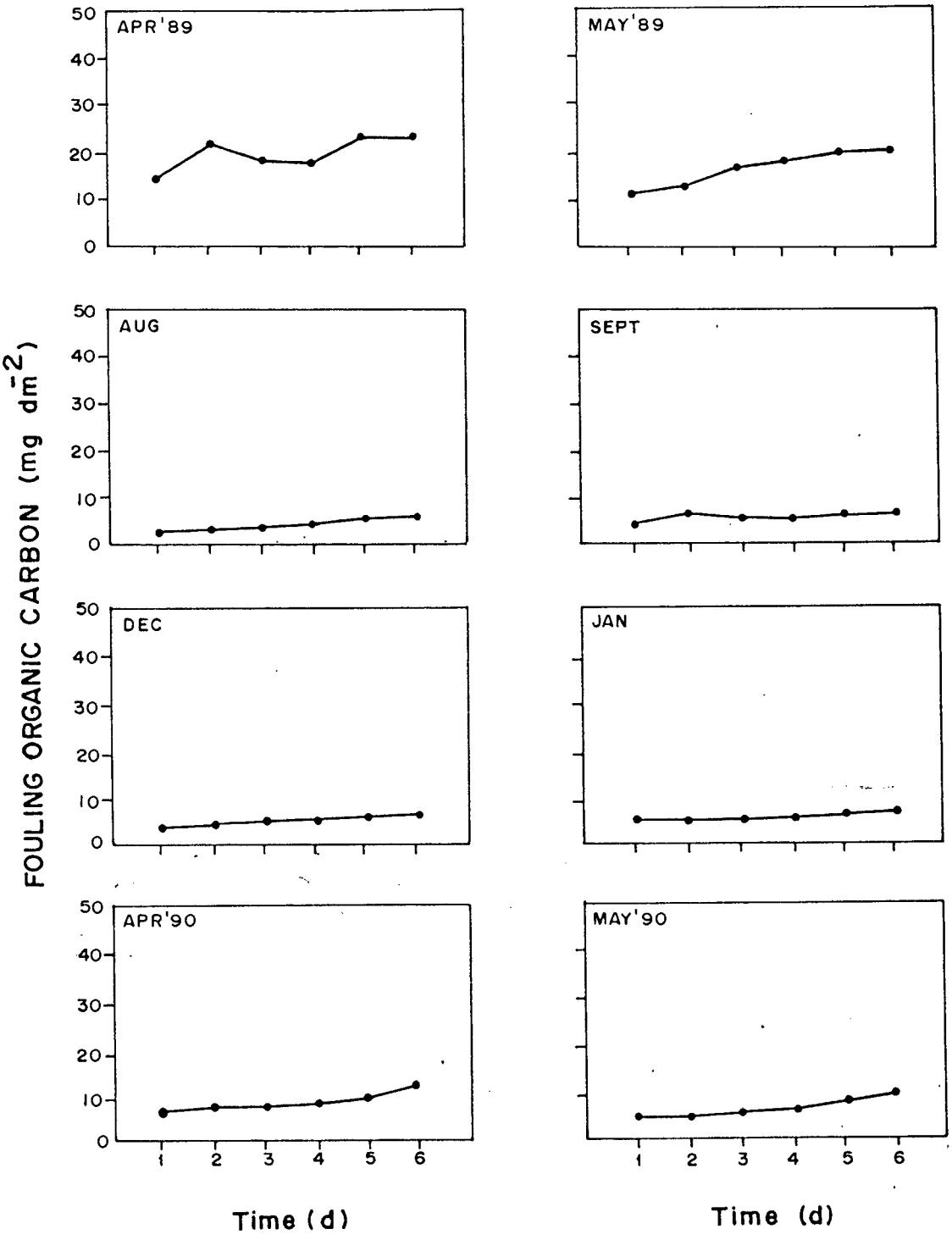

Fig 6.4 DAILY VARIATION IN ORGANIC CARBON DEVELOPED ON FIBREGLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

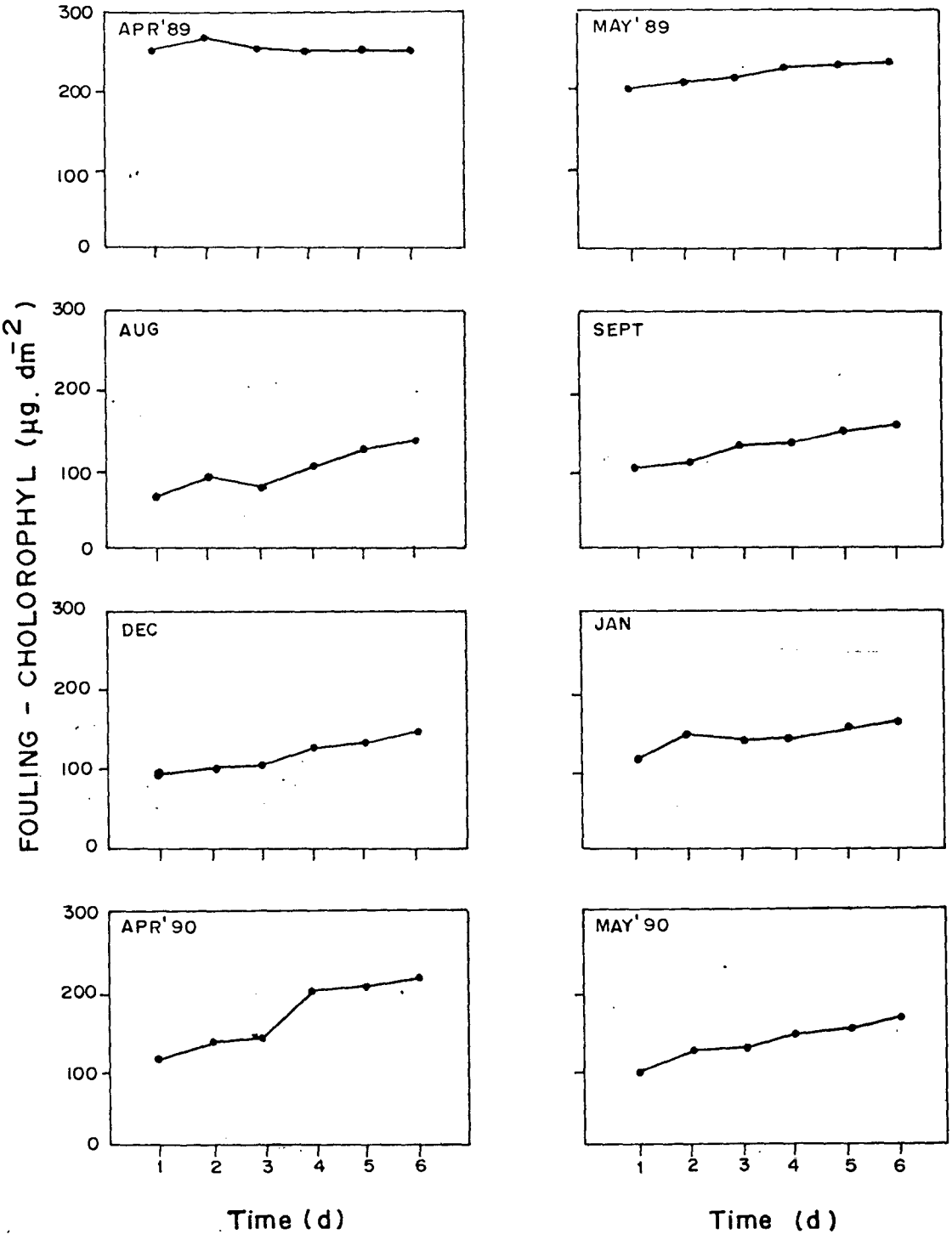

Fig.6.5 DAILY VARIATION IN CHLOROPHYLL DEVELOPED ON FIBREGLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

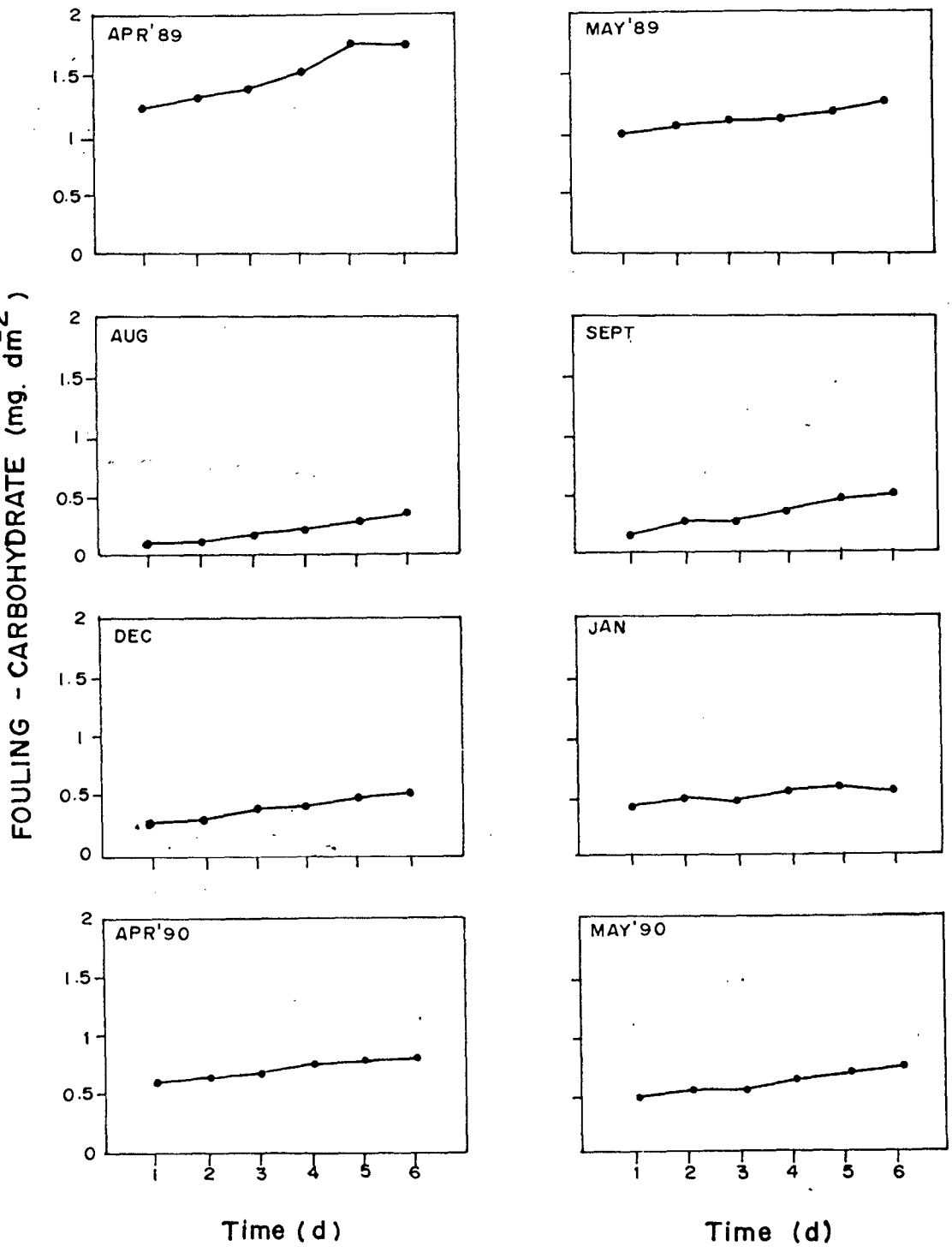

Fig. 6.6 DAILY VARIATION IN CARBOHYDRATES DEVELOPED ON FIBREGLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

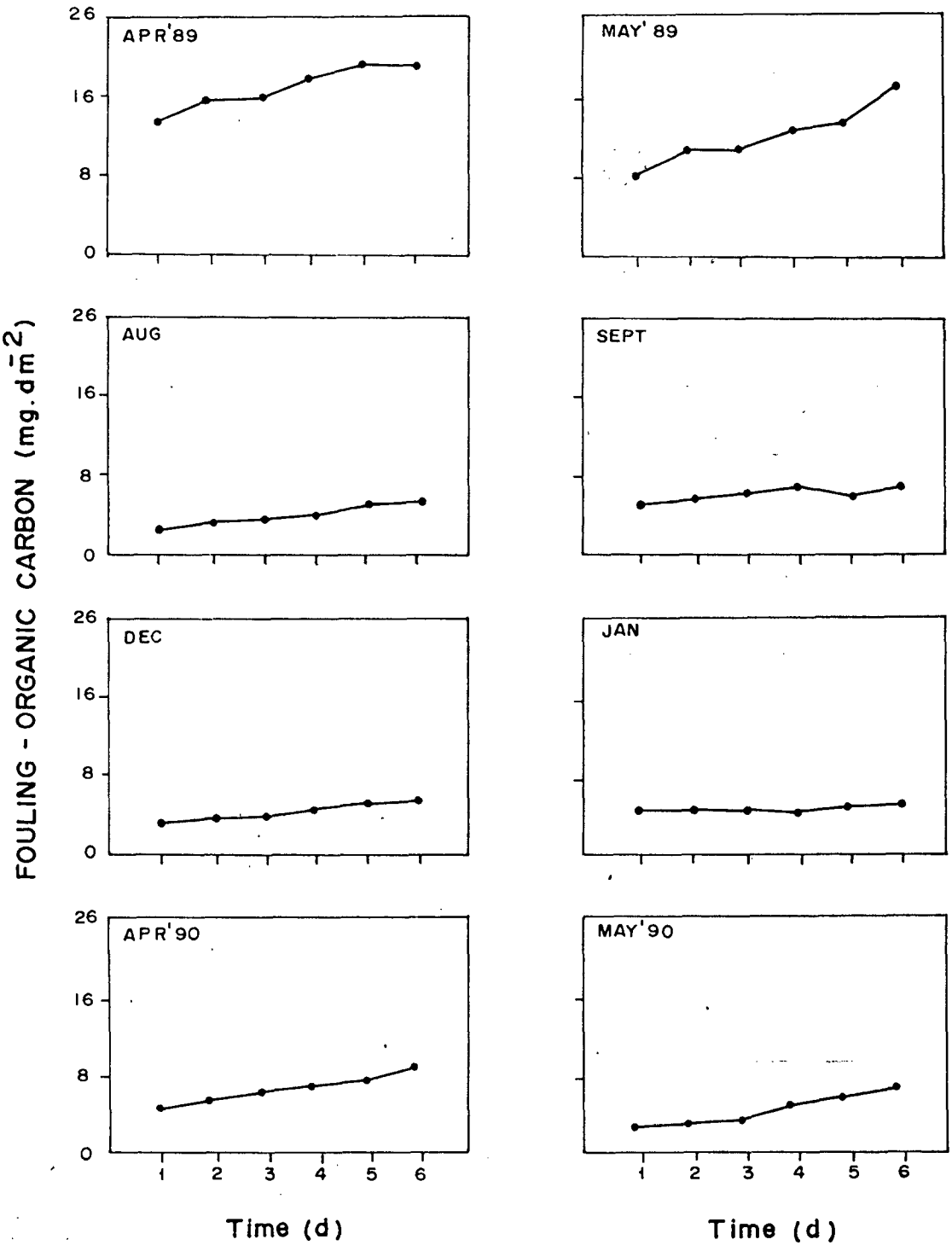

Fig.6.7 DAILY VARIATION IN ORGANIC CARBON DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

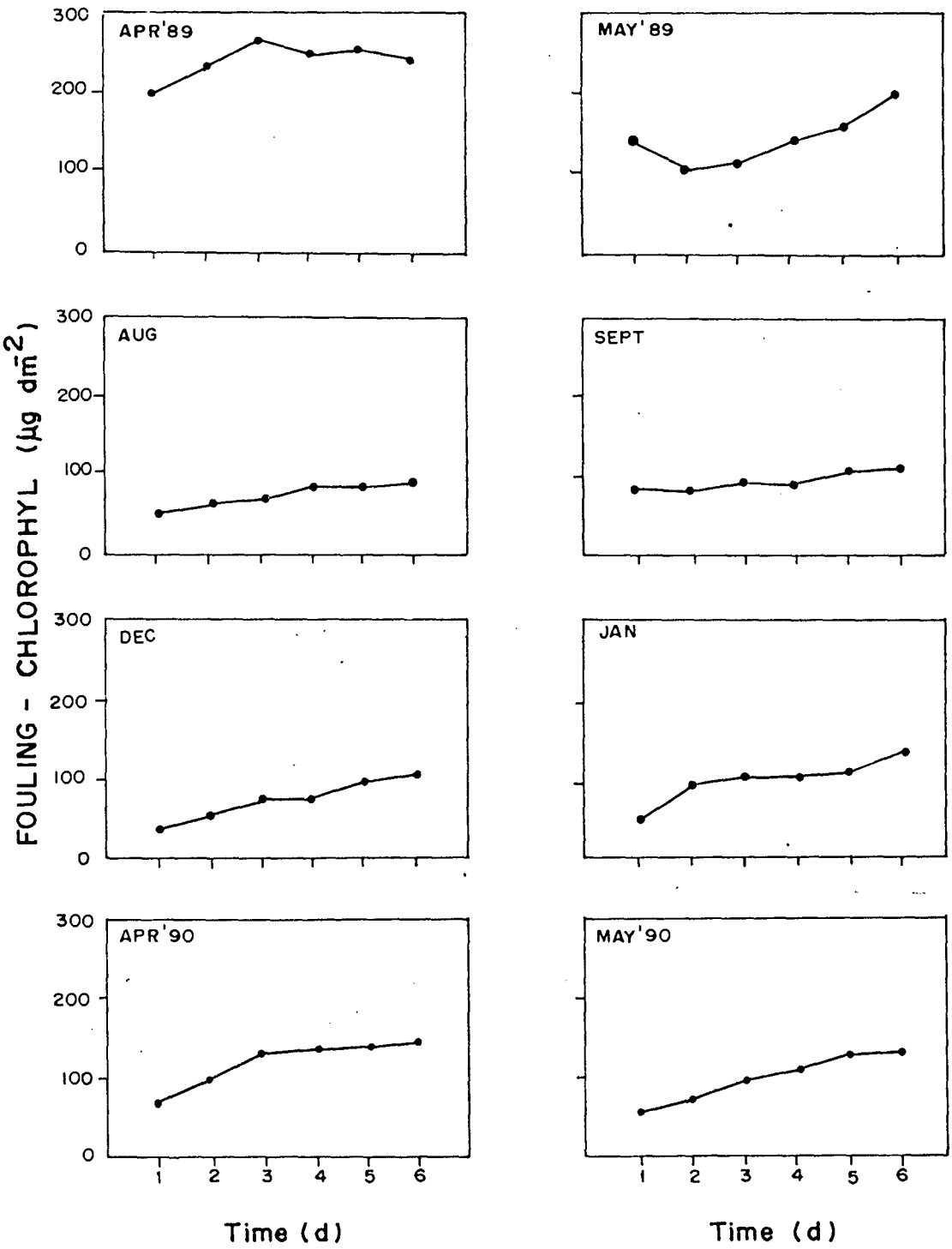

Fig.6.8 DAILY VARIATION IN CHLOROPHYLL DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

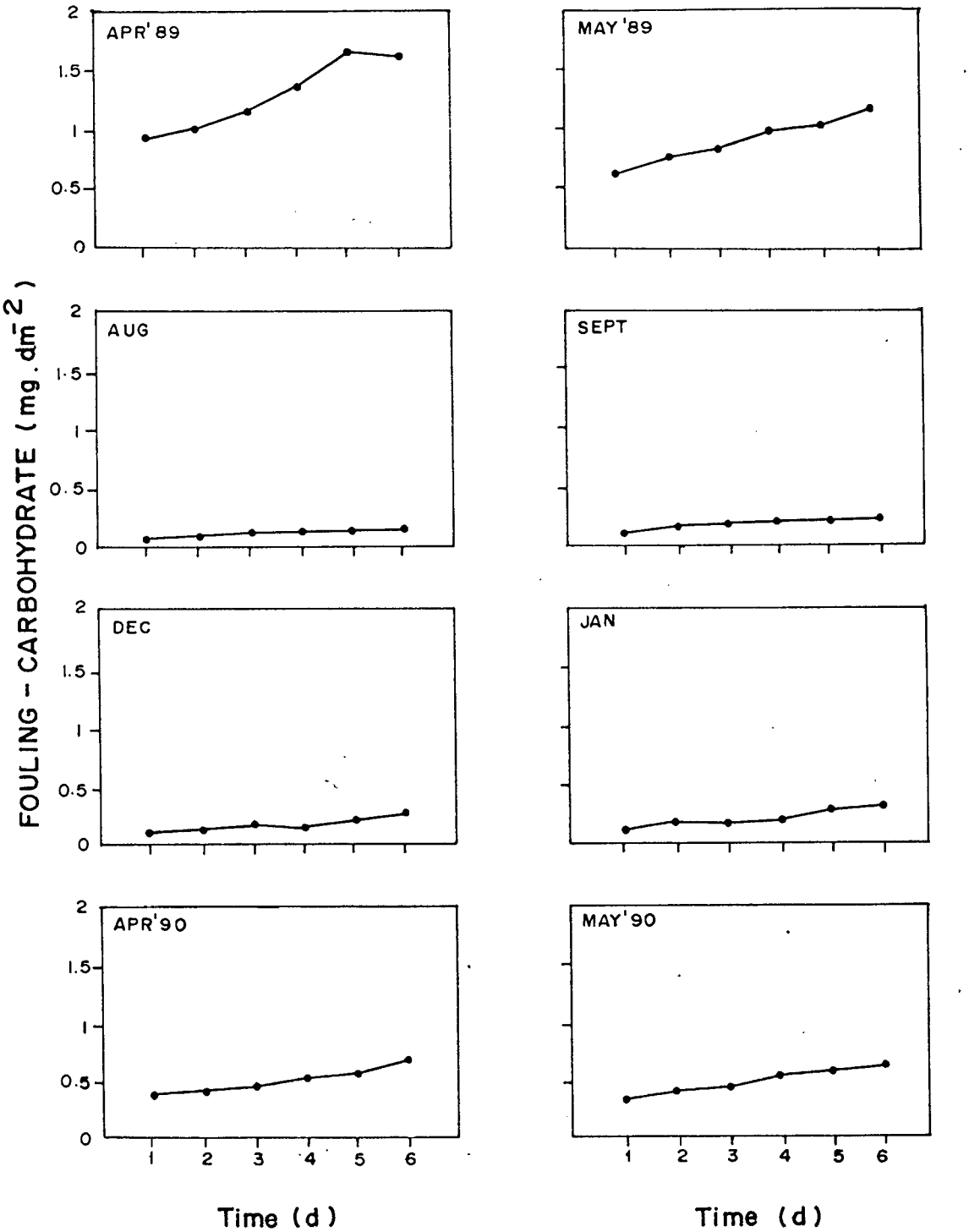

Fig.6.9 DAILY VARIATION IN CARBOHYDRATES DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA

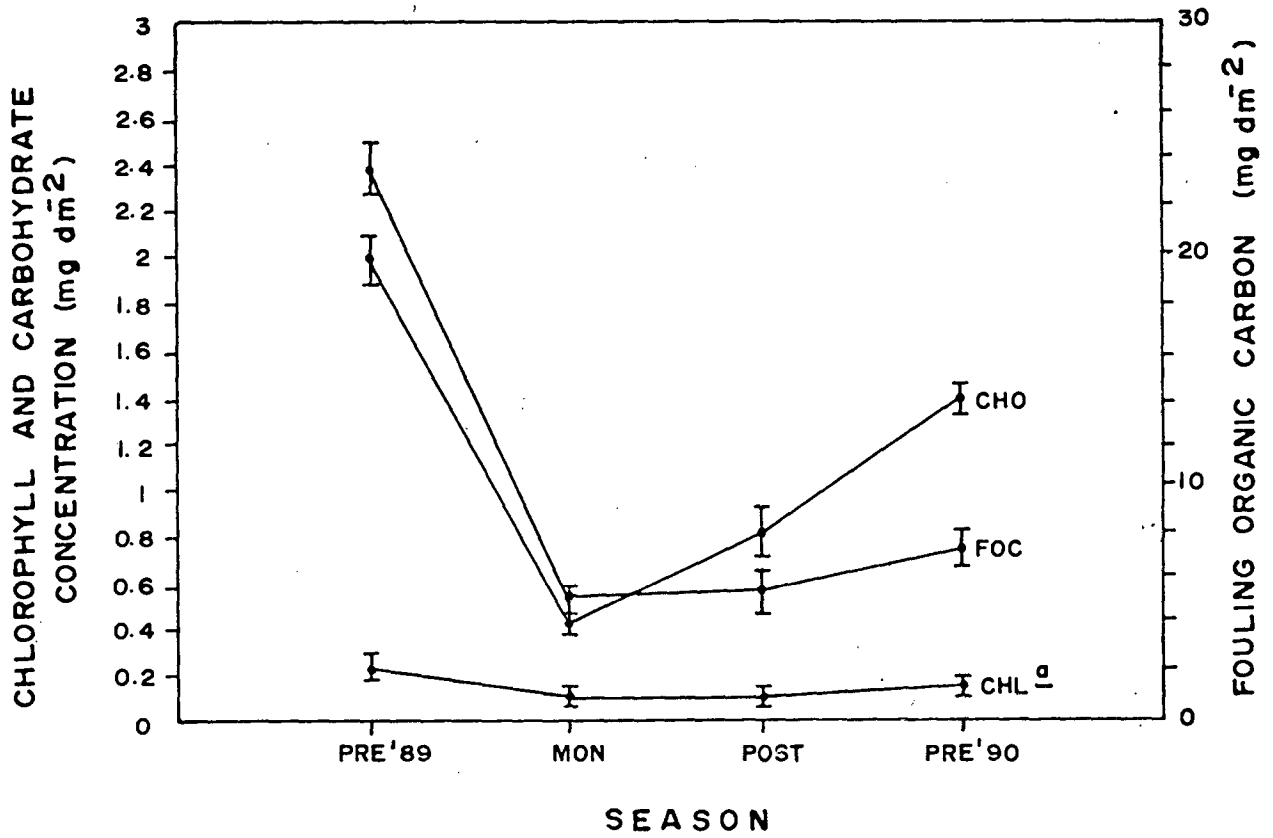

Fig. 6.10 SEASONAL VARIATION IN ORGANIC CARBON, CARBOHYDRATES & CHLOROPHYLL DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

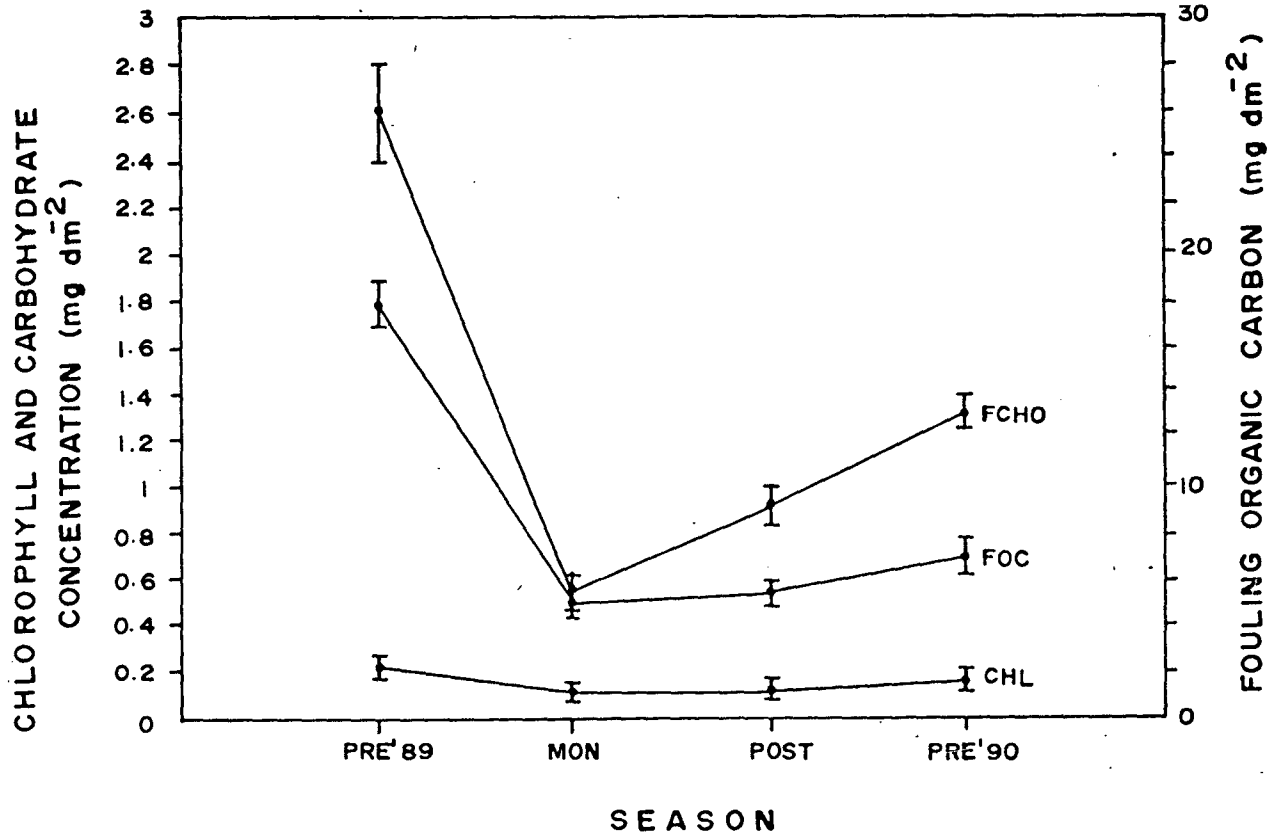

Fig.6.11 SEASONAL VARIATION IN ORGANIC CARBON, CARBOHYDRATES & CHLOROPHYLL DEVELOPED ON FIBREGLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

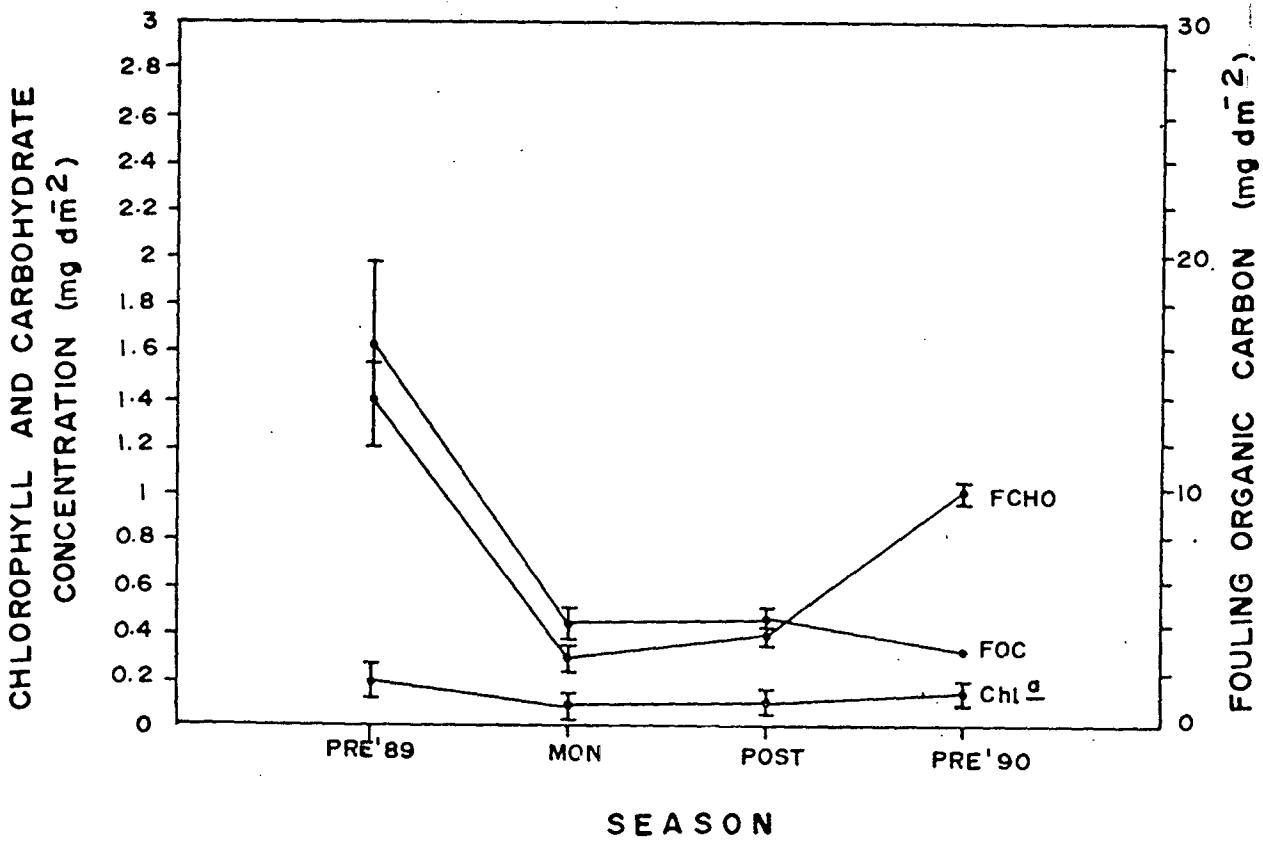

Fig.6.12 SEASONAL VARIATION IN ORGANIC CARBON, CARBOHYDRATES & CHLOROPHYLL DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

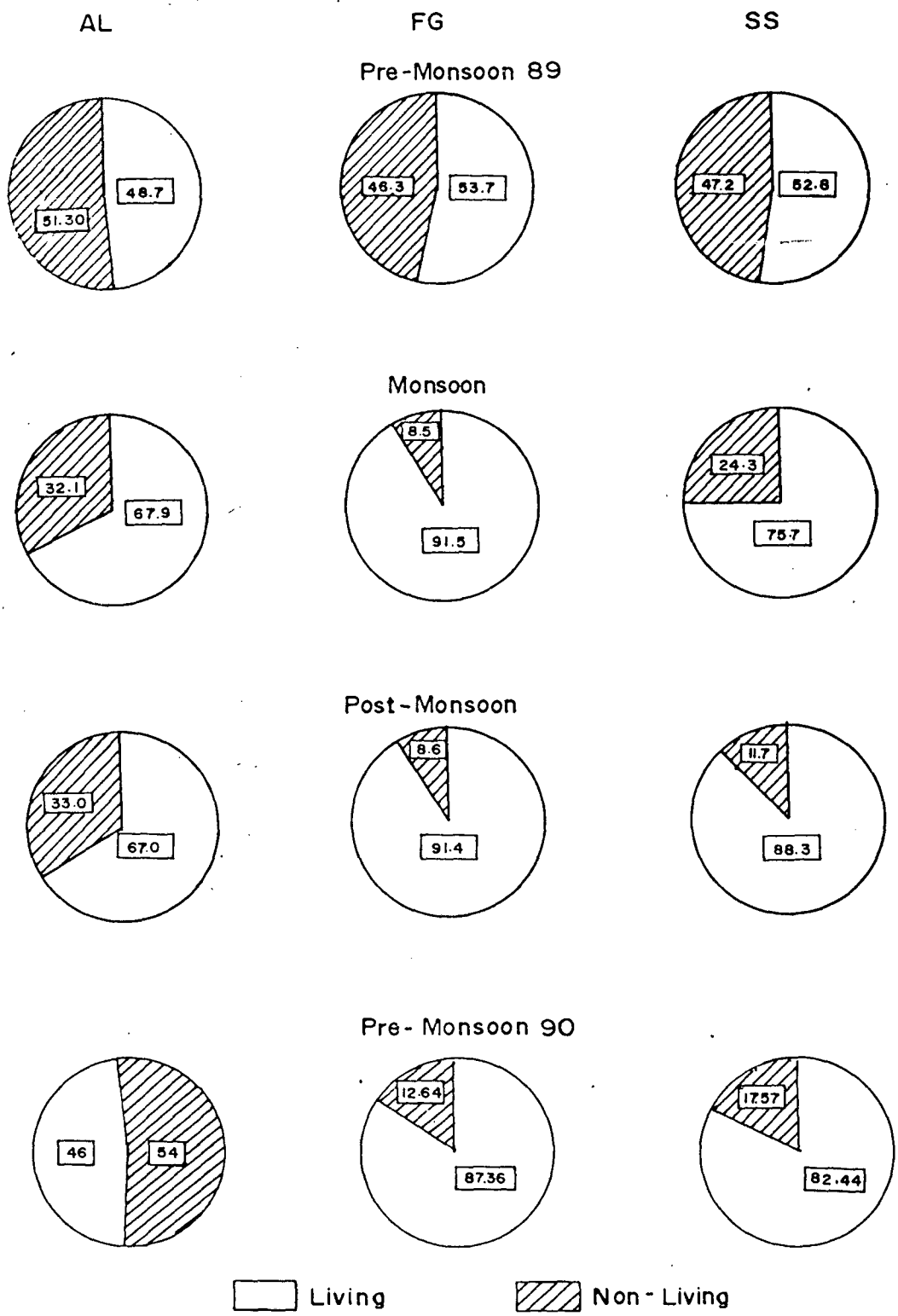

Fig.6.13 VARIATION IN LIVING & NON-LIVING CARBON DEVELOPED ON AL UMINIUM, FIBRE GLASS & STAINLESS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

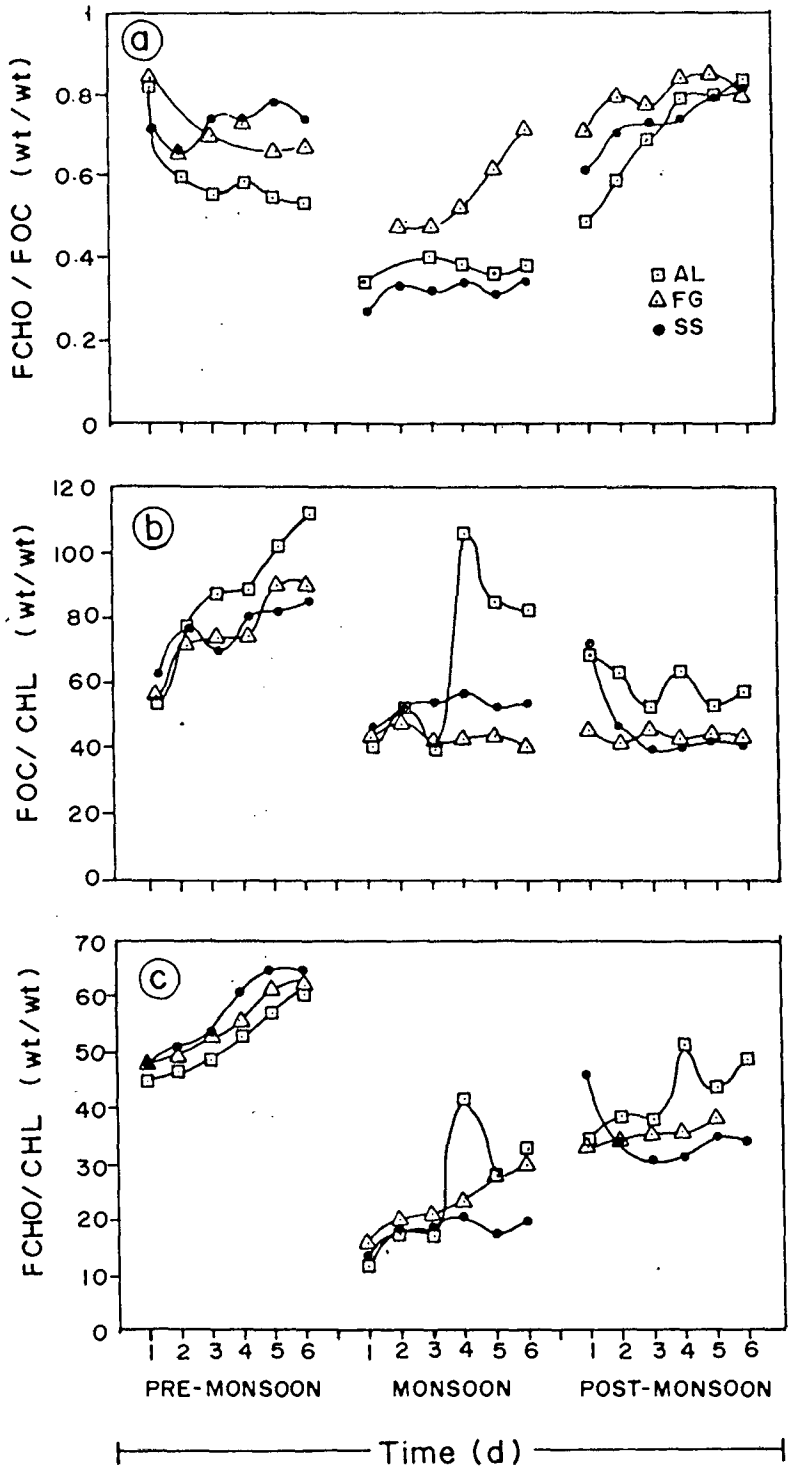

Fig.6.14 DAILY VARIATION IN FCHO/FOC, FOC/CHL & FCHO/CHL RATIOS AS A FUNCTION OF IMMERSION PERIOD

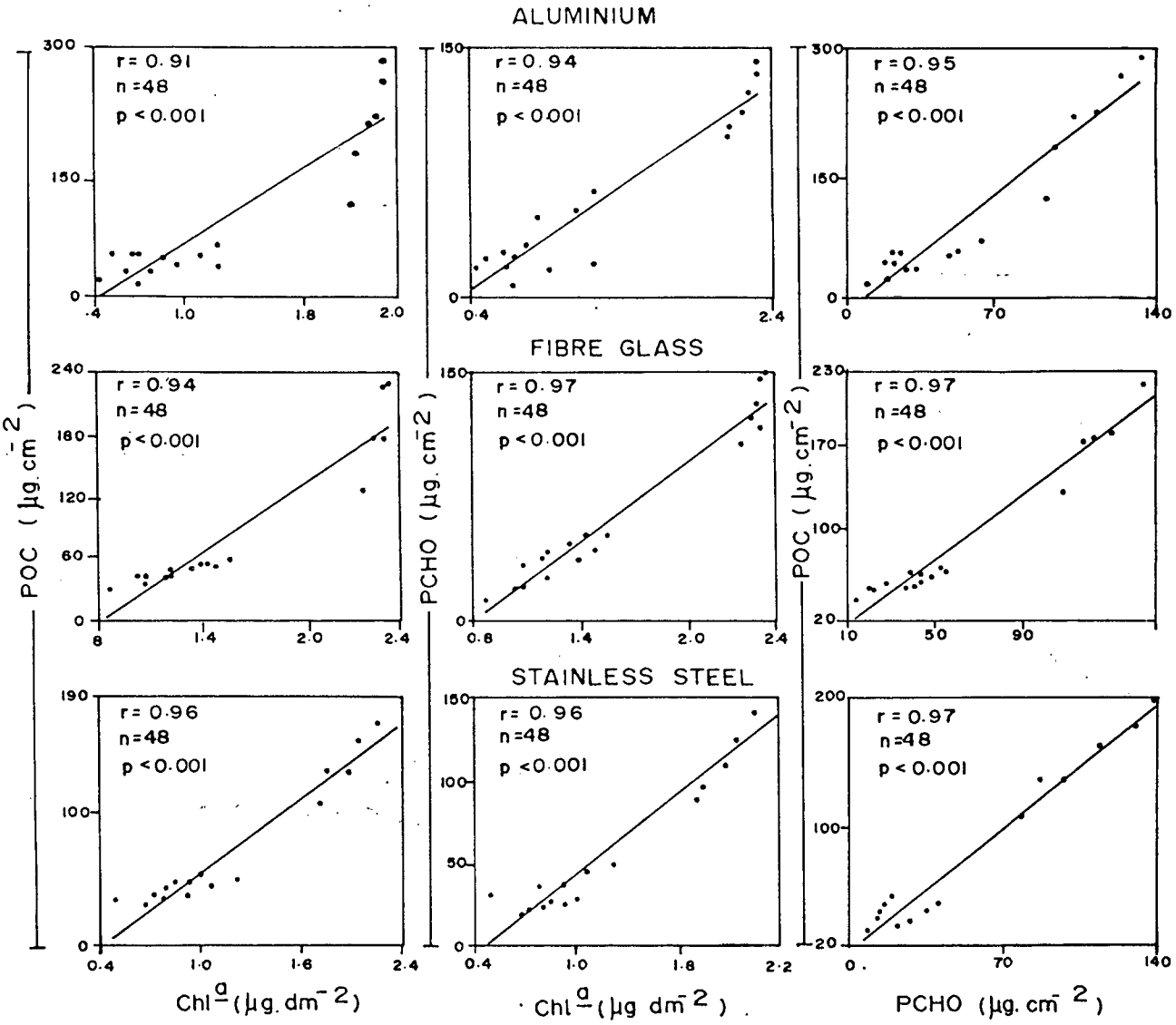

Fig. 6.15 RELATIONSHIPS BETWEEN ORGANIC CARBON, CHLOROPHYLL AND CARBOHYDRATES FOR THE DAILY SAMPLING.

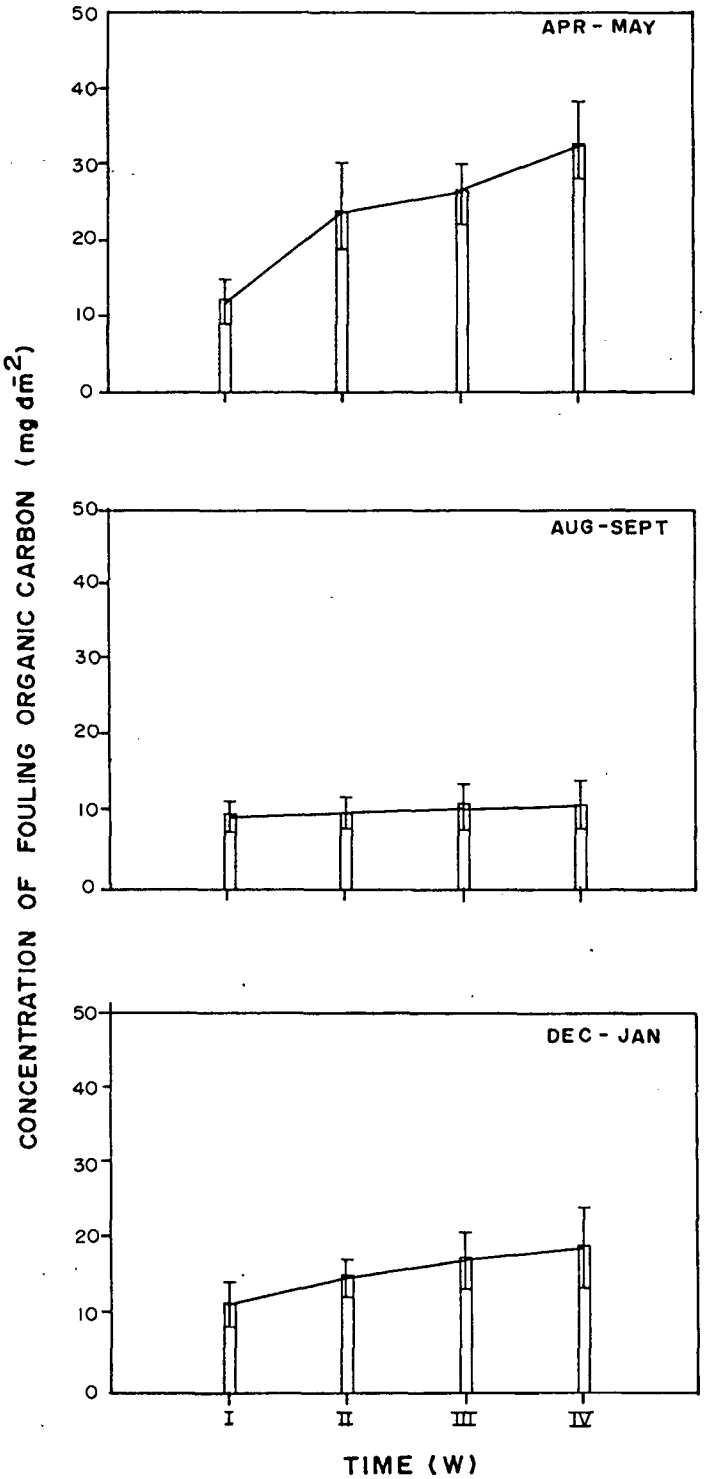

Fig.6.16 WEEKLY VARIATION IN ORGANIC CARBON DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

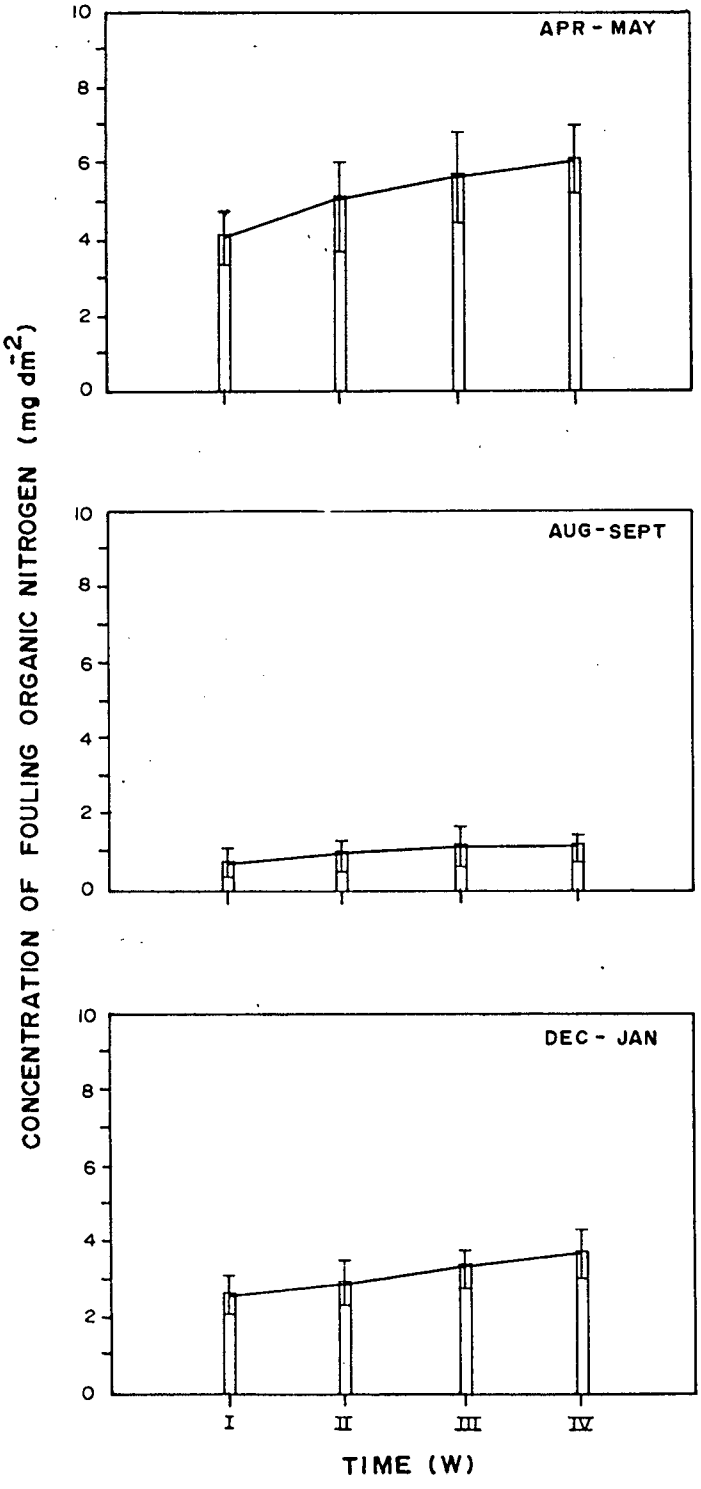

Fig.6.17 WEEKLY VARIATIONS IN ORGANIC NITROGEN DEVELOPED ON ALUMILIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

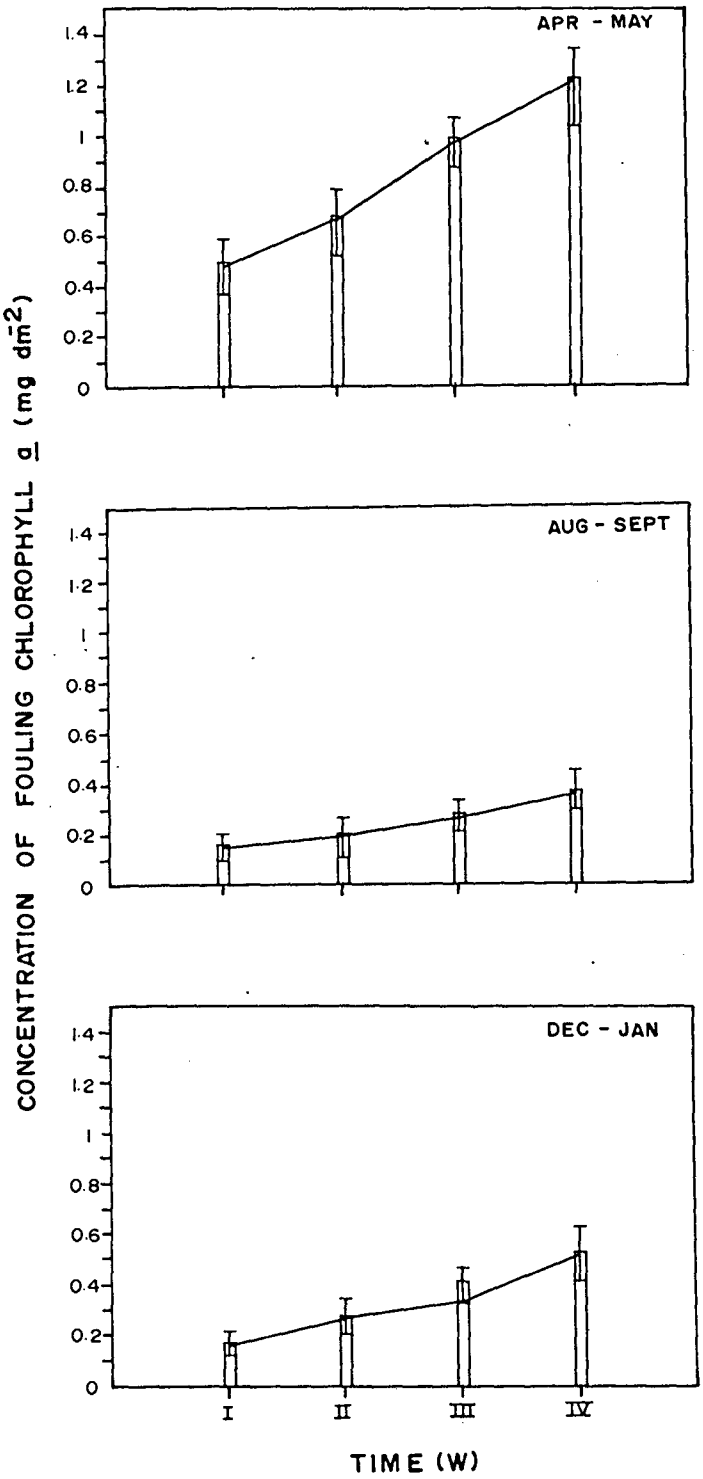

Fig 6.18 WEEKLY VARIATION IN CHLOROPHYLL DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

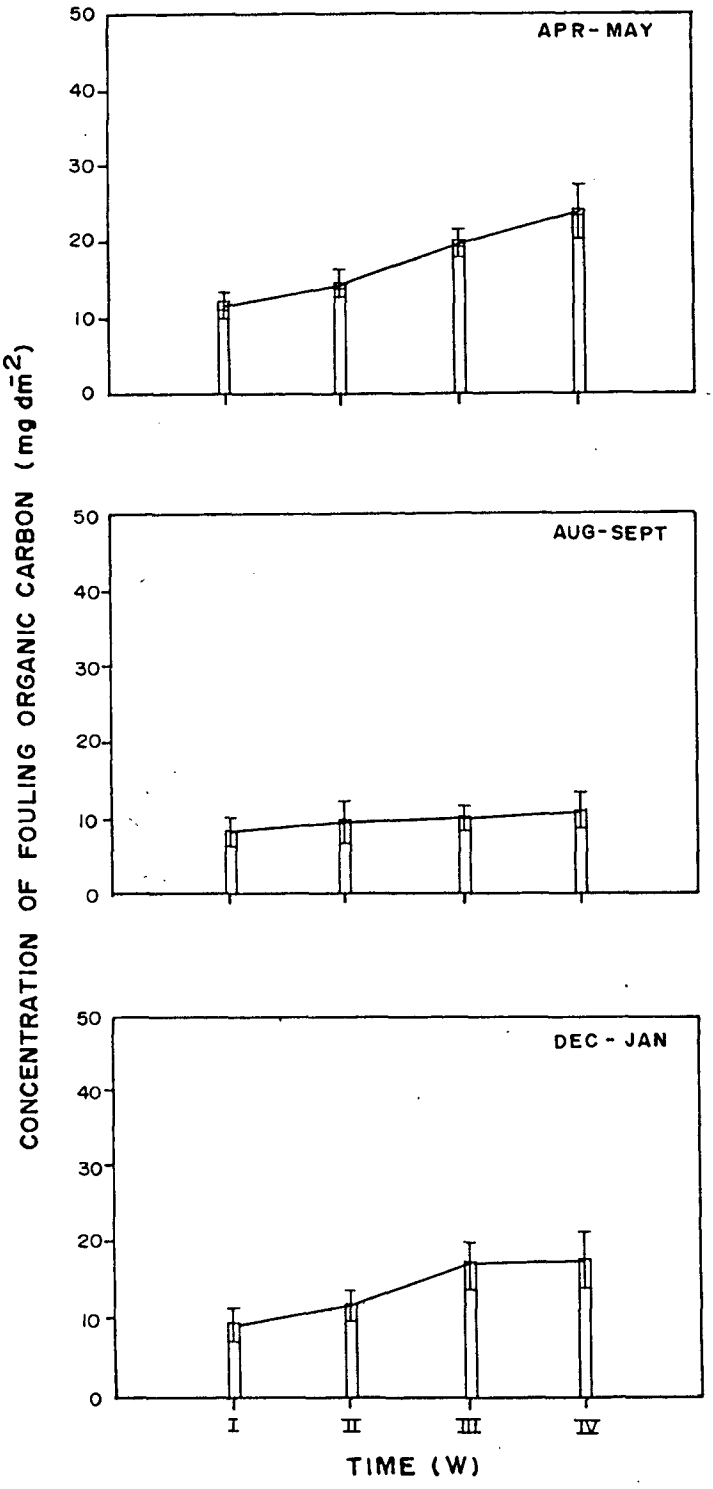

Fig.6.19 WEEKLY VARIATION IN ORGANIC CARBON DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

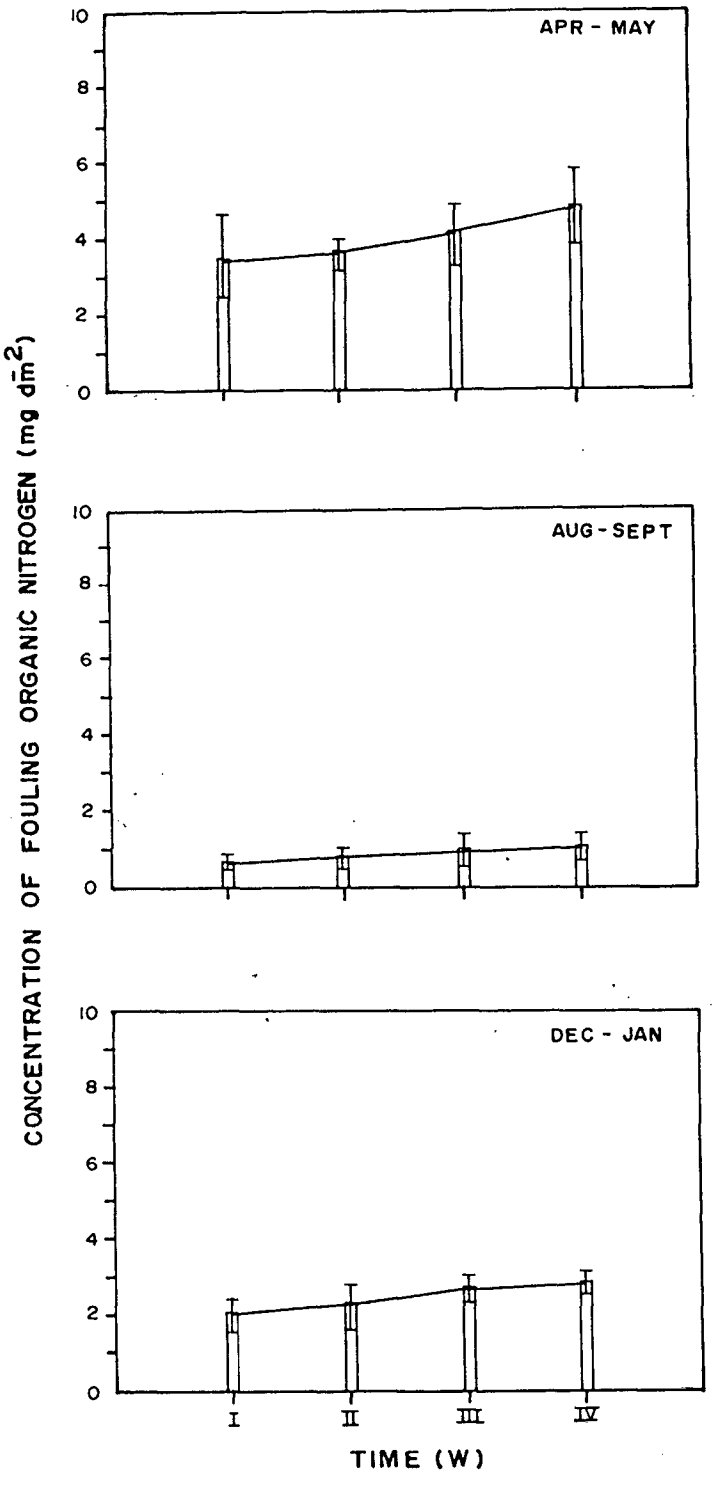

Fig.6.20 WEEKLY VARIATION IN ORGANIC NITROGEN DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

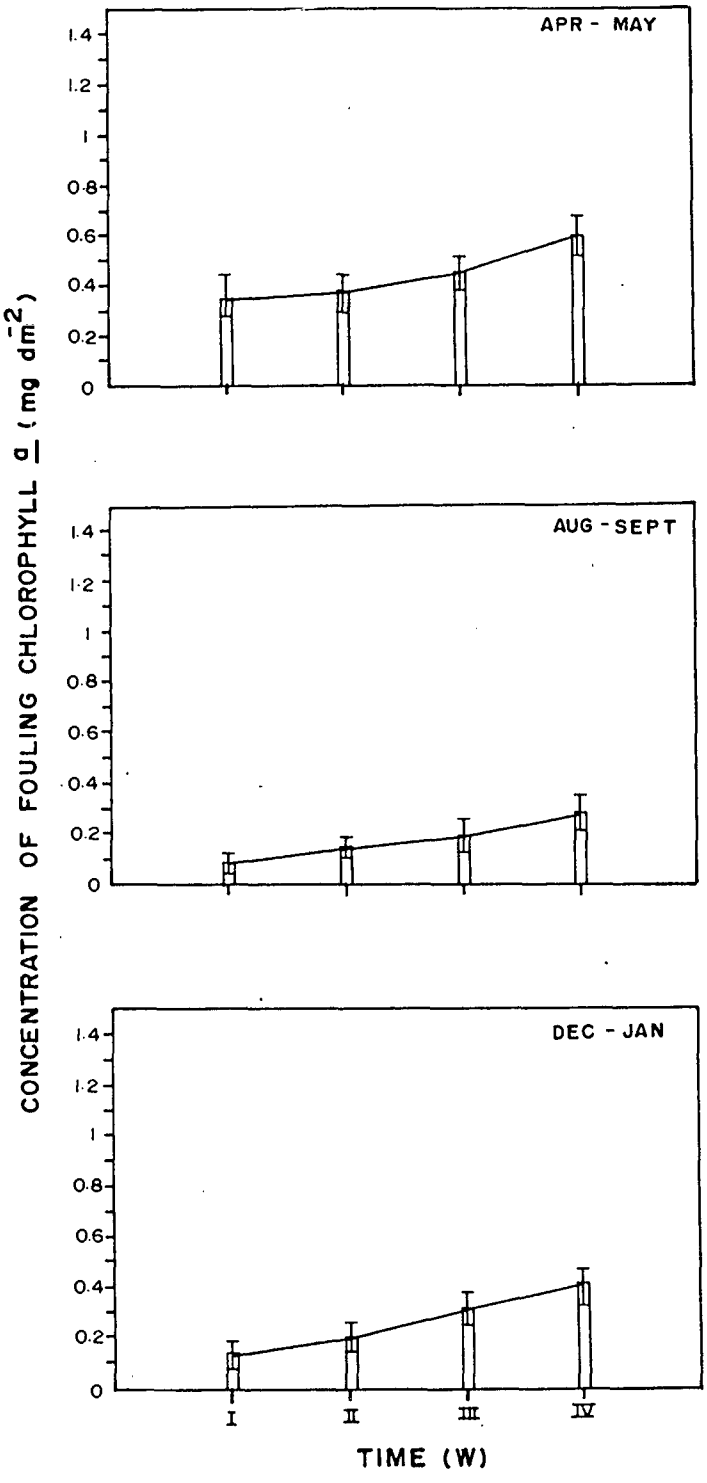

Fig. 6.21 WEEKLY VARIATION IN CHLOROPHYLL DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

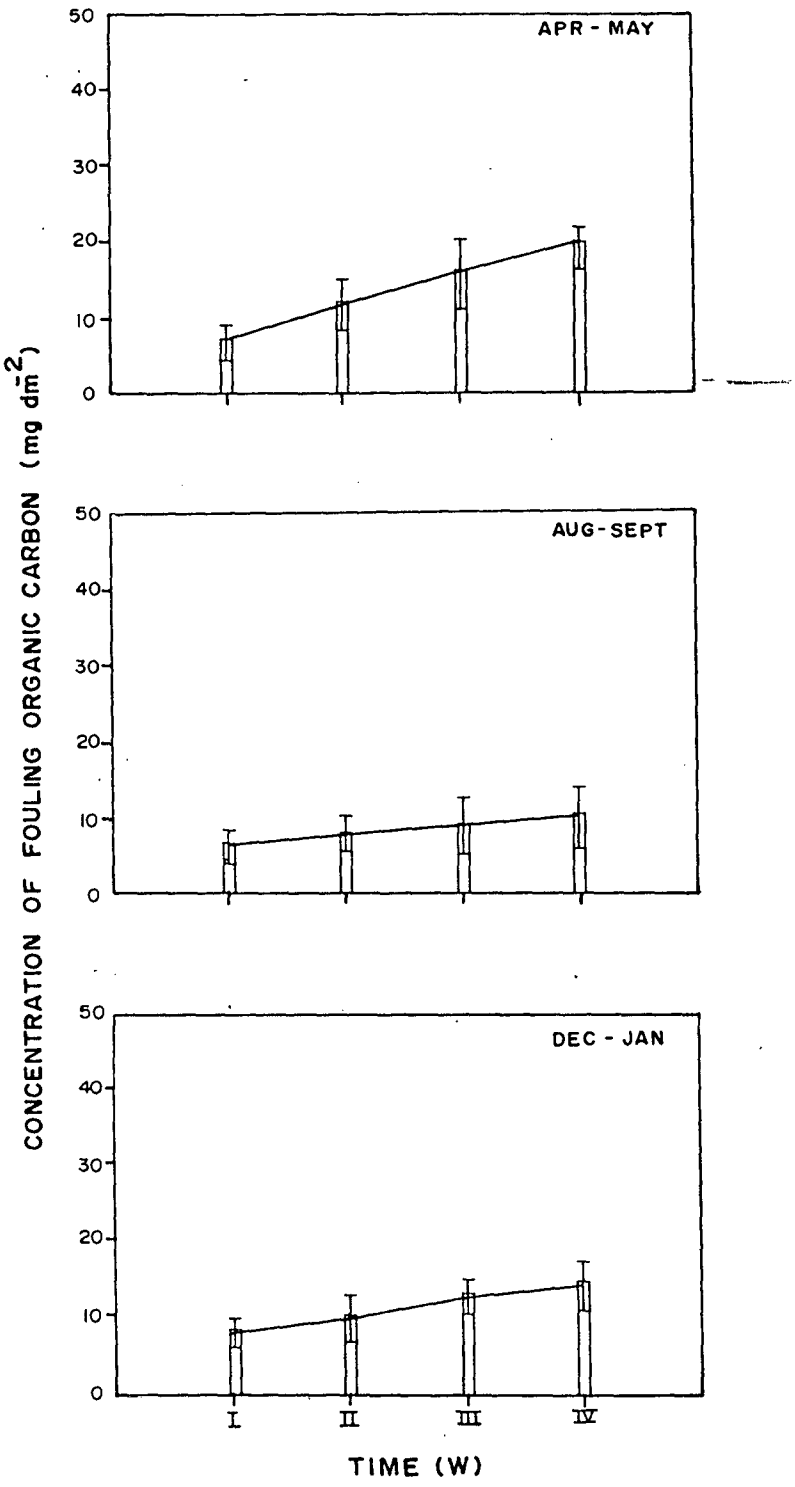

Fig.6·22 WEEKLY VARIATION IN ORGANIC CARBON DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

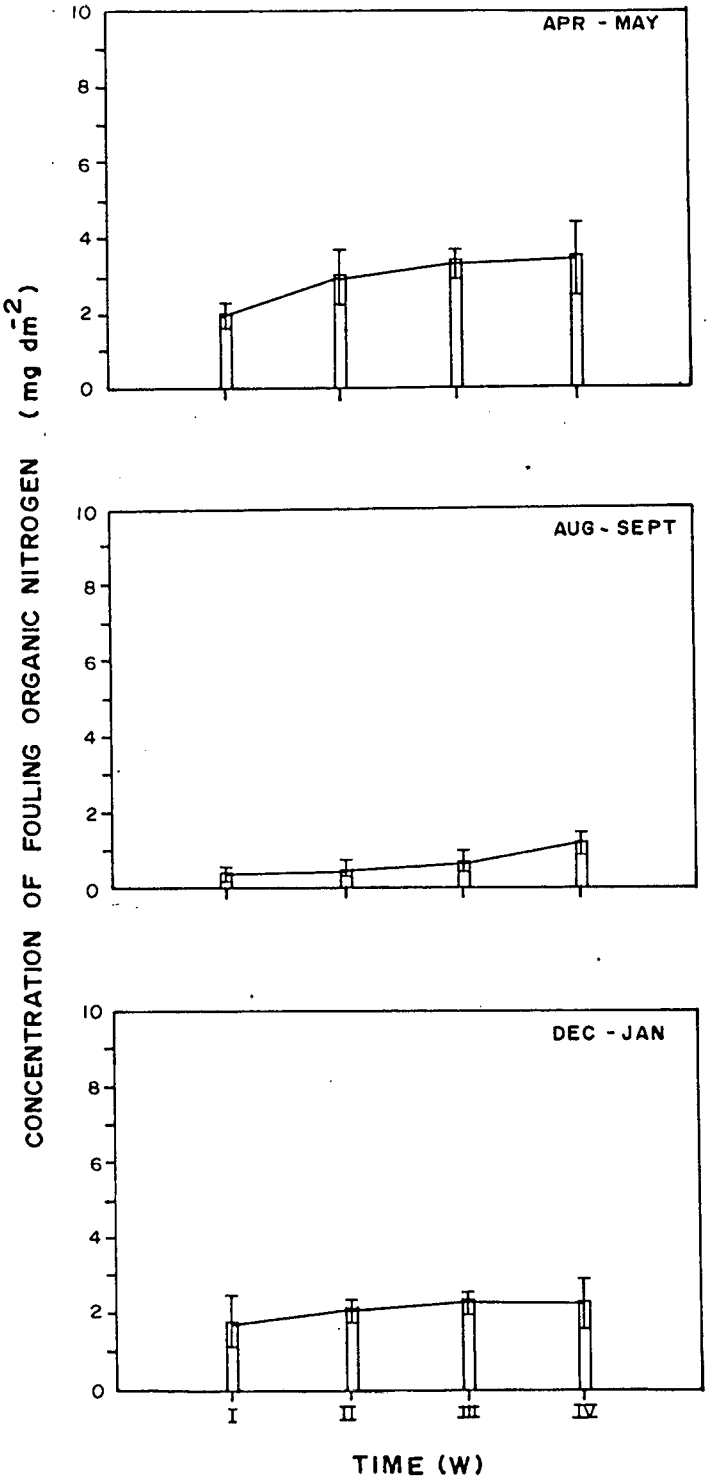

Fig.6.23 WEEKLY VARIATION IN ORGANIC NITROGEN DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED TO THE SUB-SURFACE WATER OF THE STUDY AREA

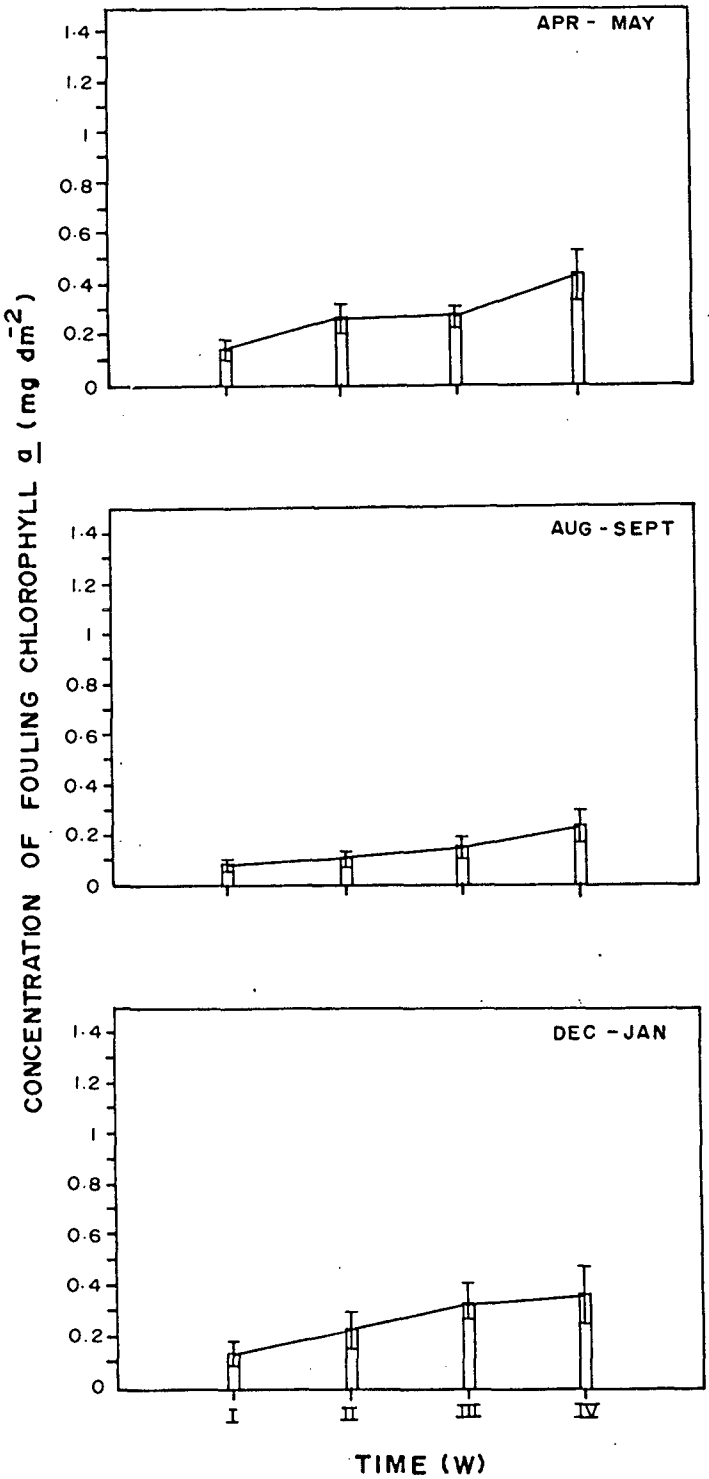

Fig 6.24 WEEKLY VARIATION IN CHLOROPHYLL DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED TO THE SUB-SURFACE WATER OF THE STUDY AREA.

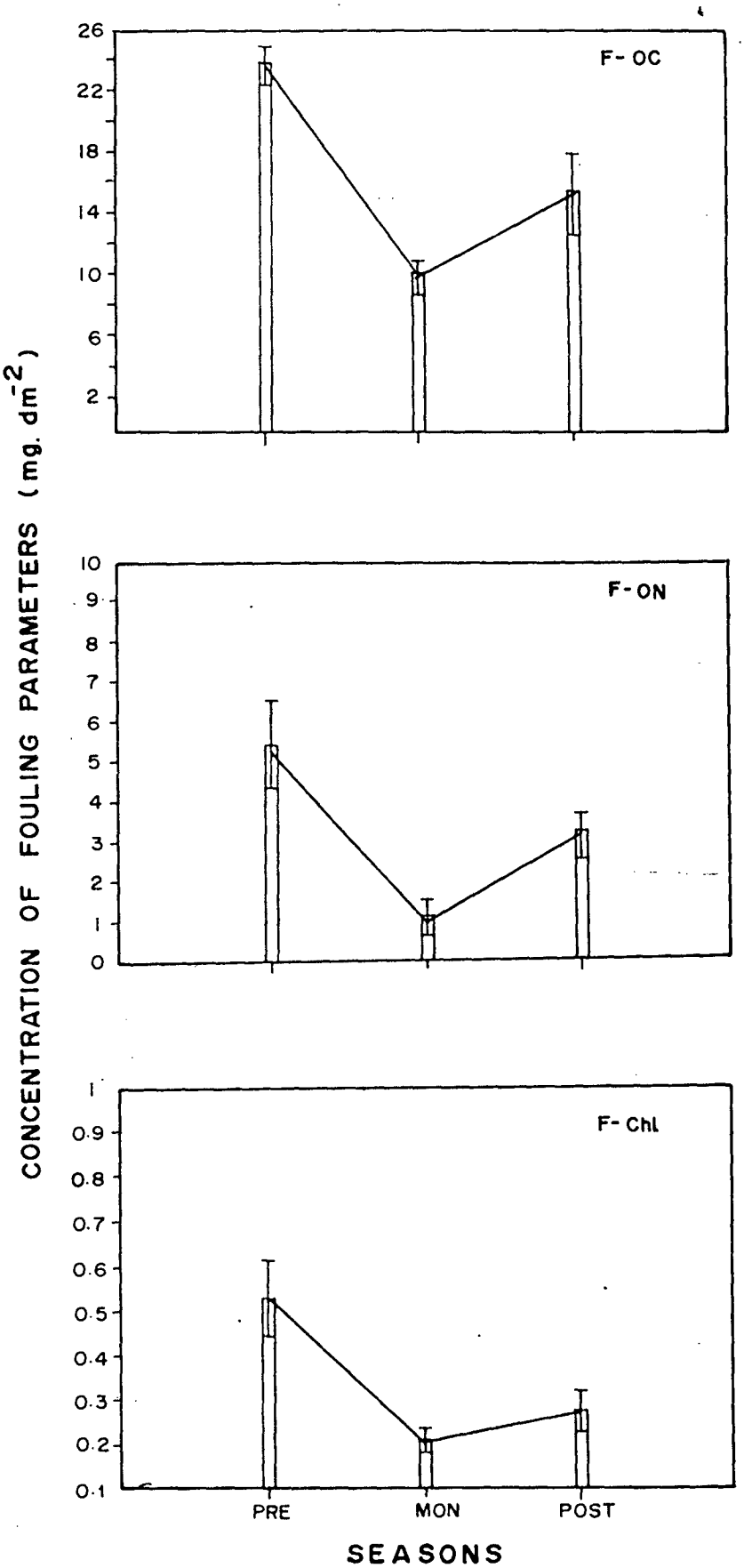

Fig.6.25 SEASONAL VARIATION IN ORGANIC CARBON, NITROGEN & CHLOROPHYLL DEVELOPED ON ALUMINIUM PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

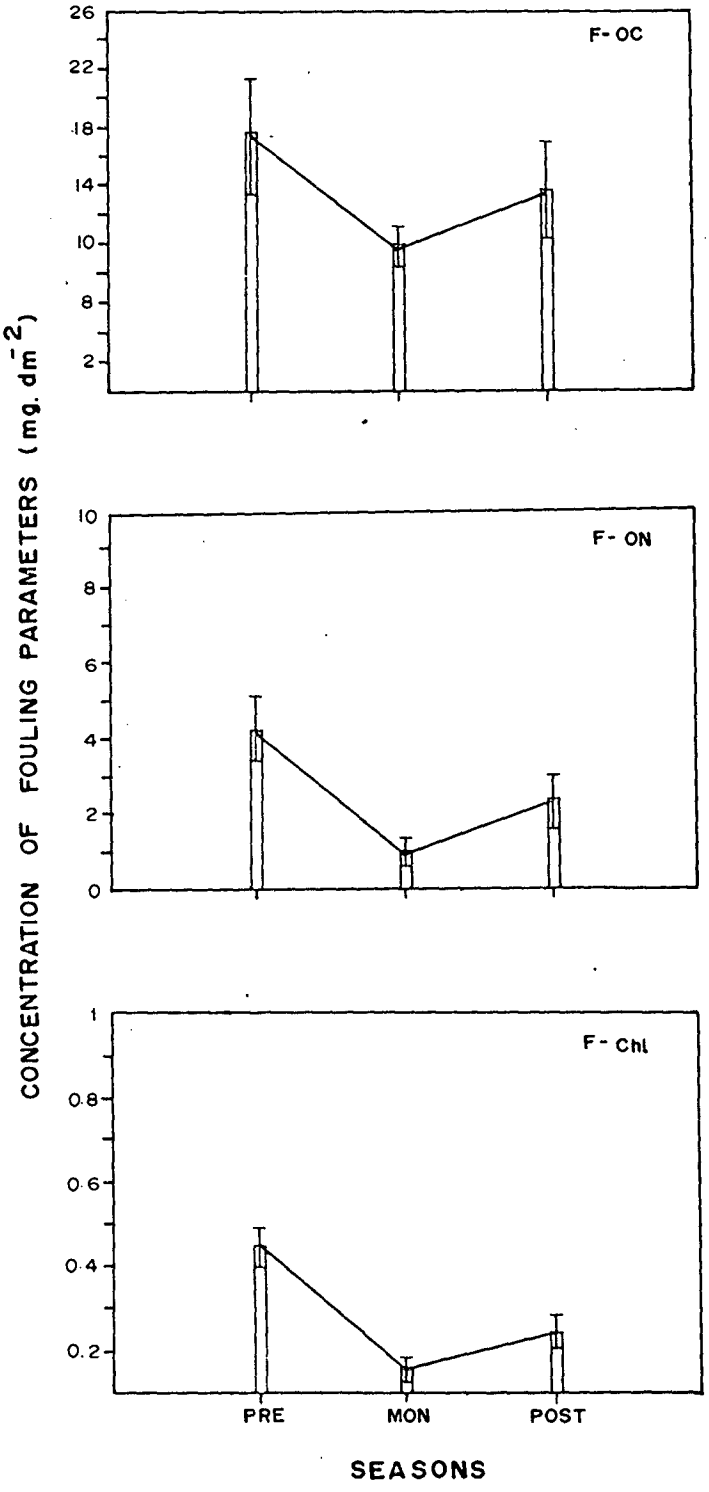

Fig.6.26 SEASONAL VARIATION IN ORGANIC CARBON, NITROGEN & CHLOROPHYLL DEVELOPED ON FIBRE GLASS PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

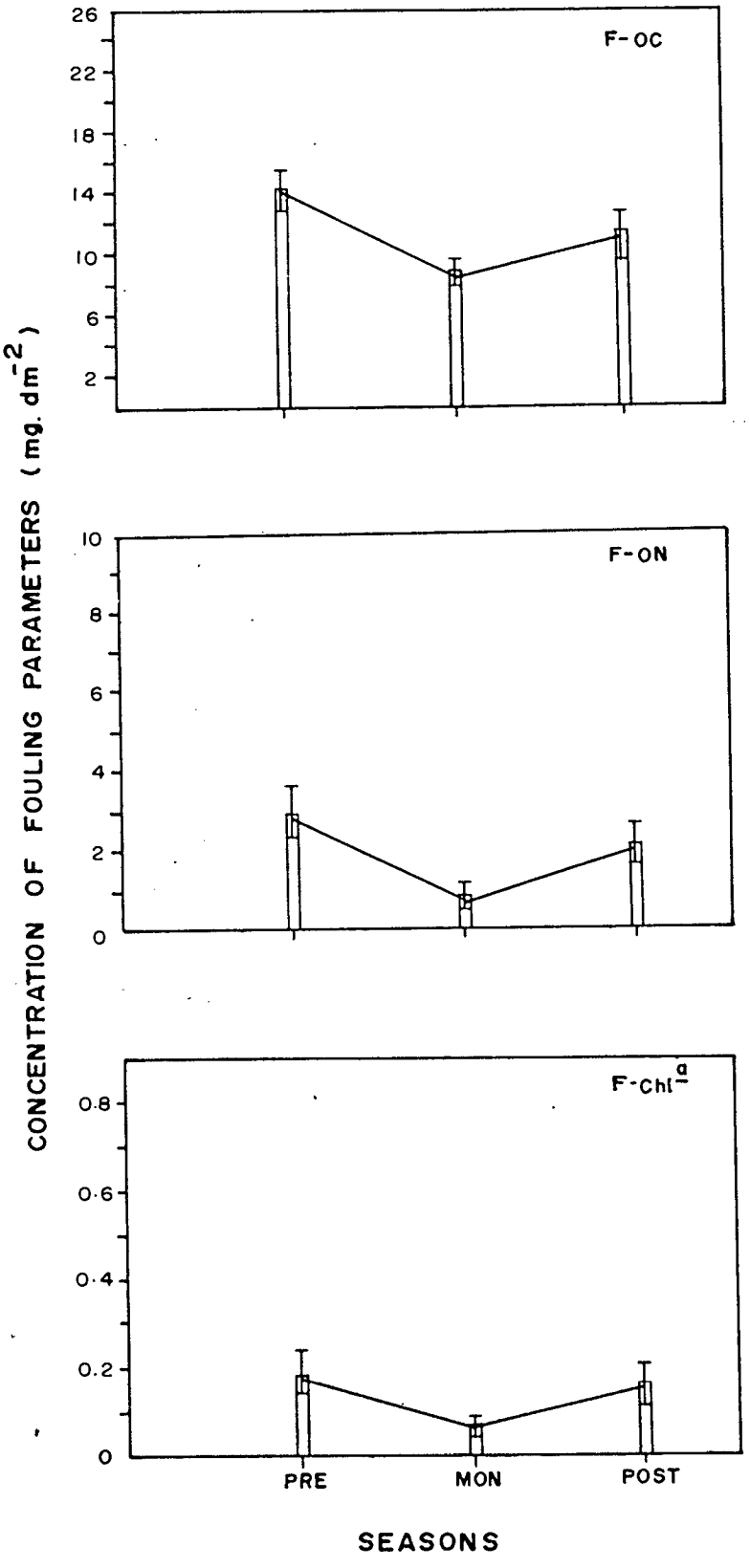

Fig.6.27 SEASONAL VARIATION IN ORGANIC CARBON, NITROGEN & CHLOROPHYLL DEVELOPED ON STAINLESS STEEL PANELS WHEN IMMERSED IN THE SUB-SURFACE WATER OF THE STUDY AREA.

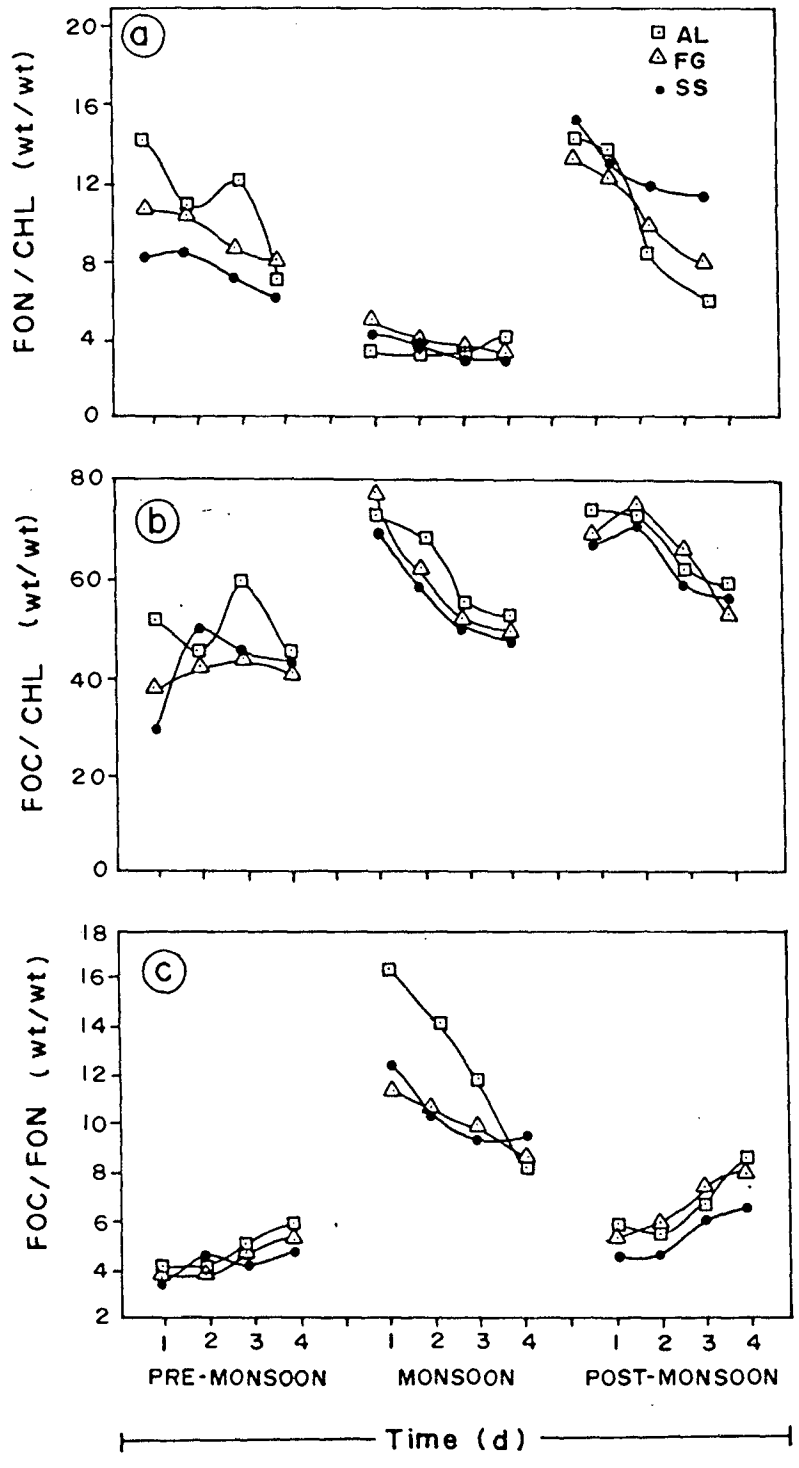

Fig.6.28 WEEKLY VARIATION IN FON/CHL, FOC/CHL AND FOC/FON RATIOS AS A FUNCTION OF IMMERSION PERIOD.

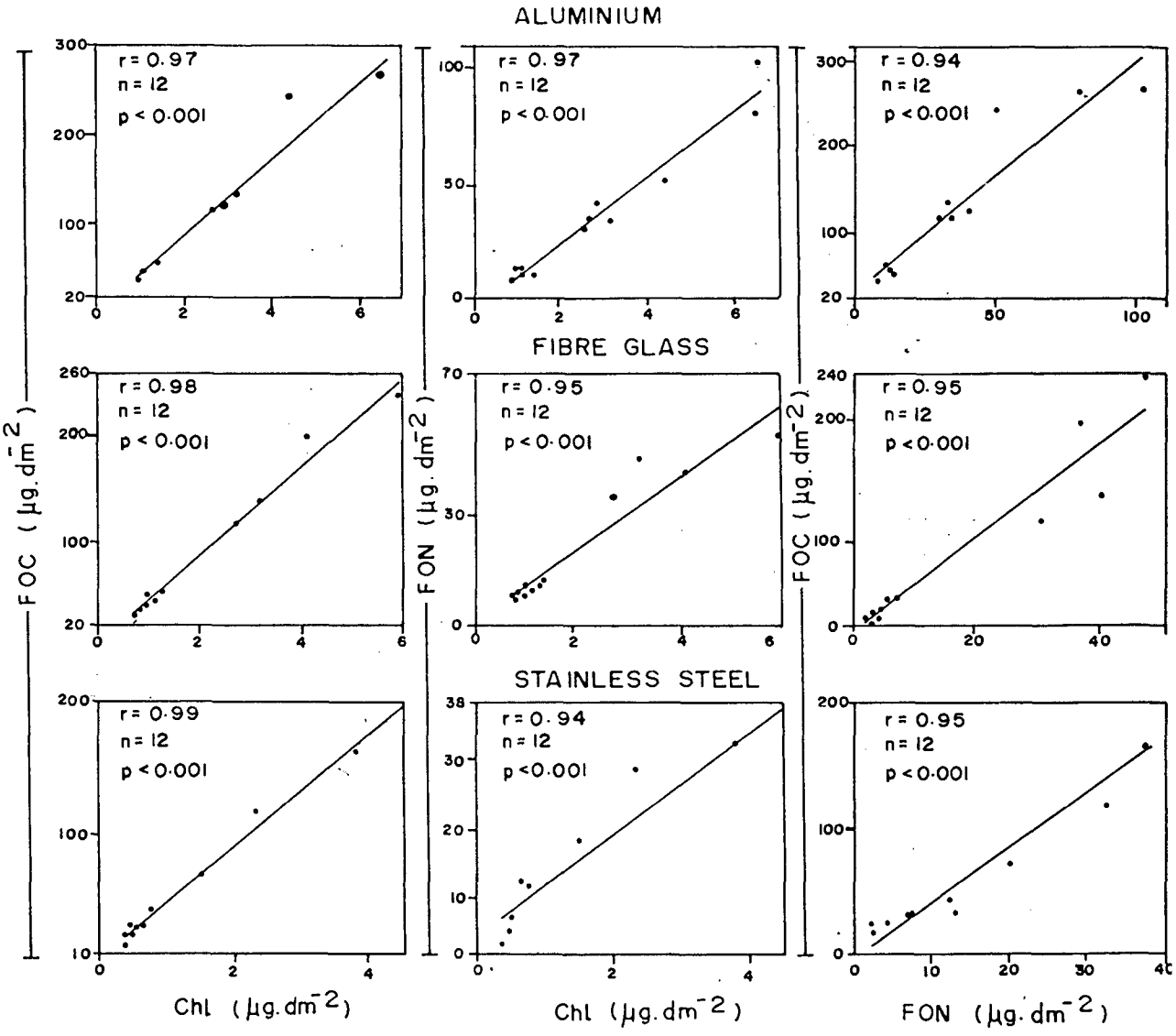

Fig.6.29 RELATIONSHIPS BETWEEN ORGANIC CARBON, CHLOROPHYLL AND ORGANIC NITROGEN FOR THE WEEKLY SAMPLING.

MOLECULAR CHARACTERIZATION OF MICROFOULING MATERIAL WITH SPECIAL REFERENCE TO AMINO ACIDS

1 INTRODUCTION

Microbial cells have a tendency to adhere to almost any surface submerged in aquatic environment. Mechanisms of microbial attachment involve physico-chemical interactions of the cell-surface macromolecules with a conditioned surface. Conditioning of surfaces immersed in aquatic environment occurs by the adsorption of organic molecules onto surfaces. In natural environment, all exposed surfaces rapidly absorb a proteinaceous film. Micro-organisms attach to conditioned surfaces with the help of organelles such as fimbriae, flagella and exopolymeric substances (EPS), (Costerton et al,1987; Van Loosdrecht et al, 1990). Such attachment and growth of micro-organisms on surfaces is generally known as microfouling.

Amino acids are organic molecules, which form the building blocks of proteins. They comprise one of the important components of the microfouling material and particulate matter in the water column. They are released mainly by phytoplankton and other living organisms during

their growth and decomposition (Jorgenson, 1982; Gocke, 1970; Whittle, 1977; Mopper & Lindroth, 1982). The nature and production by phytoplankton have been reviewed by Hellebust (1974). The characteristics of the release process have been extensively discussed by Sharp, (1977); Mague et al, (1980) and Fogg, (1983). It was shown that various physical and chemical factors encountered by phytoplankton cells in field or culture conditions, could modify the release of amino acids at different stages in their life cycle (Berland et al, 1970; Hammer et al, 1981; Hammer & Eberlein, 1981). The released amino acids are rapidly utilized by phytoplankton (Wheeler et al, 1974 & Dortch, 1982) or bacteria (Amano et al, 1982).

Thus, their occurrence in sea water is partly due to the equilibrium between release and uptake, related to the physiological activity of the various organisms of the nano and micro plankton community (Fogg, 1977; Smith et al, 1977; Larsson & Hagstrom, 1979).

Amino acids represent a very important source of carbon and nitrogen for microfouling organisms. Since, the low molecular fraction is an important substrate for microbes, much attention has been paid to study the dynamics involved in the formation and utilization of these compounds. In order to understand the pathway of these

organic compounds, during their transformation and utilization, a detailed knowledge about its chemical quantification is essential. Hence, in the present chapter, quantification, identification and detailed variation of amino acids are reported and discussed. This study was undertaken due to the importance and abundances of amino acids in the microfouling material. Moreover, to the best of my knowledge, this is the first report on the composition of amino acids, especially from the microfouling material. In addition to this, the change in the concentration of amino acids from the suspended matter of the sub-surface waters, was also assessed, so as to study the influence of amino acids on the development of microfouling material.

2 MATERIAL AND METHODS

2.1 Test panels

Aluminium, fibreglass and stainless steel test panels (10 x 15cms) were used for the study. They were cleaned and deployed as described in earlier chapters.

2.2 Retrieval

Replicate test panels of each substratum were retrieved every day (24h) for a period of 6 days during the month of April, 1989; August, 1989 and December, 1989. Although sampling was done for 6 days, analysis of the samples was selectively carried out for the 1st, 3rd and 5th day. This was done after analysis of one set of samples, which showed that for all the sampling days of the month the concentration of the total amino acids was found to show a gradual increase without any abnormalities.

2.3 Extraction of microfouling material

On reaching the laboratory, each panel was rinsed lightly with filtered seawater and scraped with a known volume (400ml) of the same. A nylon brush was used to remove the microfouling material. Aliquots (150 ml) of the scraping were filtered through ashed (450^{o}C, 4h) GF/C, glass fibre filter papers (47mm, 1um pore size). These filter papers were then dried in an oven at 40^{o}C for 24h and preserved in a desiccator until analysis was done.

2.4 Water sampling

During retrieval of test panels, water samples were also collected using a niskin water sampler. Two litres of sub-surface water was filtered using GF/C filter papers, similar to those used for microfouling samples. The filter papers were then dried and preserved in the same manner as mentioned above.

2.5 Determination of blank

Clean aluminium, fibreglass and stainless steel panels, not immersed in seawater, were scraped in a manner similar to those for experimental panels. This procedure helped in determining the amino acid contamination associated with clean panels as well as solvents and chemicals used for the digestion and isolation of amino acids from experimental panels.

2.6 Analysis

A system Gold, Beckmann, High performance liquid chromatograph (HPLC), was used for separation and quantification of amino acids.

2.7 Mobile phase

All solvents used for the present study were of high purity and the water used was of nanopure quality, which is subsequently referred to as distilled water in the course of this chapter. The acetate buffer (0.05 M), which was one of the mobile phases was prepared from HPLC grade sodium acetate, in distilled water. The pH of the acetate buffer (0.05M) was adjusted to 6.5 with acetic acid and 2% tetrahydrofuran was added to it. The other mobile phase was HPLC grade methanol. Mobile phases used were filtered through 0.2um filters and degassed prior to use.

Buffered reagent

40mg of ophthaldehyde (OPA) was dissolved in 1ml methanol. To it was added 10ml of 0.4M borate buffer (pH 9.5) + 150ul Brij solution (30% H_2O), 50ul mercaptoethanol and made to 50ml with buffer. The reagent mixture was allowed to age for 24h and filtered using 0.4um nucleopore filters prior to use. The reagent strength was maintained by addition of 1ul of 2-mercaptoethanol per ml of OPA reagent.

2.9 Solvent gradient

For the gradient run, sodium acetate buffer and methanol were used in percentage as shown in the Table 7.1 for a total duration of 30 minutes.

2.10 Shutdown and conditioning procedures

The following overnight shutdown procedures were employed. After the initial gradient run, the methanol content of the mobile phase was increased to 100% at a rate of 10%/min. The column was flushed with methanol for 10-15min, it was then flushed with water for a similar period. The latter step was found to be particularly essential to clean any precipitates of the buffer used from the column.

2.11 Standard solution

Standard stock solution of amino acid was prepared by weighing a known quantity of each L-amino acid (Sigma Chemicals Co.). These amino acids were then dissolved in 0.1 N HCl solution prepared in distilled water and made to 50ml after addition of 1ml of 1% $HgCl_2$ sol. The stock solution (0.2ml) contained 200nmoles of each amino acid.

2.12 Calibration

From the above stock solution, six different concentrations (10, 20, 30, 40, 50 & 60 nmoles) of working solutions were taken into a 10ml volumetric flask. To this, 1ml (100nmoles) of internal standard (∝-amino-butyric acid) was added and the total volume was adjusted to 10ml with distilled water to make the working solution. From the above working solution, 25µl was taken and mixed with 25ul of OPA. From this mixture, 40µl was injected into the column at the end of 2 minutes via the sampling valve. Triplicate runs of standard were made for each set of samples prior to analysis. The gradient used was the same over the course of study. The linearity of each amino acid was obtained by a correlation analysis of peak area and concentration as shown in Fig 7.1

2.13 Extraction of amino acids from the samples

Filter papers containing the microfouling material, as well as the particulate matter were treated in glass ampoules using 2ml of 6N HCl. Nitrogen gas was next passed through the mixture to free it from air. Ampoules were then sealed immediately and digested in an oven at 110° C for 24h. On cooling, the ampoules were broken and the

contents filtered (0.4um pore size) into a pear shaped evaporation flask. The filtrate was then evaporated to dryness under vacuum. After the flasks were freed from acid, they were desiccated overnight. Next day, 1ml of distilled water was added to dissolve the amino acids. From this 0.2ml of sample was transferred to 10ml volumetric flask. To this 1ml of internal standard was added and the volume was adjusted to 10ml.

2.14 Derivatization procedure for samples

From the diluted sample obtained as above, 25ul was taken and mixed with 25ul of OPA reagent. After completion of 2min, subsample (40ul), of the mixture was injected into the column. Individual amino acids were separated using the gradient system described above. Samples were identified by comparing their retention time with that of authentic standards. Quantifications of the components were achieved by peak area integration of HPLC results using data handling software of the instrument. Response factors for amino acids were assumed to be equal on weight basis and were compared to the response for alpha-aminobutyric acid (internal standard) for absolute quantification.

Blank papers without microfouling material were treated in a similar manner and comparison between the blank and standard graphs gave the possible contaminants in the procedure undertaken. The gradient run with the blank and standard is shown in Fig 7.2.

3 RESULTS

After trying several gradient composition of the mobile solvents the one used during the separation was found to be most suitable for amino acid separation. A series of standards were used to calibrate the amino acids, to know their linearity and to find out the response of the detector. The significant correlation obtained in the regression analysis of the individual amino acids against peak areas is shown in Fig 7.1. They show highly significant correlation at the existing condition.

3.1 Amino acids of the suspended particulate matter

Amino acid concentration of the suspended particulate matter (SPM) for the study period varied from 1150.32 to 2783.71nmoles/l.

Water samples were analysed for the 1st and 5th days. The value of total amino acid did not show significant

difference between the samples on both days. The 1st day and the 5th day samples of April, 1989, showed values ranging from 2783.71 to 2156.36nmoles/l. The 1st and 5th day samples of August, 1989, (1476.01 & 1150.32) and samples of December, 1989, (1727.60 & 2262.32) also did not vary appreciably.

Concentration of individual amino acids present in the samples collected during April, 1989, is shown in Fig 7.3. They being: aspartic, glutamic, serine, histidine, glycine, threonine, argenine, alanine, tyrosine, valine, phenylalanine, isoleucine, leucine and lysine. The change in the individual amino acid concentration and mole percentage contribution of amino acids with time, during April, 1989, is shown in Table 7.2. For the samples on the 1st day, concentration of amino acids ranged between undetectable quantities of tyrosine and methionine to 828.44 nmoles/l of glycine. When calculated as mole percentage it was observed that glycine was the dominant amino acid for the 1st day of April, 1989. It contributed, 29.53%, to the total amino acids. This was followed by serine (11.40%) which was less than two times the percentage contribution by glycine. The concentration of aspartic followed, contributing to about 9.02%. The other amino acids like alanine, threonine, lysine, glutamic,

valine, argenine, phenylalanine and isoleucine, were present in quantities between 6 & 3%. Histidine was present in traces (1%), while, tyrosine and methionine were undetected for the 1st day samples of April, 1989.

High concentration of glycine (312.49nmoles/l) was observed even for the samples on the 5th day, of the same month. Although, the concentration of glycine was high, the percentage contribution was lower than that observed for the 1st day (13%). Alanine (11.02%) and aspartic (10.66%) amino acids were less abundant as compared to glycine. The other amino acids showed percentage contribution to be less than 7% of the total amino acid. Tyrosine was undetected for the samples on the 1st day, while, it was present in trace quantities, on the 5th day (0.81%). Methionine remained undetected even for the samples on the 5th day.

When grouped into various fractions like acidic (aspartic acid, glutamic acid), basic (lysine, argenine and histidine), neutral (serine, glycine, threonine, valine, isoleucine and leucine), aromatic (tyrosine and phenyl alanine) and sulphur-containing amino acids (methionine), it was found that neutral amino acids were higher than all other groups. It contributed about 68% to the total amino acids for the 1st day samples. For the samples on the

5th day neutral amino acids contributed about 60%. Although the contribution by the neutral amino acids was lower on the 5th day, this fraction continued to remain high as compared to the other fractions. Acidic amino acids present in the samples on the 1st and 5th days varied between 14 and 18%, and basic between 12 and 14%. Aromatic amino acids showed a contribution between 3 and 5% for the samples on the 1st and 5th days respectively. Thus, all the groups for both the days were comparable, with minimum changes, as shown in Table 7.5.

During the month of August, 1989, as seen in Table 7.3, the percentage contribution by glycine to the total amino acids was highest for the samples on the 1st day (12%) as well as on the 5th day (13%). The next major amino acid in the 1st day samples, was aspartic (11.82%) and alanine (10.76%), which were similar to that in the samples on the 5th day (11.93 & 10.47% resp). The other amino acids contributed <8% for both the days. Tyrosine and methionine could not be detected for both the days. During this month, the variation in the individual amino acid compositions were very small (Fig 7.4).

Group compositions as seen in Table 7.5 were quite similar for both the days, with neutral amino acids predominating (59%). Acidic amino acids for both the days

were 19%, and basic amino acids were 11% on the 1st day and 12% on the 5th day. Finally, aromatic amino acids contributed 5% on the 1st day and 4% on the 5th day. Similarities in the group composition indicates a thorough mixing and uniformity in the sub-surface waters.

The samples on the 1st and 5th days of December, 1989, showed a greater variation in the composition of amino acids as seen in Table 7.4. Just like the sampling during April, 1989 and August, 1989, in December, 1989 too, the percentage contribution by glycine was high for samples on the 1st day (22.51%) as well as on the 5th day (24.56%). Phenylalanine (14.60%), followed glycine on the 1st day and its contribution was almost half of that of glycine. The contribution by other amino acids were as follows: serine 12.15%, aspartic 11.46%, alanine 11.30%, valine 7.29%, threonine 6.40% leucine 6.31% and isoleucine 4.10%.

The samples on the 5th day showed a similar kind of variation except for phenylalanine which was present in small quantities (6.09%) as compared to the samples on the 1st day (Fig 7.5).

As seen in Table 7.5, neutral amino acids were highest for samples on both the days in December, 1989, (70.05% & 68.56%). The neutral fraction was followed by

aromatic (14.60%) and acidic (11.45%) amino acids for 1st day samples. High percentage of aromatic amino acids was due to significant contribution by phenylalanine. Basic amino acids were totally absent for samples on the 1st day.

Samples on the 5th day showed neutral amino acids to be most dominant. The next highest groups of amino acids were acidic (19.55%) and aromatic (6.08%). The basic fraction was not totally absent as seen for the 1st day, but its contribution was about 5.78% to the total amino acid content. This change in the group composition of amino acids was mainly due to the difference in the concentration of individual amino acids in the suspended particulate matter sampled.

3.2 Amino acid composition of microfouling material

The results obtained for the total amino acids, from the microfouling material, varied between 65.58 & 2185 nmoles.dm-2, for various surfaces during the study period. As observed for the microfouling material, the concentration of total amino acids generally increased with increase in immersion period.

Fifteen different amino acids were detected from the spectrum for microfouling material (Fig 7.15). They

being: aspartic, glutamic, serine, histidine, glycine, threonine, argenine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine. Materials like aluminium, fibreglass and stainless steel were used as test panels. Microfouling settlement on each substratum is reported with respect to short term variations. Group composition of amino acids on various surfaces is also reported.

3.2a ALUMINIUM

The total amino acid concentration of the microfouling material, developed on aluminium test panels, ranged between 300.54 & 2185.37 nmoles.dm-2 for the study period.

During April, 1989, total amino acid concentration observed for samples on the 1st day was 300.34nmoles.dm-2, whereas, for the 3rd day the value increased to about seven times and was 2156.36nmoles.dm-2 (Table 7.6). However, for samples on the 5th day, the concentration was only marginally higher than that observed for the 3rd day samples (2185.37nmoles.dm-2). Thus, samples on both, the 3rd and 5th days, showed amino acid concentration to be greater by seven times than that on the 1st day (Table 7.6).

As observed in Fig 7.6, glycine was the major contributor to the total amino acids, for samples of all the days. Besides glycine, serine (12.8%), aspartic (10.31%), alanine (9.24%), glutamic (8.9%), threonine (8.52%), lysine (7.01%), leucine (6.58%), argenine (6.5%) were also present in the samples of the 1st day. On the other hand, for samples obtained on the 3rd day, alanine (11.45%), aspartic (11.09%), leucine (8.036%), serine (7.52%), threonine (7.17%), glutamic (7.11%), lysine (7.08%) and valine (6.72%) were abundant. The amino acid spectrum for the 5th day sample, was similar to that observed on the 3rd day. Alanine was the most abundant amino acid contributing 11.91% to the total amino acid. Threonine (9.48%), serine (9.25%), aspartic (9.11%), lysine (8.5%), glutamic (7.63%), leucine (6.54%), and argenine (6.17%) were also present. Various amino acids like phenylalanine, isoleucine, valine (for all three days), tyrosine (for 3rd & 5th day) and histidine (for the 5th day) were present in trace quantities, contributing below 5%, to the total amino acid. There were some amino acids which were undetected like histidine and tyrosine for the samples on the 1st day and methionine for samples on all the three days (Fig 7.6).

Table 7.9 shows the various group composition of

amino acids. Neutral amino acids were dominant for samples on all the three days (61.43%, 60.97% and 58.58%). There was a clear decrease in amino acid concentration with increase in immersion time, for the neutral group of amino acids. Acidic amino acids for samples on the 1st day showed 19.21%, whereas, for the 3rd and 5th days, acidic amino acid contribution was 18.20 & 17.75% to the total amino acid concentration. This group also showed a decrease with time. On the other hand, basic amino acids for the samples on the 1st, 3rd & 5th days, showed higher concentration, with increase in immersion time (13.50, 14.93 & 18.20%). Aromatic amino acids for the samples on all three days showed lower percentage without any significant trend (5.58, 5.90 & 5.47%). S-Containing amino acids, were not present in the microfouling material, developed on aluminium during April, 1989.

As shown in Table 7.7, amino acids of the microfouling material developed on aluminium during August, 1989, showed values ranging between 412.42 and 806.8 nmoles.dm-2. The variation in the concentration of total amino acids was only marginal for the samples on all three days.

The absolute amounts of individual amino acids showed glycine to be most dominant, with percentage contribution

of 15.46% on the 1st day. Next in abundance were alanine (13.17%), serine (12.79%), aspartic (10.76%), threonine (8.86%), glutamic (8.07%) and lysine (7.21%), contributing to the total amino acid in the decreasing order. Various other amino acids like leucine, argenine, isoleucine, phenylalanine, valine and methionine were present in trace quantities (<6%). The peak for tyrosine could not be traced as evident from Fig 7.7 A.

Samples on the 3rd day also showed predominance of glycine, making a contribution of 27.92% which was almost two times higher than that observed for the 1st day samples. However, the less abundant amino acids were: argenine (10.87%), serine (10.47%), valine (8.34%), alanine (7.17%), and leucine (6.31%). Glutamic, aspartic, phenylalanine, methionine, isoleucine were present in trace quantities (<6%). Histidine, threonine and tyrosine were undetected in the amino acids spectrum (Fig 7.7B).

Just as in the case of samples on the 3rd day, the 5th day samples also showed a high contribution of glycine (24.79%), followed by serine (10.34%), alanine (10.09%), argenine (9.9%), aspartic (3.05%), leucine (6.76%) and glutamic (6.36%). Amino acids which were present in trace quantities were: phenylalanine, lysine, methionine, isoleucine and tyrosine (<5%). Histidine, threonine and

valine were not detected on the spectrum for the samples on the 5th day (Fig 7.7C).

Group composition of amino acids as observed in Table 7.9, showed neutral amino acids to vary between 64.43%, 63.15 & 56.09% for the samples of the 1st, 3rd and 5th days. These amino acids formed a major fraction of the total amino acid content. Contribution by acidic amino acids on the other hand, was almost five times lower than the contribution made by neutral amino acids. Basic amino acids were high for samples on the 1st day (12.43%). The concentration decreased marginally, for samples of the 3rd day (11.05%) and increased once again for samples of the 5th day (15.18%). Samples obtained on the 1st & 3rd days showed aromatic amino acids to contribute 3.85% and 5.53% respectively. The contribution increased to 8.6% for the samples of the 5th day. S-containing amino acids which formed the minor fraction was as low as 1.79% for samples of the 1st day as compared to the samples of the 3rd (5.28%) and 5th (4.94%) days. Results obtained for the present study showed a marked increase in the case of neutral and aromatic amino acids with time. On the other hand, acidic, basic and S-Containing amino acids did not show any significant trend.

Sampling during December, 1989, showed the concentration of amino acids to vary between 402.74 and 1339.55 nmoles.dm-2 (Table 7.8). Total amino acid concentration observed for samples of the 5th day was more than two times that observed on the 1st day. Although, there was no marked variation in the concentration of total amino acids, absolute amounts of amino acid compositions was highly variable. For example, the 1st day showed argenine to be most dominant (26.84%), followed by valine (18.64%), aspartic (11.68%), isoleucine (9.65%), lysine (8.8%) and tyrosine (5.31%). Several amino acids like glutamic, serine, histidine, glycine, threonine, alanine, methionine, phenylalanine and leucine were undetected (Fig 7.8A). Thus, on the 1st day, nine amino acids from the microfouling material remained undetected. However, those amino acids that were traced, contributed significantly (> 5%) to the total amino acids.

In the 3rd day samples, unlike those of the 1st day, glycine was the most abundant amino acid, making a contribution of 15.45%. Amino acids to follow glycine, were serine (11.75%), aspartic (11.66%), threonine (8.91%), lysine (8.21%) and glutamic (8.1%). Those amino acids which were present in trace quantities and contributed below 6% were: leucine, argenine, phenylalanine,

isoleucine, valine and alanine. Only three amino acids remained undetected in the samples of the 3rd day (histidine, tyrosine & methionine) as is evident from the spectrum (Fig 7.8B).

Amino acids as shown in Fig 7.8C for the samples of the 5th day, like those of the 3rd and unlike those of the 1st days showed predominance of glycine (12.54%). Alanine (10.79%), lysine (10.32%), aspartic (9.78%) serine (8.87%), glutamic (8.82%), threonine (8.18%), argenine (7.22%) and leucine (6.46%) contributed significantly to the samples of this day. Trace quantities of phenylalanine, isoleucine, histidine, valine and tyrosine were observed for the 5th day samples. The only amino acid to remain undetected was methionine.

Peak values of neutral amino acids were observed for all the days (Table 7.9). Samples on the 1st, 3rd and 5th days, showed the contribution to the total amino acid to be 34.95%, 56.50% and 54.70% respectively. Acidic amino acids contributed a high percentage for samples of the 1st, 3rd and 5th days (11.43, 22.72 & 18.72%). Basic amino acids for samples contributed a high percentage to the total amino acids on the 1st day (44.05%). The 3rd and 5th day samples made a low contribution of 8.68% to the total amino acid. Aromatic group of amino acids was the

lowest contributor to the total amino acids (6.56, 4.84 & 5.76%). The group composition during December, 1989, did not show any significant pattern with exposure time.

3.2b FIBREGLASS

Microfouling material developed on fibreglass test panels showed total value of amino acid to vary between 268.17 & 859.88nmoles.dm-2 for the study period (Table 7.10).

Samples of the 1st day of April, 1989, showed the total amino acid value to be 268.17 nmoles.dm-2 which increased almost two times on the 3rd day (496.81nmoles.dm-2) and by three times on the 5th day (859.88 nmoles.dm-2), respectively.

The spectrum of individual amino acids from the microfouling material of Dona Paula waters, showed amino acids to vary largely for the samples on all the three days (Fig 7.9). Samples of all the days showed glycine to be the most abundant amino acid. Percentage contribution of glycine decreased from the 1st day (20.13%), till the last day (18.64%). The other amino acids which were present in relatively high quantities were aspartic, (11.95, 10.85 & 11.28%), serine (10.61, 10.08 & 8.29%) and alanine (9.43, 10.56 & 10.78) for samples of all three days. Histidine,

tyrosine and methionine were marked as ND, because their concentrations were not high enough to show quantitative separation. The list of amino acids to contribute between 7 to 4% were: glutamic, threonine, argenine, valine, leucine, lysine and phenylalanine (Fig 7.9).

Results of the group composition showed neutral amino acids to dominate, with a percentage of 64.91%, on the 1st day, 65.78%, on the 3rd day and 65.05% on the 5th day respectively. Neutral amino acids were followed by the acidic, basic and aromatic amino acids for the 1st, 3rd and 5th day samples. No particular trend was observed in any of the groups, with increase in the exposure period (Table 7.13).

Total amino acid concentration of the microfouling material developed on fibreglass during August, 1989, is given in Table 7.11.

Low concentration of individual amino acid was observed for samples of the 1st day (235.61 nmoles.dm-2). The value was only marginally higher for samples of the 3rd day (344.27 nmoles.dm-2) and 5th day (360.22 nmoles.dm-2) during August, 1989.

Glycine was the major amino acid for samples of the 1st day (17.54%). The other abundant amino acids were

serine (12.94%), aspartic (12.22%), alanine (10.02%) and glutamic (10.00%). Lower quantities of threonine (8.07%), lysine (7.13%), leucine (5.45%), phenylalanine (5.01%), argenine (4.94%), isoleucine (3.58%) and valine (3.12%). Histidine, tyrosine and methionine were undetected during the 1st day (Fig 7.10A).

Fig 7.10B shows, the spectrum for individual amino acids for samples of the 3rd day, wherein, alanine was the major amino acid, making a contribution of 14.16% to the total amino acid concentration. It was followed by glycine (13.83%), aspartic (11.55%) and threonine (10.11%). Smaller quantities of glutamic (9.13%), serine (9.10%) and lysine (7.84%) are reported for the above period. The other amino acids were present in trace quantities. Here too, like the samples of the 1st day, the undetected amino acids were the same namely, histidine, tyrosine and methionine. Samples of the 5th day, unlike the 1st and 3rd day showed aspartic to be the major contributor to the total amino acid. This was closely followed by alanine (10.87%), serine (10.46%) and threonine (9.65%). The other amino acids contributed between 8 & 3% (Fig. 10C). The undetected amino acids were identical with those for samples of the 1st and 3rd days.

Group compositions showed a sequence of neutral> acidic> basic and finally the aromatic amino acids in the decreasing order of abundance (Table 7.13). The neutral fraction contributed between 55 to 61% for all the day's samples. Acidic group of amino acids was two times lower than the neutral fraction, (22.21%, 20.69% & 21.91%). The basic amino acids made a contribution of 17.15%, 21.99% & 17.33% to the 1st, 3rd & 5th day's samples. Aromatic group of amino acids showed a consistent 5% contribution and was almost 12 times lower than the neutral amino acids for all the days.

Table 7.12 shows the total amino acid content in the microfouling material during December, 1989. Variation in the total concentration for samples of the 1st day (263.85 nmoles.dm-2), 3rd day (393.35 nmoles.dm-2) and the last day (772.63 nmoles.dm-2), were evident for this surface.

The spectral identification of the individual amino acids is graphically represented in Fig 7.11. Glycine dominated for samples collected on the 1st day (20.22%), 3rd day (17.73%) and 5th day (21.79%) of December, 1989. This was followed by other amino acids like alanine, aspartic and serine for samples of all three days. Trace quantities of glutamic, lysine, leucine, threonine, valine, isoleucine, phenylalanine and argenine were observed for

the samples of all the days. Histidine, tyrosine & methionine remained undetected in the amino acid spectrum for samples of all the days. Glycine, serine and glutamic acid showed non-linear change with time (Table 7.12). The other amino acids showed a marked increase with the increase in the study period, for example, aspartic (10.78, 11.17 & 12.93%), leucine (6.91, 7.37 & 7.89%), threonine (6.24, 7.03 & 8.61%), alanine (9.46, 12.98 & 15.52%), valine (5.94, 7.39 & 8.13) and lysine (6.28, 7.04 & 7.92%).

For samples in December, 1989, the variations in the groups of the amino acids were identical with those mentioned for April, 1989 & August, 1989 (Table 7.13). An increasing percentage contribution was seen for neutral amino acids (65.96, 66.33 & 67.17%), for the three days samples. No such trend was evident in the case of acidic and basic amino acids. Aromatic group amino acids however, formed a small portion of the total amino acid and remained almost consistent with time (<4%).

3.2c STAINLESS STEEL

Large fluctuations in the total concentration and relative composition of amino acids were observed for

samples on stainless steel surface (148.21 to 966.38 nmoles.dm-2).

Table 7.14 shows low concentration of the total amino acids for samples of the 1st day (148.21%) during April, 1989. The values increased marginally for samples of the 3rd day (207.06 nmoles.dm-2) and then three times for samples of the 5th day (966.38 nmoles.dm-2), of the same month. Such a distinct change in the total concentrations was also manifested in the individual amino acids (Fig 7.12).

During April, 1989, for samples on stainless steel test panels, only four amino acids were detected in the spectrum. Phenylalanine contributed to 43.18% which was the highest, for the study period. This was followed by aspartic (25.98%), alanine (15.94%) and glutamic (14.91%). Thus, the contribution by each amino acid to the total amino acid concentration was comparatively higher than that observed for the samples of the other days (Fig 7.12A). The 3rd day samples showed glycine to be the most dominant amino acid (20.11%). The other amino acids which made a contribution of less than half of that made by glycine were aspartic (10.73%), phenylalanine (10.37%), serine (9.45%), leucine (8.73%), alanine (8.33%), glutamic (7.67%), and isoleucine (6.74%). The other amino acids which were

present in trace quantities were threonine, valine and lysine. Four amino acids which were quantitatively insignificant were histidine, argenine, tyrosine and methionine (Fig 7.12B).

Glycine was the most dominant amino acid of the microfouling material of the 5th day (27.40%) samples. The percentage contribution of glycine increased from undetectable quantity for samples of the 1st day to 20.11% on the 3rd day samples and 27.40% of the 5th day samples. Serine was next in abundance (11.61%), however, it contributed less than half of that contributed by glycine. Alanine (9.65%), aspartic (9.15%), leucine (6.57%), threonine (6.07%), glutamic (5.70%), lysine (5.53%), valine (5.50%), argenine (4.91%), phenylalanine (4.36%) and isoleucine (3.57%) were lower in quantity. The undetected amino acids were same as those reported for the samples of the 3rd day. The abundant amino acids like aspartic, glutamic, serine etc., showed a linear rise with increase in time as seen in Fig 7.12. The other amino acids showed an irregular pattern.

With reference to Table 7.17, the group compositions of different amino acids for samples of the 1st day showed aromatic amino acids to be most dominant (43.18%). This was closely followed by acidic amino acids (40.89%).

However, the neutral group of amino acid contributed to less than half of the total concentration made by either acidic or aromatic amino acids (15.93%). S-Containing and basic amino acids were unidentified. This was the first case in which basic amino acids were totally absent and in which aromatic amino acid formed the major contributor to the total amino acid content.

Neutral amino acids were dominant for samples obtained during the 3rd (65.23%) and 5th (70.36%) days, contributing significantly, as compared to the 1st day's samples. Acidic (18.40%), aromatic (10.37%) and basic (5.43%) group of amino acids contributed to the total amino acid for samples on the 3rd day, in decreasing order of abundance. For samples of the 5th day, the order was acidic (14.84%)> basic (10.43%) > aromatic (4.36%) amino acids. Neutral amino acids showed a significant increase in the samples of the 1st to the 5th day. Basic amino acids were absent for the 1st day samples. For the samples of the 3rd day, basic amino acid contributed to 5.43% and for the 5th day to 10.43% respectively. A decrease in the contribution of acidic and aromatic amino acids was evident for the microfouling samples formed on stainless steel during April, 1989.

Total amino acid concentration for samples collected during August, 1989, showed a marked variation (Table 7.15). A value of 167.89 nmoles.dm-2 was observed for samples of the 1st day, which showed a decrease on the 3rd day samples to 65.58 nmoles.dm-2. The value further increased by three times on the 5th day (615.14nmoles.dm-2). Such an irregular change was observed on stainless steel surface unlike for any other surface. These changes are mainly attributed to the differences in the amino acid composition.

The individual amino acids showed a marked variation as seen in the spectrum (Fig 7.13A), with values ranging from undetectable quantities of histidine, threonine, argenine, tyrosine and methionine to highest percentage in the case of glycine (25.85%). All the amino acids which were present contributed significantly (>5%) to the total amino acids, they being, serine (14.89%), aspartic (12.34%), phenylalanine (10.86%), alanine (9.70%), glutamic (8.18%), leucine (7.83%), isoleucine (6.17%) and valine (5.50%).

Samples of the 3rd day showed a marked variation in the concentration of individual amino acids. Eight amino acids were totally unidentified due to their minute quantities (Fig 7.13B). Among the six amino acids which

were detected, phenylalanine was most dominant (23.32%). The other amino acids were aspartic (21.33%), alanine (17.98%), glutamic (14.17%), leucine (11.70%) and isoleucine (11.51%), contributing significantly to the total amino acid.

For the samples of the 5th day, the concentration ranged from undetectable quantities of histidine, threonine, tyrosine and methionine to highest concentration of glycine. Thus, it is evident from Table 7.15 that glycine was most dominant with a percentage contribution of 30.23%. This was followed by serine (14.75%) argenine (11.37%) and alanine (10.67%). However, amino acids like aspartic, glutamic, valine, phenylalanine, isoleucine, leucine and lysine made contributions below 6%. to the total amino acid. A lot of discrepancies were observed in the concentration of individual amino acids, for the samples of all the days of the microfouling material formed on stainless steel surface.

The group composition of amino acids is shown in Table 7.17. It was observed that neutral amino acids, dominated in samples of all the days collected during August, 1989. The percentage contribution of samples of the 1st and 5th days was greater than 69%, whereas, on the 3rd day it was only 41.19%. This was followed by acidic

and aromatic amino acids for samples of the 1st and 3rd days, while basic amino acid was totally absent. Samples of the 5th day showed basic > acidic > aromatic amino acids. Basic amino acids showed a scattered distribution, with absence of amino acids for samples of the 1st and 3rd days and presence on the 5th day samples. There was no regular pattern for amino acids group fractions with time during the study period (August). Samples of the 1st day, showed aromatic amino acid to be lower than the neutral amino acids by six times, twice for the samples of the 3rd day and thirteen times for the samples of the 5th day. Contribution by acidic amino acids was lower for samples of the 1st, 3rd and 5th days, by thrice, once & six times respectively. This clearly shows the wide discrepancy in the total, individual as well as the various group fractions of amino acids in the microfouling material developed on stainless steel during August, 1989.

Short term variations in amino acid concentration showed the value of the 1st day samples to be low, (145.32 nmoles.dm-2) during December, 1989. The concentration increased marginally for samples of the 3rd day (162.55 nmoles.dm-2). However, the value of total amino acid of the 5th day samples was three times higher than that of the

amino acids remained to be detected for samples of the 3rd day.

The amino acid spectrum for the samples of the 5th day showed glycine to be the most dominant amino acid (16.11%), closely followed by aspartic (12.08%) and alanine (11.26%). The less significant amino acids contributing between 8 to 4% were phenylalanine, leucine, lysine, glutamic, serine, threonine and argenine. During this sampling also three amino acids namely histidine, tyrosine and methionine were not detected (Fig 7.14B).

Neutral amino acids were the major contributor, especially for samples of the 1st (61.88%) and 5th (60.73%) days. Samples of the 3rd day also, neutral amino acids to be dominant, but their concentrations were very much lower than that observed for the other two days (38.37%) samples. This was followed by acidic (23.65%) and basic (14.46%) amino acids for samples of the 1st day. On the other hand for samples on the 3rd day, the order of decreasing abundance for group fractions were basic (24.40%), acidic (19.61%) and aromatic (17.63%). Samples of the 5th day showed acidic (19.05%)> basic (11.73%) > aromatic (8.49%) amino acid groups. Aromatic amino acids were totally undetected for samples of the 1st day and the contribution of basic amino acids were low.

3.3 Relative comparison between surfaces (Fig 7.15)

Total amino acid content (sum of all amino acids) of the microfouling material ranged from 301.34 to 2185.37, 235.17 to 859.88 and 65.58 to 966nmoles.dm-2, for aluminium, fibreglass and stainless steel respectively (Table 7.15). The relative amino acid composition of the microfouling material showed large variations for the three substrata during the period of immersion (Fig 7.15)

Aluminium test panels showed a total number of twelve to fifteen individual amino acids for the entire study period. Aluminium was the only surface to show the presence of sulphur-containing amino acids. That too, this fraction was observed only for samples during the month of August, 1989.

Fibreglass, however, showed twelve to thirteen individual amino acids. The amino acids observed on aluminium and not seen on fibreglass were histidine, tyrosine, and methionine.

The number of individual amino acids on stainless steel surface was still less, (< 12). Amino acids like glycine, phenylalanine and serine were more abundant on this surface. Stainless steel as compared to the other

surfaces showed wide variations with respect to total as well as individual amino acids.

In most cases, for all surfaces, glycine was the most abundant amino acid. The other abundant amino acids were aspartic and alanine serine etc. When the amino acid fractions were compared, most of the time all the surfaces showed neutral amino acids to be most dominant. This fraction was followed by acidic, basic, aromatic and sulphur-containing amino acids.

Statistical correlation analysis between the total amino acid of the microfouling material with the various hydrographic parameters and biotic parameters are shown in Table 7.18).

4 DISCUSSION

The present gradient conditions used is most favourable for the separation and identification of the amino acid composition. Highly purified solvents used during the course of extraction, derivatization and separation, lead to minimal contamination and gave highly reliable results. Calibration of standard amino acids were carefully carried out and linearity of each amino acid was significantly derived ($p<0.001$) by analysis

between peak areas and concentration (Fig 7.1). Analysis of amino acids using HPLC involves fewer transfer steps and rapid derivatization which are important for their accurate quantification (Lindroth & Mopper, 1979). A blank test to check contaminants in the procedure showed that alanine was the only contaminant (Fig 7.2).

Organic matter in seawater consists of small fractions of chemically unstable substances, one such compound is amino acid. Amino acids are released by living organisms and are subjected to rapid biological and chemical transformation (Fogg, 1975; Bada Lee, 1977). The variation in the total amino acid distribution pattern is caused by a net result of two processes i.e., its release by planktons & detrital materials and its utilization by heterotrophic organisms (Wanjersky, 1978).

Examination of the quantitative data for the suspended particulate matter (SPM) from the subsurface waters revealed that, the total amino acids for the study period ranged between 1150.32 & 2783.71nmoles/l. Studies on amino acid concentration in both coastal and offshore waters were reported earlier by Daumas, (1976); William & Yentsch (1976); Dawson & Pritchard, (1978); Garrasi et al, (1979); Jorgensen, (1982) and Mopper & Lindroth, (1982). Concentrations obtained for the present study tallied well

with reports made by Daumas, (1976) for coastal waters of the Gulf of Marseille; Jorgensen, (1982) for shallow estuary on the east coast of Jutland, Denmark (189-1861nmoles) and Siezen & Mague, (1978), for the coastal waters of the Pacific (370-2260nmoles). On the other hand, open waters like Irish Sea, Baltic Sea and Pacific Ocean, showed amino acid concentration to be less than 300nmoles (Riley & Segar, 1970; Dawson & Gocke, 1978; Siezen & Mague, 1978). These observations clearly indicate coastal waters to be highly productive as compared to open sea or offshore waters.

Amino acids from the microfouling material formed on test surfaces showed values ranging between 65.58 & 2185nmoles.dm-2, for the period under study. Comparison of this data with other microfouling data was not possible due to the absence of such studies. It was found that the total concentration of amino acids obtained in the present study agreed with data published by Pocklington (1971), Williams & Le B (1975), Whittle (1977) for phytoplanktons.

The change in the total amino acids with time and substratum is mainly attributed to the change in its various individual amino acid composition. Glycine, alanine, aspartic, leucine, serine, glutamic, valine, lysine, isoleucine, threonine, argenine, phenylalanine,

histidine and tyrosine (in the order of decreasing abundances) formed the major amino acids in the SPM of the sub-surface waters (~1m) of Dona Paula. In addition to the above mentioned amino acids from SPM, methionine was also quantified for the microfouling material.

The contribution of these amino acids to the total amino acid of the microfouling material varied with the substratum used and the period of immersion (Fig 7.15). It is obvious that microfouling biomass is influenced by environmental factors, biological factors and by components of the surface substratum including surface chemistry and surface topography (Martinez et al, 1984; Yanshun et al, 1984; Characklis & Escher, 1988). All these factors must have influenced the development of microfouling on the substrata used, thereby, affecting the amino acid composition of the microfouling material.

The abundant amino acids like glycine, alanine, aspartic, serine, etc., observed for the present study was also reported by Daumas (1976), for phytoplanktons. Higher quantities of serine, glycine, threonine could be due to their association with polyphenols which renders the amino acid resistant to thermal decomposition (Degens, 1970; Siezen & Mague, 1978).

The concentration of glutamic acid was significant, both in the sub-surface waters and in the microfouling material from surfaces. Glutamic acid is known to play an essential role in ammonium uptake mechanisms of phytoplankton (Syrett, 1981).

The contribution made by aspartic acid to the total amino acid was found to be much higher than glutamic acid in the SPM of the subsurface waters of Dona Paula as well as on surfaces. Selective utilization of glutamic acid by bacteria, could probably be one of the reason for the above feature. Similar studies, on the utilization of glutamic acid by bacteria was shown by Gocke, (1970) in a culture medium and by William and Yentsch, (1976) in natural populations.

Threonine, valine, alanine, leucine etc. are components of diatomaceous cell wall (Hecky et al, 1973). Low concentration of valine was observed on test surfaces whereas, the abundance of valine was evident in the water samples. Such a high concentration of valine in the sub-surface waters is due to the contribution of this amino acid by zooplanktons. Zooplanktons also contains tyrosine and methionine. Hence it appears that valine, tyrosine and methionine are good indicators of zooplankton origin, which was scarce on test surfaces. Thus amino acids can be used

as biomarkers of the source of microfouling material.

The present study for the microfouling material, unlike for the water samples revealed increase in the concentration of amino acids with time as shown in Fig 7.15, for all the surfaces. This may probably suggest the age of the settled organisms to influence the variation in concentration. Similar variation in the amino acid composition and physiological state of a cell have been shown in enclosure and insitu experiments under natural light conditions. For example by Hammer et al, (1981), for Thalassiosira botula and Poulet & Jezequel, (1983) for Chaetoceros debile. These authors mentioned a pronounced release of amino acid during the exponential growth phase. Hence a paralleled variation was observed between amino acid concentration and cell density. These results may very well be compared with our studies, where, instead of taking an individual culture we consider a mixed culture of several organisms on the surface of test panels and probably the 3rd and 5th day of sampling, representing the exponential phase of these organisms.

Undetectable or low concentrations of some of the amino acids on surfaces, was observed for the initial sampling period. Similar condition was observed during a laboratory experiment containing a pure rotula culture,

when no or few plankton cells were present (Hammer & Eberlein, (1981). In contrast to this, there are several amino acids which increased with the period of exposure, probably indicating increase in age and number of cells with time (Poulet & Jezequel, 1983).

The present study showed the following order of abundance for group fractions: neutral> acidic> basic> aromatic> sulphur-containing amino acids. Neutral amino acids formed a high percentage for the SPM (59 to 69%) as well as on surfaces (34 to 70%). This is mainly due to the abundance of the same constituents in planktonic material (Daumas, 1976), and also due the refractory nature of these amino acids in the microfouling material (Faganeli, 1989).

Among the neutral amino acids, threonine, although quite abundant, is not very stable all by itself. Relative abundance of threonine in the microfouling material may probably be due to its association with polyphenols which renders the amino acids more resistant to thermal decomposition (Degens, 1970). Serine and glycine to some extent also combined with phenols due to the presence of a free hydroxyl group. Hence these compounds are also quite resistant to environmental degradation (Siezen & Mague, 1978). Neutral amino acids, like valine, leucine and

isoleucine which have a branched chain structure are least stable in the marine environment (Gonzalez, 1983). Their concentration on test panels showed a non linear relation with time due to their instability.

In most cases, acidic group of amino acids (aspartic & glutamic) followed the neutral amino acids. The percentage distribution (mole%) of acidic amino acids, varied between 11 to 20% for the suspended particulate matter (Table 7.5) and 11 to 40% for the microfouling material (Table 7.9, 7.13 & 7.17).

Acidic amino acids are reported to be abundant in planktons (Gonzalez, 1983); bacteria (Henriques & Farrington, 1980); in suspended matter (Siezen & Mague, 1978); terrestrial humic hydrolysate (Khan & Sowden, 1972) and to some extent in land plants (Akiyama & Johns, 1972; Kemp & Mundrachova, 1973). In lower algae like diatoms, the concentration of aspartic amino acid was higher than glutamic acid, whereas, in higher algae, like those of rhodophyta, phaeophyta and chlorophyta, glutamic acid concentration was found to be several times higher than that of aspartic as reported by Munda & Gubensek, (1986).

From the above discussion, the most likely source of amino acid in the microfouling material developed on

various substrata are bacteria, algae (diatom), protozoa, fungi larvae of macrofoulers and detritus material derived from marine sources and terrestrial vascular plants. It is therefore, to be expected that atleast, during the period of study, the amino acid in the microfouling material are derived mainly from these sources. However, since microscopic observation of the fouling material did not reveal the presence of fungi, protozoa and larvae of macrofoulers, it can be concluded that these were not the major source of amino acids. Thus, amino acids from the microfouling material must have been derived from bacteria, diatom and terrestrial sources.

The aspartic/glutamic amino acid ratio is a sensitive indicator of microalgal contribution (Liebezeit & Bodungen, 1987). In the present study this ratio was found to be less than one for the Northern Adriatic. Microfouling material predominant in glycine and having a low aspartic/glutamic amino acid ratio (< 1), implies that the microfouling material was relatively abundant in diatomaceous material (Munda & Gubensek, 1986). This was further supported by the abundance of glycine in the microfouling material, because Hecky et al, (1973) earlier reported that diatom cell wall generally contain glycine as the most abundant amino acid.

The basic amino acids which constituted of lysine, argenine and histidine, contributed 11 to 14% to the total amino acid, for the SPM and 5 to 24% for the microfouling material. In most cases, for the present study, acidic amino acids were dominated over the basic amino acids. However, a predominance of basic over acidic amino acids for offshore samples was reported by Gonzalez, (1983). Histidine, a basic amino acid was suggested to be a decay product of phytoplanktons (Daumas, 1976). A low level of histidine, indicated that microfouling material contained more of fresh and living material.

Aromatic amino acids which comprised of tyrosine & Phenylalanine were the least detected amino acids ranging between 4 to 15% for SPM and 3 to 23% for the microfouling material. These amino acids may also be used as indicator of the age or freshness of the particulate matter (Montani & Okaichi, 1985). The small quantities of this group of amino acids on surfaces, either indicate that they are least stable or they are largely utilized in the marine ecosystem.

Sulphur-containing amino acid (methionine) was undetected for the water samples and contributed to less than 5% to the total amino acid for the microfouling material. This variation in the sulphur-containing amino

acid suggests that amino acids are rapidly recycled in the marine environment. Methionine is also a good indicators of zooplanktons, suggesting thereby, that the zooplankton contribution to the microfouling material was near to minimum.

Microfouling is known to be influenced by the hydrographic parameters. However, statistical analysis of the total amino acids showed no significant correlation with hydrographic parameters (Table 7.18). On the other hand, Liu & Hellebust (1974); Wheeler et al, (1974, 1977), showed negative relationships, in which they concluded that, deprivation of nitrogen in the growth medium produces a marked increase in the rate of amino acid uptake by phytoplankton.

Total amino acid was significantly correlated with diatom as well as bacterial cell abundance from surfaces, (except for stainless steel which showed poor correlation). This probably suggests that, the above mentioned organisms are the major contributors of amino acids on surfaces. Thus, indicating biological agents to be a significant source of these compounds.

In summary, the present data suggests a large variation in the concentration of individual amino acids in the microfouling material with time and surface. It

appears that diatoms, bacteria and detrital material are the major contributors of amino acids on various substrata.

Table 7.1

The gradient conditions used for the seperation of amino acids.

Time (min)	Flow	Composition %A	%B	Duration (min)
0.01	1.50	85	15	2.00
2.02	1.50	76	24	0.50
2.54	1.50	76	24	2.40
5.00	1.50	55	45	7.00
12.02	1.50	55	45	3.00
15.04	1.50	30	70	8.00
23.06	1.50	13	87	2.00
25.08	1.50	13	87	3.00
28.10	1.50	00	100	0.50
30.OO	1.50	00	100	3.00

%A = acetate buffer solution
%B = methanol

Table 7.2

Short term variations in the concentration of amino acids of the particulate matter from the subsurface waters (-1m) of Dona Paula during April, 1989.

	First day		Fifth day	
AMINO ACIDS	A	B	A	B
ASPARTIC	253.14	9.02	239.04	10.66
GLUTAMIC	153.57	5.47	153.31	6.84
SERINE	319.64	11.40	162.23	7.24
HISTIDINE	48.48	1.73	51.24	2.29
GLYCINE	828.44	29.53	312.495	13.94
THREONINE	161.09	5.74	154.63	6.90
ARGENINE	136.74	4.87	118.18	5.27
ALANINE	209.02	7.45	246.98	11.02
TYROSINE	ND	ND	18.11	0.81
METHIONINE	ND	ND	ND	ND
VALINE	139.3	4.97	144.81	6.46
PHENYLALANINE	105.43	3.76	109.195	4.87
ISOLEUCINE	93.27	3.33	120.29	5.37
LEUCINE	180.14	6.42	173.25	7.73
LYSINE	155.45	5.54	152.60	6.81
TOTAL	2783.71		2156.36	

ND = Not detected
A = nmoles/l
B = Mole%

Table 7.3

Short term variations in the concentration of amino acids of the particulate matter from the subsurface waters (~1m) of Dona Paula during August, 1989

	First day		Fifth day	
AMINO ACIDS	A	B	A	B
ASPARTIC	182.25	11.82	142.93	11.93
GLUTAMIC	115.01	7.46	90.30	7.54
SERINE	125.24	8.12	94.74	7.91
HISTIDINE	ND	ND	ND	ND
GLYCINE	193.49	12.55	164.46	13.73
THREONINE	104.96	6.81	88.17	7.36
ARGENINE	67.59	4.38	58.78	4.91
ALANINE	165.89	10.76	125.50	10.47
TYROSINE	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND
VALINE	106.84	6.93	75.60	6.31
PHENYLALANINE	91.80	5.95	55.87	4.66
ISOLEUCINE	92.48	6.00	73.11	6.10
LEUCINE	124.45	8.07	91.35	7.62
LYSINE	106.01	6.88	89.51	7.47
TOTAL	1476.01		1150.32	

ND = Not detected
A = nmoles/l
B = Mole%

Table 7.4

Short term variations in the concentration of amino acids of the particulate matter from the subsurface waters (~1m) of Dona Paula during December, 1989

	First day		Fifth day	
AMINO ACIDS	A	B	A	B
ASPARTIC	205.95	11.46	258.32	11.42
GLUTAMIC	ND	ND	184.13	8.14
SERINE	218.41	12.15	227.34	10.05
HISTIDINE	ND	ND	ND	ND
GLYCINE	404.47	22.51	555.67	24.56
THREONINE	115.10	6.40	148.32	6.56
ARGENINE	ND	ND	ND	ND
ALANINE	203.09	11.30	240.70	10.64
TYROSINE	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND
VALINE	131.05	7.29	132.99	5.88
PHENYLALANINE	262.45	14.60	137.77	6.09
ISOLEUCINE	73.67	4.10	92.56	4.09
LEUCINE	113.41	6.31	153.68	6.79
LYSINE	ND	ND	130.83	5.78
Total	1727.60		2262.31	

ND = Not detected
A = nmoles/l
B = Mole%

Table 7.5

Short term variation in the group composition of amino acid of the particulate matter from the surface waters (~1m) of Dona Paula during April, August and December, 1989.

AMINO ACIDS	1st day	5th day
APRIL, 1989		
NEUTRAL	68.83	60.98
ACIDIC	14.49	18.19
BASIC	12.14	14.93
AROMATIC	3.75	5.90
AUGUST, 1989		
NEUTRAL	61.89	61.99
ACIDIC	20.14	20.27
BASIC	11.76	12.89
AROMATIC	6.21	4.85
DECEMBER, 1989		
NEUTRAL	72.89	68.56
ACIDIC	11.92	19.55
BASIC	ND	5.78
AROMATIC	15.19	6.08

ND= Not detected

Table 7.6

Short term variation in the concentration of amino acids from the microfouling material developed on aluminium surface when exposed to the sub-surface waters (~1m) of Dona Paula during April, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	31.06	10.31	239.04	11.08	199.00	9.11
GLUTAMIC	26.83	8.90	153.31	7.11	166.77	7.63
SERINE	38.58	12.80	162.23	7.52	202.14	9.25
HISTIDINE	ND	ND	51.24	2.38	54.13	2.48
GLYCINE	46.28	15.36	312.495	7.17	207.11	13.80
THREONINE	25.67	8.52	154.63	5.48	207.11	9.48
ARGENINE	19.58	6.50	118.18	7.17	134.94	6.17
ALANINE	27.83	9.24	246.98	11.45	260.34	11.91
TYROSINE	ND	ND	18.11	0.84	24.83	1.14
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	11.02	3.66	144.81	6.72	82.27	3.76
PHENYLALANINE	16.82	5.58	109.195	5.06	94.7	4.33
ISOLEUCINE	15.92	5.28	120.29	5.58	97.66	4.47
LEUCINE	19.84	6.58	173.25	8.03	142.87	6.54
LYSINE	21.11	7.01	152.6	7.08	185.85	8.50
TOTAL	300.54		2156.36		2185.37	

ND = Not detected
A= nmoles.dm-2
B= Mole%

Table 7.7

Short term variation in the concentration of amino acids from the microfouling material developed on aluminium surface when exposed to the subsurface waters (~1m) of Dona Paula during August, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	44.38	10.76	36.68	5.58	65.61	8.05
GLUTAMIC	33.29	8.07	37.82	5.75	51.82	6.36
SERINE	52.74	12.79	68.92	10.49	84.21	10.34
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	63.78	15.46	183.50	27.92	201.97	24.79
THREONINE	36.53	8.86	ND	ND	ND	ND
ARGENINE	21.56	5.23	71.44	10.87	80.63	9.90
ALANINE	54.33	13.17	47.12	7.17	82.18	10.09
TYROSINE	ND	ND	ND	ND	29.34	3.60
METHIONINE	8.12	1.97	34.73	5.28	40.23	4.94
VALINE	12.88	3.12	55.09	8.38	ND	ND
PHENYLALANINE	15.89	3.85	36.36	5.53	40.72	5.00
ISOLEUCINE	15.77	3.82	18.89	2.87	33.58	4.12
LEUCINE	23.42	5.68	41.47	6.31	55.06	6.76
LYSINE	29.73	7.21	25.51	3.88	41.47	5.09
TOTAL	412.42		657.53		806.82	

ND = Not detected
A= nmoles.dm-2
B= Mole%

Table 7.8

Short term variation in the concentration of amino acids from the microfouling material developed on aluminium surface when exposed to the sub-surface waters (~1m) of Dona Paula during December, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	58.13	11.68	72.96	11.66	131.89	9.78
GLUTAMIC	ND	ND	50.68	8.10	118.90	8.82
SERINE	ND	ND	73.47	11.75	119.60	8.87
HISTIDINE	ND	ND	ND	ND	42.18	3.13
GLYCINE	ND	ND	96.66	15.45	169.14	12.54
THREONINE	ND	ND	55.71	8.91	110.38	8.18
ARGENINE	133.6	26.84	34.92	5.58	97.40	7.22
ALANINE	ND	ND	2.94	0.47	145.53	10.79
TYROSINE	26.42	5.31	ND	ND	20.39	1.51
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	92.76	18.64	20.33	3.25	46.03	3.41
PHENYLALANINE	ND	ND	26.37	4.22	56.78	4.21
ISOLEUCINE	48.01	9.65	22.08	3.53	54.94	4.07
LEUCINE	ND	ND	36.53	5.84	87.17	6.46
LYSINE	43.82	8.80	51.35	8.21	139.22	10.32
TOTAL	402.74		544.54		1339.55	

ND = Not detected
A= nmoles.dm-2
B= Mole%

Table 7.9

Short term variation in the group composition of amino acids from the microfouling material developed on aluminium surface when exposed to the surface waters (~1m) of Dona Paula during April, August & December, 1989

AMINO ACIDS	1st day	3rd day	5th day
	APRIL, 1989		
NEUTRAL	61.43	60.97	58.56
ACIDIC	19.21	18.20	17.75
BASIC	13.50	14.93	18.20
AROMATIC	5.58	5.90	5.47
S-CONTAINING	ND	ND	ND
	AUGUST, 1989		
NEUTRAL	64.43	63.15	56.09
ACIDIC	12.50	11.34	14.41
BASIC	12.43	11.05	15.18
AROMATIC	3.85	5.53	8.60
S-CONTAINING	1.79	5.28	4.94
	DECEMBER, 1989		
NEUTRAL	34.95	56.56	54.70
ACIDIC	14.43	22.72	18.72
BASIC	44.05	15.85	20.81
AROMATIC	6.56	4.84	5.75
S-CONTAINING	ND	ND	ND

ND= Not detected

Table 7.10

Short term variation in the concentration of amino acids from the microfouling material developed on fibre-glass surface when exposed to the subsurface waters (~1m) of Dona Paula during April, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	31.94	11.91	54.38	10.85	96.88	11.28
GLUTAMIC	20.30	7.57	35.73	7.13	60.62	7.06
SERINE	28.44	10.61	50.54	10.08	71.24	8.29
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	53.99	20.13	95.48	19.05	160.16	18.64
THREONINE	15.70	5.85	32.99	6.58	58.11	6.76
ARGENINE	3.76	5.13	26.35	5.26	47.62	5.54
ALANINE	25.30	9.43	52.95	10.56	92.62	10.78
TYROSINE	ND	ND	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	16.43	6.13	34.09	6.80	62.18	7.24
PHENYLALANINE	10.73	4.00	20.89	4.17	36.42	4.24
ISOLEUCINE	13.83	5.16	25.51	5.09	45.19	5.26
LEUCINE	20.39	7.60	35.28	7.04	72.81	8.47
LYSINE	17.36	6.47	32.62	6.51	56.03	6.52
TOTAL	268.17		496.81		859.88	

D = Not detected
A = nmoles.dm-2
B = Mole%

Table 7.11

Short term variation in the concentration of amino acids from the microfouling material developed on fibreglass surface when exposed to the sub-surface waters (~1m) of Dona Paula during August,1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	28.78	12.22	39.81	11.55	44.72	12.41
GLUTAMIC	23.55	10.00	31.47	9.13	28.47	7.90
SERINE	30.49	12.94	31.34	9.10	37.67	10.46
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	41.33	17.54	47.65	13.83	28.58	7.93
THREONINE	19.02	8.07	34.82	10.11	34.76	9.65
ARGENINE	11.64	4.94	17.24	5.00	25.63	7.12
ALANINE	23.60	10.02	48.79	14.16	39.14	10.87
TYROSINE	ND	ND	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	7.34	3.12	14.4	4.18	13.11	3.64
PHENYLALANINE	11.81	5.01	17.39	5.05	18.41	5.11
ISOLEUCINE	8.43	3.58	14.36	4.17	12.67	3.52
LEUCINE	12.84	5.45	20.00	5.80	18.57	5.16
LYSINE	16.8	7.13	27.00	7.84	32.25	8.95
TOTAL	235.63		344.27		360.22	

ND = Not detected
A = nmoles.dm-2
B = Mole%

Table 7.12

Short term variation in the concentration of amino acids from the microfouling material developed on fibreglass surface when exposed to the sub-surface waters (~1m) of Dona Paula during December, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	28.45	10.78	43.92	11.17	87.06	12.93
GLUTAMIC	19.02	7.21	25.42	6.46	53.41	7.93
SERINE	31.77	12.04	36.46	9.27	71.66	10.64
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	53.34	0.22	69.73	17.73	146.69	21.79
THREONINE	16.46	6.24	27.64	7.03	57.97	8.61
ARGENINE	12.92	4.90	18.63	4.74	30.79	4.57
ALANINE	24.97	9.46	51.05	12.98	104.52	15.52
TYROSINE	ND	ND	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	15.67	5.94	29.05	7.39	54.71	8.13
PHENYLALANINE	12.85	4.87	16.80	4.27	29.20	4.34
ISOLEUCINE	13.62	5.16	17.91	4.55	30.20	4.49
LEUCINE	18.22	6.91	29.05	7.39	53.12	7.89
LYSINE	16.56	6.28	27.68	7.04	53.30	7.92
TOTAL	263.85		393.34		772.63	

ND = Not detected
A = nmoles.dm-2
B = Mole%

Table 7.13

Short term variation in the group composition of amino acids from the microfouling material developed on fibre glass surface when exposed to the sub-surface waters (~1m) of Dona Paula during April, August & December, 1989.

AMINO ACIDS	1st day	3rd day	5th day
	APRIL, 1989		
NEUTRAL	64.91	65.78	65.05
ACIDIC	19.48	18.13	18.33
BASIC	11.60	11.86	12.06
AROMATIC	4.00	4.20	4.24
S-CONTAINING	ND	ND	ND
	AUGUST, 1989		
NEUTRAL	60.71	61.34	55.65
ACIDIC	22.21	20.69	21.91
BASIC	12.07	21.99	17.33
AROMATIC	5.01	5.05	5.11
S-CONTAINING	ND	ND	ND
	DECEMBER, 1989		
NEUTRAL	65.96	66.33	67.17
ACIDIC	17.99	17.63	18.18
BASIC	11.17	11.77	10.88
AROMATIC	4.87	4.27	3.77
S-CONTAINING	ND	ND	ND

ND= Not detected

Table 7.14

Short term variation in the concentration of amino acids from the microfouling material developed on stainless steel surface when exposed to the sub-surface waters (~1m) of Dona Paula during April, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	38.51	25.98	22.57	10.73	88.41	9.15
GLUTAMIC	22.10	14.91	16.13	7.67	55.08	5.70
SERINE	ND	ND	19.88	9.45	112.16	11.61
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	ND	ND	42.3	20.11	264.77	27.40
THREONINE	ND	ND	10.81	5.14	58.62	6.07
ARGENINE	ND	ND	ND	ND	47.41	4.91
ALANINE	23.62	15.94	17.51	8.33	93.22	9.65
TYROSINE	ND	ND	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	ND	ND	12.05	5.73	53.11	5.50
PHENYLALANINE	63.99	43.18	21.82	10.37	42.17	4.36
ISOLEUCINE	ND	ND	14.17	6.74	34.53	3.57
LEUCINE	ND	ND	18.36	8.73	63.50	6.57
LYSINE	ND	ND	11.40	5.42	53.40	5.53
TOTAL	148.21		207.00		966.38	

ND= Not detected
A = nmoles.dm-2
B = Mole%

Table 7.15

Short term variation in the concentration of amino acids from the microfouling material developed on stainless steel surface when exposed to the subsurface waters (~1m) of Dona Paula during August, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	20.44	12.34	13.99	21.33	37.75	6.13
GLUTAMIC	13.54	8.18	9.29	14.17	26.77	4.35
SERINE	24.66	14.89	ND	ND	90.79	14.75
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	42.80	25.85	ND	ND	186.00	30.23
THREONINE	ND	ND	ND	ND	ND	ND
ARGENINE	ND	ND	ND	ND	69.96	11.37
ALANINE	16.07	9.70	11.79	17.98	65.65	10.67
TYROSINE	ND	ND	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	9.11	5.50	ND	ND	32.46	5.27
PHENYLALANINE	17.99	10.86	15.29	23.32	31.32	5.09
ISOLEUCINE	10.22	6.17	7.55	11.51	15.401	2.50
LEUCINE	12.97	7.83	7.67	11.70	35.67	5.79
LYSINE	ND	ND	ND	ND	23.37	3.80
TOTAL	167.80		65.58		615.14	

ND= Not detected
A = nmoles.dm-2
B = Mole%

Table 7.16

Short term variation in the concentration of amino acids from the microfouling material developed on stainless steel surface when exposed to the sub-surface waters (~1m) of Dona Paula during December, 1989

	First day		Third day		Fifth day	
AMINO ACIDS	A	B	A	B	A	B
ASPARTIC	19.41	13.35	18.61	11.45	68.91	12.08
GLUTAMIC	14.96	10.29	13.26	8.16	39.83	6.98
SERINE	21.54	14.82	ND	ND	35.24	6.18
HISTIDINE	ND	ND	ND	ND	ND	ND
GLYCINE	39.80	27.38	ND	ND	91.92	16.11
THREONINE	ND	ND	ND	ND	36.65	6.42
ARGENINE	9.46	6.51	ND	ND	26.31	4.61
ALANINE	15.40	10.60	19.3	11.87	64.25	11.26
TYROSINE	ND	ND	ND	ND	ND	ND
METHIONINE	ND	ND	ND	ND	ND	ND
VALINE	ND	ND	11.73	7.22	41.88	7.34
PHENYLALANINE	ND	ND	28.65	17.63	48.43	8.49
ISOLEUCINE	ND	ND	15.17	9.33	31.88	5.59
LEUCINE	13.19	9.08	16.17	9.95	44.73	7.84
LYSINE	11.56	7.95	39.66	24.40	40.65	7.12
TOTAL	145.32		162.55		570.68	

ND= Not detected
A = nmoles.dm-2
B = Mole%

Table 7.17

Short term variation in the group composition of amino acids from the microfouling material developed on stainless steel surface when exposed to the sub-surface waters (~1m) of Dona Paula during April, August & December, 1989

AMINO ACIDS	1st day	3rd day	5th day
	APRIL, 1989		
NEUTRAL	15.93	65.80	70.36
ACIDIC	40.89	18.40	14.84
BASIC	ND	5.43	10.43
AROMATIC	43.18	10.37	4.36
S-CONTAINING	ND	ND	ND
	AUGUST, 1989		
NEUTRAL	69.02	41.19	69.24
ACIDIC	20.25	35.50	10.49
BASIC	ND	ND	15.17
AROMATIC	10.72	23.32	5.09
S-CONTAINING	ND	ND	ND
	DECEMBER, 1989		
NEUTRAL	61.88	38.37	60.73
ACIDIC	23.65	19.61	19.05
BASIC	14.46	24.00	11.73
AROMATIC	ND	17.63	8.49
S-CONTAINING	ND	ND	ND

ND= Not detected

Table 7.18

Statistical correlation between total amino acids of the microfouling material with various hydrographic and some biotic parameters, (n=9)

	Aluminium	Fibreglass	Stainless steel
Temperature	r=0.44 p<0.1	r=0.50 p=0.1	r=0.36 p=NS
Salinity	r=0.36 p=NS	r=0.46 p=NS	r=0.27 p=NS
Dissolved oxygen	r=-0.44 p<0.1	r=-0.53 p<0.1	r=-0.34 p=NS
Nitrate	r=0.14 p=NS	r=0.30 p=NS	r=0.05 p=NS
Silicate	r=0.2 p=NS	r=0.31 p=NS	r=0.12 p=NS
F-Diatom number	r=0.81 p<0.01	r=0.06 p=NS	r=0.69 p<0.02
F-Bacterial number	r=0.80 p<0.01	r=0.38 p=NS	r=0.86 p<0.01

r= correlation coefficient
p= level of significance
F= fouling
NS= not significant

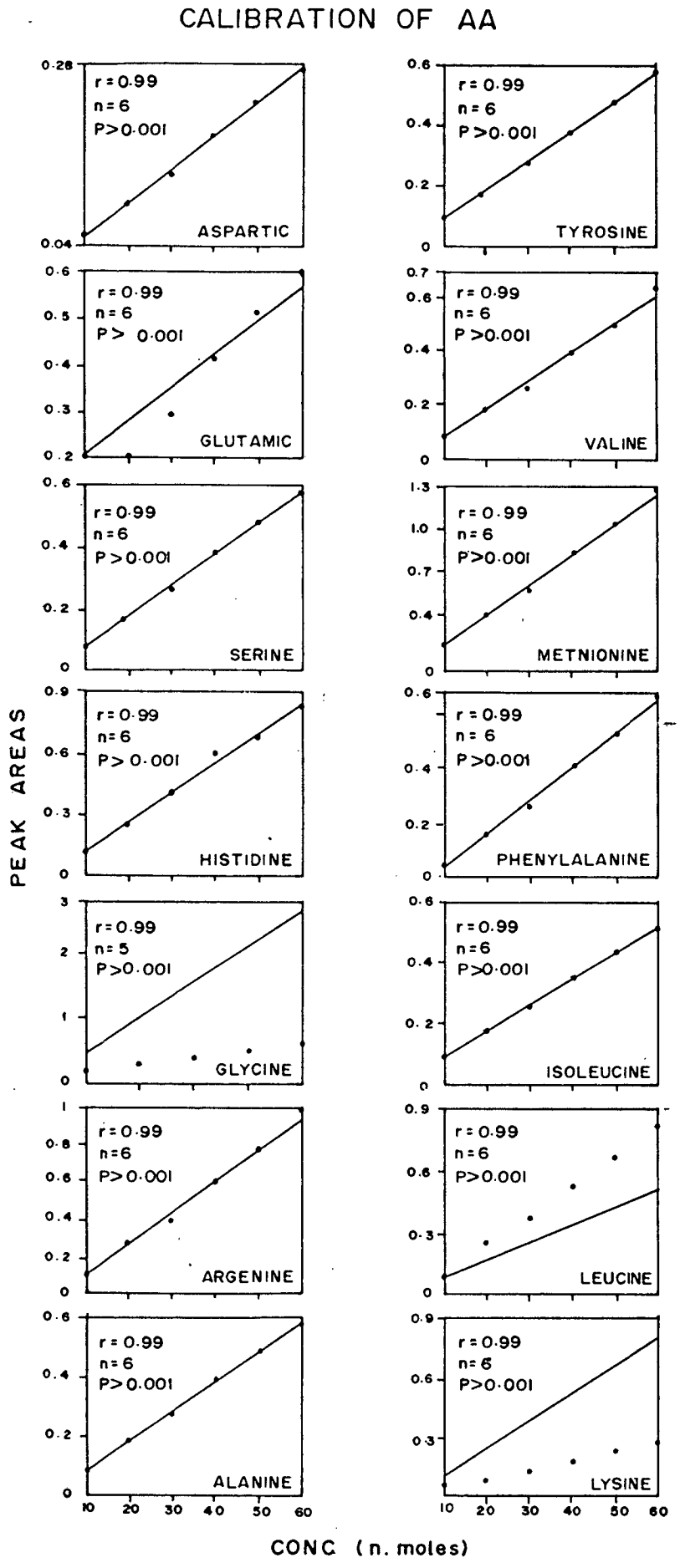

Fig. 7.1 RELATIONSHIP BETWEEN THE PEAK AREA & CONCENTRATION OF THE INDIVIDUAL AMINO ACID'S.

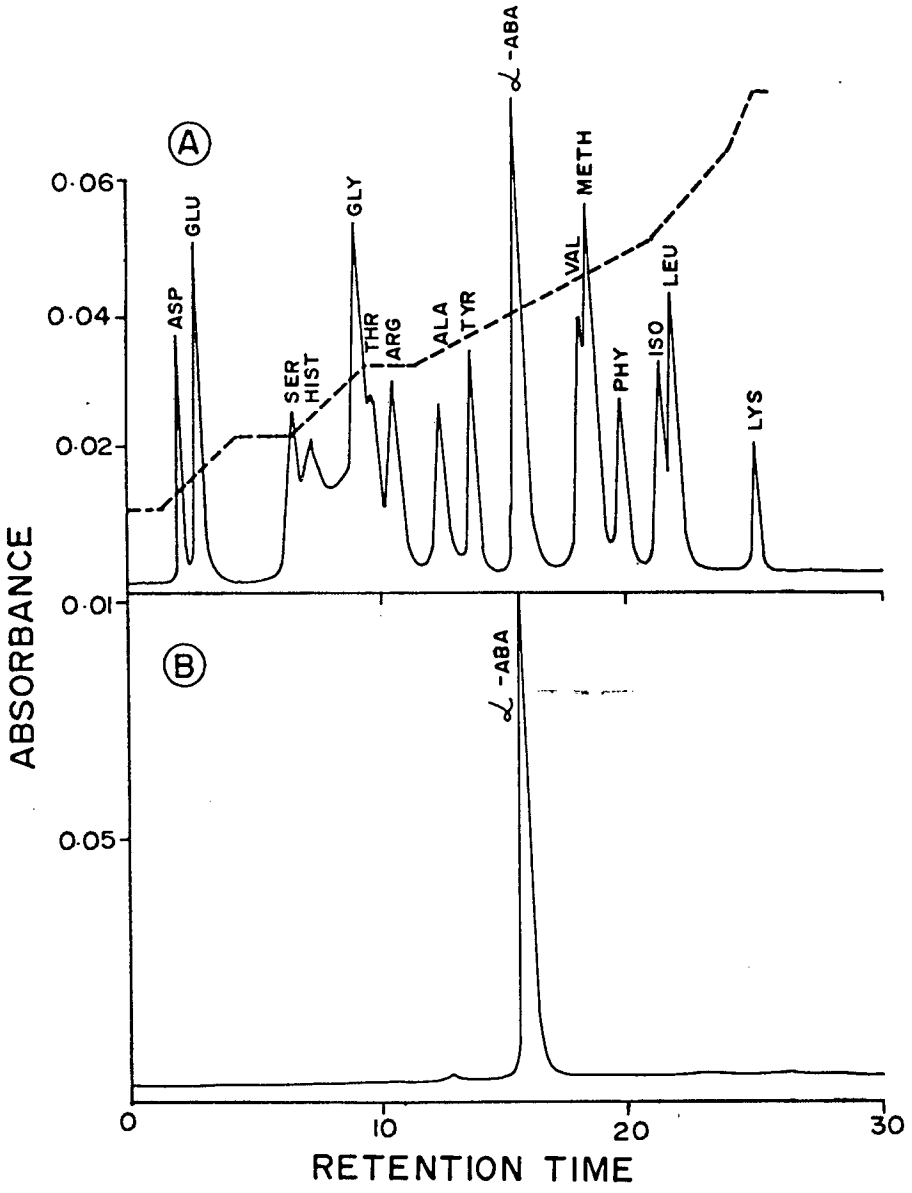

Fig.7.2 A- HPLC ANALYSIS OF STANDARD AMINO ACID'S
B- BLANK

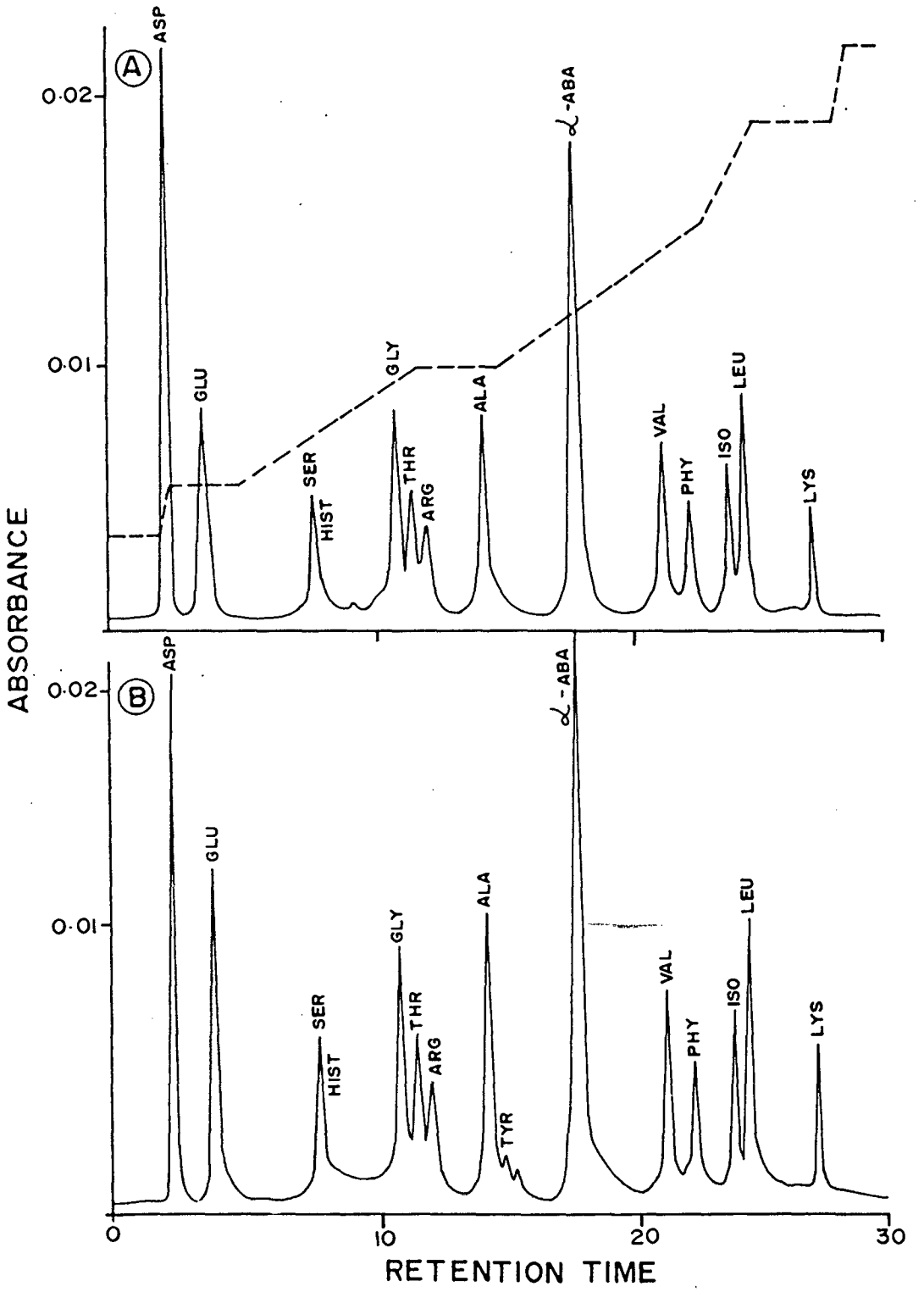

Fig.7.3 AMINO ACIDS OF SUSPENDED PARTICLES FROM THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st. (A) & 5th. (B) DAY OF SAMPLING DURING APRIL '89

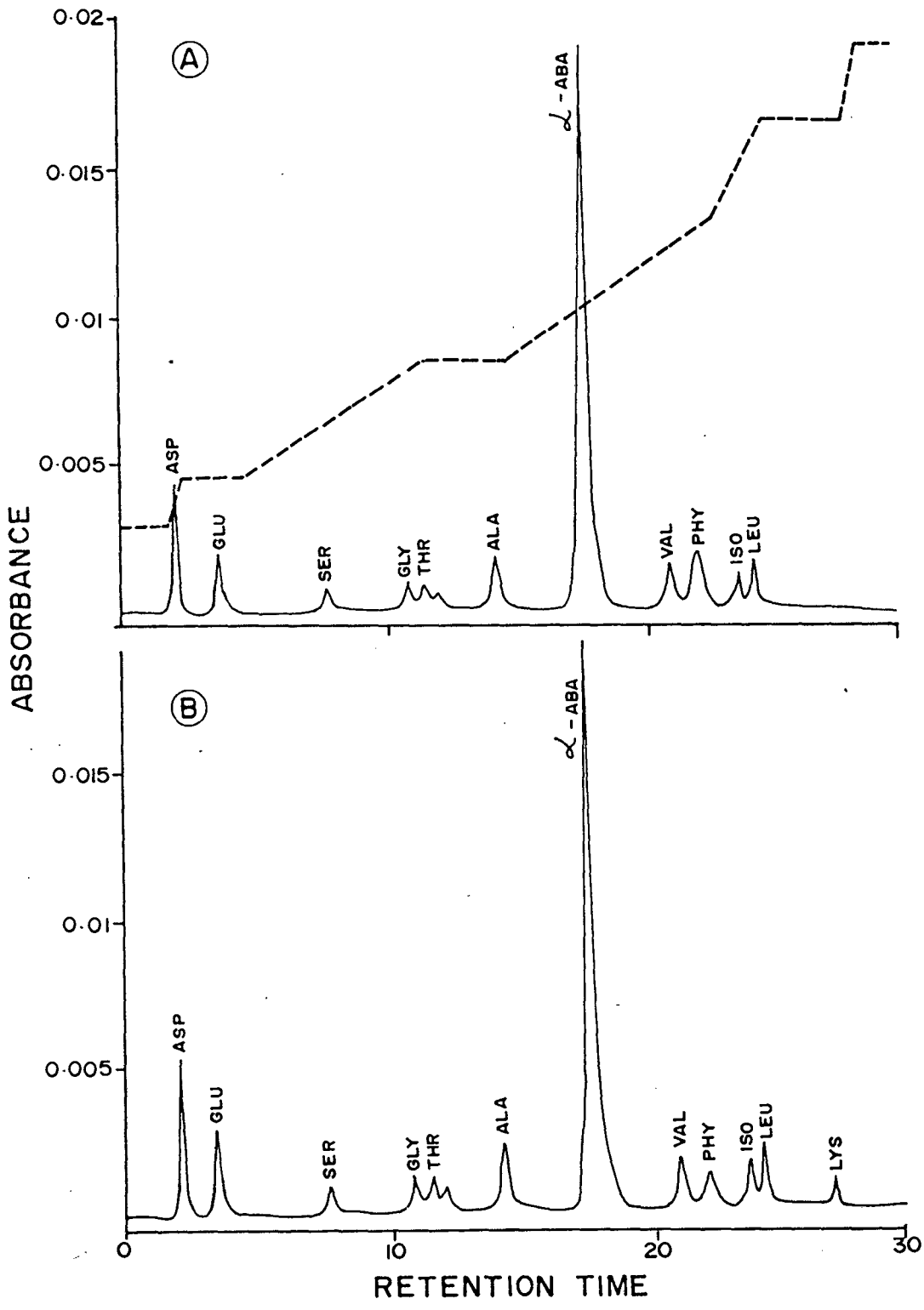

Fig.7.5 AMINO ACIDS OF SUSPENDED PARTICLES FROM THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st. (A) & 5th. (B) DAY OF SAMPLING DURING DECEMBER '89

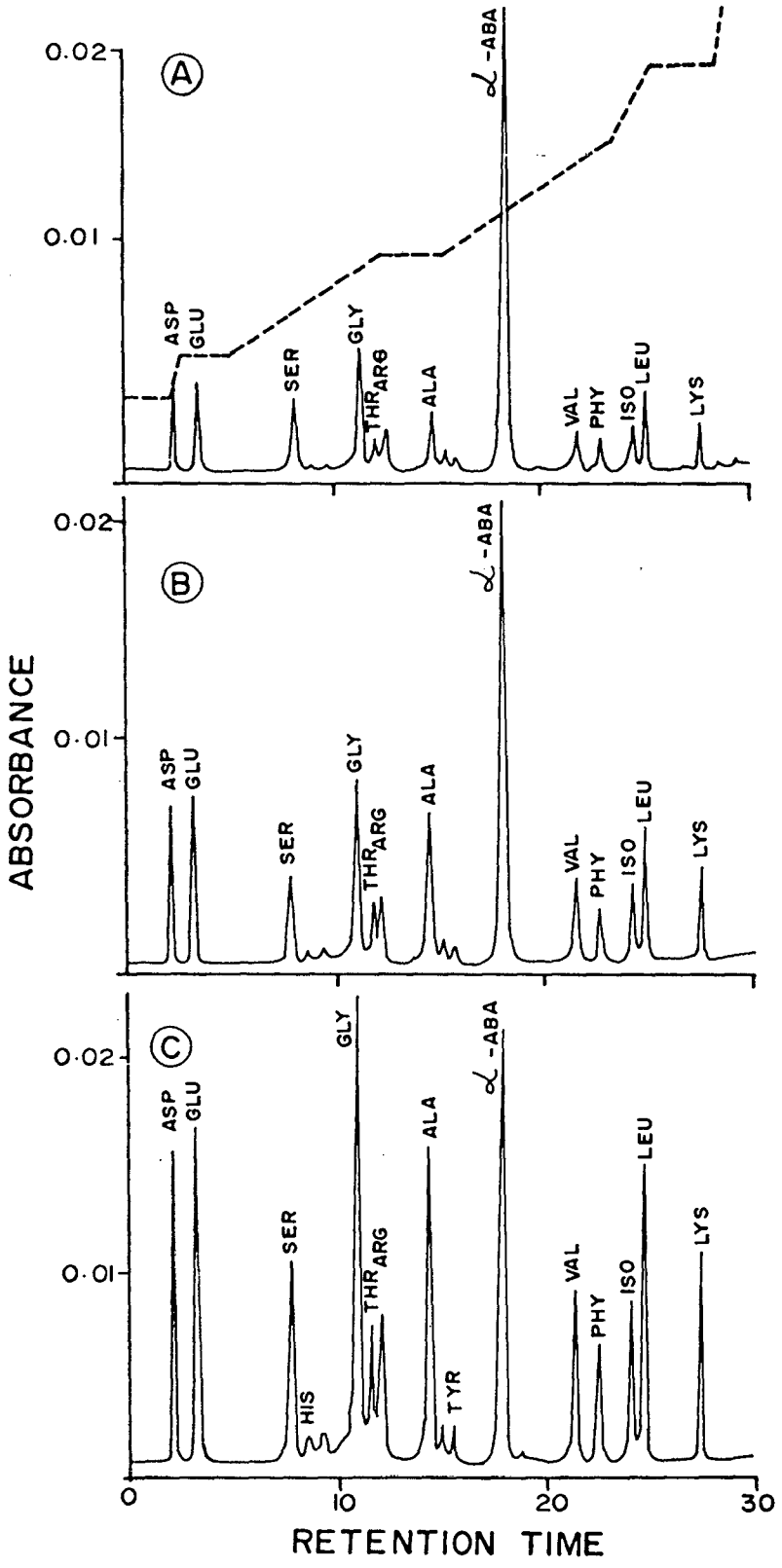

Fig. 7.6 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON ALUMINIUM PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd.(B) & 5th (C) DAY OF SAMPLING DURING APRIL '89.

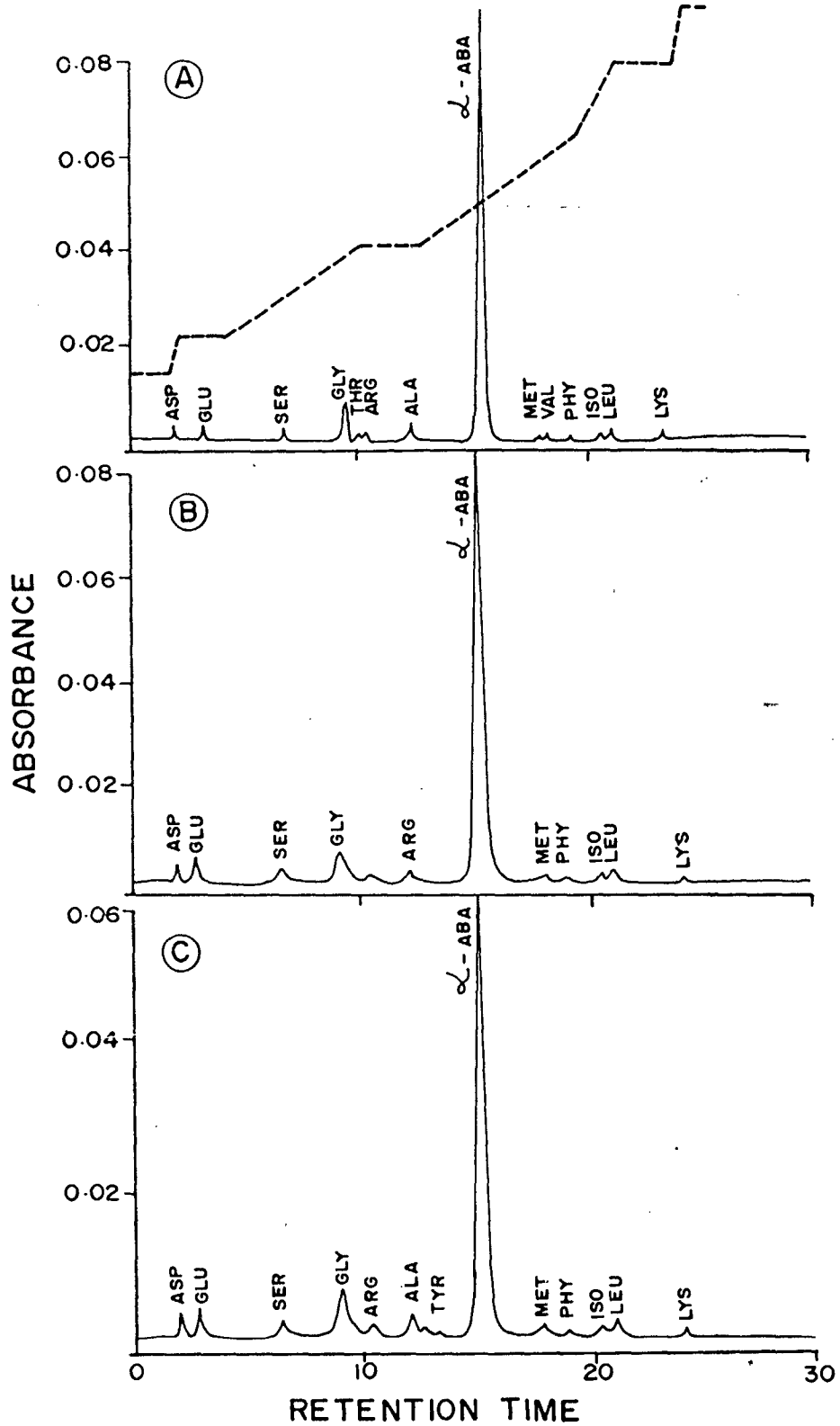

Fig. 7·7 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON ALUMINIUM PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd.(B) & 5th (C) DAY OF SAMPLING DURING AUGUST '89

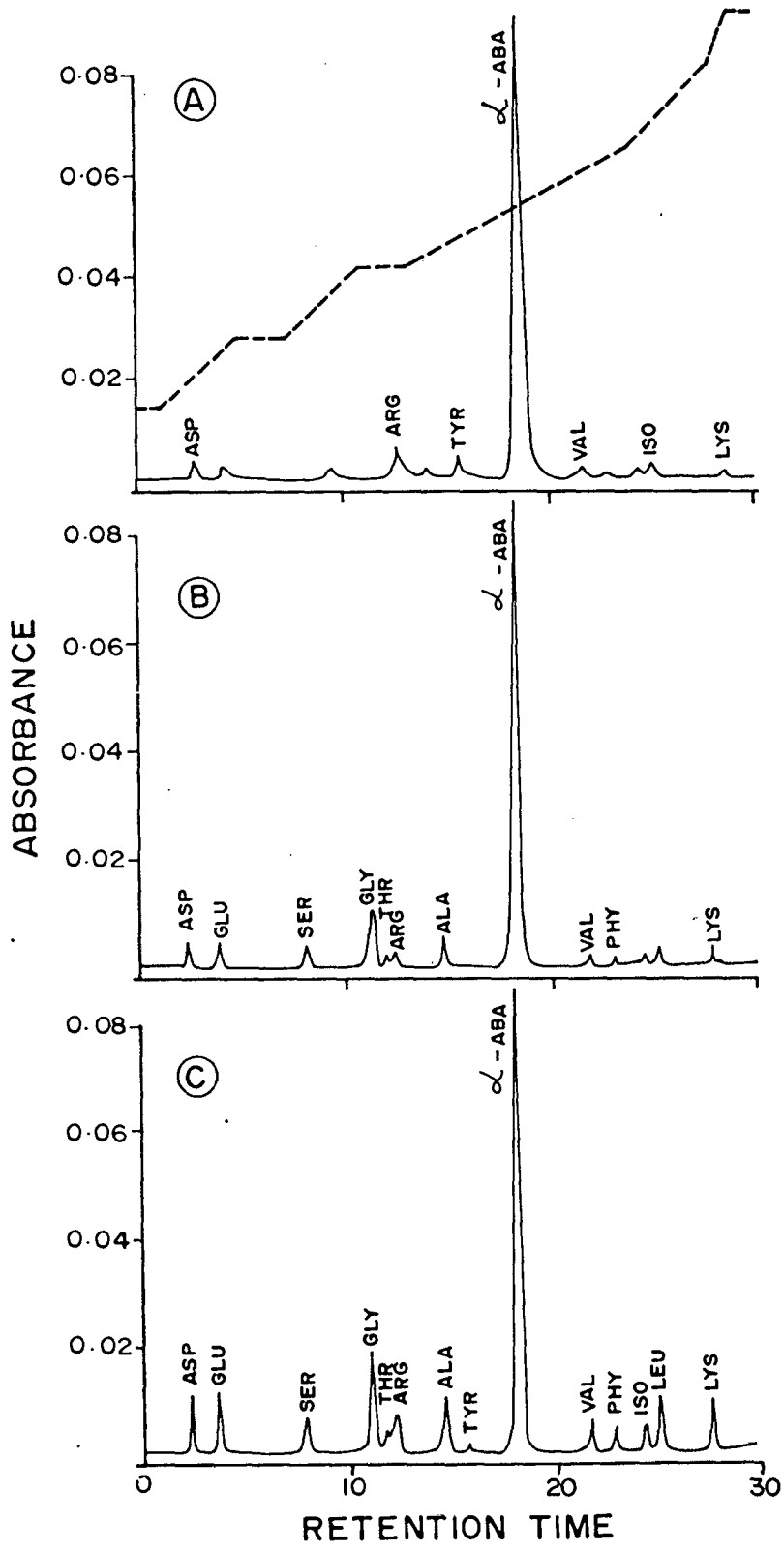

Fig. 7.8 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON ALUMINIUM PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd.(B) & 5th.(C) DAY OF SAMPLING DURING DECEMBER'89.

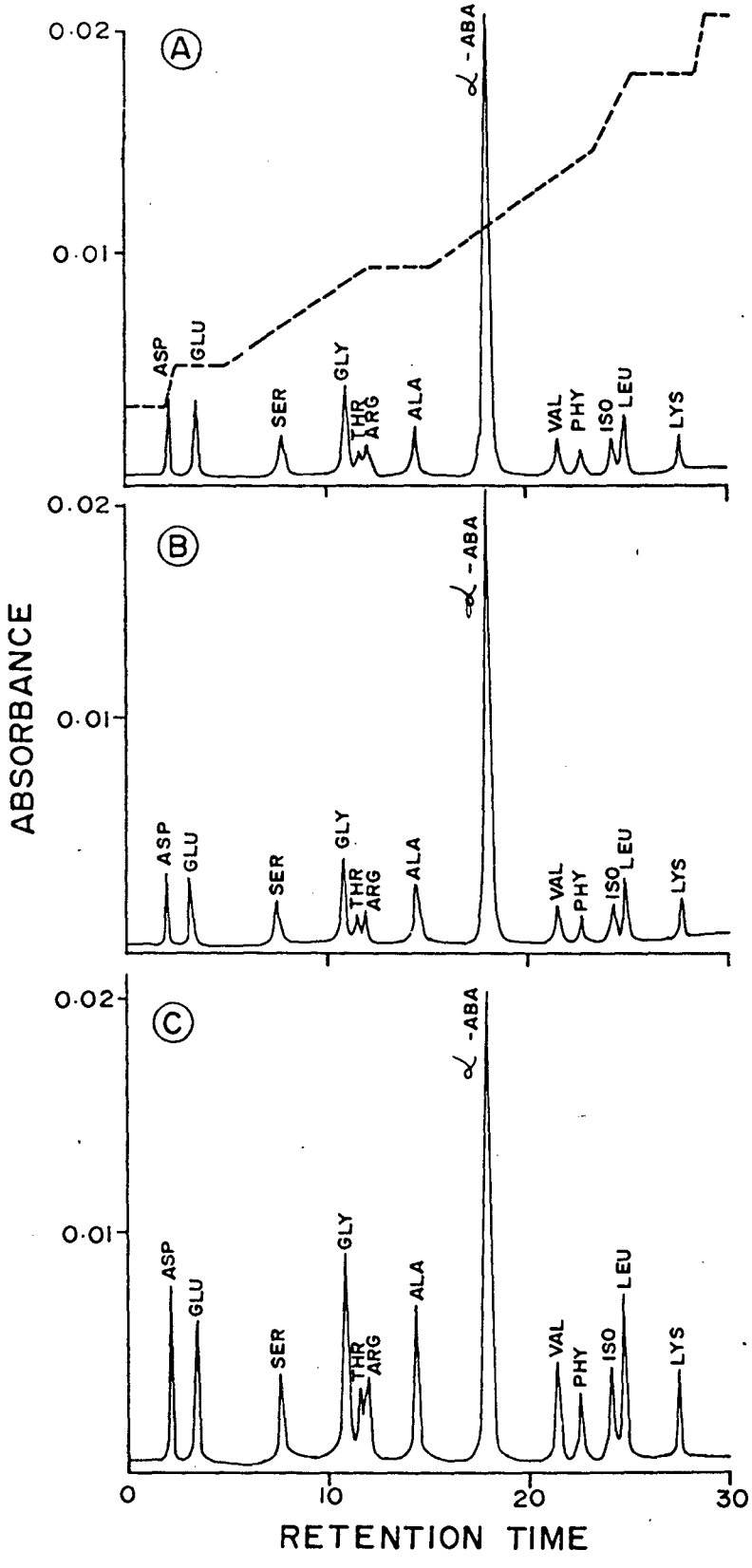

Fig. 7.9 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON FIBRE GLASS PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd.(B) & 5th (C) DAY OF SAMPLING DURING APRIL '89

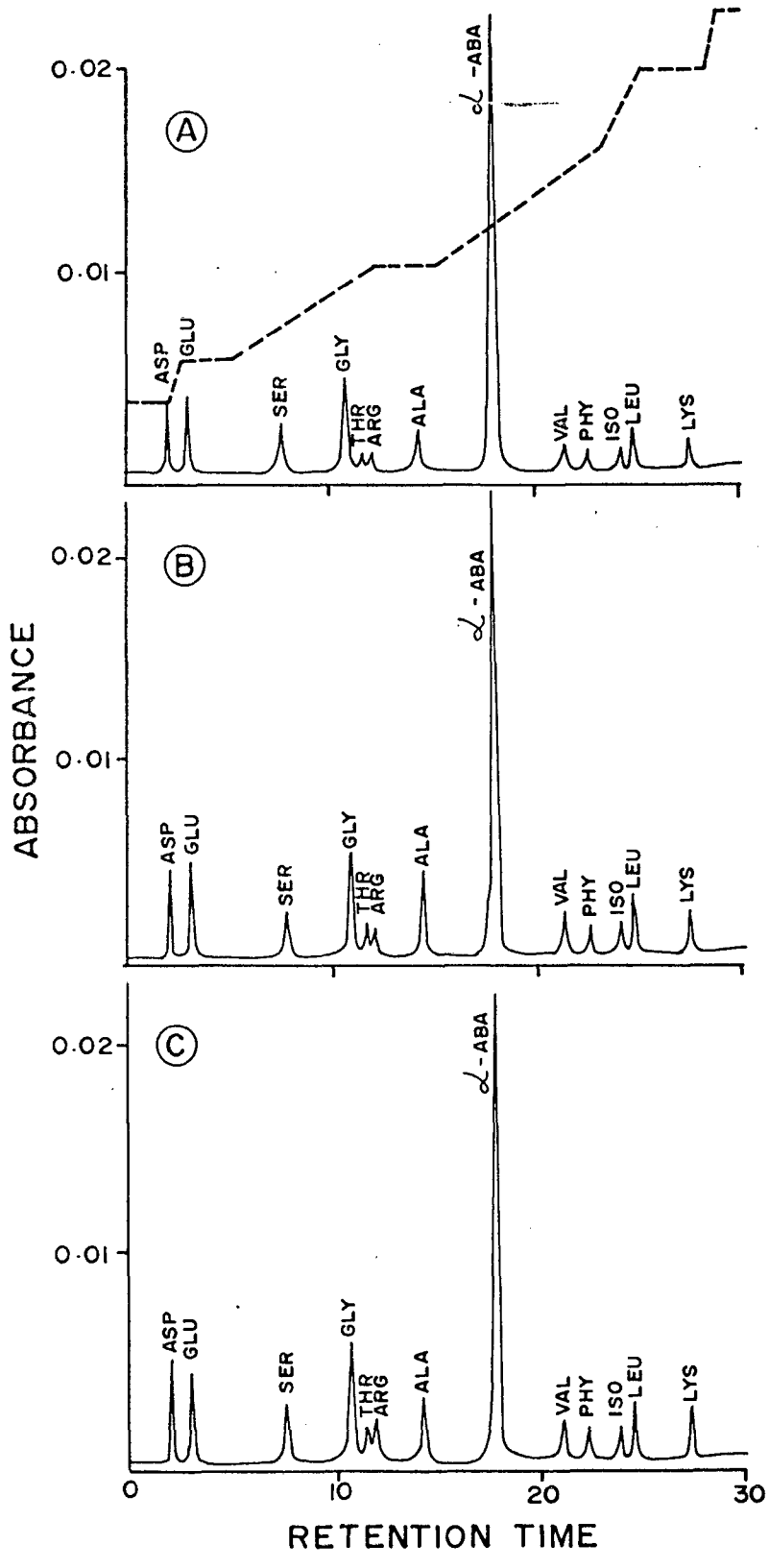

Fig. 7.10 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON FIBREGLASS PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd.(B) & 5th (C) DAY OF SAMPLING DURING AUGUST '89

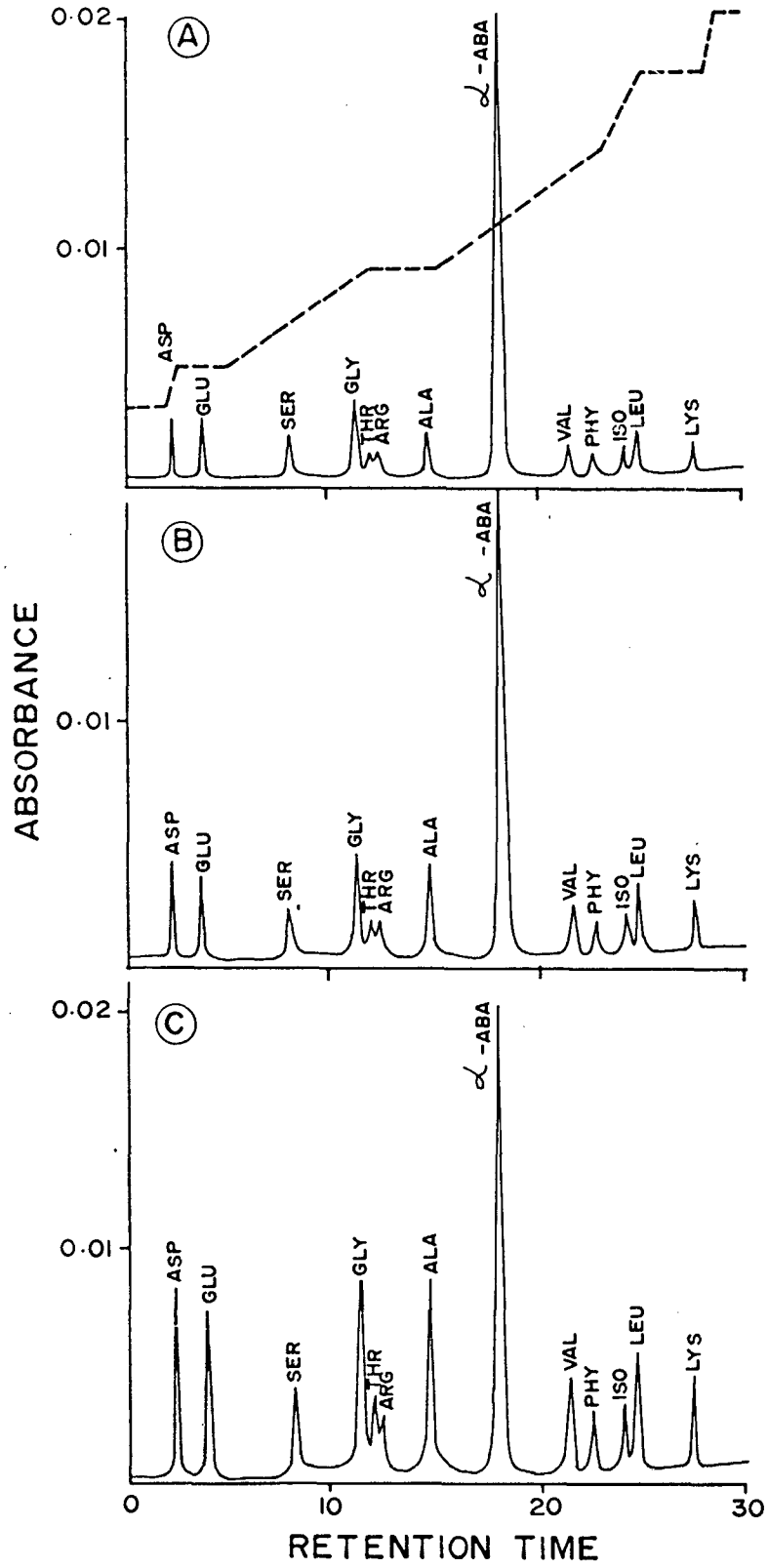

Fig.7.11 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON FIBRE GLASS PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd.(B) & 5th(C) DAY OF SAMPLING DURING DECEMBER '89

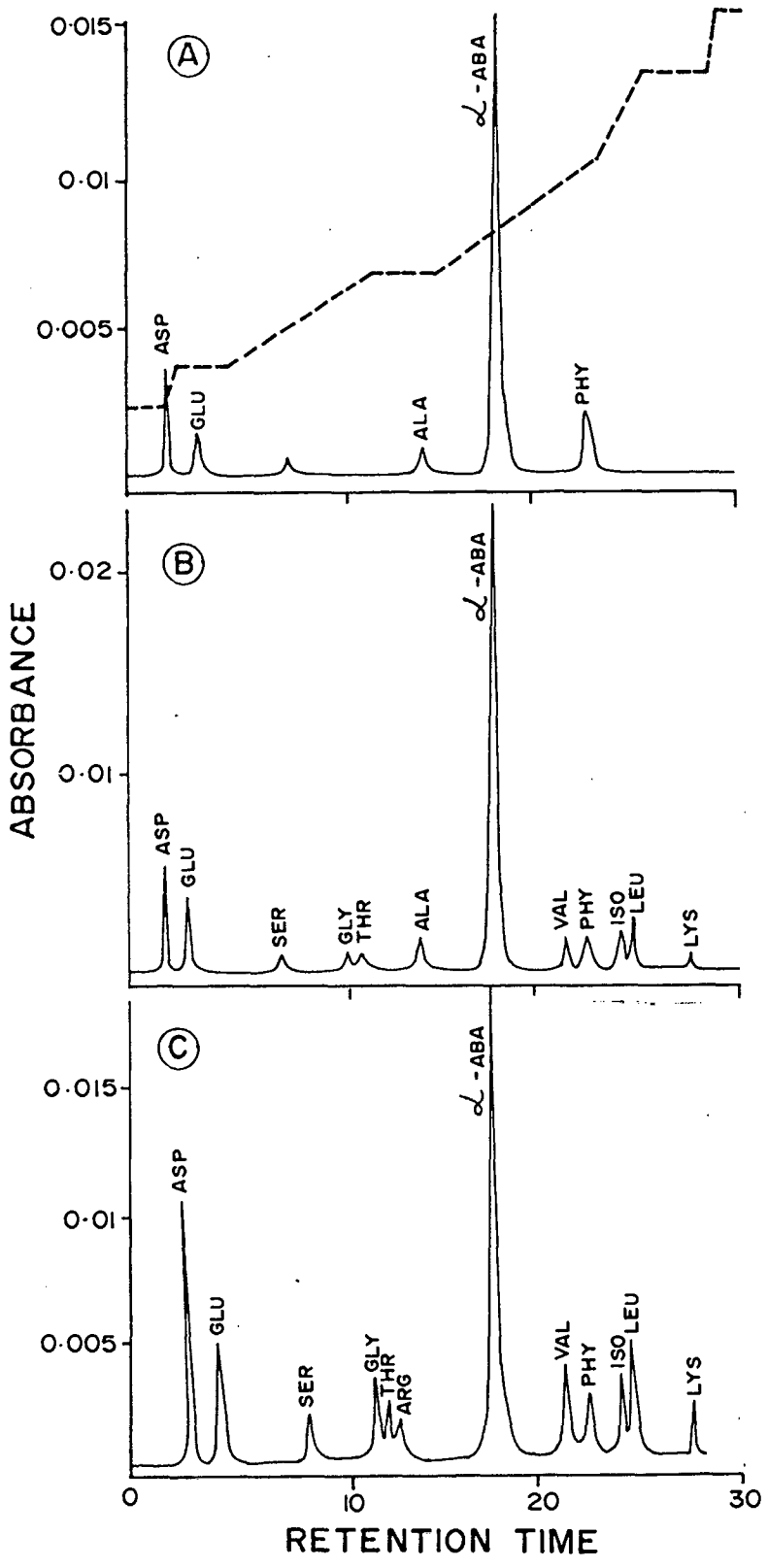

Fig. 7.12 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON STAINLESS STEEL PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st. (A), 3rd. (B) & 5th (C) DAY OF SAMPLING DURING APRIL '89

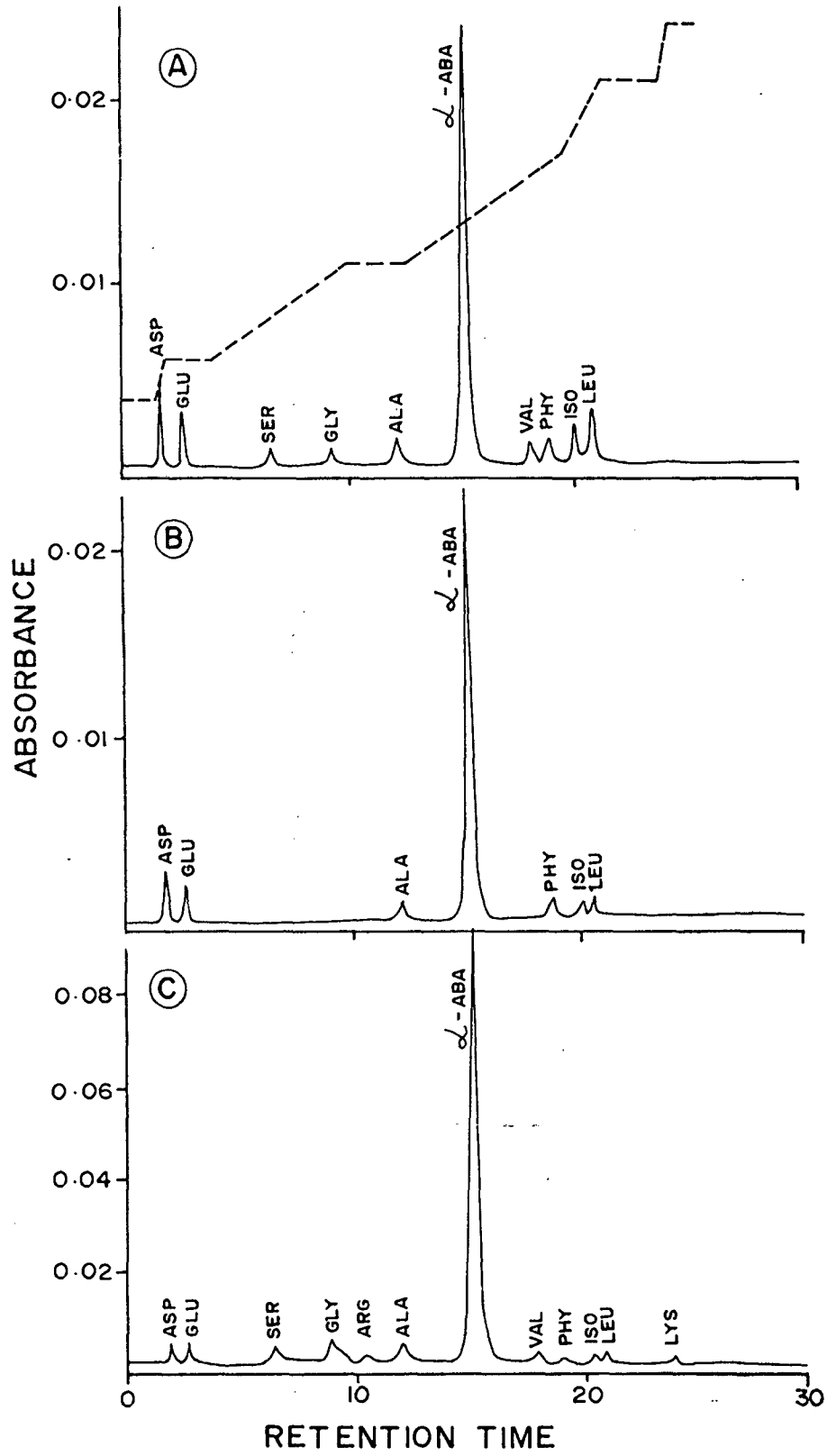

Fig. 7.13 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON STAINLESS STEEL PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1st.(A), 3rd. (B) & 5th (C) DAY OF SAMPLING DURING AUGUST '89

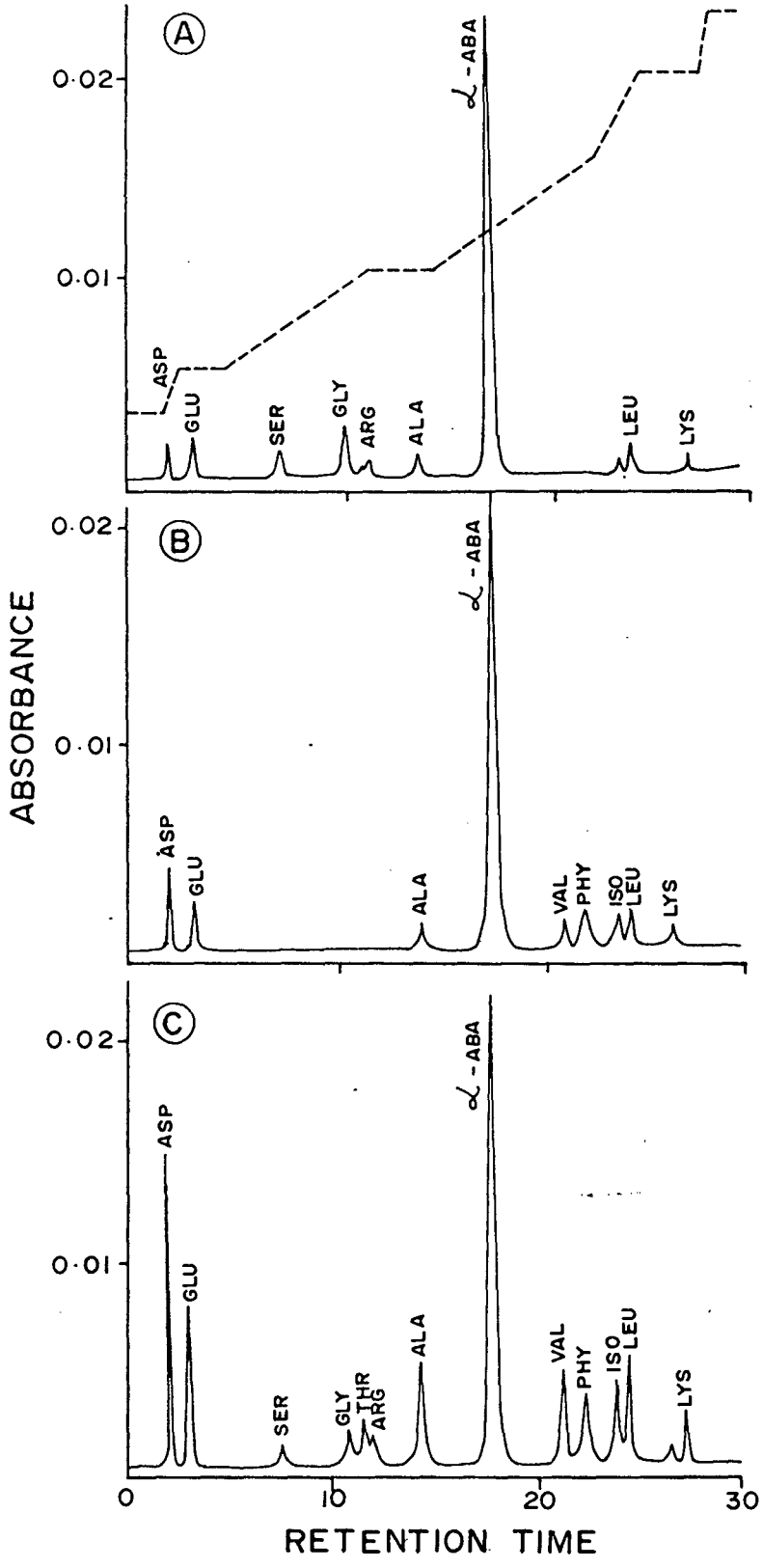

Fig. 7.14 AMINO ACIDS OF THE MICROFOULING DEVELOPED ON STAINLESS STEEL PANELS WHEN EXPOSED TO THE SUB-SURFACE WATER OF THE STUDY AREA FOR THE 1 st. (A), 3 rd. (B) & 5 th (C) DAY OF SAMPLING DURING DECEMBER '89

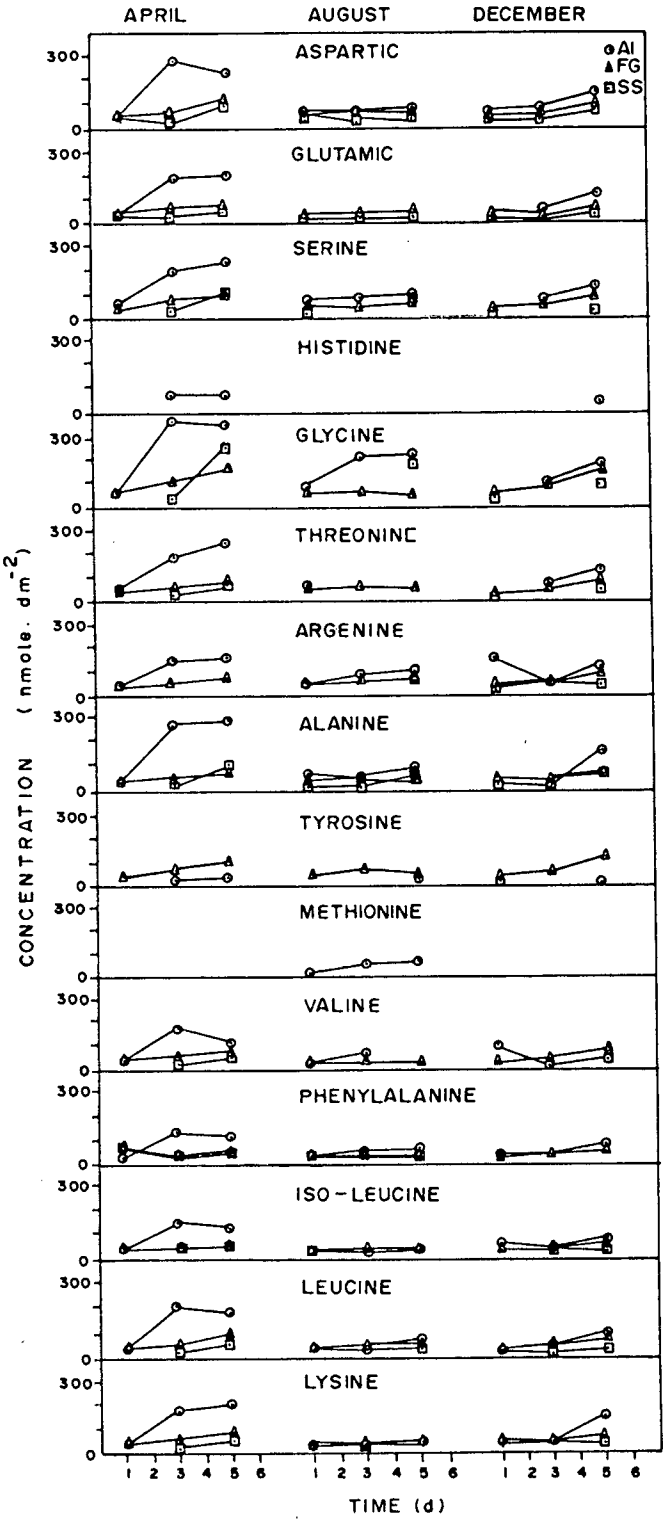

Fig. 7.15 COMPOSITION OF AMINO ACIDS

Chapter 8

DISCUSSIONS

DISCUSSION

Microfouling is the initial stage of material deterioration (Eggins & Oxley, 1980). It involves attachment and growth of microorganisms and subsequently the production of exopolymers (Neihof & Loeb, 1972; Baier, 1975; Loeb & Neihof, 1977; Characklis & Escher, 1988; Zutic & Tomaic, 1988). The fully developed microfouling layer generally consists of living and/or dead cells as well as inorganic and organic detrital material enmeshed in a fibrilar matrix or glycocalyx (Marshall, 1992).

It is obvious that the development of microfouling is influenced by environmental factors such as temperature, salinity, dissolved oxygen, nutrient levels, suspended matter and biological factors (Yanshun et al, 1984; Characklis & Escher, 1988).

The present study area receives large amount of fresh water discharge (150-400m^3/sec) from the rivers, Mandovi & Zuari, especially during the monsoon season, while low (10m^3/sec) discharge occurs during the pre-monsoon and post-monsoon seasons (Shetye & Murty, 1987).

Temperature is known to affect the metabolic and physiological activities of organisms (Levinton, 1982).

High temperature (29-32° C) was observed during the pre-monsoon season as compared to the monsoon (27-28° C) and post-monsoon (28-29° C) season. This was comparable to the observation made by De Souza (1977) for the Zuari estuary and by Dehadrai (1970) for the Marmugao Bay.

Salinity is another important hydrographic parameter which may influence the growth of microorganisms. The salinity was low (11.98- 29.65‰), during the monsoon season. Low salinity values for the monsoon seasons have been reported for the surface waters of the Zuari estuary (Qasim & Sen Gupta, 1981). During the pre-monsoon (34.47-37.89‰) season, the salinity values were comparatively higher.

There is a complex relationship between temperature and salinity, whereby, changes in salinity can modify the effects of temperature and vice versa (Kinne, 1963). The variations in these parameters may affect the organism's metabolic processes like diffusion, osmosis as well as water density, gas solubility and viscosity (Guillard, 1962; Chlebowics, 1988). Such a relationship between the temperature and salinity was also reported by Ganapati & Ramasarma, (1965); and De Souza et al, (1981).

The nutrients and dissolved oxygen content of the surface waters of Dona Paula also showed marked variations during the period of study. Peak concentrations (4.32-5.61ml/l) of dissolved oxygen was observed during the monsoon season. This was due to the low temperature and low salinity recorded during the season. Similar observation has been reported for the Vellar and the Mahanadi estuaries (Chandran & Ramamoorthy, 1984; Sai Sastry & Chandran, 1990). It is well known that low salinity increases the solubility of gases including oxygen (Head, 1985)

Nutrients showed a large variation throughout the year. The major input of nutrients could be due to river runoff, effluent discharge into seas and from insitu decomposition of organic matter. Nitrite-nitrogen was lower than nitrate-nitrogen throughout the study period. Nitrite-nitrogen is generally present as an intermediate product of microbial reduction of nitrate or oxidation of ammonium (Spencer, 1975). It could also be present as an excretion product of phytoplanktons (Head, 1985). These processes are activated in the marine environment by biological agents (Spencer, 1975). On the other hand, major sources of nitrate are, river runoff, land drainage and precipitation. Both these nutrients were minimum during the monsoon season which could be due to utilization

for biological productivity by primary producers (Boyton et al, 1982; Dehadrai, 1970), in addition to dilution of waters from the study area.

Concentration of phosphate and silicate were high during the monsoon season. High concentration of phosphate for the monsoon season could be due to massive water resources brought in by the river (Simpson et al, 1977). Similar observations were made by Sarla Devi (1991) for the Periyar river estuary and by Mishra et al, (1992) for the Bamuda estuary (Orrisa).

Another reason for the differences in the concentration of various nutrients could be due to the low concentration of nitrites and nitrates and subsequent high concentration of phosphates and silicates in the river water itself (De Souza et al, 1981)

During the pre-monsoon season, peak concentrations of nitrite and nitrate occurred with minimum concentrations of silicates and phosphates. Such high concentrations of nitrite and nitrate during the pre-monsoon season suggest that high temperature, high salinity and well aerated waters occurring during the pre-monsoon season favour the removal of phosphates showing a minimum concentration during the said season (Jitts, 1959; Pomeroy, 1975). Low

concentration of silicates during the same season may be due to its utilization by plankton and/or possible removal by non-biological sources including adsorption onto suspended particulates (Liss & Spencer, 1970).

Suspended particulate matter (SPM) of Dona Paula waters, showed peak values during the monsoon season. Intermediate values of SPM was observed during the post-monsoon season and minimum values occurred during the pre-monsoon season. Similar observations were reported by De Souza et al, (1981) for the same area.

Suspended particulate matter of the sub-surface waters contain inorganic matter as the major constituent and organic matter as the minor component. This is in agreement with the findings by Cadee (1982). Since the organic fraction is more susceptible to degradation, its concentration showed a wide variation with time. According to Toth and Lerman (1977) particulate organic matter first looses nitrogen, followed by phosphorous and oxygen. This was due to the higher activation energy required for the cleavage of C-C and C-H bonds as compared to C-N, C-P and C-O bonds.

POC content of the surface waters of Dona Paula was relatively high and varied from 0.62 to 4.93 mg/l. High concentration of POC in estuarine and coastal waters has

been reported by several workers (Wallace & Duce, 1975; Faganeli & Malej, 1981; Verlencar & Qasim, 1985). For example, POC in the estuarine (0.28 to 5.24 mg/l) and coastal waters (0.52 to 2.51 mg/l) of Goa, in the Gulf of Triest (0.1-3.46mg/l), in the north Adriartic (0.14 to 3.33 mg/l), in the Narrazalsette Bay (0.32 to 0.85 mg/l). POC values in the eastern harbour of Alexandria, (1.0 to 6.0mg/l) were relatively higher than those observed in this study. When expressed as as a percentage of the total suspended particulate matter, POC accounted for 10-16% of SPM. These values were higher than those reported for the coastal waters of other areas. For example, Biggs (1970), reported 7.8% of POC, whereas, Hobson & Menzel (1969) observed 5 to 7% of POC in the northern Chesapeak Bay and off the east coast of South America, respectively. Similarly, very low values were observed for the Mahi and Alaskan estuaries (Lodar & Hood, 1972; Bhosle et al, 1985).

Seasonal distribution showed maximum POC concentrations during the monsoon season. Such high concentration of organic carbon during the monsoon season could be due to the increased river runoff containing higher amount of re-suspended particulate material relatively rich in organic carbon. This corroborated well with findings of others (Cadee, 1982; Sreepada et al,

1993).

As observed for several other parameters PON concentrations were also higher during the monsoon season. PON contribution to the total suspended particulate matter ranged from 0.7 to 4.70%. Whereas, ratio of POC/PON was found to be >13% indicating the source to be of terrestrial origin (Parson et al, 1961).

Chlorophyll content of the waters, is the measure of phytoplankton biomass (Hitchcock, 1977; Ittekkot, 1982). Chlorophyll· concentration was higher during the pre-monsoon and the post-monsoon seasons as compared to the monsoon season. Higher chl content in waters during the pre-monsoon and post-monsoon seasons was due to low turbidity of surface waters and greater light intensity (Murrugan & Ayyakannu, 1993). Phytoplankton carbon calculated from chl content is comparable with the values obtained for the coastal waters (3.27%) by Hung et al, (1982) and (5.30%) by Cadee (1984).

Particulate carbohydrate (P-CHO) was lowest during the pre-monsoon season and highest during the monsoon season. Carbohydrate contribution to POC varied from 1.85 to 12.87%. Carbohydrate chemistry of these surface waters like any other estuary is complex due to multiplicity of carbohydrate sources, including, sewage and industrial

effluents, river runoff, planktonic and benthic processes. These sources may introduce P-CHO into the surface waters of Dona Paula. The fresh water discharge into the Bay during the monsoon probably explains the higher concentrations of P-CHO during this season (Kamat, 1976).

Amino acids are chemically unstable and form an important constituent of total organic matter. These compounds are released by living organisms, especially phytoplankton and are subjected to rapid biological and chemical transformation (Fogg, 1975; Bada Lee, 1977). Thus, the measure of amino acid concentration at any point of time may be a net result of two processes, i.e. release by phytoplankton and utilization by heterotrophic organisms (Fogg, 1977; Smith et al, 1977).

Amino acid concentrations varied from 1198.14 to 2805.04 nmoles.l^{-1}. Amino acid concentration for coastal and offshore waters were reported from different areas by Daumas (1976); Williams and Yentsh (1976); Dawrson & Pritchard (1978); Garrasi et al., (1979); Jorgensen (1982); Mopper and Lindroth (1982). Concentrations of amino acids of the present study area are comparable with those reported for the coastal waters of the Gulf of Marseille, (Daumas, 1976), the shallow estuary on the east coast of Jutland, Denmark (Jorgensen, 1982) and for the coastal

waters of the Pacific (Siezen & Mague, 1978). However, our values are higher than that of open ocean waters of the Irish Sea, the Baltic Sea and the Pacific Ocean. (Riley & Segar, 1970; Dawrson & Gocke, 1978; Siezen & Mague, 1978). These indicates that the coastal waters have higher amino acids than the open sea waters.

High concentration of amino acid corroborated with high concentration of chlorophyll especially during April (pre-monsoon season). Similar coincidence of high chl and high concentration of amino acids has been reported by Crawford et al., (1974) in the Pamlico River Estuary during a dinoflagellate bloom and in the York River when a red tide occurred (Wood, 1966).

From this it appears, that the concentration of amino acids largely depend on the abundance of phytoplankton population.

Apparent changes in the composition of amino acid compositions were observed during the study period. Glycine, alanine, aspartic, leucine, serine, glutamic, valine, lysine, isoleucine, threonine, argenine, phenylalanine, histidine and thyrosine (in the order of decreasing abundance) formed the major amino acids in the sub-surface waters of Dona Paula. Glycine was the major

contributor followed by aspartic, serine, glutamic, leucine and lysine. Abundance of these amino acids have been observed in living and non-living organic matter (Dawmas, 1976).

Group composition of amino acid showed the predominance of neutral amino acids followed by acidic, basic, aromatic and sulphur-containing amino acids.

In order to better understand the possible sources and distribution of various constituents in the study area linear regression analyses were carried out. Significant positive relationship was observed for temperature/salinity for both daily ($r=0.61$, $p<0.001$, $n=48$), as well as weekly ($r=0.64$, $p<0.001$, $n=12$) sampling periods. This suggests that these two parameters varied with respect to each other. Increase in temperature was always associated with the increase in salinity and vice versa. The influence of temperature and salinity on dissolved oxygen was assessed and it was observed that Temperature/dissolved oxygen ($r=-0.70$, $p<0.001$, $n=48$), as well as salinity/dissolved oxygen ($r=-0.66$, $p<0.001$, $n=48$), showed inverse relationships. This indicates that the temperature and salinity influenced the distribution of dissolved oxygen in these waters.

Nutrients like nitrite with nitrate and phosphate with silicate were well correlated indicating a common source of utilization and production for these nutrients. In order to predict the possible source of nutrients in the study area, nutrients were correlated with salinity. It was observed that nitrite/salinity ($r=0.59$, $p<0.001$, $n=48$) and nitrate/salinity ($r=0.70$, $p<0.001$, $n=48$) showed significant positive correlations. This suggests that these nutrients were not of riverine origin. Thus, the probable source be the in situ degradation of marine organic matter (De Souza, 1983). In contrast to this phosphate/salinity ($r=-0.52$, $p<0.001$, $n=48$) and silicate/salinity ($r=-0.72$, $p<0.001$, $n=48$), showed inverse relationships. This implies that these nutrients were brought in by the river runoff and/or autochthonous sources. Similar observations were reported by others (Qasim & Sen Gupta, 1981; Chandran & Ramamoorthy, 1984).

The observed inverse relationship between POC/salinity ($r= -0.59$, $p<0.01$, $n=12$) indicated that POC is mainly transported to this area through riverine discharge and/or other allochthonous sources. This agrees well with the findings reported by Gardner et al, (1989) and Sreepada et al, (1993). These authors observed that the POC content of the coastal waters of South California (Georgetown, USA) and along the east coast of India was

mainly of allochthonous origin.

An inverse relationship was obtained between PON/salinity ($r=-0.90$, $p<0.001$, $n=12$), indicating riverine origin of the particulate organic nitrogen.

An inverse relationship for P-CHO/salinity explains the probable allochthonous detrigenous sources. Low values during the post and pre-monsoon seasons could be due to the active utilization by the filter feeders and heterotrophic bacteria (Bordovsky, 1965). Biggs & Wetzell, (1968), reported similar behaviour of P-CHO for the Chesapeake Bay waters.

While studying the various aspects of microfouling, the influence of hydrographic parameters on microfouling was assessed using linear analysis.

The removal of microfouling material from the study surfaces is the most critical step in estimating microfouling biomass. Sharma *et al*, (1990), have shown that the use of nylon brush was the most appropriate for scraping microfouling material from surfaces.

Various parameters such as dry weight, total carbon, total nitrogen, protein, ATP, chlorophyll *a* and bacterial density have been employed to quantify the extent of

microfouling (Aftring & Taylor, 1979; Mayack et al, 1984; Bhosle et al, 1989, 1990). In the present study dry-weight, total carbon, nitrogen, CHL*a*, diatom and bacterial cell density were used. These methods were found to comparatively simple, sensitive, less time consuming and produced data and hence were used to assess microfouling on test substrata (Sharma et al, 1990).

Amongst the three surfaces, used fibreglass was hydrophobic whereas, the other two surfaces (aluminium, stainless steel) were hydrophilic. In general hydrophilic surfaces supported high microfouling biomass as well as bacterial and diatom numbers, atleast during the initial 24 hours. Such observations were also made by Fletcher (1988), Bhosle, et al, (1989), Srivastava et al, (1990), Raveendran et al, (1991). Amongst these surfaces, aluminium showed low biomass initially, however, with increase in immersion period, biomass increased significantly. Stainless steel showed low microbial abundance throughout the sampling period. This was probably due to its electropolished surface. Moreover, stainless steel is known to be more resistant to microbial attack as compared with rough surfaces (Dunsmore et al, 1981; Zoltai et al, 1981).

Microfouling biomass developed on aluminium, fibreglass and stainless steel varied considerably with exposure period and showed a linear increase over the period of immersion. Similar increase with time has been reported from many environments. For example, Bhosle et al, (1990), while working in the Arabian-Sea, Srivastava et al, (1990), for the Bombay waters and Raveendran et al, (1991), for Agatti waters of Lakshadweep Island. The increase observed in the microfouling biomass in the present study suggests irreversible attachment of the adsorbed organisms, their growth and reproduction. Occasional decrease in the fouling biomass on some of the surface was probably due to shearing and/or sloughing of adsorbed material (Marshall et al, 1971; Marshall, 1976; Characklis et al, 1984). It could also be due to grazing by aquatic organisms (Harding et al, 1987).

The sequential developments of organisms on surfaces appear to be a subject of considerable debate. For example, biological characterisation of the microfouling film was studied by several workers who suggest bacteria to be the first organisms to attach to surfaces (Corpe, 1970; Sieburth, 1979; Bhosle et al, 1990, Bhosle et al, 1993). Such bacterial films may induce diatom settlement (Miyachi et al, 1989). There are others who are of the opinion that diatoms colonize prior to bacteria (Skerman, 1956; O'Neil &

Wilcox, 1971; Paul et al, 1977). It was also opined that diatoms, fungi & cyanophytes can occur at any one stage before or after bacterial proliferation. Probably each group of organisms may help in the attachment and growth of other forms resulting in the development of a microfouling community.

In the present study, biological characterization of the microfouling film was carried out with special reference to diatoms and bacterial cell density. High abundance of diatoms and bacteria during the first 24 hour period of exposure was observed for all the surfaces. Their numbers reduced drastically for the next 24 hours. Such irregular growth pattern of diatoms and bacteria, especially in the early stages of microfouling, seems to be a regular phenomena on various surfaces immersed in marine environments (Corpe, 1972; Yanshun et al, 1984). This could probably be, due to the reversible attachment of microorganisms. Such reversibly attached organisms are removed by very small changes in water velocity, current, wind speed and other forms of disturbances. After the cells are reversibly attached, a subsequent growth was observed as a function of the immersion time. This probably suggests that the cells which were irreversibly attached, start growing. This was evident from the

subsequent increase in cell numbers of diatom and bacteria.

Among the different diatoms Nitzschia sp. was found to be the most dominant form in the surface waters as well as on various substrata. In the order of abundance this was followed by Navicula sp. and Pleurosigma sp. In addition to these diatoms, aluminium panels also showed the presence of Gramatophora sp. in large numbers. Predominance of Navicula sp. and Lycmophora sp. from the microfouling material has been reported from the temperate and tropical environments (Cooksey et al, 1984; Bhosle et al, 1989). Of the 8 genera of diatom species recorded from various substrata immersed in the Dona Paula waters, 5 belonged to pennate forms and 3 to centrales. From this it is evident that pennate diatoms were most abundant microalgae on these substrata. Such pennate diatoms are known to foul man-made structures in temperate and tropical waters (Characklis & Cooksey, 1983; Cooksey et al, 1984; Bhosle et al, 1989; Raveendran et al, 1991). Dominance of pennate forms have also been reported from microfouling material developed on test surfaces exposed to the open ocean waters of the Arabian Sea (Bhosle et al, 1989; Kelkar, 1989). These observations suggests a common occurrence of pennate diatoms on the surfaces placed in both the offshore and near shore waters.

Scanning electron microscopic observations showed the abundance of coccoid bacteria on fibreglass, aluminium and stainless steel panels. These test surfaces showed a two-tired layer of the microfouling community comprising of bacteria and diatoms enmeshed in exopolymeric material within 72h.

These observations suggest that there was a coexistence of both diatoms as well as bacteria during the period of observation. This was further confirmed, from the significant correlation between the diatoms and bacteria settled on aluminium, ($r=0.86$, $p<0.001$, $n=36$), fibreglass ($r=0.83$, $p<0.001$, $n=36$) and stainless steel ($r=0.80$, $p<0.001$, $n=36$). Such a coexistence could be due to their interdependence on each other. Bacteria grow by consuming the organic material produced by the algae. Thus the algal community to some extent influence the composition of bacteria (Riquelme et al, 1987). The algae inturn depends on bacterial products such as vitamins (Furuki et al, 1985), siderophores (Murphy et al, 1976) and metabolic products.

The abundance of bacteria and diatoms was also evident from the C/N ratio of the microfouling material. C:N (Wt/Wt) ratio is an important indicator of the nature of organic matter in the marine environment (Pocklington &

Leonard, 1979). Low C/N ratio was obtained for the microfouling material which ranged between 3 to 4.5. The value was similar to those reported for the organic matter originated from the biogenic material derived from algae and bacteria (Fenchel & Blackburn, 1979; Abdel-Moati, 1990).

In addition to biological characterization the chemical nature of the microfouling material was extensively studied. Such chemical characterization of the microfouling material showed the presence of large amount of organic carbon and nitrogenous compounds which may serve as a food source for other fouling organisms.

Earlier such a detailed study was difficult due to the chemical complexicity and minute quantities of the materials involved. However, with the development of advanced instrumentation and sophisticated techniques, it is now possible to determine nanogram quantities of substances at the molecular level. Fouling dry weight (F-DW), fouling inorganic matter (F-IM), fouling organic matter (F-OM), fouling organic carbon (F-OC), fouling organic nitrogen (F-ON), fouling chlorophyll *a* (F-CHL *a*), fouling carbohydrates (F-CHO) and fouling amino acids (F-AA) were some of the parameters monitored.

Chemical analysis revealed that above 60% of the microfouling material comprised of inorganic matter. This corroborates with the earlier studies by Berger & Little, (1980). Srivastava et al, (1990), have shown that the inorganic content of the microfouling film on any surface was much higher in both polluted as well as in unpolluted waters. In polluted water, although the concentration of organic matter in the environment was much higher, the microfouling film developed higher concentration of inorganic matter. High concentration of the inorganic matter could be due to the presence of a large number of fouling diatoms. The diatom frustules contain a high percentage of inorganic silica (Kelkar, 1989). Other terrigenous and detrital material also contributed significantly. In addition, microbial colonies which produced polysaccharides (Fletcher & Floodgate, 1973; Hoagland et al, 1993) increased the inorganic matter by adsorbing cations due to their anodic nature and hence lead to the binding of metal ions and other inorganic nutrients (White & Bensen, 1984; Ford et al, 1987).

F-CHL was routinely monitored to assess the living phytoplankton biomass (Steele & Baird, 1961). Primary production contributes significantly to the formation of organic particulates on surfaces. From the F-CHL*a* values

lowering the salinity to about 10.5‰ and also lowering the nitrite and nitrate of the surface waters. On the other hand, nutrients like phosphate and silicate are brought into the Bay. It also increases the suspended particulate matter of the waters. This causes increase in the turbidity of the water, reducing light penetration and thus directly influencing the diatom as well as bacterial counts on test panels. The dissolved oxygen concentration in the water column was comparatively high during this season due to the mixing of water. Moreover, physical forces were also strong during the monsoon season, resulting in the loss of microfouling material by shearing and/or sloughing.

On the other hand during the pre-monsoon season, the water temperature rose to about 31.5° C and salinity was as high as 37.89‰. Nutrients such as phosphates and silicates were low and nitrites and nitrates were high. The turbidity of the sub-surface waters during the pre-monsoon was much lower as compared to monsoon or post-monsoon season. This permitted good light penetration and thus, increase in the number of diatoms and also bacteria onto surfaces during April-May. There was a simultaneous increase in the chlorophyll concentration both in the water column as well as on surfaces. The post-monsoon season showed intermediate values which was closer to the

pre-monsoon season as compared to those of the monsoons.

The changes in the hydrographic parameters of the waters of the study area produced changes in microfouling biomass values. In this regard, correlation between microfouling biomass and physico-chemical and biological parameters helped to some extent to determine which parameter influenced microfouling significantly.

Temperature as well as salinity of the subsurface waters showed a significant correlation with diatom and bacterial cell numbers. It was earlier reported by White et al (1991), that bacterial abundance was significantly correlated with temperature in the water column. Similarly, microfouling biomass (F-DW, F-OM, F-IM, F-OC, F-ON, F-chl) were also significantly correlated with temperature and salinity. The above observations probably suggest that these two abiotic factors influence microfouling settlement directly on substrata. Similarly, the productivity of the water column are also high when temperature and salinity were optimum i.e. during pre-monsoon season. This probably suggests that the subtropical conditions occurring during the premonsoon season supports maximum microfouling settlement (Haugh, 1984; Yanshun et al, 1984; Karande, 1987).

Dissolved oxygen showed an inverse relationship with microfouling biomass. Inverse relationship with dissolved oxygen could probably be due to the formation of oxidised radicals of water like .OH & .HO_2 in the presence of oxygen which subsequently formed H_2O_2, all of which tend to have a lethal action (Norris & Ribbons, 1969).

Similarly nutrients showed a poor relationship with microfouling biomass. The probable reason for this relation could be due to the dependence of the microfoulers on both the particulates from the substrata as well as the ambient waters (Marshall, 1976; Ladd et al, 1979). Till date, it was generally speculated that nutrients of the water column greatly enhanced microfouling settlement, which now may not be a totally accepted fact. From the present study this does not appear to be the case. Since nutrient deficiency could also increase the carbohydrate production on surfaces to give higher adhesion values for both hydrophillic and hydrophobic surfaces (Kjelleberg, 1986). Such an observation was confirmed in our study by the high concentration of F-CHO observed during the period of nutrient deficiency.

Hence it can be seen that nutrients as well as dissolved oxygen from the subsurface water need not necessarily affect settlement at least over a longer period

of exposure. Because when nutrients supply is scarce in the immediate environment, surfaces may provide an advantage by assessing the capture and/or uptake of scarce nutrients (Marshall, 1976; Ladd et al, 1979).

Another point worth discussing is the significant but inverse relationship observed between the microfouling biomass on surfaces and suspended load of the subsurface waters. This was contrary to the earlier studies made by Bhosle et al, (1990), wherein they observed a highly significant correlation between biomass from surfaces and particulate matter of the water column from the offshore environment for one particular season. An inverse relationship for seasonal studies is an accepted phenomena because when the suspended load of the water column during the monsoon season was maximum, microfouling settlement during this season was minimum. On the other hand, when the suspended load of the water column was low (premonsoon season), the microfouling biomass was maximum. The post-monsoon season, however, formed an intermediate stage for the concentration of suspended load of the subsurface waters and biomass on test panels. However, it may not be advisable to compare this study with those of Bhosle et al, (1990), because their samples were obtained from the water column of oceanic environments and the study was restricted to one particular season of the year. The present study on

the other hand was carried out from the sub-surface waters of the near shore environments for three cumulative seasons of the year.

All these parameters (DW, FIM, F-OM, F-OC, F-ON, F-chl$\underline{a}$) were significantly correlated with diatom and bacterial density. Thus indicating that diatoms and bacterial abundances form the major source of these compounds in the microfouling material. Although such a simple correlation analysis can be done to explain the interrelation to a certain extent, we cannot ascertain the process of microfouling to occur due to one or even two hydrographic parameters. All the hydrographic parameters greatly influence the other parameters and in such a complex ecosystem the various parameters taken together attributes to the microfouling process. However we may conclusively suggest that the pre-monsoon season of our sub-tropical environments provides all the optimum conditions for the attachment and growth of the microfoulers and hence development of the microfouling community.

Carbohydrates and amino acids which were important constituents of the microfouling material were also studied. Carbohydrates are one of the main constituents of the microfouling material developed on solid substrata

(Bhosle et al, 1989, 1990). These compounds are produced by microorganisms during, as well as after their adhesion to solid surfaces (Fletcher & Floodgate, 1973). They also form an important constituent of terrestrial matter that settle on substrata. Hence, in view of their importance and abundance, their concentration in the microfilm was studied. Carbohydrate showed highly significant correlation with diatom and bacterial cell number as well as chl*a*. Thus the most likely source of carbohydrate must be from living organisms (Diatoms, fungi, protozoa and larvae of macrofoulers). However, microscopic observations did not reveal the presence of protozoans, fungi and larvae of macrofoulers. Hence carbohydrates of the microfouling material could have been derived from diatoms, bacteria and to a certain extent from detrital material.

In addition to this, when the percentage of F-CHO present in 100mg of F-POC was calculated, it was observed that values below 20 was obtained. This was another clear indication to prove the biogenic source of origin of the microfouling material.

Amino acid concentration of the microfouling material ranged from 65.58 to 2185nmoles.dm-2 for the study period. Amino acids form an important source of carbon and nitrogen for the microfouling community. No comparison of this data

with other microfouling data was possible from surfaces due to the absence of such studies.

Glycine, alanine, serine, aspartic, leucine, valine, lysine, threonine, glutamic, argenine, phenylalanine, isoleucine, histidine, tyrosine and methionine were the amino acids in the order of decreasing abundances present on surfaces. The major contributors to the total amino acids were also the major constituents of phytoplankton and most of them were quite stable in the marine environment. In addition to this, serine can be stabilised by reaction with phenolic compounds (Degens, 1970).

Studies on the amino acid composition of the microfouling material from the field conditions are totally absent, a comparison may be made with various laboratory studies. For example, according to Poulet & Jezequel (1983), high level of individual and total amino acids were observed during the stationary phase and senescent phase i.e., when growth activity is reduced. For example during the stationary phase the total concentration of amino acids were three times higher than those observed during the active growth. Similar variation in the amino acids composition and physiological state of a cell have been shown in enclosed and insitu experiments under lab conditions (Hammer et al., 1981; Poulet and Jezequel,

1983). These results may be compared with our study for mixed culture on test surfaces in field condition. Hence initial low concentrations of amino acid in the lab condition (Hammer & Eberlein, 1981) could be also compared to initial low values of amino acids on test surfaces in the field conditions. This may be due to comparatively fewer phytoplankton cells present on test surfaces as in the experimental flasks. On the other hand with increase in exposure time of test panels the concentrations of amino acids increased indicating the simultaneous increase in the number of cells on surfaces with time. This was also the case with pure cultures.

Even in the microfouling material neutral amino acids were the major contributors to the total amino acids (34-70%) followed by acidic (11-40%), basic (5-24%), aromatic (3-23%) and sulphur containing (< 5%) in most cases, although there was slight demarcation from the general trend for some of the surfaces.

Statistical analysis of the total amino acid with hydrographic parameters were also attempted. Good correlation of amino acids with temperature and inverse relation with dissolved oxygen was observed. However, poor correlation was observed with nutrients. Similar poor correlation with nutrients was also reported by North &

Stephens (1971); Liu & Hellebust (1974); Wheeler et al (1974, 1977), who saw that deprivation of nutrients in the growth medium produced a marked increase in the rate of amino acid uptake by phytoplankton.

Good correlation existed between amino acids and diatoms as well as bacterial cell density from surfaces. Once again indicating that these could be the major contributor to the amino acid concentration from surfaces. Moreover, aspartic:glutamic ratio (Wt/Wt) is a sensitive indicator of microalgal contribution (Liebezeit & Bodungen, 1987). In the present study this ratio was found to be <1. Microfouling material predominant in glycine and having a low aspartic:glutamic ratio (<1), implies that the source was relatively abundant in diatomaceous material.

Better understanding on how microfouling film functions on various surfaces might lead to some important advances in the fields of biotechnology and biochemistry. For example using microfouling film to extract minerals from low grade ores, in an area that could be promising. Maintaining microfouling and preventing macrofoulers could be of use in preventing the surface of metals from deteriorating.

Chapter 9

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REFERENCES

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