STUDIES ON PARTICLE-ASSOCIATED BACTERIA IN A TROPICAL ESTUARINE SYSTEM

THESIS SUBMITTED TO GOA UNIVERSITY

FOR THE DEGREE OF

Certified that all corrections suggested by the Examiners have been incorporated in the Thesis.

DOCTOR OF PHILOSOPHY

IN

MARINE SCIENCES

30/1/2001

BY

Ms. MARIA-JUDITH B.D. DE SOUZA, M.Sc.

UNDER THE GUIDANCE

OF

Dr. P.A. LOKA BHARATHI

SCIENTIST

BIOLOGICAL OCEANOGRAPHY DIVISION

NATIONAL INSTITUTE OF OCEANOGRAPHY

DONA PAULA – 403 004, GOA, INDIA

MAY 2000

CERTIFICATE

This is to certify that all corrections suggested by the viva-voce executive board have been incorporated in the thesis.

(Prof. I. Karunasagar)
External examiner

Dr. P. A. Loka Bharathi
(Research Guide)

STATEMENT

As required under the University ordinance 0.19.8 (vi), I state that the present thesis entitled **"STUDIES ON PARTICLE-ASSOCIATED BACTERIA IN A TROPICAL ESTUARINE SYSTEM"** is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Maria - Judith De Souza

(Maria-Judith De Souza)

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON PARTICLE-ASSOCIATED BACTERIA IN A TROPICAL ESTUARINE SYSTEM"** submitted by **Ms. Maria-Judith B. D. De Souza** for the award of the degree of Doctor of Philosophy in Marine Science is based on her original studies carried out by her under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any university or institution.

Place: Dona Paula
Date: 31/5/2000

Dr. P.A. Loka Bharathi
Research Guide
Assistant Director
Biological Oceanography Division
National Institute of Oceanography
Dona Paula-403 004, Goa

Dedicated

To

My Parents

ACRONYMS

ATP	Adenosine triphosphate
BARC	Bhabha Atomic Research Centre
BP	Bacterial production
CFU	Colony forming units
Cha	Chlorophyll *'a'*
D	Depth
DABA	Diaminobenzoic acid
DNA	Deoxyribonucleic acid
DOC	Dissolved organic matter
FLB	Free-living bacteria
'g'	Mortality
μ	Growth rate
HCl	Hydrochloric acid
IC	Inorganic content
K_t+S_n	Transport constant + natural substrate concentration
MB	Microbial biomass
OC	Organic content
PAB	Particle-associated bacteria
PABP	Particle-associated bacterial production rates
PAM	Particle-associated microbes
PCA	Principal component analysis
PN	Particle number

POC	Particulate organic carbon
PON	Particulate organic nitrogen
PP	Primary production
PSU	Practical salinity units
R	RNA:DNA ratio
RC	Retrievable counts
RNA	Ribonucleic acid
S	Salinity
SPM	Suspended particulate matter
SRB	Sulphate reducing bacteria
T	Temperature
TB	Total bacteria
TC	Total counts
TODB	Thiosulfate-oxidizing denitrifying bacteria
TONRB	Thiosulfate- oxidizing nitrate-reducing bacteria
T_t	Turnover time
TVC	Total viable counts
VC	Viable bacterial counts
V_{max}	Uptake potential

CONTENTS

ACKNOWLEDGEMENTS

The Lord is my strength and my shield, in whom I trusted and found help.

Psalm 28:7

I praise and humbly thank God for the gift of enlightenment, courage and help given to me to pursue my entrusted research work.

I wish to convey my immense gratitude to my Research guide Dr. P.A. Loka Bharathi, Scientist of Microbiology department, N.I.O for the generous surrender of her precious time to guide me in my research work. I thank her for the keen interest, her encouragement and untiring efforts during the course of my research.

Dr. D. Chandramohan, Head of Microbiology department, N.I.O, has been my project co-ordinator and leader. I avail of this great opportunity, to express my sincere thanks to him for his constant advice and invaluable guidance.

I am indebted to Dr. Shanta Achuthankutty, Scientist Microbiology department, N.I.O. for her genuine concern, constructive criticism and zealous help especially during the final stages of my thesis.

My deep sense of gratitude to Dr. Ehrlich Desa, Director, N.I.O., for permitting me to do my Ph.D by giving the necessary facilities and encouragement.

I thank Prof.G.N. Nayak, H.O.D. of Marine Science & Marine Biotechnology, Goa University, for his concern and encouragement. I extend my thanks to Prof. Sangodkar, Dean of Applied Sciences, Goa University for his support.

My immense thanks to Prof. Udupa of Mangalore University for rendering his valuable suggestions in statistics and having spared his precious time.

I am grateful to Dr. James Coyer of USA whose expertise helped me familiarize with molecular techniques. I also appreciate his help and fruitful discussions.

I thank:
Dr. Karl Banse, Scientist of Seattle, USA for his inspiring suggestions.

Dr. P.C. Rao of G.O.D., N.I.O. for his suggestions and help in mineralogy.
Dr. Shetye P.O.D., N.I.O. for his useful discussions.
Dr. Ramaswamy and Dr. P.S. Rao of G.O.D., N.I.O. for their keen interest and help during the sample analysis.

Dr. Usha Muraleedharan of Marine Biotechnology department, Goa University, for her help in isotopic analysis.

Dr. Sugandhani, C.O.D., Dr. Prabhu Matonkar and Mr. Subrahmanyam of B.O.D. for their kind help rendered during the operation of various instruments.

Dr. Krishnakumari of B.O.D., N.I.O for providing me the relevant data on primary production.

Dr. Sarma (France), Dr. Thamban (G.O.D) and Mr. Vishwa Kiran (M.C.M.R.D.) of N.I.O. for rendering help and assistance in the use of various instruments.

The personnel of photography section, workshop and drawing section for their assistance and cooperation. I also acknowledge all the staff at N.I.O. and Goa University library for their readiness in providing assistance.

Office of Naval Research (U.S.A) for funding the Indo-US project under which the present study had been carried out.
Dr. Bernard Zahuranec (O.N.R - USA) for co-ordinating the project.

I express my heartfelt thanks to my parents for their great affection, good advice, selfless sacrifice, constant encouragement and help, which gave me the determination to fulfil my research studies.

My deep gratitude to Mrs. Pushpa Chandramohan for her kind love, generous hospitality and care.

I treasure the friendship, goodwill and moral support of Mr. Vasanth Samaga, Ms. Rochelle Fernandes, and Ms. Yashoshri Shrivastava. I thank Ms. Pythia Barreto for her help during the final stages of my thesis.

I appreciate the support and help extended by all at N.I.O particularly, the senior staff and colleagues of the microbiology laboratory during the course of my research. I am thankful to Mr. Pradeep Ram, Ms. Usha Chinchkar and Mr. Manguesh Gauns for rendering their timely help.

I thank all my colleagues at the field works for helping, and encouraging me in so many ways.

Lastly I extend my thanks to all those who showed concern and rendered a helping hand in some way or the other during the course of my research work.

Maria-Judith de Souza

1.0 INTRODUCTION

Estuaries and coastal regions are regarded as complex ecosystems influenced by physical and chemical factors both temporally and spatially (Mc Cluskey, 1989). They are among the most productive ecosystems in the world (Mc Cluskey, 1989). They form the dynamic boundary between fresh and salt water and are thought to be an important interface for biological reactions (Zimmermann, 1977). They are often characterized by wetlands, marshes and swamps mostly with mangrove vegetation submerged in water for a greater period of the day (Odum, 1988). Large number of aggregates, which are fragile, amorphous microscopic particles of different forms and sizes (Grossart and Simon, 1993), in the water body are a conspicuous feature of the estuarine environment (Eisma, 1992). Particles are defined as biogeochemical molecules or subdivisions of matter characterized by properties of mass and observable position in space and time. These particles in aquatic ecosystems may be classified as "organic" comprising viruses (0.02 – 0.3μm), bacteria (>0.22-1 μm), picoplankton (<5 μm), polysaccharide strings and sheets (3 to >100 μm) and detritus (0.5 – 2500 μm) and "inorganic" comprising clay minerals (200-1000 nm), aluminosilicates (200-1000 nm) and iron colloids (100 – 500 nm) or a combination of both. Most aggregates are formed by the physical coagulation of smaller particles including microaggregates, phytoplankton, fecal pellets, organic debris and clay mineral particles (Alldredge, 1989). They also include organisms, like bacteria, protozoans and metazoans which are components of the aggregates in

estuaries (Goulder, 1976, 1977; Grieser, 1988; Eisma, 1992; Laybourne-Parry *et al.*, 1992; Rogerson and Laybourne-Parry, 1992a, b; Kies 1995; Zimmermann and Kausch, 1996), each with their own communities of attached micro-organisms in various nutritional states. This physical aggregation process may be enhanced by exudates, exopolymers and products of cell lysis generated by attached microorganisms which increase the stickiness of colliding particles and form a mucilaginous matrix in which many components become embedded (Alldredge, 1989). Flocculation is an important process in controlling the settling velocities of particles and during the tidal cycles there may be changes in the particle size distribution in estuaries (Dyer, 1986). Most of the aggregated materials are in the turbid zone where the freshwater in the river mixes with seawater (Kies, 1995). In estuarine waters, phytoplankton, plankton detritus and heterotrophic consumers provide an obvious source of suspended particles (Laane, 1982). The organisms living in and on aggregates form an important part of the plankton ecosystem, aggregating small particles and breaking down preferentially larger ones (Jackson *et al.*, 1987; Gorsky *et al.*, 2000). The nature of particles and their adsorption capacities determine the magnitude of the bacterial colonization (Hoppe, 1984). By providing a substrate for organisms, the aggregates may influence heterotrophic turnover of organic material. Their abundance may be a sign that there is a more complex food web in pelagic waters. Caron *et al.*, (1982) thought that aggregates are important loci of microbial activity in the water column, similar to the much

larger oceanic 'marine snow' aggregates. Particles play a significant role as transport agents as microorganisms associated with them sink at rates, which are potentially several orders of magnitude higher than a single unattached cell. However, the abundance and size of aggregates show considerable seasonal (Kies, 1995; Zimmermann and Kausch, 1996) and spatial variation (Kies, 1995).

Particle associated bacteria may be considered as a separate population in estuaries. The proportion of total bacterial abundance attached to particles is generally less than 10% (Zimmerman, 1978; Kirchman and Mitchell, 1982) but can vary from a few percent to 98% of total bacterial abundance in different aquatic regions (Iriberri *et.al.*, 1987). Such large variations in the percentage of the attached bacteria are being attributed to physical (viz. turbidity, salinity, temperature, light, turbulence) and chemical characteristics (distribution of nutrients) of the water column (Almeida and Alcantara, 1992). Particle bound bacteria are also relatively numerous in some fresh water and estuarine environments (Ducklow, 1982). Evidence is accumulating in studies on rivers, that as the freshwater flows into the sea the degree of attachment decreases with increasing salinity (Goulder *et al.*, 1980; Wright, 1978).

Bacteria attached to particles are considered to be very important for at least three reasons. First, the metabolic activity of associated bacteria differs

from that of the unattached bacteria (Crump *et al.*, 1998). These particle-associated bacteria are generally larger and more active per cell than unattached bacteria (Goulder, 1977; Kirchman and Mitchell, 1982; Unanue *et al.*, 1992). Secondly, particle bound bacteria may contribute to re-mineralization of particulate carbon. Particles are known to be favorable microenvironments for bacteria compared to the surrounding water because of the availability of potential substrates for growth which is facilitated by organic components of particles, adsorbed matter and possibly an increased flux of the dissolved nutrients during sinking (Reviewed by Fletcher, 1991; Kirchman, 1993). It is an important step for bacteria to attach to the surface of particle in the degradation of particulate organic matter (Kato, 1984). In many estuaries a significant fraction of total bacterial carbon production is due to particle attached bacteria (Griffith *et al.*, 1994). Thirdly, bacteria attached to particles (> 1 μm diameter) are larger prey and thus may be important for bacteriovores. Moreover, particle bound bacteria may improve the nutritional quality (Heinle *et al.,* 1977) of the detrital particles for grazing by zooplankton (Kirchman, 1983). In the Columbia River estuary (USA), the principal consumers of bacterial biomass are detritivorous metazoans (Simenstad *et al.*, 1994a). Particle attached bacteria can have a very different role in a food web because they may be directly grazed on by larger metazoans, bypassing consumption by protozoan grazers and "short-circuiting" the microbial loop (Baross *et. al.*, 1994)

As highlighted above, the particle-associated dynamics is a significant component of the temperate estuaries. Most of the studies in the temperate waters have been limited to one or two factors. Obviously the role of the particle-associated bacteria in microbial trophic dynamics is not yet well understood. Hence information on the microorganisms on aggregates in estuarine environments is very much limited (Laybourne-Parry *et. al.,* 1992; Rogerson and Laybourne-Parry, 1992a, b; Crump and Baross, 1996). The tropical estuaries are very different as they are marked by the monsoonal effect and have less variation in temperature. It would therefore be highly pertinent to elucidate the role of particle-associated bacteria in the carbon flux of our estuaries.

This study deals with the two estuarine systems along the Goa coast, the Mandovi and the Zuari and their converging point off Cabo meeting the Arabian Sea. The Mandovi-Zuari estuarine system consists of the Mandovi-Zuari rivers, their tributaries and the man-made channel, the Cumbarjua canal which joins both the rivers upstream about 15 km from the mouth of Zuari. They are not only influenced by tides but also by the mangroves, an unique ecosystem where three different pathways (i.e. particulate, DOC and leachate) lead to bacterial production. The Mandovi and Zuari rivers characterized by mixed semi-diurnal tides, strong tidal currents and their perennial connections with the Arabian Sea, support a life line of 11.69 lakhs (1991 census) who depend on these estuaries mainly for fisheries and other

activities like navigation etc. Moreover this region receives very heavy annual rainfall (107cm) especially during the monsoon season (June – September). This ecosystem thus becomes one of the most variable environments for the study of trophic relationships. The problem is viewed in a holistic manner involving factorial matrix both direct and indirect in order to understand the role of these bacteria in the overall carbon flux in the estuaries. The present work investigates:

1. The factors affecting particle-associated dynamics.
2. The significance of particle associated bacteria in a tropical estuarine system.
    a) The importance and dominance of particle-associated bacteria
    b) The role of particle-associated bacterial biomass production
    c) The metabolic activity of associated bacteria.
3. The parameters responsible for variation in the estuarine network and development of a regression model.

The present work is the first composite information on the seasonal dynamics of particle-associated bacteria in the tropical estuaries. It envisages using the regression models to predict some of the microbial parameters so as to aid the development of better management practices for these systems. In fact this study forms the first report for India.

2.0 REVIEW OF LITERATURE

2.1 HISTORY

Man has long been aware of microbial attachment to solid surfaces in the sea and in freshwaters. Suspended in all natural waters, from the smallest mountain stream to the deepest ocean, are assemblages of small particles of different sizes and shapes, which make up what we call seston or suspended particulate matter (SPM). They may occur as single particles, as aggregates (Mel'nikov, 1976) or in flocs (Eisma and Cadee, 1991). Yet, amidst this confusion is the very source of life, since very small particles comprising SPM, such as bacteria, plankton and detritus play a major role in the carbon cycle of the aquatic ecosystem (Billiones *et al.,* 1999).

In 1943, Zobell pointed out that the influence of solid surfaces upon bacterial growth depended upon both the concentration and type of organic matter present. Slime layers formed by attached microorganisms and their associated polymers are easily detected by touch (Fletcher and Marshall, 1982). It was way back in 1959 that Jannasch and Jones made a study of the attached bacteria in the Pacific ocean. Bacterial attachment has received growing attention for past 28 years as workers became aware of the significance of attached bacteria and new methods were devised or adapted to focus on the problem. In fact, the study on bacteria attached to particles was initiated in the seventies. The predominant opinion was that marine bacteria were present mainly in aggregates or attached to detritus particles (Seki, 1970). Over the years with the advent of epifluorescent microscopy

the importance of particle-attached bacteria in trophic dynamics became evident (Bell and Albright, 1981; Sieracki and Viles, 1992). Wiebe and Pomeroy (1972) and Zimmermann (1977) were the first investigators offering evidence that only a few percent of total bacterial abundance in oceanic and brackish water (Baltic Sea) could be attributed to attached bacteria. At times bacteria attached to particles can account for as much as 90% of the total bacterial abundance in some freshwater lakes and estuaries (Kirchman and Ducklow, 1987). Some workers have reported that particle-bound bacteria usually make up only less than 10% of the total assemblage (Kirchman and Ducklow, 1987).

Studies on particle-associated bacteria were carried out at different geographical locations as seen in the graph (Fig. 1). Most of the works have been concentrated on the pelagic waters (Kirchman *et al.;* 1991, Bidle and Fletcher, 1995; Almeida and Alcantara, 1992). Investigations have also been carried out on different types of ponds and marshes (Kirchman and Mitchell, 1982) from the Hudson River Plume during a diatom spring bloom (Ducklow and Kirchman, 1983) and from coastal kelp environments (Linley and Field, 1982), all in temperate regions. In aquatic systems factors controlling bacterial biomass, growth and production rates have been studied in less detail except in Urdaibi estuary near Bay of Biscay (Revilla *et al.,* 2000) been carried out in temperate regions. In the tropical areas there are virtually no study describing particle-associated bacterio-plankton dynamics. The prime factors controlling the production and growth rates in aquatic

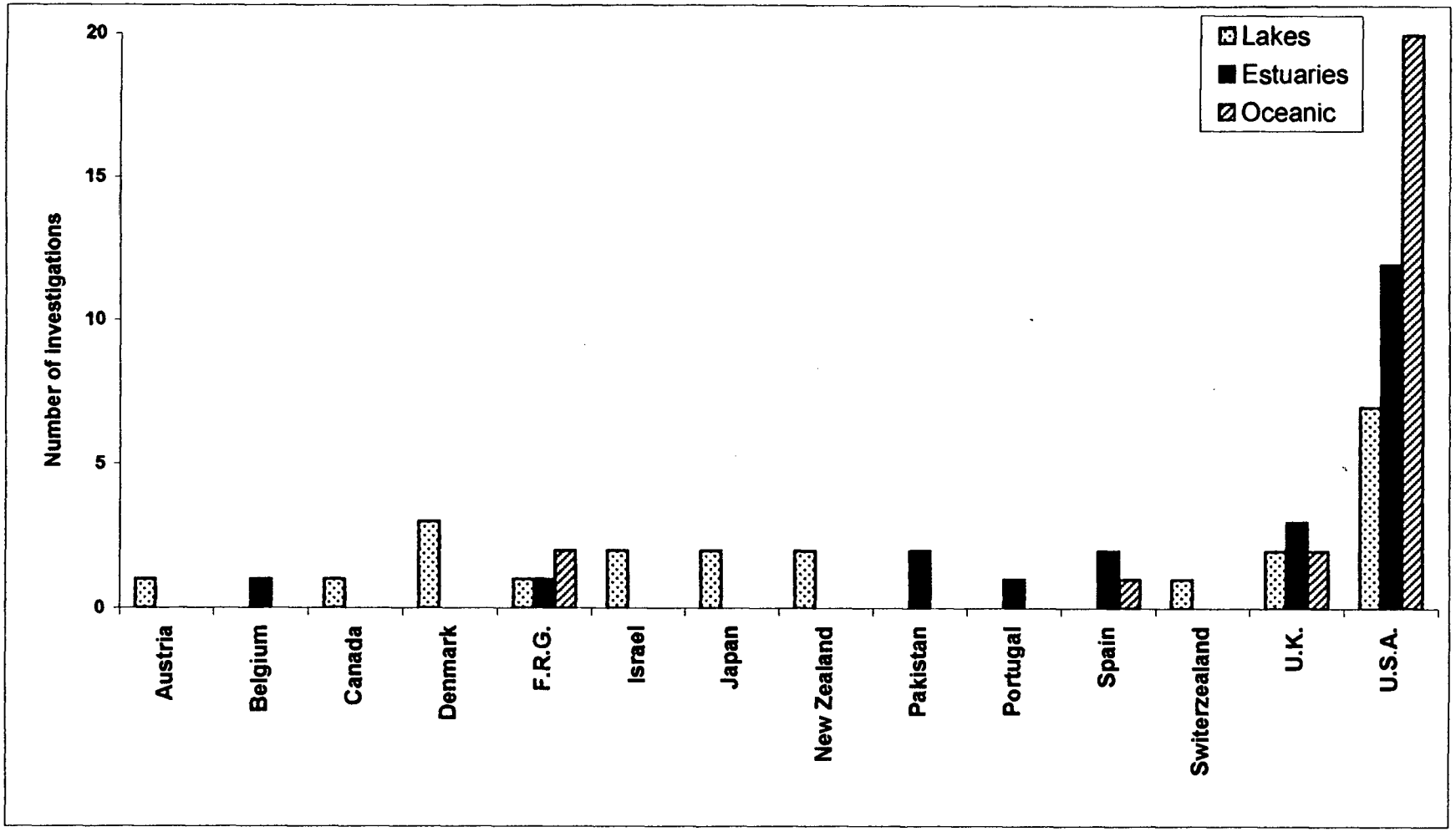


Fig. 1: International scenario of studies carried out on particle-associated bacteria.

systems have been found to be dissolved organic carbon (allochthonous and/or autochthonous), temperature, inorganic nutrient concentrations or viral mortality (White *et al.*, 1991; Kirchman and Rich, 1997; Goosen *et al.*, 1997). Nevertheless, we are yet to understand how the various environmental parameters interact and influence these particle-associated bacteria. This review brings out the significance and role of particle associated bacteria in the trophic dynamics along with controlling factors.

2.2 PARTICLE - ASSOCIATED BACTERIA AND PARTICLE SIZE

Historically, particles were typically thought of as readily observed phytoplankton cells and large detritus (e.g. >10 μm). which are present at 10^2-10^3 ml^{-1} (Azam *et al.*, 1995). Koike *et al.*, (1990) found 10^7 particles of 0.3 - 1.3 μm in size per ml; Wells and Goldberg (1991,1993) found 0.05 – 0.12 μm colloidal particles at 10^7 - 10^{10} ml^{-1} range and 0.2 - 2 μm colloidal aggregates at 10^4 – 10^5 ml^{-1} range, Alldredge *et al.*, (1993) found 10^2-10^4 ml^{-1} polysaccharide particles, including strings, sheets and bundles, 3 -100 μm in size. Goulder (1976, 1977) found that most (>75%) of the suspended solid particles at Hull (England) are aggregates and that the most frequent size category (usually >60%) is 5 to 14 μm while <20% of the particles exceed 20 μm. Colonized particles (~ 40 μm) showed 2 to 80 bacterial cells per particle agreeing with results of Fergusson and Rublee (1976) and Marsh and Odum (1979) but contrasting with the values of 2.9 to 6.4 cells per particle obtained

by Iriberri *et al* (1987) in coastal waters. Kirchman (1983) found that heavily colonized particles were always >10 μm in diameter.

Most particles in the River Danube (Austria) were found to be in the size class of >9 to 100 μm^2, accounting for 60% of the total number of particles, while the largest size class (>100 to 1125 μm^2) accounted for only 11%. The colonized particles were on an average 13 μm in diameter (Berger *et al.,* 1996). In the River Danube (Austria), smaller particles exhibited higher bacterial abundance per unit surface area than the larger particles. The percentage of particles colonized by bacteria was high when the total number of particles was low. In the Donaukanal estuary (Austria) mean particle diameter was 16 μm; Particles of the same size were found in suspended solids in the Ogeechee River (USA) (Carlough, 1994). At both sampling sites the smallest fraction of attached bacteria was found in the smallest particle fraction (Berger *et al.,* 1996).

Increased microbial activity was found associated with estuarine turbidity maxima (ETM) of the Columbia River estuary (USA) (Baross *et al.,* 1994) with a significant fraction associated with particles retained on a 20 μm screen (Crump and Baross, 1996). In the same estuary less than 50% of the bacterial carbon production in the ETM was associated with particles retained by a 20 μm screen (Crump and Baross, 1996). This implies that particles that host the active population of bacteria both in and outside the ETM were between 3 and 20 μm in size.

Almeida and Alcantara (1992) arranged particles in 4-size classes >1 to 3, >3 to 10, >10 to 40 and >40 to 140 μm and found that irrespective of tide the mean number of bacteria per colonized particle in surface water (634 particles) and sediments (2862 particles) was very similar averaging 2 to 3 cells on > 1 to 3 μm particles, 5 to 6 cells on >3 to 10μm particles, 13 to 21 cells on >10 to 40 μm particles and 40 to 59 cells on >40 to 140 μm particles. They suggested that bacterial colonization of large particles is more erratic than small ones and that particle size may play an indirect role on bacterial growth on particles. Up to 66% of total bacterial biomass was found attached to particles larger than 10μm (Becquevort *et al.*, 1998). Highly variable bacterial production values for the >3 μm fraction in eight Danish lakes have also been reported (Letarte *et al.*, 1992).

Analysis of the extent of colonization of particles of different sizes revealed that the average number of bacteria per particle varied from 2 to 192 (Almeida and Alcantara, 1992). Studies in different aquatic systems indicate that with decreasing particle size, microbial biomass and activity increases (Wallace *et al.*, 1982; Kondratieff and Simmons, 1985; Almeida and Alcantara, 1992). A similar relation between colonization density and particle size was recently detected by Passow and Alldredge (1994) and Schuster and Herndl (1995).

2.3 NATURE

Studies on the nature of SPM indicate a very heterogeneous nature (Mel'nikov, 1976; Kranck, 1980). This heterogeneity presents problems in the characterization and classification of the particles. Particles also exist as living organisms, detritus, fecal matter, exuvia, carcasses and plankton hardparts (Fowler and Knauer, 1986). Long and Azam (1996) found Coomassie stained, protein containing particles abundant in the sea. A class of transparent particles called TEP (transparent exopolymer particles) are abundant in the ocean (Alldredge *et al.,* 1993). TEP are presumably produced from dissolved carbohydrate polymers exuded by phytoplankton and bacteria. They range from a few microns to hundreds of microns (Passow and Alldredge, 1994) in size. Mostajir *et al.,* (1995) studied the non-living organic particles in nano and pico -size range (largest dimensions 1-20 μm) and identified them as DAPI yellow particles which were mainly detrital.

The complexity and heterogeneity of SPM are most evident in estuaries. Inputs from river, land and sea, turbulence and limited depth result in the characteristic high SPM load of the estuarine ecosystem. Many rivers are characterized by high concentrations of suspended inorganic and organic particles that enter the estuaries (Findlay *et al.,* 1991). Estuarine systems are also characterized by the high degree of interactions with bordering terrestrial ecosystems, which provide an important source of detritus (Pomeroy, 1980). A 20-80% quantity of

suspended particles in estuarine and riverine environments consist of detritus (Poulet, 1983) which are mostly allochthonous. Freshwater phytoplankton are a major source of organic matter to the estuary (Small *et al.,* 1990) and have been observed microscopically to be a significant component of ETM particles.

Compared to suspended particles, the colloidal fraction was relatively pure organic material 32 $\pm$ 4% (organic and 3.6 $\pm$ 0.6% N) throughout the Potomac and the Patuxent estuaries (USA) in agreement with published CHN data for colloids for Chesapeake Bay and its subestuaries (Sigleo *et al.*, 1982, 1983; Means and Wijayaratne,1984; Sigleo and Macko, 1985). The C/N atomic ratios in suspended particles ranged from 4 to 12, colloids ranged from 9 to 12 and the ultrafiltrates ranged from 1.6 to 12. Ratios near or below 10 are indicative of single-cell organisms such as phytoplankton and bacteria, whereas ratios from 20 to 100 are typical values for vascular plants (Redfield *et al.*, 1963).

In the Columbia River estuary (USA) particles in the ETM region have contributed 3 to 10% organic material by weight (Reed and Donovan, 1994) and appear to undergo some microbial transformation in a sub-oxic environment (Prahl and Coble, 1994). In Danube river (Austria), Hoch *et al.,* (1995) found inorganic particles with an organic coating in the size range of 2 to 15 μm in diameter to be abundant.

Most particles (except flocculent aggregates) are sparsely colonized by bacteria (Kirchman, 1983; Hoppe, 1984). Many particles and interfaces

may not provide the conditions necessary for the successful competition of attached bacteria with bacteria in the water phase (Hoppe, 1984). Obviously, the nature of particles and their adsorption capacities determine the magnitude of bacterial colonization (Hoppe, 1984). Bacteria attached to particles were able to utilize not only the organic compounds that made up the particles but also the dissolved compounds concentrated on particle surface (Jannasch and Pritichard, 1972). Between 86 and 100% of epibacteria in Lakes Sempach and Lucerne (Switzerland) were attached to organic particles. In lakes Dunstan and Wakatipu (New Zealand) 44 to 100% of the epibacteria were attached to organic particles (Friedrich *et al.*, 1999).

Macroscopic organic aggregates, known as "marine snow", are rich in inorganic and organic nutrients (Shanks and Trent 1979, Herndl, 1992), making them important sites for biological processes of production, decomposition and nutrient recycling in the water column. Marine snow aggregates are colonized by algae and a rich and diverse detrital community of bacteria and protozoans, usually at concentrations many-fold higher than in the surrounding water (Alldredge and Cox, 1982, Prezelin and Alldredge, 1983). Knowledge on the dynamics of attached bacteria indicates that colonizing phytodetritus bacteria give rise to highly dynamic microenvironments, which form and transform through microbial processes.

2.4 PARTICLE - ASSOCIATED BACTERIAL ABUNDANCE

Particle-bound bacteria have been enumerated in different pelagic environments by staining with fluorochrome dyes and then counting the stained bacteria by epifluorescence microscopy (Zimmermann and Meyer – Reil, 1974; Hobbie *et al.*, 1977). Particle-bound bacteria usually make up less than 10% of the total assemblage (Zimmermann, 1978; Kirchman and Mitchell, 1982). At times, bacteria attached to particles can account for as much as 90% of total bacterial abundance in some freshwater lakes and estuaries. This has been demonstrated repeatedly in many aquatic environments as summarized in Tables 1A, 1B and 1C.

In the River Danube (Austria) the abundance of attached bacterial density varied from 0.1 to 1.4 x 10^6 cells.ml^{-1} and was on average 9.5 $\pm$ 8.3% of total bacterial density. Attached bacterial densities are well within the range observed for other rivers. The abundance of particle-bound bacteria varied by a factor of 14. On an average only 9.5% were found attached to particles Hoch *et al.*, (1995). In the same river, in another observation bacteria were found to colonize 39% of the suspended particles (Hoppe 1984). Bacterial colonization of particles varied considerably in the River Danube (Austria), ranging from 3 to 17 bacteria per particle (Berger et al., 1996). In Donaukanal (Austria) the abundance of attached bacteria varied from 0.2 to 2.2 x 10^6cells.ml^{-1} and accounts for 12 $\pm$ 9 % of total bacterial abundance on an average. These variations were 5 times in the Dan River in Virginia (USA). Kondratieff and Simmons (1985) reported a variation by a

Table 1A. Summary of reports on the proportion of total bacterial abundance attached to particles in marine environments.

ENVIRONMENT	ATTACHED (%)	REFERENCE
Pacific	20 – 50	Jannasch & Jones (1959)
Pacific	33 - 50	Sorokin (1971)
Atlantic (N)	<1	Hobbie *et al.* (1972)
Coastal Atlantic (N)	Very few	Wiebe & Pomeroy (1972)
North Carolina coast (USA)	15	Ferguson & Rublee (1976)
Off Long Island (USA)	Very few	Ferguson & Palumbo (1979)
Strait of Georgia (USA)	24	Bell & Albright (1981)
New York Bight (USA)	13	Ducklow and Kirchman (1983)
Gulf Stream (USA)	0.1 – 4.4	Alldredge *et al.* (1986)
South California coast (USA)	1 - 3	Alldredge *et al.* (1986)
Off Bilbao (Spain)	1 - 22	Iriberri *et al.* (1987)
Mediterranean (NW)	0.8 – 2.8	Turley & Stutt (2000)

Table 1B. Summary of reports on the proportion of total bacterial abundance attachec to particles in estuaries and salt marshes.

ENVIRONMENT	ATTACHED (%)	REFERENCE
North Inlet Marsh (USA)	35	Wilson & Stevenson (1980)
Palo Alto Salt Marsh (USA)	22	Harvey & Young (1980)
York River estuary (USA)	85	Ducklow (1982)
Little Sippewissett Salt Marsh (USA)	1.7	Kirchman & Mitchell (1982)
Eel and Mill Ponds (USA)	1.3	Kirchman & Mitchell (1982)
Nobska Pond (USA)	6.0	Kirchman & Mitchell (1982)
Great Sippewissett Salt Marsh (USA)	1.9	Kirchman *et al.* (1984)
Newport River estuary (USA)	3.3	Palumbo *et al.* (1984)
Estuarine & nearshore waters of Georgia (USA)	30	Griffith *et al.* (1990)
Urdaibai estuary (USA)	12 -72	Revilla *et al.* (2000)
Fraser River Plume (Canada)	69	Bell & Albright (1981)
Aquatic sites at Southwest British Columbia (Canada)	44	Bell & Albright (1982)
Bay of Fundy (Canada)	10	Cammen & Walker (1982)
Humber estuary (England)	87	Goulder (1977)
Kiel Bight (FRG)	3.1	Zimmermann (1978)
Ria de Aveiro Portugal	1 - 49	Almeida & Alcantara (1992)
River Danube (Austria)	8.2	Hoch *et al.* (1995)
River Danube (Austria)	9.5	Berger *et al.* (1996)
Donaukanal (Austria)	3-21	Berger *et al.* (1996)
Indus river (Pakistan)	4 - 92	Bano *et al.* (1997)
Indus river (Pakistan)	14 - 84	Bano *et al.* (1997)

Table 1C. Summary of reports on the proportion of total bacterial abundance attached to particles in fresh water environments.

ENVIRONMENT	ATTACHED (%)	REFERENCE
Coastal ponds North East United States	<10	Kirchman & Mitchell (1982)
Lake Mendota (USA)	32	Pedros-Alio & Brock (1983)
Ice House Pond (USA)	3	Kirchman (1983)
A small pond (USA)	<1-10	Kirchman (1983)
Bay of Quinte (Canada)	17.5	Burnison (1975)
Frazer River (Canada)	82	Bell & Albright (1981)
Lake Tanning (Denmark)	73	Riemann (1978)
Lake Mosso (Denmark)	17	Riemann (1978)
Swiss Lake	1.3 – 5.7	Friedrich *et al.* (1999)
A small pond (Japan)	30 - 50	Konda (1984)
Lake Biwa (Japan)	< 1 - 3	Nagata (1987)
Lakes Dunstan & Wakatipic (New Zealand)	4.2 – 11.6	Friedrich *et al.* (1999)

factor of 13 in bacterial abundance for particle-bound bacteria whereas in the York River estuary (USA), Ducklow (1982) obtained a 3- and 7- fold variation.

Bacterial colonization of particles in five freshwater ponds ranged from 3 to17 per particle (Kirchman and Mitchell, 1982) similar to other aquatic systems (Hoppe, 1984). Interestingly in an Ice House Pond (USA) the abundance of particle-bound bacteria varied by a factor of 1000 (Kirchman, 1983).

All these observations indicate that attached bacteria vary in abundance over a wide range. This variation was observed over the year and between different environments. The large variations in the percentage of attached bacteria have been attributed to physical (viz. turbidity, salinity, temperature, light, turbulence) and chemical characteristics (distribution of nutrients) of the water column (Palumbo *et al.*, 1984; Kondratieff and Simmons, 1985; Almeida and Alcantara, 1992).

2.5 BIOMASS, PRODUCTION AND ACTIVITY

Biomass and cell production by attached bacteria accounted for 10 to 73% and 2 to 68% of total bacterial community respectively (Becquevort *et al.*,1998). The particle-attached bacterial carbon production ranged from 0.13 to 4.50 $\mu g.l^{-1}.h^{-1}$ and represented an average of 90% of total bacterial production Table 2. Thymidine incorporation rate was 10 to 100 times (4.79×10^{-8}) higher than that of free-living bacteria (2.8×10^{-9} pmol TdR $cell^{-1}h^{-1}$).

Table 2. Summary of reports on particle-associated bacterial production

Environment	% on total bacteria production	Reference
MARINE		
Atlantic Ocean	2.3 – 26	Alldredge *et al.* (1986)
Atlantic ocean (NE)	1.8	Turley & Mackie (1994)
Pacific Ocean	1.3 – 13.4	Alldredge *et al.* (1986)
Mediterranean (NW)	3-12	Turley &Stutt (2000)
Antarctic Scripps Pier	9	Furhman & Azam (1980)
Coral reef (South west Pacific)	30 – 50	Moriarty *et al.* (1985)
ESTUARIES		
Rhode River Estuary (USA)	7-22	Rublee *et al.* (1984)
Estuarine waters of Georgia (USA)	59	Griffith *et al.* (1990)
Columbia River Estuary (USA)	90	Crump *et al.* (1998)
Urdaibai estuary (Spain)	17-84	Revilla *et al.* (2000)
Nearshore of Georgia (USA)	22	Griffith *et al.* (1990)
San Francisco Bay (USA)	38	Hollibaugh & Wong (1999)
Central Bay (USA)	26	Murrell *et al.* (1999)
Suisun Bay (USA)	42	Murrell *et al.* (1999)
Indus River Delta (Pakistan)	73 - 96	Bano *et al.* (1997)
Sacramento River (USA)	22	Murrell *et al.* (1999)
New York Bight (USA)	5	Ducklow & Kirchman (1983)
FRESHWATER		
A small pond Mass.	1.2 – 5.7	Kirchman (1983)
Lake Norrviken, (Sweden)	1.5 – 59.1	Bell *et al.* (1983)
Little Crooked Lake, Ind	0.3 – 11.7	Lovell & Konopka (1985)
Lake Constance (FRG)	0.7 – 3.6	Gude (1986)

(Crump *et al.*,1998). Crump *et al.*, (1998) found that the specific thymidine incorporation rates in estuarine water samples (salinity>1) were significantly higher than in freshwater samples for both groups of bacteria (attached and free living). They suggested that riverine particles undergo changes when mixed with the estuary that affects bacterial carbon production rates. These changes involve some form of interaction between riverine particles and particles generated in the estuary for sufficient time to acquire an estuarine bacterial flora. These estuarine particles may host faster-growing bacteria, giving a higher average rate of production when combined with riverine particles.

The relative contribution of particle-attached bacteria to bacterial carbon production in coastal and estuarine systems is variable and depends on the concentration and growth rate of particle-attached bacteria (Griffith *et al.*, 1990; Unanue *et al.*, 1992). An average of 90% of the bacterial carbon production in the water column of the Columbia River estuary (USA) was due to particle-attached bacteria. Crump *et al.*, (1998) suggest that free-living bacteria and dissolved substances are rapidly flushed into and out of the estuary, but particles and particle-attached bacteria are retained in the estuary by ETM. This was true not only in the ETM (estuarine turbidity maxima), where total bacterial carbon production was enhanced, but also in less turbid estuarine water and freshwater (Crump *et al.*, 1998). Other river estuaries where attached bacteria dominate total bacterial activity in the water column, such as Humber and the Tamnar in England are generally

more turbid than the Columbia River estuary (USA) (Goulder, 1977; Bent and Goulder,1981; Plummer *et al.,* 1987; Uncles and Stephens, 1993).

In the Columbia river estuary (USA) highest levels of particle-attached bacterial carbon production were found at intermediate salinity and elevated turbidity. High turbidity ETM, with associated high bacterial carbon production was most often found at intermediate salinities. Lower-turbidity samples with unusually high bacterial carbon production were also found at these intermediate salinities. In freshwater and at higher salinity, turbidity and particle-attached bacterial carbon production were reduced (Crump *et al.*, 1998). A similar result was found at higher salinities in the plume of the Hudson River estuary (USA), where particle-attached bacterial activity was correlated with POC concentration but not with salinity (Ducklow and Kirchman, 1983). Seasons are also known to affect the production of particle associated bacteria. In the upper Chesapeake Bay (USA), particle-attached bacterial production was highest during the summer months, but the relative contribution of particle-attached bacteria to total bacterial production was highest in winter, when free-living bacterial production was the lowest (Griffith *et al.,* 1994). In spring and in summer, the Columbia River estuary (USA) had high particle-attached bacterial carbon production and low free-living bacterial carbon production (Crump and Baross, 1996; Crump *et al.*, 1998). Flocculation has shown to make some organic material more available to bacteria (Tranvik and Sieburth, 1989). It is possible that flocculation of dissolved organic material and aggregation with other

particles in brackish water promotes bacterial colonization and growth. Another possibility is that organic material available to heterotrophic bacteria could be more labile in the estuary than in the river, accelerating bacterial growth rates. Although the freshwater phytoplankton can survive at low salinity (Jackson *et al.*, 1987) they probably perish at higher salinity' and provide fresh, labile organic material to bacteria in the estuary. Particle attached bacterial concentration varied from < 2.60×10^6 (detection limit) to 5.10×10^9 cells.l^{-1} and were positively correlated with turbidity (OBS- optical backscatter sensor and SPM- suspended particulate matter) and POC (Crump *et al.*, 1998).

Kirchman and Mitchell (1982) found that the percent assimilation of ^{14}C-glucose by particles of >3.0 μm size was as high as 74 % in the brackish marsh and as low as 0% in the salt marsh. They also found that ^{14}C-glutamate incorporation ranged from 8.9 to 23% and suggested that although the number of epibacteria in relation to unattached bacteria is small, epibacterial populations can contribute significantly to the total turnover of dissolved organic compounds in aquatic ecosystems. They also carried out work on the size fraction larger than 35 μm and found that the ^{14}C-glucose uptake was small (<5%) in all water sampled. They further showed that the uptake by the fraction larger than 8 μm was at most 5 to 10% of total assimilation. A large percentage of assimilated ^{14}C-glucose and ^{14}C-glutamate was taken up by the 1.0 to 3.0 μm fraction in some environments. They also sampled at Little Sippewissett marsh (USA) during

high and low tides but did not detect any differences in epibacterial populations along the entire channel. As seen in Tables 3A, 3B and 3C the heterotrophic activity of > 3 μm particle-associated bacteria had a wide variation irrespective of the aquatic system. In general it ranged from 0% at Gulf of Marseilles, (France) (Chretiennot-Dinnet and Vacelet, 1978) and Lake Mendota (USA) (Kirchman and Mitchell (1982), Pedros-alio and Brock (1983)) to as high as 99% in the Humber Estuary (England) (Goulder, 1977; Bent and Goulder, 1981) and Salt marsh (USA) (Harvey and Young,1980).

2.6 CHARACTERISTICS OF BACTERIA ASSOCIATED WITH PARTICLES

The size of attached bacteria was significantly larger than that of free-living bacteria (Biddanda, 1986; Biddanda & Pomeroy, 1988; Azam *et al.*, 1993; Becquevort *et al.*, 1998). Bacteria attached to particles and larger phytoplankton cells are likely to sink out of the mixed layer faster than the free-living (motile) bacteria (Wangersky, 1984; Kirchman *et al.*, 1982). Attached bacteria above the thermocline were adapted to warm temperatures by showing optimal activity at 17 ^{0}C (Fuhrman and Azam - unpublished) while bacteria attached to particles from deeper depths and showed optimal activity at higher temperatures, suggesting recent origin from the above thermocline (Azam *et al.*, 1983).

Table 3A. Summary of previous reports on heterotrophic activity of associated bacteria in freshwaters

Environment	Compound	% uptake of compound by fraction >3.0 μm	References
Clear Lake (USA)	Glucose	28	Paerl (1978)
Lake Tahoe (USA).	Glucose, acetate,glycine	12 - 33	Paerl (1980)
Mirror Lake (USA)	Glucose	13 – 30	Jordan & Likens (1980)
Five ponds & two marshes (USA)	Glucose	0 – 74	Kirchman & Mitchell (1982)
Five ponds & two marshes (USA).	Glutamate	9 – 23	Kirchman & Mitchell (1982)
Freshwater pond (USA)	Glucose	4 – 43	Kirchman (1983)
Freshwater pond (USA)	Acetate	3 - 11	Kirchman (1983)
Freshwater pond (USA)	Glutamate	4 – 18	Kirchman (1983)
Lake Mendota, (USA)	Acetate	15 – 90	Pedros-alio & Brock (1983)
Lake Mendota (USA)	Sulfate	0 – 80	Pedros-alio & Brock (1983)
Bay of Quinte, (Canada)	Glucose	17	Burnison (1975)
Frome & Hook rivers, (England)	Glucose	50 – 72	Campbell & Baker (1978)
Four Danish lakes	Glucose	7 – 73	Riemann (1978)
Four New Zealand lakes	Glucose	11 – 39	Paerl (1978)
Lake Rotongaio (New Zealand)	Glucose, acetate, glycine	15 – 42	Paerl (1980)
Lake Kinneret (Israel)	Glucose	<10	Berman &Stiller (1977)

Table 3B: Summary of previous reports on heterotrophic activity of associated bacteria in estuaries and salt marshes

Environment	Compound	% uptake of compound by fraction >3.0 μm	References
Salt marsh estuary (USA)	Glucose	80	Hansen & Wiebe (1977)
Salt marsh, Palo Alto (USA)	Tetrazolium salts	63 - 99	Harvey & Young (1980)
Newport River Estuary (USA)	Amino acids	3 – 25	Palumbo *et al.* (1984)
Humber Estuary (England)	Glucose	56 – 99	Goulder (1977), Bent & Goulder (1981)

Table 3C. Summary of previous reports on heterotrophic activity of associated bacteria in oceans

Environment	Compound	% uptake of compound by fraction >3.0 μm	References
Atlantic (NE)	Amino acids	47	Williams (1970)
Atlantic (NE)	Glucose	24	Williams (1970)
Gulf of California (USA)	Glucose	<10	Berman (1975)
Gulf of California (USA)	Glucose	<10	Azam & Hodson (1977)
Gulf Stream (USA)	Glucose	20	Hanson & Wiebe (1977)
Saanich Inlet (Canada)	Serine	<10	Azam & Hodson (1977)
Mediterranean	Amino acids	38	Williams (1970)
Mediterranean	Glucose	28	Williams (1970)
Kiel Bight (FRG)	Glucose	<10	Hoppe (1976)
Gulf of Marseilles, France	Glucose	5 – 45	Chretiennot-Dinnet & Vacelet (1978)
Gulf of Marseilles, France	Protein hydrolysate	0 – 68	Chretiennot-Dinnet & Vacelet (1978)
Off Bilbao, (Spain)	Glucose	10 – 38	Iriberri *et al.* (1987)
Off northwest Africa	Acetate	<10	Azam & Hodson (1977)

High biovolume of attached bacteria could reflect the capability of bacteria to store carbon such as carbohydrates during N- and / or P- limiting conditions when cell division is no longer possible (Kanopka, 1992).

The difference in cell size implies that attached bacteria grow faster than unattached bacteria. This inference is based on the correlation between average cell size and specific growth rate observed in laboratory experiments (Pierucci, 1978; Larsson and Hagstrom, 1982). The average specific biomass and growth rate of particle-attached bacteria was very high i.e. 60 fg.C.cell^{-1} and 0.28 h^{-1} respectively (Becquevort *et al.*, 1998) in spite of their low abundance. Particle-attached bacteria exhibit lower turnover rates than free-living bacterioplankton (Hoppe *et al.,* 1993) and incorporate more glucose, glutamate (per cell) (Kirchman and Mitchell, 1982), dissolved ATP (Hodson *et al.*, 1981), and phosphate (Paerl and Merkel, 1982). There is little information on whether attached bacteria differ from unattached bacteria in the utilisation of various dissolved organic matter (DOM). Azam and Hodson (1977) found no difference in the retention of assimilated glucose, serine and acetate on Nucleopore filters having pore size of 1μm. Although the total biomass of attached bacteria is usually low relative to unattached bacteria, the attached bacteria are metabolically more active than unattached bacteria. Thus the cells may have a large volume but divide less frequently (Kirchman *et al.,* 1983). Palumbo *et al.,* (1984) found that all particle associated bacteria in Newport River estuary (USA) have a higher activity on a per cell basis.

Moreover, there is accumulating evidence that attached bacteria, despite their lower yield, exhibit higher extracellular enzymatic activity (Kirchman, 1983; Hoppe *et al.*, 1988, 1993; Karner and Herndl, 1992, Smith *et al.*, 1992). This was supported by Delong *et al.*, (1993) who found that particle-attached bacteria were phylo-genetically distinct from free-living bacteria and were able to degrade a wide range of polymeric compounds. In the Columbia River estuary (USA) extracellular enzyme activity was detected principally on particles suggesting that particulate organic material was the primary polymeric food source for bacteria in the estuary. The relatively high activity of particle-attached bacteria and their close association with particles suggested that they produced most of these enzymes and were therefore, probably responsible for most of the bacterial degradation of POC in the estuary (Crump *et al.*, 1998).

2.7 TAXONOMY

Corpe (1973) reported that early colonizing bacteria were predominantly gram negative rods of which 50-90% were *Pseudomonas* species and 10-49% were pigmented *Flavobacterium* species or non-pigmented, non-motile *Achromobacter* speciess. Gram positive bacteria were rarely detected. Marshall *et al.*, (1971 b) and Corpe (1973) found that initial colonisation by rod-shaped bacteria was followed by other species such as *Caulobacter, Hyphomicrobium* and *Saprospira* after 24 to 72 h. Some bacteria for example, *Escherichia coli* and *Salmonella typhimurium*

may have lipopolysaccharide (LPS) which enter into adhesion interactions (Shands 1966). The addition of cations has been found to promote the attachment of marine Pseudomonads (Marshall *et al.,* 1971a). De Long *et al.*, (1993) detected that the most abundant phylogenetic types in macroaggregate–associated bacterial population fell within the *Cytophaga/Flavobacteriaum* group. The other genera found were *Bacillus* and *Agrobacter.*

2.8 ROLE OF PARTICLE-ASSOCIATED BACTERIA

NUTRIENT RECYCLING

Bacteria attached to particles were able to utilize not only the organic compounds that made up the particle, but also dissolved organic compounds concentrated at the particle surface (Jannasch and Pritchard, 1972). The role of 'microbial loop' including microzooplankton is likely to be particularly important in rapidly recycling nutrients above the thermocline. This is because bacteria attached to particles and larger phytoplankton cells are likely to sink out of the mixed layer faster than the free-living (motile) bacteria (Wangersky, 1984; Kirchman *et al.*, 1982).

It has been demonstrated in the case of marine snow that enzymes associated with the particles hydrolyze much more POC than is required by particle-attached bacteria, possibly providing nutrients for free-living bacteria (Smith *et al.* 1992). However, contribution of particle-attached bacteria to the degradation of organic matter depends on the size and organic

composition of aggregates (Delong *et al.*, 1993). Processes associated with macroscopic organic aggregates should be generally regarded as common and important constituents not only in the sea but also in limnetic environments (Grossart *et al.*, 1998). The importance of biogenic aggregate (>0.5 mm in diameter) has been studied in detail by Fowler and Knauer (1986). They are responsible for the vertical flux of nutrients and transport of attached bacteria to the deep sea floor. These PAB contribute about 48% of total bacterial production in the deep waters (Turley and Stutt, 2000). Ducklow *et al.*, (1982) have measured settling rates of 0.1 to 1.0 $m.d^{-1}$ for attached bacteria in the Hudson river plume and have estimated that 3 to 67% of the total daily bacterial production settled out of the water column. Macroscopic organic aggregates in the ocean as well as in lakes are decomposed by microorganisms. These aggregates are not only vehicles of vertical transport of POM but also provide a recycling of detrital material to higher trophic levels of the aquatic food web

FOOD WEB

Particle-associated bacteria may improve the nutritional quality of the detrital particles for grazing by zooplankton. Bacteria attached to a particle (>1μm) are larger prey and thus may be more important than unattached bacteria for bacteriovores (Ferguson and Rublee, 1976). These microaggregates (phytoplankton colonized by microorganisms) in turn actively interact with free-living bacteria and particle feeders by providing

them with labile substrates (Azam and Cho, 1987; Herndl, 1988) and particles with high nutritional value (Chrost, 1991). Particle-attached bacteria can have a very different role in a food web compared to free-living bacteria because they may be directly grazed by larger metazoans, bypassing consumption by protozoan grazers and 'short-circuiting' the microbial loop (King *et al.*, 1980; Baross *et al.*, 1994). This appears to be the case in the Columbia river estuary where epibenthic copepods were found to feed directly on particle-attached bacteria (Simenstad *et al.,* 1994). Crump *et al.,* (1998) found that direct consumption of particles and particle-attached bacteria by metazoans was potentially the key step in the transfer of detrital organic matter into the food web of the Columbia river estuary. In the same estuary the concentration of rotifers correlated with SPM and bacterial carbon production (Crump and Barros, 1996). In low salinity waters of the Elbe estuary 50 to 75% of the rotifers were found to be associated with suspended aggregate particles (Zimmermann and Kausch, 1996). Filter-feeding organisms can also be inhibited in their activity by high concentrations of suspended matter (Koenings *et al.,* 1990).

Through the ingestion of the lake snow aggregates, a rapid and efficient transformation of protein rich microbial biomass to higher trophic levels could be mediated providing a short - cut between the 'microbial loop' (Azam *et al.*, 1983) and the top levels of the classical food chain. Particularly, in the littoral zone of lakes and the ocean, where the abundance of aggregates is high they may contribute a substantial portion of food to

juvenile fish. Marine snow is also a food source for large particle feeders such as fish and zooplankton (Alldredge, 1979; Urrere and Knauer, 1981; Larson and Shanks, 1996).

3.0 MATERIAL AND METHODS

3.1 DESCRIPTION OF SAMPLING SITES

Three sites were chosen for studying particle-associated bacteria (PAB) in the tropical estuarine system. The sites were Ribandar in Mandovi, Chicalim in Zuari estuary and a station off Cabo in the coastal waters of Goa (Fig. 2).

3.1.1 MANDOVI ESTUARY

The River Mandovi originates from the Parwa Ghat (Karnataka) of the Sahyadri hills, traverses a stretch of about 70 kms before joining the Arabian Sea through Aguada Bay near Panaji. Its width at the estuary is 3.2 km (upstream), and in some places it narrows down up to 0.25 kms. It is fed by monsoon precipitation and the discharge from a catchment area of 1530 km^2. Its pre-monsoon and post-monsoon flow is regulated by the semi-diurnal tides. The Mandovi has a long tributary system and along its coast it has a number of islands, narrow bends and shallow depths. The intrusion of seawater into the river is about 67 km in May and reduces to 10 km in the monsoon months (June-July). The Ribandar station in the Mandovi estuary which is at 15°30.323N and 73°52.430E is influenced by mangrove vegetation and is used for navigation, essentially for iron ore transport by barges.

3.1.2 ZUARI ESTUARY

The characteristics of the Zuari estuary differ markedly from the low run off-season during November-May to the heavy run off period of the southwest monsoon from June to October. During November-May the estuary is vertically

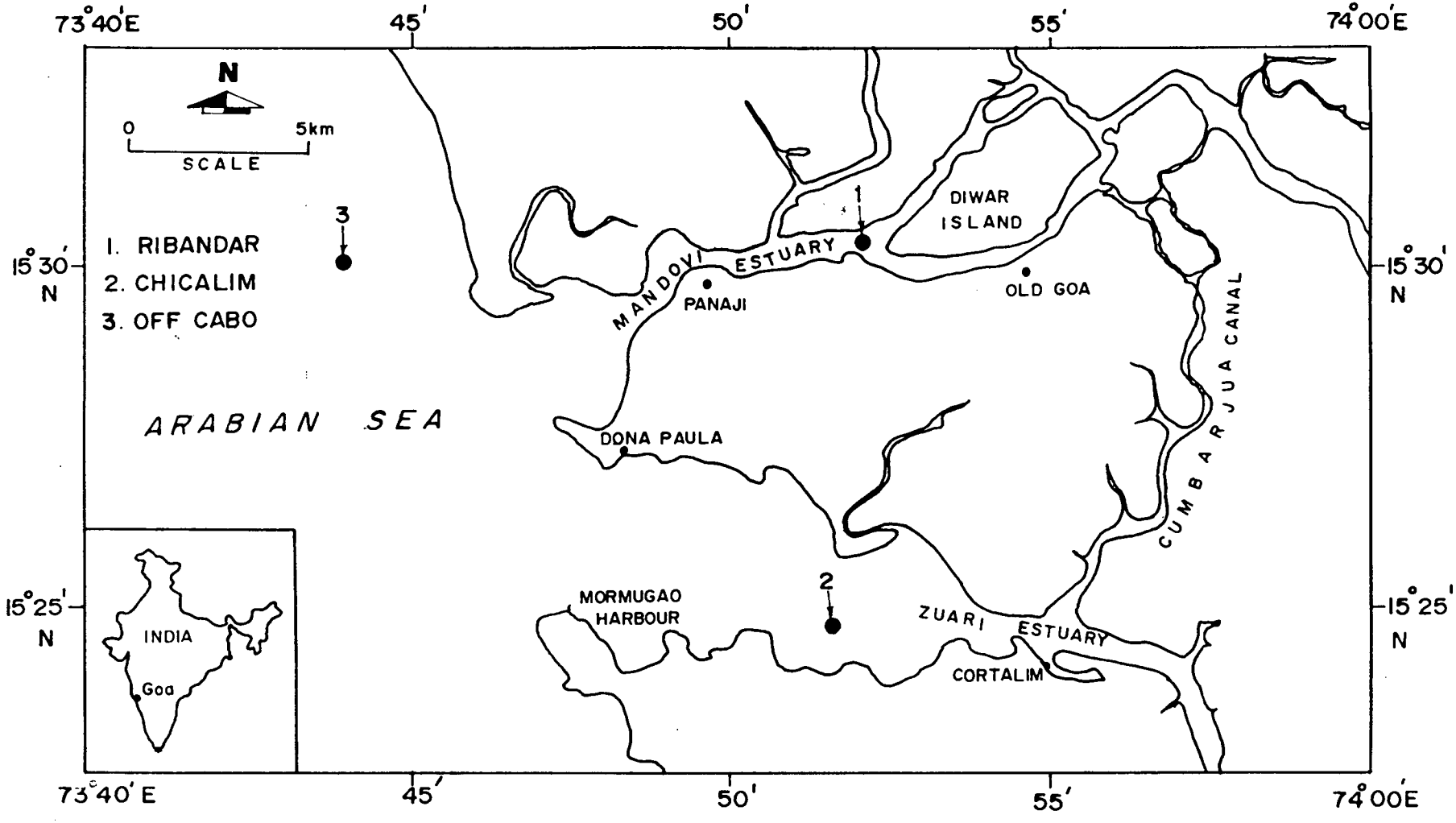


FIG.2. LOCATION OF STATIONS

mixed and the two processes controlling the transport of salt are 1) run off induced advective transport out of the estuary and 2) Tide induced diffusive transport into the estuary. The magnitude of the latter is about 20% larger, leading to a salinity rise in the estuary. In this estuary the intrusion of salt water is seen as far as 65 kms upstream in May which is reduced to a minimum of 20 km in June (Qasim and Sen Gupta, 1981). With the onset of the southwest monsoon, the run off increases dramatically and the estuary loses about 75% of its salt during the first two months. Because the estuary is partially stratified during June-October, gravitational circulation is expected to play a role in addition to tidal diffusion and run off. The average depth up to 40km from the mouth is approximately 5m. The bottom topography is marked by a few trenches as deep as 10m. The cross-sectional area of the estuary decreases exponentially. This decrease is more rapid in the first 20 km from the mouth. The two main rivers Kushavati and Sanguem contribute freshwater run off to the Zuari. The Chicalim station in the Zuari estuary is located at 15°25.107N and 73°51.472E. The Zuari estuary is connected to the Mandovi by the Cumbarjua canal, which lies 15 kms away from the mouth of Zuari. The Marmagoa harbour is situated near the mouth of the Zuari estuary.

3.1.3 COASTAL STATION

The Off Cabo station in the coastal waters is the meeting point of the Mandovi and Zuari estuaries to the Arabian sea. It is at 15°30.323N and 73°44.024E. This coastal station lies 5 kms away from the coastline, 18 kms from the Ribandar station and 14 kms away from Chicalim station. The mouth of

the Zuari and Mandovi estuaries are separated by promontory between them at Cabo (Technical report no. 02/79 NIO, 1981).

3.2 SAMPLING METHOD AND FREQUENCY

Water samples were collected using the Niskin sampler from the surface and near bottom. Samples were gently pre-screened through 220 μm bolting silk into carbuoys, which were transferred to the laboratory with minimum disturbance and time delay for further analysis.

Monthly samples were collected for a period of 14 months from September 1997 to October 1998. The coastal station could not be accessed for a period of 4 months (June –September) due to rough sea and bar formation at the mouth of the river Mandovi during the monsoons.

3.3 PHYSICOCHEMICAL PARAMETERS

Water temperature and salinity were recorded on board. Salinity was measured using an AUTOSAL (8400 A) and expressed in terms of psu.

pH was measured using a pH meter.

Suspended Particulate Matter (SPM)

Suspended particulate matter (SPM) was determined gravimetrically on pre-weighed filters (Krey, 1964). Water samples were filtered on pre-combusted (450°C, 3h) glass microfibre filters, GF/F (Whatman). Filters with retained material were rinsed with distilled water (0.22 μm filtered) to remove salts. The filter papers were then dried at 60°C for 24h, allowed to cool in a dessicator and

weighed on a Metler analytical balance (model AE 200) to a precision of 10^{-4}g in the presence of silica gel. Filters were then ignited at 450°C for 3h and re-weighed after cooling in order to determine the inorganic content (IC) of particles. The organic content (OC) was calculated by subtracting IC values from SPM. Ash weight was calculated by taking the percentage of IC over SPM.

Total Organic Carbon (TOC)

Sample were preserved in 0.5% of 50% orthophosphoric acid (JGOFS protocol 1994). Total organic carbon was measured by High Temperature Catalytic Oxidation method (HTCO) using TOC 5000 (Shimadzu) instrument. In this method, the organic carbon in the sample is oxidized to CO_2 by 1.5% platinum-coated Al_2O_3 granules as catalyst at 680°C. The amount of carbon dioxide released was measured by using a non-dispersive infrared (NDIR) detector. High purity compressed air of zero carbon dioxide grade (Boruka gases Ltd., Bangalore, India) was used as carrier gas. Further purification method was achieved by using a column purification that removed any traces of CO_2. Ultrapure (zero carbon blank) water was prepared by re-distilling the double distilled water with sodium persulphate (1.0 $g.l^{-1}$) followed by its exposure to UV light for 24h.

Particulate Organic Carbon (POC)

The particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined using a Perkin Elmer Elemental CHN analyzer (Model 2400). Water samples was filtered on GF/F (Whatman) glass fibre filter paper

and dried at 60°C., The filters were acid (Conc. HCl) fumed for 24 h to remove carbonates and dried prior to analysis (Hedges and Stern 1984).

Concentration of SPM, PIC, particulate organic carbon (POC) and particulate organic nitrogen (PON) were calculated as a function of the known volume of water filtered. Dissolved organic carbon (DOC) values were derived by subtracting the POC values from the total organic carbon (TOC).

3.4 PARTICLE PARAMETERS

3.4.1 NUMBERS

The total numbers of particles present in the water sample was counted using a Coulter counter (TAIL Model) having an orifice of 140 μm (2.8 – 56 nominal particle size) and 560 μm (11.2-216 nominal particle size).

3.4.2 SIZE

3.4.2.1 Gravimetry Method

Water samples were sequentially filtered through pre-weighed 3, 2.7, 1.2, 0.7, 0.45 and 0.22 μm pore size polycarbonate filter papers (Millipore), under low vacuum(<100 mm Hg). The filtered samples were then rinsed with 200 ml of membrane (0.22μm) filtered distilled water, dried at 40^0C and placed in a dessicator before being re-weighed on a Metler balance (Model AE200) up to a precision of 10^{-4}g in the presence of silica gel.

3.4.2.2 Laser Counter method

The size and distribution of particles in water samples were analyzed using a laser based particle size analyzer (Malvern 3600E). Distilled water

filtered through polycarbonate filters was used to calibrate the granulometer and for particle-suspension dilutions. Sample dilution if necessary was performed with membrane (0.22 μm) filtered 50% seawater under gentle magnetic stirring for 5 minutes. For blanks, membrane (0.22 μm) filtered seawater was maintained.

3.4.2.3 Scanning electron microscopy

Specimen samples were fixed with 2% glutaraldehyde after gently filtering through a 3 μm filter and then washed with 0.1 M phosphate buffer solution (Please see appendix). Acetone at concentration of 30, 50, 70, 90 and 95% was prepared with distilled water. The samples were then sequentially subjected to different concentration of acetone for a time period of 10 minutes individually, before transferring it to 100% acetone for 15 minutes to dehydrate it. The samples were processed by critical point drying (CPD) in a high-pressure container filled with CO_2 at room temperature. The specimens were then fixed on to the stubs with adhesive tapes and sputter coated (with gold) for 30 seconds before mounting it on to the stage for scanning through the JEOL Scanning electron microscope (5800 LV).

3.4.3 NATURE

3.4.3.1 Mineral Estimation

Mineral studies were conducted on the 3 to 220μm fractions of the particles present in the water column. The samples were made free of carbonate and organic matter by treating the sample with 1.0 ml acetic acid and 2.0 ml hydrogen peroxide respectively. Excess of acid was removed by washing the

samples with distilled water. Oriented slides were then prepared by pipetting 1.0 ml of the concentrated suspensions on to the glass slides and allowing them to air dry. The samples were then glycolated by exposing the slides to ethylene glycol vapors at 100°C for 1 hour.

X-ray diffraction studies were carried out on the glycolated samples from 3° to 25° 2θ at 1.2° 2θ/minute on a Philips X-ray diffractometer (1840 Model) using nickel-filtered Cu Kα radiation, operated at 20mA and 40 kV. The peaks for different mineral groups like kaolinite, chlorite, illite, montmorillonite, goethite and gibbsite were identified besides quartz and feldspar. The areas of major minerals were calculated by using the glycolated X-ray diffractograms. The quantificaton of different clay minerals was done by measuring the ratios between the length of the peak from the background and the half height width of the peak. The principle peak areas of kaolinite + chlorite, illite and montmorillonite were multiplied by the weighting factors 2, 4 and 1 respectively, and weighted peak area percentages were calculated following the semi-quantitative method of Biscaye (1965). Mineral analysis was carried out on 42 samples from 3 stations.

3.4.3.2 DNA Estimation

DNA concentration was measured with 3,5-diaminobenzoic acid dihydrochloride (DABA.2HCl) by fluorometric reaction (Kissane and Robins 1958; Holm-Hansen *et al.* 1968). The filters were dried at 60^{0}C for an hour. To the centre of each dried filter, 100µl of DABA.2HCl was added. Subsequently the samples were covered tightly and again placed at 60^{0}C for 1 hour, followed

by the addition of 3 ml of HCl (1M). The samples were vortexed for 1 min, and the fluorescence was determined (Ex: 405nm; Em: 520nm) in a F2000 spectrofluorometer (Hitachi).

3.4.3.3 RNA estimation

RNA concentrations were measured with the cupric ion-catalyzed orcinol colorimetric reaction (Lin and Schjeide, 1969). After the DABA-DNA fluorescence was measured the samples were quantitatively transferred to a clean glass tube and exactly 1.5 ml was added in a test tube for subsequent determination of RNA. Each sample (1.5 ml) was diluted with an equal volume of orcinol reagent, which was prepared by dissolving 1g of reagent grade orcinol (Sigma) and 0.15g $CuCl_2.2H_2O$ in 100 ml of concentrated HCl (11.6M). The samples were tightly sealed and incubated at 100^0C for 30 min. The samples were allowed to cool for about 30 min and the absorbance at 666nm was recorded using a UV-1201 spectrophotometer (Shimadzu). Standard curve was prepared with commercially available yeast RNA (Sigma).

3.5 BIOLOGICAL PARAMETERS

3.5.1 PRIMARY PRODUCTION

Primary productivity measurements were made using the ^{14}C technique (Steeman-Neilsen,1952). The samples were passed through 220 μm nylon bolt net to minimize the possibility of grazing by zooplankton during incubation. The radioactive carbon (^{14}C) in the form of sodium bicarbonate (Sp. Act.: 5

μCi/μmole, BARC, Mumbai) was added to (in duplicate) samples in light and dark bottles. Appropriate neutral density filters were used for bottom samples. *In situ* incubations were carried out, by exposing the samples to natural temperatures and light levels (both intensity and spectral quality) for 4-6 hours on board. To maintain the temperature water was exchanged at frequent intervals during the period of incubation. After incubation the contents were filtered through a membrane filter (0.45μm pore size). The filters were analyzed for ^{14}C-uptake rate using Tri-CARB 2500 TR Packard liquid scintillation counter and dioxane based scintillator cocktail. The values are expressed as $\mu g.C.l^{-1}.h^{-1}$.

3.5.2 CHLOROPHYLL *'a'*

A known volume of water sample (250ml) was filtered through a 0.45 μm Whatman GF/F filter paper for estimation of chlorophyll *'a'*. Pigment extraction was done by keeping the filters overnight in dark in 10 ml of 90% acetone. Fluorescence was measured in a Turner Design Fluorometer using Chlorophyll *'a'* (Sigma) as a standard. Chl *'a'* concentration is expressed as $\mu g.l^{-1}$. Phytoplankton contribution to POC was calculated assuming a C:Chl *'a'* ratio of 30 (Banse, 1977).

3.6 MICROBIAL PARAMETERS

Particle associated microbial parameters were estimated by differential method. Water samples that were pre-filtered through 220 μm pore size nylon mesh were assumed to have the total microbial load. Samples were further

filtered through 3.0 μm filter (Millipore) using low vacuum. Particle associated microbial parameters were derived by subtracting the <3 μm size population from the total population.

3.6.1. TOTAL MICROBIAL BIOMASS

The determination of adenosine triphosphate (ATP) in the particulate matter of seawater is of value as an indicator of the quantity of living material, (microbial biomass). The estimation was done as described by Parsons *et al.*, (1984).

Samples (500ml) filtered (3.0μm) and unfiltered were re-filtered onto a 0.22 μm pore size 47-mm Millipore filter. The filter was then extracted in 3.0 ml of boiling Tris buffer (pH 7.7 to 7.8 adjusted with 20% HCl) for 5 minutes. The extract was transferred into a clean dry glass vial. The contents of the vial were cooled to room temperature, and few drops of Tris buffer were added to make up the volume to 4.0 ml. Contents were mixed and stored at -20^0C till it was analysed.

The enzyme mixture used for this estimation was prepared by adding 5.0 ml of Tris buffer to a vial of about 50 mg of lyophilized firefly lantern extracts (Sigma). The vial was allowed to stand at room temperature for 2-3 hours, centrifuged and the clear solution was decanted into a clean dry test tube. This extract was allowed to stand for further 60 minutes and then used within the next 3 hours.

Into a glass vial, 0.2 ml of the enzyme preparation was pipetted out and then placed into the sample holder followed by 0.2 ml of the sample. After 30

seconds of integration, time readings were taken using a Turner TD 20e Luminometer. The light emitted was proportional to the amount of ATP present and calculated against a standard curve. Biomass ($\mu g.C.l^{-1}$) was calculated from ATP values using a conversion factor of 250.

3.6.2. TOTAL BACTERIAL COUNTS (TC)

The total number of bacteria were measured by the acridine orange direct count method (Hobbie *et al.*, 1977) The number of bacteria associated with particles was not determined by counting the number of bacteria per particle and the number of particles per microscope field. This was because their abundance is under estimated by about 45% due to bacterial colonization inside or underneath particles (Kirchman and Mitchell, 1982). The number of bacteria was calculated by subtracting <3 μm fraction from the total.

Triplicate sub-samples from the polycarbonate bottle were preserved with formaldehyde (2% final concentration). One 2.0 ml portion of each sub-sample was stained with 0.22μm filtered acridine orange (final concentration 0.01% w/v) for 5 minutes and then filtered on to black-stained Nucleopore filter with a pore size of 0.22 μm. Samples were counted at 1250X with Epifluorescence Olympus (BH) microscope using 515 barrier filter. At least 10 fields of >30 bacteria $field^{-1}$ were counted. The number of preserved sub-samples (3), stained filters prepared from each sub-sample (1), and microscope fields examined (10) for determining the abundance of unattached bacteria follows the statistical analysis of Kirchman *et al.*, (1982). Blanks were routinely checked for bacteria. Bacterial carbon was calculated from bacterial abundance by assuming a per cell carbon content of 20

fg (Lee and Fuhrman, 1987). Cell volumes were estimated from epifluorescence preparations by measuring the shortest and longest axes of a bacterium with an ocular micrometer; the bacteria were classified as either spheres or cylinders. Fuhrman, (1982) determined that cell volumes are best determined from epifluorescence preparations. Rod shaped cells were treated as cylinders and cocci as spheres. Simultaneous count were taken for orange (active), green (dormant) and the dividing bacteria (Frequency of Dividing cells, FDC). Numbers were expressed per litre basis.

3.6.3 VIABLE COUNTS (VC)

The viable count (VC) method (Kogure *et al.,* 1987) has been proposed as a method that can be used to surmount problems of overestimation of the number of viable bacteria when direct microscopic methods are used (Hobbie *et al.,* 1977). It would also overcome the problem of underestimation of the number of viable cells when culture methods of enumeration are used (Buck, 1979). Nalidixic acid (0.02% w/v), piromidic acid (0.001% w/v), pipemidic acid (0.01% w/v) and yeast extract substrate (0.025%) were added, the seawater samples were incubated at room temperature for 7 hours followed by fixation (with 0.22 μm pore size filtered) formalin (2% final concentration). Subsequent acridine orange staining of these preparations, followed by epifluorescence microscopy, allowed the enumeration of elongated cells as an estimate of the direct viable population.

3.6.4 RETRIEVABLE COUNTS (Colony forming units)

3.6.4.1 Total Aerobic Counts

Water samples (200ml) were filtered through 3.0 μm pore size polycarbonate (Millipore) filter. The filter paper was then transferred in a flask containing 200 ml of filtered and autoclaved 50% seawater to which 2.0 ml of Tween 80 was added and kept on a shaker for 15 min. The samples were sonicated for 10 seconds in order to reduce hydrophobic interactions (Yoon and Rosson, 1990) and to dislodge the bacteria from the particles. The suspension was then serially diluted with sterile 50% seawater. Depending on the dilution 0.1 ml and 0.05 ml were surface-plated on nutrient agar medium (Himedia, Mumbai) and the plates (in triplicates) were incubated and counted after 24 h incubation at room temperature (28 ± 2°C). The colony forming units were expressed as $cfu.l^{-1}$.

3.6.5 HYDROLYTIC ACTIVITIES

Plating was done to estimate the density of different biochemical groups associated with the particles. Water samples (200ml) were filtered through 3.0 μm pore size polycarbonate (Millipore) filter. The filter paper was then transferred to a flask containing 200 ml of filtered sterilized, autoclaved 50% seawater to which 2.0 ml of Tween 80 was added. The flask was kept on a shaker for 15 min and then sonicated for 10 seconds in order to reduce hydrophobic interactions (Yoon and Rosson, 1990) and to dislodge the bacteria from the particles. The suspension was then serially diluted with sterile 50% seawater. Of the diluted solution 0.1 ml and 0.05 ml were surface plated on

Chitinase, Dnase, Phosphatase, Lipase, Amylase, Protease media and the plates were incubated for 24 h at room temperature (28 ± 2 °C).

3.7 BACTERIAL PRODUCTION

Bacterial carbon production was measured by the incorporation of the nucleoside [^{3}H-methyl] thymidine (Fuhrman and Azam, 1982). Production of both total water sample and filtered fraction (<3μm) were estimated to determine the production by the particle associated bacterial population.

Three sub-samples of 30 ml of each sample, filtered and unfiltered were incubated for an hour with ^{3}H-thymidine (specific activity=52Ci mmol^{-1}, BARC, Mumbai) at a final concentration of 10nM. Incubations were terminated with 2% neutral buffered formalin. The samples were then filtered through 0.22 μm polycarbonate filters (pre-soaked 5% TCA), extracted in cold 5% TCA and ethanol rinsed. The dried filter papers were then placed in scintillation vials and filled with 3 ml of dioxane based scintillator (Sigma). The samples were then radioassayed using the Tri-CARB 2500 TR PACKARD scintillation counter. The rate of ^{3}H-thymidine incorporation was converted to bacterial production using the factor 2 x 10^{18} cells (Kirchman *et al.*,1982). The bacterial production rates were expressed as μg.C.l^{-1}.h^{-1}. The turnover time of bacteria was calculated by dividing the bacterial production rates with bacterial abundance (Zweifel, 1999). Detrital carbon was calculated by subtracting the phytoplankton carbon from POC concentrations Kirchman (1983). Turnover time of detritus carbon by PAB

as calculated by dividing the concentration of detrital carbon by the estimated bacterial production in the >3 μm fraction (Kirchman, 1983).

3.8 HETEROTROPHIC ACTIVITY

The relative heterotrophic activity of the total and free-living bacteria was measured by using labeled glucose and glutamic acid substrate (Wright and Hobbie, 1965). Three sub-samples were analyzed for uptake of each substrate measurement. In this study the compounds used were [U-^{14}C]-D-glucose (Specific activity=53 mCi mmol^{-1} BARC, Mumbai) and [U-^{14}C]-L-glutamic acid (Specific activity=190 mCi mmol^{-1} BARC, Mumbai). The substrates, glucose and glutamate were added separately to sub-sample volume of 30 ml in a narrow mouth 100 ml flask. To each of the flask 10, 50 and 100μl of each substrate was added. A strip of zigzag paper (Whatman No.1) was soaked in about 150μl of ethanolamine and placed in a scintillation vial. The vials were suspended into the flask containing the substrate and the sample. The flask was tightly corked and incubated in the dark for 2 hours. The vials were removed and scintillation cocktail was added to it before counting to give estimates of respiration rates. The reaction was then terminated by addition of 0.5 ml of concentrated HCl. The samples were then filtered through 0.22 μm polycarbonate filter paper (Millipore). The filters were rinsed with 50% seawater (0.22 μm filtered). Dried filters were placed in scintillation vials to which 3.0ml of dioxane based liquid scintillation cocktail (Sigma) was added. The samples were radio-assayed using a Tri-CARB

2500 TR Packard Liquid Scintillation Counter. Applying Michaelis-Menton kinetics the V_{max}, T_t and K_m values were calculated.

The metabolic state (specific activity per cell) of the associated bacteria was evaluated by dividing the value of ^{14}C-glucose assimilated by each fraction by the number of attached bacteria. Here an assumption was made like Kirchman and Mitchell (1982) that the specific activity of the radiolabeled compound was the same in the microenvironments around attached bacterial cells.

3.9 INSTANTANEOUS GROWTH RATE(μ)/GENERATION TIME/REMOVAL (g)

Experiments were designed to estimate instantaneous growth rate μ, mean generation time, and removal (g) of PAB with natural sea water samples using dilution methods (Ducklow et al., 1992). Diluted (20% unfiltered+80% 0.22 μm filtered) and untreated whole sea water samples were incubated upto 40 hour and sampled periodically for cell numbers. Growth and removal rates are calculated from the slope of the regression of the cell numbers on time. Generation time is calculated as 1/μ. All units are expressed per hour.

3.10 IDENTIFICATION OF PARTICLE-ASSOCIATED BACTERIA

Aerobic heterotrophic bacterial flora associated with particles was isolated seasonally from the 3 stations for identification. Morphologically, different colonies were isolated randomly, purified and subjected to various biochemical

and physiological tests (Gerhardt, 1981). The cultures were identified up to generic level based on the identification key of Oliver , (1982).

3.11 STATISTICAL ANALYSIS

Correlation matrix was calculated for all the parameters and tested for significance. Necessary transformations were carried out wherever required. Multiple regression analysis was carried out using MINITAB statistical software, version 12, for identifying the best set of parameters to explain the predictor variable. Principal component analysis (PCA) was done using MINITAB package for the best set of parameters to identify the different factors affecting variability.

4.0 RESULTS

4.1. HYDROLOGICAL PARAMETERS

The hydrological parameters (depth, temperature, pH and salinity) of the three stations are shown in Figures 3 to 7.

4.1.1 DEPTH

The Ribandar station in the Mandovi and Chicalim in the Zuari estuary did not show any significant variation in depth during the period of study. The maximum was recorded during the monsoon. Both the stations were generally less than 10m deep. The coastal station off Cabo was deeper with an average depth of 15m (Fig 3).

4.1.2 TEMPERATURE

Temperature did not show much difference between surface and bottom water. It normally ranged from 26°C in July to 33.5°C in May. Seasonal changes were more evident in the estuaries than in the coastal station (Fig 4).

4.1.3 pH

pH varied from 6.0 to 8.4. Water samples from Zuari were above neutrality in all months except in the month of February and March (Fig. 5). During these months the pH of the water in all the three stations was around 6.0.

4.1.4 SALINITY

Salinity values did not show much difference between the surface and bottom waters except Zuari (Fig 6).

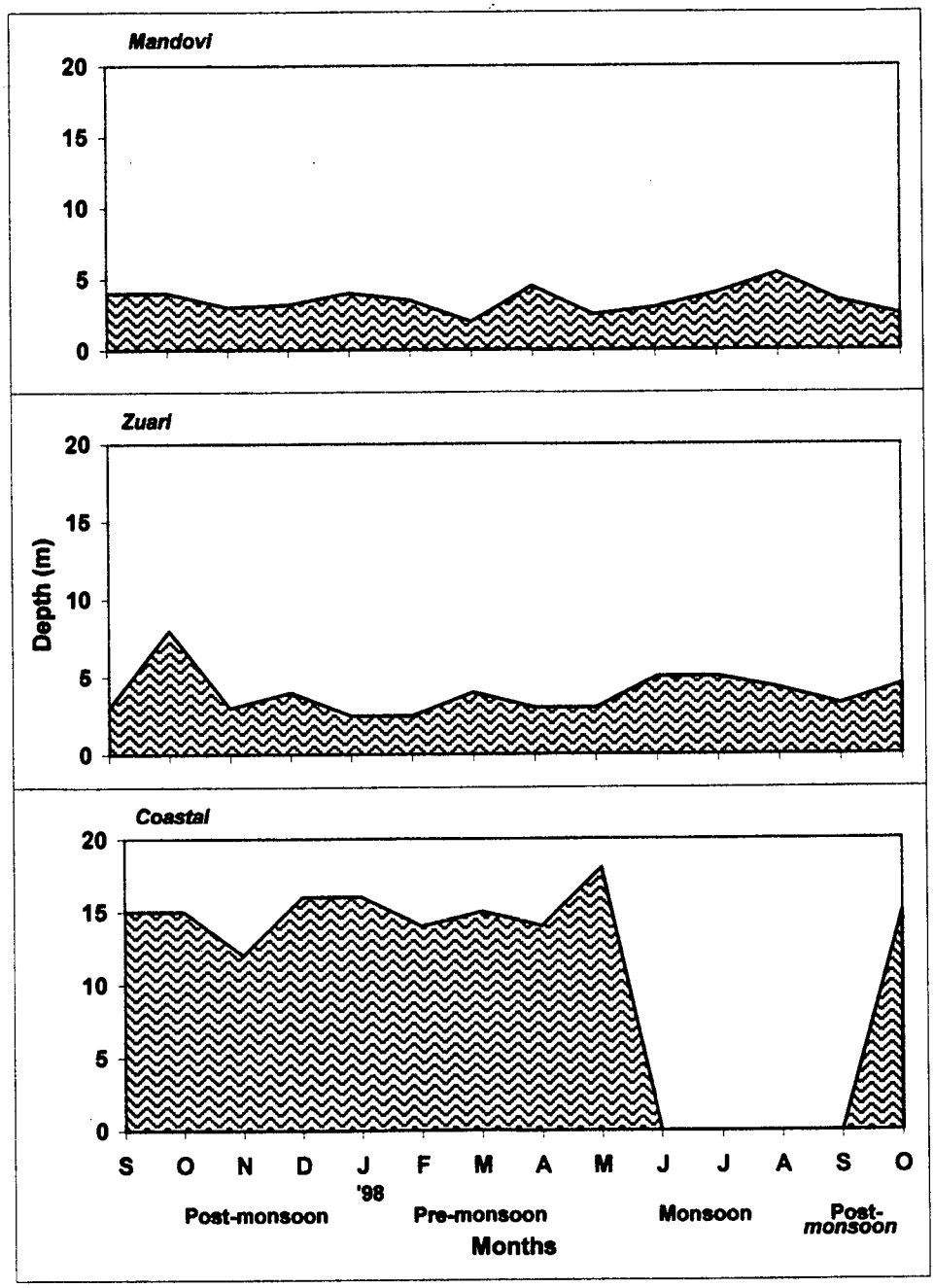


Fig. 3: Variation in depth at the sampling stations

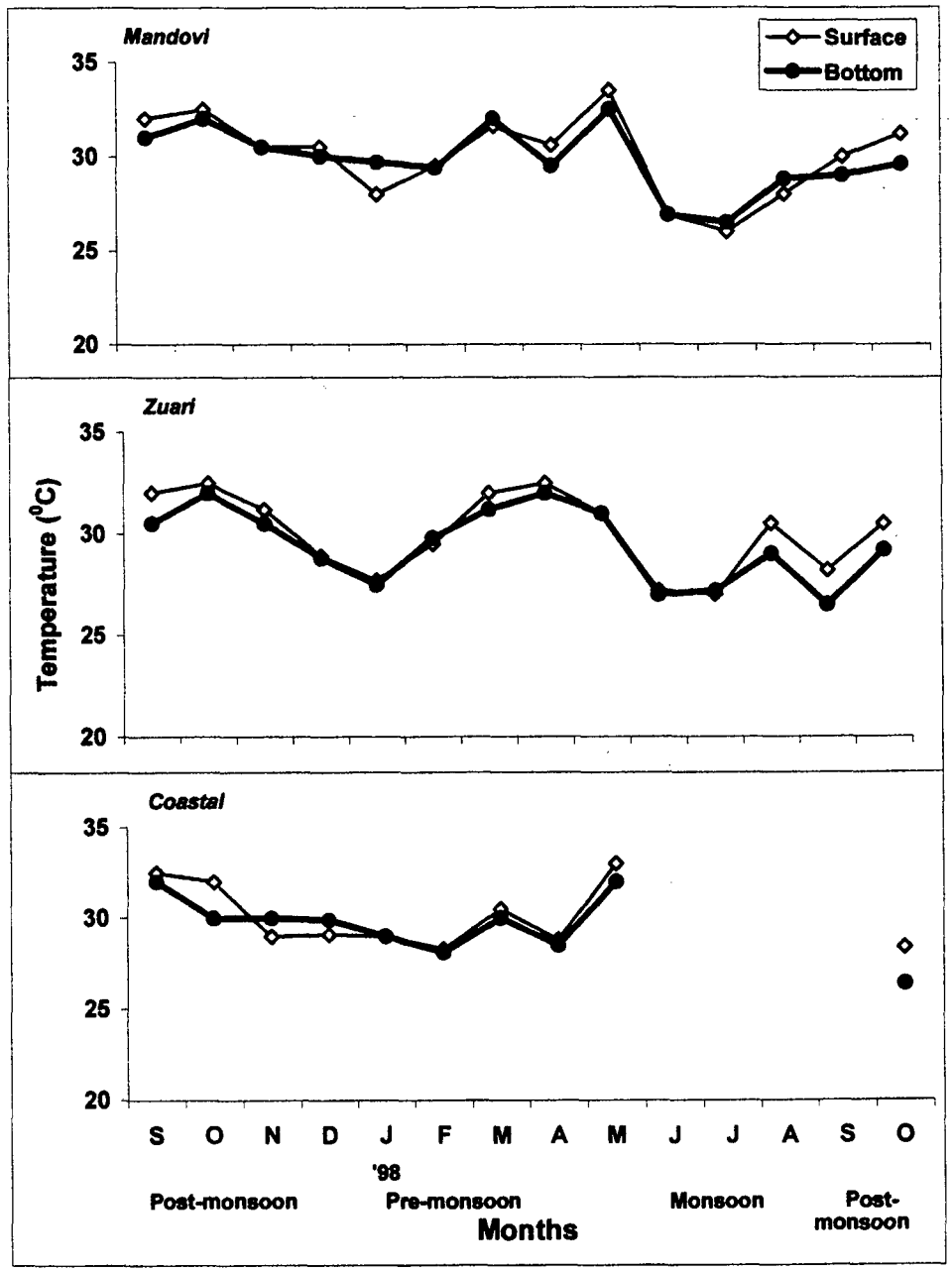


Fig. 4: Variation in water temperature at the three stations

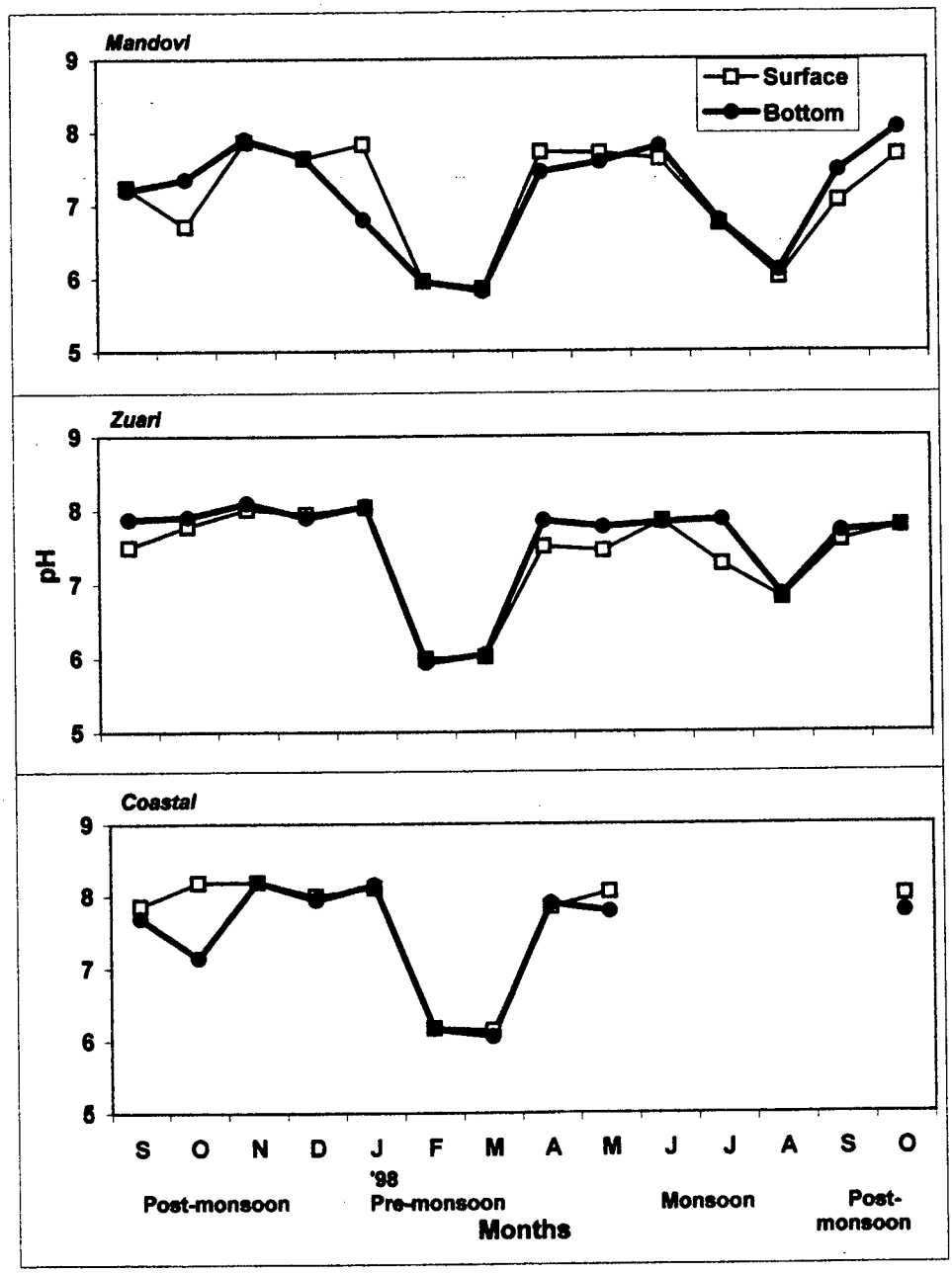


Fig. 5: Variation in pH at the sampling sites

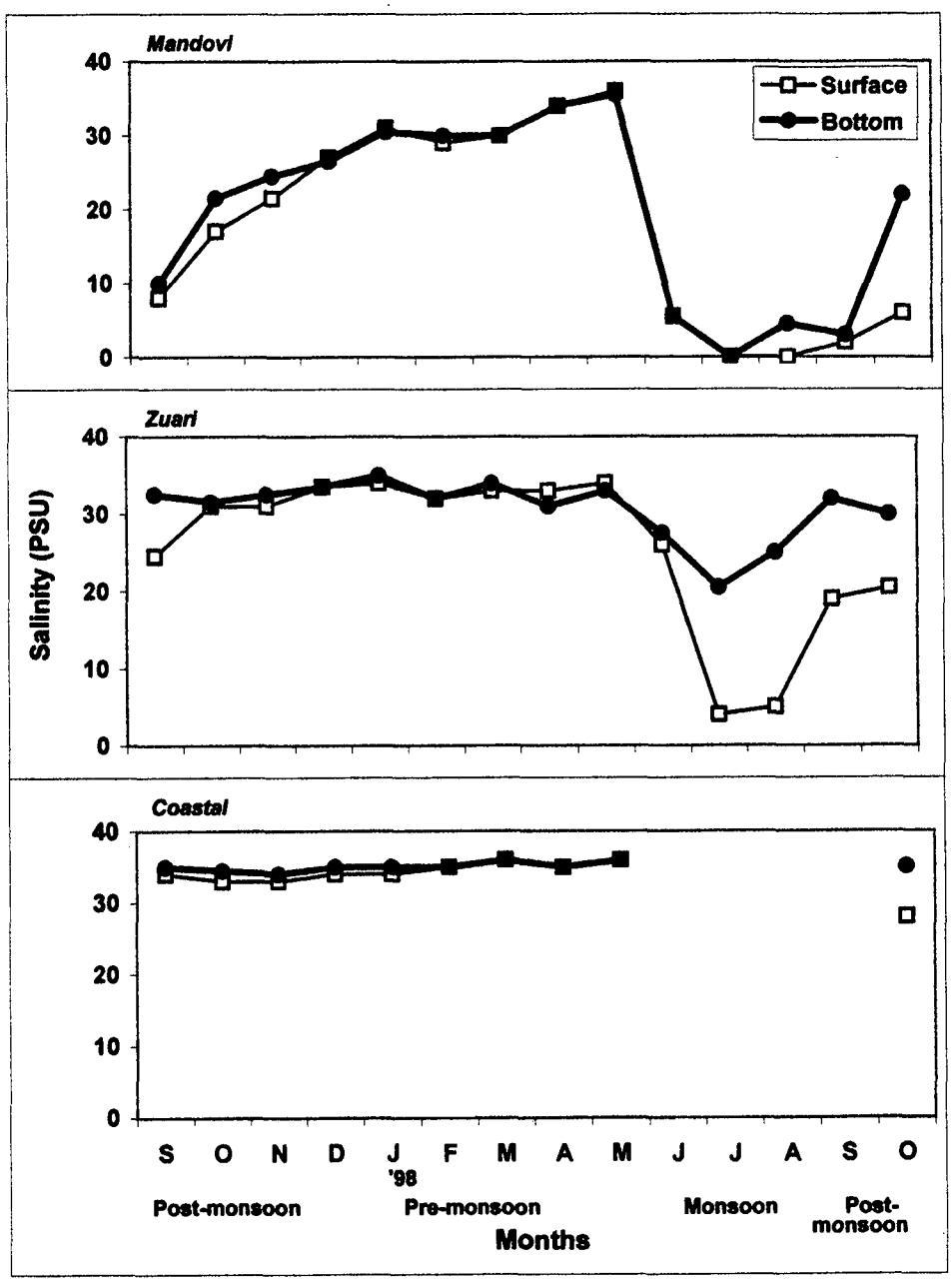


Fig. 6: Variation in salinity at the sampling sites

Though the three stations were situated apart, hydrologically there was little variation in parameters like temperature and depth. A comparison of the three systems during the three seasons clearly shows that there is very little difference in salinity between the surface and bottom waters during the pre-monsoon. The differences were more striking during the monsoon season and the stratification was evident by differences in the salinity values especially at Zuari in the surface and bottom. Stratification was also evident in the post-monsoon at Mandovi (11.67%) and Zuari (24.33%) (Fig. 7).

4.1.5 SUSPENDED PARTICULATE MATTER (SPM)

The suspended load varied from 0.002 to 0.52 $g.l^{-1}$ in the surface and from 0.006 to 1.16 $g.l^{-1}$ in the bottom waters of the estuary. In the coastal station, the variation was from 0.002 to 0.436 $g.l^{-1}$ (Fig 8). The load increases from December to February and this declines to low levels and then increase again during monsoon period (Fig 9). The suspended load was always high at the bottom and varied significantly ($p<0.05$).

4.1.6 PARTICULATE ORGANIC CARBON (POC)

Unlike the suspended load, the POC concentration was higher at surface. Monthly fluctuations were more evident in Mandovi than in Zuari. Unusually higher inputs were observed in October and June. Compared to the estuaries the POC levels at the coastal station were low and the distribution was rather steady (Fig. 10).

4.1.7 PARTICULATE ORGANIC NITROGEN (PON)

The concentration of PON during the period of study is presented in Table 4. As can be seen, the minimum concentration of 60 $\mu g.l^{-1}$ was always

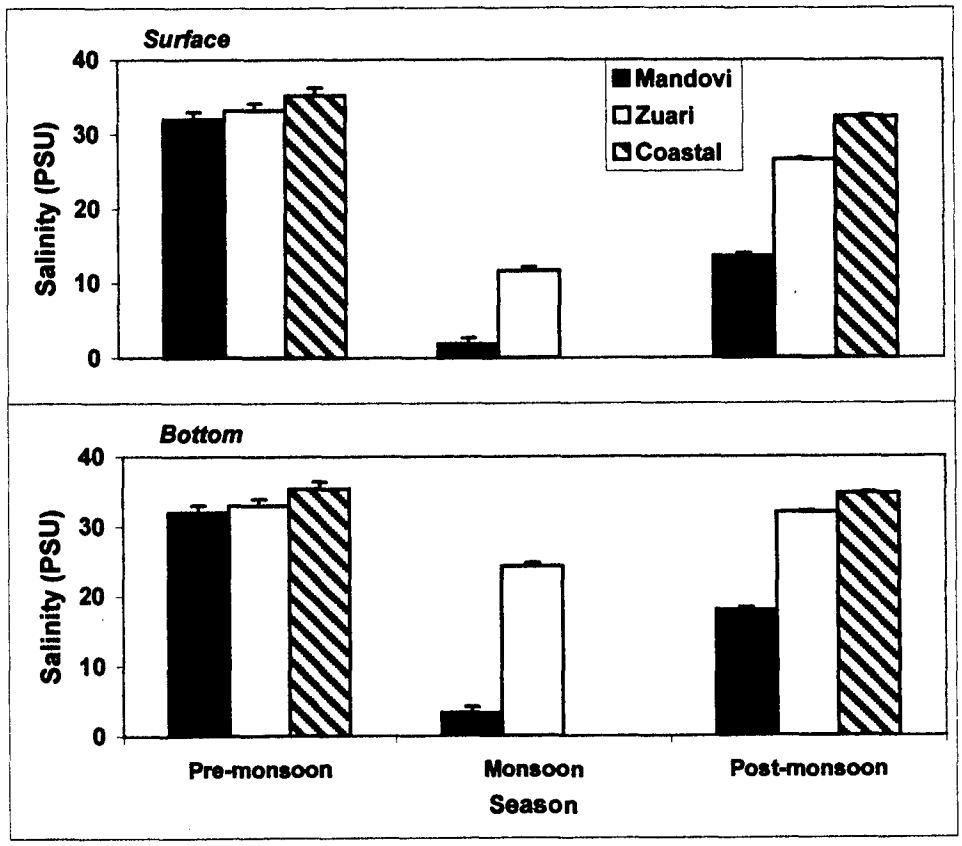


Fig. 7: Seasonal salinity at the three study sites

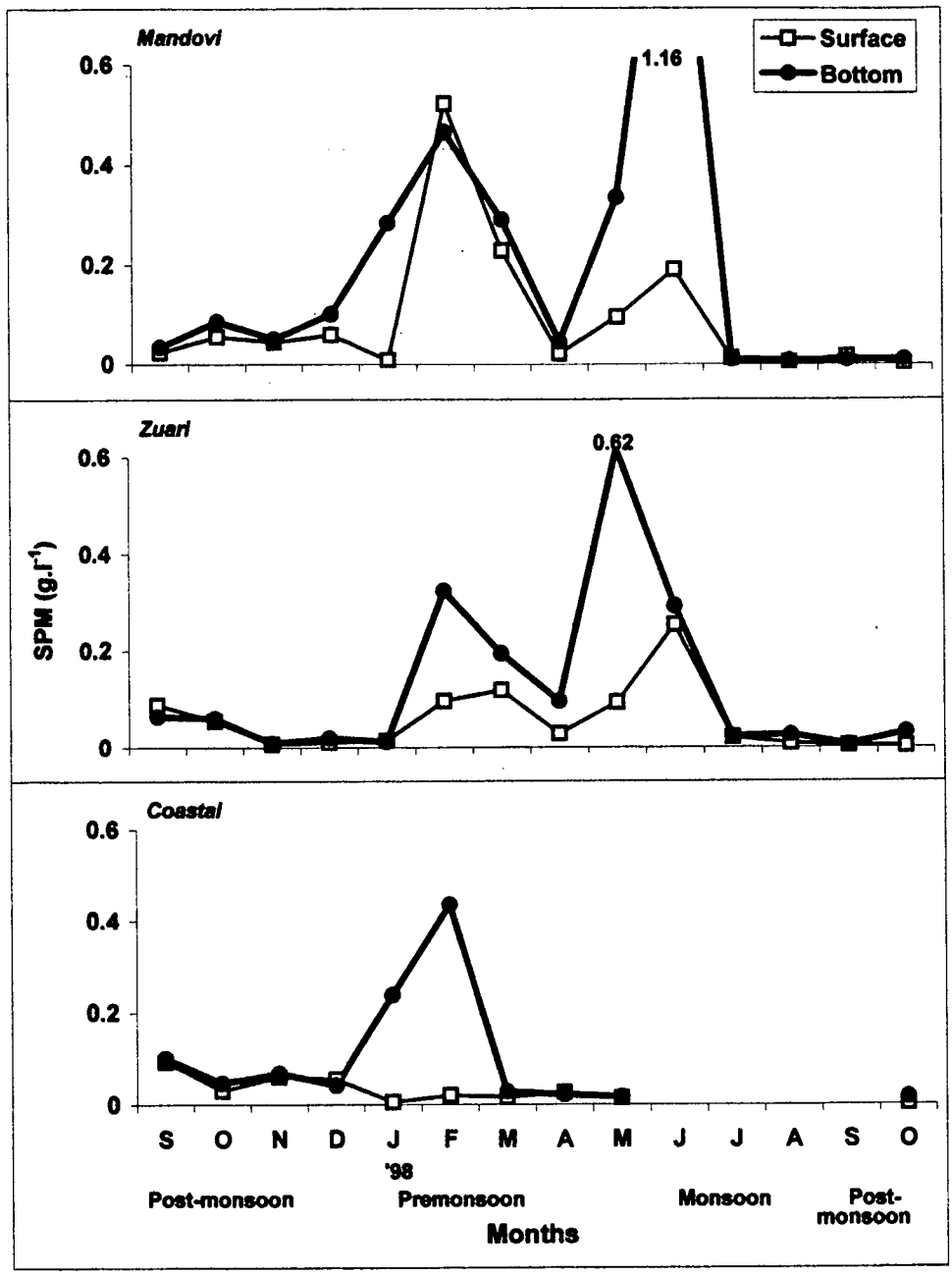


Fig. 8: Variation in suspended particulate matter (SPM) at the three stations

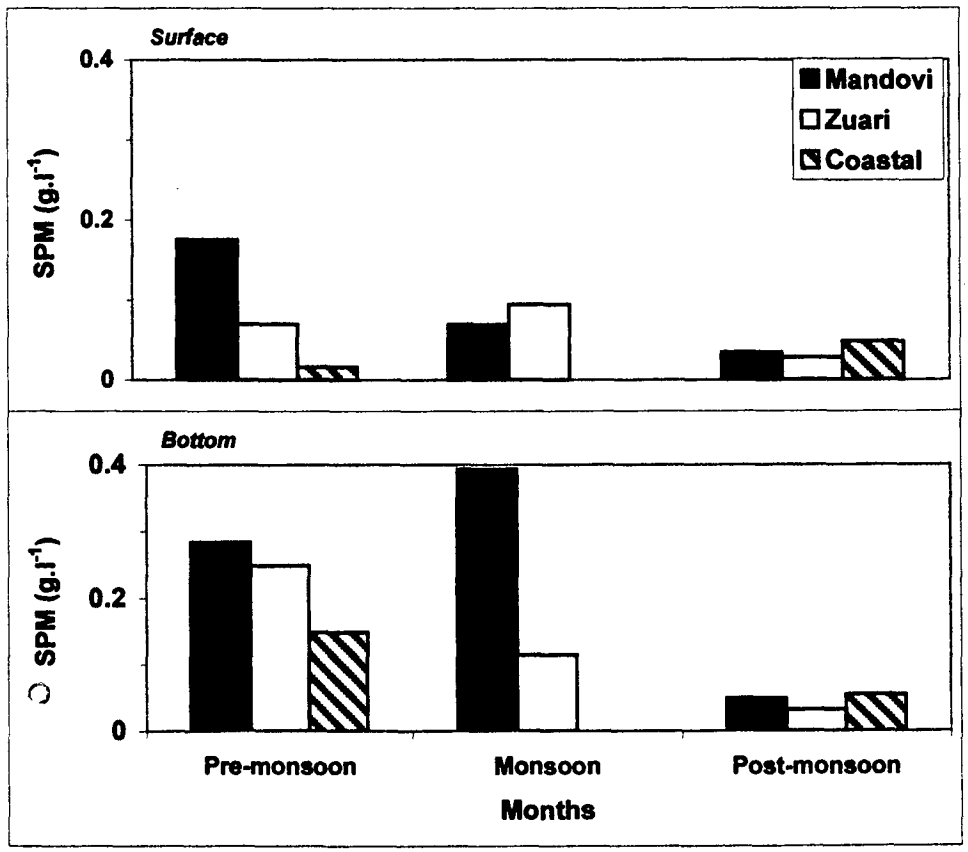


Fig. 9: Suspended particulate matter (SPM) in the estuarine network

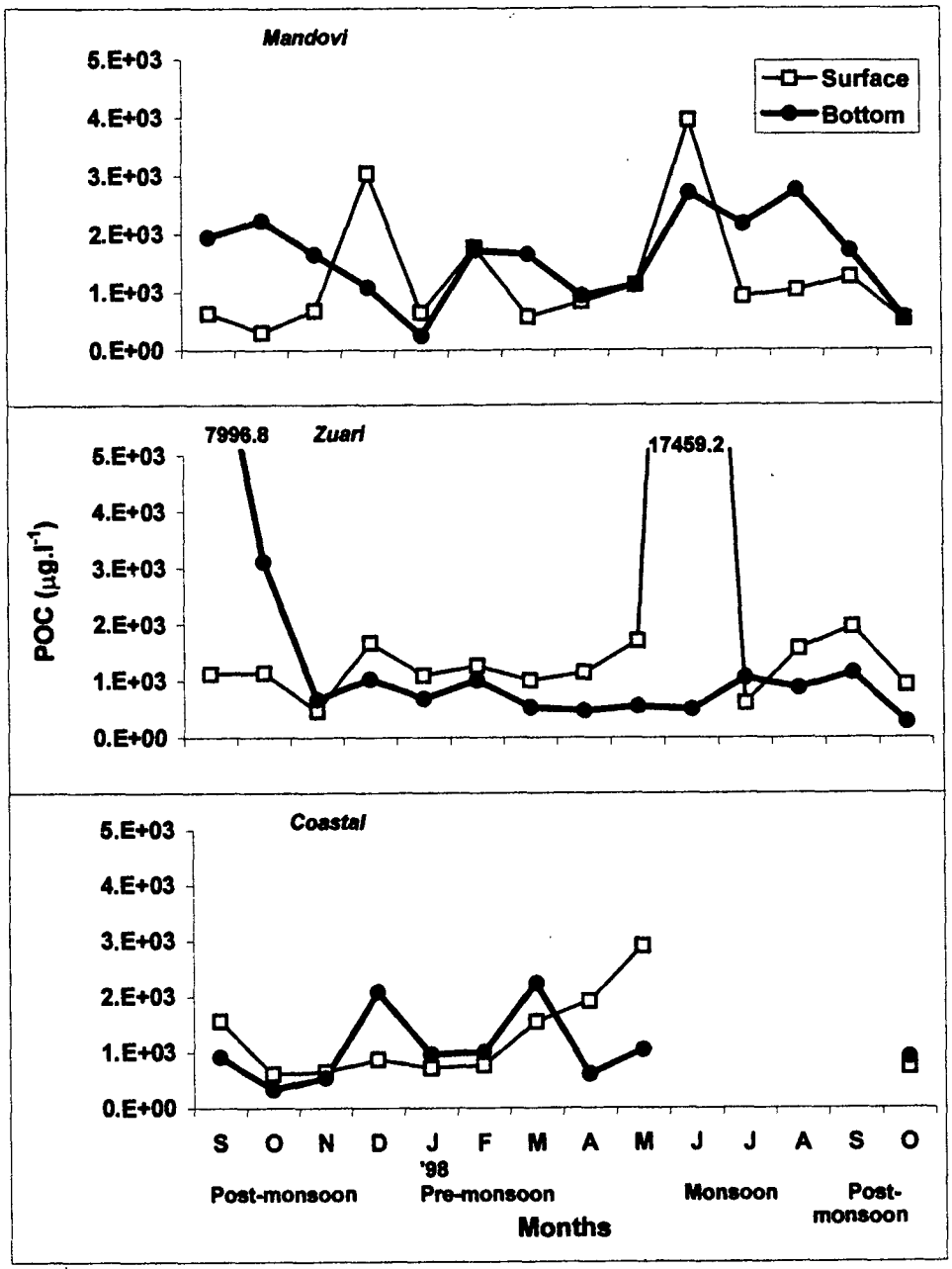


Fig. 10: Particulate organic carbon (POC) in the estuarine network

Table 4: Particulate organic nitrogen (PON) in the estuarine network

Particulate organic nitrogen ($\mu g.l^{-1}$)														
Stations	Months													
	S	O	N	D	J	F	M	A	M	J	J	A	S	O
Mandovi														
Surface	64.0	98.2	30.1	66.6	82.4	489.6	139.2	185.6	220.8	536.0	141.4	138.6	378.6	226.6
Bottom	384.0	200.0	99.2	441.6	342.4	243.2	227.2	192.0	352.0	308.8	102.0	128.0	364.8	84.8
Zuari														
Surface	422.4	247.3	96.7	65.0	72.9	435.2	75.2	531.2	298.6	392.0	325.4	200.0	285.4	240.0
Bottom	454.4	92.8	19.2	18.6	192.0	704.0	123.2	603.2	1494.4	566.4	120.0	216.0	275.2	168.0
Coastal														
Surface	224.0	177.5	106.2	569.0	60.2	116.8	142.4	168.0	160.0	ND*	ND	ND	ND	226.6
Bottom	166.4	24.0	84.8	302.4	234.5	166.6	441.6	83.2	80.0	ND	ND	ND	ND	204.8

*ND – Not done

noted during the post monsoon months whereas the maximum value of 500 was noted in different months with no particular seasonal pattern. The values observed in the bottom waters were generally higher then the surface except at the Mandovi station in February. The PON was found to be related significantly with suspended load both at the surface and bottom waters of Mandovi estuary.

4.1.8 C:N RATIO

The ratios were low in the Mandovi estuary during the pre monsoon season and high in the Zuari estuary during the post-monsoon period (Table 5) (Fig. 11).

4.1.9 PHYTOPLANKTON AND DETRITAL CARBON

The phytoplankton carbon in the Mandovi estuary was maximum during the post-monsoon period with the peak in October (130 $\mu g.C.l^{-1}$). The lowest concentration was observed in the monsoon month of July. Similar trend, albeit lower concentration was noted in the bottom waters. Apparently the maximum contribution to the POC is through the detrital carbon (Table 6). Nevertheless, the bottom waters contained very high concentrations of POC ranging from 435 $\mu g.C.l^{-1}$ in November to 1958 $\mu g.C.l^{-1}$ in September.

In the Zuari estuary the phototrophic and detrital inputs were less variant as compared to the other estuary. The bottom waters reflected the surface trend (Table 7).

At the coastal station the phytoplankton contribution was more than the two estuaries both in the surface and bottom waters without much variation

Table 5: C/N ratio of particles at the sampling stations during different seasons

Station	Pre-monsoon		Monsoon		Post-monsoon	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Mandovi	5.00	4.74	7.09	7.97	14.53	5.59
Zuari	10.30	7.42	5.40	5.30	6.51	21.10
Coastal	5.58	7.09	N.D*	N.D	3.46	7.41

* N.D —Not done

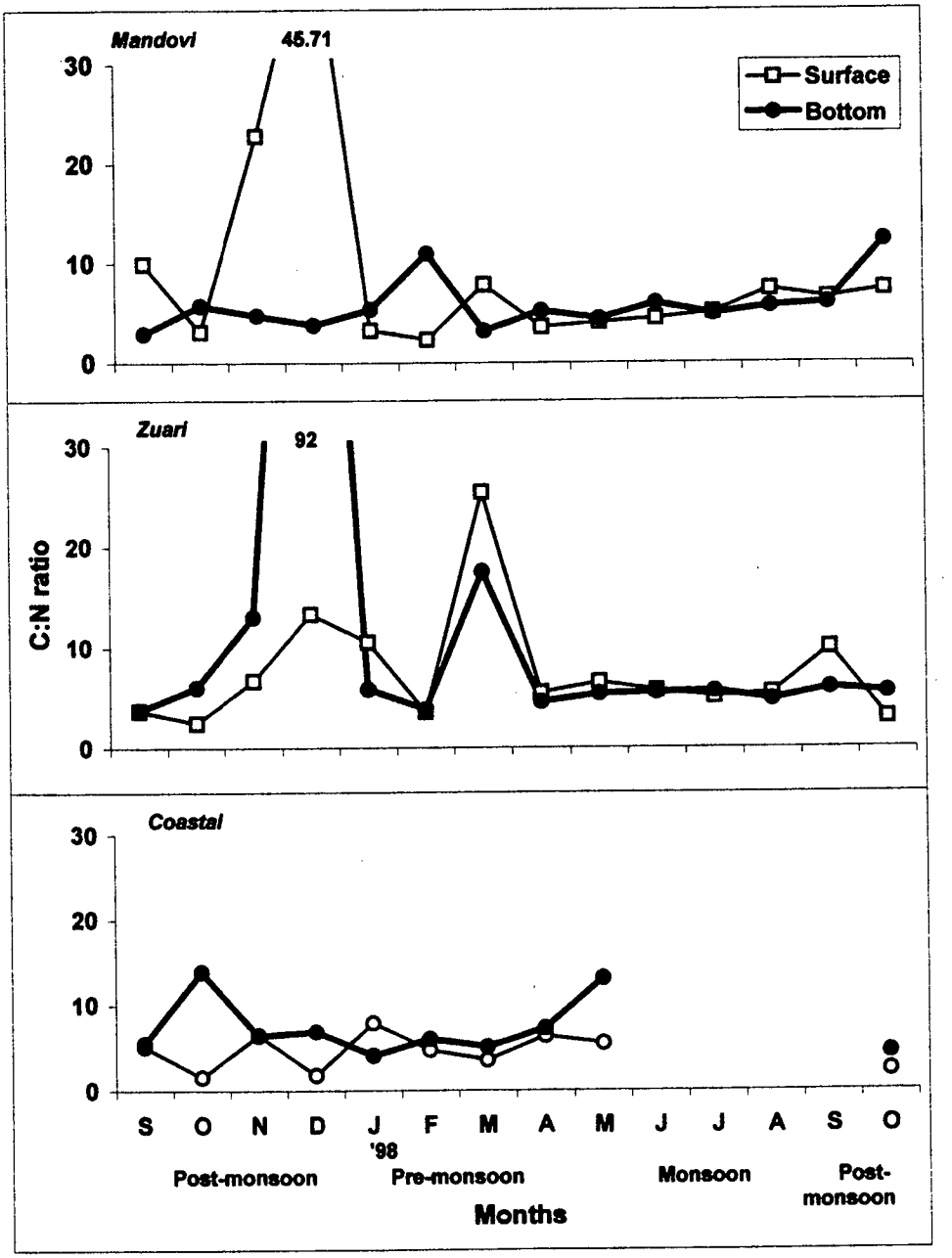


Fig. 11: C:N ratio at the three sampling sites

ıle 6: Annual variation of phytoplankton and detrital carbon in the Mandovi estuary

nths	Phytoplankton carbon ($\mu g.l^{-1}$)													
	S	O	N	D	J	F	M	A	M	J	J	A	S	O
face	113.7	129.9	44.7	100.8	26.1	21	76.5	45.6	46.2	33.0	0.6	3.3	11.4	31.5
tom	16.2	59.4	32.4	65.1	34.1	42.3	54.0	27.6	55.2	16.5	0.3	0.9	6.9	25.8
	Detrital carbon ($\mu g.l^{-1}$)													
face	523.5	174.8	640.9	2941.5	619.6	1739.0	488.3	780.0	1069.0	3916.1	917.2	1022.1	1236.6	497.9
tom	1110.2	1087.8	434.8	1611.7	1065.9	1226.5	952.4	1121.2	1656.8	17442.7	608.6	1573.5	1957.9	905.4

ble 7: Annual variation of phytoplankton and detrital carbon in the Zuari estuary

nths	Phytoplankton carbon ($\mu g.l^{-1}$)													
	S	O	N	D	J	F	M	A	M	J	J	A	S	O
rface	92.7	118.5	35.7	90.9	30.9	26.1	58.5	43.5	52.2	29.4	5.4	78.0	56.7	43.2
ttom	43.8	70.5	22.8	71.7	58.5	32.4	48.9	11.1	42.3	26.1	5.4	12.6	4.8	21.0
	Detrital carbon ($\mu g.l^{-1}$)													
rface	1465.7	490.9	608.7	775.8	735.9	1516.1	1855.1	2863.7	1898.4	2204.8	1645.5	1008.6	669.1	669.5
ttom	1661.8	486.3	226.8	1646.7	1061.5	2663.6	2111.1	2732.9	7954.5	3087.5	668.5	1021.0	1633.6	905.4

(Table 8). The difference between the surface and bottom waters was less distinct as compared to the estuaries.

4.1.10 Dissolved Organic Carbon (DOC)

The DOC varied from 33.23 to 242.9 mg.C.l^{-1} at the surface and from 51.35 to 377.79 at the bottom in Mandovi (Fig12). At the Zuari station, the corresponding values were low between 30.3 to 226.1 and from 56 50 to 329.4 mg.C.l^{-1}. At the coastal station, the concentrations ranged between 68.4 and 180 mg.C.l^{-1}. The relationship of POC to DOC is shown in Fig. 13, 14 and 15. The DOC concentration is higher in all the three stations during the pre-monsoon period in the surface waters when compared to the other seasons. In the bottom waters of the estuary, seasonality was absent with sporadic peaks. On the whole, DOC formed about 98% of total organic carbon (TOC).

Annual averages showed that in Mandovi the POC value was 1.3 times that of the surface which was in contrast to that seen in the Zuari, where the surface value was 1.7 times the values at the bottom (Fig 16).

The POC in surface and bottom waters is directly related to the detrital carbon ($p<0.001$). In the bottom waters the POC is also related to salinity and particle numbers ($p<0.05$) and PON ($p<0.001$). Chlorophyll *a* carbon had a positive relation with suspended load ($p<0.05$) in the surface waters. Detrital carbon relates negatively to DOC ($p<0.05$) and positively to particles (3–15 μm) in the surface and bottom ($p<0.05$) waters.

The DOC and TOC were positively correlated with salinity in the Mandovi (surface). In Zuari DOC was negatively correlated to PON. In the coastal waters DOC was negatively correlated with depth.

ıle 8: Annual variation of phytoplankton and detrital carbon in the Coastal station

nths	Phytoplankton carbon ($\mu g.l^{-1}$)													
	S	O	N	D	J	F	M	A	M	J	J	A	S	O
face	80.4	79.5	26.1	61.8	16.2	27.9	14.7	18.6	50.4	ND*	ND	ND	ND	61.8
tom	32.7	65.4	71.7	68.4	14.1	21.6	2.7	2.7	9.0	ND	ND	ND	ND	68.4
	Detrital carbon ($\mu g.l^{-1}$)													
face	1068.4	196.5	661.98	952.2	457.1	522.5	486.1	1044.7	829.6	ND	ND	ND	ND	509.9
tom	882.5	267.4	470.7	2010.0	940.3	980.0	2224.1	602.1	1037.4	ND	ND	ND	ND	900.6

D – Not done

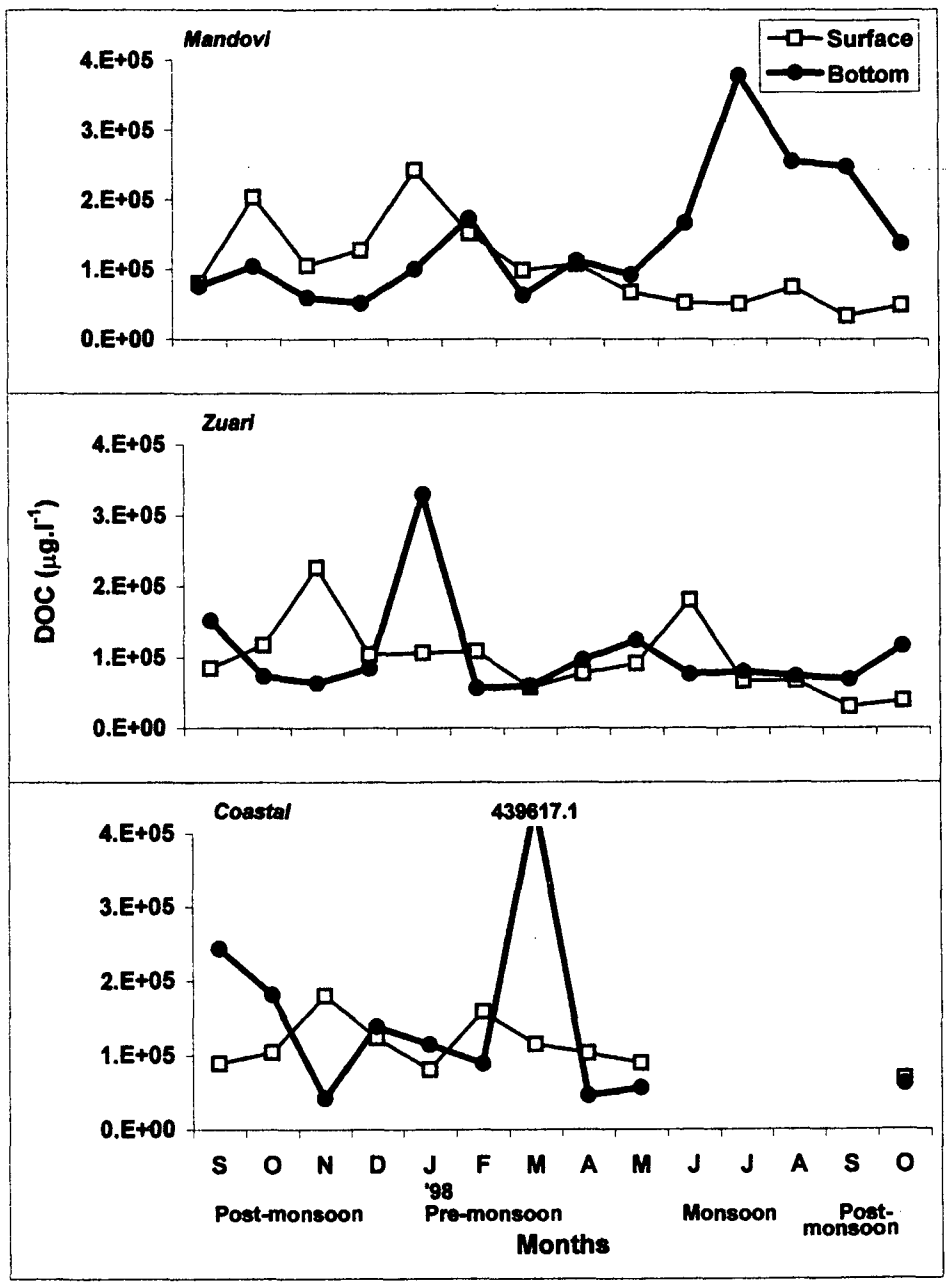


Fig. 12: Dissolved organic carbon (DOC) in the estuarine network

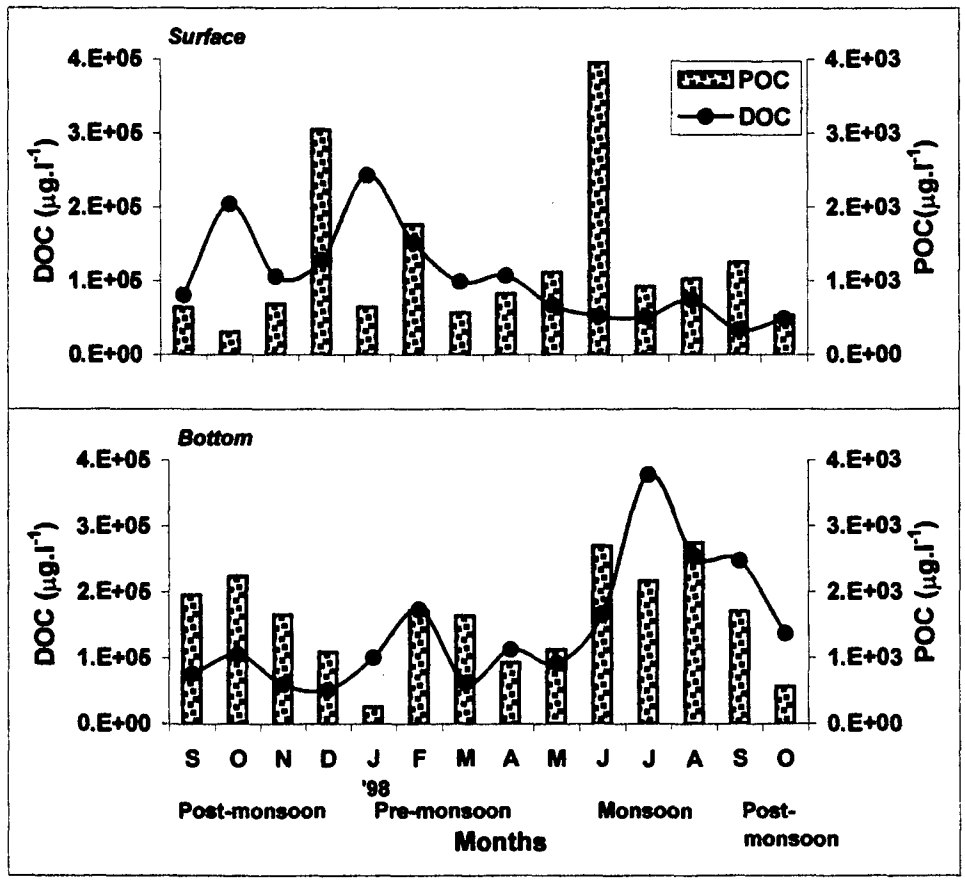


Fig. 13: POC and DOC content of water in the Mandovi estuary

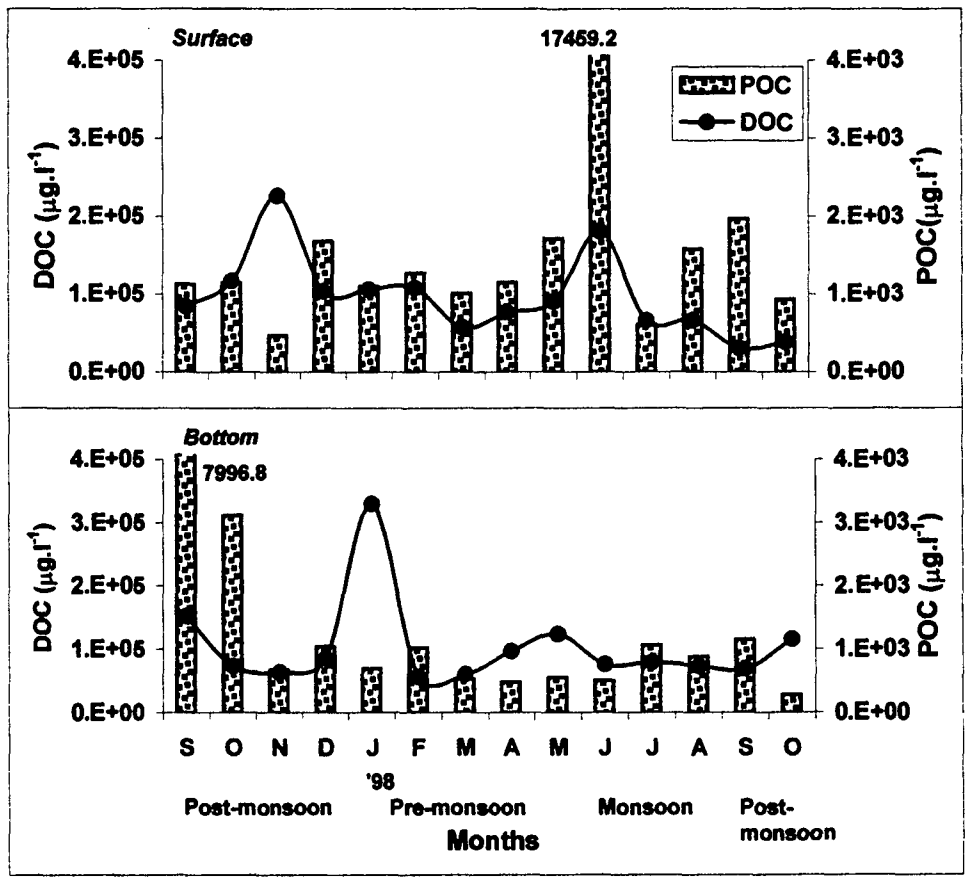


Fig.14: POC and DOC content of water in the Zuari estuary

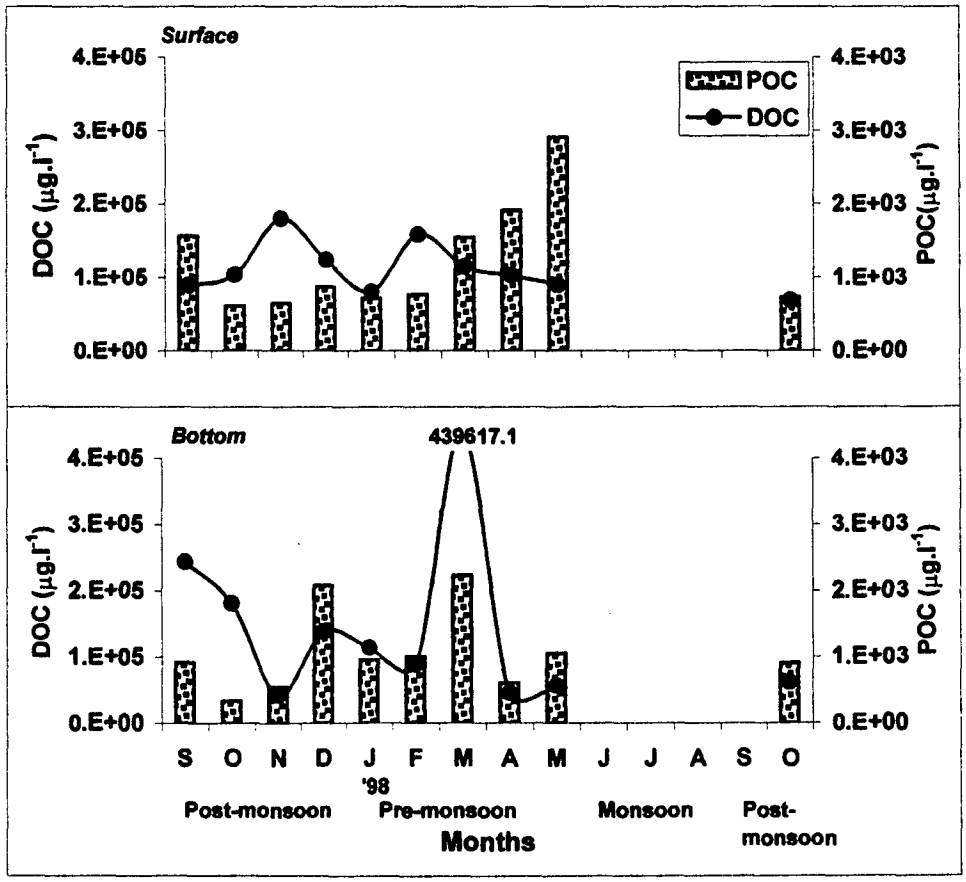


Fig. 15: POC and DOC of water in the coastal station

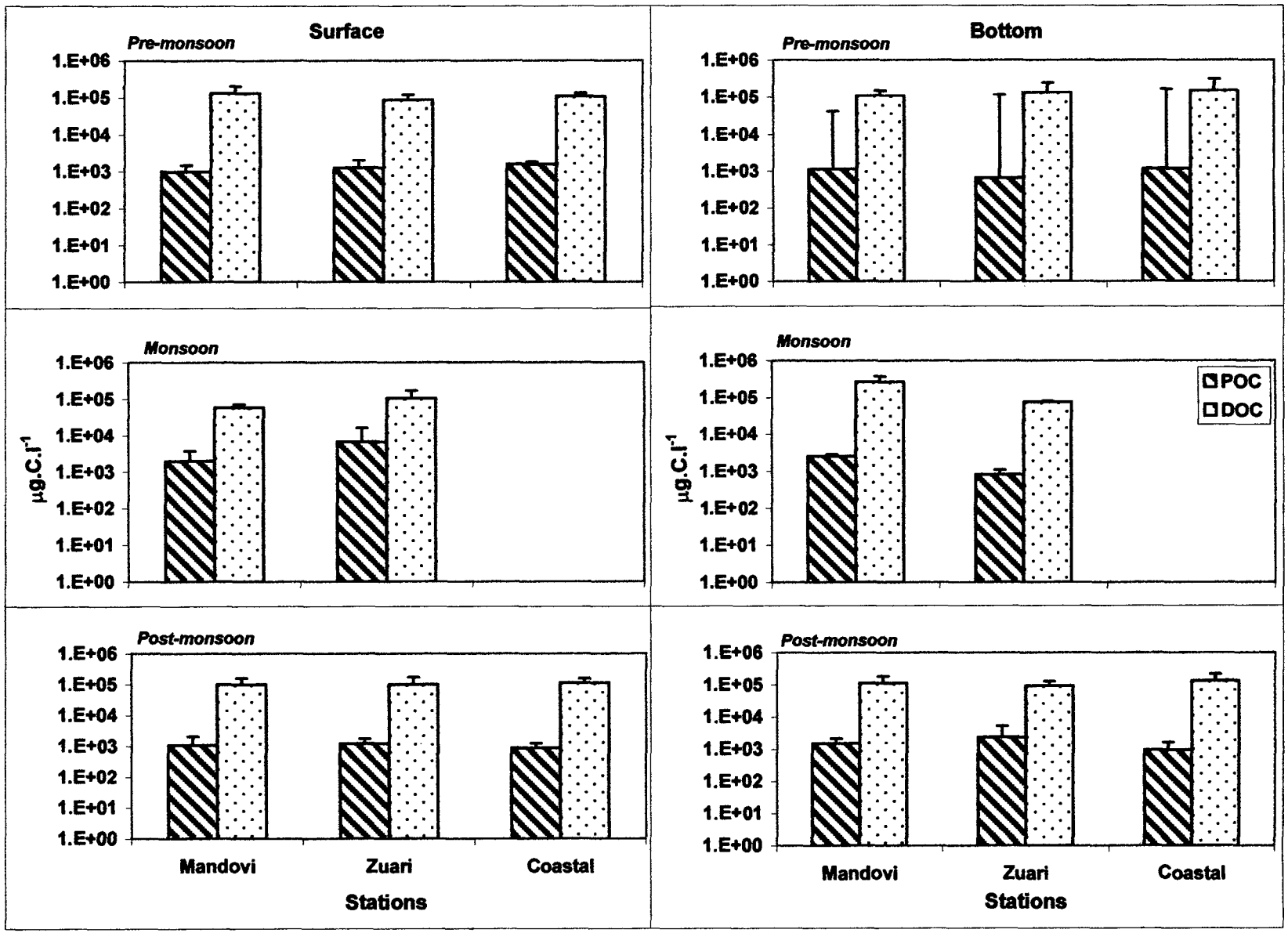


Fig. 16: Dissolved and particulate organic carbon in the estuarine network

4.2 PARTICLE CHARACTERISTICS

4.2.1 PARTICLE NUMBERS

Mandovi

Particle numbers oscillated between orders of 10^7 to $10^{10}.l^{-1}$ with the surface waters showing values thrice that of the bottom ($2.07 \times 10^9.l^{-1}$). The largest variation was observed during the post-monsoon from $2.14 \times 10^9.l^{-1}$ to $6.56 \times 10^{10}.l^{-1}$ in the surface waters and a variation from $4.0 \times 10^7.l^{-1}$ to $2.95 \times 10^9.l^{-1}$ in the bottom waters (Fig. 17).

The laser based particle analysis showed that most of the particles were >3μm in size. In both surface and bottom waters, all the fractions were present during the months of March, April and May except in the surface waters of June while in the surface waters they were also present in these months except June. The 3 -15 μm fraction dominated over other fractions (Table 9). Particle numbers in the size range of 3 - 15 μm were strongly influenced by salinity in both surface ($p<0.001$) and bottom waters ($p<0.05$).

Zuari

Low particle numbers of $0.8 \times 10^9.l^{-1}$ in the surface and $1.42 \times 10^9.l^{-1}$ in the bottom waters were observed in July. Maximum number of particles was observed during the pre-monsoon month of April ($2.82 \times 10^9.l^{-1}$) at the surface and in January ($6.56 \times 10^{10}.l^{-1}$) at the bottom (Fig 17). In the pre-monsoon and post-monsoon seasons, the bottom waters showed an order higher ($10^{10}.l^{-1}$) than the surface waters.

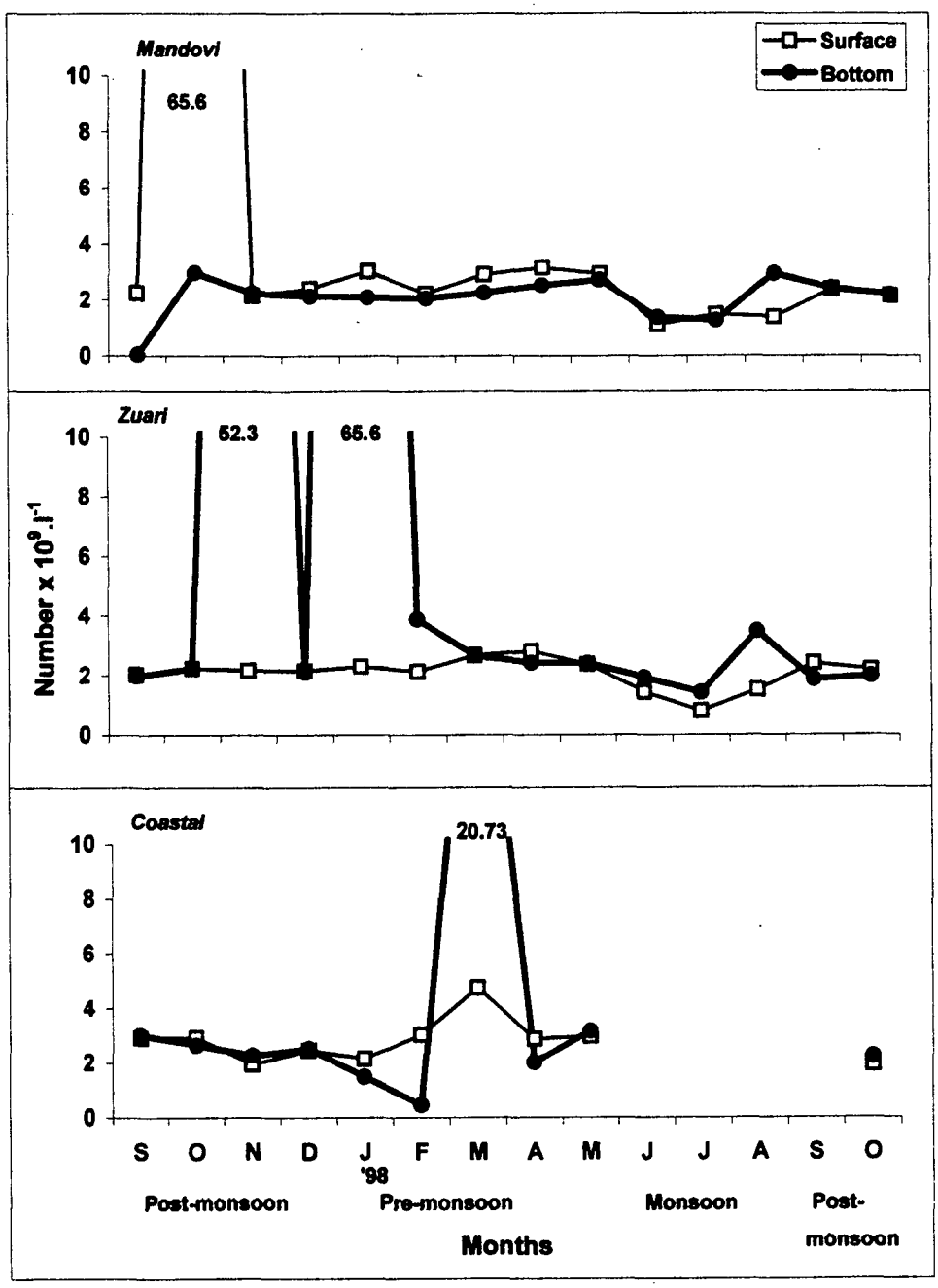


Fig. 17: Total number of particles at the sampling sites

Table 9: Particle distribution in Mandovi estuary

Particle number x $10^5.l^{-1}$												
Months	Size fractions (μm)											
	3---15		15.1-25		25.1—50		50.1—80		80.1—100		100>	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
S	22300	440	34.9									
O		29500			7780		303000		345000			
N	21400	21300		842								
D	23700	20900	194	328								
J	27700	20200	417	328		53						
F	21800	19500	360	2790	0.4	107						
M	27800	20700	796	1330	924	267	120	23	6.3	2.5	21.9	1.3
A	29200	23500	7.1	148	1200	112	424	13.4	150	1.4	400	0.7
M	28200	24200	728	675	204	455	53.8	86.2	8.2	1.9	0.3	0.6
J	10900	13400	278	275	30.1	81.8	2.3	19.2	0.3	0.7		0
J	14200	7890	293	62.5	135	55.3	45	7	0.9	0.2		
A	13700	15200	13.3	15.1	15	16.9	8.3	6				
S	23500	22600	192	167								
O	21400	21400										

Laser based particle analysis showed that a major fraction of the particles were >3μm (Fig. 10). In the pre-monsoon months all the 6 size fractions were represented in the bottom waters. The bottom waters had more number of particles in the size range of 15 - 100 μm whereas, in the surface water 3 -25 μm fraction was the most dominant (Table 10). In the bottom waters the above fraction dominated in all the months (except November and December).

Coastal

Particle numbers ranged from 1.93 x $10^9.l^{-1}$ in October'98 to 4.74 x $10^9.l^{-1}$ in March at the surface and from 0.47 x $10^9.l^{-1}$ in February to 20.73 x $10^9.l^{-1}$ in March in the bottom waters. In general higher number of particles was observed during the pre-monsoon in the surface (3.15 x $10^9.l^{-1}$) and bottom waters (5.57 x $10^9.l^{-1}$) (Fig. 17).

Particle numbers were positively related to salinity in the surface waters only ($p<0.05$). Particles (3–15 μm) also correlated with temperature in both surface and bottom ($p<0.05$) waters. Total particle numbers correlated negatively with DOC ($p<0.001$) and POC ($p<0.05$) in the bottom waters.

Size fractionation of particles indicated that the 3–15μm size dominated in all the months except October'97 in the surface (Table 11) and September'97 in the bottom waters (Table 11). The pre-monsoon months of March, April and May showed the presence of all the five size fractions of particles in the surface and bottom waters.

Table 10 Particle distribution in Zuari estuary

Particle number x 10^5.l^{-1}												
Months	Size fractions (μm)											
	3—15		15.1-25		25.1-50		50.1-80		80.1-100		100>	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
S	20300	19100		624								
O	44600	22300		1.3								
N	21300	514000	351									
D	21400	21100	67.3									
J	22900	287000	92.5	322000		47300						
F	21000	23300	294	3540	83.4	7160	0.8	3340		1320		2260
M	22800	23100	2180	2290	845	981	348	91.7	79	49.1	66.4	122
A	26500	22000	235	34500	55.4	116	17.5	25.8	1.6	1.9	0	0.5
M	21200	21700	267	309	92.8	321	11.3	143	1.1	14.2	0.1	1.5
J	14400	19100		140		25.2		3.4				
J	11900	13800	525	498	158	42.7	30.6	1.8				
A	25600	32300	2790	1990	455	467		55.4				
S	23000	18500	33	6.7		115		41.1		8.6		7.3
O	21800	172	169	148								

Table 11 Particle distribution in the coastal station

Particle number x $10^5.l^{-1}$												
Months	Size fractions (μm)											
	3—15		15.1–25		25.1–50		50.1–80		80.1—100		100>	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
S	29000					30100						
O		24200	28400	2170	759	36.5						
N	19500	22600		73.9								
D	24100	25100	393									
J	21500	14875										
F	12300	4650	15800		1050							
M	22200	175000	3750	14300	9610	12100	5600	6120	2210	258	3790.0	19.3
A	28300	19800	197	242	130	110	98.4	64.4	15.6	2.8	2.3	0.2
M	28700	30700	669	471	198	299	57.2	71.9	20.2	15.9	18.3	21.9
J	ND	ND										
J	ND	ND										
A	ND	ND										
S	ND	ND										
O	19300	38.5		33.9								

4.2.2 SIZE

Mandovi

Particles were fractionated into six groups viz. (i) >3<220 μm, (ii) >2.7<3 μm, (iii) >1.2<2.7 μm, (iv) >0.7<1.2 μm, (v) >0.45<0.7 μm and (vi) 0.22<0.45 μm. On an annual average basis size fractionation by gravimetry showed that 50% of the particles were >3 μm and <220μm range. In the surface waters (Sept '98), 94% of the particles were in this size range (Fig. 18).

Zuari

Size fractionation by gravimetry showed that around 43% of the particles in the surface and 51% of the particles in the bottom waters ranged between 3 μm and 220 μm size. As viewed in Fig. 19 more than 80% of the particles were in the >3 < 220 μm size in the month of May.

Coastal

Size fractionation by gravimetry showed that in the bottom waters 40% of the particles were >3 μm. However maximum values of 58.01% and 64.12 % were recorded in November and December respectively (Fig. 20).

In surface waters, 3–15 μm size was related to POC ($p<0.05$). The 3–15 μm fraction of particles in the bottom waters were related to ($p<0.05$) POC, PON and detrital carbon.

During pre-monsoon, the highest number of particles ($1.54 \times 10^{10}.l^{-1}$) was recorded in the Zuari bottom waters. However, in the surface waters of

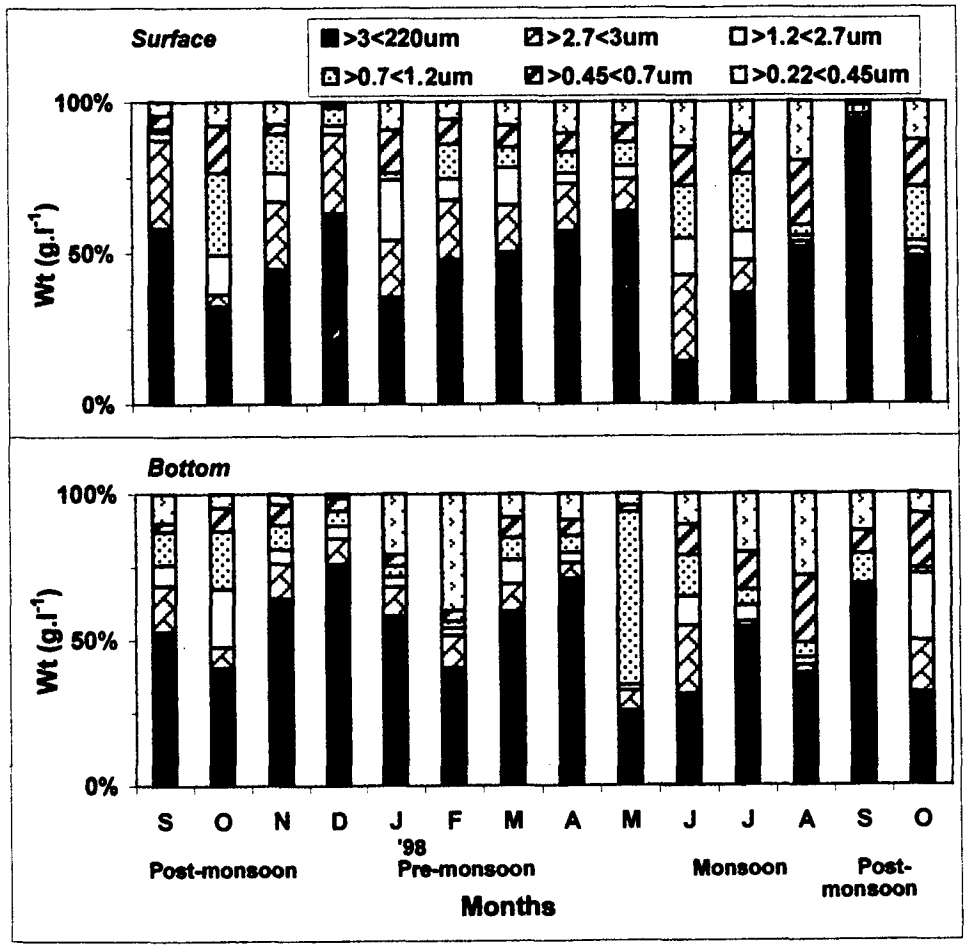


Fig. 18: Size fractionation of particles present in the Mandovi estuary based on gravimetric analysis

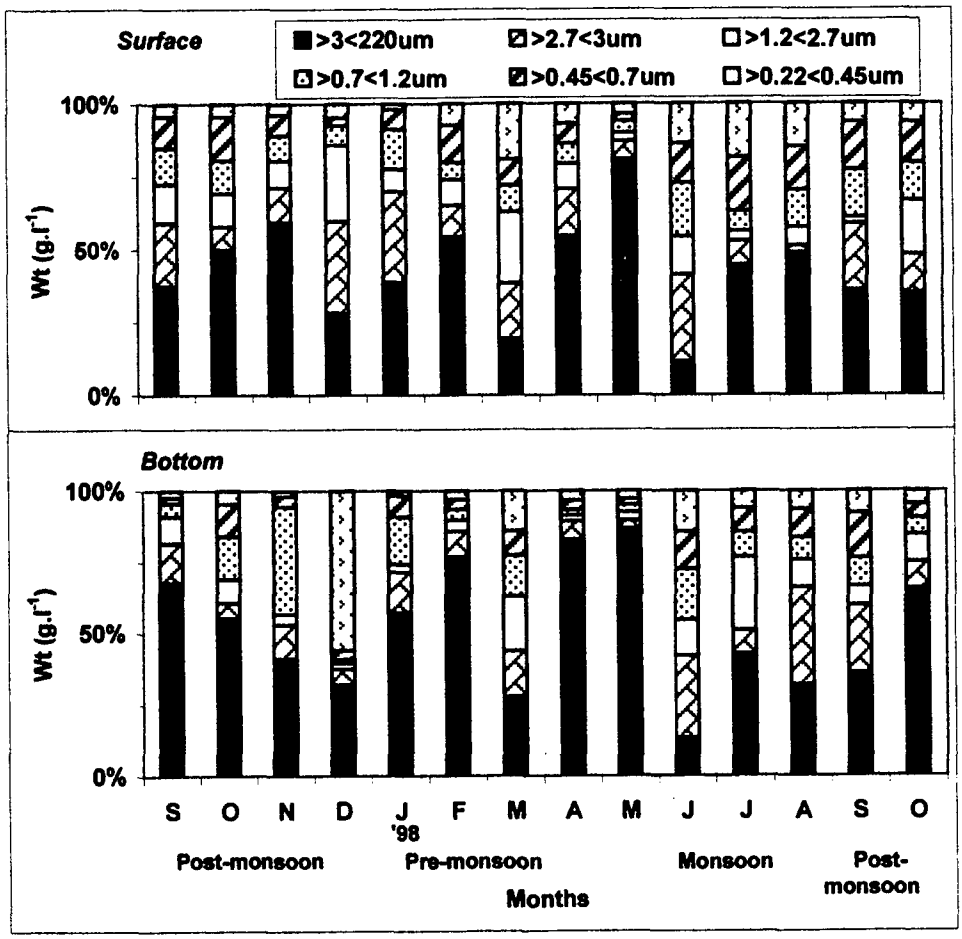


Fig. 19: Size fractionation of particles present in the Zuari estuary based on gravimetric analysis

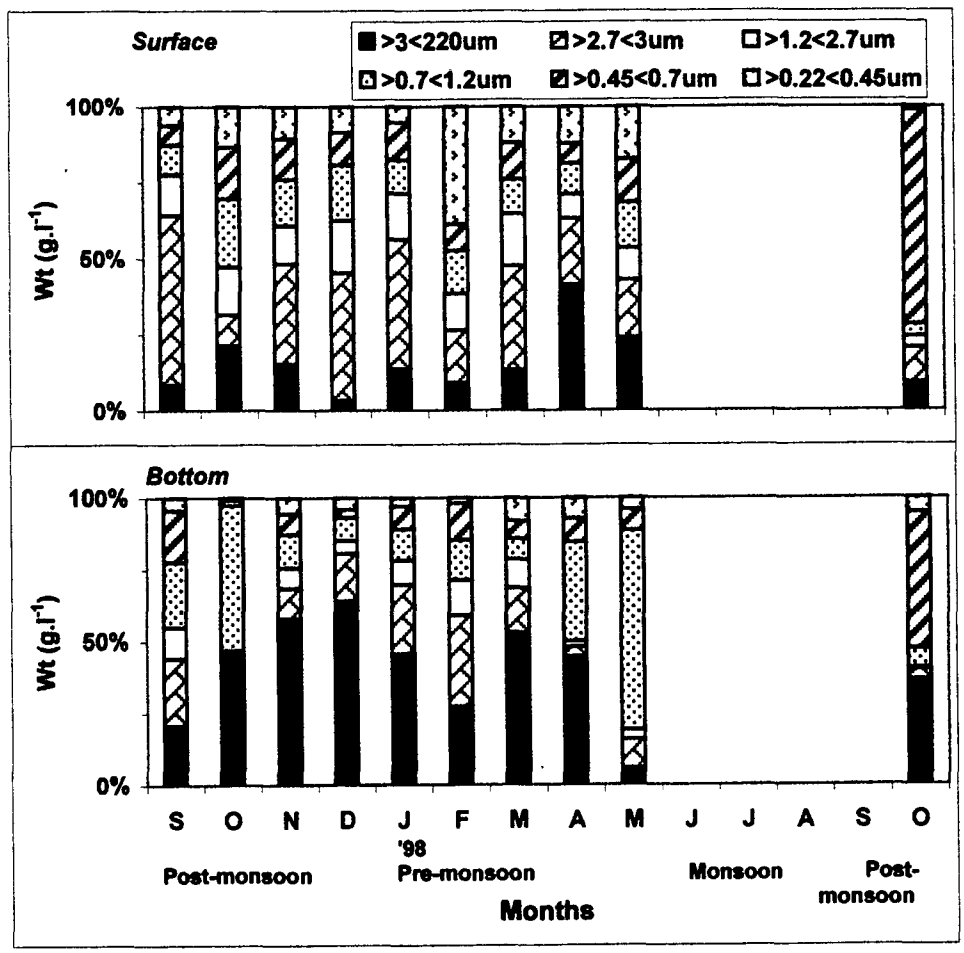


Fig.20: Size fractionation of particles present in the coastal station based on gravimetric analysis

Mandovi most number of particles (1.28 x 10^{10}.l^{-1}) were observed during the post-monsoon (Fig. 21).

It was observed that during the months of March, April and May, all the fractions of particles were present at all the stations. Maximum number of particles was found in the 3-15 μm fraction at all seasons and stations except during the post-monsoon at Mandovi (surface) where, 80-100 μm fraction dominated.

In the pre-monsoon, particles in the size range of >3<220 μm were maximum in the Mandovi (56%), Zuari (surface- 50%, bottom- 66%) and the coastal station (surface – 20%, bottom- 35%) (Fig. 22).

4.2.3 CHEMICAL CHARACTERISTICS

4.2.3.1 Mandovi

Inorganic Content (IC)

At the Mandovi station, the inorganic content of the particles ranged from 1 x 10^{-6} g.l^{-1} (Oct.'97 and Sept.'98) to 0.15 g.l^{-1} (February) in the surface waters and 1 x 10^{-6} g.l^{-1} (Sept'98) to 0.33 g.l^{-1} (May) in the bottom waters. High values of inorganic content were recorded in the surface (0.049 g.l^{-1}) and the bottom waters (0.199 g.l^{-1}) during the pre-monsoon and low values during the monsoon seasons (Fig. 23).

The annual average inorganic content (percent) of particles did not show much difference between the surface and bottom waters, the values being, 41.6% and 42.8% respectively. However, the bottom waters were 1.52 times the surface value (50.29%) during the pre-monsoon season. The lowest

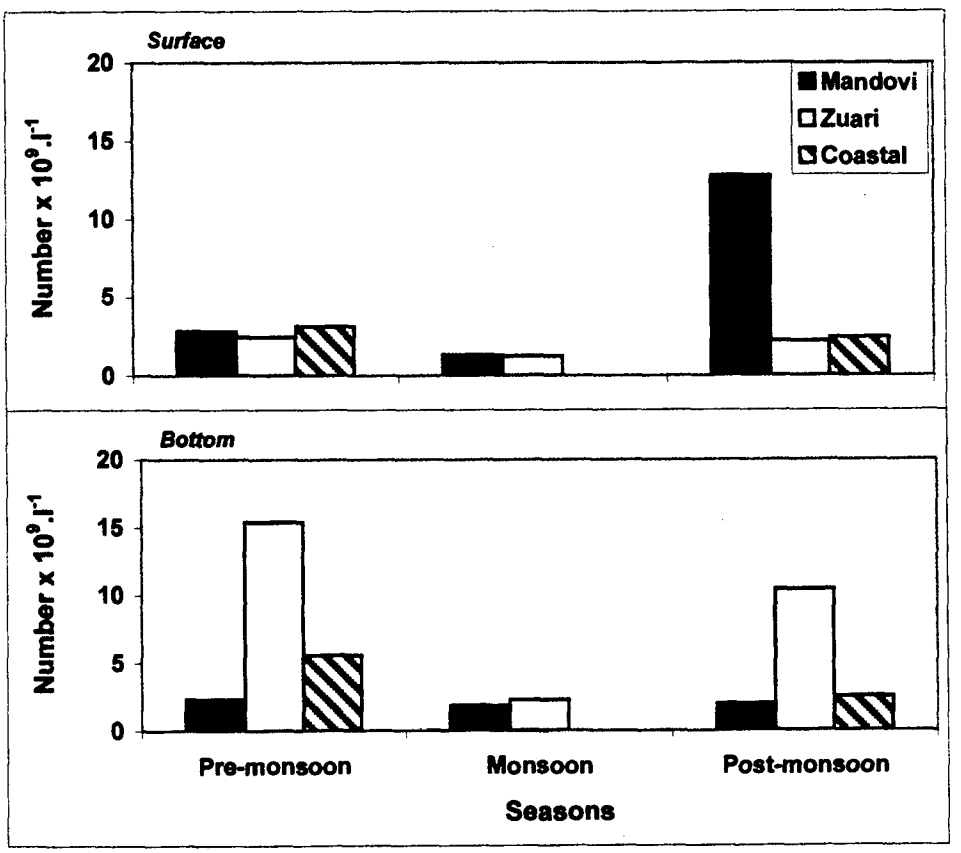
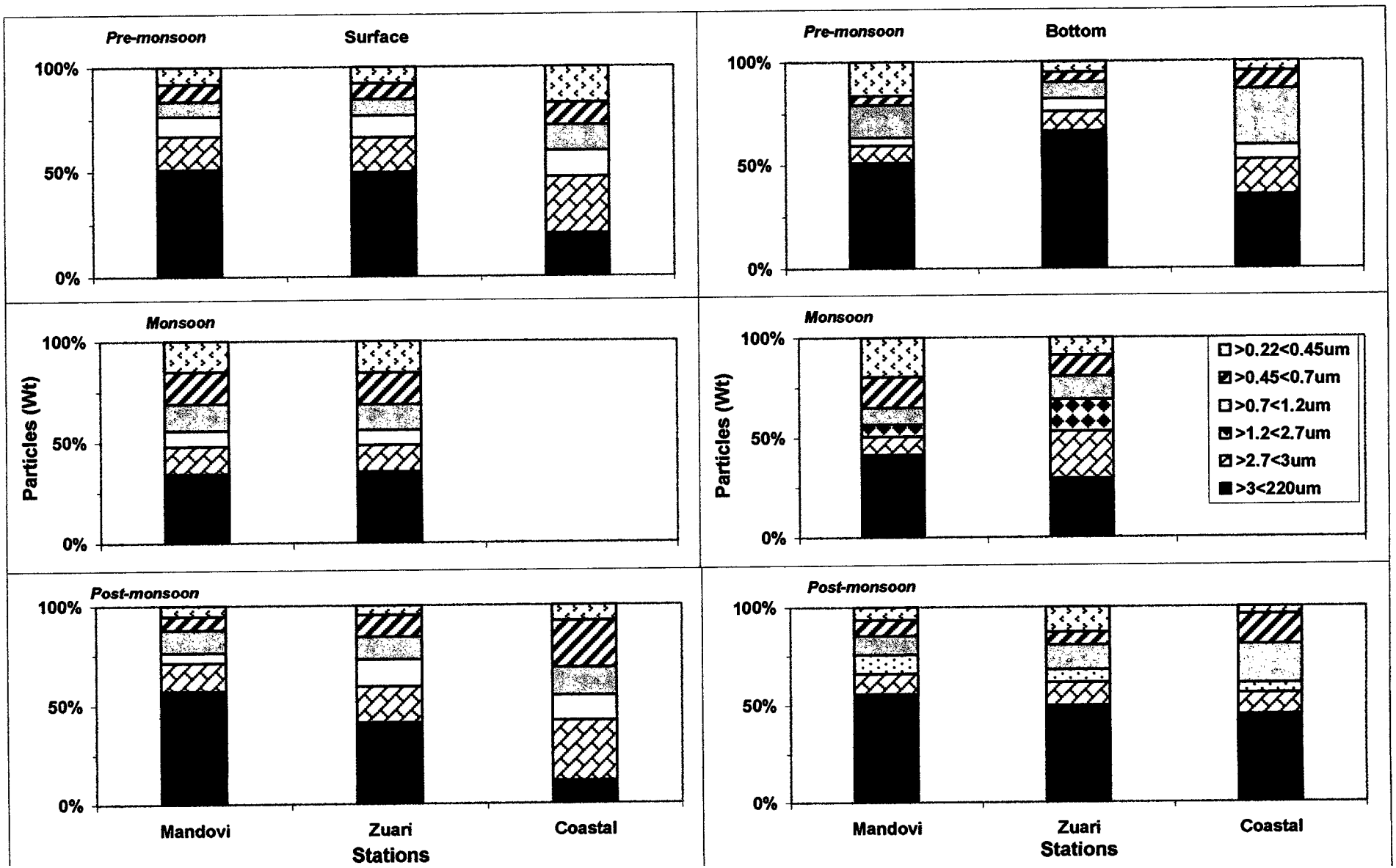


Fig. 21: Seasonal variation in particle numbers at three stations


Pre-monsoon
Surface
100%
50%
0%
Pre-monsoon
Bottom
100%
50%
0%
Monsoon
Particles (Wt)
100%
50%
0%
Monsoon
Particles (Wt)
100%
50%
0%
>0.22<0.45um
>0.45<0.7um
>0.7<1.2um
>1.2<2.7um
>2.7<3um
>3<220um
Post-monsoon
100%
50%
0%
Mandovi
Zuari
Coastal
Stations
Post-monsoon
100%
50%
0%
Mandovi
Zuari
Coastal
Stations

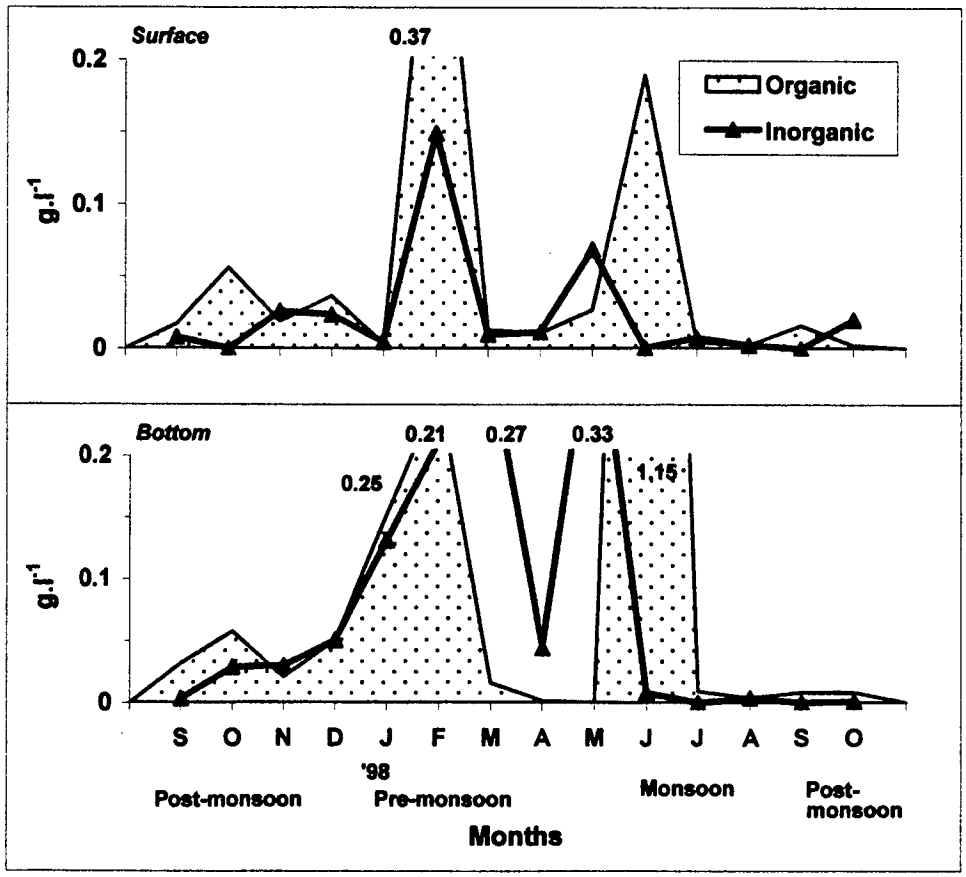


Fig. 23: Organic and inorganic content of particles in the Mandovi estuary

average of 16.9% was recorded in the bottom waters during the monsoon. The months of November (59.5%) and August (50%) showed equal distribution of inorganic content in the surface and bottom waters. The bottom waters contained > 90% ash weight in the pre-monsoon months except, February.

The inorganic content of the particle was positively related ($p<0.05$) to the suspended load in the surface waters. In the bottom, the organic content showed positive correlation ($p<0.001$) with the suspended load thereby influencing the whole water column.

Mineral content

Particles were mainly composed of clay minerals like montmorillonite, illite, kaolinite + chlorite, gibbsite and goethite. The particles were dominated by kaolinite + chlorite during all the seasons especially in the post-monsoon. However, the particles in the surface waters during the pre-monsoon showed a high percentage of montmorillonite (35%) compared to other minerals (Table 12).

Organic Content (OC)

The organic content varied between 2.2×10^{-3} $g.l^{-1}$ (Oct. '98) to 0.37 $g.l^{-1}$ (February) in the surface waters and showed large oscillation between 1×10^{-6} $g.l^{-1}$ (May) to 1.15 $g.l^{-1}$ (June) in the bottom waters. Surface waters exhibited high values (0.085 $g.l^{-1}$) during the pre-monsoon season and bottom waters, during the monsoon (0.389 $g.l^{-1}$) (Fig. 23). Organic content of the particles was found to be significantly related to suspended load in both surface and bottom waters ($p<0.001$).

Table 12: Mineral content of particles at the three stations expressed in percentage (w/w)

Mineral	Station	Seasons					
		Pre-monsoon		Monsoon		Post-monsoon	
		Surface	Bottom	Surface	Bottom	Surface	Bottom
Montmorillonite	Mandovi	35.52	27.70	21.92	21.71	19.04	12.97
	Zuari	41.44	45.42	40.93	22.45	32.71	44.85
	Coastal	28.08	35.43	ND*	ND	43.26	69.75
Illite	Mandovi	8.85	14.86	10.07	3.74	9.12	16.43
	Zuari	4.66	6.07	7.27	8.12	9.70	9.73
	Coastal	26.75	8.48	ND*	ND	16.74	8.35
Kaolinite + Chlorite	Mandovi	31.63	33.92	37.97	31.28	48.53	40.47
	Zuari	33.14	30.22	32.00	29.76	37.58	32.55
	Coastal	38.94	35.69	ND	ND	27.21	16.99
Gibssite	Mandovi	5.59	7.30	8.43	5.45	8.95	9.41
	Zuari	6.39	4.71	3.87	5.54	3.63	2.92
	Coastal	3.61	7.49	ND	ND	3.49	2.38
Goethite	Mandovi	18.42	16.22	21.61	37.81	14.36	20.73
	Zuari	14.37	13.58	15.92	34.12	16.38	9.95
	Coastal	2.62	12.91	ND	ND	9.30	2.53

* ND – Not done

4.2.3.2. Zuari

Inorganic Content

The inorganic content of the particles varied from 1 x 10^{-6} g.l^{-1} (February, July and Sept. '97) to 0.08 g.l^{-1} (May) at the surface waters; while in the bottom waters, it ranged from 1x 10^{-6} g.l^{-1} (Sept. '97) to 0.18 g.l^{-1} (March). Highest IC was recorded in the surface (0.02 g.l^{-1}) and bottom waters (0.093 g.l^{-1}) during the pre-monsoon period. The minimum inorganic content was observed during the monsoon, which was of an order lower than the other seasons (Fig. 24).

An annual average of 41% was recorded for the Zuari estuary. Except for the pre-monsoon season, the surface waters of the monsoon (16.8%) and post-monsoon (48.08%) periods had higher ash content than the bottom waters. The monsoon season had the lowest ash content in the surface (16.8%) as well as bottom (12.61%) waters. The particles of the bottom waters during October'97, March and April,'98 contained more than 80% ash while in the surface waters it was observed in the month of May.

Mineral content

The particles in these waters consisted of montmorillonite, illite, kaolinite+chlorite, gibbsite and goethite. These particles were dominated by montmorillonite followed by kaolinite + chlorite in all the seasons (Table 12).

Organic content

The organic content of particles showed a minimum value of 1 x 10^{-3} g.l^{-1} (Oct. '98) to 0.25 g.l^{-1} (June) at the surface waters. Bottom waters showed a peak of 0.55 g.l^{-1} in May and a dip of 3.4 x 10^{-3} g.l^{-1} in November.

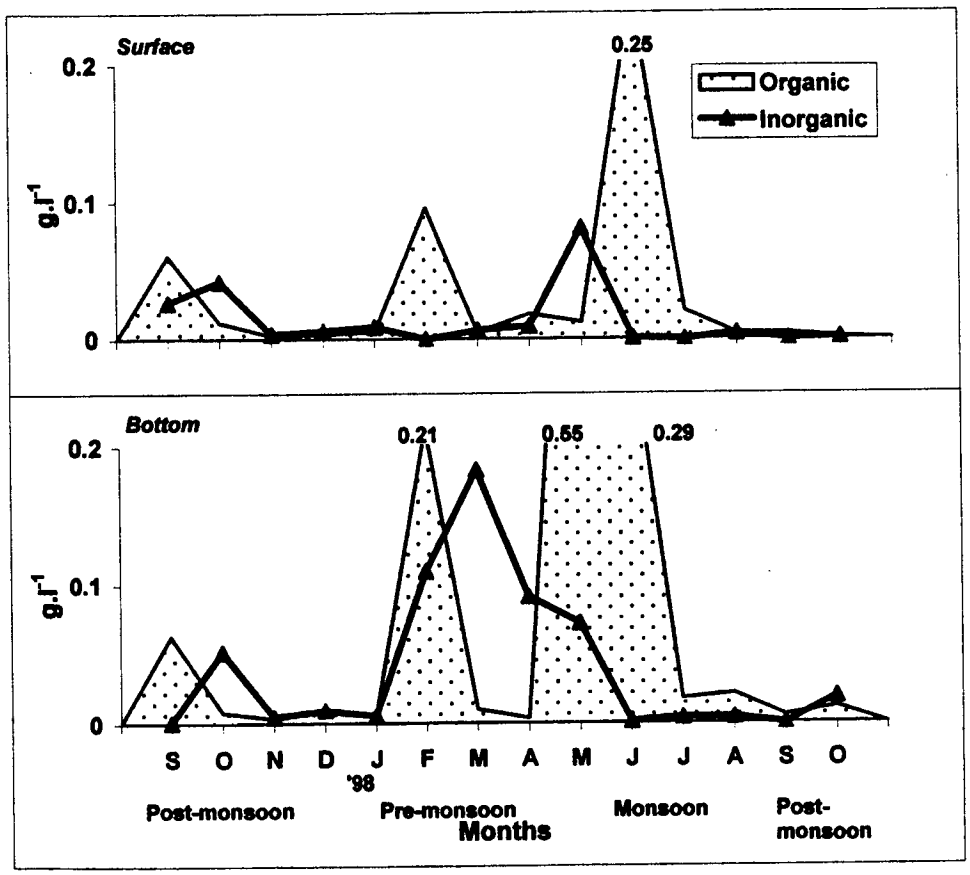


Fig. 24: Inorganic and organic content of particles in the Zuari estuary

Highest organic content was observed during the monsoon (0.09 $g.l^{-1}$) at the surface while the bottom waters it during the pre-monsoon (0.16 $g.l^{-1}$). Domination by organic content over inorganic was observed in all the seasons. However monthly observations showed the inorganic content to be more than the organic content in the months of Oct. '97 and 98, November '97, December '97 and March '98 in the surface and bottom waters (Fig 24).

4.2.3.3 Coastal

Inorganic Content

The inorganic content of particles varied from 0.0013 $g.l^{-1}$ (Oct.'98) to 0.043 $g.l^{-1}$ (Sept.'97) in the surface and 0.0 $g.l^{-1}$ (April and Oct. '98) to 0.093 $g.l^{-1}$ (February) in the bottom waters (Fig 25). The surface and bottom water of the post-monsoon period showed 2.7 and 1.4 times the inorganic content of the pre-monsoon. The inorganic content of particles correlated ($p<0.001$) well with the suspended load in the bottom waters.

The percentage of ash ranged from 29.59% (February) to 87.11% (April) in the surface and 0% (October and April) to 99.99% (Oct.'97) in the bottom waters. The percentage of ash was highest in the surface waters during the pre-monsoon (62.4 $\pm$ 24.2%) and post-monsoon (59.93 $\pm$ 9.3) seasons, when compared to the bottom waters (pre-monsoon–40.05 $\pm$ 35%, post-monsoon– 48.03 $\pm$ 37%).

Mineral Content

The suspended load contained five major groups of clay minerals. They were montmorillonite, illite, kaolinite + chlorite, gibbsite and goethite. During the post-monsoon season, montmorillonite content was about 50% of

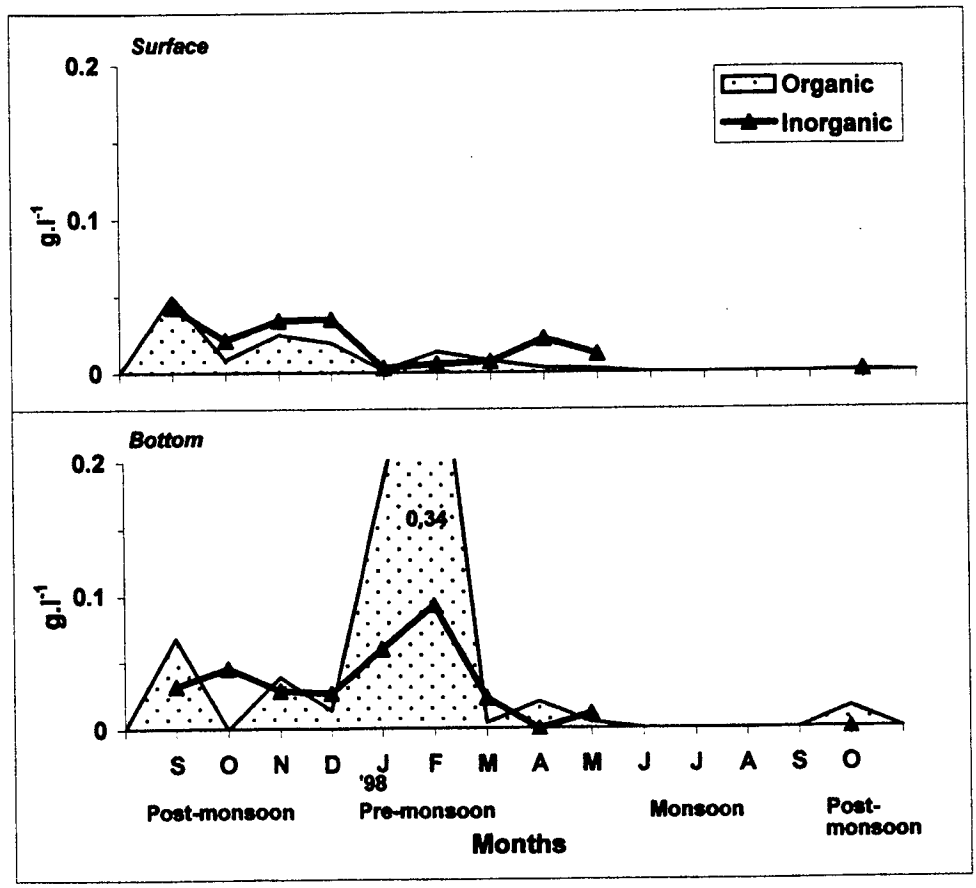


Fig. 25: Organic and inorganic content of particles in the coastal station

the total minerals. This was more obvious in the bottom waters (69.75%); while in the pre-monsoon season kaolinite + chlorite dominated in both the surface and bottom waters (Table 12).

Organic Content

The Organic Content ranged from 0.00088 $g.l^{-1}$ (Oct'98) to 0.05 $g.l^{-1}$ (Sept'97) at the surface and 0.00001 $g.l^{-1}$ (Oct'97) to 0.343 $g.l^{-1}$ (February) in the bottom waters (Fig 25). During the post-monsoon period the surface waters contained 3.56 times the OC of the pre-monsoon (0.0058 $g.l^{-1}$), while during the pre-monsoon the bottom waters contained 4.07 times the average organic matter of the post-monsoon (0.027 $g.l^{-1}$)

The annual average organic content (0.069 $g.l^{-1}$) was twice that of the inorganic content (0.032 $g.l^{-1}$) in the bottom waters. During the pre-monsoon, the organic and inorganic content in the bottom waters was higher than that during the post-monsoon, while in the surface waters, the reverse was true. The annual averages showed the bottom waters to contain higher OM and IM than the surface waters (5.2 and 1.73 times) respectively.

4.2.3.4. Mandovi

Deoxyribonucleic acid (DNA)

A high DNA content of particles was observed in December in the surface (2.23 $mg.l^{-1}$) and bottom waters (1.67 $mg.l^{-1}$). Minimum values of 0.21 $mg.l^{-1}$ at the surface and 0.17$mg.l^{-1}$ in the bottom waters were recorded in June and April respectively (Fig. 26). The trend of the DNA content of particles in the surface and bottom waters was pre-monsoon <monsoon<post-monsoon. In the surface waters the particles recorded the highest DNA

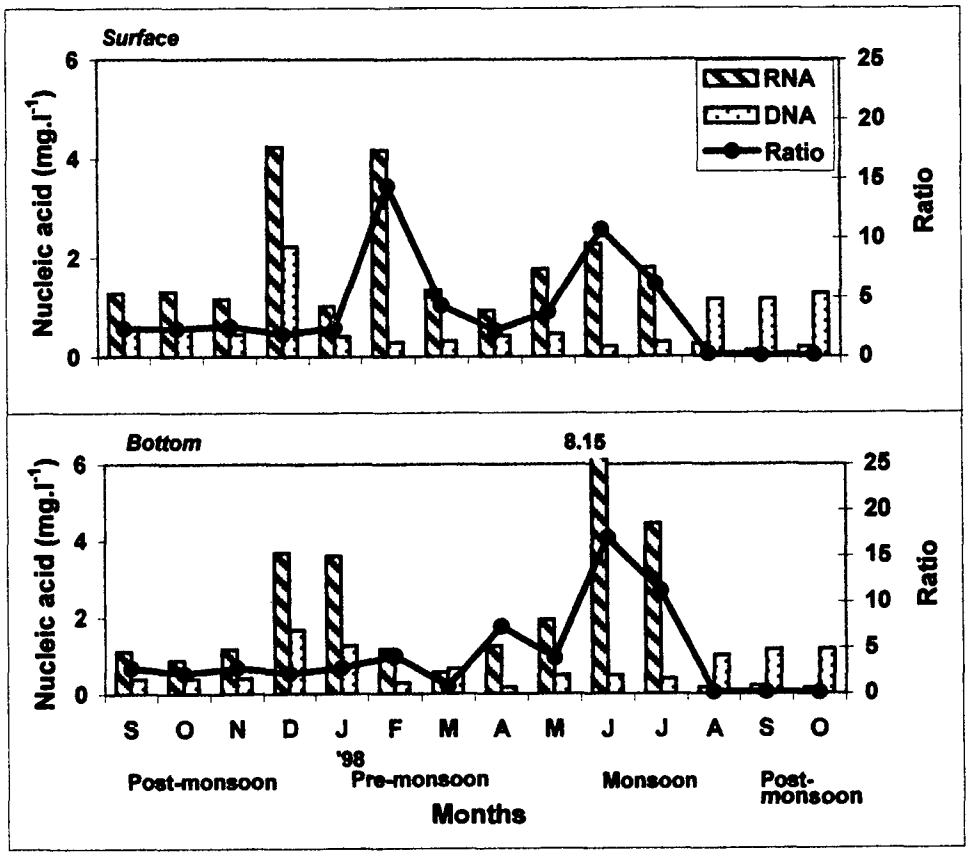


Fig. 26: Particle-associated DNA, RNA and RNA:DNA ratio in Mandovi estuary

content (1035.77 $\mu g.l^{-1}$) during the post-monsoon which was 1.2 times the bottom values. The minimum seasonal average value of 380.48 $\mu g.l^{-1}$ was recorded in the surface waters during the pre-monsoon.

The DNA correlated negatively to suspended load ($p<0.01$) in the surface waters. By integrating the surface and bottom values, the DNA showed a negative relationship to the suspended load ($p<0.05$). While DNA strongly correlated with the organic content ($p<0.001$) in the bottom waters, a negative correlation with the inorganic content ($p<0.01$) was seen in the surface waters.

Ribonucleic Acid (RNA)

The RNA content of particles varied from 0.12 $mg.l^{-1}$ (September) to 4.24 $mg.l^{-1}$ (December) in the surface waters and from 0.14 (Oct. '98) to 8.16 $mg.l^{-1}$ (June) in the bottom waters (Fig. 26). The surface waters followed a trend of pre-monsoon>monsoon>post-monsoon while in the bottom waters it was monsoon> pre-monsoon > post-monsoon. Highest concentration of 4.25 $mg.l^{-1}$ was seen during the monsoon in the bottom waters, which was 2.9 times that of the surface. The lowest value of 1199.78 $\mu g.l^{-1}$ was noted in the bottom waters during the post-monsoon.

The RNA showed a negative relation with the DNA in the surface ($p<0.001$) and bottom waters ($p<0.05$). RNA had a positive relation with the inorganic content ($p<0.05$). In the bottom waters the RNA was related to the suspended load ($p<0.001$), organic content ($p<0.001$) and PON ($p<0.001$). By integrating the surface and bottom values, the RNA essentially correlated with the suspended load, organic content and the PON ($p<0.001$).

RNA:DNA ratio

The annual average RNA/DNA ratio was 14.4 in the bottom waters as compared to 3.84 in the surface waters. Season-wise, the monsoon showed the highest ratio of 5.68 in the surface and 9.46 in the bottom waters. The post-monsoon season showed the lowest seasonal values of 1.58 in the surface and 1.73 in the bottom waters. During the pre-monsoon period the ratio was in contrast to that observed during the monsoon and post-monsoon seasons. In this season, the surface waters appeared to harbour more active groups (5.44) than the bottom waters (3.82) (Fig. 26).

Significant relationship between RNA/DNA ratio and suspended load was observed ($p<0.001$) in the bottom waters.

4.2.3.5. Zuari

Deoxyribonucleic acid (DNA)

A high value of 0.1202 $mg.l^{-1}$ was recorded during September'98 at the Zuari station (surface). The DNA content in the bottom waters was much higher, the value being, 0.1572 mg. l^{-1} (Oct. '98) (Fig. 27). The lowest DNA value was noticed during the monsoon at the surface and during the pre-monsoon in the bottom waters. DNA related well to total suspended load ($p<0.05$).

Ribonucleic Acid (RNA)

The RNA concentration reflects the active biomass of the environment. Minimum RNA value (0.121 $mg.l^{-1}$) was observed in October'98 at the surface and in the bottom waters it was observed in October'97 (0.026 $mg.l^{-1}$). The maximum concentration of RNA (5.19 mg. l^{-1}) was observed in June in the

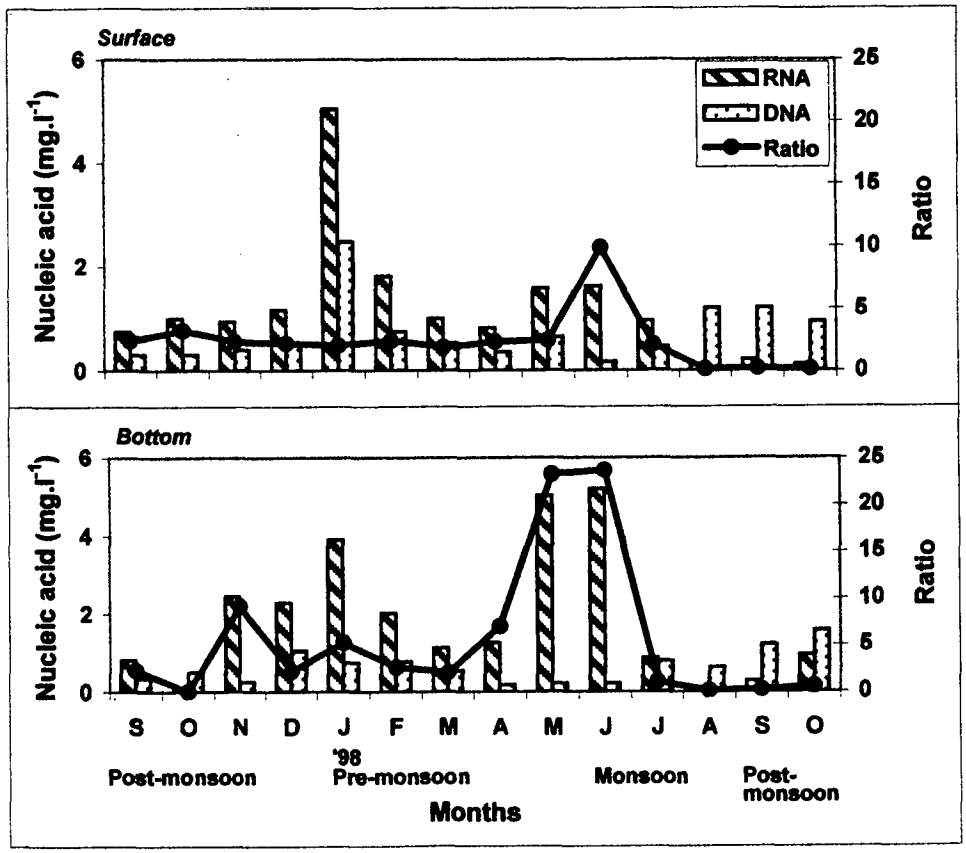


Fig. 27: Particle-associated DNA, RNA and RNA:DNA ratio in Zuari estuary

bottom waters, whereas at the surface, the maximum (5.03 mg l^{-1}) value was observed in January (Fig. 27). The pre-monsoon season showed an increase in the RNA content compared to other seasons. The RNA content was 0.1 to 10 times higher than that of DNA in the surface waters. RNA showed a significant correlation with the suspended load in the bottom waters ($p<0.001$).

RNA:DNA ratio

The Zuari surface waters had ratios ranging from 0.1 (Sept. '97) to 14.37 (February) while, in the bottom waters it ranged from 0.05 (Oct. '97) to 23.7 in June (Fig. 27). The post-monsoon season recorded the lowest ratios of 1.75 and 2.43 in the surface and bottom waters while the monsoon season had higher ratios of 4.03 and 19.52 respectively. Seasonal analysis showed that bottom waters always had higher ratios than the surface.

4.2.3.6.Coastal

Deoxyribonucleic acid (DNA)

The DNA content of particles (>3μm<220μm fraction) varied between 0.22 mg.l^{-1} (February) to 1.52 mg.l^{-1} (Oct. '98) at the surface and from 0.35 mg.l^{-1} (April) to 1.54 mg.l^{-1} (December) in the bottom waters. During the post-monsoon period the surface (0.65 mg.l^{-1}) and bottom waters (0.80mg.l^{-1}) showed 1.4 times higher values than that observed in the pre-monsoon. However, the bottom waters carried particles with more DNA than the surface waters during both the seasons (Fig. 28).

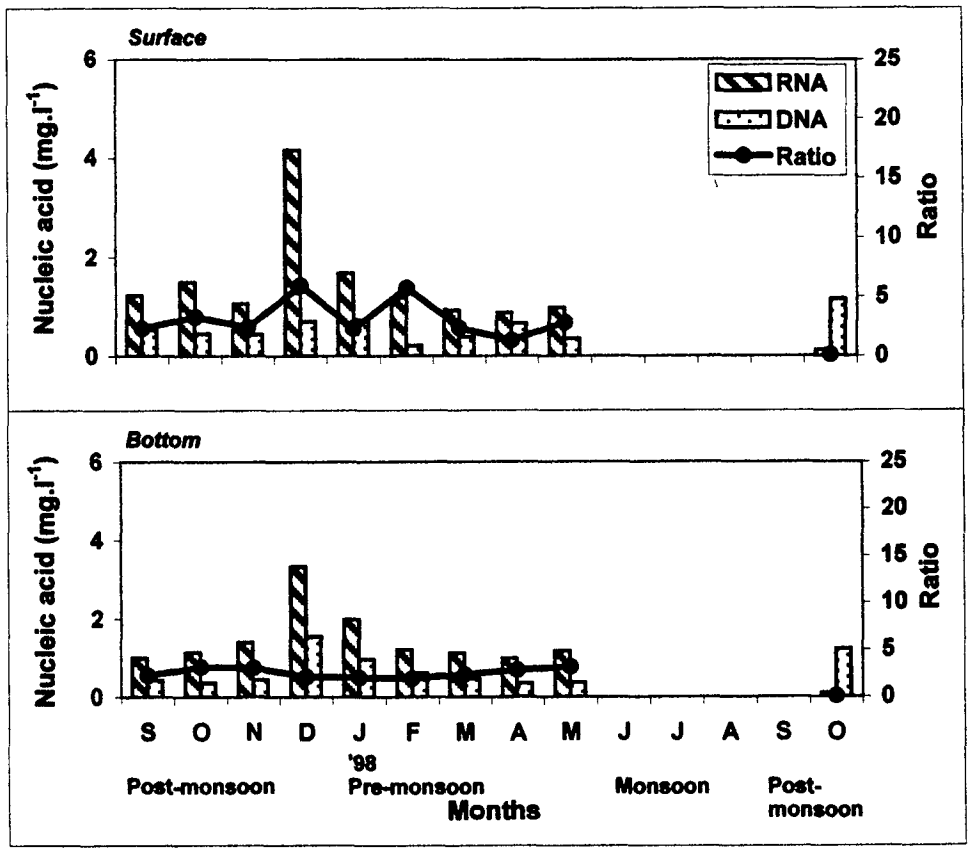


Fig. 28: Particle-associated DNA, RNA and RNA:DNA ratio in the coastal station

A negative correlation was seen between DNA and salinity ($p<0.01$) in the surface waters.

Ribonucleic Acid (RNA)

The RNA content of the particles showed minimum value of 0.12 $mg.l^{-1}$ and 0.1 $mg.l^{-1}$ (Oct. '98) at the surface while, maximum values of 4.16 $mg.l^{-1}$ and 3.33 $mg.l^{-1}$ were observed in December, in both, surface and bottom waters respectively. Seasonal averages showed the surface waters of post-monsoon to be 1.16 times higher than its bottom waters (1.39 $g.l^{-1}$) and 1.43 times the value of the surface waters during the pre-monsoon. However, in the pre-monsoon season the RNA content of particles in the bottom waters was 1.15 times that of the surface waters (Fig. 28).

RNA:DNA ratio

The RNA:DNA ratio of these particles showed minimum values in Oct. '98 in the surface (0.1) and bottom waters (0.08). However the maximum values of 5.97 (December) at the surface and 3.21 (May) at the bottom were observed. The two seasonal averages showed the ratio to be 2 to 3 in the surface and bottom waters (Fig. 28).

On comparing the three systems for their biochemical feature, it was observed that the maximum inorganic content was seen during the pre-monsoon and minimum during the monsoon at all the stations. The highest value was observed in the bottom waters of Mandovi (0.199 $g.l^{-1}$) during the pre-monsoon, while the minimum was at Zuari surface waters (0.0017 $g.l^{-1}$) during the monsoon. The coastal station during the post-monsoon showed

higher inorganic content (0.027g.l^{-1}) as compared to that of the other two stations (Fig. 29).

Annual averages showed the coastal surface waters to have high percent ash (61.17%). During the pre-monsoon season the percent ash content was maximum at all the stations and the minimum was observed during the monsoon.

All the five minerals i.e. montmorillonite, illite, kaolinite + chlorite, gibbsite, goethite, besides traces of quartz and feldspar were present at all the stations. During the post-monsoon, a high percentage (surface – 43.3%, bottom – 69.8%) of montmorillonite was seen at the coastal station, while this mineral was detected at comparatively lower percentages at Mandovi. During the same season at the Mandovi station kaolinite + chlorite dominated (surface 48.5%, bottom 40.5%). Montmorillonite was also the dominating mineral in Zuari especially during the pre-monsoon period. A higher percentage of goethite was observed at both estuarine stations at the coastal station.

The organic content was maximum during the monsoon season in the bottom waters of Mandovi (0.39 g.l^{-1}). It was minimum at all the three stations during the post-monsoon season. However, the least organic content (0.006 g.l^{-1}) was in the surface waters of coastal station during the pre-monsoon period. On an annual average the bottom waters higher organic content (more than twice) than that of the surface waters (Fig. 30).

The surface waters of Mandovi were rich in DNA during the post-monsoon having a seasonal average of 1035.77 μg.l^{-1}. The same station

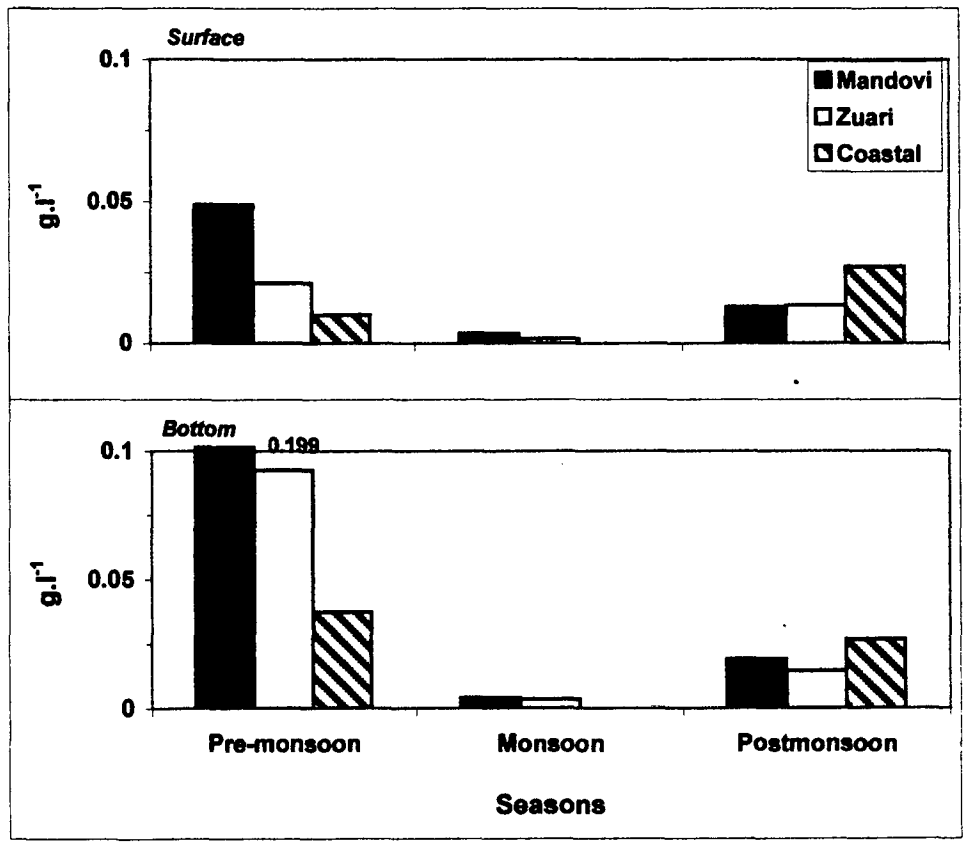


Fig. 29: Seasonal variation of inorganic content of particles at the three stations

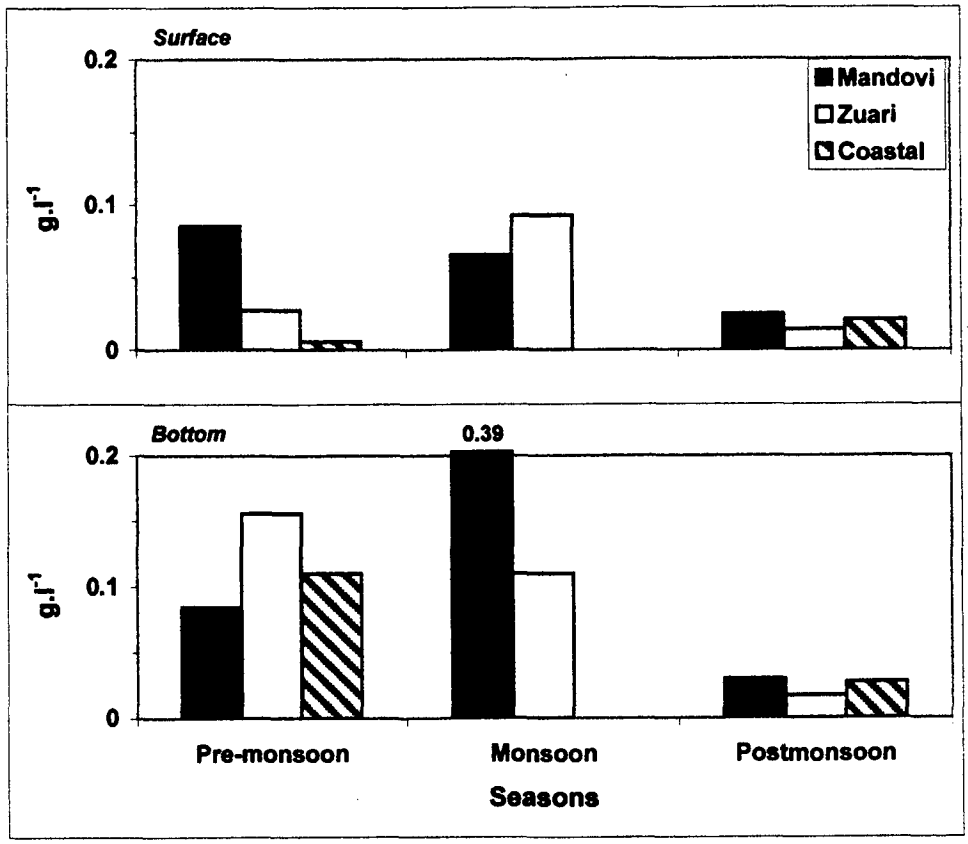


Fig. 30: Seasonal variation of organic content of particles at the three stations

showed the lowest estimated value of 380.48 $\mu g.l^{-1}$ during the pre-monsoon. In general during the post-monsoon season the waters in all stations had particles with high DNA content when compared to other seasons (Fig. 31).

RNA content of particles was exceptionally high in the bottom waters during the monsoon. Zuari station recorded a value as high as 4502.67 $\mu g.l^{-1}$. Interestingly the minimum value (700.33 $\mu g.l^{-1}$) was also recorded at the same station during the post-monsoon period. However, on an annual average the Mandovi bottom waters showed to be rich in RNA (2515.56 $\mu g.l^{-1}$).

As it was observed with RNA values the RNA:DNA ratio was maximum in the bottom waters during the monsoon with Mandovi showing a ratio of 9.46 and Zuari a ratio of 19.52. The Zuari station (bottom waters) showed a high ratio averaging 9:9 annually, while Mandovi (surface waters) showed a value of only 3.84.

4.2.4. Scanning Electron Microphotographs Of Particles

Inorder to understand the physical nature of the particles, an attempt was made to examine some of the particles collected in the Zuari estuary by scanning electron microscopy (Plate 1-5). As it can be seen the particles consisted both of biological and non biological materials. The particles were found to be colonised by bacteria at different densities and they were mostly rod shaped. In fact the size was much larger than their counterparts in the surrounding waters. On an average 10% of the particles surface area was colonised by the bacteria.

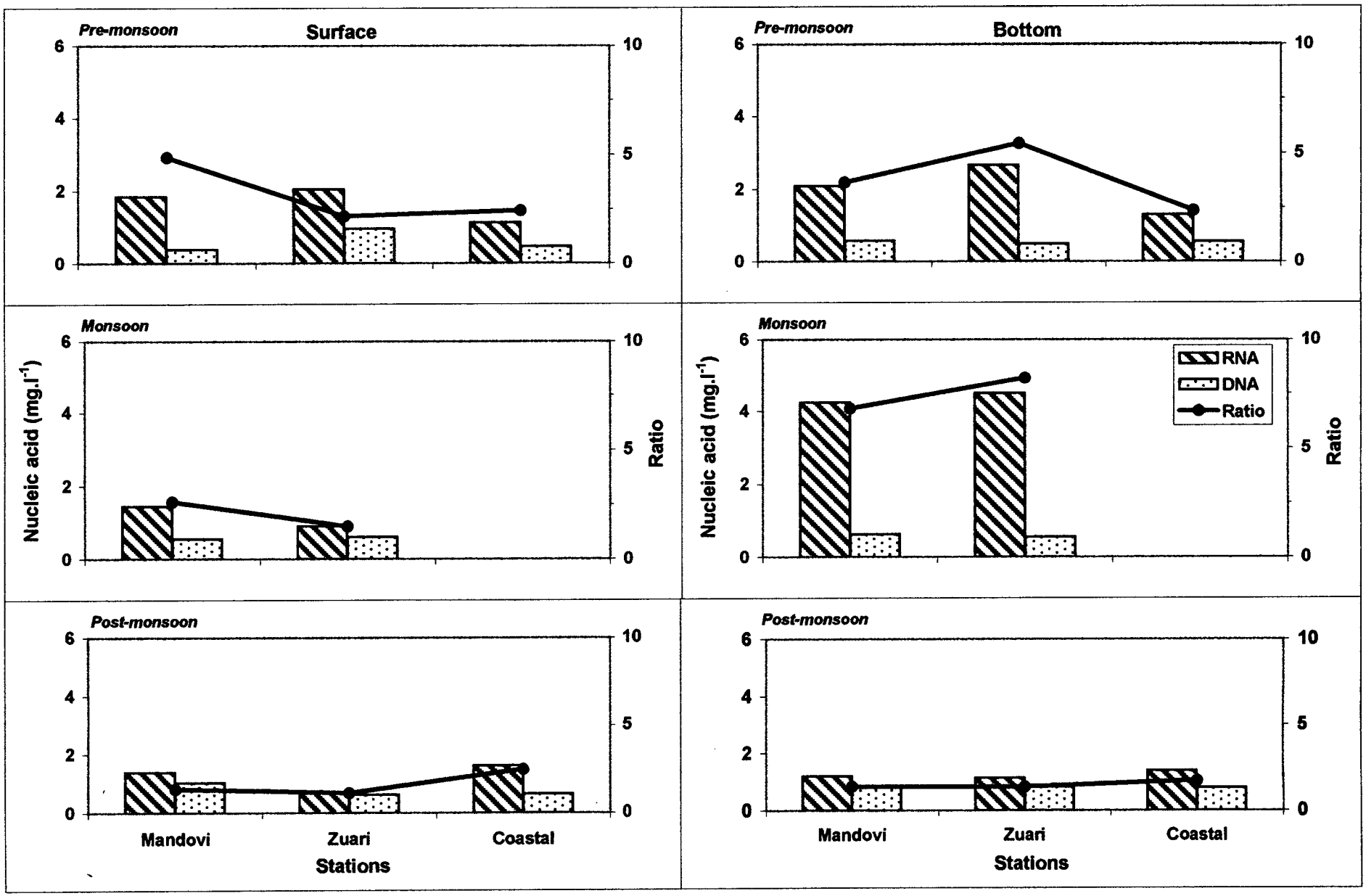
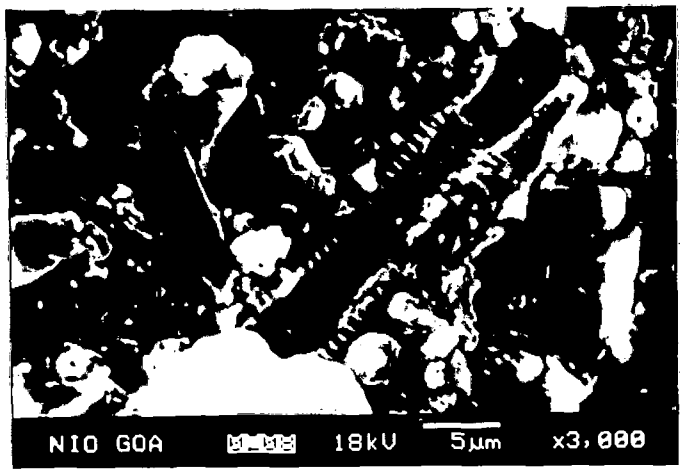


Fig. 31 Seasonal variation of RNA and DNA contents of the particles with their ratio at the three stations



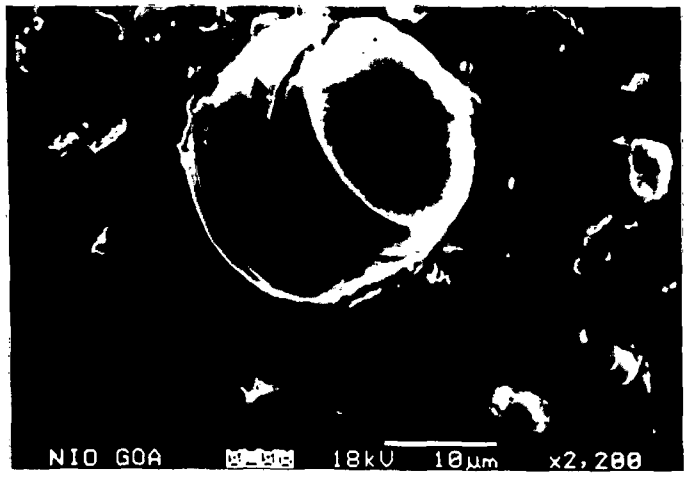
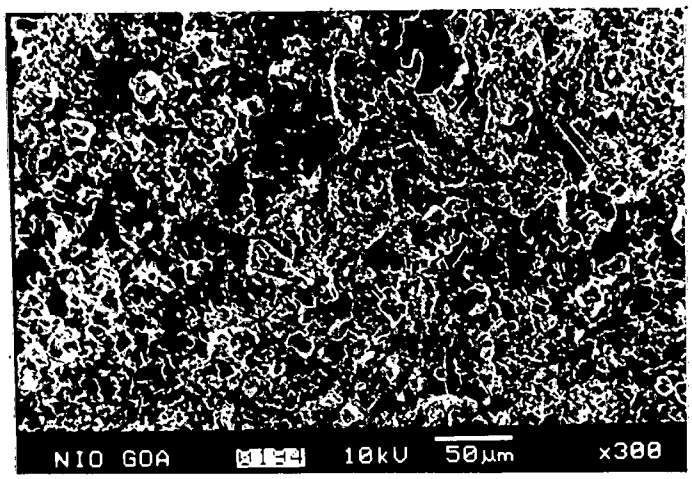
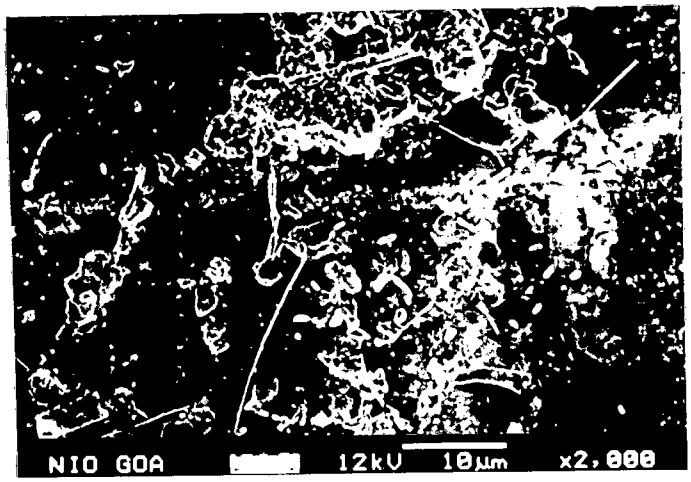


Plate - 1: Type of particles





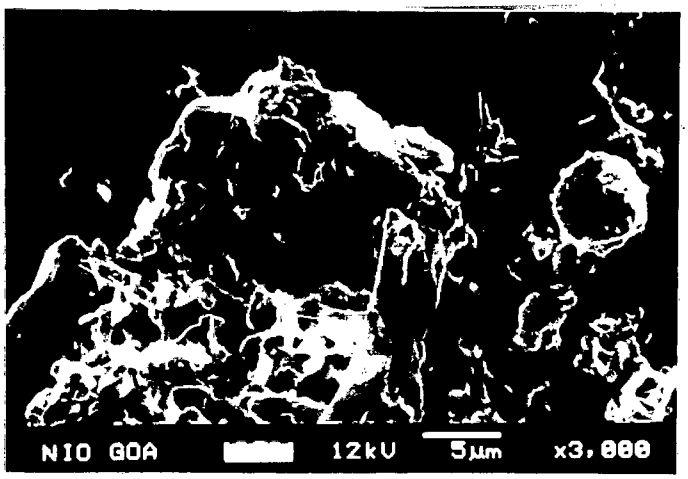
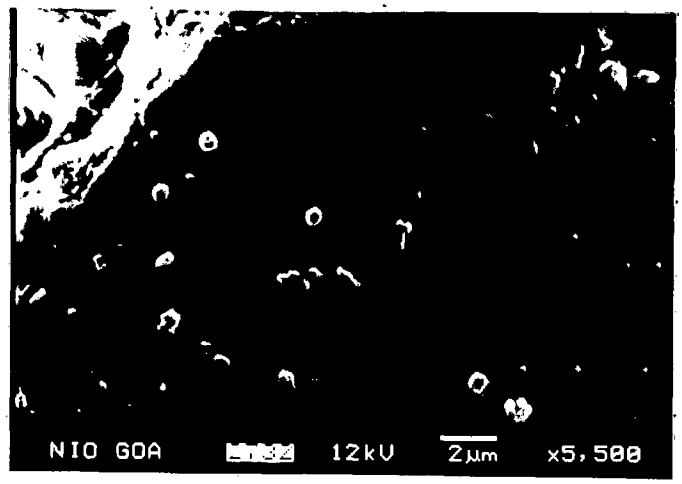
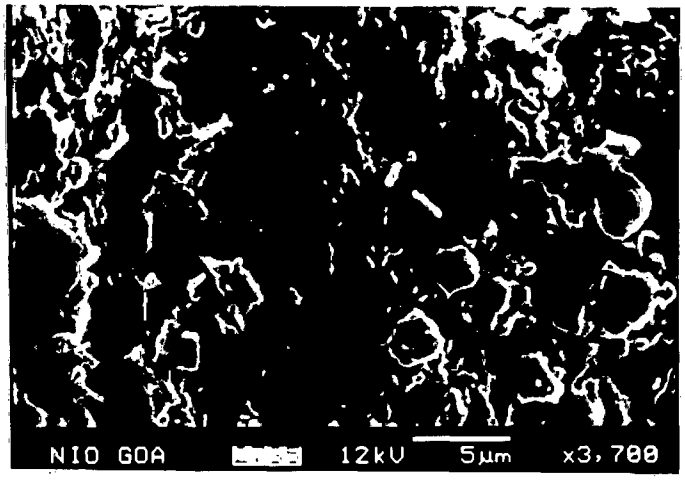


Plate - 2: Size of particles





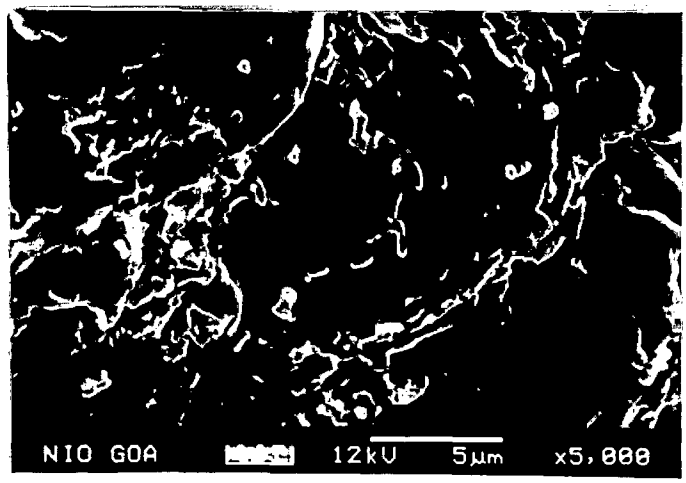
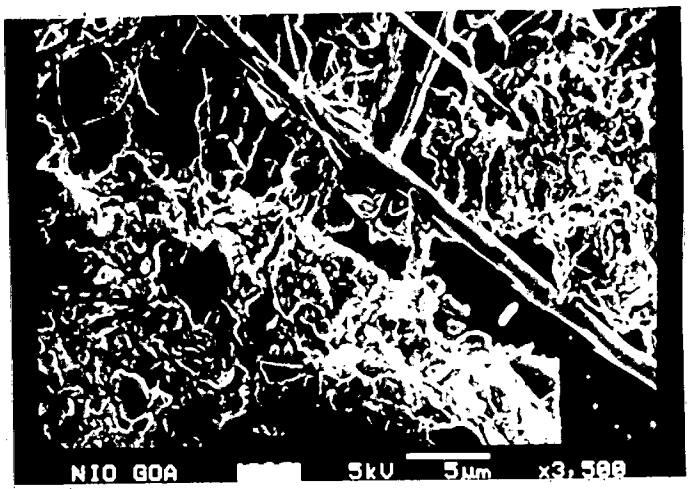
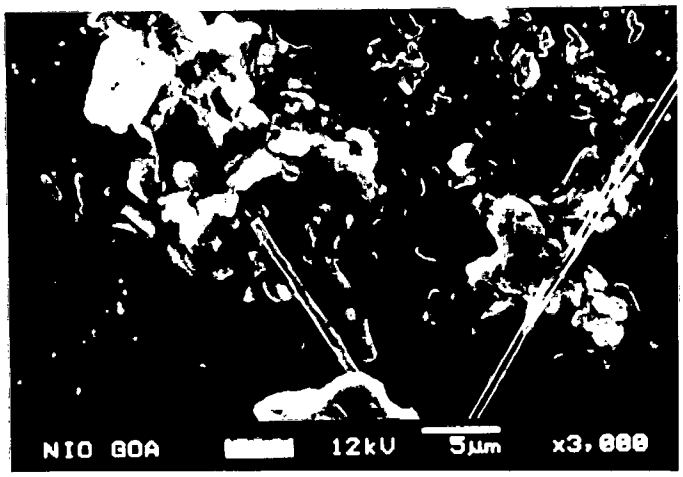


Plate-3: Thinly populated particles





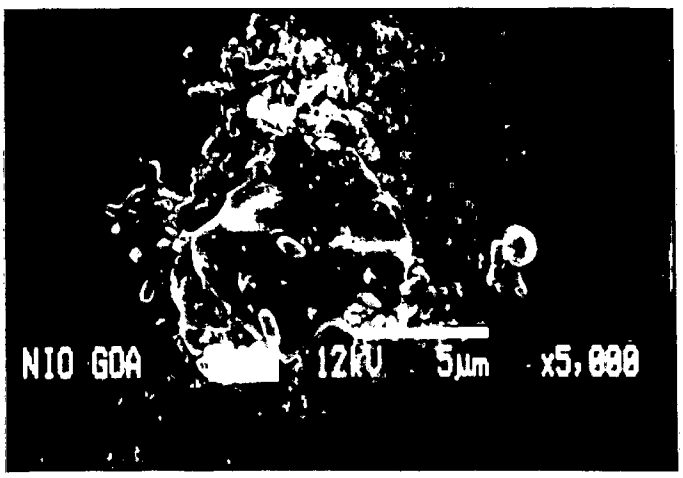
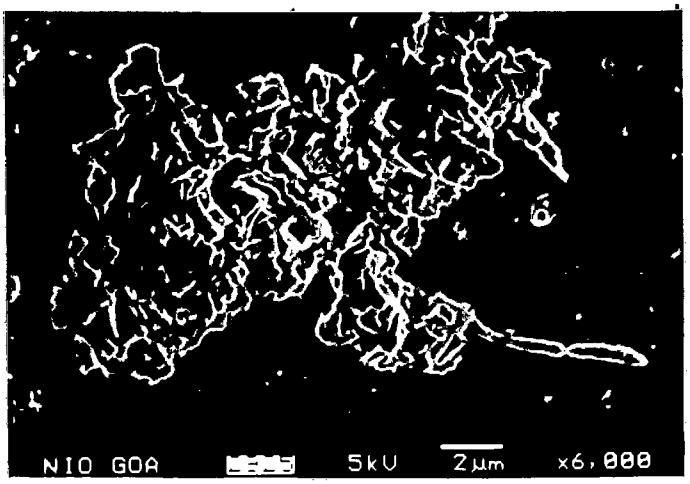
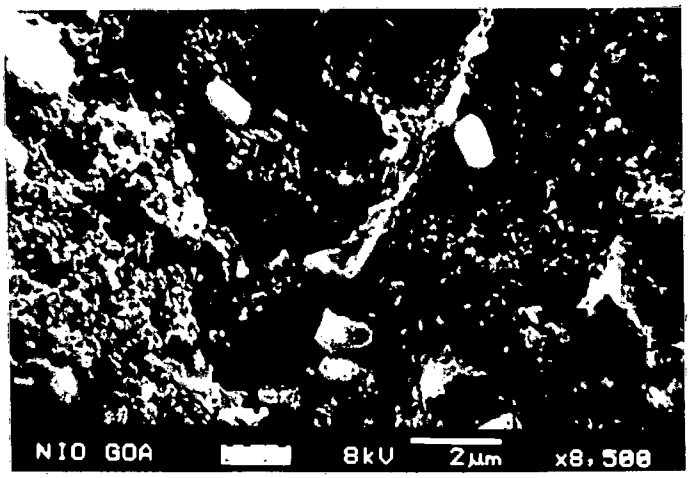


Plate-4: Thickly populated particles





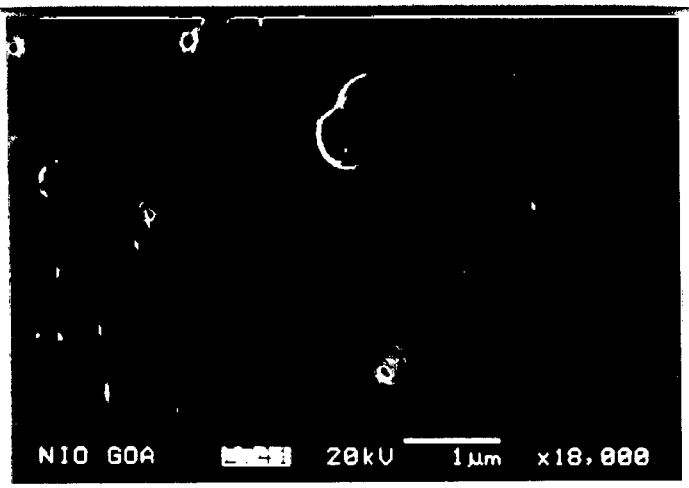


Plate-5: Different types of particle associated bacteria

4.3. MICROBIOLOGICAL PARAMETERS

4.3.1 MICROBIAL BIOMASS

Mandovi

The microbial biomass associated with particles varied from 18.86 $\mu g.C.l^{-1}$ to 1862.15 $\mu g.C.l^{-1}$ with a peak in the month of December and August. At the bottom the peaks were observed in December, June and September. The minimum value in the bottom waters was observed during the post-monsoon (4.04 $\mu g.C.l^{-1}$) while in the surface waters the maximum value of 1862.2 $\mu g.C.l^{-1}$ was seen during the same season. In the bottom waters, the PAM biomass showed a large range (41.8 to 903.8 $\mu g.C.l^{-1}$) in the pre-monsoon. No major fluctuation was noticed, during the pre-monsoon season. The bottom waters recorded higher values during the pre-monsoon (267.67 $\mu g.C.l^{-1}$) and monsoon (330.07 $\mu g.C.l^{-1}$) when compared to the surface waters values during the pre-monsoon (181.33 $\mu g.C.l^{-1}$) and monsoon (298.33 $\mu g.C.l^{-1}$). However on an average the trend of PAM biomass in the surface waters was post-monsoon>monsoon>pre-monsoon (430.19>298.08>181.33 $\mu g.C.l^{-1}$) and in the bottom waters it was monsoon > post-monsoon > pre-monsoon (330.07>319.21>267.67 $\mu g.C.l^{-1}$). The PAM biomass in the surface waters contributed >80% of the total microbial biomass whereas at the bottom it was almost 100% (except during the post-monsoon month of December). However, the average PAM biomass contribution to the total microbial biomass (471.3 $\mu g.\ C.l^{-1}$) at the surface was 62.42% and in the bottom waters (543.3$\mu g.\ C.l^{-1}$) it was 55.79% (Fig. 32). The contribution of PAB biomass to

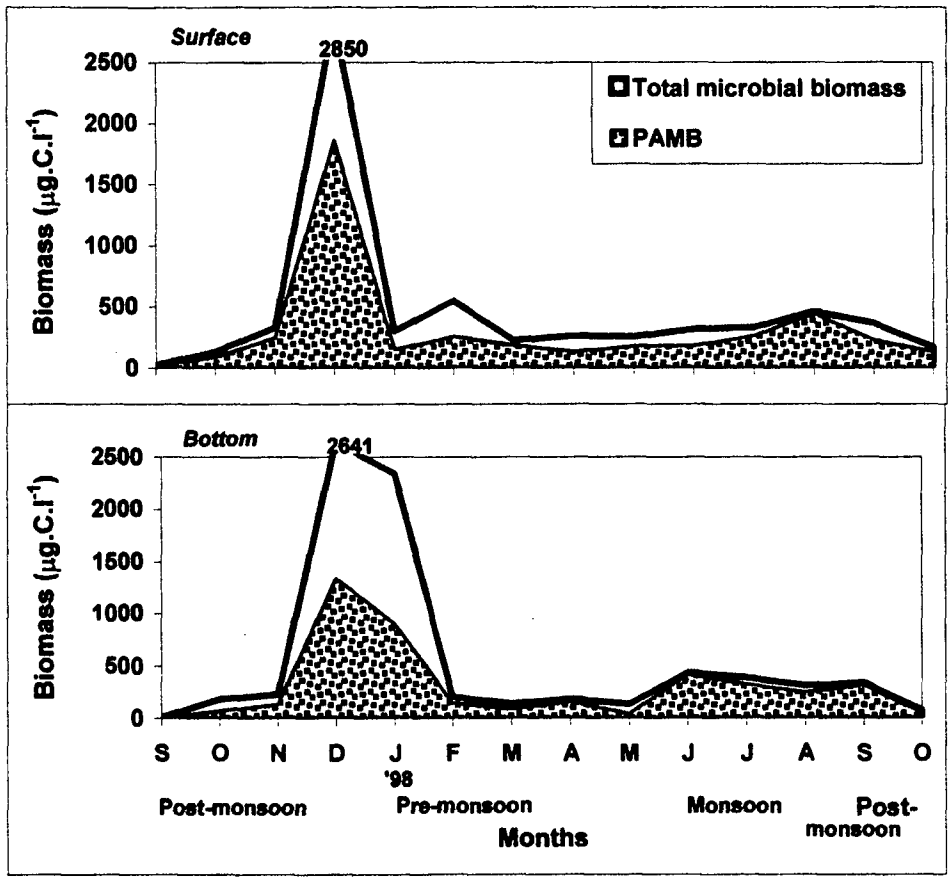


Fig. 32: Particle-associated microbial biomass (PAMB) and total microbial biomass in Mandovi estuary

the total particulate organic pool (surface and bottom waters) was 25.4% and 18.91% respectively.

Zuari

The particle associated microbial biomass fluctuated between 7.13 (Sept. '97) and 1632.95 $\mu g.C.l^{-1}$ (January) at the surface. In the bottom waters the microbial biomass ranged from 14.62 to 958.2 $\mu g.C.l^{-1}$ (Fig. 33). The surface waters had comparatively more (> 1.5 times) PAM biomass than the bottom during the pre monsoon and monsoon periods. Maximum load of PAM biomass (524.24 $\mu g.C.l^{-1}$) in the surface waters was observed during the pre-monsoon, whereas in the bottom waters it was observed during the post monsoon (335.68 $\mu g.C.l^{-1}$).

The total microbial biomass ranged from 22.52 $\mu g.C.l^{-1}$ (Sept.'97) to 2777.20 $\mu g.\ C.l^{-1}$ (January) at the surface and 24.74 $\mu g.C.l^{-1}$ (Sep. '97) to 2251.08 $\mu g.C.l^{-1}$ (December) in the bottom waters. The PAM biomass contributed a significant portion of the total microbial biomass in the surface water during the pre-monsoon (65.91%) and monsoon (80%) in the bottom waters. During the post monsoon, the contribution was 41.6% and 56.2% at surface and bottom respectively. PAM biomass contributed 14.86% in the surface and 19.90% in the bottom waters to the total POC pool. The DOC values (maximum) fitted well with the microbial biomass (maximum) average of 524 $\mu g.C.l^{-1}$ during the pre-monsoon at the surface and 336 $\mu g.C.l^{-1}$ at the bottom during the post-monsoon.

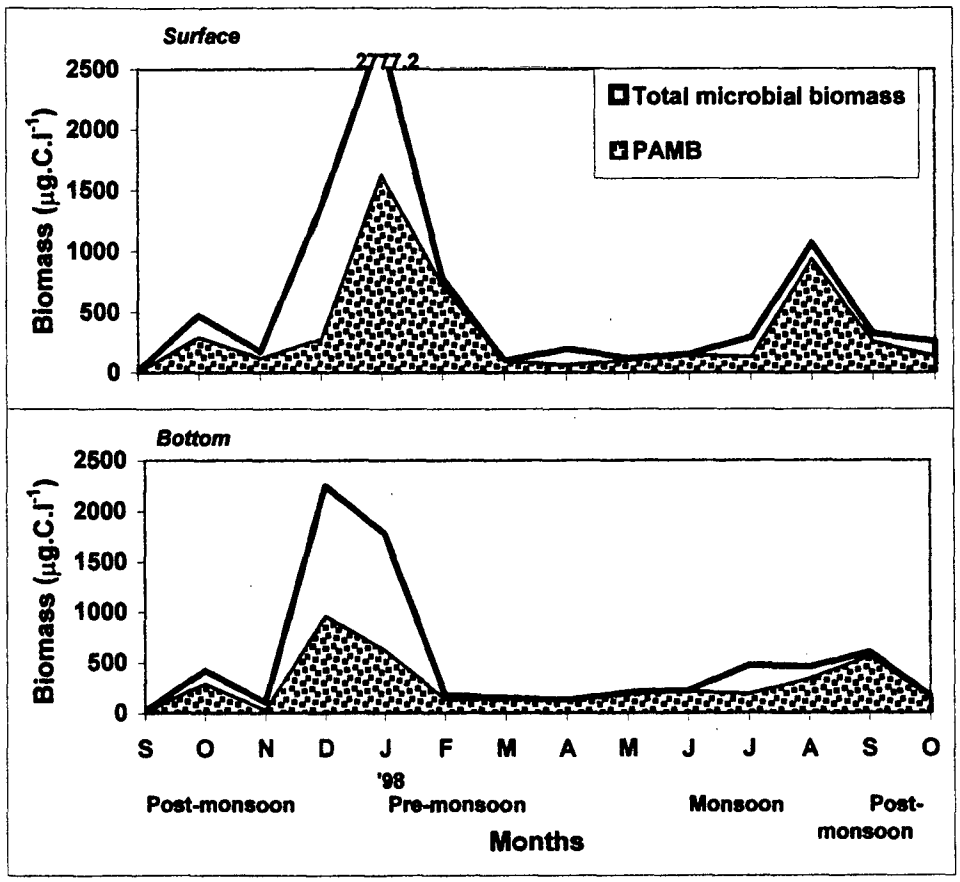


Fig. 33: Particle-associated microbial biomass (PAMB) and total microbial biomass in Zuari.

Coastal

The microbial biomass associated with particles (PAM) was 40.12 $\mu g.C.l^{-1}$ (Sept.'97) to 722.25 $\mu g.C.l^{-1}$ (January) in the surface waters and from 6.26 $\mu g.C.l^{-1}$ (Sept. '97) to 1380.15 $\mu g.C.l^{-1}$ (December) in the bottom waters. The bottom waters were 1.5 and 1.93 times the surface PAM during the pre-monsoon (246.25 $\mu g.C.l^{-1}$) and post-monsoon (197.54 $\mu g.C.l^{-1}$) respectively. The PAM biomass showed the least contribution of 42.5% and 60.2% to the total microbial biomass in December at the surface (1463.5 $\mu g.C.l^{-1}$) and bottom waters (2292.5 $\mu g.C.l^{-1}$) respectively (Fig. 34).

The PAM biomass constituted 80.69% of the total microbial biomass (305.20 $\mu g.C.l^{-1}$) during the pre-monsoon in the surface waters whereas the bottom waters showed 65.61% and 59.76% during the pre-monsoon (554.56 $\mu g.C.l^{-1}$) and post-monsoon (636.39 $\mu g.C.l^{-1}$) seasons respectively.

The PAM-biomass constituted 8.67% of the POC pool at the surface (1224.72 $\mu g.C.l^{-1}$) and 25.19% in the bottom waters (1061.0 $\mu g.C.l^{-1}$).

In the estuarine network, the particle-associated microbial biomass ranged from a low value of 181.3 $\mu g.C.l^{-1}$ at Mandovi (surface) to a high value of 524.2 in the Zuari surface waters during pre monsoon season. The post-monsoon season carried the maximum load of PAMB. During the pre-monsoon and post-monsoon the contribution of the PAM biomass to the total microbial biomass was around 50% ($\pm$ 10) at all stations with an exception at the coastal station (surface waters) where it was 80.7% in the pre-monsoon. PAM biomass contributed significantly (80%) to the total microbial biomass,

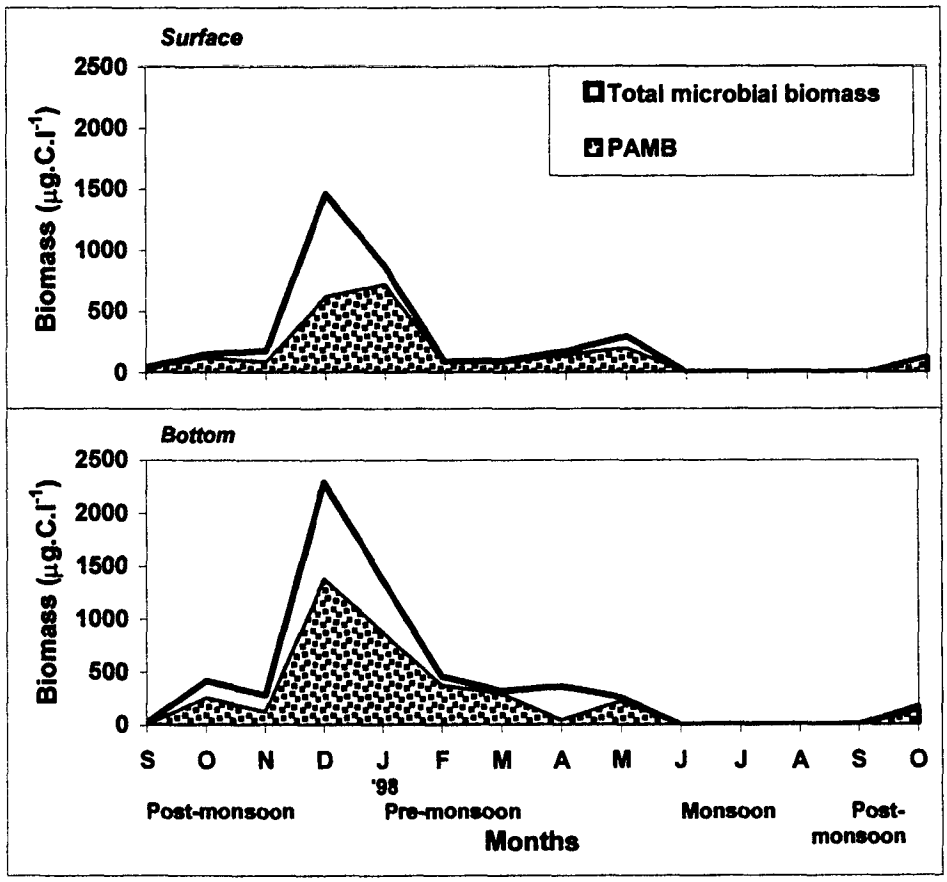


Fig. 34 : Particle-associated microbial biomass (PAMB) and total microbial biomass in the coastal station

except in the Zuari bottom waters (64.8%) during the monsoon season (Fig. 35).

4.3.2 BACTERIAL NUMBERS

4.3.2.1 Total Direct Counts (TC -PAB)

Mandovi

The annual average of TC-PAB was 1.18 x $10^{10}.l^{-1}$ (± 21.63) in the surface while in the bottom waters it was 0.98 x $10^{10}.l^{-1}$ (± 15.72). The post-monsoon season was marked by high numbers of PAB in both the surface (1.68 x $10^{10}.l^{-1}$) and bottom waters (1.19 x $10^{10}.l^{-1}$). During the pre-monsoon, the bottom waters (8.35 x $10^{9}.l^{-1}$) showed values twice that of the surface, whereas during the monsoon season the bottom values were 1.6 times lower than the surface load of 1.34 x $10^{10}.l^{-1}$.

In the surface waters of the Mandovi estuary the PAB population constituted 81% of the total bacterial population (1.66 x $10^{10}.l^{-1}$) during the monsoon season. In the bottom waters, the PAB numbers constituted 74% of the total bacterial numbers (1.13 x $10^{10}l^{-1}$) during the pre-monsoon. The PAB population constituted >50% of the total bacterial population during all seasons in both the surface and bottom waters (Fig. 36).

The colour of the bacterial cells (stained with acridine orange) while enumerating under epifluourescence microscope can be classified as dormant green cells and active orange cells. The green cells ranged from 1.0 x 10^{8} (June) to 1.45 x $10^{10}.l^{-1}$ (August) at the surface and 5.0 x 10^{6} (September) to 2.83 x $10^{10}.l^{-1}$ (December) in the bottom waters (Fig. 37). Seasonally the bottom waters showed lesser numbers of green cells than the surface waters.

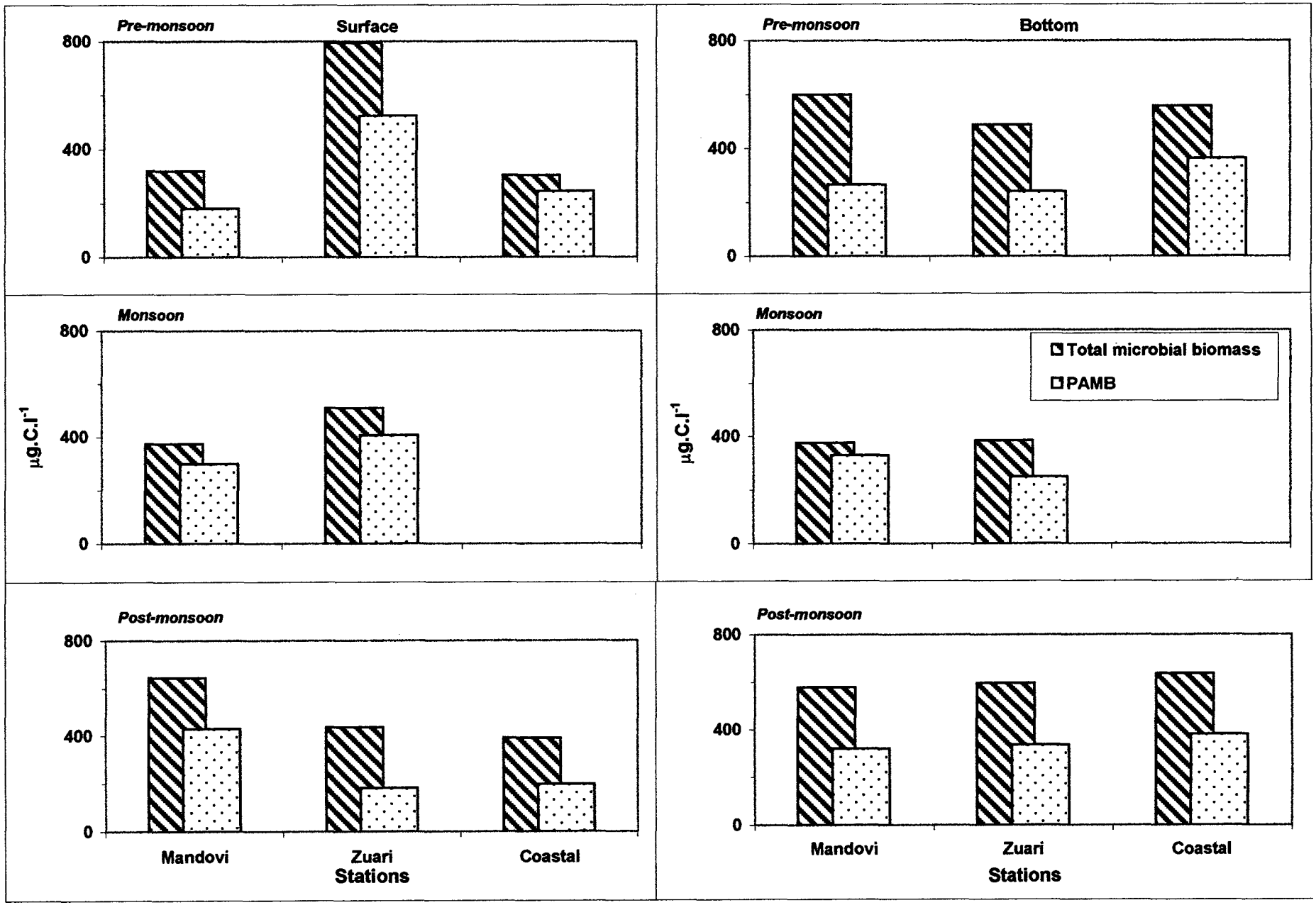


Fig. 35: Seasonal variation of total microbial and particle-associated microbial biomass (PAMB) in the estuarine network.

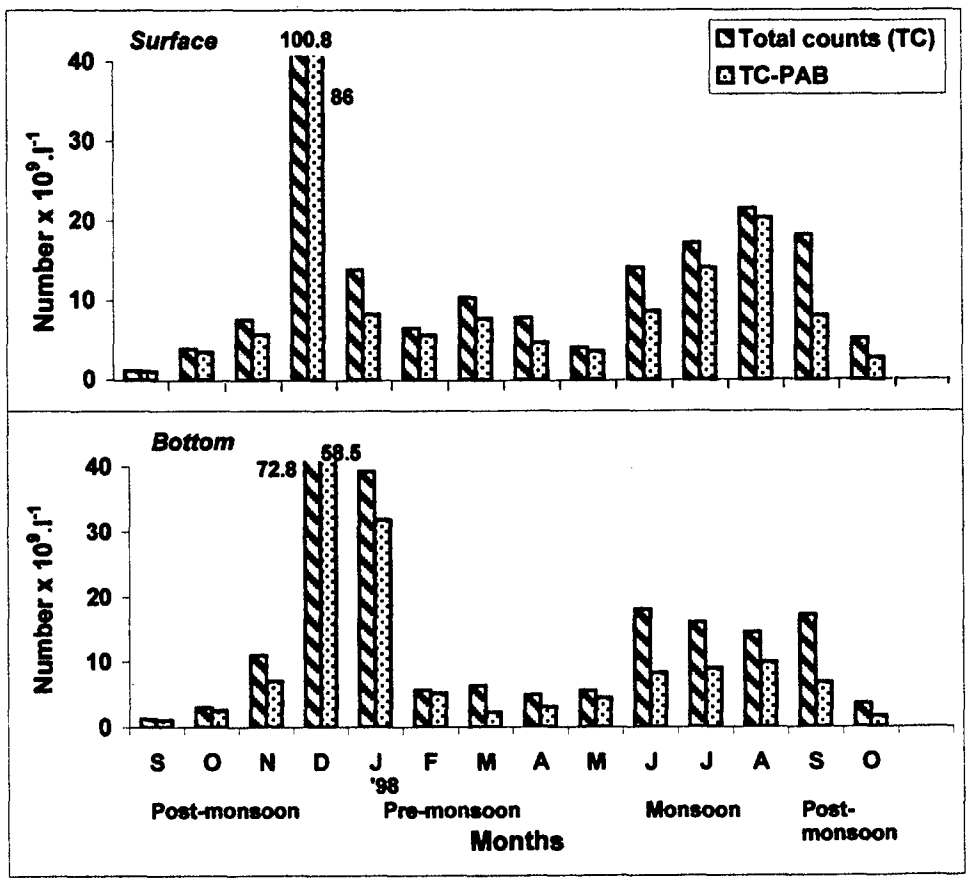


Fig. 36: Seasonal changes in the total and particle-associated bacterial population at the Mandovi station

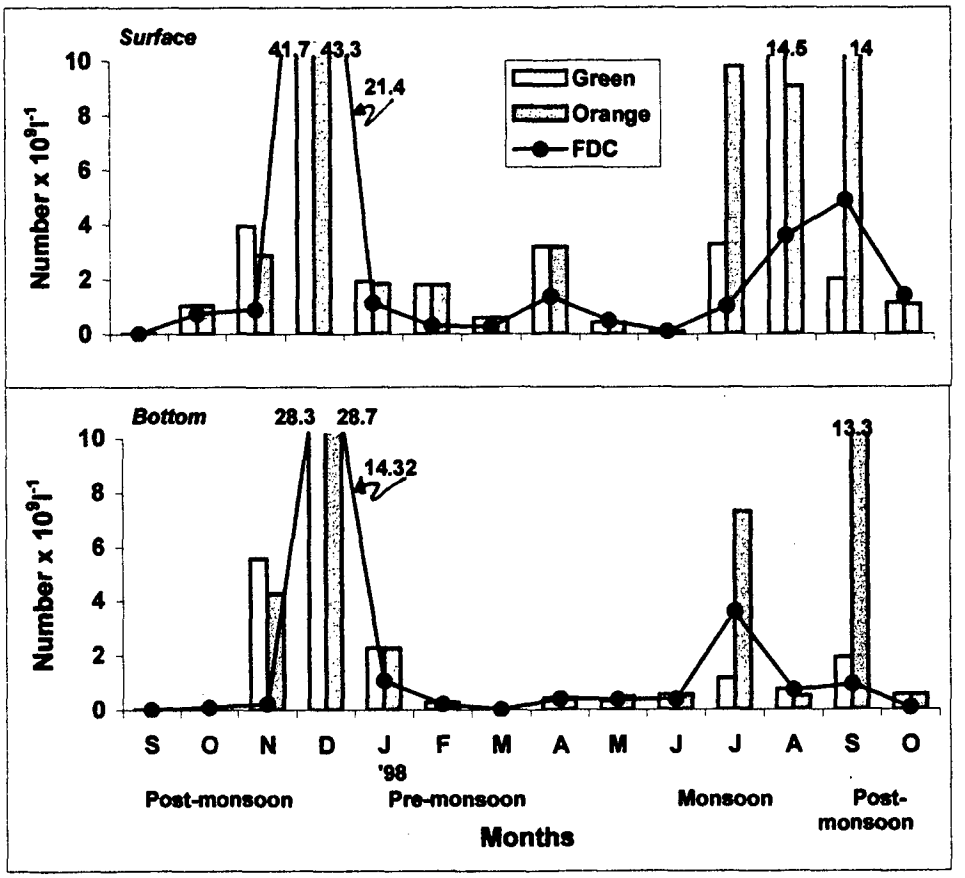


Fig. 37: Counts of green, orange and FDC aaociated with particles in the Mandovi estuary

Among the seasons, the post-monsoon showed the maximum values with the surface and bottom waters showing, 8.31 x $10^9.l^{-1}$ and 6.08 x $10^9.l^{-1}$ respectively. Low number of green cells were observed in the bottom waters during the monsoon (8.2 x $10^8.l^{-1}$). The orange cells varied from 1.0 x $10^8.l^{-1}$ (June) to 4.33 x $10^{10}.l^{-1}$ (December) in the surface and 5.0 x $10^6.l^{-1}$ (Sept. '97) to 2.87 x $10^{10}.l^{-1}$ (December) in the bottom waters (Fig. 37). Surface waters carried higher load than the bottom waters during all the seasons. The post-monsoon season carried a high number of 1.04 x $10^{10}.l^{-1}$ in the surface and 7.82 x $10^9.l^{-1}$ in the bottom waters. The orange cells exerted it dominance over green cells in all the seasons.

In the surface, FDC showed a low value of 1.3 x $10^7.l^{-1}$ and this value September'97 and reached a high of 2.14 x $10^{10}.l^{-1}$ in December. In the bottom waters, the lowest value of 3 x $10^6.l^{-1}$ was also observed in September'97 which later peaked to 1.43 x $10^{10}.l^{-1}$ in December. The seasonal trend was post-monsoon>monsoon>pre-monsoon in the surface and bottom waters. In the post-monsoon the FDC of surface waters was 1.87 times higher than that of bottom waters (2.61 x $10^9.l^{-1}$). No difference in FDC values was seen in the monsoon. As illustrated in Fig.37 the surface waters in general contained more number of FDC than the bottom waters.

FDC showed a significant relationship with POC ($p<0.05$) in the surface waters. A significant positive relation ($p<0.001$) was also observed between the FDC and TC-PAB.

BIOMASS

The PAB-biomass, not only constituted a major portion of the total bacterial biomass, but also of the microbial biomass. On an annual average in the surface waters, Mandovi carried a higher PAB biomass of 236.86 $\mu g.C.l^{-1}$ when compared to the bottom values of 196.29 $\mu g.C.l^{-1}$.In the bottom waters the PAB biomass was 1.7 times higher than that of the surface value (99 $\mu g.C.l^{-1}$) during the pre-monsoon. However, in the other two seasons the surface waters recorded higher values than the bottom waters (161.06 $\mu g.C.l^{-1}$, 238.3 $\mu g.C.l^{-1}$). A minimum value of 0.2 $\mu g.C.l^{-1}$ was observed during the post-monsoon (Sept.'97) in the bottom waters. However a peak was observed in the surface waters (1699.61 $\mu g.C.l^{-1}$) in the same season. A large range seen in the PAB biomass during the post-monsoon in the surface (0.74 -1699.6 $\mu g.C.l^{-1}$) and bottom waters (0.2 - 1150.4 $\mu g.C.l^{-1}$) was due to the high values observed in the month of December. Less fluctuations in the bottom waters was observed during the monsoons (146 -177.4 $\mu g.C.l^{-1}$). However, the surface waters (monsoon) showed a variation from 152 to 387.6 $\mu g.C.l^{-1}$. The pre-monsoon showed more stable conditions in the bottom waters (24.8 to 82.8 $\mu g.C.l^{-1}$ exception Jan - 616.6 $\mu g.C.l^{-1}$) than the surface waters (51.4 to 144.4 $\mu g.C.l^{-1}$) (Fig.38).

The PAB biomass dominated the total bacterial biomass in both the surface and bottom waters throughout the study period.

Considering the whole water column at Mandovi, PAB showed a positive correlation ($p<0.001$) with the PAM biomass.

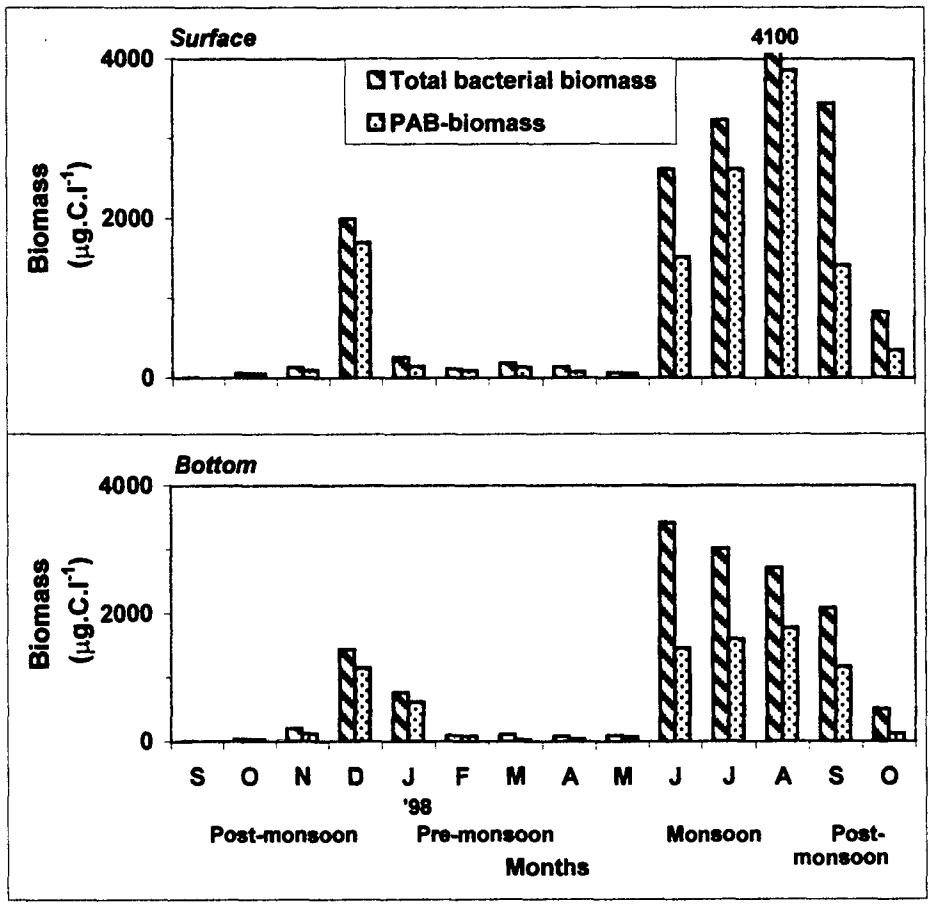


Fig. 38: Seasonal changes in the total and particle-associated bacterial biomass in the Mandovi estuary

Zuari

The variation of PAB load was from 8.8 x $10^7.l^{-1}$ to 9.62 x $10^9.l^{-1}$ in the surface, and, from 1.1 x $10^8.l^{-1}$ to 3.91 x $10^{10}.l^{-1}$ in the bottom; i.e., the variation was by over three orders - both the minimum (Sept. '97) and maximum (December) being noted during the post-monsoon. The bottom waters carried a higher load of PAB than the surface during all seasons. The monsoon season recorded similar values as that of the post-monsoon season which showed maximum PAB numbers in the surface (3.5 x $10^9.l^{-1}$) and bottom waters (9.7 x $10^9.l^{-1}$). However, the pre-monsoon season observations yielded minimum values of 2.3 x $10^9.l^{-1}$ and 5.44 x $10^9.l^{-1}$ in the surface and bottom waters respectively (Fig. 39).

The TC-PAB, which forms a major part of the total bacterial numbers ranged from 4.4 x $10^8.l^{-1}$ to 5.41 x $10^{10}.l^{-1}$ in the surface and 2.2 x $10^8.l^{-1}$ to 9.54 x $10^{10}.l^{-1}$ in the bottom waters. In the monsoon the PAB contributed around 55% to the total bacterial numbers. PAB formed a significant portion (64%) of the total bacterial load in the bottom waters during the pre-monsoon whereas in the post-monsoon the PAB in the surface waters accounted for only 26.6%.

Annually the green cells ranged from 1.0 x $10^7.l^{-1}$ to 2.59 x $10^9.l^{-1}$ in the surface and 1.1 x $10^8.l^{-1}$ to 8.04 x $10^9.l^{-1}$ in the bottom waters. Maximum number of green cells (1.26 x $10^9.l^{-1}$ and 6.7 x $10^9.l^{-1}$) were recorded in the surface and bottom waters of the post-monsoon season (Fig. 40). In the bottom waters the green cells dominated except, during the monsoons. The

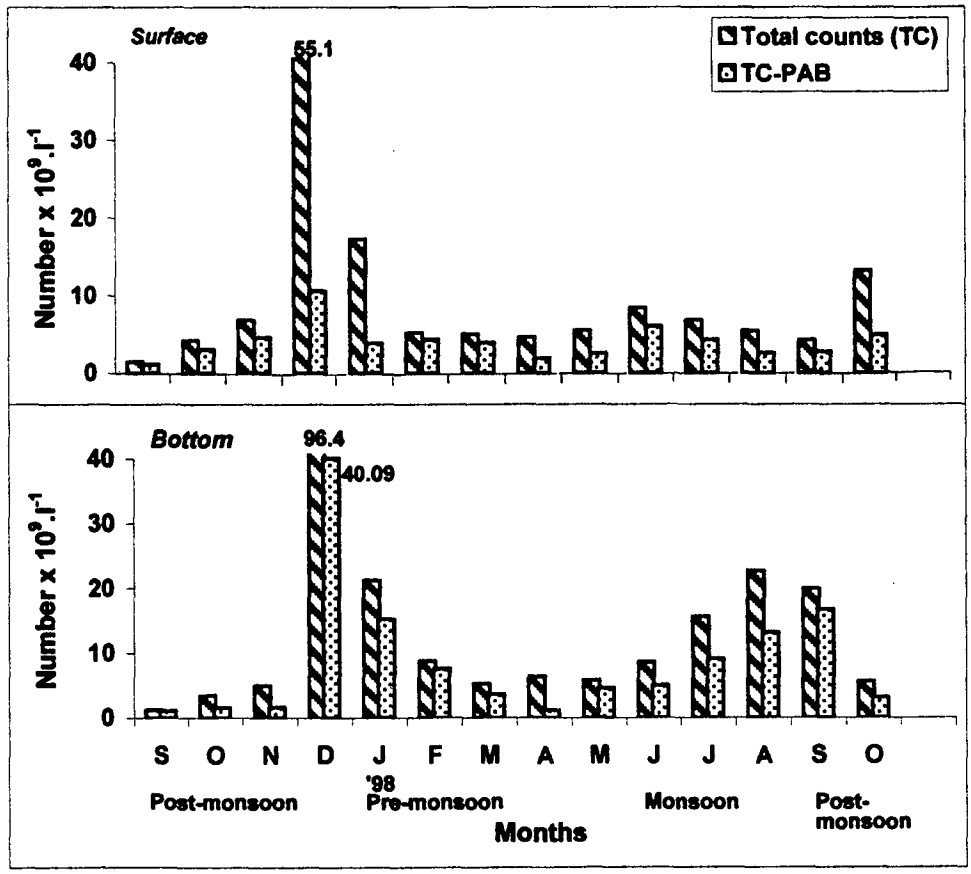


Fig. 39: Seasonal changes in the total and particle-associated bacterial population at the Zuari station

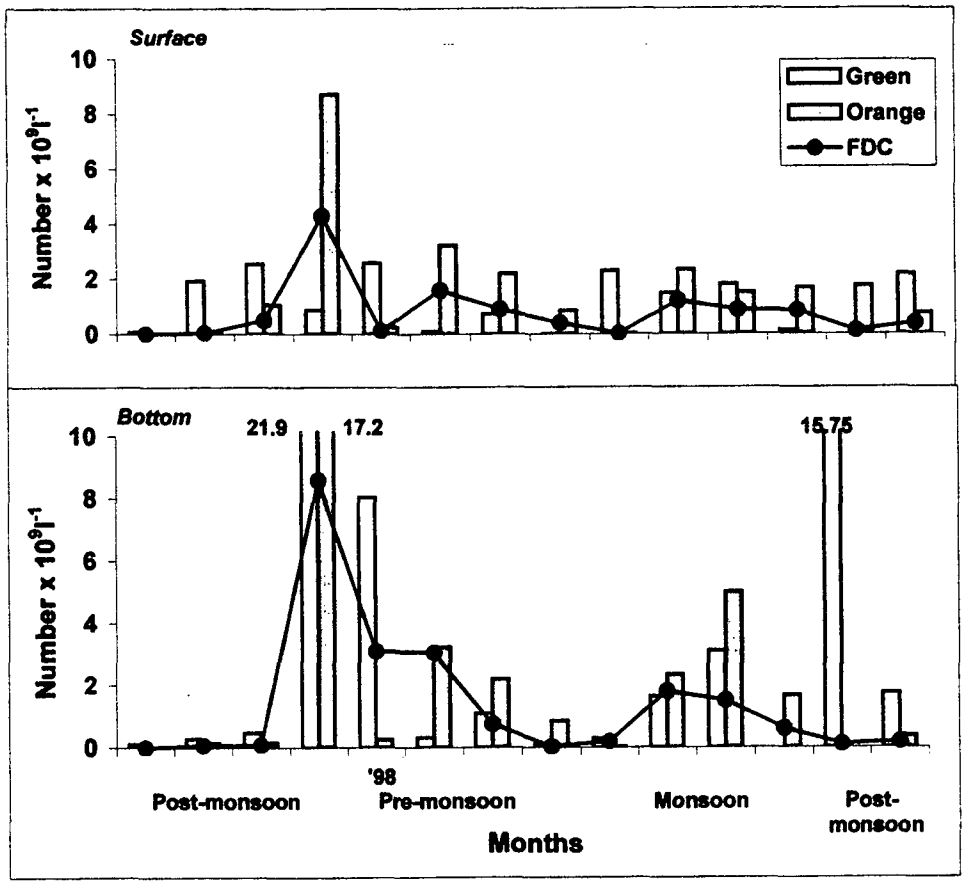


Fig. 40: Counts of green, orange and FDC aaociated with particles in the Zuari estuary

orange cells varied from 2.0 x $10^7.l^{-1}$ to 3.23 x $10^9.l^{-1}$ in the surface and 2.0 x $10^7.l^{-1}$ to 3.23 x $10^9.l^{-1}$ in the bottom waters. In the monsoons the orange cells were 1.9 times more than the green cells.

In the Zuari estuary FDC ranged from 9 x 10^6 to 4.29 x $10^9.l^{-1}$ in surface and 4.0 x 10^6 to 8.58 x $10^9.l^{-1}$ in bottom waters. Both the minimum and maximum values were observed in September'97 and December respectively. From annual averages it was calculated that the bottom waters had 1.77 times higher FDC load than the surface waters. The bottom waters had the highest FDC count of 1.5 x $10^9.l^{-1}$ during the post-monsoon. The monsoon season had a value of 9.77 x $10^8.l^{-1}$ which was the maximum seasonal average for the surface waters (Fig 40).

While the FDC showed a highly significant relationship to TC-PAB ($p<0.001$) in the surface waters it was with ATP ($p<0.05$) in the bottom waters.

BIOMASS

The bacterial biomass on the particles varied from 1.76 to 192 $\mu g.C.l^{-1}$ in the surface and 2.16 to 781.8 $\mu g.C.l^{-1}$ in the bottom waters (Fig. 41). The bacterial biomass associated with particles was always higher in the bottom waters with highest in the post-monsoon season.

Coastal

The total PAB-population ranged from 10^7 to $10^{10}.l^{-1}$ at the coastal station. The annual surface and bottom values (average) were 5.3 ($\pm$ 8.0) x $10^9.l^{-1}$, and 13.36 ($\pm$ 21.7) x $10^9.l^{-1}$ respectively. The post-monsoon season recorded higher numbers of TC-PAB when compared to the pre-monsoon (1.4 times). During the pre-monsoon, the TC-PAB (5.1 x $10^9.l^{-1}$) accounted for 50%

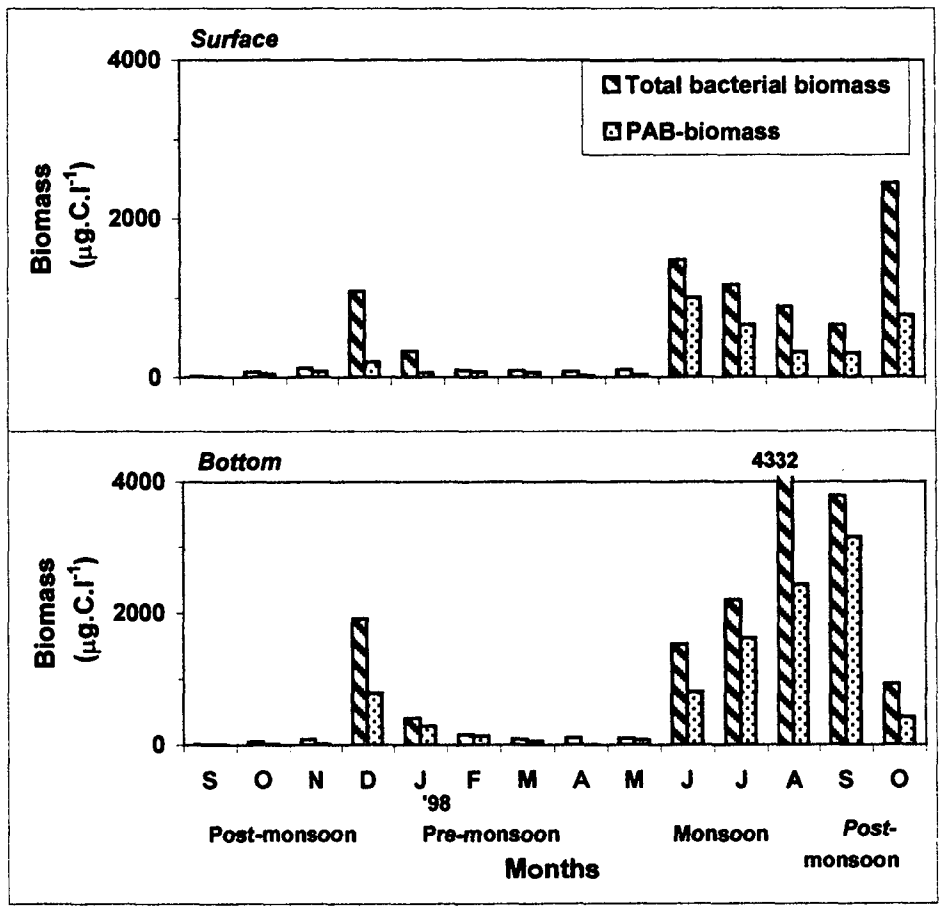


Fig. 41: Seasonal changes in the total and particle-associated bacterial biomass in the Zuari estuary

of the total bacteria ($10.19 \times 10^9.l^{-1}$) in the surface waters and 71% of the total bacteria ($15.43 \times 10^9.l^{-1}$) in the bottom waters. Similar trend was observed in the post-monsoon season also (Fig. 42).

The total bacterial-PAB correlated significantly with the microbial biomass ($p<0.001$) in the surface and bottom waters. TC-PAB also showed a good relation with RNA ($p<0.05$) in the surface waters.

The green cells varied between $1.66 \times 10^8 l^{-1}$ (Sept. '97) to $9.96 \times 10^9 l^{-1}$ (December) in the surface and from $2.0 \times 10^7 l^{-1}$ (May) to $3.9 \times 10^{10} l^{-1}$ (December) in the bottom waters. During the pre-monsoon the bottom waters ($4.95 \times 10^9 l^{-1}$) carried 2.63 times the load of the surface water. The number of orange cells ranged from nil (March) to $1.77 \times 10^{10} l^{-1}$ (January) at the surface and from 2.5×10^7 (Sept. '97) to $2.78 \times 10^{10} l^{-1}$ (December) in the bottom waters. The bottom waters ($6.6 \times 10^9 l^{-1}$) recorded 2.25 times that of the surface during the post-monsoon and 1.45 times that of the surface ($3.43 \times 10^9 l^{-1}$) during the pre-monsoon (Fig. 43).

Green cells correlated well with TC-PAB ($p<0.01$) in the surface and bottom waters. The orange cells showed good correlation with the POC ($p<0.01$) and TC-PAB ($p<0.01$) in the bottom waters.

While the FDC associated with particles varied from $3.0 \times 10^7 l^{-1}$ to $8.84 \times 10^9 l^{-1}$ (January) at the surface, those in the bottom waters showed a variation of three orders of magnitude $1.3 \times 10^7 l^{-1}$ (Sept.'97) to $1.36 \times 10^{10} l^{-1}$ (December). In the pre-monsoon season the surface (average) value was $1.97x\ 10^9 l^{-1}$, that was 1.28 times of the bottom waters (Fig. 43). Statistically

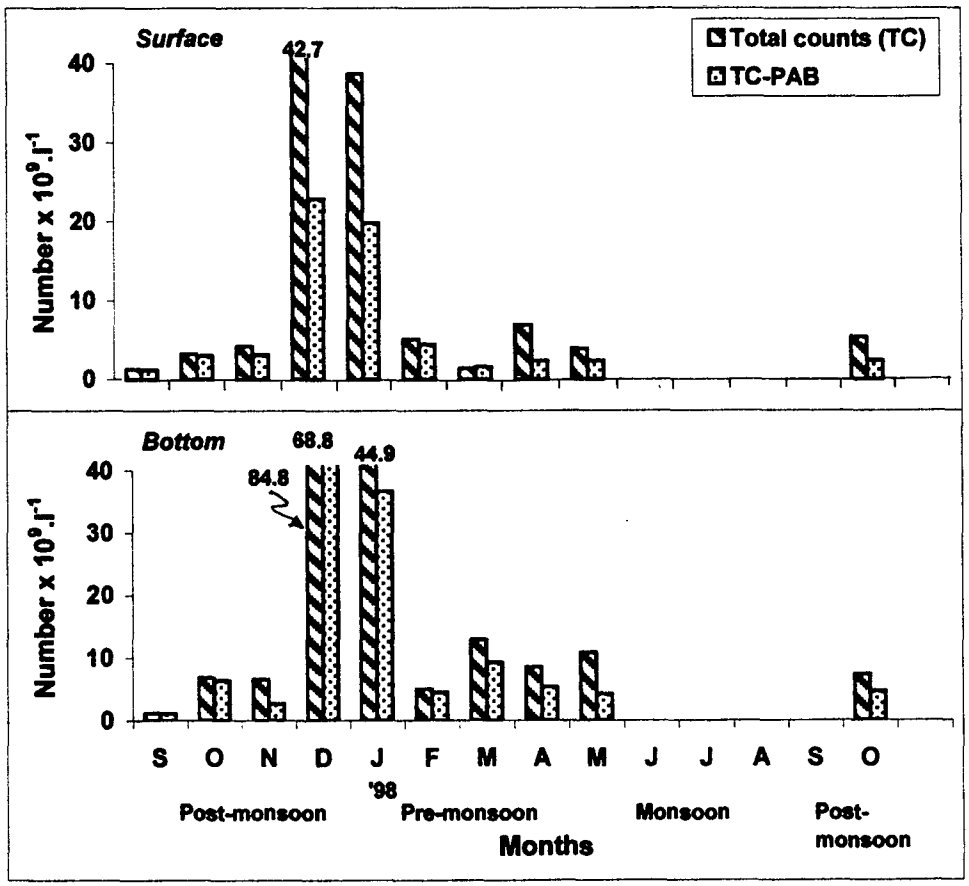


Fig. 42: Seasonal changes in the total and particle-associated bacterial population at the coastal station

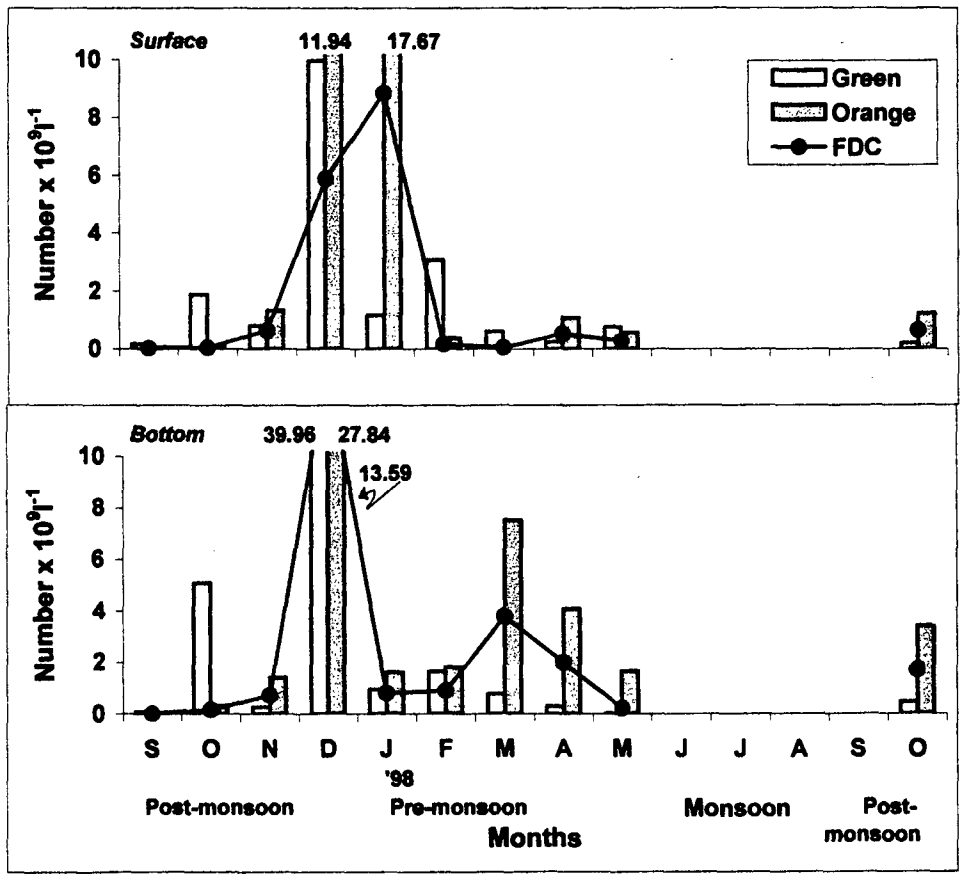


Fig. 43: Counts of green, orange and FDC aaociated with particles in the coastal station.

significant relationship was found between FDC and POC ($p<0.01$) in the bottom waters.

BIOMASS

The annual average of PAB biomass was 106.2 $\mu g.C.l^{-1}$ in the surface and 267.31 $\mu g.C.l^{-1}$ in the bottom waters (Fig. 44). The post-monsoon season showed higher values than the pre-monsoon season. The PAB-biomass contributed as much as 71.29% of the total biomass (308.62 $\mu g.C.l^{-1}$) in the bottom waters during the pre-monsoon. However this contribution was low (29.71%) during the post-monsoon in the surface waters.

PAB biomass value in the bottom waters ($3.2 \times 10^9 l^{-1}$) was 2.2 times higher than its surface value during the post-monsoon. PAB showed good relation with the FDC ($p<0.001$) in the surface waters. Among the total counts the orange cells related significantly with PAB ($p<0.001$) in the surface waters.

The overall view of the estuarine system in relationship to the particle associated bacterial abundance and biomass brings out that the Zuari generally harbored a low PAB load with an annual average of $3.03 \times 10^9.l^{-1}$ at the surface and $7.84 \times 10^9.l^{-1}$ at the bottom. However, the surface waters of Mandovi ($1.18 \times 10^{10}.l^{-1}$) and bottom waters of the coastal station ($1.34 \times 10^{10}.l^{-1}$) harbored maximum PAB population. Seasonal averages show that surface waters of Mandovi had high values of $1.68 \times 10^{10}.l^{-1}$ during the post-monsoon while minimum values of $2.3 \times 10^9.l^{-1}$ at the Zuari station was observed during the pre-monsoon period. The post-monsoon season harboured a high particle-associated bacterial load at all the stations.

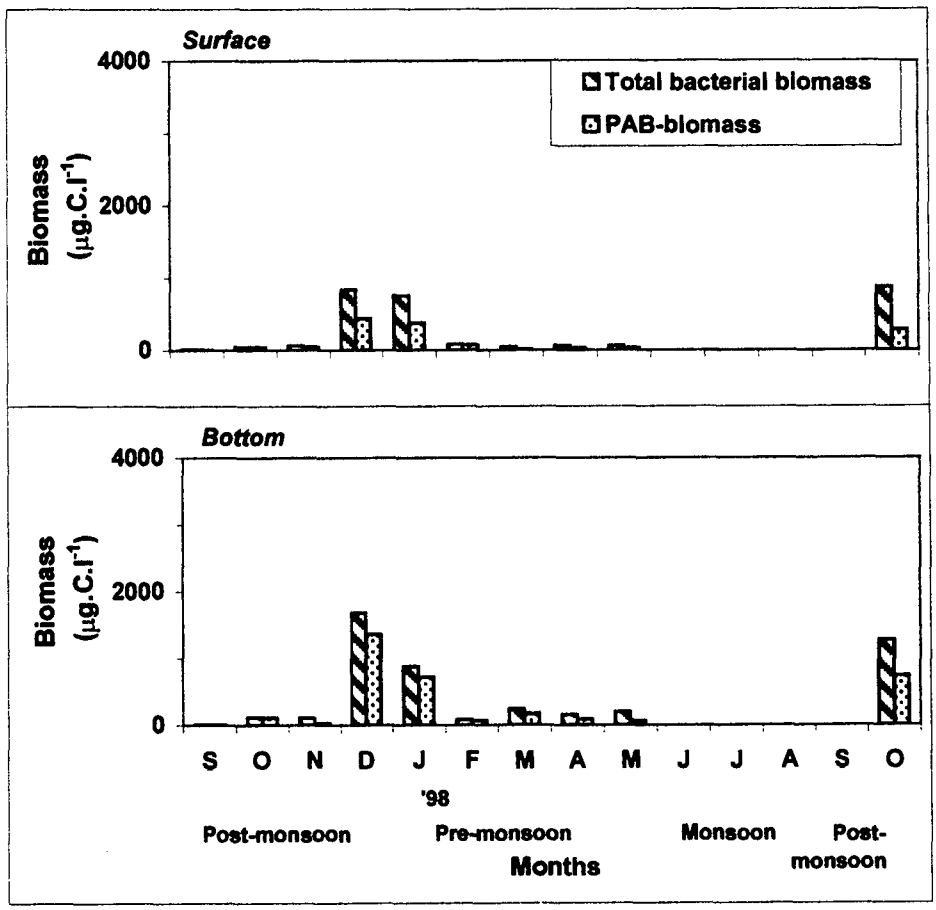


Fig. 44: Seasonal changes in the total and particle-associated bacterial biomass in the coastal station

PAB formed a significant portion of the total bacterial population in the surface waters of Mandovi (76%) and in the bottom waters at the coastal station (74.7%). However, the contribution of PAB to the total was the lowest (32%) at the Zuari station (Fig.45). In the surface waters the contribution by PAB to PAM was maximum during the monsoon season. In the bottom waters it was observed during the pre-monsoon except for the coastal station where it was seen during the post-monsoon.

At Zuari and the coastal station the bottom waters carried a higher number of green cells when compared to the surface waters throughout while at Mandovi the surface waters carried higher load especially during the monsoon where the surface value was 7.3 times that of the bottom (0.82 x $10^9.l^{-1}$). Season-wise, the post-monsoon had the highest load of green cells at all stations. Station-wise the Mandovi showed the highest load of green cells at all seasons (Fig. 45).

The FDC of PAB were maximum during the post-monsoon season at all stations. The coastal station showed the maximum number of FDC-PAB at all seasons except in the surface waters during the post-monsoon season where the maximum value of 4.89 x $10^9.l^{-1}$ was recorded at Mandovi.

The PAB biomass, was the highest in the coastal bottom waters (267.3 $\mu g.C.l^{-1}$) and the lowest in the Zuari surface waters (60.7 $\mu g.C.l^{-1}$). The PAB biomass was maximum during the post-monsoon and minimum during the pre-monsoon period at all stations. A major contribution of PAB biomass to the total bacterial biomass was seen in the Mandovi surface waters during the

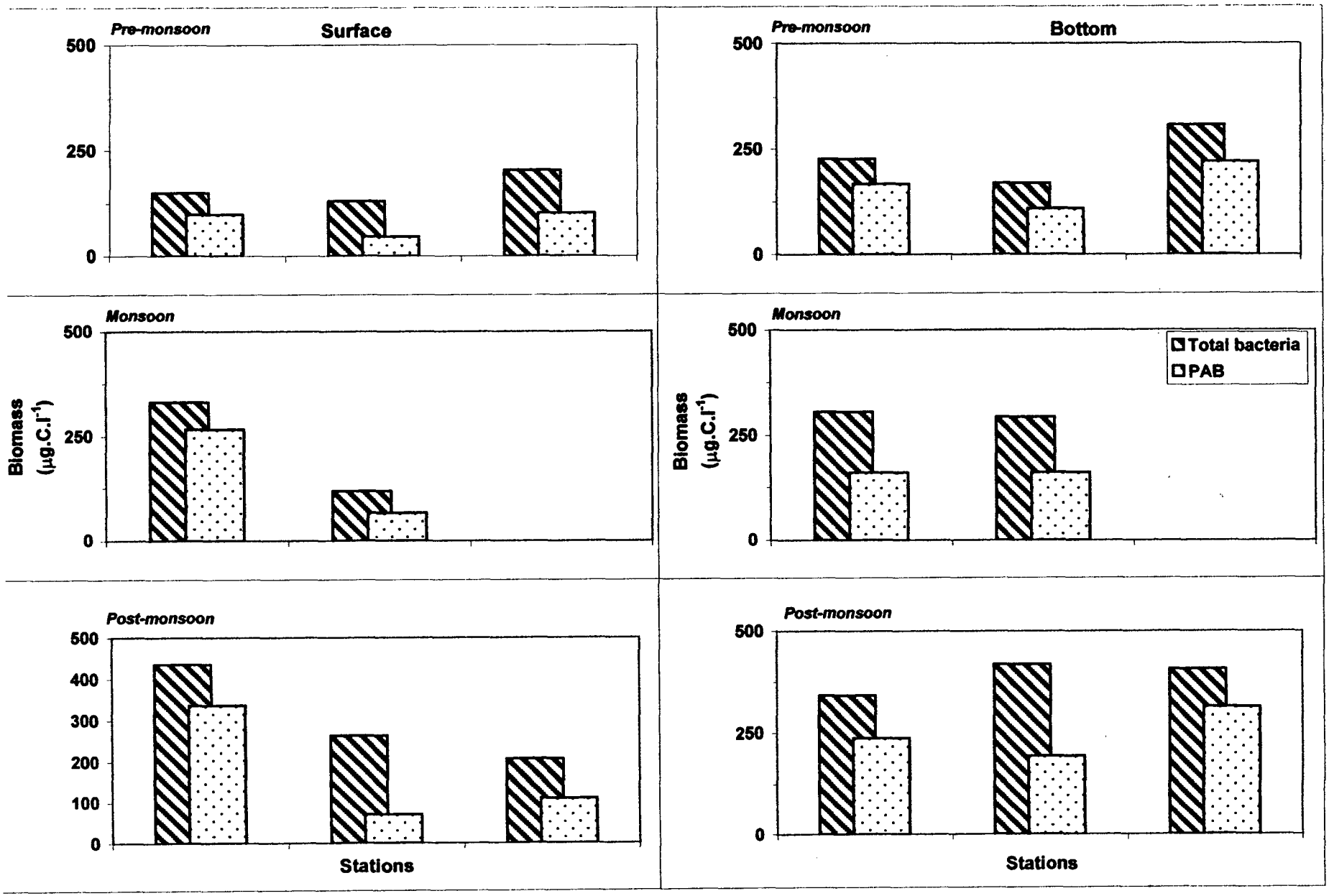


Fig. 45: Seasonal variation of total and particle-associated bacterial biomass in the estuarine network.

monsoon season (80.6%). However, at Zuari station, a low value of 17% was recorded during the post-monsoon in the surface waters.

4.3.2.2. Viable counts (VC-PAB)

Mandovi

The viable PAB ranged from 1.0 x $10^7.l^{-1}$ to 2.21 x $10^{10}.l^{-1}$ with an annual average of 2.52 x $10^9.l^{-1}$ in the surface waters and 4.0 x $10^7.l^{-1}$ to 8.62 x $10^9.l^{-1}$ with an average of 2.27 x $10^9.l^{-1}$ in the bottom waters. It was noticed that the bottom waters underwent less variation (two orders) as compared to that of the surface waters (three orders). Among the three seasons, the highest VC-PAB in the surface waters was observed during the post-monsoon period (4.17 x $10^9.l^{-1}$) as compared to the pre-monsoon (1.93 x $10^9.l^{-1}$) and monsoon (1.8 x $10^8.l^{-1}$) seasons. This post-monsoon value was also 1.9 times higher than its bottom value. Seasonally the surface and bottom waters collected during the monsoon season showed the occurrence of cells an order lower ($10^8.l^{-1}$). In the bottom waters the highest number of viable cells of PAB (3.21 x $10^9.l^{-1}$) was observed during the pre-monsoon season. The particle-associated viable bacteria in the post-monsoon showed lot of fluctuations even to the extent of three orders in the surface (0.01 to 22.05 x $10^9.l^{-1}$) and bottom waters (0.04 to 8.62 x $10^9.l^{-1}$). However during the pre-monsoon season the variation was only by an order in the surface (0.27 to 4.35 x $10^9.l^{-1}$) and bottom (0.63 to 6.38 x $10^9.l^{-1}$) waters.

In the surface waters, the VC-PAB constituted 65.23% of the total VC-PAB population, while in the bottom waters it constituted 69.55%. A significant contribution to the extent of 75.75% by VC-PAB to the total VC

($2.55 \times 10^9.l^{-1}$) was seen in the surface waters during pre-monsoon, while a low value of 39% was observed ($4.6 \times 10^8.l^{-1}$) during the monsoon. The bottom waters exhibited a different trend showing major fractions of VC-PAB to the TVC ($9.4 \times 10^8.l^{-1}$) during the monsoon period (89.4%) and lowest during the post-monsoon (61.3%) (Fig 46).

The maximum contribution of VC-PAB to the TC-PAB was observed during the pre-monsoon in the surface (39%) and bottom (38%) waters; whereas the minimum contribution was observed during the monsoons in the surface (1.35%) and bottom (10.45%) waters.

The VC density correlated well with the microbial biomass, TC and FDC in the surface and bottom waters ($p>0.001$). Among the hydrological parameters, salinity appeared to control the viable population both in the surface and bottom.

Zuari

The viable PAB varied between $8 \times 10^7.l^{-1}$ in May and June to $8.62 \times 10^9.l^{-1}$ in December at the surface and from $9.9 \times 10^7 l^{-1}$ in September'97 to $1.39 \times 10^{10}.l^{-1}$ in January in the bottom waters (Fig. 47). The bottom waters showed a higher viable count than the surface waters as seen from the total PAB. Maximum viable cell were observed during the pre-monsoon in the bottom waters ($4.23 \times 10^9.l^{-1}$). Least number of viable cells was noted in the surface ($1.99 \times 10^8.l^{-1}$) and bottom waters ($3.25 \times 10^8.l^{-1}$) during the monsoon season.

The VC-PAB made a significant contribution of (81.7%) to the total viable population ($5.18 \times 10^9.l^{-1}$) in the bottom waters during the pre-monsoon

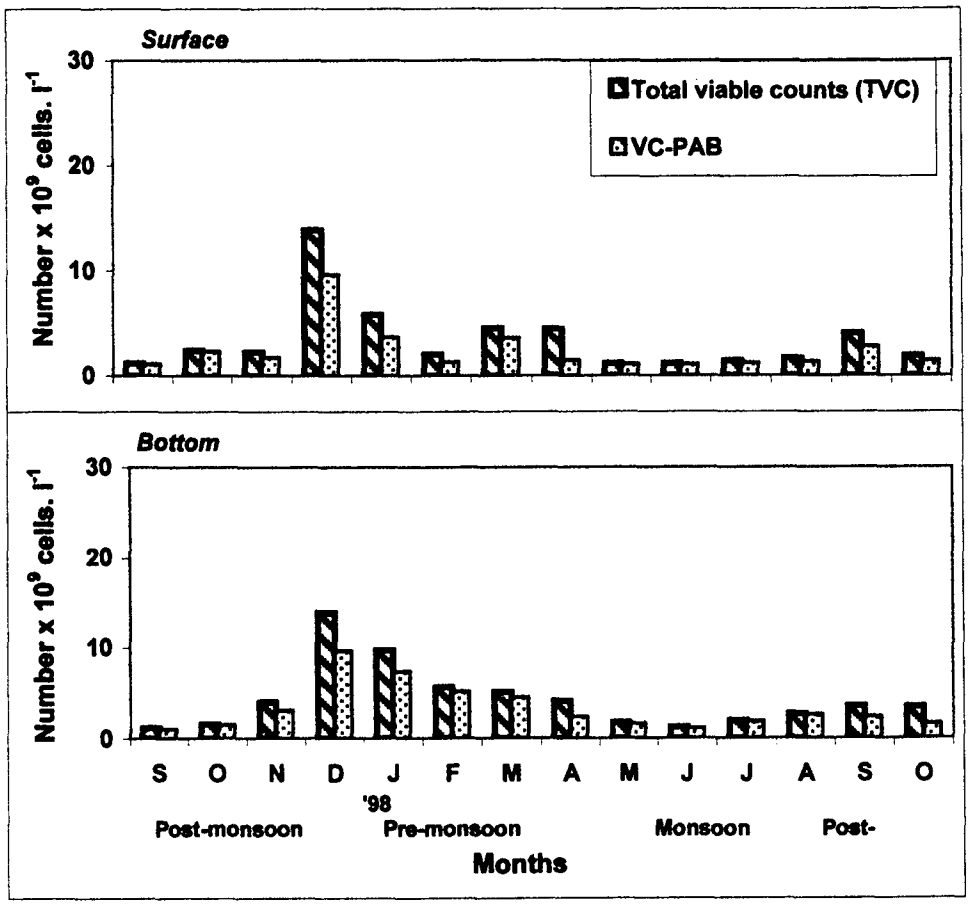


Fig. 46: Total and particle-associated viable bacterial population in the Mandovi estuary

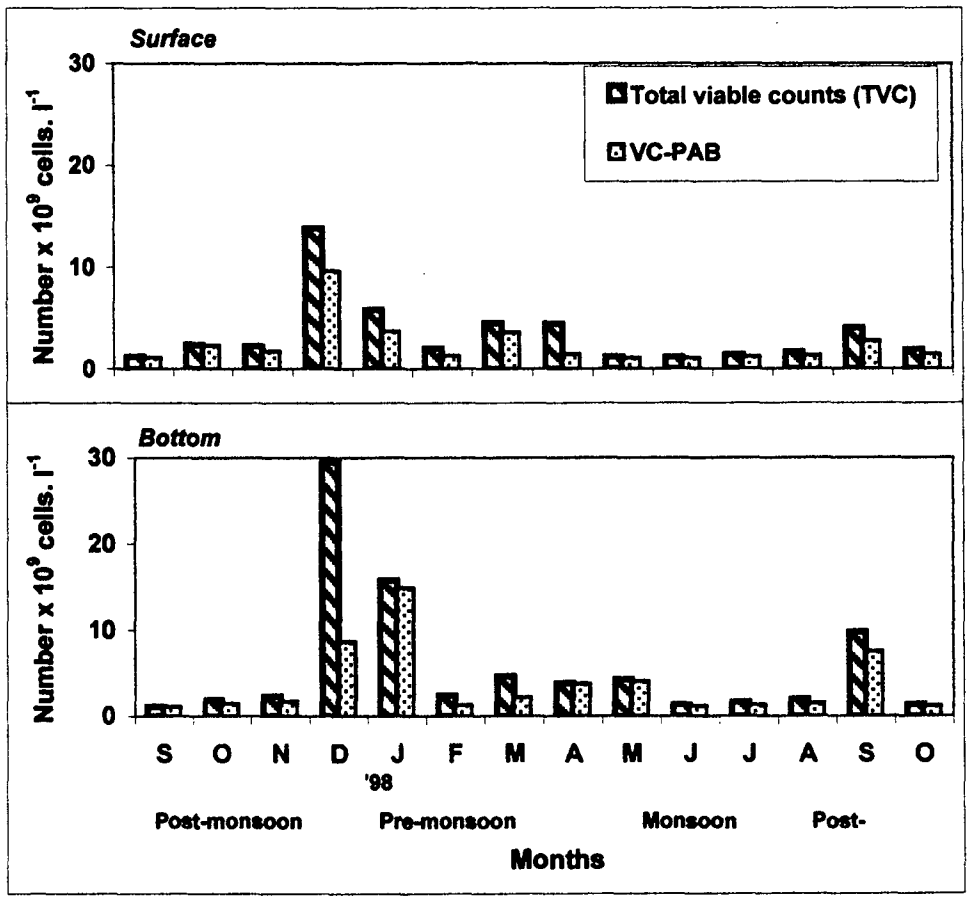


Fig. 47: Total and particle-associated viable bacterial population in the Zuari estuary

season. During the monsoon the level of VC-PAB contribution to the total viable bacterial population got reduced to 50% in the surface ($4.0 \times 10^8.l^{-1}$) and bottom waters ($6.5 \times 10^8.l^{-1}$). This level further got reduced to 39.2% during the post-monsoon period in the bottom waters.

The VC-PAB constituted a significant portion (77.8%) of the TC-PAB ($5.44 \times 10^9.l^{-1}$) in the bottom waters during the pre-monsoon season. In the surface waters, the VC-PAB formed 52.8% of the total PAB population during the pre-monsoon ($2.3 \times 10^9.l^{-1}$) and 61.56% during the post-monsoon ($3.5 \times 10^9.l^{-1}$) seasons. However, during the monsoon season the VC-PAB formed a trivial portion of only 6 and 4% of the TC-PAB at the surface ($3.3 \times 10^9.l^{-1}$) and bottom ($8.08 \times 10^9.l^{-1}$) respectively. The VC-PAB however showed significant relationship with particles ranging in size from >25 to < 220μm ($p<0.001$).

The maximum viable PAB population especially in the bottom waters ($1.15 \times 10^{10}.l^{-1}$) was encountered at the coastal station. A low number of viable cells ($1.4 \times 10^9.l^{-1}$) was recorded in Zuari surface waters. In general, viability of the total and PAB was low in both the estuaries during the monsoon season ($10^8.l^{-1}$). Coastal bottom waters recorded the highest number of VC-PAB ($1.43 \times 10^{10}.l^{-1}$) during the post-monsoon which formed a sizeable fraction ($\geq$ 80%) of the viable count. Even in the surface waters their numbers were high (82.97%). The contribution of VC-PAB to VC was generally high (75.32 to 87.15%) at all stations during the pre-monsoon. During the monsoon season, Zuari (both surface and bottom waters) showed almost the same level of contribution (50%). In the case of Mandovi, while the

surface waters showed a low percentage of 39.3 the bottom waters exhibited a high percentage of 89.5%.

Both the estuaries recorded minimal contribution of VC-PAB ($\leq$ 10%) to the TC-PAB during the monsoon. The VC-PAB at the coastal station made a significant contribution (90%) to the TC-PAB in the surface and bottom waters during the post-monsoon season. During the pre-monsoon, the percentage contribution in the surface and bottom waters was almost the same (38%), while in the same season Zuari showed more than 50% and at the coastal station surface and bottom waters it was accounted for 15% and 78.4% respectively.

Coastal

The annual average of the VC-PAB population ranged from 4.0×10^7 to $6.78 \times 10^{10}.l^{-1}$. During the pre-monsoon period, the surface waters harbored an order lower ($7.67 \times 10^8.l^{-1}$) than the bottom waters ($8.63 \times 10^9.l^{-1}$).

The VC-PAB contributed more than 80% of the VC in the surface and bottom waters during both seasons, except the surface waters of the pre-monsoon where the contribution was only up to 40% (Fig. 48).The proportion of VC-PAB to TC-PAB was the highest in the post-monsoon (90%).

VC-PAB showed a significant correlation with the TC-PAB ($p<0.05$) in the surface waters and bottom waters ($p<0.001$). The VC-PAB also correlated significantly with the microbial biomass ($p<0.001$) both at the surface and bottom. Besides it had a positive correlation ($p<0.01$) with RNA-PAB ($p<0.01$), PON ($p<0.001$) at the surface and PON ($p<0.05$) in the bottom waters.

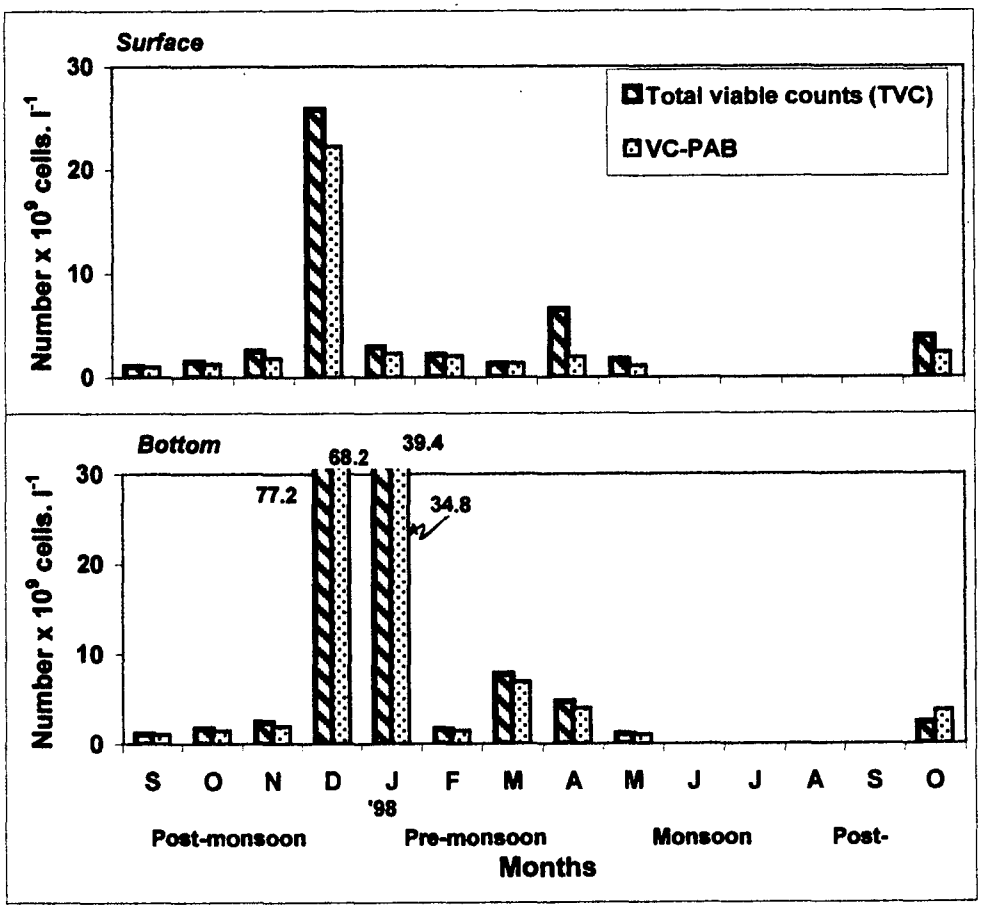


Fig. 48: Total and particle-associated viable bacterial population in the coastal station

In general, the VC-PAB on the particles were higher in the bottom waters with the maximum population of $1.15 \times 10^{10}.l^{-1}$ at the coastal station. A low number of viable cells ($1.4 \times 10^{9}.l^{-1}$) was recorded in the Zuari surface waters. In both the estuaries the viability of the total and particle-associated bacteria was low during the monsoon ($10^{8}.l^{-1}$). Coastal bottom waters recorded the highest number of VC-PAB ($1.43 \times 10^{10}.l^{-1}$) during the post-monsoon season which also formed a sizeable fraction (82.97%) of the VC at the surface waters. Maximum contribution of VC-PAB to VC was observed during the pre-monsoon (75.32 to 87.15%) at all the stations. The contribution of VC-PAB to VC in both Mandovi and Zuari waters was formed to be 89.5% (Fig. 49)

Minimal contribution ($\leq$ 10%) was recorded in both the estuaries during the monsoon season. The VC-PAB at the coastal station made a significant contribution (90%) to the TC-PAB in the surface and bottom waters during the post-monsoon period. During the pre-monsoon, the percentage contribution in the surface and bottom waters was almost the same (38%) in the Mandovi, while Zuari showed more than 50%. In the coastal waters it was 15% and 78.4% at the surface and bottom respectively (Fig. 49).

4.3.2.3 Retreivable counts (RC-PAB)

Mandovi

The RC-PAB population varied from $5.0 \times 10^{5}.l^{-1}$ to $1.18 \times 10^{8}.l^{-1}$ at the surface and $9.0 \times 10^{5}.l^{-1}$ to $1.93 \times 10^{8}.l^{-1}$ in the bottom waters. During the monsoon the number of RC-PAB was an order higher in the surface waters

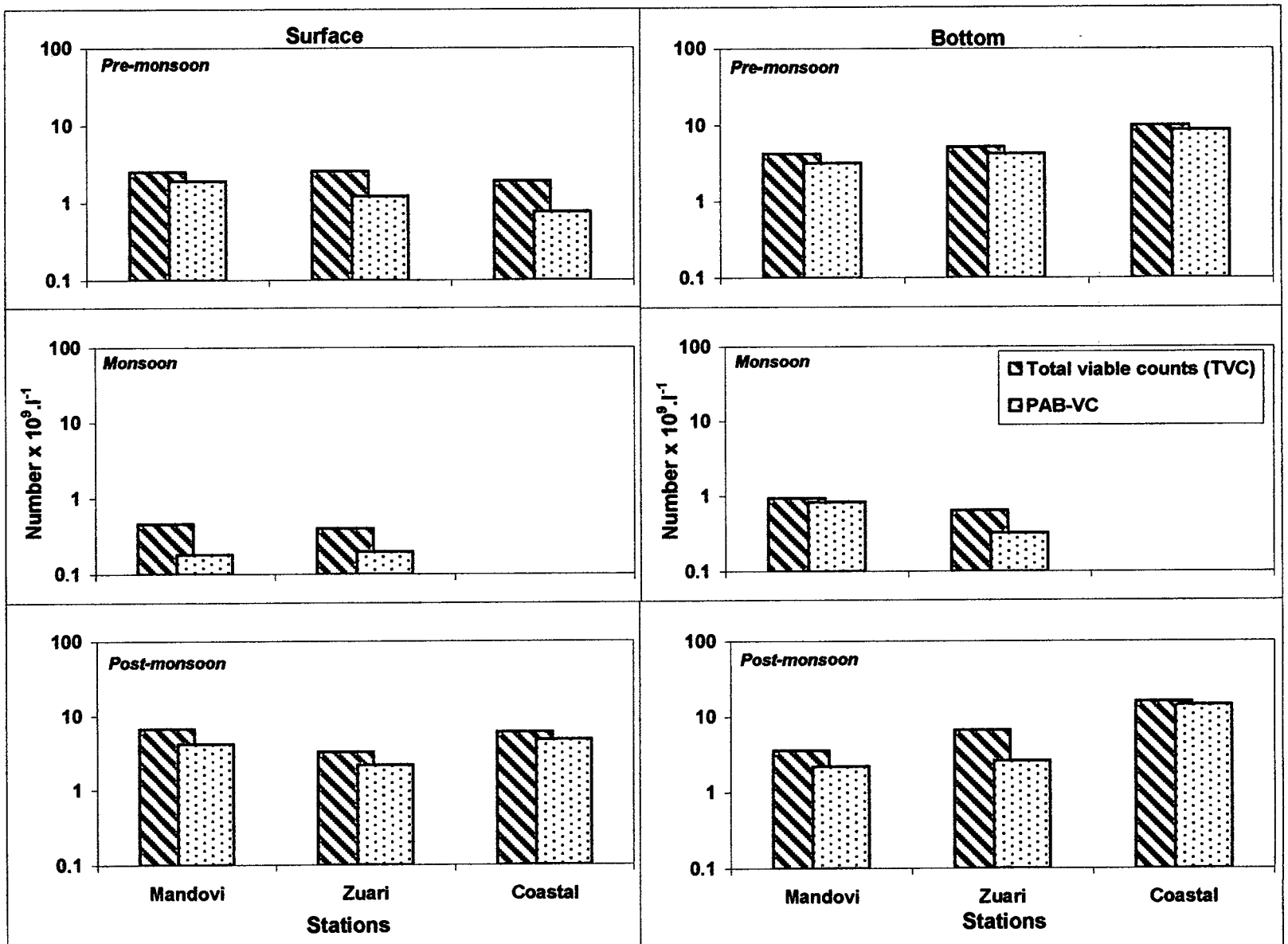


Fig. 49: Comparison of the viable particle-associated bacteria (VC-PAB) with the total viable bacterial population (TVC) in the estuarine network

than the bottom waters. In the pre-monsoon months their numbers in the surface waters were 2.6 times higher than that of the bottom waters (1.2 x $10^7.l^{-1}$). The trend reversed in the post-monsoon season with the bottom waters showing 2.3 times higher than the surface value (Fig. 50).

RC-PAB showed a positive correlation with TC-PAB and VC-PAB ($p<0.001$). A negative relationship between RC-PAB and salinity ($p<0.05$) was noticed in the surface waters.

Zuari

The RC-PAB population varied from 7.3 $x10^5 l^{-1}$ to 9.0x $10^7 l^{-1}$ in the surface and 4.4 $x10^5 l^{-1}$ to 1.08 $x10^8$ l^{-1} in the bottom waters. During the monsoon the surface waters (4.1 $x10^7$ l^{-1}) harbored higher numbers (an order) than the bottom waters ($4.0x10^7$ l^{-1}). The same trend was noticed in the post-monsoon also. The retrievable number (CFU) accounted for 0.92% and 0.13% of the TC-PAB and 1.9 and 0.39% of the VC-PAB in the surface and bottom waters respectively (Fig. 50). The retrievable counts while positively correlated with the particle DNA content in the surface waters ($p> 0.05$) it negatively correlated with RNA and salinity. In the bottom waters the retrievable counts was related to PP.

Coastal

The highest RC-PAB population was recorded during the post-monsoon (November) in the surface waters (7.0 x $10^8.l^{-1}$). The RC-PAB numbers in the surface waters (8.0 x $10^7.l^{-1}$) were 4.2 times higher than that of the bottom waters (1.9 x $10^7.l^{-1}$) (Fig. 50).

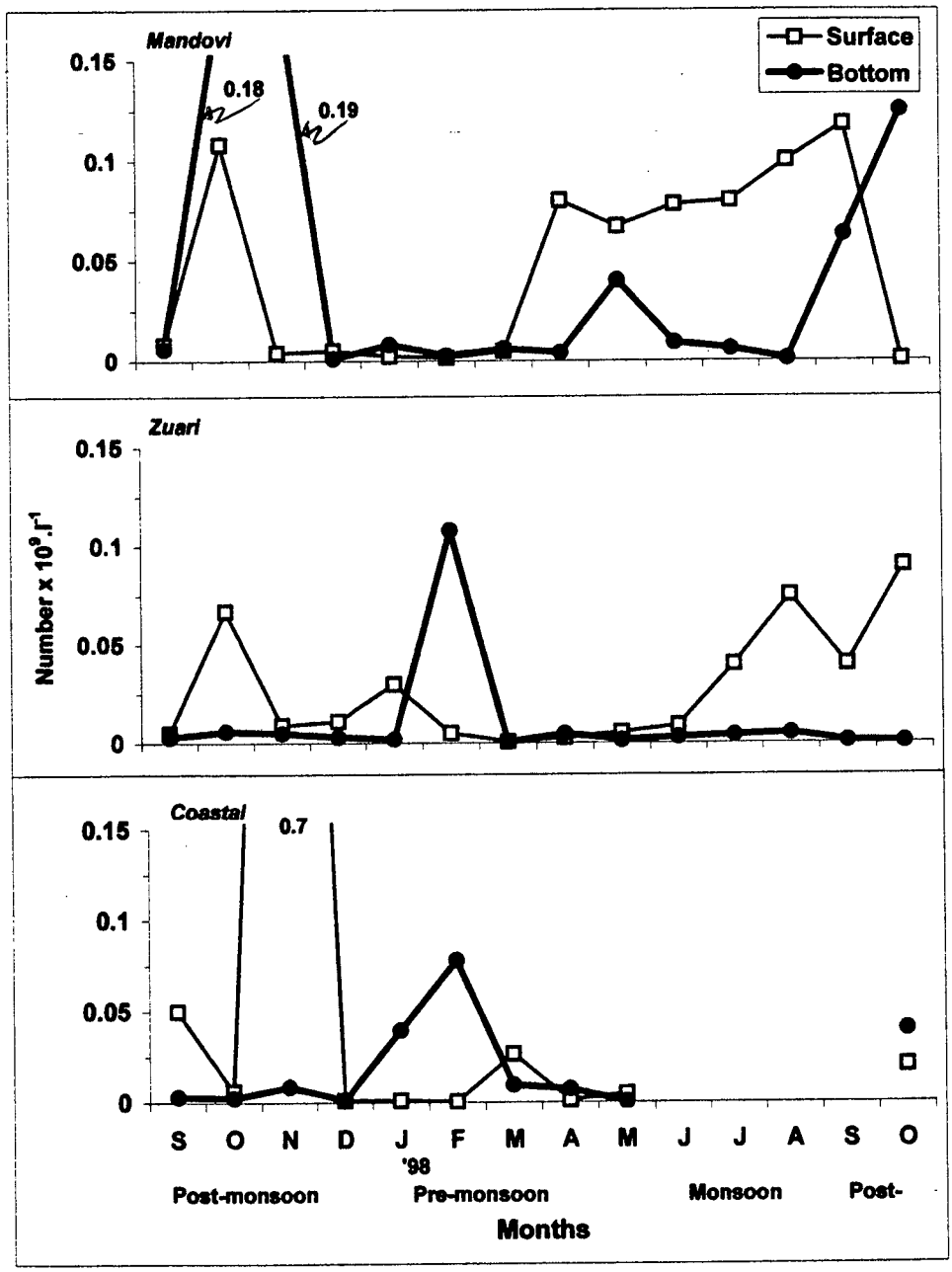


Fig. 50: Retrievable counts of PAB at the three stations

The RC-PAB correlated significantly with the suspended load ($p<0.01$) in the bottom and DOC ($p<0.05$) in the surface waters.

At all the three stations, the retrievable PAB counts were two orders lower ($10^7.l^{-1}$) than the TC-PAB. The RC-PAB formed 0.13% (Zuari bottom) to 1.5% (Coastal surface) of the TC-PAB. At the coastal station it contributed 0.16% to 2.49% of the VC-PAB in the bottom and surface waters respectively. Seasonal averages revealed that the maximum RC-PAB ($1.55 \times 10^8.l^{-1}$) was noticed at the coastal surface waters and the minimum ($3.1 \times 10^6.l^{-1}$) in Zuari bottom waters during the post-monsoon. The Mandovi station had the highest number of RC-PAB (Fig.51).

The distribution of TC, VC and RC on PAB were in the order 10^6 to 10^9. l^{-1}. The highest numbers of TC-PAB, VC-PAB and RC-PAB were recorded during the post-monsoon season. During the same season Mandovi surface waters showed the highest number of TC-PAB ($1.68 \times 10^{10}.l^{-1}$), the coastal bottom waters showed high numbers of VC-PAB ($1.43 \times 10^{10}.l^{-1}$) and at the coastal surface waters maximum number of RC-PAB ($1.55 \times 10^8.l^{-1}$).

The abundance of various biochemical groups at Mandovi and coastal surface waters showed a lot of gelatinase and phosphatase activity. Lipase activity was also observed in the coastal surface waters. In the bottom waters of Mandovi and Zuari the PAB showed a high phosphatase activity (98.52% and 71.02% respectively). The PAB at all the stations were enzymatically active, degrading substrates to different extent (Table 13). The Mandovi bottom waters were very active showing a high percentage of substrate degradation.

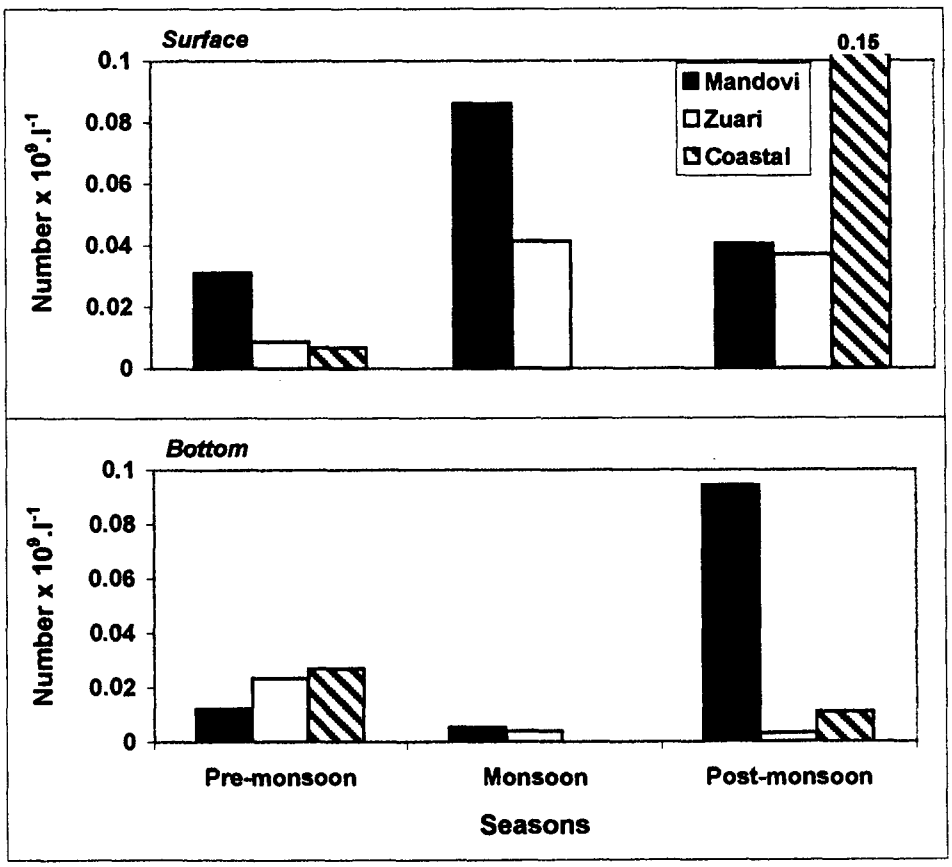


Fig. 51: Seasonal changes in retrievable counts of particle-associated bacteria at the three stations

Table 13: Enzymatic activity of the retrievable population

Enzyme	*Percent enzymatic activity					
	Mandovi		Zuari		Coastal	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Gelatinase	57.72	99.97	7.96	17.59	43.83	25.18
Lipase	11.45	88.64	11.35	40.63	57.16	42.36
Chitinase	27.44	43.22	14.77	31.02	29.35	40.11
Phosphatase	59.67	98.52	11.43	71.02	58.30	39.63
Amylase	31.93	69.19	12.09	18.94	35.19	35.64
Dnase	32.33	39.70	7.73	36.49	33.60	11.74

* 100 % = total number of retrievable PAB

During the pre-monsoon season the PAB-biomass contributed > 54.6% to the PAM biomass in the surface (181 $\mu g.C.l^{-1}$) and 62.39% in the bottom waters (268 $\mu g.C.l^{-1}$). During the monsoon season the PAB biomass formed 89.63% of the PAM biomass (298 $\mu g.C.l^{-1}$) in the surface waters while in the bottom waters it was 49%. Although the contribution of the PAB-biomass was 49% of the PAM-biomass, the total bacterial biomass dominated by 89% and 81% of the total microbial biomass in both the surface (373.3 $\mu g.C.l^{-1}$) and bottom (376.8 $\mu g.C.l^{-1}$) waters respectively during this season. Similar conditions were observed during the post-monsoon period also. However during the pre-monsoon though the total bacterial biomass contributed 47% (320.6 $\mu g.C.l^{-1}$) in surface waters and 38% (Total bacterial biomass-226.7 $\mu g.C.l^{-1}$; total microbial biomass-600.1 $\mu g.C.l^{-1}$) in the bottom waters, the PAB-biomass contributed > 54% to the PAM-biomass in both the surface and bottom waters. The above situation is unique to the pre-monsoon season in this estuary, as the monsoon and post-monsoon don't show the above trend. During the monsoon and post-monsoon seasons not only the PAB-biomass had a major effect on the PAM-biomass but also the total bacterial biomass which constituted more than 80% of the total microbial biomass. During the post-monsoon, the PAM-biomass was dominated by the PAB-biomass constituting 78.24% and 74.65% of the PAM-biomass in both the surface and bottom waters respectively.

The PAB biomass constituted 19.22% of the POC in the surface and 12.24% in the bottom waters. TC-PAB showed good correlation with the POC

($p<0.05$) only at the surface. In both the surface and bottom waters of Mandovi, the PAB showed a significant positive relation ($p<0.001$) with the PAM biomass.

Hence the contribution by the minimum bacterial load to the microbial biomass of the surface waters amounts to 24.68% and in the bottom waters 14.77%. Though the bottom waters carried a higher load of microbial and bacterial biomass, bacteria accounted for 55.28% of the microbial biomass. In the bottom waters during the post-monsoon PAB contributed to an extent of 33.5% of the total bacterial biomass, 33% of the total microbial biomass and 58% of the particle-associated microbial biomass.

The contribution of PAB biomass to the POC pool was 2.5% and 11.0% in the surface and bottom waters respectively.

The PAB biomass constituted 82.72% and 60.56% of the PAM biomass during the post-monsoon and pre-monsoon respectively in the bottom waters, while in the surface waters contribution of 41.47% and 55.83% during the pre-monsoon and post-monsoon respectively was noticed. The highest contribution (49.44%) by PAB biomass to the total microbial biomass (636.39 $\mu g.C.l^{-1}$) was recorded in the bottom waters during the post-monsoon period. Besides the lowest PAB biomass percentage of 28.20 to the total microbial biomass (391.13 $\mu g.C.l^{-1}$) was in the surface waters during the same season. The PAM-biomass constituted 18.12% and 35% of the POC pool in the surface and the bottom waters respectively. The PAB biomass accounted for as much as 89.6% (Mandovi surface during monsoon) and as low as 8.8% (Zuari surface pre-monsoon) of the PAM biomass. At all stations and

seasons the contribution by PAB to PAM biomass in the bottom waters exceeded that of the surface waters except, during the monsoon when the surface percentage was 1.8 times higher than that of the bottom (48.8%) (Fig.52).

GENERIC COMPOSITION

Taxonomic studies of PAB showed the presence of following genera: *Vibrio, Enterobacteriaceae, Bacillus, Cornybacterium, Moraxella, Flavobacterium, Pseudomonas, Aeromonas and Cytophaga.* (Table 14).

4.3.3 BACTERIAL PRODUCTION RATES ON PARTICLES

Mandovi

The total bacterial production in the surface waters ranged from 1.5 $\mu g.C.l^{-1}$ in July to 66.7 $\mu g.C.l^{-1}$ in October'98 and 0.6 $\mu g.C.l^{-1}$ in April to 54.2 $\mu g.C.l^{-1}h^{-1}$ in September'98 in the bottom waters. The annual average PAB production at Mandovi showed trivial difference in the surface (3.08 $\mu g.C.l^{-1}$) and bottom values (3.37 $\mu g.C.l^{-1}$) (Fig 53). The bacterial production rates on particles in the surface waters dropped to a low of 0.5 in August but in the month of Oct '98 the value increased to 13.3$\mu g.C.l^{-1}$. In the bottom waters the PAB productivity was 0.2 $\mu g.C.l^{-1}$ in April and peaked to 9.46 $\mu g.C.l^{-1}$ in September '98.

The variation observed during the post-monsoon in the surface (4.88 $\pm$ 5.61) and bottom waters (4.87 $\pm$ 3.68) was mainly due to high values for PAB production rates in the months of October. In the bottom waters maximum

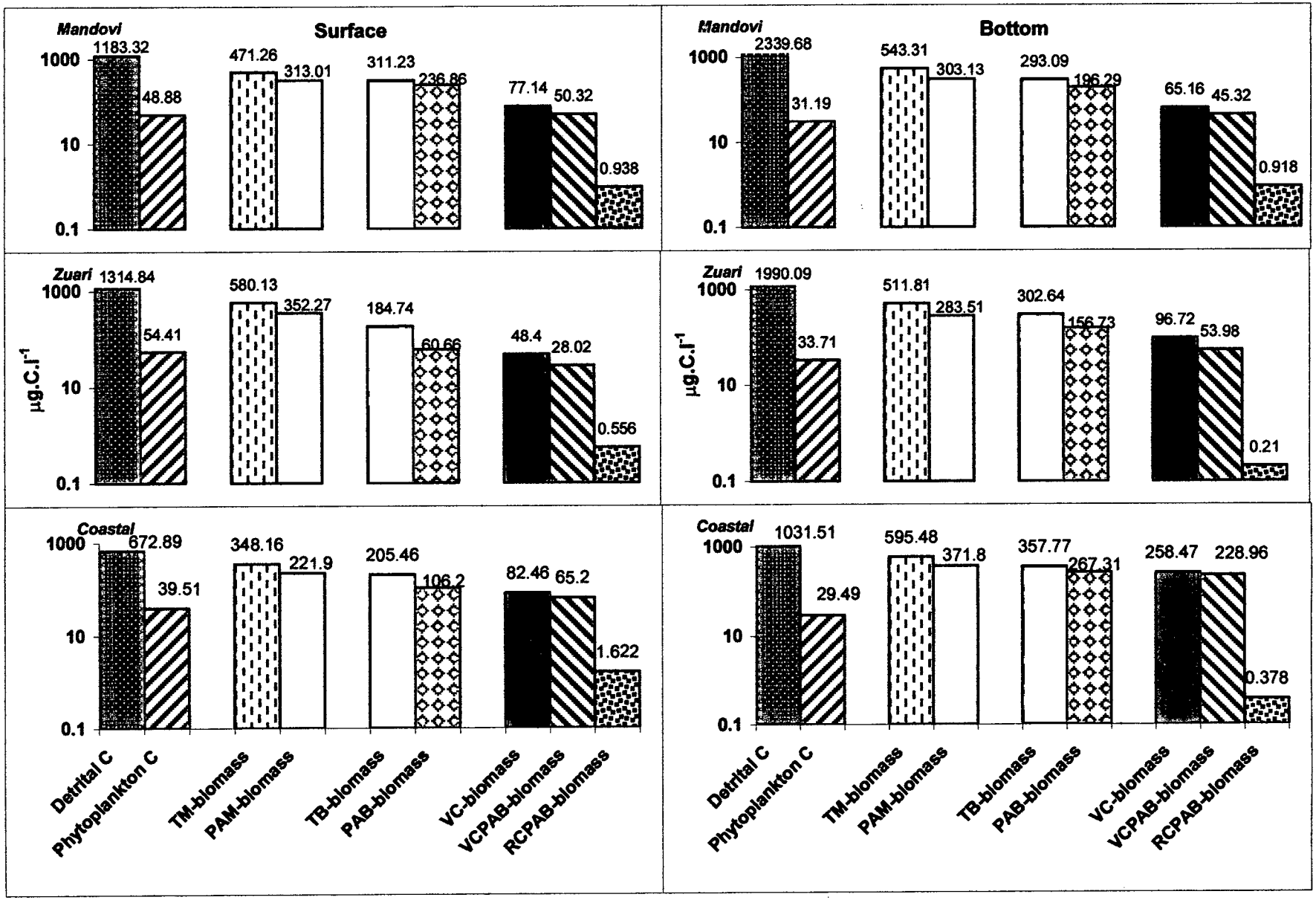


Fig. 52: Secondary bacterial carbon input to the POC

Table 14: Seasonwise distribution of bacterial genera on the particles in the study area.

Season	Bacterial genera		
	Mandovi	Zuari	Coastal
Pre-monsoon	*Bacillus* (86)* *Corynebacterium* (6) *Cytophaga* (8)	*Corynebacterium* (81) *Cytophaga* (11) *Flavobacterium* (5) *Vibrio* (3)	*Corynebacterium* (26) *Chromobacter* (48) *Enterobacteriaceae* (17) *Pseudomonas* (3) *Flavobacterium* (6)
Monsoon	*Bacillus* (30) *Corynebacterium* (25) *Enterobacteriaceae* (45)	*Bacillus* (14) *Corynebacterium* (62) *Enterobacteriaceae* (24)	*Bacillus* (8) *Chromobacter* (62) *Flavobacterium* (23) *Pseudomonas* (7)
Post-monsoon	*Bacillus* (53) *Corynebacterium* (20) *Enterobacteriaceae* (16) *Moraxella* (11)	*Corynebacterium* (58) *Moraxella* (11) *Pseudomonas* (8) *Vibrio* (23)	*Aeromonas* (21) *Corynebacterium* (12) *Chromobacter* (37) *Flavobacterium* (14)

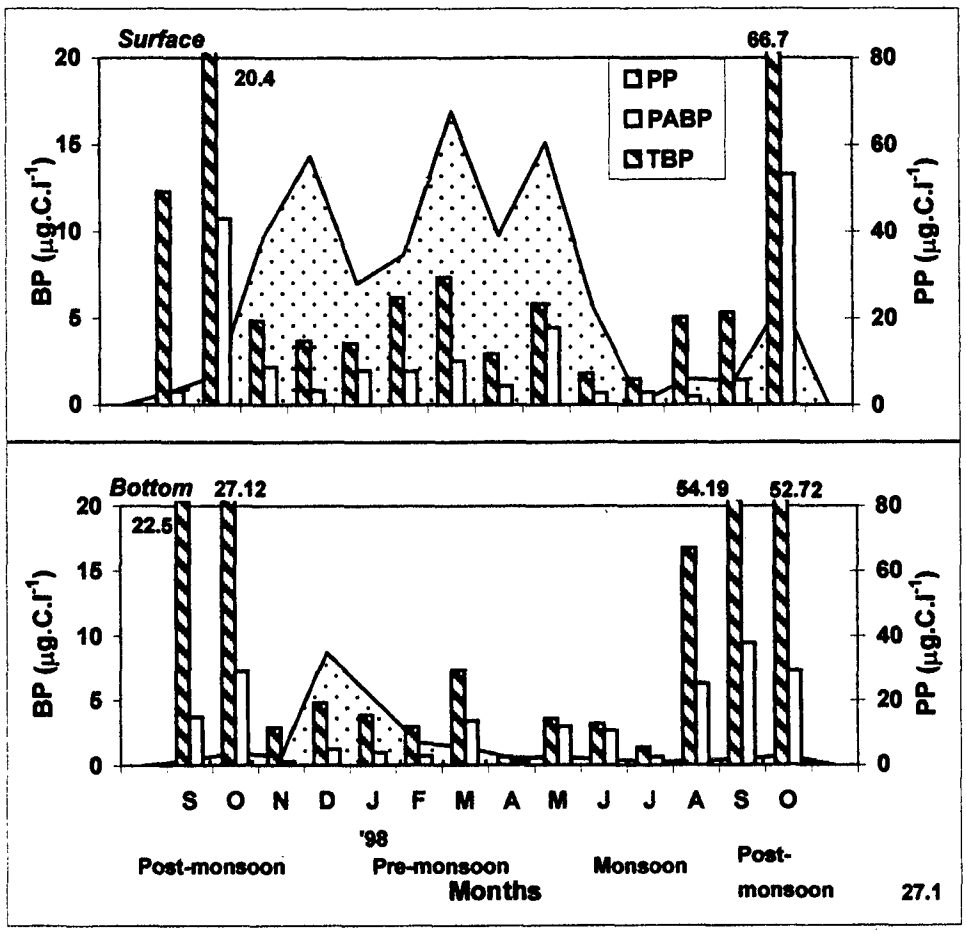


Fig. 53: Annual variation in particle-associated (PABP), total bacterial production rates (TBP) and primary production in the Mandovi estuary

variation by an order of magnitude was observed during the monsoon season (0.65 to 6.29 $\mu g.C.l^{-1}$) and the minimum variation was observed during the pre-monsoon (0.2 to 3.4 $\mu g.C.l^{-1}$) period. Seasonally, the production rates were post-monsoon > pre-monsoon > monsoon in the surface and post-monsoon > monsoon > pre-monsoon in the bottom waters.

The seasonal contribution of the PAB-production to the total bacterial production in the surface waters during the pre-monsoon was 46.5%. In the bottom waters the contribution during the pre-monsoon and monsoon season was 45%. The annual contribution by PAB production to the total bacterial production (TBP) was 29.3% in the surface (TBP – 10.54) and 23.1% in the bottom waters (TBP- 14.57 $\mu g.C.l^{-1}$).

The primary production ranged from 0.54 to 67.7 $\mu g.C.l^{-1}.h^{-1}$ in the surface and 1 to 3.86 $\mu g.C.l^{-1}.h^{-1}$ (except month of December 55.69 $\mu g.C.l^{-1}.h^{-1}$) in the bottom waters. PAB production rates also formed a significant fraction of the primary production especially in the bottom waters. In the surface waters the secondary production was more than the primary production.

Though PAB production did not correlate with the DNA in the surface waters, it showed strong correlation ($p<0.001$) in the bottom waters. However after integrating the surface and bottom values, the PAB production was significantly related to the DNA ($p<0.01$). Production rates on PAB and the total production related to the DNA content ($p<0.001$) of the particles.

Cell specific PAB production

The specific PAB production i.e. per cell production at the Mandovi surface waters was 2.65 $fg.C.cell^{-1}.h^{-1}$. In the bottom waters the annual average of cell specific PAB production rates was 28.24 $fg.C.cell^{-1}.h^{-1}$. (Table 15).

Turnover time of the PAB biomass

The turnover time for PAB ranged from 0.0001 to 1.054 in the surface waters and 0.001 to 18.6 in the bottom waters (Table 16). Thus the surface water PAB had a faster growth rate as compared to the bottom waters. However, the seasonal pattern of the turnover time was monsoon < pre-monsoon < post-monsoon.

Detrital carbon to PAB carbon

The turnover rate of the detrital carbon by PAB was on an average of 1200 h. in the surface and 1460 h. in the bottom waters. The turnover rates of detrital carbon were different in the surface and bottom. The difference was conspicuous during the premonsoon (Fig. 54).

Zuari

In the surface waters low PAB production rates of 0.66 $\mu g.C.l^{-1}.h^{-1}$ was recorded in January and the highest value of 38.31$\mu g.C.l^{-1}.h^{-1}$ in Sept. '98 (Fig. 55). The bacterial production rates on particles were higher at the bottom and ranged from 0.054 to 36.85 $\mu g.C.l^{-1}.h^{-1}$. The average surface production of 5.5 was slightly lower than the bottom production of 6.4 $\mu g.C.l^{-1}h^{-1}$. Seasonal averages showed that the post-monsoon season had the highest

Table 15: Cell specific productivity of the particle-associated bacteria at the sampling stations

Months	Cell specific productivity (µg.C.l^{-1}.h^{-1})					
	Mandovi		Zuari		Coastal	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
S	21.08	372.0	29.09	10.74	82.19	47.61
O	4.36	4.86	1.97	7.93	2.28	0.04
N	0.47	0.05	0.30	0.53	0.70	0.67
D	0.01	0.02	0.36	0.08	0.18	0.02
J	0.27	0.03	0.23	0.07	0.1	0.05
F	0.43	0.17	0.66	0.25	1.05	0.58
M	0.38	2.74	0.63	0.08	4.34	0.24
A	0.30	0.09	0.53	0.34	3.87	0.21
M	1.73	0.87	1.76	0.09	1.90	0.19
J	0.09	0.37	0.28	0.32	ND*	ND
J	0.05	0.08	0.28	0.27	ND	ND
A	0.02	0.71	8.58	0.13	ND	ND
S	0.20	1.62	21.77	2.34	ND	ND
O	7.64	11.74	0.93	16.71	43.64	22.66

*ND – Not done

Wrong we have to replace

Table 16: Turnover time of particle-associated bacterial carbon at all three stations

Station	Seasons					
	Premonsoon		Monsoon		Post-monsoon	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Mandovi	0.031	0.039	0.003	0.019	0.281	3.252
Zuari	0.038	0.008	0.152	0.012	0.454	0.319
Coastal	0.113	0.013	ND*	ND	1.290	0.710

* ND –Not done

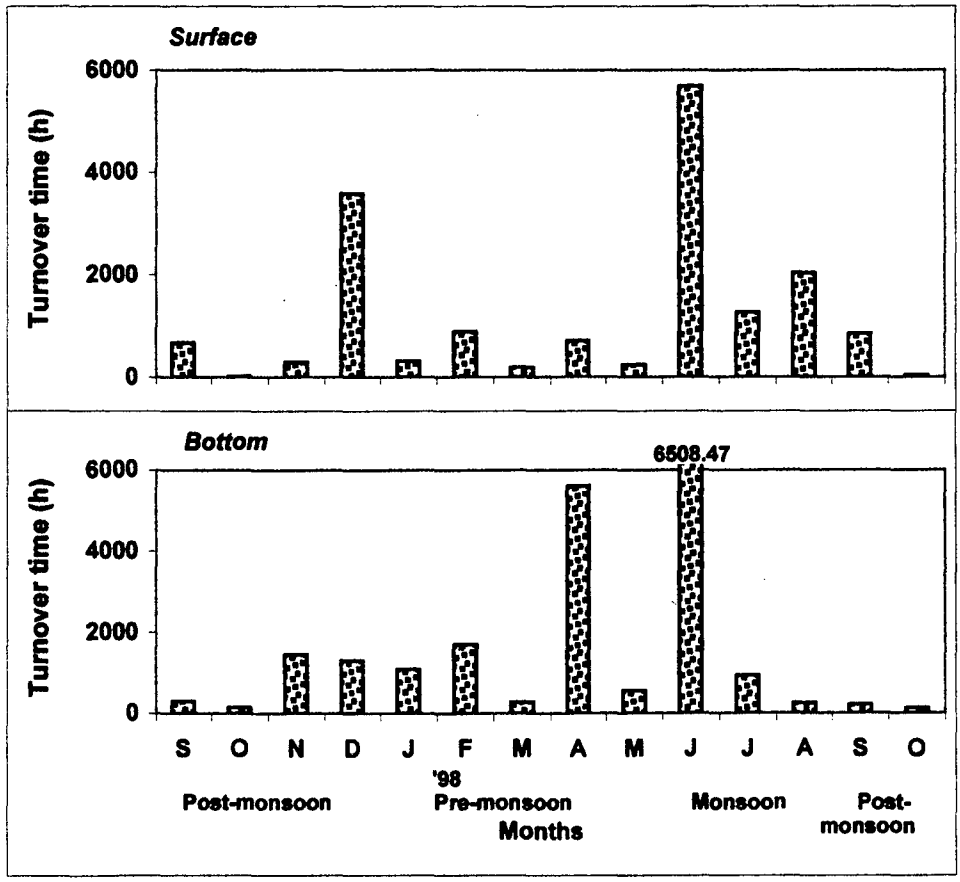


Fig. 54: Turnover rates of detrital carbon by particle-associated bacteria in the Mandovi estuary

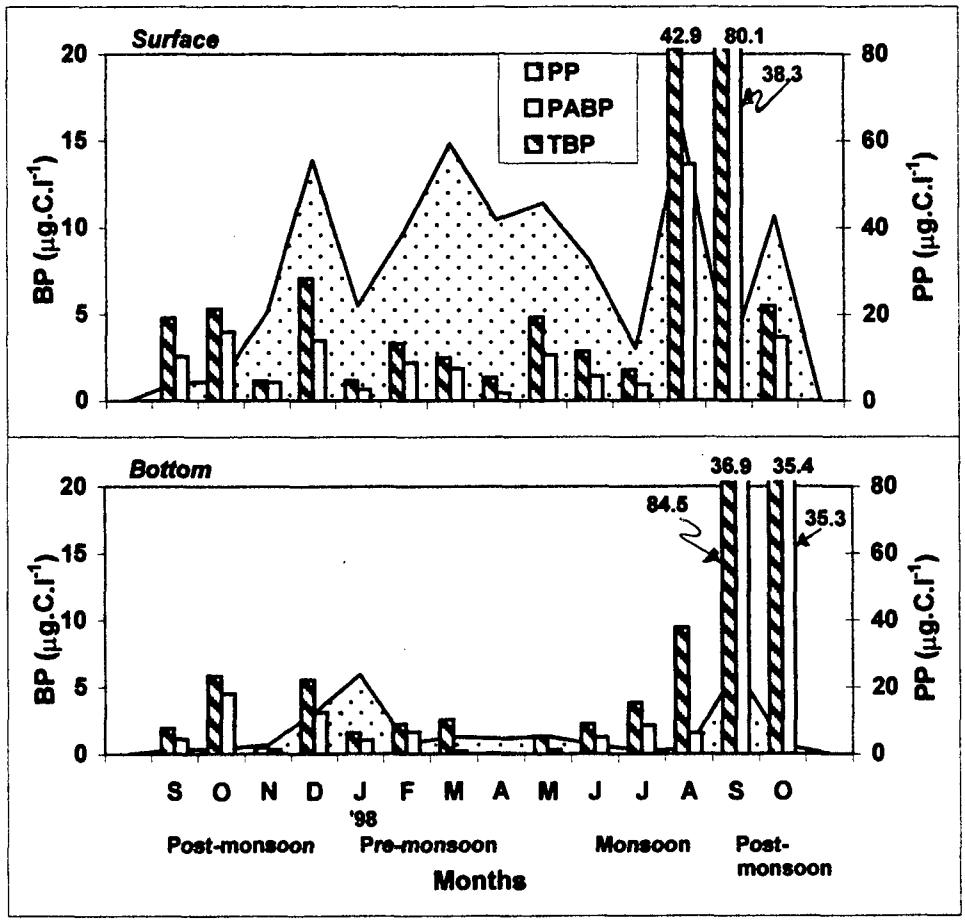


Fig. 55: Annual variation in particle-associated (PABP), total bacterial production rates (TBP) and primary production in the Zuari estuary

production rates at the surface and bottom with the latter recording 13.54 $\mu g.C.l^{-1}.h^{-1}$, which was 1.5 times the surface value.

The total PAB production ranged from 1.16 $\mu g.C.l^{-1}.h^{-1}$ to 80.14 $\mu g.C.l^{-1}.h^{-1}$ in the surface and from 0.068 $\mu g.C.l^{-1}.h^{-1}$ to 84.5 $\mu g.C.l^{-1}.h^{-1}$ in the bottom. The PAB production amounted to 46.8% of the total bacterial production (11.76$\mu g.C.l^{-1}.h^{-1}$) at the surface and 56% at the bottom (11.22 $\mu g.C.l^{-1}.h^{-1}$). Though minimum PAB-production rates were recorded during the pre-monsoon in the surface (1.58 $\mu g.C.l^{-1}.h^{-1}$) and bottom (0.66 $\mu g.C.l^{-1}.h^{-1}$) waters the contribution of PAB-production rates was 59.61% to the total bacterial production in the surface waters (2.64 $\mu g.C.l^{-1}.h^{-1}$) and 42.3% to the total production in the bottom waters (1.57 $\mu g.C.l^{-1}.h^{-1}$). The PAB production rates formed a major portion of 60.7% of the total bacterial production in the bottom waters during the post-monsoon.

The primary production values ranged from 4.12 $\mu g.C.l^{-1}.h^{-1}$ to 63.24 $\mu g.C.l^{-1}.h^{-1}$ in surface and from 1.17 $\mu g.C.l^{-1}.h^{-1}$ to 25.4 $\mu g.C.l^{-1}.h^{-1}$ in bottom waters. In the bottom waters the percentage bacterial production of primary production was much higher than the surface waters. Bacterial production rates on particles in the surface waters accounted for nearly 3.8% in the pre-monsoon, 14.8% in the monsoon and 38.8% in the post-monsoon of the primary production. During the pre-monsoon the PAB production formed 8.9% of primary production but during the monsoon and post-monsoon it formed 211% and 200.8% respectively of the primary production values.

Cell specific PAB production

The cell specific production rates on particles was 0.23 to 29.09 fg.C.l^{-1}.h^{-1} in the surface and 0.07 to16.71 fg.C.l^{-1}.h^{-1} in the bottom waters.. Maximum cell-specific PAB production rates were observed during the post-monsoon season in the surface (9.07 fg.C.l^{-1}.h^{-1}) and bottom waters (6.39 fg.C.l^{-1}.h^{-1}). The PAB had a high cell specific production during the post-monsoon and in the surface waters during the pre-monsoon. (Table 15).

Turnover time of PAB biomass

The growth rate of PAB was found to be 18.6 h. A maximum seasonal average of 0.45 was calculated at the surface waters during the post-monsoon while the least value of 0.008 h was at the bottom waters during the pre-monsoon. Seasonal averages showed that the PAB of the bottom waters had shorter growth rates than those of the surface (0.142 h). As seen from the annual average the PAB of the surface waters had a growth rate 1.7 times greater than that of the bottom (Table 16).

Detrital carbon to PAB carbon

The turnover times of detrital carbon by PAB ranged from 17.46 h (Sept'98) to 6092.98 h (8.5 months - April) in the surface waters and 25.69 h (Oct'98) to 50609.26 h (70.3 months-April) in the bottom waters. In the surface waters the pre-monsoon season recorded the maximum turnover times of 1919 (2.7 months) while the lowest of 279.81 (11.6 h) during the post-monsoon. In the bottom waters, the turnover times during the pre-monsoon (17418.68h) was 37 times that during the post-monsoon (470.75 h).

Except for the months of October'97, December, July, Sept. '98 and October'98 the bottom waters had higher turnover times than the surface waters (Fig. 56).

Coastal

The annual average PAB production was 10.48 $\mu g.C.l^{-1}.h^{-1}$ in the surface and 9.83 $\mu g.C.l^{-1}.h^{-1}$ in the bottom waters, which accounted for 60.6 and 67.5% of the total bacterial production respectively. At the coastal station, the PAB production ranged from 1.5 (November) to 61.1 $\mu g.C.l^{-1}.h^{-1}$ (October) at the surface and 0.2 (October'97) to 82.9 $\mu g.C.l^{-1}.h^{-1}$ (October'98) in the bottom waters. During the post-monsoon the PAB-production rates amounted to 17.8 and 18.2 $\mu g.C.l^{-1}.h^{-1}$ which accounted for 58 and 67% of the total bacterial production rates at the surface and bottom respectively. Though the PAB-production rate on particles is lower during the pre-monsoon by an order than the post-monsoon, the PAB-production rate contributed a higher percentage to the total bacterial production rates (83% - surface and 76.4% - bottom waters (Fig. 57).

In the coastal station, the percentage of secondary production was higher than primary production. PAB production and the total production correlated negatively with salinity ($p<0.001$) in the surface waters. In the bottom waters production of PAB showed negative correlation with temperature ($p<0.001$). The production rates on particles showed strong positive relationship with DNA-PAB ($p<0.001$), and negative relation with

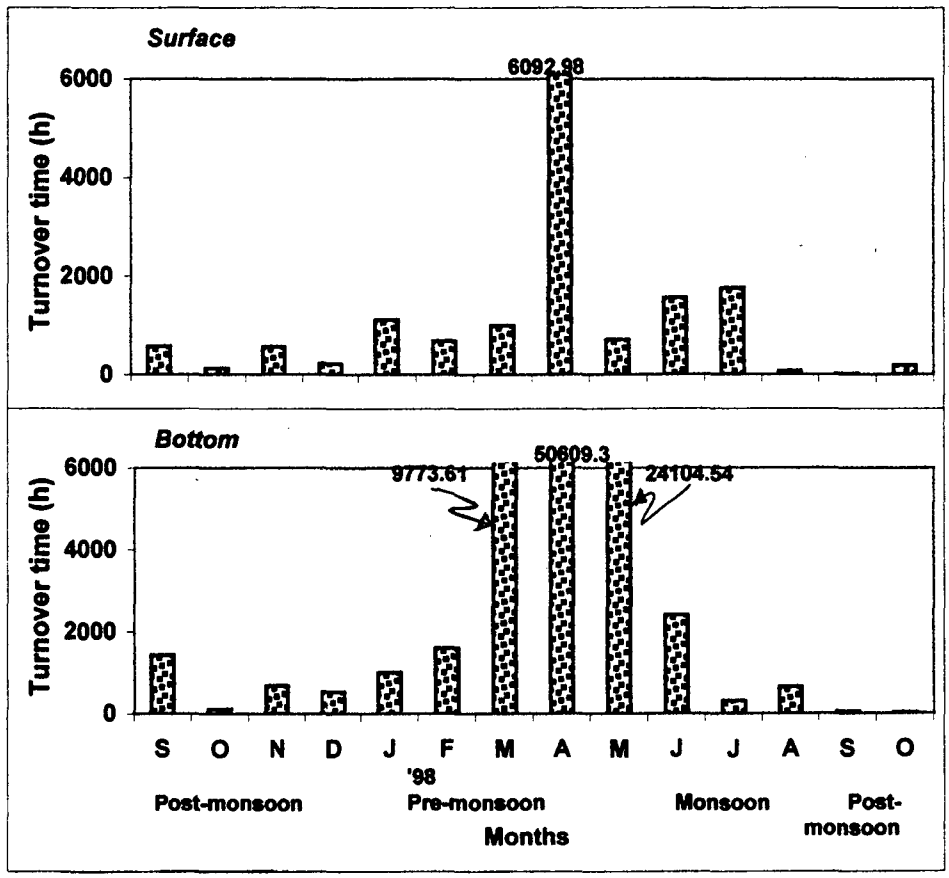


Fig. 56 Turnover rates of detrital carbon by particle-associated bacteria in tne Zuari estuary

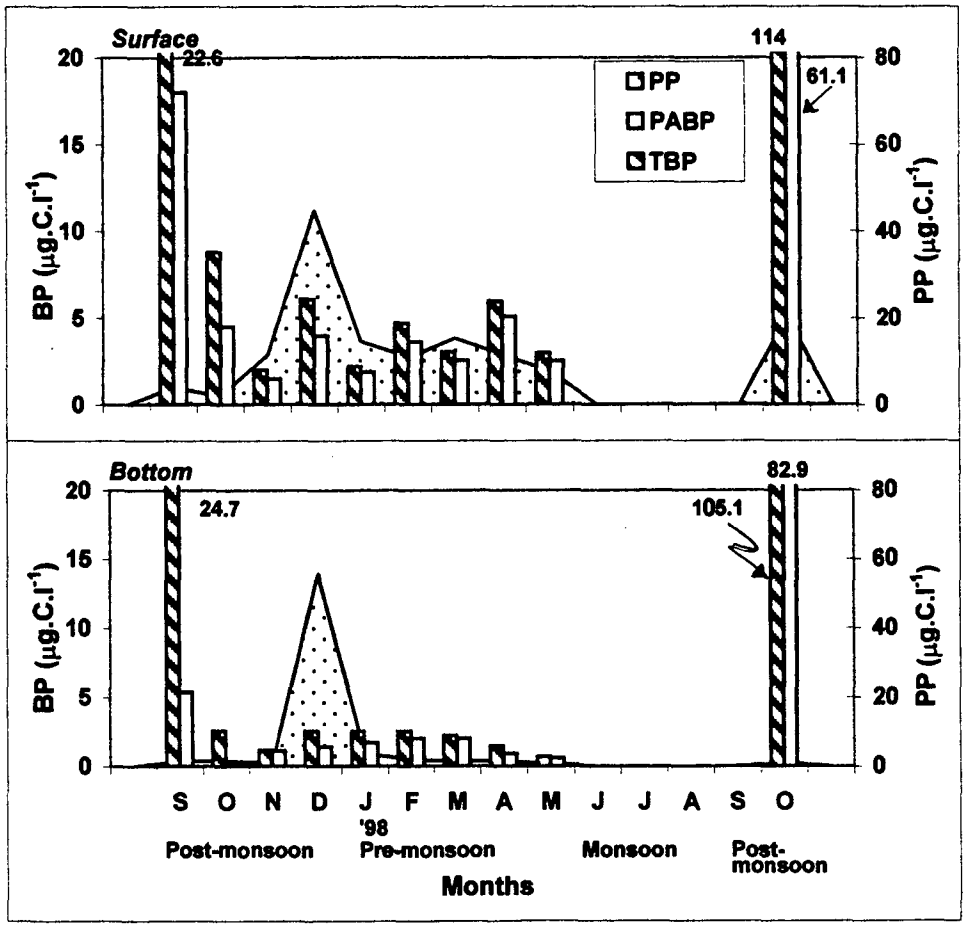


Fig. 57: Annual variation in particle-associated (PABP), total bacterial production rates (TBP) and primary production in the coastal station

salinity ($p<0.001$) in the surface waters. The production rates were also negatively related to temperature and RNA ($p<0.001$).

Cell specific PAB production

The cell specific production rates on particles ranged from 0.02 $fg.C.cell^{-1}.h^{-1}$ to 57.97 $fg.C.cell^{-1}.h^{-1}$ in the surface and 0.01 to 1015.79 $fg.C.cell^{-1}.h^{-1}$ in the bottom waters (Table 15). Annual averages of values in the surface water showed the cell specific PAB production to be 1.42 times that of FLB (9.85 $fg.C.cell^{-1}.h^{-1}$). The cell specific production rates in the surface waters during the post-monsoon was 11.5 times that during the pre-monsoon (2.25 $fg.C.cell^{-1}.h^{-1}$). In the bottom waters, the post-monsoon showed 55.7 times higher values than that seen during the pre-monsoon (0.25 $fg.C.cell^{-1}.h^{-1}$). Cell specific production rates on particles related with chlorophyll *a* carbon ($p<0.05$) in the surface waters.

Turnover time of PAB biomass

The mean growth rate ranged from 0.005 to 4.11 h in the surface and 0.001 to 2.38 h in the bottom waters. PAB had a higher growth rate (0.405 h.) in the surface waters than in the bottom waters (0.36 h.). In the post-monsoon season the PAB growth rate was an order higher than that observed during the pre-monsoon. The surface waters had higher growth rates (an order) than the bottom waters in the pre-monsoon. It was about 1.8 times the value of post-monsoon seasons (Table 16).

Detrital carbon to PAB carbon

The turnover rates of detrital carbon by PAB ranged from 8.35 (October'98) to 441.32 h. (Nov'97) in the surface and 10.86 (October'98) to 1700.7 h.

(May'98) in the bottom waters (Fig. 58). The pre-monsoon season showed 3.3 and 1.3 times higher turnover time than that calculated for the post-monsoon in the surface (160.4 h. = 6.68 days) and the bottom waters (672.8 h.). However the bottom waters had higher turnover times than the surface waters.

It was observed that during the premonsoon period the surface waters were highly productive. Zuari station had the longest turnover time irrespective of the season.

4.3.4. HETEROTROPHIC ACTIVITY

The bacterial uptake potential was estimated by using ^{14}C labeled glucose (a monomer of carbohydrate) and glutamic acid (a monomer of protein).

4.3.4.1 MANDOVI

Glucose

The annual average uptake velocity of glucose by PAB ranged from 0.064 (October'98) to 155.9 $\mu g.C.l^{-1}.h^{-1}$ (February) in the surface waters, while in the bottom waters it ranged from 0.092 (October'98) to 211.8 $\mu g.C.l^{-1}.h^{-1}$ (February). The bottom waters showed values twice that of the surface in the pre-monsoon (Fig. 59).

The uptake potential for glucose showed positive correlation with the suspended load ($p<0.05$) in the whole water column.

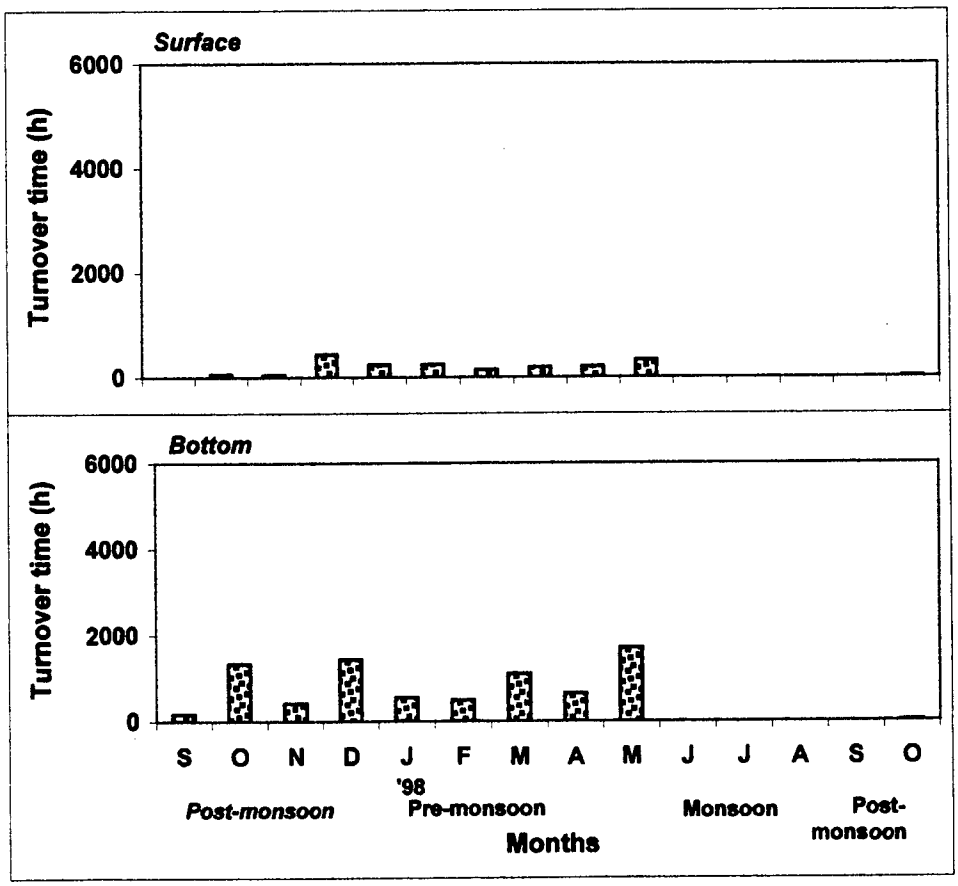


Fig.58: Turnover rates of detrital carbon by particle-associated bacteria in the coastal station

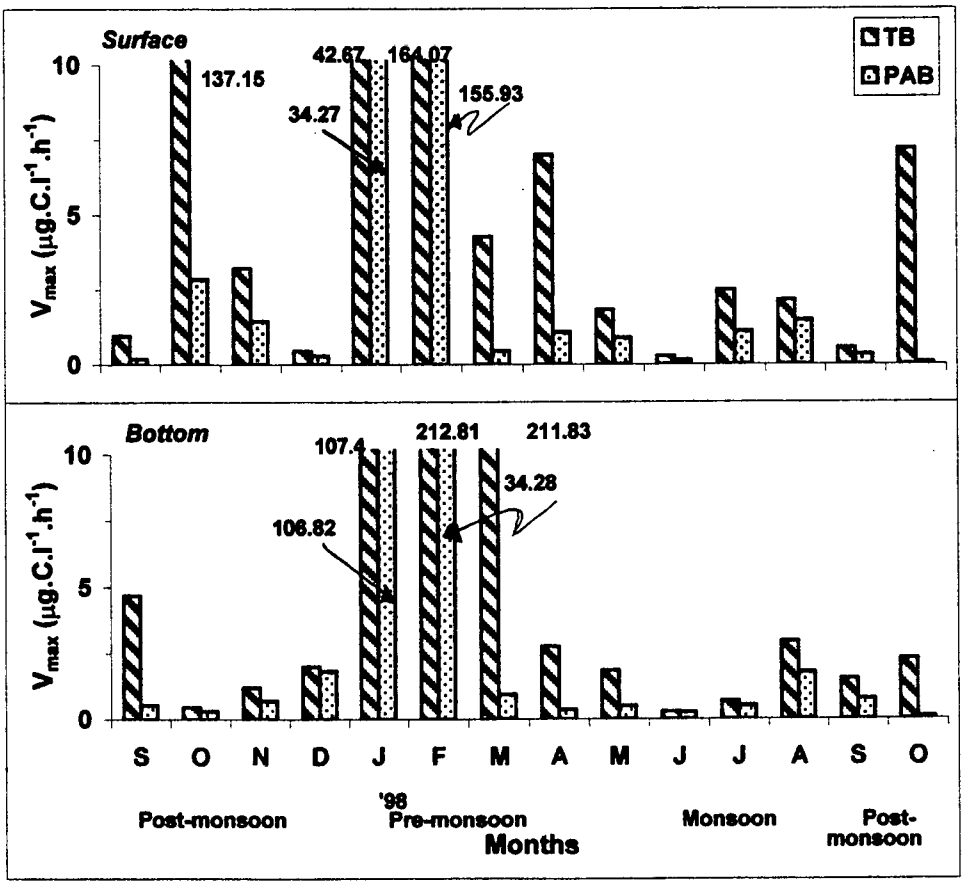


Fig. 59: Uptake potential (V_{max}) of total (TB) and particle-associated bacterial population (PAB) in the Mandovi estuary using ^{14}C-glucose.

K_t+S_n value of the Mandovi surface waters ranged from 3.70 $\mu g.C.l^{-1}.h^{-1}$ (December) to 5770.56 $\mu g.C.l^{-1}.h^{-1}$ (February) whereas in the bottom waters it ranged from 9.66 $\mu g.C.l^{-1}.h^{-1}$ (June) to 1917.11 $\mu g.C.l^{-1}.h^{-1}$ (February). PAB contributed as much as 93.51% (surface) and 88.25% (bottom) of the K_t+S_n to that of TB (Table 17).

The turnover time (T_t) of glucose by PAB was 53.65 h in the surface waters as compared to 39.8h in the bottom waters. In general monsoon season showed shorter T_t at the surface (Fig. 60).

The annual average uptake per cell (cell specific activity) of PAB for glucose was 3.3 $\mu g.C.cell^{-1}.h^{-1}$ in the surface and 7.83 $\mu g.C.cell^{-1}.h^{-1}$ in the bottom waters. (Table 18).

Glutamic acid

The uptake potential of PAB for glutamic acid ranged from 0.003 (January) to 2.78 $\mu g.C.l^{-1}.h^{-1}$ (October'97) in the surface waters and from 0.0045 (December) to 0.18 $\mu g.C.l^{-1}.h^{-1}$ (July) in the bottom waters (Fig. 61). The post-monsoon season showed the highest uptake rates for glutamic acid by PAB (0.51 $\mu g.C.l^{-1}.h^{-1}$) in the surface waters. The maximum contribution of 99.67% and 84.34% of the V_{max} of TB by PAB was observed in Oct. '97 in the surface and bottom waters respectively.

The K_t+S_n of surface waters ranged from 3.71 $\mu g.C.l^{-1}.h^{-1}$ to 120.51 $\mu g.C.l^{-1}.h^{-1}$ and that of the bottom waters ranged from 3.82 $\mu g.C.l^{-1}.h^{-1}$ to 369.25 $\mu g.C.l^{-1}.h^{-1}$. Maximum values were observed in February in the surface and bottom waters respectively (Table 17). PAB contributed 96.8%

Table 17: K_t+S_n of glucose and glutamic acid in the Mandovi estuary.

Months	K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)			
	Glucose		Glutamic acid	
	PAB	TB	PAB	TB
S	6.52 ***87.41**	21.99 **336.71**	4.06 **0.38**	6.75 **4.98**
O	7.71 **2.33**	1871.51 **25.63**	10.84 **5.65**	19.28 **7.11**
N	13.56 **27.58**	38.26 **28.17**	5.78 **8.53**	21.17 **369.25**
D	1.97 **68.32**	3.70 **75.61**	2.33 **0.99**	3.94 **3.82**
J	799.71 **985.2**	842.55 **996.36**	0.43 **9.48**	8.2 **29.16**
F	5721.72 **1902.08**	5770.56 **1917.11**	116.65 **17.97**	120.51 **54.51**
M	7.34 **15.43**	24.49 **204.93**	92.04 **3.93**	106.17 **17.39**
A	153.43 **23.09**	498.11 **164.48**	0.77 **1.57**	5.78 **5.17**
M	14.87 **4.3**	26.52 **37.5**	3.38 **1.24**	9.67 **63.1**
J	1.6 **6.79**	15.71 **9.66**	3.47 **1.97**	5.76 **5.45**
J	12.97 **13.86**	137.2 **21.33**	25.45 **7.08**	52.27 **47.68**
A	36.31 **24.1**	44.59 **34.73**	2.62 **0.84**	5.5 **12.71**
S	1.56 **6.75**	7.82 **27.86**	3.59 **12.41**	14.12 **25.39**
O	26.12 **9.87**	208.76 **15.64**	1.24 **8.30**	3.17 **64.22**

* Figures in bold indicate bottom values

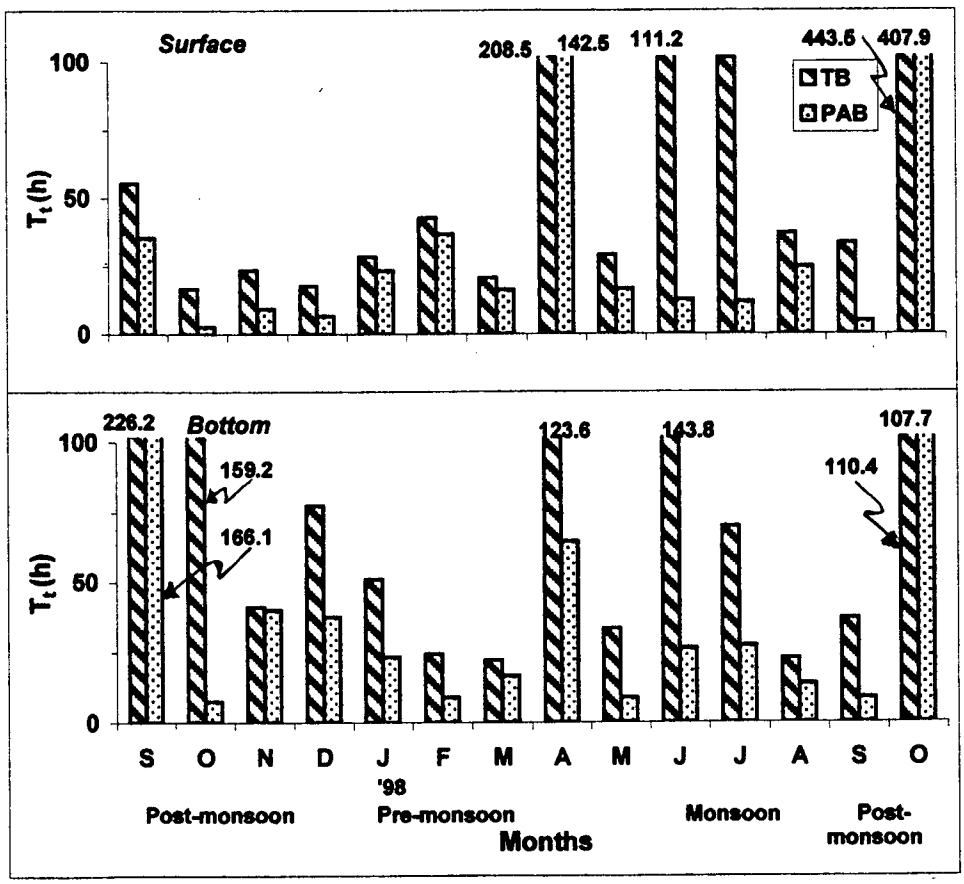


Fig. 60: Turnover time (Tt) of ^{14}C- glucose by total (TB) and particle-associated (PAB) populations in the Mandovi estuary.

Table 18: Cell specific particle-associated bacterial activity in the Mandovi estuary.

Months	Cell specific activity ($\mu g.C.l^{-1}.h^{-1}$)			
	Glucose		Glutamic acid	
	Surface	Bottom	Surface	Bottom
S	4.973	53.000	2.649	0.580
O	1.159	0.208	1.130	0.094
N	0.309	0.114	0.019	0.006
D	0.003	0.031	0.0001	0.0001
J	4.747	3.465	0.0004	0.004
F	33.972	51.167	0.105	0.006
M	0.067	0.758	0.033	0.054
A	0.293	0.173	0.044	0.063
M	0.346	0.142	0.002	0.006
J	0.017	0.036	0.002	0.006
J	0.084	0.064	0.012	0.001
A	0.076	0.198	0.003	0.002
S	0.047	0.132	0.002	0.031
O	0.037	0.148	0.042	0.045

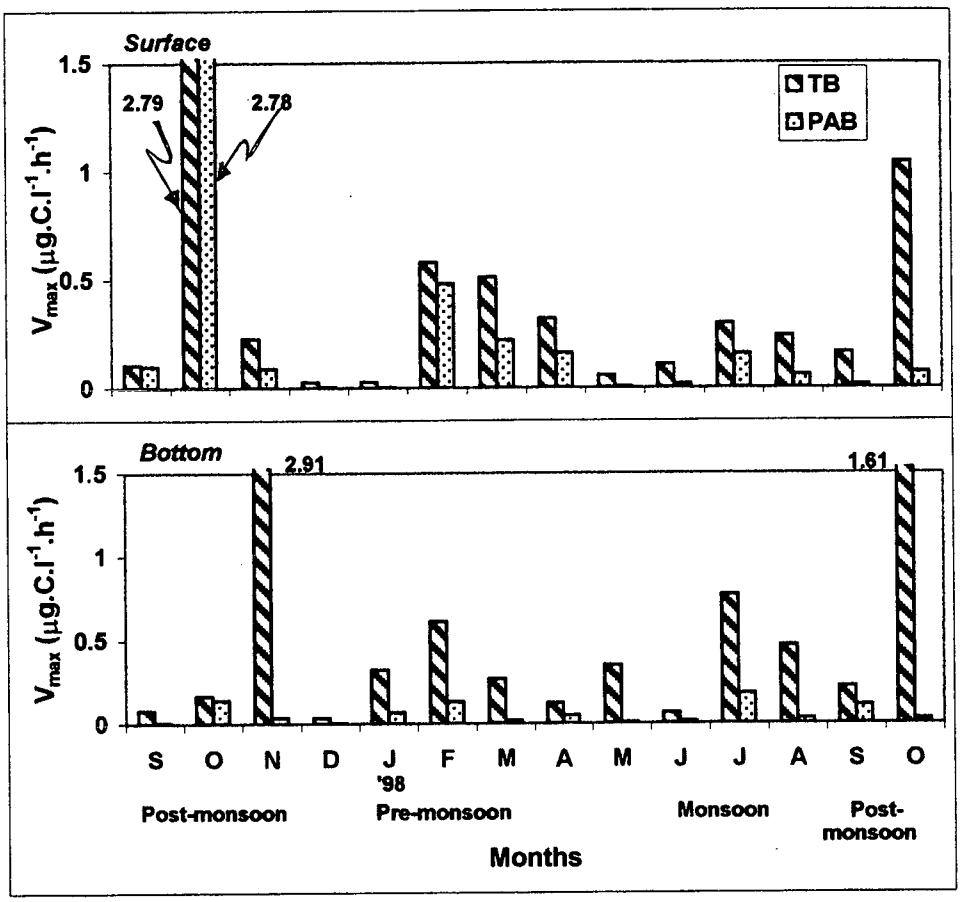


Fig. 61: Uptake potential (V_{max}) of total (TB) and particle-associated bacterial population (PAB) in the Mandovi estuary using ^{14}C-glutamic acid.

(February) and 79.47% (October'97) of the transport constant to that of TB in the surface and bottom waters respectively.

The turnover time (T_t) of glutamic acid by PAB ranged from 3.9 h (October'97) to 565.3 h (May), at the surface and from 29.5 (August) to 313.8h (October), in the bottom waters. The bottom waters showed shortest time (138 h) than the surface (184 h) (Fig. 62). Turnover time of glutamic acid with the VC-PAB ($p<0.05$). However, a negative correlation was observed with cell specific production by PAB ($p<0.05$).

The average (annual) cell specific uptake potential for glutamic acid was 0.289 $fg.C.cell^{-1}.h^{-1}$ at the surface and 0.064 $fg.C.cell^{-1}.h^{-1}$ in the bottom, with highest value recorded in post-monsoon season in both surface and bottom waters (0.64 and 0.13 $fg.C.cell^{-1}.h^{-1}$) (Table 18).

High uptake potential for glucose than glutamic acid (by one to two orders) by the PAB suggests its strong preference for glucose in all the seasons investigated in the present study.

4.3.4.2 Zuari

Glucose

The glucose uptake potential of PAB for varied from 0.015 (March) to 55.32 $\mu g.C.l^{-1}.h^{-1}$ (August) in the surface and 0.13 (December, January and June) to 33.34 $\mu g.C.l^{-1}.h^{-1}$ (March) in the bottom waters.

While, higher V_{max} was encountered in the surface waters of monsoon (18.80 $\mu gC.l^{-1}.h^{-1}$), the bottom waters showed maximum (7.17 $\mu g.C.l^{-1}.h^{-1}$) during the pre-monsoon months.

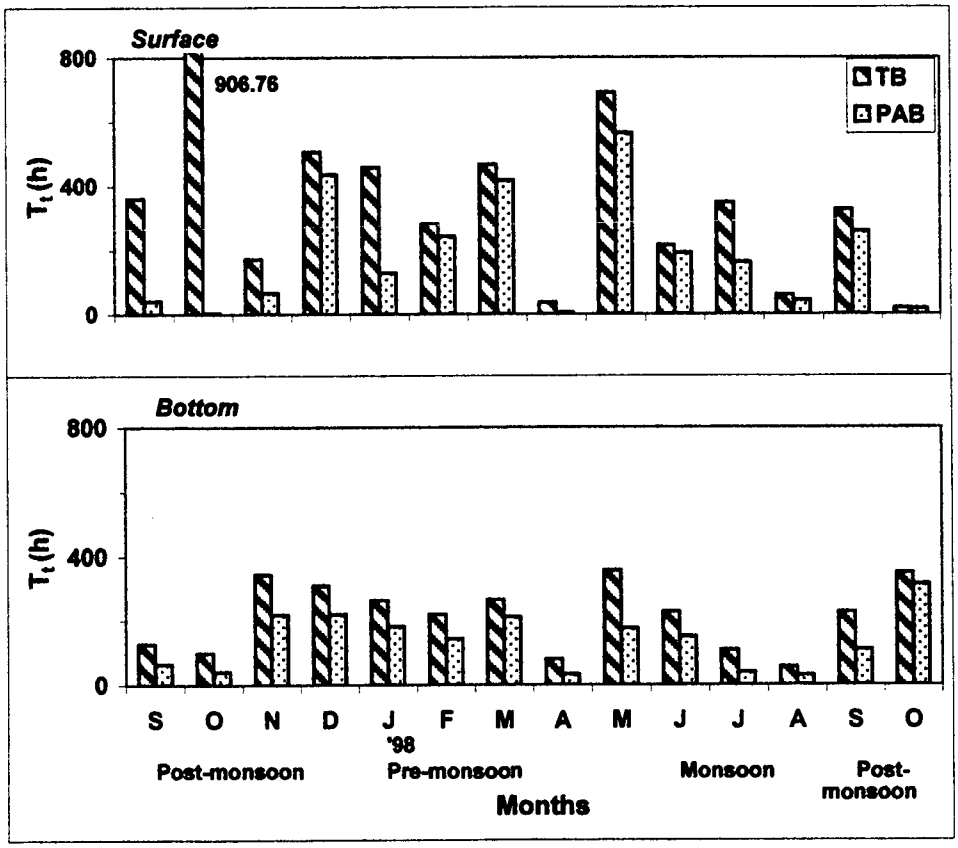


Fig. 62: Turnover time (T_t) of ^{14}C- glutamic acid by total (TB) and particle-associated (PAB) populations in the Mandovi estuary.

The total bacterial uptake potential for glucose ranged from 0.088 $\mu g.C.l^{-1}.h^{-1}$ (March) to 55.67 $\mu g.C.l^{-1}.h^{-1}$ (August) (Fig. 63). The uptake potential by PAB to that of TB accounted for 99.11% (monsoon) at the surface and 83.05% (pre-monsoon) in the bottom waters.

The K_t+S_n for glucose ranged from 0.18 $\mu g.C.l^{-1}.h^{-1}$ (June) to 881.98 $\mu g.C.l^{-1}.h^{-1}$ (August) in the surface and 0.43 $\mu g.C.l^{-1}.h^{-1}$ (December) to 189.5 $\mu g.C.l^{-1}.h^{-1}$ (March) in the bottom waters (Table 19). PAB showed a maximum contribution of 50.25% to the K_t+S_n of TB in the surface (85.93 $\mu g.C.l^{-1}.h^{-1}$) and 56.8% in the bottom (79.39 $\mu g.C.l^{-1}.h^{-1}$) waters.

The shortest T_t was observed at the surface (8.67 h) and bottom (10.10 h) waters during the monsoon period while the pre-monsoon season showed highest T_t times of 57.91 and 23.93 for surface and bottom waters. (Fig. 64). The T_t by PAB here constituted around 30% of that of TB in the monsoon and post-monsoon seasons.

The surface waters of the monsoon season showed a high cell specific activity of 11.7 $fg.C.l^{-1}.h^{-1}$ while, the bottom waters recorded a low of 0.062 $fg.C.l^{-1}.h^{-1}$ (Table 20). In the surface waters the cell specific PAB activity was high in all seasons when compared to the bottom waters.

Glutamic acid

Using glutamic acid as the substrate the PAB uptake potential varied from 0.0032 $\mu g.C.l^{-1}.h^{-1}$ (September'97) to 0.53 $\mu g.C.l^{-1}.h^{-1}$ (August) in the surface and from 0.0011 $\mu g.C.l^{-1}.h^{-1}$ (December) to 0.49 $\mu g.C.l^{-1}.h^{-1}$ (February) in the bottom waters. While maximum V_{max} was observed during the pre-

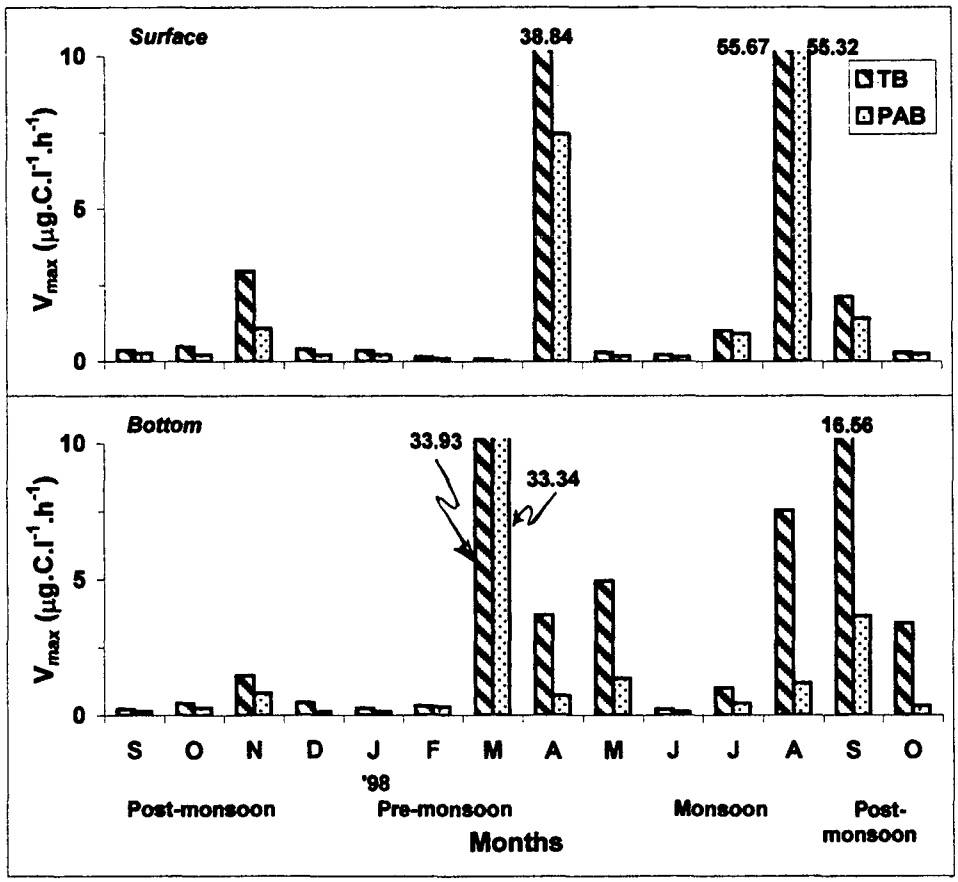


Fig. 63: Uptake potential (V_{max}) of total (TB) and particle-associated bacterial population (PAB) in the Zuari estuary using ^{14}C-glucose.

Table 19: K_t+S_n of glucose and glutamic acid in the Zuari estuary.

Months	K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)			
	Glucose		Glutamic acid	
	PAB	TB	PAB	TB
S	12.3 ***87.41**	17.36 **9.12**	1.023 **0.38**	5.86 **4.98**
O	1.53 **2.33**	2.4 **3.0**	1.26 **5.65**	226.26 **7.11**
N	84.06 **27.58**	114.06 **43.63**	1.48 **8.53**	49.68 **369.25**
D	0.19 **68.32**	0.77 **4.21**	5.29 **0.99**	9.17 **3.82**
J	4.44 **985.2**	11.08 **3.34**	20.52 **9.48**	209.29 **29.16**
F	0.24 **1902.08**	2.68 **2.82**	184.66 **17.97**	191.59 **54.51**
M	2.36 **15.43**	11.77 **197.58**	5.32 **3.93**	16.81 **17.39**
A	636.78 **23.09**	3246.46 **369.28**	1.05 **1.57**	3.90 **5.17**
M	3.86 **4.30**	8.52 **47.09**	4.35 **1.24**	8.06 **63.1**
J	0.18 **6.79**	3.33 **3.09**	0.11 **1.97**	14.26 **5.45**
J	8.09 **13.86**	8.97 **11.97**	1.31 **7.08**	386.95 **47.68**
A	881.98 **24.1**	882.1 **18.33**	16.29 **0.84**	28.62 **12.71**
S	21.38 **6.75**	23.96 **149.26**	5.24 **12.41**	16.57 **25.39**
O	9.72 **9.87**	15.45 **21.36**	14.95 **3.31**	50.24 **22.69**

* Figures in bold indicate bottom values

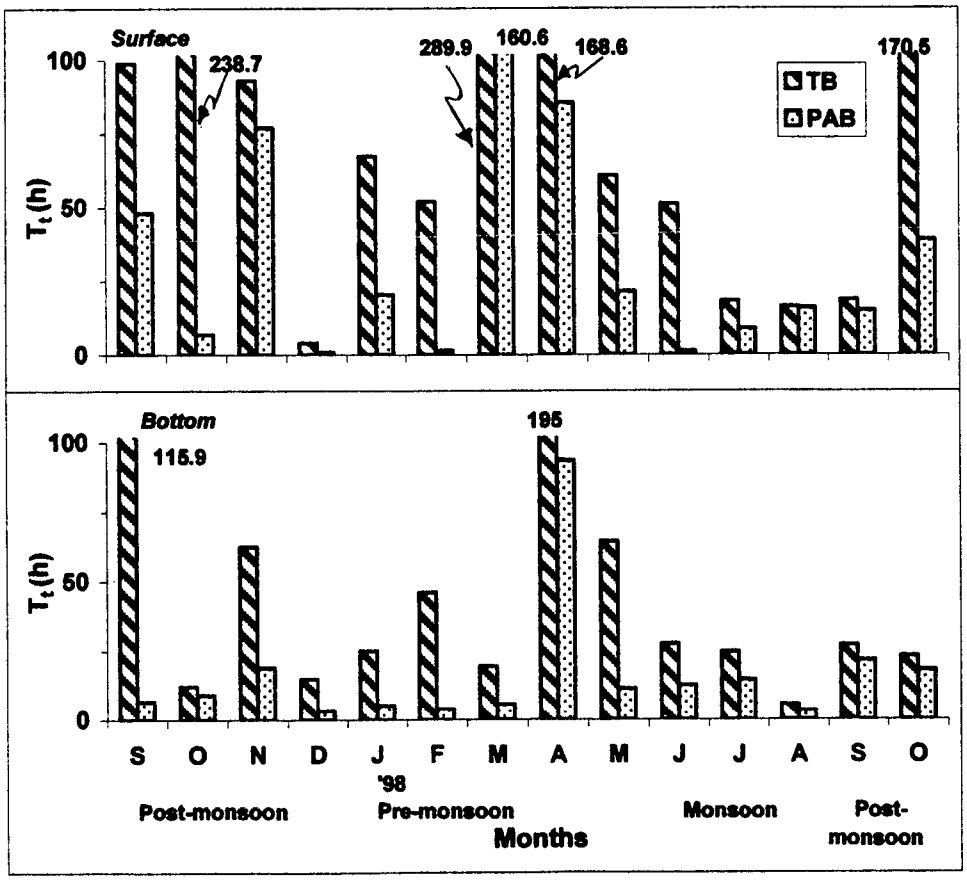


Fig. 64: Turnover time (Tt) of ^{14}C- glucose by total (TB) and particle-associated (PAB) populations in the Zuari estuary.

Table 20: Cell specific particle-associated bacterial activity at Zuari estuary.

Months	Cell specific activity ($\mu g.C.l^{-1}.h^{-1}$)			
	Glucose		Glutamic acid	
	Surface	Bottom	Surface	Bottom
S	2.955	1.389	0.036	0.130
O	0.108	0.474	0.046	0.263
N	0.303	1.286	0.002	0.049
D	0.023	0.003	0.003	0.0001
J	0.076	0.009	0.042	0.014
F	0.030	0.046	0.084	0.011
M	0.005	12.677	0.010	0.024
A	8.466	4.563	0.284	3.063
M	0.118	0.376	0.005	0.006
J	0.030	0.032	0.001	0.097
J	0.277	0.054	0.006	0.001
A	34.792	0.098	0.333	0.005
S	0.807	0.232	0.022	0.001
O	0.064	0.161	0.032	0.099

monsoon season in the surface (0.137) and bottom (0.195) waters, the post-monsoon season showed the least values (Fig. 65).

The K_t+S_n showed an annual average of 18.78 $\mu g.C.l^{-1}.h^{-1}$ in the surface and 20.48 $\mu g.C.l^{-1}.h^{-1}$ in the bottom waters. The pre-monsoon season showed the highest K_t+S_n in the surface (43.18 $\mu g.C.l^{-1}.h^{-1}$) and bottom waters (45.1 $\mu g.C.l^{-1}.h^{-1}$). During the post-monsoon the K_t+S_n of the bottom waters was 1.75 times that of the surface (Table 19).

T_t of PAB was the shortest during the monsoon period as it contributed 39.9% and 49.61% to the TB in the surface (88.88 h) and bottom (96.13 h) waters respectively (Fig. 66).

The cell specific activity for glutamic acid in the bottom waters of Zuari was found to be 0.269 $fg.C.l^{-1}.h^{-1}$, The highest cell specific activities of 0.11 $fg.C.cell^{-1}.h^{-1}$ (surface) and 0.62 $fg.C.l^{-1}.h^{-1}$ (bottom) were observed in the monsoon and pre-monsoon seasons (Table 20).

4.3.4.3 Coastal

Glucose

The glucose uptake rates of PAB (V_{max}) for ranged from 0.07 $\mu g.C.l^{-1}.h^{-1}$ (April) to 11.5 $\mu g.C.l^{-1}.h^{-1}$ (Oct'97) in the surface and 0.31$\mu g.C.l^{-1}.h^{-1}$ (Oct'98) to 12.2 $\mu g.C.l^{-1}.h^{-1}$ (March) in the bottom waters (Fig. 67). In the month of January the V_{max} of PAB accounted for 95.1% and 90% of the V_{max} of TB in the surface (5.91 $\mu g.C.l^{-1}.h^{-1}$) and bottom waters (0.97 $\mu g.C.l^{-1}.h^{-1}$) respectively.

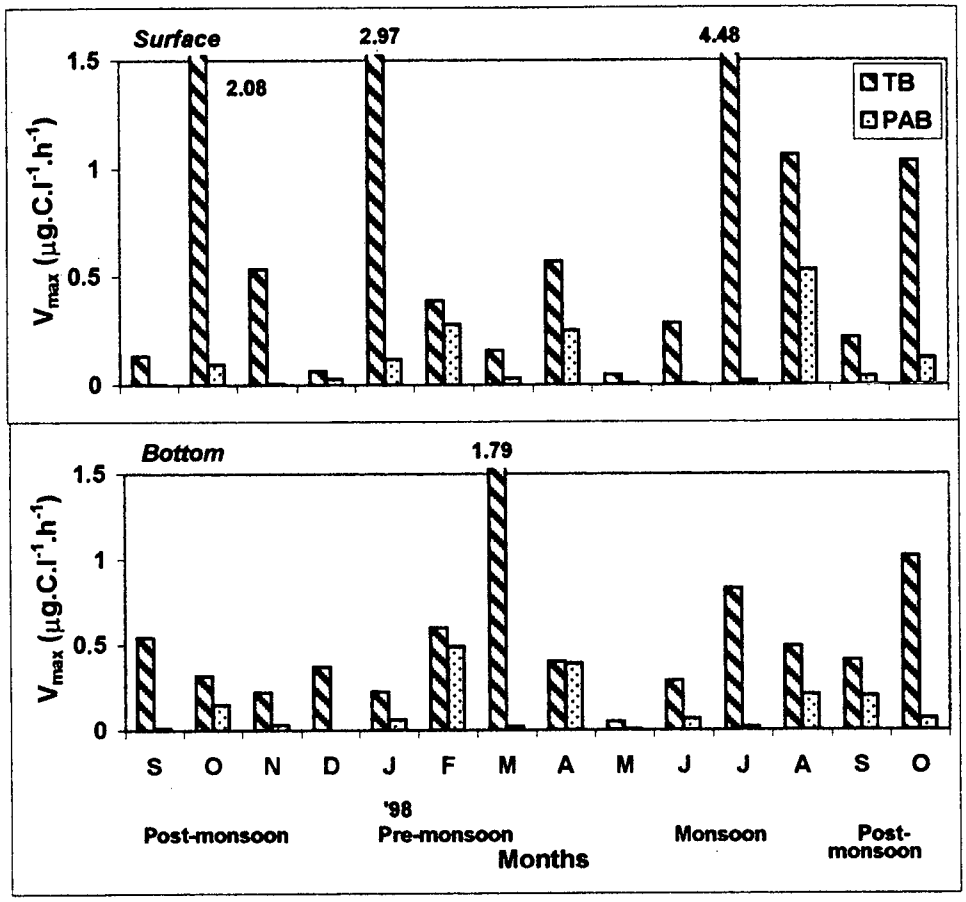


Fig. 65: Uptake potential (V_{max}) of total (TB) and particle-associated bacterial population (PAB) in the Zuari estuary using ^{14}C-glutamic acid.

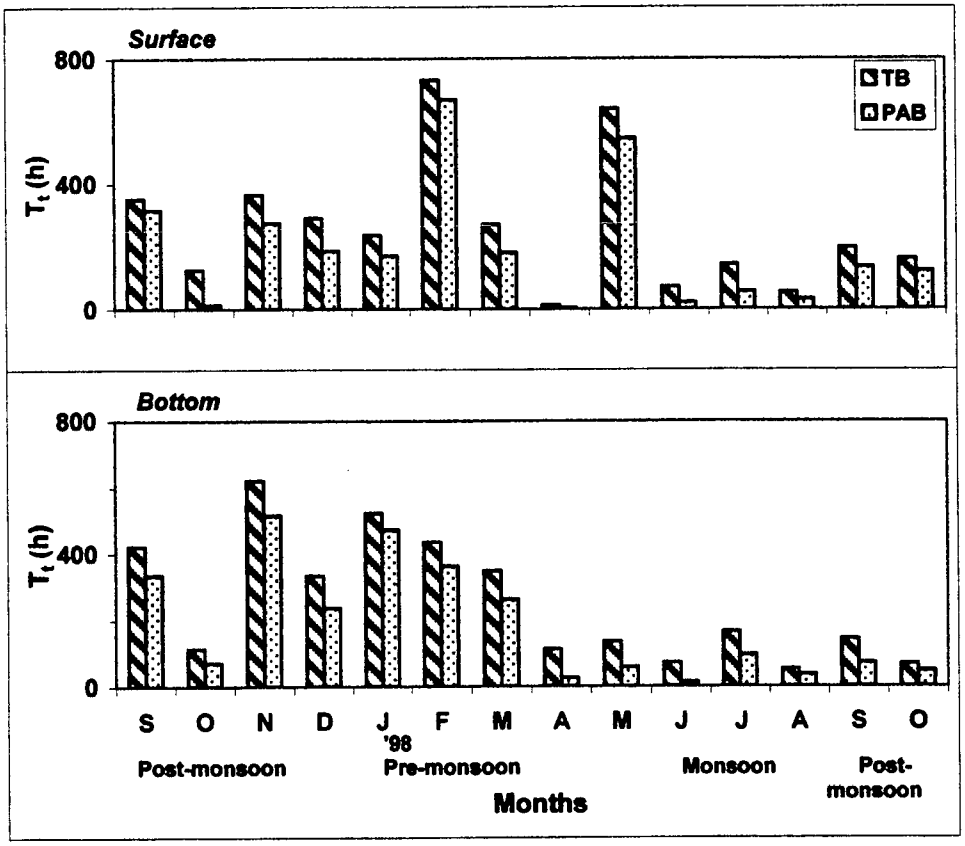


Fig. 66: Turnover time (T_t) of ^{14}C- glutamic acid by total (TB) and particle-associated (PAB) populations in the Zuari estuary.

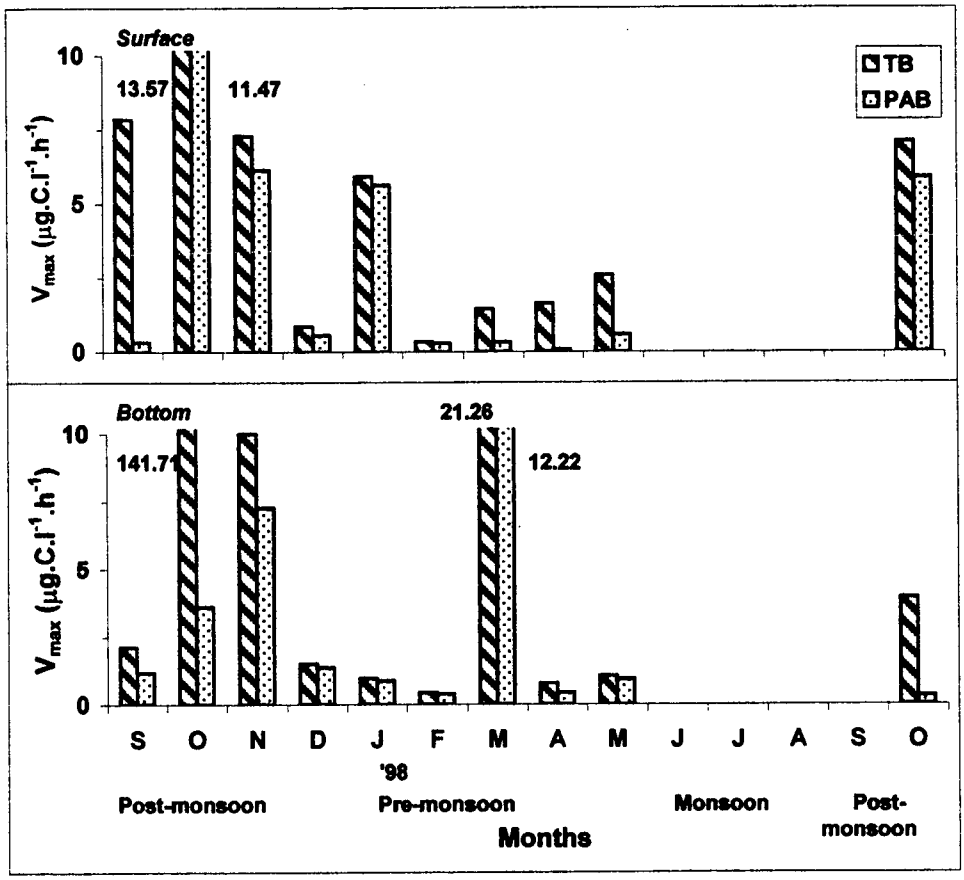


Fig. 67: Uptake potential (V_{max}) of total (TB) and particle-associated bacterial population (PAB) in the coastal station using ^{14}C-glucose.

The coastal station showed maximum values of K_t+S_n in October'97 in the surface (187.32 $\mu g.C.l^{-1}.h^{-1}$) and in the bottom waters (287.17 $\mu g.C.l^{-1}.h^{-1}$) in September'97 (Table 21). The post-monsoon showed higher K_t+S_n than that seen during the pre-monsoon. However seasonal values showed a maximum contribution of 48.46% by PAB to the K_t+S_n of TB (131.75 $\mu g.C.l^{-1}.h^{-1}$) in the bottom waters during the pre-monsoon.

T_t of PAB at Mandovi surface waters during the post-monsoon and Zuari surface waters during the monsoon formed 35.85% and 39.92% of the respectively (Fig. 68).

The specific activity of PAB during the post-monsoon was an order higher (2.89 $fg.C.l^{-1}.h^{-1}$) than that of the pre-monsoon in the surface (0.288 $fg.C.l^{-1}.h^{-1}$) and bottom waters (0.398 $fg.C.l^{-1}.h^{-1}$) (Table 22).

Maximum cell specific activity of 11.70 $\mu g.C.l^{-1}.h^{-1}$ for glucose by PAB was observed at Zuari surface waters during the monsoons and at Mandovi bottom waters (11.14 $\mu g.C.l^{-1}.h^{-1}$) during the pre-monsoon (Fig. 68).

Glutamic acid

The annual uptake potential of PAB for glutamic acid ranged from 0.004 (December) to 0.28 $\mu g.C.l^{-1}.h^{-1}$ (February) with higher activity in the pre-monsoon than the post-monsoon (Fig. 69). Seasonally the PAB population showed maximum contribution of 33.44% and 46.30% in the pre-monsoon months.

Table 21: K_t+S_n of glucose and glutamic acid in the coastal station.

Months	K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)			
	Glucose		Glutamic acid	
	PAB	TB	PAB	TB
S	1.68 ***287.17**	1343.48 **440.37**	0.19 **5.07**	0.72 **5.92**
O	187.32 **35.76**	198.42 **1257.86**	8.10 **2.14**	14.95 **9.33**
N	50.57 **54.56**	66.7 **105.95**	1.12 **0.15**	4.08 **42.71**
D	3.55 **13.93**	5.04 **16.81**	0.50 **1.23**	12.27 **2.50**
J	22.25 **8.45**	204.25 **11.21**	3.31 **2.57**	49.31 **9.72**
F	0.81 **2.97**	4.85 **5.61**	61.31 **3.91**	83.28 **16.93**
M	1.74 **263.82**	29.31 **530.49**	14.27 **2.95**	34.41 **92.39**
A	9.64 **31.92**	127.79 **80.29**	8.86 **11.93**	9.77 **14.45**
M	5.68 **18.63**	87.36 **31.15**	10.11 **7.29**	20.55 **14.32**
O	67.11 **9.03**	71.09 **116.85**	27.20 **5.66**	98.51 **104.01**

* Figures in bold indicate bottom values

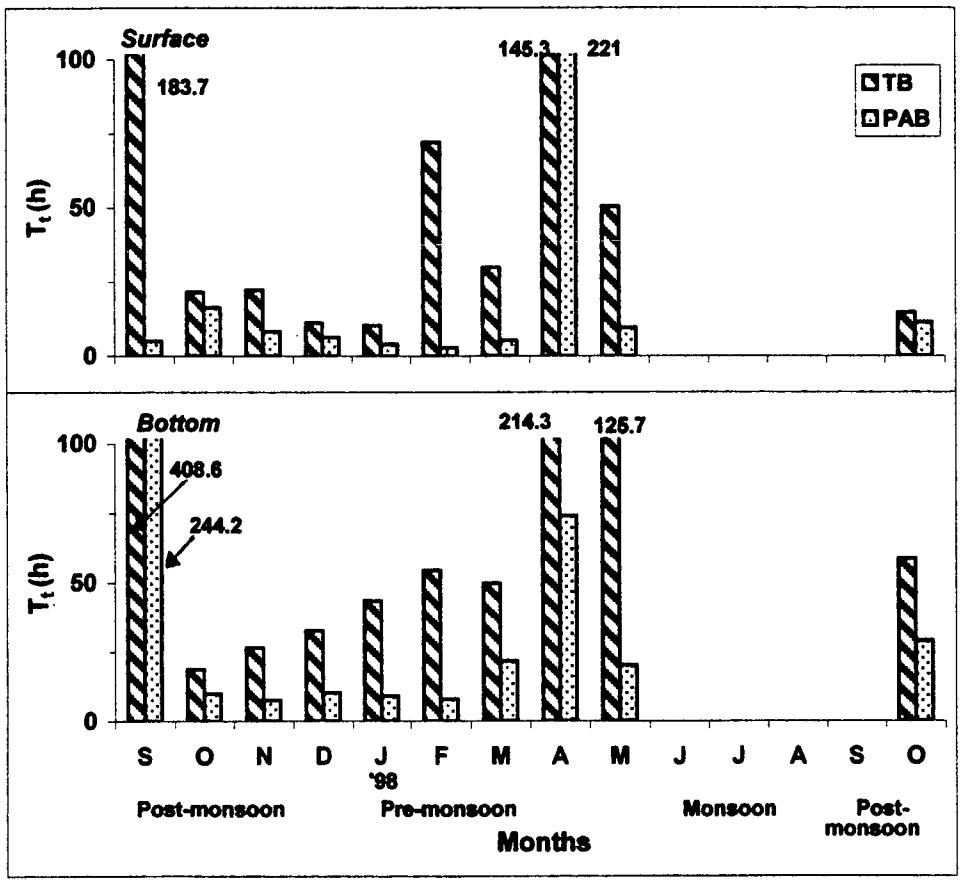


Fig. 68: Turnover time (Tt) of ^{14}C- glucose by total (TB) and particle-associated (PAB) populations in the coastal station.

Table 22: Cell specific particle-associated bacterial activity at the coastal station.

Months	Cell specific activity ($\mu g.C.l^{-1}.h^{-1}$)			
	Glucose		Glutamic acid	
	Surface	Bottom	Surface	Bottom
S	1.507	10.442	0.228	0.549
O	5.822	0.669	0.005	0.003
N	2.883	4.339	0.012	0.012
D	0.026	0.020	0.0002	0.00001
J	0.298	0.025	0.0008	0.0003
F	0.084	0.110	0.081	0.005
M	0.559	1.472	0.069	0.0007
A	0.053	0.098	0.048	0.314
M	0.444	0.286	0.019	0.001
O	4.200	0.085	0.079	0.014

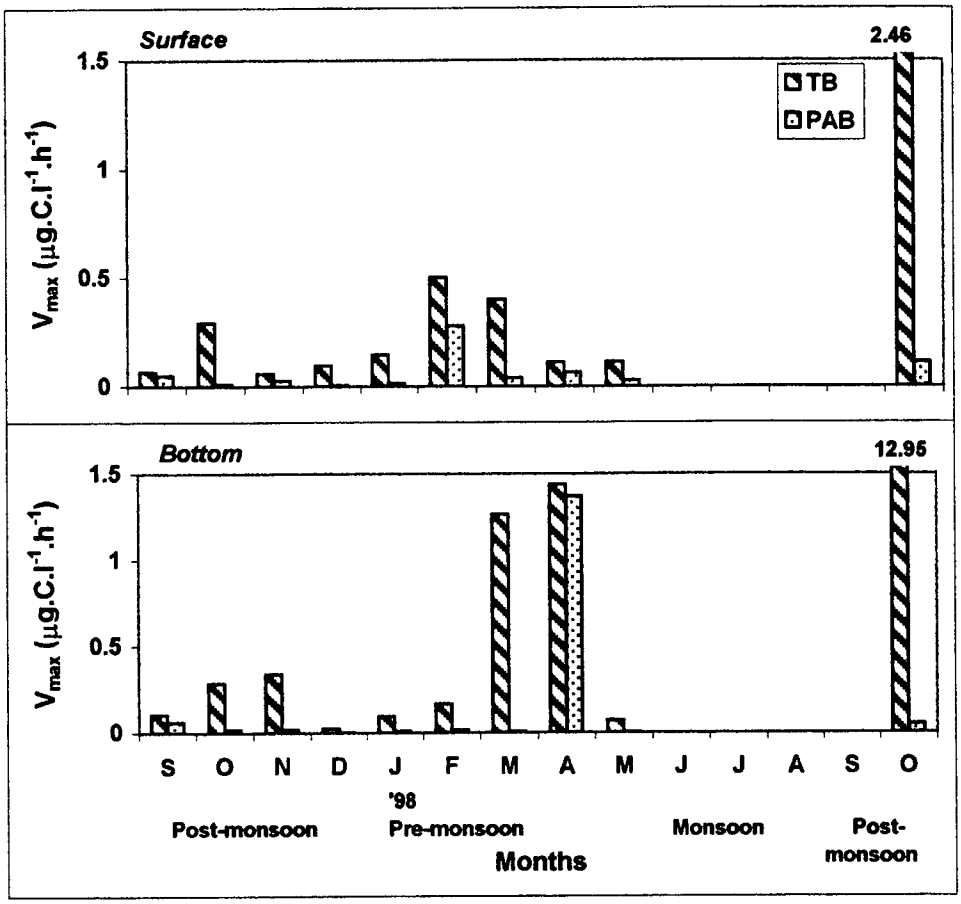


Fig. 69: Uptake potential (V_{max}) of total (TB) and particle-associated bacterial population (PAB) in the coastal station using ^{14}C-glutamic acid.

K_t+S_n of PAB for glutamic acid ranged from 0.19 $\mu g.C.l^{-1}.h^{-1}$ (September'97) to 61.3 $\mu g.C.l^{-1}.h^{-1}$ (February) in the surface and 0.15 $\mu g.C.l^{-1}.h^{-1}$ (November) to 11.93 $\mu g.C.l^{-1}.h^{-1}$ (April) in the bottom. (Table 21).

At the coastal station, K_t+S_n of TB varied from 0.72 $\mu g.C.l^{-1}.h^{-1}$ to 104.01 $\mu g.C.l^{-1}.h^{-1}$ with PAB constituting a high of 90.69 and 82.6% in the surface and bottom waters in the pre monsoon months.

Short turnover time of PAB (9.5 h) was observed during the post-monsoon in the surface waters (Fig. 70). The turnover time of PAB for glutamic acid varied from 1.4 h (April) to 790.1 h (October'97), at the surface, while in the bottom waters it varied from 7.13 (November) to 1789.7 h (April) .In the bottom waters salinity correlated positively with the T_t of glutamic acid ($p<0.05$). The PAB showed shortest T_t at the surface (April) and bottom waters (November), constituting 6.9% and 5.02% of the TB.

The activity ranged from 0.00018 x to 0.23 $fg.C.l^{-1}.h^{-1}$ in the surface and 0.00004 x to 0.55 $fg.C.l^{-1}.h^{-1}$ in the bottom waters. The post-monsoon season showed higher values than the pre-monsoon (Table 22).

The V_{max} values for glucose in the three systems during the pre-monsoon were always higher at the bottom than the surface. The heterotrophic potential of the estuarine bacteria clearly indicate that their system of uptake is more suited for a carbon source like glucose than a nitrogen source like glutamic acid.

The average ratio of uptake velocity at Mandovi, Zuari and the coastal station for glucose was found to be 4.6:1.6 :1 and for glutamic acid 4.9:1.8:1 in the

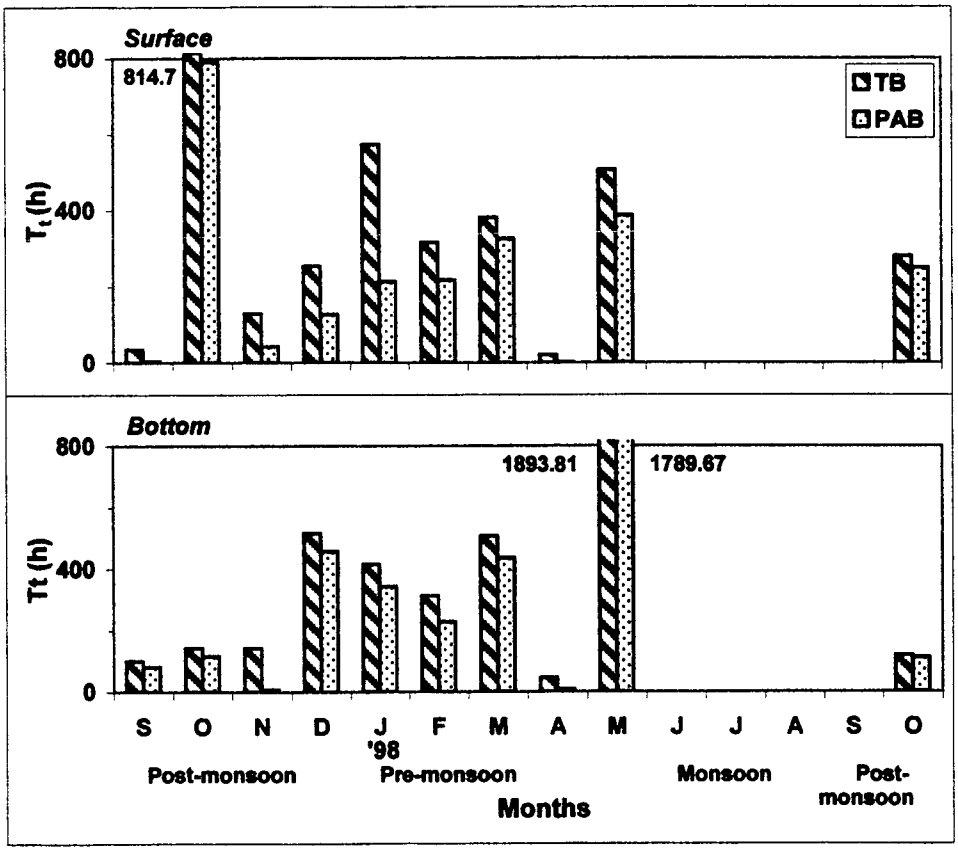


Fig. 70: Turnover time (T_t) of ^{14}C- glutamic acid by total (TB) and particle-associated (PAB) populations in the coastal station.

surface waters; while in the bottom waters it was 8.2 : 1.07 : 1 for glucose and 1 : 2.1 : 2.7 for glutamic acid. On an annual average the mangrove dominated Mandovi, showed higher uptake velocities for both the substrates in the surface waters when compared to the Zuari and coastal station. However the bottom waters showed high uptake rates at Mandovi for glucose, while the coastal bottom waters showed higher uptake for glutamic acid as compared to other stations. Annual averages showed the Mandovi station had the maximum K_t+S_n at the surface (486.09) and bottom (226.94) waters (Table 23 and 24). The cell specific activity of the three stations is tabulated in Table 25.

4.4. FUNCTIONAL MODE OF PAB

Mandovi

While comparing the production and activity rates of PAB it was observed that during pre-monsoon the PAB were more active than productive and during other seasons they were found to be more productive than active (Table 26).

ZUARI

In general the Zuari estuary was found to be both productive and active (Table 27).

Coastal

As illustrated in Table 28, it was observed that the PAB population at the coastal station in general was more productive than active. This was obvious specially during the post-monsoon which showed a

Table 23: Turnover time and K_t+S_n of glucose by particle-associated bacteria (PAB) and total bacteria (TB) during different seasons

Station	Pre-monsoon				Monsoon				Post-monsoon			
	K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)		T_t (h)		K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)		T_t (h)		K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)		T_t (h)	
	PAB	TB	PAB	TB	PAB	TB	PAB	TB	PAB	TB	PAB	TB
Mandovi	1339.41 **1586.02***	1432.45 **1664.08**	47.08 **24.44**	65.90 **50.99**	16.96 **14.92**	65.83 **21.91**	16.33 **22.43**	83.23 **78.86**	9.57 **33.71**	358.67 **84.94**	77.78 **61.30**	96.67 **108.58**
Zuari	129.54 **55.02**	1656.10 **124.02**	57.91 **23.93**	127.74 **69.95**	296.75 **3.95**	298.13 **11.13**	8.67 **10.11**	28.58 **19.19**	21.53 **17.38**	39.00 **38.43**	31.19 **12.98**	103.99 **42.61**
Coastal	8.02 **65.16**	90.71 **131.75**	33.39 **(26.51)**	76.71 **97.56**	ND@	ND	ND	ND	62.05 **80.09**	336.95 **387.57**	9.47 **60.09**	50.70 **108.95**

* Values in bold indicate bottom values
@ND – Not done

Table 24: Turnover time and K_t+S_n of glutamic acid by particle-associated bacteria (PAB) and total bacteria (TB) during different seasons

Station	Pre-monsoon				Monsoon				Post-monsoon			
	K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)		T_t (h)		K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)		T_t (h)		K_t+S_n ($\mu g.C.l^{-1}.h^{-1}$)		T_t (h)	
	PAB	TB	PAB	TB	PAB	TB	PAB	TB	PAB	TB	PAB	TB
Mandovi	42.65 **6.84***	50.07 **33.87**	271.68 **148.86**	386.45 **237.11**	10.15 **3.30**	21.18 **21.95**	131.99 **73.01**	207.60 **130.67**	4.64 **6.04**	11.50 **79.13**	137.00 **161.47**	382.11 **242.64**
Zuari	43.18 **45.10**	85.93 **79.39**	314.08 **236.28**	378.19 **310.79**	5.91 **3.34**	143.28 **27.95**	35.48 **47.69**	88.88 **96.13**	4.87 **8.54**	59.63 **32.51**	174.58 **212.91**	248.63 **283.52**
Coastal	19.57 **5.73**	39.46 **29.56**	229.41 **561.03**	359.74 **634.78**	ND@	ND	ND	ND	7.42 **2.85**	26.11 **32.89**	242.45 **154.56**	302.21 **204.19**

* Values in bold indicate bottom values
@ND – Not done

Table: 25 Cell specific activities of the PAB using glucose and glutamic acid as substrate at the study sites during different seasons

Station	Water Column	Cell specific activity (fg.C.cell^{-1}.h^{-1})					
		Pre-monsoon		Monsoon		Post-monsoon	
		Glucose	Glutamic acid	Glucose	Glutamic acid	Glucose	Glutamic acid
Mandovi	Surface	7.88	0.04	0.06	0.006	1.09	0.64
	Bottom	11.14	0.03	0.10	0.003	8.94	0.13
Zuari	Surface	1.79	0.08	11.70	0.11	0.71	0.02
	Bottom	3.53	0.62	0.06	0.03	0.59	0.09
Coastal	Surface	0.29	0.04	ND*	ND	2.89	0.06
	Bottom	0.39	0.06	ND	ND	3.11	0.11

* ND – Not done

Table 26: Mode of functioning of the PAB in Mandovi estuary

Station	$\mu g.C.l^{-1}.h^{-1}$					
	Water Column	Production rates	V_{max} (Glucose)	V_{max} (Glutamic acid)	Productivity: Activity*	PAB mode of function
Mandovi	Surface	3.089	14.32	0.29	17:83	Activity>Productivity
	Bottom	3.369	23.37	0.06	13:87	Activity>Productivity
Zuari	Surface	5.499	4.84	0.11	52:48	Productivity>Activity
	Bottom	6.395	3.07	0.12	67:33	Productivity>Activity
Coastal	Surface	10.477	3.13	0.06	77:23	Productivity>Activity
	Bottom	9.833	2.86	0.16	76:24	Productivity>Activity

* Sum of production rates and activity assumed to be 100%

Table 27: Mode of functioning of the PAB in the Zuari estuary

Season	$\mu g.C.l^{-1}.h^{-1}$					
	Water Column	Production rates	V_{max} (Glucose)	V_{max} (Glutamic acid)	Productivity: Activity*	PAB mode of function
Pre-monsoon	Surface	1.58	7.17	0.14	3:97	Activity>Productivity
	Bottom	0.66	1.59	0.20	8:92	Activity>Productivity
Monsoon	Surface	5.33	18.80	0.19	22:78	Activity>Productivity
	Bottom	1.67	0.58	0.10	71:29	Productivity>Activity
Post-monsoon	Surface	8.85	0.58	0.05	93:7	Productivity>Activity
	Bottom	13.54	0.89	0.08	93:7	Productivity>Activity

* Sum of production rates and activity assumed to be 100%

Table 28: Mode of functioning of the PAB in the coastal station

Season	$\mu g.C.l^{-1}.h^{-1}$					
	Water Column	Production rates	V_{max} (Glucose)	V_{max} (Glutamic acid)	Productivity: Activity*	PAB mode of function
Pre-monsoon	Surface	3.14	1.38	0.09	68:32	Productivity>Activity
	Bottom	1.46	2.97	0.28	31:69	Activity>Productivity
Post-monsoon	Surface	17.81	4.88	0.04	78:22	Productivity>Activity
	Bottom	18.21	2.75	0.03	87:13	Productivity>Activity

* Sum of production rates and activity assumed to be 100%

productivity:activity ratio of 78:22 for the surface waters and 87:13 for the bottom waters.

An analysis of carbon production rates in the three systems showed that each system is unique by itself (Table 29). At Mandovi the PAB production constituted 25.7% of the TBP and 19.8% of primary production. The PAB production was 3.23 $\mu g.C.l^{-1}.hr^{-1}$, but the uptake potential of glucose was 18.85 $\mu g.C.l^{-1}.h^{-1}$ and that of glutamic acid was 0.18 $\mu g.C.l^{-1}.h^{-1}$; thus showing that this station to be more active than productive. At the Zuari station, the PAB production constituted 51.8% of the total bacterial production and 31.3% of primary production. The PAB production rate was 5.95 $\mu g.C.l^{-1}.h^{-1}$ and the uptake potential rate for glucose was 3.96 $\mu g.C.l^{-1}.h^{-1}$ and glutamic acid 0.12 $\mu g.C.l^{-1}.h^{-1}$. This concludes that this system is both productive and active.

At the coastal station, PAB production rates constituted 64% of the total bacterial production, and 88% of the primary production. The PAB-production was 10.2 $\mu g.C.l^{-1}.h^{-1}$ and the uptake potential was 3 $\mu g.C.l^{-1}.h^{-1}$ for glucose and 0.1 $\mu g.C.l^{-1}.h^{-1}$ for glutamic. Thus, this coastal station was more productive than active. Hence, it is inferred that the PAB in the mangrove-dominated Mandovi estuary is more active mineralizer than productive; the coastal station in the Arabian Sea is more productive than active; and the Zuari estuary that is influenced by mangroves and marine environment imbibes the properties of both the above systems and therefore, is equally productive and active.

Table 29: Annual outlook of the mode adopted by PAB in the three systems

Stations	$\mu g.C.l^{-1}.h^{-1}$				
	Production rates	V_{max} (Glucose)	V_{max} (Glutamic acid)	Productivity: Activity* Ratio	PAB mode of function
Mandovi	3.230	18.850	0.180	14:86	Activity> Productivity
Zuari	5.950	3.960	0.117	59:41	Productivity>Activity
Coastal	10.150	3.000	0.117	77:23	Productivity>Activity

* Sum of production rates and activity assumed to be 100%

4.5. STATISTICAL ANALYSIS

4.5.1 MODELLING

The multiple regression analyses were performed on independent variables using bacterial biomass and production rates as the response separately. The software avoids highly related variables and thus prevents multi-colinearity.

Modeling of various parameters to derive a predictive model for PAB biomass and PAB production rates on particles lead to the inclusion of 12 essential parameters as best regression set can be seen in the various equations. These parameters can be classified into three groups as physico-chemical parameters, biochemical parameters and primary production. The regression equation has been derived on the assumption that the observed values would repeat for three annual cycles without variation for purpose of multiple regression analysis.

MANDOVI

The multiple regression equations for total particle-associated bacterial biomass (**B**) and particle-associated production rates (**PBP**) for Mandovi surface and bottom waters are:

Surface waters:

Log B = 1430 + 30.8 T – 423 pH + 5.81 S – 5303 SPM – 0.000442 PN + 166 OC – 3.08 RNA + 4.49 DNA + 177 R +1.68 MB + 7.53 PP + 3.61 Cha

Bottom waters:

Log B = 2606 – 93.8 D – 93.5 T + 3.40 pH + 0.850 S + 15.0 SPM + 0.020 PN + 0.993 RNA + 0.256 DNA – 7.06 R + 0.868 MB + 13.8 PP – 7.64 Cha

Surface waters:

PBP = – 45.18 + 1.20 T – 1.54 pH – 0.159 S + 0.000016 PN – 110 SPM – 19.5 OC – 0.126 RNA + 0.158 DNA + 5.73 R – 0.00422 MB + 0.477 PP + 0.130 Cha

Bottom waters:

PBP = 80.48 – 5.19 T – 5.71 D + 10.6 pH – 0.0816 S + 0.000692 PN – 17.5 SPM – 0.00309 RNA – 0.00906 DNA + 0.369 R + 0.00668 MB + 0.658 PP – 0.125 Cha

Zuari

The regression equation for total particle-associated bacterial biomass **(B)** and particle-associated bacterial production rates **(PBP)** for Zuari surface and bottom waters.

Surface waters:

Log B = – 1190.5 + 32.4 T – 4.74 pH + 3.48 S + 622 SPM – 0.0128 PN – 1582 OC – 3.32 RNA + 6.45 DNA + 247 R– 0.130 MB + 17.0 PP– 2.98 Cha

Bottom waters:

Log B = –132 + 250 D – 315 T + 780 pH + 111 S + 1955 SPM + 4741 OC – 0.808 RNA – 7.39 DNA– 10.8 R – 1.29 MB – 9.46 PP– 10.2 Cha

Surface waters:

PBP = 873.5 – 19.7 T + 776 OC + 1.33 RNA – 2.19 DNA + 2.67 S – 739 SPM – 107 R – 22.2 pH – 6.09 PP + 1.58 Cha + 0.000603 PN + 0.00464 MB

Bottom waters:

PBP = – 46.4 + 10.5 T – 8.47 D + 0.0686 AM + 0.00618 RNA + 0.496 DNA – 2.45 S – 82.8 SPM + 0.113 R – 23.9 pH + 0.0401 PP – 20.8 OC + 0.0976 Cha

Coastal:

The regression models were drawn using PAB-biomass (**B**)and PAB-production (**PBP**), as predictors for the surface and bottom waters. The regression predictive equations are given below.

Surface waters:

Log B = 2280 – 25.3 T – 134 pH – 34.1 S + 3219 SPM – 0.575 RNA + 3.36 DNA + 92.9 R + 2.22 MB

Bottom waters:

Log B = 25545 + 358 D – 254 T – 586 S – 2978 SP + 37.2 RNA – 39.4 DNA– 1730 R – 0.581 MB

Surface waters:

PBP = 173.3 – 3.51 T – 13.4 pH – 6.37 S + 318 SPM – 0.421 RNA + 0.602 DNA + 14.9 R + 0.0852 MB

Bottom waters:

PBP = 115 – 0.184 T + 1.27 D – 4.06 S – 25.8 SPM – 0.191 RNA + 0.801 DNA + 2.70 R – 0.00981 MB

Comparative analysis:

Multivariate analysis of the parameters showed that the regression equation derived for the predictive model included the same parameters for the response variable PAB biomass and PAB production. The R-sq. Adj. = 100%. However station-wise there was a minor difference in the variables responsible for the response (Table. 30)

Table 30: Comparison of parameters present in the regression equation for PAB biomass and PAB production rates at all the stations

Parameters	Mandovi		Zuari		Coastal	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Depth		✓		✓		✓
Temperature	✓	✓	✓	✓	✓	✓
pH	✓	✓	✓	✓	✓	
Salinity	✓	✓	✓	✓	✓	✓
Particle number	✓	✓	✓			
Suspended load	✓	✓	✓	✓	✓	✓
Organic content	✓		✓	✓		
RNA content of particles	✓	✓	✓	✓	✓	✓
DNA content of particles	✓	✓	✓	✓	✓	✓
RNA:DNA ratio	✓	✓	✓	✓	✓	✓
PAM biomass	✓	✓	✓	✓	✓	✓
Primary production rates	✓	✓	✓	✓		
Chlorophyll *a* carbon	✓	✓	✓	✓		

4.5.2 PRINCIPAL COMPONENT ANALYSIS (PCA)

Mandovi

At Mandovi station PCA analysis was done using 15 parameters and gave 5 factors in the surface waters (Table 31) with a cumulative percentage variation to an extent of 84.3%, while in the bottom waters (Table 32) 84.4% of the variation was by four factors. The master group for the surface waters which accounted for 30.9% of the variation included suspended load, primary production, salinity, (positive loadings) and DNA-PAB (negative loadings), while the factor 2 contained total PAB number (positive loading) chlorophyll *a* carbon and PAB production rates (negative loadings) which accounts only for 19.3% of the variation. In the bottom waters the master group which is responsible for 32.8% of the variation includes RNA-DNA-PAB, RNA-PAB, (positive loadings) and pH, temperature (negative loadings),. The factor 2, which causes 21% of variation, includes salinity and chlorophyll *a* carbon (negative loadings). From this it is obvious that the master groups of both the surface and bottom waters contain different parameters that are responsible for the variation in the surface and bottom waters.

Zuari

At Zuari station principal component analysis was done using 15 variables and gave 5 factors in the surface (Table 33) and 6 factors in the bottom waters (Table 34) accounting for 85.2% and 92% of the variation respectively. The master group which accounts for 28.2% in the surface includes suspended load organic content of particles, RNA:DNA-PAB (positive loadings) and DNA-PAB (negative loadings). The second factor,

Table 31: Principal component analysis of the parameters at the surface waters of the Mandovi estuary

Parameters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Temperature	-0.278	-0.206	0.215	**0.338**	0.245
pH	-0.085	-0.230	-0.026	**0.649**	0.149
Salinity	**0.329**	-0.299	-0.230	0.053	0.117
Suspended load	**0.357**	0.103	0.169	-0.278	0.304
Particle number	-0.095	-0.336	0.242	**-0.345**	0.070
Organic	0.079	0.143	**0.415**	0.339	-0.139
RNA content of particles	0.324	-0.182	**0.329**	0.144	-0.224
DNA content of particles	**-0.378**	0.032	-0.255	-0.084	0.172
RNA:DNA ratio	0.342	0.177	**0.354**	-0.056	0.206
PAM biomass	0.297	-0.057	-0.145	0.114	**0.596**
Total PAB	0.040	**0.442**	-0.124	-0.021	-0.055
Viable PAB	0.259	-0.181	**-0.411**	0.060	-0.332
PAB production rates	-0.167	**-0.334**	0.006	-0.240	0.322
Primary production rates	**0.339**	-0.178	-0.305	0.067	-0.054
Chlorophyll *a* carbon	0.006	**-0.481**	0.209	-0.199	-0.301
Percent variation	30.9	19.3	15.5	11.6	6.9
Cumulative percentage	30.9	50.3	65.8	77.4	84.3

Table 32: Principal component analysis of the parameters at the bottom waters of the Mandovi estuary

Parameter	Factor 1	Factor 2	Factor 3	Factor 4
Depth	0.038	0.204	0.283	**-0.352**
Temperature	**-0.354**	-0.156	-0.279	0.000
pH	**-0.410**	-0.038	0.074	0.094
Salinity	-0.258	**-0.423**	-0.049	0.017
Total suspended load	0.291	-0.299	-0.106	**0.326**
Particle number	-0.234	-0.059	0.192	**0.435**
RNA content of particles	**0.366**	-0.225	-0.144	0.200
DNA content of particles	-0.238	0.328	0.090	**0.391**
RNA:DNA ratio	**0.400**	-0.119	-0.100	0.251
PAM biomass	-0.074	-0.309	**0.356**	-0.118
Total PAB	0.135	-0.152	**0.472**	0.094
Viable PAB	-0.176	-0.271	**0.377**	-0.034
PAB production rates	-0.136	0.387	-0.085	**0.415**
Primary production rates	-0.135	-0.030	**-0.439**	-0.319
Chlorophyll *a* carbon	-0.239	**-0.383**	-0.235	0.131
Percent variation	32.8	21.0	19.9	10.7
Cumulative percentage	32.8	53.8	73.7	84.4

Table 33: Principal component analysis of the parameters in the surface waters of Zuari estuary.

Variables	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Temperature	-0.156	-0.326	**0.400**	-0.068	0.052
pH	0.114	0.039	0.196	0.368	**-0.583**
Salinity	0.099	**-0.505**	-0.021	0.230	-0.141
Particle number	-0.183	**-0.415**	-0.415	0.076	0.335
Total suspended load	**0.404**	-0.061	0.060	0.163	0.360
Organic content	**0.411**	0.145	-0.021	0.324	0.175
RNA content of particles	0.158	**-0.390**	-0.060	-0.353	-0.104
DNA content of particles	**-0.381**	0.250	-0.052	0.120	-0.026
RNA/DNA ratio	**0.459**	-0.027	0.011	0.187	0.170
PAM biomass	-0.148	-0.077	**-0.467**	-0.043	0.293
Total PAB	0.199	0.130	**-0.346**	0.124	-0.274
Viable PAB	-0.210	-0.211	-0.305	**0.364**	0.072
PAB production rates	-0.259	0.259	0.015	**0.448**	0.106
Primary production rates	-0.048	-0.262	**-0.494**	0.043	0.047
Chlorophyll *a* carbon	-0.178	-0.146	0.332	0.190	**0.483**
Percent variation	28.2	21.3	18.2	9.7	7.8
Cumulative percentage	28.2	49.5	67.7	77.5	85.2

Table 34: Principal component analysis of the parameters in the bottom waters of Zuari station

Parameters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Depth	-0.015	-0.171	0.322	**0.489**	0.078	0.356
Temperature	0.109	**0.427**	0.337	-0.034	-0.129	0.077
pH	**-0.399**	0.160	0.142	-0.151	0.257	-0.011
Salinity	0.024	**0.451**	-0.207	0.154	-0.265	0.059
Total suspended load	**0.373**	0.085	-0.169	-0.155	-0.221	0.352
Organic content	**0.350**	-0.327	-0.093	0.201	-0.023	-0.004
RNA content of particles	**0.356**	0.121	-0.196	-0.231	-0.292	-0.086
DNA content of particles	-0.360	-0.173	0.027	-0.155	-0.345	**0.412**
RNA:DNA ratio	0.319	**-0.340**	-0.071	0.233	0.014	-0.040
PAM biomass	-0.212	0.124	**-0.444**	0.328	0.087	0.113
Total PAB	-0.136	-0.246	**-0.427**	-0.091	0.283	0.203
Viable PAB	-0.127	0.196	**-0.478**	0.142	0.040	-0.270
PAB production rates	-0.303	-0.191	-0.115	0.066	**-0.588**	0.175
Primary production rates	0.158	0.061	-0.134	-0.427	0.358	**0.524**
Chlorophyll *a* carbon	0.111	0.362	0.000	**0.441**	0.153	0.359
Percent variation	28.3	19.9	18.2	11.0	7.7	6.7
Cumulative Percentage	28.3	48.2	66.4	77.5	85.2	92.0

which was responsible for 21.3% variation, included RNA-PAB, salinity and particle number (negative loadings). The master group for the bottom waters accounted for 28.3% which included suspended load, organic content of particles, RNA-PAB (positive loadings) and pH (negative loading), while the second factor showed 19.9% of the variation exhibited by temperature, salinity (positive loadings) and RNA:DNA (negative loading). The Zuari surface waters more of less have the influence of common parameters like SPM and organic content to the bottom waters.

Coastal

Principal component analysis was performed for the surface (Table 35) and bottom waters (Table 36) using 11 parameters and gave 4 factors which accounted for 92.3% of the variation for surface waters and 85.8% of the variation due to 3 factors in the bottom waters. The first master group accounted for 42.7% of the variation, which included parameters like RNA (positive loadings) with bacterial production rates on particles and DNA (negative loadings). The second factor consisting of total-PAB and viable-PAB numbers (negative loadings) was responsible for only 26.3% of the variation. In the bottom waters for the master group parameters like temperature, RNA, RNA:DNA (positive loadings), bacterial production rates on particles and DNA (negative loadings) an accounted for 42% of variation, while 26.2% variation in factor 2 was due to the same parameters as those of the surface waters with additional parameter, PAM biomass all of which are with negative loadings.

Table 35: Principal component analysis of various parameters at the coastal surface waters.

Parameters	Factor 1	Factor 2	Factor 3	Factor 4
Temperature	0.252	0.339	-0.100	**0.548**
PH	0.035	-0.208	**-0.754**	0.071
Salinity	0.392	-0.009	**0.398**	-0.063
Total suspended load	0.154	0.355	**-0.368**	-0.221
RNA content of particles	**0.419**	0.022	-0.175	-0.103
DNA content of particles	**-0.435**	0.141	-0.031	0.145
RNA:DNA ratio	0.340	-0.169	-0.196	**-0.375**
PAM biomass	0.173	-0.403	0.030	**0.575**
Total PAB	0.016	**-0.560**	-0.087	0.161
Viable PAB	-0.280	**-0.414**	0.050	-0.321
PAB production rates	**-0.411**	0.139	-0.216	0.100
Percent variations	42.7	26.3	13.3	10.1
Cumulative percentage	42.7	69.0	82.3	92.3

Table 36: Principal component analysis of various parameters in the bottom waters of the coastal station

Parameters	**Factor 1**	**Factor 2**	**Factor 3**
Depth	0.001	-0.116	**0.639**
Temperature	**0.434**	0.050	0.149
Salinity	0.003	-0.066	**0.640**
Total suspended load	0.032	-0.296	**-0.318**
RNA content of particles	**0.435**	-0.126	-0.142
DNA content of particles	**-0.453**	-0.014	-0.055
RNA:DNA ratio	**0.443**	0.057	0.018
PAM biomass	0.031	**-0.564**	-0.132
Total PAB	-0.044	**-0.542**	0.125
Viable PAB	-0.119	**-0.497**	0.031
PAB production rates	**-0.450**	0.120	0.035
Percent variations	42.0	26.2	17.6
Cumulative percent	42.0	68.2	85.8

Thus, comparing the parameters responsible for the variation in the first 2 major groups, it was observed that 5 of the parameters were responsible for variation in both the surface and bottom waters, except the parameters like temperature, RNA:DNA and PAM biomass which cause variations in the bottom waters exclusively. Hence, not much variation is observed between the surface and bottom waters of the coastal station.

Comparative analysis:

Communality of variables as seen in Table 37 showed that pH was the main factor responsible for variation at the surface waters of all stations. Apart from this PAM biomass was also responsible for variation at Mandovi and coastal surface waters, while at Zuari the particle numbers influenced the variation.

The bottom waters of Mandovi were influenced by the viable PAB and PAB production rates, Zuari by DNA content of particles and PAB production and in the coastal station depth and salinity played a major role in the variation.

The general anaerobic counts of particle-associated bacteria were 2.32 x $10^5.l^{-1}$ and 1.33 x $10^6.l^{-1}$ in the bottom waters. The SRB population in the bottom water was found to be five times the value (1 x $10^3.l^{-1}$) of the surface. The nitrate reducers showed a higher population in the bottom water of 2.1 x $10^7.l^{-1}$, which was, more than an order higher than its surface value. The denitrifiers were 2.2 x $10^5.l^{-1}$ at the surface and 1 x $10^5.l^{-1}$ in the bottom waters. The thiobacillus like organisms showed surface values to be more than twice than that of the bottom value (2.0 x $10^5.l^{-1}$). All the anaerobic PAB

Table 37: Proportionality of total variables

Variables	Communality of variables					
	Mandovi		Zuari		Coastal	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Depth		0.25		0.53		**0.42**
Temperature	0.34	0.23	0.30	0.54	0.49	0.21
pH	**0.50**	0.18	**0.53**	0.93	**0.62**	
Salinity	0.27	0.29	0.34	0.39	0.32	**0.41**
Particle number	0.30	0.25	**0.50**			
Suspended load	0.34	0.28	0.33	0.98	0.33	0.19
Organic content	0.33		0.33	0.86		
RNA content of particles	0.32	0.24	0.32	0.91	0.22	0.23
DNA content of particles	0.25	0.27	0.23	**1.06**	0.23	0.21
RNA:DNA ratio	0.32	0.25	0.28	0.81	0.32	0.20
PAM biomass	**0.48**	0.25	0.33	0.76	**0.52**	0.34
Total PAB	0.22	0.25	0.27	0.64	0.35	0.31
Viable PAB	0.38	**0.33**	0.32	0.62	0.36	0.26
PAB production rates	0.30	**0.35**	0.35	**1.04**	0.24	0.22
Primary production rates	0.25	0.31	0.32	0.92		
Chlorophyll *a* carbon	0.41	0.28	0.43	0.70		

formed more than 80% of the total bacterial population except in the bottom waters where the PAB nitrate reducers and denitrifiers formed 55.26% and 16.67% of the total bacterial population respectively.

5.0 DISCUSSION

Food webs in estuarine systems revolve around particles, which are mostly detrital in nature. The associated bacteria thrive on terrestrial and fluvial organic matter that passes through these systems to the oceans. One of the common features of tropical estuaries specially when compared with oceanic environment is the high abundance of particles and the bacteria attached to them. The contribution of particle associated bacteria in the tropical estuarine system is much more significant than in lakes, rivers, open seas and temperate estuaries. Their role can be even more substantial than those in shelf waters (Wangersky, 1984). The tropical Indian estuarine systems are more dynamic as they come under the influence of monsoon and experience a wind field, which is energetic and at times possesses a well-marked annual cycle (Shetye *et al.,*1985).

The present study focuses on the bacteria associated with particles of > 3 μm in size. It discusses their abundance, activity and productivity in relationship to a variety of factors, which are known to affect them. It deals with the role of bacteria associated with particles and develops regression models to predict bacterial abundance and productivity in Mandovi and Zuari estuaries and the coastal ecosystem. Further, it attempts to explain the extent of influence of different variables through principal component analyses. Finally, it projects the importance of PAB in the estuarine food web and traces the flow of carbon through them.

5.1 BACTERIAL ABUNDANCE

In Mandovi and Zuari estuaries the PAB numbers ranged from 0.08 x $10^9.l^{-1}$ to 1.88 x $10^{10}.l^{-1}$. There has been no discernible variation in the PAB numbers between the surface and bottom waters. Particle associated bacteria are relatively numerous in some of the freshwater and estuarine environments (Wilson and Stevenson 1980, Bell and Albright, 1982 and Ducklow, 1994). In river Danube in Austria, the attached bacterial density varied from 0.1-1.4 x $10^9.l^{-1}$ (Hoch *et al.*, 1995). The bacterial abundance and variation on particles in Zuari and Mandovi estuaries was higher than in the temperate waters. The variation in the estuaries of higher latitudes was by a factor of 3-14. (Ducklow 1982, Kondratiff and Simmons 1985, Hoch *et al.*, 1995). This variation have been attributed to factors like temperatures and salinity. Temperature affects directly by increasing the growth rate of bacteria associated with particles. The effect of salinity is indirect. In Columbia river estuary (Crump *et al.,* 1998) maximum abundance was observed in the ETM (Estuarine Turbidity Maximum) region. In this region where fresh water meets the marine, there is increased turbidity where particles and the associated bacteria accumulate.

In the Mandovi and Zuari estuaries, the variation in abundance of PAB is much more i.e. by a factor of 100. This variation is perhaps due to the variation in the particle dynamics which in turn gets influenced by the changes in the hydrological parameters. Unlike the temperate estuaries, the variation in temperature in the tropical one is less marked. In Mandovi and Zuari estuaries, temperature showed little variation which, indicates that they are typical tropical

systems. Nevertheless, an unusual negative relationship was observed between temperature and the abundance of PAB. This negative relationship is because temperature is a wide variable, which could be used as a proxy for sunlight, which influences the bacterial population negatively. The effect of sunlight is mitigated when the waters are turbid as in the case of Mandovi.

The abundance of PAB is influenced by the salinity factor as in the temperate region. The effect is indirect here also and linked to the monsoons. There is a lot of terrigenous particles associated with the fresh water inflow which not only trigger the multiplication of the autochthonous bacteria but also add allochthonous ones. This accounts for the high PAB observed in the monsoon season especially in Mandovi. These numbers build up to an all time high in the post monsoon.

Another factor that could influence abundance of PAB of the particle is the size. Bacterial colonization on particles in the size range of >1 –140 μm varied from 2 – 192 cell per particle and on particles of the size of ≤ 40 μm, it varied from 2-80 cells (Almeida and Alcantara, 1992). In this study the numbers ranged from 1-27 per particle which are higher to the range of 2.9 - 6.4 cells per particle obtained by Irriberri *et al.*, (1987) in coastal waters.

Bacteria occupied about 3.26μm^2 of the surface area of the particle colonizing about 10.6% of the surface. Most of the particles in the network were in the size range of 3 - 15μm and accounted for 55% of the total particles. A similar observation was made in the Humber estuary where >60% of the

particles were in the range of 5-14μm and maximum colonization were observed on these particles (Goulder 1976, 1977). However, colonisation of smaller particles is also not uncommon (Nagata 1987, Almeida and Alcantra 1992, Kirchman, 1983, Berger *et al.,* 1996).

Besides number and size, nature of particles dictate the preference and extent of colonization. SEM scan showed that selective particles are colonised. Sieburth (1968) observed no colonisation on the surface of diatomsand certain phytoplankton. Likewise, particles rich in inorganic content harbour less number of bacteria. In these tropical estuarine systems it was found that the inorganic content ranged from 0.001 to 1.0 $mg.l^{-1}$. This was less than the amount seen in Danube estuary (Hoch *et al.*, 1995)) where inorganic content ranged from 2.2 – 13.2 $mg.l^{-1}$. In Mandovi and Zuari estuaries particles rich in abundance of inorganic content drastically reduced the colonization. The absence of relationship between suspended load and PAB could be due to inorganic nature of the particles. Inorganic particles associated with organic matter was much higher than the inorganic particles alone in Danube (Hoch *et al.*, 1995 and Berger *et al.*, 1996) However, when inorganic particles are associated with the organic matter, colonisation is effected. In San Francisco Bay particles are largely composed of mineral grains, bound together with organic matter. This organic coating is also responsible for glueing several of these discrete particles together and forming larger units. The degradation of these units is very fast showing clearly that bacteria have a preference for

attaching to particles. This organic matter may consist of carbohydrate, protein and nucleic acids.

The bacterial distribution depends to some extent on the organic matter as the organic content of particles promote bacterial colonization. There was a fluctuation in the amount of organic carbon from 10 to 40 $mg.l^{-1}$ in these estuaries. However, the concentrations did not co-vary with the abundance. It could be due to complex nature of matter constituting POM. Organic matter is a complex size continuum of largely uncharacterized mixture of monomers and polymers including humic acid like material not utilized by bacteria (Azam *et al.*,1995, Wafar *et al.*, 1997). From the analyses of the nature of particles, for example organic content in totality did not give any meaningful relationship with PAB abundance though it is an important parameter. Hence analysis of utilizable organic substrate could be more meaningful (Azam *et al.*, 1995). With new and sophisticated techniques being introduced it could be possible to analyze the spectrum of compounds utilized by bacteria in nature.

The higher proportion of PAB at all the stations especially in the Mandovi could not only be attributed to the high organic content but also to the smaller particle size (Almeida and Alcantara, 1992). The influence is conspicuous during the monsoons when the POC content increased. Positive relation of TC with POC ($r=0.5172$: $p<0.05$) at Mandovi surface suggests that 25% of the variation is due to this factor. In the other stations the relationship was absent or negative for example as in the bottom waters of the estuaries indicating that the bottom dwelling diatoms can have an antagonistic role.

Monsoon triggered an increase in bacterial load in the post-monsoon season. This could be due to the increase in the allochthonous carbon input when freshwater meets seawater. Besides the Mandovi station is the shallowest which means an additional sedimentary organic exchange into the water column.

Nucleic acids are important constituents of particles, which are a source for N and P (Paul *et al,*. 1987). These are present at a lower concentration as compared to other molecules and could be derived in the form of dead and living matter. The concentration of DNA was abundant in post-monsoon together with the increase in bacterial numbers.

DNA and RNA concentrations generally displayed negative relationship with each other ($p<0.001$) at the surface ($p<0.05$) and at the bottom waters. These nucleic acids are known to generally display opposite temporal pattern which are likely to be dependant on the nature and characteristics of DNA and RNA molecules (Dell'anno *et al.*, 1999). The contrasting abundance could be due to the difference in the activity/productivity mode of the particle-associated bacteria as discussed later in detail under section on productivity. Holm-Hansen and Booth (1966) opine that majority of the DNA was detrital, but Fabiano *et al.*, (1993) argue that DNA:RNA ratio is a relative measure of the metabolic state of the suspended particles. DNA is abundant when the population is productive and RNA when active. Such differences have been noticed in the network both temporally and spatially. Besides, DNA has lower

turnover (Karl *et al.*, 1988) and longer degradation rates than RNA (Novitsky, 1986).

The proportion of the PAB to the total bacterial load was much higher to those reported for temperate waters. Their abundance was generally < 10% (Ducklow, 1982, Bell and Albright, 1982, Kirchman and Mitchell 1982, Hoch *et al.*, 1995, Berger *et al.*, 1996). However, there have been reports of values that are close to the present values. The Mandovi estuary harbored the maximum amounting to 76% of TB almost similar to that observed in Humber estuary in England, Goulder (1977) or York river estuary in U.S.A (Ducklow 1982), or Frazer river plume in Canada (Bell and Albright, 1981). The only report of high PAB was from a tropical creek of Indus valley (Bano *et al.*,1997).

These data show that PAB not only constitute a significant portion of the total bacterial population but also continue to be significant throughout the annual cycle in both the surface and bottom waters. Attached bacteria were variable during different sampling periods becoming dominant at times. Monsoonal peak was more dominant in Mandovi thus showing a seasonal pattern in abundance. Temporal variations are less marked in the tropical estuaries as in for example Indus river delta (Bano *et al.*, 1997), when compared to temperate waters, where seasonality in bacterial abundance is well established.

The interrelationship between parameters influencing the nature of particles differs in the three systems. POC is highly related to detritus indicating that most of these are from sources other than phytoplankton. The strong

relationship between organic content and detritus suggest that it is mostly organic. The PON in the coastal surface perhaps derives the nitrogen from the nucleic acids. In the coastal station PAB is related to both PON and POC. Therefore it is apparent that the distribution of PAB is dependant on the definite parameters in the coastal station whereas in the estuaries these parameters are not limiting as the estuarine system is complex and the environmental parameters not directly related to their distribution. Nevertheless the organic content is an important parameter that influences the PAB distribution.

5.2 BACTERIAL PRODUCTIVITY

One of the most important variables for the assessment of the bacterial trophic role is their production rate. Like PAB abundance (secondary standing stock), production showed a spatio-temporal variation in the present study. The annual average values were 77.5, 142.8 and 248.8 $\mu gl^{-1}d^{-1}$ at Mandovi, Zuari and coastal stations respectively, with exceptionally high values during certain months. The post monsoon recorded the highest bacterial production. Such wide variations have been recorded by Nagata (1987) who calculated bacterial production rates for <1um fraction which ranged from 5-50$\mu g.l^{-1}d^{-1}$ in Biwa lake in Japan. Rublee *et al.,* (1984) found it to be 1-13 $\mu gl^{-1}d^{-1}$ in New port estuary. In Columbia estuary, the production rate varied from 14.16 to 108.0 $\mu gl^{-1}d^{-1}$ (Crump *et al.,* 1998). These estimates are much lower than the values recorded in the tropical estuaries. Higher values than the values in the present

study has been reported for Loire estuary (803 $\mu g.l^{-1}d^{-1}$) in temperate regions in Bietri Bay, Ivory coast (2184 $\mu g.l^{-1}d^{-1}$) and in a tropical mangrove tidal creeks of Indus river Delta in Pakistan (900 $\mu g.l^{-1}d^{-1}$) in the tropical regions (Bano *et al.*, 1997, Ducklow and Shiah, 1993). This is very similar to the Hudson river estuary which is highly productive (Findlay *et al.*, 1991). Based on high productivity values, estuaries under study can be classified as heterotrophic ecosystems. This heterotrophic nature is further confirmed by the high bacterial turn over rates which has been is discussed later in this chapter.

The contribution of the bacterial production to the total production also showed a wide variation. The average annual contribution of PAB productivity to the total ranged from 23 to 57% with sporadic peaks and which was higher than the previously recorded values of 7-22% in New port estuary (Rublee *et al.*, 1984). The values recorded at Columbia estuary (90%) by Crump *et al.* (1998) or mangrove delta (73-93%) were higher than the values observed by Bano *et al.*, (1997). These tropical estuaries had a constantly high contribution to the total productivity throughout the year and formed a significant component. Higher productivity of 60-67% in the coastal station as compared to the estuarine stations is due to the spatial shift of organic input from the estuaries to the coast. Such a shift is also continuous by the vertical flux of the PAB to the bottom layer. Ducklow *et al.*, (1982) have measured the settling rates of 0.12 – 1m d^{-1} for attached bacteria in Hudson river plume and have estimated that 3-67% of the daily production settles out of the water column. No data are available on the settling rate in tropical estuarine systems. Yet, it is presumed

that the setting may be taking place at a good rate as there was no clear stratification seen throughout the year. As particle bound bacteria are a major component of the estuarine system, it is presumed that there would be potential loss of a significant fraction of the total productivity with sinking particles from the water column to the sediments. This variability may be seasonal and spatial as observed in the present study. Besides, sedimentary inputs of organic matter of apparently labile nature could augment the bacterial number in the bottom water.

Season-wise, bacterial production on particles was the highest during the post monsoon period which, may be a consequence of a large in put of suspended matter in to the estuaries during the monsoon, and the coastal station recorded the highest. The predominance of high productivity in the coastal station is due to the estuary borne import of the detrital carbon into the coastal system. The variability in productivity could be due either to bacterial abundance or growth rates or both (Griffith *et al.*, 1990 and Unanue *et al.*, 1992).

The pattern of bacterial production was neither parallel to the abundance of bacterial standing stock nor to the total viable fraction on particles. Similar absence in relationship has been discussed by Wehr *et al.*, (1998). As productivity is related to abundance, factors that affect abundance also affect productivity. The other factors affecting the abundance in the Mandovi and Zuari estuaries have been discussed at length in the preceding section.

It is therefore possible that productivity in this estuarine water was more dependent on growth rate. PAB have a higher growth rate than the unattached bacteria in both lacustrine and marine realms (Crump *et al.*,1998). The growth rate of bacteria attached to particles was high as they were observed to be large. Large bacteria are active and they grow faster (Goosen *et al.*, 1995). This high growth rate is in turn dependant on both temperature and substrate. These factors have long been recognized as dominant factors in regulating bacterial growth rates in aquatic systems (Cole *et al.*, 1988). Temperature affects growth rate more in the temperate waters (White *et al*,,1991; Hoch and Kirchman,1993, Shiah and Ducklow, 1994 a,b). It gains more importance and becomes a major regulating factor when nutrients are non-limiting. In tropical estuaries, the variation in temperature was not as wide as in the temperate region. Still it was enough to affect estuarine production during the monsoon when temperature dropped as nutrients were non limiting. Other important factors that affects the growth rate are the quality and availability of substrates. Though the substrate availability and bacterial standing stock was highest during the monsoon the productivity was the lowest. This is because, bacteria colonise on the particles but there was not sufficient time to acquire an estuarine adaptation to increase their growth rate. Besides, the available substrate may not be labile enough to accelerate the growth. In the estuaries, the quantity and quality of the organic substrates would explain most of the variations in bacterial production while temperature had only a seasonal effect.

In a given population the cell specific productivity is dependent not only on the growth rate cell *per se* but also the number of productive cells in a population. The cell specific productivity is calculated on the gross abundance and it varied from < 1 to 82. An average ratio of 2.6 has been reported from Columbia estuary (Crump *et al.*, 1998) though others have reported higher ratio (Kirchman and Mitchell, 1982, Kirchman and Ducklow, 1987). The rate of thymidine incorporation per cell was high 30 $fg.C.cell^{-1}.h^{-1}$. Bacterial production per cell has also been found to be higher in attached bacteria in some estuarine and coastal system (Iriberri *et al* .,1987, Crump *et al* 1998 , Griffith *et al.*, 1994.) but not in others (Ducklow and Kirchman, 1983). The tendency of organic matter to absorb on to particle allows this material to be available to attached bacteria (Pedros Alio and Brock ,1983). This may explain the high thymidine incorporation per cell.

The variation in specific activity could be due to the variation in the viable fraction (Crump *et al*, 1998). A high ratio of thymidine incorporation rate to cell abundance could also be due to the existence of large population of active cells among attached bacteria (Crump *et al.*,1998). However, one failed to find a relationship between viable cells and productivity. Hence it is inferred that this lack of relationship could be due to the differences in individual cells of the viable fraction. Therefore a large variability in this property reflects that the productivity is dependent more on growth rate of individual viable cells than on the total viable bacteria on particles. Besides, PAB were large and showed a variation from 0.1 to 4.7 μm^3. In nutrient rich environment bacteria have been

reported to be larger in size (Iriberri *et al.*, 1987). Consequently, there would be large variation in carbon content cell $^{-1}$. The use of a conservative factor of 20 fg (Lee and Fuhrman, 1987) would have under estimated the productivity in these waters. Bell *et al.*, (1983) recommended the use of 30 fg.C.cell^{-1} for environments with high load of nutrients. Moreover, some conversion factors for incorporated thymidine to bacterial cell number obtained emperically in the riverine water are higher (6-8 x10 ^{18} cells per mole (Iriberri *et al.*, 1990). In general, the productivity of this system was limited by the growth rate of the actively growing cells as depicted by the relatively high V_{max} and K_t+S_n (vide section on heterotrophic activity). The seasonal variation in this eutrophic ecosystem is regulated by temperature and nature of the substrate available with the former playing a lesser role.

Bacterial specific growth or turnover time measured in estuaries in riverine plumes and deltas generally ranges from 0.02 to 1.5 d^{-1}, although higher specific growth rates (> 24 d^{-1}) have been measured in tropical salt marsh. The range of bacterial specific growth rate observed in the present work (0.01 to 1 h) is not in agreement with other estuarine waters.

The bacterial production in Mandovi and Zuari estuaries is not related to primary production and is supported to a greater extent by allochthonous input. Statistical analyses further emphasized the fact that bacterial production was more dependant on suspended load and other abiotic parameters like temperature, and not on primary production related parameters like primary productivity rates and chlorophyll a concentrations. Hence the trophic state of

the estuaries is heterotrophy with a high turnover rate of bacterial biomass ranging from less than a few hours to a maximum of 1 d^{-1} coupled with high growth rate. This is reflected in the high abundance of attached bacteria (Crump *et al.,* 1998). Therefore the system is not only heterotrophic but also a very dynamic one. Unlike the Schelde estuary (Goosen *et al.*, 1997) in the temperate region where bacterial production is controlled by 1 or 2 factors, such as temperature for lower estuary and primary productivity for higher estuary, Mandovi and Zuari estuaries are more complex with many factors limiting the abundance and productivity. Multiple regression analyses showed that there are more than 10 factors (biotic and abiotic) controlling the bacterial production. Thus Mandovi and Zuari estuaries are more complex where variability is not dependant upon 1or 2 factors but a combination of different factors with different weightage.

In order to understand the influence of the variables on this estuarine system and to reduce the complexity, Principal Component Analysis were carried out. The present study has examined 71 parameters on annual cycle to understand the role of particle-associated bacteria in trophic dynamics. An examination of the correlation between these parameters has shown that they are significant at 0.05 levels of probability and are responsible for remarkably augmenting the productivity or activity and yet others influence these parameters negatively e.g. primary production enhances bacterial abundance at the coastal station ($r= 0.81$ $p<0.01$) or SPM decreases DNA content ($r=-0.75$ $p<0.01$) at Mandovi. However in order to understand the combined influence of

these parameters on the total variability in the estuarine network, it is needed to understand the factors of different parameters. Linear combination of the original variables are chosen such that each component explains a maximum of the variants left unexplained by preceding components, subject to the constraint that all components are orthogonal to each other (Cuadarado and Perillo, 1997).

The principal component analysis of the parameters of the three systems shows that there are only 2 parameters in the first factor that is common to all the stations. DNA content of particles for all the three stations and SPM for the estuarine stations. The DNA content of particles exerts a contrasting negative influence on the total variation of all these systems.

In Mandovi besides DNA, salinity, SPM and PP form the first factor, which is responsible for 30.9% of the variation. In Zuari, SPM and RNA:DNA ratio are other two variables besides DNA in the first factor contributing up to 28.2% variation. At the coastal station RNA and PAB production are the other parameters in the first factor responsible for 42.7% of the variation. It is probable that the DNA in the system has a contrasting effect compared to the other variables in the component.

The contrasting effect of DNA to the other variables in the major component could be due to the following reasons: (I) the bottom waters are equal or more productive than the surface, it is therefore probable that the DNA content at the surface is relatively more recalcitrant as compared to the bottom waters. As the variation in DNA content is much less as compared to other

parameters and is more refractory than RNA it displays opposite temporal and spatial pattern as compared to RNA which is more dynamic and varying. However RNA can also have contrasting effects like the DNA under certain circumstances like that of the Zuari surface waters, it is inferred that such circumstances could be accounted for when the concentration of RNA can get relatively low (26 $\mu g.l^{-1}$). The concentration of RNA in the surface waters is 1226 $\mu g.l^{-1}$ which is the lowest in the surface waters of all the stations. Besides RNA the other variables in the second factor that contribute 21.3% of variability are salinity and particle numbers. These also have negative loadings indicating that their contribution is analogous to RNA content on particles. It is also because these variables show least variation at the surface of this station. The second major factor in the case of Mandovi station is PAB-production rates and chlorophyll *a* carbon. At the coastal station both productivity and activity are high, temporally marked by stability and little variation.

The variation in the bottom waters is not governed by the same parameters as that of the surface. Whereas in the other estuarine system, of Zuari though deeper, is less heterogeneous than Mandovi system because SPM and organic carbon form two common variables in the first component. The factors that influence the variation in three systems are different as there is a gradation in the variation. In Mandovi the surface is very different from the bottom as none of the variables overlap in the first principal factor. At Zuari there is a certain amount of commonality in variables that influence the surface

and bottom waters. Whereas, at the coastal station 3 parameters that influence the surface also influence the bottom waters.

At the coastal station RNA and DNA content of particles are the main parameters in the major factor. These variables are responsible for bacterial productivity.

The gradation that was observed from the three systems from the least homogenous to the most, corroborates with the classification from the least productive and most active and the least active to the most productive.

PCA of the parameters of the surface waters of the three systems indicate that the variation is commonly governed by different groups of parameters at all the stations at different levels of variations in estuarine systems. PCA showed that the deeper coastal waters are much more homogenous than the shallow estuarine waters.

Further, regression analyses and modeling were carried out to be able to redefine these interrelationships between various parameters and also to be able to predict either the secondary standing stock or production. Regression analysis assumed that the independent variables are measured with out error. However, in practice the measurement of any continuous variable are unlikely to be error free. Nevertheless regression models can be used for predictive purposes if the variability between true replicates is small relative to the range of observations under a given condition and time at a given place provided there are no disturbance factors of environmental and anthropogenic origin (Harkantra and Parulekar, 1991).

In our regression analysis we have assumed that one set of values are repeated for 3 consecutive times assuming the data to be repetitive. Simulation modeling has been *an albeit adhoc* component of the field of aquatic microbial ecology for the past 2 decades (Hopkinson and Vallino, 1994). Models of microbial food web are important as bacteria are energetic subsidy for higher trophic levels leading to harvestable fisheries (Ducklow, 1994). It would be difficult to have a single generic model to address all questions concerning the microbial food web in this estuarine system because of the inherent complexity. However several models have been explained since the publication of Pomeroy's bioscience article (Pomeroy, 1974) that first conceptualized the significance of DOM and bacteria in food webs. The earlier models were mostly descriptive (Pace *et.al.*1984, Wetzel and Christian, 1984) while the latter ones tried to explain the mechanism that controlled the system (Fasham *et al.,* 1990, Malloney and Field, 1991, Wright and Coffin, 1983). Each model addresses different questions at varying levels of complexity. These models also differ on the degree of mechanistic control included (Hopkinson and Vallino, 1994). Ducklow (1994) rightfully highlights the value of modeling and the inherent limitations. In the present study are presented regression models for the estuarine network to address the following questions 1.What are the physical and biological parameters that control PAB biomass and productivity? 2.Whether this model could be predictive for a given time and space frame?

An examination of the 3 systems shows that of the 71 parameters studied over an annual cycle about 13 of them control the above two bacterial

parameters. Of these 13 parameters 7 of them are physico-chemical namely depth, temperature, pH, salinity, particle number, suspended particulate matter and organic content; four biochemical parameters like RNA, DNA, the ratio between these two and PAM biomass. The important biological parameters are primary production and chlorophyll *a* carbon. Almost all these parameters influence all the three systems except particle numbers, organic content, primary production and chlorophyll a carbon which do not form a part of the equation modeling the coastal ecosystem. Between the surface and the bottom waters particle number and organic content does not affect the bottom waters of Zuari and Mandovi respectively. Like wise pH does not have an effect in the bottom waters of the coastal station.

The microbial loop paradigm has been legitimized by a large amount of work following the introduction of the Pace *et al.* model (1984) (Ducklow and Carlson, 1992). However there is still comparatively few suitable data sets with which these models could be calibrated and verified (Ducklow, 1994).

It is emphasized here that the regression model presented above could be at best workable under the condition at which the observations have been made. It would perhaps be more reliable to examine these models with more data sets or sub model could be examined with fewer numbers of parameters with larger number of replicates. This sub model could be a component of a larger model to evaluate the effects of quality and quantity of organic matter, inorganic nutrient inputs on estuarine food web structure and efficiency (Hopkinson and Vallino, 1994).

5.3 HETEROTROPHIC ACTIVITY

It has been established in the previous section that Mandovi and Zuari estuarine systems could be heterotrophic because of the abundance of POM and the factors that govern its concentrations. Hence it is important to understand the heterotrophic potential of the system as mere standing stock would not indicate their potential activity. The potential activity was measured using labeled glucose and glutamic acid. These are only two of the many organic substrates that are utilized by bacteria and it is assumed that these are representative of the total metabolism. The uptake potentialities depend on kinetic parameters like maximum velocity (V_{max}) turnover time (T_t) and natural concentration and transport constant of the substrate (K_t+S_n). The maximum uptake velocity V_{max} for glucose was found to vary from 0.015 to 212.81μg $C^{-1}h^{-1}$ with Mandovi showing the maximum activity. This system reflects the eutrophic nature as it has high maximum velocity (Gocke, 1977). The measured activity in the estuary was the highest for glucose and low for glutamic acid. Although V_{max} was the highest in Mandovi the maximum cell specific activity was in the coastal water. High V_{max} in Mandovi could be due to the high availability of the substrate and the low specific activity could be attributed to the large number of inactive forms in the standing stock as discussed under section on bacterial standing stock. Published literature shows that the glucose uptake was 10.4 x10-8 to 7.2 μg $cell^{-1}h^{-1}$ (Meyer-Reil *et al* , 1978, Seki *et al.,* 1975) Glucose uptake was higher than that of the amino acid. Kirchman and Mitchell (1982) also found less incorporation of glutamate to particle

attached bacteria as compared to glucose. Bell and Albright (1982) are of the opinion that amino acid uptake was more associated with attached bacteria.

There was variation in glucose uptake and activity with season and depth. Season-wise, the estuaries were most active during the pre-monsoon unlike the coastal station where it was active during the post-monsoon. Bent and Goulder (1981) showed that V_{max} was not only different spatially but also temporally. Albright (1977) found higher V_{max} in winter and lower in summer. Moshiri *et al.*, (1979) found high V_{max} during winter and spring.

During the wet season, the particles from the terrestrial run-off are rich in organic matter but not all is labile. Hence the bacteria have to colonise and actively degrade the complex material to simpler forms. This is reflected in the high activity during the subsequent dry seasons in these tropical estuaries.

Thus the potentialities are largely dependent on the supply of degradable organic compounds. The K_t+S_n values may not only vary with different substrates but also with different locations. Higher values for glucose were invariably recorded for both the estuaries. However, K_t+S_n was higher for glucose than glutamate. This variation may be attributed to the natural concentration or to the transport constant of the substrate. Several authors have reported varying concentration of substrate depending on the geographical variation. The high uptake value of glucose indicates good adaptation of the bacterial population to take up such substrates even in low concentration. The uptake of different substrates also depends on the heterotrophic potential of the individual cells. It is a common assumption that

bacterial species cannot be successful competitors at both high and low substrate concentrations. Andrews and Harris (1986) affirm that bacteria that have high maximum growth rates and that which require abundant substrate concentration to support this rapid growth are r –strategists. The others which have lower maximum growth rate with lower concentration of the substrate are K strategists. Maximum growth rate (μ_{max}) is viewed as the primary determinant of competitiveness when resources are abundant whereas substrate affinity Ks becomes more important when the substrate concentrations are low (Velicer *et al.*, 1999). From the present observation of V_{max} and K_t+S_n for glucose and glutamate uptake it is deciphered that these microbes in the estuaries may be r→ K strategists as they were adept at both lower and higher concentrationof substrates. This was further verified with laboratory experiments on some of the particle associated isolates. It is probable that these microbes are versatile and are able to adapt themselves to changing concentration of the substrate and also to a variety of allochthonous source of carbon. (Munster, 1991).

The flux of a substrate depends on T_t and S_n concentration (Gocke, 1977). The turnover time of the substrate studied showed considerable variation both in space and time. Generally, estuarine waters exhibited high T_t when compared to the coastal station. The other difference is the overall preference to glucose. This is further supported by the K_t+S_n values. It can be seen from the results that T_t was not constant but showed variation which are mainly due to temporal factor. The difference in the T_t of the two substrates suggests the differences in the availability and presumably the physiological

and genotypical adaptation of the planktonic community. The two substrates may be used differently for biosynthesis and energy requirement at different seasonal situations. In Lake Constance , Germany the turnover of glucose was constantly larger than those of the amino acid except in the surface waters from mid-July to September (Navarro *et al.*, 1993) Therefore the dynamics of both the substrate classes are different. It is thus evident that PAB are highly active in mineralising organic compounds. This is further corroborated by the high elaboration of different enzymes by the PAB

Environmental parameters may also dictate the differences in heterotrophic potential. Significant correlation were found between V_{max} of attached bacteria and some environmental variables. The correlation with suspended load concentration, POC, POM and phaeophytin may be a result of these variables being independently correlated with the density of attached bacteria (Bent and Goulder, 1981). In estuarine waters of intermediate salinities, Bell and Albright (1981) found a relationship for bacterial heterotrophic activity and attributed it to a favorable regime where freshwater and salt water mix. Stevenson and Erkenbrecher (1976) in a review on estuarine bacteria found that heterotrophic potential of these cells to be significantly greater than those in the sea or fresh water environment. Though fresh water and marine systems have not been compared in the present study, it is indicated that PAB have high heterotrophic potentialities with Mandovi showing maximum of the three. This is partly because the salinity drops to almost limnetic conditions and resembles fresh water system. The relationship

between V_{max} of attached bacteria and temperature is positive. However, Bent and Goulder, (1981) obtained negative relationship with temperature. They explain that the factor relates negatively to suspended solids and therefore indirectly to the density of bacteria.

Besides heterotrophic glucose uptake measurements, the percent active population in the total abundance also reflects their potential activity. Thus the percentage of active population among the particles assessed by enumerating acridine orange stained active orange cells showed that the maximum in these active cells matched with the post-monsoon maximum in number in December. Besides, frequency of actively dividing cells (FDC) were more abundant in the surface waters than the bottom perhaps suggesting that abundance were linked to POC ($p<0.05$ in surface waters).

The viable numbers are also a measure of the potential activity of the PAB population. It ranged from $\leq$ 10% of TC-PAB to a significant 90%. In general viable numbers were more at the bottom in case of Mandovi. This is suggestive of the dependence of viability on organic content from sediments. RC-PAB varies from 10^5 to $10^8.l^{-1}$ which is 2 orders lower than the TC-PAB. Many of these RC were active and were able to express a number of enzymatic activities as stated earlier. The activities were better expressed in the bottom waters suggesting a distinctly active function to these forms. Thus these estuaries had a relatively high number of PAB with an appreciable number being viable and active cells with marked seasonality.

5.4 FOOD WEB

It is now possible to understand that PAB are an important trophic source not only because of their abundance but also because of their potential for higher productivity and activity . The increasing understanding of microbial interaction based on numerous studies in different aquatic systems has lead to a gradual transformation of the original microbial loop concept (Azam *et al* .,1983) into the microbial food web (Sherr *et al* ., 1988) which is now spreading from the sub-micron viruses to metazoan invertebrates (Legendre and Rassoulzadegan, 1995). PAB have a very significant role in the food web because they may be directly grazed by the metazoans, bypassing consumption by protozoan grazers and short-circuiting the microbial loop (Baross *et al*, 1994). In Elbe estuary, 50-75 % of rotifers was found to be associated with suspended aggregate particles. (Zimmermann and Kauch, 1996). Studies on Columbia estuary have shown that epibenthic copepods directly feed on particle attached to bacteria. In the Mandovi- Zuari estuarine system the annual POC input into the estuary has been estimated to be almost entirely detrital which is nearly 2 orders (x100) more than phytoplankton carbon. Cole *et al*.,(1988) had shown that decomposition of phytoplankton by attached bacteria is unimportant and over-emphasizes that phytoplnkton's contribution is negligible. Gauns (2000) has shown that microzooplankton to be an important component of the estuarine food web, which may depend on detrital carbon. Bacteria are high quality food sources and when they are attached to particle they increase the quality of the detrital material as food (Heinle *et al*., 1977).

Detrivorous metazoans may therefore be the principal consumers of PAB biomass in our systems.

The percentage of bacteria attached to particles is very high, which reduces the recalcitrance and improves the nutritive value of particles. In our estuaries, the C:N ratio was lower than 17 which is in agreement with Russell-Hunter, (1970) who postulates that animals have protein nutritional requirement which corresponds to this value of 17. These bacteria have been found to have high turnover rates and are highly heterotrophic with broad enzyme profile. The PAB thus converts the low quality terrigenous to high quality (low C:N ratio) biomass as food for grazers. As PAB are major component of the system the contribution is highly significant. The percentage of attached bacteria was variable and amounted to as high as 80% of the total number. As most of the particles were in the suitable size range for detrivores (Crump and Baross,1996) this bacterial production would be directly available to them. Moreover bacteria have a low C:N ratio ~ 4 (Lee and Furhman, 1987). Such transfer of bacterial production to the higher trophic levels is probably quite significant. Thus the dominance of PAB in our estuarine network suggest that the contribution of bacteria to the estuarine food web is mediated by particle dynamics.

5.5 CONTROL OF BACTERIAL MEDIATED CARBON

Bacterial and phytoplankton biomass and production rates have been expressed in units of carbon in order to compare the two trophic groups. The

average bacterial carbon: phytoplankton was 4.8% at Mandovi surface. This high value is indicative of eutrophic system (Dortch and Packard, 1989, Simon *et al* .,1992). In a eutrophic system the low ratio would reflect the dominance of the grazer food chain.

The bacterial production was on an average 4.8% of the net primary production at the Mandovi surface. Other authors have found values between 30-35% (Williams,1981, Cole *et al.*, 1988). The Mandovi- Zuari estuaries despite high load of detrital carbon and high bacterial net production is also the area that support higher rate of primary production, except bottom water where it reaches values of 100% of NPP, the carbon flux in estuaries is mostly mediated by bacteria attached to particles. The sources of carbon could be intrinsic from phytoplankton or extraneous from terrestrial inputs. The extent of control exercised by the particulate organic carbon and covarying parameters varies with the station and season. Thus the carbon flow in Mandovi estuary represents the sum of many complex processes that vary in space and time. The average amount of bacterial biomass in terms of carbon is nearly 20 times more than the primary biomass. The average secondary bacterial production is around 3 $\mu g\ C.l^{-1}h^{-1}$ as compared to the primary production of 28 $\mu g\ C.l^{-1}h^{-1}$. The net amount of organic carbon is sufficient to support bacterial production in the photic zone in this estuary. However, given that the allochthonous input of carbon is important in this estuary it is not clear at present to what proportion the 2 carbon sources (allochthonous and phytoplankton derived) are being utilized by bacteria. Moreover, secondary production do not correlate to the

primary production and therefore it appears that they are uncoupled in this estuary. The trophic state of the estuary appears to be less autotrophic though it has a higher primary production rates than the other two systems. The high turnover of biomass in this estuary due to short turn over time ranged from 0.003 to 0.3h. This further supports the contention that this estuarine system could be heterotrophic with a substantial amount of detrital carbon flowing through particle associated bacteria.

To summarize, the abundance, activity and productivity studies of the Mandovi, Zuari station along with the adjoining coastal station demonstrate that bacteria associated with particles are not only a significant component but also heterotrophically dynamic. Among the three stations, the mangrove dominated Mandovi estuary is more active than productive. The marine coastal station is more productive than active as it is supported by the estuarine input. The Zuari estuary is intermediate in nature and so is as active as well as productive. The system is relatively more balanced than the other two. In short the above study has given a picture of the sources and sinks of particle attached bacterioplankton production in this estuarine system and underscores the importance of terrestrial carbon sources as opposed to those derived from *in situ* primary production.

6.0 SUMMARY AND CONCLUSIONS

Particles form a significant component of estuarine aquatic systems. The bacteria inhabiting these systems are able to convert the dissolved organic matter to particulate organic matter and their ultimate fate will be greatly influenced by the material to which they are attached. The importance of the attached state is associated with the flow of bacterial biomass in the food web as the free-living and attached bacteria may be predated selectively by different species. Particle attached bacteria can have a very different role in a food web than free living bacteria because they may be directly grazed by larger metazoans, bypassing consumption by protozoan grazers and "short-circuiting" the microbial loop (Baross *et. al.*, 1994). While a lot work has been carried out in the temperate regions there are virtually no study describing bacterioplankton production dynamics of the tropical estuaries like Zuari and Mandovi

While the contribution of PAB to the total bacterial production and heterotrophic activity, in temperate aquatic ecosystems has received a great deal of attention there has been a paucity of information in tropical areas especially Indian waters. The present work deals with various parameters that could play a role in the particle-associated bacterial dynamics in an integrated manner.

The study aims to examine the factors affecting particle-associated dynamics and attempts to elucidate the significance of PAB in a tropical estuarine system in terms of biomass, productivity and activity. Particle-

associated bacterial parameters were calculated by subtracting the values of 3.0 μm pore size filtered water from the unfiltered water.

For the purpose of this study three stations Ribandar in Mandovi estuary, Chicalim in Zuari estuary and Cabo in coastal were examined in detail for a period of 14 months from September 1997 to October 1998.

The stations were generally shallow with a maximum depth of 8 m in the estuaries and 18 m at the coastal station. While temperature showed little variation, salinity ranged from 1.8 to 35.4‰ and the pH from 5.9 to 8. Suspended load is a significant component of this estuarine ecosystem at all seasons. During monsoon, bottom water samples carried higher suspended load accounting for as much as 5 times the surface values. The particle size of the suspended load mainly ranged from 1.75 μm to 50 μm with 3 μm particles dominating both in number and weight. These particles are mainly composed of three groups, organic, inorganic and clay minerals. The organic component was 3 orders greater than the inorganic during the monsoons. The clay mineral component of the particle showed a combination of montmorollinite, gibbsite, goehite, illite, chlorite, kaolinite besides other non-clay minerals like feldspar and quartz.

The particle-associated bacterial (PAB) density ranged from 10^7 to 10^{10} l^{-1}. The minimum and maximum densities were recorded during the pre-monsoon and post- monsoon period. There was a significant difference in abundance between stations. The percentage contribution of PAB to the total was from 66 to 81% at Mandovi, 27 to 56% at Zuari and 50 to 53% at the

coastal station. While the viable bacterial population on particles was an order less, the retrievable population was two orders lower than the total PAB. The trend in the distribution of the viable and retrievable fractions was similar to that of the total. The particles not only harbored a high bacterial density but also an equally high microbial biomass (7.1-1862.15 $\mu g.C.l^{-1}$). This particle-associated biomass sometimes accounts for 55% (237 $\mu g.C.l^{-1}$) of the total microbial biomass.

The PAB carbon production rates ranged from 0.4 to 61 $\mu g.C.l^{-1}.h^{-1}$ in the surface waters while that at the bottom they ranged from 0.2 to 83 $\mu g.C.l^{-1}.h^{-1}$. The contribution of carbon production rate by PAB to the total bacterial carbon production was 38% at Mandovi while at the other stations it was 64%.

The assimilation capacity (heterotrophic uptake rate) for PAB was high with specific preference for glucose over glutamic acid. Studies on cell specific activity showed that PAB had higher activities than that of FLB using glucose a substrate while the FLB dominated the PAB uptake rates when glutamate was used as substrate.

The average uptake potential of glucose by PAB was maximum at Mandovi in the bottom (23.37 $\mu g.C.l^{-1}.h^{-1}$) and surface waters (14.32 $\mu g.C.l^{-1}.h^{1}$). At the bottom waters of all stations the contribution of the V_{max} of PAB to the total bacterial population was high during the pre-monsoon being 89.24% at Mandovi, 83.05% at Zuari and 59.59% at the coastal station.

In general the uptake potential for glutamate was maximum during the pre-monsoon seasons at all the stations. The PAB of Mandovi surface waters had the highest uptake rates for glutamate compared to other stations. At the Mandovi surface waters the V_{max} of PAB: V_{max} of TC was 0.7, while in the coastal bottom waters it was 0.46 by PAB V_{max}.

The metabolic state of the PAB as estimated by the RNA/DNA ratios suggest that they are highly active with the surface PAB being more active throughout the year. The size of the PAB ranged from 0.1 to 4.7μm^3.

At the coastal station the contribution of bacterial biomass was 24.64% to the POC (1142.86 $\mu g.C.l^{-1}$) pool of which 16.93% was due to bacteria associated with particles. At Mandovi the total bacterial biomass contributed 21.32% to POC (1417.44 $\mu g.C.l^{-1}$) of which 15.28% was by PAB biomass. The PAB At Zuari where the dissolved organic carbon was the lowest (0.1 $g.l^{-1}$), the total bacterial biomass accounted for 12.84% of the POC (1897.65 $\mu g.C.l^{-1}$) of which 5.73% was by PAB biomass.

The bacterial production (BP) on particles ranges from 0.05 to 82.9 $\mu g.C.l^{-1}.h^{-1}$ and carbon production was as high as 62% of the primary production (PP) in the system. At the coastal station the average PAB production rates contributed 64% to the total bacterial production and 88% of the carbon that is produced by primary production.

The viable bacterial growth on particles was an order less than the total. Retrievable counts, were two orders low. Retrievable counts as CFU were

generally higher on the particles. The aerobes formed 62% and the general anaerobes formed 96% of the total retrievable population.

Classical taxonomic analyses showed PAB to be dominated by *Comybacterium and Bacillus* followed by *Enterobacteria, Pseudomonas, Aeromonas, Vibrio, Flavobacterium and Cytophaga.*

In general PAB were found to elaborate various enzymes like chitinase, phosphatase, lipase, amylase, gelatinase and Dnase (31, 56, 42, 33, 42 and 27% of the retrievable PAB which was $38.52 \times 10^9.l^{-1}$).

Laboratory experiments with estuarine samples from Zuari showed that nearly all of the PAB in these waters get grazed. Though the generation time of PAB is low i.e. 8.3 h at low ambient substrate concentration, it is highly probable that PAB are continuously being replenished from the total bacterial population which have a generation time of 2.8 h. This is complemented by the high turnover of PAB biomass. Typical PAB were more of *r* strategist and can build up their lowered stocks whenever the substrate availability increases.

Multiple regression analysis demonstrated that the variables like temperature, ATP-PAB, RNA-PAB, DNA-PAB, salinity, RNA:DNA ratio, suspended load and pH were important independent variables explaining in part the variability in PAB biomass and PAB production rates. The regression models indicated the involvement of 12 variables for predicting the particle associated bacterial biomass and productivity at R-sq Adj=100%.

Principal component analysis indicates that the factors that influence the variation in three systems are different, as there is a gradation in the variation.

In Mandovi estuary the surface water is very different from the bottom water as none of the variables overlap in the first principal factor. At Zuari estuary there is a certain amount of commonality in variables that influence the surface and bottom waters. Whereas at the coastal station 3 parameters that influences the surface influence the bottom waters also.

Communality of variables showed that pH was the main factor responsible for variation at the surface waters of all stations. Apart from this PAM biomass was also responsible for variation at the Mandovi and coastal surface waters, while at Zuari the particle numbers influenced the variation. The bottom waters of Mandovi were influenced by the viable PAB and PAB production rates, Zuari by DNA content of particles and PAB production and in the coastal station depth and salinity played a major role in the variation.

CONCLUSIONS

- Particles form a significant part of this estuarine system and the associated bacteria account for 26 to 81% of the total bacterial biomass in these waters. This dominance of PAB in the Mandovi-Zuari estuarine complex suggests that the contribution of bacteria as biomass to the estuarine food web is mediated by particle dynamics.

- In the whole estuarine network the PAB account for ~50% of total bacterial carbon production in the POC pool. Interestingly at the coastal station and Mandovi their production rates are ~50% of the primary productivity.

- The ratio of carbon conversion by PAB bacteria per day from mangrove: phytoplankton : other sources, is 10 : 1 : 32 at Mandovi, 5 : 2 : 22 at Zuari and 10 : 1 : 45 at the coastal station.

- The bacterial load on particles was not only high, but also showed high metabolic potential. The coastal bottom waters were very productive and accommodated a high level of viable and total PAB. This perhaps indicates that the flux from the estuaries is made available for bacterial regenerative cycle in the much more stable bottom waters in this station.

- During the post-monsoons, the PAB in the coastal bottom waters showed high viability and productivity. This indicates that this station contributed significantly to the higher trophic level during this season as the quality of a particle as a food source is dependant not only on the type of bacteria colonizing them but also on their activity and productivity.

- Cell specific uptake rates of PAB showed them to have a preference for glucose. The system is therefore nitrogen limited.

- PCA showed the three systems to be unique by themselves. The Mandovi besides being influenced by physico-chemical parameters was also influenced by factors like PAB production rates, primary production, and

chlorophyll *a* carbon; at Zuari variation was by particle number, their organic content and the physico-chemical parameters. The factors that influenced the variation at the coastal station were microbial and bacterial biomass, production rates and macromolecular content.

- The mangrove-dominated Mandovi estuary is more active than productive; the marine-dominated coastal station in the Arabian Sea is more productive than active; and the Zuari estuary that is influenced by mangroves and marine environment imbibes the properties of both the above systems and is as productive as active.

- Irrespective of season, the Zuari estuarine complex contributes more towards the carbon flux than the nitrogen. It is apparent that the Mandovi-Zuari estuarine complex supports a productive coastal ecosystem. Particle linked food web is a major nexus of energy in this tropical estuarine network.

REFERENCES

Albright .L. J.(1977) Heterotrophic bacterial dynamics in the lower Fraser River, Its estuary and Georgia strait, British Columbia, Canada. Mar. Biol. 39, 203-211.

Alldredge A.L. (1979) The chemical composition of macroscopic aggregates in two neritic seas. Limnol. Oceanogr. 24, 855-866.

Alldredge A.L(1989) The significance of suspended detrital aggregates of marine snow as microhabitats in the pelagic zone of the ocean. In: (eds): Hattori T., Ishida Y., Maruyama Y., Morita R.Y. and Uchida A., Recent Advances in Microbial Ecology, Proceedings of the 5th International Symposium on Microbial Ecology (ISME 5) pp 724.

Alldredge A.L. and Cox J.L. (1982) Primary productivity and chemical composition of marine snow in surface waters of the Southern California Bight J. Mar. Res. 40, 517-527.

Alldredge A.L., Cole J.J. and Caron D.A. (1986) Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnol. Oceanogr. 31, 68-78.

Alldredge A.L., Passow U.and Logan B.E. (1993) The abundance and significance of a class of large transparent organic particles in the ocean. Deep-Sea Res. 40, 1131-1140.

Almeida M.A. and Alcantara F. (1992) Bacterial colonization of seston particles in brackish waters (Ria de Aveiro, Portugal). Mar. Ecol. Prog. Ser. 89, 165-173.

Andrews J.H. and Harris R.F. (1986) *r*- and *k* selection in microbial ecology. Adv. Microb. Ecol. 9:99-147.

Azam F. and R.E. Hodson. (1977) Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. 22: 492-501.

Azam F. and Cho B.C. (1987) Bacterial utilization of organic matter in the sea. *In:* Fletcher M., Gray M., Jones T.R.G. (eds) Ecology of microbial communities. Cambridge University Press, Cambridge pp 261-281.

Azam F., Fenchel T., Field J.G., Gray J.S, Meyer-Reil L.A. and Thingstand F. (1983) The ecological role of water column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Azam F., Martinez J. and Smith D.C. (1993) Bacteria-organic matter coupling

on marine aggregates. *In:* Guerro R., Pedros-Alio C. (eds) Trends in microbial ecology, Spanish Society for Microbiology, Barcelona, pp 410-414.

Azam F., Smith D.C., Long R.A. and Steward G.F. (1995) Bacteria in oceanic carbon cycling as a molecular problem. In: Molecular Ecology of aquatic microbes edited by Ian Joint, NATO ASI series, Series of ecological science 38, 39-54.

Bano N, Nisa M-U, Khan N., Saleem M., Harrison P.J., Ahmed S.I. and Azam F. (1997) Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus river delta, Pakistan Mar. Ecol. Prog. Ser. 157, 1-12.

Banse K (1977) determining the carbon to chlorophyll a ratio of natural phytoplankton. Mar. Biol. 41, 199-212.

Baross J.A., Crump B.C. and Simenstad C.A. (1994) Elevated 'microbial loop' activities in the Columbia River estuarine turbidity maximum. In: Dyer K.R., Orth B.J. (eds) Changes in fluxes in estuaries: implication from science to management (ECSA22/ERF Symposium, Plymouth, September 1992). Olsen and Olsen, Fredensborg pp 459-464.

Becquevort S., Rousseau V. and Lancelt (1998) Marine and comparable roles for free-living and attached bacteria in the degradation of Phaeocystis-derived organic matter in Belgian coastal waters of the North Sea. Aquat. Microb. Ecol. 14, 39-48.

Bell C.R and Albright L.J. (1981) Attached and free-floating bacteria in the Fraser River estuary, British Columbia, Canada. Mar. Ecol. Prog. Ser. 6: 317-327.

Bell C.R. and Albright L.J. (1982) Attached and free-floating bacteria in diverse selection of water bodies. Appl. Environ. Microb. 43: 1227-1237.

Bell R.T., Ahlgren G.M. and Ahlgren I. (1983) Estimating bacterioplankton production by measuring [^{3}H] thymidine incorporation in a eutrophic Swedish lake. Appl. Environ. Microbiol. 45, 1709-1721.

Bent E.J. and Goulder R. (1981) Planktonic bacteria in the Humber Estuary: seasonal variation in population density and heterotrophic activity. Mar. Biol. 62:35-45.

Berger B., Hoch B., Kavka G. and Herndl G. (1996) Bacterial colonization of suspended solids in the River Danube. Aquat. Microb. Ecol. 10, 37-

Berman, T. (1975) Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake. Mar. Biol. 33, 215-220.

Berman, T., and Stiller M. (1977) Simultaneous measurement of phosphorus and carbon uptake in Lake Kinneret by multiple isotopic labeling and differential filtration. Microb. Ecol. 3, 279-288.

Biddanda B.A. (1986) Structure and function of marine microbial aggregates. Oceanol Acta 9, 209-211.

Biddanda B.A., and Pomeroy L.R. (1988) Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. I. Microbial succession. Mar. Ecol. Prog. Ser. 42, 79-88.

Bidle K.D and Fletcher M.(1995) Comparison of free-living and particle-associated bacterial communities in the Chesapeake Bay by stable low-molecular-weight RNA analysis. Appl.Environ. Microbiol, 61, 944-952.

Billiones R.G., Tackx M.L. and Daro M.H. (1999) The Geometric features, shape factors and fractal dimensions of suspended particulate matter in the Scheldt Estuary (Belgium). Estuar. Coast. Shelf Sci. 48, 293 - 305.

Biscaye P.E. (1965) Mineralogy and sedimentation of deep-sea clay in the Atlantic Ocean and adjacent sea and oceans. Bulletin of Geological Survey of America. 76, 803-832.

Buck J.D. (1979) the plate count in aquatic microbiology. *In:* J.W. Costerton and R.R. Colwell (ed.), Native aquatic bacteria: enumeration, activity and ecology. American Society for Testing Materials, Philadelphia. pp. 19-28.

Burnison, B.K. (1975) Microbial ATP studies. Verh. Int. Verein. Limnol. 19:286-290.

Cammen L.M. and Walker J.A. (1982) Distribution and activity of attached and free-living suspended bacteria in the Bay of Fundy. Can. J. Fish. Aquatic Sci. 39, 1655-1663.

Campbell, P.G.C., and Baker J.H. (1978) Estimation of bacterial production in freshwaters by the simultaneous measurement of ^{35}S-sulfate and D-^{3}H-glucose uptake in the dark. Can. J. Microbiol. 24, 939-946.

Carlough L.A. (1994) Origins, structure and trophic significance of amorphous seston in a black-water river. Freshwat. Biol. 13, 227-231.

Caron D.A., Davis P.G., Madin L.P. and Sieburth JMcN (1982) Heterotrophic bacteria and bacteriovorous protozoa in oceanic macroaggregates. Science 218, 795-797.

Chretiennot'Dinet M.J.and Vacelet E. (1978) Seperation fractionee du phyto plancton et estimation de l'assimmilation autotrophe et heterotrophe de ^{14}C. Oceanol. Acta 1,407-413.

Chrost (1991) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. *In:* Chrost R. (ed) Microbial enzyme in aquatic environments. Series in contemporary bioscience, Brock/Springer Verlag, Berlin, pp29-59.

Cole J.J., Findlay S. and Pace M.L. (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. 43, 1-10.

Corpe W.A. (1973) Microfouling: the role of primary film-forming bacteria. *In:* R.F. Acker, B.F. Brown, J.R. De Palma and W.P. Iverson (eds) Proc. 3rd Int. Congr. Mar. Corrosion Fouling, Northwestern Univ. Press, Evanston, Ill. pp.598-609.

Crump B.C. and Baross J.A. (1996) Particle-attached bacteria and heterotrophic plankton associated with the Columbia River estuarine turbidity maxima. Mar. Ecol. Prog. Ser. 138, 265-273.

Crump B.C., Baros J.A., and Simenstad C.A. (1998) Dominance of particle-attached bacteria in the Columbia River estuary, USA Aquat. Microb. Ecol. 14, 7-18.

Cuadarado D.E. and Perillo G.M.E. (1997) Principle component analysis applied to geomorphologic evolution. Estuar. Coast. Shelf. Sci. 44, 411-419.

De Long E.F., Franks D.G. and Alldredge A.L. (1993) Phylogenetic diversity of aggregate-attached vs free-living marine bacterial assemblages. Limnol. Oceanogr. 38, 924-934.

Dell'anno A.. Fabiano M., Mei M.L. and Danovaro R. (1999) Pelagic-benthic coupling of nucleic acids in an abyssal location of the northeastern Atlantic ocean. Appl. Environ. Microbiol. 65, 4451-4457.

Dortch O. and Packard T.T. (1989) Differences in biomass structure between oligotroph and eutrophic marine systems. Deep. Sea. Res. 36, 223-240.

Ducklow H.W. (1982) Chesapeake Bay nutrient and planktonic dynamics. 1. Bacterial biomass and production during spring tidal destratification in the York River, Virginia estuary. Limnol. Oceanogr. 27: 651-659.

Ducklow H.W. (1994) Modeling the microbial food web. Microb. Ecol. 28, 303-319.

Ducklow H.W. and Carlson C.A. (1992) Oceanic bacterial production. Adv. Microb. Ecol. 12, 113-181.

Ducklow H.W. and Kirchman D.L. (1983) Bacterial dynamics and distribution during a spring diatom bloom in the Hudson River plume, U.S.A. J. Plankt. Res. 5, 333 – 355.

Ducklow H. W. and Shiah F.K. (1993) Bacterial production in estuaries, *In* :Aquatic Microbiology : An ecological approach. (Ford, T.G., ed.), Blackwell Scientific Publications, Boston, pp. 261-287.

Ducklow H.W., Kirchman, D.L. and Rowe G.T. (1982) Production and Vertical Flux of Attached Bacteria in the Hudson River Plume of the New York Bight as Studied with Floating Sediment Traps. Appl. Environ. Microbiol. 43, 769-776.

Ducklow H.W., Kirchman, D.L. and Quinby H.L. (1992) Bacterioplankton cell growth and macromolecular synthesis in seawater cultures during the North Atlantic spring phytoplankton bloom, May 1989. Microb. Ecol. 24, 125-144.

Dyer K.R. (1986) Coastal and estuarine sediment dynamics. John Wiley and Sons Great Britain pp. 342.

Eisma D (1992) Suspended matter in the aquatic environment. Springer-Verlag, Berlin.

Eisma D. and Cadee G. (1991) Particulate matter process in estuaries. *In* :Degens,E.T.,Kempe,S. and Richey,J.E. (eds), Biogeochemistry of Major World Rivers, 283-296.

Fabiano M., Povero P., and Danovaro R. (1993) Distribution and composition of particulate matter Organic matter in the Ross Sea (Antarctica). Polar Biol. 13, 525-533.

Fasham M.J.R., Ducklow H.W. and McKelvie S.M. (1990) A nitrogen based model of plankton dynamics in the oceanic mixed layer. J.Mar. Res. 48, 591-639.

Ferguson R.L. and Palumbo A.V. (1979) Distribution of suspended bacteria in neritic waters South of Long Island during strafied conditions. Limnol. Oceanogr. 24,697-705.

Ferguson R.I.and Rublee P. (1976) Contribution of bacteria to coastal microplankton standing crop. Limnol. Oceanogr. 21,141-145.

Findlay S., Pace M. and Lints D. (1991) Variability and transport of suspended sediment, particulate and dissolved organic carbon in the tidal freshwater Hudson River. Biogeochemistry 12, 149-169.

Fletcher M. (1991) The physiological activity of bacteria attached to solid surfaces. Adv. Microb. Physiol., 32, 53-85.

Fletcher M. and Marshall K.C. (1982) Are solid surfaces of ecological significance to aquatic bacteria? In: (ed): Marshall K.C., Advances in microbial ecology 6, pp 239.

Fowler S.W. and Knauer G.A. (1986) Role of large particles in the transport of elements and organic compounds through the oceanic water column. Prog. Oceanogr. 16, 147-194.

Friedrich U., Schallenberg M. and Holliger C. (1999) Pelagic bacteria – particle interactions and community – specific growth rates in four lakes along a trophic gradient. Microb. Ecol. 37, 49 – 61.

Fuhrman J.A. (1982) Influence of method on the apparent size distribution of bacterioplankton cells: epifluourescence microscopy compared to scanning electron microscopy. Mar. Ecol. Prog. Ser. 5, 103-106.

Fuhrman J.A. and Azam F. (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39, 1085-1095.

Fuhrman J.A. and Azam. F. (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface. Mar. Biol. 66: 109-120.

Gauns M. (2000) Role of microzooplankton in the food chain dynamics of some tropical marine environments. Ph.D Thesis. Goa University.

Gerhardt P. (ed.) 1981 Manual of method for general bacteriology. American Society of Microbiology, Washington, DC.

Gocke K. (1977) Heterotrophic activity. *In:* G. Rheinheimer (ed.) Microbial ecology of a brackish water environment.. Springer- Verlag Berlin-Heidelberg.

Goosen N.K. Rijswijk P van, and Brockmann U. (1995) Comparison of Heterotrophic bacterial rates in early spring in the turbid estuaries of the Scheldt and the Elbe. Hydrobiologia 311, 31-42.

Goosen N.K., van Rijswijk P., Kromkamp J. and Peene J. (1997) Regulation of annual variation in heterotrophic bacterial production in the Schelde estuary (SW Netherlands). Aquat. Microb. Ecol. 12, 223 -232.

Gorsky G., Picheral M. and Stemmann L. (2000) Use of the underwater video profiler for the study of aggregate dynamics in the north Mediterranean. Estuar. Coast. Shelf Sci. 50, 121-128.

Goulder R. (1976) Relationships between suspended solids and standing crops and activities of bacteria in an estuary during a neap-spring neap tidal cycle. Oceologia 24: 83-90.

Goulder R. (1977) Attached and free bacteria in an estuary with abundant suspended solids. J. Appl. Bacteriol. 43, 399-405.

Goulder R., Bent E.J. and Boak A.C. (1980) Attachment to suspended solids as a strategy of estuarine bacteria. *In:* N.V. Jones and W.J. Wolff (ed.) Feeding and survival strategies of estuarine organisms, pp 1-15. Proceedings of a joint meeting of the estuarine and brackish water sciences association, The Nederlandse Oceanografenclub and the Hydrbiologische Vereniging held at the University of Hull, Hull, U.K., 1980.

Greiser N. (1988) Zin Dynamik von Schwebstoffen und ihren biologischen Komponenten in de Elbe bei Hamburg. PhD thesis, Univ. Hamburg.

Griffith P.C., Douglas D.J. and Wainright S.C. (1990) Metabolic activity of size-fractionated microbial plankton in estuarine, nearshore and continental shelf waters of Georgia. Mar. Ecol. Prog. Ser. 108, 1-10.

Griffith P., Shiah F.K., Gloersen K., Ducklow H.W. and Fletcher M. (1994) Activity and distribution of attached bacteria in Chesapeake Bay. Mar. Ecol. Prog. Ser. 108, 1-10.

Grossart H.P. and Simon M. (1993) Limnetic macroscopic organic aggregates (like snow): occurrence, characteristic and microbial dynamics in Lake Constance. Limnol. Oceanogr. 38, 532-546.

Grossart H.P., Berman T., Simon M. and Pohlmann K. (1998) Occurrence and microbial dynamics of macroscopic organic aggregates (like snow) in Lke Kinneret Israel, in fall. Aquat. Microb. Ecol. 14, 59-67.

Gude H. (1986) Loss processes influencing growth of planktonic bacterial population in Lake Constance. J. Plankt.Res.8, 795-810.

Hansen R.B. and Wiebe W.J. (1977) Heterotrophic activity associated with particulate size fractions in a *Spartina alterniflora* salt –marsh estuary, Sapelo island, Georgia, USA and the continental shelf waters. Mar. Biol. 42, 321-330.

Harkantra S.N. and Parulekar A.H. (1991) Interdependence of environmental parameters and sand dwelling benthic species abundance: A multivariate approach. Ind. J. of Mar.Sci. 20. 232-234.

Harvey R.W. and Young L.Y. (1980) Enumeration of particle bound and unattached respiring bacteria in the salt marsh environment. Appl. Environ. Microbiol. 40, 156 – 160.

Hatchikian E.C. (1972) Mecanisms d'oxydo-reduction chez les bacteries sulfato-reductrices. Ph.D Thesis., Marseilles.

Hedges J.I. and Stern J.H. (1984) Carbon and nitrogen determinations of carbonate-containing solids. Limnol. Oceanogr. 29, 657-663.

Heinle D.R., Harris R.P. Ustach J.F. and Flemer D.A. (1977) Detritus as food for estuarine copepods. Mar. Biol. 40, 341-353.

Herndl G.J. (1988) Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. II Microbial density and activity in marine snow and its implication to overall pelagic processes. Mar Ecol. Prog. Ser. 48, 265-275.

Herndl G.J. (1992) Marine snow in the Northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. Mar. Microb. Food Webs 6, 149-172.

Hobbie, J.E., R.J. Daley, and S. Jasper. (1977). Use of Nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228.

Hobbie J.E., Holm-Hansen O., Packard T.T., Pomeroy L.R., Sheldon R.W., Thomas J.P. and Wiebe W.J. (1972) A study of the distribution and activity of microorganisms in ocean water. Limnol. Oceanogr,17, 544 - 555.

Hoch M.P. and Kirchman D.L. (1993) Seasonal and inter-annual Variability in bacterial production in a temperate estuary. Mar. Ecol. Prog. Ser., 98, 283-295.

Hoch B.M., Berger B., Kavka G. and Herndl G.J. (1995) Remineralization of organic matter and degradation of the organic fraction of suspended solids in the River Danube. Aquat. Microb. Ecol. 9, 279-288.

Hodson R.E., Maccubbin A.E. and Pomeroy L.R. (1981) Dissolved adenosine triphosphate utilization by free-living and attached bacterioplankton. Mar. Biol. 64: 43-61.

Hollibaugh J.T. and Wong P.S. (1999) Microbial processes in the San Francisco Bay estuarine turbidity maximum. Estuaries 22, 848-862.

Holm Hansen O. and Booth, C.R. (1966) The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr., 11,510-519.

Holm-Hansen O., Sutcliffe Jr. W.H. and Sharp J. (1968) Measurement of deoxyribonucleic acid in the ocean and it ecological significance. Limnol. Oceanogr. 13, 507-574.

Hopkinson Jr. C.S. and Vallino J.J. (1994) Towards the development of generally applicable models of the microbial loop in aquatic ecosystems. Microb. Ecol. 28, 321-326.

Hoppe H.G. (1984) Attachment of bacteria: advantage or disadvantage for survival in the aquatic environment. *In:* Marshall KC (ed) Microbial adhesion and aggregation. Springer, Berlin pp. 283-301.

Hoppe H.G., Ducklow H. and Karrasch B. (1993) Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Mar. Ecol.Prog. Ser., 93, 277-283.

Hoppe H.G., Kim S.J. and Gocke K. (1988) Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. Appl. Environ Microbiol. 54,784-790.

Iriberri J., Unanue M., Barcina I. and Egia L. (1987) Seasonal variation in population density and heterotrophic activity of attached and free-living bacteria in coastal waters. Appl. Environ. Microbiol. 53, 2308-2314.

Jackson M.L.W., Light M.P.R. and Ayers, W.B., Jr. (1987) Geology and coproduction potential of submarine-fan deposits along the Gulf Coast of East Texas and Louisiana. J. PET. TECHNOL.39, 473+ vp.

Jannasch H.W. and Jones G.E. (1959) Bacterial population in seawater as determined by different methods of enumeration. Limnol. Oceanogr. 4, 128-139.

Jannasch H.W. and Pritchard P.H. (1972) The role of inert particulate matter in the activity of aquatic microorganisms. Mem. 1st Ital. Idrobiol. Suppl. 29, 289 – 308.

JGOFS (1994) Core measurement protocols. IOC Manual and guides 29. UNESCO

Jordan M.J. and Likens G.E. (1980) Measurement of plankton bacterial production in an oligotrophic lake. Limnol. Oceanogr. 25, 719 –732.

Kanopka A. (1992) Accumulation of polysaccharides by hotspring phototrophs during light-dark transition. FEMS Microbiol. Ecol. 102, 27-32.

Karl D.M., Knauer G.A. and Martin J.H. (1988) Downward flux of particulate organic matter in the ocean: A particle decomposition paradox. Nature 332, 438-441.

Karner M. and Herndl G. (1992) Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. Mar. Biol. 113, 341-347.

Kato K. (1984) Functional difference in the utilization of phytoplankton cell materials and dissolved organic compounds in free living and particle associated bacteria in an aquatic system. Arch. Hydrobiol. 100, 521-522.

Kies L. (1995) Algal snow and the contribution of algae to suspended particulate matter in the Elbe estuary. *In:* Wiessner W., Schnepf E., Starr R.C. (eds) Algae, environment and human affairs. Biopress Limited, Bristol pp. 93 -121.

King K.R., Hollibaugh J.T., and Azam F. (1980) Predator-prey interactions between the larvacean *Oikopleura dioica* and bacterioplankton in enclosed water columns. Mar. Biol. 56, 49-57.

Kirchman D. (1983) The production of bacteria attached to particles suspended in a freshwater pond. Limnol. Oceanogr. 28, 858-872.

Kirchman D.L. (1993) Particulate detritus and bacteria in marine environments *In*: T.E Ford. (ed) Aquatic microbiology – an ecological approach. Blackwell, Oxford, pp. 321-341.

Kirchman D.L. and Ducklow H.W. (1987) Trophic dynamics of particle-bound bacteria in pelagic ecosystems: A review, pp. 54- 82. *In*: D.J.W. Moriarty and R.S.V. Pullin (eds.) Detritus and microbial ecology in aquaculture. ICLARM Conference Proceedings 14, 420 p. International Centre for Living Aquatic Resource Management, Manilla, Philippines.

Kirchman D. and Mitchell R. (1982) Contribution of particle bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl. Environ. Microbiol. 43: 200-209.

Kirchman D.L. and Rich J.H. (1997) Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific ocean. Microb. Ecol. 33, 11-20.

Kirchman D.C., Ducklow H. and Mitchell R. (1982) Estimation of bacterial growth from changes in uptake rates and biomass. Appl. Environ. Microbiol. 44: 1296-1307.

Kirchman D., Peterson B.J. and Juers D. (1984) Bacterial growth and tidal variation in bacterial abundance in the Great Sippewissett Salt Marsh. Mar. Ecol. Prog. Ser. 19,247 – 259.

Kirchman D.L., Suziki Y., Garside C., and Ducklow H.W. (1991) High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. Nature 352, 612-614.

Kissane J.M. and Robins E. (1958) The flourometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. J. Biol. Chem. 233, 184-188.

Koenings J.P., Burkett R.D., and Edmundson J. (1990) The exclusion of limnetic Cladocera from a turbid glacier meltwater lake. Ecology 71, 57-67.

Kogure K, Simidu U, Taga N, Colwell R.R. (1987) Correlation of direct viable counts with heterotrophic activity for marine bacteria. Appl. Environ. Microbiol. 53, 2332-2337.

Koike I, Shigemitsu H, Kazuki T, and Kogure K. (1990) Role of sub-micrometer particles in the ocean. Nature 345, 242 – 244.

Konda T. (1984) Seasonal variations in four bacterial size fractions from a hypertrophic pond in Tokyo, Japan. Int. Rev. Ges. Hydrobiol. 69, 843-858.

Kondratieff P.F.and Simmons, G.M. Jr (1985) Microbial colonization of seston and free bacteria in an impound river. Hydrobiologia 128, 127-133.

Kranck, K. (1980) Variability of particulate matter in a small coastal inlet. Can. J. Fish. Aquatic Sci. 37,1209-1215.

Krey I. (1964) Die mittlere Tiefenverteilung von seston Mickrobiomase und detritus in mordlichen Nordatlantik. Kieler Meeresforsch 20, 18-29.

Laane R.W.P.M. (1982) Chemical characteristics of the organic matter in the water phase of the Ems-Dollart estuary Ph.D. Thesis, Univ. Groningen p 50.

Larson E.T., and Shanks A.L. (1996) Consumption of marine snow by two species of juvenile mullet and its contribution to their growth. Mar. Ecol Prog. Ser. 130, 19-28.

Larsson U. and Hagstrom A. (1982) Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. Mar. Biol. 67, 57-70.

Laybourne – Parry J, Rogerson A. and Crawford D.W. (1992) Temporal patterns of protozooplankton abundance in the Clyde and Loch Striven. Estuar. Coast. Shelf Sci. 35, 533 - 545.

Lee S and Fuhrman J.A. (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. 53, 1298-1303.

Legendre L. and Rassoulzadegan, F. (1995) Plankton and nutrient dynamics in marine waters. Ophelia 41: 153-172.

Letarte Y., Hansen H.J., Soendergaard M. and Pinel Alloul B. (1992) Production and abundance of different bacterial size-classes: Relationships with primary production and chlorophyll concentration. Arch. Hydrobiol., 126,15-26.

Lin R,I.S. and O.A. Schjeide (1969) Micro estimation of RNA by the cupric

ion catalyzed orcinol reaction. Anal. Biochem. 27: 473-483.

Linley E.A.S., Field J.G. (1982) The nature and ecological significance of bacterial aggregation in a nearshore upwelling ecosystem. Estuar.Coast. Shelf. Sci., 21, 272-273

Loka Bharathi P.A. (1989) The occurrence of denitrifying colourless sulphur oxidizing bacteria in marine waters and sediments as shown by the agar shake technique. FEMS Microbiol. Ecol. 62, 335-342.

Loka Bharathi P.A. and Chandramohan D. (1985) Sulfate-reducing potential in an estuarine beach. Ind. J. Mar. Sci. 14, 187-191.

Loka Bharathi P.A., Chandramohan D. and Nair S. (1988) A preliminary study of anaerobic thiosulphate oxidizing bacteria as denitrifiers in the Arabian Sea. Geomicrobiol. 6, 195-207.

Long, R.A. and Azam, F. (1996) Abundance protein containing particles in the sea. Aquat. Microb. Ecol. 10, 213-221.

Lovell C.R. and Konopka A. (1985) Thymidine incorporation by free-living and particle-bound bacteria in a eutrophic limnetic lake. Appl. Environ. Microbiol. 49, 501-504.

Malloney C.L. and Field J.G. (1991) The size-based dynamics of plankton foodwebs. 1. A simulation model of carbon and nitrogen flux. J. Plankt. Res. 13, 1003-1038.

Marsh D.H and Odum W.E. (1979) Effect of suspension and sedimentation on the amount of microbial colonization of salt marsh microdetritus. Estuaries 2, 184-188.

Marshall K.C., Stout R. and Mitchell R. (1971a) Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. 68, 337-348.

Marshall K.C., Stout R. and Mitchell R. (1971b) Selective sorption of bacteria from seawater. Can. J. Microbiol. 17, 1413-1416.

Marshall P.A., Loeb G.I., Cowan M.M., and Fletcher M. (1989) Response of microbial adhesive and biofilm matrix polymers to chemical treatments as determined by interference reflection microscopy and light section microscopy. Appl. Environ. Microbiol. 55, 2827-2831.

Mc Cluskey D.S. (1989) The estuarine ecosystem. Blackie, Glasgow.

Means J.C. and Wijayaratne R.D. (1984) Chemical characterization of estuarine colloidal organic matter: Implications for adsorptive processes. Bull. Mar. Sci. 35, 449-461.

Mel'nikov I.A. (1976) Morphological characteristics of organic detritus particles. Oceanologia 16, 401-403.

Meyer-Reil L.A., Dawson R., Liebezeit and Tiedge H. (1978) Fluctuations and interactions of bacterial activity in sandy beach sediments and overlying waters. 48, 161-171.

Moriarty D.J.W., Pollard P.C. and Hunt W.G. (1985) Temporal and spatial variation in bacterial production in the water column over a coral reef. Mar. Biol. 85, 285-292.

Moshiri G.A., Crumpton W.G. and Aumen N.G. (1979) Dissolved glucose in a bayou estuary, possible sources and utilization by bacteria. Hydrobiologia 62, 71-74.

Mostajir B., Dolan J.R. and Rassoulzadegan F. (1995) Seasonal variations of pico- and nano-detrital particles (DAPI Yellow Particles, DYP) in the Ligurian Sea (NW Mediterranean). Aquat. Microb. Ecol., 9, 267-277.

Munster U. (1991) extracellular enzyme activity in eutrophic and polyhumic lakes. *In:* Chrost RJ (ed) Microbial enzymes in Aquatic Environments. Springer-Verlag, New York, 96-122.

Murrell M.C. ,Hollibaugh J.T., Silver M.W, and Wong P.S (1999) Bacterioplankton dynamics in northern San Francisco Bay: Role of particle association and seasonal freshwater flow. Limnol. Oceanogr. 44, 295-308.

Nagata T. (1987) Production rate of planktonic bacteria in the North Basin of Lake Biwa, Japan. Appl. Environ. Microbiol. 53, 2872-2882.

Navarro J.M., Closing E., Urrutia G., Asencio G., Stead R. and Herrera C. (1993) Biochemical composition and nutritive value of suspended particulate matter over a tidal flat of Southern Chile. Estuar. Coast. Shelf. Sci., 37, 59-73.

NIO Technical Report No. 02/79 (Aug.1981). Master plan for Pollution Control of the rivers Zuari and Mandovi. Sponsored by Central Board For the Prevention and control of Water Pollution.(Govt. of India).

Novitsky J.A. (1986) Degradation of dead microbial biomass in a marine sediment. Appl. Environ. Microbiol. Rev. 22, 79-103.

Odum W.E. (1988) Comparative ecology of tidal freshwater and salt marshes.

Oliver J.D. (1982) Taxonomic scheme for the identification of marine bacteria. Deep Sea Res. 29, 795-798.

Pace M.L., Glasser J.E. and Pomeroy L.R. (1984) A simulation analysis of continental shelf food webs. Mar. Biol. 82, 47-63.

Paerl H.W. (1978) Microbial organic carbon recovery in aquatic ecosystems. Limnol. Oceanogr. 23, 927-935.

Paerl H.W. (1980) Attachment of microorganisms to living and detrital surfaces in fresh water systems. *In:* G.Bitton and K.C. Marshall (eds.). Adsorption of microorganisms to surfaces. John Wiley and Sons, New York.

Paerl H.W. and Merkel S.M. (1982) Differential phosphorus assimilation in attached vs. unattached microorganisms. Arch. Hydrobiol. 93: 125-134.

Palumbo A.V., Randolph L.F. and Rublee P.A. (1984) Size of suspended bacterial cells and association of heterotrophic activity with size fractions of particles in estuarine and coastal waters. Appl. Environ. Microb. 48,157-164.

Parsons T.R., Maita Y. and Lalli C.M. (1984) A manual of chemical and biological methods for seawater analysis pp. 80-84.

Passow u., and Alldredge A.L. (1994) Distribution size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. Mar. Ecol. Prog. Ser. 113,185-198.

Paul J.H., Jeffrey W.H. and DeFlaunn M.F. (1987) Dynamics of extracellular DNA in the marine environment. Appl. Environ. Microbiol. 53, 170-179.

Pedros-Alio C. and Brock T.D. (1983) The Importance of attachment to particles for planktonic bacteria. Arch. Hydrobiol.98, 354-379.

Pfennig N., Widdel F. and Trupper H.G. (1981) The dissimilartory sulfate-reducing bacteria. Pages 926-940 *In:* M.P. Starr, H. Stolp, H.G. Trupper, A. Balows and H.G. Schlegel (eds). The prokaryotes. Vol. I. Springer Verlag, Berlin, Heidelberg.

Pierucci O. (1978) Dimensions of *Escherichia coli* at various growth rates: models for envelope growth. J. Bacteriol. 135, 559-574.

Plummer D.H., Owens N.J.P. and Herbert R.A. (1987) Bacteria -particle interactions in turbid estuarine environments. Cont. Shelf. Res. 7, 1429-1433.

Pomeroy L.R. (1974) The ocean's food web: a changing paradigm. BioScience 24, 499-504.

Pomeroy L.R. (1980) Detritus and its role as a food source. *In:* Barnes R.K. and Mann K.H. (eds), Fundamentals of aquatic ecosystems Blackwell Publishing p 84-102.

Poulet S.A. (1983) Factors controlling utilization of non-algal diets by particle grazing copepods: A review Oceanologica Acta 6, 221-234.

Prahl F.G. and Coble P.G. (1994) Input and behaviour of dissolved organic carbon in the Columbia River estuary. *In:* Dyer K.R., Orth B.J. (eds) Changes in fluxes in estuaries: implication from science to management (ECSA22/ERF Symposium, Plymouth, September 1992). Olsen and Olsen, Fredensborg pp. 451-457.

Prezelin B.B. and Alldredge A.L. (1983) Primary production of marine snow during and after an upwelling event. Limnol. Oceanogr. 28, 1156-1167.

Qasim S.Z. and Sen Gupta R (1981) Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. Estuar. Coast. Shelf. Sci. 13, 557-578.

Redfield A.C., Ketchum B.H. and Richardo F.A. (1963) The influence of organisms on the composition of seawater. *In:* Hill M.N (ed). The Sea Vol.2, Wiley and Sons, New York. pp.26-77.

Reed D.J., and Donovan J. (1994) The character and composition of the Columbia River estuarine turbidity maximum. *In:* Dyer K.R., Orth B.J. (eds) Changes in fluxes in estuaries: implication from science to management (ECSA22/ERF Symposium, Plymouth, September 1992). Olsen and Olsen, Fredensborg pp. 445-450.

Revilla M., Iriarte A., Madariaga I. and Orive E. (2000) Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuar. Coast. Shelf Sci. 50, 297 – 313.

Riemann B. (1978) Differentiation between heterotrophic and photosynthetic plankton by size fractionation, glucose uptake, ATP and chlorophyll content. Okios 31, 358-367.

Rodina A.G. (1972) Methods in studying microorganisms of the nitrogen cycle. *In:* Colwell R.R. and Zambruski M.S. (eds.). Methods in Aquatic Microbiology, p 251-322, University Park Press, Baltimore.

Rogerson A. and Laybourn–Parry J. (1992a) Aggregate dwelling protozooplankton communities in estuaries. Arch. Hydrobiol 125, 411-422.

Rogerson A. and Laybourn–Parry J. (1992b) The abundance of marine naked amoebae in the water column of the Clyde estuary. Estuar. Coast. Shelf Sci. 34, 187-196.

Rublee P.A., Merkel S.M. Faust M.A. and Miklas J. (1984) Distribution and activity of bacteria in the headwaters of the Rhode River Estuary, Maryland, USA. Microb. Ecol. 10: 243-255.

Russel Hunter W.D. (1970) Aquatic Productivity: An introduction to some basic aspect of Biological Oceanography and Limnology. Collier-Mac Millan, London.

Schuster S. and Herndl G.J. (1995) Formation and significance of transparent exopolymeric particles in the northern Adriatic Sea. Mar. Ecol. Prog. Ser. 124, 227-236.

Seki H. (1970) Microbial biomass on particulate organic matter in sea water of the euphotic zone. Appl. Microbiol. 19, 960- 962.

Seki H., Yamaguchi Y. and Ichimura S. (1975) Turnover rate of dissolved organic materials in a coastal region of Japan at summer stagnation period of 1974. Arch. Hydrobiol. 75, 297-305.

Shands J.W. (1966) Localization of somatic antigen on gram-negative bacteria using ferritin antibody conjugates. Ann. N.Y. Acad. Sci. 133, 292-298.

Shanks A.L., and Trent D. (1979) Marine snow: microscale nutrient patches. Limnol. Oceanogr. 24, 850-854.

Sherr B.F., Sherr E.B. and Hopkinson C.S. (1988) Trophic interactions within pelagic microbial communities: indications of fedback regulation of carbon flow. Hydrobiologia 159, 19-26.

Shetye S.R., Shenoi S.C., Antony M.K. and Krishnakumar V. (1985), Procd. Indian Acad. Sci (Earth Planet Sci.), 94,129.

Shiah F. K. and Ducklow H.W. (1994 a) Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Mar. Ecol. Prog. Ser., 103, 297-308.

Shiah F.K, and Ducklow H.W. (1994b) Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnol.Oceanogr., 39,1243-1258.

Sieburth J.McN. (1968) Observations on planktonic bacteria in Narragansett Bay, Rhode Island. A resume. Misaki. Mar. Biological Instit., Kyoto University 12, 49-64.

Sieracki M.E and C.L. Viles. (1992) Distributions and fluorochrome staining properties of sub micrometer particles and bacteria in the North Atlantic. Deep Sea Res. 39: 1919-1929.

Sigleo A.C. and Macko. (1985) Stable isotope and amino acid composition of estuarine dissolved colloidal material. *In:* Sigleo A.C. and Hattori A (eds.) Mar. Estuar. Geochem. pp. 29-46.

Sigleo A.C., Hare, P.E. and Helz G.R. (1982) Composition of estuarine colloidal material: Organic components. Geochim. Cosmochim. Acta. 46, 1619-1626.

Sigleo A.C., Hare, P.E. and Helz G.R. (1983) The amino acid composition of estuarine colloidal material. Estuar. Coast. Shelf. Sci. 17, 87-96.

Simenstad C.A., Morgan C.A., Cordell J.R., and Baross J.A. (1994) Flux, passive retention and active residence of zooplankton in Columbia River estuarine turbidity maxima. *In:* Dyer K.R., Orth B.J. (eds) Changes in fluxes in estuaries: implication from science to management (ECSA22/ERF Symposium, Plymouth, September 1992). Olsen and Olsen, Fredensborg pp. 473-484.

Simon M., Cho B.C and Azam F. (1992) Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. Mar. Ecol. Prog. Ser. 86, 103-110.

Small L.F., McIntire C.D., MacDonald K.B., Lara-Lara J.R., Frey B.R., Amspoker MC and Wenfield T. (1990) Primary production, plant and detrital biomass and particle transport in the Columbia River estuary. Prog. Oceanogr. 25, 175-210.

Smith D.C., Simon M., Alldredge A.L., and Azam F. (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359, 139-142.

Sorokin, Y.I. (1971) Bacterial populations as components of oceanic ecosystems. Mar. Biol. 11, 101-105.

Steeman-Nielsen E, 1952. The use of radioactive carbon (^{14} C) for measuring organic production in Sea. Journal Du Conseil pour Exploration de la Mer. *18*:117-140.

Stevenson L.H. and Erkenbrecher C.W. (1976) Activity of bacteria in the estuarine environment. *In:* Wiley M (ed) Estuarine processes, Vol. 1. Uses, stresses and adaptation to the estuary. Academic Press, New York. pp. 381-394.

Tranvik L.J. and Sieburth J. Mc.N. (1989) Effects of flocculated humic matter on free and attached pelagic microorganisms. Limnol. Oceanogr. 34, 688-699.

Turley C.M. and P.J. Mackie. (1994) Biogeochemical significance of attached and free living bacteria and the flux of particles in the NE Atlantic Ocean. Mar. Ecol. Prog. Ser. 115,191-203.

Turley C.M. and Stutt E.D. (2000) Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the Northwest Mediterranean. Limnol. Oceanogr. 45, 419-425.

Unanue M., Ayo B., Azua I, Barcina I and Iriberri J. (1992) Temporal variability of attached and free-living bacteria in coastal waters. Microb. Ecol. 23, 27-39.

Uncles R.J., and Stephens J.A. (1993) Nature of the turbidity maximum in the Tamar Estuary, U.K. Estuar. Coast. Shelf Sci. 36, 413-431.

Urrere M.A., and Knauer G.A. (1981) Zooplankton fecal pellets fluxes and vertical transport of particulate organic material in the pelagic environment. J. Plankton Res. 3, 369-387.

Velicer G.J., Schmidt T.M. and Lenski R.E (1999) Application of traditional and phylogenetically based comparative methods to test for a trade off in bacterial growth rate at low substrate concentration. Microb. Ecol. 38, 191-200.

Wafar S., Untawale A.G., and Wafar M. (1997) Litterfall and energy flux in a mangrove ecosystem. Estuar. Coast. Shelf. Sci. 44: 111-124.

Wallace J.B., Ross D.H. and Meyer J.L. (1982) Seston and dissolved organic carbon dynamics in a southern Appalachian stream. Ecology 63, 824-838.

Wangersky P.J. (1976) Particulate organic carbon in the Atlantic and Pacific Oceans. Deep Sea Res. 23, 457-465.

Wangersky P.J. (1984) Organic particles and bacteria in the ocean. *In:* Hobbie J.E., Williams P.J. and Le B (eds.) Heterotrophic activity in the sea. Plenum, New York, pp. 263-288.

Wehr J.D., Hole D.A., Macdonald M.M. and Lonergran S.P. (1998) Effects of different organic carbon sources on a freshwater plankton community. Can. J. Aquat. Sci. J. Can. Sci. Halieut. Aquat. 5, 2150-2160.

Wells M.L. and Goldberg E.D. (1991) Occurrence of small colloids in seawater. Nature 353, 342-344.

Wells M.L. and Goldberg E.D. (1993) Colloid aggregation in seawater. Mar. Chem. 41, 353-358.

Wetzel R.L. and Christian R.R. (1984) Model studies on the interactions among carbon substrates, bacteria and consumers in a salt marsh estuary. Sym. On detritus dynamics in aquatic ecosystems, Savannah, USA. 35, 576-586.

White P.A., Kalff J., Rasmussen J.B. and Gasol J.M. (1991) The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microb. Ecol. 21, 99-118.

Wiebe W.J. and Pomeroy L.R. (1972) Microorganisms and their association with aggregates and detritus in the sea: a microscopic study. Mem. 1st Ital. Idrobiol. 29, 325-352.

Wright R.L. and Coffin R.B. (1983) Planktonic bacteria in estuaries and coastal waters of northern Massachusetts: spatial and temporal distribution. Mar. Ecol. Prog. Ser. 11, 205-216.

Williams P.J.le B. (1970) Heterotrophic utilization of dissolved organic compounds in the sea. I. Size distribution of population and relationship between respiration and incorporation of growth substrates. J. Mar. Biol. Ass. U.K. 50, 859-870.

Williams P.J.le B. (1981) Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food. Web. Kieler Meeresforsch. Sonderh. 5, 1-28.

Wilson C.A., and Stevenson L.H. (1980) The dynamics of the bacterial population associated with a salt-marsh. J. Exp. Mar. Biol. Ecol. 48, 123-135.

Wright R.T. (1978) Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36, 297-305.

Wright R.T. and Hobbie J.E. (1965) The uptake of organic solutes in lake water. Limnol. Oceanogr. 10, 22-28.

Yoon W.B. and Rosson R. (1990) Improved method of enumeration of attached bacteria for study of fluctuation in the abundance of attached bacteria and free living bacteria in response to diel variation in a seawater turbidity. Appl. Environ. Microbiol. 56, 595-600.

Zimmermann R. (1977) Estimation of bacterial numbers and biomass by epifluorescence microscopy and scanning electron microscopy. *In:* G. Rheinheimer (ed). Microbial Ecology of a brackish water environment pp.103-120. Springer Verlag, Berlin.

Zimmermann R. (1978) Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. *In*: G.Rheinheimer (ed.) Microbial ecology of a brackish water environment. Springer-Verlag, New York.

Zimmermann H. and Kausch H. (1996) Microaggregates in the Elbe estuary: structure and colonization during spring. Arch. Hydrobiol. Spec. Issues Advance Limnol. 48, 85-92.

Zimmermann R. and Meyer-Reil L.A. (1974) A new method for fluorescence staining of bacterial populations on membrane filters. Kiel. Meeresforsch. 30, 24-26.

Zobell C.E. (1943) The effect of solid surfaces upon bacterial activity. J. Bacteriol. 46, 39-56.

Zweifel U.L. (1999) Factors controlling accumulation of labile dissolved organic carbon in the Gulf of Riga. Estuar. Coast. Shelf Sci. 48,357-370.

8.0 APPENDIX

MEDIA

A. NUTRIENT AGAR (HIMEDIA, BOMBAY)

Peptone .....5.0 g

Sodium chloride.....1.5 g

Beef extract.....1.5 g

Yeast extract.....1.5 g

Agar.....15.0 g

50% sea water.....1000.0 ml

pH.....7.4

B. AMYLOLYTIC MEDIA

Nutrient agar.....28.0 g

Starch..... 2.0 g

50% sea water.....1000.0 ml

C. PROTEOLYTIC MEDIUM

Nutrient agar.....28.0 g

Gelatin.....4.0 g

50% sea water.....1000.0 ml

After the colonies have grown overlay the plate with $HgCl_2$ solution

$HgCl_2$.....15.0 g

HCl (Conc.).....20.0 ml

Distilled water.....100.0 ml

D. PHOSPHATASE MEDIUM

Nutrient agar.....28.0 g

50% sea water.....1000.0 ml

After autoclaving and just before pouring the substrate filter sterilized p-nitrophenyl phosphate (Sigma) was added to the medium so as to obtain a final concentration of 0.02%.

E. LIPOLYTIC MEDIUM

Peptone.....10.0 g

Sodium chloride.....5.0 g

$CaCl_2$.....0.1 g

*Tween.....10.0 ml

Agar.....15.0 g

Distilled water.....1000.0 ml

pH.....7.0 to 7.4

* Tween was autoclaved separately and added to the medium before pour-plating.

F. DNASE TEST AGAR

Dnase test agar (Himedia, Bombay).....42.0 g

Toludine blue (Himedia, Bombay).....0.1 g

Distilled water.....1000.0 ml

G. CHITINASE MEDIUM

Zobell's marine agar 2216 (Himedia, Bombay)..27.55 g

Agar.....7.5 g

Chitin (Sigma).....5.0 g

50% sea water.....250.0 ml

Distilled water.....750.0 ml

H. MODIFIED LIESKE'S MEDIUM

NaCl..... 31.00 g

$Na_2S_2O_3.5H_2O$.....5.00 g

KNO_3.....5.00 g

*$NaHCO_3$..... 1.00 g

K_2HPO_4.....0.20 g

$MgCl_2.6H_2O$.....0.10 g

NH_4Cl.....2.00 g

$CaCl_2.6H_2O$.....0.01 g

$FeCl_3.6H_2O$..... 0.01 g

Agar .....8.00 g

pH.....7.8-8

(*Bicarbonate was filter sterilized and added to the medium just before inoculation)

I. SRB MEDIUM

Liquid medium

Yeast extract .....1.0 g

NH_4Cl.....2.0 g

K_2HPO_4.....0.2 g

SL4.....5.0 ml

Sodium acetate.....1.0 g

Sodium lactate.....7.5 ml

50% seawater.....1000.0 ml

pH.....7.5-7.8

Last minute addition

5% Na_2S.....2.5 ml

$FeSO_4.7H_2O$ (0.4%) .....0.4 g

(acidified with 0.1ml of 4N H_2SO_4)

Solid medium

SRB liquid medium 1000.0ml

Sodium thioglycollate(3%)..... 5.0ml

0.5N NaOH..... 4.0 ml

$FeSO_4.7H_2O$(10%) 5.0ml

Agar 8.0g

REAGENTS

SL4 (TRACE ELEMENT SOLUTION)

$ZnSO_4.7H_2O$.....10.0 mg

$MnCl_2.4H_2O$.....3.0 mg

H_3BO_3.....30.0 mg

$CoCl_2.6H_2O$.....20.0 mg

$CuCl_2.2H_2O$.....1.0 mg

$NiCl_2.6H_2O$.....2.0 mg

$Na_2MoO_4.2H_2O$.....3.0 mg

$CaCl_2.2H_20$.....20.0 mg

DW.....1000.0 ml

0.1M PHOSPHATE BUFFER SOLUTION

Solution A: 0.2 M sodium phosphate monobasic solution

$NaH_2PO_4.H_2O$.....27.6 g

DW.....1000.0 ml

Solution B: 0.2 M sodium phosphate dibasic anhydrous solution

Na_2HPO_4.....28.4 g

DW.....1000.0 ml

Mix 19 ml of solution A with 81 ml of solution B . The mixture had a pH of 7.4 and osmotic pressure of 210 mOsm.

TRIS BUFFER (FOR ATP)

Tris hydroxymethylaminomethane.....2.5 g

Distilled water.....1000.0 ml

pH..... 7.7-7.8

pH was adjusted using 20% HCl.

PREPARATION OF 3,5-DIAMINOBENZOIC ACID DIHYDROCHLORIDE

The DABA.2HCl was prepared from DABA (Sigma) as follows: Sufficient amount of DABA was dissolved in 6N HCl so that the solution is almost saturated (about 10g of DABA per 100ml of HCl). One to 2 g of Norit A was added and stirred for a few minutes. The charcoal was removed from the suspension by filtration through a coarse filter paper (Whatman no. 42) on a buchner funnel. During filtration the suspension was maintained

near boiling point and the filter and the holder was preheated to prevent crystallization of DABA. The DABA. 2HCl was crystallized out of solution by cooling of the filtrate at 5^0C for 2-3 hours. The slurry was than filtered through the sintered glass base of a Millipore filtration apparatus. The crystal of DABA.2HCl were then dried at 60^0C for 2 hours, ground in a mortar and stored in a glass stoppered bottle at 5^0C. (Holm-Hansen *et al.*, 1968)

TOTAL ANAEROBIC COUNTS

Culturable anaerobic heterotrophic bacteria (AnPAB) were estimated using nutrient agar medium (prepared in 50% seawater) which was supplemented with filter sterilized 0.03% sodium thioglycolate.

FUNCTIONAL GROUPS

Sulphate Reducing Bacteria (SRB)

SHAKE AGAR METHOD.

SRB were enumerated on modified Hatchikian's medium (Hatchikian 1972, Loka Bharathi and Chandramohan, 1985). SRB were quantified using the agar shake technique (Pfennig *et al.* 1981). Here 14 ml screw-capped culture tubes containing 12 ml medium and inoculum were gently tilted to allow mixing and then allowed to set. A sterile mixture of paraffin wax and oil (2:1v/v) was then poured on top to maintain anaerobiosis. SRB were enumerated after 10-15 days of incubation at room temperature. The numbers are expressed as averages of triplicate tubes.

Thiobacillus like organisms

The enumeration of thiosulfate-oxidizing denitrifying bacteria (TODB) and thiosulfate-oxidizing nitrate-reducing bacteria (TONRB) was carried out

on modified Lieske's medium. A combination of agar shake technique and most probable number (MPN) technique (Rodina 1972) was employed to enumerate anaerobic thiosulphate oxidizing bacteria (TOB) (Loka Bharathi 1989, Loka Bharathi *et al.*, 1988). Direct aliquots from water samples (maximum 5 ml) were distributed into 15 ml screw capped tubes. For PAB, water samples were filtered on to 3.0 μm Millipore filter. The filter was then shaken in 5 ml of sterile seawater and used as inoculum. Freshly autoclaved and cooled medium to which sterilized $NaHCO_3$ had been added was introduced into the tubes containing inocula to fill up to 5/6 of the tube volume. The tubes were than tilted to mix the inoculum, allowed to set and a mixture of paraffin wax: paraffin oil in the ratio of 1:2 was poured on top to fill the remaining $1/6^{th}$ portion of tube volume. The same set of tubes was used to estimate TODB by the MPN method and TONRB by counting of colony forming units (CFU) in the tubes. All the tubes showing gas formation (bubbles or crack formation in the agar) were considered positive for TODB. Their MPN was calculated using McCready's tables. For the estimation of TONRB, CFU in all tubes were counted and the average populations were expressed as number per litre irrespective of the presence or absence of gas. Samples were recognized by gas formation in tubes with in ten to fifteen days' time. Visible CFU were however noted only after prolonged incubation (30 to 35 days).