STUDIES ON THE ECOPHYSIOLOGY OF SOME HETEROTROPHIC AND INDICATOR BACTERIA IN THE MARINE ENVIRONMENTS OF KERALA

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DOCTOR OF PHILOSOPHY

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CERTIFICATE

This is to certify that this thesis is an authentic record of the work carried out by Smt. V. Chandrika, under my supervision at the Central Marine Fisheries Research Institute, Cochin and that no part thereof has been presented for any other degree in any University.

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DECLARATION

I hereby declare that this thesis entitled "Studies on Ecophysiology of some Heterotrophic and Indicator Bacteria in the Marine Environments of Kerala" has not previously formed the basis of the award of any degree, diploma, associateship-fellowship or other similar title or recognition.

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PREFACE

Marine bacterial population can be split into two groups. The indigenous heterotrophic bacteria and the accidental flora, composed of saprophytic, pathogenic species and bacterial pollution indicators. Whenever, the faecal pollution levels were measured it is customary to conduct, the heterotrophic bacterial assays of the same environment. The measurement of sewage pollution using biological/bacterial indicators in the marine environment has been an important investigation extensively undertaken in many parts of the world during the last few decades. Technically faecal pollution levels are measured by analysing the total coliform, faecal coliform and faecal streptococci counts and by constructing faecal index - a ratio between the counts of faecal coliform and faecal streptococci. A survey of literature showed lack of information regarding the pathways and fate of sewage pollutants from sewage and land drainage in the inshore environment of Cochin and other marine environments. In order to find out sewage pollution levels in marine environment, Central Marine Fisheries Research Institute with which the candidate is associated, initiated a programme of research for three years on environmental pollution in the estuarine and inshore environment of Cochin.

A planned programme of research for over 3 years during 1972-73, 1974-75 and 1975-76 were conducted in the estuarine and inshore environment of Cochin. Short-term investigations in the littoral waters at Trivandrum, Vizhinjam and Kovalam were carried out to find out the pollution levels of these waters, during 1977.

The first part of the thesis deals with quantitative and qualitative analysis of heterotrophs and the four indicators isolated from Cochin and pattern of distribution in different seasons. Decomposition pattern of *Salvinia molesta* by the various zymogenous microflora with physico-chemical factors were dealt along with faecal pollution level during January to October, 1978.

Second part deals with eco-physiological studies of bacterial strains isolated from Trivandrum (Shanmukhom), Kovalam and Vizhinjam waters. This includes a comparative study of 50 marine bacteria and 60 human bacteria isolated from clinical specimens and their biochemical variations. In addition *in vitro* study of antibiotic sensitivity of the bacteria isolated from human beings and from littoral waters near Trivandrum coast are reported. Selected strains of *E. coli* type-I (230 nos.) isolated from estuarine environment of Cochin during 1975-76 were subjected to serological studies and 7'O'-serotypes have been encountered. The above studies such as the comparative biochemical activities of human strains against marine bacterial strains, the antibiotic sensitivity studies and serological typing of *E. coli* type-I from marine environment are the first of its kind in marine bacteriological work. These studies have in a large measure added to the understanding of pollution microbiology of estuarine and marine environments of Kerala which sustain a rich and variegated fauna including edible fishes but at the same time has a high rate of sewage pollution during a greater part of the year. Values comparable to the highest rate of heterotrophic bacteria and faecal pollution measured anywhere in the marine environment were recorded from some inshore stations.

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CHAPTER I.

INTRODUCTION.

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INTRODUCTION

Concern about marine bacterial pollution has grown steadily in recent years. Damage to marine organisms and ecosystems, deterioration of human health by direct contact with polluted water and consumption of contaminated sea food are among its diverse effects. Associated with these environmental aspects are economic costs and various social implications. Many effects of marine faecal pollution are unknown and the magnitude of those known to occur is often undetermined. Nowadays many parts of the polluted marine environment have been examined for indicators of bacterial pollution and the effect of faecal pollution on marine ecology is one of the important line of bacteriological study today. Faecal pollution of beaches by indicator bacteria has been studied by Stevenson (1953), Moore (1954, 1959), Berger *et al.* (1963), Bonde (1969, 1968), Shuval *et al.* (1968), Grunnet *et al.* (1970), Regnier Park (1972) and Tinker (1976).

Published reports on the occurrence of heterotrophs and bacterial indicator organisms of faecal pollution from the marine environment of India are very few.

Venkataraman and Sreenivasan (1954) have studied bacteriology of the offshore areas of the west coast. Studies on the hydrology and bacteriology of the estuarine systems also have not received much attention. In Malabar area, the Korapuzha estuary was surveyed for certain coliform types in mussel culture fields by Venkataraman and Sreenivasan (1955). Bacteria in the inshore environment at Mandapam (Gulf of Mannar) was studied by Velankar (1955). Incidence of coliforms and enterococci in natural waters was studied by Sastry *et al.* (1969) in Bhopal city. Studies by Mathur and Ramanathan (1966) and Sen and Ghosh (1970) in the unfiltered water supply of Calcutta have indicated many advantages of using enterococci index in place of coliform index. Studies on heterotroph and indicator organisms of faecal pollution in the Cochin Backwaters include the work of Santhakumari (1966), Gore (1971, 1976, 1979a, 1979b), Chandrika and Pillai (1974), Raveendran *et al.* (1978). Published reports on bacterial contamination of Indian beaches include Gore and Singbal (1973) and Dwivedi *et al.* (1974). Gore *et al.* (1979) studied quantitative occurrence of indicator organisms like coliforms in surface water and sediment samples of the estuary. They found non-human type of faecal pollution, originating from land drainage, discharge of organic waste and also from sewage throughout the investigation period except in January, March and July, 1977. Gore et al. (1980) also observed Salmonella sp. after bacteriological analysis of sand samples of some of the beaches of Kerala during July-October 1978, and they also found some relation between coliforms, E. coli and the presence of Salmonella.

Recently many authors have emphasised that the potential hazards to public health are associated with the pollution of coastal areas by the direct or indirect discharge of raw or partially treated sewage. Sewage contributes considerably to the quantity of faecal micro-organisms such as Salmonella, Shigella, coliforms and faecal coliforms in water and sediment which are carried with the coastal waters and may give rise to epidemics. It has been shown that sewage polluted water is often a common source of disease in man and animals either directly or indirectly (Crown, 1972; Geldreich, 1972; Gangarossa et al., 1972; Anonymous, 1973). Apart from pathogenic germs sewage contains great quantities of organic and inorganic nutrients which enhance mass development of pathogenic and non-pathogenic micro-organisms in controlled systems. Coliform numbers varied consistently in harbour water according to the degree of organic pollution of water (Kueh, 1974; Kueh and Chan, 1975). On the other hand the microflora is not infrequently inhibited or destroyed by poisonous substances. Thus continued addition of sewage effluents to enclosed water bodies with limited exchanges with adjoining ocean will result in eutrophication enriching the growth of pathogenic bacteria.

Human pathogenic microflora cannot grow permanently in the marine environment and die off eventually in the sea but depending on the prevailing conditions various pathogens can survive for a period and remain virulent. Studies on the survival of faecal coliform in the marine environment deal with various factors controlling the death of these organisms (Greenberg, 1956; Mitchell, 1968). It has been well documented that occurrence of toxic materials as mercury, (Klarmann, 1950), enzyme and metabolic inhibitors (Webb, 1963) and Selenate and other conservative pollutant (Ketchum *et al.*, 1952, Orlob, 1956; Jones, 1963; Nakamura *et al.*, 1964), high salt concentration and pH of sea water (Carlucci and Pramer, 1960, 1961; Chan & Li, 1977), limited nutrient supply (Greenberg, 1956), competition by native microflora (Waksman and Hotchkiss, 1937), the grazing action by protozoa and other predators (Mitchell and Morris, 1969; Engineer and Cooper, 1976), the existence of heavy metals (Jones, 1963) and lytic bacteria in sea water (Gulin *et al.*, 1967; Mitchell and Morris, 1969) are the major factors contributing to the rapid die off of coliforms in marine environment. On the otherhand, the addition of cysteine (Johannesson, 1957; Carlucci and Pramer, 1960) and chelating agents (Jones, 1963) to sea water increased the survival of *Escherichia coli*.

High concentration of coliform bacteria existed in sediments rather than in the overlying waters (Weiss, 1951; Rittenberg *et al.*, 1958) and that cells of *E. coli* were capable of utilising the nutrients released from estuarine sediments (Gerba and McLeod, 1976). Further more, presence of estuarine sediments has been found to greatly enhance the survival of *E. coli* in natural sea water under laboratory conditions (Gerba and Macleod, 1976) as the sea water and mud basically contain most of the nutrients required for the growth of micro-organisms. So the pathogens thrive themselves for a certain period after the sea water and sediments have been contaminated by sewage and drainage. With the environmental factors such as favourable pH, salinity and optimum temperature, the micro-organism readily develop and rapidly colonise in estuarine and marine environment. These observations indicate the ability of marine environment to support to a limited extent, the survival and even growth of coliform bacteria.

The survival time for most pathogenic bacteria is greater in freshwater lakes and rivers than in sea as sea water is bactericidal for non-marine bacteria. Frequently, the survival time is greater in sediments than in fresh water and again in marine sediments it is shorter than those of inland waters. Pathogenic and non-pathogenic bacteria are present in large numbers in bottom sediments (Gerba *et al.*, 1977) and may be released upon resuspension following dredging, boating storms, upwelling and other activities. The significance of marine sediments as reservoirs of human pathogenic and non-pathogenic bacteria had not been realised until recently. All pathogens are capable of remaining alive in the sediments for a relatively long time and even may become enriched there (Bonde, 1967).

It is well known that the pathogenic water microbes produce diseases such as cholera, dysentery, diarrhoea, typhoid, jaundice, leptospirosis etc. Hence sewage loaded coastal areas may be dangerous source of infection. Particularly frequent in polluted waters are pathogenic intestinal organisms like *Salmonella typhi* and *Salmonella paratyphi* which cause enteric fever. *Salmonella* infections may however, be caused by eating oysters and other shellfish and fishes cultured and harvested from sewage loaded waters. Less frequent are *Shigellae* (causative agent of dysentery) which occurs epidemically and is commonly spread by polluted water. Apart from human intestinal microbes such as coliforms, members of the genera *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Proteus*, *Pseudomonas* and spore forming bacteria, sewage also contains animal and fish pathogens. Pathogenic forms exist but the numbers and types will vary with the geographic area, the state of community health, the nature and degree of sewage treatment and the physiological state of the organism.

Whenever pathogens are present they are scarce in number when compared to non-pathogenic commensal bacteria (as 9% of faeces of human beings is considered to be the weight of bacteria itself) and this complicates the problem of their detection. So the procedures adopted for testing potability rely on the detection of the commensals of pathogenic bacteria native to the intestine of healthy human and other warm-blooded animals to indicate the presence of faeces. These are called 'indicator bacteria' which are bacterial parameters of pollution.

The Vembanad lake and connected backwaters around Cochin are well known for its role as a nursery ground for many commercially important prawns and fishes as well. Whenever the bacterial pathogens are present in prawns and fishes in considerable number they survive the processing procedures like freezing or cooking. Therefore, it is essential to understand the magnitude of faecal pollution in the environment from where they are caught, so that additional precautionary measures can be taken whenever faecal index are reported high. The Cochin Backwater receives sewage inlets and 7 drainage canals from the mainland as well as from the nearby islands. According to the 1971 census, the population of Cochin is 4,38,420 (2,24,051 males and 2,14,369 females). It takes an average of about 10,000 people in a community to produce the equivalent of 1 tonne anaerobically digested sewage sludge per day. In a year, this would be 365 tonnes approximately. It is reported that several million gallons of powerful septic sewage is discharged into the backwater daily. With all the sewage and sludge the backwater system remain as a potential environment for monitoring bacteriological quality of water as this sewage will cover 4 to 5 square miles with a depth of 2 to 3 fathoms. The daily east-west movement of the tides and north-south movement of the ocean currents enable the backwater to absorb this enormous discharge into her system without damaging the fish and other aquatic life. If the current intensity is reduced or stopped, the system will not be in a position to absorb the load. A recent study of Cochin Backwaters established the existence of organic pollution particularly where the canals join the Backwaters.

Geldreich and Clarke (1966) reported that the occurrence of indicator bacteria in water and mud signifies the presence of other enteric pathogens in the same environment. In many instances indicators of bacterial pollution signified human pathogenic viruses of faecal origin in coastal waters (Gerba and Goyal, 1978). Above information warrant a thorough study and increasing attention on the discharge of sewage and land drainage into coastal waters including the beaches, and a detailed investigation on heterotrophic flora and indicators of bacterial pollution. At present there is a general lack of information regarding the pathways of bacterial pollutants from sewage and land drainage and their fate in the water bodies of Cochin. Hence, the present study was aimed to monitor indicators of bacterial pollutants in brackish water environment of Cochin and also to investigate the effect of bacterial pollutants on fisheries resources and human health as an essential step towards evolving a scientific basis for protection and management measures.

Scope of the present study.

The results of the investigations are presented and discussed under two parts in 6 sections.

Under Part A (Ecology) in Section I quantitative and qualitative study of heterotrophic saprophytes have been carried out after isolating them by pour-plate methods from the samples collected from fixed stations in the inshore environment of Cochin. Identification of preceding groups with confirmatory tests wherever possible was made with numerous physiological and biochemical tests. Selected cultures were identified based on their biochemical activity towards different substrate and antibiotic sensitivity test and finally based on the scheme of Usio Simidu and Kayuyoshi Aiso (1962) (Table 6).

In addition, seasonal distribution of total heterotrophic bacteria in water and sediments were discussed and their generic composition was evaluated in the aquatic environment.

In Section II, isolation, identification and quantitative abundance of indicators of bacterial pollution encountered from inshore and estuarine environment have been presented and discussed.

Seasonal occurrence of faecal index as suggested by Finstein (1972) has also been included in order to understand the nature and source of faecal pollution.

Spatial and temporal variation and the interaction of bacterial population with the environmental parameters was also studied by statistical analysis of the data (Cohran and Cox, 1963, 1977).

In Section III results of studies on zymogenous heterotrophic microbes from *Salvinia molesta* in the marine environment are presented and discussed.

Six different selective media were used to assess the decomposition rate of the floating weed of *Salvinia molesta* in the study area as it receives the allochthonus organic matter during monsoon times. Biochemical differentiation of bacteria isolated from human beings and littoral waters from Trivandrum, Vishinjam, Kovalam areas are also discussed.

Under Part B (Ecophysiology) in Section I a comparison of the biochemical activity of the bacteria isolated from human beings and bacteria isolated from littoral waters of Trivandrum area have been made in order to understand the biochemical efficiency of the isolates from different environment and the results are presented and discussed.

In Section II results of *in vitro* investigations of antibiotic sensitivity have been presented and discussed. A comparative estimate of the bacteria isolated from littoral waters were made in order to understand the pattern of drug resistance as preliminary studies of antibiotic resistance pattern and levels of Mic (minimum inhibitory concentration) were considered to aid in the detection of R-factors and their transfer in enterobacteriaceae.

Results of serological studies on *Escherichia coli* are presented and discussed in Section III. *Escherichia coli* were isolated from water and sediment during the period of July 1975 to June 1976 for serological typing of enteropathogenic *E. coli* (EEC). *E. coli* type I were subjected to preliminary serological determination i.e. O-serotyping and antibiogram typing were done and 7-O serotypes were encountered. Sensitivity tests on enteropathic *E. coli* strains were carried out in the laboratory and the results of these and other studies are discussed in the light of our current knowledge of antibiogram typing and R^+ factor in bacterial strains with transferrable drug resistance.

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CHAPTER II.

HISTORICAL RESUME.

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In view of the enormous volume of published work on the marine biota, it might be supposed that marine bacteria were known from the time of the first directed studies on micro-organisms in the sea. Many early students of marine life apparently failed to recognize bacteria in their collections or misinterpreted bacteria as structures belonging to the plants or animals themselves. The first accurately described species of bacteria appears to date back to 1838 when Ehrenberg isolated and described *Spirochaeta plicatilis* from sea water. Cohn (1865) isolated and described the marine dwelling *Beggiatoa mirabilis* and Warming (1875) described *Beggiatoa minima*. A year later Warming (1876) also described *Thiospirillum violaceum*, *Thiospirillum rosenbergii* and *Achromatium mulleri*. The pioneer work of Certes (1884a), Fischer (1886), Russel (1891) and their contemporaries established that living bacteria are widely distributed throughout the ocean.

The literature from 1884 to 1914 consists principally of reports of bacterial counts from Talisman Expedition (Certes, 1884a), Planet Expedition (Gräf, 1909) in the Atlantic Ocean, and Scottish Antarctic Expedition (Pirie,

1912) conducted near Orkney Islands. Issatchenko (1914) reviewed his own and others observations in a 300 page Russian monograph which included also 420 references. Issatchenko (1914) was the first Scientist to emphasise the importance of bacteria in the sea as bio-chemical agents and also found a physiologically versatile bacteria including nitrifiers, denitrifiers, nitrogen fixers, sulphate reducers, ammonifiers in the water of Arctic seas to depths of 65 to 100 metres and in bottom mud.

The literature from 1838 to 1946 on marine micro-biology has been reviewed by Zo Bell and Upham (1944) and Zo Bell (1946a, 1947). After 1946, in spite of the difficulties which have curtailed the study of marine micro-biology, considerable research progress has been made in the transformation of sulphur compounds, influence of hydrostatic pressure on the growth and viability of bacteria, promotion of growth of marine bacteria due to high organic matter and suspended solids, bacterial denitrification, biological activity of bacteria associated with their growth, action of heavy metal ions on bacteria, anti-bacterial activity of sea water, seasonal, spatial and vertical distribution of marine bacteria and various bio-chemical potential of bacteria.

The recovery of the sulphate reducers having unique tolerance for temperature, salinity, and hydrostatic pressure from oil and sulphur wells suggested that they may be indigenous species in ancient marine sediments (Zo Bell and Rittenberg, 1948). A large percentage of the sulphate reducers isolated from Gulf coast sediments was found capable of utilising molecular hydrogen (Sisler and Zo Bell, 1950). Bacterial life at the bottom of the Phillippine trench was studied by Zo Bell (1952) and despite repeated attempts he was unable to isolate sulphur oxidising bacteria from this area. Johnson (1972) found bacterial reduction of arsenate in sea water while Sorokin (1972) found a direct relationship between the bacterial population and hydrogen sulphide oxidation in the Black Sea. Bansemir and Rheinheimer (1974) studied the formation of H_2S in the inner Kiel Fjord and found *Thiobacillus* as important sulphur oxidiser in coastal waters. During the "Orgon IV" cruise of the R.V. "Jean Charcot" in the Gulf of Aden and Oman Sea covered 50 sampling stations in all. In 72% of the cores collected the viable counts of sulphate reducing bacteria were maximal at a depth of 0 to 2 m. The distribution of heterotrophic aerobic, heterotrophic anaerobic, sulphate reducing and methane producing populations was studied from the Gulf of Aden and Oman Sea by

Marty (1981) and the co-existence of these four microflora exhibiting various and even opposite metabolic processes was explained due to distinctive microniche juxtaposition in the same sedimentary layer.

The influence of hydrostatic pressure on the growth and viability of marine bacteria was studied by Zo Bell and Johnson (1949). Zo Bell and Oppenheimer (1950) described the pressure apparatus and experimental procedures for studying the effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. Morita (1954) found millions of viable bacteria per gram of sediments taken from depths exceeding 10,000 metres on the Danish-Galathea Deep Sea Expedition which suggested that hydrostatic pressure is not a deterrent for bacterial life at great depths. Morita and Zo Bell (1956) found that the succinate dehydrogenase activity of *Escherichia coli* was rapidly destroyed at increased pressures. Zo Bell and Budge (1965) obtained *Pseudomonas perfectomarinus*, a barophilic facultative aerobe which is capable of growing at pressures higher than 600 atm in nutrient media containing little or no free oxygen. Zo Bell and Hittle (1967) found that the adverse effects of hyperbaric oxygenation on the reproduction and survival of bacteria are augmented by increased hydrostatic pressure. They studied 6 species of bacteria and all the six species thrived at 100 atm in nutrient media having an initial oxygen content of 7 µg/ml and they grew well in media with an oxygen content of 35 µg/ml at 1 atm.

Concerted studies were made on marine bacteria attacking various organic materials like sugars, starches, cellulose (Bavendamm, 1932; Waksman *et al.*, 1933), pectins, glucosides, fatty acids (Thayer, 1931), triglycerides, alcohols sterols, proteins, amino acids, chitin (Hock, 1940), lignins, agar (Stanier, 1941; Humm, 1946) and hydrocarbons (Zo Bell, 1950a). These organic substances were found to be attacked by both aerobic and anaerobic bacteria. From the Gulf of Mexico sediment Campbell and Williams (1951) isolated 20 strains of aerobic chitin decomposing bacteria, including species of *Achromobacter*, *Flavobacterium*, *Micrococcus* and *Pseudomonas*. Zo Bell (1954) working in the Gulf of Mexico found abundance of bacteria in shallow Gulf waters which greatly exceeded the bacteria in the open ocean and is believed to be attributable primarily due to high organic matter and suspended solids both of which promote the growth of bacteria. The influx of fresh water with its load of organic nutrients from land drainage is also a contributing factor along the littoral zone. Here there is a commingling of both fresh water and marine micro-organism and numerous transitional stages of both kinds. In the harbour of Naples (Italy), Jannasch (1955) found only 0.002% of micro-organisms were attached to organic matter while in Mediterranean 3% of the total bacteria were attached to particulate organic matter. Liston (1960) suggested the occurrence of maximum bacterial population correlated with plankton outbursts.

Bacteriological investigations were carried out during the cruises of R.V. "Meteor" in the Arabian Sea in March and April 1965 by Rheinheimer. The distribution was studied in the Northern Gulf of Oman and the Straits of Hormuz, as influenced by the current flowing out of the Persian Gulf and the higher counts of bacteria of the Gulf water is adduced to the greater amount of organic material. The formation of organic aggregates and their enlargement by further adsorption of organic compounds may be one of the reasons why, in large parts of the open oceans the concentration of dissolved nutrients is too low for bacterial growth. As a consequence nearly all bacteria can be found attached to particulate matter (Sieburth, 1968).

Sekii (1970) estimated the relation between microbial biomass to particulate organic matter in sea water of the euphotic zone of Australia. Studies by Ayyakkannu and

Chandramohan (1970, 1971) indicated that the major portion of potentially available phosphate is locked up as insoluble organic and inorganic phosphorus compounds in sediments and soils. They also noted a relationship between phosphate content, the numbers of phosphatase solubilizing bacteria and phosphatase activity in sediments. The association of bacterioneuston with the surface slicks have been discussed by Garrett (1970), Sieburth (1965), Tsyban (1971) who considered the marine bacterioneuston to form a distinct microbial biocenosis active in the organic chemistry of the sea surface slicks. Moebus (1972) observed seasonal changes in the bacterial activity of North Sea water and found that bacterial property of natural and synthetic sea water can be enhanced by addition of low amounts of organic matter. Sieburth and Brown (1975) observed increased heterotrophic activity in a polluted Fjord estuary. The growth kinetics of bacterial population showing different responses to dissolved organic substances were analysed by using a chemostat by Ishida and Kadota (1975 a,b). The properties and generic composition of phosphatase producing bacteria in coastal and oceanic sea water was estimated by Kobori (1929). Although mostly studied in coastal waters bacterioplankton are probably ecologically significant throughout the world ocean. Planktonic bacteria are widely assumed to regenerate nutrients to convert dissolved organic matter (DOM) to particulate organic matter (POM) and to alter the POM (Pomeroy, 1974, and 1979, Azam and Hodson, 1977 and Hollibaught *et al.*, 1980). It was found by Iturriaga (1979) that bacterial activity is related to sedimenting particulate organic matter. Quantitative studies have been made of bacterioplankton abundance (Francisco *et al.*, 1973; Howden, 1977; Hobbie *et al.*, 1977 and Watson Fuhrman and Azam, 1980) therefore, it is now possible to map regions of the ocean with respect to the distributional patterns of bacterioplankton biomass and growth as well as the more conventional uptake and turnover rates of selected organic compounds.

Denitrification by marine bacteria has not been investigated extensively as has been for certain terrigenous organisms Sreenivasan and Venkataraman (1956) observed that only five species of marine bacteria are capable of denitrification. Goering and Dugdale (1960) established that in an equatorial island bay microbial denitrification does occur in the marine environment. Brisou and Vargues (1963) found bacteria capable of reducing nitrate to nitrite in coastal waters off the coast of Algeria. Autotrophic bacteria capable of oxidizing ammonium and nitrite ions has been isolated by Watson (1962, 1963). In subsequent reports (Murray and Watson, 1963, 1965; Watson, 1965) more detailed characterizations, mainly on morphology, cytology and cultural conditions have been given of the marine ammonium oxidiser, *Nitrocystis oceanus*. Gundersen (1966) and Gundersen, Carlucci and Bostrom (1966) observed that ammonium oxidation on agar by *N. oceanus* was inhibited by an oxygen concentration of 90.0 atoms. An atmosphere with 21% oxygen gave maximum nitrification. There was ammonium oxidations with as little as 0.2% oxygen, although very little carbon-di-oxide was fixed by the cells. In addition to *N. oceanus* and those bacteria referred to in papers by the above workers, other enrichment cultures of ammonium and nitrite-oxidizing bacteria have been isolated from sea water or marine muds (Kimata, Kawai and Yoshida, 1961, 1963 a,b; Vargues and Brisou, 1963). The paper describes the isolation and purification of more marine nitrifying bacteria and the results of some laboratory experiments in which the influence of substrate concentration and temperature on nitrification has been determined. Gas chromatography was used by Barbaree and Payne (1967) to analyse the gases released by growing cultures of denitrifying marine bacterium, and they discussed the applicability of gas chromatography to analyse ammonia in the marine ecosystem. The isolation, purification and some kinetic studies of marine nitrifying bacteria isolated from the north Pacific Ocean off the Pier of Scripps Institution of Oceanography were done by Carlucci and Strickland (1968). They observed in a number of cultures subjected to both the temperature and substrate dependent experiments, the lag or induction phase of nitrite production subsequent to inoculation and suggested a chemostat study of nitrification by marine bacteria would be helpful in assessing the importance of the lag phase in nature.

Fiadeiro and Strickland (1968) concluded that nitrate reduction was responsible for the high concentration of nitrite in oxygen poor waters. Carlucci and Mcnally (1969) found chemoautotrophic nitrifying bacteria oxidising nitrite at low concentration of substrate and oxygen and concluded that high concentrations of nitrite may arise in open ocean waters from ammonia oxidation. Menon *et al.* (1972) and Taylor (1940) found a decrease in population from the 1 m level to the 5 cm level with the notable exception of nitrifying bacterial populations that increased by three orders of magnitude from the 1 m to the 5 cm level. This increased population may be related to the high ammonifying population creating a ready nutrient source. Herbert (1975) recorded heterotrophic nitrogen fixation in shallow estuarine sediments.

Biological activity of the bacteria which are associated with their growth such as temperature, salinity, oxygen consumption, acid and alkali formation were studied by Society of American Bacteriologists (1957) and detailed information are given in their Manual of Microbiological Methods. The indirect influence of temperature on metabolic activity of bacteria was observed (Orleb, 1956; Rheinheimer, 1966). Sieburth (1964 b) found '*Corynebacterium*' changing gram characteristics at different temperatures being gram-negative and pleomorphic below 16°C and gram-positive and frequently coccoid above that temperature. Morita and Haight (1964) found psychrophillic marine organisms growing between 1 and 20°C with an optimum at 15 - 16°C, but was killed by heating to 28.5°C for 6.25 h. Wiebe and Liston (1965) found only 7 obligate psychrophils among some 452 bacteria isolated from sediments. Sieburth (1965) also found a difference in the temperature relations of bacteria in estuarine waters between spring and winter whereas Nedwell and Floodgate (1971) found temperature as a factor in the seasonal selection of heterotrophic bacteria in intertidal sediment.

Bell and Dutka (1972 a,b) found membrane filter procedure somewhat superior to the pour plate procedure and majority of the heterotrophic population in the sediment are mesophilic. This heterotrophic population readily metabolises and multiplies at 4°C although the rate is less than half of that at 20°C. They also indicated that these sediments do not contain any true psychrophiles. Surface sediment heterotrophic bacterial densities in these lakes consistently range between 10^6 and 10^7 organism per gram of dry weight, no matter what counting medium has been used or what year or month the sample has been collected (Weeks, 1944; Vanderpost, 1972 and Bell and Dutka, 1972).

Heavy metal ions are important constituents of natural and artificial sea water. Jones (1964) speculated fluctuations in the concentrations of heavy metal ions which may be toxic for most non-marine bacteria. Macleod _et al._ (1954-60) have found that all the marine bacteria have got specific requirement for sodium, potassium and magnesium ions, but Carlucci _et al._ (1961), Scarpino and Pramer (1962) and Jones (1964) are of view that the action of heavy metal ions can be diminished by addition of chelating agents to sea water. Brown (1964) stated that bacteria with a low salt tolerance have two cell membranes with one polygonal layer about $100A^{0}$ across and the pleomorphism in bacteria may be described as the effort of bacteria to stabilise itself against rupture by the ionic components of the sea. Wood (1965) found that pleomorphism may occur in bacteria from other habitat but not so frequently as in marine bacteria. Daiku and Sakai (1976) conducted physiological studies on the inorganic salt requirements of marine bacteria and found salt was an essential requirement for cytochromes in the cytoplasmic membrane for effecting electron transfer in the oxidation-reduction reactions.

Seasonal changes in the antibacterial activity of sea water was recorded by Sieburth and Pratt (1962) and

Vaccarro *et al.* (1950). Rosenfield and Zo Bell (1947); Krassilinikov (1964); Burkholder *et al.* (1966) and Gauthier (1969) suggested that the antibacterial substances have been produced by bacteria to inhibit the algae. Seasonal changes in the antibacterial activity of sea water was recorded by Sieburth and Pratt. So far there are 4 key reference works in the field of marine bacteriology (Zo Bell, 1946; Brisou, 1955; Kriss, 1963 and Oppenheimer, 1963) which have got extensive information on the distribution of bacteria and their various characteristics; bio-chemical activities and hydrobiological importance in the indigenous aquatic environments.

Unio Simidu and Kayuyoshi Aiso (1962) observed variation in the bacterial counts of bacteria in the samples from low saline areas in brackish and fresh water media than in sea water media. Fluctuation in salinity of the Kieler Bucht (Rheinheimer, 1977) caused corresponding changes in the bacterial population particularly if water of low salinity from the central Baltic overlies more saline water from the Belt Sea. Macleod (1965) reviewed his former work and pointed out that he has not found any character which could be used to separate marine bacteria from bacteria found in other environments.

Pugh *et al.* (1974) analysed microbiological characteristics of two beaches and Anglessey Klaus (1974) observed the influence of salt on the distribution and activity of bacterial population from fresh and waste waters in Kiel Fjord.

Maeda *et al.* (1977) worked out the generic composition of acid hydrolysing bacteria in sea water in the Japanese coast. Ramsay (1977) isolated aerobic heterotrophic bacteria from water, mud and macrophyla of Lake Grasmere, New Zealand. Maureen (1977) *et al.* characterised lipolytic and proteolytic bacteria isolated from marine sediments. Murata *et al.* (1977) isolated Ni-tolerant bacteria in water and sediments. Investigations on the bacterial ecology in sandy beaches of the North Sea and the Baltic was conducted by Rheinheimer (1977). Joiris (1977) studied the role of heterotrophic bacteria in marine ecosystems. Joint (1978) estimated microbial production of an estuarine mudflat. Novitsky and Morita (1978) found out the possible strategy for the survival of marine bacteria under starvation conditions. Shanta Nair *et al.* (1978) surveyed the distribution of heterotrophic bacteria in marine sediments in the Goa coast.

Kitamikado and Kasama (1978) isolated aerobic collagenetic bacteria in the coastal area of Japan. Ramsay (1978) measured the direct counts of bacteria by a modified acridine orange method, in relation to their heterotrophic activity.

Weise and Rheinheimer (1979) investigated the bacterial settlement on marine sand sediments by Fluorescence Microscopy. Tejero (1979) isolated heterotrophic bacteria from the upwelling region of North-west Africa during "Altor VII" Cruise II and worked out the numerical taxonomy of the heterotrophic aerobes. Sakata and Kakimoto (1979) studied the effect of visible light on pigmented and non-pigmented bacteria and found that non-pigmented bacteria are susceptible to light while pigmented bacteria are resistant to visible light.

Fuhrman et al. (1980) correlated the distribution of bacterioplankton with the distribution of other biological parameters such as chlorophyll, primary production and organic particulates.

Schroder and Van ES (1980) surveyed the distribution of bacteria in intertidal sediments of the Ems Dollard Estuary in Netherland, and Schroder (1980) studied the influence of organic pollution on heterotrophic bacterial macrobenthic and meiobenthic populations in the intertidal flats of the same area. Simidu et al. (1980) studied heterotrophic bacterial flora of sea water from the Nanseishoto (Ryukyu Retto) area. Shiba and Taga (1980) studied heterotrophic bacteria attached to sea weeds. Yoshimura et al. (1980) established seasonal fluctuation of heterotrophic and coliform bacteria.

Bent and Goulder (1981) studied planktonic bacteria in the Humber estuary and found seasonal variation in population density and heterotrophic activity in the surface waters of the estuary. Krstulovic and Sobot (1981) studied the distribution of suspended bacteria, colony forming bacteria and H_2S producing bacteria in the coastal waters of the central Adriatic. Kimio Fukami *et al.* (1981) found out the fluctuation of the communities of heterotrophic bacteria during the decomposition process of phytoplankton; initially the counts will be moderate but in the end of decomposition process of phytoplankton, counts will be very high due to the increase in the nutrients in the vicinity of aerobic heterotrophs. Duong Van Qua *et al.* (1981) studied the occurrence and generic composition of protease producing halophilic bacteria in neritic seawater around Japan. From all these observations it has to be concluded that apart from anaerobes in aquatic environments, the aerobic heterotrophs play a good role as potential decomposers or degraders of organic matter whether it is phytoplankton, zooplankton or any other organic material.

Indicator Bacteria:

Treatises on water bacteriology are generally concerned primarily with the study of sanitary properties of domestic water supply, swimming pools and sewage. Sea water is rarely considered for various reasons. The sea water does present certain problems of interest to marine microbiologists, sanitary engineers and students of public health. Outbreaks of oyster borne typhoid, gastroenteritis and R-factor transferrence and other diseases have focussed attention on these problems in recent years. Slanetz and Bartley (1965) and Geldreich (1966) published review of the research conducted on the significance of faecal coliforms in the Basic and Applied Science programme at the Cincinnati Water Research Laboratory, Cincinnati, Ohio.

To augment primary bacteriological measurements, use of supplementary bacterial parameters are desirable to pinpoint specific hazards. Mallman and Seligman (1950); Litsky *et al.* (1955); Slanetz and Bartley (1957); Kenner *et al.* (1961); Burman (1961); Mead (1966) and Geldreich and Kenner (1969) believed the occurrence of faecal streptococci in water which suggested recent faecal pollution, whereas their absence indicated little or no warm-blooded animal contamination. Faecal coliforms were identified as *faecal coli* when lactose fermentation and indole production at 44°C is effected by the isolated strain (Bonde, 1962; 1966 a, b) or faecal coliform's whole identity is based solely on lactose fermentation at 44.5°C (Geldreich, 1966).

Many authors considered coliforms as an indirect indicator of faecal pollution and coliform index also may not be the true indicator of faecal pollution (Moussa, 1965; Mathur and Ramanathan, 1966). Enteric bacteria are found less resistant to most commonly used disinfectants than viruses (Kjellander and Lund, 1965; Kruse _et al._, 1970; and Chambers, 1971). Enteric bacteria are also known to be more resistant to environmental factors than enteric viruses (Shuval, 1957). Some authors (Andre _et al._, 1967; Rudolphs _et al._, 1950; Geldreich, 1968; Gallagher and Spino, 1968; Beard and Meadowcroft, 1935) compared the persistence of a limited number of pathogens with an indicator. From their studies, it was concluded that a few indicator bacteria survive somewhat longer than some enteric pathogenic bacteria. Mitchell (1968) studied the factors affecting the decline of non-marine micro-organisms in sea water. Sepp and Jepling (1968) applied faecal coliform concept to coastal waters of California, which indicated that the bacterial parameter was closely related to the sanitary quality of marine waters during storm water run off and to those marine waters receiving various kinds of wasteflows.

Brezenski and Russomanno (1969) and Grunnet and Nielsen (1969) isolated enteric pathogens in tidal estuary and in seventeen beaches of New York. Evidence from field investigations of Gallagher *et al.* (1969) suggested that all total coliform occurrences can be associated with faecal pollution.

The dispersion and disappearence rate of enteric organisms from a marine outfall in Israel was also estimated by Gilath *et al.* (1970). Geldreich's (1970) analysis of data compiled over three years study on Lake Michigan bathing water in the Chicago area indicated that the closing of nine metropolitan beaches resulted in 20% decrease during the season if a faecal coliform limit of 200 organisms per 100 ml were used instead of the total coliform standard. Bathing water quality standards for New York city beaches were established by Caroll *et al.* (1970) and Foster *et al.* (1971).

Available data suggest the existence of a wide range of levels with regard to minimum infectious dosage, among the various water-borne pathogens to infect a bather or consumer eating fish harvested from polluted water (Kehr and Butterfield, 1943; Dupont, 1971). Massive concentration of cells of enteropathogenic *E. coli* have been reported necessary to produce infection in adult volunteers. Gore (1971) made some observations on the bacterial flora of the beach sand and beach water and found that the major flora isolated from the beach was coliform of faecal origin apart from other heterotrophic bacteria which are capable of producing acid and gas from sugars.

In the more open coastal waters of Belgium, Pinon *et al.* (1972) and Pinon and Pisek (1972) found the faecal coliform to total coliform ratios ranging from 0.13 to 0.37 with a mean value of 0.28. Similar data observed in beach area in Southern California (Cali. State Dept. of Public Health, 1970) indicated a more rapid assimilation of faecal pollution occurring in more open coastal waters.

Bruni *et al.* (1972) worked on water and sediment from Lake Ganzirri and found relatively high counts in coastal waters than in open ocean waters. Also they found some correlation between indicators and heterotrophic bacteria. The difference in heterotrophic counts from near shore and mid-section of Volta Lake were reported by Biswas (1972). Bacterial populations was unaffected by changes in temperature but there was evidence of an inverse relationship with transparency in the surface layers.

Pollution of coastal waters by enteric pathogens has been also studied in estuarine and marine environments by Yoshpe-purer and Shuval *et al.* (1972); Paoletti (1964); Brezenski and Russomanno,(1969), Brezenski (1971) and Shuval (1972). Gerba and Schaiberger (1973) found a direct correlation between the amount of rainfall and coliforms on an ocean bathing beach.

Hashimoto *et al.* (1974) found the distribution of motile streptococci in faeces of man and animal in river and sea water in Japan. In 1974, Goldreich published microbiological concepts for coastal bathing waters.

Significant numbers of coliform bacteria have been noted (Gameson, 1975) in sea water adjacent to a colony of nesting gulls. While it is accepted that the disposal of some conservative substances to estuaries and the sea should be prevented or restricted, differences of opinion exist regarding the need for limiting by treatment the discharge of degradable wastes (Calvert, 1975 and Baalsrud, 1975) and for the regulation of water quality by the imposition of standards (Moore, 1975 and Shuval, 1975). Control has been exercised to dispose sewage into the sea in accordance with certain qualitative criteria (Agg, 1975).

Mosley (1975) doubts the potential for infectivity of enteric organisms in the sea, but even the dilution of the infective agents, will not prevent the cause of epidemiological problems that many subclinical infections result because of small infective doses. Faecal pollution of beaches by 'Indicator bacteria' like coliforms, *E. coli* and *Streptococcus faecalis* has been identified and studied by Regnier and Park (1972); Turner (1976); Bonde (1968);

Stevenson (1953); Moore (1959); Berger *et al.* (1963); Shuval *et al.* (1968); Grunnet *et al.* (1970); Cohen and Fuerer (1968) and Tinker (1976). Enteric pathogens like *Salmonella* have been reported from seafoods of India (Arul James and Iyer, 1972; Joseph Mathen and Iyer, 1976; Nerkar *et al.* (1975) and Iyer *et al.* 1975).

Water, sediment and sand from Southern Biscayne Bay were examined over a 3 months period by Buck (1976) for the indicator and potentially pathogenic bacteria and yeasts. The Miami river was found the most significant source of pollution (10^{-5} total coliforms 100/ml) but bathing beaches showed low densities of all micro-organisms sought.

Koo and Wong (1976) failed to detect any association between the incidence of infectious disease and bathing in Penang, Malaysia. Previous work has revealed that high concentrations of coliform bacteria exists in sediments rather than in the overlying waters (Weiss, 1951; Rittenberg *et al.*, 1958) and that cells of *E. coli* were capable of utilising the nutrients released from estuarine sediments (Gerba and McLeod, 1976). Furthermore, presence of estuarine sediments has been found to enhance greatly the survival of *E. coli* in natural sea water under laboratory conditions (Gerba and Macleod, 1976). Vasconcelos and Swartz (1976) studied the survival of bacteria in sea water using a diffusion chamber apparatus. An electrochemical method for early detection and monitoring of coliform in water was analytically designed by Wilkin and Boykin (1976).

Studies on enumeration of bacteria in marine water by Stanfield and Irving (1977) demonstrated that methods of enumeration are not always equivalent and that a realistic assessment of the value of a method can be made only when the organisms to be recovered have been exposed to an environment capable of stressing or attenuating the bacteria. Robinson and Stanfield (1977) have demonstrated that the variation in total coliform count can be obtained in marine waters by using different selective media and incubation procedures.

Distribution of viral and bacterial pathogens in a coasta canal community was studied by Gerba *et al.* (1977) who detected high concentrations of microbes in sediments of pollute coastal areas. Pathogenic and non-pathogenic bacteria are present in large numbers in bottom sediments and may be releas ed upon resuspension following dredging, boating, storms and other activities (Goyal *et al.*, 1977; Grimes, 1975

Tobin and Dutka (1977) found significant differences between various brands of membrane filters in their ability to recover bacteria, from pure cultures, natural waters or sewage. Apparently they also found that small changes in the incubation temperature cannot affect the coliform count obtained. A high correlation of the *Salmonella* and enterococci indices was found by Vlodavets and Kalina (1977) when a study was conducted to isolate *Salmonella* in the coastal sea water.

Bacterial population structure in polluted areas was studied by Bianchi (1977) and found that simple enumeration of bacterial indicators of faecal pollution does not offer a sufficiently realistic view of the microbial participation in pollution processes. Tennant and Reid (1978) studied lactose fermentation at 35.5°C and 44°C on coliform bacteria isolated from sea water and shellfish. [illegible] (1978) found *E. coli* satisfactory as an indicator of faecal pollution in tropical regions after determining the coliform and *E. coli* in inshore areas, embracing more than three quarters of the coastline of Penang Island.

There is adequate evidence to-date to show that enteric pathogens enter the sea in large numbers through sewage outfalls. Shuval (1978) found that all these pathogens survive long enough and in high enough concentrations to lead to the transmission of the disease to man by contaminated shellfish and by bathing in water highly contaminated by fresh sewage.

An inquiry was made in France by Lys and Lyon (1978) among tourists who go on the summer time to seaside resorts and the tourists informed that actions for the protection of environment in France are not sufficient. The probability of human diseases in people living in contact with polluted sea water is evaluated by Martin Bouyer (1978) in France. Zanoni *et al.* (1978) surveyed six faecal coliform sampling areas of Lake Michigan off the Milwaukee metropolitan area and found that the counts dropped rapidly with distance from the shore.

Historically the different aspects of the water industry have been fragmented so that water supply, waste disposal and water pollution have not been studied in a comprehensive manner. James and Evison (1979) aimed to provide a comprehensive review of the role of biological indicators in the assessment of water quality. Qualitative and quantitative abundance of indicator bacteria and their possible correlation with certain environmental parameters as abundance of fresh or decaying *Salvinia* debris and the overall pollution status of the Cochin beach are presented by Gore *et al.* (1979). The influence of pH, salinity and organic matter on the adsorption of *E. coli* and other enteric pathogens in estuarine sediment was studied by Labelle and Gerba (1979). In laboratory studies of *Escherichia coli* and enteric pathogens were shown to survive longer in the presence of sediment than in sea water alone (Gerba and Mcleod, 1976; Vandonsel and Geldreich, 1971 and La Belle and Gerba, 1979).

Thapliyal *et al.* (1972) studied standard plate counts, coliform and enterococcus densities in various natural waters in the Terai region (India) and identified 10 IMViC types in their source of water supply. They considered enterococci indicator system of greater value than the coliform count indicating water pollution. Phirke and Verma (1972) worked in river water samples in Delhi and found a linear relationship among two indicator systems.

McFeters and Stuart (1972) suggested that the survival of coliform bacteria in water is directly related to some environmental parameters. Many studies have now shown, however, that coliform bacteria are able to reproduce in enriched water and thus falsely indicate an elevated health hazard (Eliassen, 1967; Hendricks, 1972; and Dutka, 1973).

After a study of 181 waste water samples, Chang and Yates (1973) concluded that there was no correlation between concentration of coliform bacteria, faecal streptococci, coliphage, and enteric viruses. Mundt (1973) reported that 90% of the *Streptococcus faecalis* obtained from plants, wild animals and insects differ from *S. faecalis* of human origin in Litmus milk digestion and in fermentation into cellobiose.

Cabelli *et al.* (1974) indicated failure of the indicator bacterial concept when applied to virological quality of ground water. Metcalf *et al.* (1974) found shellfish cultured in polluted waters as a potential route of disease transmission.

Wolfe (1972) established the importance of shellfish from faecally polluted waters in transmission of intestinal infection.

Stephen *et al.* (1975) suggested that seafood may be responsible for the outbreaks of gastro-enteritis in children as enteropathic *E. coli* and other coliforms have a tendency to accumulate in fish, shellfish, mussels, prawns, crabs and in sediments. It has been demonstrated by Grimes (1975) that these sediment bound faecal coliforms will be released by the dredging activities.

Taylor *et al.* (1973) presented a new technique and a new medium for quantification of faecal coliforms as there is a growing recognition and use of faecal coliform group as an indicator of recent faecal contamination of surface waters.

Kenard and Vallentine (1974) have shown that the ratio of phage to faecal bacteria is about 0.7 : 1 regardless of the level of contamination. This procedure is now being tested in cities by the Atlantic Research Corporation and Public Technology in the fields. The quantity of both total and faecal coliforms in water is indicated by the specificity of bacteriophage. Presnell (1974) is of view that use of faecal coliform test avoids the undesirable risk of excluding some faecal contamination.

Huthan (1974), Bissonnette *et al.* (1974) used various recovery methods to detect coliforms in water by applying the membrane filter chamber technique. Newman and O'Brien (1975) used gas chromatographic analysis as a presumptive test for coliform bacteria in water and Abshire and Guthrie (1973) studied *E. coli* as faecal indicator by Fluorescent Antibody Method.

Carney *et al.* (1975) studied seasonal occurrence and distribution of microbial indicators and pathogens in the Rhode river of Chesapeake Bay. These microbial indicators are consistently found in harbours and their numbers varied according to the degree of organic pollution of the water (Kuch, 1974; Kuch and Chan, 1975). But recently some authors have isolated enteroviruses in the absence of indicator bacteria which supported the view, that the bacteria migrate through certain soils. Unfortunately very few studies in this line have been conducted (Gerba *et al.*, 1975 a).

Feasibility of using ozone to disinfect sea water use in controlled environment in shrimp culture were run at the Environmental Shrimp Culture Station at Puerto Penasco, Mexico (Donald and Lightener, 1979) 99.9% of the pathogens were killed within 95 minutes of minimum exposure period with ozone.

Storage techniques of *Escherichia coli* strains containing plasmid DNA in liquid nitrogen was found in U.K. by Breese and Sharp (1980). The gas production in lactose broth by *E. coli* strains are directly correlated to pH of the enrichment media (Meadows *et al.*, 1980). It was found by Levens and Tuovinen (1980) that various membrane filters used for the isolation of *E. coli* has got some inhibitory effect in the formation of colony, size of the colony etc. In Sao Paulo, Brazil, Longo *et al.* (1980) identified strains of *E. coli* from stool samples by direct fluorescent antibody tests. Holbrook *et al.* (1980) found a modified direct plate count method for counting *E. coli* and other enteric pathogens at the Unilever Research Laboratory, Colworth House, Sharnbrook, Bedfordshire. Gooch (1980) evaluated a multitest system for rapid identification of *Salmonella* and other enteric pathogens including indicators. All these methods are found extremely helpful to find out whether a sample is faecally polluted or not within 10 hrs of time.

In summary, it can be stated that the knowledge of heterotrophic bacteria and indicators of bacterial pollution in marine environment has accumulated slowly from a very late beginning when compared with progress in other areas of the parent discipline. Periodic reports of marine bacteria resulting from fortuitous discovery interspersed with "short-term" concentration on very special groups, mark the general trend of historical development of marine microbial eco-physiology. The progression of critical studies since 1960 may be prognostic of the future achievements and developments to be expected.

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CHAPTER III.

MATERIAL AND METHODS.

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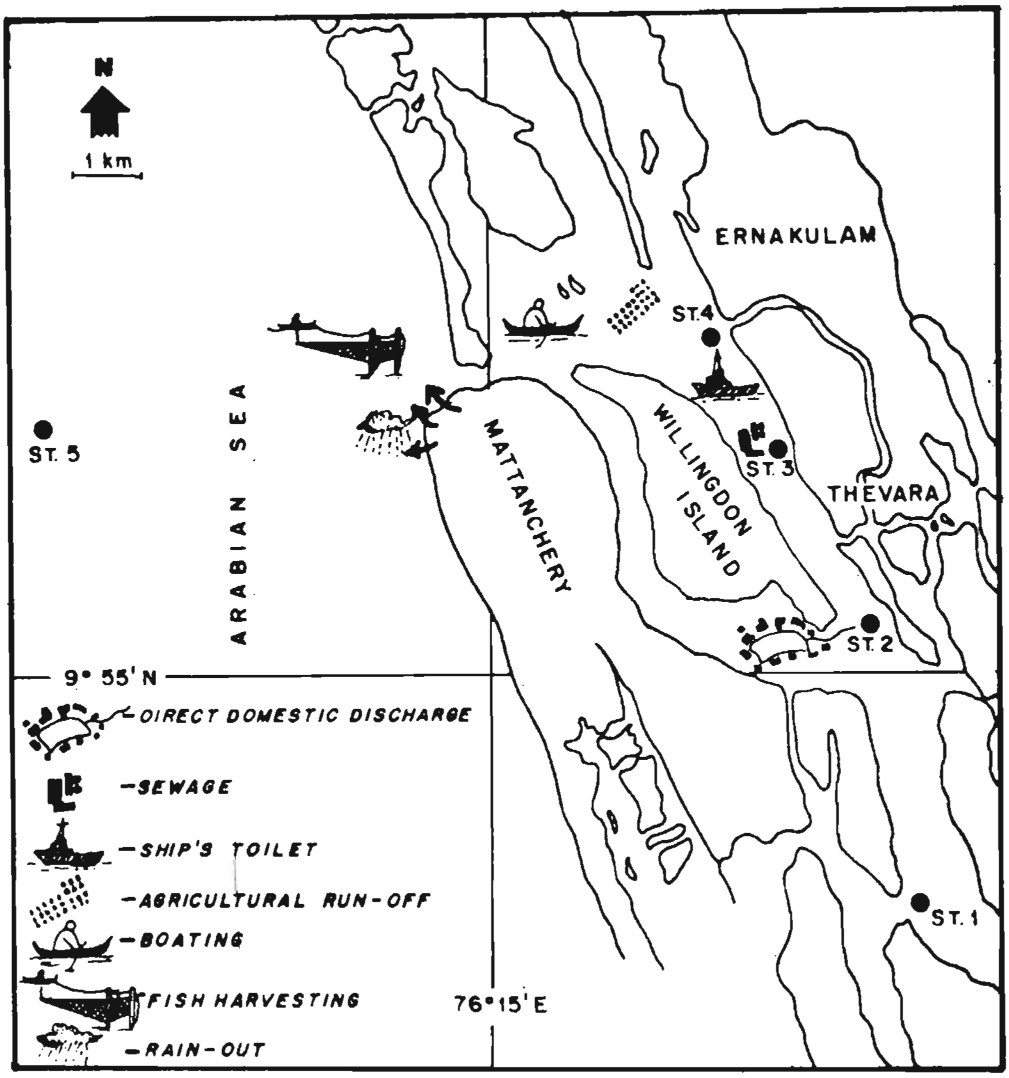
The general methods and materials are as given below. The specific ones are given at appropriate places in the text.

The sampling sites in the backwater are widely separated and have dissimilar water and sediment characteristics and directly influenced by monsoon cycle and local inputs. Altogether three sets of observations were made at stations in the area between 09°38'N - 10°00'N inside the Cochin backwater and contiguous estuarine system, and near the barmouth where marine conditions prevail, and the results are analysed and presented. First set of data were collected during May 1972 to March 1973 from 5 stations of which 4 stations were almost equidistant from one another and located inside the backwater system and one station off the barmouth of Cochin (Fig. 1). A total of 95 sea water samples and 98 sediment samples were analysed for total bacterial population, _Escherichia coli_ and phytoplankton and also for other environmental parameters such as temperature, salinity, oxygen, phosphate, nitrate, nitrite and silicate. Influence of environmental parameters on the fluctuation in the bacterial

Fig. 1. Map showing the sampling Stations during 1972-73 showing entry of pathogenic micro-organisms into an estuarine water-body.



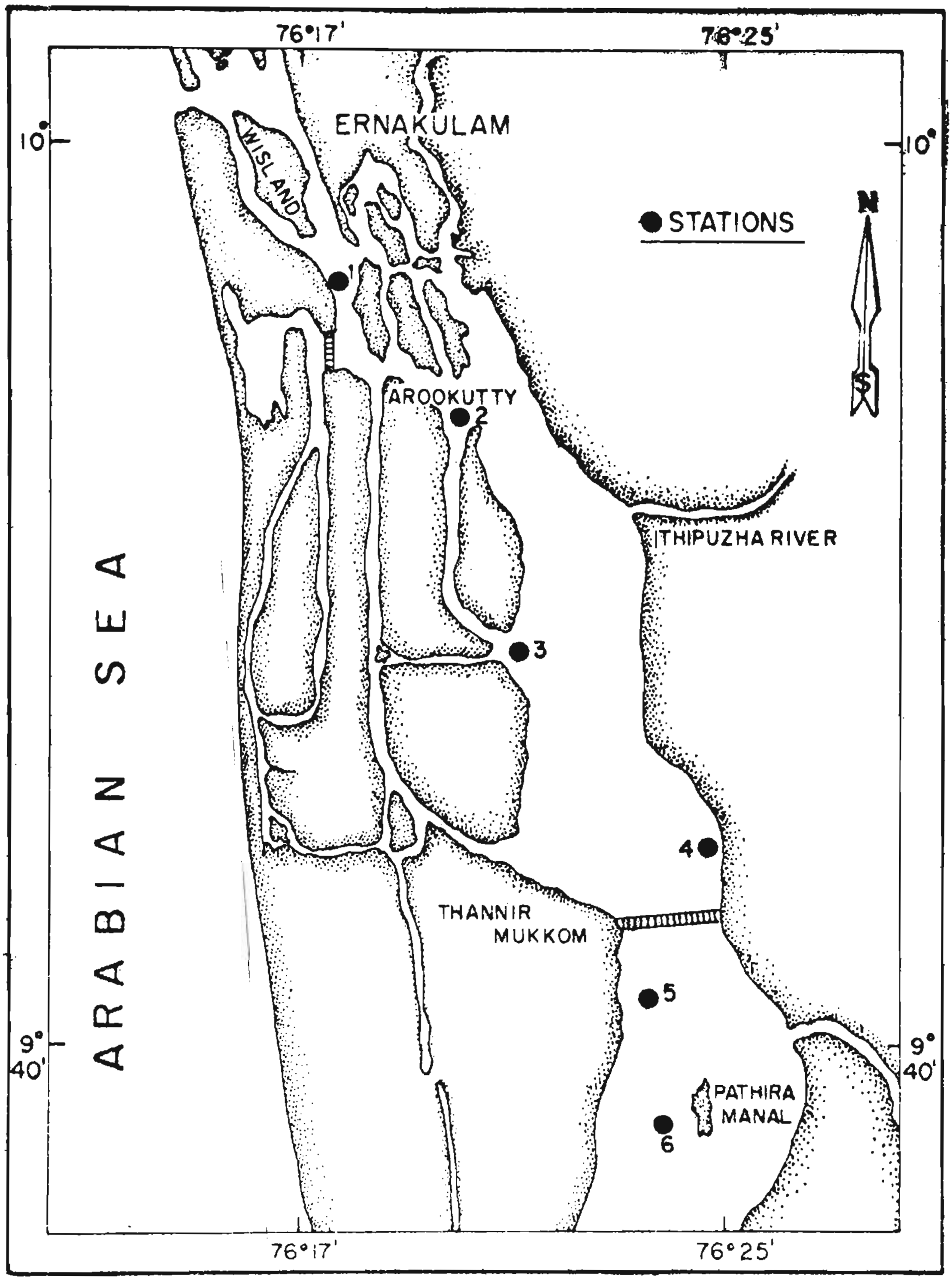
1972-1973

TRANSPORT OF PATHOGENIC MICRO-ORGANISMS TO AN ESTUARINE WATER BODY.

population was studied during this period and the results were analysed statistically.

In the second set of observations data were collected during March 1974 to February 1975 from 6 fixed stations all located completely in the backwater system (Fig. 2). Fortnightly collection of water and sediment samples were taken and totally 70 samples were collected from sea water and 72 samples were collected from sediments from the six stations. Sediments of the sampling Stations I, II, V and VI during this period were black silty clay deposits except at Station II, where the sediment was yellow sandy throughout the sampling period. The American Society for Testing Material (ASTM) Sub Committee on biological monitoring has recommended the following 9 bacteria as representative of bacteriological indicators, coliforms, faecal coliforms, (indicator of human pathogen), faecal streptococci (indicator of animal pathogen) coagulase positive staphylococci (indicator of body pollution), *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Clostridium perfringens*, *Aeromonas hydrilla* and *Candida albicans*. Out of this 9 bacterial indicators data have been collected on the first 4 indicators of faecal pollution during the period of observation. Influence of environmental parameters such as temperature, salinity, oxygen, pH, phosphate and nitrate on the fluctuation of total heterotrophs, total coliforms, *Escherichia coli*, faecal streptococci and staphylococci was

Fig. 2. Map showing the sampling stations during 1974-75.


76°17'
76°25'
10°
10°
ERNAKULAM
W ISLAND
● STATIONS
N
S
1
AROOKUTTY
2
THIPUZHA RIVER
3
ARABIAN SEA
4
THANNIR MUKKOM
5
9° 40'
9° 40'
PATHIRA MANAL
6
76°17'
76°25'

studied during this period and the results were examined by analysis of variance and product moment correlation. A ratio between *E. coli* and faecal streptococci was constructed which formed faecal index.

In the third set of observations data were collected from 3 fixed stations during July 1975 to June 1976 (Fig. 3). The first station was fixed near the mouth of the sewage effluent of Cochin city, the second at a place 5 km away from the sewage effluent with typical backwater conditions in the Mattancherry channel to know the effect of backwater on sewage bacteria and the third one in typical marine condition near barmouth to know the effect of marine environment on sewage bacteria as the mean salinity was relatively high at this station as compared to other two stations. Station I and II do not significantly differ in mean salinity. Besides other environmental parameters, data were collected on the distribution of organic carbon and organic nitrogen to ascertain the influence of these factors on the bacterial populations such as total coliforms, *E. coli*, faecal streptococci and staphylococci. The ratio between *E. coli* and faecal streptococci formed the faecal index.

In addition specialised studies on the zymogenous microbial decomposition of the floating weed *Salvinia molesta* and the effect of this allochthonous organic matter in sediment ecosystem were investigated in the areas off Cochin during January 1978 to October 1978.

Fig. 3. Map showing the sampling stations during 1975-76.

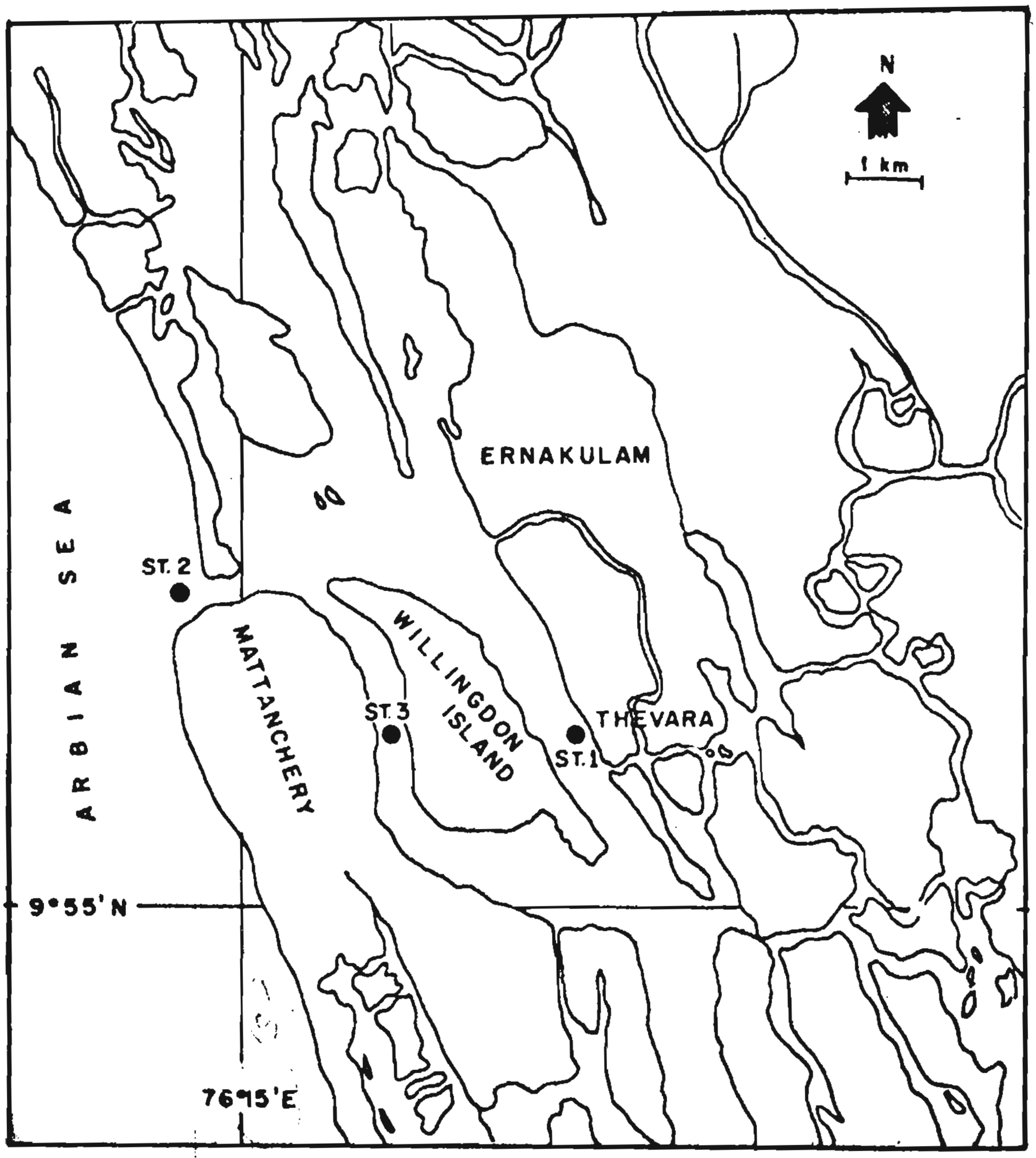

N
1 km
ERNAKULAM
ARBIAN SEA
ST. 2
MATTANCHERY
WILLINGDON ISLAND
ST. 3
THEVARA
ST. 1
9°55'N
76°15'E

fig (3)

The samples for ecophysiological studies (Part B Sections I & II) were collected from Shankumugham, Kovalam and Vizhinjam coasts. Clinical strains for comparative study of bio-chemical activity and for antibiotic sensitivity were isolated from sputum, urine, blood samples from Sree Chitra Thirunal Medical Centre, Trivandrum. *E. coli* type I for serological studies (Section III) were collected from water and sediment during the period July 1975 to June 1976 from the estuarine environment of Cochin.

Collection of water samples:

Surface sea water was collected in sterile 300 cc glass bottles in aseptic conditions and kept at 4°C until the time of bacteriological investigations in all the collections. Sediment samples were collected by the bottom grab (Van Veen grab (0.048 m^2), Barnett, 1959), for microbiological investigations the only suitable equipment for sediment collection is that one in which the cover of the grab can be opened from above. Investigations have shown that the number of bacteria and diversity of constituent groups decrease rapidly as the lower layers of the sediments are reached. Therefore, the topmost layer (in particular, the sediment water interface) should be sampled for microbiological analyses. The sample bottle and polythene bags with sediments are held at 4°C until processing, 18-24 hours later.

Identification of Bacteria:

The determination of bacteria is not possible on the basis of their morphological characteristics alone; in the great majority of cases their metabolic performance must also be taken into account. The first requirement to identify is well-developing pure cultures of the bacteria. Their growth in various nutrient media is observed and in particular, the form and pigmentation of colonies. This is followed by microscopic examination of the form, size and motility of the cells, with the help of the adsorption, adhesive capacity of bacterial strains using suitable dyes (Gram-staining, acid fastness). In addition to microscopic examination numerous physiological and biochemical tests were carried out. The following biochemical tests were carried out, among others; motility, gelatin liquifaction, nitrate reduction, H_2S formation from cysteine and sulphate, indol formation, NH_4 + formation from proteins or peptone, acid and gas formation from sugars, alcohols and glycosides, starch hydrolysis, degradation of chitin, casein and hydrocarbons, catalase and oxidase formation and antibiotic sensitivity. Temperature, salinity, oxygen, pH, nutrients were determined and their optima was found out.

All the results were collected in tables. Each bacterial strain tested thus received a 'profile' which becomes more reliable with more data it contained. With the help of these characterisation, the identification of the bacteria was attempted. The procedure for carrying out the tests is described in the various specific chapters (Part B, Section I). Bergey's Manual of Determinative Bacteriology by R.S. Breed, E.G.D. Murray and N.R. Smith (Bailliere, Tindall and Cox Ltd., London, 1957), the scheme of Usio Simidu and Kazuyoshi Aiso (1962) and also the scheme of Shewan _et al_. (1960) were referred for the classification.

Culture media:

Both synthetic and semi-synthetic types of media were used for isolation, purification, maintenance and sub-culture of heterotrophic bacteria and indicators of bacterial pollution.

Aged sea water was used in the preparation of all media for heterotrophic aerobes, and distilled water in the preparation of media for indicators of bacterial pollution. The pH of the media were adjusted to 7.2 (heterotrophs) and 6.9 (for indicators of bacterial pollution) respectively with the help of N/10 NaOH and N/10 Hcl solution.

a) <u>Sea water agar (SWA) for heterotrophic bacteria</u>:

<u>Composition</u>:

Peptone - 1%
Ferric phosphate - a pinch
Agar - 2%
Aged sea water - 100 ml
pH 7.2 15 lbs - 30 mts.

Sea water agar media was used to isolate all heterotrophic bacteria from sea water as well as sediment samples.

b) <u>Tergitol^7 Agar</u>:

<u>Composition</u>:

Proteose peptone No.3 Difco - 5 g
Bacto yeast extract - 3 g
Bacto - Lactose - 10 g
Bacto - Agar - 15 g
Tergitol^7 - 0.1 ml
Bacto Bromothymol blue - 0.025 g
Distilled water - 1 litre

Bacto Tergitol^7 Agar is a selective medium for *Escherichia coli* and members of the coliform group, prepared according to the formula given by Chapman[1]. Chapman[2] reported that the addition of Triphenyl tetrazolium chloride (TTC) to this medium permitted the confirmation of

E. coli after 10 hours of incubation and also that this medium gave excellent results for *Monilia* and other fungi. Chapman[1] reported that the addition of Tergitol[7] to an agar medium consisting of proteose peptone No.3, Bacto yeast extract, Lactose, Bromo Thymol blue permitted unrestricted development of all coliform organisms and inhibited the development of Gram negative spore-formers as well as Gram-positive micro-organisms.

Escherichia - produced yellow colonies surrounded by yellow zones.

Aerobacter - large mucoid colony surrounded by yellow zones.

Paracoli and other non-*lactose* fermenting strains - Colonies usually surrounded by blue zones.

Proteus - has no tendency of spreading.

0.5 ml of sea water or 0.5 ml of 10^{-2} diluted sediment sample is inoculated by smearing the surface with the sample if the surface of the medium is dry. Pour-plates do not give satisfactory results as these organisms are heat-sensitive.

The addition of 40 mg of TTC to a litre of Sterile Tergitol[7] Agar permitting the confirmation of *E. coli* after 10 hrs of incubation was described by Chapman[2]. *E. coli* does not reduce the dye while other coliform organisms rarely fail to do so. Surface colonies of *E. coli* on this medium are greenish yellow surrounded by a yellow halo while other coliform surface colonies are dark-red. Readings can be made following incubation at 37°C for 10 hrs.

e) KF Agar:

Faecal Streptococci:

Composition:

Proteose peptone No.3 - 10 g
Yeast Extract - 10 g
Sodium chloride - 5 g
Sodium glycerophosphate - 10 g
Maltose CP - 20 g
Lactose - 1.0
Sodium Azide - 0.4
Sodium carbonate AR - 0.636
Bromocresol purple - 0.015 (1 cc % sol)
Agar - 20 g

To each 100 ml of the cooled medium 1 ml of 1%. 2, 3, 5 triphenyl tetrazolium chloride is also added before pouring into the plates. Plates are incubated at 37°C for 48 hrs after which the red and pink colonies are counted as Faecal Streptococci.

d) **Sodium desoxycholate agar:**

Total coliforms:

Composition:

		g/L
Bacto - peptone	-	10 g
Lactose	-	10 g
Sodium chloride	-	5 g
Sodium citrate	-	2 g
Dipot - phosphate	-	2 g
Sodium desoxycholate	-	1 g
1% neutral red	-	3 ml
Agar	-	16 g

Characteristic colonies appearing in the desoxycholate medium after 24 hrs of incubation at 37°C are streaked on to EMB agar followed by confirmation by E.C. medium test.

EMB agar:

		g/L
Peptone	-	10 g
$K_2H\ PO_4$	-	2 g
Agar	-	20 g

To the sterile 100 ml of molten agar are added 1 g of lactose 2 ml of 2% Eosin and 1.25 ml of 0.5% methylene blue.

E.C. medium:

	g/L
Bacto Tryptone	- 20 g
Lactose	- 5 g
Bacto Bile Salts No.3	- 1.5 g
Dipot. phosphate	- 4.0 g
Monopot. phosphate	- 1.5 g
Sodium chloride	- 5 g

e) **Chapman's Agar:**

Composition:

	g/L
Bacto yeast extract	- 2.5
Bacto - Tryptone	- 10
Gelatin	- 30
Mannitol	- 10
Lactose	- 2
Sodium chloride	- 75
K_2HPO_4	- 5
Bromocresol purple	- 2.5 ml of 1% sol.
Agar	- 15
D.W.	- 1 lit.

Procedure:

Suspend the ingredients in 1 litre distilled water, bring to the boiling stage to dissolve completely, steri by autoclaving at 121°C for 15 minutes, disperse the precipitate by gentle agitation before pouring into petri dishes.

MEDIA AND METHODS FOR ZYMOGENOUS BACTERIA:

Method for isolation of proteolytic, amylolytic and lipolytic population:

Estimation of proteolytic population:

1. Estimation of chitinolytic population:

Mineral **Medium** (Aaronson, 1970)

K_2HPO_4 - 1.0 g

$MgSO_4$ $7H_2O$ - 0.5 g

NaCl - 0.5 g

$CaCl_2$ - 0.1 g

$Fe\ (NH_4)_2\ SO_4\ 6\ H_2O$ - 0.005 g

Agar - 15.0 g

Water - 1.0 lit.

Chitin precipitate was supplemented to the melted medium till the medium become turbid and pH was adjusted to 7.0.

Preparation of chitin precipitate:

Cleaned prawn carapace was decalcified in 1% (V/V) Hcl for four days. Hcl solution was changed everyday. The carapace was washed and placed in 2% (W/V) KoH for 10 days.

(KOH was boiled 4 times during the 10 day period). It was then washed, cut into strips and extracted four times with boiling ethanol. The chitin was dried and stored.

Chitin precipitate was prepared by dissolving chitin strips in chilled 50% (V/V) H_2SO_4 and precipitating it by a 15 fold dilution with water. The precipitate was washed free of acid, embedded in distilled water and stored in a refrigerator.

The medium was sterilised at 15 lbs. pressure for 15 minutes. The inoculated media were incubated for two weeks in darkness at 20°C. Colonies of chitinoclastic bacteria were recognised by the development of transparent halos surrounding the colonies.

2. Estimation of Ureolytic population:

Christensen's Urea Agar:

Peptone	-	1.0 g
KH_2PO_4	-	2.0 g
D. Glucose	-	1.0 g
Agar	-	20.0 g
Phenol red (0.2% solution)	-	6.0 ml
Water	-	1.0 lit.

pH - 6.8 - 7.0

The medium was sterilised by intermittent heating for 3 days and cooled to 50°C. 20% Urea solution, previously sterilised by filtration through a membrane filter, was then added to give a final concentration of 2%. The plates were incubated at room temperature (28 ± 2°C) for seven days. Ureolytic activity was detected by the change in colour of the medium from light yellow to pink.

3. Estimation of gelatinolytic population:

Frazier's gelatin agar (modified)

(Harrigon and McCance, 1972)

Peptone	-	10.0 g	2.5 g
Meat extract (Lab Lemco)	-	10.0 g	2.5 g
Gelatin	-	4.0 g	1.0 g
Agar	-	15.0 g	3.7 g
Water	-	1.0 lit.	
pH	-	7.2	

Medium was sterilised by autoclaving for 20 minutes at 115°C. The inoculated plates were incubated at room temperature (28 ± 2°C) for 8 days and tested using mercuric chloride solution of the following composition:

Mercuric chloride - 15.0 g
Conc. Hcl - 2.0 ml
Dist. water - 100 ml

The plates were flooded with 8 - 10 ml of the reagent. Unhydrolysed gelatin formed a white precipitate with the reagent. Gelatin hydrolysers were identified by the clear halos around the colonies.

4. <u>Estimation of caseinolytic population</u>:

(Harrigan and McCance, 1972)

Peptone	-	10.0 g
Meat extract (Lab Lemco)	-	10.0 g
*Casein	-	30.0 g
Agar	-	15.0 g
Water	-	1.0 lit.

* Casein (BDH) was used instead of skim-milk.

The medium was sterilised at 15 lb. pressure for 15 minutes and the inoculated medium was incubated at room temperature (28 ± 2°C) for at least 7 days. Caseinolytic bacteria were detected by the appearance of clear zones around the colonies.

5. <u>Estimation of amylolytic population</u>:

<u>Zo Bell's 2216 e medium + starch</u>

Peptone	-	5.0 g
Yeast extract	-	1.0 g
K_2HPO_4	-	0.5 g
$FeSO_4$, 7 H_2O	-	0.01 g
Soluble starch	-	2.0 g
Agar	-	15.0 g
Water	-	1.0 lit.

pH - 7.5 - 7.8

The medium was sterilised at 15 lbs. pressure for 15 minutes. The inoculated plates were incubated at room temperature (28 ± 2°C) for at least 7 days and tested with Gram's iodine solution of the following composition:

Potassium iodide - 2.0 g
Iodine - 1.0 g
Dist. water - 300 ml

The plates were flooded with 10 ml of iodine solution. Unhydrolysed starch formed a blue colour with iodine. The amylolytic colonies developed clear zones around them.

6. Estimation of lipolytic population:

Tween Agar: (Harrigan and McCance, 1972)

Peptone - 10.0 g
$CaCl_2$ - 0.1 g
Tween 80 (Sorbitol monooleate) - 10.0 ml
Agar - 15.0 g
Water - 1.0 lit.
pH - 7.0 - 7.4

Medium was sterilised at 15 lbs. pressure for 15 minutes. The inoculated plates were incubated at room temperature (28 ± 2°C) for 7 days. Lipolytic colonies were detected by the appearance of opaque zones surrounding them. Appearance of a waxy material around the colonies was the identification of the liberation of insoluble oleic acid formed as a result of lipase action.

Preparation of sensitivity discs:

1. Penicillin : 10 units/disc.

1 Vial contained - 10,000 units

Dissolved in 10 ml of phosphate buffer with pH 6.8

So that 1 ml contained 10,000 units.

1 ml of this solution diluted with 4 ml of phosphate buffer to make 2,000 units in one ml. 200 discs were wetted with this one ml so that each disc contained 10 units of Penicillin.

2. Ampicillin : 10 g/disc.

250 mg (250000 g) dissolved in 12.5 ml of phosphate buffer having a pH 5.8

1 ml contained - 2000 g

1 ml diluted with 9 ml of phosphate buffer

So that 1 ml contained 2000 g.

200 discs were wetted with this one ml so that each disc contained 10 g of Ampicillin.

3. **Streptomycin** : 50 g/disc

Dissolved 1 gm (1000000 g) in 10 ml of distilled water
1 ml contained - 10,0000 g of the antibiotic
Again diluted 1 ml with 9 ml of distilled water
so that 1 ml contained 10,000 g/ml

(1 ml was used to wet 200 discs).

4. **Kanamycin** : 50 g/disc.

Dissolved 0.5 gm in 10 ml of distilled water
0.5 gm contains 5,00,000
1 ml contained - 50,000 g.
2 ml of the antibiotic was diluted with 8 ml of
distilled water so that 1 ml contained 10,000 g.

(1 ml of the final solution was used to wet 200 discs)

5. **Tetracycline and Chloramphenicol** : 50 g/disc

1 ml - 200 discs
1 ml should contain - 10,000 g
2,50,000 g dissolved in 40% alcohol in 5 ml.
1 ml contained - 50,000 of the antibiotic
Diluted 1 ml with 4 ml of 40% alcohol so that
1 ml will contain 10,000 g/ml.
(1 ml was used to wet 200 discs.)

6. Septran : 300 g/disc.

Tablet contained 450 mg.

1 ml is used to wet 200 discs.

1 ml should contain 300 x 200 = 60,000 g/ml.

Dissolved tablets in (450,000 g) 7.5 ml of 40% alcohol

so that 1 ml will contain 60,000 g/ml.

(1 ml was used to wet 200 discs)

7. Gentamycin : 10 g/disc.

200 x 10 = 2,000 g/ml

Diluted 0.1 ml with 1.9 ml distilled water to make

40,000 g so that 1 ml will contain 2,000 g.

(1 ml was used to wet 200 discs).

8. Erythromycin : 15 g/disc.

15 x 200 = 3,000 g/ml

1 Ampule contains 250,000 g/ml

Dissolved in 8.3 ml of Absolute alcohol

1 ml = 30,000 g

Diluted 1 ml with 9 ml Absolute alcohol

1 ml contained = 3,000 g.

(1 ml was used to wet 200 discs).

9. [illegible] : 5 mg/disc.

1 ml = 200 disc = 1,000 g/ml

Dissolved 1 gm in 5 ml of distilled water = 200,000 g/ml.

Diluted 1 ml with 9 ml of distilled water

1 ml contained = 20,000 g/ml

Diluted 1 ml with 9 ml of distilled water

1 ml contained = 2,000 g/ml

Diluted 5 ml with 5 ml of distilled water

So that 1 ml contained 1,000 g/ml.

(1 ml was used to wet 200 discs).

Cleaning and Sterilisation:

Glasswares were first cleaned with detergents and then with acidified potassium dichromate ($K_2C_2O_4$) solution. They were thoroughly washed under running tap-water and finally rinsed with distilled water and left for drying.

The culture media containing glucose were sterilised at 10 lbs. pressure for 20 minutes. All the other media were sterilised at 15 lbs. pressure for 15 minutes in the autoclave.

Petri dish pair and pipettes were sterilised by keeping them inside their respective boxes in hot-air oven for 1½ hours at 160°C.

Spatula, inoculating needles, mortar and pestle and other needles were sterilised by dripping in rectified spirit and by flaming it to red heat.

The wooden inoculating chamber was sterilised by first wiping all its walls and surface with rectified spirit soaked in cotton wool and then by spraying formalin (4% formaldehyde solution in water) inside the chamber. The formalin was allowed to evaporate away before using the chamber. Some crystals of p-dichlorobenzene were also spread inside the chamber to prevent mites inside.

PLATING:

Quantitative analysis:

Individual marine microbiologists utilise different methods to estimate bacterial populations in a given sample. Every method has its advantages and disadvantages and there is a definite protocol for each method. The methods utilised in marine microbiology are discussed in several papers and books (Jannasch, 1965; Rodina, 1972; Zo Bell, 1946).

Pour-plate technique:

Depending on the anticipated bacterial numbers and the turbidity, samples were either concentrated or diluted. Plate counts were made by the pour-plate technique using a medium composed of 0.01% yeast extract, 1% peptone and 2% agar in aged sea water. The medium was found to yield higher total counts than ordinary sea water without yeast extract or nutrient agar (Hi-media) in sea water but it required a standard incubation for one week at room temperature. The pour-plate technique was found to yield higher counts than the spread plate.

The bottles are vigorously agitated, the mouths of the bottles were flamed and a dilution series is prepared, 1 ml of the sample is added to 9 ml sterilised sea water and the test tube is agitated vigorously. The number of bacteria per millilitre has now been diluted to 1/10 (= 10-1). This step is repeated with each of nine new sterile pipettes until the anticipated number of bacteria per millimetre is less than 300. An attempt was made to have no more than 300 colonies per plate, as reciprocal influence will otherwise develop.

If a series of dilutions are made of an inoculum of a particular dilution/dilutions is pour-plated, the total number of viable organisms present in a given quantity of the dilution may be determined. This method is known as the pour-plate method for the enumeration of viable organisms.

Calculation:

Averaged the counts obtained and reported as aerobic plate count/ml/gm. Total plate count per gm of the sample was computed as follows:

$$\left.\begin{matrix}\text{No. of bacteria}\\ \text{/gm/ml}\end{matrix}\right\} = \frac{\text{No. of colonies/ml/gm x Reciprocal of dilution x 1}}{\text{Weight of the sample in grams.}}$$

Qualitative analysis:

Since it was impossible to examine in detail all the colonies which grew on the count plate a limited number of bacterial strains were isolated with different morphological appearances and preserved in sea water agar slopes for further morphological and biochemical investigations. Well separated colonies were selected for isolation and each isolate was streaked 3 times to check the purity before the testing program was carried out.

Cultural characteristics and biochemical reactions were studied by standard procedures (Karthiyani and Mahadeva Iyer, 1967) using aged sea water in media preparation. Each of the isolate was examined for colony morphology, cell morphology, Gram reaction and motility. Gram-staining was carried out using Hucker's modifications. The inoculum for Gram-staining was taken from 24 hr. cultures. Motility was ascertained using hanging drop preparation of young broth cultures as well as inoculating into the top of 5 cm of Hugh & Leifson's medium, stabs, incubating at room temperature and examining for spreading of turbidity. Generic classification of bacterial isolates was done according to the scheme of Usio Simidu and Kazuyoshi Aiso (1962).

Along with bacterial analysis determination of water, temperature, oxygen, salinity, nutrients and phytoplankton enumeration were also made. Oxygen was estimated by Winkler's method. Salinity was determined by using Harvey's method. Estimation of nutrients was done according to the methods suggested by Strickland and Parson (1968). Formalin preserved samples were used for quantitative enumeration of phytoplankton.

Determination of number of Indicator Organisms:

1. Spread plate counting technique:

Spread plating is perhaps the most commonly used technique for determining total viable counts (TVC) of *E. coli*. The success of the technique is dependent on the medium, the accuracy of the dilutions, the precision of pipetting and the care used in spreading the inoculum on the surface of the plates. Care was taken to ensure that hot glass spreaders are not used to spread temperature-sensitive bacteria.

0.5 ml of the original sample of sea water was poured on to plates and spreaded the inoculum evenly using sterile hockey sticks. In the case of sediment 0.5 ml of the 10^{-2} dilution was poured on T^7 agar plates and spreaded the inoculum evenly until the inoculum was absorbed completely into the media. Then the plates were inverted and incubated at 37°C for 24 hrs. and colonies were counted after 24 hrs.

Isolation and identification of Escherichia coli:

Procedure for enumeration:

i) The preparation of the test samples was the same as given for the total plate count. Here only 10^{-1} and 10^{-2} of the inoculum was used for the enumeration of the organisms. Using 1 ml of the inoculum from each of the above dilutions, at least two plates of $Tergitol^7$ agar medium were poured, each plate containing about 20 ml of the medium to ensure a good growth of the organisms.

ii) If found necessary, the plates were dried at 55°C for about 45 minutes. One ml of the inoculum was pipetted on the surface of the dried $Tergitol^7$ agar plates and the plate was streaked with a sterilised bent glass rod till the inoculum get absorbed into the medium.

iii) Incubated the plates for 16 - 18 hours at 37°C. Colonies having the following characteristics were considered as *E. coli*:

a) Circular, non-mucoid and flat colony.

b) The colony forms a regular and well defined circle having a *yellow 'halo' with pinkish tinge colour* and occupying major portion (more or less 3/4th of the colony diameter) of the colony.

c) The margin of the colony, which usually measures more or less 1/4th of the colony diameter, is grey in colour.

d) Normally the diameter of the colony does not exceed 4 mm.

Suspicious colonies on Tergitol 7 agar plates were subcultured into Nutrient agar plates which were incubated for 24 hours at 37°C. Each culture was subjected to the following biochemical tests:-

i) Eijkman test
ii) Indole production test
iii) Methyl red test
iv) Voges-proskauer (V-P) test
v) Citrate utilisation test
vi) Slide agglutination test using polyvalent *E. coli* "O" anti-serum.

i) *Eijkman test*:

This was carried out as follows:

Inoculated MacConkey broth, warmed to 37°C, and incubated at 44 ± 0.1°C for 48 hours. A positive result is indicated by the production of both acid and gas. *E. coli* gave positive results in the test. If the organism produces both acid and gas in MacConkey broth within 48 hours at the above temperature, the following confirmatory tests were carried out.

ii) *Indole test:*

Peptone water (5 ml) was inoculated with 24 hours nutrient agar culture and incubated for 48 hours at 37°C. After incubation 0.5 ml Kovac's reagent was added, shaken well and examined after 1 and 5 minutes. A red colour in the reagent layer indicated the presence of Indole. *E. coli* produces Indole in this test i.e. positive reaction.

Methyl red (MR) and Voges-Proskauer (V-P) tests:

For both these tests a loopful of 24 hours nutrient agar culture was inoculated into 2 tubes of Glucose-Phosphate medium (Clark & Lub's broth) and incubated them for 48 hrs at 37°C.

iii) MR test:

To one of the above incubated tubes 3-4 drops of 0.04 methyl red solution was added. A magenta red colour, showing the presence of acid, is regarded as a positive reaction, a yellow colour negative and an orange colour showed an equivocal (±) result. *E. coli* gave a positive result in this test.

iv) V-P test:

To the second tube of the duplicate culture added 2-3 drops of Creatine solution (1% creatine in 0.1 N-HCl) and 1 ml of 40% KOH aqueous solution. The tube was shaken vigorously, and kept in a sleping position and examined after 1 and 4 hours. The development of an eosin pink colour indicated a positive reaction, a yellow colour denoted a negative reaction and a light deep orange brown colour indicated an equivocal (±) result.

If found necessary, the tube, before the addition of reagents, were warmed gently to about blood-heat (in a water bath at 37°C). *E. coli* gave negative result in this test.

v) **Citrate Utilisation test:**

This test was carried out as follows:

All glasswares were made chemically clean and alkali-free.

Sub-cultured Tergitol[7] Agar culture into a Nutrient Agar plate and incubated the plate at 37°C for 24 hours. Using the 24 hour nutrient agar culture, a light suspension was made in sterile water or saline and inoculated the tube of the Citrate medium with a straight wire. The tube was at 37°C and examined daily up to 7 days for turbidity. A tube of the sterile medium (not inoculated with the organism) should also be incubated along with the inoculated tube to be used as control.

As *E. coli* does not utilise citrate, turbidity will not develop in the medium in the presence of this organism and thus the inoculated tube will appear as clear as the un-inoculated one.

Slide Agglutination test using polyvalent *E. coli* "O" antiserum was also performed. This test was carried out in the manner as follows:

Serological test:

Pretested all *E. coli* serological antisera with known test cultures to ensure reliability of results with unknown cultures. Viable cultures were handled carefully to prevent contaminating environment.

Polyvalent somatic (O) slide or plate test:

Using wax pencil, marked 2 sections 1 x 2 cm on inside of the petri dish. Placed 1/2 of 3 mm loopful of culture from 24 or 48 hours Nutrient agar slant on dish in upper part of each marked section. Added 1 drop of saline solution, to lower part of each marked section. With clean, sterile transfer loop or needle emulsified the culture in saline solution for 1 section and repeated for other section. Added 1 drop of *E. coli* polyvalent somatic (O) antisera to a section of emulsified culture and mixed with clean, sterile transfer loop or needle. Tilted the mixture in both sections back and forth 1 minute and observed against dark background. Any degree of agglutination was considered as positive reaction.

Classified polyvalent somatic (O) test as:

(a) Positive - Agglutination in culture-saline-serum mixture and no agglutination in culture-saline mixture.

(b) Negative - No agglutination in culture-saline-serum mixture.

(c) Non-specific-both mixtures agglutinate. Requires additional testing as in identification of Enterobacteriaceae.

Isolation of indicators using selective media:

The enteric indicator test:

Total coliform:

'Coliform count' of coliform bacteria colonies grown on Sodium desoxycholate agar. Red or Rose colonies after 48 hours of incubation at 37°C.

Result:

The presence of coliforms in water indicates possible faecal contamination.

Coliform morphology and physiology:

1. Non spore-forming, rod shaped, some flagellate, mostly fimbriate, approximately 2 - 4 um by 0.5 um.

2. Coliforms take a negative gram-stain, "Gram-negativ

3. Coliforms ferment lactose, with the production of gas and acid.

4. Coliforms are aerobic and facultatively anaerobic.

5. There are 16 coliform IMViC types.

Coliform colony characteristics:

Colour:- Basically pink to dark-red plus a golden metallic sheen.

Size:- Variable.

Sheen size:- The metallic sheen may cover an entire colony or it may consist of a concentrate in the centre of the colony.

Genesis:- It is assumed that one coliform organism on the surface of the medium produces one visible colony.

Faecal Coliform test:

'Faecal coliform count' of faecal coliform bacteria grown on the surface of a Tergitol[7] Agar.

Result:

The presence of faecal coliforms in water specifically indicates faecal waste contamination by warm-blooded animals.

Faecal coliforms morphology:

Identical to the typical coliform morphology.

Physiology:-

It varies according to IMViC classification test for coliforms. Faecal coliforms are by ++-- (typical E. *coli* variety I) with +--- and -+-- type as well.

Faecal Streptococcus test:

'Faecal Strep count' for faecal streptococcus colonies grown on the surface of KF Agar.

Result:

The presence of faecal streptococcus bacteria specifically indicates faecal waste contamination by warm-blooded animals.

Morphology and Physiology:

1. Spherical or oval cells. Arranged in pairs. Each cell 1 mu in diameter non-motile, non-spore forming some capsulate.
2. Gram-positive.
3. Growth at 45°C in 40% con. of bile.
4. Marked resistance to heat, alkalinity and high conc. of salt.
5. Aerobic.
6. Produces acid but not gas in mannitol and lactose.

The Faecal coliform - Faecal Strep. Ratio:

Construction:

With the increase in investigation and use of the Faecal Streptococcus test a significant analytical tool was developed. It was formulated from data concerning faecal coliform and Faecal Streptococcus counts at the same sampling station. The two counts were set in proportion as follows and ratio between Faecal coliform and Faecal Streptococci will give the faecal index.

$$\text{Faecal index} = \frac{\text{Faecal coliforms}}{\text{Faecal Strep.}} = \text{Ratio}$$

Isolation and identification of Staphylococcus - procedure for enumeration:

(i) The preparation of the test samples were the same as given for the total plate count. Here, only the inoculum was used for the enumeration of the organisms. At least two plates of staphylococcus medium-110 were poured, each plate containing about 20 ml of the medium to ensure a good growth of the organism.

(ii) The plate was dried at 56°C for about 45 minutes, pipetted 1 ml of the inoculum on the surface of the dried plates and the inoculum was spread over the surface using sterile bent glass streaking rods.

(iii) Incubated the plates for 48 hours at 37°C. Only colonies with orange yellow colour were counted as pathogenic staphylococcus.

(iv) Well isolated suspected colony was picked up and streaked into a nutrient agar plate. Incubated at 37°C for 24 hours and each suspected colony was subjected to the following test:

(a) Microscopical examination:

When viewed under the microscope, the cells of staphylococci were Gram-positive cocci arranged in grape-like clusters on solid media and in pairs, small groups or short chains in liquid media.

(b) Coagulase test by slide technique:

Two drops of water were taken on a clean microscopic slide. In each drop emulsified a suspected colony to produce a thick and homogeneous suspension with the minimum of spreading. Added a drop of undiluted human or rabbit plasma to one drop and mixed gently. Coagulate-positive staphylococci produced Microscopic clumping within 5 - 15 seconds. Delayed clumping does not constitute a positive reaction. The second suspension served as a control. The clumping may be noted by comparing the test suspension with the control one. Simultaneously, Gram-stained preparation of the 48 hours staphylococcus medium 110 plate culture of the suspected organism was examined under the microscope. Gram-positive cocci arranged in pairs (usually in clusters) was regarded as staphylococci.

Most-probable number method for coliforms:

1. Presumptive coliform test:

Inoculated the water and sediment samples after mixing weighed sediment sample in 100 ml of sterilised sea water as follows:

Specimens	No. of tubes	Types of medium
50 ml	Two	Double strength McConkey broth
10 ml	Five	-do-
5 ml	Five	Single strength McConkey broth
0.1 ml	Five	-do-

Inoculated the tubes at 37°C for 18 - 24 hours and observed for gas production, and if negative, incubated further 24 hours.

Tubes showing gas production) Presumptive coliform
in Durham's tubes) positive

2. Differential coliform test (Eijkman test):

Single strength McConkey broth. (To differentiate *E. coli* from atypical coliforms). The positive presumptive tubes were taken and warmed them to 37°C. The tubes were subcultured into McConkey broth (Single strength) and incubated at 44°C for 24 hours in a water bath.

Result:

Tubes with gas production are considered as positive (Eijkman's test). If no gas - at 44°C = Atypical coliforms. Some types of atypical coliform also produce gas at 44°C. This can be identified by their failure to produce Indole at 44°C. Hence parrallelly Indole test was done at 44°C (in peptone water).

Confirmed typical coliform counts were done by using different volumes of media as done in presumptive test. As a control a positive and negative can be included. Tests were made simultaneously both at 37°C and at 44°C. Typical *E. coli* were identified finally by Bio-chemical tests like MR test, V-P test, Indole test and Citrate test.

Using the positive and negative test tubes the count per ml of the sample was computed by using Thomas's simple formula when following MPN method:

$$\text{MPN/100 ml} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{ml sample in negative tubes} \times \text{ml sample in all tubes.}}}$$

MAINTENANCE OF STOCK CULTURE ON SOLID MEDIA:

Each of the cultures isolated was kept in stock in duplicates one on sea water agar slant and another in Zo Bell 2216 agar slants. *E. coli* was kept in stock in nutrient agar slants. Well growing and sporulating cultures were sealed by dipping their plugged ends in melted wax. These were then stored in refrigerators at 4 ± 2°C.

Slants of SWA, Zo Bell's 2216 and also all media in bulk were stored in the refrigerators. The neck of the containers were tied up with polythene sheets to prevent them from drying up.

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CHAPTER IV.

PART A. ECOLOGY.

SECTION I.

HETEROTROPHIC MICROBES ASSOCIATED WITH ESTUARINE AND MARINE ENVIRONMENT.

CHAPTER IV.

PART A: ECOLOGY.

I. HETEROTROPHIC MICROBES ASSOCIATED WITH ESTUARINE AND MARINE ENVIRONMENTS:

The importance of heterotrophs and bacterial indicators of pollution in fisheries research has not received much attention in the country. In general the heterotrophic bacterial counts were found to increase in proportion to the counts of the pathogens or indicators of pathogens. So whenever the faecal pollution levels were measured it was customary to conduct, the heterotrophic bacterial assays of the same environment. Hence the numerical abundance and seasonal variation of some heterotrophic bacteria, their generic composition and biochemical and physiological reactions were studied for three years during 1972-73, 1974-75, 1975-76 in the estuarine, and inshore marine environment of Cochin in order to assess their ecological importance in aquatic environments. An attempt was also made to correlate their seasonal variations in density with some of the physico-chemical factors such as temperature, salinity, oxygen, pH, rainfall, nutrients (phosphate, nitrate and silicate), organic carbon and organic nitrogen and with the seasonal variation of phytoplankton.

The investigations consisted mainly of a quantitative and qualitative examination of sea water and sediment samples collected fortnightly during the study period. The purpose of the study is to describe numerically and taxonomically the heterotrophic bacteria present in the water column and sediment at different loci in estuarine environment of Cochin by sampling twice a month for three years (1972-73, 1974-75 and 1975-76). Seasonal percentage variation of abundant bacterial genera was studied and their enzymatic potential was assessed by using various biochemical and physiological experiments.

Area of study:

The study area in Cochin Backwater comprises a chain of shallow brackish water lagoons and swamps, is situated between lat. 9°28' and 10°N and long. 76°13' and 76°31'E (Fig. 1). The length of the area studied is about 65 km and the width varies from 0.5 to about 15 km. A channel of about 500 m width at Cochin makes a permanent connection with the Lakshadweep Sea. The major source of the fresh water for the backwater is from two large rivers, Periyar in the north and Pampa in the south. Four other small rivers viz., Achankoil, Manimala, Meenachil and Moovattupuzha also flow into the backwaters. The depth of the backwater varies considerably. It is deeper in the harbour area close to the sea, the depth being about 12 m and shallower in the upper reaches with a depth of about 1-5 m.

The area of study during 1972-73 is confined to these areas of the backwater system, the first 4 stations being situated between Aroor and the barmouth and the 5th one in neritic waters in the inshore environment of Cochin. (Fig. 1). All the sampling stations during 1974-75 were situated in the estuarine environment of Cochin Backwater. Altogether 6 fixed stations were studied, 4 of them situated in the brackish water, whereas 2 of them were almost in fresh water condition (Fig. 2). During the third year of study, 3 stations were fixed between 9°28' and 10°N in the estuarine area - the first one was fixed near the sewage outlet. Only when the distance from source of pollution is short, a significant, systematic difference between stations can be demonstrated. The distance between sewage and Station I is about 1/2 kilometre and this station is influenced by nearby sewage and drainage outlets almost exclusively. After a distance of about 3 km the effect of dilution, sedimentation and decay are evident and after 3-4 hour passage of sewage along north-west oceanic currents the faecal pollution in other two stations off the sewage outlet is reduced to less than 10% of the original amounts. Although Station II is situated near the barmouth actually it is in estuarine environment dominated by marine conditions which was evident from bacterial and chemical parameters. The mean salinity is relatively high at this station, as compared to other two stations. Stations II and III do not differ significantly in mean salinity.

For many years the possible effects of sewage pollution in fishing and public health gave rise to the increasing concern among technologists, scientists and others. In a recent study of Cochin Backwaters it has been shown that the discharge of untreated sewage into water bodies can do a lot of damage. The study points out that, organic pollution, to a large extent, exists in Cochin Backwaters especially where the canals join the backwater system. With all these points taken into consideration this area was selected as it forms a potential environment to monitor indigenous heterotrophic bacteria as well as exotic bacterial indicators and pathogens.

viz., pre-monsoon period (February to May) of high salinity, monsoon period (June to September) of very low salinity and post-monsoon period (October to January) of rising and fluctuating salinity. Hydrographical studies were not conducted in Station IV. Data were not available on dissolved oxygen and nutrients in surface sediments of all the stations.

Temperature:

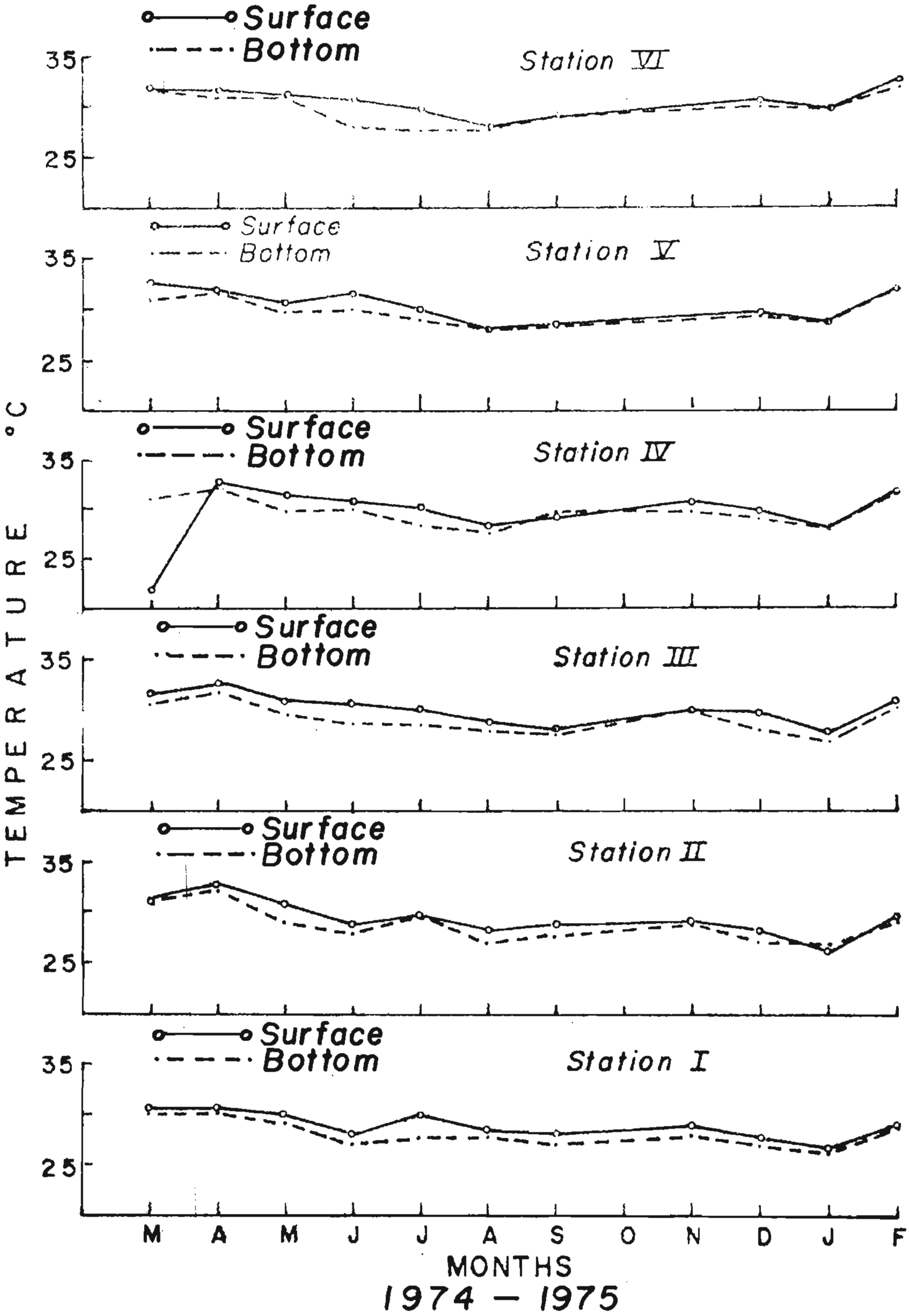
1972-73:

Surface and bottom water temperature were observed at the 4 stations (Table 1). Lowest temperature of surface and bottom water were recorded (27.5°C and 27.2°C respectively at stations near Thevara during June and the highest (31.5°C and 31.2°C respectively) at Station I near Aroor during March. A sudden decrease in temperature was observed during monsoon and the highest temperature was recorded during the pre-monsoon (Fig.12 a & b).

1974-75:

Records of seasonal changes in temperature are shown in Fig. 4. An examination of the curves shows that, during the pre-monsoon months, temperature remains uniform both in the surface as well as in bottom water and remains at the maximum. With the onset of monsoon in May the change in the temperature becomes apparent.

Fig. 4. Showing temperature (°C) of the water and sediment in the sampling stations during 1974–75.


Surface
Bottom
Station VI
Station V
Station IV
Station III
Station II
Station I
35
25
TEMPERATURE °C
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974 – 1975

1975-76:

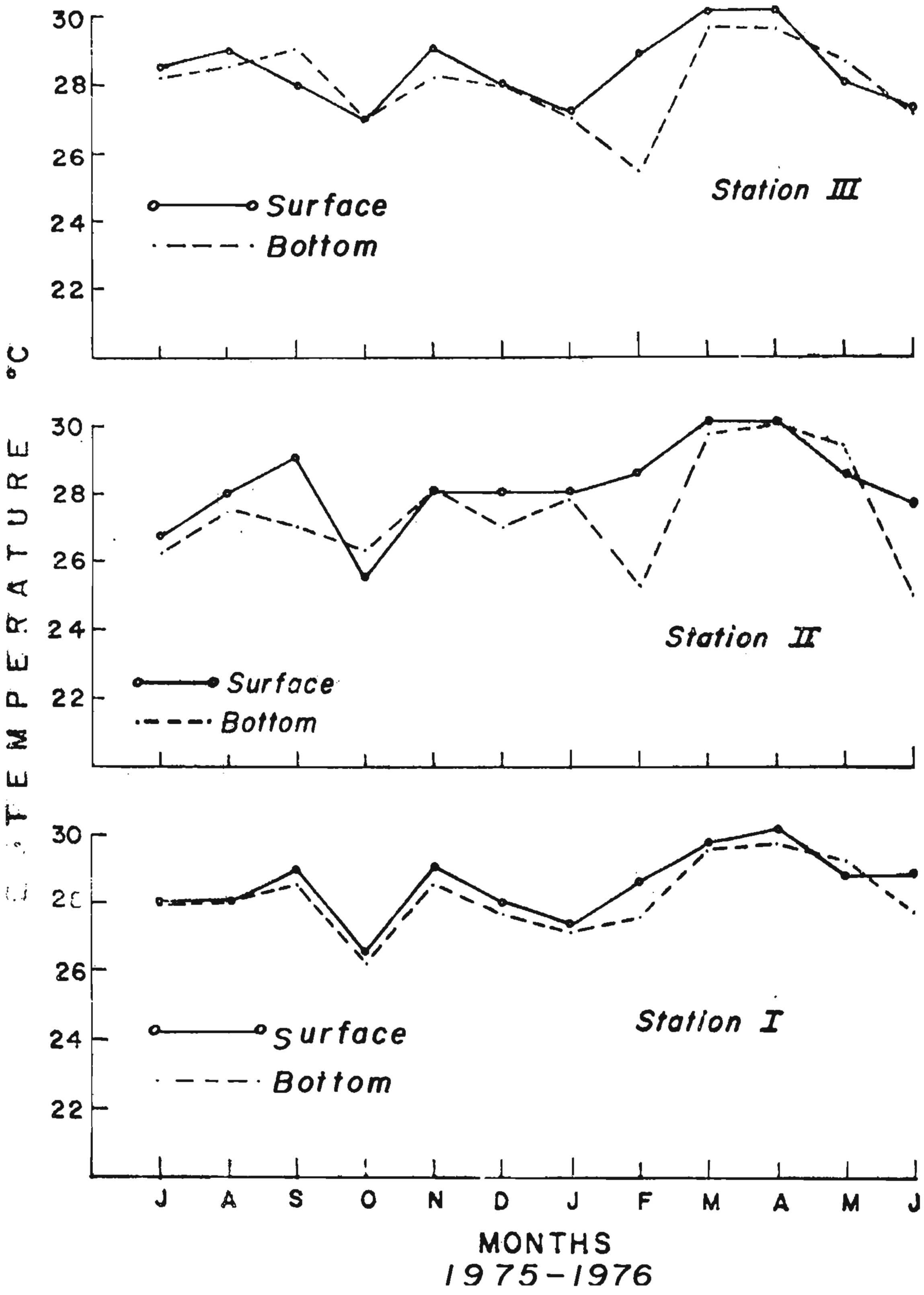
Maximum temperature of sea water was recorded (30.1°C) in Stations I and II during March and April in the pre-monsoon months and in sediments during April (30.0°C). Minimum temperature was recorded in water and sediments (25.5°C and 25.0°C respectively) during October and June. [Fig.5]

Salinity:

1972-73:

Salinity was found to be the most fluctuating factor among all the hydrographical parameters studied (Table 1). The SW monsoon season is characterised by heavy rainfall and there was a significant decrease in salinity values at all stations. During the monsoon period very low saline conditions prevailed at all stations from June to September (0.44 - 4.09 ‰) when the rainfall was at its maximum. Pre-monsoon was dry with less rainfall and the maximum salinity was observed at all stations. Maximum salinity (of 34.5 ‰ and 33.48 ‰) were recorded during May and March at Stations II and IV, V respectively near the bar-mouth. In the estuarine stations highest salinity recorded was 32.26 ‰ during March. A steady increase in salinity at all stations was observed during post-monsoon period (Fig.12 a & b).

Fig. 5. Showing temperature (°C) of the water and sediment in the sampling stations during 1975-76.



30
28
26
24
22
Surface
Bottom
Station III
30
28
26
24
22
Surface
Bottom
Station II
30
28
26
24
22
Surface
Bottom
Station I
TEMPERATURE °C
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975-1976

1974-75:

Like temperature, during pre-monsoon months the salinity showed vertical homogeneity and with the commencement of monsoon rain in May the surface salinity is considerably reduced up to September, from thereafter salinity increases and attains a peak in January 1975 (Fig. 6).

1975-76:

Maximum salinity was recorded in April 1976 (33.31‰) in Station I and the minimum (1.40 ‰) was observed in July 1975 in Station I, in sea water (Fig. 7). In sediments maximum salinity (33.53 ‰) was observed in March in the Station II and the minimum (19.28 ‰) was recorded in the month of October in Station I. Salinity at all stations, like temperature showed inverse relationship with rainfall.

Dissolved Oxygen:

1972-73:

During the SW monsoon a high value of dissolved oxygen (4.96 ml/l) was recorded in September at Station V (Table 1). Dissolved oxygen came to a minimum of 0.20 ml/l in November at Station II near Aroor. A clear inverse

Fig. 6. Showing salinity (%o) of the water and sediment in the sampling stations during 1974-75.

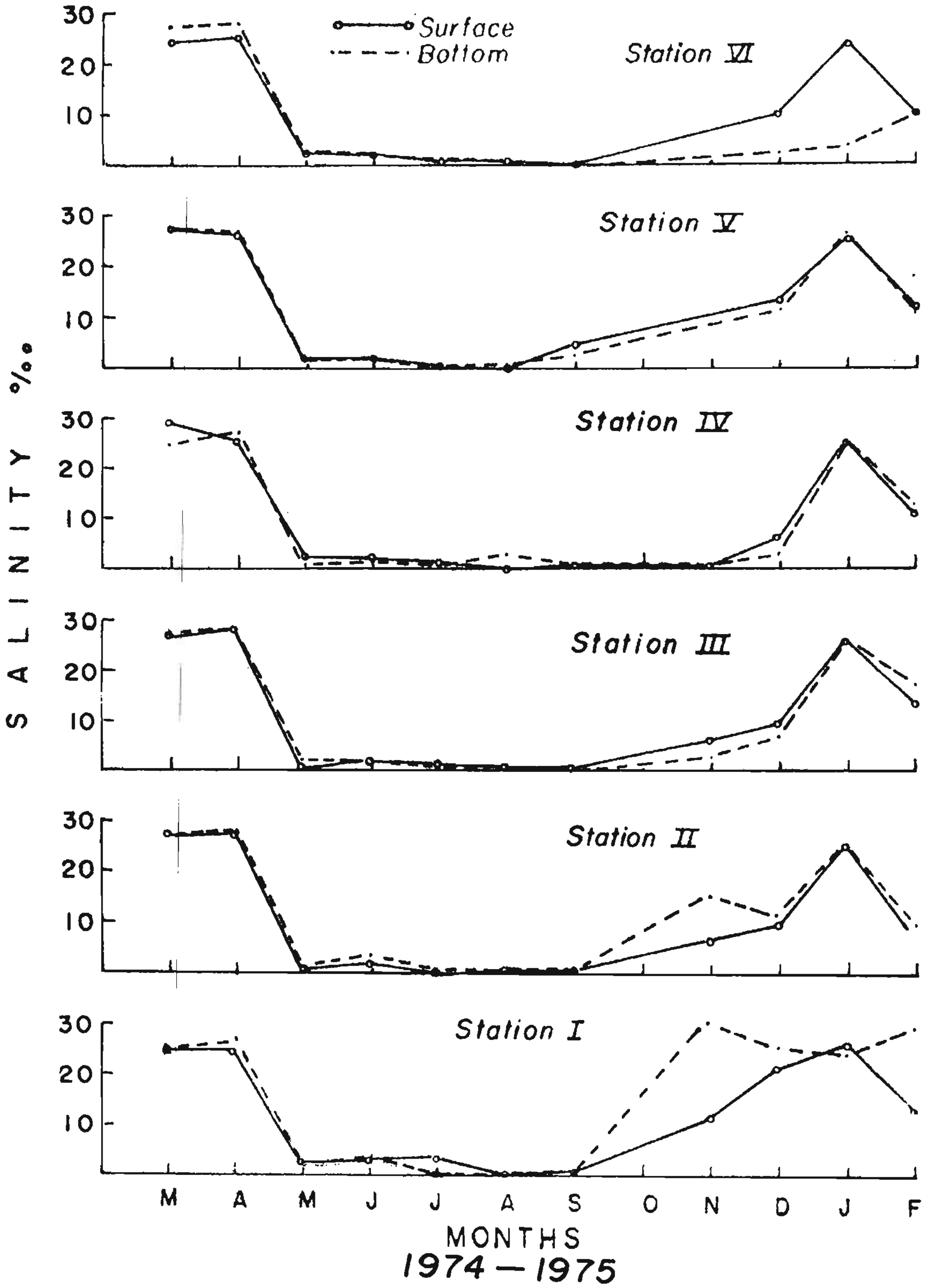
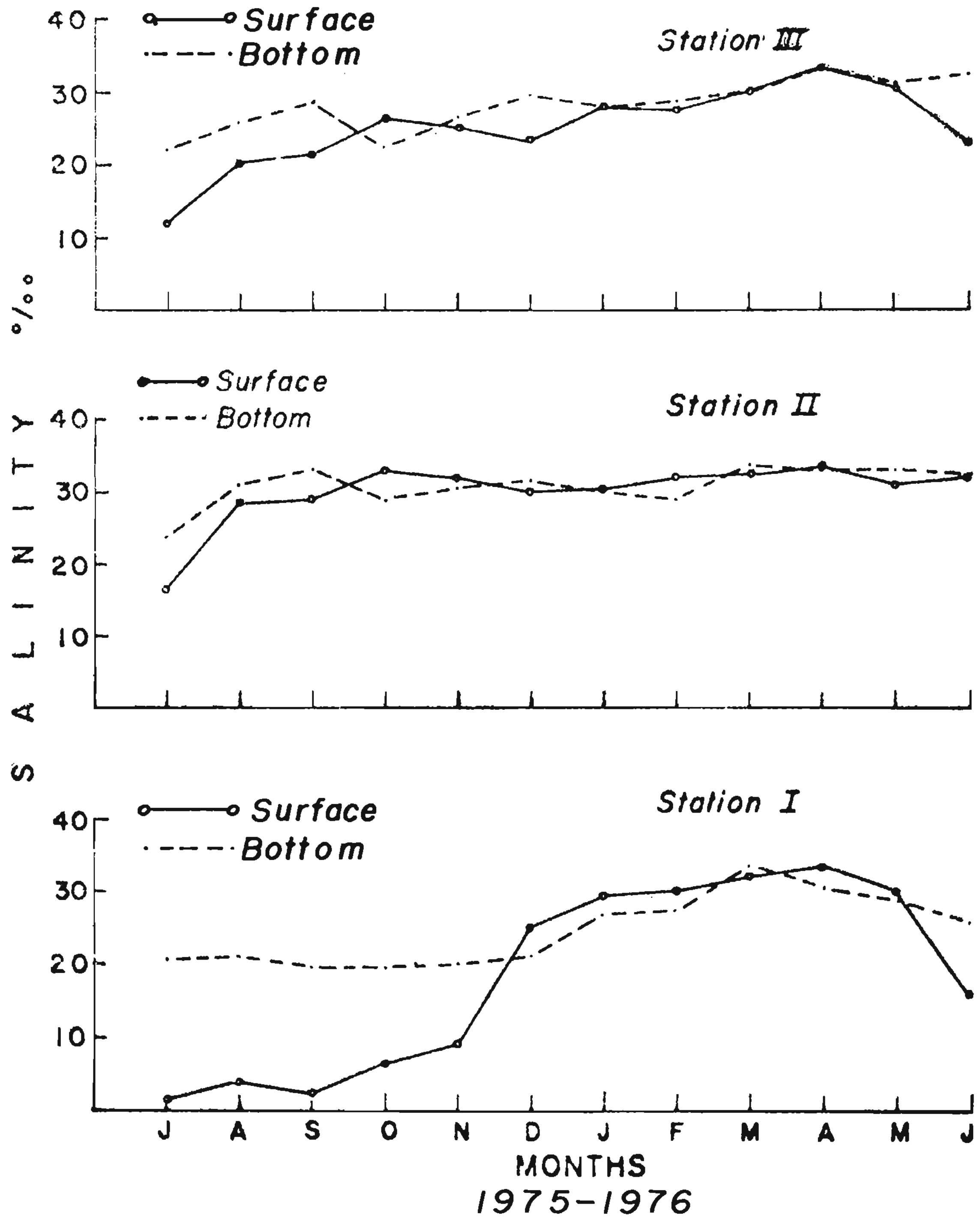

Surface
Bottom
Station VI
Station V
Station IV
Station III
Station II
Station I
30
20
10
SALINITY ‰
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974 — 1975

Fig. 7. Showing salinity (‰) of the water and sediment in the sampling stations during 1975-76.


Surface
Bottom
Station III
40
30
20
10
°/oo
SALINITY
Surface
Bottom
Station II
40
30
20
10
Surface
Bottom
Station I
40
30
20
10
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975-1976

relationship between dissolved oxygen and salinity was also observed.

1974-75:

In surface water dissolved oxygen values are subjected to little fluctuation but at bottom water a rapid decrease in oxygen value was noticed during the monsoon months. O_2 was found significantly ($P<0.01$) negatively correlated with temperature in Station III (Table 10). [Fig : 8]

1975-76:

Maximum dissolved oxygen value was recorded in January in Station II (5.79 ml/l) and the minimum in September in Station III (3.29 ml/l) (Fig. 9). In sediments in Station II during July 1975 minimum value was recorded (2.094 ml/l) and maximum in Station II in the month of December (5.83 ml/l).

pH:

Only in 1974-75 pH was included in the observations as environmental parameters and pH showed some fluctuation (7.00 to 7.90) in surface water (Fig. 10). During monsoon months values in both surface and bottom pH decreased

Fig. 8. Showing dissolved oxygen (ml/l) of the water and sediment in the sampling stations during 1974-75.

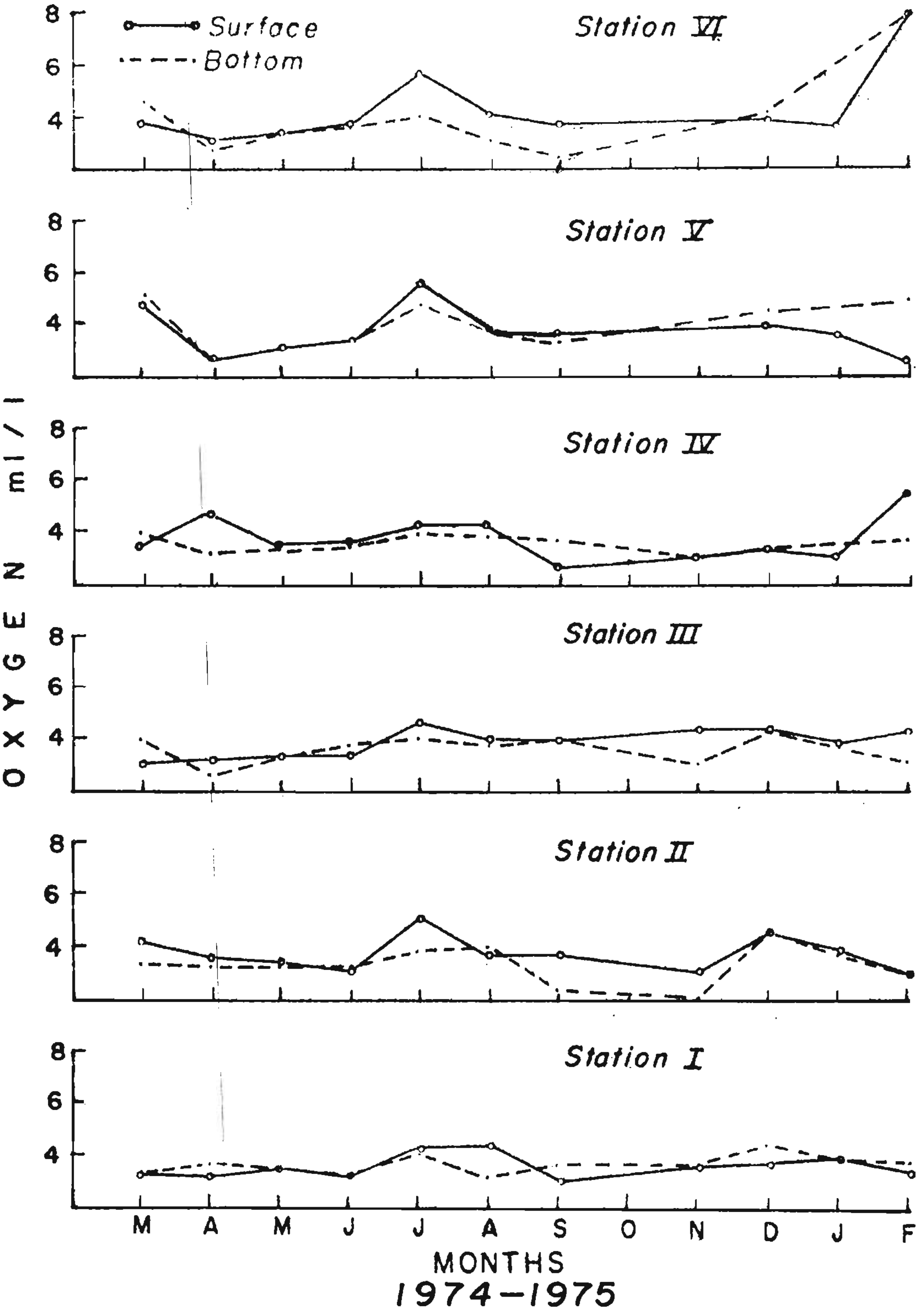

Surface
Bottom
Station VI
Station V
Station IV
Station III
Station II
Station I
8
6
4
OXYGEN ml/l
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974–1975

Fig. 9. Showing dissolved oxygen (ml/l) of the water and sediment in the sampling stations during 1975-76.

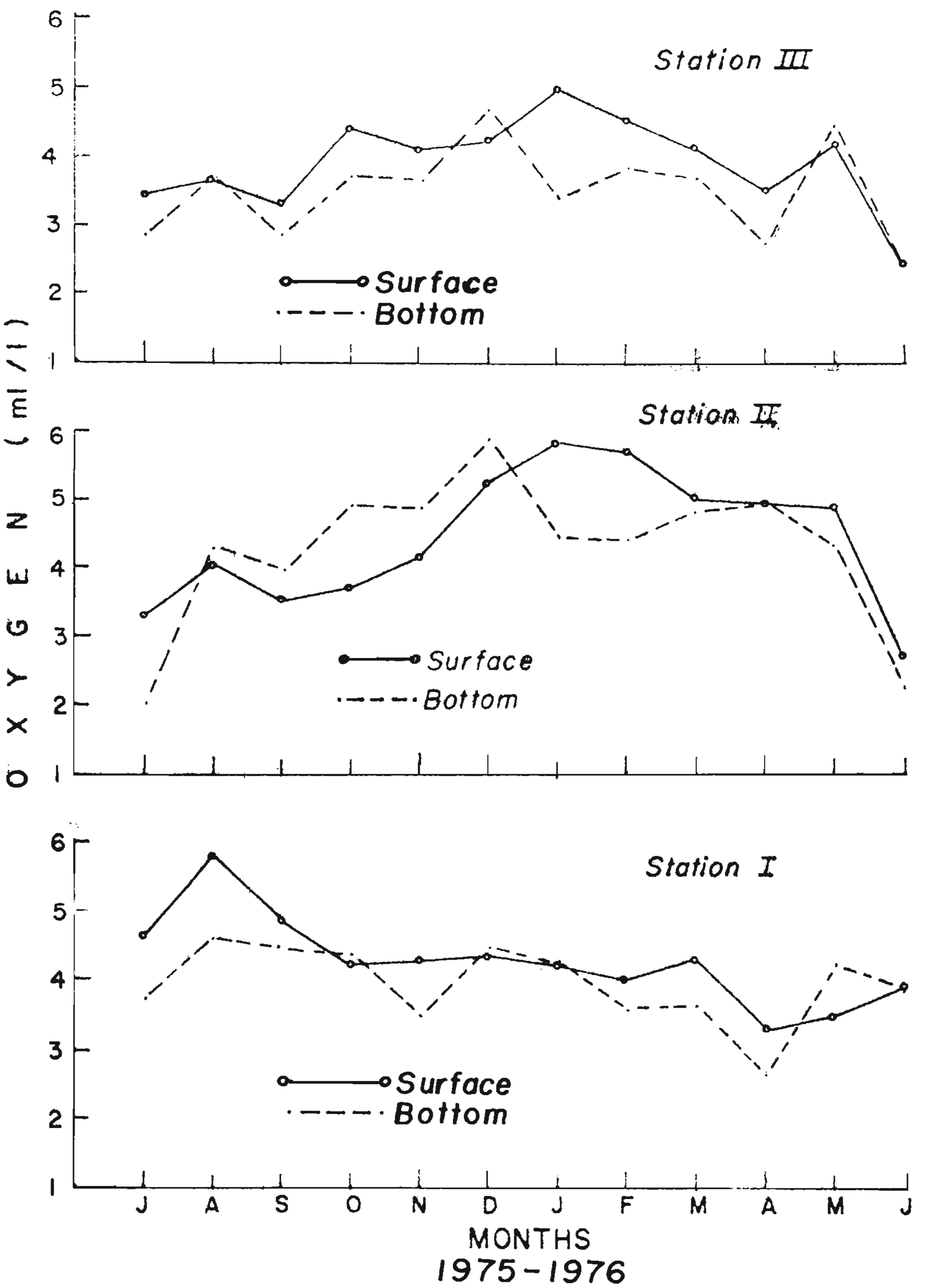
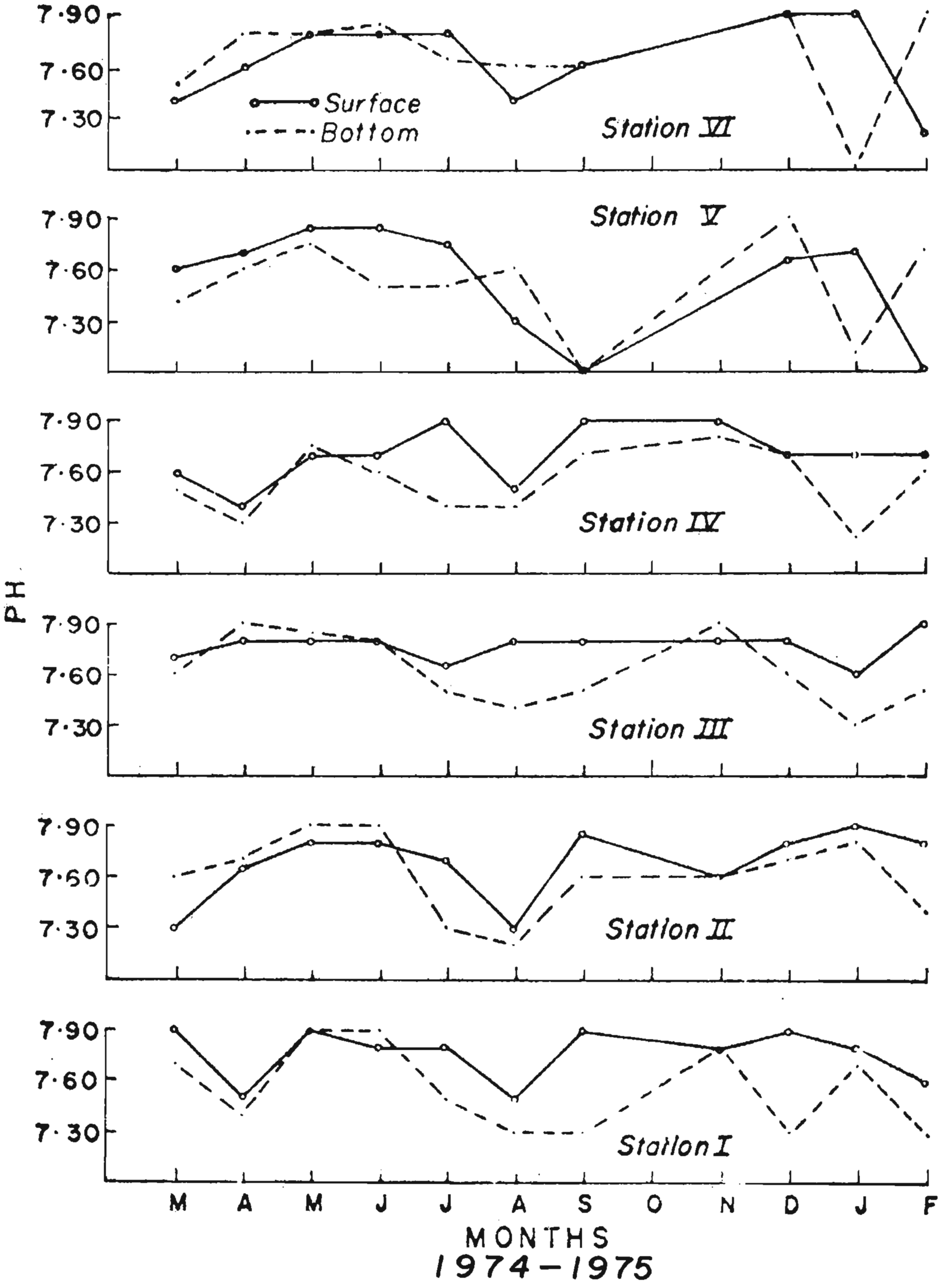

Station III
Surface
Bottom
Station II
Surface
Bottom
Station I
Surface
Bottom
OXYGEN (ml/l)
6
5
4
3
2
1
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975-1976

Fig. 10. Showing pH of the water and sediment in the sampling stations during 1974-75.


Surface
Bottom
Station VI
Station V
Station IV
Station III
Station II
Station I
7·90
7·60
7·30
PH
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974–1975

reaching a minimum during August and September. It is interesting to note that a clear stratification such as that noticed in temperature, salinity and oxygen was not observed in regard to pH values. The pH of the entire column either decreases or increases simultaneously.

In sea water pH was found significantly ($P < 0.05$) positively correlated with nitrate-nitrogen in Station I at 5% level but in Stations IV and V the reverse was the case, the pH being significantly ($P < 0.05$) negatively correlated with nitrate Tables 8, 11 and 12).

In sediments, pH was found significantly positively correlated with PO_4-P ($P < 0.01$) in Stations I and III (Tables 14 and 16) at 1% level. It is also evident from Table 15, that pH showed significant ($P < 0.05$) positive correlation with *E. coli* in Station II. In Station IV positive correlation of pH with heterotrophic bacteria was also evident (Table 17) and negative correlation was seen with oxygen values ($P < 0.05$). From the Table 11 it is seen that pH was significantly ($P < 0.05$) negatively correlated with salinity which reflects the mixing with fresh water.

Rainfall:

Rainfall data was available only for the years 1974-75 and 1975-76.

1974-75:

Maximum rainfall was recorded during the monsoon season (497 mm) with the highest rainfall (922 mm) in July. Post-monsoon period recorded next highest rainfall (307 mm) in December 1974 and Premonsoon season got the lowest amount of rainfall out of the three seasons studied. Minimum rainfall was noted in January and February 1975 (1 mm).

1975-76:

Maximum rainfall (721 mm) was recorded in the month of December 1975 and the minimum rainfall (1 mm) was recorded during January and February 1976. In all the seasons post-monsoon period received the lowest rainfall (185.75 mm).

Nutrients:

The seasonal variability of the nutrients in the backwater demands an understanding of the freshwater discharge into the system, which is chiefly controlled by the spectacular rainfall regime during the monsoon months. This provides a general mechanism underlying not only the nutrient distribution, but also the other environmental features. The area under investigation remains dominated by marine condition for about 6 months and then, rather suddenly becomes freshwater-dominated for the rest of the year. The other feature of importance is the short-term changes brought about by tidal oscillations, but these remain reasonably constant throughout the year except perhaps during extraordinary changes in meteorological conditions.

Phosphates:

1972-73:

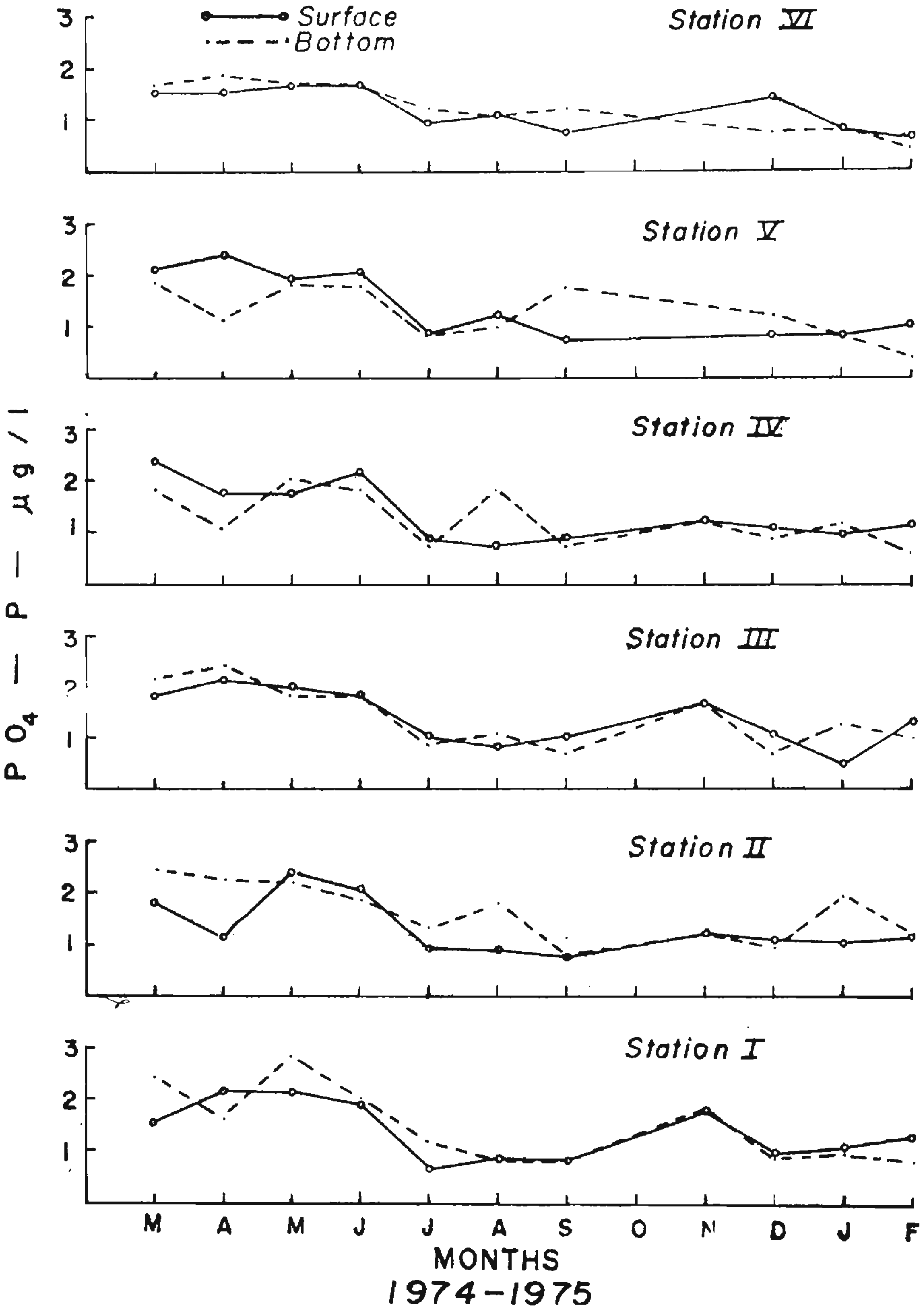
Maximum phosphate values were recorded at Stations II and III in January (11.81 µg/l and 10.79 µg/l respectively) in seawater and the minimum values were recorded (2.20 µg/l) in May in seawater at Station V. During May to September, phosphate values are low and there is very little difference in the values at different stations. Phosphorous contributions to the estuary is largely dependent upon external sources such as land drainage and freshwater run-off. If a direct relationship between the inorganic phosphorous and freshwater discharge does exist, one would expect maximum values at the surface during monsoon period. However, from the data obtained, the reverse seems to be true, indicating that there may be some other mechanism (like bacterial utilisation of phosphorous as a substrate for their metabolism) for the phosphorous depletion in surface waters in the estuary.

1974-75:

Maximum values of phosphate in sea water was observed at Stations IV and V in the months of March and April 1974 which may be due to influx of freshwater. Minimum values were observed during January, 1975 (Fig. 11). The consistent distribution of phosphates in the water column may be because of the distribution of suspended material. One would expect higher values of phosphate at the surface where the brackish water remains undisturbed and decreasing values towards the bottom. However, here also the reverse seems to be true, as higher values (2.80 µg/l) were obtained at Station I near the sewage outlet during May 1974 indicating that there may be some other mechanism for phosphorous enrichment in the estuary.

In sea water phosphate showed significant ($P < 0.05$) negative correlation with oxygen (Table 7 a) in Station I. In Stations III, and V phosphate was significantly ($P < 0.05$) positively correlated with temperature (Tables 7c, 7e).

Fig. 11. Showing phosphate content (μg/l) of the water and sediment during 1974-75.


Surface
Bottom
Station VI
Station V
Station IV
Station III
Station II
Station I
3
2
1
PO_4 — P — µg / l
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974–1975

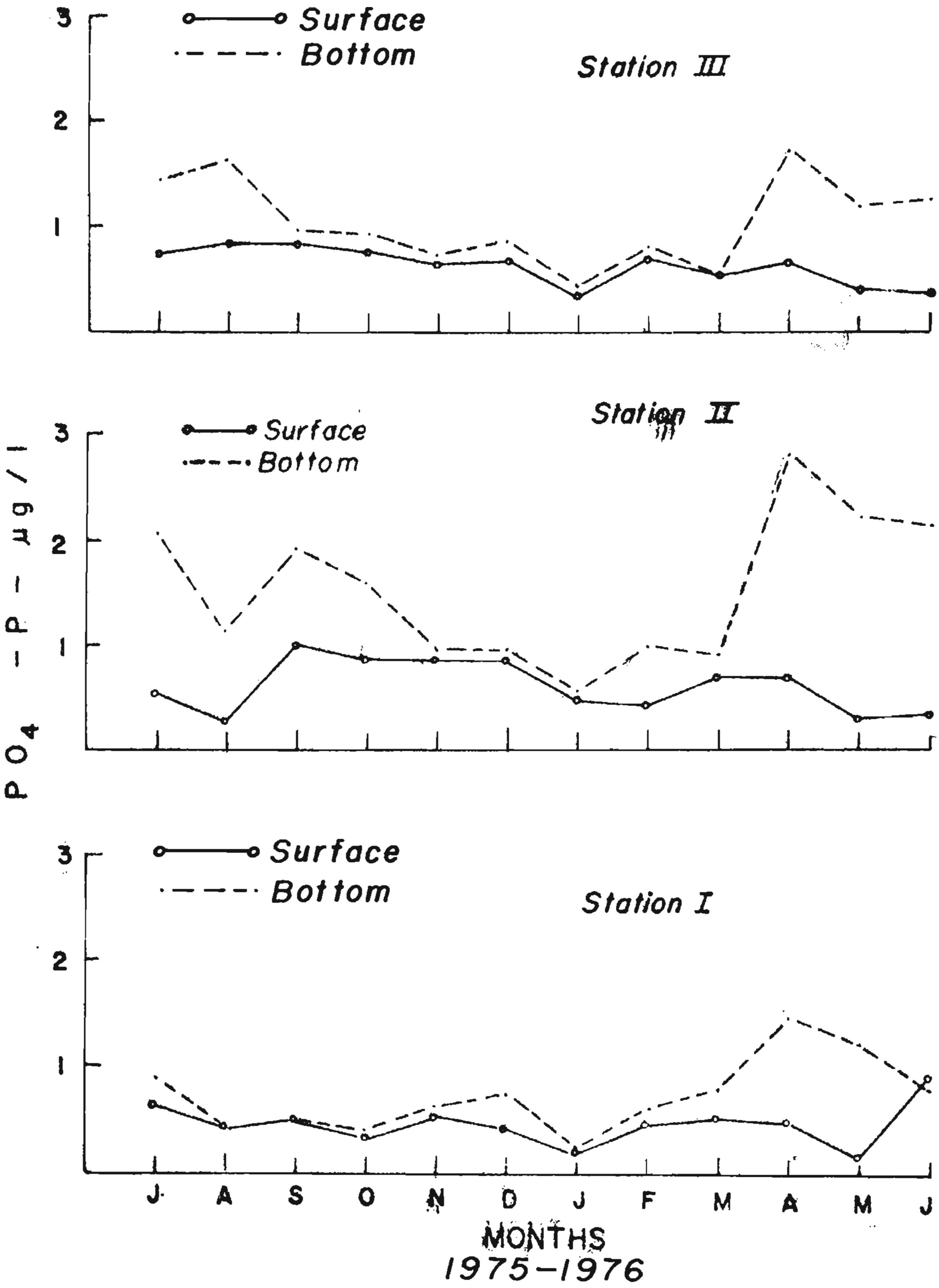
In sediment also significant ($P < 0.05$) positive correlation was seen with temperature but oxygen was found to be significantly ($P < 0.05$) negatively correlated with phosphate.

1975-76:

In this year also maximum value (2.846 µg/l) of phosphate was recorded in the sediment during April 1976 at Station II and the minimum at Station I during January 1976 (Fig. 12). In sea water maximum value reached only upto 0.986 µg/l in the month of September at Station II and the minimum value recorded was in the month of August 1975 at Station II. A greater occurrence of organic phosphorous at the bottom provides evidence that phosphates remain bound to the stirred up sediment.

Sea water, both in Station I near the sewage outlet and in Station II near fairway buoy the values of phosphate was significantly ($P < 0.05$ and $P < 0.01$ respectively) correlated with organic carbon. In sediments near the sewage outlet phosphate was found significantly (PO_4-P) positively correlated with temperature at 1% level ($P < 0.01$) and negatively correlated with oxygen ($P < 0.05$) at 5% level.

Fig. 12. Showing phosphate content (µg/l) of the water and sediment during 1975-76.


Surface
Bottom
Station III
Surface
Bottom
Station II
Surface
Bottom
Station I
PO4 - P - µg/l
3
2
1
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975–1976

Nitrate-Nitrogen:

1972-73:

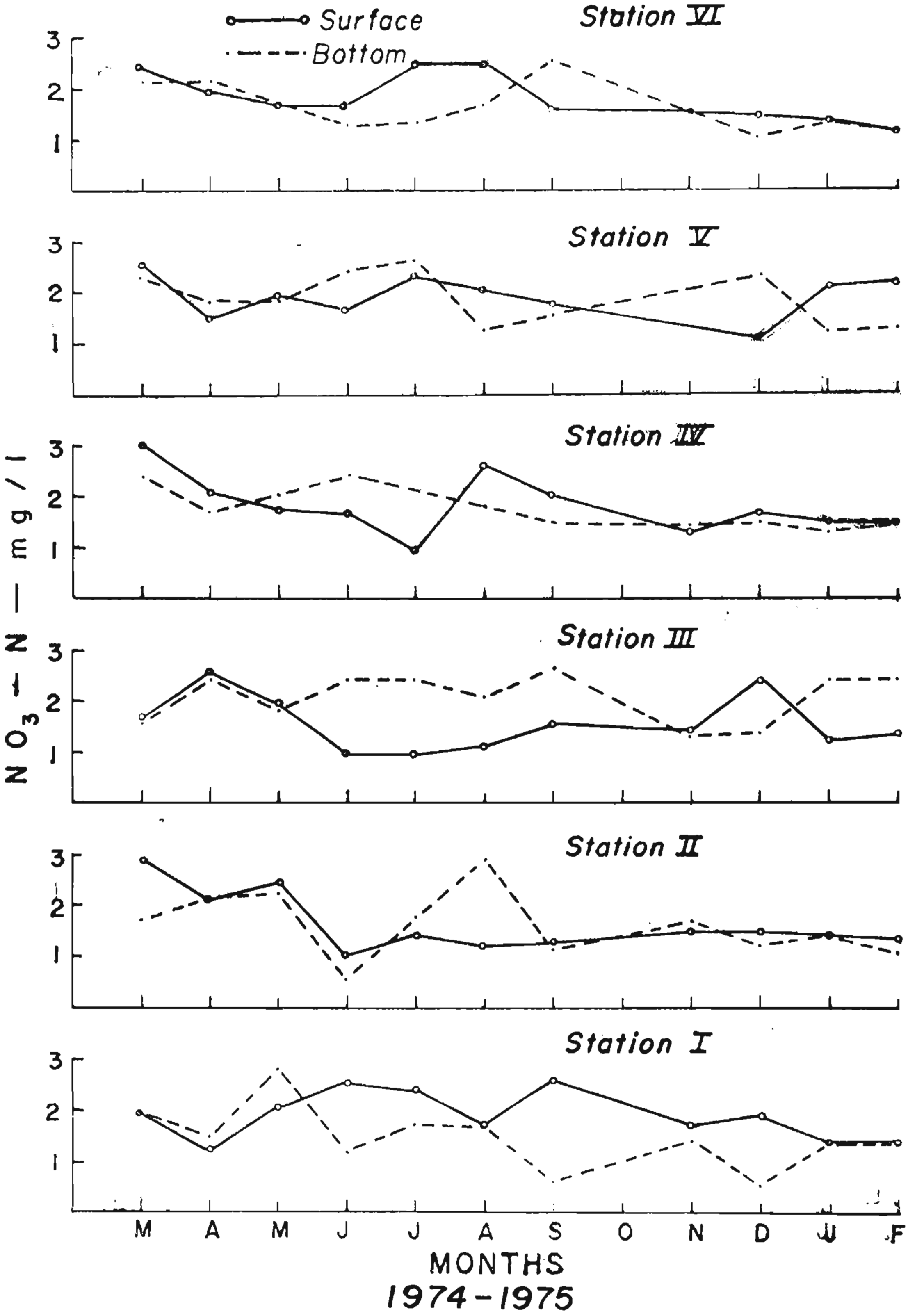
Seasonal changes in Nitrate-N values have been indicated in Table 1. The occurrence of abnormally high values of Nitrate-N in September, October and November suggest that these are associated with the early part of the monsoon when the freshwater influx is maximum. Minimal values of Nitrate-N when the conditions in the estuary are predominantly marine, suggest that the contribution of nitrogen from the sea is very meagre in these months. From November onwards the values begin to rise reaching as high as 10 µg/at/l during December and January.

1974-75:

It is clear from the figure that only very little nitrate-N was recorded for most of the months (Fig. 13). Minimal values of nitrate-N was noticed, when the conditions in the estuary are predominantly marine. Maximum values were observed in Station IV in pre-monsoon months.

In sea water, nitrate was found to be significantly ($P < 0.05$) negatively correlated with salinity in Station I (Table 8) and in Station II nitrate was significantly ($P < 0.05$) positively correlated with temperature. However,

Fig. 13. Showing nitrate content (mg/l) of the water and sediment during 1974-75.


Surface
Bottom
Station VI
Station V
Station IV
Station III
Station II
Station I
3
2
1
NO₃ – N — mg/l
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974-1975

in Station IV nitrate showed significant ($P < 0.05$) negative correlation with temperature.

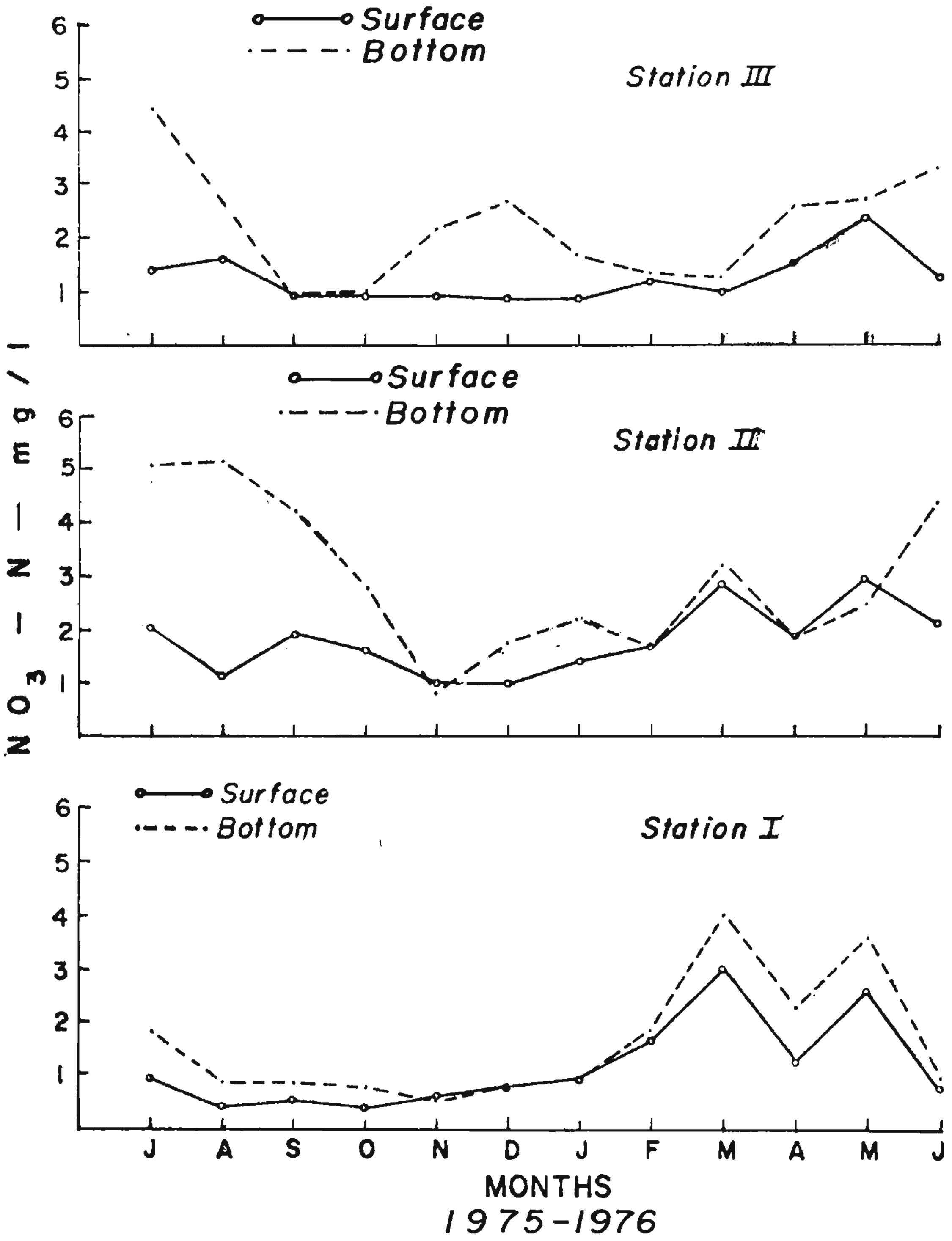
In sediment, nitrate was significantly ($P < 0.05$) positively correlated with phosphate in Station I (Table 14) and in Station IV ($P < 0.01$) (Table 11). In Station VI significant positive correlation was seen at 5% level between nitrate and total plate count.

1975-76:

High nitrate.nitrogen values were recorded (2.9 mg/l) at Station I in the month of March. Minimum values 0.38 mg/l were found in October in Station I. In sediment higher values were obtained during August in Station II (5.1 mg/l) and minimum values in November at Station I (0.5 mg/l) (Fig. 14). There is little difference between the nitrogen cycle observed at the surface and bottom suggesting that the distribution of nitrate was homogenous throughout the water column in all seasons.

In sea water nitrate showed significant ($P < 0.05$) positive correlation with salinity in Station I near the sewage outlet. Significant ($P < 0.05$) negative correlation was seen with organic nitrogen in the same station in sea water.

Fig. 14. Showing nitrate content (mg/l) of the water and sediment during 1975-76.


Surface
Bottom
Station III
Surface
Bottom
Station II
Surface
Bottom
Station I
NO3 - N - mg/l
6
5
4
3
2
1
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975-1976

In the estuarine sediments nitrate showed positive correlation with each of temperature ($P < 0.05$), salinity ($P < 0.01$) and phosphate ($P < 0.05$). In the inshore sediments, nitrate exhibited significant ($P < 0.05$) negative correlation with oxygen. In the brackish water area nitrate showed significant positive correlation with phosphate.

Nitrite:

1972-73:

The values of nitrite-N are homogenous throughout the sampling area except during the period when the conditions in the estuary are marine i.e. from December to March. During the monsoon months the values at the surface become significantly lower when compared to other seasons, which cannot be explained in terms of freshwater discharge alone. The values of nitrite-N are much lower than those of nitrate-N in the present study which suggest that the nitrite-N may be formed as a result of decomposition of organic nitrogen.

Silicate-Silicon:

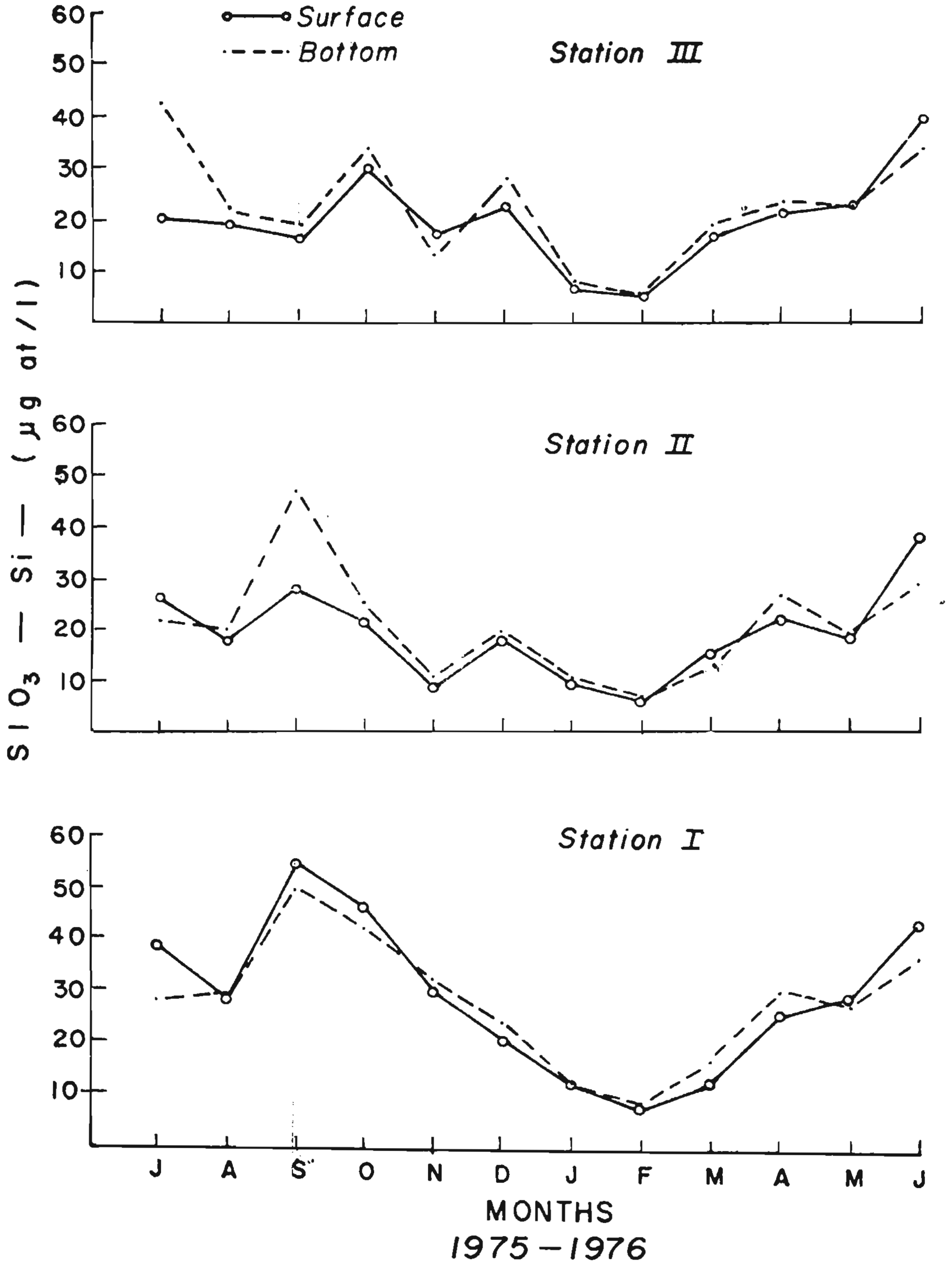
1975-76:

In sea water maximum values of silicate were obtained in the month of September (55.96 µg/l) and the minimum at

Station III during February (5.25 μg/l) (Fig. 15). In the sediment maximum values were recorded (90.02 μg at/l) in the month of September and minimum values were obtained in the month of February at Station III (4.35 μg at/l). Maximum values recorded during September and October indicate that silicon is associated with heavy silt load of the estuary. The values gradually decreased from surface to bottom showing a typically inverse relationship with salinity profile (Table 14a).

The foregoing account clearly indicates that the cycle of chemical factors in the estuary was fairly regular making the year divisible into 3 distinct seasons: 1. The premonsoon season, 2. Monsoon season and 3. Postmonsoon season. During premonsoon season the estuary showed simple stable and uniform conditions. The freshwater discharge is reduced to a minimum and the estuary becomes merely an extension of the adjoining sea, the environmental factors being almost similar to that of coastal waters. The monsoon season was associated with sudden changes from typically marine to brackish water conditions and extremely significant changes occur in the environmental features of the estuary. The heavy rainfall brings about marked changes in temperature, salinity, dissolved oxygen, pH and nutrients of water and sediment. each of these parameters has its own characteristics. Nutrient distribution is largely dependent upon 2 main

Fig. 15. Showing silicate content (mg/l) of the water and sediment during 1975-76.


Surface
Bottom
Station III
Station II
Station I
S I O$_3$ — Si — (µg at/l)
60
50
40
30
20
10
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975 – 1976

components - the marine influence and freshwater discharge. During the period when the system remains predominantly marine, the nutrient concentrations are low and remain homogeneous throughout the water column, but during the period of freshwater discharge high concentrations of nutrients occur within the system. Large quantities of organic matter brought into the estuary by the sewage and drainage canals have a marked influence on distribution of nutrients.

In surface water silicate showed significant ($P < 0.01$) negative correlation at 1% level with salinity in Station I (Table 23). In Station II silicate was significantly negatively correlated with oxygen ($P < 0.01$) and positively correlated with nitrate ($P < 0.001$) (Table 24). Also, in Station III, significant negative correlation was seen with oxygen at 5% level (Table 25). Coliforms were significantly ($P < 0.05$) positively correlated with silicate.

In sediments, significant ($P < 0.05$) positive correlation was seen with phosphate (in Station II) near Fairway Buoy (Table 27). Organic carbon was significantly positively correlated with silicate at 5% level whereas (in Station I) near the sewage outlet (Table 26), organic carbon was significantly ($P < 0.01$) positively correlated with salinity at 1% level.

Organic substances:

Organic substances, dissolved or suspended in sea water, are particularly important as food for C - heterotrophic micro-organisms. The size and composition of bacterial populations of a water body depend to a large degree on the concentration and composition of these substances. Organic compounds act as activating and inhibiting factors. So observations of total organic carbon and total organic nitrogen were made during the period July 1975 to June 1976 and the values are given in Figs. 16 & 17.

Total organic carbon:

The values of organic matter are somewhat higher compared to the already reported value of 2.5% (Murthy and Veerayya, 1972) in the month of June 1976 in Station I near the sewage outlet (2.81%) and in Station II in the month of September 1975 (3.16%) and in Station III in September 1975, May and June 1976 (2.92%, 3.14% and 3.26% respectively) (Fig. 16). In mud deposits, the values exceeded the reported one during July, September and October 1975 in Station I (2.89%, 4.21% and 3.27% respectively), in Station II in September 1975 (3.28%) and in Station III in August 1975 (4.69%). Murthy *et al.* (1969) have attributed the higher organic content in the shallower area to

Fig. 16. Showing organic carbon content (%) of water and sediment in the area of study during 1975-76.

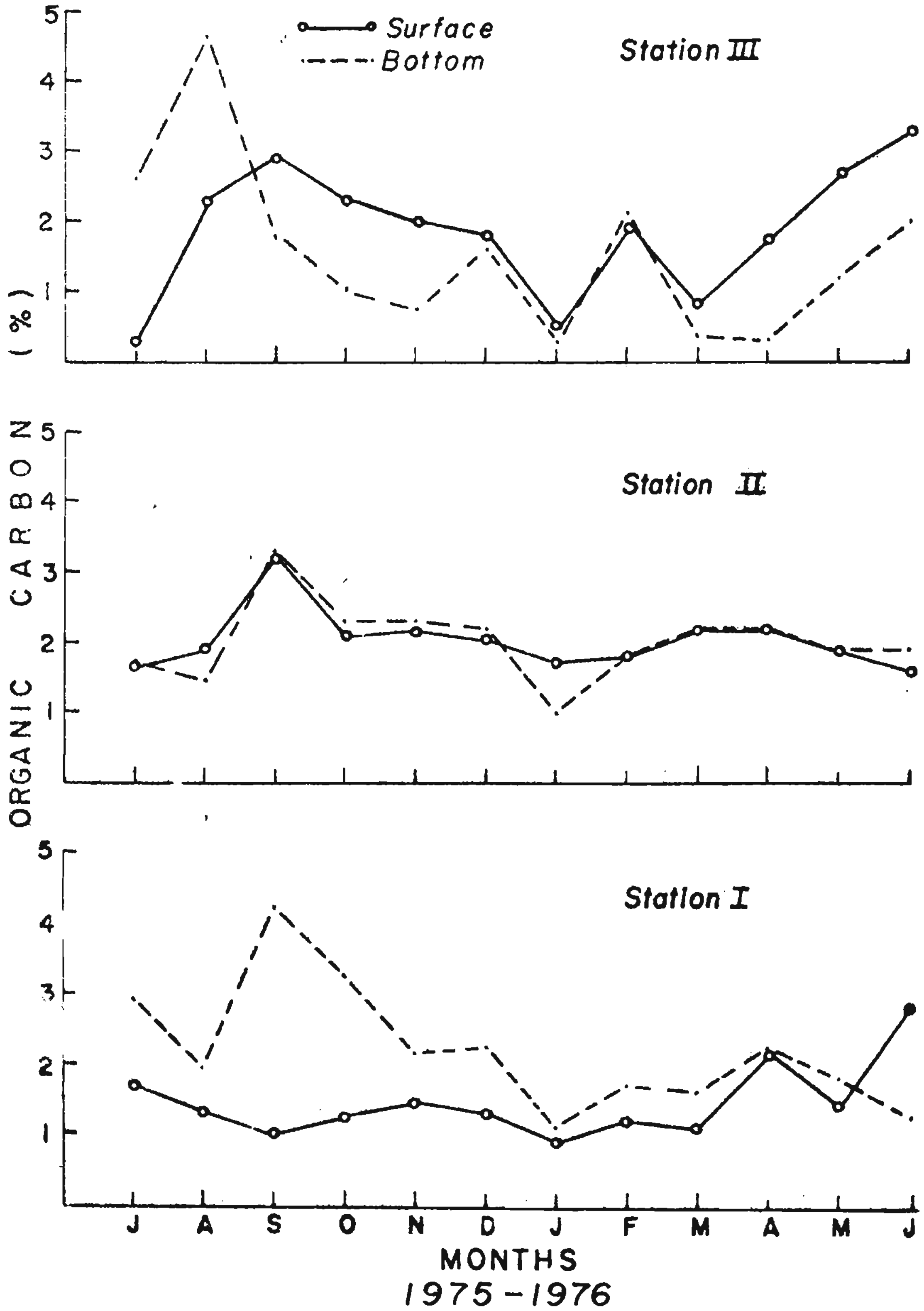
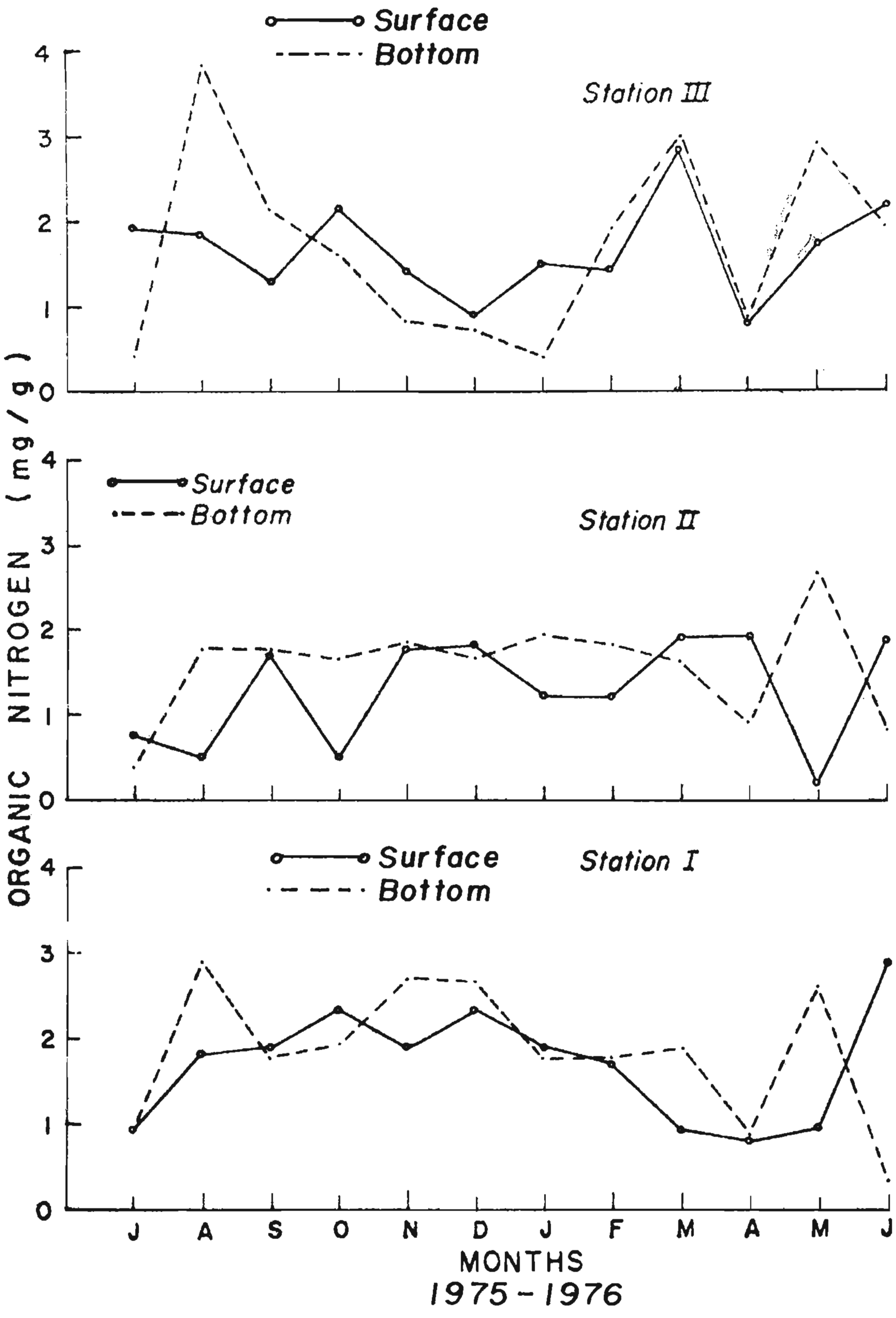

Surface
Bottom
Station III
Station II
Station I
ORGANIC CARBON (%)
5
4
3
2
1
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975 - 1976

Fig. 17. Showing organic nitrogen content (mg/g) of water and sediment in the area of study during 1975-76.


Surface
Bottom
Station III
Station II
Station I
ORGANIC NITROGEN (mg/g)
0
1
2
3
4
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975 - 1976

the contribution of terrigenous sources and this reasoning seems to be applicable to Station II near barmouth in the present study. Sarala Devi *et al.* (1979) also have reported high organic loads at barmouth based on water quality study.

In sea water, at Station I and Station II organic carbon was positively correlated with nitrate ($P < 0.05$ and $P < 0.01$ respectively). In sediments at Station I organic carbon showed significant negative correlation with salinity ($P < 0.05$). In Stations I and II, in sediment organic carbon showed significant positive correlation with silicate at 1% and 5% level ($P < 0.05$ and $P < 0.01$ respectively).

Organic Nitrogen:

Seasonal variations in organic nitrogen were not very pronounced at Station II, however, the values showed larger variation. Station I recorded maximum variation showing high value in the months of October, December 1975 and in June 1976 (2.32, 2.33 and 2.86 mg/g respectively). The highest value of organic nitrogen (2.86 mg/g) was recorded at Station I in June (Fig. 17). In sediments, Station I showed high values during November and December 1975 and June 1976 (2.66, 2.65 and 2.60 mg/g)respectively). In Stations II and III only in June 1976 higher values were recorded (2.63 and 2.83 mg/g respectively) and the nitrogenous wastes from the sewage and other effluents discharged through drainage canals may be responsible for this high value. In the product-moment correlation coefficient computed for organic nitrogen with other parameters showed significant positive correlation at 5% level with oxygen ($P < 0.05$) only in sediments at Station II near barmouth.

Distribution and composition of heterotrophic bacterial flora - 1972-73:

The estuarine microbial flora generally consist of marine, brackish water, fresh water and intermediate forms. The important bacterial genera encountered during the present investigations were *Alcaligenes*, *Vibrio*, *Pseudomonas*, *Aeromonas*, *Flavobacterium* and *Micrococci*. In addition *Bacillus* spp. was found in surface sediments. The bacterial population based on numerical counts showed wide variation in their distribution in different stations both in sea water and in surface sediments. Table 1 gives bacterial counts along with chemical factors during 1972-73. Quantitative distribution of bottom flora as determined by numerical abundance in surface sea water and sediments in all stations is given in Figs. 18 a & b. Seasonal cycle in

Fig. 18 a. Showing total plate count (No. x 10^6) in water along with percentage occurrence of the 6 genera isolated during 1972-73.

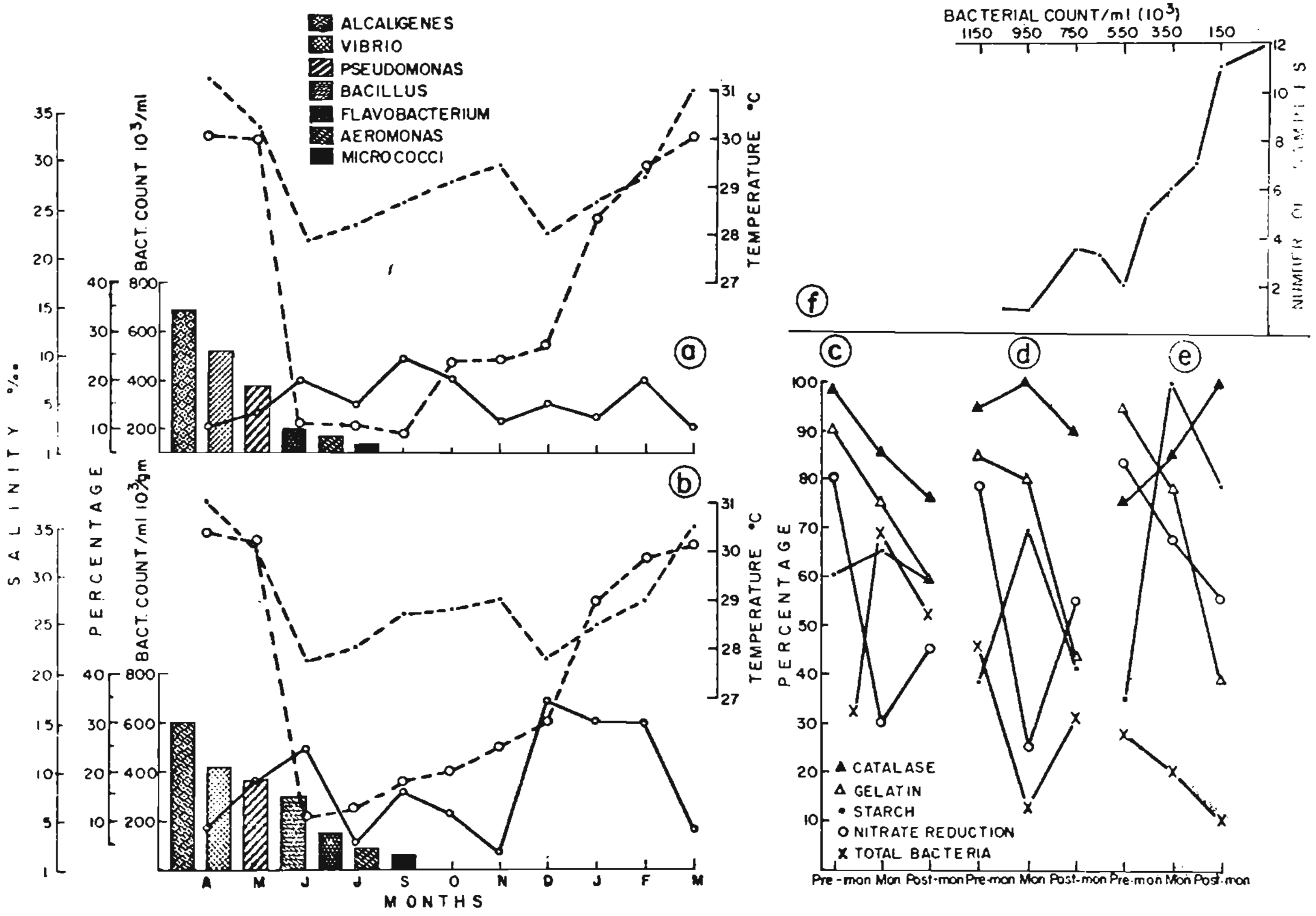
Fig. 18 b. Showing total plate count (No. x 10^6) in sediment along with percentage occurrence of the 7 genera isolated during 1972-73.

Fig. 18 c. The seasonal percentage variation of the genera *Alcaligenes* during pre-monsoon, monsoon and post-monsoon seasons.

Fig. 18 d. The seasonal percentage variation of the genera *Vibrio* during pre-monsoon, monsoon and post-monsoon seasons.

Fig. 18 e. The seasonal percentage variation of the genera *Pseudomonas* during pre-monsoon, monsoon and post-monsoon seasons.

Fig. 18 f. Showing the sampling distribution of the bacterial density during 1972-73.


ALCALIGENES
VIBRIO
PSEUDOMONAS
BACILLUS
FLAVOBACTERIUM
AEROMONAS
MICROCOCCI
SALINITY ‰
PERCENTAGE
BACT. COUNT 10³/ml
BACT. COUNT/ml 10³/gm
TEMPERATURE °C
a
b
A M J J S O N D J F M
MONTHS
BACTERIAL COUNT/ml (10³)
1150 950 750 550 350 150
NUMBER OF SAMPLES
f
c
d
e
PERCENTAGE
▲ CATALASE
△ GELATIN
• STARCH
○ NITRATE REDUCTION
X TOTAL BACTERIA
Pre-mon Mon Post-mon Pre-mon Mon Post-mon Pre-mon Mon Post-mon

the total bacterial flora revealed the highest count (656 x 10^{-3}/ml) in the month of December 1972 and the minimum (95 x 10^{-3}/ml) during November 1972. The period of maximum abundance of bacteria was encountered during December to March. In all the stations except the fourth, the minimum total counts were recorded during the monsoon period. In Station IV, minimum counts were observed during the premonsoon period, with an increasing trend from the premonsoon to the postmonsoon seasons. In Stations III and V, the total counts did not show large fluctuations indicating that the bacterial flora were not subjected to large seasonal variations in abundance. The abundance attained the maximum during the postmonsoon season for all the stations except Station V, where the difference between the lowest and highest count was not large.

Bacterial Taxonomy:

Marine bacteria cannot be identified in the same manner as other micro-organisms such as phytoplankton. Their microscopic size and lack of morphologic diversity necessitate the application of physiological criteria for identification. Cultural methods and the morphological and physiological taxonomic criteria must be selected to minimise procedural bias. So the generic classification

TABLE 2. Morphological and biochemical characteristics of 319 bacterial strains isolated from Cochin Backwater area from April 1972 to March 1973.

Characteristics		Frequency of occurrence (%)
1. Gram		
	Positive	0.6
	Negative	99.11
2. Motile		
	PM	47.7
	PT	30.0
3. Pigmented		3.2
4. O/F Dextrose fermentation		
	Oxidative	40.7
	Fermentative	30.0
	No reaction	9.5
5. Proteolytic		78.3
6. Amylolytic		44.5
7. Nitrate reducers		60.8
8. H_2S Producers (SO_4 reducers)		45.7
9. Indole Producers		20.3
10. Sugar Fermentation		-
11. Sucrose		75.0
12. Lactose		nil
13. Maltose		55.0
14. Mannitol		65.0

of bacterial isolates was done according to a modified scheme of Usio Simidu and Kayuyoshi Aiso (1962). [TABLE-6]

Qualitative analysis:

Altogether 319 strains were isolated, identified and briefly studied physiologically and classified into 6 genera. Almost all of the isolates (99.4%) were asporogenous gram-negative rods usually somewhat pleomorphic (Table 2). Gram-positive bacteria were rare, only 0.7% were isolated. Only 47.7% of the isolates had polar lophotrichous flagella and 30% had peritrichous flagella. Pigmented bacteria isolated formed 3.2% of the total isolates. Most of the non-pigmented forms were dull-white and a few of these included opaque colonies. All the strains isolated were, actively motile except the *micrococci*. Dextrose was fermented oxidatively (40.7%) and fermentatively (30.0%). The rest of the isolates were unable to utilise dextrose. 60.8% of the isolates were capable of reducing nitrate into nitrite. 78.3% of the total isolates liquified gelatin indicating active proteolytic activity of the flora isolated. Approximately 44.5% of the isolates hydrolysed starch and 45.7% strains reduced sulphates forming H_2S. None of the isolates fermented lactose and produced gas from any of the sugars. Most of the isolates fermented sucrose, maltose and mannitol. The above observations indicate that the heterotrophic micro-organisms are actively involved in the degradation and total turn-over of organic matter in the Cochin Backwater.

Percentage occurrence of each genus is given in Figs. 18 a and 18 b. *Alcaligenes*, *Vibrio*, *Pseudomonas*, *Bacillus*, *Flavobacterium*, *Aeromonas* and *Micrococci* constituted the genera occurring both in sea water and sediment. The percentage occurrence of Gram-positive bacteria like *Micrococci* and *Bacillus* was more in sediment than in sea water.

In the absence of any detailed work on the percentage occurrence of bacteria at generic level in Cochin area, the present data have been compared with the data available elsewhere (Table 3). A perusal of the table shows that the genus *Alcaligenes* predominates the inshore environment of Cochin, while *Vibrio* was found to be the dominant genus in Chesapeake Bay and Kamogawa Bay. *Pseudomonas* was generally encountered in moderate numbers except in the Long Island Sound. The *Flavobacterium* occurred as a predominant genus in Narragansett Bay, (Rhode Island), while it was seen in lesser quantities in other environments. The *Micrococcus* was found to be the only genus moderate to rare in all the environments included in Table 3 which

TABLE 3. Comparison of generic percentage distribution of heterotrophic bacteria isolated from different environments by various investigators with the present study (1972-73).

Genus	Cochin Backwater		Chesapeake Bay		Long Island Sound Conn.	Narragansett Bay R.I.	Kamogawa Bay Japan
	Seawater (Chandrika, 1972)	Mud	Bay (Lovelace *et al.*, 1968)	Mid	(Conover, 1956)	(Murchelano, 1967)	(Simidu & Aiso, 1962)
			1	2			
1. *Alcaligenes*	32.0	28.0	13.0	42.0	28.6	12.2	21.3
2. *Vibrio*	22.0	22.0	56.0	17.0	4.9	13.3	37.3
3. *Pseudomonas*	18.0	16.0	18.0	-	40.6	28.3	29.8
4. *Bacillus*	7.0	14.0	-	-	0.1	0.5	5.5
5. *Flavobacterium*	8.0	8.0	6.0[b]	8.0[b]	23.1	40.7[b]	2.1
6. *Aeromonas*	7.0	5.0	-	-	-	-	-
7. *Micrococcus*	2.4	5.0	-	-	0.3	1.2	0.4

indicated that it may not be an indigenous genera but may be an exotic one which survive in the marine environment. *Bacillus*, the spore bearing, gram-positive rods, the user of refractory organic compounds has been recorded only in summer months in all the marine environments by the various investigators.

It is difficult to integrate the data of Table 3 and present meaningful quantitative synopsis of the bacterial genera indigenous to sea water from coastal environments. It may not be desirable to do so because varied procedural methodologies may somewhat affect the outcome of bacterial taxonomic studies. But the observed generic diversity is more likely caused by environmental and seasonal diversity rather than by any other factor. The six genera represented a very small percentage of Eubacteriales and Pseudomonadales although their relative abundance varied considerably, due to various environmental and biological factors, but they are consistently found in coastal waters.

Generic composition:

Alcaligenes:

Among various bacterial groups *Alcaligenes* constituted the bulk of the flora in terms of number at all stations throughout the year. *Alcaligenes* accounted for

32% of the total in surface water and 28% of the total in sediments (Table 3). During the monsoon and post-monsoon months *Alcaligenes* predominated and difference in the physiological activities during the different seasons of the annual cycle is also observed (Fig. 20). The catalase and gelatinolytic activity was very high in pre-monsoon months and was in the decreasing order in the monsoon and post-monsoon months. The amylolytic bacterial counts were very high in monsoon months and a clear inverse relationship between total number of *Alcaligenes* and nitrogenase activity was observed during the three seasons.

Vibrio:

Vibrio was the second most abundant genera forming 22% of the total in both water and sediment (Table 3). 100% catalase activity was seen during the monsoon period (Fig. 18 e). Gelatinolytic activity was maximum in pre-monsoon months. Nitrate reduction was very low in monsoon period but high in the pre-monsoon period. The inverse relationship between the counts of *Vibrio* spp. and amylolytic activity (starch hydrolysis) was also observed.

Pseudomonas:

18% of the total isolates turned to be *Pseudomonas* in water and 16% in sediment. Marchelano and Brown (1970) have reported the abundance of *Pseudomonas* in summer than at any other time of the year. In the present study also their density decreased in the monsoon period (Fig. 18 e) indicating that the seasonal distribution of *Pseudomonas* is directly influenced by temperature. Calalase activity was recorded high in the post-monsoon period. Gelatinolytic activity of *Pseudomonas* decreased steadily throughout the year but exhibited the same pattern like *Alcaligenes* and *Vibrio*. More aerobic isolates utilised nitrate in the pre-monsoon period and there was a gradual decrease in the nitrate reduction during monsoon and post-monsoon periods. Amylolytic activity was low in pre-monsoon period and attained a peak during monsoon and the activity declined again in post-monsoon season.

Bacillus:

Bacillus was dominant in sediments being 14% whereas in sea water it occurred only 9% of the total.

Flavobacterium:

The pigmented *Flavobacterium* was found only during the months of April, May and July connected with phytoplankton blooms and formed only 8% of the total both in water and sediment.

Aeromonas:

This genus formed 7% of the total heterotrophs in water and 9% of the population in sediment.

Micrococci:

The gram-positive *Micrococci* were very scarce in number forming only 2.4% of the total in water and 5% in mud.

This pigmented genera was found to increase associated with phytoplankton blooms and during red tide, as red tide was found to contain discoloured aggregations of algae, bacteria, ciliates, diatoms, flagellates and other pigmented organisms. Total heterotrophic counts in Station V corresponding to the period of red water was found to decrease (63×10^3) when compared to the counts recorded in the month of June (732×10^3) in the monsoon months (Table 1). Steeman Nielsen (1955) has suggested that algal antibiotics keep down bacteria, but phagotrophs such as dinoflagellates may well require bacteria to reach bloom proportions. Oppenheimer (1963) found that bacterial numbers were high in the *PRorocentrum* red tides of California but the meaning of this is equivocal. The bacteria can live on the excerines from the dinoflagellates and also can provide growth factors such as Vitamin B_{12}. In the present case, the decline of bacterial counts during red tide may be due to production of toxin by dinoflagellates.

The distribution of the bacterial counts showed some clustering 'to judge' whether the clustering is in space or in time the method of 'analysis of variance' was applied to the logarithm of the counts. From the results given in Table 4, the variation between months (significant at 5% level) is found to be more than that between the stations. Thus the clustering appears to be relatively more in time than in space.

Assuming that 1 ml of surface water used for counting the bacteria from 5 stations are random samples, the sampling distribution of the bacterial density is given in Fig. 18f. This distribution has a mean (m) of 414 counts/ml with a variance (s^2) of 495 - 594. As $s^2 > m$ the distribution is characterised by overdispersion. When overdispersion is present, a clustering of the samples in some ranges of the counts can be expected. It can be seen from Fig. 18f that more samples occurred with counts at 50 and between the counts 650 and 850 on an average rate. Cassie (1971) has described an approximate relationship between the mean and variance of distribution with over-dispersion. This gives s^2 in terms of m as approxi-

TABLE 4. Analysis of variance of logarithm of bacterial counts* (1972-73).

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares
Between months	3.7017	9	0.4113
Between stations	0.4532	4	0.1133
Error	7.8346	36	0.2176
Total	11.9895	49	

* Counts for all the stations were available only for 10 months.

mately $s^2 \backsim m + cm^2$ where 'c' is a constant which gives the characteristic of the population. The value of 'c' works out to 2.9 in the present case. This coefficient can be used to compare different bacterial populations, as larger values of 'c' indicate a greater overdispersion.

The correlation coefficients (Table 5 a) have been worked out separately for each station because it is possible that the linear relationship between the variables might change from station to station. The coefficients between the bacterial counts and the nitrite showed high positive values at three stations (one coefficient is significant and another highly significant). As can be seen from Table 5 a, phosphates showed positive correlation with the bacterial abundance (one significant coefficient and three high values). While at Stations I and II, the nitrates showed some positive relationship, it did not show similar relationship for the rest. In the case of silicates, the coefficients for Stations I and II were negative and rather high. Though temperature showed a negative correlation (significant at 5% level with the bacterial abundance at Station I), it is not significant at this level for the other stations. This can largely happen when the temperature tolerance of different species of bacteria differ and when different types of bacteria become abundant at different stations. For arriving at definite conclusions

TABLE 5 a. Correlation coefficient between bacterial counts and physico-chemical factors in 4 stations of the Cochin Backwater (1972-73).

Station No.	Correlation coefficient between bacterial counts and							
	Phytoplankton	Salinity	Temperature	Oxygen	Phosphates	Nitrites	Silicates	Nitrates
I	-0.3179	-0.1136	-0.6242*	-0.0322	0.5006	0.8377*	-0.4562	0.3947
II	-0.2111	-0.1983	-0.07995	0.2266	0.5192	0.6045*	-0.3984	0.5657
IV	-0.1734	-0.1620	-0.3106	-0.1734	0.3471	0.5119	0.0712	-0.3298
V	-0.3390	-0.2500	-0.2460	0.0664	0.6803*	-0.0763	-0.2066	0.0499

* Significant at 5% level.

(In Station No. III the data are not available) further study of the various parameters affecting the individual species should be made, as the abundance of phytoplankton itself may depend on other factors noted above. It is possible that elevated phytoplankton pigments (Phaeopigments) are indicative of high zooplankton grazing on phytoplankton (Shuman and Lorenzen, 1975) which may make more organic matter available for bacterial growth. Further, it is likely that the different species of bacteria may prefer different species of phytoplankton. But according to Smith (1977) increased photosynthesis will cause increased bacterial growth and Fuhrman *et al.* (1980) is of the view that the routes between photosynthetic carbon fixation and bacterioplankton production are quite complex. Marchalano and Brown (1970) found the annual bacterial cycle positively correlated with the cycle of phytoplankton. In the present case it is not possible to conclude statistically whether there is a negative or positive relationship between total phytoplankton and bacterial counts. Oxygen does not show appreciable linear relationship with the bacterial counts as judged from the correlation coefficients.

Only at Station V, salinity showed (though not significantly) some correlation with the bacterial counts. This may perhaps be due to the mixing of sea water.

Applying the test for 'homogeneity of correlation coefficients' (Rao, 1952), the correlation coefficients were found to be homogeneous. Therefore, by pooling the stations a single coefficient was worked out for each parameter (Table 5 b). Here, only the nitrates showed a highly significant correlation with bacterial counts. However, with phosphate and temperature, the correlation coefficient was also rather high (significant at 5% level).

In the present study the viable bacterial counts showed seasonal fluctuations in numbers at different seasons giving a maximum in postmonsoon and premonsoon months (December 1972 to March 1973). The bacterial counts of the present study were of similar magnitude as given by Zo Bell (1948) from the Southern California coast, Velankar (1955) from the Palk Bay and Gulf of Mannar, Civlic (1955) in the Adriatic Sea and Kriss (1961) in the Black Sea. Also no definite seasonal trend was noted as also reported by Velankar (1955) and Lloyd (1930). Wood (1959) found irregular seasonal distribution in the waters of Lake Macquire but in the waters off Sydney, Brown (1964) recorded higher bacterial counts during summer and spring than at other seasons.

The results of the generic distribution of the microflora showed variation in the percentage composition of various bacterial genera in the two layers of water. As reported by Marchelano and Brown (1970) *Alcaligenes*,

TABLE 5 b. Correlation coefficient between bacterial counts and physico-chemical factors of the Cochin Backwater (1972-73).

(Calculated by pooling the four stations)

Factors	Correlation coefficient
1. Phytoplankton	- 0.2007
2. Salinity	- 0.0531
3. Temperature	- 0.2778
4. Oxygen	- 0.0028
5. Silicates	- 0.0326
6. Nitrates	- 0.0399
7. Phosphates	0.2690
8. Nitrites	0.5528*

* Significant at 1% level.

Vibrio and *Pseudomonas* dominated the bacterial flora. Wood (1953) remarked the abundance of *Micrococci* in Australian waters which was found contradictory in the present study as *Micrococci* contributed very little to the total bacterial flora.

The enzymatic potential of the isolates of the three dominant genera assayed roughly by using the two substrates such as Gelatin and Starch indicated that although there was seasonal percentage variation of the three abundant heterotrophic bacterial genera, the enzymatic potential remained essentially constant. Lewis (1971) also reported the ability of the organisms to liquify gelatin, produce indole and H_2S and decompose urea to form ammonia can be used for qualifying the spoilage potential of that organism. The three dominant genera were found actively degrading gelatin and reducing sulphur indicating enzymatic spoilage potential of the three genera.

Velankar (1955) reported absence of any relationship between temperature and bacterial population. Temperature showed a negative correlation (significant at 5% level with bacterial abundance at Station I) in the present investigation. Brown (1964) found no correlation between bacterial counts and soluble organic phosphorous and counts of phytoplankton in waters off Sydney but Gundersen *et al.* (1972)

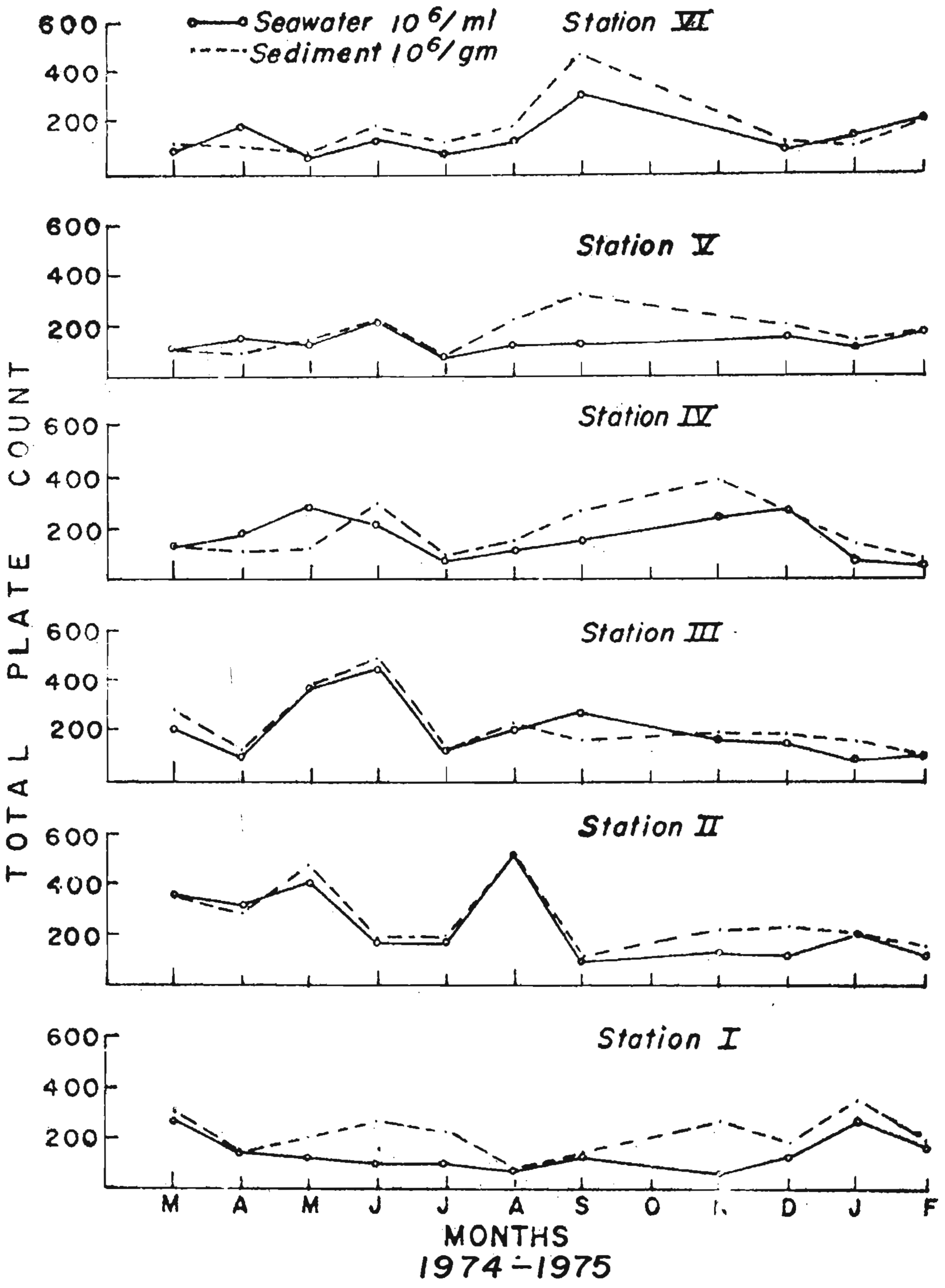
reported a close correlation between the concentration of proteins and heterotrophic bacteria. In the present study, in addition to the significant correlation between the bacterial counts and nitrite and phosphate, somewhat high correlation between bacterial counts and temperature supported the view (Gundersen *et al.*, 1972) that the distribution of nutrients and temperature play an important role in the distribution of bacteria.

Distribution and composition of heterotrophic bacterial flora during 1974-75:

Quantitative analysis:

Seasonal cycle of total heterotrophic bacterial flora (Fig. 19) revealed the highest count (512×10^6/gm) at Station II in sediment and in sea water (496×10^6/ml) in the month of August 1974 and a minimum during February 1975 in Station IV in sea water (46×10^6/ml) and in sediment (84×10^6/gm). The counts of saprophytic heterotrophs were generally high during monsoon months in all the stations when fresh water influx is maximum and that any further discharge in subsequent months does not add to bacterial contribution. Altogether 296 strains were isolated, identified and classified into 7 genera. [TABLE:6] In addition, 36 spore-forming *Bacillus* strains were isolated using specific methodologies from mud samples as they are generally indicators of refractory organic compounds and is being discussed

Fig. 19. Showing total plate count (No. x 10^6) in water and sediment in the area of study.[1974-75]


Seawater 10^6/ml
Sediment 10^6/gm
Station VI
Station V
Station IV
Station III
Station II
Station I
600
400
200
TOTAL PLATE COUNT
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974-1975

separately in this chapter.

Qualitative analysis:

Morphological and physiological characteristics of the isolates are studied using various biochemical reactions (Fig. 20 a). The figure representing frequency of occurrence, are self-explanatory and need no further discussion.

Frequency of occurrence of the 7 bacterial genera in 4 different families are illustrated in Fig. 21. *Alcaligenes* was the dominant bacterial genus isolated during the sampling period (1974-75). Together, *Pseudomonas*, *Vibrio*, *Flavobacterium*, *Photobacterium*, *Aeromonas* comprised 92.3%. *Bacillus* and *Micrococcus* accounted for only 7.3%.

Genera	Pre-monsoon		Monsoon		Postmonsoon	
	Seawater	Mud	Seawater	Mud	Seawater	Mud
Alcaligenes	28	46	36	56	32	94
Pseudomonas	8	17	15	16	13	9
Vibrio	8	10	16	14	18	12
Flavobacterium	7	8	8	13	12	12

The numbers of *Pseudomonas*, *Vibrio* and *Flavobacterium* in sea water were small in pre-monsoon period. In mud only, the numbers of *Vibrio* and *Flavobacterium* were small and did show seasonal changes. All the genera are comparatively more in sediments than in sea water.

Fig. 20. Showing biochemical and physiological activity of bacterial strains isolated during 1974-75 (a) and during 1975-76 (b).

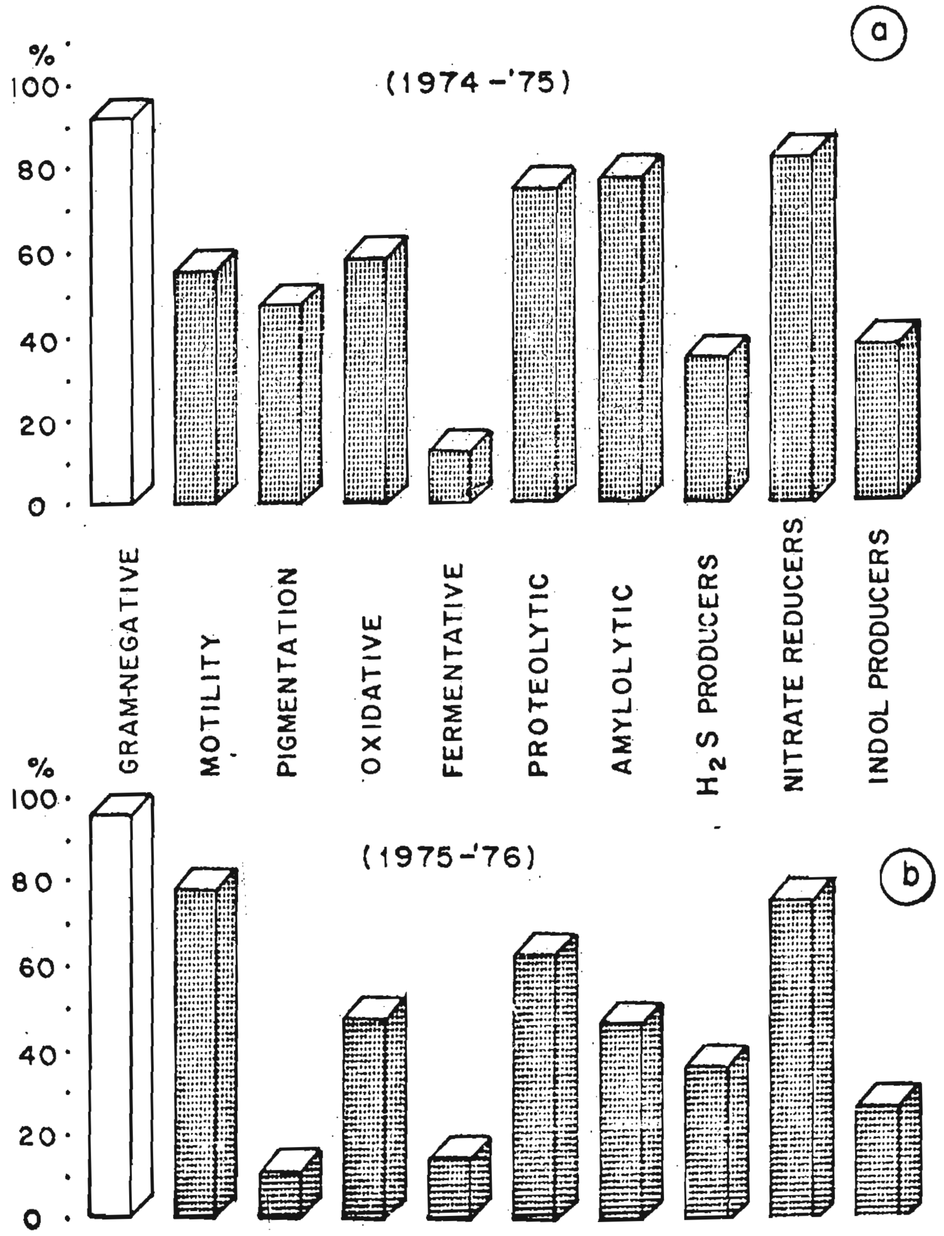
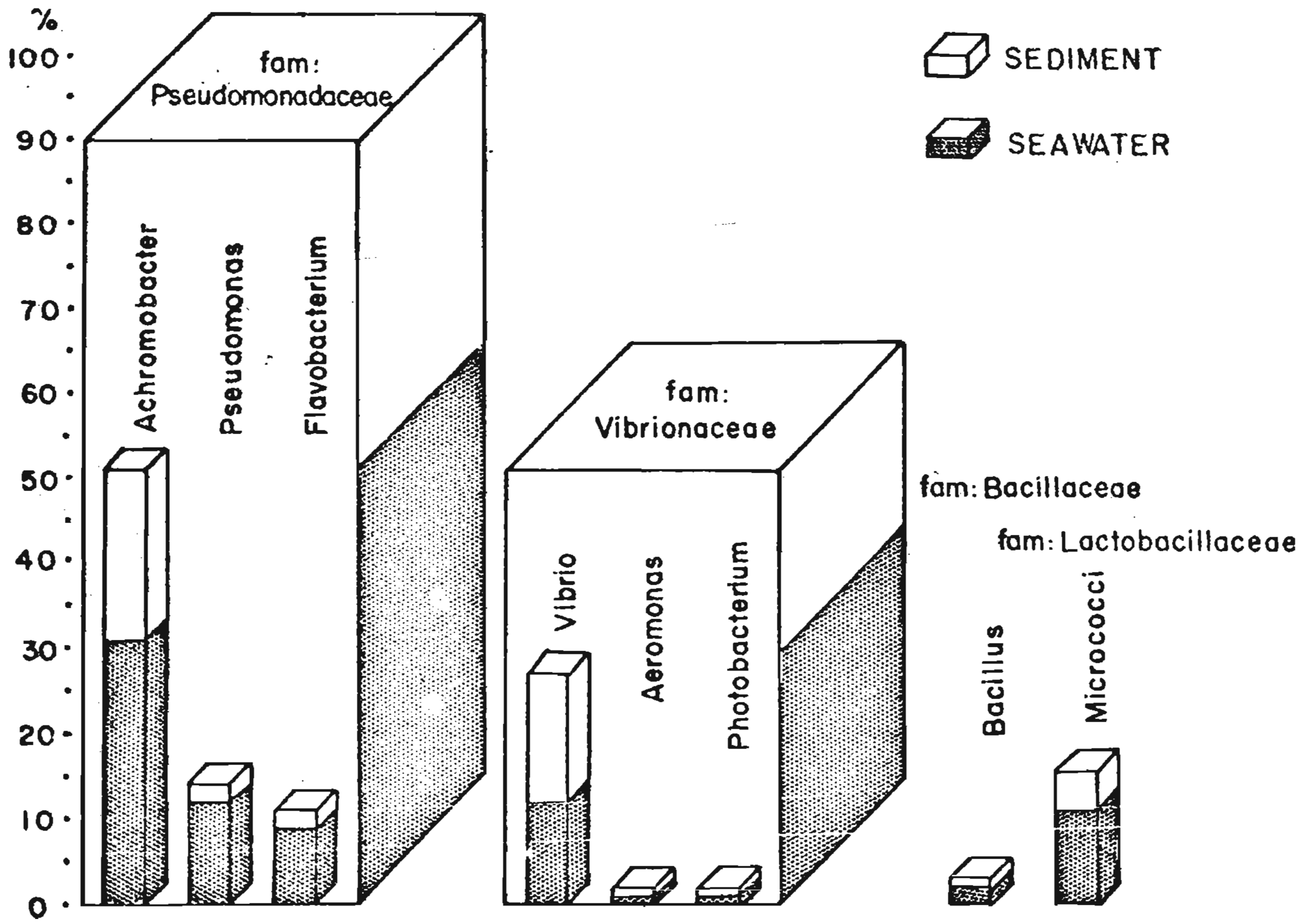
a
(1974 -'75)
%
100
80
60
40
20
0
GRAM-NEGATIVE
MOTILITY
PIGMENTATION
OXIDATIVE
FERMENTATIVE
PROTEOLYTIC
AMYLOLYTIC
H_2S PRODUCERS
NITRATE REDUCERS
INDOL PRODUCERS
%
100
80
60
40
20
0
(1975-'76)
b

Fig. 21. Showing generic composition of 296 bacterial strains isolated during 1974-75.


%
100
90
80
70
60
50
40
30
20
10
0
fam: Pseudomonadaceae
Achromobacter
Pseudomonas
Flavobacterium
fam: Vibrionaceae
Vibrio
Aeromonas
Photobacterium
fam: Bacillaceae
fam: Lactobacillaceae
Bacillus
Micrococci
SEDIMENT
SEAWATER

Motility was recorded only in 46% of the total isolates during the sampling period (1974-75) which may be due to the non-motile characteristics of the genera *Alcaligenes* and *Flavobacterium*. *Alcaligenes* was predominant both in sea water (32%) and sediment (52%) in all the seasons.

Analysis of variance test:

The study of the distribution of bacteria along with environmental parameters was subjected to 'Analysis of variance' to test whether the correlation is with space or time. The results of analysis of variance were given in the Table 7. There was no significant difference in the total plate count between stations, months and regions.

Product-moment correlation coefficient:

The purpose of the study was to see which parameters were correlated to the bacterial parameters and which were not, in an attempt to identify some of the mechanisms which influences or are influenced by bacterioplankton. To test whether there were significant independency of the hydrological factors and the different types of bacteria, the product-moment correlation coefficient between each factor with others were calculated using the formula:

TABLE 7. Analysis of variance of logarithm of bacterial counts (1974–75).

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of Squares	Variance ratio (F)
Total heterotrophic bacteria	7.9685	127	-	-
Between stations	0.5990	5	0.1198	2.11
Between months	0.8627	10	0.0863	1.52
Between regions	0.2011	1	0.2011	3.54
Error	6.3058	111	0.0568	-

* Significant at 5% level ($P < 0.05$)

** Significant at 1% level ($P < 0.01$)

$$r = \frac{\Sigma(x - \bar{x})\ (y - \bar{y})}{n\sigma x \sigma y} \quad \text{where}$$

$\bar{x}$ = mean of x values, $\bar{y}$ = mean of y values

σx = SD of x values = $\sqrt{\frac{\Sigma x - \bar{x})^2}{n}}$

σy = SD of y values = $\sqrt{\frac{\Sigma y - \bar{y})^2}{n}}$

and n = no. of pairs of observation on x and y. The matrix of correlation framed for each station for surface and bottom water for the year 1974-75 were presented in Tables 8 to 13 for sea water, and 14 to 19 for sediments. The significance of the correlation coefficient were tested using 't' test.

$$t = \frac{r\sqrt{n - 2}}{\sqrt{1 - r^2}}$$, where r is the coefficient correlation.

The degrees of freedom of 't' is (n - 2). The significant correlation coefficient were marked in the correlation matrix as a, b and c where,

a = represents significance at 5% level ($P < 0.05$)

b = represents significance at 1% level ($P < 0.01$)

c = represents significance at 0.1% level ($P < 0.001$)

Significant positive correlation implies that as one factor increases the other also increases and significant negative correlation implies that as one factor increases, the other factor decreases.

In Sea Water:

From the Table 8 it is clear that in Station I, total heterotrophic bacteria was significantly positively correlated with salinity at 5% level. The positive correlation may be due to dominance of fresh water at Station I, which is situated in the southern side of Thanneermukkom Bund. Salinity recorded for this station was very low during the monsoon period. Heterotrophic bacteria indigenous to the marine environment will prefer higher salinity which resulted in the development of positive relationship between salinity and total heterotrophs only in this particular station.

Negative correlation of total heterotrophic bacteria with pH was significant at 5% level at Station II (Table 9). It is well known, that heterotrophs prefer acidic medium of water or sediment in marine environment the reason why they have developed on inverse relationship with pH of the environment at Station II which was dominated by brackish water conditions.

Whenever the heterotrophs increases indicators of bacterial pollution also increases. In Station III, the total heterotrophs showed significant positive correlation with staphylococci at 1% level (Table 10).

TABLE 8. Product moment correlation coefficients of all measured parameters in sea water (1974–75) – Station I.

Parameters	Temp.	Sali.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. cocci*	Faecal index
Temp.	1.00											
Salinity	0.13	1.00										
O_2	−0.27	−0.18	1.00									
PO_4-P	0.41	0.28	−0.60^a	1.00								
NO_3-N	−0.06	−0.71^a	−0.14	−0.26	1.00							
pH	−0.03	−0.12	−0.29	−0.09	0.61^a	1.00						
TPC	−0.04	0.71^a	−0.15	0.06	−0.33	0.21	1.00					
Coliforms	−0.35	0.43	−0.14	0.29	−0.14	0.41	0.29	1.00				
E. coli	0.21	−0.19	−0.18	0.66^a	−0.42	−0.47	−0.31	−0.16	1.00			
S. faecalis	0.02	−0.03	0.13	−0.06	−0.02	0.43	−0.00	0.33	−0.45	1.00		
S. cocci	−0.07	−0.26	0.10	0.34	−0.22	−0.32	−0.57	−0.09	0.44	−0.01	1.00	
Faecal index	0.38	−0.02	−0.34	0.52	0.07	−0.21	0.11	0.01	0.55	−0.49	−0.31	1.00

TABLE 9. Product moment correlation coefficients of all measured parameters in the sea water (1974-75) - Station II.

Parameters	Temp.	Sali.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. cocci*	Faecal index
Temp.	1.00											
Salinity	0.12	1.00										
O_2	-0.01	0.20	1.00									
PO_4-P	0.35	-0.08	-0.29	1.00								
NO_3-N	0.67[a]	0.42	0.18	0.51	1.00							
pH	-0.33	-0.04	-0.11	-0.00	-0.40	1.00						
TPC	0.30	-0.08	0.15	0.32	0.48	-0.65[a]	1.00					
Coliforms	-0.47	0.97	-0.10	0.19	0.03	0.22	-0.25	1.00				
E. coli	-0.58	0.07	-0.04	-0.01	-0.16	0.09	0.28	0.46	1.00			
S. faecalis	0.10	-0.04	-0.26	0.21	-0.03	-0.90	0.53	-0.02	0.05	1.00		
S. cocci	-0.94	-0.32	-0.19	0.00	-0.26	-0.10	0.30	0.21	0.73[a]	0.43	1.00	
Faecal index	0.07	0.08	0.01	-0.05	-0.07	0.25	-0.14	-0.13	-0.06	-0.40	0.57	1.00

TABLE 10. Product moment correlation coefficients of all measured parameters in sea water (1974–75) – Station III.

Parameters	Temp.	Sali.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. cocci*	Faecal index
Temp.	1.00											
Salinity	0.31	1.00										
O_2	-0.48	-0.44	1.00									
PO_4-P	0.90[a]	0.15	-0.60	1.00								
NO_3-N	0.45	0.42	-0.28	0.42	1.00							
pH	0.31	-0.54	0.07	0.39	0.25	1.00						
TPC	0.07	-0.54	-0.35	0.45	-0.23	0.24	1.00					
Coliforms	-0.50	0.19	0.10	-0.12	-0.26	-0.44	-0.06	1.00				
E. coli	0.11	0.35	-0.43	0.23	-0.04	-0.03	0.04	0.25	1.00			
S. faecalis	-0.41	0.29	0.07	-0.16	0.31	-0.12	-0.25	0.42	-0.27	1.00		
S. cocci	-0.26	-0.36	-0.39	0.14	-0.36	0.18	0.82[b]	0.12	0.25	-0.09	1.00	
Faecal index	0.17	0.10	-0.49	0.50	-0.12	0.13	0.44	0.19	0.49	-0.40	0.54	1.00

In Stations IV and V heterotrophs showed significant negative correlation with oxygen in sea water (Tables 11 & 12). But Marty(1981) provided the result opposing this view that heterotrophic aerobic bacteria were detected by him in the Arabian Sea sediments in the Gulf of Aden and Oman Sea (in November, 1978) even beyond 100 cm in core samples. Because of their facultative or microaerophilic nature, generally, it is believed that the aerobic heterotrophs are highly tolerant to anoxic conditions. The negative correlation between bacterial counts and oxygen recorded at Stations IV and V may be due to cumulative effect of some unknown factors.

In Station VI, significant positive correlation was seen at 1% level between heterotrophic bacteria and temperature (Table 13). Hedwell and Floodgate (1971) also found a positive correlation between temperature and seasonal selection of heterotrophic bacteria in an intertidal environment. However, Chan and Kueh (1976) worked out the distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour, Hong Kong and found temperature was not a limiting factor on the bacterial population even during summer months.

TABLE 11. Product moment corellation coefficients of all measured parameters in sea water (1974–75) – Station IV.

Parameters	Temp.	Sali.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S.aureus*	Faecal index
Temp.	1.00											
Salinity	-0.41	1.00										
O_2	0.30	0.29	1.00									
PO_4-P	-0.27	0.44	-0.11	1.00								
NO_3-N	-0.65^a	0.38	-0.13	0.42	1.00							
pH	0.06	-0.54	-0.44	-0.35	-0.61^a	1.00						
TPC	0.22	-0.21	-0.63^a	0.31	-0.01	0.04	1.00					
Coliforms	0.01	0.10	0.45	-0.57	-0.48	0.31	-0.71^a	1.00				
E. coli	0.09	-0.26	-0.45	-0.47	-0.19	0.45	0.00	-0.04	1.00			
S. faecalis	0.32	-0.16	0.38	-0.79^b	-0.18	-0.09	-0.48	0.47	0.34	1.00		
S.aureus	-0.20	-0.19	-0.33	-0.57	0.19	0.18	-0.23	0.10	0.77^b	0.48	1.00	
Faecal index	0.20	-0.08	0.22	0.33	-0.31	0.19	0.16	-0.01	-0.38	-0.53	-0.61^a	1.00

TABLE 12. Product moment correlation coefficients of all measured parameters in sea water (1974-75) - Station V.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. sonnei*	Faecal index
Temp.	1.00											
Salinity	0.38	1.00										
O_2	-0.24	-0.13	1.00									
PO_4-P	0.69^a	0.27	-0.29	1.00								
NO_3-N	0.10	0.02	0.43	0.03	1.00							
pH	0.21	0.10	0.17	0.47	-0.61^a	1.00						
TPC	0.77^b	-0.08	-0.67^a	-0.21	-0.54	-0.01	1.00					
Coliforms	0.42	0.73^a	-0.42	0.13	0.03	-0.12	0.30	1.00				
E. coli	-0.73^a	-0.14	0.18	-0.33	-0.19	-0.30	0.06	-0.32	1.00			
S. faecalis	-0.42	0.20	0.04	-0.60^a	0.28	-0.24	-0.37	0.22	-0.08	1.00		
S. sonnei	-0.55	-0.29	0.09	-0.22	-0.07	-0.56	-0.03	-0.36	0.83^b	-0.03	1.00	
Faecal index	-0.32	-0.39	0.43	-0.34	0.05	0.20	-0.38	-0.50	-0.15	0.32	-0.25	1.00

TABLE 13. Product moment correlation coefficients of all measured parameters in sea water (1974-75) - Station VI.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. cocci*	Faecal index
Temp.	1.00											
Salinity	0.46	1.00										
O_2	0.30	-0.13	1.00									
PO_4-P	0.31	0.07	-0.59	1.00								
NO_3-N	0.28	-0.14	-0.05	0.35	1.00							
pH	-0.29	0.03	-0.53	0.28	-0.09	1.00						
TPC	-0.11	-0.08	-0.05	-0.55	-0.59	-0.54	1.00					
Coliforms	-0.04	0.62[a]	-0.18	-0.10	-0.36	0.09	0.17	1.00				
E. coli	0.07	0.12	-0.43	0.58	-0.21	0.35	-0.33	-0.28	1.00			
S. faecalis	0.10	0.29	0.22	-0.23	0.38	0.01	-0.33	0.43	-0.69[a]	1.00		
S. cocci	-0.41	-0.56	-0.01	-0.23	-0.27	0.03	0.34	-0.65	0.26	-0.45	1.00	
Faecal index	0.30	0.52	-0.49	-0.13	-0.45	0.32	-0.33	0.40	0.63[a]	-0.15	-0.02	1.00

In Sediment:

In Stations I and IV total heterotrophic counts were significantly ($P < 0.05$) positively correlated with hydrogen ion concentration at 5% level (Tables 14 & 17). What is often termed normal sea water is of about 35 ‰ salinity and is of alkaline, but the degree of alkalinity varies with numerous factors, including proximity of the water to land mass. pH should vary diurnally as well as seasonally, but in the present study the variation was much less. Generally, in the sub-surface sea water the pH range from 7.5 to 8.4 but in surface water, the pH range from 8.1 to 8.3. The latter range occurs when the CO_2 in the surface water is in equilibrium with that in the atmosphere. The H_3O^+ ion concentration is directly related to carbon-di-oxide in sea water. If the CO_2 content decrease, pH will increase and waters above pH 7.5 have most of the carbon-di-oxide in various combined forms rather than as dissolved gas. In estuaries, under some conditions, the pH of the water may exceed 8.5. In the present investigation pH ranged in surface as well as in sub-surface water between 7.0 and 7.90 which may be due to high freshwater influx as mixed fresh and sea water may drop towards neutrality or still below the acid range.

No significant correlations were seen in Stations III and V (Tables 16 - 18) between total heterotrophic

TABLE 4. Product moment correlation coefficients of all measured parameters in sediments (1974-75) - Station I.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. sonnei*	Faecal index
Temp.	1.00											
Salinity	0.29	1.00										
O_2	-0.38	0.31	1.00									
PO_4-P	0.58	-0.04	-0.51	1.00								
NO_3-N	0.53	-0.20	-0.47	0.70^a	1.00							
pH	0.07	-0.05	-0.37	0.80^b	0.52	1.00						
TPC	-0.12	0.21	0.07	-0.32	0.10	0.68^a	1.00					
Coliforms	-0.20	0.16	-0.23	0.33	0.04	0.70^a	0.69^a	1.00				
E. coli	0.02	0.24	0.28	-0.23	-0.02	0.02	-0.27	0.05	1.00			
S. faecalis	-0.37	-0.69^a	-0.18	-0.12	-0.06	-0.07	-0.30	0.23	0.13	1.00		
S. sonnei	-0.03	-0.35	-0.25	0.51	0.39	0.50	-0.09	0.26	0.28	0.46	1.00	
Faecal index	0.04	0.53	0.28	-0.43	-0.14	-0.42	-0.05	0.05	0.69^a	-0.10	-0.37	1.00

TABLE 15. Product moment correlation coefficients of all measured parameters in sediments (1974-75) - Station II.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TFC	Coliforms	*E. coli*	*S. faecalis*	*S. cocci*	Faecal index
Temp.	1.00											
Salinity	0.47	1.00										
O_2	-0.25	0.01	1.00									
PO_4-P	0.41	0.46	0.10	1.00								
NO_3-N	0.20	0.01	0.18	0.38	1.00							
pH	-0.08	0.27	-0.12	0.32	-0.41	1.00						
TFC	0.05	-0.04	0.29	0.61^a	0.26	-0.09	1.00					
Coliforms	0.05	0.73^a	0.23	0.21	-0.28	0.47	-0.23	1.00				
E. coli	0.32	0.36	-0.29	0.03	-0.05	0.61^a	-0.15	0.33	1.00			
S. faecalis	-0.49	-0.27	0.22	-0.09	-0.53	0.22	-0.27	0.07	-0.26	1.00		
S. cocci	-0.10	0.10	0.11	0.48	-0.04	0.16	0.44	0.12	-0.34	0.45	1.00	
Faecal index	0.28	0.15	-0.38	-0.20	-0.03	0.26	-0.33	-0.05	0.77^b	-0.40	-0.72^a	1.00

TABLE 16. Product moment correlation coefficients of all measured parameters in sediments (1974-75) - Station III.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S. faecalis*	*S-sonnei*	Faecal index
Temp.	1.00											
Salinity	0.42	1.00										
O_2	-0.76^b	0.34	1.00									
PO_4-P	0.73^a	0.48	-0.69^a	1.00								
NO_3-N	-0.14	0.09	-0.04	0.05	1.00							
pH	0.67^a	-0.09	-0.62^a	0.67^a	-0.34	1.00						
TPC	-0.07	0.35	0.11	0.39	-0.13	0.37	1.00					
Coliforms	-0.25	0.27	-0.10	0.08	-0.21	-0.93	0.01	1.00				
E. coli	0.23	0.49	-0.25	0.31	-0.41	0.11	-0.24	0.49	1.00			
S. faecalis	-0.21	-0.43	0.29	-0.36	-0.18	0.09	-0.30	0.31	0.04	1.00		
S-sonnei	0.53	0.47	-0.11	0.59	-0.25	0.46	0.47	0.03	0.43	-0.02	1.00	
Faecal index	0.04	0.17	0.02	-0.22	-0.17	-0.19	-0.46	0.53	0.72^a	0.08	-0.20	1.00

TABLE 17. Product moment correlation coefficients of all measured parameters in sediments (1974-75) - Station IV.

Para-meters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coli-forms	*E. coli*	*S. fae-calis*	*S. aureus*	Faecal index
Temp.	1.00											
Salinity	0.44	1.00										
O_2	-0.23	0.08	1.00									
PO_4-P	-0.05	-0.01	-0.00	1.00								
NO_3-N	0.25	-0.00	0.29	0.80[b]	1.00							
pH	0.11	-0.68[a]	-0.39	0.05	0.04	1.00						
TPC	-0.13	-0.48	0.03	0.01	0.19	0.61[a]	1.00					
Coli-forms	-0.14	0.37	-0.56	-0.44	-0.44	-0.48	-0.34	1.00				
E. coli	-0.08	0.75[b]	-0.58	-0.08	-0.14	-0.69[a]	-0.33	0.55	1.00			
S. faecalis	0.02	-0.08	0.17	0.05	0.10	0.31	-0.24	0.20	0.03	1.00		
S. aureus	-0.28	-0.13	-0.33	0.25	0.32	-0.43	-0.02	-0.25	0.33	-0.40	1.00	
Faecal index	-0.12	-0.01	0.08	-0.39	-0.75[b]	-0.28	-0.43	0.53	0.32	0.24	-0.56	1.00

TABLE 18. Product moment correlation coefficients of all measured parameters in sediments (1974-75) - Station V.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coliforms	*E. coli*	*S.faecalis*	*S.sonnei*	Faecal index
Temp.	1.00											
Salinity	0.47	1.00										
O_2	0.12	0.11	1.00									
PO_4-P	-0.11	-0.08	-0.35	1.00								
NO_3-N	0.11	-0.12	0.25	0.39	1.00							
pH	0.37	-0.18	0.12	-0.16	0.28	1.00						
TPC	0.52	-0.22	0.37	0.55	0.29	0.55	1.00					
Coliforms	0.63^a	0.50	0.90^a	0.07	0.26	0.57	-0.06	1.00				
E. coli	0.35	0.20	0.44	-0.11	0.47	0.40	0.00	0.34	1.00			
S. faecalis	-0.44	-0.24	-0.04	-0.36	-0.63^a	-0.42	-0.19	-0.21	-0.59	1.00		
S.sonnei	0.51	-0.06	0.63^a	0.23	0.71^a	0.35	0.13	0.07	0.41	-0.52	1.00	
Faecal index	0.46	0.38	0.16	-0.48	0.13	0.43	-0.19	0.71^a	0.65^a	-0.46	-0.18	1.00

TABLE 19. Product moment correlation coefficients of all measured parameters in sediments (1974-75) - Station VI.

Para-meters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC	Coli-forms	*E. coli*	*S. fae-calis*	*S.aureus*	Faecal index
Temp.	1.00											
Salinity	0.58	1.00										
O_2	0.41	0.11	1.00									
PO_4-P	0.08	0.21	-0.33	1.00								
NO_3-N	0.19	0.25	-0.54	0.57	1.00							
pH	0.15	-0.44	0.35	0.14	-0.11	1.00						
TPC	0.40	-0.26	0.22	0.16	0.62^a	0.44	1.00					
Coli-forms	0.66^a	0.65^a	0.03	0.39	0.03	0.63^a	0.26	1.00				
E. coli	0.46	-0.04	0.45	0.07	0.10	0.46	0.02	0.09	1.00			
S. fae-calis	-0.75^b	0.12	-0.45	-0.49	-0.68^a	-0.78^b	-0.58	-0.34	-0.57	1.00		
S.aureus	0.46	-0.25	0.24	0.42	0.25	0.50	-0.05	-0.16	0.53	-0.61^a	1.00	
Faecal index	0.25	0.52	0.28	0.11	0.25	0.38	0.11	0.47	0.52	-0.47	-0.20	1.00

counts and any of the physico-chemical or bacteriological parameters.

The total counts were significantly ($P < 0.05$) positively correlated with phosphate and nitrate in Stations II and VI respectively eventhough the concentration of phosphate and nitrate was recorded in very low amounts (Tables 15 & 19). The complexity and variability of estuarine waters and their biota has a control over the establishment of nitrate-phosphate concentration in these waters (Johnson and Sparrow, 1970). Only one over all aspect of these nutrients in the general type of estuary seems established; both of these nutrients are ordinarily in greater abundance in estuaries than in the surface waters of the open ocean. However, Chan and Kueh (1976) have collected data from 10 stations in Tolo Harbour, Hong Kong and showed that the horizontal distribution of the bacterial population increased proportionately with the availability of dissolved phosphates and nitrates. Studies by Ayyakkannu and Chandramohan (1970, 1971) indicated a relationship between phosphate content and phosphate solubilising heterotrophic bacteria. Ishida and Kadota (1975 a,b) analysed bacterial flora by using chemostat, and the growth kinetics of bacterial population showed response only to dissolved organic substances as in the present study, but Brown (1964) found no correlation between bacterial counts and organic phosphorus in waters off Sydney.

Bacillus - indicators of refractory organic compounds:

Wolf and Barker (1973) suggest the following definition of the genus:

"Rod-shaped organisms which are spore bearing, usually Gram-positive, catalase producing and capable of sporulating aerobically". Often spore-forming bacteria are used as an indicator for the presence of refractory organic compounds not utilised by non-spore formers.

Bacillus are of the utmost interest to such scientists who are interested with control of infection and efficiency in cleaning procedure. Although pathogenicity is restricted to a few species, *Bacillus* has received new importance as possible infective agents in "sterile" disposable equipment (Bonde, 1966, 1973; Curtis *et al*., 1967). Laboratories controlling the quality of foods and water are well acquainted with *Bacillus* either because of their sanitary significance or their interference with bacteriological methods. In non-selective media for coliforms *Bacillus* may inhibit the growth of coliforms by competitive growth or by producing antibiotics (Bonde, 1967, 1968).

Ford (1916) stated "It is still an important problem of modern hygiene to study the *Bacillus* group which profoundly influences all sorts of substances which affect man's physical conditions". Being frequent inhabitants of filter sands *Bacillus* are unavoidably present in samples of drinking water (Bonde, 1972) and may as "rapid liquefiers of gelatin" or by abundant growth in the hot count" be the occasion of injustified rejection of water supplies.

Pathogenic strains are largely found in the group *B. cereus* and *cereus* variants producing powerful toxins, which are still the objects of examination (Johnson & Bonventre, 1967; Kim & Goepfert, 1971; Nikodemusz & Gonda, 1966; Spira and Goepfert, 1972; Stamatin & Anghelescu, 1969).

Besides anthrax and food poisoning other infection have also been associated with *Bacillus cereus* as well as with other *Bacillus* strains (Elter, 1966). Being ubiquitous these spore-bearers have attracted great interest in work on ecology and may indicate pollution (Bonde, 1965, 1971, 1973; Puleo *et al.*, 1970).

Aerobic sporers do not seem to occur widely in sea water. Their importance is greater in sediments. Thus Zo Bell and Upham (1944) were able to isolate several species of the genus *Bacillus* from the marine mud around the Pacific coast.

Only very few studies have been performed on the occurrence of spore-forming heterotrophic microflora in the offshore and estuarine waters of Cochin Backwater (Gore, 1971, 1972, 1979) and that too on aerobic non-spore formers. The present investigation on aerobic spore formers, that is on *Bacillus genera*, was motivated by the increasing importance of this genus to fishing industry and public health as well as by the variability and diversity of morphological colony formation which have always confronted the microbiologists and taxonomists.

For general classification of *Bacillus* strains the following literature were referred. Besides those already mentioned by Gibson (1938), Smith *et al.* (1952) and Breed *et al.* (1957) also included were those of Wilson and Miles (1964), Cowan and Steel (1965), Jepsen (1960) and Wolf and Barker (1968).

Material and Methods:

The sediments were first partially dried at room temperature to induce sporulation of vegetative cells of the spore-forming bacteria. Counts obtained by heating were less when compared to counts obtained after drying. An appropriate amount of sediment (1 g) rich in organic matter was partially dried in sterile weighing bottles. A suspension was prepared by dilution and 3 - 5 ml were transferred to small sterile test tubes which were placed in a rack in a water bath set at 80°C for 10 minutes.

Inoculations from a pasteurised suspension were made in the usual way by pour-plate method. The culture medium used for spore-forming species was a mixture of beef-peptone and sea water agar combined in a proportion of 1:1. The pH was maintained at 7.0 to 7.2. The inoculated medium was incubated at 25 - 30°C for 2 - 3 days and after which colonies were counted.

Different species of spore-formers yielded characteristic colonies on the medium used (Plates 1,2 & 3). A rough estimate of the number of bacterial species was made on the basis of morphology of the colonies. The plates were retained for additional 4 - 5 days at room temperature after estimating the total counts of heterotrophs.

Results:

Colonies of spore-formers most often observed belong to the following species.

1. *Bacillus megaterium* Bary: Smooth white butyrous, shiny colonies consisting of typical rod-shaped colonies, consisting of typical Gram-positive rod-shaped cells.

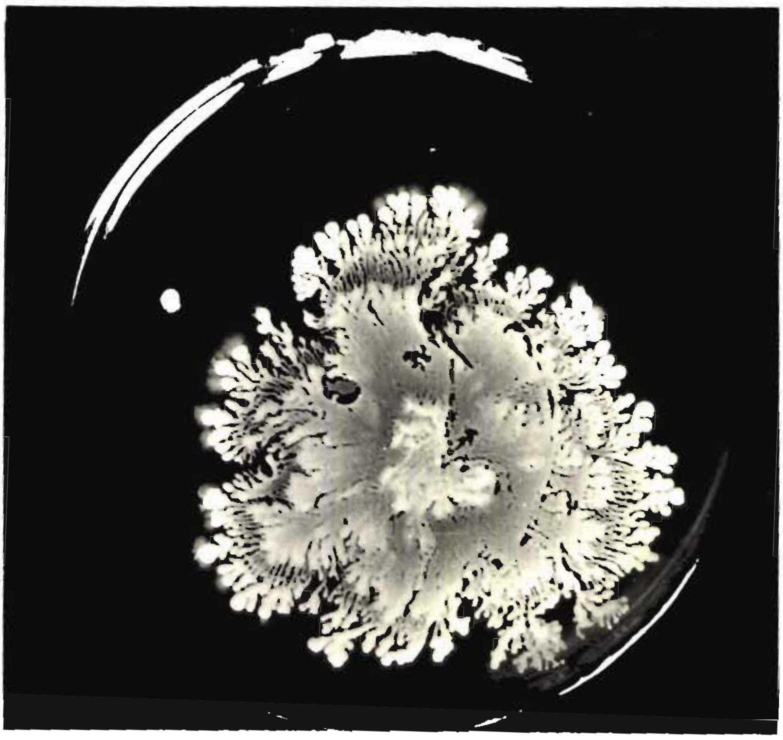
2. *Bacillus cereus*: sub sp. mycoides (Flüge) Smith *et al.* (spreading over the surface, with curving filaments radiating from the colony).

PLATE I.

A, B, C: *Bacillus cereus subsp. mycoides* (Flugge) Smith *et al.*

Morphological Characters:

Spreading over the surface, with curving filaments radiating from the colony.



A

B

C

PLATE II.

A & B : *Bacillus cereus* Frankland and Frankland.

Morphological Characters:

Flat colonies with flat center, weakly wavy edges, and a powdery surface.

C & D : *Bacillus idosus* Burchard

Morphological Characters:

Dry, lusterless colonies, laminated and wrinkled.

A

B

C

D

PLATE III. *Bacillus intricatus* Migula

Morphological Characters:

Widely spreading, whitish colonies, flat mycelium like, in growing into the agar, containing filaments with numerous septa.

A - Showing colony appearance on 7th day of incubation.

B - Showing colony appearance on 12th day of incubation (8.2 cm dia.).

A

B

3. _Bacillus mucosus_: Zimmermann: mucoid semitransparent resembling drops of pastes.

4. _Bacillus agglomeratus_ (Migula): small greyish round colonies.

5. _Bacillus cartilagineus_: Krasilinikov: Thick, round compact colonies which can be lifted from the agar in their entirety.

6. _Bacillus cereus_: Frankland and Frankland: Flat colonies with flat center, weakly wavy edges and a powdery surface.

7. _Bacillus idosus_ Burchard: Dry lusterless colonies, laminated finely wrinkled.

8. _Bacillus intricatus_ Migula: Widely spreading, whitish colonies, flat, mycelium like, ingrowing into the agar, containing filaments with numerous septa.

All the _Bacillus_ strains included in this investigation were collected from sediments of the different estuarine stations of Cochin Backwater during 1974-75.

36 random colonies with different morphological appearance was isolated and was maintained on slants of stock culture agar for doing further bio-chemical tests. The tests and percentage occurrence of positive reactions were given in the Table 20.

TABLE 20. Bio-chemical characteristics of Bacillus strains.

Test	Result	% Positive
Grams-strain	Gram + long rods	100.0
Motility	+	96.9
Nitrate reduction	+	82.6
Citrate utilisation (christensen)	+	91.8
Indole	-	0
Voges-Proskauer	+	89.8
Urease (Christensen)	-	2.6
Gelatin liquifaction	+	100.0
Casein hydrolysis	+	100.0
Starch hydrolysis	+	88.9
Acid from: Glucose	+	100.0
Lactose	-	8.0
Sucrose	+	95.0
Mannitol	-	60.0
Maltose	+	10.0

+ = positive in 80-100% of strains tested.

- = negative in 80-100% of strains tested.

Characters of the isolates:

Spore-forming rods, motile forming chainlets. The spores were oval-cylindrical and not strictly localised. Gram-positive, growth in fluid media was classified as

a) Clearing with sediment

b) Uniform turbidity.

Formation of pigments were estimated from growth on nutrient agar. In sea water agar they gave a finely wrinkled, dry streak with mat surface. In Zo Bell's 2216, colonies are found as a mat with folded surface and two types of colonies were found. One is transparent and difficult to remove from the agar with an even margin, others more dense with villi on the margin.

In sea water agar with 1% glucose, growth occurred on the surface as wrinkled whitish film. The agar around the colonies turned brown or black.

Fermentation of sugars:

Some 4 strains fermented the sugars like glucose, sucrose, and mannitol in 6 days and all the 12 strains fermented the sugars after the 8th day. Lactose and maltose were not fermented by most of the strains. Hydrolysis of starch was noted in 88.9% strains within 72 hrs. and Gelatin was liquefied by almost all the strains.

82.4% of the strains reduced nitrate. Growth in sodium chloride (5% and 10%) were found satisfactory.

Hugh and Leifson's results were oxidative and fermentative and urease activity of the strains was comparatively negative forming only 2.6% of the total strains. Indole production was completely absent but 100% casein hydrolysis was seen among these isolates. Citrate was utilised by 91.8% of the isolates and 89.8% were positive for Vogesproskaur reaction.

For the present investigation, *Bacillus* sp. isolated only from sediments were included. Totally 36 isolates were selected based on their morphological appearance and their bio-chemical activities were studied. Wood (1953) described a pink *Bacillus subtilis* like organism frequently in some East Australian estuarine sediments. Cinnamon coloured bacillus was isolated in plenty in the present investigation. Hilen (1923) recorded seven species of *Bacillus* from fouled surface from marine environment. Apart from *Mycoplana* and *Staphylococcus* both non-pigmented, a pink bacillus with central spores were isolated from low leaching rate copper and from mercury but not from high leaching-rate copper paint. These cultures were resistant to copper and mercury and there was some correlation between the resistance to the two metals. Subcultures on copper and mercury agars showed increased tolerance, suggesting that to some extent resistance is selective. Venkataraman and Sreenivasan (1955 b,c) found nine *Bacillus* spp. some pigmented from marine environments in Calicut area and from fresh shark. Only eight species of *Bacillus* were found in the present investigation from the Backwater sediments.

Wood (1940) encountered 10% *Bacillus* spp. in spoiling fish from market and retail shop but in trawled fish only 9% *Bacillus* was isolated. Out of 72 bacterial strains isolated by Venkataraman and Sreenivasan (1954) from sea water, 40.3% were of *Bacillus* from Calicut seawater whereas 50.8% *Bacillus* were isolated from 65 bacterial strains isolated from mackerel. Wood (1940) isolated 37% of *Bacillus* from market surfaces, 35% from gut of fishes, 12% from sea water and market air, 9% from gills and slime of fish and 8% from tap water. Colwell isolated in Chesapeake Bay 6% *Bacillus* from 152 isolates isolated from the oysters caught from natural and controlled environments of Pacific coasts. Buck (1973) isolated 7% *Bacillus* from Connecticut river in the prethermal and thermal discharge from Connecticut Yankee Nuclear Plant. In Lake Macquarie Wood (1959) encountered the genus *Bacillus* with extreme proteolytic capacity 22% from the surface water, 39.5% from 1 metre from bottom and 45% from bottom sediments.

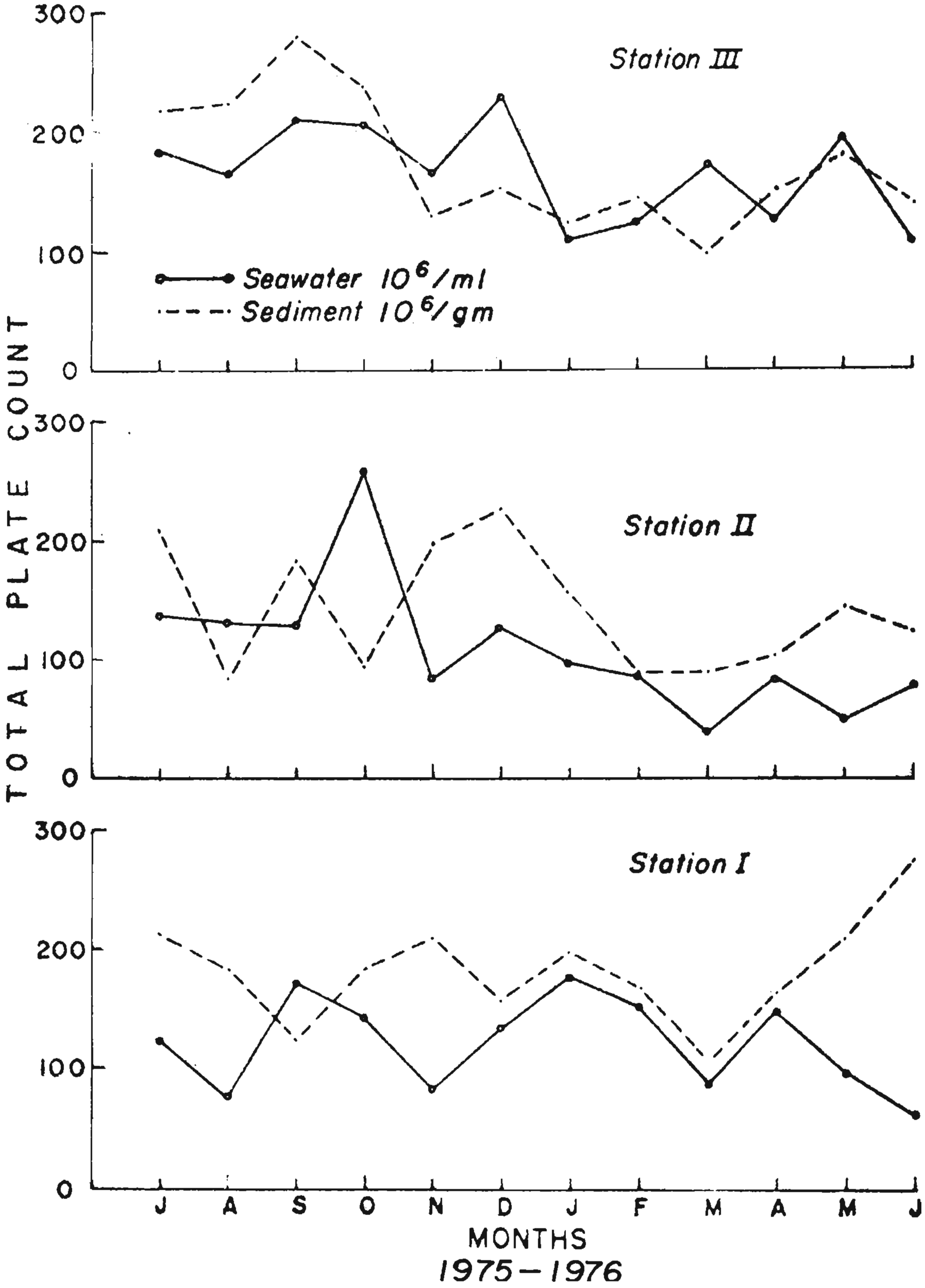
All these results show clearly that only sediment is exclusively the habitat of the genus *Bacillus* and it can be isolated in higher numbers from sea water where there is busy transport by ships or by dredging. In Calicut sea water studied by Venkataraman and Sreenivasan [1954 more *Bacillus* was found to be present. 10% *Bacillus* was isolated from Cochin Backwater sediments and 2.6% of the total isolates from sea water turned to be *Bacillus* spp. in the p st Litchfield (1966) found that *Bacillus* produced higher enzyme yields when protein was suspended in medium than when it was dissolved in the medium. *Bacillus* spp. isolated from Cochin Backwater sediments were biochemically active. Proteins like gelatin, casein were actively utilised by the isolated strains (Table 20). Naturally occurring refractory compounds are generally considered to include the less readily degraded components of plant and animal residues such as cellulose, chitin, pectin, lignin and humus. Cycling of refractory materials in the aquatic environment can be considered to take place chiefly at the water-sediment interface. The surface sediments of the aquatic ecosystem act as a boundary between the circulating dynamic medium primarily dominated by the properties of water and its solutes and the structurally more stable medium the sediment, with properties much like soil. This boundary area was found to be the site of intense microbial activity in most natural waters. A variety of bacteria including *Bacillus* was isolated from surface sediments in large numbers relative to the overlying water column. The mud water interface contain most of the organic matter sedimented from the surface water. Observations of Barber (1968) and others indicate that particulate matter in deep waters below the photic zone comprises humus like material which are slowly degraded by *Bacillus* strains.

In conclusion, the presence of 1% *Bacillus* strains in the sediments of Cochin Backwater suggests that cycling of refractory organic compounds does occur in this estuary mainly with the help of spore-forming microbes like *Bacillus*. However, the data are fragmentary and the pattern of true 'cycles' of these refractory organic compounds and the bacteria connected with it remain to be studied in detail.

Distribution and composition of heterotrophic bacterial flora during 1975-76:

The total heterotrophic counts from all water and sediment samples in a given month, were averaged from fortnightly values and presented in Fig. 22. The highest count was encountered in October, 1975 in Station II (296 x 10^6/ml) and the lowest in March, 1976 (36 x 10^6/ml) in the same station near the Cochin harbour. The area was predominated by marine conditions in most of the times eventhough this station was situated in the estuarine area.

Fig. 22. Showing total plate count (No. x 10^6) in water and sediment in the area of study during 1975-76.


300
200
100
0
Station III
Seawater 10⁶/ml
Sediment 10⁶/gm
300
200
100
0
Station II
300
200
100
0
Station I
TOTAL PLATE COUNT
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975 – 1976

In sediment, highest value of heterotrophic aerobes were obtained in June, 1976 in Station I, near the sewage outlet (274×10^6/ml) and the lowest was encountered in August, 1975 in Station II (84×10^6/ml) situated in marine environment. An important aspect in assessing the water quality of an estuarine area zoned for fishing activities may be given by the bacteriology of sediment. The sediment deposits provided a stable index of the general quality of the overlying water, particularly in this estuary, where there is great variability in the bacterial quality of water, due to tidal flow, underwater currents, fresh water run-off and sewage and drainage outflow.

One relevant segment of marine bacterial ecology needing investigation is that of seasonal population fluctuations. The seasonal variations of heterotrophs in sea water, was meagre, whereas in sediments it was prominent during monsoon (Station I) and postmonsoon months (Stations II and III).

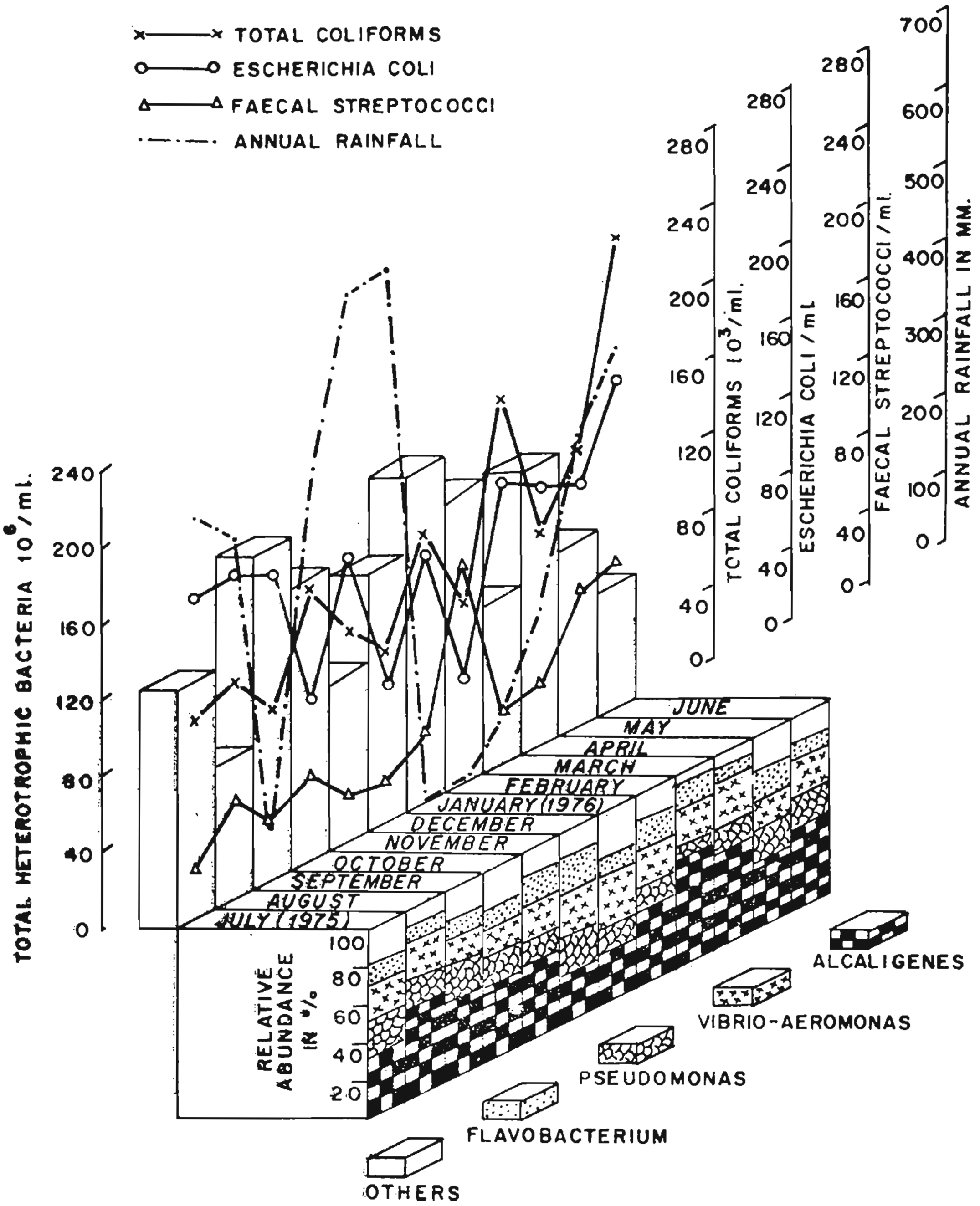
The total counts in monsoon months were less when compared to other two seasons. The decrease in heterotrophic microbial activity during monsoon months is probably due to a reduction in the availability of organic nutrients due to dilution rather than reduced water temperature.

Heterotrophic populations were found to be highest in mud and the numbers remain relatively constant at all stations in spite of the variations in environmental parameters. The number of bacteria varied according to the site of sampling and seasons and ranged between 30 and 300 colonies in the 10^{-6} dilution.

Altogether, 282 pure strains were maintained after isolation for further identification and for a brief study physiologically. The morphological and physiological characteristics of the isolates are summarised in the Fig. 20 b. Almost all of the isolates (270, 96.%) were asporogenous gram-negative rods usually pleomorphic. Gram-positive bacteria isolated were only 4.%. Motile bacteria were more abundant (78.2%) than non-motile bacteria and 220 isolates were motile when grown in semi-solid agar. Gelatinolytic activity was found to be more than starch hydrolysis and the genus <u>Alcaligenes</u> was found to be very active in the proteolytic process.

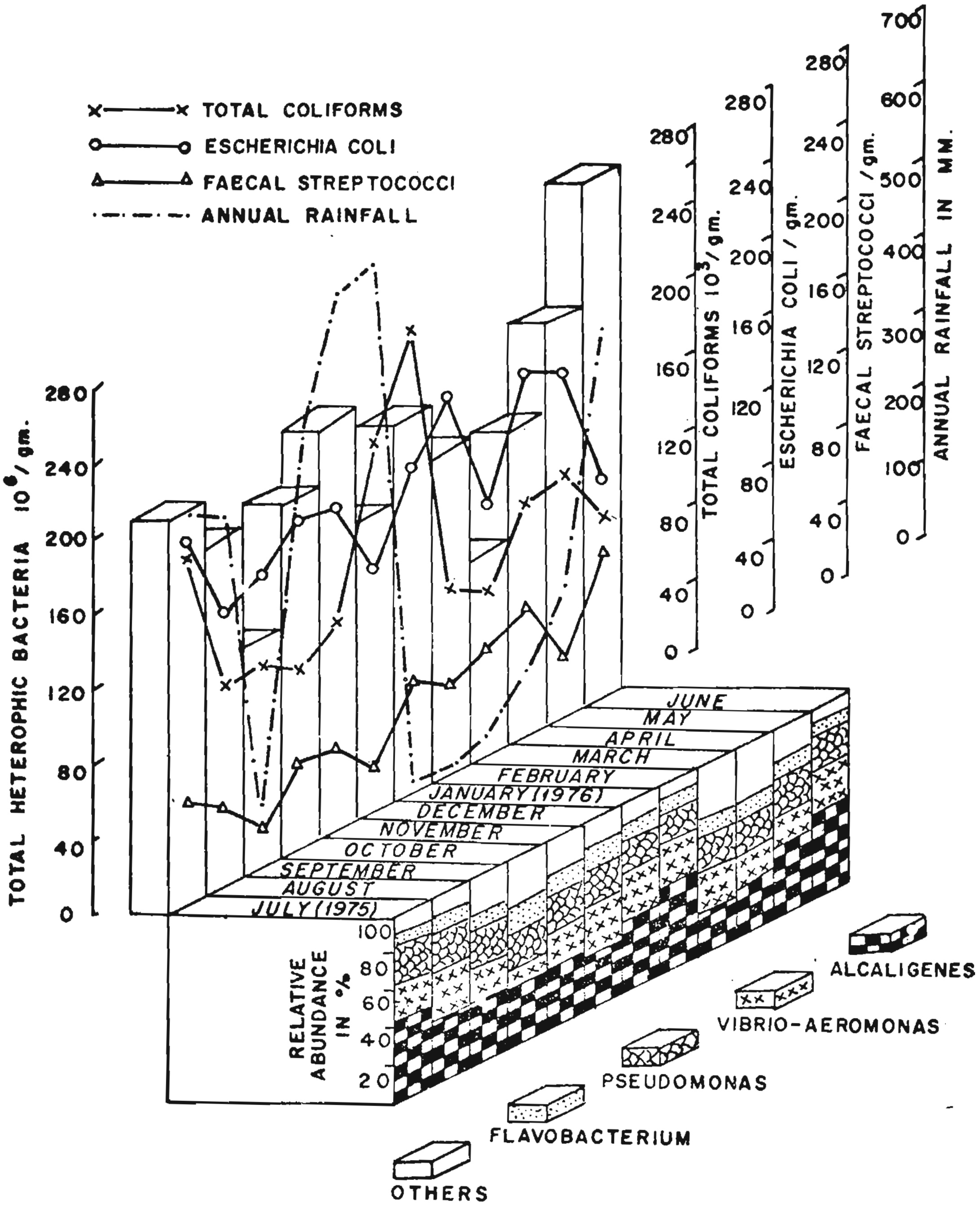
The relative abundance of four dominant genera in percentage isolated from sea water and sediment in each station are illustrated in Figs. 23a-f. Approximately 90-95 bacterial strains were isolated from each station from both water and sediments and maintained in the laboratory for further identification, during 1975-76.

Fig. 23 a. Showing total plate count, relative percentage of predominant genera, Total coliforms, *Escherichia coli* and Faecal streptococci in Station I in surface water together with rainfall data for Cochin AP during 1975-76.



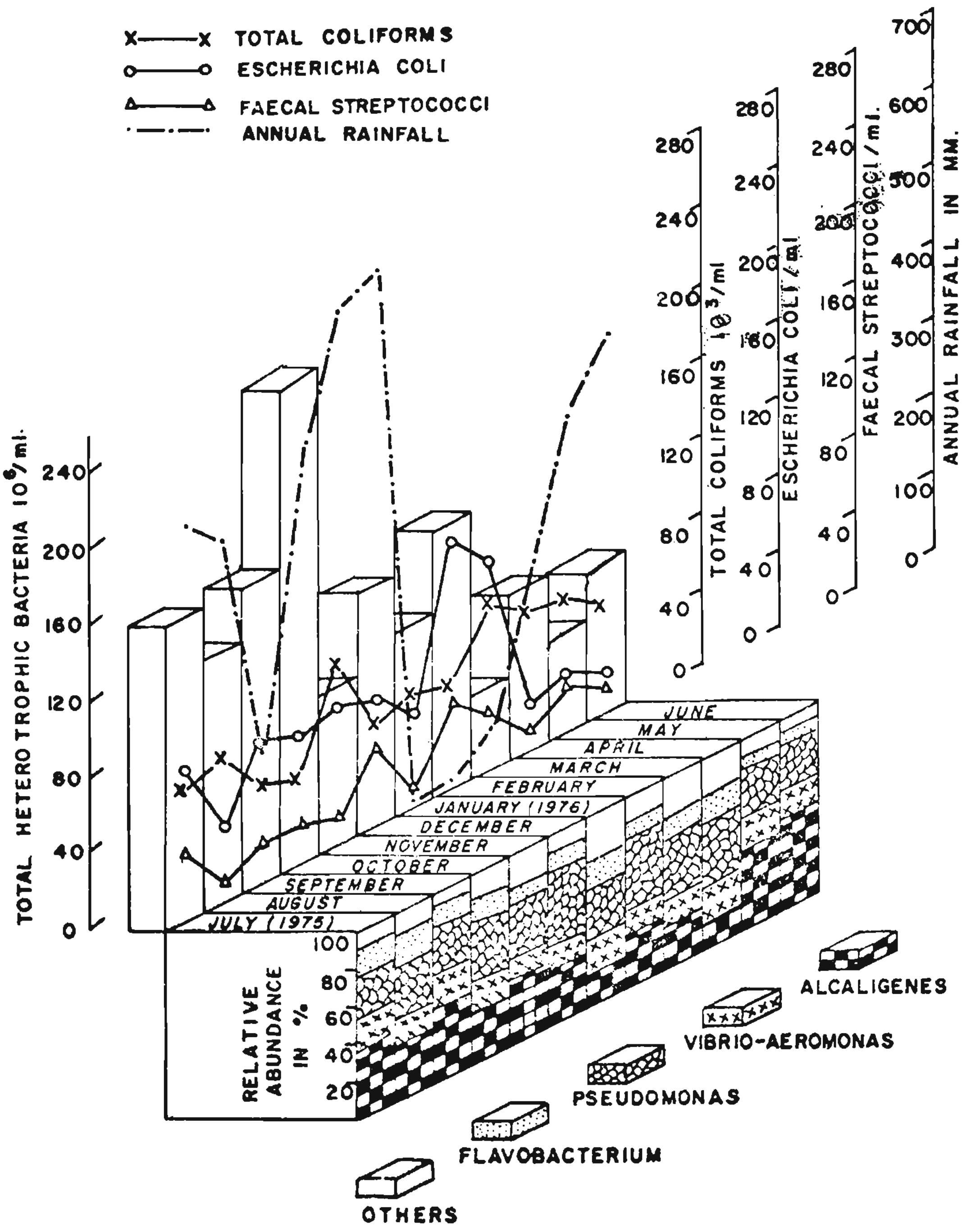
TOTAL COLIFORMS
ESCHERICHIA COLI
FAECAL STREPTOCOCCI
ANNUAL RAINFALL
TOTAL HETEROTROPHIC BACTERIA 10^6/ml.
0
40
80
120
160
200
240
TOTAL COLIFORMS 10^3/ml.
0
40
80
120
160
200
240
280
ESCHERICHIA COLI / ml
0
40
80
120
160
200
240
280
FAECAL STREPTOCOCCI / ml.
0
40
80
120
160
200
240
280
ANNUAL RAINFALL IN MM.
0
100
200
300
400
500
600
700
JULY (1975)
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1976)
FEBRUARY
MARCH
APRIL
MAY
JUNE
RELATIVE ABUNDANCE IN %
20
40
60
80
100
ALCALIGENES
VIBRIO-AEROMONAS
PSEUDOMONAS
FLAVOBACTERIUM
OTHERS

Fig. 23 b. Showing total plate count, relative percentage of predominant genera, Total coliforms, *Escherichia coli* and Faecal streptococci in Station I sediment together with rainfall data for Cochin AP during 1975-76.



TOTAL COLIFORMS
ESCHERICHIA COLI
FAECAL STREPTOCOCCI
ANNUAL RAINFALL
TOTAL HETEROPHIC BACTERIA 10^6/gm.
0
40
80
120
160
200
240
280
TOTAL COLIFORMS 10^3/gm.
ESCHERICHIA COLI / gm.
FAECAL STREPTOCOCCI / gm.
ANNUAL RAINFALL IN MM.
0
100
200
300
400
500
600
700
JULY (1975)
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1976)
FEBRUARY
MARCH
APRIL
MAY
JUNE
RELATIVE ABUNDANCE IN %
20
40
60
80
100
ALCALIGENES
VIBRIO-AEROMONAS
PSEUDOMONAS
FLAVOBACTERIUM
OTHERS

Fig. 23 e. Showing total plate count, relative percentage of predominant genera, Total coliforms, *Escherichia coli* and Faecal streptococci in the surface water in Station II together with rainfall data for Cochin AP during the period 1975-76.



X——X TOTAL COLIFORMS
o——o ESCHERICHIA COLI
Δ——Δ FAECAL STREPTOCOCCI
·—·—· ANNUAL RAINFALL
TOTAL HETEROTROPHIC BACTERIA 10⁶/ml.
0
40
80
120
160
200
240
TOTAL COLIFORMS 10³/ml
0
40
80
120
160
200
240
280
ESCHERICHIA COLI /ml
0
40
80
120
160
200
240
280
FAECAL STREPTOCOCCI / ml.
0
40
80
120
160
200
240
280
ANNUAL RAINFALL IN MM.
0
100
200
300
400
500
600
700
JULY (1975)
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1976)
FEBRUARY
MARCH
APRIL
MAY
JUNE
RELATIVE ABUNDANCE IN %
20
40
60
80
100
ALCALIGENES
VIBRIO-AEROMONAS
PSEUDOMONAS
FLAVOBACTERIUM
OTHERS

Fig. 23 d. Showing total plate count, relative percentage of predominant genera, Total coliforms, *Escherichia coli* and Faecal streptococci in the sediment in Station II together with rainfall data for Cochin AP during the period 1975-76.

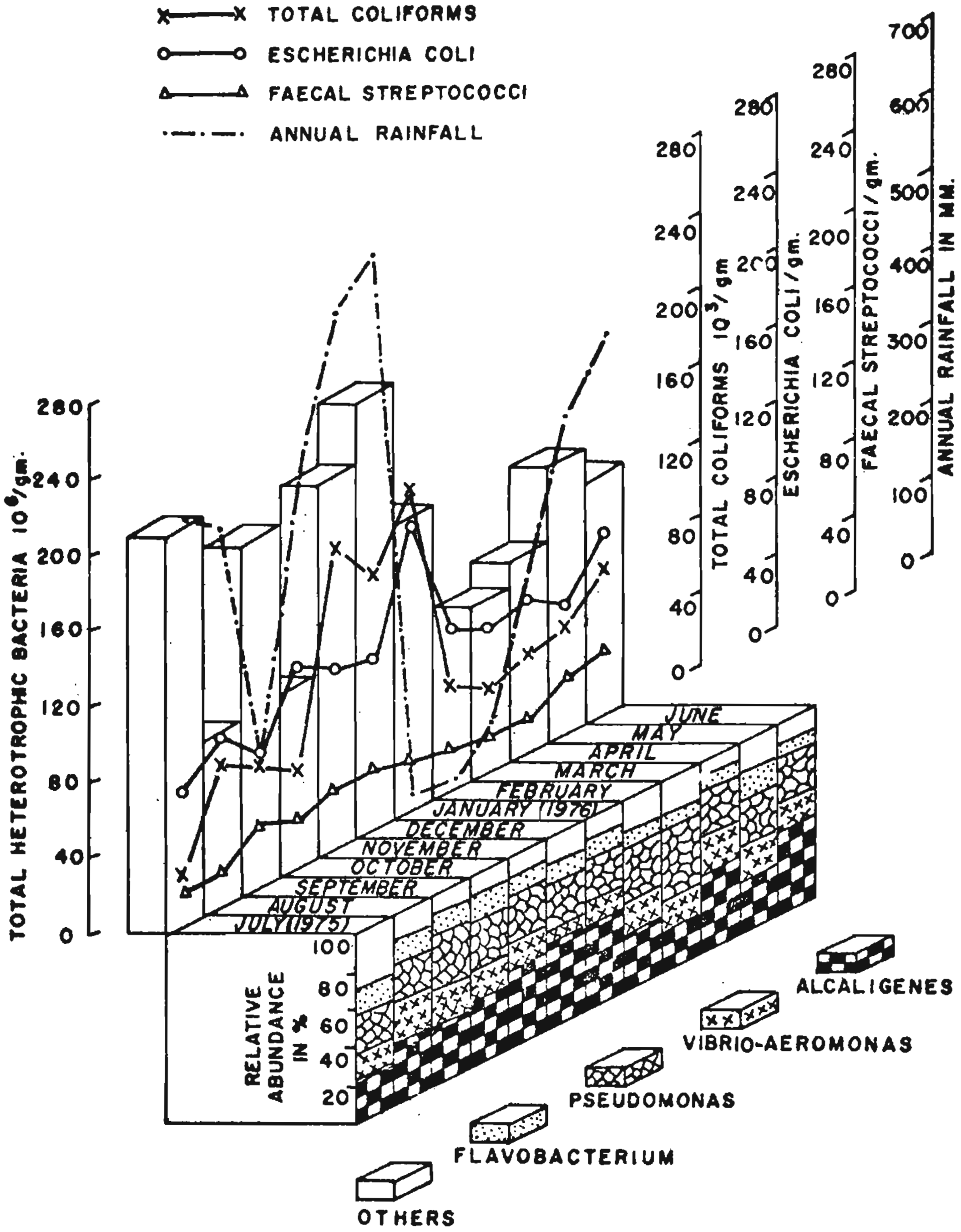

TOTAL COLIFORMS
ESCHERICHIA COLI
FAECAL STREPTOCOCCI
ANNUAL RAINFALL
TOTAL HETEROTROPHIC BACTERIA 10^6/gm.
0
40
80
120
160
200
240
280
TOTAL COLIFORMS 10^3/gm
ESCHERICHIA COLI/gm.
FAECAL STREPTOCOCCI/gm.
ANNUAL RAINFALL IN MM.
0
100
200
300
400
500
600
700
JULY (1975)
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1976)
FEBRUARY
MARCH
APRIL
MAY
JUNE
RELATIVE ABUNDANCE IN %
20
40
60
80
100
ALCALIGENES
VIBRIO-AEROMONAS
PSEUDOMONAS
FLAVOBACTERIUM
OTHERS

Fig. 23 c. Showing total plate count, relative percentage of predominant genera, Total coliforms, *Escherichia coli* and Faecal streptococci in surface water in Station III together with rainfall data for Cochin AP during the period 1975-76.

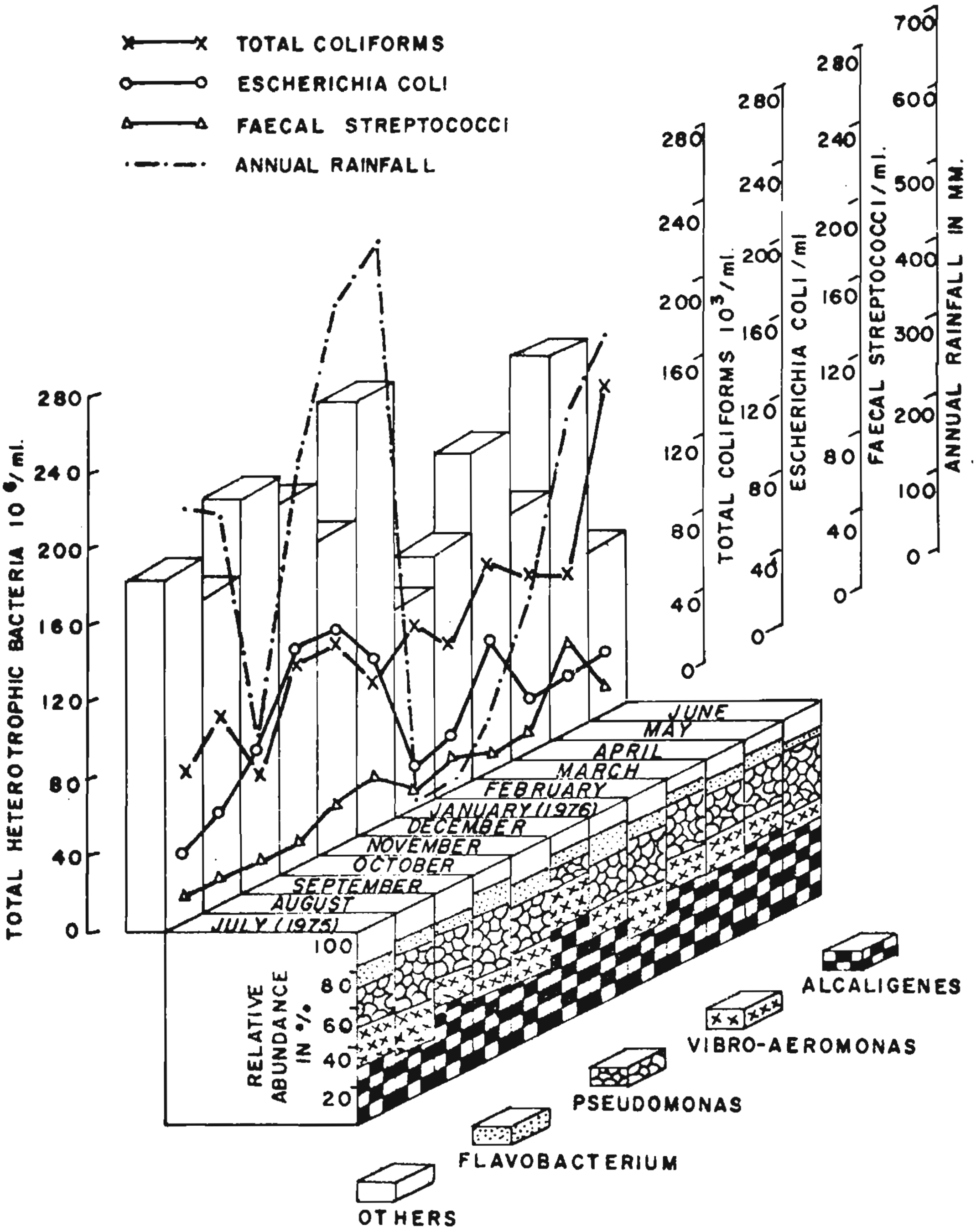
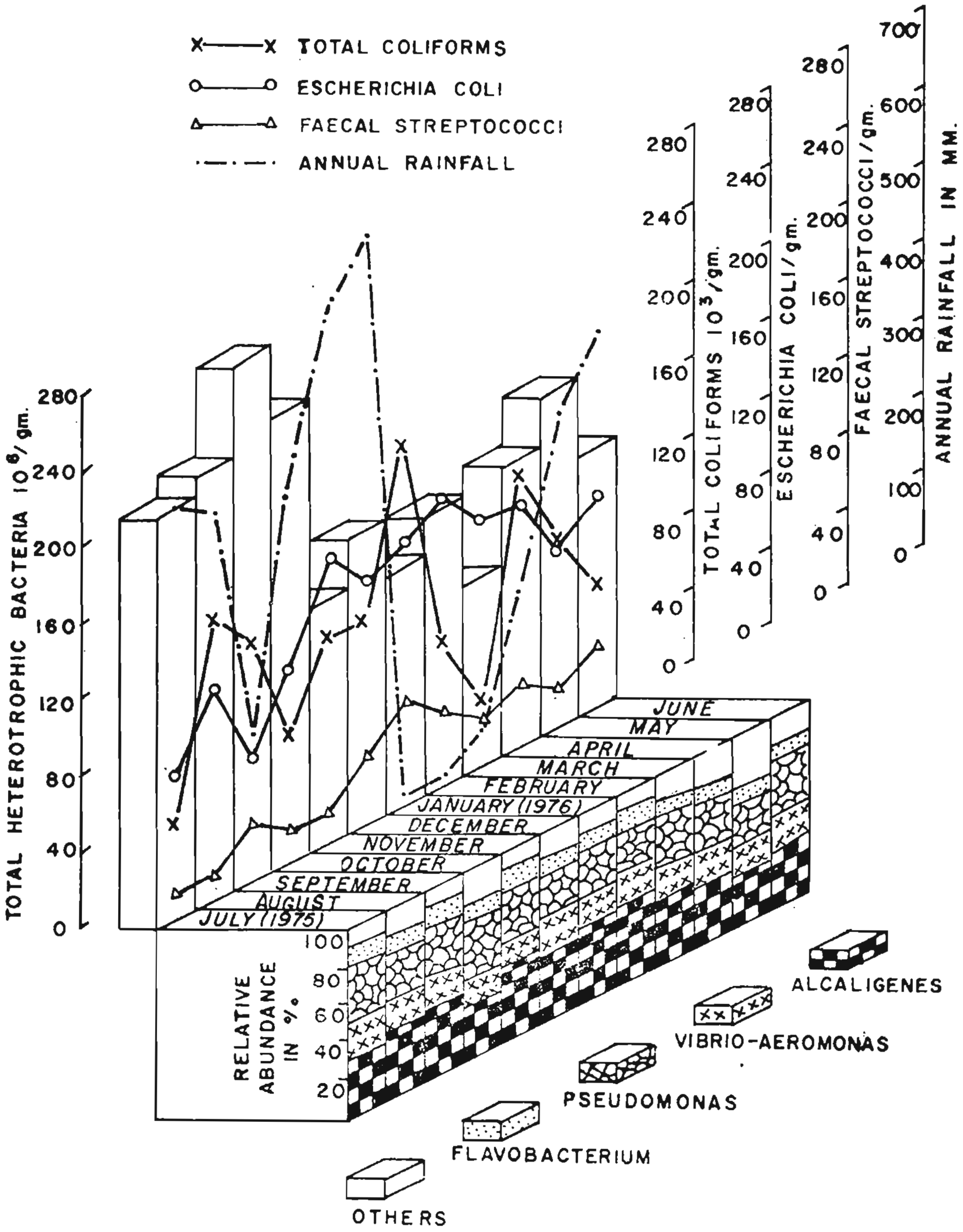

TOTAL COLIFORMS
ESCHERICHIA COLI
FAECAL STREPTOCOCCI
ANNUAL RAINFALL
TOTAL HETEROTROPHIC BACTERIA 10^6/ml.
0
40
80
120
160
200
240
280
TOTAL COLIFORMS 10^3/ml.
ESCHERICHIA COLI /ml
FAECAL STREPTOCOCCI /ml.
ANNUAL RAINFALL IN MM.
0
100
200
300
400
500
600
700
JULY (1975)
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1976)
FEBRUARY
MARCH
APRIL
MAY
JUNE
RELATIVE ABUNDANCE IN %
20
40
60
80
100
ALCALIGENES
VIBRO-AEROMONAS
PSEUDOMONAS
FLAVOBACTERIUM
OTHERS

Fig. 23 f. Showing total plate count, relative percentage of genera, Total coliforms, *Escherichia coli* and Faecal streptococci in sediment in Station III together with rainfall data for Cochin AP during the period 1975-76.


TOTAL COLIFORMS
ESCHERICHIA COLI
FAECAL STREPTOCOCCI
ANNUAL RAINFALL
TOTAL HETEROTROPHIC BACTERIA 10^6/gm.
0
40
80
120
160
200
240
280
TOTAL COLIFORMS 10^3/gm.
ESCHERICHIA COLI/gm.
FAECAL STREPTOCOCCI/gm.
ANNUAL RAINFALL IN MM.
100
200
300
400
500
600
700
JULY (1975)
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1976)
FEBRUARY
MARCH
APRIL
MAY
JUNE
RELATIVE ABUNDANCE IN %
20
40
60
80
100
ALCALIGENES
VIBRIO-AEROMONAS
PSEUDOMONAS
FLAVOBACTERIUM
OTHERS

Alcaligenes:

In sea water, *Alcaligenes* exceeded 40% of the total heterotrophs in 8 of the 12 months in Station I i.e. near the sewage (Fig.23 a). In sediment only in May and August, *Alcaligenes* exceeded 40% of the total (Fig. 23 b) and almost half of the isolates produced indol. In marine conditions i.e. in Station II, *Alcaligenes* was dominant only in the monsoon months (May, June, 1975) in sea water, whereas it was encountered above 40% in all the post-monsoon months in sediment. Apart from this in April and June, 1976 also *Alcaligenes* was isolated above 40% in sediment. In brackish water environment *Alcaligenes* exceeded well above 38% in the months of December, March, April, May and June which showed their preference of pH (7.0 to 7.9) and other chemical factors in this station. *Alcaligenes* was predominant in all the seasons showing their capacity to adapt themselves in marine environment.

Pseudomonas:

The next important group encountered was *Pseudomonas*. Isolates producing greenish fluorescent pigment was totally absent. Venkataraman and Sreenivasan (1955) isolated 2 fluorescent pigmented *Pseudomonas* which did not grow at 37°C, but grew slowly at 28-30°C. They also found only the non-fluorescent *Pseudomonas* were marine forms and the two fluorescent ones which grew in fresh water media with equal facility must be of fresh water origin. Since they isolated this greenish fluorescent Pseudomonas more frequently from green mussels, the fluorescent one must be considered as non-marine types. Generally the distribution of Pseudomonas was abundant in sea water when compared to sediment.

Near the sewage outlet (Station I) Pseudomonas exceeded 30% of the total in January, when lowest rainfall was recorded, which showed that these isolates were indigenous flora and not an exotic one (Fig. 23 a). 90% of the isolates were powerful denitrifiers, producing gaseous nitrogen from nitrates, but negligible fermentative activity was seen.

In Station II, Pseudomonas was recorded more than 35% in February and April, 1976 (Fig. 23 c), and the distribution had an inverse correlation with rainfall. In Station III, Pseudomonas formed 38% in sea water in the month of January, 1976 (Fig. 23 e).

In sediments, Pseudomonas was encountered less, and near the sewage outlet (Station I), the percentage of Pseudomonas exceeded 20% in 10 of the 12 months period of observation (Fig. 23 b). In marine conditions (Station II) Pseudomonas exceeded 30% of the total isolates during September 1975 and in March 1976 whereas in brackish water conditions (Station III) (Fig. 23 f) Pseudomonas were encountered 28% in December 1975 and 29% in April 1976. All these isolates were found to grow equally well on sea water as well as on fresh water media. Gelatinolytic activity was very meagre in this genera in the present study but Zo Bell and Upham (1944) found that some 60 strains of marine bacteria (members of *Pseudomonadales* and *Eubacteriales*) can carry out *Proteolysis* and 47 could liquefy gelatin. Starch breakdown (48%) was mainly carried out by *Bacillus* and *Pseudomonas* spp. and these genera were found to be rich in the exo-enzyme amylases.

Vibrio - Aeromonas:

In sea water 15 - 16% of the total isolates were found to be *Vibrio* - *Aeromonas* in the present study. In Station I, *Vibrio* - *Aeromonas* groups attained 16% during the months of July, August and May (Fig. 23 a). In Station II these two genera in the family *Vibrionaceae* ranged between 15 and 16% during September, October, November, January, April, May (Fig. 23 c). In brackish water the *Vibrio* - *Aeromonas* group exceeded this range in August, December 1975, January, February, March and April 1976 (Fig. 23 e). Differentiation between *Vibrio* and *Aeromonas* spp. was made on the basis of gas production in Barsiekow medium (glucose broth) containing a Durham's tube. Gas producers were assigned to the genus *Aeromonas*. Morphologically, most of the *Aeromonas* strains thus identified ranged from Cocco-bacilli to short, stout rods.

In sediments *Vibrio* - *Aeromonas* group formed 20% of the total isolates during August and December 1975, February and June 1976 in Station I near the sewage outlet (Fig.23b). In marine conditions (Stn. II) 20% recovery of this group was possible during April, March and May (Fig. 23 d). In brackish water, *Vibriomonas* was abundant during October 1975 and June 1976 (Fig. 23 f).

The percentage of *Vibrio* - *Aeromonas* did not show very great seasonal changes in the distribution of *Vibriomonas* in water and sediments. In biochemical activities much difference was not observed between *Vibrio* and *Aeromonas* except in the metabolism of glucose. Metabolism of glucose by *Aeromonas* was fermentative with copious production of gas. But the metabolism of *Vibrio* was oxidative.

Flavobacterium:

The pigmented *Flavobacterium* was the predominant genera found to produce orange, red/yellow pigments, the counts always being high in sea water than in sediments. Pigments are not soluble in the medium, the hue was produced often depending upon the nutrient medium. Red and rose pigments were evident next, belonging to *Micrococcus* and *Bacillus* respectively. *Flavobacterium* strains failed to grow in fresh water media and may be considered to be marine according to Zo Bell (1946). They exhibited very poor saccharolytic tendencies as noted in the case of "truly marine" bacteria. *Flavobacterium* strains were inert, never acting on sugars or on proteins and this confirm their unimportant role in the spoilage of fish (Castell and Mapplebeck, 1952). Sakata and Kakimoto (1979) found that non-pigmented bacteria were susceptible to visible light whereas pigmented bacteria were resistant to visible light, the reason why *Flavobacterium* was encountered more in surface water in the present study.

Marine bacterial flora in general are characterised by the presence of only a few types without much diversity with restricted distribution pattern, (Venkataraman and Sreenivasan, 1957) and the poverty of bacterial genera in marine environment may be due to the specific bactericidal nature of sea water.

Others:

This comprised, spore-forming Gram-positive rods *Bacillus* and Gram-positive *Micrococci*. Like the 3 predominant genera these 2 genera also exhibited very poor saccharolytic tendencies as in the case of '*truely marine*' bacteria. But none of the *Bacillus* and *Micrococci* were 'marine' forms in the above sense as well as in their Gram-positive nature.

Venkataraman and Sreenivasan (1957) after working in the offshore sea water of the west coast isolated the following genera, *Bacillus*, *Micrococcus*, *Sarcina*, *Flavobacterium*, *Achromobacter*, *Bacterium*, *Cornebacterium* and *Alcaligenes*. In the present investigation *Sarcina*, *Bacterium* and *Cornebacterium* were completely absent, instead of *Pseudomonas*, *Vibrio* and *Aeromonas* were encountered in the decreasing order of abundance.

Velankar (1955, 1957) attempted to classify the bacterial strains isolated by him into 4 different groups based on their nature of Gram-staining and sugar fermentation for convenience of discussion. Table 21 illustrates comparison of bacterial genera isolated in the present study with the description of characters of marine strains by Velankar (1955).

All the 7 genera identified fit very well into the description of characters of the isolates and exhibited all the physiological reactions.

TABLE 21. Comparison of bacterial genera isolated during 1975-76 with description of characters of marine strains by Velankar (1955).

Genera isolated in the present study.	Description of characters by Velankar (1955)
Alcaligenes *Vibrio* *Aeromonas*	Gram-negative, non-sporing, achromic rods, which produced acid from one or more sugars.
Pseudomonas	Gram-negative non-sporing, achromic rods, which cannot ferment any of the sugars.
Flavobacterium	Gram-negative non sporing, chromogenic rods.
Bacillus	Gram-positive spore-forming rods.
Micrococci	Gram-positive cocci and a few other organisms.

Analysis of variance test:

To test whether there is any significant difference in the bacterial counts between stations, months and regions, data were analysed statistically using the analysis of variance technique. For the purpose of analysis, the bacterial counts were converted to their log values after adding 1 to all observations wherever necessary. The observations from the analysis (Table 22) showed that the total plate count has shown significant difference between stations ($P < 0.01$) and between regions ($P < 0.01$). Station III was having significantly higher counts in bottom water and in surface sea water. The variation between months were not significant at 5% level. Also, none of the first order interactions was significant at 5% level.

Product-moment correlation coefficient:

The matrix of correlation framed, for each station, for surface and bottom water bacteria during 1975-76 were presented in Tables 23 to 25 for sea water and 26 & 27 for sediments. In sea water, in Station I, no correlation was seen between any of the chemical and microbiological parameters with total heterotrophic counts (Table 23). In Station II, significant ($P < 0.01$) (Table 24) negative correlation was seen with temperature, which showed the

TABLE 22. Analysis of variance of logarithm of total heterotrophic bacterial counts (1975-76).

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	Variance ratio (F)
Total heterotrophic bacteria	2.1883	71	-	-
Between stations	0.3063	2	0.1532	6.96**
Between regions	0.2279	1	0.2279	10.36**
Between months	0.5316	11	0.0483	2.20
Between stations x regions	0.1026	2	0.0513	2.33
Between stations x months	0.3318	22	0.0151	0.69
Between regions x months	0.2051	11	0.0186	0.85
Error	0.4835	22	0.0220	-

* Significant at 5% level ($P < 0.05$)

** Significant at 1% level ($P < 0.01$)

TABLE 23. Product moment correlation coefficients of all measured parameters in sea water (1975-76) - Station I.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	SO_3-Si	Org. Carbon	Org. Nitrogen	TPC	Coliforms	*E.coli*	*S.faecalis*	*S.sonnei*	Faecal index
Temp.	1.00													
Salinity	0.38	1.00												
O_2	-0.36	-0.68[a]	1.00											
PO_4-P	0.37	-0.27	0.03	1.00										
NO_3-N	0.50	0.69[a]	-0.51	-0.15	1.00									
SO_3-Si	-0.15	-0.76[b]	0.22	0.28	-0.52	1.00								
Org. Carbon	0.97[a]	0.13	-0.30	0.69[a]	0.05	0.04	1.00							
Org. Nitrogen	-0.48	-0.32	0.21	0.26	-0.62[a]	0.30	-0.02	1.00						
TPC	-0.38	0.27	-0.05	-0.48	-0.11	-0.33	-0.28	0.06	1.00					
Coliforms	0.10	0.16	-0.24	0.43	0.27	0.06	0.37	0.26	-0.05	1.00				
E.coli	0.23	-0.37	0.21	0.25	-0.15	0.43	0.32	-0.20	-0.00	0.25	1.00			
S. faecalis	-0.05	0.23	-0.22	0.04	0.22	-0.20	0.12	0.16	-0.01	0.14	-0.33	1.00		
S.sonnei	-0.08	-0.19	-0.20	0.02	-0.07	0.28	0.33	-0.16	-0.13	-0.15	0.23	-0.19	1.00	
Faecal index	0.09	-0.40	0.42	0.15	-0.19	0.09	-0.06	-0.08	-0.05	-0.25	0.26	-0.73[b]	0.27	1.00

true phychrophilic nature of the marine bacteria isolated from Cochin harbour area. Moreover, this type of inverse association analysis may indicate groups of bacteria particularly useful for comparative physiological ecology. Although, both normal and inverse forms of association analysis may be ecologically meaningful as separate analysis, it may be of interest to examine the extent to which these saprophytic heterotrophs are tied up with their habitat.

In Station III, significant ($P < 0.05$) positive correlation was seen between total heterotrophic bacteria and *Escherichia coli* (Table 25). Whenever *E. coli* or faecal coliforms were found above the permitted level, usually total heterotrophic bacteria were also found in high numbers. In many cases, the total heterotrophs themselves are considered to be an important index to determine the hygienic quality of the environment.

In sediment:

In sediment, no correlation was seen between any of the chemical and microbiological parameters with total heterotrophic bacteria in sediments in all the stations (Tables 26 to 28).

The significance of marine sediments as reservoir of heterotrophic bacteria and other micro-organisms has not been realised until recently. The bacterial population

TABLE 24. Product moment correlation coefficients of all measured parameters in sea water (1975-76) - Station II.

Para-meters	Temp.	Sal.	O$_2$	PO$_4$-P	NO$_3$-N	SO$_3$-Si	Org. Carbon	Org. Nitrogen	TPC	Coli-forms	*E.coli*	*S.fae-calis*	*S.sonnei*	Faecal index
Temp.	1.00													
Salinity	0.36	1.00												
O$_2$	0.43	0.36	1.00											
PO$_4$-P	-0.00	0.12	-0.08	1.00										
NO$_3$-N	0.32	-0.02	-0.14	-0.26	1.00									
SO$_3$-Si	-0.16	-0.23	-0.81^b	0.01	0.99^a	1.00								
Org. Carbon	0.35	0.20	-0.11	0.71^b	-0.00	0.10	1.00							
Org. Nitrogen	0.94	0.30	0.05	0.47	-0.13	0.09	0.31	1.00						
TPC	-0.77^b	-0.18	-0.34	0.37	-0.43	0.18	0.10	-0.38	1.00					
Coliforms	0.42	0.03	0.03	-0.08	0.12	-0.26	-0.02	0.18	-0.96	1.00				
E.coli	0.03	-0.10	0.23	0.34	-0.03	-0.45	0.17	0.16	0.05	-0.05	1.00			
S.fae-calis	0.08	-0.27	0.22	0.06	0.10	-0.16	-0.13	-0.02	0.00	-0.36	0.66^a	1.00		
S.sonnei	-0.11	0.18	0.22	0.13	0.17	-0.01	-0.29	0.01	-0.05	0.00	-0.06	0.28	1.00	
Faecal index	-0.03	0 .21	-0.20	0.14	-0.33	-0.11	0.09	0.23	-0.01	0.33	0.09	-0.55	-0.40	1.00

TABLE 25. Product moment correlation coefficients of all measured parameters in sea water (1975-76) - Station III.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	SO_3-Si	Org. Carbon	Org. Nitrogen	TPC	Coliforms	*E.coli*	*S.faecalis*	*S.sonnei*	Faecal index
Temp.	1.00													
Salinity	0.24	1.00												
O_2	-0.04	0.37	1.00											
PO_4-P	0.24	-0.40	-0.06	1.00										
NO_3-N	0.14	0.07	-0.23	-0.13	1.00									
SO_3-Si	-0.30	-0.16	-0.66^a	-0.07	0.17	1.00								
Org. Carbon	-0.26	0.11	-0.50	0.11	0.24	0.52	1.00							
Org. Nitrogen	-0.02	-0.11	-0.08	-0.21	0.00	0.25	-0.12	1.00						
TPC	-0.11	-0.29	0.11	0.53	-0.01	0.18	0.11	-0.03	1.00					
Coliforms	-0.18	0.10	-0.41	-0.51	-0.08	0.59^a	0.25	0.48	-0.52	1.00				
E.coli	-0.07	-0.08	0.18	0.42	-0.55	0.21	0.14	0.09	0.61^a	0.00	1.00			
S.faecalis	-0.14	0.11	0.24	-0.11	0.56	0.00	0.23	-0.20	0.43	-0.17	0.03	1.00		
S.sonnei	0.39	0.19	-0.23	0.16	0.31	-0.13	-0.37	-0.19	0.09	-0.47	-0.37	0.20	1.00	
Faecal index	0.25	0.24	-0.24	-0.05	-0.16	0.40	0.21	0.61^a	0.23	0.33	0.42	-0.18	-0.09	1.00

TABLE 26. Product moment correlation coefficients of all measured parameters in sediments (1975-76) - Station I.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	SO_3-Si	Org. Carbon	Org. Nitrogen	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. sonnei*	Faecal index
Temp.	1.00													
Salinity	0.97	1.00												
O_2	-0.53	-0.51	1.00											
PO_4-P	0.71^b	0.50	-0.65^a	1.00										
NO_3-N	0.66^a	0.79^b	-0.35	0.99^a	1.00									
SO_3-Si	-0.01	-0.55	0.19	0.01	-0.35	1.00								
Org. Carbon	-0.08	-0.62^a	0.18	-0.08	-0.25	0.73^b	1.00							
Org. Nitrogen	-0.01	-0.26	0.50	-0.30	-0.04	-0.12	-0.02	1.00						
TPC	-0.31	-0.16	0.01	0.05	-0.31	0.08	-0.23	-0.32	1.00					
Coliforms	-0.26	-0.13	0.21	-0.12	-0.21	-0.35	-0.13	0.03	0.12	1.00				
E. coli	0.03	0.07	-0.41	0.19	0.19	-0.16	0.13	-0.10	0.00	0.03	1.00			
S. faecalis	-0.12	0.43	-0.55	0.13	0.05	-0.19	-0.50	-0.73^b	0.43	-0.09	0.06	1.00		
S. sonnei	0.16	0.03	-0.17	0.43	0.20	0.23	0.08	-0.61^a	0.40	0.16	-0.10	0.30	1.00	
Faecal index	0.12	-0.33	0.42	0.03	0.01	0.33	0.59^a	0.46	-0.34	0.05	-0.04	-0.90^c	-0.17	1.00

TABLE 27. Product moment correlation coefficients of all measured parameters in sediments (1975-76) - Station II.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	SO_3-Si	Org. Carbon	Org. Nitrogen	TPC	Coliforms	*E. coli*	*S. faecalis*	*S. [illegible]*	Faecal index
Temp.	1.00													
Salinity	0.50	1.00												
O_2	0.47	0.39	1.00											
PO_4-P	0.12	0.12	-0.42	1.00										
NO_3-N	-0.30	-0.19	-0.70[a]	0.27	1.00									
SO_3-Si	-0.12	0.23	-0.29	0.63[a]	0.47	1.00								
Org. Carbon	0.06	0.33	0.16	0.31	-0.04	0.66[a]	1.00							
Org. Nitrogen	0.31	0.41	0.58[a]	-0.42	-0.40	-0.25	0.02	1.00						
TPC	-0.11	-0.29	-0.08	-0.04	-0.10	0.17	0.17	-0.09	1.00					
Coliforms	0.02	0.08	0.39	-0.59[a]	-0.52	-0.33	-0.25	0.35	0.44	1.00				
E. coli	-0.15	-0.15	0.20	-0.51	-0.30	-0.41	-0.58[a]	0.11	-0.07	0.65[a]	1.00			
S. faecalis	-0.30	0.25	-0.02	-0.03	-0.13	0.33	0.28	0.43	0.46	0.46	0.10	1.00		
S. [illegible]	-0.68[a]	-0.29	-0.49	-0.06	0.34	-0.05	0.02	-0.34	-0.22	-0.45	-0.14	-0.06	1.00	
Faecal index	0.26	-0.17	0.15	-0.21	-0.05	-0.45	-0.52	-0.26	-0.50	-0.01	0.51	-0.77[b]	-0.10	1.00

TABLE 28. Product moment correlation coefficients of all measured parameters in sediments (1975-76) - Station III.

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	SO_3-Si	Org. Carbon	Org. Nitrogen	TFC	Coli-forms	*E.coli*	*S.faecalis*	*S.sonnei*	Faecal index
Temp.	1.00													
Salinity	0.24	1.00												
O_2	-0.10	-0.06	1.00											
PO_4-P	0.31	0.06	-0.34	1.00										
NO_3-N	0.09	-0.05	-0.18	0.58^a	1.00									
SO_3-Si	0.23	-0.00	-0.24	0.55	0.46	1.00								
Org. Carbon	-0.12	-0.03	0.02	0.51	0.36	0.26	1.00							
Org. Nitrogen	0.22	0.16	0.18	0.18	0.08	-0.08	0.45	1.00						
TFC	0.09	-0.49	-0.11	0.41	-0.07	0.15	0.49	0.20	1.00					
Coli-forms	0.04	0.19	0.05	0.07	-0.08	-0.42	-0.01	-0.18	-0.01	1.00				
E. coli	-0.34	0.21	0.34	-0.53	-0.18	-0.60^a	-0.27	-0.09	-0.83^c	0.08	1.00			
S.faecalis	-0.44	0.33	-0.14	-0.38	-0.16	-0.38	-0.13	-0.44	-0.34	0.31	0.22	1.00		
S. sonnei	-0.09	0.63^a	-0.03	-0.11	-0.12	-0.23	-0.39	0.05	-0.01	0.06	-0.26	0.17	1.00	
Faecal index	0.16	-0.26	0.29	-0.02	-0.00	-0.14	0.11	0.26	-0.18	0.06	0.40	-0.73^b	-0.33	1.00

of the sediments can influence the static and dynamic composition of the bacterial populations of sea surface water as well as other marine organisms found in the estuarine, inshore and offshore regions.

It can be assumed that the significant abundance of *Alcaligenes* populations found in the water column of this area are directly related to the *Alcaligenes* population in the sediment deposits in the same ecosystem. In the latter case *Alcaligenes* spp. comprised upto 40 percent and above of the total heterotrophic bacterial population. For a more complete understanding of these population interactions, additional studies of the behaviour of selected bacterial species associated with transport of watermass by underwater currents and tides must be done.

The predominance of the *Pseudomonadaceae* in coastal sea water and sediments has been reported by various investigators in several countries (Marchelano Brown, 1970; Altschuler and Riley, 1967; Zo Bell, 1946 and Wood, 1953) whereas Lovelace *et al.* (1968) reported the dominance of *Vibrionaceae* in *Crassostrea virginica*, as a commensal microbe in Chesapeake Bay and Simidu *et al.* (1980) also reported the predominance of *Vibrionaceae* in the heterotrophic bacterial flora of the sea water from the Nansei Shoto (Ryukyo Retto) area in Kuroshio current which runs between the Pacific Ocean and the east China Sea. The mechanisms resulting in the predominance of *Pseudomonadaceae* in coastal waters and sediments are not clear, but there is some evidence that suggest that nutrients derived by phytoplankton blooms and nutrients of animal origin, derived from zooplankton and fishes can accelerate the growth of *Alcaligenes* in sea water. In the Cochin Backwater large quantities of nutrients entering via several sewage and drainage canals, also support the growth of these abundant micro-organisms especially *Pseudomonas* covering the whole trophic levels. In this context, conditions for microbial growth in the Cochin Backwater are similar to those of Narragansett Bay (Rhode Island, USA), Kamogawa Bay (in Japan, 1962) and Long Island Sound (New York, USA), where predominance of *Pseudomonadaceae* has been documented and the occurrence of *Alcaligenes*, *Pseudomonas* and *Aeromonas* were reported.

An unequivocal relationship was not observed between the relative abundance of *Alcaligenes* spp. and members of the other bacterial genera with the chemical and hydrographic parameters examined in this study. The highest heterotrophic counts indicated the abundance of one or more predominant genera. Whenever, higher counts of total heterotrophs were observed at Station II in the inshore region that observation was coincided with that of lowest temperature and lowest salinity of the sea water and sediment samples analysed. The total heterotrophic counts were low in brackish water area at Station III whenever salinity and temperature was recorded high. The high salinity at the time of sampling (32.84‰ in sea water and 33.53‰ in sediments) (Fig. 7) suggests that inflow by underwater currents or high tide may have occurred at that time.

Although the data indicate that 3 of the 4 dominant genera (*Alcaligenes*, *Pseudomonas*, *Vibrio* spp.) may dominate the bacterial flora at different times of the year, Fig.20b shows that the biochemical activity is essentially uniform for the entire year. The result also, suggests, although the generic composition of the bacterial flora changes with season, there are no significant differences, in numbers of proteolytic and amylolytic, zymogenous bacterial flora. If the major ecological function of bacterial heterotrophs is the degradation of non-living organic matter then, at all times of the year, bacteria must be present which can metabolise the substrate present. It is interesting to note that, although certain bacterial genera are dominant at specific times of the year, the physiological activities of the bacterial population present at any one time are essentially constant.

A thorough analysis of the data concerning morphology, physiology and biochemical activity of micro-organisms has revealed that they are structurally and biochemically complex organisms. They may rapidly adapt themselves to different environmental conditions, because of their ability to form adaptive (induced) enzymes produced by the influence of new substrates of the surrounding environment. Consequently, the production of adaptive enzymes may cause a change in the character of metabolism and biological functions of the new variants which needs a further study.

SECTION II.

INDICATORS OF BACTERIAL POLLUTION ENCOUNTERED IN THE ESTUARINE AND MARINE ENVIRONMENT

II. INDICATORS OF BACTERIAL POLLUTION ENCOUNTERED IN THE ESTUARINE AND MARINE ENVIRONMENT:

The release of faecal pollution into Cochin Backwater can introduce a wide variety of intestinal pathogens. The primary habitat of *E. coli* or other coliforms is the intestinal tract of warm-blooded animals. With domestic sewage many pathogenic micro-organisms get into coastal waters and may give rise to epidemics. Added to these are great quantities of organic and inorganic nutrients which cause mass development of bacteria. The sewage has a characteristic microflora. The organisms found were mostly putrefying bacteria like *Pseudomonas*, *Proteus*, *Bacillus*, *Aerobacter* and pathogens like *Salmonella* spp., *Leptospira*, *E.E. coli*, *Francisella*, *Mycobacterium*, *Vibrio cholerae*, pathogenic amoebae, viruses and numerous bacteriophages. Relatively high is the proportion of coliform bacteria which is an important indicator of pollution of the water with faecal material of warm-blooded animals.

Human pathogenic bacteria cannot grow permanently in the brackish water or sea, but depending upon the kind of water and prevailing conditions various pathogens can survive for a period. During that period it may contaminate the fishes by adhering to gills, slime, fins and other parts of body of fishes. Possibility of a direct contamination of fishes with faeces is rather remote, if at all happens it does not often exceed 25 mg of faecal matter/10 kg of fish which in turn can deposit 100 enterobacteriaceae, 10 group D streptococci and a few clostridia in fish. But whatever may be the type of contamination when once, the organisms have entered into fishes in considerable numbers it is very difficult to get rid of them completely. Those fishes which are contaminated by the pathogenic or sewage bacteria spoil quickly soon after the catch. Moreover, these pathogens, whenever they are present in large number they survive freezing and cooking. So consumption of contaminated fishes is a source of infection.

Significance of faecal indicator organisms:

At one time when the detection and enumeration of pathogenic organisms like *Salmonella* and *Shigella* was a difficult task, the use of the socalled faecal indicator organisms like *E. coli* and Faecal streptococci was the only possible method of assessing the sanitary conditions of aquatic environment. The underlying principle was that if the indicator organisms are absent, it is possible that the pathogenic types may also be absent. The indicator organism originally suggested by Scardinger (1892) for this purpose was *Escherichia coli* first isolated by Escherich (1887) from the intestinal content of human beings and later on supported by many workers by isolating the same organism from human and animal faeces in considerable proportions. Another organism which is also universally approved as an indicator organism is faecal streptococci which is present in the stools of man and many warm-blooded animals.

But now there are many quick and reliable methods for the direct determination of these pathogenic organisms the question naturally arises whether these indicator organisms have any important role in modern hygiene. But because of the following reasons the validity of the test for pathogens is not universally accepted.

First, *Salmonella*, as a rule is so heterogenously distributed in the environment that a negative outcome of its detection has only limited significance.

Secondly, apart from the classical pathogens, of the genera *Salmonella* and *Shigella*, other organisms, may also spread through the environment. Many of these especially viruses and intestinal worms can be detected only with rather complicated methods beyond the scope of many microbiological laboratories.

Thirdly, the detection of *Salmonella* in the presence of an overwhelming majority of other bacteria in the sample is difficult.

Therefore, a test to detect *Salmonella* should not be interpreted to signify occurrence or absence of other pathogens. Since, pathogens could be present in polluted water during the absence of *Salmonella*, there is no complete assurance that the incidence of other water-borne diseases will correlate with *Salmonella* occurrence alone.

These points clearly indicate that indicator organisms have still a place in respect of faecal pollution significance, but questions are often raised to distinguish faecal contamination from human faeces from those of animals because these organisms are also present in the animal and bird excreta. But from the hygienic point of view, all sorts of faecal contamination are equally dangerous whether originated from man or animals. Perhaps, the estimated per capita output of these organisms/24 hr period is more from animal faeces than from human faeces. There is also authentic proof to the effect that pigs and birds are more frequent carriers of *Salmonella* than man. All these facts focus to the point that all sorts of faecal indicator bacteria in aquatic environment are equally objectionable irrespective of their source.

Factors judging the suitability of indicator organisms in the marine environment:

Total coliforms:

Coliform bacteria traditionally have been the bacteriological tool used to measure the occurrence and intensity of faecal contamination for marine pollution investigations. As defined in Standard Methods for the Examination of Water and Wastewater (1975) - "The coliform group includes all of the aerobic and facultative anaerobic, gram-negative, non-spore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C". From this definition, it becomes immediately apparent that this bacterial grouping is somewhat artificial in that it embodies a heterogenous collection of bacterial species, having only a few broad characteristics in common. Yet, for practical applications to marine pollution studies, this grouping of total coliform group has proved to be a workable arrangement.

The total coliform group merits consideration as an indicator of pollution because these bacteria are always present in the normal intestinal tract of humans and other warm-blooded animals and are eliminated in large numbers in faecal wastes. Thus the absence of total coliform bacteria is evidence of a bacteriologically safe water. Unfortunately, some strains included in the total coliform group, have a wide distribution in the environment but are not common in faecal material. Some coliforms surviving sewage chlorination may increase one or two logs within one or two days travel downstream. This phenomenon is known as after growth.

Other indicator systems have been proposed from time to time including certain pathogenic bacteria, anaerobic spore-formers, and total bacterial population, but for a variety of reasons these proposed indicator systems have been found to be unsatisfactory. However, recent investigation into faecal coliform sub-group of the total coliform bacteria has shown great promise for sharpening the bacteriological tools used to detect evidence of faecal pollution.

Escherichia coli:

It is a gram-negative, rod-shaped, non-spore-forming bacteria. Its presence in the marine environment is accepted as an indicator of faecal contamination as the primary habitat of *E. coli* is the intestinal tract of many warm-blooded animals. Coastal waters get contaminated with *E. coli* either by direct contact or by mixing up with terrestrial sewage. All fishes and shell-fishes will accumulate these indicators in their gills, fine slime and in the body surface. When once the organisms have entered into the fishes and shell fishes in considerable numbers it is very difficult to get rid of them completely. Even if the organisms are completely removed by some chemical treatment, the wholesomeness of the food, cannot be guaranteed as many of the viruses and intestinal worms which are comparatively resistant to such treatments will be present in the product in viable forms. Hence it is very essential to protect the potential marine environment from faecal contamination in order to control the quality of fish, shellfish, prawns and also other fishery products. Offshore water generally does not contain *E. coli*, whereas incidence of this organism is usually noted in nearshore waters.

Faecal streptococci:

They are gram-positive, non-spore-forming and non-motile cocci which are found in human and animal faeces in large numbers and hence their presence in marine environment has been well accepted as an indicator of faecal contamination. Just like *E. coli*, faecal streptococci are also absent in offshore waters.

Experiments have given clear indication that faecal streptococci are comparatively resistant to many adverse conditions. Moreover, faecal streptococci is also useful in determining the post process proliferation of faecal contamination in marine environment which cannot be detected by the use of much less resistant *E. coli*. These point to the superiority of faecal streptococci as an index of faecal contamination in the environment.

Staphylococcus aureus:

Food poisoning caused by *Staphylococcus aureus* is very common in fishery products. The causative organism is present, on human skin, in boils, carbuncles, ulcers, sweat, eargum, throat etc. It has been estimated that about 30% of the human beings are carriers of *Staphylococcus aureus*. Hence human element is an important factor in sanitation of coastal area. A few staphylococci may be harmless, but food poisoning outbreaks may happen in the fishery products if multiplication of these organisms happens in dangerous proportions. Eventhough, *Staphylococcus aureus* are destroyed in higher temperature during cooking of the fish, the toxin formed already can withstand 100°C for more than 3 hours and hence are present in the material and if this exceeds 4 µg/g (4 microgram) of the product food poisoning takes place. Vomitting, diarrhoea, general malaise, prostration etc are the general symptoms which start within 1-6 hours after consuming the infected food.

A valuable index of the sanitary conditions of the marine environment is the estimation of faecal coliforms, faecal streptococci, *Staphylococci*, and *Clostridium perfringens*. The presence of the latter indicates an earlier faecal contamination. Usually, the 'coliform group' are used to assess the quality of a variety of waters, eg. drinking water, effluents, marine beaches, fresh water beaches, and irrigation waters.

In sewage and drainage receiving waters as in Cochin Backwater all coliform organisms cannot be considered as indicators because most of them may not be faecal origin but belong to soil or plant origin. For that reason only *E. coli* type I is considered in the present study as a proper faecal indicator because only that group can be estimated quantitatively with an adequate precision.

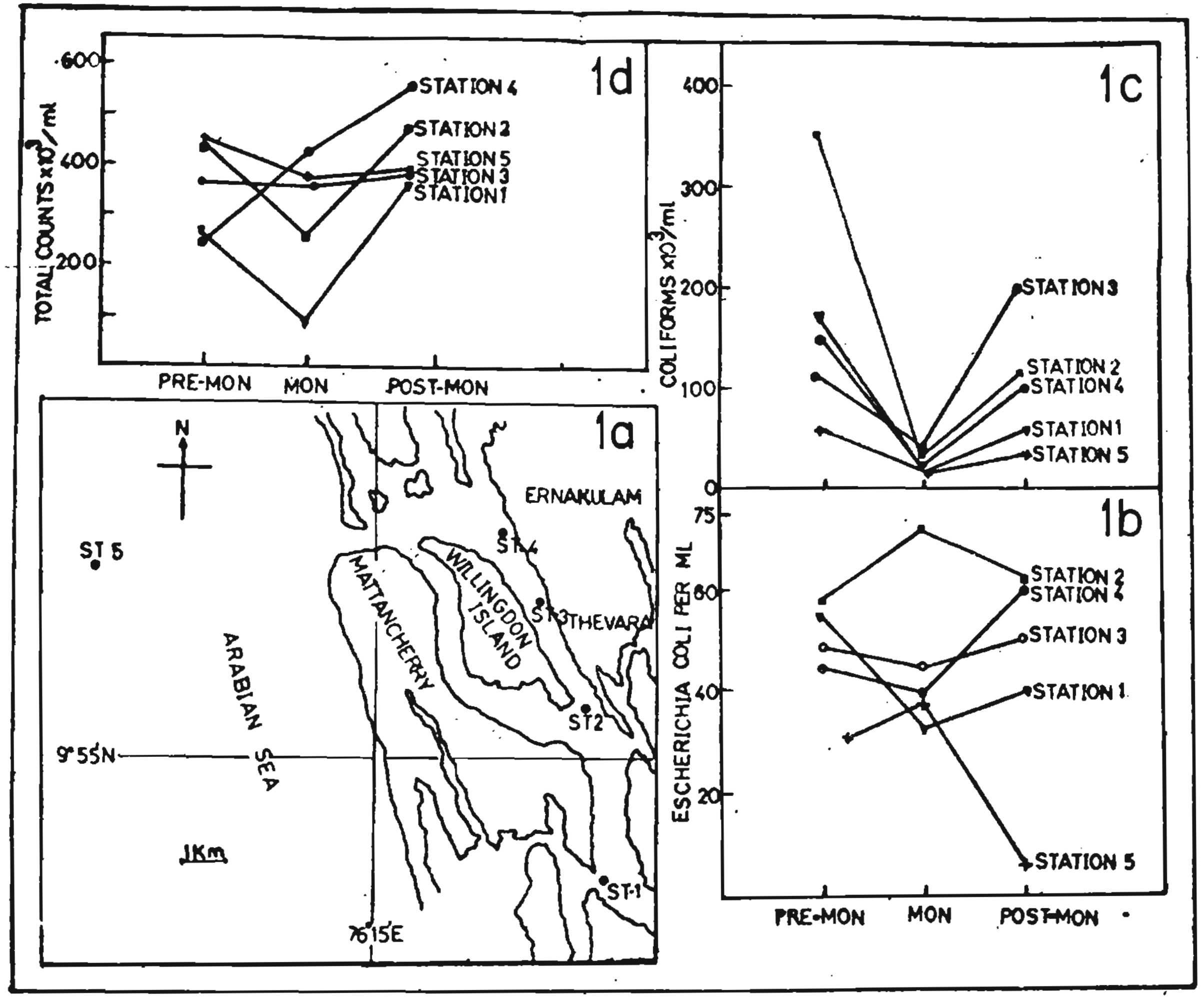
1972-73:

From the 5 stations fixed (Fig. 31a) for 1972-73 investigations in the marine and estuarine conditions, only water samples were analysed for total colifotms and *E. coli* during 1972-73 and the results are as follows:

Quantitative analysis:

Table 1 gives the counts of total bacteria, coliforms, *E. coli* and the data on dissolved oxygen, salinity and

Fig. 24. (1a) Map showing the sampling stations during 1972-73.
(1b) Seasonal distribution of *Escherichia coli*,
(1c) Seasonal distribution of Total coliforms,
(1d) Seasonal distribution of Total heterotrophs, -
in surface water in the area of study
during 1972-73.


1d
TOTAL COUNTS x10³/ml
600
400
200
PRE-MON
MON
POST-MON
STATION 4
STATION 2
STATION 5
STATION 3
STATION 1
1c
COLIFORMS x10³/ml
400
300
200
100
0
STATION 3
STATION 2
STATION 4
STATION 1
STATION 5
1b
ESCHERICHIA COLI PER ML
75
60
40
20
PRE-MON
MON
POST-MON
STATION 2
STATION 4
STATION 3
STATION 1
STATION 5
1a
N
ST 5
ARABIAN SEA
9°55'N
1Km
76°15'E
MATTANCHERRY
WILLINGDON ISLAND
ERNAKULAM
ST 4
ST 3
THEVARA
ST 2
ST-1

1972 – 1973

temperature for the period May 1972 to March 1973. The figures for each month are the averages of the values given by the fortnightly collections. Total coliform levels were always higher than faecal coliforms (*E. coli*) levels which is not surprising since total coliforms can originate from non-faecal sources such as plants and soils. The coliform counts fluctuated between 12 x 10^3/ml in October to 428 x 10^3/ml in March, the counts being minimal during the monsoon period for all the stations. Minimum fluctuations in the counts during the three seasons were observed in Station V. While the total heterotrophic counts remained more or less the same during the pre and post monsoon periods in Station II, the coliform counts were very high during the premonsoon period in the same station.

The *E. coli* counts ranged from 12/ml in February to 93/ml in March. In the case of *E. coli* while the counts were minimum during the monsoon period for Stations I, III and IV, for Stations II and V, the counts were maximum during this period. At Station V, the minimum count was recorded during the postmonsoon period and for Station II the abundance did not differ significantly during the pre- and post- monsoon periods. Thus, as there is no uniformity in the trends of occurrence and abundance in the different stations, which lead us to presume that the seasonal variations in the environment do not affect the abundance of

E. coli in the estuary. The degree of contamination of water is generally determined by the coli-index. The coli-index is the number of individuals of *E. coli* found in 1 litre of water. Water is considered to be of good quality if the coli-index is 2-3. The coli-index ranged from 6000-144000 in the month of September in Stations I and V respectively.

Seasonal occurrence of total heterotrophs total coliforms, *E. coli* in the three seasons is shown in Fig. 2+ 1b,1c,1d. In all the stations except the fourth, the minimum total counts was recorded during the monsoon period. This is a bit unusual because one would expect the number to be higher in monsoon months than any other season. The reduction in bacterial number during monsoon months may be probably due to reduction in the availability of organic nutrients due to dilution and reduced water temperature. Heterotrophic bacterial survival in estuarine water was strongly influenced by temperature and even if fresh water run-off was greater in monsoon months heterotrophic numbers were found to be less, because of their rapid die off owing to organic nutrients dilution or depletion.

In Stations III and V, the total counts did not show large fluctuations indicating that the bacterial flora are not subjected to large seasonal variations in abundance.

In Station IV, the minimum counts were observed during the premonsoon period, with an increasing trend from the premonsoon to the postmonsoon seasons. The abundance attained the maximum during the postmonsoon season for all the stations except Station V, where the difference between the lowest and the highest count was not large.

Total coliforms also followed the same pattern of seasonal distribution like heterotrophs (Fig. 24, 1c). Goyal *et al.* (1977) also found the number of total coliforms higher in winter months than in summer months.

The number of *E. coli* was always higher in the monsoon season (Fig. 24, 1b). In Station II, the counts were higher in all the 3 seasons. In the stations located in the marine environment (Station V) *E. coli* counts were lowest in the premonsoon and in postmonsoon seasons. In monsoon months, the counts exceeded the *E. coli* counts at Station I which is located near the drainage canals. It is very clear from the survival study of *E. coli* by Greenberg (1956), Carlucci and Pramer (1959) and Mitchell (1968) that die off of coliforms in marine waters is a rapid event that is controlled by variety of factors including toxicity due to high salt concentration, predation, competition by native microflora and limited nutrient supply. If the pollution is remote *E. coli* cannot survive more than 7 days in sea water to give higher counts at Station V. The higher counts of *E. coli* obtained in an offshore area in the present study established the fact that fresh faecal pollution has been taking place during the time of sampling from sewage and drainage and from ship's toilet. In addition, the heavy transport, fishing activity and dredging activity may resuspend the coliform flora that are present in the sediment into the water column which may be another cause of higher counts during monsoon months in the offshore area.

A comparison of the counts for the estuarine (Table 29 - Stations I - IV) and marine (Table 29 - Station V) environments showed that the environmental differences in temperature, salinity and dissolved oxygen do not largely affect the total counts. However, the coliform counts and the *E. coli* counts were found to be less at Station V.

The *E. coli* counts did not show any correlation with total coliform counts nor any seen between coliforms and total counts.

The lowest temperature was recorded during the monsoon period and the maximum during the premonsoon period. The monthly temperature values did not show wide variations. The total counts, coliform counts and *E. coli* counts did not show any correlation with the temperature values. Salinity was highly variable. During the SW monsoon period, owing to increased land drainage and resultant flood waters, it becomes as low as that in fresh water. The trends in the

TABLE 29. Total bacteria, coliforms and *E. coli* for Stations I-IV and V (1972-73).

Month	Bacterial counts (10^3/ml)		Colifirms (10^3/ml)		*E. coli* Type I /ml	
	Stations I-IV	Station V	Stations I-IV	Station V	Stations I-IV	Station V
May	420	90	22.7	17.0	24.5	20.0
June	403	732	27.2	nil	23.6	nil
July	139	68	29.0	16.0	25.5	nil
September	334	328	14.5	13.0	88.5	6.0
October	274	122	13.5	7.0	48.7	31.0
November	93	-	386.6	-	72.0	-
December	765	225	123.0	72.0	34.5	32.0
January	507	720	31.0	24.0	62.0	52.0
February	79	496	163.0	115.0	17.6	10.0
March	450	840	417.0	75.0	100.0	64.0

dissolved oxygen are somewhat similar to those in salinity. The bacterial counts (total counts, coliform counts and E. coli counts) did not show any linear relationship with salinity or dissolved oxygen.

A positive correlation was reported by Velankar (1955) in the inshore environment at Mandapam between total counts and coliforms. Iyer et al. (1974) found no correlation between coliforms and total counts (personal communication). In the present investigation also no correlation could be established between coliform counts and total counts.

Venkataraman and Sreenivasan (1954) reported that the offshore waters of the west coast off Tellicherry and off Calicut are devoid of coliform bacteria. Velankar (1955) also did not encounter any coliform bacteria in the offshore waters of Mandapam. According to Ketchum et al. (1949), the viability of coliform organisms is relatively less in sea water than in fresh water. The results of the present observations agree with this. However, the E. coli and other coliform counts as well as total counts are relatively low during the monsoon period, though the salinity is negligible. The dilution of the estuarine waters owing to heavy precipitation may be a causative factor. In contrast to these findings, Venkataraman and Sreenivasan (1955) found the peak of pollution immediately following the advent of the SW monsoon in the mussel beds at Korapuzha estuary, Calicut.

According to Wood (1965) absence of coliforms in an aquatic environment may be due to the fact that they do not occur in uncontaminated waters and even if they do so, they do not persist for long as the coliforms are sensitive indicator species. But Andre *et al.* (1967); Gallagher (1968); Geldreich *et al.* (1968) and Rudolphs (1950) studied the survival rate of coliforms and enterococci and has shown that coliform persists somewhat longer than some enteric pathogenic bacteria. Wherever coliforms are detected they signify the potential presence of other enteric pathogens in the same environment (Geldreich, 1966). Gore (1971) working along the Cochin Beach and beach sand reported that the coliforms represented a major flora, owing to the constant contamination by human excretion. In the present investigation it was found that both *E. coli* and other coliforms occurred in large numbers in the estuarine and marine environment of Cochin which themselves have epidemiological significance as causative agents of infectious gastro-enteritis. This probably indicates constant warm-blooded animals' faecal pollution through sewage, land drainage and through other source in this estuary. The preceding results indicate the necessity to take extra precautions to eliminate pathogenic organisms while processing fishery products in season when coli index is on the higher side, as fishes and prawn caught from polluted areas may invariably show high numbers of such organisms.

Occurrence and distribution of bacterial indicators during 1974-75:

Apart from the isolation and enumeration of total plate count, total coliforms and *Escherichia coli*, occurrence and distribution of faecal streptococci and staphylococci are also included during the period (1974-75 and 1975-76) of study as these indicators will assist in differentiating the source of pollution.

The population of total coliforms in the water column was found to vary between 12 x 10^3/ml in Station I in July 1974 and 210 x 10^3/ml at Station III in January 1975 (Fig. 25) and that of faecal coliforms was between 2/ml in Station V during February 1975 to 144/ml in December 1974 in Station IV (Fig. 26).

The concentration of faecal streptococci ranged from 2/ml in May 1974 at Stations IV and V to 69/ml in Station V during September 1974 (Fig. 27). The faecal index was worked out as suggested by Finstein (1972) and the faecal index ranged between 0.85 during November 1974 at Station I to 13.50 in June 1974 at Station III (Fig. 28). The ratio between faecal coliform and faecal streptococci

Fig. 25. Showing Total coliforms counts (No. x 10^3) of the water and sediment in the area of study during 1974-75.

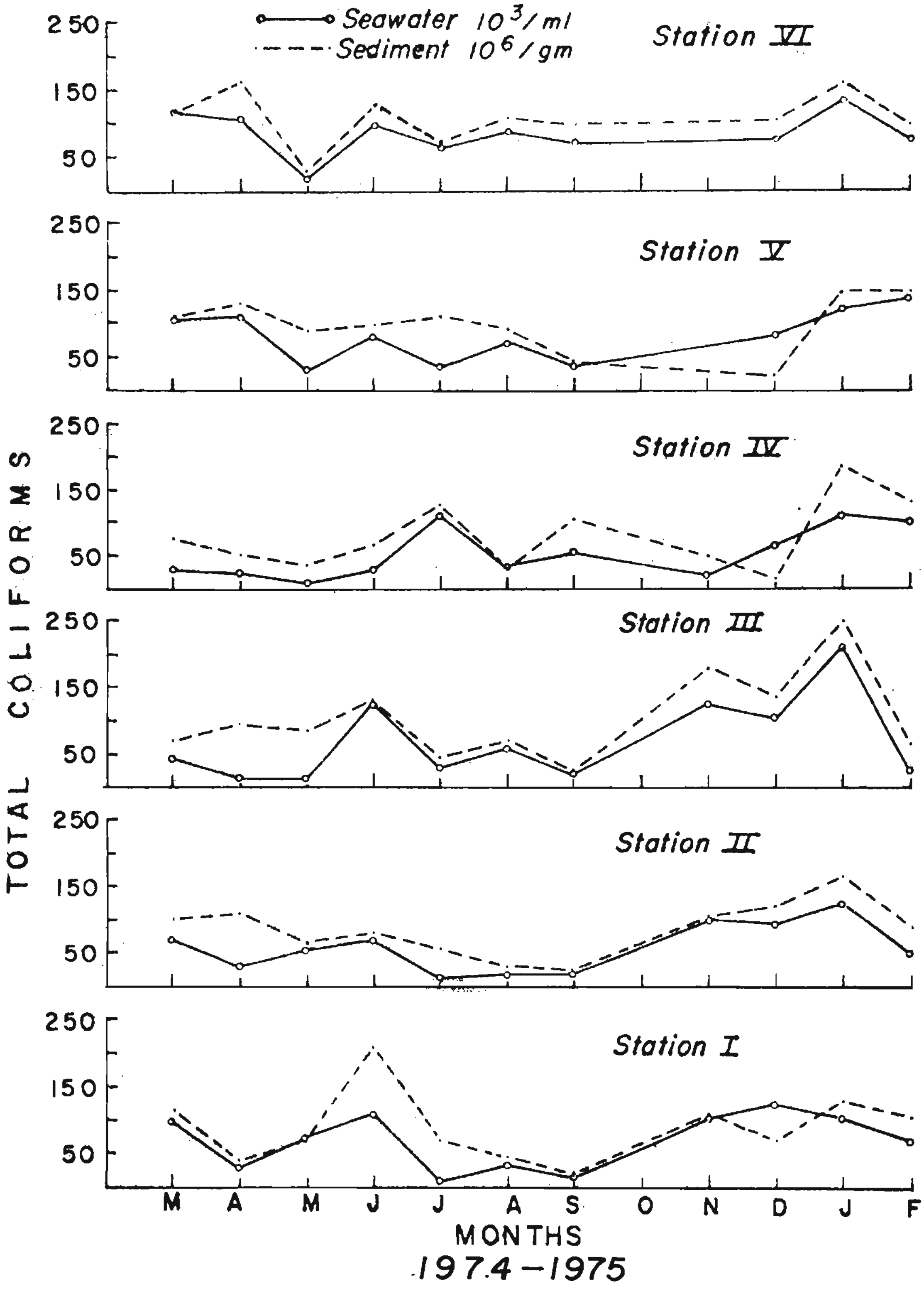

Seawater 10^3/ml
Sediment 10^6/gm
Station VI
Station V
Station IV
Station III
Station II
Station I
250
150
50
TOTAL COLIFORMS
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974-1975

Fig. 26. Showing *Escherichia coli* counts (No./ml) in water and sediment in the area of study during 1974-75.

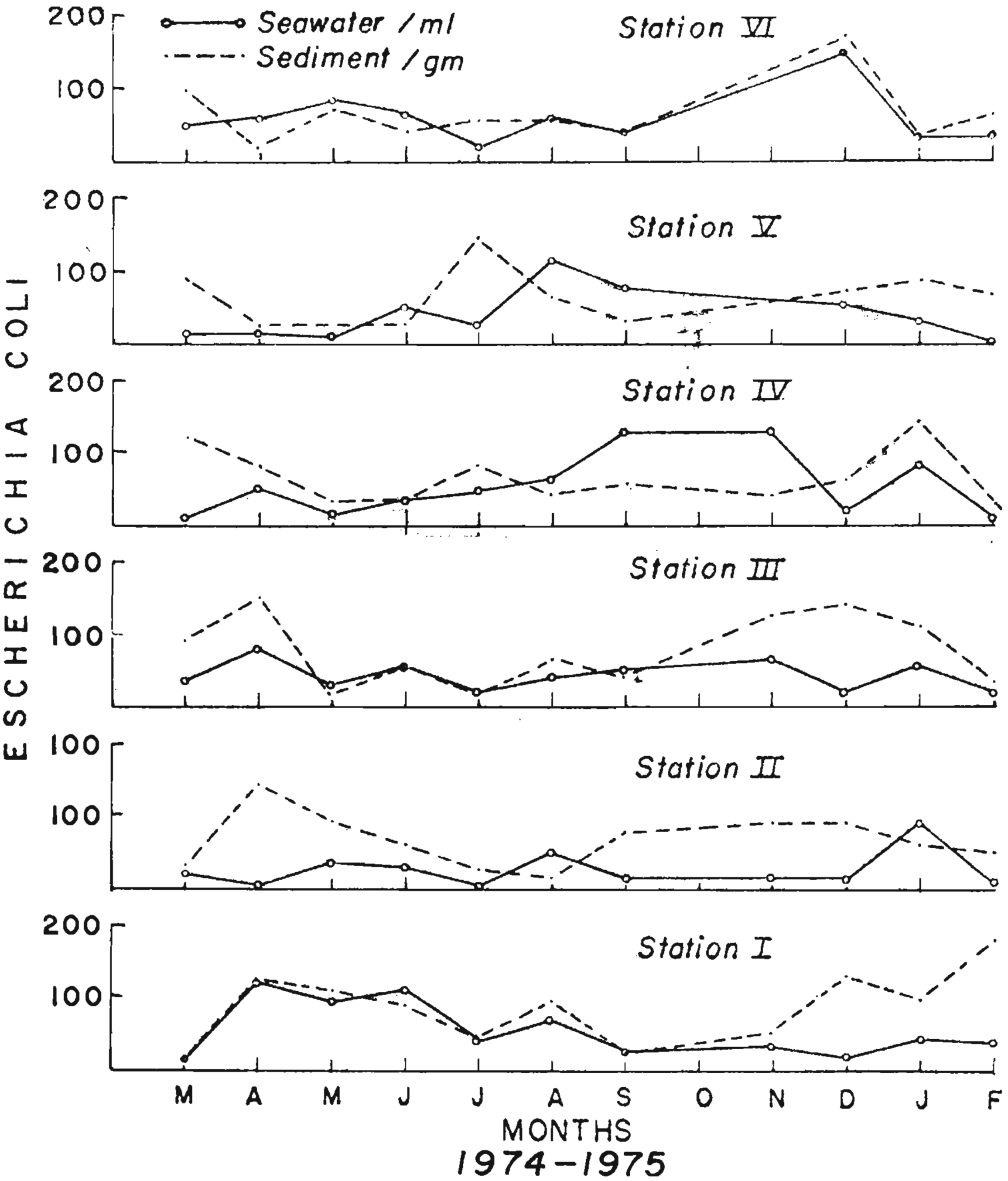

Seawater /ml
Sediment /gm
Station VI
Station V
Station IV
Station III
Station II
Station I
200
100
ESCHERICHIA COLI
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974–1975

Fig. 27. Showing *Streptococcus faecalis* counts (No./ml) in water and sediment in the area of study during 1974-75.

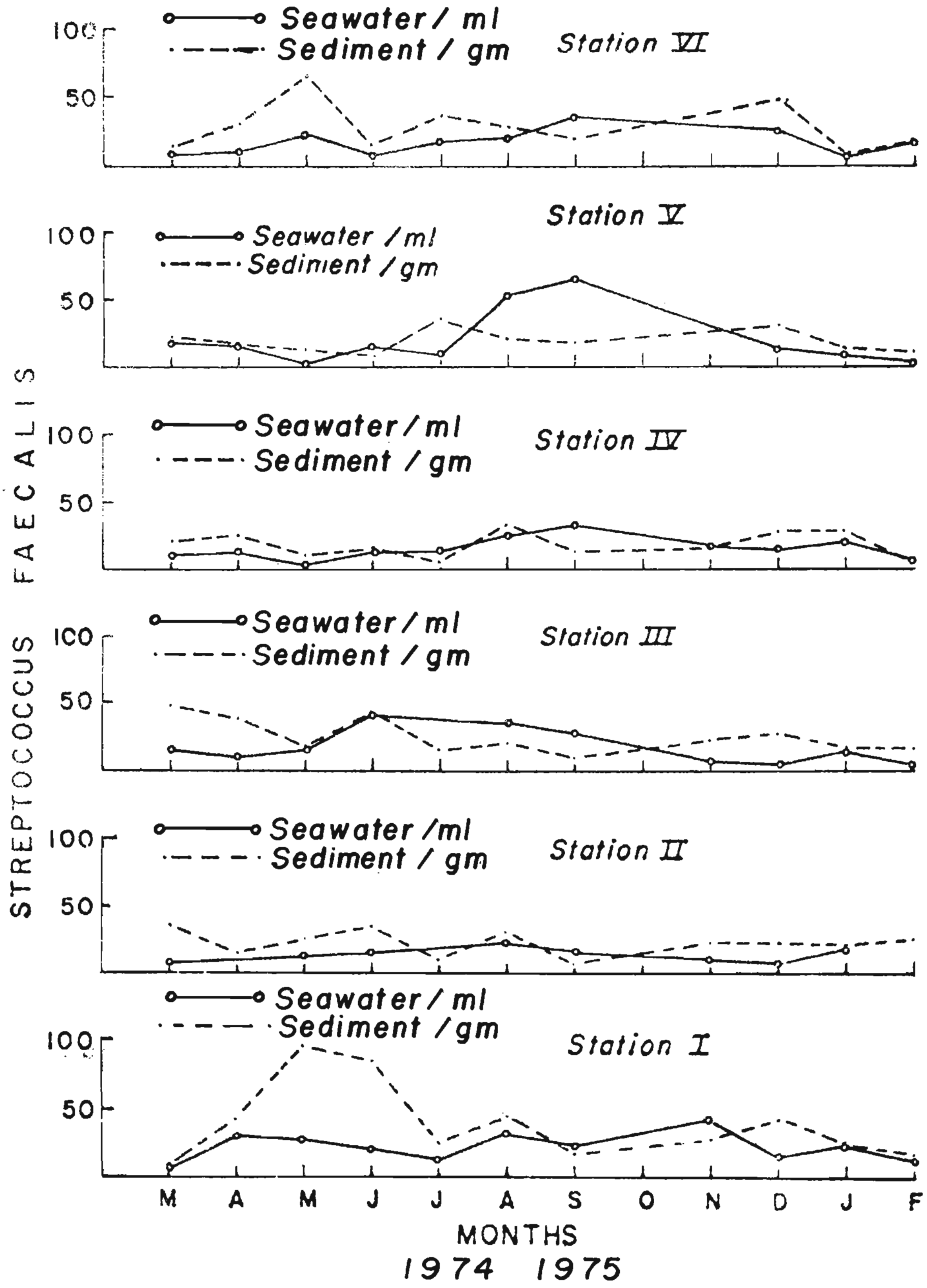
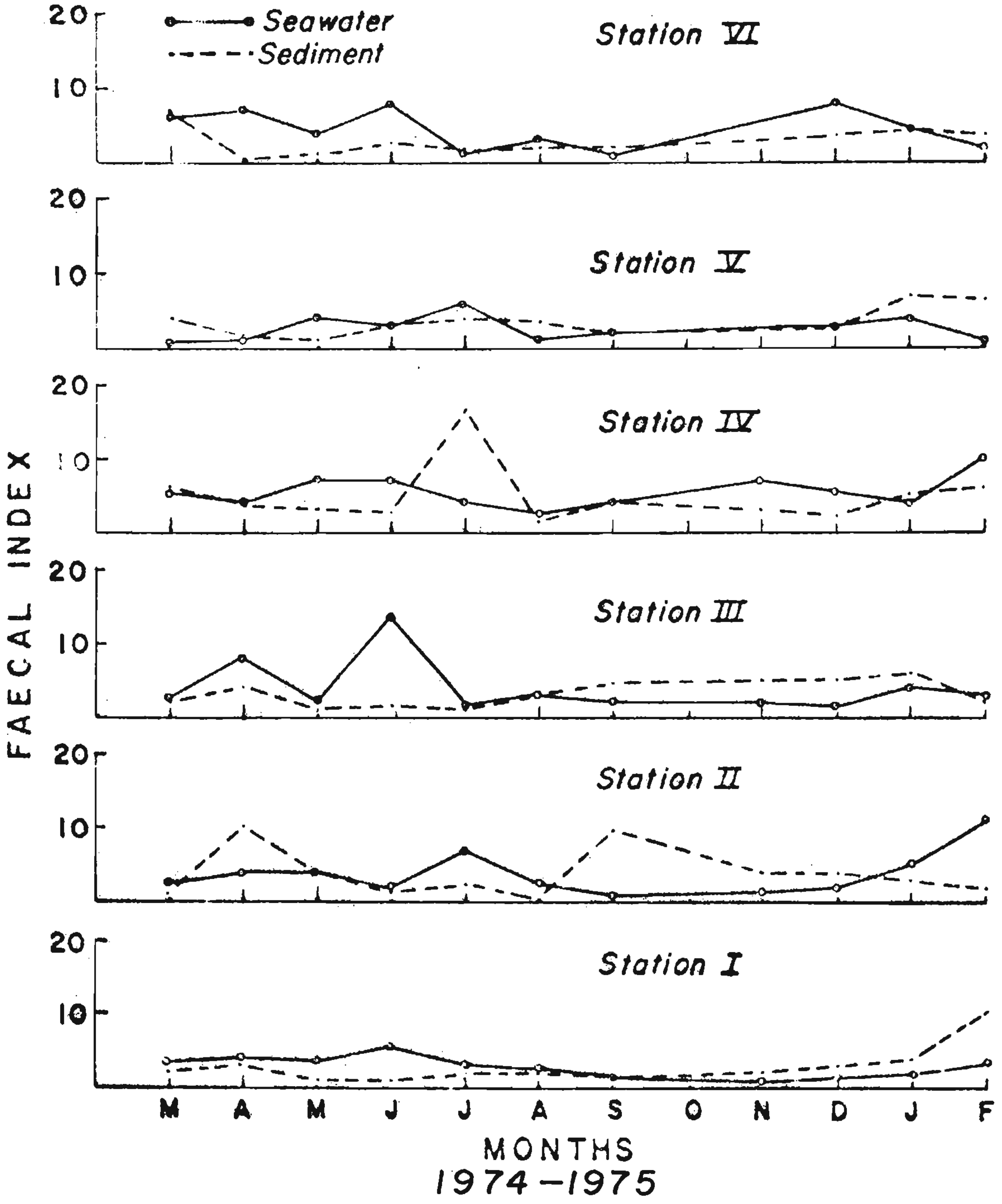

Seawater / ml
Sediment / gm
Station VI
Station V
Station IV
Station III
Station II
Station I
100
50
STREPTOCOCCUS FAECALIS
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974 1975

Fig. 28. Showing faecal index of the water and sediment in the area of study during 1974-75.

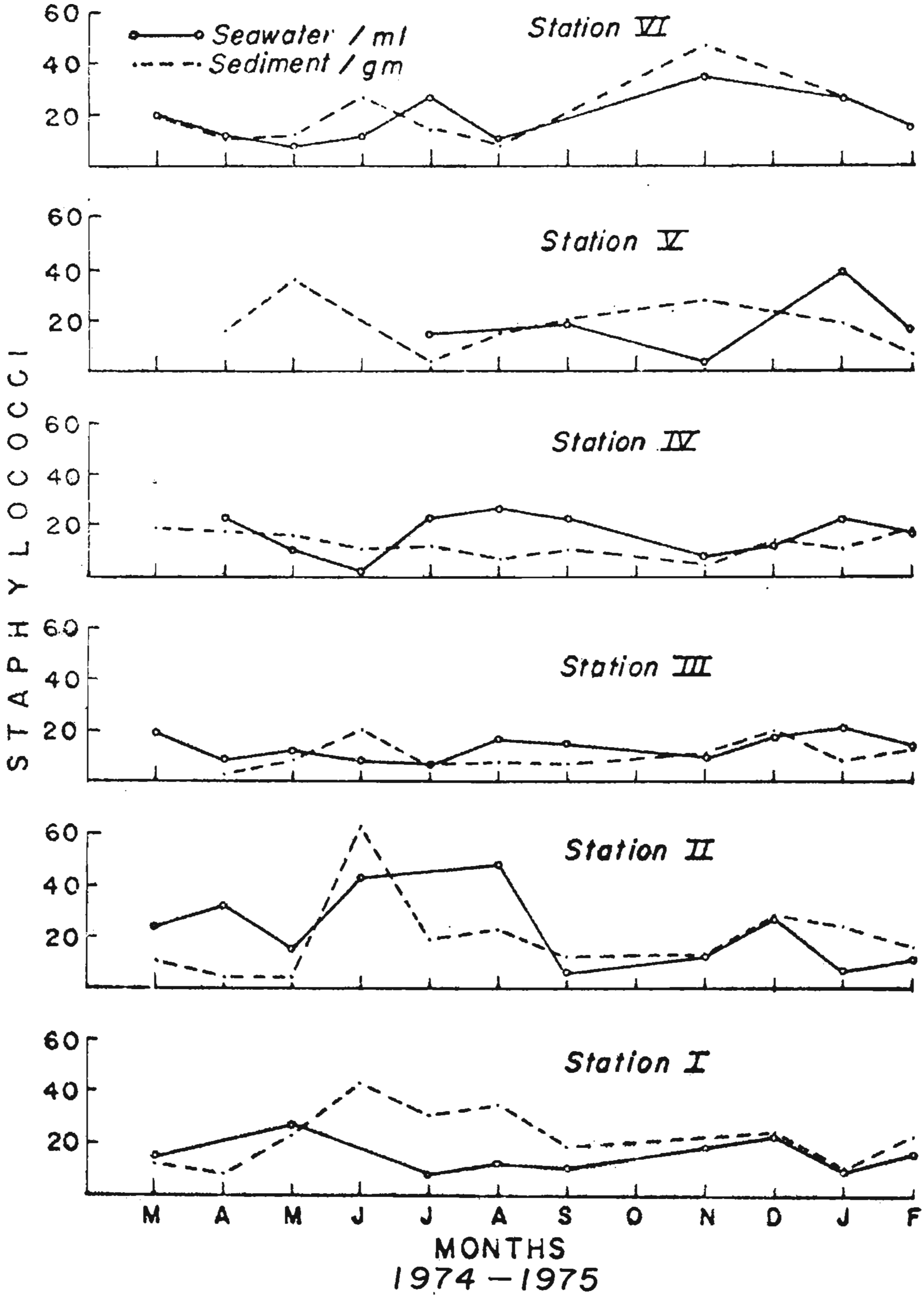

Seawater
Sediment
Station VI
Station V
Station IV
Station III
Station II
Station I
20
10
FAECAL INDEX
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974–1975

(i.e. faecal index) formed 4 or above 4, it has denoted that the source of pollution is exclusively from human faecal wastes. In the present case, the source of pollution during September 1974 was completely from animal wastes. In August 1974, the faecal index showed predominance of human faecal wastes in mixed pollution. In sea water, the faecal index obtained showed that 50% of the faecal pollution in the Cochin backwater was contributed by human faecal wastes. Staphylococci was generally found very rare and varied between 2/ml in Station IV (Fig. 29) during June 1974 to 48/ml in Station II during August 1974.

In sediments, higher total coliform counts were obtained in Station III in January 1975 (250×10^3/ml) and lower counts were recorded during December 1974 (16×10^3/ml) (Fig. 25) and that of faecal coliforms (*E. coli*) ranged from 14/ml in August 1974 in Station II, to 180/ml in February 1975 in Station I (Fig. 26).

Faecal streptococci (*Streptococcus faecalis*) ranged from 5/ml in July 1974 at Station IV, to 96/ml in May 1974 at Station I (Fig. 27). Staphylococci ranged from 2/ml in April 1974 at Station III to 62/ml at Station II during June 1974 (Fig. 29). No appreciable seasonal variation was seen in any of the indicators of bacterial pollution examined in the period 1974-75.

Fig. 29. Showing the counts (No./ml) of *Staphylococci* in water and sediment in the area of study during 1974-75.


Seawater / ml
Sediment / gm
Station VI
Station V
Station IV
Station III
Station II
Station I
60
40
20
STAPHYLOCOCCI
M
A
M
J
J
A
S
O
N
D
J
F
MONTHS
1974 – 1975

The faecal index in the mud varied between 0.68 in Station VI in April 1974 to 16.50 in Station IV in July 1974 (Fig. 28). The source of pollution in May and June 1974, was completely from animal faecal wastes, eventhough the predominance of human faecal wastes was seen in some stations. Only 32% of faecal pollution was contributed by human faecal wastes in sediments through the sewage, drainage and freshwater run-off.

Seasonal distribution of total coliforms, *E. coli*, *Streptococcus faecalis*, faecal index and *Staphylococci* at the six stations under study are shown in Figs. 25-29. All the four groups of organisms were encountered in higher numbers in sediment than in sea water. The bacterial content of surface waters and sediments fluctuated widely, but there was no evidence of rhythmic seasonal variation.

The data of total heterotrophic counts and *E. coli* in sea water and sediment from all the six sites were subjected to correlation analysis. At Stations II and V, a slight negative correlation was seen between total heterotrophic count and *E. coli* in sea water (Fig. 30 a). No significant correlation was seen between the two parameters in sediment samples from all the sites as expected (Fig. 30 b).

Fig. 30 a. Showing correlation analysis of heterotrophic bacteria against *Escherichia coli* in surface water during 1974-75.

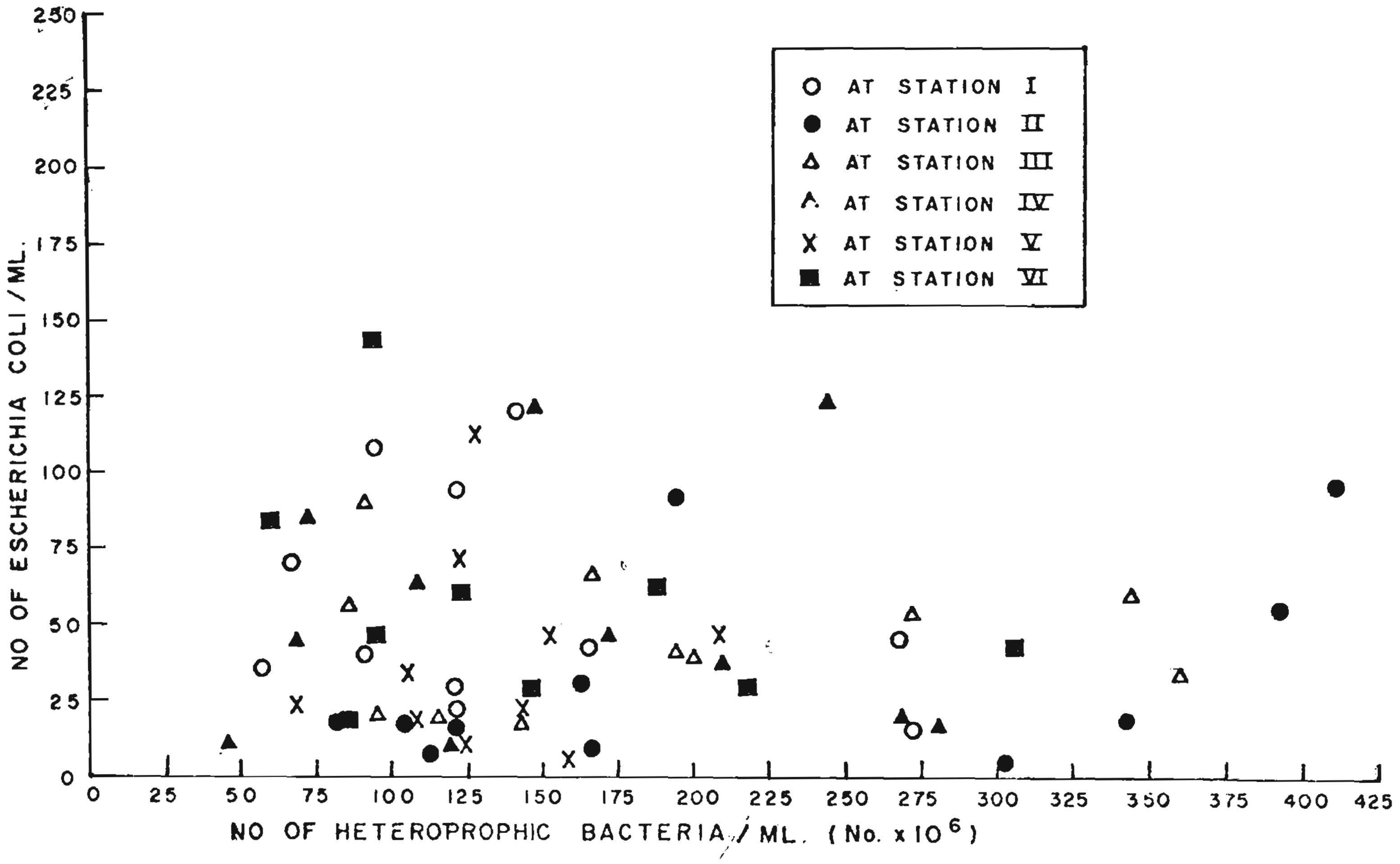
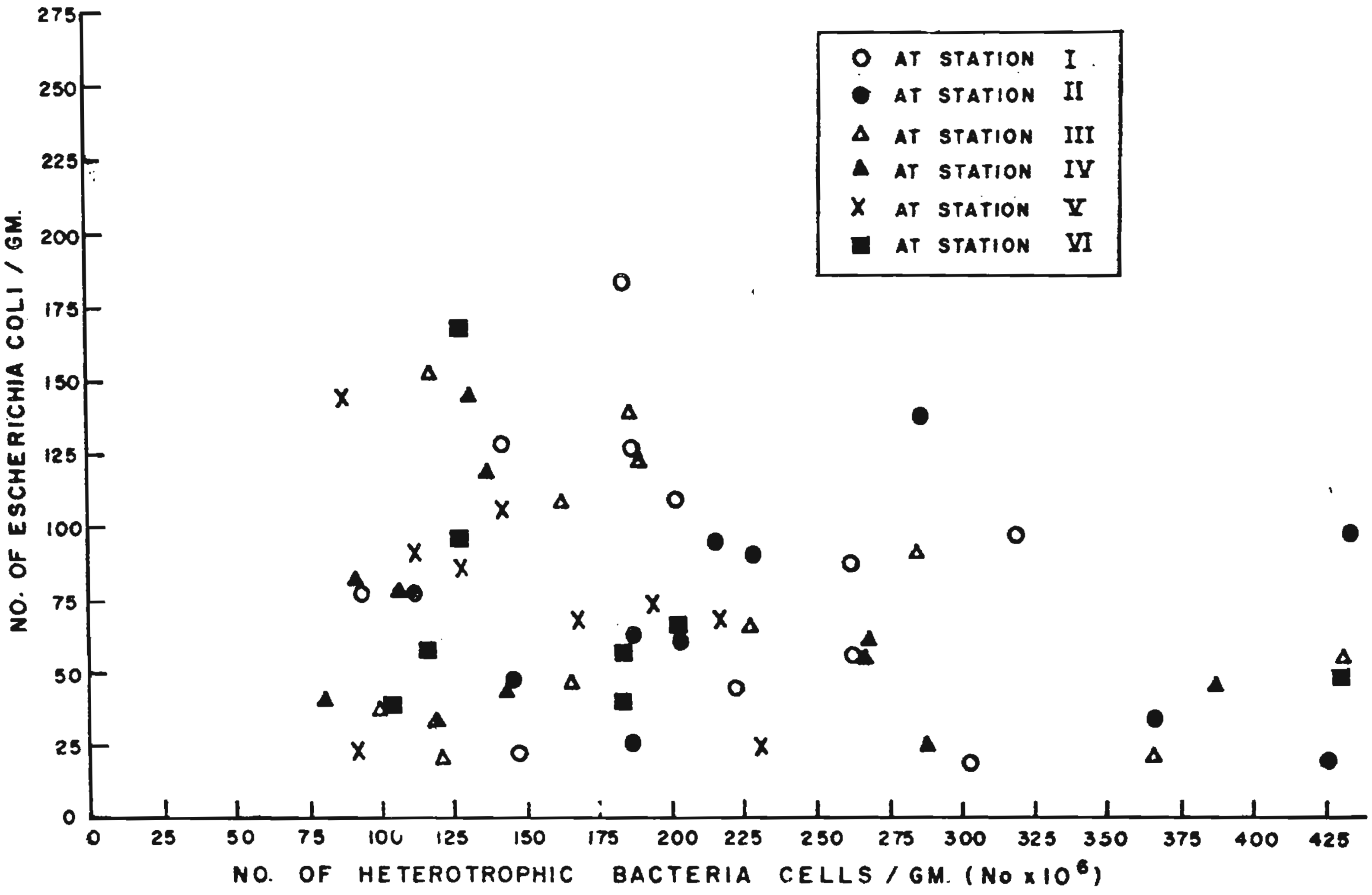

NO OF ESCHERICHIA COLI / ML.
0
25
50
75
100
125
150
175
200
225
250
NO OF HETEROPROPHIC BACTERIA / ML. (No. x 10^6)
0
25
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
○ AT STATION I
● AT STATION II
△ AT STATION III
▲ AT STATION IV
X AT STATION V
■ AT STATION VI

Fig. 30 b. Showing correlation analysis of heterotrophic bacteria against *Escherichia coli* in sediment during 1974-75.


NO. OF ESCHERICHIA COLI / GM.
0
25
50
75
100
125
150
175
200
225
250
275
0
25
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
NO. OF HETEROTROPHIC BACTERIA CELLS / GM. (No x 10^6)
○ AT STATION I
● AT STATION II
△ AT STATION III
▲ AT STATION IV
X AT STATION V
■ AT STATION VI

The analysis of variance test showed the following results (Tables 30-34).

Coliforms:

The counts differed significantly between stations ($P < 0.05$), between months ($P < 0.01$) and between regions ($P < 0.01$). The presence of coliforms was more in sediment than in sea water (Table 30).

Escherichia coli:

E. coli counts showed significant difference ($P < 0.01$) between regions. Significantly higher counts were observed in sediment than in sea water (Table 31).

Faecal index:

Faecal index showed significant difference between stations ($P < 0.01$) and between months ($P < 0.05$). Station IV was having significantly higher faecal index compared to other stations in both sea water and sediment (Table 32).

Faecal streptococci:

Streptococci counts showed significant difference between stations ($P < 0.05$), months ($P < 0.05$) and regions ($P < 0.05$). The count was significantly high in back-

TABLE 30. Analysis of Variance Table (Total coliforms) (1974-75).

Source	Sum of squares	Degrees of freedom	Means square	Variance ratio 'F'
Total coliforms	12.7318	127		
Between stations	0.8695	5	0.1739	2.63*
Between months	3.8168	10	0.3817	5.78**
Between regions	0.7209	1	0.7209	10.93**
Error	7.3245	111	0.0660	

TABLE 31. Analysis of Variance Table (*Escherichia coli*)(1974-75).

Source	Sum of Squares	Degrees of freedom	Means square	Variance ratio 'F'
Total *E. coli*	16.1255	126		
Between stations	0.9803	5	0.1961	1.87
Between months	1.6098	10	0.1610	1.54
Between regions	2.0256	1	2.0257	19.36**
Error	11.5098	110	0.1046	

TABLE 32. Analysis of Variance Table (Faecal Index) (1974-75).

Source	Sum of squares	Degrees of Freedom	Means square	Variance ratio 'F'
Total Faecal index	11.1871	129		
Between stations	1.4538	5	0.2908	4.08**
Between months	1.9980	10	0.1998	2.24*
Between regions	0.0984	1	0.0884	1.24
Error	8.0470	113	0.0712	

water than in marine conditions. Station I was having higher streptococci counts than other stations both in sea water and sediment compared to other stations (Table 33).

Staphylococci:

There is no significant difference between the counts of staphylococci between months, stations and regions as far as *Staphylococci* was concerned (Table 34).

Product-moment correlation coefficients were computed between bacterial counts and other physico-chemical parameters of the water and sediment samples from six sites. Tables 8 to 19 illustrate the results of product-moment correlation coefficients of all measured parameters in sea water and sediment (bottom water).

In sea water *E. coli* showed significant ($P < 0.05$) positive correlation with phosphate in Station I (Table 8). In Station II, *Staphylococci* showed significant positive correlation with *Escherichia coli* (Table 9). The correlation is significant at 5% level in August 1974 and January 1975. No correlation was seen among the other bacterial and chemical parameters in Stations I and II. In Station III, also no correlation was established between the observed chemical and microbiological factors (Table 10).

TABLE 33. Analysis of Variance Table
(*Streptococcus faecalis*) (1974-75).

Source	Sum of squares	Degrees of freedom	Means square	Variance ratio 'F'
Total *Streptococcus faecalis*	11.2706	123		
Between stations	0.8776	5	0.1755	2.39*
Between months	1.9473	10	0.1947	2.11*
Between regions	0.9363	1	0.9863	13.43**
Error	7.8594	107	0.0735	

TABLE 34. Analysis of Variance Table (*Staphylococci*) (1974-75).

Source	Sum of Squares	Degrees of Freedom	Means Square	Variance ratio 'F'
Total *Staphylococci*	9.8672	111		
Between stations	0.6276	5	0.1255	1.43
Between months	0.8350	10	0.0835	0.95
Between regions	0.0468	1	0.0468	0.53
Error	8.3578	95	0.0880	

In Station IV, coliforms showed significant ($P < 0.05$) negative correlation with total heterotrophic counts at 5% level (Table 11). *Streptococcus faecalis* showed significant ($P < 0.01$) negative correlation at 1% level with phosphate. Also, significant positive correlation was seen between staphylococci and *Escherichia coli* at 1% level in Station IV.

Faecal index showed significant ($P < 0.05$) negative correlation with staphylococci. In Station V significant ($P < 0.05$) negative correlation was seen between *E. coli* and temperature at 5% level (Table 12). Significant ($P < 0.05$) negative correlation was also seen between *Streptococcus faecalis* and phosphate. Staphylococci showed significant ($P < 0.01$) positive correlation with *E. coli*. In Station VI mixed pollution was evident based on faecal index (as it ranged between 2.20 and 3.80 in May, July, August, September 1974 and February 1975) and *Streptococcus faecalis* showed significant ($P < 0.05$) negative correlation with *E. coli* at 5% level which may be due to the predominance of animal wastes in mixed pollution in this station (Table 13). Also faecal index showed significant positive correlation with *E. coli* ($P < 0.05$).

In sediments, in Station I, coliforms were significantly ($P < 0.05$) positively correlated with both pH and total heterotrophic plate counts (Table 14). *Streptococcus faecalis* showed significant ($P < 0.05$) negative correlation with salinity. The faecal index of the mud showed significant positive correlation with *E. coli* at 5% level.

There was no effect of pH on any of the variables studied except on *E. coli* in sediments, which showed significant positive correlation with pH ($P < 0.05$). Faecal index was significantly ($P < 0.01$) positively correlated with *E. coli* ($P < 0.01$) and negatively correlated with *Staphylococci* at 5% level. In Station III, also faecal index was significantly positively correlated with *E. coli* (Table 16).

A negative correlation was found between *E. coli* and pH at 5% level and positive correlation at 1% level was seen at Station IV (Table 17). In Station V, coliforms were positively correlated at 5% level, and with oxygen at 0.1% level. *Streptococcus faecalis* was significantly ($P < 0.05$) negatively correlated with nitrate whereas *Staphylococci* showed significant ($P < 0.05$) positive correlation with oxygen and nitrate at 5% level.

In Station VI, total coliforms showed significant ($P < 0.05$) positive correlation with temperature, salinity, and pH (Table 19). *Streptococcus faecalis* was significantly negatively correlated with temperature ($P < 0.01$), nitrate ($P < 0.05$) and pH ($P < 0.01$). *Staphylococci* also showed significant ($P < 0.05$) negative correlation with *Streptococcus faecalis*.

Both heterotrophic bacterial counts and indicator *E. coli* counts reached maximum at the most polluted stations. Faecal coliform levels were lower than total coliform levels, as encountered during 1972-73 which can originate from non-faecal sources such as plants and soils. In general, the number of indicator organisms at all the sampling sites was lower for water than for sediment samples. Also, there was comparatively less fluctuation in the bacterial concentration in sediments rather than in water samples. According to Greenberg (1956) adsorption and sedimentation tend to remove organisms from suspension and concentrate them on bottom deposits where they continue an active existence. Rittenberg *et al.* (1958) found high coliform levels in mud extending several miles from marine sewage outfalls discharging primary effluent, suggesting that survival of bacteria will increase after sedimentation. Van Donsel and Geldreich (1971) examined a wide variety of sediments and found that total coliforms, faecal coliforms, streptococci concentrations were 100 to 1,000 times higher in sediments than overlying water. The presence of large number of coliforms in sediments is hazardous in light of observation by Grimes (1975) who revealed that there is every possibility of getting increased faecal coliform counts in surface water followed by the disturbance and relocation of bottom sediments by dredging. The observation by Geldreich (1972) that pathogen can survive in the bottom deposits of a river or lake for several weeks before they die, also supports the above theory. The ability of enterobacteria to utilise nutrients released from sediments has been shown by Hendricks and Morrison (1967). Gerba and McLeod (1976) concluded on the basis of laboratory results that *E. coli* could survive and grow in unsterile natural sea water only when sediment was present during the experiment. Pathogens adsorbed to sediments poses danger by adhering to fish catch during trawling and also resuspension of sediments in response to currents, storms, boat traffic, dredging can result in release of adsorbed bacteria into overlying water, thus posing a hazard to human health.

Sayler *et al*. (1975) and Gore *et al*. (1975) found lower number of indicator organisms in sediment than in water samples. The stations sampled for their study, were far off from the nearest shore eventhough the depth (8-12 m) was found to be the same, as in the present study.

Deeper water would require the actual suspension of sediment material to cause a concurrent increase in the number of organisms in the water column, whereas in shallow water, sediment-associated bacteria could also be resuspended by a decrease in salinity. The shallowness of the area and the limited flushing action, and the nearness of sewage and drainage outlets probably build up more nutrients, allowing more favourable conditions for coliform persistence in sediments than in surface water along coastal area. The area of present study is a very shallow one, the maximum depth range being 10 - 12 metres.

Seasonal occurrence of indicator organisms and faecal index in water and sediments at the six sites is shown in the Figs. 23 a-f. Total coliforms had maximum count in postmonsoon season. Faust et al. (1975) found that coliform survivals in estuarine water was strongly influenced by temperature, with die-off increasing rapidly with elevated temperatures. In the present study, product moment correlation coefficient computed showed direct relationship between coliforms and temperature. In environments, less stable than the oceans or where there are no other limiting factors, temperature act as most important ecological factor. Thus, eventhough amount of faecal coliforms and total coliforms were discharged along with faecal wastes, during the months of January, February, March, faecal coliform levels encountered were less because of their rapid die-off which may be due to ecological factors other than temperature.

In Stations II (in August 1974 and January 1975), IV and V *Staphylococci* showed positive correlation with *E.coli* which indicated that the source of pollution in these stations was from human wastes.

An inverse correlation found between faecal *streptococci* and phosphate was rather unusual, as one would expect *Streptococci* to prefer phosphate as one among growth factors. Also, an inverse correlation was seen between survival of faecal *streptococci* and salinity of sediment at Station I at 5% level. The observed increase in the number of organisms may be due partly to desorption of bacteria after a heavy rainfall. Actual suspension of sediment material must have taken place due to currents and rainfall to cause a concurrent increase in the number of 'faecal streps' in sediment.

Although bacterial standards for judging the potential health hazards have never been unequivocably agreed upon, because of the need for epidemiological studies arbitrary bacterial standards have been utilised in the United States. The National Technical Advisory Committee on water quality criteria has recommended a limit of 1,000 total coliforms per 100 ml and 200 faecal coliforms per 10 ml of water.

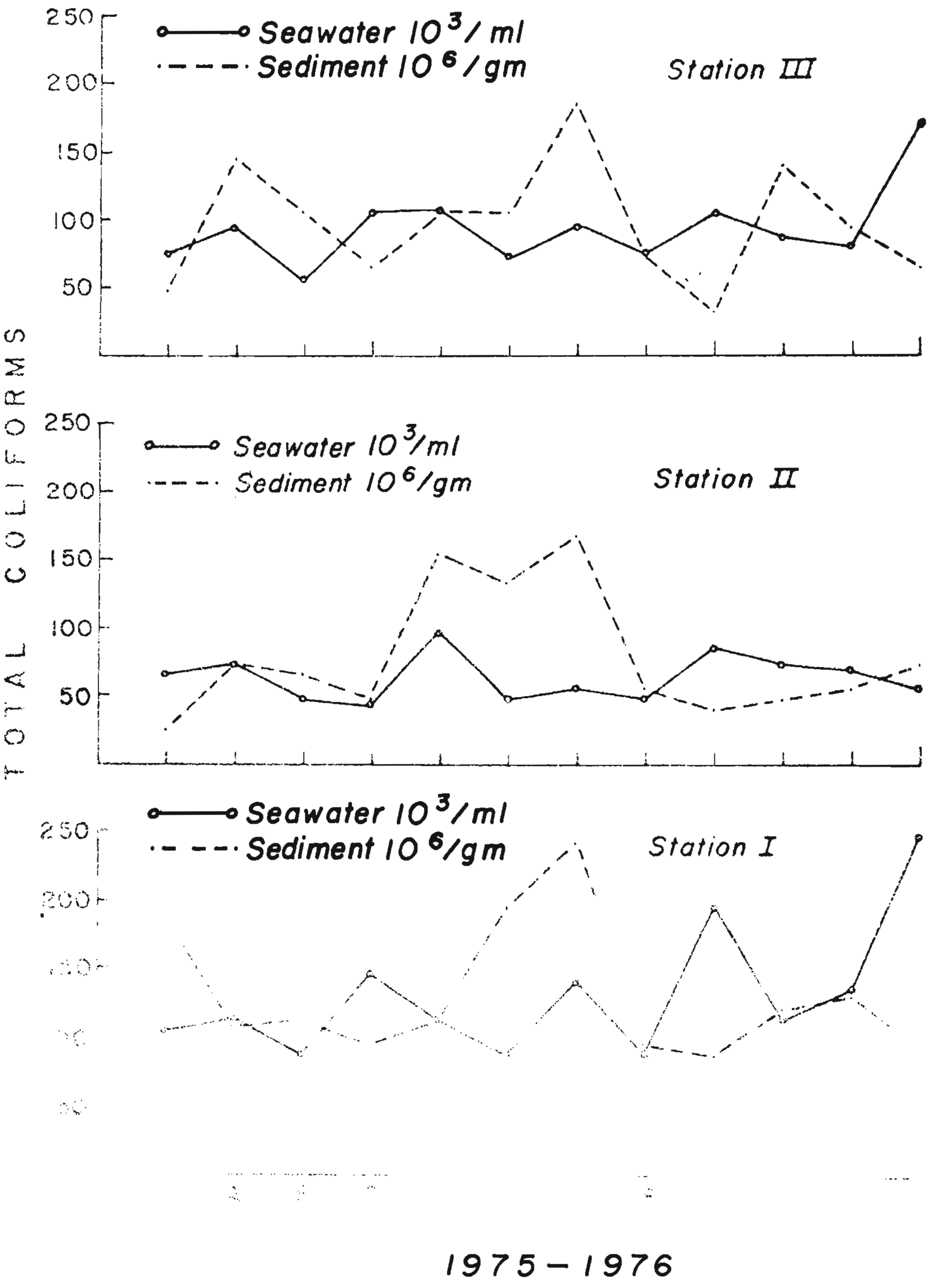
In India, Indian Standards Institution has recommended the tolerance limits for bacterial pollutants in surf zones subject to effluent discharge 2,500 total coliform per 100 ml for bathing, recreation, shellfish culture and salt manufacture (Table 52). This value was exceeded in almost all the stations in all the months in sea water and especially in sediments. In sea water in Station I, the total coliforms were below 2,500 in the month of July and September. In Station II during July, August, and September lowest counts were recorded. During April, May and September the counts exceeded the tolerance limit in Station III. In April, May and November 1974, lowest counts were encountered at Station IV which was found dominated by fresh water conditions. Station V, was found most polluted by animal wastes having higher counts in all the months and Station VI was equally polluted as per the coliform counts except in the month of May 1974.

Occurrence and distribution of bacterial indicators during 1975-76.

Total coliform:

Total coliform counts ranged from 42×10^3/ml in Station II in the month of October 1975 to 248×10^3/ml in the month of June 1976 in sea water (Fig. 31). In sediment the counts ranged from 24×10^3/ml in Station II during

Fig. 31. Showing Total coliforms (No. x 10^3) in water and sediment in the area of study during 1975-76.


Seawater 10^3/ml
Sediment 10^6/gm
Station III
250
200
150
100
50
TOTAL COLIFORMS
Seawater 10^3/ml
Sediment 10^6/gm
Station II
250
200
150
100
50
Seawater 10^3/ml
Sediment 10^6/gm
Station I
250
200
1975 – 1976

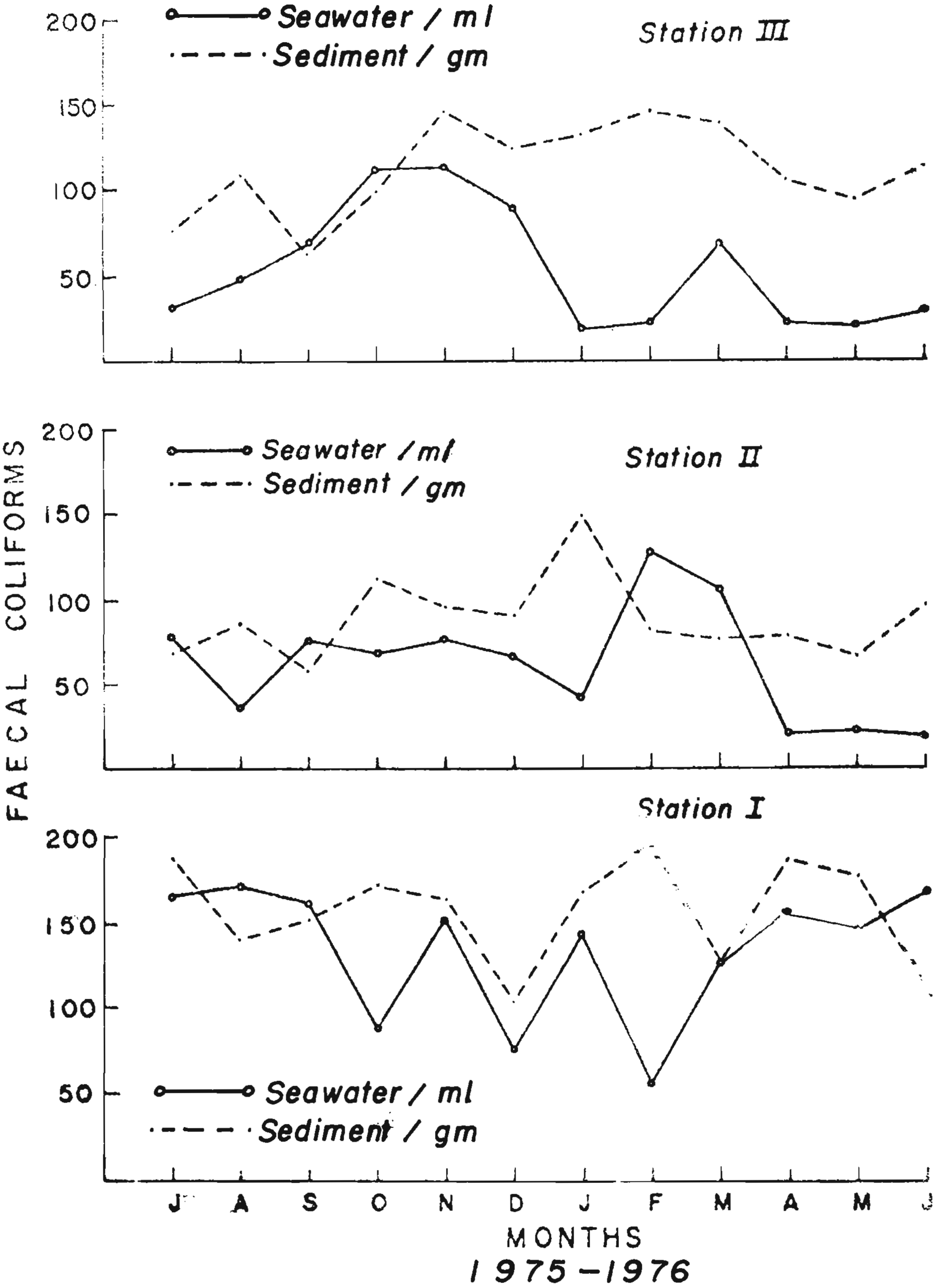
July 1976. The highest count was recorded in the month of June 1976, in sea water and the lowest was seen in sediment. The abundance of total coliforms in surface water may be due to recent faecal pollution from sewage as well as pollution from soil or plant origin.

Escherichia coli:

E. coli ranged from 18/ml in Station II in the month of June and in Station III in the month of January 1976 to 168/ml in the month of June at Station I near the sewage outlet (Fig. 32). In sediment the counts ranged from 62/ml in Station II in the month of September to 188/ml in Station I in the month of July 1975 and April 1976.

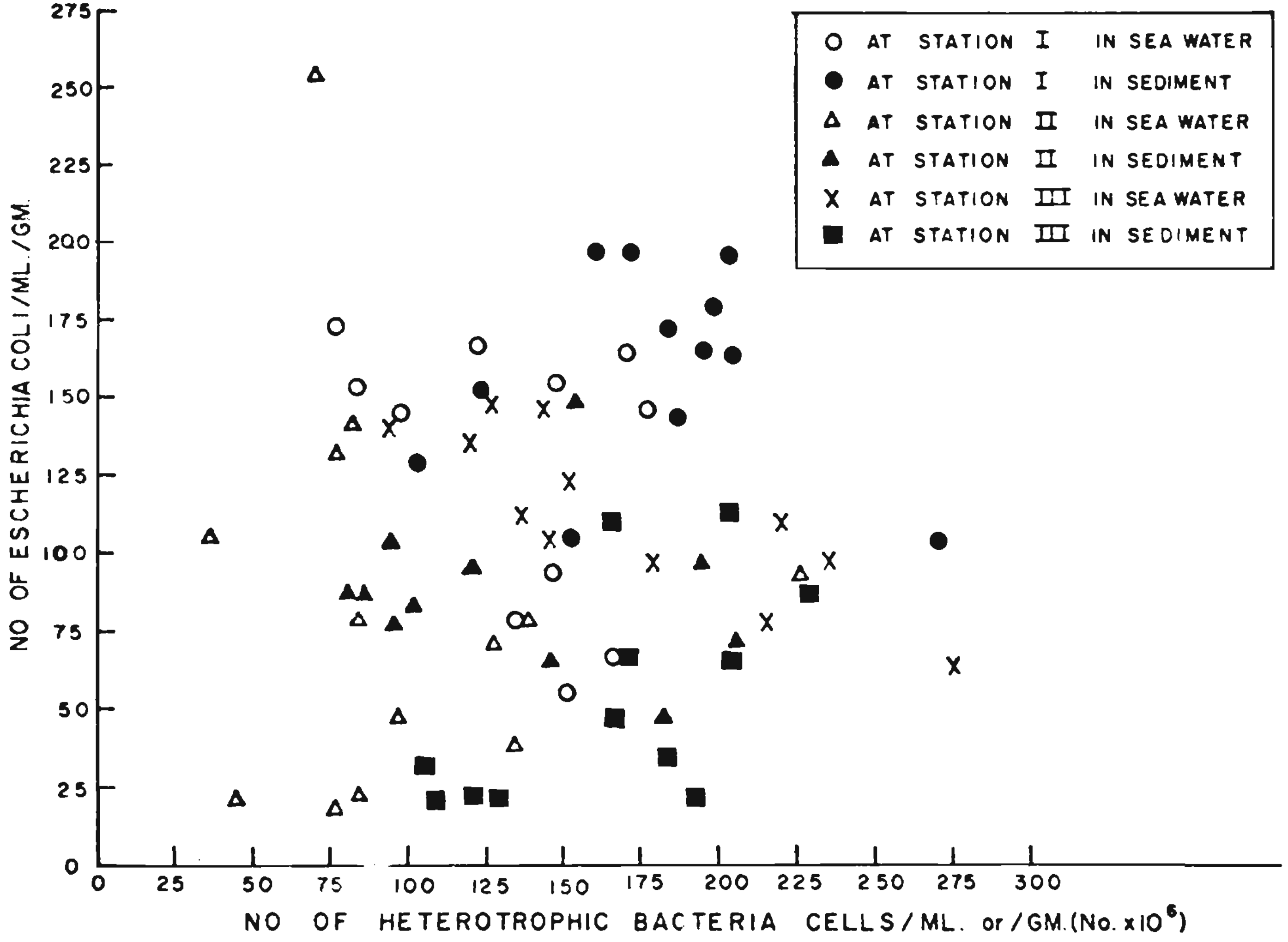
The correlation analysis of *E. coli* and Heterotrophic bacterial counts showed that the sediments at Station I in all the months harboured highest *E. coli* as well as heterotrophic counts (Fig. 33). In Station II, *E. coli* was recorded high in sea water (250/ml), when heterotrophic counts were recorded very low (75×10^6/ml) which denotes fresh faecal pollution due to dredging activities or from ship's toilet near Cochin harbour area. *E. coli* as well as heterotrophic counts were recorded very low in sediments at Stations II and III.

Fig. 32. Showing faecal coliforms (*Escherichia coli*) (No./ml) in water and sediment in the area of study during 1975-76.



Seawater / ml
Sediment / gm
Station III
Station II
Station I
200
150
100
50
FAECAL COLIFORMS
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975-1976

Fig. 33. Showing correlation analysis of heterotrophic bacteria against *Escherichia coli* in water and sediment during 1975-76.



275
250
225
200
175
150
125
100
75
50
25
0
NO OF ESCHERICHIA COLI/ML./GM.
0
25
50
75
100
125
150
175
200
225
250
275
300
NO OF HETEROTROPHIC BACTERIA CELLS/ML. or /GM.(No. x10⁶)
○ AT STATION I IN SEA WATER
● AT STATION I IN SEDIMENT
△ AT STATION II IN SEA WATER
▲ AT STATION II IN SEDIMENT
X AT STATION III IN SEA WATER
■ AT STATION III IN SEDIMENT

Nearly 446 pure strains of *E. coli* from sea water and sediments isolated were classified based on the scheme into *E. coli* type I (Table 35). All these strains were subjected to serological groupings and antibiogram typing and seven 'O' serotypes has been encountered. Some 230 strains were considered as enteropathic *E. coli* and details are given in Part B, Section III.

Streptococcus faecalis:

In sea water the '*faecal strep*' ranged from 5/ml in Station II during April 1976 to 122/ml in February 1976 in Station I (Fig. 34). In sediment the highest count was encountered in June (72/ml) in Station I, near the sewage outlet and lowest count was obtained in Station II during March and April 1976. Altogether, 48 pure strains of *Streptococcus*, isolated in the selective media (KF agar) were tested based on the scheme (Fig. 35) as defined in standard methods for the examination of water. 75% of the strains (36 nos.) turned to be *Streptococcus faecalis* (by growing in brain-heart infusion broth within 2 days at 45°C and 10°C, being catalase negative, shown growth in 40% bile broth). Also, these 36 *Streptococcus* strains showed growth at 6.5% Sodium chloride. Starch was not hydrolysed, peptonisation of litmus milk was negative. A positive litmus milk peptonisation and negative starch hydrolysis

Fig. 34. Showing distribution of *Streptococcus faecalis* (No./ml) in water and sediment in the area of study during 1975-76.

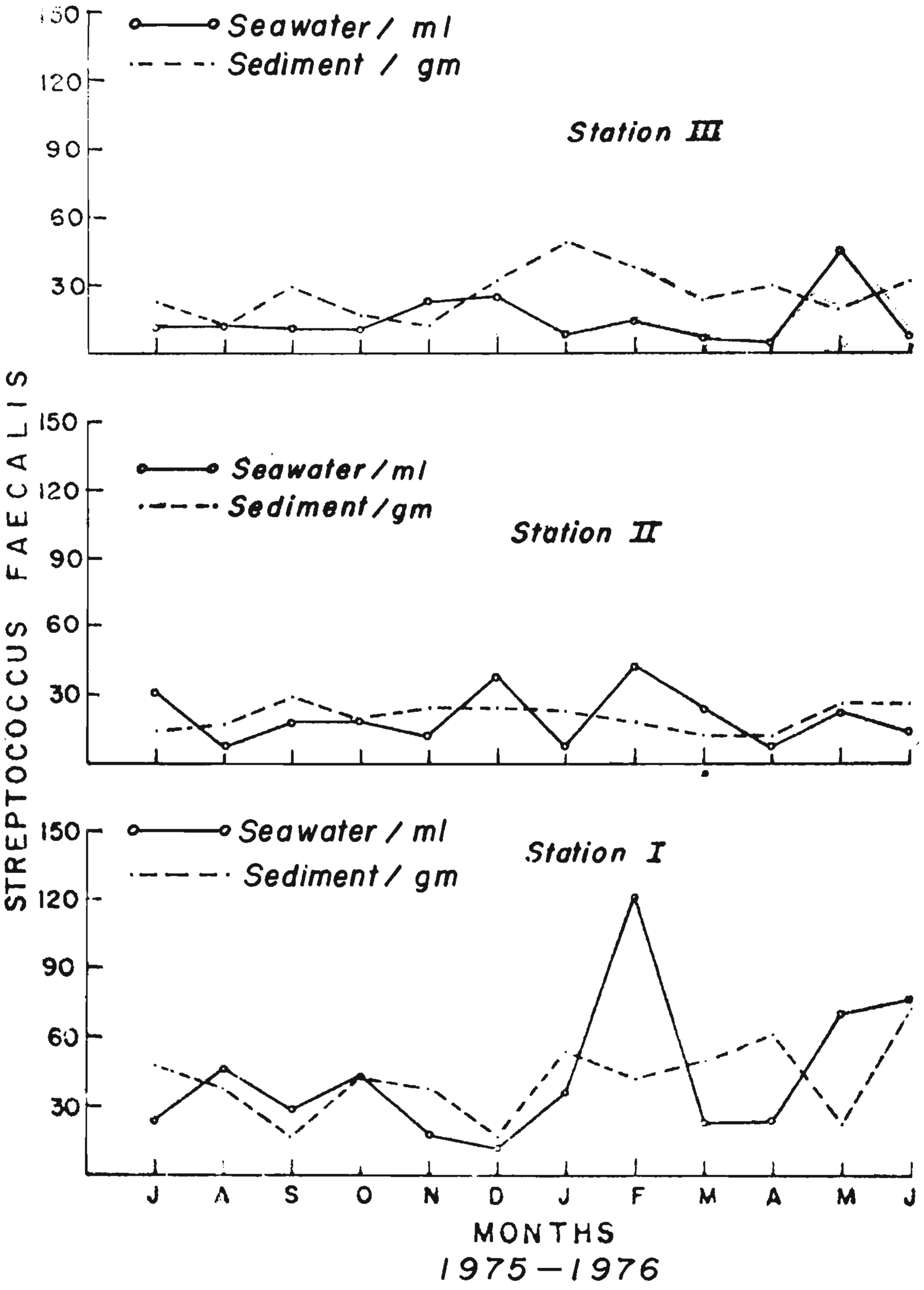
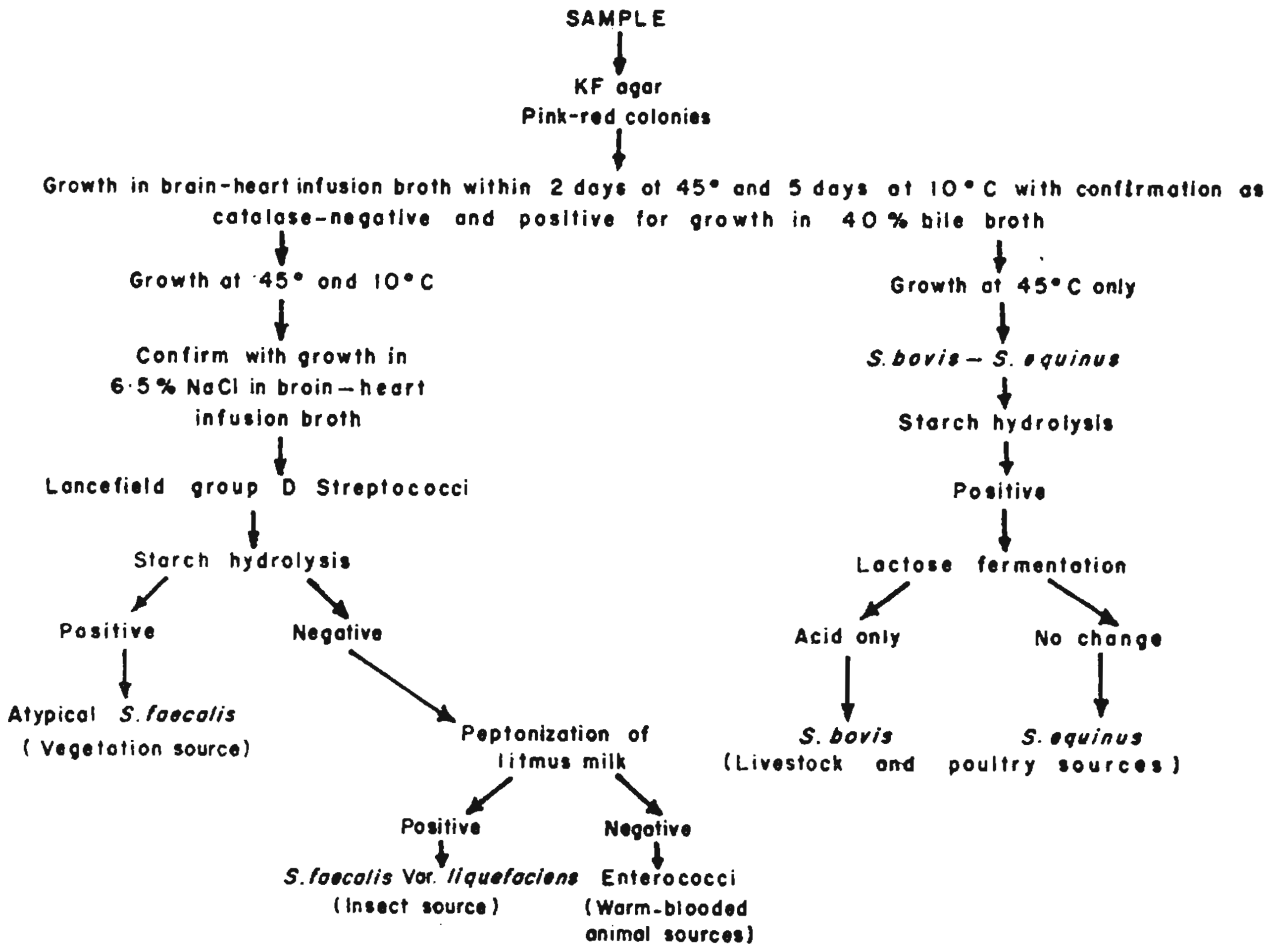

Seawater / ml
Sediment / gm
Station III
Station II
Station I
STREPTOCOCCUS FAECALIS
150
120
90
60
30
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975 – 1976

Fig. 35. Schematic outline for identification of faecal streptococci.



Note: A small percentage of unclassified strains (biotypes) was found since faecal streptococci vary greatly in their biochemical reactions.

Schematic outline for identification of faecal streptococci.

was seen in four strains which turned to be *Streptococcus faecalis var. liquefaciens*. The occurrence of *Streptococcus faecalis var. liquefaciens* in various environmental sources is given in Fig. 36. In all the other 8 strains, growth was seen only at 45°C in brain-heart infusion broth, hydrolysed starch and fermented lactose without gas production which confirmed their identification as *Streptococcus bovis* showing their origin from Livestock faecal wastes. *Streptococcus equinus* was completely absent in the present study which indicated meagre faecal pollution from poultry sources during the time of sampling period. The percentage occurrence of *Streptococcus bovis* and *Streptococcus equinus*, the faecal streptococcus population in warm-blooded animal faeces is given in Fig. 37. Geldreich and Kenner (1969) found that the detection of these two species, *S. bovis* and *S. equinus* in water indicated very recent farm animal waste contamination, which was found meagre in the present investigation.

Staphylococci:

An advantage of *Staphylococci* as indicators of potential hazards in an environment is that they are more resistant to chlorination than coliforms and thus their presence or absence is a measure of the efficiency or chlorination procedure (Robinson, Mood and Elliott, 1957). Evans (1977) in a recent review of the use of coagulase positive *Staphylococci*

Fig. 36. Occurrence of *S. faecalis* var. *liquefaciens* in various environmental sources.

OCCURRENCE OF *S. faecalis* VAR. *liquifaciens* IN VARIOUS ENVIRONMENTAL SOURCES

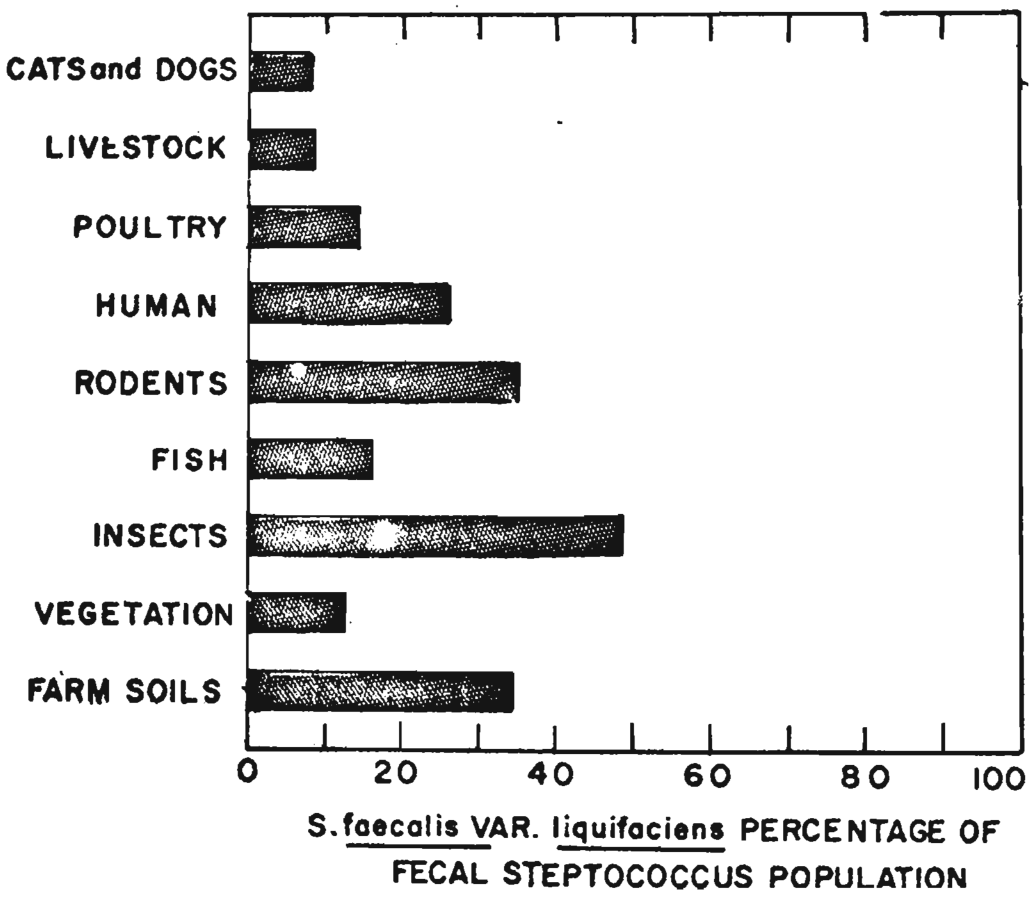
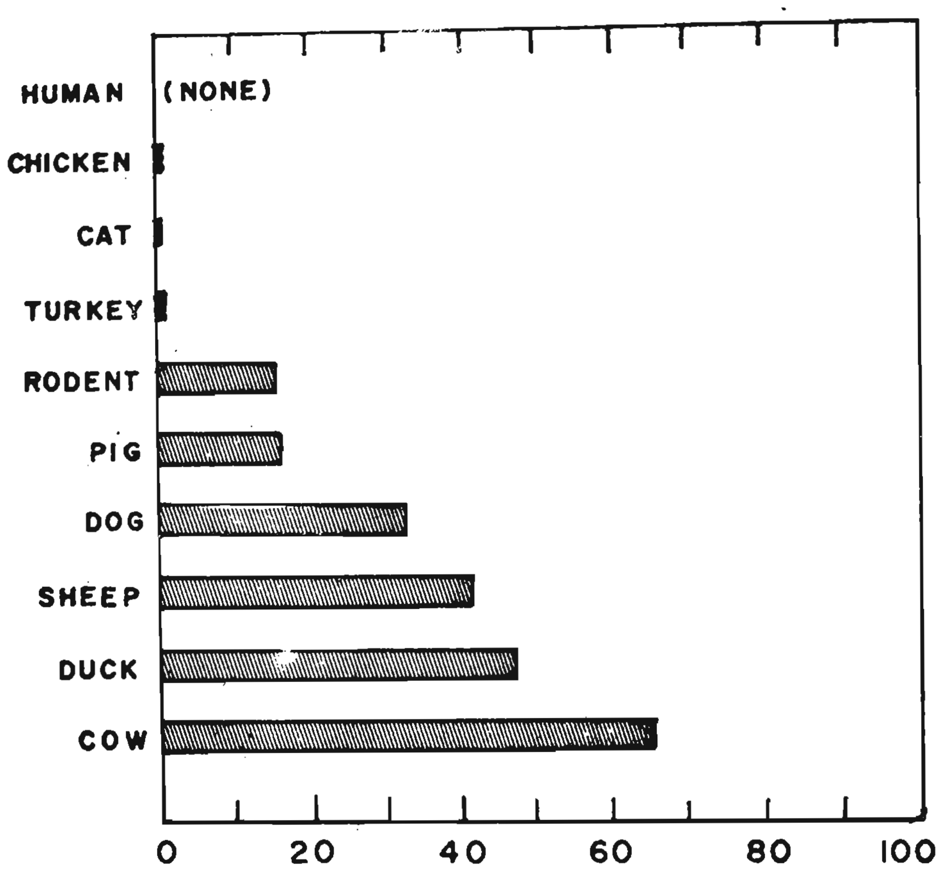


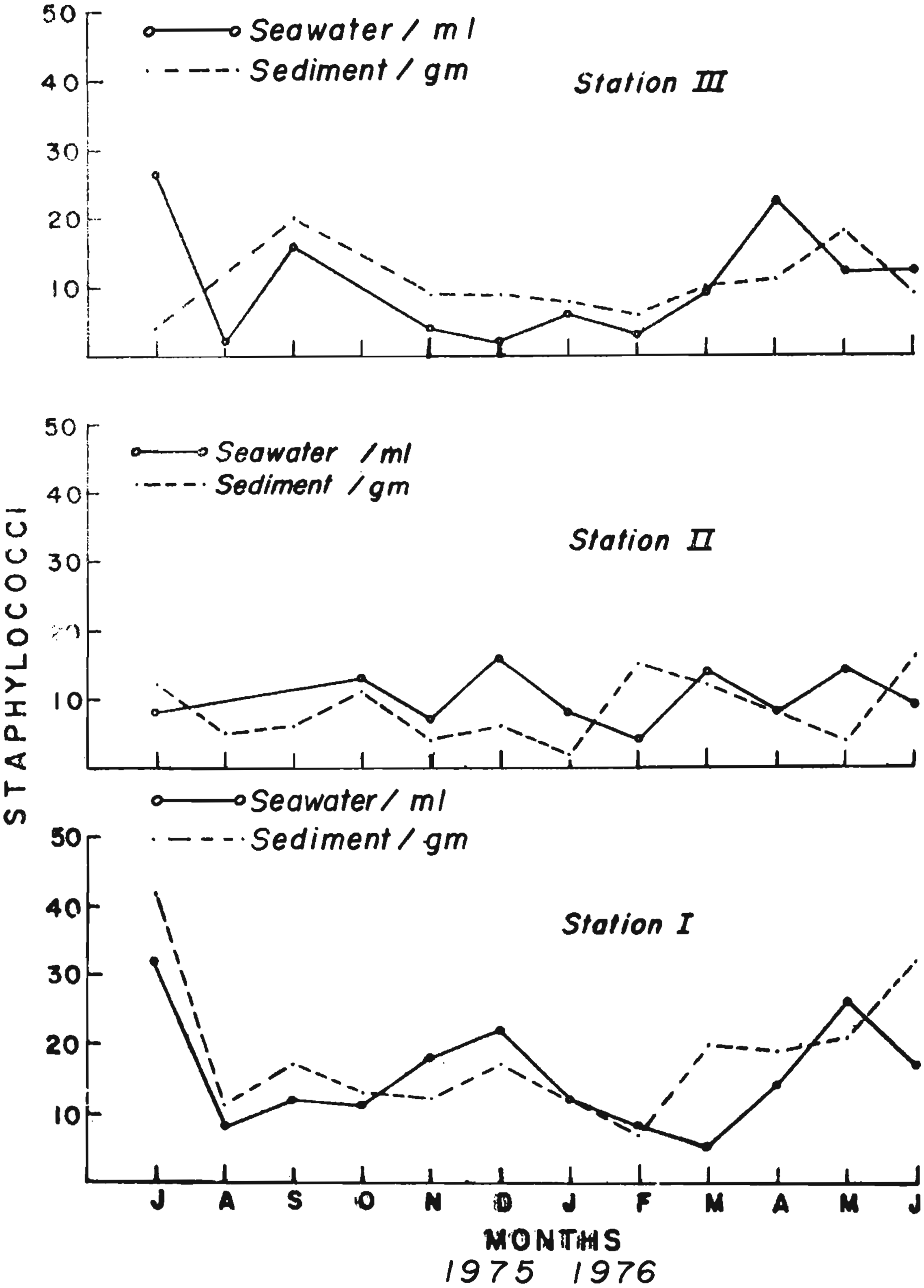
Fig. 37. Percentage of *S.* *bovis* and *S.* *equinus* of faecal streptococcus population in warm-blooded animal faeces.



S. BOVIS-*S. EQUINUS* PERCENTAGE OF FECAL STREPTOCOCCUS POPULATION IN WARM-BLOODED ANIMAL FECES as indicators of potential health hazards in water, noted the lack of information on the effectiveness of media and methods for the selective enumeration of *Staphylococci*. Some laboratories are reporting success with Baird-Parker (Difco) media and find it satisfactory for enumerating *Staphylococcus aureus* from hydrotherapy pools by the membrane filteration procedure. In the present study, isolation of *Staphylococcus aureus* by pour-plating procedure using Chapman's Agar was found satisfactory. Colony verification with coagulase test showed that all the suspected colonies in the selective media were *S. aureus* by giving coagulase-positive reaction. *Staphylococci* were major bacterial contaminants in all the aquatic environments with body contact waters. This is especially true that *Staphylococci* are major bacterial contaminants of swimming pool and recreational waters, that many of these *Staphylococci* are the pathogen *S. aureus* and that the enteric indicators do not provide an index of their hazard.

The counts of *Staphylococci* in sea water ranged from 2/ml in December 1975 to 32/ml in July 1975. No *Staphylococci* was found in most of the months at Stations II and III (Fig. 38). In sediment, the counts ranged from 2/ml in Station II during January 1978, to 44/ml in Station I during July 1975. *Coagulase positive Staphylococcus* is similar to the indicators *Pseudomonas aeruginosa* and *Candida*

Fig. 38. Showing *Staphylococci* (No./ml) in water and sediment in the area of study during 1975-76.


Seawater / ml
Sediment / gm
Station III
Station II
Station I
STAPHYLOCOCCI
50
40
30
20
10
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975 1976

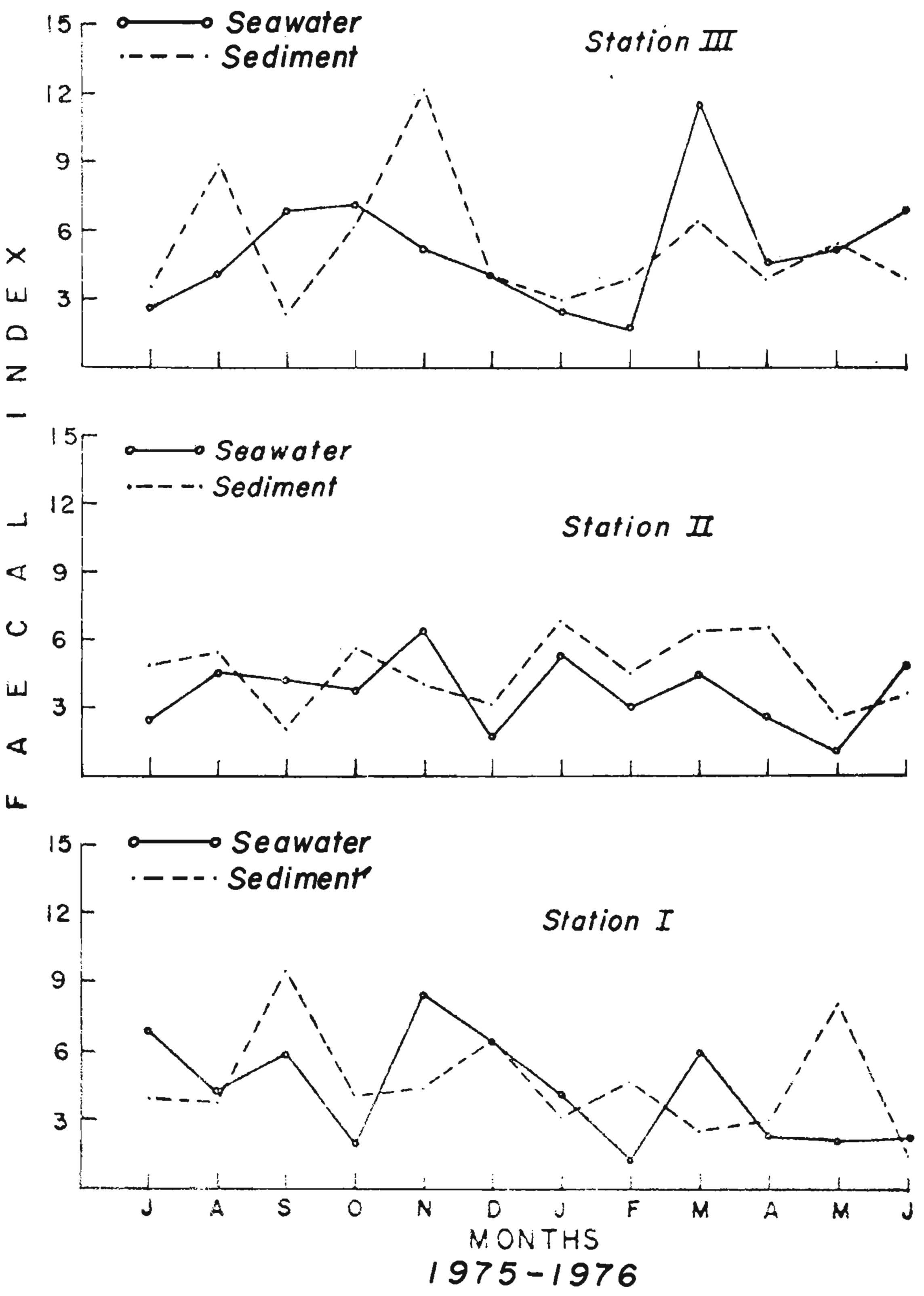
albicans in that it is a known pathogen and its presence in water is apparently directly related to man's activities.

Faecal index:

There is no universally accepted convenient method to distinguish further as to which of the sources, human or animal is primarily responsible for organic pollution. However, studies by Geldreich (1962, 1964) indicated that proportions of faecal coliforms to faecal *Streptococci* (faecal index) can be used with proper caution, for source differentiation i.e. ratios of less than 0.6 should suggest that the pollution is from animal wastes, while for human wastes it should be higher about 4. Using the above considerations as guidelines the ratios of faecal coliforms were computed and evaluated in relation to the information on potential sources of pollution. The results in the Fig.39 shows the ratios of counts of E. *coli* and faecal *Streptococci* from water and sediment. The ratios of E. *coli* to faecal *Streptococcci* ranged from 1.0 in Station II in May 1976 to 11.3 in March at Station III in sea water. In sediment, the faecal index ranged from 2.1 in September 1975 at Station II to 12.1 during November 1975 in Station III.

The use of FC-FS ratios to ascertain whether pollution of waters is of human or animal origin (Geldreich and Kenner, 1969) has been questioned by McFetters, and others (1974)

Fig. 39. Showing faecal index of water and sediment in the area of study during 1975-76.


Seawater
Sediment
Station III
Station II
Station I
FAECAL INDEX
15
12
9
6
3
J
A
S
O
N
D
J
F
M
A
M
J
MONTHS
1975-1976

who pointed out, that the ratios depended upon differential die-away rates of faecal coliforms and faecal streptococci. These workers doubted the reliability of the ratios particularly when applied to bacteria from domestic sewage. However, Feachem (1975) suggested that differential die-away rates may increase the value of FC-FS ratios in determining the source of pollution.

The results of analysis of variance test is given in Table 36 a-e. The main observations from the analysis of total coliforms showed significant difference between stations ($P < 0.01$) and between months ($P < 0.05$) (Table 36 a). Station I was having significantly higher count compared to other stations in both sea water and bottom sediments. Among the first order interractions, region against month showed significance at 5% level indicating that the occurrence of coliforms differed in different months in the two different environment i.e. water and sediment. There is significant difference also in *E. coli* counts between stations ($P < 0.01$) and between regions ($P < 0.01$) (Table 36 b). In Station I significantly higher counts were encountered in both sea water and bottom sediments when compared to other stations. The *E. coli* count was significantly higher in sediments than in sea water.

The counts of *S. faecalis* differed significantly between stations ($P < 0.01$) and between regions ($P < 0.05$) (Table 36c).

TABLE 36. Analysis of Variance Table (1975-76).

36 a. Coliforms:

Source	ss	df	ms	F
Total coliforms	2.9527	71		
Between Stations	1.0115	2	0.5058	31.62**
Between Regions	0.0013	1	0.0013	0.08
Between Months	0.5087	11	0.0462	2.89*
Station x Region	0.0106	2	0.0053	0.33
Station x Months	0.3321	22	0.0151	0.94
Region x Months	0.7361	11	0.0669	4.18*
Error	0.3524	22	0.0160	

36 b. *Escherichia coli*:

Source	ss	df	ms	F
Total *E. coli*	5.5102	71		
Between Stations	1.6341	2	0.8171	23.08**
Between Regions	1.0030	1	1.0030	28.33**
Between Months	0.4200	11	0.0382	1.08
Station x Region	0.3122	2	0.1562	4.41*
Station x Months	0.8200	22	0.0373	1.05
Region x Months	0.5424	11	0.0493	1.39
Error	0.7784	22	0.0354	

36 c. Faecal index:

Source	ss	df	ms	F
Total faecal index	3.9490	71		
Between Stations	0.0877	2	0.0439	1.40
Between Regions	0.0777	1	0.0788	2.49
Between Months	0.6846	11	0.0622	1.99
Station x Region	0.0302	2	0.0151	0.48
Station x Months	1.4032	22	0.0638	2.04
Region x Months	0.5735	11	0.0521	1.66
Error	0.6880	22	0.0313	

36.d. Staphylococci:

Source	ss	df	ms	F
Total Staphylococci	11.3026	71		
Between Stations	2.3306	2	1.1653	13.06**
Between Regions	0.0472	1	0.0472	0.53
Between Months	2.1785	11	0.1980	2.22
Station x Region	0.0327	2	0.0164	0.18
Station x Months	3.7848	22	0.1720	1.93
Region x Months	0.9664	11	0.0849	0.99
Error	1.9624	22	0.0892	

36 e. *[illegible] [illegible]*:

Source	ss	df	ms	F
Total *S. [illegible]*	5.8712	71		
Between Stations	1.6245	2	0.8123	17.10**
Between Regions	0.2676	1	0.2676	5.63*
Between Months	0.6214	11	0.0565	1.19
Station x Region	0.2501	2	0.1251	2.63
Station x Months	1.2846	22	0.0584	1.23
Region x Months	0.7777	11	0.0707	1.49
Error	1.0453	22	0.0475	

Station I showed significantly higher counts when compared to other stations both in sea water and sediments. Among the two regions, sediments showed significantly higher counts than sea water. None of the first order of interactions were significant at 5% level.

No significant difference has been found in the faecal index between stations, regions and months (Table 36 c).

The counts of *Staphylococci* showed significant difference between stations ($P < 0.01$) at 1% level (Table 36 d). Station I was having significantly higher count of *Staphylococci* than the other two stations. There was no significant difference in the counts between regions and months. None of the first order interactions were significant at 5% level.

Product-moment correlation coefficients were calculated between bacterial counts and other physico-chemical parameters of the water samples in sea water and sediment at the three selected sites (Fig. 3) and the results are given in Tables 23 to 28.

In Station I, *Streptococcus faecalis* was significantly ($P < 0.05$) negatively correlated with faecal index (Table 23) whereas in Station II, significant ($P < 0.05$) positive correlation was seen with *Escherichia coli* (Table 24). In Station III *E. coli* showed positive correlation with total heterotrophs (Table 25). Goldreich (1968) found only sporadic coliform isolation during summer and autumn and high counts usually occurred only when sediment counts were high over, 1,00,000 per gram. Faecal index showed significant ($P < 0.0$ positive correlation with organic nitrogen. The importance of organic content of water as a factor which affects the survival of coliform bacteria in the Black Sea was stressed by Krassilnikov (1938), but it has never been demonstrated by Nusbaum and Garver (1955) that faecal coliforms are capable of utilising nutrients present in estuarine water or sediment. It is evident from the present data that coliforms utilise nutrients especially organic nitrogen in the estuarine water for their survival.

In sediments, organic nitrogen showed significant negative ($P < 0.01$) correlations with *Streptococcus faecalis* at 1% level and with *Staphylococci* at 5% level in Station I. Faecal index showed significant ($P < 0.05$) positive correlation with organic carbon at 5% level and significant ($P < 0.01$) negative correlation was seen at 0.1% level with *Streptococcus faecalis* (Table 26). In Station II coliforms were significantly ($P < 0.05$), negatively correlated with phosphate at 5% level. *E. coli* showed significant negative correlation with organic carbon at 1% level and positive correlation at 5% level with coliforms. *Staphylococci* was significantly ($P < 0.05$) negatively correlated with temperature. Faecal index showed negative correlation at 1% level with *S. faecalis* (Table 27).

In Station III, *E. coli* showed significant negative correlations with silicate and total heterotrophs (Table 28). This may be due to excessive growth of general heterotrophic bacterial population that occurred during the time of sampling which will affect the distribution of faecal coliforms at logarithmic period of heterotrophic growth. Faecal index showed significant negative correlation with *S. faecalis* at 1% level which indirectly indicated the source of pollution at Station III was mainly due to human faecal matter. *Staphylococci* showed significant positive correlation with salinity ($P < 0.05$) at 5% level, with decreasing salinity there was corresponding increase in the value of *Staphylococci* both in water and sediment samples. The observed increase may be due to dilution of sea water by rainfall and also may be due to fresh water run-off during the rainy season, which may bring additional *Staphylococci* into the system. Robinson and Mood (1966), Palmquist and Tankow (1973), Boccai and Montanaro (1974) have supported the use of staphylococci and more specifically *S. aureus* as an indicator of body pollution in swimming pool hazards. Another advantage of staphylococci as indicators of potential hazards in swimming pool or any other aquatic environment is that they are more resistant to chlorination than coliforms. Evans (1977) in a recent review of the use of coagulase positive staphylococci as indicators of potential health hazards in water, noted the lack of information on the effectiveness of media and methods for the selective enumeration of staphylococci. In the present study Chapman's Agar was found suitable as a selective media for isolation of *Staphylococcus aureus*. The isolated cultures were confirmed by coagulase test as *S. aureus* contains bacterial enzyme capable of coagulating citrated or oxalated plasma.

The significance of *E. coli* and other coliforms, faecal streptococci and staphylococci in the sea is contingent upon two important considerations. First, how low do indicator bacteria of faecal origin survive in the sea and secondly, do the fish act as carriers of these bacteria in their body or in the intestinal tract?

In the present investigations all the four bacterial indicators were recorded invariably in all the samples investigated which showed that these indicators are capable of survival for long periods in sea water. In addition large numbers of intestinal bacteria are introduced daily in the backwater system through sewage and land drainage.

The importance of an understanding of the factors involved in the destruction of enteric micro-organisms in sea water has been emphasised in recent years (Mitchell and Morris, 1969). But the high counts encountered during the present study may be the result of any one or any combination of various inter-related physico-chemical and biological factors including the adsorption and sedimentation of bacteria. If the rate of adsorption and sedimentation is low and, if there is penuity of toxic substances or bacteriophages and if there is abundant nutrients and less sunlight that will not affect or reduce the coliform or intestinal population. From the more recent investigations it appears that factors which show thermal instability are the most likely causes of this bacterial die-off (Roper & Marshall, 1978). Temperature variations in the present study is not too sharp to affect the indicators as it ranged only between 25.0°C and 30.1°C and only during monsoon time lowest temperature was recorded.

Mitchell *et al.* (1967), Enzinger and Cooper (1976), Roper and Marshall (1977) have emphasised the increase in *E. coli* die-off rates by predator organisms. The role of the indigenous microbial population in the decline of *E. coli* in marine and estuarine water was investigated by McCambridge and Mc Meekin (1979). The result of their studies showed that the survival of *E. coli* was mainly dependent on the presence of protozoan predators and not on the presence of predacious bacteria. The removal of indigenous protozoa by filtration or the use of antibiotics resulted in a reduced destruction of the *E. coli* population in their experiment. Increasing the protozoan concentration caused an increased reduction in *E. coli* numbers. All these results indicated that the predatory protozoan organisms are very limited ~~may~~ be in their distribution in the backwater.

Warren and Rawn (1938) estimated that enough coliform bacteria are discharged by sewage effluents along the west coast of the United States each day to give over a hundred for every litre of water in the North Pacific Ocean, if evenly distributed. Comparable sanitary conditions were found by Weston (1938) on the east coast of United States also. However, such organisms are found only in the tide water, harbours, and bays, which are often badly polluted. California State Bureau of Sanitary Engineering Commission (1943) found that only in solids or greases, coliform bacteria were found to survive for long periods of time in the sea. Buttiaux (1958) and Rittenberg *et al.* (1958) also revealed the high concentration of coliform bacteria around three marine sewage outfalls in California. Enteric pathogen isolation and identification from estuarine and marine environments were reported by Buttiaux and Leurs (1953) and Paoletti (1964), but Beard and Meadowcroft (1935) are of view that a few indicator bacteria survive somewhat longer than some enteric pathogenic bacteria.

Many studies have now shown that coliform bacteria are capable of reproduction in enriched waters and thus by this after growth falsely indicate an elevated health hazard. (Eliassen, 1967; Henricks, 1972; Dutka, 1973). But faecal streptococci cannot reproduce in the sea, the reason why 'faecal strep' and 'faecal index' have been considered in the present study. The desire for an indicator system which unequivocally denotes the presence of faecal material and the existence of a potential health hazard has stimulated recent research along two lines, bacteriological and biochemical. Unlike biological indicators, biochemical indicators like the faecal sterol, coprostanol do not appear to be affected by chemical disinfectants or toxic waste discharge. Furthermore the presence of coprostanol, in water could indicate the existence of faecal pollution in situations where an industrial waste predisposed the use of conventional bacterial indicators. But, several problems have been noted concerning the use of coprostanol as an absolute indicator of faecal pollution. One of these, is laborious procedure required to process each sample (Dutka, Chan, Coburn, 1974). Under ideal conditions only 10 - 12 samples per day can be processed. Another problem is the lack of knowledge concerning the relationship between pathogens (bacteria - Virus), indicators and coprostanol. Dutka and El Shaarawi (1975) have already noted that there appears to be no consistent relationship between faecal sterols and indicator bacteria. The finding of faecal sterol invariably indicates particle bound faecal material, and pathogens (bacteria and viral) associated with polluted water. So, only if, the disinfecting process during sewage treatment makes the use of bacterial indicators infeasible, the presence of potentially dangerous faecal material could still be detected by bio-chemical means.

The natural microbial flora found in sea water are not free swimmers but are usually found in association with plankton, fishes and prawns. The flora nearshore and at harbour mouths contain varying proportion of terrestrial or sewage bacteria. Backwaters also contain varying proportion of freshwater bacteria as well as bacteria of marine origin. Fish harbours bacteria mainly at three sites, (1) the surface slime (10^3 to 10^5/g), (2) the gills (10^5 to 10^6/g), (3) guts (10^5 to 10^8/g), especially in a fish. The muscle or flesh or other internal organs like liver, heart etc. of a healthy fish are sterile. As long as the fish is alive, the activity of these bacteria is under check and they cannot act on fish muscle. But once fish is dead, these bacteria start attacking the flesh, thereby hastening the phenomenon of microbial spoilage. In the case of prawns, the bacterial load is higher than fish and are susceptible to bacterial spoilage much faster than fishes. The pathogenic bacteria which are likely to contaminate the fish are coliforms, *E. coli*, faecal streptococci, *Salmonella*, *Staphylococci*, *Clostridia* etc. All these groups of bacteria are of human origin except perhaps the *Clostridia* whose natural habitat is soil. Apart from indicators, the presence of single cell of *Salmonella* makes the fish unfit for human consumption. These organisms generally do not grow at 0°C but above such temperature they are likely to grow in foods under favourable conditions and infection is ensured by consuming such contaminated food. While *Salmonella* causes infective typhoid fever, the case of *Clostridia* and *Staphylococci* are different. They produce powerful toxin which is lethal to human beings. The fact that fishes caught from polluted sea water may be the carrier of infection was suggested by Stephen *et al.* (1975) after an outbreak of gastro-enteritis in children owing to the enteropathic *E. coli*, as these enteropathogenic

E. coli and coliforms have a tendency to accumulate in fish, shellfish, prawns, crabs and in sediments.

Role of fish as conveyers of micro-organisms in aquatic environments was studied by Peter and Baker (1961). The free swimming fishes and prawns normally carry a population of commensal bacteria, the nature of which has been the subject of investigation by workers in the northern temperate zone (Snow and Beard, 1939; Reay and Shewan, 1949; Georgala, 1958; Colwell and Liston, 1960). A knowledge of the characteristics of these bacteria is very important in understanding the role of these organisms in bringing about spoilage in sea foods. Being marine in origin, the data on the nature and distribution of these micro-organisms in the sea are of fundamental significance to workers studying the correlation between the flora on the fish and of the fishing grounds. Colwell and Liston (1960) have established that a distinct commensal bacterial flora is associated with Pacific Oyster (*Crassostrea gigas*) and that habitat conditions, to a limited extent affect the nature of the microflora in the species.

Some earlier work on the east coast of India refers to the quantitative and qualitative nature of the bacteria in the sea off Madras and Mandapam coasts (Velankar, 1950, 1957). A description of the activities of certain physiological groups of bacteria off Tuticorin coast had been presented by Venkataraman and Sreenivasan (1956). The same authors (1954) had collected some data on the bacteriology of offshore waters of Calicut, but no attempt was made to correlate the flora of fish with microbial flora of environment from where it was caught. But Karthiayani and Mahadeva Iyer (1975) collected data in Cochin coast on the distribution of heterotrophic bacterial flora of offshore water and sediment and also microflora of fresh fish and prawns caught therein, and found no correlation between the two. But positive correlation can be expected only between bacterial indicators and fish as they are of animal origin and definitely show some affinity to animal protein. Wolfe (1972) established the importance of shellfish from faecally polluted waters in transmission of intestinal infection.

Total heterotrophs showed significant negative correlation with temperature at 1% level at Station II in surface water. Biswas (1972) also found in Volta Lake, that the bacterial populations were unaffected by changes in temperature but there was evidence of an inverse relationship only with transparency in the surface layers. It was reported by George and Gaudy (1973) and Guthrie *et al.* (1974) that bacterial populations are generally sensitive to temperature changes only in low nutrient concentration of sea water. Klein and Wu (1974) also found that starvation for nutrients could increase the susceptibility of heterotrophic bacteria to slight temperature changes. Furthermore, as Klein and Wu's conclusions were drawn from experiments with resting cells the type of stress exerted is not readily comparable with that imposed on starved but growing cells. They also stated that a decline of coliforms at high temperature was prevented by additional nutrients. This finding also corresponds to those of Verstraete *et al.*'s (1975) observation that faecal bacteria are favoured at high temperatures and nutrient levels. Negative correlation between heterotrophic bacteria and temperature may be due to poor nutrient concentration of sea water at Station II.

Mc Cambridge and Mc Meekin (1979) reported that the survival rate of *E. coli* was reduced during the summer months as compared to early spring. This reduction was due to the increase in temperature of the water which resulted in increased ecological interactions (Verstraete and Voets, 1976). Similar results were noted in two further sampling sites in the Derwent estuary (Mc Cambridge, 1977).

In the present study, reliable data on the distribution of indicator organisms and pathogens in the estuarine environment was obtained only at Station I. This reliability was due to the location of the Station I near the sewage outlet. But in all the three stations the faecal index was always above 4, which showed that the source of pollution is mainly from human wastes. Studies on the sources of bacteria in the estuarine environment of Cochin by Gore and Raveendran (1979) indicated that the bacteria were predominantly of non-human origin.

The observation during 1975-76 also showed more counts in sediments than in sea water. Bruni _et al_. (1972) worked in water and sediment from Lake Cranzirri (Italy) and found relatively high counts in sediments than in sea water. Also high counts were encountered by them in coastal water than in open ocean waters. Bruni _et al_. (1972) also found some correlation between indicators and heterotrophic bacteria. In the present study in Station III (Table 25) _E. coli_ was significantly ($P < 0.05$) positively correlated with total heterotrophic counts in sea water.

The occurrence of several fold more indicator bacteria in sediment samples in the estuarine environment as compared to those in water samples is not surprising in view of a previous study in which Gerba and Mc Leod (1976) showed that _E. coli_ survived for a longer period in sediment than in sea water. Occurrence of a greater number of micro-organisms of faecal origin in bottom sediments has also been reported previously (Van Donsel and Geldreich, 1971; Hendricks, 1971; Goyal _et al_., 1977). The presence of a greater number of enteric bacteria in sediments may be due to several factors -

(1) die-off may be more at the surface where U.V. radiation is more. (2) Sediments may be rich in organic nutrients. (3) Bacteria adhering to particulate matter may settle down, because of sedimentation process. (4) Adsorption of bacteri to marine silt and other solids may occur.

The sediments rich in pathogens may easily be resuspended following storms, boating and dredging. Since, a significant proportion of both pathogenic and non-pathogenic bacteria and other micro-organisms is present in sediments (Gerba *et al*., 1977; Goyal *et al*., 1977), they may pose a potential health problem on resuspension. This problem is compounded by the occurrence of drug resistance in a large number of isolates from sediment.

The presence of large number of coliforms or faecal coliforms in the absence of bacterial pathogens is of no consequence because these organisms are usually considered as harmless indicators of water quality. However, this is not necessarily true if the bacteria in question possess transferable drug resistance. Once, these organisms enter the gastro-intestinal tract of humans, they may colonise the human gut themselves, transfer their resistance to already colonised bacteria or transfer their R-factors to the sensitive pathogens with which their host may become infected (Smith, 1969; Farrar *et al*., 1972). R^+ bacteria may survive longer or as long as the antibiotic-sensitive bacteria in certain water environments (Smith *et al.*, 1974). Coliforms with R-factors were shown to increase in maturation ponds from 0.86 to 2.45 % during treatment of conventionally purified sewage (Grabow *et al.*, 1973, 1975). R$^+$ faecal coliforms are not killed in sea water nor do they have a detectably different survival rate in sewage contaminated sea water to drug-sensitive faecal coliforms (Smith, 1971). In a limited laboratory study, Goyal *et al.*, (1979) found, R$^+$ bacteria to survive as long as the antibiotic sensitive ones in sea water containing sediment material.

It can be concluded that coastal and estuarine waters contaminated to the extent of those of the present study may serve as a reservoir of R$^+$ bacteria (as evidenced by the serological and antibiotic resistance work in Part B, Section III), which may find their way into human beings through consumption of raw shellfish, prawns, fishes caught or cultured in sewage contaminated water.

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SECTION III.

ZYMOGENOUS HETEROTROPHIC MICROBES ASSOCIATED WITH *SALVINIA MOLESTA* AUBLET IN THE INSHORE ENVIRONMENT.

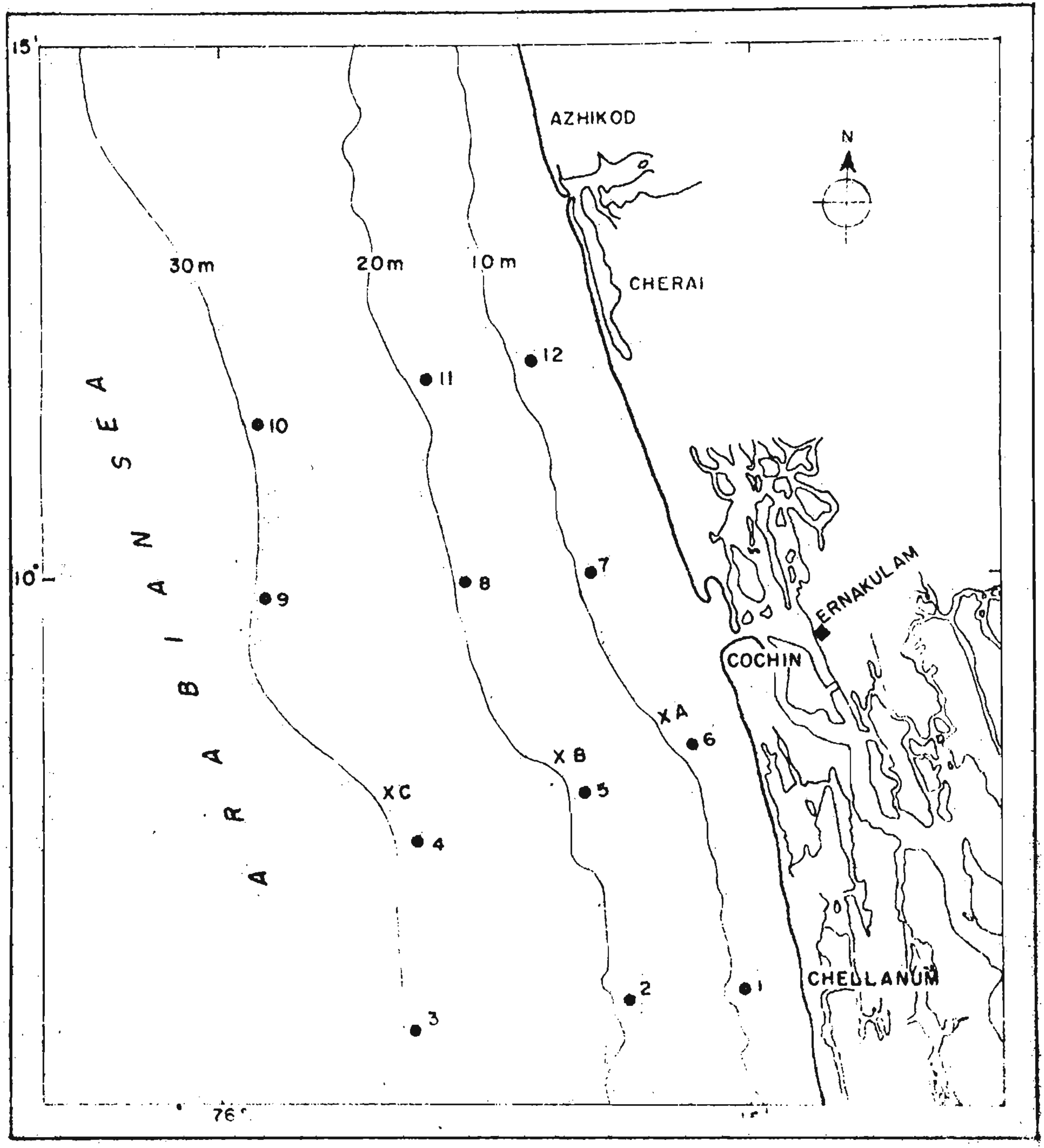
III. ZYMOGENOUS HETEROTROPHIC MICROBES ASSOCIATED WITH SALVINIA MOLESTA AUBLET IN THE ESTUARINE ENVIRONMENT.

The water fern *Salvinia molesta* Aublet locally known as "African Payal" is originally a native of Central and South America (Mitchell, 1969). It has radiated to different parts of the world and in recent years has successfully colonised in Kerala waters to the extent of emerging as a serious aquatic weed problem (Menon, 1971). The weeds are transported into backwaters during the monsoon and in the postmonsoon months when the rivers run low, tidal water sweeps large quantities of the weed upstream, building up stagnant blankets over backwaters where they undergo decay owing to salinity and get deposited at the bottom. Thus Cochin Backwater system receives allochthonous organic matter, primarily from the floating weed during monsoon and postmonsoon periods. Nutritional significance of detritus along with associated micro-organisms in estuarine ecosystem has been studied by various authors (Odum, 1967; Fenchel, 1970). Although there were a few reports on the fauna associated with *Salvinia molesta* (Gopalan and Sreekumaran Nair, 1975) and microbial decomposition of the floating weed (Radhakrishnan *et al.*, 1979) there is no information on the enrichment of zymogenous bacterial population in response to the allochthonous organic matter in the sediment ecosystem and its effect on the living resources of the inshore waters.

This section presents results of the investigations on the short term effects of weed deposits in the inshore areas off Cochin during January 1978 to October 1978. Data includes hydrographic properties as well as zymogenous aerobic heterotrophic bacteria isolated from decomposing weed in the backwaters. The structure, bio-chemical activity and physiological groups of bacteria were also studied as this would provide an understanding of the impact of the organic detritus on microbial populations within the sea water - sediment ecosystem.

During 1978 January to October, from 12 stations indicated in the Fig. 40 in a grid of four transects at the respective depths of 10, 20 and 30 metres water samples were collected from surface and bottom and analysed for salinity, dissolved oxygen and nutrients by standard methods. Water and sediment samples were suitably diluted and plated on Zo Bell's medium "2216", Kuster's Agar, and Martin's rose Bengal agar for the enumeration of bacteria, actinomycetes and fungi respectively. All the zymogenous bacteria were isolated in their selective media in suitable dilutions.

Fig. 40. Area of study during January - October 1978.


15'
10°
76°
15'
AZHIKOD
CHERAI
N
30m
20m
10m
ARABIAN SEA
ERNAKULAM
COCHIN
CHELLANUM
XA
XB
XC
1
2
3
4
5
6
7
8
9
10
11
12

Colonies weere counted after the respective incubation period at RT. Counts for the sediments were averaged and expressed in the basic unit weight of oven-dried mud. In total 45 pure strains were isolated and stored on stock culture agar for further bio-chemical and physiological reactions. The generic classification of bacterial isolates was done according to a modified scheme of Usio Simidu and Kazuyoshi Aiso (1962). An assessment of the proteolytic activity of the bacteria was made by growing them on agars enriched with 4 nitrogenous source. Chitinolytic bacteria were isolated in mineral medium (Aaronson, 1970). Ureolytic population in Christensen's urea agar (2%). Gelatinolytic population was enumerated on Frazier's nutrient gelatin agar after flooding the plates with 0.1% acidic mercuric chloride. Caseinolytic population was detected by the appearance of clear zones around the colonies in casein meat agar. Amylolytic micro-organisms were enumerated on starch mud extract agar (3 g starch/litre), after flooding the plates with Grams iodine solution. The lipolysis of Tween 80 was determined by noting the presence of insoluble Oleic acid precipitated around the colony; pH of the mud was measured with 1 : 2 mud : water ratio.

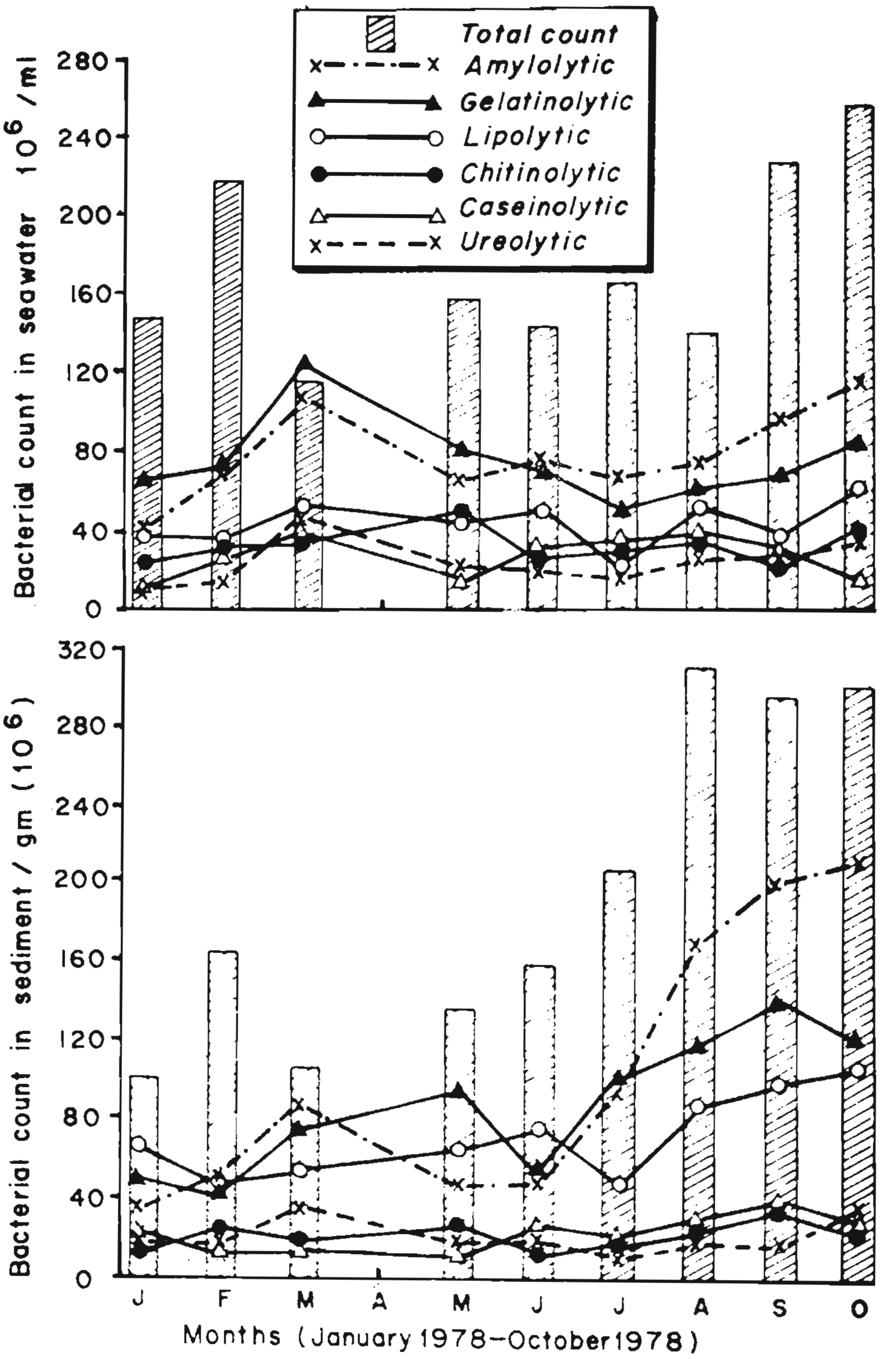
The nutritional grouping techniques of the predominant bacteria were a modification of the method of Lochhead and Chase (1943) with minor modifications. 45 bacterial colonies from the greatest dilution of sea water and mud samples were transferred to the slants after 5 days incubation. Three day old inocula from the slants incubated at (RT) were suspended in 2.5 ml sterile saline and transferred aseptically to sterile centrifuge tubes. Cells were harvested by centrifugation at 2000 rpm for 5 minutes and resuspended in 2.5 ml sterile saline. One loopful of washed cells was inoculated into each tube of broth of an increasing nutritional complexity series. The amino acid mixture was prepared using 1 g/litre vitamin free casamino acids (DIFCO). Tubes were incubated at (RT) in the dark and were considered positive if turbidity developed from an originally clear tube after 10 days incubation. Growth in mud extract broth served as a control for culture viabili

Coliform and faecal coliform determinations were made by the most probable number method (MPN) according to standard methods for the examination of water and waste water (Am. Water Works Assoc. *et al.*, 1976). Lactose broth was used for the coliform test and Ec broth was used for faecal coliform test. All of the analytical methods were those described by Strickland and Parsons (1968).

The moisture content ranged from 10.0 to 10.8% and the pH ranged from 7.23 to 8.71. Within these ranges it was concluded that variations in populations were not caused by variations in sediment moisture or pH.

The occurrence of zymogenous bacterial pattern is given in Fig. 41 along with total bacterial population. The monthly total bacterial count per ml of sea water varied within a very limited range indicating the existence of a fairly constant level of population in the surface waters and in the detritus rich sediments. The standing crop of bacteria which are responsible for degradation of *Salvinia* at various depths is given in the Table 37. Zymogenous bacteria (proteolytic, amylolytic and lipolytic) were recorded more in sediment than in water even in 30 m depths. The total bacterial population ranged from 99.36 x 10^6 to 265.32 x 10^6/gm in the sea water and in the sediments from 137.2 x 10^6 to 232.6 x 10^6/ml; 9 genera of bacteria belonging to six families *viz.* Neisseriaceae (25%); Pseudomonadaceae (10%); Vibrionaceae (25%); Micrococcaceae (5%); Bacillaceae (5%) and Enterobacteriaceae (25%) and Contaminants (5%) were found associated with the weed deposits. *Alcaligenes*, *Pseudomonas* and *Vibrio* occurred in abundance in all the three seasons. There was no marked pattern of distribution among the microflora, but all the zymogenous microflora exhibited the maxima in the postmonsoon period. The interrelationship of different microbial flora is given in Table 38. From the present investigation it is evident that marine microbes play a significant role in biodegradation

Fig. 41. Proteolytic, Amylolytic, lipolytic and chitinolytic activity of heterotrophic bacteria isolated during 1978.



Total count
Amylolytic
Gelatinolytic
Lipolytic
Chitinolytic
Caseinolytic
Ureolytic
Bacterial count in seawater 10^6/ml
0
40
80
120
160
200
240
280
Bacterial count in sediment / gm (10^6)
0
40
80
120
160
200
240
280
320
J
F
M
A
M
J
J
A
S
O
Months (January 1978–October 1978)

TABLE 37. Standing crops of the bacteria which are responsible for the processes of degradation of Salvinia in various water depths.

Bacterial process in Salvinia degradation in sediments and waters	Water/cells/ml				bottom sediments (cells/g)			
	10 m	20 m	30 m	40 m	10 m	20 m	30 m	40 m
1. Total heterotrophs	10^3-10^5	10^3-10^5	10^2-10^4	10^0-10^3	10^4-10^5	10^4-10^5	10^3-10^5	10^5-10^6
2. Proteolytic bacteria	10^1-10^4	10^1-10^3	10^1-10^3	10^1-10^2	10^2-10^6	10^3-10^5	10^0-10^4	-
3. Amylolytic bacteria	10^2-10^5	10^2-10^4	10^0-10^3	10^0-10^3	10^3-10^7	10^4-10^5	10^3-10^5	10^5-10^6
4. Lipolytic bacteria	10^2-10^5	10^2-10^3	10^2-10^4	-	10^3-10^7	10^4-10^5	10^3-10^5	10^5-10^6
5. Chitinolytic bacteria	10^1-10^1	10^1-10^1	-	-	10^2-10^4	10^3-10^4	10^2-10^4	10^0-10^2
6. Caseinolytic bacteria	10^0-10^1	10^1-10^1	-	-	10^0-10^2	10^1-10^3	10^5-10^2	10^1-10^1
7. Ureolytic bacteria	10^0-10^1	10^0-10^1	-	-	10^0-10^1	10^0-10^1	-	-

TABLE 38. Occurrence of microflora and their inter-relationship in the surface water and sediments in the inshore area of Cochin.

Months		Bacteria (10^6/ml)	Actinomycetes (10^6/ml)	Fungi (10^6/ml)	Inter-relationship between different microflora		
					B : F	B : A	F : A
January	SR	99.36	21.63	28.34	3.50	4.59	1.31
	SD	145.60	42.81	8.75	16.64	3.40	0.20
February	SR	166.60	28.46	44.63	3.73	5.85	1.56
	SD	197.00	56.46	16.40	12.01	3.48	0.29
March	SR	107.33	32.50	18.40	5.83	3.30	0.56
	SD	116.63	62.64	24.50	4.76	1.86	0.39
April	--	-	-	-	-	-	-
May	SR	137.40	40.40	19.48	7.05	3.40	0.48
	SD	152.66	80.10	22.46	6.79	1.90	0.28
June	SR	158.60	31.80	25.36	6.25	4.98	0.79
	SD	140.80	64.74	18.46	7.62	2.17	0.28
July	SR	186.60	44.32	60.42	3.08	4.21	1.36
	SD	166.40	72.16	37.20	4.47	2.30	0.51
August	SR	265.32	66.16	66.16	4.01	4.01	1.00
	SD	137.16	68.28	24.50	5.59	2.00	0.35
September	SR	252.60	64.20	63.21	3.99	3.93	0.98
	SD	212.86	66.16	22.62	9.41	3.21	0.34
October	SR	256.40	52.62	71.64	3.57	4.87	1.36
	SD	232.64	82.06	32.20	7.22	2.83	0.39

SR = Surface; SD = Sediments; B : F = Bacteria : Fungi;

B : A = Bacteria : Actinomycetes; F : A = Fungi : Actinomycetes.

of water weeds like _Salvinia_. Strains isolated from sea water has got equal potency in the biochemical activity when compared to the bacterial strains isolated from the sediment. Fungi and actinomycetes were found to be only secondary disintegrators of organic matter in the marine environment.

The morphological, physiological and biochemical activities of the 45 bacterial strains isolated during the study are given in Table 39. The table incorporates the details of the 20 biochemical tests made on the strains and percentage incidence of amino acid requiring bacteria. All isolates were viable since they all grew when transferred to mud extract broth. Almost all of the isolates were asporogenous gram negative rods. 45% of the isolates liquified gelatin, hydrolysed starch and fermented dextrose. 60% of the isolates reduced nitrates and utilised casamino acids as their nitrogenous source.

Statistical analysis:

The results of statistical data are given in Tables 40 to 49. The coefficient of correlation between the total count and each bacterial flora with the hydrological parameters like salinity, temperature, oxygen and nutrients were

TABLE 39. Morphological and Physiological characteristics of 45 bacterial strains isolated from the inshore region of Cochin from January to June 1978.

Characteristics	Frequency of occurrence (%)
Gram Positive	2.20
Negative	97.70
Motility	66.60
Pigmented	26.66
O/F media	44.44
Oxidative	11.11
Fermentative	22.22
Alkaline	22.22
Gelatin hydrolysers	42.22
Starch hydrolysers	44.44
Nitrate reducers	60.00
H_2S producers	6.66
Oxidase	97.77
Catalase	93.33
Urease	97.77
Citrate reducers	73.33
Indole producers	20.00
M.R.	15.55
V.P.	40.00
Glucose	42.22
Lactose	44.40
Sucrose	44.40
Mannitol	40.00
Penicillin Resistance test Single disc method (20 I.U.)	97.70

TABLE 40. Correlation coefficient between zymogenous bacterial count and environmental parameters in the Stations north of Cochin Barmouth in the month of January 1978 (Sea water). (Station Nos. 7 - 12).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatinolytic	Caseinolytic	Amylolytic	Lipolytic
Temperature	0.62326	-0.48479	0.99220[c]	0.43294	-0.66118	0.97394[b]	0.55055
Salinity	0.20830	-0.43692	0.27861	-0.29618	0.63556	-0.19862	0.23456
Oxygen	0.97795[b]	-0.43070	0.81545	-0.44853	-0.55049	0.63049	0.94818[a]
Phosphates	0.18334	-0.29478	-0.16233	0.41468	-0.92103[a]	0.50446	0.07554
Nitrites	-0.17415	-0.52577	-0.47116	0.97179[b]	-0.35858	0.42289	0.32029
Nitrates	-0.57549	0.37155	-0.59669	-0.16309	0.50995	-0.69535	-0.56497

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level

TABLE 41. Correlation coefficient between zymogenous bacterial count and environmental parameters in the stations (Nos. 7 - 12) north of Cochin in the month of January 1978 (Sediments).

Zymogenous bacteria / Environmental parameters	Total count	Chitino-lytic	Ureolytic	Gelatino-lytic	Caseino-lytic	Amylolytic	Lipolytic
Temperature	0.69772	0.46418	-0.02129	0.75317	0.57551	0.69831	-
Salinity	0.35599	-0.08838	-0.99940^c	-0.43513	-0.07521	0.25519	-
Oxygen	0.69106	0.49158	0.65794	0.67311	0.02625	-0.18289	-
Phosphates	0.93258^a	0.89561	0.51967	0.91030^a	0.58502	0.26115	-
Nitrites	0.10442	0.10959	-0.78492	-0.24973	-0.39965	-0.10977	-
Nitrates	0.76900	-0.75218	-0.03423	-0.66943	-0.31897	-0.10977	-

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level

TABLE 42. Correlation coefficient between zymogenous bacterial count and environmental parameters in the Stations (Nos. 7 - 12) north of Cochin Barmouth in the month of February 1978 (Sea water).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatinolytic	Caseinolytic	Amylolytic	Lipolytic
Temperature	0.61683	0.86943[a]	0.79295	0.60491	-0.09330	0.59760	-0.13959
Salinity	0.88237[a]	0.39327	0.80346	0.89409	-0.57960	0.61325	-0.01690
Oxygen	-0.18371	-0.71578	-0.79482	-0.55204	0.27603	-0.27639	0.48736
Phosphates	-0.22999	-0.82790	-0.59428	-0.67054	0.19341	-0.49596	0.48370
Nitrites	0.22460	-0.53088	-0.07939	-0.13316	-0.31981	-0.48017	0.92643
Nitrates	-0.17984	-0.58901	0.27158	0.35075	-0.83089[a]	-0.22941	-0.47792

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level.

TABLE 43. Correlation coefficient between zymogenous bacterial count and environmental parameters in the Stations (Nos. 7 - 12) north of Cochin Barmouth in the month of February 1978 (Sediments).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatinolytic	Caseinolytic	Amylolytic	Lipolytic
Temperature	0.73366	0.89465^a	0.24472	0.58779	-0.59094	0.87491^a	0.26123
Salinity	-0.16271	-0.27625	0.62981	0.68134	-0.17260	-0.11411	0.57898
Oxygen	-0.53351	-0.52179	-0.38098	-0.23729	0.29878	-0.30229	0.78681
Phosphates	0.14646	-0.34518	-0.35189	-0.19298	-0.57430	0.29755	0.48963
Nitrites	0.28929	0.06211	0.37389	0.59828	-0.79491	0.45417	0.48724
Nitrates	0.00156	-0.00724	-0.05094	0.62335	0.06419	0.10404	0.86636^a

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level

TABLE 44. Correlation coefficient between zymogenous bacterial count and environmental parameters in Stations (Nos. 7 - 12) north of Cochin Barmouth in the month of March 1978 (Sea water).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatino-	Caseinolytic	Amylolytic	Lipolytic
Temperature	0.36904	0.64108	0.61748	-0.051045	0.44976	0.19333	0.71694
Salinity	0.20975	-0.42980	-0.10820	+0.05530	-0.09493	-0.08547	-0.59851
Oxygen	0.037745	-0.53538	-0.64430	0.40141	0.10384	0.31991	0.21861

TABLE 45. Correlation coefficient between zymogenous bacterial count and environmental parameters in Stations (Nos. 7 - 12) north of Cochin Barmouth in the month of March 1978 (Sediments).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatinolytic	Caseinolytic	Amylolytic	Lipolytic
Temperature	-0.21087	-0.50911	0.15847	0.01267	-0.47971	0.79483	-0.14545
Salinity	-0.14374	-0.70393	0.48271	-0.50835	0.63634	-0.41880	0.09793
Oxygen	0.65946	0.78383	-0.72280	0.67847	-0.45941	0.26606	0.19978

TABLE 46. Correlation coefficient between the six zymogenous bacteria and the physico-chemical factors in Stations (Nos. 1 - 6) south of Cochin Barmouth during January 1978 (Sea water).

Zymogenous bacteria / Physico-Chemical factors	Total count	Chitino-lytic	Ureolytic	Gelatino-lytic	Caseino-lytic	Amylolytic	Lipolytic
Temperature	-0.33079	-0.058717	-0.45347	-0.46588	0.18044	0.35817	0.01827
Salinity	0.88434	0.92369^a	0.80690	0.60358	0.34340	0.79640	0.30530
Oxygen	-0.45828	-0.72750	-0.34118	0.10049	-0.58076	-0.77705	-0.70589
Phosphates	-0.48896	-0.47706	-0.58733	-0.33772	0.34713	0.25830	-0.37915
Nitrites	-0.71844	-0.70786	-0.69423	-0.21123	-0.67629	-0.54062	-0.78206
Nitrates	0.58334	-0.34540	0.63684	0.75126	-0.42484	0.86901	-0.21763

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level

TABLE 47. Correlation coefficient between the six zymogenous bacteria and the physico-chemical factors in Stations (Nos. 1 - 6) south of Cochin Barmouth during January 1978 (Sediments).

Zymogenous bacteria / Physico-chemical factors	Total count	Chitino-lytic	Ureolytic	Gelatino-lytic	Caseino-lytic	Amylolytic	Lipolytic
Temperature	-0.93235^{a}	-0.79407	0.88342^{a}	0.84293	0.35319	-0.78651	
Salinity	0.37033	0.92434^{a}	-0.43085	-0.60239	0.30468	-0.99834^{c}	
Oxygen	0.88162^{a}	0.30861	-0.69139	-0.53007	-0.53301	0.83161	
Phosphates	-0.11927	0.057025	-0.57716	-0.59908	-0.88100^{a}	0.63061	
Nitrites	0.94551^{a}	0.82179	-0.88034^{a}	-0.85007	-0.86421	0.76949	
Nitrates	-0.66176	0.11101	0.31794	-0.10692	0.41232	0.37295	

a = Significant at 5%level
b = Significant at 1% level
c = Significant at 0.1% level

TABLE 48. Correlation coefficient between zymogenous bacterial count and environmental parameters in the Stations (Nos. 1 - 6) south of Cochin Barmouth in the month of February 1978 (Sea water).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatinolytic	Caseinolytic	Amylolytic	Lypolytic
Temperature	-0.49484	-0.41815	-0.01225	0.01233	0.02969	-0.79328	-0.46777
Salinity	-0.87767	0.06957	-0.12465	0.95360	-0.46595	-0.59003	-0.01827
Oxygen	0.71254	-0.10322	0.17547	-0.84403^a	-0.12910	0.30540	- 0.57664
Phosphates	-0.00093	-0.21830	-0.09896	0.03749	-0.48884	-0.11179	-0.19842
Nitrites	-0.05584	-0.58942	-0.21923	0.03351	-0.66705	0.11706	-0.09706
Nitrates	0.89772^a	-0.61655	-0.18028	-0.44911	0.27936	0.87692^a	0.50934

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level

TABLE 49. Correlation coefficient between zymogenous bacterial count and environmental parameters in the Stations (Nos. 1 - 6) south of Cochin Barmouth in the month of February 1978 (Sediments).

Zymogenous bacteria / Environmental parameters	Total count	Chitinolytic	Ureolytic	Gelatinolytic	Caseinolytic	Amylolytic	Lypolytic
Temperature	-0.41379	-0.49237	0.41710	0.00608	-0.56770	0.059373	-0.71719
Salinity	-0.22309	-0.27237	-0.29508	-0.00061	-0.38722	-0.17291	0.07387
Oxygen	0.94083^b	0.69391	-0.62364	-0.18610	0.35377	0.33255	0.29702
Phosphates	-0.34520	-0.70142	0.28793	0.25208	-0.82026^a	-0.08487	-0.52740
Nitrites	0.17197	-0.53533	0.33551	0.83096	-0.49453	-0.06740	0.04249
Nitrates	0.71043	0.61425	-0.80230	-0.60538	0.25319	0.60378	0.37476

a = Significant at 5% level
b = Significant at 1% level
c = Significant at 0.1% level

calculated for the months of January, February and March for samples of sea water and sediments obtained from two sets of stations located south and north of Cochin barmouth.

During January in sea water except for the significant positive correlation between ureolytic and amylolytic bacteria with temperature, somewhat high correlation was observ between Gelatinolytic bacteria with nitrates. Total counts and lipolytic bacteria also showed high positive correlation with oxygen in north stations. Significant negative correlation was observed between caseinolytic bacteria with phosphate (Table 40).

In south stations significant positive correlation was found only in the case of chitinolytic bacteria with salinity and all other parameters were found to be non-significant (Table 46). The sediment samples collected from stations in the north showed (Table 41) significant positive correlation of total counts and Gelatinolytic bacteria with phosphates. Significant negative correlation was observed between Ureolytic bacteria with salinity. Samples collected from stations located in the south showed significant positive correlations between total count with temperature, oxygen and nitrites, chitinolytic bacteria with salinity, ureolytic bacteria with temperature and nitrite and caseinolytic bacteria with phosphate (Table 47).

Velankar (1955) reported the absence of any relationship between temperature and bacterial population. In the present study significant negative correlation was observed between bacterial count with temperature, lipolytic, amylolytic and ureolytic bacteria with salinity, phosphates and nitrite respectively.

During February sea water samples from stations north of Cochin showed significant positive correlation (Table 42) between chitinolytic bacteria and temperature and total counts showed significant positive correlation with salinity (at 5% level). Significant negative correlation at 5% level was observed between caseinolytic bacteria with nitrates. In the south stations samples (total count, and amylolytic bacteria) showed significant positive correlations at 5% level with nitrates. The relationship between gelatinolytic bacteria with oxygen was negative and significant (Table 48). All these results show that the temperature and salinity was favourable for the growth of chitinolytic bacteria and total bacterial population in the sea water. The level of significance varied between 0.1 to 5%.

The sediment samples from north during February showed significant positive correlation between chitinolytic and amylolytic bacteria with temperature, whereas lipolytic microbes showed positive correlation with nitrates (Table 43).

The sediment flora of southern region showed significant positive correlation between total count and oxygen. Significant negative correlation was found between caseinolytic bacteria with phosphates (Table 49).

During March, samples collected from stations in the north showed no significant correlations between hydrological parameters and the six zymogenous bacterial flora in sea water and in sediment samples (Tables 44 & 45). Brown (1964) also found no correlation between bacterial counts with temperature and phytoplankton in the waters off Sydney. Nutrients especially nitrates and nitrites showed positive correlation with all the strains isolated in the present study. Gundersen et al. (1972) also support the correlation between the concentrations of nutrients and bacteria.

In the present findings the coefficient of correlation of bacteria with chemical parameters like nitrate, nitrite and phosphate are rather high than with physical parameters thus supporting the view that the nutrient concentration play an important role in the distribution of bacteria.

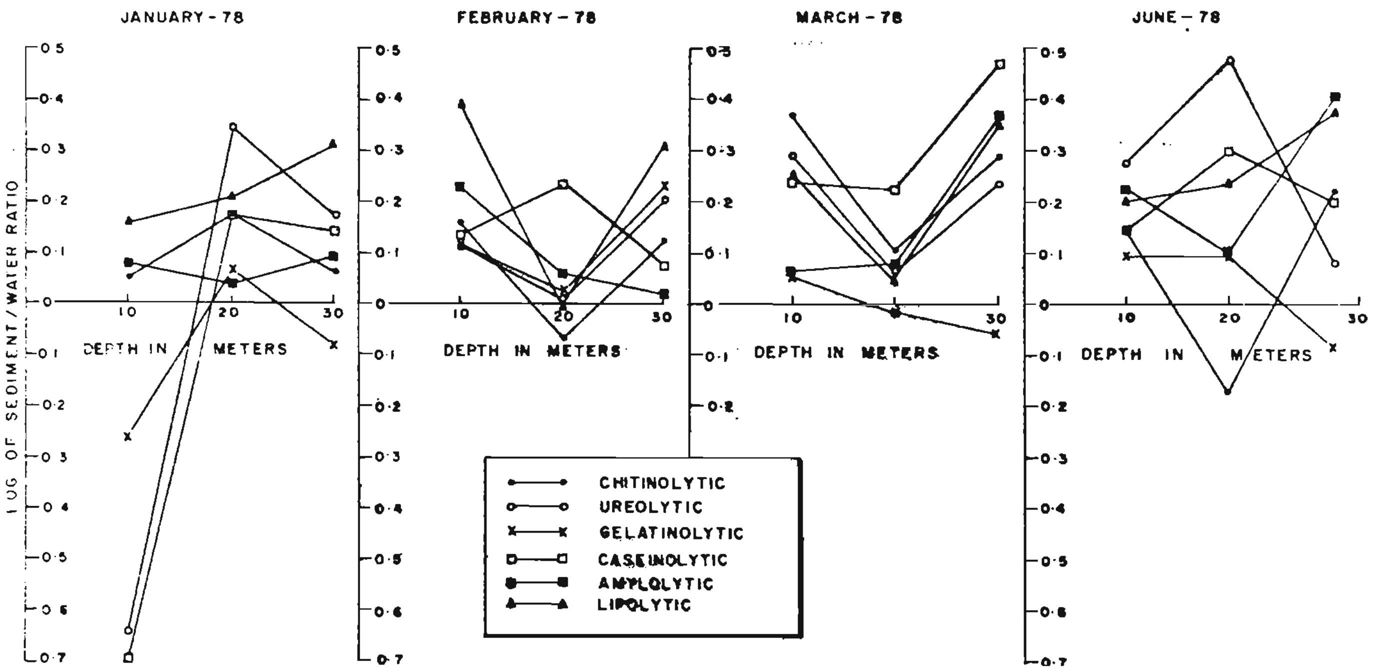
To test whether the total bacteria in north and south stations differ significantly in sea water and sediment data 't' test was employed. It was observed that the north and south stations do not differ significantly as far as total count is concerned (for sea water samples t = 2.075, degrees of freedom = 20 for sediment samples t = 1.46 with degrees of freedom = 20).

The sediment/water ratio was constructed to compare the community structure of physiological groups of micro-organisms within the sampling area receiving the Salvinia weed deposits. The distribution pattern of the six zymogenous bacteria in 10, 20 and 30 m depth during the sampling period from January to June is given in the Fig.42.

During January the sediment : water ratio (S/W) of proteolytic bacteria were at 20 m depth whereas S/W ratio of amylolytic bacteria were encountered less. S/W ratio of lipolytic bacteria were encountered only in moderate numbers in 20 m depth. The S/W ratio of the 6 zymogenous bacteria were generally less at 30 m when compared to their S/W ratio at 10 m and 20 m depth range in January. In February except chitinolytic bacteria all the other proteolytic microbes were encountered more at 20 m depth. Amylolytic activity was more at 10 m moderate at 20 m and less at 30 m. Gelatinolytic activity was more at 30 m and less at 20 m and moderate at 10 m. S/W ratio of caseinolytic bacteria was more at 20 m and less at 30 m.

In March S/W ratio of lipolytic and casein degrading bacteria were encountered in the same pattern as in the previous months. There is a similarity in the S/W ratio of chitinolytic and ureolytic bacteria in all the observations. The S/W ratio of amylolytic and gelatinolytic bacteria were abundant in the reverse order.

Fig. 42. Sediment/water ratio of chitinolytic, ureolytic, gelatinolytic, caseinolytic, Amylolytic and lipolytic organisms isolated during 1974-75.


JANUARY - 78
FEBRUARY - 78
MARCH - 78
JUNE - 78
LOG OF SEDIMENT / WATER RATIO
0·5
0·4
0·3
0·2
0·1
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
10
20
30
DEPTH IN METERS
CHITINOLYTIC
UREOLYTIC
GELATINOLYTIC
CASEINOLYTIC
AMYLOLYTIC
LIPOLYTIC

In June the S/W ratio of caseinolytic microbes were encountered more at 20 m and less at 30 m transect and moderate at 10 m. The S/W ratio of amylolytic bacteria were more at 30 m and in 10 m. At 20 m they were only in moderate numbers. From the studies of organic production and the food chain of Cochin Backwaters it is known that a major portion of energy is available in the form of detritus (Qasim, 1970). The detritus constitutes an additional pathway between organic production and animal nutrition increasing the efficiency of energy transfer from one trophic level to other. The production of *Salvinia* detritus is seasonal in that the fresh weeds come into the estuarine system in monsoon months undergo decomposition leaving detritus which accumulates in the bottom. It is observed that the southern stations were seen densely covered with decayed weed masses indicating the effect of southward water currents. Fenchel (1970) has stated that the slowly decomposing plant body may enable the ecosystem to continue functioning even when the primary producers are temporarily removed. In the present investigation it was observed that the floating weed *Salvinia* was continuously attacked by the zymogenous bacteria in sediments which may prove beneficial especially in the absence of other primary producers.

From the overall picture obtained by the observations, the fishing ground off Cochin in the depth range of 10 to 20 m appeared to be more dynamic and productive when compared to other transects investigated in the inshore region.

The indicator bacteria observed using MPN methods during this study in sediments and in overlying water are given in Tables 50 and 51. Sea water from southern area to the Cochin harbour contained 4370 to 15,000/100 ml of total coliforms (Table 50). Northern stations to the Cochin harbour ranged from 4870 to 12,000 total coliforms in sea water and 2,50,000 to 30,00,000/100 ml in sediments. E. coli ranged from 160 to 18,000/100 ml in sea water and 10,000 to 8,40,000/100 ml in sediments (Table 51). The southern stations recorded a range of 160 to 20,000/100 ml of E. coli in sea water and 50,000 to 24,00,000/100 ml in sediments. The pollution microbes were encountered more in southern stations than in northern stations of the Cochin harbour.

The bacterial standard for the tolerance limits for shellfish and commercial fish culture has been given by Indian Standard Institution (Table 52). The National Technical Advisory Committee on water quality criteria has also recommended a limit of 1000 total coliforms per 100 ml and 200 faecal coliforms per 100 ml for recreational water (Table 52a). These values were exceeded in 2 out of 3 monthly samples taken during the course of the sampling period in this study.

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TABLE 59. Coliform (average) faecal coliform values per 100 ml of water or wet sediment in south Stations.

Sampling Stations	Coliforms				Faecal coliforms			
	Water		Sediment		Water		Sediment	
1	4,370	12,000	1,70,000	13,00,000	20	20,000	20,000	24,00,000
2	220	6,400	3,20,000	36,00,000	80	670	50,000	90,000
3	420	1,400	25,000	2,40,000	160	950	4,900	1,60,000
4	120	1,200	2,200	22,000	30	160	700	2,800
5	620	6,200	18,000	7,40,000	40	1,800	150	92,000
6	2,110	15,000	45,000	18,00,000	30	1,800	1,100	26,000

TABLE 51. Average coliform, faecal coliform values per 100 ml of water or wet sediment in north Stations.

Sampling Stations	Coliforms				Faecal coliforms			
	Water		Sediment		Water		Sediment	
7	4,870	11,000	80,000	12,00,000	80	18,000	10,000	8,40,000
8	880	5,600	2,90,000	30,00,000	80	1,100	47,000	60,000
9	1,200	2,800	15,000	2,80,000	160	350	3,200	1,80,000
10	240	1,800	1,900	19,800	50	3,200	900	18,000
11	560	5,200	9,000	46,000	60	200	120	81,000
12	1,800	12,000	26,000	2,80,000	20	1,500	1,300	26,000

TABLE 92. Tolerance limits for pollutants in surf zone subject to effluent discharges.

Characteristics	Tolerance limits for			
	Bathing, Recreation Shellfish culture and salt manufacture	Commercial fish culture	Harbour water	Navigation
1. pH value	6.5 to 8.5	6.5 to 8.5	6.5 to 9.0	6.5 to 9.0
2. Free ammonia (as N) mg/l Max	1.2	1.2	no limit	no limit
3. Dissolved oxygen mg/l Min	40% saturation value or 3 mg/l whichever is higher	-	3 mg/l	2 mg/l
4. Phenolic compounds mg/l Max	0.1	0.1	-	-
5. Insecticides, pesticides, herbicides fungicides mg/l	+	+	-	-
6. Arsenic (as As), mg/l Max	0.01	0.01	-	-
7. Floating material	No visible floating material of sewage or industrial waste origin.			
8. Colour and odour	No noticeable colour or offensive odour			
9. BOD for 5 days at 20°C mg/l Max	5	-	5	-
10. Bacterial count, coliform organisms, MPN per 100 ml Max	2500	-	2500	-
11. Suspended solids	No visible suspended solids of sewage or industrial waste origin.			
12. Bioassay test	No less than 90% test animals shall survive in 96 hr test			
13. Mercury mg/l Max	+	-	-	-

TABLE 52 a. Recommended limits of total and faecal coliform (FWPCA 1968).

Type of water	Total coliform/100 ml		Faecal coliform/100 ml	
	Desirable	Permissible	Desirable	Permissible
Primary contact water (Swimming)	1,000	2,000	200	1,000
Secondary contact water (Boating, Fishing)	5,000	10,000	1,000	5,000
Shellfish culture	1,000	2,500	200	1,000

Treated sewage effluent coliform levels should not exceed those of water receiving the discharge.

TABLE 58. Coliform (average) faecal coliform values per 100 ml of water or wet sediment in south Stations.

Sampling Stations	Coliforms				Faecal coliforms			
	Water		Sediment		Water		Sediment	
1	4,370	12,000	1,70,000	13,00,000	20	20,000	20,000	24,00,000
2	220	6,400	3,20,000	36,00,000	80	670	50,000	90,000
3	420	1,400	25,000	2,40,000	160	550	4,900	1,60,000
4	120	1,200	2,200	22,000	30	160	700	2,800
5	620	6,200	18,000	7,40,000	40	1,800	150	92,000
6	2,110	15,000	45,000	18,00,000	30	1,800	1,100	26,000

TABLE 51. Average coliform, faecal coliform values per 100 ml of water or wet sediment in north Stations.

Sampling Stations	Coliforms				Faecal coliforms			
	Water		Sediment		Water		Sediment	
7	4,870	11,000	80,000	12,00,000	80	18,000	10,000	8,40,000
8	880	5,600	2,90,000	30,00,000	80	1,100	47,000	60,000
9	1,200	2,800	15,000	2,80,000	160	380	3,200	1,80,000
10	240	1,800	1,900	19,800	50	3,200	900	18,000
11	560	5,200	9,000	46,000	60	200	120	81,000
12	1,800	12,000	26,000	2,80,000	20	1,500	1,300	26,000

PART B. ECOPHYSIOLOGY

SECTION I.

BIOCHEMICAL DIFFERENTIATION OF BACTERIA ISOLATED FROM HUMAN BEINGS AND MARINE BACTERIA.

PART B

ECO-PHYSIOLOGY

I. BIOCHEMICAL DIFFERENTIATION OF BACTERIA ISOLATED FROM HUMAN BEINGS AND MARINE BACTERIA:

This section deals with the bacterial flora isolated, identified from Trivandrum beach water together with a comparative study of the biochemical properties of marine bacteria with strains of bacteria isolated from clinical specimens.

Isolation of marine strains:

The bacterial flora of the 13 beach samples from the three beaches (Vishinjam, Kovalam and Shankumughom) were analysed according to standard methods. Twenty seven strains were isolated from 6 water samples from Shankumughom, 12 strains from 5 water samples from the Kovalam coast and 4 isolates from 2 water samples collected from Vishinjam beach. The result of bio-chemical investigations indicated the existence of 5 genera of marine flora according to the scheme of Shewan (1960), 4 genera of medical bacteria,(strains identified as per Cowan and Steel (1974) of 15 each, were selected, identified and preserved in nutrient agar for further tests. Clinical strains were isolated from sputum, urine, blood of human beings.

Media:

All isolations were made by spreading 0.1 ml of beach water on the surface of a suitably dried MacConky Agar, Blood Agar and Nutrient Agar. Temperature and duration of incubation were 37°C, 24 hrs respectively. Selected isolates were examined for colony morphology, gram stained and sub-cultured in peptone water. Stock cultures were maintained at 4°C on semisolid nutrient agar. Cultures grown in peptone broth for 4 to 6 hours were examined by 'Hanging drop method' for motility and shape of the living cells.

Characterization of strains:

Morphology:

The isolated cultures were regularly checked for cell morphology and purity by examination of Gram-stained smears. The selected 43 marine isolates and 60 clinical isolates were examined for colony morphology, gram-stained and sub-cultured in peptone water. Stock cultures were maintained at 4°C on semisolid nutrient agar.

Pigment production:

Strains were observed for production of pigments. Altogether 8 *Pseudomonas* spp. were isolated from marine environment out of which 2 isolates produced a green soluble pigment whereas all the 15 clinical *Pseudomonas* isolates were strong green pigment producers.

Motility:

A wet film prepared from 4 to 6 hours culture were examined by 'Hanging drop method' for motility and shape of living cells. Strains were also stab inoculated into semi-solid (Motility test medium) which was then incubated until growth was visible. The medium contained 0.3% agar in ordinary sea water peptone water for marine bacteria and peptone water for clinical isolates.

Oxidase test:

A freshly prepared 1% solution of tetramethyl-p-phenylene-diamine dihydrochloride in distilled water was poured on filter paper in a petri dish. Small amount of culture was smeared on the reagent paper and a blue colour indicated oxidase production.

Catalase test:

Seventy two hours agar slant culture was flooded with 10% hydrogen peroxide solution; a stream of bubbles of O_2 arose from colonies of catalase producing organisms.

Hydrogen sulphide production:

A strip of lead acetate paper was suspended during incubation in the neck of a cysteine broth tube inoculated with the cultures. Blackenning of the paper indicated H_2S production.

Nitrate reduction:

This was tested in Nitrate broth (KNO_3 200 μg/ml). The presence of nitrite ions was indicated by a deep red colour when 0.5 ml of nitrate solution A and 0.5 ml of nitrate solution B (Cruickshank, 1968) were added to 48 hours cultures.

Indole production:

Indole was detected by adding 0.5 ml of xylene to the liquid supernate of 72 hours nutrient broth culture and after vigorous shaking a few drops of Kovac's reagent was also added. A pink colour indicated the presence of Indole.

Positive and negative control strains were included in each batch of tests.

Gelatinase test:

A charcoal-gelatin disc was prepared in the laboratory by a modification of Kohn's method (Kohn, 1953) : 12.5 g gelatin (Difco) was dissolved in 100 ml of nutrient broth; 5 g of finely powdered charcoal was added and the mixture was poured into metal petri dishes and allowed to solidify at 4°C. The charcoal-gelatin was held in 10% formalin at room temperature for 5 days and then cut into discs 1 cm in diameter. The discs were washed in running tap water for 48 hours at 4°C and pasteurised by heating at 70°C in sterile distilled water for 20 minutes.

The charcoal gelatin disc in sea water broth and nutrient broth was inoculated and observed for digestion of the disc during incubation for 4 days and strains showing slow liquifaction were retained for 7 days. In positive reaction of hydrolysis of gelatin the charcoal disc may be observed below the test tubes after the liquifaction.

Oxidative and fermentative breakdown in glucose:

Dissimilation of glucose was tested by the methods of Hugh and Leifson (1953). For each isolate duplicate tubes of MOF medium (Hi-media) were inoculated and allowed to set. A few drops of sterile glycerine was added to one of the duplicates to form a seal. The results were read after 24 - 48 hours of incubation.

Citrate utilization:

Tubes of Koser's citrate medium were inoculated by straight wire moistened with the saline suspension of bacteria and incubated for 24 hours. A bright blue colour was recorded as positive.

Urease activity:

About 0.5 ml of a non-sterile solution of 2 g of urea and 2 ml of 0.04% phenol red in 100 ml of distilled water was introduced into each tube and then heavily inoculated with a loopful of 24 hours old nutrient agar culture. The tubes were incubated at room temperature (28 ± 2°C) for 7 days. Ureolytic activity was detected by the change in colour of the medium from light yellow to pink.

Starch hydrolysis:

The organisms were grown for 48 hours in nutrient agar basal medium with 0.2% starch. If starch was hydrolysed the amylolytic colonies developed clear zones around them when Iodine crystals were kept in the cover of the petri dish and slightly warmed the Iodine crystals over the flame (Mahadeva Iyer, 1968). Brown colour indicated partial hydrolysis as starch is in the form of erythrodextrin. Unhydrolysed starch formed a blue colour with Iodine starch complex.

Aesculin hydrolysis:

The isolates both marine and pathogenic were grown for 48 hours in nutrient broth containing 1% aesculin. If aesculin was hydrolysed a black discolouration developed when 0.5 ml of a 1% aqueous solution of ferric ammonium citrate was added.

Reaction in triple sugar iron agar:

Ten grams of each lactose and sucrose were added to a litre of peptone broth with 1 g of glucose, 5 g of sodium chloride and distributed in tubes and prepared the slants after sterilisation. Fermentation of either sucrose or lactose or both will give rise to an acid reaction throughout the medium whereas fermentation of only glucose will produce acid in the 'butt' but not in the slant. The addition of 0.2 g of ferrous sulphate and 0.3 g of sodium thiosulphate of the above formula allows the determination of the production of hydrogen sulphide in the same medium, cultures producing H_2S showed an extensive blackening of the agar due to iron sulphide precipitation (Salkin and Willett, 1940).

The Methyl red and Voges-Proskauer reaction:

The glucose-phosphate (MR) medium was inoculated and incubated at 37°C for 2 days. Two drops of methyl red solution was added to the cultures. Red colour showed positive reaction; orange was found in intermediate stage. Yellow colour for negative reaction. Voges Proskauer (VP) test for acetyl methyl carbinol or acetoin production was carried out on the same tube of culture. The test was mainly used to distinguish various coliform organisms from each other.

Carbohydrate-fermentation tests:

Acid production from carbohydrates was tested using 1% of the substrate and 1% Andrade's Indicator in a peptone water medium. The 11 substrates used were Glucose, Lactose, Sucrose, Mannitol, Mannose, Dulcitol, Inositol, Adonitol, Salicin, Zylose and Arabinose. Results were recorded after 24 and 48 hours of incubation.

Eijkman's test:

Single strength MacConkey broth was prepared and inoculated by all 43 marine strains for the elevated temperature test. The cultures were incubated in a water bath maintained at 44°C. The results were recorded after 24 hours of incubation.

The taxonomic scheme employed for classification was that of Shewan *et al.* (1960) for marine bacteria, Cowan and Steel (1974) for bacteria isolated from clinical specimens and Bergy's manual of Determinative Bacteriology (1974) for general reference.

Antibiotic sensitivity:

Nearly 10 antibiotics namely Penicillin, Ampicillin, Chloramphenicol, Kanamycin, Erythromycin, Streptomycin, Terramycin, Gentamycin, Septran and Cephoran impregnated filter paper discs were placed on 1.5% Nutrient Agar Plate and the results were recorded after 24 hours of incubation at 37°C.

Cell morphology:

All strains were Gram-negative rods non-sporing, motile or non-motile aerobic bacteria. Pleomorphism was common. Cell shape varied from filamentous to cocco-bacillary, often in the same smear. Some strains formed chains, some had pointed ends and others rounded ends. Cell morphology varied further with the culture medium and had little discriminatory value. Marine bacterial cells were comparatively smaller when compared to pathogenic strains.

Colony morphology:

Colonies on blood agar differed in size from pin-point to 3 - 4 mm in diameter. No rhizoid colonies were produced in nutrient or MacConkey Agar. Thirteen lactose fermenting and 5 non-lactose fermenting strains isolated from MacConkey were further studied for their biochemical characteristics.

Results of biochemical tests:

All the strains were catalase positive except 2 *Aeromonas* and one *Enterobacter* strains. Almost all the results were clearly positive or negative. Table 53 gives the types of bacterial genera isolated from the beach water samples. All the five bacterial genera namely *Aeromonas*, *Pseudomonas*, *Vibrio*, *Paracolons*/*E. coli* and *Alcaligenes* were present in Shankumughom beach water. *Vibrio*, *Aeromonas* and *Pseudomonas* were not encountered in Vizhinjam beach whereas *E. coli* / *Paracolons* were comparatively more than the other two beaches. *Vibrio* spp. were not encountered

TABLE 53. Types of bacterial genera isolated from the beach water near Trivandrum.

Sl. No.	Place of collection	No. of samples	*Aeromonas*	*Pseudomonas* (No. %)	*Vibrio* (No. %)	*Paracolons*/ *E.coli* (No. %)	*Achromobacter*	Total isolates
1.	Shankumughom Beach	6	10	6	2	2	7	27
2.	Vizhinjam Coast	2	0	0	0	3	1	4
3.	Kovalam Beach	5	5	2	0	2	3	12
	Total	13	15	8	2	7	11	43

in Kovalam beach and only 2 strains were isolated from Shankumughom waters. This was supported by the investigation by Karthiayani and Iyer (1975) who reported the paucity of *Vibrio* spp. in surface water and bottom mud whereas percentage of this genera on fresh sardines and prawns seems to be fairly high. *Streptococcus faecalis* was isolated in Kovalam and Shankumughom beach waters. Absence of *Micrococci* in the 13 sea water samples was a striking observation. It was reported (Karthiayani and Mahadeva Iyer, 1975) that gram-positive organisms were prevalent in the bottom mud and their paucity in surface waters. Altogether 43 bacterial strains were isolated from 13 random samples of beach water and maximum isolates were from Shankumughom Beach water.

Table 55 illustrates the results of 30 biochemical tests done with the 43 marine bacterial cultures. Details of the reactions of the 26 biochemical reactions of the 43 marine bacterial strains are given in Table 55a. Final identification of marine bacteria is given in Tables 55b and 55c. Most of the isolates were highly motile and small in size when compared to bacteria isolated from clinical specimens. About half of the isolates were highly fermentative regarding the sugars like glucose, lactose, sucrose, mannose, mannitol, xylose and salicin. One fourth of the

TABLE 54. Results of 30 biochemical tests with 43 marine bacteria isolated from the beach water near Trivandrum.

Sl. No.	Name of the Test	Positive strains	%	Sl. No.	Name of the Test	Positive strains	%
1.	Motility test	29	67.4	16	V.P. test	18	41.9
2.	Gas production	20	46.5	17	Citrate utilisation	32	74.4
3.	Glucose fermentation	20	46.5	18	Urease test	24	55.8
4.	Lactose "	15	34.9	19	Oxidase test	25	58.1
5.	Sucrose "	19	44.2	20	H_2S production	2	4.6
6.	Mannose "	20	46.5	21	Nitrate reduction	26	60.4
7.	Mannitol "	21	48.8	22	O-F reaction	20	46.5
8.	Dulcitol "	6	13.9	23	Ejkmann test	14	32.2
9.	Adonitol "	7	17.3	24	Aesculin hydrolysis	29	67.4
10.	Inositol "	12	27.9	25	Gelatin hydrolysis	19	44.1
11.	Salicin "	17	39.5	26	Starch hydrolysis	18	41.8
12.	Arabinose "	14	32.6	27	Pigment production	2	4.6
13.	Xylose "	16	37.2	28	Catalase test	40	92.0
14.	Indole production	7	17.3	29	Sensi. to 2.5 I.U. Penicillin	4	9.2
15.	M-R test	9	20.9	30	Sensi. to 10 mg Terramycin	29	67.4

TABLE 55a. Identification tests of bacteria isolated from littoral waters of Trivandrum (Marine Bacteria).

Test No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Strain No. / Name of tests	Motility	Gas	Glucose	Lactose	Sucrose	Mannitol	Mannose	Dulcitol	Inositol	Salicin	Xylose	Adonitol	Indole	M-R	V-P	Citrate	Urease	Catalase	Oxidase	H_2S	Nitrate	O.F. med.	Kligman's test	Starch hydrolysis	Aesculin	Gelatin
MB - 1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	++	-	+	-	-	-	-	+
MB - 2	+	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+	-	+	OF	-	-	+	-
MB - 3	+	+	+	-	+	+	+	-	+	(+)	+	-	-	-	+	+	+	+	+	-	+	OF	-	-	+	+
MB - 4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	O	-	-	-	-
MB - 5	+	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+	-	+	OF	-	-	+	-
MB - 6	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	OF	-	-	+	ND
MB - 7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	O	-	-	+	+
MB - 8	-	+	+	-	-	-	+	-	-	-	(+)	-	-	-	-	+	-	+	-	-	-	O	-	-	-	+
MB - 9	+	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+	-	+	OF	-	+	+	-
MB - 10	++	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	OF	+	+	+	-
MB - 11	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	+	+	+	-	+	OF	-	+	+	-

+=Positive; A=Acid only; (+)=Weak/delayed; O=Oxidation; F=Fermentation; AM=Actively motile; SM=Slowly motile; ND=Not done.

TABLE 55a. (Contd.)

Test No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Name of tests / Strain No.	Motility	Gas	Glucose	Lactose	Sucrose	Mannitol	Mannose	Dulcitol	Inositol	Salicin	Xylose	Adonitol	Indole	M-R	V-P	Citrate	Urease	Catalase	Oxidase	H_2S	Nitrate	O.F. med.	Killmann's test	Starch hydrolysis	Aesculin	Gelatin
MB - 12	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	OF	+	+	+	-
MB - 13	+	+	+	+	+	+	+	+	-	(+)	+	-	+	+	+	-	-	+	+	-	+	O	+	-	+	-
MB - 14	-	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	OF	+	-	+	-
MB - 15	(+)	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	OF	+	-	+	+
MB - 16	+	+	+	+	+	-	+	-	+	+	+	-	-	-	+	+	-	+	-	-	-	OF	+	+	+	+
MB - 17	++	+	+	-	+	+	(+)	-	-	+	-	-	-	-	+	+	-	-	+	-	-	OF	+	+	+	+
MB - 18	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	+
MB - 19	++	-	-	-	-	-	-	-	-	-	-	-	+	-	-	(+)	-	-	-	-	+	-	+	+	+	+
MB - 20	++	-	+	+	-	+	+	+	-	(+)	+	-	-	-	-	-	-	+	-	-	+	O	+	+	+	-
MB - 21	+	-	+	+	-	+	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	OF	+	+	+	-
MB - 22	++	-	+	+	-	+	-	-	(+)	+	+	-	-	+	-	-	-	+	-	-	+	OF	+	+	+	-

+=Positive; A=Acid only; (+)=Weak/delayed; O=Oxidation; F=Fermentation; AM=Actively motile;
SM=Slowly motile; ND=Not done.

TABLE 95a. (Contd.)

Test No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Name of tests / Strain No.	Motility	Gas	Glucose	Lactose	Sucrose	Mannitol	Mannose	Dulcitol	Inositol	Salicin	Xylose	Adonitol	Indole	M-R	V-P	Citrate	Urease	Catalase	Oxidase	H_2S	Nitrate	O.F. med.	Killmann's test	Starch hydrolysis	Aesculin	Gelatin
MB - 23	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	(+)	+	-	-	-	-	+	-	+	-
MB - 24	+	-	+	-	+	-	-	-	-	-	-	+	-	+	-	+	+	+	+	-	-	-	-	-	+	+
MB - 25	++	-	+	-	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	OF	-	+	+	+
MB - 26	-	-	+	-	-	-	-	-	-	-	(+)	-	-	-	-	+	-	+	-	-	-	OF	-	-	-	-
MB - 27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	+	+	-	-	-	-	-	-	-	-
MB - 28	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	+	-	-	-	+	-	+
MB - 29	+	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-	A	+	(+)	-	+	OF	-	-	+	-
MB - 30	-	-	-	-	-	-	-	-	-	-	(+)	(+)	-	-	-	+	-	+	-	-	-	-	-	-	-	-
MB - 31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	++	-	-	-	-	-	-	-
MB - 32	+	+	+	-	+	-	-	-	-	-	-	(+)	-	-	+	+	-	+	-	-	+	OF	-	-	ND	+
MB - 33	-	-	+	+	A	+	-	-	-	-	(+)	-	+	+	-	-	+	+	-	-	+	-	-	-	+	+

+=Positive; A=Acid only; (+)=Weak/delayed; O=Oxidation; F=Fermentation; AM=Actively motile; SM=Slowly motile; ND=Not done

TABLE 55a. (Contd.)

Test No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Strain No. / Name of tests	Motility	Gas	Glucose	Lactose	Sucrose	Mannitol	Mannose	Dulcitol	Inositol	Salicin	Xylose	Adonitol	Indole	M-R	V-P	Citrate	Urease	Catalase	Oxidase	H_2S	Nitrate	O.F. med.	Eijkmann's test	Starch hydrolysis	Aesculin	Gelatin
MB - 34	+	-	-	-	-	-	-	-	ND	A	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+
MB - 35	-	+	+	+	(+)	-	-	-	ND	-	-	-	-	-	+	+	+	+	+	-	+	OF	-	+	+	+
MB - 36	+	+	+	+	-	(+)	ND	-	-	(+)	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	+
MB - 37	+	+	-	-	-	-	ND	-	ND	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-	-
MB - 38	-	+	-	+	+	+	ND	-	ND	+	-	-	-	-	+	+	+	+	+	-	+	OF	+	-	+	-
MB - 39	-	-	-	+	+	+	ND	-	ND	+	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-
MB - 40	+	+	-	-	+	-	ND	-	ND	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+	+	+
MB - 41	-	+	-	-	-	+	ND	+	ND	-	-	-	-	+	-	+	-	+	-	-	+	OF	-	+	+	-
MB - 42	AM	-	-	-	-	-	ND	-	ND	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+
MB - 43	-	-	-	-	-	-	ND	-	ND	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-

+=Positive; A=Acid only; (+)=Weak/delayed; O=Oxidation; F=Fermentation; AM=Actively motile; SM=Slowly motile; ND=Not done.

TABLE 55b. Identification of Marine Bacteria
(Shewan, 1960).

Strain No.	Motility	Oxidase	O-F	Identification
SMB 1	+	+	NF	*Pseudomonas*
" 2	+	+	F	*Aeromonas*
" 3	+	+	F	*Aeromonas*
" 4	-	-	NF	*Achromobacter*
" 5	+	+	F	*Aeromonas*
" 6	+	+	NF	*Pseudomonas*
" 7	+	+	NF	*Pseudomonas*
" 8	-	-	NF	*Achromobacter*
" 9	+	+	F	*Aeromonas*
" 10	++	+	F	*Vibrio*
" 11	+	+	F	*Aeromonas*
" 12	+	+	F	*Aeromonas*
" 13	+	+	F	*Aeromonas*
" 14	-	+	F	*Aeromonas*
" 15	+	+	F	*Aeromonas*
" 16	-	-	F	*E. coli*
" 17	+	+	F	*Aeromonas*
" 18	+	-	NF	*Achromobacter*
" 19	++	-	NF	*Achromobacter*
" 20	++	-	F	*E. coli*
" 21	++	-	F	*E. coli*
" 22	+	-	F	*E. coli*
" 23	+	-	NF	Unidentified
" 24	+	+	F	*Aeromonas*
" 25	++	+	F	*Vibrio*
" 26	-	-	NF	*Achromobacter*
" 27	-	-	NF	*Achromobacter*
" 28	++	++	NF	*Pseudomonas*

Contd....

TABLE 55b (Contd.)

Strain No.		Motility	Oxidase	O-F	Identification
SMB	29	+	+	F	*Aeromonas*
"	30	-	-	NF	*Achromobacter*
"	31	-	++	NF	*Pseudomonas*
"	32	+	-	F	*E. coli*
"	33	-	-	F	*E. coli*
"	34	+	+	NF	*Pseudomonas*
"	35	-	+	F	*Aeromonas*
"	36	+	+	F	*Aeromonas*
"	37	+	+	NF	*Pseudomonas*
"	38	-	+	F	*Aeromonas*
"	39	-	-	F	*E. coli*
"	40	+	-	NF	*Achromobacter*
"	41	-	-	NF	*Achromobacter*
"	42	+	+	NF	*Pseudomonas*
"	43	-	-	NF	*Achromobacter*

Strain Nos. 1 to 16; 25 to 32; 40 to 42 } Isolated from Shanmughom beach.

Strain Nos. 17 to 19; 23 & 24; 33 to 37; 38 & 39 } Isolated from Kovalam beach resort

Strain Nos. 20 to 22 & 43 } Isolated from Vizhinjam beach

TABLE 55e. Number and Percentage of identified strains of marine bacteria isolated from littoral waters of Trivandrum.

Identified strains	No. of strains	Percentage (%)
Pseudomonas	8	19
Achromobacter	10	23
Vibrio	2	5
Aeromonas	15	38
E. coli	7	16
Unidentified	1	2

isolates were highly fermentative of Inositol and Arabinose. Out of the 11 sugars tested none of the sugars was attacked by 15 marine bacterial strains (Table 56). Marine strains were also found to be inefficient in fermenting dulcitol and adonitol.

All the isolates were non-pigment producers except two species of _Pseudomonas_. Only 2 oxidative _Pseudomonas_ sp. isolated from marine environment produced green fluorescent diffusible pigment, which were grouped in _Pseudomonas_ group I. All the other oxidative, non-pigmented organisms were grouped under _Pseudomonas_ group II. Only two _Vibrio_ spp, produced H_2S in TSI agar slants. Most of the marine strains possessed the enzyme catalase whereas urease and oxidase were found only in 25 marine strains. About 32 isolates utilised citrate as their carbon source.

Glucose was metabolised fermentatively by 46.5% of the strains in the Hugh and Leifson's medium. The remaining 8.2% has oxidatively metabolised glucose in this medium.

Nitrate was reduced to nitrite by 26 of the 43 strains. The methyl red reaction was positive in 9 strains, four giving weak result. Aesculin hydrolysis was more when compared to Gelatin and Starch.

TABLE 56. Biochemical differentiation of fermentative and non-fermentative strains of marine bacteria isolated from litteral waters of Trivandrum.

Sl. No.	Name of the Test	Fermentative strains (24 Nos.)		Non-fermentative strains (19 Nos.)	
		No. of positive strains	Percentage	No. of positive strains	Percentage
1.	Indole production	3	12.5	4	21.1
2.	M-R test	7	29.1	2	10.5
3.	V-P test	16	66.6	2	10.5
4.	Citrate utilization	17	70.8	15	79.0
5.	Urease test	14	53.3	10	52.6
6.	Catalase test	23	95.5	17	89.4
7.	Oxidase test	17	70.8	8	42.1
8.	H_2S production	0	0.0	2	10.5
9.	Nitrate reduction	22	91.7	4	21.1
10.	O-F reaction	17	70.8	3	15.7
11.	Ejkman test	11	46.2	3	15.7
12.	Starch hydrolysis	12	50.4	6	31.4
13.	Aesculin hydrolysis	21	88.2	8	32.1
14.	Gelatin hydrolysis	10	44.1	9	47.3
15.	Sensitivity to Penicillin (2.5 I.U./Disc.)	2	8.3	2	10.5
16.	Sensitivity to Terramycin	19	79.1	10	52.6

Results of Tolerance tests:

Only 6% of the total isolates were found to tolerate bile salts illustrating their origin and source from intestine of animals or human beings. No definite pattern of tolerance was observed but inhibition of growth by desoxycholate was not prevented by the presence of taurocholate in these isolates. On this medium the growth of non-intestinal organisms is inhibited. The presence of colonies is presumptive evidence of faecal contamination. The lactose fermenting coliform species appear as red colonies and the typhoid-dysentry group as white or blue colonies.

Results of antibiotic disc resistance tests:

Only 4 strains were sensitive to 2.5 I.U. penicillin whereas 29 strains were sensitive in 10 mg terramycin, 22 strains were insensitive to 10 gm penicillin but sensitive to ampicillin (22), terramycin (36), chloramphenicol (32), cephoren (24). Most of the isolates were sensitive to septran (40), gentamycin (39), streptomycin (38). There was not any definite pattern in the strains insensitive to streptomycin, gentamycin and septran.

The biochemical reactions such as the ability of the organisms to liquefy gelatin, produce indole and H_2S and decompose urea to form ammonia can be used for qualifying the spoilage potential of that organism (Lewis *et al.*, 1971). In this respect the spoilage potential of the marine organisms were more when compared to bacteria isolated from clinical specimens.

Table 56 shows the differentiation in biochemical activity between fermentative and non-fermentative strains. The fermentative strains were very active in fermenting glucose in oxidation-fermentation media and also had high percentage of catalase enzyme. Gelatin was hydrolysed actively while H_2S production was low in fermentative strains. High positive oxidase and urease were noted with non-fermentative marine strains. Mostly fermentative strains were biochemically active in all the reactions.

Table 57 illustrates some 16 bio-chemical tests of the clinical strains based on which the clinical strains were identified after initial isolation.

Table 57 a incorporates comparison of the biochemical tests of marine bacteria and also bacteria isolated from clinical specimens. Glucose fermentation was very high in human bacteria whereas citrate utilisation was very high in marine bacteria. H_2S production was very low both in marine bacteria and also strains isolated from clinical specimens. Indole production was high in strains isolated from marine environment than in human strains.

TABLE 57. Identification tests of 50 bacteria isolated from clinical samples from Sree Chitra Thirunal Medical Centre, Trivandrum.

Sl. No.	Name of the strain	Motility	Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin	Xylose	Citrate	Urease	MR	VP	H_2S	Indol	Oxidase
1.	*Pseudomonas aeruginosa*	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
2.	*Klebsiella pneumoniae*.	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-
3.	*Proteus mirabilis*	+	+	-	-	-	-	-	-	(+)	-	+	-	-	+	-	-
4.	*Mima polymorpha*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*E. coli*	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	-
6.	*Klebsiella pneumoniae*	-	+	+	-	+	-	+	+	+	-	-	-	-	-	+	-
7.	*E. coli*	+	+	+	+	+	+	+	(+)	(+)	(+)	-	+	-	-	+	-
8.	*E. coli*	+	+	+	+	+	+	+	+	(+)	(+)	-	+	-	-	+	-
9.	*Citrobacter freundii*	+		-			-	-		-	+		+	-	-	(+)	-
10.	*Klebsiella pneumoniae*	-	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-
11.	*Pseudomonas aeruginosa*	+		-	-	-	-	-	-	-	+	-	-	-	-	-	+
12.	*Klebsiella pneumoniae*	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-

Contd..

TABLE 77 (Contd.)

Sl. No.	Name of the strain	Motility	Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin	Xylose	Citrate	Urease	MR	VP	H_2S	Indole	Oxidase
13.	*Pseudomonas*	+		-	-	-	-	-	-	-	+	?+	-	-	-	-	+
14.	*E. coli*	+	+	+	-	+	+	+	-	+	-	-	+	-	-	+	-
15.	*Klebsiella pneumoniae*	-	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-
16.	*Pseudomonas*	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
17.	*E. coli*	+	+	+	-	+	+	+	-	+	-	-	+	-	-	+	-
18.	*E. coli*	+	+	+	-	+	+	+	-	+	-	-	+	-	-	+	-
19.	*E. coli*	+	+	+	-	+	+	+	-	+	-	-	+	-	-	+	-
20.	*Alcaligenes faecalis*		-	-	-	-	-	-	-	-	-	-			-	-	+
21.	*Alcaligenes faecalis*		-	-	-	-	-	-	-	-	-	-			-	-	+
22.	*Bacterium anitratum*		-	-	-	-	-	-	-	-	+	-			-	-	-
23.	*Citrobacter freundii*	+		-	+	+	+	-	+	+	-	+			-	-	-
24.	*Klebsiella pneumoniae*	-	+	+	+	+	-	+	+	+	+	?+	-	+	-	-	-
25.	*Pseudomonas*	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
26.	*Pseudomonas*	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+

Contd..

TABLE 97 (Contd.)

Sl. No.	Name of the strain	Motility	Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin	Xylose	Citrate	Urease	MR	VP	H_2S	Indole	Oxidase
27.	*Atypical E. coli*	+	+	?-	-	+	+	+	-	+	-	-	+	-	-	+	-
28.	*Pseudomonas*	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
29.	*Klebsiella*	-	+	+	+	+	-	+	+	+	+	+	-	+	-	-	-
30.	*Proteus mirabilis*	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-
31.	*Klebsiella*	-	+	+	+	+	-	+	+	+	+	+	-	+	-	-	-
32.	*Klebsiella*	-	+	+	+	+	+	-	+	+	-	-	-		-	-	-
33.	*Alcaligenes faecalis*	-	-	-	-	-	-	-	-	-	-	+	-	-	late		
34.	*E. coli*	+	+	+	+	+	+	-	-	-	-	+	-	-	-		
35.	*Pseudomonas*	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+
36.	*Pseudomonas*	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+
37.	*Alcaligenes faecalis*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38.	*E. coli*	+	+	?+	-	+	+	+	-	+	-	-	+			+	-
39.	*Alcaligenes faecalis*		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
40.	*Klebsiella*	-	+	+	+	+	-	+	+	+	+	+	-	+	-	-	-

Contd..

TABLE 57 (Contd.)

Sl. No.	Name of the strain	Motility	Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin	Xylose	Citrate	Urease	MR	VP	H_2S	Indole	Oxidase
41.	*Pseudomonas*	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+
42.	*Klebsiella*	-	+	+	+	-	+	+	+	+	+	-	+	-	-	-	-
43.	*Bacterium anitratum*	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
44.	*Pseudomonas*	+		-	-	-	-	-	-	-	+	+	-	-	-	-	+
45.	*E. coli*	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-
46.	*Proteus mirabilis*	+			-	-	-	-		-	+	+	+	-	-	-	-
47.	*Klebsiella*	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-
48.	*E. coli*	+	+	+	-	+	+	+	+	-	-	-	+	-	-	+	-
49.	*Mima polymorpha*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50.	*Bacterium anitratum*	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+

TABLE 57a. Comparison of the results of 16 biochemical tests with marine bacteria (43) and bacteria isolated from clinical specimens (50).

Sl. No.	Name of the Test	Percentage of positive strains (Marine)	Percentage of positive strains from clinical specimens.
1.	Motility	67.4	59.0
2.	Glucose fermentation	46.5	72.0
3.	Lactose "	34.9	48.0
4.	Sucrose "	44.2	34.0
5.	Mannitol "	48.8	46.0
6.	Dulcitol "	13.9	32.0
7.	Arabinose "	32.6	44.0
8.	Salicin "	39.5	36.0
9.	Xylose "	37.2	50.0
10.	Citrate utilisation	74.4	56.0
11.	Urease test	55.8	42.0
12.	M.R. test	20.9	36.0
13.	V.P. test	41.9	18.0
14.	H_2S production	4.6	4.0
15.	Indole production	17.3	28.0
16.	Oxidase test	58.1	16.0

Table 58 shows reports of marine bacterial genera isolated by various investigators in different parts of the world. A perusal of the table shows that *Pseudomonas* predominates North Sea and is completely absent in N. Cape Norway and occurs in all the other areas of lesser quantities. *Aeromonas* was encountered only by Simidu *et al.* (1971) in inshore waters of Japan. *Vibrio* dominated in Chesapeake Bay but completely absent in Australian waters. Enterobacteriaceae (*E. coli*) were found in inshore water of Japan. *Alcaligenes* was encountered in all parts of the area studied in moderate numbers.

Table 59 incorporates reports of bacterial genera from Calicut, Cochin and Trivandrum coastal waters. *Pseudomonas* occurred in all the 3 places whereas *Aeromonas* was encountered only in Trivandrum coastal waters. *Vibrio* and *E. coli* were dominant in the coastal waters of Cochin. *Alcaligenes* occurred in moderate numbers in Calicut, Cochin and Trivandrum coastal waters.

From the results of the present investigation it was noted that there is not much differentiation in the genera of bacteria in different areas of the sea though they differ much in biochemical characters. The 5 genera isolated from Trivandrum coastal water occurred only in moderate numbers in the other parts of the world. Much differentiation was

TABLE 58. Reports of marine bacterial genera expressed as percentage isolated by various authors in different coastal waters of the world.

Sl. No.	Place of study	Author	Pseudo-monas	Aero-monas	Vibrio	Paracolon/ E.coli	Achromo-bacter
1.	Australia	Wood	10.0	0.0	0.0	0.0	26.0
2.	Australia	Wood	0.9	0.0	0.0	0.0	0.0
3.	North Sea	Shewan & Hodgkiss	94.0	0.0	0.0	0.0	6.0
4.	N. Cape, Norway	Shewan & Hodgkiss	0.0	0.0	0.0	0.0	14.0
5.	Narragansett Bay, R.I.U.S.A.	Marchellano	28.3	0.0	13.3	0.0	12.2
6.	Kamogawa Bay, Japan.	Simidu & Aiso	29.8	0.0	37.3	0.0	21.3
7.	Long Island Sound Connecticut, USA	Marchellano & Brown	40.6	0.0	4.9	0.0	28.6
8.	Inshore water of Japan	U. Simidu Ashino and Kaneko.	28.1	9.4	34.4	3.1	15.6
9.	Chesapeake Bay, Md.	Lovelace, Tubiash and Colwell.	18.0	0.0	56.0	0.0	13.0

TABLE 99. Reports of marine bacterial genera (in percentage) isolated from coastal waters of Kerala, India.

Sl. No.	Place of study	Author	Pseudo-monas	Aero-monas	Vibrio	Paracolon/ E.coli	Achromo-bacter
1.	Calicut, India.	Venkataraman and Sreenivasan.	18.0	0.0	1.4	0.0	11.6
2.	Cochin, India.	Gore	18.5	0.0	24.1	31.4	9.2
3.	Trivandrum, India*	Chandrika, Shanmugham and Ravindranath.	19.0	38.0	5.0	16.0	21.0

* Present Report.

not observed regarding biochemical characters of marine bacteria and human bacteria except that marine bacteria were more sensitive to antibiotics than human bacteria. In the present study only fermentative strains were biochemically active and fermentative strains were mostly isolated from clinical specimens. The abundance of *Aeromonas*, *Pseudomonas*, *Paracolons* / E. *coli* in beach sea water is an indication that the three beaches are intensely polluted and there was a contamination of sand with human faeces. Brezenski and Russomanno (1969) and Grunnet and Nielsen (1969) also isolated E. *coli* and other enteric pathogens from seventeen beaches of New York, but Zo Bell (1941b) failed to find out coliform bacteria in any of 961 samples of sea water collected at stations remote from possibilities of terrigenous contamination although large numbers of E. *coli* were found in polluted bays and estuaries. The factors contributing to high occurrence of pathogens in beach water may be due to:

1. Temperature of the water.
2. Presence of organic matter, easily decomposed.
3. Continuous supply of bacteria to the recipient water, through sewage, rainfall, fresh water run-off and drainage.

The present finding is that though there is much biochemical differentiation between marine bacteria and human bacteria both are equally active in all biochemical reactions except their sensitivity pattern towards antibiotics.

SECTION II.

ANTIBIOGRAM OF BACTERIA FROM HUMAN BEINGS AND MARINE ENVIRONMENT.

II. ANTIBIOGRAM OF BACTERIA FROM HUMAN BEINGS AND MARINE ENVIRONMENT:

The basis of antibiotic resistance and the mechanism of antibiotic activity has become a lively branch of cell biology and genetics. Antibiotics are natural organic compounds produced by micro-organisms as secondary and terminal metabolites which are not essential to the life of the producing cell. In the last 40 years over 3,000 antibiotics have been discovered. But very few of them have been found to have the right combination of properties, high activity against the invading organism, low toxicity in mammals and physical and metabolic stabilities to justify their use in man. Those with enduring clinical value include the antibiotics such as gentamycin, the erythromycin and the family of tetracycline. The early penicillins were succeeded by a variety of modified penicillin that proved to be effective against bacterial strains that had developed penicillin resistance. These compounds together with early penicillins are now collectively known as the beta-lactam-antibiotics as the key to the antibacterial properties of these compounds lies in the beta-lactam-ring. The resistant bacteria can inactivate the beta-lactams in one of the three ways: (a) by opening the beta-lactam ring - a reaction which is catalysed by enzymes called penicillinases or beta-lactamases; (b) by splitting off the side chain - a reaction which is catalysed by amidases and (c) by the removal of the acetyl group in the 3-position in the substituted beta-lactams. The last reaction is catalysed by esterases. All these enzymes are widely distributed in the microbial world. The widespread, often indiscriminate use of the antibiotics has resulted in a strong selection for resistant bacteria which have the capacity to produce one or more of these enzymes.

A number of workers have reported on the presence of drug-resistant coliform bacteria in surface waters (Smith, 1970 b, 1971; Feary *et al.*, 1972; Grabow *et al.*, 1975). The principal source being raw and treated hospital and municipal waste (Sturtevant and Feary, 1969; Sturtevant *et al.*, 1971; Grabow and Prozesky, 1973; Linton *et al.*, 1974). R + bacteria has also been isolated from rivers (Smith, 1970 b; Feary *et al.*, 1972) and coastal bathing and canal waters (Smith, 1971; Smith *et al.*, 1974; Sagar M. Goyal *et al.*, 1979). Even fresh water mussel (*Hyridella menziesii*) collected from lakes in New Zealand which were known to be remote from domestic and agricultural wastes were found to harbour R + bacteria (Cooke, 1976 b). Such studies were performed in sediments of marine environments by Sagar M. Goyal *et al.*, in 1979 along the Gulf coast of

Texas which has led to a concern for the water quality of coastal canals along Texas, U.S.A.

Wood (1963) classified marine micro-organisms into Autotrophic, Heterotrophic and contaminants. The latter presents a continuous threat to coastal environment particularly in sewage contaminated areas (Higgins and Burns, 1975; Gameson and Pike, 1968a; McCoy, 1971). Transfer of antibiotic resistance from fishes, animals and human strains of *E. coli* to resident *E. coli* in the alimentary tract of man is a potential public health problem: the presence of bacteria with transferable drug-resistance (R +) in coastal canal water in Houston, U.S.A. was studied by Goyal *et al.* (1979). R - plasmids are extra chromosomal genetic elements determing resistance to antimicrobial drugs. Numerous genera of bacteria including *Pseudomonas*, *Aeromonas*, *Vibrio*, *Streptococcus* and *Enterobacteriaceae* have been found to carry transferable drug resistance.

It has been shown that pathogenic, non-pathogenic bacteria and human enteroviruses are present in large numbers in sewage-polluted marine environment and is a source of disease in man and animals either directly or indirectly (Craun, 1972; Geldreich, 1972; Gangarosa *et al.*, 1972).

In this section comparison of the antibiotic sensitivity of 43 marine strains with 60 human bacterial strains isolated from clinical specimens at the same period was made as the preliminary study of the antibiotic pattern will be of help in future working for the presence of R - factors and their transfer in enterobacteriaceae. Further, determination of the prevalence and distribution of antibiotic - resistant bacteria in the coastal areas are important since they have been associated with water borne epidemics in man (Day *et al*., 1974; Gangarosa *et al*., 1972; Schaeffer and Moriarty, 1974).

Technique employed for sensitivity tests towards Beta-lactam and other antibiotics:

The most widely accepted, most easily performed and least time consuming procedure for determining the susceptibility of bacteria to antibiotics is the disc-agar diffusion method. Following the identification of a bacterial strain it is often necessary to carry out *in vitro* test to determine the susceptibility of that pathogen to each of a range of appropriate antibiotics. The results of such tests assist the marine microbiologists in the classification of the strain and the clinician in the selection of optimally active agents for chemotherapy.

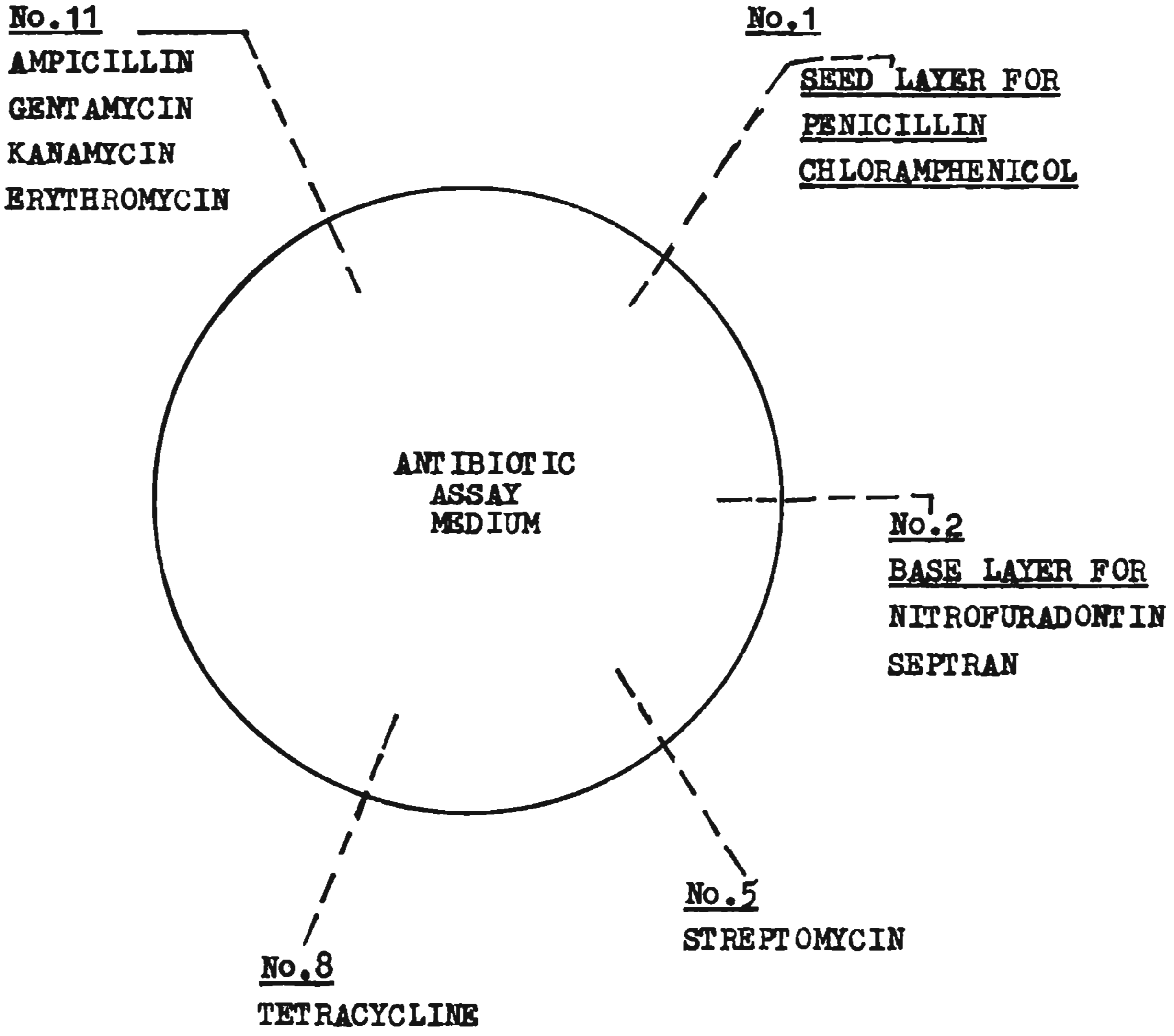
A flood plate is initially prepared from a diluted broth culture of the bacterial strain to be tested and several small absorbent paper discs - each impregnated with a different antibiotic are placed on the surface of the agar.

During subsequent incubation the antibiotics diffuse out into the surrounding agar and zones of growth inhibition occur around those discs which contain antibiotics to which the organism is sensitive.

Different antibiotic sensitivity agars (Table 60) was used for different type of antibiotics as sensitivity agars should not contain constituents which excessively enhance or reduce the inhibitory or lethal effects of the particular antibiotics being tested. (For example, the use of agar containing blood or serum tends to reduce the inhibitory activity of those antibiotics which show a high degree of protein binding). The other important factors are the pH of the test medium as is the inoculum size and period of incubation. In the present investigation 5 types of seed layer were used for the 10 different antibiotics as shown in Table 60. The standardised method "Kirby-Bauer" procedure as described by Bauer *et al.* (1966) was followed in the preparation of antibiotic discs. Discs were prepared in the laboratory in 3 different concentrations of all the ten antibiotics and stored in the refrigerator until further use. Details of preparation of disc has been given in general material and methods.

Antibiotic assay medium from Hindustan dehydrated media (Hi-media) was prepared by adding the required distilled water and sterilised at 15 lbs. pressure (121°C) for 15 minutes and the medium was poured to a depth of 4 mm

TABLE 60. Various medias used as a base for the sensitivity tests by "Kirby and Baur's" Procedure from "Hindustan-dehydrated-media".



in a 100 mm (10 cm) diameter plate. The plates were allowed to dry for at least 30 minutes at 35 - 37°C or at RT for 1 hour before inoculation. Plates were used within 72 hours of preparation for the experiments.

Marine bacteria isolated from littoral waters (43 nos.) and bacteria isolated from clinical specimens (60 nos.) were emulsified in nutrient broth. Inoculation of plates was done within 15 minutes of emulsifying broth culture so that standardisation remains correct. A sterile swab was dipped into the culture and surplus suspension was removed from the swab by rotating it against the side of the tube before plates are seeded. The suspension is streaked evenly in 3 directions on to the surface of the medium. The petri dish was kept aside for drying for at least 5 minutes but not longer than 30 minutes. Appropriate antibiotic disc was dispensed manually on the inoculated plate and gently pressed it down with sterile forceps flamed and cooled between each disc. Discs were placed 10-25 mm from each other to avoid overlapping zones.

Plates were incubated immediately or within 30 minutes for overnight at 35 - 37°C. Best results were obtained after 18 hours of incubation. Zone of inhibition equal to size of disc or even slightly greater eg. 6.5 - 7 mm is taken as negative and zone of inhibition more than 12 - 15 mm was taken as positive meaning that the test organism is sensitive to the given antibiotic. The zone diameter was measured, the end point is taken as complete inhibition of growth as determined by the naked eye. Zones of the control cultures were noted whether their range falls within the ranges indicated in the chart (Difco) to assure the accuracy of the assay.

Antibiotic assay:

A total of 103 strains of bacteria isolated from marine environment and from clinical specimens were screened initially for resistance to selected antibiotics.

To determine the minimal concentration of antibiotic needed to inhibit the microbial growth *in vitro* 3 different concentrations of 10 different antibiotics were tested so that the minimum inhibitory concentration (MIC) of the antibiotic can be determined. The range of inhibitions observed from the MIC upwards, a quantitative assay of the antibiotic is obtained. These two parameters the MIC and the quantitative assay are of immense importance in chemotherapy in determining the efficiency of the antibiotic. The lower the MIC the higher will be its potency. Preliminary studies of antibiotic resistance pattern and levels of MIC were considered to be of help in further working for the identification and classification of bacteria.

The pattern of drug sensitivity of marine bacteria observed during the investigation is given in Table 61. Multiple drug resistance was more prevalent than resistance to one or two drugs among marine bacteria. None of the strains was resistant to Gentamycin and only one strain was resistant to Septran. Tetracycline was found to be resistant to only 2 strains and 3 strains were resistant to Streptomycin. Five strains were resistant to Kanamycin and 7 strains to Chloramphenicol. A higher rate of resistance was found in Penicillin (22), Ampicillin (16), Erythromycin (16), Gentamycin (18), Septran (11) and Cephoron (19). Most common antibiograms encountered during the investigations in marine isolates are given in Table 62. The prevalent pattern as given in the table were PAC (Penicillin, Ampicillin, Chloramphenicol), PAEC (Penicillin, Ampicillin, Erythromycin, Chloramphenicol), PACKESC (Penicillin, Ampicillin, Chloramphenicol, Kanamycin, Erythromycin, Septran, Cephoron) of this order.

In all the antibiotics tested out of 3 concentrations of 10 different antibiotics the lower concentration showed higher rate of resistance in *Klebsiella*, *Pseudomonas* and *E. coli* strains isolated from clinical material though not much differentiation in sensitivity was noted between middle

TABLE 61. Sensitivity pattern of 43 marine bacterial strains* isolated from littoral water near Trivandrum.

Antibiotics	No. of sensitive strains	No. of moderately sensitive strains	No. of resistant strains
1. Penicillin	15 (34.8%)	4 (9.7%)	22 (51.1%)
2. Ampicillin	22 (51.1%)	3 (6.9%)	16 (36.8%)
3. Chloramphenicol	32 (74.4%)	2 (4.6%)	7 (16.1%)
4. Kanamycin	33 (75.9%)	3 (6.9%)	5 (11.5%)
5. Erythromycin	23 (52.9%)	2 (4.6%)	16 (36.8%)
6. Streptomycin	38 (87.4%)	-	3 (6.9%)
7. Tetracycline	36 (82.8%)	3 (6.9%)	2 (4.6%)
8. Gentamycin	22 (50.6%)	1 (2.3%)	18 (41.4%)
9. Septran	24 (55.2%)	-	17 (39.1%)
10. Cepharon	21 (48.3%)	1 (2.3%)	19 (43.7%)

* 15-*Aeromonas*, 8-*Pseudomonas*, 2-*Vibrio*, 7-*Paracolons* and *E. coli*, 11-*Achromobacter*.

TABLE 62. Most common antibiograms encountered in marine bacterial isolates.

Type of bacteria	Most common resistant pattern found	Frequency of occurrence (%)
Achromobacter	All sensitive	8
Pseudomonas	P, Am, E, C	3
	P, Am, C, K, E, S, T, C	1
	P, Am, C	1
	P, Am, C, K, E, S, C	1
	E, T	1
Aeromonas	P, Am, C,G,	2
	P, C, E, C	1
	Am, E, C	2
	P	2
	P, Am	1
	E	1
	P, E	1
	K	1
	Am, E	1
	C	2
	P, A	1
	P, S	1
E. coli	all resistant	2
	P, Am	2
	P, E, S, C	1
	P, K, E, C	1

and higher concentration (Fig. 43). Staphylococcus showed higher rate of resistance only at middle and higher concentration of the antibiotics tested.

Sensitivity pattern of four pathogenic bacteria like Staphylococcus spp., Pseudomonas spp., Escherichia coli, Klebsiella spp. and 43 marine bacteria were studied and the numbers of sensitive and resistant bacteria of both categories were given in Fig. 44. A higher rate of resistance against commonly used antibiotic was noted among human pathogens. Table 63 illustrates sensitivity pattern of bacteria of clinical significance against different antibiotics in vitro. A perusal of the table shows 83.3% of penicillin resistant strains, 63.3% of ampicillin resistant strains and 60% tetracycline resistant strains. Lowest resistance was found with Gentamycin (0%), Kanamycin (17%) and Streptomycin (20%). In total the number of sensitive strains were more (50.8%) when compared to the resistant strains (43%). The percentage of pathogenic strains sensitive and resistant is given in Table 64. Data in Table 64 indicates 36% of Escherichia spp. and 38% of Klebsiella spp. isolated commonly from clinical samples, were resistant to almost all of the antibiotics. The number of sensitive and resistant pathogenic bacteria is illustrated in the Fig. 45. Details of the sensitivity pattern of 43 marine strains is illustrated in Table 65. If the zone of inhibition was below

Fig. 43. The resistant rate of human pathogens like (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, (c) *Klebsiella* spp. and (d) *Staphylococcus* in three different concentrations.

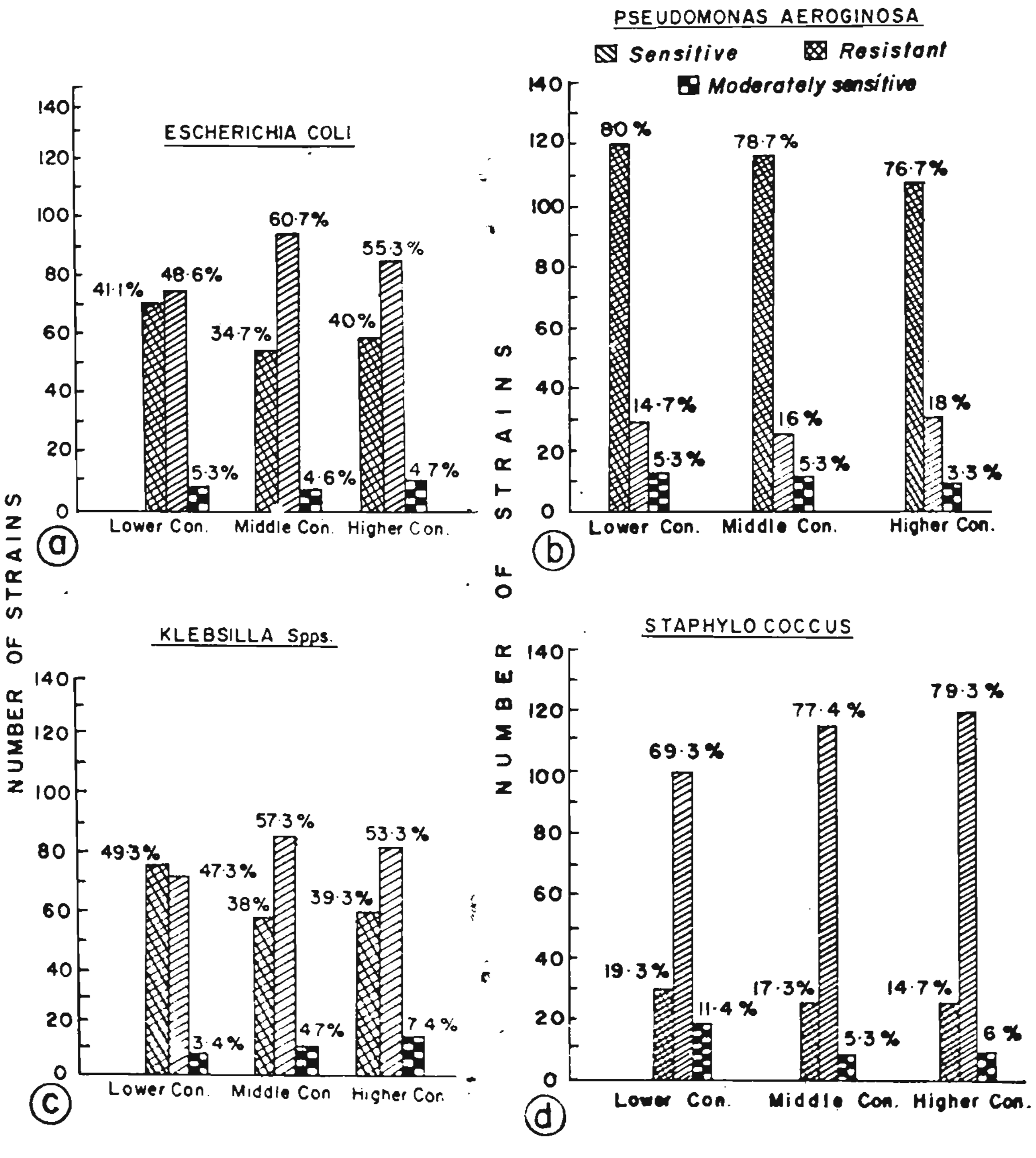

PSEUDOMONAS AEROGINOSA
▨ Sensitive
▩ Resistant
▣ Moderately sensitive
NUMBER OF STRAINS
ESCHERICHIA COLI
0
20
40
60
80
100
120
140
41·1%
48·6%
5·3%
34·7%
60·7%
4·6%
40%
55·3%
4·7%
Lower Con.
Middle Con.
Higher Con.
a
80%
14·7%
5·3%
78·7%
16%
5·3%
76·7%
18%
3·3%
b
KLEBSILLA Spps.
49·3%
47·3%
3·4%
38%
57·3%
4·7%
39·3%
53·3%
7·4%
c
STAPHYLO COCCUS
19·3%
69·3%
11·4%
17·3%
77·4%
5·3%
14·7%
79·3%
6%
d

Fig. 44. Number of sensitive (a) and resistant (b) human as well as marine bacteria against 11 commonly used antibiotics.

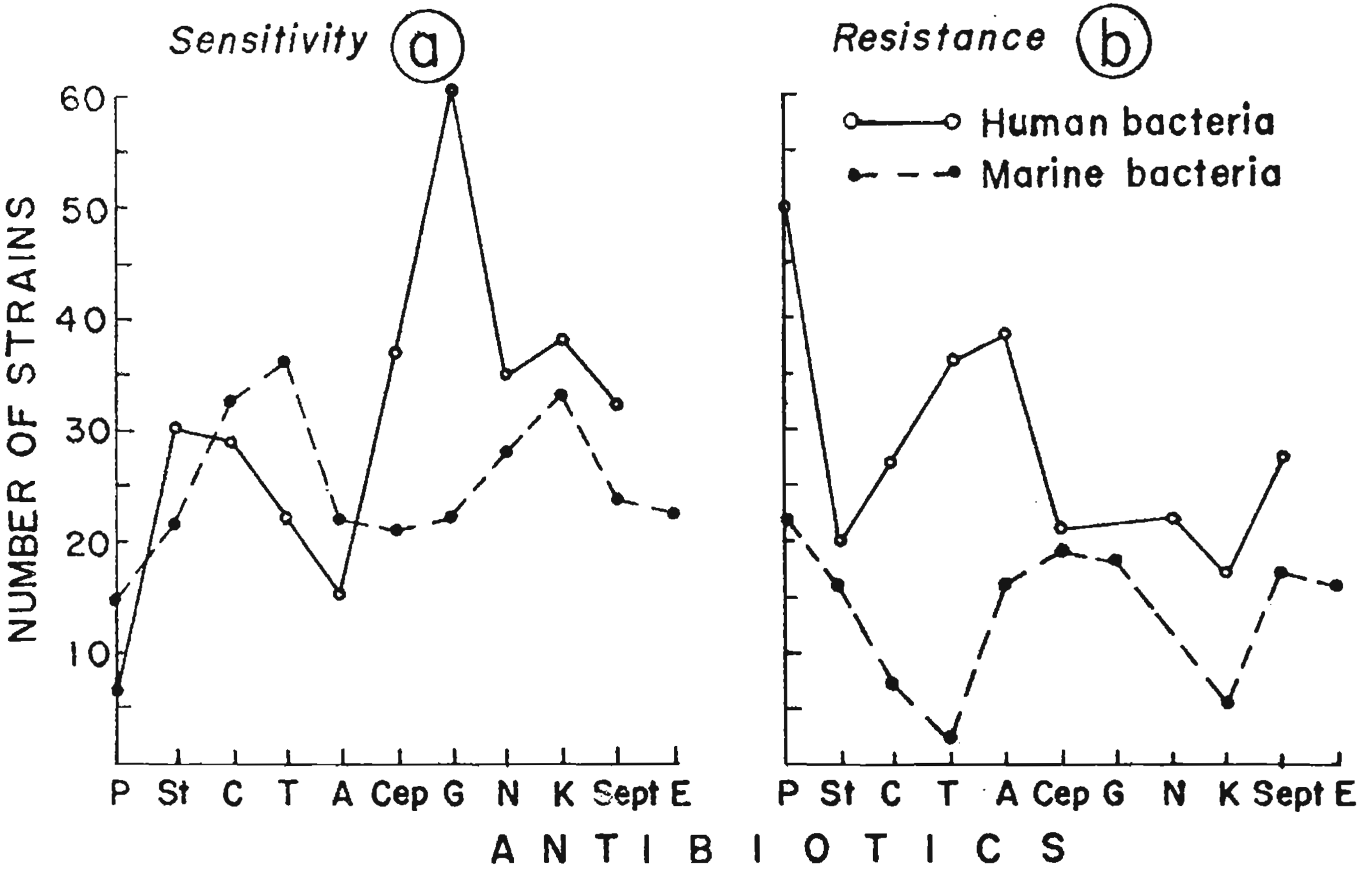

Sensitivity
ⓐ
Resistance
ⓑ
Human bacteria
Marine bacteria
NUMBER OF STRAINS
60
50
40
30
20
10
P St C T A Cep G N K Sept E
P St C T A Cep G N K Sept E
ANTIBIOTICS

TABLE 63. Sensitivity pattern of human pathogens* against different antibiotics commonly used in clinical practice by *in vitro* techniques.

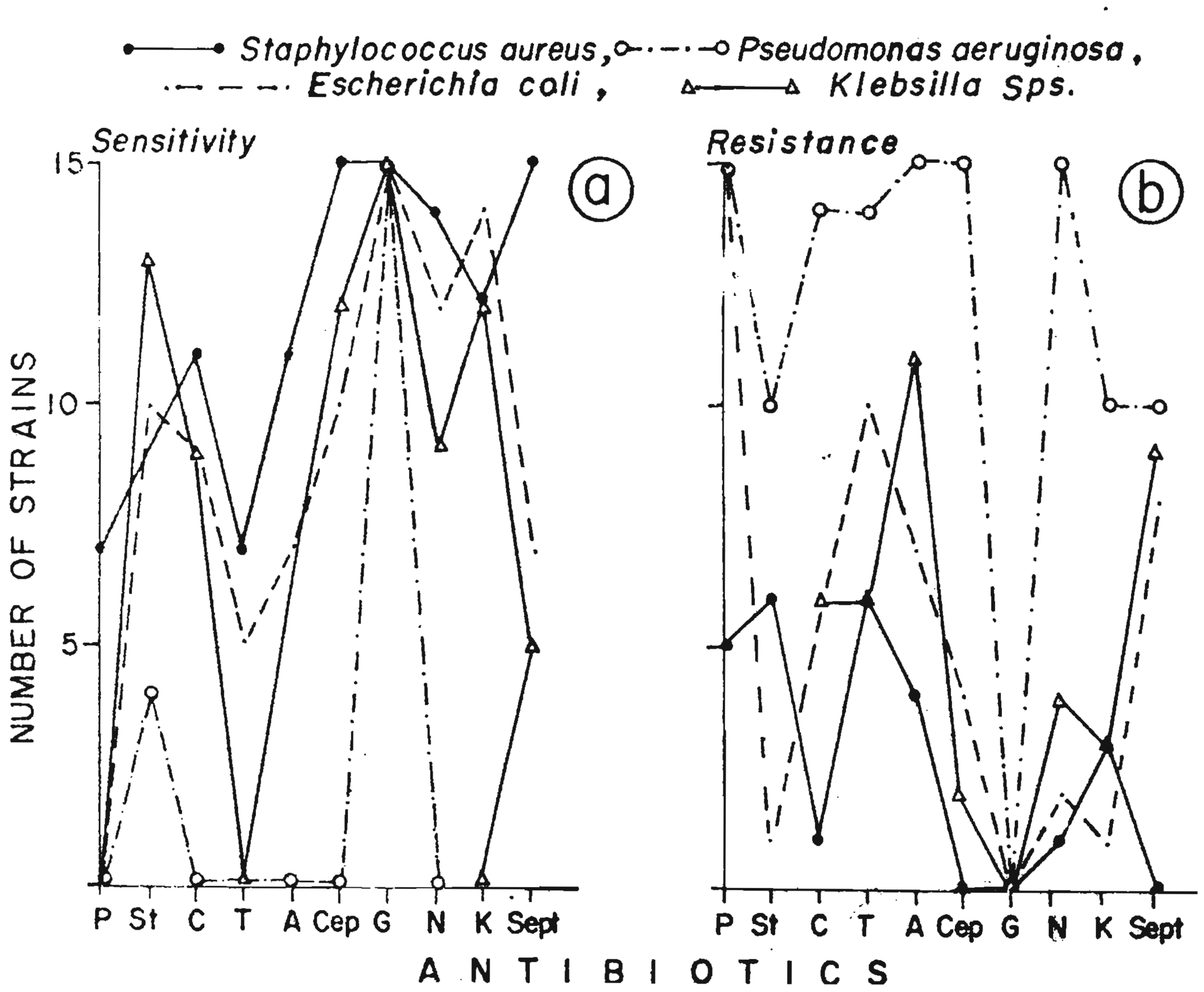
Name of antibiotics	Con./disc	No. of resistant strains	No. of sensitive strains	No. of mode sensitive
1. Penicillin	10 units	50 (83.3%)	7 (11.7%)	3 (5%)
2. Streptomycin	50 µgm	20 (33.3%)	30 (50%)	10 (15%)
3. Chloramphenicol	50 µgm	27 (45%)	29 (48.3%)	4 (6.7%)
4. Tetracycline	50 µgm	36 (60%)	22 (36.7%)	2 (3.3%)
5. Ampicillin	10 µgm	38 (63.3%)	15 (25%)	7 (11.7%)
6. Cephaloridine	5 µgm	21 (35%)	37 (61.7%)	2 (3.3%)
7. Gentamycin	10 µgm	0	60 (100%)	0
8. Nitrofurantoin	100 µgm	22 (36.7%)	35 (58.3%)	3 (5%)
9. Kanamycin	50 µgm	17 (28.3%)	38 (63.3%)	5 (8.4%)
10. Septran	300 ugm	27 (45%)	32 (53.3%)	1 (1.7%)
Total		258 (43%)	305 (50.8%)	37 (6.2%)

* 15 strains each of *S. aureus*, *P. aeruginosa*, *E. coli* and *Klebsiella* species.

TABLE 64. Sensitivity pattern of four pathogenic bacteria commonly isolated from clinical specimens.

Antibiotics	*Staphylococcus* spp.		*Pseudomonas* spp.		*Escherichia coli*		*Klebsiella* spp.	
	No. of sensit.	No. of resist.	No. of sensit.	No. of resist.	No. of sensit.	No. of resist.	No. of sensit.	No. of resist.
1. Penicillin	7(46.7%)	5(33.3%)	-	15(100%)	-	15(100%)	-	15(100%)
2. Streptomycin	9(60%)	6(40%)	4(26.7%)	10(66.7%)	10(66.7%)	1(6.7%)	13(86.7%)	1(6.7%)
3. Chloramphenicol	11(73.3%)	1(6.7%)	-	14(93.3%)	9(60%)	6(40%)	9(60%)	6(40%)
4. Tetracycline	7(46.7%)	6(40%)	-	14(93.3%)	5(33.3%)	10(66.7%)	9(60%)	6(40%)
5. Ampicillin	11(73.3%)	4(26.7%)	-	15(100%)	7(46.7%)	7(46.7%)	-	11(73.3%)
6. Cephaloridine	15(100%)	-	-	15(100%)	10(66.7%)	4(26.7%)	12(80%)	2(13.3%)
7. Gentamycin	15(100%)	-	15(100%)	-	15(100%)	-	15(100%)	-
8. Nitrofurantoin	14(93.3%)	1(6.7%)	-	15(100%)	12(80%)	2(13.3%)	9(60%)	4(26.6%)
9. Kanamycin	12(80%)	3(20%)	-	10(66.7%)	14(93.3%)	1(6.7%)	12(80%)	3(20%)
10. Septran	15(100%)	-	5(33.3%)	10(66.7%)	7(46.7%)	8(53.3%)	5(33.3%)	9(60%)
Total	116(77.3%)	26(27.3%)	24(16%)	118(78.7%)	89(59.3%)	54(36%)	84(56%)	57(38%)

Fig. 45. Number of sensitive (a) and resistent (b) pathogenic isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. against 10 commonly used antibiotics.


Staphylococcus aureus,
Pseudomonas aeruginosa,
Escherichia coli,
Klebsilla Sps.
Sensitivity
Resistance
(a)
(b)
NUMBER OF STRAINS
15
10
5
P
St
C
T
A
Cep
G
N
K
Sept
ANTIBIOTICS

6.5 - 7 mm it was taken as negative and if the zone of inhibition was more than 12 - 15 mm it was taken as positiv reaction.

Regarding marine isolates 32.1% of the isolates were found to be resistant to all of the antibiotics tested. 100% resistance was seen in the human isolates to only penicillin (Table 64) whereas in marine isolates 100% resistance was found with three antibiotics i.e. Penicillin, Chloramphenicol and Cephoron (Table 66). These values are higher than those reported by Fontaine and Hoadley (1976) for total coliforms from domestic sewage (55.5%) but closer to values for hospital sewage (90.2%). Clinical and marine bacteria that have gained resistance to β-lactam antibiotics like Penicillin and Ampicillin and related compounds often arise by the acquisition of a plasmid that produces a β-lactamase. An increase in β-lactamase activity has also been shown to cause resistance in some mutants isolated in the laboratory by Sprat *et al.* (1977). In other cases β-lactamase activity is not the cause of resistance and only in gram-negative bacteria alteration of the cell envelope resulting in decreased penetration of the antibiotic to the targets responsible for lethality in cytoplasmic membrane has been proposed. Several investigators have used selective media containing antibiotics to isolate antibiotic resistant bacteria (Koditschek and Guyre, 1974; Sturtevant *et al.*, 1971) from raw or treated sewage and poorly processed sludge but in the present investigation this approach yielded poor results when plated with sea water. It is also observed that certain bacteria especially those naturally present in the intestine of man, when introduced into an aqueous environment become stressed and debilated. This observation has been reported previously by Bissonnette *et al*. (1975); Maxey (1970); and Stuart *et al*. (1977). These workers demonstrated that selective media used to enumerate stressed or debilated coliforms could cause significant loss of these indicator bacteria which are resistant to antibiotics. In the present investigation it was found that approximately 2% of these colonies appearing in MacConky agar were resistant to penicillin, ampicillin and cephoron.

Table 67 illustrates comparative sensitive pattern of bacteria of marine and human origin against different antibiotic used in clinical practice tested by *in vitro* method. Penicillin resistance was found to be more prevalent both in bacteria of human origin (83%) as well as in marine bacteria (51.2%). Ampicillin came second in this category under β-lactam antibiotics in human bacteria whereas marine bacteria were more resistant to Cephaloridine (43.9%) and Gentamycin (41.4%). 100% sensitiveness was seen in human bacteria with the antibiotic Gentamycin (10 μgm). Tetracycline was found to be the fourth one in the order of resi-

TABLE 67. Comparative sensitivity pattern of bacteria of marine and human origin against different antibiotics used in clinical practice (tested by *in vitro* method).

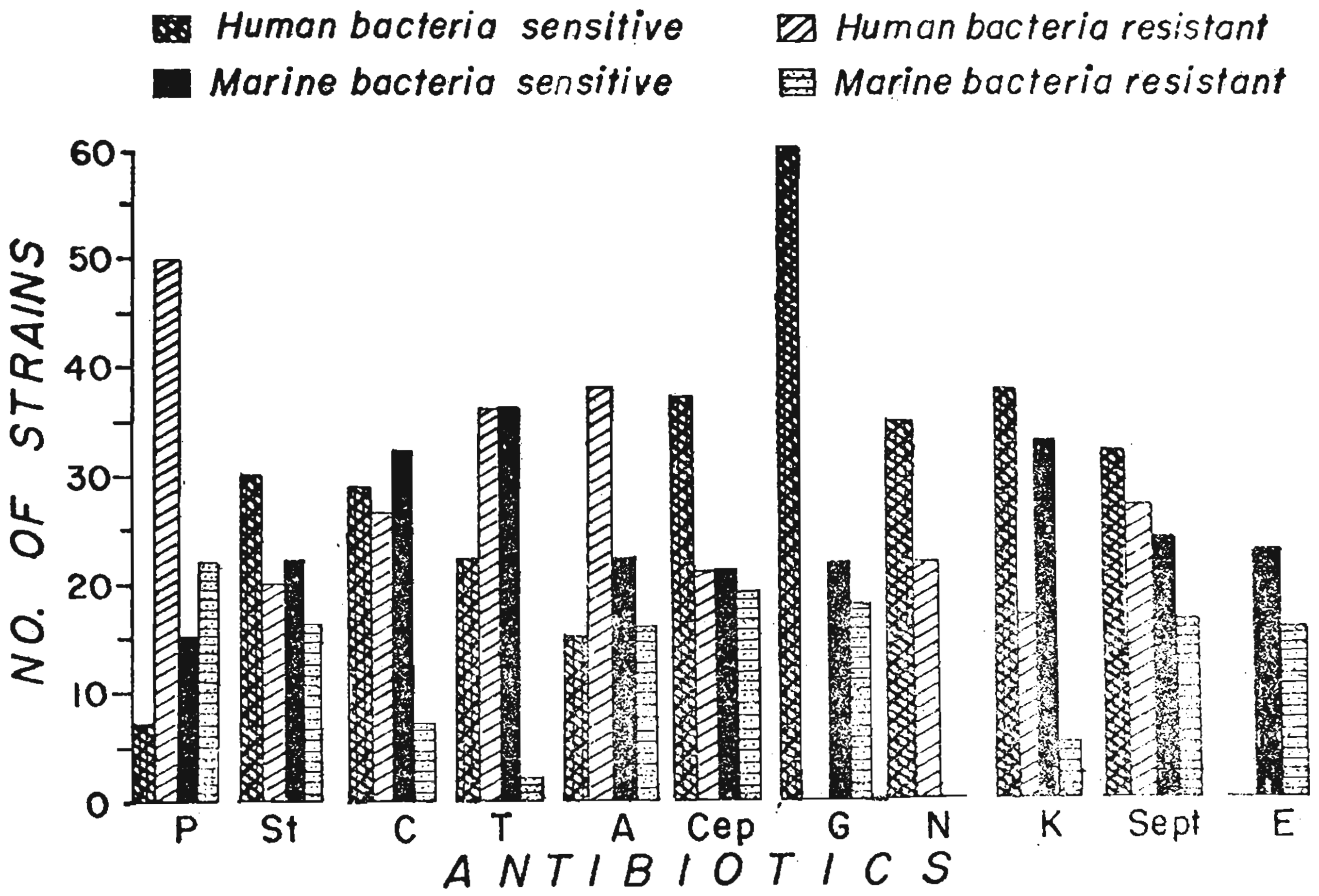
Antibiotics	Conc./disc	Human bacteria (60 strains)		Marine bacteria (43 strains)	
		No. of sensitive	No. of resistant	No. of sensitive	No. of resistant
1. Penicillin	10 units	7 (11.7%)	50 (83%)	15 (34.8%)	22 (51.2%)
2. Streptomycin	50 µgm	30 (50%)	20 (33.3%)	22 (51.1%	16 (36.8%)
3. Chloramphenicol	50 µgm	29 (48.3%)	27 (45%)	32 (74.4%)	7 (16.1%)
4. Tetracycline	50 µgm	22 (36.7%)	36 (60%)	36 (82.8%)	2 (4%)
5. Ampicillin	10 µgm	15 (25%)	38 (63.3%)	22 (51.1%	16 (36.8%)
6. Cephaloridine	5 µgm	37 (61.7%)	21 (35%)	21 (48.3%)	19 (43.7%)
7. Gentamycin	10 µgm	60 (100%)	0	22 (50.6%)	18 (41.4%)
8. Nitrofuradantin	100 µgm	35 (58.3%)	22 (36.7%)	-	-
9. Kanamycin	50 µgm	38 (63.3%)	17 (28.3%)	33 (75.9%)	5 (11.5%)
10. Septran	300 µgm	32 (53.3%)	27 (45%)	24 (55.2%)	17 (39.1%)
11. Erythromycin	15 µgm	-	-	23 (52.9%)	16 (36.8%)
Total		305 (50.8%)	258 (43%)	250 (58.1%)	138 (32.1%)

stance and 60% of the isolates were resistant to Tetracycline (50 µgm). In marine bacteria resistance towards (Fig. 46) Tetracycline (4%), Kanamycin (11.5%) and Chloramphenicol (16.1%) was less when compared to other aminoglycoside antibiotics.

Based on *E. coli* distribution the Vishinjam beach was considered to be most polluted of all the three beaches studied. The intensity of *E. coli* in Shankumughom and Kovalam was found to be the same.

Eventhough Vishinjam beach possessed more faecal coliforms than the beaches of Shankumughom and Kovalam, the Shankumughom beach possessed antibiotic resistant microflora distinct from that of other sites and had an average of 16 *E. coli* /ml in spread plate count technique. These data support the view that the three beaches were considerably polluted by human waste during the study period and quantity of antibiotic resistant microflora support the view that urban communities living in and around Trivandrum may harbour significantly greater numbers of resistant organisms than rural communities in the areas of Kovalam and Vishinjam which are less exposed to the administration of antibiotics for their ailments.

Fig. 46. Comparative sensitive pattern of bacteria of marine and human origin against different antibiotics used in clinical practice. (tested by *in vitro* method).


Human bacteria sensitive
Marine bacteria sensitive
Human bacteria resistant
Marine bacteria resistant
NO. OF STRAINS
60
50
40
30
20
10
0
P
St
C
T
A
Cep
G
N
K
Sept
E
ANTIBIOTICS

The high rate of incidence of multiple drug resistant organisms in the littoral waters of Trivandrum indicates the possibility of R-factor mediated drug resistant bacteria also in this environment. Apparently these drug resistant bacteria are discharged through raw or treated sewage into these waters whose composition reflects previous and immediate selective pressures. If these bacteria are ingested along with fish caught in this area they could theoretically transfer their antibiotic resistance to other gram-negative pathogens of different genera in the intestine (Davies and Round, 1972). Goyal *et al*. (1979 found approximately 53% of their coliform isolates could transfer their resistance pattern to other gram-negative pathogens. Certain *E. coli*, carrying plasmid linked genes for resistance to various antimicrobial agents can transfer these genes and the gene coding for enterotoxin production to a suitable recipient bacterial cell (Gyles *et al*., 1977; Smith, 1967). Such a transfer would create a pathogen with formidable capabilities, possessing enhanced infectivity and virulence. Since the use of new and old antibiotics is constantly increasing both for clinical, agricultural and maricultural purposes it is necessary to carry out an area wide survey to assess the incidence and extent of the multiple drug-resistance. Otherwise we may reach a situation when the future of chemotherapy can appear dubious.

Selection of R-factors in culture system:

Regarding usage of polluted water for Piscicultural studi it is believed that situations such as those mentioned above contribute measurably as causes responsible for the recovery of R-factor carrying organisms. In natural ecosystem the initial introduction of an antibiotic resistant bacterial population in fish is of very little or no consequence since it may occur at a low frequency in the total bacterial population even in the absence of antibiotic pressure (Smith, 1973). The potential danger that exists in any system lies in the indiscriminant use of antibiotics particularly tetracycline as "cure all". The misuse of antibiotics in culture system also may result in the selection of R-factor carrying clones of pathogenic bacteria. Ultimately an antibiotic resistant bacterial population may result in a fish disease problem that would not respond to usual antibiotic therapy. This same bacterial population may also serve as a source of potential transfer of antibiotic resistance to other similar micro-organisms. Resistance to different aminoglycoside antibiotics like Streptomycin, Kanamycin etc is due to several enzymes produced by different R-factors (Panse, 1981). Several enzymes have been characterised which modify different groups of aminoglycoside antibiotics. Cautious and responsible use of antibiotics will aid in minimising the development and spread of potential R-factors carrying organisms that may confer antibiotic resistance to otherwise antibiotic sensitive bacterial species.

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SECTION III.

SEROTYPING OF *ESCHERICHIA COLI* ISOLATED FROM ESTUARINE ENVIRONMENT.

III. SEROTYPING OF ESCHERICHIA COLI : ISOLATION OF ENTEROPATHOGENIC ESCHERICHIA COLI FROM SEAWATER AND SEDIMENT:

Investigations on indicators of the pathogenic microbial flora of sea water and sediments revealed the presence of enteropathic *Escherichia coli*. A total of 446 *E. coli* strains were isolated from 3 stations from sea water and sediment during the period of July 1975 to June 1976. Fig. 47 illustrates isolation and screening of *E. coli*. Only 230 isolates were subjected to preliminary serological determination, O-serotyping and antibiogram typing and seven 'O'-serotypes has been encountered. Table 68 gives the details of isolates, isolated from sea water and sediment. Serotype 026, 055, 086, 0111, 0119, 0127 and 0128 were identified in *E. coli* isolated from sediment samples. Except the O-serotype 026, all the other serotypes were isolated also from sea water. Sensitivity tests on enteropathic *E. coli* strains were carried out in the laboratory and the results are discussed. A high insidence of antibiotic resistance was found. The results suggest that marine environment also can be identified as potential reservoir of antibiotic resistant bacteria with transferrable drug resistance.

Fig. 47. Isolation and screening procedure of *Escherichia coli* for serological typing of the species.

A variety of pathogens can be regularly detected in sea water and sediments contaminated by sewage outlets by using methods which are sensitive enough. Usually, these pathogens or indicators of pathogens will reflect the current state of community health. *E. coli* isolated repeatedly from both human and animal sources are found widely distributed in the marine environment. Although *E. coli* is one of the more common groups of organisms in our environment, and usually non-pathogenic it can cause clinical infections in man. *E. coli* has been found to be etiological agent in cases of cystitis, pyelitis, pyelonephritis, gall bladder infections, septicaemia, meningitis, endocarditis, summer diarrhoea of children and adults and epidemic infant diarrhoea. Of these infections, epidemic infant diarrhoea is of foremost clinical significance.

The extensive scheme for serotyping *E. coli* is a complex and time consuming method and is carried out only in specialised laboratories. The scheme in use is based on the work begun by Kauffmann in the early 1940s (Kauffmann 1943 and 1944). It is based on the study of three antigens the O somatic antigen, the K capsular antigen and the H flagellar antigen. The modern internationally accepted serotyping scheme for strains of *E. coli* has been described by Edwards *et al.* (1955) and Kauffmann (1969).

The O antigen is a thermostable somatic antigen. Its specificity is related to the arrangement of the sugar residues in the cell wall and the cross-reactions occurring between different O-antigen have been related to the presence of the same sugars (Ørskov *et al.*, 1967). About 150 antigens are recognised at present but it is probable that there are others. Most of the recognised strains have been defined in Europe and there is considerable geographical variation in the distribution of *E. coli* serotypes.

Because of the large number of antisera necessary to type *E. coli*, these are generally grouped together into 'pools' of antisera with similar cross-reactions being put together. The reaction of the antigen with the constituent members of the pools is then determined. The final identification of the serotype of the organism was done by determining the titre of the sera whose cross-reactions being put together. The reaction of the antigen with the constituent members of the pools was then determined. The final identification of the serotype of the organism was done by determining the titre of the sera whose cross-reactions have been investigated with the organisms or by the use of absorbed sera.

Prior to the development of serological methods for the identification of pathogenic *E. coli*, biochemical tests were used in an attempt to differentiate them from *E. coli* isolated from different sources of environment. Biochemical tests alone were inadequate in making such a distinction, because non-pathogenic and pathogenic *E. coli* may possess different antigenic identities but they may have identical biochemical characteristics.

The delineation of enteropathogenic cultures from the "non-pathogens" was facilitated by the work of Kauffmann *et al.* (1947) which resulted in a serological typing system for *E. coli*. Later work of Ørskov (1956) and studies of Ewing and Edwards (1955) and Ewing (1956) contributed to the development of an antigenic scheme for this group of organisms which made possible the identification and characterisation of non-pathogenic *E. coli* and those serotypes of pathogenic *E. coli*. Nearly 150 serotypes were identified so far. But only 14 serological types are pathogenic causing diarrhoea and dehydration.

Epidemic or sporadic gastro-enteritis has been frequently reported from sea fish and fresh water mussels (Stephen *et al.*, 1975) which was caused by enteropathic *E. coli*. *Salmonell* and *Shigella* have been incriminated in this type of infection and occasionally bacteria of other groups or viral agents have also been suspected. However, the majority of such out breaks have been attributed to enteropathic *E. coli*. The use of serological methods privide evidences of presence of enteropathic *E. coli*.

Ørskov (1951) drew the attention first to the association of certain serotypes with human and animal enteritis. Mackel *et al.* (1960) were able to show an association between domestic cats and infantile diarrhoea, the serotypes involved being 055, 0111, 0112 and 0126. Recently Tolgai *et al.* (1971) reported the occurrence of enteropathic *E. coli* strains in the alimentary canal of *Rattus rattus* and *R. norvegicus*. Stephen *et al.* reported the isolation of enteropathic *E. coli* from the sea fishes like mackerels (*Rastrelliger kanagurta*), sardines (*Sardinella longiceps*) and fresh water mussels (*Lamellidens marginalis*).

The work reported in this section is an attempt to establish that enteropathic *E. coli* and antibiotic resistant organisms are contaminated into the marine environment by sewage and drainage and to survey how frequently *E. coli* of the same serotype found in sea water could also be isolated from the sediment in the same sampling stations.

Isolation of faecal coliforms from sea water and sediments:

On arrival at the laboratory, the sea water and sediment samples which were collected aseptically were plated on MacConkey Agar. Tergitol[7] Agar and nutrient agar and incubated aerobically for 16 to 48 hours at 37°C. About 10 discrete colonies from each MacConkey Agar plate were picked up from each sample and subjected to all the biochemical tests. Colonies producing indole in peptone water, and acid and gas in MacConkey broth after overnight incubation in a water bath at 44°C were considered to be E. *coli* faecal type I .

Isolates not strictly E. *coli* type I were not considered. The purity of the isolates was checked by sub-culture on MacConkey Agar. After being satisfactorily identified each isolate was stored on a nutrient agar slope at room temperature in the dark.

It is essential that appropriate media must be used for detecting, isolating and differentiating micro-organisms on morphological and physiological characteristics before applying serological techniques. The differential media, MacConkey Agar, will inhibit the growth of gram-positive micro-organisms for at least [illegible] hours. Blood agar is a non-selective media and is used to assure the growth of those strains of E. *coli* which do not grow well on differential media. The use of these media is also important in obtaining accurate slide agglutination results. If E. *coli* directly isolated from MacConkey agar is subjected to slide agglutination, the results cannot be accurate as bile salts in MacConkey agar or acid from lactose fermentation may result in erroneous slide agglutination patterns. All biochemically confirmed *E.coli* were plated on blood agar for slide agglutination test with the polyvalent '0' antiserum against the common enteropathic serotypes. This was followed by slide agglutination with antisera against specific serotypes. The methods described by Taylor (1960) was stricly be followed for the identification of enteropathic *E. coli*. Antibiotic sensitivity of the isolated enteropathic *E. coli* was tested *in vitro* for their sensitivity towards different antibiotics as per the paper disc diffusion method of Cruickshank (1968).

Totally 436 *E. coli* both pathogenic and non-pathogenic strains were isolated in the period July 1975 to June 1976 from Cochin Backwater out of which 230 strains were turned into enteropathic *E. coli* belonging to seven different serotypes *viz.* 026, 055, 086, 0111, 0119, 0127 and 0128. From the 14 different distinct serotypes of *E.E. coli* (Dart and Stretton (1980) only seven serotypes were encountered in the present study. Table 68 illustrates the enteropathic *E. coli* and other bacterial isolates from sea water and sediments. In total, 102 sea water samples and 104 sediment samples were analysed for enteropathic *E. coli*, non-pathogenic *E. coli*, *Klebsiella* sp., *Proteus mirabilis*, *Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staph-aureus* and *Providence* groups in order to understand the bacterial population structure in polluted areas. Apart from non-pathogenic and enteropathic *E. coli* 716 isolates were identified by doing conventional biochemical tests. Mention must be made regarding the variants of *E. coli* serotypes encountered during the study period. H_2S production was noted in 12 *E. coli* strains isolated from sediments during the onset of monsoon period in 1976. Trelearen (1960) encountered some strains of *E. coli* producing spurious hydrogen sulfide isolated from the presence of an anaerobe (*Eubacterium lentum*) growing in synergism with the Enterobacteria and producing H_2S. This fact suggests, that *E. coli* producing H_2S encountered in the present investigation may also contain synergistic anaerobes capable of producing H_2S. A few strains fermented lactose only after 48 hours of incubation on MacConkey agar and hence appeared as non-lactose fermenting serotypes. Some lactose fermenting strains were found to be non-motile. On the whole each *E. coli* serotype had a particular fermentation pattern. These findings are in conformity with that of Trelearen (1960) and Taylor (1960).

Some of the 0 types found in this survey were present in relatively few other animals on environment while others occurred more frequently in human faecal matter. The data indicated that these 7 types occurred in sea water at the level of 44.34% and in the sediment at the level 55.6%. Within these O-types 76% were found to be antibiotic resistant isolates and it is of interest that with one exception (026) all these O-types have been isolated from man (Hartley et al., 1975).

It is not understood why certain O-types are more abundant in sediments than in sea water but it may be due to their ability to colonise in the sediment. The suggestion that certain serotypes are strong in certain environment has been made by Cooke (1974). When certain *E. coli* O-types appeared in the faecal matter of Gastroenteritis affected infants, adults or animals they were likely to persists in sewage (Gauge, Gunther & Spaulding, 1961) as well as a similar situation could occur in sea water and sediment whenever sewage is let into the backwater or sea. In Japan *E. coli* was isolated in marine sediments and from fish stomach and gut contents upto 3 months after the sewage was let into the marine environment. In sea water the *E. coli* serotypes are less which may be due to presence of inorganic salts and absence of organic matter whereas sediments are rich in nutrient rich organic matter. Another possible reason for abundance of specific O-serotypes is that a high proportion of isolates are drug-resistant.

A wide scatter of seven O-types have been found to occur in both sea water (102) and sediment (128). Six O-types were found to be common to both (Table 68) the environment. The figures indicate that there is no demarcation between animal '*E. coli*' and human '*E. coli*' in the strains isolated, eventhough the source of pollution of this faecal coli into the marine environment may be given by the faecal index. Survey of marine fishes and fresh water mussels also showed considerable overlap with O-types of human origin (Stephen *et al.*, 1975). A high adaptation was found in *E. coli* serotypes O142 and O149 to different temperature of sea water by Alton (1980) in the experimental study in Russia. The study revealed that the adapted strains did not die in sea water though the growth of their colonies at 37°C ceased. The growth of colonies at lower temperatures lasted for a longer period. Only a very little percentage of *E. coli* O142 and O149 cells did not adapt to the temperature or the chemical composition of sea water, being inactive. From the present investigation it is clear that temperature is not the factor for the distribution of *E. coli* in sea water or sediment as *E. coli* and *E.E. Coli* were encountered as usual in low temperature in June (25°C) as well as at high temperature in March and April (30.1°C).

The O-types isolated from sea water and sediment were further divided on the basis of the distribution of antibiotic sensitivity and resistance. Resistance pattern with Penicillin (P), Erythromycin (E), Ampicillin (A), Oxytetracycline (OTC), Streptomycin (ST), Chloramphenicol (C), Kanamycin (K), Neomycin (N), Gentamycin (G) and Sulphamethoxazole (S) were determined and the results are presented in Table 69.

The resistant pattern revealed that with the exception of 62 isolates the remaining 168 strains were resistant to Gentamycin and only 24 strains were sensitive to Neomycin. All the 230 strains were uniformly resistant to Penicillin and Erythromycin but showed varying degrees of susceptibility to other drugs like Chloramphenicol, Ampicillin, Gentamycin, Streptomycin, Kanamycin, Oxytetracycline and Sulphamethoxazole (Table 69). The number of drug resistant strains were relatively high, which indicate the possibility of exchange of genetic material ('R' factor) between enteric allochthonous bacteria and marine autochthonous micro-organisms.

Of the total number (230) of isolates tested, very low percentage of resistance was seen to oxytetracycline (15.2%) and Streptomycin (15.6%). Overall sensitivity revealed that Neomycin (89.5%), Gentamycin (73%) and Kanamycin (61.3%) were most effective followed in order of efficacy by Sulfamethoxazole

TABLE 69. Antibiotic Sensitivity of 230 Enteropathic *Escherichia coli*.

Antibiotics	Antibiotic content	Strain inhibited from seawater	No. of strains inhibited from sediment	Total No. of strains inhibited	% of strains inhibited
1. Penicillin	10 units	0	0	0	100.0
2. Ampicillin	10 mgm	36	17	53	23.0
3. Chloramphenicol	50 mgm	24	40	64	27.8
4. Erythromycin	30 mgm	0	0	0	100.0
5. Kanamycin	50 mgm	72	69	141	61.3
6. Neomycin	50 mgm	93	113	206	89.5
7. Oxytetracycline	50 mgm	5	30	35	15.21
8. Trimethoprim/ Suphamethoxazole	1.25 mgm/23.75 mgm	56	65	121	52.6
9. Gentamycin	10 mgm	88	80	168	73.0
10. Streptomycin	50 mgm	24	12	36	15.6

(52.6%), Chloramphenicol (27.8%), and Ampicillin (23%). The high percentage of micro-organisms resistant to commonly used antibiotics indicate need for resistance testing before taking any remedial measures.

In sea water 51% (52/102) of the O-type included resistant isolates and 49% (50/102) were sensitive isolates. A similar evaluation of sediment data revealed that 50.8% (65/128) were resistant strains and 49.2% (63/128) were sensitive strains. These figures are comparable suggesting a similar qualitative distribution of drug resistance and sensitivity throughout the O-types isolated. Some O-types were invariably resistant, others sensitive but a much large proportion include sensitive and resistant strains.

Although a similar number of O-type isolated from each environment were found to include resistant isolates, the actual proportion of resistant to sensitive isolates in the sediment was higher than that found in sea water. However, it was rare to isolate resistant strains from marine sediment on non-selective media whereas in sea water 60% of all strains isolated without antibiotic selection pressure were antibiotic resistant. Studies on sea fish and other fresh water mussels published (Stephen *et al.*, 1975) revealed a similar pattern and it may be argued that this is a reflection of the considurable pressure exerted by widespread use of antibiotics in animals and human beings in previous years.

It is reported that antibiotic resistant *E. coli* in the intestine of man is considerably less when compared to the intestine of animals is a matter of some encouragement. It may indicate that the disputed flow of resistance factors from the sewage reservoir to man is rather limited but the data of the present study reveals that the level of resistance of the total R^+ *E. coli* flora in sediment constitutes a potentially important source of R^+ factors. The abundance of antibiotic resistant strains is an indication that an increased percentage of isolates harbouring an R^+ factor is closely associated with antibiotic resistance strains. Bourque *et al.* (1980) compared the ability of strains harbouring already an R^+ factor to receive another R^+ factor plasmid, with isolates not harbouring such a plasmid and observed no significant difference in their ability to receive the plasmid containing new R^+ factors. Kaul in collaboration with Waling (1978) isolated from a sample of 123 healthy individuals 110 drug-resistant *E. coli* strains of which nearly 46% were multiple drug resistant *E. coli* strains. Several patterns of multiple drug resistant (MDR) were observed and an interesting finding of this survey was a much lower MDR was observed among individuals from rural areas near Bombay where exposure to antibiotics is less. Obviously frequent expostures to antibiotics is responsible for the high occurrence of MDR strains among the urbanites. The pathway of resistant enteropathic E. <u>coli</u> through food chain is shown in Table 70.

TABLE 70. Pathway of Bacteria R^+ E.E. <u>coli</u> into man from sewage contaminated marine environment.

R^+ E.E. <u>coli</u> from sewage	→	Sensitive and resistant E. <u>coli</u> in sea water and sediments. It exists above 10^3/ml OR/gm	→	Through cultured fish or oysters & fishery products	→	Through food chain ↓ Man.

The fishes that feed in the polluted areas can pick up contamination on the surface of their gill or skin as well as in the intestine. The transport of such bacteria by fish migrating into and out of polluted areas is poorly understood and little is known of their transfer to man through food chain or by other means. It is known that potentially pathogenic bacteria such as R^+ E.E. <u>coli</u>, <u>Salmonella</u>, <u>Shigella</u>

and *Clostridium perfringens* can gain entrance to marine and estuarine areas from sewage and land run-off and consequently comprise a route for the potential transmission of R$^+$ factors from fishes to man. There is every possibility that pathogens found in the marine environment could also be isolated from marine fish. Therefore R$^+$ factors of E.E. *coli* orginating from sediments which are deposited by sewage may contribute the flow of R-plasmids from sea water to fishes and to man accordingly. Workers involved in cleaning in the fish processing factory or in the household area are also exposed to possible risk of infection by these resistant organisms. This possibility is supported by the view that urban communities harbour significantly greater numbers of resistant organisms than rural communities who are less exposed to the use of antibiotics for ailments. Whichever view is accepted about the spread of R$^+$ factors it is agreed that the use of antibiotics has the effect of selecting R$^+$ bacteria. Quite disturbingly all the transmissible drug resistance factors from resistant E. *coli* could also be transmitted to sensitive E. *coli* strains which may behave as causative agent for gastro-enteritis. Also, there is every possibility of exchange of genetic material between enteric allochthonous bacteria and marine autothonous bacteria. In order to maintain the *status quo* of marine environment it is very essential to restrict the use of antibiotics as much as possible for human, animal and poultry ailments and to treat the sewage for bacteria free nature before letting it into the vital marine environment - the backwaters, inshore waters as well as offshore waters. With the available data the estuarine environment of Cochin can be identified as potential reservoir of R^+ bacteria with transferrable drug resistance.

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CHAPTER V.

SUMMARY.

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1. "Studies on the ecophysiology of heterotrophic and indicator bacteria in the marine environments of Kerala" were carried out based on the samples collected in the Cochin backwater system during April 1972 to February 1973, March 1974 to February 1975, July 1975 to June 1976 and in the inshore area during January to October 1978. An account of the heterotrophic bacterioplankton and indicator bacteria are given with intensity charts and tables.

2. The distribution of microbes in the Cochin backwater system is found to be affected mainly by monsoons when the organic and inorganic matter brought down into the coastal waters through the Pampa river in the south and Periyar in the north of Cochin backwater is found to be of considerable importance in the maintenance of microbial life in the inshore environment.

3. Samples from all the stations contained significant quantities of heterotrophs and faecal pollution indicators. Fluctuation in the number of heterotrophic

bacteria by the standard count at RT and coliform bacteria at 37°C depended on the concentration of organic substances as pollutants at each station, salinity and water temperature.

4. The highest counts of total heterotroph and indicator bacteria were encountered at Station III during 1972-'73 and at Station I during 1975-'76 which were situated around 2 to 3 kilometres from the sewage outlet.

5. The monsoon was characterised by general decline in the population of *E. coli* and coliforms and the decrease in the number of coliforms, faecal coliforms, faecal streptococci and *Staphylococcus* sp. was more than that of heterotrophic bacteria.

6. As regards the seasonal distribution of bacteria in 1972-'73 the maximum number of heterotrophic bacteria had been observed during the postmonsoon period. The total counts between one station and the other did not vary as much as the counts between months did. The distribution was characterised by overdispersion. Six genera were identified during 1972-'73 and *Achromobacter* was found to be the dominant genus in all the seasons.

The highest count was encountered in the month of December and the minimum during November. In all the stations except the fourth, the minimum total counts were recorded during the monsoon period. In Station IV minimum counts were observed during the premonsoon period, with an increasing trend from the premonsoon to postmonsoon seasons.

During 1974-'75, maximum counts were recorded during monsoon months. No significant difference was noted in the total plate count (TPC) between stations, months and regions.

During 1975-'76, seasonal variation in sea water was meagre, whereas in sediments variations were prominent during monsoon in Station I, and in postmonsoon months at Stations II and III. The total plate counts (TPC) in monsoon months were less when compared to other two seasons.

7. The quantitative distribution of the heterotrophic flora in all the stations in the estuary was characteristic of its own which was influenced by the complex microbial and chemical processes to which the station in question was exposed. Nevertheless, the bacterial content of the sediments sampled was to a large extent governed by the various sediment properties

and appeared more to be a function of the content of utilisable organic matter.

8. In total, 8 genera namely *Alcaligenes*, *Pseudomonas*, *Vibrio*, *Aeromonas*, *Photobacterium*, *Flavobacterium*, *Micrococci* and *Bacillus* were encountered year after year as the main constituent of bacterio-plankton in the surface water as well as sediments. The occurrence of *Micrococci* and *Bacillus* seems to be a characteristic feature of sediments rather than surface water.

9. The composition of bacterial flora was much influenced by fresh water from rivers and by waste water from sewage.

10. All bacteria, heterotrophs as well as indicators were isolated more from sediments than from water. Clayey sediments harboured more indigenous heterotrophs whereas sandy sediments harboured pollutional organisms.

11. Considerable uniformity was noted, in the bio-chemical reactions of a number of strains taken from a single water sample and there was considerable variation in the reactions of strains taken from a series of samples either in the same locality or at different stations at different times.

12. Marine bacteria as a group, with their high enzymatic potential revealed by various biochemical tests were found, equipped to break down almost any organic matter that occur in the sea, and the variability may be necessary to adapt themselves readily to different substrates.

13. The physico-chemical quality of sea water was less influenced by sewage water and a predominant part of the sediments in all the stations sampled was silt brought by rivers. However, during 1975-'76 at Station I, the extent of eutrophication due to silt was very heavy resulting in lack of oxygen. But all representatives of indicator bacteria from warm-blooded animals were encountered indicating that all these forms are microaerophilic in nature.

14. The spreading of enteric bacteria in sea water and sediments from sewage outlet to offshore stations was extensive by stratification, tides and under water currents.

15. Large proportion of bacteria in the sediment samples were total coliforms (which represent both human and non-human sources) rather than *E. coli*. *E. coli* is considered more responsible for faecal contamination of sea water column than of the sediment.

16. No predictable maximum microbial density could be correlated with rainfall. So the unpredictable and intermittent appearance of *E. coli* and other intestinal bacterial community may be exclusively due to sewage and drainage and not due to rain water.

17. The presence of *E. coli* and *Streptococcus faecalis* in sea water and sediments indicated that these organisms can probably survive and multiply when water temperatures are between 27.1 and 32.6°C, and at 3%o salinity of sea water.

18. No appreciable seasonal variation was seen in any of the indicators of bacterial pollution examined. The amount of coliforms discharged along with faecal wastes were large at times but coliform levels are somewhat uniform, may be because of their rapid die-off due to strong influence of factors like temperature, salinity, oxygen and bactericidal action of sea water and due to bacteriophage attack. An inverse relationship between the concentration of indicators and salinity of water was found to occur at a 99.9% level of significance. The source of faecal pollution was of human origin in most of the months as per the faecal index.

19. Gram-negative rods constituted 95% of the isolates in all the three years of observations.

20. *Pseudomonas*, the potential degrader of organic matter in the marine ecosystem dominated the premonsoon flora during 1974-'75 whereas *Alcaligenes* and *Vibrio* were most abundant during monsoon times. *Flavobacterium*, the orange pigmented bacteria were predominant in the phytoplankton blooms in the surface waters whenever high salinity and low surface oxygen values were recorded. Apart from 296 strains of heterotrophic bacteria isolated and identified, 36 spore-forming *Bacillus* were isolated and identified into 9 genera during 1974-'75.

21. Bacterial species varied seasonally, but proteolytic, lipolytic and amylolytic activity appeared independent of season. More isolates were proteolytic, than lipolytic or amylolytic. Enzymatic activities did not vary significantly, seasonally and were not a reflection of the bacterial genera present in the water at the time, as no strain possessed all the enzymes.

22. Samples collected during 'Red tide' from Station I in August 1974 constituted mainly by pigmented bacteria *Flavobacterium* and *Bacillus*. *E. coli* was completely absent in sea water samples during 'red tide', but was present in

sediment samples. The high salinity and low surface oxygen values observed during that period and the inhibiting influence of certain forms of phytoplankton such as *Gymnodinium*, the blue-green alga, *Trichodesmium* and diatoms such as *Pleurosigma* and *Thallasiosira* on bacterial distribution is suggested.

23. The distribution of individual species of heterotrophic micro-organisms showed that some of them were distributed throughout in the surface water and bottom sediments. This indicated that a number of heterotrophic species developing on the laboratory media find organic substances suitable for their use throughout the entire water mass, from the surface to the bottom and that this was responsible for their wide distribution. Total heterotrophs were positively correlated with nitrate and phosphate at 5% level.

24. The average mean ratios relating pathogens to indicators were 1 *Salmonella* to 45 total coliforms, 13 faecal coliforms and 9 faecal *Streptococci* for water samples. Data collected from the inshore stations during January-October 1978 showed that *Salmonella* isolations occurred with nearly 100% frequency when the faecal coliform concentration was 2000/100 ml. In 82% of the observations faecal coliforms exceeded 2000 for 100 ml suggesting the

possibility of potential presence of *Salmonella* in these waters.

25. The variety and number of genera in the *Salvinia* deposits were maximum in the postmonsoon months and scanty during monsoon months. Population of the 6 zymogenous bacteria in *Salvinia* rich inshore sediments collected during January-October 1978 increased with decreasing depth, but the enzymatic activities were similar in all the months.

26. All the 6 zymogenous bacteria from *Salvinia* deposits had high positive correlation with temperature and nutrients especially phosphates.

27. Sediment rich in *Salvinia* harboured highest population of 6 zymogenous bacteria irrespective of depth in shallow regions. But a decline in the population was observed based on sediment/water ratio of the count at 30 m.

28. Population responses to *Salvinia* deposits were generally greater in sediments than in water samples. The reason for the microfloral response in sediments is due to the liberation of decomposing nutrients from the dead and decayed *Salvinia* remains, which indicated the role of microbenthos in the organic cycle of Cochin backwaters.

29. The pollution microbes isolated from the inshore station during January to October 1978 were recorded more in the southern stations when compared to northern stations. Generally 100 - 1000 times more coliforms were detected in the sediment than in the water column. No significant relationship was observed between the concentration of organisms with temperature and pH of the water.

30. Faecal index was constructed with the *Enterobacteriaceae* isolated and the relationship between total bacterial population and faecal index was found significant.

31. Random samples from the beaches of Shankumugham, Vishinjam and Kovalam were collected for ecophysiological studies of marine bacteria during January to June 1977. Human bacterial strains were isolated from clinical specimens in the Sree Chitra Thirunal Medical Centre, Trivandrum and an account of the comparative study of marine bacteria and strains isolated from human beings was given on biochemical variations and antibiogram typing. *E. coli* type I isolated from the samples collected during 1975-'76 from three fixed stations from Cochin backwater was subjected to serological typing to know their enteropathogenicity and R-factor (RTF - resistance transfer factor) to certain antibiotics. The significance of R-factor suggests that human pathogens in water may become resistant

to common antibiotic therapy once the bacteria infect man or animals through sea-food from polluted sea water.

32. Most of the isolates, encountered in the three beaches near Trivandrum (Shankumughom, Kovalam and Vishinjam) belong to *Pseudomonas* spp. (37.2%), Coliforms (37.2%) and *Aeromonas* spp. (20.9%). All the isolates were non-pigment producers except 2 species of *Pseudomonas*. Citrate utilisation (74.4%) was found to be very high in marine strains.

33. Maximum isolates, including enterobacteria were isolated from Shankumughom beach water.

34. About 75 bacterial strains isolated from clinical specimens showed high rate of saccharolytic action.

35. Comparative studies of marine bacteria with human pathogens isolated from clinical specimens showed that both were bio-chemically active and the only difference found was their sensitivity pattern towards antibiotics.

36. The results of antibiogram of marine bacteria showed that multiple drug resistance was more prevalent than resistance to one or two drugs among marine bacteria. The prevalent pattern were PAC (Penicillin, Ampicillin, Chloramphenicol), PAEC (Penicillin, Ampicillin, Erythromycin, Chloramphenicol), PACKESC (Penicillin, Ampicillin, Chloramphenicol, Kanamycin, Erythromycin, Septran, Cephoron) in this order.

37. None of the marine strains were resistant to Gentamycin and only one strain was resistant to Septran. Tetracycline was found to be resistant to only 2 strains, 3 strains were resistant to Streptomycin, 5 strains were resistant to Kanamycin and 7 strains to Chloramphenicol.

38. 100% resistance was encountered in isolates from marine environment to only Penicillin, whereas isolates from clinical specimens showed 100% resistance to three antibiotics, i.e. Penicillin, Erythromycin and Cephoran. A higher percentage of resistant organisms were noted among human pathogens ranging from 50% to 60%, while the resistant rate was only 34% among marine bacteria.

39. Out of 3 concentrations of 10 different antibiotics used, the lower concentration showed higher rate of resistance with most of the Gram-negative bacterial strains from clinical specimens tested though much differentiation in sensitivity was not noted between middle and higher concentration.

40. The resistant rate was higher in *Aeromonas* and *Pseudomonas* than in *E. coli*, *Achromobacter* and *Vibrio* strains among marine strains. The resistant rate among human pathogens was higher in *Klebsiella pneumoniae* than *E. coli* strains. A higher rate of resistance against commonly used antibiotics was noted among human pathogens.

41. Gram-positive, coagulase positive *Staphylococcus* spp. isolated from clinical specimens tested in three different concentrations of 10 antibiotics showed higher rate of resistance to the highest concentration of the antibiotics.

42. The high rate of incidence of multiple drug resistant organisms in the littoral waters of Trivandrum indicated the possibility of R$^+$-factor mediated drug-resistant bacteria also in this environment, which could transfer their resistance pattern to other gram-negative pathogens. Such transfer would create a pathogen with formidable capabilities possessing enhanced infectivity and virulence.

43. Out of 446 *E. coli* strains isolated from sediments of Cochin backwater only 230 isolates were subjected to preliminary serological determination of O-serotyping and antibiogram typing and seven *E. coli* 'O' serotypes encountered and serotypes 026, 055, 086, 0111, 0119, 0127 and 0128 were identified as enteropathic *E. coli*. 7% of the 0111 *E. coli* serotype was inagglutinable in O-antiserum unless first heated at 100°C. Except O-serotype 026, all the other serotypes were isolated also from sea water.

44. The data also indicated that these 7 types occurred in sea water at the level of 44.34 % and in the sediment

at the level of 55.6%. Within these O-types 76% were found to be antibiotic resistant isolates and it is of interest that with one exception (O26) all these 'O' types have been isolated from human faecal matter.

45. Sensitivity tests on enteropathic E. coli strains isolated from Cochin Backwater showed a high incidence of antibiotic resistance. Based on E. coli distribution the Vizhinjam beach was considered to be most polluted of all the three beaches studied. Eventhough Vizhinjam beach possessed more E. coli than the beaches of Shankumughom and Kovalam, the Shankumughom beach possessed more antibiotic resistant microbes distinct from that of other sites. All these results suggest that the marine environments of Kerala can also be identified as potential reservoir of antibiotic resistant bacteria with transferable drug resistance.

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CHAPTER VI.

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A P P E N D I X X.

CHANDRIKA, V., 1976. INDICATORS OF BACTERIAL POLLUTION IN COCHIN BACKWATERS. *PROCEEDINGS OF THE NATIONAL SEMINAR ON ENVIRONMENTAL POLLUTION, COCHIN*: PAGES 290-294.

Mrs V. Chandrika, stood second in the University of Madurai in 1969, for her M.Sc. Examination. She joined the Central Marine Fisheries Research Institute in 1971 and is working on the problem of Bacterial Pollution in Cochin Backwaters in the Marine Biology and Oceanography section of the Institute.

INDICATORS OF BACTERIAL POLLUTION IN THE COCHIN BACKWATERS

Marine Bacteriology recently has attracted considerable interest, and many parts of the polluted marine environment have been examined for indicators of bacterial pollution. As a branch of applied bacteriology the estimation of marine faecal pollution and the effects of faecal pollution on marine ecology is one of the most important line of bacteriological study to-day.

The Cochin Backwater located along 09° 58′ N and 76° 15′ E is constantly receiving large quantities of pollutants by land drainage, industrial effluents and by sewage outflow. The pools for retting coconut husk and also the tidal flow dump large amount of organic wastes into the backwater. With all these pollutants Cochin Backwater remains a suitable study place for indicators of bacterial pollution. Preliminary reports of some bacteriological examinations of Cochin beach sand have been given by Santhakumary (1966) and Gore (1971, 72) and even these are incomplete in the sense that sampling was done at random and did not cover an annual cycle. Hence, the present work was aimed to study the occurrence, distribution and seasonal variations of three indicator bacteria namely coliforms, faecal coliforms and faecal streptococci in surface water and bottom deposits of Cochin backwater. The quantitative composition and interrelationships betweed different microforms have also been discussed.

Sample collection

The sampling sites in the backwater is widely separated and have dissimilar sediment characteristics and is directly influenced by monsoon cycle and local inputs. Samples were collected from 6 fixed stations from the Cochin Backwater from a depth of 10 m with a Petersen grab. The central portions of the samples were immediately and aseptically transferred to sterile petri dishes. Sediments of the sampling stations I, II, IV, V, VI are black silty clay mud deposits except station III where the sediment was yellow sandy throughout the sampling period. The petri dishes with samples were kept in a sterile plastic bag and held at 4°C until processing 18–24 hours later.

Sample processing

Petri dishes containing and samples were weighed to take the initial weight of the mud. Then approximately 5 grams were taken in a sterile mortar and pestle. The mud was ground well and mixed with 100 ml of sterile sea water and plated according to standard methods. The quantitative composition of and interrelationship between different microorganisms (B, F, A) occurring in sediment samples were determined. The three basic 'indicator organisms' Total coliform, faecal coliform (*E. coli* type I) and faecal streptococci counts were taken. The faecal coliform and faecal streptococcus ratio offer a valuable investigative tool in the estimation of the source of pollution in these waters.

Seasonal cycle of indicator bacteria in surface water

The distribution of coliform bacteria in the surface waters of Cochin Backwaters was studied for a period of one year. The *E. coli* counts ranged from 12/ml in February to 93/ml in March. The coliform counts fluctuated between 12×10^3/ml in October to 425×10^3/ml in March. The total bacterial count varied between 95×10^3/ml in November to 656×10^3/ml in December. The total bacterial count and that of *E. coli* were found to be high during the post-monsoon months, whereas the coliforms were abundant during the pre-monsoon months. The monsoon was characterised by general decline in the populations of *E. coli* and coliforms. The coli index (*E. coli* type I) was found to be high during September, November, January and March.

Seasonal cycle of general microflora in sediments

Fig. I gives the log counts of Total bacteria, fungi, actinomycetes, total coliform, faecal coliform (*E. coli* type I) and faecal streptococci in the sediments during the pre-monsoon, monsoon and post-monsoon seasons. High values of total bacterial counts were recorded in post-monsoon period Fungal counts followed the same pattern seasonally as total bacterial counts but always lower than the bacterial counts. Actinomycetes were in the increasing trend from pre-monsoon to post-monsoon season. However, the period of maximum abundance of bacteria, fungi and actinomycetes appears to be during the post-monsoon season.

The total microbial populations ranged from 1.67×10^6/gm in September 1974 to 56.46×10^6/gm in January 1975 (Table I). Maximum microbial population was recorded in post-monsoon season.

The bacterial flora was maximum in August (88.04%). The predominance of bacteria over the other organisms in the sediment samples have been found in all the seasons of the period of study. The maximum number of fungi was recorded during the post-monsoon season, the minimum being in the monsoon period. The actinomycetes were poor in surface sediments in pre-monsoon months but was present in all the months. The actinomycetes were recorded in all stations only in June.

The maximum B/F (Table I) ratio was observed in December indicating the dominance of bacteria over fungi. Such a dominance of bacteria over other micro-organisms was reported by Last and Deighton (1965) in the surface of living leaves. The maximum B/A and F/A ratio were recorded during May. Detailed studies are needed to understand the nature of interactions between the micro-organisms in the sediments of Cochin Backwater.

Seasonal cycle of bacterial indicators in sediments

Fig. I also shows the seasonal cycle of bacterial indicators. Seasonal cycle of total coliform is somewhat different from the seasonal cycle of other microbes.

Peak values were recorded in the premonsoon season. Faecal coliforms (*E. coli* type I) were found to be abundant in the post-monsoon period. Faecal streptococci values were somewhat same for both pre-monsoon and monsoon period and were in the slightly increasing trend from pre-monsoon to post-monsoon season. Generally high values of coli-index has been recorded in July, August and September 1974 and also in February 1975, the maximum being in September. Faecal streptococci were encountered in high numbers in January (Table II) than faecal coliforms but generally encountered in lower numbers than faecal coliforms.

Maximum total coliforms were recorded in June (88.63×10^2/gm) and the minimum being in November (45.92×110^2/gm). Faecal coliforms ranged from 10.42/gm in April to 39.21/gm in September. whereas faecal streptococci ranged from 0.17/gm in June to 27.35/gm in January.

Table I

Occurrence of microflora and their interrelationship in the Sediments of Cochin Backwater.

Month	Total Microbial Popula-tion (105)	% to total microflora			Interrelation between different microflora		
		Bacteria	Fungi	Actinomycetes	B:F	B:A	F:A
JANUARY	56·46	77.84	13 63	8·51	5.71	9.14	1.60
FEBRUARY	9.04	82.30	11.06	6.63	7.44	13.31	1.67
MARCH	—	—	—	—	—	—	—
APRIL	5.45	80.73	14.31	4.95	5.64	16.31	2·89
MAY	4.33	85.63	11.68	2.77	7.33	30.91	4.21
JUNE	11·81	82.21	12.44	5.33	6.61	15.42	2 33
JULY	11.28	86.96	8.68	4.34	10.01	20.03	2.00
AUGUST	23.99	88.04	7.46	4.50	11 80	19.56	1 65
SEPTEMBER	1.67	54.49	32.33	13.17	1.68	4.14	2.45
OCTOBER	—	—	—	—	—	—	—
NOVEMBER	4.79	82.88	10.85	6.26	7.64	7.64	1.73
DECEMBER	17.42	81·17	3.04	15.78	26 70	5.14	0·19

Faecal coliform - Faecal streptococci relationship in sediments

The ratio between faecal coliforms and faecal streptococci ranged from 0.8 in January to 6.2 in June (Table II). Densities of both faecal coliforms and faecal streptococci reflected considerable faecel pollution entering the Cochin Backwater. Station HI was found to be highly polluted where high faecal coliform counts were encountered throughout the sampling period. This may be due to the mixing of polluted fresh water from Ithi-puzha river system. The ratio between faecal coliforms to faecal streptococci were above 4 during April, June, July and August 1974 and February 1975 (pre-monsoon and monsoon months) which shows that the source of pollution is mainly from human wastes. The source of pollution for the rest of the months is from animal wastes.

Faecal coliforms ranged from 10.42/gm to 39.21/gm and total coliforms ranged from 45.9 x 10^2/gm to 88.6 x 10^2/gm in June. Sediment which contains more than 1000 *E. coli*/100 ml will harbour *Salmonella* species also.

CONCLUSION

The result of the present study shows that apart from 100% sewage treatment by digestion and filtering through sand pebble-bed by the sewage treatment plant, Cochin Backwater is constantly polluted by faecal matter of human origin by the seven drainage canals and by other sources such as untreated sewage from extraneous source and overflow soakpits.

The microbial flora of the Cochin Backwater is dominated by pollutional organisms of enteric fresh water and soil origin. Sandy sediments harboured more pollutional organisms whereas clayey sediments harboured more indigenous organisms.

The presence of these indicator organisms in all the months indicated that these organisms can probably survive and multiply when water temperature is between 27.6 and 37.09°C. So it is suggested that all the effluent discharge including the storm water during the monsoon months should be properly treated before letting into the backwaters in order to ensure the quality of such a vital environment.

Table II

Occurence of Bacterial pollution indicator organisms in the Cochin Backwater for the period (February 1974 to January 1975) together with ratios between Faecal coliform and Faecal streptococci

	Month	TOTAL COLIFORM 102/gm	FAECAL COLIFORM/gm	FAECAL STREPTO-COCCI/gm	FC: FS
1.	January	51.74	20.93	27.35	0.76
2.	February	58.35	34.22	7.42	4.61
3.	March	—	—	—	—
4.	April	86.72	10.42	2.05	5.08
5.	May	79.77	17.65	6.13	2.87
6.	June	88.63	10.54	0.17	6.20
7.	July	53.16	35.50	7.50	4.73
8.	August	63.69	30.08	7.05	4.26
9	September	46.40	39.21	14.37	2.72
10.	October	—	—	—	—
11.	November	45.92	27.85	26.35	1.06
12.	December	52.60	27.03	20.36	1.32

DISCUSSION

Sri. Mia Khan

I wonder whether the work present could be made more useful. Heavy pollution by E.coli or streptococci would affect the fish or the prawn. Has any study been made on the bacterial pollution either in the prawn, fish etc.

Mrs. V. Chandrika

Counts of E.coli and streptococci are done by our sister organisation and hence I am unable to give an answer.

Sri S. Sundaram

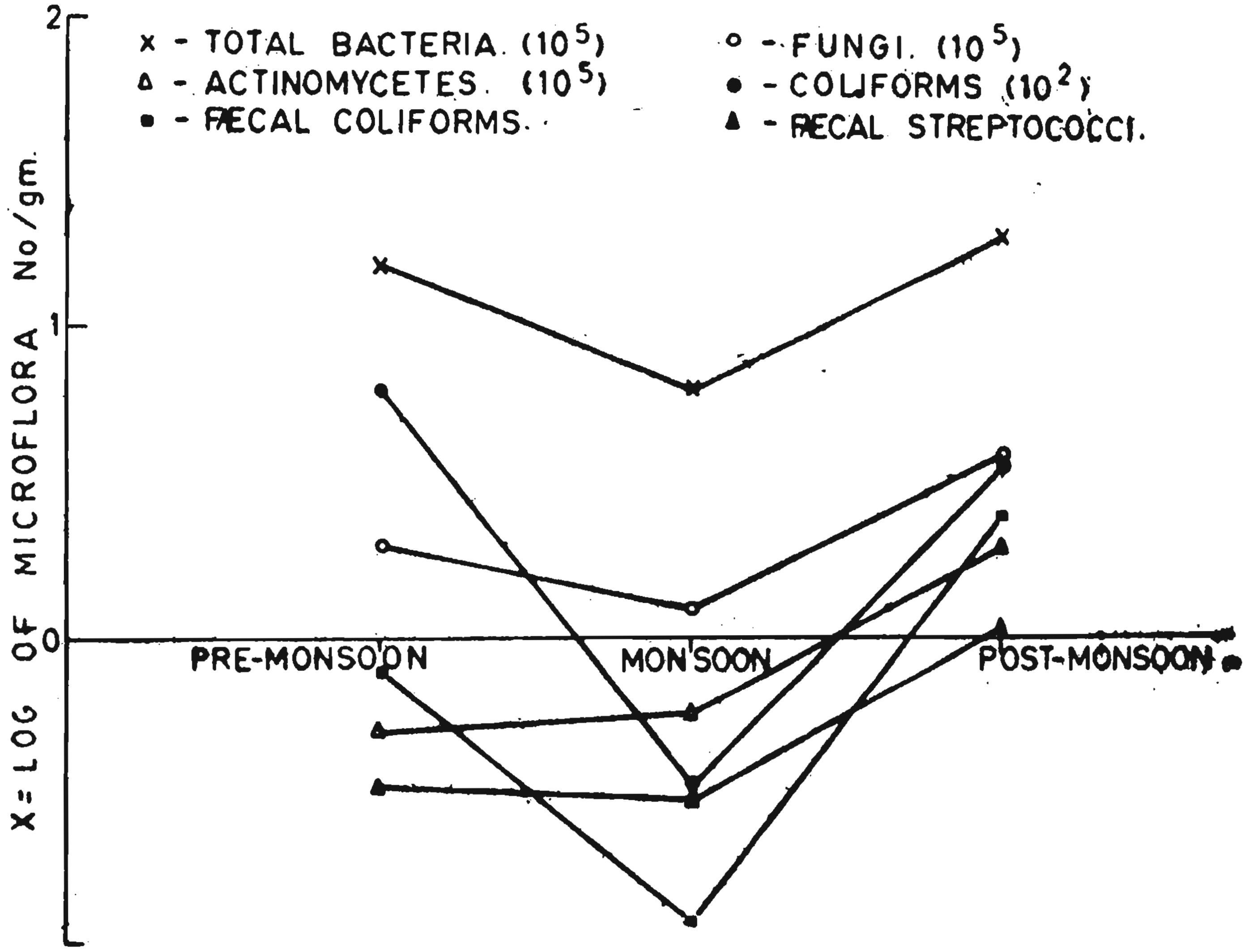
Apart from E.coli etc. any observation made on the relationship between the intake of saline water] vs. bacteria.

Mrs. V. Chandrika

Regarding salinity tolerance, we have not made studies.

General comments by Dr. Sekharan

Presence of pendric form of bacteria in food fishes are known. We are still in the process of identifying the problems. Bacterial flora study is being done.



SEASONAL DISTRIBUTION OF SOME MICROFLORA IN THE SEDIMENTS OF COCHIN BACKWATERS DURING THE PERIOD APRIL 1974 TO FEB. 1975.

APPENDIX II.

PILLAI, V. KUNJUKRISHNA, V. CHANDRIKA, C.P. GOPINATHAN, A. REGUNATHAN AND P.V. RAMACHANDRAN NAIR, 1981. INVESTIGATIONS ON THE ECOLOGICAL EFFECTS OF *SALVINIA* WEED DEPOSITS IN THE INSHORE WATERS OFF COCHIN. *PROC. 8TH ASIAN PACIFIC WEED SCI. SOC. CONF.*, VOL. II, PP. 175-183.

INVESTIGATIONS ON THE ECOLOGICAL EFFECTS OF *SALVINIA* WEED DEPOSITS IN THE INSHORE WATERS OFF COCHIN

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ABSTRACT

The problem of pollution from *Salvinia* weed has been attracting considerable attention in this region for the last two decades. The spontaneous growth of this weed choking the waterways, lakes and estuaries during the monsoon and post-monsoon seasons has become a menace. The immediate effect of the spreading weeds is the physical interference in the aquatic environment affecting the fishing operations as well as the inland navigation, which naturally attracts more attention. Although there were a few reports on the effect of *Salvinia* as a biological pollutant, there is no information as to its effect on the ecosystem and the living resources of the inshore waters.

The paper presents the results of investigations on the short-term as well as long-term effects of weed deposits in the inshore areas off Cochin during 1976–78. Data include hydrographic properties, distribution of benthic population, primary productivity and also microbiological aspects.

The period of weed deposition starts immediately after the onset of monsoon season and continues till January. Along with the flood waters enormous quantities of *Salvinia* reach the estuarine areas. As soon as the weeds come into contact with the saline water they start to decay. The tidal movement brings the major part of them to the inshore areas and the decayed weeds settle to the bottom in large quantities. This process continues till January–February. However, the peak period is between October–January. This phenomenon invariably results in a decrease in dissolved oxygen contents of the benthic area. The comparatively low rate of primary production observed in the waters of the benthic region during this period along with high dark fixation indicate abnormal conditions prevailing there. The long-term effects appear to be the interference on the benthic population, especially the filter feeding animals.

Nine genera of bacteria in the weed deposits were isolated and the morphological, biochemical and physiological characters of representative isolates and their seasonal abundance were studied. The variety and number of genera in the weed deposits were maximum in the post-monsoon season and scanty during the monsoon season. Three major micro-organisms – bacteria, fungi and actinomycetes absorbed in the *Salvinia* detritus rich sediments were recorded in all the three seasons. The association of the micro-organisms with the decaying weed indicates the role of microbenthos in the organic cycle in the shore waters off Cochin.

Although these effects appear to be seasonal in nature, the phenomena continues every year with cyclic regularity. The possible environment damage and its consequences to the ecosystem in general and to the fishery of this area in particular are also discussed.

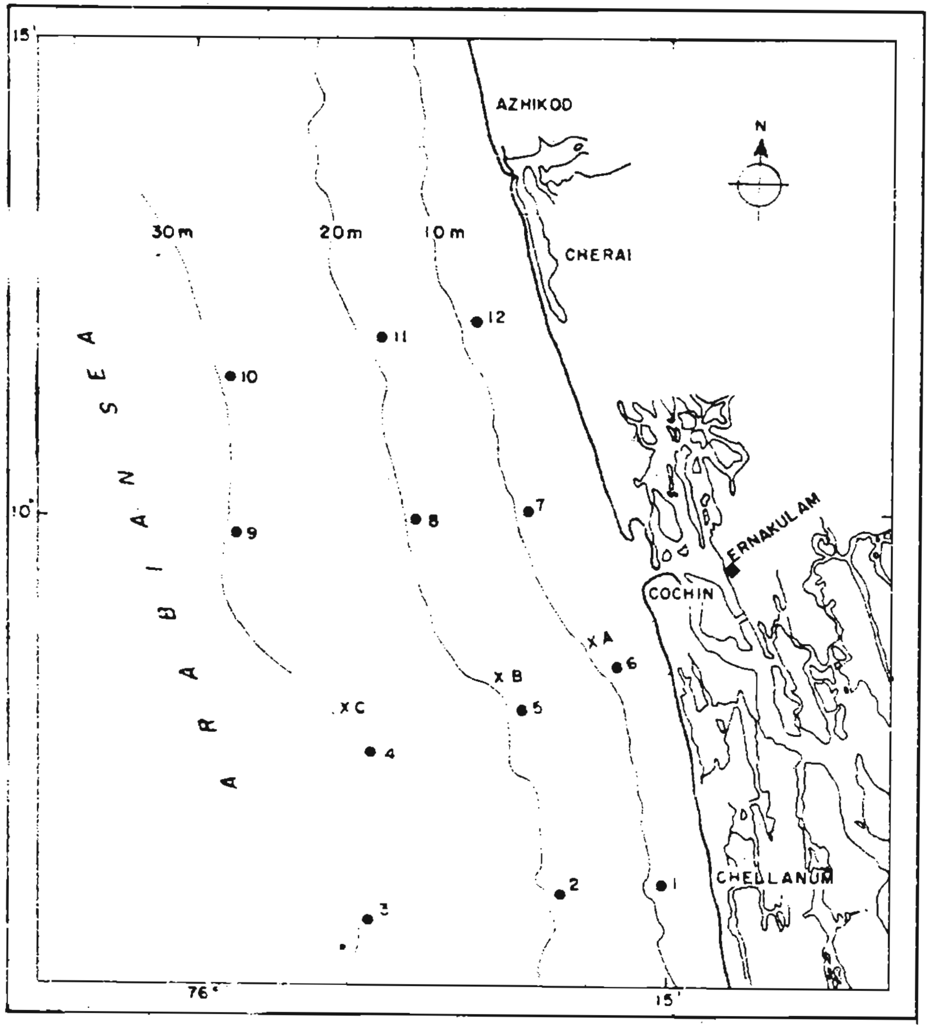

Fig. 1 Map showing the area investigated and the station positions.

INTRODUCTION

The massive growth and subsequent proliferation of the aquatic weed *Salvinia molesta* Mitchell has become a menace in the rivers, backwaters and inshore areas of Kerala (south west coast of India) for the last several years. Family Salviniaceæ (pterydophyta) consists of two main living genera : *Salvinia* and *Azolla*. The plants are inhabitants of tropical fresh waters. It is a free floating hydrophyte and the stem is a branched rhizome attaining a length of about 10 cm. The method of reproduction is both by vegetative and sexual methods. However, the chief method of propagation is by vegetative means. This paper deals with the results of investigations conducted to assess the adverse effects, if any of the large quantities of weed on the living resources of the inshore areas of Cochin.

MATERIAL AND METHODS

During 1976–77 samples were collected from three stations at depths 10, 20 and 30 m and in 1978 from 12 stations in a grid of four transects at the respective depths of 10, 20 and 30 m. The station positions are given in Fig. 1. Water samples were collected from surface and bottom and analysed for salinity, dissolved oxygen and nutrients by standard methods. Dredge samples were collected during a dredge of 45 cm x 12 cm size and grab samples by a Van–Veen type grab of 0.05 m^2 and analysed for the benthic fauna. Primary productivity of the water from the surface as well as just above the benthic region was estimated by C^{14} method. For the microbiological studies, surface water samples for bacterial assay were collected in a sterile 250 cc glass bottles in aseptic conditions. Sediment samples were collected by using a grab. Central portion was collected in sterile petridishes using a sterile spatula and transported to the laboratory. Analyses were carried out within 30 hrs. The samples were kept at 4°C until the time of analysis. Autoclaved sea water was used for making serial dilutions. Water and sediment samples were suitably diluted and plated on ZoBells "medium 2216"; Kurters Agar and Martins Rose bengal agar for the enumeration of bacteria, actinomycetes and fungi respectively. All the zymogenous bacteria were isolated in their selective media in suitable dilutions. Colonies were counted after the respective incubation period at RT. The generic classification of bacterial isolates was done according to a modified scheme of USIO SIMIDU and Kayuyoshi Aiso in 1962.

RESULTS

Hydrography

In general, the hydrographic parameters like water temperature, salinity, dissolved oxygen and nutrients did not show much variations except for the very low dissolved oxygen values recorded at the benthic regions during the months of January, July and November 1976, which coincided with the presence of heavy weed deposits in the inshore area. However, this feature was not noticed during 1978. The extremely low values of dissolved oxygen were recorded during January 1976, July and November 1977 (Table 1) which coincided with the presence of decaying weeds at the benthic areas and is possibly due to the high rate of organic decomposition in the area at the respective stations. Since the rate of weed deposition and degree of decomposition are not consistent and also considering the effect of water currents, it is rather difficult to interpret such variations in hydrographic parameters. However, it is observed that in the monsoon and post-monsoon seasons the beaches extending to several kilometers to south of Cochin are seen densely covered with decayed weed masses indicating the effect of southward after currents.

(1976, 77 & 78)

Period	Parameters	Stns.	I 10 m	II 20 m	III 30 m
January '76	Temperature	S	29.0 °C	29.5 °C	28.5 °C
		B	28.5	28.4	28.0
	Salinity ‰	S	22.5	30.5	28.0
		B	28.7	30.0	28.5
	D oxygen ml/l	S	2.35	1.94	2.28
		B	1.98	1.81	1.23
July '76	Temperature °C	S	28.5	28.20	–
		B	26.5	25.50	–
	Salinity ‰	S	34.67	35.34	–
		B	35.0	34.10	–
	D. oxygen ml/l	S	3.64	4.60	–
		B	3.42	4.0	–
November '76	Temperature °C	S	29.50	28.70	29.20
		B	28.50	28.20	28.80
	Salinity ‰	S	34.72	34.86	32.53
		B	34.86	34.23	34.16
	D. oxygen ml/l	S	4.14	3.94	3.70
		B	4.00	4.30	3.80
January '77	Temperature °C	S	29.0	29.2	29.5
		B	28.5	28.5	28.7
	Salinity ‰	S	32.18	32.10	32.5
		B	32.30	30.88	33.0
	D. oxygen ml/l	S	3.91	3.94	4.2
		B	4.11	3.90	3.0
July '77	Temperature °C	S	26.5	25.50	–
		B	26.0	25.00	–
	Salinity ‰	S	12.48	18.80	–
		B	32.80	31.60	–
	D. oxygen ml/l	S	4.94	4.96	–
		B	2.42	2.50	–
November '77	Temperature °C	S	30.0	30.0	30.50
		B	29.5	29.0	28.50
	Salinity ‰	S	32.7	32.0	31.10
		B	33.9	35.10	35.15
	D. oxygen ml/l	S	2.78	3.75	3.81
		B	2.40	2.73	1.51
January '78	Temperature °C	S	29.1	29.5	29.5
		B	28.0	27.8	27.8
	Salinity ‰	S	32.1	32.0	31.5
		B	34.0	34.0	34.0
	D. oxygen ml/l	S	4.30	5.0	4.7
		B	3.6	4.2	4.0

S = Surface, B = Bottom

of Cochin

Months	St. No.	Benthic production	Dark assimilation	Dark assimilation ratio
1978 January	1	3.66	8.67	2.36
	3	4.23	5.95	1.40
February	1	3.38	13.61	4.02
March	3	3.38	6.03	1.78
	4	3.10	6.81	2.19
April	1	4.0	11.89	2.97
	2	4.54	6.42	1.41
	3	3.19	5.38	1.68
	4	3.32	6.21	1.87
	5	2.99	3.70	1.23

Primary productivity

Primary productivity data collected during 1976, 1977 and 1978 by C^{14} method showed that the rate of production at the surface layers in the area was uniformly high compared at the benthic region, (Surface: 55–125 mg $C/m^3/hr$; Benthic: 10 mg $C/m^3/hr$). One interesting feature observed was that at certain periods, especially during January–April 1978, high dark fixation rates were observed in coincidence with the presence of heavy weed deposits at the bottom (Table 2).

Benthic fauna

Qualitative distribution pattern of the benthic biomass for the period 1976–77 is given in Table 3. Although the data do not

Table 3: Abundance of benthic biomass at 10 m off Cochin [Samples collected with a dredge (45 cm x 12 cm) towed for 15 minutes]

Station	Groups				1976			1977						
		Jan.	Mar.	Apr.	May	July	Aug.	Nov.	Dec.	Jan.	Feb.	Mar.	May	July
	Squilla sp.	1		1				1	3		3	1		
	Cynoglossus sp.	1	1		1			1		2	7	2		
	Crabs			2						1	6			
	Sciænid sp.		2	1	2						5			
	Gastropods	103	45		8	10		1	14	2	85		15	7
	Dentalium sp.	5							1				300	
	Tureteua sp.	25			4	24	1			13	15	6	38	14
	Bivalves	2	4	15	116	3				1	120	5	14	2
	Murex sp.										45			
	Nudibranchs					2	1							
	Echroid sp.	12												
	Echinoderms			10	5	1				5		1	11	2
	Polychætes			5	1									1
	Acetes sp.										5			

provide a full representation of the benthos due to the inherent limitations of the dredge sampling, it gives a general picture about the distribution and abundance of major groups in the inshore areas. Conspicuous absence of filter feeding organisms like the bivalve molluscs during monsoon is probably due to the physical disturbance to them by the cellulose deposits accumulated during the disintegration of the decaying weeds. Besides, the dominance of polychæte worms and molluscs such as *Tellina* met which are indicative of abnormal conditions.

Microbiological studies

Microbiological studies were carried out during January to October 1978 only. The occurrence of zymogenous bacterial pattern is given in Fig. 2 along with total bacterial population. The monthly total bacterial count per ml of sea water varied within a very limited range indicating the existence of a fairly constant level of population in the surface waters and in the detritusrich sediments. The total bacterial population ranged from 99.36×10^6 to 265.32×10^6/g in the sea water and in the sediments from 137.2×10^6 to 232.6×10^6/ml; 9 genera of bacteria belonging to six families viz., Neisseriaceæ (25%); Pseudomonadaceæ (10%); Vibrionaceæ (25%); Nicrococcaceæ (5%); Bacillaceæ (5%) and Enterobacteriaceæ (25%) and Contaminants (5%) were found associated with the weed deposits. *Alcaligenes*, *Pseudomonas* and *Vibrio* occurred in abundance in all the three seasons. There was no marked pattern of distribution among the microflora, but all the zymogenous microflora exhibited the maxima in the post-monsoon period. The inter-relationship of different microbial flora is given in Table 4. From the present investigation, it

Table 4: Occurrence of microflora and their inter-relationship in the surface water and sediments in the inshore area of Cochin

Months		Bacteria 10^6/ml	Actinomycetes 10^6/ml	Fungi 10^6/ml	Inter-relationship between different microflora		
					B:F	B:A	F:A
January	SR	99.36	21.63	28.34	3.50	4.59	1.31
	SD	145.60	42.81	8.75	16.64	3.40	0.20
February	SR	166.60	28.46	44.63	3.73	5.85	1.56
	SD	197.00	56.46	16.40	12.01	3.48	0.29
March	SR	107.33	32.50	18.40	5.89	3.30	0.56
	SD	116.83	62.64	24.50	4.76	1.86	0.39
April	-	-	-	-	-	-	-
May	SR	137.40	40.40	19.48	7.05	3.40	0.48
	SD	152.66	80.10	22.46	6.79	1.90	0.28
June	SR	158.60	31.80	25.36	6.25	4.98	0.79
	SD	140.80	64.74	18.46	7.62	2.17	0.28
July	SR	186.60	44.32	60.42	3.08	4.21	1.36
	SD	166.40	72.16	37.20	4.47	2.30	0.51
August	SR	265.32	66.16	66.16	4.01	4.01	1.00
	SD	137.16	68.28	24.50	5.59	2.00	0.35
September	SR	252.60	64.20	63.21	3.99	3.93	0.98
	SD	212.86	66.16	22.62	9.91	3.21	0.34
October	SR	256.40	52.62	71.64	3.57	4.87	1.36
	SD	232.64	82.06	32.20	7.22	2.83	0.39

SR = Surface, SD = Sediments, B = Bacteria, A = Actinomycetes, F = Fungi

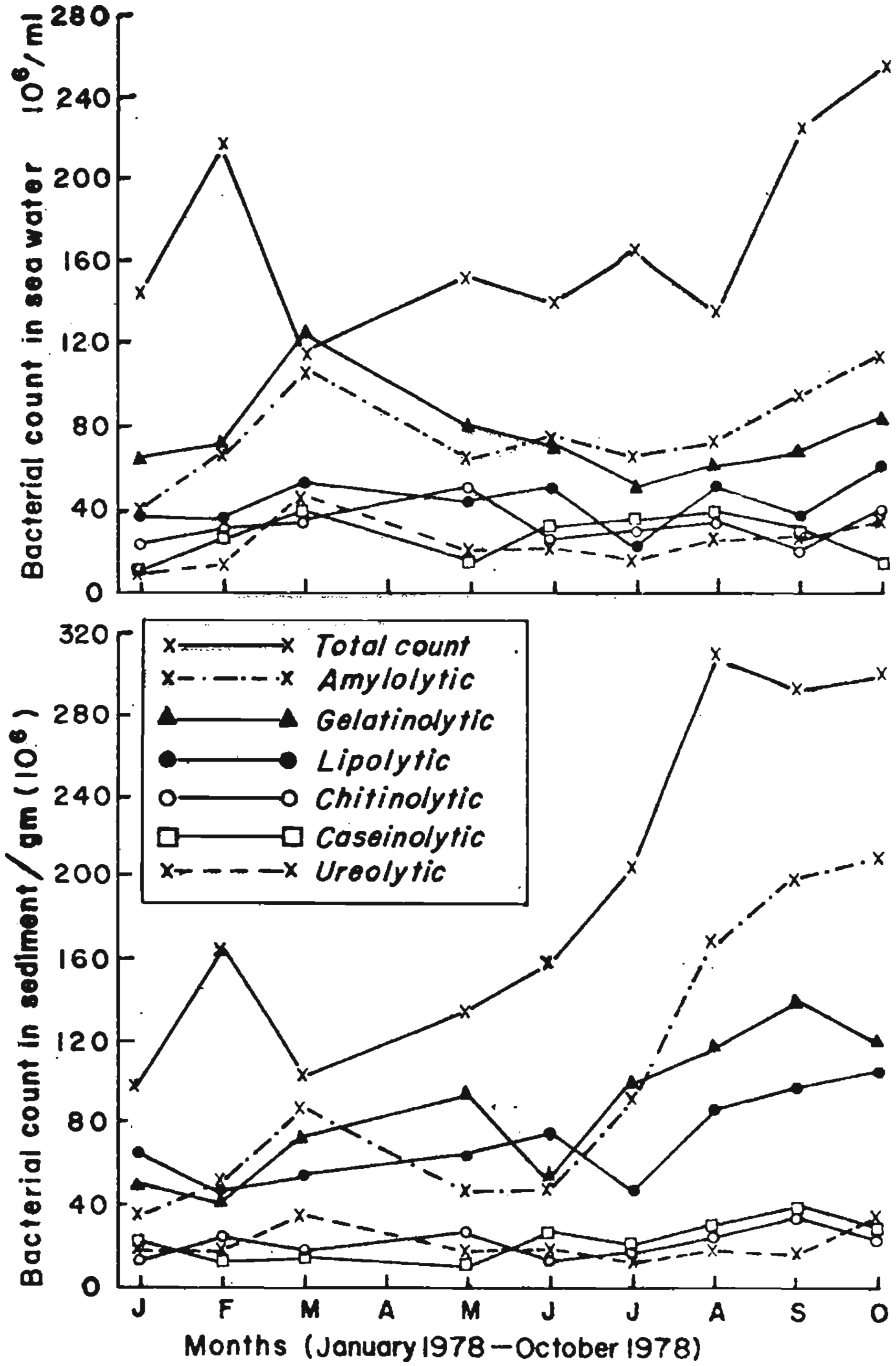

Fig. 2 The interrelationship of different microbial flora in waters and sediment off Cochin.

is evident that marine microbes play a significant role in biodegradation of water weeds like *Salvinia*. Bacteria isolated from sea water is equally virulent in the biochemical activity when compared to the bacteria isolated from the sediment. Fungi and actinomycetes were found to be only secondary disintegrators of organic matter in the marine environment.

DISCUSSION

The vast expanse of fresh and brackish water areas available in this region provides an ideal situation for the aquatic plants such as *Salvinia* to grow and flourish. The process is accelerated by the presence of excess nutrients, especially phosphate reaching the backwaters from the adjacent agricultural lands through rivers and streams. These nutrients promote the growth of water plants which are often limited by a natural shortage of nutrients. Considering the ecological peculiarities of this area, and the availability of plenty of water resources in the form of rivers, lakes and backwaters, the weed growth and subsequent problems caused by it are of great significance.

The effect of this phenomena on the aquatic resource in general and on the fisheries in particular is of considerable interest. The weeds utilize most of the available nutrients thus restricting the survival and growth of other useful plants and animals. Further, the free use of water ways is disturbed and also the flow of water is reduced. When they remain as a thick mat, the penetration of light is reduced to a considerable extent. The floating weeds interferes with the operation of fishing gears like stake nets and dip nets in the estuarine areas and also interferes with the trawl net operations in the inshore fishing areas. As soon as the weeds come into contact with saline water they start to decay and eventually settles at the bottom.

The present observations agree with the previous reports on the ecology of *Salvinia* in estuarine waters of Cochin in certain aspects by Gopalan and Nair (1975). They stated that the density of fauna of the level bottom under the weed mat is considerably poorer than the open area and the oxygen level of bottom waters in the weed covered areas was 2.82 ml/l whereas that of adjacent open waters was 4.31 ml/l in November. The present record of very low oxygen scale weed deposition at the bottom probably explains that the same conditions were prevailing in the inshore waters at that time.

There is practically no information on the effect of *Salvinia* and its decomposition products on the ecosystem. When the weed floats on the surface as a compact mass it prevents vertical mixing, shade out the phytoplankton and allow increase in free CO_2 and consequent decrease in pH. With the subsequent settling and decay of the weeds the pH in the sediment tend to rise. Present studies on primary productivity at the benthic area revealed that high dark fixation values (upto 100%) are present on several occasions. In normal sea water the dark fixation due to chæmosynthetic bacteria is usually found to vary between 1–2%, but as reported by Nielsen and Jensen (1957) it can be as high as 5% in certain specialised ecosystems. Nielsen (1960) has shown that dark fixation by bacteria tend to ascend rapidly instead of reaching a saturation plateau thereby the relative dark fixation exceeding 100% is attained for a six hour experiment. It is likely that the high bacterial population generated by the decaying weeds when enclosed in a bottle with C^{14} showed exhorbitantly high values as compared to phytoplankton production measured from the sea surface waters. The occurrence of ærobic chitin decomposing bacteria such as *Alcaligenes* and *Pseudomonas* in the sediments with a high rate of degradation potential as evidenced by their biochemical activities is a significant factor.

The fishery of this area is composed of both pelagic as well as benthic groups, of which shrimps dominate in the landings to a great extent. The period of weed deposition in the inshore area is in the period June–January which is repeated every year. The effect of weed deposition at the inshore area on the living resources is not yet fully understood. However, the phenomenon is of some significance since the monsoon season is the period of breeding of most of the commercially important species in these waters. Any disturbance in the ecosystem adversely affect the activities of the organisms. The effect of weed deposits on the benthic population appears to be transitional. Since the phenomena is repeated every year for a long time, it is quite possible that there will be a shift in the pattern of benthic population by the replacement of a resistant group in the benthic area of the inshore waters of this region which can, in the long run, even alter the food chain relationship.

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