STUDIES ON HETEROTROPHIC BACTERIA IN THE MANGROVE ECOSYSTEM NEAR COCHIN

DISSERTATION SUBMITTED BY Shri SURENDRAN V.
IN PARTIAL FULFILMENT FOR THE DEGREE
OF MASTER OF SCIENCE (MARICULTURE)
OF THE
UNIVERSITY OF COCHIN

OCTOBER 1985

CENTRE OF ADVANCED STUDIES IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN-682 035

C E R T I F I C A T E

This is to certify that this Dissertation is a bonafide record of work carried out by Shri Surendran, V. under my supervision and that no part thereof has been presented before for any other degree.

V. chandrika

(DR. V. CHANDRIKA)
Scientist,
Central Marine Fisheries
Research Institute,
COCHIN.

Countersigned by

Director,
Central Marine Fisheries
Research Institute,
COCHIN.

C O N T E N T S

		PAGE
1.	PREFACE	1 - 3
2.	INTRODUCTION	4 - 11
3.	MATERIALS AND METHODS	12 - 23
4.	RESULTS	24 - 48
5.	DISCUSSION	49 - 59
6.	SUMMARY	60 - 62
7.	REFERENCES	63 - 71

.....

P R E F A C E

Mangroves are typical coastal vegetation of tropical and subtropical areas with a characteristic assemblage of varied faunal communities drawn from the adjoining marine, estuarine and terrestrial habitats. The importance of mangrove ecosystem to coastal aquaculture and fisheries is fast gaining momentum as it has created a growing awareness among the scientific community throughout the world to study their ecological significance and benefits to mankind.

From the fisheries point of view mangrove creaks and swamps form important nursery and feeding ground for both finfish and shellfish. In the Cochin estuarine system, mangrove vegetation has colonised in areas of traditional aquaculture fields as well as the areas potentially suitable for aquaculture.

Mangrove swamps comprising of foliage as a major organic material support a detrital type of food chain in the tropical marine environment (Odum and Heald, 1975). Microbial populations in the mangrove area function as primary decomposers of organic foliage. Heterotrophic bacterial activity is important in decomposition, degradation and solubilization of organic matter leading to detritus

formation. The regeneration and recycling of nutrients in the mangrove ecosystem is influenced to a great extent by the existing heterotrophic bacterial population which in turn enhances the productivity of the ecosystem, thus making this a potential area for mariculture.

Realising the importance of decomposition pattern in this environment and its significance in mariculture, a study was undertaken for understanding the distribution of heterotrophic bacterial population in the tropical mangrove ecosystem. The investigations are aimed at quantitative estimation of bacterial population, and, isolation and identification of bacteria from these microbiotopes. As this mangrove ecosystem undergo well marked seasonal and spatial variation in the physico-chemical parameters which may affect the distribution of heterotrophic bacterial flora, investigations are also made on the seasonal variation of some of these parameters such as soil temperature, soil pH, soil Eh, organic carbon content of soil, available nitrate in soil, available phosphorus in soil etc. All these estimated environmental parameters and bacterial parameters are subjected to statistical analysis, to know how far the environmental factors influence the distribution of heterotrophic bacteria in this biotope.

I wish to express my sincere gratitude to Dr. (Mrs.) V. Chandrika, Scientist, Central Marine Fisheries Research Institute, Cochin, and my Supervising Teacher, without whose able guidance and sincere co-operation this work would not have been a reality. I would like to express my sincere gratitude to Dr. E.G. Silas, former Director, Central Marine Fisheries Research Institute, Cochin not only for providing necessary facilities for my work, but also for his constant encouragement given to me during his tenure. I wish to take this opportunity to thank Dr. P.S.B.R. James, Director, Central Marine Fisheries Research Institute, Cochin for providing all facilities for the successful completion of my work. My thanks are due to Mr. Srinath, Scientist, Central Marine Fisheries Research Institute, Cochin for his sincere help in statistical analysis of the data. I also thank Mr. R.V. Singh for his timely helps. To Mr. Muralidharan, C.M. are my special thanks for his help provided during the initial stages of my work. I stand in appreciation for Mr. Nandakumar's prompt help in procuring the required materials and instruments. I sincerely acknowledge all my classmates, juniors and seniors for their help rendered at various stages of this work. Finally, I acknowledge the Indian Council of Agricultural Research for providing me with Junior Research Fellowship for my post-graduate work in Mariculture.

.....

I N T R O D U C T I O N

Concern about mangrove ecosystem has grown steadily in recent years by the increased awareness about its ecological significance and benefits to mankind. Many aspects of this ecosystem is still unknown, and the magnitude of those known to occur, are often undetermined. Now-a-days, there is a growing interest in all parts of the world to study this ecosystem and to preserve its integrity.

Limited investigations are being made in India during the past on the general ecology of mangroves. Blasco (1975) made an extensive study on the mangroves of India. Some observations on the distribution, ecology and the environmental features of the mangrove from two major estuarine systems of Goa have been reported by Untawale et al. (1973). Dwivedi et al. (1975) studied the ecology of mangrove swamps of the Mandovi estuary, Goa. Untawale et al. (1977) studied the structure and production in a detrital rich estuarine mangrove swamp in Kollur estuary near Coondapoor (Karnataka) along the central west coast of India. The distribution of trace elements in the Pichawaram mangroves was done by Ramdhas et al. (1975). Muralidharan (1984) studied the colonisation of a mangrove plant Acanthus ilicifolius in relation to various physico-chemical parameters, in the mangrove ecosystem near Cochin.

Mangrove swamps comprising of foliage as a major organic material support a detrital type of food chain in the tropical marine environment (Odum and Heald, 1975). These detrital food webs existing in the ecosystem have been the focus of many studies (e.g. Fenchel and Jorgensen, 1977). The role of microorganisms in natural systems, such as, decomposition, nutrient regeneration and cycling, and production of particulate matter are of extreme significance in high productive systems like mangroves and salt marshes, where, distinct grazing of primary production by herbivores is not important. Bacteria are a key component of the detrital microbial community because of their rapid colonisation and high metabolic activity in association with detrital material (Fenchel and Jorgensen, 1977; Morrison et al., 1977; Rublee, 1978).

Published reports on the occurrence of heterotrophs from mangrove environment of India are very few. Matondkar et al. (1980a) studied seasonal variation of micro flora from the mangrove swamps of Goa situated along the Mandovi-Zuari estuary. Studies were conducted on heterotrophic bacterial flora by the same authors in 1981. Marine microbiological studies of mangrove swamps of Killai backwaters has been conducted by Venkatesan and Ramamurthy (1971) and they reported the presence of physiologically active group of bacteria such as starch hydrolysers, pectin degraders, gelatin liquifiers etc., from the same ecosystem. Matondkar

(1981) observed similar physiologically active groups from mangrove swamps of Goa.

Alagu Ravi (1984) studied the sulphur bacteria in the prawn culture ecosystem near Cochin. Humnadkar and Agate (1985) isolated 21 bacterial species from mud and water collected from mangroves of Sindhudurg and Malvan area in Konkan, Maharashtra. Chandrika et al. (1985) encountered green sulphur bacteria responsible for detritus decomposition from mangrove mud of Karuthedum near Cochin.

Studies on heterotrophic and indicator organisms of faecal pollution in the Cochin backwater include the work of Santhakumari (1966), Gore (1971, 1976, 1979a, 1979b), Chandrika (1976), Raveendran et al. (1978).

Considerable work has been carried out on the heterotrophic bacteria in the past by various Indian workers from east and west coasts of India. Velankar (1950, 1955, 1957) has done some pioneering work from the east coast of India by studying the heterotrophic bacteria of Madras waters, surveying the bacterial population of the inshore waters at Mandapam and by isolating a number of bacteria during the course of his work. Venkataraman and Sreenivasan (1954, 1954b, 1956), Gore (1972, 1973, 1974) studied the bacterial flora from the west coast of India. The eco-physiology of heterotrophic and indicator bacteria in the marine environments of Kerala was studied by Chandrika (1983). The distribution of heterotrophic bacteria in marine sediments

along Goa coast was surveyed by Shanta Nair et al. (1978). Shanta Nair and Lokabharathi (1980) studied the heterotrophic activity in tropical sandy beaches of the west coast of India.

Quite a lot of investigations are being made on heterotrophic bacteria in marine and similar environments, abroad. Murchilano and Brown (1970) investigated heterotrophic bacteria in Long Island Sound. Hakim et al. (1981) studied heterotrophic bacteria in intertidal sediments of the Karnafuli estuary, Bangladesh. The distribution of aerobic heterotrophic bacteria in Cananeia estuary, Brazil was investigated by Watanabe (1980). Kwong-Yu Chan and Kueh (1976) studied the horizontal and vertical distribution of heterotrophic bacteria in relation to temperature, pH, dissolved oxygen, salinity and available nutrients in Tolo harbour. Park A. Rublee (1982) investigated the seasonal distribution of bacteria in salt marsh sediments in North Carolina. Rheinheimer (1968, 1971, 1980), Ahrens (1969), Meyer-Reil (1973) studied the microbial flora of Baltic Sea which represents one of the largest brackish water areas of the world. Bernard Baleux and Mare Trousellier (1982) studied the spatial distribution and sampling of heterotrophic bacteria in surface brackish lagoon sediments.

Rheinheimer (1981) investigated the role of bacteria in the food web of the western Baltic and studied the transformation of organic material from the primary producers to

bacteria and from these to zooplankton and zoobenthos during the annual cycle. Iturriaga and Hoppe (1977) observed the heterotrophic activity on photo assimilated organic matter. Meyer-Reil and Faubel (1980) studied the uptake of organic matter by meiofauna organisms and their interrelationships with bacteria. Rieper (1976) investigated the relationships between algal blooms and bacterial populations in the Schlei Fjord (Western Baltic Sea). Teal (1962) observed the energy flow in the salt marsh ecosystem of Georgia. Particulate organic detritus in a Georgia salt marsh estuarine system was studied by Odum and De La Cruz (1967). Studies conducted by Heald and Odum (1969) revealed the contribution of mangrove swamps to Florida fisheries. The production of organic detritus in a South Florida estuary was investigated by Heald (1971).

Importance of bacteria in the chemistry and fertility of the marine environment is well established. Heterotrophic bacteria - their activities and relative abundance reflect the hydrological structure and nutrient levels in the marine environment. Aerobic heterotrophic bacteria are known to be deeply involved in the process of regeneration of nutrients for primary producers such as algae (Zo Bell, 1963; Wood, 1967; Rheinheimer, 1980; Sibert and Brown, 1975). Yasuhiko Tezuka (1966) observed the commensalism between the sulphate reducing bacterium Desulphovibrio desulphuricans and other

heterotrophic bacteria. Density and composition of heterotrophic bacterial population in North Sea sediments were studied by Boeye et al. (1975). Meyer-Reil et al. (1978) studied the interrelationship between microbial and chemical parameters of sandy beach sediments. The heterotrophic bacterial flora in the sea water samples from Bengal Bay and the South China Sea was studied by Usio Simidu et al. (1982). Hobbie and Williams (1984) defined marine heterotrophic activity as the process by which carbon autotrophically fixed into organic compounds by photosynthesis is transformed and respired.

Ayyakkannu and Chandramohan (1971) noted relationship between phosphate content, the number of phosphate solubilising bacteria and phosphatase activity in marine sediments at Porto Novo. Although mostly studied in coastal areas, heterotrophic bacterioplankton are ecologically significant throughout the marine and estuarine environment. Schroder and Van Es (1980) studied the influence of organic pollution on macrobenthic and meiobenthic heterotrophic bacterial population in the intertidal flats of the Enis Dollard estuary, Netherland. Simidu et al. (1980) studied heterotrophic bacterial flora of sea water from the Nanseishoto (Rynkyu Rethi) area.

Bent and Goulder (1981) studied planktonic heterotrophs in the Humber estuary and found seasonal variation in population density and heterotrophic activity in surface waters of the estuary. Fuhrman et al. (1980) observed fluctuation

of the communities of heterotrophs during decomposition process of phytoplankton.

Decomposition of organic matter and nutrient regeneration in the marine environment has been studied by Sorokin (1978). The importance of microbial populations composed of bacteria, actinomycetes, yeasts and moulds in the metabolism and productivity of aquatic ecosystems have been studied by Zo Bell (1946), Rodina (1951), Kuznetsov (1959, 1970), Seki (1965b), Sorokin (1971b), Strickland (1971). But the knowledge of microbial biomass, distribution, productivity and in situ metabolism in the mangrove ecosystem is scanty when compared to all other marine ecosystems.

A specific feature of bacterial decomposers is their ability to metabolise - and include into the ecosystem's energy budget - energy from sources and concentrations which are barely or not at all, accessible to other organisms (Zo Bell, 1946). The external metabolites excreted by the marine microflora are an important factor in the formation and integration of marine biocenoses (Lucas, 1947; Johnston, 1955). This role of the marine microflora is demonstrated most clearly in the epibiotic communities of sessile microalgae and bacteria covering solid surface areas and detritus particles (Khailov and Gorbenko, 1967; Khailov and Finenko, 1968).

In regard to productivity studies and in respect to biogeochemistry, the process of microbial production itself is no less important than the process coupled with it, namely, microbial decomposition which has attracted far more attention (Rittenberg, 1963; Sorokin, 1972d). Microbial synthesis transforms dead organic matter and carbon dioxide into microbial cell protein (Parsons and Strickland, 1961). This process includes into cell synthesis - and thus into the food chain as well as - an appreciable number of elements such as nitrogen, phosphorus, sulphur, iron, manganese, cobalt and some trace metals.

.....

MATERIALS AND METHODS

Study area:-

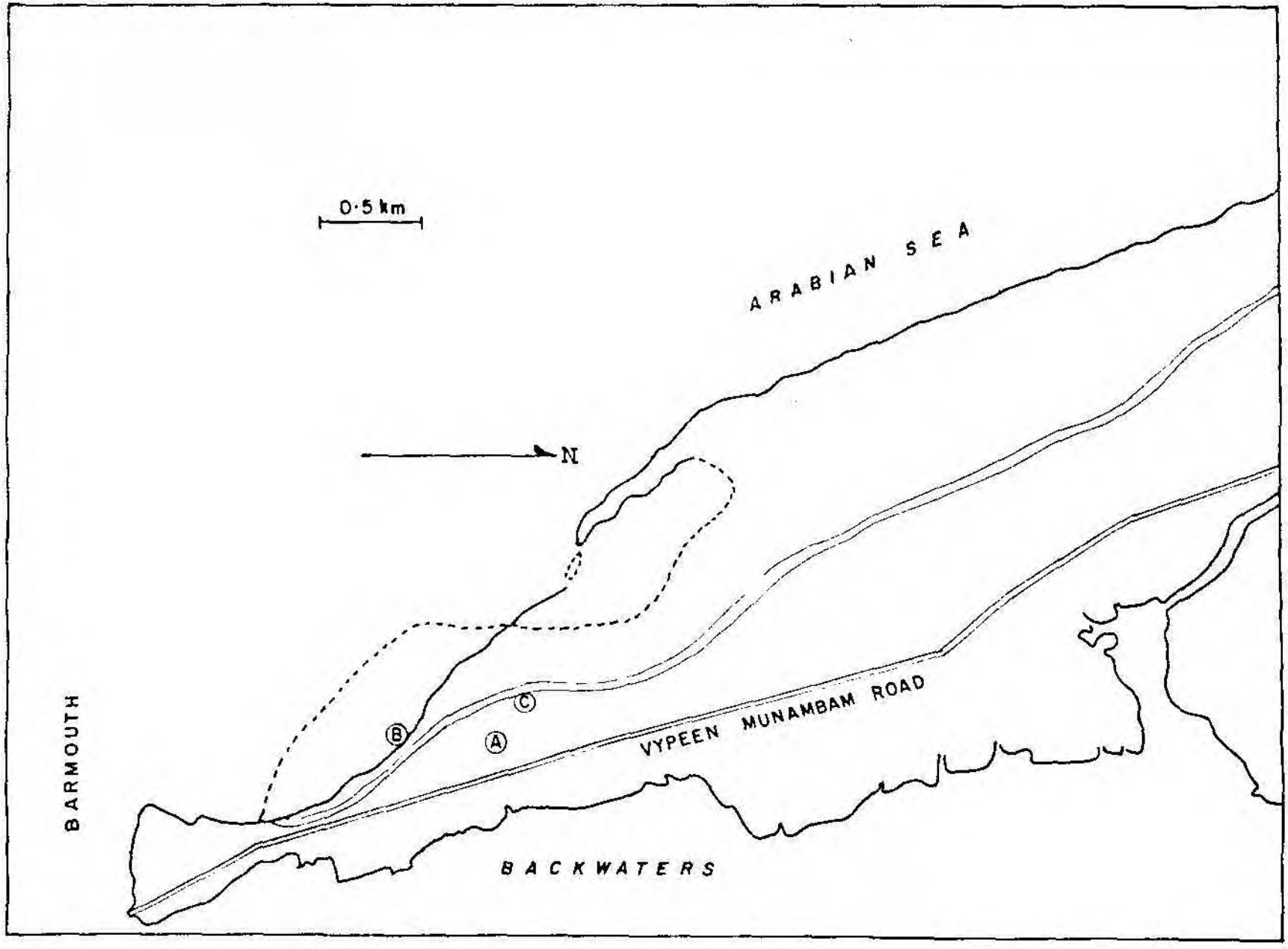
The studies were conducted for a period of 6 months, during March-August 1985, around Murikinpadam area in Vypeen Island near Cochin. The area is a typical tropical mangrove ecosystem with an intricate system of channels, creeks and canals which suffers tidal flux, and, with an abundant and varied flora of mangroves. Three Stations (Station A, Station B, and Station C) were selected in the area for the present study, as indicated in Fig. 1.

Station A was an area which was always influenced with tidal inundation and dominated by a single species of mangrove flora namely Acanthus ilicifolius (Plate I).

Station B - This station was about 3 km southwest of Station A, and was comparatively less influenced with tidal inundation. The area was occupied with mangrove vegetation dominated by two genera namely Acanthus and Avicennia (Plate 2).

Station C was a shallow prawn filtering pond situated in the mangrove ecosystem (Plate 3). This was located about 2 km north of Station B. As this pond had direct connection to the tidal canal, the influence of tides were maximum here.

Fig. 1. Map of Vypeen Island showing study area (Stations A, B & C).


0·5 km
ARABIAN SEA
N
BARMOUTH
VYPEEN MUNAMBAM ROAD
BACKWATERS
A
B
C

Parameters of study:-

Quantitative as well as qualitative studies of the bacterial population in the 3 stations were carried out for a period of 6 months (March-August 1985). Seasonal variations of some important environmental parameters such as rain fall, soil temperature, soil pH, soil Eh, electrical conductivity of soil, organic carbon, available nitrate and available phosphorus present in the soil were also investigated. Rainfall data was obtained from the Naval Meteorological Office.

Collection of samples:-

Fortnightly sampling of soil from the above 3 stations was done throughout the period of study. Sampling were done between 0700 and 0900 hours invariably in all sampling dates. Soil samples were collected, from surface up to 5 cm depth, using a plexiglass mud corer aseptically, and transferred quickly to sterile polyethene bags. Samples were taken from more than 3 places from each station and mixed well after transferring to polyethene bags. Care was taken not to contaminate the soil samples by any means.

Soon after collection, the samples were transferred to an ice-box and transported immediately to the Bacteriology Laboratory at Central Marine Fisheries Research Institute, Cochin. The samples were subjected to bacteriological investigations within 3 hours of sampling.

Soil samples for physico-chemical investigations were collected separately from the above stations in similar manner.

Bacteriological investigations:-

Both quantitative and qualitative analysis of bacteria were done in the soil samples of the above said stations during the period of study (March-August 1985).

Quantitative analysis:-

Enumeration of total viable heterotrophic bacteria:- The viable count of total heterotrophic bacteria was determined by pour plating method (Rodina, 1972).

Approximately 1 g of the soil sample was aseptically transferred to a sterilized glass mortar, ground well with pestle, and mixed well with 99 ml of sterile aged water which was collected from the same ecosystem. After thorough mixing serial dilutions were made by adopting standard procedures given by Rodina (1972). One ml of the inoculum was transferred to 10 mm diameter sterile glass petri dishes and pourplated.

The sea water agar medium was used for pourplating.

The plates were prepared in duplicate and incubated at room temperature (28 $\pm$ 2°C) for 48 hours, in a bell jar (Plate 4). The colonies developed in the petri dishes

(Plate 5) were counted after 48 hours and represented on dry weight basis.

All the inoculation procedures were carried out in an inoculation chamber sterilized with ultraviolet radiations.

Qualitative analysis:-

Qualitative analysis of bacteria was carried out during pre-monsoon and monsoon periods.

All the colonies randomly selected from the primary plates were isolated and subcultured both in peptone broth and agar slants. Further purification was done by repeated streaking on the sea water agar medium of the same composition. All the purified isolates were subsequently examine for colony characteristics, cell morphology and gram stain. They were then subjected to a battery of biochemical tests for identification purpose. The various tests employed in identification procedure are briefly mentioned below:

Gram staining:

Hucker's modified technique was employed to stain the isolated strains. Broth cultures were used for staining the heat fixed smear. The heat fixed smear after staining with Hucker's ammonium oxalate crystal violet for 1 minute and washing in tap water for a second, was flooded with Lugol's Iodine (Gram's modification) solution for 1 minute.

Then the smear was washed in tap water, decolourised with 95% ethyl alcohol and blot dried. The smear was then counterstained for 1 minute in saffranin solution, washed in tap water, air dried and observed under an oil immersion objective of a microscope (Plates 6 & 7).

Motility test:

Hanging drop preparations were made and motility was observed directly under microscope.

Test for detecting nitrate reduction:

This test was conducted to detect the reduction of nitrate by bacteria. The test was done by adding two drops of sulphanilic acid and two drops of a-naphthyl amine solution to a 48 hour peptone broth culture of bacteria. The presence of nitrite was indicated by a pink red colour and the presence of ammonia was confirmed by the reddish brown precipitate when treated with Nesler's reagent.

Gelatin liquifaction test:

Ability to liquify gelatin is a diagnostic criterion of some value because the proteolytic activity of a bacterial species may be measured in a gelatin medium (Rodina, 1972).

The test was carried out by inoculating gelatin media with 24 hour broth culture, incubating the tubes at 37°C for 48 hours, refrigerating the tubes for about 1 hour and observing the solid nature of gelatin.

Test for amylolytic activity of bacteria:

The test was employed for detection of the enzyme amylase in bacteria (enzyme involved in the hydrolysis of starch). The bacterial strain was streaked on a beef-extract agar plate containing 0.2% of soluble starch and incubated at room temperature for 2 days. To make the test 2-3 iodine crystals were placed on the petri dish cover after inverting the plates and warmed. The clear zone, developed was taken as an indication of starch hydrolysis.

The indole test:

This test demonstrates the ability of certain bacteria to decompose the aminoacid tryptophan to indole which accumulates in the medium. The cultures were inoculated into tryptone broth medium and incubated at room temperature (28 ± 2°C) for 72 hours. A positive reaction was indicated by the appearance of a red ring on addition of Kovac's reagent.

Test for detecting hydrogen sulphide formation:

The enzymatic decomposition of proteins or peptones composed of sulphur containing aminoacids, by bacteria, results in the formation of hydrogen sulphide. The test was done by inoculating the bacterial culture in L-cysteine broth above which strips of filter paper, soaked with a saturated solution of lead acetate, dried were suspended. The presence of hydrogen sulphide was indicated by a black colour on the lead acetate paper. The colour was due to the formation of lead sulphide by the action of escaping hydrogen sulphide on lead acetate paper.

Sugar media to test carbohydrate fermentation:

The following sugars were selected for the study:

i) Glucose, ii) Lactose, iii) Maltose, iv) Mannitol and v) Sucrose.

The sugar solutions were prepared in peptone water base to get a final concentration of 1%. The media distributed in sterilized test tubes were steamed for 3 consecutive days and inoculated with bacterial culture. They were observed for gas formation in the first 18-36 hours.

Hugh and Leifson's test or 'Oxferm' test:

The test was employed to distinguish between oxidative and fermentative utilization of carbohydrates. Carbohydrate media was prepared with phenol red as an indicator in duplicate tubes and were inoculated with bacterial test strains. They were then incubated for 24 hours - one aerobically while the other one anaerobically by sealing the surface of the medium with 2 cm of liquid paraffin. The results were read as:

Oxidative metabolism	-	acid in aerobic tube only (Yellow colour)
Fermentative metabolism	-	acid in both tubes.

The Oxidase test:

The test was conducted to detect the presence of certain oxidases in the bacteria that will catalyse the transport of electrons between the electron donors in the bacteria and redox dye - N'N'N' Tetramethyl paraphenylene diammine dihydrochloride. The dye was reduced to a deep purpose colour in a positive reaction.

The Catalase test:

Catalase is an enzyme capable of decomposing hydrogen peroxide into water and molecular oxygen and the enzyme is produced by many bacteria, especially aerobic bacteria. The presence of the enzyme was detected by adding hydrogen peroxide to the bacterial culture and noting the evolution of oxygen.

Test of sensitivity towards Penicillin:

Antibiotic sensitivity of isolated bacterial cultures towards penicillin was tested with Penicillin discs (10 I.U.).

Identification of bacterial genera:

A total of 108 strains of bacteria were isolated and identified up to genus level by following the scheme given by Usio Simidu and Kayuyoshi Aiso (1962). The results were compared and verified with Bergey's manual of determinative bacteriology (1975).

Physico-Chemical investigations: -

As the mangrove ecosystem undergoes seasonal and spatial variations in the physico-chemical characteristics, which may affect the bacterial flora, an attempt has been made to study some important physico-chemical parameters and to find out their possible relationship if any, with the bacterial population.

The following parameters have been studied:

i) Rainfall

ii) Soil temperature

iii) Soil pH

iv) Soil Eh

v) Electrical conductivity of soil

vi) Organic carbon in soil

vii) Available nitrate in soil

viii) Available phosphorus in soil.

Rainfall data was obtained from Naval Meteorological Office.

Soil temperature was estimated from the study area itself by using a Jennson delux alcoholic thermometer of range 0 - 110°C, each division being 1°C.

After transportation to the laboratory, the soil samples were immediately subjected to pH and Eh estimation using an Elico pH meter (Model LI-10 T) with pH and Eh electrodes. The pH and Eh of soils were determined in the wet condition itself.

The soil samples were air dried by spreading on polyethene sheets in trays and then ground well using mortar and pestle, and sieved through 0.425 mm sieve for chemical analysis (Dewis and Freitus, 1970). These samples were stored in wide-mouthed plastic jars with screw lid.

Electrical conductivity of soil was estimated by making soil extract in the ratio of 1 : 2 with distilled water and subsequent determination using Biochem conductivity bridge (Dewis & Freitus, 1970).

Determination of organic carbon in the soil was done by adopting Walkley and Black's rapid titration method.

Available nitrate present in the soil was estimated by colourimetric method described by Mackereth (1957).

Available phosphorus in soil was estimated colorimetrically by modified Olsen's method, where, ascorbic acid - ammonium molebdnate reagent was used instead of stanncus chloride - ammonium molebdnate complex, for sodium bicarbonate extract of soil (Khanna, 1977).

Statistical analysis:-

To study the relationship between different environmental parameters and total heterotrophic bacterial count, correlations were computed and tested for significance. The homogenity of relationship between the environmental parameters and bacterial count among the three stations was tested by Z-test of homogenity (Snedecor and Cochran, 1967).

.....

PLATE-3 A VIEW OF STATION-C

PLATE-2 A VIEW OF STATION-B

PLATE-1 A VIEW OF STATION-A

PLATE-4 AEROBIC INCUBATION AT ROOM TEMPERATURE (28 ± 2°C) IN A BELL JAR

PLATE-5 HETERTROPHIC BACTERIAL COLONIES DEVELOPED ON SEA WATER AGAR PLATES

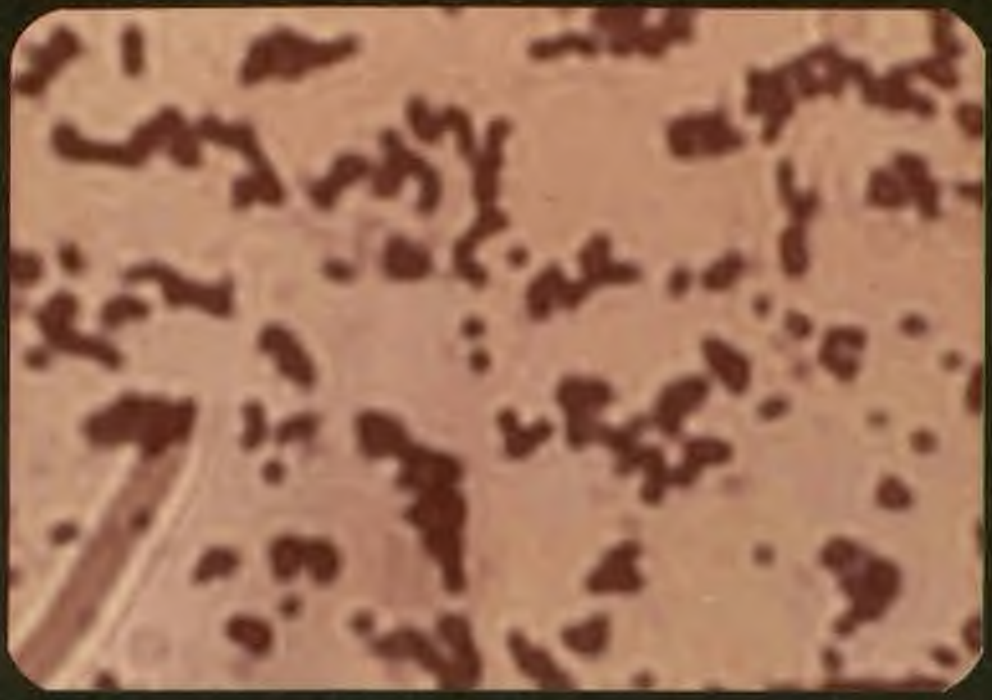


PLATE-6 GRAM-POSITIVE COCCI

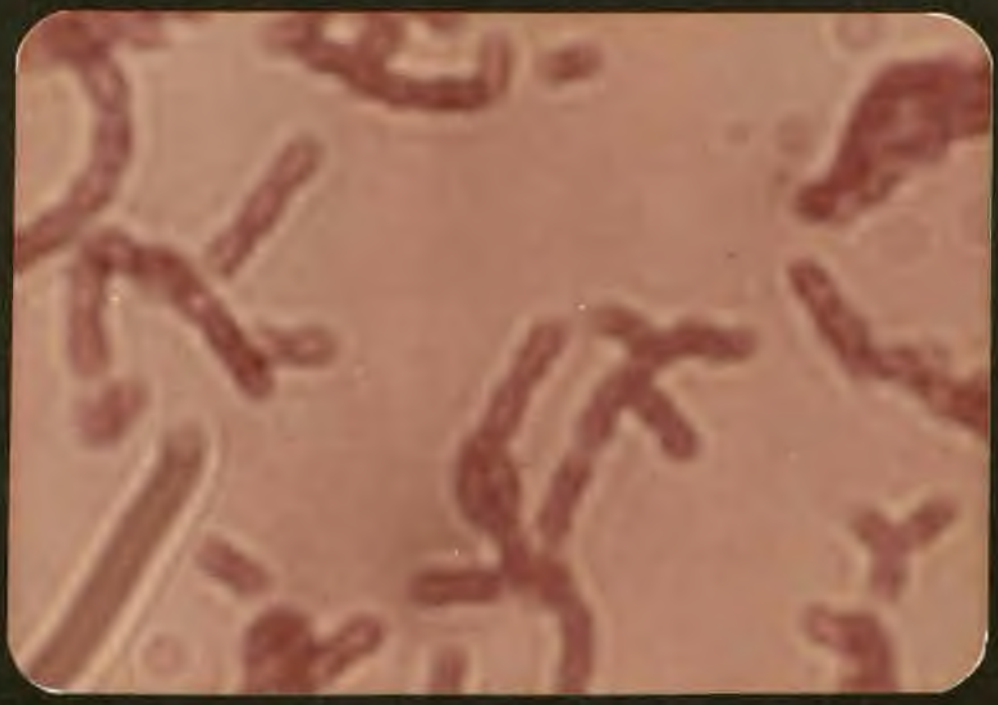


PLATE-7 GRAM-NEGATIVE RODS

R E S U L T S

Bacteriological observations:-

Quantitative analysis of total heterotrophs:-

Total heterotrophs didn't show much variations in Stations A, B and C (Figs. 3, 4 & 5). Still, comparatively higher average counts have obtained in Station A (13.6 x 10^6/g soil) and Station B (9.8 x 10^6/g soil) than in Station C (8.9 x 10^6/g soil).

Seasonal variation in total heterotrophic bacterial count could be observed in all the three stations. The count was maximum during summer months and gradually showed a decline and became minimum during peak monsoon. A gradual increase in bacterial count was noted after the peak monsoon till August. In all the stations, the minimum count was recorded during June and maximum during March. In Station A it varied from 3.33 x 10^5/g soil to 52.00 x 10^6/g soil. Total heterotrophs in Station B varied from a minimum of 6.59 x 10^5/g soil to a maximum of 29.23 x 10^6/g soil. In Station C the minimum count observed was 10.00 x 10^5/g soil and the maximum 30.75 x 10^6/g soil.

Qualitative analysis:-

A total of 108 strains (36 strains from each station) were isolated and identified up to genera level with the help of the scheme given by Usio Simidu and Aiso (1962). The

results were compared with Bergey's Manual of Determinative Bacteriology (1975). The analysis were carried out both in pre-monsoon and monsoon period in all the three stations.

Morphological and Biochemical observations:

Morphological and biochemical characteristics of all the isolates were investigated and the results are summarised in Table 4.

In Station A, gram positive strains were rare (2.8 %) when compared to gram negative strains (97.2 %). 97.2% of the total isolates from this station was found to be motile. Pigmented forms were recorded as 36.1 %. Only 11.1 % of total isolates were found to be producing indole. The saccharolytic activity of all the isolates was observed to be very poor in all the three stations. Hugh and Leifson's test employed to distinguish between oxidative and fermentative utilization of carbohydrates revealed that oxidative metabolism was more common among the isolates in Station A (52.8 %). 13.9 % of the isolates were fermentative and 33.3 % showed no reaction for this test. None of the isolates were found to be alkaline. All the isolates were catalase positive. 72.2 % oxidase positive forms and 61.1 % penicillin sensitive forms were also recorded in this station.

Out of the isolates from Station B, 5.6 % were gram positive and 94.4 % were gram negative. Motile forms were recorded as 94.4 % and pigmented forms as 36.1 %. Biochemical

tests revealed that 80.6 % were nitrate reducers, 61.1 % gelatin liquifiers, 55.6 % starch hydrolysers, 19.4 % indole producers and 58.3 % H_2S producers. Hugh and Leifson's test showed that 38.9 % were oxidative forms, 25 %were fermentative, and 36.1 % not reactive; no alkaline forms were recorded. All the isolates were catalase producing forms as in Station A. 77.8 % of the isolates were oxidase positive and 50 % were noted as penicillin sensitive.

Similar results were obtained for the isolates in Station C also. 2.8 % of the isolates were gram positive and 97.2 % were gram negative. 97.2 % of the isolates were motile and 41.7 % were pigmented forms. Of all the isolates, 58.3 % were nitrate reducers, 61.1 % gelatin liquifiers and 91.7 % starch hydrolysers. Indole producers were very limited in number (2.8 %). 72.2 % of total isolates were found to be producing hydrogen sulphide. The result of Hugh and Leifson's test was recorded as follows: Oxidative forms 58.3 %, fermentative forms 2.8 %; no alkaline forms were recorded; 38.9 % of the isolates were found to be not reactive. 66.7 % of isolates were oxidase positive. All the isolates were catalase producers in this station also. Penicillin sensitivity was observed in 88.9 % of the isolates.

Identification of genera:

The following six genera are identified out of the 108 strains isolated from the three stations during the study period (March-August 1985):

Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Pseudomonas and Micrococcus.

Relative abundance of taxonomic groups:

The relative abundance of all these genera varied seasonally in the three stations (Fig. 6).

Alcaligenes was predominant in Station A during pre-monsoon period (33.3 %), followed by Pseudomonas (25 %), Vibrio (25 %), Flavobacterium (8.3 %), Micrococcus (8.3 %); and Cytophaga was not recorded. During monsoon also Alcaligenes remained as the predominant group (33.3 %) followed by Pseudomonas (29.2 %), Cytophaga (25 %), Flavobacterium (8.3 %), Vibrio (4.2 %), and Micrococcus could not be isolated during this time.

In Station B, the predominant group during pre-monsoon was Pseudomonas (33.3 %), followed by Alcaligenes (25 %), Flavobacterium (16.7 %), Cytophaga (8.3 %), Vibrio (8.3 %) and Micrococcus (8.3 %). During monsoon, Alcaligenes was found to be dominating in this station (33.3 %), followed by

Pseudomonas (29.2 %), Vibrio (25 %), Flavobacterium (4.2 %), Cytophaga (4.2 %) and Micrococcus (4.2 %).

Like in Station A, Alcaligenes was the dominant group in Station C also during pre-monsoon (58.3 %). It was followed by Flavobacterium (25 %), Cytophaga (8.3 %) and Pseudomonas (8.3 %). Vibrio and Micrococcus were not encountered from the soil samples in the pre-monsoon period. Alcaligenes remained predominant during monsoon also (54.2 %). This was followed by Cytophaga (20.8 %), Flavobacterium (8.3 %), Pseudomonas (8.3 %), Vibrio (4.2 %) and Micrococcus (4.2 %).

The relative abundance of different genera in the study area in general (taken as the average of the three stations) is given as follows: Alcaligenes (39.6 %), Pseudomonas (22.2 %), Flavobacterium (11.8 %), Cytophaga (11.1 %), Vibrio (11.1 %) and Micrococcus (4.2 %).

Distribution of physiologically active isolates:

Distribution of physiologically active isolates in these three stations were also investigated (Table 6).

In Station A, during pre-monsoon period 66.7 % of the total isolates reduced nitrate to nitrite, 58.3 % hydrolysed starch, 91.7 % liquified gelatin, and 91.7 % produced H_2S from sulphur containing aminoacids. During monsoon 87.5 % of total isolates were found to be nitrate reducers. About 66.7 % starch hydrolysing heterotrophs were encountered.

Only 62.5 % of total isolates liquified gelatin and 58.3 % produced H_2S.

Physiological activity was found to be slightly different in Station B. During pre-monsoon 75 % of the isolates were nitrate reducers. Same abundance was noticed for starch hydrolysers. Gelatin liquifiers and H_2S producers were reported as 100 % and 66.7 % respectively. During monsoon, 83.3 % of total isolates were nitrate reducers. 45.8 % of isolates hydrolysed starch, 41.7 % liquified gelatin and 54.2 % produced hydrogen sulphide.

In Station C, the relative abundance of various physiologically active isolates during pre-monsoon was as follows: 25 % nitrate reducers, 83.3 % starch hydrolysers, 91.7 % gelatin liquifiers and 66.7 % H_2S producers. During monsoon, it was observed that 75 % of total isolates reduced nitrate and 95.8 % hydrolysed starch. Gelatin liquifiers and hydrogen sulphide producers were recorded as 45.8 % and 75% respectively.

Environmental Parameters:-

Rainfall:

The rainfall data obtained for Cochin area showed a marked seasonal variation. The peak rainfall was noted during the month of June (963.2 mm) and the lowest in March (57.8 mm). The onset of monsoon could be observed in May itself, having

a rainfall of 523.4 mm. The monsoon persisted for the rest of study period till August with a gradual decrease (Fig. 2).

Soil Temperature:

Though temperature didn't vary much among the three stations, it showed a well marked seasonal variation. Initially in March-April months temperature was maximum in the three stations (29.5°C, 30.5°C and 29.5°C in Stations A, B & C respectively). Temperature declined considerably by the onset of monsoon and reached to a minimum in July (23.8°C, 23°C and 24°C in the Stations A, B and C respectively - Figs. 3, 4 & 5).

Soil pH

The soil pH didn't show any drastic seasonal variation in any of the three stations. Still, slightly higher values were obtained during monsoon months. In Station A, a minimum pH of 7.1 was recorded during March and a maximum of 7.8 during August. In Station B, the minimum pH was 7.25 during March and maximum 8.20 during August. In Station C, the pH varied fom 7.20 to 8.25 during the months of March and August respectively(Figs. 3, 4 & 5).

Soil Eh:

No drastic variation in soil Eh could be recorded from any of the stations during the study period. Moreover it showed negative values in all the 3 stations throughout the study period. Soil Eh in Station A varied from -120 mv to -215 mv during the months of May and June respectively. In Station B it varied from -30 mv to -110 mv during March and April respectively. A variation from -165 mv to -190 mv was noted in Station C. The Eh of -165 mv was recorded during March and -190 mv during May and first fortnight of March (Figs. 3, 4 & 5).

Electrical conductivity of Soil:

Electrical conductivity of soil, which is directly related to salinity, showed an obvious seasonal variation in all the three stations. Electrical conductivity of soil was maximum during summer months and the peaks were reported as 91.624 millimhos (April), 97.732 millimhos (May) and 65.664 millimhos (March) in Stations A, B and C respectively. As monsoon progressed, electrical conductivity was found to be decreasing. A sudden decline of this parameter was observed during June in all the stations, and the values become as low as 11.53 millimhos, 2.138 millimhos and 7.636 millimhos during July in the Stations A, B and C respectively. Since then soil conductivity remained at a low level till August (Figs. 3, 4 & 5).

Organic carbon in soil:

Organic carbon neither showed considerable seasonal variation nor it varied much from station to station. However, slightly higher organic carbon content was observed during monsoon period in Stations A and B whereas in Station C maximum value was observed during March. In Station A it varied from 3.133 % to 4.6 % and was recorded during the months of March and June respectively. Station B also showed a slight variation in organic carbon content, from 3.815 % (July) to 4.704 % (June). A variation from 2.77 % to 4.285 % was observed in Station C during the months of July and March respectively. Of all the stations the organic carbon content came as low as 2.77 % during July in Station C and as high as 4.704 % during July in Station B (Figs.3,4 &5).

Available nitrate in soil:

Available nitrate could be detected in traces only, in all the three stations and it also maintained a steady level except during the months of April and May. The lowest value of 0.0095 ppm was recorded during July, August months in Stations A, B and C. The value went up to 0.077 ppm (April), 0.077 ppm (May), 0.0385 ppm (March, April) in Stations A, B and C respectively (Figs. 3, 4 & 5).

Available phosphorus in soil:

Available phosphorus in the soil from all the three stations was much higher when compared to available nitrate content and it showed seasonal variations also. Phosphorus content was higher during summer months with peaks as 104.4 ppm (March), 96.45 ppm (March) and 76.45 ppm (March, April) in Stations A, B and C respectively. By the onset of monsoon, the phosphorus content in soil was found to be reduced considerably, and reached almost a steady state during June, July, August months. The minimum values obtained for the above three stations were 76.45 ppm (June), 60.3 ppm (May) and 52.95 ppm (August) respectively. Phosphorus content was higher in Stations A and B when compared to that of Station C (Figs. 3, 4 & 5).

Statistical Analysis:

The correlation between different environmental parameters and total heterotrophic bacterial count was statistically analysed and the results summarised are in Tables 7, 8 and 9. Available phosphorus content in soil was found to be the only factor which had significant relationship with total heterotrophic bacterial count in all the three stations. However, in Station C, apart from available

phosphorus content, soil temperature, electrical conductivity of soil and available nitrate in soil are also found to be having significant relationship with total heterotrophic bacterial count. The Z-test which was carried out to study the homogenity of relationship between the environmental parameters and bacterial count among the 3 stations gave values well below 1.96 in all the 3 stations which indicated that the Stations A, B and C are more or less homogenous.

.....

TABLE - 1

ENVIRONMENTAL AND BACTERIOLOGICAL PARAMETERS OF STATION - A

Month	Rainfall (mm)	Soil temperature °C	Soil pH	Soil Eh (mv)	Electrical conductivity of soil (m.mhos)	Organic Carbon%	Available Nitrate (ppm)	Available phosphorus (ppm)	Total heterotrophic bacterial count No./g soil
MAR	057.8	27.9	7.5	-150	60.318	3.133	0.0385	104.4	48×10^6
		27.0	7.1	-165	67.19	3.397	0.0385	94.1	52×10^6
APR	071.1	29.0	7.4	-165	62.61	3.344	0.0385	102.95	24.44×10^6
		29.0	7.2	-170	91.624	4.076	0.077	82.35	8.57×10^6
MAY	523.4	29.0	7.3	-130	84.752	3.502	0.077	77.9	17.5×10^5
		27.5	7.35	-120	69.482	3.554	0.058	88.25	37.14×10^5
JUN	963.2	24.0	7.7	-215	31.304	3.554	0.0385	76.45	3.33×10^5
		26.5	7.4	-180	22.142	4.6	0.0385	82.35	6.25×10^5
JUL	416.5	23.8	7.39	-190	11.53	3.293	0.0095	79.4	40×10^5
		24.0	7.45	-200	15.27	3.815	0.0095	77.95	37.77×10^5
AUG	299.1	26.0	7.5	-150	18.324	3.711	0.019	80.9	74×10^5
		24.5	7.8	-185	15.652	3.606	0.0095	79.4	80.8×10^5

TABLE - 2

ENVIRONMENTAL AND BACTERIOLOGICAL PARAMETERS OF STATION - B

Month	Rainfall (mm)	Soil temperature °C	Soil pH	Soil Eh (mv)	Electrical conductivity of soil (m.mhos)	Organic Carbon %	Available Nitrate (ppm)	Available Phosphorus (ppm)	Total heterotrophic bacterial count No./g soil
MAR	057.8	30.5	7.35	-30	61.082	3.983	0.0385	96.45	18.82×10^6
		27.0	7.25	-65	91.524	3.972	0.0385	92.05	29.23×10^6
APR	071.1	27.0	7.55	-90	76.354	3.972	0.0385	90.6	28.75×10^6
		27.8	7.30	-110	83.988	4.181	0.048	88.45	3.84×10^6
MAY	523.4	29.5	7.40	-60	78.644	4.129	0.077	60.3	38.88×10^5
		27.0	7.35	-50	97.732	4.129	0.058	60.3	40×10^5
JUN	963.2	24.0	8.0	-50	3.664	4.704	0.0385	72.05	6.59×10^5
		24.8	7.9	-90	4.276	4.181	0.019	63.25	11.30×10^5
JUL	416.5	23.0	7.51	-90	4.428	3.815	0.0095	64.7	54×10^5
		24.0	7.55	-40	2.138	4.547	0.0095	67.75	36.31×10^5
AUG	299.1	25.5	7.65	-60	5.574	4.129	0.0095	81.45	85×10^5
		24.0	8.20	-80	3.97	4.285	0.019	80.00	92.3×10^5

TABLE - 3

ENVIRONMENTAL AND BACTERIOLOGICAL PARAMETERS OF STATION - C

Month	Rainfall (mm)	Soil temperature °C	Soil pH	Soil Eh (mv)	Electrical conductivity of soil (m.mhos)	Organic Carbon %	Available Nitrate (ppm)	Available Phosphorus (ppm)	Total heterotrophic bacterial count No./g soil
MAR	057.8	28.5	7.45	-165	57.264	4.089	0.0385	76.45	30.76×10^6
		29.0	7.20	-190	65.664	4.285	0.0385	75.0	21.66×10^6
APR	071.1	29.5	7.4	-180	51.156	3.188	0.019	76.45	17.64×10^6
		28.5	7.35	-180	52.684	3.763	0.0385	58.8	10×10^6
MAY	523.4	28.5	7.40	-190	35.122	2.979	0.019	61.75	23.63×10^5
		29.0	7.35	-190	50.392	3.136	0.019	64.7	54.28×10^5
JUN	963.2	25.0	7.9	-170	16.798	3.763	0.019	61.75	10×10^5
		27.0	7.75	-170	13.362	3.711	0.0095	55.9	13.33×10^5
JUL	416.5	24.0	7.71	-180	7.636	3.188	0.0095	58.8	30×10^5
		24.5	7.70	-140	7.94	2.770	0.0095	58.8	24.28×10^5
AUG	299.1	26.5	7.70	-170	15.27	3.867	0.0095	52.95	60×10^5
		26.0	8.25	-150	10.536	3.920	0.0095	54.4	63×10^5

TABLE - 4

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF HETEROTROPHIC BACTERIA ISOLATED FROM THE THREE STATIONS (A, B & C) IN THE MANGROVE ECOSYSTEM

Characteristic	Frequency of occurrence (%)		
	Station - A	Station - B	Station - C
Gram			
Positive	2.8	5.6	2.8
Negative	97.2	94.4	97.2
Motility	97.2	94.4	97.2
Pigmentation	36.1	36.1	41.7
Nitrate reduction	80.6	80.6	58.3
Gelatin liquifaction	72.2	61.1	61.1
Starch hydrolysis	63.9	55.6	91.7
Indole production	11.1	19.4	2.8
H_2S production	69.4	58.3	72.2
Carbohydrate fermentation			
Glucose	2.8	16.7	5.6
Lactose	2.8	0	5.6
Maltose	2.8	16.7	5.6
Mannitol	2.8	16.7	2.8
Sucrose	2.8	16.7	2.8
Hugh & Leifson's test			
Oxidative	52.8	38.9	58.3
Fermentative	13.9	25	2.8
Alkaline	0	0	0
No reaction	33.3	36.1	38.9
Oxidase test	72.2	77.8	66.7
Catalase test	100	100	100
Penicillin sensitivity	61.1	50	88.9

TABLE - 5

RELATIVE ABUNDANCE OF DIFFERENT BACTERIAL GROUPS DURING PRE-MONSOON AND MONSOON PERIODS IN THE THREE STATIONS - A, B & C IN THE MANGROVE ECOSYSTEM

	Station - A		Station - B		Station - C	
	Pre-monsoon	Monsoon	Pre-monsoon	Monsoon	Premonsoon	Monsoon
No. of isolates	12	24	12	24	12	24
Bacterial Groups	% Bacterial groups					
Alcaligenes	33.3	33.3	25	33.3	58.3	54.2
Flavobacterium	8.3	8.3	16.7	4.2	25	8.3
Cytophaga	-	25	8.3	4.2	8.3	20.8
Vibrio	25	4.2	8.3	25	-	4.2
Pseudomonas	25	29.2	33.3	29.2	8.3	8.3
Micrococcus	8.3	-	8.3	4.2	-	4.2

TABLE - 6

DISTRIBUTION OF PHYSIOLOGICALLY ACTIVE ISOLATES FROM MANGROVE ECOSYSTEM

Station	Period	Total No. of isolates	Nitrate reducers	Starch hydrolysers	Gelatin liquinfiers	H_2S producers
			% positive isolates			
A	Pre-monsoon	12	66.7	58.3	91.7	91.7
	Monsoon	24	87.5	66.7	62.5	58.3
B	Pre-monsoon	12	75	75	100	66.7
	Monsoon	24	83.3	45.8	41.7	54.2
C	Pre-monsoon	12	25	83.3	91.7	66.7
	Monsoon	24	75	95.8	45.8	75

TABLE - 7

CORRELATION MATRIX OF DIFFERENT CHARACTERS IN STATION - A

	Soil temperature	Soil pH	Soil Eh	Electrical conductivity of soil	Organic carbon	Available nitrate	Available phosphorus	Total heterotrophic bacterial count
Soil temperature	1							
Soil pH	* -0.5799	1						
Soil Eh	* -0.7235	* -0.4276	1					
Electrical conductivity of soil	* 0.8814	* -0.6298	* 0.617	1				
Organic carbon	NS -0.018	NS -0.0775	NS -0.2036	NS -0.1619	1			
Available nitrate	* 0.8039	NS -0.5349	NS 0.5508	* 0.9032	NS 0.1387	1		
Available phosphorus	NS 0.5570	NS -0.2592	NS 0.3751	NS 0.4169	NS -0.4469	NS 0.1178	1	
Total heterotrophic bacterial count	NS 0.3425	NS -0.3266	NS 0.2039	NS 0.3441	NS -0.5031	NS -0.0002	* 0.8183	1

* - Significant at 5% level

NS - Not significant.

TABLE - 8

CORRELATION MATRIX OF DIFFERENT CHARACTERS IN STATION - B

	Soil temperature	Soil pH	Soil Eh	Electrical conductivity of soil	Organic carbon	Available nitrate	Available phosphorus	Total heterotrophic bacterial count
Soil temperature	1							
Soil pH	* -0.6394	1						
Soil Eh	NS 0.2286	NS -0.1071	1					
Electrical conductivity of soil	* 0.8036	* -0.7567	NS -0.0665	1				
Organic carbon	NS -0.3679	NS 0.5289	NS 0.3202	NS -0.4457	1			
Available nitrate	* 0.7338	NS -0.4447	NS 0.0929	* 0.7874	NS -0.0728	1		
Available phosphorus	NS 0.3948	NS -0.2115	NS -0.0581	NS 0.3664	NS 0.2617	NS -0.06	1	
Total heterotrophic bacterial count	NS 0.1734	NS -0.3420	NS 0.0109	NS 0.5150	NS -0.5232	NS 0.0524	* 0.7508	1

* - Significant at 5% level

NS - Not Significant.

TABLE - 9

CORRELATION MATRIX OF DIFFERENT CHARACTERS IN STATION - C

	Soil temperature	Soil pH	Soil Eh	Electrical conductivity of soil	Organic carbon	Available nitrate	Available phosphorus	Total heterotrophic bacterial count
Soil temperature	1							
Soil pH	* -0.7296	1						
Soil Eh	* -0.6023	* 0.697	1					
Electrical conductivity of soil	* 0.8884	* -0.8289	* -0.5952	1				
Organic carbon	NS 0.2029	NS 0.067	NS -0.0362	NS 0.294	1			
Available nitrate	* 0.6355	* -0.6719	NS -0.4025	* 0.8728	NS 0.4994	1		
Available phosphorus	* 0.6226	* -0.6390	NS -0.362	* 0.7981	NS 0.1633	* 0.661	1	
Total heterotrophic bacterial count	* 0.5891	NS -0.4896	NS -0.1392	* 0.7597	NS 0.5145	* 0.7509	* 0.8270	1

* - Significant at 5% level

NS - Not Significant.

Fig. 2. Summary of variations in rainfall and bacterial population.

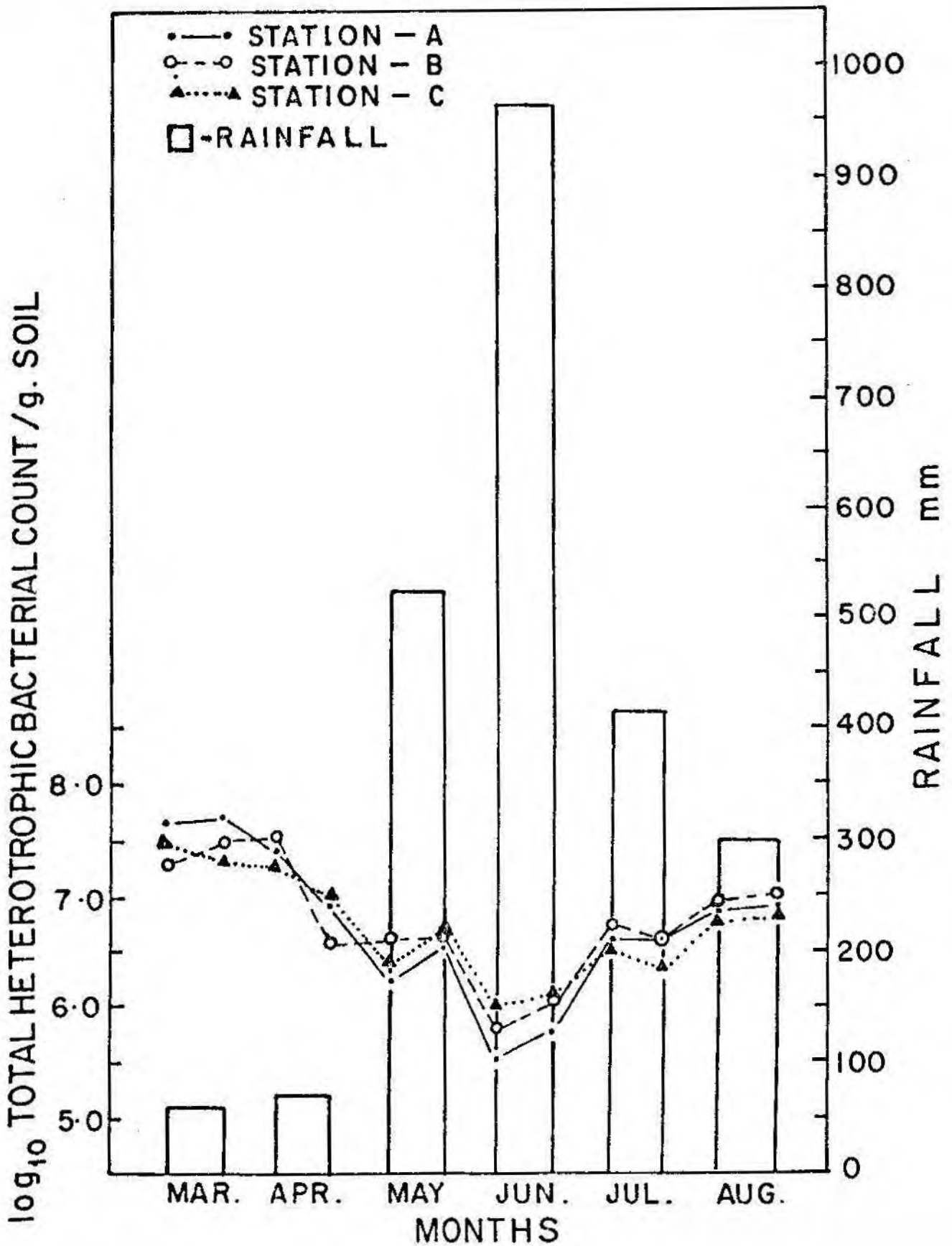

STATION – A
STATION – B
STATION – C
RAINFALL
log10 TOTAL HETEROTROPHIC BACTERIAL COUNT / g. SOIL
8·0
7·0
6·0
5·0
1000
900
800
700
600
500
400
300
200
100
0
RAINFALL mm
MAR.
APR.
MAY
JUN.
JUL.
AUG.
MONTHS

Fig. 3. Summary of variations in environmental parameters and bacterial population in Station A.

STATION— A

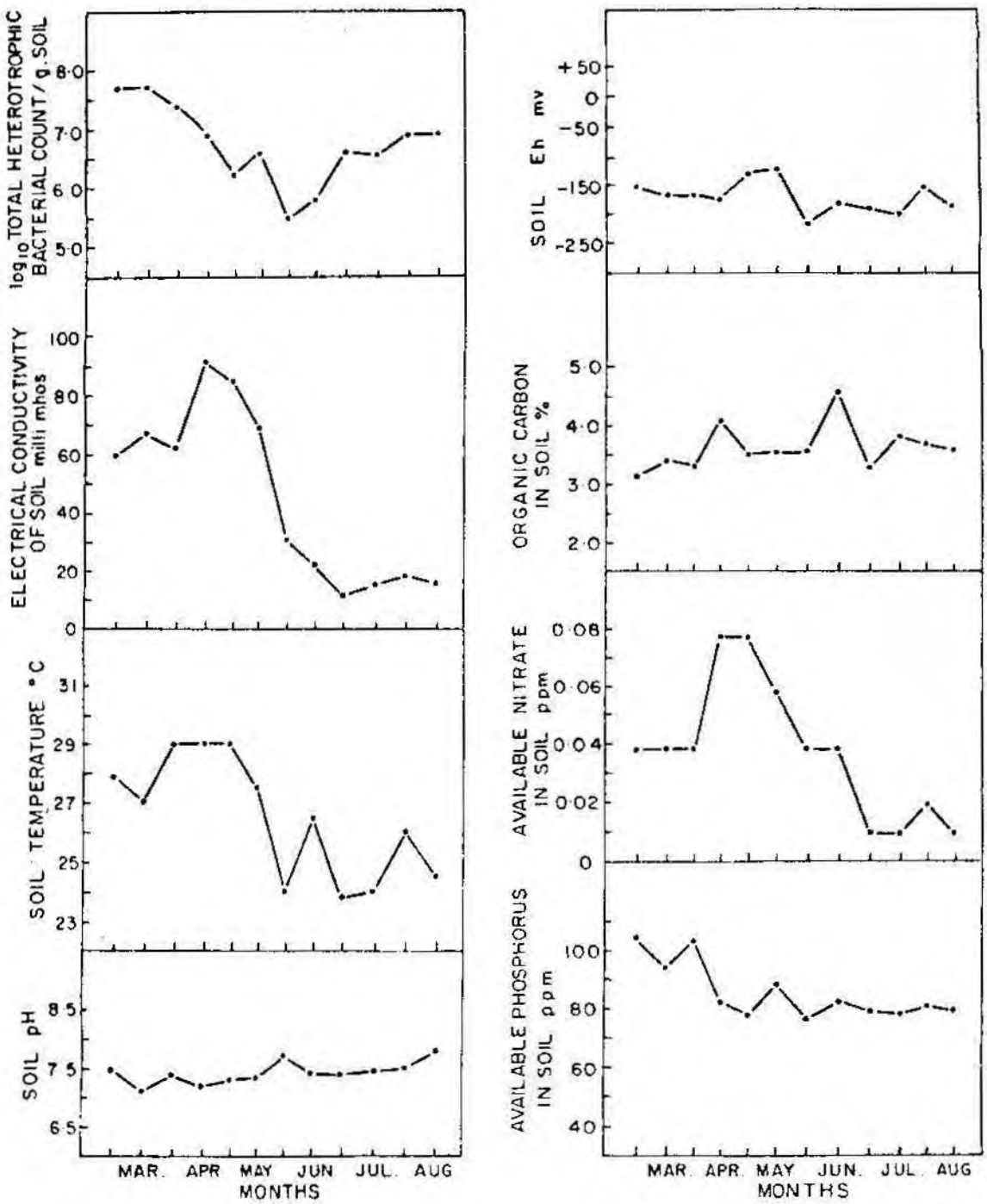

log10 TOTAL HETEROTROPHIC BACTERIAL COUNT / g. SOIL
8·0
7·0
6·0
5·0
ELECTRICAL CONDUCTIVITY OF SOIL milli mhos
100
80
60
40
20
0
SOIL TEMPERATURE °C
31
29
27
25
23
SOIL pH
8·5
7·5
6·5
MAR. APR MAY JUN JUL. AUG
MONTHS
SOIL Eh mv
+50
0
-50
-150
-250
ORGANIC CARBON IN SOIL %
5·0
4·0
3·0
2·0
AVAILABLE NITRATE IN SOIL ppm
0·08
0·06
0·04
0·02
0
AVAILABLE PHOSPHORUS IN SOIL ppm
100
80
60
40
MAR. APR. MAY JUN. JUL. AUG
MONTHS

Fig. 4. Summary of variations in environmental parameters and bacterial population in Station B.

STATION– B

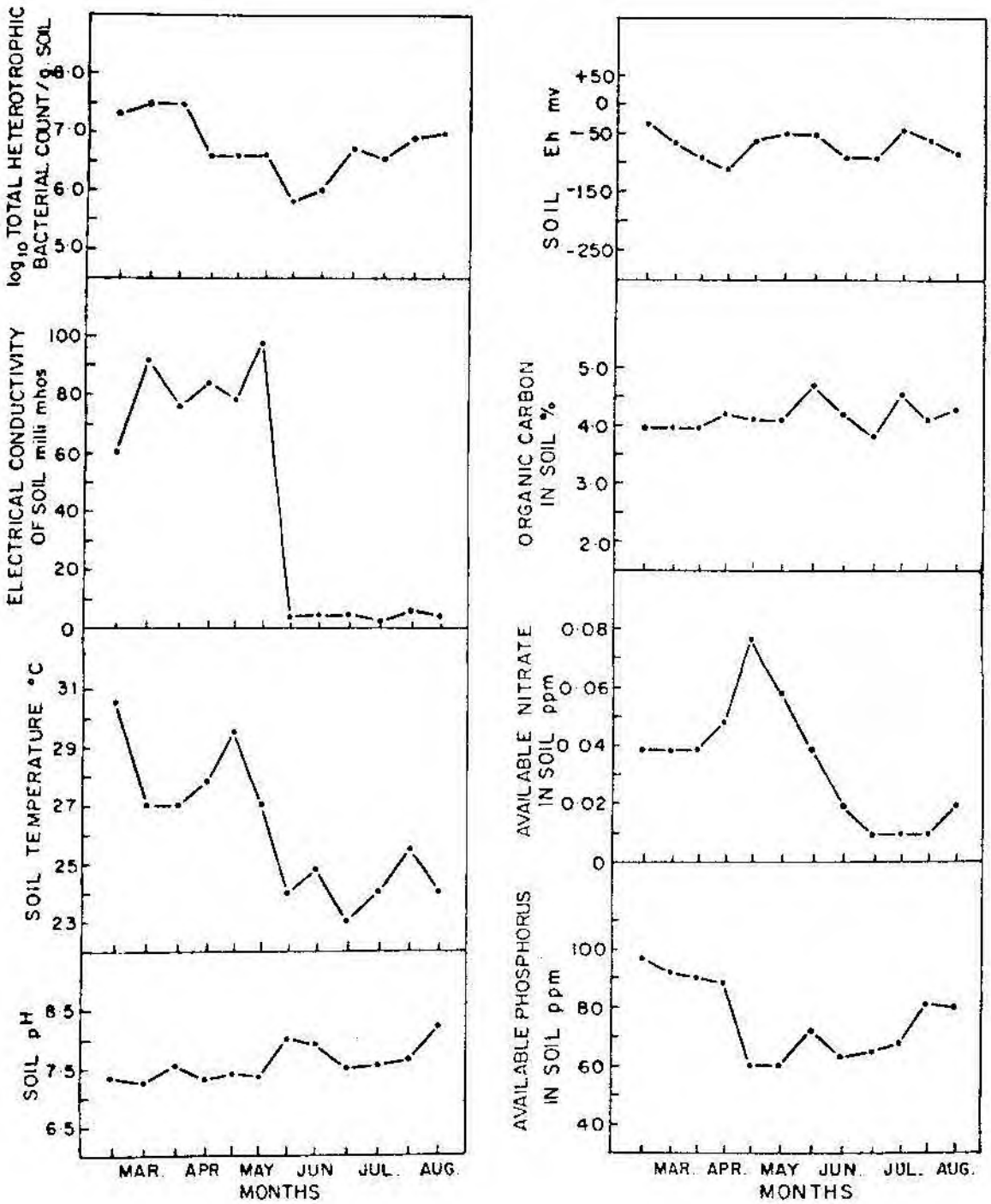

log10 TOTAL HETEROTROPHIC BACTERIAL COUNT/g. SOIL
8·0
7·0
6·0
5·0
ELECTRICAL CONDUCTIVITY OF SOIL milli mhos
100
80
60
40
20
0
SOIL TEMPERATURE °C
31
29
27
25
23
SOIL pH
8·5
7·5
6·5
MAR. APR. MAY JUN. JUL. AUG.
MONTHS
SOIL Eh mv
+50
0
-50
-150
-250
ORGANIC CARBON IN SOIL %
5·0
4·0
3·0
2·0
AVAILABLE NITRATE IN SOIL ppm
0·08
0·06
0·04
0·02
0
AVAILABLE PHOSPHORUS IN SOIL ppm
100
80
60
40
MAR. APR. MAY JUN. JUL. AUG.
MONTHS

Fig. 5. Summary of variations in environmental parameters and bacterial population in Station C.

STATION – C

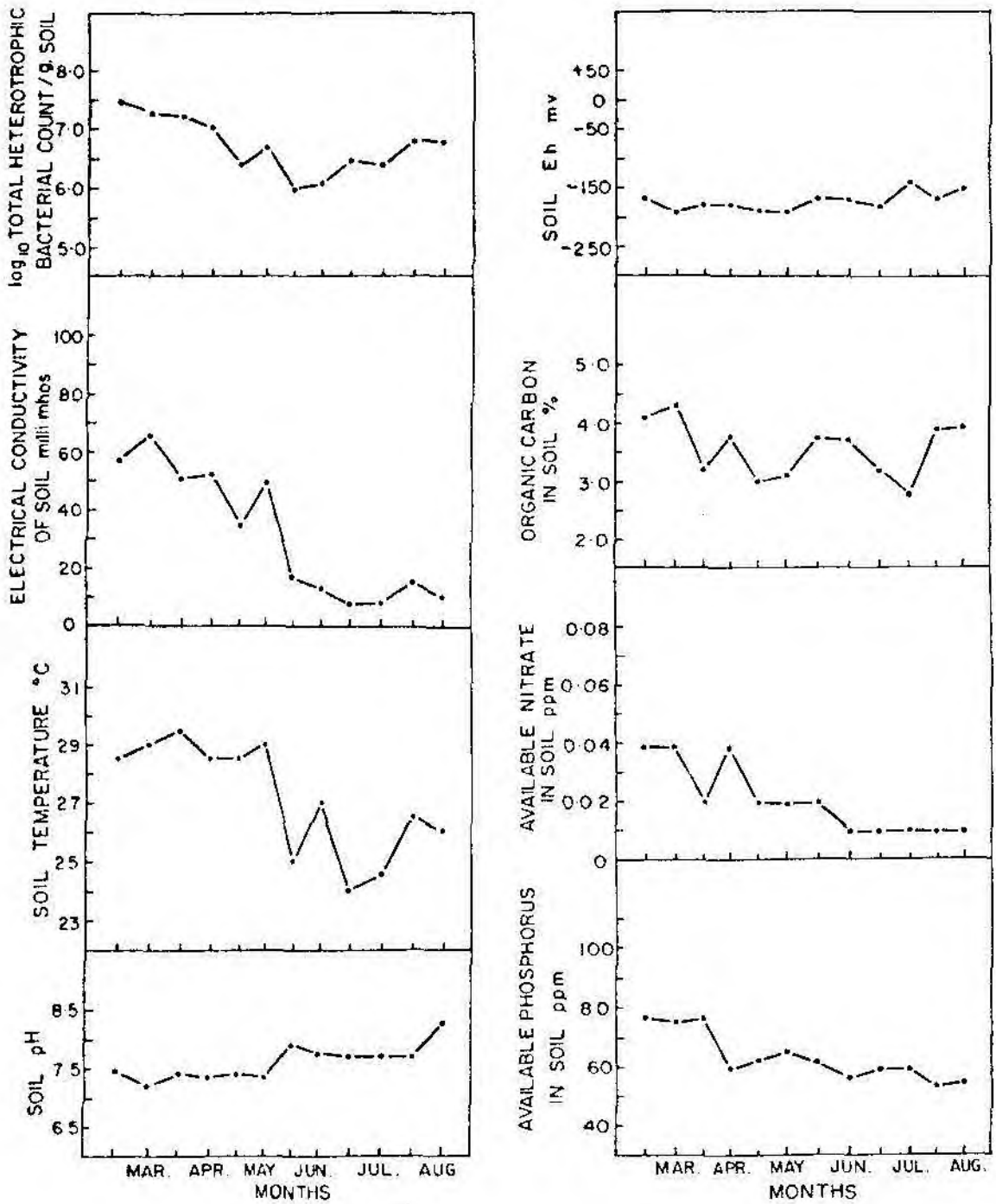
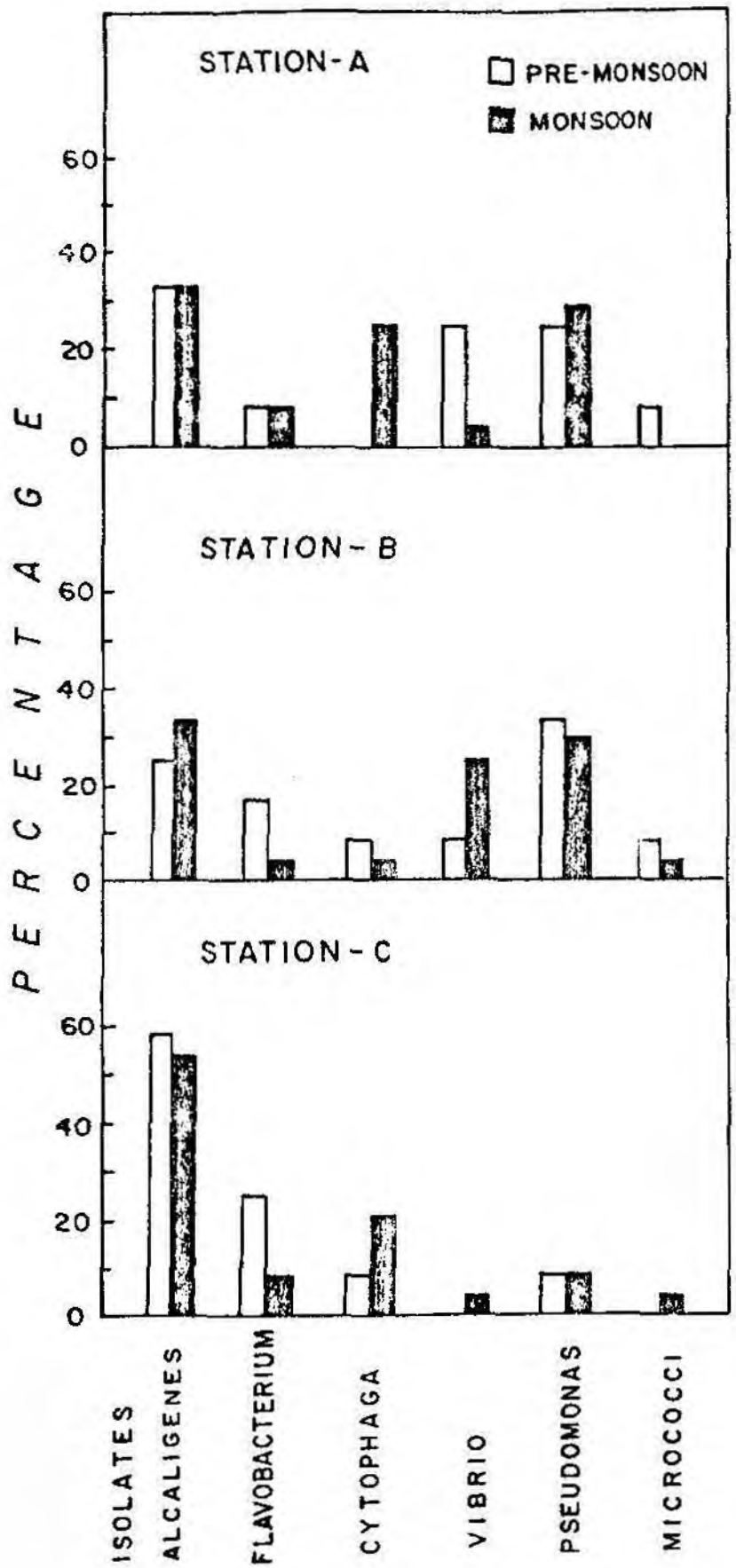

log10 TOTAL HETEROTROPHIC BACTERIAL COUNT / g. SOIL
8·0
7·0
6·0
5·0
ELECTRICAL CONDUCTIVITY OF SOIL milli mhos
100
80
60
40
20
0
SOIL TEMPERATURE °C
31
29
27
25
23
SOIL pH
8·5
7·5
6·5
MAR. APR. MAY JUN. JUL. AUG
MONTHS
SOIL Eh mv
+50
0
-50
-150
-250
ORGANIC CARBON IN SOIL %
5·0
4·0
3·0
2·0
AVAILABLE NITRATE IN SOIL ppm
0·08
0·06
0·04
0·02
0
AVAILABLE PHOSPHORUS IN SOIL ppm
100
80
60
40
MAR. APR. MAY JUN. JUL. AUG.
MONTHS

Fig. 6. Summary of distribution pattern of different taxonomic groups of bacteria in Stations A, B & C.


STATION-A
PRE-MONSOON
MONSOON
STATION-B
STATION-C
PERCENTAGE
0
20
40
60
ISOLATES
ALCALIGENES
FLAVOBACTERIUM
CYTOPHAGA
VIBRIO
PSEUDOMONAS
MICROCOCCI

D I S C U S S I O N

In the present study the distribution pattern of heterotrophic bacteria was analysed both quantitatively and qualitatively. The viable heterotrophic bacterial counts showed seasonal variation giving the maximum in pre-monsoon months; and, all the three stations exhibited the similar pattern.

Not much variation was recorded in heterotrophic bacterial population among the three stations. However the population was found to average from 13.6 x 10^6/g soil in Station A to 8.9 x 10^6/g soil in Station C. In Station B an average count of 9.8 x 10^6/g soil was recorded. This indicates the presence of highest bacterial population in Station A followed by Station B and then Station C.

A conspicuous seasonal variation of more or less similar pattern could be observed in all the three stations. Maximum counts were obtained during pre-monsoon months. The bacterial population was found declining during monsoon; a gradual recovery was also noted as the monsoon weakened and the count ranged from 3.33 x 10^5/g soil to 52.00 x 10^6/g soil during the months of June and March respectively.

Literature to support the present observation are scanty as there is no substantial work on the bacteriology of tropical mangroves. Matondkar et al. (1981) observed that viable heterotrophic counts were ranging from 4.8×10^4 to 1.5×10^6 in the soil from mangrove swamps of Goa situated along Mandovi-Zuari estuary. Park A. Rublee (1981) reported bacterial count ranging from 0.8 to 2.2×10^6 cells/g dry wt. of soil in the salt marsh sediment in North Carolina. Wood (1953) observed that bacterial population per gram of mud in the continental shelf of Port Hucking varied from 1×10^5 to 3×10^6 and that per ml estuarine mud in the oyster-growing region of Botany Bay ranged from 8×10^4 to 2.5×10^7. Zo Bell (1959) observed that in recently deposited marine sediments, the bacterial population varied from 10^5 to 10^8/g of wet material. Similar pattern was observed by Chandrika (1983) in the distribution of total heterotrophs in Cochin backwater sediments; the population density was found to be varying according to the site of sampling and seasons, and ranged between 30 - 300 colonies in 10^6 dilution. Slight variation in the range of bacterial counts recorded in various observations may be due to the existence of varied environmental conditions at different places and different methodology followed by various authors.

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 Palk Bay and Gulf of

Mannar, Cviic (1955) in the Adriatic Sea and Kriss (1961) in the Black Sea. 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. Hakim et al. (1981) studied the heterotrophic bacterial population in intertidal sediments of the Karnafuli estuary and reported that bacterial counts per gram of dry sediment varied from 2.103×10^6 to 6.612×10^6 and 2.016×10^6 to 4.420×10^6 in the nutrient agar medium and Zo Bell medium - 2216 respectively.

Six genera of heterotrophic bacteria were identified from a total of 108 strains, based on various morphological and biochemical characteristics. They are:

Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Pseudomonas and Micrococcus.

Morphological and biochemical characteristics of bacteria isolated from this mangrove environment expressed close similarity with that of marine bacteria. More than 90% of the isolates were Gram-negative, motile forms (Table 4). About 80% of the marine bacterial species catalogued by Zo Bell and Upham (1944) are Gram-negative rods. Zo Bell (1946) opined that more than 95% of the bacteria occurring in the sea are Gram-negative rods. Zo Bell (1946) also observed that majority of the bacteria found in the sea are actively motile.

Pigmented bacteria were found to range from 36.1 % to 41.7 % among the three stations (Table 4). Relatively high percentage occurrence of pigmented bacteria has been reported from marine environment also. An examination by Zo Bell and Feltham (1934) of several thousand colonies developing on nutrient agar inoculated with sea water or marine mud showed that 69.4 per cent of them produced pigments. High proteolytic activity, poor saccharolytic activity and weak indole production capacity of the bacteria isolated from the mangrove area (Table 4) agrees with the observations by Zo Bell and Upham (1944) on the biochemical and physiological properties of marine bacteria. The weak fermentative power of marine bacteria has been mentioned by Coupin (1915). Bacterial isolates from the mangrove ecosystem are also found to be poor fermentators (Table 4).

The relative abundance of various taxonomic groups of bacteria identified has shown in Fig. 6. Alcaligenes was the dominant group isolated during both pre-monsoon and monsoon periods in Stations A and C while in Station B during pre-monsoon, Pseudomonas was found to be dominating. The percentage frequency of occurrence of the genera like Pseudomonas, Flavobacterium, Cytophaga and Vibrio are found to be subjected to seasonal and spatial fluctuations. Generally, Alcaligenes was found to be the predominant group in this ecosystem (39.6%) followed by Pseudomonas (22.2%), Flavobacterium (11.8%),

Cytophaga (11.1 %), Vibrio (11.1 %) and Micrococcus (4.2 %).

Matondkar et al. (1980) reported the presence of microbial groups like Corynebacterium, Bacillus, Planococcus, Staphylococcus, Streptococcus, Micrococcus, Pseudomonas etc. from the mangrove swamps of Goa, situated along the Mandovi-Zuari estuary. The presence of similar groups of organisms belonging to Bacillus, Corynebacterium, Micrococcus and Pseudomonas has been reported from mangrove swamps of Thailand (Daengshuba, 1979). Boeye et al. (1975) while studying the density and composition of heterotrophic bacterial population in North Sea sediment observed that the genera like Bacillus, Flavobacterium, Achromobacter, Micrococcus, Brevibacterium etc., were present in that ecosystem. Murchelano and Brown (1970) studied the heterotrophic bacteria in the water column of Long Island Sound and found that Pseudomonas was the predominant genus followed by Achromobacter, Vibrio, Cytophaga, Micrococcus and Bacillus. Watanabe (1980) while studying the distribution of aerobic heterotrophic bacteria in Cananeia estuary, Brazil, reported that Pseudomonas, Vibrio and Corynebacterium were more frequently recovered from non-sea water media, whereas Flavobacterium, Myxobacteriales, Hyphomicrobiales and Actinomycetales composed the major part of isolates from the sea water media. The heterotrophic bacteria isolated from Tolo Harbour was mainly represented by marine

genera like Pseudomonas, Aeromonas, Flavobacterium, Beneckea, Achromobacter, Vibrio and Clostridium (from mud flora) (Kwong-Yu Chan and C.S.W. Kueh, 1976).

Observations during present study differ in certain aspects with the previous observations by various authors. The results obtained indicates the non-occurrence of genera like Bacillus and Corynebacterium in this ecosystem, which were recorded to be present in appreciable quantities in mangrove swamps of Goa (Matondkar et al., 1981). This may be due to the ecological variations of the two study areas. Also, the genus Micrococcus was found to be present in very low quantities and could not even isolated from Station A during monsoon and from Station C during pre-monsoon period.

Generic diversity observed during the study period is more likely caused by varying environmental conditions. Still, as this was a short term study, it is difficult to correlate bacterial generic diversity with ecological variables. However, this may be possible on the availability of sufficient data by a long term observation.

The distribution of physiologically active isolates such as nitrate reducers, starch hydrolysers, gelatin liquifiers and hydrogen sulphide producers was studied and the results are summarised in Table 6. The data clearly indicate the occurrence of various physiologically active bacteria in appreciable quantities in this particular ecosystem. Their

percentage abundance of occurrence did not show any remarkable variation either spatially or seasonally. However, the occurrence of nitrate reducers and gelatin liquifiers are found to be more in Stations A and B when compared to that of Station C. Starch hydrolysers were recorded to be present low in Station A, but was found to be high in Station C. Relatively higher abundance of hydrogen sulphide producers were recorded in Station A followed by Station C and Station B.

Seasonal variation of the occurrence of physiologically active isolates were not prominent over the period of study in all the stations. Nitrate reducers were found to be increasing in their abundance during monsoon in Stations A, B and C. Similar increase was observed in the case of starch hydrolysers in Stations A and C, but, in Station B during monsoon amylolytic activity was found to be decreasing. A decrease in the abundance of gelatin liquifiers was noted during monsoon in all the three stations. Hydrogen sulphide producers were also found to be decreasing during monsoon in Stations A and B but the activity was recorded high in Station C.

Matondkar et al. (1981) reported the presence of similar physiologically active groups from mangrove swamps of Goa. Chandrika (1983) reported the enzymatic potential of the three dominated genera Alkaligenes, Vibrio and Pseudomonas isolated from Cochin backwaters and found that although there was

seasonal percentage variation in the genera isolated the enzymatic potential remained essentially constant. Various physiologically active groups of bacteria such as nitrifying bacteria, nitrate reducers etc. , were reported by Gore (1974) from the inshore environment at Karwar and Vengurla.

Among the environmental parameters studied, rainfall ranged from 57.8 mm to 963.2 mm and was recorded during the months of March and June respectively (Fig. 2). Temperature showed a well marked seasonal variation with a decline during monsoon and ranged from 30.5°C to 23°C (Figs. 3, 4 & 5). pH did not vary much and ranged between 7.1 and 8.25 (Figs. 3, 4 & 5). Data obtained on Eh gave a clear indication about the prevailing reducing conditions of the soil. Eh remained negative throughout the study period and did not exhibit any considerable seasonal variations (Figs. 3, 4 & 5). A conspicuous seasonal variation could be noted for the electrical conductivity of soil which is directly related to salinity, and it was found to range from 97.732 millimhos to 2.138 millimhos (Figs. 3, 4 & 5). Organic carbon in soil remained at a steady state in all the three stations without any considerable seasonal variation (Figs. 3, 4 & 5). Though available nitrate could be detected only in traces from soil in the three stations, available phosphorus content was found to be considerably high (Figs. 3, 4 & 5).

Attempts have been made to establish any possible relationship between the above mentioned environmental para-

-meters and varying total heterotrophic bacterial count. Rainfall and bacterial count were found to be constituting an inverse relationship (r = ⁻0.6887, ⁻0.8597 and ⁻0.7680 for Stations A, B and C respectively). Considerable decline in bacterial count observed during monsoon may be due to a reduction in the availability of organic nutrients due to dilution. Statistical analysis revealed that, except available phosphorus content in soil, no other factor constitutes significant relationship with bacterial population in Stations A and B (Tables7 & 8). However in Station C, it was observed that apart from available phosphorus content, soil temperature, electrical conductivity of soil and available nitrate content in soil also have significant relationship with bacterial count (Table 9). These differences in observations in Station C may account for its difference in existing environmental conditions from the other two stations (Station C was a man-made prawn filtering pond in the mangrove ecosystem, whereas Stations A & B were natural mangrove areas with a thick vegetation). However, the Z test of homogenity conducted to test the homogenity of relationship between the physico-chemical parameters and bacterial count among the three Stations, revealed that Stations A, B and C are more or less homogenous.

Zo Bell (1946, 1967), Carlucci (1974) reported that one of the important factors governing the distribution of marine bacteria is the availability of nutrients particularly phosphates and nitrates. It is well established that mineralisation of organic phosphates and nitrates by marine bacteria operates at a higher rate (Gunnesson, 1963). Data obtained for available phosphorus and nitrate (Tables 1, 2 and 3) show that higher concentration of the above nutrients are present in Station A followed by Station B and Station C. As indicated earlier the same gradation is noted for bacterial population also. Higher counts of heterotrophic bacteria in Stations A and B than in Station C may attribute to the higher concentrations of phosphate and nitrate in the respective stations. Similar result reported by Kwong-Yu Chem and C.S.W. Kuch (1976) in Tolo Harbour supports the present observation. Chandrika (1983) reported that in addition to the significant correlation between total heterotrophs with nitrite and phosphate somewhat high correlation between bacterial counts and temperature in the Cochin backwater.

Hakim et al. (1981) found that organic carbon, nitrogen content and salinity of the sediments are having much effect on the distribution of bacteria. Shanta Nair and Loka Bharathi (1980) while studying the distribution of bacterial flora of three sandy beaches of the west coast of India observed that there was no correlation between bacterial population and

chemical characteristics of the beaches such as pH, PO_4-P, NO_3-N and NH_4^+. They also noted that the heterotrophic distribution of tropical sandy beaches to some extent was independent of sand temperature.

Lack of correlation between heterotrophic bacterial population and many of the chemical characteristics observed during the present study may be due to the fact that the system may not be simply controlled by an equilibrium level of nutrients (Pugh et al., 1974). Moreover, though variation in heterotrophic bacterial counts was conspicuous during the study period, considerable variation did not occur in the case of many of the environmental parameters. This may be again one of the reasons for not able to establish a direct correlation between the bacterial count and many of the environmental parameters.

It is noteworthy that despite generic diversity, various physiological activities in this ecosystem remained more or less stable without considerable fluctuations. This gives an indication that bacterial degradation of environmental substrates may not depend upon the activities of particular genera of bacteria (Murchilano and Brown, 1970).

The present study may be helpful in giving a brief idea about the distribution of heterotrophic bacteria in this mangrove ecosystem. Better understanding of the bacterial ecology of this specialised ecosystem of tropics will be possible by a long term study.

.....

S U M M A R Y

1. Studies on heterotrophic bacteria in the mangrove ecosystem near Cochin were conducted for a period of six months (March-August 1985). Three Stations (Stations A, B and C) with different ecological conditions were selected in Murikinpadom area for this study and the distribution of heterotrophic bacteria was studied both quantitatively and qualitatively, along with physico-chemical characteristics. The results are given in the form of intensity charts and tables.

2. Samples from all the stations contained significant quantities of total heterotrophs. The distribution of heterotrophs did not show much variation among the three stations, but, seasonal variation of more or less similar pattern could be observed in all the three stations. The heterotrophic bacterial count was found to be ranging from 3.3×10^5 to 52.0×10^6 in the soil during the months of June and March respectively.

3. A total of 108 strains were isolated and six genera were identified from these isolates - Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Pseudomonas and Micrococcus.

4. Alcaligenes was found to be the predominant genus in this ecosystem. The relative abundance of different taxonomic groups are observed as Alcaligenes (39.6 %), Pseudomonas (22.2 %), Flavobacterium (11.8 %), Cytophaga (11.1 %), Vibrio (11.1 %) and Micrococcus (4.2 %).

5. Gram-negative rods constituted 95.8 % of the total isolates.

6. The percentage abundance of occurrence of various physiologically active isolates did not show any remarkable variation either spatially or seasonally.

7. All the isolates from mangrove soil showed poor saccharolytic activity as marine isolates are generally poor in saccharolytic activity.

8. The total heterotrophic count showed inverse relationship with rainfall.

9. Statistical analysis revealed that available phosphorus content in soil is the only factor having significant relationship with bacterial population in Stations A and B. However, in Station C, apart from available phosphorus content, soil temperature, electrical conductivity of soil and available nitrate in soil were

also found to constitute significant relationship with total heterotrophic bacterial count.

10. The relationship between the physico-chemical parameters and bacterial count revealed that Stations A, B and C are more or less homogenous.

.....

R E F E R E N C E S

*AHRENS, R., 1969. Okologische Unter suchungen an sternbildended Agrobacterium - Arten aus der ostsee. Kieler Meeresforch 23:127 - 136.

ALAGURAVI, S., 1984. Studies on sulphur bacteria in the prawn culture ecosystem. M.Sc. dissertation submitted to the University of Cochin.

AYYAKKANNU, K., and CHANDRAMOHAN, 1971. Occurrence and distribution of phosphate solubilising bacteria and phosphatase in marine sediments at Porto Novo. Mar. Biol., 2 (3): 201 - 206.

BALEUX, B., and M. TROUSSELLIER, 1982. Spatial distribution and sampling of heterotrophic bacteria in surface brackish lagoon sediments. J. Fr. Hydrol. vol. 13, no.2 (38): 125 - 139.

BENT, E.J., and R. GOULDER, 1981. Planktonic bacteria in the Himber estuary: Seasonal variation in population density and heterotrophic activity. Mar. Biol., 62 (1) 35 - 47.

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY, 1975. 8th Edition (Buchanan, R.E., and N.E. Gibbons, Eds.) Williams and Wilkin Co., Baltimore.

BLASCO, F., 1975. The mangroves of India. Institut Francais de Pondichery. Travaux de la section scientifique at Technique. No.14, 175 pp.

BOEYE, A., WAYEN BERGH, M., and M. AERTS., 1975. Density and composition of hetertrophic bacterial populations in North Sea sediments. Mar. Biol., 32: 263 - 270.

BROWN, A.D., 1964. Seasonal variation in heterotrophic bacterial population in waters of Sydney. Aust. J. Mar.Freshw. Res., 15 (1): 73 - 76.

CARLUCCI, A.P., 1974. Nutrients and microbial response to nutrients in sea water. p. 245 - 248, In: Colwell, R.R., and R.Y. Morita (eds), Effect of the Ocean Environment Baltimore, MD., U.S.A.

CHAN, K., and C.S.W. KUEH., 1976. Distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour. Int. J. Ecol. Environ. Sci., 2 (1): 47 - 57.

CHANDRIKA, V. 1983. Studies on the ecophysiology of some heterotrophic and indicator bacteria in the marine environments of Kerala. Ph.D. Thesis submitted to the University of Cochin.

CHANDRIKA, V., RAMACHANDRAN NAIR, P.V., and L.R. KHANDEKAR., 1985. Distribution of phototrophic thionic bacteria in the anaerobic and micro-aerophilic strata of mangrove ecosystem of Cochin. Abstract-EN. P - 3 - 26th Annual Conference of the Association of Microbiologists of India.

CVIIC, V., 1955. Distribution of bacteria in the waters of the mid - Adriatic Sea. Ren. M.V. "Hvar" Cruises. Split, Yugoslavia), Vol.4, Pl.1.

DAENGSHUBA, W.S., 1979. A priliminary study of aerobic heterotrophic bacteria in the mangrove swamp at Amphoe Khlung on Mangrove and Estuarine vegitation in South East Asia, Indonesia, 121 - 124.

DAVIS, J., and F. FREITUS, 1970. Physical and chemical methods of soil and water analysis. FAO Soil Bulletin No. 10. pp. 275.

*FENCHEL, T.M., 1970. Studies on the decomposition of organic detritus derived from the turtle grass Thalassia testudinum. Limnol. Oceanogr., 15: 14 - 20.

FENCHEL, T., and B.B. JORGENSEN, 1977. Detritus food chains of aquatic ecosystems: The role of bacteria. In: advances in Microbial Ecology. Volume - I (Alexander, M., ed.). Plenum Press, New York, pp. 1 - 58.

FUHRMAN, J.A., ZIMMERMAN, J.W., and F. AYAM., 1980. Bacterio-plankton in the coastal euphotic zone: Distribution, activity and possible relationship with phytoplankton. Mar.Biol., 60 (4): 201 - 209.

GORE, P.S., 1971. Some observations on the bacterial flora of the beach sand and beach sea water. Environ Hlth., 13 (2): 115 - 119.

GORE, P.S., 1972. Some bacteria isolated from marine plankton and mud. Fish. Technol., 9 (1): 48 - 63.

GORE, P.S., 1974. Bacteria in the inshore environment at Karwar and Vengurla. Fish. Technol., 11 (1): 34 - 42.

GORE, P.S., RAVEENDRAN, O., and R.V. UNNITHAN, 1979. Pollution in Cochin Back waters with reference to Indicator Bacteria. Indian J. mar. Sci., 8 (1): 43 - 46.

GORE, P.S., RAVEENDRAN, O., and R.V. UNNITHAN, 1979. Pollution status of Cochin beach. Indian J. mar. Sci., 8: 187 - 190

GORE, P.S., and S.Y.S. SINGBAL, 1973. Ecology and Production in two sandy beaches of Goa. Part A - Distribution of bacteria in Colva and Siridao in relation to certain environmental parameters. Fish. Technol 10 (1): 55 - 60.

*GUNNESSON, C.G., 1963. Mineralisation of organic matter in Santa Monica Bay, California. p. 641 - 653. In: Oppenheimer C.H. (ed.) Symposium on Marine Microbiology. Charles Thomas Publ., Springfield III, U.S.A.

HAKIM, M.A., KHAN, Z.U.M., and M.A. RAHMAN, 1981. A note on the heterotrophic bacterial population in intertidal sediments of the Karnafuli estuary. Mahasagar, Vol. 14, no.1, pp. 79 - 81.

HEALD, E.J., 1971. The production of organic detritus in a South Florida estuary. University of Miami Sea Grant Technical Bulletin, 6: 110 pp.

HEALD, E.J., and W.E. ODUM., 1969. The contributions of mangrove swamps to Florida fisheries. Proc. Gulf Caribb. Fish. Inst. 22 Ann. Sess : 130 - 135.

HOBBIE E. JOHN and P.J.I. WILLIAMS (eds.), 1984. Heterotrophic activity in the sea. Plenum Press, New York. 569 pp.

HUMNADKAR, V.S., and A.D. AGATE, 1985. Ecology of estuarine mangrove ecosystem with reference to autotrophic bacteria. Abstract - E.N O 7. 26th Annual Conference of the Association of Microbiologists of India.

ITURRIAGE, R. and H.G. HOPPE, 1977. Observations of heterotrophic activity on photoassimulated organis matter. Mar. Biol. 40: 101 - 108.

JOHNSTON, R., 1955. Biologically active compounds in the sea. J. mar. biol. Ass. U.K., 34: 185 - 195.

*KHAILOVE, K.A., and Z.Z. FINENKO., 1968. Interaction of detritus and high molecular weight components of dissolved organic matter of sea water. Okeanologiya, 8: 980 - 990.

*KHAILOVE, K.A., and A. YU GORBENKO, 1967. The external metabolic regulation in the system: periphyton micro organisions - dissolved organic matter of sea water. Dokl. (Proc.) Acad. Sci. USSR. 133: 1434 - 1437.

KHANNA, S.S. and D.V. YADAV, 1979. Practical manual for introductory courses in soils. Haryana Agricultural University, Hissar.

KRISS, 1961. Marine Microbiology: Deep Sea. Translated from Russion by J.M. Shewan.

*KUZNETSOV, S.I., 1959. Die Rolle dev Micro organismen in Stoffreislauf der seen, VEB, Berlin.

*KUZNETSOV, S.I., 1970. Microflora of lakes and its Geochemical activity, Nauka, Leningrad.

LUCAS, C.E., 1947. The ecological effects of external metabolites Biol. Rev., 22: 270 - 295.

MACKERETH, F.J.H., 1957. Water analysis for limnologists (revised). Fresh water Biological Association, Ambleside. West Morel and.

MATONDKAR, S.G.P., MAHTANI, S and S. MAVINKURVE, 1980a. Seasonal variations in the microflora from mangrove swamps of Goa. Indian. J. mar. Sci., 9: 119 - 120.

MATONDKAR, S.G.P., MAHTANI, S., and S. MAVINKURVE, 1981. Studies on mangrove swamps of Goa: 1. Heterotrophic bacterial flora from mangrove swamps. Mahasagar - Bulletin of the National Institute of Oceanography. 14 (4) : 325 - 327.

*MEYERREIL, L.-A., 1973. Untersuchungen uber die salzanspruche von Ostseebakterien. Bot. Mar. 16: 65 - 76

MEYERREIL, L.-A., DAWSON, R., LIEBEZEIT, G., and H. TIEDGE, 1978. Fluctuations and interactions of bacterial activity in sandy beach sediments and overlying waters. Mar. Biol. 48: 161 - 171.

MAYERREIL, L.-A., and A. FAUBEL, 1980, Uptake of organic matter by meiofauna organisms and interrelationships with bacteria, Mar. Ecol. 3: 251 - 256.

MORRISON, S.J., KONG, J.D., BORRIE, R.J., BECHTOLD, R.E., and D.C. WHITE., 1977. Evidence for microfloral succession on allochthonous plant litter in Apalachicola Bay, Florida, U.S.A. Marine Biology 41: 229 - 240.

MURALIDHARAN, C.M., 1984. Colonisation of the mangrove Acanthus ilicifolius LINNAEUS in the sea accreted regions near Cochin. M.Sc. dessertation submitted to the University of Cochin.

MURCHELANO, R.A., and C.BROWN., 1970. Heterotrophic bacteria in long Island Sound. International Journal on Life in Oceans and Coastal Waters, Vol.7, No.1: 1 - 6.

ODUM, E.P., and A.A. DE LA CRUZ., 1967. Particulate organic detritus in a Georgia salt marsh estnarine ecosystem. In: Estuaries, G.H. Lauff Ed. American Association for the advancement of science. Publications, 83, Washington, 383 - 388.

ODUM, W.E., and E.J. HEALD., 1975. The detritus based food web of an estuarine mangrove community. In: Estuarine Research, edited by L.E. Cronin, Academic Press, New York, 1: 265 - 286.

OLSEN., 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. Circ. U.S. Dept. Agri., pp 19 - 39.

PARSONS, T.R., and J.D.H. STRICKLAND., 1961. On the Production of particulate organic carbon by the heterotrophic processes in seawater. Deep Sea Res., 8: 211 - 220.

PUGH, K.B., ANDREWS, A.R., GIBBS, C.F., DAVIS, S.J., and G.D. FLOODGATE., 1974. Some physical, Chemical and microbiological characteristics of two beaches of Anglesy. Journal of Experimental Marine Biology and Ecology, 15: 305 - 333

RAMADHAS, V., RAJENDRAN, A., and V.K. VENUGOPALAN, 1975. Studies on trace elements in Pichavaram mangroves. Proceedings of the International symposium on Biology and Management of Mangroves. Walsh, Snedakar and Teas (Eds.), Vol. 1: 96 - 114.

RAVEENDRAN, O., GORE, P.S. and R.V. UNNITHAN., 1978. Observations on faceal contamination of Cherai beach in Kerala. Indian J. mar. Sci. 7: 128.

*RHEINHEIMER, G., 1968. Beobachtungen Uber den Einflu B von Salzgehaltsschwankungen auf die Bakterien flora in der westlichen Ostsee, Sarsia 34: 253 - 262.

*RHEINHEIMER, G., 1971. Uber das Vorkomen Von Brackwasserbakterien in der Ostsee. Vie et Milieu. Suppl. 22: 281 - 291.

RHEINHEIMER, G., 1980. Aquatic Microbiology, 2nd Edition, London, New York: Wiley.

RHEINHEIMER, G., 1981. Investigations on the role of bacteria in the food web of the western Baltic. Kieler Meeresforsch. Sonderh. 5, 284 - 290.

*RIEPER, M., 1976. Investigations on the relationships between algal blooms and bacterial populations in the Schele Fjord (Western Baltic Sea). Helgol. Wiss. Meeresunters. 28: 1 - 18.

RITTENBERG, S.C., 1963. Marine bacteriology and the problem of mineralistation. In: C.Oppenheimer(Ed), Symposium on Marine Microbiology. C.C. Thomas, Springfield, Illinois. pp. 48 - 60.

RODINA, A.G., 1951. On the role of different groups of bacteria in the productivity of the water bodies. (Russ.) Trans. Prohl. Thematic Conf. Zool. Inst. Acad. Sci. USSR, 1: 21 - 36

RODINA, A.G., 1972. Methods in aquatic microbiology. University Park Press, Baltimore MD., USA pp. 461.

RUBLEE, P. 1978. Bacteria in a North Carolina Salt Marsh: Standing crop and importance in the decomposition of Spartina alterniflora. Ph.D. Thesis, North Carolina State University, Raleigh. 80 pp.

RUBLEE, P.A., 1982. Seasonal distribution of bacteria in saltmarsh sediments in North Carolina. Estuar, Coastal. & Shelf Science 15 (1): 67 - 74.

SANTHAKUMARI, R. 1966. Some bacterial characteristics of the Cochin Backwater. Curr. Sci., 35: 310.

SCHRODER, H.G.J., and F.B. VAN Es., 1980. Distribution of bacteria in intertidal sediments of the Enis Dollard estuary. Neth. J. Sea Res., 14(2): 268 - 287.

*SEKI, H., 1965 b. Studies on microbial participation to food cycle in the sea. II. J. Oceanogr. Soc. Japan, 20:24.

SHANTA NAIR, et. al., 1978. Distribution of heterotrophic bacteria in marine sediments. Indian J. Mar. Sci. 7: 18 - 22.

SHANTA NAIR and P.A. LOKANARAMI., 1980. Heterotrophic bacterial population in tropical sandy beaches. Mahasagar - Bulletin of the National Institute of of Oceanography, 13 (3): 261 - 267.

SIBERT, J. and T.J. BROWN., 1975. Characteristics and potential significance of heterotrophic activity in a polluted fjord estuary. J. exp. mar. Biol. Ecol 19: 97 - 104.

SIMIDU, U., and K. AISO, 1962. Occurrence and distribution of heterotrophic bacteria in seawater from the Kamogawa Bay. Bull. Jap. Soc. Sci. Fish. 28: 1133 - 1141.

SIMIDU, U., TAGA, N., COLWELL, R.R. and F.R. SCHARARY., 1980 Heterotrophic bacterial flora of the seawater from the Nanseishoto (Rynkyu Rethi) area. Bull. Jap. Soc. Sci. Fish., 46(4) 505 - 511.

SIMIDU, U. TSUKAMOTO, K., and Y. AKAGI., 1982. Heterotrophic bacterial population in Bengal Bay and the South China Sea. Bull. Jap. Soc. Sci. Fish., 48 (3) : 425 - 431.

SNEDECOR, G.W., and W.C. COCHRAN., 1967. Statistical methods 6th Ed. Iowa State University Press, Iowa.

*SOROKIN, Yu.I., 1971 b. Bacterial populations as components of oceanic ecosystems. Mar. Biol., 11, 101 - 105.

*SOROKIN, Yu.I., 1972d. Microbial activity as a biochemical factor in the ocean. In: D. Dryssen and D. Jagner(Eds.), The Changing Chemistry of the Oceans. Almquist, Stockholm. pp. 189 - 204.

SOROKIN, Yu.I., 1978. Decomposition of organic matter and nutrient regeneration. In: Otto Kinne(Ed.), Marine Ecology. Vol. VI - Dynamics. John Wiley & Sons New York. pp. 501 - 616.

STRICKLAND, J.D.H., 1971. Microbial activity in aquatic environments. Symp. Soc. gen. Microbiol., 21: 231 - 253.

TEAL, J.M., 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology, 43: 614 - 624.

TEZUKA, Y., 1966. A commensalism between the sulphate - reducing bacterium Desulphovibrio desulphuricans and other heterotrophic bacteria. Bot. Mag. Tokyo 79: 174 - 178.

UNTAWALE, A.G., BALASUBRAMANIAN, T., and M.V.M. WAFAR., 1977. Structure and production in a detritus rich estuarine mangrove swamp. Mahasagar - Bulletin of the National Institute of Oceanography. 10(3 & 4): 173 - 177.

UNTAWALE, A.G., DWIVEDI, S.N., and S.Y.S. SINGBAL., 1973. Ecology of mangrove in Mandovi and Zuari estuaries and the interconnecting Cumbarjua Canal of Goa. Indian J. mar. Sci., 2: 47 - 53.

VELANKAR, N.K., 1950. Bacteriological survey of seawater from the coast of Madras City (Bay of Bengal). Proc. Indian Acad. Sci., 32: 30

VELANKAR, N.K., 1955. Bacteria in the inshore environment at Mandapam. Indian J. Fish. 2: 96 - 112.

VELANKAR, N.K., 1957. Inhibition of bacteria from marine sources by aureomycin. Proc. 44th India. Sci. Cong., Part III, Abst., 321.

VENKATARAMAN, R., and A. SREENIVASAN., 1954 Bacteriology of inshore seawater and of mackerels off Tellichery (Malabar). Proc. Indian Acad. Sci. 20(B): 651 - 656.

VENKATARAMAN, R., and A. SREENIVASAN., 1954. Bacteriology of offshore water of the west coast. Proc. Ind. Acad Sci., 40 B : 161 - 165.

VENKATESAN, V., and V.D. RAMAMURTHY., 1971. Marine microbiological studies of mangrove swamps of Killai backwaters. Journal of the Oceanographic Society of Japan. 27: 51 - 55.

WALKLEY, A., and I.A.BLACK., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29 - 38.

WATANABE, K., 1980. Distribution of aerobic heterotrophic bacteria in Cananeia estuary, Brazil. Acta Vet (Belgr) 30 (5/6): 253 - 276.

WOOD, E.J.F., 1953. Heterotrophic bacteria in the marine environment of eastern Australia. Austr. J. Mar. Freshwater Res. 4: 160 - 200.

WOOD, E.J.F., 1959 a. Some aspects of marine microbiology. Indian J. Mar. Biol., 1: 26 - 32.

WOOD, E.J.F., 1967. Microbiology of Oceans and Estuaries. Amsterdam: Elsevier Publishing Co., pp. 319.

*ZOBELL, C.E., 1939. Occurence and activity of bacteria in marine sediments. (Recent Marine Sediments), Amer. Assoc. Petrol. Geol. Tulsa, Okla., pp. 416 - 427.

ZOBELL, C.E., 1946. Marine Microbilogy: a Monograph on Hydrobacteriology, Chronica Botanica, Waltham, Mass.

ZOBELL, C.E., 1963. Domain of the marine microbiologist. In: Symposium on Marine Microbiology ed. Oppenheirmer, C.H. Springfield: Charles C. Thomas.

ZOBELL, C.E., 1967. Bacteriology of the Sea. In: Fundamental Principles of Bacteriology by A.J. Salle. McGraw - Hill Book Company, New York.

*ZOBELL, C.E., and FELTHAM, CATHERINE, B., 1934. Preliminary studies on the distribution and characteristics of marine bacteria. Bull. Scripps Inst. Oceanogr., Tech. Ser., 3: 279 - 296.

*ZOBELL, C.E., and S.C. RITTENBERG., 1948. Sulphate reducing bacteria in marine sediments. J. mar. Res. (Sears Found Marine Res.), 7: 602 - 617.

ZOBELL, C.E., and H.C. UPHAM, 1944. A list of marine bacteria including description of sixty new species. Bull. Scripps Inst. Oceanogr., 5: 239 - 292.

---

* Not refered in original.