BIODEGRADATION OF PHENOLIC COMPOUNDS IN DIFFERENT ECOSYSTEMS IN COCHIN

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Dedicated to

My Teachers

DECLARATION

I hereby declare that this thesis entitled **"BIODEGRADATION OF PHENOLIC COMPOUNDS IN DIFFERENT ECOSYSTEMS IN COCHIN"** is a record of original and bonafide research carried out by me under the supervision and guidance of **Dr. V. Chandrika**, Senior Scientist, Central Marine Fisheries Research Institute (CMFRI), Cochin and that no part there of has been presented before for any other degree in any University.

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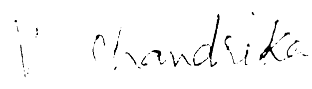
CERTIFICATE

This is to certify that this thesis entitled **"BIODEGRADATION OF PHENOLIC COMPOUNDS IN DIFFERENT ECOSYSTEMS IN COCHIN"** embodies the research of original work conducted by **IMELDA JOSEPH** under my supervision and guidance. I further certify that no part of this thesis has previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.



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PREFACE

The processes that affect the fate of an organic chemical in the environment include chemical hydrolysis, photolysis, sorption and biodegradation. Biodegradation is probably the most difficult process to study quantitatively since it is carried out by living organisms in a dynamic environment. With their catabolic versatility, bacteria, fungi and actinomycetes play major roles in the ultimate degradation of pollutants that enter the environment. The degradative activity of bacteria and fungi is based on their ability to catalyse the initial steps in degradation with variety of enzymes to produce metabolites that can enter existing metabolic pathways.

Cycling of carbon in nature requires the existence of organisms, predominantly micro-organisms, that can degrade molecules produced by biosynthesis. Micro-organisms reproduce very rapidly and have a high rate of mutation that allows them to evolve enzyme systems able to catalyze the metabolism of a great variety of organic structures.

Most aerobic bacteria that utilize aromatic compounds as respiratory substrates attack them through one or another of the two convergent branches of ß-ketoadipate pathway. Through these reactions, the six carbon atoms of the aromatic nucleus in the primary substrate the converted to the six carbon atoms of an aliphatic acid, ß-keto adipic acid. This is in turn cleaved to acetyl-s-CoA and succinic acid, both of which can immediately enter the Tricarboxylic acid cycle (TCA).

Information on the mechanism of degradation of organic chemicals by marine isolates is very little. The studies conducted at the United States Environmental Protection Agency (US EPA) Laboratory, Gulf Breeze, Florida, suggest that degradative process in estuarine and marine environments may differ significantly from those in terrestrial and fresh water environments.

Major advances have been made in the last few years in the methodology for the study of detrital based food webs. Phenols are formed as intermediates in the microbial degradation of organic matter derived from plant and animal residues and humus. These compounds can influence the rates of consumption and decay of organic matter and thus they alter rates of regeneration and transformations of nitrogen. The grazing and binding of proteins by phenolics may favour retention of nitrogen within an ecosystem, in live and dead plant biomass. The presence of phenolics may also reduce losses of dissolved inorganic nitrogen. Inhibition of nitrification may lower losses of inorganic nitrogen from an ecosystem, since ammonium is less susceptible to being leached or transported away by water than nitrate. Further, ammonium is taken up preferentially to nitrate by marine producers. Inhibition of nitrification and preferential uptake could reduce export of nitrogen from an ecosystem. Phenols also have the ability of chelate heavy metals with quite different affinities. The role of phenolics could be of more importance in ecosystems dominated by vascular plants and higher algae, as is evident by occurrence of biologically more active compounds.

Detection of phenolics play an important role in knowing the cycling of nutrients in the environment. Nitrogenous compounds will form complexes or

polymers with phenols rendering the nitrogen containing substances less susceptible to digestion. Proteins complexed with polyphenols and amino acids linked with phenols or polyphenols are resistant to microbial attack by contrast with free proteins and amino acids, rendering a decrease in mineralization rate and velocity of nutrient release affecting nutrient cycles.

Sublethal concentrations of phenols are often responsible for imparting 'flesh tainting', an unpleasant taste to fish flesh even when present in very low concentrations. Flesh tainting is nearly as detrimental to the fisheries as a complete mortality.

The purpose of this study was to determine the ability of specifically adapted bacteria to degrade phenol and to quantify the rate of biodegradation at different concentrations by mixed as well as individual isolates. Regular quantitative analysis of phenolics and aerobic phenololytic heterotrophs from five different ecosystems were done during 1990-1991, and the ability of micro-organisms isolated from those areas, to utilize phenol, o-cresol and orcinol was also studied. In addition, data on environmental parameters like temperature, dissolved oxygen, salinity, pH, organic carbon and nutrients were also collected during the period of study.

Laboratory studies on biodegradation of pollutants are best conducted by using mixed cultures isolated from the field samples. The goal of such studies is to use degradation rates measured in the laboratory to predict degradation rates in the environment. A number of factors such as

temperature, salinity, pH, microbial biomass and prior exposure can affect the degradation rates and thus, the fate of a pollutant.

Multispecies tests support both qualitative (screening) and quantitative assessment of biodegradation of a pollutant. Screening biodegradation tests using microbial communities commonly follow the disappearance or metabolism of a chemical in samples of water, sediment, or sewage taken directly from the field and incubated experimentally. Insight into modes of biodegradation in natural environment that could not have been gained from pure culture studies is often obtained from multispecies tests. Knowledge of the ability of sediment associated bacteria to degrade chemicals faster than water associated bacteria has come from such studies (Lee and Ryan 1979). Similar enhancement of knowledge (relative to pure culture studies) about toxic effects of chemicals or micro-organisms and biogeochemical processes they mediate has also been obtained in mixed culture. The present study is one of its first kind in natural aquatic environment and has aimed to bring out some idea about the potential phenol biodegraders in such environments where the phenol concentration is beyond permitted level.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

The present study 'BIODEGRADATION OF PHENOLIC COMPOUNDS IN DIFFERENT ECOSYSTEMS IN COCHIN' is concentrated on estimation of phenolic compounds in different ecosystems during different seasons. Various micro-organisms like bacteria and actinomycetes active in biodegradation of phenol were also screened in all the seasons. Environmental parameters were estimated to know the effect of physico-chemical parameters on biodegradation of phenol. Laboratory experiments were conducted in order to determine the rate of biodegradation at different concentrations of phenol using mixed and individual strains of bacteria. The viability of aerobic heterotrophs in selected phenolic compounds were also tested at different concentrations.

Phenols are one of the major groups of secondary metabolites in plants. Phenolic acids in soil naturally may be formed during humic acid breakdown (Alexander 1961). Three possible sources of phenols reported are

i. Phenolic materials such as flavanoides leached from the plant debris.
ii. Phenolic compounds formed during lignin decomposition and
iii. Phenolic substances synthesized by soil micro-organisms which may have seen utilizing carbohydrates (Burges *et al* 1963; Burges 1967).

Whitehead (1964) identified various phenolic compounds from different types of soils. During the course of coconut husk retting, polyphenols from the

husks constantly get leached out into the ambient water. Polyphenols constitute about 75-76 g/kg of the husk material (Jayashankar 1966; Bhat 1969). Presence of phenolic compounds in mangroves of Goa were reported by Karanth *et al* (1975) and Gomes and Mavinkurve (1982). Different types of phenolic compounds were reported to be present in marine organisms (Higa 1981). Several types of phenolic compounds, especially bromophenol, are reported from brown seaweeds (Zandovi and Jensen 1981; Steinberg 1984). Phenolic compounds found in coastal marine environments may be naturally produced by marine organisms or enter the ocean from anthropogenic sources.

Apart from natural phenolics formed from plant degradations, synthetic phenols occur in environment from effluents of oil refineries, glasswool manufacturers, several chemical producing factories and from manufacturers of phenolic resins (Jorgensen 1971). Cresols are used in the manufacture of resins, plasticizers, dyes, explosives, lysol disinfectant and creosote. The waste water from all these industries contain cresols in addition to other pollutants.

Due to high volatility and water solubility, phenols impart taste and odour to the aquatic environment even at part/billion levels (Thomas 1973). A wide range of phenol concentrations (0.08 to 1800 mg/l) pose serious pollution problem, adversely affecting the organisms of food chain and fish population by interfering with carbohydrates, proteins and lipid metabolism, ions transport, nerve conduction and energy production at bimolecular levels due to uncoupling of oxidative phosphorylation in fishes (Desiah 1978; Gupta 1985a). A wide range of phenol concentrations has been reported to be harmful to fish (EIFAC 1972). Acute levels directly cause the fish mortality. The

available data for phenol indicate that toxicity to marine organisms occurs at concentrations as low as 5850 μg/l and would occur at lower concentrations among species that are more sensitive than those tested (US EPA 1980). Desired level for the protection of public health is 3.5 mg/l. For controlling undesirable taste and odour qualities of ambient water, the estimated level is 0.3 mg/l (US EPA 1980).

Micro-organisms possess a remarkable adaptive capacity and can develop resistance to any toxic organic compounds. The present study was aimed also to screen heterogenous microflora exhibiting degradative enzymes and active phenol degradation. During breakdown of phenol, large quantities of organic compounds are released causing high bacterial production. The bacteria are grazed by zooplankters mainly flagellates and ciliates. Thus dissolved organic compounds are brought back into food web.

For biodegradation to occur at all, the environment concerned must contain at least one population with the appropriate catabolic metabolisms. The rate of biodegradation depends on the initial interaction between the compound and the organism, the kinetic properties of the metabolic process (largely determined by the concentration of the degrading population and the compound concentration), and the physico-chemical conditions which can be important in determining the fate of a compound in the biosphere (Plimmer 1978).

An understanding of the individual pathways and enzymes involved in the biodegradation of many compounds is fundamentally important in

describing it in natural environment. Dagley (1967) reported cleavage methods for aromatic compounds. Evans (1963) described the salient features of microbial aromatic ring metabolism. It was reported that phenol is directly hydroxylated through hydroxylases by micro-organisms to catechol (Sleeper and Stainer 1950). Catechol is converted by oxygenases, hydrolases, dehydrogenases and aldolases to pyruvate and acetaldehyde or succinate and acetyl CoA (Kilby 1948; Ribbons and Chapman 1968; Fiest and Hegeman 1969).

The Pseudomonads are characterized by their ability to catabolize a great variety of unusual organic compounds by special metabolic pathways. Enzymes for a number of these special pathways are determined by plasmid genes. In each case a plasmid determines the enzyme of a single catabolic pathway or a portion thereof. It is also clear that a shift in metabolic pattern for a compound can occur as a result of relatively few mutational events, from meta to ortho pathway in *Pseudomonas putida* (Fiest and Hegeman 1969).

Although an organism possess all the necessary enzymes and pathways to degrade a particular compound, degradation may not occur because the compound is unable to enter the cell. Similarly, an organism may lack an appropriate enzyme or set of enzymes to convert the compound to intermediates of central metabolism. That is why all micro-organisms are not able to biodegrade organic compounds (Slater and Somerville 1979). Only some enzymes called constitutive enzymes present in an organism in physiologically significant amount under all conditions. Others are formed as and when needed only. The biodegradation of a compound depends on its ability to induce

the formation of concerned degradative enzymes and on the permeability of the compounds in the cell. In the mixed culture subjected to a new compound, only a few of different types of organism present, may have the genetic capacity to form the inducible enzyme necessary for the breakdown of the new compound. It would then be necessary to make the micro-organisms to adapt to these compounds to facilitate the induction of enzyme. Predominance of such acclimatized micro-organisms in a mixed culture will effectively remove the pollutant from the environment (Sastry 1986).

The isolation of a specific culture capable of degrading a particular compound is often done by the method of enrichment or selective culture technique, which has been successfully used to study the biodegradation of a wide variety of compounds. With this idea, the present study on biodegradation of phenolic compounds in different ecosystems around Cochin was undertaken. The ecosystems selected include a coconut husk retting area at Chittoor, a mangrove ecosystem, Mangalvana, a backwater system, at Thykoodam and perennial and seasonal aquaculture ponds, at Narakkal.

A planned programme of research for determination of phenolic compounds and various other physico-chemical parameters were conducted during March 1990 to November 1991. Laboratory experiments for determining biodegradation were also done during the period of research.

The thesis is presented in 6 Chapters, Chapter-1 **INTRODUCTION** to the study undertaken, a **REVIEW** of the status of biodegradation study of phenolic compounds in order to bring out the present level of knowledge in the

subject and the relative importance of such a study in brackish water environments with other systems like terrestrial and fresh water ecosystems which are well documented. Also a brief review on ecological aspects is included in this chapter.

Chapter 2 - is on **MATERIALS AND METHODS** used for sampling, estimation and data analysis of physico-chemical parameters, bacteriological observations and laboratory experiment technique followed for biodegradation studies. The chapter is presented in five sections. Section 2.1 gives the details of the study area, 2.2 - determination of environmental variables, 2.3 - viability of aerobic heterotrophs in phenolic compounds, 2.4 - biodegradation studies 2.5 - statistical analysis carried out based on the data collected during the period of study.

In Chapter 3, the results obtained are given in five sections. The observations of physico-chemical parameters and aerobic heterotrophs are given in Section 3.1. along with results of statistical analyses. Section 3.2 gives the results of viability study conducted with aerobic heterotrophs. The results of biodegradation studies using mixed cultures from different stations are presented in section 3.3. Section 3.4 is on the genera involved in biodegradation of phenol and those which can thrive in o-cresol and orcinol upto 500 ppm. The results of biodegradation studies of phenol using individual isolates of bacteria are given in Section 3.5 along with the results of statistical analysis.

In Chapter 4, a discussion and overall assessment of results obtained during the present study are given in two sub-heads. Section 4.1 discuss the results of environmental data and 4.2, those of biodegradation of phenol.

An executive summary of the results obtained during the present study is given in Chapter 5 and Chapter 6 follows it with a detailed list of literature cited on the subject of present investigation.

REVIEW OF LITERATURE

Ecological aspects

The general hydrography and biology of Cochin backwaters have been discussed by many workers (George 1958; George and Kartha 1963; Ramamritham and Jayaraman 1963; Sankaranarayanan and Quasim 1969; Murthy and Veerayya 1972; Pillai and Ravindran 1988). There are also numerous studies on coconut retting reported (Pandalai *et al* 1957; Jayashankar and Menon 1961; Jayashankar and Bhat 1964, Bhat *etal* 1973; Remani *et al* 1980; Ambikadevi 1988). Ecology of retting grounds was first studied by Abdul Aziz and Nair (1976, 1978 1986) at Edava-Nadayara Paravur backwaters in Kollam District. Studies on hydrology and sediment parameters in coconut retting areas of Cochin have done by Remani *et al* (1981) and Ambikadevi (1988). The microbiological aspects of coconut retting and the micro-organisms in the area were studied by Jayashankar and Menon (1961), Bhat (1966), Jayashankar (1966) and Bhat *et al* (1973).

The ecological and microbiological aspects of the mangrove ecosystem are studied by many workers. Chandrika *et al* (1989) studied distribution of phototrophic thionic bacteria in the anaerobic and microaerophilic strata of mangrove ecosystem of Cochin and found that thionic microbes have a profound influence on mangrove soil fertility. Certain aspects of the Indian mangroves were discussed by Krishnamurthy *et al* (1975). The suitability of mangroves for aquaculture was discussed by Sunderraj (1978) and Murthy and Jayaseelan (1986). Odum and Heald (1975) reported detritus based food-web of estuarine mangrove systems. Rajagopalan *et al* (1980), described the productivity of different mangrove ecosystems in Cochin area, Venkatesan and Ramamurthy (1971) have discussed on the microbiology of mangrove swamp of Killai backwaters. The microbiology of the mangroves in Thailand was studied by Daengshuba (1979). The seasonal variation in microflora from mangrove swamps of Goa was reported by Matondkar *et al* (1980a, 1981). The sediment phenolics in the mangrove swamps of Goa are reported by Karanth *et al* (1975) and Gomes and Mavinkurve (1982). Nair *et al* (1988) reported the physico-chemical parameters of seasonal aquaculture ponds in Cochin backwaters. The enumeration of aerobic heterotrophs in seasonal and perennial aquaculture ponds was done by Santhi Thirumani (1992).

Biodegradation Studies

The occurrence of micro-organisms in sewage or other decomposing matter containing phenols was first reported by Fowler *et al* (1991). Wagner (1914) and Thornton (1923) found out that phenols were not only non-lethal to certain micro-organisms, but also can be decomposed by certain genera of them

by utilizing it as a source of carbon and energy. Dagley (1967), reported that, though benzene nucleus is resistant to the attack of any corrosive reagents, it is opened and metabolized by micro-organisms under neutral conditions in normal temperature. Results of earlier studies indicate that micro-organisms are able to degrade ring compounds (Buddin 1914; Sen Gupta 1921; Tattersfield 1928; Gray and Thornton 1928; Zobell 1946, 1950). The occurrence and degradation of phenolic compounds in marine sediments by natural populations of marine bacteria have been recently reported by Boyd and Carlucci (1993).

Phenol is oxidized faster under aerobic conditions. The aerobic degradation of aromatic compounds by micro-organisms has been studied in detail by Evans (1963), Ornston and Stainer (1964), Dagley and Gibson (1965), Dalgey (1967), Fiest and Hegeman (1969), Dagley (1971) and Shivaraman and Parhad (1985). Aerobic bacteria utilize the tricarboxylic acid cycle (TCA) broadly for two purposes (i) for generating pyridine nucleotides, a process equivalent to harnessing the energy made available by oxidation, and (ii) for generating aspartate and glutamate from which proteins, nucleic acids and other constituents to the cells are ultimately synthesized.

Anaerobic biodegradability of phenols and cresols by methanogens have been reported by Healy and Young (1978, 1979), Boyd *et al* (1983) and Ehrlich *et al* (1982).

Oxidation of phenolics by micro-organisms depend on various physico-chemical factors. Several studies have been reported pertaining to the

utilization of phenols and cresols by mesophilic and thermophilic micro-organisms (Dagley 1971; Buswell and Twomey 1975; Buswell 1975; Antai and Crawford 1983).

Temperature play an important role in phenol biodegradation (Hamdy *et al* 1954; Itturiaga and Rheinheimer 1972; Ermolaev 1979). pH (Hamdy *et al* 1956; Jayashankar and Bhat 1966; Boto and Bunt 1981), oxygen concentration (Boto and Bunt 1981; Stringfellow 1984), inoculum size and source (Stolbonov 1971; Zoledziowska 1973; Ermolaev and Mironov 1975), chemical structure of the test compound (Czekalowski and Skarzynski 1948; Alexander and Lustigman 1966; Ermolaev 1979), concentration of test compound (Tabak *et al* 1981; Rubin *et al* 1982) and adaptation of the microbial population to degrade the test compound (Tabak *et al* 1964; Jones and Carrington 1972; Haller 1978; Spain *et al* 1980) also determine the rate of biodegradation of phenol.

The quantity of phenolics that can be utilized by micro-organisms is reported by Gray and Thronton (1928), Czekalowski and Skarzynski (1948), Bennet (1962), Wase and Hough (1966), Dagley (1967) and Visser *et al* (1977).

Eventhough organic materials stimulate the oxidation of phenols (Shimp and Pfaender 1984), inorganic salts may be necessary, at optimum levels (Ermolaev 1979; Shimp and Pfaender 1984).

Many observations have been reported on isolation of micro-organisms that can oxidise phenols. Gray and Thronton (1928) were the first to isolate

various types of bacteria from soil which were capable of utilizing phenol or cresol as the sole source of carbon. Phenol utilizing bacteria can be grown in mineral slats medium, as specified by Gray and Thornton (1928), Tabak *et al* (1964) and Aaronson (1970).

Micro-organisms of various families have been shown to utilize phenolic compounds as the sole source of carbon for growth in mineral salts medium. Amongst bacteria, members of the families Micrococcaceae, Mycobacteriaceae, Pseudomonidaceae, Spirillaceae, Bacteriaceae and Bacillaceae (Fuhs 1961; Dagley 1967; Ermolaev and Mironov 1975; Parhad *et al* 1981, Sastry 1986) are reported to be as phenololytic organisms. These organisms are widely distributed in the sediment and water, and are useful in metabolizing phenolic compounds harmful to the aquatic life.

Stainer (1947, 1948) described the technique of simultaneous adaptation for determining metabolic pathways in the bio-oxidation of aromatic compounds and also showed how fluorescent Pseudomonads attack many compounds. Subsequent studies on the mechanism, optimal conditions for degradation and the intermediate products of the metabolism of aromatic compounds by micro-organisms are reviewed by Happold (1950) and later further reports on the aspect were of Cain *et al* (1961), Evans (1963); Ribbons (1966); Fiest and Hegeman (1969); Dutton and Evans (1969) and Hughes and Bayley (1983).

The bacterial strains most commonly used for biochemical studies of phenolic metabolism are *Pseudomonas.* Stainer *et al* (1966), Durham (1956),

Marr and Stone (1961), Dagley and Gibson (1965), Bayley and Wigmore (1973) and Andreoni and Besetti (1986) reported the utilization of phenolic compounds by different species of *Pseudomonas*.

Breakdown and utilization of phenol and related compounds by different species of *Archromobactor* has been reported by Czsekalowski and Skarzynski (1948), Dagley *et al.* (1965), Jones and Carrington (1972) and Kramer and Doetch (1950) *Alcaligenes* sp. was reported to metabolize phenol and cresol via catechol meta-cleavage pathway (Hughes and Bayley, 1983). *Mycobacterium* was reported to utilize phenolic compounds by Marr and Stone (1961), Buswell and Twomey (1975) and Buswell (1975), Crawford (1975) and Parhad *et al.* (1981) reported the growth of *Bacillus* sp. on phenol and the isomeric cresols, and the ability of intact bacteria to oxidize a variety of aromatic compounds. *Micrococcus* sp. was reported to degrade phenolic compounds by Jayashankar and Bhat (1966), Kramer and Doetch (1950). *Vibrio* sp. was tested for phenol biodegradation of Krammer and Doetch (1950). Divanin *et al* (1977) determined the biochemical changes during phenol degradation by *Bacterium* sp.

The ability to degrade phenolic compounds is not confined to bacteria alone. Thus, these reactions also take place in certain soil and wood rotting fungi, and in species of *Aspergillus, Penicillium, Neurospora, Oospora, Nocardia, Haphomycetes, Trichosporon, Candida* etc. (Henderson and Farmer 1955; Fuhs 1961; Henderson 1961; Harris and Rickettes 1962; Shivaraman *et al* 1978; Sastry 1986). The biodegradation of phenol and related compounds by *Streptomyces* sp. were reported by Crawford and Olson (1978). Pometto *et al* (1981); Sutherland *et al* (1981) and Antai and Crawford (1983).

Biodegradation of chlorophenols and nitrophenols have also been reported (Boyd and Shelton 1983; Spain *et al* 1980).

In the degradation of recalcitrant substances such as phenols, lignin or cellulose, frequently there is likewise an interaction by various micro-organisms. After the development of special type micro organisms which possess enzyme systems for the decomposition of such substances, other microbes which can utilise the intermediate products follow. In that way the special type microbes create the pre-conditions for the development of this latter group of organisms, by whose activities an accumulation of harmful metabolic products is often avoided. A co-operation of this kind of synergistic actions by different organisms is known as 'metabiosis' and this phenomenon is widespread in nature and are highly influenced by physico-chemical factors of the environment.

CHAPTER 2

MATERIALS AND METHODS

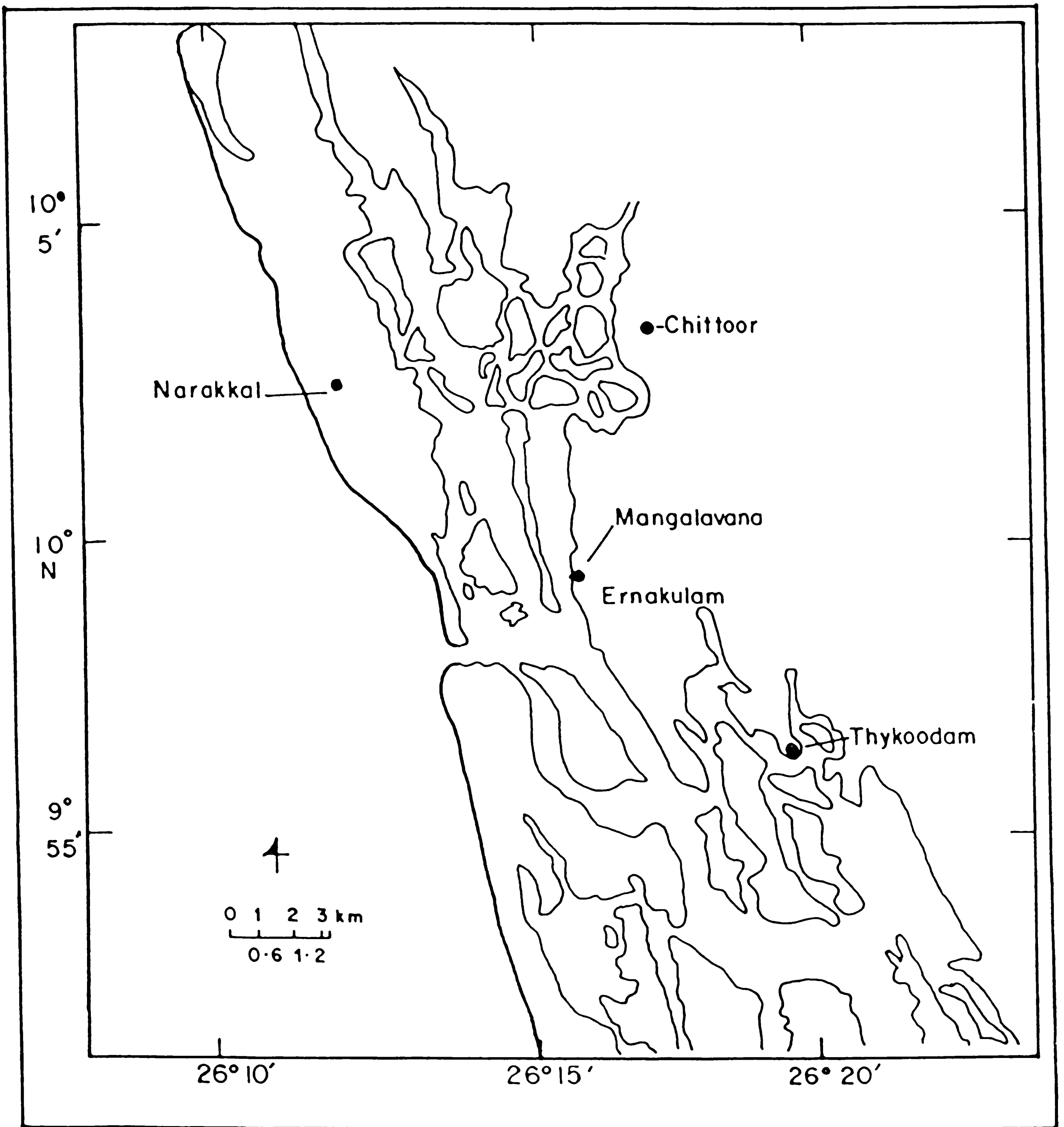
2.1 Study area

The Cochin back water system is located along 9°58'N latitude and 76° 15'E longitude. The present investigation was carried out in and around Cochin at five different aquatic ecosystems located between 10°03'N latitude and 76° 14'E longitude (Fig.1). The five stations were selected in order to study the extent of pollution caused by phenolics in these areas in relation to the environmental parameters and total aerobic heterotrophs and rate of biodegradation of phenolic compounds.

Station I, a coconut husk retting area located at Chittoor, is inlet of the main back water stream. In this area, which covers about 6 acres, nearly 100 retting pits, each about 8 x 5 x 1.8 m in size are operated. About 10,000 to 15,000 husks are dumped in each pit and kept immersed in each pit for a period of 6 to 12 months, depending on the quality of the husk.

Station II, Mangalavana, a patchy mangrove area, located near CMFRI is dominated by plants like *Avicennia* sp. and *Acanthus* sp. It is drawing many of its ecological characteristics from the back water and the terrestrial ecosystem. The surface soil of the swamp is alternately inundated, and drained by the tides. It supports a variety of aquatic fauna like crabs, amphibians, fishes, prawns etc. and their juveniles. The terrestrial fauna is

Fig.1 Map showing the sampling stations during March 1990 to November 1991



10°
5'
-Chittoor
Narakkal
Mangalavana
10°
N
Ernakulam
Thykoodam
9°
55'
0 1 2 3 km
0·6 1·2
26° 10'
26° 15'
26° 20'

dominated by the migratory birds, whose regular visit is pronounced in the ecosystem and is preserved as a bird sanctuary.

Station III is a flowing back water system at Thykoodam which is influenced by tides only. This station was selected as a comparatively non-polluted area.

Stations IV and V are aquaculture ponds, seasonal and perennial ponds at Narakkal. Seasonal ponds are used for paddy cultivation during monsoon months (June - October) and for prawn filtration during summer months (November - April). The seasonal ponds chosen were about 0.4 ha in area. The perennial ponds selected were 0.6 ha in area and located near the seasonal ponds with fish/prawn culture throughout the year. Both seasonal and perennial ponds selected are connected to the back water system by the same feeder canal.

From the above five stations, water and sediment samples were collected at monthly interval from March 1990 to November 1991, to estimate the physico-chemical parameters like temperature, salinity, dissolved oxygen, pH, nitrate-nitrogen, phosphate-phosphorous, silicate, organic carbon, organic matter, sediment phenolics and total aerobic heterotrophs. Biodegradability studies were also conducted with aerobic heterotrophs isolated from the five stations, in order to find out the quantity of phenol oxidized by environmental bacterial isolates. Samples were regularly taken during early hours of the day in all the five stations. The samples were transported to the laboratory as early

as possible, as shorter the time between collection and observation the more accurate will be the results.

2.2 Environmental variables

2.2.1 Hydrological Parameters

Temperature

Temperature of water was noted at the site of collection itself using a 0-50°C high precision thermometer.

Salinity

Salinity was estimated by the classical Mohr titration (Strickland and Parsons 1968).

Procedure

10 ml water samples were titrated against silver nitrate solution with potassium chromate as indicator. Silver nitrate was standardised for every set of titrations using standard sea water supplied by the Oceanography Institute, Copenhagen. The mean tire value of the samples was taken. From the mean value, salinity was calculated using the formula.

$$\text{Salinity } (S_2) \text{ (o/oo)} = \frac{V_1 S_1}{V_2}$$

where, V_1 = Volume of silver nitrate used for 10 ml standard seawater.

V_2 = Volume of silver nitrate used for 10 ml water samples

S_1 = Salinity of standard sea water.

Dissolved oxygen

Dissolved oxygen samples were collected using 125 ml 'corning' bottles with BOD stopper. Traditional winkler method with azide modification was used for determination.

Procedure

To the bottle in which water samples filled without air bubbles, 1 ml manganous sulphate (Winkler -A) and 1 ml alkali-iodine - azide solution (Winkler - B) were added at the sampling site itself. The bottle is stoppered and brought to the laboratory. The precipitated formed inside the bottle was dissolved using 2 ml concentrated sulphuric acid.

From this 10 ml samples poured into a 250 ml conical flask and titrated with standard sodium thiosulphate (6.3 g/l) solution to a pale straw colour. Few drops of starch indicator solution was added to this and titrated until the blue colour disappears. The amount of dissolved oxygen is calculated as follows.

$$\text{Dissolved oxygen} = \frac{(T).\ (N).\ (8000)}{S}$$

where,

T = Volume in ml thiosulphate used

N = Normality of thiosulphate

S = Volume of sample in ml

Hydrogen ion concentration (pH)

A battery operated 'Toshniwal' pH meter having a combined electrode was used for determination of hydrogen ion concentration with accuracy.

Water samples collected in 125 ml plastic bottles were used for the determination of pH. The instrument was calibrated with the help of pH buffers (4.2 and 9.1). After taking the pH meter reading, the *in situ* pH was calculated using for the formula (FAO 1975).

pH *in situ* = pH measured + 0.0118 $(t_2 - t_1)$

where,

t_1 = temperature *in situ*

t_2 = measured temperature

Nutrients

The samples were collected in 150 ml plastic bottles and analysis were done within 3 hours of collection.

Nitrate - nitrogen

Nitrate - nitrogen of water samples were determined by method as described by Parsons *et al* (1984).

Procedure

25 ml samples was measured out and 1 ml buffer reagent (phenol + NaOH) is added and mixed well. With rapid mixing 0.5 ml reducing agent (Cu SO_4 $5H_2O$ + Hydrazine Sulphate) was added. The samples were kept in a dark place for 20 hrs. Then 1 ml acetone, and after 2 minutes 0.5 ml sulphanilamide were added. After 2 minutes 0.5 ml NNED solution was also added and mixed. Compared the colour with standard potassium nitrate solution treated similarly, at 545 nm in an Erma colorimeter. The value is expressed in ppm.

Calculations

$$\text{Concentration of sample } (\mu\text{gatom/ml}) = \frac{\text{Concentration of standard x OD of sample}}{\text{OD of standard}} \times \frac{1000}{\text{ml sample}}$$

$$\mu\text{gatom/l} \times \frac{14.006}{1000} = \text{concentration in ppm.}$$

Phosphate - phosphorous

Method of Murphy and Riley (1962) was followed for the estimation.

Procedure

To 50 ml sample, 5 ml mixed reagent (ammonium molybdate + concentrated H_2SO_4 + potassium antimony tartarate) was added and after 5 minutes, the extinction of the solution was measured at 885 nm against

distilled water blank. With potassium hydrogen phosphate, standard phosphate readings were taken. The concentrations are expressed in ppm.

Calculations

$$\text{Concentration of sample } (\mu\text{gatom/ml}) = \frac{\text{Concentration of standard x OD of sample}}{\text{OD of standard}} \times \frac{1000}{\text{ml sample}}$$

$$\mu\text{gatom/l} \times \frac{30.97}{1000} = \text{concentration in ppm}$$

Silicate - silicon

Mullin and Riley method (1955) was followed for silicate determination of water sample.

To 25 ml sample 10 ml ammonium molybdate was added and mixed thoroughly. After 10 minutes, 15 ml reducing agent was added and mixed well. Allowed to stand for 2 to 4 hrs. Standard was also prepared using sodium silico fluoride and absorbance was measured at 810 nm against distilled water blank. The concentration is expressed in ppm.

Calculations

$$\text{Concentration of sample } (\mu\text{gatom/ml}) = \frac{\text{Concentration of standard x OD of sample}}{\text{OD of standard}} \times \frac{1000}{\text{ml sample}}$$

$$\mu\text{gatom/l} \times \frac{28.09}{1000} = \text{concentration in ppm}$$

2.2.2 Sediment parameters

Temperature

Sediment temperature was noted at the site of sampling using 0.50°C mercury thermometer.

Hydrogen ion concentration (pH)

pH was measured for wet soil by immersing the electrode to it. Calculations was done as for determination of water pH.

2.2.3 Phenolics

Sediment phenolics was determined by the 4-amino antipyrine method as described in APHA (1975). Phenol itself had been selected as a standard for colorimetric procedures and any colour produced by the reaction of other phenolic compounds were reported as phenol. The samples were analysed within 24 hrs after collection.

Procedure

Distillation of sediment sample

About 500 gm of sediment was taken in a 1 distillation flask and 500 ml distilled water was added. About 500 ml of distillate was collected and analysed for total phenol content.

Direct photometric method

100 ml of distillate was taken in a 250 ml beaker and 2 ml ammonium chloride (5%) was added. The pH was adjusted with 1 N NaOH to 10.0 $\pm$ 0.2. Then 0.2 ml 4-aminoantipyrine (2%) was added, mixed and 2 ml potassium ferricyanide (2%) added. A 100 ml distilled water blank and a series of 100 ml phenol standards containing 0.1, 0.2, 0.3, 0.4 and 0.5 mg phenol were also treated in the similar way as sample. After 15 minutes, the absorbance of the sample and standards against the blank at 510 nm were read. The concentration of phenol was calculated as follows

$$\text{Concentration of phenol (mg/l)} \quad \frac{CD}{E} \times \frac{1000}{B}$$

Where,

C = mg standard phenol solution

D = absorbance of sample

E = absorbance of standard phenol solution

B = ml original sample

Organic carbon

This was estimated by chromic acid method (FAO 1964; Khanna and Yadav 1979).

To 1 g soil sample, 10 ml potassium dichromate and 20 ml concentrated H_2SO_4 were added and mixed well. Kept for 30 minutes and diluted to 200 ml. One spoonful sodium fluoride and diphenylamine were added. Presence of blue

colour indicates organic carbon content in the sample. This was titrated against N/2 ferric ammonium sulphate to a brilliant green colour. A blank without soil should be run simultaneously.

Calculation

$$\text{Percentage organic carbon} = (S-T) \times \frac{.003}{2} \times \frac{100}{\text{weight of soil}}$$

where,

S = volume of N/2 ferric ammonium sulphate used for blank

T = volume of ferric ammonium sulphate used for sample.

Organic matter

The percentage organic carbon was multiplied by a factor 1.172 and expressed in percentage for organic matter.

2.2.4 Aerobic heterotrophic bacteria-enumeration, isolation and identification

Collection of sediment samples for bacteriological observations

Sediment samples were collected before 7.00 A.M. aseptically into sterile polythene covers from surface layer of the sediment, as investigations have shown that the number of bacteria and diversity of constituent groups decrease rapidly, as sediment depth increases. The samples were kept at 4°C until the time of bacteriological investigations, 18 to 24 hrs later.

Individual microbiologists utilise different methods to estimate bacterial populations in a given sample. The methods used in marine microbiology are discussed in several standard publications (Rodina, 1972; Zo Bell, 1946.)

Culture methods

Zo Bells 2216 medium (Himedia Lab. Pvt. Ltd.) was used for the culture of heterotrophs for total plate count (TPC). Seawater agar was used for isolating individual bacterial colonies.

Media

Composition

Seawater agar

Peptone - 1%

Agar - 2%

Ferric phosphate - 1 pinch

Seawater - 100 ml

pH 7.2 15 lbs 30 mts

Sea water peptone

Peptone - 1

Potassium nitrate - 0.2%

Ph 7.2 15 lbs 30 mts

Sterilization of media and glassware

The culture media were sterilized at 15 lbs for 30 minutes in the autoclave. Glasswares were first cleaned with detergents and then with dilute

phenol solution/potassium dichromate. They were thoroughly washed with running tap water and finally rinsed in distilled water and left for drying. They are then sterilized by keeping in their respective cans in hot-air oven for 1 hr at 160°C. The inoculation needle and spatula were sterilised by flaming it to red hot.

Plating

Quantitative analysis

Sample dilution

99 ml aged sea water was taken in a 150 ml conical flask and sterilized. About 1 g sediment sample was asceptically transferred to it. The flask was shaken well for 5 mts. in a mechanical shaker to ensure the thorough mixing of sample with dilution. 1 ml from it was transferred to 9 ml sterile seawater blank in test-tubes. The dilution was continued for the required number of dilutions (upto 10^8). Depending on the anticipated bacterial numbers and the turbidity, samples were either concentrated or diluted while preparing serial dilutions.

Pour-plate technique

1ml sample from the required dilution was transferred into a petridish. The petri-dishes were labelled correctly indicating the sample code, medium used, the dilution and date. Duplicate plates were poured for each sample in each dilution for standardisation. About 15-20 ml medium (Zo Bells 2216) was poured into the petri-dishes at 40-45°C. The dishes were rotated clockwise and

anticlockwise direction for through mixing. After the medium is solidified the petri-dishes were incubated at room temperature in an inverted position. The number of bacterial colonies were counted on the 5th day. Plates showing total number of colonies between 30 and 300 were taken as standard plates. The weight of the sediment sample was determined after filtration and drying the sediment inoculated into 99 ml dilution. The calculations were done as follows.

$$\text{No. of bacteria/g sediment} = \frac{\text{No. of colonies/g x Reciprocal of dilution x 1}}{\text{Weight of sediment in g.}}$$

Counts in duplicate plates were averaged and reported as aerobic plate count/g.

Culture of micro-organisms and their maintenance

Two types of culture media used for the isolation and identification of heterotrophs were Sea Water Agar (SWA) and Sea Water Peptone (SWP) Agar slants were made with S.WA and sub-culturing was done with a loop. Cultural characteristics such as pigment production were more readily observed in slant culture. Simultaneously sub-culturing was done in SWP/broth medium also. The maintenance of pure culture was done by periodical sub-culturing of strains on agar slants.

Physiological and biochemical tests for identification of heterotrophs

Gram-staining

A drop of water was placed on a clean glass slide and a loopful of 24 hr. young bacterial culture was transferred to the water drop with inoculation

needle and emulsified. The drop was spread uniformly over the slide to form a thin rectangular smear and the slide was passed rapidly through the flame 3 times for fixing the smear.

Stains

Ammonium - oxalate crystal violet

Solution A

Crystal violet (90%) dye	-	2g
Ethyl alcohol	-	20 ml

Solution B

Ammonium oxalate	-	0.8 g
Distilled water	-	80 ml

Solution A and Solution B are mixed

Grams' modification of Lugol's iodine solution

Iodine	-	1 g
Potassium iodide	-	2 g
Distilled water	-	300 ml

Counter stain

Saffrannin '0' 2.5 solution in 95% ethyl

Alcohol	-	10 ml
Distilled water	-	10 ml

Procedure

1. The smeared slide placed on a stain rack and crystal violet was poured. It was kept for 2 minutes without drying.
2. The stain was drained and then washed gently with tap water.
3. Gram's iodine solution was poured after 1 minute, the excess solution drained off.
4. The smear was washed and blotted dry.
5. Decolourised for 30 seconds with gentle agitation in 95% ethyl alcohol, then washed with water and air dried.
6. The slide was examined under oil immersion objective and observations were recorded.

Results

Gram-positive bacteria - purple or violet

Gram-negative bacteria - pink or red

Motility

Hanging - drop technique

Cultures of 18 to 24 hrs. old strains grown in broth was taken. Clean, dry cover slip was taken and a little vaseline was smeared on the edges of it. A small loopful of culture was transferred into the centre of cover slip. A cavity slide was inverted over the cover slip, so that the drop of the culture was hanging in the centre of the cavity. The preparation was examined under

microscope using high power objective to observe motility of different bacterial strains.

Action on nitrates

The term nitrate reduction includes all processes in which nitrate disappears under the influence of bacterial action and appears in less oxidized state. The test is of value in identifying and classifying bacteria.

To young cultures of 24 hours nutrient broth added 2 drops of sulphanilic acid reagent and 2 drops of α-naphthylamine solution. The presence of nitrate was indicated by a pink or red colour.

Hugh and Leifson's test or Oxferm test

This test was done to distinguish aerobic and anaerobic breakdown of carbohydrate by bacteria.

Medium

Peptone	- 1%
Glucose	- 1%
K_2HPO_4	- 0.3%
Agar	- 0.3%
Sea water	- 100 ml

Phenol red 1 cc/100 cc of 0.1 solution

Procedure

1. Medium heated to dissolve, adjusted the pH to 7.4 and 1.5 ml, 0.2% bromothymol blue and sterile dextrose to give a final 1%. It was then poured asceptically into narrow test-tubes (1 cm).
2. Heated two tubes of H & L medium in boiling water for 10 minutes to drive off oxygen, cooled and inoculated.
3. Incubated one tube aerobically and the other anaerobically. Sealed the surface of the medium with 2 cm liquid paraffin to provide anaerobic conditions.

Results

Oxidative metabolism	-	Acid in aerobic tube only
Fermentative metabolism	-	Acid in both tubes
Yellow colour medium	-	Acid produced
Bubble in test-tube	-	Gas formed

Catalase test

Catalase is an enzyme capable of decomposing hydrogen peroxide into water and molecular oxygen. The presence of this enzyme was demonstrated by adding hydrogen peroxide to a culture and noting the evolution of oxygen.

Oxidase test

This test was aimed to detect the presence of certain bacteria that will catalyse the transport of electrons between the electrons donors in the bacteria and redox dye, tetra-methyl-paraphenylene diamine dihydrochloride. This dye is reduced to a deep purple colour.

Procedure

The organisms were inoculated into the SWA plates and incubated at 37°C for 24 hrs. Then the organisms were scrapped off from the plates and rubbed on the filter paper.

Production of indole

Indole is a putrefactive compound produced by the action of some bacteria on the amino acid, tryptophan. Since tryptophan is the only naturally occurring amino acid containing the indole ring, the test is specific for this compound.

Procedure

Inoculated two tubes of tryptone/peptone broth with 24 hrs culture and incubated at 37°C for 4 days. After incubation, 5 cc of the Kovac's reagent was added to the tubes. The appearance of deep cherry red colour in the reagent layer indicated presence of indole.

Kovac's reagent

Para-dimethyl-amino benzaldehyde	-	5 g
Amyl alcohol or Butyl alcohol	-	75 cc
Concentrated hydrochloric acid	-	25 cc

Antibiotic - sensitivity test

This test was done to determine whether the bacteria is sensitive to penicillin (2.3 IU/disc), or not.

Antibiotic agar

Peptone	-	1%
Agar	-	2%
Sea Water	-	100 ml

Procedure

The bacterial strains were thickly inoculated in the antibiotic medium and the discs were spotted at various points on the agar surface. The antibiotic diffuses through the agar occupying a circular zone around the original spot. Size of the zone is related to concentration of antibiotic. The scheme used for identification of aerobic heterotrophs was of Simidu and Aiso (1962), (Table 24).

2.3 Viability of aerobic heterotrophs in phenolic compounds

Viability of heterotrophic micro-organisms in increasing concentrations of phenol (0.05 to 1.0%) in mineral salts agar media being studied using all the isolates from the five different stations. Mineral media plates without phenol served as control. Each of the microbial isolates was spot-inoculated initially on 0.05% phenol agar and subsequently those showing growth were sub-cultured on increasing concentrations of phenol media. The cultures which could tolerate 1% phenol in the media, were further screened for growth on o-cresol and orcinol used in the media at a concentration of 0.05% (Gomes and Mavinkurve 1982).

2.4 Biodegradation experiments

2.4.1 Selection and adaptation of bacteria to utilize phenol

Micro-organisms were obtained from the five selected ecosystems around Cochin. Methods used in selecting or adapting organisms to degrade these compounds were static flask culture (Tabak *et al* 1981), primary enrichment on shaker (Tabak *et al* 1964) etc. In all instances, the material containing organisms subjected to preliminary enrichment was eventually inoculated in conical flask (sterilized cotton plugged) containing 50 ml Mineral Salts Medium (MSM) (Gray and Thornton 1928; Aaronson 1970) to which 0.25 μg of vitamin B_{12} had been added. The medium was prepared asceptically.

2.4.2 Composition of mineral salts medium

K_2HPO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.2 g
NaCl	-	15.0 g
$CaCl_2$	-	0.1 g
$FeCl_2$	-	0.02 g
$(NH_4)_2 SO_4$	-	1.0 g
Distilled water	-	1 litre
Phenol	-	at required concentration
pH	-	7.0 to 7.2

Procedure

1 g sediment was inoculated into the conical flask and incubated for 3 days at room temperature. Subcultures in the same medium were made periodically to acclimatise the culture to grow on phenol as sole source of carbon as per the duration of the experiment. All traces of organic nutrients as debris carried in the original inoculation were rapidly lost, and all non-bacterial forms soon disappeared. As soon as the oxidative capacity of the selected bacteria could be demonstrated in media containing low concentration of phenolic substrate, 2 to 25 ml of this material were subcultured into media containing progressively increasing concentrations of the compound. The increase in concentration of the substrate throughout the culture enrichment period ranged from 100 ppm to 1000 ppm for phenol, 100 to 500 ppm for

o-cresol and orcinol. Compounds used were analytical grades suitable for most laboratory syntheses.

2.4.3 Isolation and identification of micro-organisms

The mixed cultures were pour plated in mineral salts agar (same as MSM with 2% agar) and SWA. The colonies were isolated in agar slants and examined for motility reaction to gram-stain, ability for nitrate production from nitrate, capacity to produce indole, catalase, Hugh and Leifson's oxidative fermentative test, antibiotic sensitivity test etc. The presence of *Pseudomonas* strains were confirmed with the help of cytochrome oxidase test (Gaby and Hadley 1957; Gaby and Free 1958). The scheme used for bacterial identification was the same as for heterotrophs (Simidu and Aiso 1962).

2.4.4 Determination of recalcitrant phenols

For the analysis of the remaining phenol, solids were separated by centrifugation, the supernatant was filtered through Whatman No.1 filter paper. The 4-amino antipyrine method (APHA 1975) was used colorimetrically to determine residual concentration of phenol. The solutions of test compound from the respective uninoculated control flasks were also analysed to verify that any loss of substrate in the test flask was not due to volatalisation or chemical oxidation. To follow the increase in oxidative ability of organism and make periodic determination of the rate of dissimilation of phenols, the same 4-amino antipyrine method was used.

2.4.5 Use of individual micro-organisms for biodegradation studies

From the isolated, identified mixed culture, the most dominant ones were selected for individual biodegradation studies. *Pseudomonas, Alcaligenes, Vibrio* and *Streptomyces* were selected for the study. The experimental procedure was similar as in the mixed culture experiments. The concentrations used were from 100-500 ppm of phenol, o-cresol and orcinol. The percentage biodegradation of phenol and viability count of bacteria in the 3 selected compounds were determined.

2.5 Statistical analysis

The results obtained during the present investigation were processed statistically to obtain sample mean and standard deviation of parameters for water and sediment in all the five stations. Results of computer analysis of two-way ANOVA were used to test the significance of seasons and stations on different environmental parameters. Correlation analysis was carried out (Snedecor and Cochran 1967) to find out the interrelation among different parameters. Multiple regression analysis was carried out to arrive at a regression equation. Two-way ANOVA for biodegradability of phenol and viable count of bacteria was also done in experimental studies. The results are given either in the form of graphic intensity charts or tables for better presentation.

CHAPTER 3

RESULTS

3.1 Environmental variables

3.1.1 Hydrological parameters

Temperature

Station - I

During March 1990 - to August 1991, the temperature values ranged between 28.0°C to 32.03°C in the Station. The values decreased during monsoon months June/July due to the commencement of South-West monsoon. the lowest temperature recorded for the period was in December 1990 (28°C) and highest in March 1990 (32.0°C). Monsoon ad post-monsoon seasons encountered minimum mean temperature (29.5°C). The seasonal mean and standard deviation of the values are given in Table 1. Fig. 2 shows the monthly variation of water temperature in the station during the period of study.

Station - II

In Station II, the maximum temperature recorded was 31.5°C in April 1990, and minimum in July 1991 (25.75°C). The lowest mean temperature was recorded in monsoon (28.16°C) and highest during pre-monsoon (30.17°C) seasons. The monthly variation in temperature for the station is shown in

Fig..2. The seasonal mean and standard deviation in temperature is given in Table 2.

Station - III

During the period from March 1990 to August 1991, highest temperature was recorded in July'91 (31°C) and lowest in June '91 (27.5°C). Fig. 2 and Table 3 gives the temperature variations and standard deviation for the period of study during pre-monsoon, monsoon and post-monsoon seasons.

Station - IV

The temperature values in this station showed a variation between 25.75°C to 33.75°C, The highest temperature recorded was during September '90, when the paddy cultivation was going on in the seasonal paddy field and lowest during June '91, in the monsoon season. The monthly variation, seasonal mean and standard deviation of water temperature of the station is given in Fig. 2 and Table 4.

Station - V

In perennial aquaculture ponds, the water temperature ranged between 26.75°C and 33.25°C. The highest value was recorded during October '90 and minimum during October '91. This was prior to the start of north-east monsoon. The seasonal mean of temperature along with standard deviation and monthly variation are given in Fig. 2 and Table 5.

Salinity

Station - I

Salinity values of the station varied between 0.23-14.04 o/oo. Maximum salinity was encountered in post-monsoon months and minimum in monsoon month. Fig.3 shows the monthly variation of salinity values. Seasonal mean and standard deviation are given in Table 1.

Station - II

Maximum salinity was recorded during March '90 and (23.3 o/oo) and minimum during July '91 (0.26 o/oo). The monthly variations in salinity values are shown in Fig.3 and seasonal means and standard deviation are given in Table 2.

Station - III

Salinity values ranged between 0.07 and 23.3 o/oo in this station. Post monsoon months recorded maximum values and monsoon months minimum. The monthly variation in salinity values are given in Fig.3 and seasonal mean and standard deviation are given in Table 3.

Station - IV

The salinity values in this station ranged between 0.8 o/oo and 17.68 o/oo. Maximum was encountered in pre-monsoon month and minimum in monsoon month. The monthly variation, seasonal mean and standard deviation are given in Fig.3 and Table 4.

Fig. 2 : Showing temperature (°C) of the water in the sampling stations during 1990-91

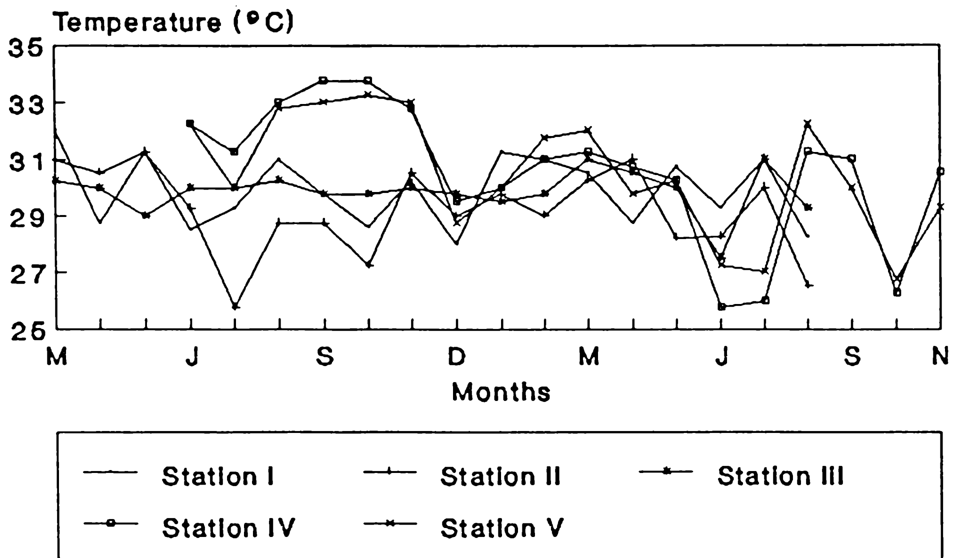
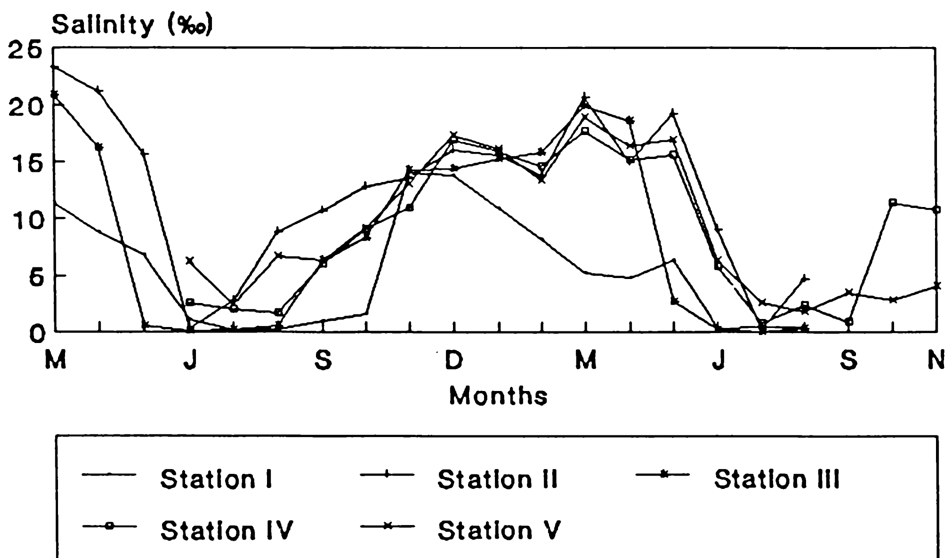


Fig. 3 : Showing the Salinity (‰) of the water in the sampling stations during 1990-91



Station - V

In perennial aquaculture ponds salinity ranged between 1.8 and 18.9 o/oo. Maximum value was recorded during pre-monsoon and minimum during monsoon months. The variation in salinity over the period '90-'91 are given in Fig.3 and seasonal mean and standard deviation are given Table 5.

Dissolved oxygen

Station - I

Dissolved oxygen value was found almost nil in the station, which is a coconut husk retting area, during April-to July and November, December '90. The values ranged between 0 and 4.45 ml/l. The anoxic condition prevailed during April '90 may be accounted for the hectic husk retting process that was going on in that location. The monthly variation, seasonal mean and standard deviation are given in Fig.4 and Table 1.

Station - II

During 1990-'91 period, the range in dissolved oxygen for mangrove area was between 0.4 and 8.3 ml/1. Lowest values were encountered during pre-monsoon and highest in monsoon months. The seasonal mean and standard deviation of dissolved oxygen is given in Table 2 and monthly variation of the values are given in Fig.4.

Table 1 **Seasonal mean and standard deviation of Physico-chemical parameters in station I**

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Water			
Temperature (^{0}C)	30.46 $\pm$ 1.21*	29.5 $\pm$ 1.09	29.5 $\pm$ 1.4
Salinity (^{o}/00)	7.3 $\pm$ 2.2	0.632 $\pm$ 0.38	10.07 $\pm$ 5.66
Dissolved Oxygen (ml/l)	1.97 $\pm$ 1.44	1.205 $\pm$ 0.815	1.04 $\pm$ 0.80
pH	7.15 $\pm$ 0.21	7.28 $\pm$ 0.5	7.7 $\pm$ 0.8
Phosphate phosphorous (ppm)	0.063 $\pm$ 0.047	0.045 $\pm$ 0.04	0.019 $\pm$ 0.034
Nitrate-nitrogen	0.004 $\pm$ 0.025	0.043 $\pm$ 0.07	0.019 $\pm$ 0.034
Silicate (ppm)	0.917 $\pm$ 0.28	1.235 $\pm$ 0.74	0.92 $\pm$ 0.62
Sediment			
Temperature (^{o}C)	31.46 $\pm$ 1.1	29.45 $\pm$ 1.01	29.6 $\pm$ 0.44
pH	7.24 $\pm$ 0.44	7.30 $\pm$ 0.71	7.90 $\pm$ 0.77
Organic Carbon (%)	2.61 $\pm$ 0.34	3.27 $\pm$ 0.39	2.77 $\pm$ 0.31
Organic matter (%)	4.49 $\pm$ 0.59	5.62 $\pm$ 0.68	4.76 $\pm$ 0.545
Phenolics (ppm)	3.41 $\pm$ 3.93	14.63 $\pm$ 4.64	0.825 $\pm$ 0.207
Heterotrophic Bacterial count (No. x 10^4)	138.7 $\pm$ 38.72	72.5 $\pm$ 29.6	150.37 $\pm$ 18.30

* Standard deviation

Station - III

For this station maximum value for dissolved oxygen was recorded during August '91 (7.09 ml/1) and minimum in April '90 (1.12 ml/1) Pre-monsoon months recorded minimum O_2 value and monsoon months recorded maximum. The monthly variation over March '90 to August '91 seasonal mean and standard deviation for dissolved oxygen are given in Fig.4 and Table 3.

Station - IV

A range between 1.48 and 6.63 ml/1 was encountered for dissolved oxygen in this station. Maximum was encountered in monsoon months and minimum in post-monsoon months. Monthly variation seasonal mean and standard deviation for dissolved O_2 in the station are given in Fig.4 and Table 4.

Station - V

The maximum and minimum values for dissolved oxygen was observed during post-monsoon months of '91. The minimum O_2 value recorded, was 1.1 ml/1 and maximum was 7.45 ml/1. The monthly variation, seasonal mean and standard deviation are given in Fig. 4 and Table 5.

Hydrogen ion concentration (pH)

Station - I

The pH ranged between 6.45 and 8.15 in this station. Maximum was recorded in post monsoon months and minimum in monsoon months. The seasonal mean values, standard deviation and monthly variation in pH are given in Table 1 and Fig.5.

Station - II

The pH in this station varied between 5.7 and 8.3. Maximum was encountered in December '90 and minimum in January '91. The monthly variation of pH values, seasonal mean and standard deviation are given in Fig 5 and Table 2.

Station - III

In Thykoodam pH ranged between 6.05 and 8.4, maximum being in post-monsoon month and minimum in pre-monsoon month. The seasonal mean values for pH and standard deviation are given in Table 3 and monthly variation in pH is shown in Fig.5.

Station - IV

The pH ranged between 5.6 and 8.3 in seasonal aquaculture ponds. The maximum and minimum were recorded in monsoon months. The seasonal mean, standard deviation and monthly variation in pH values for the station are given in Table 4 and Fig.5.

Station - V

In perennial aquaculture ponds, the pH ranged between 6.75 and 8.1. August and November '90 recorded maximum values. The minimum being recorded in July 90. The monthly variation, seasonal mean and standard deviation are given in Fig.5 and Table 5.

Fig. 4 : Showing Dissolved Oxygen (ml/l) of the water in the sampling stations during 1990-91

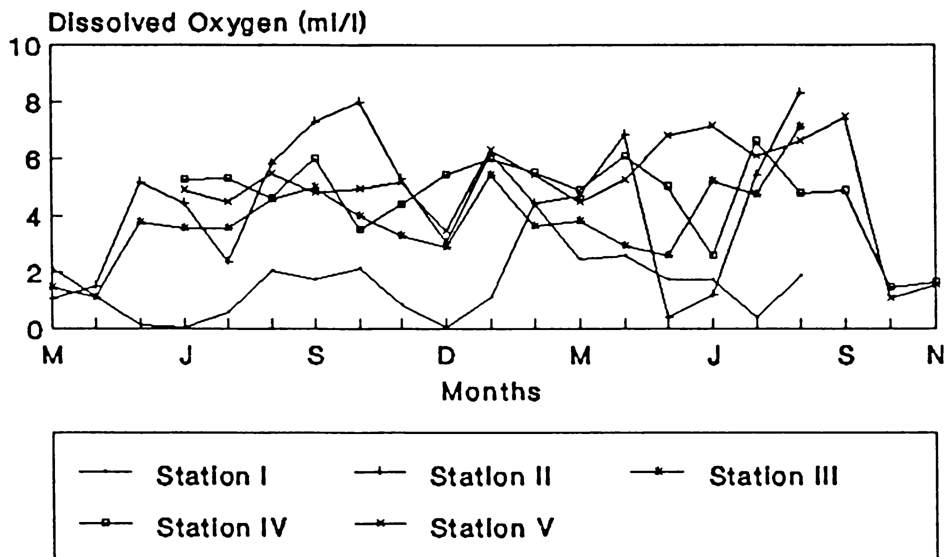


Fig. 5 : Showing pH of the water in the sampling stations during 1990-91

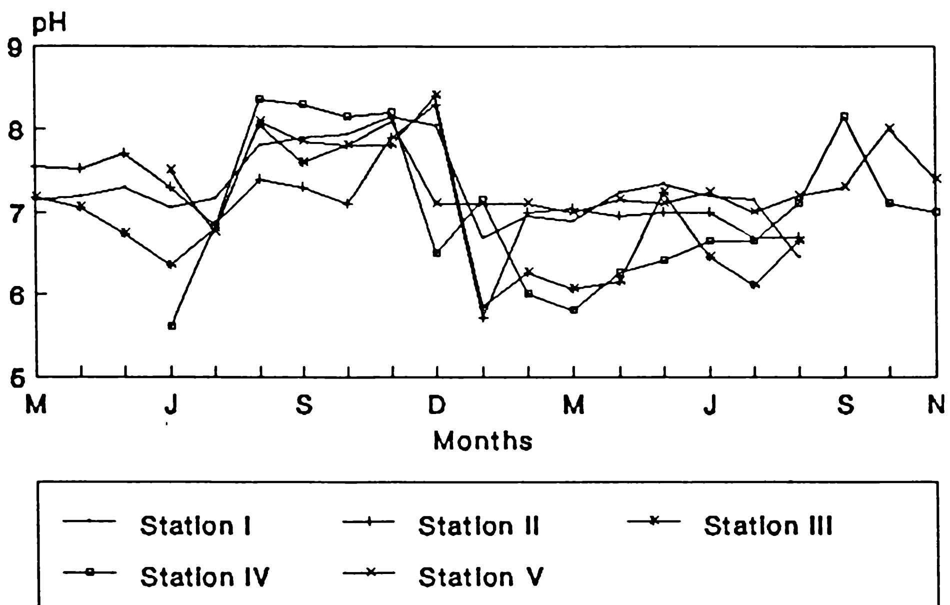


Table 2 Seasonal mean and standard deviation of physico-chemical parameters in station II

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Water			
Temperature (^{0}C)	30.17* ± 1.27	28.16 ± 1.51	29.28 ± 1.47
Salinity (^{0}/00)	18.38 ± 3.54	5.30 ± 4.1	14.45 ± 1.57
Dissolved Oxygen (ml/l)	3.45 ± 2.36	5.0 ± 2.6	5.63 ± 1.9
pH	7.25 ± 0.346	7.4 ± 0.32	7.25 ± 1.07
Phosphate-phosphorous (ppm)	0.112 ± 0.08	0.54 ± 0.56	0.37 ± 0.09
Nitrate-nitrogen	0.042 ± 0.03	0.078 ± 0.058	0.044 ± 0.024
Silicate (ppm)	0.105 ± 0.59	1.345 ± 0.44	1.125 ± 0.61
Sediment			
Temperature (^{0}C)	29.88 ± 1.55	28.3 ± 1.22	29.0 ± 1.65
pH	7.2 ± 0.36	7.136 ± 0.24	7.4 ± 0.9
Organic Carbon (%)	2.93 ± 0.16	2.0 ± 1.4	3.58 ± 0.53
Organic matter (%)	5.38 ± 1.07	3.85 ± 3.36	5.99 ± 0.01
Phenolics (ppm)	1.61 ± 1.03	10.78 ± 5.19	1.155 ± 0.35
Heterotrophic Bacterial count (No. x 10^4)	86.35 ± 20.97	49.78 ± 28.09	60.75 ± 14.25

* Standard deviation

Phosphate - phosphorous

Station - I

In coconut retting area, the phosphate-phosphorous ranged between 0 and 0.201 ppm. The maximum being recorded in pre-monsoon month and minimum during monsoon. The monthly variation, seasonal mean and standard deviation are given in Fig. 6 and Table 1.

Station - II

In the mangrove ecosystem phosphate-phosphorous ranged between 0.01 and 1.3 ppm. The maximum value was encountered in monsoon month and minimum in pre-monsoon month. The variation in the value, seasonal mean and standard deviation are given in Fig.6 and Table - 2.

Station - III

In Thykoodam, the value of phosphate-phosphorous ranged between 0.185 and 0.699 ppm. The maximum was recorded during monsoon and minimum during pre-monsoon month. The monthly variation in phosphate value, seasonal mean and standard deviation are given in Fig.6 and Table 3.

Station - IV

In this station which is seasonal aquaculture ponds, the range of phosphate-phosphorous was between 0.005 and 0.326 ppm. The maximum was recorded in monsoon and minimum in pre-monsoon months. The monthly

variation in phosphate values, seasonal mean and standard deviation are given in Fig.6 and Table 4.

Station - V

The phosphate-phosphorous in perennial ponds ranged between 0.006 and 0.109 ppm. The lowest value was recorded in monsoon months (June 90 and June 91). The monthly variation in phosphate-phosphorous is given in Fig.6. and seasonal mean and standard deviation are given in Table 5.

Nitrate-nitrogen

Station - I

The values of nitrate-nitrogen in coconut retting area ranged between 0 and 0.159 ppm. Absence of nitrate-nitrogen was observed in post-monsoon month and maximum during monsoon month. The monthly variation, seasonal mean and standard deviation are given in Fig.7 and Table.1.

Station - II

In the mangrove area the values of nitrate-nitrogen ranged between 0.004 and 0.62 ppm. Maximum was obtained in monsoon month and minimum during post-monsoon month. Monthly variation of nitrate-nitrogen in the station is given in Fig.7 and seasonal mean values and standard deviation in Table 2.

Fig. 6 : Showing Phosphate-phosphorous (ppm) of the water in the sampling stations during 1990-91

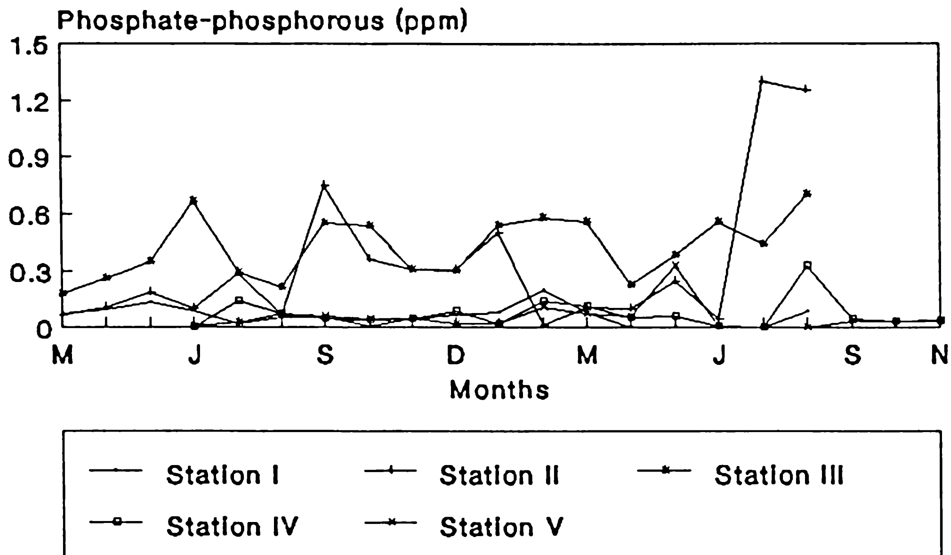
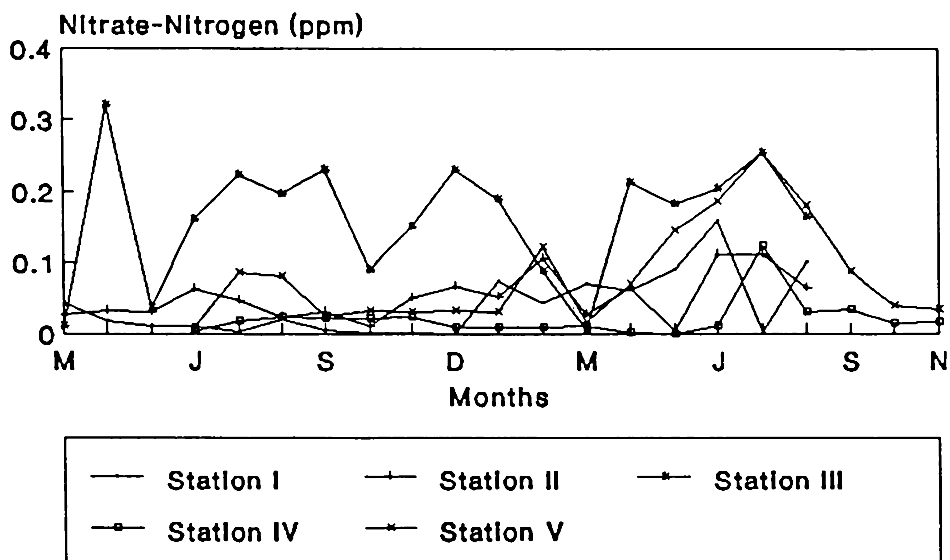


Fig. 7 : Showing Nitrate-Nitrogen (ppm) of the water in the sampling stations during 1990-91



Station - III

In Thykoodam area the value of nitrate-nitrogen ranged between 0.006 and 0.32. Maximum and minimum values were obtained during pre-monsoon months. The seasonal mean values and their standard deviation are given in Table 3 and monthly variation of nitrate-nitrogen in Fig.7.

Station - IV

The lowest recorded nitrate-nitrogen in this station and 0.001 ppm and highest 0.123 ppm. The maximum value was observed in monsoon and minimum in pre-monsoon. The monthly variation in nitrate nitrogen is shown in Fig.7 and seasonal mean values and standard deviation in Table 4.

Station - V

The nitrate-nitrogen value ranged between 0.0095 and 0.255 ppm in perennial ponds. Maximum and minimum values were recorded in monsoon months. The variation in nitrate-nitrogen for 1990 - '91 are given in Fig. 7 and seasonal mean values and standard deviation are given in Table 5.

Silicate-silicon

Station - I

The silicate value for coconut retting area ranged between 0.495 and 2.42 ppm. The maximum value was recorded in monsoon months (July and August '90 and '91 respectively). The monthly variation in silicate values are

shown in Fig.8. seasonal mean values and standard deviation are given in Table 1.

Station - II

In this station, the values of silicate ranged from 0.42 to 2.04 ppm. Maximum value was recorded during pre-monsoon and minimum during post-monsoon months. The monthly variation of the values are shown in Fig.8 and seasonal mean values and standard deviation are given Table 2.

Station - III

The silicate value ranged from 0.102 to 2.46 ppm. The maximum value was obtained in monsoon month and minimum during post-monsoon month. The monthly variation in silicate concentration is shown in Fig.8 and seasonal mean values and standard deviation are given in Table 3.

Station - IV

The values of silicate in seasonal aquaculture ponds ranged between 0.07 and 1.06 ppm. The maximum value was obtained twice, once in monsoon and another in post-monsoon. The minimum value is recorded during pre-monsoon months. The variation in silicate concentration for each month is shown in Fig.8 and seasonal mean values and standard deviation are given in Table 4.

Table 3 Seasonal mean and standard deviation of physico-chemical parameters in station III

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Water			
Temperature (^{0}C)	30.07* ± 0.7	29.6 ± 1,17	29.75 ± 0.53
Salinity (^{0}/00)	14.54 ± 7.38	1.14 ± 2.20	12.99 ± 3.01
Dissolved Oxygen (ml/l)	2.7 ± 1.13	4.8 ± 1.2	3.48 ± 0.55
pH	6.67 ± 0.5	6.89 ± 0.73	7.46 ± 1.1
Phosphate-phosphorous (ppm)	0.36 ± 0.16	0.455 ± 0.17	0.42 ± 0.13
Nitrate-nitrogen (ppm)	0.081 ± 0.081	0.204 ± 0.048	0.165 ± 0.068
Silicate (ppm)	0.648 ± 0.25	0.90 ± 0.90	0.564 ± 0.24
Sediment			
Temperature (^{o}C)	29.78 ± 0.58	29.45 ± 1.12	29.25 ± 0.6
pH	6.77 ± 0.412	7.09 ± 0.68	7.6 ± 1.03
Organic Carbon (%)	0.59 ± 0.24	2.51 ± 0.81	0.38 ± 0.037
Organic matter (%)	1.02 ± 0.42	4.32 ± 1.4	0.66 ± 0.06
Phenolics (ppm)	1.83 ± 2.7	0.916 ± 0.31	0.59 ± 0.08
Heterotrophic Bacterial count (No. x 10^4)	87.2 ± 28.5	61.0 ± 27.5	115.63 ± 35.3

* Standard deviation

Station - V

The silicate value for perennial aquaculture ponds ranged between 0.126 and 2.38 ppm. The maximum and minimum values were recorded during monsoon months. The monthly variation in silicate values is shown in Fig.8 and standard deviation and seasonal mean values are given in Table 5.

3.1.2 Sediment parameters

Temperature

Station - I

During '90 and '91, the minimum temperature was recorded during monsoon months and maximum during pre-monsoon months. The temperature values of the station ranged between 28.5°C and 31.5°C. The monthly variation in temperature is shown in Fig.9 and seasonal mean and standard deviation are given in Table 1.

Station - II

During the period of observation, it is found that the temperature of this station varied between 26.15°C and 31.5°C. The maximum temperature was encountered during pre-monsoon month and minimum during monsoon months. The variation observed for temperature in each month is shown in Fig.9 and seasonal mean and standard deviation are given in Table 2.

Station - III

The temperature values ranged between 28.5°C and 31.25°C. The minimum range was recorded in monsoon and post-monsoon months. The variation in temperature for the station is shown in Fig.9 and seasonal mean and standard deviation in Table 3.

Station - IV

The temperature values in the seasonal aquaculture pond ranged between 26.25°C and 33.25°C. The high value was observed in September '90 when the paddy cultivation was going on. Minimum temperature was recorded in monsoon months. The seasonal mean and standard deviation of sediment temperature is given in Table 4 and the monthly variation recorded for sediment temperature is shown in Fig.9.

Station - V

In this station the temperature variation observed was between 27.5°C and 33.25°C. Maximum temperature was recorded in post-monsoon months in '90 and minimum during post-monsoon months of '91. The monthly variation in temperature recorded is shown in Fig,9 and seasonal mean and standard deviation are given in Table 5.

Fig. 8 : Showing Silicate (ppm) of the water in the sampling stations during 1990-91

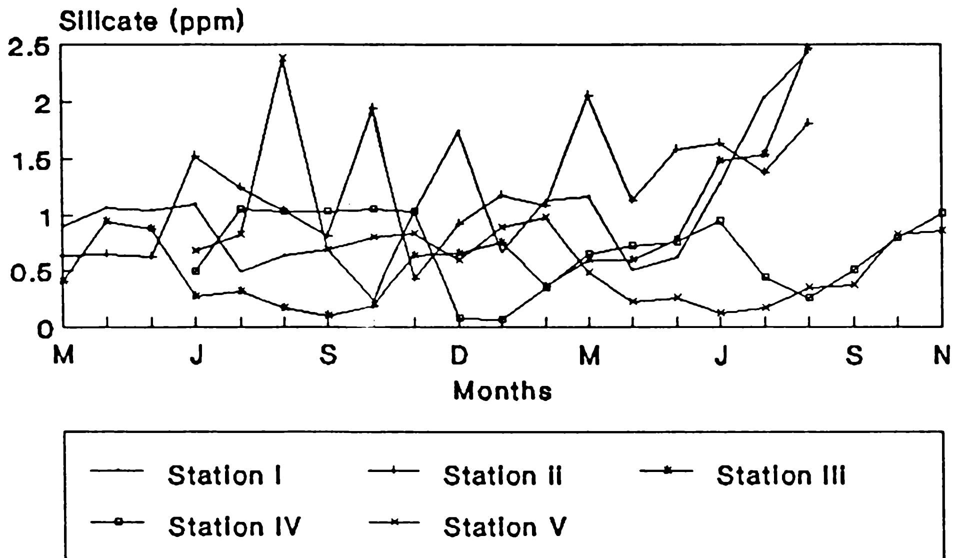
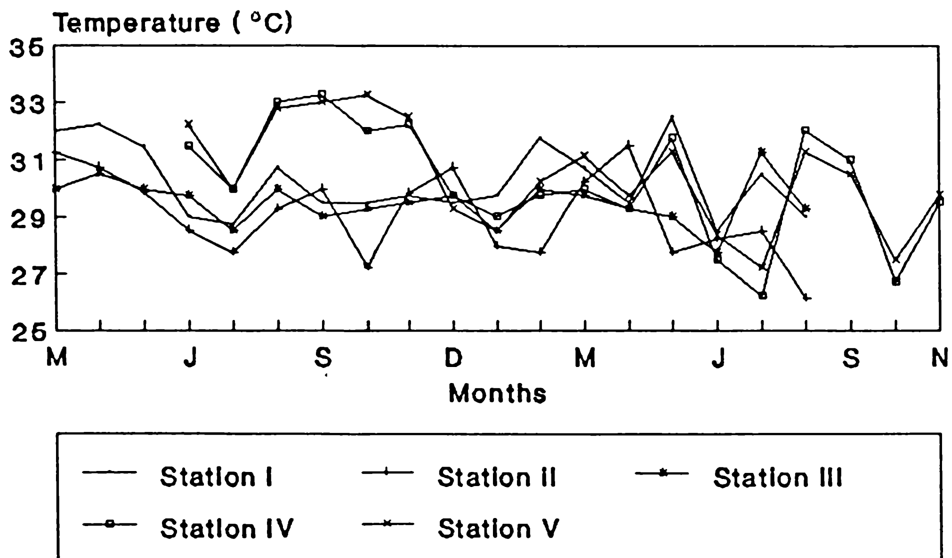


Fig. 9 : Showing Temperature (°C) of the sediment in the sampling stations during 1990-91



Hydrogen ion concentration (pH)

Station - I

A range between 6.4 and 8.6 was recorded for sediment pH in the coconut husk retting area. The high value of pH was recorded in post-monsoon month and minimum in pre-monsoon month. The variation in the values over months is given in Fig.10 and seasonal mean and standard deviation are given in Table 1.

Station - II

The sediment pH varied between 6.7 and 8.0 in the mangrove station. The pH was lower during monsoon months and higher during post-monsoon months. The monthly variation in sediment pH for the station is given in Fig.10 and seasonal mean and standard deviation are given in Table 2.

Station - III

Here the pH value ranged between 6.0 and 8.25. The pH was lower during pre-monsoon and more during post-monsoon months. The variation in pH value over months are shown in Fig.10 and seasonal mean and standard deviation are given in Table 3.

Station - IV

The pH value in seasonal aquaculture ponds varied between 4.63 and 8.3. Minimum pH was recorded in pre-monsoon months and maximum during post-monsoon months. The monthly variation in sediment pH for the station

is given in Fig.10 and seasonal mean and standard deviation are given in Table 4.

Station - V

In perennial aquaculture ponds, the variation in sediment pH ranged between 6.2 and 8.3. Lower pH were encountered in pre-monsoon months and higher values during post-monsoon months. The variation in sediment pH for the investigation period is shown in Fig.10 and seasonal mean and standard deviation in Table 5.

Organic carbon

Station - I

The values for organic carbon in the station ranged between 2.4 and 3.75%. The lower values were recorded in pre-monsoon months and higher values during monsoon months. The variation in organic carbon from March'90 to August '91 is given in Fig.11 and seasonal mean and standard deviation are given in Table 1.

Station - II

The organic carbon percentage in the mangrove station varied between 0.95 and 4.18. The higher values were encountered in post-monsoon months and lower values during monsoon months. The monthly variation in organic carbon, seasonal mean and standard deviation are given in Fig.11 and Table 2 respectively.

Table 4 Seasonal mean and standard deviation of physico-chemical parameters in station IV

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Water			
Temperature (^{0}C)	30.81* ± 0.46	30.53 ± 2.96	30.45 ± 2.54
Salinity (^{0}/00)	15.71 ± 1.55	2.85 ± 2.0	12.46 ± 3.09
Dissolved Oxygen (ml/l)	5.38 ± 0.52	4.95 ± 1.134	3.74 ± 1.82
pH	6.1 ± 0.48	7.2 ± 0.96	7.35 ± 0.66
Phosphate-phosphorous (ppm)	0.093 ± 0.07	0.082 ± 0.106	0.05 ± 0.02
Nitrate-Nitrogen (ppm)	0.006 ± 0.005	0.045 ± 0.058	0.015 ± 0.007
Silicate (ppm)	0.62 ± 0.198	0.72 ± 0.32	0.67 ± 0.47
Sediment			
Temperature (^{0}C)	30.18 ± 1.1	30.56 ± 2.55	29.9 ± 1.95
pH	5.97 ± 0.99	7.4 ± 0.69	7.5 ± 0.58
Organic Carbon (%)	1.57 ± 0.114	3.92 ± 0.45	3.0 ± 0.21
Organic matter (%)	2.71 ± 0.18	6.74 ± 0.77	5.14 ± 0.36
Phenolics (ppm)	0.54 ± 0.079	0.68 ± 0.51	0.79 ± 0.64
Heterotrophic Bacterial count (No. x 10^4)	77.1 ± 25.08	49.63 ± 15.77	75.8 ± 13.5

* Standard deviation

Station - III

In this station, the values of organic carbon ranged between 0.36 and 4.18%. Lowest value was recorded in pre-monsoon month and highest in monsoon month. The monthly variation in the values, seasonal mean and standard deviation are given in Fig.11 and Table 3.

Station - IV

The percentage organic carbon in this station ranged between 1.45 and 4.35%. The higher values were encountered in monsoon months and lower values in pre-monsoon months. The monthly variation in organic carbon for the Station is given in Fig.11 and seasonal mean and standard deviation are given in Table 4.

Station - V

The variation in organic carbon for the period June '90 to November '91 ranged from 0.34 to 2.05%. The values were more in pre-monsoon and less in post-monsoon months. The monthly variation, seasonal mean and standard deviation are given in Fig.11 and Table 5 respectively.

Organic matter

Station - I

The organic matter content of the sediment in this station varied from 4.13 to 6.08%. The values were higher during monsoon months and lower

Fig. 10 : Showing pH of the sediment in the sampling stations during 1990-91

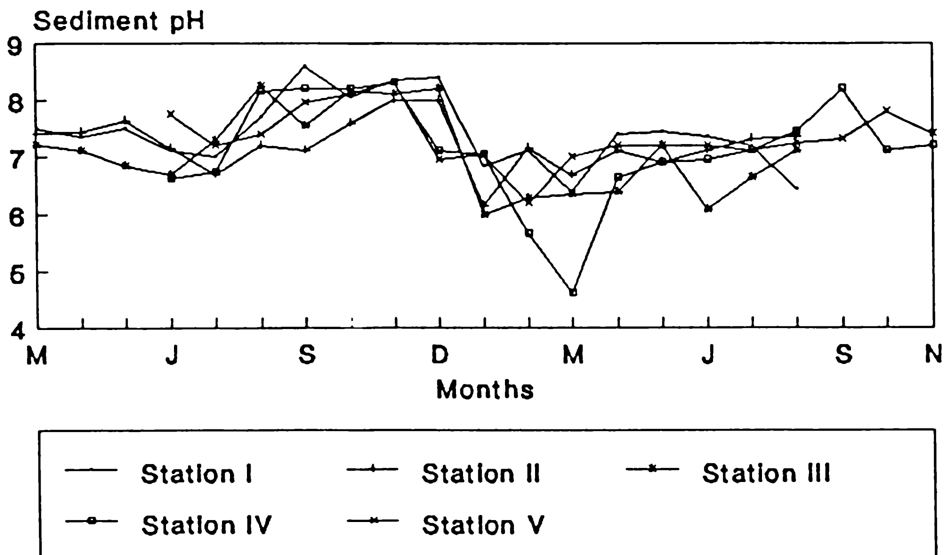
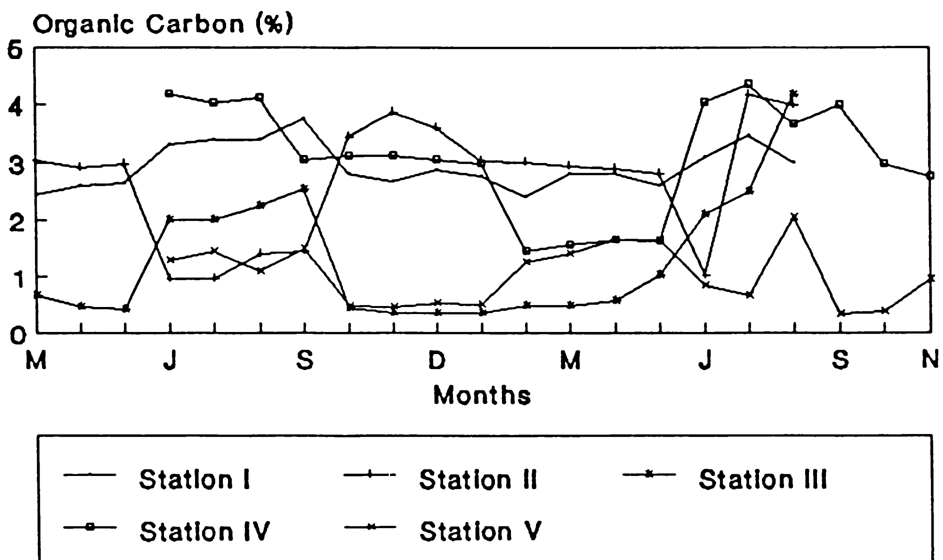


Fig. 11 : Showing Organic Carbon (%) of the sediment in the sampling stations during 1990-91



during pre-monsoon months. The monthly variation is shown in Fig.12 and seasonal mean and standard deviation are given in Table 1.

Station - II

The sediment in the mangrove system showed a variation between 1.64 and 7.19% in organic matter content. The maximum and minimum values were recorded in July'90 and '91 respectively. Higher values were recorded in post-monsoon months and lower values during monsoon months. The percentage variation is shown in Fig.12 and seasonal mean and standard deviation in Table 2.

Station - III

In Thykoodam, the organic matter content in the sediment ranged between 0.62 and 4.325%, The higher values were encountered during monsoon months and minimum during post-monsoon months. The monthly variation in the organic matter content, seasonal mean and standard deviation are given in Fig.12 and Table 3 respectively.

Station - IV

During the period of June'90 to November'91, the organic matter content of the seasonal aquaculture pond varied between 2.54 and 7.47%.. The values were higher during monsoon and lower during pre-monsoon seasons. The monthly variations, seasonal mean and standard deviation are given in Fig.12 and Table 4 respectively.

Station - V

In the perennial aquaculture pond, the organic matter content varied between 0.67 and 3.53%. The maximum value was encountered in August'91 and minimum during October'91. The monthly variation of organic matter for the study period is shown in Fig.12 and seasonal mean and standard deviation are given in Table 5.

Sediment phenolics

Station - I

In the coconut husk retting area, the sediment phenolics varied between 0.41 and 19.64 ppm. The maximum value was recorded in June'90 and minimum during March'91. Higher values were recorded during monsoon months and lower values during post-monsoon months. The monthly variation in phenol content in the station is shown in Fig.13a. The seasonal mean and standard deviation are given in Table 1.

Station - II

In the mangrove ecosystem, the sediment phenolics ranged between 0.018 and 16.75 ppm. The maximum value was recorded in June'90 and minimum during February'91. The higher values were recorded during monsoon months and lower values during post-monsoon months. The monthly variation in phenol concentration in the sediment is shown in Fig. 13a. Seasonal mean and standard deviation are given in Table 2.

Fig. 12 : Showing Organic Matter (%) of the sediment in the sampling stations during 1990-91

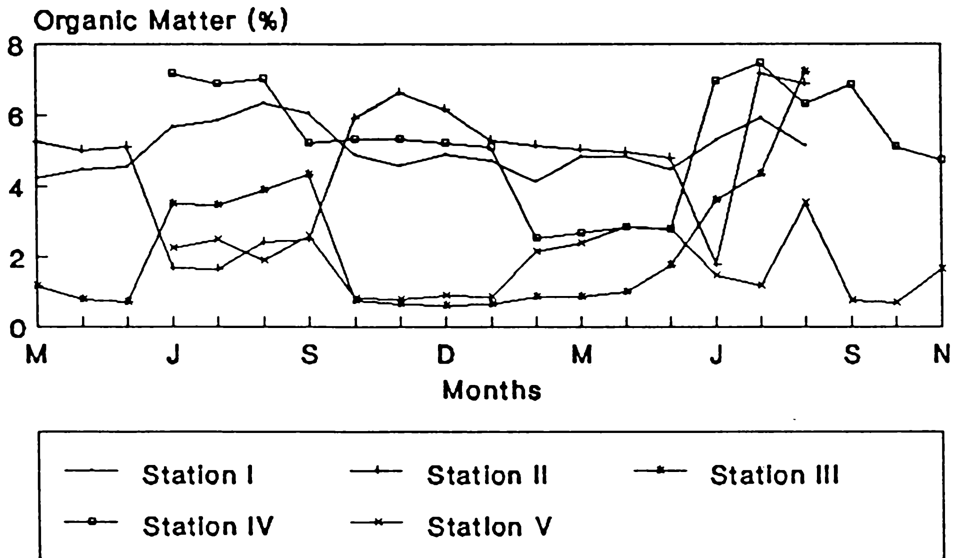
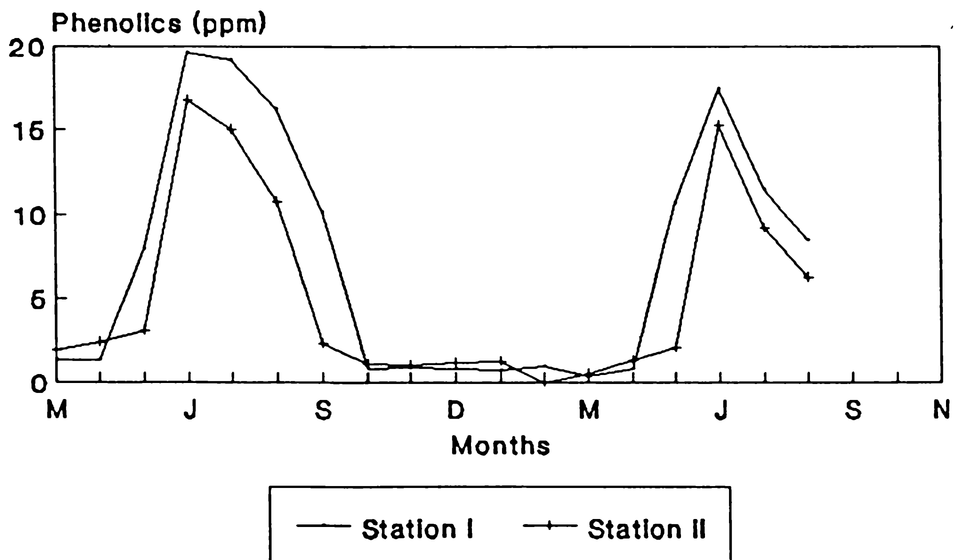


Fig. 13a : Showing Phenolics (ppm) of the sediment in the sampling stations I and II during 1990-91



Station - III

At Thykoodam, the highest value for phenol was recorded in March'90 (8.2 ppm). The minimum value recorded was 0.55 ppm during June'90. The monthly variation in sediment phenolics of the stations is shown in Fig.13b and seasonal mean and standard deviation are given in Table 3.

Station - IV

In seasonal aquaculture ponds, the sediment phenolics varied between 0.12 and 1.55 ppm. The highest value was encountered in November'90 and lowest during August'91. The monthly variation in the values are shown in Fig.13b, seasonal mean and standard deviation are given in Table 4 for sediment phenolics in the station.

Station - V

In perennial aquaculture ponds, the sediment phenolics ranged between 0.37 and 1.73 ppm. The highest value was recorded in August'90 and lowest during December'90. Higher values were recorded in monsoon months and low values during post-monsoon months. The monthly variation, seasonal mean and standard deviation are given in Fig.13b and Table 3 respectively for sediment phenolics.

Table 5 **Seasonal mean and standard deviation of physico-chemical parameters in station V**

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Water			
Temperature (°C)	30.94 ± 1.05*	30.56 ± 2.34	30.16 ± 2.43
Salinity (°/00)	16.36 ± 2.18	4.06 ± 1.99	10.38 ± 5.8
Dissolved Oxygen (ml/l)	5.49 ± 0.93	5.86 ± 1.1	3.83 ± 1.91
pH	7.08 ± 0.11	7.39 ± 0.46	7.58 ± 0.45
Phosphate-phosphorous (ppm)	0.144 ± 0.194	0.028 ± 0.29	0.037 ± 0.013
Nitrate-Nitrogen (ppm)	0.09 ± 0.05	0.112 ± 0.087	0.033 ± 4.05
Silicate (ppm)	0.485 ± 0.33	0.71 ± 0.70	0.797 ± 0.12
Sediment			
Temperature (°C)	30.85 ± 1.05	30.69 ± 2.0	30.13 ± 2.17
pH	6.95 ± 0.49	7.4 ± 0.38	7.6 ± 0.57
Organic Carbon (%)	1.49 ± 0.25	1.17 ± 0.53	0.49 ± 0.2
Organic matter (%)	2.56 ± 0.44	2.01 ± 0.91	0.95 ± 0.33
Phenolics (ppm)	0.696 ± 0.185	1.036 ± 0.42	0.448 ± 0.116
Heterotrophic Bacterial count (No. x 10^4)	67.63 ± 29.7	59.25 ± 16.48	87.16 ± 12.3

* Standard deviation

Aerobic heterotrophs

Total Plate Count (TPC)

Station - I

The microbial flora in the coconut husk retting area generally consist of brackish water, freshwater and intermediate forms. The total plate count varied between 36 x 10^4/gm in monsoon month to 179.5 x 10^4/gm in the pre-monsoon month. Definite seasonal cycle was observed and maximum abundance of bacteria was encountered during pre-monsoon and minimum during monsoon months. Large fluctuations in number were observed with seasonal variation and abundance. The monthly variation in TPC is given in Fig.14 and seasonal mean and standard deviation are given in Table 1.

Station - II

The period of maximum abundance of aerobic heterotrophs in this station was during pre-monsoon months. The pattern of seasonal cycle was the same as in Station I, eventhough the counts between maximum and minimum was not large. The counts ranged between 24.5 x 10^4/gm to 110 x 10^4/gm. The maximum was observed in post-monsoon season. The monthly variation in TPC for the station is given in Fig.14 and seasonal mean and deviation in Table 2.

Station - III

The TPC for Thykoodam station ranged from 37.5 x 10^4/gm to 148 x 10^4/gm. The maximum abundance was observed in post-monsoon months and minimum during monsoon months. The variation was clear with seasons.

The monthly variation is shown in Fig.14 and seasonal mean and standard deviation are given in Table 3.

Station - IV

In seasonal aquaculture ponds, the TPC varied between $30x10^4$/gm to $106x10^4$/gm. The maximum count was recorded during pre-monsoon month and minimum during monsoon months. The monthly variation in TPC is shown in Fig.14 and seasonal mean and standard deviation in Table 4.

Station - V

As in the other stations, here also the minimum count was recorded during monsoon season. The maximum count was encountered in February '91. The TPC in this station ranged between $34x19^4$/gm and 137.10^4/gm. The monthly variation, seasonal mean and standard deviation are given in Fig.14 and Table 5 respectively for TPC of the station.

3.1.3 Statistical analyses

3.1.3.1 Analysis of variance

To test the significance of effect of seasons over stations for different physico-chemical parameters, the results of Two-way Analysis of Variance was used.

F - value for water temperature between stations was significant ($p<0.01$) while that between seasons was not significant (Table 6).

F - value for salinity was significant between stations ($p<0.05$) and between seasons ($p<0.01$) (Table 7).

Fig. 13b : Showing Phenolics (ppm) of the sediment in the sampling stations III, IV and V during 1990-91

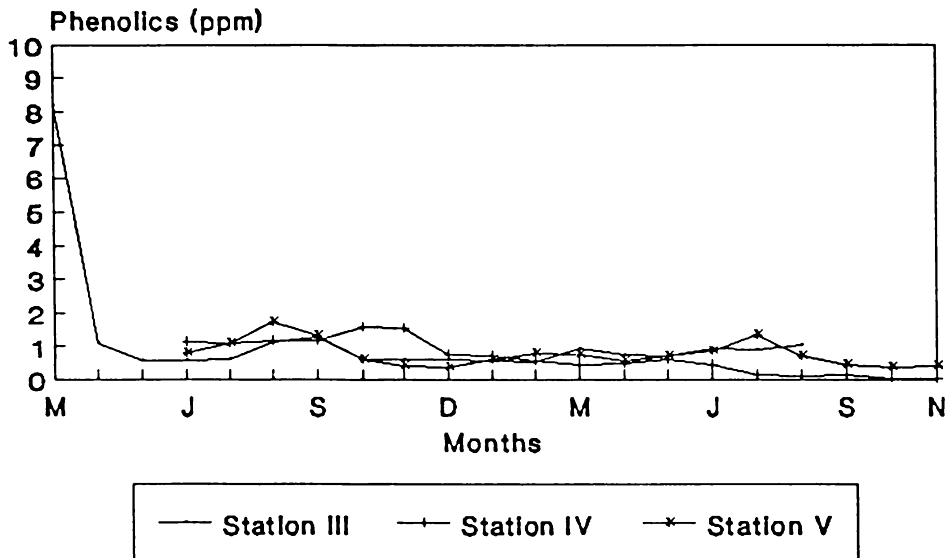


Fig. 14 : Showing Total Plate Count (TPC = No x 10) of the sediment in the sampling stations during 1990-91

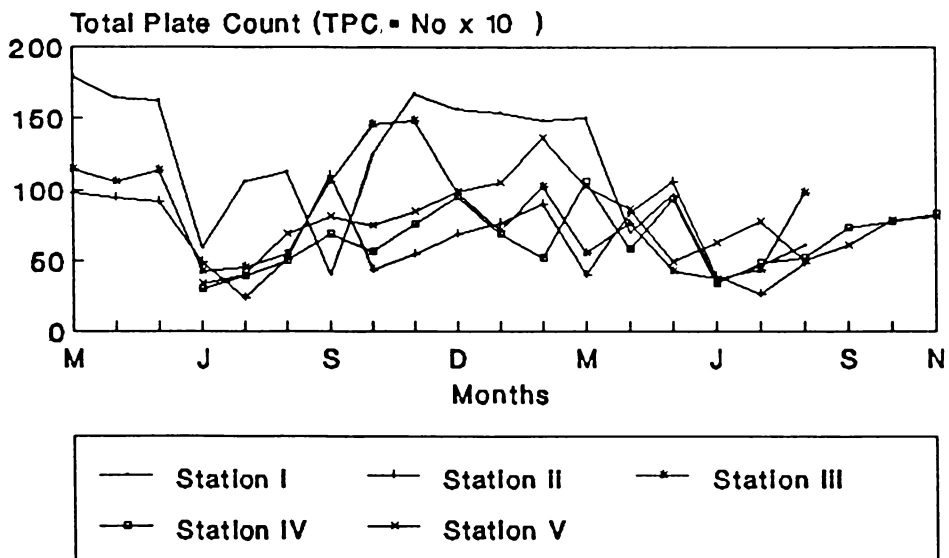


Table 6 **Two - way ANOVA for water temperature over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	4.34	1.085	7.38	SIG (1%)
Season	2	1.88	0.940	6.39	NS
Error	8	1.18	0.147		
Total	14	7.40			

Table 7 **Two - way ANOVA for salinity over stations andover seasons**

Source	DF	SS	MS	F	Remarks
Station	4	70.91	17.728	4.72	SIG (5%)
Season	2	370.91	185.174	49.28	SIG (1%)
Error	8	30.06	3.757		
Total	14	471.32			

Table 8 **Two - way anova for dissolved oxygen over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	26.64	6.660	7.41	SIG (1%)
Season	2	1.77	0.884	0.98	NS
Error	8	7.19	0.899		
Total	14	35.60			

F - value for dissolved oxygen between stations was significant ($p<0.01$), while between seasons it was non-significant (Table 8).

F - value for water pH between stations was not significant, while between seasons, it was significant ($p<0.05$) (Table 9).

F - value for phosphate-phosphorous was significant between stations ($p<0.05$) and non-significant between seasons (Table 10).

F - value for nitrate-nitrogen was significant between stations ($p<0.01$) and not significant between seasons (Table 11).

F - values for silicate between stations ($p<0.01$) and seasons ($p<0.05$) were significant (Table 12).

F - values for sediment temperature was significant between stations ($p<0.05$) while it was non-significant between seasons (Table 13).

F - values for sediment pH showed no significance between stations, while it was significant between seasons ($p<0.05$) (Table 14).

F - values for organic carbon between stations and seasons wee non-significant (Table 15). Similarly, for organic matter also F-value showed no significance between stations and seasons (Table 16).

F - values for sediment phenolics were non-significant between stations as well as seasons (Table 17).

F - value for aerobic heterotrophs (TPC) between stations and seasons were significant ($p<0.05$) (Table 18).

Table 9 **Two - way ANOVA for water pH over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	0.56	0.140	1.81	NS
Season	2	0.95	0.475	6.13	SIG (5%)
Error	8	0.62	0.077		
Total	14	2.13			

Table 10 **Two - way ANOVA for phosphate-phosphorous over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	0.34	0.084	6.01	SIG (5%)
Season	2	0.01	0.004	0.27	NS
Error	8	0.11	0.014		
Total	14	0.45			

Table 11 **Two - way ANOVA for nitrate-nitrogen over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	0.03	0.008	8.49	SIG (1%)
Season	2	0.01	0.003	3.39	NS
Error	8	0.01	0.001		
Total	14	0.04			

Table 12 **Two - way ANOVA for silicate over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	0.70	0.176	16.83	SIG (1%)
Season	2	0.14	0.070	6.68	SIG (5%)
Error	8	0.08	0.010		
Total	14	0.93			

Table 13 **Two - way ANOVA for sediment temperature over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	4.47	1.117	4.16	SIG (5%)
Season	2	2.22	1.111	4.14	NS
Error	8	2.15	0.268		
Total	14	8.83			

Table 14 **Two - way ANOVA for sediment pH over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	0.48	0.121	1.17	NS
Season	2	1.56	0.779	7.50	SIG (5%)
Error	8	0.83	0.104		
Total	14	7.40			

Table 15 **Two - way ANOVA for organic carbon over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	10.69	2.673	3.61	NS
Season	2	1.45	0.723	0.98	NS
Error	8	5.92	0.740		
Total	14	18.06			

Table 16 **Two - way ANOVA for organic matter over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	32.01	8.002	3.66	NS
Season	2	4.47	2.233	1.02	NS
Error	8	17.49	2.186		
Total	14	53.96			

Table 17 **Two - way ANOVA for sediment phenolics over stations and over seasons**

Source	DF	SS	MS	F	Remarks
Station	4	80.06	20.015	1.59	NS
Season	2	66.90	33.448	2.65	NS
Error	8	100.80	12.600		
Total	14	247.76			

Table 18 Two - way ANOVA for total acrobic heterotrophs over stations and over seasons

Source	DF	SS	MS	F	Remarks
Station	4	6326.99	1581.747	5.94	SIG (5%)
Season	2	4486.37	2243.184	8.42	SIG (5%)
Error	8	2131.18	266.397		
Total	14	12944.53			

3.1.3.2 Correlation coefficient analysis

In order to assess the correlation and influence of each parameter studied and to find the extent of influence of other environmental parameters on sediment phenolics and total aerobic heterotrophs the following characters were selected.

1. Water temperature
2. Salinity
3. Dissolved oxygen
4. pH
5. Phosphate-phosphorous
6. Nitrate-nitrogen
7. Silicate-silicon
8. Sediment temperature
9. Sediment pH
10. Organic carbon
11. Organic matter
12. Sediment phenolics
13. Total aerobic heterotrophs

Station - I

The results of this analysis for the period March 1990-August 1991, in Station I are presented as correlation matrix (Table 19).

Water temperature is correlated with sediment temperature (r = 0.58490) and it is also correlated with the total aerobic heterotrophs (r = 0.37550) of the sediment.

The salinity of the water in coconut retting area is correlated with phosphate-phosphorous (r = 0.33821). It is negatively correlated with organic carbon (r = -0.53808), organic matter (r = -0.57048) and sediment phenolics (r = 0.70746). The salinity is again correlated with the total aerobic heterotrophs (r = 0.76783).

The dissolved oxygen of the station showed no correlation with any of the parameters studied.

The pH of the water showed negative correlation with nitrate-nitrogen (r = -0.44566) and influenced sediment pH positively (r = 0.89564).

The values of phosphate-phosphorous was positively correlated with the total aerobic heterotrophs in the station (r=0.46584).

Nitrate nitrogen had negative influence on sediment pH (r = -0.46193).

Water silicate had no significant relation with the parameters studied in this station.

Sediment temperature was negatively correlated with organic carbon (r = -0.46160) and sediment phenolics (r = -0.33636). It has positive relation with aerobic heterotrophs of the station (pH = -0.49171).

Table 19 CORRELATION MATRIX of environmental parameters for Station I

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.00000												
2.	0.24585	1.00000											
3.	0.26081	-0.08567	1.00000										
4.	-0.17279	0.16239	-0.17658	1.00000									
5.	0.08567	0.3381*	0.06234	-0.28411	1.00000								
6.	-0.00901	-0.14021	0.32218	-0.44566*	-0.02849	1.00000							
7.	-0.14354	-0.00636	-0.19245	-0.24746	0.27482	0.21907	1.00000						
8.	0.58490*	0.35170*	0.18179	-0.09999	0.22341	-0.20223	-0.07325	1.00000					
9.	-0.14914	0.24931	-0.16975	0.89564*	-0.18171	-0.46193*	-0.29001	-0.04258	1.00000				
10.	-0.22322	-0.53808	-0.26097	0.23155	0.19551	0.02785	0.12439	-0.49149*	0.08695	1.00000			
11.	-0.20350	-0.57048*	-0.21808	0.22786	-0.17030	-0.00614	0.09398	-0.46160	0.11588	0.96098*	1.00000		
12.	-0.11598	-0.70746	0.28419	-0.03330	-0.22130	0.07934	0.03115	-0.33636	-0.09737	0.61238*	0.62181*	1.00000	
13.	0.37550	0.76783*	0.01473	0.16124*	0.46584*	-0.28232	-0.22974	0.49171*	0.16752	0.47268*	-0.46451*	-0.64346*	1.00000

Critical value (2-tail, 0.05) = +/- 0.32860 * Significant correlation

The organic carbon content of the sediment was directly influenced by the organic matter (r = -0.96098) and sediment phenolics (r = -0.61238), whereas the total aerobic heterotrophs were negatively influenced by it (r = -0.47268).

The organic matter was influencing sediment phenolics positively (r = -0.62181) and aerobic heterotrophs negatively in the station (r = -0.46451).

The sediment phenolics had a native influence on the aerobic heterotrophs (r = -0.64346).

The environmental parameters which had significant correlation with phenolics content in the sediment were further analysed statistically and a multiple regression equation was fitted (Table 20). From the values it show that salinity had maximum influence ($\hat{r}2$ = 0.2972) on the distribution of phenolics, then organic matter ($\hat{r}2$ = 0.1260). The coefficient of determinants (R^2) of the three variables (salinity, organic, matter and sediment temperature) was about 57.11%. Similarly the parameters which had correlation with total aerobic heterotrophs were also analysed for multiple regression equation (Table 21). Salinity had maximum influence on total heterotrophs. The other factors were water temperature, phosphate-phosphorous, sediment temperature and organic matter. The coefficient of determinants (R^2) of the variables was 69.12%.

Table 20 REGRESSION ANALYSIS - Sediment phenolics Station - I

Dependent Variable_Phenolics

Variables	Regression Coefficient	SE	T(DE=32)	Prob	Partial $\hat{r}2$
Salinity	-0.7622	0.2072	-3.679	0.00086	0.2972
Org.Mat	2.7211	1.2666	2.148	0.03936	0.1260
Sed.Temp.	-0.0214	0.6972	-0.031	0.97572	2.94021 E-05
Constant	-1.8259				

Std.Error of Est = 4.8836
Adjusted R squared = 0.5309
R Squared = 0.5711
Multiple R = 0.7557

Table 21 REGRESSION ANALYSIS - Total aerobic heterotrophs - Station I

Dependent variable_ Heterotrophs

Variables	Regression Coefficient	SE	T (DF = 30)	Prob.	Partial $\hat{r}2$
Wat Temp.	3.8981	4.6417	0.840	0.40766	0.0230
Salinity	5.9892	1.2518	4.784	0.00004	0.4328
PO_4 - P	240.7705	124.8088	1.929	0.06322	0.1104
Org.Mat.	1.7747	7.3681	0.241	0.81130	0.0019
Sed.Temp.	6.1418	4.8844	1.257	0.21830	0.0501
Constant	-240.4672				

Std.Error of Est = 28.0399
Adjusted R squared = 0.6398
R Squared = 0.6912
Multiple R = 0.8314

Station - II

The correlation matrix for station II is given in Table 22 for the physico-chemical parameters studied during 1990-91 period.

Water temperature had significant correlation with salinity (r = 0.47320) and total heterotrophs (r = 0.38741). There was an indirect correlation with silicate content in the water (r = -0.48720).

Salinity of the water was negatively correlated with nitrate-nitrogen (r = -0.56558) and sediment phenolics (r = -0.76103). It had significant correlation with sediment temperature (r = 0.50659).

The dissolved oxygen in the mangrove ecosystem had significant influence on the phosphate-phosphorous (r = 0.51921).

The hydrogen ion concentration (pH) of the station had significant correlation with the sediment pH (r = 0.73536) and sediment temperature (r = 0.49433). It had shown a negative correlation with silicate content of the water (r = -0.38966).

Phosphate-phosphorous of the station had significant correlation with sediment temperature (r = 0.40812), organic carbon (r = 0.45085) and organic matter (r = 0.45034).

The nitrate-nitrogen of the station showed significant influence on sediment phenolics (r = 0.51853) and inverse influence on total aerobic heterotrophs (r = -0.33586).

Table 22 CORRELATION MATRIX of environmental parameters for Station II

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.00000												
2.	0.47320*	1.00000											
3.	-0.10519	-0.33630*	1.00000										
4.	0.23051	0.22480	-0.25861	1.00000									
5.	-0.28151	-0.46549*	0.51921*	-0.29069	1.00000								
6.	-0.03773	-0.56558*	0.03867	0.05859	0.13614	1.00000							
7.	-0.48270*	-0.24103	0.21469	-0.38966*	0.22882	0.19803	1.00000						
8.	0.75032	0.50659*	-0.23071	0.49433*	0.40812*	0.20473	0.53523*	1.00000					
9.	0.12564	0.06539	0.05563	0.73536*	0.04596	0.03698	0.24864	0.22369	1.00000				
10.	0.28694	0.29605	0.29696	0.02912	0.45085	-0.13674	0.00653	0.02576	0.33680*	1.00000			
11.	0.28813	0.29687	0.29698	0.02922	0.45034	-0.13696	0.00676	0.02592	0.33707*	0.9999*	1.00000		
12.	-0.40995*	-0.76103*	-0.18614	-0.10032	0.03045	0.51853*	0.21157	0.34244*	0.15363	0.64752*	0.64811*	1.00000	
13.	0.38741*	0.64046*	-0.17680	0.17140	0.22281	-0.33586*	0.45813*	0.35437*	0.04780	0.07858	0.07916	-0.58696*	1.00000

Critical value (2-tail, 0.05) = +/- 0.32860 * Significant correlation

Silicate value showed significant positive influence on aerobic heterotrophs (r = 0.45813) of the station. The sediment temperature had significant correlation with sediment phenolics (r = 0.34244) and aerobic heterotrophs (r = 0.35437).

The sediment pH of this station was significantly influencing the organic carbon (r = 0.33680) and organic matter (r = 0.33707). Organic carbon influenced organic matter significantly (r = 0.9999) and also sediment phenolics (r = 0.64752). In this station, phenolic content negatively correlated with the aerobic heterotrophs (r = -0.58496). The organic matter had significant negative influence on sediment phenolics of the station.

The physico-chemical parameters which had significant influence with phenolics of the station were further analysed and multiple regression equation was obtained (Table 23). The values obtained show that organic matter had maximum correlation ($\hat{r}2$ = 0.4522) followed by salinity ($\hat{r}2$ = 0.3638), nitrate-nitrogen ($\hat{r}2$ = 0.626). The coefficient of determinants (R^2) of the variables was about 79.12%. Likewise, in the case of total aerobic heterotrophs also, multiple regression analysis was done (Table 24). Salinity had maximum influence ($\hat{r}2$ = 0.3165) followed by water silicate ($\hat{r}2$ = 0.1955), sediment temperature ($\hat{r}2$=0.3638) and nitrate-nitrogen ($\hat{r}2$ = 0.0104). The coefficient of determinants (R^2) of the above variables was 53.42%.

Station - III

The influence of various environmental parameters is given in the correlation matrix Table 25.

Table 23 REGRESSION ANALYSIS - Sediment phenolics station - II

Dependent Variable-Phenolics

Variables	Regression Coefficient	SE	T(DE=32)	Prob	Partial $\hat{r}2$
Wat.Temp.	0.0416	0.5051	0.082	0.93488	2.26247E-04
Salinity	-0.4241	0.1024	-4.142	0.00026	0.3638
No_3-N_2	19.0617	13.4727	1.415	0.16741	0.0626
Org.Mat	-1.4376	0.2889	-4.976	0.00002	0.4522
Sed.Temp.	-0.1685	0.5115	-0.329	0.74410	0.0036
Constant	19.6134				

Std.Error of Est = 2.7823
Adjusted R squared = 0.7564
R Squared = 0.7912
Multiple R = 0.8895

Table 24 REGRESSION ANALYSIS - Total aerobic heterotrophs-Station - II

Dependent Variable-Heterotrophs

Variables	Regression Coefficient	SE	T(DF= 30)	Prob	Partial r2
Wat Temp	0.5041	3.5281	0.143	0.88734	6.70986
Salinity	2.8070	0.7531	3.727	0.00080	0.3165
NO_3 - N_2	57.1137	101.5205	0.563	0.57790	0.0104
Silicate	-21.2962	7.8881	-2.700	0.01129	0.1955
Sed. Temp	-3.9020	3.6429	-1.071	0.29265	0.0368
Constant	152.7506				

Std. Error of Est = 20.5110
Adjusted R Squared = 0.4565
R Squared = 0.5342
Multiple R = 0.7309

Water temperature of the station had significant correlation with sediment temperature (r = 0.59115) and silicate (r = -0.33069).

Salinity of the station was significantly correlated with aerobic heterotrophs (r = 0.41832), dissolved oxygen (r = 0.57424), nitrate-nitrogen (r = -0.43885), organic carbon (r = -0.67815), organic matter (r = -0.67865) and sediment phenolics (r = 0.35491).

The dissolved oxygen content of the water had been shown to be significantly influence organic carbon (r = 0.72158) and organic matter (r = 0.72190) content of the station.

The water pH of the station was significantly correlated with sediment pH (r = 0.91793) and aerobic heterotrophs (r = 0.41636). Phosphate-phosphorous was not significantly correlated with any of the factors. Nitrate-nitrogen had significant influence on organic carbon (r = 0.46796), organic matter (r = 0.46640), sediment phenolics (r = -0.38288) and aerobic heterotrophs (r = -0.41911).

Silicate content of the water showed significant positive correlation on organic carbon (r = 0.48540) and organic matter (r = 0.48616). The sediment temperature had a significant negative influence on aerobic heterotrophs (r = - 0.37756). Organic carbon had significant influence on organic matter (r = 0.9998) and aerobic heterotrophs (r = 0.37786). Organic matter influenced aerobic heterotrophs significantly (r = -0.37756).

Table 25 CORRELATION MATRIX of environmental parameters for Station III

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.00000												
2.	0.31222	1.00000											
3.	-0.29308	-0.57424*	1.00000										
4.	-0.01121	-0.05700	-0.11148	1.00000									
5.	-0.26408	-0.20263	0.65482*	-0.34163*	1.00000								
6.	-0.15220	-0.43885*	0.35085*	0.07922	0.02617	1.00000							
7.	-0.33069*	-0.28520	0.50281*	-0.31100	0.35676*	0.12520	1.00000						
8.	-0.59115*	0.14156	-0.21849	0.09049	-0.29963	-0.30547	-0.01100	1.00000					
9.	0.14161	-0.10211	-0.06718	-0.91793*	-0.33624*	0.09760	-0.28996	0.13063	1.00000				
10.	-0.16368	-0.67815*	0.72158*	-0.06160	0.31616	0.46796*	0.48540*	-0.09139	0.01520	1.00000			
11.	-0.16441	-0.67865*	0.72190*	-0.06131	0.31665	0.46640*	0.48616*	-0.09182	0.01489	0.9998*	1.00000		
12.	-0.11696	-0.35491*	-0.35932*	0.09094	-0.32133	-0.38288*	-0.10871	0.14975	0.05381	0.066770	0.06665	1.00000	
13.	-0.00722	0.41832*	-0.16402	0.41636*	-0.03799	-0.41911*	-0.13639	0.16756	0.45968*	-0.37786*	-0.37756	0.19636	1.00000

Critical value (2-tail, 0.05) = ± 0.32860; * Significant Correlation

Table 26 REGRESSION ANALYSIS - Sediment phenolics-Station-II

Dependent Variable-Phenolics

Variables	Regression Coefficient	SE	T(DF=30)	Prob	Partial r2
Diss O_2	-0.2454	0.2442	-1.005	0.32250	0.0306
Salinity	0.0285	0.0439	0.648	0.52144	0.0130
NO_3 - N_2	-5.2466	3.5842	-1.464	0.15300	0.0628
Constant	2.6396				

Std. Error of Est = 1.6194
Adjusted R Squared = 0.1410
R Squared = 0.2146
Multiple R = 0.4633

Table 27 REGRESSION ANALYSIS - Total aerobic heterotrophs-Station III

Dependent Variable-Heterotrophs

Variables	Regression Coefficient	SE	T(DF=30)	Prob	Partial r2
Wat pH	-7.5747	14.6341	-0.449	0.65646	0.0067
Salinity	1.4007	0.7886	1.776	0.08583	0.0952
NO_3 - N_2	-132.2029	61.0090	-2.167	0.03831	0.1353
Org. Mat	-0.6553	3.3879	-0.193	0.84793	0.0012
Wed. pH	32.7338	15.6589	2.027	0.05168	0.1204
Constant	-86.9335				

Std. Error of EST = 26.7512
Adjusted R Squared = 0.4389
R Squared = 0.5190
Multiple R = 0.7204

The environmental parameters showing significant correlation with sediment phenolics were analysed to obtain a multiple regression equation as is given in Table 26. Nitrate-nitrogen had maximum influence ($\hat{r}2$ = 0.0628) on the distribution of phenolics, then dissolved oxygen ($\hat{r}2$ = 0.0306) and salinity ($\hat{r}2$ = 0.0130). The coefficient of determinants (R^2) of the above three variables was about 21.46%. The multiple regression analysis for parameters which had significant influence on the distribution of total aerobic heterotrophs has shown the order, of importance as nitrate - nitrogen ($\hat{r}2$ = 0.1353), sediment pH ($\hat{r}2$ = -0.1204), salinity ($\hat{r}2$ = 0.0952), water pH ($\hat{r}2$ = 0.0067) and organic matter ($\hat{r}2$ = 0.0012). The coefficient of determinants (R^2) was found to be 51.90% for the independent variables (Table 27).

Station - IV

The correlation matrix for station IV is given in Table 28.

Water temperature of the station, significantly influenced the pH (r = 0.37457), sediment temperature (r = 0.89641), sediment phenolics (r = 0.63300) and nitrate-nitrogen (r = -0.48922).

The water salinity had significant correlation with nitrate-nitrogen (r = 0.37594) water pH (r = -0.36990), sediment pH (r = -0.47046) organic carbon (r = -0.84254), organic matter (r = -0.84254), and total aerobic heterotrophs (r = 0.66553). The dissolved oxygen in the station showed significant correlation with silicate content of the water (r = 0.41605) and aerobic heterotroph count (r = 0.8060).

Table 28 CORRELATION MATRIX of environmental parameters of Section IV

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.00000												
2.	-0.03767	1.00000											
3.	0.27120	-0.00498	1.00000										
4.	0.37457*	-0.35990*	-0.14750	1.00000									
5.	0.09281	0.16493	0.11073	0.13443	1.00000								
6.	-0.48922*	0.37594*	-0.04994	0.05497	-0.18782	1.00000							
7.	0.24685	-0.22878	-0.41605*	0.38835*	0.08682	0.09809	1.00000						
8.	0.89641*	-0.14341	0.22794	0.42190*	0.30074	-0.45430*	0.21980	1.00000					
9.	0.25763	-0.47046	0.12437	0.84031*	-0.13062	0.09422	0.30239	0.39934*	1.00000				
10.	-0.14049	0.84254	-0.06163	0.35053*	-0.28303	0.46312*	0.06962	-0.01884	0.50762*	1.00000			
11.	-0.14179	-0.84254	-0.06246	0.34843*	0.28325	0.46389*	0.06805	0.02001	0.50650*	0.9995*	1.00000		
12.	0.63300*	-0.03309	-0.22336	0.32889*	0.12590	0.11877	0.39411*	0.58716*	0.33519*	0.10454	0.10470	1.00000	
13.	0.04121	0.66553	-0.0806	0.01722	0.22381	0.34319	-0.11734	0.06282	-0.16133	-0.55115*	-0.55416*	-0.18737	1.00000

Critical value (2-tail, 0.05) = ± 0.32860; * Significant Correlation

The pH of the seasonal aquaculture pond showed significant influence on silicate (r = 0.38835), sediment temperature (r = 0.42190), sediment pH (r = 0.84031), organic carbon (r = 0.35053), organic matter (r = 0.34843) and sediment phenolics (r = 0.32889). The phosphate-phosphorous in the ponds did not show any significant influence on any of the parameters studied. Nitrate-nitrogen showed significant influence on organic carbon (r = 0.46312), organic matter (r = 0.46389), aerobic heterotrophs (r = 0.34319) and sediment temperature (r = -0.45430). The silicate content of the water had no significant influence on any of the factors studied.

The sediment temperature significantly influenced both sediment pH (r = 0.39931) and sediment phenolics (r = 0.58715). The hydrogen ion concentration (pH) of the sediment had significant influence on organic matter (r = 0.50650), organic carbon (r = 0.50762) and sediment phenolics (r = 33519), organic carbon was significantly correlated with organic matter (r = 0.9995) and total aerobic heterotrophs (r = -0.55115). Organic matter of the sediment had a significant negative correlation on the total aerobic heterotrophs of the station (r = -0.55416).

The physico-chemical parameters which had significant correlation with sediment phenolics of the seasonal aquaculture pond were again statistically analysed for obtaining a multiple regression equation Table 29. From the results it is known that water temperature ($\hat{r}2$ = 0.1332), sediment pH ($\hat{r}2$ = 0.0534) water = pH ($\hat{r}2$ = 0.0165) and sediment temperature ($\hat{r}2$ = 0.043) had significant influence on phenol distribution of the station. The coefficient determinants (R^2) for the above variables was obtained as 44.25%. Similarly

Table 29 REGRESSION ANALYSIS - Sediment phenolics-Station-IV

Dependent Variable-Phenolics

Variables	Regression Coefficient	SE	T(DF=30)	Prob	Partial r2
Wat Temp.	0.1496	0.0685	2.183	0.03674	0.1332
Wat pH	-0.1063	0.1476	-0.720	0.47674	0.0165
Sed pH	0.1903	0.1439	1.322	0.19577	0.0534
Sed Temp	-0.0298	0.0814	-0.366	0.71698	0.0043
Constant	-3.5934				

Std Error of Est = 0.3973
Adjusted R Squared = 0.3706
R Squared = 0.4425
Multiple R = 0.6652

Table 30 REGRESSION ANALYSIS - Total aerobic heterotrophs-Station-IV

Dependent Variable-Heterotrophs

Variables	Regression Coefficient	SE	T(DF=30)	Prob	Partial r^2
Salinity	2.4672	0.8657	2.850	0.00759	0.2025
NO_3 - N_2	-63.1001	75.7070	0.833	0.41075	0.0212
Org. Mat	1.1032	3.2919	0.335	0.73973	0.0035
Constant	38.3550				

STD Error of Est = 16.7674
Adjusted R Squared = 0.4038
R Squared = 0.4549
Multiple R = 0.6745

the variables influencing distribution of aerobic heterotrophs of the station were analysed for multiple regression equation. It is found that salinity ($\hat{r}2$ = 0.2025), nitrate - nitrogen ($\hat{r}2$ = 0.0212) and organic matter ($\hat{r}2$ = 0.0035) had influenced distribution of aerobic heterotrophs in the station as in the given order. The coefficient determinants (R^2) for the parameters was found to be 45.49% (Table 30).

Station - V

The influence of various physico-chemical parameters for perennial aquaculture pond is given in correlation matrix Table 31. The water temperature of the pond had significant influence on water pH (r = 0.35911), nitrate-nitrogen (r = 0.40793), silicate (r = 0.40163) and sediment temperature (r = 0.90658). The salinity had significant correlation with sediment phenolics (r = 0.36927) and total aerobic heterotrophs (r = 0.40388).

The dissolved oxygen of the station had significantly influenced nitrate-nitrogen (r = 0.52645) alone. Water pH had a significant correlation with silicate (r = 0.45341), sediment temperature (r = 0.41337) and sediment pH (r = 0.7644).

The phosphate-phosphorous of the water had no significant influence on any of the parameters investigated during the period of study. Nitrate-nitrogen had significant correlation with silicate (r = -0.34703), sediment temperature (r = -0.36848), sediment pH (r = 0.36752) and aerobic heterotrophs (r = -0.35478). Silicate in the water had significant correlation with sediment temperature (r = -0.33445) and sediment phenolics (r = 0.41320). Sediment

Table 31 CORRELATION MATRIX of environmental parameters for Station V

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.00000												
2.	0.14788	1.00000											
3.	0.16906	0.06930	1.00000										
4.	0.35911*	-0.28813	-0.21950	1.00000									
5.	0.09017	0.29280	0.09208	-0.02923	1.00000								
6.	-0.40793*	-0.31268	0.52645*	-0.35619*	0.04432	1.00000							
7.	0.40163*	-0.21216	-0.25150	0.45341	-0.02386	-0.34703*	1.00000						
8.	0.90658*	0.09182	0.16259	0.41337*	0.23715	-0.36848*	0.33445*	1.00000					
9.	0.29406	-0.26669	-0.16556	0.7644*	-0.02895	-0.36752*	0.15445	0.44426*	1.00000				
10.	0.32539	0.04283	0.18469	0.26461	0.19128	0.21548	0.11575	0.33030*	-0.18759	1.00000			
11.	0.32534	0.04055	0.18239	0.26193	0.19167	0.21683	0.11651	0.33201*	-0.18521	0.99992*	1.00000		
12.	0.17147	0.36927*	0.25445	0.05371	0.09219	0.31563	0.41320*	0.18133	0.00314	0.27191	0.27179	1.00000	
13.	0.13943	0.40388*	-0.27900	0.09817	-0.15016	-0.35478*	0.00765	-0.22831	0.11062	-0.40960*	-0.41302*	-0.19721	1.00000

Critical value (2-tail, 0.05) = ± 0.32860; * Significant Correlation

temperature had significant influence on sediment pH (r = 0.44426), organic carbon (r = 0.33030) and organic matter (r = 0.33201) of the station. The sediment pH had no significant influence on any of the parameters studied. Organic carbon content influenced organic matter (r = 0.9992) of the station and also total aerobic heterotrophs (r = 0.40960) organic matter had significant correlation with total aerobic heterotrophs. The sediment phenolics had no significant influence on any of the parameters studied.

The multiple regression analysis of parameters which had significant influence on total phenolics of the sediment in perennial aquaculture ponds had shown that salinity ($\hat{r}2$ = 0.0901) and nitrate-nitrogen ($\hat{r}2$ = 0.0514) are more important Table 32 The coefficient determinants (R^2) had given a value of 18.08% .

Factors influencing aerobic heterotrophs significantly were also analysed for obtaining a multiple regression equation Table 33. Salinity ($\hat{r}2$ = 0.1567), nitrate-nitrogen ($\hat{r}2$ = 0.0877), organic matter ($\hat{r}2$ = 0.0863) and sediment temperature ($\hat{r}2$ = 0.0831) were the parameters which influenced aerobic heterotrophs of perennial aquaculture pond. The coefficient determinants (R^2) of these variables was obtained as 42.09% .

Table 32 REGRESSION ANALYSIS - Sediment phenolics-Station-IV

Dependent Variable-Phenolics

Variables	Regression Coefficient	SE	T(DF=30)	Prob	Partial r2
Salinity	-0.0912	0.0106	-1.808	0.07974	0.0901
NO_3 - N_2	1.2217	0.9135	1.337	0.19021	0.0514
Constant	0.8419				

STD Error of Est = 0.3658
Adjusted R Squared = 0.1311
R Squared = 0.1808
Multiple R = 0.4252

Table 33 REGRESSION ANALYSIS - Total aerobic heterotrophs-Station-V

Dependent Variable-Heterotrophs

Variables	Regression Coefficient	SE	T(DF=30)	Prob	Partial r2
Salinity	1.2700	0.5291	2.400	0.02257	0.1567
NO_3 - N_2	-91.1234	52.7917	-1.726	0.09426	0.0877
Org. Mat	6.6682	3.8962	-1.711	0.09698	0.0863
Sed Temp	3.2912	1.9640	-1.676	0.10384	0.0831
Constant	178.7989				

STD Error of Est = 0.3658
Adjusted R Squared = 0.1311
R Squared = 0.4209
Multiple R = 0.6488

3.1.4 Distribution and composition of aerobic heterotrophs

Random colonies were selected from the culture plates, sub-cultured and maintained in agar slants for further identification purposes. The scheme of Simidu and Aiso (1962) Table 34 was followed for identification upto generic level, of the isolates. The microbial flora in the sampling Stations (I to V) are constituted of brackish water, fresh water and intermediate forms.

Station - I

The genera isolated from the coconut retting area are *Aeromonas, Alcaligenes, Bacillus Enterobacteriaceae, Flavobacterium, Pseudomonas* (Group III and IV), *Vibrio* and *Micrococcus*. The observations showed that *Pseudomonas* and *Vibrio* were the most frequently occurring genera in the station. *Bacillus* was the least abundant genera. All the genera were not present in all the seasons. The generic diversity was more during post-monsoon months. The percentage distribution of aerobic heterotrophs in coconut retting area is given in Fig.15.

Station - II

In the mangrove ecosystem, the micro-flora identified include all those genera found present in Station - I. Apart from them, *Pseudomonas* Group II was also isolated. The most frequent ones were *Vibrio* and *Pseudomonas*. *Alcaligenes* was very few in abundance and occurrence. More generic diversity was observed during pre-monsoon month. The percentage distribution of aerobic heterotrophs in Station II is given in Fig.16.

Fig. 15 : Showing monthly percentage distribution of aerobic heterotrophs in station I

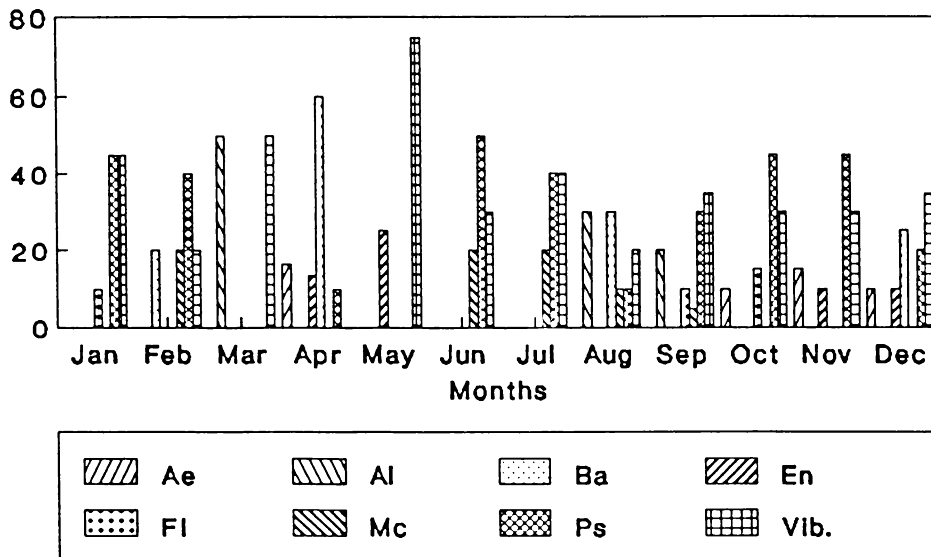
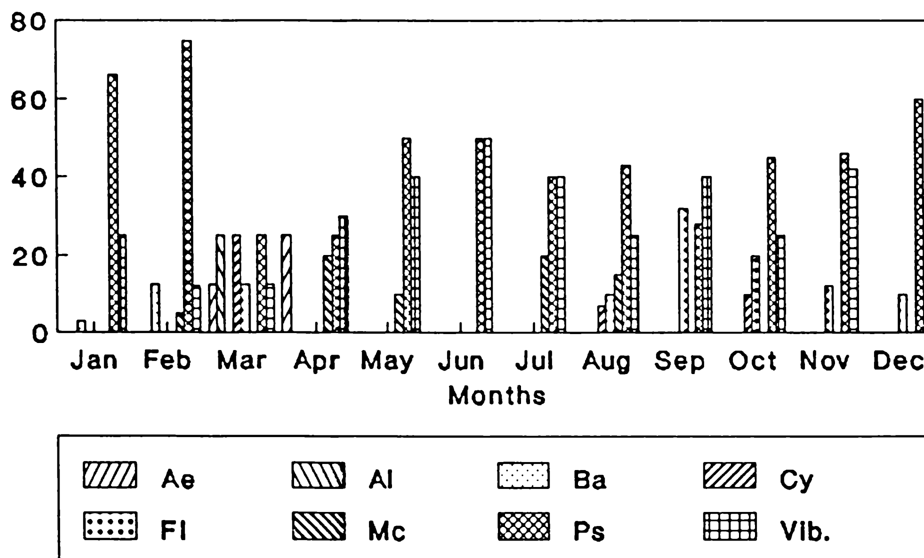


Fig. 16 : Showing monthly percentage distribution of aerobic heterotrophs in station II



Ae *Aeromonas* ; Al - *Alcaligenes* ; Ba - *Bacillus* ; Cy - *Cytophaga* ; En - *Enterobacteriaceae* ; Fl - *Flavobacterium* ; Mc - *Micrococcus* ; Ps - *Pseudomonas* ; Vib - *Vibrio*

Station - III

In this station, maximum generic diversity of heterotrophs were found during post-monsoon months. *Pseudomonas, Vibrio* and *Alcaligenes* were reported more frequently. *Aeromonas* was very few in number in this station. Percentage distribution of aerobic heterotrophs in this station is given in Fig.17.

Stations - IV and V

Since the seasonal aquaculture ponds and perennial ponds were of close proximity to each other, the heterotrophic diversity were of similar pattern in these stations. The generic distribution was almost similar as in other three stations. But here, *Alcaligenes* and *Flavobacterium* were found to be more dominant than other genera. The percentage distribution is given in Fig.18.

3.1.5 Generic composition of aerobic heterotrophs

A total of 153 isolates were identified and briefly studied biochemically and physiologically and classified 10 genera from the five stations during September 1990 - August 1991, using the scheme of Simidu and Aiso (1962).

Gram negative asporogenous rods predominated forming 73.8% and highly pleomorphic in nature. Gram positive cocci were rare and formed only 13.0% of the total Gram positive long rods *(Bacillus)* formed 13.7%. Among the gram negative small rods *Alcaligenes* (14.38%) and *Pseudomonas* Group IV (14.38%) predominated among other genera. The genera *Alcaligenes* had peritrichous flagella, but all *Pseudomonas* had polar lophotrichous flagella.

Fig. 17 : Showing monthly percentage distribution of aerobic heterotrophs in station III

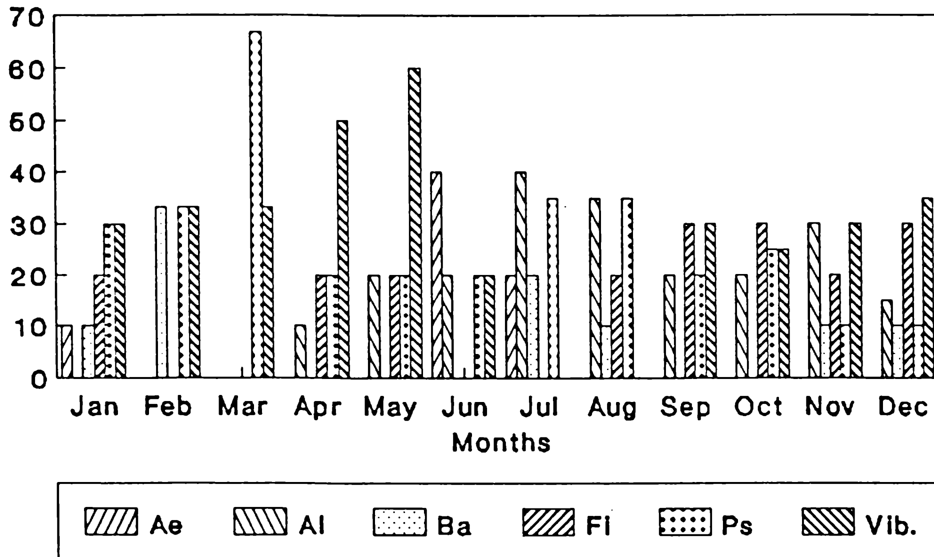
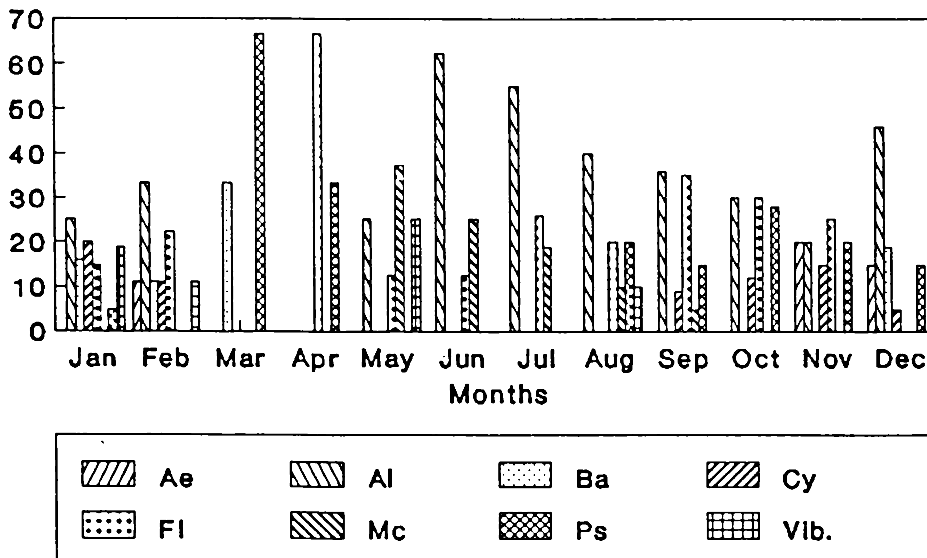
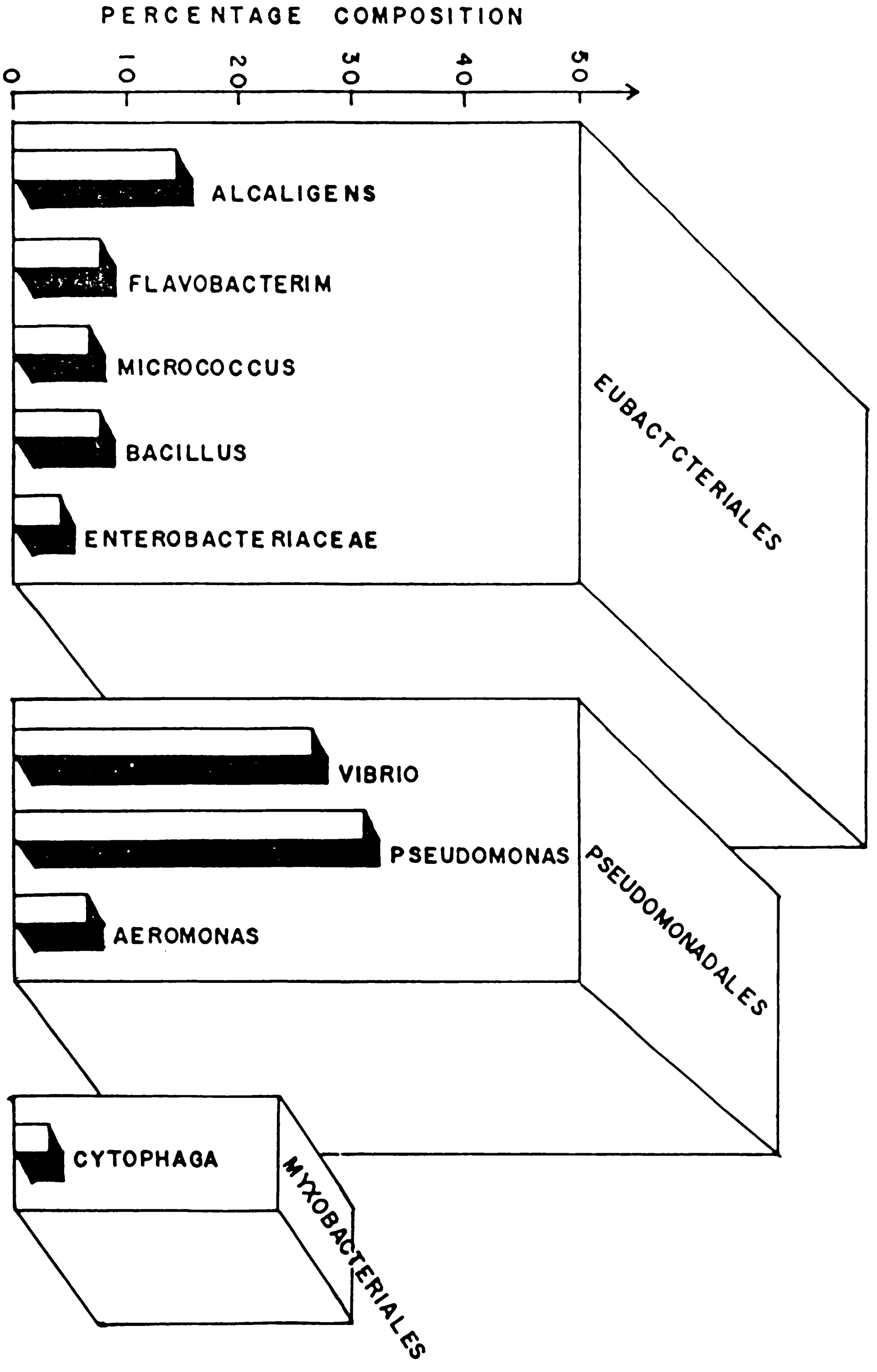


Fig. 18 : Showing monthly percentage distribution of aerobic heterotrophs in stations IV and V



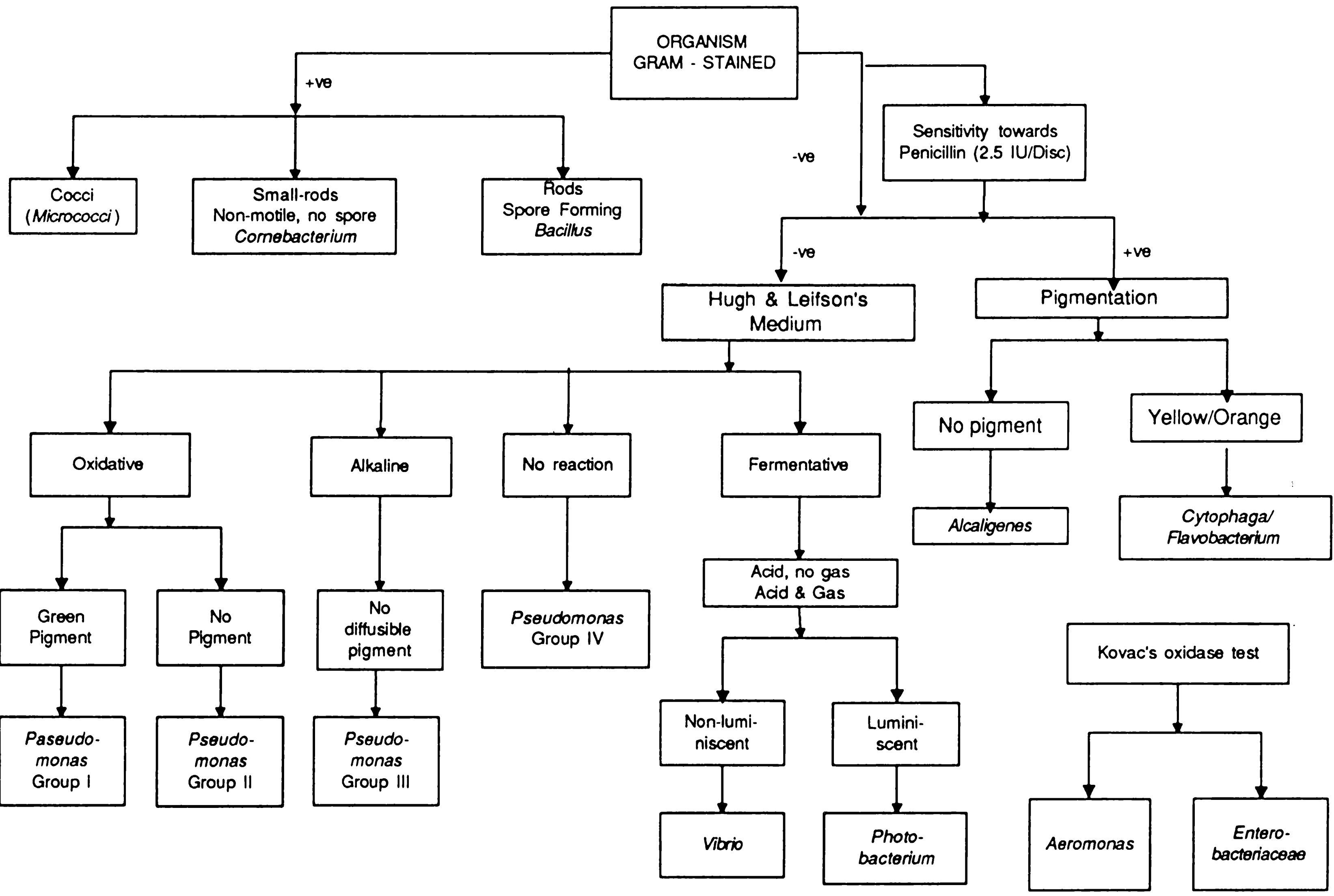
Ae *Aeromonas* ; Al - *Alcaligenes* ; Ba - *Bacillus* ; Cy - *Cytophaga* ; En - *Enterobacteriaceae* ; Fl - *Flavobacterium* ; Mc - *Micrococcus* ; Ps - *Pseudomonas* ; Vib - *Vibrio*

Fig.19 Showing generic composition of 153 bacterial strains isolated from the sampling stations during 1990-91



PERCENTAGE COMPOSITION
0
10
20
30
40
50
ALCALIGENS
FLAVOBACTERIM
MICROCOCCUS
BACILLUS
ENTEROBACTERIACEAE
EUBACTCTERIALES
VIBRIO
PSEUDOMONAS
AEROMONAS
PSEUDOMONADALES
CYTOPHAGA
MYXOBACTERIALES

Table 34 The outline of procedure for screening of cultures (Marine bacteria). Scheme of Usio Simidu and Kayuyoshi Aiso (1962)



Among *Pseudomonas*, 3 groups were identified. Group II formed 8.5% and Group III 7.19%. In total, *Pseudomonas* was outstandingly predominant compared to other genera (30.1%) and Vibrio formed 21.57% of the total Fig.19.

3.1.5.1 Percentage variation of bacterial genera

Aeromonas sp. was most abundant during monsoon month of June (40%) in Station III, whereas in other stations it appeared during post-monsoon months. Its occurrence was least in Station II. The monthly percentage distribution of *Aeromonas* sp.is given in Table 35.

Alcaligenes sp.the gram-negative small rods were more abundant in Stations IV and V which are culture ponds. It was abundant in Station III also, while in Stations I and II it was less frequent. The maximum percentage was observed in Station IV during June (62.5%). The monthly percentage variation of *Alcaligenes* sp.is given in Table 36.

The gram positive long rods, *Bacillus* sp. was not of much frequent occurrence in any of the stations. It was observed maximum from Station IV during March (33.4%). In Stations I and II it was very rare in occurrence. The monthly variation of the genera is given in Table 37.

Monthly percentage variation of *Cytophaga* sp. is given in Table 38. It was absent in Stations I and III during the period of observation.

Enterobacteriaceae had appeared only in Stations I and II Table 39. Of all the genera *Flavobacterium* was the most frequently occurred one in all the

Table 35 Monthly percentage variation of *Aeromonas* sp.

Months	Stations				
	I	II	III	IV	V
January	-	-	10	-	-
February	-	-	-	11.1	-
March	-	12.5	-	-	-
April	16.4	25	-	-	15
May	-	-	-	-	-
June	-	-	40	-	-
July	-	-	20	-	-
August	-	-	-	-	-
September	-	-	-	-	-
October	10	-	-	-	-
November	15	-	-	20	20
December	10	-	-	15	18

Table 36 Monthly percentage variation of *Alcaligenes*

Months	Stations				
	I	II	III	IV	V
January	-	-	-	25.0	25
February	-	-	-	33.3	-
March	50	25	-	-	-
April	-	-	10	-	20
May	-	-	20	25.0	20
June	-	-	20	62.5	55
July	-	-	40	55	40
August	30	-	35	40	35
September	20	-	20	36	20
October	-	-	20	30	30
November	-	-	30	20	17
December	-	-	15	46	30

Table 37 Monthly percentage variation of *Bacillus* sp.

Months	Stations				
	I	II	III	IV	V
January	-	3	10	16	20
February	20	12.5	33.3	11.1	20
March	-	-	-	33.1	28
April	-	-	-	-	20
May	-	-	-	-	-
June	-	-	-	-	-
July	-	-	20	-	-
August	-	-	10	-	-
September	-	-	-	-	-
October	-	-	-	-	-
November	-	-	10	-	-
December	-	-	10	19	12

Table 38 Monthly percentage variation of *Cytophaga*

Months	Stations				
	I	II	III	IV	V
January	-	-	-	20.0	10
February	-	-	-	11.1	-
March	-	12.5	-	-	-
April	-	-	-	-	-
May	-	-	-	-	-
June	-	-	-	-	-
July	-	-	-	-	-
August	-	7.0	-	-	-
September	-	-	-	9	12
October	-	10.0	-	12	10
November	-	-	-	15	15
December	-	-	-	5	10

Table 39 Monthly percentage variation of *Enterobacteriaceae*

Months	Stations				
	I	II	III	IV	V
January	-	-	-	-	-
February	-	-	-	-	-
March	-	12.5	-	-	-
April	13.6	-	-	-	-
May	25.0	-	-	-	-
June	-	-	-	-	-
July	-	-	-	-	-
August	-	-	-	-	-
September	-	-	-	-	-
October		-	-	-	-
November	10	-	-	-	-
December	10	-	-	-	-

Table 40 Monthly percentage variation of *Flavobacterium sp.*

Months	Stations				
	I	II	III	IV	V
January	10	-	20	15	20
February	-	-	-	22.2	-
March	-	-	-	-	60
April	60	-	20	66.6	25
May	-	-	20	12.5	20
June	-	-	-	12.5	15
July	-	-	-	26	40
August	30	10	20	20	20
September	10	32	30	35	35
October	15	20	30	30	30
November	-	12	20	25	28
December	25	10	30	-	10

Table 41 Monthly percentage variation of *Micrococcus*

Months	Stations				
	I	II	III	IV	V
January	-	-	-	-	-
February	20	5	-	-	-
March	-	-	-	-	-
April	-	20	-	-	-
May	-	10	-	37.5	30
June	20	-	-	25	35
July	20	20	-	19	20
August	10	15	-	10	15
September	5	-	-	5	15
October	-	-	-	-	-
November	-	-	-	-	-
December	-	-	-	-	-

Table 42 Monthly percentage variation of *Pseudomonas* Group II

Months	Stations				
	I	II	III	IV	V
January	-	16	-	5	-
February	-	12.5	-	-	66
March	-	-	-	66.6	-
April	-	-	-	33.4	20
May	-	40	-	-	-
June	-	-	-	-	-
July	-	10	-	-	-
August	-	18	-	20	20
September	-	10	-	15	18
October	10	20	-	28	30
November	15	15	-	20	20
December	10	20	-	15	15

Table 43 Monthly percentage variation of *Pseudomonas* Group III

Months	Stations				
	I	II	III	IV	V
January	-	-	-	-	-
February	-	-	-	-	-
March	-	12.5	-	-	-
April	-	-	33.34	-	-
May	-	20	-	-	-
June	-	50	-	-	-
July	20	30	-	-	-
August	-	25	-	-	-
September	-	18	-	-	-
October	-	25	-	-	-
November	-	15	-	-	-
December	-	-	-	-	-

Table 44 Monthly percentage variation of *Pseudomonas* Group IV

Months	Stations				
	I	II	III	IV	V
January	45	56	30	-	-
February	40	62.5	33.3	-	-
March	-	12.5	33.3	-	-
April	10	25	20	-	-
May	-	-	-	-	-
June	50	-	20	-	-
July	20	-	20	-	-
August	10	-	35	-	-
September	30	-	20	-	-
October	45	-	25	-	-
November	45	16	10	-	-
December	20	40	10	-	-

Table 45 Monthly percentage variation of *Vibrio* sp.

Months	Stations				
	I	II	III	IV	V
January	45	25	30	19	20
February	20	12.0	33.34	11.2	-
March	50	12.5	33.30	-	-
April	-	30	50	-	-
May	75	40	60	25	25
June	30	50	20	-	-
July	40	40	-	-	-
August	20	25	-	10	10
September	35	40	30	-	-
October	30	25	25	-	-
November	30	42	30	-	-
December	35	30	35	-	-

Table 46 Morphological and biochemical characteristics of 153 bacterial strains isolated from the sampling stations during september 1990 to august 1991

Characteristics		Frequency of occurrence (%)
1.	Gram negative rods	73.8
2.	Gram positive rods	13.7
3.	Gram positive cocci	13.0
4.	Motility	92.8
5.	No_3 reduction	66.0
6.	Pigmented strains	9.8
7.	Glucose metabolism	
	a. Oxidative metabolism	39.86
	b. Alkaline metabolism	7.19
	c. Fermentative "	38.56
	d. No. reaction "	14.37
8.	Indole positive	6.50
9.	Catalase metabolism	16.99
10.	Oxidase metabolism	70.59
11.	Penicillin sensitive	24.18

five stations. It was recorded maximum from Station IV during April (66.6%). The monthly percentage variation is given in Table 40.

The gram-positive cocci, *Micrococcus* sp. was absent in Station III. It was more abundant during monsoon season. The percentage variation of *Micrococcus* sp. is given in Table 41.

Pseudomonas sp. groups II, III and IV were reported from different stations during the period of study. Groups III and IV were absent in Stations IV and V in all the months. Group II was absent in Station I. Station II harboured maximum of *Pseudomonas* groups II and III. The monthly variations of *Pseudomonas* Groups II, III and IV are given in Tables 42, 43 and 44.

Vibrio sp. was present in all the five stations with maximum occurrence in Stations I and II. During post-monsoon months it was absent in Stations IV and V. The percentage variation of the genera is given in Table 45.

3.1.6 Morphological and biochemical characteristics of aerobic heterotrophs

All the strains isolated were motile except (8.2%) *Flavobacterium* and *Micrococcus*. 66.0% of the isolates were capable of reducing nitrate. Pigmented bacteria isolated formed 9.8% of the total isolates. Dextrose was fermented oxidatively (39.86%) and fermentatively (38.56%). Of the total isolates, 7.49% showed alkaline reaction and 14.37% had no reaction at all.

Indole was found to be positive in 72 hr. Culture in tryptone broth which may be due to the presence of *Enterobacteriaceae* (3.92%) among the isolates. Catalase production was there in 16.99% of the total isolates, and oxidase positive cultures formed 70.59%. Penicillin was found to be sensitive towards 24.18% of the isolates.

The morphological and biochemical characteristics of 153 bacterial strains isolated from various study areas from September 1990 to August 1991 is given in Table 46.

The relative percentage of predominant bacterial genera along with sediment phenolics, temperature, organic matter and total plate count for the five sampling stations are given in Figs.20,21,22, 23 and 24. In Stations I and II, when the bacterial count was low, the sediment phenolics increased along with organic matter content. In Stations III, IV and V, the sediment phenolics and temperature did not show much variation during the period of study, whereas, the organic matter content and total plate count showed a seasonal fluctuation with an inverse relationship with each other. The relative abundance of bacterial genera showed no seasonal pattern in any of the five stations.

3.2 Viability of aerobic heterotrophs in phenolic compounds

To assess the phenol degrading ability of aerobic heterotrophs 153 strains of bacteria isolated from the five sampling stations during September 1990 to August 1991, were tested for their viability at increasing concentrations of phenol (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0%).

Fig.20 Showing total plate count (TPC), relative percentage of predominant genera, sediment phenolics, temperature and organic matter for Station I

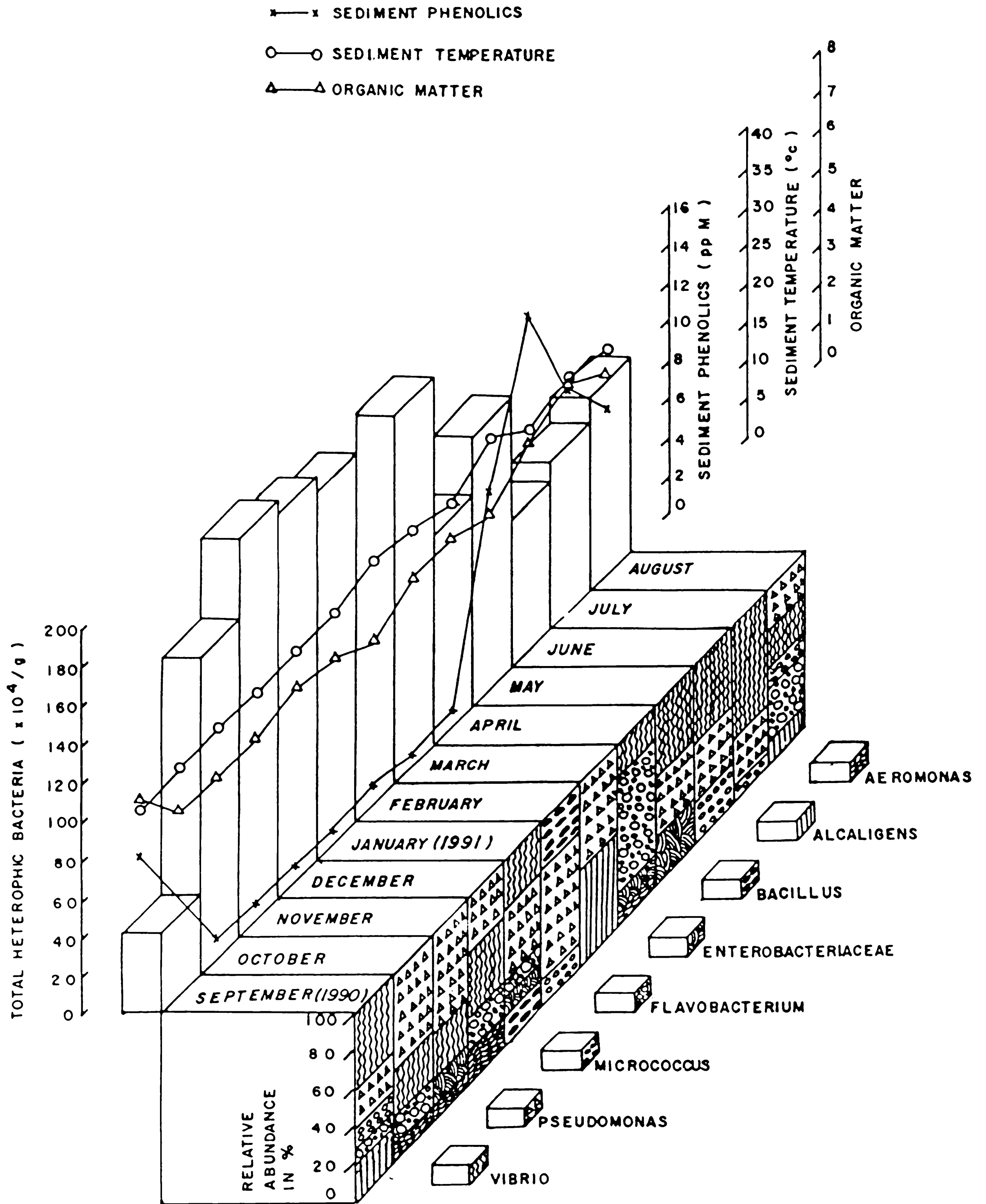
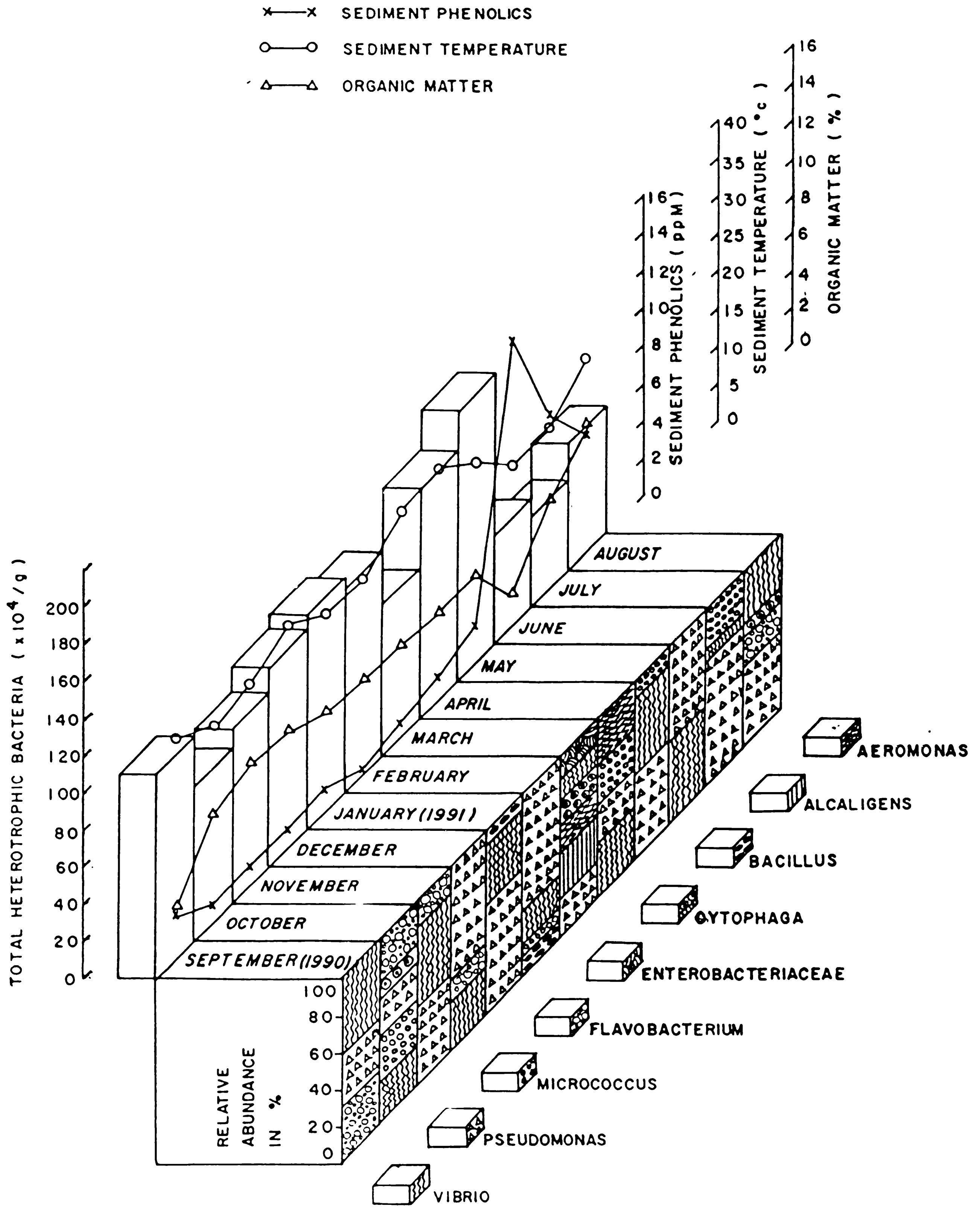

SEDIMENT PHENOLICS
SEDIMENT TEMPERATURE
ORGANIC MATTER
SEDIMENT PHENOLICS (pp M)
SEDIMENT TEMPERATURE (°c)
ORGANIC MATTER
TOTAL HETEROPHIC BACTERIA (x 10⁴/g)
SEPTEMBER (1990)
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1991)
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
RELATIVE ABUNDANCE IN %
AEROMONAS
ALCALIGENS
BACILLUS
ENTEROBACTERIACEAE
FLAVOBACTERIUM
MICROCOCCUS
PSEUDOMONAS
VIBRIO

Fig.21 **Showing total plate count (TPC), relative percentage of predominant genera, sediment phenolics, temperature and organic matter for Station II**



SEDIMENT PHENOLICS
SEDIMENT TEMPERATURE
ORGANIC MATTER
SEDIMENT PHENOLICS (ppM)
SEDIMENT TEMPERATURE (°c)
ORGANIC MATTER (%)
TOTAL HETEROTROPHIC BACTERIA (x 10⁴ / g)
SEPTEMBER (1990)
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1991)
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
RELATIVE ABUNDANCE IN %
AEROMONAS
ALCALIGENS
BACILLUS
CYTOPHAGA
ENTEROBACTERIACEAE
FLAVOBACTERIUM
MICROCOCCUS
PSEUDOMONAS
VIBRIO

Fig.22 **Showing total plate count (TPC), relative percentage of predominant genera, sediment phenolics, temperature and organic matter for Station III**

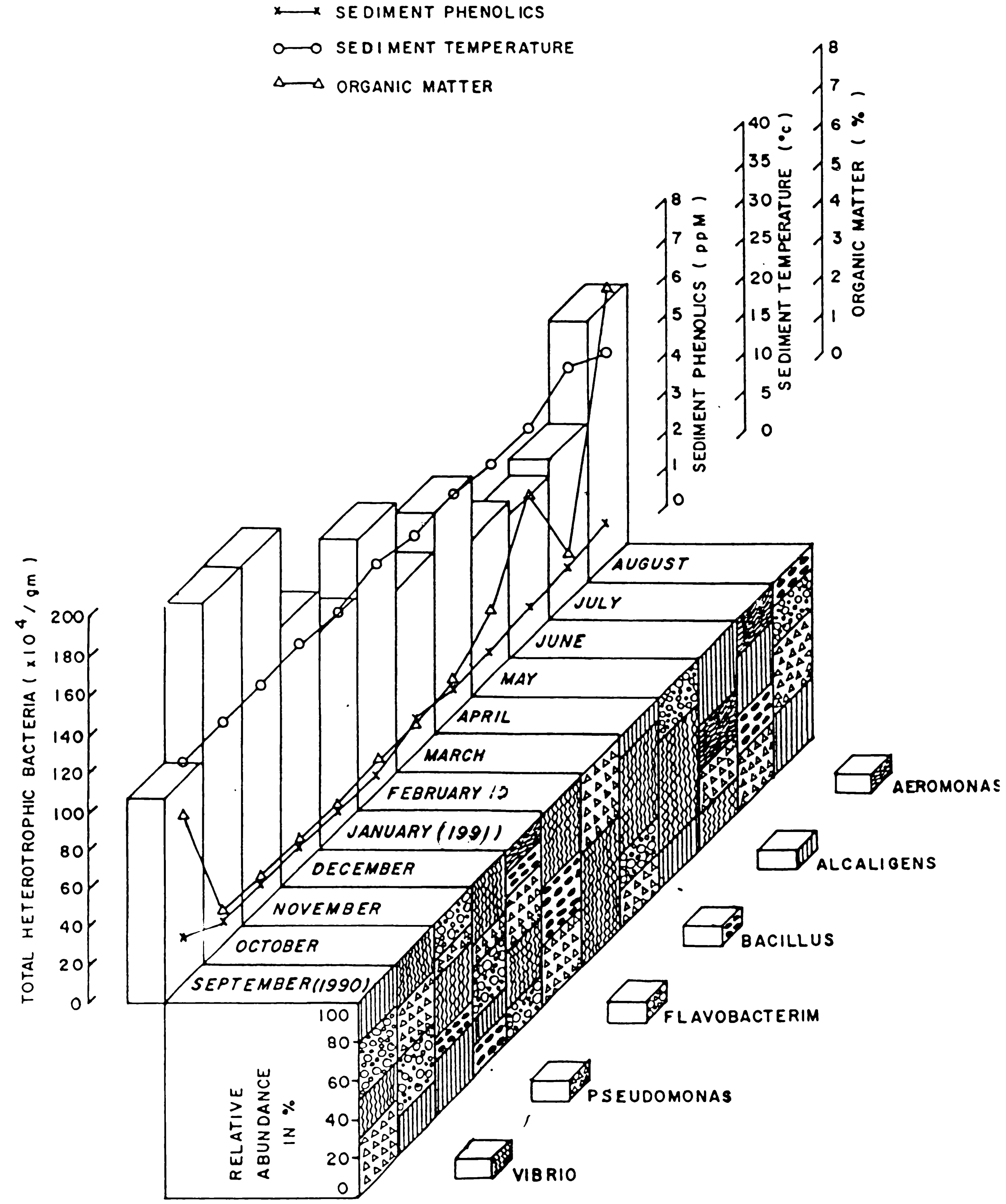
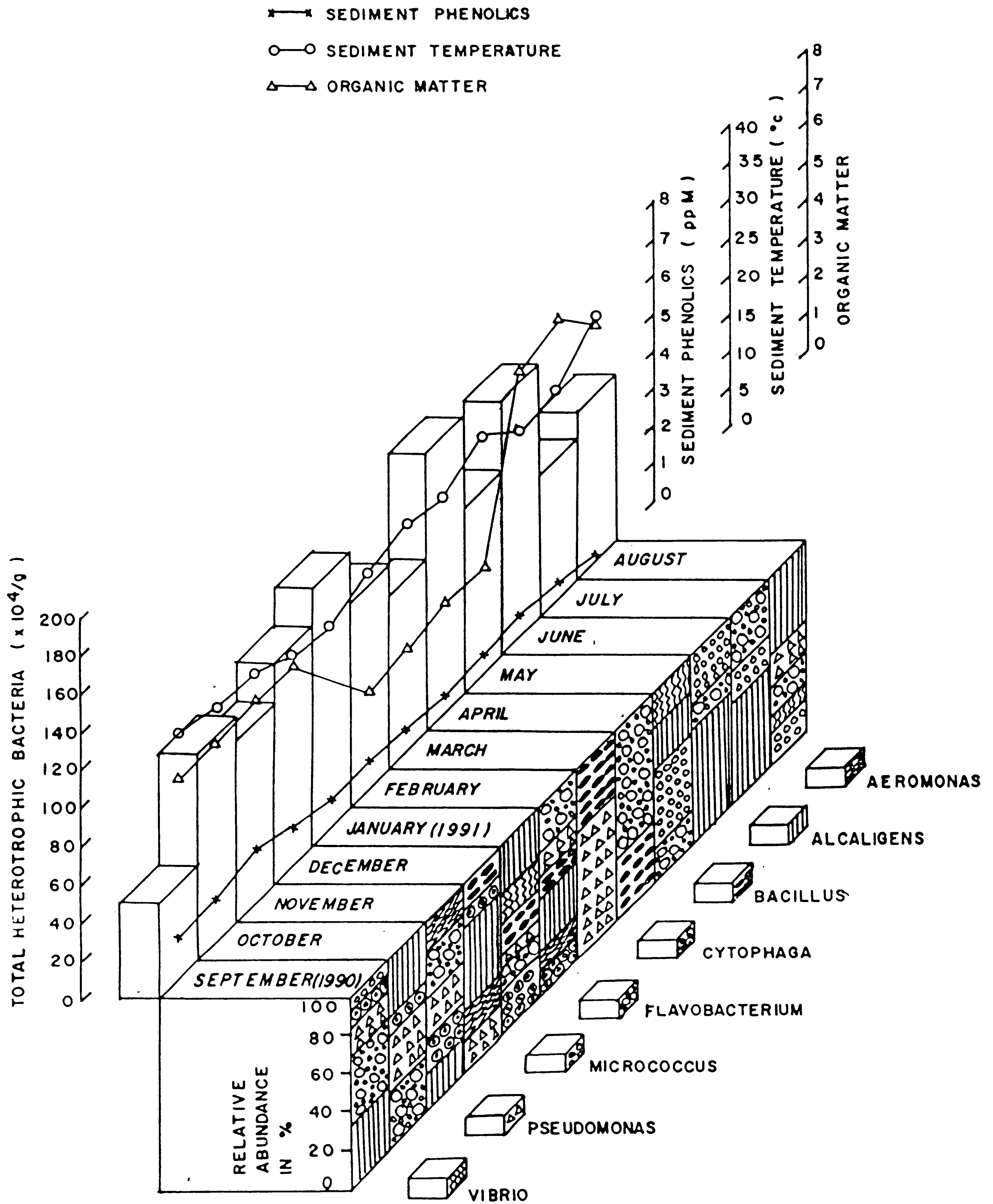
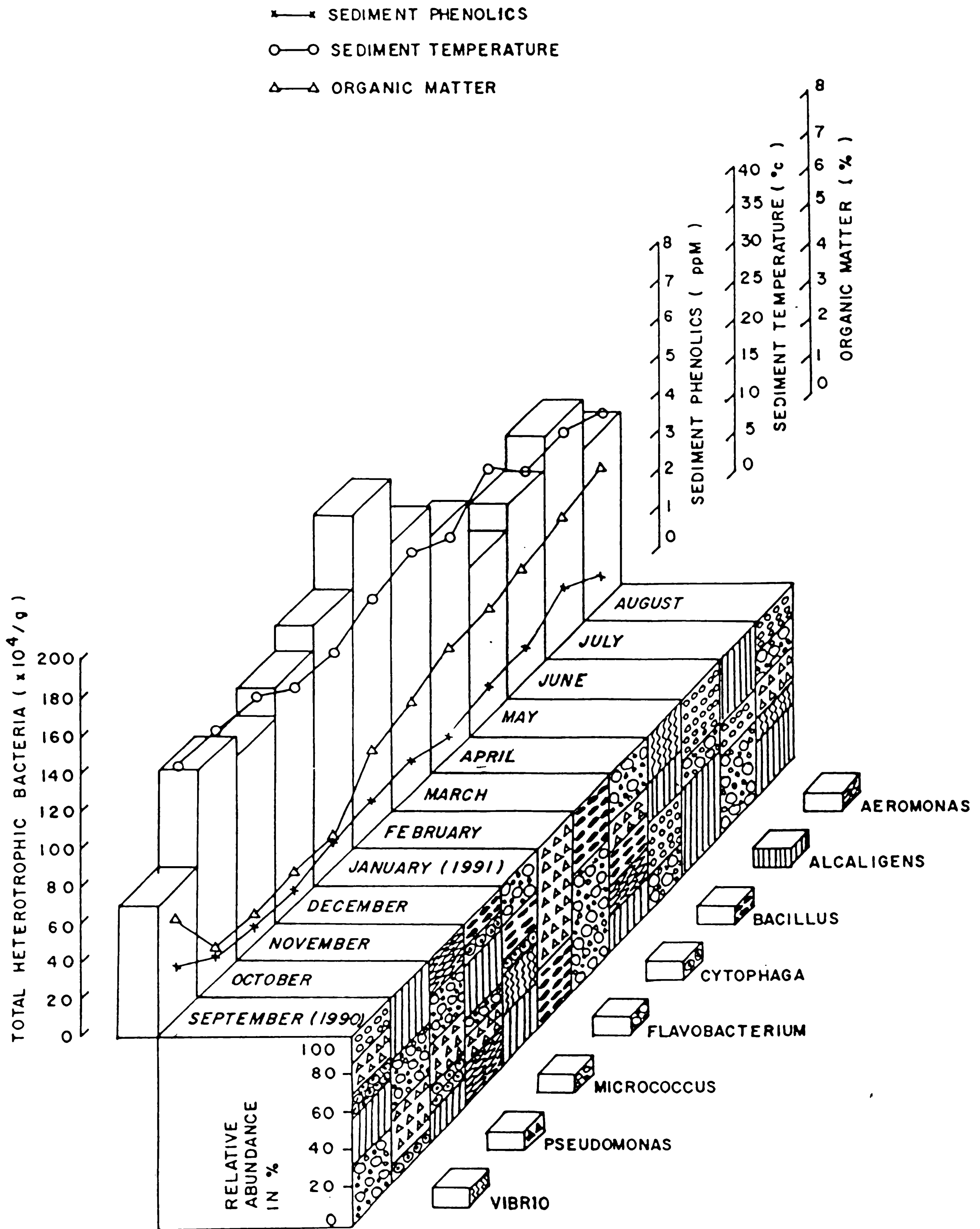

SEDIMENT PHENOLICS
SEDIMENT TEMPERATURE
ORGANIC MATTER
TOTAL HETEROTROPHIC BACTERIA (x10⁴ / gm)
0
20
40
60
80
100
120
140
160
180
200
SEDIMENT PHENOLICS (ppM)
0
1
2
3
4
5
6
7
8
SEDIMENT TEMPERATURE (°c)
0
5
10
15
20
25
30
35
40
ORGANIC MATTER (%)
0
1
2
3
4
5
6
7
8
SEPTEMBER(1990)
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1991)
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
RELATIVE
ABUNDANCE
IN %
0
20
40
60
80
100
AEROMONAS
ALCALIGENS
BACILLUS
FLAVOBACTERIM
PSEUDOMONAS
VIBRIO

Fig.23 **Showing total plate count (TPC), relative percentage of predominant genera, sediment phenolics, temperature and organic matter for Station IV**



SEDIMENT PHENOLICS
SEDIMENT TEMPERATURE
ORGANIC MATTER
SEDIMENT PHENOLICS (ppM)
SEDIMENT TEMPERATURE (°c)
ORGANIC MATTER
TOTAL HETEROTROPHIC BACTERIA (x 10⁴/g)
SEPTEMBER(1990)
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1991)
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
RELATIVE ABUNDANCE IN %
AEROMONAS
ALCALIGENS
BACILLUS
CYTOPHAGA
FLAVOBACTERIUM
MICROCOCCUS
PSEUDOMONAS
VIBRIO

Fig.24 **Showing total plate count (TPC), relative percentage of predominant genera, sediment phenolics, temperature and organic matter for Station V**



SEDIMENT PHENOLICS
SEDIMENT TEMPERATURE
ORGANIC MATTER
SEDIMENT PHENOLICS (ppM)
SEDIMENT TEMPERATURE (°c)
ORGANIC MATTER (%)
TOTAL HETEROTROPHIC BACTERIA (x 10⁴ / g)
RELATIVE ABUNDANCE IN %
SEPTEMBER (1990)
OCTOBER
NOVEMBER
DECEMBER
JANUARY (1991)
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
AEROMONAS
ALCALIGENS
BACILLUS
CYTOPHAGA
FLAVOBACTERIUM
MICROCOCCUS
PSEUDOMONAS
VIBRIO

The results of this study indicate that a number of micro-organisms in different aquatic ecosystems could tolerate phenol at higher concentrations. Of the 153 isolates, 81 could tolerate 0.05% phenol. Viability of aerobic heterotrophs in increasing concentrations of phenol is shown in Fig.25. As phenol concentrations increased, the viability of cultures from all the groups was reduced (Gram-positive rods, Cocci and Gram-negative rods). About 28% gram positive rods, 30% cocci and 15% gram negative rods could tolerate 1% of phenol. All these isolates were tested on 0.05% of o-cresol and Orcinol and found 100% survival.

3.3 Biodegradation studies

3.3.1 Biodegradation of phenol by static-culture-flask-screening-procedure using mixed culture

The biodegradability data from the static-culture-flask biodegradation-screening test and primary enrichment studies have demonstrated that phenol was significantly biodegraded with rapid adaptation periods necessary to achieve optimum biodegradation rates. The biodegradability data are expressed in Table 47 for Station I. Significant biodegradation with rapid acclimation was observed, with a range of average biodegradation from 60 to 100% after 9 days of incubation. Upto 300 ppm of phenol concentrations, the biodegradation was complete (100%) within the first 3 days of incubation. From the results, it is understood that biodegradation is comparatively high and reaches maximum percentage in the third subculture.

In Station II, the percentage biodegradation was lesser compared to Station I which is a polluted area. The range of average degradation was 48 to

Fig. 25 : Showing viability of aerobic heterotrophic bacteria in increasing concentrations of phenol (0.05-1.0%)

Table 47 Biodegradability of phenol using mixed culture from Station I

Conc.of phenol (ppm)	Average of 3 flasks (biodegradation of phenol in 9 days (%)		
Conc.	I SC	II SC	III SC
100	100	100	100
200	100	100	100
300	100	100	100
400	90	100	100
500	80	95	100
600	78	79	85
700	74	78	80
800	70	72	74
900	65	69	72
1000	60	63	66

Table 48 Biodegradability of phenol using mixed culture from Station II

Conc.of phenol (ppm)	Average of 3 flasks (biodegradation of phenol in 9 days (%)		
Conc.	I SC	II SC	III SC
100	100	100	100
200	100	100	100
300	100	100	100
400	80	92	100
500	78	85	95
600	75	77	79
700	70	73	78
800	62	64	70
900	55	58	65
1000	48	52	60

Table 49 Biodegradability of phenol using mixed culture from Station III

Conc.of phenol (ppm)	Average of 3 flasks (biodegradation of phenol in 9 days (%)		
Conc.	I SC	II SC	III SC
100	100	100	100
200	100	100	100
300	100	100	100
400	75	90	100
500	70	80	90
600	68	74	80
700	65	69	76
800	60	68	72
900	50	60	62
1000	48	53	59

Table 50 Biodegradability of phenol using mixed culture from Stations IV and V

Conc.of phenol (ppm)	Average of 3 flasks (biodegradation of phenol in 9 days (%)		
Conc.	I SC	II SC	III SC
100	100	100	100
200	100	100	100
300	85	95	100
400	72	83	98
500	70	76	80
600	65	70	73
700	63	66	68
800	55	60	65
900	51	56	60
1000	46	50	55

100%. In this station also, biodegradability was faster and complete upto 300 ppm in 3 days incubation period. Sub-culture III was giving maximum biodegradation percentage (Table 48).

At Thykoodam which is a comparatively non-polluted station, the percentage biodegradation expressed in III sub-cultures were almost similar to that in the mangrove ecosystem. The range of degradation was from 48 to 100% (Table 49). The comparatively slower degradation rate attributes to the slower acclimation of bacterial cultures used for the experiment.

The seasonal and perennial aquaculture ponds were considered together for biodegradation studies, because both these stations were inhabiting almost similar micro-flora under normal conditions of environmental parameters and aquaculture practices. The range in biodegradation rate was from 46-100% Table 50 upto 1000 ppm of phenol concentrations. In this experiment also, maximum rate of biodegradation was obtained in the Sub-culture III.

3.3.2 Biodegradability of phenol from 100 to 1000 ppm concentrations using mixed cultures

The biodegradation of phenol at 100 ppm concentration using mixed cultures is shown in Fig.26. The maximum degradation rate was shown by cultures from Station I and II which are areas of possible phenolic pollution. 100% degradation was obtained in two days of incubation in these stations. Whereas it look 3 to 5 days for complete biodegradation in the other two stations.

Fig. 26 : Showing biodegradability of phenol at 100 ppm using mixed cultures from the sampling stations during '90-91

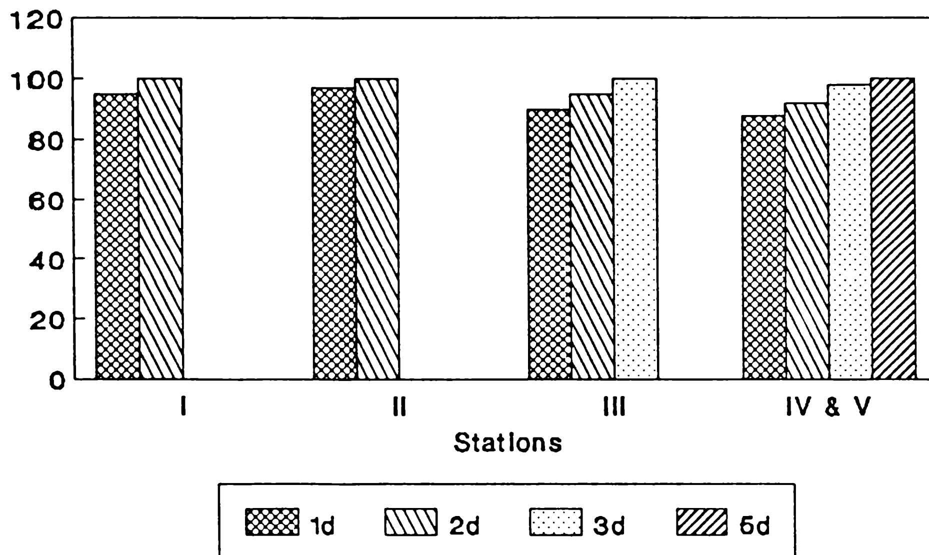
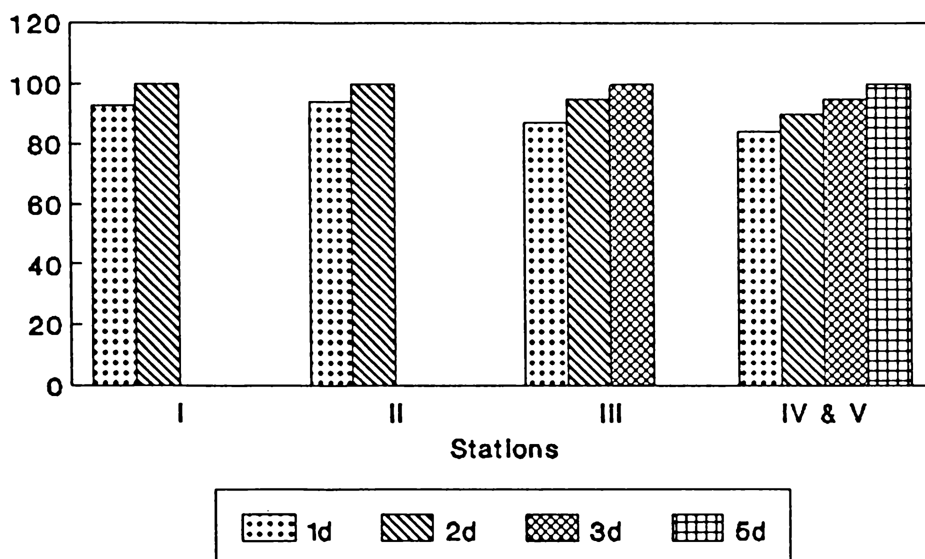


Fig. 27 : Showing biodegradability of phenol at 200 ppm using mixed cultures from the sampling stations during '90-91



The degradation at 200 ppm concentration of phenol also showed the same trend in percentage utilization. It look 2 to 5 days for 100% biodegradation of the test compound (Fig.27).

At 300 ppm concentration, biodegradability was 100% from 5 to 9 days of culture period in the four set of experiments using cultures from four different stations (Fig.28).

For 400 ppm, 100% biodegradation could be attained only with cultures from three sources and not from the aquaculture ponds (Fig.29).

At 500 ppm of phenol concentration the culture from coconut husk retting area only could attain 100% biodegradability. The rate was decreased considerably at this concentration for other stations (Fig.30).

At 600 ppm, the biodegradation rate ranged from 73-85% using the four different set of micro-organisms (Fig.31).

The biodegradation rate of phenol ranged from 68-80% in the four sets of experiments at 700 ppm. The degradation rate was more with cultures from Station I, then Station II, and so on (Fig.32).

The utilization of phenol at 800 ppm, ranged from 65-74% in four sets of experiments (Fig.33) in 9 days.

At 900 ppm, which is a very high concentration for microbes to be completely degraded, a range of 60-72% was obtained. The trends of

Fig. 28 : Showing biodegradability of phenol at 300 ppm using mixed cultures from the sampling stations during '90-91

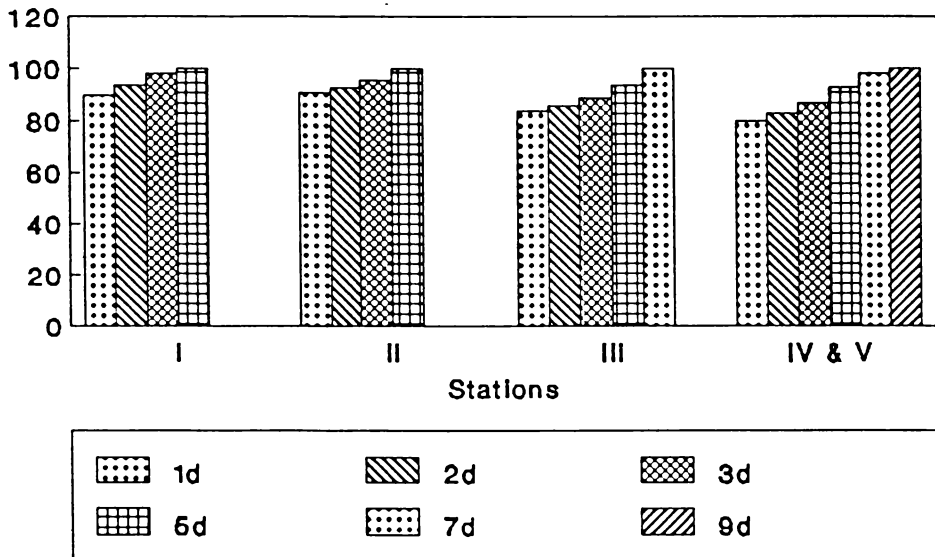


Fig. 29 : Showing biodegradability of phenol at 400 ppm using mixed cultures from the sampling stations during '90-91

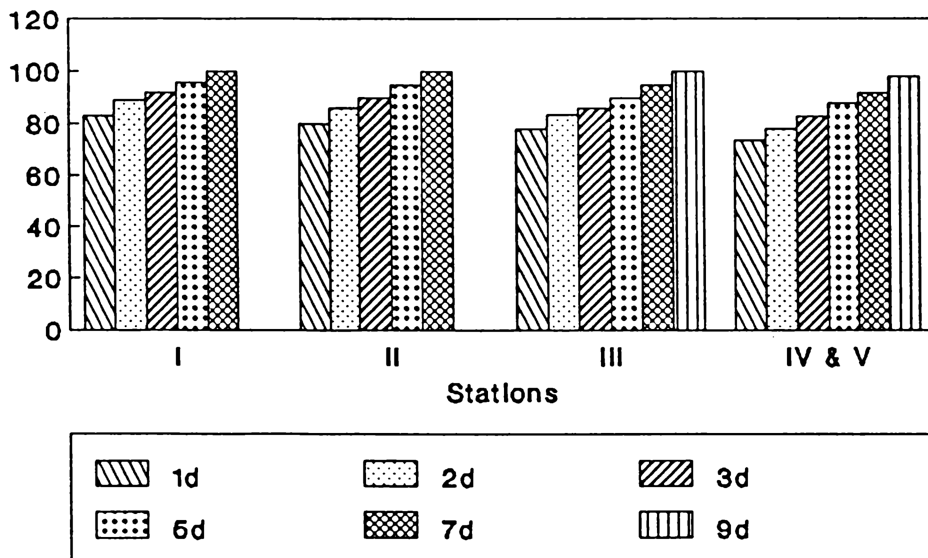


Fig. 30 : Showing biodegradability of phenol at 500 ppm using mixed cultures from the sampling stations during '90-91

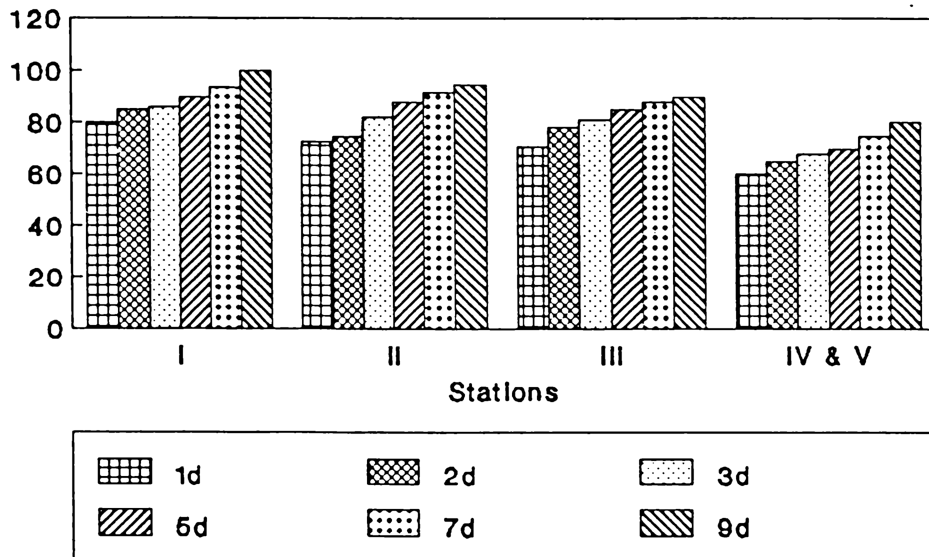


Fig. 31 : Showing biodegradability of phenol at 600 ppm using mixed cultures from the sampling stations during '90-91

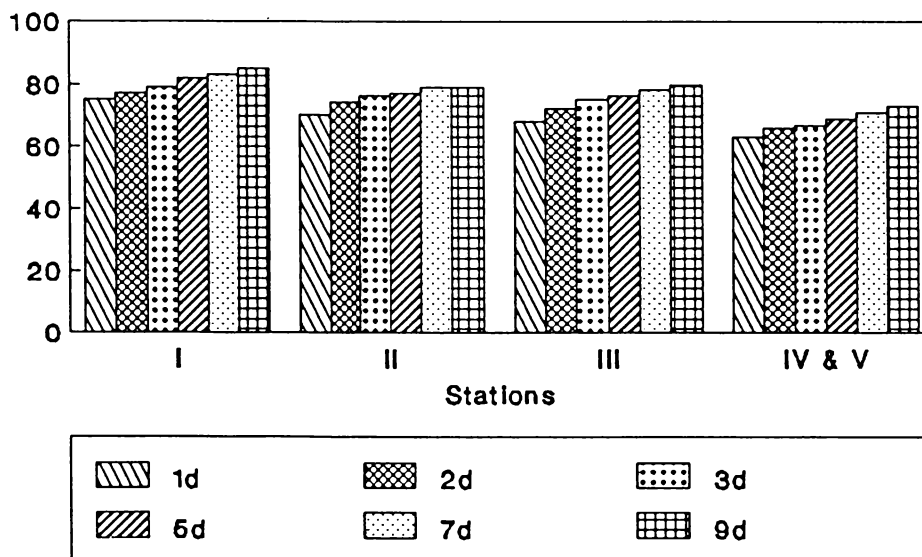


Fig. 32 : Showing biodegradability of phenol at 700 ppm using mixed cultures from the sampling stations during '90-91

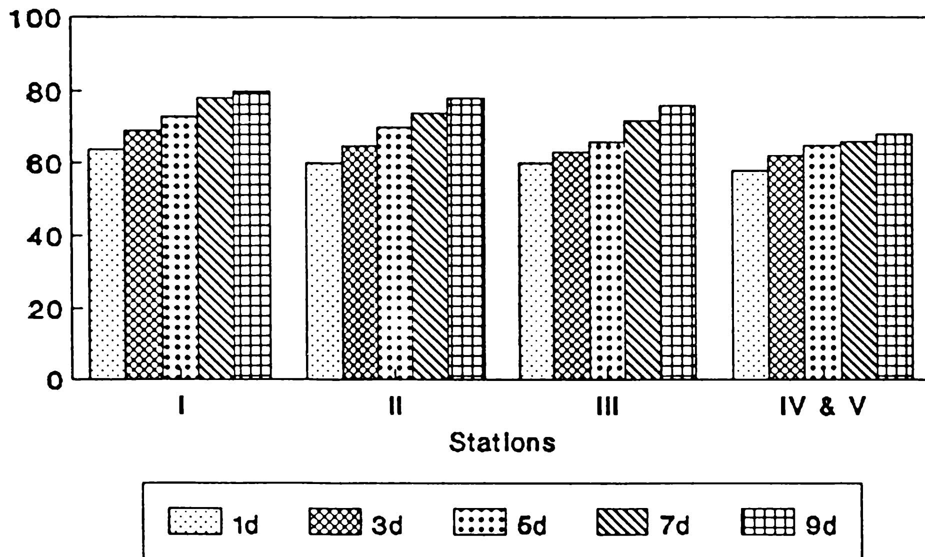
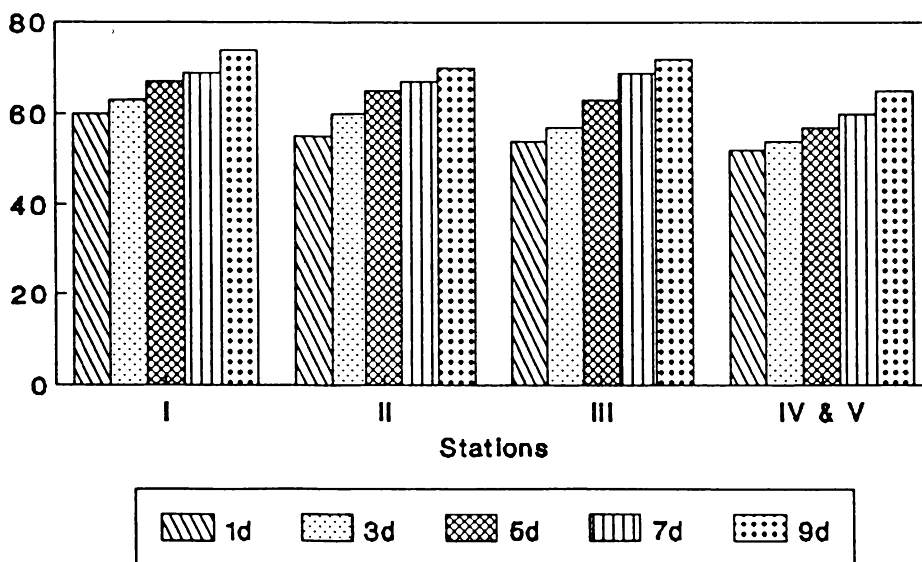


Fig. 33 : Showing biodegradability of phenol at 800 ppm using mixed cultures from the sampling stations during '90-91



utilization for different set of experiments using cultures from four sources are shown in Fig.34.

As per the present investigation, the final concentration of phenol selected was 1000 ppm. Though more than 50% biodegradation was observed at this concentration, the over all metabolism of phenol was comparatively poor (Fig.35).

At all concentrations, and cultures from all four sources, the maximum biodegradation was observed during the first phase of the experiment. In the proceeding observations, the rate of biodegradation decreases considerably giving only marginal increase in percentage utilization of the compound.

3.3.3 Statistical Analysis

In order to find out the significance of rate of biodegradation, results of two way ANOVA over sub cultures, from I to III and over concentration of phenol from 100 ppm to 1000 ppm were used.

F - Values for Station I between sub cultures ($P<0.05$) and between concentrations ($P<0.01$) were significant (Table 51).

F - Values for Station II showed significance between subcultures ($P<0.01$) and concentrations ($P<0.01$) (Table 53).

F - values for Station III showed significance between sub-cultures ($P < 0.01$) and concentrations ($P < 0.01$) (Table 53).

F - Values for aquaculture ponds were also showed significance between subculture and concentrations.($P<0.01$) (Table 54).

Fig. 34 : Showing biodegradability of phenol at 900 ppm using mixed cultures from the sampling stations during '90-91

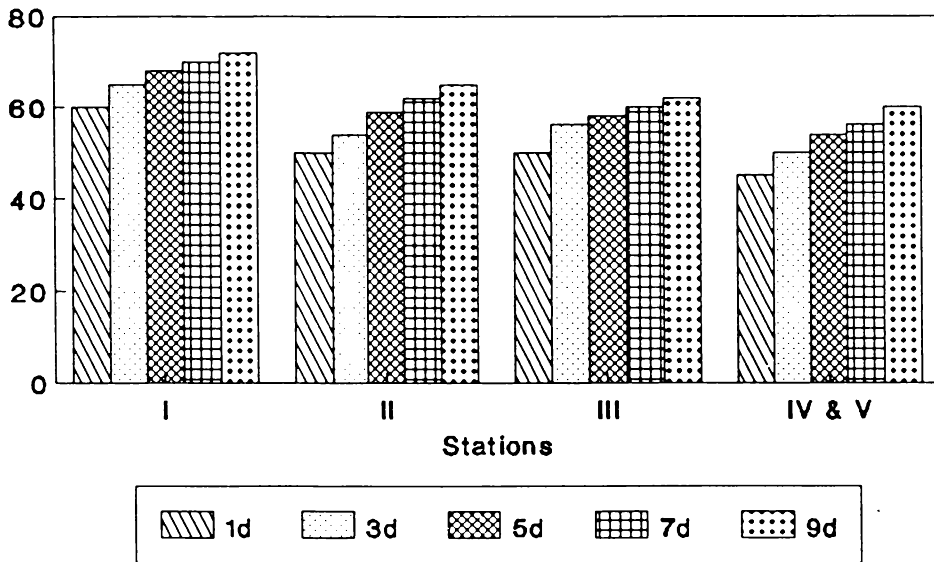


Fig. 35 : Showing biodegradability of phenol at 1000 ppm using mixed cultures from the sampling stations during '90-91

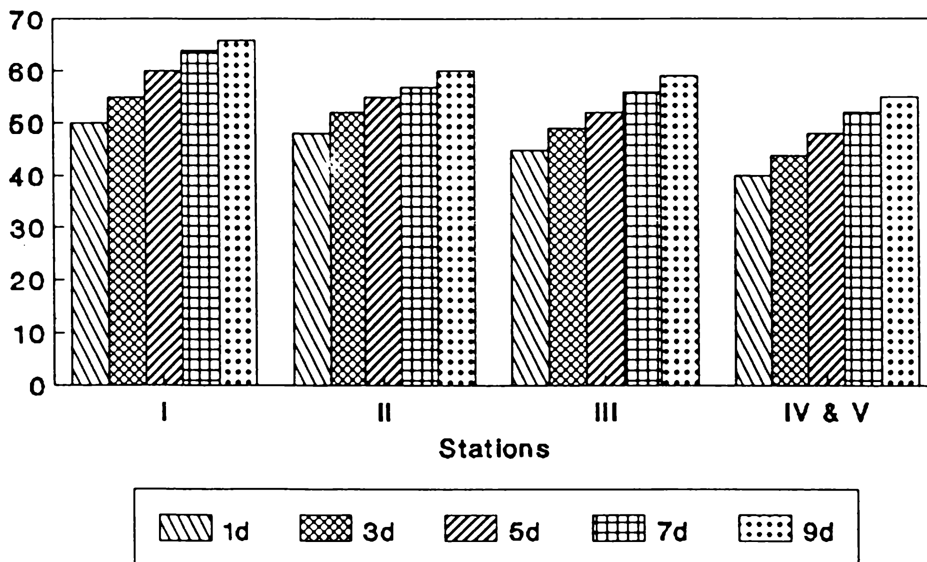


Table 51 Two - way ANOVA for biodegradability of phenol over sub-cultures and over concentrations - Station I.

Source	DF	SS	MS	F	Remarks
Sub-culture	2	214.32	107.158	4.72	SIG (5%)
Concentration	9	6410.30	712.256	31.57	SIG (1%)
Error	18	408.68	22.704		
Total	29	7033.30			

Table 52 Two - way ANOVA for biodegradability of phenol over sub-cultures and over concentrations - Station II.

Source	DF	SS	MS	F	Remarks
Sub-culture	2	232.22	116.110	6.58	SIG (1%)
Concentration	9	7742.66	860.295	48.75	SIG (1%)
Error	18	371.62	17.646		
Total	29	8292.50			

Table 53 Two - way ANOVA for biodegradability of phenol over sub-cultures and over concentrations - Station III.

Source	DF	SS	MS	F	Remarks
Sub-culture	2	321.58	160.789	7.68	SIG (1%)
Concentration	9	8006.31	889.590	42.50	SIG (1%)
Error	18	376.81	20.934		
Total	29	8704.69			

To assess the significance of rate of biodegradation, results of two way ANOVA over stations and over days were used.

The F-values for biodegradability of phenol at 100 ppm concentration show that it was not significant between stations and between days (Table 55).

The F-values for biodegradability of phenol at 200 ppm showed that between stations it was non-significant while it was significant between days ($P < 0.01$) (Table 56).

The F-values for phenol biodegradation at 300 ppm showed significance between stations ($P < 0.01$) and days ($P < 0.01$) (Table 57).

At 400 ppm, the F - values were showing significance between stations and between days ($P < 0.01$) (Table 58).

For biodegradability at 500 ppm F-value showed significance both between stations and days ($P < 0.01$) (Table 59).

The F-values for phenol biodegradation at 600 ppm, showed significance between stations and days ($P < 0.01$) (Table 60).

F-values for biodegradation of phenol at 700 ppm, 800 ppm, 900 ppm, and 1000 ppm showed significance between stations and days ($P < 0.01$) (Tables 61, 62, 63, and 64).

In all the analysis, the ANOVA, was carried out after transforming the percentage values to Sin^{-1} (or arc sine).

Table 54 **Two - way ANOVA for biodegradability of phenol over sub-cultures and over concentrations - Stations IV and V**

Source	DF	SS	MS	F	Remarks
Sub-culture	2	301.24	150.621	7.99	SIG (1%)
Concentration	9	7546.57	838.508	44.46	SIG (1%)
Error	18	339.50	18.861		
Total	29	8187.31			

Table 55 **Two - way ANOVA for biodegradability of phenol- 100 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	134.17	44.723	3.27	NS
Days	5	771.45	154.289	11.27	SIG (1%)
Error	15	205.42	13.695		
Total	23	1111.04			

Table 56 **Two - way ANOVA for biodegradability of phenol- 200 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	188.10	62.700	3.26	NS
Days	5	1095.25	219.049	11.39	SIG (1%)
Error	15	288.47	19.232		
Total	23	1111.04			

Table 57 **Two - way ANOVA for biodegradability of phenol- 300 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	357.61	119.204	11.45	SIG (1%)
Days	5	1617.93	323.587	31.07	SIG (1%)
Error	15	156.20	10.413		
Total	23	2131.74			

Table 58 **Two - way ANOVA for biodegradability of phenol_ 400 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	324.67	108.224	16.59	SIG (1%)
Days	5	1887.44	377.487	57.86	SIG (1%)
Error	15	97.87	6.524		
Total	23	2309.97			

Table 59 **Two - way ANOVA for biodegradability of phenol_ 500 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	792.62	264.207	33.22	SIG (1%)
Days	5	841.07	168.214	21.15	SIG (1%)
Error	15	119.29	7.953		
Total	23	1752.99			

Table 60 Two - way ANOVA for biodegradability of phenol- 600 ppm, over station and over days

Source	DF	SS	MS	F	Remarks
Station	3	193.49	64.495	239.03	SIG (1%)
Days	5	123.94	24.787	91.86	SIG (1%)
Error	15	4.05	0.270		
Total	23	321.47			

Table 61 Two - way ANOVA for biodegradability of phenol- 700 ppm, over station and over days

Source	DF	SS	MS	F	Remarks
Station	3	84.05	28.017	22.49	SIG (1%)
Days	4	222.17	55.543	44.59	SIG (1%)
Error	12	14.95	1.246		
Total	19	321.17			

Table 62 Two - way ANOVA for biodegradability of phenol- 800 ppm, over station and over days

Source	DF	SS	MS	F	Remarks
Station	3	73.96	24.652	31.30	SIG (1%)
Days	4	203.59	50.847	64.56	SIG (1%)
Error	12	9.45	0.788		
Total	19	286.79			

Table 63 **Two - way ANOVA for biodegradability of phenol_ 900 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	181.40	60.467	222.25	SIG (1%)
Days	4	150.49	37.623	138.28	SIG (1%)
Error	12	3.26	0.272		
Total	19	355.16			

Table 64 **Two - way ANOVA for biodegradability of phenol_ 1000 ppm, over station and over days**

Source	DF	SS	MS	F	Remarks
Station	3	109.30	36.432	120.24	SIG (1%)
Days	4	171.65	42.911	141.62	SIG (1%)
Error	12	3.64	0.303		
Total	19	284.58			

3.4 Bacteria utilizing phenolic compounds

Bacteria utilizing phenolic compounds were obtained from all the five environments (Stations I to V) sampled. The percentage distribution of bacteria at 100 to 1000 ppm of phenol in biodegradation experiments is shown in Fig.36. Upto 1000 ppm, in the culture conditions provided, only *Pseudomonas* sp. and *Vibrio* sp. were able to be isolated. All the isolates except *Micrococcus* sp. were gram-negative, non-sporulating aerobic small to medium sized rods. *Bacillus* a gram-positive rod was identified in 100 ppm concentration. Optimal growth was recorded in room temperature (30-32°C). Both motile and non-motile forms were observed. On the basis of colony and cell morphology, gram-stain, pigment production, and biochemical tests, isolates were distinguished for the following genera. *Alcaligenes* sp. *Bacillus, Cytophaga, Enterobacteriaceae, Flavobacterium, Micrococcus, Pseudomonas* and *Vibrio.* Beyond 500 ppm, only *Vibrio* and *Pseudomonas* could be isolated. The identification was carried out using the scheme of Simidu and Aiso (1962) (Table 34).

The percentage distribution of bacteria at 100 ppm to 500 ppm of o-cresol and orcinol were also studied during the present investigation (Fig.37 and Fig.38).

Different groups of *Pseudomonas* (II, III and IV) were observed to contribute maximum percentage of the population. The genera which were present in phenol were present in o-cresol and orcinol also.

Fig. 36 Showing % distribution of bacteria at different concentrations of phenol (100-1000 ppm)

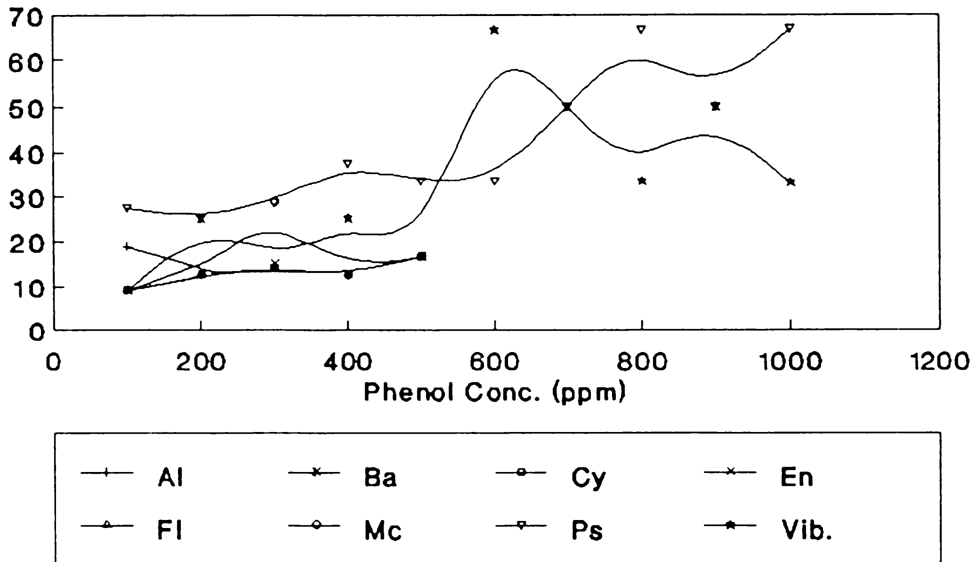
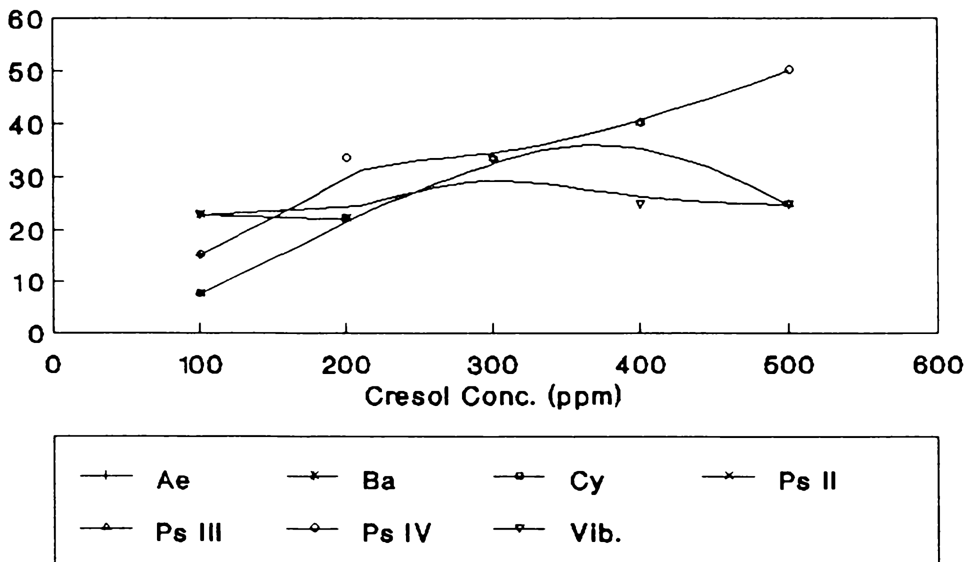
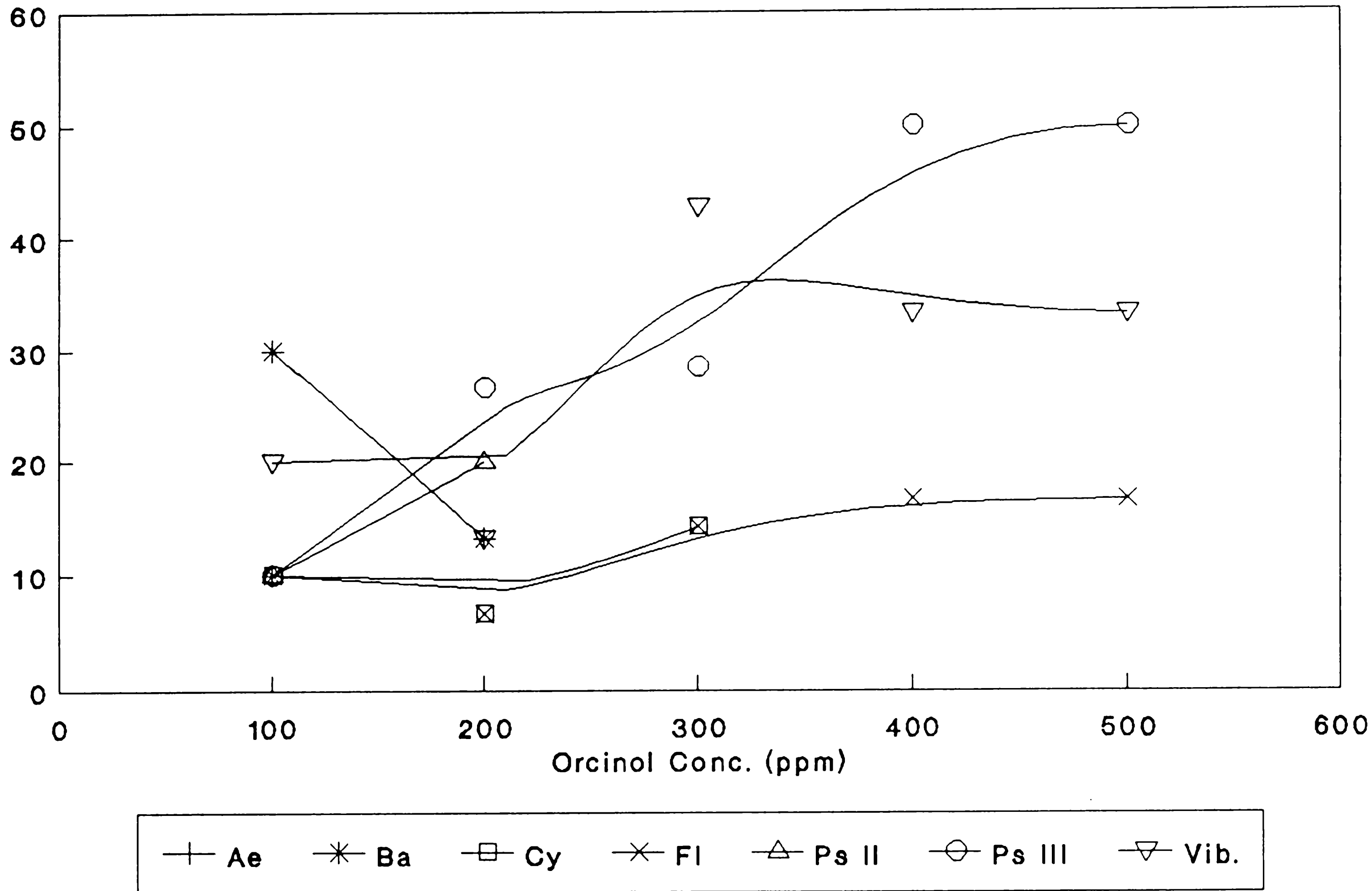


Fig. 37 Showing % distribution of bacteria at different concentrations of o-cresol (100-500 ppm)



Ae- *Aeromonas* ; Al - *Alcaligenes* ; Ba - *Bacillus* ; Cy - *Cytophaga* ;
En - *Enterobacteriaceae* ; Fl - *Flavobacterium* ; Mc - *Micrococcus* ;
Ps II - *Pseudomonas* Group II ; Ps III - *Pseudomonas* Group III ; Vib - *Vibrio*

Fig. 38 Showing % distribution of bacteria at different concentrations of orcinol (100-500 ppm)



Ae - *Aeromonas* ; Ba - *Bacillus* ; Cy - *Cytophaga* ; Fl - *Flavobacterium* ;
Ps II - *Pseudomonas* Group II ; Ps III - *Pseudomonas* Group III ;
Vib - *Vibrio*

3.5 Biodegradation of phenol using individual microbial isolates

3.5.1 *Alcaligenes* sp.

Bacteria isolated from mixed cultures were used for biodegradation studies using individual isolates. The strains already adapted to phenol were plated on mineral salts agar with 0.01, 0.02, 0.03, 0.04 and 0.05% of phenol for preparation of pure cultures. The adaptation of selected bacteria to higher concentrations of phenol was accomplished by sub-culturing in mineral salts medium (Aaronson 1970), containing higher concentrations of phenol. The sub-cultures were done in every week. Those multiplying in 0.05% phenol were used for biodegradation experiments.

The results of the experiments show that *Alcaligenes* could readily grow in medium with 100 ppm to 500 ppm of phenol. In 9 days, it showed 60% biodegradation in 100 ppm phenol and 48% in 500 ppm. The viable count taken on 5th and 10th day of the experiment showed that in 100 ppm, 100 x 10^4/ml on 5th day and 112 x 10^4/ml on 10th day were there. In 500 ppm on 5th day there was a count of 85 x 10^4/ml and 80 x 10^4/ml on 10th day. The utilization of phenol from 100 ppm to 500 ppm by *Alcaligenes* sp. is shown in Fig.39.

Two-way ANOVA over concentration and over days showed that the rate of biodegradation by *Alcaligenes* sp. was significant between, concentrations and days ($P < 0.01$) (Table 65). The F - value for viable count of *Alcaligenes* between concentrations was significant ($P < 0.05$) and non-significant between days (Table 66).

Table 65 Two - way ANOVA for biodegradability of phenol by *Alcaligenes* sp. over concentrations and over days

Source	DF	SS	MS	F	Remarks
Concentration	4	101.04	25.259	9.61	SIG (1%)
Days	4	2701.22	675.305	257.05	SIG (1%)
Error	16	42.03	2.628		
Total	24	2844.29			

Table 66 Two - way ANOVA for viable count of *Alcaligenes* sp. over different phenol concentrations and over days

Source	DF	SS	MS	F	Remarks
Concentration	4	637.40	159.350	7.29	SIG (5%)
Days	1	67.60	67.600	3.09	NS
Error	4	87.40	21.850		
Total	9	792.40			

Table 67 Two - way ANOVA for biodegradability of phenol by *Pseudomonas* sp. over concentrations and over days

Source	DF	SS	MS	F	Remarks
Concentration	4	1279.38	319.845	7.28	SIG (1%)
Days	4	6396.70	1599.174	36.39	SIG (1%)
Error	16	703.12	43.945		
Total	24	8379.20			

3.5.2 *Pseudomonas* sp.

Phenololytic *Pseudomonas* sp. were isolated in the similar way as *Alcaligenes* sp. from the mixed culture. The culture media, bacteriological and chemical methods employed were all the same as for *Alcaligenes* sp. the utilization of phenol from 100 ppm to 500 ppm concentrations of phenol by *Pseudomonas* is shown in Fig.40. It showed 100% biodegradation in 9 days upto 500 ppm concentration. The viable count ranged from 95 x 10^4/ml in 500 ppm in 5 days to 115 x 10^4/ml in 100 ppm in 5 days.

The two-way ANOVA results for biodegradation of phenol by *Pseudomonas* sp. between concentrations and between days were found to be significant ($P < 0.01$) (Table 67).

The F values for viable count between concentrations and days were also found to be significant ($P<0.01$) (Table 68).

3.5.3 *Vibrio* sp.

Vibrio sp. which were capable of utilizing phenol was isolated from the mixed culture. The methodology was the same as for the other two species. The utilization of phenol from 100 ppm to 500 ppm by *Vibrio* sp. is shown in Fig.41. It showed 35-60% biodegradation in 9 days at different concentrations studied. The viable count had ranged from 115 x 10^4/ml in 100 ppm in 5 days and 90 x 10^4/ml in 500 ppm in 5 days.

Fig. 39 Showing percentage utilization of phenol at different concentrations by *Alcaligenes* sp. (100-500 ppm)

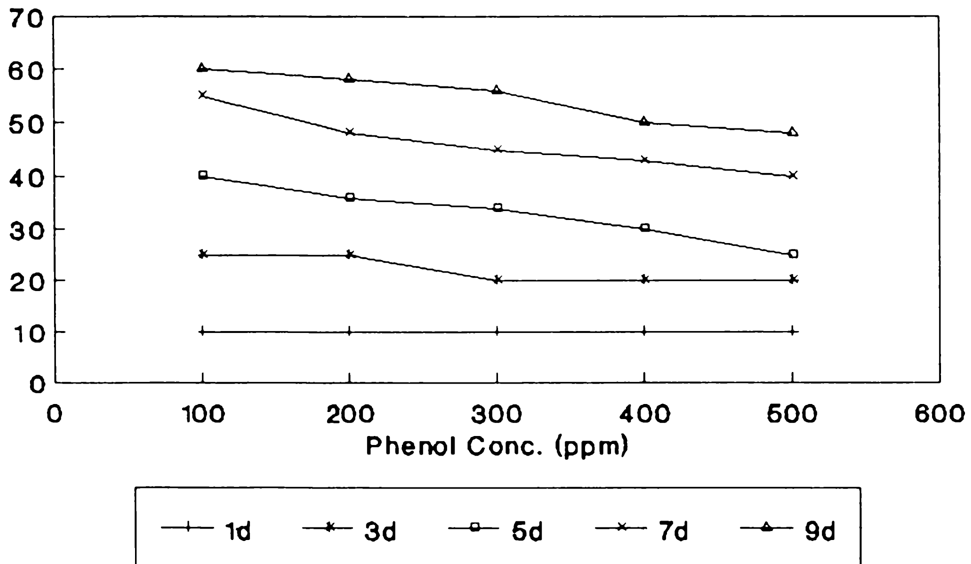


Fig. 40 Showing percentage utilization of phenol at different concentrations by *Pseudomonas* sp. (100-500 ppm)

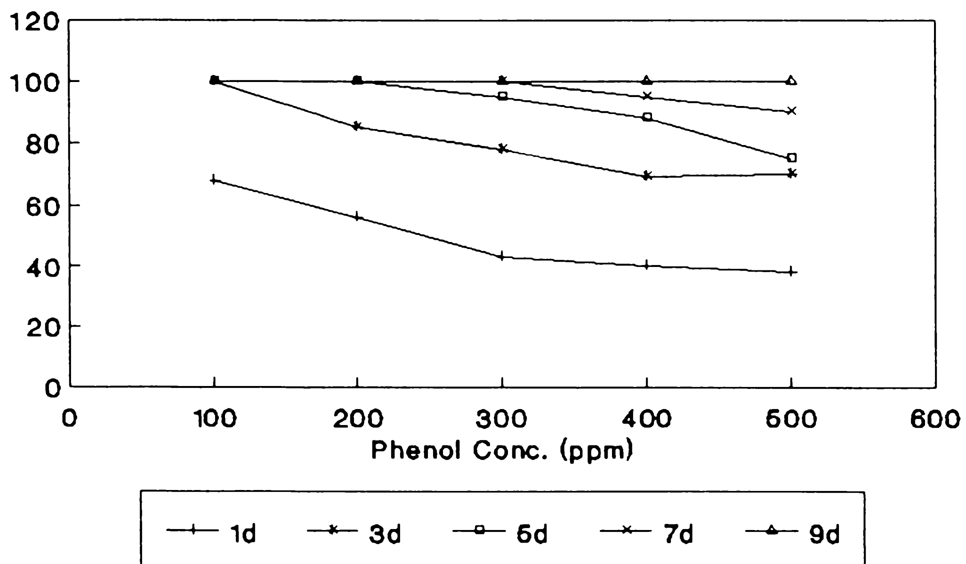


Table 68 **Two - way ANOVA for viable count of *Pseudomonas* sp. over different phenol concentrations and over days**

Source	DF	SS	MS	F	Remarks
Concentration	4	615.40	153.850	41.03	SIG (1%)
Days	1	160.00	160.00	42.67	SIG (1%)
Error	4	15.00	3.750		
Total	9	790.40			

Table 69 **Two - way ANOVA for biodegradability of phenol by *Vibrio* sp. over concentrations and over days**

Source	DF	SS	MS	F	Remarks
Concentration	4	242.41	60.604	19.90	SIG (1%)
Days	4	1936.97	484.242	159.04	SIG (1%)
Error	16	48.72	3.045		
Total	24	2228.10			

Table 70 **Two - way ANOVA for viable count of *Vibrio* sp. over different phenol concentrations and over days**

Source	DF	SS	MS	F	Remarks
Concentration	4	1040.00	260.00	104.00	SIG (1%)
Days	1	2.50	2.50	1.00	NS
Error	4	10.00	2.50		
Total	9	1052.50			

The F value obtained by two-way ANOVA for biodegradation of phenol by *Vibrio* sp. showed significance between concentrations and days (P < 0.01) (Table 69).

The F-value of two-way ANOVA for viable count of *Vibrio* sp. between concentrations was significant (P < 0.01) and it was not significant between days (Table 70).

3.5.4 *Streptomyces* sp.

Steptomyces sp. was isolated from the environment. Four isolates were tried for viability in phenol medium. The only one, of the four, which could survive and utilise phenol was selected for individual biodegradation study. Mycelia from a 14 day old culture were inoculated into the mineral salts medium as in the case of bacterial isolates. It used phenol as the sole source of carbon and energy.

The *Streptomyces* sp. could utilize 100% of phenol in 3 days in 100 ppm concentration. In 9 days it could degrade 500 ppm phenol. The growth and utilization of phenol at different concentrations (100-500 ppm) by *Streptomyces* is shown in Fig.42.

The viable count was maximum in 10 days at 100 ppm phenol concentration (65 x 10^4/ml). The lowest count was 25 x 10^4/ml in 500 ppm in 10 days. The two-way ANOVA for utilization of phenol by *Streptomyces* sp. shows that the F - value was significant between concentrations as well as between days (P < 0.01) (Table 71). The F - value for viable count between concentrations was significant (P < 0.01) while it was non significant between days (Table 72).

Fig. 41 Showing percentage utilization of phenol at different concentrations by *Vibrio sp.* (100-500 ppm)

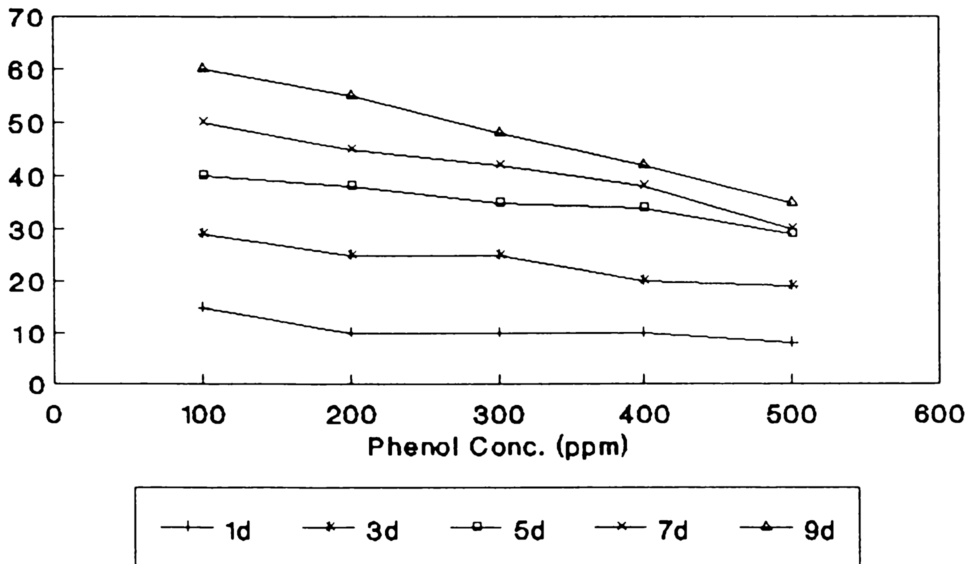


Fig. 42 Showing percentage utilization of phenol at different concentrations by *Streptomyces* sp. (100-500 ppm)

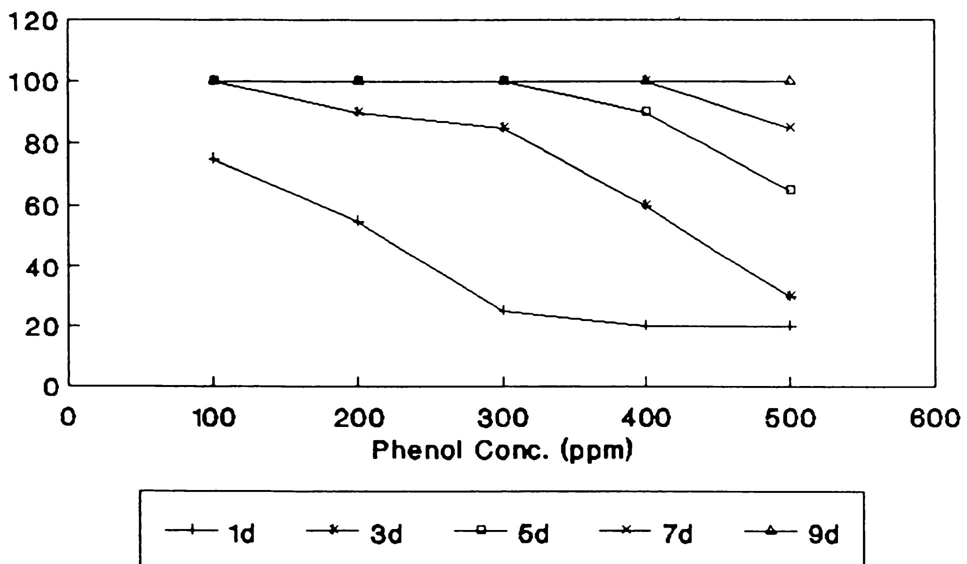


Table 71 Two - way ANOVA for biodegradability of phenol by *Streptomyces* sp. over concentrations and over days

Source	DF	SS	MS	F	Remarks
Concentration	4	2668.86	667.215	6.82	SIG (1%)
Days	4	8908.71	2227.178	22.75	SIG (1%)
Error	16	1566.25	97.891		
Total	24	13143.82			

Table 72 Two - way ANOVA for viable count of *Streptomyces* sp. over different phenol concentrations and over days

Source	DF	SS	MS	F	Remarks
Concentration	4	1910.00	477.500	191.00	SIG (5%)
Days	1	2.50	2.500	1.00	NS
Error	4	10.50	2.500		
Total	9	1922.50			

CHAPTER 4

DISCUSSION

A growing interest in mariculture, and thus the production and export of fishes and prawns, has led to tremendous achievements in fisheries sector in the recent years in India and abroad. Detailed information about the types and numbers of bacteria in polluted as well as non-polluted aquatic environments is essential if abnormal conditions, such as may be attributed to adverse water quality factors or the onset of favourable disease conditions, are to be recognized and corrected. An investigation on the occurrence of phenol and phenol-degrading bacteria in the aquatic environment is important for ecological and applied reasons. As nitrogenous compounds will form polymers with phenols or polyphenols rendering them less susceptible to microbial degradation, detection of phenolics and its role in nutrient cycles is very essential. Simultaneously, with the determination of bacterial group in question and their ability to degrade phenolic compounds, other environmental parameters and nutrients should be determined to find out which factor is enhancing the biodegradation. However, only in balanced system, where continuously small amounts of organic substances are added, we can expect a stable environmental parameters and microbial concentrations. Considering all these, the present study on 'BIODEGRADATION OF PHENOLIC COMPOUNDS IN DIFFERENT ECOSYSTEMS IN COCHIN' was initiated and five stations - a coconut retting area, a mangrove, a back water system, and two aquaculture systems were selected and water and sediment samples were

analysed for environmental parameters including sediment phenolics and total aerobic heterotrophs. Laboratory experiments to determine the rate of degradation of phenol at different concentrations, using mixed cultures and individual isolates from the selected environments were also conducted. The results of the investigation are discussed as follows:

Located along 9°58'N and 76°15'E on the south-east coast of India, the Cochin back water system forms a tropical positive estuary which provides excellent conditions for rich aquatic fauna. Cochin corporation sewage system empties its municipal waste and other particulate organic matter into the back waters through many canals which results in organic pollution of the system. Coconut husk retting is considered as one of the important sources of organic pollution in Cochin back waters. As a result of retting, large quantities of organic substances including pectin, pectosan, fat, tannin and also toxic polyphenols are liberated into the water by the activity of micro-organisms (Jayashankar 1966). Such an area, where coconut retting is done throughout the year was selected as Station I, in the present study.

The patchy mangroves in the coastal area of Cochin forms a unique ecosystem, harbouring a variety of flora and fauna. The mangroves with its high organic detritus content, inhabit juveniles of many fishes and prawns, thus forming a nursery ground for them. Heald (1971) pointed out that many commercially important finfish and shellfish live in the mangrove environment and that vascular plant detritus is the primary source of food for many estuarine organisms. The mangrove sediments are rich in organic debris and humic substances, the decomposition of which results in the formation of

polyphenols. These polyphenols are degraded to simpler forms which can be utilized by other organisms by bacteria and fungi. The Station II selected for the study was one such system where there was a variety of mangroves, dominated by *Avicennia* sp. and *Acanthus* sp.

Station III formed a back water system, which was a comparatively non-polluted area with free exchange of tidal water. The sources of phenol in this area could be of terrestrial or biological origin.

The seasonal and perennial aquaculture ponds were selected as Station IV and Station V respectively. These stations were considered in order to find out whether the permissible limit of phenol concentration exceeds in the water and sediments, here.

4.1 Environmental variables

The Vembanad lake and the adjacent back waters are influenced by monsoons as stated by Pillai *et al* (1975) and Sankaranarayanan *et al* (1980). Rajagopalan *et al* (1980) reported that environmental parameters of mangroves showed considerable seasonal fluctuations due to monsoons prevailing in Kerala. So, the investigation period was classified into three seasons, pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January) seasons.

The highest temperature for Station I was recorded during March '90 (32°C) and the lowest during December '90 (28°C). During pre-monsoon season, the average temperature was highest (30-36°C) and for monsoon and post-

monsoon it was the same (29.5°C). The higher temperature in pre-monsoon was reduced with the onset of south-west monsoon and did not show wide range during post monsoon months. In Station II, highest temperature was encountered in May '90 (31.25°C) and lowest in July '91 (25.75°C). The annual temperature had shown a clear cut seasonal trend with highest temperature during pre-monsoon (30.18°C) and lowest during monsoon (28.16°C) seasons. In Station III, the temperature values showed a similar trend as in Station II. But here, the highest (31°C) and lowest (27.5°C) temperature values were recorded during monsoon months. This may be due to the intermittent monsoon showers prevailed then. The temperature was higher during pre-monsoon seasons (30.07°C), which was slightly lesser than in Station II. In Stations IV and V, there was not much variation in seasonal mean values of temperature. Still, pre-monsoon seasons encountered the highest temperatures (30.81°C and 30.94°C for Stations IV and V respectively). The changes in temperature indicated the freshwater discharge and incursion of large volume of cold water from the sea to the estuary. It is reported that distribution of marine organisms are influenced by salinity and low temperature (Akagi *et al* 1980).

The salinity of the water also showed seasonal fluctuation in all the five stations which varied from fresh water to saline- conditions. Rajagopalan *et al* (1980) reported that the extreme range in salinity was mainly due to monsoons and tidal influence. In Station I, the highest salinity was recorded in November '90 (14.04 o/oo) and lowest in July '90 (0.23 o/oo). During monsoon seasons, the salinity showed a steep fall and reached the lowest and then

slowly increased. The average salinity values showed that the post-monsoon season recorded the highest (14.07 o/oo). This may be due to the minimum tidal exchange in to the retting pits during post-monsoon season and high evaporation rate. The annual range of salinity obtained for Remani *et al* (1981) was 0.21 to 25.82 o/oo. As they reported, the process of retting did not show any effect on the salinity of the water. In Station II the highest salinity was recorded during March '90 (23.3 o/oo) and lowest during July '90 (0.26 o/oo). The seasonal mean was lowest during monsoon (5.3 o/oo) and highest during pre-monsoon (18.38 o/oo) seasons. The salinity decreased from pre-monsoonal peak to almost fresh conditions during monsoon and then a rise during post-monsoon months. Station II showed wide salinity fluctuations during seasons. The highest salinity was recorded in March '90 (20.86 o/oo) and lowest during July '91 (0.07 o/oo). The seasonal mean values showed vide variation in salinity as pre-monsoon (14.57 o/oo) monsoon (1.4 o/oo) and post monsoon (12.99 o/oo) seasons. The steep fall in salinity during monsoon months can be attributed to the land run-off and heavy fresh-water influx. In Station IV, the highest salinity was recorded in March'91 (17.68 o/oo) and lowest in July '91 (0.8 o/oo) whereas in Station V, the highest salinity recorded was in December '90 (17.3 o/oo) and lowest in August '91 (1.8 o/oo). In seasonal ponds (Station IV), during monsoon season due to paddy cultivation there was no access for brackish water in and the salinity remained very low then; while in perennial ponds, the lower salinity recorded was due to heavy influx of rain water. The higher salinity in aquaculture ponds in due to the influx of tidal water to prawn culture systems through the feeder canals.

Since dissolved oxygen is an important factor in water quality determination (Faerenbach 1969), depletion of oxygen in the water affects the total system of the medium. In Station I, anoxic to 4.5 ml/l of dissolved oxygen concentrations were recorded during the study period. Anoxic conditions in retting area is also reported by Abdul Aziz and Balakrishnan (1986). This was explained as due to highly restricted circulation process and organic decomposition. The immense quantities of coconut husks regularly burried in and the resulting stagnant conditions were found responsible for anoxic conditions. The mechanical action of the gas bubbles that rise from the bottom is also suspected to induce oxygen depletion in retting areas. The highest range obtained in the present observation was the same as reported by Remani *et al* (1981) from retting grounds at Cochin. Von Brand (1946) observed that critical oxygen concentrations were attained faster at higher temperatures due to quicker decomposition of organic matter. In the mangrove ecosystem, the dissolved oxygen values ranged from the lowest 0.4 ml/l to the highest 8.3 ml/l. The minimum value was nearly anoxic which may be due to the heavy decomposition of organic debris in the sediment, which resulted in maximum utilization of oxygen. Murthy and Jayaseelan (1986) reported a range of 3.0 ml/l to 7.6 ml/l of oxygen from a mangrove eco-system. The high productivity in the mangroves may be responsible for the high concentration of oxygen. The phytoplankton in the water contribute appreciable to the synthesis of organic matter (Teixeira and Kutner 1963; Teixera *et al.*, 1969 and Watanabe and Kutner 1965). Diatoms were reported to be dominant numerically by Walsh (1967) in Hawai and Bacon (1971b) in Trinidad. The dissolved oxygen levels of Station IV varied between 1.48 ml/l and 6.63 ml/l.

The oxygen level was lower during post-monsoon season due to the decomposition of paddy stumps, which resulted in high microbial activity (Sankaranarayanan *et al* 1986). In Station V also lower oxygen levels were reported during post-monsoon season. The lowest being 1.1 ml/l in October '91 and highest 7.15 ml/l in June '91. Higher oxygen concentrations during monsoon season is reported by Remani *et al* (1981).

In Station I, the highest pH ion concentration was recorded in November '90 (8.15) and lowest in August '91 (6.45) Lower values of pH after rains was reported by Abdul Aziz and Balakrishnan (1986). The seasonal mean showed higher pH value during post-monsoon (7.7) and lower during pre-monsoon (7.15) seasons. In mangrove ecosystem, the pH value ranged between 5.7 and 8.3. The average value was higher during monsoon (7.4) season. The high pH values reported during monsoon season may be due to the river discharge and flow from the adjacent sea (Abdul Aziz and Balakrishnan 1986). In Stations III, IV and V lower pH values were recorded during pre-monsoon season and higher values during post-monsoon season. This often results due to the north-east monsoon effect and land drainage. Low pH during monsoon season was reported by Sankarananrayanan *et al* (1986).

In aquatic systems, the limiting factors are dissolved nutrients such as nitrogen and phosphorous which are converted to particular form by plant growth. The nutrients are derived by their cycling by producers and consumers. In Station I, the phosphate-phosphorous content of the water was higher during pre-monsoon season and it ranged from 0 to 0.201 ppm during the period of investigation. The seasonal cycle of phosphate was discussed by

Panikkar and Jayaraman (1956). The values did not show much variations between seasons. The phosphate concentration in pre-monsoon months was high in coconut retting area, as reported by Abdul Aziz and Balakrishnan, (1986). In Station II, the phosphate value ranged between 0.017 ppm and 1.3 ppm. The highest mean value was observed in monsoon (0.54 ppm) and lowest in pre-monsoon (0.112 ppm) From the lower values in pre-monsoon season, phosphate showed a rise in monsoon and a slow decreasing trend towards the end of post-monsoon seasons. In Station III also, high phosphate value was observed during monsoon season (0.455 ppm). The pre-monsoon season showed lowest mean (0.36 ppm). In Station IV the phosphate value ranged from 0 to 0.326 ppm. In Station V, the range was between 0.006 and 0.109 ppm. Through out the period of study this Station gave lower values only indicating typical oligotrophic nature of the environment. It was not indicating dearth of nutrients but as soon as nutrients are released it will be utilised by the standing crop in the perennial pond. Higher mean value was recorded in pre-monsoon (0.144 ppm) and lower value during monsoon (0.037 ppm) seasons. The high phosphate values recorded during monsoon season in Station II, III and IV are supported by the reports of Panikkar and Jayaraman, (1956); Sankarananyanan and Quasim (1969); Abdul Aziz and Balakrishnan (1986) and Sankarananyanan *et al* (1986). Dharmaraj *et al* (1980) reported that inorganic nutrients exhibited high values during the north-east monsoon or by the diminishing stages of south-west monsoon. On considering the interchange of phosphorous between bottom and the overlying water, Moore (1940) observed that the phosphorous content of estuarine mud is so high and is about 50 times more than that of water above. Depending on the degree of

organic matter degradation, different amount of humic substances are accumulated which are considered to be important in chemical removal of phosphate (Smith and Longmore 1980). Harvey (1955) observed that antilytic enzymes of bacteria in sediments releases considerable amounts of phosphate into the water. Pomeroy *et al* (1963); Phillips (1964); Barlow and Bishop (1965) and Harrison *et al* (1972) reported that the exchange of phosphate from the water is caused by bacteria and they are capable of storing phosphates under aerobic conditions.

In coconut retting area (Station I), the nitrate nitrogen concentration ranged between 0 and 0.159 ppm. Higher values were obtained during pre-monsoon and monsoon months. The mean nitrate level was lower in retting zone, which has been attributed to a process called denitrification, initiated by bacteria in the absence of oxygen (Abdul Aziz and Balakrishnan 1986). In Station II, III, IV and V also higher nitrate values were recorded during monsoon and lower values during pre-monsoon seasons. This observation is in confirmity with those of Panikkar *et al* (1956); Rao and George (1959); Sankaranarayanan and Quasim (1969); Sreedharan and Salih (1974); Murthy and Jayaseelan (1986); Abdul Aziz and Balakrishnan (1986) and Sankarananrayanan *et al* (1988). The role of birds towards contribution of fertility of mangrove sediment and water has not been investigated adequately (Walsh 1974). Mangalavana, the Station II, was one such ecosystem where large number of birds including ergets, herons, storks, spoonbills etc. visit regularly and is preserved as a bird sanctuary. Birds in mangroves have been described by Cawkell (1964), Parkes and Dickerman (1967); Field (1968),

Dickerman and Juarex (1971) and Ricklefs (1971). By birds which roost in mangrove trees, but fed else where, nutrients are brought into the swamps and functions of such nutrients need investigation.

Chandrika (1984) reported that total heterotrophs were positively correlated with nitrate and phosphate at 5% level. Bacteria require nitrogen and phosphate for their growth, whether these nutrients are completely assimilated or released into the water during bacterial growth depends upon whether they are present in the substrate in excess of the nutrient capacities of bacterial protoplasm. If sufficient nitrogen or phosphorous is available for maximum bacterial growth, assimilation of inorganic nutrients from the surrounding environment occurs. It is referred to as nutrient immobilisation, a mechanism by which bacteria reduce the quantity of soil nutrients available to plants (Alexander 1961).

In all the five stations, the higher silicate values were recorded during monsoon months. The values ranged from 0.495 ppm to 2.42 ppm in Station I. The pre-monsoon months recorded minimum values (0.917 ppm). In Station II the silicate load ranged between 0.42 and 2.05 ppm. In Station III lowest recorded value is 0.102 ppm in September '90 and highest in 2.46 ppm in August '91. Station IV showed comparatively lower silicate values within a range of 0.073 to 1.06 ppm. In Station V, the silicate concentration was higher and ranged between 0.126 and 2.38 ppm. The heavy silt load entering into the backwater system due to the land run-off during monsoon season was responsible for the high concentration of silicate in these ecosystems (Sankaranarayanan and Quasim 1969). When productivity is increased,

nutrient content is reduced. The increase in nutrients in the systems are due to the turn over rate within which exceeds the advective supply from external sources. If the system is shallow, it is believed that the major factor which governs distribution of nutrients may be the variations in the regenerative property of the bottom mud rich in organic matter due to biological oxidation.

The sediment temperature followed same pattern as water temperature and recorded more during pre-monsoon season (32-46°C) and less during monsoon (29.45°C) in Station I. In Station II also, a similar pattern of temperature distribution was observed. The highest temperature was recorded in April '91 (31.5°C) and lowest in August '91 (26.15°C). Station III showed a temperature range between 28.5°C and 31.25°C. Station IV showed highest temperature in September '90 (33.25°C) and lowest during July '91 (26.25°C). In Station V, the highest temperature recorded in October '90 (33.25°C) and lowest in July '91 (27.25°C). In Stations III and IV, the seasonal mean values were lower in post-monsoon months than in monsoon. The low temperature values indicated the lesser influence of monsoon on sediment temperature in these stations. Only in shallow areas, the sediment temperature is affected faster by the change in temperature. But it was observed that a positive correlation exists between water and sediment temperature in all the five stations.

Sediment pH followed the same pattern as water pH and ranged between 6.4 and 8.6 in Station I. The lowest value was recorded in March 91 and highest in September 1990. During monsoon season, the mean value for sediment pH was higher (7.09) and lower for pre-monsoon. The other stations

also showed the same trend. The high pH recorded during monsoon season can be attributed to the heavy influx of fresh water to the systems. Shankaranarayanan *et al* (1986) recorded low pH during monsoon season in the Cochin back water systems.

The sediments of all the five stations showed varying amount of organic carbon during the period of study. Sediments of retting yard were black in colour with foul smell of hydrogen sulphide which can be called sulfuretta. Pith and fibres were present in it. The mangroove sediments also were rich in organic matter with decomposing leaves and twigs. The organic carbon content in Station I was highest in September '90 (3.75%) and lowest during February '91) (2.4%). The organic matter of the station ranged between 4.13% to 6.08%. The value was high for post-monsoon (5.99%) and low for monsoon (3.85%) Remani *et al* (1980) reported an average of 3.5% for the Cochin back waters, while the values of Murthy and Veerayya (1972) stand at 2.5%. The enrichment of organic content of the sediments at the retting grounds, without any other major sources of inputs, may be attributed to the process itself. In Station II, organic carbon content ranged from 0.95% to 4.18%. In '90 the values were lower during monsoon, while in 91 it was higher. In this station also pre-monsoon season recorded higher values (3.58%) and monsoon months lower (2.0%). The organic matter content also showed the same trend in its distribution. In Station II, organic carbon content ranged from 0.95% to 4.18%. In '90 the values were lower during monsoon, while in '91 it was higher. In this station also pre-monsoon season recorded higher values (3.58%) and monsoon months lower (2.0%). The organic matter content also showed the

same trend in its distribution. In Station III, the highest value was recorded in August '91 (4.18%) and lowest in December 90 (0.36%). The organic carbon content was high during pre-monsoon season as reported by Remani *et al.*, (1980). In seasonal field (Station IV) also highest value was observed in monsoon month, July '91 (4.35%), whereas in the perennial pond, it was lowest in September, 91 (0.34%) and highest in August 91 (2.05%). The distribution of organic matter also showed a similar trend of organic carbon distribution in these stations. An increase in organic matter may result from the break down of a heavy plankton bloom which totally sunk to the bottom due to the absence of zooplankton (Bodungen *et al* 1975). They reported an enrichment of organic matter in the sediment surface leading to a considerable increase in the decomposition rates of proteins and carbohydrates and creation of eutrophic conditions.

The monthly variation in sediment phenolics in different aquatic ecosystems have not been studied in detail by any workers. Though plant polymers are degraded to simpler substances of environmental concern, studies regarding it are very few. The coconut husk retting is purely a biological process in which large quantities of polyphenols are leached to the sediment (Jayashankar 1966). The mangrove swamps of Goa showed the presence of various phenolic compounds in the sediment samples (Karanth *et al* 1975; Gomes and Mavinkurve 1982). In the present study also it was found that the coconut retting area at Chittoor and Mangrove station, Mangalvana showed very significant seasonal changes in phenolic content of the sediment. The other three stations also showed presence of phenolic compounds in the

sediment at decreased levels, compared to Stations I and II. The maximum concentration was recorded in June '90 (19.64 ppm) and lowest was 0.8 ppm in Station I. The monsoon season recorded maximum (14.63 ppm) and post monsoon (0.825) minimum. Station II also showed a similar trend in phenolic concentration. In mangroves of Mandovi-Zuari estuary, Gomes and Mavinkurve (1982) reported only 0.26 to 1.01 ppm of phenolics. The highest phenol content in Station III was during pre-monsoon months. A clayey sediment with high organic matter content gave high phenol concentrations. Sandy sediment, as in perennial pond did not give much concentration of phenol. The accumulation of lignin and humus known to be resistant to bacterial attack was suspected to be responsible for the high phenol concentrations in Station II. The maximum phenol content recorded in aquaculture ponds during the study period did not exceed 1.5 ppm, which may not have any effect on other aquatic organisms.

The monthly variation in total aerobic heterotrophs showed significant variation over seasons in all the stations. The maximum count was obtained in Station I during pre monsoon season (138.7×10^4). In Station II and IV also pre-monsoon months recorded highest counts (86.35×10^4 and 77.1×10^4 respectively). In Stations III and V highest bacterial counts were retrieved during post monsoon season (115.63×10^4 and 87.16×10^4 respectively). The counts were minimum during monsoon season in all the stations. It was reported that, in monsoon when the salinity is low and leaf content is high, the microbial flora in mangroves were largely consisted of fungal populations (Mantondkar *et al* 1980). In other stations also dominance of fungal population

may be one of the reasons for fewer numbers of aerobic heterotrophs during monsoon season. Haeckel and Rheinheimer (1983) reported that most of the bacteria increased with higher summer temperature in Angeran, Sweden.

The correlation coefficients have been worked out separately for each station (Tables 19, 22, 25, 28 and 31) because it is possible that linear relationship between variables might change from station to station.

The correlation analysis indicated that the observed correlations between sediment phenolics and total aerobic heterotrophs with other environmental parameters were based on some fundamental relationships to a certain extent. To find out the importance of influence of those parameters which had correlation with sediment phenolics and total aerobic heterotrophs and to fit a regression equation, multiple regression analysis was also carried out.

The effect of temperature on micro-organisms is well known (Morita 1974). In the present study, temperature had positive correlation with bacterial count in stations I and II and a negative correlation in Station II. Bent and Goulder (1981) found out significant correlation between density of attached bacteria and temperature and salinity. Various species are known to be adapted to seasonal temperature variations (Susan and Riley 1967) and adaptation of microbial population could play an important role in the ecology of the sediment (Nedwell and Floodgate 1971). Increase in the number of bacteria with higher summer temperature was reported by Haeckel and Rheinheinher (1983). A positive correlation between aerobic heterotrophs and

salinity was observed in all the five Stations during the period of study. Rheinheimer (1979) reported changes in bacterial population as a function of salinity. With increasing salinity, the proportion of halophilic bacteria was reported to increase. In contrary to fresh water and terrestrial bacteria, marine and brackish water bacteria require sodium to live. Whereas most bacteria from fresh water, waste water and soil are normally killed within a short time by the bactericidal action of brackish water (Szwerinski 1981). Chandrika (1984) found no significant difference in total plate count (TPC) between Stations months and regions. Seasonal variations in sea water was meagre and maximum number was observed during past-monsoon period. Seasonal variations was prominent during monsoon in sediments.

The total counts between one station and the other did not vary as much as the counts between months and regions. During monsoon, minimum of halotolerant species and during pre-monsoon and post-monsoon seasons maximum of them being reported (Pugh *et al* 1974). Jana *et al* (1979 and 1980) found that population size of bacteria is correlated with temperature and dissolved oxygen. In the present study also, a correlation was exhibited between dissolved oxygen and heterotrophic bacterial count. Nutrients showed varying degree of correlation with aerobic heterotrophs. Jana (1977) has, however demonstrated a positive correlation between seasonal variation in phosphate and that of heterotrophic bacteria in some hot water springs in West Bengal. A similar relation was observed in Station I between heterotrophs and phosphate content in water. By the process of immobilization the nutrients available in water is reduced by bacteria (Alexander 1961). This

may be the reason for an inverse relationship between total heterotrophs and nitrate content in Stations II, III and V. Silicate showed positive correlation with heterotrophs in Station V which may be due to silicate solubilizing property of some bacteria (Purushothaman *et al* 1974).

The organic matter content of the sediment showed negative correlation with bacterial count in all the five Stations. This has been supported by the views of Bodungen *et al* (1975). They observed a decrease in microbial biomass during the enrichment of organic material in sediment, thus questioning the predominant role of microbial extracellular enzymes in the early stages of decomposition process. This is due to the absence of suitable substrates for microbial growth by the decomposition of proteins and carbohydrates in the sediment. The common interpretations of the correlation of organic carbon with microbes is that, an abundance of food leads to abundance of consumers (Longbottom 1970). According to Novobrantzev, (1932) bacterial population is directly proportional to the organic matter. The phenolic content of the sediments in Stations I and II showed negative correlation with heterotrophs which may be due to the antimicrobial activity of phenols as reported by Bennet (1962) and Valiela (1991).

The multiple regression analysis determined for finding out a regression equation has shown that in Station I, II, IV and V salinity was the most important variable affecting heterotrophic count in those stations. The next important variables were phosphate-phosphorous (Station I), silicate (Station II) and nitrate-nitrogen for Station III, IV and V. Temperature pH

and organic matter were also found to influence heterotrophic count in the five stations at different levels.

In the correlation analysis for sediment phenolics with different physico-chemical parameters, it was seen that temperature had a negative correlation with phenolic concentration in Station II. At higher temperature due to microbial activity phenolics in the sediment gets degraded (Itturiaga and Rheinheimer 1972). The correlation between salinity shows that biodegradation of phenolic compounds is faster in saline water than in fresh water, as may be due to the presence of halotolerant micro-organisms in more numbers than other types which can degrade phenolics. Organic matter also showed a positive correlation with phenolic content of the sediments in Stations I and II. Most of the organic debris is comprised of plant remains and on degradation by certain fungi turns to phenolic sub-units (Burges 1967 and Stout *et al* 1976).

The regression analysis have shown that phenolics content of sediments in Stations I and V were mainly dependent on salinity. In Station II, organic matter was the prime factor determining sediment phenolics. The other factors influencing phenolics in sediment were inorganic nutrients especially nitrate nitrogen temperature, pH and dissolved oxygen.

The aerobic heterotrophic bacterial genera isolated from the five stations include *Aeromonas, Alcaligenes, Bacillus, Cytophaga, Enterobacteriaceae, Flavobacterium, Micrococcus, Pseudomonas* Groups II, III and IV and *Vibrio.* Stations I and II showed striking similarity in heterotroph distribution. Such

a similarity in the two environments is perhaps due to the fact that, both the ecosystems foster the decomposition of plant material in an estuarine system. The presence of *Bacillus, Micrococcus* and *Pseudomonas* has been observed in mangrove swamps of Goa (Matondkar *et al* 1980 a) and Thailand (Daengshuba 1979) and in the coconut retting liquor (Bhat and Nambudri 1971). The significance of these micro-organism is well established (Eklund and Gyllenberg 1979). Similarly Stations IV and V were almost alike in heterotrophic bacterial distribution. Here abundance of *Fluvobacterium* and *Alcaligenes* is also noticeable. The predominance of *Pseudomonas* in coastal sea water and sediments has been reported by various investigators in several countries : (Zo Bell 1946; Wood 1953; Brown 1970). Simidu *et al.,* (1980) reported predominance of *Vibrionaceae* in Nauseishoto area in Kuroshio current which runs between the pacific ocean and East-China sea. Conditions for microbial growth in all the five stations were similar to that of Kamogawa Bay, Japan (Simidu and Aiso 1962) and Long Island Sound, New York (Susan and Riley 1972) where predominance of *Pseudomonas* has been documented and the occurrence of *Alcaligenes, Aeromonas* and *Micrococci* were reported. In the present study, halotolerant species were found to be more during pre-monsoon and post-monsoon seasons, when warm conditions prevail. Temperature governs all biological processes and it is thus a prime factor of concern to bacteria. Each micro-organism has an optimum temperature for growth and a range outside which growth and multiplication ceases. The occurrence or non-occurrence of a particular genus is thus dependent on seasons as is observed in the present study. As in coastal areas, predominance of gram negative rods was observed in all the five stations. Chandrika (1984)

found that 95% of the isolates during a three year period of study was constituted of gram negative rods. With their high enzymatic potential revealed by various biochemical tests, marine bacteria were equipped to degrade any organic compound that occur in the sea as the marine bacterial enzymes are very stable in brackish water and fresh water environments.

It is also interesting to note that although certain bacterial genera are dominant at specific times of the year, the biochemical and physiological activity were recorded essentially constant, indicating the maintenance of nutrient level in these environments by other bacterial genera. Chandrika (1984) observed seasonal variation in bacterial genera, but proteolytic, lipolytic and amylolytic activity appeared independent of seasons.

In the present investigation, it is observed that a number of aerobic heterotrophic bacteria from different aquatic environments could tolerate and even utilize phenol at high concentrations. Of the 153 bacterial isolates, 53% could grow well in the presence of 0.05% phenol in the medium. These cultures were inoculated to higher concentrations (0.1, 0.2, 0.4 0.6 0.8 and 1%) of phenol. Gram-positive cocci were more tolerant to phenol than gram-negative rods and gram-positive rods. A similar observation was reported by Gomes and Mavinkurve (1982) with isolates, from mangrove swamps of Goa. They also found that whenever the concentration of phenol is increased in the medium, the viability of micro organism is reduced. Similarly, in the present observations also, as the concentration of phenol has increased, the percentage of viable bacteria has decreased. As the results is shown (Fig.25) 6 gram-positive rods, 6 gram-positive cocci, and 17 gram-negative rods could

tolerate 1% of phenol. ie. 15.6% out of the 153 isolates could tolerate in 1% of phenol. These cultures were also found to grow well on o-cresol and orcinol also.

4.2 Biodegradation studies

In the present investigation for isolation of phenololytic bacteria, enrichment culture, batch culture, selective culturing, (Tabak *et al* 1964) were the techniques followed. Biodegradation rates were determined by static culture flask procedure (Bunch and Chambers 1967, and Tabak *et al* 1981).

Selective culturing or enrichment techniques have been the classical tool and form the initial step in studying the existence and role of specific metabolic groups of micro-organisms in a given natural environment. By adding a particular substrate, as phenol in the present study, to a natural population, or by increasing the concentration of a nutrient or energy source, those micro-organisms engaged specifically in its transformation will increase in numbers and become amenable to subsequent isolations for pure culture studies. Small growth advantages, which are not detectable in short lived closed culture systems, are amplified in such runs. Thus organisms competing for the same substrate but growing at a different rate can be separated by continuous culture enrichment procedures (Jannasch 1977).

The static culture flask biodegradation studies followed in the present experiments using mixed as well as individual bacterial isolates from different aquatic environments, enabled to determine the biodegradability of phenol at

different concentrations ranging from 100 ppm to 1000 ppm. A similar methodology was followed by Bunch and Chambers (1967) and Tabak *et al* (1981) with slight variations in set-up.

The oxidation of phenol by micro-organisms have been reported by many workers (Fowler *et al* 1911; Wagner 1914; Gray and Thornton 1928; Evans 1963; Shivaraman and Parhad 1985). They found that phenol could serve as a source of carbon and energy for soil and aquatic bacteria.

Since biodegradation of phenol is reported to be faster under aerobic conditions (Evans 1963; Ornston and Stainer 1964; Dagley and Gibson 1965; Fiest and Hegeman 1969) the present investigation was carried out in a partially aerobic condition.

Different workers have noted the effect of temperature on the biodegradation of phenol. Hamdy *et al* (1954, 1956) found that a temperature range of 37°C to 55°C as optimum for oxidation of phenol by sewage bacteria. Itturiaga and Rheinheimer (1972) reported that microbial decomposition of phenol takes place mainly during the warm season at water temperature above 10°C. The biodegradation rate was reported to increase with increase in temperature. Ermolaev (1979) also reported that temperature had significant influence on phenol biodegradation rate. Buswell and Twomey (1975) found out that *Bacillus stearothermophillus* could oxidize phenols at a temperature range of 37°C to 55°C. Based on these observations the present experiments were maintained at room temperature of 30°C - 32°C.

The effect of pH on the rate of phenol biodegradation has also been studied. (Hamdy *et al* 1956; Jayashankar and Bhat 1966; Boto and Bunt 1981). They have found that a pH of 7.0 to 7.5 is optimum for oxidation of phenol by bacteria. So, pH was maintained at 7.5 in the medium. But as the experiment proceeded, the pH was found to fall from 7.5 to 6.5 by the end of experiment duration (9 days). A similar observation was reported by Tabak *et al* (1964) and this was attributed to the reactions taking place in the medium and due to production of organic acids during biodegradation.

Inorganic compounds affect the rate of biodegradation as well (Ermolaev 1979; Shimp and Pfaender 1984). Pawlaczyk (1965) found that glucose or other carbohydrates in the medium caused an inhibition of phenol decomposition by *Pseudomonas fluorescens* but on the other hand, urea was found to be stimulatory. Mineeva (1974) found that *Azotobacter agile* and *Pseudomonas denitrificans* are capable of decomposing phenol both when it is the only carbon source in the medium and in the presence of other organic substances like glucose for *A.agile* and citric acid for *P.denitrificans*. But in the present study only phenol was added as carbon and energy source in the mineral salts medium (Aaronson 1970).

The source of inoculum also showed considerable influence on biodegradation rate in the present study. Stolbonov (1971) found that the highest biochemical activity occurred in areas where sewage is discharged from sources of phenol contamination. In moving away from the source, the phenol degrading ability seemed to decrease. He observed positive correlation between phenol degrading ability of water and its content of phenol degrading bacteria

as well as a positive strong correlation between the amount of saprophytic and phenol utilizing bacteria in water. Ermolaev and Mironov (1975) studied abundance and species composition of phenol degrading micro organisms and rate of biochemical oxidation in shelf waters of Black Sea and recorded a significant increase in micro organisms and their activity in polluted areas. They also observed that in areas characterised by constant polluted river discharge, as insignificant number of well adapted micro-organisms with high phenol degrading activity. Emrolaev (1979) reported that the number and activity of phenol degrading bacteria were dependent on the phenol content in the water determined chemically. In the present batch culture experiment also, it was found that the inoculum from coconut retting area (Station I), where maximum concentrations of phenol were recorded had shown maximum rate of biodegradation.

Adaptation of microbial population to the test compound had significant correlation with biodegradation of phenol. Tabak *et al* (1964) found that bacteria should be well adapted to metabolize phenol, hydroxy phenols, when cultured in mineral salts medium with the specific substrate as the sole source of carbon. Spain *et al* (1980) after studying adaptation effects on biodegradation rates has given possible explanations for the differences in adaptation between Range point samples and Escambia river samples like (i) the bacterial biomass in the salt marsh samples may be low (ii) the higher salinity or lower concentrations of nutrients have been limiting in salt marsh or (iii) absence of specific organisms able to mineralise the test compounds in the salt marsh samples might have precluded adaptation. In the present

investigation also, biodegradation studies were conducted after adapting the micro-organisms from different environments by primary enrichment technique (Tabak *et al* 1964) and also on determining biodegradation rates following static culture flask procedure, it was found that the rate of biodegradation was maximum in sub-culture III when compared to sub-cultures I and II. A similar observation was reported by Tabak *et al* (1981) in their study on biodegradation of priority pollutants. Visser *et al* (1977) found that many bacteria can tolerate phenol concentration as high as 2400 ppm after adaptation.

Several studies have been reported pertaining to the quantity of phenol that can utilized by bacteria. Some of these investigations have shown that approximately 1000 ppm of phenol was optimum for oxidation (Gray and Thornton 1928; Czekalowski and Skarzynski 1948). Itturiaga and Rheinheimer (1972) found that a group of bacteria and fungi can attack phenols, provided the concentration should not exceed 2000 ppm. So in the present study concentration ranging from 100 ppm to 1000 ppm of phenol were used to study the biodegradability by mixed bacterial isolates. Under normal conditions the concentration levels of any pollutant will be significantly smaller than those used in biodegradation studies, and as such may not elicit an induction enzyme formation response by the micro-organism screened (Tabak *et al* 1981). In the present study maximum concentration of phenolics obtained was only 19.64 ppm from coconut retting area (Station I), the most polluted station studied. But higher concentrations were used to study the biodegradability, with mixed (100 to 1000 ppm) as well as individual

(100-500 ppm) bacterial isolates from the genera which can be used as potential biodegraders in a phenol polluted aquatic ecosystem. Usually biological methods are preferred for pollutant treatment as they are economical (Shivaraman and Parhad 1985). The results obtained indicate that phenol adapted bacteria could utilize 100% phenol in the medium in two or three days upto 300 ppm. A similar trend was reported by Tabak *et al* (1964). It was also observed that as the concentration of the test compound (phenol) increased in the medium, the rate of biodegradation has decreased. Rubin *et al* (1982) found that the rate of phenol degradation is a linear function of concentrations below 1 ppm, falls off between 1 ppm and 100 ppm and is again high at levels above 100 ppm. These findings reflect the activity of oligotrophs at lower concentrations and eutrophs at higher concentrations Wase and Hough (1966) reported that at the highest contents of phenol in the medium (12 μ moles/ml), the yeast adapted readily and produced considerable amounts of riboflavin. In the present investigation the activity of heterotrophs was found to decrease with increase in phenol concentration in the medium.

At 100 ppm above, the biodegradability was not significant over stations and over days. From 200 ppm upto 1000 ppm, the F-values showed significance at 1% level over stations and over days. Visser *et al* (1977) found that over 70% of the micro-flora in river was able to tolerate phenols at a concentration of 150 ppm and the number of phenol tolerating bacteria was found to increase from a relatively unpolluted to the most polluted part of the river by approximately 40%. The present finding also showed that as the concentration of phenol is more, the tolerance of bacteria to phenol was also

high. Alexander (1961) reported that biodegradation rate was more during the first phase of the incubation period. Similarly, in the present study, a trend was obtained with maximum degradation of phenol in the initial 2-3 days and then only marginal hike in biodegradation in the proceeding days.

Phenololytic micro-organisms belong to bacteria fungi and actinomycetes. Amongst bacteria those belonging to genera *Azotobacter, Alcaligenes, Pseudomonas, Flavobacterium, Bacillus brevibacterium, Clostridium, Micrococcus, Vibrio, Sarcina* etc. were reported to utilize phenol as sole source of carbon and energy (Fuhs 1961; Dagley 1967; Ermolaev 1975; Visser *et al* 1977; Parhad *et al* 1981; Sastry 1986). In the present study bacterial genera which could tolerate phenol at different concentrations were, *Alcaligenes, Bacillus, Cytophaga, Enterobacteriaceae, Flavobacterium, Micrococcus, Psedumonas* and *Vibrio*. The results indicate that these genera occur in all the five stations selected for the study. Also it was seen that *Pseudomonas* and *Vibrio* readily adapt themselves to metabolise phenol at higher concentrations (> 500 ppm). Same strains of bacteria were obtained in o-cresol and orcinol at concentrations ranging from 100-500 ppm.

A few selected phenol tolerant strains of bacteria were isolated from the mixed culture and purified and maintained in mineral salts agar with 0.05% phenol in it. The methodology followed for purification and maintenance was the same as that of Czekalowski and Skarzynski (1948). There are lot of studies on the metabolism of phenolic compounds by *Pseudomonas* sp. In the present study, three genera of bacteria and a *Streptomyces* sp. were used for biodegradation experiments using individual isolates. The concentration of

phenols ranged from 100 to 500 ppm. The oxidation of phenols and cresols by *Pseudomonas* sp. have been reported by many workers (Hamdy *et al* 1956; Durham 1956; Ribbons 1966; Bayley *et al* 1966; Fiest and Hegeman 1969; Bayley and Wigmore 1973; Spain and Gibson 1988). In the present investigation, the *Pseudomonas* strain could degrade 500 ppm of phenol in 9 days. The analysis of variance test showed significance over phenol concentration and over days. The *Alcaligenes* sp. could degrade only 60% phenol at 100 ppm in 9 days. The viable count also showed a decrease with increasing concentrations. The biodegradability by *Alcaligenes* was significant over concentrations and over days ($P < 0.01$). The viable count showed significance over concentrations, but the relationships were insignificant over days. *Vibrio* also showed a similar trend in biodegradability and viable count. *Streptomyces* sp. utilised 100 ppm of phenol in 3 days and 500 ppm in 9 days, indicating *Streptomyces* is also a potential phenololytic strain like *Pseudomonas*. The viable count was very low in the case of *Streptomyces* when compared to other bacterial strains. The results of test of significance for biodegradability showed significance over concentrations and over days. But, the F-value was not significant over phenol concentrations in viable count as in the case of bacteria. The biodegradability of aromatic compounds by *Streptomyces* sp. was reported by Sutherland *et al* (1981) and Pometto *et al* (1981). Antai and Crawford (1982) reported that phenol could be degraded by *Streptomyces setonii* at lower concentrations. But, the optimum quantity of phenol that could be utilized by *Streptomyces* is not reported by any workers so far.

As in the case of mixed culture, a neutral pH (7.5) and room temperature (30-32.5°C) were found to be better for growth and phenol oxidation by different microbial strains used. This is in confirmity with the reports of Hamdy *et al* (1956) who found out that phenol oxidizing *Pseudomonas* had optimum growth and utilization of phenol at neutral pH and room temperature. The decrease in rate of biodegradation and viable count with increase in phenol concentrations were common to all the strains used. This can be attributed as substrate inhibition property. Substrate inhibition on the growth of *Bacterium* NCIB 8250 by Phenol was reported by Jones *et al* (1973). A study by Divanin *et al* (1977) shows that , during growth on phenol as a sole carbon and energy source, a rearrangement of the metabolifes of the cells occur and similar organisms are responsible for the degradation of complex substances like hydrocarbons in marine environment. The degradation of phenol by *Alcaligenes faecalis* and *Pseudomonas acidivorans* isolated from soil has been reported by Shivaraman and Parhad (1985). They used 500 ppm of phenol in the basal medium for biodegradability studies.

In overall, results of biodegradation studies showed that rate of biodegradation with mixed cultures was more efficient than that with individual isolates. Zo Bell (1962) had reported that individual isolates of micro organisms were even unable to grow in mineral salts medium, while mixed cultures could degrade upto 1% phenol in the medium.

In the biodegradation of particular pollutants like phenol in sediments, extracellular enzymes are involved which are secreted from living cells (mainly micro-organisms) as well as liberated during the lysis of cells. Unless the

enzymes are released in close contact to substrate, their fate is uncertain. Processes like absorption, decomposition and denaturation greatly influence the efficiency of the enzymes released. According to studies carried by Berns, (1980) some of these enzymes remain active in soil by the formation of humic enzyme complexes. These active enzymes contribute to biodegradation as well. In the present study also, it may be attributed that biodegradation has occurred at different concentrations of phenol due to the micro-organisms inoculated to the medium though direct evidences have not observed to find out the enzymes involved in the process.

Microbial degradation of organic compounds is often investigated in the laboratory to measure biodegradation rate constants that can be used to predict potential biodegradation rates in the environment. It is difficult, however to extrapolate results obtained in the laboratory systems to predict the fate of organic pollutants in the environment where conditions may be different from those in the laboratory (Spain *et al* 1980). In the present investigation also, we cannot expect the results to be extrapolated for field prediction, but a fundamental idea about rate of biodegradation and the bacteria involved in the process could be obtained, which can be further modified and made fool proof for future use. A number of reasons for our inability to rely on laboratory generated data to predict field results can be noted, and usually is related to laboratory design (high nutrients, optimized oxygen concentration etc.) or the nature of the micro organisms found in the field. The environment in which an organisms exists play an important role in its function, as observed in the present study. Generally organic compounds including phenols are degraded more rapidly under aerobic conditions, therefore oxygen level can affect the rate at which a compound degrades.

Other environmental conditions include pH, temperature and salinity. Low nutrient levels, both organic and inorganic can retard or accelerate biodegradation. Sometimes it stimulates degradation and other times may retard degradation. The chemical structure and concentration of the substrates are important considerations in biodegradation (Bourquin 1984). Though chemical and some biological process bring about some changes in chemical structures, it may not alter the toxicity of the product. Whereas microbial degradation can produce major changes in the chemical structure of the introduced chemical mineralization leading to detoxicity is often end result of microbial activities.

CHAPTER 5

SUMMARY

1. The present investigation on 'BIODEGRADATION OF PHENOLIC COMPOUNDS IN DIFFERENT ECOSYSTEMS IN COCHIN', was carried out on the basis of data collected from five different aquatic ecosystems-a coconut husk retting area, a mangrove, a back water system and seasonal and perennial aquaculture ponds, over a period from March 1990 to November 1991, to understand the seasonal variations in sediment phenolics' content and aerobic heterotrophs in relation to other physico-chemical parameters. A preliminary study was undertaken to screen aerobic phenololytic organisms from the selected environments. Selection of suitable micro-organisms (bacteria and actinomycetes) from sediment samples, which can cause efficient degradation of phenolics resulting in release of simple organic substances which provide nutritional support to the growth of phytoplankton or other higher organisms was also done.
2. The physico-chemical parameters like temperature, salinity, dissolved oxygen, pH, nutrients, organic carbon and organic matter had shown varying degrees of seasonal fluctuations during the period of study.
3. Temperature had positive correlation at 5% level with aerobic heterotrophs in Station-I ad II, whereas, in Station - III, IV and V, there was no significant correlation observed.
4. Salinity showed significant direct relationship with total aerobic heterotrophs in all the five stations during the period of study.

5. Of all the stations, only Station IV recorded an inverse relationship between dissolved oxygen and aerobic phenololytic organisms and relationship was recorded with other parameters.
6. Hydrogen ion concentration had shown significant positive correlation with aerobic heterotrophs in Station-III. No other stations had shown significant correlation between pH and aerobic heterotrophs.
7. Only Station-I showed significant positive correlation between phosphate-phosphorous and aerobic heterotrophs.
8. Nitrate-nitrogen had shown significant correlation with aerobic heterotrophs in Stations-II, III and V. Station-IV had shown a significant negative correlation with it.
9. In Station-II alone, silicate-silicon had significant correlation with aerobic heterotrophs.
10. Organic carbon and organic matter showed significant inverse relationship with total aerobic heterotrophs in all the five stations observed.
11. Sediment phenolics had significant negative correlation with aerobic heterotrophs in Station-I and II. Other three stations had shown no correlation between phenolics and heterotrophs.
12. The correlation between sediment phenolics and other physico-chemical parameters revealed that temperature had direct influence on sediment phenolics in Stations-II and IV.
13. Salinity was indirectly correlated with sediment phenolics in Stations-I, II and V and directly in Station-III.
14. Dissolved oxygen had indirect influence on sediment phenolics in Station-III alone.
15. pH had positive correlation with phenolics only in Station-IV.

16. Nitrate-nitrogen was directly correlated with phenolics in Stations II and III and silicate in Stations-IV and V.
17. Sediment organic carbon and organic matter were significantly influencing phenolics in Station I and II.
18. The sediment phenolic content was not significant over seasons and over stations.
19. Total aerobic heterotrophic counts were significant over stations and over seasons.
20. The bacterial genera isolated from sediment samples were *Aeromonas, Alcaligenes, Bacillus, Cytophaga, Enterobacteriaceae, Micrococci, Pseudomonas* Groups II, III and IV, and *Vibrio* .
21. Gram-negative rods predominated the phenololytic isolates.
22. Of the total aerobic isolates, 52.94% could tolerate 0.05% phenol and 15.6% could survive in 1% phenol.
23. Micro-organisms isolated from sediment samples degraded phenol considerably at different concentrations (100-1000ppm) under laboratory conditions.
24. The biodegradation rate was higher in the beginning of decomposition, but thereafter declined gradually.
25. Biodegradation rate was more with mixed cultures than with individual isolates.
26. Individual isolates were less tolerant to higher concentrations of phenol in the medium, while mixed cultures could degrade higher concentrations.
27. The biodegradability of phenol with mixed culture over sub-cultures and over days were significant at all the ten selected concentrations.
28. *Pseudomonas* and *Streptomyces* were found to be the most potential phenol degraders in hte present study.

29. The biodegradation of phenol and viable counts of *Alcaligenes, Pseudomonas, Vibrio* and *Streptomyces*, were found to be significant over different phenol concentrations and over days of observations.
30. As the concentration of phenol increased in hte medium, the rate of biodegradation was found to be decreased.
31. The formation of specific extracellular enzymes to resist the toxic substrates like phenol by the micro-organisms was established indirectly in the laboratory experiments conducted.
32. It is also concluded that, though chemical oxidation bring about change in the structure .of the pollutant like phenol, the toxicity of the compound may remain as such. But in biological oxidation, as once structure is changed, the toxicity of the pollutant is also removed completely.
33. The results of the study suggests that the addition of microbial inoculum to various concentrations of phenol in the medium enhances the rate of biodegradation, thereby reducing the total period required for degradation. These findings also indicate that, if optimum conditions are provided, the biodegradation can be maximised, in the shortest possible period under experimental conditions. Whenever, extreme conditions are observed in the natural environment, they were more often the result of water masses brought about by under water currents and high tides than response of phenololytic bacteria to changing environmental conditions.
34. The quantity and quality of phenol in every station sampled was dependent on variety of factors, however the relations between phenol, fertility of the environment and bacterial development are dependant mainly on the hydrologic conditions.

CHAPTER 6

REFERENCES

Aaronson, S., 1970. Procedures for the enrichment and/or isolation of micro-organisms. *In* Experimental Microbial Biology, Academic Press, New York and London, pp.65-227.

Abdul Aziz, P.K. and **N.Balakrishnan Nair,** 1976. The problem of pollution in the retting zones of the back waters of Kerala Natl. Environ. Pollut. Conference, May 6-8, Cochin, *Souvenir,* 295 pp.

Abdul Aziz, P.K. and **N.Balakrishnan Nair,** 1978. The nature of pollution in the retting zones of the back waters of Kerala. *Aquatic Biology,* **3** : 41-62.

Abdul Aziz, P.K. and **N.Balakrishnan Nair,** 1986. Ecology of the coconut husk retting grounds in Kerala. *Proc.Symp.Coastal Aquaculture* 1986, pp.1115-1130.

Akagi, Y., U.Simidu and **N.Taga,** 1980. Growth response of oligotrophic and heterotrophic marine bacteria in various substrate concentrations and taxonomic studies on them. *Can.J.Microbiol.,* **26** : 800-806.

Akagi, Y, N.Taga and **U.Simdu,** 1977. Isolation and distribution of oligotrophic marine bacteria. *Can.J.Microbiol.,* **23** : 981-987.

Alexander, M. 1961. Lignin decomposition. *In* Introduction to soil microbiology.

Alexander, M. and **B.K.Lustigman,** 1966. Effect of chemical structure on microbial degradation of substituted benzenes. *J.Agric.Food.Chem.,* **14**: 410-413.

Ambika Devi, M., 1988. Ecological studies on coconut husk retting areas in the Cochin backwaters and its relation to fish seed availability. M.Sc. Dissertation, Cochin Univ. Sci. Tech. 82 pp.

American Public Health Association (APHA), 1975. Standard Methods for the examination of water and waste water ed.14, American Public Health Association Inc., New York, pp.574-577.

Andreoni, A. and **G.Besetti,** 1986. Comparative analysis of different *Pseudomonas* strains that degrade cinnamic acid. *Appl.Environ.Microbiol.,* **52** : 930-934.

Antai, S. and **H.Crawford,** 1983. Degradation of phenol by *Streptomyces setonii. Can. J. Microbiol.,* **29** : 142-143.

Bacon, P.R., 1971b. The maintenance of a resident population of *Balanus eburneus* (Gould) in relation to salinity fluctuations in a Trinidad mangrove Swamp. *J.Exp.Mar.Biol.Ecol.,* **6** : 189-198.

Barlow, J.F. and **J.W.Bishop,** 1965. Phosphate regeneration by zooplankton in Cayuga Lake. *Linnol.Oceanogr.,* **10** (Suppl.) : R15-R24.

Bayley, R.C., S.Dagley and **D.T.Gibson,** 1966. The metabolism of cresols by species of *Pseudomonas. Biochem. J.,* **101** : 293-301.

Bayley, R.C. and **G.J. Wigmore,** 1973. Metabolism of phenols and cresols by mutants of *Pseudomonas putida. J.Bacteriol.,* **113** : 1112-1120.

Bennet, E.O., 1962. Factors affecting the antimicrobial activity of phenols. *Bact.Rev.,* **14**: 123-140.

Bent, E.T. and **R.Goulder,** 1981. Planktonic bacteria in the Lumber estuary. Seasonal variation in population density and heterotrophic activity. *Mar.Biol.,* **62** : 35-45.

Bhat, J.V., 1966. Pattern of bacterial and yeast populations of coir rets. *Coir,* **10** : 20-23.

Bhat, J.V., 1969. Souvenir, Third International Congress on the Global Impacts of Applied Microbiology, Bombay, 39 pp.

Bhat, J.V., K.G.Kuntala and **Pramila Varadaraj,** 1973. Studies on microbiological softening of coir fibre. *Coir,* **18** : 19-23.

Bhat, J.V. and **A.M.D. Nambudiri,** 1971. Uniquity of coir retting. *J.Sci.Ind.Res.,* **30** : 720-728.

***Bodungen, B.Von. K von Brockel, V.Smetaceck** and **B.Zeitschel,** 1975. Ecological studies on the plankton in the Kiel Bright. I. Phytoplankton Meerentutkimuslait Julk Havsforskiningsinst. *Skr.,* **239** : 179-186.

Boto, K.G. and **J.S.Bunt,** 1981. Dissolved oxygen and pH relationships in Northern Sustralian mangrove waterways. *Linnol. Oceanogr.,* **26** : 1176 - 1178.

Boucher, G. and **S.Chamroux,** 1976. Bacteria and Meiofauna in an experimental sand ecosystem. I. Material and preliminary sand ecosystem. I.Material and preliminary results. *J.Exp.Mar.Biol.Ecol.,* **22** : 237-249.

Bourquin, A.W., 1984. Biodegradation in the estuarine, marine environments and the genetically altered microbe. *In* Genetic control of environmental pollutants. ed. by O.S.Gulbert and H.Alexander, Plenum Press, New York, pp.97-115.

Boyd, T.J. and **A.F.Carlucci,** 1993. Degradation rates of substituted phenols by natural populations of marine bacteria. *Aquatic Toxicology,* **25** : 71-82.

Boyd, S.A. and **D.R.Shelton,** 1983. Anaerobic biodegradation of chlorophenols in fresh and activated sludge. *Appl.Environ.Microbiol.,* **47** : 272-277.

Boyd, S.A., D.R.Shelton, D.Berry and **J.M.Tiedje,** 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. *Appl.Environ.Microbiol.,* **46** : 50-54.

Brown, J.P. 1970. Susceptibility of the cell walls of some yeasts to lysis by enzymes of *Helix pomatia can.J. Microbiol.,* **17** : 205-208.

Buddin, W., 1914. Partial sterilization of soil by volatile and non-volatile antisceptics. *J.Agric.Sci.,* **6** : 417-455.

Bunch, R.L. and **C.W.Chambers,** 1967. A biodegradability test for organic compounds *J.Water Pollut.Control Fed.,* **39** : 181-184.

Burges, A., 1967. The decomposition of organic matter in soil. *In* Soil Biology ed. by A.Burges and E.Raw. Academic Press, London, pp.15-47.

Burges, A., H.M.Hurst and **S.B.Walkden,** 1963. Nature of humic acids. *Nature* (London) **199** : 696-697.

***Berns, R.G.,** 1980. Microbial adhesion to soil.

Buswell, J.A., 1975. Metabolism of phenol and cresols by *Bacillus stearothermophilus*. *J.Bacteriol.,* **124** : 1077-1083.

Buswell, J.A. and D.G.Twomey, 1975. Utilization of phenol and cresols by *stearotheromphilus J.Gen.Microbiol.,* **87** : 377-379.

Cain, R.B., D.W.Ribbons and **W.C.Evans,** 1961. The metabolism of protocatechuic acid by certain micro-organisms. *Biochem.J.,***79** : 312-316.

Cawkell, E.M., 1964. The utilization of mangroves by African birds. *Ibis,* **106** : 251-253.

Chandrika, V., 1984. Studies on the eco-physiology of some heterotrophic and indicator bacteria in the marine environments of Kerala. Ph.D. Thesis, Cochin Univ. Sci. Tech., 304 pp.

Crawford, H., 1975. Novel pathway for degradation of protocatechuic acid in *Bacillus* sp. *J.bacteriol.,* **121** : 531-536.

Crawford, R.L. and **P.Polson,** 1978. Microbial catabolism of vanillate. decarboxylation to guaiacol. *Appl.Environ.Microbiol.,* **36** : 539-543.

Czekalowski, J.W. and **B. Skarzynski,** 1948. The breakdown of phenols and related compounds by bacteria. *J.Gen.Microbiol.,* **2** : 231-238.

Daengshuba, W.S., 1979. Preliminary study of aerobic heterotrophic bacteria in the mangrove swamp of Amphoe Khlung, Changwat Chan-Tabari, Thailand. *In* Symposium on Mangrove and Estuarine vegetation of South-East Asia, Indonesia, pp.121-124.

Dagley, S., 1967. The microbial metabolism of phenolics. *In* Soil Biochemistry, ed. by A.C.McLaren and G.H.Peterson, Edward Arnold, London, pp.287-317.

Dagley, S. 1971. Catabolism of aromatic compounds by micro-organisms. *Adv. Microbiol. Physiol.,* **6** : 1-46.

Dagley, S., P.J. Chapman and **D.T.Gibson,** 1965. The metabolism of ß-phenyl propionic acid by an *Achromobactor. Biochem. J.,* **97** : 643.

Dagley, S., W.C.Evans and **D.W.Ribbons,** 1960. New pathways in the oxidative metabolism of aromatic compounds by micro-organisms. *Nature* (London). **188**, 560-566.

Dagley, S. and **D.T.Gibson,** 1965. The bacterial degradation of catechol. *Biochem.J.,* **95** : 466-474.

Dale, N.G., 1974. Bacteria in intertidal sediments - Factors related to their distribution. *Linnol. Oceanogr.,* **19** : 509-518.

Desaiah, D., 1978. Effect of penta chlorophenol on the ATPase in rat tissues. *Pentachlorophenol,* **12** : 277-283.

Dharmaraj, K., N.Balakrishnan Nair and **K.Padmanabhan,** 1980. Studies on the hydrographical features of vizhinjam Bay. Symp. Coastal Aquaculture.

***Dickerman, R.W.** and **C.Juarez,** 1971. Nesting studies of the boat billed beron *Cochlearius cochlearius* at San Blas, Nayarit, Mexico. *Ardea.,* **59**: 1-16.

Divanin, I.A, K.K.Ermolaev and **O.G.Mironov,** 1977. Studies on some biochemical indices of phenol degradation by *Bacterium album* *Rapp.Pv.Reun.Cons.Int.Explor.Mer.,* **171** : 126-128.

Durham, N.N., 1956. Bacterial oxidation of p-aminobenzoic acid by *Pseudomonas fluorescens J.Bacteriol.,* **72** : 335-336.

Dutton, P.L. and **W.C.Evans,** 1969. The metabolism of aromatic compounds by *Rhodopseudomonas palustris.* A new reductive method of aromatic ring metabolism. *Biochem. J.,* **113** : 525-536.

Ehrlich, G.G., D.F.Goerlitz, E.M.Godsy and **M.F.Hult,** 1982. Degradation of phenolic contaminants in ground water by anaerobic bacteria, St.Louis Park, Minnesota. *Ground Water,* **20** : 703-710.

EIFAC/T 15, 1972. Water quality criteria for European fresh water fish. Report on monohydric phenols and inland fisheries. EIFAC Tech. Paper., No.15, Rome.

***Eklund, E.** and **H.G.Gyllenberg,** 1974. Bacteria. *In* Biology of plant hitter decomposition, ed. by C.H.Dickinson and C.J.F. Pugh, Vol.2, Academic Press, London, 249-272.

Ermolaev, K.K., 1979. Investigation on the ability of the Black sea phenol-decomposing micro-organisms to utilise mono, di, and tri-atomic phenols. Biol. Morya (Kiev), **50** : 28-30.

Ermolaev, K.K., 1979. Investigation of microbial degradation of phenols in the Black sea. *Biol.Morya* (Kiev), **50** : 30-39.

Ermolaev K.K. and **O.G.Mironov,** 1975. The role of phenol destroying micro-organisms in the process of phenol degradation in Black sea. *Mikrobiologia,* **44** : 928-932.

Evans, W.C., 1963. The microbiological degradation of aromatic compounds. *J.Gen.Microbiol.,* **32** : 177-184.

Faerenbach, J., 1969. Pollution and eutrophication of Great South Bay, Long Island, New York. *J.Water.Pollut.Control.Fed.,* **41** : 1456-1466.

FAO, 1975. Manual of methods in aquatic environment research part I. Methods for detection, measurement and monitoring of water pollution *Fao.Fish.Tech.Paper,* No.137.

Field, G.D., 1968. Utilization of mangroves by birds on the Freetown peninsula, Sierra Leone. *Ibis,* **110**, 354-357.

Fiest, C.J. and **G.D.Hegeman,** 1969. Phenol and benzoate metabolism by *Pseudomonas putida.* Regulation of tangential pathways. *J.Bacteriol.,* **100** : 869-877.

Fowler, G.J., E.Ardern and **W.T.Lockett,** 1911. The Oxidation of phenol by certain bacteria in pure culture. *Proc.Roy.Soc.* (London) B, **83** : 149-153.

Fuhs, G.W., 1961. Der mikrobiele Abbauron kohlenwasserstoffen. *Ark.Microbiol.,* **39** : 374-422.

Gaby, W.L. and **E.Free,** 1958. Differential diagnosis of *Pseudomonas* like micro-organisms in the clinical laboratory. *J.Bacteriol.,* **76** : 442-444.

Gaby, W.L. and **C.Hadley,** 1957. Practical laboratory test for the identification of *Pseudomonas aeruginosa J.Bacteriol.,* **74** : 356-358.

George, M.J., 1958. Observation on the plankton of Cochin back water. *Indian.J.Fish.,* **5** : 375-407.

George, M.J. and **K.N.Kartha,** 1963. Surface salinity of Cochin back water with reference to tide. *J.Mar.Biol.Ass.India,* **5** : 178-184.

Gomes, H.P. and **S.mavinkurve,** 1982. Studies on mangrove swamps of Goa-II. Micro-organism degrading phenolic compounds. *Mahasagar Bull.Natl.Inst.Oceanogr.,* **15** : 111-115.

Gopinathan, C.P., P.V.R.Nair and **A.K.K.Nair,** 1978. Quantitative ecology of phytoplankton in the Cochin backwater. *J.Mar.Biol.Ass.India,* 325-326.

*__Gray, P.H.H.__ and **H.G.Thornton,** 1928. Soil bacteria that decompose certain aromatic compounds *Zenter, Bacteriol Parasitenk.,* Abt.II, 73-74.

Gupta, S., 1985a. Effects of phenolic compounds on alkaline phosphatase activity in certain tissues of *Notopterus notopterus. Proc.Symp.Assess.Environ.Pollution,* The Academy of Environmental Biology, India, Muzaffarnagar, pp.151-157.

*__Haeckel, A.M.__ and **G.Rheinheimer,** 1983. Studies on the annual cycle of bacteria and fungi in the Angerian, a coastal stream in northern sweden. *Aquilo Ser. Zool.,* **22** : 51-56.

Haller, H.D., 1978. Degradation of monosubstituted benzoates and phenols by waste water. *J.Waer Pollut. control. Fed.,* **50** : 2771-2777.

Hamdy, M.K., Sherrer, E.L., H.H.Weiser and **W.D.Sheets,** 1954. Microbiological factors in the treatment of phenol wastes. *Appl.Microbiol.,* **2** : 143-148.

Hamdy, M.K. E.L.Sherrer, C.I.Randles, H.H.Weiser and **W.D.Sheets,** 1956. Some characteristics of a phenol-oxidizing *Pseudomonas*. *Appl.Microbiol.,* **4** : 71-75.

Happold, F.C. 1950. Biological oxidation of aromatic rings. *Biochem.Soc.Symp.,Cambridge*, England, No.5.

Harris, G. and **R.W. Ricketts,** 1962. Metabolism of phenolic compounds by yeasts. *Nature* (London), **195** : 473-476.

Harrison, M.J., E.Robert and **R.Y.Morita,** 1972. Solubilisation of inorganic phosphates by bacteria isolated from upper Klamath Lake sediment. *Linnol.Oceanogr.*, **17** : 50-55.

Harvey, H.W., 1955. The chemistry and fertility of seawater. Cambridge Univ. Press, 224 pp.

Heald, E., 1971. The production of organic detritus in a South Florida estuary. Univ. Miami, *Sea Grant. Tech.Bull.,* **6** : 110 pp.

Healy, J.B. and **L.Y.Young,** 1978. Catechol and phenol degradation by a methanogenic population of bacteria. *Appl.Environ.Microbiol.,* **35** : 216-218.

Healy, J.B. and **L.Y.Young,** 1979. Anaerobic biodegradation of eleven aromatic compounds to methane. *Appl.Environ.Microbiol.*, **38** : 84-89.

Henderson, M.E.K., 1961. The metabolism of aromatic compounds related to lignin by some *Hyphomycetes* and yeast - like fungi *J.Gen.Microbiol.,* **26**: 155-165.

Henderson, M.E.K. and **V.C.Farmer,** 1955. Utilization by soil fungi and p-hydroxybenzaldehyde, feulic acid, syringaldehyde and vanillin. *J.Gen.Microbiol.,* 12 : 37-46.

Higa, T., 1981. Phenolic substances. *In* Marine natural products - Chemical and Biological perspectives ed. by R.H.Scheuer. Academic Press, New York, pp.93-145.

Hugh, E.J.L. and **R.C.Bayley,** 1983. Control of catechol meta-cleavage pathway in *Alcaligens eutrophus J.Bacteriol.,* **154** : 1363-1370.

Itturiaga, R. and **G. Rheinheimer,** 1972. Untersuchugen uber das vorkommen von phenol abbauenden mikro-organismen in Gewassern and sedimenten. *Kieler Meeresforschung,* 28.

Jana, B.B., 1977. The influence of environmental parameters on the bacterial populations of thermal springs in West Bengal, India. *Biol.J.Linn.Soc.,* **9** : 243-257.

Jana, B.B., 1979. Primary production and bacterioplankton in fish ponds with mono and polyculture. *Hydrobiologia,* **62** : 81-87.

Jana, B.B., G.N.Patel, S.K.Roy and **U.K.De,** 1980. Growth characteristics of heterotrophic bacterial population of water and bottom sediments in the tanks under different trophic conditions. *Hydrobiologia,* **75** : 231-239.

Jannasch, H.W., 1967. Enrichment of aquatic bacteria on continuous culture *Archiv. fur Mikrobiologie,* **59** : 165-173.

Jannasch, H.W., 1977. Growth knetics of quatic bacteria. *In* Aquatic Microbiology. The society for applied bacteriology symposium series No.6., ed. by F.A. Skinner and J.M. Shewan. Academic Press, New York and London, pp.55-68.

Jannasch, H.W., and **G.E. Jones**, 1959. Bacterial populations in sea water as determined by different methods of enumeration. *Limnol. Oceanogr.,* **4** : 128-139.

Jayashankar, N.P., 1966. Studies on aerobic microflora associated with the retting of coconut husks. *Coir*, **9** : 16-19.

Jayashankar, N.P., and **J.V. Bhat,** 1964. The role of *Micrococcus* in coir fermentation. *Curr. Sci.,* **33** : 369-372.

Jayashankar, N.P., and **J.V. Bhat,** 1966. Mode of attack on phenol by *Micrococcus* sp. isolated from coir rets. *Can. J. Microbiol.,* **12** : 1031-1034.

Jayashankar, N.P., and **K.P.V. Menon**, 1961. Microbiological flora of a few coconut retting areas. *Coir*, **5** : 33-36.

Jones, G.L. and **E.G. Carrington**, 1972. Growth of pure and mixed cultures of micro-organisms concerned in the treatment of carbonization waste liquors. *J. Appl. Bacteriol.,* **35** : 395-404.

Jones, G.L., F. Jansen and **A.J.McKay**, 1973. Substrate inhibition of the growth of *Bacterium* NCIB 8250 by phenol. *J. Gen. Microbiol.,* **74** : 139-148.

Jorgensen, S.E., 1971. How to treat phenolic waste water? *Vatten*, **27** : 434-440.

Karanth, N.G., P.A. Lokbharathi and **S. Nair,** 1975. Distribution of phenolic acids in soils from two mangrove areas in Goa. *Indian J. Mar. Sci.,* **4** : 215-217.

Khanna, S.S. and **D.V. Yadav,** 1979. Practcal mannual for introductory courses in soils. Haryana Agricultural University, Hissar, pp 33-43, 107-110, 117-120.

Kilby, B.A., 1948. The bacterial oxidation of phenol to ß-ketoadipic acid. *Biochem. J.,* **43** : V-VI.

Kramer, N. and **R.H. Doetsch,** 1950. The growth of phenol utilizing bacteria on aromatic carbon sources. *Arch. Bochem.,* **26** : 401-405.

Krishnamurthy, K., V. Sunderraj and **S. Santhanam,** 1975. Aspects of an Indian mangrove forest. *Proc. Int. Symp. Biol., Management Mangroves,* Honolulu, Hawaii, **1** : 88-95.

Lee, R.F. and **C.Ryan,** 1979. In Proceedings of the workshop. Microbial degradation of pollutants in marine environments. EPA-600/9-79-012, U.S. Environmental protection Agency, Cincinnati, Ohio, pp.443-450.

Longbottom, M.R., 1970. The distribution of *Arenicola marina* (L) with particular reference to the effects of particle size and organic matter of the sediments. *J.Exp.Mar.Biol.Ecol.,* **5** : 138-157.

Marr, E.K. and **R.W.Stone,** 1961. Bacterial Oxidation of benzene. *J.Bacteriol.,* **81** : 425-430.

Matondkar, S.G.P., 1981. Studies on mangrove swamps of Goa. I.Heterotrophic bacterial flora from mangrove swamps. *Mahasagar-Bull.Natl.Inst.Oceanogr.,* **14** : 325-327.

Matondkar, S.G.P., S.Mahanti and **S.Mavinkurve,** 1980a. Seasonal variations in the microflora from mangrove swamps of Goa. *Indian J.Mar.Sci.,* **9**: 119-120.

Meir Reil, L.A., R.Dawson, G.Leibezeit and **H.Tiedge,** 1978. Fluctuations and interactions of bacterial activity in sandy beach sediments and overlying water. *Mar.Biol.,* **48** : 161-171.

Mineeva, N.M. and **N.A.Laptema,** 1974. Phenol oxidation by *Azotobactor agile* and *Pseudomonas denitrificans* cultures. *Biol.Vnutr.Vod.,* **23** : 10-13.

Moore, H.B., 1940. The mud of the Clyde sea area - phosphate and nitrogen contents. *Mar.Biol.Ass.U.K.,* **16** : 595-607.

Morita, R.Y., 1974. Temperature effects on marine environment and on microbial activities. ed. by R.R.Colwell and R.Y.Morita, *Univ.Parks.Press,* Baltimore, pp.75-79.

Mullin, J.B. and **J.P.Riley,** 1955. The colarimetric determination of silicate with special reference to sea and natural waters. *Anal.Chim.Acta.,* **27**: 162-175.

Murphy, J. and **J.P.Riley,** 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal.Chim.Acta.,* **27**: 31-36.

Murthy, R. and **M.J.P.Jayaseelan,** 1986. Prospects of aquaculture in a mangrove ecosystem. *Proc.Symp.Coastal Aquaculture,* 1986, **4** : 1039-1067.

Murthy, P.S.N. and **M.Veerayya,** 1972. Studies on the sediments of Vembanad lake, Kerala state. Part I. Distribution of organic matter. *Indian.J.Mar.Sci.,* **1** : 45-51.

Nair, K.K.C. V.N,Sankaranaryanan, T.C.Gopalakrishnan, T.Balasubramanian, C.B.Lalithambikadevi, P.N.Aravindakshan and **M.Krishnakutty,** 1988. Environmental conditions of some paddy-cum-prawn culture fields of Cochin back waters. South West coast of India. *Indian J.Mar.Sci.,* **17** : 24-30.

Nedwell, D.B. and **G.D.Floodgate,** 1971. The seasonal selection by temperature of heterotrophic bacteria in an intertidal sediment. *Mar.Biol.,* **11** : 306-310.

Novobrantzev, P.V. 1932. The development of bacteria in lakes depending on the presence of easily assimilable organic matter. *Microbiology* (Moscow), **6** : 28-36.

Odum, W.E. and **E.H.Heald,** 1975. The detritus based food-web of an estuarine mangrove community. *In* Estuarine Research, ed. by L.E. Cronin, Academic Press, New York, pp.265-286.

Ornston, L.N. and **R.Y.Stainer,** 1964. Mechanism of ß-ketoadipate formation by bacteria. *Nature* (London), **204** : 1279-1283.

Pandalai, K.M. U.K.Nair and **K.P.V.Menon,** 1957. Quality of water in relation to the retting of coconut husks. *Coir,* **1** : 30-33.

Panikkar, N.K. and **R.Jayaraman,** 1956. Some aspects of productivity in relation to fisheries of Indian neritic waters. *Proc. 8th Pacific Sci.Congress,* III : 1111-1122.

Parhad, N.M., P.Kumaran and **N.Shivaraman,** 1981. Microbial degradation of waste waters from the manufacture of mettallurgical and domestic coke,. *Proc.Natl.Workshop Microbial degrad. Indus. Wastes.,* NEERI, Nagpur.

***Parkes, K.C.** and **R.W.Dickerman,** 1967. A new subspecies of mangrove warbler (*Dendroica petechia*) from Mexico. *Ann.Carnegie Mus.,* **39** : 85-90.

Parson, T.R., Yoshiak and **C.M.Lalli,** 1984. A manual of chemical and biological methods for seawater analysis, Pergamon Press, Oxford,283pp.

Pawlaczyk, M., 1965. Effect of glucose andurea on the rate of phenol degradation by *Pseudomonas fluorescence. Acta. Mikrobiologia Polonica,* **14** : 207-214.

Phillips, J.E., 1964. The ecological role of phosphorous in waters with special reference to micro-organisms. *In* Principles and applications in aquatic microbiology ed. by H.heukelekian and N.C.Dondero. Wiley. pp.61-81.

Pillai, A.G. and **K.Ravindran,** 1988. Hydrography of the Cochin Harbour. *Fish.Tech.,* **25** : 83-86.

Plimmer, J., 1978. *In* Microbial degradation of pollutants in marine environments. ed.by P.H. Pritchard and A.W.Bourquin, Washington, U.S.Environmental Protection Agency.

Pomeroy, R., H.M. Mathews and **H.S.Min,** 1963. Excretion of phosphate and soluble organic phosphorous compounds by zooplankton. *Limnol.Oceanogr.,* **8** : 50-55.

Pometto III, A.L., J.B.Sutherland and **D.L.Crawford,** 1981. *Streptomyces setonii* : Catabolism of vanillic acid via guaiacol and catechol. *Can.J. Microbiol.,* **27** : 636-638.

Pugh, K.B., A.R.Andrews, C.F.Gibbs, S.J.Davis and **G.D.Floodgate,** 1974. Some physical, chemical and microbiological characteristics of two beaches of Auglessey. *J.Exp.Mar.Biol.Ecol.,* **15** : 305-333.

Purushothaman, A., D. Chandramohan and **R.Natarajan,** 1974. Distribution of silicate dissolving bacteria in Vellar estuary. *Curr.Sci.,* **43** : 282-283.

Rajagopalan, M.S. C.P.Gopinathan and **V.Balachandran,** 1980. Productivity of different mangrove ecosystems. *Symp.Coastal Aquaculture,* p16.

Ramamritham, C.P. and **R.Jayaraman,** 1963. Some aspects of hydrological conditions of the back water around Willington Island, Cochin, *J.Mar.Biol.Ass.India,* **5** : 170-177.

Rao, S.V.S. and **P.C. George,** 1959. Hydrology of the Korapuzha estuary, Malabar, Kerala State. *J.Mar.Biol.Ass.India,* **1** : 212-223.

Remani, K.N., P.Venugopal, K.Saraladevi and **R.V.Unnithan,** 1981. Sediments of retting yard.

Remani, K.N., P.Venugopal, K. Saraladevi and **R.V.Unnithan,** 1980. Retting of coconut husk as a source of organic pollution in Cochin back waters.

Rheinheimer, G., 1979. Estuarine bacterial populations and their role in the decomposition of organic material. *In* Proceedings of WSCOR/ACMRR/ECOR/IHAS/UNESCO/LMG/IABO/IAPSO/Review and workshop held at FAO Headquarters, Rome, Italy, 26th-30th March, 1979 ed. by J.M.Martin, J.D. Burton and D.Eisma.

Ribbons, D.W., 1966. Metabolism of o-cresol by *Pseudomonas aeruginosa* Strain T1. *J.Gen.Microbiol.*, 44 : 221-231.

Ribbons, D.W. and **P.J. Champman,** 1968. Bacterial metabolism of orcinol. *Biochem. J.,* **106** : 44-45.

Ricklefs, R.E., 1971. Foraging behaviour of mangrove swallos at Barrow, Colorado Island, *Auk*, **88** : 635-651.

Rodina, A.G., 1972. Methods in aquatic microbiology. Univ. Park Press, Baltimore.

Rogoff, M.H., 1961. Oxidation of aromatic compounds by bacteria. *Adv.Appl.Microbiol.,* **3** : 193-219.

Rubin, H.E., R.V.Subba Rao and **M.Alexander,** 1982. Rates of mineralisation of trace concentrations of aromatic compounds in lake water and sewage samples. *Appl.Environ. Microbiol.,* **43** : 1133-1138.

Sankaranarayanan, V.N. and **S.V.Panampunnayil,** 1979. Studies on organic carbon, nitrogen and phosphorous in sediments of Cochin back waters. *Indian J.Mar.Sci.,* **8** : 27-30.

Sankaranarayanan, V.N., S.Sukumaran, T.Balasubramanyan, S.Rosamma and **S.V.Panampunnayil,** 1980. Studies on the environmental conditions of the tidal ponds in the Ramanthuruth Island, Cochin, *Symp.Coastal Aquaculture,* **1** : 362-368.

Sankaranarayanan, V.N. and **S.Z.Quasim,** 1969. Nutrients of the Cochin backwaters in relation to environmental characteristics. *Mar.Biol.,* **2** : 236-247.

Santa Nair and **P.A.Lokbharathi**, 1980. Heterotrophic bacterial population in tropical sandy beaches. *Mahasagar Bull.Natl.Inst.Oceanogr.,* **13** : 261-267.

Santhi Thirumani, S., 1992. Heterotrophic bacterial activity in selected aquaculture systems near Cochin. Ph.D. Thesis, Cochin Univ.Sci. and Tech. (Unpublished).

Sastry, C.A., 1986. Industrial waste biodegradation. Part I. *Encology,* **1** : 29-33.

Sen Gupta, N.N., 1921. Dephenolisation in soil. *J.Agric.Sci.,* **2** : 136-158.

Shimp, R.J. and **F.K.Pfaender,** 1984. Influence of easily degradable naturally occurring substrates on biodegradation of mono-substituted phenols by aquatic bacteria. *Appl.Environ.Microbiol.,* **49** : 394-401.

Shivaraman, N., P.Kumaran and **N.M.Parhad,** 1978. Phenol degradation by *Candida tropicalis* and influence of other toxicants. *Indian J.Environ. Hlth.,* **20** : 101-111.

Shivraman, N. and **N.M.Parhad,** 1985. Biodegradation of phenol and cyanide by pure and mixed cultures. *Indian J.Microbiol.,* **25** : 79-82.

Simidu, U. and **K.Aiso,** 1962. Occurrence and distribution of heterotrophic bacteria in sea water from the Kamogawa Bay. *Bull.Jap.Soc.Sci.Fish.*, **28** : 1135-1141.

Simidu, U., N.Taga, R.R. Colwell and **T.R.Scharary,** 1980. Heterotrophic bacterial flora of the seawater from the Nauseishoto (Ryukyu Rettp) area. *Bull.Jap.Soc.Sci.Fish.*, **46** : 505-511.

Slater, J.H. and **H.J.Somerville,** 1979. Microbial aspects of waste treatment with particular attention to the degradation of organic compounds. *In* Microbial technology, ed. by A.T.Bull, D.C.Ellwood and (Ratledge, Cambridge Univ. Press, Cambridge.

Sleeper, B.P. and **R.Y.Stainer,** 1950. The bacterial oxidation of aromatic compounds. I. Adaptive patterns with respect to polyphenolic compounds. *J.Bacteriol.*, **59** : 117-127.

Smith, J.D. and **A.R.Longmore,** 1980. Behaviour of phosphate in estuarine water. *Nature* (London), **270** : 532-534.

Snedecor, G.W. and **W.G.Cochran,** 1967. Statistical methods. Oxford and IBH Publ. Co.593 pp.

Spain, J.C. and **D.T.Gibson,** 1988. Oxidation of substituted phenols by *Pseudomonas putida* F1 and *Pseudomonas* sp. Strain JS6.

Spain, J.C. P.H. Pritchard and **A.W.Bourquin,** 1980. Effects of adaptation on biodegradation rates in sediment/water cores from estuaries and fresh water environments. *Appl. Environ. Microbiol.,* **40** : 726-734.

Sreedharan, M. and **K.V.M.Salih,** 1974. Distribution characteristics of nutrients in the estuarine complex of Cochin. *Indian J.Mar.Sci.,* **3** : 125-130.

Sreenivasan, A., 1964. The limnology, primary production and fish production in a tropical pond. *Limnol. Oceanogr.,* **9** : 391-396.

Stainer, R.Y., 1947. Simultaneous adaptation : a new technique for the study of metabolic pathways. *J.Bacteriol.,* **54** : 339-348.

Stainer, R.Y., 1948. The oxidation of aromatic compounds by fluorescent pseudomonads. *J.Bacteriol.,* **55** : 477-494.

Stainer, R.Y., N.J.Palleroni and **M.D.Daudroff,** 1966. The aerobic *Pseudomads,* a taxonomic study. *J.Gen.Microbiol.,* **43** : 159-271.

Steinberg, P.D., 1984. Algal chemical defence against herbivores. Allocation of phenolic compounds in the kelp *Alaria margimata. Science,* **223** : 405-406.

*__Stolbonov, A.K.,__ 1971. Phenol and rhodane destructing microflora as a factor for natural purification of the Dnieper reservoir. *Gidrobiol.,* Zh., **7** : 11-19.

Stout, D.J., R.K. Tate and **F.L.Molloy,** 1976. Decomposition processes in New Zealand soils with particular respect to rates and pathways of plant degradation. *In* The role of terrestrial and aquatic organisms in decomposition processes ed. by J.M.Anderson and A.Macfodyer. 17th symposium of the British Ecological society, Blackwell Scientific Publications, London, pp.97-141.

Strickland, J.D.H. and **T.R. Parsons,** 1968. A practical handbook of seawater analysis, *Bull. Fish. Res. Bd. Can.,* **167** : 2-311.

Stringfellow, W.T. and **S.G.Horner,** 1984. The effect of phenol dosage on parameters of microbial activity in sediments. Presented at 84th Annual Meeting of American Society for Microbiology. *In* Abstracts of the American Society for Microbiology, ASM Publ., Washington (USA) p.211.

Sunderraj, V., 1978. Suitability of a mangrove biotype for brackish water aquaculture. *Seafood Exp. J.,* **10** : 23-27.

Susan, J.A. and **G.A.Riley,** 1967. Microbiological studies in Long Island Sound. *Bull. Bingham Oceanogr Coll.,* **19** : 81-88.

Sutherland, J.B. D.L. Crawford and **A.L.Pometto,** 1981. Catabolism of substituted benzoic acids by *Streptomyces* species. *Appl.Environ. Microbiol.,* **41** : 442-448.

Szwerinski, H., 1981. Investigations on nitrification in the water and sediment of the Keil Bight (Baltic sea) *Kieler Meeresforsch Sonderh,* **5** : 396-407.

Tabak, H.H., C.W. Chambers and **P.W. Kabler,** 1964. Microbial metabolism of aromatic compounds. I. Decomposition of phenolic compounds and aromatic hydrocarbons by phenol adapted bactria. *J. Bacteriol.,* **87**: 910-919.

Tabak, H.H., A.S. Quave, I.C. Mashmi and **F.E. Barth,** 1981. Biodegradability studies with organic priority pollutant compounds. *J. Water Pollut. Control Fed.,* **53**: 1503-1518.

Tattersfield, F., 1928. The decomposition of naphthalene in the soil and the effect upon its insecticide action. *Ann. Appl. Biol.,* **15**: 57-80.

***Teixeira, C.J. Tunidsi** and **J. Santaro Ycaza,** 1969. Plankton studies in a mangrove environment. VI. Primary production, 300 plankton standing stock and some environmental factors. *Int. Rev.Ges. Hydrobiol.,* **54**: 289-301.

Thomas, N.A., 1973. Assessment of fish flesh tainting substances. Biological methods for the assessment of water quality. ASTM. STP **52B**: 178-193.

Thornton, H.G., 1923. The destruction of aromatic antisceptics by soil bacteria. *Nature* (London), **111**: 347-350.

United States Environmental Protection Agency (U.S.EPA), 1980. Ambient water quality criteria for phenol. EPA 440/5-80-066, October, 1980, A1- C33 pp.

Unnithan, R.V., M. Vijayan and **K.N. Remani,** 1975. Organic pollution in Cochin back waters. *Indian J.Mar. Sci.,* 4: 39-42.

Valiela, I. and **R. Buchsbaun,** 1991. The role of the phenolics in marine organisms and ecosystems. *In* Bioactive compounds from marine marine organisms with emphasis on the Indian. An Indo. U.S. Symposium. ed by M.F. Thompson, R. Sărojani and R. Nagabhushanam pp 23-28.

Venkatesan, V. and **V.D. Ramamurthy,** 1971. Marine microbiological studies of mangrove swamps of Killai back waters. *J.Oceanogr.Soc.Jap.,* **27**: 51-55.

Visser, S.A., G. Lamontagne, V.Zoulalian and **A. Tassier,** 1977. Bacteria active in the degradation of phenols in polluted waters of the St. Lawrence river. *Environ. Contam. Toxicol.,* **6**: 455-469.

Von Brand, T., 1946. Anaerobiasis in invertebrates. *Biodynamics,* Normandy, M.O. 328.

***Wagner, R.,** 1914. Veber benzol-bakterien. Z.f. *Gar.Physiol.,* **4** : 289-292.

Walsh, G.E., 1967. An ecological study of a Hawaiian mangrove swamp. *In* Estuaries ed. by G.H. Lauff, AAAS Publ., **83**, Washington, pp.420-431.

Walsh, G.E., 1974. Mangroves : A review. *In* Ecology of halophytes. ed. by R.Reimold and W.Queen, Academic Press, New York, pp.51-74.

Wase, D.A.J. and **J.S.Hough,** 1966. Continuous culture of yeast on phenol. *J.Gen.Microbiol.,* **42** : 13-23.

Watanabe, K. and **M. Kutner,** 1965. Plankton studies in a mangrove environment III. Bacterial analysis of waters in Cananeia *Biol. Inst. Oceanogr.,* **14** : 43-51.

Whitehead, D.C., 1964. Identification of p-hydroxy-benzoic, vanillic, p-coumaric and ferulic acids in soils. *Nature* (London), **202** : 417.

Wood, E.J.F., 1953. Heterotrophic bacteria in marine environments of eastern Australia. *Aust.Mar.Freshw.Res.,* **4** : 160-200.

Zandovi, K.N. and **A. Jensen,** 1981. Studies on phenolic compounds of some brown algae. *Proc.Int.Seaweed Symp.,* **8** : 655-660.

Zo Bell, C.E., 1946. Action of micro-organisms on hydrocarbons. *Bacteriol. Rev.,* **10** : 1-49.

Zo Bell, C.E., 1950. Assimilation of hydrocarbons by micro-organisms. *Adv.Enzymol.,* **10** : 443-486.

Zo Bell, C.E., 1962. Microbial modification of crude oil in the sea.

Zo Bell, C.E., and **C.W.Grant,** 1942. Bacterial activity in dilute nutrient solutions. *Science*, **96** : 189.

***Zoledziowska, J.** and **F.Edeline,** 1973. Study of biodegradability of small concentrations of phenol in river water. *Trib. Cebedeau*, **354** : 231-234.

***Not referred in original.**