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STUDIES ON FUNGAL FLORA WITH SPECIAL REFERENCE TO YEASTS IN THE COCHIN BACKWATER

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DOCTOR OF PHILOSOPHY

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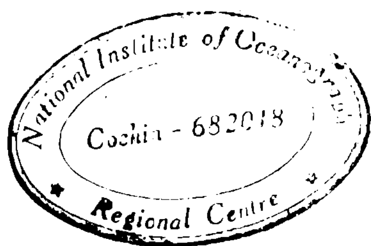
APRIL, 1990

IN LOVING
MEMORY
OF
MY FATHER

This is to certify that this thesis is an authentic record of the work carried out by Shri. N. Prabhakaran, M.Sc., under my supervision at the Regional Centre of the National Institute of Oceanography (Council of Scientific and Industrial Research), Cochin and that no part thereof has been presented for the award of any other degree.



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DECLARATION

I hereby declare that the thesis entitiled ''Studies on Fungal Flora with Special Reference to Yeasts in the Cochin Backwater'' is an authentic record of the work carried out by me at the Regional Centre, National Institute of Oceanography, Cochin - 18, under the supervision of Dr. P. Sivadas, Assistant Director and has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

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PREFACE

Man's concern with environmental deterioration is one of the major reasons for the increased interest in marine and estuarine microbes. Microbes form an important link in the biogeochemical cycling and their cyling activites often determine to a large measure the potential productivity of an ecosystem. Anthropogenic pollution of streams, rivers, estuarine and marine habitats can disturb the dynamic equillibria between the various forms of cycled materials and hence the composition of the biota. Developments in modern technology has led man to exploit the vast and varied oceanic resources of the pelagic as well as the benthic regions. All these activities have made it increasingly important to understand better the marine ecosystem, an environment in which fungi are ubiquitous and important members of biota. Until recently, this was a neglected group, much of the attention being drawn by the bacterial flora. It was only after 1940 mycologists became increasingly attracted by the aquatic fungi.

The Cochin backwater has a detritus dominated food chain (Qasim, 1970 ; Qasim and Sankaranarayanan, 1972). The supply of detritus is from both autochthonous and allochthonous sources. In the recycling of the nutrients in the estuary, bacteria and fungi therefore play a particularly significant role. The allochthonous plant materials contain biopolymers such as cellulose, lignin, humus etc., that are difficult to

degrade into simpler substances. The fungi have the ability to degrade substances, thereby making them available for cycling within the system. There is only scattered information on the estuarine and microbial populations of India and practically no work has been done on the fungal populations of the Cochin backwater except one or two occasional papers (Jones, 1968 ; Nair, 1970). The present study was therefore devoted to composition and the activity of mycopopulations of Cochin backwater. For convenience the thesis is divided into eight chapters. The opening chapter briefly reviews the literature and projects the importance of work and the main objectives. Second chapter discusses the materials and methods. In the third chapter the systematics and taxonomy of estuarine yeasts are examined in detail since this information is scarcely available for our waters. The general ecological aspects of the yeasts and filamentous fungi in the area of study are examined in the fourth chapter using appropriate statistical techniques. A special reference to the fungi in a small mangrove ecosystem is attempted in the fifth chapter. The biochemical studies are discussed in the sixth chapter and the penultimate chapter provides an overall discussion. In the last chapter the summary of the work is presented.

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CHAPTER 1

INTRODUCTION

In recent years mycological research have attracted the attention of many marine ecologists, physiologists and others especially those working on microbial degradation of chemical substances and organic matter within the ecosystem. Marine fungi represent a vast nutritional and ecological array of heterotrophic microorganisms. There are obligatory forms which live and flourish exclusively in the marine environment while many others are facultatively marine and can be found in terrestrial environment also. Fungi transported from terrestrial and fresh water regions are also common in the estuarine and marine environment and can be considered as euryplastic. The filamentous forms of Ascomycetes and Deuteromycetes occur on exposed pilings, plant and other woody materials while yeasts are associated with decaying organic materials. Both filamentous forms and yeasts can be found as epiphytes, saprophytes and also as pathogens. The lower fungi, Phycomycetes are a heterogenous group and many of them are parasites on plants and animals. Fungi are also found in marine sediments and water.

Microbial role in the transformation of matter and regeneration of nutrients has invited the attention of marine researchers to describe the various processes taking place in the marine environment. In most of the cases the studies on bacteria are highlighted and often the role of fungi have

been neglected (Fenchel, 1972; Hanson and Wiebe, 1977). The ecological studies of fungi and their role in marine and marine dominated systems have hardly progressed beyond the descriptive phase with strong emphasis on distributional ecology. The information on marine fungal ecology is so fragmentary that meaningful conclusions regarding the relationships of fungi to either substrate or environmental parameters can rarely be made (Hughes, 1975). This lack of information has apparently led some observers like Fenchel (1972), Hanson and Wiede (1977) and others to comment that fungi are unimportant in marine systems.

As discussed by Jones (1974) the most important and potential function of marine fungi is the decomposition of plant litter. The mycological literature adequately documents the ability of fungi to decompose plant litter in non-marine environments (Stark, 1972; Kirk, 1973; Witkamp, 1974; Jackson, 1975; Kaushik, 1975; Parkinson, 1975; Swift, 1977; Bärlocher et al., 1978). Fungi virtually always occur in autochthonous and allochthonous plant litter in marine system. The fungi are well suited for the breakdown of plant material by the formation of hyphae which along with the production of extracellualar enzymes enable the effective penetration in to plant cells (Harley, 1971). Relying on direct observations rather than cultural techniques has provided evidences for in situ fungal reproduction on coastal marine plant litter (Kohlmeyer, 1977; Kohlmeyer and Kohlmeyer, 1979). Presently it is known that in coastal

waters all groups of fungi take part in minerlization of dead organic matter and recycling of nutrients. However the precise role of fungi in these processes have hardly been investigated (Raghukumar and Rao, 1986).

1.1 Literature review

The existance of fungi, or what are called moulds by the common man has been recognized almost since the beginning of man's recorded experiences and impressions of nature. Before the invention of microscope itself natuaralist's attention was invited by the larger fungi. Thousands of species of fungi are known from the terrestrial habitat and their roles in the nature have been widely recognized. Although a large number of fungi do exist in the marine environment this fact went unnoticed.

Marine and estuarine mycological studies

Filamentous fungi

The early history of marine mycology starts with the report of Saccardo (1883), Ellis and Everhart (1885) who reported species of *Ophiobolous* on plant remains in marine environments. In the beginning of twentieth century Petersen (1905) made a study of Chytridiaceous forms parasitic on algae. He found that there are true marine fungi which are active in the destruction and disintegration of living autotrophic marine plant. In the successive years, Cotton (1907) and Sutherland (1915a,b,c, 1916) added new

reports of fungi occurring in marine environment.

Major impetus to isolate fungi from marine waters, intertidal soil and benthic sediments were made since 1930 and large number of papers describing these species have been published. Most investigators used standared isolation techniques such as plating or dilution plate methods or baiting. All these expriments resulted in the isolation of several terrestrial fungi with a few marine or facultative marine species. Elliott (1930) using dilution plate techniques isolated species of ubiquitous terrestrial fungi from the marshy soils of England and recorded lesser number of fungal propagules. In 1937, Sparrow conducted a preliminary investigation of mycoflora of mud samples collected from Buzzard's Bay, Vineyard Sound and the Gulf of Maine, considerably distant from land. He used plating method and recorded many terrestrial forms.

The discovery by Barghoorn and Linder (1944) that fungi showed remarkable adaptations for aquatic mode of life and the potential role of these fungi as wood degraders created much interest among mycologists. They carefully conducted a series of investigations on the various microbiological, chemical and physical factors involved in the decomposition and preservation of submerged plant materials and isolated several fungi specific to the marine environment from wood submerged in the sea.

Johnson and Sparrow (1961) compiled the list of fungi

isolated from sea water and sediments in their monumental book "Fungi in Oceans and Estuaries". The following authors have used marine sediment or soil for the isolation of fungi which resulted in the frequent report of terrestrial species from this environment: Saito (1952,1955), Höhnk (1952a,b, 1953, 1955, 1956, 1958, 1959, 1962, 1967), Gaertner (1954), Harder and Uebelmesser (1955), Nicot (1958a,b), Te Srake (1959), Siepmann (1959a,b), Pugh (1960, 1962, 1966, 1968, 1974), Borut and Johnson (1962), Pugh *et al.* (1963), Dabrowa *et al.* (1964), Apinis and Chesters (1964), Steele (1967), Kishimito (1969), Park (1972), Cowley (1973), Schaumann (1974b, 1975), Pitts and Cowley (1974), Moustafa (1975), Moustafa and Al-Musallam (1975), Moustafa *et al.* (1976), Abdel-Fattah *et al.* (1977) and Abdel-Hafez *et al.* (1977).

Higher fungi from sea water were isolated using the aforesaid methods by Höhnk (1959), Roth *et al.* (1964), Meyers *et al.* (1967b), Schaumann (1974b), Muntanola Cvetkovic and Ristanovic (1980) and others.

Woody subtrates often find their way into the sea. Besides, man deliberately introduces wood in the marine environment in the form of fishing craft and structures such as jetties. Several Ascomycetes and Deuteromycetes produce a vast array of wood degrading enzymes. Kohlmeyer and Kohlmeyer (1979) reviewed the higher lignicolous fungi from wood and other cellulosic materials in their book, "Marine Mycology, the Higher Fungi". Since this review, several publications describing lignicolous fungi have been published

(Rees *et al.*, 1979; Kohlmeyer, 1980, 1981a,b, 1984, 1985; Vrijmodel *et al.*, 1982, 1986; Hegarty and Curran, 1982; Koch, 1982; Jones *et al.*, 1983; Booth, 1983; Zanial and Jones, 1984; Miller *et al.*, 1985; Grasso *et al.*, 1985; Vanzanella *et al.*, 1985, Koch and Jones, 1986).

The degradative process of marine fungi involving the production of intra and extracellular enzymes have received considerable study. Meyers and Reynolds (1959a,b,1960,1963), Meyers and Scott (1968), Meyers *et al.* (1960) were among the first to study the cellulolytic activity of marine lignicolous fungi in detail, which included both Ascomycetes and Deuteromycetes. Meyers (1968) and Jones and Irvine (1972) discussed the degradative role of filamentous marine fungi in the marine environment. Pisano *et al.* (1964) screened 14 marine fungi for the gelatinase activities and found such activity in the culture filtrates of 13 isolates. The enzyme systems in several marine fungi were examined by Sguros and his co-workers (1970). Rodriguese *et al.* (1970) studied the dehydrogenase patterns in marine filamentous fungi, while Vembu and Sguros (1972) examined citric acid cycle and glycoxylate by pass in glucose-grown filamentous marine fungi.

Schaumann (1974a) demonstrated in 20 marine fungi, the production of cellulase by applying the viscocimetric and agar plate methods. He used sodium carboxymethyl cellulose as substrate for the test. The clearing of cellulose-containing agar by 14 marine fungi was also used by

Hennigsson (1976) as a measure of cellulase and xylanase production. Nilsson (1974) employed several methods to assay the enzymatic activities of 36 lignicolous fungi. He found that marine fungi like *Humicola alopallonella* were unable to degrade pure cellulose substrates in culture, but produced characteristic soft-rot patterns. Leightley and Eaton (1977) demonstrated the ability to degrade wood cell wall components of several marine fungi belonging to the genera *Cirrenalia*, *Halosphaeria*, *Humicola*, *Niaculcitalna* and *Zalerion*. They compared them with fresh water and terrestrial fungi and found production of cellulase, xylanase and mannanase in all species tested.

Detailed information on the extracellular enzyme production by marine fungi has been provided by Molitoris and Schaumann (1986) and Schaumann *et al*. (1986).

Mangrove trees are fascinating study objects for any marine mycologist. The bases of their trunks and pneumatophores are permanently or intermittendly submerged in salt water. Terrestrial fungi occupy the upper part of the trees and marine species, the lower part. At the edge of the intertidal area there is an overlap between marine and terrestrial fungi. The majority of manglicolous marine fungi are omnivorous and found mostly on dead and decaying cellulosic substrates. Most of the literature on higher fungi of mangroves were descriptions of new species, new host records, on the geographical distribution, taxonomy etc., but much less in their important role in nutrient cycling etc.

The first account of marine fungi occurring on mangroves was by Cribb and Cribb (1955,1956) in Australia. They were the pioneer mycologists to observe marine fungi *in situ* on mangroves. Kohlmeyer and Kohlmeyer (1979) reviewed the higher manglicolous fungi. Since this review several publications describing manglicolous fungi have been published (Aleem, 1980; Kohlmeyer, 1980,1984,1985; Kohlmeyer and Schatz, 1985; Kohlmeyer and Vittal, 1986; Koehm and Garrison, 1981; Schatz, 1985; Hyde *et al.*, 1986; Crane and Shearer, 1986; Hyde and Borse, 1986a,b; Hyde and Jones, 1986, 1987, 1988; Jones and Tan, 1987 and Hyde and Mouzouras, 1988). Hyde and Jones (1988) compiled the list of fungi from mangroves.

A few researchers have studied the mycoflora in mangal soil. Stolk (1955) reported two new species from Eastern African mangrove soil. Swart (1958, 1963) examined the culturable mycoflora of mangrove soils of Eastern Africa. He reported *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Phoma*, *Septonema*, *Robillarda* and *Periconia* from mangrove soils and noted the absence of Basidiomycotina and the rare occurrence of Ascomycotina and Phycomycotina. Swart (1970) reported a new *Penicillium* species from Australian mangrove soil. Lee and Baker (1972a,b, 1973) investigated soil microfungi in Hawaiian mangrove swamps. They used plating techniques to isolate fungi from the surface of roots of *Rhizophora mangle*, from macerated root tissue and from rhizosphere soil.

Newell (1973, 1976) made an extensive study of the microbial colonization on mangrove seedlings. He investigated the mycofloral succession on submerged seedlings of *Rhizophora mangle*. He made direct observation of fungi fruiting at the time of collection and species developing on the seedlings after damp chamber incubation. Newell also applied culture techniques to find species not sporulating on incubated seedlings and reported altogether 84 species of marine fungi.

Mangrove leaf tissue seems to be the most intensively investigated mangrove substratum for understanding the role of fungi in the degradation processes (Fell and Master 1973, 1975, 1980; Fell *et al.*, 1975, 1980, 1984; Cundell *et al.*, 1979; Wannigama *et al.*, 1981 and Findlay *et al.*, 1986).

While the higher marine fungi in the mangroves have attracted considerable interest, little effort has been devoted to the lower fungi. The most detailed studies were those of Ulken (1970, 1972, 1975, 1981, 1983, 1984, 1986), Fell and Master (1980) and Findlay *et al.* (1986).

Yeasts

Although yeasts are higher fungi, the marine species are less studied by mold specialists. The confusing nature of yeasts taxonomy is one of the main reasons discouraging investigations on their ecology (Fell, 1976). Fell (1976) and Kohlmeyer and Kohlmeyer (1979) provide upto date reviews of the available information on their taxonomy, distribution

and ecology. Mycological examinations of estuarine and open ocean environments have revealed the occurrence of diverse populations of yeasts of various taxa and physiological groups.

The occurrence of yeasts in the seas has often been reported as incidental during the study of other micro-organisms. The discovery of marine yeasts goes back to 1894 when Fischer separated red and white yeast from the Atlantic Ocean. Fischer and Brebeck (1894), Tsiklinsky (1908), Gräf (1909), Issatchenko (1914), Hunter (1920), Nadson and Burgwitz (1931), ZoBell and Feltham (1934) and ZoBell (1946) were the early investigators who reported the occurrence of yeasts along with moulds and bacteria in the sea. Since then many researchers have reported the occurrence of yeasts and yeast like fungi in the pelagic environment, on shrimp, in the fish gut, gut contents of marine mammals and birds and on decomposing algae (Kriss et al., 1952; Phaff et al., 1952; Kriss and Novozhilova, 1954; Kriss 1959; Johnson and Sparrow, 1961; van Uden and ZoBell, 1962; Siepmann and Hohnk, 1962; Shinano, 1962; Capriotti, 1962; van Uden and Castelo Branco, 1963 and Kawakita and van Uden, 1965).

van Uden and Fell (1968) and Ahearn et al. (1968) emphasized the widespread occurrence of yeasts in oceans and estuaries. Goto et al. (1974) and Vaatamen (1976) studied the distribution of yeasts in Pacific Ocean and Northern Baltic Sea respectively. While investigating the distribution of yeasts of the North Sea, Meyers et al. (1967a)

observed that certain yeast populations showed noteworthy concentration in association with various stages of development of the dinoflagellate, *Noctiluca miliaris*. Kriss *et al*. (1967) concluding the work carried out as a part of Russian Oceanic research in Indian Ocean and other regions reviewed their efforts in describing marine yeasts. Fell (1967) studied the distribution of yeasts in the Indian Ocean and discussed the relationship to hydrographic and biological conditions. Morris (1968) presented an excellent review of the various isolation techniques of marine yeasts and also discussed their possible use as indicators of water masses, fish populations, pollution etc..

The majority of the yeasts in marine habitats are probably general saprophytes with few exceptions as pathogens. Some of the yeast species are pollution indicators. *Candida tropicalis*, *C.krusei* and *C.parapsilosis* are usually found in estuarine regions and rarely occur in oceans (van Uden and Fell, 1968; Fell, 1976).

Sechadri and Sieburth (1971) evaluated various yeast media while quantitatively estimating yeasts on sea weeds. Gunkel *et al*. (1984) found the increase of yeast population during the degradation of *Desmarestia viridis* in model sea water microecosystems.

Fell *et al*. (1960) were the first researchers to study the distribution of yeasts in benthic environment. They obtained a total of 179 yeast isolates from 45 sampling

stations in the course of a qualitative yeast survey in Biscayne Bay, Florida. Fell and van Uden (1963) used coring device to study the marine yeasts. Yeast population were found confined to upper 2 cm of sediment at water depths of 540m.

The first major discussion about the yeasts found in estuaries and other inshore regions was by van Uden (1967). Kriss *et al*. (1952), Roth *et al*. (1962), van Uden and Castelo Branco (1963) and Fell (1965) found denser yeast populations in littoral zones than in adjacent open seas. The estuaries of the rivers Tagus, Sado and Guadiana, in Portugal were studied for yeast populations by Taysi and van Uden (1964) and van Uden (1967). Qualitative studies of yeasts in the Miami river were attempted by Capriotti (1962). Suehiro (1963) found a maximum of 2000 viable yeast units per gram of intertidal mud at two stations from the coast of Kyushu, Japan. Meyers *et al*. (1971) counted very high concentrations of viable cells in sediments of *Spartina alterniflora* marshes at the Louisiana coast. Ahearn (1973) studied the effect of environmental stress on aquatic yeast populations. Volz *et al*. (1974) found that the frequency of isolation and number of yeasts species were greater in sands and sediments than in a few invertebrates that they studied in Bahamas.

In the following years further literature were added to the study on marine yeasts. Yamagata and Fujita (1977), in Uragami sea and basin of the Ota river; Cheng and Lin (1977)

in the western coast of Taiwan, Hinzelin and Lectard (1978) in the Moselle waters, Mujdaba Apas (1978, 1980) in the Romanian Black sea coast, Vishniac and Hempfling (1979) in the Antarctic soil, Hinzelin *et al.* (1980) in the French saline waters, Paula *et al.* (1983) in the beaches of Sao Paulo, Brazil, Kolesritskaya and Maksimova (1983) in southern Baikal waters, Brunni *et al.* (1983) in the Dnieper River waters, isolated and studied the yeast populations.

Candida albicans is the most facultatively common and versatile marine yeast, frequently reported as a pathogen causing candidiasis in marine animals. The studies on yeasts with special reference to *C. albicans* were made by several authors. Crow *et al.* (1977) isolated and studied the atypical strains of *C. albicans* from the North Sea and found that such atypical isolates are likely to be misidentified by normal taxonomic procedures. Buck and Bubucis (1978) described a membrane filter procedure for the enumeration of *C. albicans* in natural waters. Buck (1980, 1983, 1986) examined the occurrence of *C. albicans* in relation to fecal matter of dolphins and sea gulls. Bossart (1982) and Dunn *et al.* (1984) reported candidiasis in dolphins and pinnipeds.

The isolation and identification of *C.albicans* from polluted aquatic environments are facilitated by the inclusion of a selective medium to detect the reduction of 2,3,5-triphenyl tetrazolium chloride (Cooke and Schlitzer, 1981). They observed that *C. albicans* occurred commonly in low numbers in sewage effluents, rivers and streams. The

distribution of this yeast as a pollution indicator organism has been studied by Robertson and Tobin (1983) and Ekundayo (1983). Safer and Ghannous (1983) observed morphological alterations in _C. albicans_ by sea water.

In situ exposure of _C. albicans_ to three streams containing acid mine drainage was accomplished using membrane diffusion chamber by DePasquale _et al_.(1984). _C. albicans_ was extremely tolerant of the acid stress as reflected by average decreases in survivors of less than two logs during a three day exposure period.

Yeasts are found to be associated with oil pollution. They are known for the production of single cell protein (SCO - single cell oil, current usage) from hydrocarbons which are useful for combating oil pollution. Turner and Ahearn (1970) reported increase in population of hydrocarbonoclastic yeasts in a fresh water stream after the incidental discharge of waste oil from an asphalt refinery into the stream. Yeast population increased within the five day period following the spill from an initial 30-200 c.f.u./ml to $10^{2}-10^{5}$ c.f.u./ml. Ahearn _et al_.(1971a) studied the effect of oil on Louisiana marshland yeast populations. Ahearn _et al_. (1971b) also studied the Louisiana crude oil and its distillates being the sole source of carbon for the growth of yeasts isolated from various marine habitats. _Debaryomyces hansenii_, _Candida parapsilosis_ and _Rhodotorula glutinis_ were the predominant species assimilating the carbon from the above source. Meyers and Ahearn (1972) investigated biodegradative

processes of oil in the *Spartina* ecosystem, with particular emphasis on the ecological role of yeasts and filamentous fungi. The selective effect of oil in developing yeast population in estuarine marshland was noted by Ahearn and Meyers (1972). After few months of periodic controlled enrichment of the field plots with crude oil, the dominant species were found to be hydrocarbonoclastic strains of *Trichosporon* and *Pichia*. Ahearn and Meyers (1976) presented an excellent review of research work on fungal degradation of oil in the marine environment.

Crow *et al*. (1980) studied on the hydrocarbon utilizing yeasts *Candida maltosa* and *C. lipolytica*. Both were capable of reducing recoverable amounts of branched chain and aromatic hydrocarbons in a mixture of naphthalene, tetradecane, hexadecane and pristane. Fedorak *et al*. (1984) isolated 74 yeasts from marine water and sediment samples from the strait of Juan de Fuca and Northern Puget Sound. When these yeasts were grown in the presence of Prudhoe Bay crude oil only three yeasts were able to degrade some or all the n-alkanes. Gruettner and Jenson (1984) recorded the physiological composition of the microbial community involved in oil degradation in Kalundborg Fjord, a Danish marine area. Ahearn and Crow (1986) reviewed and dealt in detail, the metabolism of alkanes and alkene by fungi including yeasts.

Nutritional evaluation of marine yeasts in raising aquaculture and rearing the bio-feeds is attaining accelerated momentum. Recent investigations have indicated

the importance of marine yeasts as feed in aquaculture (Al-Hajj *et al.*,1983; Aujero *et al.*,1984; Higashiuhara *et al.*, 1984; La Ferla and Zaccone,1985 and Al Hinty and James,1986).

Marine and Estuarine Mycological Studies in India

Filamentous fungi

The marine habitats in India have received hardly any attention in the field of mycology as compared with other branches of marine science. There have been only a few records of fungi from the marine habitats of India and they were mostly terrestrial forms transported to estuaries, mangroves and intertidal beaches. A little work has been done on obligate marine fungi from Indian waters.

The publication of Becker and Kohlmeyer (1958) on the presence of soft rotting fungi on small fishing crafts was one of the first marine mycological studies in India. The only species named was *Halosphaeria quadricornuta*. Later a few more lignicolous fungi have been reported by Kohlmeyer (1959). Almieda (1963) made a preliminary investigation of microorganisms on timber in Indian coastal waters. In his report he listed *Aspergillus* sp., *Cladosporium* sp., *Halosphaeria quadricornuta* and a number of bacteria. Kohlmeyer *et al.* (1967) reported three more lignicolous fungi from India. Jones (1968) reported *Humicola* sp. and *Cirrenalia macrocephala* belonging to Deuteromycotina and *Lulworthia floridana*, *L. purpurea* and *H. quadricornuta* belonging to Ascomycotina. He could not find any

successional pattern of fungi and the number of fungi recorded was low due to the very rapid deterioration of the wood by the animal borers and bacteria.

While studying the problem of timber destroying organisms along the Indian Coasts Nair (1970) recorded five species of wood infesting fungi from the Cochin backwater, viz. Gnomonia longirostris, Halosphaeria quadricornuta, Torpedospora radiata, Corrollospora pulchella and Lulworthia sp.. They were all obligatory marine fungi with cellulolytic properties. He felt that there was apparently a softening of the timber by such hydrolytic processes which enhances the activities of the timber destroying organisms.

Raghukumar (1973) studied the lignicolous marine fungi in and around Madras, east coast of India during 1967-1971. He recorded twelve Ascomycetes and six Fungi Imperfecti from drift wood and wood submerged in the sea. Patil and Borse (1982) reported two species of Halosarpheia, viz. H. fibrosa and H. ratnagiriensis sp.nov.,from Maharashtra, west coast of India. The former species was a new record for India and the later was a few species to science.

In the course of marine mycological survey of the coast of Maharashtra, Borse (1985) collected a Basidiomycetes fungus Nia vibrissa from a dead and decaying intertidal wood. Six more Ascomycetes were collected from the same area, some of which were found to be rare and not previously reported from India (Borse,1987).

More recently while studying the distribution of lignicolous marine fungi in the Vellar estuary, east coast of India, Ravikumar and Purushothaman (1988a,b) recorded *Cirrenalia tropicails*, a hypomycete and *Corollospora intermedia*, an Ascomycete which were new records for India.

Pawar and Thirumalachar (1966) were the first Indian mycologists to study the ecology of higher fungi in soils of marine environments. While studying the intertidal beach and marshy soils of Bombay they found a low number of fungal propagules for marine soils. They compared the growth of pure cultures of marine and terrestrial isolates of the same species of soil fungi and concluded that most of the marine isolates grew better on sea water agar than on a distilled water medium, whereas the terrestrial isolates of the same species showed the reverse rection. They maintain that the only differentiation between marine and terrestrial fungi is that the former is better adapted to grow and tolerate saline conditions. Later Subramanian and Raghukumar (1974) conducted similar studies in soils of marine and brackish environments in and around Madras. They isolated eighty six species of fungi, most of them were common terrestrial forms. Upadhyay *et al*. (1978) studied the ecology of microfungi in a coastal sand belt near Kanyakumari (Cape Comorin) with special reference to soil microenvironment. *Aspergilli* and *Penicillia* were the commonest components of beach and sand dunes.

Freitz *et al*. (1979) studied the microfungi from coastal waters of Bombay and Goa. Fungi with different physiological activities were isolated from immersed timber panels, sediments, mangrove vegetation and algae from the brackish water in Bombay and Goa. Patil and Borse (1983a) reported three arenicolous fungi viz. *Arenariomyces trifurcatus*, *Corollospora lacera* and *C. maritima* from the foam samples, collected from sandy beaches in Maharashtra.

The marine fungi in relation to their physiological actvities were also studied by a few authors. Desai and Betrabet (1971) studied the cellulolytic activity of fungal isolates from Bombay waters. Nair and Lokabharathi (1977) observed the degradation of hydrocarbons by a *Fusarium* sp. isolated from tar balls accumulated in Goa beaches. Nair *et al*. (1977) studied the distribution and activity of L-asparaginase producing fungi in the marine environment of Porto Novo, east coast of India. Araujo *et al*. (1981) screened marine fungi for their phosphorus solubilizing ability. Namboori *et al*. (1980) investigated the fungal transformation of Pregneolone and Progesterone with the marine fungus *Cladosporium herbarum*. Ranu Gupta and Ravindran (1988) determined the ultimate compressive stress of preservative treated wood samples exposed to fungal attack. All the fungal isolates were cellulolytic lignicolous forms from decaying fishing craft.

The fungal population and ecology of Indian mangrove swamps are also very poorly investigated. The earlier papers

dealt with the descriptions of single species isolated from mangrove soils; Rai and Tewari (1963) on *Preussia* isolates, Pawar *et al.* (1963) on a *Monosporium* and Pawar *et al.* (1967) on *Phoma* spp.. Additional investigations on Indian mangal soils were conducted by Pawar and Thirumalchar (1966), Padhye *et al.* (1967), Rai *et al.* (1969), Venkatesan and Ramamurthy (1971), Rai and Chowdhery (1975,1976) and Chowdhery (1979).

The relationships between salinity and cellulolytic activity of mangrove fungi were studied by Rai and Chowdhery (1976) and Garg (1982). They found that the cellulose degrading activity decreased with increase in the salinity except in a few species.

Chowdhery and Rai (1980) descibed five species of aquatic oomycetes which were new records from Indian mangroves.

Matondkar *et al.* (1980a, b) studied the seasonal variations in the microflora of mangrove swamps of Goa and for various exoenzyme activities. Matondkar (1980) while studying the role of heterotrophic microorganisms in mangrove ecosystem found the dominance of *Monilia*, *Mucor*, *Syncephalastrum*, *Aspergillus* and *Trichothecium*. Sheilla De Velho and Joe D'Souza (1982) isolated a total of 52 fungal cultures from the mangrove swamps of Chapora, Mandovi, Sal and Zuari estuaries of Goa and screened for pectinase activity.

Chowdhery *et al.* (1982) investigated the Sunderban mangrove swamps, West Bengal and isolated a good number of fungi from rhizosphere, rhizoplane and non-rhizosphere zones of mangroves. Highest number of fungi were isolated from rhizosphere zone. Ascomycetes were frequent in rhizoplane and Zygomycetes in rhizosphere; while Basidiomycetes were absent. They observed the active growth of many terrestrial species in mangrove swamps by direct microscopic method. Garg (1983) observed the frequent occurrence of *Aspergilli* and *Penicilli* in Sunderban mangrove mud while studying the vertical distribution of mycoflora through direct and dilution plate methods.

Recently more reports on manglicolous marine fungi were published from Maharashtra. Most of the species were new records to India from mangrove habitat (Patil and Borse, 1983b, 1985; Borse, 1984, 1987a,b,c,d). A recent work related to the ecology of fungi in mangrove swamps was conducted by Misra (1986). By using soil plate techniques he isolated twenty fungal species belonging to 12 genera with the dominance of *Aspergilli* and *Penicillia* from the mangrove muds of Andaman-Nicobar islands. Prabhakaran *et al.* (1987) investigated a mangrove swamp of Cochin backwater and recorded thirty one fungal species from the mud and twenty seven from decaying leaves, stems and roots of *Avicennia officinalis* and *Acanthus illicifolius*. The dominant fungal genus was *Aspergillus* followed by *Penicillium*, *Fusarium* and *Trichoderma*.

Yeasts

In India it was Bhat and Kachwalla (1955), who made the first attempt to investigate the marine yeasts. They collected sea water samples off the coast of Bombay and collected over 80 isolates by the enrichment culture methodology. In the same year Bhat *et al*. (1955) studied the different aspects of the nutrition of marine yeasts and their growth. After a decade Sechadri *et al*. (1986) further added to the yeast studies by their work in the marine and estuarine waters of Porto Novo. Patel (1975) found that actively growing algae contain lesser number of yeasts per gram of algae than yeasts found per ml of surrounding sea water. Godinho *et al*. (1978a,b) developed techniques to isolate hydrocarbon assimilating yeasts from the marine environment and conducted nutritional studies on hydrocarbon degrading yeasts of marine origin.

Glenda D'Souza and Joe D'Souza (1979), Emilia Da Costa and Joe D'Souza (1979a,b) Nelson D'Souza and Joe D'Souza (1979a,b) and Naik *et al*. (1982a,b) isolated a good number of yeasts from Goan estuaries including mangroves and studied various physiological activities of the isolates.

1.2 Need to take up fungal studies in the Cochin backwater

Cochin backwater, a tropical estuary has a detritus dominated food chain. The estuarine system is highly productive due to the supply of detritus from both autochthonous and allochthonous sources (Qasim, 1970; Qasim

and Sankaranarayanan, 1972). The role of fungi is important in detritus dominated ecosystems. A lot of allochthonous materials is added up into the backwater by mangroves and other macrophytes bordering the backwater. It is established that in marine coastal systems macrophytes form the major producers and are the basic source of energy that supply to the animals of commercial and sport fisheries (Mann, 1976). Herbivores consume about 5% of the macrophyte material (Fenchel, 1972; Odum et al.,1973). All the remaining material must be converted to microbial biomass prior to utilization by the primary consumers (Hargrave, 1976; Yingst, 1976; Heinle et al., 1977 and Tenore, 1977). Most animals of the ecosystem including many economically important ones such as prawns and detritus feeding fish cannot assimilate fresh macrophyte vegetation. Fungi and bacteria decompose the vegetation and make them assimilable for detritivores. Their activities bring an enrichment of nitrogen in detritus, refelected by a low carbon to nitrogen ratio of the detritus in comparison to fresh undecomposed detritus. This is highly suitable for the nutrition of detritus feeders. Cochin backwater is well known for it's traditional farm fishery which is directly linked to the constant availability of nutrients, where fungi must be playing an important role.

Presently Cochin backwater is exposed to various hazards of industralization. Sewage and Oil pollution are common and the estuary often shows the symptoms of eutrophication. Many microbial populations especially yeasts are good pollution

indicators. Thus the quality of the water can be determined based on the distribution of yeats. It is found that yeasts like *Candida tropicalis*, *C. krusei* and *C. parapsilosis* rarely occur in oceans but are usually found in estuarine regions where pollution is common (Fell, 1976). *Candida* species convert hydrocarbons into single cell protein (Meyers and Ahearn, 1972). They are resistant than bacteria to UV rays, fluctuations in osmotic pressure and salinity. The studies on the role of hydrocarbonoclastic yeasts are called for as the above conditions prevail in the Cochin backwater along with traces of oil pollution.

Virtually no work has been done on the mycopopulations of the backwater system except one or two occasional investigations (Jones, 1968; Nair, 1970). Work on general systematics of higher fungi from Indian waters are meagre. The importance of microbial taxonomy and ecology have been increasingly recognized in recent years in view of their significant role in the cycling of nutrients, in ecosystem productivity, in combating pollution, because of their potential in biotechnological applications etc..

Systematics of filamentous fungi can more easily be studied as they are mainly based on cultural and morphological charcteristics. Taxonomy of yeasts is much more difficult and require examination of cultural, morphological, physiological and biochemical characteristics.

In the present study yeasts were therefore given greater importance especially with respect to their systematics besides the studies on ecology, biochemical activity etc., taken along with filamentous fungi. Throughout, the two groups are treated separately so as to see more clearly their distinctive features. In brief the broad objectives of this work are:

(1) A general survey of the mycoflora (both filamentous and yeasts) present in the water, mud and decaying mangrove vegetation to ascertain the kind of mycoflora that is found in the Cochin backwater,

(2) To record their occurrence and also their abundance in different sites in backwater,

(3) To take up a detailed study of the taxonomy and systematics of estuarine yeasts,

(4) To examine general ecology and distribution and

(5) To contribute to the understanding of their possible role in the biogeochemical cycling in the backwater system.

CHAPTER 2

MATERIALS AND METHODS

2.1 Area of study

The Cochin backwater (between 09° 58'N - 10° 10'N and 76° 15'E - 76° 25'E) is a shallow, semienclosed extensive body of brackish water running parallel to the coastline located in the tropical zone. There is a regular influx of water from tributaries and canals into the backwater. The system also encloses many islands. It is connected to the sea by the 450m wide entrance at Cochin which is also the main shipping channel to the Cochin Port and also by another opening further north at Azhikode. The estuarine system is connected with the Arabian Sea throughout the year and hence a free flow of sea water into the estuary and a counterflow of freshwater into the sea during all the seasons. Pamba, Meenachil and Muvattupuzha rivers join the main body on its southern limb and Periyar joins the northern limb. Since these rivers flow into the system at its northern and southern extremities, a large quantity of fresh water is added to the system especially during the monsoon season. The influx of saline water is most felt around the entrance to Cochin Port. The system in general is shallow relative to the width and has a dendritic shoreline. The tidal range around the bar mouth is about 1m. The surrounding coast is relatively low. The system is of a positive type with the freshwater inflow and precipitation exceeding evaporation (Pillai *et al.*,1973).

The backwater is exposed to various anthropogenic pollution. A number of chemical and metallurgical industries located at Udyogamandal regularly discharge their effluents into the Periyar to be carried to the backwaters. The backwater also receives directly or indirectly the sullage water and muncipal sewage from the Cochin city (Saraladevi, 1986). The ecosystem is also undergoing man-made shrinkage at an alarming rate by bunding and reclamation for agriculture, aquaculture, harbour and urban development etc. (Gopalan *et al.*, 1983).

2.2 Sampling procedure

In order to study the mycoflora of Cochin backwater seven sites were selected (Fig. 2.1). Fishing harbour (station 1) anchors a large number of fishing boats and small quantities of fish wastes are often thrown from the harbour. Bar mouth (station 2) is the deepest station where maximum salinity is observed. Cochin Oil Terminal Jetty (station 3) and North Tanker Berth (station 4) are adjacent stations, where oil spilling is common. Station 5, Narakal is a shallow area surrounded by pokkali fields and vestiges of mangroves. Edavanakadu (station 6) is also a shallow station, situated in the main channel which receives saline water from Azhikode bar mouth. The station 7, Mangalavanam is a small area connected to the backwater by a feeder canal and surrounded by mangroves, where decaying vegetation is always abundant. Depth of the stations 1 to 4 ranged between

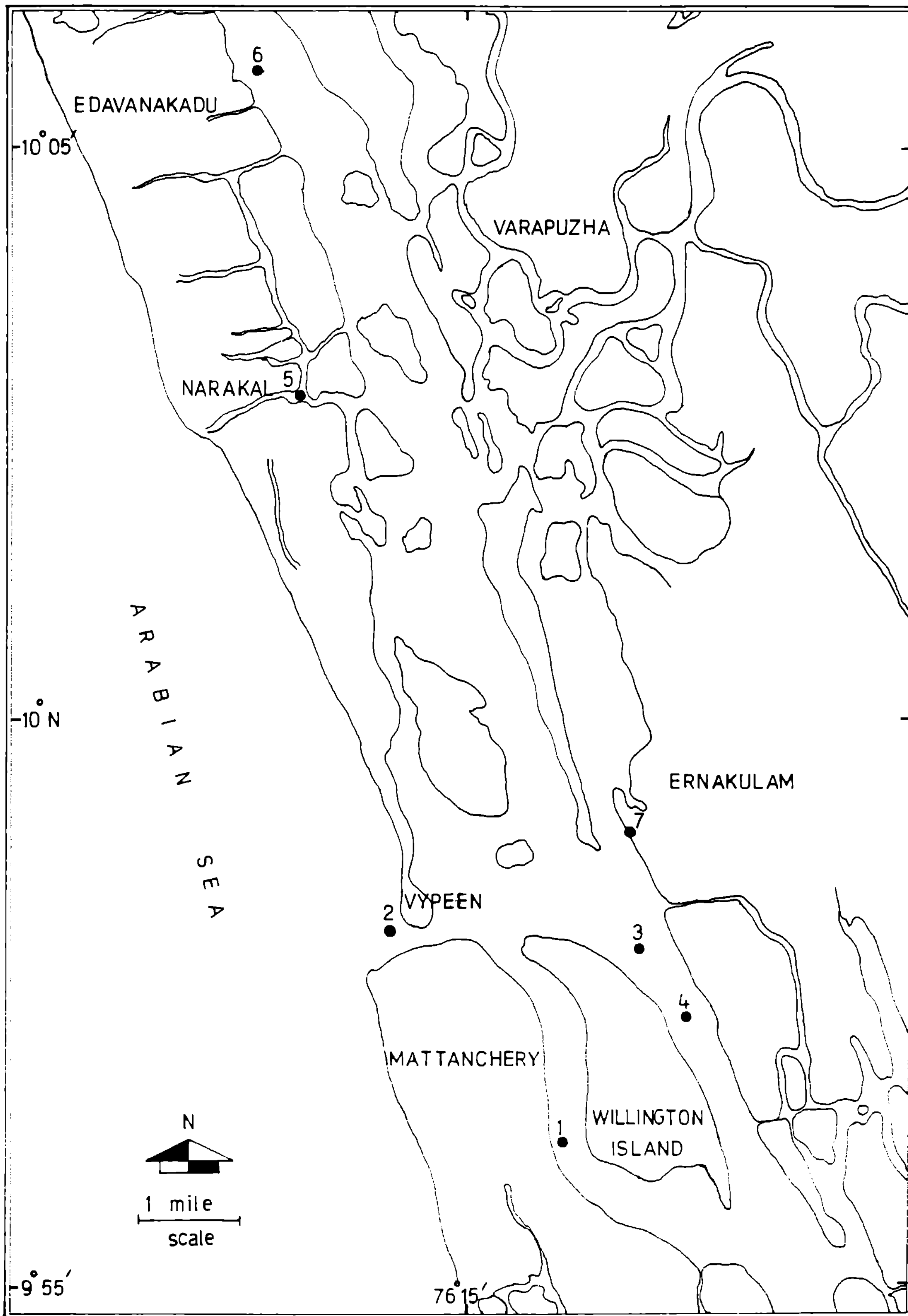

Fig. 2.1 Map showing the location of stations

5.5m to 9m and of stations 5 to 7, between 1 to 2m during high tide.

Water and mud samples were collected bimonthly from the seven sampling stations for two years during 1986 and 1987. In addition monthly samplings of mud and decaying mangrove vegetation were conducted at station 7, as part of a more detailed investigation of mangrove mycoflora for the two years.

Collection of water samples

To avoid aero-aquatic interface microbial populations, whose abundance according to Crow *et al*. (1975) can be two orders of magnitude more than those at 10cm depth, water samples were always collected one metre below the surface level. A locally fabricated ZoBell's microbiological water sampler (Fig.2.2) was used for the same. The bottle and its acessories of the sampler were steam sterilized for about an hour before use and was free from air contamination.

Collection of mud samples

The mud samples were collected using a van Veen grab (0.05 m^2). To avoid possible terrestrial contamination the inner walls of the grab were sterilized with absolute alcohol. Mud samples were directly transferred into alcohol sterilized polythene bags.

Water and mud samples were collected for mycopopulation studies as well as for estimating physico-chemical

parameters. During each sampling, collection of materials were completed within six hours. To avoid possible microbiological errors the samples were immediately transferred into an ice box maintaining a temperature of about $4 \pm 2^{\circ}$ C. Physico-chemical parameters were estimated using standard methods. Salinity was estimated by using Mohr titration and dissolved oxygen by fixing the Winklers reagents in the field and titrating in the laboratory (Strickland and Parsons, 1965). Temperature was recorded using an ordinary thermometer with 0 - 50° C graduation. The pH and Eh of the samples were recorded using electrodes (Century - Chandigarh, India). BOD_5 was determined in accordance with the procedure of American Public Health Association (APHA - 1983). The organic carbon of mud was estimated by the method of El Wakeel and Riley (1957).

2.3 Mycological methods

Isolation of fungi from water samples

In the laboratory known quantities of water samples were filtered through 0.45µm porosity cellulose acetate membranes using a sterile millipore filtration unit in an aseptic chamber. Samples were run in triplicates in 100 ml aliquots. After filtration the membranes were transferred into Petri dishes containing isolation media. The medium employed to isolate filamentous fungi was GY-Agar (Johnson and Sparrow, 1961) and for yeasts YM-Agar (Wickerham, 1951) (Appendix Ia & b). After sterilization an antibiotic mixture of

Chlortetracycline HCl 10mg%, Chloramphenicol 2mg% and Streptomycin sulphate 2mg% (filter sterilized) was incorporated to the medium to prevent bacterial growth.

To isolate filamentous fungi, all the experimental Petri plates were incubated at 28±2°C in an unilluminated BOD incubator for two weeks. For yeasts the Petri plates were incubated for three weeks at about 15° C to permit the development of yeasts and to keep development and proliferation of mould colonies on the membrane surface to a minimum (Fig. 2.3). Colony counts were taken microscopically and expressed in numbers per litre. Filamentous fungi were isolated and planted into fresh Petri plates for further purification, while representative yeasts were subcultured into fresh plates to insure uniclonal development and purified by dilution method at laboratory temperature.

Isolation of fungi from mud samples

Enumeration and isolation of fungi were accomplished by dilution pour plate technique.

In order to prevent possible contamination, the material used for the isolation purpose was taken from the central portion of the mud. Suspensions at 1:100 dilutions were prepared using sterile distilled water. One ml of each dilution was pipetted into the isolation medium (GY-Agar for filamentous fungi and YM-Agar for yeasts, prepared with 50% aged sea water and an antibiotic mixture mentioned previously). To isolate filamentous fungi the plates were

Fig. 2.2 ZoBell's Microbiological water sampler

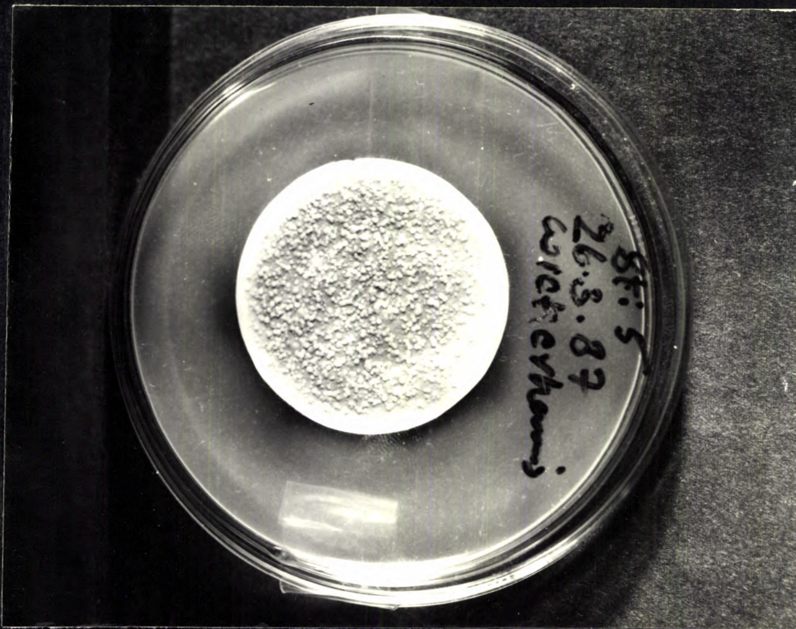

Fig. 2.3 Plate showing development of yeast colonies

incubated at 28±2°C in an unilluminated BOD incubator for two weeks, while for yeasts the plates were incubated at about 15°C for three weeks. Colony counts were determined microscopically and expressed in numbers per gram mud. The representative fungi were isolated and purified by dilution plate technique.

The filamentous fungi isolated from water and mud were maintained either in Emerson's YpSs-Agar medium or GPYS medium and yeasts in GPY-Agar or in Wickerham's medium (Appendix,I 2a-d). All the stock cultures were stored at 4°C in the laboratory and subcultured every three months into fresh medium.

Investigation on mangrove mycoflora

Sampling procedure

Mud samples were collected aseptically in triplicate by inserting a sterile 33cm hollow cylinder to a depth of about 15cm from which the subsurface mud was taken for mycological studies. Decaying fallen leaves, stems, roots and pneumatophores of *Avicennia officinalis* and *Acanthus illicifolius* were also collected in sterilized polythene bags. Physico-chemical parameters were determined using standard methods.

Isolation of fungi

Fungi were isolated from mud samples by dilution plating technique as mentioned earlier. The methodology adopted to

isolate fungi from decaying plant substrate was that of Fell and Master (1973). The decaying plant parts were aseptically cut into small pieces. These were then washed well with sterilized sea water collected from the sampling site and dipped into 0.01% $HgCl_2$ solution for 3 minutes for surface sterilization. The pieces were then washed well with sterilized sea water four times and placed on plates containing isolation medium. The use of $HgCl_2$ solution for surface sterilization is specifically designed to isolate filamentous fungi which penetrate into the internal layers of the substrate. Other microorganisms like bacteria, yeasts and certain Phycomycetes are excluded by the procedure. The plates were incubated at $28 \pm 2^\circ$ C for seven days. Quantitative data were collected by dilution plating method. However the quantitative data collected from decaying mangrove vegetation were not considered for ecological studies since surface sterilization was used. The fungal counts were reliable only as estimates of relative abundance of fungal species. The fungal colonies that showed up were purified and maintained at 4°C, subculturing every three months into fresh medium.

Identification of filamentous fungi

The isolates were identified according to different standard schemes described by Raper and Thom (1949), Raper and Fennell (1965), Gilman (1967), Barron (1968), Barnett and Hunter (1972), Ainsworth, Sparrow and Sussman (1973a,b), Ellis (1976), Kohlmeyer and Kohlmeyer (1979) and Hawksworth, Sutton and Ainsworth (1983). Identification of filamentous

fungi is much simpler than yeasts and is largely based on morphological characteristics and hence did not involve studies of physiological and biochemical characteristics etc.

Classification and Identification of Yeasts

The yeast isolates were identified based on the detailed cultural, morphological, physiological and biochemical examinations. The methodology adopted are mostly taken from Kreger van Rij (1984); Lodder (1970) and Barnett *et al.* (1979) were also referred for identification.

Pure cultures of isolates were routinely obtained by replating on either YM-Agar or Malt-extract Agar (Appendix I 3a & b).

Characteristics of vegetative cells

Growth in liquid medium :-

The cellular morphology and mode of reproduction of strains were studied in liquid culture, either in malt extract or in 2% (W/V) glucose-yeast extract-peptone water (Appendix I 4a & b). The organism was inoculated from an actively growing slant in 30ml of malt extract or in 2% glucose-yeast extract-peptone water in 100ml cotton plugged Erlenmeyer flasks and incubated for 2-3 days in the dark at 25° C or 28° C. The shape and mode or reproduction, the occurrence of cells and other characteristics were studied. The length and width of cells were measured and the extreme values obtained from the measurement of at least 20 cells

were recorded. The cultural characteristics were noted after 2-3 days.

Growth on solid medium :-

The isolates were examined for their cultural characteristics on either malt-extract agar or 2% glucose-yeast extract-peptone agar (Appendix I 4c & d). Actively growing organism was inoculated as a streak culture on slants in plugged tubes and incubated at 25 or 28°C for one month. The cultural characteristics were noted.

Formation of pseudomycelium and true mycelium :-

Slide culture and the Dalmau plate techniques were used. In slide culture technique the strain was inoculated in one or two lines along a slide containing agar medium (corn meal agar, malt extract agar or potato agar, Appendix I 5a & b) kept in a Petri dish. A sterile coverslip was placed over part of the lines. Incubated at 25°C for 4-5 days and examined microscopically. In Dalmau plate technique a single streak inoculation was made near one side of one-two days old poured plate (corn meal agar or potato agar). Two point inoculations were made near the other side of plates. The central section of the streak and one of the point inoculation were covered with sterile coverslips. The plates were incubated at 25° C for 7-10 days and examined microscopically.

Microscopical examination for ascospores :-

The test material was first brought to a state of active growth by subculturing either on YM-Agar or malt agar for 1-2 days at 25-28° C. Then the organism was inoculated on sporulation media (modified Gorodkowa agar, malt extract agar, YM-Agar or acetate agar, Appendix I 6a-d). The plates were incubated at 25-28° C for 3 days before being examined microscopically for the first time. Material which showed no sporulation was then maintained at room temperature and examined at weekly intervals for at least 4-6 weeks.

Ascospores were observed by staining the slide preparations. In Schaeffer-Fulton's modification of the Wirtz method, heat fixed preparations were flooded with 5% aqueous malachite green for 30-60 seconds and heated to steaming three or four times. The excess stain was rinsed off under running water for about half a minute. The preparations were then counterstained with 0.5% safranine for about 30 seconds. The mature ascospores stained blue green and the vegetative cells red. In modified Kufferath carbol-fuchsine staining method slide preparations were heat-fixed and flooded with Ziehl-Neelsen carbol-fuchsine and steamed gently for about 2-5 minutes; decolourized with either 2% lactic acid or 95% ethanol containing 1% conc.HCl. The slides were rinsed in water and counterstained with either 1% methylene blue, thionin or Nile blue hydrochloride. The mature ascospores stained red and vegetative cells blue.

Physiological and biochemical characteristics

Fermentation of carbohydrates :-

For identification purposes the ability to ferment glucose, galactose, sucrose, maltose, lactose and raffinose were routinely tested. The fermentation of sugars was tested in 2% (W/V) (raffinose, 4% (W/V)) solutions in Durham tubes. The sugars were dissolved in 0.05% solution of commercial powdered yeast extract. 5-6ml aliquots of the solution of (filter sterilized) were dispensed into plugged sterile tubes (150 x 12mm) carrying insert tubes. Blank without sugar was maintained as control. The tubes were inoculated directly from actively growing slant cultures by means of a stout platinum loop. The tubes were incubated at 28°C in the dark and regularly shaken and observed for the accumulation of gas in the insert tubes over a period of 14 days. Fermentation was rated based on the time required for the formation of visible amounts of gas. The tests were conducted in triplicates. The results were recorded as indicated below:

+ Fermentation strong, gas filling the insert tube within 1-3 days,

+W Fermentation weak, gas filling the insert tube only partially,

+VW Fermentation very weak, only a buble formed in the insert tube,

+S Fermentation slow or delayed, still gas filling the insert tube and

_ Fermentation absent.

Assimilation of carbon compounds :-

Assimilation test was conducted with 18 specific carbon compounds mentioned under the description of species (Chapter 3). In certain cases for confirmation tests, additional compounds were used. The ingredients of nitrogen basal medium (Appendix I 7a) and the appropriate amount of the carbon compound were dissolved in demineralized water. The pH was adjusted to 5.6 and sterilized by filteration. Aliquots of 0.5ml of the sterile solution were pipetted aseptically into plugged rimless test tubes containing 4.5ml sterilized demineralized water. Actively growing organism was throughly dispersed in about 3ml sterile tap water in 16mm tube. The suspension was aseptically diluted with sterile water until the black lines approximately 3/4mm wide drawn on white cardboard became visible through the tube as dark bands. Each of the tubes containing the different carbon sources was then inoculated with one drop of such a suspension from a Pasteur pipette. Blank tube containing the basal medium with deleted carbon source served as control. After inoculation the tubes were incubated in the dark for 3 weeks at 28°C in an upright position. The tubes were shaken manually and examined weekly. The tests were conducted in triplicates.

The degree of assimilation was determined by placing vigorously shaken tubes against a white card bearing lines of 3/4mm wide, drawn with Indian ink. If growth in the tubes completley obliterated the lines it was recorded as 3+; if

the lines appeared as diffuse bands, the growth was rated as 2+; if the bands were distinguishable with indistinct edges it was recorded as 1+; while the absence of growth was indicated as -. A 3+ or 2+ reaction within three weeks was considered positive and a 1+ reaction as very weak. The results were recorded as indicated below:

+ rapid

+W weak

+S slow

_ absent

Splitting of arbutin :-

This test was conducted on arbutin-agar slants (Appendix I.8). Slants were inoculated with an actively growing culture of the yeast and incubated at 28°C. Development of dark brown colour within 2-7 days indicated the splitting of arbutin.

Assimilation of nitrogen compounds :-

The ingredients of the nitrogen medium (Appendix I.7b) and the nitrogen source to be tested were dissolved in demineralized water. pH was adjusted to 5.6 and sterilized by filteration. 0.5ml aliquots of the sterile medium was pipetted into plugged rimless test tubes containing 4.5ml sterilized demineralized water. 0.5ml of filter sterilized basal medium served as blank. The tubes received the same inoculum as was used in the carbon assimilation tests and incubated in the dark at 28°C for three weeks. Tests were conducted in triplicates. The degree of growth was assessed

as mentioned under carbon assimilation test.

Growth in vitamin-free medium :-

0.5ml of the filter sterilized basal medium (Appendix I.7c) was aseptically added into 4.5ml sterile water in 16mm plugged tubes. The tubes were inoculated as mentioned under carbon assimilation test and were incubated at 25° C. The tests were conducted in triplicates. The degree of growth was assessed as mentioned under carbon assimilation test.

Growth on 50% glucose-yeast extract agar :-

The 50% glucose-yeast extract agar (Appendix I.9) slants were lightly inoculated from an actively growing culture. Incubated at 25°C and examined after 5 days for growth.

Growth in 10% NaCl plus 5% glucose in yeast nitrogen base :-

To the 16mm plugged tubes containing 10% NaCl plus 5% glucose medium (Appendix I.10), 0.5ml of filter sterilized bacto yeast nitrogen base was added and inoculated as mentioned under carbon assimilation test. Incubated at 25°C for three weeks and the degree of growth was recorded as in carbon assimilation test.

Growth at 37°C :-

The organism under test was grown on YM-Agar at 37 C up-to 5 days. Results were recorded after 5 days.

Formation of extracellular amyloid compounds-starch test :-

The organism under test was streaked on agar plates (Appendix I.11) and incubated for two weeks at 25 C. Tested

for starch by flooding with Lugol's iodine solution (Appendix IIa). Positive results indicated colour reactions varying in intensity from blue to purple.

Urease test :-

The test was conducted on Christensen's urea agar medium (Appendix I.12). The organism under test was inoculated on slants and incubated at 25°C. Cultures were observed daily upto 5 days. Positive reactions were indicated by the appearance of a deep pink colour.

2.4 Ecological studies

After completing the identification and total counts, the data were examined by quantitative methods for ecological interpretations. Appropriate statistical methods were used for this purpose and they include Tukey's test of additivity and Analysis of Varience (Snedecor and Cochran, 1963), Duncan's multiple 't' test (Federer, 1967), Sander's index of affinity (Sanders, 1960), Shanon Weaver diversity index (Monte Lioyd and Ghelardi, 1964), Heip's measure of evenness index (Heip, 1974), Point correlation coefficient (Cole, 1949) and Cluster analysis (Sanders, 1978). The data did not permit the use of statistical methods for attempting environmental correlations.

2.5 Biochemical activities

Filamentous fungi

The following procedures were subjected in common to all the biochemical activities (except cellulolytic activity) mentioned below. The biochemical screening tests were conducted on solid media in Petri plates. In all the tests the medium devoid of test substrate served as control. The inoculum always consisted of a 5mm disc of mycelium and agar obtained by using a sterile cork borer. Each dish was inoculated with the mycelium side of the inoculum on the agar. All the plates were incubated in the dark at 28±2°C in an unilluminated BOD incubator for 5 days except where noted. The activity was determined by measuring the radius of the zones formed in the agar after 24, 48, 72, 96, and 120 hrs incubation. Results were expressed as the average of three replicates.

Cellulolytic activity

To test cellulolytic ability a preliminary test has been conducted prior to final test. The selected cultures were inoculated on YpSs slants containing 10cm Whatman filter-paper strips. Screening was done by observing their growth on filter-paper strips after 15 days at 28°C. The positive cultures were further subjected to agar diffusion assay of cellulolytic ability based on method by Tansey (1971).

The cellulose substrate used in the clearing test was prepared according to the procedure of Walseth (1952) and

Rautela and Cowling (1966). The assay medium (Appendix I.13a) was dispensed into 20 x 150mm culture tubes. Each tube contained a six cm column of medium and autoclaved at 121 C for 15 minutes. Cultures for inoculum were grown at 28° C on plates of Emerson's YpSs-agar. The inoculum consisted of 12mm discs of mycelium and agar obtained by using a sterile cork borer. Three replicate tubes and controls were incubated either at 28° C or 37° C in an unilluminated BOD incubator. Culture tubes were placed upright in beakers containing sterilized distilled water and these were sealed in a polythene bag.

Depth of clearing (DC) was measured at seven-day intervals. Clearing was recorded to the nearest millimeter, where possible and represented the average of three replicates.

Amylolytic activity

To detect amylolytic activity, the medium described by Hankin and Anagnostakis (1975) was used (Appendix I 13b). The ability to degrade starch was used as the criterion for determination. After incubation the plates were flooded with an iodine solution (Appendix IIb) and a yellow zone around the colony in an otherwise blue medium indicated amylase production.

Pectolytic activity

To detect pectolytic activity, the medium described by Hankin *et al.* (1971) was used (Appendix I 13c). Incubated plates were flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide (Emerck). The clear zones around the colony in an otherwise opaque medium indicated degradation of pectin.

Chitinolytic activity

The solid medium formulated by Hankin and Anagnostakis (1975) was used to test the chitinolytic fungi (Appendix I 13d). The chitin was prepared by treatment of chitin as described by Campbell and Williams (1951). Incubation was continued for 5 days. The activity was determined by noting clear zones in the opaque agar around colonies.

Lipolytic activity

The solid medium formulated by Hankin and Anagnostakis (1975) was used to detect the production of lipolytic enzymes (Appendix I 13e). The lipolytic activity was determined by a visible precipitate due to the formation of crystals of the calcium salt of the lauric acid liberated by the enzyme, or as a clearing such a precipitate around the colony due to complete degradation of the salt of the fatty acid.

Proteolytic activity

Caseinase activity :-

A medium that contained skim milk powder (Appendix I 13f)was used to detect the production of caseinase (Rajamani and Hilda, 1987). After incubation 5ml of trichloroacetic acid (TCA) were added to each Petri dish over the agar surface. The clear zones around the colony in an otherwise opaque medium indicated degradation of casein. After 10 minutes the measurements were taken.

Gelatinase activity :-

A solid medium contained gelatin as the protein substrate (Appendix I 13g) was used to detect the production of proteolytic enzyme (Hankin and Anagnostakis, 1975). After incubation the plates were flooded with $HgCl_2$ solution in HCl (Appendix II c). Gelatinase production was indicated by the formation of clear zones around the colony.

Phosphate solubilization test

To detect the phosphate solubilization modified Pikovskaya's (1948) medium was used (Appendix I 13h). The phosphate solubilization was observed by the clear zone that detected around the colony.

Yeasts

Hydrocarbon assimilation

The liquid nutrient medium designed by Chatterjee *et al.* (1978) was used for the test (Appendix I 14). The medium was dispensed in 16mm tubes (5ml each) and sterilized. Hydrocarbons to be tested were sterilized separately and 0.5ml was added in each tube. Medium devoid of carbon source served as control. A loopful of organism from an actively growing slant was inoculated into each tube. The inoculated tubes were shaken occassionally and incubated at 28°C for about a week. The hydrocarbon utilization was assessed by the proliferation and growth of yeast in the oil-medium interface.

Pectinase activity

To test pectinase activity 1% pectin-Wickerham's medium (Appendix I 15) was used. The medium devoid of pectin served as control. Point inoculations of yeast isolates were done and the plates were incubated at 28°C upto 5 days. Following incubation the dishes were flooded with 1% aqueous solution of hexadcyltrimethyl ammonium bromide (Emerck). Enzymatic activity was determined by observing clear zones around the inoculum.

APPENDIX

I. MEDIA

1. Isolation Media

Filamentous fungi

(a) GY-Agar (Johnson & Sparrow, 1961)

Glucose	1.0g
Yeast extact	0.1g
Agar	18.0g
50% aged Sea water	1 litre
pH 6.5	

Yeasts

(b) YM-Agar (Wickerham, 1951)

Glucose	10.0g
Peptone	5.0g
Yeast extract	3.0g
Malt extract	3.0g
Agar	20.0g
50% aged Sea water	1 li.
pH 6.5	

2. Maintenance Media

Filamentous fungi

(a) Emerson's YpSs-Agar

Yeast extract	4.0g
Starch (soluble)	15.0g
Dipotassium Phosphate	1.0g
Magnesium sulphate	0.5g
Agar	20.0g
50% aged sea water	1 litre
pH 6.5	

(b) GPYS-Medium

Glucose	1.0g
Peptone	0.5g
Yeast extract	0.1g
Agar	16.0g
50% aged Sea water	1 li.
pH 6.5	

Yeasts

(c) GPY-Agar

Glucose	20.0g
Peptone	10.0g
Yeast extract	5.0g
Agar	20.0g
50% aged sea water	1 litre
pH 6.5	

(d) Wickerham's Medium

(See YM-Agar)

3. Classification and Identification of yeasts

Plating Medium

(a) YM-Agar

Yeast extract	3.0g
Malt extract	3.0g
Peptone	5.0g
Glucose	10.0g
Agar	20.0g
Dist. water	1 litre

pH adjusted to 6

(b) Malt-extrat Agar

Prepared 10%(w/w)solution of powdered malt extract in demineralized water to which added 2% agar. pH adjusted to 6.

4. To study the characteristics of vegetative cells

(a) Malt extract

Dissolved 15% powdered malt extract in demineralized water by heating in a water bath. pH adjusted to 5.4.

(b) Glucose-Yeast extract-peptone water

Glucose	20.0g
Peptone	10.0g
Yeast extract	5.0g
Dist. water	1 li.

pH not adjusted

(c) Malt-extract agar

Prepared malt-extract agar by adding 2% agar in broth.

(d) Glucose-yeast extract-Peptone agar

Prepaed GYP-Agar by adding 2% agar in broth.

5. To study the formation of pseudomycelium and true mycelium

(a) Corn meal agar

Stirred 12.5g corn meal in 300ml water and heated in a water bath at 60 C for 1 hr and filtered. To 300ml filtrate added 3.8g agar. Autoclaved at 15 lbs over pressure for 15min. Refiltered through absorbent cotton wool and autoclaved for 15min at 15 lbs overpressure.

(b) Potato agar

Socked 100g peeled potatoes in 300ml tap water for several hours in a refrigerator. Filtered through cloth and autoclaved for 1 hour at 15 lbs overpressure. Added 230ml of extract to 770ml tap water containing 20g glucose and 20g agar. Autoclaved at 15 lbs overpressure for 15 min.

6. Microscopical examination for ascospores

(a) Modified Gorodkowa agar medium (van der Walt, 1970)

Dissolved 0.1% (W/V) glucose, 1% (W/V) peptone, 0.5% (W/V) Sodium chloride and 2% (W/V) Agar in hot tap water and autoclaved for 15min at 15 lbs overpressure.

(b) Malt-extract agar

Dissolved 12g agar in 400ml demineralized water by steaming and added 20g malt extract in hot solution. Autoclaved for 15min at 15 lbs overpressure.

(c) YM-Agar (Wickerham, 1951)

(See 3.(a))

(c) Acetate agar (McClary, Nutty & Miller, 1959)

Potassium acetate	9.8g
Glucose	1.0g
Sodium chloride	1.2g
Magnesium sulphate	0.7g
Yeast extract	2.5g
Agar	20.0g
Tap water	1 li.

7. Composition of basal media totest the assimilation of carbon and nitrogen compounds and the growth in vitamin free medium for yeasts

Ingredients	a. Carbon assimilation test	b. Nitrogen assimilation test	c. Vitamin free yeast base
Nitrogen Compound			
Ammonium sulphate	5g	-	5g
Carbon compound			
Dextrose	-	10g	10g
Amino acids			
L-Histidine monohydrochloride	10mg	1mg	10mg
DL-Methionine	20mg	2mg	20mg
DL-Tryptophan	20mg	2mg	20mg
Vitamins			
Biotin	20µg	20µg	-
Calcium panthothenate	2000µg	2000µg	-
Folic acid	2µg	2µg	-
Inositol	1000µg	1000µg	-
Niacin	400µg	400µg	-
p-Aminobenzoic acid	200µg	200µg	-
Pyridoxine hydrochloride	400µg	400µg	-
Riboflavin	200µg	200µg	-
Thiaminehydrochloride	400µg	400µg	-

(cont./-)

Appendix 7. (continued)

Compounds supplying trace elements			
Boric acid	500μg	500μg	500μg
Copper sulphate	40μg	40μg	40μg
Potassium iodide	100μg	100μg	100μg
Ferric chloride	200μg	200μg	200μg
Manganese sulphate	400μg	400μg	400μg
Sodium molybdate	200μg	200μg	200μg
Zinc sulphate	400μg	400μg	400μg
Salts			
Potassium phosphate (monobasic)	850mg	850mg	850mg
Potassium phosphate (dibasic)	150mg	150mg	150mg
Magnesium sulphate	500mg	500mg	500mg
Sodium chloride	100mg	100mg	100mg
Calcium chloride	100mg	100mg	100mg

Dissolved the basal media in 100ml demineralized water by warming. For carbon assimilation test after dissolving the carbon compound the pH was adjusted to 5.6 and sterilized by filteration. For nitrogen assimilation test when nitrogen source was nitrate, used 0.78g potassium nitrate; when it was nitrite, used 0.26g sodium nirite. Adjusted the pH to 5.6 and sterilized by filteration.

8. Splitting of Arbutin

Dissolved 0.5% (W/V) arbutin and 2% agar in yeast infusion and dispensed into tubes and autoclaved for 15 min at 15 lbs overpressure. After sterilization 2-3 drops of sterile 1% ferric ammonium citrate solution was added.

9. Growth on 50% glucose-yeast extract-agar

Dissolved 50g glucose in 50ml of yeast infusion and added 3% agar. Autoclaved at 10 lbs overpressure for 10min.

10. Growth in 10% NaCl plus 5% glucose in yeast nitrogen base

Dissolved 5g glucose and 10g NaCl in 100ml demineralized water. Dispensed in tubes and autoclaved for 15min at 15 lbs overpressure. Added 0.5ml filter sterilized bactoyeast nitrogen base to each tube before use.

11. Starch Test

Dissolved 0.2% ammonium sulphate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulphate heptahydrate and 2% glucose in demineralized water. Adjusted the pH to 4.5. Prepared equal volume of 4% agar and autoclaved for 15min at 10 lbs overpressure. Mixed well and prepared plates

in Petri dishes containing 1 drop of 20% (W/V) commercial yeast extact solution and one drop of conc. vitamin solution.

12. Christensen's urea agar medium for urease test

Peptone	1.0g
Glucose	1.0g
Sodium chloride	5.0g
Potassium dihydrogen phosphate	2.0g
Phenol red	0.012g
Demineral. water	1 litre
pH 6.8	

Dissolved 20g agar and dispensed in tubes. Autoclaved for 15 min at 15 lbs overpressure. Added 0.5ml of 20% filter sterilized urea solution, mixed well and slanted.

13. Studies on Biochemical activities of fungi

(a) Cellulolytic activity

Cellulose	5.00g (dry weight)
Ammonium dihydrogen phosphate	2.00g
Potassium dihydrogen phosphate	0.40g
Magnesium sulphate	0.89g
Yeast extract	0.50g
Adenine	4.00g
Adenosine	8.00g
Agar	17.00g
Thimine hydrochloride	100ug
Aged sea water (50%)	1 litre
pH 6	

(b) Amylolytic activity

Nutrient broth	0.8g
Agar	18.0g
Starch (soluble)	2.0g
Aged sea water (50%)	1 litre
pH 6	

(c) Pectolytic activity

* Mineral salt solution	500ml
Yeast extract	1.0g
Agar	15.0g
Pectin (citrus)	5.0g
Aged sea water (50%)	500ml
pH 6	

* The mineral salts solution contained per litre:

(cont./-)

Appendix 13 (c)- (continued)

Ammonium sulphate - 2g; Potassium dihydrogen phosphate - 6g; di-Sodium hydrogen phosphate - 6g; Ferrous sulphate - 0.2g; Calcium chloride - 0.001g; Boric acid -10μg; Manganeese sulphate -10μg; Zinc sulphate -70μg; Copper sulphate -50μg; Molybdenum oxide -10μg

(d) Chitinase activity

Mineral salts solution (see 13c)	500ml
Yeast extract	0.2g
Purified chitin	24.0g
Agar	15.0g
Aged sea water (50%)	500ml
pH 6	

(e) Lipolytic activity

Peptone	10.0g
Sodium chloride	5.0g
Calcium chloride	0.1g
Agar	20.0g
Aged sea water (50%)	500ml
pH 6	

Autoclaved tween 20 (Sorbitan monolaurate) separately and added 1ml per 100ml of sterile cooled basal medium.

(f) Caseinase activity

Mixed 10g skim milk powder in 100ml distilled water. Dissolved 10% agar in 100ml of 50% aged sea water. Prepared 300ml 0.05M sodium dihyrogen phosphate, di-sodium hydrogen phosphate (buffer 7); sterilized the milk solution with flowing steam. Autoclaved agar and buffer solution separately. All three solutions mixed aseptically and plated.

(g) Gelatinase activity

The medium consisted of nutrient agar, 0.4% gelatin and 50% aged sea water. Autoclaved 8% gelatin solution and added to the nutrient agar at the rate of 5ml per 100ml of medium.

(h) Tricalcium phosphate solubilization test

Tricalcium phosphate	5.0g
Ammonium sulphate	0.5g
Sodium chloride	0.2g
Potassium chloride	0.2g
Magnesium sulphate	0.1g
Manganeese	traces
Ferrous sulphate	,,

(cont./-)

Appendix 13 (h) - (continued)

Glucose	10.0g
Yeast extract	0.5g
Agar	18.0g
Aged sea water (50%)	1 litre

14. Hydrocarbon assimilation -yeasts

Potassium dihydrogen phosphate	3.0g
di-Sodium hydrogen phosphate	6.0g
Sodium chloride	5.0g
Magnesium sulphate	0.1g
Manganeese chloride	traces
Ferrous sulphate	,,
Calcium chloride	,,
Ammonium molybdate	,,
Aged sea water (50%)	1 litre

pH 7

Dispensed in 16mm tubes (5ml each) and sterilized. Sterilized the hydrocarbons separately and added 0.5ml in each tube.

15. Pectinase activity - yeasts

Pectin	10.0g
Peptone	5.0g
Malt extract	3.0g
Yeast extract	3.0g
Agar	20.0g
Aged sea water (50%)	1 litre

pH 6

II. REAGENTS

(a) Lugol's iodine solution I

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

(b) Lugol's iodine solution II

Iodine	3.0g
Potassium iodide	15.0g
Dist. water	1000ml

(c) Mercuric chloride solution

Mercuric chloride	15.0g
Dist. water	100ml
Conc. HCl	20ml

CHAPTER 3

TAXONOMY OF ESTUARINE YEASTS AND IDENTIFICATION OF YEASTS AT SPECIES LEVEL

Present day classification of the yeasts is based on strains. The characteristics used in classifying yeasts are cultural, morphological, reproductive, physiological and biochemical features. The system of classification of the yeasts followed in this study is that adapted by Kreger van-Rij (1984). The mode of identification is described under materials and methods.

3.1 Classification and list of yeast species identified

Division : Ascomycotina

Class Hemiascomycetes

Order Endomycetales

Family Saccharomycetaceae

Sub family Saccharomycetoideae

Genus *Debaryomyces*

(1) *Debaryomyces hansenii*

(2) *Debaryomyces marama*

(3) *Debaryomyces vanriji*

Genus *Hansenula*

(4) *Hansenula anomala*

Genus *Kluyveromyces*

(5) *Kluyveromyces marxianus*

Genus *Pichia*

(6) *Pichia bovis*

(7) *Pichia guilliermondii*

Genus *Saccharomyces*

(8) *Saccharomyces cerevisiae*

(9) *Saccharomyces exiguus*

(10) *Saccharomyces kluyveri*

Division : Deuteromycotina

Order Blastomycetes

Family Cryptococcaceae

Genus *Candida*

(11) *Candida albicans*

(12) *Candida atmospherica*

(13) *Candida halophila*

(14) *Candida intermedia*

(15) *Candida krusei*

(16) *Candida membranaefaciens*

(17) *Candida parapsilosis*

(18) *Candida pseudointermedia*

(19) *Candida sake*

(20) *Candida solani*

(21) *Candida tropicalis*

Genus *Cryptococcus*

(22) *Cryptococcus laurentii*

Genus *Geotrichum*

(23) *Geotrichum candidum*

Genus *Rhodotorula*

(24) *Rhodotorula aurantiaca*

(25) *Rhodotorula glutinis*

(26) *Rhodotorula graminis*

(27) *Rhodotorula lactosa*

(28) *Rhodotorula minuta*

(29) *Rhodotorula rubra*

Genus *Trichosporon*

(30) *Trichosporon aquatile*

(31) *Trichosporon cutaneum*

(32) *Trichosporon penicillatum*

Family Sporobolomycetaceae

Genus *Sporobolomyces*

(33) *Sporobolomyces roseus*

3.2 Taxonomy and systematic discussion

Division Ascomycotina

Class Hemiascomyctes

Order Endomycetales

Family Saccharomycetaceae

Mycelium, pseudomycelium, budding cells and arthrospores side by side or alone. Vegetative reproduction by fission or by budding. Sexual reproduction by isogamous or heterogamous conjugation. Ascospores of various shapes.

Sub Family Saccharomycetoideae

Mycelium and budding cells, pseudomycelium and (or) single budding cells; vegetative reproduction by fission and budding or budding only; ascospores of various shapes, dissimilation oxidative to fermentative.

Genus *Debaryomyces* Lodder et Kreger van Rij nom.cons.

General characters : Vegetative reproduction by multilateral

budding. A primitive or occasionally well developed pseudomycelium may be present. Sexual reproduction is by heterogamous or isogamous conjugation. Spherical or oval spores, one to four spores per ascus. Fermentation slow, weak or absent. Nitrate not assimilated, but nitrite may be assimilated.

Taxonomic treatment of Debaryomyces species from the Cochin backwater :

DEBARYOMYCES HANSENII (ZOPF) LODDER ET KREGER-VAN RIJ

Figs 3.1a,b, 3.34

Description :

Growth in malt extract : After 3 days at 25°C, the cells are spherical to short oval (2 - 5.5) x (2.5 -6) µm; single, in pairs and in short chains. A sediment and a ring are formed after one month.

Growth on malt agar : After one month at 25°C the streak culture is greyish white to yellowish, dull and the margin is entire.

Slide culture on potato agar : Pseudomycelium is not formed. Ascospores are observed on Gorodkowa agar at 25° C. One warty ascospore is observed per ascus.

Fermentation:

Glucose	+VW	Galactose	-	Sucrose	+VW
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds:

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	+	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalsoe	+	D-Ribose	+	Citric acid	-
Lactose	+	L-Rhamnose	-	Inositol	-

Splitting of arbutin : +

Assimilation of nitrate : -

Assimilation of nitrite : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : +

Growth at 37°C : +

DEBARYOMYCES MARAMA DI MENNA

Figs 3.2a,b, 3.35

Description :

Growth in malt extract : After 3 days at 25°C, the cells are spherical to short oval (2.1 - 3.75) x (3.5 - 4.9) μm, single and in pairs. A sediment and a ring are present after one month.

Growth on malt agar : After one month at 25°C the streak culture is yellowish-white, soft, shiny to dull and smooth with entire margin.

Slide culture on potato agar : Pseudomycelium is absent. One to four ascospores per ascus are observed on malt agar at 25°C.

Fermentation :

Glucose	-	Galactose	-	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	+
Sucrose	+	Sol.starch	+	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	+
Lactose	+	L-Rhamnose	-	Inositol	-

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : +

Growth at 37°C : -

DEBARYOMYCES VANRIJI (VAN DER WALT ET TSCHEUSHNER)

ABADIE, PIGNAL ET JACOB VAR.VANRIJI

Figs 3.3a,b, 3.36

Description :

Growth in malt extract : After 3 days at 25°C the cells are spherical to short oval (3.1 -6.1) x (3.5 - 7.1) µm; single. A sediment and a pellicle are formed after one month.

Growth on malt agar : After one month at 25°C the streak culture is yellowih-brown, dull, smooth with a sinuous margin.

Slide culture on potato agar : Pseudomycelium absent. One to four ascospores per ascus are observed on malt agar at 25°C.

Fermentation :

Glucose	-	Galactose	-	Sucrose	+VW
Maltose	-	Lactose	-	Raffinose	+W

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	+
Sucrose	+	Sol.starch	+	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	+
Lactose	-	L-Rhamnose	-	Inositol	-

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : +

Growth at 37°C : +

Genus : Hansenula H.et P. Sydow

General characters : Asexual reproduction by multilateral budding and spheroidal, ellipsoidal or elongate cells. Pseudohyphae or true hyphae may be present. One to four hat shaped, hemispheroidal, or saturn-shaped ascospores per ascus. Sugars may or may not be fermented and nitrate assimilated.

Taxonomic treatment of Hansenula species from the Cochin backwater :

HANSENULA ANOMALA (HANSEN) H.ET. P. SYDOW

Figs 3.4a,b, 3.37

Description:

Growth in malt extract : After 3 days at 25°C, the cells are spheroidal to elongate (2.1 - 3.1) x (2.1 - 6.1) µm; single, in pairs and in small clusters. After one month a pellicle is formed.

Growth on malt agar : After one month at 25°C the streak culture is cream coloured, smooth with entire margin.

Slide culture on potato agar : Pseudomycelium is absent. Hat shaped ascospores were observed on 5% malt extract agar.

Fermentation :

Glucose	+	Galactose	-	Sucrose	+
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Raffinose	-	Erythritol	+
Sucrose	+	Sol.starch	+	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	-	Succinic acid	+
Trehalose	+	D-Ribose	-	Citric acid	+
Lactose	-	L-Rhamnose	-	Inositol	-
L-Sorbose	-	Inulin	-	Salicin	+
Melibiose	-	D-Arabinose	-	DL-Lactic acid	+
Melezitose	+	α Methyl D-glucoside			+

Assimilation of nitrate : +

Growth in vitamin-free medium : +

Growth in 10% NaCl plus 5% glucose in yeast nitrogen base : +

Growth at 37° C : +

Genus : Kluyveromyces van der Walt emend. van der Walt

General characters : Vegetative reproduction by budding, cells spheroidal, ovoid, ellipsoid, cylindrical to elongate; Evanescent asci, uni- to multispored. Ascospores crescentiform, reniform oblong, ellipsoidal or spheroidal. Fermentation present. Nitrate not assimilated.

Taxonomic treatment of Kluyveromyces species from the Cochin backwater:

KLUYVEROMYCES MARXIANUS (HANSEN) VAN DER WALT var. DROSOPHILARUM

Figs 3.5a,b, 3.38

Description :

Growth in malt extract: After 3 days at 25°C the cells are spheroidal to ellipsoidal (2.8 - 4.9) x (2.8 - 6.5) µm, reproduction by budding; occur singly, in pairs and in short chains. After one month, a ring, sediment and a thin pellicle are formed.

Growth on malt agar: After one month at 25°C the streak culture is cream-coloured, dull, smooth with undulating margin.

Dalmau plate culture on corn meal agar: Pseudomycelium absent.

One to four reniform ascospores per ascus are observed on malt extract agar.

Fermentation :

Glucose	+	Galactose	+	Sucrose	+
Maltose	-	Lactose	-	Raffinose	+

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	+	D-Xylose	+S	D-Mannitol	+
Cellobiose	-	L-Arabinose	-	Succinic acid	+
Trehalose	-	D-Ribose	-	Citric acid	+
Lactose	-	L-Rhamnose	-	Inositol	-

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +

Genus : Pichia Hansen

General characters: Asexual reproduction by multilateral budding. Cells are spheroidal, ellipsoidal, or elongate. Pseudohyphae present. One to four hat-shaped, hemispheroidal, spheroidal or saturn shaped with a smooth surface ascospores per ascus. Sugars may or may not be fermented. Nitrate not assimilated.

Taxonomic treatment off Pichia species from Cochin backwater:

PICHIA BOVIS VAN UDEN ET DO CARMO-SOUSA

Figs 3.6a,b, 3.39

Description :

Growth in malt extract : After 3 days at 25°C the cells are spheroidal to long ovoidal (1.9 - 4.97) x (2.8 - 8.8) µm and occur in single, paired or in small clusters. After one month dry climbing pellicle is observed.

Growth on malt extract-agar : After one month at 25° C, the streak culture is tannish-white coloured and growth is butyrous, smooth with an entire margin.

Dalmau plate culture on potato agar : After one week at 25° C moderately well developed pseudohyphae are observed.

Two hat shaped ascospores per ascus are observed on YM-Agar.

Fermentation :

Glucose	+	Galactose	-	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compunds :

Galactose	-	Raffinose	-	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	-	Citric acid	+
Lactose	-	L-Rhamnose	-	Inositol	-
L-Sorbose	-	D-Arabinose	-	Salicin	+
Melibiose	-	D-glucosamine HCl	-	Pot.D-gluconate	+
Melezitose	+	Glucitol	+	DL-Lactic acid	+
Inulin	-	αmethyl D-glucoside	+		

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract : -

Growth in 10% NaCl plus 5% glucose in yeast nitrogen base : -

Growth at 37°C : +

PICHIA GUILLIERMONDII WICKERHAM

Figs 3.7a,b, 3.40

Description :

Growth in malt extract : After 3 days at 25°C the cells are ovoidal to elongate (2.1 - 4.9) x (3.1 - 7.1) μm; occur in single, paired and in short chains. After one month a ring is observed.

Growth on malt extract agar : After one month at 25°C the streak culture is tannish-white in colour, flat, smooth with lobate margin.

Dalmau plate culture on corn meal agar : After one week at 25° C well branched pseudohyphae bearing whorls of blastospores are observed.

One to four hat-shaped spores per ascus are observed on 5% malt extract agar.

Fermentation :

Glucose	+	Galactose	-	Sucrose	+
Maltose	-	Lactose	-	Raffinose	+

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-
L-Sorbose	+	D-Arabinose	+	Salicin	+
Melibiose	+	D-glucosamine HCl	+	Pot.D-gluconate	+
Inulin	-	αMethyl-D-glucoside	+		

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : +

Growth in 10% NaCl plus 5% glucose in yeast nitrogen base : -

Growth at 37°C : +

Genus : Saccharomyces Meyen ex Reess

General characters : Vegetative reproduction by multilateral budding; cells globose, ellipsoidal or cylindrical, pseudohyphae may be formed. One to four smooth walled ascospores per ascus-globose to short ellipsoidal. Fermentation - vigorous. Nitrate not assimilated.

Taxonomic treatment of Saccharomyces species from the Cochin backwater :

SACCHAROMYCES CEREVISIAE MEYEN EX HANSEN

Figs 3.8a,b, 3.41

Description :

Growth in malt extract : After 3 days at 25°C the cells are globose to subglobose (5.0 - 7.1) x (5.0 - 7.8) µm and ellipsoidal to cylindrical (3.1 - 7.1) x (4.5 - 8.5) µm ; occur in singly, in pairs and in short chain. After one month at 25°C a sediment and a ring are observed.

Growth on malt agar : After one month at 25° C the streak culture is butyrous, cream to slightly brownish, slightly raised and smooth with light striations..

Slide culture on corn meal agar : Rudimentary pseudohyphae are observed.

One to four globose ascospores per ascus are observed on acetate agar.

Fermentation :

Glucose	+	Galactose	-	Sucrose	+
Maltose	+	Lactose	-	Raffinose	+

Assimilation of carbon compounds

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	+	D-Xylose	-	D-Mannitol	-
Cellobiose	-	L-Arabinose	-	Succinic acid	-
Trehalose	-	D-Ribose	-	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-

Assimilation of nitrate : -

Growth in vitamin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : +

SACCHAROMYCES EXIGUUS REESS EX HANSEN

Figs 3.9a,b, 3.42

Description :

Growth in malt extract : After 3 days at 25°C the cells are subglobose to ellipsoidal (2.5 - 5.0) x (3.5 - 5.7) µm ; occur singly and in pairs. After one month, a sediment and a ring are present.

Growth on malt agar : After one month at 25° C the streak culture is butyrous, cream coloured, smooth, flat and spreading, glossy with entire margin.

Slide culture on corn meal agar : Pseudohyphae are not formed. One to four globose ascospores per ascus are observed on acetate agar.

Fermentation :

Glucose	+	Galactose	+	Sucrose	+
Maltose	-	Lactose	-	Raffinose	+

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	-	D-Xylose	-	D-Mannitol	-
Cellobiose	-	L-Arabinose	-	Succinic acid	-
Trehalose	+	D-Ribose	-	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

SACCHAROMYCES KLUYVERI PHAFF, MILLER ET SHIFRINE

Figs 3.10a,b, 3.43

Description :

Growth in malt extract : After 3 days at 25° C, the cells are globose to ellipsoidal (3.0 - 7.0) x (4.0 - 8.0) µm; occur in singly, in pairs and in clusters. After one month a sediment and a ring are present.

Growth on malt agar : After one month at 25° C the streak culture is cream coloured, flat and smooth, glossy to dull with lobate margin.

Slide culture on corn meal agar : Pseudohyphae absent. One to four globose ascospores per ascus are observed on acetate agar.

Fermentation :

Glucose	+	Galactose	+	Sucrose	+
Maltose	+W	Lactose	-	Raffinose	+

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	+	D-Xylose	-	D-Mannitol	-
Cellobiose	-	L-Arabinosen	-	Succinic acid	+
Trehalose	+	D-Ribose	-	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-

Assimilation of nitrate : -

Growth in vitamin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +

Division : Deuteromycotina

Order : Blastomycetes

Family : Cryptococcaceae

Budding yeast cells always present. Pseudomycelium, true mycelium and arthrospores may be formed. Cells hyaline, coloured due to carotenoid pigments. Dissimilation strictly oxidative or oxidative and fermentative.

Genus: Candida Berkhout

General characters : Reproduction by multilateral budding ; cells globose, ovoid, cylindrical to elongate in shape. Pseudomycelium absent, rudimentary or well developed ; true mycelium may be present. Extracellular polysaccharides may be formed. Fermentation absent or present.

Taxonomic treatment of Candida species from the Cochin backwater :

CANDIDA ALBICANS (ROBIN) BERKHOUT

Figs 3.11a,b, 3.44

Description ;

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are globose to short ovoid and long-oval to elongate, (2.9 - 7.1) x (3.1 - 8.5) µm ; occur singly, budding, in short chains and in clusters. After one month a ring and a sediment are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25° C the streak culture is cream coloured,

glistening waxy, soft and smooth ; margin with a mycelial border.

Dalmau plate culture on corn meal agar : Mycotorula type pseudomycelium present.

Fermentation :

Glucose	+	Galactose	+W	Sucrose	+
Maltose	+	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Melezitose	+	Ribitol	+S
L-Sorbose	+	Sol.starch	+	Galacitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	-	D-Glucitol	+
Cellobiose	-	D-Arabinose	-	Salicin	-
Trehalose	+	D-Ribose	-	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	-
Melibiose	-	Glycerol	-	Citric acid	-
Raffinose	-	Erythritol	-	Inositol	-

Splitting of arbutin : -

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth at 37°C : -

CANDIDA ATMOSPHERICA SANTA MARIA

Figs 3.12a,b, 3.45

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are globose, ovoid to cylindrical (3.0 - 5.0) x (4.26 - 6.3) μm ; occur singly, in pairs and in clusters. After one month a film and a ring are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C, the streak culture is cream coloured, smooth, raised and the border fringed with pseudohyphae.

Dalmau plate culture on corn meal agar : A pseudomycelium consisting branched chains of cells giving a wavy appearance and having a few blastospores.

Fermentation :

Glucose	+	Galactose	-	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Melezitose	+	Ribitol	+
L-Sorbose	+S	Sol.starch	-	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	+	D-Glucitol	+
Cellobiose	+	D-Arabinose	+	Salicin	+S
Trehalose	+	D-Ribose	+	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	+
Melibiose	-	Glycerol	+	Citric acid	+
Raffinose	-	Erythritol	+	Inositol	-

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth at 37°C : -

Urease : -

CANDIDA HALOPHILA YARROW ET MEYER

Figs 3.13a,b, 3.46

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are globose to subglobose (2.0 - 3.9) x (2.0 - 4.8) µm ; occur singly and budding. After one month a ring and a sediment are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C the streak culture is white, flat smooth and waxy in appearance with entire margin.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation :

Glucose	+	Galactose	+	Sucrose	+
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+S	Melezitose	-	Ribitol	-
L-Sorbose	-	Sol.starch	-	Galacititol	-
Sucrose	+S	D-Xylose	-	D-Mannitol	+
Maltose	-	L-Arabinose	+S	D-Glucitol	-
Cellobiose	+	D-Arabinose	-	Salicin	+
Trehalose	+S	D-Ribose	-	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	+S
Melibiose	-	Glycerol	+S	Citirc acid	+S
Raffinose	-	Erythritol	-	Inositol	-

Splitting of arbutin : -

Assimilation of nitrate : +

Growth in vitamin-free medium : -

Growth at 37°C : -

Urease : -

CANDIDA INTERMEDIA (CIFERRI ET ASHFORD) LANGEROM ET GUERRA

Figs 3.14a,b, 3.47

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are ovoid (2.5 - 5.0) x (4.2 - 6.9) µm ; occur in budding and in chains. After one month a pellicle is formed.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C the streak culture is cream coloured, dull, soft, smooth and margin fringed with pseudohyphae.

Dalmau plate culture on corn meal agar : The pseudomycelium consists of short pseudohyphae arranged in a tree like manner, bearing blastospores.

Fermentation :

Glucose	+	Galactose	+	Sucrose	+
Maltose	-	Lactose	-	Raffinose	+

Assimilation of carbon compounds :

Galactose	+	Melizitose	+	Ribitol	-
L-Sorbose	+	Sol.starch	+	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	-	D-Glucitol	+
Cellobiose	+	D-Arabinose	-	Salicin	+
Trehalose	+	D-Ribose	-	DL-Lactic acid	-
Lactose	+	L-Rhamnose	-	Succinic acid	-
Melibiose	-	Glycerol	-	Citirc acid	-
Raffinose	+	Erythritol	-	Inositol	-

Splitting arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast-extract agar : -

Growth at 37°C : +W

Urease : -

CANDIDA KRUSEI (CASTELLANI) BERKHOUT

Figs 3.15a,b, 3.48

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are ovoid, elongate and cylindrical (2.2 -5.5) x (4.3 - 8.2) µm ; occur in singly, budding and in chains. Pseudomycelium present. After one month at 25° C there is a dry creeping pellicle observed.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C the streak culture is off-white dull, soft, smooth with an irregular margin, fringed with pseudomycelium.

Dalmau plate culture on corn meal agar : Pseudomycelium well developed, consists of chains of elongated cells with frequent branching ; clusters and chains of blastospores occur along the pseudohyphae.

Fermentation :

Glucose	+	Galactose	-	Sucrose	-
maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	-	Melezitose	-	Ribitol	-
L-Sorbose	-	Sol.starch	-	Galactitol	-
Sucrose	-	D-Xylose	-	D-Mannitol	-
Maltose	-	L-Arabinose	-	D-Glucitol	-
Cellobiose	-	D-Arabinose	-	Salicin	-
Trehalose	-	D-Ribose	-	DL-Lactic acid	+
Lactose	-	L-Rhamnose	-	Succinic acid	+
Melibiose	-	Glycerol	+	Citric acid	+
Raffinose	-	Erythritol	-	Inositol	-

Splitting of arbutin : -

Assimilation of nitrate : -

Growth in vitamin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +

Urease : -

CANDIDA MEMBRANAEFACIENS (LODDER ET KREGER VAN RIJ) WICKERHAM ET BURTON

Figs 3.16a,b, 3.49

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are spherical to short ovoid (3.0 - 5.5) x (3.5 - 6.0) μm ; frequently occur in groups. After one month islets formed by clustering of cells are observed.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C the streak culture is cream coloured, dull glistening soft fringed with pseudomycelium.

Dalmau plate culture on corn meal agar : The pseudomycelium consists of ramified chains of long pseudohyphae bearing chains and clusters of roundish blastospores in verticillated positions ; the pseudohyphae may be terminally swollen, club like or swollen.

Fermentation :

Glucose	+	Galactose	-	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Melezitose	+	Ribitol	+
L-Sorbose	+	Sol.starch	-	Galactitol	+
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	+	D-Glucitol	+
Cellobiose	+	D-Arabinose	+	Salicin	+
Trehalose	+	D-Ribose	+	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	-
Melibiose	+	Glycecrol	+	Citric acid	-
Raffinose	+	Erythritol	+	Inositol	-
Inulin	+				

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : +S

Growth at 37°C : +

CANDIDA PARAPSILOSIS (ASHFORD) LANGERON ET TALICE

Figs 3.17a,b, 3.50

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are oval, elliptical and elongated (2.6 - 4.2) x (3.2 - 6.2) μm and occur singly, in pairs and in short chains and clusters. After one month a sediment and a ring are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25° C the streak culture is cream coloured, glistening smooth and soft, margin fringed with pseudo-mycelium.

Dalmau plate culture on corn meal agar : Pseudomycelium consists of branched chains of elongated cells with chains and clusters of round to oval blastospores forming at intervals along the hyphae.

Fermentation :

Glucose	+	Galactose	-	Sucrose	+W
Maltose	+W	Lactose	-	Raffinose	+

Assimilation of carbon compounds :

Galactose	+	Melezitose	-	Ribitol	+
L-Sorbose	+	Sol.starch	-	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	-	D-Glucitol	+
Cellobiose	-	D-Arabinose	-	Salicin	-
Trehalose	+	D-Ribose	+	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	-
Melibiose	-	Glycerol	+	Citric acid	-
Raffinose	-	Erythritol	-	Inositol	-

Splitting of arbutin : -

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : +

Growth at 37° C : +

Urease : -

<u>CANDIDA</u> <u>PSEUDOINTERMEDIA</u> NAKASE, KOMAGATA ET FUKAZAWA

Figs 3.18a,b, 3.51

Descriptions :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C cells are globose, short-oval to long oval (3.0 - 6.5) x (4.3 - 6.3) µm and occur singly and in pairs. After one month a sediment and pellicle are formed.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C the streak culture is white to yellowish dry, dull raised fringed with mycelium.

Dalmau plate culture on corn meal agar : pseudomycelium consists of tree like branched chains of pseudohyphae bearing ramified chains of blastospores in verticillate positions.

Fermentation:

Glucose	+	Galactose	+	Sucrose	+
Maltose	+W	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Melezitose	+	Ribitol	+
L-Sorbose	+	Sol.starch	+	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	-	D-Glucitol	+
Cellobiose	+	D-Arabinose	-	Salicin	+
Trehalose	+	D-Ribose	+	DL-Lactic acid	-
Lactose	-	L-Rhamnose	+S	Succinic acid	+
Melibiose	-	Glycerol	-	Citric acid	+

Raffinose	+	Erythritol	-	Inositol	-
Inulin	-				

Splitting of arbutin : +

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +

CANDIDA SAKE (SAITO ET OTA) VAN UDEN ET BUCKLEY

Figs 3.19a,b, 3.52

Description :

Growth on glucose-yeast extract-peptone water : After 3 days at 25 C the cells are globose to oval and long oval to cylindrical (2.8 - 4.5) x (4.2 - 7.5) µm ; they occur singly, in pairs and in short chains ; pseudomycelium observed. After one month a pellicle and sediment are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25° C the streak culture is cream coloured, glistening, soft, smooth, folded fringed with pseudomycelium.

Dalmau plate culture on corn meal agar : The pseudomycelium consists of branched chains of cells with a few blastospores to well developed pseudohyphae with many blastospores and long, curved cells with few blastospores giving wavy appearance.

Fermentation :

Glucose	+	Galactose	+S	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Melezitose	-	Ribitol	-
L-Sorbose	+	Sol.starch	-	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	-	D-Glucitol	+
Cellobiose	+	D-Arabinose	-	Salicin	+
Trehalose	+	D-Ribose	-	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	+
Melibiose	-	Glycerol	+	Citric acid	-
Raffinose	-	Erythritol	-	Inositol	-

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth at 37°C : -

CANDIDA SOLANI LODDER ET KREGER-VAN RIJ

Figs 3.20a,b, 3.53

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are short ovoid (2.0 - 4.3) x (2.8 - 5.3) μm ; occur singly, in pairs and in clusters. After one month at 25°C a pellicle is present.

Growth on glocose-yeast extract-peptone agar : After one month at 25°C the streak culture is greyish white, dull, soft margin fringed with pseudomycelia.

Dalmau plate culture on corn meal agar : The pseudomycelium consists of ramified chains of short pseudohyphae bearing chains of oval and cylindrical blastospores.

Fermentation :

Glucose	+	Galactose	-	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	-	Melezitose	+	Ribitol	-
L-Sorbose	+	Sol.starch	-	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	-
Maltose	+	L-Arabinose	-	D-Glucitol	-
Cellobiose	+	D-Arabinose	-	Salicin	+
Trehalose	+	D-Ribose	-	DL-Lactic acid	+
Lactose	-	L-Rhamnose	-	Succinic acid	+
Melibiose	-	Glycerol	+	Citric acid	-
Raffinose	-	Erythritol	-	Inositol	-

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth at 37°C : -

CANDIDA TROPICALIS (CASTELLANI) BERKHOUT

Figs 3.21a,b, 3.54

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C the cells are globose, short-ovoid to long ovoid (4.3 - 5.9) x (5.9 - 8.5) µm. After one month a sediment, a ring and islets are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25 C the streak culture is cream coloured, dull, soft, smooth with mycelial border.

Dalmau plate culture on corn meal agar : Pseudomycelium and true mycelium present. Pseudomycelium consists of long, branched pseudohyphae bearing blastospores singly,in short chains and clusters.

Fermentation :

Glucose	+	Galactose	+	Sucrose	+
Maltose	+	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Melezitose	-	Ribitol	+
L-Sorbose	+	Sol.starch	+	Galactitol	-
Sucrose	+	D-Xylose	+	D-Mannitol	+
Maltose	+	L-Arabinose	-	D-Glucitol	+
Cellobiose	-	D-Arabinose	-	Salicin	-
Trehalose	+	D-Ribose	-	DL-Lactic acid	-
Lactose	-	L-Rhamnose	-	Succinic acid	+
Melibiose	-	Glycerol	+	Citric acid	+
Raffinose	-	Erythritol	-	Inulin	-

Splitting of arbutin : +

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth at 37°C : +

Genus : Cryptococus Kutzing emend. Phaff et spencer

General characters : Reproduction by multilateral budding. Cells spheroidal ovoidal, elongate, amoeboid or polymorphic and capsulated. Carotenoid pigments may be produced ; pseudomycelium absent or rudimentary. Ring and sediment produced. Fermentation absent. Inositol assimilated.

Taxonomic treatment of Cryptococcus species from the Cochin backwater :

CRYPTOCOCCUS LAURENTII (KUFERATH) SKINNER

Figs 3.22a,b. 3.55

Description :

Growth in malt extract : After 3 days at 25° C the cells are spheroidal to ovoidal to elongate (2.0 - 5.3) x (3.0 - 6.9) µm ; occur singly, in pairs and in short chains. A thin ring and sediment are present. After one month thick slimy ring and a heavy sediment are found.

Growth on malt agar : After one month at 25°C the streak culture is cream coloured turning pinkish. The surface is smooth and glossy with slimy texture. The border is entire.

Slide culture on potato agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	+
Sucrose	+	Sol.starch	+	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	+
Lactose	+	L-Rhamnose	+	Inositol	+
Melibiose	+				
Sodium glucuronate	+				
Glucono-α-Lactose	+				

Assimilation of nitrate : -

Assimilation of nitrite : -

Growth in vitamin free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +

Starch formation : +

Genus : Geotrichum Link

General characters : The formation of branched septate hyphae breaking up into arthrospores. No loose budding cells and blastospores. Fermentation present or absent.

Taxonomic treatment of Geotrichum species from the Cochin backwater :

GEOTRICHUM CANDIDUM LINK EX PERS.

Figs 3.23a,b, 3.56

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25° C arthrospores (2.5 - 6.0) x (4.3 - 9.5) µm and true mycelium are present. Loose budding cells and blastospores are absent. After one month a thick folded pellicle and loose sediment are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25° C the streak culture is dull white with a brownish tinge, folded, powdery, folded border, fringed with mycelium.

Slide culture on potato agar : Arthroconidia in chain within primary filaments abundantly formed.

Fermentation :

Glucose	+	Galactose	-	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Raffinose	-	Erythritol	-
Sucrose	-	Sol.starch	-	Ribitol	+
Maltose	-	D-Xylose	+	D-Mannitol	+
Cellobiose	-	L-Arabinose	-	Succinic acid	-
Trehalose	-	D-Ribose	+	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-
Sorbitol	+				

Assimilation of nitrate : -

Growth in vitamin-free medium : +

Growth at 37° C : -

Urease : -

Genus : Rhodotorula Harrison

General characters : Reproduction by multilateral budding ; cells spheroidal, ovoidal to elongate. Red or yellow carotenoid pigments are produced, may be due to capsule formation ; dry or wrinkled. Inositol not assimilated. Fermentation absent.

Taxonomic treatment of Rhodotorula species from the Cochin backwater :

RHODOTORULA AURANTIACA (SAITO) LODDER

Figs 3.24a,b, 3.57

Description :

Growth in malt extract : After 3 days at 25°C the cells are elongate to cylindrical (3.0 - 4.5) x (6.0 - 8.5) μm ; occur singly and pairs. After one month an orange ring and sediment are present.

Growth on malt agar : After one month the streak culture at 25°C is orange to reddish, smooth, soft, semi-glossy, flat to low conves with an entire border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	-	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	-	L-Arabinose	-	Succinic acid	+
Trehalose	-	D-Ribose	-	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-
Melezitose	+	Melibiose	-		

Assimilation of nitrate : +

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +W

Starch formation : -

Urease : +

RHODOTORULA GLUTINIS (FRESENIUS) HARRISON

Figs 3.25a,b, 3.58

Description :

Growth in malt extract : After 3 days at 25°C the cells are ovoidal to globose (2.3 - 4.5) x (4.0 - 6.5) μm. A thin ring and a little sediment present. After one month salmon coloured ring and a heavy sediment present.

Growth on malt agar : After one month at 25° C the streak culture is salmon coloured. Surface is smooth, glossy, appearance with pasty texture, flat with entire border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	+	D-Xylose	+	D-Mannitol	-
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	-
Lactose	-	L-Rhamnose	+	Inositol	-
Melezitose	+	Melibiose	-		

Assimilation of nitrate : +

Growth in vitmin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : +

Starch formation : -

Urease : +

RHODOTORULA GRAMINIS DI MENNA

Figs 3.26a,b, 3.59

Description:

Growth in malt extract : After 3 days a t 25°C the cells are globose to ovoidal or elongate (2.5 - 5.0) x (4.0 - 6.25) µm ; occur in single, in pairs and in clusters. A thin ring and a little sediment are present.

Growth on malt agar : After one month at 25°C the streak culture is coral red, smooth, glossy, soft and flat with irregular border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	-
Maltose	-	D-Xylose	+W	D-Mannitol	-
Cellobiose	-	L-Arabinose	-	Succinic acid	+
Trehalose	+	D-Ribose	-	Citric acid	+W
Lactose	-	L-Rhamnose	-	Inositol	-
Melezitose	-	Melibiose	-		

Assimilation of nitrate : +

Growth in vitamin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

Starch formation : -

Urease : +

RHODOTORULA LACTOSA HASEGAWA

Figs 3.27a,b, 3.60

Description :

Growth in malt extract : After 3 days at 25°C the cells are short ovoidal to long ovoidal (3.0 - 4.0) x (4.5 - 5.7) µm ; occur singly, in pairs and in budding. There is a thin ring and a little sediment. Growth is slow. After one month there is a thin ring and a sediment.

Growth on malt agar : Larger cells than in liquid malt extract. After one montn at 25°C the streak culture is pink, smooth, highly glossy, soft, convex and little spreading with entire border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	-	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	+S
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+S	D-Ribose	-	Citric acid	+S
Lactose	-	L-Rhamnose	+	Inositol	-
Melezitose	+	Melebiose	+		

Assimilation of nitrate : +

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

Starch formation : -

Urease : +

RHODOTORULA MINUTA (SAITO) HARRISON

Figs 3.28a,b, 3.61

Description :

Growth in malt extract : After 3 days at 25°C the cells are ovoidal to globose (2.3 - 4.2) x (3.5 - 5.9) µm ; occur singly and in pairs. There is a thin pink ring and a little sediment. After one month orange to red ring and a light sediment are present.

Growth on malt agar : After one month at 25 C the streak culture is pink, smooth, glossy, soft and the cross section flat to low convex with the entire border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	-	Erythritol	+
Sucrose	-	Sol.starch	-	Ribitol	+
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	-	L-Arabinose	+	Succinic acid	+W
Trehalose	+	D-Ribose	+	Citric acid	-
Lactose	+	L-Rhamnose	-	Inositol	-
Melezitose	+	Melibiose	-		

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

Starch formation : -

Urease : +

RHODOTORULA RUBRA (DEMME) LODDER

Figs 3.29a,b, 3.62

Description :

Growth in malt extract : After 3 days at 25°C cells vary from short ovoidal to elongate (2.0 - 5.5) x (2.5 - 6.5) μm and often longer (4.5 - 9.5) μm. There is an incomplete ring and little sediment. After one month there is a pink coloured ring and a mode sediment are present.

Growth on malt agar : After one month at 25°C the streak culture is deep coral to pink coloured, glistening, smooth, soft, low convex to flat with entire border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	-	Ribitol	+
Maltose	-	D-Xylose	+	D-Mannitol	-
Cellobiose	-	L-Arabinose	-	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	-
Lactose	-	L-Rhamnose	+	Inositol	-
Melezitose	-	Melibiose	-		

Assimilation of nitrate : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

Starch formation : -

Urease : +

Genus : Trichosporon Behrend

General characters : Pseudomycelium well developed or reduced. Budding cells of various shapes. Mycelium (septate)and arthrospores present. Ring and pellicle may be formed. Asexual endospores may be formed. Fermentation may be present or absent.

Taxonomic treatment of Trichosporon species from the Cochin backwater :

TRICHOSPORON AQUATILE HEDRICK ET DUPONT

Figs 3.30a,b, 3.63

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C branched septate hyphae are present and many arthrospores of all sizes. A white dry pellicle and some loose sediment are formed. After one month a dry pellicle and a loose sediment are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25°C the streak culture is white dry, folded tough and fringed with mycelium.

Slide culture on potato agar : Septate, branched hyphae abundant arthrospores of various lengths are formed. Aerial mycelium present.

Ferementation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	-	Erythritol	+
Sucrose	+	Sol.starch	+	Ribitol	-
Maltose	+	D-Xylose	+	D-Mannitol	-
Cellobiose	+	L-Arabinose	+	Succinic acid	+
Trehalose	+	D-Ribose	+	Citric acid	+W
Lactose	+	L-Rhamnose	-	Inositol	-

Assimilation of nitrate : -

Assimilation of nitrite : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : +

Growth at 37°C : -

Urease : +

TRICHOSPORON CUTANEUM (DE BEURM., GOUGEROT ET VAUCHER) OTA

Figs 3.31a,b, 3.64

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25°C true mycelium and arhtrospores of various sizes are present. Budding cells are scarce. The yeast cells are spheroidal to oval (3.5 - 5.5) x (3.5 - 7.0) µm. After one month a pellicle and sediment are present.

Growth on glucose-yeast extract-peptone agar : After one month at 25° C the streak culture is white to yellowixh, smooth, folded dull, moist slimy with tough texture and entire border fringed with mycelium.

Slide culture on corn meal agar : The mycelium abundant; arhtrospores of variable sizes abundant.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	+
Sucrose	+	Sol.starch	+	Ribitol	-
Maltose	+	D-Xylose	+	D-Mannitol	-
Cellobiose	+	L-Arabinose	+	Succinic acid	-
Trehalose	+	D-Ribose	+	Citric acid	-
Lactose	+	L-Rhamnose	+	Inositol	+

Splitting of arbutin : -

Assimilation of nitrate : -

Assimilation of nitrite : -

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : +W

Growth at 37°C : +

Urease : +

<u>TRICHOSPORON</u> <u>PENICILLATUM</u> (DO CARMO-SOUSA) VON ARK.

Figs 3.32a,b, 3.65

Description :

Growth in glucose-yeast extract-peptone water : After 3 days at 25° C abundant truemycelium and arthrospores are present. A thin dry dull pellicle is formed. The yeast cells are (3.0 - 4.1) x (4.2 - 9.5) μm.

Growth on glucose-yeast extact-peptone agar : After one month at 25°C the streak culture is white to whitish cream coloured, glossy of filamentous texture, raised with an entire border fringed with mycelium.

Dalmau plate culture on corn meal agar : Abundant true mycelium and arthrospores are formed.

Fermentation :

Glucose	+W	Galactose	+W	Sucrose	-
Maltose	-	Lactose	-	Raffinose	-

Assimilation of carbon compounds :

Galactose	+	Raffinose	-	Erythritol	-
Sucrose	-	Sol.starch	-	Ribitol	-
Maltose	-	D-Xylose	+	D-Mannitol	-
Cellobiose	-	L-Arabinose	-	Succinic acid	-
Trehalose	-	D-Ribose	-	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-

Assimilation of nitrate : -

Assimilation of nitrite : -

Growth in vitamin-free medium : +

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

Starch formation : -

Urease : -

Division : Deuteromycotina

Order : Blastomycetes

Family : Sporobolomycetaceae

Sporobolomycetaceae

Mycelium, pseudomycelium and budding yeast cells; vegetative reproduction by fission and budding. The vegetative cells may form aerial sterigmata, single or bifurcated. Ballistospores may form, dissimilation strictly oxidative.

Genus : Sporobolomyces Kluyver et van Niel

General characters : Pink, red or orange coloured colonies on soild media, vegetative reproduction by budding, by pseudomycelium and true mycelium and by ballistospores. Fermentation absent ; urease positive.

Taxonomic treatment of Sporobolomyces species from the Cochin backwater :

SPOROBOLOMYCES ROSEUS KLUYVER ET VAN NIEL

Figs 3.33a,b, 3.66

Description :

Growth in malt extract : After 3 days at 25°C the cells are ovoidal to elongate (3.0 - 6.5) x (5.0 - 7.5) µm; occur singly and in pairs. There is a thin ring and a light

sediment. After one month there is a thin ring and heavy sediment.

Growth on malt agar : After one month at 25°C the streak culture is jasper pink, smooth, glossy, soft flat to raised with entire border.

Dalmau plate culture on corn meal agar : Pseudomycelium absent.

Fermentation : -

Assimilation of carbon compounds :

Galactose	+	Raffinose	+	Erythritol	-
Sucrose	+	Sol.starch	+	Ribitol	-
Maltose	+	D-Xylose	+	D-Mannitol	+
Cellobiose	-	L-Arabinose	-	Succinic acid	-
Trehalose	+	D-Ribose	-	Citric acid	-
Lactose	-	L-Rhamnose	-	Inositol	-
Melezitose	-	Melibiose	-		

Assimilation of nitrate : +

Growth in vitamin-free medium : -

Growth on 50% (W/W) glucose-yeast extract agar : -

Growth at 37°C : -

Starch form tion : -

Urease : +

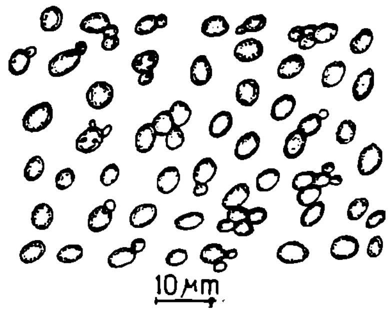

Fig. 3.1a *Debaryomyes hansenii*
After 3 days in malt extract.

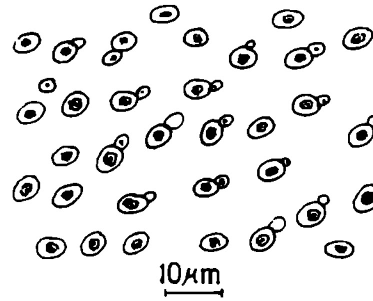

Fig. 3.1b *Debaryomyces hansenii*
After 15 days on Gorodkowa – agar at 25°C.

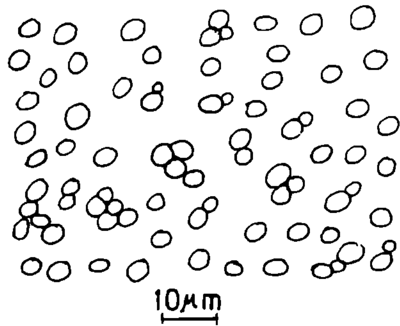

Fig. 3.2a *Debaryomyces marama*
After 3 days in malt extract.

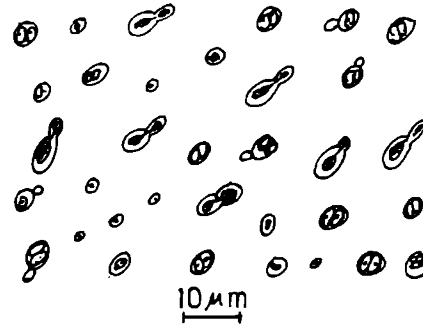

Fig. 3.2b *Debaryomyces marama*
After 15 days on malt agar (25°C)

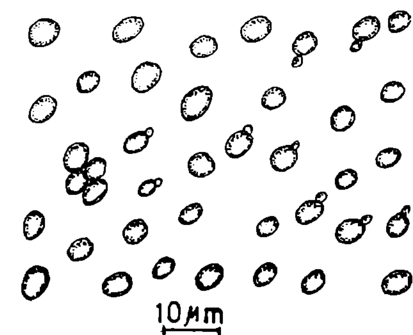

Fig. 3.3a *Debaryomyces vanriji*
After 3 days in malt extract

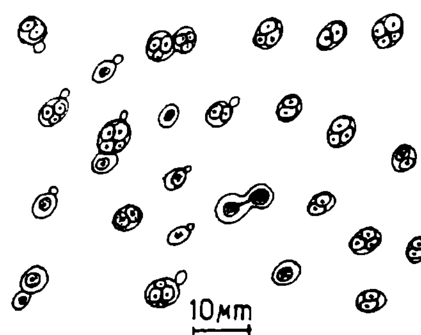

Fig. 3.3b *Debaryomyces vanriji*
After 15 days on malt agar at 25°C

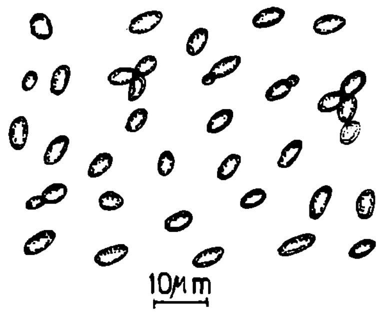

Fig.3.4a Hansenula anomala
After 3 days in malt extract

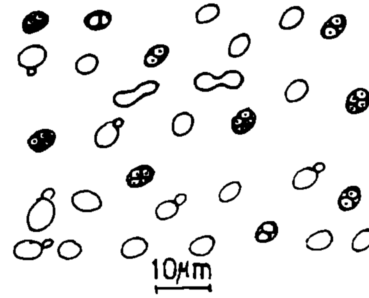

Fig.3.4b Hansenula anomala
After 15 days on malt extract agar

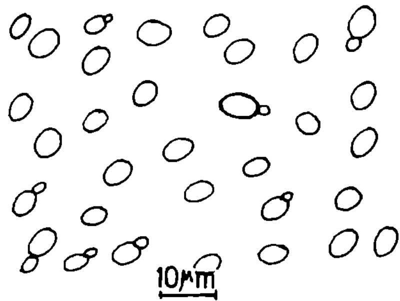

Fig.3.5a Kluyveromyces marxianus var. drosophilarum
After 3 days in malt extract

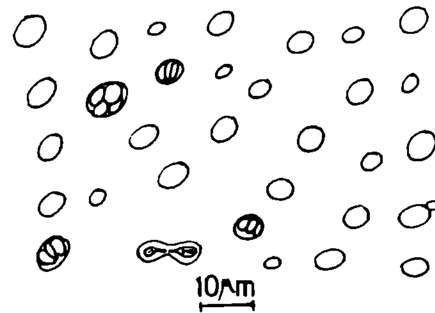

Fig. 3.5b Kluyveromyces marxianus var. drosophilarum
After 15 days on malt extract agar

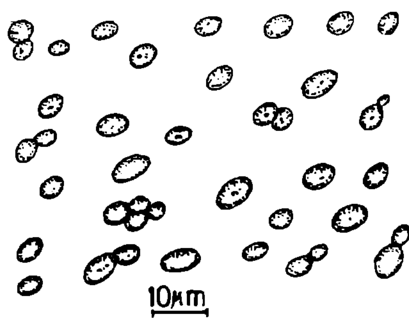

Fig.3.6a Pichia bovis
After 3 days in malt extract

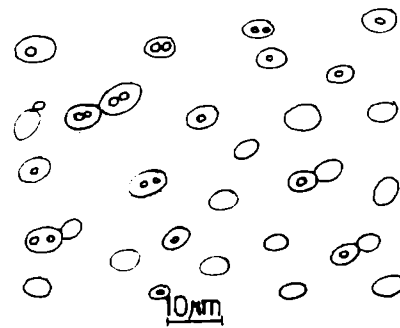

Fig. 3.6b Pichia bovis
After 7 days on malt extract agar

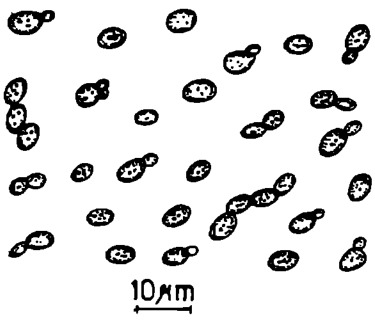

Fig. 3.7a *Pichia guilliermondli*
After 3 days in malt extract

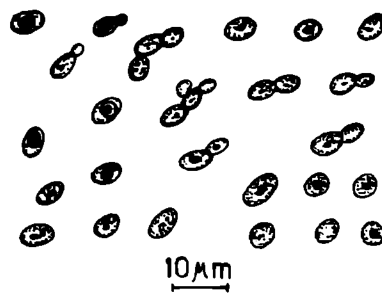

Fig. 3.7b *Pichia guilliermondii*
After 15 days on malt extract agar

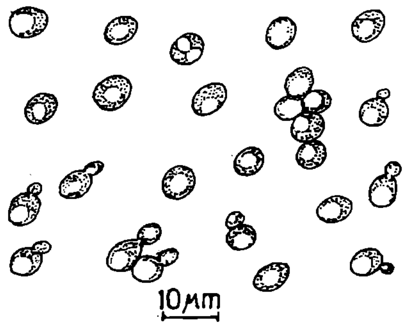

Fig. 3.8a *Saccharomyces cerevisiae*
After 3 days in malt extract

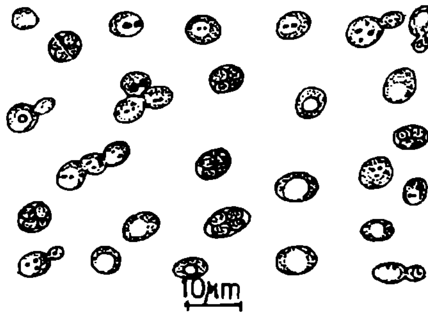

Fig. 3.8b *Saccharomyces cerevisiae*
After 15 days on Gorodkowa agar

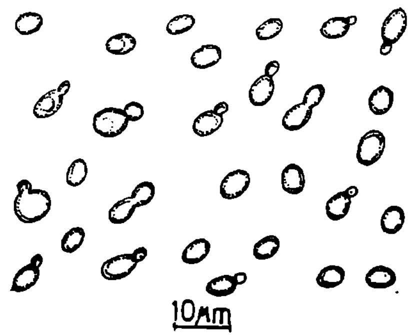

Fig. 3.9a *Saccharomyces exiguus*
After 3 days in malt extract

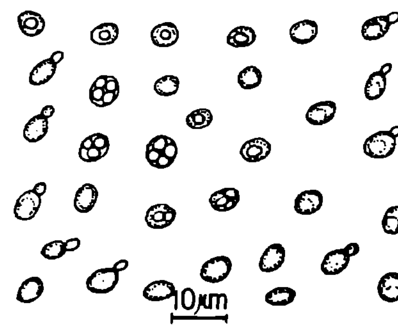

Fig. 3.9b *Saccharomyces exiguus*
After 15 days on malt extract agar

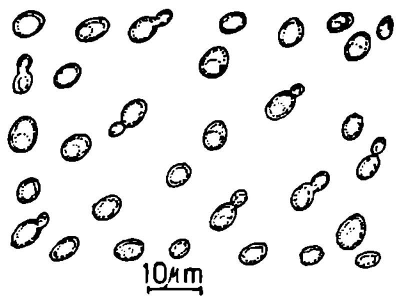

Fig. 3.10a *Saccharomyces kluyveri*
After 3 days in malt extract

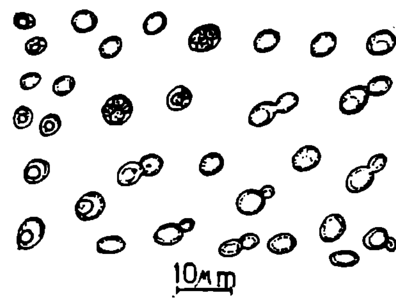

Fig. 3.10b *Saccharomyces kluyveri*
After 15 days on malt extract agar

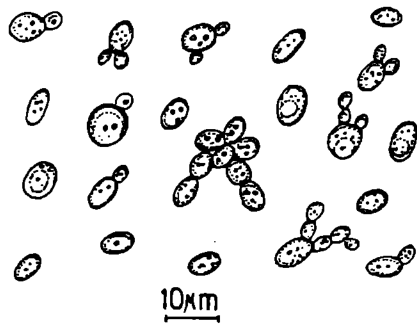

Fig. 3.11a *Candida albicans*
In glucose-yeast extract-peptone-water (after 3 days)

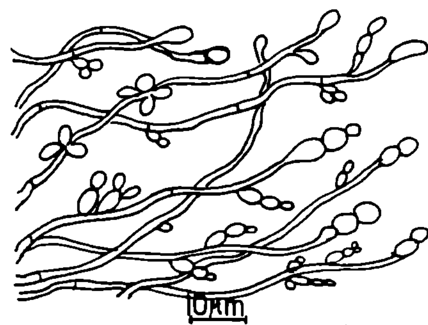

Fig. 3.11b *Candida albicans*
After 15 days on glucose-yeast extract-peptone agar

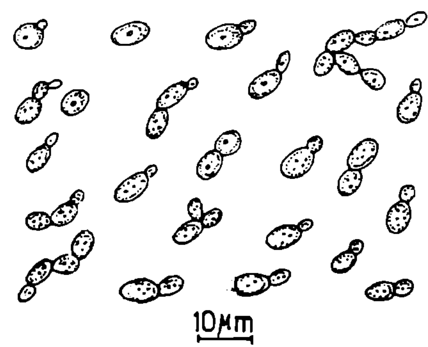

Fig. 3.12a *Candida atmospherica*
In glucose - yeast extract - peptone water (after 3 days)

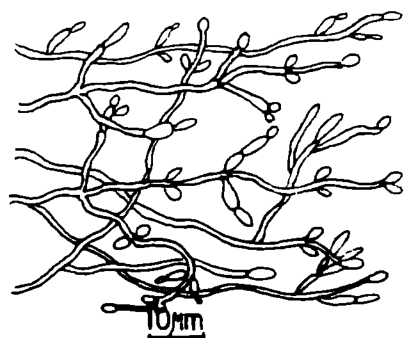

Fig. 3.12b *Candida atmospherica*
After 15 days on glucose-yeast extract-peptone agar

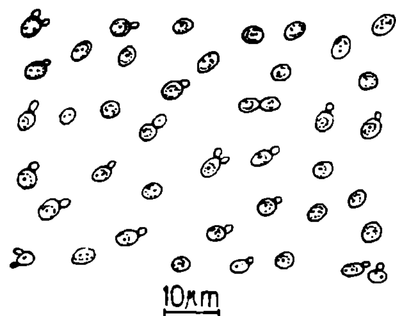

Fig. 3.13a *Candida halophila*
In glucose-yeast extract-peptone-water (after 3 days)

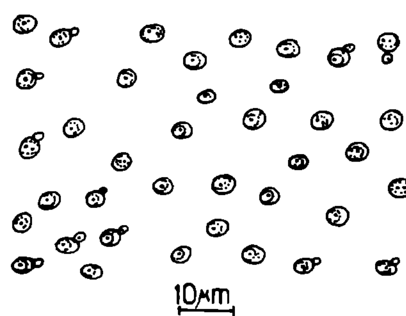

Fig. 3.13b *Candida halophila*
After 15 days on glucose-yeast extract-peptone agar

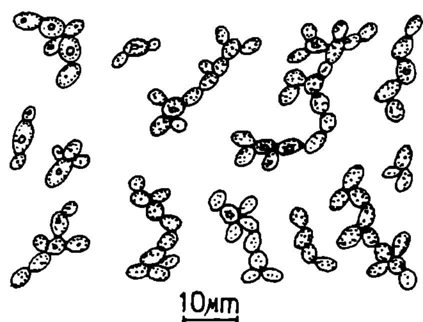

Fig.3.14a *Candida intermedia*
In glucose-yeast extract-peptone-water (after 3 days)

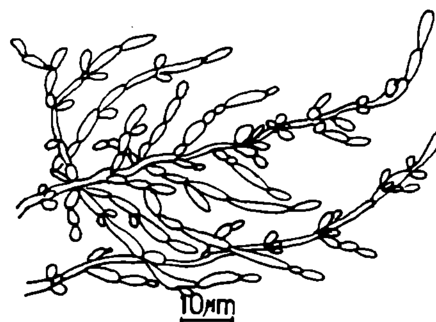

Fig.3 14b *Candida intermedia*
After 15 days on glucose-yeast extract-peptone agar

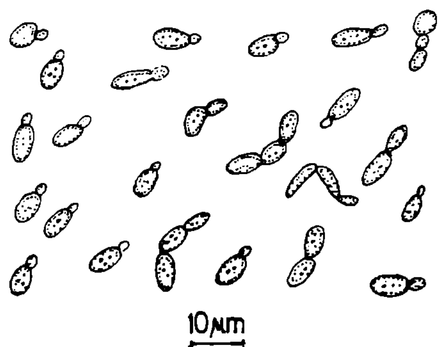

Fig.3.15a *Candida krusei*
In glucose-yeast extract-peptone-water (after 3 days)

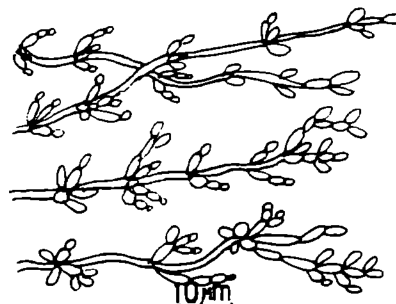

Fig.3.15b *Candida krusei*
After 15 days on glucose-yeast extract-peptone agar

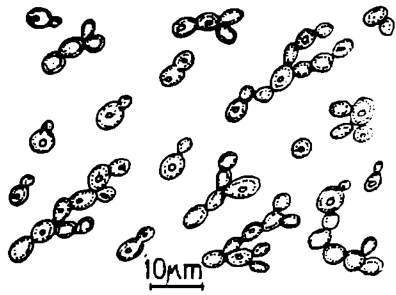

Fig. 3.16a Candida membranaefaciens
In glucose-yeast extract-peptone water
(after 3 days)

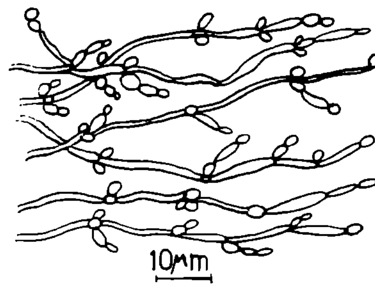

Fig.3.16b Candida membranaefaciens
After 7 days on glucose-yeast extract-
peptone agar

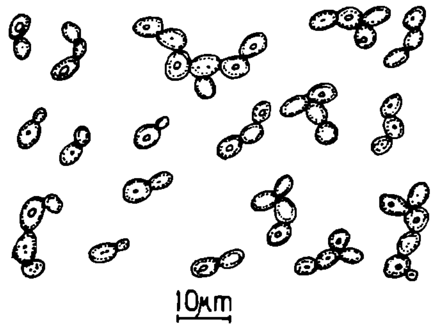

Fig. 3.17a Candida parapsilosis
In glucose-yeast extract-peptone-
water (after 3 days)

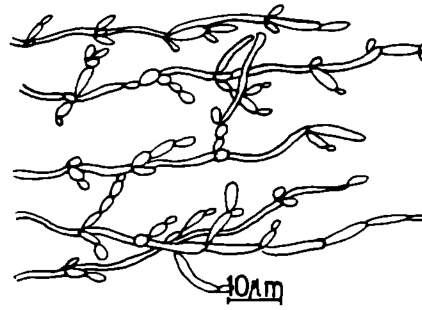

Fig. 3.17b Candida parapsilosis
After 15 days on glucose-yeast extract-
peptone agar

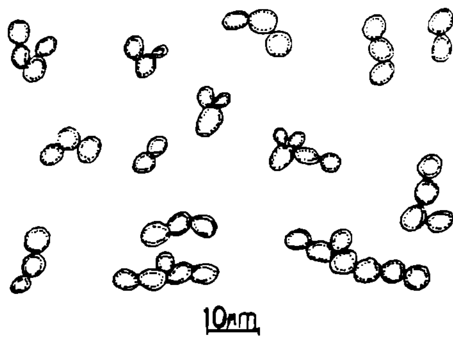

Fig. 3.18a Candida pseudointermedia
In glucose-yeast extract-peptone-water
(after 3 days)

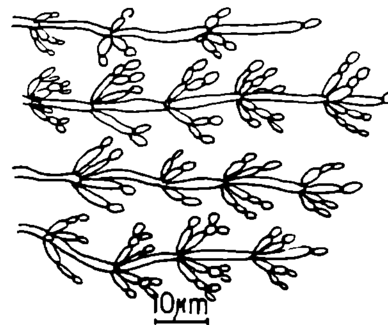

Fig. 3.18b Candida pseudointermedia
After 7 days on glucose-yeast extract-
peptone agar

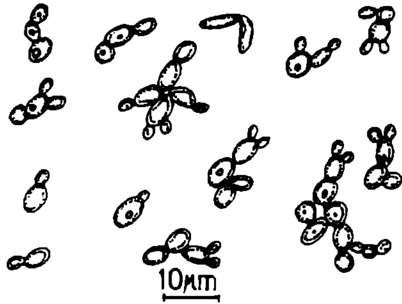

Fig.3 19a *Candida sake*
In glucose-yeast extract-peptone-water (after 3 days)

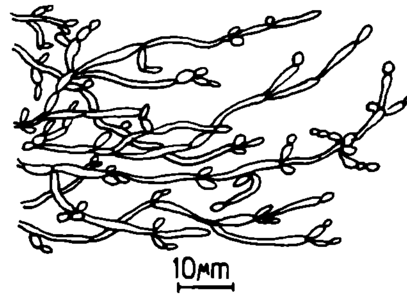

Fig.3 19b *Candida sake*
After 7 days on glucose-yeast extract-peptone agar

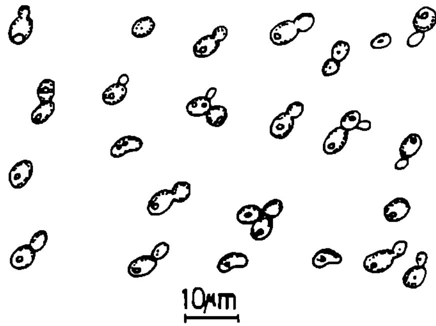

Fig.3.20a *Candida solani*
In glucose-yeast extract-peptone-water (after 3 days)

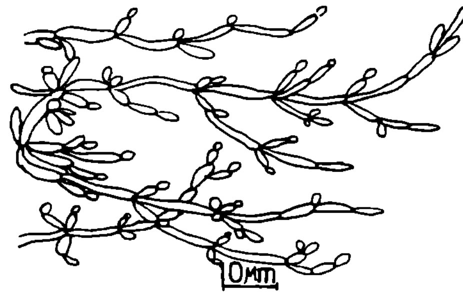

Fig.3.20b *Candida solani*
After 7 days on glucose-yeast extract-peptone agar

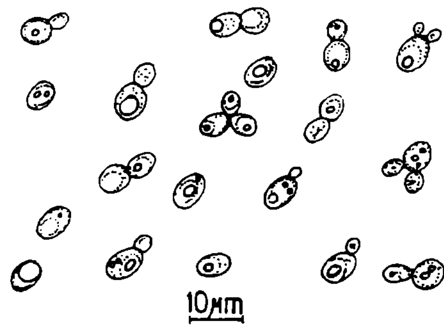

Fig. 3.21a *Candida tropicalis*
In glucose-yeast extract-peptone-water (after 3 days)

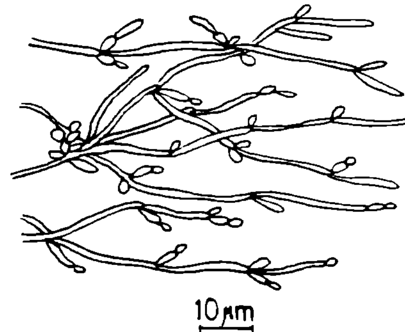

Fig. 3.21b *Candida tropicalis*
After 7 days on glucose-yeast extract-peptone agar

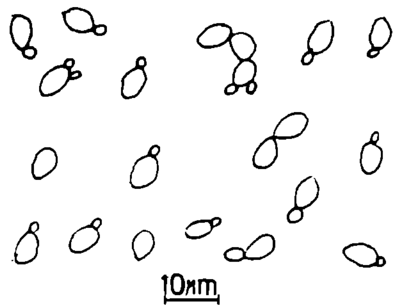

Fig. 3.22a *Cryptococcus laurentii*
After 3 days in malt extract

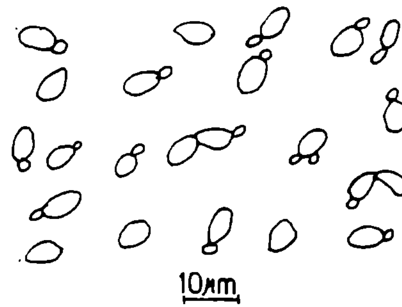

Fig. 3.22b *Cryptococcus laurentii*
After 7 days on malt extract agar

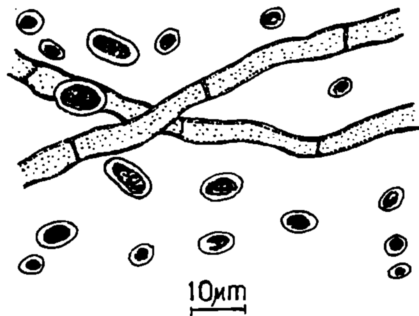

Fig. 3.23a *Geotrichum candidum*
After 3 days in glucose-yeast extract-peptone water

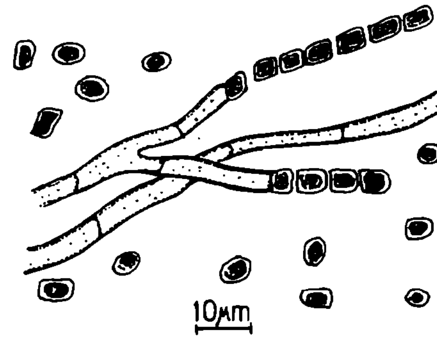

Fig. 3.23b *Geotrichum candidum*
After 7 days on glucose-yeast extract-peptone agar

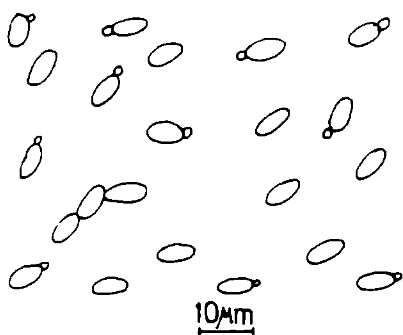

Fig. 3.24a *Rhodotorula aurantiaca*
After 3 days in malt extract

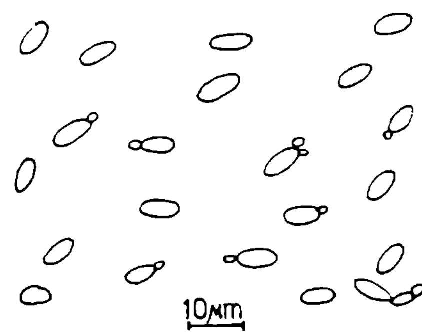

Fig. 3.24b *Rhodotorula aurantiaca*
After 7 days on malt agar

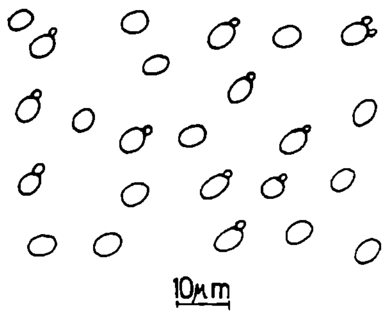

Fig. 3.25a *Rhodotorula glutinis*
After 3 days in malt extract

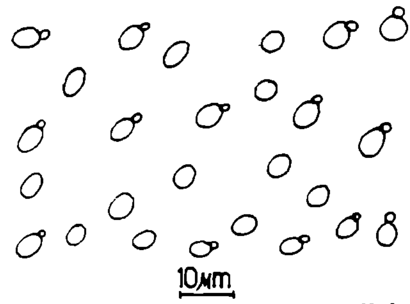

Fig. 3.25b *Rhodotorula glutinis*
After 7 days on malt agar

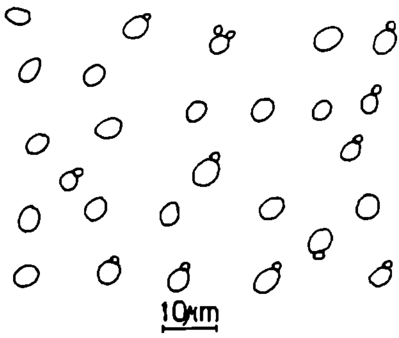

Fig. 3.26a *Rhodotorula graminis*
After 3 days in malt extract

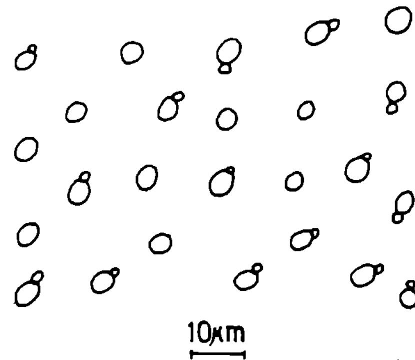

Fig. 3.26b *Rhodotorula graminis*
After 15 days on malt agar

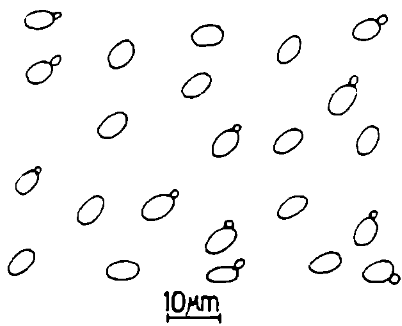

Fig. 3.27a *Rhodotorula lactosa*
After 3 days in malt extract

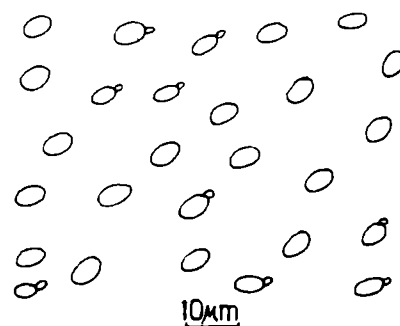

Fig. 3.27b *Rhodotorula lactosa*
After 7 days on malt agar

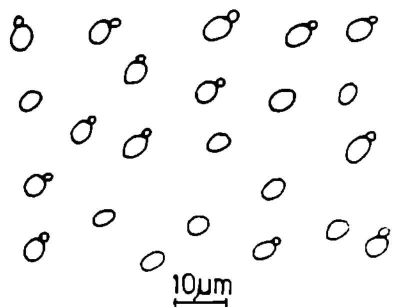

Fig. 3.28a Rhodotorula minuta
After 3 days in malt extract

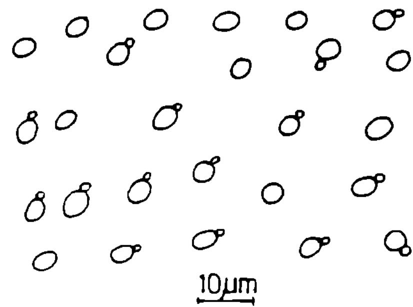

Fig. 3.28b Rhodotorula minuta
After 7 days on malt agar

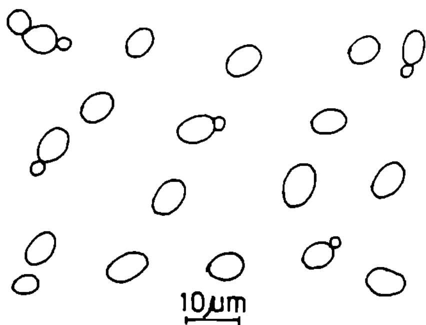

Fig. 3.29a Rhodotorula rubra
After 3 days in malt extract

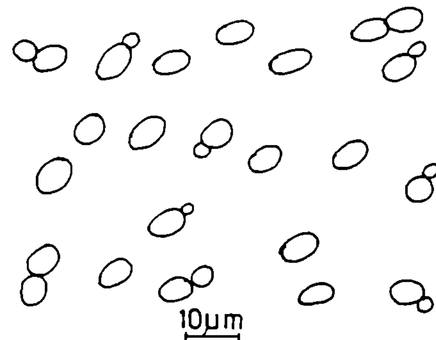

Fig. 3.29b Rhodotorula rubra
After 7 days on malt agar

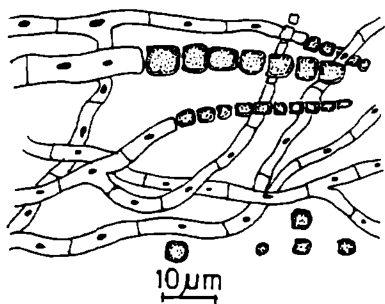

Fig. 3.30a Trichosporon aquatile
After 3 days in glucose-yeast extract-peptone water

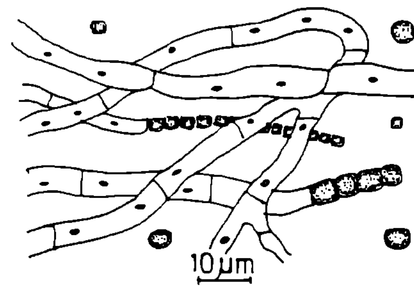

Fig. 3.30b Trichosporon aquatile
After 7 days on glucose-yeast extract-peptone agar

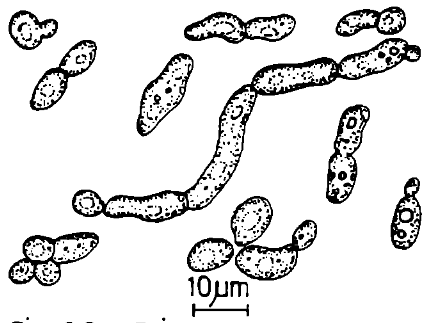

Fig. 3·31a Trichosporon cutaneum
After 3 days in glucose-yeast extract peptone water

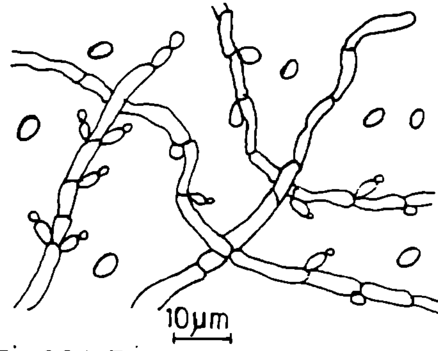

Fig. 3·31b Trichosporon cutaneum
After 15 days on glucose-yeast extract-peptone agar

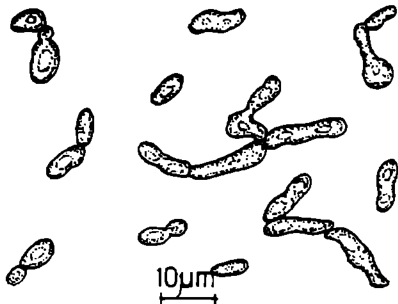

Fig. 3 32a Trichosporon penicillatum
After 3 days in glucose-yeast extract-peptone water

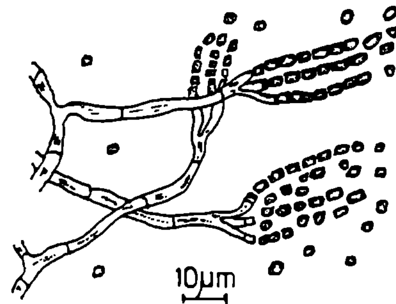

Fig. 3 32b Trichosporon penicillatum
After 7 days on corn meal agar

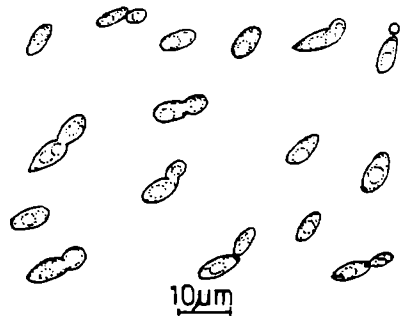

Fig. 3.33a Sporobolomyces roseus
After 3 days in malt extract

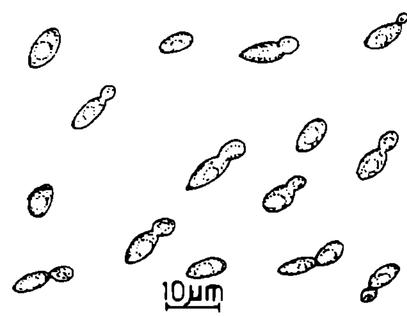

Fig. 3·33b Sporobolomyces roseus
After 7 days on malt agar

Fig. 3.34 Debaryomyces hansenii

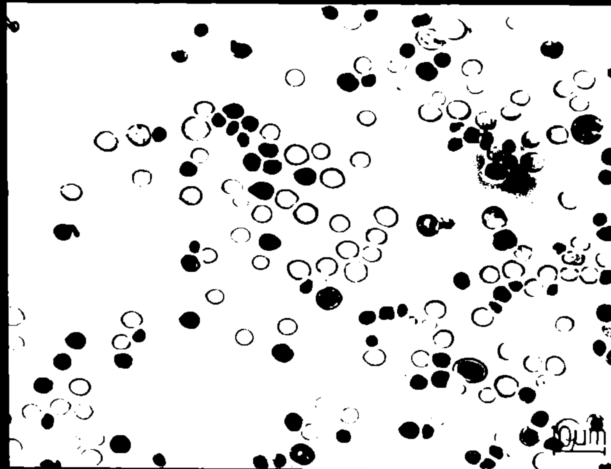

Fig. 3.35 Debaryomyces marama

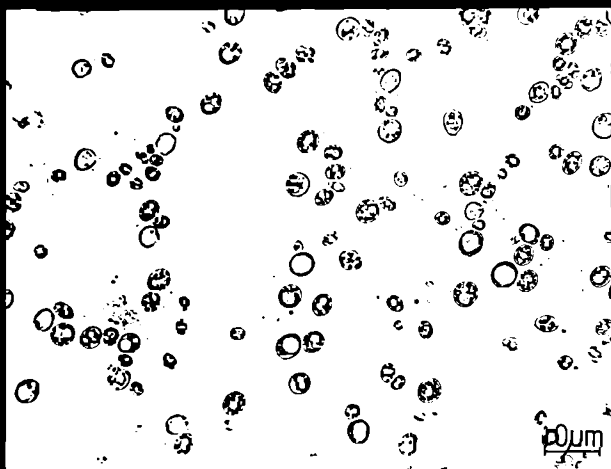

Fig. 3.36 Debaryomyces vanriji

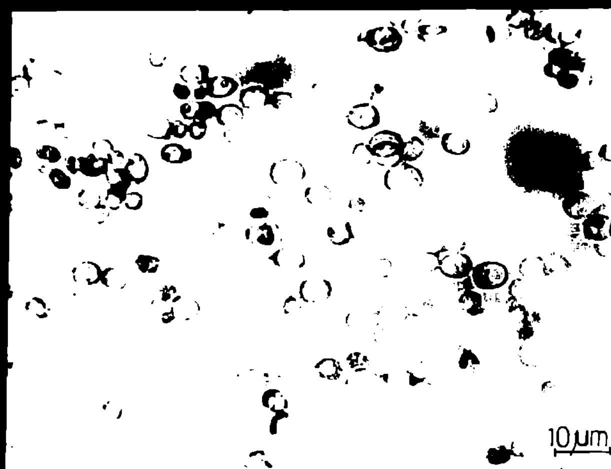

Fig. 3.37 Hansenula anomala

Fig. 3.38 Kluyveromyces marxianus

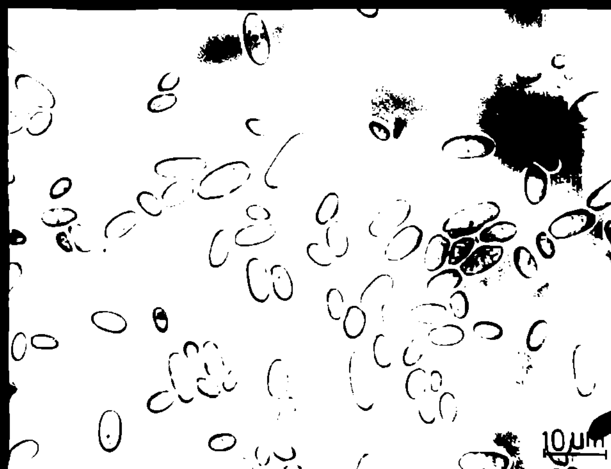

Fig. 3.39 Pichia bovis

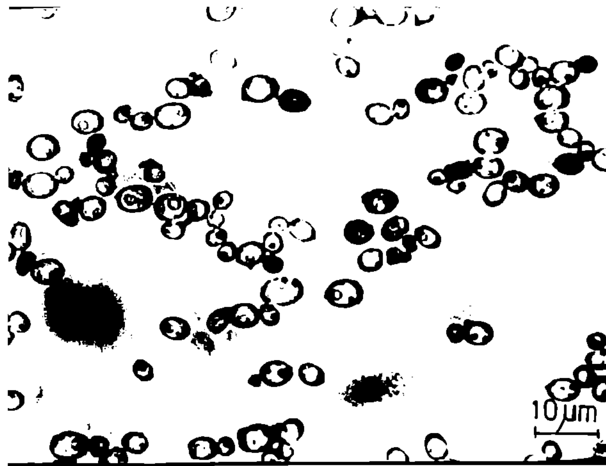

Fig. 3.40 *Pichia guilliermondii*

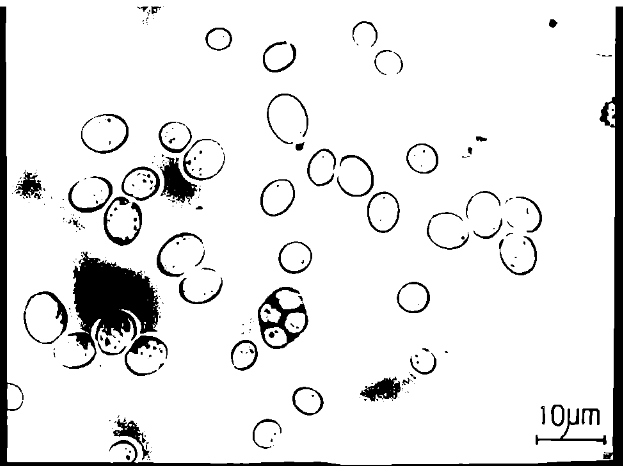

Fig. 3.41 *Saccharomyces cerevisiae*

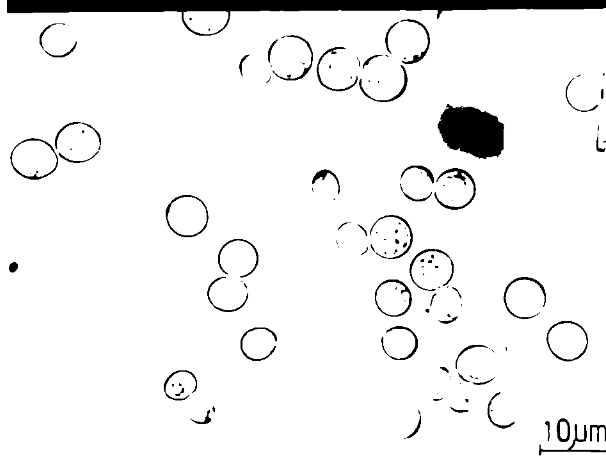

Fig. 3.42 *Saccharomyces exiguus*

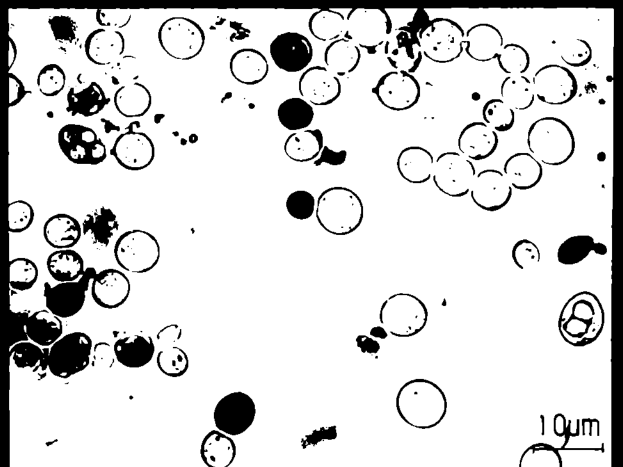

Fig. 3.43 *Saccharomyces kluyveri*

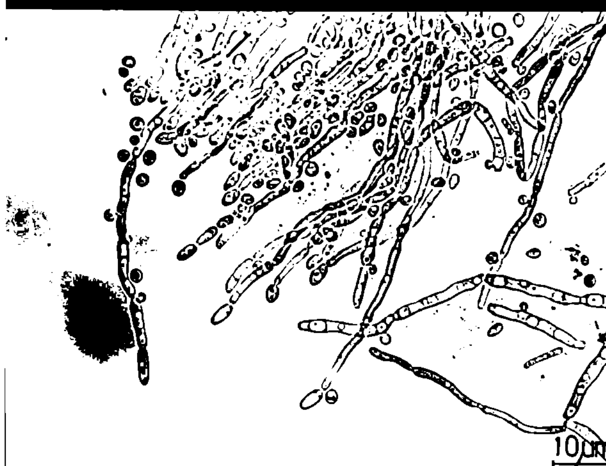

Fig. 3.44 *Candida albicans*

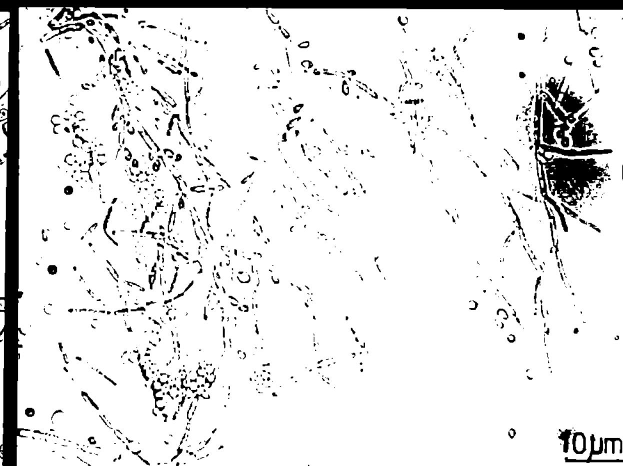

Fig. 3.45 *Candida atmospherica*

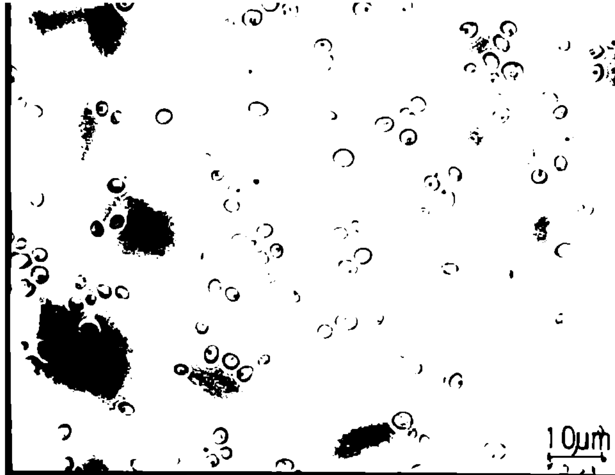

Fig. 3.46 Candida halophila

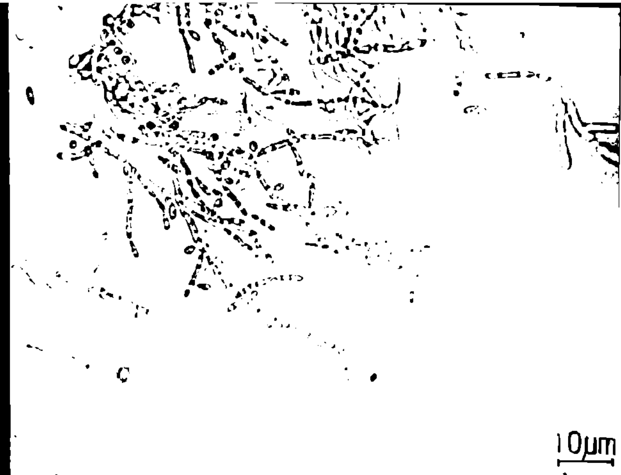

Fig. 3.47 Candida intermedia

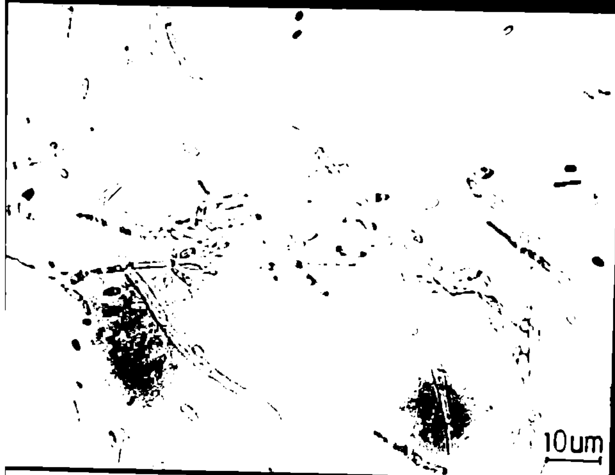

Fig. 3.48 Candida krusei

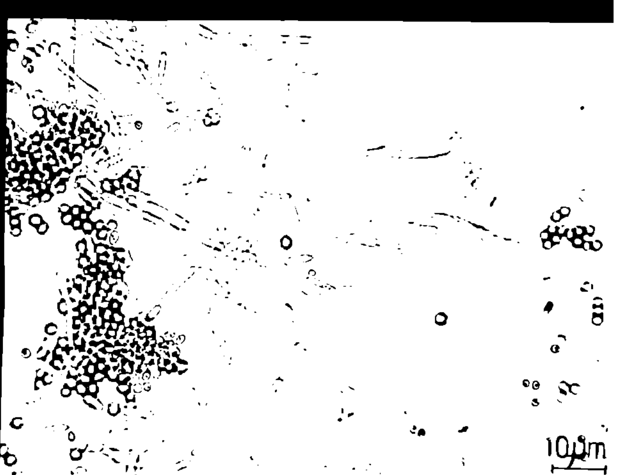

Fig. 3.49 Candida membranaefaciens

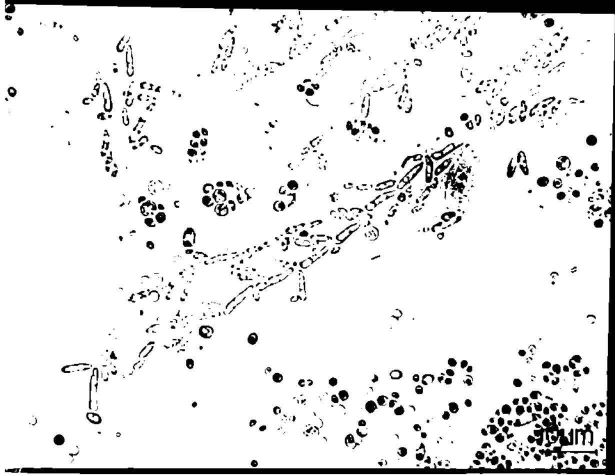
Fig. 3.50 Candida parapsilosis

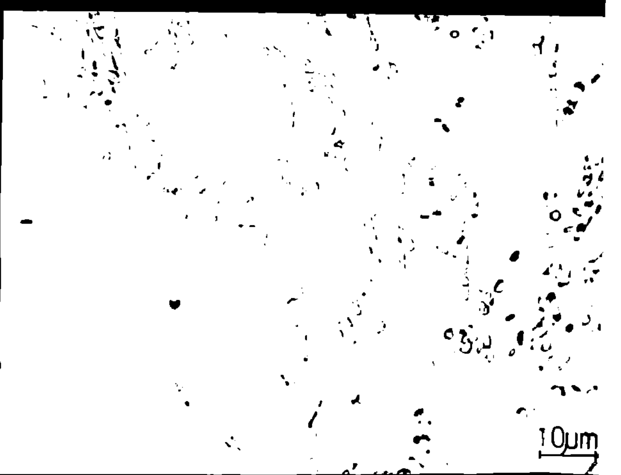

Fig. 3.51 Candida pseudointermedia

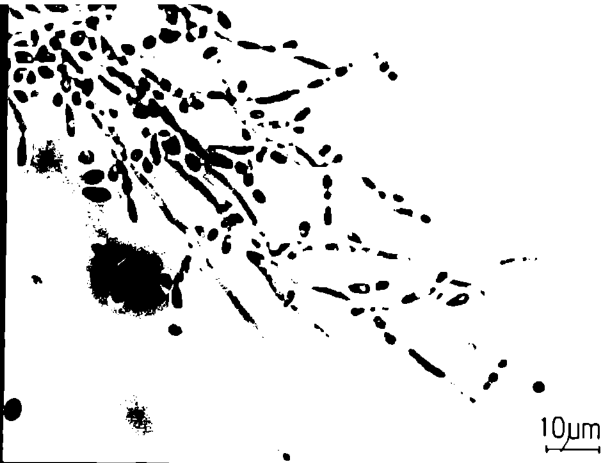

Fig. 3.52 *Candida sake*

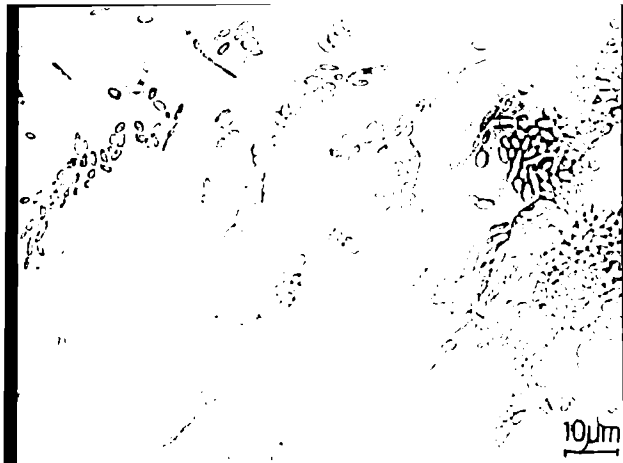

Fig. 3.53 *Candida solani*

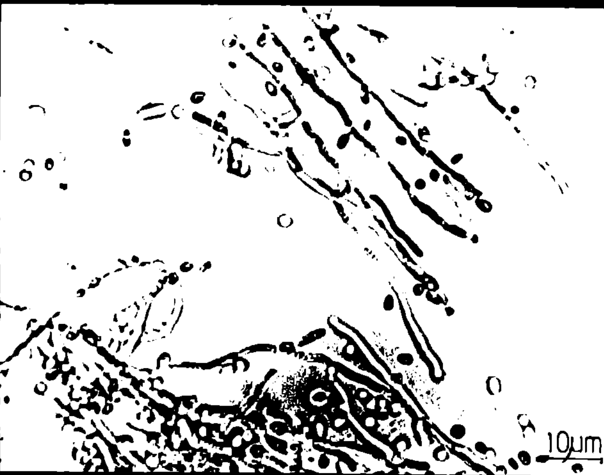

Fig. 3.54 *Candida tropicalis*

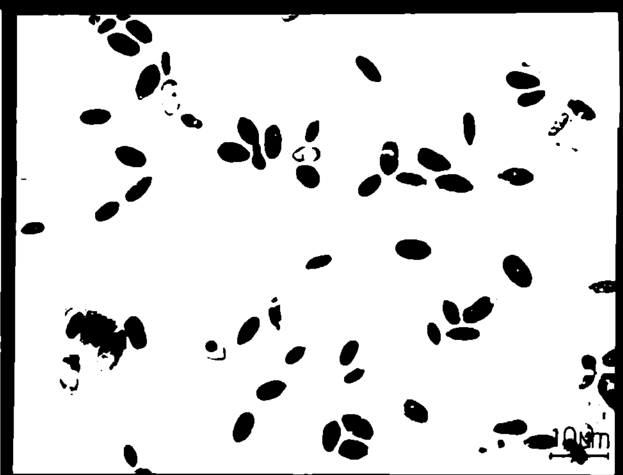

Fig. 3.55 *Cryptococcus laurentii*

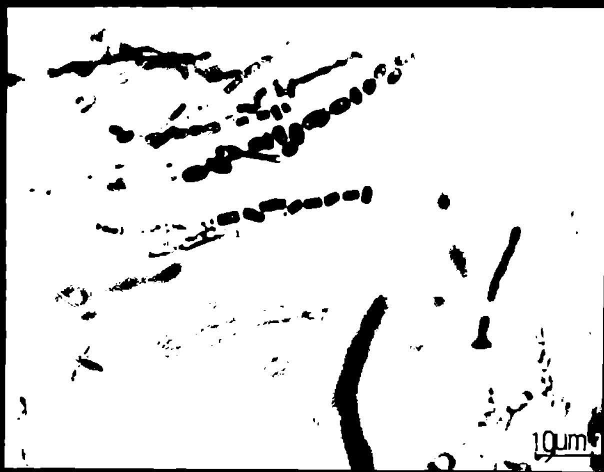

Fig. 3.56 *Geotrichum candidum*

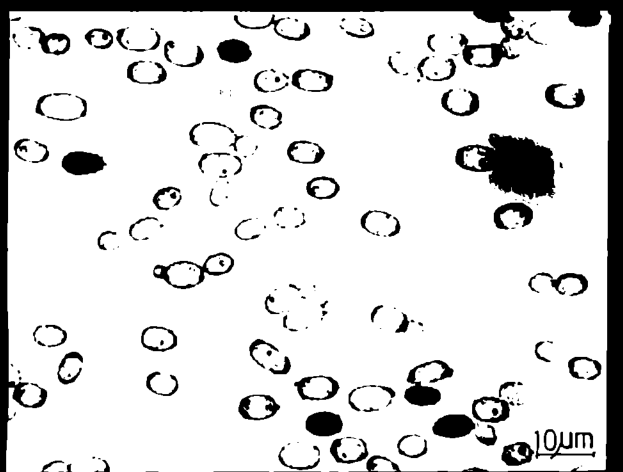

Fig. 3.57 *Rhodotorula aurantiaca*

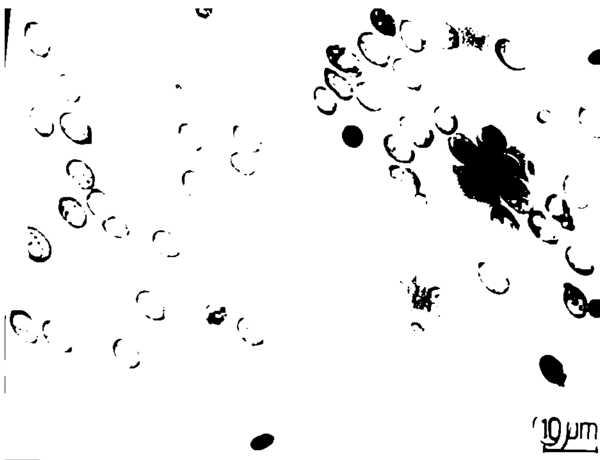

Fig. 3.58 Rhodotorula glutinis

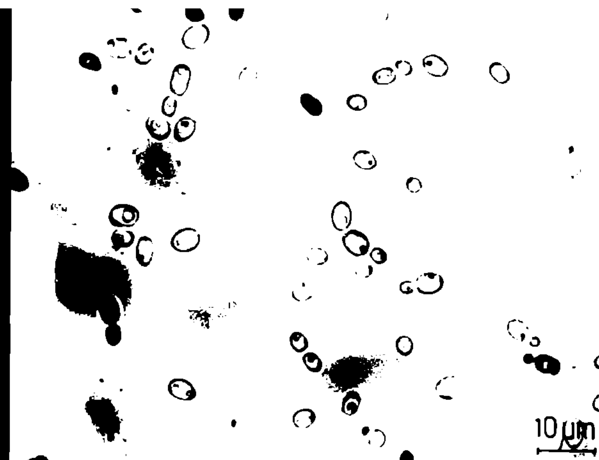

Fig. 3.59 Rhodotorula graminis

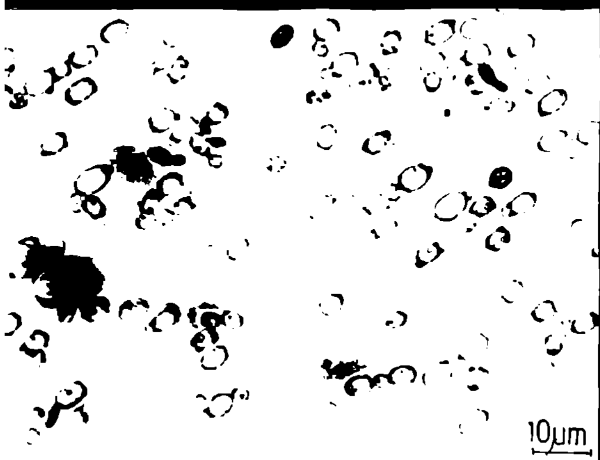

Fig. 3.60 Rhodotorula lactosa

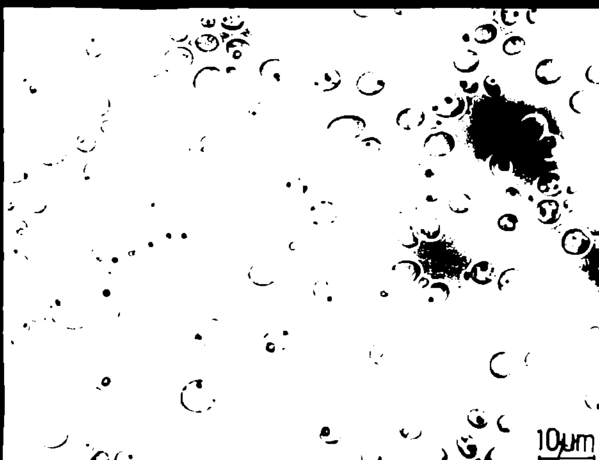

Fig. 3.61 Rhodotorula minuta

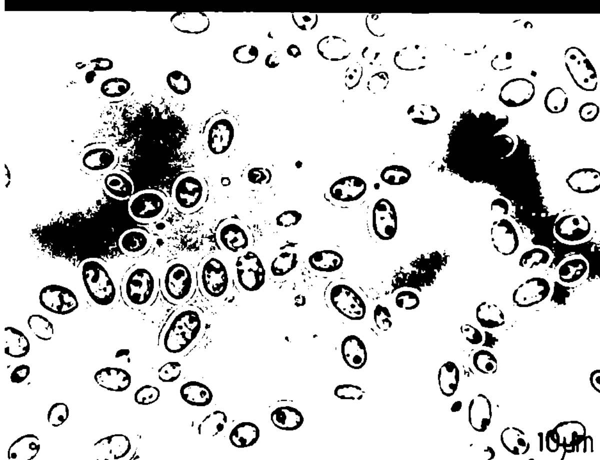

Fig. 3.62 Rhodotorula rubra

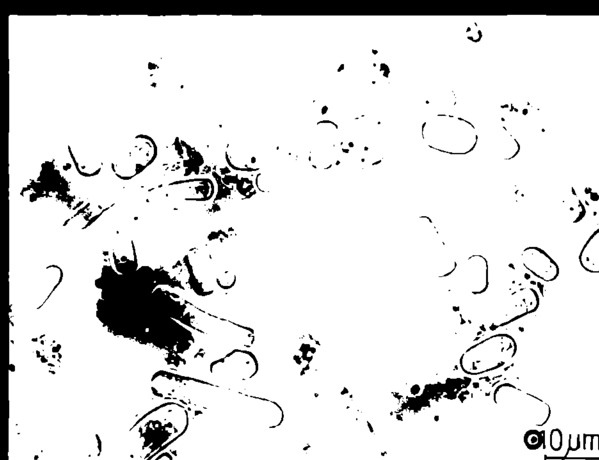

Fig. 3.63 Trichosporon aquatile

Fig. 3.64 Trichosporon cutaneum

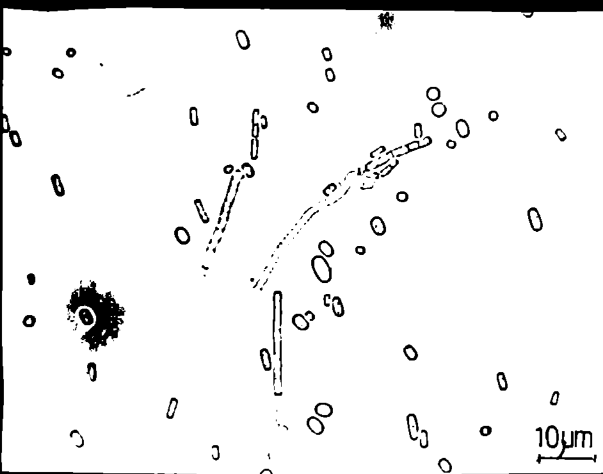

Fig. 3.65 Trichosporon penicillatum

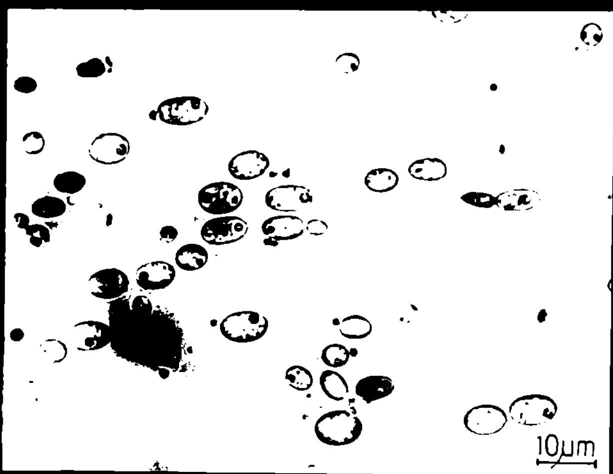

Fig. 3.66 Sporobolomyces roseus

CHAPTER 4

ECOLOGY AND DISTRIBUTION OF FUNGI

The present investigation being a general survey of the group as a whole with considerable emphasis on their systematics, biochemical activities etc., only limited attempt could be made to collect their ecological and environmental characteristics. However efforts to collate this information from the available data have given some insight on their ecology. Proper study of microbial ecology under field conditions is difficult because of their unique ecological characteristics and their extreme variations in abundance both in space and time. It also requires detailed laboratory studies under controlled conditions. Application of powerful statistical methods to correlate microbial abundance with the environmental parameters as is generally done with the macrofauna and flora can lead spurious conclusions unless detailed data are available (Atlas and Bartha, 1981). It will also necessitate micro-scale sampling in time and space. Statistical methods were therefore employed to examine the extent of species association, co-existence etc. and to infer therefrom the environmental influence on their occurrence and abundance.

4.1 Environmental factors

The environmental parameters examined in this study include salinity, dissolved oxygen, temperature, pH, Eh, BOD and organic carbon of mud.

Variations in hydrographic features in this estuary are governed by the tidal and monsoonal regimes. Variations in major physico-chemical parameters during the years 1986 and 1987 at different stations in the Cochin backwater are shown in Figs 4.1 to 4.5.

Salinity

Pronounced seasonal variations were observed in the distribution of salinity with high values during premonsoon months and low values during monsoon months at all the seven stations for the two years. In general it varied from 0.99×10^{-3} to 34.45×10^{-3} (Fig. 4.1). Distribution of salinity showed the same trend in both the years but the values in stations 1 to 4 and 7 were strikingly different in July and November during the two years with a maximum difference of 23.67×10^{-3} in station 2 during July. This was due to monsoonal variations, it being weak and delayed in 1987. Distribution of salinity at station 6 was also influenced to some extent by saline water incursion through Azhikode inlet and hence had higher salinity than station 5, located four miles down. Station 7 recorded higher salinity in all the sampling months during 1987 and is because of less efficient tidal flushing than in other stations. Station 2 near the bar mouth maintained the highest salinity range during the two years, with the salinity range of 5.61×10^{-3} to 34.45×10^{-3} in 1986 and 6.33×10^{-3} to 33.20×10^{-3} in 1987. Similarly station 5 had the lowest range with the values from

0.99×10^{-3} to 24.55×10^{-3} in 1986 and from 3.09×10^{-3} to 26.97×10^{-3} in 1987.

Dissolved oxygen

Bimonthly estimations of dissolved oxygen content of water showed fluctuations during both the years of sampling and varied within the range of 2.20 to 6.70 mg/l (Fig. 4.1). But most of the values were above 3 mg/l. The general annual trend was more or less the same at all the stations although at station 5 some disparity in the dissolved oxygen values between the two years was present from January to September. At station 7 fluctuations were minimum and during the two years the DO values remained within 3.16 to 4.16 mg/l. High DO values were recorded in July at stations 1,3,4 and 5. The peak oxygen values were reported at station 2 in March for the two years (6.08 mg/l in 1986 and 6.48 mg/l in 1987) and also in station 6 (6.70 mg/l) during 1987.

Temperature

Temperature varied from 26 to 34° C for water and 26.9 to 32.9° C for mud (Fig. 4.2). Although the annual range of temperature was more less the same during the two years, striking monthly variations characterized the temperature distribution of 1986 and 1987. In 1986 sharp variations in temperature were noted during the onset and withdrawl of SW monsoon, high values were recorded during premonsoon months (Jan - May) with a steep fall to the lowest values during monsoon months at all the stations. This trend was almost absent in 1987 in majority of the stations. This was due to

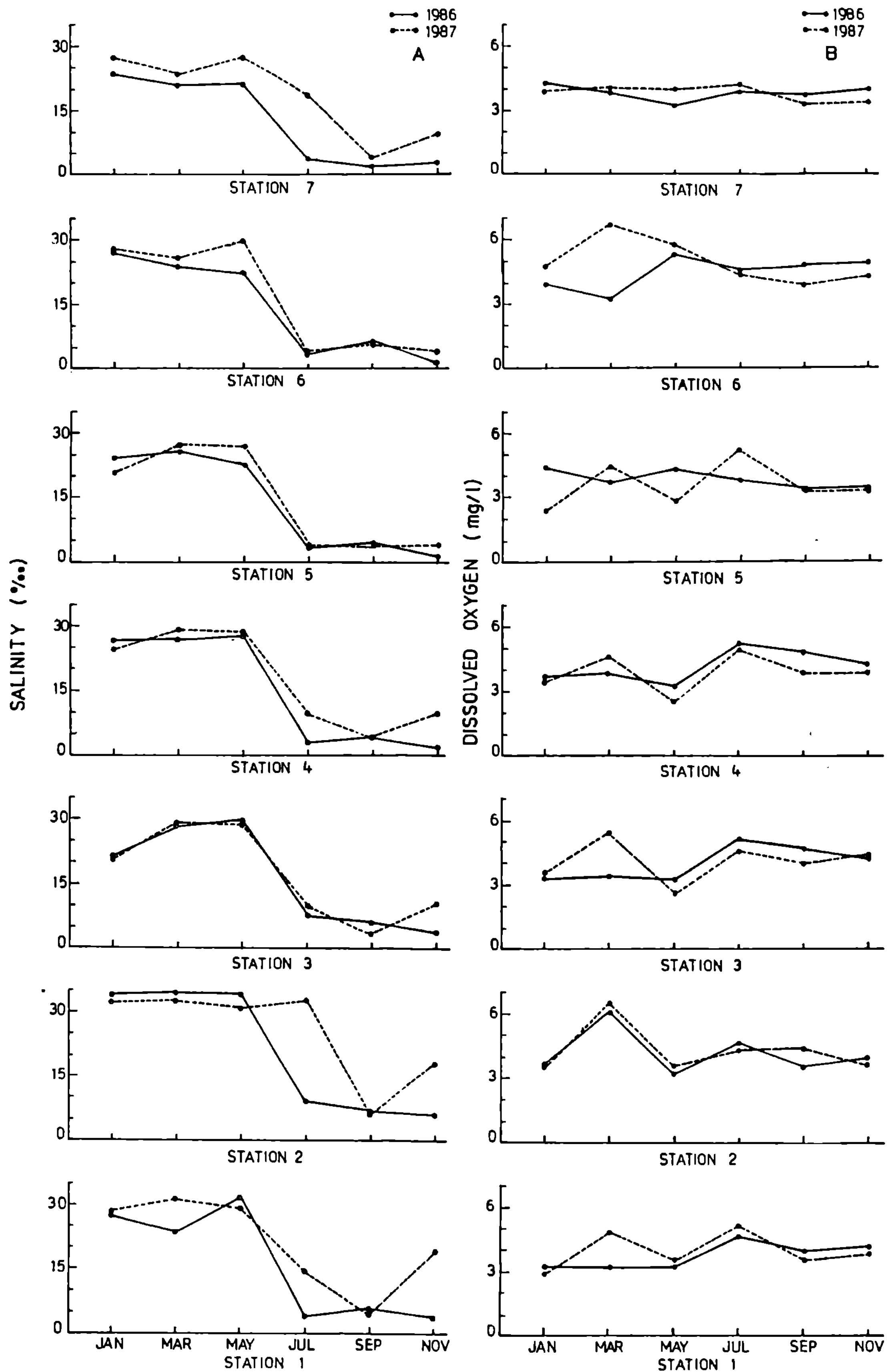

Fig. 4.1 Bimonthly variations in A. salinity and B. dissolved oxygen at different stations for the years 1986 & 1987.

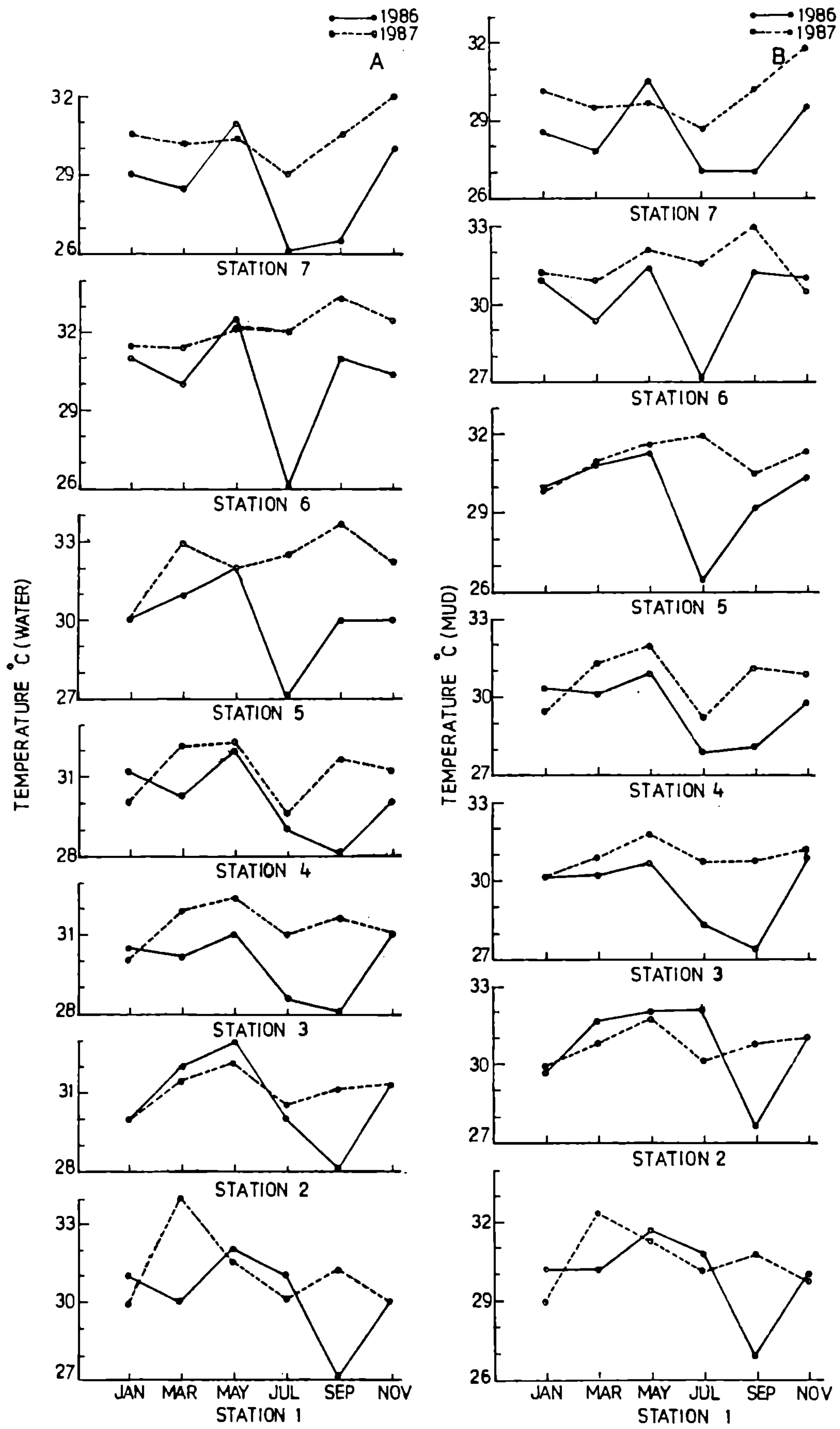

Fig. 4.2 Bimonthly variations in temperature of A. water and B. mud at different stations for the years 1986 & 1987

weak and delayed monsoon in 1987. The temperature difference between the two years was more pronounced at stations 3, 4, 5, 6 and 7 in both water and mud. Stations 5, 6 and 7 showed maximum temperature difference of 5.5° C between the two years in July and in the remaining stations the maximum temperature difference was in September having a range of 27°C to 31.2°C.

The distribution pattern of salinity and temperature recorded in the different stations during the two years also showed considerable difference (Figs 4.1 and 4.2).

pH

The pH of water ranged from 6.90 to 8.31 and that of mud from 6.59 to 8.23 for the years 1986 and 1987 (Fig. 4.3). The pH of water was generally high at all stations except the two values of 6.90 and 6.99 obtained in September 1986 and 1987 at station 5. No striking difference was noticed in the general trend of pH values for the two years. Comparatively high pH values during premonsoon months and low pH values during monsoon-postmonsoon months were recorded at stations 1, 2, 3, 4, and 5. This is due to incursion of sea water during premonsoon months and freshwater dominance during monsoon - postmonsoon months. The pH values of water at station 7 showed least variations. The pH of the bottom mud remained above neutral at all stations during premonsoon months except at station 7 in 1986.

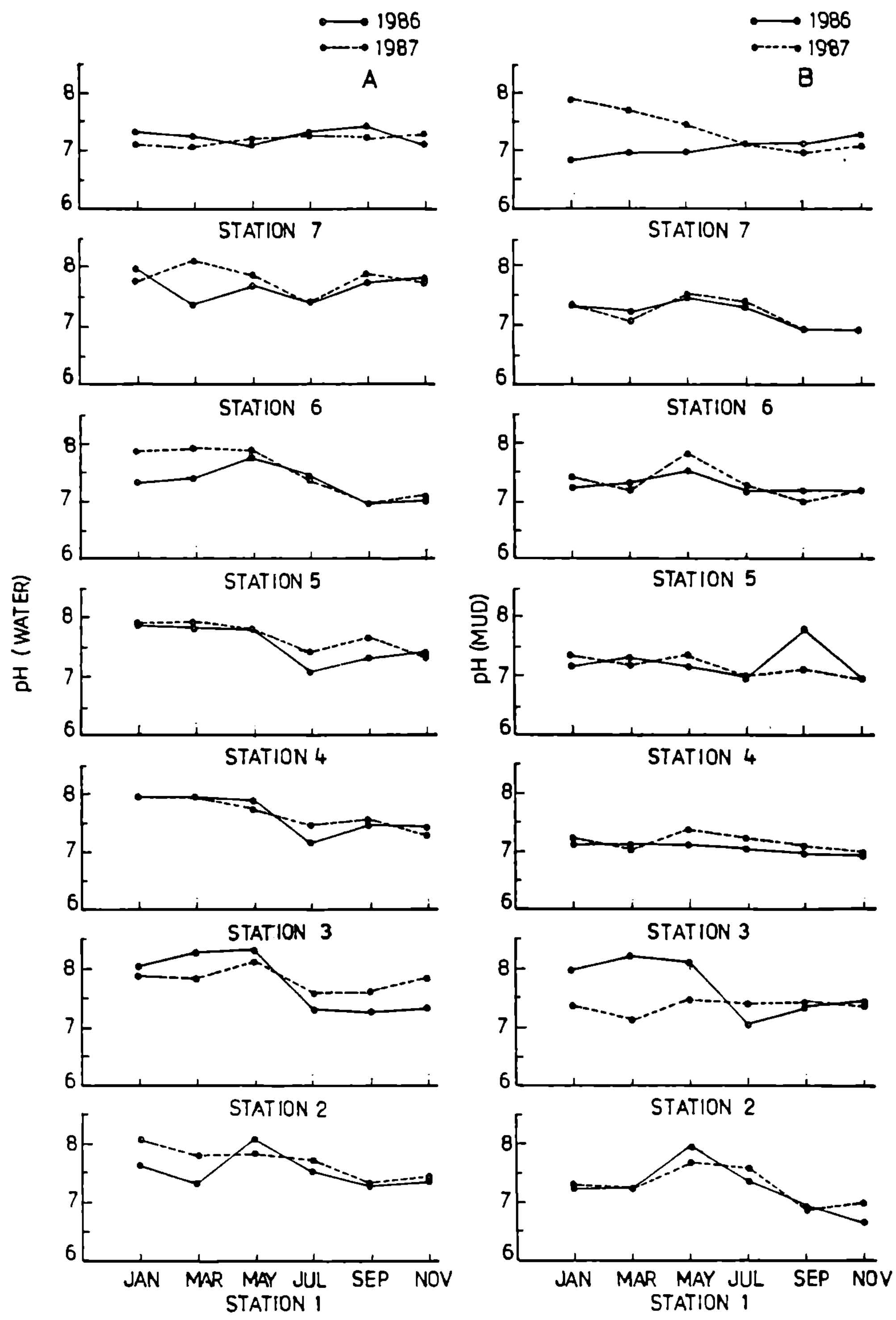

Fig. 4.3 Bimonthly variations in pH of A. water and B. mud at different stations for the years 1986 & 1987

Eh

The redox potential (Eh) is greatly influenced by the presence or absence of molecular oxygen. For water Eh ranged from +2mv to +540mv and for mud it varied from -31mv to -290mv (Fig. 4.4). Here also a clear trend was noticed for the two years like salinity and DO. In water samples low +ve Eh values were recorded in premonsoon months and high values in monsoon - postmonsoon months. A reverse trend was seen in the mud samples, where high -ve values during premonsoon months and low -ve values during monsoon-postmonsoon months were obtained.

BOD_5

BOD_5 values are generally related to the amount of suspended or dissolved organic matter in the water. In general the values ranged in between 0.06 to 5.38 mg/l for the two years (Fig. 4.5) and are well within the specified limit for natural waters. Low BOD_5 values were recorded in January, March and May in both the years and peak values in July. The sharp increase in BOD_5 values at all stations during this month in both the years can be due to extraneous inputs like the initial rain water runoff and associated anthropogenic inputs. Except for July for all other months BOD_5 values were uniformly low in both the years. Nearly identical distribution of BOD_5 values for the different months was seen in all stations during the two years.

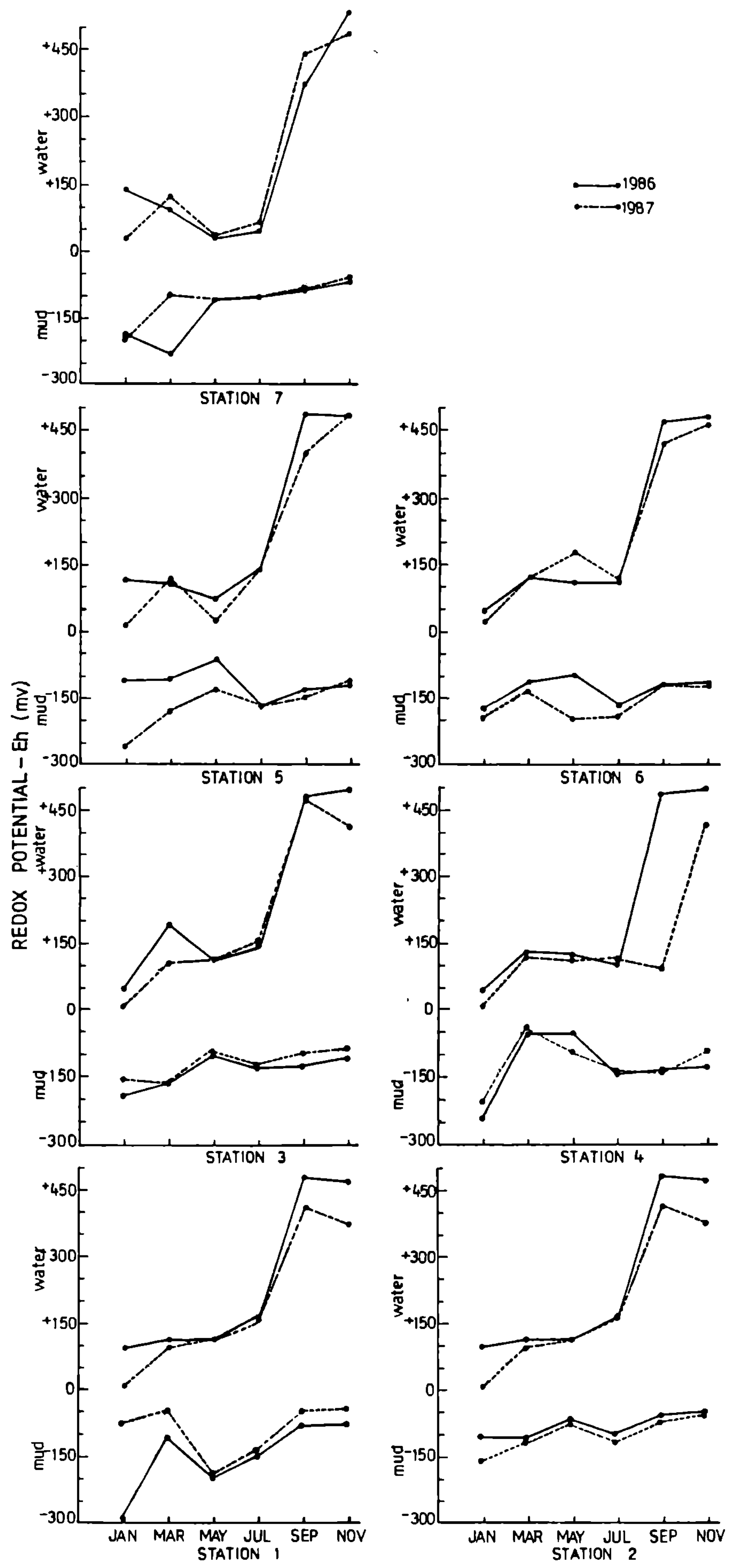

Fig. 4·4 Bimonthly variations in Eh of water and mud at different stations for the years 1986 & 1987

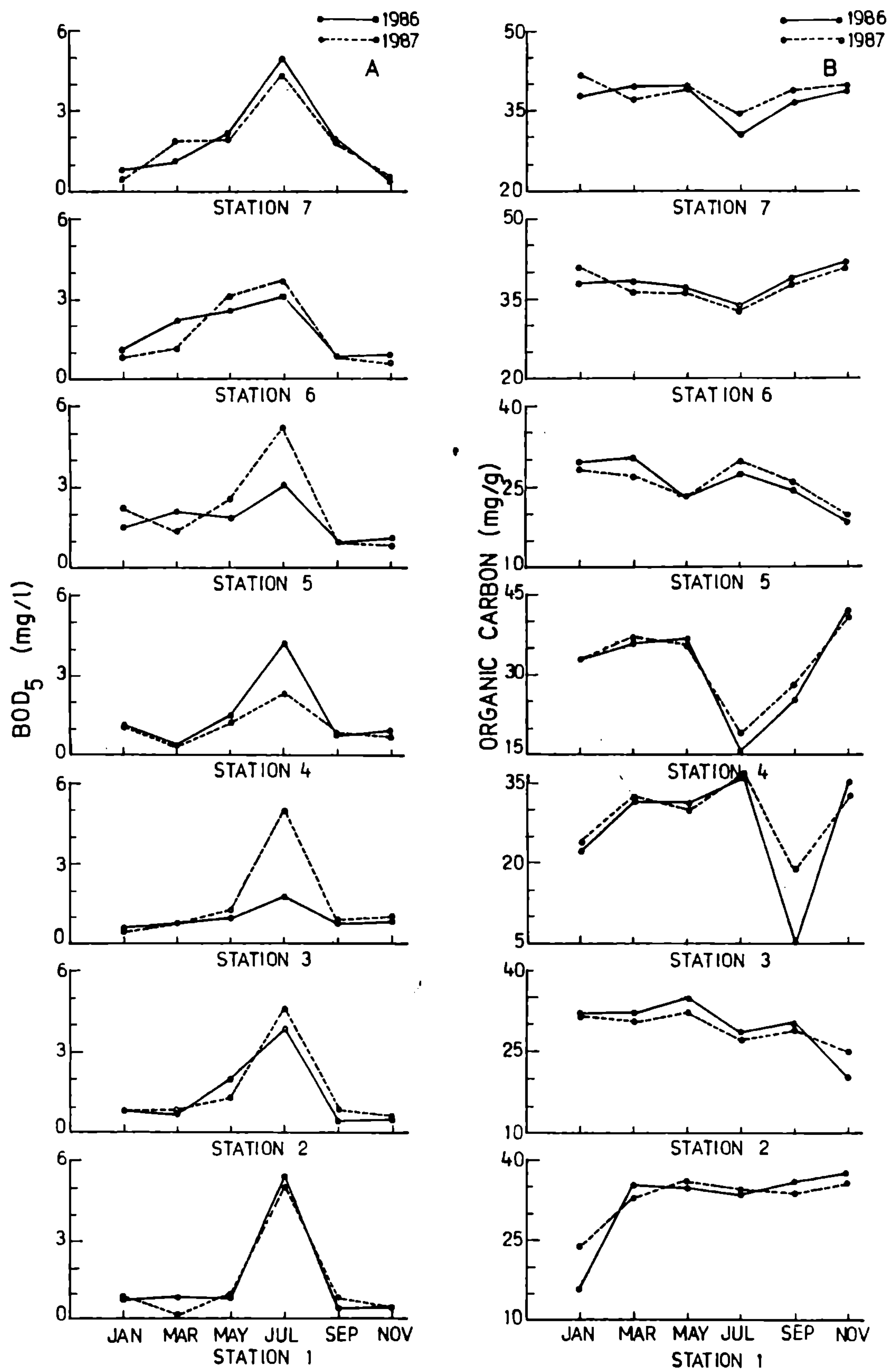

Fig. 4·5 Bimonthly variations in A. BOD_5 values of water and B. organic carbon of mud at different stations for the years 1986 & 1987

Organic carbon

In the present study the organic carbon content of mud was found to vary 4.37 mg/g to 41.40 mg/g (Fig. 4.5). Unlike the BOD_5 values of the water, the organic carbon content of the mud showed differences in the pattern of distribution between stations, but in each station it showed the same pattern during the two years. In station 1 the organic carbon content showed a steep rise from January (in 1986, 15.63 mg/g and in 1987, 23.69 mg/g) to March (in 1986, 34.96 mg/g and in 1987, 32.89 mg/g) in both the years and remained steady until November. In stations 4 and 5 organic carbon content registered a steady increase from January followd by a steep fall in July (station 4) and September (station 5) in both the years. Stations 6 and 7 had nearly identical distribution. The organic content of mud varied from 4.37 mg/g to 41.40 mg/g. Stations 6 and 7 had its own source of organic input because of the presence of dense macrophytes. The highest value of 41.40 mg/g was recorded in station 7 in 1987.

4.2 Mycoflora

Ninety six species of filamentous fungi belonging to 39 genera and 35 species of estuarine yeasts belonging to 11 genera could be isolated from water and mud during the two year survey (1986 and 1987). Fifty species of filamentous fungi belonging to 25 genera were recorded from water samples while 86 species belonging to 35 genera were obtained from mud samples. Thirty five species of estuarine yeasts

belonging to 11 genera were recorded from water samples and 16 species belonging to 7 genera from mud samples.

Filamentous fungi

General observations

The list of fungi isolated from seven localities is given in Table 4.1. Tables 4.2 to 4.5 show the occurrence of various species of fungi in the different stations for two years. Most striking feature is that although many species were represented in the samples collected from different stations only a few were of regular occurrence. Among the various genera, *Aspergillus* was the most dominant represented by 16 species, followed by *Penicillium* with 14 species. *Fusarium* was represented by nine species and *Cephalosporium* and *Trichoderma* with four species each. Both in water and mud *Aspergillus fumigatus*, *A. niger* and *A. terrus* were the most common species.

Considerable variations in the specieswise and numerical abundance were also seen with respect to water and mud samples as well as their annual, bimonthly and stationwise occurrence. Table 4.6 shows the number of species represented in water and mud samples from different stations during 1986 and 1987. Figs 4.6 and 4.7 similarly indicate spatio-temporal variations in the total counts. Propagules were better represented in the mud samples both qualitatively and quantitatively. In water samples number of propagules per litre varied as much from 4 to 1.14×10^3 and in the

Table 4.1 Species of filamentous fungi isolated from the Cochin backwater

Acrothecium sp.
Absidia cylindrospora Hagem
Absidia sp.
Achlya racemosea Hildebrand
Achlya sp.
Allescheriella sp.
Allomyces sp.
Alternaria fasciculata Cooke and Ellis
A. humicola Oudemans
A. tenuis Nees
Aspergillus candidus Link
A. chevalieri (Mangin) Thom and Church
A. flavipes Bainier and Sartory
A. flavus Link
A. fumigatus Fresenius
A. glaucus Link
A. humicola Chaudhuri
A. janus Raper and Thom
A. nidulans (Eidam Winter
A. niger van Tieghem
A. oryzae (Ahlburg) Cohn
A. sydowi (Bainierr and Sartory) Thom and Church
A. terrus Thom
A. ustus (Bainier) Thom and Church
A. versicolor (Vuillemin) Tiraboschi
Aspergilus sp.
Aureobasidium sp.
Botryodiplodia theobromae Patouillard
Botrytis terrestris Jensen
Cephalosporium acremonium Corda
C. humicola Oudemans
C. roseo-griseum Saksena
Cephalosporium sp.
Ceratocystis sp.
Chalara sp.
Chaetomium cristatum Ames
C. globosum Kunz
C. nigricolor Ames
Cladosporium herbarum (Persoon Link
C. resinae (Lindau) de uries
Colletotrichum gloeosporoides (Penzig) Penzig and Sacc.
Curvularia geniculata (Tracy and Earle) Boedijn
C. lunata (Walkeri) Boedijn
C. tetramera (McKinney) Boedijn
Drechslerra halodes (Drechsler) Subramanian et Jain
Drechslera sp.
Emericella nidulans (Eidam) Vuill
Eupenicillium sp.
Fusarium lini Bolley
F. moniliforme Sheldon
F. neoceras Wollenweber and Reinking
F. oxysporum Schlechtendahl
F. redolens Wollenweber
F. sambucinum Fukel
F. semitectum Berkeley and Ravenel
F. solani (Martius) Appel and Wollenweber
Fusarium sp.
Gliocladium penicilloides Corda
Helminthsporium sp.
Monilia brunnea Gilman and Abott
Mucor hiemalis Wehmer
Mucor sp.
Myrothecium verrucaria (Albertini and Schweinitz) Ditmer
Paecilomyces varioti Bainier
Paecilomyces sp.
Penicillium albidum Sopp
P. brefeldianum Dodge
P. chrysogenum Thom
P. citrinum Thom
P. janthinellum Biourge
P. javanicum van Beyma
P. levitum Raper and Fennell
P. liliacinum Thom
P. lividum Westling
P. luteum Zukel
P. parvum Rapper and Fennell
P. pinophillum Hedgecock
P. monoverticillate symmetrica
Penicillim sp.
Phialophora sp.
Phoma sp.
Phomopsis sp.
Phytophthora sp.
Polyschema indica Behera Mukerji & Sharma apud Sharam, Behera and Mukerji
Pythium sp.
Rhizopus nodosus Namyslowski
R. oryzae Went and Gerrlings
Rhizopus sp.
Saprolegenia sp.
Sporotrichum roseum Link
Trichoderma album Preuss
T. glaucum Abbott
T. koningi Oudemans
T. viride pers. ex Fr.
Verticillium sulphurellum Sacc.
Verticillium sp.
Unidentified - 12 isolates

Table 4.2 Species of fungi isolated from the water samples of Cochin backwater and their occurrence in different stations for the year 1986

Sl.No.	Mycoflora	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	<u>Absidia cylindrospora</u>									x																																	
2.	<u>Achlya</u> sp.																	x																									
3.	<u>Allomyces</u> sp.																																				x						
4.	<u>Alternaria fasciculata</u>																						x																				
5.	<u>A</u>. <u>humicola</u>										x		x																														
6.	<u>A</u>. <u>tenuis</u>				x		x										x						x	x				x						x									
7.	<u>Aspergilus flavus</u>																																		x								
8.	<u>A</u>. <u>fumigatus</u>	x	x	x	x	x	x	x	x	x				x	x	x				x	x	x							x	x		x			x	x		x					
9.	<u>A</u>. <u>janus</u>																																										
10.	<u>A</u>. <u>niger</u>				x		x					x						x							x		x									x	x		x	x	x	x	
11.	<u>A</u>. <u>oryzae</u>																																x										
12.	<u>A</u>. <u>terrus</u>						x												x						x		x			x			x						x		x	x	x
13.	<u>A</u>. <u>versicolor</u>																																										
14.	<u>Botrytis terrestris</u>																																							x			
15.	<u>Cephalosporium acremonium</u>																																								x		
16.	<u>C</u>. <u>roseo-griseum</u>																	x																									
17.	<u>Ceratocystis</u> sp.																																					x					
18.	<u>Chalara</u> sp.																																					x		x			
19.	<u>Chaetomium cristatum</u>																																										
20.	<u>C</u>. <u>globosum</u>																																										
21.	<u>Cladosporium herbarum</u>					x																			x						x												x
22.	<u>C</u>. <u>resinae</u>																														x										x		
23.	<u>Colletotrichum gloeosporoides</u>																																						x				
24.	<u>Curvularia lunata</u>					x																																	x				
25.	<u>Drechslera halodes</u>																																										
26.	<u>Drechslera</u> sp.																																										
27.	<u>Fusarium oxysporum</u>																																							x			
28.	<u>F</u>. <u>sambucinum</u>																																										
29.	<u>F</u>. <u>semitectum</u>																																										
30.	<u>F</u>. <u>solani</u>					x	x																	x				x	x					x	x					x	x	x	x
31.	<u>Gliocladium penicilloides</u>																														x												
32.	<u>Helminthosporium</u> sp.																						x																				
33.	<u>Monilia brunnea</u>																																										
34.	<u>Mucor hiemalis</u>	x		x				x			x										x	x	x			x						x	x										
35.	<u>Mucor</u> sp.																																					x					
36.	<u>Paecilomyces varioti</u>																x																										
37.	<u>Paecilomyces</u> sp.																																										
38.	<u>Penicillium citrinum</u>																																			x							
39.	<u>P</u>. <u>janthinellum</u>				x													x	x										x	x		x	x					x	x	x		x	x
40.	<u>P</u>. <u>levitum</u>																																										
41.	<u>P</u>. <u>luteum</u>																															x											
42.	<u>P</u>. <u>parvum</u>																																										
43.	<u>P</u>. <u>pinophillum</u>																								x										x								
44.	<u>Pythium</u> sp.																																										
45.	<u>Rhizopus nodosus</u>											x						x																									
46.	<u>R</u>. <u>oryzae</u>																												x														
47.	<u>Trichoderma koningi</u>				x																																					x	
48.	<u>T</u>. <u>viride</u>																																										
49.	<u>Verticillium sulphurellum</u>																																										
50.	<u>Verticillium</u> sp.																																										

x Unidentified isolates

Table 4.3 Species of fungi isolated from the water samples of Cochin backwater and their occurrence in different stations for the year 1987

Sl.No.	Mycoflora	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	Absidia cylindrospora																																										
2.	Achlya sp.																																										
3.	Allomyces sp.																																										
4.	Alternaria fasciculata																																										
5.	A. humicola																																										
6.	A. tenuis			x	x					x	x					x	x						x					x						x							x		
7.	Aspergilus flavus																																										
8.	A. fumigatus	x	x	x	x	x	x	x	x	x							x			x		x	x					x							x				x	x	x	x	x
9.	A. janus	x																																									
10.	A. niger		x		x		x		x			x			x			x			x						x						x			x			x			x	x
11.	A. oryzae																																x										
12.	A. terrus					x	x	x	x		x	x			x							x			x		x		x	x	x		x						x	x		x	x
13.	A. versicolor																												x														
14.	Botrytis terrestris																																										
15.	Cephalosporium acremonium																																								x		
16.	C. roseo-griseum																																										
17.	Ceratocystis sp.																																										
18.	Chalara sp.																																							x			
19.	Chaetomium cristatum													x																													
20.	C. globosum																															x											
21.	Cladosporium herbarum			x		x						x																x						x			x	x					
22.	C. resinae						x			x			x			x			x			x		x	x						x							x			x		x
23.	Colletotrichum gloeosporoides																																						x				
24.	Curvularia lunata																																										
25.	Drechslera halodes																															x											
26.	Drechslera sp.													x																													
27.	Fusarium oxysporum																																	x						x	x		
28.	F. sambucinum	x																																									
29.	F. semitectum							x																																			
30.	F. solani			x						x													x						x						x		x			x		x	x
31.	Gliocladium penicilloides																									x																	
32.	Helminthosporium sp.																																										
33.	Monilia brunnea									x																x												x		x			
34.	Mucor hiemalis								x		x		x										x										x										
35.	Mucor sp.																																										
36.	Paecilomyces varioti																x																										
37.	Paecilomyces sp.	x																																									
38.	Penicillium citrinum																																		x	x							
39.	P. janthinellum		x		x				x									x				x								x			x								x	x	
40.	P. levitum													x																													
41.	P. luteum																																							x			
42.	P. parvum																												x														
43.	P. pinophillum					x																																					
44.	Pythium sp.																																					x					
45.	Rhizopus nodosus																																x										
46.	R. oryzae		x						x			x			x					x	x						x																
47.	Trichoderma koningi																																										
48.	T. viride																									x						x											
49.	Verticillium sulphurellum	x																																									
50.	Verticillium sp.																									x																	

x Unidentified isolates

Table 4.4 Species of fungi isolated from the mud samples of Cochin backwater and their occurrence in different stations for the year 1986

Sl.No. Mycoflora	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1. Acrothecium sp.													x																								x					
2. Absidia cylindrospora																	x																									
3. Absidia sp.																																		x								
4. Achlya racemosa																																			x							
5. Achlya sp.																																			x							
6. Allescheriella sp.																																										
7. Alternaria fasciculata																																								x		
8. A. humicola					x																																					
9. Aspergillus candidus																																			x			x			x	
10. A. chevalieri	x																																									
11. A. flavipes	x																																									
12. A. flavus																																										
13. A. fumigatus	x	x	x	x	x	x	x			x				x	x	x	x	x	x				x	x	x	x	x			x	x	x	x			x		x				
14. A. glaucus									x				x																													x
15. A. humicola																																			x							x
16. A. janus																																										
17. A. nidulans																												x												x	x	
18. A. niger						x							x							x													x				x		x	x	x	
19. A. oryzae											x																		x													
20. A. sydowi	x																						x		x																	
21. A. terrus						x				x	x		x						x			x															x					
22. A. ustus																									x													x				
23. A. versicolor									x																																	
24. Aspergillus sp.	x			x																																						
25. Aureobasidium sp.																	x																									
26. Botryodiplodia theobromae																																										
27. Cephalosporium acremonium												x							x																							
28. C. humicola																																										
29. C. roseo-griseum																									x																	
30. Cephalosporium sp.																																										
31. Ceratocystis sp.																x																										
32. Chaetomium globosum																																										
33. C. nigricolor																																										
34. Cladosporium herbarum						x					x	x																												x	x	
35. C. resinae				x		x				x														x																		
36. Colleto. gloeosporoides																																										
37. Curvularia geniculata																																										
38. C. lunata	x																							x														x		x		x
39. C. tetramera																														x												x
40. Drechslera sp.																	x																									
41. Emericella nidulans																																										
42. Eupenicillium sp.																																										
43. Fusarium lini					x																																					
44. F. moniliforme																																			x							
45. F. neoceras																																								x		
46. F. oxysporum																																										
47. F. redolens																																										
48. F. semitectum																			x																							
49. F. solani																		x							x											x	x	x				
50. Fusarium sp.																			x																			x				
51. Helminthosporium sp.												x																														x
52. Monilia brunnea																																										
53. Mucor hiemalis			x				x	x				x				x										x		x			x	x										x
54. Myrothecium verrucaria																																										
55. Paecilomyces variotii										x						x						x						x							x							
56. Paecilomyces sp.																																										
57. Penicillium albidum																																										
58. P. brefeldianum																																										
59. P. chrysogenum	x																												x													
60. P. citrinum																x																							x	x		
61. P. janthinellum	x	x					x		x					x	x			x		x				x		x	x				x	x							x			
62. P. javanicum																																										
63. P. lilacinum									x																																	
64. P. lividum																																										
65. P. luteum																															x	x	x				x	x				
66. P. parvum																																		x								
67. P. pinophillum						x																														x						
68. P. mono.symmetrica																	x																									
69. Penicillium sp.																																										
70. Phialospora sp.																																										
71. Phoma sp.										x							x																									
72. Phomopsis sp.				x																																						
73. Phytophthora sp.																			x																							
74. Polyschema indica																		x																								x
75. Pythium sp.																	x																									
76. Rhizopus nodosus				x																																						
77. R. oryzae											x																															
78. Rhizopus sp.																																										
79. Saprolegenia																							x																			
80. Sporotrichum roseum																									x																	
81. Trichoderma album												x																														
82. T. glaucum																																										
83. T. koningi			x		x						x											x	x						x					x		x			x		x	
84. T. viride																																										
85. Verticillium sulphurellum																																										
86. Verticillium sp.																																										
X. Unidentified isolates																																										

Table 4.5 Species of fungi isolated from the mud samples of Cochin backwater and their occurrence in different stations for the year 1987

Sl.No.	Mycoflora	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	*Acrothecium* sp.																																								x		
2.	*Absidia cylindrospora*																																										
3.	*Absidia* sp.																																		x								
4.	*Achlya racemosa*																																										
5.	*Achlya* sp.														x												x											x					
6.	*Allescheriella* sp.																										x																
7.	*Alternaria fasciculata*																																										x
8.	*A. humicola*			x	x								x									x						x						x									
9.	*Aspergillus candidus*																																x						x			x	
10.	*A. chevalieri*																																										
11.	*A. flavipes*																																										
12.	*A. flavus*																																					x					
13.	*A. fumigatus*	x	x	x	x	x	x	x	x	x					x					x			x				x		x				x				x				x	x	x
14.	*A. glaucus*																																							x			
15.	*A. humicola*																																								x		
16.	*A. janus*	x																																									
17.	*A. nidulans*																																										
18.	*A. niger*	x		x	x		x			x	x			x		x	x	x	x									x						x	x	x	x			x		x	
19.	*A. oryzae*		x						x																																		
20.	*A. sydowi*	x																																									
21.	*A. terrus*			x	x	x	x	x	x	x			x		x	x						x						x		x		x		x			x			x			
22.	*A. ustus*																					x																					
23.	*A. versicolor*																																										
24.	*Aspergillus* sp.																									x																	
25.	*Aureobasidium* sp.																																										
26.	*Botryodiplodia theobromae*																																					x					
27.	*Cephalosporium acremonium*																				x																				x		x
28.	*C. humicola*													x																													
29.	*C. roseo-griseum*																																										
30.	*Cephalosporium* sp.																									x																	
31.	*Ceratocystis* sp.																																										
32.	*Chaetomium globosum*								x																		x																
33.	*C. nigricolor*																									x																	
34.	*Cladosporium herbarum*	x		x		x						x																x		x				x		x					x	x	
35.	*C. resinae*						x											x				x		x																			x
36.	*Colleto. gloeosporoides*																																						x				
37.	*Curvularia geniculata*																																					x					
38.	*C. lunata*																														x										x		x
39.	*C. tetramera*																																										
40.	*Drechslera* sp.																																										
41.	*Emericella nidulans*														x																												
42.	*Eupenicillium* sp.														x																	x											
43.	*Fusarium lini*																																										
44.	*F. moniliforme*																																										
45.	*F. neoceras*																																										
46.	*F. oxysporum*	x																					x																				
47.	*F. redolens*																																					x					
48.	*F. semitectum*																																										
49.	*F. solani*	x		x																											x			x		x						x	x
50.	*Fusarium* sp.																																										
51.	*Helminthosporium* sp.												x																														
52.	*Monilia brunnea*							x						x								x										x		x						x			
53.	*Mucor hiemalis*				x						x		x				x	x											x										x			x	x
54.	*Myrothecium verrucaria*																									x																	
55.	*Paecilomyces varioti*																x																										
56.	*Paecilomyce* sp.																			x																							
57.	*Penicillium albidum*		x								x															x						x											
58.	*P. brefeldianum*																															x											
59.	*P. chrysogenum*													x																													
60.	*P. citrinum*															x					x					x			x														
61.	*P. janthinellum*		x			x		x	x		x	x		x	x	x	x	x			x	x		x	x			x	x				x	x							x		
62.	*P. javanicum*		x																																								
63.	*P. liliacinum*																																										
64.	*P. lividum*																				x																						
65.	*P. luteum*																																x			x							
66.	*P. parvum*				x		x													x																							
67.	*P. pinophillum*																		x																								
68.	*P. mono.symmetrica*																																						x				x
69.	*Penicillium* sp.	x																																									
70.	*Phialospora* sp.	x																																									
71.	*Phoma* sp.																																										
72.	*Phomopsis* sp.			x																	x		x			x	x																
73.	*Phytophthora* sp.																																										
74.	*Polyschema indica*																																										
75.	*Pythium* sp.																										x						x					x	x				
76.	*Rhizopus nodosus*										x										x													x									
77.	*R. oryzae*		x							x						x																								x			
78.	*Rhizopus* sp.																																x										
79.	*Saprolegenia*																																										
80.	*Sporotrichum roseum*																																										
81.	*Trichoderma album*																																										
82.	*T. glaucum*														x											x	x																
83.	*T. koningi*		x	x	x				x	x		x	x					x					x		x		x	x	x		x				x					x		x	
84.	. *viride*																																						x				
85.	*Verticillium sulphurellum*																			x																							
86.	*Verticillium* sp.																									x																	
X.	Unidentified isolates																																										

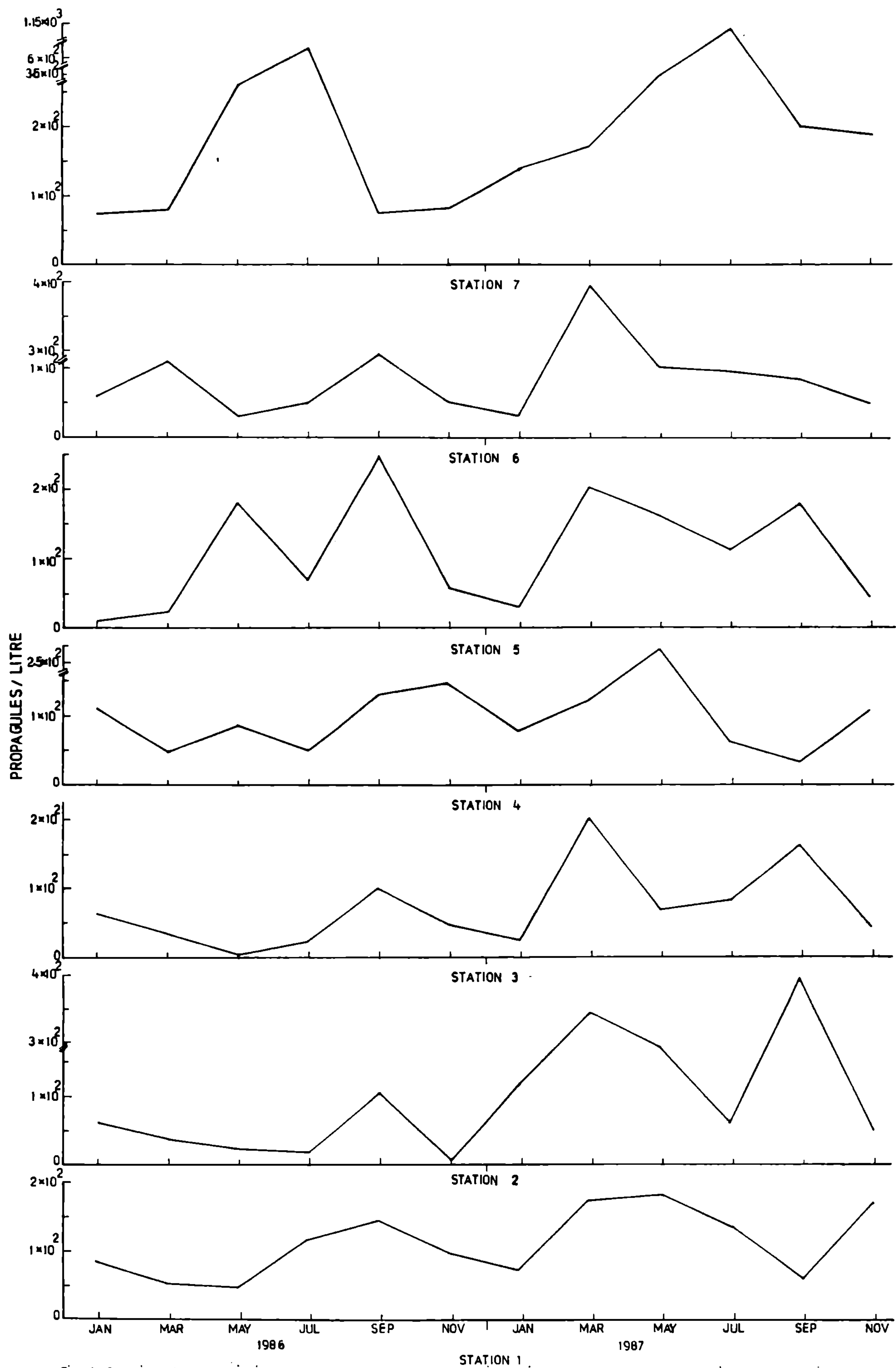

Fig. 4·6 Bimonthly variations of total fungal populations in water samples at different stations for the years 1986 & 1987.

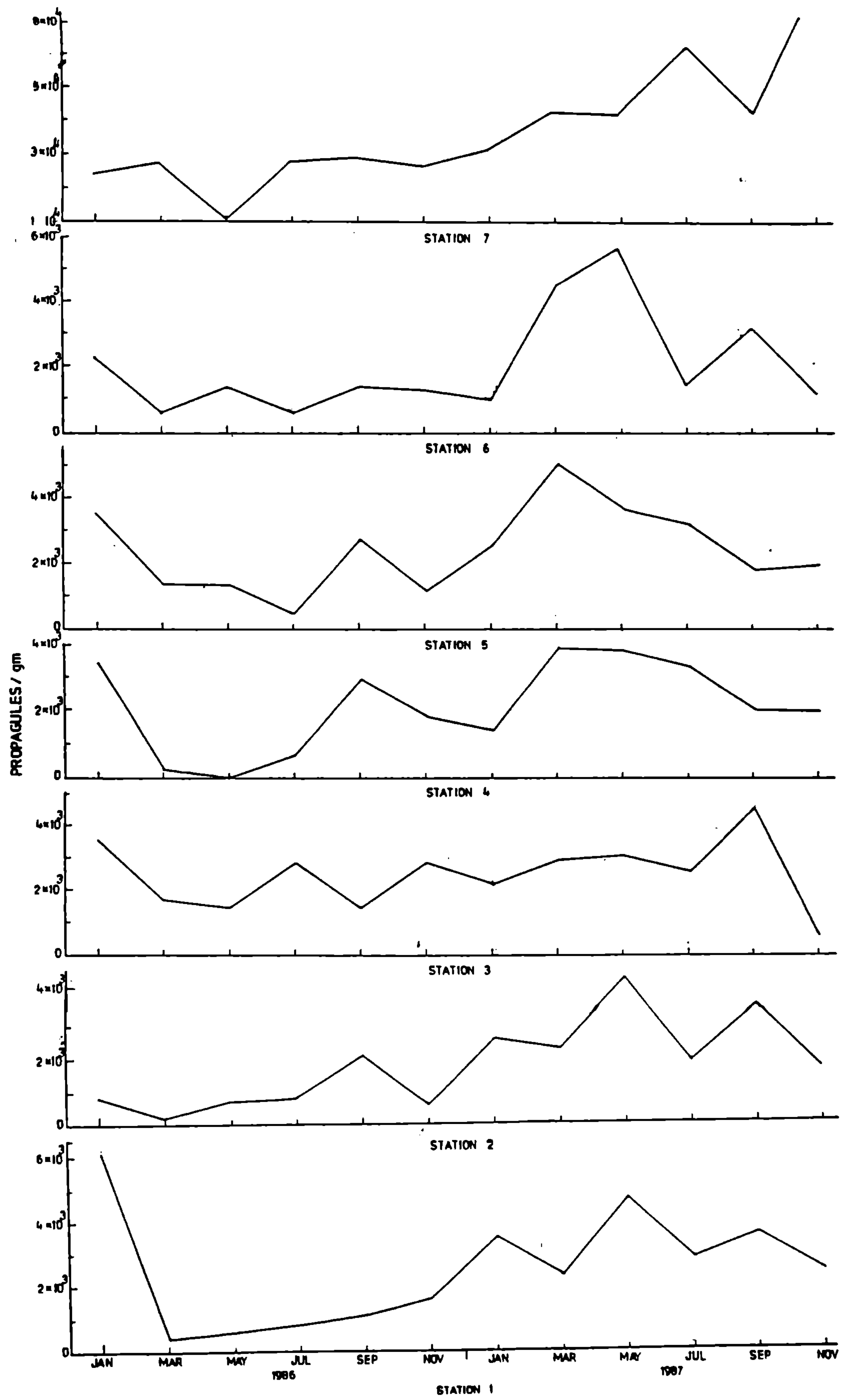

Fig. 4.7 Bimonthly variations of total fungal populations in mud samples at different stations for the years 1986 & 1987

Table 4.6 Data on the fungi isolated from water and mud samples of Cochin backwater collected during 1986 and 1987

No.of fungal species isolated	Water		Mud	
	Genera	Species	Genera	Species
Station 1	11	17	11	29
Station 2	9	15	13	23
Station 3	10	14	21	29
Station 4	8	13	15	25
Station 5	10	16	18	32
Station 6	11	19	14	26
Station 7	14	20	19	37

mud samples it ranged from 0 to 8.1×10^{4} propagules per gram. In all stations fungal counts were less in 1986 than in 1987. A decreasing trend in their abundance during the premonsoon months was also noticed in 1986 in many stations which was more evident in the mud samples. Histogram showing the abundance of various species in stations 1 to 7 during 1986 and 1987 is given in Figs 4.8 to 4.11. Overall abundance was again seen to be distinctly more in 1987 in all stations and also represented by more number of species.

Quantitative studies

Spatio-temporal and qualitative distribution of the fungal counts were examined by ANOVA and Trellis diagram. Since the extremely high counts in the mud samples in station 7, stood out distinctly from the rest of the samples, only the mud samples from the other six stations were subjected to these tests. The water samples, however from all the seven stations could be taken together for all

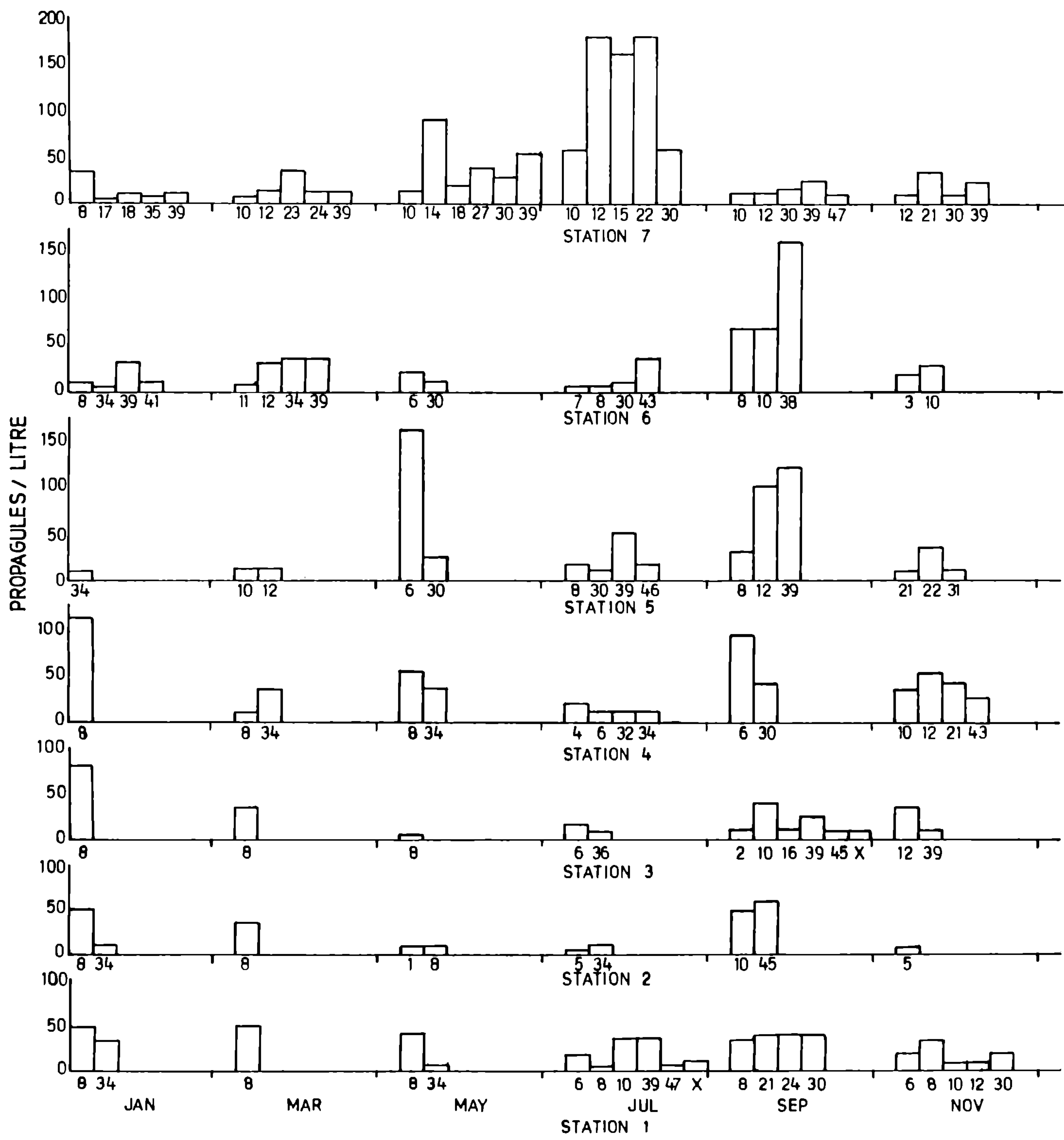

Fig. 4·8 Monthly distribution of fungal species in water samples at different stations for the year 1986 (Numbers on 'x' axis refer to the species; vide Table 4·2)

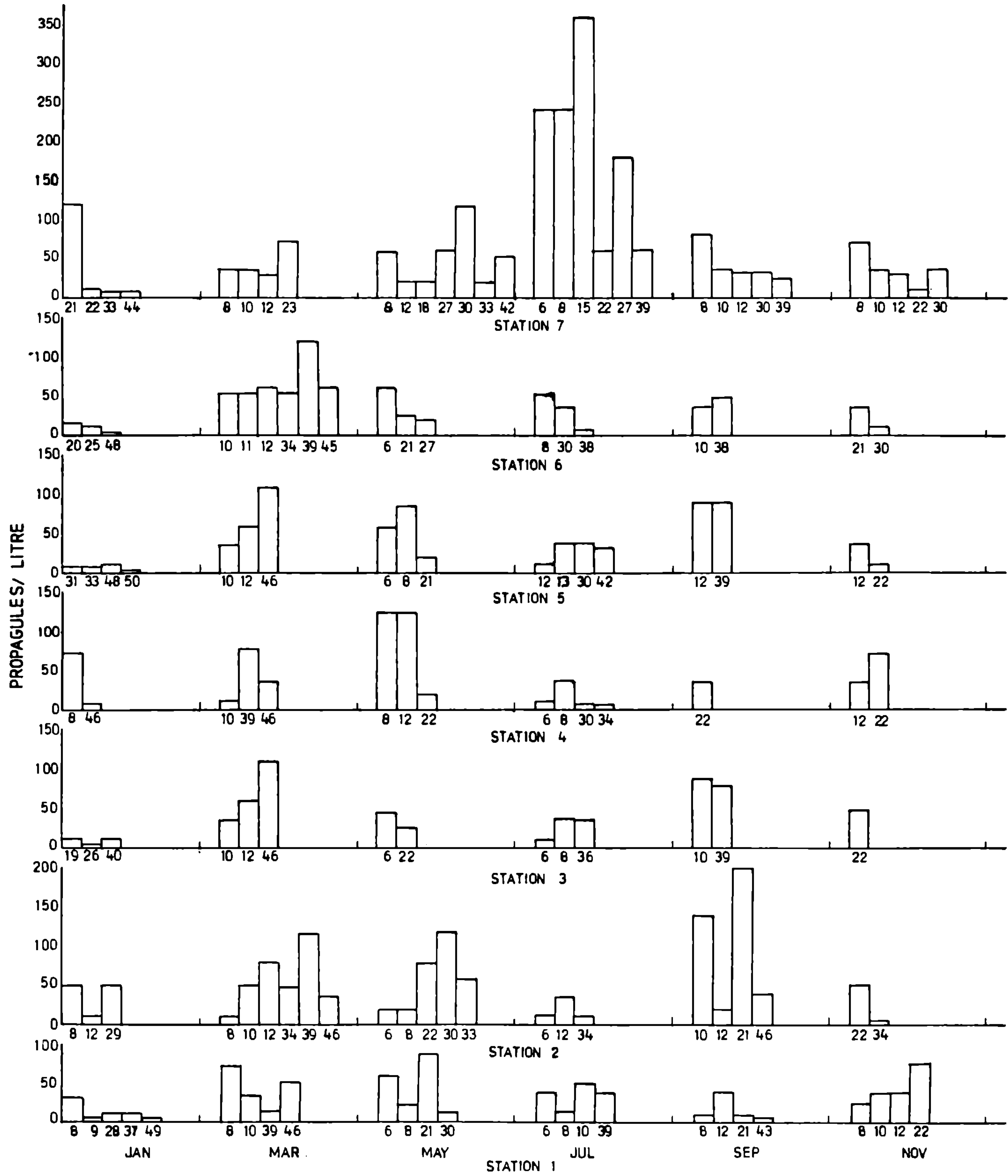

Fig. 4·9 Monthly distribution of fungal species in water samples at different stations for the year 1987 (Numbers on 'x' axis refer to the species; vide Table 4·3)

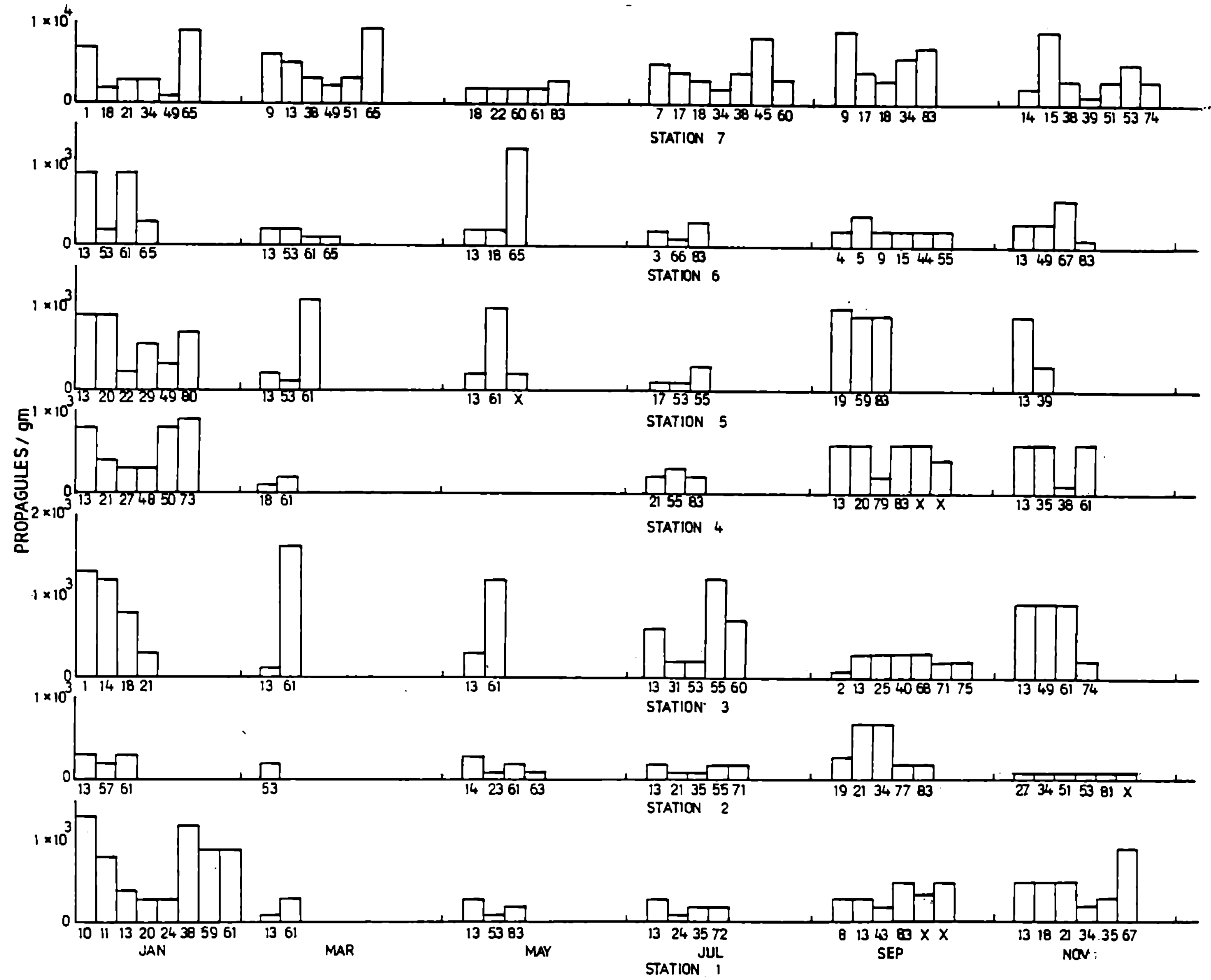

Fig. 4.10 Monthly distribution of fungal species in mud samples at different stations for the year 1986 (Numbers on 'x' axis refer to the species; vide Table 4.4)

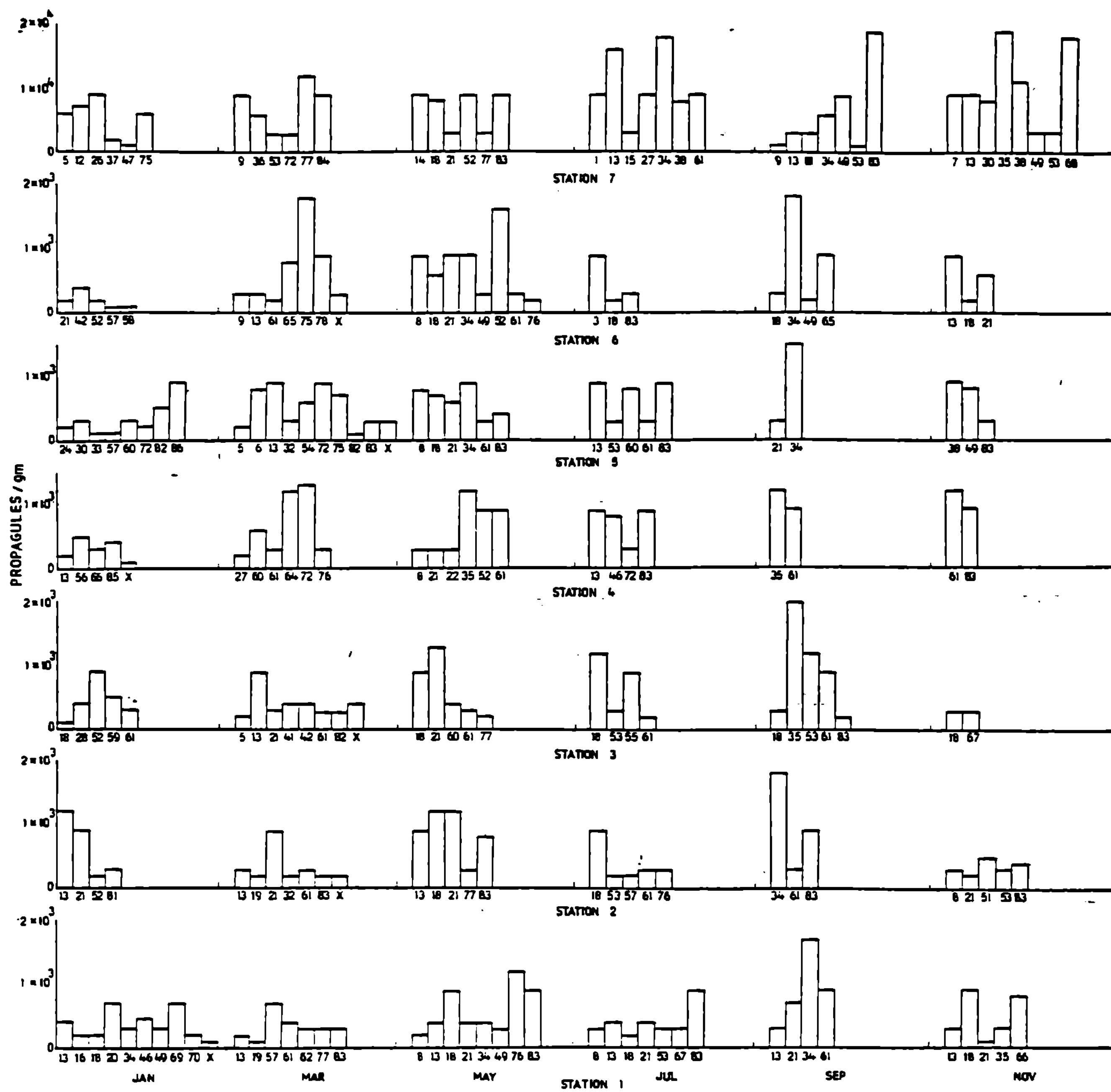

Fig. 4-11 Monthly distribution of fungal species in mud samples at different stations for the year 1987 (Numbers on 'x' axis refer to the species; vide Table 4-5)

statistical analysis.

Since the data showed wide variability, before subjecting them to statistical analysis, necessity of suitable transformation was examined by Tukey's test of additivity. It was found that for water samples of both years the transformation $Y = 1/X^{0.6}$ will establish homogenity of variance. For mud samples of 1986 logarithmic transformation was required only at 10% confidence level and no transformation was required for 1986 samples. Since the F-values in both cases are non-significant at 5% level, ANOVA test was performed on the actual data.

The data were next subjected to a 3-way ANOVA test using the total counts after the necessary tranformation to examine stationwise, monthly and annual difference (Tables 4.7 and 4.8). In both water and mud samples the F-ratio was highly significant ($P < 0.005$) for years, stations and months, the greatest variablity being for the years (Tables 4.7 and 4.8).

Similarly the interaction effects between years and stations and especially between years and months were highly significant. The interaction effect between stations and months was comparatively less although it was found significant for the mud samples. A two-way ANOVA test was therefore applied separately for the two years (Tables 4.9 - 4.12). The F-test showed no significant between station difference for both the years whereas, except for one F-ratio significant between-month difference of the fungal

Table 4.7 3-way classification for testing significance of difference between (1) years (2) stations and (3) months for water samples after transformation

Source	S.S.	d.f.	M.S.S.	F-ratio
Total	0.29141	83	0.02665	
Years (A)	0.02665	1	0.00636	56.3319*
Stations (B)	0.03817	6	0.00306	13.4503*
Months (C)	0.01528	5		6.4609*
(AB)	0.08976	13		
(BC)	0.06584	41		
(AC)	0.20173	11		
(A x B)	0.02494	6	0.00416	8.7886*
(B x C)	0.01239	30	0.00041	0.8734
(A x C)	0.15981	5	0.03196	67.5708*
Error	0.01418	30	0.00047	

* - F is significant at 0.5% level

Table 4.8 3-way classification for testing significance of difference between (1) years (2) stations and (3) months for mud samples+

Source	S.S.	d.f.	M.S.S.	F-ratio
Total	13245.11	71.00	186.55	
Years (A)	2787.56	1.00	2787.56	276.55**
Stations (B)	676.78	5.00	135.36	13.43**
Months (c)	1205.11	5.00	241.02	23.91**
(AB)	3854.11	11.00	350.37	
(BC)	3231.11	35.00	92.32	
(AC)	7789.78	11.00	708.16	
(A x B)	389.78	5.00	77.96	7.73**
(B x C)	1349.22	25.00	53.97	5.35**
(A x C)	3797.11	5.00	759.42	75.34**
Error	252.00	25.00	10.08	

** - F is significant at 1% level

\+ ANOVA is carried out on original data, since F-ratio in Tukey's test of additivity was non-significant at 5% level.

Table 4.9 2-way classification for transformed data of filamentous fungi from water samples collected during 1986

Source	S.S.	d.f.	M.S.S.	F-ratio
Total	0.226868	41		
Stations	0.055993	6	0.009332	1.94267
Months	0.026760	5	0.005350	1.11413
Error	0.144114	30	0.004804	

Table 4.10 2-way classification for transformed data of filamentous fungi from water samples collected during 1987

Source	S.S.	d.f.	M.S.S.	F-ratio
Total	0.037899	41		
Stations	0.007118	6	0.001186	1.94036
Months	0.012438	5	0.002488	4.06869**
Error	0.018343	30	0.0006114	

** - F is significant at 1% level

Table 4.11 2-way classfication for transformed data of filamentous fungi from mud samples collected during 1986

Source	S.S.	d.f.	M.S.S.	F-ratio
Total	4.603350	35	0.131524	
Stations	0.807336	5	0.161467	2.18157
Months	1.945667	5	0.389134	5.25757**
Error	1.850345	25	0.074014	

** - F is significant at 1% level

Table 4.12 2-way classification for the original data of filamentous fungi from mud samples collected during 1987

Source	S.S.	d.f.	M.S.S.	F-ratio
Total	5031.88889	35	143.76825	
Stations	161.88889	5	32.37778	0.348581
Months	2547.88889	5	509.57778	5.486147*
Error	2322.1111	25	92.88444	

* - F is significant at 5% level

counts of both the water and mud samples were noticed and it was quite pronounced during 1987 as is seen from the relative F-values from the tables for the respective years for both water and mud samples. Thus the monthly effect on the total counts appears strikingly more important than the station difference and the high interaction effect between year and months in Tables 4.7 and 4.8 seems to be contributed largely by the factors that prevailed in 1987. The somewhat greater contribution to the total variability by the station difference during 1986 than during 1987, evenif statistically not significant (Tables 4.9 to 4.12) also suggest that 1986 and 1987 were ecologically different from each other for the fungal populations.

Duncan's multiple 't' test for water samples collected during 1986 showed the average abundance in stations 2 and 3 were relatively similar with the highest overall abundance in station 7 (202.00) and lowest in stations 2 and 3 (40.38 and 48.17). The similarity with total counts between stations 1,4,5,6 and 7 was suggested by Duncan's test although this is

not clearly evident from Fig. 4.6. September (156.57) and March (55.43) were the months of maximum and minimum overall abundance.

For 1987 grouping of stations were (a) stations 1 and 2, (b) stations 3,4,5 and 6 and (c) station 7 which also registered the highest average fungal abundance (364.67) as against the lowest at station 3 (100.67); July (241.71) and January (72.00) being the respective periods of maximum and minimum overall abundance. Likewise the grouping of months were (a) January, (b) March and May and (c) July and September.

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As in water samples the overall fungal counts in mud samples were also lowest in station 2 (08.67 in 1986 and 26.83 in 1987) and 3 (26.83 in 1987) although the highest count for both the years were in station 1 (21 in 1986 and 32.17 in 1987). The lowest count in the mud during 1986 was also in March (07.67) and the highest in January (31.50) but in 1987 maximum average abundance was in May (42.50) and the minimum in November (17.50).

Some refinements in the conclusions on similarities and differences between stations, months etc., based on ANOVA and Duncan's multiple 't' test using gross total counts was possible when the data is further examined in terms of richness of species. From Table 4.13, 1986 March was a distinctly lean month in terms of number of species and their abundance in samples whereas in 1987 largest number of

species and uniformly high relative abundance were recorded in March although richness of species in May was also equally high. Monthly differences between the two years were also more striking during the first half of the year. Such comparison of the species counts for each station during the two years do not show clear station difference although overall abundance of species and their counts in all stations were higher in 1987.' The ANOVA test in total counts

Table 4.13 Total number of isolates and their abundance for different months in water and mud samples collected at different stations during 1986 and 1987*

Stations	Jan	Mar	May	Jul	Sep	Nov
			Water samples 1986			
1	2(84)	1(52)	2(44)	6(116)	4(140)	5(92)
2	2(60)	1(36)	2(20)	2(16)	2(105)	1(8)
3	1(76)	1(36)	1(4)	2(24)	6(101)	2(48)
4	1(112)	2(48)	2(88)	4(50)	2(130)	4(144)
5	1(12)	2(24)	2(180)	4(96)	3(250)	3(60)
6	4(60)	4(112)	2(32)	4(52)	3(294)	2(52)
7	5(72)	5(80)	6(260)	5(640)	5(76)	4(84)
			1987			
1	5(68)	4(168)	4(178)	4(132)	4(56)	4(164)
2	3(116)	6(344)	5(60)	3(60)	4(397)	2(56)
3	3(28)	3(208)	2(72)	3(84)	2(166)	1(48)
4	2(80)	3(124)	3(270)	4(64)	1(36)	2(108)
5	4(32)	3(204)	3(165)	4(116)	2(180)	2(48)
6	3(32)	6(396)	3(105)	3(96)	2(84)	4(48)
7	4(248)	4(172)	7(350)	6(1140)	5(200)	5(188)
			Mud samples 1986			
1	8(61)	2(4)	3(6)	4(8)	6(21)	6(26)
2	3(8)	1(2)	4(7)	5(8)	5(21)	6(6)
3	4(36)	2(17)	2(15)	5(29)	7(12)	4(29)
4	6(35)	2(3)	0	3(7)	6(30)	4(19)
5	6(36)	3(14)	3(14)	3(5)	3(28)	2(12)
6	4(23)	4(6)	3(16)	3(6)	6(18)	4(13)

Table 4.13 (cont./)..

Table 4.13 (continued)

Stations	Jan	Mar	May	Jul	Sep	Nov
			Mud samples 1987			
1	10(35)	7(23)	8(47)	7(28)	4(36)	5(24)
2	4(26)	7(23)	5(44)	5(19)	3(30)	5(17)
3	5(22)	8(30)	5(31)	4(26)	5(46)	2(6)
4	5(15)	6(36)	6(42)	4(29)	2(21)	2(21)
5	8(26)	10(51)	6(37)	5(32)	2(18)	3(20)
6	5(10)	7(46)	8(55)	3(14)	4(32)	3(17)

* - Numbers in parenthesis indicates total counts per litre for water samples and counts in units of 10^2/gm for mud samples

has also brought out the more distinct monthly difference than the station difference in the fungal composition and abundance in the samples. As per Duncan's multiple 't'test in 1987, July was the month of overall abundance for water samples and May for mud samples; from Table 4.13 and Shannon Weaver diversity index which gives weightage both to the number of species and their uniformly high representation, March, May and July provided almost equally favourable conditions for mycopopulations in the backwater.

The diversity indices given in Figs 4.12 and 4.13 also indicate certain other interesting features. The year 1986 was a period of relatively low diversity and also subjected to more extreme monthly fluctuations. In 1987 high values of diversity indices between 2.5 and 3 were recorded in many stations. Annual and monthly fluctuations did not show any definite trend except that station 7 is distinct from all the

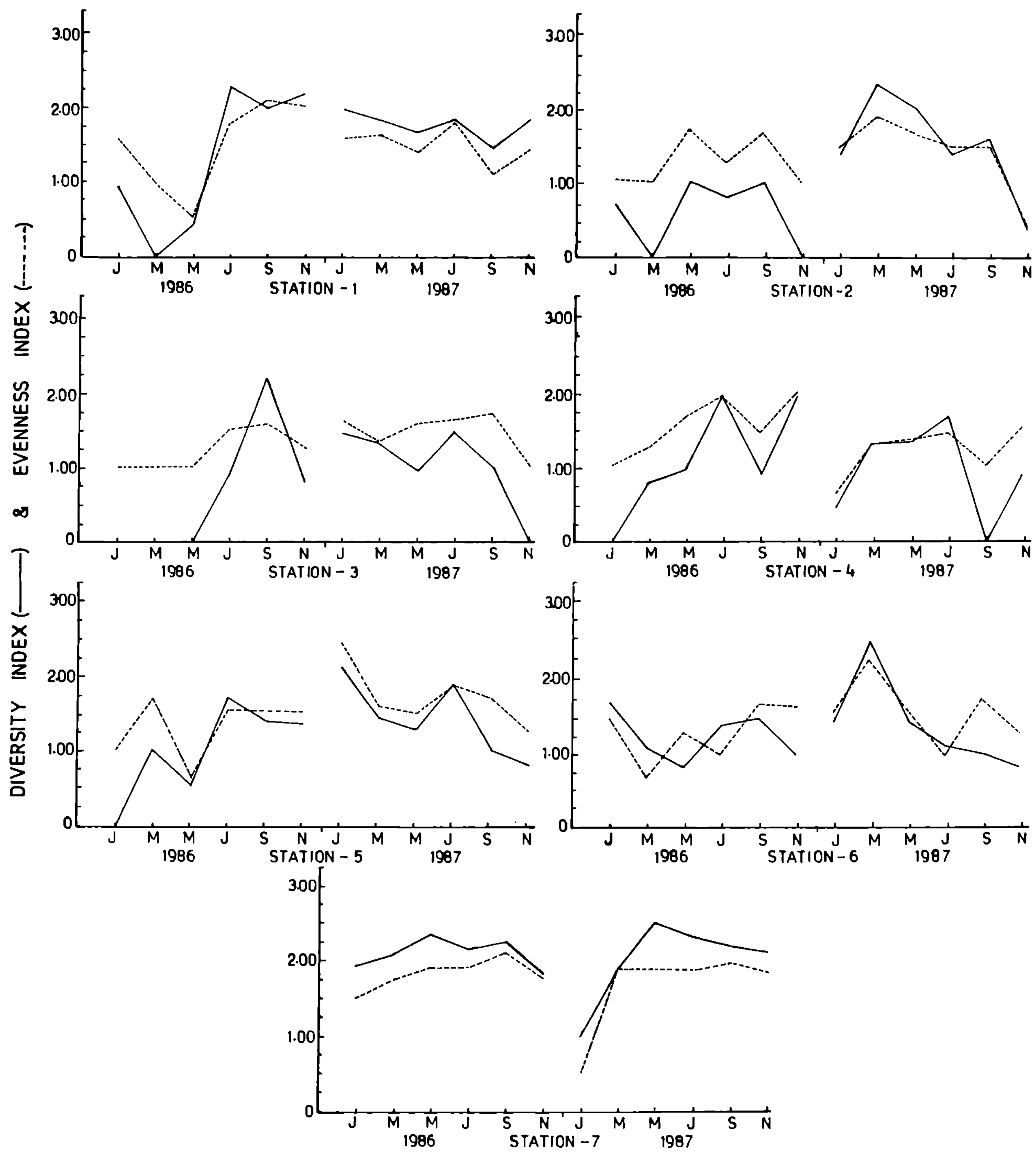

Fig. 4.12 Species diversity and evenness index for fungal species in water samples collected at different stations during 1986 & 1987.

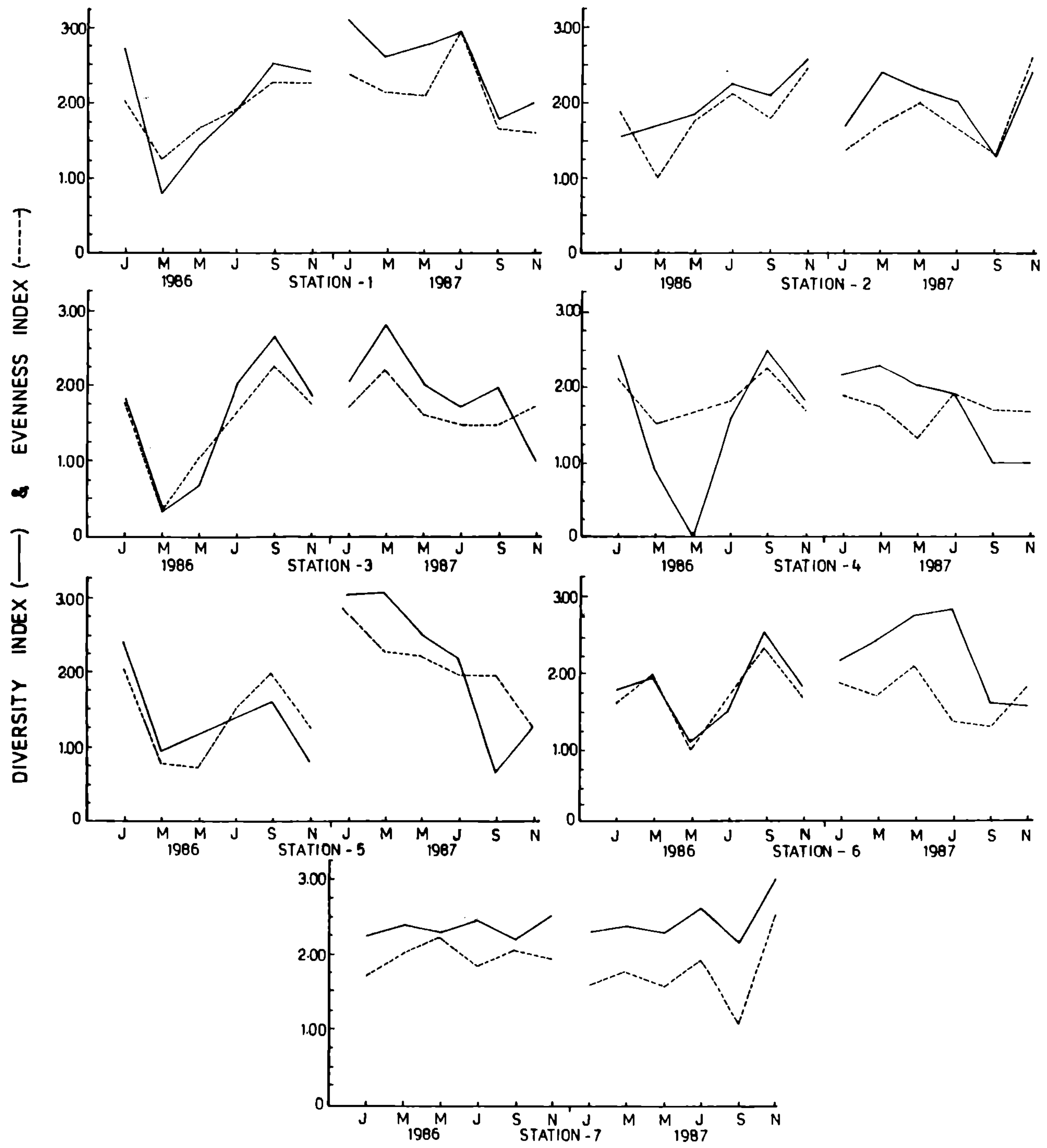

Fig. 4.13 Species diversity and evenness index for fungal species in mud samples collected at different stations during 1986 & 1987.

other stations with a high degree of uniform values in both the years. Comparison of diversity indices for the two years also suggest that in 1986 lower values were recorded during the premonsoon months, increasing sharply with the onset of monsoon. In 1987 a reverse trend was suggested by the graphs both in water and mud samples, another important feature that makes this year distinct from 1986 for the mycoflora. Conditions in station 7 seems to be favourable for the occurrence of more number of species (Figs 4.8 - 4.11).

Heip's evenness index estimated separately for each station for the two years also showed high uniformity in the distribution of the species and their numerical abundance in station 7 (Figs 4.12 and 4.13). In all other stations the evenness index calculated for each month showed more or less similar pattern as that of the diversity index.

The similarity of stations based on commonness of species and their relative abundance was examined by the Trellis diagram (Figs 4.14 to 4.17), separately for water and mud samples. The affinity indices indicated by the shaded circles showed strikingly different pattern of distribution for the years 1986 and 1987 both with respect to stations and months. During 1986 lower percentage of common occurrence between stations was noticed during months when the number of species were rather high in the samples. But such a correlation was not seen in 1987.

From 1986, January showed highest affinity index for the

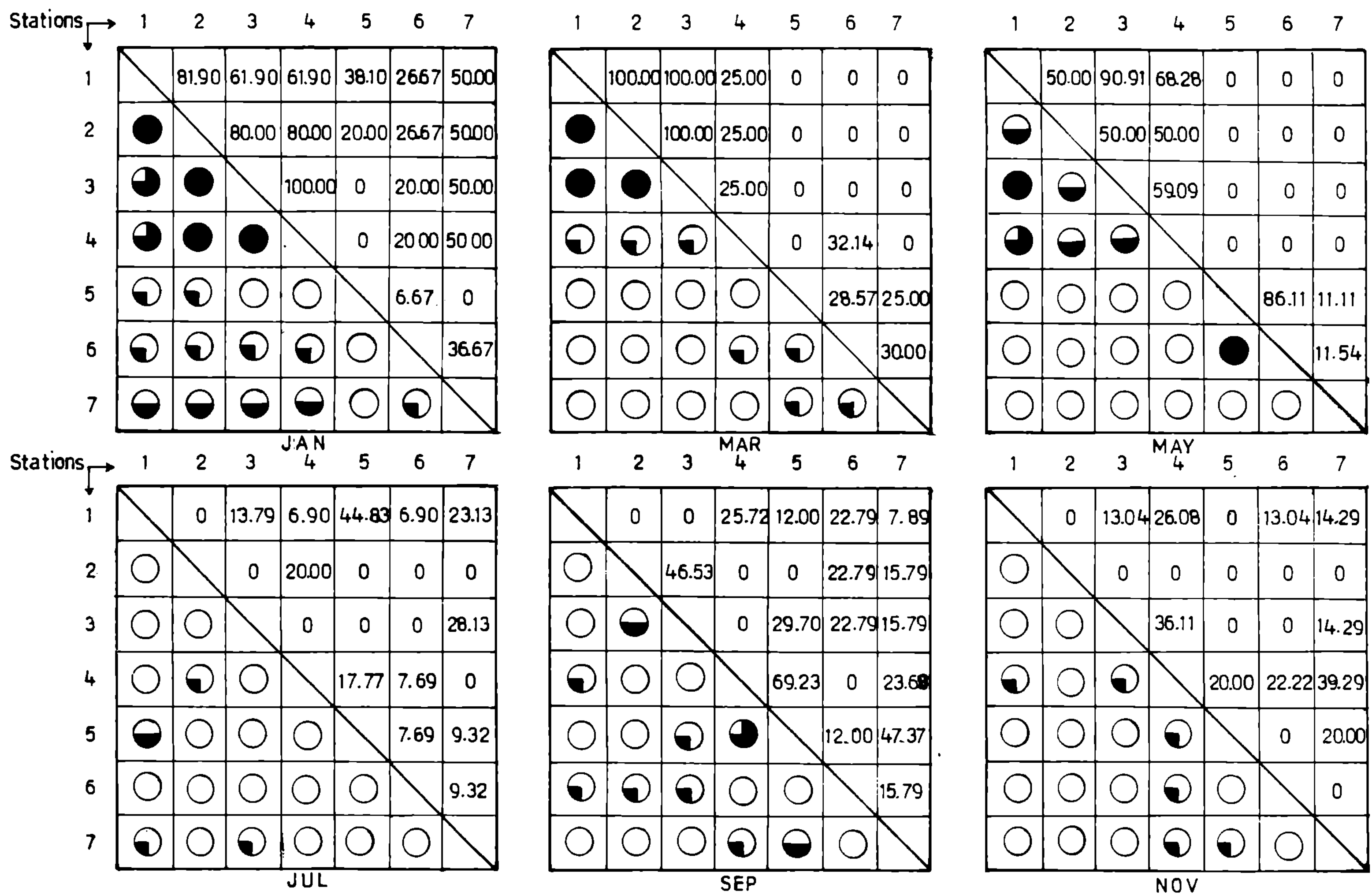

Fig. 4.14 Trellis diagram showing percentage affinity index between stations for the fungi in water samples – 1986

○ <20% ◔ 20 – 40% ◑ 40–60% ◕ 60-80% ● 80 – 100%

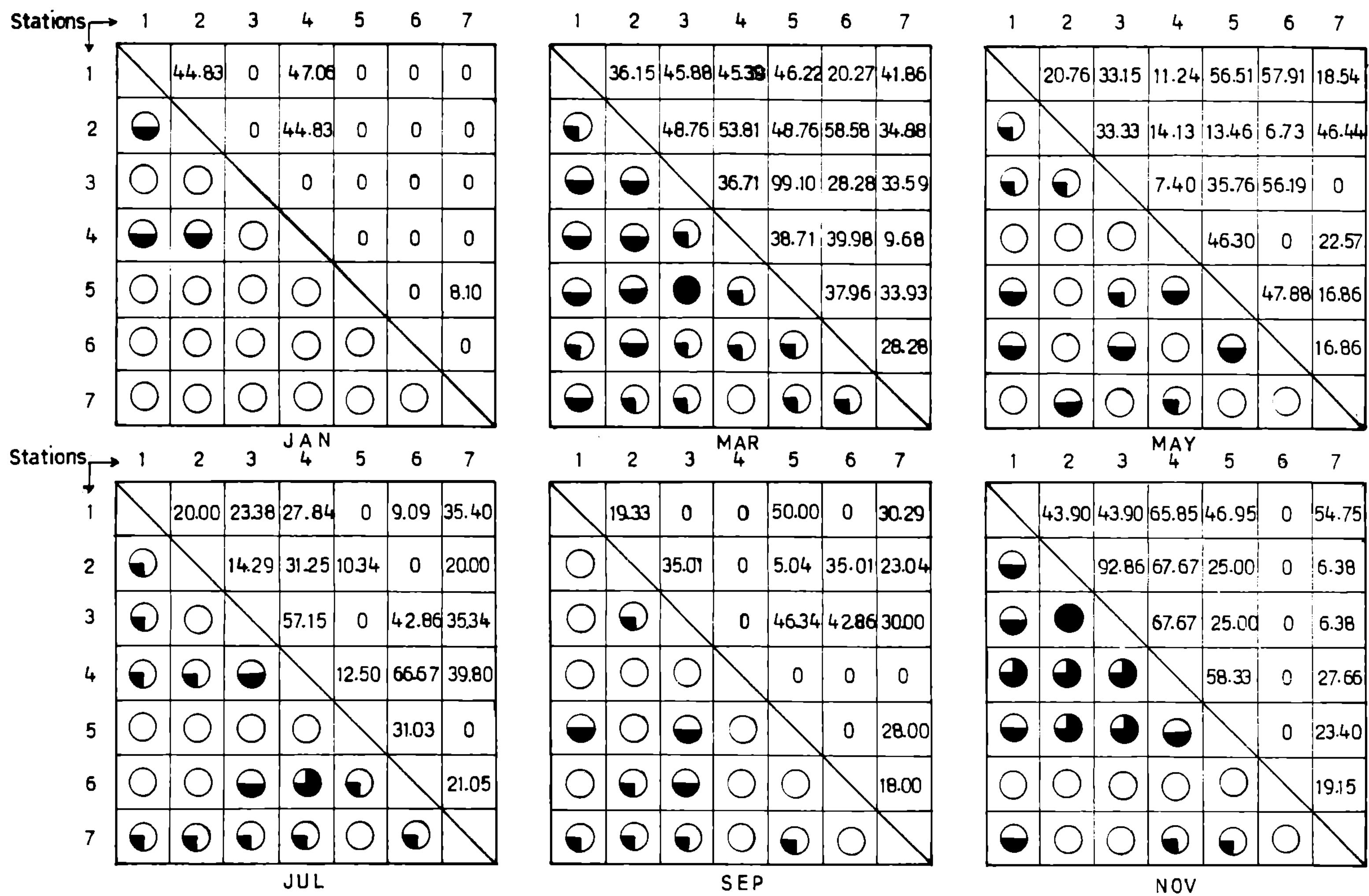

Fig. 4.15 Trellis diagram showing percentage affinity index between stations for the fungi in water samples — 1987

○ 20% ◔ 20 – 40% ◑ 40-60% ◕ 60-80% ● 80 -100%

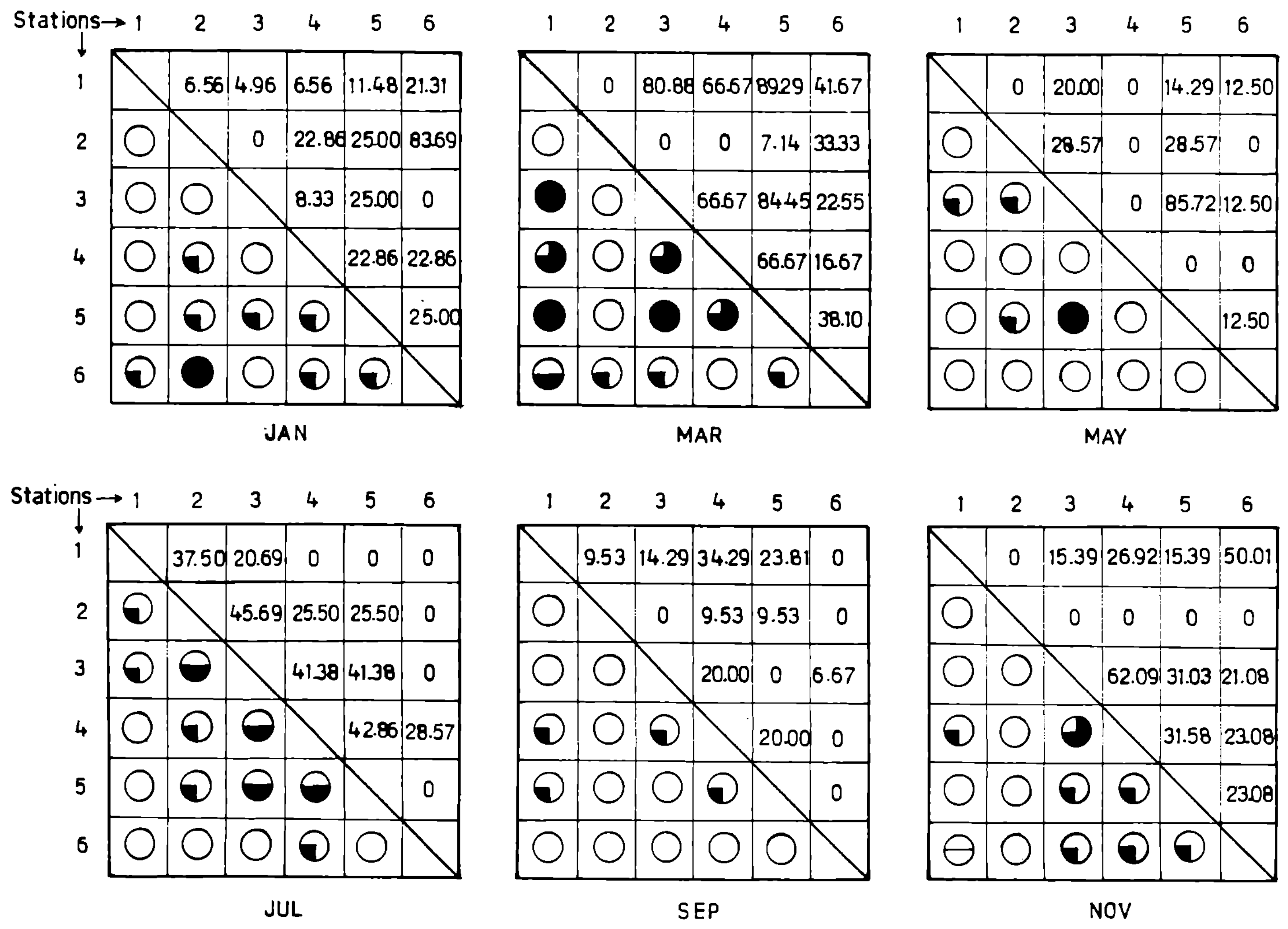

Fig. 4.16 Trellis diagram showing percentage affinity index between stations for the fungi in mud samples – 1986

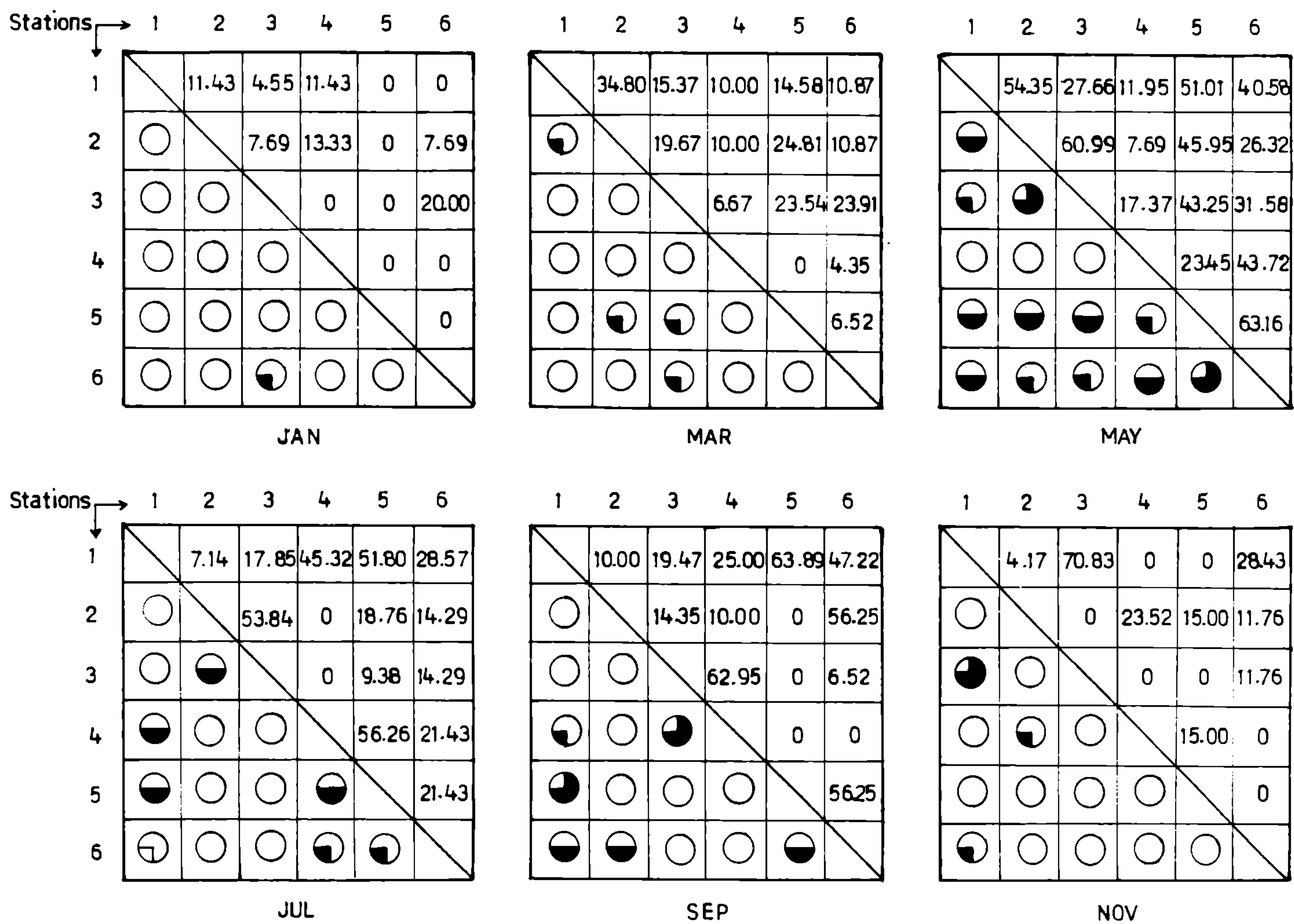

Fig. 4.17 Trellis diagram showing percentage affinity index between stations for the fungi in mud samples – 1987

○ < 20% ◔ 20–40% ◑ 40–60% ◕ 60–80% ● 80–100%

water samples and March for the mud samples. From Table 4.13 these two months also had the minimum representation of fungal species in the water and mud samples. Affinity index for the water samples was lowest during the more abundant months, July, September and November. In 1987 on the other hand the number of species were significantly higher throughout the year in all stations. Percentage of affinity indices between stations estimated for the different months for water and mud samples did not show any relation with the corresponding diversity index.

The negative correlation between the number of species and percent common occurrence in 1986 and the absence of any such relation in 1987 despite the higher richness of species referred above suggest independent occurrence of fungal species both in water and mud samples. Because of the large number of fungal species in the samples, species association could not be examined statistically. However the occurrence and relative abundance of different species given in Figs 4.8 to 4.11 also support this conclusion since no pairs of species were seen together in 50% of the water or mud samples collected during any of the two years (Fager, 1957).

The above analysis of the fungal composition and abundance over the two years in the Cochin backwater clearly indicate that 1987 was ecologically different from 1986 and provided more favourable and relatively uniform environment for the mycopopulations. However an examination of the various environmental factors did not reveal any striking

difference in the range and distribution of various environmental parameters such as DO, pH, Eh etc., during the two years except for temperature which was significantly higher in certain months in 1987 especially during March, July, September and November and to a lesser extent in salinity. This was due to the weak and delayed monsoon in 1987. Hughes (1960) made many hydrographical measurements throughout the period of his study, but found the variations in DO content, pH, nitrate and phosphate concentration of the water had no apparent effect on the distribution of species within the estuary. Höhnk (1952a,b) however found an obvious distribution pattern of aquatic Phycomycetes in estuarine waters, which could be correlated to salinity. Subramanian and Raghukumar (1974) also could not correlate the fungal populations with environmental factors. The striking difference in the abundance between the two years may perhaps be due to presence or absence of adequate microbial substratum such as decaying plant litter. Although organic carbon content of the mud in the two years were very similar, the contribution of decaying plant litter accumulating in and around Cochin bar mouth region may be more in 1987, tidal and monsoonal flushing being less during this year.

Yeasts

The qualitative study of yeast flora of the estuarine system revealed a total of 35 species belonging to 11 genera (Table 4.14). *Candida* was the dominant genus represented

by 12 species followed by *Rhodotorula* with six species. Both in water and mud the dominant genera were *Candida* and *Rhodotorula*. The occurrence of yeast species for different months were recorded for each station (Table 4.15 to 4.18). Unlike the filamentous fungi yeasts showed certain characteristics in their occurrence and distribution. Yeasts were also predominantly independent in their distribution but unlike filamentous fungi they were found to occur together more frequently in the samples. Certain species were found to be confined to certain stations. Environmental differences such as variations in salinity, presence of pollutants etc., also exerted greater influence on the distribution of yeasts in different months and stations. Of the 35 species isolated from water samples 28 species were recorded in more than 3 stations. Among the 16 yeast species isolated from the mud samples, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Geotrichum candidum*, *Rhodotorula minuta* and *R. rubra* occurred in more than three stations. Species such as *C. tropicalis* and *C. albicans* were the most common species in the water samples but not so in the mud samples. The white yeasts *Debaryomyces marama*, *D. vanriji* and *C. krusei* appeared only in stations 1 and 2 whereas *Pichia guilliermondii* in stations 2 and 5,

Table 4.14 Species of yeasts isolated from different sampling sites of Cochin backwater during 1986 and 1987

Ascosporogenous yeasts

Debaryomyces hansenii (Zopf) Lodder et Kreger-van Rij
D. marama Di Menna
D. vanriji (van Der Walt et Tscheuschner) Abadie, Pignal et Jacob var. *vanriji*
Hansenula anomala (Hansen) H. et P. Sydow
Kluyveromyces marxianus (Hansen) van Der Walt var. *drosophilarum*
Pichia bovis van Uden et Do Carmo-Souza
P. guilliermondii Wickerham
Saccharomyces cerevisiae Meyen et Hansen
S. exiguus Reess ex Hansen
S. kluyveri Phaff, Miller et Shifrine
Saccharomyces sp.

Imperfect yeasts

Candida albicans (Robin Berkhout
C. atmospherica Santa Maria
C. halophila Yarrow et Meyer
C. intermedia (Ciferri et Ashford) Langeron et Guerra
C. krusei (Castellani) Berkhout
C. membranaefaciens (Lodder et Kreger van-Rij) Wickerham et Burton
C. parapsilosis (Ashford) Langeron et Talice
C. pseudointermedia Nakase, Komagata et Fukazawa
C. sake (Saito et Ota) van Uden ey Buckley
C. solani Lodder et Kreger-van Rij
C. tropicalis (Castellani) Berkhout
Candida sp.
Cryptococcus laurentii (Kufferath) Skinner
Geotrichum candidum Link ex Pers.
Rhodotorula aurantiaca (Saito) Lodder
R. glutinis (Fresenius) Harrison
R. graminis Di Menna
R. lactosa Hasegawa
R. minuta (Saito) Harrison
R. rubra (Demme) Lodder
Trichosporon aquatile Hedrick et Dupont
T. cutaneum (De Berum, Gougerot et Vaucher) Ota
T. penicillatum (Do Carmo-Souza) von Arx
Sporobolomyces roseus Kluyver et van niel

C. halophila, *C. solani* and *Trichosporon aquatile* occurred only in station 2. These species not only showed restricted occurrence but also failed to build up populations. They may

Table 4.16 Species of yeasts isolated from the water samples of Cochin backwater and their occurrence in different stations for the year 1987

Sl.No.	Taxa	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	Debaryomyces hansenii	x	x	x				x	x	x					x	x					x						x	x														x	
2.	D. marama	x								x	x																																
3.	D. vanriji			x						x																																	
4.	Hansenula anomala		x							x																																	
5.	Kluyvermyces marxianus			x				x	x	x	x				x	x																											
6.	Pichia bovis			x				x	x		x															x																	
7.	P. guilliermondii							x	x																																		
8.	Saccharomyces cerevisiae							x	x	x					x								x			x													x			x	
9.	S. exiguus			x	x									x																													
10.	S. kluyveri															x				x						x		x										x					
11.	Saccharomyces sp.				x									x		x				x					x	x		x										x					
12.	Candida albicans	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x				x	x					x	x			x	x	x		x	x	x
13.	C. atmospherica		x							x					x	x					x					x	x																
14.	C. halophila									x																																	
15.	C. intermedia		x							x	x									x													x										
16.	C. krusei		x							x																																	
17.	C. membranaefaciens			x																													x						x				
18.	C. parapsilosis		x	x							x				x	x					x				x		x	x															x
19.	C. pseudointermedia			x						x						x																											
20.	C. sake									x																												x					
21.	C. solani									x																																	
22.	C. tropicalis	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x		x	x	x	x	x		x		x		x	x	x	x	x
23.	Candida sp.											x		x								x						x														x	
24.	Cryptococcus laurentii					x	x					x	x			x						x						x				x		x		x	x						
25.	Geotrichum candidum		x			x									x		x	x	x										x	x									x	x			
26.	Rhodotorula aurantiaca							x	x	x		x																					x										
27.	R. glutinis	x						x	x	x																																	
28.	R. graminis							x	x	x																																	
29.	R. lactosa							x	x	x																							x										
30.	R. minuta	x		x		x	x		x	x			x			x						x				x	x	x			x	x	x	x	x	x	x						
31.	R. rubra	x		x	x		x		x	x	x	x	x	x			x		x			x		x	x		x	x	x	x			x	x		x							
32.	Sporobolomyces roseus	x	x						x	x																	x	x					x	x									
33.	Trichosporon aquatile									x																																	
34.	T. cutaneum		x			x									x		x		x	x		x	x									x							x	x			
35.	T. penicillatum		x																x														x						x	x			

Table 4.15 Species of yeasts isolated from the water samples of Cochin backwater and their occurrence in different stations for the year 1986

Sl.No.	Taxa	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	Debaryomyces hansenii	x	x	x					x	x				x	x	x										x	x	x						x									
2.	D. marama								x	x	x																																
3.	D. vanriji		x	x					x	x																																	
4.	Hansenula anomala	x	x							x						x																											
5.	Kluyvermyces marxianus			x							x				x	x				x																							
6.	Pichia bovis				x			x							x											x																	
7.	P. guilliermondii							x																		x																	
8.	Saccharomyces cerevisiae							x	x						x	x			x				x																			x	x
9.	S. exiguus	x	x	x					x	x																x								x									
10.	S. kluyveri	x	x					x						x						x														x									
11.	Saccharomyces sp.			x	x					x																x																x	
12.	Candida albicans	x	x	x	x			x	x	x				x	x	x			x	x	x		x		x	x	x	x			x		x	x		x	x	x	x	x		x	x
13.	C. atmospherica	x	x								x			x	x					x	x						x	x					x					x					x
14.	C. halophila								x	x																																	
15.	C. intermedia	x						x		x				x		x																x	x										
16.	C. krusei																																										
17.	C. membranaefaciens			x					x	x				x																			x										
18.	C. parapsilosis	x	x	x					x	x	x				x					x	x			x			x	x										x				x	
19.	C. pseudointermedia			x						x						x				x																							
20.	C. sake		x						x																			x										x					
21.	C. solani							x																																			
22.	C. tropicalis			x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x		x	x	x				x	x	x		x	x			x	x	x	x	x
23.	Candida sp.									x				x								x		x	x				x			x		x	x			x	x		x		
24.	Cryptococcus laurentii					x	x					x	x			x						x				x						x		x		x	x						
25.	Geotrichum candidum	x					x								x		x				x	x				x				x		x		x									
26.	Rhodotorula aurantiaca					x			x																					x			x										
27.	R. glutinis		x							x																			x														
28.	R. graminis	x	x							x		x																	x														
29.	R. lactosa	x	x					x	x	x																				x			x										
30.	R. minuta			x		x	x		x	x	x	x	x			x			x			x				x	x	x		x	x		x		x	x	x						
31.	R. rubra	x	x	x	x	x		x		x	x	x	x					x						x	x		x	x	x	x				x									
32.	Sporobolomyces roseus																																	x									
33.	Trichosporon aquatile																																										
34.	T. cutaneum			x													x		x			x		x				x				x											
35.	T. penicillatum																															x											

Table 4.17 Species of yeasts isolated from the mud samples of Cochin backwater and their occurrence in different stations for the year 1986

Sl.No.	Taxa	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	*Debaryomyces hansenii*																																					x					
2.	*Saccharomyces cerevisiae*				x	x											x				x	x							x										x				
3.	*Candida albicans*																																										
4.	*C. intermedia*																																										
5.	*C. membranaefaciens*															x																											
6.	*C. parapsilosis*															x																											
7.	*C tropicalis*				x	x		x			x									x	x					x													x			x	
8.	*Candida* sp.																x																										
9.	*Cryptococcus laurentii*																																	x									
10.	*Geotrichum candidum*																													x						x		x	x	x	x		x
11.	*Rhodotorula graminis*																																	x									
12.	*R. lactosa*																											x															
13.	*R. minuta*	x	x		x	x	x				x	x			x			x					x	x			x	x	x	x	x	x	x									x	
14.	*R. rubra*					x		x	x	x	x							x					x	x		x																	
15.	*Trichosporon aquatile*																																										
16.	*T. penicillatum*																																							x			

Table 4.18 Species of yeasts isolated from the mud samples of Cochin backwater and their occurrence in different stations for the year 1987

Sl.No.	Taxa	Station 1						Station 2						Station 3						Station 4						Station 5						Station 6						Station 7					
		J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N	J	M	M	J	S	N
1.	*Debaryomyces hansenii*												x																														
2.	*Saccharomyces cerevisiae*												x				x				x	x							x										x				x
3.	*Candida albicans*									x																																	
4.	*C. intermedia*	x								x																																	
5.	*C. membranaefaciens*									x																																	
6.	*C. parapsilosis*																																										
7.	*C tropicalis*	x									x									x	x																		x	x	x		
8.	*Candida* sp.															x																											
9.	*Cryptococcus laurentii*																																				x						
10.	*Geotrichum candidum*								x																					x					x			x	x	x	x	x	x
11.	*Rhodotorula graminis*																																										
12.	*R. lactosa*																																										
13.	*R. minuta*		x		x	x		x	x		x	x	x		x			x	x			x	x	x		x	x	x	x	x	x	x	x	x	x	x	x						
14.	*R. rubra*	x	x		x	x		x																x		x		x	x														
15.	*Trichosporon aquatile*																																					x					
16.	*T. penicillatum*								x																																		

be more stenoplastic to the variations in the backwater compared to other species. Except *D. marama* all the above mentioned species may be stenohaline because they appeared only during premonsoon months.

Among red yeasts *Rhodotorula minuta* and *R. rubra* were present both in water and mud samples from most of the stations and hence appears more tolerant to environmental variations. On the other hand the species *R. aurantiaca*, *R. graminis*, *R. glutinis*, *R. lactosa* and *Sporobolomyces roseus* were least common. However they occurred in stations 1 and 2 including during monsoon months. These were totally absent from the stations 3, 4 and 7. They also occurred sporadically in stations 5 and 6.

Considerable variations in the species composition and total yeast counts were also seen in both the water and mud samples. Table 4.19 gives the number of species represented

Table 4.19 Data on the yeasts isolated from water and mud samples of Cochin backwater during 1986 & 1987

No. of yeast species isolated	Water		Mud	
	Genera	Species	Genera	Species
Station 1	11	29	3	5
Station 2	10	32	6	10
Station 3	10	22	3	6
Station 4	8	17	3	4
Station 5	9	23	4	6
Station 6	8	18	3	4
Station 7	5	14	6	7

in water and mud samples from different stations during 1986 and 1987. Figs 4.18 and 4.19 similarly indicate spatio-temporal variations in the total number of colony forming units (c.f.u.). It was higher in water samples and varied from 1.6×10^{2} to 1.6×10^{4} c.f.u. per litre and in the mud samples it ranged from 0 to 9×10^{3} c.f.u. per gram. In stations 1 and 2 species density as well as their total counts in water samples were high in both the years with a maximum of 1.6×10^{4} c.f.u. in May 1987. The counts were much lower in stations 3,5 and 6. In all stations yeast counts in the water samples were more abundant in 1986 during premonsoon period. The same trend was also seen in 1987 with less variations at stations 3,5 and 6. Yeast counts showed a different pattern in the mud samples. The species composition was uniformly low in all stations (Table 4.19) but counts as high as 9×10^{3} c.f.u. were found in station 7 but were much lesser in the remaining stations (Fig. 4.19). In contrast to water samples greater abundance in mud samples was noticed during monsoon-post monsoon months at stations 1 to 6, but in station 7 maximum abundance was recorded during the premonsoon months.

For the statistical examination the yeasts were broadly divided into two groups viz., white yeasts comprising species of the genera *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Candida*, *Cryptococcus*, *Geotrichum* and *Trichosporon* and red yeasts (producing pigments) comprising the species of the genera *Rhodotorula* and *Sporobolomyces*.

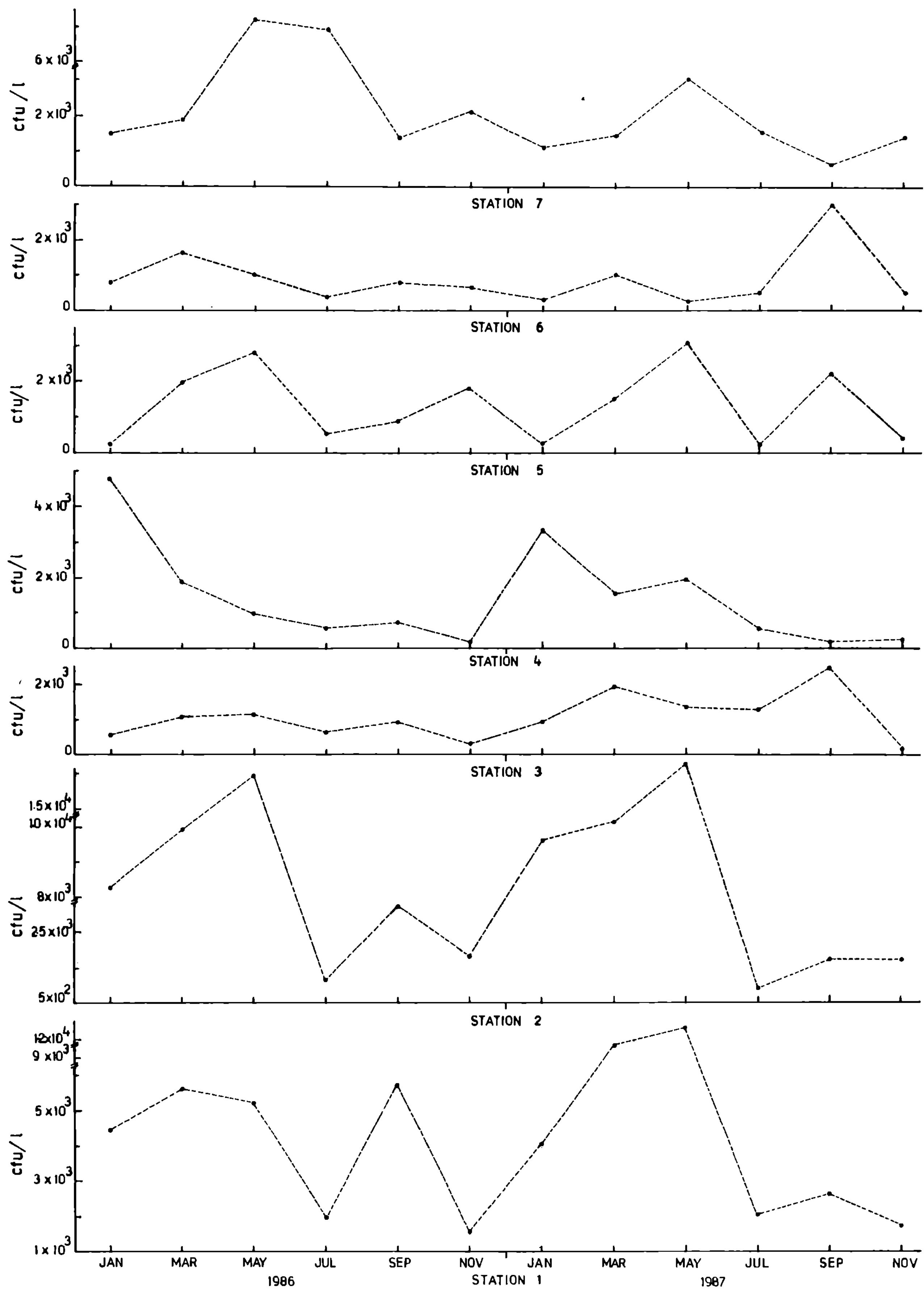

Fig. 4·18 Bimonthly variations of total yeast populations in water samples collected at different stations during 1986 & 1987

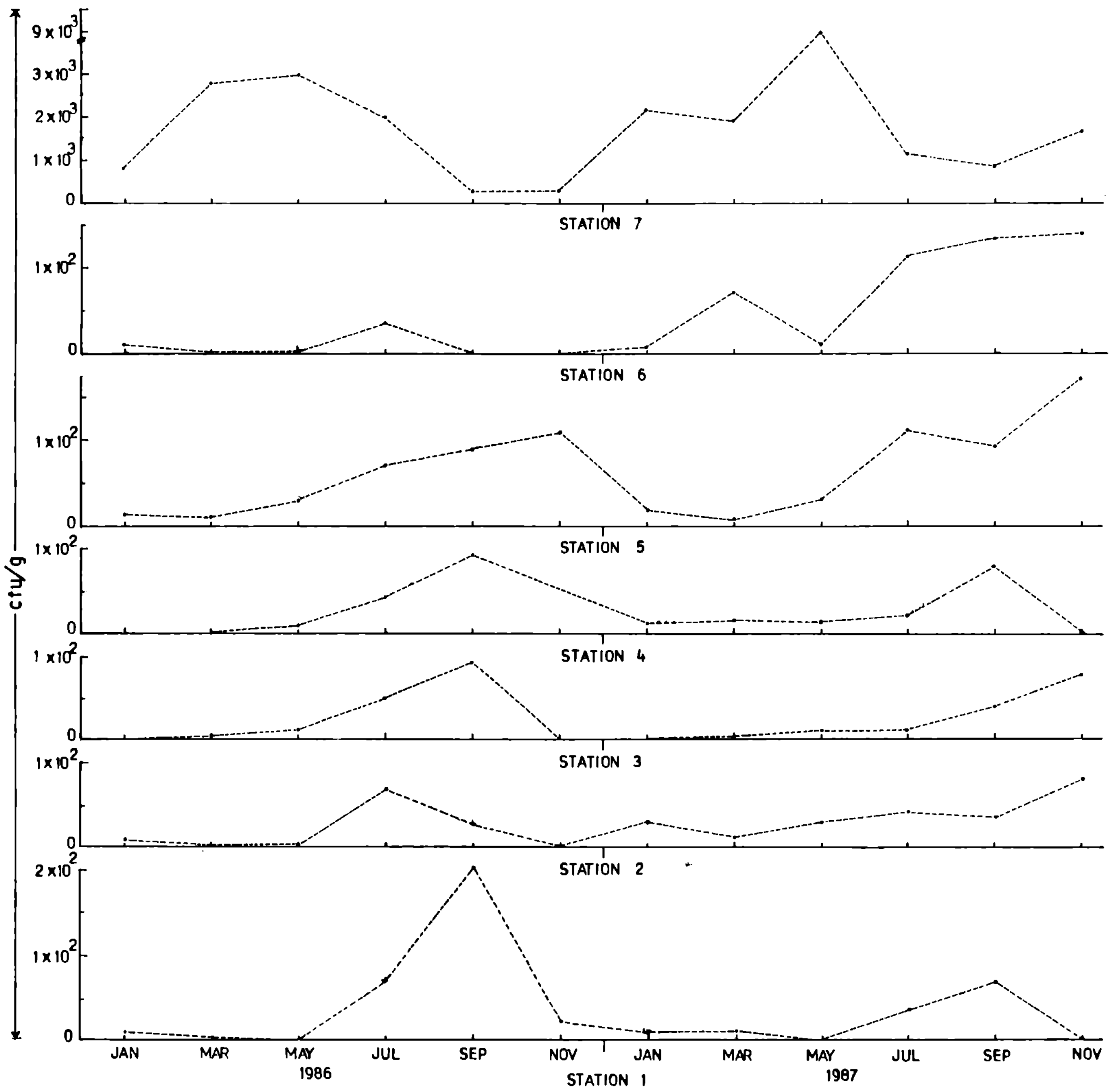

Fig. 4.19 Bimonthly variations of total yeast populations in mud samples collectted at different stations during 1986 & 1987

Individual species counts could not be taken as it was not possible to ascertain visually whether all yeast colonies having the same appearance belong to the same species. Sometimes isolates from colonies with slightly different appearance were found to belong to the same species (Beech and Davenport, 1971).

Percentage of red and white yeasts for water and mud samples for 1986 and 1987 (Figs 4.20 and 4.21) showed different distributional pattern. The white yeasts were present in all stations but the red yeasts were completely absent from station 7 in both years except mud samples collected in September, 1986. Total yeast counts were highest in stations 1 and 2 followed by station 7. Yet white yeasts contributed only a small percentage to the yeast count in stations 1 and 2. Red yeasts always formed the largest percentage in stations 1 and 2 in both the years followed by stations 5, 6, 3 and 4. Hinzelin and Lectard, (1978) in their study on River Mossele found that in clean and less polluted water, the white yeasts were absent or insignificantly found and only red pigmented yeasts were present. According to them abundance of red yeasts indicate less organic pollution. Station 7 is located in a eutrophicated region while stations 1 and 2 are subjected to greater tidal flushing. Stations 3 and 4 are both located near oil tanker jetties and are also under the influence of muncipal sewage discharge.

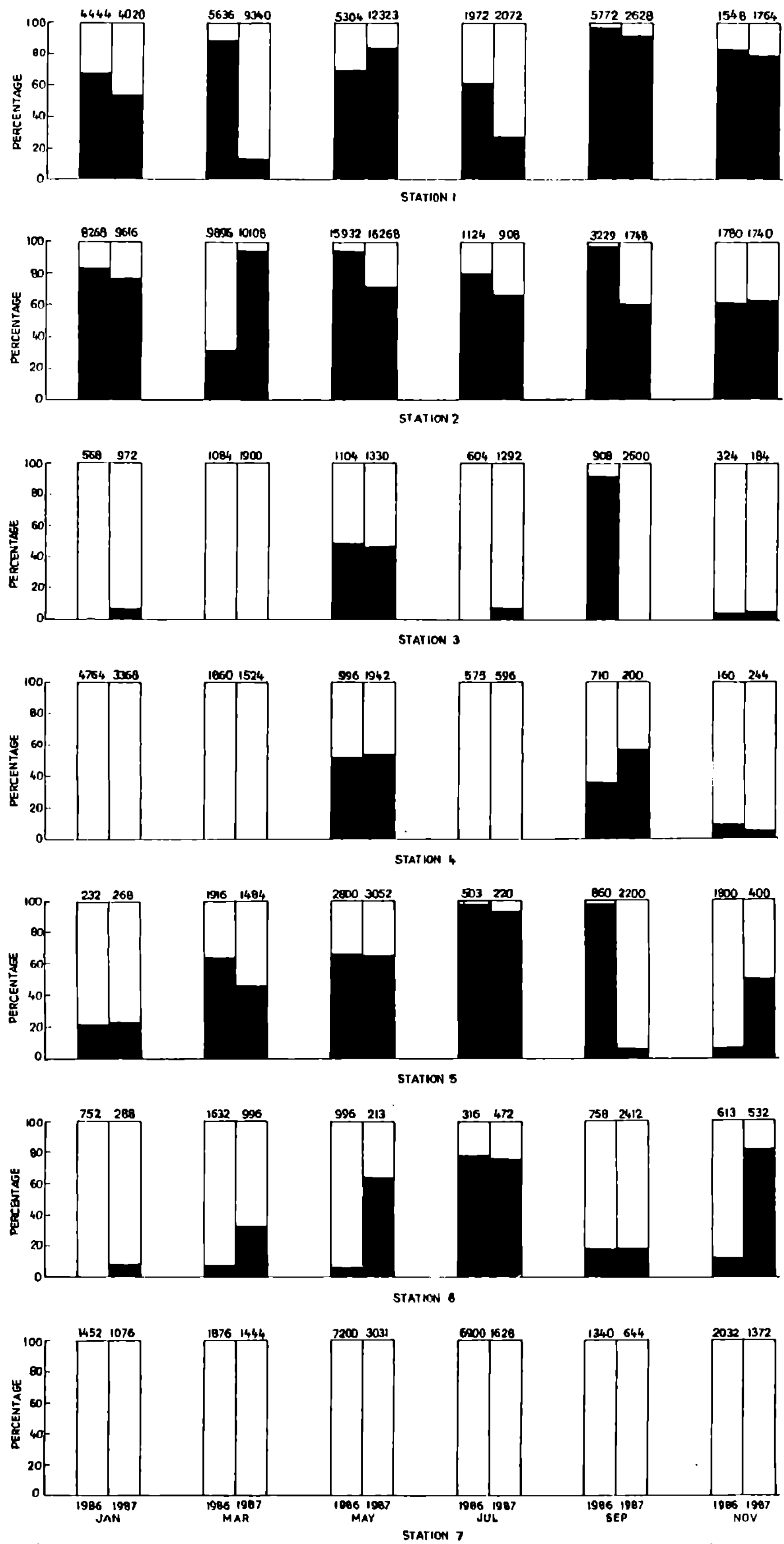

Fig. 4·20 Percentage bar diagram showing the occurrence of white and red yeasts in water samples at different stations, collected for the years 1986 & 1987.
■ Red yeasts; □ White yeasts and numbers above the bar diagram refer to total counts per litre

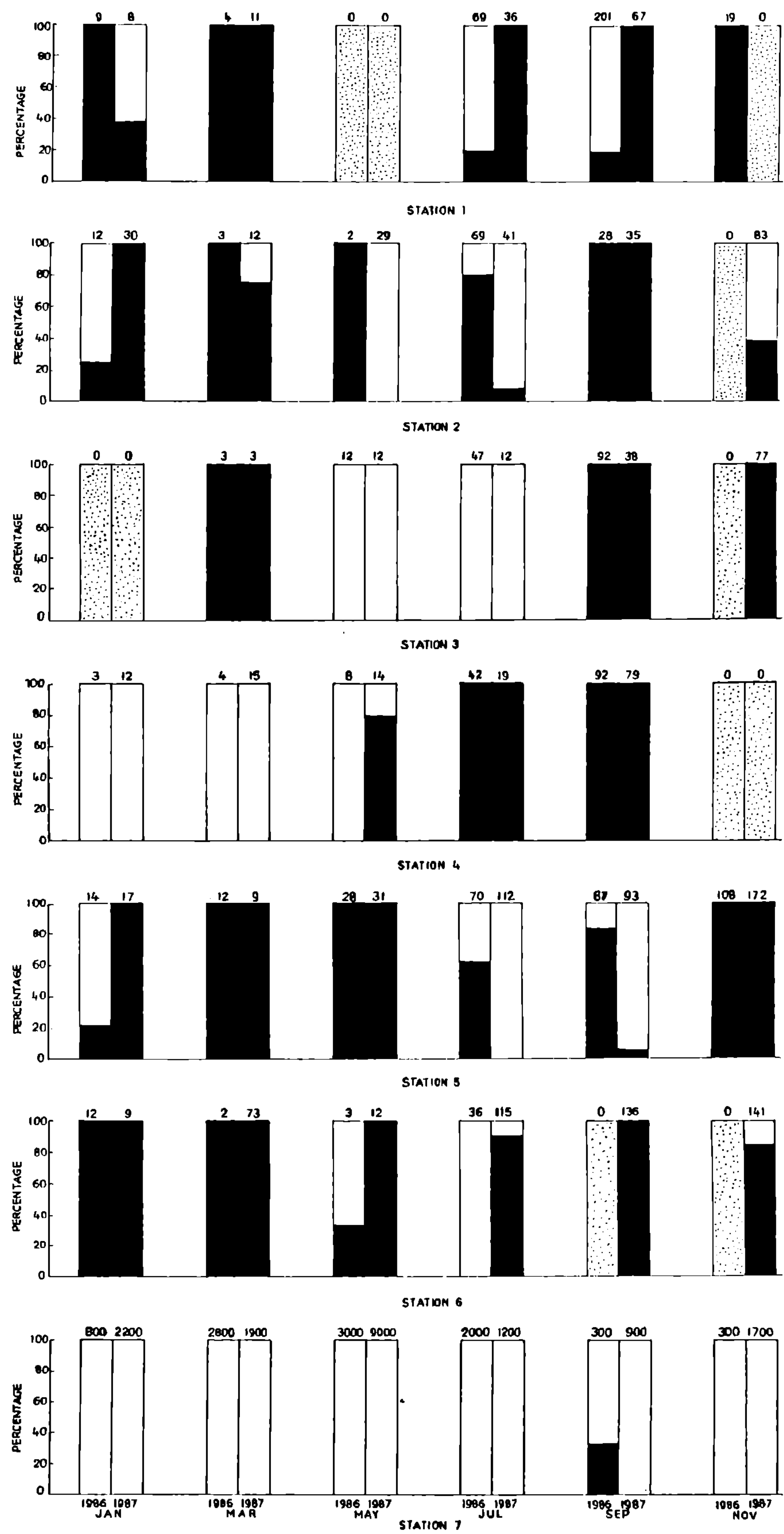

Fig. 4.21 Percentage bar diagram showing the occurrence of white and red yeasts in mud samples at different stations, collected for the years 1986 & 1987.
■ Red yeasts; □ White yeasts; ▒ No yeasts and numbers above the bar diagram refer to total counts per gram

Tukey's test of additivity carried out on white and red yeast counts separately for water and mud samples for the years 1986 and 1987 suggested appropriate transformation. The following transformation of the data were suggested for water and mud samples.

For water samples

$Y = (X + 1)^{-0.52}$ for white yeasts, 1986

$Y = (X + 1)^{-1.16}$ for white yeasts, 1987

$Y = (X + 1)^{-0.17}$ for red yeasts, 1986

$Y = \log_{10}(X + 1)$ for red yeasts, 1987

For mud samples

$Y = (X + 1)^{-0.29}$ for white yeasts, 1986

$Y = (X + 1)^{-0.86}$ for white yeasts, 1987

$Y = (X + 1)^{0.42}$ for red yeasts, 1986

$Y = (X + 1)^{0.15}$ for red yeasts, 1987

The data were next subjected to a 2-way ANOVA test using the total counts after the necessary transformation to examine stationwise and monthly differences for white and red yeasts separately (Table 4.20). Except for one significant F-value in 1987, the station and monthwise differences both in water and mud samples in respect of white and red yeasts were non-significant, whereas in 1986 significant station and monthwise differences were obtained for white and red yeasts in both the water and mud samples. As in the case of filamentous fungi, 1987 provided more uniform environment for yeasts.

Table 4.20 F-values from 2-way ANOVA for water and mud samples estimated separately for white and red yeasts for the two years, 1986 & 1987

		F-ratio 1986	F-ratio 1987
Water samples			
White yeasts			
	Stations	4.4168**	1.06190
	Months	10.94755**	1.01428
Red yeasts			
	Stations	10.2167**	11.68041**
Mud samples			
White yeasts			
	Stations	0.205624	0.867759
	Months	3.1004	0.7514691
Red yeasts			
	Stations	2.6705*	2.41507
	Months	3.2434*	1.6028

** - F is significant at 1% level
* - F is significant at 5% level

F-values also indicated distinct habitat differences between mud and water, since all the highly significant F-values were confined to water samples. Station and monthwise differences were more conspicuous in the water samples than in the mud. The latter was thus providing a more uniform environment. Significant station- and month-wise differences in the total counts were not shown by the white yeasts except for the water samples in 1986. Red yeasts on the otherhand showed highly significant stationwise differences in both the years for the water samples but not monthly differences. The station and monthly differences for the mud samples in 1986

however was significant at 5% level. Stationwise differences thus seems to be a more important factor for the abundance of red yeasts than the monthly differences.

Although certain species of yeasts were found to occur more frequently in different stations compared to filamentous fungi, Cole's point correlation coefficient analysis to examine their co-existence (Fig 4.22) showed only few pairs of species having high positive correlation value. It was also found that the same group did not occur consistently in any two stations, the groups being formed by different species. As in the case of filamentous fungi, yeasts also therefore exhibited independent occurrence.

From the above analysis of the data some striking features in respect of the distribution and abundance of filamentous fungi and yeasts were noticed. Independent occurrences in samples were shown by both the groups even when as many as 96 species of filamentous fungi and 35 species of yeasts could be included in the analysis. While majority of the species of filamentous fungi occurred at one time or other in all the stations, a more definite stationwise distribution was shown by yeast populations. Similarly, when the white yeasts were present in all the stations, its dominance in station 7 and the absence of red yeasts were very conspicuous. In fact it was found that the relative percentage of red and white yeasts along with their numerical abundance in different stations seems to be a good ecological index indicating the extent of organic pollution.

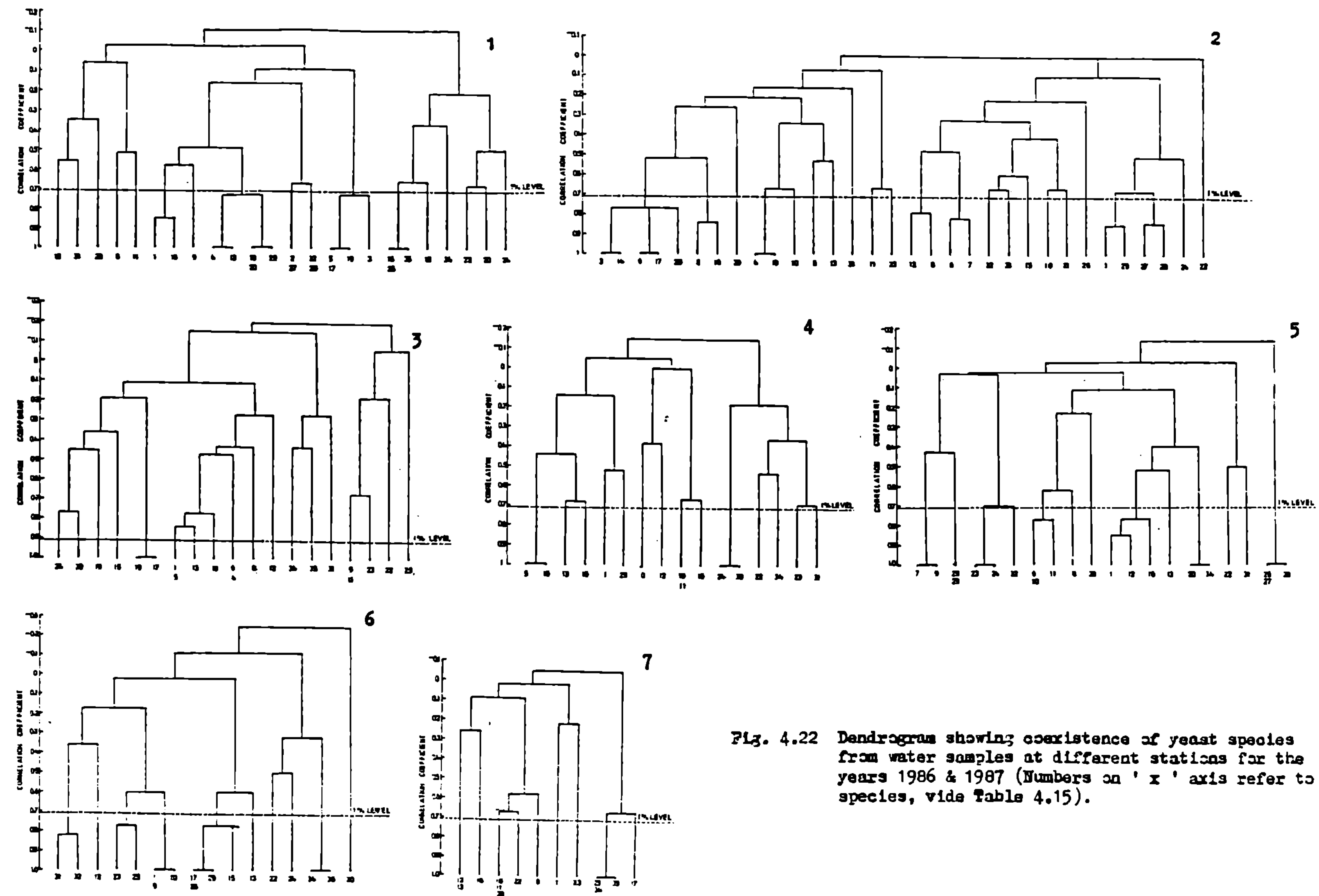

Fig. 4.22 Dendrograms showing coexistence of yeast species from water samples at different stations for the years 1986 & 1987 (Numbers on ' x ' axis refer to species, vide Table 4.15).

Filamentous fungi were more abundant in mud samples, while yeasts were much more abundant in the water samples, both in terms of species and numerical abundance. The year 1987 was conspicuous by the greater abundance of filamentous fungi. In the case of yeast populations this distinction could be brought out clearly only by ANOVA test.

CHAPTER 5

STUDIES ON MYCOFLORA WITH SPECIAL REFERENCE TO A MANGROVE ECOSYSTEM

Mangrove systems generally grow in areas that are protected from wave action, such as estuaries, bays and lagoons. They have a higher tolerance for salt and are characterized by the presence of pneumatophores, prop roots and vivipary. Mangrove forests serve as ecotones between land and sea where they create a living buffer and act as nutrient filter as well as synthesizer of organic matter (Garg *et al.*, 1984). They contribute nutrients, organic matter and detritus to the adjoining coastal ecosystems and thus support the various pelagic and benthic communities of the waters of the continental shelf. They are often associated with rich fisheries and often nicknamed as " nutrient banks." They are excellent abodes for marine macro- and microorganisms. The extensive anastomosing net work of pneumatophores and prop roots cause the deposition of mud and silt brought by rivers and this leads to the formation of land. The significance of mangroves lies not only in building islands but also in protecting shores from erosion.

The mangrove mud is a mixture of plant litter and soil brought by rivers. The soil is usually deep black, soft in nature and rich in organic matter which are reduced to detritus by microbial decomposition. The biodegradation of

mangrove vegetation leads to the release of organic and humic substances which are partly dissolved in the surrounding water and as both particulate and dissolved forms transported to the sea through estuaries and other inlets. Thus mangrove ecosystem serves as a sanctuary and nutritional bank for coastal aquatic ecosystem.

The role of fungi in mangrove ecosystem has been highlighted in Chapter 1. In studying the mangrove fungi Kohlmeyer and others have conducted extensive and meticulous search for higher marine mangrove fungi, inhabiting the woody tissues of mangroves, while the fungi from sediments, especially from the rhizosphere region were studied in detail by Swart, Lee and Becker and others. From the Mangalavanam region (station 7) monthly samples of mud and decaying leaves, stems and roots of *Avicennia officinalis* and *Acanthus illicifolius* were collected for two years during the period of 1986 and 1987 to examine the mycoflora contained in them along with some of their ecological aspects.

5.1 Description of the study area

Mangalavanam is a small shallow mangrove ecosystem of the Cochin backwater characterized by dense growth of mangal vegetation (Figs 5.1 and 5.2). Although located in the midst of fast growing urban region, it is a small pocket of flourishing mangrove system which also provides permanent habitat to a large bird population. It is connected to the main backwater by a feeder canal and has an area of 2.8

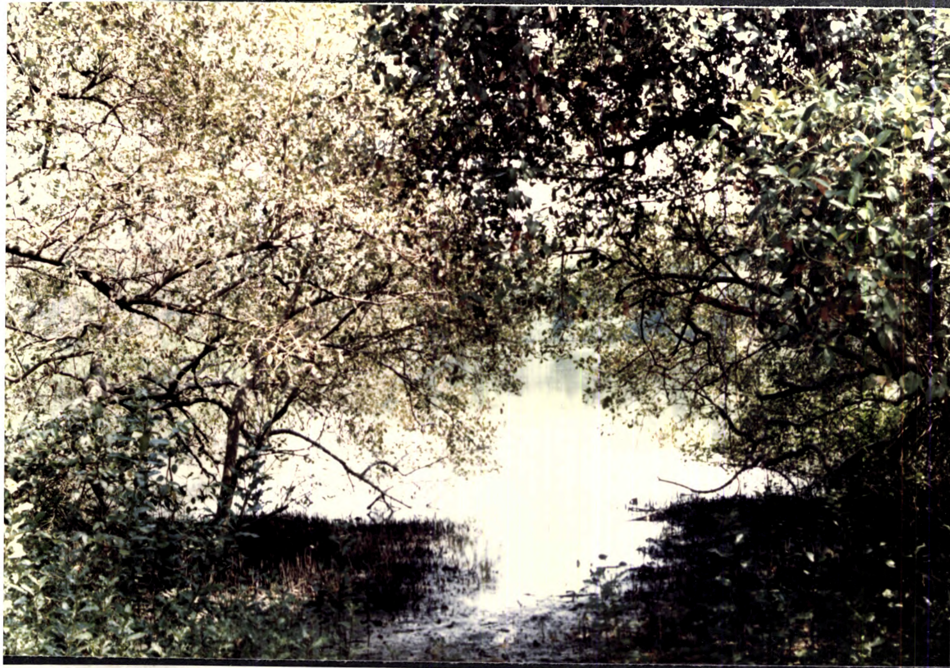

Fig. 5.1 The mangrove ecosystem. A view of the inner side of Mangalvanam during low tide showing the sampling location.

Fig. 5.2 Avicennia officinalis and its pneumatophores.

hectares. It is in the form of a shallow muddy pond of average depth of less than 1 m and with a small green island at the centre and dense mangrove vegetation at the periphery. During low tide part of the muddy substratum is exposed. The dominant macrophyte is *Avicennia officinalis* followed by *Acanthus illicifolius* and a few *Rhizophora mucronata*. The withered and yellowing mangrove leaves fall directly upon water and later settle below. Decaying stems, roots and fallen leaves of the macrophytes add up considerably to the detritus of the ecosystem. In the monsoon, postmonsoon and early premonsoon season free floating plants *Eichhornia crassipes*, *Salvinia molesta* and *S. rotundifolia* brought in during the high tide from extensive mats in the Cochin backwater and not flushed out from the mangrove area decay there adding to the detritus.

5.2 Physico-chemical features

The environmental parameters examined in this study include salinity, DO, temperature, pH, Eh, BOD_5 and organic carbon of mud. Variations in hydrographic parameters at Mangalavanam for the period of observation (January, 1986 to December, 1987) are given in Fig. 5.3.

Salinity

Salinity values ranged between 2 to 27.9×10^{-3} over the two years. They were higher from December to May for both the years and showed a steep fall in June. Low salinities ($< 4 \times 10^{-3}$) were recorded from June to November,

1986. From January to July distribution of salinity showed the same trend in both the years. However from August to November significant difference was noticed between the years with a range of 3.90×10^{-3} to 15.10×10^{-3} in 1986 and 18.18×10^{-3} to 20.30×10^{-3} in 1987. This was due to delayed and insufficient monsoonal rain in 1987. A comparison of temporal variations in salinity for the years shows clearly the influence of the monsoonal cycle in the area of study.

Dissolved oxygen

Variations in dissolved oxygen content of water ranged between 2.3 to 3.99 mg/l. Values fell below the average level of 3 mg/l only in April, 1986 and February, August and September, 1987. August, 1986 recorded the peak value of 3.99 mg/l as against the lowest value in August, 1987 (2.3 mg/l). The year 1987 showed lower DO compared to 1986 probably associated with higher ambient temperature and salinity. This system however did not experience severe oxygen depletion at any time.

Temperature

Temperature varied from 25.3 to 31.5°C for water and 25.2 to 30.58°C for mud. High values were recorded from January to June in 1986 both in water and mud and in most of the months of the year 1987. In 1986 fluctuation was minimum in water from June ti Septemeber and in mud from July to November. The highest values for water (31.5°C) and mud (30.5° C) were recorded in January, 1986. While the lowest

values (25.3°C for water and 25.2°C for mud) were recorded in June, 1987. This shallow system did not experience sharp temperature gradients during the entire period of study.

pH

The pH of water did not show much variation (range 6.98 to 7.49) over the period of study, with slightly higher values in 1987 as compared to 1986, probably associated with the prevailing higher salinities. The same trend was also noticed for the pH of mud (6.53 to 7.20). The peaty mud showed values at the neutral level or just below it during the entire period of study with the exception of September, 1986.

Eh

For the two years the Eh of water fluctuated within a range of +53 to +340 mv. In both the years high positive values were obtained from July to December (monsoon and postmonsoon). For the two years the Eh of mud varied within a range of -69 to -210. Similar trend was noticed for the two years.

BOD_5

BOD_5 values in general showed to build up during the pre- and postmonsoon culminating in peak values in June (4.42 mg/l in 1986 and 5.54 mg/l in 1987). Instantaneous inputs like land runoff seem to be responsible for these high values in June. The same reasoning could be extended to the erratic fluctuations in BOD_5 during 1986. The subsequent

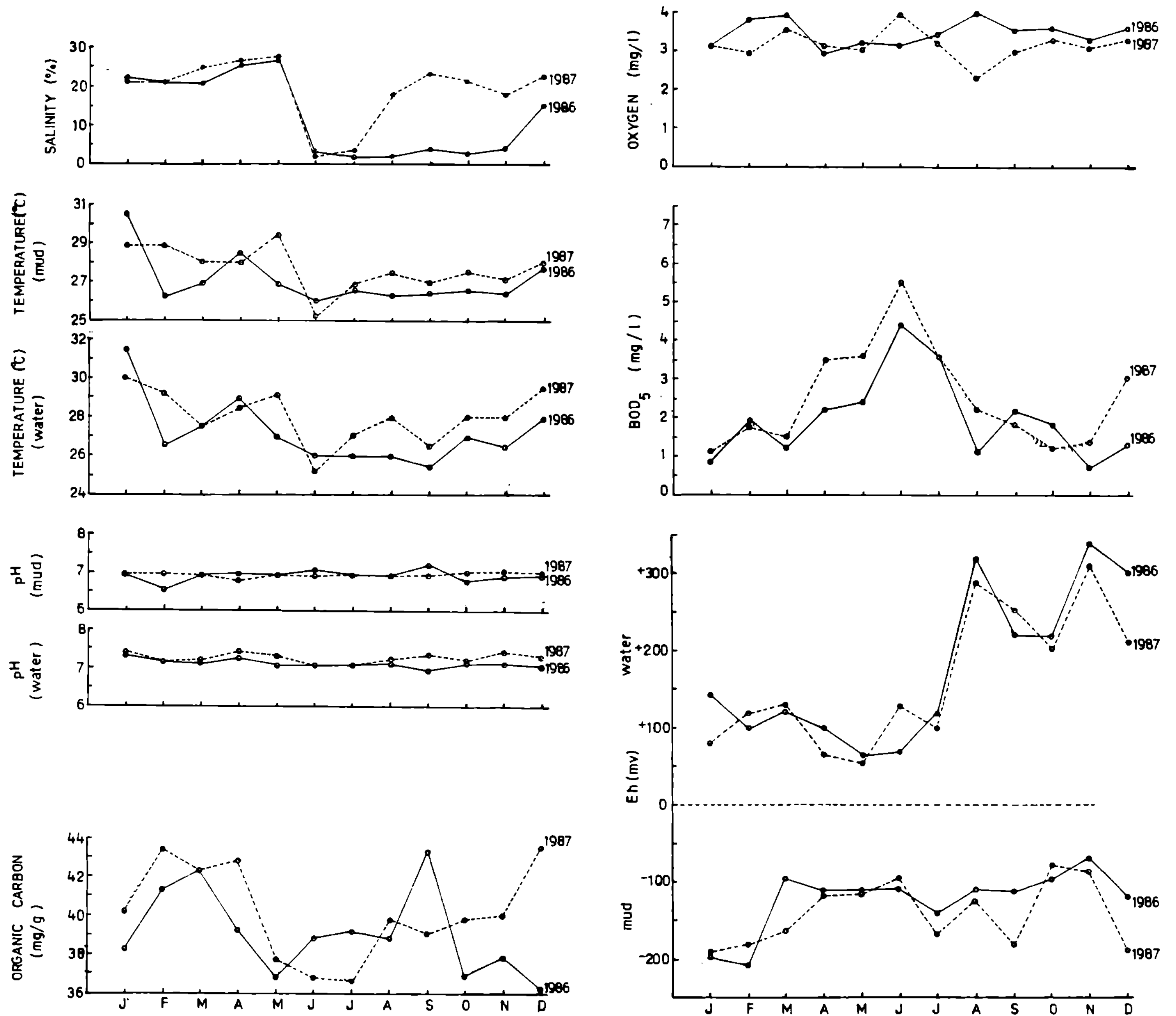

Fig. 5·3 Distribution of various physico-chemical parameters in Mangalvan

year which was drier showed a steady decrease in BOD_5 values as the monsoon season progressed. The lowest value of 0.72 mg/l was recorded in November, 1986. Notable upward trend in BOD_5 was noted for both the years during the transition period.

The graph of BOD_5 and Eh distribution of water during 1986 showed a clear negative correlation but this was not so clearly evident in 1987 except in the months of April, May, July and December. In the mud the Eh values were almost independent of the BOD_5 values of the water in both the years and also showed nearly similar trend except for few erratic values in 1987 especially during March and September. BOD_5 of water remained fairly high in both the years during April to July with the maximum in June and a steep fall in August. A lesser peak in BOD_5 values were noticed in December during 1986 and 1987. Low Eh values of water also occurred during this period. Low negative values of Eh were also uniformly maintained from March to November in 1986.

Organic carbon

In general organic carbon in mud was high at station 7 as compared to the adjacent estuary (average 39.71 mg/g). Detrital material from mangroves seems to cause the enrichment. Values for 1986 ranged between 43.44 mg/g (September) to 36.32 mg/g (December). Those for 1987 were between 36.66 mg/g (July) to 43.56 mg/g (December). Organic carbon content in general tended to be lower in the monsoon months, the exception being a sharp rise in 1986 especially

in September. It also showed a fall in December 1986 as against a sharp increase for the same month in 1987.

5.3 Mycoflora

General observations

A qualitative study of the mycoflora of mud and decaying mangrove vegetation revealed a total of 71 species belonging to 35 genera from the mangrove ecosystem for the years 1986 and 1987 (Table 5.1). Fifty four species belonging to 26 genera could be isolated from mud samples, whereas fifty one fungal species belonging to 28 genera were recorded in the decaying plant materials. Microscopical observations just after the collection revealed the presence of active hyphae in mud samples and a good number of sporulating Deuteromycetes in plant material, associated with large number of small invertebrates including ciliates, foraminifera, flat worms, nematodes, polychaetes and copepods. Most of the leaves collected for study were fragile and the epidermal layers could be easily torn apart while agitating with water. Transverse sections of living plant materials did not show the presence of fungal hyphae. But the decomposing plant material collected from the mud surface could be found inhabited by a number of microfungi.

Tables 5.2 and 5.3 show the occurrence of various species of fungi in the different months for the two years. The striking observation was that although many species were represented in the monthly samples, only few were of regular

Table 5.1 Species of fungi isolated from the mangrove mud and decaying vegetation of mangrove ecosystem during the years 1986-1987

Acrothecium sp.
Absidia cylindrospora Hagem
Alternaria fasciculata Cooke and Ellis
A. humicola Oudemans
A. tenuis Nees
Aspergillus candidus Link
A. chevalieri (Mangin) Thom and Church
A. fumigatus Fresenius
A. glaucus Link
A. nidulans (Eidam) Winter
A. niger van Teighem
A. oryzae (Ahlburg) Cohn
A. sydowi (Bainier and Sartory) Thom and Church
A. terrus Thom
A. ustus (Bainier) Thom and Church
A. versicolor (Vuillemin) Tiraboschi
Botrytis terrestris Jensen
Cephalosporium acremonium Corda
C. roseo-griseum Saksena
Cephalosporium sp.
Cirrenalia pseudomacrocephala Kohlmeyer
Chalara sp.
Chaetomium cristatum Ames
C. globosum Kunze
Cladosporium herbarum (Persoon) Link
C. resinae (Lindau) de Uries
Colletotrichum gloeosporoides (Penzig) Penzig and Sacc.
Curvulaia geniculata (Tracy and Eaele) Boedijn
C. interseminata (Berkeley and Ravenel)
C. lunata (Walker) Boedijn
Dendryphiella sp.
Didymosphaeria enalia Kohlmeyer
Drechslera halodes (Drechsler) Subramanian et Jain
Fusarium neoceras Wollenweber and Reinking
F. oxysporum Schlechtendahl
F. poae (Peck) Wollenweber
F. solani (Martius) Appel and Wollenweber
F. vasinfectum Atkinson
Geotrichum candidum Link
Gliocladium penicilloides Corda
Helminthosporium sp.
Humicola alopallonella Meyers et Moore
Kymadiscus haliotrepus (J. Kohlmeyer et. Kohlmeyer)J. Kohl. et. E. Kohl.
Monilia brunnea Gilman ans Abott
Mucor hiemalis Wehmer
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar
Paecilomyces varioti Bainier
Penicillium albidum Sopp
P. citrinum Thom
P. claviforme Bainier
P. chrysogenum Thom
P. funiculosum Thom
P. herquei Bainier and Sartory
P. janthinellum Biourge
P. luteum Zukel
P. monoverticillate symmetrica
P. parvum Raper and Fennell
P. pinophillum Hedgecock
Philophora sp.
Phoma humicola Gilman and Abott
Phompsis sp.
Rhizopus arrhizus Fischer
R. oryzae Went and Gerlings
R. nodosus Namyslowski
Sporormia minima Auerswald
Sporotrichum sp.
Trichoderma glaucum Abbott
T. koningi Oudemans
T. viride Pers. ex Fr.
Verticillium sulphurellum Saccardo
Zalerion maritimum Linder Anastasiou
Sterile mycelium (Dark)
Phycomycete (Unidentified)

Table 5.2 Occurrence of fungi in the mud samples of Mangalvanam during different months of 1986 and 1987

Sl.No.	Mycoflora	1986												1987											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
1.	Acrothecium	x					x								x				x						
2.	Absidia cylindrospora																					x			
3.	Alternaria fasciculata		x					x							x					x					
4.	A. humicola																			x					
5.	A. tenuis																	x							
6.	Aspergillus candidus			x			x			x	x					x			x			x			
7.	A. chevellieri												x			x									
8.	A. fumigatus		x	x					x						x						x		x	x	x
9.	A. glaucus											x													
10.	A. nidulans							x			x											x			
11.	A. niger	x	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x	x
12.	A. oryzae									x												x			
13.	A. sydowi	x					x												x						
14.	A. terrus	x			x		x					x		x	x			x	x	x		x		x	
15.	A. ustus					x	x				x														
16.	Botrytis terrestris														x										
17.	Cephalosporium acremonium															x		x	x	x			x	x	
18.	Chalara sp.												x												
19.	Cladosporium herbarum	x					x	x			x		x	x		x				x			x		x
20.	C. resinae													x			x					x		x	
21.	Colletotrichum gloeosporoides																x								
22.	Curvularia lunata			x				x				x				x	x			x					x
23.	Dendryphiella sp.												x												
24.	Drechslera halodes															x									
25.	Fusarium neoceras				x			x			x														
26.	F. oxysporum										x	x					x						x	x	
27.	F. poae																			x	x	x			
28.	F. solani	x		x			x			x			x	x		x	x	x					x		x
29.	F. vasinfectum		x						x			x					x								
30.	Geotrichum candidum	x		x					x	x	x		x	x							x		x		x
31.	Helminthosporium sp.		x	x				x				x												x	
32.	Monilia brunnea													x				x						x	
33.	Mucor hiemalis											x										x			
34.	Penicillium citrinum					x					x				x										
35.	P. claviforme																			x					
36.	P. chrysogenum																				x				
37.	P. funiculosum															x									
38.	P. herquei																				x				
39.	P. janthinellum		x			x			x				x			x		x		x	x	x	x		x
40.	P. luteum	x		x			x		x	x		x		x						x					
41.	P. monoverticillate symmetrica							x																	
42.	P. pinophillum								x												x				
43.	P. albidum																						x		
44.	Paecilomyces varioti																						x		
45.	Phoma humicola																				x				
46.	Phialophora sp.																								x
47.	Phomopsis sp.																						x		
48.	Rhizopus arrhizus				x					x			x												
49.	R. oryzae																								x
50.	Sporotrichum sp.									x															
51.	Trichoderm glaucum					x			x			x	x								x	x			
52.	T. koningi		x		x				x		x				x						x	x	x	x	x
53.	T. viride																	x							
54.	Verticillium sp.													x							x				
x	Sterile mycelium (dark)																								

Table 5.3 Occurrence of fungi in the decaying mangrove vegetation of Mangalvanam during different months of 1986 and 1987

Sl.No.	Mycoflora	1986												1987											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
1.	Alternaria fasciculata							x																x	x
2.	A. humicola																					x			
3.	A. tenuis																						x		
4.	Aspergillus candidus						x			x															
5.	A. fumigatus		x			x			x																
6.	A. glaucus											x													
7.	A. nidulans													x											
8.	A. niger	x	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x		x	x	x
9.	A. oryzae									x															
10.	A. terrus	x		x	x							x		x	x		x			x	x				
11.	A. versicolor															x									
12.	Botrytis terrestris													x		x	x			x					
13.	Cephalosporium acremonium															x									
14.	C. roseo-griseum															x									
15.	Cephalosporium sp.															x									
16.	Cirrenalia pseudomacrocephala			x												x									
17.	Chalara sp.				x																				
18.	Chaetomium cristatum			x												x									
19.	C. globosum																				x				
20.	Cladosporium herbarum						x		x		x		x		x	x	x	x	x		x			x	x
21.	Colletotrichum gloeosporoides												x			x		x	x	x					x
22.	Curvularia geniculata																					x			
23.	C. interseminata														x										
24.	C. lunata			x				x													x				
25.	Dendryphiella sp.																x								
26.	Didymosphaeria enalia																x	x							
27.	Drechslera halodes																			x			x		
28.	Fusarium neoceras				x			x																	
29.	F. oxysporum	x				x					x			x											
30.	F. solani	x		x			x				x		x	x									x		x
31.	F. vasinfectum																	x	x	x	x		x	x	x
32.	Geotrichum candidum	x		x																				x	
33.	Gliocladium penicilloides																				x		x	x	
34.	Helminthosporium sp.		x	x				x							x					x					
35.	Humicola alopallonella			x																					
36.	Kymadiscus haliotrepus														x		x								
37.	Mucor hiemalis											x													
38.	Myrothecium verrucaria																x					x	x		
39.	Penicillium citrinum														x			x							
40.	P. janthinellum		x			x								x								x			
41.	P. luteum	x		x			x		x	x		x							x	x					
42.	P. monoverticillate symmetrica							x																	
43.	P. parvum																							x	
44.	Paecilomyces varioti																					x			
45.	Rhizopus nodosus																								x
46.	Sporormia minima				x																				
47.	Sporotrichum sp.												x												
48.	Trichoderma glaucum																x								x
49.	T. koningi		x		x				x	x								x	x		x	x	x		x
50.	T. viride																					x			
51.	Zalerion maritimum																x	x							
x	Sterile Mycelium															x		x	x					x	
x1	Phycomycete (Unidentified)															x									

occurrence. Among the various genera isolated from mud samples both *Aspergillus* and *Penicillium* were dominant having 10 species in each. They were followed by *Fusarium*, *Alternaria* and *Trichoderma*. In decaying mangrove vegetation *Aspergillus* was the dominant genus with 8 species followed by *Penicillium* with 5 species. Typical marine forms like *Cirrenalia pseudomacrocephala*, *Dendryphiella* sp., *Didymosphaeria enalia*, *Drechslera halodes*, *Humicola alopallonella*, *Kymadiscus haliotrepus* and *Zalerion maritimum*, could be isolated from the dead plant material only during premonsoon seasons of 1986 and 1987. This may be due to the fact that marine fungi grow and sporulate favourably under high salinity. These fungi except *Dendryphiella* sp. and *D. halodes* could not show up in mud samples. Both in mud and decaying mangrove samples *Aspergillus niger* was the most frequent species, which appeared 21 times out of 24 monthly samplings.

Considerable variations in the occrrence and relative abundance of fungi were also observed in the samples collected from mud and decaying vegetation. Fig.5.4 shows temporal variations in the total counts for 1986 and 1987. In mud samples number of propagules per gram varied from 1×10^4 to 5.3×10^4 and in decaying mangrove vegetation it ranged from 1.6×10^4 to 5.4×10^4 per gram. Generally low number of propagules were registered from January to May (premonsoon months) in mud samples in both the years. Similar trend was also observed in decaying mangrove

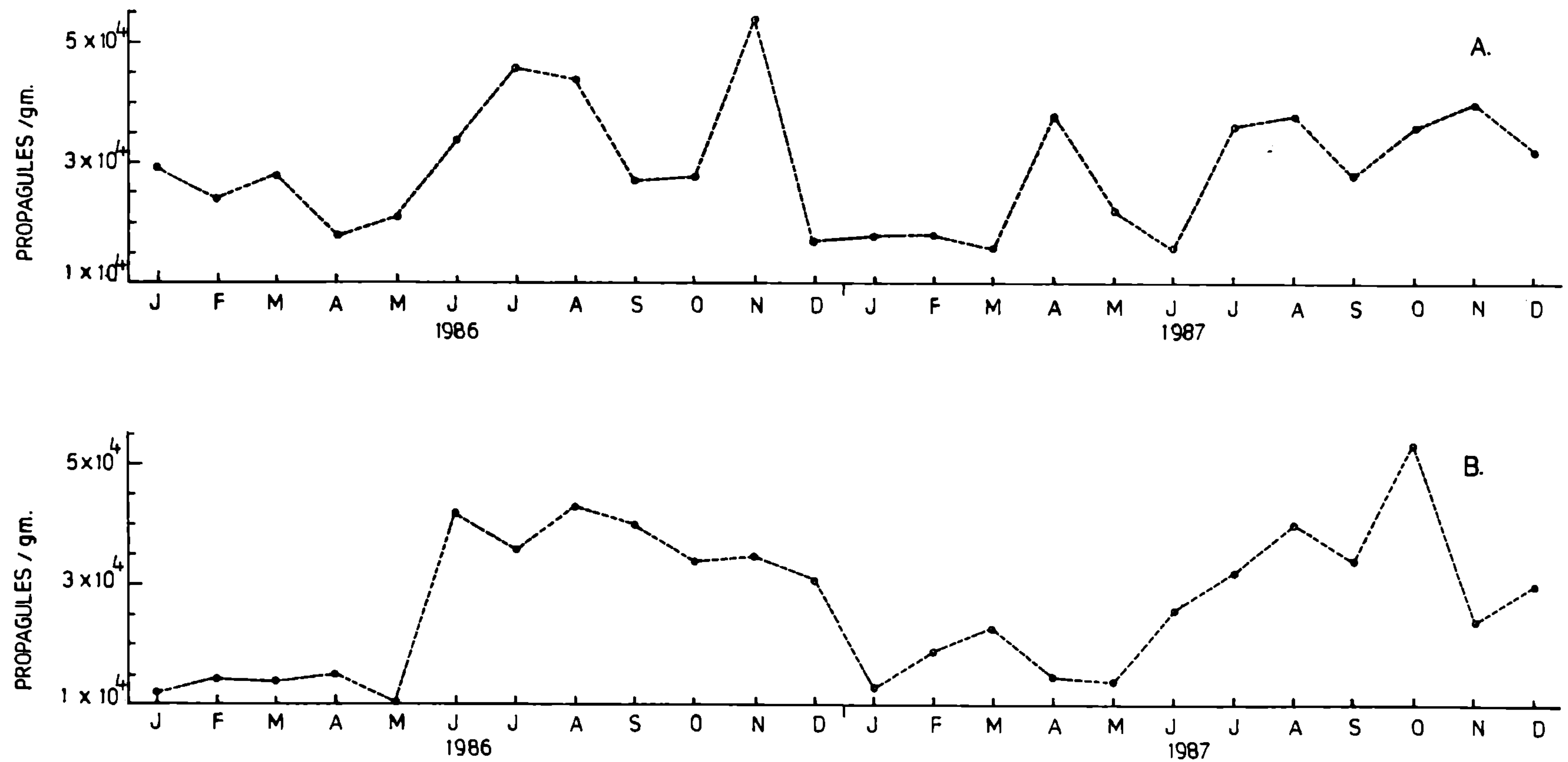

Fig. 5·4 Monthly variations of total fungal populations in A. decaying mangrove vegetation and B. mud samples at Mangalvan – 1986 & 1987

vegetation except for one high value during April, 1987. Table 5.4 presents the average number of propagules and the number of species appeared during different seasons for 1986 and 1987 in mud and decaying vegetation. In general the values were lower during premonsoon period, whereas monsoon and postmonsoon periods registered higher values. The same was seen with respect to the number of species although it was not evident in decaying vegetation. Histogram showing

Table 5.4 Monthly average number of propagules and number of fungal species in Mangalavanam mud and decaying mangrove vegetation during the three seasons in 1986 and 1987

	Average no. of fungal propagules per gram		Average no. of fungal species appeared	
	1986	1987	1986	1987
<u>Mud</u> <u>samples</u>				
Premonsoon (Jan-May)	1.30×10^{4}	1.68×10^{4}	4.2	5.4
Monsoon (Jun-Sept)	4.03×10^{4}	3.30×10^{4}	6.5	7.0
Postmonsoon (Oct-Dec)	3.30×10^{4}	3.57×10^{4}	7.7	6.3
<u>Mangrove</u> <u>vegetation</u>				
Premonsoon (Jan-May)	2.40×10^{4}	2.24×10^{4}	3.4	5.2
Monsoon (Jun-Sept)	3.70×10^{4}	2.95×10^{4}	3.3	4.8
Postmonsoon (Oct-Dec)	3.30×10^{4}	3.60×10^{4}	3.3	5.0

the abundance of various species in Mangalavanam during 1986 and 1987 is given in Figs 5.5 to 5.8. The year 1987 was characterized by a large number of species in both mud and decaying mangrove vegetation. A qualitative difference in the species composition during the premonsoon months compared to other two seasons was noticed in both the years. More obligatory forms were recorded only during this period.

The data collected in mud samples were statistically examined by ANOVA, Duncan's mutiple 't' test and Trellis diagram based on the fungal counts in each sample. However data collected from decaying mangrove vegetation and used for Trellis diagram were based on the presence or absence of the species without considering their numerical abundance. In addition to these analysis, data were also examined by diversity and evenness indices and Cole's point correlation coefficient.

Tukey's test of additivity caried out on fungal counts for mud based on the two year data for 1986 and 1987 showed that the treatment effects were additive, so no transformation of data was required. The data were next subjected to a 3-way ANOVA test using the fungal counts to examine monthwise, specieswise and annual differences (Table 5.5) from the respective mean abundance. The F-values did not show any significant difference in the total fungal counts between the two years, but monthly and species differences were significant at the 5% level as also the interaction effects between months and species and species

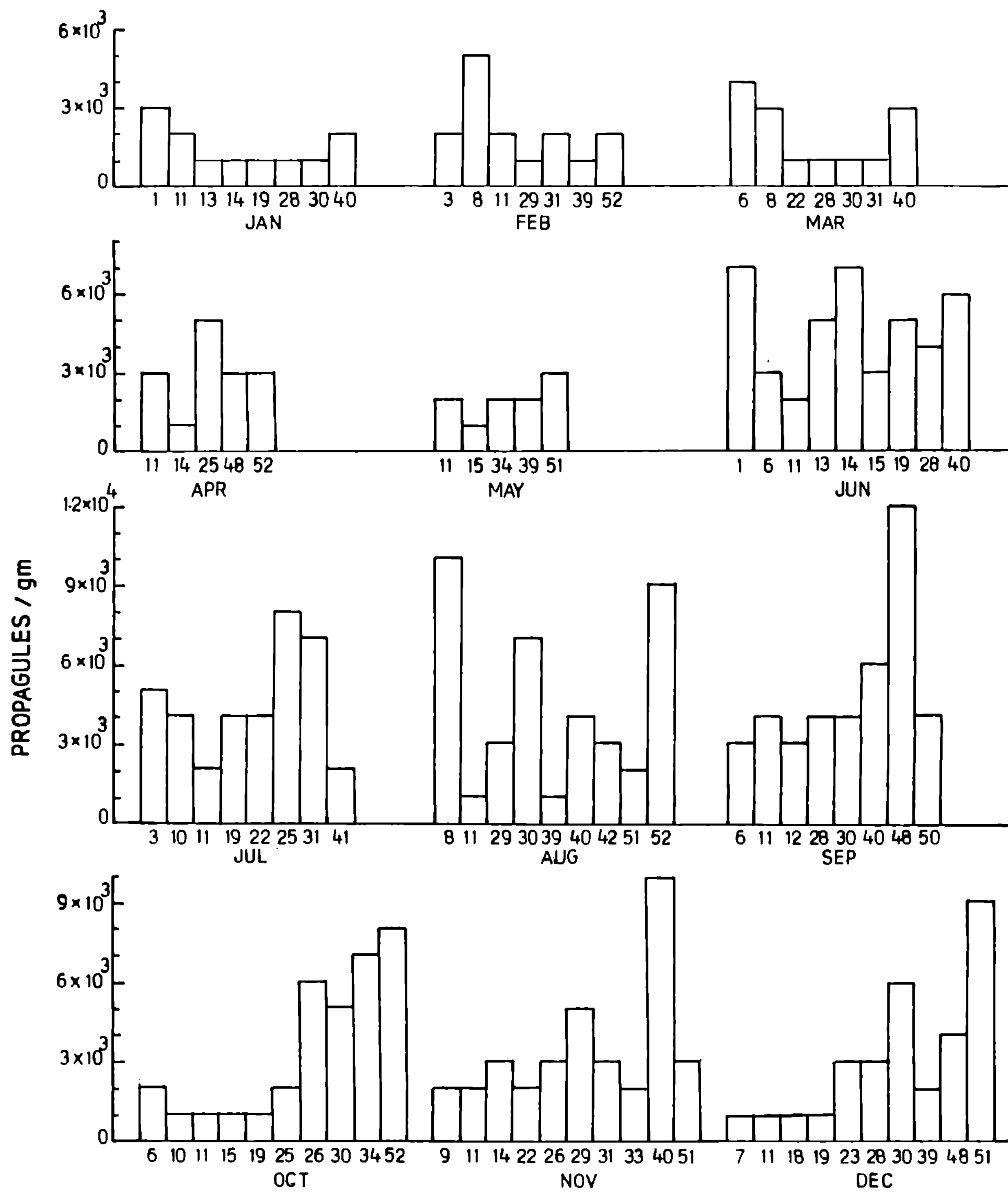

Fig. 5.5 Monthly distribution of fungal species in mud samples at Mangalvan for the years 1986 (Numbers on 'x' axis refer to the species; vide Table 5.2)

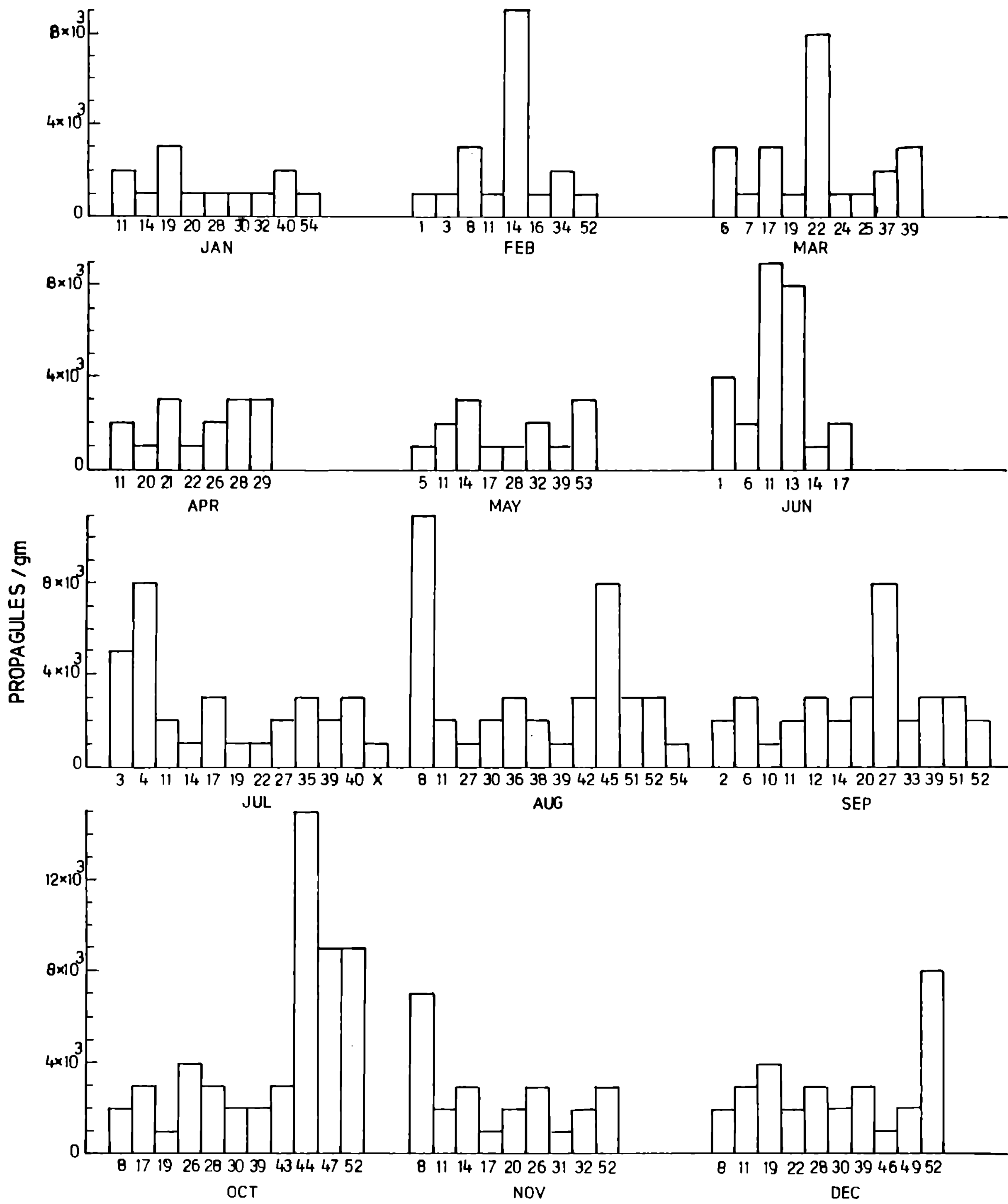

Fig. 5.6 Monthly distribution of fungal species in mud samples at Mangalvan for the year 1987 (Numbers on 'x' axis refer to the species; vide Table 5.2)

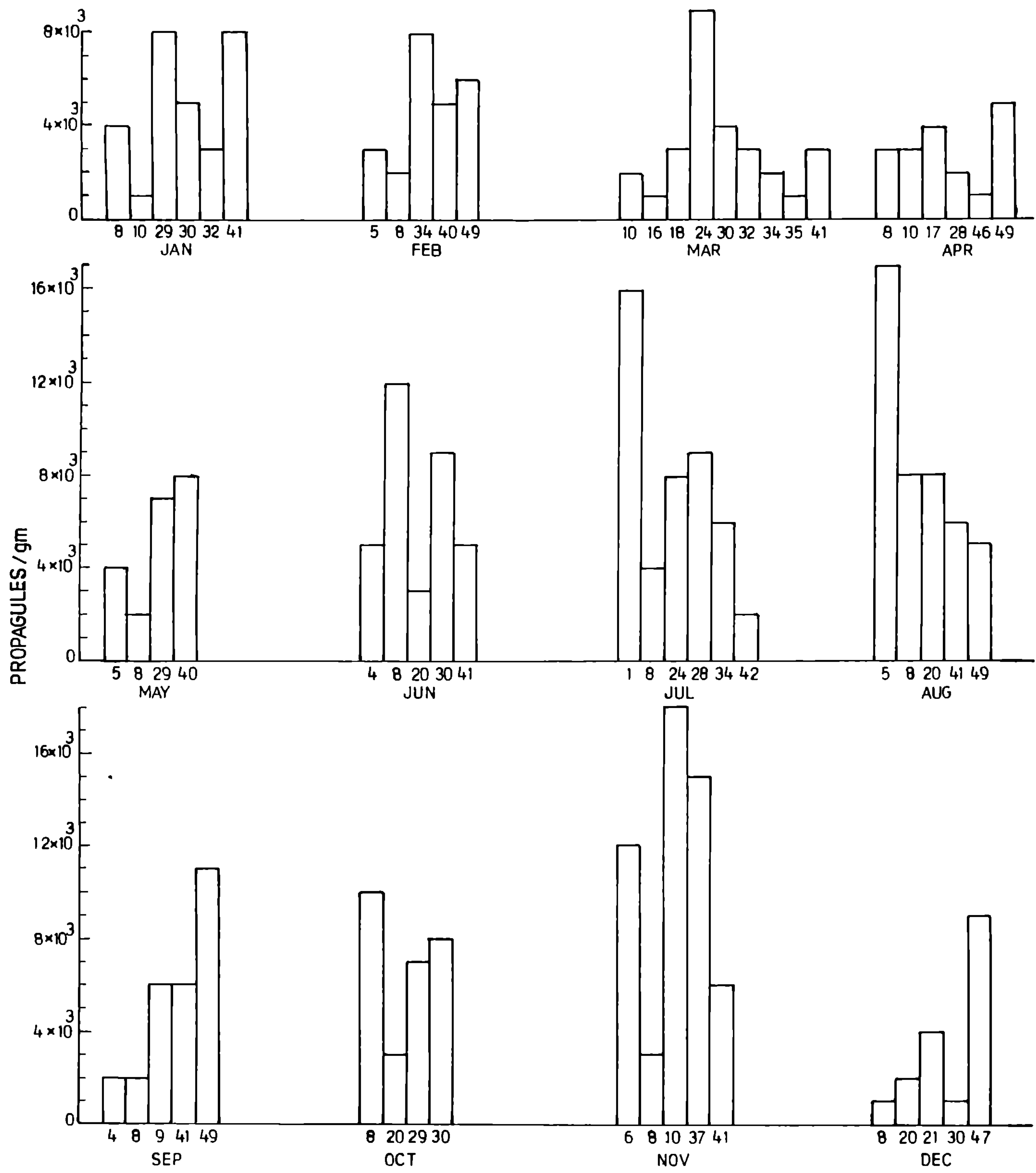

Fig. 5·7 Monthly distribution of fungal species in decaying mangrove vegetation samples at Mangalvan for the year 1986 (Numbers on 'x' axis refer to the species; vide Table 5·3)

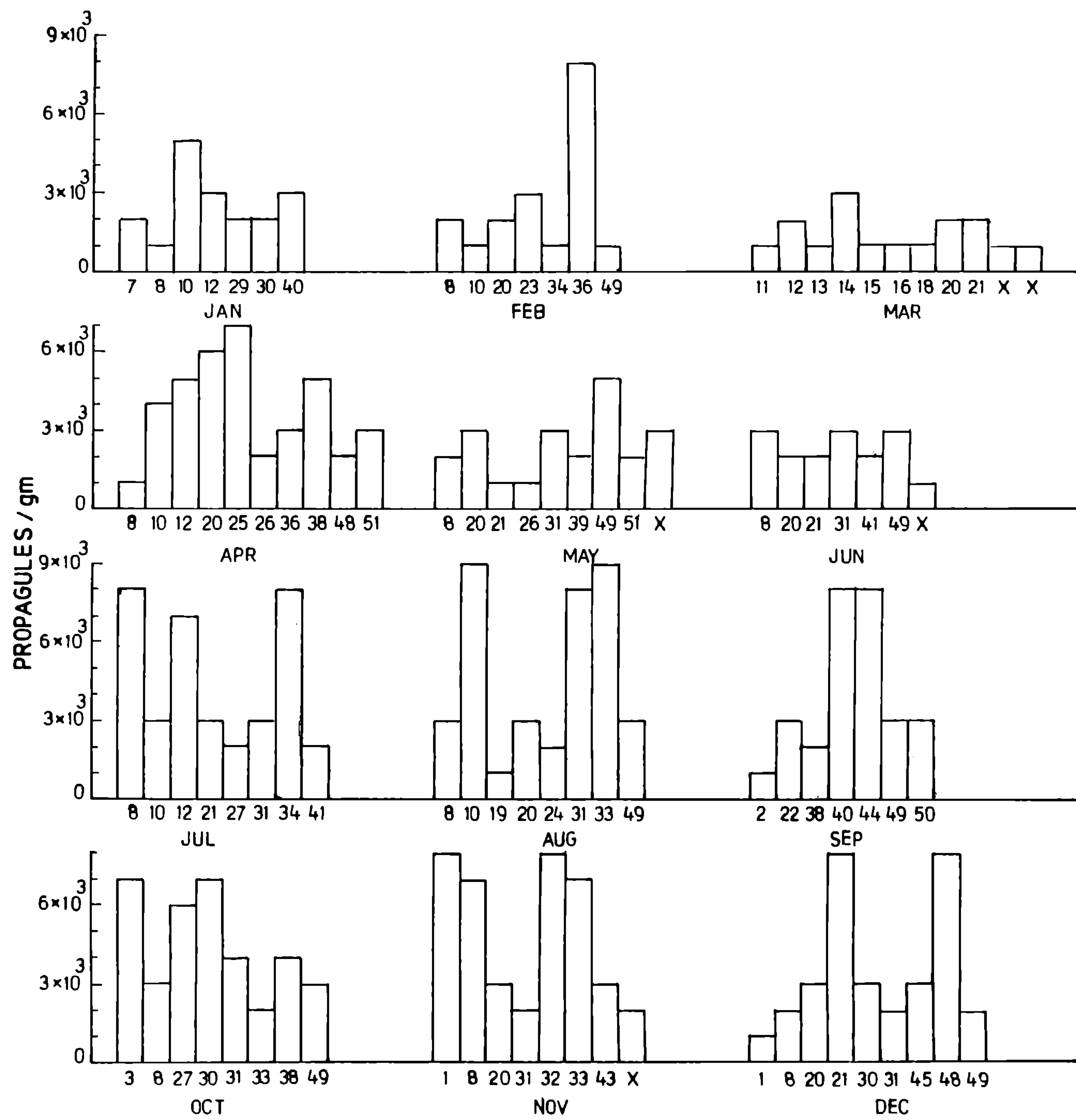

Fig. 5.8 Monthly distribution of fungal species in decaying mangrove vegetation samples at Mangalvan for the year 1987 (Numbers on 'x' axis refer to the species; vide Table 5.3)

and years. The interaction effect between months and years is not significant which suggests more or less similar counts between the same months in the two years.

Table 5.5 3-way classification for testing significance of difference between months, species and years in mud samples of Mangalavanam

Source	S.S.	d.f.	M.S.S.	F-ratio
Months (A)	40.01993	11	3.6382	4.01515**
Species (B)	428.71340	72	5.9544	6.5713**
Years (C)	0.00509	1	0.00509	0.00501
(AB)	2138.58842	875		
(BC)	612.75509	145		
(AC)	46.3419	23		
(A x B)	1669.8551	792	2.10840	2.3269**
(B x C)	184.0366	72	2.55606	2.8209**
(A x C)	6.31683	11	0.57426	0.6338
Error	717.6415	792	0.90611	
Total	3046.58842	1751		

** - F is significant at 1% level

Duncan's multiple 't'test for mud samples collected during 1986 showed that March and May, July and August were more or less similar period of average species abundance. In 1987, April and May, June and July similarly registered closer agreement in species abundance (Fig. 5.9).

The diversity index was uniformly high in both mud and decaying vegetation for both the years (Figs 5.10 and 5.11) as also observed for station 7 in Chapter 5 from routine bimonthly collections. However in 1987 the diversity index for decaying mangrove vegetation was distinctly higher than

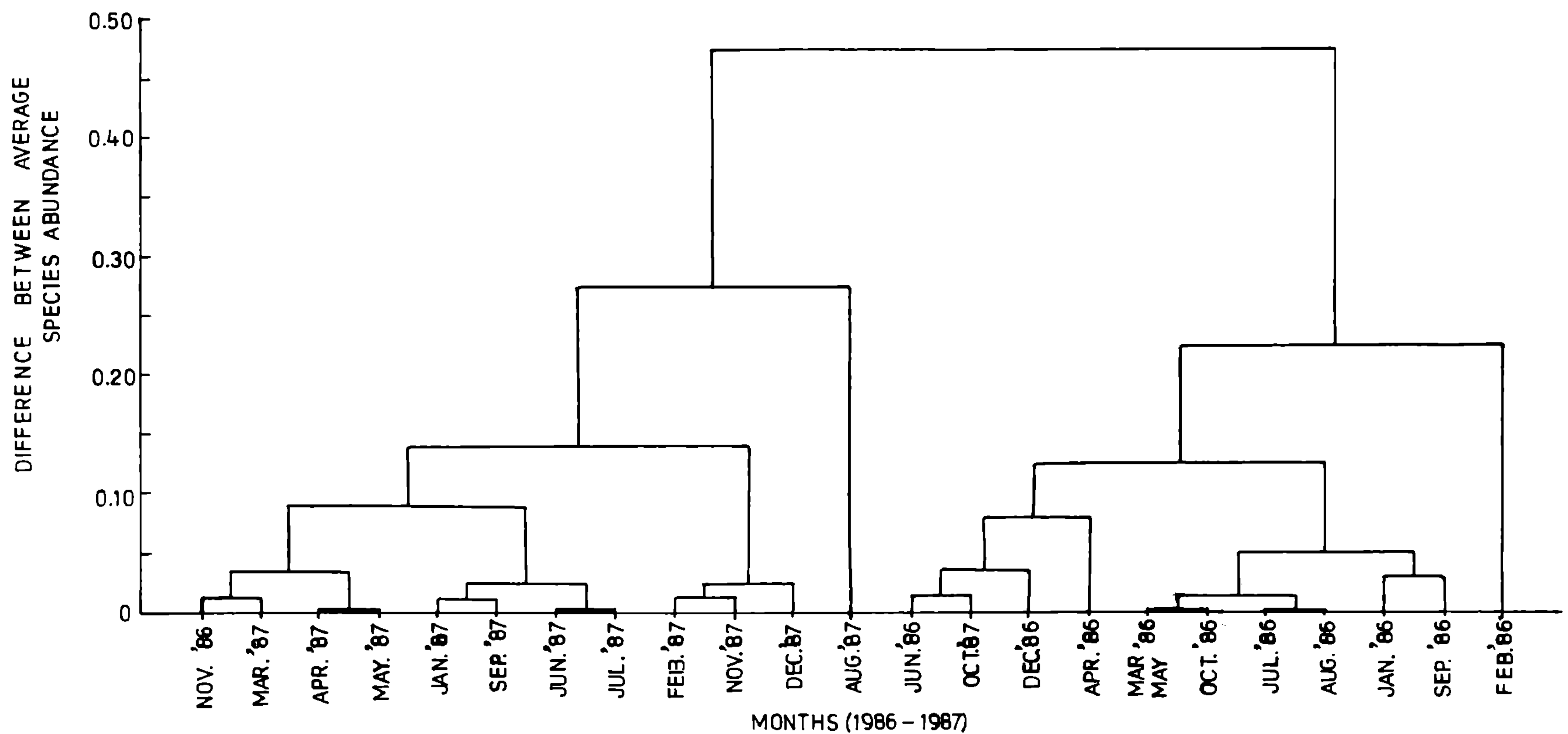

Fig. 5.9 Grouping of months based on difference between the months average species abundance

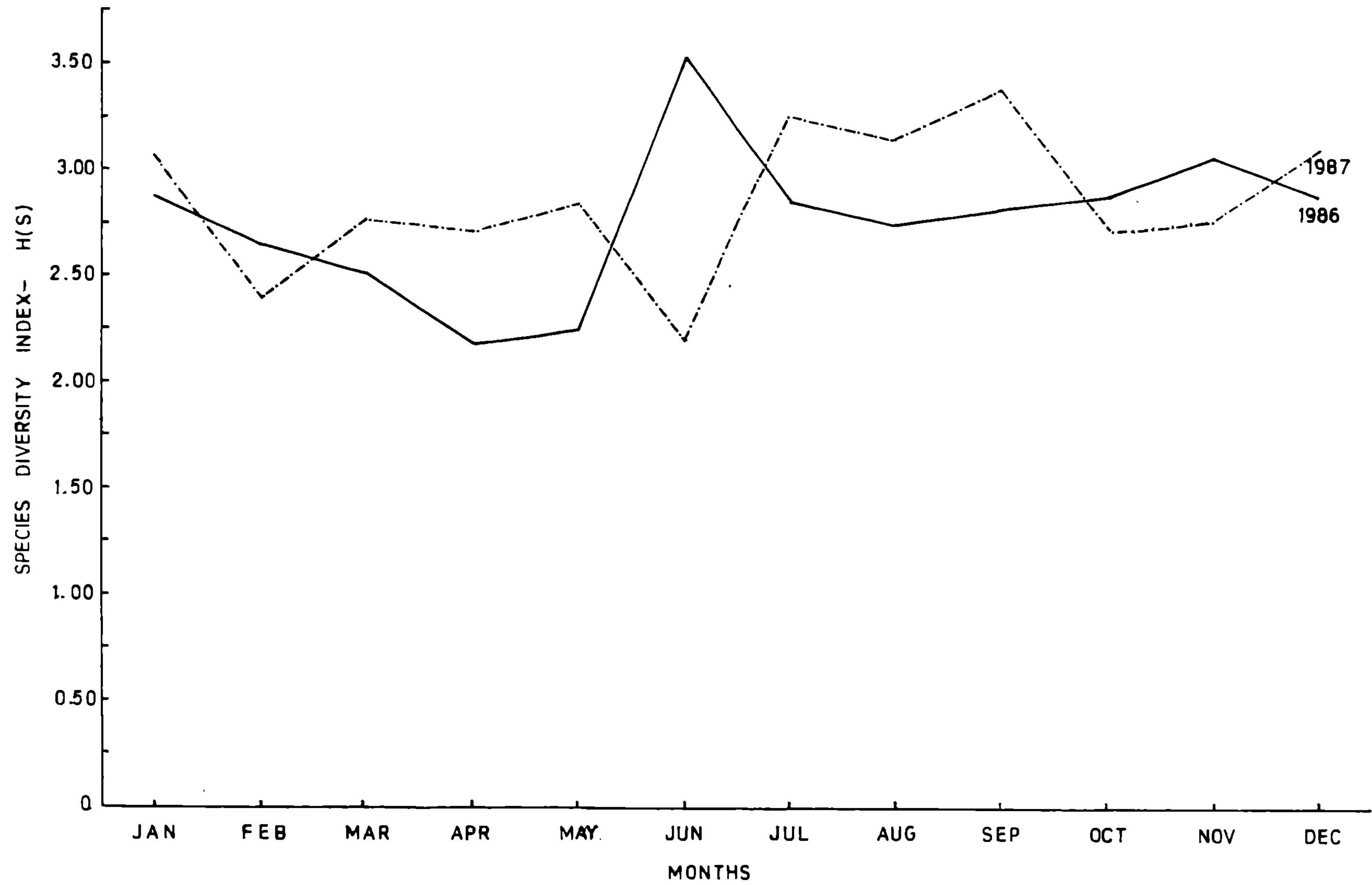

Fig. 5.10 Shannon Weaver information index H(S) for fungal species in Mangalvan mud samples

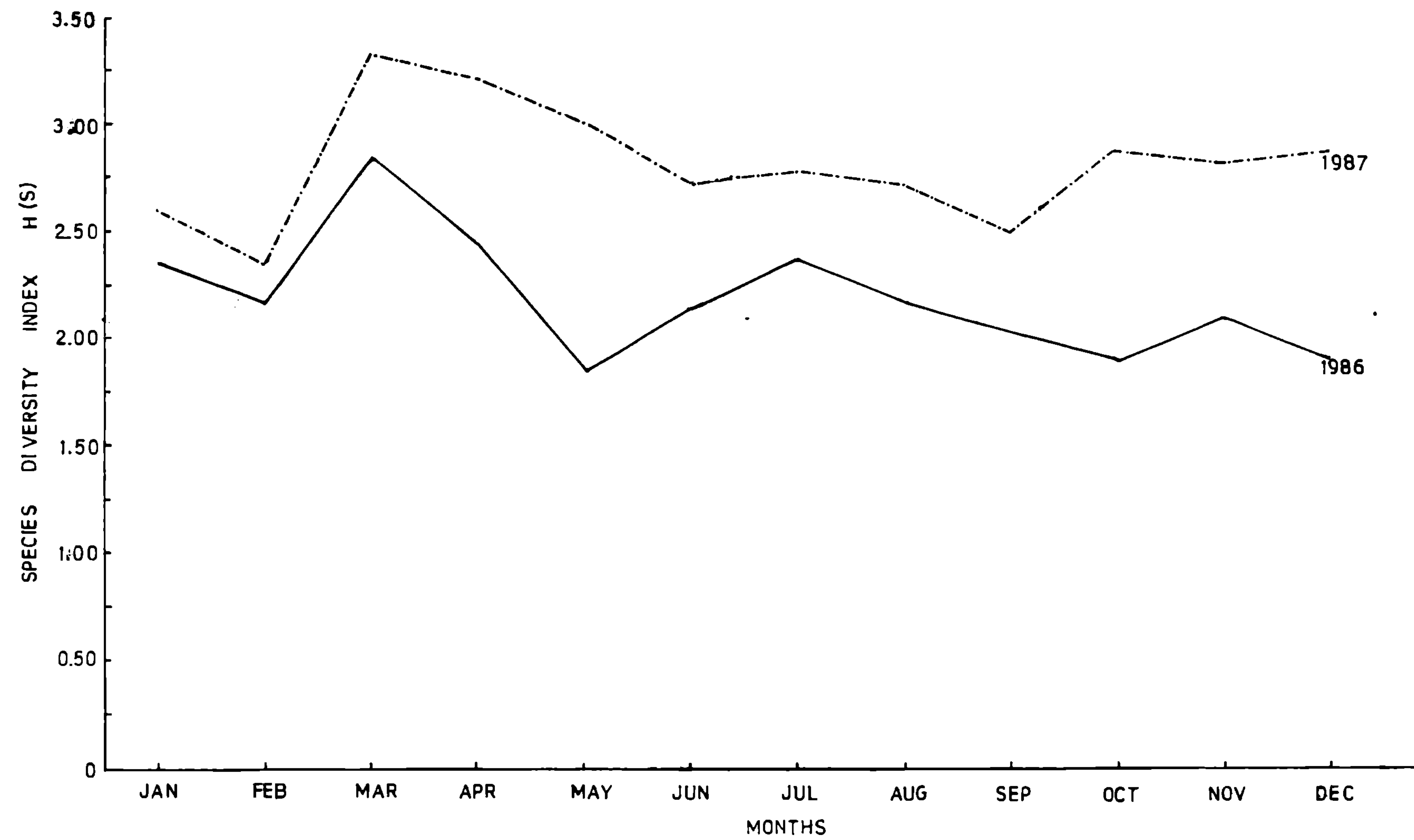

Fig. 5.11 Shannon Weaver information index H (S) for fungal species in decaying mangrove vegetation

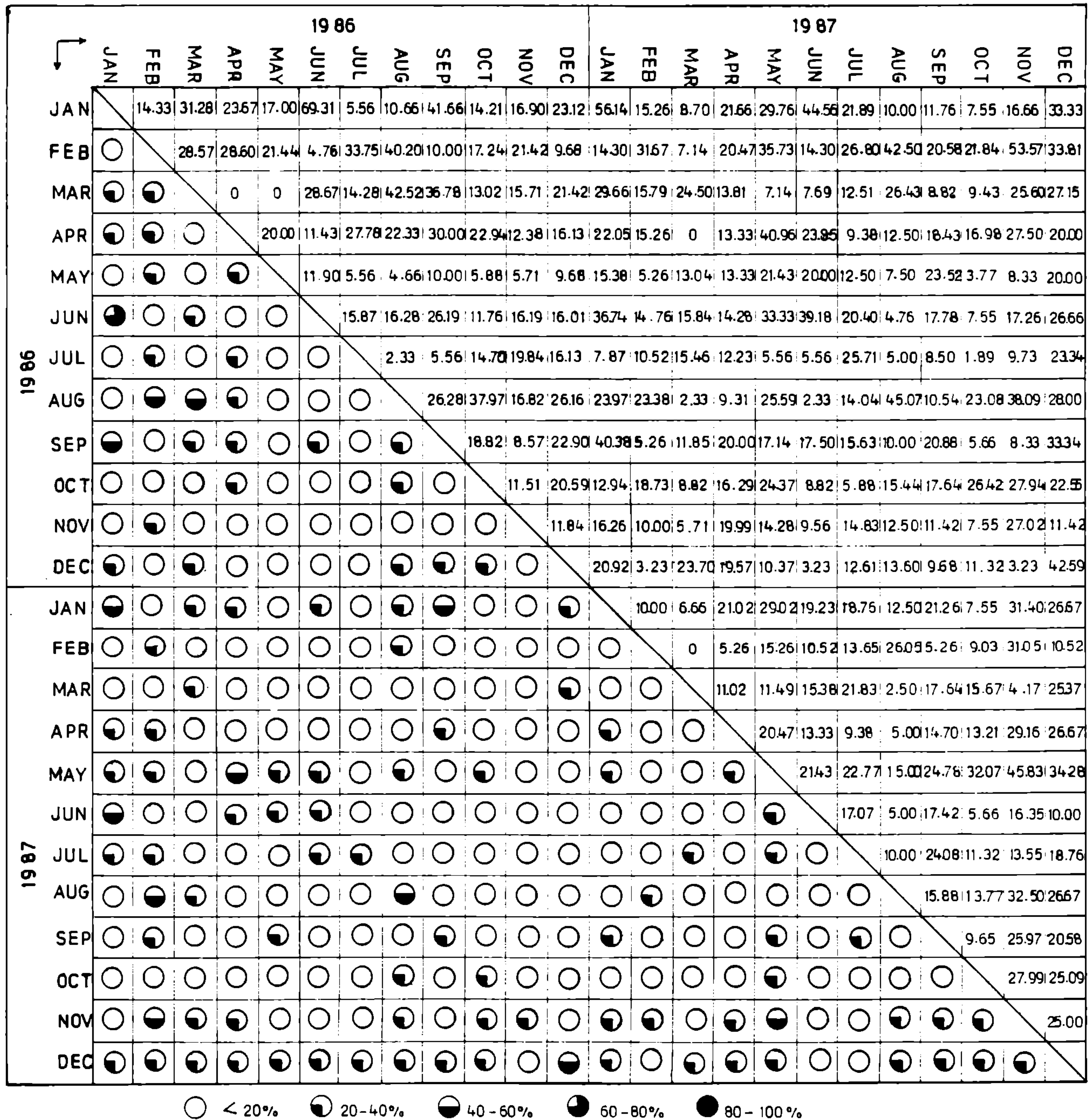

Fig. 5.14 Trellis diagram showing percentage affinity index between months for the fungi in Mangalvan mud samples

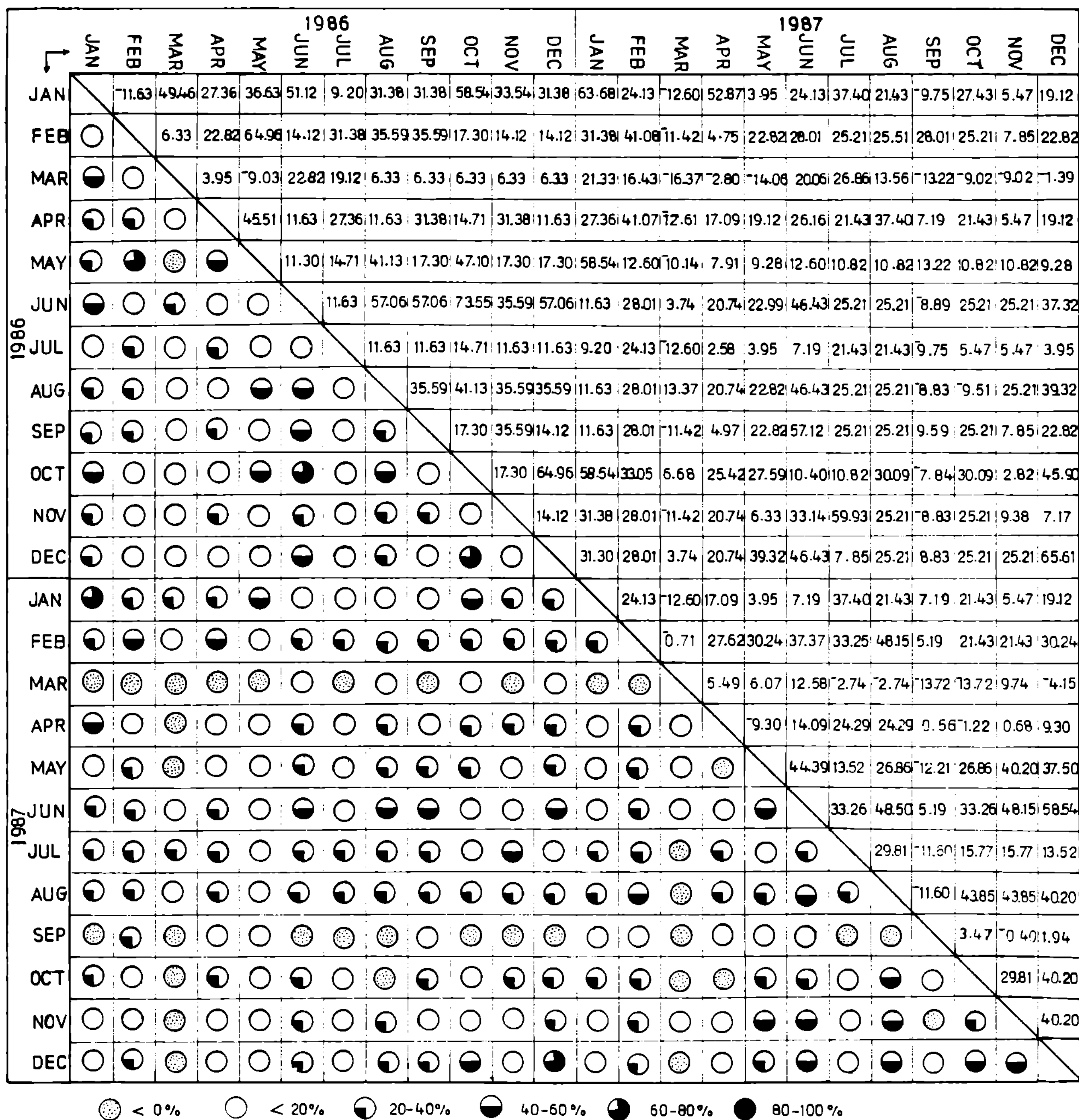

Fig. 5.15 Trellis diagram showing percentage affinity index between months for the fungi in decaying mangrove vegetation

in 1986 during all the months, the range of values for the two years being 1.82 and 3.32. The values for the mud samples were more or less in the same range for both years (2.25 to 3.54) although diversity indices were generally higher in 1987 except for February, June, October and November. The two years also showed alternation of periods of lower and higher values, the maximum value of 3.54 in 1986 coincinding with the onset of monsoon.

Heip's evenness index calculated for each month showed more or less similar pattern as that of the diversity index. From the Figs 5.12 and 5.13 it can be seen that greater the diversity index, greater the evenness index which therefore implies that there was consistency in the species distribution.

The similarity on the common occurrènce of individual species and their relative abundance in mud samples in different months was examined by the Trellis diagram. In Fig. 5.14 affinity indices of all combinations of pairs of months for the mud samples during 1986 and 1987 have been estimated and plotted and it distintly shows poor similarity of months based on commonness of species and their abundance. Most of the circles lie below the range of 20 - 40% affinity index. The Trellis diagem (Fig. 5.15) was also drawn for fungal species in decaying mangrove vegetation, but it was done only on the basis of presence or absence of the species since the quantitative data could not be useed as a reliable index of abundance, as already mentioned under methodology.

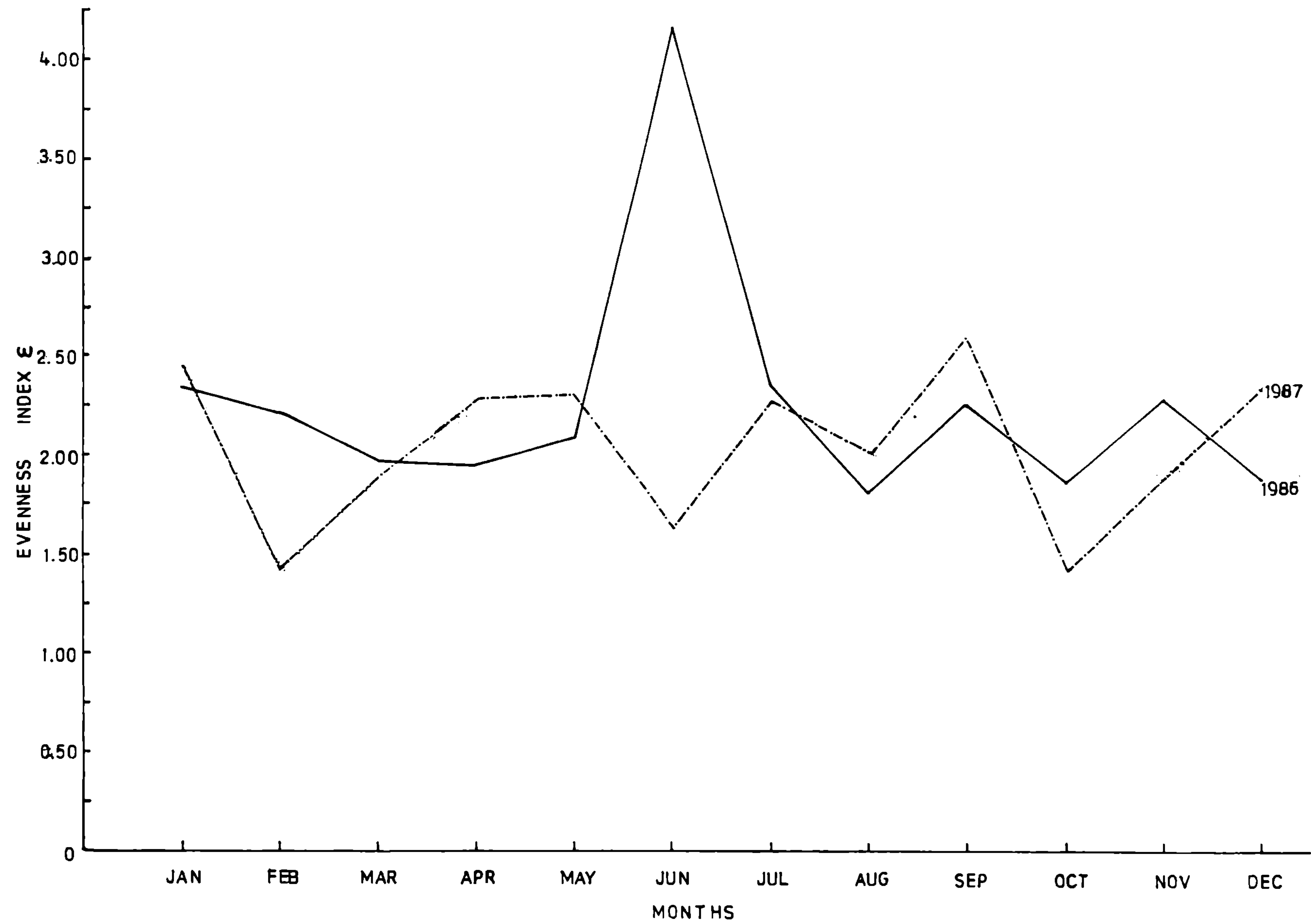

Fig. 5.12 Heips evenness index ε for fungal species in Mangalvan mangrove mud samples

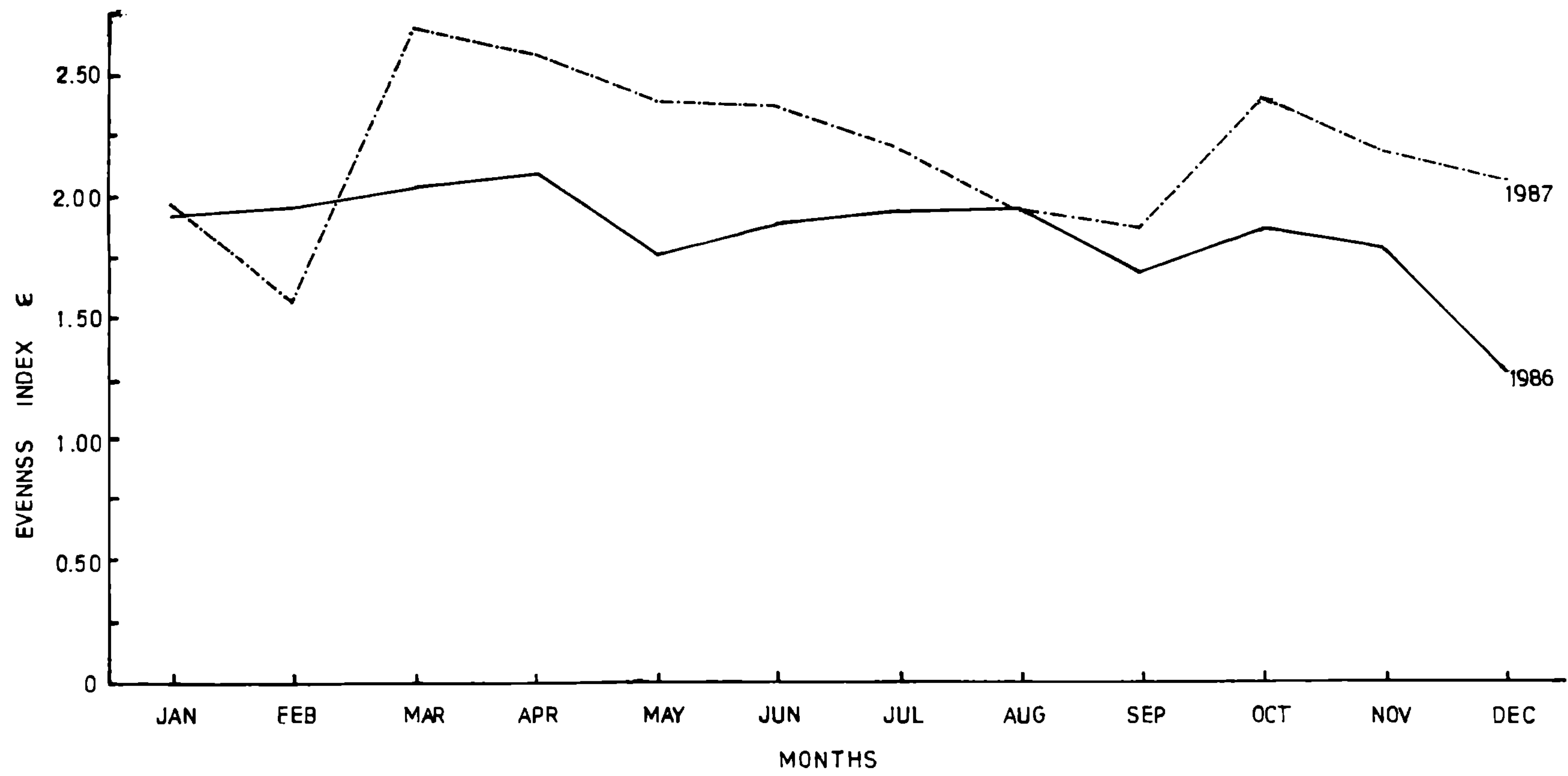

Fig. 5.13 **Heips evenness** index ε for fungal species in **decaying mangrove vegetation**

Affinity indices between months were again low for both the years. Most of the negative correlations (affinity $< 0\%$) were seen in 1987 despite the highest diversity index as can be seen from 5.11. An examination of affinity index between species for both mud and decaying mangrove vegetation (Figs 5.16 and 5.17) also strongly indicate the independent occurrence of fungal species, since the frequency distribution is strongly centered in the range close to zero. Most of the species correlation were between -14.6% and 0.0% for the mud and it was between -0.11% and -0.056% for decaying vegetation.

The more intensive study of the occurrence and abundance of filamentous fungi from station 7 (Mangalavanam) confirms the major conclusions obtained during the routine bimonthly study from different stations in the Cochin backwater discussed in the earlier Chapter. Inspite of relatively high uniform fungal abundance maintained at Mangalavanam, the availability of suitable organic substratum such as decaying plant litter might have contributed to the difference in the species and numerical abundance during 1986 and 1987. The uniformly higher diversity index observed throughout the year 1987 compared to 1986 for decaying mangrove vegetation (Fig.5.11) may also indicative of greater availability of organic substratum of plant origin, in excess of what was contributed by the local mangrove vegetation. The statistical analysis of the data such as dendrograms, Trellis diagram and species association test strongly indicate the

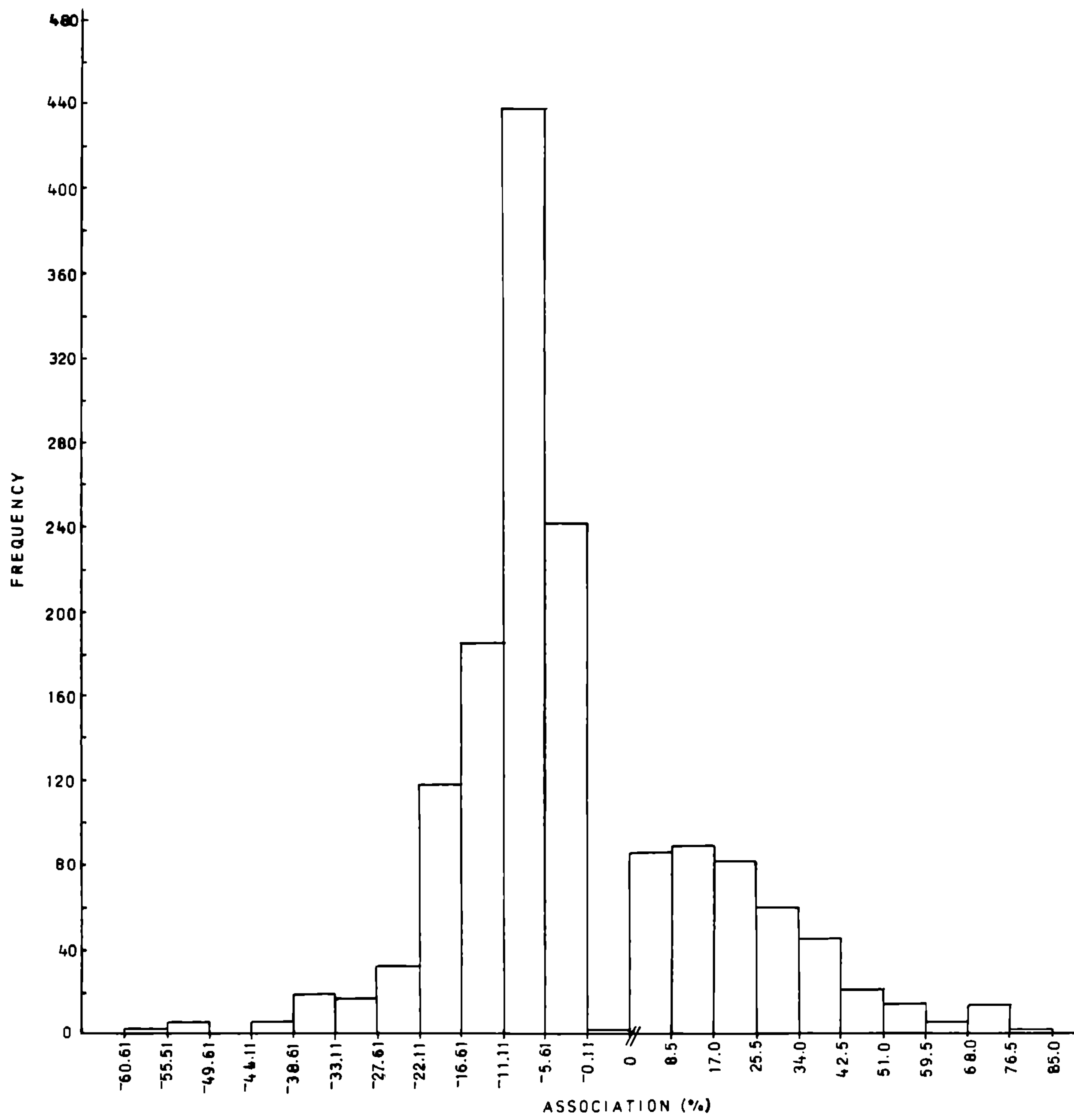

Fig. 5.16 Frequency distribution of the species association in mangrove mud samples

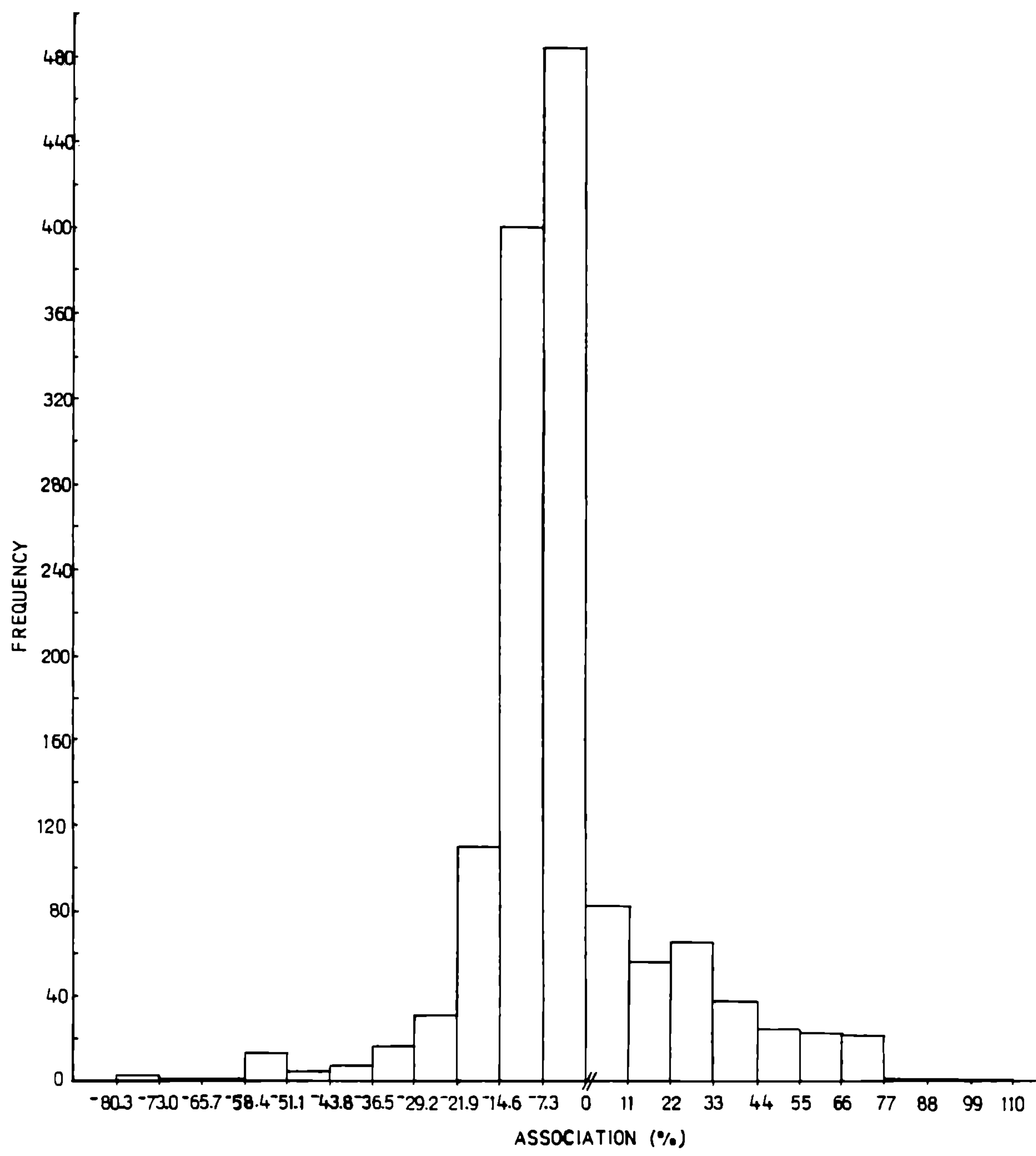

Fig.5.17. Frequency distribution of the species association in decaying mangrove vegetation

independent occurrence of fungal species. This is more striking when we consider that station 7 was also environmentally distinct from the other stations providing more specialized uniform and favourable conditions for the mycoflora except for the red yeasts.

CHAPTER 6

BIOCHEMICAL ACTIVITIES

Commercial exploitation of fungal enzymes for industrial purposes has increased in recent years. Upto 300 tones of glucoamylase are produced annually (Molitoris and Schaumann, 1986). Terrestrial species are so far utilized for enzyme production for which they are grown in submerged culture. According to the above authors it is possible to select fungal strains having greater potential for enzyme production from species naturally adapted to grow in liquid media.

Besides their potential for such commercial exploitation, their important role in the degradation of complex molecules in aquatic and especially estuarine and marine environments are much less documented. Fungi are known to secrete a series of exoenzymes which breakdown the more complex natural materials into simpler substances (Harley, 1971 ; Alexander, 1983). Jones (1974) and Odum and Heald (1975) have pointed out the importance of mangrove detritus as the energy base for an extensive food web. The first link is provided by fungi and bacteria which convert the relatively indigestable lignin and cellulose of vascular plant tissue into protein source that can be digested by the organisms in the second link of the food web. A great part of this activity is due to the degradative activity of many higher fungi. This prompted the studies on biochemical activities of filamentous fungi for which isolates obtained

from the mangrove area (Mangalavanam), where organic material of plant origin is added continously into the detrital system were chosen. Unlike filamentous fungi, yeasts do not breakdown complex natural substances like cellulose and starch, but depend on other organisms for the initial breakdown processes (Cooke, 1979). Their hydrocarbonoclastic activities were examined since petroleum products are introduced into the estuary from the tanker jetties and neighbouring petroleum companies. Yeast isolates collected from different stations were examined for their ability to utilize some of the common forms of hydrocarbon. They were also tested for their ability to utilize pectin as they are known to have this property like the filamentous fungi.

In all 51 fungal and 35 yeast isolates were screened for their various biochemical activities.

6.1 Fungal activity in mangrove ecosystem

Interest in mangrove fungi is mainly based on the economic importance of the mangrove community throughout the tropics (Saeenger *et al.*, 1983). The role of fungi in the mangrove ecosystem is less well known although it is presumably similar to that of other forest and swamp ecosystems (Findlay *et al.*, 1986). Heald and Odum (1970) showed that the food web of the Florida Bay estuarine ecosystem is largely based on plant detritus and attendant microbes comprising fungi, bacteria and protozoa. The microbes convert the refractory detrital material of plant

origin into microbial biomass, which nutrionally enrich the detrital particles for the detritivores at the base of the food web (Odum, 1971). Kaushik and Hynes (1971) have demonstrated that the fungi are the main agents behind this phenomenon in aquatic ecosystems.

Research on marine fungi has been mainly concerned with their isolation, cultivation, morphology, taxonomy and systematics. Recently increasing number of papers appear on the physiology of marine fungi related to enzymatic aspects (Schaumann, 1974a ; Nilsson, 1974 ; Wainwright and Sherbrock -Cox, 1981 ; Torzilli, 1982 ; Benner et al.,1984 and others). But these papers deal only with single species or isolated physiological aspects of fungi. A broad investigation of enzymology of selected isolates of mangrove fungi based on a larger and more representative number of species from different taxonomic and ecological groups is therefore attempted in this study. The present programme examines the degradative exo-enzymes produced by fungi which are important in host-infection, deterioration of materials and breakdown of organic matter. The purpose is to determine the function of the mycofloral community through the investigation of their enzymatic activities. Information about these activities would allow a better understanding of the role of fungi in the marine influenced backwater system especially around the industrial belt of Cochin. This will also be a pointer for a more specific and elaborate studies in future.

Selection of species

In order to obtain information on a wide spectrum of fungi, selected isolates from two ecological groups (Mud or sediment inhabiting forms and litter degrading forms) and different taxonomic groups (Deuteromycotina, Ascomycotina and Phycomycotina) were studied.

Selection of enzymes

The enzymes investigated in this programme were selected depending on their metabolic and industrial importance, ecological significance and the availability of simple, quick qualitative or semiquantitative tests on agar plates or tubes. The use of solid media permits the rapid screening of large population of fungi for the absence or presence of specific enzymes and allow search for genetic variants to be made more precisely. Such media are also useful in ecological studies where enzymatic capabilities of fungi are to be compared with other microorganisms (Hankin and Anagnostakis, 1975).

A total of seven enzymic abilities and phosphate solubilizing ability of selected isolates were screened. The enzymic ability tests conducted were cellulase, amylase, pectinase and chitinase (C - metabolism), gelatinase and caseinase (N - metabolism) and lipase (fat -metabolism). Amylase can breakdown natural substrates such as plant starch and is used in sugar, bakery, textile and paper industry. Cellulase acts upon plant and animal cellulose and is used in paper industry, in pollution control and in the use of waste

material, while pectinase breaksdown pectin, a component of plant cell walls and is used in food industry and beverages. The chitinase breaksdown natural polysaccharides such as chitin of animals and fungi and are very useful in the biological control of pathogens. The two proteases - gelatinase and caseinase breakdown both plant and animal proteins. Gelatinase is presently used as a detergent, additive to beer, drugs and cosmetics while caseinase for pollution control and in the use of waste material. The lipase is valued in food industry and often used as an additive for extractions. This enzyme breaksdown the natural substances such as animal and plant lipids. In addition to these, phosphate solubilizing ability of fungi were aslo studied since phosphorus is often a limiting factor in an ecosystem and it is important to identify the agents that are able to release them back to the system.

The ability of several fungal species to produce enzymes on solid media is shown in Table 6.1. The term enzyme production is here intended to mean both synthesis of the enzyme by the fungus and activity of the enzyme in the medium after it is produced. From the data presented in Table 6.1 it is apparent that majority of fungi produce more than one enzyme.

Cellulase activity

During a typical 35 day test for cellulase, clearing of swollen cellulose is observed (Fig. 6.1; Table 6.2). Of the 51 isolates screened for cellulase production 49 were

Table 6.1 The enzymatic abilities and phosphate solubilization of fungal species isolated from mangrove ecosystem

Mycoflora	Amylase	Cellulase	Pectinase	Chitinase	Lipase	Caseinase	Gelatinase	Phosphate solubilization
Acrothecium sp.	+	+	+	-	+	-	+	-
Absidia cylindrospora	-	+	+	-	+	-	-	-
Alternaria fasciculata	-	+	+	-	+	-	+	-
A. humicola	-	+	+	-	+	-	+	-
A. tenuis	-	+	+	-	+	-	+	-
Aspergillus candidus	+	+	+	-	-	-	-	+
A. chevalieri	+	+	+	-	+	+	-	-
A. fumigatus	-	+	-	-	+	+	+	+
A. nidulans	-	+	-	-	+	-	-	-
A. niger	+	+	-	-	+	-	-	+
A. oryzae	+	+	+	-	+	-	+	-
A. sydowi	+	+	-	-	+	+	+	-
A. terrus	+	+	+	-	+	+	+	+
A. ustus	+	+	+	-	+	+	+	-
A. versicolor	+	+	+	-	+	+	-	-
Botrytis terrestris	+	+	+	-	-	-	-	-
Cephalosporium acremonium	+	+	-	-	+	+	-	-
C. roseo-griseum	-	+	+	-	-	+	+	-
Chalara sp.	-	-	-	-	-	-	+	-
Chaetomium cristatum	-	+	+	-	+	+	-	-
C. globosum	-	+	-	-	+	-	-	-
Cladosporium herbarum	+	+	+	-	+	+	+	-
C. resinae	+	+	+	-	+	+	+	-
Colletotrichum gloeosporoides	-	+	+	-	+	-	+	-
Curvularia geniculata	-	+	+	-	+	-	-	-
C. interseminata	+	+	-	-	+	-	+	-
C. lunata	-	+	-	-	+	-	-	-
Dendryphiella sp.	+	+	+	-	+	+	-	-
Drechslera halodes	+	+	-	-	-	-	-	-
Fusarium neoceras	-	+	+	-	+	+	+	-
F. oxysporum	+	+	+	-	+	+	+	-
F. solani	-	+	+	-	+	+	-	-
Geotrichum candidum	-	-	+	-	-	-	-	-
Gliocladium penicilloides	-	+	-	-	+	-	-	-
Helminthosporium sp.	-	+	+	-	-	-	-	-
Humicola alopallonella	+	+	+	-	+	-	+	-
Monilia brunnea	-	+	-	-	+	+	-	-
Mucor hiemalis	-	+	-	-	+	-	-	-
Myrothecium verrucaria	-	+	+	-	+	-	+	-
Paecilomyces varioti	+	+	+	-	-	+	+	-
Penicillium citrinum	+	+	+	-	+	+	-	-
P. claviforme	+	+	-	-	+	-	+	-
P. janthinellum	+	+	+	-	+	+	-	+
P. mono. symmetrica	+	+	+	-	+	-	-	-
Phoma humicola	-	+	+	-	+	-	+	-
Sporormia minima	-	+	+	-	+	+	-	-
Trichoderma glaucum	-	+	+	-	+	-	-	-
T. koningi	-	+	-	-	+	-	-	+
T. viride	-	+	-	-	+	-	-	-
Verticillium sulphurellum	+	+	-	-	-	-	-	-
Zalerion maritimum	+	+	+	-	-	-	-	-

\+ Activity - No activity

positive to the test, which constitutes 96.1%. Majority of the isolates showed maximum cellulase activity at 37°C while

Table 6.2 Depth of clearing of acid-swollen cellulose suspension in agar column by mangrove fungi

Mycoflora	Depth of clearing (mm)				
	Days				
	7	14	21	28	35
Acrothecium sp.	4.0	5.0	6.0	8.0	9.0
Absidia cylindrospora	3.0	3.5	4.0	7.0	8.2
Alternaria fasciculata (28°C)	2.0	6.0	10.0	15.0	21.0
A. humicola (28°C)	2.0	4.0	5.0	6.0	7.2
A. tenuis	6.0	10.0	13.0	23.0	26.0
Aspergillus candidus	4.0	6.0	12.0	16.0	18.0
A. chevalieri (28°C)	1.0	2.0	5.0	12.0	15.0
A. fumigatus	4.3	7.0	15.0	24.5	28.0
A. nidulans	2.0	3.5	6.0	8.0	9.5
A. niger	3.0	3.5	6.0	7.0	18.0
A. oryzae (28°C)	2.0	4.0	8.0	12.0	14.0
A. sydowi	2.0	2.5	4.0	6.0	10.0
A. terrus	7.0	10.0	13.0	20.0	28.0
A. ustus	4.0	6.0	9.0	12.0	16.0
A. versicolor	3.0	5.5	8.0	15.0	17.0
Botrytis terrestris	3.0	5.0	7.0	11.0	13.0
Cephalosporiun acremonium	4.0	7.6	11.0	15.0	20.0
C. roseo-griseum	3.5	8.3	15.0	25.0	30.1
Chaetomium cristutum	5.0	11.0	16.0	23.0	31.0
C. glbosum	2.5	4.3	6.0	11.0	15.0
Cladosporim herbarum	3.5	5.0	8.0	9.0	11.0
C. resinae (28°C)	0.0	0.0	2.0	3.0	3.0
Colletotrichum gloeosporoides	7.0	8.3	12.0	16.0	20.0
Curvularia geniculata (28°C)	3.0	11.0	13.0	20.0	25.0
C. interseminata	5.0	8.6	13.5	18.0	22.0
C. lunata (28°C)	0.0	0.0	5.0	11.0	15.0
Dendryphiella sp.	7.0	8.7	10.0	12.0	15.0
Drechslera halodes (28°C)	2.0	6.0	7.0	10.0	12.0
Fusarium neoceras	6.2	11.0	13.0	20.0	22.0
F. oxysporum (28°C)	0.0	0.0	0.0	0.0	10.0
F. solani	5.0	7.5	12.0	17.0	20.0
Gliocladium penicilloides	4.0	6.0	8.0	12.0	18.0
Helminthosporium sp.(28°C)	3.0	8.5	12.0	20.0	25.0
Humicola alopallonella(28°C)	2.0	5.3	9.0	14.0	16.0
Monilia brunnea	6.3	10.0	16.0	23.0	26.0

(cont./-)

Table 6.2 (continued)

Mycoflora	Depth of clearing (mm)				
	Days				
	7	14	21	28	35
Mucor hiemalis	1.0	2.0	4.0	7.0	8.5
Myrothecium verrucaria	9.0	11.0	15.0	18.0	22.0
Paecilomyces varioti	1.5	2.0	2.0	4.5	6.0
Penicillium citrinum	3.0	3.5	7.5	12.0	18.0
P. claviforme	0.0	0.0	1.0	1.0	1.5
P. janthinellum	6.0	10.0	12.0	16.0	20.0
P. mono. symmetrica (28°C)	1.0	2.5	3.5	5.0	5.5
Phoma humicola (28°C)	3.0	6.0	10.0	13.5	15.0
Sporormia minima	6.0	10.0	13.0	15.0	18.0
Trichoderma glaucum	7.0	10.0	15.0	19.0	24.0
T. koningi	7.5	11.0	15.0	20.0	24.5
T. viride	6.0	10.6	16.0	24.0	27.0
Verticillium sulphurellum	4.0	6.0	10.0	16.0	18.0
Zalerion maritimum	5.0	7.0	9.0	11.0	12.0

Temperature 37°C, unless mentioned

a few at 28°C. Maximum depth of clearing of acid-swollen cellulose was shown by Chaetomium cristatum followed by Cephalosporium roseo-griseum, Aspergillus fumigatus and A. terrus at 37° C. Alternaria tenuis, C. acremonium, Colletotrichum gloeosporoides, Curvularia geniculata, C. interseminata, Fusarium neoceras, F. solani, Helminthosporium sp., Monilia brunnea, Myrothecium verrucaria, Penicillium janthinellum, Trichoderma glaucum, T. koningi and T. viride showed good cellulolytic activity. Except C. geniculata and Helminthosporium sp. all aforementioned isolates preferred 37°C.

Amylase activity

From the results given in Table 6.3, it can be seen that 25 out of 51 mangrove fungal isolates (49%) possess the amylolytic ability. Amylolytic activity is shown in Fig. 6.2.

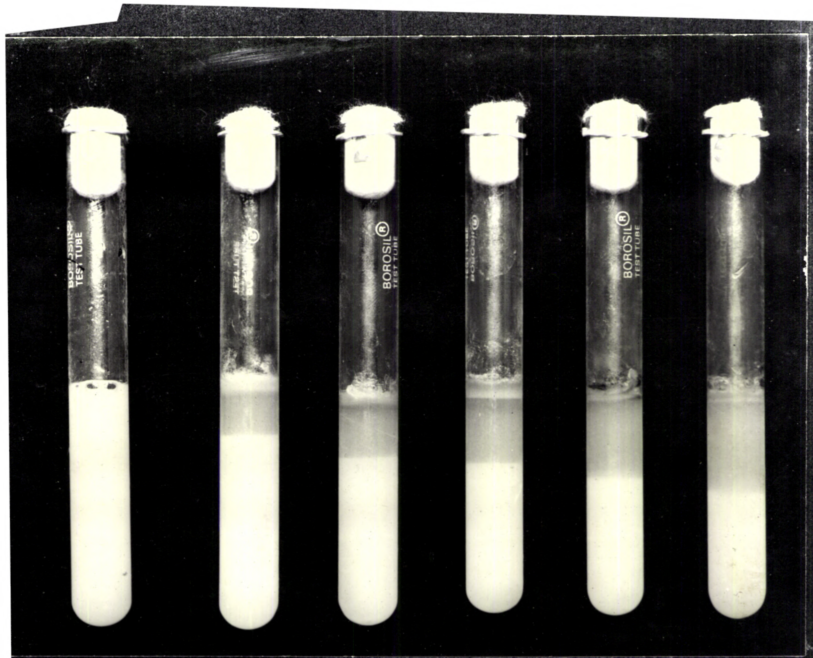

Fig. 6.1 Composite plate showing clearing of swollen cellulose by NIO C-251 Chaetomium cristatum. From left to right, the different tubes have been incubated for 0, 7, 14, 21, 28 and 35 days respectively. Depth of clearing can be seen in the photograph.

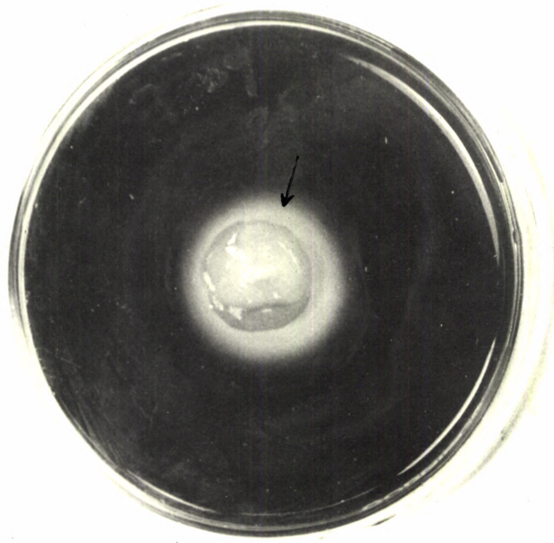

Fig. 6.2 Plate showing lysis of starch by NIO C-136 Humicola alopallonella. Arrow indicates clear zone.

From the Table it can be seen that Aspergillus chevalieri had the maximum ability. The other fungi which showed good amylase activity were Paecilomyces varioti, Penicillium claviforme and Verticillium sulphurellum. Aspergillus

Table 6.3 The amylolytic ability of fungal species of isolated from mangrove area

Mycoflora	Radius of the lysed zone (mm) and and period of incubation				
	24hr	48hr	72hr	96hr	120hr
Acrothecium sp.	0.5	1.5	1.6	1.9	1.9
Aspergillus candidus	2.5	3.0	3.5	3.6	3.6
A. chevalieri	5.5	8.0	10.0	10.5	10.8
A. niger	0.0	0.5	0.8	0.8	0.8
A. oryzae	1.0	4.0	4.0	4.05	4.1
A. sydowi	1.0	3.0	4.0	4.05	4.5
A. terrus	3.3	4.0	4.0	4.0	4.1
A. ustus	0.0	0.8	1.1	1.5	1.2
A. versicolour	0.0	1.5	1.8	1.8	2.0
Botrytis terrestris	0.0	1.0	1.0	1.1	1.1
Cephalosporium acremonium	2.5	3.8	4.1	4.2	4.3
Cladosporium herbarum	2.0	2.5	2.6	2.6	2.7
C. resinae	0.5	1.1	1.5	1.5	1.5
Curvularia interseminata	1.0	1.5	1.5	1.5	1.8
Dendryphiella sp.	2.0	3.0	3.3	3.5	3.7
Drechslera halodes	0.5	2.0	2.0	2.1	2.1
Fusarium oxysporum	0.5	1.0	1.0	1.0	1.0
Humicola alopallonella	0.0	1.5	1.8	1.8	1.8
Paecilomyces varioti	1.5	3.5	4.8	5.5	5.6
Penicillium citrinum	0.5	3.0	3.0	3.1	3.1
P. claviforme	2.5	3.5	3.6	5.5	5.8
P. janthinellum	1.0	3.0	3.0	3.1	3.1
P. mono.symmetrica	3.5	4.0	4.1	4.1	4.2
Verticillium sulphurellum	3.0	5.0	5.3	5.3	5.5
Zalerion maritimum	1.3	1.5	2.0	2.3	2.5

candidus, A. orzae, A. sydowi, A. terrus, Cephalosporium acremonium, Cladosporium herbarum, Dendryphiella sp., Penicillum citrinum, P. janthinellum, P. monoverticillate symmetrica and Zalerion maritimum showed moderate activity.

Both A. niger and Fusarium oxysporum showed poor amylase activity.

Pectinase activity

The pectinolytic activity by the mangrove fungi is given in Table 6.4. Fig. 6.3 shows the pectinolytic activity. Of the 51 isolates screened 34 isolates (66.7%) showed positive results. Marine fungus Dendryphiella sp., showed excellent

Table 6.4 The pectinolytic ability of fungal species isolated from mangrove area

Mycoflora	Radius of the lysed zone (mm) and period of incubation				
	24hr	48hr	72hr	96hr	120hr
Acrothecium sp.	2.2	3.0	3.5	3.8	3.8
Absidia cylindrospora	1.5	2.3	2.5	2.8	2.9
Alternaria fasciculats	5.3	6.0	6.3	6.5	6.6
A. humicola	2.5	2.8	3.0	3.1	3.2
A. tenuis	3.0	3.4	3.7	3.8	3.8
Aspergillus candidus	1.0	1.8	2.5	2.6	2.8
A. chevalieri	2.8	3.0	3.3	3.5	3.6
A. oryzae	2.0	2.5	2.5	2.6	2.6
A. terrus	2.0	2.3	3.0	3.3	3.3
A. ustus	2.6	3.0	3.1	3.3	3.3
A. versicolor	2.3	3.0	3.1	3.3	3.2
Botrytis terrestris	1.5	1.8	2.0	2.5	2.6
Cephalo. roseo-griseum	2.0	2.8	3.5	3.7	3.7
Chaetomium cristatum	1.0	2.1	2.5	2.7	2.7
Cladosporium herbarum	3.5	5.0	6.0	6.3	6.3
C. resinae	3.0	3.3	3.5	4.0	4.1
Colleto. gloeosproides	4.0	4.3	4.5	4.6	4.6
Curvularia geniculata	2.3	2.4	2.8	3.0	3.2
Dendryphiella sp.	5.0	6.0	6.8	7.0	7.1
Fusarium neoceras	4.5	4.5	5.0	5.3	5.3
F. oxysporum	1.5	2.5	3.0	3.3	3.3
F. solani	6.0	6.0	6.5	6.7	6.8
Geotrichum candidum	1.5	2.5	3.0	3.4	3.5
Helminthosporium	2.0	2.7	3.0	3.1	3.6

(cont./-)

Table 6.4 (continued)

Mycoflora	Radius of the lysed zone (mm) and period of incubation				
	24hr	48hr	72hr	96hr	120hr
Humicola alopallonella	2.5	4.0	4.6	5.0	5.1
Myrothecium verrucaria	1.5	2.0	2.5	2.8	3.0
Paecilomyces varioti	2.3	2.5	3.0	3.3	3.5
Penicillium citrinum	2.0	3.0	3.2	3.2	3.2
P. janthinellum	3.0	3.5	3.8	4.0	4.1
P. mono.symmetrica	2.5	2.8	3.0	3.0	3.0
Phoma humicola	3.0	3.5	4.0	4.1	4.2
Sporormia minima	1.5	3.0	4.5	4.8	5.1
Trichoderma glaucum	1.0	2.0	2.8	3.0	3.0
Zalerion maritimum	1.5	3.5	4.5	5.0	5.3

activity, while Alternaria fasciculata, Cladosporium herbarum, Fusarium neoceras, F. solani, Humicola alopallonella, Sporormia minima and Zalerion maritimum showed comparatively good activity.

Chitinase activity

Although 51 isolates were screened for chitinase none of them showed its production (Table 6.1).

Lipase activity

Forty one isolates (80.40%) showed lipase activity. The results are presented in Table 6.5. Fig.6.4 illustrates the lipase activity. Maximum activity was shown by Acrothecium sp. followed by Aspergillus versicolor and Fusarium neoceras. Cladosporium resinae and Phoma humicola showed good activity.

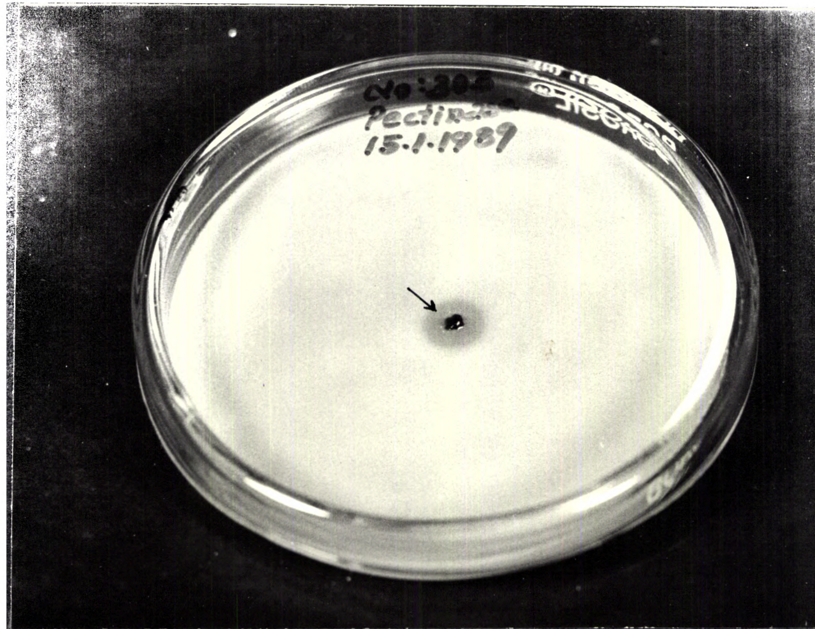

Fig. 6.3 Plate showing pectin degradation by NIO C-309 *Zalerion maritimum*. Arrow indicates clear zone.

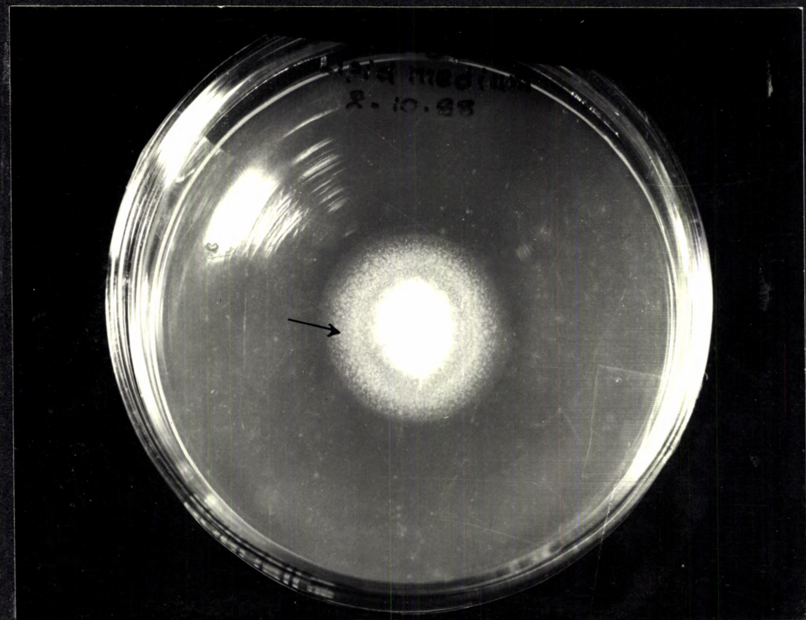

Fig. 6.4 Plate showing lipase activity by NIO C-239 *Fusarium oxysporum*. Arrow indicates crystals of the calcium salt of the lauric acid liberated by the enzyme.

Table 6.5 The lipolytic ability of fungal species isolated from mangrove area

Mycoflora	Radius of the lysed zone (mm)and period of incubation				
	24hr	48hr	72hr	96hr	120r
Acrothecium sp.	2.0	3.8	5.0	7.0	8.0
Absidia cylindrospora	0.7	1.5	2.0	2.5	3.3
Alternaria fasciculata	1.4	1.8	2.5	3.0	3.0
A. humicola	0.8	1.5	1.9	2.5	2.6
A. tenuis	2.3	3.3	4.0	4.6	4.9
Aspergillus chevalieri	1.1	2.1	3.0	4.5	4.6
A. fumigatus	1.0	1.3	3.0	4.6	4.6
A. nidulans	0.5	1.1	2.3	3.0	3.4
A. niger	0.5	1.5	2.5	3.0	3.3
A. oryzae	1.5	3.0	4.3	4.5	4.9
A. sydowi	0.6	1.9	3.3	4.0	4.1
A. terrus	1.6	2.5	3.1	3.5	3.9
A. ustus	1.3	2.7	4.0	4.5	4.7
A. versicolor	1.8	3.3	4.5	6.5	7.9
Cephalosporium acremonium	1.5	2.8	4.0	4.5	4.5
Chaetomium cristatum	0.0	0.1	1.0	1.3	1.3
C. globosum	1.0	2.0	2.8	2.8	3.0
Cladosporium herbarum	1.5	3.0	3.8	5.5	4.5
C. resinae	2.0	4.0	4.8	5.5	5.7
Colleto. gloeosporoides	0.8	1.3	1.3	1.5	1.5
Curvularia geniculata	0.0	0.0	0.5	1.0	1.5
C. interseminata	1.7	2.6	3.5	3.8	3.8
C. lunata	0.0	0.5	0.5	1.1	1.2
Dendryphiella sp.	0.8	1.6	2.6	3.0	3.3
Fusarium neoceras	2.3	3.9	5.5	7.6	7.7
F. oxysporum	2.0	3.0	3.5	4.3	4.3
F. solani	0.5	1.5	2.3	3.0	3.2
Glio. penicilloides	0.0	0.5	1.3	1.5	1.8
Humicola alopallonella	1.0	1.5	2.0	3.0	3.0
Monilia brunnea	1.0	1.5	3.0	3.3	3.3
Mucor hiemalis	1.6	2.5	3.4	4.0	4.1
Myrothecium verrucaria	1.3	2.3	3.0	3.5	3.5
Penicillium citrinum	1.5	2.3	3.0	3.8	4.0
P. claviforme	1.1	1.9	3.0	4.1	4.1
P. janthinellum	1.0	1.6	2.9	4.5	4.8
P. mono.symmetrica	1.3	2.0	3.5	3.8	4.0
Phoma humicola	2.0	3.3	4.3	6.3	6.8
Sporormia minima	0.0	0.3	0.5	1.3	1.3
Trichoderma glaucum	0.3	1.5	3.0	3.8	4.2
T. koningi	0.3	0.5	1.0	2.0	2.0
T. viride	0.0	0.5	0.5	1.0	1.0

Caseinase activity

The caseinolytic activity is shown in Table 6.6. Fig. 6.5 illustrates the activity. Only 20 isolates (39.2%) were able to show the positive results. *Paecilomyces varioti* showed maximum lysis of casein followed by *Cladosporium herbarum*. The other isolates that showed good activity were *Aspergillus chevalieri*, *A. fumigatus*, *A. versicolor*, *Cephalosporium acremonium*, *C. roseo-griseum*, *Dendryphiella* sp., *Fusarium neoceras* and *F. solani*.

Table 6.6 The caseinolytic activity of fungal species isolated from mangrove area

Mycoflora	Radius of the lysed zone (mm) and period of incubation				
	24hr	48hr	72hr	96hr	120hr
Aspergillus chevalieri	1.5	2.5	4.0	4.3	4.5
A. fumigatus	3.0	3.5	3.5	3.6	3.6
A. sydowi	1.5	1.8	2.5	2.8	3.0
A. terrus	2.5	3.1	3.1	3.1	3.3
A. ustus	3.0	3.0	3.1	3.2	3.2
A. versicolor	3.2	3.5	3.5	3.6	3.6
Cephalo. acremonium	1.8	2.6	3.0	3.5	3.8
C. roseo-griseum	2.0	3.5	4.3	4.5	4.5
Chaetomium cristatum	1.0	2.5	2.5	2.8	2.8
Cladosporium herbarum	1.8	2.8	5.0	6.8	8.3
C. resinae	0.0	0.3	0.5	0.8	0.9
Dendryphiella sp.	1.5	3.0	3.5	3.8	4.0
Fusarium neoceras	2.3	3.5	3.8	3.8	3.8
F. oxysporum	0.0	1.0	1.3	1.5	1.5
F. solani	2.5	3.0	3.5	3.5	3.6
Monilia brunnea	0.5	1.0	1.0	1.3	1.5
Paecilomyces varioti	2.0	5.0	7.0	9.0	10.0
Penicillium citrinum	2.0	3.0	3.1	3.3	3.3
P. janthinellum	2.0	3.0	3.0	3.3	3.3
Sporormia minima	1.5	2.0	2.5	2.8	3.0

Gelatinase activity

The results are shown in Table 6.7 and Fig. 6.6 illustrates the gelatinase activity. Twenty two isolates (43.1%) were able to show this ability. Paecilomyces varioti showed excellent result followed by Acrothecium sp. and Cephalosporium roseo-griseum.

Table 6.7 Gelatinase ability of fungal species isolated from mangrove area

Mycoflora	Radius of the lysed zone (mm) and period of incubation				
	24hr	48hr	72hr	96hr	128hr
Acrothecium sp.	4.0	7.0	8.0	10.5	13.0
Alternaria fasciculata	3.0	4.0	4.3	4.4	4.5
A. humicola	3.0	3.5	4.0	4.1	4.2
A. tenuis	2.0	3.0	3.3	3.5	3.8
Aspergillus fumigatus	1.0	2.0	2.1	2.1	2.5
A. oryzae	1.0	1.5	2.0	2.0	2.1
A. sydowi	0.3	0.5	1.0	1.1	1.3
A. terrus	1.5	1.8	2.1	2.1	2.2
A. ustus	3.5	4.0	4.0	4.1	4.3
Cephalo. roseo-griseum	4.0	6.0	9.0	10.0	11.5
Chalara sp.	0.5	1.0	1.0	1.0	1.1
Cladosporium herbarum	2.5	5.5	7.0	8.0	9.0
C. resinae	0.5	1.5	2.3	2.3	2.5
Colleto. gloeosporoides	0.5	1.0	1.5	1.5	1.6
Curvularia interseminata	4.0	4.5	4.8	4.8	5.3
Fusarium neoceras	2.0	3.0	3.2	3.3	3.3
F. oxysporum	1.0	1.5	1.8	1.8	2.0
Humicola alopallonella	0.5	1.0	1.8	1.8	1.9
Myrothecium verrucaria	0.8	1.5	2.0	4.0	4.3
Paecilomyces varioti	5.5	7.5	9.5	13.5	16.0
Penicillium claviforme	3.0	5.0	5.3	7.0	7.3
Phoma humicola	1.0	2.0	2.5	2.8	2.9

Fig. 6.5 Plate showing lysis of milk protein by NIO C-80 *Paecilomyces varioti*. Arrow indicates clear zone

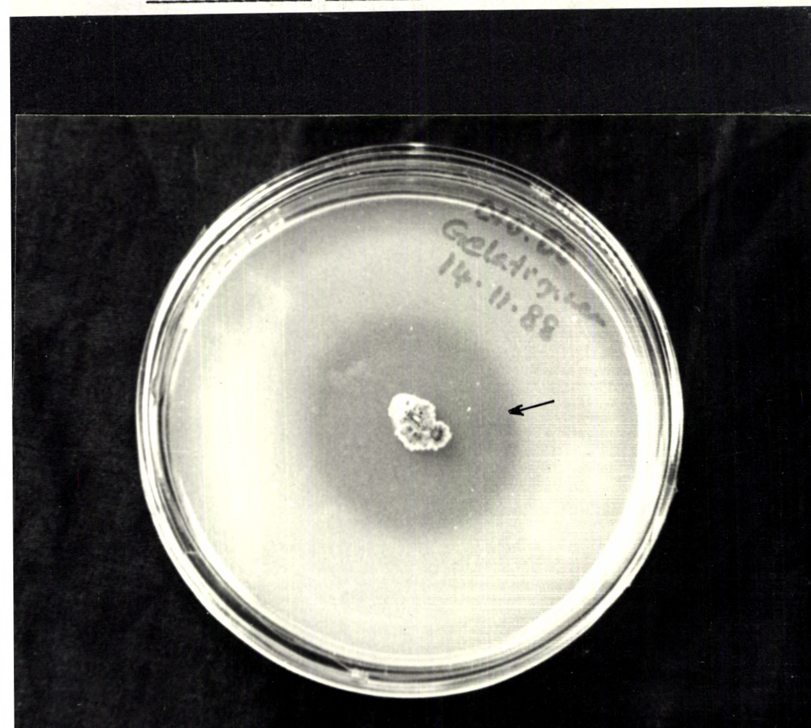

Fig. 6.6 Plate showing gelatinase activity by NIO C-80 *Paeilomyces varioti*. Arrow indicates clear zone.

Phosphate solubilizing acitivity

The results are shown in Table 6.8 and Fig. 6.7 illustrates the activity. Of the 51 isolates screened only 6 isolates (11.8%) were able to solubilize the tricalcium phosphate. Excellent activity was shown by *Aspergillus candidus*. *A. niger* and *A. fumigatus* showed good activity while *Trichoderma koningi* showed low activity.

Table 6.8 Phosphate solubilization by fungal species isolated from mangrove area

Mycoflora	Radius of the lysed zone (mm) and period of incubation				
	24hr	48hr	72hr	96hr	120hr
Aspergillus candidus	4.5	6.0	7.0	9.5	9.5
A. fumigatus	2.0	3.0	4.0	4.0	4.3
A. niger	2.0	3.0	4.3	4.5	4.5
A. terrus	0.0	0.0	1.5	2.0	2.3
Penicillium janthinellum	1.5	2.0	3.0	3.0	3.0
Trichoderma koningi	0.0	1.0	1.5	1.5	1.8

6.2 Activity of estuarine yeasts

Hydrocarbon assimilation

The result of hydrocarbon assimilation by the selected isolates is presented in Table 6.9. Fig. 6.8 illustrates the assimilation test. Photomicrographs (Figs 6.9 to 6.11) show the growth of yeast cells on oil globules. From the Table it can be seen that out of 35 isolates 26 (74.3%) were able to assimilate kerosene and 27 (77.1%) were able to assimilate diesel. About 16 isolates showed questionable growth and 8 isolates (23.9%) showed clear growth in crude oil. The

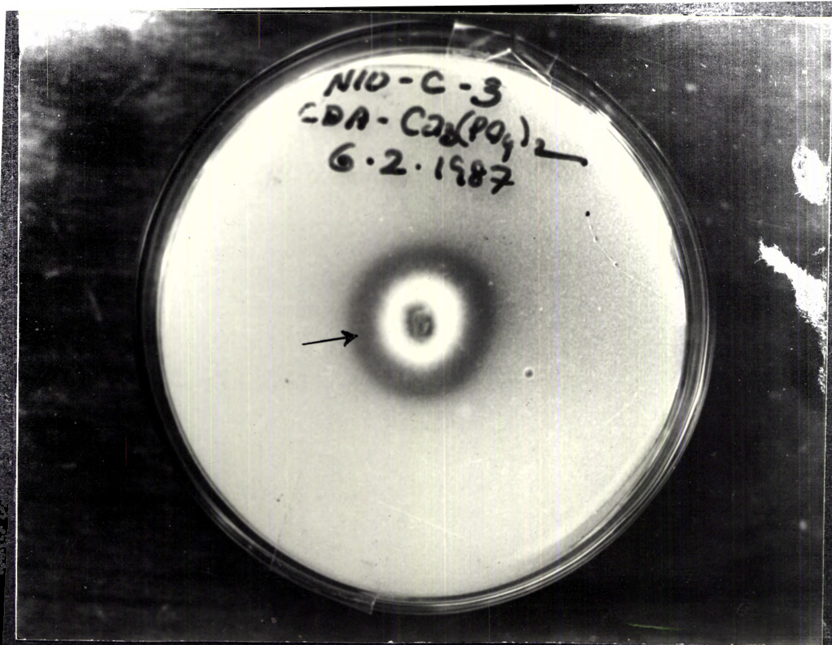

Fig. 6.7 Plate showing phosphate solubilization by NIO C-3 Aspergillus candidus. Arrow indicates clear zone.

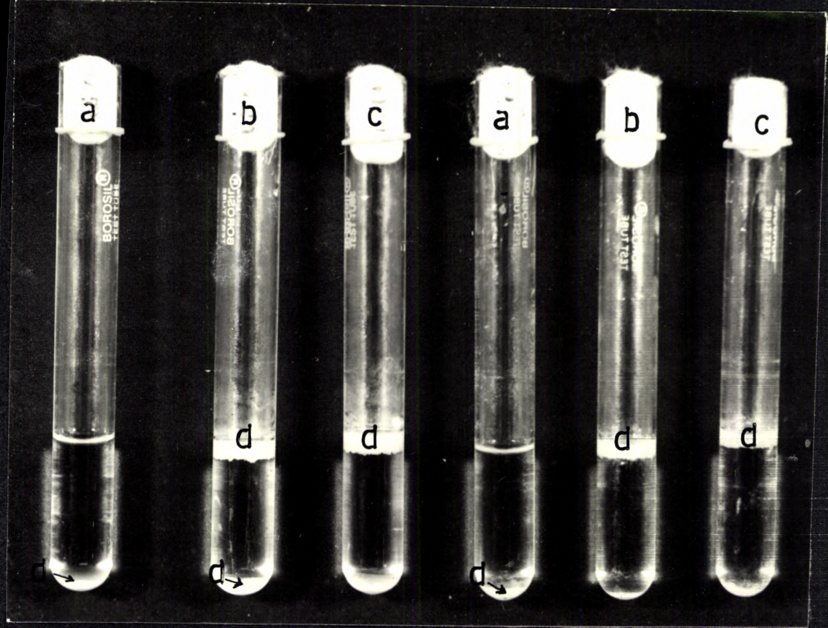

Fig. 6.8 Composite plate showing hydrocarbon assimilation by NIO C-62 Candida tropicalis. (a) Control, (b) Kerosene, (c) Diesel & (d) Organism after 4 days & 7 days respectively.

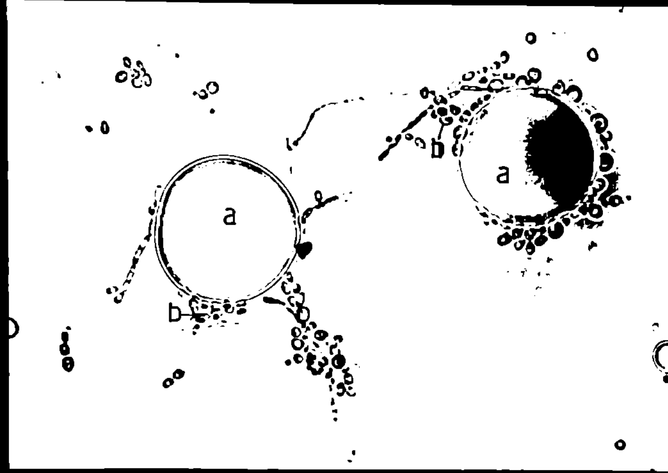

Fig. 6.9 Photomicrograph showing 3 days old growth of NIO C-62 Candida torpicalis cells on diesel globule - under low magnification. (a) Oil droplet and (b) yeast cells.

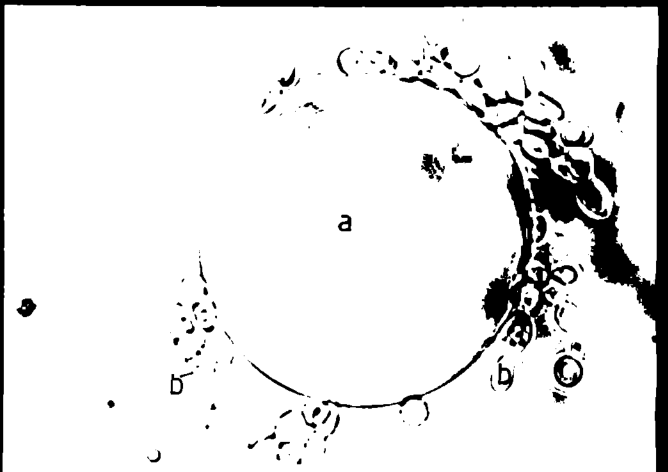

Fig. 6.10 Photomicrograph showing 3 days old growth of NIO C-62 Candida tropicalis cells on diesel globule - under high magnification. (a) Oil droplet and (b) yeast cells.

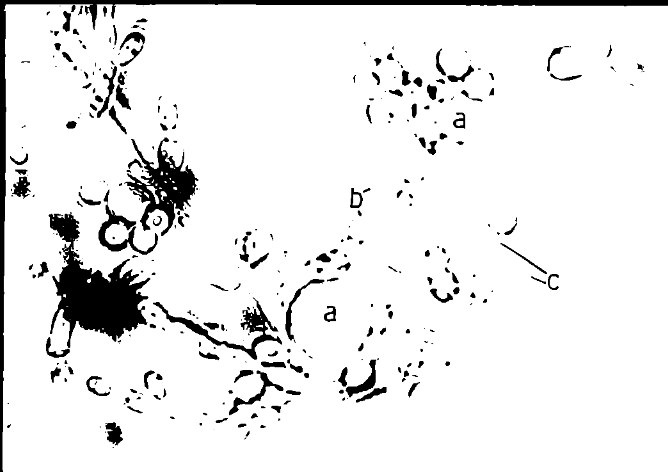

Fig. 6.11 Photomicrograph showing 7 days old growth of NIO C-62 Candida tropicalis showing complete entrapment of diesel oil droplets. (a) Oil droplet, (b) yeast cells & (c) pseudohyphae.

growth rate varied for each isolate and was observed in water-oil interface.

Table 6.9 Hydrocarbon assimilaation and pectinase activity of yeasts isolated from Cochin backwater

Organism	Hydrocarbon assimilation Crude oil	Kerosene	Diesel	Pectinase activity
Debaryomyces hansenii	-	-	-	+
D. marama	+	+	+	+
D. vanriji	+	+	+	+
Hansenuala anomala	*	+	+	+
Kluyveromyces marxianus	-	+	+	+
Pichia bovis	*	+	+	+
P. guilliermondii	+	+	+	+
Saccharomyces cerevisiae	-	-	-	+
S. exiguus	*	+	+	+
S. kluyveri	-	-	-	+
Saccharomyces sp.	-	-	-	+
Candida albicans	*	+	+	-
C. atmospherica	+	+	+	+
C. halophila	*	+	+	+
C. intermedia	*	+	+	+
C. krusei	*	+	+	+
C. membranaefaciens	+	+	+	+
C. parapsilosis	*	+	+	+
C. pseudointermedia	*	+	+	+
C. sake	*	+	+	+
C. solani	*	+	+	+
C. tropicalis	+	+	+	+
Candida sp.	-	-	-	+
Cryptococcus laurentii	-	-	-	+
Geotrichum candidum	*	+	+	+
Rhodotorula aurantiaca	*	+	+	+
R. glutinis	*	+	+	+
R. graminis	-	+	+	+
R. lactosa	-	+	+	+
R. minuta	*	+	+	+
R. rubra	+	+	+	+
Trichosporon aquatile	-	-	-	+
T. cutaneum	*	-	+	+
T. penicillatum	+	+	+	+
Sporobolomyces roseus	-	-	-	+

+ Growth - No growth * Questionable growth

Pectinase activity

The result is shown in Table 6.9. Fig. 6.12 illustrates the pectinase activity by yeasts. Of the 35 yeasts screened for pectinase, 34 (97.1%) showed positive activity.

The ability of so many species of filamentous fungi from the Cochin backwater to secrete a series of exoenzymes essential for the breakdown of compounds such as cellulose, starch etc., strongly indicate their significance in releasing the energy locked up in complex natural substances especially of plant origin. Mycopopulations thriving so successfully in the backwater, and clearly seen in 1987 in all the the stations show that they play a meaningful role in the food chain at that trophic level. These and other important aspects of higher fungi in detritus dominated estuarine system are discussed in the next section.

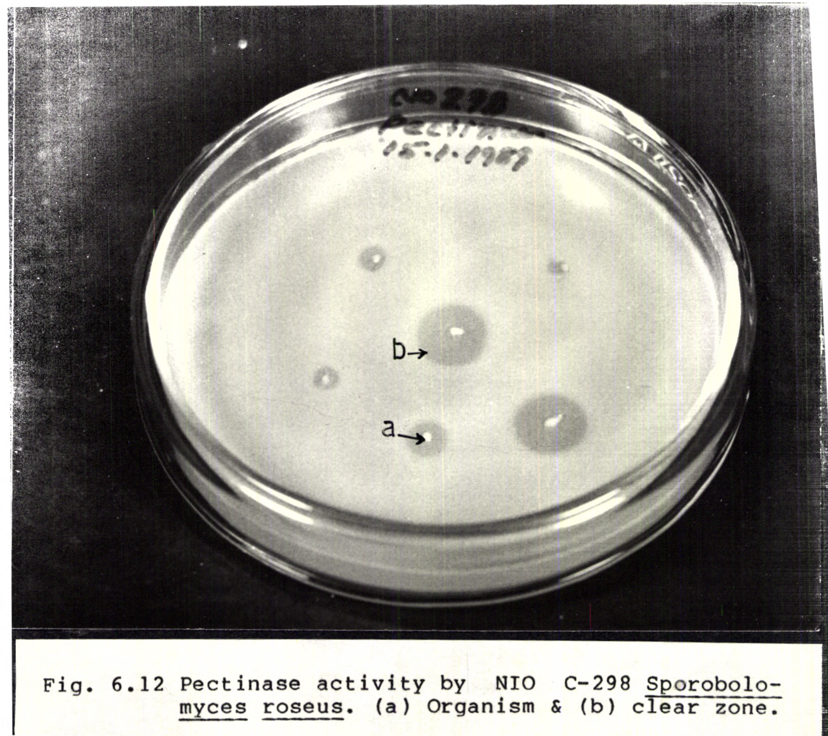

Fig. 6.12 Pectinase activity by NIO C-298 *Sporobolomyces roseus*. (a) Organism & (b) clear zone.

CHAPTER 7

GENERAL DISCUSSION

In the present investigation both yeasts and filamentous fungi inhabiting the water and mud have been studied for the general survey of fungi in Cochin backwater and adjacent areas. Species composition of yeasts showed presence of 35 species belonging to 11 genera and the majority of the species were found to be Deuteromycetes with dominant genera of *Candida* and *Rhodotorula*. In all, 24 species of Deuteromycetes and 11 species of Ascomycetes were isolated from the samples. Most of the species showed good growth at 37°C and also assimilative versatility in the utilization of various carbon compounds. The ability for assimilation of diverse carbon compounds is beneficial for heterotrophic yeasts living in the marine environment where the supply of nutrients and carbon compounds becomes critical at certain times (van Uden and Fell, 1968).

Although yeasts have been isolated from the estuary, they have not been differentiated into terrestrial and aquatic. Hundreds of yeast species may be regularly introduced into the system from terrestrial runoff, air, rivers, sewage, ships, birds etc.(van Uden and Castelo-Branco, 1963; Kawakita and van Uden, 1965; van Uden, 1967; van Uden and Fell, 1968). However, only a few forms seem to be capable of building up populations. *Debaryomyces hansenii*, *Pichia guilliermondii* and other species of the genera *Candida*,

Rhodotorula and *Cryptococcus* were frequently noticed in the samples. These species are well known in terrestrial habitats and are also apparently adapted to aquatic habitats which include marine environment. *Candida tropicalis* and *Candida albicans* were the most common forms in the back-waters of Cochin. These species alongwith *C. krusei*, *C. parapsilosis* and *G. candidum* occur predominantly in estuaries as well as in association with man and other animals (van Uden, 1958, 1960, 1963). Even healthy human beings will have detectable levels of *C. albicans*, *C. tropicalis* in the feces (APHA, 1985). In general, *C. albicans* *C. tropicalis* and *T. cutaneum* are common in areas of high of organic content, which may be due to a variety of causes such as sewage pollution and terrestrial runoff. In fact, their occurrence is indicative of sewage pollution in backwaters of Cochin.

High yeast densities were noticed in water when compared with mud samples at different sampling sites in the study except at station 7 in mangrove area of Mangalavanam. Generally yeasts are considered to be saprobes which depend on organic content of the system. Being a detritus dominated estuary, Cochin backwater is a productive system and there need be no surprise for higher yeast density and diversity. In the ocean waters increased population densities related to the productivity of the region and in particular corresponded with increased concentration of invertebrates although causal relationships were not determined (Fell, 1967). It is likely

that high concentration of plankton will also excrete end-products which become available to stimulate the blooming of yeasts. Differences can be found not only in population densities, but also in species composition.

Another striking ecological feature observed during the study was the relative percentage of red and white yeasts along with their numerical abundance in different stations. The high relative percentage of red yeasts at stations 1 and 2 shows the prevailing pollution free environment in these regions due to greater tidal flushing. Red yeasts were totally absent in water samples collected at station 7, which is located in a eutrophicated region. Being located near the oil tanker jetties and also under the influence of sewage discharge stations 3 and 4 showed higher percentage of white yeasts. These diverse observations therefore suggests the significance of relative percentage of red and white yeasts as biological indicators of organic pollution of the system.

Even the restricted studies on bio-degradation of organic compounds by yeasts collected from different stations in the Cochin backwater apparently show that they have a useful role in the degradation of petroleum products and also of natural polymers present in the system. Majority of the 35 selected isolates tested showed growth in kerosene and diesel oil. In crude oil clear indications of growth were registered by eight isolates and questionable growth by sixteen.

Growth of hydrocarbon utilizing microorganisms and their

ability for biodegradation of petroleum products are influenced by environmental parameters such as pH, salinity, temperature, oxygen and nutrients (Bartha and Atlas, 1977). Under favourable conditions yeasts (especially *Candida* sp.) covert paraffin hydrocarbons and other petroleum fractions into single celll protein. Although bacteria have been proposed for the destruction of petroleum effluents, the hydrocarbonoclastic yeasts and moulds have not been examined intensively as *in situ* biodegrdation agents, in spite of the wealth of excellent experimental laboratory data (Meyers and Ahearn, 1972). Ahearn *et al*. (1971b) tested representatives from marine habitats that grew on Louisiana crude oil and found *Debaryomyces hansenii*, *Candida parapsilosis* and *Rhodotorula glutinis* showing good activity.

Meyers and Ahearn, (1972) have discussed the application of yeasts to mediate oil decomposition. Yeasts are more resistant than bacteria to UV rays and to fluctuations in osmotic pressure and salinity and hence may have a potential role in the degradation of effluents in shallow areas such as estuaries where biological treatment methods are preferred.

Emilia Da Costa and D'Souza (1981) isolated most common yeasts belonging to the genera *Debaryomyces*, *Pichia*, *Saccharomyces*, *Candida*, *Cryptococcus*, *Geotrichum*, *Kloeckera*, *Rhodotorula*, *Trichosporon* and *Pullularia*. Only thirty percent of the isolates were found to be degrading hydrocarbon (diesel oil) and they commented that their presence in the estuaries appears to be advantageous in

reduing oil pollution.

Hydrocarbonoclastic yeasts are widespread in the neritic environment and may ocur in high densities in surface slicks (Ahearn and Meyers, 1976). In some cases selective growth of certain of the indigenous yeasts have been reported on surface slicks, although it has been noted that sustained increase in yeast biomass does not always occur, eventhough ample organic substrate in the form of oil is readily available (Ahearn and Meyers, 1976). Information is lacking regarding this fact. Considerable studies have to be be made for the selection of yeast species, which when introduced into oil dominated environments can effectively accelerate the degradation of oil.

It is also noteworthy that most of the yeasts isolated from the Cochin backwater also had high pectinase activity although degradation of complex substrates like cellulose and other polysaccharides are largely carried out by filamentous fungi and bacteria. Nelson D'Souza and D'Souza (1979a,b) also found that the majority of yeasts isolated from estuarine mangrove environment showed pectinase activity. Since estuarine yeasts are found to possess high pectinase activity, tapping this ability for industrial purposes seem to merit more serious investigation.

The present study also reported 96 species of filamentous fungi belonging to 39 genera from water and mud samples collected at seven stations of the backwaters of

Cochin which included mangrove ecosytem of Mangalavanam. Among them, 40 species were common in both water and mud samples. In comparison with yeasts, more number of filamentous fungal species are noticed in mud than in water saamples. Species composition shows that the majority of the isolated fungi are common in terrestrial habitats. Many of these genera have been reported from a variety of substrates including terrestrial soils (Gilman, 1967; Barron, 1968 and others). This study therefore confirms the earlier observations regarding the occurrence of terrestrial fungal propagules in estuarine and marine habitats. Like yeasts, filamentous fungi are also introduced into the aquatic ecosystem through the various allochthonous sources such as plant litter and other organic materials, erosion and runoff from soil etc. Subramanian and Raghukumar (1974) consider this occurrence as ' invasion ' of the marine habitat in the form of dormant propagules. This is likely to be more common in estuaries than in other non-estuarine marine locations, since estuaries receive daily inputs from rivers and diurnal tidal inputs from the ocean (Atlas and Bartha, 1981).

Many of the filamentous fungi described from different stations are reported by various authors (Pawar and Thirumalachar, 1966 ; Subramaniam and Raghukumar, 1974) as being relatively broad in ecological tolerance and having a high capacity for physiological adaptive responses. the fungal species from the two habitats - terrestrial and marine environment, could be morphologically alike but may differ in

their physiological adaptation such as tolerance to salinity etc. Many microorganisms found in estuaries are euryhaline, able to grow under conditions of low salinity typical of fresh water and under conditions of higher salinity typical of marine water (Atlas and Bartha, 1981).

Different species of filamentous fungi isolated during the survey indicate that in the backwater *Aspergillus* and *Penicillium* form the dominant genera. The most common species was *Aspergillus fumigatus*.

Fungal study of Mangalavanam area (mangrove ecosytem) showed presence of 71 species grouped under 35 genera. These forms were isolated from mud and decaying mangrove plant material. Most of the genera found in Mangalavanam area are also encountered in backwaters of Cochin. Majority of the species isolated from these samples were ubiquitous saprophytes with the dominance of Deuteromycetes, often associated with the breakdown of plant material. Similar occurrence of many of these terrestrial species from marine mangrove soil were recorded by many authors (Swart, 1958 ; Rai *et al*., 1969 ; Rai and Chowdhery, 1976 ; Matondkar *et al*., 1980b, Garg, 1983 ; Misra, 1986). As pointed out by Chandramohan (1984) the fungi of saline mangrove sediments are largely representative of typical soil mycoflora except for Basidiomycetes and Zygomycetes which are rare or absent. The present investigation therefore corroborates the previous observations regarding the occurrence of terrestrial fungal species in mangrove swamps.

A few typical marine species such as *Cirrenalia pseudomacrocephala*, *Dendryphiella* sp., *Didymosphaeria enalia*, *Drechslera halodes*, *Humicola alopallonella*, *Kymadiscus haliotrepus* and *Zalerion maritimum* were isolated from decaying plant litter from mangrove area, but could not be isolated from the backwater. They were represented in the samples only during high saline premonsoon months.

In this study microscopical examination of samples after each collection showed actively growing fungal mycelia. The isolation of many terrestrial fungi in active condition (from Mangalavanam) shows that these fungi posses a great degree of adaptability and they become natural inhabitants of this habitat. The presence of actively growing mycoflora in mangrove swamps which afford a favourable habitat for the growth and proliferation of soil fungi is not surprising, since mangrove environment is known to be very rich due to high amount of dissolved and particulate orgaic matter (Garg *et al.*, 1984).

As noticed in other stations of backwater both *Aspergillus* and *Penicillium* also showed their dominance in this mangrove swamp. *Aspergillus fumigatus* was common in backwaters while *A. niger* in Mangalavanam area. These groups are primary invaders, often called as sugar fungi which prefer simple organic compounds. Mangrove swamps are rich in simple carbohydrates and nitrogen. As stated by Swart (1958) this may be the reason for the dominance of *Aspergilli* and *Penicillia* in mangrove swamps. Garg (1983)

and Misra (1986) reported the domiance of *Aspergilli* over Mucorales and *Penicillia* in the mud of mangrove swamps of Sunderbans and Andamans. Garg (1983) suggests that the isolation of *Aspergillus* species particularly the members of *A. glaucus* group in greater number and frequency is due to high nutrient levels in swamps. They prefer a medium with high osmotic concentration and compete very easily with other mycofloral components. Raper and Fennell (1965) have also reported that certain non-osmophilic species of *Aspergillus* may grow luxuriantly under halophytic conditions.

Seasonal changes in fungal distribution pattern were not detected at any of the sites studied except to a certain extent in mangrove area of Mangalavanam. Both mud and decaying mangrove vegetation collected even from the same spot showed distinctive features in the distribution of species in qualitative and quantitative terms. This reflects the importance of microhabitats in microbial eology. The complex of environmental factors of these microhabitats involved in influencing fungal community composition and structure is till imperfectly known, but presumably this is due to physical and chemical features of the mud, the character of the decaying vegetation, mud microclimate and a vast array of specific biotic interactions (Christenson, 1981).

The two years of bimonthly sampling at different stations and the more intensive monthly collections at Mangalavanam provided certain clues on the ecology of

filamentous fungi. The year 1986 was a period of relatively low species diversity for water and mud samples collected from backwater compared to year 1987, which on the other hand showed high diversity values at many stations. However, Mangalavanam was distinct from other stations in maintaining uniformly high species diversity and species richness in both the bimonthly and monthly samples during the two years. This shows that Mangalavanam provided more uniform and favourable conditions for mycopopulations evenwhen the conditions at other stations were subjected to wider fluctuations. The data also clearly showed that between the two years, species diversity and richness were distinctly higher in 1987 than in 1986 even at Mangalavanam. The weaker monsoonal flushing in the year 1987 would have caused greater accumulation of plant litter in the system. This might have contributed microsubstrates for the growth of diverse fungal populations. Another interesting feature revealed by the samples from the dfferent stations is the strongly independent occurrence of the fungal species in the Cochin backwater. More conclusive evidence of this is also provided by the monthly samples taken from the same spot during the two years as part of a more intensive study of the Mangalavanam - an area characterized by more uniform and favourable conditions for mycopopulations. Independent occurrence of species thus appears to be a characteristic feature of the fungal distribution as evidenced from the present investigaton.

Biochemical studies show that the heterogenous fungal floa present in the Cochin backwater are capable of producing different types of degradative exoenzymes which can act upon organic substrates and release nutrients from them. Majority of them were actively producing more than one enzyme. In the laboratory the majority of isolates examined grew and caused hydrolysis of various substrates in the agar medium.

The maintenance of community structure and function in estuaries depend on inputs of organic matter derived from allochthonous and autochthonous sources and their subsequent degradation through enzymic and other means. Although some of these exogenous and endogenous materials are water soluble, the majority are biopolymers in which much of the energy is locked up in recalcitrant substance like cellulose and lignin. These substances are further protected by tanins, polyphenols or even outright poisons that actively depress the palatability and digestibility of the substrate. These biopolymers are enzymatically degraded to their constituent monomers accomplished by extracellular hydrolases secreted by microorganisms that cleave peptides, esters or glycosidic bonds (Matile, 1975). As a component of the estuarine microbial community, microfungi play a major role in the decomposition of litter inputs through mineralization and enhancement of invertebrate feed on decomposing litter. Many small invertebrates feed only on litter previously colonized by microorganisms and appear to derive the majority of their nutrition from the microorganisms associated with

the ingested litter (Odum and Heald, 1975). Kaushik and Hynes (1971) Jones (1974) and others have demonstrated the singinificance of fungal activity during the initial stages of decomposition. Although the role of individual groups of microorganisms in the decomposition of litter is just beginning to emerge, the ubiquity of fungi in marine influenced estuarine environment suggests that they posses certain unique characteristics which allow them favourably to compete with other microorganisms.

Information on the distribution of the enzymes among the members of fungal community also provides a measure of the communities biochemical diversity. From the Table 6.1 it can be seen that majority of 51 selected isolates produced more than one enzyme. About 96.1% of isolates produced cellulase. It was followed by lipase (80.4%), pectinase (66.7%), amylase (49%), gelatinase (43.1%) and caseinase (39.2%). About 11.8% of isolates were able to solubilize tricalcium phosphate (Fig. 7.1). Matondkar <u>et al</u>. (1980b) found each of the 74 isolates they tested elaborated at least one of the hydrolytic enzymes like amylase, cellulase or pectinase, majority of them producing more than one enzyme.

Cellulose is a prominent carbonaceous constituent of higher plants and probably the most abundant organic compound in nature. The decomposition of this polysaccharide has a special significance in the biological cycle of carbon. Investigations made by Rai and Chowdhery (1976) and Garg (1982) have revealed that the mangrove isolates generally

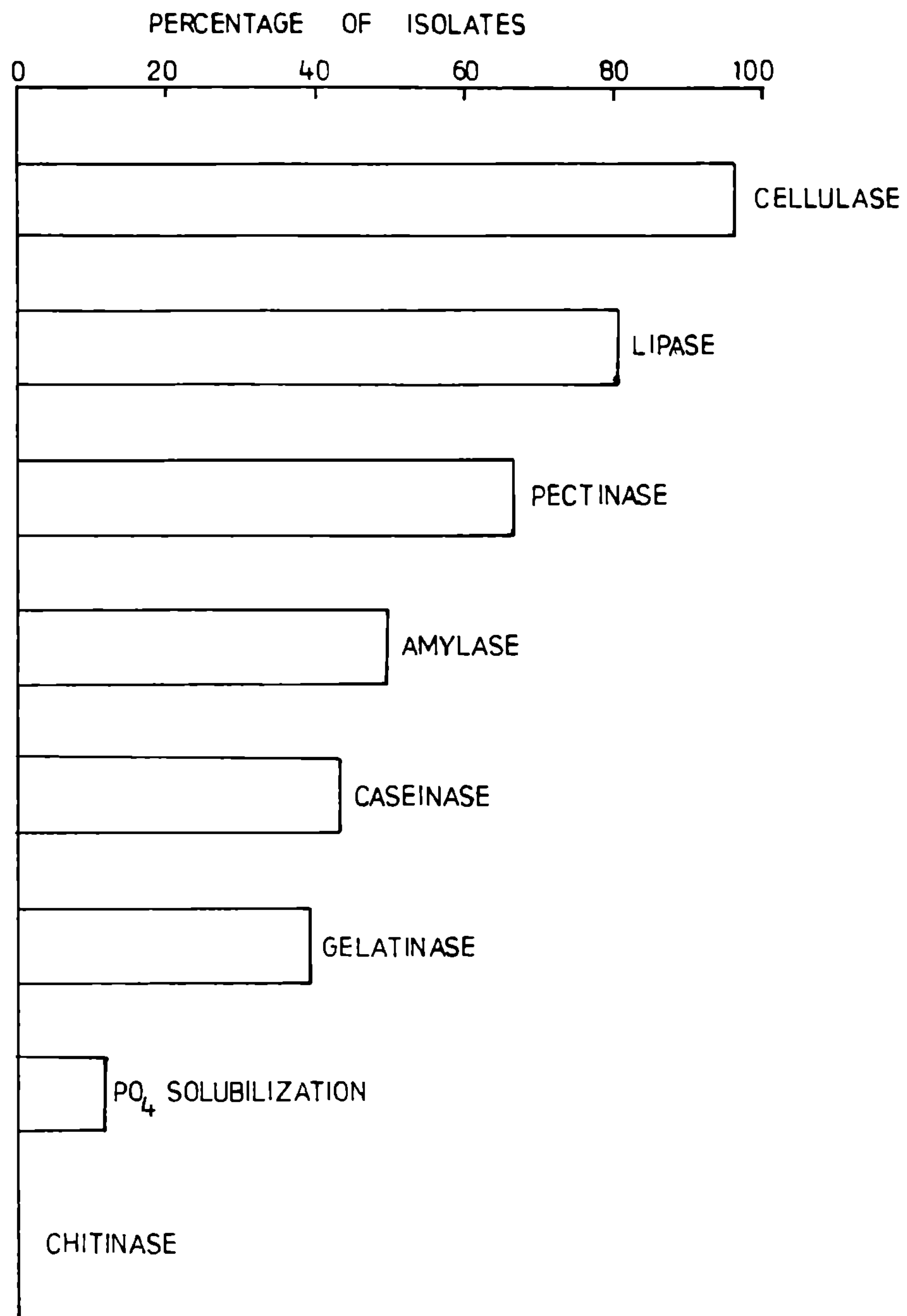

Fig. 7.1 Percentage of physiologically active fungal isolates in mangrove ecosystem.

produce a higher amount of cellulolytic enzymes as compared to the fertile soil counterparts. The organic content of the soil and other conditions in the mud of the mangrove ecosystem may be the main influencing factors contributing to the high cellulolytic activity of the fungal populations (Garg, et al., 1984). The organic content in the mud samples of the Mangalavanam station was also the highest (Figs 4.5 and 5.3) as also the cellulolytic activity.

Although pectic substances never make up a large portion of the dry matter of plants, they are important polysaccharides binding the individual cells together. The significance of pectinolytic fungi in mangrove swamps has been studied by Sheilla De Velho and D'Souza (1982) in Goan estuaries. Present study showed that many fungi exhibited significantly high pectinolytic activity and hence may aid in the degradation of pectic substances, added constantly to the ecosystem.

Chitin is a common polysaccharide found in the skeletons of a number of invertebrate animals, in the cell walls of filamentous fungi and as cellular structure in some protozoa and algae (Alexander, 1983). As a structural constituent it gives mechanical strength to organisms containing it. The reports on chitinolytic higher fungi are rare. Ulken (1983) reported chitinolytic lower fungi from mangrove swamps. While there are instances of higher fungi from terrestrial habitats showing chitinolytic activity (Alexander, 1983), similar reports from aquatic habitats are meagre. The

absence of chitinolytic activity in all the facultative species, some of which are reported to exhibit this property in terrestrial habitat (Alexander, 1983) suggests that production of chitinase is perhaps inhibited in sea water medium as is seen in the present study and possibly in freshwater also though such studies are wanting.

Though lipolytic activity is rarely reported from mangrove ecosystem the fungal isolates from Mangalavanam showed high lipase activity. This corroborates with Cochrane's (1958) statement that most fungi have the capability to produce lipase.

The proteolytic activity of mangrove fungi was tested by screening caseinase and gelatinase activities. Results showed a good number of them were able to degrade both casein and gelatin, revealing that mangrove fungi play an equally significant role in the degradation of protein substrates. Many terrestrial fungi readily decompose protein which include the genera *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium* and *Rhizopus*. Undoubtedly the fungi occupy a dominant position in proteolysis, particularly in acid localities (Alexander, 1983).

Considerable work has been done on the physiology of phosphate solubilizing microorganisms of the terrestrial habitat, but such studies for the marine environment are a few. In the present study of the 51 isolates of fungi screened for the phosphate solubilizing property six showed

positive results, genus *Aspergillus* exhibiting fairly high activity. Arujo *et al*. (1981) reported several species of fungi to be able to solubilize the phosphorus in the coastal waters of Bombay. Devendran *et al*. (1974) showed the higher phosphatase activity and increased numbers of phosphate solubilizing bacteria may be among the determining factors responsible for the higher primary productivity in the mangrove region. It thus appears that certain mangrove and marine fungal isolates play a complimentary role to those of bacteria in making phosphate available as a nutrient in the biogeochemical cycles of marine and estuarine systems.

The above account based on *in vitro* studies in the laboratory provided conditions and substrates quite different from what the organisms may encounter in nature. Nevertheles it is useful in evaluating their potential hydrolysing abilities. Laboratory nutritional studies using pure substrates give an indication of physiological capabilities of fungi and provide indication of their activity in natural habitats. In natural organic matter same constitutents may be complexed with one another, making enzymatic attack more difficult or impossible. At the same time pure culture studies also do not allow for synergistic interactions between decomposers of differing biochemical potential (Swift, 1976).

In conclusion, the present study shows that the presence of rich and varied mycoflora with ability to act on biopolymers and hydrocarbons have a potential role in the

cycling of nutrients in the detritus dominated estuary and also in imparting limited resiliency against organic pollution. The data also strongly suggest that significant ecological differences especially at the microbial level can exist between two adjacent years besides short term variations as evidenced from the qualitative and quantitative differences in the abundance of the mycoflora during 1986 and 1987 and, by the highly significant F-values for monthly differences in the fungal counts compared to station differences.

CHAPTER 8

SUMMARY

1. This thesis is based on detailed studies on the fungal flora with speical reference to yeasts in the Cochin backwaters during the years 1986 and 1987. In this study importance is given to the systematics of yeasts as these are not worked out in any detail in the estuaries of India. The ecology and distribution of the mycoflora for a period of two years were carried out. Laboratory studies for the biochemical activities of selected isolates were also conducted.

2. Thirty three species of yeasts belonging to 11 genera could be identified on cultural, morphological, physiological and biochemical studies. This include 10 Ascomycetes and 23 Deuteromycetes. Their systematics is presented describing the salient features of the different species.

3. The distribution and other ecological aspects are separately examined for filamentous fungi and yeasts. Ninety six species of filamentous fungi belonging to 39 genera from water and mud samples collected at seven stations of the backwaters of Cochin are reported. Majority of them were ubiquitous terrestrial forms adapted to estuarine environment dominated by Deuteromycetes. Although many species were represented in the samples from different stations only a few were of

regular occurrence. Aspergillus was the most dominant genus followed by Penicillium.

Considerable species and numerical variations were seen with regard to water and mud samples as well as their annual, bimonthly and stationwise occurrence.

Fungal propagules were better represented in the mud samples both qualitatively and quantitatively. In water samples number of propagules per litre varied as much from 4 to 1.14×10^{3} and in the mud samples it ranged from 0 to 8.1×10^{4} propagules per gram.

The quantitative analysis of the fungal composition and abundance over the two years clearly indicated that 1987 was ecologically different from 1986 and provided more favourable environment for the mycopopulations. Overall abundance and species abundance were seen to be distinctly more in 1987 in all stations. The analysis also showed strongly independent occurrence of the fungal species in the backwater.

Species composition of yeasts in the backwater revealed a total of 35 species belonging to 11 genera. Candida was the dominant genus followed by Rhodotorula. Yeasts were also predominantly independent in their distribution although a few species occurred together more frequently in the samples than the filamentous fungi. The white yeasts Debaryomyces marama, D. vanriji and Candida krusei appeared only in stations 1 and 2,

whereas *Pichia guilliermondii* in stations 2 and 5, *C. halophila*, *C. solani* and *Trichosporon aquatile* occurred in only station 2.

Considerable variations in the species composition and total yeast counts were also seen in both the water and mud samples. Unlike filamentous fungi, yeast density was higher in water samples and varied from 1.6×10^{2} to 1.6×10^{4} c.f.u. per litre and in the mud samples it ranged from 0 to 9×10^{3} c.f.u. per gram. The species composition in mud samples was uniformly low in all stations. While yeast counts in the water samples were more during premonsoon period, greater counts were noticed in the mud samples during monsoon-postmonsoon months in stations 1 to 6. However in station 7 maximum abundance was recorded during the premonsoon months.

Percentage of white and red yeasts for 1986 and 1987 in water and mud samples showed difference in distribution pattern. The white yeasts were present in all stations but the red yeasts were completely absent from station 7 in both years except the September, 1986 mud samples. Red yeasts were particularly abundant in station 1 and 2. This observation may be considered as an ecological index for the extent of organic pollution in the backwater.

4. An intensive investigation of the filamentous fungi of the mud and decaying vegetation of a mangrove ecosystem

showed presence of 71 species grouped under 35 genera. Majority of the species isolated from these samples were ubiquitous saprophytes. Maximum number of species were also recorded from station 7.

Many species were represented in the monthly samples and only a few were of regular occurrence. Among the various genera isolated from samples *Aspergillus* and *Penicillium* were dominant. Seven typical marine forms isolated during the two years were reported from the decaying mangrove vegetation only. They were seen during the high saline premonsoon months.

Fungal counts were distinctly higher in station 7 and were subjected to monthly variations as in other stations. In mud samples number of propagules per gram varied from 1×10^4 to 5.3×10^4 and in decaying plant litter it ranged from 1.6×10^4 to 5.4×10^4 per gram. In general less number of propagules were registered in premonsoon months while monsoon and postmonsoon periods registered higher values. The same was seen with respect to the number of species. However it was not evident in decaying mangrove vegetation.

Although higher qualitative and quantitative abundance of fungal species were observed at station 7 during the period of investigation highest values of diversity index were recorded throughout the year, 1987.

Greater abundance of mycopopulations in 1987, despite high organic content and their similar distribution pattern during the two years suggest that greater percent of plant litter probably contributed to the detritus in 1987 than in 1986.

Uniform and favourable ecological conditions prevailed during both the years at Mangalavanam enabled the occurrence of different species of filamentous fungi. These fungi showed independence in their occurrence. Similar independent occurrence of fungal populations have been reported from other stations studied. This seems to be a unique characteristic of fungal distribution in the Cochin backwater.

5. Biochemical studies pertaining to the hydrolytic activities were carried out for 51 selected fungal isolated from mangrove ecosystem. Majority of the isolates produced more than one enzyme. The cellulase producing fungi were of higher magnitude in the percentage of isolates followed by lipase, pectinase, amylase, gelatinase and caseinase. Only few isolates showed the ability to solubilize phosphate. None of the tested isolates was showing the ability to degrade chitin.

   Thirty five selected yeast isolates were also screened for pectinase and hydrocarbon assimiltaion activities. Thirty four isolates showed pectinase production. Out of 35 yeast isolates 26 were able to

assimilate kerosene and 27 were able to assimilate diesel. About 16 isolates showed questionable growth and 8 isolates showed good growth in crude oil.

6. Community interrelationship, ecology and biochemical activities of the mycoflora of Cochin backwater fully amplify the potential role of these mycopopulations in the production, utilization and recycling of the detritus in addition to their important role in combating organic and industrial pollution.

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