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* have been observed in these areas.
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 backwaters 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 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 suggest 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 cell 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 biodegradation 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 hydrocarbons (diesel oil) and they commented that their presence in the estuaries appears to be advantageous in
reducing oil pollution.

Hydrocarbonoclastic yeasts are widespread in the neritic environment and may occur 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, even though 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 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
In Cochin which included mangrove ecosystem 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 samples. 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; Subramanian 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 ecosystem) 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 Basidomycetes 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 pseudo-macrocephala, Dendryphiella sp., Didymosphaeria entalia, 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 possess 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 organic 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 dominance 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 ecology. 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 even when 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 different 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 investigation. 
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 tannins, 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 et al. (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. Nevertheless 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 constituents 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.