

## Fungal activity in Mangalvan : an estuarine mangrove ecosystem

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**Abstract.** This paper presents a systematic study of the fungal flora and their decaying activity for the three seasons (premonsoon, monsoon and postmonsoon of 1986) prevailing in Cochin and their possible role in nutrient regeneration in Mangalvan- a mangrove area of Cochin backwater. Consequent to decay and decomposition of the mangrove vegetation by these fungi it adds to the detritus load, contributing substantially to the food web of this important estuarine ecosystem. Thirty one fungal isolates were recorded from the soil and 27 from decaying leaves, stems, roots and pneumatophores of the mangrove macrophytes while 7 from the decaying free floating plants. *Aspergillus* was the dominant fungal genus followed by *Penicillium*, *Fusarium* and *Trichoderma*. Most of these estuarine fungi are terrestrial forms adapted to wide salinity fluctuations and some of them showed more activity at low salinity in monsoon season. Marine fungi *Humicola alopallionella* and *Cirrenalia pseudomacrocephala* became active at high salinity of the premonsoon season decaying *Avicennia* stem. *Conoplea* sp. and *Cylindrotrichum* sp. are new records from decaying *Salvinia* leaves. From 31 soil isolates screened for phosphate solubilizing activity, 9 showed activity, 2 good activity and NIO-C-3 *Aspergillus candidus* excellent activity indicating another possible role of these active fungi in the nutrient regeneration of the ecosystem by solubilizing insoluble phosphorus compounds making it available to other organisms present there.

**Keywords.** Mangalvan; fungi; decaying vegetation; phosphate solubilization

### 1. Introduction

Mangalvan is a small shallow mangrove ecosystem of the Cochin backwater situated between approximately 9°59'N to 9°60'N lat. and 76°16' E to 76°18' E long. (figure 1). It is connected to main backwater by a feeder canal and has an area of 2.8 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*. In the monsoon and early postmonsoon season free floating plants, *Eichhornia crassipes*, *Salvinia molesta* and *Salvinia rotundifolia* form extensive mats moving with the tide and the ones that are not flushed out decay there adding up to detritus. Decaying stems, roots and fallen leaves of the macrophytes also add up considerably to the detritus of this ecosystem.

Microorganisms play a major role in the cycling of nutrients and fungi are more important in the breakdown of plant materials (Nair 1980). Mangalvan is rich in mangrove vegetation and according to Odum and Heald (1975) and Newell (1973) the fallen mangrove leaves are turned to detrital particles by microbial activity which support a large population of detrital consumers present there.

Although fungi play an important role in the mangrove ecosystem very little work has been carried out

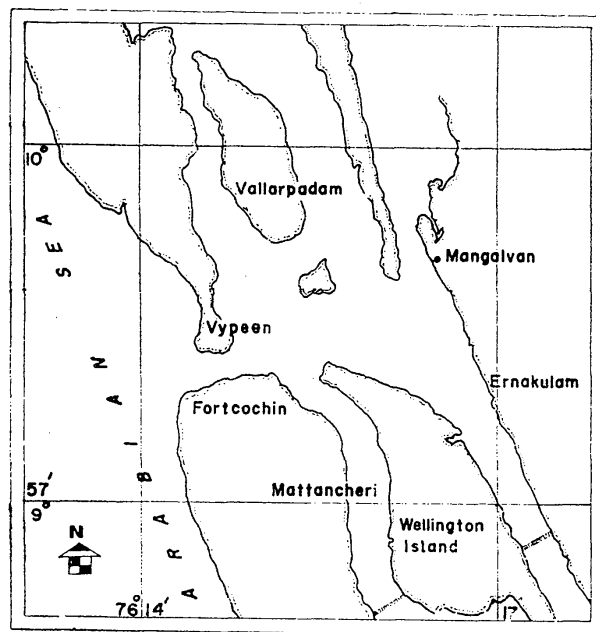


Fig. 1 Map of Cochin backwater showing the position of Mangalvan - estuarine mangrove ecosystem

in India on their species composition and role in the decaying of vegetation. Some contributions in this line in India are those of Rai *et al* (1969), Untawale *et al* (1977), Subramanian *et al* (1984), Gomes and Mavinkurve (1982), De V Velho and D'Souza (1982), Matondkar *et al* (1980), Chowdhury (1979) and Misra (1986).

The present study attempts to examine some of the microbial activities at the base of the food chain in the above mangrove system with special reference to the fungal flora of the soil and the decaying mangrove macrophytes, their saprophytic activities and possible role in the nutrient regeneration from the soil. This necessarily involves an initial survey of the fungal flora and hence this study is taken up as part of a comprehensive investigation in the Cochin backwater.

## 2. Materials and methods

### 2.1. Collection of samples

Collection of mud samples and decaying vegetation were made every month at low tide from places having a thin film of water. Mud samples were collected aseptically in triplicate by inserting a 33 cm hollow cylinder to a depth of about 15 cm and then scooping out the core. Decaying fallen leaves, stems, roots and pneumatophores of *Avicennia* and *Acanthus* were collected in sterilized polythene bags. Decaying plant parts of the free floating plants, *Eichhornia* and *Salvinia* were also collected in sterilized polythene bags. For each sample, separate polythene bags were used.

Temperature, pH, dissolved oxygen and salinity values were recorded for the water and mud from the collection sites at the same time applying standard methods (Strickland and Parsons, 1965).

### 2.2. Fungal isolation from the mud samples

Fungi were isolated from the mud samples by dilution plating technique. GPY-agar (Uden Van and Fell 1968) and YpS's agar (Kohlmeyer and Kohlmeyer 1979) media were used for planting. The plates were incubated at  $28 \pm 2^\circ\text{C}$  within 48 h to 72 h the fungal colonies showed up. These colonies were counted for biomass calculation for each fungal species in the plate. The colonies were purified by transferring them into fresh plates and finally taken up in fresh slants.

### 2.3. Fungal isolation from decaying vegetation

The decaying plant parts were aseptically cut into small pieces. These were then washed well with sterilized sea water to remove dirt adhering on them. Then they were dipped into 0.01% mercuric chloride solution for 3 minutes for surface sterilization of the pieces. The pieces were then washed well with sterilized sea water to remove all the traces of mercuric chloride solution and these pieces were then placed on plates with YpS's — agar and GPY-agar media and incubated at  $28 \pm 2^\circ\text{C}$ . Within 24 h to 72 h the fungal colonies showed up and were purified as in the previous case.

The sea water used in these processes were collected from the collection sites and to inhibit bacterial growth an antibiotic mixture (chlortetracycline HCl 10 mg%, chloramphenicol 2 mg %, streptomycin sulphate 2 mg%, (Uden Van and Fell 1968) was used in both the isolating media.

### 2.4. Phosphate solubilizing test

All the 31 soil isolates were tested for phosphate solubilizing activity. The test was carried out qualitatively. The medium was modified Czapeck's Dox-agar medium (Araujo *et al* 1981) and modified Pikovskaya's medium (Pikovskaya 1948) in petriplates. The phosphate source was tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ). On these plates fungi were inoculated at different spots on the medium. The plates were incubated at  $28 \pm 2^\circ\text{C}$  for 3 to 6 days. When a clear zone of phosphate solubilizing around the active fungal colonies were seen while the rest of the medium in the plate remained turbid due to insoluble tricalcium phosphate.

## 3. Results and discussion

### 3.1. Environmental characteristics

Of the physico-chemical parameters examined, pH remained between (6.86 and 7.24). The temperature

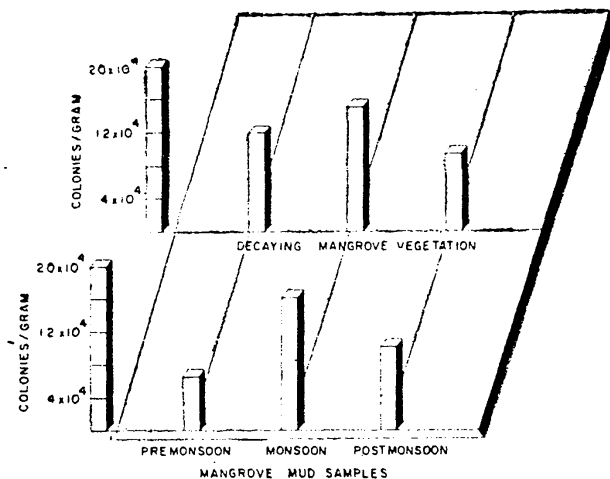


Fig. 2 Species composition of microfungi in Mangalvan soil at different seasons

1. *Acrothecium* sp., 2. *Alternaria fasciculata*, 3. *Aspergillus candidus*, 4. *A. chevalieri*, 5. *A. fumigatus*, 6. *A. glaucus*, 7. *A. niger*, 8. *A. nidulans*, 9. *A. oryzae*, 10. *A. sydowii*, 11. *A. terreus*, 12. *A. ustus*, 13. *Chalara* sp., 14. *Cladosporium herbarum*, 15. *Curvularia lunata*, 16. *Fusarium neoceras*, 17. *Fusarium oxysporum*, 18. *F. solani*, 19. *F. vasinfectum*, 20. *Geotrichum candidum*, 21. *Helmithosporium* sp., 22. *Mucor hiemalis*, 23. *Penicillium-citrinum*, 24. *P. janthinellum*, 25. *P. luteum*, 26. *P. monoverticillate symmetrica*, 27. *P. pinophilum*, 28. *Rhizopus arrhizus*, 29. *Sporotrichum* sp., 30. *Trichoderma koningi*, 31. *Trichoderma* sp.

fluctuations were minimal with narrow ranges of 26.1 to 28.3°C. Dissolved oxygen also showed little variations with values between 3.40 and 3.52 mg l<sup>-1</sup>. However there was wide fluctuations in salinity with 2‰ in the monsoon season (July, August) and 27‰ in late premonsoon season (April, May).

### 3.2. Species composition and fungal activities

In figure 2 the species composition in mud samples of Mangalvan for the 3 seasons premonsoon, monsoon

and postmonsoon is presented. Figure 3 shows the fungal species composition in decaying vegetation at the above 3 seasons. Figure 4 and 5 indicate respectively the nature of fungal viability in soil and activity in decaying vegetation in Mangalvan and the phosphate solubilizing activity of the active soil isolates.

The results indicate an interesting pattern of decaying abilities by the fungi during the 3 seasons. Plating indicated high viability of *Aspergillus niger*,

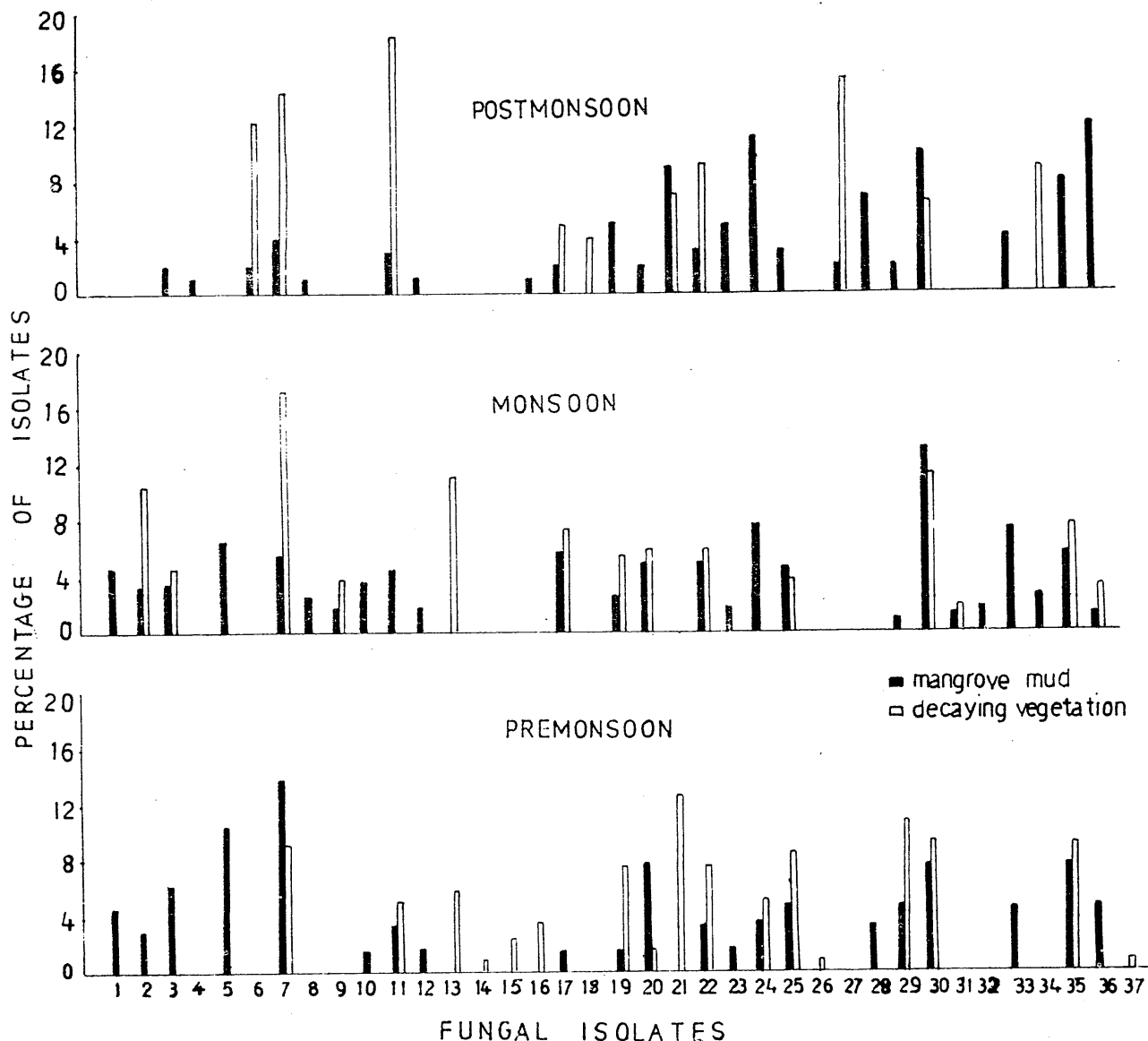


Fig. 3 Species composition of microfungi in decaying vegetation in Mangalvan at different seasons

1. *Aspergillus candidus*, 2. *A. glaucus*, 3. *A. niger*, 4. *A. oryzae*, 5. *A. terreus*, 6. *Aspergillus* sp., 7. *Alternaria fasciculata*, 8. *Cirrenalia pseudomacrocephala*, 9. *Chaetomium* sp., 10. *Chalara* sp., 11. *Cladosporium herbarum*, 12. *Colletotrichum gloeosporoides*, 13. *Curvularia lunata*, 14. *Fusarium neoceras*, 15. *F. oxysporum*, 16. *F. solani*, 17. *Geotrichum candidum*, 18. *Helmithosporium* sp., 19. *Humicola alopallonella*, 20. *Mucor hiemalis*, 21. *Penicillium janthinellum*, 22. *P. luteum*, 23. *P. monovercillate symmetrica*, 24. *Sporotrichum* sp., 25. *Trichoderma koningi*, 26. *Trichoderma* sp., 27. *Sporormia* sp.

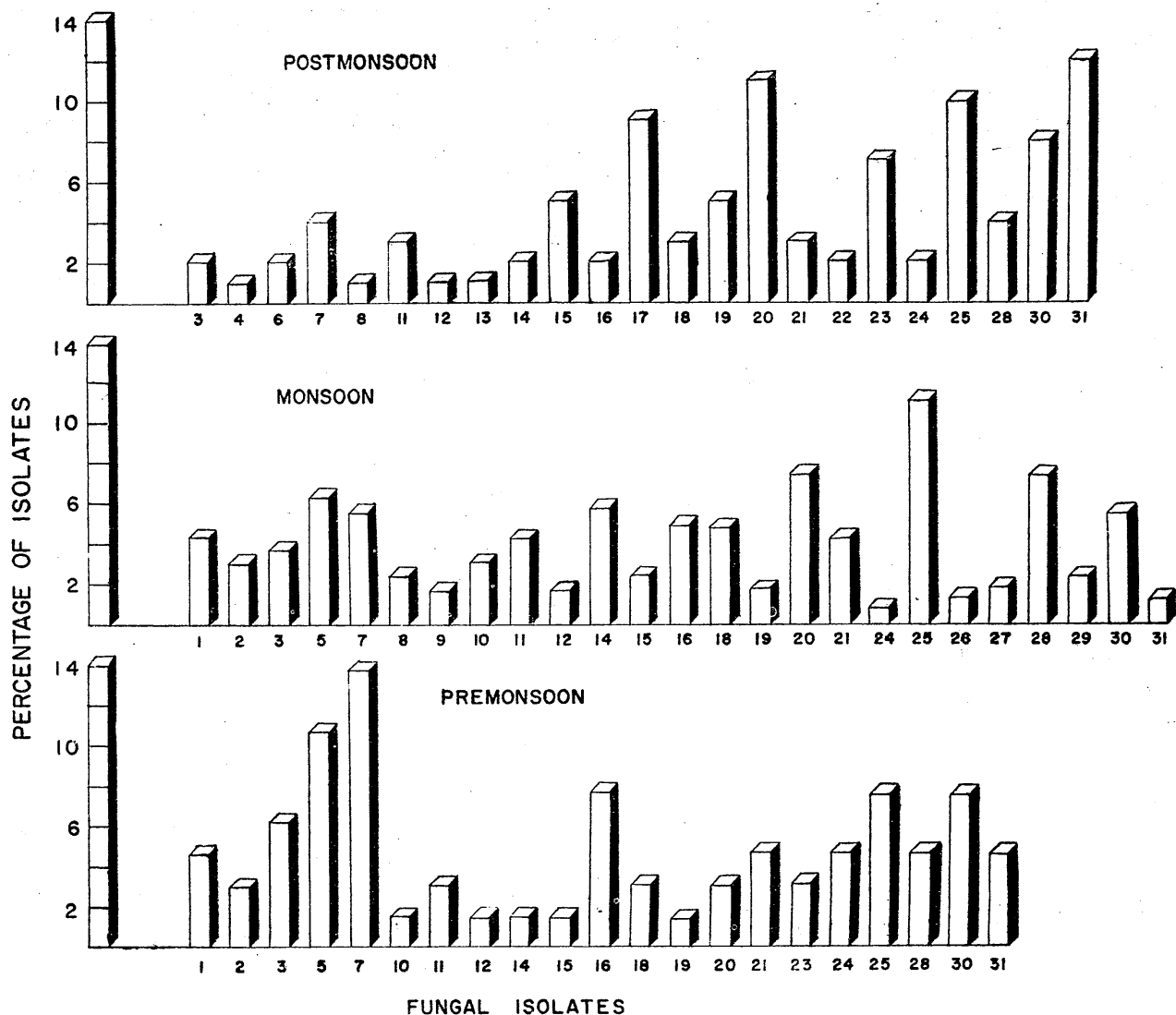


Fig. 4 Comparison of total fungal biomass in mangrove soil and decaying vegetation at different seasons

*Fusarium solani* and *Penicillium luteum* in the soil and high activity in the decaying vegetation throughout the year irrespective of the wide salinity fluctuations. On the contrary the marine fungi *Cirrenalia pseudomacrocephala*, *Humicola alopallonella* and *Sporormia* sp. could not be isolated in viable form from the mud samples; however when the salinity reached 26.9‰ in the late monsoon (May) they showed up actively decaying *Avicennia* stem. Likewise *Aspergillus oryzae* and *Penicillium monovercillate symmetrica* could be isolated in viable form from the mud and showed decaying activity only in the monsoon season when the salinity was very low (2-3‰).

Fungi exhibiting different combinations of these properties at different seasons were also encountered.

*Helminthosporium* sp. *Penicillium janthinellum*, *Trichoderma koningi* and *Aspergillus candidus* could be isolated from the soil in viable form in all the seasons but showed its decaying activity only during the low salinity regime. *Aspergillus ustus*, *Fusarium vasinfectum* and *Rhizopus arrhizus* could be isolated from the soil at all the seasons in viable form but did not show activity in decaying vegetation at all. Species exhibiting an array of other combinations of these two properties could also be seen from figure 5.

The free floating plants washed into the backwater by the tide during the monsoon and early postmonsoon months contribute to the decaying vegetation in the mangrove during this period. Fungi isolated from the *Salvinia molesta* leaves were *Fusarium vasinfectum*,

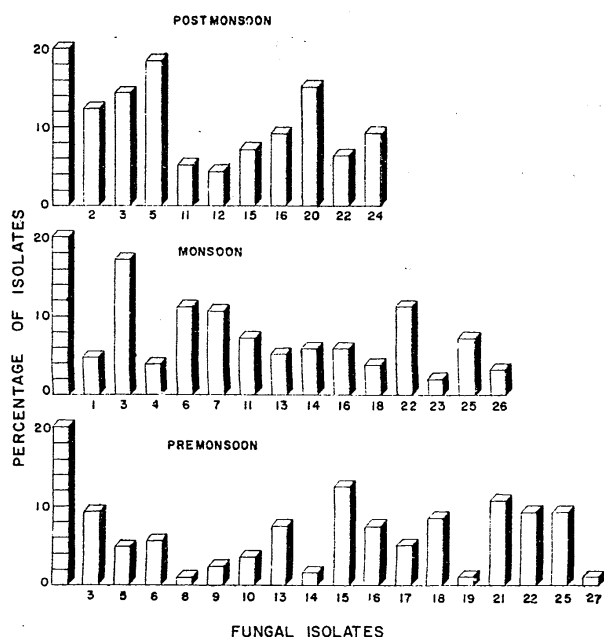


Fig. 5 Comparison of fungal species composition in mangrove mud & decaying vegetation at different seasons

1. *Acrothecium* sp., 2. *Alternaria fasciculata*, 3. *Aspergillus candidus*, 4. *A. chevalieri*, 5. *A. fumigatus*, 6. *A. glaucus*, 7. *A. niger*, 8. *A. nidulans*, 9. *A. oryzae*, 10. *A. sydowi*, 11. *A. terreus*, 12. *A. ustus*, 13. *Aspergillus* sp., 14. *Cirrenalia pseudomacrocephala*, 15. *Chaetomium* sp., 16. *Chalara* sp., 17. *Cladosporium herbarum*, 18. *Colletotrichum gloeosporoides*, 19. *Curvularia lunata*, 20. *Fusarium neoceras*, 21. *F. oxysporum*, 22. *F. solani*, 23. *F. vasinfectum*, 24. *Geotrichum candidum*, 25. *Helminthosporium* sp., 26. *Humicola laopallonea*, 27. *Mucor hiemalis*, 28. *Penicillium citrinum*, 29. *P. janthinellum*, 30. *P. luteum*, 31. *P. monoverticillate symmetrica*, 32. *P. pinophilum*, 33. *Rhizopus arrhizus*, 34. *Sporotrichum* sp., 35. *Trichoderma koningi*, 36. *Trichoderma* sp., 37. *Sporormia* sp.

*Phoma* sp., *Pythium* sp., *Cylindrotrichum* sp. and *Conoplea* sp. The last two fungi are new records from this substrate. *Pythium* sp. and *Colletotrichum gloeosporoides* were isolated from decaying stem and bladders of *Eichhornia crassipes*.

In addition to the decaying activities exhibited by these fungi associated with plant material, study of the soil fungi showed that some of the species also possess the ability to act on insoluble phosphorus compounds (table 1). *Aspergillus candidus* (NIO-C-3) showed excellent activity for phosphate solubilization. While *A. terreus* (NIO-C-47) and *A. candidus* (NIO-C-29) showed good activity *A. terreus* (NIO-C-20), *A. niger* (NIO-C-13), *Trichoderma koningi* (NIO-C-18) *A. fumigatus* (NIO-C-36) and *Penicillium janthinellum* (NIO-V-39) showed moderate activity and low activity by *A. niger* (NIO-C-12) thus showing the possible major role of fungi in the cycling of nutrients by

releasing this important element in the soil in soluble form.

Table 1: Qualitative screening of fungi for phosphate solubilizing ability

Culture No.	Organism	Solubilizing ability
3	<i>Aspergillus candidus</i>	+++ +
12	<i>Aspergillus niger</i>	+
13	<i>Aspergillus niger</i>	++
18	<i>Trichoderma koningi</i>	++
20	<i>Aspergillus terreus</i>	++
29	<i>Aspergillus candidus</i>	+++ +
36	<i>Aspergillus fumigatus</i>	++
39	<i>Penicillium janthinellum</i>	++
47	<i>Aspergillus terreus</i>	+++ +

++++ excellent    +++ good    ++ moderate    + poor

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#### References

- Araujo A, D'Souza J and Karande A A 1981 Phosphorus solubilization by some marine fungi; *Mahasagar* 14 1 67-70
- Chowdhery H J 1979 A new species of *Aspergillus* from mangrove swamps of West Bengal, India; *Curr. Sci.* 48 953
- De V Velho Sheilla and D'souza J 1982 Studies on pectinolytic fungi from the mangrove sediments; *Mahasagar* 15 3 167-173
- Gomes R H and Mavinkurve S 1982 Studies on Mangrove swamps of Goa: II micro-organisms degrading phenolic compounds; *Mahasagar* 15 2 111-115
- Kohlmeyer J and Kohlmeyer E 1979 Marine mycology the higher fungi 20, Academic Press, (New York)
- Matondkar S G P, Mahtani S and Mavinkurve S 1980 The fungal flora of the mangrove swamps of Goa; *Mahasagar* 13 3 281-283
- Matondkar S G P, Mahtani S and Mavinkurve S 1980 Seasonal variations in the microflora from mangrove swamps of Goa; *Indian J. Mar. Sci.* 9 119-120

- Misra J K 1986 Fungi from the mangrove muds of Andaman Nicobar Islands; *Indian J. Mar. Sci.* **15** 185-186
- Nair N B 1980 Marine Ecology, Macmillan company (India Ltd.) 266 p
- Newell S Y 1973 Succession and role of fungi in degradation of red mangrove seedlings ; *Estuarine Microbial Ecology*, (ed) Sterenson H and Colwell R Belle, W Baruch Symposia in Marine Science, Univ. of South Carolina Press (Columbia) 455-465
- Odum W F and Heald E J 1975 Mangrove forests and aquatic productivity: In coupling of land and watersystems; *Ecological studies*, Springer Verlang, (New York)
- Pilovskaya R I 1948 *Mikrobiologia* **17** 362
- Rai M Tewari J P and K Mukerji K G 1969 Mycoflora of mangrove mutf; *Mycopathologia et Mycologia Applicata* **38** 17-31
- Strickland J D H and Parsons T R 1965 A Manual of Sea water Analysis; *Fisheries Research Board of Canada* 2nd edition 203
- Subramanian P, Jeyaseelan M J P and Krishanmurthy K 1984 Thw nature of biodegradation of vegetation in Mangrove Ecosystem; *Chemistry in Ecology* **2** 47-58
- Uden Van N and Fell J W 1968 Marine yeasts; *Advances in Mlcro biology of the sea*, Academic Press, (New York) **1** 167-201
- Untawale A G, Bhosle N B, Dhargalkar V K, Matondkar S G P and Sayeeda Bukhari 1977 Biochemical chance in mangrove foliage during growth and decomposition ; *Indian J. Mar. Sci.* **6** 104-106