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Microflora of Some Soils of Sri Lanka and Ecological Considerations

Soil flora and Ecological Considerations

by

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SUMMARY This paper considers the occurrence and distribution of microflora of some soils in Sri Lanka (Ceylon) which could be potentially destructive to articles of value such as microscopes, cameras, electrical resistances etc. At the same time an attempt is made to examine the responses of some micro-organisms to local environmental conditions and assess the extent to which application of a knowlege of these responsess can provide an ecological approach to biodeterioration.

Ceylon was divided intoWet, Dry and Intermediate zones on the basis of rainfall, and soil sampling was done only once in each locality in thse zones at diffrent times of the year. Thirty-one different fungi have been isolated fron the soils. Twelve of these were found on the optical systems of microscopes and cameras in Sri Lanka (Nagamuttu,1967) while these and many others among the isolates were reported by other scientists on optical materials in the tropics. The commonest genera isolated are the *Aspergillus* and *Penicillium*. The high total count of colonies had a large number of species represented. It appears that the soluble salt content in the soil has a greater influence on the fungi population than its organic matter. Also, there is a drop in the number of organisms occurring at lower levels of the soil. The present survey is a continuation of the earlier study. The findings were not published but were reported in the University of Ceylon Annual Report for the period 1968/69. Peries, Liyanage and Liyanage (1979) have subsequently recorded the isolation of some of the identical fungi in the rubber-growing soils from theWet zone and Matale. Of the isolattes in the present study, seven species of fungi have n ot been reported to-date.

INTRODUCTION

The scientific study of soil began with Adamatz in 1886 (Ranzoni, 1968) when he collected eleven species of fungi from experimental fields for biological studies, but it was after the first World War that the importance of fungi in agriculture and pharmaceutical industry was realised. Since then, microflora of soil attracted the attention and stimulated interest in many scientists such as Jensen (1931) in Denmark; Trester, Backus and Curits (1934), Miller, Giddens and Foster (1957), Chritensen and Backus (1961) and Ranzoni (1968) in the United States of America; Nour (1956) in Sudan; Bisbay, James and Timonin (1935) in Canada; Farrow (1954) in Panama and Costa Rica; Yousef Al Doory, Tolba and and Al Ani (1959) in Iraq; Eicker (1969) in Zululand; Nagamuttu (1968, unpublished) in Sri Lanka;Singh (1976) in Newfoundland; Peries *et al* (1979) in Sri Lanka. It has been reported (Benjamin, 1969) that more than 150,000 fungi and more than 25,000 new taxa have been found and still more continue to be identified, while the Iowans have stated that there are as many fungi as there are flowering plants. In addition to the economic importance of some of these fungi, there is also a broad spectrum of micro-organisms of the soil involved in the biodeterioration of scientific and industrial apparatus in the tropics. Identification of these organisms can give a timely warning as to whether a species encountered in the field is beneficial or is destructive and whether appropriate measures are to be planned.

Climate

Fig. 1 shows the division of Sri Lanka into Wet, Dry and Intermediate zones and its mean annual rainfall in inches. The Wet zone is bounded by line CD; Intermediate zone within ABCD and the part of the country on the right of AB is the Dry zone. The Wet zone is influenced by the South West monsoon with May to July being the wettest and January to March the driest months. The climate changes with altitude and within this tropical belt in the mountain regions, there may exit a temperate climate. Fig. 2 gives the monthly mean maximum and minimum temperature in degrees Celcius.

Materials and Methods

The materials used were sterilized trowels, screw-capped bottles, spoons, spatulas and double-layered plastic bags. Soil samples were collected by digging a hole two inches beneath the surface which had been previously scraped off of litter and thrusting a sterilized bottle into the side of the hole. This method ensured collection of true fungi free from aerial contamination. More than one sample was taken from each locality. In addition to these samples at two inches depth, a small quantity of soil at the same site was taken at a depth of six inches to study the microbial activity at this depth. All samples were taken to the laboratory and stored in a safe place to avoid contamination of live-cultures of parasites and bacteria maintained in the laboratory for teaching purposes. Plant and fibre materials were picked up by sterilized forceps before inoculating the soil into the culture media. Direct soil planting method of Warcup (1950) was adopted.

Czapeck-Cox agar medium with 0.5% yeast extract and Eggins-Pugh medium (personal communication, 1968) was used for inoculating the soil.

Eggins - Pugh medium

Potassium dihydrogen phosphate	1.0	g
Ammonium sulphate	0.5	,,
Potassium chloride	0.5	,,
L-asparagine	0.5	,,
Yeast extract	0.5	,,
Magnesium sulphate (Hydrated)	0.2	,,
Glucose	20.0	,,
Starch	5.0	,,
Distilled water	1000	cc

An insignificant deviation from Eggins-Pugh medium was the inclusion of 20 g glucose instead of 5 g as it was the experience of the author in a previous study (Nagamuttu, 1967) that a high precentage of sugar increased the osmotic pressure of the substrate, accelerated the sporulation of xerophilic fungi such as *Aspergillus glaucus* and also reduced the cultural period.

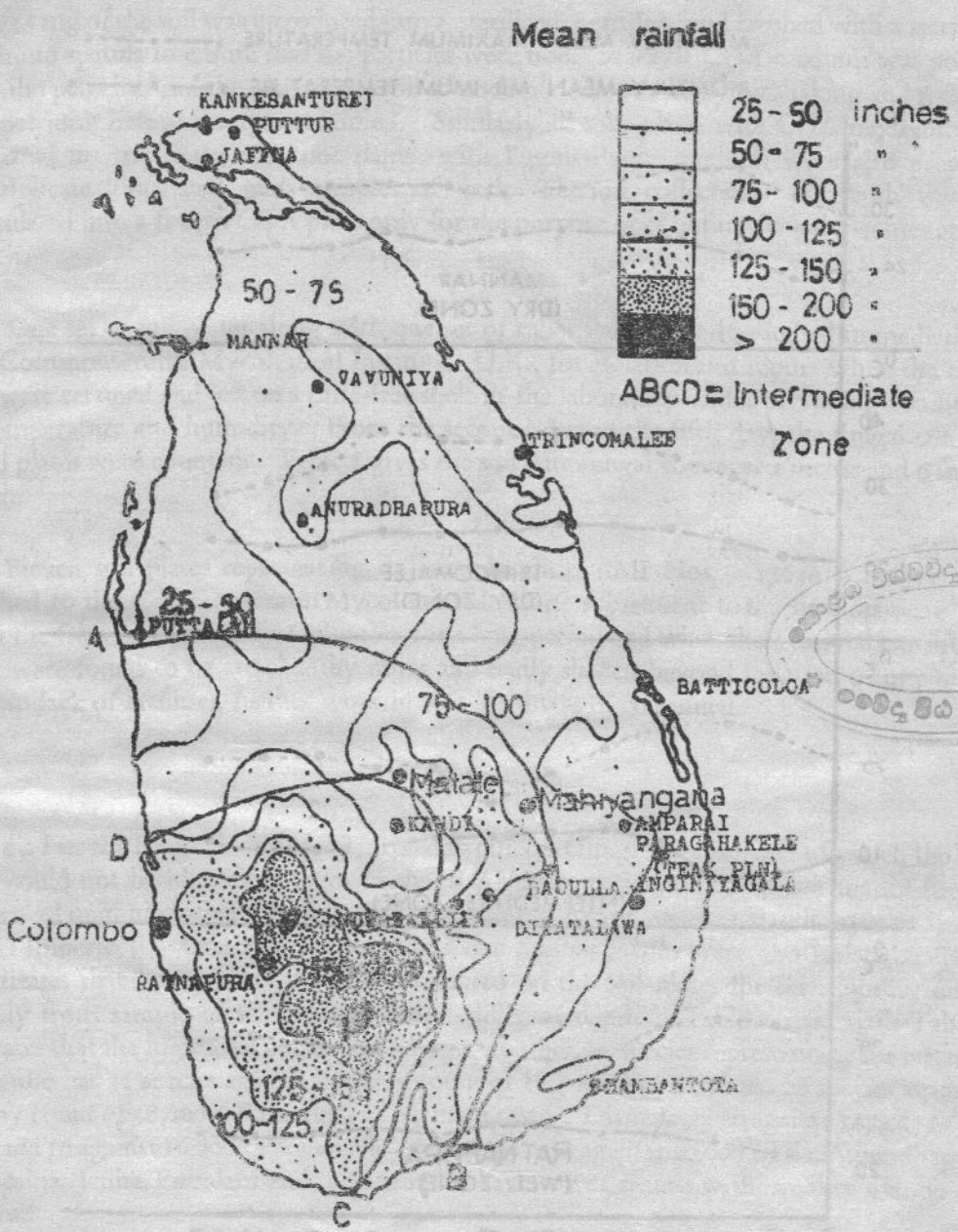
Mean rainfall
25 - 50 inches
50 - 75 "
75 - 100 "
100 - 125 "
125 - 150 "
150 - 200 "
> 200 "
ABCD = Intermediate Zone
KANKESANTURET
PUTTUR
JAFFNA
MANNAR
VAVUNIYA
TRINCOMALEE
ANURADHAPURA
PUTTALAM
BATTICOLOA
Matale
Mahiyangana
KANDY
AMPARAI
PARAGAHAKELE
TEAK PLNT
BADULLA
DIYATALAWA
Colombo
RATNAPURA
A
B
C
D
50 - 75
25 - 50
75 - 100
100 - 125
125 - 150

Fig.

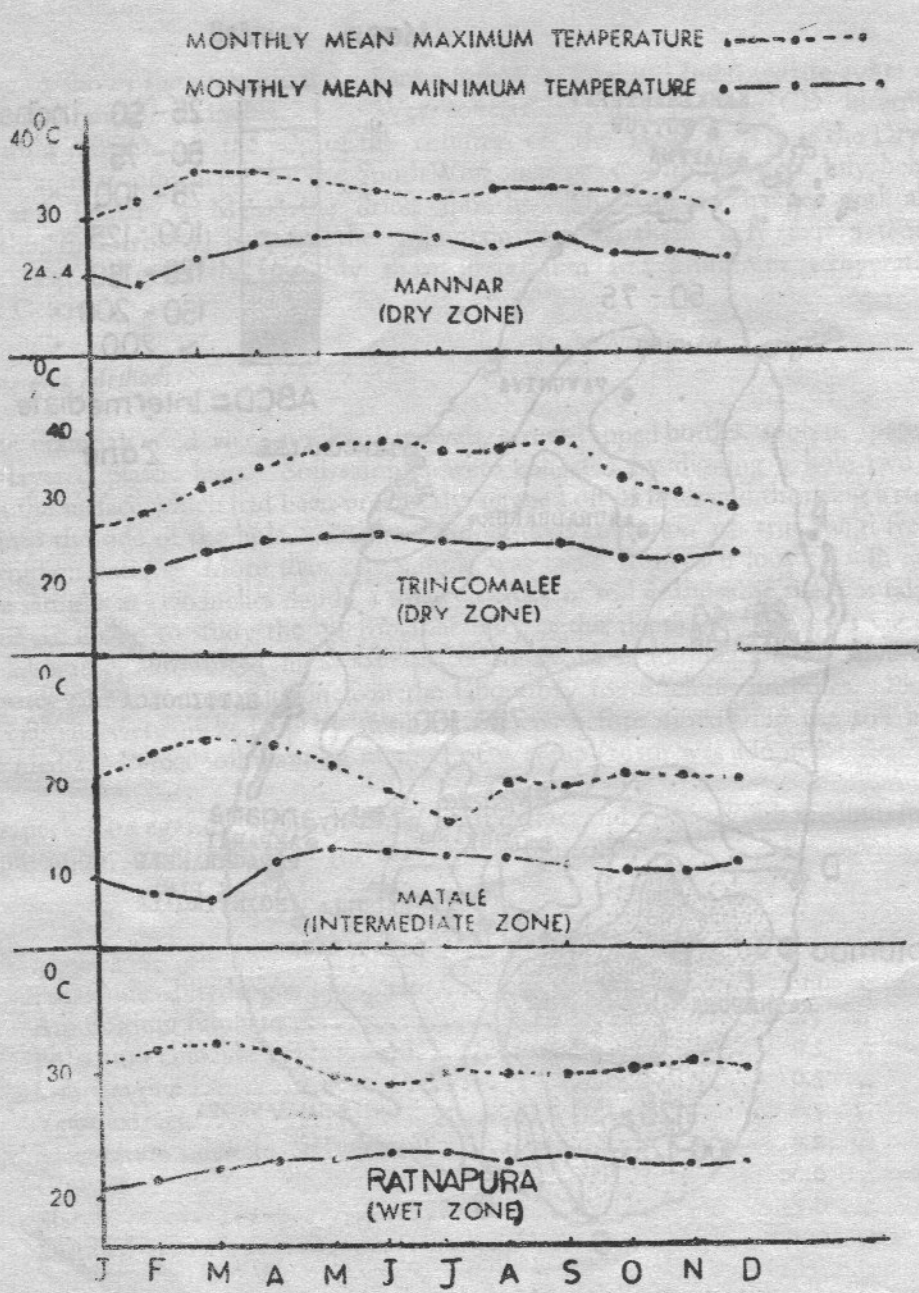
MONTHLY MEAN MAXIMUM TEMPERATURE
MONTHLY MEAN MINIMUM TEMPERATURE
40 °C
30
24.4
MANNAR
(DRY ZONE)
°C
40
30
20
TRINCOMALEE
(DRY ZONE)
°C
30
10
MATALE
(INTERMEDIATE ZONE)
°C
30
20
RATNAPURA
(WET ZONE)
J F M A M J J A S O N D

Fig. 2

15 mg of the soil was introduced into a sterilized petridish and crushed with a sterilizep platinum spatula to ensure that the particles were fine. 10 ml of CDA medium was poured into the petridish and the particles of soil were dispersed uniformly by shaking and rotating the petridish before the agar solidified. Similarly all soil plates with CDA medium were prepared in triplicate and tube slants with Eggins-Pugh medium were also prepared in triplicate inoculated with samples of soil. The soil collected at 6" depth was also inoculated into a fourth CDA plate only for the purpose of counting fungal colonies at this depth.

One set of soil plates along with one set of tube slants was despatched immediatly to the Commonwealth Mycological Institute, U.K., for isolation and report while the other sets were retained and left on a mite-free shelf in the laboratory under prevailing conditions of temperature and humidity. From the seconed day to the fifth day, the fungal colonies in all plates were counted. Table 1 gives the soil data fungal counts at 2 inches and 6 inches depth.

Eleven soil plates representing different localities (IMI Nos. 156361 - 156371) despatched to the Commonwealth Mycological Institute subsequent to the first lots were held up at the General Post Office, Colombo, for a long period and when these arrived late in U.K. they were found to be attacked by mites and badly shaken beyond isolation of organisms. Due to lack of facilities, further work in this field was discontinued.

RESULTS

Table 2 lists the fungi identified. Thirty-one different fungi were isolated of which the first four could not be identified down to the sub-species as identification is a matter for the expert. Fungi identified include 7 Phycomycetes, 2 Ascomycetes, 2 Basidiomycetes and 21 Fungi Imperfecti. One Actinomycete and one *Bacillus subtilis* were also isolated. Table 1 indicates that the number of colonies counted on the soil plates for each locality differs greatly from sample to sample and also at different depths. Table 1 read with Table 2 indicates that the high total count had a large number of species represented; for instance, Colombo has 31 species against a colony count of 18,400: Vavuniya has 28 species against a colony count of 18,000 ; Ratnapura 21 against 14,200 ; Diyatalawa 20 against 14,800; Mahiyangana 16 against 16,200 ; Parahakelle Teak gardens 11 again st 10,600 while Anuradhapura, Batticaloa, Jaffna, Puttalam and Trincomalee have lower counts with smaller numbers of species.

Among the isolates in this study, the seven fungi not recorded to-date in Sri Lanka are *Rhizopus arrhizus* (Cohn) Schroet, *Rhizopus arrhizus* Fischer, *Syncephalastrum racemosum* (Cohn)Schroet, *Monascus ruber* van Teigh, *Aspergillus glaucus*, *A. penicilloides* Speg, *A. terreus* Thom and *P. rugulosum* Thom.

DISCUSSION

In this study, soil sampling was done only once in each site across the three zones. Of the 26 sites sampled, only 14 were experimented on. Some months elapsed between the dates of collection and isolation of fungi. This would have hindered the germination of organisms in the mycelial state. Identification of organisms in the mycelial states is difficult as one mycelium would seem similar to another in this state. Hence the smallness in the fungi population isolated. Peries *et al* (1979) have isolated a large number of fungi as they studied the rubber soils each mor th in the year subject to climatic variations. In this study, Colombo and Amparai soils showed the highest percentage of organic matter and these have yielded a greater number of *Aspergillus* than the soils with a lesser amount of organic matter. This is in agreement with Christensen and Backus (1961) but is different from the findings of Peries *et al* (1979) in respect of Matale soil which has a higher percentage of organic matter than the other soils studied by them.

While Peries *et al* (1979) sifted the soil before dilution and inoculation into the culture medium, in our study it was decided not to sift the soil as, on a previous occasion, unsifted the soil yielded as many genera as sifted soil. This is also the view taken by Rabzoni (1968). The dilution method was not used and the direct soil plating method of Warcup (1950) was adopted as this technique is less laborious than dilution method and also has yieled a larger number of genera than the dilution methods. In this study emphasis was laid on the kinds of fungi rather than the numbers of individual ones. The paucity of fungi population can also be attributed to the limitations of isolation techiques and culture media used.

In this study, *Absidia* has been isolated in three out four soils at acid pH which is in agreement with Peries *et al* (1979) who have recorded the same in five out of six soils at acid pH. This is similar to the findings of Jensen (1931) who has recorded *Absidia cylindrospora* as growing best in pure culture in the pH range 2.53 - 5.52. Warcup (1951) has listed *Absidia orchidis* in the PH range 3.8 - 4.6 On the other hand, Nicholls (1956) had isolated *Absidia cylidrospora* in five out of eight soils at pH 7.0 or more. warcup (1951 had isolated *A.glauca* in the pH range 6.4 -8.4 In our srudy, it is noted from Table 2 that *Mucor* exceeds *Absidia, Mortierella* and *Cunninghamella* in the warm soils. This is in agreement with Peries *et al* (1979) *et al* (1957) in America and Nour (1956) in Sudan have recorded that *Absidia* and *Cunninghamella* have exceeded Mucor in their warm soils. Thus these findings are the result of the reaction of organisms to changes in physical factors. This makes the results more difficult to interpret and less consistent.

Soils have been collected from the teak plantations in Anuradhapura and Amparai District (Parahakelle) for our study. The ground conditions here resemble closely those in a pine forest or Hevea plantations as the canopy greatly reduces the light reaching the soil. In *Phytophthora* germination, darkness favours the germination of oospores and inhibits sporangia while bright light favours the formation of sporangia and inhibits oospores, Light of low intensity favours the formation of both in all apecies of *Phytophora* (Harnish 1965). However, *Phytophthora* could not be isolated from thse soils whereas Peries *et al.*

(1979) isolated this fungus in two wet rubber districts which isolation they considered was the first in any soil microbiological studies. However earlier, Miller *et al* (1957) had isolated this fungus in the pine soil of Georgia while there is also a reference to isolation of *Phytophthora* by Cambell (1949).

The main genera isolated in this study are the *Aspergillus* and *Penicillium* which are reputed to be the commonest genera of fungi all over the world (Nour, 1965; Ranzoni, 1968; Eicker, 1969; Singh, 1976). In this study, more *Aspergillus* than *Pencillium* has been isolated in the Dry zone. This tendency for *Aspergillus* to replace *Pencilium* in warm soils is borne out by Miller *et al* (1957) in American soils, Nour (1956) in Sudan and Farrow (1954) in Panama and Costa Rica. But in the wet zone, *Aspergillus* and *Pencillium* as isolated by us and Peries *et al* (1979) are almost equally represented. Borut (1960) working on the soils in Northern Negev (Israel) also found *Aspergillus* and *pencillium* frequent. He plotted curves of growth at different temperatures for species of *Penicillium* and *Aspergillus* and a number of other isolates. All species of *Pencillium* had peek of optimum growth at 26° C. The Aspergilli were more variable, but the majority had higher optium, 36, 36, 30 and 26° C. The shape of curves for Aspergilli showed a less marked fall at temperatures just below the optimum than was the case for those *Pencillium*. This indicates that *Aspergillus* not only tolerates higher temperatures but also shows near optimal growth at temperatures which are optimal for the Pencillia. Hence the almost equal representation of both these species in the tropical wet zone of Sri Lanka as shown by our studies. Conversely, *P. lilacinum* as demonstrated by Borut (1960) showed an extended range of temperature above the optimum and is expected to be the most active species of *Pencillium* at higher temperatures at which most other species of *Pencillum* would be inhibited. Hence Peries *et al* (1979) isolated *P. lilacinum* in Matale soil at a frequency of 46 which means that the air and soil temperature of Matale would have been high at the time of sampling and has not been conducive to the germination of other species of *Pencillium*. In our studies we have isolated *Pencillium* and *Trichoderma* in acid soils. Peries *et al* too (1979) have isolated these two genera in soils at acid pH. We therefore agree with Warcup (1951) in his generalisation that *Pencillium* and *Trichoderma* have come to be associated with soils at acid conditions. The paucity of *Pencillium* in Matale soil may be due to the fact that the pH of Matale soil was near neutral (6.4) as recorded by Peries *et al* (1979). In contrast, Ranzoni (1968) has isolated *Pencillium* and Trichoderma in the Sonaran Desert soil in southwestern United States and northwestern Mexico at pH 7.4 and above and at temperature as high as 41° C.

In our study, we have isolated *Aspergillus aculeatus*, *A. flavus*, *A. niger* and *A. glaucus* in Colombo where the mean temperature during the day is 31°C; during night 24.3° C and relative humidities 74% during the day and 87% during night. The same fungi have been isolated at Ratnapura where the mean temperature during the day is 32.16°C; 22.9°C at night and relative humidities 76% and 94%. In Vavuniya in the Dry zone these fungi have been isolated at 32°C during day; 22.6°C during night and rH 64% and 84%. *P.citrinum* too appeared in Colombo and Ratnapura under identical conditions as for *Aspergillus*. These findings are in agreement with those of Peries *et al* (1979) but do not conform to the studies of Chen and Griffin (1966. 1966a) on Australian soils and Bisby *et al* (1935) on Manitoba soils. In the studies of Chen and Griffin (1966,1966a) the effects of both temperature and humidity were investigated by adding sterile hair to the soil; it was found that at the

same temperature but different humidities, most of the fungal flora colonised the hair only under relative humidities of 95% or more while below 95% the colonising flora consisted of *Aspergillus* and *Penicillium* only. When both temperature and humidity were varied, *A. niger* was active at 15 and 20°C under rH 100% while under rH 85% it was found to be active at 25 and 30°C. *P. citrinum* was found to be active at 15°C under rH 85%; under rH 80% it was active at 20 and 25°C and under rH 75% it was active at 30°C Bisby *et al* (1935) selected different isolates by incubating replicate soil plates at 37, 25 and 6°C. Of 49 recorded isolates of *Aspergillus* spp., 27 were tolerant to 37°C. The only other organism regularly isolated at this temperature was Trichoderma *viride*. At 6°C some species of *Penicillium* and Mucorales appeared regularly and *Cladosporium* less frequently. These observations show that different species not only tolerate different humidities at different temperatures but also differ from one another in their activities in the soil.

Studies of soil microbiology thus clearly indicate that groups of organisms vary from soil to soil in a way related to physical environments. Various experiments have been carried out under simulated tropical environments in order to elucidate a generality for groups of organisms active in the soil. These conceptions were fallacious. The diversities of these views and the failure of these conceptions to receive wide acceptance or recognition indicate that they are not accurate. The tropical environment of Sri Lanka varies widely as regards temperature, humidity and H relationships from place to place and from season to season. Experiments designed to simulate tropical environments must not only consider temperatures as high as 36°C with the lower order of relative humidity values, but also the effects of rapid and considerable drops in temperature at night on any of the moulds under investigation. There is, therefore, a need for more studies on the three-dimensional relationships of pH, rH and temperature to fungal growth. This should be supported by several isolation techniques and identification on soil plates at different pH, rH and temperature and with varied nutrient supply in the culture-media. As the pH varies, the availability of certain nutrientions in the medium may lead to secondary effects. A dynamic step to study the ecology of soil microbiology would be the creation of model systems containing a number of known common organisms whose activities on pure culture might be checked in competition with the soil work on the field. Such investigations could lead to the prediction of fungi likely to be active in soils of different reactions. Unless these factors are explored and a generality arrived at, the problem of biological deterioration which still baffles the world-scientists cannot be solved.

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TABLE 1

Localities where soil samples were collected.		pH	No. of colonies in dry soil at 2" depth 6	%Organic matter	%Soluble salts
AMPARAI	C.S	5.8	14,600+ 13,800++	2.11	0.20
ANURADHAPURA	C.S	5.4	11,600+ 10,900++	1.80	0.60
BATTICALOA	C.S	7.3	11,700+ 11,000++	1.73	0.70
COLOMBO (Campbell Park)	G.S	5.2	18,400+ 17,200++	2.6	0.20
DIYATALAWA	C.S.	6.0	14,800+ 13,200++	1.30	0.30
JAFFNA	G.S	5.80	10,200+ 10,000++	1.20	0.65
KANDY	G.S.	6.3	14,000+ 13,800++	0.70	0.30
MAHIYANGANA	F.S	5.80	16,2000+ 15,600++	1.20	0.20
MANNAR	C.S.	5.4	9,800+ 7,000++	1.20	0.20
PARAHAKELLE TEAK Gdns.	F.S.	7.80	10,600+ 10,000++	1.20	0.16
PUTTALAM	G.S.	5.4	9,500+ 9,000++	1.20	0·70
RATNAPURA	G.S.	5.8	14,200+ 13,900++	1.40	0.20
TRINCOMALEE	F.S	7.1	10,600+ 10,000++	1.20	0.70
VAVUNIYA	C.S.	5.1	18,000+ 16,000++	2.20	0.18

C.S. - Cultivated soil; G.S - Grass soil; F.S - Forest soil

TABLE 2

Frequency of occurrence of fungi in each locality. (If one or more than one species of the same fungus appeared in a plate, it counted as one frequency)

Classification	AMPARAI	ANURADHAPURA	BATTICALOA	COLOMBO	DIYATALAWA	JAFFNA	KANDY	MAHIYANGANA	MANNAR	PARAHAKELLE TEAK Gdns.	PUTTALAM	RATNAPURA	TRINCOMALEE	VAVUNIYA
PHYCOMYCETES														
Absidia sp.	1	—	—	2	—	—	—	—	—	1	—	—	—	1
Cunningha mellas p.	1	—	—	1	1	—	—	—	—	1	—	1	—	1
Mortieretta sp.	1	—	—	1	1	—	1	—	—	1	—	1	—	1
Mucor sp.	1	—	1	1	1	—	—	1	—	—	—	1	—	1
Rhizopus arrhizus (Cohn) Schroet.	—	—	—	1	1	—	—	—	—	—	—	—	—	2
Rhizopus arrhizus Fischer.	1	—	—	1	1	—	—	—	—	—	—	—	—	2
Syncephalasterum racemosum (Cohn) Schroet.	—	—	—	1	—	—	—	—	—	—	—	—	—	—
ASCOMYCETES														
Chaetomium sp.	1	—	1	1	—	—	—	—	—	1	—	2	—	1
Monaseus ruber vmTeigh.	—	—	—	1	—	—	—	—	—		—		—	1
BASIDOMYCETES														
Basidomycetes I	—	—	—	1	—	—	—	—	—	—	—	—	—	1
FUNGI IMPERECTI														
Alternaria sp.	—	—	—	1	1	—	—	—	—	—	—	1	—	—
Aspergillus aculeatus Izuka.	1	—	—	2	1	—	—	1	—	—	—	—	—	1
A.flavus Link ex Fr.	2	1	1	2	2	1	1	1	—	2	1	1	1	2
A. glaucus group.	1	—	—	1	—	—	—	1		1		—	—	1
A.niger van Teigh.	1	1	—	2	2	—	2	1		2	—	2	—	1
A.penicilloides Speg.	—	—	—	1	—	—	—	—	—	—	—	—	—	1
A.sydowi(Bain & Sart) Thom & Church.	1	—	—	1	1	—	1	—	—	1	—	2	—	1
R.terreus Thom.	1	—	—	1	1	2	—	—	—	—	—	—	—	2
R.verisicolor(Vuill.) Tiraboschi.	—	—	—	1	1	—	—	—	—	—	—	2	—	2
Aspergillus sp. I	1	1	—	1	1	1	—	1	—	1	1	1	—	1
Aspergillus sp. II	—	—	—	1	—	—	1	1	1	—	—	1	—	1
Botryodiplodiea theobromae Patou	—	—	—	1	—	—	1	—	—	—	—	—	—	1
Fusarium sp.	1	1	1	1	1	—	1	1	—	—	—	1	1	1
Penicillium citrinum Thom.	1	—	—	2	1	—	—	—	—	—	—	2	—	2
P.funiculum Thom	—	—	—	1	1	—	1	—	—	—	—	—	—	—
P.lilacinum Thom	1	—	—	2	1	1	1	1	1	1	1	2	—	2
P.rugulosum Thom	1	—	—	1	1	—	1	1	—	—	—	—	—	—
Penicillium sp. I	1	1	—	1	1	—	2	—	—	1	—	1	—	1
Penicillium sp. II	—	—	—	2	—	—	1	—	—	—	—	—	—	1
Trichoderma harzianum mifai.	1	1	—	1	1	1	1	1	—	—	1	1	—	1
Trichoderma viride ex S.F.Gray.	1	1	—	2	—	—	1	1	1	—	—	—	—	1
Trichoderma viride agg. sensu *mifai.*	—	—	—	1	—	—	—	1	—	—	—	—	—	1
Dark sterile mycelium	1	1	—	1	—	—	—	1	1	—	—	—	—	1
White mycelium	1	—	—	1	—	—	—	—	—	—	—	—	—	1
Yellow mycelium.	—	—	—	1	—	—	—	—	—	—	—	—	—	1