THE FLORA AND ECOLOGY OF SOIL MUCORALES IN A DRY UPLAND FOREST AND SOME GRASSLAND ECOSYSTEMS IN KENYA

by

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DECLARATION

I hereby declare that this thesis and the work described herein are original, except where indicated by reference or otherwise, and has not previously been submitted for any degree at this or any other University.

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DEDICATED TO MY PARENTS, HUSBAND AND CHILDREN

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I remember with gratitude my father Jasper, mother Edita, and other members of my family, for their support and encouragement through the years. Finally, I thank my husband Peter and children: Philip, Michael and Jasper Emmanuel for their support and patience during the course of this exercise.

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GENERAL ABSTRACT

The characterization and ecology of microorganisms is a pre-requisite for their eventual conservation and manipulation in applied aspects of science. Mucoraceous fungi, for example, are known for their biodegradation and fermentation properties. These properties have been applied commercially in the production of organic acids, vitamins and foodstuffs. However such research on microbial biotechnology has concentrated on direct or indirect use of microorganisms with little definition of the populations involved in their natural habitat. Further, the release of genetically modified organisms could become a new and serious environmental hazard. Hence the need to:

(i) have a taxonomic framework for the microorganism

(ii) acquire knowledge on the ecology of the organism and show possible correlations between the organisms and their environment.

This study was carried out in a dry upland forest and grassland zones in Kenya. Soil samples were collected from Karura forest, Kisamis wooded grassland, Athi River Plains and Machakos shrubland ecosystems. Mucoraceous fungi were recovered from a portion of the soil samples, characterised and identified using morphological characters. The fungal communities associated with different vegetation types were compared using the coefficient of community. Analyses of variance tests were performed to determine the variation of mucorales occurrence in relation to the environmental factors.

The major environmental factors that influenced the distribution of mucoraceous fungi among the different ecological sites were soil moisture content, soil temperature and

levels of K. N. Mg and Pb in the soil. These fungi showed a decline in frequency of occurrence and species diversity with increasing lead pollution load in the soil. However. *Cunninghamella* sp. were predominantly present in the heavily contaminated soils and showed good in vitro growth in lead agar medium. This indicated a potential for *C. elegans* to be utilized as a lead pollution bio-indicator.

In conclusion. soil mucoraceous fungi present a correlation between changes in the physical and chemical properties of the soil and changes in the fungal populations present. These variations in the soil environment occur as a result of seasonal changes imposed upon the soil and differences in cover vegetation. Mismanagement of the environment. for example. destruction of vegetation cover and introduction of harmful materials to the environment. therefore. means loss of diversity in mucoraceous populations.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Studies on soil biology has contributed to the knowledge of agriculture, medicine and biology. In the soil are the roots of plants, the burrowing mammals, insects and other animals, bacteria and fungi. To the soil are returned the dead remains of both plants and animals, and there these are decomposed to materials which are again used by plants and animals.

Fungi play a useful part in the maintenance of terrestrial life through their widespread activity on and in the soil. Members of the soil fauna break up the plant remains into smaller fragments, which are thus more quickly degraded by the heterotrophic soil bacteria and fungi which are surface feeders. But it is the soil fungi that are typically the pioneer colonizers of dead plant tissues. They are fitted for this pioneering role by their physical organization into a network of mycelium.

The final result of this breakdown of dead plant tissue is the liberation of the essential mineral nutrients needed by higher plants in a soluble form. Apart from this, the other functions of fungi in the soil ecosystem includes; elemental storage against leaching, facilitation of transport of essential elements and water from soil to plant roots, modification of soil permeability and promotion of aggregation, modification of soil ionic exchange and water holding capacities, synthesis of humic substances, participation in saprophytic food chains, instigation of parasitic symbioses, instigation of mutualistic symbioses, predation, production of environmental biochemicals, enhancement of seed germination through seed coat erosion, cultivation for enzymes or food.

The recognition of the soil fungi, together with their ecology, therefore becomes an important factor in the improvement of agriculture, medicine and chemical industries. The ultimate success of biotechnology is dependent upon advances in and support for the fundamental sciences which underpin it. It would be extremely short-sighted to neglect studies of microbial taxonomy and descriptive ecology. The bottle-neck which would otherwise result is the lack of awareness by the applied scientist and technologist of the richness of microbial types and activities. On the other hand, characterization of microorganisms is the first step in biotechnology risk assessment of an environmental introduction. Despite this potential, studies on soil mycoflora have not been done in East Africa.

Hence the broad aims of this work is to:

(i) recover mucoraceous fungi from the soil, characterise them and provide taxonomic keys for their identification,

(ii) examine the distribution of mucoraceous fungi in parts of Kenya and determine the environmental factors that influence their occurrence.

Members of the order Mucorales were recovered from soil samples collected from Karura forest, Machakos shrubland, Kisamis wooded grassland and Arthi River plains (grassland). The fungi were characterized and identified using morphological characters and identification keys constructed. The effect of vegetation cover, physical (rainfall, temperature, soil moisture and texture) and chemical (Na, K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb, N, C and pH) factors, on the distribution and abundance of these fungi, was also studied.

CHAPTER 2

FLORA OF SOIL MUCORALES IN KARURA FOREST, A DRY UPLAND FOREST

ABSTRACT

Members of the order Mucorales are mostly saprobes reported to be of world-wide distribution. In Africa, however, very little work has been done in a way of taxonomic study or enumeration of these fungi. The delimitation of families and genera is mainly based on the vegetative, anamorphous structures, the sporangia or conidia and rarely on the teleomorph the zygospores. This is because most of the Mucorales are heterothallic and form zygospores only in mating experiments. This limitation has meant overdependence on vegetative structures leading to changes in the classification of these organisms now and again. Further the existing identification keys are often too short or vague. It is in view of these factors that this work was carried out. Mucoraceous fungi were recovered from the soils of Karura Forest using the soil and dilution plate techniques, and identified using morphological characters. Twenty nine species were isolated and identification keys constructed.

INTRODUCTION

The order Mucorales consists mostly of saprobes, or rarely gall-forming or contact mycoparasites These fungi form extensive mycelia, branched, coenocytic when young, sometimes becomes septate with age. Asexual reproduction is by columellate or acolumellate sporangia, sporangiola or merosporangia, or rarely by chlamydospores, or arthrospores, or blastospores. The sporangiospores are one-celled, hyaline, colourless or pigmented, smooth or roughened. Sexual reproduction is by zygospores formed on opposed or apposed suspensors.

Although the majority of mucorales have a world-wide distribution (Christenberry, 1940; Boedijn, 1958), very little work has been done in respect to taxonomy or enumeration of these fungi in Africa. The classification of members of this order has undergone changes as shown by the works

of Benny (1991, 1992), Benny and Benjamin (1975), Benny *et al* (1992), Morton and Benny (1990). Further, the existing description or notes available in form of keys are often too short or vague. In this study, an attempt has been made to construct a working flora of soil mucorales of Karura Forest.

OBJECTIVE

To isolate and identify Mucorales from the soils of Karura forest and construct keys for identification of these fungi.

MATERIAL AND METHODS

Description of study area

Karura forest is a dry upland forest patch located within Nairobi Province (Lind and Morrison, 1974). The forest extends for an area of 1,041.3 hectares and is traversed by rivers Thigirii, Karura, Ruiruaka and Gitathuru. The forest has blocks of plantations as shown in Figure 1.

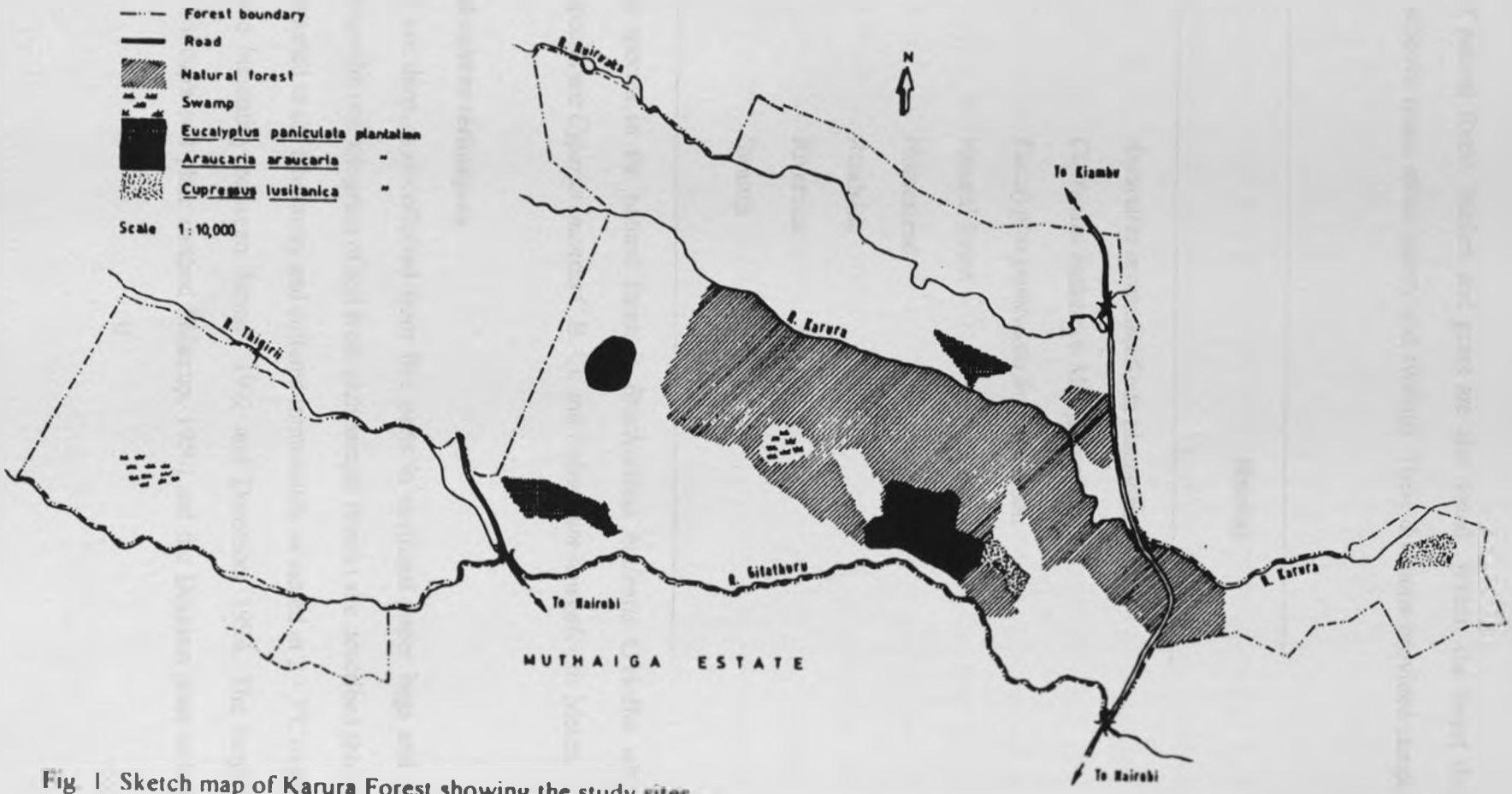

Fig. 1 Sketch map of Karura Forest showing the study sites.

However areas of natural forest. bushes and grass are also found. Within the forest there are residential areas. schools. roads. stone quarry and swamps. These divisions provided sample sites as shown below:

Block	Habitat
Block 1a. b	*Araucaria araucana* Koch plantation
Block 2a. b	*Cupressus lusitanica* Mill plantation
Block 3a. b	*Eucalyptus paniculata* Sm. plantation
Block 4a. b	Natural forest
Block 5a. b	Homestead
Block 6a. b	Roadside
Block 7a. b	Riverside
Block 8a. b	Swamp

The common tree species in the natural forest is *Brachylaenia huilensis* O'Hoffin while the common swamp species are *Cyperus imensus* C.B. Cl. and *Polygonum senegalensis* Meizn.

Soil sampling and culture techniques

Top soil. up to 2 cm deep. was collected from five parts in sterilized paper bags and mixed thoroughly ending up with one kilogram of soil from each sample (block) site described above. The samples were transported to the laboratory and cultured immediately or stored at 2 - 3°C overnight. Sampling was done bimonthly between January 1992 and December 1994. The fungi were recovered by the Warcup's soil plate method (Warcup. 1950) and the Dilution plate techniques

(Chester and Thornton, 1956). The isolates were identified using morphological characters and sent to the International Mycological Institute, Kew, Surrey, England, for proof identification.

RESULTS

Table 1 shows the list of mucoraceous fungi recovered from the soils of Karura forest.

DESCRIPTIONS OF TAXA

MUCORALES

The mycelium of the entire order may be divided into the substrate mycelium and the aerial mycelium. The substrate mycelium consist of non-septate coenocytic hyphae usually embedded in the substratum. Septa may be formed in old hyphae but these cut off multinucleate segments. Aerial hyphae arise from the substrate mycelium and bear the sporangia. These hyphae may vary from simple, unbranched hyphae to luxuriant much branched aerial mycelium. The sporangia are usually terminal but sometimes lateral and contain many non-motile spores. In some genera the sporangium is reduced and contains only a few spores and is termed a sporangiolum; and in others there is just one spore which functions as a conidium. Sexual reproduction consists of the fusion of two multinucleate gametangia. Most members of the order are isogamous and heterothallic, but some are either heterogamous or homothallic or both. The zygospores germinate to form a germ sporangium which contains spores similar to those of a typical sporangium.

Key to the families

1. Only sporangia present: sporangia columellate, many-spored with fugacious or deliquescent walls; zygosporangia typically borne between opposed suspensors; zygospores not enclosed in hyphal envelope, rough 2

   Sporangia present or absent, sporangiola present in

all cases 3

2. Sporangiospores appendaged ..... Gilbertellaceae

Sporangiospores not appendaged ..... Mucoraceae

3. Sporangia may be present or absent: sporangiola present in all cases 4

Only sporangiola present 5

4. Sporangia columellate: sporangiola columellate or acolumellate. zygospores striate or warty. borne on tongs-like or opposed suspensors..... 6

Sporangia acolumellate: sporangiola acolumellate, zygospores smooth or angular. borne on opposed suspensors..... Mortierellaceae

5. Sporangiola (merospsorangia) with uniseriate sporangiospores. borne on vesicles. zygospores warty. borne on opposed suspensors ..... Syncephalastraceae

Unispored sporangiola (not merosporangia). borne on vesicles. sporangiospores never uniseriate. zygospores warty. borne on opposed suspensors ..... Cunninghamellaceae

6. Sporangiola acolumellate. borne on separate and distinct

sporangiophores: zygospores striate. borne on tongs-like

suspensors ..... Choanephoraceae

Sporangiola columellate. borne on the same or separate.

morphologically identical sporangiophores. zygospores

warty. borne on opposed suspensors ..... Thamnidiaceae

Table 1: Mucoraceous fungi recovered from Karura Forest.

Order:	**MUCORALES**
Family:	Choanephoraceae *1. Choanephora cucurbitarum*
Family:	Cunninghamellaceae *1. Cunnunghamella bainieri* *2. C. echinulata* *3. C. elegans*
Family:	Gilbertellaceae *1. Gilbertella persicaria*
Family:	Mortierellaceae *1. Mortierella bainieri* *2.Mortierella* sp.
Family:	Mucoraceae I. *Absidia* 1. *A. cylindrospora* 2 *A. spinosa* 3. *A. zychae* II. *Actinomucor* *1. A. elegans* III. *Circinella* 1.*C. simplex* 2. *Circinella* sp. IV. *Mucor* 1*M. ambiguus* 2*M. falcatus* 3*M. fragilis* 4*M. globosus* 5*M. hiemalis* 6*M. microsporus* 7*M. mucedo* 8*M. plumbeus* 9*M. racemosus* 10*M. spinosus* 11 *M. subtilissimus* 12. *M. zychae* 13. *M. lamprosporus* V. *Rhizopus* 1. *R. echinatus* 2. *R. nigricans* 3. *R. oryzae*
Family:	Syncephalastraceae 1. *Syncephalastrum racemosum*
Family:	Thamnidiaceae I. *Helicostylum* 1. *Helicostylum elegans* II. *Thamnidium* 1. *Thamnidium elegans*

Choanephoraceae J. Schrot, 1958. In *Engler and Prantl, Die naturl. Pflanzenfam.* 1 (1): 131. In O'Donnell, 1979. Zygomycetes in culture.

Sporophores arising directly from the substrate mycelium or aerial hyphae, simple or branched, producing either sporangia or sporangiola, never both. Sporangia large, multispored and columellate, or smaller, few-spored and acolumellate, wall persistent echinulate, with a weak longitudinal line of rapture; sporangiospores with long, hyaline, polar appendages, and a brown, longitudinally striate wall. Sporangiola uni- or multispored wall persistent of two types: 1) wall entire, appears inseparable from spore wall; sporangiospores without appendages, brown, smooth or more or less longitudinally striate; 2) wall raptures longitudinally and separates from the spore wall; sporangiospores with hyaline, polar appendages, and a brown, striate wall. Zygospores having a thin, hyaline, relatively smooth zygosporangial wall and a thicker-walled, brown, striate zygospore; appendages opposed; often entwined, without appendages.

***Choanephora* Currey, *J. Linn. Soc., Bot.* 13: 578. 1873 *ut nom. nov. pro Cunninghamia* Currey 1873. In O'Donnell, 1979. Zygomycetes in culture.**

Sporangiola always present, usually accompanied by sporangia; sporangiole indehiscent, wall not separable, without longitudinal lines of weakness.

Choanephora cucurbitarum **(Berkeley and Broome) Thaxter. Rhodora 5, 97, 1903. In Boedijn, 1958, Sydowia, 12: 321 - 361.** Fig.2

Colony white becoming cream on aging, mycelium cottony, with aerial growth. Sporangiophores arising from surface hyphae, erect, unbranched, hyaline, 1.2 x 15 µm in diam, ending at the tip in a globose swelling, 36 - 48 µm in diam. From this swelling arise stalks that end in vesicular swellings 28 - 50 µm in diam. On these vesicles unispored sporangiola are borne on short sterigmata; sporophore in some cases may not have a swollen globose tip, but rather a fairly enlarged cylindrical tip with the sporangiola arising from all over the enlarged surface. Sporangiola pale, Rusty Tawny brown, elliptic, acuminate abtuse, with longitudinal striations by low sparingly anastomosing ribs, 16.8 - 18.8 x 9.0 - 11.3 µm. Sporangiophores unbranched, mostly shorter than the sporophores, 18 - 22 µm in diam, more or less strongly bent at the tip. Sporangia globose, black, 89 - 120 µm in diam; sporangial wall covered with short spines 1 - 1.5 µm long, persistent splitting at maturity. Columella subglobose, with a distinct collar at the base, 22 - 30 µm in diam (Boedijn, 1958; also pear-shaped, 27 - 119 x 24 - 98 µm; globose, 18 - 36 µm in diam). Sporangiospores elliptic, one pole broadly oval, striate in longitudinal direction, pale, Rusty Tawny brown, 17.2 - 19 x 8 -10 µm (Boedijn, 1958; one pole of the sporangiospores is broadly oval or with irregular outline, usually paler than the sporangiola, sometimes spores wholly smooth, 15 - 27 x 6 - 10 µm, with a bundle of very delicate threads 15 - 36 µm long at both poles).

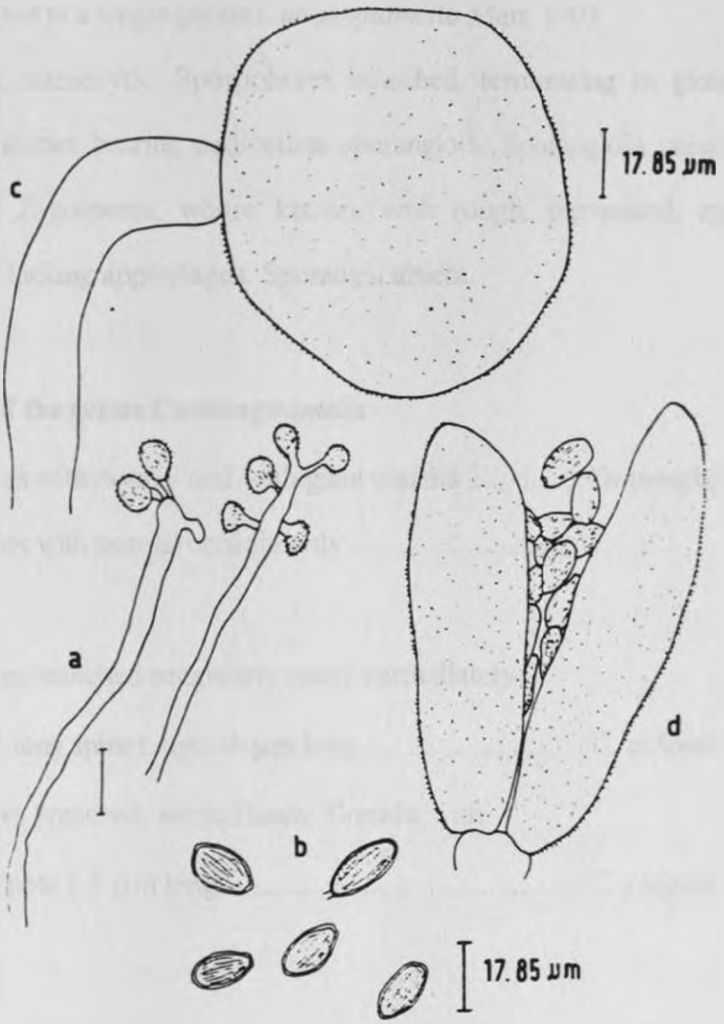

Fig. 2: *Choanephora cucurbitarum*, (a) Sporangiophores showing sporangiolar vesicles with exposed sterigmata, (b) Striate unispored sporangiola, some with sterigmata attached, (c) Circinately borne sporangia, and (d) Circumscissile sporangial dehiscence showing striate sporangiospores.

Cunninghamellaceae R.K. Benjamin, Aliso 4: 415. 1959; emend. Benny, R.K. Benjamin and P.M. Kirk. In Benny G.L., R.K. Benjamin and P.M. Kirk, Mycologia, 84 (5) 1992, pp. 615 - 641.

This family is restricted to a single genus *Cunninghamella* Matr. 1903.

Mycelium branched, coenocytic. Sporophores branched, terminating in globose to obovoid or obpyriform fertile vesicles bearing pedicellate sporangiola. Sporangiola unispored, acolumellate, smooth or spinose. Zygospores, where known, with rough, pigmented, zygosporangial wall, suspensors opposed, lacking appendages. Sporangia absent.

Key to the species of the genus *Cunninghamella*

1. Fertile vesicles with normal and with giant conidia ..... *Cunninghamella bainieri.*

   Fertile vesicles with normal conidia only 2

2. Conidiophores branched irregularly rarely verticillately.

   Conidia with long spines, upto 4 µm long ..... *C. echinulata*

   Conidiophores branched, verticillately. Conidia with

   short spines, upto 0.5 µm long ..... *C. elegans*

***Cunninghamella bainieri* Naumov 1939, Cles Mucorinees, 107. In Baijal and Mehrotra, 1980. Sydowia 33: 1 - 16.** Fig.3

Colonies on PDA and Czapek Dox Agar pale smoke grey to pale Ochraceous Buff, at first floccose

later granular. Conidiophores 11 - 12.5 µm in diam. verticillately to pseudoverticillately branched: terminal vesicles globose to subglobose. 33 - 40 µm in diam (Baijal and Mehrotra. 1980: 31.5 - 42 µm). secondary vesicles of the same shape but smaller in size. 13.8 - 25 µm in diam (14 - 35 µm). Conidia of two types. normal and giant; normal conidia mostly globose to oval. hyaline. globose 6 - 8.9 µm in diam (Baijal and Mehrotra. 1980: 6 - 10.5 um) or oval 8.5 - 14.2 x 8.2 - 12 µm (9.75 x 9 - 12 µm). echinulate. spines 2 - 3 µm in length: giant conidia globose brown. thick walled upto 26 um in diam (Baijal and Mehrotra. 1980: upto 28 µm). borne either singly or in groups of few on generally verrucose secondary vesicles. echinulate, sometimes smooth. spines 1.5 - 2 µm in length.

Cunninghamella echinulata **(Thaxter) Thaxter 1903, Rhodora 5 : 98. In Baijal and Mehrotra, 1980. Sydowia 33: 1 - 16. 1980.** Fig.4

Colonies on PDA and CZ agar. at first while later pale Ochraceous Buff in colour; rhizoids present. Conidiophores up to 11 µm in diam. mostly branched. branches arising singly monochasial cymosely. irregularly. oppositely or rarely verticillately; primary vesicles oval to broadly clavate or pyriform. sometimes globose. 23 - 34.5 µm in diam (Baijal and Mehrotra. 1980. 21 - 41 µm) secondary vesicles of same shape as primary vesicles but smaller in size. 14 - 32 x 13 - 24 µm (Baijal and Mehrotra. 1980: 13.5 - 32 x 9 - 25 µm). Conidia. borne on sterigmata up to 1.5 µm in length. produced on the entire or upper part of the vesicle. hyaline to pale brown in colour. mostly globose 5.4 - 14.2 µm. rarely obovate or ellipsoidal 10.4 - 12.8 x 9.1 - 10.71 µm in diam (Baijal and Mehrotra. 1980: 5.5 - 22.5 µm). verrucose to strongly echinulate. spines up to 4 µm in length. numerous short lateral branches. simple or branched with a blunt end or a very short vesicle up to 7.5 µm in diam. bearing one to few conidia. arising from conidiophores or aerial mycelium.

Cunninghamella elegans **Lendner. 1907. Bull. Herb. Boissier 7 : 250. In Baijal and Mehrotra. 1980. Sydowia Annal. Mycol. ser. 2, 33: 1 - 13.** Fig.5

Colonies on PDA and CZ agar at first white later on grey in colour. pale Smoke Grey. rhizoids present. Conidiophores up to 12 um in diam. branched verticillately; terminal vesicles subglobose to oval. 21 - 32 µm in diam (Baijal and Mehrotra. 1980: up to 38.5 µm). lateral vesicles of same shape but smaller. upto 20 µm in diam. Conidia globose. 5.4 - 9.3 µm (Baijal and Mehrotra. 1980: 6 - 10 µm). subglobose to obovate 6.4 - 14.3 x 5.4 - 8.9 µm (Baijal and Mehrotra. 1980: 6.6 - 12.1 x 4.4 - 8.8 µm) brown at maturity. punctate to spinulose. spines up to 0.5 µm in length.

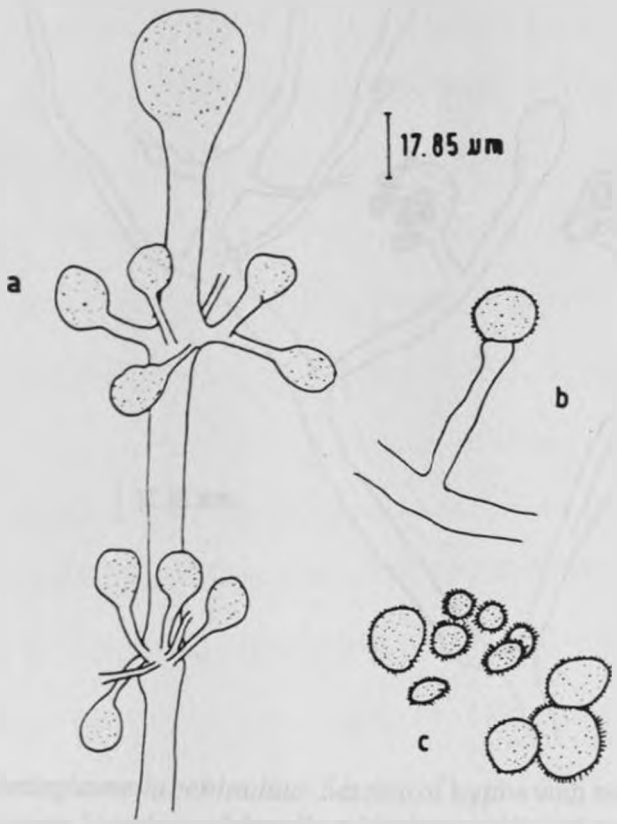

Fig. 3: *Cunninghamella bainieri*. (a) Upper portion of verticillately branched conidiophore with sporangiola that have seceded leaving naked sterigmata; (b) A short lateral branch with a very short vesicle and a giant conidium; and (c) Giant and normal conidia

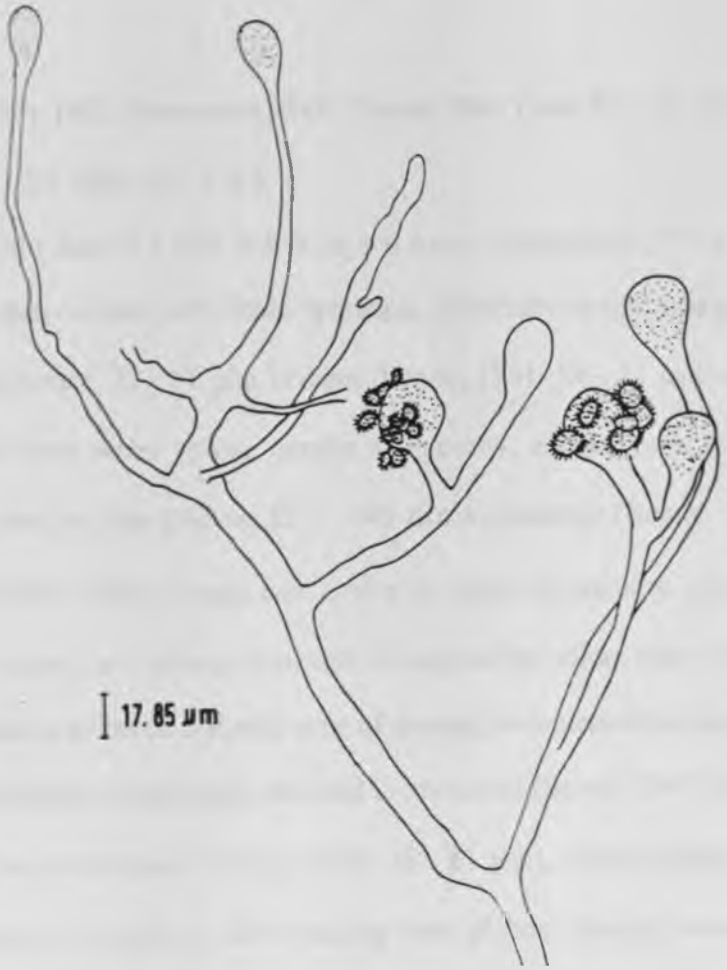

Fig. 4: *Cunninghamella echinulata*. Section of hypha with branched conidiophores and rhizoids. Vesicles with heavily echinulate conidia and those with naked sterigmata

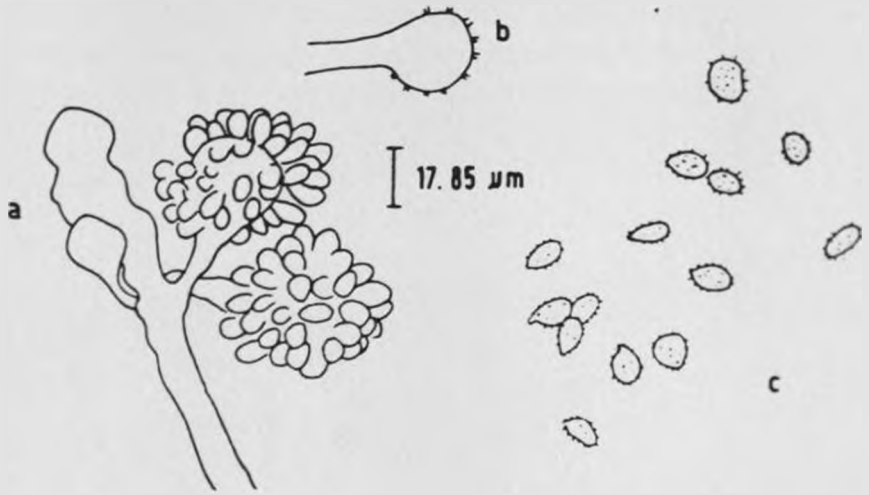

Fig. 5: *Cunninghamella elegans*. (a) Branched conidiophore with unispored sporangiola borne on fertile vesicles; (b) Vesicle with naked sterigmata after release of sporangiola, and (c) Spiny conidia showing sterigmata attachment scars.

Gilbertellaceae Benny, 1991. Benny, G.L., Mycologia 83 (2), 1991, pp. 150 - 157.

The family is restricted to a single genus and a single species.

***Gilbertella persicaria* Eddy 1925. Hesseltine, Bull. Torrey Bot. Club 87 : 24. 1960. In Benny, G.L. 1991. Mycologia 83 (2): 150 - 157.** Fig.6

Colony on PDA filling Petri dish (8.5 cm) in five days at room temperature (25°C), turf 3 - 8 mm high, lax, white at first, then covered with black sporangia, especially around margin of culture at maturity. Sporangiophores erect, 22 - 31 µm in diam (Benny, 1991: 19 - 33 µm) arising directly from the substrate, apex bent when young, upright at maturity, simple, each terminating in a sporangium. Sporangia more or less globose 82.5 - 148 µm in diameter (Benny, 1991: 45 - 170 µm) yellow-brown to brown when young, dark brown to balck at maturity, multispored; wall persistent, covered with spines, and having at maturity a longitudinal suture where it separates into two more or less equal halves to reveal a lighter zone of sporangial contents that later become more liquid and drop-like; columellae obpyriform, obovoid to obconical (Benny, 1991: also cylindrical) 74 - 79 µm in diam at the widest point (Benny, 1991: 36 - 81 µm), smooth-walled, with a slight basal collar. Sporangiospores irregular in shape ranging from globose through broadly fusiform or ellipsoid to ovoid 7.1 - 16.1 x 4.8 - 9.2 µm (Benny, 1991: (5.1-) 7.6 - 11.4 (-17.8) x (3.8-) 6.4 - 8.9 (-12.7), hyaline, smooth-walled with 2 - 3 hyaline appendages, up to 22.8 µm long (Benny, 1991 : 24 µm), arising from the ends of the spore.

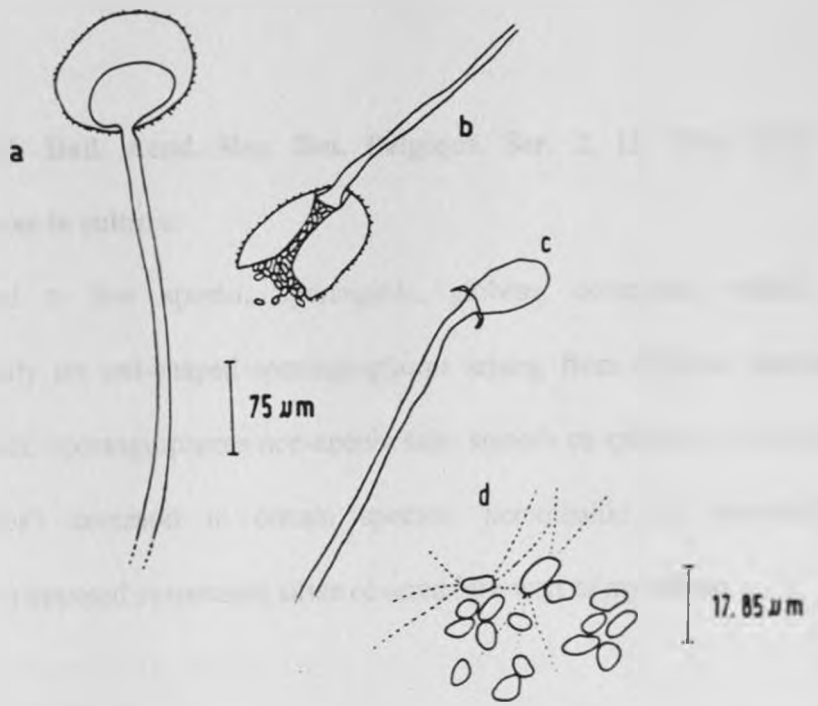

Fig. 6: *Gilbertella persicaria*. (a) A mature sporangium before spore dehiscence. The sporangial wall is spinulose and columella is visible; (b) Sporangium after separation of the wall along its longitudinal suture showing the enclosed spores; (c) Columella and subtending collar after sporangiospore and sporangial wall dehisce; and (d) Hyaline sporangiospores showing the origin of their thin, hyaline appendages.

Mortierellaceae A. Fischer, 1892. In Rabenhorst, *Krypt. Fl.* 1 (4) : 268. In O'Donnell 1979. Zygomycetes in culture.

Mycelia fine, often arachnoid, branched, coenocytic at first. Colony white often zonate and/or producing a garlic-like odour. Sporangia one- to many-spored, with a rudimentary columella, which is septum-like or slightly bulged or dome-shaped. Smooth or rough chlamydospores often formed. Zygospores smooth or dimpled; suspensors opposed, unequal naked or with a hyphal envelope.

***Mortierella* Coemans, 1863. Bull. Acad. Roy. Bot. Belgique, Ser. 2, 15: 536. 1863. In O'Donnell 1979, Zygomycetes in culture.**

Sporangia and/or unispored to few spored. Sporangiola, globose, columella reduced or acolumellate, borne terminally on awl-shaped sporangiophores arising from delicate arachnoid hyphae of determinate growth; sporangiophores non-apophysate; smooth or spinose to echinulate chlamydospores ('stylospores') common in certain species; homothallic or heterothallic; zygosporangia borne between opposed suspensors, often covered by a weft of mycelium.

***Mortierella bainieri* Constantin Kuhlman E.G., 1969. Can. Journ. Bot. 47: 1719 - 1723.** Fig.7

Colonies growing in moderately fast filling Petri plate (8.5 cm) in 7 days, turf white growing high up to the lid of Petri plate. Hyphae anchored to the substratum by branched rhizoids and forming sporangiophores. Sporangiophores erect, branching sympodially with up to six branches, with one axis; 10 - 15 µm wide at base tapering to 3 - 5 µm at tip. Sporangia globose 25 - 38 µm in diam. Sporangiospores cylindrical, 4 - 6 x 7 - 10 µm thin walled. Chlamydospores present 15 - 20 µm, thick walled, intercalary or terminal. Zygospores not seen.

***Mortierella* sp.** Fig.8

Colonies growing moderately fast filling Petri plate (8.5 cm) in 7 days, turf white growing high up to lid of Petri plate. Hyphae anchored to the substratum by branched rhizoids and forming sporangiophores. Sporangiophores erect, single or branched, 3 - 5 µm in diam, anchored by rhizoids. Sporangia globose, 27 - 40 µm in diam, many-spored on dehiscence leaving an inconspicuous collarette. Columellae absent. Sporangiospores globose, smooth walled 1.8 - 2.1 µm in diam. Chlamydospores thick-walled 14 - 18 x 14 - 18 µm, numerous, both intercalary and terminal; some in very long chains. Zygospores not seen.

Notes

The description given above is close to that given by Gams in Persoonia **9:** 111 - 140, 1976; for *Mortierella jenkinii* (A.L. Sm.) Naumov. The manner of sporangiophore branching and sporangial size of this isolate is similar to that described by Gams. However the difference comes in the size and shape of the sporangiospores and the amount and shape of the chlamydospores. Gams observed short-ellipsoidal to cylindrical spores with smooth walls and 3.5 - 4.0 (-0.5) x 2.0 - 2.5 µm in size. Chlamydospores were absent or tardily produced and lemon-shaped.

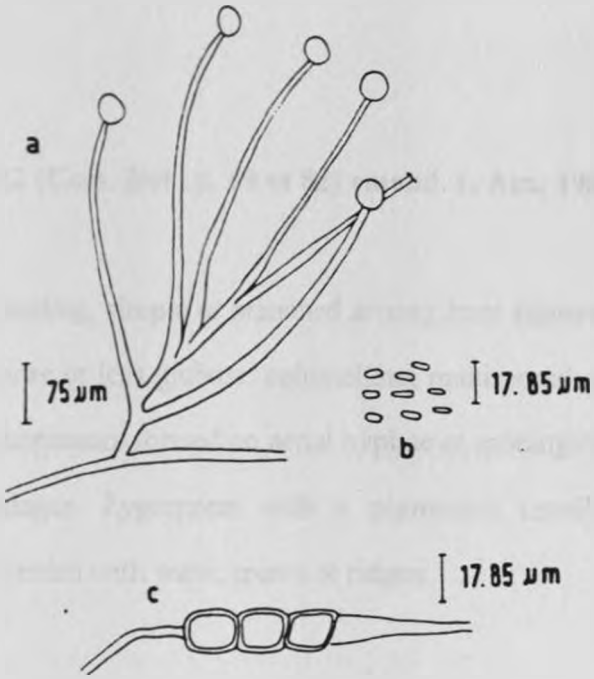

Fig. 7: *Mortierella bainieri*. (a) Sporangiophores bearing sporangia. Note the collar behind after dehiscing of one of the sporangia. Note also the branching habit of sporangiophore, (b) Sporangiospores; and (c) Chlamydospores.

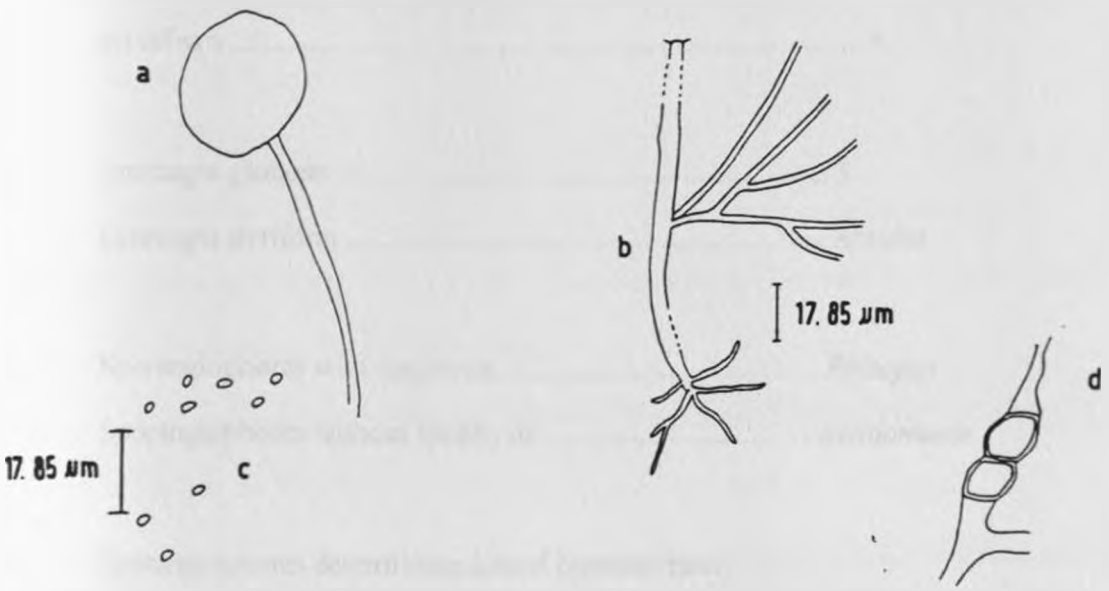

Fig. 8: *Mortierella* sp. (a) Sporangiophore bearing many-spored sporangium; (b) Sporangiophore showing general characteristics of the branching habit. Also showing collar at the tip of sporangiophore; (c) Sporangiospores; and (d) Intercallary chlamydospores.

Mucoraceae Dumortier 1822 (Com. Bot., p. 69 et 82) emend. v. Arx, 1983, Sydowia 35 : 10 - 26.

Sporangiophores erect or ascending, simple or branched arising from substrate mycelium or aerial hyphae. Sporangia similar, more or less globose, columellate, multispored, without an apophysis; wall diffluent or persistent. Suspensors formed on aerial hyphae or sporangiophore, opposed, equal or unequal, without appendages. Zygospores with a pigmented usually dark, thick-walled zygosporangium that is ornamented with warts, spines or ridges.

Key to genera of the Mucoraceae

1. Sporangiophores arising from stolons which form rhizoids at point of contact with substratum 2

   Sporangiophores arising directly from vegetative mycelium 4

2. Sporangia globose 3

   Sporangia pyriform ..... *Absidia*

3. Sporangiophores with apophysis ..... *Rhizopus*

   Sporangiophores without apophysis ..... *Actinomucor*

4. Sporangiophores determinate, lateral branches rarely circinate ..... *Mucor*

Sporangiophores indeterminate. lateral branches

circinate ..... *Circinella*

Absidia **van Tieghem, *Ann. Sci. Nat. Bot.*, Ser. 6, 4: 350. 1876. In O'Donnell, K. 1979. Zygomycetes in culture.**

Sporangia pyriform. columellate. relatively small. borne terminally on sporangiophores arising along stolons; sporangiophores apophysate; zygosporangia borne between opposed suspensors which often bear a whorl of circinate appendages on one or both suspensors.

Key to the species of *Absidia*

1. Heterothallic. sporangiospores ovoid. no more than 3.5 µm in length. up to 2 sporophores from the same place on the stolon (mostly single) ..... *A. zychae*

   Heterothallic or homothallic. sporangiospores cylindrical. 3 - 5 µm in length. sporophores in whorls of up to 4 (sometimes 5) 2

2. Heterothallic ..... *A. cylindrospora*

   Homothallic ..... *A. spinosa*

Absidia cylindrospora **Hagem var. nigra Hesseltine and Ellis, var. nov. In Hesseltine and Ellis, 1964. Mycologia 56: 568 - 601.** Fig.10

Colonies pale Smoke Grey becoming Mouse Grey at two weeks. rapidly growing. filling Petri dishes (8.5 cm) to their lids in 5 days at 25°C. hyphae 6 - 15 µm in diam bearing the sporangiophores 2 - 7 µm in diam. hyaline to light brown. smooth. erect. Sporangiophores simple or in whorls of up to 4 (-5) from the stolons. a few sporangiophores branched and arising from the

substrate mycelium. Sporangia 18 - 25 µm in diam (Hesseltine and Ellis, 1964: 10 - 30 µm), deliquescing, pyriform. Columellae semi-globose, hyaline, 8 - 20 µm in diam (Hesseltine and Ellis, 1964: 6.5 - 24 µm), usually with a short slender appendage 2 - 4 µm long and a funnel shaped apophysis, collar present. Sporangiospores 3.8 - 4.2 x 2.4 - 3.8 µm (Hesseltine and Ellis, 1964: 3.5 - 4.5 x 2 - 3.5 µm), cylindrical, smooth, hyaline. Zygospores not seen.

***Absidia spinosa* Lender, Bull. Herb. Boiss., Ser. 11, 7 : 250. 1907. In Hesseltine and Ellis, 1964. Mycologia 56: 568 - 601.** Fig.11

Colony on PDA floccose, up to 1.5 cm high, completely filling Petri dish (8.5 cm) in 7 days at 25°C, rapidly growing, white at first, then Smoke Gray. Hyphae 7 - 15 µm in diam bearing the sporangiophores 2 - 7 µm in diam, hyaline, always with a septum below the sporangium, erect; sporophores simple or in whorls of up to 4 (-5) from the stolons, generally unbranched; stolons 9.5 - 12 µm in diam (Hesseltine and Ellis, 1964: 4.5 - 25 µm), hyaline to brown in colour, smooth, usually unseptate. Sporangia 12 - 40 µm in diam (Hesseltine and Ellis, 1964: 12 - 30 (-42) µm, hemispherical with a funnel-shaped apophysis, with collars; projections usually present on upper surface of columellae, varying in shape from a short spine to a pointed or rounded cylinder to a rounded club measuring 1.8 - 4.5 x 0.5 - 1 µm (Hesseltine and Ellis, 1964: 1.5 -4.5 x 0.5 - 1), often lacking on the largest columellae. Sporangiospores 3.8 - 5.1 x 2.1 - 2.2 um (Hesseltine and Ellis, 1964: 3.5 - 5 x 2 - 2.5 (-3.7) µm), infrequently oval up to 5.0 x 5.5 µm, hyaline, smooth-walled, typically short cylindrical with rounded ends. Chlamydospores not seen. Zygospores 48 - 58 µm in diam (Hesseltine and Ellis, 1964: 40 - 75 µm), always present, scattered throughout the aerial hyphae, spherical to globose, hyaline when young, soon Chestnut Brown, later dark brown; zygospore wall coarsely verrucose, covered with blunt stellate projections up to 4.1 um in length (Hesseltine and Ellis: 4.5 µm); suspensors typically unequal and with fingerlike appendages from

the larger suspensors. when equal. both suspensors larger and with appendages. at first hyaline. later brown. smooth. hemispherical to cone-shaped: diameter of larger suspensor 24 - 29 µm in diam (Hesseltine and Ellis. 1964: (21 -) 25 - 36 (-38) µm) diam. of smaller suspensor 12.6 - 18 µm (Hesseltine and Ellis. 1964: (7-) 14 - 21 µm): frequently hyphae bearing the suspensors extend as sterile projections of variable length: fingerlike appendages 4.6 - 8 µm in diameter (Hesseltine and Ellis. 1964: 4.5 - 8 µm) near the base. forming simultaneously from the suspensors soon after fusion. at first hyaline. later brown. generally 9 - 12 per suspensor. variable in length but of the same approximate length for only one suspensor. ½ to 3 times the circumference of the mature zygospore in length. tapering slightly toward the extremity. tips rounded or bluntly pointed. non-septate; when appendages shorter closely surrounding the zygospores. when longer extending outward around and past the zygospore as a loose sheath. Homothallic.

Absidia zychae **Hesseltine and Ellis, sp. nov. Hesseltine and Ellis, Mycologia 58: 761 - 785. 1966.** Fig.9

Colonies Smoke Grey. 1 - 2 mm high. very resrticted. only 2.4 cm in diam at three weeks at 25°C. never covering all the surface of the agar in a Petri dish (8.5 cm). growth irregular. odor faint but distinct. somewhat like cheese. Sporangiophores 5.1 - 7.5 µm in diam (Hesseltine and Ellis. 1966: 3 - 8 µm) erect from stolons. hyaline. smooth walled. unbranched (Hesseltine and Ellis. 1966: occasionally sympodially branched. occasionally two sporangiophores from the same place on the stolon but mostly single). Sporangia 25 - 30 µm in diam (Hesseltine and Ellis. 1966: P2 - 32 µm) hyaline. deliquescent. pyriform (Hesseltine and Ellis. 1966: pyriform in the large sporangia to globose in the smaller ones). many-spored: sporangial wall smooth. transparent. Columellae 18 - 23

µm in diam (Hesseltine and Ellis. 1966: 15 - 25 µm). semi-globose. hyaline to tan. with a distinct collar. smooth-walled. without a projection at the apex. spores often remaining attached to columella wall. Sporangiospores 2.0 - 3.2 x 1.6 - 2.0 µm in diam (Hesseltine and Ellis. 1966: 1.6 - 3.3 x 1.6 - 2.2 µm). oval to cylindrical. smooth. hyaline. Zygospores not seen. Sucker-type mycelium present in great abundance in the substrate. thin-walled. very twisted and with short. blunt branches.

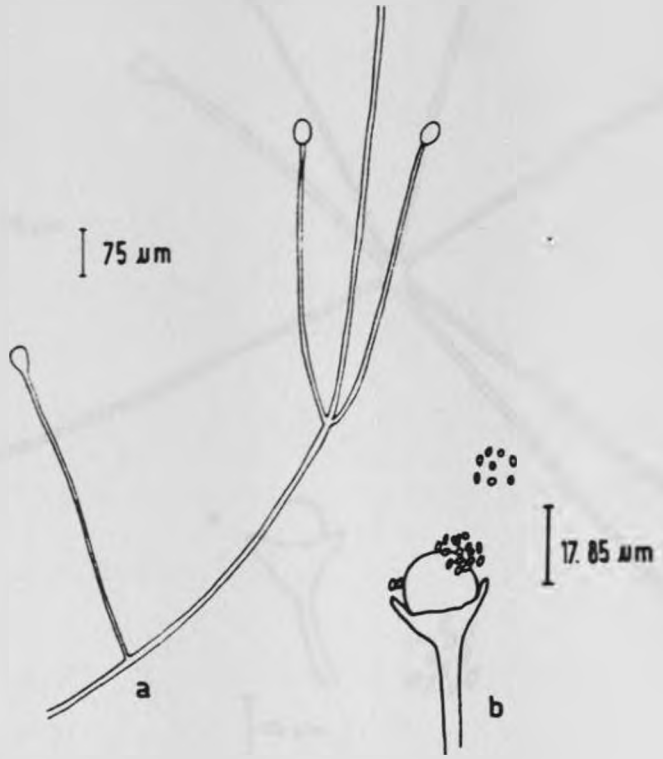

Fig. 9: *Absidia zychae*. (a) Sporophores bearing globose to pyriform sporangia; and (b) Very small sporangiospores and columella.

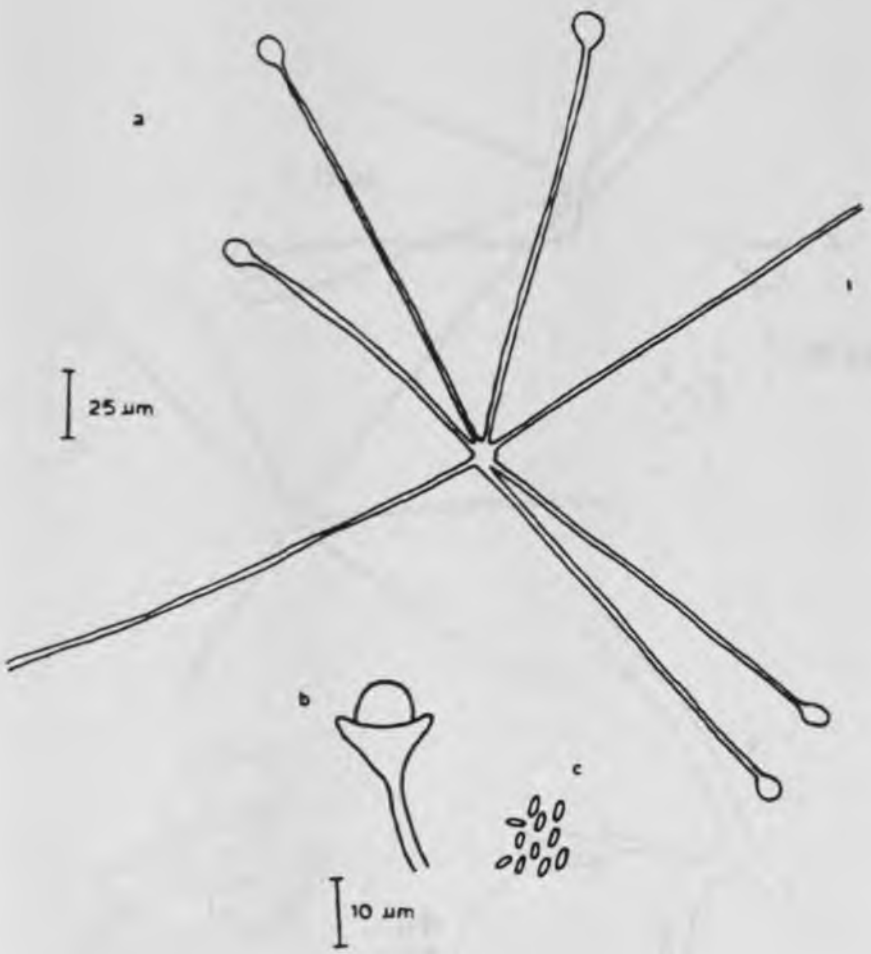

Fig. 10: *Absidia cylindrospora*; (a) Sporangiophores in a whorl of 5 from the stolon; (b) Funnel-shaped apophysis with columella; and (c) Sporangiospores.

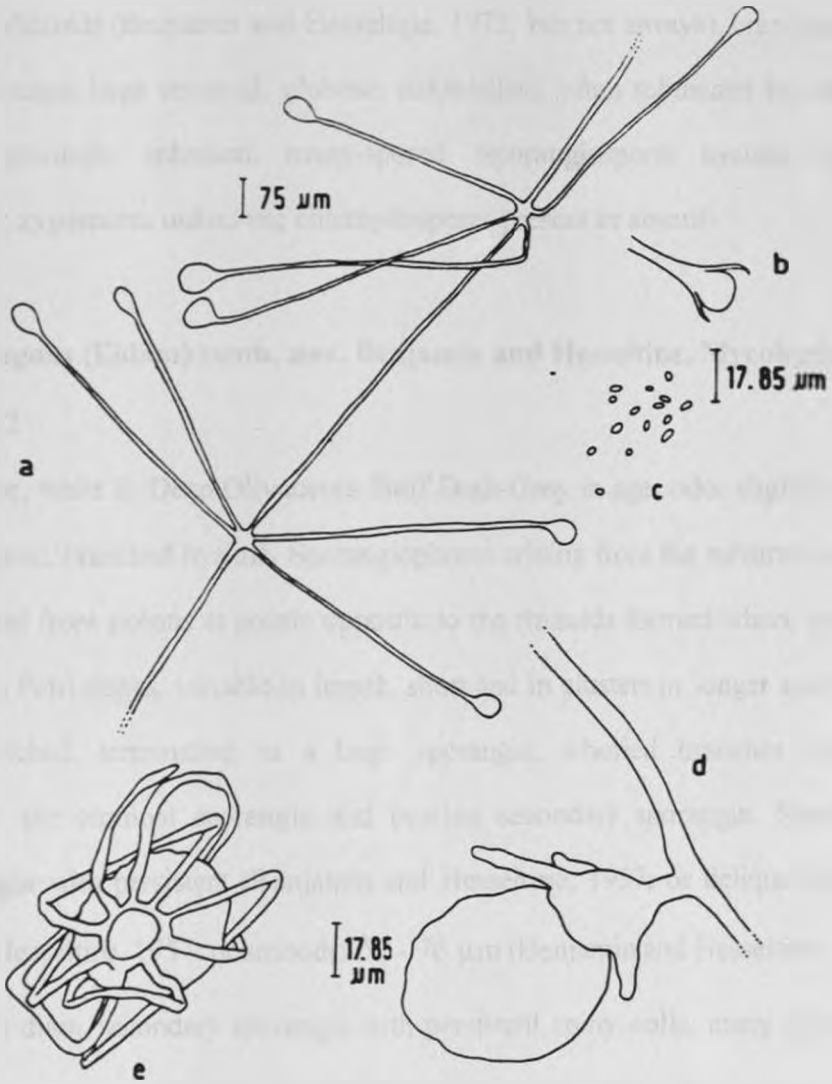

Fig. 11: *Absidia spinosa*. (a) Sporangiophore in whorls from the stolon; (b) Columella with collar; (c) Sporangiospores; (d) Young zygospore; and (e) Mature zygospore.

Actinomucor **Schostak. *Ber. Deut. Bot. Ges.* 16: 155, pl. IX, figs. 1 - 13, 1898. In Benjamin and Hesseltine, Mycologia 49: 240 - 249. 1957.**

Mycelium branched: stolons and rhizoids present, both branched. Sporangiophores arising long stolons opposite rhizoids (Benjamin and Hesseltine, 1975: but not always), branched, septate, non-apophysate. Sporangia large terminal, globose, columellate, often subtended by whorl of smaller sporangia; all sporangia spherical, many-spored. Sporangiospores hyaline. (Benjamin and Hesseltine, 1957: zygospores unknown; chlamydospores present or absent).

Actinomucor elegans **(Eidam) comb. nov. Benjamin and Hesseltine, Mycologia 49: 240 - 249. 1957.** Fig.12

Colonies floccose, white to Deep Olivaceous Buff Drab-Grey in age; odor slightly yeasty; stolons and rhizoids present, branched hyaline. Sporangiophores arising from the substrate mycelium, from aerial hyphae, and from stolons at points opposite to the rhizoids formed where the stolons touch the substrata and Petri dishes, variable in length, short and in clusters or longer and verticillately or racemosely branched, terminating in a large sporangia, whorled branches originating short distances below the terminal sporangia and bearing secondary sporangia. Sporangia globose; terminal sporangia with persistent (Benjamin and Hesseltine, 1957: or deliquescent) spiny walls (Benjamin and Hesseltine, 1957: or smooth), 52 - 76 µm (Benjamin and Hesseltine, 1957: less than 80 µm (-120) in diam, secondary sporangia with persistent spiny walls, many spored, 44.3 - 45.2 µm in diam (Benjamin and Hesseltine, 1957: 20 - 50 µm). Columellae of the large sporangia pyriform, hyaline, 54.2 - 56.9 x 32 - 35 µm; columellae of the secondary sporangia globose to sub-globose, 13.1 - 15 µm in diam (Benjamin and Hesseltine, 1957: 12 - 30 µm). Sporangiospores smooth, heavy-walled, singly hyaline in mass grey-black, globose, 7.1 - 7.4 µm in diam (Benjamin and Hesseltine, 1957: 6 - 8 µm).

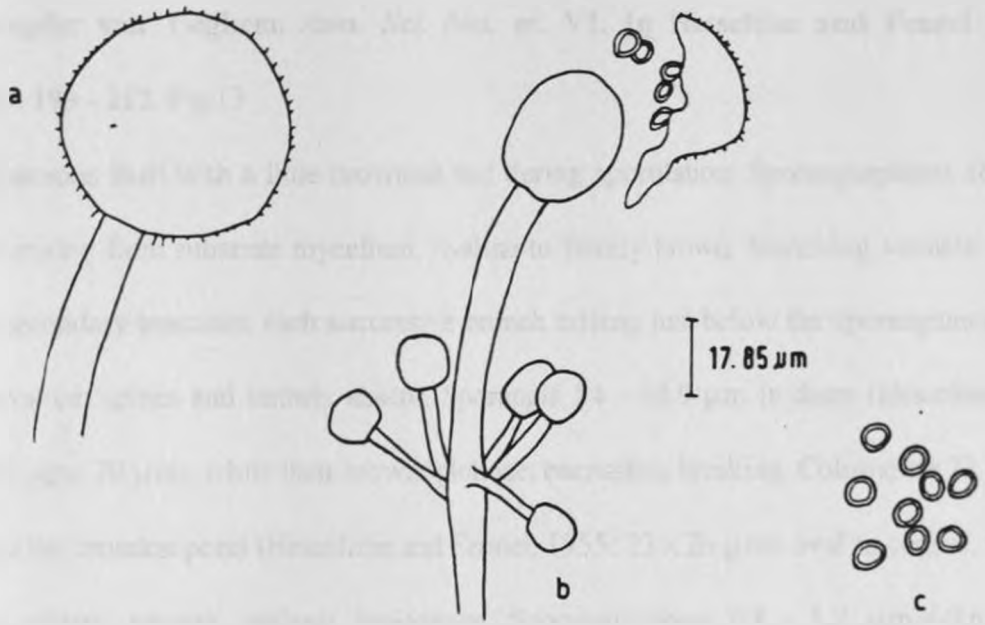

Fig. 12: *Actinomucor elegans*. (a) Mature globose sporangium with spiny sporangial wall; (b) Sporangiophore with terminal sporangium subtended by whorl of smaller sporangia; sporangiospores have been released and columellae of terminal and secondary sporangia still attached to sprangiophore; portion of a broken sporangium showing the rough nature of sporangial wall; and (c)Thick walled sporangiospores.

Circinella **van Tieghem and Le Monnier, 1873. Ann. Sci. Nat. ser. V. 17: 298. In Hesseltine and Fennell 1955. *Mycologia* 47: 193 - 212.**

Mycelium hyaline or coloured. Sporangiophores branched sympodially, indefinite in length: branches with one or many sporangia (Hesseltine and Fennel, 1955: sometimes with a sterile spine). Sporangia always borne circinately at the end of branches, globose, with a persistent sporangial wall, many-spored, columellate with a well-defined collar. Sporangiospores globose, smooth (Hesseltine and Fennell, 1955: zygospores produced between equal and unadorned suspensors).

Circinella simplex **van Tieghem. *Ann. Sci. Nat. er.* VI. In Hesseltine and Fennel 1955. Mycologia 47: 193 - 212.** Fig.13

Colonies Olivaceous Buff with a little brownish tint during sporulation. Sporangiophores 16 - 18 µm in diam, arising from substrate mycelium, hyaline to faintly brown, branching variable, often with several secondary branches, each successive branch arising just below the sporangium in the region of curvature, spines and umbels absent. Sporangia 54 - 68.9 µm in diam (Hesseltine and Fennell, 1955: upto 70 µm), white then brown, globose, encrusted, breaking. Columellae 23 - 24.9 µm in diam at the broadest point (Hesseltine and Fennel, 1955: 23 x 26 µm), oval to conical, with well defined collars, smooth, without projections. Sporangiospores 4.8 - 5.2 µm (-9.6 µm) (Hesseltine and Fennell, 1955: 3 - 5.5 µm (-11 µm)), in length, very irregular, some roughly oval and angled, others curved, smooth. Chlamydospores and zygospores not seen.

Circinella **sp.** Fig.14

Colonies at first white then pale smoke grey, sparse odorless. Sporangiophores 15 - 17 µm in diam, arising from the substrate, characteristically twisted or in loose spirals, always terminating in 1

sporangium. branching alternately to produce sporangium-bearing stalks; short stalks 7.5 - 8.2 µm towards the base of the sporangiophore 16.6 - 78 (-86) µm. Sporangia (25-) 53.5 - 75 (-89.3) in diam. black. globose to slightly pyriform. smaller sporangia at the tip of the sporangiophore; sporangial wall encrusted. dark. not allowing spores to be clearly seen within. extremely resistant to breaking. deliquescing when mature. Columellae 25 - 34 µm in diam. smooth. sub-globose. Sporangiospores 3.6 - 7.1 x 1.8 - 3.6 µm in diam. ovoid but slightly angled as a result of mutual pressure in the sporangium. smooth light bluish black often adhering together when released fron the sporangium. Chlamydospores 8.6 - 15.2 x 14.9 - 23.3 µm. oval to broadly ellipsoidal. Zygospores not seen.

Notes:

The description above does not fit in any of the descriptions of *Circinella* species outlined by Hesseltine and Fennel (1955). Sporangiophores of the isolate arise from the substrate and are characteristically twisted (open spiral) like in *C. mucoroides* Saito. 1907. Centbl. f. Bakt. 11. 17: 159 (Hesseltine and Fennel. 1955 Mycologia. **47:** 193 - 212). The diameters of the sporangiophore are also similar. However. the sporangiophore branching and manner of sporangial production is different. The sporangiophore of *C. mucoroides* branches sympodially to produce either sterile spines or circinate sporangium-bearing stalks or both.

The sporangia of the above described isolate are similar to those produced by *C. rigida* Smith. 1951. (Trans. Brit. Mycol. Soc. **34:** 19. In Hesseltine and Fennell. 1955. Mycologia. **47:** 193 - 212. In shape. colour and wall type (incrusted wall). However *C. rigida* produce smaller sporangia. 20 - 72 µm in diam that are circinately borne. and globose sporangiospores unlike the above isolate that forms larger sporangia (25-) 53.3 - 75 (-89.3) that are alternately formed. and ovoid sporangiospores.

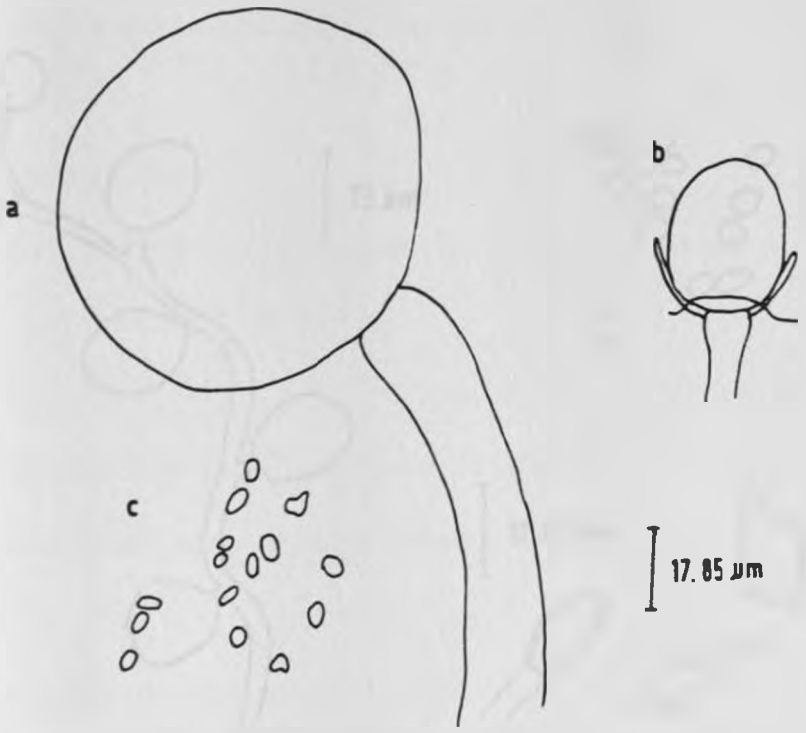

Fig. 13: *Circinella simplex*. (a) Incrusted sporangia, does not allow spores to be seen through its wall; (b) Oval columella with collar and subsporangial swelling; and (c) Sporangiospores.

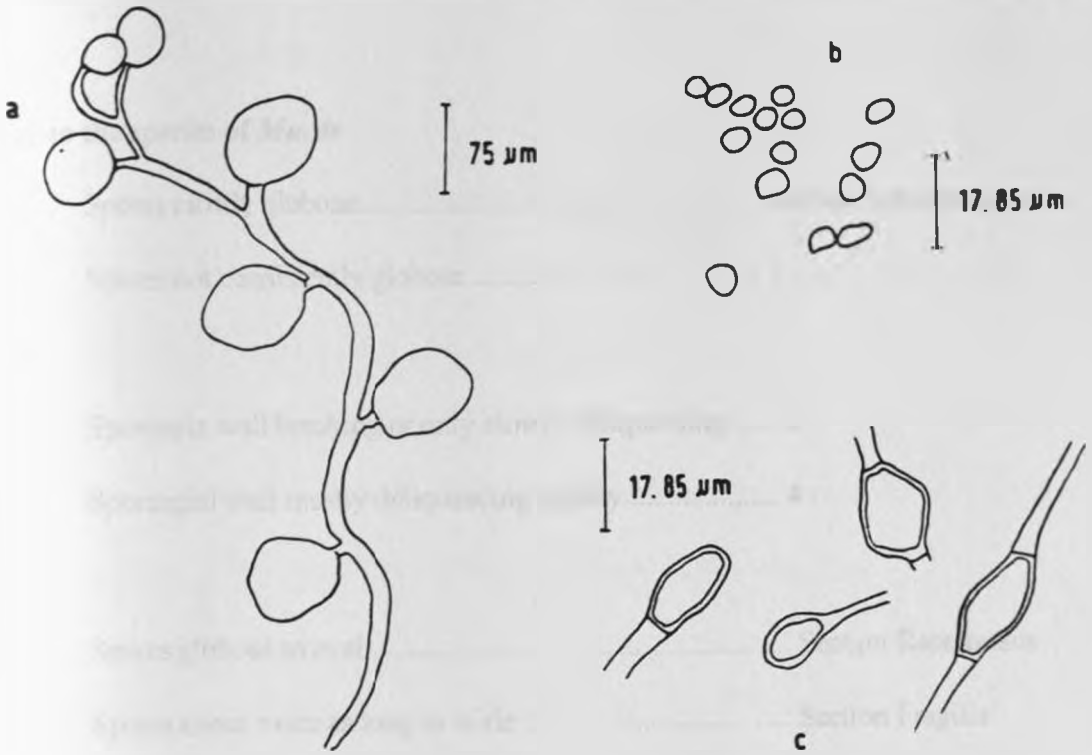

Fig. 14: *Circinella* sp. (a) Twisted sporangiophore bearing stalked sporangia produced alternately; (b) Sporangiospores; and (c) Intercallary and tip chlamydospores.

Mucor **Micheli. Nova plant. gen., p. 215. 1729. Fries, Syst. Mycol. 3: 317. 1832 non L. 1753. In O'Donnell, 1979. Zygomycetes in culture.**

Substrate mycelium much branched. aerial mycelium simple or branched. bearing either sporangia or zygospores. or both. Sporangiophore simple or branched. Sporangia terminal. globose. columellate: sporangial wall diffluent or breaking open. Columella variously shaped. non-apophysate. Homothallic or heterothallic. Zygosporangia borne between opposed suspensors.

Key to the species of *Mucor*

1. Spores mostly globose..... section Sphaerosporus

   Spores not consistently globose 2

2. Sporangia wall breaking or only slowly deliquescing 3

   Sporangial wall mostly deliquescing rapidly 4

3. Spores globose to oval ..... Section Racemosus

   Spores about twice as long as wide ..... Section Fragilis

4. Sporangia smaller than 100 µm in diam ..... Section Hiemalis

   Sporangia 100 µm or more in diam 5

5. Sporangia of two types: larger sporangia from tall

   sporangiophores: smaller sporangia from short

sporangiophores ..... Section Mucedo

Sporangia of only one type arising from high sporangiophores more or less sympodially branched ..... Section Flavus

A. Section Sphaerosporus

1. Columella usually with spines ..... *M. spinosus*

   Columella smooth 2

2. Spores larger than 8 µm, sporangiola usually present ..... *M. lamprosporus*

   Spores smaller than 8 µm, sporangiola absent ..... *M. globosus*

B. Section Racemosus

Sporangiophores unbranched or irregularly and lightly branched; globose spores up to 4 µm in diam, ovoid spores less than 8 µm long; spores smooth ..... *M. racemosus*

Sporangiophores branched, globose spores more than 4 µm in diam, ovoid spores up to 4 µm in diam, ovoid spores up to 9 µm long; spores spiny ..... *M. plumbeus*

C. Section Flavus

Sporangiophores with a sterile, sickle-shaped branch at the base.

sporangia often up to 100 µm (-130) in diam, sporangiospore

mainly globose up to 10 µm in diam ..... *M. falcatus*

D. Section Fragilis

1. Sporangia up to 53 µm in diam ..... *M. ambiguus*

   Sporangia larger than 53 µm in diam ..... *M. fragilis*

E. Section Hiemalis

1. Sporangiophores with giant-cells ..... *M. zychae*

   Sporangiophores without giant-cells 2

2. Spores long and narrow (1: 2 - 3) ..... *M. subtilissimus*

   Spores oblong (1: 1.5 - 2) 3

3. Spores rounded-off cylindrical-ellipsoidal, very uniform, up to 4 µm

   (at most 5 µm) long ..... *M. microsporus*

   Spores ellipsoidal, very variable in shape and dimensions, up to

   7 µm long ..... *M. hiemalis*

F. Section Mucedo

Sporangia echinulate. spores regularly cylindrical to ellipsoidal ..... *M. mucedo*

***Mucor ambiguus* Vuill. Etud. biol. s.l. Champ. In Saccardo P.A., 1891. Sylloge Fungorum 9: 335.**

Colonies at first white, later grey to brown. Sporangiophores strongly branched on the lower part. Spiral, above straight. Sporangia globose 43 - 53 µm in diam, columellate: sporangial wall diffluent. Sporangiospores ovate, 7 x 4 - 5 µm.

Mucor fragilis Bainier in Ann. Scienc. Natur. 1884. 6 ser. 19: 209. In Saccardo and Sydow, 1988. Sylloge Fungorum 7: 191.

Colonies Mouse Grey turning brown with age, filling Petri plates (8.5 cm) to their lids in 7 days at 25°C. Sporangiophores erect, strongly branched with smaller sporangia at the apex. Sporangia globose 54 - 68 µm in diam. Columellae obovoid. Sporangiospores ovoid 7.3 - 4.2 x 2.1 - 3.5 µm (Saccardo and Sydow 1988: 4 x 2 µm).

***Mucor globosus* Fischer In Rabenhorst Krypt. Flora 1. 202. 1892. In Boedijn, 1958. Sydowia X11: 321 - 362.**

Colonies 2 cm or more high, very pale yellowish in colour, about Cartridge Buff till Cream Buff. Sporangiophore branched 15 - 30 µm in diam (Boedijn, 1958: 7 - 33 µm). Sporangia globose, dark coloured, 6.5 - 124 µm in diam (Boedijn, 1958: 60 - 125 µm). Columella globose till oval, sub-hyaline with a low collar at the base: 53 - 58 µm in diam (Boedijn: 54 - 60) or 98 - 104 x 76 - 79 µm (Boedijn: 90 - 140 x 75 - 80 µm). Spores sub-hyaline, globose, till sub-globose 4 -6.8 µm in

diam (Boedijn. 1958: 3.5 - 7 µm). Chlamydospores intercallary in the hyphae. mostly cylindrical with granular contents. 52 - 59 x 22 - 28 µm (Boedijn. 1958: 50 - 85 x 18 - 29 µm).

***Mucor hiemalis* Wehmer. *Ann. Mycol.* 1. 39. 1903.**

Colonies soft pale grey. about pale Olivaceous Buff till pale Buff with a yellowish hue. turf up to 2 cm high. fitting the Petri plate (8.5 cm) in 5 days. Sporangiophore simple at first. afterwards more or less sympodially branched; wall finely granulated; contents yellowish oil. 9 - 17.2 µm in diam (Boedijn. 1958: 5 - 17 µm). Sporangia globose 32.1 - 35.7 µm in diam (Boedijn. 1958: 22 - 130). Sporangial wall deliquent. Columella globose to obovoid. 35 - 42 µm in diam. with a distinct collar. Sporangiospores elliptical. very variable in shape and dimensions. Snuff Brown in mass.

singly hyaline 2.8 - 6.8 x 1.5 - 2.8 µm. Chlamydospores especially in old cultures. yellowish in colour. occur single in short chains. both intercalary and terminal. globose or elongated 15 - 22 µm in diam or 12.6 - 18.2 x 9 - 12 µm; wall 1.5 - 2.0 µm thick.

Mucor lamprosporus Lendner, Mucor. Swisse p.92. 1908. In Christenberry Dec. 1940, Journal of the Mitchele Society, 333 - 366. Fig.15

Colony white turning pale Sienna brown with age. Sporangiophores bent at the tip. bearing sporangia or sporangiola. Sporangia globose. 65 - 80 µm in diam. sporangial wall with very short spines. deliquescing slowly. Sporangiola globose; sporangial wall thick and persistent. resistant to breaking but later dissolves away. light coloured and allows spores to be seen through easily. 32.8 - 34 µm in diam. Columellae subglobose. smooth. Sporangiospores globose. but slightyly angled as a result of mutual pressure in the sporangiolum. double walled. smooth. 11.25 - 15 µm in diam.

Mucor falcatus **Schipper Antonie van Leeuwenhoek, 33: 195. 1967. In Schipper, M. A. A., 1978. Stud. Mycol. 17: 1 - 52.** Fig.16

Colonies pale Smoke Grey with a slightly yellowish tinge: slightly aromatic. Sporangiophores up to 18 µm in diam. readily collapsing, once or repeatedly sympodially branched, the main stalk soon bending aside and remaining short and sterile, the first branch taking its place, either growing tall and bearing a sporangium or the branching is repeated in the same way. Sporangia yellowish to brown 72 - 110 µm in diam (Schipper, 1978: up to 100 (-130) µm), with encrusted deliquescent walls. Columellae conical or cylindrical 42 - 58 x 35 - 49 (Schipper, 1978; 60 x 55 µm), with or

without yellowish-brown contents, collarate. Sporangiospores mostly globose, a few ellipsoidal and/or deformed, 6 - 9.2 µm in diam (Schipper, 1978: 5.5 - 10 µm) with granular contents, pale Straw-yellow.

Mucor microsporus **Namyslowski Bull. int. Acad. Sci. Lett. Cracovie, ser. B. Sci. nat. p. 517. 1910. In Schipper, M. A. A., 1978. Stud. Mycol. 17: 1 - 52.** Fig.17

Colonies vinaceous Buff. Sporangiophores unbranched. Sporangia hyaline when young, later yellowish-brown 76.5 - 80.2 µm in diam (Schipper, 1978: up to 80 (-100) µm); walls diffluent. Columellae obovoid to ellipsoidal 62 - 52 µm in diam (Schipper, 1978: 70 x 55 (80 x 65) µm), with yellowish contents, collarate. Sporangiospores ellipsoidal to cylindrical 4 - 5.2 x 2.1- 2.3 µm.

***Mucor mucedo* Linn. Spec. Plant. 11, 1655. Saccardo and Sydow, 1888. Sylloge Fungorum. 7 : 191.**

Colonies Mouse Grey, rapidly growing, filling Petri plates (8.5 cm) to their lids in 7 days at 25°C. Sporangiophores of two types: tall simple, erect sporangiophores with large sporangia; smaller simple or lightly branched sporangiophores with small sporangia near the substrate. Sporangia globose, echinulate, pale yellow to ash grey, 36 - 172.4 µm in diam. Columella ovoid, pale yellow to brownish grey). Sporangiospores regularly cylindrical to ellipsoidal, 6 - 11 x 3 - 4 µm (Saccardo and Sydow, 1988 also ovoid-ellipsoid, 6 - 9 x 3 - 4 µm).

***Mucor subtilissimus* Oudemans Contrib. FL. Mycol. Pays-Bas XVI, p. 15. In Saccardo and Sydow, 1902. Sylloge Fungorum 16: 385.** Fig.18

Colonies pale grey to pale Olivaceous Buff, mycelia with oil globules deep golden in colour. Sporangia globose, hyaline, 40.8 - 48 µm (Saccardo and Sydow, 1902 : 40 - 45 µm); sporangial wall not persistent. Columellae globose, hyaline, 26 - 33 µm in diam (Saccardo and Sydow, 1902 : 25 - 35 µm), sporangiospores elliptical to cylindrical, hyaline 6.8 - 7 x 2.8 - 3.1 µm (Saccardo and Sydow, 1902 : 7 x 3).

***Mucor plumbeus* Bon. Abhandle. aus dem Gebiete Mykol. 11, p. 109, Rivolta Parass. veg. p. 494, f. 233. In Saccardo P.A. and Sydow, 1888. Sylloge Fungorum. 7: 191.** Fig.19

Colonies dull white turning grey, showing mass of black headed sporangia. Sporangiophores erect, branched, rarely simple. Sporangia globose, dark grey to black, with spines, 30.0 - 52.5 µm in diam. Columellae oblong to obovate, apex often with blunt projections, collarate dirty brown. Sporangiospores globose 4.282 - 7.140 to oval 5.355 - 6.426 x 6.783 - 8.925, dark grey to black, spiny. Chlamydospores sometimes present.

Mucor racemosus **Fres. Beitr. Mykol. p. 12. 1850. Scroet, Krypt. Flor. Schles. Pilze. p. 204. In Saccardo and Sydow, 1988. Sylloge Fungorum 7: 192.** Fig.20

Colonies white turning yellow-brown. Aerial mycelium absent after 4 weeks on PDA. Sporangiophores unbranched or irregularly and lightly branched (Saccardo and Sydow, 1988: 0.4 - 0.5 cm tall). Sporangia globose, 40.5 - 52.2 µm in diam, yellowish to light orange (Saccardo and Sydow, 1988: 40 - 66 µm). Columellae oval, collarate, sporangiospores globose, 3.60 - 3.96, to ovoid 3.60 - 3.96 x 3.57 - 6.783 (Saccardo and Sydow, 1988: 5.8 x 5 µm), hyaline, smooth.

Mucor zychae **Baijal and B. S. Mehrotra, 1965. Sydowia 19:204. In Schipper, M. A. A., 1978. Stud. Mycol 17: 1 - 52.** Fig.21

Colonies Grey Olivaceous, zonate: substrate hyphae with swollen regions and globose giant-cells 62 - 68 µm in diam, filled with droplets. Sporangiophores up to 7 µm in daim at first erect but soon drooping, unbranched. Sporangia hyaline with a yellowish tinge 56 - 59 µm in diam (Schipper, 1978: up to 60 (-75), with slightly encrusted, transparent deliquescent walls. COlumellae subglobose to conical, 27.1 - 28 x 18.9 - 20.2 µm (Schipper, 1978: 28 x 21 (32 x 36) µm). Sporangiospores ellipsoidal 18 - 21.5 x 11 - 12 µm (Schipper, 1978: (9.5) 13.5 - 23 x (7.2-) 10.1 - 13.5 µm) with granular contents, pale yellowish-grey.

Mucor spinosus **van Tiegh. Mem. sur le Muc. p. 391. In Ann. des Scienc. Nat. 1876, Schroeter Krypt. Flor. Schles. Pilze. p. 205. In Saccardo and Sydow, 1988. Sylloge Fungorum 7: 191.**

Colonies dull white. Sporangiophores irregularly branched (Saccardo and Sydow : 0.5 - 1 cm tall).

Sporangia globose. wall spiny. yellowish-brown to dirty brown. globose 56.5 - 57.2 µm in diam. Collumelae ovoid with up to 11 spines at the apex (Saccardo and Sydow 1988: 10 - 12 spines). Sporangiospores globose. deep brown. spiny. 6 - 8 um in diam (Saccardo and Sydow. 1988 : 4 - 9 µm in diam).

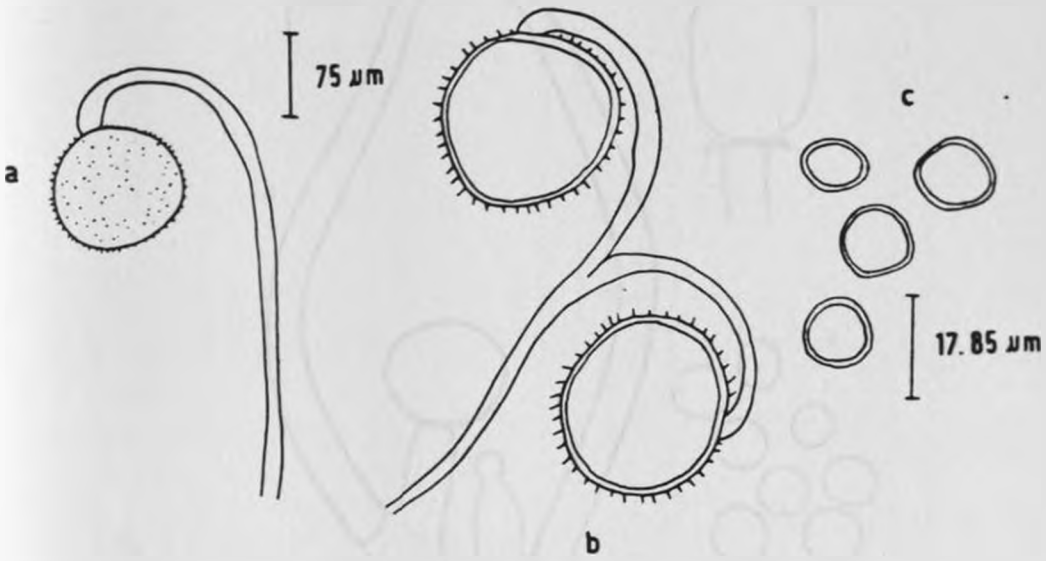

Fig. 15: *Mucor lamprosporus*. (a) Drooping sporangium; (b) Portion of sporangiophore to show mode of branching and sporangiolum; and (c) Spores.

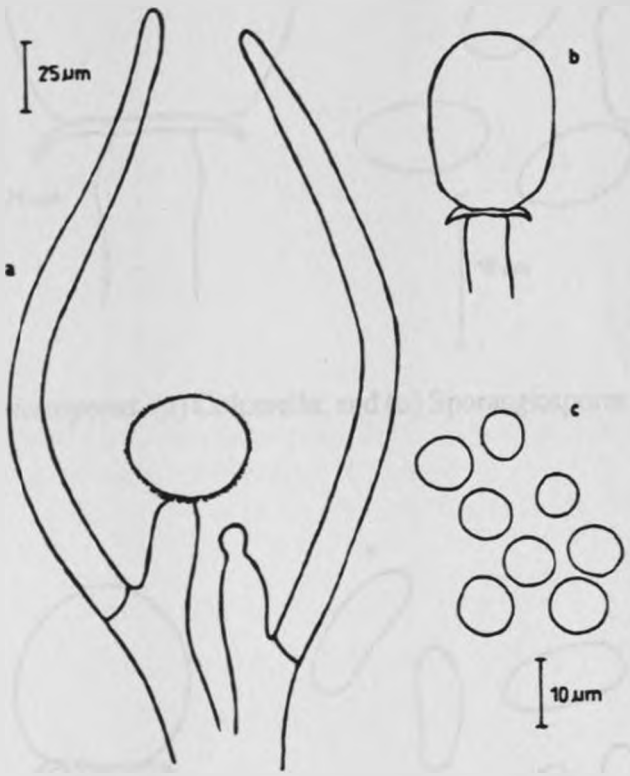

Fig. 16: *Mucor falcatus*. (a) Young sporangiophores; (b) Columella; and (c) Sporangiospores.

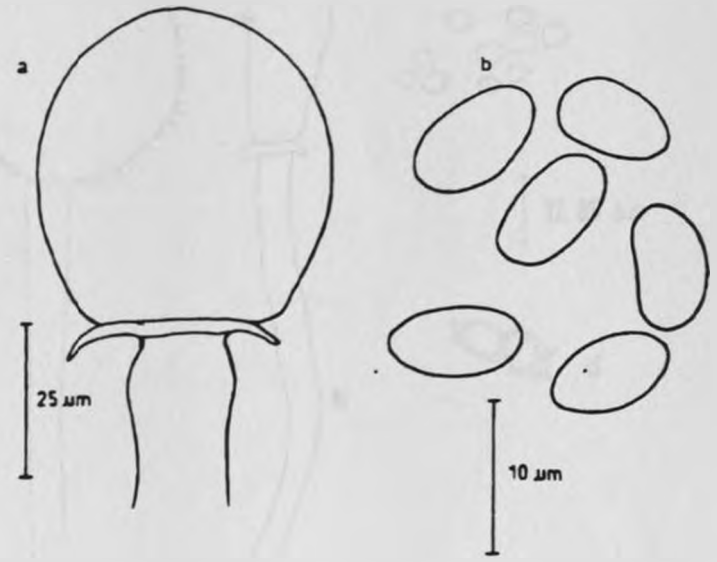

Fig 17: *Mucor microsporus*. (a) Columella, and (b) Sporangiospores

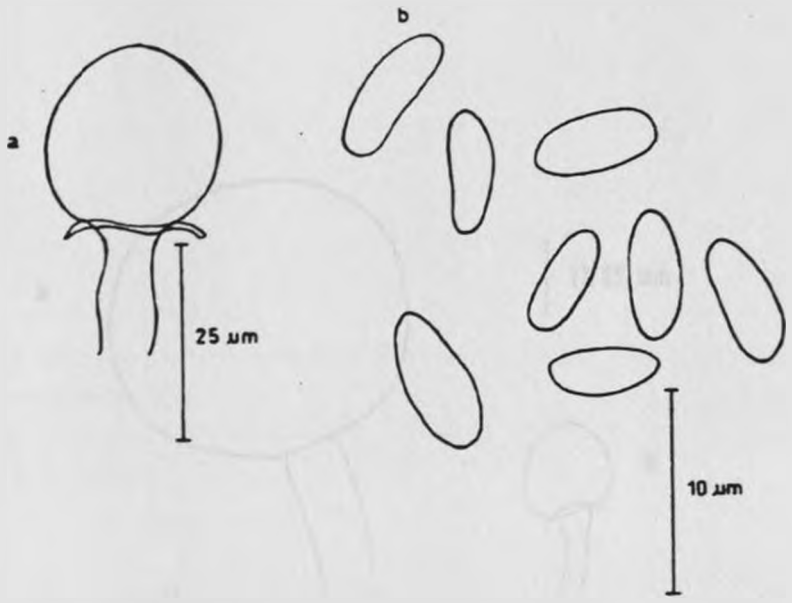

Fig 18: *Mucor subtilissimus*. (a) Columella, and (b) Sporangiospores

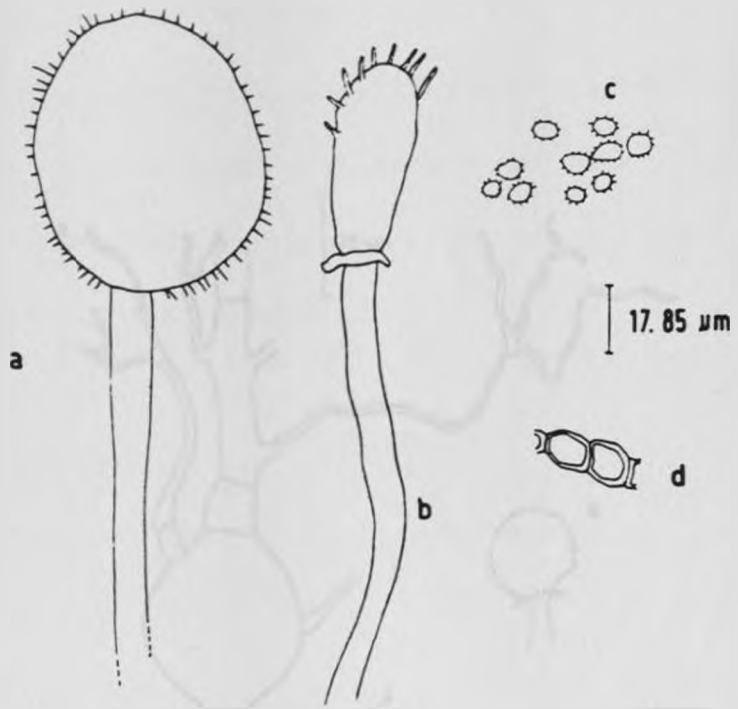

Fig. 19: *Mucor plumbeus*. (a) Spiny sporangium; (b) Columella showing blunt projections; (c) Echinulate sporangiospores; and (d) Chlamydospores.

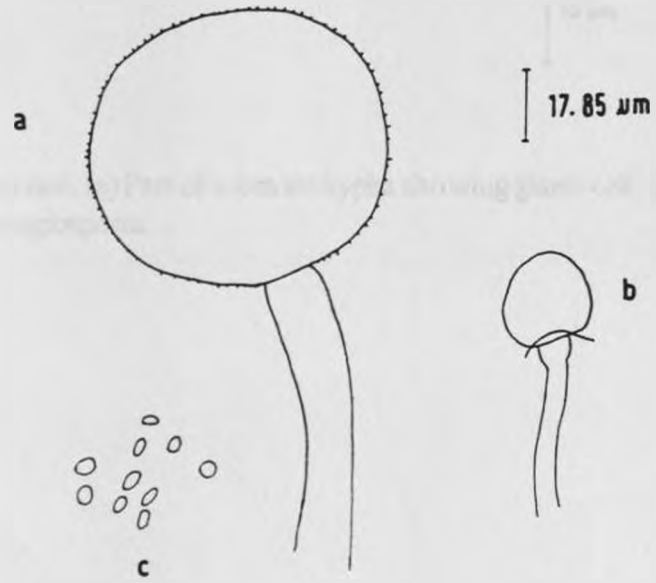

Fig. 20: *Mucor racemosus*. (a) Spiny sporangium; (b) Columellae showing collar and subsporangial swelling; and (c) Sporangiospores.

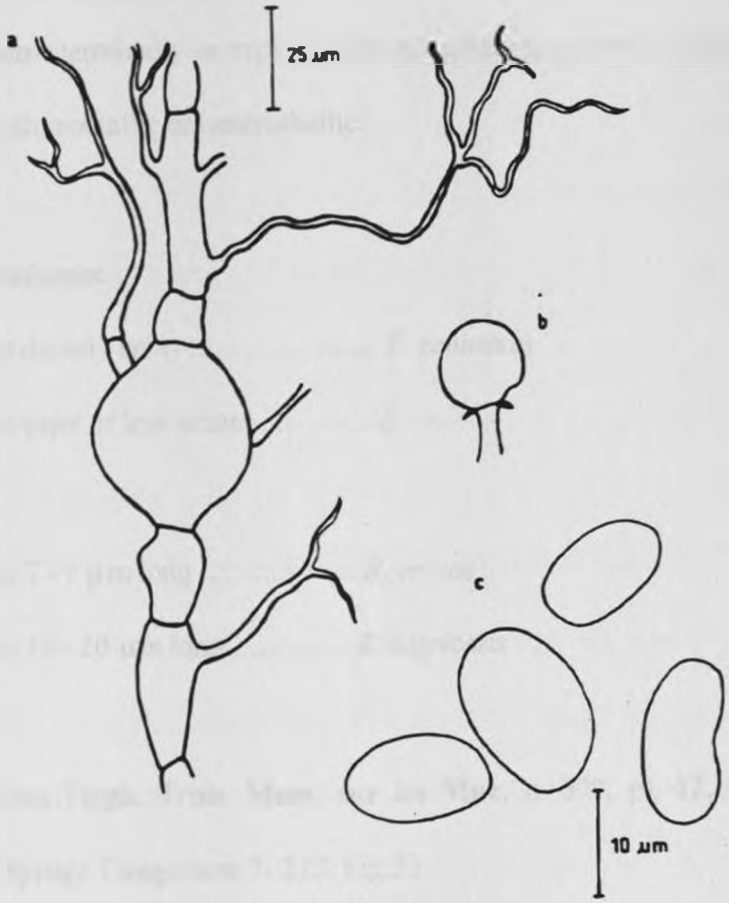

Fig. 21: *Mucor zychae*. (a) Part of substrate hypha showing giant- cell; (b) Columella; and (c) Sporangiospores.

Rhizopus **Ehrenberg, Nova Acta. Acad. Leop. 10, 1: 198. 1820. In Christenberry, 1940. *Journ. of the Mitchell Society*, December 1940 : 333 - 366.**

Aerial mycelium forming arching stolons which bear at each node a fascicle of sporangiophores attached by rhizoids to the substratum. Sporangiophores apophysate. Sporanga globose to sub-globose, columellate, borne terminally or typically simple sporangiophores. Columella collapsing, bearing no apical spine. Homothallic or heterothallic.

Key to the species of *Rhizopus*.

1. Sporangiospores densely spiny ..... *R echinatus*

   Sporangiospores more or less striate 2

2. Sporangiospores 7 - 9 µm long ..... *R. oryzae*

   Sporangiospores 10 - 20 µm long ..... *R. nigricans*

Rhizopus echinatus **Van Tiegh. Trois Mem. sur les Muc. p. 370, pl. 12, fig. 64 - 68. In Saccardo P.A., 1888. Sylloge Fungorum 7: 213.** Fig.22

Colony dull white fast growing filling Petri plates (8.5 cm) to their lids in 3 days. Sporangiophore erect, simple, in fascicles of 3 - 4. Sporangia globose 30 - 62 µm in diam, apophysate, verrucose. Sporangiospores globose with dense spines (Saccardo, 1888 : 15 µm).

***Rhizopus nigricans* Ehrenberg De Mycetogenesi in Nova Acta X, p. 198. tab. II, *Mucor stolonifer* Ehrenb. Sylvae Mycol. 13, p. 25, Scroeter Krypt. Flor. Schles Pilze P. 206, Ascophora mucedo Tode Fung. Mecklemb. fasc. 11, Bon. G. der Mykol. pag. 44, a Rhyzopus Ehr., A. cordana Bon. p. 44, A. coenansi Bon. l.c. p. 44. In Saccardo P.A., 1888. Sylloge Fungorum 7 : 213.** Fig.23

Colony dull white, fast growing filling Petri plates (8.5 cm) to their lids in 3 days. Sporangiophores erect, simple, 3 - 10 fascicle, rarely solitary, non-septate, base of fascicle provided with rhizoids connected by long creeping stolons. Sporangia globose, Olivaceous-black, granular, 120 - 245 µm. Columella semi-globose, sporangiospores globose to elliptical, 14 - 16 x 9 - 11 µm, grey.

***Rhizopus oryzae* Went, Beobacht. Hefeart. etc der Arackfabr. p. 16. In Saccardo P.A., 1895. Sylloge Fungorum 11: 240.** Fig.24

Colonies pale grey in colour fast growing filling Petri plates (8.5 cm) to their lids in 3 days, dotted by the Olivaceous Grey sporangia. Sporangiophores sometimes single but mostly in fascicles of 2 - 6, usually 2 - 3, often sprouting from a short trunk-like base at the end of which rhizoids originate; sub-hyaline to brownish. Occasionally brown coloured vesicles 23 - 32 µm in diam are formed in the hyphae, from those vesicles 2 or more sporangiophores arise. Sporangia globose, black, 125 - 240 µm (Saccardo, 1895 : 17 - 240 µm). Columella sub-hyaline to pale brown semi-globose, globose to oval with a broad apophyse collarate. Spores globose, sub-globose to oval, grey, striate, 3.57 - 7.14 x 3.927 - 8.925 µm.

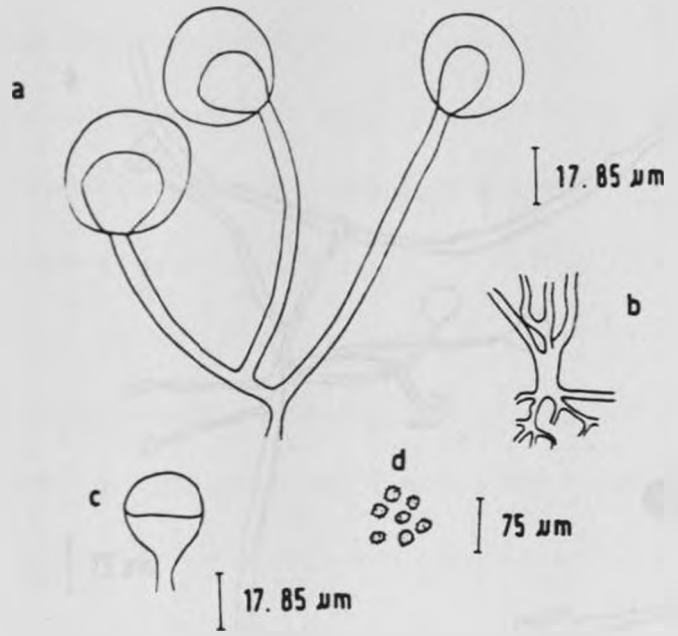

Fig. 22: *Rhizopus echinatus*. (a) Fascicle of sporangia; (b) Base of such a fascicle; (c) Apophysis and columella; and (d) Spiny sporangiospores.

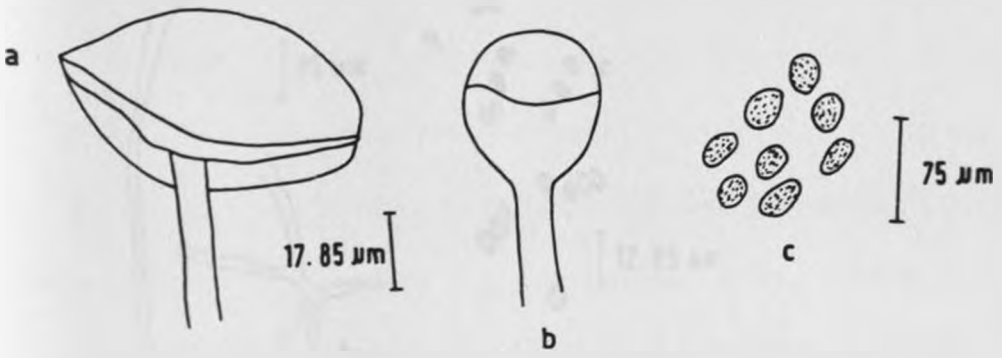

Fig. 23: *Rhizopus nigricans* (a) Columella showing typical collapsed condition; (b) Columella showing apophysis after shedding spores; and (c) Striate sporangiospores.

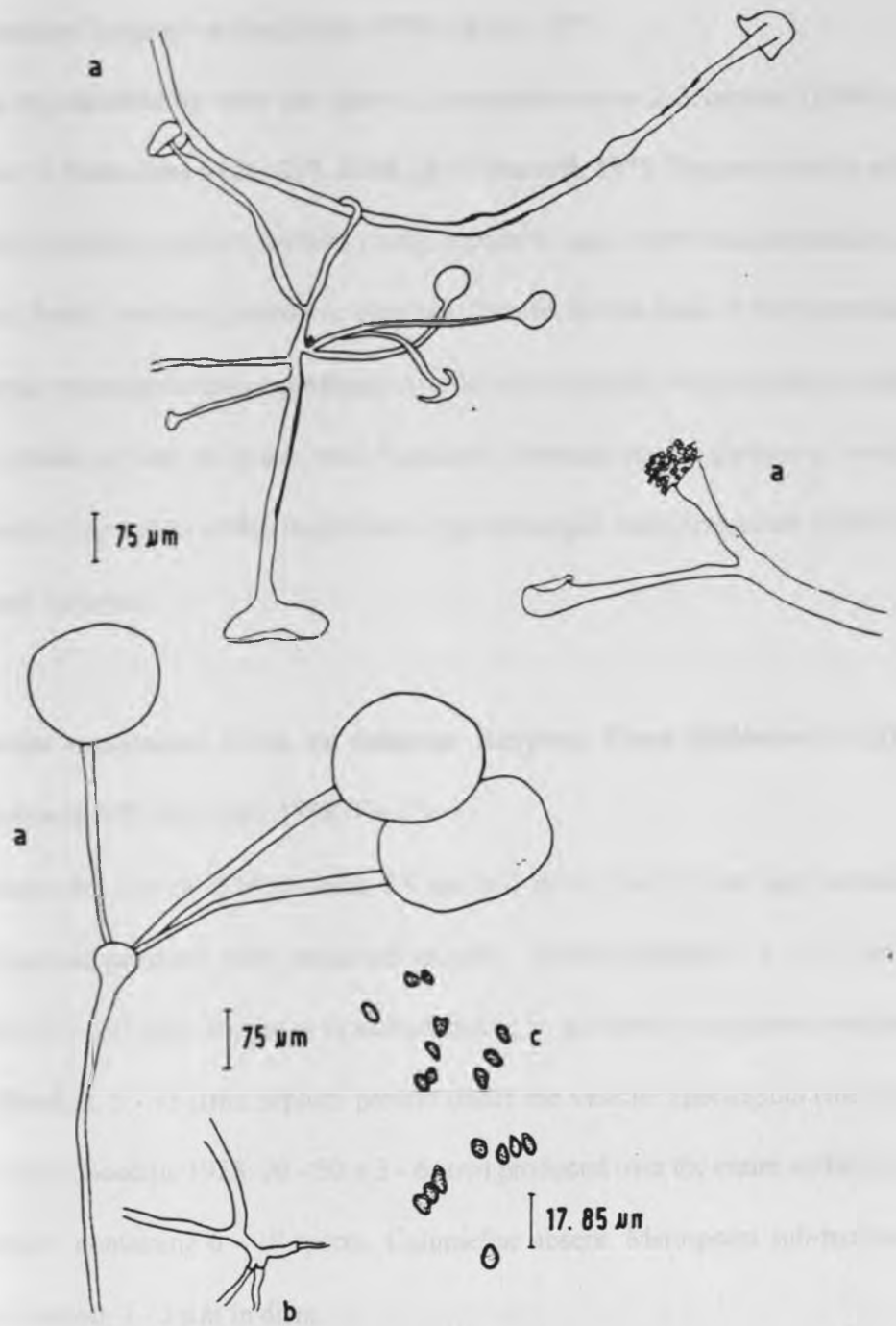

Fig. 24: *Rhizopus oryzae*. (a) Sporangiophore habit: different modes of sporangiophore branching and vesicles (bulbous structures) formed along parts of hyphae; Collapsed columellae; (b) Base fascicle; and (c) Striate sporangiospores.

Syncephalastraceae Naumov ex Benjamin, 1959 Aliso 4 : 327.

This family is represented by only one genus, *Syncephalastrum* J. Schroter. (1886) in Cohn, Kryptog. Flora V. Schlesiens 3 (2) : 217. 1886. In O'Donnell, 1979. Zygomycetes in culture.

Somatic hyphae branched, coenocytic when young, septate in age, stolon-like, adventitious rhizoids often produced. Fertile vesicles globose or obovoid, formed on the apex of the sporophore or its branches; bearing sporangiola (mesosporangia) over its entire surface. Merosporangia acolumellate containing a variable number of spores; wall fugacious. Sporangiospores globose to ovoid usually borne uniseriately. Zygospores with a rough, dark zygosporangial wall, suspensors slightly unequal, non-appendaged, opposed.

***Syncephalastrum racemosum* Cohn ex Schroter Kryptog. Flora Schlesiens 3.1.217. 1886. Boedijn in Sydowia XII: 321 - 362. 1958.** Fig.25

Colonies Olivaceous Grey on PDA growing 6.9 cm in 7 days. Turf 1.2 cm high, mycelium non-septate with stolons provided with branched rhizoids. Sporangiophores 10 - 25 um in diam (Boedijn, 1958: 1.5 - 30 µm), simple or branched ending in globose or subglobose vesicles 12 -35 um in diam (Boedijn, 5 - 75 µm); septum present under the vesicle. Sporangiola (merosporangia) 20 - 35 x 4 - 6 µm (Boedijn, 1958: 20 - 50 x 3 - 6 µm) produced over the entire surface of terminal vesicle, uniseriate, containing 6 - 10 spores. Columellae absent. Merospores sub-hyaline, dark in mass, globose, smooth 3 - 5 µm in diam.

Thamnidiaceae Brefeld. Benny and Benjamin, 1975. Aliso 8 No. 3, 301 - 351.

Sporophore erect or ascending, rarely repent, simple or branched, arising directly from the substrate mycelium or from stolons; producing large terminal, columellate, multispored sporangia having a deliquescent wall, or sporangia absent; always producing pedicellate, uni- or mulitspored sporangiola having a persistent but separable wall. Sporangia and sporangiola apophysate or non-apophysate. Sporangiospores thin-walled, smooth as viewed with the light microscope. Zygospores roughened, usually dark colored, borne between opposed, equal or unequal suspensors that lack appendages.

Key to the genera of Thamnidiaceae.

1. Some sporangiophores terminating in a sterile spine-like projections 2

   No sporangiophores terminating in a sterile spine-like projection 2

2. Sporangiola borne laterally either singly or in whorls on a fertile vesicle,

   vesicle usually terminating in a sterile spine-like extension 3

   Sporangiola pedicellate, borne laterally on dichotomous branches 3

3. Zygosporangia borne between opposed or tong-shaped suspensors ..... *Helicostylum*

   Zygosporangia borne between opposed suspensor ..... *Thamnidium*

Helicostylum **Corda, Icon. Fung. 5: 55. 1842. In O'Donnell, 1979. Zygomycetes in culture.**

= Chaetostylum van Tieghem and Le Monnier, *Ann. Sci. Nat. Bot.*, Ser. 5, 17 : 328. 1873 [fide hythgoe, 1958].

Sporangia and sporangiola borne on same sporangiophore: sporangia globose to sub-globose, columellate, borne terminally on simple or branched sporangiophores, some sporangiophores terminating in sterile spine-like projections; sporangiophores non-apophysate; sporangiola few-spored, globose to sub-globose, columellate, borne laterally either singly or in whorls on a fertile vesicle, vesicle usually terminating in a sterile spine-like extension; heterothallic, where known; zygosporangia borne between opposed or tong-shaped suspensors.

Helicostylum elegans **Corda, Icones Fung. 5 : 18, 55. 1842. In Upadhyay, 1973 Mycologia 65: 733 - 751.** Fig.26

Colonies colorless at first, becoming pale Grey-Olivaceous, with a fruity odor, margin irregular. Sporangiophores arising from substrate mycelium, hyaline, simple or branched, erect 20.9 - 25 µm in diam (Upadhyay, 1973 : (10-) 12 - 18 (-30) µm), ending in a large sporangium (Upadhyay, 1973: rarely in a sterile spine in large sporangiophores), constricted below the sporangium, terminal sporangia always formed in advanced of sporangiola, globose, brown, multispored, diffluent. Columellae pyriform or ovoid, 42 - 44.8 µm in diam (Upadhyay, 1973 : 45 - 90 x 40 - 80 µm); smaller ones up to 35 µm in diam), hyaline, smooth with or without a basal collar; branches bearing sporangiola and stiff sterile spines arising singly or in whorls from the sporangiophore; primary branches simple, ending in a sporangiole (Upadhyay, 1973 : 30 - 130 (-260) x 2.4 - 5.6 µm), or racemosely or verticillately branched, the branchlets variable in length (Upadhyay, 1973 : 3.8 - 7.6 µm in diam); secondary branches simple. Sporangiola globose 16.2 - 18.1 µm in diam

(Upadhyay. 1973 : (18-) 11 - 17 (-24) µm) with a sub-globose columella 6.5 - 8.2 µm in diam. sporangiola wall persistent. Sporangiospores from terminal sporangia and sporangiola alike. ellipsoidal. hyaline. smooth. 9 - 11.4 µm x 3.6 - 4.2 µm (Upadhyay. 1973: 6.5 - 12 (-14) x 3.5 - 7.8 (-9) µm).

Thamnidium **Link ex Gray (1821) Benny G.L., 1992. Mycologia, 84 (b): pp 834 - 842.**

The genus is restricted to a single taxon. *T. elegans* that produces deliquescent-walled sporangia at the apex of a primary sporophore or its branches along with persistent-walled sporangiola on lateral dichotomous branchlets. Sometimes only dichotomous branchlets arise singly. in pairs. or in verticels of three or more.

Thamnidium elegans **Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gestammten Naturk. 3 : 31. 1809. In Benny G.L, 1992. Mycologia, 84 (6) : 834 - 842.** Fig.27

Colonies on PDA 6.4 cm in diam in 7 days at 25°C. turf sparse. colourless; fruiting heads grey. Sporangiophores erect. 12 - 15 µm in diam (Benny. 1992: 10 - 20 µm). arising directly from the substrate mycelium. constricted below sporangium. coenocytic when young. irregularly septate at maturity. hyaline to light yellow. simple or sympodially branched. of three types: (1) main axis and each primary branch usually terminating in a sporangium and producing one or more lateral dichotomous branchlets which bear terminal sporangiola. (2) only sporangia formed. or (3) only fertile branchlets bearing sporangiola produced. Sporangia globose to subglose 50 - 54.5 µm (Benny. 1992: 38 - 103 µm) in diam. hyaline to light brown: wall deliquescent: columellae obovoid to globose 29.8 - 32.1 µm in diam at the widest part (Benny. 1992: 29 - 50 x 28 - 51 µm). basal collar present. Lateral fertile branchlets arising from the sporangiophore singly. in pairs. or in

verticles of three or more. Branchlets three-dimensionally dichotomously branched, length and diam of the branches diminishing with each successive dichotomy. Sporangiola globose to sub-globose 17.2 - 19.1 µm in diam (Benny, 1992: 10 - 32 µm), few-spored (Benny, 1992. 1 - 12 or more spores) wall persistent, smooth, hyaline to light brown, deciduous: columellae globose, 5.2 - 7.6 µm in diam (Benny, 1992: 1 - 12.5 µm), smooth. Sporangiospores ovoid to ellipsoid, 5.4 - 7.8 x 8.9 - 13.4 µm (Benny, 1992: 5 - 8.5 x 6.5 - 14 µm) hyaline to light yellow.

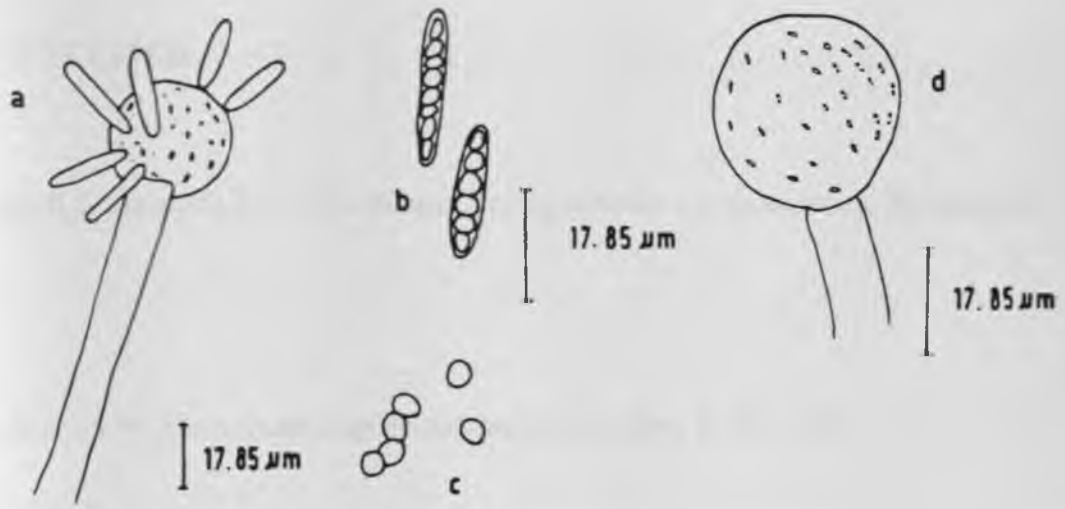

Fig. 25: *Syncephalastrum racemosum*. (a) Fertile vesicle bearing cylindric merosporangia; (b) Merosporangia containing merospores; (c) Merospores singles and in chain; and (d) Fertile vesicle with scars.

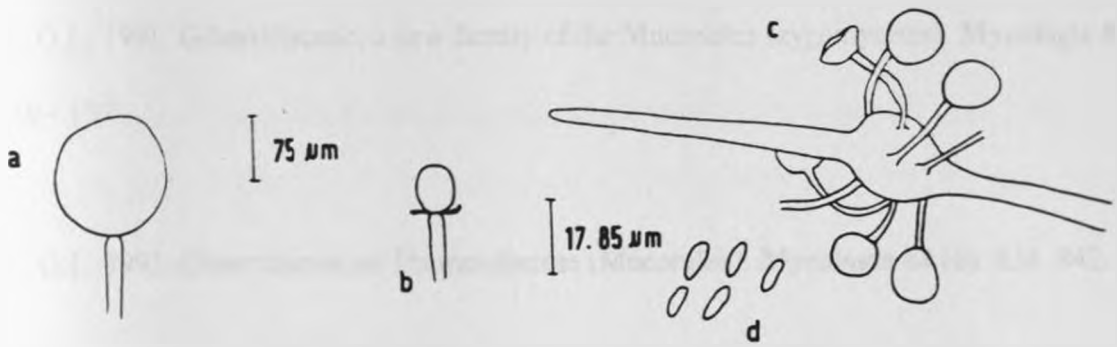

Fig. 26: *Helicostylum elegans*. (a) Terminal sporangium with incrusted wall; (b) Obovoid columella of sporangium; (c) Fertile vesicle with whorl of pedicellate sporangiola. Also showing sporangiola columella and sterile spine at the end of the branch; and (d) Spores.

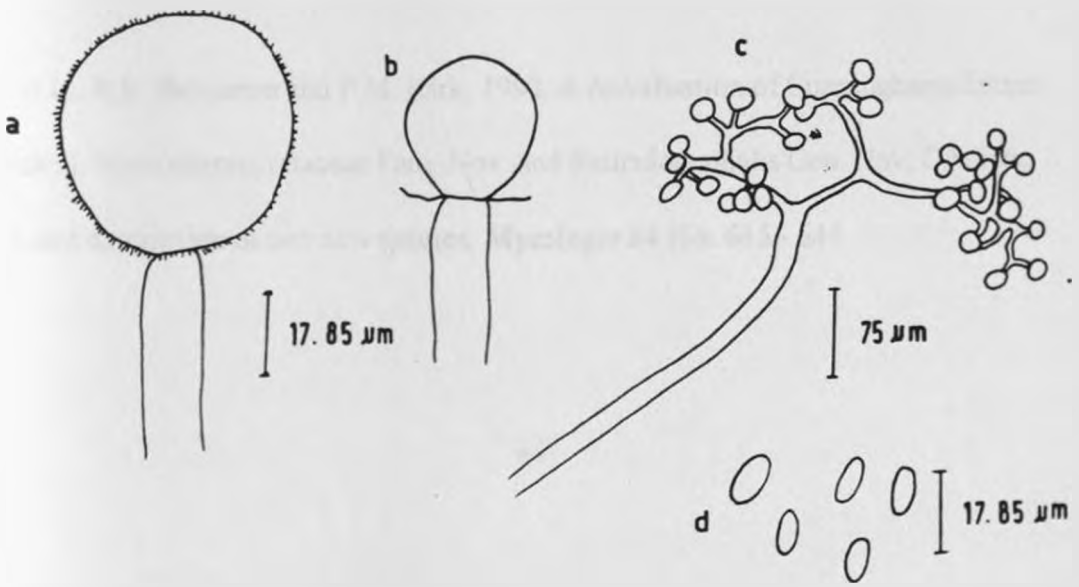

Fig. 27: *Thamnidium elegans*. (a) Terminal sporangium; (b) A columella from a sporangium; (c) Dichotomous sporangiolar branch; and (d) Sporangiospores from a sporangium.

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CHAPTER 3

THE DISTRIBUTION OF MUCORALES IN SOME PARTS OF KENYA

ABSTRACT

The study was based on the hypothesis that patterns of mucorales in the soil are directly influenced by plant communities and human activities. To test this hypothesis, the distribution and occurrence of mucorales in forest, grassland, wooded grassland, and shrubland were investigated. Top soil and litter were aseptically collected from these sample sites. Mucoraceous fungi were recovered from the soil using soil plate and dilution methods. Sampling was done monthly for three years.

Thirty three species belonging to the genera *Mucor, Gilbertella, Absidia, Circinella, Rhizopus, Cunninghamella, Helicostylum, Mortierella, Syncephalastrum, Actinomucor, Thamnidium and Choanephora* were isolated from the forest habitats. No mucoraceous fungi were isolated from the wooded grassland and shrubland. From the grasslands, these fungi were isolated only four times. *Mucor* species were predominant and the most frequently isolated species was *Mucor plumbeus*. The total population per species of the mucoraceous fungi in different vegetational units varied significantly at 99.9% level of probability with an F-value of 8.3462. The natural forest had the highest species diversity compared to plantation forests of *Cupressus lusitanica, Araucaria araucana and Eucalyptus paniculata*. However, some species e.g. *Mucor plumbeus, M. hiemalis and M. racemosus* were found in all the forest habitats while others such as *Cunninghamella echinulata, C. bainieri, C.elegans, Absidia cylindrospora, A. spinosus, A. zychae, Choanephora cucurbitarum* were limited to certain vegetational units.

INTRODUCTION

The distribution patterns of microfungi in litter and soil has been investigated for many years, but most of the work has been done with a major bias towards arctic and temperate ecosystems. Qualitative and quantitative variation in species composition in relation to aerial vegetation has been reported (Christensen, 1989; Clarke and Christensen, 1981; Christensen and Whittingham, 1965; Novak and Whittingham, 1968). According to these reports, vegetation determines the litter type, which forms the substrate for the soil fungi. Subsequently, the substrate type determines the composition of a specific fungal flora (Baath, 1981; Kjoller and Struwe, 1982; Ranzoni, 1968). Soil depth has also been reported to influence the occurence of fungi in the soil. The frequency of occurence of most taxa is reported to be inversely related to soil depth (Bissett and Parkinson, 1979a; Bissett and Parkinson, 1979b; Christensen *et al.*, 1962; Stenton, 1953; Warcup, 1951). The influence of seasons have also been noted as causing a shift in fungal community composition from one extreme in winter to another in summer (Bissett and Parkinson, 1979a; Bissett and Parkinson, 1979c; Widden, 1986; Widden and Parkinson, 1973). The available data, however, are too few to provide a reliable and world-wide mapping of these microfungi. The fungal community as a whole is thought to exhibiting two categories of characteristics:

(a) geographical distributions of the constituent species, habitat specificity, species diversity in the community, community structure and mechanisms involved in species replacements (Frankland, 1981; Griffin, 1992).

(b) behavioural characteristics. These features relate to the ecosystem function hence the significance and need for such data in the planning and management of ecosystems. It is in view of these facts, that this survey was carried out.

OBJECTIVE

To study the pattern of distribution of mucoraceous fungi in relation to vegetation types.

MATERIALS AND METHODS

Description of study areas

Figure 28 shows the vegetation zones of Kenya. The sample sites within these zones are:

(a) Karura forest

Sample sites are as described in Chapter 1.

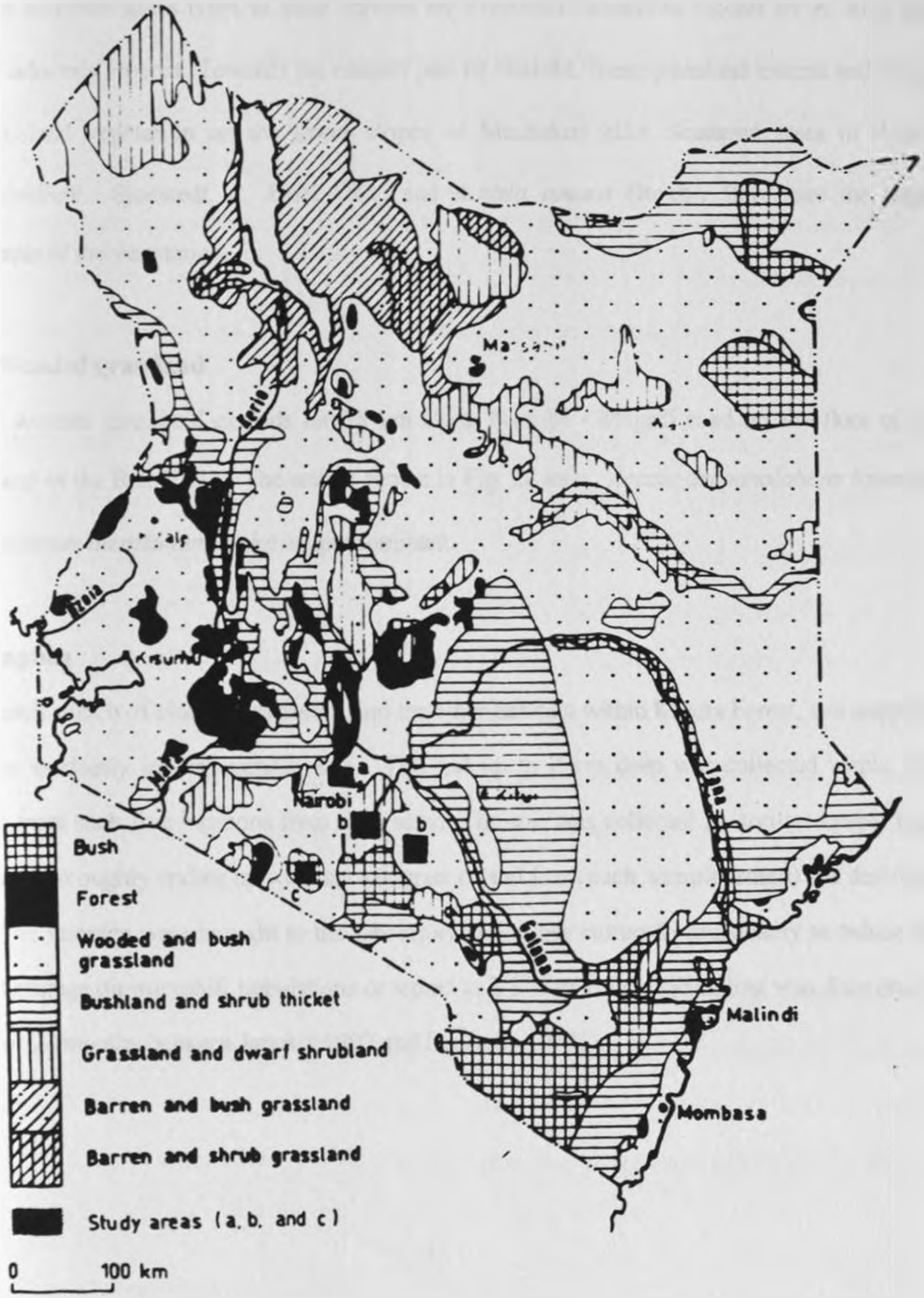

Fig 28 Vegetation zones of Kenya Forest and Grassland study sites

(b) Grassland and Shrubland

The representative study area for the grassland ecosystem was Athi River plains region depicted in Fig.28 as b. The grassland covers some 1, 163.63 hectares to the south and south-east of Nairobi. The most common grass types in these regions are *Eragrostis tenuifolia* Hochst ex A. Rich. and *Aristida adoensis* Hochst. Towards the eastern part of Nairobi, these grassland extend and merge into shrubland vegetation on the lower slopes of Machakos hills. Scattered trees of *Acacia drepanoloblium* Sjoestedt, *A. kirkii* Oliv. and *Albizia amara* (Roxb.) Boiv. are the major components of the vegetation.

(c) Wooded grassland

Kisamis wooded grassland extends for 28 km along Nairobi - Magadi road on the floor of the eastern arm of the Rift Valley. The area is shown in Fig. 28 as c. *Acacia drepanolobium* Sjoestedt and *Pennisetum menzianum* Leeke are predominant.

Soil Sampling

Within each stretch of plant communities and the other habitats within Karura Forest, soil sampling was done randomly in homogenous areas. Top soil up to 2 cm deep was collected within $1m^2$ quadrats from such five locations from each stand. The soil was collected in sterilized paper bags and mixed thoroughly ending up with one kilogram of soil from each sample (stand) site described above. The samples were brought to the laboratory and either cultured immediately to reduce the effect of storage on microbial populations or stored at 2-3°C overnight. Sampling was done once a month for 28 months between January 1992 and December 1994.

Estimation of Mucorales Populations

The fungi were recovered from the soil by the Warcup's soil plate method (Warcup, 1950) and the Dilution plate techniques (Chesters and Thornton, 1956). The dilution levels used were 1:50, 1:5,000 and 1:10,000. Five grams of soil was added to 250 ml to give an initial dilution of 1:50. This primary suspension was shaken end-over-end in a rotary shaker for five minutes. The suspension was allowed to stand for thirty seconds before 1 ml portions were transferred to 99 ml sterile distilled water to give and approximate dilution of 1:5,000. This was shaken for 1 minute by hand and two ml portions removed. 1 ml was placed in a sterile Petri dish, the other was added to 2 ml of sterile distilled water to give a final dilution of 1:10,000. The final dilution aliquot was shaken by hand for one minute and a 1 ml sample transferred to a sterile Petri dish.

Previously melted and cooled agar medium (15-20 ml) was poured into each Petri dish and the contents thoroughly mixed by gentle rotation of the dish on the bench. The following media were used: Czapek's (Dox) Agar (CZ), Malt Extract Agar(MA), Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), (Smith and Onions, 1983) The antibiotic Rose Bengal was added to the media at isolation stages. Pure cultures for identification were, grown on antibiotic-free media. Duplicates were sent to the International Mycological Institute, United Kingdom, for proof identification. The two methods of isolation and the range of media, were used in the expectation that they would reduce the bias introduced by any single technique or medium.

Inoculated plates were incubated at room temperature and observed daily for fungal growth. The average number of colonies per dish was multiplied by the dilution factor to obtain the number of propagules per gram in the original soil sample. These absolute counts provide data on species abundance and were treated with ANOVA statistical test. The Warcup soil plates and the dilution

plates. both. provided data that were used to calculate the frequency of isolation of each species using the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of times isolated}}{\text{Total number of isolations performed}} \times 100$$

Mucoraceous fungal communities associated with different microhabitats (vegetation types) were compared using the coefficient of community (CC), based solely on presence or absence of species (Gauch, 1982; Mueller-Dombois, 1974):

$$\text{Coefficient of Community (CC)} = \frac{2c}{a + b}$$

a = total number of species in the first community being considered

b = total number of species in the second community and

c = number of species common to both communities.

The value of CC ranges from 0 (when no species are present in both communities to 1 (when all species are present in both communities).

RESULTS

Mucoraceous fungi belonging to the genera *Absidia*, *Actinomucor*, *Choanephora*, *Circinella*, *Gilbertella*, *Helicostylum*, *Mortierella*, *Mucor*, *Rhizopus*, *Syncephalastrum* and *Thamnidium* were recovered from various habitats within Karura Forest. Figure 29 shows the frequency of isolation of these fungi. Members of the genus *Mucor* appeared with the highest frequency and in all sample sites. *Cunninghamella* sp. also appeared with a high frequency only in *Eucalyptus*, *Cupressus*, homestead and roadside stands. The same pattern was shown by *Absidia* sp. which

was prevalent in the natural forest. *Eucalyptus*, *Cupressus* and homestead stands and *Choanephora* sp. in the homestead stand only. The remaining genera appeared with very low frequency (less than 50%). No member of the order Mucorales was recovered from the wooded grassland and shrubland soils. From the grassland soils, *Mucor plumbeus* was isolated only once, while *Mortierella* sp. were recovered three times.

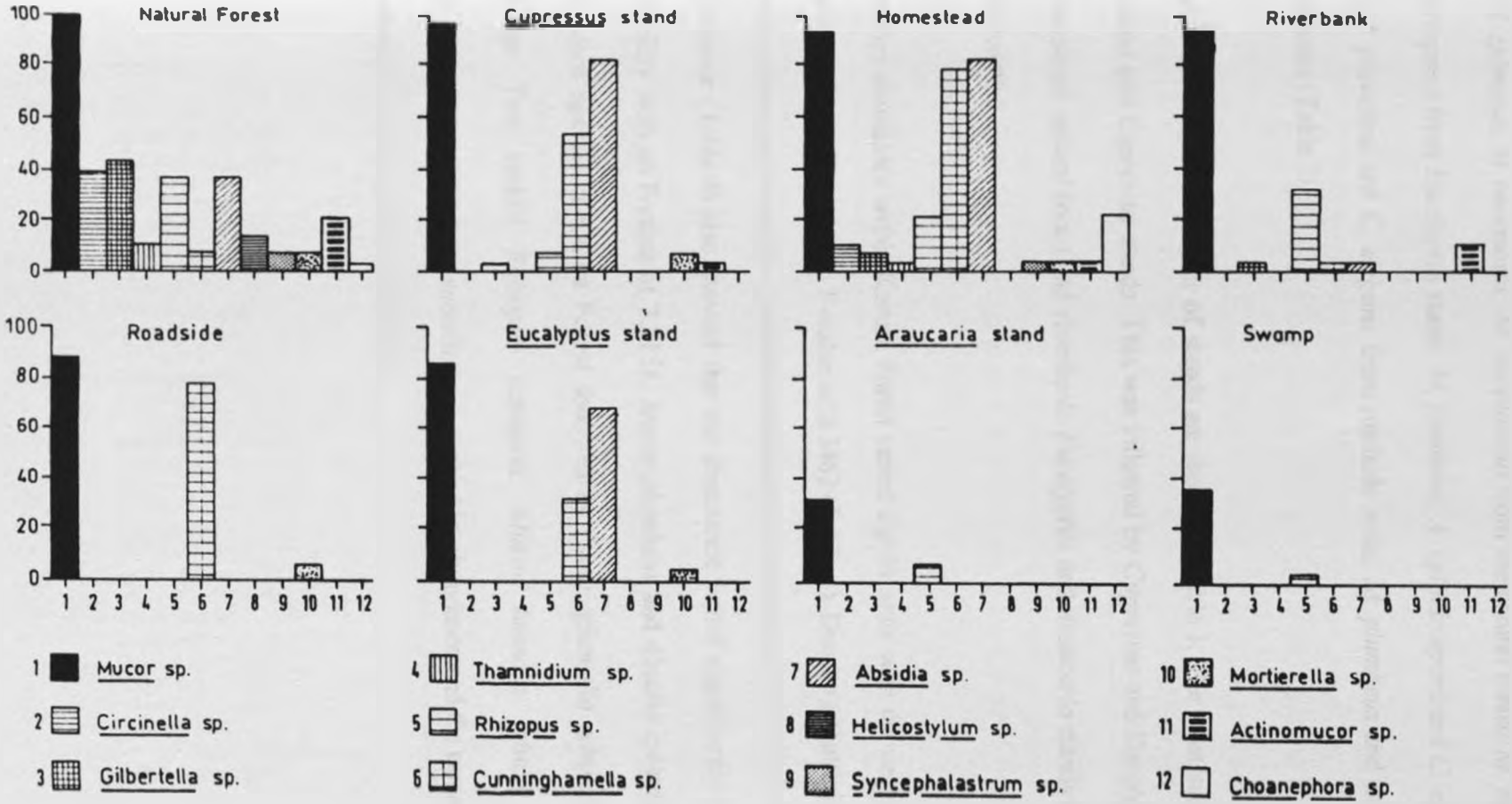

Fig. 29 Frequency of isolation of mucoraceous genera from various habitats in Karura Forest

Table 2 is a list of the 32 mucoraceous species recovered from the various habitats within Karura Forest. Seven occurred at frequencies of 50% or more and are considered dominants: *M. plumbeus*, *M. hiemalis*, *M. spinosus*, *M. racemosus*, *M. subtillismus* from the natural forest: *M. plumbeus* and *Absidia cylindrospora* from *Eucalyptus* stand: *M. plumbeus*, *A. cylindrospora* and *C. elegans* in the village soils: *M. plumbeus* and *C. elegans* from roadside soils: *M. plumbeus* and *M. racemosus* from riverbank soils (Table 2).

Coefficient of community for each pair of stands are shown in Table 3. The most similar habitats are the homestead and *Cupressus* stands. This was followed by *Cupressus* and *Eucalyptus*; natural forest and homestead: natural forest and riverbank. *Eucalyptus* and *Araucaria* stands had the least similarity coefficient.

Mucorales species abundance within Karura Forest varied significantly with the vegetation type at the 99.9% level of probability with an F-value of 8.3462 (Table 4). Duncan's Multiple Range Test ranked the natural forest as having the greatest abundance and swamp soil, the least (Table 5). The analysis of variance (Table 4) also showed that the abundance varied significantly at the 99.9% level of probability with an F-value of 7.3151. *Mucor plumbeus* and *Absidia cylindrospora* were the most abundant species in Karura Forest followed by *Cunninghamella echinatus*. Duncan's Multiple Range Test ranked *Rhizopus echinatus*, *Mucor hiemalis*, *Mucor racemosus*, *Cunninghamella bainieri* and *Mucor subtillisimus* as third in abundance and the remaining species as the least abundant, ranking fourth (Table 6).

Table 2: Mucoraceous fungi isolated from various habitats in Kenya

Species	HABITATS										
	Natural Forest	*Cupressus lusitanica* stand	Home-stead stand	River-bank stand	Road-side stand	*Eucalyptus paniculata* stand	*Araucaria araucana* stand	Swamp	Grass-land	Wooded Grassland	shrub-land
Mucor plumbeus	●●	●●	●●	●●	●●	●●	●	●	●	○	○
Absidia cylindrospora	●	●●	●●	○	○	●●	○	○	○	○	○
Cuuninghamella elegans	●	●●	●●	○	●●	●	○	○	○	○	○
Mucor racemosus	●●	●	●●	●●	●	●	●	●	○	○	○
Mucor hiemalis	●●	●	●	●●	●	●	●	●	○	○	○
Mucor spinosus	●●	●	●	●	●	●	○	○	○	○	○
Mucor subtilissimus	●●	●	●	●	●	●	●	●	○	○	○
Absidia zychae	●	●	●	●	○	●	○	○	○	○	○
Actinomucor elegans	●	●	●	●	○	○	○	○	○	○	○
Helicostylum elegans	●	○	○	○	○	○	○	○	○	○	○
Choanephora cucurbitarum	○	○	●	○	○	○	○	○	○	○	○
Circinella sp.	●	○	●	○	○	○	○	○	○	○	○
Circinella simplex	●	○	●	○	○	○	○	○	○	○	○
Cunninghamella bainеri	○	●	○	○	●	●	○	○	○	○	○
Cunninghamella echinulata	○	●	●	○	●	○	○	●	○	○	○
Gilbertella persicaria	●	●	○	●	○	○	○	●	○	○	○
Mortierella bainieri	●	●	●	○	●	●	○	●	●	○	○
Mucor ambiguus	●	○	○	○	○	○	○	●	○	○	○
Absidia spinosus	○	●	○	○	○	○	○	○	○	○	○
Mucor falcatus	●	●	●	○	○	●	○	●	○	○	○
Mucor fragilis	●	●	○	●	○	●	○	●	○	○	○
Mucor globosus	●	●	●	○	○	○	○	●	○	○	○
Mucor lamprosporus	○	○	○	○	○	○	○	○	○	○	○
Mucor mucedo	●	○	○	○	○	○	○	○	○	○	○
Mucor zychae	●	●	●	○	○	●	○	○	○	○	○
Rhizopus echinatus	●	○	○	●	○	○	●	○	○	○	○
Mucor microsporus	●	●	●	●	○	○	○	○	○	○	○
Rhizopus nigricans	●	○	○	○	○	○	○	○	○	○	○
Rhizopus oryzae	●	○	●	●	○	○	○	○	○	○	○
Syncephalastrum racemosus	●	○	●	○	○	○	○	○	○	○	○
Thamnidium elegans	●	○	●	○	○	○	○	○	○	○	○
Mortierella sp.	●	○	○	○	○	○	○	○	○	○	○

○ 0% Frequency of isolation ● <50% Frequency of isolation ●● >50% Frequency of isolation

Table 3: Coefficient of community for the various habitats within Karura Forest ecosystem

Vegetational stand	Swamp	Riverbank	Roadside	Homestead	*Araucaria araucana*	*Eucalyptus paniculata*	*Cupressus lusitanica*	Natural Forest
Natural Forest	0.324	0.714	0.388	0.734	0.364	0.564	0.636	
Cupressus lusitanica	0.37	0.688	0.616	0.82	0.348	0.758		
Eucalyptus paniculata	0.454	0.592	0.66	0.648	0.236			
Araucaria araucana	0.5	0.476	0.534	0.286				
Homestead	0.25	0.68	0.58					
Roadside	0.422	0.5						
Riverbank	0.4							
Swamp								

Table 4: Vegetation and fungal distribution: Analysis of variance table

K Value	Source	Degree of Freedom	K Value	Probability
1	Replication	1	3.5089	0.0612ns
2	Factor A (months)	11	4.2920	0.0000***
4	Factor B (vegetation)	7	8.3462	0.0000***
6	AB	56	1.6775	0.0013***
8	Factor C (fungi type)	31	7.3151	0.0000***
10	AC	341	1.0345	0.3458
12	BC	271	3.1221	0.0000***
14	ABC	2387	0.9290	
-15	Error	3050		

Table 5: Duncan's Multiple Range Test ranking sampling units according to their mucorales abundance.

Sample site	Mean	Similarity	Rank Number
Natural Forest	15112.0	**A**	1
Cupressus lusitanica	1074.0	**AB**	2
Homestead	1009.0	**AB**	2
Eucalyptus paniculata	663.2	**BC**	3
Riverbank	308.6	**CD**	4
Roadside	272.9	**CD**	4
Araucaria araucana	93.55	**D**	5
Swamp	25.08	**D**	5

*Letter **A** = highest abundance*
*Letter **C** = lowest abundance*

Table 6: Duncan's Multiple Range test ranking mucoraceous species according to their abundance in Karura Forest

Species	Mean	Similarity*
Mucor plumbeus	4286.0	A
Absidia cylindrospora	4189.0	A
Cunninghamella elegans	2029	B
Rhizopus echinatus	1193.0	BC
Mucor hiemalis	1165.0	BC
Mucor racemosus	1058.0	BC
Cunninghamella bainieri	1010.0	BC
Mucor subtilissimus	928.8	BC
Mucor spinosus	722.2	C
Rhizopus nigricans	697.9	C
Cunninghamella echimulata	590.3	C
Rhizopus oryzae	531.3	C
Gilbertella persicaria	433.0	C
Circinella simplex	422.2	C
Helicostylum elegans	319.4	C
Mucor zychae	288.2	C
Mucor globosus	284.4	C
Choanephora cucurbitarum	277.8	C
Mucor mucedo	213.2	C
Mortierella bainieri	104.2	C
Syncephalastrum racemosus	69.44	C
Actinomucor elegans	69.44	C
Mucor lamprosporus	55.90	C
Absidia zychae	45.14	C
Mucor ambiguus	8.681	C
Mucor falcatus	6.944	C
Rhizopus nigricans	6.944	C
Mucor fragilis	6.944	C
Thamnidium elegans	6.944	C
Absidia spinosus	5.556	C
Circinella sp.	3.819	C
Mortierella sp.	3.472	C

* Species with the same lettering are equal in abundance
Letter **A** = highest abundance
Letter **C** = Lowest abundance

DISCUSSION

The qualitative and quantitative differences in species composition among the stands reveal the direct influence of plant communities and human activities on the distributional patterns of these fungi. Most of the mucoraceous fungi recovered from the soil, in this study, were from the forest ecosystem. These results indicate that the mucoraceous fungi were rare in grassland ecosystems. Several investigators have shown a correlation between microfungal species diversity and abundance and vascular plant diversity and type (Apinis, 1958; Bissett and Parkinosn, 1979; Christensen, 1969; Christensen and Whittingham, 1965; Christensen *et al.*, 1962; Curtis, 1959; Orput and Curtis, 1957; Tresner *et al.*, 1954; Widden, 1986); concomitant high species diversity for vascular plants and soil microfungi. The effect of site differences is further exemplified by data obtained within the forest ecosystem. Figure 2. Table 2. The natural forest had the highest species diversity and abundance compared to the single vegetational stands. and areas affected by human activities.

High species diversity of vascular plants as found in natural forest ecosystems is undoubtedly an environment that supports a high percentage of mucoraceous fungi if not a higher percentage of fungi. These results suggest the possible usage of mucorales distribution in the biomonitoring of environmental degradation. Environmental degradation caused by, for example, clearing the natural forests for settlement, construction of roads, plantation farming adversely affect the functioning of the soil ecosystems by interfering with the soil inhabitants.

The distribution of heterotrophic fungi in the soil are largely determined by the availability of suitable substrates as well as an appropriate physical environment, both of which are largely determined by the nature of the vegetation cover. Habitat selectivity found in this study conform to

the patterns found by Widden (1979). Individual species had marked preference for some sites: species of *Cunninghamella* preferred the warmer more exposed sites (homesteads and roadsides) to the cooler habitats of the natural forest and *Araucaria* stand. Such preference could be due to the physical environment. The genera *Absidia* and *Choanephora* also portrayed such behaviour.

Although the data presented have clearly demonstrated that vegetation cover influence distribution pattern of mucorales. vegetation variation may not be the prime factor. There must be influence from climatic factors and soil chemistry working together within the vegetaion cover and type. The use of multivariate statistical procedures on such data would give an idea on the interrelatedness of these environmental effects. The results of these analyses are reported in the later chapters.

CONCLUSION

Vegetation cover is a major factor in determining mucorales distribution at generic and species levels. *Mucor plumbeus* was the most abundant and frequently isolated member of the order mucorales within Karura forest. Specificity of choice of niche within the forest was also observed.

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CHAPTER 4

EFFECT OF ABIOTIC FACTORS ON THE SEASONAL COMPOSITION AND ABUNDANCE OF SOIL MUCORACEOUS FUNGI IN FOREST, GRASSLAND AND SHRUBLAND ECOSYSTEMS IN KENYA.

ABSTRACT

The knowledge of relationships between soil fungi and their environment is a pre-requisite for their eventual manipulation in applied aspects of science. To understand these relationships, detailed information on the correlation between changes in the physical, chemical and biological properties of the soil and changes in the fungal populations present is required. The species composition and relative abundance of soil mucoraceous fungi in different ecological conditions in Kenya was studied in relation to rainfall, air temperature, humidity and soil moisture content. Mucoraceous fungi were recovered from the soil using soil plate and dilution methods. The soil moisture content was obtained from the subsamples of the soil used for mucoraceous fungal isolations. The results revealed that site differences in relation to rainfall and soil moisture were a major factor in determining the occurrence of these fungi. While mucoraceous fungi were common in forest soils, they were rare or absent in grassland, wooded grassland and shrubland soils. Soil moisture was the major variable shown to contribute to site differences and, in turn, to mucorales distribution.

Seasonal changes in mucoraceous fungal composition and abundance were related to rainfall pattern which in turn influenced the moisture status of the soil while *Cunninghamella* and *Absidia* species occurred abundantly during relatively dry seasons. *Mucor* sp. on the other hand did not appear to respond linearly to this trend and were isolated throughout the sampling period. Rare

genera like *Choanephora, Thamnidium, Mortierella, Gilbertella, Chaetostylum, Syncephalastrum, Circinella* and other less common species of *Mucor* like *M. corticolus, M. ambiguus, M. falcatus, M. saturinus, M. fragilis* were also recovered during the dry season.

INTRODUCTION

As the science of biotechnology evolves and management of species become more sophisticated, there is a need for science to have knowledge of the relationship between the organism and its natural habitat. The initial knowledge of the diversity of these organisms, the associations they form within their ecosystem and the environmental influence on them, is the basis for successful exploitation of their environmental biotechnological potential. There is lack of data on the functioning of these complex microbial communities in rich tropical environment. For example, little is known about the environmental conditions required for an optimal ecosystem functioning and interactions among the micro-organisms.

Mucoraceous fungi are a major component of soil microbial communities in many ecosystems (Moubasher and El-Dohlob, 1970; Wicklow and Carrol, 1981; Widden and Parkinson, 1973). Nevertheless mucoraceous species composition and relative abundance in different ecological conditions is poorly understood in most tropical ecosystems. Studies on environmental influence on soil fungi have concentrated on temperate ecosystems while little is known about the tropics.

The existing studies have revealed some characteristics of fungal species composition and distribution in relation to temperature and soil moisture. A shift in species composition from one

extreme in summer to another in winter has been observed (Bissett and Parkinson 11: 1979; Clarke and Christensen. 1981; Widden 11: 1986). Contrary to their observation. Gams and Domsh (1969) Widden and Parkinson (1973). and Parkinson and Balasooriya (1969) have recorded no such seasonal cycles in the occurrence of soil fungi regardless of the climatic extremes. This suggests that a set of environment conditions of a habitat is the key determinant of the characteristics of a given soil fungal population. Moubasher and El-Dohlob (1970) reported a rich period in fungal population and genera when the water content of the soil was moderate and a low average maximum temperature of 19 - 35°C. The poorest period in fungal population and genera was recorded when the temperature was relatively high with a monthly average maximum of 37 - 40°C and low soil water content.

In view of the above facts. this study was formulated to test the hypothesis that the species diversity and abundance of mucoraceous fungi in a habitat are influenced by the combined effect of rainfall. air temperature. relative humidity and soil water content regimes.

OBJECTIVE

To determine the effect of rainfall. air temperature. humidity and soil water content regimes on the occurrence and composition of mucoraceous fungi.

MATERIALS AND METHODS

Soil samples were collected from forest. grassland. wooded grassland and shrubland communities. described earlier in Chapter 2.

Estimation of mucoraceous fungi populations in the soil.

Sampling was carried out once a month for 28 months between January 1992 and December 1994. Soil samples were collected randomly from homogenous areas within a vegetational stand. The soil samples constituted top soil, litter and ripe fruits collected from five locations, in sterilized paper bags. The soil was mixed up thoroughly, ending up with about one kilogram top soil and litter. The fungi were recovered from the soil samples within two days of collection to minimize the effect of storage on microbial populations.

The Warcup's soil plate method (Warcup, 1950) and the Dilution Plate method (Chesters and Thornton, 1956) were used to isolate the fungi from the soil. The latter method provided data on the abundance of the mucorales. This technique is fully describes in Chapter 2.

The following media were used in each collection for the above methods: Czapek's (Dox) Agar (CZ), Malt Extract Agar (MA), Potato-Dextrose Agar (PDA), (Smith and Onions, 1983). Rose bengal was added to the media as an antibiotic against bacteria, during the isolation stages. Pure cultures were maintained on antibiotic-free media.

The fungi were characterized using morphological similarities among the species of each genus (Chapter 1).

Environmental data

Rainfall and air temperature data for 1992 - 1994. for all the sample sites. were obtained from the Meteorological Department. Within Karura forest. air temperatures were recorded from the following vegetational stands: *Araucaria*. *Cupressus* and the natural forest stands. The temperatures were recorded twice a week at two hourly intervals from 8 a.m. to 6 p.m. for fourteen months. Dry and wet bulb thermometers were used. The humidity was calculated from the readings from the dry and wet bulb thermometers.

The soil moisture content was determined each time samples were collected for fungal isolation using the method described by van Reevwijk (1987). However samples from Karura forest were analysed twice a week for one year to give a close comparison. Analyses of variance tests were performed to determine the variation of mucorales occurrence in relation to environmental factors.

RESULTS

(a) Influence of rainfall, temperature and soil moisture on mucorales occurrence in forest, grassland, wooded grassland and shrubland soils.

Figure 30. 31 and 32 show that rainfall and air temperature did not influence the occurrence of mucoraceous fungi in grassland. wooded grassland and shrubland communities. Though the trend in rainfall pattern was the same for all the stands with the highest monthly totals falling between March and May. 1992: October 1992 and May 1993: November and December 1993: March and May 1994: October and December 1994 (Figure 30(a). 31(a). 32(a). mucoraceous fungi were mostly recovered from Karura forest throughout the three years (Figure 30c. 31c. 32c).

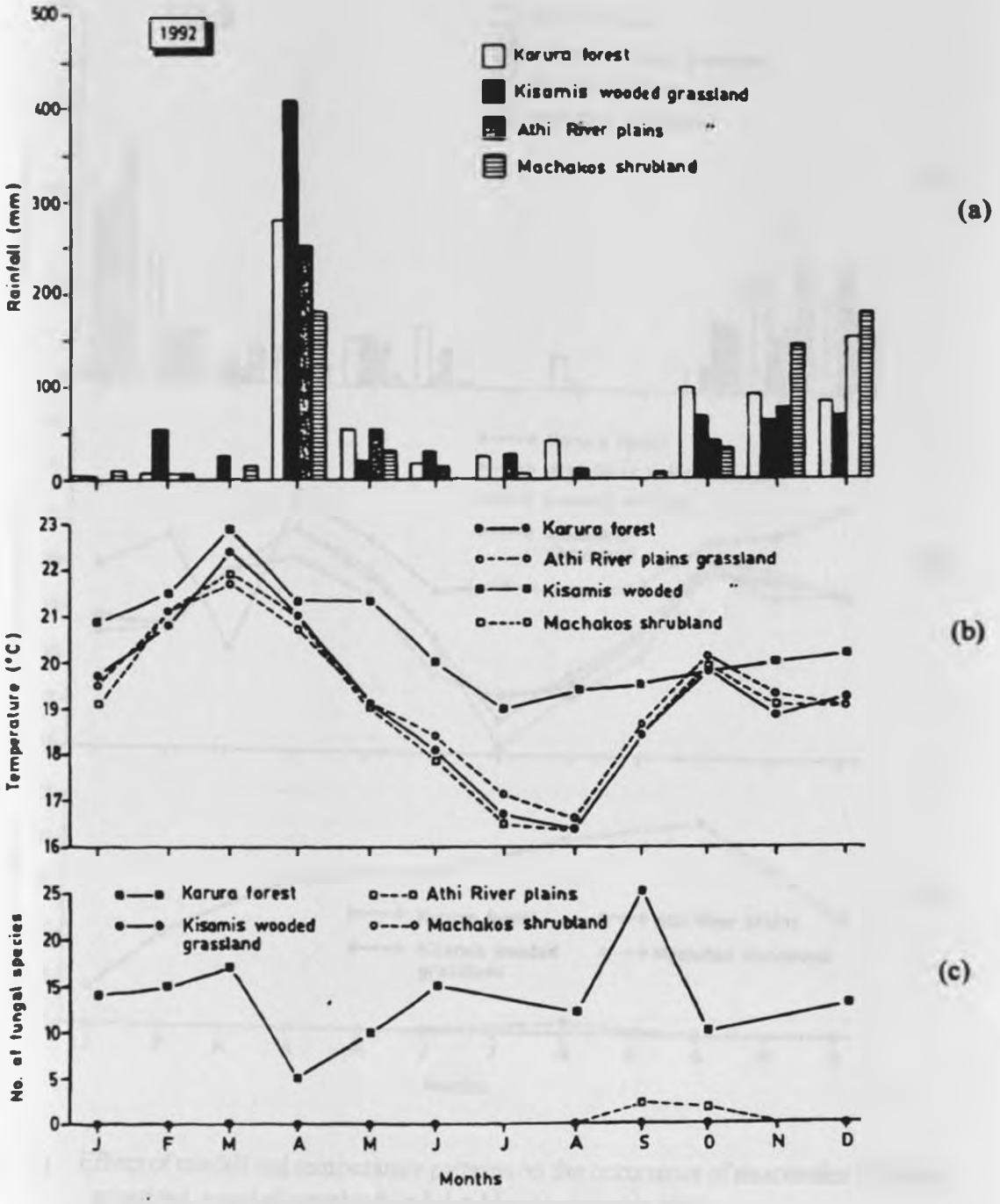

Fig. 30: Effect of rainfall and temperature patterns on the occurrence of mucorales in forest, grassland, wooded grassland and shrubland habitats in 1992.

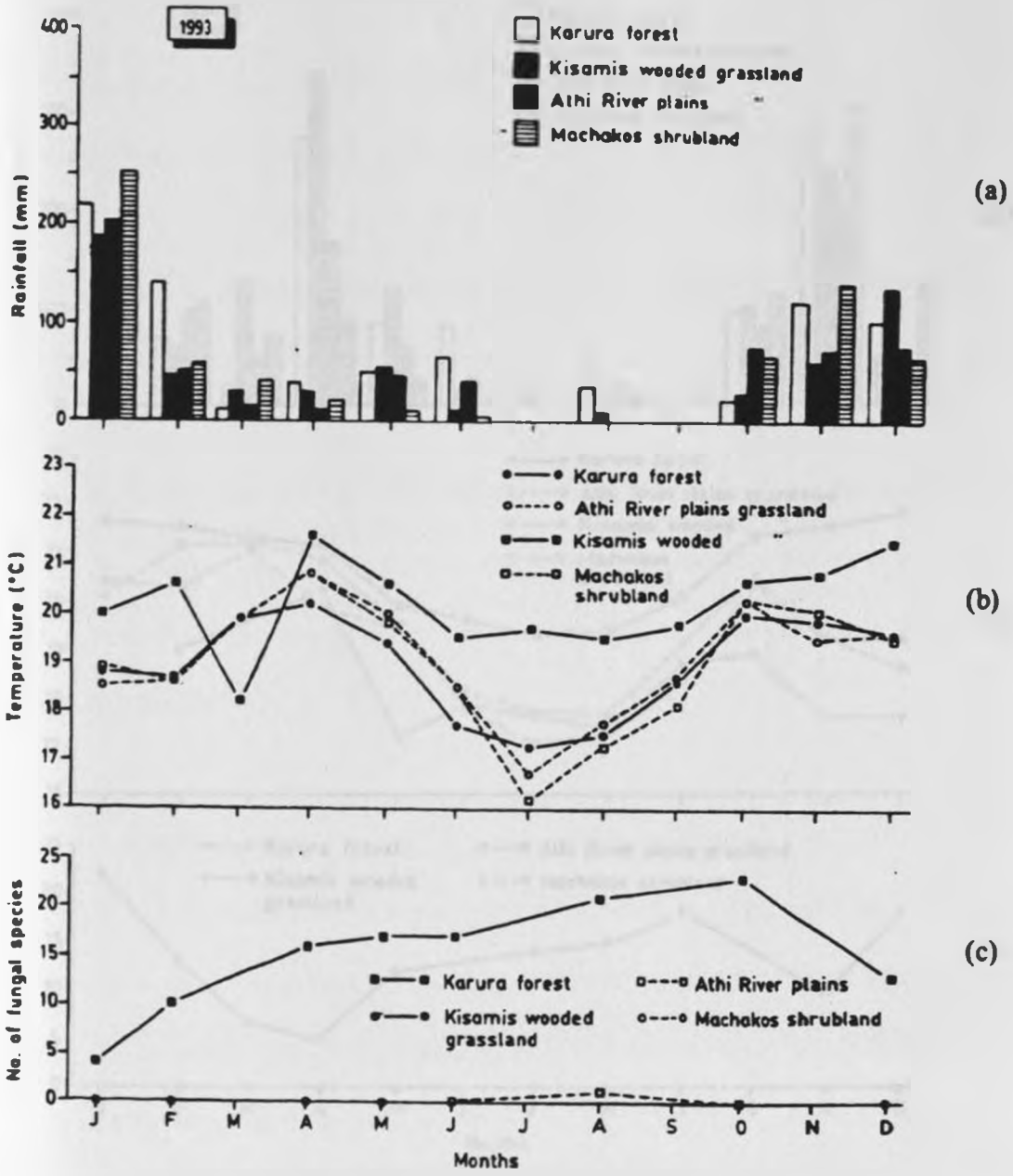

Fig. 31 : Effect of rainfall and temperature patterns on the occurrence of mucorales in forest, grassland, wooded grassland and shrubland habitats in 1993.

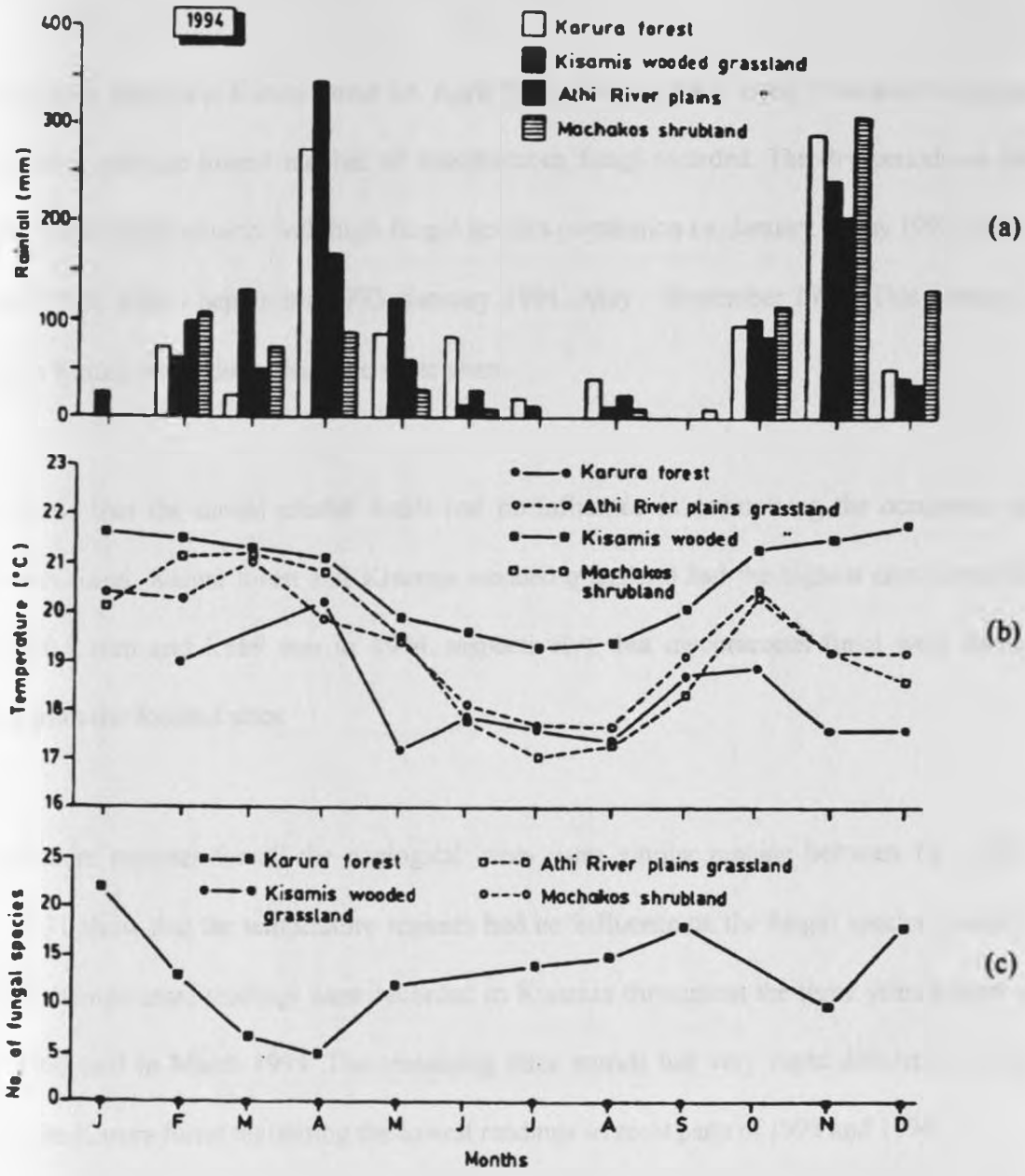

Fig. 32 : Effect of rainfall and temperature patterns on the occurrence of mucorales in forest, grassland, wooded grassland and shrubland habitats in 1994.

Mucor sp. and *Mortierella* sp. were recovered on only three occasions (September and October 1992, August 1993 - Figures 30c, 31c) from the grassland stand (Athi River plains) and none at all from Kisamis wooded grassland and Machakos shrubland.

The peak rainfall seasons in Karura forest i.e. April 1992, January 1993, April 1994 and November 1994 coincides with the lowest number of mucoraceous fungi recorded. The dry periods on the other hand correlated positively with high fungal species population i.e. January - May 1992, June - September 1992, May - September 1993, January 1994, May - September 1994. This pattern is portrayed in Karura forest throughout the three years.

Table 7 shows that the annual rainfall totals had no influence in determining the occurrence of mucoraceous fungi. Karura forest and Kisamis wooded grassland had the highest annual rainfall totals (1,020.9 mm and 1,169 mm in 1994, respectively), but mucoraceous fungi were mostly recovered from the forested sites.

Air temperature regimes for all the ecological areas were similar ranging between 16 - 20°C. Figures 30 31 show that the temperature regimes had no influence on the fungal species diversity. The highest temperature readings were recorded in Kisamis throughout the three years except in October 1992 and in March 1993. The remaining three stands had very slight differences in the readings with Karura forest registering the lowest readings in most parts of 1993 and 1994.

Table 7: Comparison of rainfall yearly totals (mm) for Karura forest, Athi River Plains, Kisamis wooded grassland and Machakos shrubland with mucorales occurrence

STAND	Karura forest		Kisamis wooded grassland		Athi River Plains, grassland		Machakos shrubland	
YEAR	Total rainfall (mm)	Number of species isolated	Total rainfall (mm)	Number of species isolated	Total rainfall (mm)	Number of species isolated	Total rainfall (mm)	Number of species isolated
1992	703.4	136	749.9	0	641.9	0	613.8	0
1993	821.8	121	602.7	0	598.8	2	634.7	0
1994	1,020.9	134	1,169	0	748.7	0	875.7	0

It is obvious from Figure 33 that soil moisture is the most significant abiotic factor that influenced the occurrence of mucoraceous fungi in the different vegetational communities. Karura forest had the highest mean of 22.250 ^{w}/w % followed by Kisamis wooded grassland with 6.339 ^{w}/w %, Machakos shrubland with 4.585 ^{w}/w % and lastly Athi grassland with 3.605 ^{w}/w %. The difference was significant at the 99.9% level of probability with an F-value of 58.1962. Soil moisture content below 10 ^{w}/w % could not sustain most of the species. The fungi were isolated only from Karura forest where the soil moisture content was above 15 ^{w}/w %. However, the highest diversity of mucoraceous fungi were observed in the months of October 1993 and January 1994 when the soil moisture content was relatively low i.e. 15.533 ^{w}/w % and 17.214 ^{w}/w % respectively.

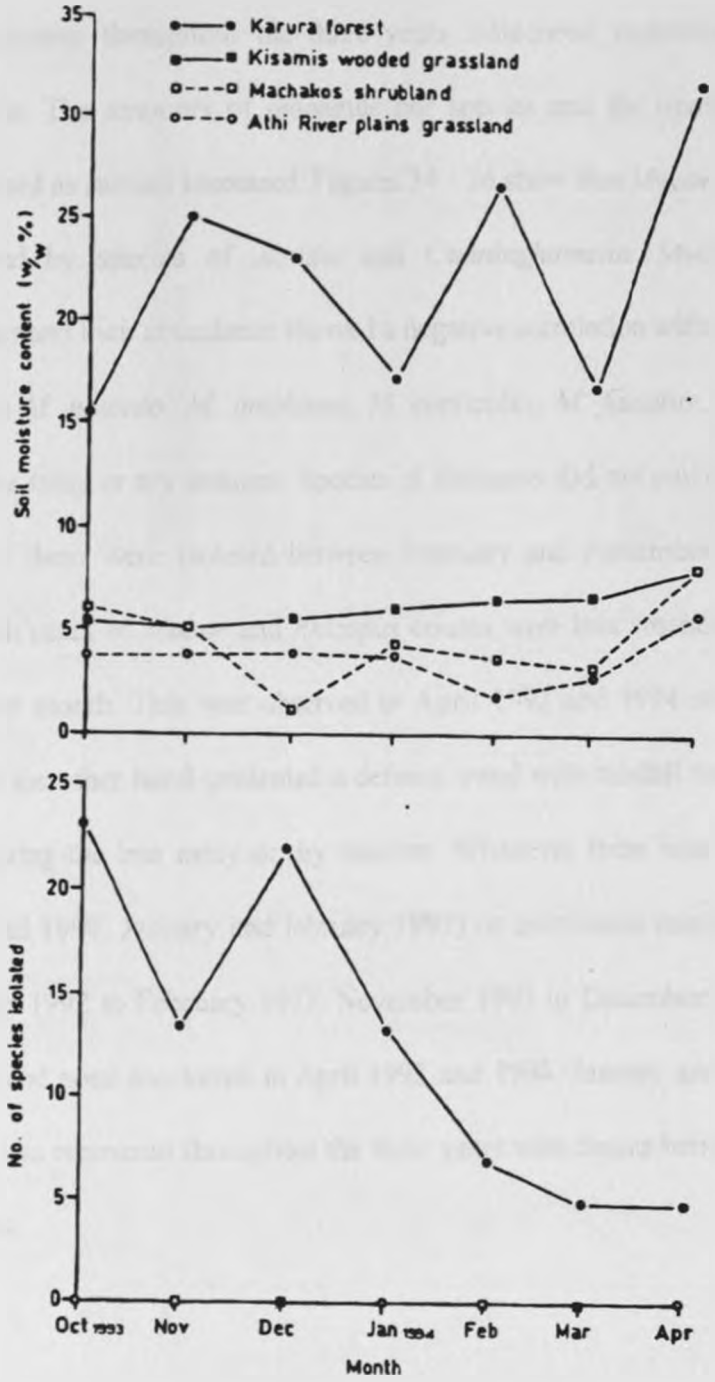

Fig. 33: Effect of soil moisture content on the occurrence of mucoraceous fungi.

(b) Influence of rainfall, temperature, humidity and soil moisture on mucorales abundance within Karura forest

Variation in rainfall amounts throughout the three years influenced mucorales richness and diversity in Karura forest. The amounts of propagule per species and the numbers of different species recovered decreased as rainfall increased. Figures 34 - 36 show that *Mucor* species were the most dominant followed by species of *Absidia* and *Cunninghamella*. *Mucor* sp. occurred throughout the three years and their abundance showed a negative correlation with rainfall. The less frequent species such as *M. mucedo*, *M. ambiguus*, *M. corticolus*, *M. falcatus*, *M. fragilis* were recovered during the less rainy or dry seasons. Species of *Rhizopus* did not particularly show any trend, however most of them were isolated between February and November 1993 when the rainfall was low. In both cases of *Mucor* and *Rhizopus* counts were low whenever rainfall totals were above 200 mm per month. This was observed in April 1992 and 1994 and January 1993. *Cunninghamella* sp. on the other hand presented a definite trend with rainfall totals. These fungi occurred abundantly during the less rainy or dry seasons. Whenever there was a rise in rainfall amounts (April 1992 and 1994, January and february 1993) or continuous rainfall more than 80 mm per month (October 1992 to February 1993; November 1993 to December 1994) the counts decreased significantly and none recovered in April 1992 and 1994, January and February 1993). *Absidia cylindrospora* was recovered throughout the three years with counts being high during the less rainy or dry seasons.

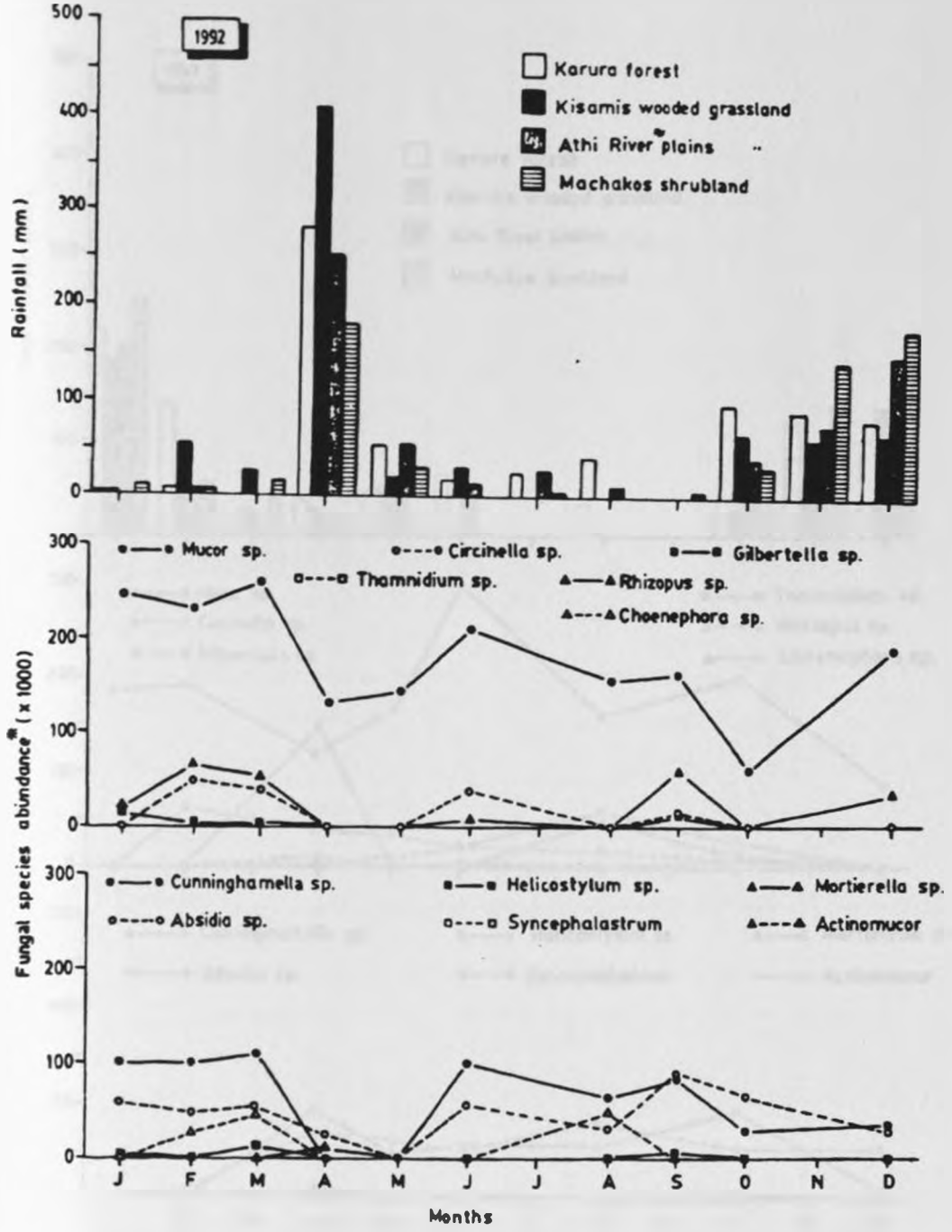

Fig. 34 : Variation of mucorales species abundance*, in Karura Forest, with rainfall totals in 1992.

* Abundance based on propagule density (pooled) calculated from a number of colonies per dilution level from dilution plates.

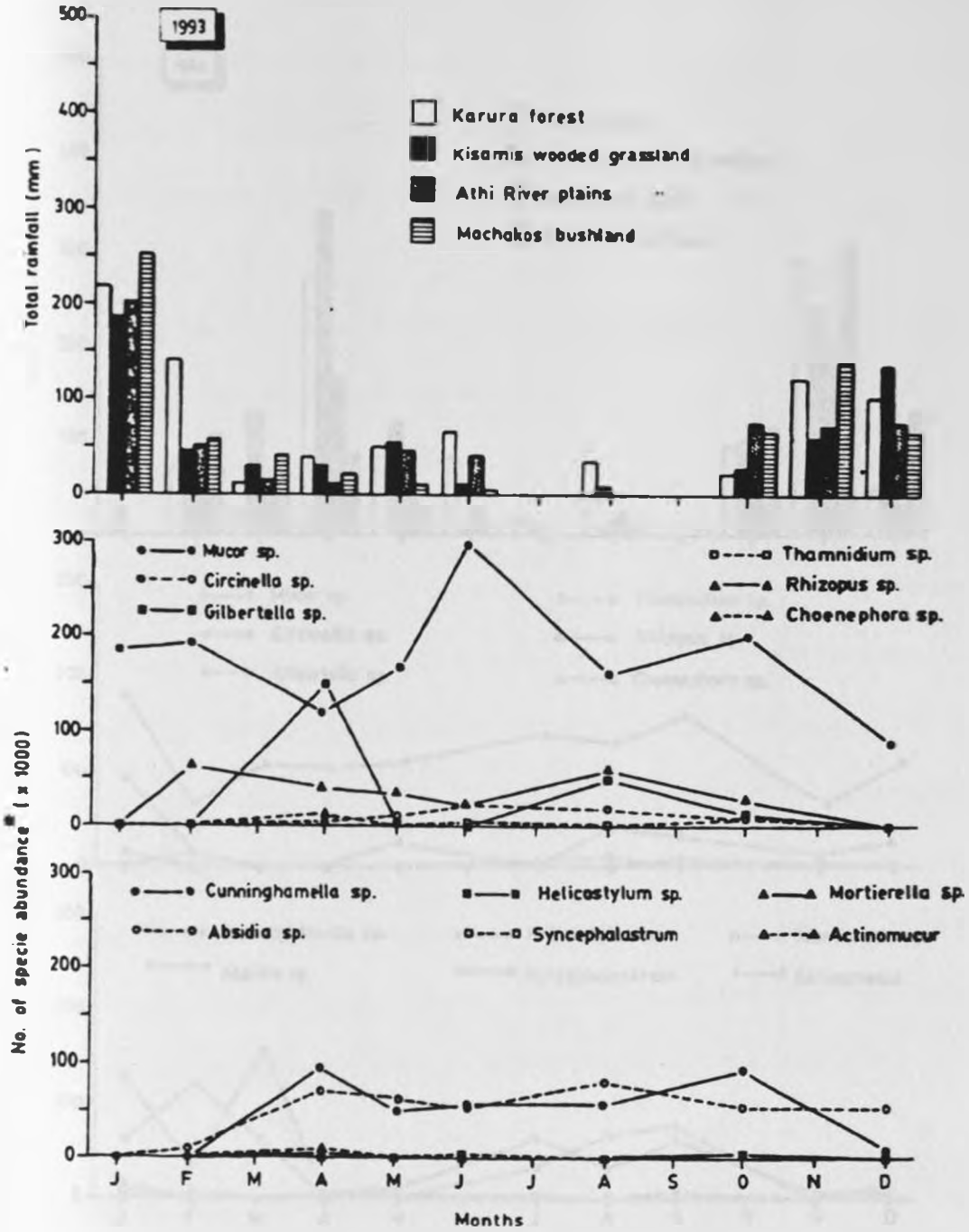

Fig. 35: Variation of mucorales species abundance*, in Karura Forest, with rainfall totals in 1993

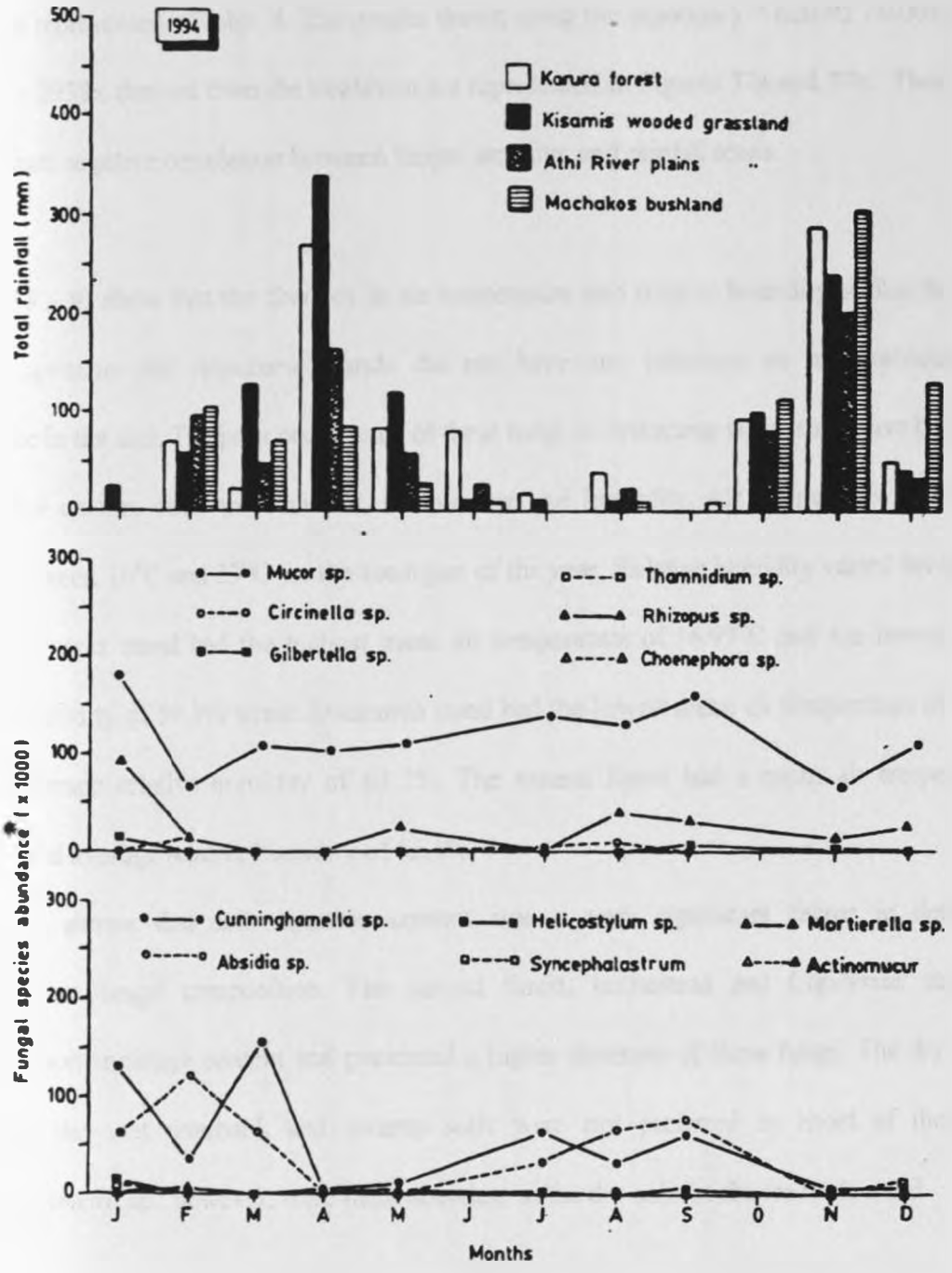

Fig. 36: Variation of mucorales species abundance*, in Karura Forest, with rainfall totals in 1994

The less common species. *Absidia zychae* was mostly isolated from the soil during the dry months. This was also true for the infrequent genera such as *Circinella* sp. *Gilbertella* sp., *Thamnidium* sp., *Helicostylum* sp., *Syncephalastrum* sp., *Mortierella* sp., *Choanephora* sp. and *Actinomucor* sp.

The same data on species richness was treated using coefficient of correlation analysis and the results are represented in Table 8. The graphs drawn using the equation y = 620.95 - 0.0041x and y = 941.11 - 2938x derived from the treatment are represented in Figures 37a and 37b. These graphs also indicate negative correlation between fungal amounts and rainfall totals.

Figures 38 - 40 show that the changes in air temperature and relative humidity within the natural forest, *Cupressus* and *Araucaria* stands did not have any influence on mucoraceous fungal abundance in the soil. The poor occurrence of these fungi in *Araucaria* stand must have been due to some other reason, other than rainfall, temperature and humidity. Air temperature in the forest ranged between 16°C and 23°C for the most part of the year. Relative humidity varied between 40 - 80%. *Cupressus* stand had the highest mean air temperature of 16.97°C and the lowest average relative humidity of 59.1% while *Araucaria* stand had the lowest mean air temperature of 16.45°C and an average relative humidity of 63.3%. The natural forest had a mean air temperature of 16.72°C and average relative humidity of 65.1%.

Figure 41 shows that soil moisture content was a very significant factor in determining mucoraceous fungal composition. The natural forest, homestead and *Cupressus* stands had moderate soil moisture content and presented a higher diversity of these fungi. The dry roadside soils and the wet riverbank and swamp soils were not preferred by most of these fungi. *Cunninghamella* sp., however, were most abundant in the dry soils of the roadside stand.

Table 8: Correlation Table showing the variation of fungal amounts with rainfall totals

	Covariance	Correlations	Intercept	Slope	Standard	Student	Probation	Equation
Replication 1	-105227.45	-0.041	620.95	-1.832	0.904	2.027	0.043	y = a + bx y = 620.95- 0.041x
Replication 2	-16801.54	-0.037	941.11	-2.938	1.613	1.822	0.069	y = a + bx y = 941.11 - 2.938x

Where x = amount of rain
y = Mucorales richness

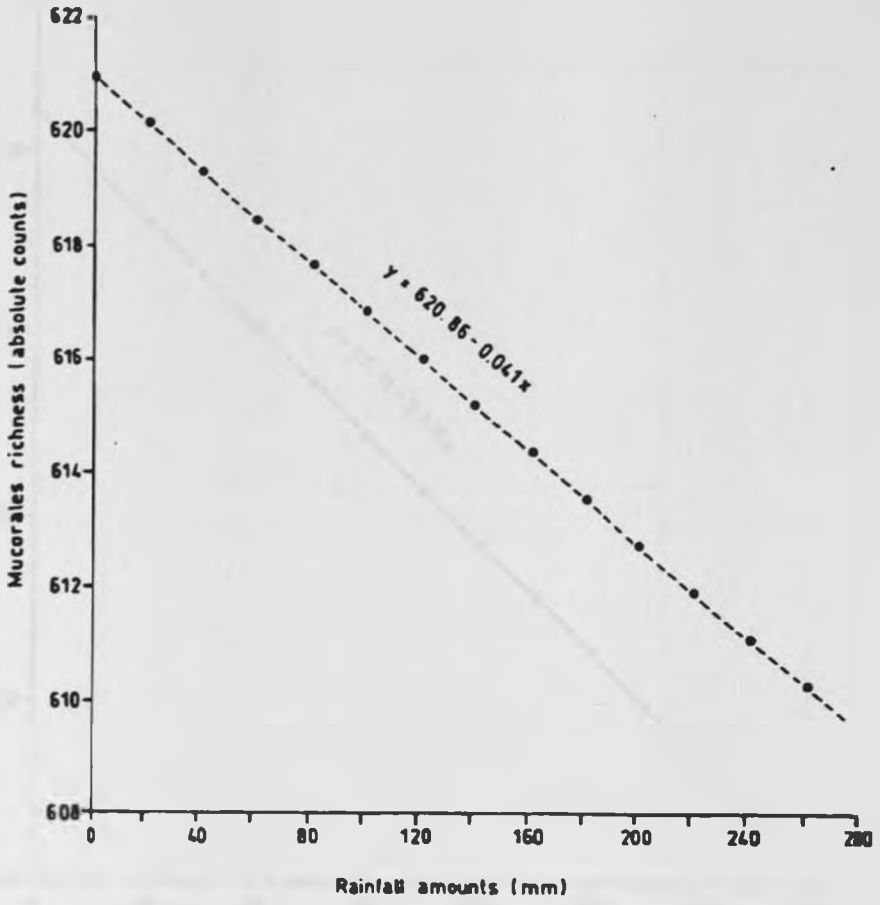

Fig. 37a: Effect of rainfall amounts on Mucorales species richness in Karura Forest, Jan 1992 - Dec 1994.

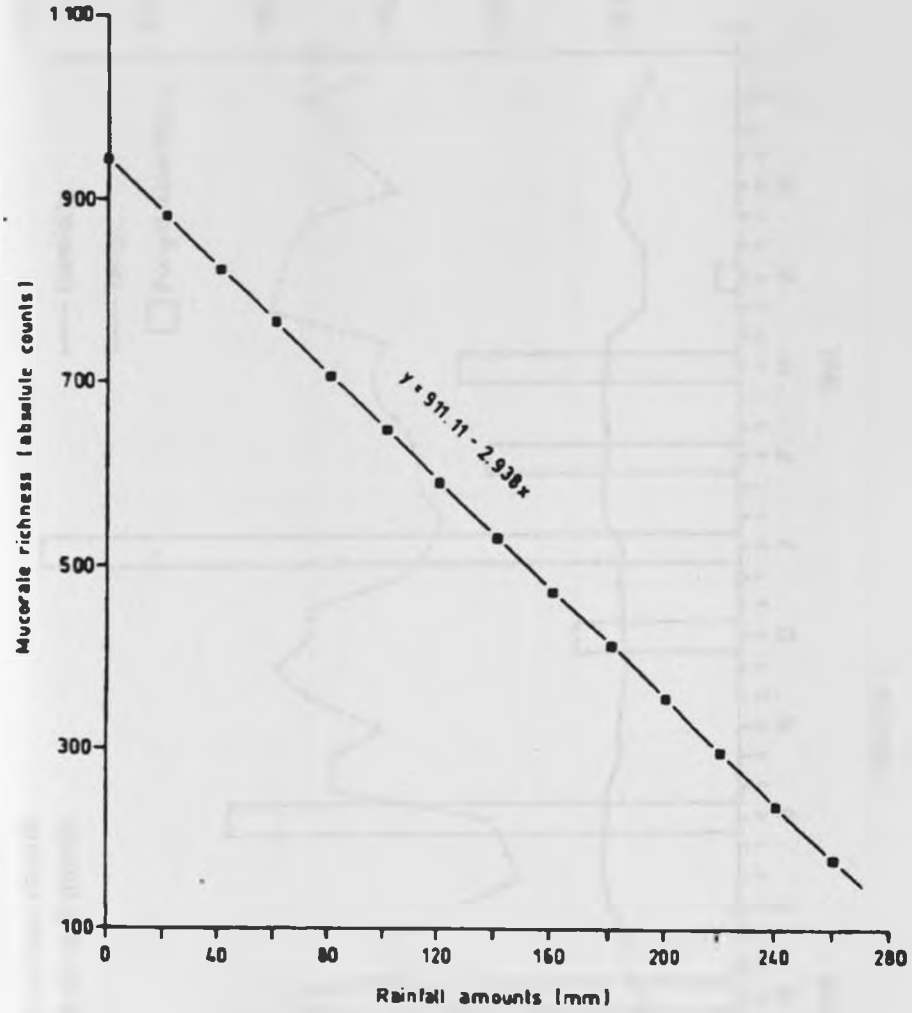

Fig. 37b: Effect of rainfall amounts on Mucorales species richness in Karura Forest, Jan 1992 - Dec 1994.

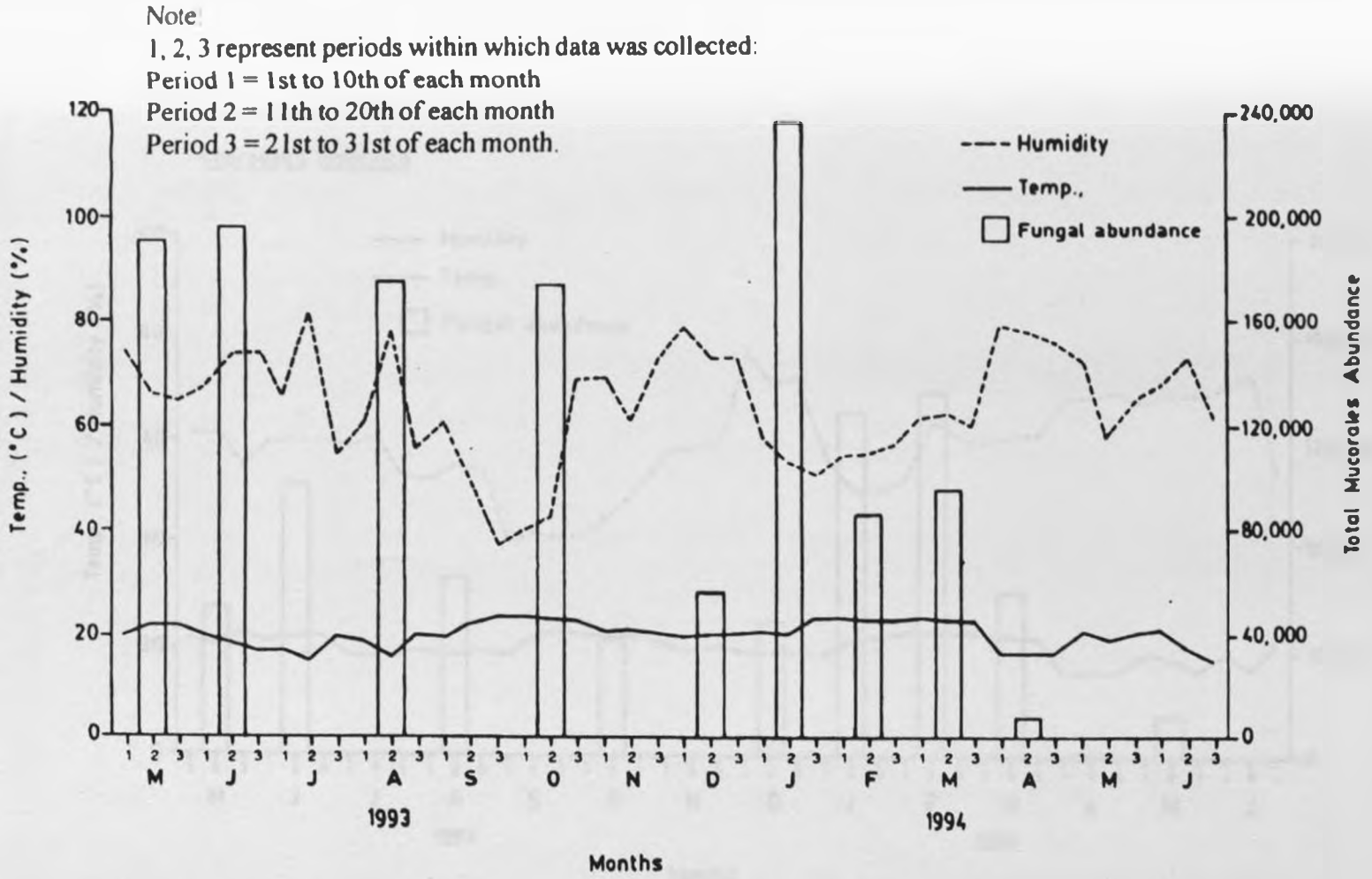

Fig. 38: Variation of temperature and humidity within the natural forest and their effects on mucorales abundance.

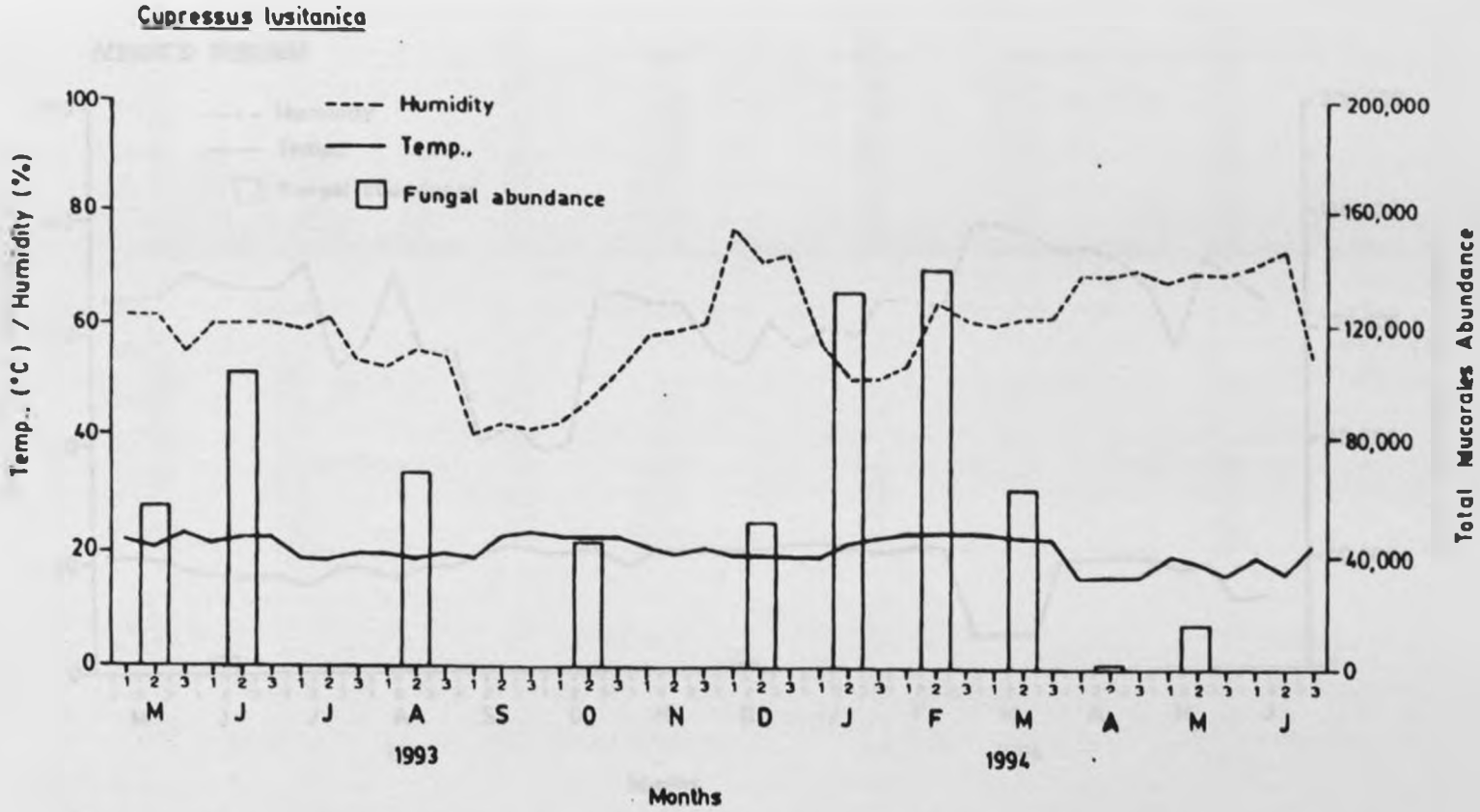

Fig. 39: Variation of temperature and humidity within *Cupressus lusitanica* stand and their effect on mucorales abundance

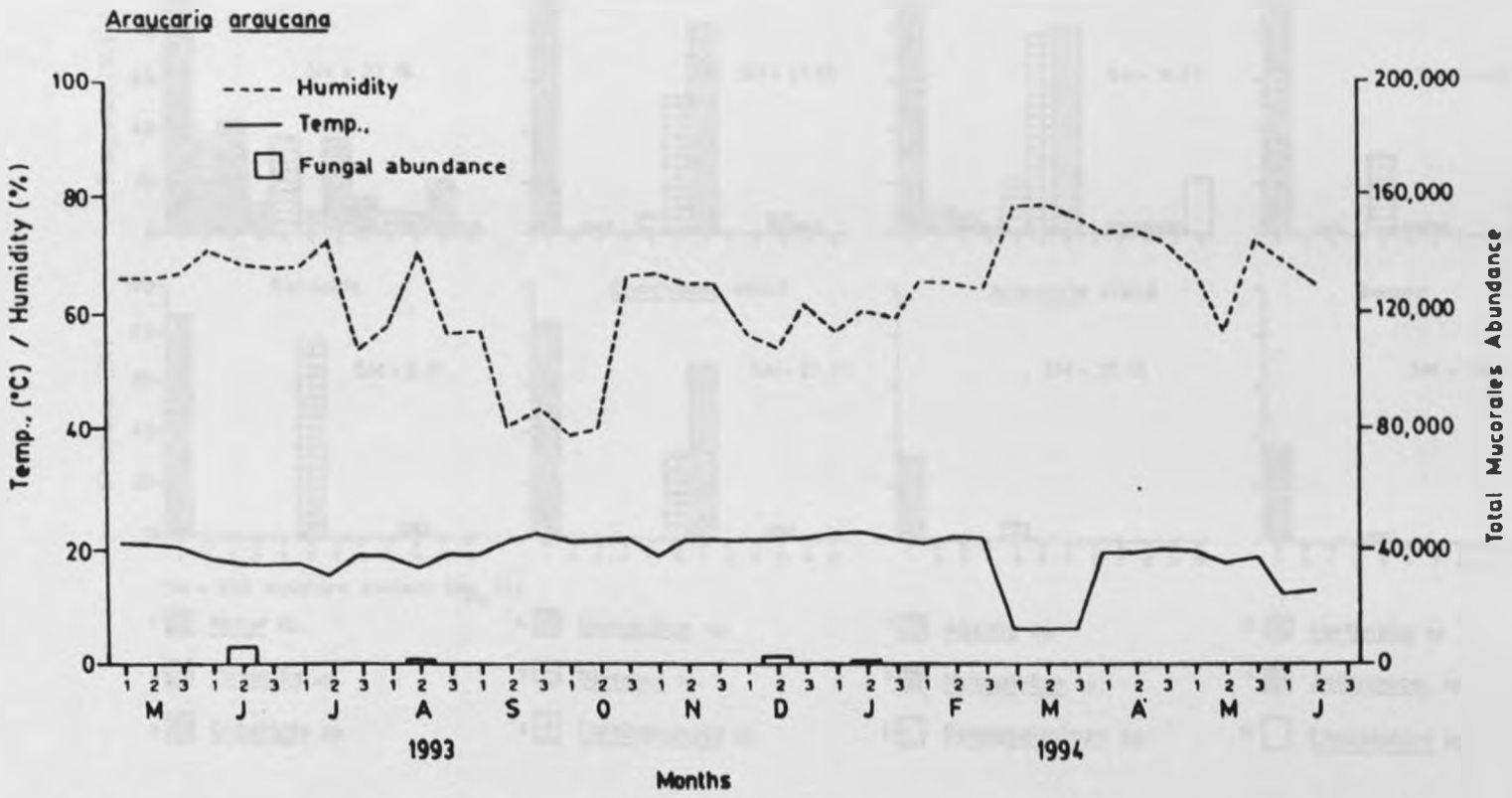

Fig. 40: Variation of temperature and humidity within *Araucaria araucana* stand and their effect on mucorales abundance

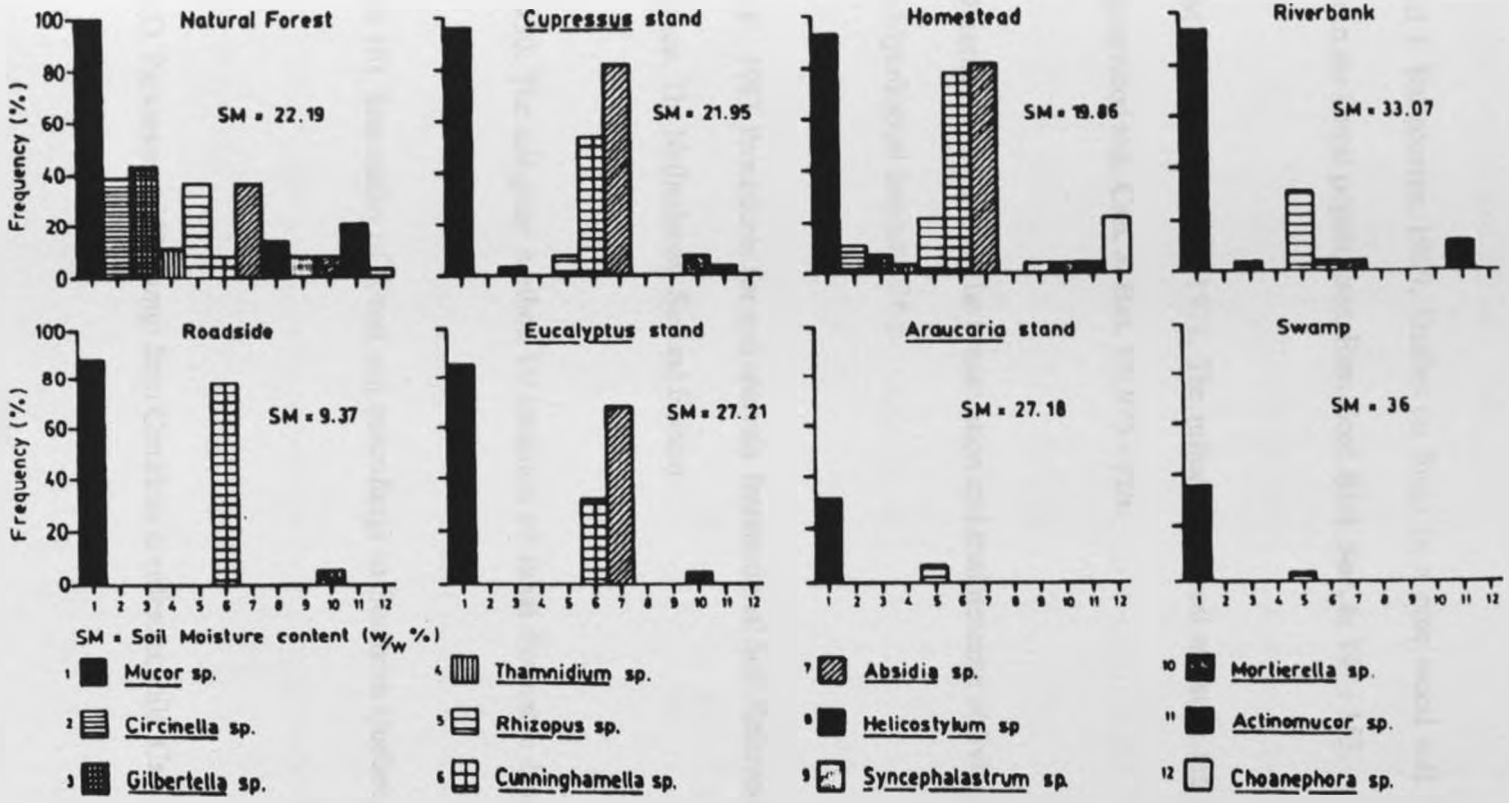

Fig. 41: Effect of soil moisture content on the frequency of isolation of mucoraceous genera from various habitats in Karura Forest.

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Despite the moderate soil moisture content. humidity and air temperature within *Araucaria* stand. mucoraceous fungal occurrence within the stand was poor. The limiting factor was therefore a different variable. probably vegetation cover. (Chapter 2) other than soil moisture. humidity and air temperature.

CONCLUSION

The differences among the sites and seasonality are the major sources of variation in the data. While the differences in site determined mostly the presence or absence of soil mucoraceous fungi (and this could be seen among the different vegetational stands - forest. grassland. wooded grassland. shrubland - and also within Karura forest itself) seasonality influenced the diversity and abundance of these fungi.

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also be affected by the rain downpour directly. The damp conditions in the A_1 layer during the heavy rains could dampen the mycelial fragments reducing their viability. This means that most of the isolations done during this season will be growth from spores as compared to the less rainy seasons. The possibility of the fungal propagules remaining dormant during the unfavourable conditions cannot be ignored.

Soil moisture content was also highly correlated with species composition among the sites (Fig. 41). Most of mucoraceous fungi were recovered from soils with moderate moisture content - Natural forest (22.19 ^{w}/w %) *Cupressus* (21.95 ^{w}/w %) and Homestead stands (19.86 ^{w}/w 5). *Cunnighamella* species however appeared to be adapted to low moisture and were dominant in the roadside soils (9.37 ^{w}/w %). Similar results were obtained by Moubasher and El-Dohlob (1970). and Shameemulah and Parkinson (1971).

Air temperature and humidity were moderate (16 - 23°C and 40 - 80% respectively) within the forest and were not limiting factors. Temperature and humidity fluctuations also did not influence mucorales occurrence and abundance. Moubasher and El-Dohlob (1970) also obtained rich fungal population from soil samples when average maximum temperature ranged between 19°C and 35°C and the poorest period in fungal population was when the temperature was relatively high (monthly average maximum 37 - 40°C).

researchers in the temperate regions have also reported such trends in which soil fungi in general are more abundant in spring and fall and fewer in summer and winter (Christensen.1969; Gochenaur. 1978. 1984; Ishi. 1969; Kuter. 1986; Moubasher and El-Dohlob. 1970; Widden II: 1986). Attili and Tank-Tormsielo (1994) while studying the occurrence of microfungi during leaf litter decomposition. observed a changing community composition with seasons. Other workers have indicated that no clear cyclical trends in the abundance of fungal species occur (Gams and Dormsch. 1969; Parkinson and Balasooriya. 1969; Dickinson and Kent. 1972; Widden and Parkinson. 1973). Widden (II: 1986) observed a tendency for the fungal community to shift from a summer community towards a characteristic winter community during the colder periods of the year. This. to some extent. was also true in Karura forest where rare genera were only isolated during the dry seasons. Species of *Cunninghamella* also disappeared completely during the wet periods. Such season-species interactions can be attributed to competition. the less vigorous isolates being abundant only during the dry seasons. This observation agrees with that of Widden (11: 1986) who suggested that such a trend may exist because fungi abundant during relatively unfavourable times of the year are not good competitors and therefore should be considered as survivors. Clarke and Christensen (1981) also reported heightened activity in population of infrequent taxa in the spring.

Soil fungi have been known to exist in the soil in the form of both spores and mycelial fragments which ramify the soil. When soil is plated. the colonies arise from both spores and mycelial fragments (Harley and Waid. 1955: Christensen. 1989; Garret. 1981). Therefore. mucorales being saprophytes found mostly in the upper 5 cm form of mineral soil (A_1 horizon) (Chapter 4) could

DISCUSSION AND CONCLUSION

The results on the effect of the abiotic factors on species composition of mucoraceous fungi reveal that the major source of variation in the distribution of these fungi among the different ecological sites was soil moisture content (Figure 33). Soil moisture availability was a limiting factor in the grassland. wooded grassland and shrubland ecosystems resulting in poor occurrence of mucoraceous fungi. Karura forest was rich in mucoraceous fungal population and had the highest average soil moisture content of 22.250 $^{w}/_{w}$ %. The other ecological sites had each a mean lower than 7 $^{w}/_{w}$ %.

Dowding and Widden (1974) concluded that pH. temperature and moisture were the most important factors affecting the composition of the mycoflora over 21 arctic and alpine tundra sites. Bissett and Parkinson (11: 1979) also reported that temperature. moisture. K. and pH were the most important variables affecting fungal composition in the alpine environment. An examination of Figures 30 - 32 shows that rainfall totals and patterns. and air temperature differences among these ecological sites were comparatively small and so could not be the reason for the absence of these fungi in the wooded grassland and shrubland soils.

Further studies within Karura forest showed that rainfall seasonal pattern and the soil moisture content were the most important factors in determining mucoraceous species abundance and diversity. Clear cyclical changes in the qualitative and quantitative nature of populations of mucorales were observed. Many species declined during the wet periods of the year. some disappearing altogether. and tended to increase in the dry periods (Figures 34 - 36). Some

CHAPTER 5

THE INFLUENCE OF SOIL PHYSICAL AND CHEMICAL FACTORS ON THE DISTRIBUTION OF MUCORALES

ABSTRACT

Soil physical and chemical properties and the availability of suitable nutrients are some of the factors that determine the distribution of fungi. These factors, in turn, depend on the composition of the vegetation cover. The replacement of the Natural forests with plantations has raised the suspicion that such interferences with the environment has affected the population of the soil biota. To test this hypothesis, the variation of soil physical and chemical properties with vegetation cover and depth was studied in relation with the distribution and abundance of mucoraceous fungi.

About 1 kg of soil was collected from the natural forest, *Eucalyptus*, *Cuppressus*, and *Araucaria* plantations in Karura forest at the following depths: 0 - 2, 10 - 15, 25 - 30 cm. Mucoraceous fungi were recovered from the soil samples and the remaining portion of the soil used to determine the moisture content, temperature, pH, concentration of Na, K, Ca, Mg, P, Mn, Fe, Cu, Zn, N and C. Analyses of variance tests were performed on each of the variables to test the significance of the differences observed and these were compared with the distribution and abundance of mucoraceous fungi. The following factors showed significant variation with site and depth: K, Ca, Cu, N, Mg, pH, temperature and moisture. However, K, N, Mg, moisture and temperature were the most important factors that positively correlated with the occurrence of mucoraceous fungi. The natural forest that had the greatest frequency of isolation of mucoraceous fungi, also had the highest amount of K, N and Mg in the soil and moderate temperature decreasing with depth. The

accumulation of litter in the plantations indicated a negative effect on the occurrence of the fungi. *Araucaria* plantation presented the thickest litter mat and the lowest species diversity and abundance.

The results proved the contribution of vegetation cover on the nature of the soil environment and subsequently on mucoraceous fungal distribution and abundance. The conservation of the existing natural forests is therefore a priority and their destruction means loss of habitat and biodiversity.

INTRODUCTION

Global changes in land use are affecting the biological composition of terrestrial ecosystems. Internationally, many developing countries are under severe economic pressure to turn fields and forests into monocropped estates of crops mainly for export. Madeley and Warnock (1995) exclaimed that when higher export earnings are possible, the value of the biodiversity being lost may be forgotten. The conversion of more and more land to intensive agriculture, urban centres, roads has become a threat resulting into the disappearance of tropical forests and loss of habitats. According to Madeley and Warnock (1995) tropical forests are being destroyed at a rate of at least 15.4 million hectares a year. Karura forest is one of the few remaining forest patches in Kenya. A large portion of this forest, however, has been replaced with modern residential buildings, roads and plantations. This implies that the forest, as a natural habitat for some organisms is endangered.

The major source of soil organic substrates is plant litter. Heal *et al.*, (1989) and Tian (1992) stated that the amount and nature of nutrients released during decomposition of these substrates, to the

soil, depends mostly on litter type. The type of litter on the other hand is determined by vegetation cover. Facelli and Pickett (1991), while studying dynamics of plant litter, reported that litter alters the physical and chemical environment directly and indirectly. The availability of suitable nutrients and the soil physical and chemical properties, however, are some of the factors that have been reported to influence fungal distribution and abundance (Angel and Wicklon, 1975; Bissett and Parkinson, 1979 I; Kjoller, A. and Strume, S. 1982, 1989; Mishusten, 1975; Park, 1976a, b; Shameemullah, 1971; Stenton, 1953; Stephenson, 1988, 1989; Warcup, 1951; Wicklan, D.T. and Carroll, G.C., 1981; Widden, 1986 I, III;).

Replacement of the natural forests with plantations in Karura forest has therefore raised some concern for such activities have disrupted physical and chemical properties of the soil and in turn affecting the occurrence of mucoraceous fungi. The changes in these factors with soil depth have also been studied in relation to the distribution of these fungi.

OBJECTIVES

(a) To study the variation of the following soil factors with vegetation types and soil depth in Karura forest:

(i) Physical factors of the soil:

Soil moisture

Soil temperature

Soil texture

(ii) Chemical factors of the soil:

pH, concentration of exchangeable Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), available Phosphorus (P), extractable Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Total Nitrogen (N), Organic Carbon (C).

(b) To relate the distribution of mucorales in Karura forest to the physical and chemical factors listed in (a) above and also with depth.

MATERIALS AND METHODS

Soil samples were collected between April 1992 and April 1993, on a monthly basis. The samples were obtained from square pits, 30 cm by 30 cm and 30 cm deep. The litter cover was measured against the pit using a ruler. The following sites were sampled: plantations of *Cupressus lusitanica*, *Eucalyptus paniculata* and *Araucaria araucana* and the natural forest.

Approximately 1 kg of soil was taken from the following range of depths: 0 - 2 cm; 10 - 15 cm and 25 - 30 cm, using sterilized metal spades, beginning from the depth 25 - 30 cm to reduce contamination from above. The soil samples were collected in sterilized paper bags and brought to the laboratory for isolation of mucoraceous fungi using the soil plate and the dilution plate methods (Chesters and Thornton, 1956; Warcup, 1950). The remaining soil samples were used for physical and chemical analyses as described below. The texture of soil from all the sample sites was tested by pipette methods (Garrett, 1981; Oloitan and Lambin, 1984; Griffin, 1992).

The soil moisture content was determined each time samples were collected for fungal isolation. It was determined by weighing 0.001 g accuracy. The soil was dried overnight at 103°C with the lid removed. The dried soil was removed from the oven. lid closed. and cooled in a desiccator and weighed. The moisture content was taken to be the loss in weight of the sample (A - B) expressed as a percentage of the original weight of the soil (A - tare tin). van Reevwijk. 1987:

$$\text{Moisture (\%)} = \frac{\text{A - B}}{\text{A - tare tin}}$$

The soil pH was obtained by adding 50 ml water to 20 g soil. The mixture was stirred. for 10 min. left to stand for 30 min and stirred again for 2 min. The pH of the supernatant liquid was then measured using pH meter.

Organic carbon was determined by the Watley-Black method using the procedure outlined by van Reevwijk. 1987. This involved a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid. After the reaction. the residual dichromate was titrated against ferrous sulphate.

Total nitrogen was estimated by the Kjeldahl method. The sample was digested in sulphuric acid and organic nitrogen converted to ammonium sulphate. The solution was then made alkaline and ammonia (NH_3) distilled. The evolved NH_3 was trapped in boric acid and titrated with standard acid (van Reevwijk. 1987).

The Oslen method was used to determine available P. The sample was extracted with a sodium

bicarbonate solution of pH 8.5. The concentration of exchangeable K and Mg in the soil was determined by atomic absorption spectrometry (van Reeuwijk, 1987).

Extractable Fe and Mn were determined by dithionite extraction method. The samples were shaken with a complexing and reducing buffer of sodium citrate and sodium dithionite. Fe and Mn were measured in the extract by atomic absorption spectrometer. Extractable calcium was determined by shaking the sample with water, and selectively precipitating calcium from the extract by adding acetone. The precipitate was re-dissolved in water and the calcium determined by measuring its concentration in the solution (van Reeuwijk, 1987).

The soil temperature in these sites was obtained by inserting soil thermometers (model - Negretti and Zambia, Casella London) at the following depths: 5, 15, 25 cm. The thermometers were inserted in place and left to settle for an hour before readings could be taken. The remaining portion of the thermometers above the ground were covered with aluminium foil. The soil temperatures were taken twice a week at two hour intervals from 8.00 a.m. to 6.00 p.m.

Analyses of variance were performed on each of the soil physical and chemical variables to test the significance of differences observed among the means and Duncan's Multiple Range Test done to compare the relative magnitude of variation with depth within and among the sites.

RESULTS

Distribution of mucoraceous fungi in Karura Forest.

The soil was a red-brown earth with a clay texture in all the sample sites. The litter layer varied in size from site to site as shown in Table 9. *Eucalyptus* plantation had the thickest litter cover of 10 cm while the Natural forest had the thinnest cover of 2 cm. Within the Natural Forest, the litter cover was at different stages of decomposition while in *Eucalyptus*, *Cupressus* and *Araucaria* stands, the litter cover consisted mostly of undecomposed branches, leaves and fruits of the tree. In *Eucalyptus* and *Araucaria* stands, the litter cover formed a mat covering most of the ground.

Table 9 shows that the total number of species recovered from the Natural forest was greater (36 species) than those from *Cupressus* (26 species), *Eucalyptus* (23 species) and *Araucaria* (10 species) plantations. These differences were significant at the 99.9% level of probability with an F-Value of 11.0007 (Table 10). The frequency of isolation of the common species, *Mucor* sp. was also higher in the Natural forest than in other stands. The variation in distribution of mucoraceous fungi with depth was also significant at the 99.9% level of probability with an F-value of 52.4202 (Table 10).

Table 9: Frequency (%) of isolation of mucoraceous fungi in Karura Forest at varying soil depths.

Habitat / Soil depth / Mucoraceous fungi	Natural forest				*Cupressus lusitanica* plantation				*Eucalyptus paniculata* plantation				*Araucaria araucana* plantation			
	Litter cover 2 cm	0 - 2 cm deep	10 - 15 cm deep	25 - 30 cm deep	Litter cover 5 cm	0 - 2 cm deep	10 - 15 cm deep	25 - 30 cm deep	Litter cover 10 cm	0 - 2 cm deep	10 - 15 cm deep	25 - 30 cm deep	Litter cover 8 cm	0 - 2 cm deep	10 - 15 cm deep	25 - 30 cm deep
Absidia cylindrospora	0	57.1	14.3	0	0	85.7	42.9	42.9	0	71.4	42.9	0	0	14.3	0	0
Absidia zychae	0	0	0	0	0	28.6	0	0	0	0	0	0	0	0	0	0
Actinomucor elegans	0	14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Circinella sp.	0	14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Circinella simplex	0	14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cunninghamella echinulata	0	42.9	0	0	0	14.3	0	0	0	14.3	0	0	0	0	0	0
Cunninghamella elegans	0	14.3	14.3	0	0	28.6	0	0	0	14.3	0	0	0	0	0	0
Gilbertella persicaria	0	0		0	0	57.1	14.3	0	0	71.4	42.9	0	0	0	0	0
Helicostylum elegans	0	57.1		0	0	14.3	0	0	0	0	14.3	0	0	0	0	0
Mortierella bainieri	0	14.3	0	0	0	28.6	0	0	0	0	0	0	0	0	0	0
Mucor ambiguus	0	0	0	0	0	0	0	0	0	0	14.3	0	0	0	0	0
Mucor fragilis	0	14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mucor globosus	0	28.6	0	0	0	14.3	0	0	0	28.6	0	0	0	0	0	0
Mucor hiemalis	0	71.4	14.3	14.3	0	28.6	28.6	0	0	42.9	28.6	0	0	0	0	0
Mucor lamprosporus	0	14.3	14.3	0	0	0	0	0	0	5	0	0	0	0	0	0
Mucor mucedo	0	14.3	14.3	0	0	0	14.3	0	0		0	0	0	0	0	0
Mucor plumbeus	0	100	0	42.9	0	100	42.9	28.6	0	14.3	42.9	42.9	0	14.3	28.6	14.3
Mucor racemosus	0	71.4	0	14.3	0	71.4	42.9	0	0	0	14.3	14.3	0	28.6	0	0
Mucor spinosus	0	42.9	42.9	28.6	0	28.6	28.6	0	0	71.4	0	0	0	14.3	0	0
Mucor subtilissimus	0	57.1	42.9	14.3	0	28.6	14.3	14.3	0	28.6	0	14.3	0	57.1	0	14.3
Mucor varians	0	14.3	28.6	0	0	0	0	0	0	28.6	0	0	0	0	0	0
Mucor zychae	0	0	14.3	0	0	0	0	0	0	14.3		0	0	0	0	0
Rhizopus echinatus	0	42.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus microsporus	0	0	0	0	0	14.3	0	0	0	14.3	0	3	0	14.3	0	0
Rhizopus nigricans	0	28.6	14.3	0	0	0		0	0	14.3	0	0	0	0	0	0
Rhizopus oryzae	14.3	14.3	0	0	0	0		0	0	0	0	0	0	28.6	0	0
Total number of species isolated	1	21	10	5	0	14	8	4	0	13	7	3	0	7	1	2

Table10: Vertical Distribution of Mucoraceous fungi in soil: Analysis of Variance Table

K Value	Source	Degrees of Freedom	F Value	Probability
1	Replication (Vegetation stands)	3	11.0007	0.0000 ***
2	Factor A (Depth)	2	52.4202	0.0000 ***
4	Factor B (Time)	6	0.3426	
6	AB	12	0.2869	
8	Factor C (Fungi)	23	8.4434	0.0000 ***
10	AC	46	6.9312	0.0000 ***
12	BC	138	0.6733	
14	ABC	276	0.6180	
-15	Error	1509		

Figure 42 shows a marked falling off in the frequency of isolation of these fungi with depth in all the sample sites. Most of the less frequent species. *Absidia zychae. Actinomucor elegans. Helicostylum elegans. Circinella rigida. C. simplex. Mucor lamprosporus. M. varians. M. zychae. Rhizopus nigricans*. were recovered from the top soil (Table 9). Similar trend was observed with species abundance as displayed in Figure 43. All the species decreased significantly with depth some disappearing altogether at the depth of 25 - 30 cm e.g. *Circinella. Cunninghamella* and *Gilbertella* species. No isolate was obtained from the litter layer in all the sites except in the Natural forest that *R. nigricans* was recovered only once from rotting fruits.

Physical and chemical characters of the soil.

Table 11 shows that significant differences with depth within the sites were observed for Na. K. Mn and Fe in the Natural forest: Na. K. Ca. Mg. Fe. Cu and Zn in *Araucaria* stand: P. N. C. soil moisture and temperature in the *Cupressus* stand and K. Mg. C and soil temperature in the *Eucalyptus* stand. Figure 44 shows that all these variables decreased with depth except for Fe and Cu in *Araucaria* stand. and soil moisture in *Cupressus* stand which increased with depth. The population of mucoraceous fungi also decreased with depth in all the sites. Significant difference with depth among sites were observed for certain elements only and for soil moisture and temperature. Table 12 shows that K. Ca. Cu and soil moisture varied significantly with site at all depths. N varied significantly with depth at the levels of 0 - 2 cm and 10 - 15 cm while pH and Mg were significant at the depths of 10 - 15 cm and 25 - 30 cm. Temperature varied with depth at the levels of 0 - 2 cm and 25 - 30 cm.

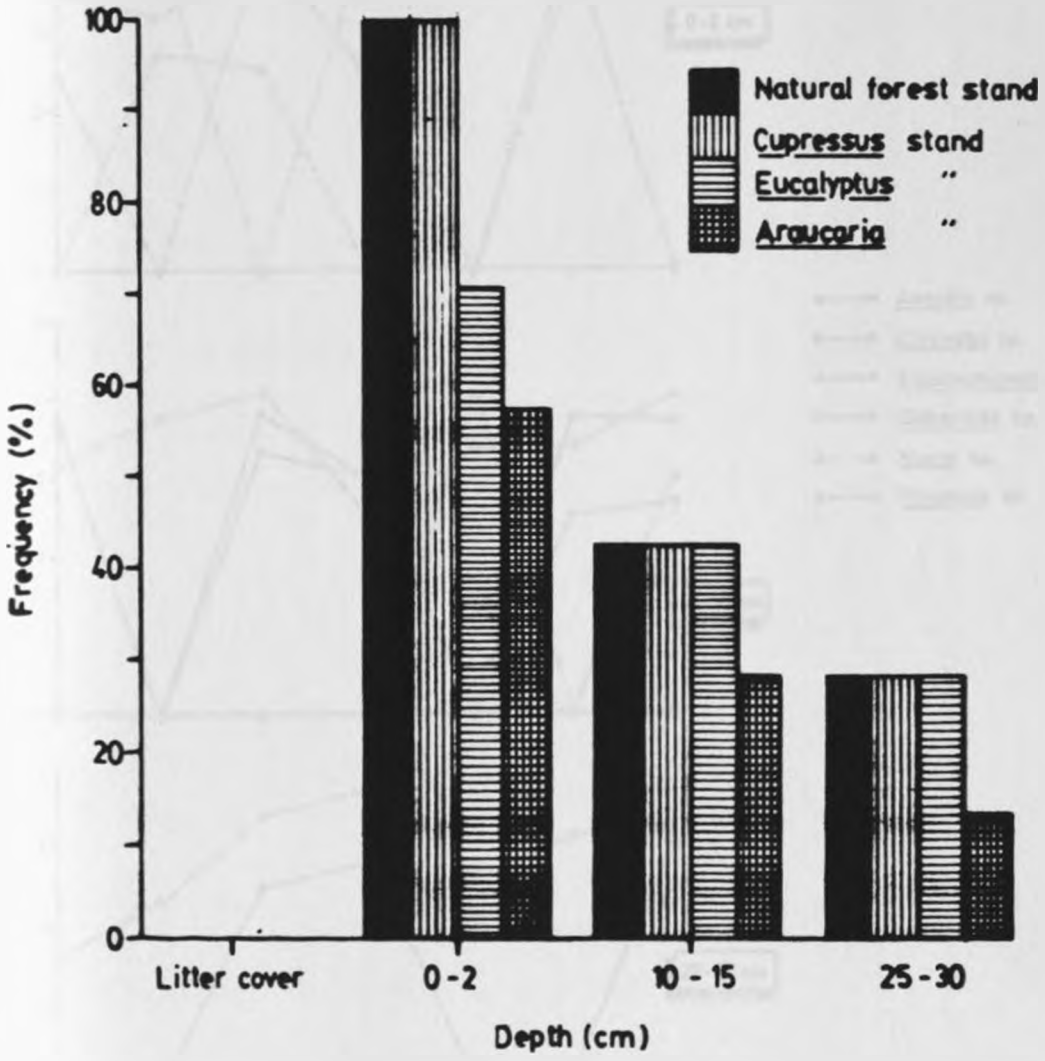

Fig. 42: Frequency of isolation of mucoraceous fungi in Karura Forest at varying soil depths.

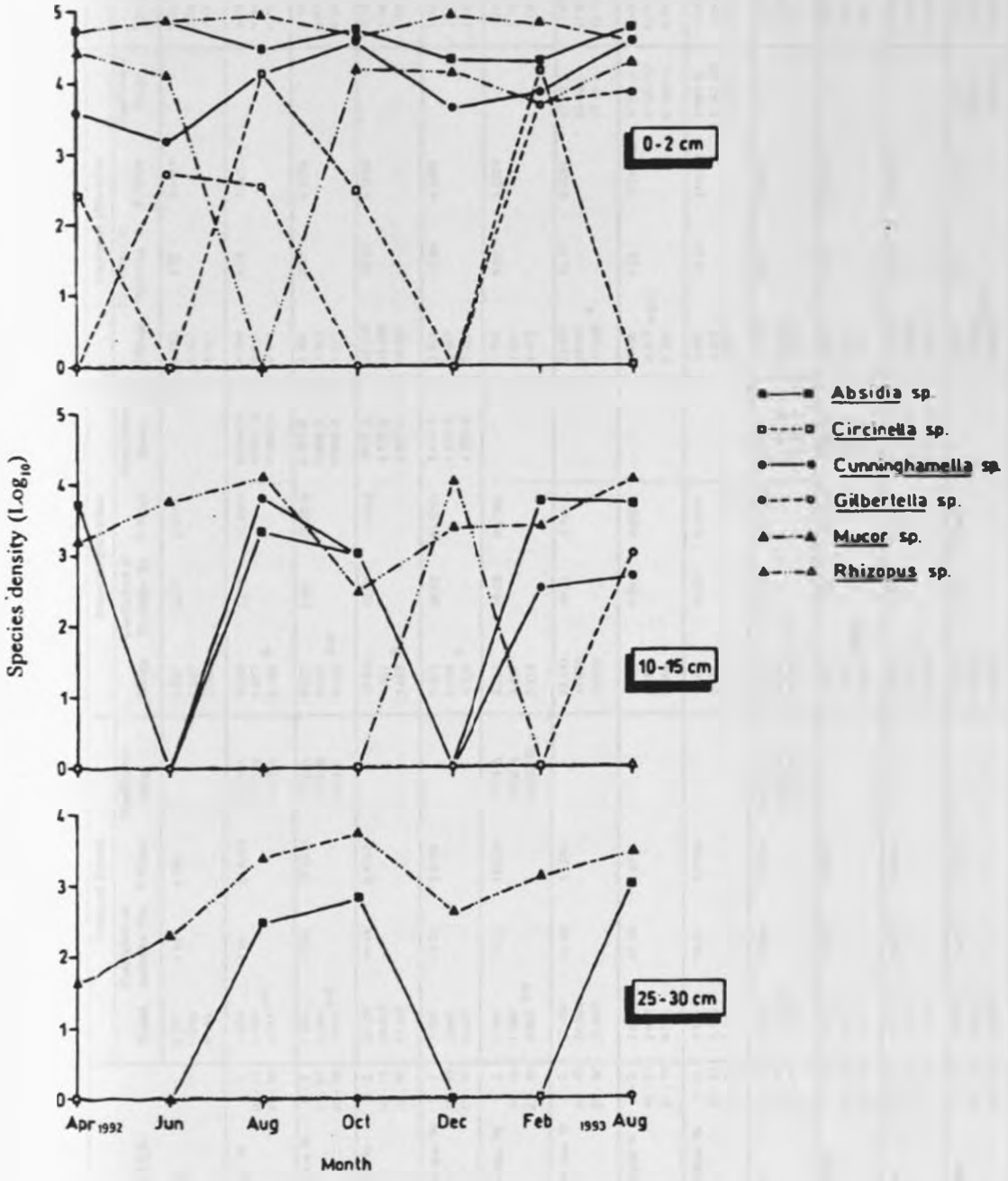

Fig. 43: Variation of dominant mucorales species abundance* with depth

Table 11: Variation of physical and chemical characters with depth: Analysis of Variance Table

PARAMETER		Natural forest				*Araucaria* plantation				*Cupressus* plantation				*Eucalyptus* plantation			
		Means	Coefficient of variation (%)	F-Value	Significance Range	Means	Coefficient of Variation %)	F-Value	Significance Range	Means	Coefficient of Variation %	F-Value	Significance Range	Means	Coefficient of Variation %	F-Value	Significance Range
pH		6.733 6.600 6.533	1.33	4.000	-	6.867 5.833 5.500	12.37	2.7041	-	6.867 6.467 6.167	5.72	2.6747	-	5.667 5.700 5.433	4.61	0.9500	-
Sodium m.e°. %	0 - 2	1.033	6.19	23.6123	1.033 A	0.690	12.79	11.4343	0.6900 A	0.853	6.75	5.8120	-	0.740	29.51	0.0651	-
	10 - 15	0.837			0.837 B	0.457			0.4567 B	0.783				0.867			
	25 - 30	0.737 **			0.731 B	0.460 *			0.4600 A	0.707				0.783			
Potassium m.e. %	0 - 2	1.980	12.01	19.2347	1.980 A	0.977	8.60	21.3879	0.977 A	1.500	7.17	6.3376	-	0.973	14.54	14.4704	0.971 A
	10 - 15	1.540			1.540 B	0.660			0.660 B	1.367				0.877			0.8767 A
	25 - 30	1.053 **			1.053 C	0.680 **			0.680 B	1.217				0.500 *			0.5000 B
Calcium m.e. %	0 - 2	21.267	16.53	0.1717	-	19.333	49.83	11.1135	19.33 A	22.133	32.44	4.1056	-	4.900	45.31	3.9375	-
	10 - 15	19.467				4.533			4.533 B	13.600				2.600			
	25 - 30	15.400				3.667 *			3.667 B	10.833				1.800			
Magnesium m.e. %	0 - 2	3.397	11.78	3.4154	-	3.633	28.02	11.7024	3.633 A	3.367	14.89	4.5329	-	2.700	19.72	15.3338	2.700 A
	10 - 15	3.047				1.933			1.933 B	2.943				1.533 *			1.533 B
	25 - 30	2.637				1.200 *			1.200 B	2.320				1.163 *			1.163 B
Manganese m.e. %	0 - 2	1.950	17.51	13.8541	1.950 A	2.033	10.15	0.1750	-	1.710	8.99	2.4524	-	2.333	12.40	4.9631	-
	10 - 15	1.680			1.680 A	2.067				1.693				1.343			
	25 - 30	0.867 **			0.8667 B	2.133				1.473				1.080			
Phosphorus m.e. %	0 - 2	32.000	21.69	3.1290	-	25.333	29.11	4.908	-	25.000	11.15	8.7885	25.00 A	13.667	38.73	1.4370	-
	10 - 15	24.000				15.000				22.667			22.69 B	21.667			
	25 - 30	21.000				13.000				17.000 *			17.00 B	14.333			
Total Nitrogen %	0 - 2	1.673	41.36	4.8741	-	0.060	0.00	0.048	-	0.750	1.26	1951.0029	0.700 A	0.630	43.21	4.0043	-
	10 - 15	1.013				0.280				0.527 ***			0.5267 B	0.273			
	25 - 30	0.543				0.060				0.360			0.3600 C	0.350			
Organic carbon %	0 - 2	7.343	38.21	3.4528	-	4.743	26.07	6.4370	-	3.987	11.65	13.0276	3.987 A	4.530	33.39	14.3780	4.530 A
	10 - 15	4.893				2.933				3.227			3.227 AB	1.460			1.460 B
	25 - 30	3.167				2.297				2.427 *			2.427 B	1.380 *			1.38 B
Iron p.p.m.	0 - 2	48.467	10.94	8.7549	48.47 A	68.590	0.47	232.5873	68.59 C	57.247	8.08	0.5034	-	113.150	71.79	0.9531	-
	10 - 15	40.820			40.82 AB	71.507			71.507 B	54.247				57.240			
	25 - 30	33.207 *			33.21 B	74.460 ***			74.460 A	58.420				61.03			
Copper p.p.m.	0 - 2	1.373	18.85	4.0187	-	2.140	1.49	19.9300	2.140 B	1.420	7.48	1.7500	-	2.147	28.54	0.8830	-
	10 - 15	1.097				2.247			2.247 A	1.320				1.660			
	25 - 30	0.887				2.310 ***			2.310 A	1.270				1.657			
Zinc p.p.m.	0 - 2	33.723	20.54	6.6916	-	30.810	0.07	8.6529	30.810 A	29.570	18.48	4.8622	-	21.317	18.08	1.8454	-
	10 - 15	21.317				24.313			24.313 B	27.550				17.877			
	25 - 30	19.767				20.640 **			20.640 C	18.447				16.180			
Soil moisture	0 - 2	23.407	5.64	3.1016	-	17.410	5.48	2.8493	-	16.867	3.36	15.4995	16.867 C	16.595	10.33	3.0227	-
	10 - 15	22.424				17.267				17.708			17.708 B	14.911			
	25 - 30	23.944				18.272				18.424 ***			18.424 A	16.549			
Soil temperature	0 - 2	16.998	5.27	0.3599		16.612	35.82	0.6581	-	17.313	3.65	163.2019	17.31 A	18.286	4.24	196.5661	18.29 A
	10 - 15	16.896				16.610				16.624 ***			16.62 B	17.169			17.17 B
	25 - 30	16.015				16.055				16.218			16.22 C	16.895 ***			16.89 C

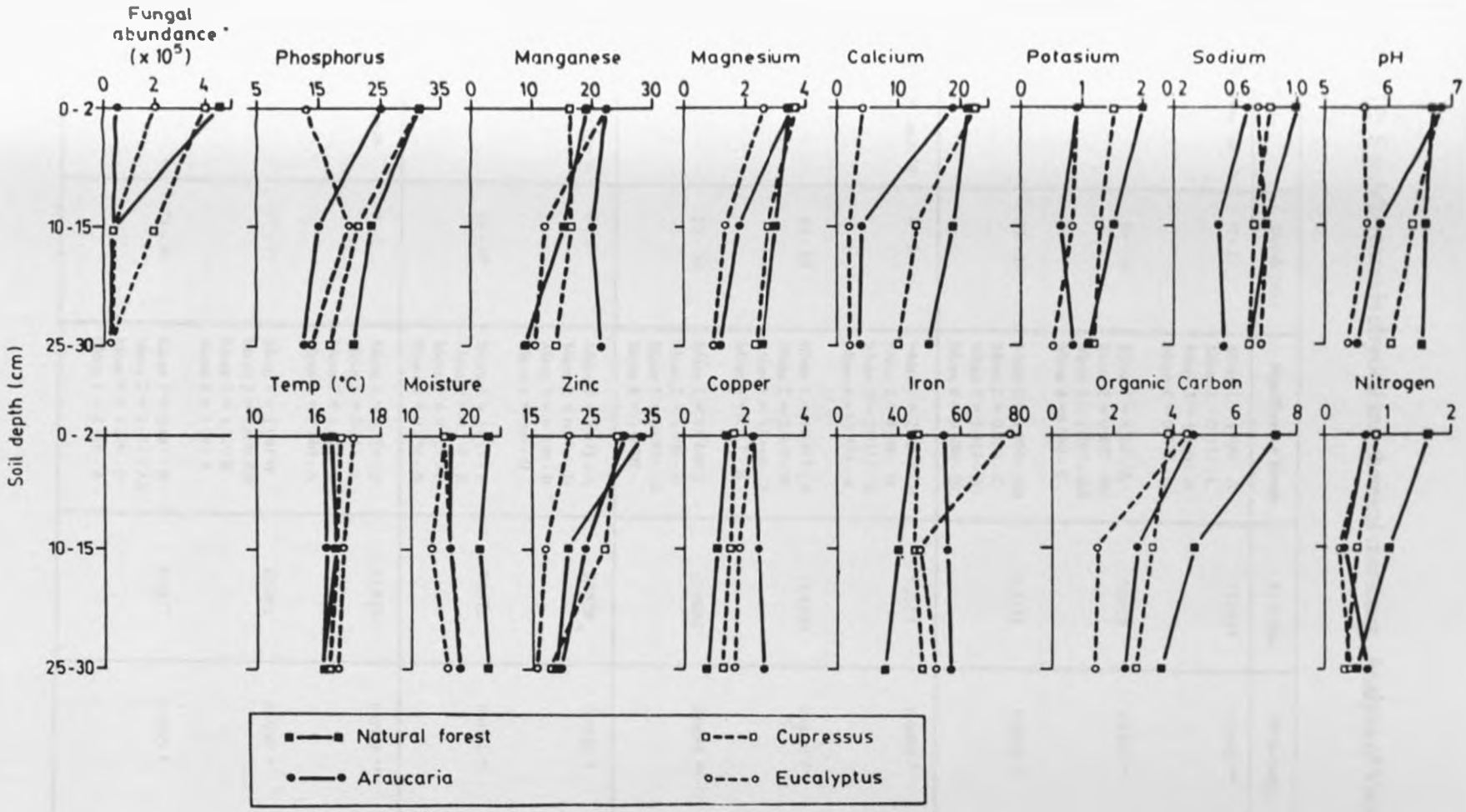

Fig. 44: Effect of soil physical and chemical factors on mucoraceous fungal abundance*

* Abundance based on propagule densities (pooled) calculated from number of colonies per dilution level from dilution plates.

Table 12: Site differences in physical and chemical characters: Analysis of Variance Table

Source	Depth (cm)	Significance Range	F- Value	Probability
Potassium m.e %	0 - 2	Mean 1 = 1.980 - A Mean 2 = 0.973 - C Mean 3 = 1.500 - B Mean 4 = 0.977 - C	13.8023	0.0042 **
	10 - 15	Mean 1 = 1.54 - A Mean 2 = 0.827 - BC Mean 3 = 1.367 - AB Mean 4 = 0.660 - C	5.6028	0.0367 *
	25 - 30	Mean 1 = 1.053 - AB Mean 2 = 0.500 - C Mean 3 = 1.217 - A Mean 4 = 0.680 - BC	6.1253	0.0294 *
Calcium m.e. %	0 - 2	Mean 1 = 21.267 - A Mean 2 = 4.900 - B Mean 3 = 22.133 - A Mean 4 = 19.333 - A	5.2517	0.0408 *
	10 - 15	Mean 1 = 19.467 - A Mean 2 = 2.600 - B Mean 3 = 13.600 - A Mean 4 = 4.533 - B	13.8790	0.0042 **
	25 - 30	Mean 1 = 15.400 A Mean 2 = 1.800 - B Mean 3 = 10.833 - A Mean 4 = 3.667B	13.9682	0.0041 **
Total Nitrogen %	0 - 2	Mean 1 = 1.673 - A Mean 2 = 0.630 - B Mean 3 = 0.700 - B Mean 4 = 0.60 - B	5.6828	0.0346 *
	10 - 15	Mean 1 = 1.013 - A Mean 2 = 0.237 - B Mean 3 = 0.527 - B Mean 4 = 0.280 - B	7.8975	0.0166 *
Copper p.p.m.	0 - 2	Mean 1 = 1.373 - B Mean 2 = 2.147 - A Mean 3 = 1.420 - B Mean 4 = 2.140 - A	13.8425	0.0042 **
	10 - 15	Mean 1 = 1.097 B Mean 2 = 1.660 AB Mean 3 = 1.320 B Mean 4 = 2.247 A	6.3002	0.0277 *
	25 - 30	Mean 1 = 0.887 - B Mean 2 = 1.657 - AB Mean 3 = 1.270 - B Mean 4 = 2.310 - A	6.3857	0.0269 *

Table 12 Cont.

Magnesium m.e. %	10 - 15	Mean 1 = 3.047 - A Mean 2 = 1.533 - B Mean 3 = 2.943 - A Mean 4 = 1.933 - B	10.7183	0.0080 ***
	25 - 30	Mean 1 = 2.637 A Mean 2 = 1.163 B Mean 3 = 2.320 AB Mean 4 = 1.200 B	4.9968	0.0453 *
pH	10 - 15	Mean 1 = 6.600 A Mean 2 = 5.700 C Mean 3 = 6.467 AB Mean 4 = 5.833 BC	5.0580	0.0441 *
	25 - 30	Mean 1 = 6.533 A Mean 2 = 5.433 B Mean 3 = 6.167 A Mean 4 = 5.500 B	11.6312	0.0065 **
Soil temperature	0 - 2	Mean 1 = 16.998 C Mean 2 = 18.286 A Mean 3 = 17.313 B Mean 4 = 16.672 D	76.0190	0.0000 ***
	25 - 30	Mean 1 = 16.015 C Mean 2 = 16.895 A Mean 3 = 16.618 B Mean 4 = 16.055 C	24.2239	0.0000 ***
Soil moisture	0 - 2	Mean 1 = 23.407 A Mean 2 = 16.595 B Mean 3 = 16.867 B Mean 4 = 17.410 B	98.5769	0.0000 ***
	10 - 15	Mean 1 = 22.424 A Mean 2 = 14.922 C Mean 3 = 17.708 B Mean 4 = 17.267 B	122.6114	0.0000 ***
	25 - 30	Mean 1 = 23.944 A Mean 2 = 16.549 C Mean 3 = 18.424 B Mean 4 = 18.272 B	156.7805	

'm.e = milliequivalent
* - significant at 95% level of probability
** - very significant at 99% level of probability
*** - very highly significant at 99.9% level of probability

A B C Duncan's Multiple Range Test ranging the level of difference

Mean 1 = Natural Forest
Mean 2 = *Eucalyptus* stand
Mean 3 = *Cupressus* stand
Mean 4 = *Araucaria* stand

The remaining elements i.e. Na. Mn. P. Fe and Zn did not show any significant difference with site at all the three depths tested.

(a) Potassium

The amount of K in the soil at the depths 0 - 2 cm and 10 - 15 cm was highest in the Natural forest (at 0 - 2 cm = mean of 0.977 m.e.% and 10 - 15 cm = mean of 0.877 m.e%) stands. However at 25 - 30 cm depth. the amount of K was high in *Cupressus* stand and least in *Eucalyptus* stand (Table 12).

b) Calcium

The amount of calcium in *Eucalyptus* stand was very low (4.900 m.e.%) at the top soil as compared to other stands. The Natural forest (21.267 m.e.%) *Cupressus* (22 - 133 m.e.%) and *Araucaria* (19.333 m.e.%) stands. did not show any significant differences in their amount of the element. Calcium showed a decrease with depth for all the stands but this was drastic in *Araucaria* stand (10 - 15 cm = mean 4.533 m.e.% 25 - 30 cm = mean 3.667 m.e.%). The Natural forest and *Cupressus* stands. however had the greatest amount of the element at all the soil depth levels (Table 12).

(c) Copper

Araucaria stand had the highest amount of Cu at all the three soil depths tested. Table 12.

(d) Nitrogen

Total nitrogen in the soil was not significantly different with sample sites at the soil depth of 25 - 30 cm. However at the levels 0 - 2 cm and 10 - 15 cm deep. it was highest in the Natural forest (1.673% and 1.013% respectively) but similar in the other stands. Table 12.

Magnesium

The Natural forest and *Cupressus* stands had the highest levels of magnesium in the soil. Table 12.

pH

The soil pH was the same at the top soil for all the sample sites. However with depth. *Eucalyptus* and *Araucaria* stands became more acidic compared to the other stands. Table 12.

Soil temperature

Soil temperature decreased with depth in all the sample sites. *Eucalyptus* stand had the highest at all depths and *Araucaria* stand the least.

Soil moisture

The Natural forest had the highest soil moisture content and *Eucalyptus* stand the least.

DISCUSSION

The vegetation cover influenced greatly the physical and chemical conditions of the soil. This together with soil depth affected the abundance and diversity of mucoraceous fungi thus confirming the hypothesis. Mucoraceous fungi were abundant in the top soil of the Natural forest as compared to the *Eucalyptus*, *Cupressus* and *Araucaria* plantations, decreasing with depth in all the sample sites.

The soil variables that showed significant variation with vegetation cover and depth were K, Ca, Cu, N, Mg, pH, temperature and moisture. However K, N and moisture were the variables that correlated with the occurrence of mucoraceous fungi. Soils from the natural forest had the highest amount of K, N and moisture, decreasing with depth. The same trend was portrayed by

mucoraceous fungi. The other variables Ca. Cu. Mg. pH and temperature. though significantly different with vegetation cover at certain depths. did not present a pattern similar to that shown by the fungi (Table 12).

These results agree with those of Widden (III. 1986) who reported temperature. moisture. K. N and to a lesser extent pH. as factors that determine the distribution of fungi. Bissett and Parkinson (III. 1979) also reported temperature. moisture. K and pH as factors influencing fungal composition in alpine tundra. Dowding and Widden (1974) concluded that temperature. moisture and pH were the most important factors affecting the composition of the mycoflora over 21 arctic and alpine tundra sites. The importance of soil pH. temperature and moisture either separately or in combination. on the distribution of soil fungi has been found in many other studies (Bissett and Parkinson. 1979b: Brown. 1958; Christensen. 1969; Gochenaur. 1978; Morall. 1974; Sewell. 1959; Shammeemullah and Parkinson. 1971; Warcup. 1951; Widden and Abitbol. 1980;).

The trends in variation of Na. Mn. Fe. P. Zn and C with vegetation cover were not significant and therefore did not contribute to the differences observed in the distribution of mucoraceous fungi among the sites. However these elements varied significantly with depth within individual stands. These within differences could have contributed to the distribution pattern of these fungi observed within the sites. The natural forest showed variation of Na. Mn and Fe with depth. Soil temperature. P and C in *Cupressus* plantation decreased with depth. Several workers have also reported a decrease in frequency of isolation of fungi with depth relating it to a drop in nutrient content of the soil with depth (Bissett and Parkinson (I). 1979; Soderstrom. 1975; Stenton. 1953; Warcup. 1951; Wicklow *et al*., 1974).

The accumulated litter in the plantations must have also contributed to the relatively low population of mucoraceous fungi in these sites. The thick mat, apart from determining the compounds released to the soil, must have influenced the soil environment physically by intercepting light, modifying the soil temperature and water dynamics of the soil and also acting as a mechanical barrier. The accumulation of litter in the plantations further suggested that the rate of decomposition in these sites was slow, a process that depends on the chemical composition of the litter. Berendse *et al.*, (1987) and Choudhury (1988) state that long-lived organs usually have more lignin and secondary chemicals than short-lived organs. Mucoraceous fungi are generally not lignin decomposers and such litter type would not favour them. The very low population of these fungi in *Araucaria* plantation indicates the influence of yet another variable. Production of phytotoxins either by leaching or decomposition of litter is suggested.

The soil pH and texture, however, were the same throughout the forest and were not limiting factors.

CONCLUSION

The composition of vegetation determines litter type which in turn affects the physical and chemical nature of the soil. These factors influence the occurrence of mucoraceous fungi and probably soil biota as a whole. The Natural forests produce litter which is composed of a wide variety of plant species and therefore nutrient-rich, as compared to the monocropped plantations with only one type of plant residue. Replacement of the Natural forests with plantations means destruction of habitats and interferences with soil nutrient dynamics. The soil physical and chemical characteristics also vary with depth.

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CHAPTER 6

SOIL LEAD (Pb) CONCENTRATION AND EFFECT ON THE DISTRIBUTION OF MUCORACEOUS FUNGI

ABSTRACT

Lead is one of the major heavy metal environmental pollutants. Automotive exhaust fumes are one of the leading sources of high lead levels in the environment. Much of this is deposited close to the roads. The tremendous increase in traffic density in Kenya raises the suspicion that the emission of harzadous levels of lead into the environment is a daily phenomena. Microorganisms could be good indicators of such pollution hence this study on the implications of lead from motor vehicles on the occurrence of mucoraceous fungi.

The study was carried out along Kiambu road which is a heavy traffic exit from the city of Nairobi. The fungi were recovered from the top soil collected from the roadside habitat and forest stand using soil plate and dilution plate methods. The soil lead content was determined by atomic absorption spectrometry. The effect of lead on the occurrence, growth and sporulation of the fungi was studied by incorporating different concentrations of the heavy metal into the culture media.

Lead concentration of up to 160 $\mu g\ g^{-1}$ was recorded from the roadside, habitat soils. This decreased with increase in distance away from the road. The mucoraceous fungi showed a decline in frequency of occurrence and species diversity with increasing lead pollution load in the soil. However, *Cunninghamella* sp. were predominantly present in the heavily contaminated soils and showed good *in vitro* growth in lead agar medium with upto $100\mu g\ g^{-1}$ of lead. The principal conclusion from this study is that there is potential for *C. elegans*, the common species of *Cunninghamella*, to be used as a lead pollution indicator.

INTRODUCTION

Conservation of biodiversity is a major theme of research among scientists today. The major source of the current decline in biodiversity is human activity through mismanagement of the earth's resources and introduction of harmful products into the environment. Due to the high environmental level of many pollutants, there has been a considerable increase in monitoring air, water and foodstuffs and provision of maximum limits (FAO/WHO 1976; Government Printer, 1978; Philips *et al*., 1982; Talbot and Chegwidden, 1982). However these maximum limits have been set with reference to humans, while ignoring microorganisms, which also form a large component of the earth's biodiversity and are part of major food chains. Concern about interferences with the environment of microorganisms have been raised by Hawksworth (1991); Hawksworth and Colwell (1992); Younes (1992) and Madeley and Warnock (1995). However, these authors have ignored the crucial comparison of pollutants with biological effects in microorganisms. In other cases, as indicated by Masood (1996), the maximum limits set have been exceeded.

One of the main sources of environmental metal toxicity problems is lead. Heavy metals like lead are known to have cummulative effects since they are not easily detoxified, and thus pollute the environment either by direct toxicity, interference with the respiratory and photosynthetic balance or by impairment of the diversity and stability of an ecosystem (Fairey and Gray, 1970; Doelman and Haanstra, 1979; Kabata-Pendias, 1979; Morrison *et al*., 1989). High concentrations of lead have been detected in the roadside soils and dust of urban districts and this has been attributed

largely to automobile exhaust fumes (Muskett and Jones. 1980; Rodriguez and Rodriguez. 1982; Nasralla. 1984). In Kenya. the number of registered motor vehicles has been increasing steadily and as a result raising the environmental lead burden.

Consequently. the present study was undertaken to investigate the influence of lead arising from automobile exhaust fumes. on the distribution of soil mucorales. Mucoraceous fungi have been known for their decomposition and fermentation properties. These properties have been applied commercially in the production of organic acids. vitamins and foodstuffs (Gray. 1970; Lockwood. 1975; Prakash and Sarbhoy. 1993; Sharma and Sarbhoy. 1984;). Preservation of the habitat of these fungi together with their inventory becomes an essential requirement for their effective management and conservation.

OBJECTIVE

To evaluate the:

(i) implications of lead from motor vehicles on the distribution of mucoraceous fungi.

(ii) effect of lead on the in vitro growth and sporulation of mucoraceous fungi.

Hypothesis

Lead particles in soil affect the distribution of mucorales.

MATERIALS AND METHODS

Study Area

Sampling was done along Kiambu road, in Karura Forest. Kiambu road traverses Karura Forest connecting Nairobi City to Kiambu town, and has traffic volume of about 800 vehicles per hour.

Sampling

Top soil up to 2 cm deep was collected at a metre interval from the road, in a line perpendicular to the road into the forest. Background samples were taken beyond 30m into the forest. The soil samples were collected in sterilized paper bags, as described in Chapter 3, and brought to the laboratory for isolation of mucoraceous fungi and lead content analysis.

Isolation of Fungi

Mucoraceous fungi were recovered from the soil by Warcup's soil plate method (Warcup, 1950) and the Dilution Plate techniques (Chester and Thornton, 1956) as described in Chapter 3.

Quantification of Lead in Soil

Laboratory Apparatus

The glassware were soaked for 24 hours in washing detergent, rinsed with chromic acid, then rinsed with running tap water and soaked in 10% (v/v) Nitric acid (HNO_3) for 24 hours. Whenever required for immediate use, the glassware were again rinsed with tapwater and then with de-ionised water and oven-dried at 90°C.

Sample bottles (polypropylene bottles) were preconditioned by soaking in detergent solution for 24 hours, rinsing in tapwater, then leached in 10% (v/v) Nitric acid (HNO_3) for at least another 24 hours. The bottles were then rinsed with tapwater followed by deionised water and oven dried at 60°C.

Analytical procedure

(a) Reagents

All the chemicals used were of analytical reagent grade:

Hydrochloric Acid. HCL	BERK SPENCER ACIDS LTD.
Sulphuric Acid. H_2SO_4	"
Lead Nitrate ($Pb(NO_3)_2$	BDH CHEMICALS LTD.

(b) Soil Pretreatment and Digestion

Soil samples were oven-dried at 105°C for 24 hours in 100ml beakers and then sieved using a 1 mm pore sieve. The large particles, mostly stones and pebbles were discarded. Using a pestle and mortar, the fine soil was ground into a fine powder.

To 5.000 g of dry soil 10 ml hydrochoric acid (3N HCl) and 10 ml Sulphuric acid (1N H_2SO_4) were added. The boiling tubes were then placed in an aluminium hot block, which was heated on a hot plate at 90-100°C. The digests were heated with occasional stirring for 2 hours, allowed to cool,

then filtered through acid washed filter paper No. 40 into a 100 ml volumetric flask. The filter paper was washed with deionised water and the filtrate made up to 50 ml with deionised water. The filtrate was contained in preconditioned polypropylene bottles, ready for atomic absorption spectrophotometer (AAS) analysis. The residue was discarded (Perkin-Elmer, 1976).

(c) AAS Analysis

Following the pretreatment and digestion methods already described, each sample was analysed for lead in triplicate using the AAS (Perkin-Elmer Model 2380 - Perkin Elmer Co., 1980). Blanks were also analysed in triplicate. Measurements were made at 217.0 nm. The absorbance was recorded three times for each sample and the average obtained.

Calibration Standards

Stock Solution (1.000 ppm)

1.000 ppm Pb ions in 0.5 M Nitric acid (HNO_3) was prepared by weighing 1.6108 g of 99.5% lead nitrate [$Pb(NO_3)_2$] into a one litre volumetric flask. 31.70 cm^3 of 70% nitric acid (HNO_3) added. The solution was diluted to the mark by adding deionised water.

Aliquots of the stock solution were used to prepare calibration standards containing 1.000, 4.000, 8.000, 12.000, 16.000 and 20.000 ppm. Calibration standards were freshly prepared from the stock solution whenever required.

Effect of lead on mucoraceous fungal growth and sporulation

Potato dextrose agar (PDA) containing Pb was prepared by adding lead nitrate [$Pb(NO_3)_2$] to the media before autoclaving. $Pb(NO_3)_2$ was chosen as the source of Pb ions because of its high solubility in water compared with other Pb salts. The concentrations of nitrate $(NO_3)^-$ component of the salt incooperated within the media was also within the range of that found in the soils.

A litre of the following concentrations of Pb were prepared: 1, 2, 5, 10, 50, 100, 200, 300, 600, 1.000, 2.000 ppm (μgml^{-1}). 25 g of PDA was added to each flask containing the above solutions and autoclaved. A portion of the media was used in measuring pH. This was to make sure that changes in pH with addition of $Pb(NO_3)_2$ were not too high to influence fungal growth. The remaining amount of media was dispensed in Petri plates and used for culturing mucoraceous fungi as shown below:

(i) Samples of soil from the roadside (edge of road) and forest habitats were serially diluted to a concentration of 1:5.000 (Chapter 3). The dilutions were inoculated in plates with Pb concentrations mentioned above and incubated. The colonies of mucoraceous fungi that grew were identified and counted on the seventh day.

(ii) Isolates of *Mucor subtilissimus*, *Absidia cylindrospora* , and *Cunninghamella elegans* were inoculated on the plates with the different concentrations of Pb ions. Growth was observed and spore counts done on the 4th day, using an Improved Neubaer Haematocytometer - Spencer Bright-line at 400 times magnification under a microscope (Abraham and Schrot, 1970).

RESULTS

Soil mucoraceous fungal population distribution as a function of soil lead (Pb) content along Kiambu road in Karura forest.

Figure 45 shows that mucoraceous fungi were more frequently recovered from the forest soils than on the roadside stand. *Mucor* sp. were more abundant in the forest habitat while *Cunninghamella* sp. were more frequently isolated from the roadside habitat. *Absidia* sp. were absent along the road. This is illustrated in Table 13. Figure 45 also shows that the Pb levels in soil was high in the soil from the roadside habitat decreasing with increasing distance from the road and finally constant background levels are reached within the forest.

Influence of Pb on isolation of mucoraceous fungi from the soil using dilution plate technique

Table 14 shows that Pb affected the isolation of mucoraceous fungi from the soil. The number of colonies per species decreased with increasing amount of Pb in Petri plates. Plates without Pb (PDA alone) had several colonies per species while those with 200 μgml^{-1} of Pb and above showed no growth.

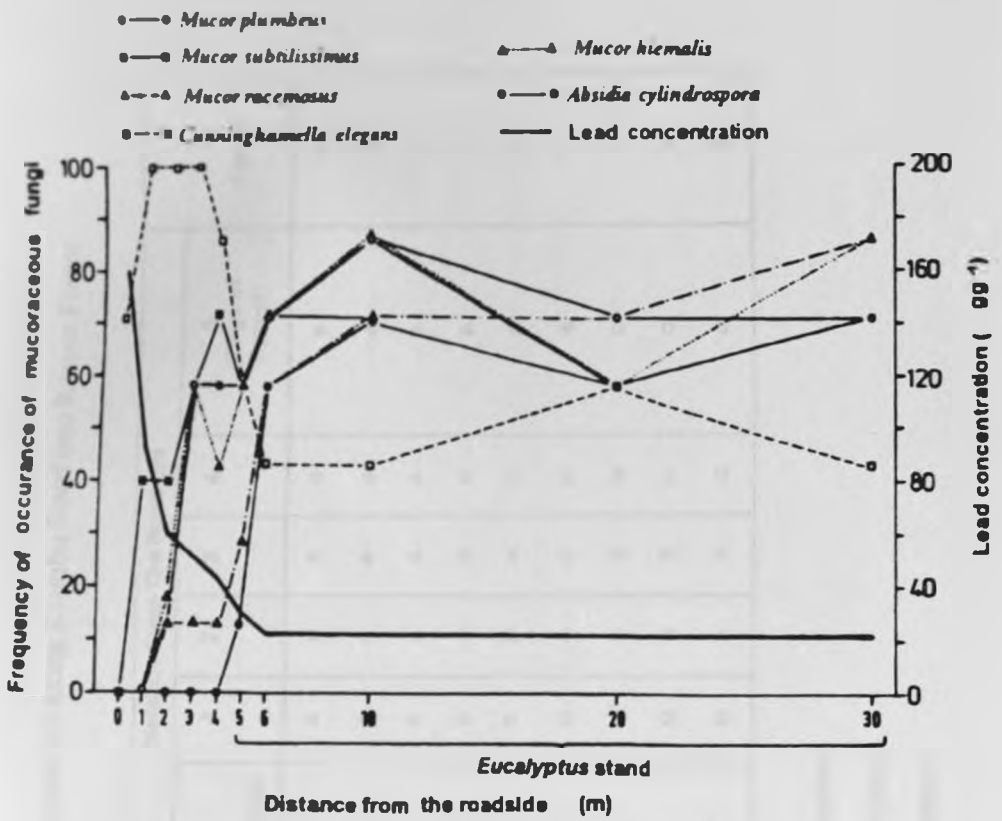

Fig. 45: Occurrence of some common soil mucoraceous fungi in relation to the amount of lead (Pb) in the soil and distance from the busy main road

Table 13: Mucoraceous fungal population along Kiambu Road into Karura Forest.

Species	Distance From The Road (m)						Forest
	0 (Edge of road)	1	2	3	4	5 (Edge of Forest)	
Mucor plumbeus	▫	▲	▲	●	●	●	●
Cunninghamella elegans	●	●	●	●	▲	▲	▲
Mucor hiemalis	▫	▲	▲	▲	▲	▲	▲
Mucor subtilissimus	▲	●	▲	▫	▫	▲	▲
Mucor racemosus	▫	▫	▫	▲	▲	▲	▲
Absidia cylindrospora	▫	▫	▫	▫	▫	▲	●
Cunninghamella echinatus	▫	▫	▲	▲	▫	▫	▫
Absidia zychae	▫	▫	▫	▫	▫	▫	▲
Rhizopus oryzae	▫	▫	▲	▲	▫	▫	▫

● > 50% frequency of isolation

▲ < 50% frequency of isolation

▫ 0% frequency of isolation

Table 14: Effect of lead (Pb) on the recovery of some mucoraceous fungi from the soil using the dilution plate method.

Habitat (Source of sample soil)	Mucoraceous fungi isolated from the sample soil	Number of colonies per Petri dish*			
		PDA	PDA with 50 µgml $^{-1}$ of Pb	PDA with 100 µgml $^{-1}$ of Pb	PDA with 200, 300, 600, 1, and 2,000 µgml $^{-1}$ of P
Eucalyptus stand	*Absidia* sp.	3	2	0	0
	Cunninghamella sp.	2	1	1	0
	Mucor sp.	14	11	2	0
Roadside stand	*Absidia* sp.	0	0	0	0
	Cunninghamella sp.	6	4	2	0
	Mucor sp.	4	3	1	0

* Mean of 6 readings

Effect of Pb on the growth and sporulation of some mucoraceous fungi.

Addition of Pb into growth media resulted in decreased growth rate and sporulation of *Absidia cylindrospora*, *Cunninghamella elegans* and *Mucor subtilissimus*. Petri plates with Pb concentration of 1-10 ppm had good growth similar to the control while those with concentration of 50 ppm and above presented poor growth as shown in Plate 1. Plates subjected to 50 and 100 μgml^{-1} of Pb exhibited sparse (thin) and slower growth rate compared with the control, which had thick luxuriant growth. Plates with 200 μgml^{-1} of Pb and above had no growth (Table 15). This difference in growth rate was significant at 99.9% level of probability and with an F-value of 156.8249 as indicated in Table 16. Duncan's Multiple Range Test displayed in Table 17 indicates that the growth rate of the different isolates remained the same in the control and the Petri plates with Pb up to 10 μgml^{-1} but slowed down with increase in the amount of Pb in the media.

Similar effects of Pb on spore production by these fungi is indicated in Table 18.

Changes in pH of the growth media due to addition of Pb $(NO_3)_2$ was small. Petri plates with 1, 2, 5, 10, 50, 100, 200, 300, 600, 1,000, 2,000, 0 (control) ppm of Pb presented pH of 4.90, 4.90, 4.60, 4.70, 4.80, 4.80, 4.75, 4.75, 4.55, 4.55, 4.50, 5.2 respectively.

Table 15: Effect of lead (Pb) on the radial growth of some mucoraceous fungi

Taxa / Media	Radial Growth (mm)*		
	Absidia cylindrospora	*Cunninghamella elegans*	*Mucor subtilissimus*
PDA (Control)	7.45	7.70	8.42
PDA with:			
1 µgml $^{-1}$ of Pb	7.40	7.70	8.50
2 µgml $^{-1}$ of Pb	7.383	7.70	8.40
5 µgml $^{-1}$ of Pb	7.50	7.717	8.55
10 µgml $^{-1}$ of Pb	7.467	7.70	8.40
50 µgml $^{-1}$ of Pb	6.533	6.117	6.933
100 µgml $^{-1}$ of Pb	5.40	4.283	4.183
200, 300, 600 1,000 and 2,000 µgml $^{-1}$ of Pb	0	0	0

* Average of six readings

Table 16: Effect of Pb on the radia growth of some mucoraceous fungi: Analysis of Variance Table

Source	Degrees of Freedom	Sum of Squares	Mean Square	F - Value	Probability
Replication	5	0.075	0.015	2.1252	0.0685
Media	6	167.681	27.947	3963.6422	0.0000***
Fungi	2	10.874	5.437	771.1494	0.0000***
Media x Fungi	12	13.269	1.106	156.8249	0.0000***
Error	100	0.705	0.007		
Total	125	192.604			

Coefficient of variation 1.16%

Table 17: Duncan's Multiple Range Test ranking the variation in radial growth rate of some mucoraceous fungi with addition of lead (Pb) in the culture media.

Taxa / Media	*Absidia cylindrospora*		*Cunninghamella elegans*		*Mucor subtilissimus*	
	Mean Radial Growth (mm)*	Similarity Code	Mean Radial Growth (mm)*	Similarity Code	Mean Radial Growth (mm)*	Similarity Code
PDA (Control)	7.450	DE	7.700	C	8.417	B
PDA with:						
1 $\mu g ml^{-1}$ of Pb	7.383	DE	7.700	C	8.500	AB
2 $\mu g ml^{-1}$ of Pb	7.383	E	7.700	C	8.400	B
5 $\mu g ml^{-1}$ of Pb	7.500	D	7.717	C	8.550	A
10 $\mu g ml^{-1}$ of Pb	7.467	DE	7.700	C	8.400	B
50 $\mu g ml^{-1}$ of Pb	6.533	G	6.117	H	6.933	F
100 $\mu g ml^{-1}$ of Pb	5.400	I	4.283	J	4.183	K
200, 300, 600 1,000 and 2,000 $\mu g ml^{-1}$ of Pb	0	L	0	L	0	L

*Mean with the same similarity code are the same. For example, A = A; AB is in a transition between A and B.

Table 18: Effect of lead (Pb) on sporulation of some species of mucorales

Taxa	Media	Average Number of Spore Counts on Haemocytometer*	Number of Spores per ml of the Suspension (x 10^4)
Absidia cylindrospora	PDA (Control)	81	20.25
	PDA with:		
	1 µ gml $^{-1}$ of Pb	84	21
	2 µ gml $^{-1}$ of Pb	79.5	19.875
	5 µ gml $^{-1}$ of Pb	81.4	20.35
	10 µ gml $^{-1}$ of Pb	82	20.5
	50 µ gml $^{-1}$ of Pb	18.4	4.6
	100 µ gml $^{-1}$ of Pb	4	1
Cunninghamella elegans	PDA (Control)	46.2	11.55
	PDA Plus:		
	1 µ gml $^{-1}$ of Pb	45	11.25
	2 µ gml $^{-1}$ of Pb	44.2	11.05
	5 µ gml $^{-1}$ of Pb	45.1	11.275
	10 µ gml $^{-1}$ of Pb	44	11
	50 µ gml $^{-1}$ of Pb	8.6	2.15
	100 µ gml $^{-1}$ of Pb	5.2	1.3
Mucor subtilissimus	PDA (Control)	96.2	24.05
	PDA Plus:		
	1 µ gml $^{-1}$ of Pb	89.2	22.3
	2 µ gml $^{-1}$ of Pb	76.1	19.025
	5 µ gml $^{-1}$ of Pb	82.4	20.6
	10 µ gml $^{-1}$ of Pb	72	18
	50 µ gml $^{-1}$ of Pb	32.5	8.125
	100 µ gml $^{-1}$ of Pb	8	2

* Average of six readings

Plate 1: Effect of lead (Pb) on the radial growth of mucoraceous fungi; (a) *Absidia cylindrospora* (b) *Cunninghamella echinulata;* and (c) *Mucor subtilissimus*

DISCUSSION

The amount of Pb in the roadside habitat increased with decreasing distance from the road. The population and diversity of mucoraceous fungi on the other hand increased with increasing distance from the road. These results suggest that the presence of the heavy metal influenced the occurrence of these fungi. Several workers have also reported that heavy metals affect the composition of microfungal flora. Nordgren *et al.*, (1985) while studying soil microfungi of coniferous forest surrounding a brass mill at Gusum in southeast Sweden, reported a decrease in isolation frequency of fungi common in coniferous forest soils close to the mill. Freedman and Hutchinson (1980), Jordan and Lechevalier (1975) and Kendrick (1962), have also reported adverse effects of copper, zinc and nickel-copper on fungal occurrence. While the frequency of isolation of all the other genera of mucorales decreased with proximity to the road, *Cunninghamella* sp. remained abundant. This suggests tolerance to some extent of this genus to an amount of Pb. This could be due to the evolution of highly successful tolerance mechanisms to this metal or low availability of the metal. This presumed tolerance of *Cunninghamella* sp. to some amount of Pb requires confirmation by *in vitro* studies with radiolabelled Pb. If such properties are confirmed, then the most common species, *Cunninghamella elegans*, could be used as a biological indicator of Pb pollution. Other workers have also recorded tolerance of fungi to heavy metals (Nordgren *et al.*, 1983; Tatsuyama *et al.*, 1975; Williams *et al.*, 1975). *Cunninghamella elegans* have also been shown to metabolise fluorene, a tricyclic aromatic hydrocarbon detected in gasoline and diesel engine exhaust by Jairaj *et al.* (1993).

Fluorene has been included on the list of priority pollutants by the U. S. Environmental Protection Agency. This fungus also metabolises other compounds, naphthalene and acenaphthene reported by Cerniglia *et al* . (1983) and Pothuluri *et al*., (1992) respectively.

The Pb concentration in soil fell off rapidly with increasing distance from the road. These results agree with those of previous investigations and support the supposition that vehicle exhausts are largely responsible for the roadside environmental Pb contamination (Branquinho and Robinson, 1976; Muskett and Jones, 1980; Rodriguez and Rodriguez, 1982; Ward *et al*.,1975). Hence there is a need to monitor the concentration of this metal in the environment since the numbers of registered motor vehicles in Kenya is on the increase.

Results from the *in vitro* studies proved strongly that Pb interferes with growth and sporulation of mucoraceous fungi. At concentrations of 200 μgml^{-1} and above, the metal inhibits spore germination and growth of the fungi. Concentrations of 50 - 100 μgml^{-1} of Pb also affected the fungi resulting in reduced rate of growth and sporulation. Lower concentrations of Pb (10 μgml^{-1} and below) seemed not to affect the fungi. Although the physiological effect of Pb on the cell contents of mucoraceous fungi was not studied, the available evidence stated above indicates that the maximum tolerable level (concentration) of the metal by the fungi lies between 10 - 50 μgml^{-1}. A more precise concentration level would be established by physiological studies of dose-response relationship, an area that is not fully understood (Morrison *et al*., 1989). These results confirm the explanation given by Morrison *et al*., that when the capacity of a cell to detoxify accumulated metal

is exceeded. damage to the cell will occur. Morrison states further that signs of intracellular metal toxicity may include ultrastructural deformities as well as reductions in cell division rate.

The use of $Pb(NO_3)_2$ in the *in vitro* studies was appropriate since the incorporation of the compound in the growth media did not change the pH significantly. The amount of nitrate [$(NO_3)^-$] part of the compound introduced into the growth media was within the range of that found in the soil (Chapter 3).

CONCLUSION

Lead is present as a contaminant of the roadside environment. Its effects on mucoraceous fungi are adverse and this could be the case with other microorganisms in the roadside ecosystem. There is a need to monitor the concentration of this heavy metal in the environment given that the numbers of registered motor vehicles in Kenya is on the increase. Provision of maximum limits based on average environmental concentrations should also take into account that not all populations are equally sensitive and about equally exposed. Soil microorganisms could be suffering a greater than average lead intake than other members of the ecosystems.

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APPENDICES

APPENDIX 1

Soil Digestion for Lead Analysis

To confirm the reliability of the use of HCl-H_2SO_4, HCl-HF digestion was also performed with the same soil samples. The soil samples were pretreated as described in the text. To 5.000 g of dry soil in a boiling tube was added 10 ml concentrated hydrofluoric acid (HF) and 10 ml of concentrated hydrochloric acid (HCl). The boiling tubes were then placed in an aluminium hot block which was heated on a hot plate at 100°C. The digests were heated with occasional stirring to dryness. The procedure was repeated with similar portions of HF and HCl acids. 5 ml of HCl and 5 ml of HF were then added to the digest and again evaporated to dryness. The residue was then dissolved in 5 ml concentrated HCl by warming. 15 ml deionised water was added to the residue, warmed and then filtered. The filtrate was made up to 50 ml ready for AAS analysis. Readings from the HF-HCl digestion ad those of HCl-H_2SO_4 digestion were similar as shown below:

Lead Content of roadside soil as a function of distance from the road: Two soil digestion methods compared.

Distance from road (Kiambu road) (m)	Soil digestion method	Lead concentration (ug g^{-1}) $HCL-H_2SO_4$ Digestion	HCL-HF Digestion
0		160.6	162.33
1		92.7	91.3
2		60.5	65.2
3		52.84	53.12
4		44.33	42.61
5		30.40	32.10
6		14.28	14.21
10		13.74	13.15
20		13.21	13.02
30		13.51	13.00

APPENDIX 2

Spore counts on Haemocytometer

The Improved Neuber Haematocytometer was used. Counts were done on the large square which has sixteen small squares. 1 small square, as indicated on the haemocytometer is 1 mm deep and has an area of $1/400mm^2$.

Average count per large square = X (16 small squares)

$$\therefore \text{1 small square will have } \frac{X \times 1}{16} \text{ counts}$$

But volume of each small square = Area x Depth

$$= 1/400mm^2 \times 0.1 \text{ mm}$$

$$= \frac{X}{16} \text{ counts are in } 1/400 \text{ mm}^3.$$

$$1.000 \text{ mm}^3 \text{ (1 ml) will have } \frac{X}{16} \times 1.000 \times 400$$

$$= \frac{1.000X \times 4000}{16 \times 1}$$

$$= \frac{4X \times 10^6}{16}$$

$= 4X \times 16^6$ counts/ml

Factor = $¼ \times 10^6$

Counts obtained within the large square (16 small squares) were multiplied by this factor ($¼ \times 10^6$) to get the actual number of spores per ml.

APPENDIX 3

PREPARATION OF LEAD NITRATE SOLUTION FOR FUNGAL CULTURES

A. Lead nitrate was chosen as a source of Pb because of its high solubility in water unlike other salts of Pb. To know the different weights of the salt to be dissolved in water in order to obtain the required concentrations. the following calculations were done:

$Pb(NO_3)_2$ molecular weight = 331.21

Pb atomic weight = 207.1%

Purity of $Pb(NO_3)_2$ used = 99.5%

1 g solute dissolved in 1L solvent 1.000 ppm (mg/L or $\mu g\ ml^{-1}$. $mg\ kg^{-1}$. $\mu g\ ml^{-1}$)

331.21 g $Pb(NO_3)_2$ = 207.19 g Pb

? = 1 g

$$= \frac{331.21 \times 1}{207.19}$$

= 1.598 g if 100% purity.

But the salt is of 99.5% purity

100% = 1.5986

99.5% = ?

$$= \frac{1.5986 \times 99.5}{100} = 1.6066 \text{ g of salt}$$

1.6066 g of salt dissolved in 1 Litre of water - 1.000 $\mu g\ ml^{-1}$ (mgl^{-1} ppm)

From this stock solution dilutions were made to obtain the required concentrations with respect to Pb.

B. It was also important that the amount of nitrate ($NO3^-$) incooperated in the agar medium was within the range found naturally in the soil. To find out the amount of nitrate in the medium the following calculations were carried out.

Molecular weight $(NO_3)_2$ = 124.02

Molecular weight Pb = 207.19

Molecular weight of $Pb(NO_3)_2$ = 331.21

$Pb(NO_3)_2$ 331.21 g = 124.02 g NO_3^-

$Pb(NO_3)_2$ (100% purity 2. 670 = 1 g NO_3^-

= 2.6840 g of NO_3^-

(99.5% purity) 1.6066 g Pb/L solution = 2.6840 g NO_3^-/L (99.5% purity)

If 1.000 ppm of $Pb(NO_3)_2$ with respect to Pb	=	1.6066 g of Pb in 1 L solution	= 2.6840 of NO_3^- in 1L solution

2 ppm (for example) = 3.2132 µg l Pb = 5.368 ppm NO_3^-

$$\frac{1.6066 \text{ g l} \times 2\text{ppm}}{1000 \text{ ppm}} = \frac{3.2132 \text{ µgml}^{-1} \times 12.6840 \text{ g l}}{1.6066 \text{ g/l}}$$

= 0.0032132 g l = 5.368 µg ml^{-1} of lead

= 3.2132 µg ml^{-1}

Others:

1. 2 ppm Pb = 5.368 $\mu g\ ml^{-1}$ (ppm) NO_3^-

2. 1 ppm Pb = 2.684 $\mu g\ ml^{-1}$ (ppm) NO_3^-

3. 5 ppm Pb = 13.42 "

4. 10 ppm Pb = 26.84 "

5. 50 " = 134.2 "

6. 100 " = 268.4 "

7. 200 " = 536.8 "

8. 300 " = 805.2 "

9. 600 " = 1610.4 "

10. 1.000 " = 4.294.4 "

11. 2.000 " = 8.588.8 "