SOIL FUNGI OF A COPPER SWAMP[1]

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Abstract

Microfungal and copper analyses were carried out on 35 soil samples derived from 10 profiles in and near a swamp containing cupriferous peat. Copper content varied from less than 1 to 68,000 parts per million (p.p.m.). While the fungus flora was generally sparse, populations of up to 9000 colonies/g of soil were found in peat horizons of high copper content, but fungi were very few or absent in leached inorganic horizons of low copper content. Thirty-one species of fungi were isolated: 12 hyphomycetes, 5 phycomycetes, 3 coelomycetes, 3 basidiomycetes, 2 pyrenomycetes, and 6 sterile forms. Thirteen species, including *Penicillium ochro-chloron*, the most prolific fungus encountered, were found exclusively in samples containing over 7500 p.p.m. of copper. Nine species, including the rare *Mucor ambiguus*, occurred only in samples containing less than 5 p.p.m. of copper. The distribution of the remaining nine species, which included several of the commoner soil saprophytes, was apparently unaffected by either high or low copper concentrations.

Introduction

Copper ore, derived from Pennsylvanian formations, was deposited in the lower layers of deep glacial drift lying beneath forest cover in the Aboushagan woods north of Sackville, New Brunswick. The water of springs emerging from the drift contained about 1 p.p.m. of copper. This copper was reprecipitated in the subsurface layer of forest peat, presumably by chelation with organic material, without disturbing the vegetation above it to any extent. About 60 years ago, a fire destroyed the vegetation overlying some of the peat. The increase in radiation to which the soil was subsequently exposed may have produced a higher soil evaporation rate, consequently drawing greater copper concentrations to the surface. Not only did vascular plants fail to re-establish themselves, but the roots of existing plants around the periphery of the open area were apparently exposed to intolerable copper concentrations and died. In the last 50 years the open area has grown in extent from about 200 square yards to over 5000 square yards, and the moss *Pohlia nutans* has become the dominant ground cover (2) (Figs. 1, 3). The peat is unusual in having an almost neutral reaction (pH 6.0–7.2) in contrast to the usual high acidity exhibited by this material. The swamp lies about 8 miles north of Sackville, at 46°00′ N. 64°21′ W.

To the mycologist, the copper swamp offers a unique opportunity to determine whether or not there exists here a community of soil fungi whose members have reached some state of equilibrium with, or tolerance to, the copper concentrations present in the peat.

Description of Terrain and Methods

It was necessary to obtain fungal counts from the different soil horizons and to plot vertical and horizontal fungal distribution in and near the swamp. A transect was accordingly chosen which ran across the swamp, passing through

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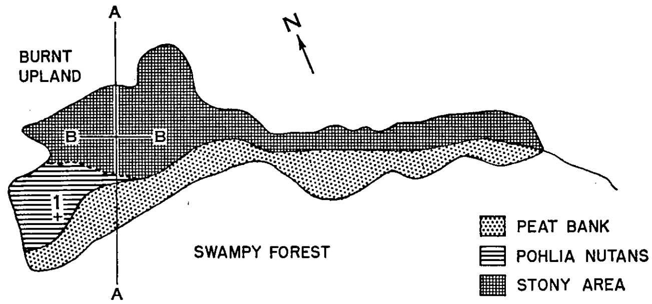

FIG. 1. Sketch map of the open area of the copper swamp (after Fraser). Scale 1:1600. A–A. Transect chosen for study. B–B. Direction of main longitudinal transect. 1. Position of profile 1 at stream source.

areas representative of the different surface features, soil profiles, and copper concentrations known to exist therein.

A longitudinal transect had previously been laid down on the open area of the swamp. This ran downhill WNW. to ESE. in the general direction of flow of the stream (Figs. 1, B–B, 3). The earlier transect was marked at 20 ft intervals by wooden pegs, and the transect for the present study (Fig. 1, A–A) was laid down perpendicular to the earlier transect at the peg marked 5180 ft. This transect ran from the swampy forest, across the exposed peat bank, stream, *Pohlia* bed, and stony area, onto the upland to the north, which was burned over in 1955. The transect crossed many representative areas at one of the widest parts of the clearing (Figs. 1, 2, 4, 5), and coincided with a transect used earlier by Fraser for the determination of copper gradients in the soil. Copper concentrations found in soil samples during the present study generally agreed well with values obtained by Fraser (Kerr-Addison Gold Mines Ltd., Toronto, private communication). However, he was primarily concerned with the deeper, undisturbed layers of the soil, and ignored the surface 10 cm in most cases, while no fewer than 21 of the samples taken during the present study were derived from this superficial layer, which is of the greatest biological significance.

Figure 2 diagrammatically represents the main features of topography and vegetation, and shows the location of 9 of the 10 profiles which were dug for sampling purposes. The 10th profile may be located on Fig. 1, and was dug at the source of the stream which normally runs along the edge of the peat bank. However, no surface water was seen during the time the samples were obtained, as the summer was exceptionally dry. This facilitated sampling, and helped to reduce contamination, especially at the stream source and in the stream bed on the transect. Profiles varied in depth, but none was deeper than 60 cm, as this depth proved quite adequate for experimental purposes. The usual precautions to ensure sterility were taken during sampling. Samples were chosen by inspection, and were taken from every visually distinguishable horizon in each profile, and at different depths in thick horizons. From three to five samples were derived from each profile, a total of 35 being collected from all 10 profiles. The points from which samples were derived are marked on Figs. 6–13. A brief description of the profiles follows.

(1) Fig. 6. At source of stream. *Vegetation*: young green *Pohlia nutans*. *Soil*: 15 cm black peat sediment; buff-colored sandy loam with hard inclusions of cemented sand grains.

(2) Fig. 7. On transect in forest. *Vegetation*: tree cover mainly *Picea mariana*, *Abies balsamea*; ground cover *Scapania nemorosa*, *Lophozia ventricosa*, *Bazzania trilobata*. *Soil*: 3 cm dark brown decaying moss with many strands of basidiomycete mycelium; 10 cm dryish friable brown organic matter with tree roots; 50 cm peat; sandy loam.

(3) Fig. 8. Discontinuous tree zone, limit of tracheophytes. *Vegetation*: scattered very young *Picea mariana*, *Abies balsamea*; no ground flora. *Soil*: 1–2 cm mixed decaying conifer litter and dried peat fragments; 50 cm peat; light reddish-brown sandy loam.

(4) Peat bank near stream. *Vegetation*: none. *Soil*: 5 cm powdery peat; 25–27 cm wet sticky peat; sandy loam.

(5) Fig. 9. Stream bed. *Vegetation*: none. *Soil*: 1–2 cm black peat sediment; 1 cm rust-colored sand; 12–13 cm blue-gray sand; light reddish-brown sand with bluish inclusions.

(6) Fig. 10. *Pohlia nutans* zone. *Vegetation*: surface mat of *Pohlia nutans*, *Soil*: 3 cm reddish-brown decomposing moss; 2 cm dark humus-infiltrated sand; 3 cm rust-colored sand; 12 cm light reddish-brown sand; 1 cm dark humus-infiltrated sand; light reddish-brown sand.

(7) Fig. 11. Stony area at 5180 ft on main transect. *Vegetation*: none, surface mostly covered by stones of various sizes. *Soil:* 5 cm dark stained sand; reddish sandy loam.

(8) Fig. 12. Stony area with *Pohlia*. *Vegetation*: dried up yellow-brown *Pohlia nutans* growing between stones. *Soil*: as profile (7).

(9) Fig. 13. Burnt upland. *Vegetation*: dead but standing *Picea mariana* and *Abies balsamea* scattered about; ground cover *Vaccinium myrtilloides* and *V. angustifolium* dominant. *Soil*: 1–1.5 cm loose sandy humus; 15 cm pale buff sand, shading to rust-colored sandy loam.

(10) Burnt upland. *Vegetation*: dead trees as profile (9); ground cover *Vaccinium myrtilloides* and *V. angustifolium*, now mixed with *Pteridium*

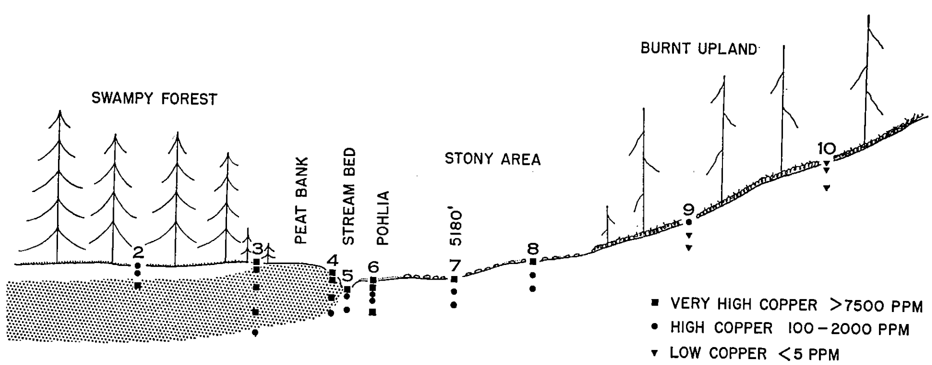

FIG. 2. Diagrammatic vertical section across the swamp along the transect, showing surface features, locations of profiles and samples, and distribution of the copper levels encountered. Scale: horizontal, 1:720; vertical (surface), 1:96; vertical (profiles), 1:48.

aquilinum and *Solidago* spp. *Soil*: 1–1.5 cm sandy humus; 7 cm pale buff sand, shading to rust-colored sandy loam.

Fungal Analysis

Samples were transported to the laboratory at the Biology Department, Mount Allison University, Sackville, on the day of collection, and 350 soil plates prepared from them. The media employed were malt extract agar, at pH 5.5, and, as a precaution against excessive bacterial growth, a modification of Martin's peptone-dextrose medium (8) in which aureomycin (30 mg/liter) was substituted for streptomycin. From each sample, five plates of each of the two media were prepared, 25 mg of soil being sprinkled onto every plate prior to the addition of agar. After incubation at room temperature (68–72°F) for 3 days, the plates were inspected and fungal colonies counted. Representatives of all apparently different isolates were subcultured onto malt extract agar slopes and brought to Ottawa for identification.

Copper Analysis

The soil samples were subsequently air-dried at 72–75° F, lightly ground in a mortar and pestle, and a 20-mg sample from each weighed into test tubes. These were shaken for 2 minutes with 5 ml of a solution containing 10% ammonium citrate and 10% hydroxylamine hydrochloride acidified to pH 2 with 1 *N* hydrochloric acid. After settling of the sediment, suitable aliquots (0.5–5 ml) were removed and more of the original extracting solution added when necessary to make up the volume to 5 ml. This solution was then shaken for 2 minutes with 5 ml of 0.001% Dithizone (diphenylthiocarbazone) in benzene, and compared with standards prepared from known amounts of copper.

For a water extraction, 100 mg of sample was shaken with 10 ml distilled metal-free water, then filtered. A 2-ml aliquot was then made up to 5 ml and treated with Dithizone as before.

Results

Wright *et al.* (14), in a study of trace element distribution in virgin soils, found that copper concentrations were usually low, and did not exceed 23 p.p.m. Values obtained in the present study ranged from about 1 p.p.m. up to 68,000 p.p.m. They are given in Table I. The values fell into three ranges: very low, 1–5 p.p.m.; high, 100–2000 p.p.m.; and very high, exceeding 7500 p.p.m. The distribution of these three levels of copper is indicated in Fig. 2.

Startling variations in vertical distribution of copper within many of the profiles are seen in Table I. It is clear from this table that all the highest values were obtained from the peat, which never contained less than 26,000

FIG. 3. General view of the open area of the swamp, looking ESE. The peat bank is on the right, the *Pohlia nutans* bed is in the left foreground, and the stony area in the left background. Profile 1 at the source of the stream is marked by the post in the foreground. FIGS. 4, 5. Views of the transect taken from the point where it crosses the main transect at 5180 ft. Fig. 4, looking NNE. across the stony area onto the burnt upland. Fig. 5, looking SSW. across the stony area, *Pohlia* bed, stream, and peat bank, into the swampy forest.

PLATE I

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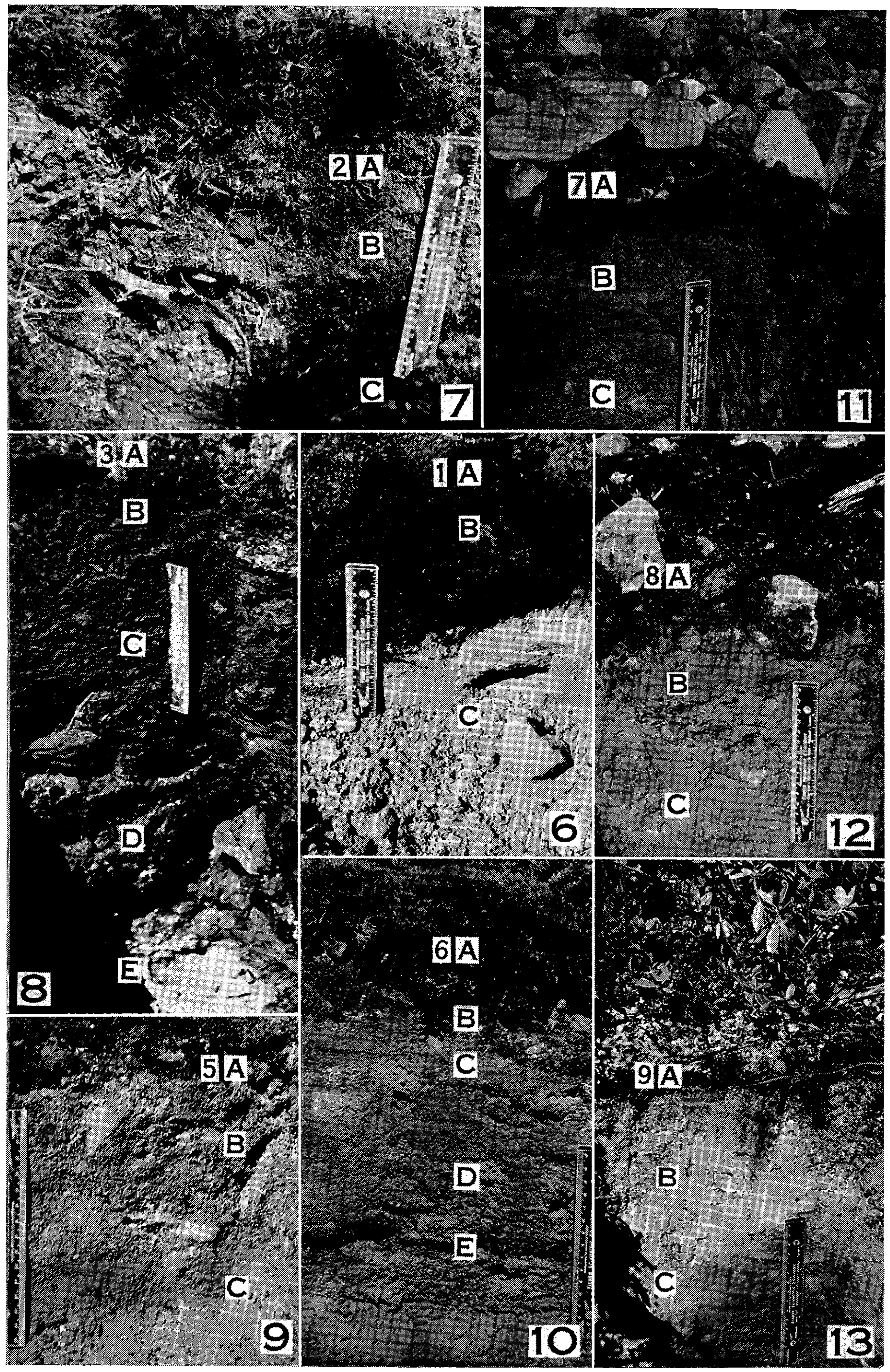
2 A
B
C
7
7 A
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3 A
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1 A
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12
5 A
B
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9
6 A
B
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13

p.p.m. of copper. Mineral horizons bordering on the peat, and the surface organic or A_0 horizon overlying the peat in the forest (profile 2) contained much smaller amounts of copper. Only in the burnt upland do copper levels become more or less normal (profile 10).

Interesting interrelationships between organic matter, copper content, and fungi emerged from the study. From no fewer than eight of the samples no fungi were isolated; all of these samples represented subsurface horizons completely lacking organic matter, and with less than 2000 p.p.m. copper. Some of the vertical distributions are highly significant. In profile 4 the surface peat, with 60,000 p.p.m. copper, yielded four species of fungi and over 8700 colonies/g. By contrast, the sandy loam underlying the peat at a depth of 35 cm contained only 350 p.p.m. copper and yielded no fungi. Although it might appear that copper content and fungal count are directly related, such is not the case. Copper and fungi are in fact both dependent, though in different ways, on the organic matter content of the soil. However, it is at first sight surprising to see copper content and fungal count fluctuating in the same, rather than in opposite directions, as might have been expected.

In profile 6, both copper concentrations and fungal population follow the distribution of the organic matter very closely. Sample 6A was derived from the surface organic layer, or A_0 horizon, which consisted of living plants and decaying remains of *Pohlia nutans*. This was found to contain 14,000 p.p.m. copper, and six species of fungi numbering 600 colonies/g. Sample 6B was taken from the humus-infiltrated subsurface inorganic or A_1 horizon, and yielded 9000 p.p.m., 6 species and about 650 colonies/g. Samples 6C and 6D from the leached inorganic or A_2 horizon were lowest in copper, with 600 and 950 p.p.m. respectively. Sample 6C appeared sterile, and only two species with a population of 16 colonies/g were found in 6D. Sample 6E was derived from the illuvial or B_1 horizon, a narrow zone of humus accumulation below the A_2. The copper content rose sharply here to 10,000 p.p.m., and the fungal count increased correspondingly to five species and 420 colonies/g. In contrast to the copper-bearing profiles discussed above, the soil of profile 10 on the burnt upland contained only negligible amounts of copper, less than 5 p.p.m. Here, as might be expected, the mycoflora showed greater diversity, sample 10A yielding 13 species, the highest number recorded from any single sample.

Discussion

X-ray analysis of the peat failed to reveal the presence of copper minerals, and the greater part of the copper appears to be chelated in the peat (5). While this may temporarily inactivate most of the metal, cold water extracts of cupriferous peat samples were found to contain appreciable amounts of copper (up to 180 p.p.m.), and it is believed that the fungi, in metabolizing the organic matter, would gradually release the chelated copper.

Fungi need copper in trace amounts for normal growth and sporulation, but their requirements rarely exceed 0.1 p.p.m., and are often much less (4). When copper levels are higher than this, toxicity soon becomes apparent. McCallan *et al.* (9) using a standardized technique, found that 5 p.p.m. of

FIGS. 6–13. Eight of the profiles, showing the various soil horizons, and the depth from which the samples were obtained; for explanation see text.

TABLE I

Descriptions of the samples, with total copper concentrations and fungal counts

Profile	Sample	Depth, cm	Description	Horizon	Copper content, p.p.m.	Fungi: No. of species isolated	Fungi: Colonies /gram of soil
1	A	0–1	Peat sediment under young *Pohlia*	A_o	34,000	6	520
1	B	5	Peat sediment	A_o	51,000	6	1,000
1	C	20	Sandy loam	C	1,200	2	16
2	A	0–3	Live and decaying moss	A_o	250	4	1,300
2	B	5	Duff	A_o	700	5	4,200
2	C	15	Peat	A_o	33,000	6	1,050
3	A	0–2	Surface peat fragments	A_o	28,000	5	1,800
3	B	5	Peat	A_o	68,000	4	40
3	C	20	Peat	A_o	44,000	1	4
3	D	40	Peat	A_o	38,000	3	16
3	E	55	Sandy loam	C	1,750	0	0
			No vegetation				
4	A	0–1	Peat	A_o	60,000	4	8,750
4	B	5	Peat	A_o	54,000	4	950
4	C	20	Peat	A_o	26,000	2	8
4	D	35	Sandy loam	C	350	0	0
			No vegetation				
5	A	0–1	Peat sediment in stream bed	A_o	45,000	4	1,300
5	B	5	Sand	C	850	5	350
5	C	15	Sandy loam	C	700	0	0
6	A	1–3	Live and decaying *Pohlia*	A_o	14,000	6	600
6	B	5	Humus-infiltrated sand	A_1	9,000	6	650
6	C	8	Sandy loam	A_2	600	0	0
6	D	15	Sandy loam	A_2	950	2	16
6	E	20	Humus-infiltrated sand	B_1	10,000	5	420
			No vegetation				
7	A	2–4	Humus-infiltrated sand	A_1	7,500	7	490
7	B	10	Sandy loam	C	1,850	0	0
7	C	20	Sandy loam	C	1,800	0	0
8	A	0–5	*Pohlia* mat and humus-infiltrated sand	A_o A_1	10,000	7	620
8	B	10	Sandy loam	C	1,300	0	0
8	C	20	Sandy loam	C	1,700	0	0
9	A	0–2	Humus-infiltrated sand	A_1	300	9	2,450
9	B	10	Sandy loam	C	3.5	5	1,500
9	C	20	Sandy loam	C	4.5	7	1,500
10	A	0–2	Humus-infiltrated sand	A_1	4	13	1,500
10	B	5	Sandy loam	C	2.5	5	700
10	C	20	Sandy loam	C	<1	2	2,100

available copper inhibited the germination of 99.9% of *Stemphylium sarcinaeforme* spores. On the other hand, it has long been known that some fungi are resistant to high copper concentrations. Trabut (13) reported a green *Penicillium* species growing in 9% aqueous copper sulphate, and Pulst (10) discussed a fungus which could grow in saturated copper sulphate solution. Bedford (1) obtained from Fehling's reagent a *Penicillium* species which grew in saturated copper sulphate at pH values ranging from 2.5 to 6.5. Starkey and Waksman (12) found two imperfect fungi which were very resistant to extreme acidity and high copper concentrations. There are also many instances of fungi producing strains with much greater than normal tolerance of copper or other toxic substances. Grover and Moore (7) recently showed that increased tolerance could be induced by incorporating gradually increasing amounts of toxicant in the medium on which the fungi were grown. The induced tolerance was, however, soon lost in the absence of the toxic agent. Gottlieb (6) suggested that tolerant strains might produce a large excess of some metabolite or metabolites—possibly hydroxy or amino acids—which could chelate and inactivate the toxic material.

During the present study 31 species of fungi were isolated. They could be divided into three groups according to their distribution between the various copper levels encountered (Table II). The first group is made up of those species isolated only from samples with high or very high copper content.

TABLE II

Fungi isolated from the copper swamp

	No. of samples	Total colonies
Group 1. Fungi isolated only from high-copper samples		
Penicillium ochro-chloron Biourge	14	4575
Phoma sp.	6	58
Sterile white mycelium	4	144
Gliocladium catenulatum Gilm. & Abott	4	21
Sterile pale brown mycelium	4	10
Penicillium restrictum Gilm. & Abbott	2	100
Penicillium sp.	2	7
Aspergillus fumigatus Fresenius	2	2
Sterile dark mycelium 1	1	3
Sterile dark mycelium 2	1	2
Sterile pink mycelium	1	1
Phoma sp.	1	1
Sterile orange basidiomycete mycelium	1	1
Group 2. Fungi isolated from high- and low-copper samples		
Penicillium spinulosum Thom	21	927
Trichoderma viride Persoon	19	292
Mucor hiemalis Wehmer	10	150
Sterile cream-colored basidiomycete mycelium	8	85
Mortierella isabellina (Oudemans) Zycha	7	93
Penicillium decumbens Thom	5	14
Gelasinospora calospora (Mouton) Moreau & Moreau	3	20
Phoma sp.	2	3
Penicillium thomii Maire	2	10
Group 3. Fungi isolated only from low-copper samples		
Mucor ambiguus Vuillemin	3	66
Alternaria sp.	3	34
Paecilomyces sp.	2	520
Sterile yellow basidiomycete mycelium	2	74
Mucor ramannianus Moeller	2	6
White mycelium with yellow 'primordia' (Plectascales?)	1	7
Mortierella pusilla Oudemans	1	5
Sordaria macrospora Auerswald	1	2
Penicillium lapidosum Raper and Fennell	1	1

These must possess some copper tolerance, which appears to confer a local advantage on them, enabling them to establish themselves in this unfavorable habitat while seemingly remaining at a disadvantage and possibly being excluded by competition where copper is absent. By far the most striking example of this group is *Penicillium ochro-chloron*, isolated from 14 of the 16 high-copper samples, and, with a total of 4575 colonies, the most prolific species encountered. This fungus has been repeatedly isolated by other workers from solutions of copper sulphate, from tentage and fabrics treated with copper naphthanate or other copper-bearing fungicides, and from sulphuric acid (11), and appears to fill an unusual ecological niche, acting as an 'indicator' species (cf. Cannon (3)).

The other two groups may, between them, comprise the 'normal' soil mycoflora (or that segment of it revealed by the technique employed). The second group is composed of species which may be termed 'adaptable', as they were isolated from both cupriferous and non-cupriferous samples. They include several common soil saprophytes, notably *Penicillium spinulosum*, *Trichoderma viride*, *Mucor hiemalis*, and *Mortierella isabellina*. These fungi have presumably developed some copper tolerance, but unlike the first group do not appear dependent on it. This seems to be just one more example of the inherent adaptability required of successful soil fungi.

The third group comprises those species apparently sensitive to the copper concentrations occurring in the swamp. None of these species was isolated from any sample containing more than 5 p.p.m. copper. The most interesting of these fungi was the rare *Mucor ambiguus*. Although isolated on only a few previous occasions, this species was obtained during the present work from three out of five low-copper samples, 66 colonies being recorded.

The soil mycoflora of the swamp appears somewhat deficient in species, but includes representatives of each of the three major divisions of the fungi. Many of the species present are physiologically adapted for survival in concentrations of available copper well above those encountered in normal soils. Some rare fungi were isolated, and two possibly undescribed species of the *Pencillium* complex were recorded.

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