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THE EFFECT OF SLUDGE ADDITIVES ON SOIL FUNGUS POPULATIONS

by

WM. BRIDGE COOKE [1])

ABSTRACT

Using pour plates and shaken-flask cultures, 56 species of filamentous and yeast-like fungi were isolated from samples of Clermont silt loam and Otokee fine sand in which the grasses *Dactylis glomerata* and *Phalaris arundinacea* were grown and in which at weekly or triweekly intervals 1/4 inch of aerobically or anaerobically digested sewage sludge was added. Of these fungi, 13 species were present in the original Clermont silt loam, 17 species in the Otokee fine sand, 18 in the aerobically digested sludge, and 12 in the anaerobically digested sludge. Samples from which fungi were isolated were taken at five and nine month intervals after the initiation of the experiment. The build-up of fungal populations indicates that in addition to a rhizosphere effect which may have been present, the fungi were actively reducing the organic matter added to the soil in the digested sewage sludge.

INTRODUCTION

The study of soil fungi has attracted the interest of a wide variety of specialists in fields of soil science, mycology, plant pathology, and other related disciplines. Organisms assigned to the category "soil fnngi" are so diverse that they may be found in all classes and many orders throughout the fungus kingdom. To this extent the term *soil fungi* is meaningless since many species found in the soil are found equally easily, or even more easily, in other habitats in which they may seem to be well adjusted to living conditions.

In BURGES' description (BURGES & RAW, 1967) of the soil system, it is pointed out that this consists of the mineral fraction, the organic fraction, the soil moisture, and the soil atmosphere. The mineral fraction is derived from the rock layers on which the soil is developed. The organic fraction is built up from residues left on the death of plants and animals and the resulting decomposition of these remains. The final degradation products are quite complex and are lumped together under the general term "humic acids". Soil moisture is determined by water tension measurements. The field capacity of the soil occurs when the gravitational pull on the water is roughly balanced by the capillary and adsorption forces exerted by the intersticies and colloidal materials in the soil. When plants

[1]) Formerly: Mycologist, Biological Treatment Activities, Advanced Waste Treatment Research Laboratory, Robert A. Taft Water Research Center, FWPCA, 4676 Columbia Parkway, Cincinnati, Ohio 45226.
Present address: 1135 Wilshire Ct., Cincinnati, Ohio 45230.
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can no longer remove water from the soil the permanent wilting point has been reached and this is thought to be fairly constant, varying only slightly for different plants. The soil atmosphere, the air occurring in the sometimes minute spaces between soil particles, through diffusion is not greatly different from the atmosphere above the soil, but because of factors of aeration and microbial growth, the relative amounts of oxygen and carbon dioxide may vary more critically. Carbon dioxide may reach 3—4 % in some instances, and for short periods it may reach as much as 10 %. However, it is only when oxygen levels drop below 5 % that micro-organisms appear to be affected adversely.

WARCUP, in BURGES & RAW (1967), has given an excellent review of the occurrence and growth of fungi in the soil. A few pertinent topics will be summarized briefly here. KÜHN, in 1858, is thought to have been the first person to show that a plant disease, stem canker of potatoes, was caused by a fungus in the soil, *Rhizoctonia solani*. However, ADAMETZ, in 1886, is said to have been the first to isolate fungi from the soil. It was not until 1916 that WAKSMAN showed convincingly that the soil furnishes a habitat for an autochthonous population of fungi, which is occasionally invaded by, or even replaced by, other fungi in response to the addition of a quickly "fermentable" substratum.

Soil fungi have been studied by direct observation, and by a variety of isolation techniques. A variety of structures produced by fungi occur in or on the soil. These include the mycelium, spores produced by the fungus at the termination of a growing period, various storage organs, and a variety of types of fruiting structures. Substrates for growth of fungi in the soil include litter lying on the surface, roots of plants growing in the soil, plant residues including cells sloughed from root surfaces, in addition to materials found in the litter, and litter which has become incorporated in the soil following various degrees of decomposition, seeds, and animal residues including animal bodies, hair, skin, and fecal matter, and fungal structures including spent mycelium, ungerminated spores, and residues of fruiting structures.

A variety of factors affect the fungi in the soil. Mycostasis and spore dormancy affect the population in ways which are becoming understood slowly. Spore germination and hyphal growth are affected by a variety of factors related to food supplies, competition, and other factors. The development of the fructification and the dispersal of spores are important factors in the biology of all types of soil fungi. These are related to available food supplies and conditions under which, and locations in which, spores are produced. Lysis, parasitism, and predation are factors involved in the destruction of fungal structures. Such factors may be related to age of the structure, its use to the fungal organism as a whole or in that part of the mycelium in which it occurs in relation to the activity of that part of the mycelium, and other factors relating to

populations of organisms capable of breaking down tissues, or capable of using certain tissues as food sources.

PARKINSON, in BURGES & RAW (1967), surveyed studies dealing with "soil microorganisms and plant roots". The growing root of a plant modifies the soil in a number of ways as it pushes its way through the soil. Not only do cells of the root cap become sloughed off, but exudates are added to the environment of the outer surface of the root. These soils and exudates are usable by soil micropopulations as food or energy sources. A variety of bacteria occurs in the immediate vicinity of the growing root, as do a number of species of fungi. The bacteria have been studied more intensively than the fungi although studies on fungi are of increasing importance. The habitat in the soil adjacent to the root is known as the *rhizosphere*, that in the area between the soil and the root surface is the *rhizoplane*, and that on the immediate surface of the root, including one or two layers of root tissue, is referred to as the *root surface*. Techniques have been, and are being, developed to study the populations of bacteria and fungi in each of these habitats. As these techniques become more refined, results of earlier studies become less and less acceptable, since it is more and more obvious that the habitats sampled in the earlier studies are less specific than was thought at the time of the study. As a result of refinement of techniques, the bacteria and the fungi within the rhizosphere are studied using different methods for each type of organism.

Various soil treatments appear to have less effect on the bacterial populations of the rhizosphere than on those of the non-rhizosphere; the bacterial populations of the rhizosphere thus appear to be under the primary influence of the root, buffered against other changes. Bacterial numbers appear to be higher in rhizospheres from drier soils. Because of the nature of root exudates and their location with relation to the growing point of the root, the qualitative nature of the fungal population of the rhizosphere varies along the length of the root. The initiation of the rhizosphere effect on the bacteria may occur in the germinating seed from which, in the spermatosphere, exudates are first liberated. While this may be postulated for the fungi, little experimental evidence has been developed to prove the point. Analyses of different compounds in artificially developed "rhizospheres" and their effects on microbiological populations has not yet yielded conclusive information on the role of root exudates in the control by root-producing plants of beneficial or harmful populations of such organisms. Studies in relation to plant disease control by such exudates are in progress. On the other hand, foliar applications of certain types of chemical sprays have been shown to have definite effects on rhizosphere populations.

More intensive work with the fungi has been carried out on the root surface. This is differentiated from the rhizoplane by restricting it exclusively to the organisms growing only on the surface of the roots. More sophisticated techniques are required, most people

today using the root washing-segment plating technique, finally plating washed 5 mm segments of roots (PETERSON, 1958). The nature of root surface populations, the development of fungi on root surfaces, and factors affecting the development of fungi on roots are the basic areas of interest in recent studies of this microhabitat. It is thought that the organisms themselves may have effects of several types in the root region. These would include possible effects on root development, effects of antibiotic production on the host roots as well as on competing microorganisms, effects on nutrient availability, and effects on root infection fungi such as the antagonism of *Trichoderma viride* to such fungi as those causing flax wilt, and *Fomes annosus* root disease of pines.

BURGES, in BURGES & RAW (1967), has presented a very interesting and useful summary of information concerning the sequence of events during the decomposition of organic matter in soils as these are understood at the present time. No one has as yet tried to follow such a series of events from its inception with the fall of leaves to the soil, which may have been preceded by the colonization of the leaf in the bud at the beginning of the preceding spring season, through the initial stages of decay on the forest floor, to the final accumulation of humic acids. Portions of this process have been studied in one type of English forest litter. Some humic acid residues have been radiocarbon dated, humic acids in prairie soils from the United States showed a mean residence of about 1,000 years, humic acids from a B horizon under *Pinus* in Sweden were about 400 years old, and humic acid from the B horizon in a podzol under a heath in East Anglia, England, ranged between 1,500 and 2,860 years old. It may take as little as a year for a leaf to lose much of its characteristic structure and as much as five years for it to completely lose its identity. Depending on the type of litter, and possibly on the amount of work expended on discovering the nature of the succession of bacteria, fungi, protozoa and microinvertebrates involved in the process, a highly complicated series of events combines to complete the decomposition process within the types of structures making up the forest litter.

The above review is presented to give an appreciation of some of the complexities of populations of microorganisms, particularly fungi, living in a soil. The studies on which these generalizations are based of necessity must be carried out on very small quantities of soil. In some cases these samples are handled with a minimum of disturbance. In other cases the samples are thoroughly mixed up in order to obtain the greatest amount of information possible from the sample.

To determine some of the effects of the potential use of undewatered digested sewage sludge, pots of two types of soil were prepared and seeded with two species of grasses, orchard grass (*Dactylis glomerata*), and reed Canary grass (*Phalaris arundinacea*). Pots were prepared in triplicate for each type of treatment and ran-

domized in position on the greenhouse benches. No attempt was made to remove weeds such as tomato plants (*Lycopersicon esculentum*), other grasses or herbs, seeds of which may have been in the original soil or may have been introduced with the digested sludge.

Between 8 A.M. and 5 P.M., five days a week, ceiling banks of fluorescent lights supplemented daylight. Soils used were Clermont silt loam obtained in Clermont Co., Ohio, and Otokee fine sand, obtained in southeastern Fulton Co., Ohio. Both soils were dried, screened, and stored dry before use. After the seeded pots were prepared in January, 1969, they were treated with sludge. An amount equal to one-fourth inch depth on the soil in the pots was added to each pot at the beginning of the experiment. Thereafter, half the pots received one-fourth inch sludge once each week, the other half received one-fourth inch once every three weeks. Between sludge treatments, pots were watered "normally" so that the soil would not dry out. Half the pots received anaerobic digested sludge, the other half received aerobic digested sludge.

Techniques

After five months, in early June, and again after nine months, in late September, samples of soil were removed from each pot aseptically, and analysed for their content of fungi. Sampling was performed without regard to root systems which were present, including weed roots in unseeded control pots. To this extent it may be said that sampling was similar to that used in early studies of plant rhizosphere populations. No attempt was made to determine the effect of plant roots, or to determine populations which might have been specifically related to the rhizosphere, rhizoplane or root surface. Soils collected from each of the triplicate pots in each type of treatment were mixed together so that no attempt was made to determine the effect of light or temperature on the green plant, resulting from the randomized positioning of pots on the benches, as these factors might have influenced the populations of soil fungi adjacent to the plant roots.

Samples were processed in the Fungus Studies Laboratory. Four media were used in two basically different types of isolation procedure. After compositing the three samples from each triplicate set, 15 ml of moist to wet soil were added to 135 ml distilled water in 250 ml Erlenmeyer flasks. These flasks were placed on a rotary shaker at approximately 150 oscillations per minute for 30 minutes. After dispersion of the soil particles in this way the flasks were removed and 5 ml of each sample were added to 45 ml distilled water. This process was repeated for a dilution of 1 : 1000, the dilution at which both sets of samples were plated.

In preparation for plating and for inoculation of liquid media, four types of media had been prepared. For pour-plates, neopeptone-dextrose agar, and neopeptone-dextrose agar with rose bengal were

prepared according to formulae given by COOKE (1963). These media were stored in 10 ml lots in aluminum capped culture tubes in a hot water bath. A set of flasks was prepared to which two types of media were added. The flasks were sterilized with cotton stoppers in place; 25 ml of a 2X strength yeast-nitrogen-base (YNB) filter-sterilized solution were added to each flask. To half the flasks, 25 ml of 2X strength filter-sterilized 1 % dextrose solution were added; to the other half, 25 ml of 2X strength filter-sterilized 20 % dextrose solution were added, using sterile automatic pipettes.

Following the dilution procedures described above for the samples, five tubes of each of the two agar media were removed from the water bath, the aluminum caps removed, 0.05 ml of a 1 mg/150 ml solution of Tetracycline added, and one ml of the 1 : 1000 dilution of the sample added. The agar was immediately poured into plastic Petri dishes 100 ml in diam., the top dish on each pile of five labeled, and each pile stored on a laboratory bench at room conditions of light and temperature for seven days incubation. Following the preparation of the pour plates, one ml of the 1 : 10 dilution of each sample was added to one each of the YNB—1 % and YNB—20 % dextrose solution flasks. These flasks were placed on the rotary shaker at low speed, approximately 150 oscillations per minute, for incubation at room conditions of light and temperature for 64 or 88 hours. At the end of that time the flasks were removed, the growth allowed to settle for four hours, and the yeast growth was then streaked onto yeast extract-malt extract-dextrose agar, or onto Diamalt agar plates. Ribbons of mold growth which formed around the glass surface at the edge of the shaken liquid, and chunks or balls of mold growth floating or remaining in suspension, were picked and planted on Diamalt agar for identification. The technique was used primarily to obtain yeasts. Yeast growth settled to the bottom of the flask while bacteria, if present and developing in these media, remained in suspension as a cloudy white or yellow growth. At the 1 : 10 dilution level, usually more than one species of yeast was present and the first series of streaked plates yielded mixed cultures. These were purified by one or more repetitions of the streaking process on Diamalt agar. Relatively pure yeast cultures were held on neopeptone-dextrose agar slants for the series of physiological and morphological tests necessary for the identification of yeast species. Additional purification of selected isolates was carried out as necessary.

Following incubation of pour plates for seven days, colonies on the plates were counted. In the set of isolations made in June, a record was kept of the number of colonies of each identifiable species, or each colony type picked for later identification study. In the set of isolations made in September, only a total colony count was made in addition to noting the identity of those colonies which could be identified at the time the plates were read. As a result, only the total colonies observed for each type of treatment,

and the kinds of fungi observed on the plates will be reported. Colony counts will be based on counts made from neopeptone-dextrose-rose bengal-Tetracycline agar plates. Species lists will be based on observations made on all four media.

RESULTS

On the basis of the isolation techniques used in this study, the numbers of colonies reported in Table I were recorded. Without

Table I.

Average numbers of colonies of fungi recovered from soil samples.

Sampled Material	Average colonies per gram oven dry weight x 10,000	
	June	September
Initial populations		
Soil: Clermont silt loam	8.3	
Otokee fine sand	7.0	
Sludge: Aerobic digested	410.0	
Anaerobic digested	170.0	
Soil and sludge only:		
Aerobic sludge:		
Clermont silt loam	7.1	20.0
Otokee fine sand	5.1	16.0
Anaerobic digested:		
Clermont silt loam	3.7	17.1
Otokee fine sand	11.0	42.3
All treatments:		
Clermont silt loam:		
Aerobic sludge	12.0	20.8
Anaerobic sludge	8.0	32.9
1/4" per week	8.3	23.5
1/4" per third week	11.9	30.2
Orchard grass	12.3	25.3
Reed Canary grass	7.9	28.3
Average	10.1	26.8
Otokee fine sand		
Aerobic sludge	8.7	20.5
Anaerobic sludge	8.3	28.8
1/4" per week	6.6	24.3
1/4" per third week	10.4	25.0
Orchard grass	8.0	27.8
Reed Canary grass	9.0	24.0
Average	8.5	25.1

regard to species composition, it is noted that the soil samples initially contained 7 to 8×10^4 cells, while sludges contained 170—410×10^4 cells. After applying sludge to the soil, without the presence of deliberately introduced plants, there was a reduction in the number of colonies recovered from the soils, with one exception which may be related to the presence of adventive plants. After three more months, however, there was a 3—4-fold increase in the numbers of colonies recovered. During the first five months, under the regular treatments imposed during the experiment, there was only a slight increase in numbers of colonies recovered over the

number present in the original soil. However, after three more months subjection to these treatments, there was a 2—3-fold increase in the numbers of colonies recovered.

In Table II are listed the extrapolated indicated number of yeast

Table II.

pH of Samples, and Yeast Growth in YNB Medium in Shaken Flask Cultures.

Sample Number	pH June	Growth in YNB-glucose media			
		June		Sept.	
		1% G	20% G	1% G	20% G
		1000	1000	1000	1000
1	7.1	>1000	>1000	>1000	>1000
2	7.2	>1000	>1000	>1000	>1000
3	7.1	>1000	>1000 (R)	>1000	none
4	6.8	>1000	>1000	>1000	>1000
5	7.2	>1000	>1000	>1000	>1000
6	7.3	>1000	>1000	>1000	>1000
7	7.35	>1000	>1000	>1000	>1000
8	7.4	>1000	>1000	>1000	>1000
9	7.25	>1000	>1000	>1000	>1000
10	7.0	>1000	>1000	>1000	>1000
11	N.T.	N.T.	N.T.	none	none
12	6.4	none	1000	1000	none
13	6.85	>1000	>1000	>1000	>1000
14	7.0	>1000	>1000	>1000	>1000
15	6.4	>1000	>1000	>1000	>1000
16	6.85	>1000	>1000	>1000	>1000
17	7.0	>1000	>1000	>1000	>1000
18	7.3	>1000	>1000	>1000	>1000
19	7.3	>1000	>1000	>1000	>1000
20	7.1	>1000	>1000	>1000	>1000
21	6.7	>1000	>1000	>1000	>1000
22	7.35	>1000	>1000	>1000	>1000
23	7.2	>1000	>1000	N.T.	N.T.
24	7.4	>1000	>1000	N.T.	N.T.
25	6.1	1000	1000 (R)	N.T.	N.T.
26	6.1	1000	1000	N.T.	N.T.

N.T. - Not Tested

(R) - Growth includes Rhodotorula.

Values in last four columns refer to minimal numbers of cells observed in flasks inoculated with 1 ml of a 1:10 dilution of the original sample. These values are extrapolated on the basis of earlier work with similar materials (Cooke, 1965).
Except for samples 11, 12, 23, 24, 25, and 26, these minimal values could have approximated 10,000, or even 100,000 in some cases.

cells recovered from these samples. In those control samples which were tested, yeast numbers are lower than those from the operational experiment.

Values for pH of the several samples tested in June are listed in Table II. These were taken from the supernatant from shaken samples of the 1 : 10 dilution of the original sample. Unfortunately, it was not practical to take pH values for the September samples early in the experiment. The delayed results were usually at least 0.4 units higher than the values obtained in June so these results were discarded.

A list of 56 species obtained from these samples is given in Table III, together with the samples from which they were recovered. The total number of species recovered from each sample is summarized in Table IV. Occurrence of each species in each sample is indicated

by presence values unweighted as to numbers of colonies observed for any one species in any one habitat. Presence of a species in the June samples is indicated by the numeral "2", those in the September samples by the numeral "3". These symbols are related to the code number used to identify the sample in bench work in the laboratory, rather than to any quantitative value which could be attributed to the occurrence of a species in the habitat.

Discussion

As a result of the isolations made from the samples of soil described above, it was hoped that some idea could be obtained of the place of fungi in soils to which large amounts of organic matter were added regularly. Bacteria and fungi include those organisms which are reducers of organic matter which has been elaborated by producers from carbon dioxide and water in the presence of light, and further rearranged by consumers, those organisms incapable of producing organic matter but consuming by ingestion that which has been produced by green plants. The fungi, and those nonphotosynthetic and nonchemosynthetic bacteria present in the habitat, utilize the waste products of the metabolism of the producers and consumers, as well as the dead tissues of all organisms, through the action of exoenzymes secreted into the environment. As the more complex molecules are digested in part by these exoenzymes, the products of this digestion process are absorbed into the cells of the reducers in which, through endoenzymes further reduction makes available to the cytoplasm of the cell essential nutrients. Through the use of these nutrients, elements or molecules of many kinds, the cells are enabled to grow, divide, and thus increase the tissues of the organisms. The resulting growth may be entirely vegetative, or, under certain circumstances, spores may be produced. In the case of most bacteria and yeasts or yeast-like fungi, this growth results in increased numbers of cells which form loose aggegations or colonies. A few species in both groups may form chains of discrete cells, and a few yeasts may produce a true mycelium. In the case of those species forming colonies of discrete cells, there is no protoplasmic connection between mature parent and daughter cell. In the case of those yeast-like fungi in which a true mycelium is formed there is probably a viable protoplasmic connection between the cells of the mycelium. At first, the daughter bud may be connected with the parent cell, but as it matures this connection is eliminated. In all other fungi isolated in this study, the mycelium originating from the spore or resting cell at the beginning of growth continues to grow as long as nutrients are available to it. In some species of fungi it is known (Burnett, 1968) that cytoplasm flows through the mycelium for relatively great distances;

Table III.

Fungus Species	Entry		Controls							
	Soils		Sludges		Operational Only sludges added*					
	Clermont Silt Loam	Otokee Fine Sand	Aerobic Digested	Anaerobic Digested	Clermont silt loam Aerobic		Anaerobic		Otokee fine sand Aerobic		Anaerobic	
	26	25	23	24	10		9		21		22	
White Yeasts	2	2	2	2	2	3	2	3	2	3	2	3
Geotrichum candidum	2	2	2	2	2	3	2	3	2	3	2	3
Mucor hiemalis	2	2	2		2	3	2	3	2	3	2	3
Penicillium spp.	2	2	2	2		3		3	2	3	2	3
Penicillium rubrum	2				2	3	2	3	2	3	2	
White Molds (Sterile &/or indet)	2	2	2		2	3			2		2	
Gliocladium roseum		2			2	3		3	2	3	2	3
Trichoderma harzianum	2	2	2		2		2		2		2	
Zygorhynchus moelleri		2	2				2	3	2			3
Rhinocladiella mansonii			2	2	2	3				3		3
Myrothecium verrucaria		2			2			3				
Phoma spp.								3				3
Rhodotorula spp.	2		2	2				3			2	3
Aspergillus niger			2			3		3		3		3
Trichosporon spp.			2	2		3		3		3		3
Gliocladium catenulatum										3		
Trichoderma hematum		2				3		3		3		3
Humicola grisea										3	2	3
Fusarium oxysporum			2		2	3	2	3	2	3	2	3
Trichoderma polysporum	2	2										
Gliocladium deliquescens		2			2		2		2			
Phoma herbarum	2		2	2	2				2			
Cephalosporium acremonium		2	2	2			2	3				
Candida spp.						3		3		3		3
Torulopsis spp.						3		3		3		3
Gliocladium fimbriatum						3		3		3		
Fusarium aqueductuum			2	2				3				
Trichosporon cuteneum								3		3		3
Sepedonium sp.						3		3				
Rhizopus arrhizus							2	3	2			3
Aspergillus ochraceus		2			2							
Penicillium lilacinum			2	2								3
Cryptococcus spp.								3				3
Penicillium variabile		2			2							
Curvularia lunata					2							
Rhizopus nigricans										3		
Trichosporon capitatum				2								
Aspergillus flavus							2					
Cladosporium cladosporioides												
Aureobasidium pullulans					2							
Peyronellaea glomerata								3				
Penicillium funiculosum												
Verticillium lateritium												
Paecilomyces marquandii												
Aspergillus flavipes												
Trichoderma koningii	2	2										
Penicillium clavigerum			2									
Cephalosporium sp.			2									
Fusarium roseum												
Cunninghamella blakesleeana	2											
Trichoderma longibrachiatum	2											
Paecilomyces varioti		2										
Aspergillus fumigatus				2								
Paecilomyces farinosus					2							
Aspergillus candidus												
Column totals	13	17	18	12	17	16	11	24	13	18	11	20
Pair totals					26		29		25		24	
Group Totals	13	17	18	12			37				30	

in other species, nutrients are utilized at the point of absorption. It is usually thought that sporulation is related to mycelial starvation. Further, it is thought that sporulation in a liquid environment is usually atypical as judged by the type of sporulation observed in colonies on primary isolation agar plates, or on slants in culture collections where the fungus is maintained under optimum conditions for sporulation.

Grasses Only#				Operation Each Treatment Regardless of Other Treatments			
Clermont Silt loam		Soil		Grass		Sludge	
Dactylis	Phalaris	/ Clermont silt loam	Otokee fine sand	/ Dactylis	Phalaris	/ Aerobic	Anaerobic
11	12	S-C	S-O	G-D	G-P	S-A	S-N
	2 3	2 3	2 3	2 3	2 3	2 3	2 3
3	3	2 3	2 3	2 3	2 3	2 3	2 3
3	2 3	2 3	2 3	2 3	2 3	2 3	2 3
3	2 3	2 3	2 3	2 3	2 3	2 3	2 3
3	2 3	2 3	2 3	2 3	2 3	2 3	2 3
	2 3	2 3	2 3	2 3	2 3	2 3	2 3
3	3	3	2 3	2 3	2 3	2 3	2 3
3	2 3	2 3	2	2 3	2 3	2 3	2
	2 3	2 3	2 3	2 3	2 3	2 3	2 3
3	3	3	2 3	2 3	2 3	2 3	2 3
3	2	2 3	2 3	2 3	2 3	2 3	2 3
3	3	2 3	2 3	2 3	2 3	2 3	2 3
		2 3	3	3	2 3	2 3	3
3		3	2 3	3	2 3	2 3	3
	2 3	3	3	3	3	3	3
	3	2 3	2 3	2 3	2 3	2 3	2 3
3	3	3	3	3	3	3	3
			2 3	2 3	2 3	2 3	2 3
3	3	2 3	2 3	2 3	2 3	2 3	2 3
		2	2 3	2 3	2 3	2	2 3
3	2	2	2	2	2	2	2
		2	2	2	2	2	2
		2	2	2	2	2	2
	3	3	3	3	3	3	3
	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3
		3	3	3	3	3	3
		3	3	3	3	3	3
	3	3	3	3	3	3	3
		2 3	2	2 3	2 3	2 3	2 3
	3	2		2	2	2	2
3	3		3	3			3
		3	3	3	3	3	3
	2	2		2	2		2
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	2						
	3						
3	3						
	3						
16	12 24 28	24 31 43	22 31 38	25 31 41	29 34 45	27 30 41	23 33 42
	29		47		47		47

In the pots of soil prepared for planting of grasses to determine the effect of sludge on grass growth, there are two major sources of fungal populations. The first is the original soil which, unless sterilized, has populations of viable fungal spores and fungal and bacterial cells. The second is the sludge which is added to the soil as an irrigant and fertilizer supplement, or merely as a means of disposal of this type of material. Minor sources of fungal spores

include the seed coats of the grasses planted in the experiment (whose fungal spores or cells in the "spermatosphere" were not sampled), and spores which are carried in the air and which continually fall-out by precipitation or by trapping on surfaces over which the air stream passes.

Table IV.

Total Species Recovered from Sludge Treated Soils

Soil	Species Recovered			
	June	Sept.	Total	
Clermont silt loam, dry sample from storage	13	NS	13	
Otokee fine sand	17	NS	17	22
Aerobic digested sludge	18	NS	18	
Anaerobic digested sludge	12	NS	12	20
Clermont silt loam				
plus aerobic sludge	11	24	28	
plus anaerobic sludge	17	17	26	36
Otokee fine sand				
plus aerobic sludge	13	18	24	
plus anaerobic sludge	11	20	23	29
Clermont silt loam				
plus Dactylis glomerata	16	NS	16	
plus Phalaris arundinacea	12	24	28	29
Clermont silt loam plus grass plus sludge	24	31	42	
Otokee fine sand plus grass plus sludge	22	31	37	46
Dactylis plus soil plus sludge	25	31	40	
Phalaris plus soil plus sludge	29	34	44	46
Aerobic sludge plus soil plus grass	27	30	40	
Anaerobic sludge plus soil plus grass	23	33	41	46
Total species recovered, all samples				56

The initial soil sample contains a certain amount of organic matter, sufficient to activate a population of fungi when the soil is watered. When seeds carried in the sewage sludge, or added deliberately in the experiment, germinate, a second source of organic matter becomes available to the fungus cells which are already developing and growing in the soil. These are the exudates from the germinating seeds, resulting from the metabolic activities in the seed tissues, and the exudates from the growing root tips, in addition to the root cap cells which are continuously and regularly sloughed off the growing root hair. An additional source of nutrients for the fungi is found in the organic matter in the sludge which contains sufficient material to support the growth of good populations of soil fungi.

Soil fungi require large amounts of water, but a completely and permanently water logged condition probably inhibits growth. It has been found that fungi such as *Fusarium oxysporum* var. *cubense* survives prolonged periods of flood fallowing of fields in

areas where this has been tried as a means of controlling the Panama disease of bananas. While flood fallowing did not completely kill the fungus, it probably reduced the amount of growth which otherwise would be produced, while permitting survival of resting cells for future inoculum. For good growth of fungi, the soil atmosphere should have a high relative humidity and this is probably controlled by good porosity permitting the irrigating water to percolate through the soil without clogging completely all of the pores of the region where plant growth is taking place.

Very little is known of the temperature requirements of soil fungi. Minimal, optimum and maximal temperatures from laboratory experiments are known; good temperatures for maintenance of growth of plants in the field and under greenhouse bench conditions are known, but the temperatures of soil at various depths and under various conditions of plant growth and fungal activity, are poorly understood. Temperatures under which the growth of the isolated fungi occurred in the soil can only be described as the ambient temperature of the heated greenhouse. Air temperature above the soil was maintained at a fairly constant level during the progress of the experiment.

On the basis of these observations, it is interesting to note that during the first five months of the experiment little change took place in the population of soil fungi recovered from the soils in the pots on the greenhouse bench, at least so far as numbers of colonies of fungi appearing on the plates is concerned (Table I). On the average, there was a slight increase in numbers of colonies during this period, and in certain types of treatment, especially the pots to which sludge was added only once in three weeks rather than once a week.

Initially, Clermont silt loam yielded a somewhat higher colony count than Otokee fine sand, and aerobic digested sludge was richer in fungi than anaerobic digested sludge. When aerobic sludge was added to the soils there was a decrease in numbers of colonies, but when anaerobic sludge was added to the soils the population of the Clermont silt loam was depressed, while that in the Otokee fine sand increased.

On checking the species lists (Table III), it will be noted that the populations of fungi present in soils and sludges are not necessarily additive when the two are mixed as when the sludge is applied to the surface of the pot. This may be a result of the fact that the population of the soil was a fixed entity while at each weekly or triweekly addition of sludge a new population was introduced to the environment. This may be among the factors which may be responsible for the initial instability of the observed populations. As these fungi become adjusted to the habitat in which they are now found, some species drop out apparently because of poor competitive capacity, others increase in number or even become dominant because of good competitive capacity, or favorable nutrition, or a

combination of these and other poorly understood factors.

Another set of characteristics may also be operative here. It will be noted that in the case of soils which have been planted with *Dactylis* or *Phalaris*, the populations of soil fungi which developed included more species than were recovered only from the untreated soil or the soil treated only with sludge. In this case, in addition to a slightly different unit of soil being used in the pots than that sampled in pour plates, the presence of the grass roots may have activated spores or resting cells of some fungal species not activated by the nutrients in the pour-plate media.

That some types of cells present in the sludges which were added to the soils developed well in the new habitat, that of wet soil, is evident from the data in Table II. Here it is noted that in control samples, where only raw soil was tested, or where soil planted with grass seeds was tested, few yeast cells were recovered when any were present at all. However, wherever sludges were added to the soils, larger numbers of yeast cells were recovered. Even where the presence of yeasts may be masked by *Geotrichum candidum*, indications are that large numbers of yeasts of several species are present in each sample. Species assignable to at least *Candida*, *Torulopsis* and *Trichosporon* were observed in primary streak plates made from shaken-flask cultures of most of the sludge-soil samples tested. *Rhodotorula* was present in at least two of the June samples.

Future studies on the effect of sludges on soils with and without plant cover should take into consideration several types of interaction: the effect of each combination of plant and sludge, the effect of cultivations as well as the effect of passive addition of sludge, the effect of plants alone and in combination with the microorganisms of sludge on the organic matter content of the sludges, the possible extent of build-up of humic acids resulting from decomposition of complex fractions in the sludge and waste products of cover-plant growth, the effect of sludge or other organic additives on the fungi and other microorganisms present on the root surface, in the rhizosphere, and in the rhizoplane in the presence of ambient and of optimum conditions of light, and other interesting factors in this complex habitat, which is highly specialized. Information developed from such studies can lead to a better understanding of the role of fungi and other reducer organisms in the recycling of organic matter in the ultimate disposal of sewage sludge.

Zusammenfassung

Mittels Platten- und Schüttelflaschenkulturen sind 56 Arten von Fadenpilzen und hefeähnlichen Organismen aus Proben von Clermont Siltlehm und von Otokee Feinsand isoliert worden, in welchen die Gräser *Dactylis glomerata* und *Phalaris arundinacea* wuchsen und zu welchen in wöchentlichen oder dreiwöchentlichen Intervallen ca 6 mm (1/4″) von aerob oder anaerob verdautes Abwassersediment hinzugefügt wurde. Von diesen Pilzen waren 13 Arten in den originalen Clermont Siltlehm, 17 Arten in Otokee Feinsand, 18 in dem aerob verdauten und 12 in dem

anaerob verdauten Abwassersediment vorhanden. Proben, von denen Pilze isoliert worden sind, wurden in Intervallen von fünf und neun Monaten nach Beginn des Experiments genommen. Der Aufbau der Pilzpopulation zeigt, daß neben dem rhizospheren Effekt, der vorhanden sein mochte, Pilze das organische Material tatsächlich verminderten, wenn sie zum verdauten Abwassersediment hinzugefügt wurden.

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