VA Mycorrhizal Colonization and Spore Populations in an Abandoned Agricultural Field after Five Years of Sludge Application[1]

P. T. ARNOLD[2] and L. A. KAPUSTKA, Department of Botany, Miami University, Oxford, OH 45056

ABSTRACT. The effects of five years of sewage sludge application on vesicular-arbuscular mycorrhizal (VAM) fungi were studied. The commercially produced sludge, Milorganite, has been applied to plots monthly during growing seasons since 1978. Urea-phosphate fertilizer was applied to other plots, with still others containing no nutrient amendment. The VAM spore counts, along with root colonization percentages of *Cirsium arvense* and *Barbarea vulgarus* grown in field plots and bioassay plants (corn and geranium) grown in a glasshouse, all showed little or no difference between the sludge-amended and unamended plots. Depressed VAM spore populations and colonization occurred in urea-phosphate fertilizer. Non-VAM fungal populations were higher in the sludge-amended plots than in the other treatments. The VAM colonization levels of the test plants were not hindered by the toxic metals in the sludge-amended soils after five years of Milorganite addition.

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INTRODUCTION

Dispersal of sewage sludge on agricultural lands is frequently the least expensive means of disposal and is thought to be beneficial as it supplies organic matter and nutrients to the soil (Milne and Graveland 1972, Webber et al. 1980). Furr et al. (1976) reported that urban sludges had nitrogen (N) levels of 1.48-5.80% (dry weight, dw) and phosphorus (P) levels of 0.96-2.72% (dw). Several urban areas, including Chicago, Illinois, Houston, Texas, Milwaukee, Wisconsin, and Schenectady, New York are selling their sludge as fertilizer.

Sludge is one of the main vectors of toxic metal to agricultural lands (Gupta and Haeni 1980). Furr et al. (1976) found that concentrations of toxic metals in sludge materials (dw) from several cities were as follows: arsenic (As), 3.3-30.0 $ug \cdot g^{-1}$; mercury (Hg), 3.4-18.0 $ug \cdot g^{-1}$; nickel (Ni), 36.4-562 $ug \cdot g^{-1}$; lead (Pb), 136-7, 627 $ug \cdot g^{-1}$; and zinc (Zn), 560-6,890 $ug \cdot g^{-1}$.

The Ecological Research Center (ERC) of Miami University, Oxford, Ohio has been the site of studies of the various effects of sludge dispersal on the ecosystems of abandoned agricultural fields (Anderson and Barrett 1982, Anderson et al. 1982, Barrett 1982, Clark et al. 1981, Maly and Barrett 1984, and Hyder and Barrett 1985). This study assessed the effect of sludge treatment on VAM colonization and the number of VAM spores in the soil. These data, along with plated non-VAM fungal populations and metal content of plants from earlier studies, were used to characterize the effect of sludge amendment.

MATERIALS AND METHODS

STUDY SITE. The ERC contains eight 0.1-ha plots: two control (no nutrient amendment), three urea-phosphate-amended, and three sludge-amended plots. The commercially produced sludge, Milorganite (6-2-0; NPK), has been applied to plots at a rate of 8,960 $ka \cdot ha^{-1} yr^{-1}$. Commercial urea-phosphate fertilizer has been applied on different plots with equivalent nutrient (N, P, K) input as the Milorganite. Both sludge and urea-phosphate were applied five times annually since 1978. The rates of sludge and urea-phosphate metal addition for each treatment are shown in Table 1. The present study began in 1983.

TABLE 1

Mean (±SE) metal contents and application rates of Milorganite and urea-phosphate fertilizer applied to plots.*

	Milorganite		Urea-Phosphate	
Metal	Content**	Rate***	Content**	Rate***
Cd	72.8(±3.6)	0.652	5.5(±3.4)	0.008
Cu	333.8(±15.8)	2.991	2.0(±0.2)	0.003
Pb	409.9(±19.3)	3.673	0.8(±0.4)	0.001
Zn	942.8(±26.6)	8.447	60.5(±37.8)	0.088

*Data provided by Dr. G. W. Barrett, Miami University from subsamples obtained from May–September, 1981.
**$ug \cdot g^{-1}$ dry weight
***$kg \cdot ha^{-1} yr^{-1}$

The soil in the study area is a moderately eroded, Xenia silt-loam on a bedrock-controlled till plain with a 2 to 6% slope. It has moderately slow permeability, moderate available water capacity, and medium runoff. The surface layer has good tilth and an organic matter content of 2-4%. It is dark grayish brown, with friable silt loam about 18 cm thick and a 74-cm-thick subsoil. The shale and interbedded limestone bedrock (approx. 2:1) is at a depth of 1.2 to 1.8 m. The soil is typically at a pH between 5 and 7 in the rhizosphere (Lerch et al. 1978).

NON-VAM FUNGAL PROPAGULES. Five soil cores (upper 15 cm) were obtained randomly from the control, urea-phosphate, and sludge-amended plots on 20 June and 5 August 1983. One gram of fresh soil (approx. 0.8 g dw) from each core was diluted with sterilized distilled H_2O, and 1 ml aliquots of 10-fold dilutions were plated on Martin's dextrose-peptone agar supplemented with streptomycin sulfate (100 $mg \cdot L^{-1}$) and Rose Bengal (33 $mg \cdot L^{-1}$). After two days incubation at 20°C, at least 20 plates yielding 30-300 colonies were counted from each treatment to determine the population of plated, non-VAM fungi. This technique underestimates the fungal population in that it does not include fungi that grow slowly or not at all on this media.

SPORE COUNTS OF VAM FUNGI. Counts of VAM spores in random soil samples collected on 5 August 1983 were made with a modification of the plate method of spore population determination (Smith and Skipper 1979). The soil from each core was well mixed to ensure a homogeneous soil sample. Ten aliquots of 100 g of soil from each treatment were sieved with a 22-μm screen, the sievings were plated onto filter paper and the spores were counted. No attempt to identify spores to taxonomic level was made.

BIOASSAY INFECTION LEVELS. Random soil samples (approx. 1 kg) from each plot were planted with either corn (*Zea mays* L.) seeds or geranium (*Pelargonium* sp.) cuttings in free draining pots (15 cm × 15 cm). Corn and geranium were chosen because they are easily colonized by VAM fungi and are representative of both a monocot and a dicot. After three months of growth in the glass-

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[2]Present address: P.O. Box 1205, Young Harris College, Young Harris, GA 30582.

root samples from each treatment-plant combination were cleared and stained with trypan blue (Phillips and Hayman 1970). Percentage VAM colonization was scored by the grid-line intersect method (Giovannetti and Mosse 1980). Wilcoxon rank-sum statistics (with tie corrections) were performed on the data.

FIELD INFECTION LEVELS. Roots of yellow rocket (*Barbarea vulgaris* R. Br.) and canadian thistle (*Cirsium arvense* (L.) Scop.) were obtained from each plot in June, 1983. These plants were chosen because they were dominant in all three treatments. Percent VAM colonization was determined as described above.

RESULTS

NON-VAM FUNGAL POPULATIONS. Total plated, non-VAM fungal populations (Table 2) were significantly ($P < 0.01$) higher (approx. 10-fold on 20 June and approx. 4-fold on 5 August 1983) in both the urea-phosphate and sludge-amended plots than in unamended soil. The fungal populations in nutrient-amended treatments, however, did not differ significantly from each other. Since fungi are highly responsive to nutrient amendment (Swift 1976), this increase in plated fungi is not surprising. This indicates that five years of sludge amendment (compared with an equivalent nutrient input) had no adverse effect on the total fungal population. Species differences in fungi (not determined in this study) among treatments could indicate if fungal species selection had occurred.

VAM FUNGAL POPULATIONS. The VAM fungal spore populations were significantly ($P < 0.05$) lower in the urea-phosphate treatment than in the control and sludge-amended plots (Table 2). Corn and geranium bioassay plants, as well as yellow rocket and canadian thistle roots from field collections (Table 3), all showed VAM colonization. Each plant species showed a significantly ($P < 0.05$) lower percentage colonization in the urea-phosphate treatment. Colonization percentages of most of the species did not differ significantly in the control and sludge-amended treatments. However, geranium had significantly ($P < 0.01$) lower colonization in the sludge-amended treatments.

DISCUSSION

The spore population data and the percentage colonization data both indicated a reduction of the number of VAM propagules with the urea-phosphate amendment. Milorganite (sludge) amendment of the same nutrient input (538 kg $\cdot$ ha^{-1} yr^{-1} N, 179 kg $\cdot$ ha^{-1} yr^{-1} P, no K) did not cause a significant change in mycorrhizal colonization or spore population compared to the control. Many investigators (e.g. Hayman 1978 and Tinker 1980) have found that high levels of phosphate can reduce the formation of VA mycorrhizae; therefore the lack of inhibition in the sludge treatment may be attributed to a slower release of available phosphate in the sludge. The lower availability of phosphate ions in the sludge may have been accentuated by the atypically dry summer (total rainfall for June-August period was 17.58 cm compared to the average of 27.28 cm for the same period; J. Klink, pers. comm.), thus slowing the release and assimilation of the nutrients even further.

The uptake of nutrients by mycorrhizae presents an interesting problem in toxic metal environments. Although the increased absorption of Zn or other necessary nutrients into the host plant by mycorrhizae is beneficial in nutrient-deficient soils (Gray and Gerdemann 1973, Bowen et al. 1974, Cooper and Tinker 1978), this same mechanism may become deleterious when the metal content of soil reaches or is near a toxic level. Although an ericoid mycorrhiza (*Pezizella*) showed an ability to prevent absorption of high concentrations of copper (Cu) and Zn into the host plants *Calluna vulgaris, Vaccinium macrocarpon,* and *Rhododendron ponticum* (Bradley et al. 1981), Gildon and Tinker (1983a, 1983b) showed that VA mycorrhizal colonization can increase the amount of Cu transported into the shoots of a host plant. Gildon and Tinker (1983a, 1983b) also suggested that strains of VA mycorrhizae present in high-level, toxic metal environments are more tolerant to metal influx. Heavy VA mycorrhizal colonization (95%) has been reported in roots of orchard grass growing in such an environment, where surface-mined areas were amended with sludge supernatant (Bohn and Liberta 1982). VAM spore counts of 11.1 spores $\cdot$ g soil^{-1} were also reported in their study, thus raising the possibility of the presence of metal-tolerant VAM strains.

A previous study (Maly and Barrett 1984) showed that significantly higher amounts of cadmium, Cu, and Zn ions were taken up into the shoots of plants in the ERC sludge plots. We have shown that these plants are mycorrhizal. It was determined in the present study that the VAM fungal colonization levels on the roots of these plants do not appear to be hindered owing to these metal concentrations. Hence, VA mycorrhizae may be translocating these metals into their hosts and facilitating the increase in concentration. This is supported by data from Killham and Firestone (1983), who showed that under conditions of acid and metal deposition, Cu, Ni, Pb, and Zn uptake was enhanced by VAM fungal colonization.

TABLE 2

Mean (±SE) VAM spore counts and total plated non-VAM fungi in the sludge, fertilizer, and control treatments.

	VAM spores per kg of soil (Dry wt.)	Total plated non-VAM fungal propagules per kg of soil (Dry wt.)	
Treatment	5 August 1983	20 June 1983	5 August 1983
Control	2400(±680)	1.424×10^{4}**	3.322×10^{4}**
Fertilizer	1940(±930)*	1.785×10^{5}	1.386×10^{5}
Sludge	2600(±510)	1.525×10^{5}	1.204×10^{5}

*Significantly different from other treatments at $P < 0.05$.
**Significantly different from other treatments at $P < 0.01$.

TABLE 3

Percent VAM colonization of geranium and corn bioassay plants and canadian thistle and yellow rocket from the field.

	Mean% colonization		
Test plant	Control	Fertilizer	Sludge
A. Bioassay plants			
Geranium	84.0**	61.8	74.1
Corn	60.5	47.2**	61.0
B. Field plants			
Canadian thistle	70.1	13.0**	74.0
Yellow rocket	36.6	23.4*	31.6

*Significantly different from other treatments at $P < 0.05$.
**Significantly different from other treatments at $P < 0.01$.

More conclusive evidence is currently being gathered regarding the role of VAM fungi in toxic metal uptake from sludge by experimentally studying inoculated mycorrhizal and non-mycorrhizal plants.

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