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VESICULAR–ARBUSCULAR MYCORRHIZAL ASSOCIATIONS WITH BARLEY ON SEWAGE-AMENDED PLOTS

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Summary—Barley plants grown on field plots amended with sewage from two sources were examined for vesicular-arbuscular (VAM) infection. Three treatments (control, 8.2 tonnes sludge ha^{-1} and 32.9 ha^{-1}) were applied annually for 8 yr with no additions during years 9 and 10. A barley crop was grown on the site during each of the 10 yr of the study. Percent VAM infection on barley roots showed no effect of nonindustrial sludge application. The urban-industrial sludge treatments, however, produced a 6-fold drop in infection rate. Little correlation was found between percent VAM infection and available soil P content. Zn concentration in the soil were found to be negatively correlated with VAM infection. This study suggests that there are factors within sludge, in addition to P, that affect mycorrhizal occurrence 2 yr after the last application of sludge to soil.

INTRODUCTION

All stable systems depend on cycling of required nutrients. We have interfered with these cycles by importing and concentrating large amounts of nutrients near urban areas where these products are a pollution source rather than a resource (Allison, 1973). The U.S. produces 2 million dry tonnes of sewage per day (Peters, 1981). A major portion of this sludge's fertilizer value lies in its phosphorous (P) content (Sommers, 1977). Information on soil–sludge interactions is needed to increase recycling as a viable alternative for this under-utilized resource.

The fertilizer potential and chemical hazard that might develop from the utilization of sludge, was evaluated on a series of field plots at the University of California, Berkeley. The 10-yr project involved the addition of varying rates of two sludge types to agronomic field plots (Williams *et al.*, 1980). In determining the effects of sewage-amended soil on plants such as barley, plant yields and composition were routinely measures and evaluated. The effects of sludge on soil microorganisms that interact with the plant are less noticeable and not usually investigated (CAST, 1980). We chose to observe the degree of occurrence of the mycorrhizal association (the most common root–fungus relationship) on barley grown on these soils.

The vesicular–arbuscular mycorrhizal association (VAM) is a symbiotic association between plant and microbe in soils with less than optimal P concentration to support plant growth (Hayman, 1975; Trappe and Fogel, 1977). The fungus obtains photosynthate while the plant derives more P absorbing capacity. This study was performed to quantify the difference in percentage VAM infection on barley due to the type of sludge and its application rate to field plots. The soil's P status and its effect on VAM infection were also examined.

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MATERIAL AND METHODS

Two sources of sludge were obtained between 1973 and 1980. One was a wet cake (25% solids content) form the East Bay Municipal Utility District treatment plant at Oakland, California. This sludge (Oakland) was anaerobically digested after vacuum filtration. The second sludge obtained from the Central Contra Costa Sanitary District at Pacheco, California, was essentially domestic or nonindustrial in origin. This sludge (Pacheco) was primary treated, composted, and then sieved before placement on the soil. The Oakland sludge has a higher P concn, and an N content three times higher than that of the sludge from Pacheco (Table 1). The Oakland sludge also had considerably higher Zn, Pb, Cu and Cr contents which attest to its industrial origin.

The two sludges were added annually for the first 8 yr to 47, 3 by 2.5-m plots on a soil classified as a Tierra loam. Before s'udge applications the soil has a cation exchange capacity (CEC) of 20.1 cmol kg^{-1} soil and a pH range of the saturated paste of 5.2–5.6 (Williams *et al.*, 1980). On each control plot and after each sludge addition, a crop of Mariout barley was grown and harvested. At the end of 8 yr no further additions of sludge were made, but for the following 2 yr barley was grown and harvested as before.

For this study, two sludge application rates were sampled; 8.2 and 32.9 tonnes sludge $ha^{-1} yr^{-1}$ as well as the unfertilized control plots. Maximum sludge application over the 8-yr period totaled 263.2 tonnes ha^{-1}.

Sampling began 2 yr after the last sludge application. Three root-soil cores were taken from each of the three replicate plots. The roots were then rinsed

Table 1. Chemical analysis of the sludges (averaged up to 1975 from Williams *et al.*, 1980)

	Oakland sludge	Pacheco sludge
	(% dry wt)	
Total N	3.3	1.1
Ammonia-N	0.2	0.03
Nitrate-N	0.05	0.25
Total P	1.64	0.4
Ca	2.26	2.18
Mg	0.62	0.57
K	0.09	0.08
Na	0.12	0.12
	($\mu g\ g^{-1}$)	
Cd	37	8
Co	13	9
Cr	1470	40
Cu	600	180
Fe	22,100	12,800
Hg	14	5
Mn	300	230
Ni	180	40
Pb	1090	250
Zn	3910	440

and cut into 1–2 cm segments before staining with acid fuchsin (Phillips and Hayman, 1970; Ambler and Young, 1977). Percentage VAM was determined on these root sections by using a modified-grid intersection approach (Giovannetti and Mosse, 1980). The intersection of the root segments and lines etched on a Petri dish marked the points of observation, approx. 80 per sample. Positive infection was identified when vesicles, arbuscules or extensive internal hyphae were visible through a 25× dissecting microscope.

Soils were sampled after harvest during the tenth yr, air-dried and sieved (<2 mm). Available soil P was extracted by the bicarbonate method (Olsen *et al.*, 1954). Total soil P was determined by a $HClO_4$ plus HNO_3 digest (Page *et al.*, 1982). A modified ignition method was used to analyze for organic P (Black, 1965). Samples for both total and organic P were ball milled for 15 min before analysis. All PO_4^{3-}-P determinantions were performed on a Lachat AutoAnalyzer (Anon, 1975). Perchloric acid digested soil samples were measured for Zn on a Varian Techtron Atomic Spectrophotometer Model AA-6 (Page *et al.*, 1982).

RESULTS

The application of Pacheco sludge (residential) had no effect on the percentage of VAM infection rate on barley (Fig. 1). There was, however, a 6-fold drop in infection rate between the controls and the Oakland sludge (urban-industrial) at the 8.2 tonnes ha^{-1} rate. Because the field was slightly sloping there was some concern about soil differences or the movement of sludge amendments outside the application site. Plants on the control plots down-slope to the treatments were significantly different ($P = 0.01$) in VAM infection than those up-slope.

Mosse (1973) established a strong negative correlation between percentage VAM infection and bicarbonate-extractable soil P concentrations. But no relationship was exhibited in Fig. 2 between available P and VAM infection rate for the Pacheco sludge.

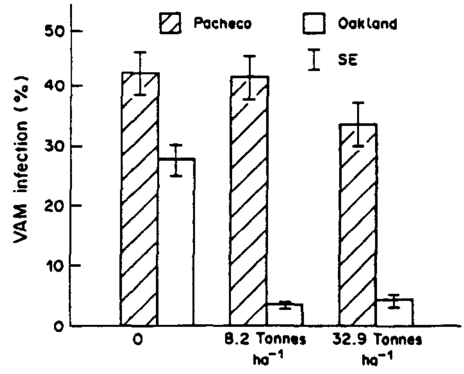

Fig. 1. The effect of sludge treatment and application rate on percent vesicular–arbuscular mycorrhizal (VAM) infection.

Available P values were significantly different, at the 5% level, between application rates but not between sludge types at the same rate (Table 2).

To determine whether another P fraction may have influenced the infection rate, total and organic P were also analyzed (Table 2). Oakland sludge-amended soil has consistently higher amounts of P in the fractions determined, except for available P in the highest application rate which was lower than the Pacheco treatment. The Pancheco sludge (1978) had higher amounts of available P but a lower total P than Oakland sludge. This lower ratio of available P to total P in the metal-containing Oakland sludge probably reflects the precipitation of metal phosphates which would decrease the concentration of extractable P (CAST, 1980).

The percentage organic P divided by total P, decreased with application rate. This illustrates that P in sewage sludge is mostly in the inorganic form (Johnson, 1981). There were no significant differences (at the 5% level) for the amounts of soil organic P between Oakland and Pacheco treatments applied at the same rate.

The fertilizer effect of the sludges was present 2 yr after the last application as shown by the increased grain yields with higher application rates (Table 3).

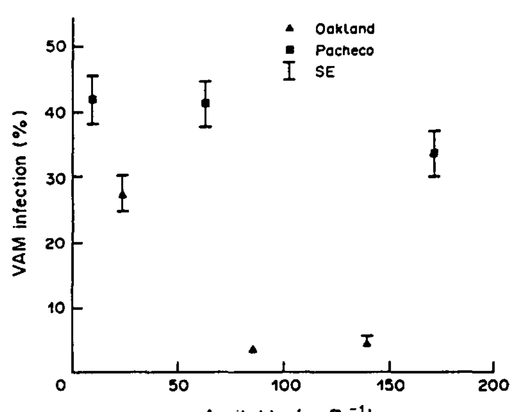

Fig. 2. The effect of available soil P on percentage VAM infection.

Table 2. Sludge effects on VAM infection and P status in soils

Treatment	Infection (%)	Total-P	Available-P (μg^{-1})	Organic-P	Organic-P / Total-P (%)
Pacheco					
Control	42a	368a	10.1a	105a	29
8.2 tonnes ha^{-1}	41a	1034b	63.8b	152a	15
32.9 tonnes ha^{-1}	33a	2211c	171.7c	245b	11
Oakland					
Control	28b	675d	24.4a	160a	24
8.2 tonnes ha^{-1}	4c	1382e	86.0b	185a	13
32.9 tonnes ha^{-1}	5c	2708c	140.1c	326b	12
Raw sludge (1978)					
Pacheco		8516	1183	742	9
Oakland		14,368	965	2029	14

Values in a column with the same letter following are not significantly different at the 5% level.

Table 3. Sludge effects on grain yield, soil pH and Zn content

Treatment	Grain yield (kg plot^{-1})	Zn ($\mu g\, g^{-1}$)	pH
Pacheco			
Control	1.04a	61.2a	6.4ab
8.2 tonnes ha^{-1}	1.85ab	11.4b	6.9a
32.9 tonnes ha^{-1}	3.03cd	206.4c	7.6a
Oakland			
Control	2.54bd	114.4b	6.1b
8.2 tonnes ha^{-1}	5.61e	246.6d	5.3d
32.9 tonnes ha^{-1}	4.61f	421.7e	4.9e

Values in a column with the same letters following are not significantly different at the 5% level.

The plots treated with nutrient-rich Oakland sludge still gave a better response than the Pacheco plots. The highest correlation of percentage VAM infection was found to be with grain yield ($r = -0.94$). This may indicate the plant's nutritional composition and tendency for infection, i.e. the healthier the plant the less need for the microsymbiont (Mosse, 1973).

Although the pH of the sludges themselves are near neutral, sludge applications changed the soil's pH as measured in a 1 : 1 soil suspension. The application of Pacheco sludge raised the pH, while Oakland's higher N content, and presumably subsequent nitrification, lowered the soil pH (Table 3). This lowering of the soil's pH by the Oakland sludge with its higher metal content may pose the risk of increasing metal availability to both plants and fungi (CAST, 1980; Hyde *et al.*, 1979).

The infection rate of a Zn-sensitive VAM on clover decreased with increased soil concentration of the metal (Gildon and Tinker, 1981). Zn concentrations were approximately 8-fold higher in the Oakland than the Pacheco sludge (Table 1). The total accumulation of Zn in the soil after 8 yr of application is reported in Table 3. This relationship gave the highest linear correlation with percentage VAM ($r = -0.84$) for any soil property measured. The lower pH and higher Zn concentrations in the Oakland plots correspond to an absence of a fungal symbiont. The combined pH–Zn effect on the inhibition of VAM produced a multiple correlation coefficient, $r = 0.93$. Low pH and high metal content of soil has been reported to diminish spore germination and VAM infection (Siqueira *et al.*, 1984).

In the process of determining VAM infection, we noted that most internal vesicles were oblong, approx. 50 × 30 μm. But at the highest application rate vesicles were smaller, about 10 μm dia. These smaller "bean-shaped" vesicles were mostly associated with the epidermal layer of the root, and were observed again on the barley the following year.

DISCUSSION

The VAM infection rates on barley grown on soils treated with two sludge types differ greatly and can not be accounted for by changes in available P in the soil. Possible explanations for the observed differences are: (1) the sludge's effect on the pH of the soil. The Pacheco sludge (residential) increased soil pH to 7.6 while Oakland (urban-industrial) decreased the pH to 4.9. This could cause a direct pH effect on the mycorrhizal association or an indirect effect by allowing more metals to become avilable and thus limit the infection rate; (2) components other than P in the Oakland sludge negatively affect the infection rate of VAM on barley; (3) different mycorrhizal associations occur on the plots. On the plots with the highest application rates of Pacheco sludge (a VAM infection rate of 33%) vesicles appear smaller as compared to the vesicles on other treatments and on the controls. The high rate of application of this sludge (Pacheco) may select for a different and successful mycorrhizal fungus, or the edaphic qualities of the plots produce morphological changes in an existing association that may interfere with its symbiosis (Mosse, 1973).

What is surprising is not that the Oakland (urban-industrial) treatment has a 6-fold drop in VAM infection, but that Pacheco sludge additions produced no change in rate of infection. It would be expected that increasing the phosphate fertility of the soil would reduce infection rate, but this did not occur in the Pacheco plots. On the Pacheco plots with the highest sludge application, a distinct mycorrhizal association was evident. Neither the high concentration of available P (172 $\mu g\, g^{-1}$) nor the moderate concentrations of Zn (206 $\mu g\, g^{-1}$) discouraged the development of the smaller VAM associations on these higher pH plots (pH 7.6). We found that 2 yr after the last sludge application there are factors, in addition to phosphate concentrations, within the sludges that affected the VAM association.

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