



## Short communication

**Effects of the land application of sewage sludge on soil heavy metal concentrations and soil microbial communities**John J. Kelly<sup>a</sup>, Max Häggblom<sup>b</sup>, Robert L. Tate III<sup>a,\*</sup><sup>a</sup>*Department of Environmental Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA*<sup>b</sup>*Department of Biochemistry and Microbiology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA*

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The ocean dumping of sewage sludge was banned in the United States as of 31 December 1991 (Hill et al., 1996). As a result, land application of sewage sludge has become an increasingly common method of disposal (McGrath et al., 1994). Long-term studies are needed to improve our understanding of the effects of land application of sewage sludge on soil biological systems (McGrath et al., 1995). In 1978 researchers at Cornell University established a sludge application field study (Pevery et al., 1994). Although the treatment plots within this study were not replicated, the site provides an interesting opportunity to examine the effects of sludge application at a well monitored, long-term field site.

A small-scale study was undertaken to determine if the effects of the sludge application on soil metal concentrations and on soil microbial communities could be detected 18 y after the application took place. Soil microbial communities were assessed by measuring microbial activity, population size, zinc resistance, and community structure. BIOLOG and phospholipid fatty acid (PLFA) assays were used to assess microbial community structure.

In 1978, a 24 m × 24 m plot was amended with 244 tonnes ha<sup>-1</sup> of dewatered sewage sludge. The soil at the site is a moderately well drained Hudson silty-clay loam. The metal contents in the sludge were: 4127 mg zinc kg<sup>-1</sup>, 1112 mg copper kg<sup>-1</sup>, 81 mg cadmium kg<sup>-1</sup>, 169 mg nickel kg<sup>-1</sup>, 111 mg chromium kg<sup>-1</sup>, and 653 mg lead kg<sup>-1</sup>. The sludge plot and an adja-

cent control plot were rototilled to 20 cm. In 1985 both plots received 12 tonnes of lime ha<sup>-1</sup>. Both plots have been under grass and dwarf apple trees since 1985 (Pevery et al., 1994). In September 1996, six soil samples (top 15 cm) were collected from spots at least 3 m apart within each of the plots.

All soil samples were sieved to 2 mm and maintained at 4°C. Soil organic matter content was determined by loss on ignition (Bear, 1955). Soil cation exchange capacity (CEC) was determined by saturation with ammonium acetate at pH 7 (Chapman, 1965). Total metals were extracted according to the EPA 3050 soil digestion method (Environmental Protection Agency, 1986). Soluble metals were extracted by shaking for 18 h with 10 mM CaCl<sub>2</sub> (Aten and Gupta, 1996). Metal concentrations were determined with a multielement direct current plasma spectrometer (Applied Research Laboratories, Valencia, CA; Spectraspan Model 7) at a wavelength of 2025 nm. Microbial biomass was estimated by chloroform fumigation (Jenkinson, 1966). Dehydrogenase activity was assessed by a modification of the method of Casida (Tate and Terry, 1980). Counts of culturable bacteria were obtained on soil extract agar plates. Counts of zinc resistant bacteria were obtained on soil extract agar plates amended with 1 mM ZnSO<sub>4</sub> (Jordan and LeChevalier, 1975). All values for physical, chemical and biological variables for the two treatments were compared by Student's *t*-test (at the 95% level). Mean values and standard errors are reported.

PLFAs were extracted by the method of White et al. (1979). PLFA profiles were obtained with the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE). Several randomly selected samples were also analyzed via GC-MS to confirm the FAME

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Table 1  
Metal content of soils

Site	Cd	Cr	Cu	Ni	Pb	Zn
<i>Total metals (mg kg<sup>-1</sup>)</i>						
Control	12.5 (0.41) <sup>a,c</sup>	39.8 (0.72) <sup>a</sup>	23.4 (0.89) <sup>a</sup>	33.4 (10.6) <sup>a</sup>	133 (8.77) <sup>a</sup>	123 (5.44) <sup>a</sup>
Sludge	44.5 (3.99) <sup>b</sup>	512 (59.4) <sup>b</sup>	341 (40.3) <sup>b</sup>	159 (16.8) <sup>b</sup>	337 (39.6) <sup>b</sup>	1506 (206) <sup>b</sup>
<i>Soluble metals (mg kg<sup>-1</sup>)</i>						
Control	0.012 (0.005) <sup>a</sup>	ND	0.066 (0.009) <sup>a</sup>	ND	0.034 (0.011) <sup>a</sup>	0.083 (0.031) <sup>a</sup>
Sludge	1.172 (0.274) <sup>b</sup>	ND	1.515 (0.247) <sup>b</sup>	ND	0.159 (0.017) <sup>b</sup>	1.966 (0.326) <sup>b</sup>

<sup>a,b</sup> Mean values which are statistically different between treatments at the 95% level, Student's *t*-test.

<sup>c</sup> Mean values (standard error).

identification by the MIDI system. An adaptation of BIOLOG for metal contaminated soils was used (Kelly and Tate, 1998a). Rate of color development on BIOLOG plates was determined by calculating an average well color development (AWCD) for each plate at each reading (Garland, 1996). PLFA and BIOLOG profiles were analyzed by principal component analysis (PCA) using SAS statistical software. BIOLOG plates with an AWCD closest to 0.25 were used for the PCA analysis (Garland, 1996).

Significant effects of sludge application on soil physical and chemical properties were observed. Sludge-treated soils increased in organic matter from 6.80% ( $\pm 0.17$ ) to 11.02% ( $\pm 0.88$ ) and increased in CEC from 12.96 ( $\pm 0.27$ ) to 17.22 ( $\pm 0.56$ ) cmol kg<sup>-1</sup>. The amounts of total and soluble metals were significantly higher for the sludge treatment, including a 100-fold increase in soluble cadmium, and greater than 20-fold increases in soluble zinc and copper (Table 1).

Sludge amendment also affected the soil microbial communities. Sludge-amended soils showed a significant increase in the counts of culturable bacteria (Table 2), but more than a 20-fold decrease in dehydrogenase activity (Table 2). Sludge amendment also resulted in changes in the structure of the soil microbial communities. There was more than a 20-fold increase in the zinc resistance of the microbial communities from the sludge-amended soils (Table 2). PCA of the PLFA profiles indicated that the treatments differed in the makeup of their microbial communities [Fig 1(a)]. Correlation coefficients for the PCA [Fig 1(b)] demonstrated relative decreases for the sludge

amendment in 18:0 10 Me, 16:1  $\omega 5c$ , 18:2  $\omega 6c$  and 20:2  $\omega 6c$ . The rate of color development on BIOLOG plates was slower for sludge-amended samples (data not shown). However, PCA of the BIOLOG profiles showed no clear separation of the treatments (data not shown).

The relative decreases in several fatty acids which were observed in this study suggest that sludge amendment may be resulting in decreases in several specific populations of soil microorganisms. The PCA analysis [Fig 1(b)] demonstrated decreases for the sludge amendment in 18:0 10 Me, which has been suggested as an indicator for actinomycetes (Frostegard et al., 1993b), 16:1  $\omega 5c$ , which has been suggested as an indicator for arbuscular mycorrhizal fungi (Haack et al., 1994), 18:2  $\omega 6c$ , which has been suggested as an indicator for fungi (Frostegard et al., 1993b), and 20:2  $\omega 6c$ , which has been found in fungi and several plant species (Zelles, 1997).

Because the control plot in this study received neither metals nor sludge, it is not possible from this study to differentiate effects of the elevated metal contents from effects of the sludge material itself. For example, the increase in the culturable microbial population in the sludge treatment is most likely due to the increase in organic matter resulting from sludge amendment. However, the significant increase in zinc resistance of the soil microbial communities does indicate that the increase in soluble metals affected the soil microbial communities. In addition, other studies have previously demonstrated that elevated metal concentrations can result in decreased dehydrogenase activity

Table 2  
Soil microbial community parameters

Site	Biomass (mg C g <sup>-1</sup> soil)	Viable Bacteria (bacteria g <sup>-1</sup> soil $\times 10^6$ )	Dehydrogenase (mg TPF g <sup>-1</sup> soil)	% Zn resistant bacteria
Control	0.295 (0.149) <sup>a,c</sup>	2.91 (0.65) <sup>a</sup>	0.315 (0.017) <sup>a</sup>	0.03 (0.01) <sup>a</sup>
Sludge	0.245 (0.103) <sup>a</sup>	8.69 (2.01) <sup>b</sup>	0.086 (0.013) <sup>b</sup>	0.67 (0.14) <sup>b</sup>

<sup>a,b</sup> Mean values which are statistically different between treatments at the 95% level, Student's *t*-test.

<sup>c</sup> Mean values (standard error).



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