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Enrichment of sewage sludge with two-metal combinations (from Cu, Ni and Zn) and short-term nitrogen transformations in soil

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Abstract

Sewage sludge may improve soil fertility but there is concern about potential adverse effects of metals, particularly on soil microorganisms. Three laboratory experiments were undertaken involving incubations of soil-sludge mixes; effects of varying sludge metal contents on N mineralization and microbial biomass N were measured and related to respiration and extractable metal data. Two metals from Cu, Ni and Zn were combined in factorial experiments at three input rates below, close to and about 50% higher than current European Community (EC) limits. Although higher inputs of Ni, and of Cu with Ni, decreased cumulative respiration at 3 weeks, respiration was generally higher in more contaminated soils over the full 7 week incubation. By comparison with low metal sludges, all three metals decreased biomass N at higher input rates (≤ 43%) and, for the most part, increased soil mineral N (≤ 60%). Responses to different metals were generally additive. However, in some cases, particularly for Ni and Zn combinations, responses to each metal were diminished where the other was applied at higher rates. For most metals, the higher mineral N concentrations in more contaminated soil/sludge mixes were explained by a combination of increased sludge mineralization and decreased microbial assimilation. Findings suggest that complex microbial responses to mixtures of metals should be considered when regulating sludge application to agricultural soils.

Key words: Incubations, metals, microbial N, mineral N, sewage sludge

Introduction

Sewage sludge contains plant nutrients and therefore, has considerable potential as a fertilizer. It also acts as a soil improver by increasing the organic matter content, thus improving the structure, nutrient and water holding capacity of some soils. However, the application of sewage sludge may affect soil quality due mainly to the various contaminants present.

The most common potentially toxic elements (PTE's) found in sewage sludge are Cd, Cr, Cu, Ni, Pb and Zn. Some of these metals are essential or beneficial micronutrients for plants, animals and microorganisms. However, all metals may be toxic at high concentrations (Baath, 1989). With repeated applications of sludge, metals accumulate in the surface layer of the soil, with little movement below the zone of sludge incorporation (Chang *et al*., 1984).

Numerous studies have shown that high levels of certain metals in soils can reduce plant growth (Logan *et al*., 1997; Neilson *et al*., 1997; Simon *et al*., 1998). However, there are also concern about effects of high metal concentrations on soil microorganisms and their activities. Various studies have reported a decrease in microbial biomass (Khan and Scullion, 1999; Kao *et al*., 2006), inhibition of enzyme activities (Kunito *et al*., 2001; Wang *et al*., 2007), changes in microbial community structure (Hu *et al*., 2007; Rajapaksha *et al*., 2004) and reductions in organic matter decomposition (Kao *et al*., 2006) in soils with elevated metal levels.

However, findings relating to effects of metals on microbial activity measured as CO_2 evolution have been less consistent. Several authors have reported significant decreases in CO_2 evolution in metal contaminated compared with uncontaminated soil (Rost *et al*., 2001). Others have reported increased CO_2 evolution in metal polluted soil (Bardgett and Saggar, 1994). Gibbs *et al*. (2006a, b) found no consistent respiration response when metals (including Cu and Zn) were applied in sludges within current EC statutory limits.

Soils treated with relatively low metal sewage sludge invariably show an increase in N mineralization (Kao *et al*., 2006). However, results from investigations of N mineralization in more heavily contaminated soils are contradictory. Increased N mineralization has been reported in such soils (Bogomolov *et al*., 1996), whilst other studies (Kao *et al*., 2006) have observed a decrease with higher metal inputs.

These conflicting findings may have arisen because nitrogen mineralization and immobilization by

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microorganisms occur simultaneously (Chang and Broadbent, 1982). The net outcome of these processes must be interpreted with caution unless both are monitored. In addition, the complex and variable nature of soils may lead to different levels of bioavailability once metals are added (Khan and Scullion, 2000). It is the soluble metal fraction which exerts the major influence on soil microbial activities and this fraction may differ with soil type, environmental conditions and time of measurement relative to metal inputs (Khan and Scullion, 2000). Mineralization of organic materials in soils may also be inhibited directly where metals are bound to them (Kao *et al*., 2006).

In the study reported here, changes in metal availability, microbial and mineral nitrogen, and respiration were measured in soil amended with sewage sludge contaminated with two elements from Cu, Ni and Zn in various combinations, over a series of short-term (7 week) laboratory incubations. Preliminary experiments (Khan and Scullion, 2002) involving a wider range of single metals had shown that these three metals affected microbial indices individually at soil concentrations close to current European Community (EC) limits for inputs in sludge (EC, 2005). It was of interest therefore to investigate microbial responses to combinations of these metals over similar input ranges. The EC limits were used as a guide to realistic metal inputs rather than to directly assess their validity. The aim was to investigate the pattern of nitrogen release from sludges of differing metal contents and the effect of these metals on the balance of nitrogen between mineral and microbial pools. Increases in soil mineral nitrogen in soils treated with high metal sludges were predicted to result from a combination of higher sludge mineralisation rates and lower assimilation of nitrogen by microorganisms.

Materials and Methods

Experimental design and conditions

Three separate experiments (Cu-Ni experiment, Ni-Zn experiment and Cu-Zn experiment) were carried out, started at 4-week intervals in order to spread the analytical workload. Each experiment involved nine treatments, including all combinations of three levels (low, medium and high) of two metals in a factorial design with three full treatment replicates. For example, in the Cu-Ni experiment treatments were Cu-low with either Ni-low, Ni-medium or Ni-high, Cu-medium with either Ni-low, Ni-medium or Ni-high and Cu-high with either Ni-low, Ni-medium or Ni-high.

Metal inputs (medium and high) were applied in a standard sewage sludge application. The ‘low’ treatment (in effect a control or no metal input treatment) was the unspiked sludge amended soil. The sludge was artificially contaminated with different levels of one or two metal salts ($CuSO_4$, $NiCl_2$, $ZnSO_4$) as appropriate. Levels of metal inputs to sludges aimed to produce total concentrations of each metal (Cu, Ni or Zn) in sludge treated soils below (medium) or slightly above (high) (Table 1) current EC soil limits (EC, 2005) at the intended rate of sludge application. Because of the very large effect of sludge on microbial processes, it was not considered useful to include a soil only control, since the main interest was microbial responses to metals.

Undigested sludge with a low metal content (Cu=183, Ni=63, Zn=450 mg kg^{-1}) was obtained from Aberystwyth sewage works and stored at 2 ^{0}C in an airtight container until required. On a dry solids basis, organic carbon and total nitrogen contents were 28.7% and 2.5%, respectively. Prior to each incubation, sludge was well mixed, then dewatered and metal salts in solution were mixed thoroughly with the sludge. All sludges were then returned to cool storage for two weeks prior to an experiment commencing in order to provide a degree of metal equilibration with the sludge.

Previous experiments (Khan, 1999) indicated that the proportions of total metals in sludge extractable by $CaCl_2$ were similarly low for added and indigenous sludge Cu and Zn. Since our study was short-term, it was considered that the $CaCl_2$ metal fraction was likely to indicate potential toxicity. Extractable metals were measured during incubations to test the assumption of microbial exposure broadly proportional to the rate of sludge metal input.

A slightly acidic soil (pH 6.1 – 6.4) was collected from 0-10 cm depth at the same grassland site shortly before each experiment commenced and when friable (10-12% moisture w/w) gently broken to pass a 4mm sieve (Table 2a). Total soil metal concentrations measured for each soil prior to sludge applications were well below EC Directive limits for sludge treated soil (EC, 2005). On the basis of measurements carried out on soil used in the initial experiment only (Table 2b), very little of the Ni content was extractable by EDTA or $CaCl_2$; although the proportions of total Cu and Zn removed by these extractants were higher than for Ni, only EDTA extractable Cu (9.2%) represented a significant fraction of the total metal pool.

Sludge was mixed into the soil at a rate equivalent to 40 g oven dry sludge per kg soil, placed in 500 ml plastic containers (for analyses at 3 weeks) or 250 ml conical flasks (for CO_2 measurements and analyses at 7 weeks); all containers were partially sealed with perforated Parafilm (American Can Inc.). The sludge input rate used was high on a rate per hectare basis but not when the uneven distribution of applied sludges at a scale relevant to microorganisms is considered. The moisture content of the soil-sludge mix was adjusted to a level equivalent to –50 kPa, as determined by centrifugation of subsamples. It was maintained at this level during incubations, carried out in the dark at 22 ± 0.5 ^{O}C, by check weighing and addition of water by pipette as required.

Soil analyses

Extractable metals, pH, microbial N and mineral N were measured after 3 and 7 weeks. CO_2 evolution was measured at intervals throughout the incubations but only cumulative data for the 0-3 and 0-7 week periods are considered here.

A range of general parameters was measured in order to characterise the soils and sludges used in the experiments. Soil pH was determined using a glass electrode in 1:2.5 w/v soil-water suspensions or directly on sludges prior to dewatering. Particle size distribution was measured by differential settling of samples dispersed by hydrogen peroxide and sodium hexametaphosphate (MAFF, 1986). Organic matter content was measured as weight loss on ignition (LOI) of oven dry soil or sludge at 400 ^{O}C for 16 hours (Ball, 1964). Soil cation exchange capacity (CEC) was measured by leaching soil with 1M ammonium acetate, removing the excess with ethanol, then measuring exchangeable ammonium extracted with 2M potassium chloride (MAFF, 1986). For 'total' metals, samples were digested for 20 hours in hot, concentrated HNO_3. For extractable metals, soil or sludge samples were shaken for 1 hour in 0.05M EDTA or for 16 hours in 0.1 M $CaCl_2$ (McGrath and Cegarra, 1992). All metal concentrations in extracts were measured by atomic absorption spectroscopy (Pye-Unicam SP9).

Soil respiration was estimated by measuring the change in CO_2 concentration in flasks temporarily sealed for 6 hours (Sparling, 1981) and data expressed as C output. Measurements started after one week and continued at 3-4 day intervals until the end of each experiment. Gas chromatography (Pye-Unicam Series 104 Chromatograph) was used to measure CO_2 concentrations. Cumulative CO_2-C evolved between 0-3 and 0-7 weeks was estimated to facilitate comparison with mineral N and biomass N data, by interpolating CO_2-C evolution rates measured on each occasion.

Total N in sludges was measured using the Kjeldahl digestion method. A fumigation-extraction method was used for estimating microbial biomass N, with extractable N converted to microbial N using a standard (x 1.85) factor (Brookes *et al*., 1985). Soil was fumigated with chloroform for 24 hours; fumigated and non-fumigated soils were then extracted with 1M K_2SO_4. Filtrates were oven dried and digested with concentrated H_2SO_4. Steam distillation followed by titration with dilute HCl was carried out for nitrogen measurements. Mineral N in the soil was extracted by 1M KCl, the extracts steam-distilled and concentrations determined as above (Keeney and Nelson, 1982).

Statistical analysis

All treatment combinations consisted of three replicates. Data were analysed separately for each experiment using two-way analysis of variance. The significance of differences between main treatment means (low, medium and high input levels of one metal averaged over all levels of the other metal) was assessed against the least significant difference test; interaction effects (between different input rates of each metal) were also evaluated in these analysis. All statistical procedures used "STATGRAPHICS Version 6.0".

Results and Discussion

Data for each measurement are presented together for all three experiments, so as to facilitate the comparison of treatment responses. Where interaction effects were statistically non-significant, only main treatment effects are given (Tables 3-5). These data are equivalent to the means generated in the two-way analysis of variance described above. Means for the nine treatment combinations in an experiment are presented (Figures 1-3), only where statistically significant treatment interactions were obtained. Responses in terms of extractable metal concentrations are considered only briefly (data not shown).

Chemical properties

Compared with initial values and with those at 3 weeks, there were marked decreases in the pH of soil-sludge mixes by the end of all experiments (overall means 5.5, 5.0 and 5.0 for Cu-Ni, Ni-Zn and Cu-Zn experiments respectively). Higher metal inputs often decreased pH at 3 weeks although these effects were small (typically < 0.15 pH units). No such trend was observed at 7 weeks (detailed pH data of 3 and 7 weeks not given).

Increasing metal inputs caused significant and progressive increases in soil EDTA and $CaCl_2$-extractable concentrations on both sampling occasions. Between week 3 and 7, there was no consistent change in levels of any metal extracted by EDTA or for Cu extracted by $CaCl_2$. Over this same period, there was a consistent increase in $CaCl_2$ extractable Ni and Zn at all input rates (10-12 and 17-49 mg kg^{-1} for high inputs of Ni and Zn, respectively).

Previous studies have suggested that spiked metals are more ‘plant available’ than indigenous sludge metals (Adams and Sanders, 1984) and their fractionation within sludges may change over periods of years (Berrow and Burridge, 1980). In these experiments, a progressively lower proportion of sludge metal inputs was present in $CaCl_2$ extractable forms as metal inputs increased (Khan and Scullion, 1999). The initial pH of the sludges in our experiments was almost a full unit higher than the thresholds for differential $CaCl_2$ extractability of Cu, Ni and Zn in unamended and metal enriched sludges (Adams and Sanders, 1984). This may have resulted in microbial exposure to added metals similar to that for indigenous sludge metals. The decrease with time in soil pH in our incubations and in other similar studies (Wong *et al*., 1998) may explain the increased levels (Zhang *et al*., 2006) of $CaCl_2$ extractable Ni and Zn at 7 weeks. However, effects of metals on pH were too small to explain microbial responses to varying metal inputs.

Microbial properties

More than 70% of the total CO_2-C evolved during each experiment was recorded during the first 3 weeks (Table 3), irrespective of metal treatment. Cumulative C evolution over 7 weeks of incubation was equivalent to around 40% of sludge C inputs; a proportion of this measured C would have derived from decomposition of soil organic matter.

Respiration responses to increased metal inputs varied between metals and with sampling period. Up to 3 weeks, additional Cu inputs decreased respiration in the Cu-Ni experiment but caused an increase in the Cu-Zn experiment; over 7 weeks higher Cu inputs increased respiration in both experiments although this effect was statistically significant only in the Cu-Zn experiment. Higher Ni inputs consistently decreased respiration over the first 3 weeks but had no effect over the entire incubation period. With higher inputs of Zn, respiration progressively increased over both sampling periods, although the effects of medium inputs were often small and again significant only in the Cu-Zn experiment. The combined effect of high inputs of Cu and Ni resulted in the maximum (9% relative to unamended sludge) reduction in respiration after 3 weeks. Over 7 weeks, combined high Cu and Zn inputs caused the largest (10%) increase in cumulative respiration relative to unamended sludge.

Interactions between metal treatments in the Cu-Ni and Cu-Zn experiments were non-significant at both sampling occasions. In the Ni-Zn experiment, highly significant and broadly similar interactions were found for both 0-3 and 0-7 week periods (Figure 1). Higher inputs of Ni or Zn markedly increased CO_2-C evolution when combined with low inputs of the other metal. However, when combined with high inputs of the other metal additional Zn, and in particular Ni, caused a reduction in respiration.

Given the carbon:nitrogen ratio (C:N ratio) (approx. 11.5) of the sludge, the effect of metals on N mineralization might be expected to be proportional to variations in CO_2 production. Thus, Ni inputs would have caused less N release from sludge whereas higher Zn inputs would have promoted this release. The maximum cumulative increase in respiration caused by varying sludge metal concentrations was less than 300 mg kg^{-1} C. So, increased N release attributable to metal effects on sludge mineralization would have been less than 30 mg kg^{-1} N.

Biomass N declined markedly from week 3 to week 7 in all three experiments (Table 4). When sampled at 3 weeks, biomass N was equivalent to between 24 and 32% of sludge N input, falling to around 7-10% at 7 weeks.

At 3 weeks, increasing inputs of all metals caused progressive decreases in biomass N. At 7 weeks, although a similar trend was observed it was much less pronounced and, in most cases, only the high input treatment differed significantly from the low input treatment. Compared with soil to which the low metal sludge had been applied, the largest reductions in biomass N were observed after 3 weeks in the high input Cu soil in the Cu-Ni experiment (-31% relative to unamended sludge) and after 7 weeks in the Cu-Zn experiment (-33% relative to unamended sludge). A single highly significant interaction between metal treatments was found in the Cu-Zn experiment at 3 weeks (Figure 2). Medium inputs of either metal caused marked reductions in biomass N only when combined with medium or high levels of the other.

Table 1. Target[a] total metal concentrations of soil treated with contaminated sludge as estimated from initial soil and sludge metal contents. EC limits[b] (arable soils) are given for information

Metal	Cu	Ni	Zn
	--------(mg kg^{-1})-------------		
Low	35	35	177
Medium	112	58	220
High	182	98	320
EC Directive limits[b]	135	75	200

[a]Concentrations varied for only two metals in each experiment. Targets for low treatments reflected soil/unamended sludge concentrations.
[b]pH ranges from 6 to 7 (EC, 2005).

Figure 1. Interaction plot between metals for cumulative CO_2-C evolved from soil amended with sewage sludge contaminated with varying levels of metals (Ni Zn experiment) a: over 3 weeks, b: over 7 weeks of incubation

a: b:

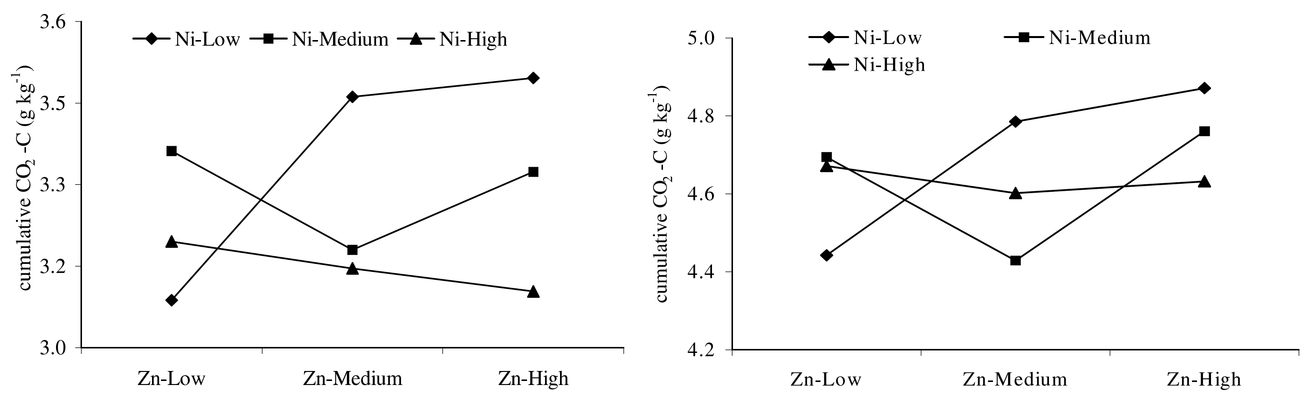


Table 2a. Some properties of soil used in the initial experiment, prior to sludge treatment

Soil series	CEC [a]	LOI [b]	Clay	Silt	Sand
	Cmol(c) kg^{-1}	------------%------------			
Denbigh	61.4	10.1	29.8	41.	28.5
USDA classification - Andic Dystrochrept					

[a] CEC = cation exchange capacity, [b] LOI = loss on ignition.

Table 2b. Mean metal concentrations in soil prior to sludge treatment

	Cu	Ni	Zn
	--------------mg kg^{-1}--------------------		
Total	28	32	159
EDTA	2.6	0.1	4.1
$CaCl_2$	0.4	<0.1	1.8

Table 3. Main treatment and interaction effects on cumulative CO_2-C evolution for three experiments over a: 3 weeks b: 7 weeks of incubation

a:

Treatment	**-Cu-Ni experiment-**		**-Ni-Zn experiment-**		**-Cu-Zn experiment-**	
	Cu	**Ni**	**Ni**	**Zn**	**Cu**	**Zn**
			g kg^{-1}			
Low	3.41a	3.40 a	3.34 a	3.22 b	3.41 b	3.36 c
Medium	3.35 a	3.33 ab	3.26 b	3.23 ab	3.48 b	3.47 b
High	3.22 b	3.24 b	3.15 c	3.30 a	3.56 a	3.63 a
Significance	**	*	***	*	***	***
Interaction	NS		***		NS	

b:

Treatment	-Cu-Ni experiment		-Ni-Zn experiment-		-Cu-Zn experiment-	
	Cu	Ni	Ni	Zn	Cu	Zn
			g kg-1			
Low	4.54	4.58	4.70	4.60 b	4.83c	4.85 c
Medium	4.57	4.54	4.63	4.61 b	4.97 b	4.94 b
High	4.61	4.60	4.65	4.76 a	5.12 a	5.14 a
Significance	NS	NS	NS	***	***	***
Interaction	NS		***		NS	

*P < 0.05, **P < 0.01, ***P < 0.001, NS = non-significant.
For each sampling occasion means in a column with a common letter suffix do not differ at a 5% level of probability (least significant difference test).

Table 4. Main treatment and interaction effects on microbial biomass N for three experiments after a: 3 weeks b: 7 weeks of incubation

a:

Treatment	-Cu-Ni experiment-		-Ni-Zn experiment-		-Cu-Zn experiment-	
	Cu	Ni	Ni	Zn (mg kg-1)	Cu	Zn
Low	317 a	295 a	289 a	288 a	270 a	269 a
Medium	306 a	288 a	269 b	273 a	247 b	239 b
High	217 b	259 b	250 c	248 b	236 c	245 b
Significance	***	***	***	***	***	***
Interaction	NS		NS		**	

b:

Treatment	-Cu-Ni experiment-		-Ni-Zn experiment-		-Cu-Zn experiment-	
	Cu	Ni	Ni (mg kg-1)	Zn	Cu	Zn
Low	124 a	128 a	120 a	119 a	111 a	105 a
Medium	123 a	118 a	114 b	116 a	101 a	96 ab
High	103 b	104 b	110 b	108 b	74 b	87 b
Significance	**	**	**	**	***	**
Interaction	NS		NS		NS	

**P < 0.01, ***P < 0.001, NS = non-significant.
For each sampling occasion means in a column with a common letter suffix do not differ at a 5% level of probability (least significant difference test).

Evidence of progressive decreases in biomass N with increasing concentrations of all three sludge metals was in line with other similar studies (Bogomolov *et al*., 1996). These effects were more pronounced than those reported by Khan and Scullion (1999) for biomass C. They suggested a shift in microbial composition from bacteria to fungi in response to metal inputs, as found by Rajapaksha *et al*. (2004).

Soil mineral N

Mineral N values up to an equivalent of 52% at 3 and 64% at 7 weeks of sludge N inputs were recorded. All high and most medium metal inputs caused significant increases in mineral N after 3 weeks incubation (Table 5). The observed increases in soil mineral N with higher metal inputs were broadly similar to corresponding reductions found in biomass N (Table 4) for Cu in both experiments and for Ni in the Ni-Zn experiment. Mineral N increases with higher Zn inputs in both the Cu-Zn and Ni-Zn experiments, and for Ni in the Cu-Ni experiment, exceeded biomass N reductions. Over the three experiments, the greatest increase in mineral N at 3 weeks (+60% relative to unamended sludge) was observed with the high Cu-Ni combination (Figure 3a). Mineral N levels after 7 and at 3 weeks a significant interaction was found between Cu and Ni; the positive effect of either metal on mineral N was marked only when applied at the high input level and in combination with the high level of the other metal (Figure 3a). At 3 and 7 weeks there were similar significant Ni-Zn interactions (Figure 3b and 3c), where higher Ni inputs caused marked increases in mineral N except when combined with high Zn inputs. Only high inputs of Zn increased mineral N, but this effect was less pronounced when combined with high inputs Ni. The patterns of these interactions differed from those obtained for respiration responses to the same metals (Figure 1).

Investigations into the effect of metals on mineral-N concentrations have produced conflicting conclusions with, for example, studies showing increases (Bogomolov *et al*., 1996) or little change (Mathur and Preston, 1981) for Cu. In our study, with a few exceptions, higher metal inputs caused progressive increases in mineral N. This finding, at least for Zn, was in contrast to that of Rost *et al*. (2001) for sludge enriched with Zn to give soil concentrations similar to those used here. It was expected that these effects would be determined by treatment differences in sludge mineralization, as indicated by CO_2 respiration, and in microbial assimilation of N. At 3 weeks, mineral N increases in response to Cu inputs could be accounted for entirely by decreases in biomass N, given the limited effect of this metal on respiration. In contrast, mineral N increases with higher Zn inputs generally exceeded decreases in biomass N, the difference being attributable to increased mineralization of sludge. There was no consistent pattern in the relative size of mineral and biomass N responses to Ni, nor could respiration effects explain these inconsistencies. Only a proportion of the reduction in biomass N between weeks 3 and 7 was recovered from the mineral pool at the end of the incubations, suggesting that some of the microbial N was incorporated into stable organic forms. Experimental conditions made losses of N from the system unlikely.

Bogomolov *et al*. (1996) considered high mineral N values in metal contaminated soils to be caused mainly by mineralization of microbial cells killed by metal treatments. Whilst this might hold for Cu in our experiments, it did not explain responses to added Zn nor some of the interaction effects obtained. A combination of sludge mineralization and differential retention of N in biomass did however, account for the observed effects of Zn, and some Ni inputs. For the most part, metal effects on biomass N appeared to have a stronger influence than those for sludge mineralization on net N mineralisation.

Effects of metal inputs on microbial and nitrogen indices were, for the most part, additive. Where interactions were found these took two forms. The effects of high levels of Ni or Zn on respiration were diminished when the other metal was also present at high concentrations, suggesting that the combined toxicity effect of these metals was such that higher metabolic quotients (Khan and Scullion, 1999) were balanced by a reduction in biomass. In the other type of interaction, all involving Cu, its combination with high inputs of Ni or Zn led to an enhanced reduction in biomass N and increase in mineral N. These findings may be attributed, at least in part, to Cu causing an increase in $CaCl_2$ extractable Ni and Zn (Khan, 1999).

Relationships between extractable metals and N indices

Correlation coefficients relating different concentrations of extractable metals to N indices are given in Table 6. EDTA and $CaCl_2$ concentrations were not consistently associated with variations in biomass and mineral N across the three experiments, nor was one extractable fraction consistently more highly correlated than the other. Where Cu was included as an experimental treatment, extractable Cu concentrations tended to have closer correlations with N indices than for the other metal. In the Ni-Zn experiment, Zn showed consistently closer correlations. These inconsistencies in associations between extractable metals and N-indices are in line with those found for C-indices by Khan and Scullion (1999) and with

Table 5. Main treatment and interaction effects on mineral N for three experiments (mg kg^{-1}) after a: 3 weeks b: 7 weeks of incubation

a:

Treatment	-Cu-Ni experiment-		-Ni-Zn experiment-		-Cu-Zn experiment-	
	Cu	Ni	Ni	Zn	Cu	Zn
			(mg kg^{-1})			
Low	425 c	428 c	454 b	438 c	474 b	467 c
Medium	457 b	458 b	448 c	463 b	471 b	478 b
High	527 a	524 a	491 a	492 a	504 a	504 a
Significance	***	***	***	***	***	***
Interaction	***		***		NS	

b:

Treatment	-Cu-Ni experiment-		-Ni-Zn experiment-		-Cu-Zn experiment-	
	Cu	Ni	Ni	Zn	Cu	Zn
			(mg kg^{-1})			
Low	520 b	550	507 b	476 b	539 ab	521 ab
Medium	538 b	557	501 b	482 b	572 a	573 a
High	641 a	591	559 a	609 a	484 b	501b
Significance	***	NS	***	***	*	*
Interaction	NS		***		NS	

*P < 0.05, ***P < 0.001, NS = non-significant.
For each sampling occasion means in a column with a common letter suffix do not differ at a 5% level of probability (least significant difference test).

Figure 2. Interaction plot between metals for microbial biomass N in soil amended with sewage sludge contaminated with varying levels of metals after 3 weeks (Cu-Zn experiment)

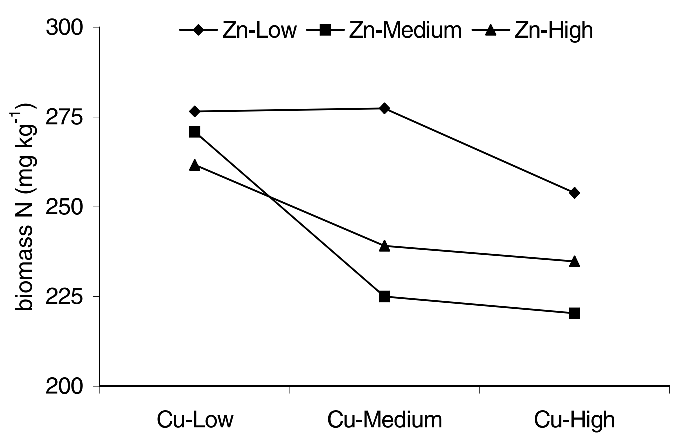
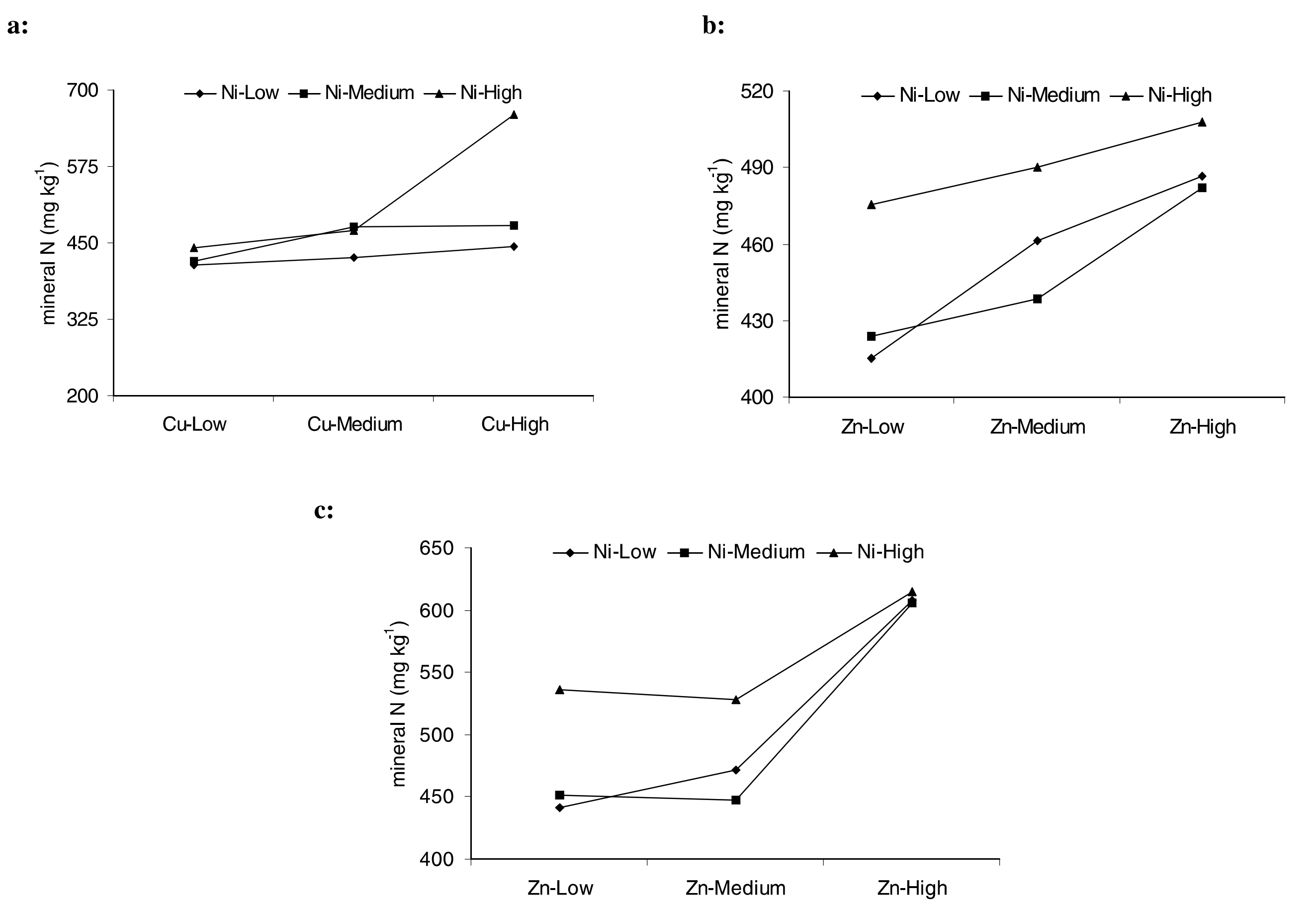


Table 6. Correlation coefficients of EDTA and $CaCl_2$-extractable metal with microbial and mineral N (n= 27)

	EDTA		$CaCl_2$	
	Biomass N	**Mineral N**	**Biomass N**	**Mineral N**
Cu-Ni experiment				
Cu	-0.447*	0.725***	-0.475*	0.638***
Ni	-0.474*	0.256	-0.434*	0.296
Ni-Zn experiment				
Ni	-0.488**	0.353	-0.488**	0.385*
Zn	-0.568**	0.836***	-0.619***	0.853***
Cu-Zn experiment				
Cu	-0.738***	0.295	-0.661***	-0.248
Zn	-0.322	-0.192	-0.279	-0.154

*P < 0.05, **P < 0.01, ***P < 0.001.

Figure 3. Interaction plot between metals for mineral N in soil amended with sewage sludge contaminated with varying levels of metals a: Cu-Ni experiment (3 weeks), b: Ni-Zn experiment (3 weeks), c: Ni-Zn experiment (7 weeks)



conclusions reached by Menzies *et al*. (2007) regarding the prediction of phytoavailabilty of metals.

Conclusions

Treating soil with metal contaminated sewage sludge leads to elevated concentrations of available metals that affect soil microorganisms and their activities. Increases in mineral N with higher sludge metal contents found here and in other studies (Hassen *et al*., 1998) may promote plant growth (Neilsen *et al*., 1997) and thus dilute metal concentrations in plant tissues (Luo and Christie, 1998). Although plants may not show any adverse response, results reported here indicate that microbial processes affecting N cycling from sludge can be influenced by inputs of metals close to current EC guidelines for sludge application to agricultural soils and that the effects of multiple metal contaminations are at least additive. Given the additive nature of most microbial responses to increased metal concentrations, and some examples where responses were more pronounced with combinations of metals, indicate that regulatory controls may be inadequate when based on concentrations of single metals. In only one example (for Ni-Zn interaction for mineral N at 7 weeks) high inputs of one metal nullified the effects of increasing inputs of the other metal. Given the persistence of metals in soil, caution must be exercised (Dahlin *et al*., 1997) in regulating metal pollution. Our data suggest that using higher metal sludges in practice would result in a more rapid release of nitrogen, perhaps leading to higher losses. The mechanisms controlling these effects vary between metals, but can be explained to some extent, by differences in their partitioning between organic matter and exchange site/aqueous pools. Recent field studies (Gibbs *et al*., 2006a, b) failed to find consistent effects of sludge metals on a range of microbial processes. Heterogeneity in microbial activity under field conditions and the potential for losses of soluble metals by leaching may explain differences between these findings and those of the above field studies.

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