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RESIDUAL EFFECTS OF ZINC, COPPER AND NICKEL IN SEWAGE SLUDGE ON MICROBIAL BIOMASS IN A SANDY LOAM

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Summary—Relationships between total metals, CaCl₂-extractable metals and soil microbial biomass were investigated in a sandy loam soil (Cuckney series) at Gleadthorpe Experimental Husbandry Farm, U.K. The metals occurred because sewage sludges, enriched either with different rates of the single metals Zn, Cu or Ni, or with combinations of the metals (Zn and Cu or Zn and Ni) at different rates, were applied in 1982 and again, in some cases, in 1986. The observed increases in total soil metal concentrations were generally in good agreement with the intended soil metal additions. However, the proportional amounts of metals extracted by CaCl₂ differed between metals. Calcium chloride extracted a maximum of about 42% of total Zn, 9% of total Cu and 26% of total Ni in the sludged soil, but very much less (3% of total Zn, 1% of total Cu and 2% of total Ni) in control soils (i.e. the soils that never received sewage sludge). Neither Zn, Cu or Ni present singly in soils at below current EC permitted total soil metal concentrations decreased the amounts of soil microbial biomass. However, Cu at about 4.9 times and Zn at about 2.3 times permitted limits decreased the amounts of soil microbial biomass by 51 and 36%, respectively, when present separately, compared to the control soil. The soils which contained either Cu or Zn separately at about 1.4 times permitted limits contained about 12% less biomass C than the control soil. In contrast, Cu and Zn in combination at about 1.4 and 1.2 times permitted limits, respectively, decreased the biomass by about 29%, and soils containing Cu and Zn in combination at 1.8 and 1.4 times the limits contained 53% less biomass than the control soil. Thus a combination of Zn and Cu decreased the amount of biomass at lower soil metal concentrations than were required when either metal was present singly, suggesting the effects were additive. Biomass C as a percentage of total soil organic C in soils contaminated singly with higher rates of Zn or Cu or with both metals in combination was less than half that in the soil which received no sludge, uncontaminated sludge or sludge contaminated with lower rates of metals. Thus, this statistic provides a sensitive indicator of the effects of heavy metals on microbial biomass.

INTRODUCTION

The supply of mineralized C, N, P and S from soil organic matter, the decomposition of plant and animal residues and the maintenance of soil structure are all dependent upon the correct functioning of the soil microbial ecosystem (De Haan *et al.*, 1989). Therefore it is important to determine and predict the adverse effects of heavy metals and other pollutants on soil microorganisms (Flemming and Trevors, 1989; Bååth, 1989).

When sewage sludges are applied to soil, their beneficial effects due, for example, to the extra organic matter, N or P supplied, may be short lived. However, any heavy metals introduced with them will persist and accumulate with increasing sludge addition. Thus Brookes and McGrath (1984); Brookes *et al.* (1986b); Reddy *et al.* (1987) and Reddy and Faza (1989) reported adverse effects of heavy metals from past applications of sewage sludge on soil

microbial biomass (measured as biomass C or ATP) and microbial activities (measured as urease or dehydrogenase).

There is concern about the increasing concentrations of heavy metals in soils. It is agreed that, if too much metal-contaminated sludge is applied, soil processes will be adversely affected. Yet, insufficient information is available upon which to frame regulations. One particular uncertainty is whether the toxic effects of combinations of metals are synergistic, additive or antagonistic (Beckett and Davis, 1982).

Much of the experimental data upon which such decisions are made comes from plant-growth studies. In contrast, little research has been done on the effects of metals on soil microorganisms, especially *in situ*. Pure culture studies have indicated that the effects of combinations of metals vary with the microbial species and the metal combinations involved (e.g. Stratton and Corke, 1979; Ainsworth *et al.*, 1980; Bååth, 1989). To determine whether the effects of different heavy metals applied in sewage sludge on soil microorganisms are synergistic, additive or antagonistic, factorial experiments with addition of sewage sludges contaminated predominantly with

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single metals or metals in particular combinations are required.

We have investigated a U.K. field experiment on a sandy loam soil of the Cuckney series at Gleadthorpe Experimental Husbandry Farm where sludges enriched with increasing concentrations of the single metals Zn, Cu or Ni and combinations of Zn and Cu or Zn and Ni were applied. This experiment provided us with an opportunity to investigate the effects of single metals and combinations of different metals on the amounts of soil microbial biomass.

MATERIALS AND METHODS

Description of the field experiment

A field trial was laid down in February 1982 at Gleadthorpe Experimental Husbandry Farm (North Nottinghamshire, England) on a sandy loam soil (9% clay) of the Cuckney Series to investigate the effects of Zn, Cu and Ni applied in sewage sludge upon the growth of agricultural crops. In order to investigate the effects of individual metals and the combinations of Zn and Cu or Zn and Ni, sludges were spiked by adding Zn, Cu and Ni salts singly or in combination to sewage effluent at the Water Research Institute, Colehill, near Birmingham. An untreated sludge was also produced. The initial experimental design called for equal quantities of organic matter to be applied to each plot to avoid any differences due to plant nutrient supply or soil structure effects. The untreated sludge was used where necessary to adjust quantities so that all sludged plots received 100 t ha^{-1} of sludge as dry solids. Soil metal concentrations achieved were lower than anticipated and so further additions of naturally-contaminated sludges with either a high Zn or Cu content or both together were made to selected plots in March 1986 (see Table 1). The plots were each $2.5 \times 7.2 \text{ m}$ and the experimental design was a randomized block with two replicates. The different metal treatments are shown in Table 1.

Various arable crops, including barley, Italian rye grass and sugar beet were grown until 1985. Then, only barley was grown until 1988 when all the plots were sown with clover to monitor long-term effects of metals under clover leys. Sulphur was added as appropriate during the winter of 1987–1988 with the aim of adjusting all the soils to about pH 7.

Sampling and preparation of soils

Both replicate plots of 21 treatments were sampled in February 1990. Ten cores (0–10 cm depth) were taken from the middle of each plot and bulked in the field. The bulked cores of moist soil from each plot were then stored separately in polythene bags overnight at 5°C in the dark and sieved ($<2 \text{ mm}$) the following day. Stones, plant materials and visible soil fauna were manually removed. The water holding capacity (WHC) of each soil was determined and the moisture content adjusted to 40% of WHC. The soils were then incubated for 7 days over soda lime at 25°C to permit uniform rewetting and to allow microbial

activity to settle down after the disturbances due to sampling and rewetting (Jenkinson, 1988). Sub-samples of each soil were then air-dried and further portions ground in a Tema mill ($<160 \mu\text{m}$) for chemical analysis.

Soil chemical analyses

Soil pH was measured with a glass electrode using a 1:2.5 soil:water ratio. Total soil N was measured by Kjeldahl digestion (Bremner, 1965) and organic C by dichromate digestion (Kalembasa and Jenkinson, 1973). Total metals in the ground portions of each sample were determined after digestion with 4:1 (v/v) concentrated HCl:HNO₃ (McGrath and Cunliffe, 1985). Metals extractable in 0.1 M CaCl₂ in air-dried and unground soil portions were determined by extracting soils using a 1:5 soil:solution ratio as described by Sauerbeck and Styperek (1985), except that a 16 h shaking period was used in our work. The extracts were analysed for metals using an inductively-coupled plasma-optical emission spectrometry (ICP) (A.R.L. 34000 instrument).

Soil microbial biomass measurements

Soil microbial biomass C measurements were made by the fumigation–extraction method (Vance *et al.*, 1987). Moist soils, equivalent to 50 g on an oven-dry weight basis (105°C , 24 h) were fumigated with ethanol-free chloroform for 24 h at 25°C . After fumigant removal, the soils were extracted immediately by shaking with 200 ml of 0.5 M K₂SO₄ for 30 min. Unfumigated soils were extracted similarly at the time fumigation commenced. The filtered soil extracts were then stored at -15°C until analysis. Organic C in the soil extracts was measured by an automated procedure, using a Dohrmann DC 80 carbon analyser (Wu *et al.*, 1990). In this procedure, 10 ml soil extract was mixed with 10 ml sodium hexametaphosphate [5% (w/v), pH 2]. Potassium persulphate was then automatically added in the carbon analyser and organic C in the extracts was oxidized to CO₂ by reaction with persulphate and u.v. light. The evolved CO₂ was then measured by infra-red analysis.

Soil microbial biomass carbon (B_c) was calculated from: $B_c = 2.22 E_c$ where $E_c = [(\text{organic C extracted from fumigated soil}) - (\text{organic C extracted from unfumigated soil})]$ (Vance *et al.*, 1987; Wu *et al.*, 1990).

Microbial biomass ninhydrin-nitrogen was measured in all soils by the method of Amato and Ladd (1988) as modified by Joergensen and Brookes (1990).

Biomass ninhydrin-N (B_{NIN}) was calculated from: $B_{\text{NIN}} = [(\text{ninhydrin-N in } 0.5 \text{ M K}_2\text{SO}_4 \text{ extracts of fumigated soil}) - (\text{ninhydrin-N in extracts of unfumigated soil})]$.

All measurements were made in duplicate in each soil sample and the mean calculated for each plot. All results given are thus the mean results from the two plots of each treatment.

Table 1. Soil organic C, total N, pH, total metals, 0.1 M CaCl₂-extractable metals, biomass C and ninhydrin-N in soils from Gleadthorpe Experimental Husbandry Farm

Soil treatments (metal rate intended)	Weight of sewage sludge applied in:		pH	Soil organic carbon (%)	Soil total nitrogen (%)	Total			CaCl ₂ -extractable			Biomass C
	1982 (t dry matter ha ⁻¹)	1986 (t dry matter ha ⁻¹)				Zn	Cu	Ni	Zn	Cu	Ni	
No sludge (control soil)	0	0	6.6	1.04	0.10	42	12	8	1.3	0.1	0.2	169
Uncontaminated sludge ^a	200	0	6.5	1.22	0.12	66	19	13	15.0	0.2	1.3	183
Zn-sludge (600 kg ha ⁻¹) ^a	200	0	6.5	1.23	0.13	220	29	12	75.8	0.2	1.0	185
Zn-sludge (1200 kg ha ⁻¹) ^a	200	0	6.3	1.35	0.13	375	23	14	142.6	0.2	0.8	172
Zn-sludge (1900 kg ha ⁻¹) ^a	200	82	6.5	1.43	0.14	457	28	16	185.6	0.2	1.5	140
Zn-sludge (2800 kg ha ⁻¹) ^a	200	82	6.6	1.55	0.15	705	29	14	281.3	0.3	1.4	108
Zn: Cu-sludge (300:250 kg ha ⁻¹) ^a	200	6	6.6	1.22	0.11	127	95	15	39.3	2.2	1.3	186
Zn: Cu-sludge (900:1200 kg ha ⁻¹) ^a	200	71	6.5	1.56	0.16	322	176	13	123.0	3.0	1.7	140
Zn: Cu-sludge (1500:1000 kg ha ⁻¹) ^a	200	110	6.6	1.82	0.19	367	191	12	144.2	4.8	1.6	120
Zn: Cu-sludge (2300:1600 kg ha ⁻¹) ^a	200	162	6.6	1.88	0.20	427	262	14	179.5	9.0	2.5	79
Cu-sludge (1200 kg ha ⁻¹) ^a	200	83	6.5	1.41	0.14	74	197	12	17.3	14.0	1.5	150
Cu-sludge (2000 kg ha ⁻¹) ^a	200	139	6.5	1.82	0.19	104	415	12	25.0	29.9	2.0	94
Cu-sludge (3000 kg ha ⁻¹) ^a	200	199	6.5	1.99	0.20	112	690	14	31.7	62.1	2.0	82
Zn: Ni-sludge (150:25 kg ha ⁻¹) ^a	200	0	6.3	1.21	0.11	89	23	20	9.1	0.3	1.2	182
Zn: Ni-sludge (300:50 kg ha ⁻¹) ^a	200	0	6.5	1.22	0.12	120	21	24	25.8	0.1	3.0	180
Zn: Ni-sludge (450:75 kg ha ⁻¹) ^a	200	0	6.6	1.21	0.11	179	25	29	38.4	0.3	4.7	183
Zn: Ni-sludge (600:100 kg ha ⁻¹) ^a	200	0	6.4	1.22	0.11	200	28	35	37.2	0.2	5.7	181
Ni-sludge (50 kg ha ⁻¹) ^a	200	0	6.4	1.20	0.11	65	23	22	4.8	0.2	5.7	184
Ni-sludge (100 kg ha ⁻¹) ^a	200	0	6.6	1.22	0.11	62	17	33	3.0	0.2	3.5	181
Ni-sludge (150 kg ha ⁻¹) ^a	200	0	6.6	1.21	0.12	68	21	39	3.0	0.2	5.5	189
Ni-sludge (200 kg ha ⁻¹) ^a	200	0	6.6	1.22	0.12	62	17	53	4.9	0.2	8.8	182
LSD (P = 0.05)			0.29	0.045	0.009	9.5	9.3	3.3	8.94	1.49	0.33	11.7
EC <i>Mandatory upper limits</i>						300	140	75				

^aActual amount of metal added per hectare.

RESULTS AND DISCUSSION

Soil pH, organic carbon and total nitrogen

Organic C, total N and pH in the soils of all the treatments are shown in Table 1. The average soil pH was about 6.5 and did not differ between treatments. The organic C and total N contents of soil given uncontaminated sludge in 1982 was about 15% higher than in the non-sludged soils. Soils given Zn-sludge at 1200 kg ha⁻¹ contained significantly more organic C (1.35% C) than soils given 600 kg Zn ha⁻¹ or soils receiving uncontaminated sludge (1.22–1.23% C). The organic C content of soils which received sludges enriched with both Zn and Ni at up to 600 and 100 kg ha⁻¹, respectively and soils contaminated with Ni alone at between 50–200 kg ha⁻¹ all contained 1.20–1.22% C. These results therefore suggest organic matter accumulation at 1200 kg Zn ha⁻¹ but not at these other rates of metal addition. With these exceptions it is not possible to attribute other differences in soil organic C contents between treatments to the different concentrations of metals given in the sludges and now in the soils. This is because all other treatments shown in Table 1 received a further sludge application in 1986 to increase the heavy metal contents further, to target levels. Thus, for example, although soil organic C increased from 1.41% C at 1200 kg Cu ha⁻¹ to 1.99% C at 3000 kg Cu ha⁻¹, the total amount of sludge applied was also increased between these two treatments, from 283 to 399 t ha⁻¹. Thus sludge addition rate and soil heavy metal contents are confounded in many treatments in this experiment and so the effects of heavy metals on the accumulation of organic matter in this experiment will not be discussed further.

Total metal concentrations of the soils

The amounts of total metals (Zn, Cu and Ni) in the soils from all treatments are shown in Table 1. The metal concentrations in the control soils are generally in reasonable agreement with other soil types in Britain (Department of the Environment, 1989). The concentrations of metals were slightly higher in soils given uncontaminated sludge than in the control soils. When sludges containing a particular metal or combination of metals were added, this did not generally cause an increase in the metal concentration in the soil other than that of the principle metal or metals added except that soil Zn concentrations were also increased in soils given Cu-contaminated sludge (Table 1). Addition of sludges enriched with metals at the highest rate increased soil metal concentrations, on average, by about 660 mg Zn, 680 mg Cu and 45 mg Ni kg⁻¹ soil whereas addition of the sludges spiked with combinations of metals (Zn:Cu, Zn:Ni) increased soil metal concentrations by about 385 mg Zn, 250 mg Cu and 27 mg Ni kg⁻¹ soil, respectively. The highest soil Ni concentration (53 mg Ni kg⁻¹ soil) was appreciably lower (by about

30%) than the current permitted maximum limits in agricultural soils (Commission of the European Communities, 1986) while the highest Zn and Cu concentrations (705 mg Zn and 690 mg Cu kg⁻¹ soil) were 2.3 and 4.9 times higher, respectively.

Calcium chloride-extractable metals in soils and their relationship with total soil metal concentrations

Calcium chloride only extracted about 3% of total Zn, <1% of total Cu and about 2% of total Ni in the control soils (Table 1). This may indicate that most of the metals were relatively unavailable to a weak extractant such as CaCl₂. These observations are in line with other reports (Sauerbeck and Styperek, 1985; Sanders *et al.*, 1987). In the sludged plots, while the amounts of metals extracted by CaCl₂ generally depended upon total metal concentrations, the extracted proportions were greater than in the control plots and differed more between metals. For example, a maximum of 9% of total Cu was extracted by CaCl₂, while up to 42% of total Zn and up to 26% of total Ni were extracted by CaCl₂ in the Zn or Ni-sludged plots, respectively (Table 1). These proportions are in close agreement with values reported by Brookes *et al.* (1986b) and Sanders *et al.* (1987). This suggests that metals added in sewage sludge may be more available in soil than native metals.

Correlation coefficients (*r*) between CaCl₂-extractable and total Zn, Cu and Ni concentrations in all sludge-treated soils were highly significant (*r* = 0.993, 0.962 and 0.910 respectively). This suggests that the amounts of metals extracted by CaCl₂ may be a useful index of total metal concentrations in sludged soils. This relationship clearly differed between control and sludged soils (see above) but there were insufficient control soils for a proper statistical analysis so these were not included.

Relationship between soil microbial biomass carbon and ninhydrin-nitrogen

There was a close correlation between biomass carbon (B_C) and biomass ninhydrin-N (B_{NIN}) be-

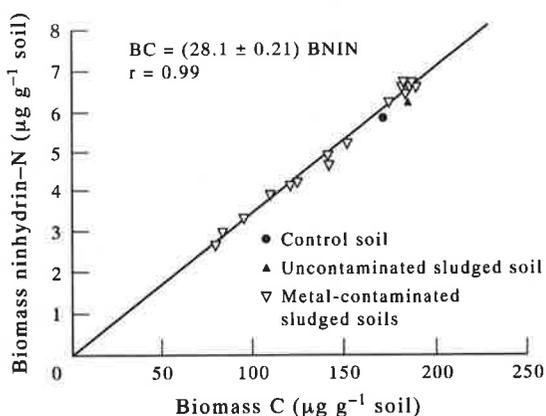


Fig. 1. The relationship between microbial biomass C and microbial biomass ninhydrin-N in Gleadthorpe soils.

tween all the treatments (Fig. 1). The regression equation was: $B_C = (28.1 \pm 0.21)B_{NIN}$; ($r = 0.99$) and the intercept was not significantly different from zero. This proportionality factor (28.1) was very close to that (28.2) reported by Chander and Brookes (1991) and within the range (15–34) reported by Joergensen and Brookes (1990). Amato and Ladd (1988) reported a linear relationship between biomass C and biomass ninhydrin-N in unamended soil ($B_C = 21 B_{NIN}$). Similarly, Carter (1991) also found a linear relationship between biomass C (measured by fumigation-incubation) and biomass ninhydrin-N (measured by fumigation-extraction) using KCl as the extractant in unamended field soils from different tillage systems and pastures and obtained the factor $B_C = 24 B_{NIN}$. Ocio and Brookes (1990) found an identical linear relationship between both unamended and straw-amended soils and obtained the factor $B_C = 31.2 B_{NIN}$. Although there are differences between the proportionality factors in these different studies, the correlations were all highly significant. Amato and Ladd (1988) used a 10 day $CHCl_3$ fumigation period while the others used 24 h, which probably accounts for Amato and Ladd's lower proportionality constant. Thus these results suggest that ninhydrin-N measurements could provide a very rapid measure of biomass in uncontaminated and metal-contaminated soils, and that the microbial biomass in metal-contaminated soils has the same biomass ninhydrin-N concentration as the biomass in the untreated soils. Thus, biomass measurements by fumigation-extraction (Vance *et al.*, 1987) can be made with reasonable confidence either by estimating biomass C or ninhydrin-N in soils contaminated with metals.

Relationship between biomass carbon and total soil metal concentrations

Addition of uncontaminated sludge increased soil biomass C by about 8% compared to amounts in the control soil (i.e. the soil that never received sludge). Addition of Ni-contaminated sludge, which did not increase soil Ni concentrations above current EC limits, increased soil biomass similarly (Table 1). Slightly elevated concentrations of total Zn and Cu also had no adverse effect on biomass C. On the other hand, the soils which contained most Cu ($690 \mu\text{g g}^{-1}$ soil; 4.9 times permitted limit) or most Zn ($705 \mu\text{g g}^{-1}$ soil; 2.3 times permitted limit) had 51 and 36% smaller biomasses, respectively, than the control soil. In addition, a combination of both Zn and Cu caused larger decreases in microbial biomass at lower soil concentrations compared to either of the single metals at much higher concentrations. For example, the soils which contained either Cu or Zn separately at about 1.4 times permitted limits had about 12% less microbial biomass compared to the control soil. However, the soils which contained *both* Cu at 1.4 times the permitted limit *and* Zn at 1.2 times the permitted limit (i.e. $191 \mu\text{g Cu}$ and $367 \mu\text{g Zn g}^{-1}$

soil, respectively) had 29% less microbial biomass. Similarly, the soil which contained *both* Cu at about 1.8 times *and* Zn at about 1.4 times the permitted limit (i.e. $262 \mu\text{g Cu}$ and $427 \mu\text{g Zn g}^{-1}$ soil) had 53% less biomass, than the control soil. These results therefore suggest that a combination of Zn and Cu was more harmful to the biomass than when either was present singly and that the effects of Zn and Cu in combination were about the same as the sum of these two metals when present individually at approximately similar soil concentrations. This provides evidence that Zn and Cu are additive in their toxic effects on soil microbial biomass. In contrast, a combination of Zn and Ni did not decrease the microbial biomass in this experiment. This was almost certainly because the concentrations of Zn and Ni in the soils of these treatments were very low (*ca* 0.3–0.7 times the permitted limits, respectively).

There appear to be no comparable reports in which the effects of metals, present singly or in combination, on soil microorganisms *in vivo* have been compared. Similarly there is little information on the suppression of growth or tolerance developed by individual microorganisms *in vivo* with respect to combinations of metals. However, there is evidence for both positive and negative interactions between metals upon their toxic effects to microorganisms *in vitro*. For example, Babich and Stotzky (1982) reported that Mg and Zn decreased the toxicity of Ni to microorganisms. Conversely, Ainsworth *et al.* (1980) found synergisms between Ni and Cd in their toxic effects towards growth and survival of *Klebsiella pneumoniae*. Singh and Dwivedi (1987) found that at 500 mg Cu l^{-1} and 500 mg Zn l^{-1} solution, the growth of *Sclerotium rolfsii* was decreased by 18.4 and 21.4% respectively, compared with 42.6% in combination, suggesting that the toxic effects of Zn and Cu were additive.

Relationship between biomass carbon and $CaCl_2$ extractable metals

In the European Community the permitted limits of metals in agricultural soils are based on total soil metal concentration (aqua regia soluble) (Department of the Environment, 1989; Commission of the European Communities, 1986). Brookes and McGrath (1984, 1987) and Brookes *et al.* (1984, 1986a, b) considered these limits with respect to the effects of metals on soil microbial biomass and microbial activities. However, there has been little work on the relationship between $CaCl_2$ -extractable metals and the amounts of microbial biomass in metal-contaminated soils. This was studied in this work.

Calcium chloride-extractable Zn above $142 \mu\text{g g}^{-1}$ soil and Cu above $14 \mu\text{g g}^{-1}$ soil caused marked decreases in the amounts of soil microbial biomass when the metals were present separately (Table 1). Moreover, when both Zn and Cu were present together in the soils, $CaCl_2$ -extractable Zn above $123 \mu\text{g g}^{-1}$ soil and Cu above $3.0 \mu\text{g g}^{-1}$ soil also

decreased the amount of microbial biomass. This suggests that Zn and Cu in combination were more harmful than either of the single metals at similar soil concentrations so that their toxic effects on the soil microbial biomass were additive. In contrast, neither a combination of Zn and Ni up to CaCl_2 -extractable concentrations of 37 and $5.7 \mu\text{g g}^{-1}$ soil respectively, nor Ni alone, up to CaCl_2 -extractable concentrations of $12.1 \mu\text{g g}^{-1}$ soil, decreased the biomasses. These trends are the same as those discussed previously in relation to total metal concentrations in soil and thus indicates that CaCl_2 extracts could be used as an indication of whether microbial biomass was likely to be affected by a particular metal concentration or combination of metals in soil.

Relationship between biomass carbon and total soil organic carbon

Generally biomass C comprises about 1–4% of total soil organic C (e.g. Jenkinson and Ladd, 1981; Insam *et al.*, 1989; Anderson and Domsch, 1989). Thus there is an approximate linear relationship between these two variables, although it may vary somewhat between soils of different physical characteristics or between soils undergoing different managements. For example, clay soils may contain several times the amount of biomass than sandy soils under similar management (e.g. Lynch and Panting, 1980; Van Veen *et al.*, 1985). Also, in general, the microbial biomass in forest and grassland soils forms a larger proportion of total soil organic matter than the biomass in arable soils (e.g. Ayanaba *et al.*, 1976; Adams and Laughlin, 1981; Gupta and Germida, 1988; Srivastava and Singh, 1988). This is in accord with the statement of Jenkinson and Ladd (1981) that situations favouring the accumulation of organic matter in soil increase both the amount of biomass and the proportion of this biomass in the total soil organic matter.

Changes in soil management cause the microbial biomass to increase or decrease much faster than the total amount of soil organic matter. Ayanaba *et al.* (1976) and Adams and Laughlin (1981) reported that changing from forest or grassland to arable management caused much greater decreases in biomass C than total soil organic C. Similarly, Powlson *et al.* (1987) reported that 18 yr of straw incorporation in Danish soils caused about a 40–50% increase in biomass C whereas total soil organic C only increased by 5%, a statistically insignificant increase. Similar results were also reported by Saffigna *et al.* (1989) for Australian soils. This, and much other similar work, supports the original idea of Powlson and Jenkinson (1976) that the biomass is a much more sensitive indicator of changing soil conditions than is total soil organic matter content so that the biomass can serve as an 'early warning' of such changes long before they may be detected in other ways. There is now accumulating evidence that heavy metals at around, or a little above, current permitted EC limits also decrease the

proportion of biomass C in total soil organic matter. Thus, Brookes and McGrath (1984) reported that soils from the Woburn Market Garden Experiment which last received metal-contaminated sewage sludge more than 30 yr ago (high-metal soils) contained up to 50% less microbial biomass than similar soils which received inorganic fertilizer or farmyard manure (low-metal soils) over this period. The biomasses in the high-metal soils, now containing heavy metals at up to three times above current EC limits, also showed no correlation with total soil organic C, unlike the low-metal soils from the same experiment. Recently, Chander and Brookes (1991) reported that biomass C as a percentage of total soil organic C was twice as large (1.5–2.0%) in non-sludged soils or soils which received non-contaminated sewage sludge than in soils which received Cu-contaminated or Zn-contaminated sewage sludge (0.7–1.0%) in U.K. field experiments at Luddington and Lee Valley experimental farms.

Similarly, in our work, amounts of biomass C as a percentage of total soil organic C in control soils and in soils given uncontaminated sludge ranged from 1.5 to 1.6 (Fig. 2). These values were within the range of those reported for other soils (see previous text), and are remarkably similar to those reported by Chander and Brookes (1991). The values of this percentage in soils containing higher concentrations of either Zn or Cu or both were also less than half (0.4–0.7) those in control soils or in soils given uncontaminated sludge (1.5–1.6).

So far, such data have only been obtained from carefully designed and controlled field experiments, with full plot replication and containing plots which have never received sewage sludge or plots which have received uncontaminated sewage sludge, so that easy comparisons can be made. It is desirable that this work should be extended to the natural environment to determine the environmental effects of heavy metals from, for example, sewage sludge disposal, mining of metal ores or metal smelting works. The collection of such data is always beset by the difficulty of obtaining valid comparable analytical results from suitable uncontaminated control soils. We suggest that the link between biomass C and total soil organic C, discussed above, may itself constitute an 'internal control' so that when soils deviate much from (biomass C):(total soil organic C) ratios perceived as normal for the particular management, soil type and climate, it may be a *preliminary indication* that some damage or change to the functioning of the soil ecosystem has occurred due, for example, to heavy metals and indicate that further work should be done.

As discussed earlier, because larger additions of sludge were applied to soils containing the higher concentrations of all metals apart from Ni it was not possible to relate soil metal concentration to soil organic matter accumulation in this experiment, as has been previously reported (e.g. Pugh and Williams, 1971; Rühling and Tyler, 1973; Tyler, 1981; Nordgren

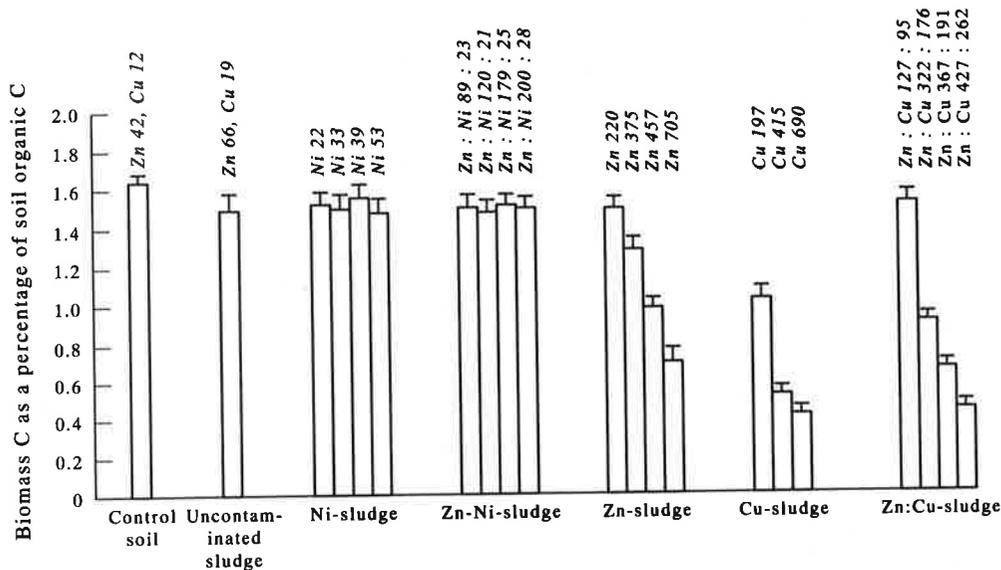


Fig. 2. Microbial biomass C expressed as a percentage of total soil organic C in Gleadthorpe soils (standard errors shown). Values given in italic text are the total soil metal concentrations ($\mu\text{g g}^{-1}$ soil).

et al., 1983; Haanstra and Doelman, 1984; Chander and Brookes, 1991). However, our finding of higher and very comparable biomass C as a percentage of total soil organic C in the other treatments given sludge but containing lower amounts of metals strongly suggests that it was the metals themselves rather than, for example, persistent and toxic organic compounds in the sludges, that were decreasing the biomass. The higher organic matter contents in the soils containing higher concentrations of, for example, Zn and Cu either separately or in combination, or Cu alone (Table 1) did not apparently prevent the metals at the higher concentrations from decreasing the biomass.

We consider that the most important findings from this work are:

- (1) The amounts of metals extracted by CaCl_2 were mainly proportional to the total soil metal concentrations. However, the proportions extracted differed between metals: calcium chloride extracted a maximum of about 42% Zn, 9% Cu and 26% Ni, expressed as a percentage of the total soil metals in the sludged soils, but very much less (3% Zn, 1% Cu and 2% Ni) in soils that never received sewage sludge.
- (2) Zn at about 2.3 times and Cu at about 4.9 times current permitted EC limits decreased the amounts of soil microbial biomass by about 36 and 51% respectively when present separately compared to the soil which never received sewage sludge (the control soil).
- (3) Zn and Cu when present separately at about 1.4 times current permitted EC limits respectively each decreased the biomass by about 12% whereas in combination (Cu at

about 1.8 times and Zn at 1.4 times EC limits) the biomass was decreased by about 53% compared to the control soil. Thus a combination of Zn and Cu decreased the microbial biomass much more than the individual metals at similar soil concentrations, suggesting the effects were additive.

- (4) Ni alone or a combination of Ni with low concentrations of Zn did not show any adverse effects on soil microbial biomass in this experiment. However, soil Ni concentrations were all below current permitted EC limits.
- (5) The ratio (biomass C):(soil organic C) in soils contaminated with higher concentrations of Zn or Cu or both was less than half that in the control soil. Thus this ratio provided a sensitive indicator of the effects of heavy metals on microbial biomass.

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