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Initial results from a long-term, multi-site field study of the effects on soil fertility and microbial activity of sludge cakes containing heavy metals

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Abstract

In a long-term study of the effects on soil fertility and microbial activity of heavy metals contained in sewage sludges, metal-rich sludge cakes each with high Zn, Cu or Cd concentrations were applied annually for 4 years (1994–1997) to nine sites throughout Britain. These sites were selected to represent agricultural soils with a range of physical and chemical properties, typical of those likely to be amended with sewage sludge. The aim was to establish individual total Zn (approx. 60–450 mg kg^{-1}), total Cu (approx. 15–200 mg kg^{-1}) and total Cd (approx. 0.2–4 mg kg^{-1}) metal dose–response treatments at each site. Sludges with low metal concentrations were added to all treatments to achieve as constant an addition of organic matter as possible. Across the nine sites, soil pH was the single most important factor controlling Zn ($P < 0.001$; $r^2 = 92\%$) and Cd extracted with 1 M NH_4NO_3 ($P < 0.001$; $r^2 = 72\%$), and total iron content the most important factor controlling Cu extracted with 1 M NH_4NO_3 ($P < 0.001$; $r^2 = 64\%$). There were also positive relationships ($P < 0.001$) between soil organic carbon (C) concentrations and soil biomass C and respiration rates across the nine sites. Oxidation of sludge C following land application resulted in approximately 45% of the digested sludge cake C and approximately 64% of the 'raw' sludge cake C being lost by the end of the 4-year application period. The sludge cake applications generally increased soil microbial biomass C and soil respiration rates, whilst most probable numbers of clover *Rhizobium* were generally unchanged. Overall, there was no evidence that the metal applications were damaging soil microbial activity in the short term after the cessation of sludge cake addition.

Keywords: Sewage sludge, biosolids, heavy metals, soil biomass C, soil respiration, *Rhizobium* most probable numbers

Introduction

Sewage sludge is a useful source of major plant nutrients (nitrogen, phosphorus, sulphur and magnesium) and organic matter, and as a result of some conditioning processes may have value as a liming material (MAFF, 1987). However, sewage sludge can contain larger concentrations of heavy metals than most soils. There is concern that once the metals have been added to agricultural land and accumulate in the topsoil, they could have long-term negative effects on soil fertility and microbial activity as they are not easily leached and crop offtakes are small.

As a result of the European Union ban on sewage sludge dispersal at sea, which ceased at the end of 1998, and tighter restrictions on direct discharges of sewage, larger quantities of sludge are being recycled to agricultural land in the UK. A UK sludge survey (Gendebien *et al*., 1999) estimated that in 1996–1997, 520 000 tonnes of sludge dry solids per year (tds $year^{-1}$) were applied to agricultural land, which was equivalent to 47% of UK sludge production (excluding dedicated sites). The total area of land receiving sludge in 1996–1997 was 80 000 ha, at an average annual application rate of 6.5 tds ha^{-1}. Reuse to agricultural land is predicted

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to remain the principal outlet (Gendebien *et al.*, 1999). A further 72 000 tds were reused in land reclamation, forestry and as a component of soil and compost products.

The maximum permitted metal concentrations in soils, laid down in the European Community Directive 86/278/EEC (CEC, 1986), have been implemented in England, Scotland and Wales by The Sludge (Use in Agriculture) Regulations – Statutory Instrument No. 1263 (SI, 1989) and as amended by The Sludge (Use in Agriculture) Amendment Regulations 1990 – Statutory Instrument No. 880 (SI, 1990). In addition, a Code of Practice for the Agricultural Use of Sewage Sludge complements the UK Regulations (DoE, 1996). The Code contains advice on soil metal limits not subject to the provisions of Directive 86/278/EEC and additional guidance concerning sludge treatment and land application. As a result of recommendations from an Independent Scientific Committee Reviewing the 'Soil Fertility Aspects of Sludge Applications to Agricultural Land' (MAFF/DoE, 1993), the topsoil advisory limit for total Zn for pH 5–7 was reduced to 200 mg kg^{-1} (a reduction from 300 mg kg^{-1} at pH 6–7 and 250 mg kg^{-1} at pH 5.5 to <6) and the total Cd limit for grassland (0–7.5 cm) was reduced from 5 to 3 mg kg^{-1}. These reductions were seen as precautionary measures (DoE, 1996) to protect soil fertility.

The soil microbial biomass is widely recognised as an important agent in soil organic matter breakdown and recycling of plant nutrients. Generally, biomass size and activity increase when organic materials are applied to soils. However, Brookes & McGrath (1984) showed that the microbial biomass in soil that had received aerobically digested sewage sludge dried in lagoons was half that in soil which had received farmyard manure (FYM). The sludge-treated soil had a much higher respiration rate per unit weight of biomass (commonly known as specific respiration rate) than the FYM-treated soil, although the adenylate energy charge of the biomass (a measure of potential metabolic activity) was high in both soils (Brookes & McGrath, 1987). Work at Luddington and Lee Valley showed that biomass C as a proportion of total soil organic C was reduced, where Zn and Cu enriched sludge had been added (Chander & Brookes, 1991). Also, the effects of Zn and Cu were found to be additive in reducing microbial biomass (Chander & Brookes, 1991). Stark & Lee (1988) also showed that biomass size generally decreased with increasing soil metal concentrations. McGrath *et al.* (1987) showed that less atmospheric N was fixed on sludge-treated plots of the Woburn Market Garden Experiment. Large decreases in fixation of atmospheric N are highly significant, if future agricultural systems are required to exploit more fully the N-fixing properties of leguminous crops (e.g. clover, peas, beans and lupins).

Based on present knowledge and available experimental sites in the UK, it was not possible to identify unequivocally which of the metals, or combination of metals, present in sewage sludges were responsible for the toxic effects noted in crops and soil microbial processes. It was unlikely to be Cr, Pb or Hg as these are largely insoluble in sludge-treated soils of near-neutral pH and above (McGrath 1987), but Zn and Cu were considered to be the most likely metals responsible for the observed effects. The involvement of Zn was confirmed by results from an experiment at Braunschweig in Germany (Chaudri *et al.*, 1993; McGrath *et al.*, 1995). However, Smith (1991) who reviewed historical data on sludge-treated soils, considered that Cd could be the important element in determining the presence of *Rhizobium* in soil.

McGrath (1990) concluded that 'at present there is not enough experimental evidence available upon which to base upper limit concentrations for soil microbial processes and that processes performed by a few organisms, for example N_2-fixation and nitrification are those most likely to be affected'. Similarly, Smith (1991) concluded that at present 'our knowledge of the effects of heavy metals in sewage sludge on soil microbial processes is insufficient to establish whether the innate fertility of agricultural soils is being significantly affected by sludge application within current maximum soil limits for metals'. Similarly, the Independent Scientific Committee Reviewing the 'Soil Fertility Aspects of Sludge Applications to Agricultural Land' (MAFF/DoE, 1993) recommended that 'further research is needed to examine the effects of heavy metals from sewage sludge on soil microorganisms'.

In order to examine the effects of individual metals (Zn, Cu and Cd) on soil microbial activity and long-term soil fertility, a series of long-term field experiments were established at nine sites throughout Britain in 1994. At each site, individual Zn, Cu and Cd dose–response treatments were established using sludge cakes containing high concentrations of either Zn, or Cu, or Cd only. In addition to the metal-rich sludge cakes, 'low' metal content sludge cakes were applied to balance organic matter additions across the metal dose–response treatments. This paper presents results from the initial metal build-up phase (1994–1997) of this long-term study.

Materials and methods

Experimental sites

Nine experimental sites [Gleadthorpe (GLE), Woburn (WOB), Watlington (WAT), Pwllpeiran (PWL), Rosemaund (ROS), Bridgets (BRI), Hartwood (HAR), Auchincruive (AUC) and Shirburn (SHI)] were selected throughout Britain to represent a range of soil physical and chemical properties, in particular clay and organic matter contents (Figure 1).

Metal-rich sludge cakes were selected to have only high Zn, or Cu, or Cd concentrations relative to the concentrations of the other metals present (Table 1). These sludge cakes which contained metal concentrations much higher than would normally be applied to agricultural land were applied annually to the nine sites over 4 years (1994–1997) to

Figure 1 Location of experimental sites in the UK.

establish the individual Zn, Cu and Cd metal dose–response treatments (Table 2). In addition to the sludge cakes with high metal concentrations, sludge cakes with low metal concentrations were added to achieve constant organic matter inputs on all the metal dose–response treatments. The digested Zn- and Cd-containing sludges were matched with a digested low metal content sludge whereas the raw (undigested) Cu-rich sludge was balanced with a low metal raw sludge. The upper limits were chosen to be above the maximum concentrations permissible in 1994 and for this reason an extra rate 5 treatment was necessary at the calcareous Shirburn site (SI, 1989). In addition, there were control treatments that received either no sludge or only sludge cake with low metal concentrations.

The treatments were replicated in three fully randomised blocks (45 plots at sites 1–8, and 60 plots at Shirburn). Individual plots ($6 \text{ m} \times 8 \text{ m} = 48 \text{ m}^2$) were bounded by permanent grass strips to prevent soil movement during cultivation. Cultivations were carried out annually using a Celli spading machine to help ensure that the sludge additions were evenly incorporated throughout the topsoil depth (0–25 cm) and to encourage organic matter breakdown of the sludge cakes. The target topsoil pH at sites 1–6 was 6.5, as recommended for arable crops in England and Wales (Anonymous, 2000), with lime additions made where necessary to maintain the pH at 6.5. For the sites in Scotland, the target topsoil pH was 5.8 at Hartwood (typical for grassland in Scotland) and pH 6.0 at Auchincruive (typical for a grass and arable rotation in Scotland). At Shirburn, the inherent pH is approximately 8.0 as a result of the calcium carbonate content of the soil.

Soil and sludge cake analysis

Topsoil samples (0–25 cm) were taken in April/May 1994 prior to the first sludge cake applications to characterise each site (Table 3) and again in October 1997 from all

Table 1 Chemical analysis of sludge cakes added from 1994 to 1997 at the nine field sites

	'Low metal' digested	'Low metal' raw	Zinc-rich	Copper-rich	Cadmium-rich
DM (%)	18.3	36.7	23.5	18.0	67.8
Org C (%ds)	38.1	42.9	31.6	37.6	12.9
N (%ds)	5.25	3.50	3.95	4.35	1.32
NH_4-N (%ds)	0.55	0.87	0.82	0.77	0.04
P (%ds)	1.98	1.61	2.87	0.76	1.70
K (%ds)	0.16	0.13	0.18	0.17	0.29
pH	7.3	7.3	7.5	5.2	6.8
Zn (mg kg^{-1})	560	490	6000	550	1100
Cu (mg kg^{-1})	590	450	1400	5050	540
Cd (mg kg^{-1})	1.8	1.7	11.2	0.7	44
Ni (mg kg^{-1})	27	16	560	39	130
Pb (mg kg^{-1})	94	140	830	1240	410
Cr (mg kg^{-1})	47	33	1400	450	430
Al (mg kg^{-1})	15 900	11 200	24 350	8450	20 050
Fe (mg kg^{-1})	9800	4800	22 750	8500	22 850
Mn (mg kg^{-1})	350	200	760	230	640
Hg (mg kg^{-1})	2.72	2.57	3.63	1.76	4.22

DM, dry matter; ds, dry solids.

Table 2 Target total topsoil (0–25 cm) metal concentrations, including background (mg kg^{-1}) in metal-rich sludge cake experiments

Target level	Zinc	Copper	Cadmium
Background range	34–140	7.4–22.6	0.12–0.95
Rate 1	150 (200[a])	50	1 (1.5[b])
Rate 2	250	100	2
Rate 3	350	150	3
Rate 4	450	200	4
Rate 5[c]	600	275	5

[a]Target rate 1 for Pwllpeiran, taking account of an elevated background soil Zn concentration (140 mg kg^{-1}).
[b]Target rate 1 for Bridgets, taking account of an elevated background soil Cd concentration (0.95 mg kg^{-1}).
[c]Rate 5 treatment only applied at the Shirburn site.

plots following the final sludge application. The topsoil samples from all the plots and sludge cake samples were analysed for total [perchloric-nitric acid digestion] (MAFF, 1986) and ammonium nitrate (NH_4NO_3) extractable (Deutsche Institute fur Normung, 1997) Zn, Cu and Cd concentrations – draft ISO standard method (ISO/CD 19730) for extraction of trace elements in soil (Tables 4 and 5). Topsoil samples were also analysed for pH (in water), organic C [potassium dichromate oxidation] (MAFF, 1986), total iron, aluminium and manganese contents [perchloric-nitric acid digestion]. The topsoil organic C values in 1997 and sludge C loadings were used to estimate the amount of sludge C remaining after the final sludge application (Table 6). Soil samples from the untreated control, low metal digested, low metal raw, Zn rate 3, Cu rate 3 and Cd rate 3 treatments were analysed for soil microbial biomass C (Vance *et al*., 1987) (Table 7), soil respiration rate (Smith & Hadley, 1990) (Table 8) and most probable numbers (MPNs) of clover *Rhizobium* (Vincent, 1970; Woomer *et al*., 1990) (Table 9). The rate 3 treatments were selected for analysis as these target soil levels represented the maximum permissible metal levels in soils treated with sludge (DoE, 1996).

Statistical analysis

Results from the soil microbial biomass C, soil respiration rate and MPNs of clover *Rhizobium* following the fourth sludge cake application were analysed by ANOVA (all six treatments), with significant results analysed further by the Dunnett's test. The ANOVA was used to test whether there were treatment differences between all of the means. The Dunnett's test was used to establish whether there were differences (i) between the three digested sludge cake treatments, and (ii) between the two raw sludge cake treatments.

In order to assess the influence of soil properties on topsoil metal extractability, stepwise multiple regression was used (GENSTAT Committee, 1993), involving all the soil variables listed in Table 3 (excluding soil $CaCO_3$ content), plus the individual plot soil pH and organic C contents measured in 1997.

Table 3 Mean physical, chemical and microbial properties of topsoil (0–25 cm) at untreated field sites in 1994

	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Sand (> 63 μm) (%)	71 (1.73)	80 (1.20)	56 (2.33)	24 (4.04)	8 (0.67)	10 (0.88)	59 (2.56)	51 (1.33)	44 (2.52)
Silt (2–63 μm) (%)	22 (1.45)	12 (0.88)	28 (1.20)	53 (4.18)	67 (0.67)	60 (2.85)	20 (1.20)	29 (0.29)	36 (3.71)
Clay (< 2 μm) (%)	7 (0.33)	8 (0.33)	16 (1.33)	23 (0.33)	25 (0.67)	30 (2.00)	21 (3.38)	20 (1.04)	20 (1.20)
FAO[a] Soil Class (%)	Cambisol	Arenosol	Cambisol	Cambisol	Luvisol	Rendzina	Cambisol	Fluvisol	Rendzina
pH (%)	7.1 (0.06)	7.2 (< 0.01)	7.4 (0.06)	5.4 (0.03)	7.0 (0.09)	6.8 (< 0.01)	5.8 (0.04)	6.0 (0.09)	8.0 (0.06)
$CaCO_3$ (%)	nd	nd	nd	nd	nd	nd	nd	nd	11.6 (nd)
Org. C (%)	1.2 (0.06)	1.3 (0.07)	1.3 (0.07)	3.3 (0.21)	1.7 (0.12)	1.5 (0.12)	4.7 (0.19)	2.5 (0.09)	3.0 (0.20)
Fe_2O_3 (%)	1.66 (0.11)	2.90 (0.09)	3.71 (0.12)	5.69 (0.30)	4.77 (0.06)	4.00 (0.24)	3.32 (0.06)	4.24 (0.10)	3.25 (0.05)
Al_2O_3 (%)	1.51 (0.09)	1.13 (0.02)	2.25 (0.26)	4.97 (0.11)	5.77 (0.12)	5.10 (0.20)	7.87 (0.22)	3.54 (0.09)	2.45 (0.10)
MnO_2 (%)	0.06 (0.01)	0.03 (< 0.01)	0.09 (< 0.01)	0.15 (0.02)	0.16 (0.01)	0.23 (0.01)	0.96 (0.20)	0.12 (< 0.01)	0.12 (< 0.01)
Total Zn (mg kg^{-1})	34.4 (0.92)	44.8 (1.06)	43.3 (0.49)	140 (4.59)	77.8 (0.29)	49.4 (0.61)	72.3 (1.87)	82.4 (0.42)	68.5 (0.94)
Total Cu (mg kg^{-1})	7.4 (0.28)	13.9 (0.36)	11.4 (0.11)	13.2 (0.74)	17.3 (0.09)	12.2 (0.24)	19.8 (0.33)	22.6 (0.14)	13.1 (0.12)
Total Cd (mg kg^{-1})	0.17 (0.01)	0.12 (< 0.01)	0.27 (0.01)	0.15 (< 0.01)	0.24 (0.01)	0.95 (0.04)	0.23 (0.01)	0.33 (< 0.01)	0.37 (0.01)
Biomass C (mg kg^{-1})	66 (3.5)	108 (4.5)	278 (10.0)	609 (23.8)	419 (10.0)	293 (11.2)	663 (24.8)	428 (6.8)	1094 (29.5)
Respiration (mg CO_2-C kg^{-1} h^{-1})	0.14 (0.01)	0.16 (0.01)	0.23 (0.01)	0.32 (0.01)	0.17 (0.01)	0.32 (0.02)	0.16 (0.01)	0.47 (0.01)	0.58 (0.02)
Rhizobium MPN ($\log_{10}$ cells g^{-1})	4.9	4.7	4.9	2.8	5.0	4.8	4.8	4.6	4.6

Values in parentheses are standard errors ($n = 3$).
MPN, most probable number; nd, not determined.
For location and key to sites, see Figure 1.
[a]FAO (1998).

Table 4 Mean total metal concentrations (mg kg^{-1}) in topsoil (0–25 cm) after fourth sludge cake application (1997)

Treatment	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Zinc									
Control	43 (1.7)	46 (1.8)	47 (1.4)	150 (7.4)	82 (0.7)	68 (0.8)	81 (3.8)	75 (4.4)	73 (2.6)
Low metal digested cake	85 (2.3)	76 (14.7)	88 (2.9)	156 (17.4)	108 (2.1)	106 (1.6)	108 (0.4)	111 (3.3)	115 (8.1)
Zn rate 1	134 (6.6)	125 (14.1)	203 (14.0)	212 (8.3)	152 (5.3)	202 (27.7)	171 (16.8)	147 (1.8)	185 (5.9)
Zn rate 2	232 (49.0)	211 (8.1)	304 (35.5)	274 (2.4)	251 (4.5)	299 (17.9)	240 (11.6)	236 (10.2)	298 (20.4)
Zn rate 3	334 (44.4)	304 (24.9)	459 (21.6)	371 (17.1)	444 (8.0)	414 (9.3)	406 (30.9)	314 (8.1)	345 (5.4)
Zn rate 4	291 (29.4)	224 (16.4)	539 (10.6)	370 (20.5)	421 (34.7)	459 (51.0)	443 (4.2)	342 (30.3)	414 (17.5)
Zn rate 5	nd	nd	nd	nd	nd	nd	nd	nd	579 (20.3)
Copper									
Control	9 (0.5)	14 (1.0)	13 (0.8)	19 (2.9)	37 (18.4)	15 (2.5)	25 (5.5)	18 (0.6)	15 (0.9)
Low metal raw cake	37 (2.2)	36 (1.1)	48 (1.0)	54 (0.8)	50 (4.0)	67 (4.6)	56 (8.6)	53 (2.3)	41 (2.4)
Cu rate 1	69 (6.8)	56 (3.3)	109 (0.3)	81 (2.9)	74 (5.5)	75 (2.7)	74 (8.4)	66 (4.2)	76 (7.2)
Cu rate 2	120 (18.3)	92 (10.3)	178 (28.1)	182 (13.7)	137 (17.7)	136 (2.4)	129 (6.6)	103 (9.1)	140 (5.9)
Cu rate 3	188 (39.4)	161 (15.0)	254 (14.1)	202 (15.3)	202 (38.7)	212 (1.0)	195 (15.7)	140 (17.0)	171 (2.1)
Cu rate 4	166 (23.6)	188 (37.6)	309 (26.4)	234 (37.9)	219 (21.8)	209 (3.8)	239 (17.1)	206 (31.6)	224 (13.3)
Cu rate 5	nd	nd	nd	nd	nd	nd	nd	nd	262 (12.6)
Cadmium									
Control	0.2 (< 0.01)	0.2 (0.02)	0.3 (0.02)	0.2 (0.02)	0.3 (0.01)	1.0 (0.01)	0.3 (0.01)	0.3 (< 0.01)	0.5 (0.03)
Low metal digested cake	0.3 (0.01)	0.3 (0.06)	0.4 (0.01)	0.3 (< 0.01)	0.3 (0.01)	1.1 (0.02)	0.4 (0.01)	0.3 (< 0.01)	0.6 (0.03)
Cd rate 1	1.0 (0.20)	0.8 (0.08)	1.3 (0.06)	1.1 (0.07)	1.0 (0.04)	1.8 (0.38)	1.2 (0.09)	1.0 (< 0.01)	1.3 (0.14)
Cd rate 2	1.9 (0.13)	1.7 (0.22)	2.5 (0.17)	2.3 (0.39)	1.7 (0.09)	2.5 (0.28)	2.0 (0.06)	2.6 (0.29)	2.1 (0.23)
Cd rate 3	2.8 (0.17)	2.9 (0.17)	4.0 (0.11)	3.4 (0.14)	2.1 (0.85)	3.4 (0.21)	3.4 (0.10)	3.4 (0.39)	3.5 (0.20)
Cd rate 4	3.3 (0.21)	3.5 (0.37)	4.8 (0.16)	4.2 (0.56)	3.7 (0.02)	4.7 (0.42)	4.6 (0.13)	3.8 (0.22)	4.1 (0.31)
Cd rate 5	nd	nd	nd	nd	nd	nd	nd	nd	5.0 (0.44)

Values in parentheses are standard errors ($n = 3$).
nd, not determined.
For location and key to sites, see Figure 1.

Results and discussion

Soil total and extractable metal concentrations

The metal-rich sludge cake additions generally achieved total metal concentrations in the topsoil close to the target levels at each site (Table 4). However, Zn concentrations on the rate 4 Zn treatments at Gleadthorpe and Woburn (291 and 229 mg kg^{-1} respectively), were lower than the target value of 450 mg kg^{-1}. These low apparent recoveries may have been associated with sludge cake incorporation difficulties during cultivation of these light-textured soils and therefore problems in obtaining a homogenous representative topsoil sample for analysis.

Topsoil NH_4NO_3-extractable Zn concentrations were consistently greater at Hartwood and smaller at Shirburn, than at the other sites (Table 5). Extractable Zn concentrations on the rate 4 treatments at Hartwood were approximately 120-fold greater than at Shirburn (ca 51 mg kg^{-1} compared with 0.4 mg kg^{-1} respectively), approximately sixfold greater than at Gleadthorpe, Woburn and Pwllpeiran (approx. 8 mg kg^{-1}) and approximately 50% greater than at Rosemaund and Auchincruive (25–37 mg kg^{-1}). Extractable Cd concentrations on the rate 4 treatments were consistently higher at Hartwood (0.12 mg kg^{-1}) compared with the other sites (0.02–0.07 mg kg^{-1}). As the total metal concentrations at Hartwood were not higher than at the other sites, the extractability of Zn and Cd from the Hartwood soil was substantially higher than those from the other soils.

Extractable Cu concentrations were approximately 2.5 mg kg^{-1} on the rate 4 treatments at Watlington and Hartwood, approximately 2 mg kg^{-1} at Shirburn and approximately 1 mg kg^{-1} at the other six sites.

Soil factors affecting metal extractability

For the nine sites in this study, between 64% and 92% of the variation in extractability of topsoil Zn, Cu and Cd could be explained ($P < 0.01$). The extractability of topsoil Zn and Cd was inversely related to soil pH ($P < 0.001$; $r^2 =$ 92% and 72% respectively, Figure 2), with none of the other soil variables in Table 1 contributing further. Extractable Zn and Cd concentrations decreased markedly across the pH range 5.5–6.5 and steadily declined thereafter to < 0.1% of total topsoil Zn and < 0.5% of total topsoil Cd concentrations. For both Zn and Cd, this strong inverse relationship with topsoil pH was expected, as many workers have shown soil pH to be the single most important factor controlling

Table 5 Mean NH_4NO_3 extractable metal concentrations (mg kg^{-1}) in topsoil (0–25 cm) after fourth sludge cake application (1997)

Treatment	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Zinc									
Control	0.2 (0.03)	0.1 (0.01)	0.1 (nd)	0.1 (0.01)	0.1 (0.05)	0.3 (0.10)	1.3 (0.34)	0.2 (0.07)	0.1 (nd)
Low metal digested cake	0.5 (0.07)	0.7 (0.07)	0.4 (0.14)	0.5 (0.10)	1.7 (0.10)	0.9 (0.05)	3.4 (0.49)	2.5 (0.10)	< 0.1 (< 0.01)
Zn rate 1	1.2 (0.17)	2.0 (0.25)	1.6 (0.40)	1.7 (0.51)	4.6 (0.88)	5.5 (2.05)	8.0 (0.76)	5.3 (0.54)	0.1 (0.01)
Zn rate 2	3.1 (0.63)	5.2 (0.76)	3.9 (1.35)	4.5 (1.71)	14.7 (4.22)	8.8 (0.39)	17.9 (2.69)	14.1 (2.95)	0.2 (0.02)
Zn rate 3	6.4 (0.84)	6.4 (0.89)	9.9 (1.52)	6.1 (1.83)	24.7 (2.60)	12.3 (3.23)	36.7 (4.31)	22.5 (5.13)	0.3 (0.01)
Zn rate 4	7.4 (0.78)	8.4 (0.98)	14.7 (6.09)	7.7 (2.24)	25.0 (2.91)	18.1 (0.51)	51.3 (1.71)	37.1 (1.59)	0.4 (0.04)
Zn rate 5	nd	nd	nd	nd	nd	nd	nd	nd	0.8 (0.04)
Copper									
Control	0.04 (0.004)	< 0.03 (nd)	< 0.03 (nd)	0.06 (0.023)	< 0.03 (nd)	< 0.03 (nd)	0.12 (0.029)	0.03 (0.003)	0.10 (0.003)
Low metal raw cake	0.49 (0.033)	0.27 (0.021)	0.29 (0.013)	0.21 (0.022)	0.32 (0.038)	0.45 (0.012)	0.38 (0.068)	0.29 (0.023)	0.30 (0.008)
Cu rate 1	0.93 (0.057)	0.56 (0.049)	0.81 (0.102)	0.44 (0.110)	0.51 (0.061)	0.43 (0.060)	0.66 (0.091)	0.30 (< 0.001)	0.60 (0.031)
Cu rate 2	1.26 (0.276)	0.80 (0.163)	1.06 (0.156)	0.66 (0.111)	0.89 (0.075)	0.68 (0.112)	1.18 (0.185)	0.44 (0.047)	1.10 (0.083)
Cu rate 3	2.33 (0.731)	1.37 (0.220)	2.67 (0.107)	0.79 (0.030)	1.01 (0.293)	1.08 (0.206)	2.20 (0.473)	0.62 (0.065)	1.47 (0.045)
Cu rate 4	1.43 (0.257)	1.45 (0.381)	2.31 (0.264)	1.06 (0.164)	1.38 (0.154)	0.76 (0.019)	2.65 (0.522)	1.05 (0.223)	1.99 (0.228)
Cu rate 5	nd	nd	nd	nd	nd	nd	nd	nd	2.76 (0.200)
Cadmium									
Control	0.004 (0.001)	0.002 (< 0.001)	0.002 (< 0.001)	0.003 (0.001)	0.004 (0.001)	0.032 (0.007)	0.023 (0.006)	< 0.030 (nd)	0.002 (< 0.001)
Low metal digested cake	0.005 (0.001)	0.005 (< 0.001)	0.005 (0.001)	0.006 (0.001)	0.009 (0.001)	0.017 (0.002)	0.019 (0.005)	0.030 (< 0.001)	0.002 (< 0.001)
Cd rate 1	0.012 (0.002)	0.013 (0.001)	0.014 (0.002)	0.014 (0.004)	0.033 (0.005)	0.030 (0.005)	0.069 (0.013)	0.030 (< 0.001)	0.005 (0.001)
Cd rate 2	0.021 (0.003)	0.020 (0.002)	0.017 (0.002)	0.030 (0.003)	0.014 (0.002)	0.028 (0.004)	0.108 (0.007)	0.060 (0.010)	0.008 (0.001)
Cd rate 3	0.021 (0.001)	0.026 (0.002)	0.029 (0.003)	0.032 (0.005)	0.036 (0.013)	0.033 (0.003)	0.119 (0.031)	0.060 (0.010)	0.009 (0.002)
Cd rate 4	0.029 (0.001)	0.028 (0.003)	0.033 (0.002)	0.039 (0.005)	0.053 (0.009)	0.031 (0.011)	0.118 (0.025)	0.070 (0.010)	0.018 (0.002)
Cd rate 5	nd	nd	nd	nd	nd	nd	nd	nd	0.019 (0.002)

Values in parentheses are standard errors ($n = 3$).
nd, not determined.
For location and key to sites, see Figure 1.

the extractability of heavy metals (Alloway & Jackson, 1991; Sauerbeck, 1991; Hooda *et al.*, 1997).

Cu extractability was strongly inversely related to total iron (Fe_2O_3) content ($P < 0.001$; $r^2 = 0.64\%$, Figure 2), with a further 14% of the variation accounted for by the inclusion of total manganese oxide (MnO_2) content ($P < 0.001$). Unlike Zn and Cd, topsoil Cu extractability was not related to topsoil pH. The relative insensitivity of Cu extractability to soil pH has been reported before (Sanders *et al.*, 1986; Smith 1994) and has been attributed to the strong adsorption of Cu to soil surfaces and organic matter (Sanders & Adams, 1987). Additionally, the high surface area and adsorption capacity of Fe oxides, coupled with the ability of Cu^{2+} to replace Fe^{2+} in some Fe-oxides (Hickey & Kittrick, 1984) is likely to have contributed to the strong relationship between topsoil iron content and extractable Cu.

Losses of organic carbon from sludges

Total carbon loadings (1994–1997) across the nine sites were on average 59 (33–81) and 62 (30–83) t ha^{-1} for the digested and raw sludge cake treatments respectively. In 1997, following the fourth sludge cake application, the average proportions of applied sludge C estimated to remain in the topsoil horizon (0–25 cm) were 55% and 36% for the digested and raw sludge cakes respectively (Table 6). Therefore, it is estimated that 45% of the digested sludge C and 64% of the raw sludge C had been lost from the topsoil by the end of 1997. These C losses were largely a result of the rapid mineralisation of added sludge organic matter. The differences between the digested and raw sludge cakes were most likely related to the anaerobic digestion pre-treatment of the digested sludge cakes which would have removed a large proportion of the readily degradable organic C which remained in the raw sludge cakes.

Microbial properties

Soil microbial biomass C concentrations following sludge cake application were generally higher on treatments receiving sludge cake compared with the untreated controls (Table 7). Similarly, Chander & Brookes (1991), Fließbach

Table 6 Summary of sludge carbon additions (1994–1997) and sludge carbon turnover following final sludge cake application

	C applied, 1994–1997 ($t\ ha^{-1}$)	Soil Org C, 1997 ($t\ ha^{-1}$, 0–25 cm[a])	Sludge C remaining, 1997 (%)
GLE			
Control	–	36	–
Digested	69	59	33
Raw	68	49	19
WOB			
Control	–	32	–
Digested	81	51	25
Raw	81	47	19
WAT			
Control	–	30	–
Digested	63	66	58
Raw	67	59	45
PWL			
Control	–	78	–
Digested	33	103	88
Raw	30	95	55
ROS			
Control	–	44	–
Digested	57	78	58
Raw	57	70	49
BRI			
Control	–	32	–
Digested	48	67	68
Raw	49	52	46
HAR			
Control	–	107	–
Digested	74	137	45
Raw	81	127	22
AUC			
Control	–	66	–
Digested	69	115	74
Raw	83	104	44
SHI			
Control	–	62	–
Digested	37	79	44
Raw	41	70	23
Mean			
Digested			55
Raw			36

For location and key to sites, see Figure 1.
[a]An adjusted soil depth was used at each site to correct for small difference in bulk density between the untreated control and the sludge-amended treatments.

et al. (1994) and Banerjee *et al.* (1997) reported that the application of sewage sludge resulted in increased soil microbial biomass concentrations, probably due to stimulation of the biomass by addition of sludge organic matter. Statistically significant biomass C concentration differences ($P < 0.05$) on the digested sludge cake treatments were only found at Gleadthorpe, with the Zn-rich treatment having a lower biomass C concentration than the low metal treatment. Similarly, biomass C concentration differences ($P < 0.05$) on the raw sludge cake treatments were only found at Gleadthorpe and Rosemaund, with the Cu-rich treatment having lower biomass C concentrations than the low metal treatment. In general, the results for the nine sites support no consistent inhibitory effects from the sludge applications on soil microbial biomass in the short term following application. These data soon after the cessation of sludge applications are in contrast to those observed by Brookes & McGrath (1984), who verified a reduction in soil microbial biomass (of up to 50%), suggesting a toxic effect due to the presence of heavy metals. Also, in an evaluation of the effects of applying sewage sludge on soil biomass, Chander & Brookes (1991) reported that the microbial biomass decreased where sewage sludge high in heavy metals was applied to soil and that the decrease was larger in sandy than in clayey soils. Khan & Scullion (2002) also reported decreases in microbial biomass on sandy soils contaminated with heavy metals. It may be that the lower number of responses within this study to date are due to the form in which the heavy metals were applied or the relatively short time period between sludge application and the biomass carbon measurements. Both Chander & Brookes (1991) and Khan & Scullion (2002) added metals as inorganic salts to sludge cakes, whereas in this study, metals were present in the sludge cake itself which may have resulted in differing effects on the soil microbial population.

Across the nine sites, there were relationships ($P < 0.001$) between soil total organic C contents and biomass C concentrations on the digested sludge cake ($r^2 = 33\%$) and the raw sludge cake treatments ($r^2 = 38\%$).

Soil respiration rates following sludge cake application were generally higher on treatments receiving sludge cake compared with the untreated controls (Table 8). The increase in respiration rates across the nine sites was probably because the activity of soil micro-organisms was stimulated by the application of sludge organic matter. At Hartwood, respiration rates on the Zn-rich treatment were higher ($P < 0.05$) than on the low metal and Cd-rich treatments. At Auchincruive, respiration rates on the Cd-rich treatment were higher ($P < 0.05$) than on the low metal and Zn-rich treatments. In contrast, at Shirburn, respiration rates on the Zn and Cd-rich treatments were lower ($P < 0.05$) than the low metal treatment. Respiration rate differences ($P < 0.05$) on treatments receiving the raw sludge cakes were only evident at Woburn, where the Cu-rich treatment had a higher respiration rate than the low metal treatment.

The respiration rate per unit of microbial biomass (i.e. specific respiration rate – qCO_2) is a parameter that is commonly used to assess soil microbial activity. The qCO_2 can be interpreted as an index of 'microbial efficiency', as it is a measurement of the energy necessary to maintain metabolic

Table 7 Mean microbial biomass carbon (mg C kg^{-1}) in topsoil (−25 cm) on selected treatments sampled in October 1997

Treatment	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Control	221 (13.4)	120 (14.7)	259 (13.7)	444 (31.5)	407 (49.4)	331 (47.4)	642 (56.4)	248 (68.5)	1116 (13.4)
Low metal digested cake	316 (28.0)	196 (12.4)	372 (44.5)	481 (48.9)	454 (40.4)	552 (54.4)	775 (48.6)	242 (73.6)	1046 (7.8)
Zn rich – rate 3	230* (25.9)	195 (4.7)	321 (26.5)	523 (26.6)	428 (57.8)	468 (34.5)	813 (87.5)	330 (38.6)	1013 (47.9)
Cd rich – rate 3	267 (27.0)	242 (14.4)	367 (12.9)	487 (12.7)	497 (69.3)	615 (23.6)	763 (14.2)	308 (121)	954 (35.0)
Low metal raw cake	458 (16.5)	322 (10.1)	624 (16.8)	488 (13.4)	672 (95.3)	843 (49.6)	1153 (135)	517 (28.5)	1157 (63.6)
Cu rich – rate 3	254* (28.4)	323 (56.2)	625 (59.0)	496 (33.6)	534* (85.1)	689 (61.1)	1094 (97.5)	427 (36.9)	1145 (36.8)
SEM	23.9	25.4	33.6	30.4	69.0	46.8	82.8	68.9	39.0
P	< 0.001	< 0.001	< 0.001	ns	ns	< 0.001	0.005	ns	0.02
LSD ($P < 0.05$)	114	121	160	na	na	222	393	na	185

Values in parentheses are standard errors ($n = 3$).
na, not applicable; ns, not significant; SEM, standard error of mean.
For location and key to sites, see Figure 1.
*Significantly different ($P < 0.05$) from the appropriate low metal sludge cake treatment (digested in the case of Zn, Cd; raw in the case of Cu).

Table 8 Mean respiration rate (mg CO_2-C kg^{-1} h^{-1}) in topsoil (0–25 cm) on selected treatments sampled in October 1997

Treatment	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Control	0.30 (0.030)	0.17 (0.013)	0.27 (0.005)	0.26 (0.044)	0.46 (0.012)	0.31 (0.010)	1.00 (0.163)	0.49 (0.023)	0.42 (0.022)
Low metal digested cake	0.52 (0.077)	0.49 (0.061)	0.71 (0.059)	0.29 (0.027)	0.60 (0.062)	0.35 (0.051)	1.48 (0.037)	0.97 (0.034)	0.73 (0.031)
Zn rich – rate 3	0.62 (0.113)	0.45 (0.030)	0.73 (0.060)	0.29 (0.070)	0.68 (0.027)	0.46 (0.057)	2.55* (0.125)	0.97 (0.006)	0.51* (0.002)
Cd rich – rate 3	0.38 (0.051)	0.57 (0.036)	0.60 (0.014)	0.25 (0.085)	0.65 (0.038)	0.34 (0.090)	1.68 (0.318)	1.22* (0.012)	0.47* (0.011)
Low metal raw cake	0.59 (0.075)	0.49 (0.063)	0.66 (0.220)	0.39 (0.025)	0.84 (0.122)	0.37 (0.026)	3.46 (0.362)	1.36 (0.102)	0.50 (0.045)
Cu rich – rate 3	0.70 (0.157)	0.70* (0.046)	1.36 (0.086)	0.35 (0.011)	0.84 (0.093)	0.31 (0.049)	5.10 (0.944)	1.34 (0.086)	0.48 (0.068)
SEM	0.094	0.045	0.102	0.051	0.070	0.053	0.441	0.058	0.037
P	ns	< 0.001	< 0.001	ns	0.02	ns	< 0.001	< 0.001	< 0.001
LSD ($P < 0.05$)	na	0.214	0.486	na	0.334	na	2.095	0.273	0.176

Values in parentheses are standard errors ($n = 3$).
na, not applicable; ns, not significant; SEM, standard error of mean.
For location and key to sites, see Figure 1.
*Significantly different ($P < 0.05$) from the appropriate low metal sludge cake treatment (digested in the case of Zn, Cd; raw in the case of Cu).

Table 9 Mean most probable numbers of *Rhizobium leguminosarum* biovar *trifolii* (log_{10} cells g^{-1}) in topsoil (0–25 cm) on selected treatments sampled in October 1997

Treatment	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Control	5.12	5.03	5.17	4.97	5.20	5.03	4.05	2.01	4.73
Low metal digested cake	4.60	4.82	4.65	4.97	4.54	4.60	3.59	3.09	4.54
Zn rich – rate 3	4.56	4.95	3.55[a]	4.90	3.83	4.79	2.76	3.11	4.33
Cd rich – rate 3	4.54	5.23	4.64	4.73	4.90	4.90	3.32	3.85	4.68
Low metal raw cake	4.73	4.48	4.88	4.54	4.95	5.17	3.57	2.12	4.45
Cu rich – rate 3	4.64	4.54	4.54	4.48	4.42	5.12	3.55	3.19	4.79
SEM	0.197	0.254	0.157	0.090	0.175	0.150	0.471	0.642	0.178
P	ns	ns	< 0.001	0.007	< 0.001	ns	ns	ns	ns
LSD ($P < 0.05$)	na	na	0.748	0.425	0.831	na	na	na	na

na, not applicable; ns, not significant; SEM, standard error of mean.
For location and key to sites, see Figure 1.
[a] > 1 log_{10} lower than the appropriate low metal sludge cake treatment (digested in the case of Zn, Cd; raw in the case of Cu).

Table 10 Mean metabolic quotient (qCO_2) in topsoil (0–25 cm) on selected treatments sampled in October 1997

Treatment	GLE	WOB	WAT	PWL	ROS	BRI	HAR	AUC	SHI
Control	0.14 (0.017)	0.15 (0.031)	0.10 (0.006)	0.06 (0.013)	0.12 (0.017)	0.10 (0.019)	0.15 (0.014)	0.22 (0.041)	0.04 (0.003)
Low metal-digested cake	0.16 (0.011)	0.25 (0.035)	0.20 (0.034)	0.06 (0.001)	0.13 (0.009)	0.06 (0.003)	0.19 (0.013)	0.47 (0.117)	0.07 (0.002)
Zn rich – rate 3	0.27 (0.037)	0.23 (0.011)	0.23 (0.011)	0.05 (0.005)	0.16 (0.018)	0.10 (0.006)	0.32 (0.018)	0.30 (0.035)	0.05 (0.003)
Cd rich – rate 3	0.15 (0.032)	0.24 (0.025)	0.16 (0.008)	0.05 (0.019)	0.14 (0.016)	0.06 (0.015)	0.22 (0.046)	0.52 (0.162)	0.05 (0.001)
Low metal raw cake	0.13 (0.021)	0.15 (0.015)	0.10 (0.033)	0.08 (0.007)	0.13 (0.038)	0.04 (0.004)	0.30 (0.008)	0.26 (0.023)	0.04 (0.006)
Cu rich – rate 3	0.27 (0.032)	0.23 (0.040)	0.22 (0.033)	0.07 (0.005)	0.16 (0.028)	0.04 (0.004)	0.46 (0.056)	0.31 (0.013)	0.04 (0.006)

Values in parentheses are standard errors ($n = 3$).
For location and key to sites, see Figure 1.

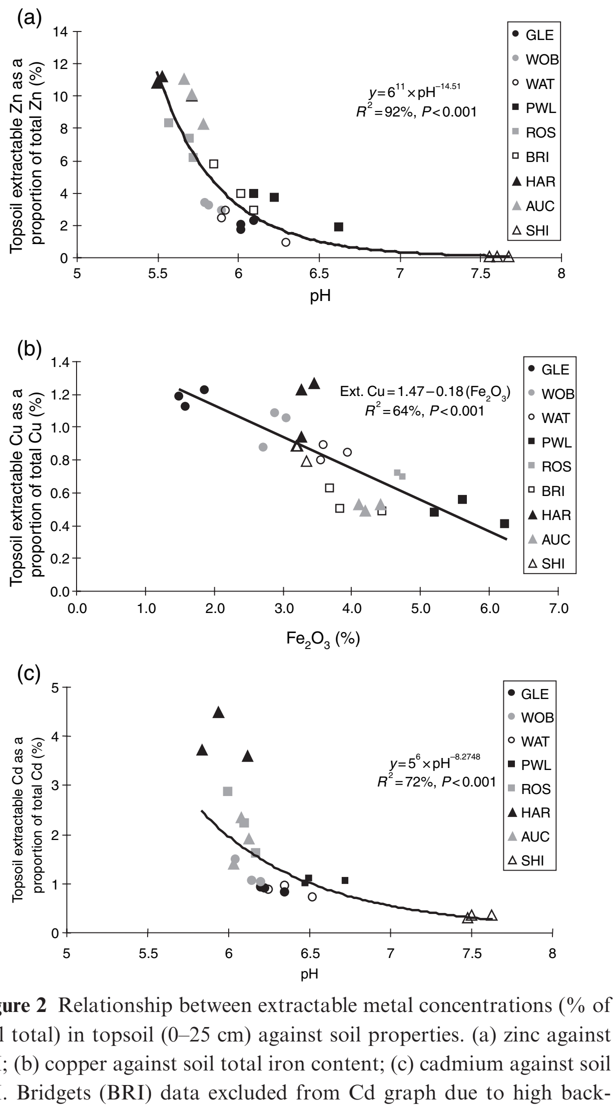

Figure 2 Relationship between extractable metal concentrations (% of soil total) in topsoil (0–25 cm) against soil properties. (a) zinc against pH; (b) copper against soil total iron content; (c) cadmium against soil pH. Bridgets (BRI) data excluded from Cd graph due to high background Cd concentration. For location and key to sites, see Figure 1.

activity in relation to the energy required to synthesise new biomass (Bardgett & Saggar, 1994). Therefore, soils under 'stress' exhibit a higher qCO_2 than 'non-stressed' soils.

In this study, qCO_2 generally increased as a result of sludge application (Table 10). The highest qCO_2 values were generally measured where metal-rich sludges were applied, in particular on the Zn- and Cu-rich sludge cake treatments. At all the sites, with the exception of Bridgets, the lowest qCO_2 values were generally measured on the untreated control treatments.

Across the nine sites, there were relationships ($P < 0.001$) between soil total organic C contents and respiration rates on the digested sludge cake ($r^2 = 34\%$) and the raw sludge cake treatments ($r^2 = 27\%$). However, there was only a weak relationship between soil microbial biomass C concentrations and respiration rates on the digested sludge cake ($r^2 = 7\%$) and raw sludge cake treatments ($r^2 = 21\%$).

Most probable numbers of clover *Rhizobium* were slightly lower on some of the treatments receiving sludge cakes compared with the untreated controls (Table 9). However, only at Watlington, on the Zn-rich treatments, were MPNs of clover *Rhizobium* $>1\ \log_{10}$ below those on the low metal-digested cake treatment. In general, there was no evidence to suggest that addition of sludge cakes had an effect on soil *Rhizobium leguminosarum* biovar *trifolii* numbers in the short term following application.

Conclusions

Across the nine sites in this study, soil pH was the single most important factor controlling Zn and Cd extractability. Copper extractability was largely controlled by the iron content of the soil, with manganese of secondary importance.

Sludge C mineralisation resulted in approximately 45% of the digested sludge and approximately 66% of the raw sludge cake C inputs being lost from the topsoil horizon by the end of the 4-year application period. Across the nine sites, there were positive relationships between soil organic C levels and microbial biomass C concentrations and soil respiration rates.

Application of sludge cakes generally increased soil microbial biomass C concentrations and soil respiration rates, with clover *Rhizobium* population sizes remaining largely unchanged. Although there were a limited number of significant responses to metal-rich sludge cake application, in the short term following the cessation of the application programme, there were no consistent effects of metals on microbial activity that could unequivocally be attributed to metal toxicity *per se*.

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