Soil Use and Management, June 2006, **22**, 180–187 doi: 10.1111/j.1475-2743.2006.00009.x

Initial results from long-term field studies at three sites on the effects of heavy metal-amended liquid sludges on soil microbial activity

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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-amended liquid sludges each with elevated Zn, Cu or Cd concentrations were applied over a 3-year period (1995–1997) to three sites in England. The experiments were sited adjacent to experimental plots receiving metal-rich sludge cakes enabling comparisons to be made between the effects of heavy metal additions in metal-amended liquid sludges and sludge cakes. The liquid sludge additions were regarded as 'worst case' treatments in terms of likely metal availability, akin to a long-term situation following sewage sludge additions where organic matter levels had declined and stabilised. The aim was to establish individual Zn (50–425 mg kg^{-1}), Cu (15–195 mg kg^{-1}) and Cd (0.3–4.0 mg kg^{-1}) metal dose–response treatments at each site, but with significantly smaller levels of organic matter addition than the corresponding sludge cake experiments. There were no differences ($P > 0.05$) in soil respiration rates, biomass carbon concentrations or most probable numbers of clover *Rhizobium* between the treatments at any of the sites at the end of the liquid sludge application programme. Soil heavy metal extractability differed between the metal-amended liquid sludge and metal-rich sludge cake treatments; Zn and Cd extractabilities were higher from the liquid sludge additions, whereas Cu extractability was higher from the sludge cake application. These differences in metal extractability in the treated soil samples reflected the contrasting NH_4NO_3 extractable metal contents of the metal-amended liquid sludges and sludge cakes that were originally applied.

Keywords: Liquid sludge, sludge cake, heavy metals, soil biomass carbon, soil respiration, *Rhizobium*

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 contains 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, because they are not easily leached and crop offtakes are small.

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Received February 2005; accepted after revision November 2005

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. Gendebien *et al.* (1999) in a survey of UK sludge production estimated that in 1996/97, 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). Reuse to agricultural land was predicted to remain the principal outlet for the foreseeable future. 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 the recycling of plant nutrients. Generally, biomass size and activity increase when organic materials are applied to soils. However, the adverse effects of heavy metals on soil microbial properties, such as biomass (Brookes & McGrath, 1984; Stark & Lee, 1988; Chander & Brookes, 1991, 1993; Khan & Scullion, 2002), specific respiration rate (Brookes & McGrath, 1987) and potential metabolic activity (Brookes & McGrath, 1987) are well documented. Symbiotic nitrogen fixation has also been shown to be sensitive to heavy metals in soils (McGrath *et al*., 1988; Chaudri *et al*., 1993) and has received particular attention because decreases in the ability of legumes to fix atmospheric N are agronomically significant, particularly as future agricultural systems may seek to exploit more fully the N fixing properties of leguminous crops (e.g. clover, peas, beans and lupins).

Based on available data from experimental sites in the UK in the early 1990s, 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 on crops and soil microbial processes.

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, e.g. 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'.

Work reported by Gibbs *et al*. (2006) describes the initial results from a long-term multi-site study of the effects on soil fertility and microbial activity of heavy metals following the application of metal-rich sludge cakes at nine experimental sites throughout Britain. At three of these experimental sites (Woburn, Watlington and Rosemaund), separate experimental plots receiving metal-amended liquid sludge applications were established adjacent to the metal-rich sludge cake experiments. The aim was to compare the effects on soil fertility and microbial activity of zinc (Zn), copper (Cu) and cadmium (Cd) additions in metal-amended liquid sludges with those from metal-rich sludge cakes.

The liquid sludge additions were used to produce 'target' soil metal concentrations with less addition of organic matter than could be achieved with metal-rich sludge cakes. They were regarded as 'worst case' treatments in terms of likely metal availability, akin to a long-term situation following sewage sludge additions where organic matter levels had declined and stabilised. It is under this latter situation that adverse effects on soil microbial properties had previously been found (McGrath *et al*., 1988, 1995; McGrath, 1990).

So as to examine the effects of individual metals (Zn, Cu and Cd) on soil microbial activity and long-term soil fertility, Zn, Cu and Cd dose–response treatments were established using liquid sludges amended with elevated concentrations of either Zn, Cu or Cd. This paper presents results from the initial metal build-up phase (1995–1997) of this long-term study.

Materials and methods

Three experimental sites were selected at Woburn-WOB (Arenosol), Watlington-WAT (Cambisol) and Rosemaund-ROS (Luvisol) adjacent to the 'main' sludge cake experiments (Gibbs *et al*., 2006).

The metal-amended liquid sludges were prepared by equilibrating Zn, Cu or Cd sulphates with a low-metal content liquid digested sludge (Davis & Carlton-Smith, 1981) and were specifically prepared to have high Zn, Cu or Cd concentrations relative to the other metals present (Table 1). The metal salts were added as sulphates because they were likely to be reduced to sulphides, which have been shown to be important forms of Zn, Cu and Cd in sludges (Stover *et al*., 1976). The liquid sludges and the metal sulphates were equilibrated over a 6–9 month period in enclosed vessels that were stirred periodically. The metal-amended liquid sludges were applied annually to each site over a 3-year period (1995–1997) to establish individual Zn, Cu and Cd metal dose–response treatments (Table 2). In addition to the metal-amended liquid sludge treatments, a low-metal content liquid sludge treatment receiving the same organic matter loading as the 'high' metal treatments was included in the design,

Table 1 Chemical analysis of liquid sludges applied from 1995–1997 at the three field sites (mean of three samples)

	Low metal	Zn-rich	Cu-rich	Cd-rich
DM (%)	2.41	2.35	2.23	2.20
Org C (% ds)	38.6	33.4	33.9	37.6
N (% ds)	6.36	7.00	7.25	7.09
NH_4–N (% ds)	2.03	2.18	2.15	2.27
P (% ds)	2.13	2.00	2.03	2.04
K (% ds)	0.39	0.51	0.52	0.55
pH	7.5	7.6	7.6	7.7
Zn (mg kg^{-1})	590	22 100	640	610
Cu (mg kg^{-1})	590	607	12 000	645
Cd (mg kg^{-1})	1.7	2.6	2.9	285
Ni (mg kg^{-1})	26	27	29	28
Pb (mg kg^{-1})	96	101	105	105
Cr (mg kg^{-1})	47	50	53	52
Al (mg kg^{-1})	18 000	15 380	16 000	16 300
Fe (mg kg^{-1})	12 560	10 650	10 600	10 700
Mn (mg kg^{-1})	380	350	355	360
Hg (mg kg^{-1})	2.7	2.9	3.3	3.1

ds, dry solids.

Table 2 Metal-amended liquid sludge experiments – target total topsoil metal concentrations (mg kg^{-1})

Target level	Zn	Cu	Cd
Background range	43–74	12–15	< 0.20–0.29
Rate 1	175	75	2
Rate 2	300	135	3
Rate 3	425	195	4

along with an untreated control. The upper limits were chosen to be above the maximum soil metal concentrations permissible in the UK at the commencement of the study (SI, 1989).

The treatments were replicated in three fully randomised blocks (33 plots). Individual plots (1.2 m × 3.5 m = 4.2 m^2) were bounded by permanent oil-tempered hardboard to prevent soil movement during cultivations. Cultivations were carried out annually using a small plot spading machine to ensure that the sludge additions were evenly incorporated throughout the topsoil depth (0–25 cm). At all the three sites, the soil pH was adjusted and maintained where necessary at a target pH of 6.5 through the addition of lime as recommended for arable crops in England and Wales (Anon, 2000).

Topsoil samples were taken prior to the first liquid sludge application to characterise each site (Table 3) and again in January 1998 from all plots following the final liquid sludge application. Samples of each liquid sludge were also taken prior to each application so as to characterise heavy metal and nutrient loadings (Table 1). The topsoil samples taken in 1998 were analysed for total (perchloric nitric acid digestion) (MAFF, 1986) and ammonium nitrate (NH_4NO_3) extractable [Deutsche Institute fur Normung, 1997 – draft ISO standard method (ISO/CD 19730) for the extraction of trace elements in soil] Zn, Cu and Cd concentrations (Tables 4 and 5), soil pH (in water) and organic carbon (C) (potassium dichromate oxidation), total iron, aluminium and manganese contents

Table 3 Mean soil physical, chemical and microbial properties of sites (1995). Standard errors in parentheses ($n = 3$)

	Woburn	Watlington	Rosemaunde
Sand (> 63 μm) (%)	83 (0.0)	50 (1.5)	8 (0.6)
Silt (2–63 μm) (%)	8 (0.3)	32 (0.9)	69 (0.0)
Clay (< 2 μm) (%)	9 (0.3)	18 (1.2)	23 (0.6)
pH	7.3 (0.06)	7.4 (0.03)	7.0 (0.07)
Organic C (%)	1.1 (0.07)	1.5 (0.06)	1.9 (0.02)
Fe_2O_3 (%)	3.4 (0.20)	3.6 (0.04)	4.4 (0.08)
Al_2O_3 (%)	1.2 (0.02)	2.5 (0.08)	5.5 (0.12)
MnO_2 (%)	0.03 (0.00)	0.10 (0.00)	0.16 (0.01)
Total Zn (mg kg^{-1})	50.4 (1.39)	42.9 (0.74)	74.2 (0.77)
Total Cu (mg kg^{-1})	15.1 (0.20)	12.1 (0.60)	15.3 (0.21)
Total Cd (mg kg^{-1})	< 0.20 (ND)	0.25 (0.01)	0.29 (0.04)
Biomass C (mg kg^{-1})	115 (1.8)	294 (9.0)	447 (12.2)
Respiration (mg CO_2–C kg^{-1} h^{-1})	0.12 (0.00)	0.30 (0.02)	0.29 (0.01)
Rhizobium MPNs (log_{10} cells g^{-1})	5.1	5.1	5.3

MPNs, most probable numbers.

Table 4 Mean topsoil total metal concentrations (mg kg^{-1}) after third application of liquid sludge application (January 1998)

Treatment	Woburn	Watlington	Rosemaunde
Zn			
Control	47 (2.8)	46 (1.0)	80 (0.9)
Low-metal sludge	59 (0.9)	56 (0.8)	86 (1.2)
Zn rate 1	178 (33.9)	209 (10.6)	164 (3.4)
Zn rate 2	206 (5.8)	262 (18.8)	268 (15.2)
Zn rate 3	314 (37.8)	417 (17.0)	372 (44.8)
Cu			
Control	14 (0.4)	13 (0.4)	17 (0.2)
Low-metal sludge	22 (1.3)	23 (1.4)	24 (0.8)
Cu rate 1	73 (13.4)	100 (5.3)	67 (5.0)
Cu rate 2	117 (13.7)	134 (6.8)	127 (2.2)
Cu rate 3	169 (20.8)	173 (16.3)	154 (14.0)
Cd			
Control	0.2 (0.02)	0.3 (0.02)	0.3 (0.04)
Low-metal sludge	0.2 (0.01)	0.3 (0.02)	0.3 (0.01)
Cd rate 1	1.6 (0.17)	2.2 (0.14)	2.0 (0.09)
Cd rate 2	2.3 (0.45)	2.9 (0.20)	2.6 (0.12)
Cd rate 3	3.5 (0.22)	3.7 (0.17)	3.4 (0.20)

Values in parentheses are standard errors ($n = 3$).

Table 5 Mean topsoil NH_4NO_3 extractable metal concentrations (mg kg^{-1}) after third liquid sludge application (January 1998)

Treatment	Woburn	Watlington	Rosemaunde
Zn			
Control	0.1 (0.01)	< 0.1 (–)	0.1 (0.04)
Low-metal sludge	0.2 (0.04)	0.1 (0.03)	0.4 (0.18)
Zn rate 1	7.8 (1.47)	3.8 (2.52)	5.5 (0.99)
Zn rate 2	12.8 (0.81)	3.7 (0.77)	24.5 (2.01)
Zn rate 3	32.6 (3.18)	28.8 (5.9)	58.3 (12.70)
Cu			
Control	0.06 (0.005)	0.05 (0.001)	0.03 (0.002)
Low-metal sludge	0.09 (0.011)	0.09 (0.010)	0.06 (0.004)
Cu rate 1	0.24 (0.042)	0.42 (0.022)	0.28 (0.024)
Cu rate 2	0.50 (0.048)	0.47 (0.014)	0.55 (0.024)
Cu rate 3	0.68 (0.064)	0.57 (0.062)	0.79 (0.084)
Cd			
Control	0.001 (0.0001)	0.001 (0.0001)	0.004 (0.0008)
Low-metal sludge	0.002 (0.0002)	0.003 (0.0002)	0.006 (0.0013)
Cd rate 1	0.034 (0.0012)	0.034 (0.0069)	0.071 (0.0193)
Cd rate 2	0.038 (0.0018)	0.044 (0.0034)	0.123 (0.0310)
Cd rate 3	0.079 (0.0117)	0.063 (0.0093)	0.175 (0.0178)

Values in parentheses are standard errors ($n = 3$).

Table 6 Summary of sludge carbon additions (1995–1997) and sludge carbon turnover following final liquid sludge application

	C applied 1994–1997 (t ha^{-1})	Soil Org C 1997 (t ha^{-1} 0–25 cm[a])	% Sludge C present 1997
Woburn			
Untreated control	–	28	–
Liquid sludge	20	39	54
Watlington			
Untreated control	–	44	–
Liquid sludge	16	59	95
Rosemaunde			
Untreated control	–	51	–
Liquid sludge	13	63	91
Mean			80

[a]An adjusted soil depth was used at each site to correct for small differences in bulk density between the untreated control and liquid sludge-amended treatments.

(perchloric nitric acid digestion) (MAFF, 1986). The topsoil organic C values in 1998 and liquid sludge C loadings were used to estimate the amount of liquid sludge C remaining after the final sludge application (Table 6). Topsoil samples from the low-metal liquid sludge, and from Zn rate 2, Cu rate 2 and Cd rate 2 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 2 treatments were selected for analysis, as their target soil levels represented the maximum metal concentrations permissible in soils treated with sludge (DoE, 1996) and also allowed comparison with the equivalent sludge cake treatments (Gibbs *et al.*, 2006).

Table 7 Mean topsoil (0–25 cm) microbial biomass carbon (mg C kg^{-1} soil) levels on selected treatments (rate 2) sampled in January 1998

Treatment	Woburn	Watlington	Rosemaunde
Control	141 (15.8)	286 (10.7)	340 (5.3)
Low-metal sludge	161 (2.8)	272 (18.9)	286 (8.1)
High-Zn sludge	123 (14.8)	278 (9.2)	311 (10.7)
High-Cu sludge	134 (19.1)	243 (34.3)	287 (14.7)
High-Cd sludge	150 (25.2)	303 (11.4)	318 (18.3)
Estimated standard error of the residual	17.5	20.9	13.5
P-statistic	ns	ns	ns

Values in parentheses are standard errors ($n = 3$).

Table 8 Mean topsoil (0–25 cm) respiration rate (mg CO_2–C kg^{-1} soil h^{-1}) on selected treatments (rate 2) sampled in January 1998

Treatment	Woburn	Watlington	Rosemaunde
Control	0.13 (0.017)	0.33 (0.056)	0.23 (0.004)
Low-metal sludge	0.24 (0.030)	0.28 (0.026)	0.18 (0.020)
High-Zn sludge	0.30 (0.066)	0.31 (0.013)	0.21 (0.015)
High-Cu sludge	0.27 (0.062)	0.32 (0.057)	0.24 (0.032)
High-Cd sludge	0.32 (0.085)	0.43 (0.122)	0.27 (0.037)
Estimated standard error of the residual	0.064	0.069	0.028
P-statistic	ns	ns	ns

Values in parentheses are standard errors ($n = 3$).

Table 9 Mean topsoil (0–25 cm) *Rhizobium leguminosarum* biovar. *trifolii* numbers (log_{10} cells g^{-1} soil) on selected treatments (rate 2) sampled in January 1998

Treatment	Woburn	Watlington	Rosemaunde
Control	4.97	5.38	5.38
Low-metal sludge	4.40	5.20	5.30
High-Zn sludge	4.54	5.20	5.30
High-Cu sludge	4.73	4.82	5.38
High-Cd sludge	4.73	5.03	5.38
Estimated standard error of the residual	0.079	0.168	0.071
P-statistic	ns	ns	ns

So as to assess differences between the liquid sludge treatments following the third liquid sludge addition, the results of the soil microbial biomass C, soil respiration rate and MPNs of clover *Rhizobium* were analysed by ANOVA (GENSTAT 5 Committee, 1993). Also, to assess the influence of soil properties on topsoil metal extractability, stepwise multiple regression was conducted involving all of the soil variables reported in Table 3, plus the plot soil pH and organic C contents measured in 1998.

Results and discussion

Soil total and extractable metal concentrations

At the three sites, the metal-amended liquid sludge additions generally achieved soil total metal concentrations close to the target values (Table 2). However, some treatments were below the target concentrations, notably at Woburn on the Zn rates 2 and 3, and Cd rate 2 treatments. The failure to reach the target metal concentrations at Woburn was also a problem encountered with the sludge cake experiment (Gibbs *et al.*, 2006). This was further evidence that these apparent anomalies may be due to a site-specific effect, most likely reflecting the difficulties of sludge incorporation and associated problems in obtaining a homogenous representative topsoil sample for analysis following the cessation of sludge additions.

Topsoil NH_4NO_3-extractable Zn and Cd concentrations were consistently the highest at Rosemaund and lowest at Watlington, which was due to the greater extractability of Zn and Cd at Rosemaund and not a reflection of higher total topsoil Zn and Cd concentrations (Table 4). Extractable Zn levels on the rate 3 treatment at Rosemaund were approximately two-fold higher than at Watlington (58 and 29 mg kg^{-1} respectively). Similarly, Cd concentrations were two- to three-fold higher at Rosemaund (0.18 mg Cd kg^{-1}) than at Woburn and Watlington (0.06–0.08 mg Cd kg^{-1}). The lower extractability of Zn and Cd at Watlington was most probably due to the slightly higher soil pH (mean = 6.6) at this site in 1998, compared with the mean pH at the other two sites of 6.4. Topsoil NH_4NO_3-extractable Cu concentrations were similar at all three sites (0.6–0.8 mg Cu kg^{-1} on the rate 3 treatments).

Topsoil NH_4NO_3-extractable Zn and Cd concentrations were consistently two- to three-fold higher ($P < 0.001$) on the liquid sludge treatments (29–58 mg Zn kg^{-1}; 0.06–0.18 mg Cd kg^{-1}) compared with the corresponding sludge cake treatments (8–25 mg Zn kg^{-1}; 0.03–0.05 mg Cd kg^{-1}) (Gibbs *et al.*, 2006). The greater topsoil extractability of Zn and Cd from the liquid sludge treatments reflected the greater NH_4NO_3-extractable Zn and Cd concentrations in the metal-amended liquid sludges (58 and 1.2 mg kg^{-1} respectively) compared with the metal-rich sludge cakes (22 and 0.26 mg kg^{-1} respectively). In contrast, topsoil NH_4NO_3-extractable Cu concentrations were consistently two- to three-fold smaller ($P < 0.001$) on the liquid sludge treatments (0.6–0.8 mg Cu kg^{-1}) compared with the sludge cake treatments (1.5–2.3 mg Cu kg^{-1}). The higher topsoil Cu extractability from the metal-rich sludge cake reflected the higher NH_4NO_3-extractable Cu concentrations in the sludge cake (approx. 340 mg kg^{-1}) compared with the metal-amended liquid sludge (approx. 135 mg kg^{-1}). These extractable levels may have been a reflection of the origin of the Cu (electronic component manufacturing) and the raw (untreated) nature of the sludge cake.

So as to evaluate the effects of soil properties on extractability of the metals added in the liquid sludge at each site, topsoil NH_4NO_3-extractable metal concentrations were calculated as a proportion of the total metal concentration minus the soil background metal concentration. Mean topsoil NH_4NO_3 extractable Zn, Cu and Cd concentrations (mean of the three application rates) as a proportion of total topsoil metal concentrations for the three sites are shown in Figure 1. The site with the largest proportions of extractable Zn and Cd was Rosemaund (13% for Zn and 5% for Cd) and the smallest Watlington (4% for Zn and 2% for Cd). At all the three sites, the proportion of total Cu extractable with NH_4NO_3 was approximately 0.5%.

Across the three sites, the proportions of soil extractable Zn and Cd on the metal-amended liquid sludge treatments (4–13% of total topsoil Zn and 2–5% of total Cd concentrations) were significantly ($P < 0.01$) higher than those on the corresponding sludge cake treatments (2–7% of total topsoil Zn and 1–2% of total Cd concentrations) (Gibbs *et al.*, 2006), reflecting the differing forms and bioavailability of the applied sludge metals.

Multiple regression analysis involving all the soil variables detailed in Table 3, plus the plot pH and organic C values measured in 1998, showed that the extractability of topsoil Zn was inversely related to soil pH ($P < 0.05$; $r^2 = 53\%$) and that the extractability of Cd was most strongly related to Al_2O_3 content ($P < 0.01$; $r^2 = 70\%$), with a further 16% of the variation accounted for by the inclusion of pH and total MnO ($P < 0.001$). The importance of soil pH in controlling Zn and Cd availability was also demonstrated in the paired sludge cake experiments (Gibbs *et al.*, 2006).

In contrast across the three sites, the proportions of Cu extractable with NH_4NO_3 were significantly higher ($P < 0.001$) following the sludge cake application (0.8–1.0% of total topsoil Cu concentrations) compared with the metal-amended liquid sludge addition (0.4–0.5% of total topsoil Cu concentrations), again reflecting differences in the form and bioavailability of the metals in the applied sludge.

The rapid oxidation of organic matter applied in the raw sludge cake (on average 64% of the applied raw sludge cake organic matter was estimated to have been lost from the topsoil (0–25 cm) horizon at the end of the sludge cake application programme) may have contributed to higher short-term Cu extractability compared with the release of Cu associated

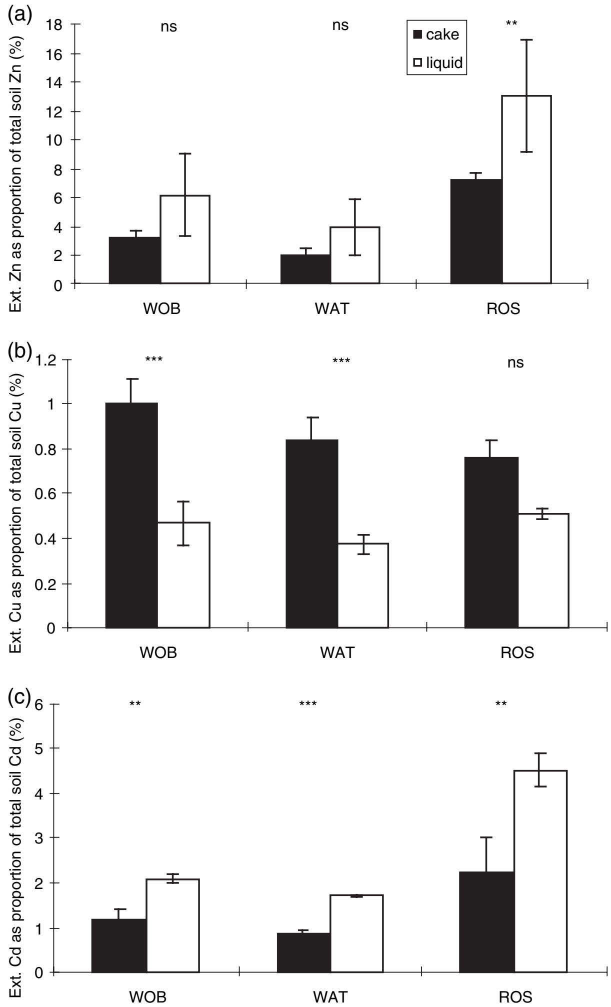

Figure 1 Ammonium nitrate extractable as a proportion of topsoil total concentrations: (a) Zn, (b) Cu, (c) Cd. Significant difference (** $P < 0.01$, *** $P < 0.001$) between liquid and cake treatments. ns, not significant ($P > 0.05$). WOB, Woburn; WAT, Watlington; ROS, Rosemaunde.

with the 'stabilised' organic matter in the liquid digested sludge. Multiple regression analysis showed that Cu extractability was positively related to total Fe_2O_3 content ($P < 0.05$; $r^2 = 53\%$), with a further 13% of the variation accounted for by soil pH and organic C content. The importance of soil Fe_2O_3 content in controlling Cu availability was also demonstrated in the paired sludge cake experiments (Gibbs *et al.*, 2006).

Sludge organic carbon

Total carbon loadings (1995–1997) across the three sites averaged 16.5 t ha^{-1} (range: 12.8–20.0). In 1998, following the final liquid sludge application, the average proportion of applied liquid sludge estimated to remain in the topsoil horizon (0–25 cm) was approximately 80% (range: 54–95%) (Table 6). Therefore, only 20% of the applied liquid sludge C had been lost from the topsoil by the end of the application phase. This rate of organic C turnover was much slower than the turnover estimated to have occurred on the companion sludge cake experiments [45 and 64% for the digested (Zn and Cd) and raw experiments (Cu) respectively] (Gibbs *et al.*, 2006).

Microbial properties

There were no differences ($P > 0.05$) following the final liquid sludge application in soil microbial biomass C concentrations between any of the rate 2 metal and low-metal sludge treatments at any of the three sites (Table 7). The results from the three sites indicate no inhibitory effects from the liquid sludge applications on the soil microbial biomass in the short term following application. These data, soon after the cessation of the liquid sludge applications, are similar to those from the paired sludge cake experiments (Gibbs *et al.*, 2006), but are in contrast to those observed by other workers (Brookes & McGrath, 1984; Chander & Brookes, 1991; Khan & Scullion, 2002) who reported long-term decreases in the size of the soil microbial biomass of up to 50%, particularly on sandy textured soils.

As expected, across the treatments sampled at the three sites, there was a strong relationship ($P < 0.001$) between soil total organic C content and biomass C concentrations ($r^2 = 87\%$). Soil microbial biomass C concentrations following the liquid sludge applications were smaller (range 123–318 mg C kg^{-1} soil) than following the sludge cake applications (range 195–672 mg C kg^{-1} soil), which reflected the smaller inputs of organic matter from the liquid sludges than from the sludge cakes.

Soil respiration rates following the final liquid sludge application showed a trend towards higher ($P > 0.05$) rates on all the treatments receiving the metal-amended liquid sludges compared with the low-metal sludge controls (Table 8). Across the three sites, there was no relationship ($P > 0.05$) between soil total organic C contents and soil respiration rates. Soil respiration rates following the liquid sludge applications were smaller (range 0.18–0.43 mg CO_2–C kg^{-1} soil h^{-1}) than following the sludge cake applications (range 0.45–1.36 mg CO_2–C kg^{-1} soil h^{-1}), which reflected the smaller organic matter inputs from the liquid sludges than from the sludge cakes.

The respiration rate per unit of microbial biomass (i.e. the 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 activity in relation to the energy required to synthesise new biomass (Bardgett & Saggar, 1994). Hence, soils exhibiting a higher qCO_2 are commonly regarded as under 'stress'. In this study, qCO_2 exhibited a trend towards increasing ($P > 0.05$) as a

Table 10 Mean metabolic quotient (qCO_2) in topsoil (0–25 cm) on selected treatments sampled in January 1998

Treatment	Woburn	Watlington	Rosemaunde
Control	0.09 (0.003)	0.12 (0.025)	0.07 (0.001)
Low-metal sludge	0.15 (0.021)	0.10 (0.014)	0.06 (0.006)
High-Zn sludge	0.26 (0.080)	0.11 (0.004)	0.07 (0.005)
High-Cu sludge	0.22 (0.069)	0.13 (0.008)	0.09 (0.016)
High-Cd sludge	0.24 (0.096)	0.14 (0.040)	0.09 (0.016)

Values in parentheses are standard errors ($n = 3$).

result of the liquid sludge applications (Table 10). The highest qCO_2 values were generally measured where metal-amended liquid sludges had been applied and the lowest qCO_2 values were generally on the untreated control treatments.

There were no differences ($P > 0.05$) in MPNs of clover *Rhizobium* between the rate 2 metal treatments and either the uncontaminated liquid sludge and untreated control treatments at any of the three sites (Table 9). Therefore, there was no evidence to suggest that the sludge cake additions had an effect on soil *Rhizobium leguminosarum* biovar. *trifolii* numbers in the short-term following application.

Conclusions

Soil heavy metal extractability differed between the metal-amended liquid sludge and metal-rich sludge cake treatments; Zn and Cd extractabilities were higher from the liquid sludge additions, whereas Cu extractability was higher from the sludge cake application. These differences in metal extractability in the treated soil samples reflected the contrasting NH_4NO_3 extractable metal contents of the metal-amended liquid sludges and sludge cakes that were originally applied.

Application of the metal-amended liquid sludges had no effects ($P > 0.05$) on soil respiration rates, biomass carbon concentrations or MPNs of clover *Rhizobium* at the current statutory limits for Zn, Cd and Cu limits in soil. In the short term following the cessation of the liquid sludge application programme, there were no consistent metal effects on microbial activity that could be attributed to metal toxicity *per se*.

Acknowledgements

The funding of this work by the Department for Environment, Food and Rural Affairs (Defra), UK Water Industry Research Limited (UKWIR), Environment Agency (EA) and Welsh Assembly Government (WAG) is gratefully acknowledged.

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