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Long-term effects of metals in sewage sludge on soils, microorganisms and plants

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SUMMARY

This paper reviews the evidence for impacts of metals on the growth of selected plants and on the effects of metals on soil microbial activity and soil fertility in the long-term. Less is known about adverse long-term effects of metals on soil microorganisms than on crop yields and metal uptake. This is not surprising, since the effects of metals added to soils in sewage sludge are difficult to assess, and few long-term experiments exist. Controlled field experiments with sewage sludges exist in the UK, Sweden, Germany and the USA and the data presented here are from these long-term field experiments only. Microbial activity and populations of cyanobacteria, *Rhizobium leguminosarum* bv. *trifolii*, mycorrhizae and the total microbial biomass have been adversely affected by metal concentrations which, in some cases, are below the European Community's maximum allowable concentration limits for metals in sludge-treated soils. For example, N_2-fixation by free living heterotrophic bacteria was found to be inhibited at soil metal concentrations of (mg kg^{-1}): 127 Zn, 37 Cu, 21 Ni, 3.4 Cd, 52 Cr and 71 Pb. N_2-fixation by free-living cyanobacteria was reduced by 50% at metal concentrations of (mg kg^{-1}): 114 Zn, 33 Cu, 17 Ni, 2.9 Cd, 80 Cr and 40 Pb. *Rhizobium leguminosarum* bv. *trifolii* numbers decreased by several orders of magnitude at soil metal concentrations of (mg kg^{-1}): 130–200 Zn, 27–48 Cu, 11–15 Ni, and 0.8–1.0 Cd. Soil texture and pH were found to influence the concentrations at which toxicity occurred to both microorganisms and plants. Higher pH, and increased contents of clay and organic carbon reduced metal toxicity considerably. The evidence suggests that adverse effects on soil microbial parameters were generally found at surpringly modest concentrations of metals in soils. It is concluded that prevention of adverse effects on soil microbial processes and ultimately soil fertility, should be a factor which influences soil protection legislation.

INTRODUCTION

Soils may become contaminated with metals from a variety of anthropogenic sources such as smelters, mining, power stations, industry and the application of metal-containing pesticides, fertilizers and sewage sludges to land. Emissions from smelters, power stations and metal industries usually result in aerial deposition giving a more diffuse type of contamination. A more concentrated type of deposition occurs when metal-contaminated sewage sludges are applied to land or where metal-containing pesticides and fertiliziers are used extensively. Heavy metals in sewage sludges originate mostly from industrial sources, but domestic waste can contain significant amounts of Zn and Cu.

Approximately 30% of the sludge produced in the United Kingdom annually is disposed of at sea, and 42% is applied to agricultural land [18]. The latter is equivalent to about 18 million tonnes (wet weight basis) annually [46] and spreading takes place on about 10% of the total farming land [1]. Sewage sludge can be a valuable source of plant nutrients such as N, P, Ca and Mg and can act as a soil conditioner. The amount disposed of onto land is likely to increase significantly in the next four years as dumping at sea from the UK will be banned in 1998. This picture is likely to be repeated in other countries as a result of international agreements to cease sea disposal of sludge.

Repeated applications of sewage sludge can result in elevated metal concentrations that persist in the plough layer [40]. Chang et al. [13] found that more than 90% of the Cd, Cu, Cr, Ni, Pb and Zn added to annual sludge treatments over a 6-year period in a field experiment remained in the cultivated layer (0–15 cm) in both sandy and loam soils. Similarly, between 71 and 96% of the metals added through sludge applications to a sandy loam soil remained in the cultivated layer (0–20 cm) even though the last application was made some 25 years earlier [39,40]. Hence, the potentially large heavy metal content of sewage sludges may be a hindrance to their long-term use as manures in agriculture.

Once the metals are in the soil they are strongly held by the soil particles and there is little removal by plant uptake or movement down the soil profile [40]. Soil erosion is probably the major pathway through which large amounts of metals may be lost from the soil. In general, heavy metals are less mobile and thus less bioavailable in soils with higher organic matter contents and clay contents [15,35]. However, they are more mobile in acid soils and hence, more available for plant uptake, but become less so as the pH is raised [45].

Some metals such as Zn, Cu, Ni, Co and Cr are essential or beneficial micronutrients for plants, animals and microorganisms [1], whereas others such as Cd, Hg and Pb have no

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known biological and/or physiological functions. However, all these metals may be toxic at higher concentrations.

The European Community and other countries have set maximum permissible values for metal concentrations in agricultural soils through the application of sewage sludge (Table 1). These values resulted from work on metal uptake by crops, animals and humans, but take little or no account of the possible effects of metals on the soil microbial populations [8,16,17,22,23]. Recently, however, concern has increased over the effects of relatively small concentrations of metals on soil microorganisms and soil microbial activity [4,20,25,26,34,42].

The long-term effects of metals added to soils in sewage sludge are very difficult to assess, as there are few such experiments and consequently a lack of good long-term data. Appropriate field experiments exist in the UK, Sweden, Germany and the USA. In this paper we review the evidence from these field experiments of the impact of metals on the growth of selected plants, soil microbial activity and long-term soil fertility.

FIELD EXPERIMENTS

Woburn, England

This experiment began in 1942. Twenty-five applications of anaerobically-digested sewage sludge, naturally contaminated with metals, from a sewage works in West London were added at 8.2 or 16.4 t organic matter (OM) ha^{-1} yr^{-1} from 1942–1961. In addition, farmyard manure (FYM) at 5.2 or 10.4 t OM ha^{-1} yr^{-1} and inorganic fertilizer were also added as treatments to control plots. Since 1961 and 1965, respectively, the sewage sludge and FYM treatments have received only inorganic fertilizers. The soil is a sandy loam with about 10% clay and 2% organic carbon. The pH was maintained around 6.5 by regular maintenance dressings with lime.

Lee Valley and Luddington, England

These sludge experiments started in 1968. The soil at Lee Valley is a heavy silt loam with 21% clay, 4% organic carbon and a pH of 5.6–5.9. That at Luddington is a sandy loam with 15% clay, 3% organic carbon and a pH of 6.5. Both experiments received the same treatments. Sewage sludges from various sewage works were used and the different treatments received either a single large dose of 125 t dry solids (DS) ha^{-1} of four sewage sludges contaminated predominantly with Zn (16 000 mg kg^{-1}), Cu (8000 mg kg^{-1}), Ni (4000 mg kg^{-1}), or Cr (8000 mg kg^{-1}), or 31 t ha^{-1} yr^{-1} of the same sludges for four years from 1968. In addition, there was a relatively uncontaminated sludge treatment and a control treatment with inorganic fertilizers only.

Gleadthorpe, England

The soil at this site is a sandy loam with 9% clay and 1–2% organic carbon (control soil). This experiment was started in 1982 and the sewage sludges used were artificially contaminated by adding metals salts to raw sewage and then dewatering. One application of Zn- or Cu- or Ni-contaminated sludge was made to all plots with a further application to some, but not all, 5 years later. Apart from these single metal treatments, mixed metal treatments of Zn plus Cu and Zn plus Ni were also applied. There were also treatments in 1982 with 'uncontaminated' sewage sludge at approximately 100 t DS ha^{-1} or inorganic fertilizers.

Ultuna, Sweden

This experiment began in 1956, and sewage sludge, naturally contaminated with metals, from sewage works in Uppsala and FYM were both added as treatments at 14 t and 10 t ha^{-1} every 2 years, respectively, from 1956–1988. Inorganic fertilizers were also added as treatments over the same period. The site has post glacial soil with 35% clay, 35% silt and 21% fine sand, and a pH of 6.2 and 6.6 in the unfertilized and FYM-treated plots respectively, and pH 5.3 in the sludge-treated plot. The organic carbon content in the sludge-treated soil was 2.7%, whereas the unfertilized and FYM-treated plots contained 1.2 and 1.9% organic carbon respectively.

Braunschweig, Germany

Two field experiments were begun in 1980 on the same field and both received the same treatments consisting of inorganic fertilizers or 'moderately' contaminated or metal-amended liquid sludge added at rates of 100 or 300 m^3 ha^{-1}

TABLE 1

Maximum concentrations of metals allowed in agricultural soils treated with sewage sludge

Country	Year	mg kg^{-1} soil						
		Cd	Cu	Cr	Ni	Pb	Zn	Hg
European Community	1986	1–3	50–140	100–150[a]	30–75	50–300	150–300	1–1.5
US[b]	1993	20	750	1500	210	150	1400	8

[a] Now withdrawn.
[b] Calculated from maximum cumulative pollutant loading limits, assuming incorporation to 15-cm depth and average soil bulk density of 1.33 g cm^{-3}, but not including the background concentrations of these elements in soils.

yr^{-1} for 10 years. These were equivalent to 5 or 16 t DS ha^{-1} yr^{-1} from 1980–1990. The moderately contaminated sewage sludges used were obtained from a local sewage works and were naturally contaminated. However, the contaminated sludges were from a different works in 1980, then from 1981–1990 the same moderately contaminated sludge was artificially contaminated with metal salts and anaerobically incubated for 6 weeks before use. One experimental site (Braunschweig 1) was on an old arable soil with plot pH values ranging from 6.0–7.0 and 0.8–1.5% organic carbon content; the other experimental site (Braunschweig 2) was on an ex-woodland soil with plot pH values ranging from 5.3–5.7 and 1.6–2.6% organic carbon content. Both soils were silty loams with 50% silt, 45% sand and 5% clay.

Fairland and Beltsville, Maryland, USA

Two field experiments were established at Fairland and Beltsville, Maryland, in 1975 and 1976 respectively. The soil at Fairland was a sandy loam with pH values in the plots ranging from 6.4–6.9. Anaerobically-digested sludge was applied at 112 t ha^{-1}. The soil at Beltsville was a fine sandy loam with two different types of sludge applications. Some plots received heat-treated sludge from Annapolis at 224 t ha^{-1} and these plots had pH values ranging from 5.1–6.0; other plots received Chicago 'Nu-Earth' sludge at 100 t ha^{-1} and had plot pH values ranging from 5.7–6.6.

RESULTS AND DISCUSSION

Biological nitrogen fixation

Fixation of atmospheric dinitrogen is only carried out by prokaryotes that possess the enzyme nitrogenase [50]. This ability is widely distributed amongst the prokaryotes and includes free-living heterotrophic bacteria, phototrophic cyanobacteria and organisms which fix nitrogen in symbiotic associations with plants, of which the most important group is the *Rhizobium*–legume symbiosis.

Effects of metals on free-living heterotrophic bacteria

Free-living heterotrophic N_2-fixing bacteria are ubiquitous in soil and include species which can fix nitrogen under aerobic, microaerophilic and anaerobic conditions. Significant decreases in acetylene reduction activity (ARA) by aerobic and microaerophilic N_2-fixers were reported in metal-contaminated soils from Woburn compared to FYM-treated soils [7]. These reductions occurred at metal concentrations close to the EC upper limits for Zn and Cu, and 3–4 times the limit for Cd (Table 1). Mårtensson and Witter [38] found heterotrophic N_2-fixation to be severely reduced in metal-contaminated soil compared to FYM-treated soil at Ultuna, in Sweden. Nitrogen-fixing activity by aerobic diazotrophs in the metal-contaminated soil decreased to 2% of that measured in the FYM-treated soil. Metal concentrations in the FYM-treated soil were at background levels, and those in the sludge-treated soil are given in Table 2. However, the pH of the sludge-treated plot was low (pH 5.3) and this may also have affected both numbers of, and N_2-fixation by, free-living heterotrophic bacteria at this site in addition to the metals.

Fließbach and Reber [20] also confirmed the great sensitivity of N_2-fixation by free-living heterotrophic bacteria to metals in the old arable soil at Braunschweig in Germany. The metal concentrations in this soil ranged from (mg kg^{-1}): Zn, 157–381; Cu, 42–102; Ni, 12–25; Cd, 0.7–2.5; Cr, 41–90; and Pb, 61–88. However, no N_2-fixation by free-living heterotrophic bacteria could be measured in the ex-woodland soil (Braunschweig 2) in the same field. The pH of the soil in these plots ranged from 5.3–5.7 and the metal concentrations were similar to those in the old arable experiment (Braunschweig 1).

Recently, Lorenz et al. [37] tested the possibility of using N_2-fixation by free-living heterotrophic bacteria as a sensitive biological indicator of metal pollution on sludge-treated soils from the experiments at Woburn, Luddington, Lee Valley and Gleadthorpe. The Woburn soils gave the same results as those obtained by Brookes et al. [7], but no activity could be found in either the Luddington or Lee Valley soils. The Gleadthorpe soils gave sporadic activity which did not correlate with the metal concentrations in these soils. In a further experiment where metal-contaminated soil and FYM-treated soil, from Woburn, were mixed in various proportions to give soils of increasing metal concentrations, free-living heterotrophic N_2-fixation was significantly inhibited at metal concentrations of (mg kg^{-1}): 127 Zn, 37 Cu, 21 Ni, 3.4 Cd, 52 Cr and 71 Pb. However, it was concluded that free-living heterotrophic bacteria were not ubiquitous or active enough to be used as indicator organisms for detecting metal pollution of soil [37].

Effects of metals on free-living phototrophic cyanobacteria

Phototrophic cyanobacteria are autotrophs which grow on soil surfaces and make use of light to fix CO_2 in photosynthesis. Twenty-five years after the last treatments ceased, cyanobacterial N_2-fixing activity was reduced by 30% on metal-contaminated soil from Woburn compared to the FYM-treated soil [6]. Also, colonization was delayed in the metal-contaminated soil. A gradient of increasing metal concentrations in soil between plots previously treated with either FYM or sewage sludge was also sampled, and a smooth decrease in nitrogenase activity with increasing metal concentrations was found [6]. Nitrogen fixation was reduced by 50% at metal concentrations given in Table 2. Even stronger inhibition of cyanobacteria has been reported [38] on the metal-contaminated soil at Ultuna compared to the FYM-treated control soil (Table 2).

Fließbach and Reber [21] reported large decreases in the counts of cyanobacteria in plots of the two field experiments at Braunschweig to which increasing amounts of 'moderately' contaminated unamended or metal-amended sludge had been added compared to the control NPK or moderately contaminated unamended low sludge-treated plots. Using the acetylene reduction assay to assess nitrogenase activity in cyanobacteria, they found that after 30 days incubation, acetylene reduction and hence N_2-fixation, was suppressed by 30 and 70% in the low and high sludge-treated plots, respectively, at the old arable site (Braunschweig 1, pH 6.1–6.8). In the ex-woodland site (Braunschweig 2, pH 5.3–5.7), the corresponding suppression was about 25 and 100% respectively (Table 2). Interestingly, annual additions of the moderately contaminated clay and organic carbon content in the sludge-treated soil may have reduced metal toxicity to rhizobia at this site, even though the pH was low.

More recently, Giller et al. [26] constructed a gradient of soil with increasing metal concentrations by mixing metal-contaminated soil and FYM-treated soil, from Woburn, in various proportions. To these soils they added equal numbers of *R. leguminosarum* bv. *trifolii* and monitored their survival over a 171-day period. At 53 and 171 days, the number of rhizobia in the 2/3 sludge soil had decreased to $<10^4$ cells g^{-1} soil, whereas those in the control FYM soil remained at $>10^6$ cells g^{-1} soil. A portion of the surviving rhizobia in the soils containing the largest amounts of sludge (i.e. $>1/2$ sludged soils), at 171 days, may have been ineffective metal-tolerant rhizobia. In earlier studies these rhizobia were found to number 10^2 cells g^{-1} soil in freshly sampled rhizosphere soil from the metal-contaminated plot at Woburn [25]. The metal concentrations in the 2/3 sludge soil, at which significant reductions in the numbers of rhizobia occurred after 53 days, were (mg kg^{-1}): 228 Zn, 67 Cu, 31 Ni, 6.9 Cd, 81 Cr and 84 Pb.

Chaudri et al. [14] sampled all the plots of two field experiments at Braunschweig and found *R. leguminosarum* bv. *trifolii* numbers decreased by several orders of magnitude, compared to the control plots, at metal concentrations well below the current EC limits for these metals (Table 3; Fig. 2). Although several metals increased simultaneously in both field experiments, these authors suggested that there seemed to have been a strong effect of Zn on the numbers of rhizobia in these soils. Also, metal toxicity to rhizobia occurred at slightly smaller concentrations in the ex-woodland site (Braunschweig 2) due to the lower pH compared to the old arable site (Braunschweig 1).

Effects of metals on other species of legumes/rhizobia

Spring beans (*Vicia faba*) grown in the metal-contaminated plots at Woburn during 1968 and 1969 (more than 7 years after sludge application ceased) showed no difference in yields compared to the control FYM-treated plots [32]. In fact, the yields from these plots were identical in both years, but both were greater than the yield from the plots treated with NPK fertilizer. The spring beans were not inoculated with rhizobia nor was nitrogen fertilizer added and therefore, must have been infected by the indigenous effective population of *R. leguminosarum* bv. *viciae* present in these soils. The yield data for the two years from these plots suggest that *R. leguminosarum* bv. *viciae* is not as sensitive to heavy metals as *R. leguminosarum* bv. *trifolii.*

Work on the effects of heavy metals, through the application of sludge, on yields and N_2-fixation in soyabean was carried out in two field experiments (Beltsville and Fairland sites) in Maryland, USA [27–29, 33]. Heckman et al. [27], using soils to which heat-treated sludge had been applied in pot experiments, found increasing yields and N_2-fixation by soybean with increasing sludge application rates. In 1983 and 1984 non-nodulating and nodulating isolines of soybean were used to determine toxic effects of metals on N_2-fixation. No metal toxicity occurred at Beltsville, but at Fairland there was

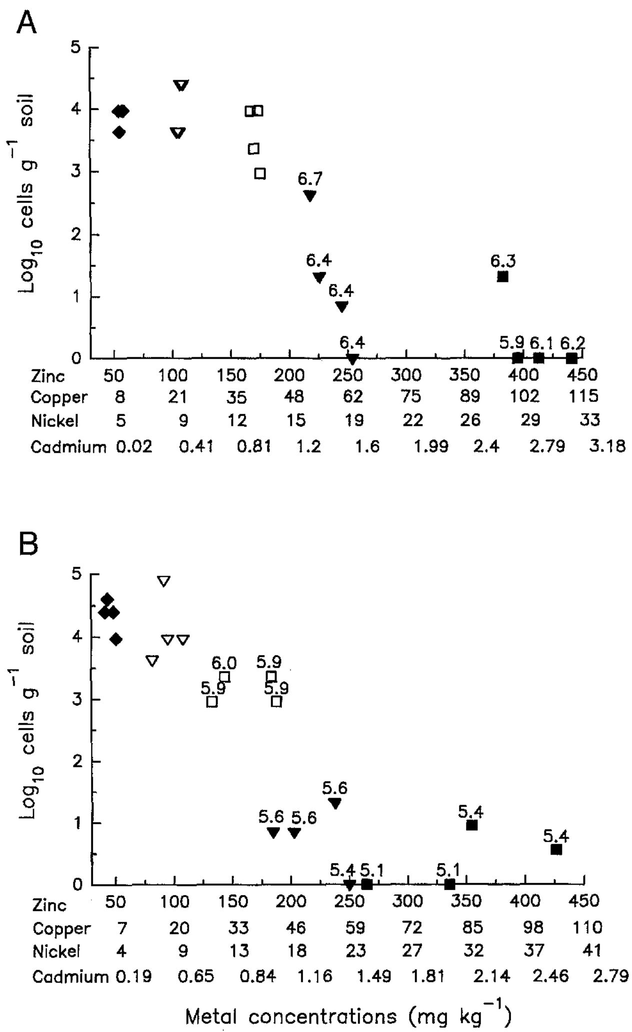

Fig. 2. Numbers of indigenous effective *R. leguminosarum* bv. *trifolii* in soils from the A) old arable experiment (Braunschweig 1) and B) ex-woodland experiment (Braunschweig 2) at Braunschweig. Treatments: ◆ inorganic fertilizers; ▽ 100 m^3 'moderately' contaminated unamended sludge ha^{-1} yr^{-1}; □ 100 m^3 metal-amended sludge ha^{-1} yr^{-1}; ▼ 300 m^3 'moderately' contaminated unamended sludge ha^{-1} yr^{-1}; ■ 300 m^3 metal-amended sludge ha^{-1} yr^{-1}. Numbers on symbols represent pH of soils.

a 12–55% decrease in the %N from N_2-fixation in soybean in the metal-contaminated plots suggesting metal toxicity [29]. Kinkle et al. [33] found that the numbers of *Bradyrhizobium japonicum* increased with increasing rates of sludge application, and the numbers of serotypes and their metal sensitivities remained the same regardless of the treatment. Neither of these studies reported metal concentrations in the sludge-treated soils at the two sites. But it is now known that all the metal concentrations in these two sites were below those in most of the European experiments, even in the most heavily contaminated plots [41]. It is therefore possible that the metals were not present in large enough concentrations to cause adverse effects on yields, N_2-fixation or on the free-living *B. japonicum* in these two experiments. The increase in numbers of *B. japonicum* with increasing applications of sludge was presumably due to the additional substrate from the organic matter in the sludge. It is important to distinguish between short-term positive effects due to substrate and longer-term negative effects due to metal toxicity.

Giller et al. [26] found that *R. loti* was present only in soil from an FYM-treated plot, at Woburn, and not in a metal-contaminated plot. No *R. meliloti* were present in either plot which is not surprising as alfalfa (*Medicago sativa*) often requires inoculation with *R. meliloti* when grown in the UK. In a further experiment, they added equal numbers of *R. loti* and *R. meliloti* to gradients of increasing metal concentrations prepared by mixing metal-contaminated soil with FYM-treated soil from Woburn. After 50 days exposure, the number of *R. meliloti* in soil mixtures containing 5/6 or more sludge soil were an order of magnitude smaller compared to the control FYM-treated soil, but were still above 10^5 cells g^{-1} for all soils. In contrast, *R. loti* numbers decreased by several orders of magnitude (i.e. 10^2 cells g^{-1} soil) in soil mixtures containing 1/2 or more sludge soil, compared to the FYM soil, which contained 10^5 cells g^{-1} soil. It was concluded that *R. meliloti* was less sensitive to heavy metals than *R. loti*, which was similar in sensitivity to *R. leguminosarum* bv. *trifolii*, but because these results were for single strains of each species, confirmation was required with a broader range of strains.

Effects of metals on the soil microbial biomass

There is a reasonably close linear positive relationship in uncontaminated soils between the organic carbon contents of soils and their biomass carbon contents [31]. Normally, the soil microbial biomass C comprises about 1–4% of the total soil organic C depending on the particular management practices, soil type and climate [31]. The soil microbial biomass is the most labile fraction of the soil organic matter and, therefore, can be a useful indicator of changes in soil conditions due to changes in soil management practices.

Brookes and McGrath [4] measured the microbial biomass in soils from the Woburn experiment some 20 years after the last treatments were applied. They found that in the high metal soils (sludge-treated) the microbial biomass was approximately half that in the low metal soils (FYM-treated). They also showed that in the low metal soils the usual linear relationship between organic carbon and biomass carbon existed, but no relationship was seen with the high metal soils. Metal concentrations at which the biomass was affected are given in Table 4. Also, the rate of respiration per unit weight of biomass (specific respiration) was much higher in the high metal soils than in the low metal soils, whereas the adenylate energy charge (thought to indicate the level of metabolic activity [19]) was high in both soils [5]. Recently, Brookes [3] suggested that the link between biomass C and total soil organic C could be used as an indicator of the functioning of the soil ecosystem. Soils deviating significantly from 'normal' (biomass C/total soil organic C) ratios measured for a particular soil type, climate or management practice may indicate a change in the functioning of the ecosystem and therefore warrant further study [3].

Chander and Brookes [10] measured the soil microbial biomass in soils from the experiments at Lee Valley and Luddington where single large doses of sewage sludge contaminated predominantly with single metals were added some 22 years earlier. They found decreased soil microbial biomass in soils containing larger concentrations of Zn and Cu in the heavier soil at Lee Valley than in the sandy loam soil at Luddington compared to the respective 'uncontaminated' sludge control soils (Table 4). There was no effect of Ni at 148 mg kg^{-1} soil on the soil microbial biomass in both soils. Similarly, Cd at 6 mg kg^{-1} soil in the 'uncontaminated sludge control' soil was found to have no effect on the microbial biomass in the silty loam soil. Unfortunately, no treatment contained Cd at the Luddington site and therefore, effects of this metal are not known at this site.

Further work by Chander [9] and Chander and Brookes [11] on soils from Woburn confirmed the higher specific respiration rate of the microbial biomass in the metal-contaminated soils first reported by Brookes and McGrath [5]. When glucose and maize straw substrates were added to the Woburn soils, proportionately more was respired as CO_2 from contaminated soils compared to soils from uncontaminated plots [11]. Consequently, less new microbial biomass was formed from the added substrate and it was concluded that the lower efficiency of conversion of carbon into biomass is one of the explanations for the lower biomass observed in the metal-contaminated soils. The fact that specific respiration is greater in the experiments on the metal-contaminated soils means that measurements of soil respiration alone cannot give a good indication of effects of metals on the size of the microbial biomass. In fact, increased respiration could be interpreted as an indication of increased stress and/or an increased death rate of microbes.

Soil microbial biomass carbon and nitrogen were found to be reduced by about 60%, in soil from the metal-contaminated sewage sludge-treated plot at Ultuna, compared to soil from the control FYM-treated plot [52]. The metal concentrations at which these reductions occurred are given in Table 4.

In both field experiments at Braunschweig, and at both the low and high rates of sludge application, the microbial biomass increased with increasing additions of 'moderately' contaminated unamended sludge organic matter each year. In contrast, increases in biomass were less pronounced or even absent in the inorganic fertilizer treatments and metal-contaminated sludge treatments, especially at the largest rates of addition. These effects on the biomass were apparent after only 7 years of sludge addition [20], at the metal concentrations shown in Table 4.

Effects of metals on mycorrhizae

Koomen et al. [34] examined the effects of metal-contaminated soil and FYM-treated control soil, from Woburn, on both native and an introduced species of vesicular-arbuscular mycorrhiza (VAM) (*Glomus mosseae*) in pot experiments. They found that in the control soil 60% of the white clover roots were infected with native VAM compared to only 1% for the metal-contaminated soil. In the experiment where VAM were introduced into the soils, the control soil had 46% and 21% mycorrhizal infection in the inoculated and non-inoculated treatments respectively. In contrast, none of the

TABLE 4

Minimum concentrations of metals in soils which negatively affected the soil microbial biomass

Experimental site	mg kg^{-1} soil					
	Zn	Cd	Cu	Ni	Pb	Cr
Woburn, UK	180	6.0	70	22	100	105
Luddington, UK	281	–	150	–	–	–
Lee Valley, UK	857	–	384	–	–	–
Ultuna, Sweden	230	0.7	125	35	40	85
Braunschweig 1, Germany	360	2.8	102	23	101	95
Braunschweig 2, Germany	386	2.9	111	24	114	105

plant roots were infected with mycorrhizae in the metal-contaminated soil from either treatment. Clover roots were also sampled in the field, at Woburn, to see if this trend was repeated. When clover roots were first sampled no mycorrhizal infection was present. But after six months 52% of the root length sampled from the FYM-treated plots and 69% of root length from the sludge-treated plots were infected. Hence, mycorrhizal infection was delayed in both the FYM-treated and sludge-treated plots.

Koomen et al. [34] suggested that the apparent contradiction between their pot and field experiments could be explained by the greater time that had elapsed during field samplings which may have allowed the infection by a small inoculum-density of mycorrhizae present in the sludge-treated plots to gradually increase to the levels observed on the FYM-treated plots. They further suggested that the metals could be delaying the development of mycorrhizal infection rather than completely suppressing it and that the infection that developed in the metal-contaminated soil after some time was likely to be due to indigenous metal-tolerant mycorrhizae present in the soil. An isolate of *G. mosseae* was found to be tolerant to large concentrations of Zn and Cd in a heavily polluted soil at Shipham in south west England. This was as effective in enhancing the growth of clover as a 'normal' isolate of *G. mosseae* in a pot experiment, with infection levels being comparable after 6 weeks in the control pots containing uncontaminated soil [24]. Reductions in VAM infections of maize with increasing metal-contaminated sludge applications have also been reported at Braunschweig, with a relative shift of mycorrhizal infection to the uncontaminated sub-soil [36] possibly to avoid the toxic effects of the metals in the top soil.

However, caution should be exercised in interpreting these results as large amounts of available-phosphorus is a general property of long-term sludge-treated soil which could have inhibited infection. Also, in some of the work discussed [36] the reductions in VAM infections occurred in plots where the pH was low and this may have affected both the infection of the roots and the growth of the roots themselves. Even after taking these considerations into account, these studies suggest that there is a strong negative effect of metals on VA-mycorrhizal infection and development.

CONCLUSIONS

The concentrations of metals in soil at which adverse effects occur on soil microbial parameters in the long-term field experiments are summarized in Fig. 3 and related to background metal concentrations. The lowest observed adverse effect concentrations (LOAECs) for Zn and Cu that adversely affected soil microbial parameters were greater than the largest background concentration for these metals in soils, except for cyanobacteria for which there were LOAEC values below the smallest background concentration for Zn (Fig. 3). For Cd, the LOAECs considerably overlapped the largest background concentrations found in soil. However, some caution should be exercised in interpreting this Figure, since all of these metals were present simultaneously in the soils from these field experiments. It is possible that the effects observed with any one metal may actually have been due to another, or to a combination of metals. In some cases the metal(s) responsible for the adverse effects can be identified even though other metals were present in the soil. For example, the metal-contaminated soils from the Woburn and Braunschweig field experiments have similar Zn and Cu concentrations, but, at the point where effects were observed, the soil from Woburn contained Cd at twice the European Community's upper limit, whereas the soils from the Braunschweig experiments were below the limit and contained <1 mg Cd kg^{-1}. *Rhizobium leguminosarum* bv. *trifolii* numbers declined significantly in both of these metal-contaminated soils compared to their respective relatively 'uncontaminated' control soils. Because the metal-contaminated soils at Braunschweig contained near-background Cd concentrations, but gave a similar decline in rhizobial numbers to that noted for the metal-contaminated soil from Woburn, and because Cu is strongly bound in soils at these concentrations, it is likely that Zn was the metal causing adverse effects on rhizobia at these two sites.

Figure 3 shows that LOAECs for each organism or group vary and soil type, organic matter content and pH may also influence the LOAECs. This is due to differences in soil type and properties: for example, Woburn and Braunschweig soils are both loam soils, with low organic matter contents. The LOAECs for rhizobia from the metal-contaminated soils from

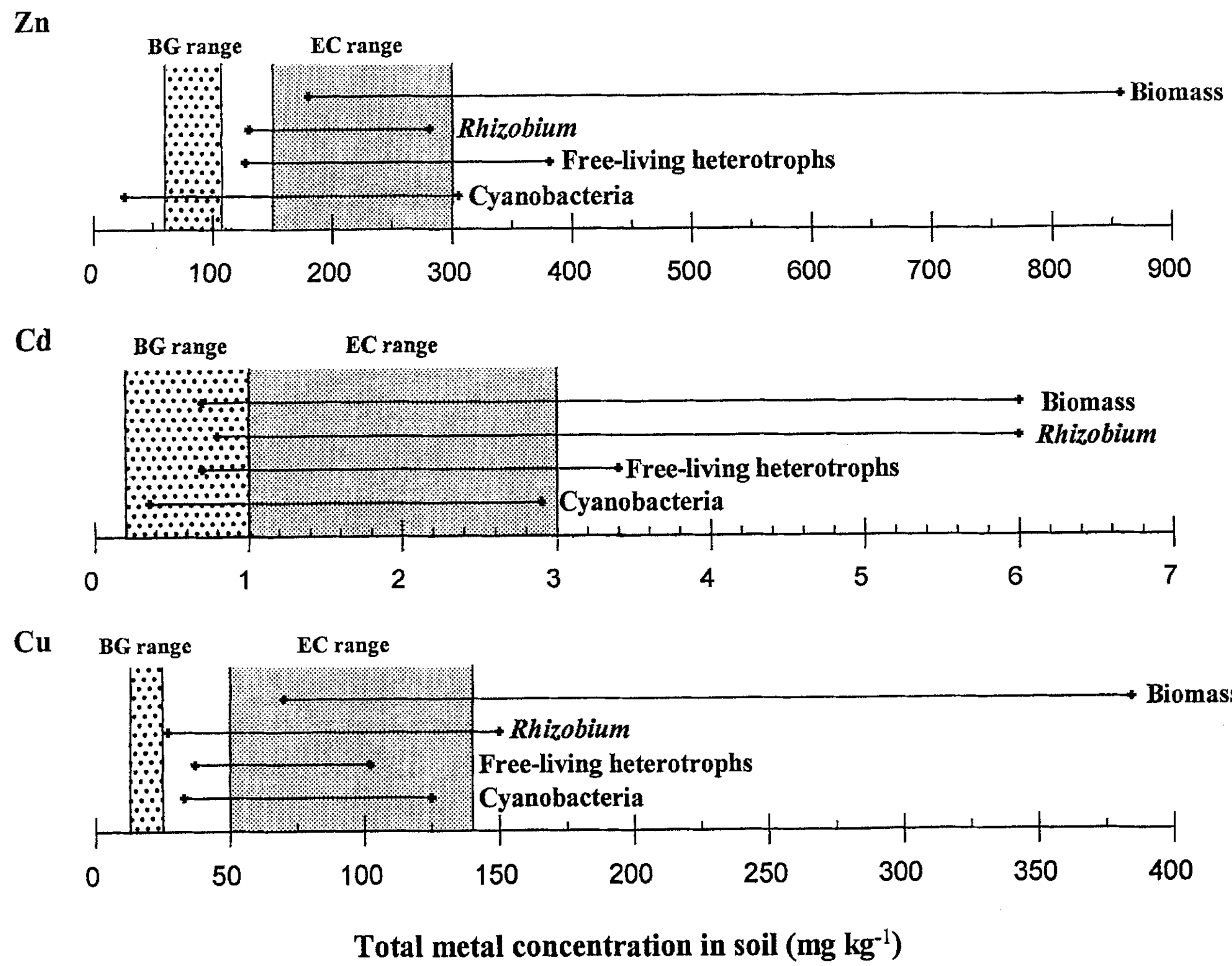

Fig. 3. Range of lowest observed adverse effect concentrations (LOAECs) of Zn, Cd and Cu on groups of microorganisms found in soils of six long-term field experiments, compared with soil background concentrations (90 percentile range, McGrath and Loveland [44]) found in UK soils. BG range = background concentration range; EC range = range of limits for sludge-treated soils in the European Community.

both these sites were similar. However, site 2 at Braunschweig had a lower pH (pH 5.3–5.7) and this resulted in smaller LOAECs for rhizobia compared to site 1 which had a higher pH (pH 6–7). In contrast, a heavy soil such as that at Lee Valley (silty clay loam with 4% organic carbon) gave a large LOAEC for adverse effects on the soil microbial biomass compared to the sandy loam soil at Woburn. Heavier soils, near-neutral to neutral pH soils and/or soils containing large amounts of organic matter may reduce the toxic effects of the heavy metals to soil microbes by binding the metals and making them less bioavailable, thus giving large LOAECs.

The reported adverse effects on soil microbial parameters were generally found at surprisingly modest concentrations of metals in soils. Indeed, these were smaller than the concentrations reported by Chang et al. [12] that were likely to decrease growth of sensitive crop species. In conclusion, we believe that the aim of soil protection legislation should be to prevent long-term effects on soil microbial processes and, ultimately, soil fertility.

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