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Short communication

Fresh additions of heavy metals do not model long-term effects on microbial biomass and activity

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Abstract

Previous work has reported adverse effects of long-term exposures of heavy metals (e.g. Cu, Ni, Cd and Zn) on soil microbial biomass (up to 50% decrease) and microbial activity at metal concentrations around current European Union permitted limits. Our aim was to see if we could model such changes in short-term (up to 50 days) laboratory incubations where soils were given a single pulse of metal salts. Such additions, however, caused only small changes in the measured variables. It was concluded that such short-term incubations are a poor model of changes in microbial biomass or activity due to chronic exposure to metals. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Microbial biomass; Heavy metals; Respiration; Specific respiration

Soils containing heavy metals (e.g. Cu, Ni, Cd, Zn) from long-term inputs of contaminated sewage sludge may contain less microbial biomass and have altered microbial activities. We tried to replicate these effects in small-scale laboratory incubations, using fresh metal additions. In particular, we wished to study the role of Cd as Smith (1996) suggested that it was frequently responsible for these effects.

We studied soils from a permanent arable and permanent grassland plot of the Highfield Ley–Arable experiment (Johnston, 1973) (Aquic Paluedelpf). The grassland soil had a pH in water of 6.84 and contained 3.80% organic C and 0.354% total N. The arable soil had a pH of 6.64 and contained 1.67% C and 0.174% N. Sampling depths were 0–23 cm (arable) and 0–10 cm depth (grassland), respectively. The moist soils were sieved <2 mm then incubated at 40% water holding capacity and 25°C prior to use.

Cd, Cu, and Zn, added as aqueous sulphates, gave final total metal concentrations of 12, 140 and 300 mg kg^{-1} soil respectively. Biomass C was measured by fumigation extraction (Wu et al., 1990). Initial measurements on the unamended soil were made at 0 days then on all treatments at 7 and 49 days. Soil CO_2-C evolved was trapped in 1.0 M NaOH and measured by autotitration. The treatments, each replicated three times, are expressed on an oven-dry soil basis.

The metals were added to both soils to give Cu and Zn at maximum and Cd at four times maximum European Union (EU) permitted rates for agricultural soils. They were each applied singly or as (Cd + Cu), (Cd + Zn), (Cu + Zn) and (Cd + Cu + Zn).

Overall, the main metal effect was to decrease the cumulative respiration rate. Single metals were less inhibitory than metal combinations. Effects were most marked in the grassland soil with depressions in respiration greater than two-fold with the (Cd + Cu + Zn) treatment (Fig. 1). This treatment caused the maximum respiratory depression in both the grassland and arable soils. Other differences were often small and non-significant.

The general order of inhibition for single metals was: Zn > Cu > Cd and grassland > arable. For metal combinations, it was (Cd + Cu + Zn) > [(Cd + Zn) and (Cu + Zn)] > (Cd + Cu) and, again, grassland > arable (Fig. 1).

Although Cd was added at four times the EU limit, when added singly it inhibited respiration less than the other metals. Even in combination with Cu, Zn or (Cu + Zn) the inhibitory effect of Cd was no larger than that of the other metals in combination (Fig. 1). Thus, this work did not support the view of Smith and Giller (1992) that Cd is the metal causing the most adverse effects on the soil microbial biomass and its activity. However, it must be noted that our results refer to acute effects caused by recent additions of

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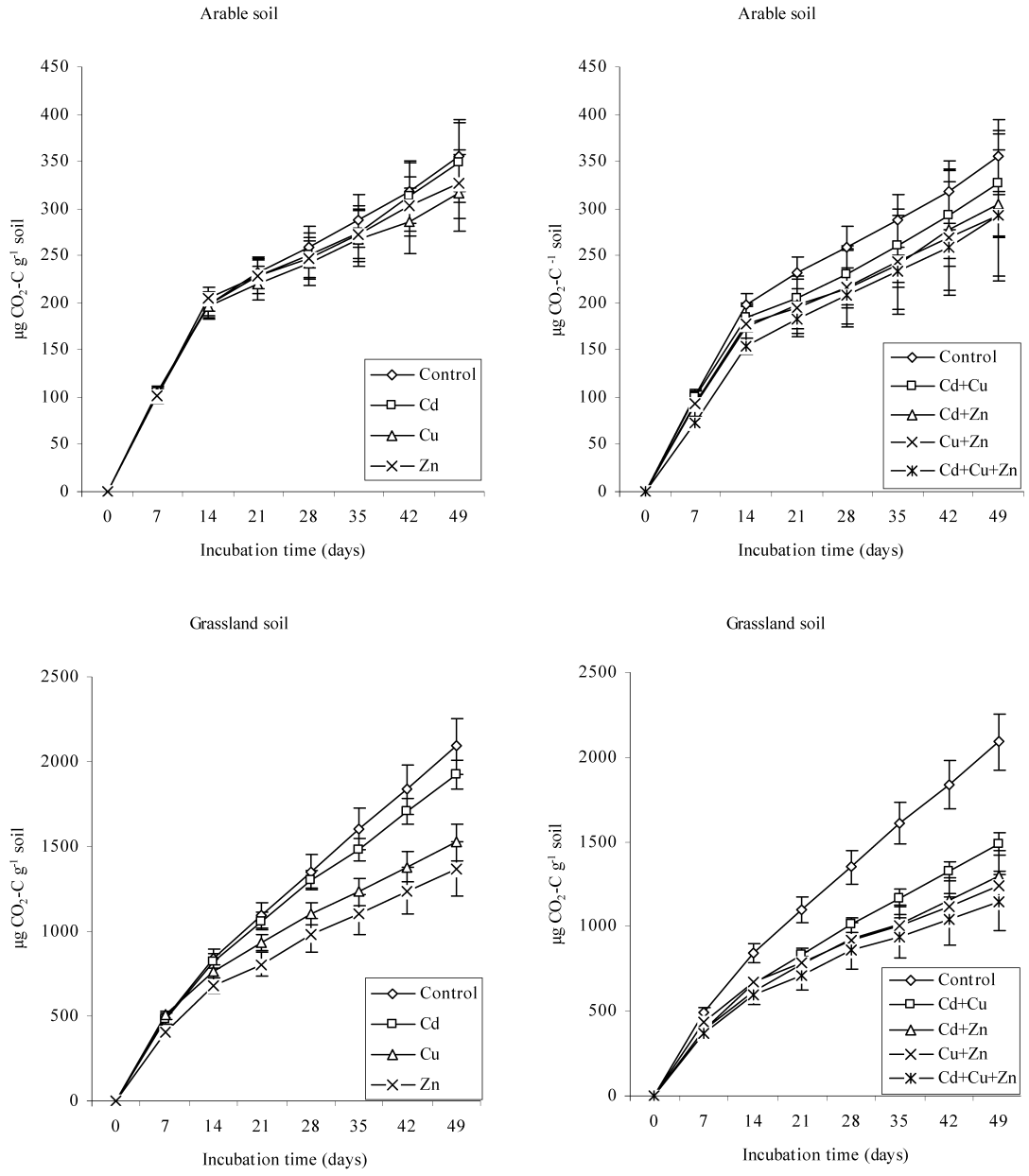

Fig. 1. Cumulative respiration of the arable and grassland soils under different heavy metal treatments. The error bar is the standard of the means.

metals while they were referring to effects on the microbial ecosystem caused by chronic exposure.

The results also differ from those of Brookes and McGrath (1984) who reported no differences in CO_2 evolution rates between uncontaminated and metal contaminated soils of the Woburn Market Garden Experiment. Instead, they more closely resemble those of Tyler (1981) who reported that decreased soil respiration was 'probably' a common feature of heavy metal pollution of soils. The metal concentrations in Tyler's organic forest soils were, however, enormous (e.g. 40 g Cu + Zn kg^{-1} soil).

Similar results were reported by, for example, Fritze et al. (1989) for a heavily metal-polluted scots pine forest soil. Maximum concentrations were approximately 3700 µg Cu cm^{-3} soil (bulk densities not given).

These results refer to highly organic soils and similar 'field' concentrations of heavy metals do not occur in agricultural soils for obvious reasons. So, a perfect comparison between our laboratory and appropriate field data is not possible.

The reason for the agreement between our results and the above results is probably because we added metal salts directly and measured respiration shortly afterward. In both cases, the fresh metal additions and the large total metal concentrations in the 'field' soils would have caused metal concentrations in soil solution to be comparatively large. Thus, a larger proportion of the freshly added metals

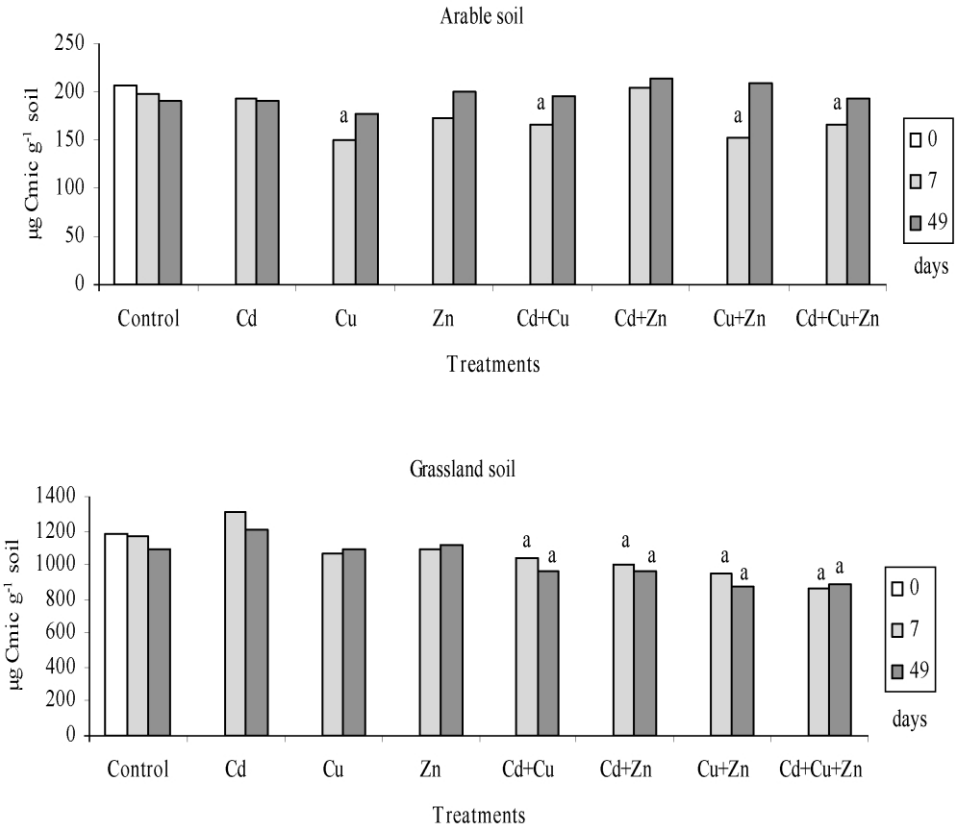

Fig. 2. Microbial biomass C of the arable and grassland soils given different heavy metal treatments. The bars with superscript indicate significant differences (see text).

would have been biologically active, unlike those in the Woburn field experiment. There, the metals were added incrementally in sewage sludge over more than 20 years. They would become less soluble, and less biologically active, during this longer equilibration.

We conclude that freshly added metals and metals from past field applications of sewage sludge invoke quite different respiratory responses from the microbial biomass.

Biomass C was measured at day 0 in the unamended soils, then at 7 and 49 days for all treatments (Fig. 2). Generally, metal-effects, either individually or in combination, were small (5–12%) and variable. The negative effect of single metals on the biomass were in the general order Cd < Zn < Cu. Any effect had occurred by 7 days after addition and there were no further negative effects up to day 49. The metals in combination again generally produced small (ca. 10%) decreases, although one of 25% occurred in the arable soil at 7 days following addition of (Cu + Zn). This was apparently followed by a recovery to the amount of biomass in the control soil by day 49. We doubt this was a true result, being almost within experimental error.

The decreases in biomass caused by the metals were much less than those observed, for example, by Brookes and McGrath (1984) or Chander and Brookes (1991a) in metal contaminated field soils. Later work showed that two mechanisms were mainly responsible. Firstly, conversion of substrate into new microbial biomass was decreased in the high- compared to low-metal soils. Secondly, C inputs by plants were decreased in high- compared to low-metal soils (Chander and Brookes, 1991b).

The situation with our work is rather different. Here, the metals were added as simple salts to the population of resting microbial cells. The main effect would be one of direct toxicity to the microbial biomass rather than one of substrate-use efficiency.

There was certainly no evidence that Cd was more toxic to the biomass than the other metals. If anything, the reverse was the case. However, again the response of the microbial biomass to the freshly added metals was quite dissimilar to its response to metal residues in long-term field experiments.

The rates of biomass specific respiration, measured at 7 and 49 days after addition of the metals, were similar within arable and grassland soils for all metals and metal combinations. This also contrasts with the work of Brookes and McGrath (1984). There, the specific respiration rate of the biomass in metal-contaminated soils from the Woburn experiment was about twice that in uncontaminated soils. Similarly, Chander and Brookes (1991b) showed that the specific respiration rate of the biomass, produced from fresh substrates, was significantly faster in contaminated than uncontaminated soils. Both results concur in showing

Table 1
Biomass specific respiration of the Highfield soils at 7 and 49 days after heavy metal addition

Incubation time (days)	Biomass specific respiration ($\mu g\ CO_2$-C g^{-1} biomass C day^{-1})							
	No metal	Cd	Cu	Zn	Cd + Cu	Cd + Zn	Cu + Zn	Cd + Cu + Zn
Arable soil								
7	0.07	0.08	0.10	0.08	0.09	0.06	0.09	0.06
49	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02
Grassland soil								
7	0.06	0.05	0.07	0.05	0.05	0.05	0.06	0.06
49	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.03

that it is the biomass synthesised in the presence of metals, rather than one subjected to a fresh, large input of metals, that undergoes physiological changes, or adaptations, to the metal contamination (Table 1).

We could not simulate long-term effects of heavy metals on microbial biomass and microbial processes by addition of similar amounts of metal salts to soil. The biomass which develops under conditions of chronic metal toxicity is apparently quite dissimilar to its response to heavy metals than one which is subjected to an acute exposure. Overall, our work supports and strengthens the general conclusion in the review by Giller et al. (1998) that responses produced after the addition of metal salts to soils 'are unpredictable and may bear little relation to results seen in the field'.

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