Biologically mediated processes in the root environment

This session examined the interaction between the root and soil processes in the rhizosphere. It considered the consequences and opportunities to exploit our understanding of these interactions in three key areas: plant-mediated processes to acquire nitrogen, sulphur and phosphorus; the influence of carbon inputs in the rhizosphere on microbial diversity, dynamics and function; and computer simulation of soil–plant–microbe interactions.

Biodiversity in the root environment

Fertile agricultural soils contain at least 10^8 bacteria g^{-1} soil, comprising numerous different taxonomic groups, as well as many fungi and other eukaryotic micro-organisms. The contribution of the majority of these species to plant growth and soil processes is unknown. The functional and genetic diversity of soil microbial populations are not necessarily the same, and their relative importance in maintaining sustainable soil systems is unclear. This session examined the role of micro-organism population diversity in soil ecosystems, the significance of changes in population structure, and the application of different techniques to further our understanding of this complex subject.

Exploitation of rhizosphere interactions

This session considered how our understanding of interactions in the rhizosphere can be exploited in the maintenance of sustainable plant production systems. Topics included an overview of the current state of biological control, particularly the use of applied biocontrol agents, modelling the spread of micro-organisms in soil and on roots and its relevance to disease management, bioremediation of polluted soil and restoration of soil fertility, and the commercial exploitation of microbial inoculants.

The guest editors for this Special Issue were Professor David Powlson, Drs Geoff Bateman, Keith Davies, John Gaunt and Penny Hirsch of IACR-Rothamsted and Dr Peter Barlow of IACR-Long Ashton.

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CADMIUM AND ZINC TOXICITY TO SOIL MICROBIAL BIOMASS AND ACTIVITY

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The effects of sewage sludge selectively enriched with zinc at the maximum permitted level and cadmium at concentration four times the current EU limits on microbial biomass and activity were tested using soils under different management in a laboratory incubation experiment. The microbial biomass and activity and the heavy metal mobility were measured over a period of six months. The results showed that neither zinc at the maximum permitted level nor the cadmium at concentrations as high as four times the current EU limits may have major adverse effects on the microbial biomass and respiration of soils.

1. INTRODUCTION

In 1986, the European Union (EU) produced a directive containing mandatory limits to prevent the accumulation of heavy metals in agricultural soils above safe limits. Particularly, the limit for cadmium is kept very low (Table 1). It is considered to be very toxic because it is not required for any known biological process, it is very mobile due to its low affinity for the soil colloids [2] and might be easily transferred to crops [16].

Table 1
Maximum permitted concentrations of some heavy metals in agricultural soils, according to the current EU regulation [9]

Total concentration (mg kg^{-1} soil)	
element	*range*
Cadmium	1-3
Chromium	100-150
Copper	50-140
Lead	50-300
Nickel	30-75
Zinc	150-300

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Over the past decade or so, there has been increasing evidence that heavy metals at or around the current EU permitted limits may have adverse effects upon the soil microbial ecosystem, while crop growth may apparently be unaltered. Thus, soils from the Woburn Garden Market Experiments at Rothamsted (UK), which last received metal-contaminated sewage sludge more than thirty years ago, now contain only about half as much total microbial biomass (measured as biomass C or ATP) as similar plots that received farmyard manure or inorganic fertilizers (NPK) over the same period. The microbial populations of the high metal plots still hold a higher specific respiration rate [3,4] which, according to the theoretical biology, is an index of stress [18]. Brookes et al [5] also reported a strong decrease of microbial acetylene reduction in the same soils indicating a decrease of the nitrogenase activity; McGrath et al [15] showed that in sludge-treated soils the N_2 fixation activity was ineffective though the nodulation of the clover roots occurred.

Since the 1960's, all plots have received NPK only and now the maximum metal concentrations in the previously sludged plots are around the maximum current EU limits, except for cadmium, which may be more than three times the limit in some plots [3]. Heavy metals are present in the human-derived sewage sludge because household and industrial wastes are currently collected by the same routes, and once in soils, they persist and are concentrated.

We set up an experiment to determine whether the microbiological properties of the high metal plots depend on the high cadmium concentration. We also investigated the occurrence of synergistic adverse effects of cadmium and zinc when their concentrations exceed and meet the current EU limits, respectively.

2. MATERIALS AND METHODS

The experiment was carried out using Rothamsted soils (Aquic Paleudalf, USDA 1992), 27% clay, $pH_{(H2O)}$ 5.2, under arable and grassland management. Soils were both amended with cadmium- or zinc-enriched sludge (12 and 300 mg kg^{-1} of metals, respectively) equivalent to a sludge application rate of 40 t ha^{-1}. The metal enrichment of the sludge was made as follows: Moist sludge cake was homogenized by hand and portions equivalent to 100 g of dry matter were placed in plastic containers and allowed to dry in fan-equipped cupboards. When 20% of water was lost, the sludge were treated with the same amount of water lost or cadmium and zinc (as sulphates) solutions. Single and bimetallic solutions were used to reach the desired metal concentrations in soils. The treated sludges were then mixed by hand and allowed to dry for two weeks until a constant weight was reached. To achieve better incorporation in the soils and rapid mineralization, all the sludges were dried and milled to <1 mm after drying. Unamended soils and soils amended with unspiked sludge served as controls. The elemental composition of the sewage sludge used is in Table 2.

The soils (120 g o.d. equivalent) were incubated in 1.1 L dark glass bottles with separate vials of water and 1 M NaOH (25 ml each). Cumulative CO_2 evolved was measured over 24 weeks by automated titration with standardized 1M HCl, according to Tinsley et al [20]. The vials of NaOH were replaced at 7 d intervals. Soil microbial biomass C was measured by fumigation extraction [21], and the cadmium and zinc mobility were determined using 1 M NH_4NO_3 [19].

Table 2.
Elemental analysis of the Banbury sewage sludge used in the experiment. The total elemental content was determined using aqua regia digestion of the dry powdered sludge, according to McGrath and Cunliffe [13] and are expressed as mg Kg^{-1} of sludge. The organic C and the total N were reported as percent of dry matter.

Organic C	Total N	Cd	Cr	Cu	Pb	Zn
48	20	0.5	36.5	46.7	73.5	17.0

The LSD (Tukey's test, $P < 0.05$) was calculated for the biomass C values to assess the statistical significance of difference of the means (n = 3).

3. RESULTS AND DISCUSSION

Neither cadmium at four times the current EU limit nor zinc at the maximum permitted level decreased the total soils microbial biomass (Figure 1) and respiration (Figure 2) of the arable and grassland soils over the period of 6 months.

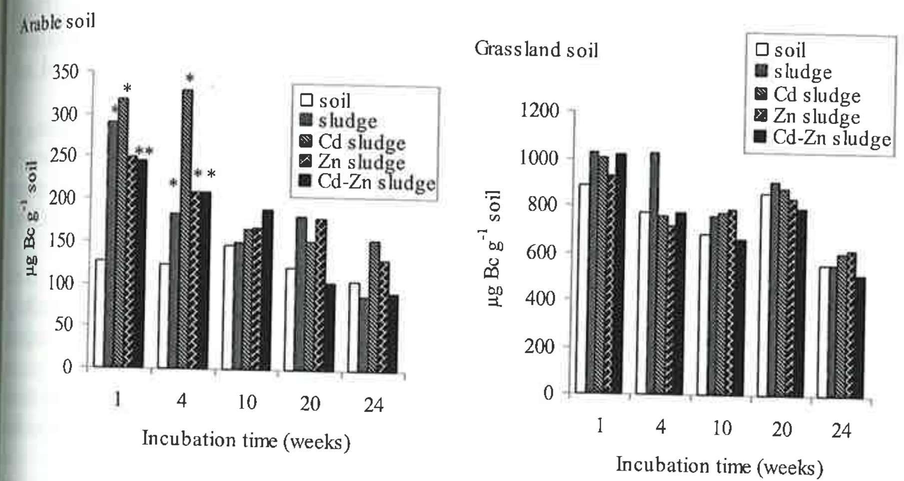

Figure 1. Microbial biomass C of the Rothamsted arable and grassland soils. * = $P<0.05$

The sludge incorporation induced a significant microbial biomass growth only in the arable soil; the increase was not significant in the grassland, where the biomass content was already very high (Figure 1). Then the biomass declined, in all soils, to values not significantly different from those of the respective controls. The trends displayed by the soil respiration were consistent with those of the biomass for all soils and treatments.

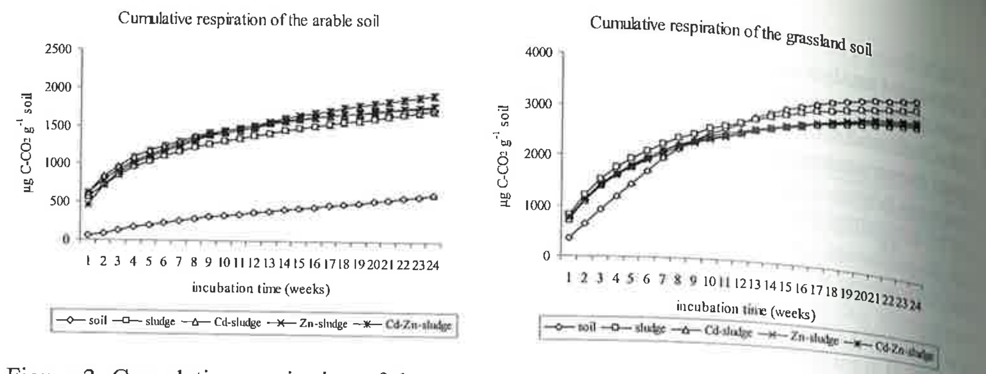

Figure 2. Cumulative respiration of the Rothamsted arable and grassland soils. The error bar is the standard error (n = 3) of the mean.

The cadmium and zinc mobility increased with time, and their mobility was different in the two soils, being generally more mobile in the arable soil (Figure 3). This was probably due to the lower organic matter content of the arable soil because it is known that soil organic matter can reduce heavy metal mobility by binding them [1]. After 24 weeks, about 10% of the total cadmium and 12% of the total zinc were NH_4NO_3-soluble in the arable soil while 3% of the total cadmium and 6% of the total zinc were NH_4NO_3-soluble in the grassland soil, approaching a plateau concentration in both the soils.

Moreover, no particular differences in heavy metal mobility were observed depending on the single- or bimetallic treatment, although it is well known that heavy metals compete for the adsorption sites when added to soils simultaneously [8] before they undergo to adsorption. Possibly the heavy metal releasing over time was due to the sludge organic matrix decomposition.

Anyway, we would exclude later effects of the metals added because an equilibrium (i.e. a minimum metal mobility) had surely been reached after about six months since the sludge addition unless dramatic changes such as rapid acidification or addition of chelating agents occurs.

The results presented suggest that cadmium, even at concentration as high as four times the current EU limit might be not toxic to soil microganisms or at least no more toxic than zinc at or a little above the current EU limits.

Chaudri et al. [7], using soils from other long-term experiments located at Braunschweig, Germany, demonstrated that the zinc concentrations of those soils was responsible for the decline in rhizobia numbers, enumerated by the MPN technique, although zinc total concentration did not exceed the maximum EU limit.

In our opinion the total content of heavy metals and the plant uptake cannot be the only criteria to be taken into account for establishing heavy metals limits in soils. For example Dahlin et al. [10], by comparing soils with different total heavy metals contents, reported adverse effects in the less polluted soils even if their total heavy metal concentrations were below the current EU limits. McGrath et al. [17] demonstrated that heavy metal toxicity, as measured by using biosensors, is better correlated with the free ion concentration rather than the total soil concentration.

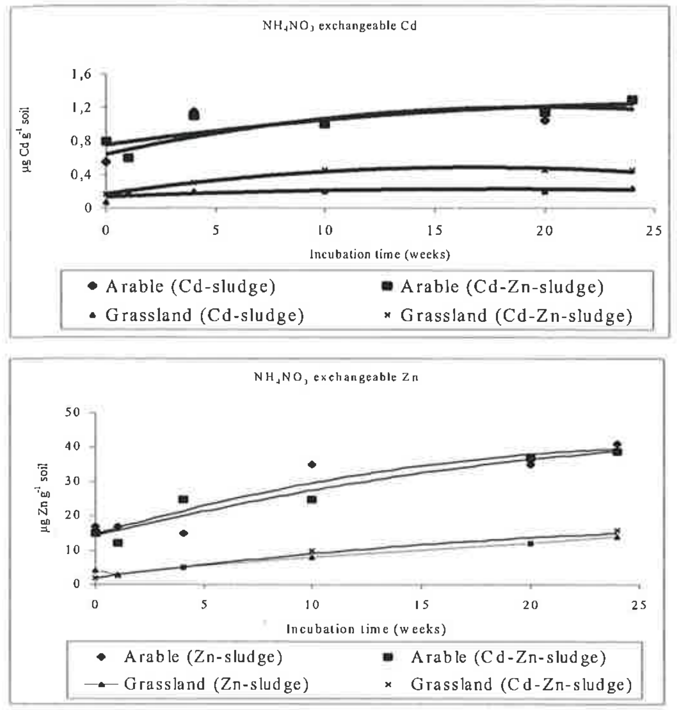

Figure 3. Cadmium and zinc mobility in the Rothamsted soils amended with Cd-, Zn-, and Cd+Zn-enriched sludge. The error bar is the standard error of the mean (n = 3).

It has been also reported that heavy metal toxicity to microorganisms might be enhanced by the synergistic action of different pollutants. It seems that microorganisms become more sensitive to some chemicals when exposed to other analogous compounds [11]. Chander and Brookes [6] described such an additive effects of heavy metals in soils from the Gleadthorpe experiments (UK). Soil amendments with single-metal (Cu, Ni, or Zn) polluted sludge had only small effects on the microbial biomass, but in plots receiving bimetallic sludge (Ni+Zn or Cu+Zn) the microbial biomass content was reduced by half compared with the reference soil.

Indeed, polluted soils usually contain more than a single heavy metal. However, such an additive effect of cadmium and zinc was not observed here and more work is needed for clarifying the mechanisms behind this phenomenon.

4. CONCLUSIONS

The use of human-derived sewage sludge as a fertilizer is a common agricultural practice and in some European countries 50% of this material produced is spread on farmlands. The sludge production is continuously increasing and, because sea disposal in the EU is illegal since 1998 and the other feasible disposal practices (e.g. incineration) are expensive and release gaseous and solid pollutants, an increase in the amounts of sludge used as fertilizer is likely.

The results presented suggest that cadmium eventually present in this material may not cause decrease of microbial biomass or alter its activity even if its content exceeds the current EU limit. Conversely, there is evidence that other heavy metals, such as copper, nickel, and zinc can cause such effects at metal concentrations at or a little above the current EU limits.

However, a better understanding of the synergistic interactions between heavy metals will enable us to use the responses of the soil microbial biomass as a reliable index of the environmental impact of the incorporation into soils of heavy metal-contaminated wastes *sensu* Brookes [3].

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