EFFECTS OF SEWAGE SLUDGE APPLICATIONS TO AGRICULTURAL SOILS ON SOIL MICROBIAL ACTIVITY AND THE IMPLICATIONS FOR AGRICULTURAL PRODUCTIVITY AND LONG-TERM SOIL FERTILITY: Phase III

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SUMMARY

Background

This report summarises research progress during Phase III (2002-2006) of a long-term series of field experiments evaluating "the effects of sewage sludge applications to agricultural soils on soil microbial activity and the implications for agricultural productivity and long-term soil fertility" at nine sites in Britain. The work involved investigating the effects of established soil heavy metal concentrations (*i.e.* Zn, Cu and Cd) on soil microbial activity and fertility, following creation of the metal dose-response treatments in Phase I (1994-1998), and builds upon soil microbial and crop quality measurements made during Phase II of the project (in 1999 and 2001). The project has focused on Zn, Cu and Cd metal additions as these were assessed as the metals most likely to be responsible for soil microbial property impacts measured in previous work. The sludge cake metal dose-response treatments were established using selected cakes that contained elevated metal concentrations (*i.e. c.*6,000 mg/kg Zn; *c.*5,000 mg/kg Cu; *c.*44 mg/kg Cd) and were applied over a 4-year period, which would have provided a high-level of 'stress' to the soil microbial community. The sludge cake loading rates and metal concentrations were *not* intended to be typical of those used in operational recycling practices to agricultural land, and were applied to achieve a range of soil metal concentrations from background levels to above statutory soil metal limit values. In operational practice it would take more than 100 years of annual applications to reach statutory soil metal limit values. However, *long-term treatments* which are more representative of operational practice have been embedded in to the experimental design.

At three sites in England, the effects of Zn, Cu and Cd in metal-rich sludge cakes were compared with those in metal-amended liquid sludge, to provide a contrast in the form of metals applied and organic matter loading rates. The metal-amended liquid sludge treatments were expected to represent 'worst-case' treatments in terms of likely metal availabilities, akin to a long-term situation where organic matter levels had declined and stabilised.

Three key soil microbial properties were measured, viz.: (i) soil respiration rate, a measurement of soil microbial activity (ii) soil microbial biomass, a measurement of the total soil microbial population and (iii) clover rhizobia numbers, a specific species responsible for nitrogen fixation.

The project was undertaken by ADAS, Rothamsted Research and WRc in England and Wales, and by the Macaulay Institute and SAC in Scotland. The project is jointly funded by the Department for Environment, Food and Rural Affairs (Defra), the Environment Agency (EA), the Welsh Assembly Government (WAG), UK Water Industry Research Limited (UKWIR) and the Scottish Executive Environment and Rural Affairs Department (SEERAD).

Policy objective

- To determine the effects of established soil heavy metal concentrations on soil microbial activity and the implications for agricultural productivity and long-term soil fertility.

Scientific objectives

- To determine the effect of zinc, copper and cadmium additions in sewage sludge on soil microbial processes - biomass carbon, soil respiration, *Rhizobium leguminosarum* biovar *trifolii* (numbers of clover rhizobia) at the nine experimental sites.

- To examine the effect of zinc, copper and cadmium additions on selected soil chemical properties and speciation of metals present in soil solution.

- To evaluate the effect of metal additions in sewage sludge on metal bioavailability to crops, agricultural productivity and long-term soil fertility.

- To continue the annual addition of metal-rich sludge cakes on the long-term treatments at maximum permissible annual loading rates.

- To compare the effects of zinc, copper and cadmium additions in metal-amended liquid sludges, with additions via contaminated sludge cakes, on metal bioavailability for micro-organisms and crops, agricultural productivity and long-term soil fertility (3 English sites only).

Project synthesis

During the four years (2002-2006) of this project, significant ($P < 0.05$) responses in soil microbial properties (i.e. rhizobia numbers and microbial biomass size) and agricultural crop quality (i.e. grain Cd concentrations) were measured following the application of metal-rich sludge cakes and metal-amended liquid sludges during Phase I (1994-1997).

The soil samples taken in spring 2003 and 2005 at all nine sites in Britain (and additionally in 1999 and 2001 during Phase II of the project) showed significant ($P < 0.05$) responses in rhizobia numbers on the Zn sludge cake treatments, and in soil microbial biomass size on the Zn and Cu sludge cake treatments.

Further soil sampling and measurements during future years of this long-term study will help to establish whether the effects measured so far are permanent and consistent over time.

Conclusions

Sludge cake experiments

Soil microbial properties

- Significant ($P < 0.05$) responses in soil microbial properties were measured following the application during Phase I (1994-1997) of metal-rich sludge cakes. The soil samples taken in 2003 and 2005

from all the treatment plots at the nine sites showed significant ($P < 0.05$) responses in soil microbial properties, which were primarily on the Zn and Cu dose-response treatments, viz.:

- Soil respiration rates (an indicator of soil microbial activity) measured in soil samples taken from all the treatment plots in the spring of 2003 and 2005 tended to be *higher* (*i.e.* increased CO_2 evolution) on the treatments receiving low metal sludge cakes compared with the untreated controls, due to the sludge cake organic matter additions which provide additional organic C for microbial respiration.

  On the metal dose-response treatments, there were significant ($P < 0.05$) inverse (*i.e.* soil respiration rate decreased with increasing metal concentration) relationships between soil respiration rates and total topsoil Zn concentrations at Hartwood in both 2003 and 2005, and for Cd at Woburn and Hartwood in 2003. For Cu, there were significant ($P < 0.05$) positive (*i.e.* soil respiration rate increased with increasing metal concentration) relationships between soil respiration rates and total topsoil Cu concentrations at Watlington and Auchincruive in both 2003 and 2005, and Rosemaund in 2003.

  Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) it is difficult to see any clear and consistent effect of metal additions on soil respiration rates (i.e. microbial activity).

- Soil microbial biomass carbon concentrations (a measurement of the size of the soil microbial population) in samples taken from all the treatment plots in 2003 and 2005 were generally higher on the treatments receiving low metal sludge cakes compared with the untreated controls, again due to the sludge cake organic matter additions which provide additional organic C for microbial assimilation.

  On the metal dose-response treatments, there were significant ($P < 0.05$) inverse relationships between soil biomass C concentrations and total topsoil Zn concentrations at four sites in both 2003 and 2005 (Woburn, Watlington, Rosemaund and Bridgets), at Hartwood and Auchincruive in 2003, and Shirburn in 2005. For Cu, there were significant relationships at three sites in both 2003 and 2005 (Woburn, Watlington and Hartwood), and at Gleadthorpe and Rosemaund in 2005. For Cd, there was no consistent effect on biomass concentrations.

  Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) at all the sites where there was a relationship between soil biomass C and total topsoil Zn or Cu, the relationship was inverse i.e. biomass levels (the size of the soil microbial population) decreased with increasing metal concentrations. These significant responses were on average c.20% lower at the UK statutory soil metal limit values than the low metal sludge cake control.

- There were significant relationships ($P < 0.001$) between clover rhizobia numbers (a specific species responsible for nitrogen fixation)

and total topsoil metal concentrations. For Zn, on the metal dose-response treatments, there were inverse relationships ($P < 0.05$) between rhizobia most probable numbers (MPNs) and Zn at six sites in both 2003 and 2005 (Gleadthorpe, Woburn, Watlington, Rosemaund, Bridgets and Hartwood), and at Pwllpeiran and Auchincruive in 2005. For Cu, there were significant relationships at Auchincruive in 2003 and at Pwllpeiran, Rosemaund, Bridgets and Hartwood in 2005. For Cd, there were significant relationships at Rosemaund and Shirburn in 2003, and at Gleadthorpe, Woburn, Pwllpeiran and Hartwood in 2005 (although three of these relationships were also significant for Zn). At all the sites (except for Cu at Shirburn in 2005) where a statistically significant relationship between rhizobia MPNs and total topsoil metals was found, rhizobia MPNs *decreased* with increasing topsoil metal concentrations.

Soil rhizobia MPNs decreased with increasing total topsoil Zn concentrations at three of the sludge cake sites in all four measurement years (1999, 2001, 2003 and 2005), and at eight of the sites in 2005. Moreover, at the Hartwood site rhizobia MPN levels were not detectable above 301 mg/kg in 2003 and above 188 mg/kg Zn in 2005. Additionally, on the Zn rate 3 treatments (i.e. 1994 – 2005) there was a trend of decreasing rhizobia MPNs at eight of the sludge cake sites compared with the low metal sludge cake control treatment. The body of evidence from the results to date indicates that Zn, rather than Cd, is likely to have been responsible for decreases in rhizobia population sizes measured in previous studies.

Soil chemical properties

- Ammonium nitrate (NH_4NO_3) extractable topsoil Zn concentrations were consistently higher at Hartwood (7% of total) than at the other non-calcareous sites, and lowest at the calcareous Shirburn site (0.1% of total). At Hartwood, this was partly due to higher total topsoil Zn concentrations, but also the greater extractability of Zn at this site, which was probably related to the lower soil pH (5.7-6.0) than the other sites (the non-calcareous sites in England and Wales are limed to a target pH of 6.5). The low extractable Zn concentrations at Shirburn were principally related to the high soil pH value (*c.*8.4) and associated free calcium carbonate (lime) content of the soil. Extractable topsoil Cu concentrations were consistently higher at Watlington (1.5% of total) than at the other sites, and lowest at Hartwood (0.25% of total). Extractable Cd concentrations were consistently higher at Auchincruive and Rosemaund (*c.*7.9% of total) than at the other sites, and lowest at Bridgets and Shirburn (*c.*0.55% of total).

  Across the nine sites there were strong relationships ($P < 0.01$) between total topsoil Zn, Cu and Cd concentrations and corresponding topsoil NH_4NO_3 extractable metal concentrations (r^2 = 44-95% for Zn, 43-97% for Cu and 14-76% for Cd); where extractable Zn and Cd concentrations were above analytical limits of detection.

Crop yields and metal concentrations

- First cut grass yields (2002 and 2004) were similar or higher on the sludge cake treatments compared with the untreated controls. There were significant ($P < 0.05$) *positive* relationship between ryegrass yields and total topsoil Zn and Cu concentrations at Auchincruive in 2002, and at Bridgets (total Zn) and Hartwood (total Cu) on 2004.

- Grain yields (2003 and 2005) at the 6 sites in England were similar or higher on the sludge cake treatments compared with the untreated controls. There was a significant ($P < 0.05$) *positive* relationship between grain yields and total topsoil Cu at Woburn in 2003. However, there were no significant relationships ($P > 0.05$) between total topsoil metal concentrations and grain yields on any of the other treatments at the six sites in 2003 and 2005.

  There were moderate to strong relationships ($P < 0.001$) between grain Zn (r^2 = 59-84%), Cu (r^2 = 32-79%) and Cd (r^2 = 45-94%) concentrations, and corresponding total topsoil metal concentrations in 2003. However, in 2005, relationships between grain metals and total topsoil metals were not as strong.

Metal-amended liquid sludge experiments

Soil microbial properties

- Significant ($P < 0.05$) responses in soil microbial properties were measured following the application of metal-amended liquid sludges during Phase I (1995-1997). The soil samples taken in 2003 and 2005 from all the treatment plots at the three sites showed significant ($P < 0.05$) responses in soil microbial properties, which were primarily on the Zn and Cu dose-response treatments, viz.:

- Soil respiration rates measured in samples taken from all the treatment plots at the three sites in spring 2003 and 2005 were generally *higher* on the treatments receiving low metal-amended liquid sludges compared with untreated controls, as a result of the sludge organic matter additions made during Phase I which provided organic C for microbial respiration.

  On the metal dose-response treatments, there were significant ($P < 0.05$) *positive* relationships between soil respiration rates and total topsoil Zn concentrations at Rosemaund in 2003, for Cu at Woburn in both 2003 and 2005, and Cd at Watlington in 2003.

  Over the course of the four measurements to date (1999, 2001, 2003 and 2005) where there were relationships between soil respiration rates and total topsoil metal concentrations, these were all positive i.e. soil respiration rates increased with increasing topsoil metal concentrations. Although, overall the sites it was difficult to see any clear and consistent effects of the metal additions on soil respiration rates.

- Soil microbial biomass C concentrations measured on samples from the three sites were generally *higher* on the treatments receiving low

metal liquid sludges compared with the untreated controls. These increases can be related to the sludge organic matter additions made during Phase I which provided organic C for microbial assimilation.

On the metal dose-response treatments, there were significant ($P < 0.05$) *inverse* relationships between soil biomass C concentrations and total topsoil Zn concentrations at Woburn in 2005, and for Cu at Watlington in both 2003 and 2005 and at Woburn in 2005.

Over the course of the four measurements to date (1999, 2001, 2003 and 2005) where there were significant relationships between total soil Zn and Cu concentrations these were always inverse i.e. biomass C concentrations decreased with increasing metal concentrations.

- On the metal dose-response treatments, there was only one significant ($P < 0.05$) inverse relationship between rhizobia MPNs and total topsoil Zn at Rosemaund in 2003, but no relationships ($P > 0.05$) for either Cu or Cd.

  Over the course of the four measurements to date (1999, 2001, 2003 and 2005) there was only one significant ($P < 0.05$) inverse relationship between rhizobia MPNs and total topsoil Zn (Rosemaund in 2003), and no relationships for Cu or Cd.

Soil chemical properties

- At all three sites, there were strong relationships between topsoil total Zn, Cu and Cd concentrations (r^2 = 73-92% for Zn, 74-98% for Cu and 31-93% for Cd) and corresponding topsoil NH_4NO_3 extractable metal concentrations.

Crop yields and metal concentrations

- First cut grass yields (2002 and 2004) and grain yields (2003 and 2005) at the three sites were similar or higher on the metal-amended liquid sludge treatments compared with the untreated controls.

- On the metal dose-response treatments, there were no relationships ($P > 0.05$) between grass yields and total topsoil metal concentrations. However, there were significant ($P < 0.05$) inverse relationships between grain yields and total topsoil Zn and Cu at Watlington in 2003, and a significant positive relationship between grain yield and total topsoil Cu at Woburn in 2003.

  There were strong positive relationships ($P < 0.05$) between grain Zn (r^2 = 62-93%) and Cd (r^2 = 87-98%) concentrations and corresponding total topsoil metal concentrations at the three sites. At Woburn in both 2003 and 2005, there were strong relationships ($P < 0.001$) between grain Cu and total topsoil Cu concentrations. However, at Rosemaund in 2003 the relationship was weak (r^2 = 26%) and for the other site years there were no relationships.

Comparison of sludge cake and liquid sludge sites

- Soil respiration rate responses on the sludge cake and metal-amended liquid sludge experiments showed *limited agreement* (only on the Zn treatments at Rosemaund in 2003 were there similar

responses). *To date in both experiments, it is difficult to see any clear and consistent respiration rate responses to metal additions*.

- Soil biomass C in the sludge cake and metal-amended liquid sludge experiments showed good agreement for Cu and Cd. For Cu, the size and number of responses were similar in both experiments, and for Cd there were no consistent responses in either experiment. However, there was little agreement for Zn, with eight responses (out of 12) on the sludge cake experiments and only two on the 'paired' Zn-amended liquid sludge experiment.

  For Cu, there appear to be consistent effects on the microbial biomass in both the sludge cake and metal-amended liquid sludge experiments. This indicates, in the case of Cu, that bioavailability to the microbial biomass is not being influenced by the added organic matter. Indeed, the soil extractability of Cu in the sludge cake experiments was higher than the Cu-amended liquid sludge experiments (as measured by NH_4NO_3 extraction). Hence, the form of Cu application appears to be secondary to total topsoil Cu concentrations *per se*. This could have major implications for the application of a wide range of materials to agricultural land *e.g.* copper sulphate fungicides, farm manures, green waste compost, paper mill crumble *etc.*

- Rhizobia MPN responses on the sludge cake and metal-amended liquid sludge experiments showed little agreement. For Zn, there were nine inverse relationships ($P < 0.05$) between rhizobia MPNs and total topsoil Zn in the sludge cake experiments and only one (out of 12) in the 'paired' liquid sludge experiments. For Cu, there were four inverse relationships ($P < 0.05$) in the sludge cake experiments and none in the 'paired' liquid sludge experiments. And for Cd, there were two inverse relationships ($P < 0.05$) in the sludge cake experiments and none in the 'paired' liquid sludge experiments.

  Conventional wisdom would suggest that metals added in metal-amended liquid sludges would be more detrimental to micro-organisms than sludge cake amendments, because the larger amount of added organic matter in the sludge cake would ameliorate metal toxicity to micro-organisms by binding metals, thus reducing bioavailability. However in this study, Zn added to soil in the metal-rich sludge cake (6,000 mg Zn/kg dry solids) had a much more adverse effect on rhizobia MPNs and the soil microbial biomass, compared with little or no measurable effects of adding Zn to soil in the metal-amended liquid sludge.

  We propose that Zn toxicity to microbial cells seen in the sludge cake experiments was due to direct toxicity through exposure to high concentrations of Zn in and around the sludge cake particles, and poisoning at a cellular level when mineralising this organic matter for cell maintenance and reproduction, leading to cell disruption. The results to date in this study discount the 'sludge organic matter protection' hypothesis proposed by some workers, but support the conclusions of other workers that in the early years after sludge

application it is the properties of the sludge that are most important rather than the bulk soil chemistry (*e.g.* Lake *et al.,*1984; Merrington *et al.,* 2003).

We believe that no detrimental effects were seen from Zn in the metal-amended liquid sludge experiments, because the metals were **not** intimately associated with the added organic matter. Thus, the Zn would be more evenly incorporated throughout the soil and not in association with organic matter 'hot spots' as in the sludge cake experiments. We hypothesise that in the liquid sludge experiments, rhizobia are mostly associated with uncontaminated organic matter from root exudates, dead roots and the small amounts of straw and grass residues incorporated in to the soil during post harvest cultivations.

- There were no clear differences in topsoil NH_4NO_3 extractable metal concentrations between the sludge cake and metal-amended liquid sludge experiments, with soil pH probably the most important factor controlling Zn and Cd extractability between sites.
- Combining grain Cd data from the sludge cake and metal-amended liquid sludge experiments (and associated inorganic metal salt studies; Defra Project SP0133) indicated that the current soil total Cd limit of 3 mg/kg was *not sufficiently protective* to produce grain below the EU maximum permissible grain cadmium concentration of 0.235 mg/kg dry matter, unless the soil pH was maintained above 6.8.

Policy context

This experimental work provides a sound scientific base for the *continued development* of policies on soil protection where heavy metals in sewage sludge are applied to agricultural land, and will directly inform the debate on setting maximum permissible heavy metal concentrations in soils as part of the revision of the EU Directive on Sludge Use in Agriculture and associated UK legislation.

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1. INTRODUCTION

1.1 Sludge recycling to land

Sewage sludge is a useful source of nitrogen, phosphorus and organic matter, and as a result of some conditioning processes has value as a liming material (MAFF, 1986). However, sewage sludge contains larger concentrations of heavy metals than most soils. Once the metals have been added they accumulate in the topsoil, as they are not easily leached and crop offtakes are small.

As a result of the ban on sewage sludge dispersal at sea, which ceased at the end of 1998, and tighter restrictions on direct discharges of sewage, more sludge is likely to continue to be recycled to agricultural land. A UK sewage sludge survey (Gendebien *et al*., 1999) estimated that in 1996/97, 520,000 tonnes of sewage sludge dry solids per year (tds/yr.) 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 1996/97 was 80,000 hectares, which represented an average annual application rate of 6.5 tds/ha. Only *c*.0.5% of agricultural land received sludge in 1996/97. A further 72,000 tonnes was reused in land reclamation, forestry and as a component of soil and compost products. In 2005, 62% of sewage sludge production was recycled to agricultural land (Water UK, 2006), equivalent to 1 million of tds, which at an average application rate of 6.5 t/ha tds would equate to *c.*150,000 ha of agricultural land receiving sludge.

To comply with guidelines laid down in the Code of Good Agricultural Practice for the Protection of Water (MAFF, 1998) applications of sewage sludge should not exceed 250 kg N/ha per annum. Within Nitrate Vulnerable Zones designated under the EC Nitrate Directive (CEC, 1991), the mandatory field application limit is 250 kg N/ha per annum (Defra, 2002).

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 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 application to grassland. As a result of recommendations from the Independent Scientific Committee Reviewing the Soil Fertility Aspects of Sludge Applications to Agricultural Land (MAFF/DoE, 1993), the total topsoil advisory limit for zinc for all pH's in the range 5 to 7 has been reduced to 200 mg/kg (a reduction from 300 mg/kg at pH 6 - 7 and 250 mg/kg for pH's 5.5-6) and that the total cadmium limit for grassland (0 - 7.5 cm) has been reduced from 5 to 3 mg/kg as a precautionary measure (DoE, 1996).

The maximum advisory (DoE, 1996) total topsoil Zn concentrations where sludge is recycled to agricultural land are 200 mg/kg for soils with pH in the range 5-7, and 300 mg/kg for soils with pH > 7 and where they contain > 5% calcium carbonate. The maximum permissible levels (SI, 1989) are 300 mg/kg for soils in pH range 6.0-7.0, 250 mg/kg for soils in pH range 5.5 < 6.0 and 200 mg/kg for soils in pH range 5.0 <_5.5, and where topsoil pH > 7.0 and it contains > 5% calcium carbonate 450 mg/kg.

The maximum advisory and permissible (DoE, 1996; SI, 1989) total topsoil Cu concentrations where sludge is recycled to agricultural land are 135 mg/kg for soils with pH in the range 6.0-7.0, 100 mg/kg for soils in pH range 5.5 < 6.0 and 80 mg/kg for soils in pH range 5.0 < 5.5, and where topsoil pH > 7.0 and it contains > 5% calcium carbonate 200 mg/kg.

The maximum advisory and permissible (DoE, 1996; SI, 1989) total topsoil Cd concentrations are 3.0 mg/kg for all soils of pH 5.0 and above.

The experimental work reported here aims to provide a sound scientific base for the *continued development* of policies on soil protection where sewage sludge is applied to agricultural land. Also, in helping to identify the pathways and fates of contaminants derived from sewage sludge and their effects on soil (microbial, chemical and physical) properties and agronomic productivity.

1.2 Soil microbial activity

The soil microbial biomass is widely recognised as the 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 and McGrath (1984) showed that the microbial biomass in soil from the classical Woburn Market-garden experiment (sandy loam soil, pH 6.5) which had received aerobically digested lagoon-dried sewage sludge (at rates of 8.2 and 16.4 t/ha per year of organic matter between 1942 and 1961) was half that in soil which had received farmyard manure - FYM (at rates of 5.2 and 10.4 t/ha per year of organic matter between 1942 and 1967). The metal contaminated soil had a much higher respiration rate per unit weight of biomass, although the adenylate energy charge of the biomass (a measure of potential metabolic activity) was high in both soils (Brookes and McGrath, 1987). At Woburn, sludge applications had increased soil metal concentrations of zinc and copper up to current maximum permissible limits for soils in the pH range 6-7 (SI, 1989), and cadmium concentrations 2 to 4 times the limit (McGrath, 1994).

Biomass measurements on the ADAS experiments at Luddington and Lee Valley showed that biomass carbon as a proportion of total soil carbon was reduced, where zinc and copper enriched sludge had been added. Measurements on the ADAS Gleadthorpe experiment (started in 1982) showed that the effects of copper and zinc were additive in reducing biomass (Chander and Brookes, 1991). Stark and Lee (1988) also showed that biomass generally decreased with increasing soil metal

concentrations. The differences in responses between the sites can most likely be ascribed to differences in soil physio-chemical properties.

McGrath *et al*. (1987) showed that on the sludge treated soil at Woburn nodulation of clover occurred, but the rhizobia were ineffective in fixing atmospheric nitrogen. However, adding inorganic fertiliser nitrogen to the sludge treated soil restored clover yields to that of the uncontaminated soil (McGrath *et al*., 1988). As isolated bacteria from the ineffective nodules were still unable to fix nitrogen in the absence of toxic heavy metals, it was concluded that their ineffectiveness in sludge treated soil was not directly due to metal toxicity (Giller *et al*., 1989) but due to the survival of only one genotype of the *Rhizobium* population (Hirsch *et al.*, 1993). This ineffective strain isolated from the sludge-treated plots was shown to have greater tolerance to metals than an effective clover rhizobia strain isolated from uncontaminated farm yard manure treated soil at Woburn (Chaudri *et al*., 1992). The ineffectiveness of rhizobia in fixing atmospheric nitrogen is highly significant from an agricultural viewpoint, if future 'low input' systems wish to more fully exploit the nitrogen fixing properties of clover, peas, beans and lupins.

1.3 Existing knowledge

Based on previous knowledge and available experimental sites in Britain, it was not possible to identify unequivocally which of the range of heavy metals present in sewage sludge was responsible for the toxic effects noted on crops and soil microbial processes. It was unlikely to be Cr, Pb and Hg as these are largely insoluble in sludge treated soil of near neutral to neutral pH, and hence are less likely to be bioavailable (McGrath, 1987). Zinc, particularly, and copper were implicated in Rothamsted and ADAS work, and results from Braunschweig implicated zinc (Chaudri *et al.*, 1993; McGrath *et al*, 1995). However, Smith (1991) who screened historically sludge treated soils, considered that cadmium could be the important element 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 *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 micro-organisms".

This work addresses Government's policy objectives of providing a sound scientific base for the *continued development* of policies on soil protection where sewage sludge is applied to agricultural land, by

evaluating the effects of heavy metal additions in sewage sludge on soil properties (microbial, chemical and physical), agricultural productivity and long-term soil fertility. The findings of the project will directly inform the debate on setting maximum permissible heavy metal concentrations in soils as part of the revision of the EU Directive on Sludge Use in Agriculture and associated UK legislation.

2. OBJECTIVES

2.1 Policy objective

- To determine the effects of established soil heavy metal concentrations on soil microbial activity and the implications for agricultural productivity and long-term soil fertility.

2.2 Scientific objectives

- To determine the effect of zinc, copper and cadmium additions in sewage sludge on soil microbial processes - biomass carbon, soil respiration, *Rhizobium leguminosarum* biovar *trifolii* (clover rhizobia most probable numbers) at the nine experimental sites.

- To examine the effect of zinc, copper and cadmium additions on selected soil chemical properties and speciation of metals present in soil solution.

- To evaluate the effect of metal additions in sewage sludge on metal bioavailability to crops, agricultural productivity and long-term soil fertility.

- To continue the annual addition of metal-rich sludge cakes on the long-term treatments at maximum permissible annual loading rates.

- To compare the effects of zinc, copper and cadmium additions in metal-amended liquid sludges, with additions via contaminated sludge cakes, on metal bioavailability for micro-organisms and crops, agricultural productivity and long-term soil fertility (3 English sites only).

3. METHODOLOGY

3.1 England, Wales and Scotland - Sludge cake experiments

3.1.1 Sites

Metal dose-response treatments were established (Phase I: 1994-1997) at seven sites in England and Wales and two sites in Scotland, with topsoil textures ranging from sands to medium loams, clay contents from 7 to 30% and initial organic carbon (C) contents from 1.2 to 4.7 % (see Appendix A for locations). Organic C contents at the end of Phase I were unchanged on the untreated controls and 'long-term' build-up treatments, and were between 0.9 to 2.0 % higher on the 'moderate rate' build-up treatments (see Section 3.1.2 for treatment details). Initial topsoil zinc (Zn), copper (Cu) and cadmium (Cd) concentrations were similar to or below 'typical' levels for uncontaminated soils in Britain, except at Pwllpeiran where the total soil Zn concentration was 140 mg/kg (typical background soil level 80 mg/kg) and at Bridgets where the total soil Cd concentration was 0.95 mg/kg (typical soil level 0.50 mg/kg).

The range in Zn, Cu and Cd concentrations achieved on the moderate rate build-up treatments at each site at the end of Phase I (1997) is shown in Table 1. A full list of treatments and target total soil metal concentrations is shown in Tables 2 and 3. Soil pH at the non calcareous sites in England and Wales (sites 1 – 6) were managed to maintain a target level of *c.*6.5, and at Shirburn (site 9) *c.*8.0 due to the presence of free calcium carbonate. In Scotland, soil pH was managed to achieve a target value of *c.*5.8 at Hartwood and *c.*6.0 at Auchincruive.

3.1.2 Treatments and design

The treatments (Tables 2 and 3) were replicated in three fully randomised blocks (69 plots at sites 1 to 8 and 84 plots at Shirburn). Individual plots (6 m x 8 m = 48 m^2) were bounded by permanent grass strips to prevent soil movement during cultivations. All cultivations, drilling and harvesting were mechanised using small plot equipment.

- Treatments 4-7 (6-10 at Shirburn) were intended to provide Zn treatments in the range *c.*50 mg Zn/kg (140 mg/kg for Pwllpeiran) up to 450 mg Zn/kg (present upper concentration limit on non-calcareous soils is 300 mg/kg) at sites 1 to 8, and 600 mg Zn/kg at the calcareous Shirburn site.
- Treatments 8-11 (11-15 at Shirburn) were intended to provide Cu treatments in the range *c.*15 mg/kg up to 200 mg Cu/kg (present upper concentration limit on non-calcareous soils is 135 mg/kg) at sites 1 to 8, and 275 mg Cu/kg at the calcareous Shirburn site.
- Treatments 12-15 (16-20 at Shirburn) were intended to provide Cd treatments in the range *c.*0.2 mg/kg (0.95 mg/kg for Bridgets) up to 4 mg Cd/kg (present upper concentration limit on all soils is 3 mg/kg) at sites 1 to 8, and 5 mg Cd/kg at the calcareous Shirburn site.
- Treatments 2A/B and 3 were 'control' treatments amended with uncontaminated digested and raw sludge cakes, respectively, to

give the same organic C inputs to treatments 4 to 15. At Shirburn, treatments 4 and 5 were additional 'control' treatments to give the same organic C inputs to treatments 10, 15 and 20.

- Treatments 16 to 20 (21 to 25 at Shirburn) to investigate the effects of more gradual metal additions with application limits based on the maximum permitted annual rates of metal addition.

Table 1. Topsoil total metal concentration ranges on the moderate rate (rates 1 to 4) build-up treatments achieved at each site following Phase I sludge cake applications (1997)

Site name (county)	Topsoil texture	Total metal concentration ranges: metal dose-response treatments (mg/kg)		
		Zn	Cu	Cd
Typical background level		80	20	0.5
1 Gleadthorpe [GLE] (Notts.)	Sandy loam	134 - 291	69 – 166	1.0 - 3.3
2 Woburn [WOB] (Beds.)	Loamy sand	125 - 224	56 – 188	0.8 - 3.5
3 Watlington [WAT] (Oxon.)	Sandy loam	202 - 539	109 – 309	1.3 - 4.8
4 Pwllpeiran [PWL] (Cered.)[+]	Clay loam	212 - 370	81 – 234	1.1 - 4.2
5 Rosemaund [ROS] (Here.)	Silty clay loam	152 - 421	74 – 219	1.0 - 3.7
6 Bridgets [BRG] (Hants)[++]	Silty clay loam	199 - 453	75 – 207	1.8 - 4.7
7 Hartwood [HAR] (N. Lanarkshire)	Sandy clay loam	171 - 443	74 - 239	1.2 – 4.6
8 Auchincruive [AUC] (Ayrshire)	Sandy clay loam	147 - 342	66 - 206	1.0 – 3.8
9 Shirburn [SHR] (Oxon.)[+++]	Calc. clay loam	185 - 579	76 – 262	1.3 - 5.0

[+] Zn target level 1 for Pwllpeiran was 200 mg/kg, to account for the increased background soil Zn concentration.
[++] Cd target level 1 for Bridgets was 1.5 mg/kg, to account for the increased background soil Cd concentration.
[+++] Includes the high rate (level 5) treatments at Shirburn.

Table 2. Treatments at sludge cake sites 1 to 8

No.	Treatment	Target total soil metal concentration (mg/kg)	Organic matter level
1A	No sludge control	Background	Background
1B	No sludge control	Background	Background
I	***Moderate rate build-up treatments*** +		
2A	Uncontaminated digested sludge cake	Low	Moderate
2B	Uncontaminated digested sludge cake	Low	Moderate
3	Uncontaminated raw sludge cake	Low	Moderate
Zinc rich sludge:		*Zinc*	
4	High Zn sludge rate 1	150 (200 ++)	Moderate
5	High Zn sludge rate 2	250	Moderate
6	High Zn sludge rate 3	350	Moderate
7	High Zn sludge rate 4	450	Moderate
Copper rich sludge:		*Copper*	
8	High Cu sludge rate 1	50	Moderate
9	High Cu sludge rate 2	100	Moderate
10	High Cu sludge rate 3	150	Moderate
11	High Cu sludge rate 4	200	Moderate
Cadmium rich sludge:		*Cadmium*	
12	High Cd sludge rate 1	1 (1.5 +++)	Moderate
13	High Cd sludge rate 2	2	Moderate
14	High Cd sludge rate 3	3	Moderate
15	High Cd sludge rate 4	4	Moderate
II	***Long term build-up treatments***		
16A	Uncontaminated digested sludge cake		Low
16B	Uncontaminated digested sludge cake		Low
17	Uncontaminated raw sludge cake		Low
18	High Zn sludge, 15 kg Zn/ha annually		Low
19	High Cu sludge, 7.5 kg Cu/ha annually		Low
20	High Cd sludge, 0.15 kg Cd/ha annually		Low

+ Sludge applied over a 4 year period (1994-1997).

++ Zn target level 1 for Pwllpeiran, taking account of increased background soil Zn concentration.

+++ Cd target level 1 for Bridgets, taking account of increased background soil Cd concentration.

Table 3. Treatments at sludge cake site 9 (Shirburn)

No.	Treatment	Target total soil metal concentration (mg/kg)	Organic matter level
1A	No sludge control	Background	Background
1B	No sludge control	Background	Background
I	***Moderate rate build-up treatments*** +		
2A	Uncontaminated digested sludge cake	Low	Moderate
2B	Uncontaminated digested sludge cake	Low	Moderate
3	Uncontaminated raw sludge cake	Low	Moderate
4	Uncontaminated digested sludge cake	Low	High
5	Uncontaminated raw sludge cake	Low	High
Zinc rich sludge:		*Zinc*	
6	High Zn sludge rate 1	150	Moderate
7	High Zn sludge rate 2	250	Moderate
8	High Zn sludge rate 3	350	Moderate
9	High Zn sludge rate 4	450	Moderate
10	High Zn sludge rate 5	600	High
Copper rich sludge:		*Copper*	
11	High Cu sludge rate 1	50	Moderate
12	High Cu sludge rate 2	100	Moderate
13	High Cu sludge rate 3	150	Moderate
14	High Cu sludge rate 4	200	Moderate
15	High Cu sludge rate 5	275	High
Cadmium rich sludge:		*Cadmium*	
16	High Cd sludge rate 1	1	Moderate
17	High Cd sludge rate 2	2	Moderate
18	High Cd sludge rate 3	3	Moderate
19	High Cd sludge rate 4	4	Moderate
20	High Cd sludge rate 5	5	High
II	***Long term build-up treatments***		
21A	Uncontaminated digested sludge cake		Low
21B	Uncontaminated digested sludge cake		Low
22	Uncontaminated raw sludge cake		Low
23	High Zn sludge, 15 kg Zn/ha annually		Low
24	High Cu sludge, 7.5 kg Cu/ha annually		Low
25	High Cd sludge, 0.15 kg Cd/ha annually		Low

+ Sludge applied over a 4 year period (1994-1997).

3.1.3 Sludges and application

Sludges were identified during Phase I that were rich in Zn, Cu and Cd, relative to the other metals present. These sludges were used together with uncontaminated digested and raw sludge cakes to establish the individual Zn, Cu and Cd metal dose-response treatments (1994-1997), and continued to be used in Phases II (1998-2002) and III (2002–2006) to add metals to the long-term build-up rate treatments receiving the maximum permissible annual metal loading rates. The sludge cakes were applied 'as received' from the sludge treatment centres (*i.e.* they received no further treatment/reconstitution) and were incorporated into the topsoil (0-25cm depth) using a spading machine. Mean metal concentrations in the sludge cakes added during Phase I are summarised in Table 4.

The selected sludge cakes had metal concentrations that were much larger than those presently used in operational recycling practices to agricultural land in Britain (*i.e. c.*6,000 mg/kg Zn vs. 600 mg/kg Zn; *c.*5,000 mg/kg Cu vs. 300 mg/kg Cu; *c.*44 mg/kg Cd vs. 1.5 mg/kg Cd). Also, annual loading rates during the four year establishment phase were typically more than 30-fold greater than occurs in operational practice (Nicholson *et al*., 2003).

Table 4. Mean metal-rich sludge cake analyses (1994-97)

	Sludge type	Source	Mean metal concentration (mg/kg dry solids)		
			Zinc	**Copper**	**Cadmium**
1.	**Zinc-rich (digested cake)**	Coleshill	**6002**	1423	11.2
2.	**Copper-rich (raw cake)**	Selkirk	553	**5049**	0.69
3.	**Cadmium-rich (digested cake)**	Perry Oaks	1087	544	**43.5**
4.	**'Uncontaminated' digested cake**	Banbury	559	589	1.78
5.	**'Uncontaminated' raw cake**	Wantage	491	453	1.74

To ensure that sufficient quantities of sludge were available to raise soil metal concentrations on the long-term build up treatments to the maximum permissible values (SI, 1989), a *sludge storage facility* was established at ADAS Gleadthorpe. In the region of 100 tonnes fresh weight (fw) of the Zn-rich sludge, *c.*70 tonnes fw of the Cu-rich sludge and *c.*60 tonnes fw of the Cd-rich sludge were progressively delivered to ADAS Gleadthorpe. These sludge quantities will provide sufficient reserves to sustain the annual application treatments up to the maximum permitted concentration limits of 300 mg Zn/kg, 135 mg Cu/kg and 3 mg Cd/kg (*c.*40 years supply). The sludges were air-dried prior to storage under cover in isolated bays, to help 'stabilise' them and facilitate future handling.

Cultivations were undertaken at least 3 times in summer 1998 (after the first grass cut) on each plot, to help ensure that the sludge additions during Phase I of the project were as evenly incorporated as possible throughout the topsoil depth (0-25 cm). The final cultivation pass used the spading machine to help ensure mixing to the full depth of soil cultivation. The annual long-term sludge cake additions were made each summer (July-September) when soil conditions were most

favourable and thoroughly cultivated into the topsoil. Cultivations continued to be undertaken *annually* during Phase III to encourage organic matter breakdown from the sludge cake additions.

3.1.4 Cropping

During Phases II and III the sites have been managed under ley/arable cropping or grassland, depending upon individual soil characteristics and climatic conditions (Table 5). During Phase III the same grass cultivar (an Italian ryegrass mixture from Cotswold Grass Seeds, 'Special Mix A') was sown at all sites in autumn of 2001 and 2003 (additionally in autumn of 2002 and 2004 at Pwllpeiran, Hartwood and Auchincruive), at a seed rate of 40 kg/ha for harvest in summer of 2002 and 2004 (and summer of 2003 and 2005 at Pwllpeiran, Hartwood and Auchincruive). Similarly, the same spring wheat variety (Chablis) was selected for harvest in 2003 and 2005. The wheat variety chosen was 'stiff' strawed to minimise lodging risks. The cropping regime at each site (*i.e.* grassland or arable) was selected to be representative of land management practices in relation to soil type/climatic conditions. The winter wheat variety (Chablis) was selected as it is a milling wheat (Group 1) variety that is used for bread making and hence needs to comply with the EU maximum grain Cd concentration limit (*i.e.* 0.235 mg/kg dry matter) to be traded in the market place.

Table 5. Cropping regime for Phase III cake sludge experiments in Britain

	Site	Cropping rotation	Harvest year			
			2002	2003	2004	2005
1.	Gleadthorpe	Ley/arable	grass	wheat	grass	wheat
2.	Woburn	Ley/arable	grass	wheat	grass	wheat
3.	Watlington	Ley/arable	grass	wheat	grass	wheat
4.	Pwllpeiran	Grassland	grass	grass	grass	grass
5.	Rosemaund	Ley/arable	grass	wheat	grass	wheat
6.	Bridgets	Ley/arable	grass	wheat	grass	wheat
7.	Hartwood	Grassland	grass	grass	grass	grass
8.	Auchincruive	Grassland	grass	grass	grass	grass
9.	Shirburn	Ley-arable	grass	wheat	grass	wheat

During harvest years 2002 and 2004, first cut ryegrass yields and metal concentrations in the harvested dry matter were determined at all nine sites. In harvest years 2003 and 2005, wheat yields and grain metal concentrations were determined at six of the sites (ryegrass yields and metal uptakes at Pwllpeiran, Hartwood and Auchincruive). Grass yields were determined using a small plot mechanised grass harvester and grain yields using a small plot combine harvester. The grass and grain metal concentrations will provide valuable information on metal bioavailability to crops.

3.1.5 Soil sampling

Soil samples were taken from all the treatment plots in spring 2003 and 2005 to complement the measurements made in October 1997 following the final sludge application in Phase I and those made in spring 1999 and spring/summer 2001 during Phase II. The selected soil physical,

chemical and microbial properties that were determined during Phase III are summarised in Appendix B.

a) Physical properties

The effects of the sludge additions on soil *bulk density* (MAFF, 1982) were measured in 2005 on selected treatments (1, 2, 3, 6, 10, 14 at sites 1 to 8; and 1, 2, 3, 8, 13, 18 at site 9).

b) Chemical properties

Total (aqua-regia soluble) and *extractable* (ammonium nitrate [NH_4NO_3]) heavy metal (Zn, Cu, Cd, Ni, Pb) concentrations, soil pH and soil organic C levels (potassium dichromate oxidation) were determined using standard procedures on the soil samples taken from all the treatment plots in spring 2003 and 2005. Additionally, total N, extractable P, K, Mg and conductivity measurements were made on all the plot samples taken in 2005, using standard procedures (MAFF, 1986). Also, the speciation of metals present in soil solution (Holm *et al.,* 1995, Knight *et al.,*1998) and cation exchange capacity (CEC) were measured on selected treatments (1, 2, 3, 6, 10, 14 at sites 1 to 8; and 1, 2, 3, 8, 13 and 18 at site 9).

The soil pH measurements were used to determine the need for future lime additions to maintain the 'target' pH values. Soil P, K and Mg concentrations were used to provide a basis for the inorganic fertiliser strategies.

c) Microbial properties

Standard methodologies were agreed between the contractors. The effects of the sewage sludge additions in Phase I on soil microbial properties were assessed on the soil samples taken in 2003 (excluding the long-term build-up treatments) and 2005 from all the treatments. The following assays were undertaken:

- biomass carbon (Vance *et al.*, 1987)

- soil respiration (Smith & Hadley, 1990)

- *Rhizobium* most probable numbers (Vincent, 1970; Woomer *et al.,* 1990)

3.1.6 Plant analysis

Zinc, Cu and Cd concentrations (MAFF, 1986), and yields were measured on first cut ryegrass harvests in 2002 and 2004 and on grain harvested in 2003 and 2005 (first cut ryegrass at Pwllpeiran, Hartwood and Auchincruive).

The plant metal uptakes provide a standard marker for the environmental availability of the soil metals. This is important for comparison with other measures of metal activity within the experiments (i.e. extractable soil metals, metal speciation) and for comparison between the sludge cake and metal-amended liquid sludge experiments, as well as other ongoing experiments. These measurements will in the longer term indicate when the sludges have equilibrated with the soil, to

the extent that plant metal availability may be taken as a reliable indicator of the long-term 'stabilisation' of sludge applications to land.

3.1.7 Sample storage

Dried soil and dried sludge cake samples from sites in England and Wales were archived at Rothamsted in sealed glass containers for future reference purposes, including potential analysis of organic pollutants. Similarly samples from Hartwood and Auchincruive were archived at the Macaulay Institute and SAC, respectively.

3.1.8 Statistical analysis

Effects of the metal dose-response treatments were initially tested by the null hypothesis using analysis of variance (ANOVA), utilising the randomised block design at each field site (see Section 3.1.2). Treatment means compared in the result tables include metal dose-response treatments and the untreated control, to investigate the overall effect of sludge as well as the effects between sludge treatments in this *initial* statistical appraisal. All computations were carried out using Genstat 8th Edition (VSN International Ltd., Hemel Hempstead, UK).

The effects of the metal dose-response treatments on soil microbial activity (respiration rate, biomass carbon, rhizobia MPNs) and crop yields were assessed using regression analysis, using data from all 15 of the metal dose-response treatment plots.

3.1.9 Slide archive

A dedicated slide archive was established at ADAS Gleadthorpe (following transfer from ADAS Boxworth) that records details of the site establishment and sludge spreading operations carried out during Phase I. The archived slides are available on CD-ROM in jpeg or bitmap formats.

3.2 England and Wales - Metal amended liquid sludge experiments

3.2.1 Sites

Metal dose-response treatments were established (Phase I: 1995-1997) at three sites on non-calcareous soils in England (Sites 2, 3 and 5, Appendix A), with topsoil textures ranging from sands to medium loams, clay contents from 9 to 23%, and initial organic C contents from 1.1 to 1.9%. Organic C contents at the end of Phase I were unchanged on the untreated controls and were between 0.4 to 0.5 % higher on treatments receiving liquid sludges (see Section 3.2.2 for treatment details) compared with the untreated controls. Initial topsoil Zn, Cu and Cd concentrations were 'typical' of uncontaminated topsoil in England.

The range in topsoil Zn, Cu and Cd concentrations achieved on the dose-response treatments at the end of Phase I (1998) at each site is shown in Table 6. A full list of treatments and target total soil metal concentrations is shown in Table 7. Soil pH was managed to maintain a target of *c.*6.5 at all three sites.

Table 6. Topsoil total metal concentration ranges on the metal dose-response treatments (rates 1 to 3) achieved at each site in England following Phase I metal-amended liquid sludge applications (1998)

Site name (county)	Topsoil texture	Total metal concentration ranges: metal dose-response treatments (mg/kg)		
		Zn	Cu	Cd
Typical background level		80	20	0.5
2 Woburn (Beds.)	Loamy sand	178 - 314	73 – 169	1.62 - 3.52
3 Watlington (Oxon.)	Sandy loam	209 - 416	100 – 173	2.22 - 3.71
5 Rosemaund (Here.)	Silty clay loam	164 - 372	67 – 154	2.00 - 3.38

3.2.2 Treatments and design

Metal-amended liquid digested sludges, prepared by separately equilibrating Zn, Cu and Cd sulphates with uncontaminated liquid digested sludge for 6-9 months, and uncontaminated liquid sludges were applied at the sites over 3 application cycles to establish the individual Zn, Cu and Cd metal-dose response treatments during Phase I (1995-97) of the experiment (Table 7). These liquid sludges received no further treatment/reconstitution (other than metal sulphate additions or no addition in the case of uncontaminated sludge and intermittent mixing before application) and were incorporated into the topsoil (0-25cm depth) using a spading machine. These treatments provide a contrast in the form of metal applied and with lower inputs of organic matter compared with the sludge cake additions. Conventional wisdom would regard these as *"worst case" treatments* in terms of likely metal availability, akin to a long-term situation where soil organic matter levels have declined and stabilised.

The treatments were replicated in three fully randomised blocks (39 plots). Individual plots (1.2 x 3.5m = 4.2 m²) are bounded by permanent barriers to prevent the movement of soil during cultivation (the wooden barriers were replaced in 2002 with durable plastic to maintain the plot integrity). All cultivations, drilling and harvesting were either mechanised using small plot equipment, or carried out by hand.

Table 7. Treatments at metal-amended liquid sludge sites

Number	Treatment	Target total soil metal concentration (mg/kg)	Annual cultivation
21A	No sludge control	Background	✓
21B	No sludge control	Background	✓
22A	Uncontaminated sludge	Low	✓
22B	Uncontaminated sludge	Low	✓
Zn amended sludge:		***Zinc***	
23	Zinc rate 1	175	✓
24	Zinc rate 2	300	✓
25	Zinc rate 3	425	✓
Cu amended sludge:		***Copper***	
26	Copper rate 1	75	✓
27	Copper rate 2	135	✓
28	Copper rate 3	195	✓
Cd amended sludge:		***Cadmium***	
29	Cadmium rate 1	2	✓
30	Cadmium rate 2	3	✓
31	Cadmium rate 3	4	✓

3.2.3 Sludges and application

The mean total metal concentrations in the metal-amended liquid sludges applied during Phase I are summarised below:

- *Zn*-amended *liquid sludge:* mean concentration 22,080 mg Zn/kg ds
- *Cu*-amended *liquid sludge:* mean concentration 12,040 mg Cu/kg ds
- *Cd*-amended *liquid sludge:* mean concentration 280 mg Cd/kg ds
- *'Uncontaminated' liquid sludge:* mean concentrations 590 mg Zn/kg, 590 mg Cu/kg and 1.7 mg Cd/kg ds.

The total metal addition rates achieved similar soil metal concentration ranges to the sludge cake sites, so that metal availability could be compared at similar soil concentrations, but with smaller amounts of organic matter.

Cultivations were undertaken at least 3 times in summer 1998 (after the first grass cut) on each treatment, to help ensure that the liquid sludge additions were as evenly incorporated as possible throughout the topsoil depth (0-25 cm). The final cultivation pass was with the small plot spading machine to help ensure mixing to the full depth of soil cultivation. During Phase III, cultivations continued to be undertaken to encourage organic matter breakdown from the liquid sludge additions.

3.2.4 Cropping

During Phases II and III, the sites were managed under ley/arable cropping (Table 8). During Phase III the same grass cultivar (an Italian ryegrass mixture from Cotswold Grass Seeds, 'Special Mix A') was sown

at all sites in autumns 2001 and 2003 at a seed rate of 40 kg/ha for harvest in summers 2002 and 2004. Similarly, the same spring wheat variety (Chablis) was sown in February/March 2003 and 2005. The wheat variety chosen were 'stiff' strawed to minimise lodging risks. Additionally, the winter wheat variety (Chablis) was selected as it is a milling wheat (Group 1) variety that is used in bread making and hence needs to comply with the EU maximum grain Cd concentration limit (0.235 mg/kg dry matter) to be traded in the market place.

Table 8. Cropping regime for Phase III of the liquid sludge experiments in England

	Site	Cropping rotation	Harvest year			
			2002	2003	2004	2005
2.	Woburn	Ley/arable	grass	wheat	grass	wheat
3.	Watlington	Ley/arable	grass	wheat	grass	wheat
5.	Rosemaund	Ley/arable	grass	wheat	grass	wheat

During harvest years 2002 and 2004, first cut ryegrass yields and metal concentrations in the harvested dry matter were determined at all three sites. In harvest years 2003 and 2005, wheat yields and grain metal concentrations were determined at all three sites. Grass yields were determined using a small plot mechanised grass harvester and grain yields using a small plot combine harvester. The grass and grain metal concentrations will provide valuable base-line information on metal bioavailability to crops.

3.2.5 Soil sampling

Soil samples were taken from all the treatment plots in spring 2003 and spring 2005 to complement the measurements made in October 1997 following the final liquid sludge application in Phase I, and those made in spring 1999 and spring/summer 2001 during Phase II. The selected soil physical, chemical and microbial properties that were determined are summarised in Appendix B.

a) Physical properties

The effects of the sludge additions on soil *bulk density* (MAFF, 1982) were measured in 2005 on selected treatments (21, 22, 24, 27, 30) at each site.

b) Chemical properties

Total (aqua-regia soluble) and *extractable* (ammonium nitrate [NH_4NO_3]) heavy metal (Zn, Cu, Cd, Ni, Pb) concentrations, soil pH and soil organic C levels (wet chemistry method) were determined using standard procedures on the soil samples taken from all the treatment plots in spring 2003 and 2005. Additionally, total N, extractable P, K, Mg and conductivity measurements were made on all the plot samples taken in 2005, using standard procedures (MAFF, 1986). Also, the speciation of metals present in soil solution (Holm *et al.,* 1995, Knight *et al.,*1998) and cation exchange capacity (CEC) were measured on selected treatments (21, 22, 24, 27 and 30) in 2005.

The soil pH measurements were used to determine the need for future lime additions to maintain the 'target' pH values. Soil P, K and Mg concentrations were used to provide a basis for the inorganic fertiliser strategies.

c) Microbial properties

Standard methodologies were agreed between the contractors. The effects of the liquid sludge metal additions in Phase I on soil microbial properties were assessed on the soil samples taken in spring 2003 and 2005 from the treatment plots. The following assays were undertaken:

- biomass carbon (Vance *et al.*, 1987)

- soil respiration (Smith & Hadley, 1990)

- *Rhizobium* most probable numbers (Vincent, 1970; Woomer *et al.,* 1990)

3.2.6 Plant analysis

Zinc, Cu, Cd and Ni concentrations (MAFF, 1986), and yields were measured on the first cut grass harvests in 2002 and 2004, and on grain harvested in 2003 and 2005.

The plant metal uptakes provide a standard marker for the environmental activity of the soil metals. This is important for comparison with other measures of metal activity within the experiments (i.e. extractable soil metals, metal speciation) and for comparison between the sludge cake and metal-amended liquid sludge experiments, as well as other ongoing experiments. These measurements will in the longer term indicate when the sludges have equilibrated with the soil, to the extent that plant metal availability may be taken as a reliable indicator of the long-term 'stabilisation' of sludge applications to land.

3.2.7 Sample storage

Dried soil samples were archived at Rothamsted in sealed glass containers for future reference purposes, including potential analysis of organic pollutants.

3.2.8 Statistical analysis

Effects of the metal dose-response treatments were initially tested by the null hypothesis using analysis of variance (ANOVA), utilising the randomised block design at each field site (see Section 3.1.2). Treatment means compared in the result tables include metal dose-response treatments and the untreated control, to investigate the overall effect of sludge as well as the effects between sludge treatments in this *initial* statistical appraisal. All computations were carried out using Genstat 5, Release 4.22 (VSN International Ltd., Oxford, UK).

The effects of the metal dose-response treatments on soil microbial activity (respiration rate, biomass carbon, rhizobia MPNs) and crop yields were assessed using regression analysis, using data from all 12 of the dose-response treatment plots.

3.2.9 Slide archive

A dedicated slide archive was established at ADAS Gleadthorpe (following transfer from ADAS Boxworth) that records details of the site establishment and sludge spreading operations carried out during Phase I. The archived slides are available on CD-ROM in jpeg or bitmap formats.

4. RESULTS

4.1 Sludge cake experiments

4.1.1 Soil microbial properties

Soil samples taken from all the treatment plots in spring 2003 and 2005 were analysed for soil respiration rates, microbial biomass carbon concentrations and *Rhizobium leguminosarum* biovar *trifolii* most probable numbers (MPNs).

Soil respiration rates

Soil respiration rates were measured on soil samples taken from all the treatment plots in spring 2003 (excluding the long-term build-up treatments) and 2005, with mean treatment respiration rates summarised in Tables 9 and 10.

Soil respiration rates in 2003 and 2005 were generally higher on the treatments receiving sludge cakes compared with the untreated controls.

2003 sampling: There were no differences ($P > 0.05$) in soil respiration rates between the Zn rich sludge cake treatments and either the uncontaminated digested or untreated controls at any of the seven sites in England and Wales. However, at Auchincruive the Zn rate 2 and 3 treatments had higher soil respiration rates ($P < 0.05$) of 0.15-0.16 mg CO_2-C/kg and at Hartwood the uncontaminated digested sludge cake treatment had a higher soil respiration rate ($P < 0.05$) of 0.35 mg CO_2-C/kg compared with the untreated control treatments.

At Watlington, the Cu rate 4 treatment had a higher soil respiration rate ($P < 0.05$) of 0.27-0.29 mg CO_2-C/kg compared with the uncontaminated raw and untreated control treatments. At Rosemaund, the Cu rate 2-4 treatments had higher soil respiration rates ($P < 0.05$) of 0.24-0.26 mg CO_2-C/kg compared with the untreated control. And at Auchincruive, the Cu rate 4 treatment had a higher soil respiration rate ($P < 0.05$) of 0.17 mg CO_2-C/kg compared with the untreated control.

At Woburn, soil respiration rates were 0.28-0.30 mg CO_2-C/kg lower ($P < 0.05$) on the Cd rate 3 and 4 treatments compared with the uncontaminated digested treatment. At Rosemaund and Bridgets, the Cd rate 1 and 2 treatments had higher ($P < 0.05$) soil respiration rates of 0.21-0.34 mg CO_2-C/kg compared with the untreated controls, and at Auchincruive the Cd rate 4 treatment had a higher ($P < 0.05$) soil respiration rate of 0.16 mg CO_2-C/kg compared with the untreated control.

Soil respiration rates were regressed against total topsoil metal concentrations (Figures 1-9). On the metal dose-response treatments, regression of the soil respiration rate measurements against total topsoil Zn concentrations identified significant inverse relationships ($P < 0.05$) at Hartwood and for Cd at Woburn and Hartwood. For Cu, there were significant positive relationships ($P < 0.05$) at Watlington, Rosemaund and Auchincruive. A summary of the regression analyses is given in Table 11.

Table 9. Mean topsoil (0-25 cm) respiration rates (mg CO_2-C/kg oven dry soil/hour) on treatments sampled in spring 2003.

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR
Control	0.60	0.32	0.23	0.60	0.22	0.23	0.84	0.27	0.44
Unc. Digested	0.71	0.62	0.51	0.44	0.37	0.40	1.19	0.42	0.52
Unc. Raw	0.76	0.52	0.25	0.64	0.38	0.33	0.96	0.34	0.55
Unc. High Dig.	-	-	-	-	-	-	-	-	0.48
Unc. High Raw	-	-	-	-	-	-	-	-	0.55
Zn rate 1	0.66	0.38	0.38	0.53	0.37	0.33	1.15	0.38	0.52
Zn rate 2	0.65	0.36	0.37	0.76	0.42	0.32	1.06	0.43	0.52
Zn rate 3	0.75	0.41	0.49	0.50	0.36	0.35	1.03	0.42	0.65
Zn rate 4	0.59	0.45	0.43	0.49	0.32	0.39	0.93	0.39	0.50
Zn rate 5	-	-	-	-	-	-	-	-	0.48
ANOVA (1) p	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.045*	*0.036*	*NS*
$LSD_{0.05}$	-	-	-	-	-	-	0.350	0.151	-
CV%	24	24	28	21	19	25	12	14	25
Cu rate 1	0.65	0.38	0.30	0.46	0.41	0.31	0.93	0.32	0.56
Cu rate 2	0.72	0.38	0.34	0.62	0.47	0.38	0.98	0.36	0.58
Cu rate 3	0.66	0.42	0.45	0.40	0.46	0.38	0.94	0.39	0.65
Cu rate 4	0.73	0.36	0.52	0.62	0.48	0.36	0.77	0.44	0.57
Cu rate 5	-	-	-	-	-	-	-	-	0.61
ANOVA (2) p	*NS*	*NS*	*0.010*	*NS*	*0.008*	*NS*	*NS*	*0.037*	*NS*
$LSD_{0.05}$	-	-	0.235	-	0.197	-	-	0.145	-
CV%	13	25	24	26	17	21	11	15	15
Cd rate 1	0.71	0.39	0.41	0.57	0.43	0.47	1.13	0.38	0.60
Cd rate 2	0.63	0.36	0.44	0.68	0.43	0.57	0.98	0.39	0.67
Cd rate 3	0.51	0.34	0.48	0.58	0.38	0.40	0.96	0.39	0.65
Cd rate 4	0.56	0.32	0.38	0.67	0.39	0.41	0.79	0.43	0.60
Cd rate 5	-	-	-	-	-	-	-	-	0.58
ANOVA (3) p	*NS*	*0.030*	*NS*	*NS*	*0.031*	*0.010*	*NS*	*0.022*	*NS*
$LSD_{0.05}$	-	0.281	-	-	0.194	0.233	-	0.132	-
CV%	21	25	24	23	19	20	17	12	20

Three ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control. The ANOVA fitted to each group was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05).

Figure 1. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Gleadthorpe (spring 2003)

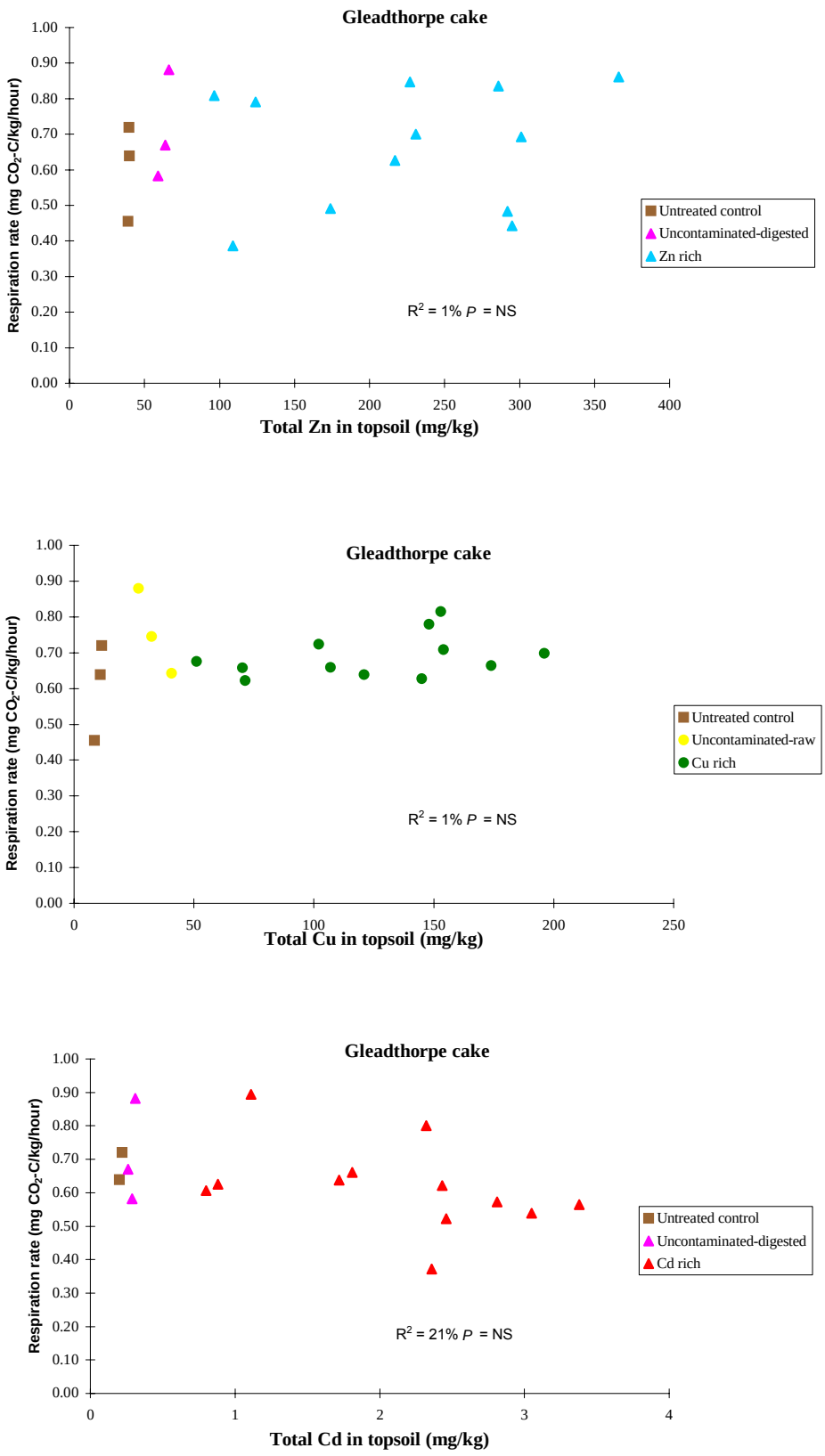
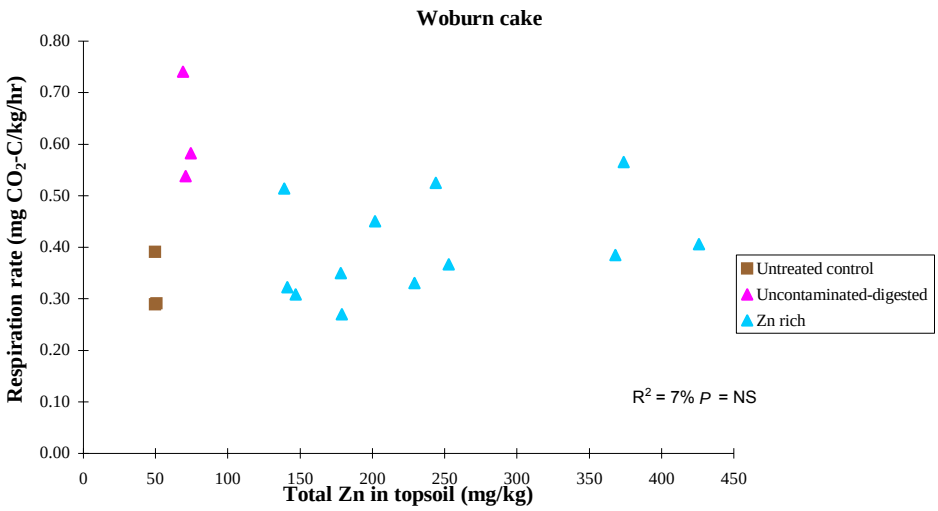
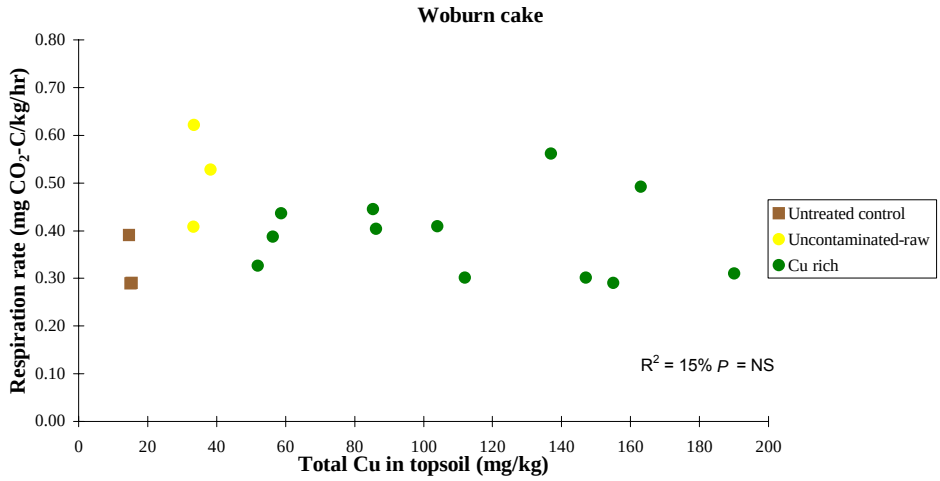


Figure 2. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Woburn (spring 2003)





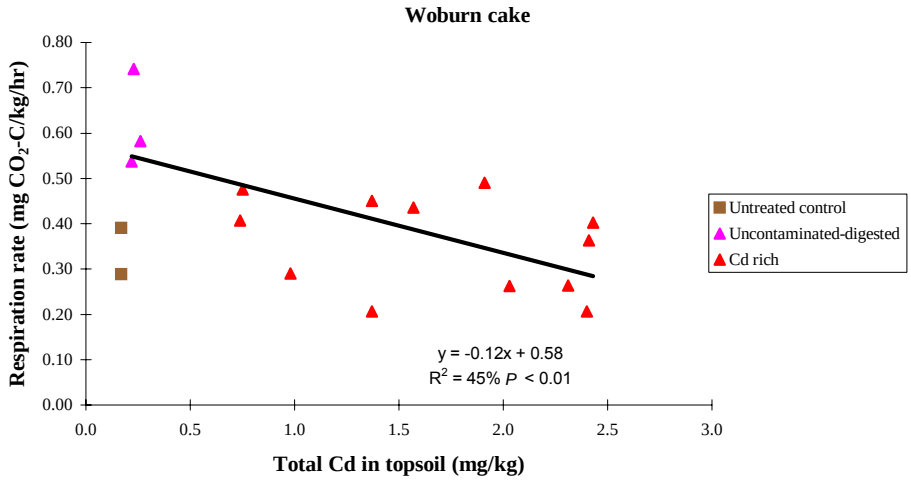
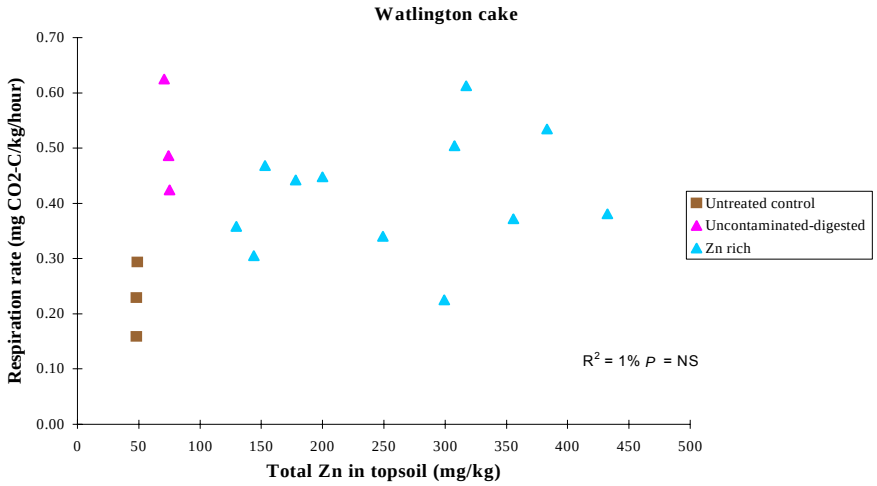
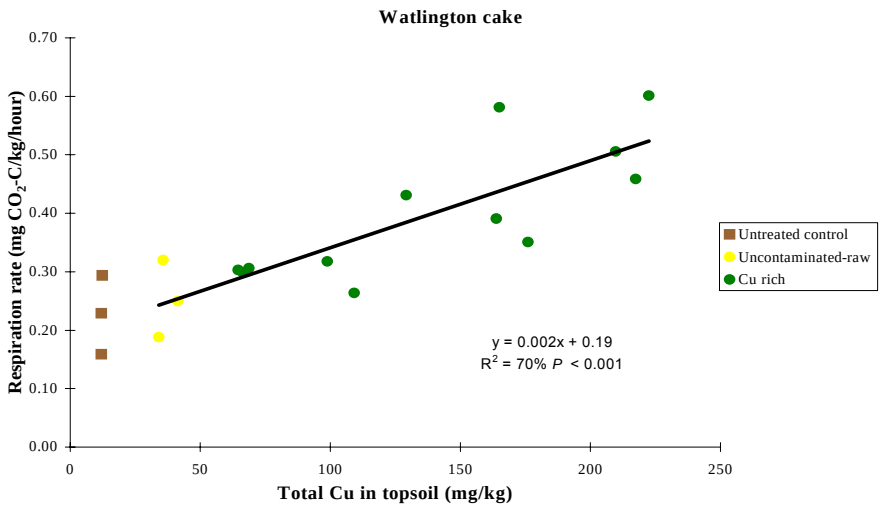


Figure 3. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Watlington (spring 2003)





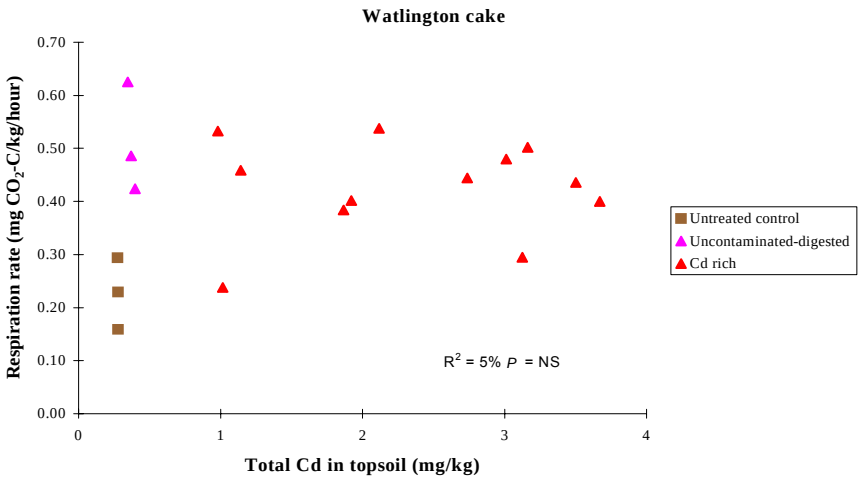


Figure 4. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Pwllpeiran (spring 2003)

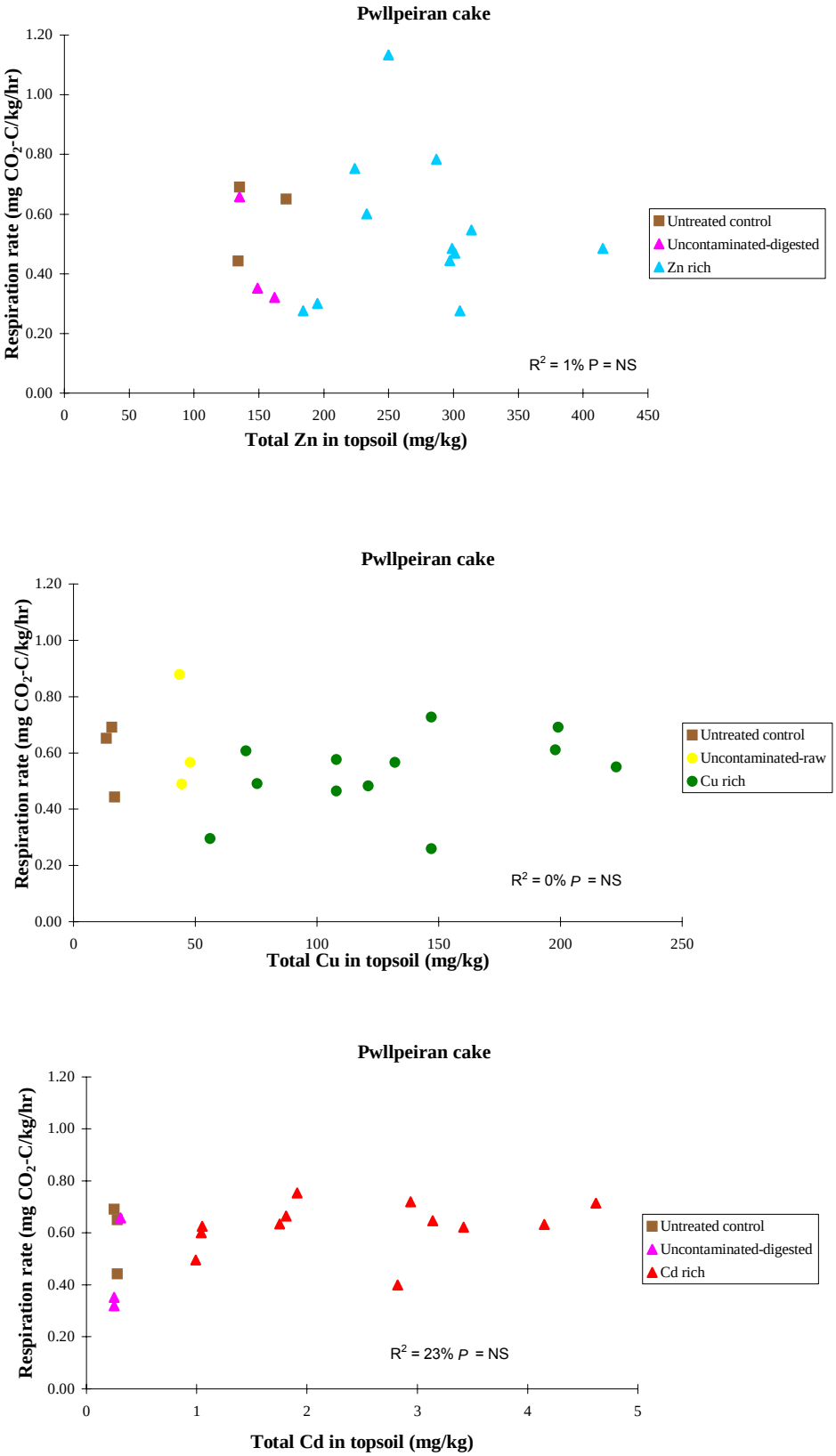


Figure 5. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Rosemaund (spring 2003)

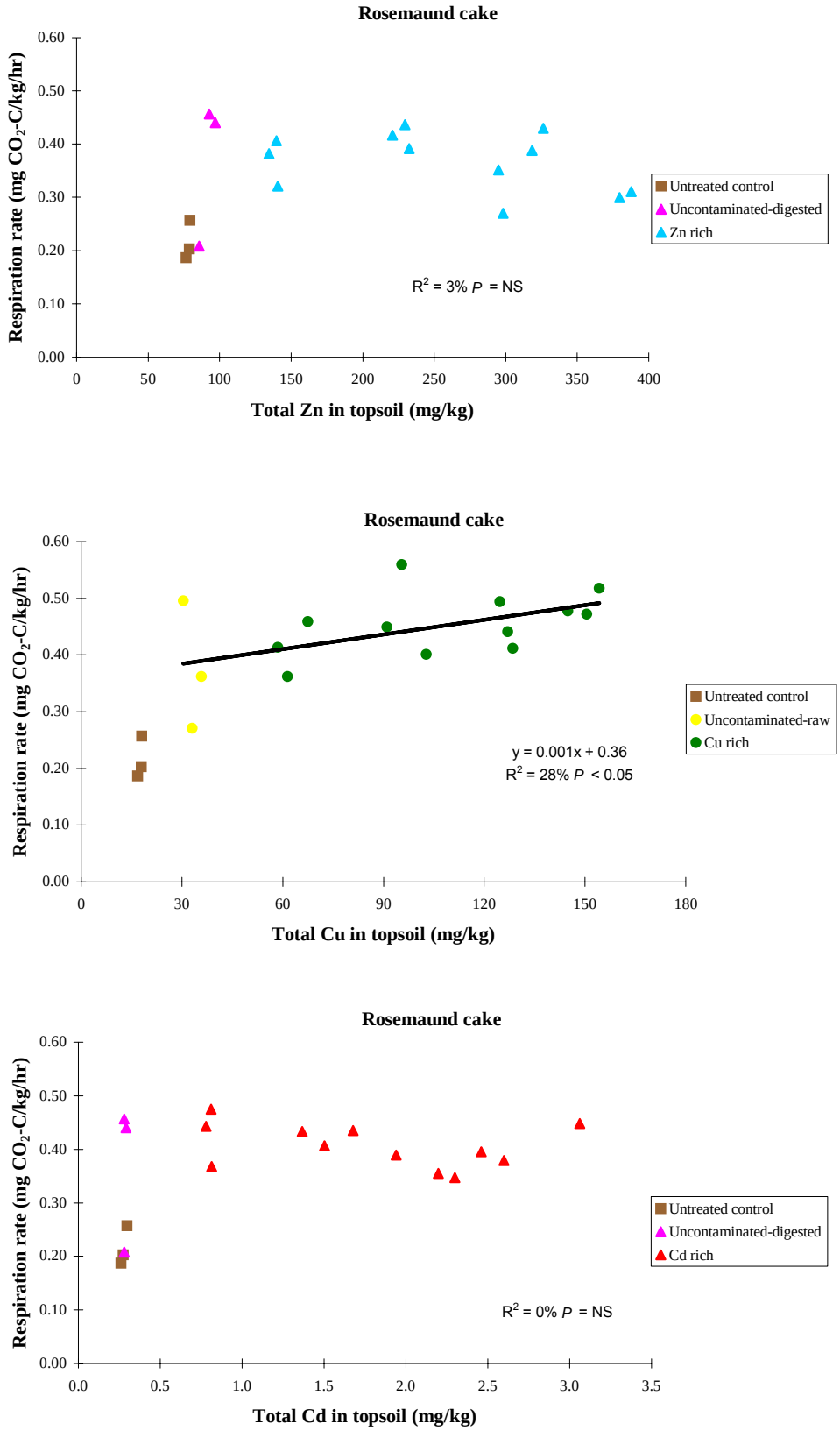


Figure 6. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Bridgets (spring 2003)

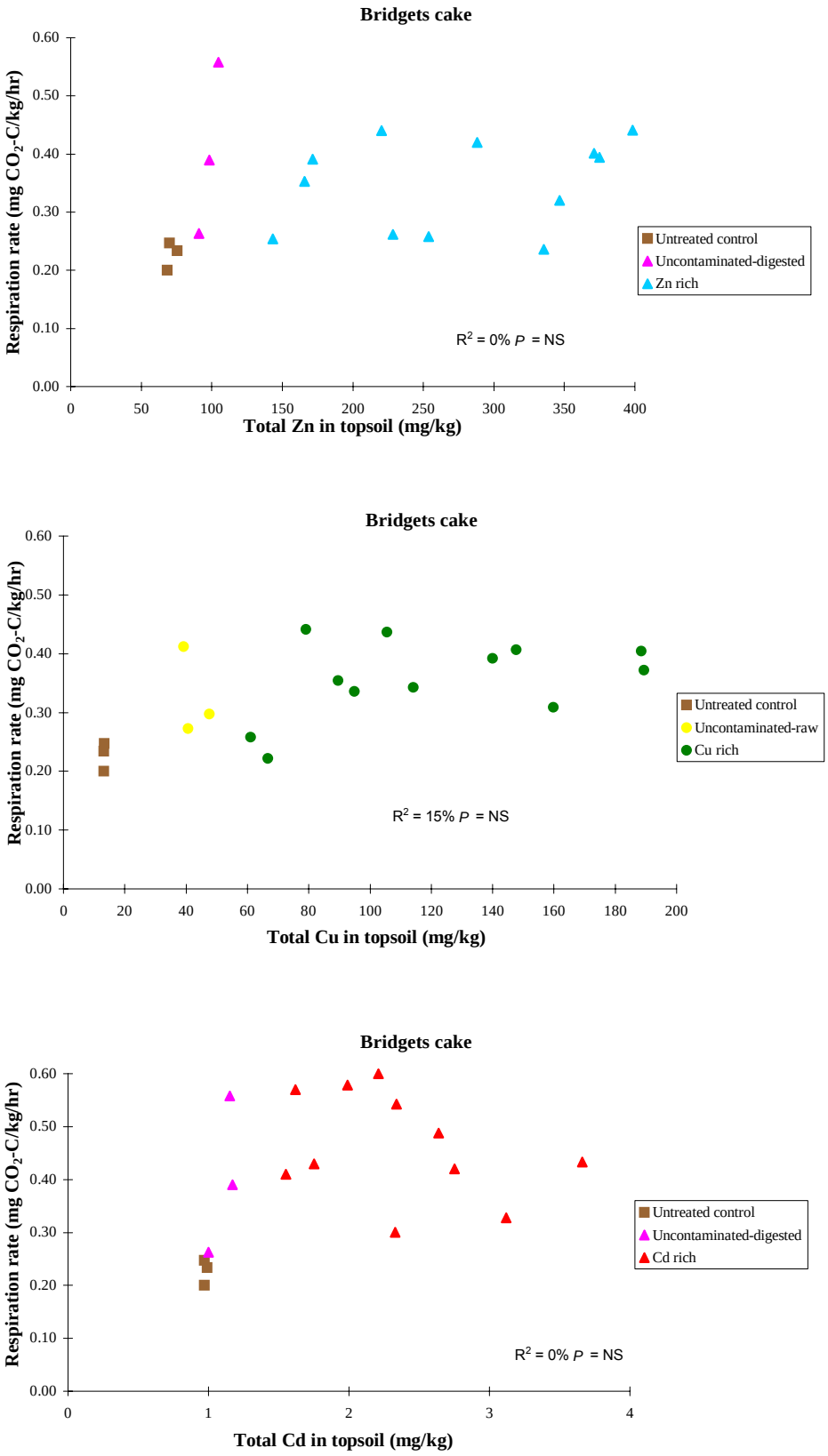


Figure 7. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Hartwood (spring 2003)

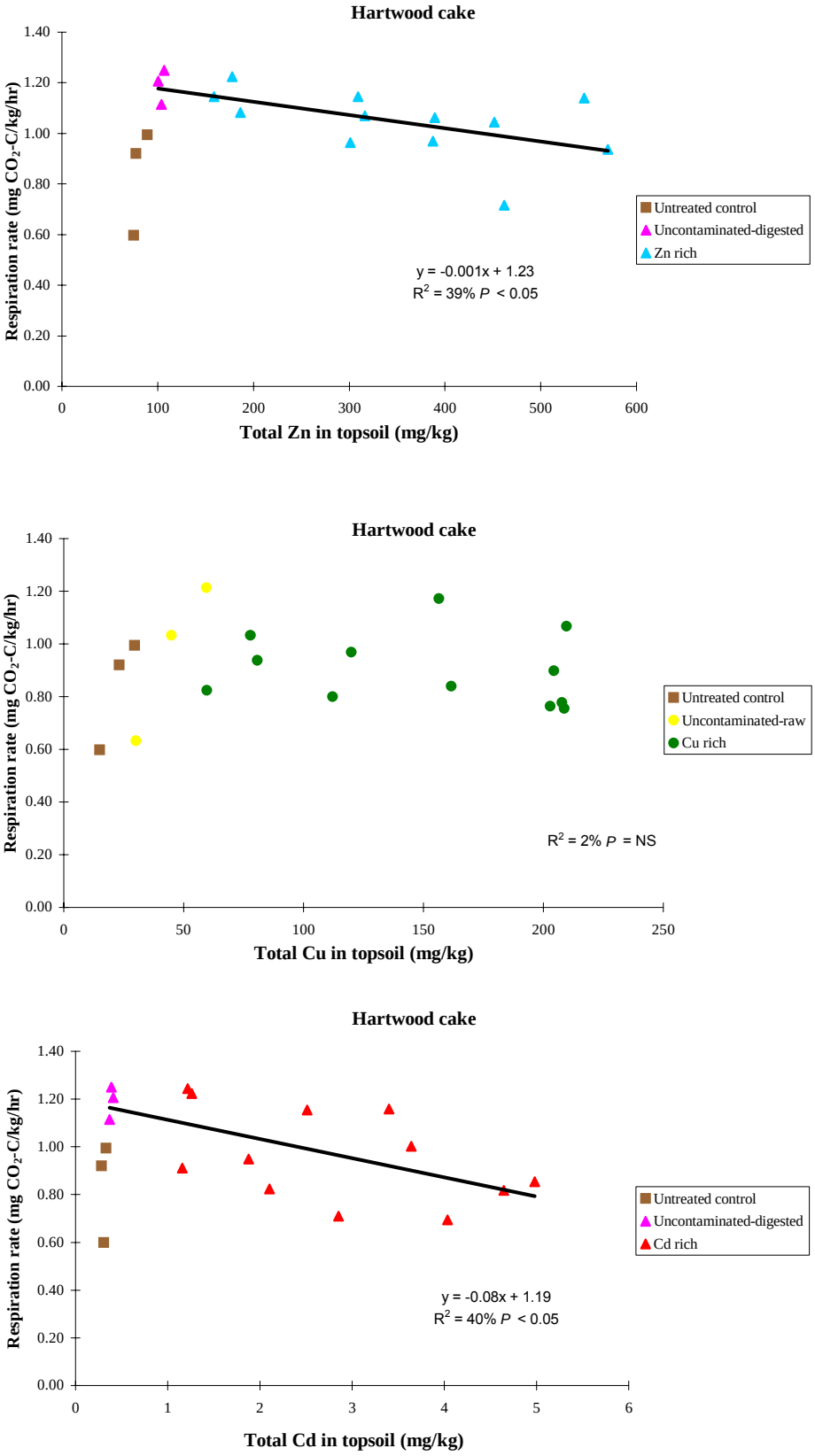


Figure 8. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Auchincruive (spring 2003)

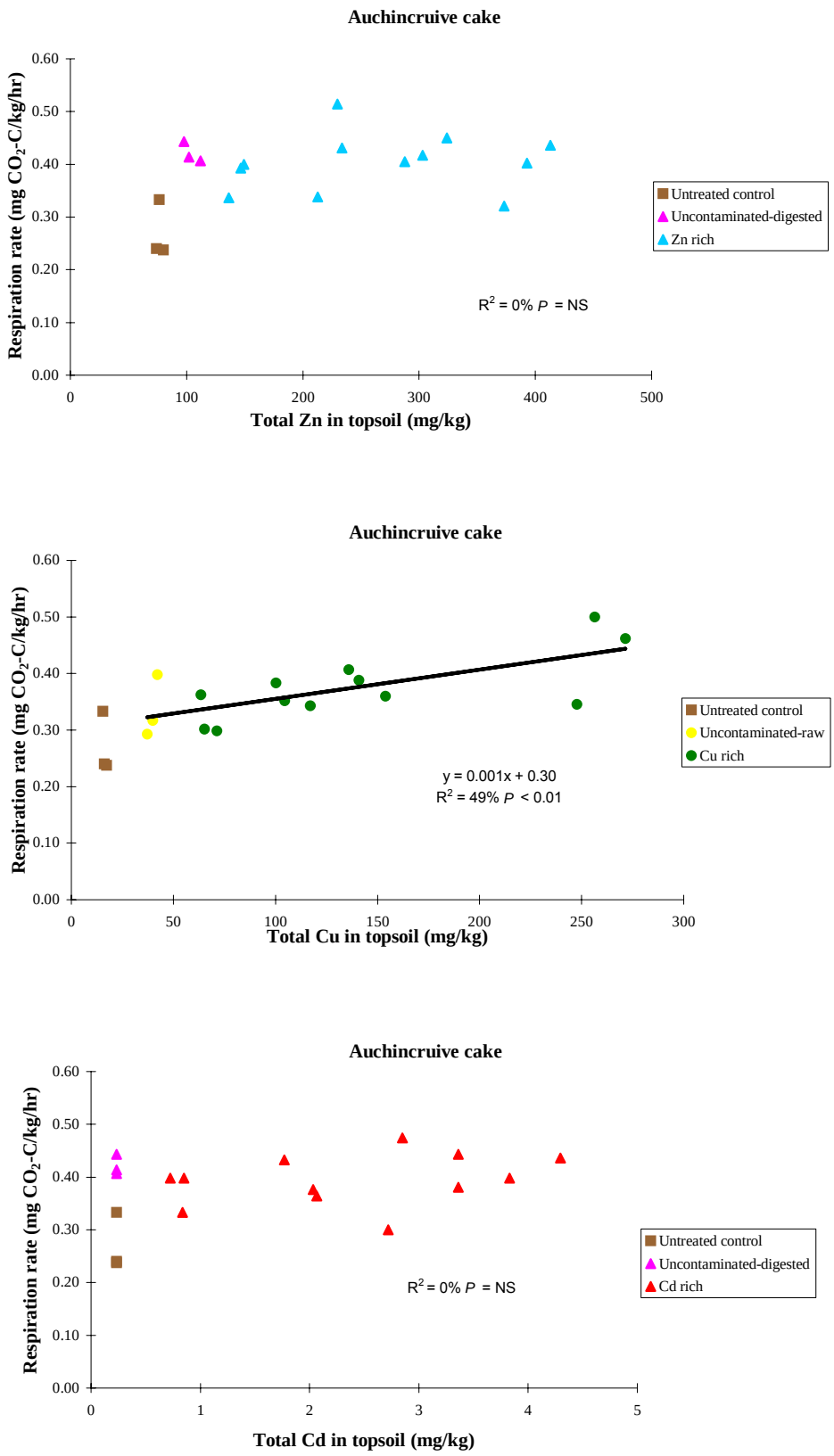
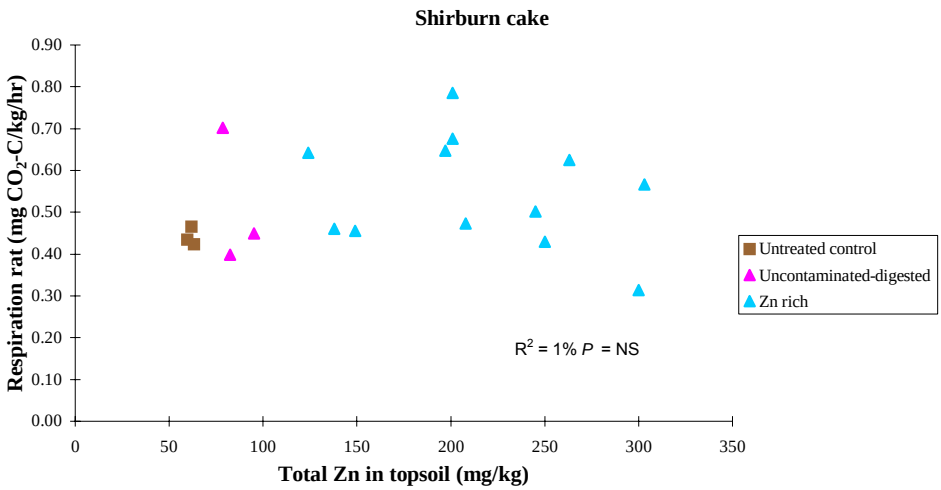
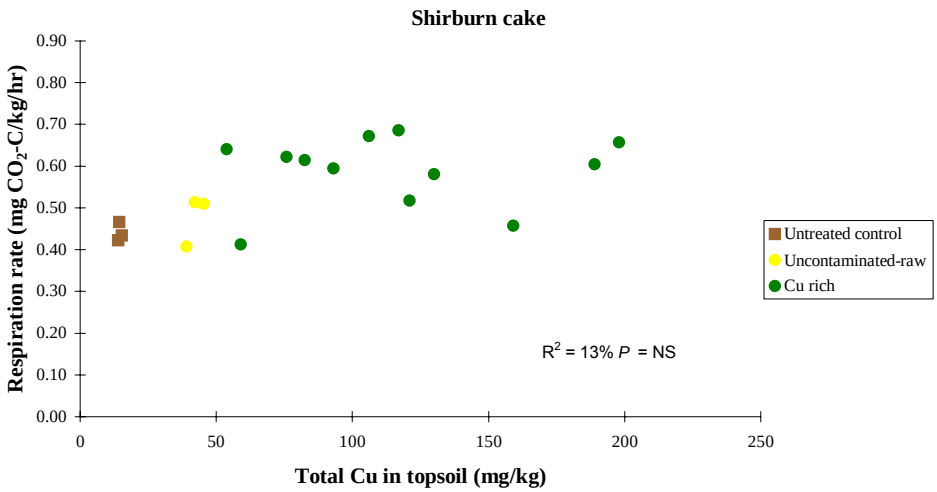


Figure 9. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Shirburn (spring 2003)





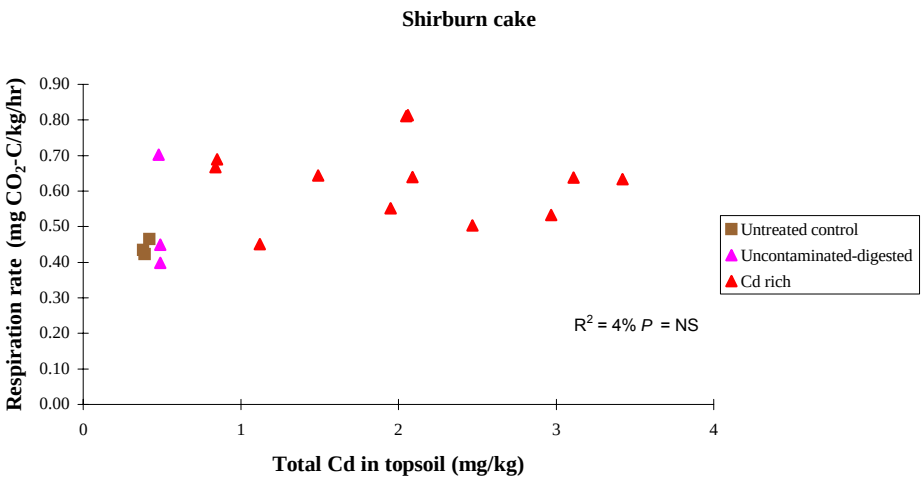


Table 10. Mean topsoil (0-25 cm) respiration rates (mg CO_2-C/kg oven dry soil/hour) on treatments sampled in spring 2005.

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR
Control	0.25	0.36	0.27	0.42	0.30	0.27	0.73	0.42	0.42
Unc. Digested	0.37	0.47	0.39	0.47	0.34	0.28	1.11	0.62	0.51
Unc. Raw	0.27	0.36	0.33	0.46	0.28	0.29	0.91	0.53	0.46
Unc. High Dig.	-	-	-	-	-	-	-	-	0.50
Unc. High Raw	-	-	-	-	-	-	-	-	0.51
Zn rate 1	0.29	0.48	0.38	0.39	0.36	0.28	1.19	0.57	0.59
Zn rate 2	0.36	0.48	0.40	0.43	0.37	0.32	0.98	0.53	0.61
Zn rate 3	0.39	0.48	0.39	0.44	0.32	0.28	0.99	0.62	0.59
Zn rate 4	0.28	0.56	0.41	0.42	0.35	0.31	0.88	0.60	0.57
Zn rate 5	-	-	-	-	-	-	-	-	0.61
ANOVA (1) p	0.012	NS	0.005	NS	NS	NS	0.027	< 0.001	0.003
$LSD_{0.05}$	0.120	-	0.099	-	-	-	0.400	0.097	0.142
CV%	13	16	28	11	10	11	14	6	9
Cu rate 1	0.36	0.44	0.36	0.43	0.29	0.31	0.99	0.50	0.59
Cu rate 2	0.32	0.47	0.36	0.48	0.29	0.34	0.92	0.50	0.67
Cu rate 3	0.30	0.47	0.37	0.50	0.28	0.30	0.89	0.55	0.71
Cu rate 4	0.32	0.46	0.40	0.50	0.31	0.29	0.85	0.67	0.63
Cu rate 5	-	-	-	-	-	-	-	-	0.66
ANOVA (2) p	NS	0.023	0.014	NS	NS	NS	NS	< 0.001	0.010
$LSD_{0.05}$	-	0.125	0.100	-	-	-	-	0.113	0.259
CV%	21	10	10	15	16	11	19	8	15
Cd rate 1	0.45	0.47	0.35	0.55	0.39	0.29	1.28	0.57	0.63
Cd rate 2	0.43	0.53	0.38	0.50	0.34	0.38	1.05	0.56	0.63
Cd rate 3	0.37	0.51	0.38	0.44	0.35	0.33	1.14	0.56	0.58
Cd rate 4	0.41	0.53	0.36	0.60	0.36	0.32	0.91	0.52	0.61
Cd rate 5	-	-	-	-	-	-	-	-	0.68
ANOVA (3) p	0.009	NS	0.006	NS	NS	NS	NS	0.001	0.001
$LSD_{0.05}$	0.140	-	0.086	-	-	-	-	0.103	0.168
CV%	13	16	9	19	13	9	25	7	20
LT Unc. Dig.	0.29	0.49	0.25	0.48	0.30	0.27	0.90	0.43	0.64
LT Unc. Raw	0.30	0.46	0.25	0.54	0.33	0.28	0.79	0.41	0.61
LT Zn	0.28	0.52	0.30	0.51	0.31	0.32	0.65	0.42	0.65
LT Cu	0.27	0.56	0.24	0.49	0.30	0.32	0.81	0.50	0.67
LT Cd	0.28	0.45	0.23	0.46	0.32	0.26	0.88	0.43	0.54
ANOVA (4) p	NS	NS	NS	NS	NS	NS	NS	NS	0.003
$LSD_{0.05}$	-	-	-	-	-	-	-	-	0.169
CV%	15	18	17	10	14	19	17	8	10

Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. The ANOVA fitted to each group was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05).

Table 11. Summary of relationships between soil respiration rates (mg CO_2-C/kg/hr) and total topsoil metals - sludge cake sites (spring 2003 samples)

	Zn	Cu (r^2 ; *P*)	Cd
GLE	NS	NS	NS
WOB	NS	NS	61%; $P < 0.01$
WAT	NS	62%; $P < 0.01$	NS
PWL	NS	NS	NS
ROS	NS	24%; $P < 0.05$	NS
BRI	NS	NS	NS
HAR	9%; $P < 0.05$	NS	18%; $P < 0.05$
AUC	NS	16%; $P < 0.01$	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship, Red = inverse relationship. Model parameters for significant responses are given in Figures 1-9.

2005 sampling: At Gleadthorpe, there was a higher soil respiration rate of 0.14 mg CO_2-C/kg/h ($P < 0.05$) on the Zn rich rate 3 treatment compared with the untreated control. At Hartwood, a higher soil respiration rate of 0.46 mg CO_2-C/kg/h ($P < 0.05$) was measured on the Zn rich rate 1 treatment compared with the untreated control. At Watlington, Auchincruive and Shirburn there were higher ($P < 0.01$) soil respiration rates of 0.11-0.14, 0.11-0.20 and 0.15-0.19 mg CO_2-C/kg/h, respectively, for the four Zn rich treatments at each site compared with the untreated controls. There were no differences ($P > 0.05$) in soil respiration rates between the Zn rich sludge cake treatments and either the uncontaminated digested or untreated controls at any of the other four sites.

On the Cu rich treatments at Woburn, the soil respiration rate on the rate 3 treatment was 0.11 mg CO_2-C/kg higher ($P < 0.05$) compared with the uncontaminated raw treatment. At Watlington, the Cu rich rate 3 and 4 treatments had higher soil respiration rates of 0.10-0.13 mg CO_2-C/kg ($P < 0.05$) compared with the untreated control. At Auchincruive, the Cu rich rate 3 treatment had a higher soil respiration rate of 0.13 mg CO_2-C/kg ($P < 0.001$) above the untreated control, and the Cu rate 4 treatment had a respiration rate which was 0.12-0.25 mg CO_2-C/kg ($P < 0.001$) above the untreated control and all the raw sludge cake treatments. At Shirburn, the Cu rich rate 2 and 3 treatments had higher soil respiration rates of 0.25-0.29 mg CO_2-C/kg ($P < 0.01$) compared with the untreated control. There were no differences ($P > 0.05$) in soil respiration rates between the Cu rich sludge cake treatments and either

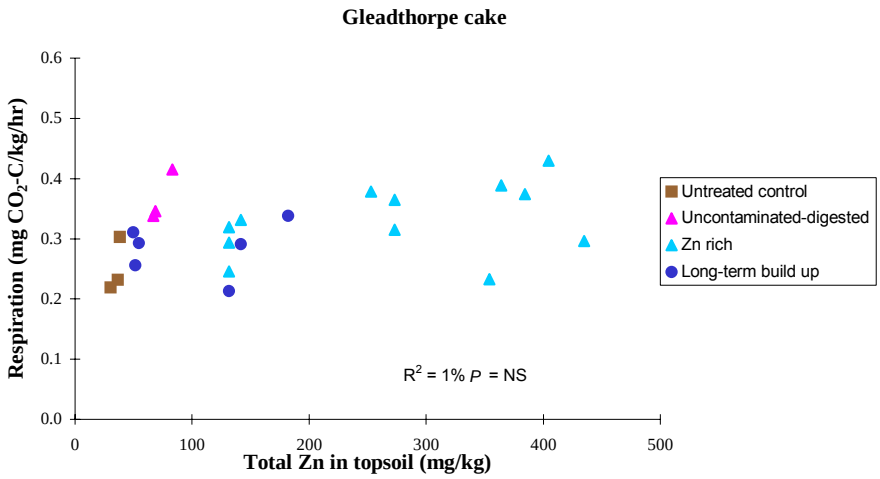
the uncontaminated raw or untreated controls at any of the other five sites.

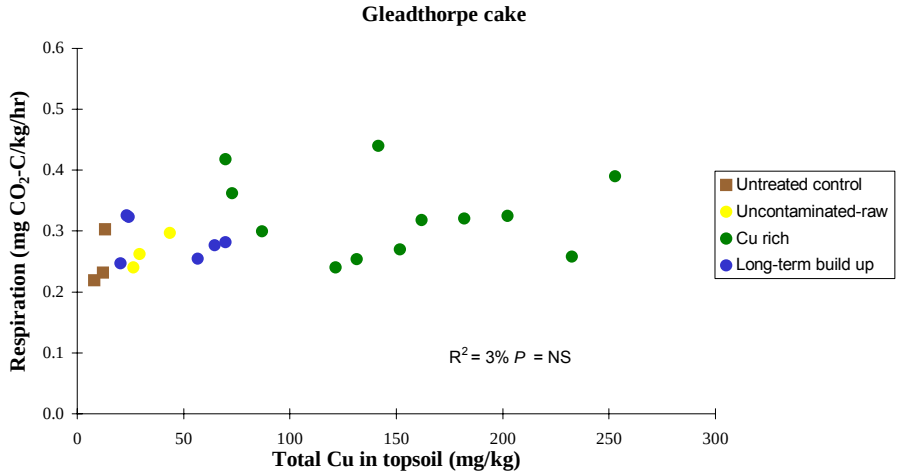
At Gleadthorpe, soil respiration rates on the Cd rich rate 1, 2 and 4 treatments were 0.16-0.20 mg CO_2-C/kg higher ($P < 0.01$) than the untreated control treatment. At Watlington, soil respiration rates on the Cd rich rate 2-4 treatments were 0.09-0.11 mg CO_2-C/kg higher ($P < 0.01$) than the untreated control treatment. At Auchincruive, soil respiration rates on the Cd rich rate 1-3 treatments were 0.14-0.15 mg CO_2-C/kg higher ($P < 0.001$) than the untreated control treatment. At Shirburn, soil respiration rates on the Cd rich rate 1, 2, 4 and 5 treatments were 0.19-0.26 mg CO_2-C/kg higher ($P < 0.001$) than the untreated control treatment, and respiration rates on rate 5 treatment were also 0.28 mg CO_2-C/kg higher ($P < 0.001$) than the high rate uncontaminated digested cake treatment. There were no differences ($P > 0.05$) in soil respiration rates between the Cd rich sludge cake treatments and either the uncontaminated digested or untreated controls at any of the other five sites.

At Shirburn, with the exception of the long-term Cd treatment, soil respiration rates on all of the long-term sludge cake treatments were 0.19-0.25 mg CO_2-C/kg higher ($P < 0.01$) than the untreated control treatment. There were no differences in soil respiration rates ($P > 0.05$) between the untreated controls and any of the long-term build up treatments at any of the other eight sites.

Soil respiration rates were regressed against total topsoil metal concentrations (Figures 10-18). On the metal dose-response treatments, regression of the soil respiration rate measurements against total topsoil Zn concentrations identified a significant inverse relationship ($P < 0.05$) at Hartwood. For Cu, there were significant positive relationships ($P < 0.05$) at Watlington and Auchincruive. However, there were no significant ($P > 0.05$) relationships between soil respiration rates and total topsoil Cd concentrations. A summary of the regression analyses is given in Table 12.

Figure 10. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Gleadthorpe (spring 2005)





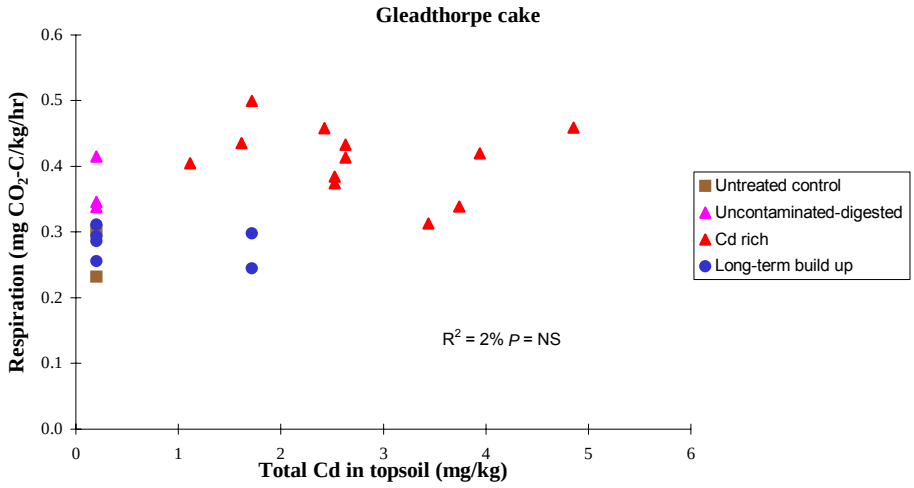
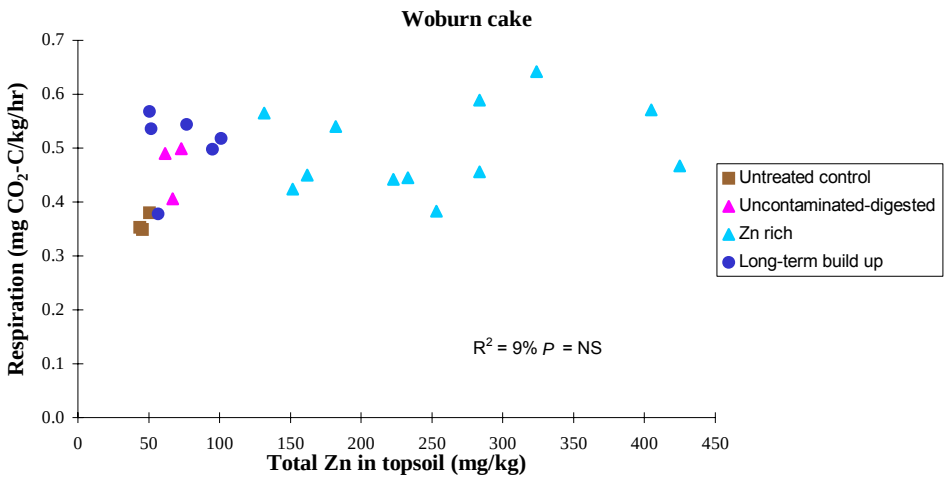
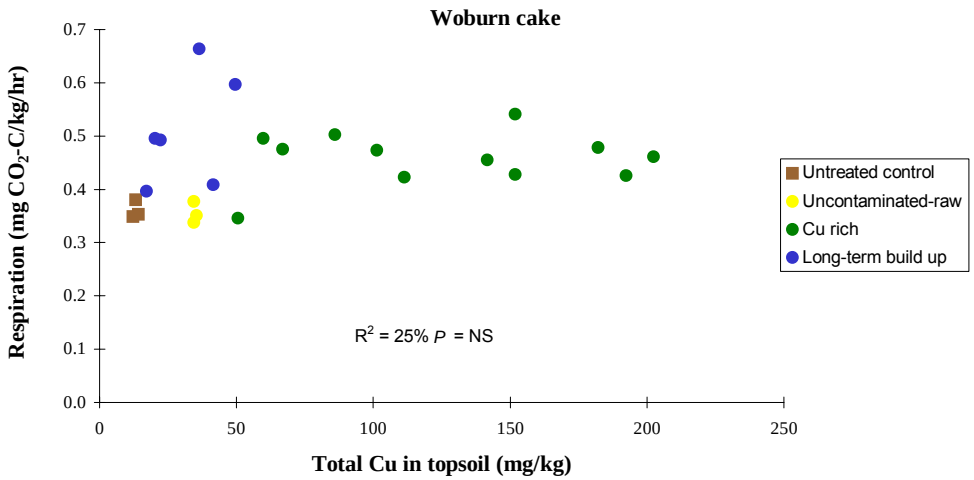


Figure 11. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Woburn (spring 2005)





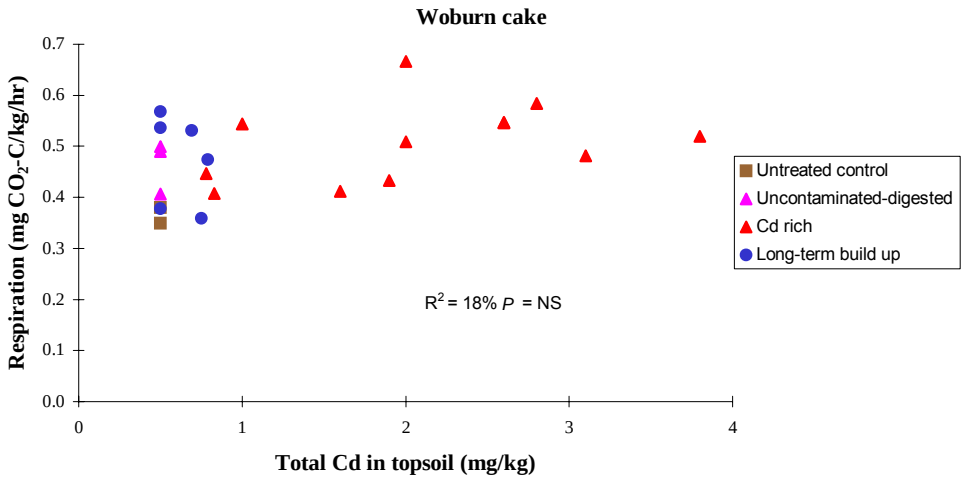
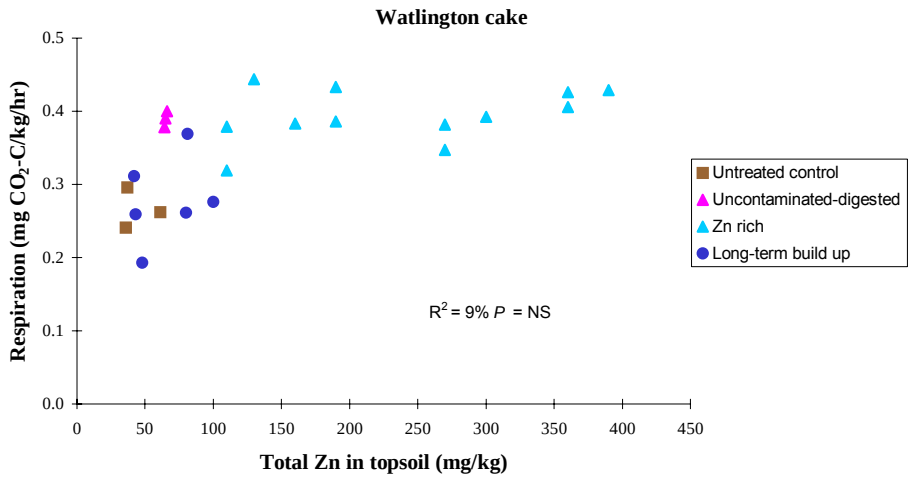
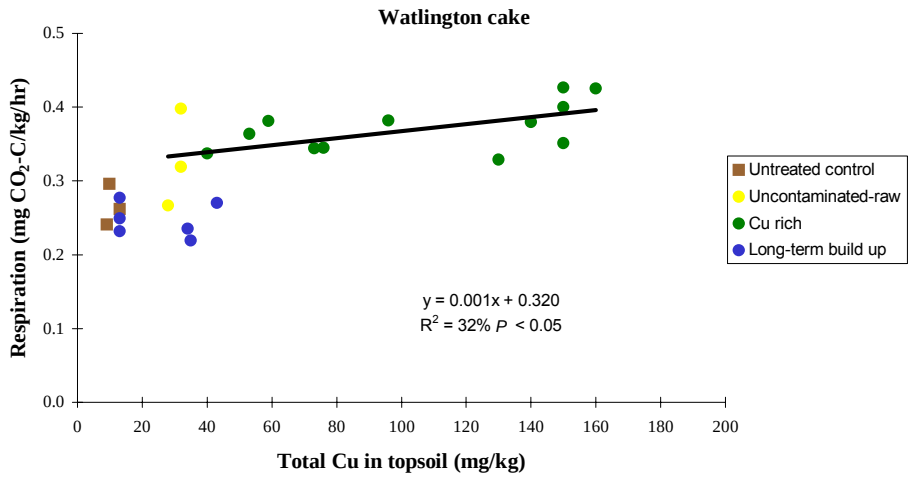


Figure 12. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Watlington (spring 2005)





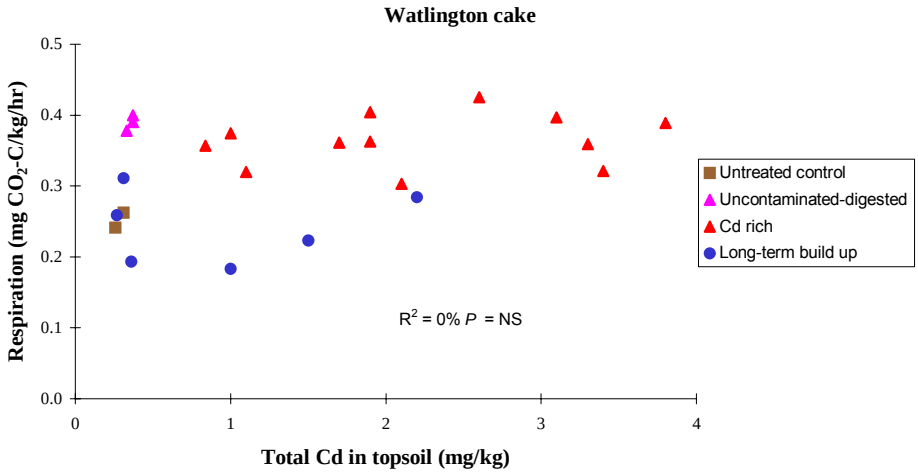
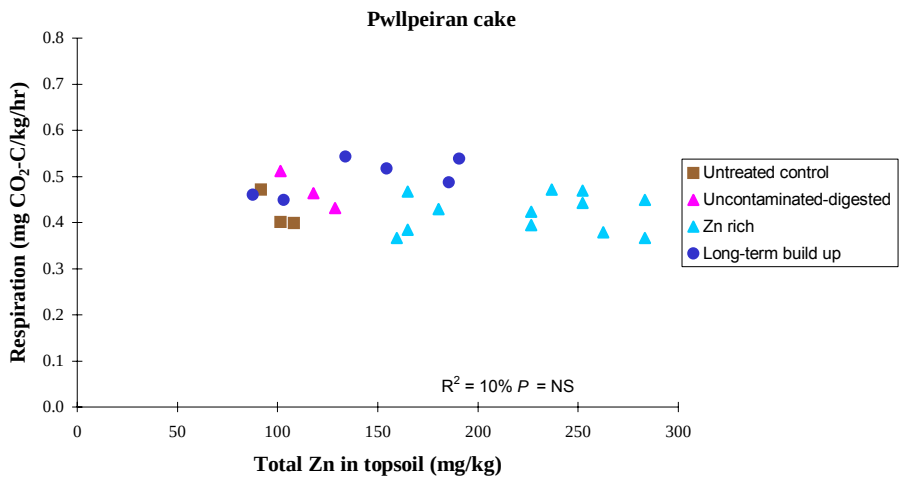
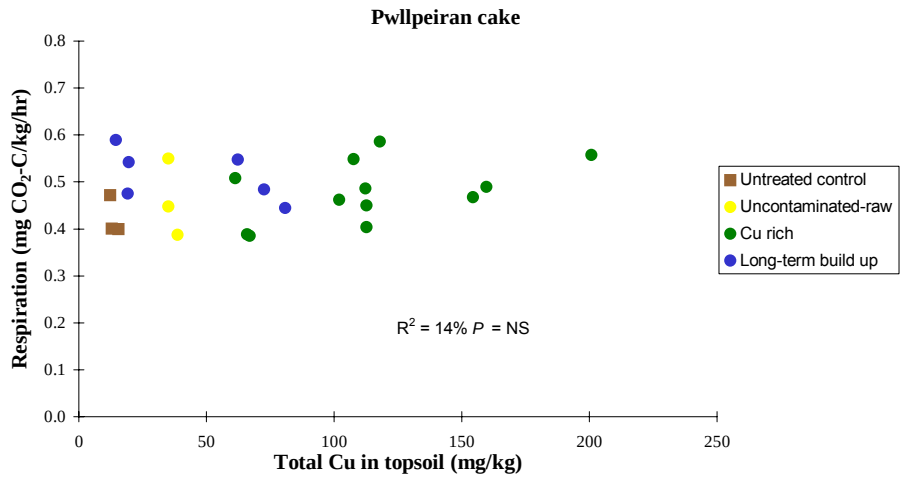


Figure 13. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Pwllpeiran (spring 2005)





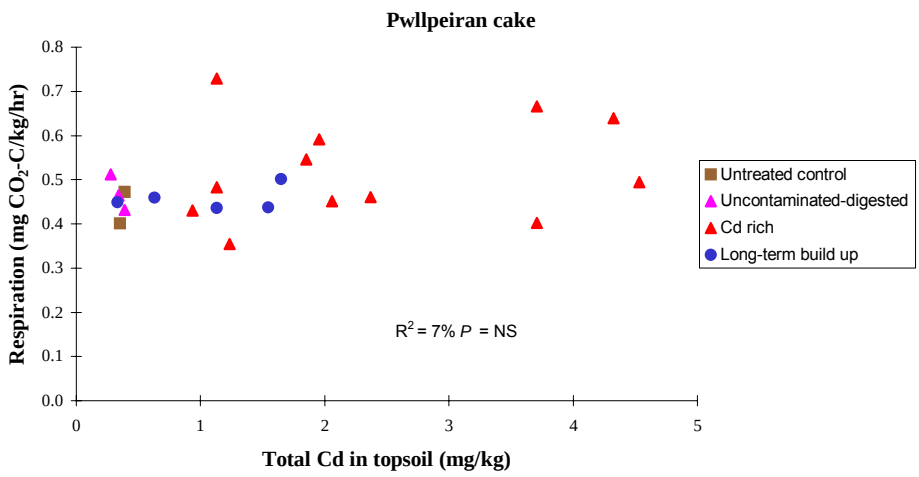
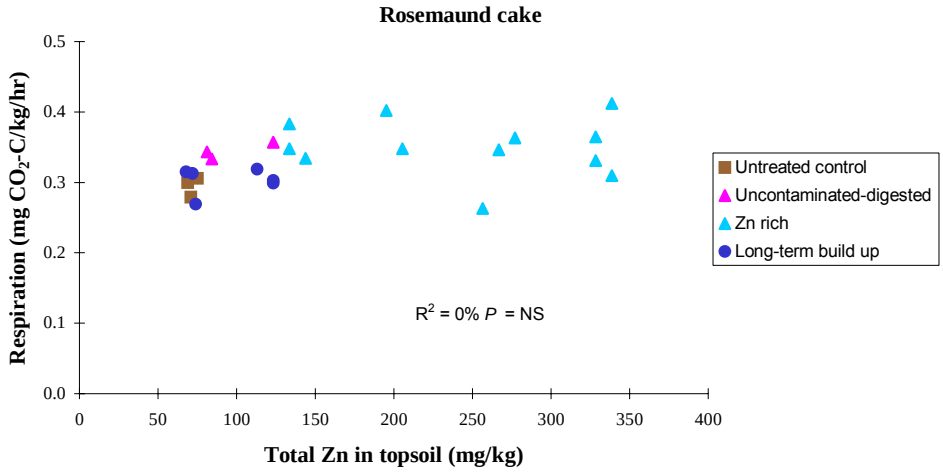
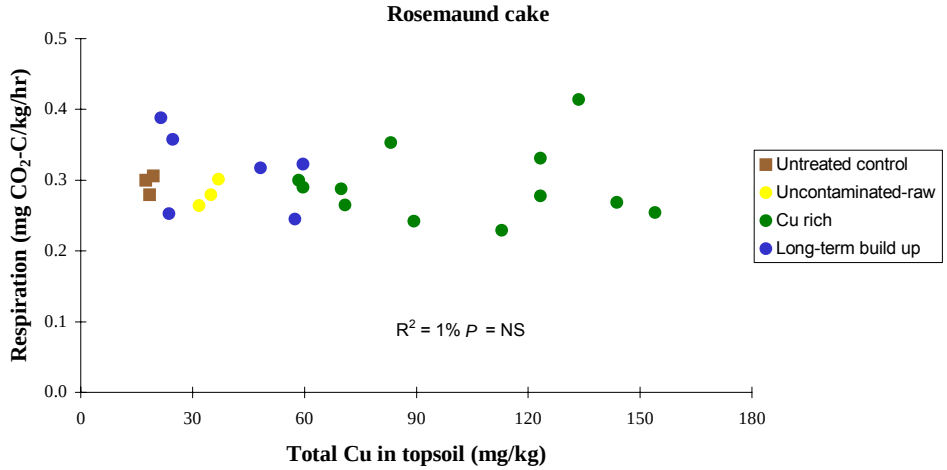


Figure 14. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Rosemaund (spring 2005)





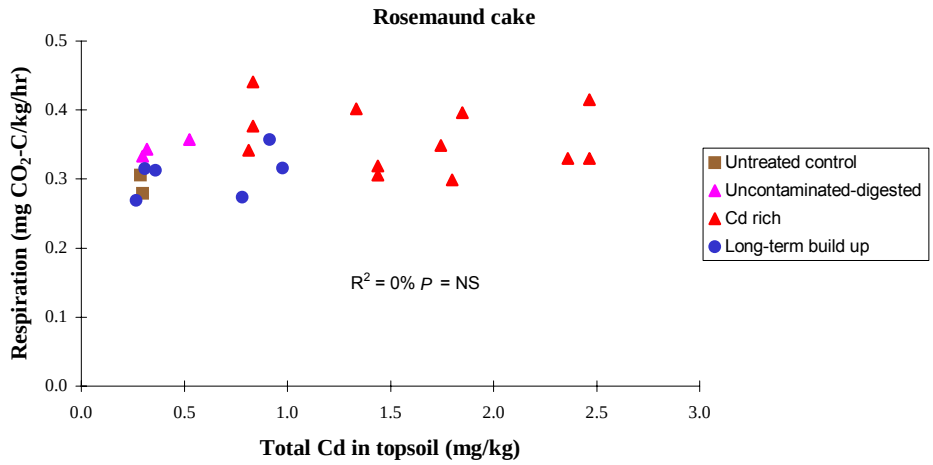


Figure 15. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Bridgets (spring 2005)

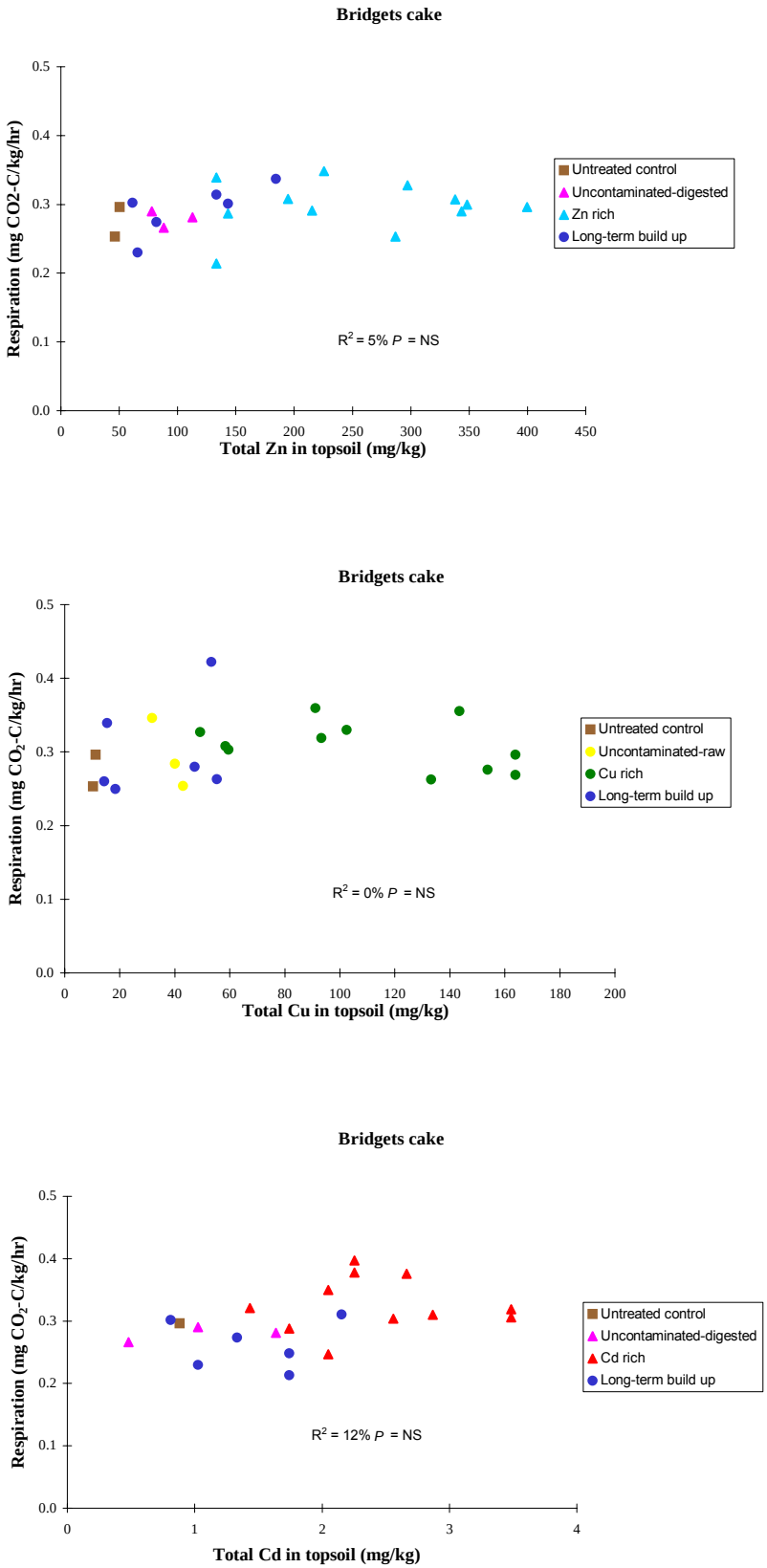


Figure 16. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Hartwood (spring 2005)

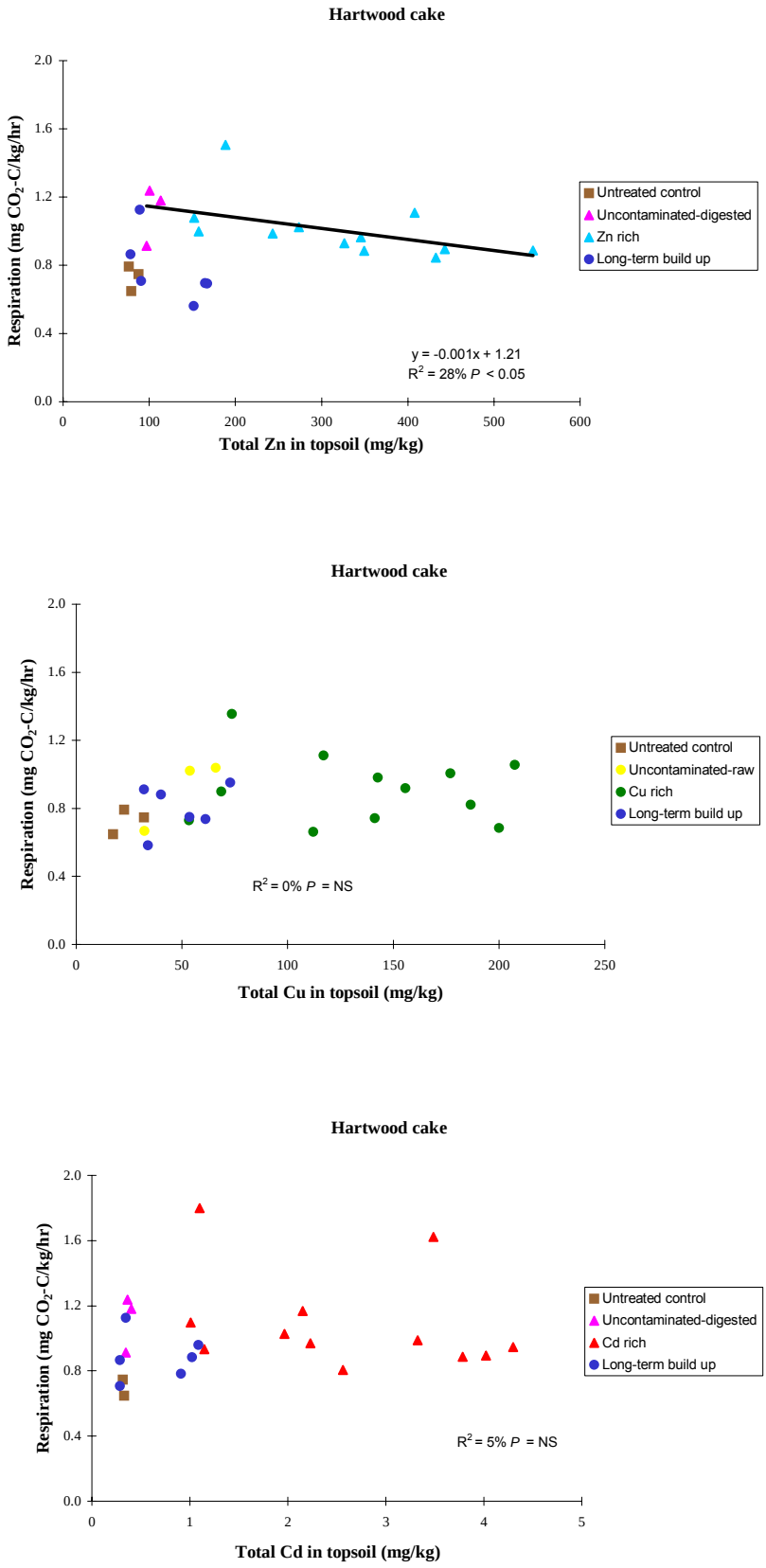


Figure 17. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Auchincruive (spring 2005)

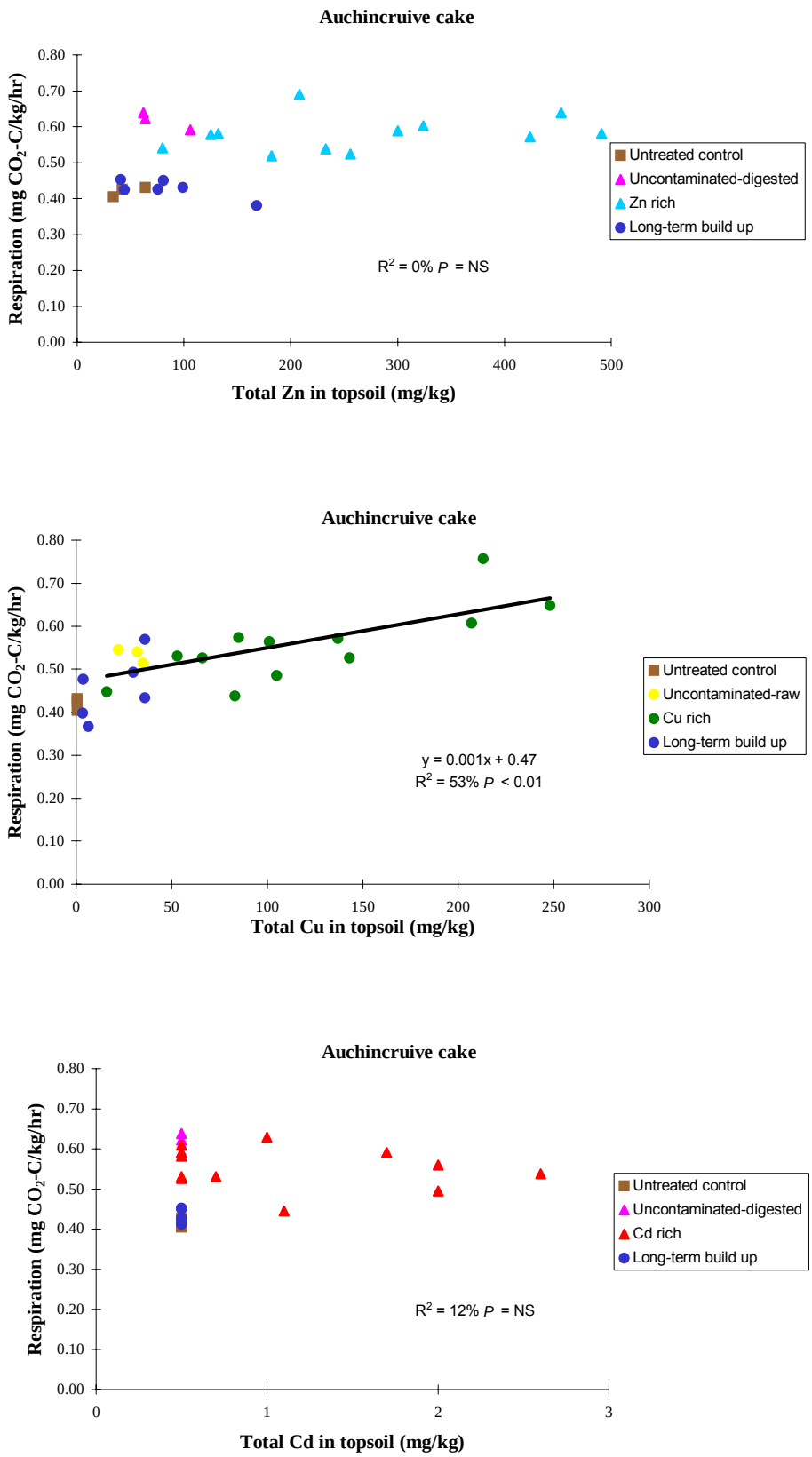
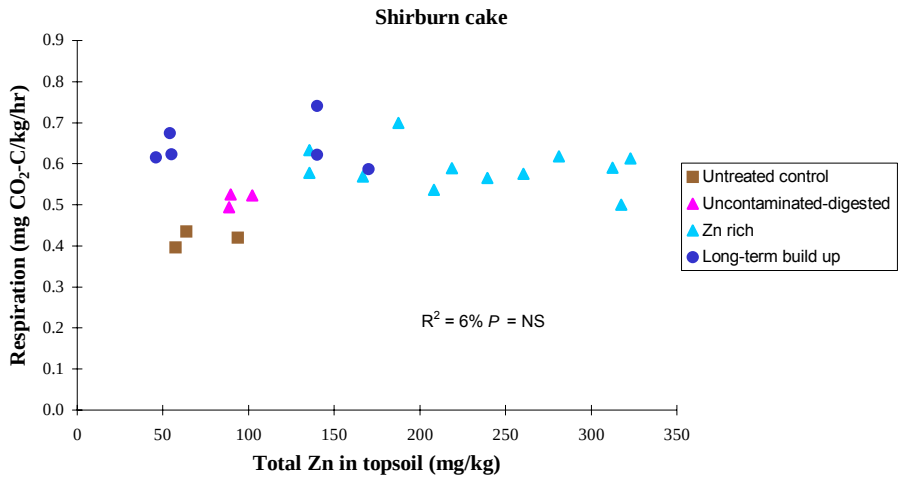
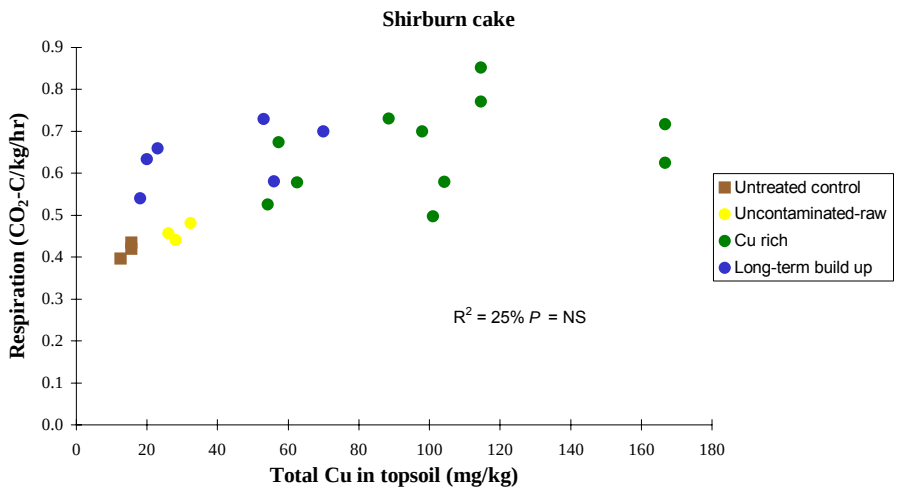


Figure 18. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) - Shirburn (spring 2005)





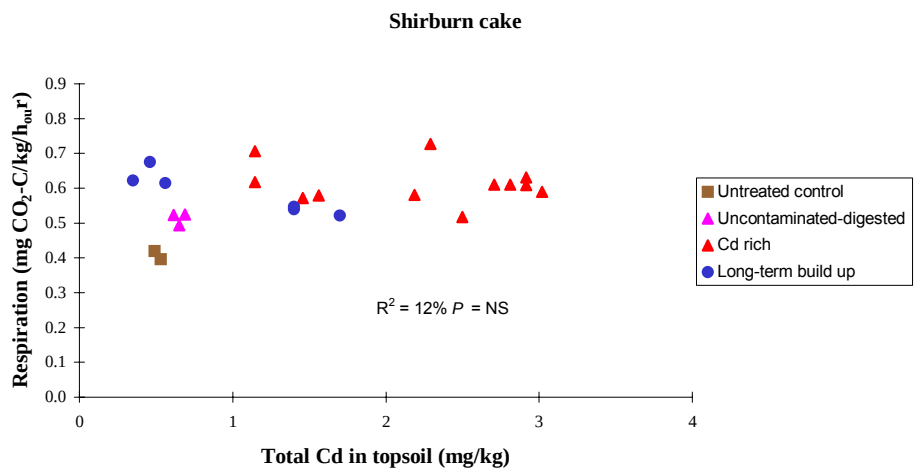


Table 12. Summary of relationships between soil respiration rates (mg CO_2-C/kg/hr) and total topsoil metals - sludge cake sites (spring 2005 samples)

	Zn	Cu (r^2 ; *P*)	Cd
GLE	NS	NS	NS
WOB	NS	NS	NS
WAT	NS	15%; $P < 0.05$	NS
PWL	NS	NS	NS
ROS	NS	NS	NS
BRI	NS	NS	NS
HAR	11%; $P < 0.05$	NS	NS
AUC	NS	18%; $P < 0.01$	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship, Red = inverse relationship. Model parameters for significant responses are given in Figures 10-18.

Significant inverse relationships (P < 0.05) between respiration rates and total topsoil Zn concentrations were evident at Hartwood in both 2003 and 2005, and for Cd at Woburn and Hartwood in 2003. For Cu, significant positive relationships were evident at Watlington and Auchincruive in both 2003 and 2005, and at Rosemaund in 2003.

Microbial biomass carbon concentrations

Soil microbial biomass carbon concentrations were measured on soil samples taken from all the treatment plots in spring 2003 (excluding the long-term build-up treatments) and 2005, with mean treatment mean microbial biomass carbon concentrations summarised in Tables 13 and 14.

Table 13. Mean topsoil (0-25 cm) microbial biomass carbon (mg C/kg oven dry soil) concentrations on treatments sampled in spring 2003.

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR
Control	231	138	277	510	391	319	522	456	803
Unc. Digested	320	216	357	493	444	412	542	518	820
Unc. Raw	317	224	462	561	445	410	572	517	844
Unc. High Dig.	-	-	-	-	-	-	-	-	771
Unc. High Raw	-	-	-	-	-	-	-	-	883
Zn rate 1	267	215	414	484	446	359	503	508	904
Zn rate 2	244	176	367	477	401	353	403	486	784
Zn rate 3	227	181	328	522	367	329	397	476	835
Zn rate 4	192	173	271	457	371	339	310	435	732
Zn rate 5	-	-	-	-	-	-	-	-	*681*
ANOVA (1) p	*NS*	*< 0.001*	*< 0.001*	*NS*	*NS*	*0.019*	*NS*	*NS*	*0.010*
$LSD_{0.05}$	-	32.9	74.6	-	-	75.0	-	-	165.5
CV%	17.0	6.0	8.0	11.0	10.0	8.0	20	10	7
Cu rate 1	325	224	429	490	475	412	541	502	846
Cu rate 2	268	210	410	521	412	338	429	520	846
Cu rate 3	250	197	386	412	443	377	392	465	768
Cu rate 4	197	173	319	521	346	337	273	429	808
Cu rate 5	-	-	-	-	-	-	-	-	*680*
ANOVA (2) p	*0.002*	*0.001*	*< 0.001*	*0.016*	*NS*	*NS*	*NS*	*NS*	*NS*
$LSD_{0.05}$	82.3	52.8	93.5	110.6	-	-	-	-	-
CV%	11.0	10.0	9.0	8.0	12.0	13.0	25	18	9
Cd rate 1	308	217	399	547	468	414	662	512	893
Cd rate 2	270	200	405	513	433	453	509	532	770
Cd rate 3	201	211	394	502	441	420	473	472	877
Cd rate 4	221	217	337	488	410	368	403	486	777
Cd rate 5	-	-	-	-	-	-	-	-	*724*
ANOVA (3) p	*NS*	*0.002*	*0.001*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
$LSD_{0.05}$		52.5	75.6	-	-	-	-	-	-
CV%	23.0	9.0	7.0	9.0	12.0	15.0	25	15	8

Three ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control and the appropriate uncontaminated sludge control. The ANOVA fitted to each group was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05).

Table 14. Mean topsoil (0-25 cm) microbial biomass carbon (mg C/kg oven dry soil) concentrations on treatments sampled in spring 2005.

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR
Control	221	192	287	389	263	325	506	498	874
Unc. Digested	316	265	372	426	345	392	593	596	961
Unc. Raw	320	268	473	412	454	420	572	586	873
Unc. High Dig.	-	-	-	-	-	-	-	-	1006
Unc. High Raw	-	-	-	-	-	-	-	-	974
Zn rate 1	237	229	359	425	393	367	424	531	860
Zn rate 2	249	251	312	411	280	389	518	517	875
Zn rate 3	244	140	301	417	318	304	464	600	867
Zn rate 4	190	143	279	420	234	316	364	490	767
Zn rate 5	-	-	-	-	-	-	-	-	808
ANOVA (1) p	*0.015*	*0.011*	*NS*	*NS*	*NS*	*0.021*	*NS*	*0.002*	*0.009*
$LSD_{0.05}$	92.2	113	-	-	-	89.5	-	80.8	181
CV%	13	20	14	8.0	22	9.0	22	5.0	7.0
Cu rate 1	281	292	417	413	345	384	525	610	946
Cu rate 2	238	278	388	444	423	416	470	569	907
Cu rate 3	231	247	364	397	350	444	418	503	844
Cu rate 4	233	189	319	397	348	357	308	597	855
Cu rate 5	-	-	-	-	-	-	-	-	736
ANOVA (2) p	*0.009*	*0.001*	*0.006*	*NS*	*0.034*	*NS*	*NS*	*NS*	*0.048*
$LSD_{0.05}$	78.6	68.7	129	-	168	-	-	-	215
CV%	11	10	12	9.0	16	12	23	8	9.0
Cd rate 1	313	252	333	433	382	448	561	572	990
Cd rate 2	296	266	396	420	338	415	494	567	873
Cd rate 3	236	238	378	430	392	411	588	509	932
Cd rate 4	264	267	382	408	335	404	394	531	844
Cd rate 5	-	-	-	-	-	-	-	-	851
ANOVA (3) p	*NS*	*NS*	*0.020*	*NS*	*NS*	*0.019*	*NS*	*NS*	*NS*
$LSD_{0.05}$	-	-	93.4	-	-	93.2	-	-	-
CV%	15	17	9.0	7.0	25	8.0	22	11	6.0
LT Unc. Dig.	245	190	378	430	343	347	451	498	940
LT Unc. Raw	254	234	311	414	341	372	453	492	861
LT Zn	198	200	278	394	288	377	329	519	884
LT Cu	209	216	314	406	312	344	480	514	911
LT Cd	210	174	342	358	293	337	455	458	882
ANOVA (4) p	*NS*	*NS*	*0.020*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
$LSD_{0.05}$	-	-	84.8	-	-	-	-	-	-
CV%	16	19	9.0	7.0	26	13	17	8.0	7.0

Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. The ANOVA fitted to each group was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05).

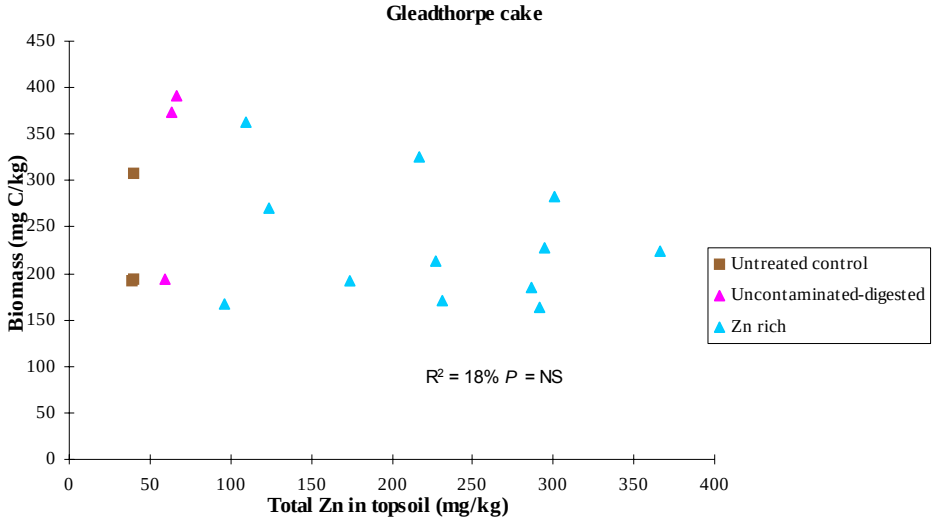
2003 sampling: At Woburn, the soil microbial biomass C concentrations on the Zn rate 1-4 treatments were 35-78 mg C/kg ($P < 0.05$) higher than the untreated control, but 35-43 mg C/kg ($P < 0.05$) lower than the uncontaminated digested treatment. At Watlington, the soil microbial biomass on the Zn rate 1 and 2 treatments was 90-137 mg C/kg ($P < 0.05$) higher than the untreated control. At Bridgets, the soil microbial biomass on the rate 4 treatment was 90 mg C/kg ($P < 0.05$) lower than the uncontaminated digested treatment. At Shirburn, the soil microbial biomass on the Zn rate 4 and 5 treatments was 172-223 mg C/kg ($P < 0.05$) lower than the Zn rate 1 treatment. There were no differences ($P > 0.05$) between the Zn rich sludge cake treatments and either the uncontaminated digested or untreated controls at any of the other five sites.

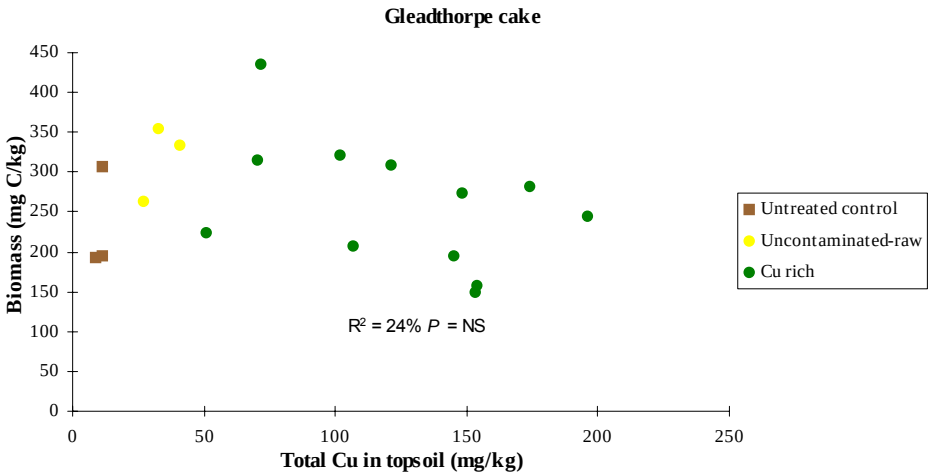
At Gleadthorpe, the soil microbial biomass carbon concentration on the Cu rate 4 treatment was 120 mg C/kg ($P < 0.05$) lower than the uncontaminated raw treatment. At Woburn, soil microbial biomass carbon concentrations on the Cu rate 1-3 treatments were 59-86 mg C/kg ($P < 0.05$) higher than the untreated control. At Watlington, microbial biomass carbon concentrations on the Cu rate 1-3 treatments were between 109 - 152 mg C/kg higher than the untreated control ($P < 0.05$), but the biomass carbon concentration on the Cu rate 4 treatment was 38 mg C/kg ($P < 0.05$) lower than the uncontaminated raw treatment. At Pwllpeiran, the soil microbial biomass carbon concentration was 149 mg C/kg lower on the Cu rate 3 ($P < 0.05$) treatment than the uncontaminated raw treatment. There were no differences ($P > 0.05$) between the Cu rich sludge cake treatments and either the uncontaminated raw or untreated controls at any of the other five sites.

At Woburn and Watlington, all of the Cd rich treatments had higher soil microbial biomass carbon concentrations ($P < 0.05$) of between 62-79 mg C/kg and 59-128 mg C/kg, than the corresponding untreated control treatments, respectively. There were no differences ($P > 0.05$) between the Cd rich sludge cake treatments and either the uncontaminated digested or untreated controls at any of the other seven sites.

Soil microbial biomass C concentrations were regressed against total topsoil metal concentrations (Figures 19-27). On the metal dose-response treatments, regression of the soil microbial biomass C values against total topsoil Zn concentrations identified significant inverse relationships ($P < 0.05$) at six sites (Woburn, Watlington, Rosemaund, Bridgets, Hartwood and Auchincruive), for Cu there were significant ($P < 0.05$) inverse relationships at three sites (Woburn, Watlington and Hartwood) and for Cd an inverse relationship at Gleadthorpe ($P < 0.05$). A summary of the regression analyses is given in Table 15.

Figure 19. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) – Gleadthorpe (spring 2003)





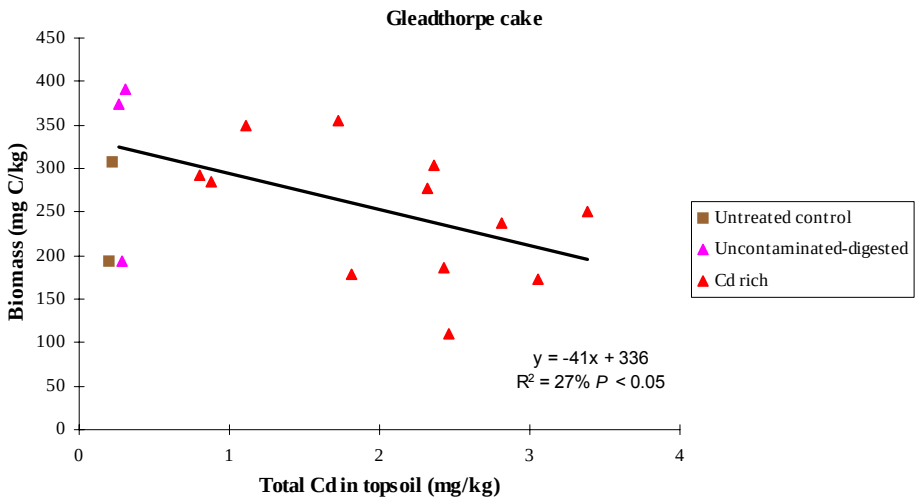
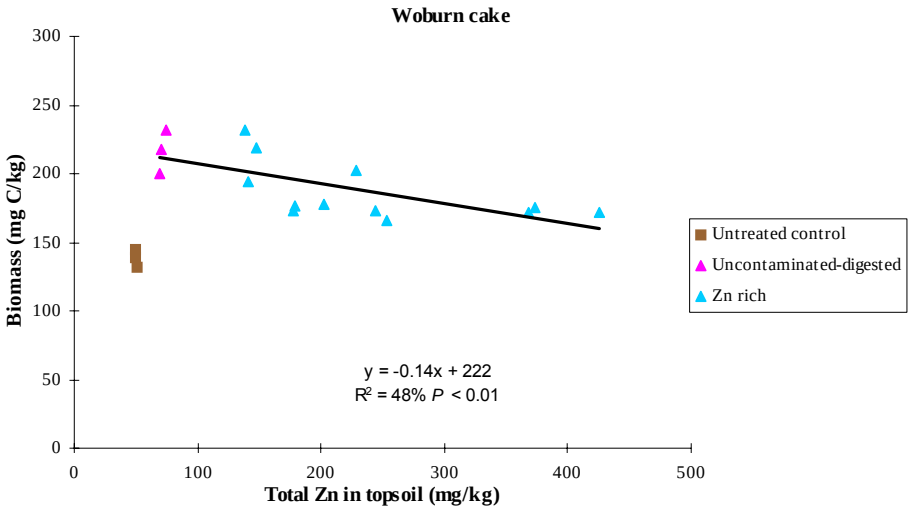
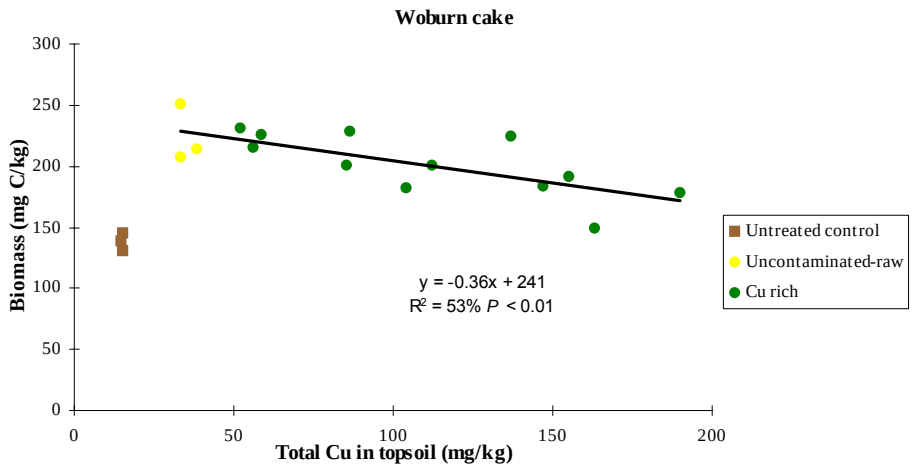


Figure 20. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Woburn (spring 2003)





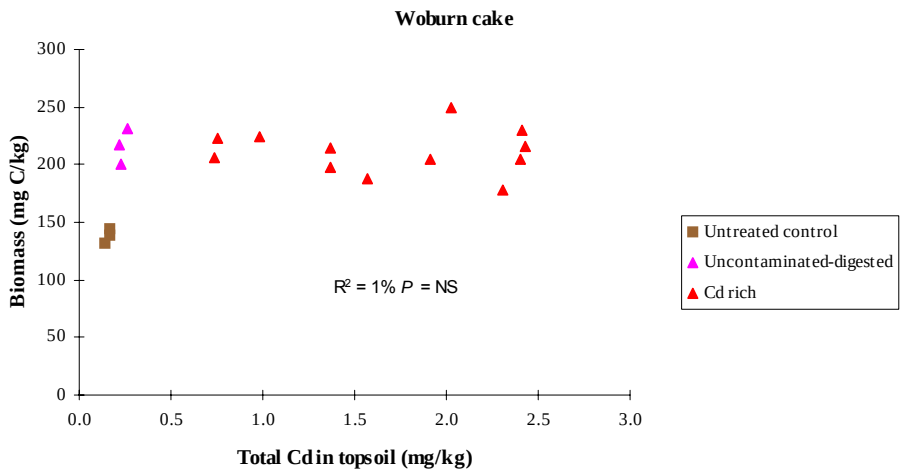


Figure 21. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Watlington (spring 2003)

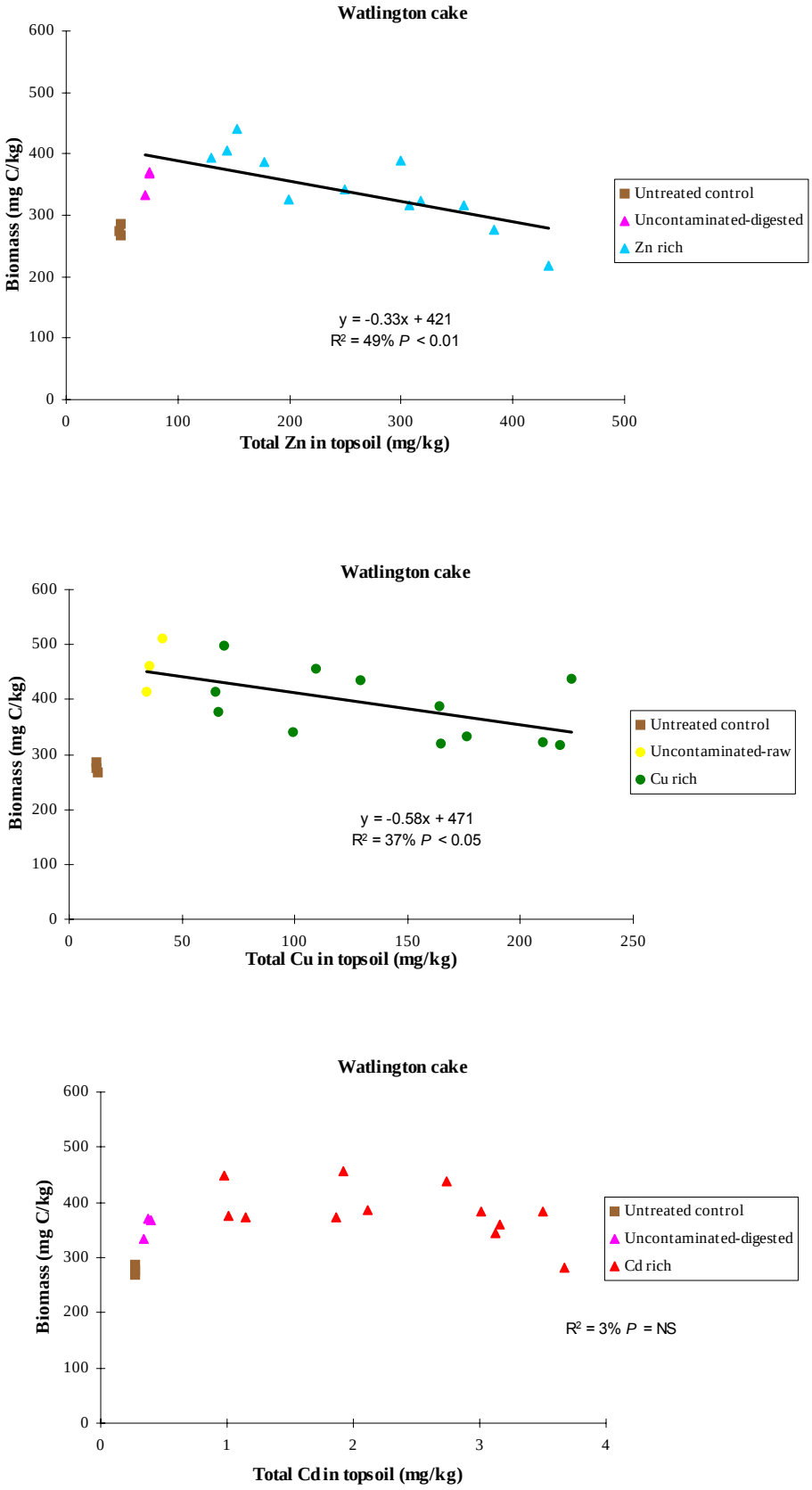


Figure 22. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Pwllpeiran (spring 2003)

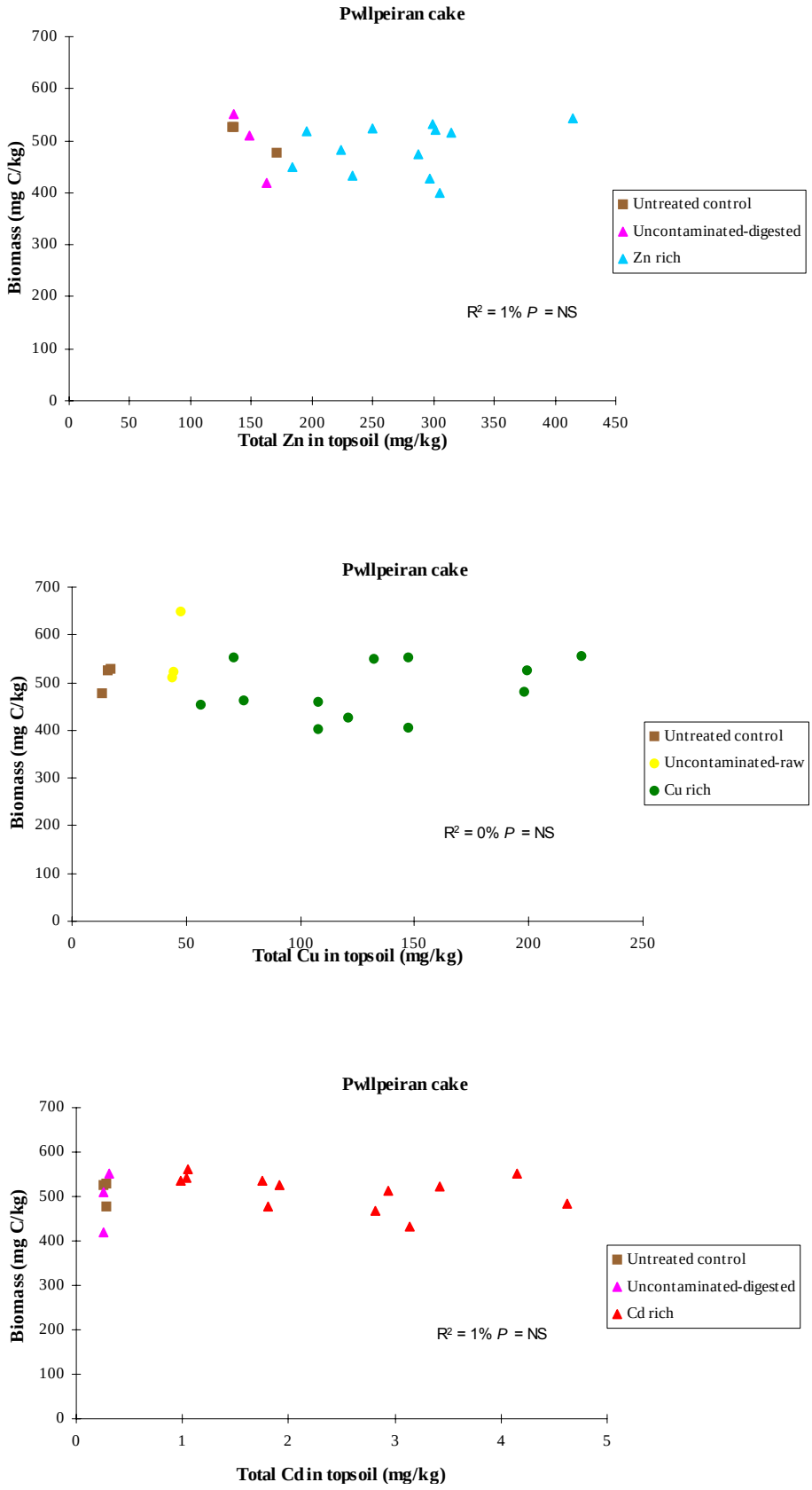


Figure 23. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Rosemaund (spring 2003)

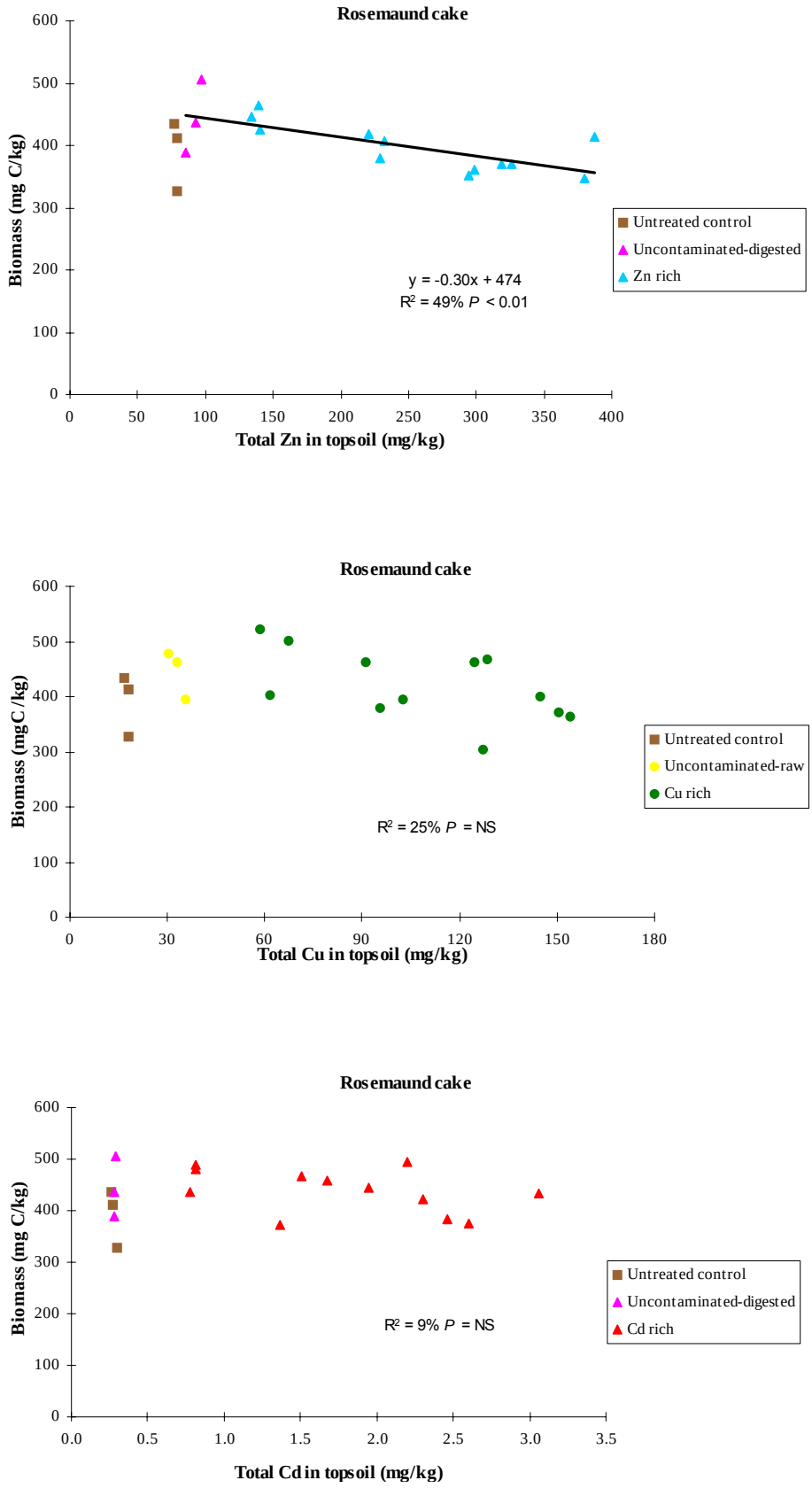
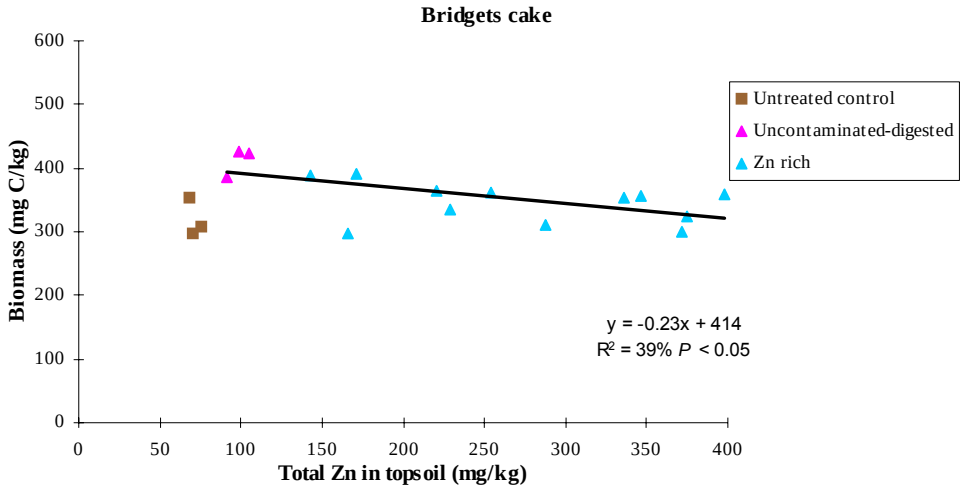
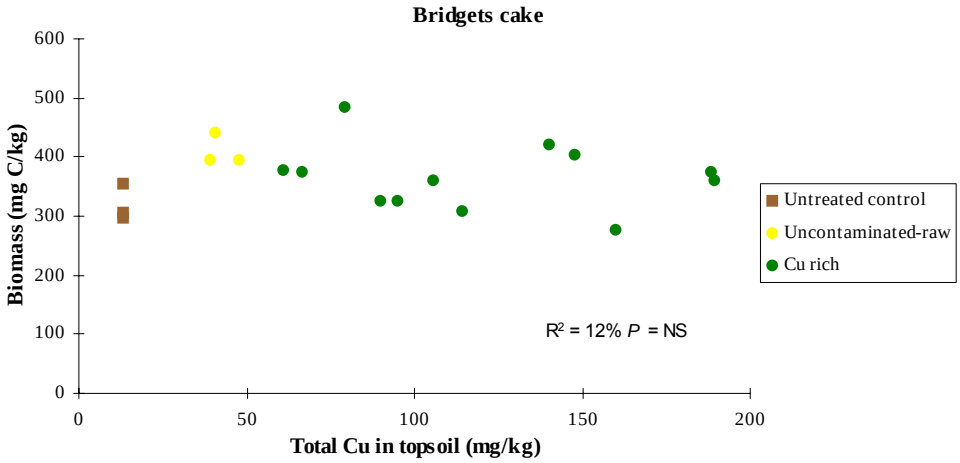


Figure 24. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Bridgets (spring 2003)





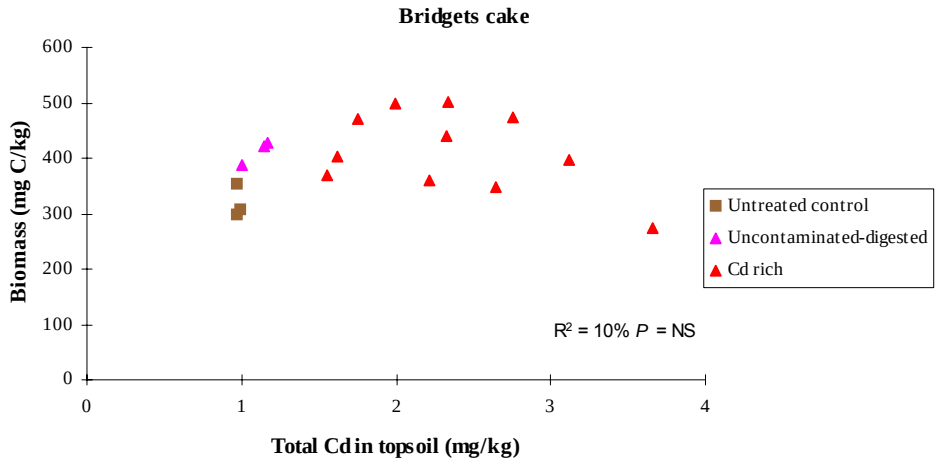
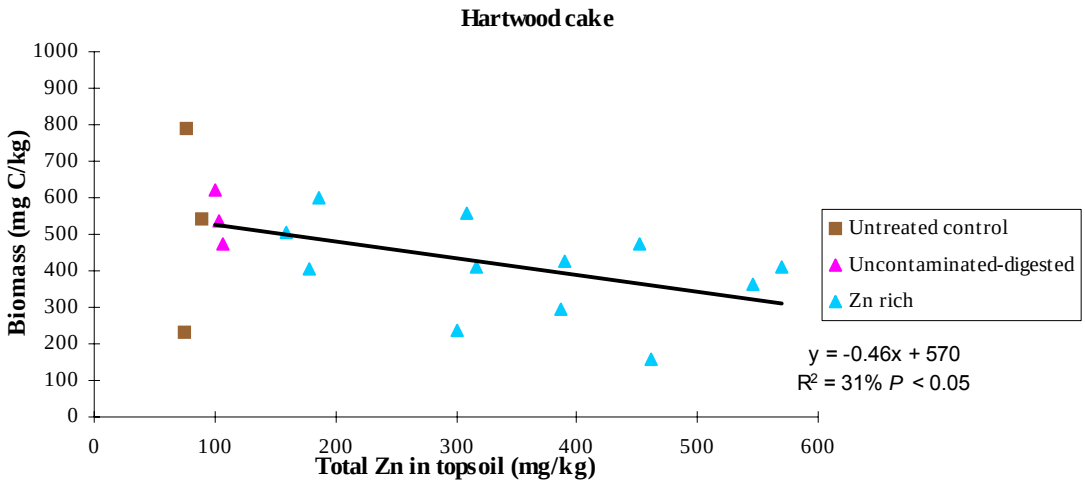
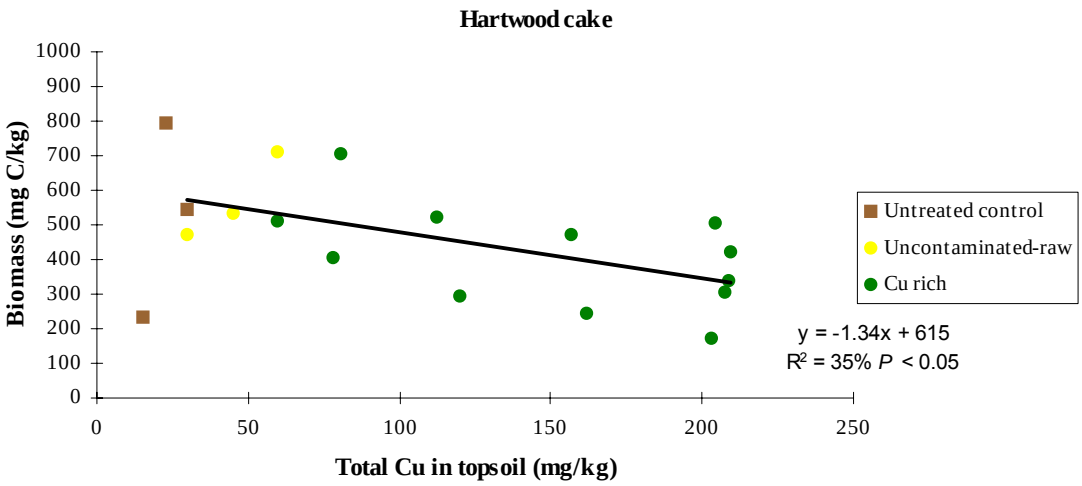


Figure 25. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Hartwood (spring 2003)





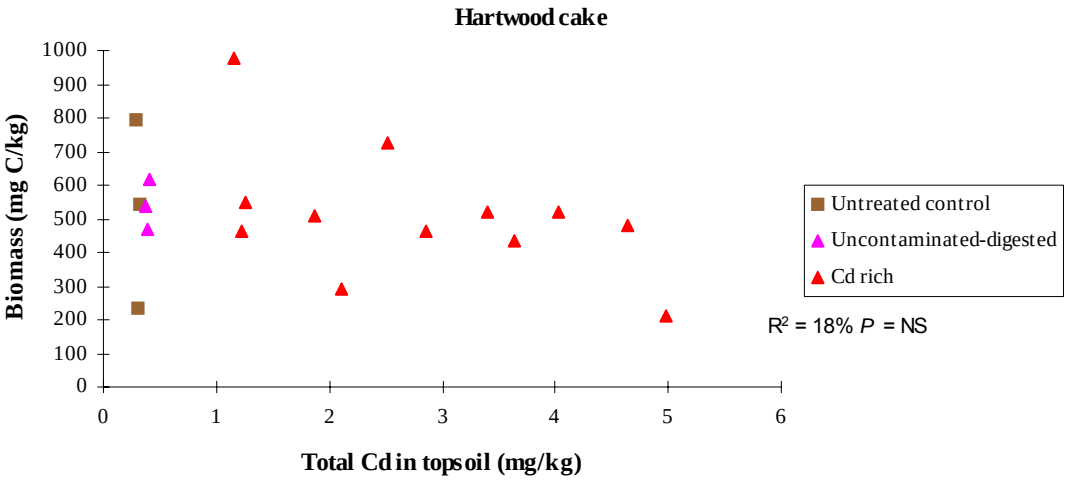
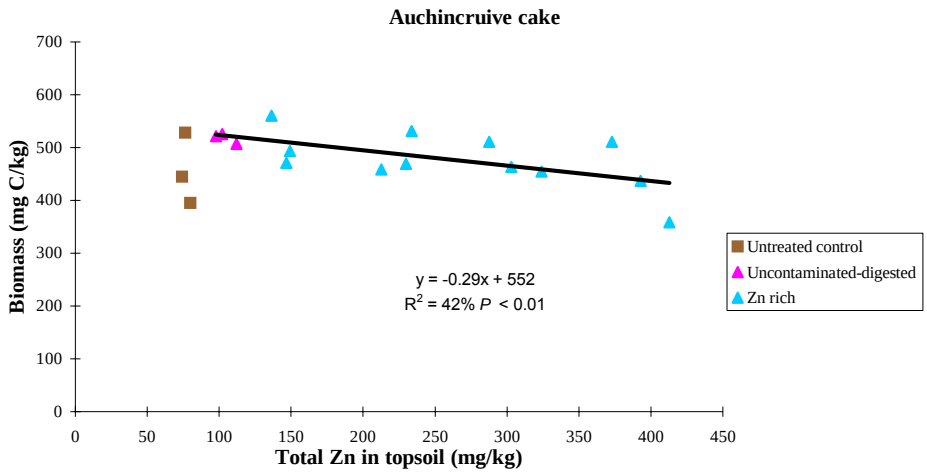
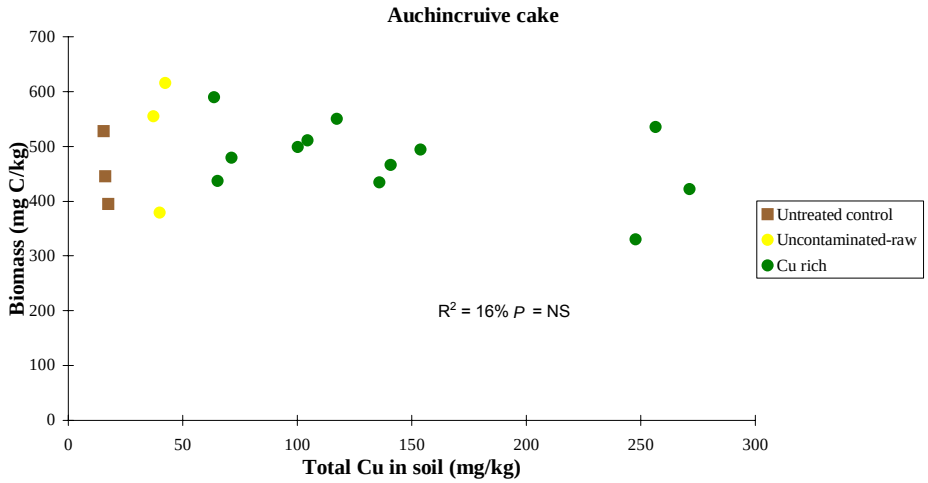


Figure 26. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Auchincruive (spring 2003)





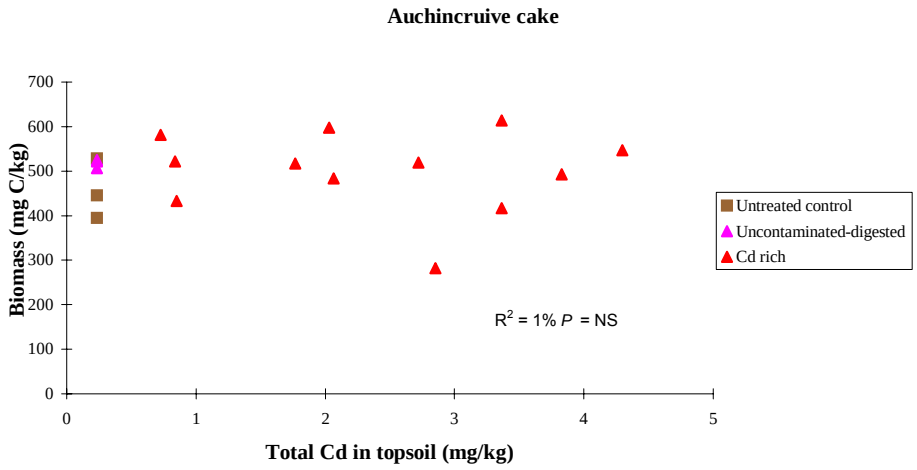


Figure 27. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Shirburn (spring 2003)

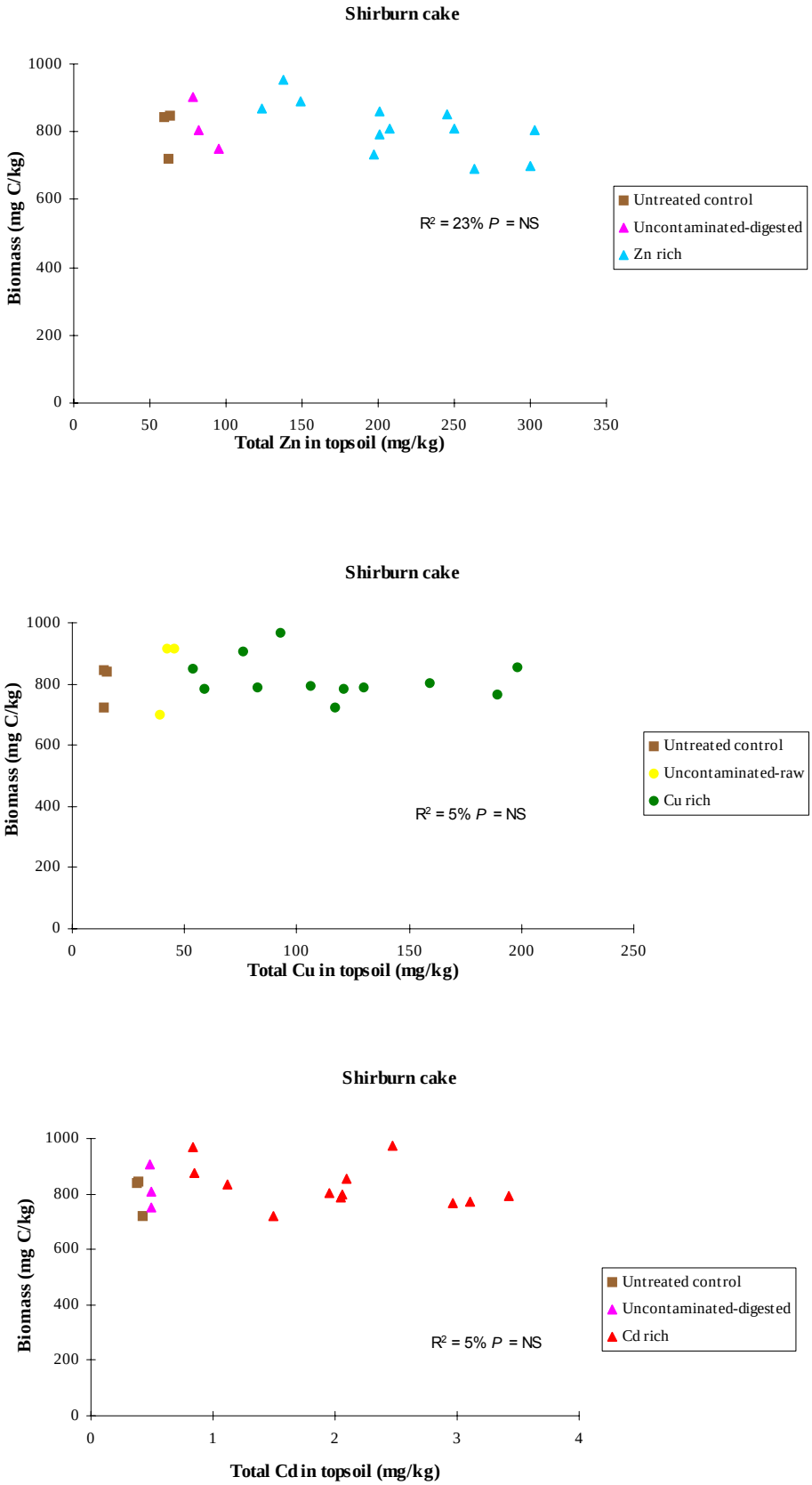


Table 15. Summary of relationships between soil biomass C concentration (mg/kg) and total topsoil metals - sludge cake sites (spring 2003 samples)

	Zn	Cu (r^2 ; *P*)	Cd
GLE	NS	NS	35%; $P < 0.05$
WOB	16%; $P < 0.01$	16%; $P < 0.01$	NS
WAT	19%; $P < 0.01$	13%; $P < 0.05$	NS
PWL	NS	NS	NS
ROS	14%; $P < 0.01$	NS	NS
BRI	12%; $P < 0.05$	NS	NS
HAR	17%; $P < 0.05$	25%; $P < 0.05$	NS
AUC	11%; $P < 0.01$	NS	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship. Model parameters for significant responses are given in Figures 19-27.

2005 sampling: At Gleadthorpe and Auchincruive on the uncontaminated digested sludge cake treatments, soil microbial biomass C concentrations were 95-98 mg C/kg higher ($P < 0.05$) than the untreated control treatments. On the Zn-rich rate 4 treatment at Gleadthorpe, soil microbial biomass carbon concentrations were 126 mg C/kg lower ($P < 0.05$) than the uncontaminated digested sludge cake treatment. At Woburn, on the Zn-rich rate 3 and 4 treatments soil microbial biomass carbon concentrations were 122-125 mg C/kg lower ($P < 0.05$) than the uncontaminated digested sludge cake treatment. At Bridgets, soil microbial biomass carbon concentrations on the Zn rate 3 treatment were 88 mg C/kg ($P < 0.05$) lower than the uncontaminated digested sludge cake treatment. At Auchincruive, soil microbial biomass carbon concentrations on the Zn-rich rate 4 treatment were 106 mg C/kg lower ($P < 0.01$) than the uncontaminated digested sludge cake treatment. At Shirburn, on the Zn-rich rate 4 treatment soil microbial biomass C concentrations were 194 mg C/kg lower ($P < 0.01$) than the uncontaminated digested sludge cake treatment, and on the Zn-rich rate 5 treatment 198 mg C/kg lower ($P < 0.01$) than the high rate uncontaminated digested sludge cake treatment. There were no differences ($P > 0.05$) between the Zn-rich sludge cake treatments and either the uncontaminated digested sludge cake or untreated control treatments at any of the other four sites.

At Gleadthorpe, Woburn, Watlington and Rosemaund on the uncontaminated raw sludge cake treatments soil microbial biomass C concentrations were 76-190 mg C/kg higher ($P < 0.05$) than the untreated control treatments. On the Cu-rich rate 2-4 treatments at

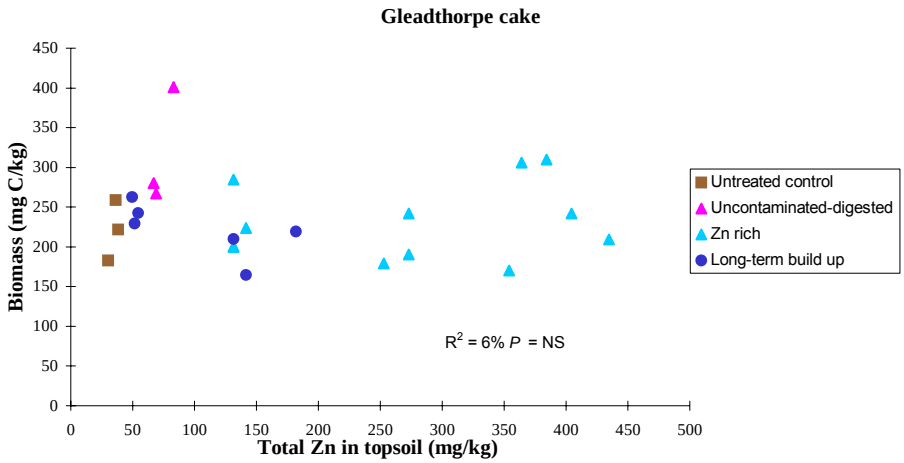
Gleadthorpe, soil microbial biomass carbon concentrations were 82-89 mg C/kg lower ($P < 0.05$) than the uncontaminated raw sludge cake treatment. At Woburn, soil microbial biomass C concentrations on the Cu-rich rate 4 treatment were 79 mg C/kg lower ($P < 0.001$) than the uncontaminated raw sludge cake treatment. At Watlington, soil microbial biomass C concentrations on the Cu-rich rate 4 treatment were 154 mg C/kg lower ($P < 0.01$) than the uncontaminated raw sludge cake treatment. At Shirburn, the soil microbial biomass carbon concentration on the Cu-rich rate 5 treatment was 238 mg C/kg lower ($P < 0.05$) than the high rate uncontaminated raw sludge cake treatment. There were no differences ($P > 0.05$) between the Cu-rich sludge cake treatments and either the uncontaminated raw sludge cake or untreated controls at any of the other four sites.

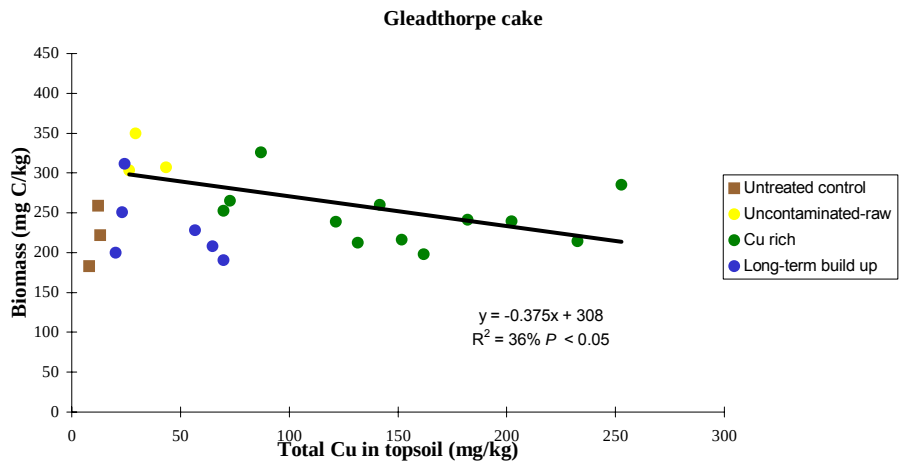
At Watlington, on the Cd-rich rate 2 and 4 treatments soil microbial biomass C concentrations were 95-109 mg C/kg higher ($P < 0.05$) than the untreated control, but not significantly ($P > 0.05$) different from the uncontaminated digested sludge cake treatment. At Bridgets, on the Cd-rich rate 1 treatment the soil microbial biomass C concentration was 123 mg C/kg higher ($P < 0.05$) than the untreated control, but again concentrations were not significantly ($P > 0.05$) different from the uncontaminated digested sludge cake treatment. There were no differences ($P > 0.05$) in soil microbial biomass carbon concentrations between the Cd-rich sludge cake treatments and either the uncontaminated digested sludge cake or untreated controls at any of the other seven sites.

There were no differences in soil microbial biomass carbon concentrations ($P > 0.05$) between the untreated controls and the long-term build-up treatments at eight of the sites. However, at Watlington the long-term build-up uncontaminated digested sludge cake treatment had a 91 mg C/kg higher soil biomass C concentration ($P < 0.05$) than the untreated control, while the soil microbial biomass C concentration on the long-term Zn build-up treatment was 100 mg C/kg lower ($P < 0.05$) than the long-term uncontaminated digested sludge cake treatment.

Soil microbial biomass C concentrations were regressed against total topsoil metal concentrations (Figures 28-36). On the metal dose-response treatments, regression of the soil microbial biomass C concentrations against total topsoil metal concentrations identified significant inverse relationships ($P < 0.05$) at five sites for both Zn (Woburn, Watlington, Rosemaund, Bridgets and Shirburn) and Cu (Gleadthorpe, Woburn, Watlington, Rosemaund and Hartwood). A summary of the regression analyses is given in Table 16.

Figure 28. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) – Gleadthorpe (spring 2005)





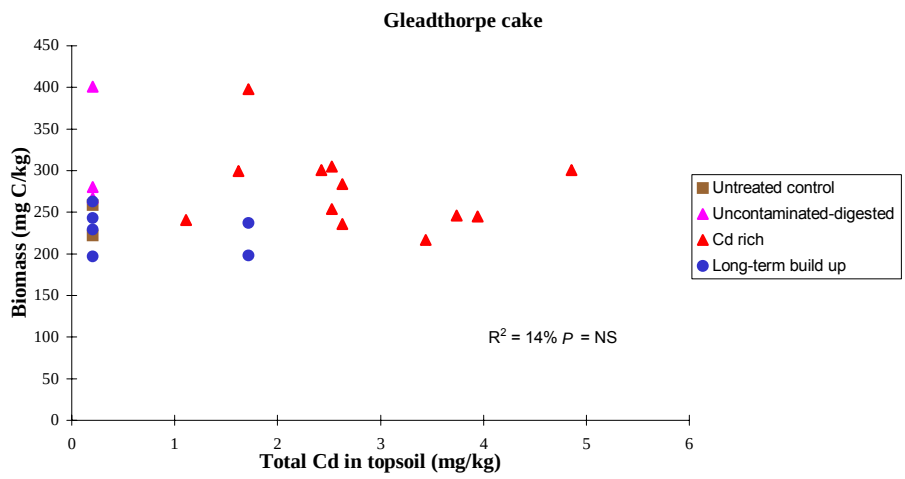


Figure 29. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Woburn (spring 2005)

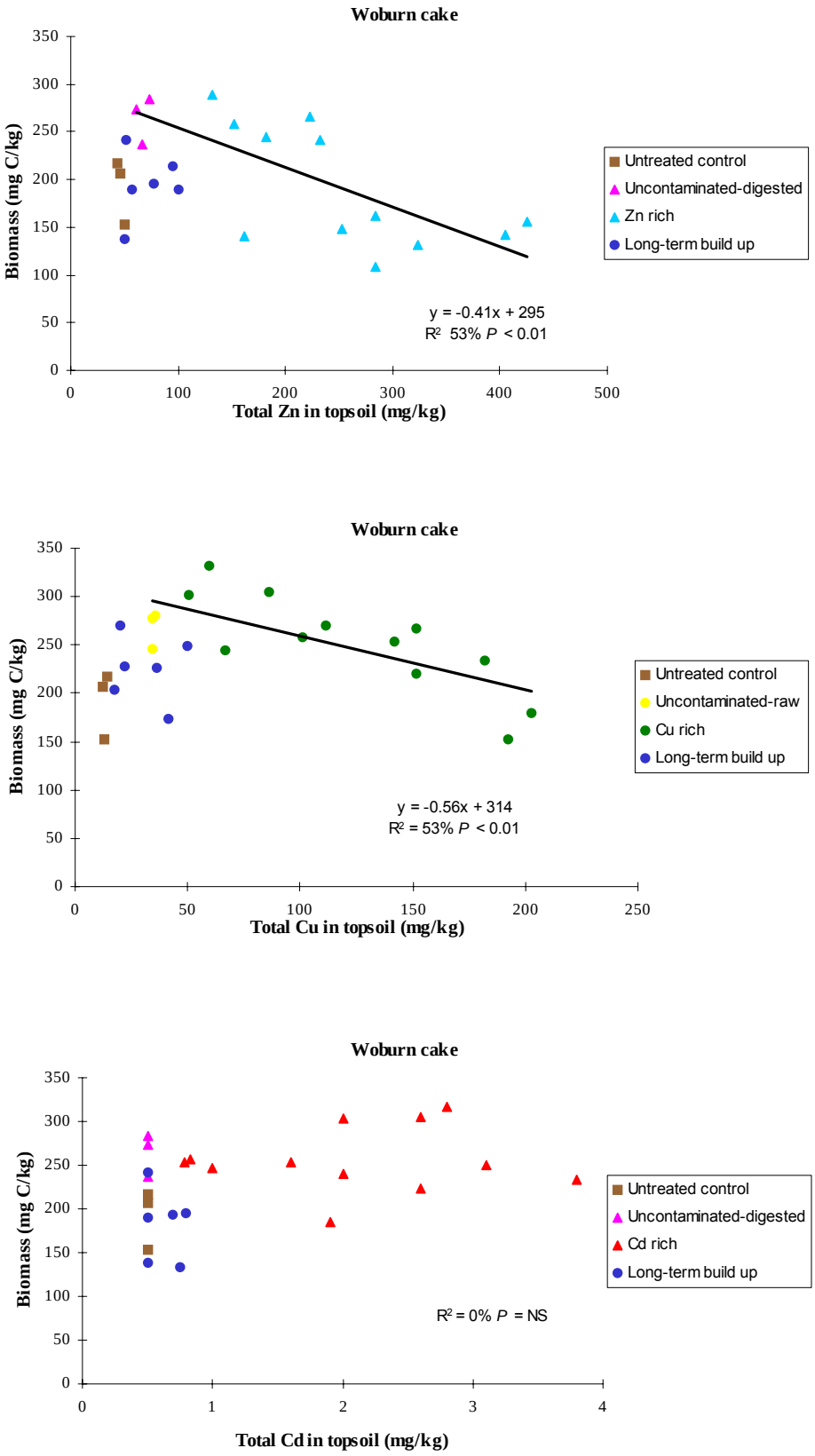


Figure 30. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Watlington (spring 2005)

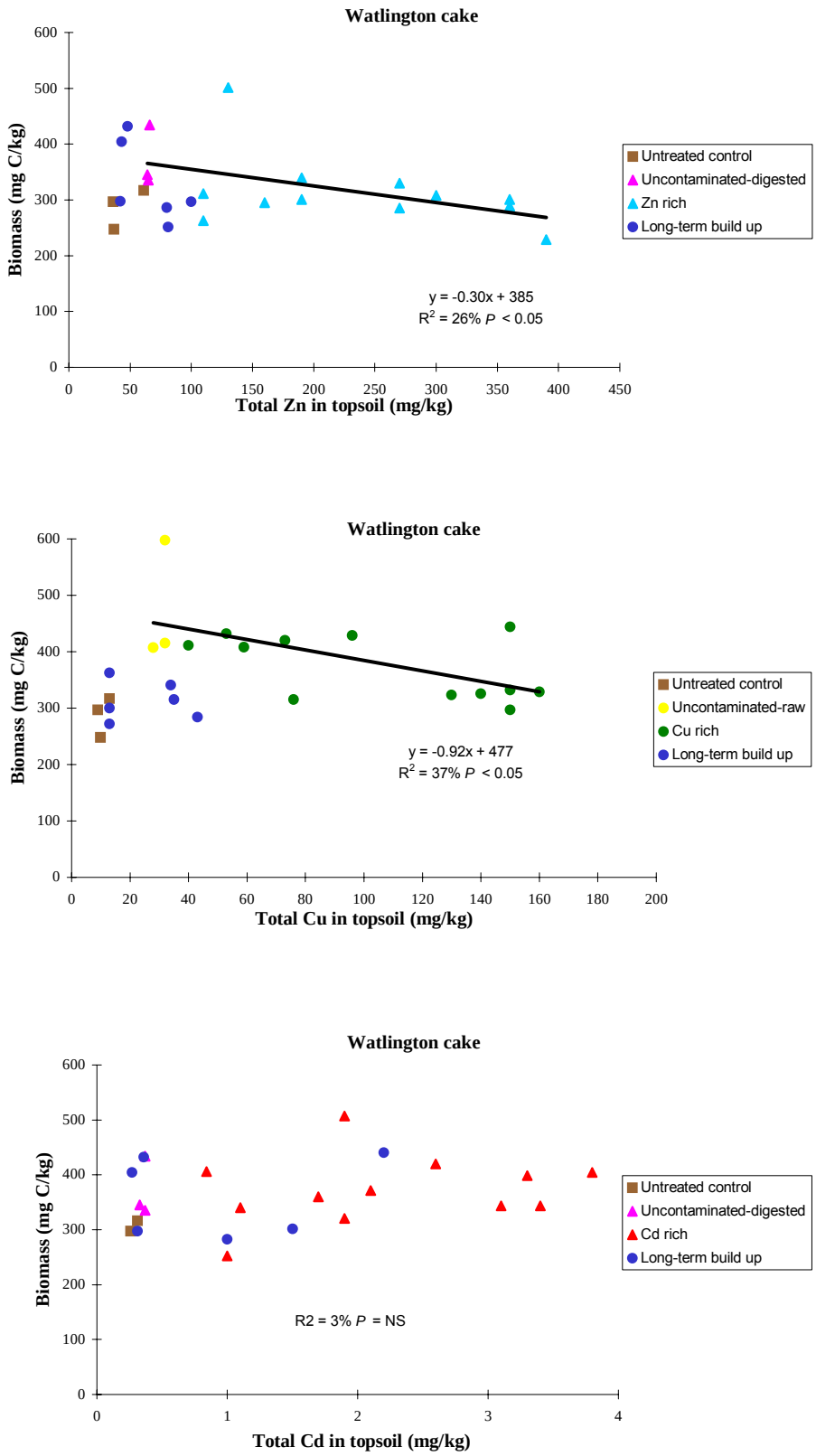


Figure 31. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Pwllpeiran (spring 2005)

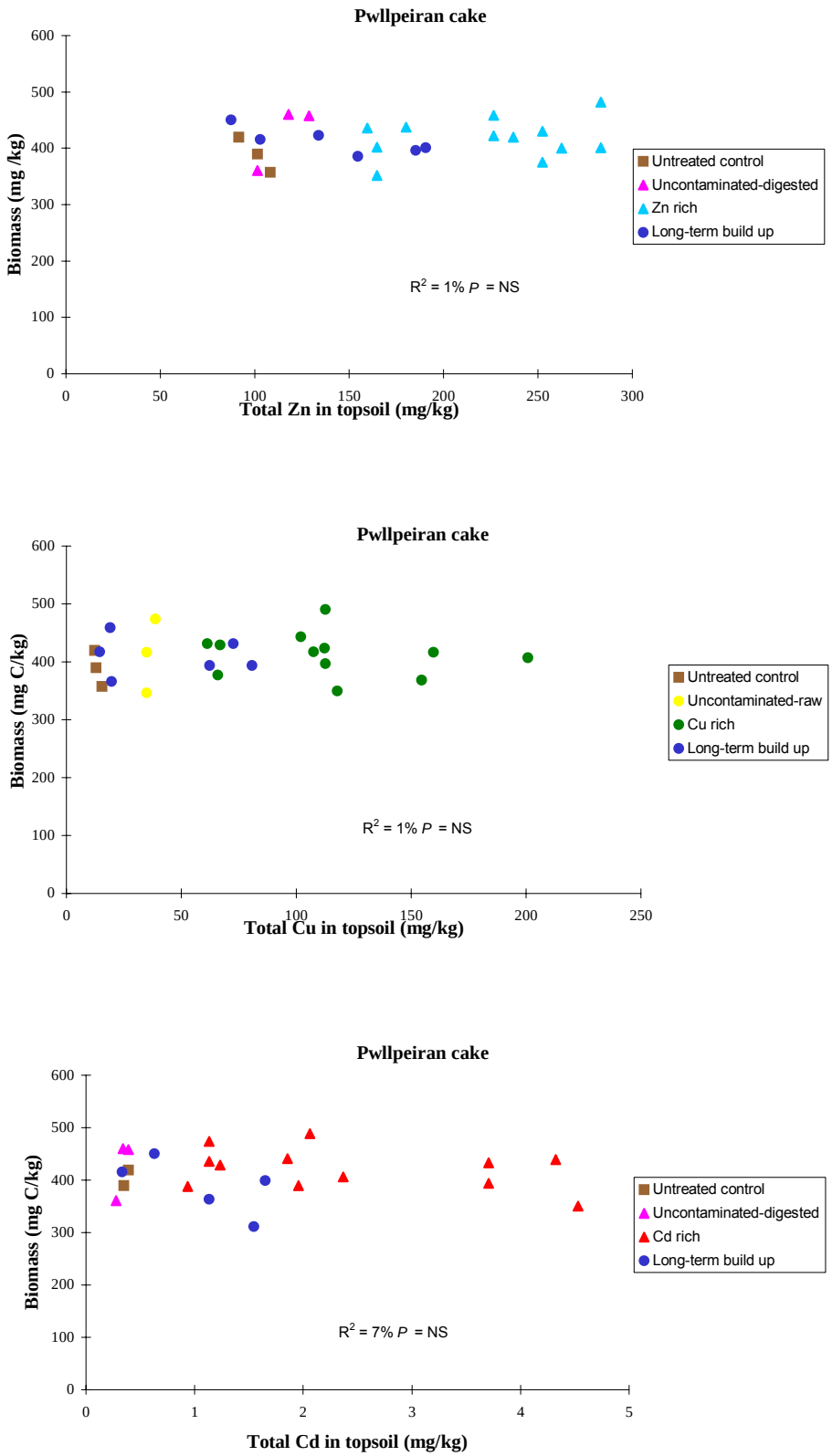


Figure 32. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Rosemaund (spring 2005)

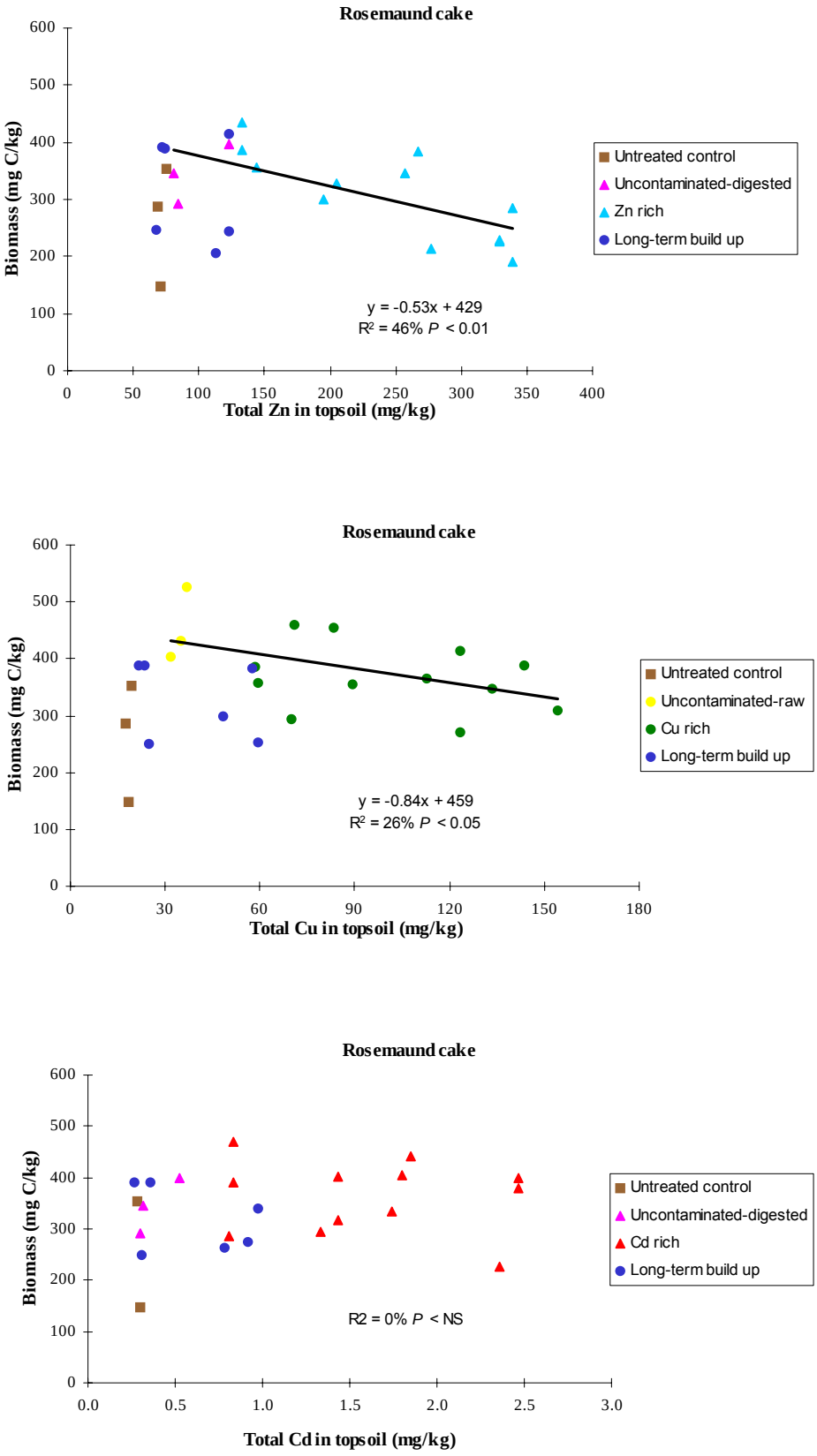
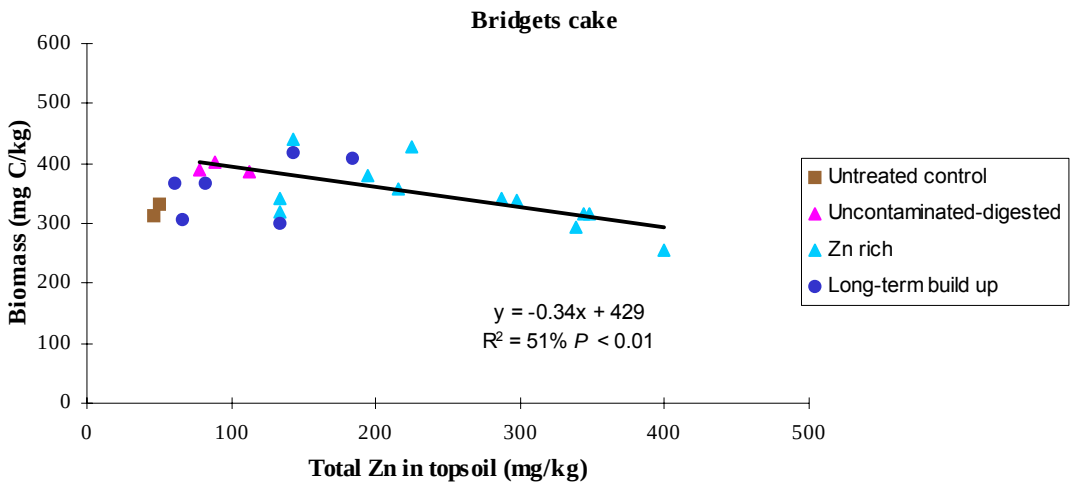
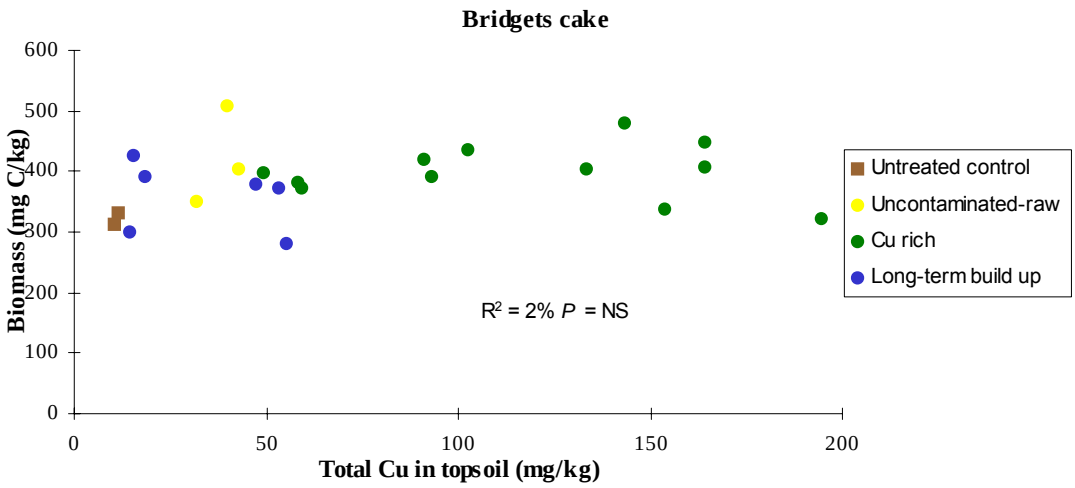


Figure 33. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Bridgets (spring 2005)





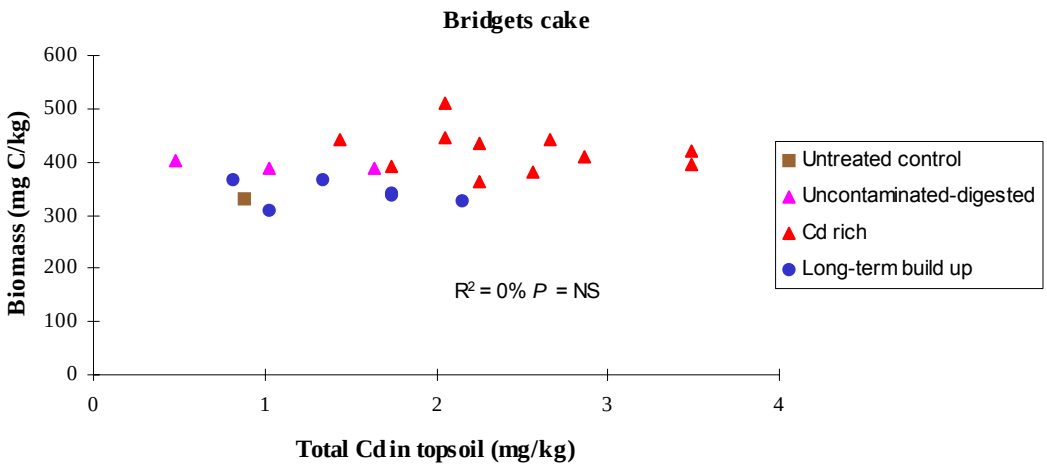
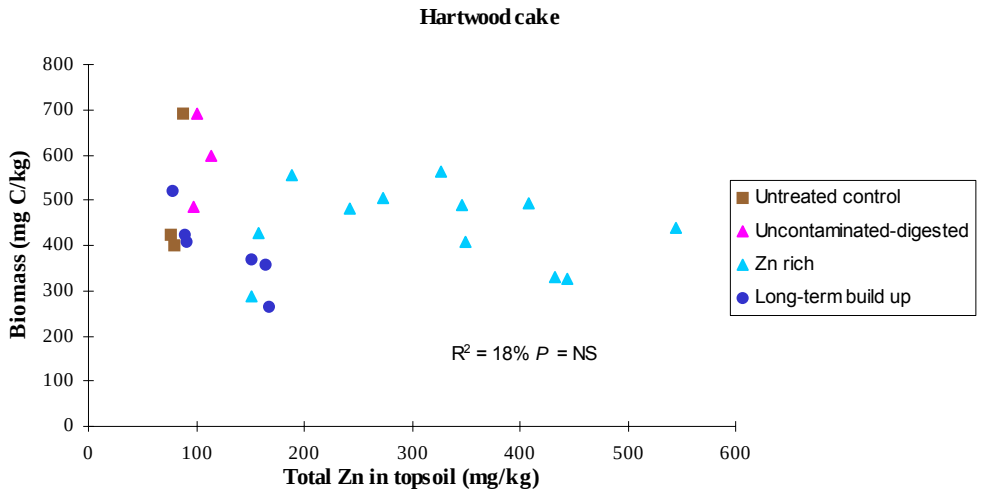
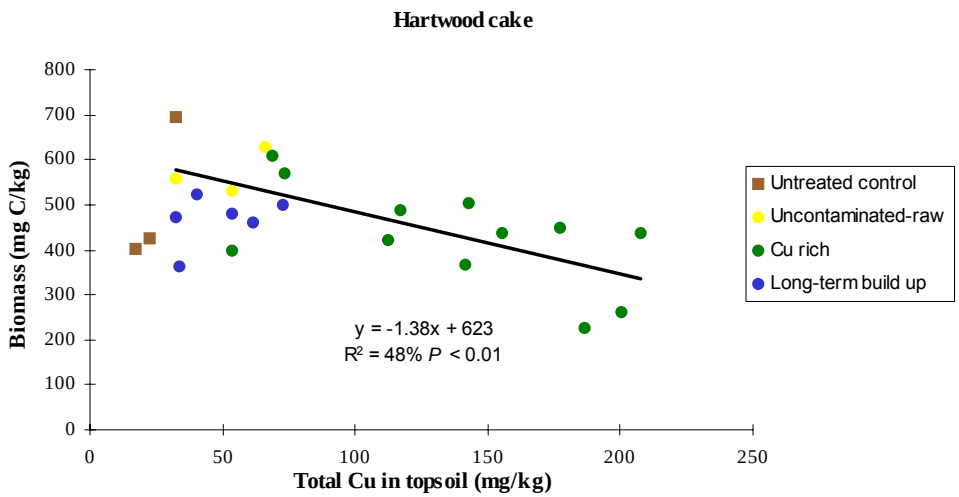


Figure 34. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Hartwood (spring 2005)





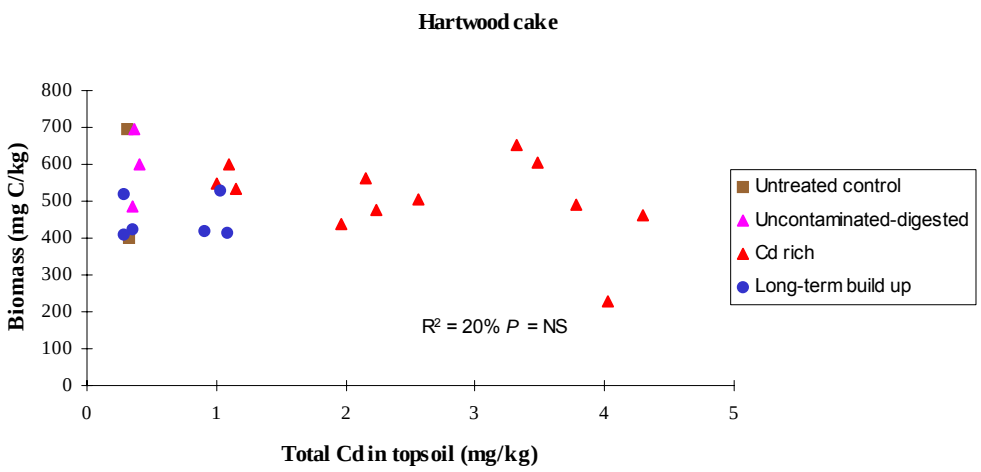
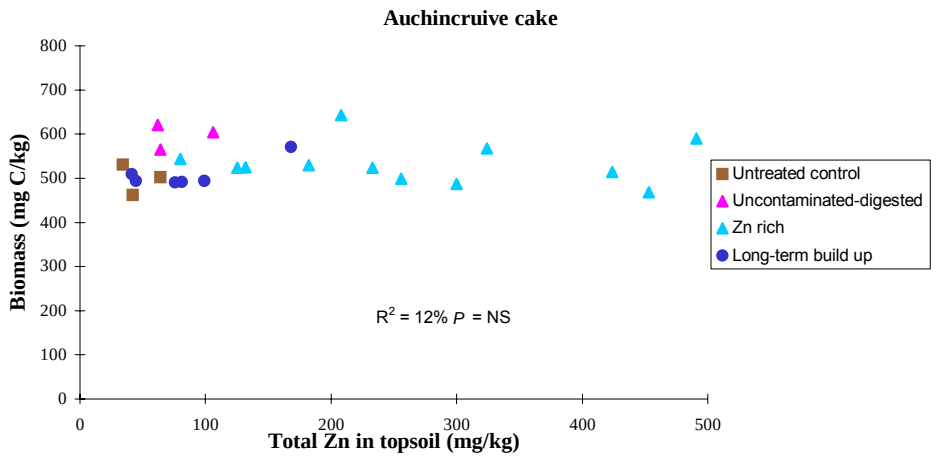
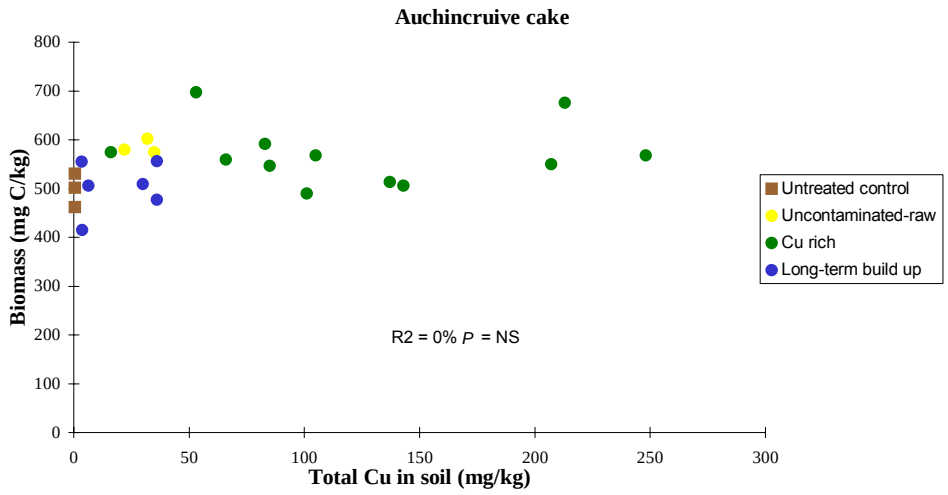


Figure 35. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Auchincruive (spring 2005)





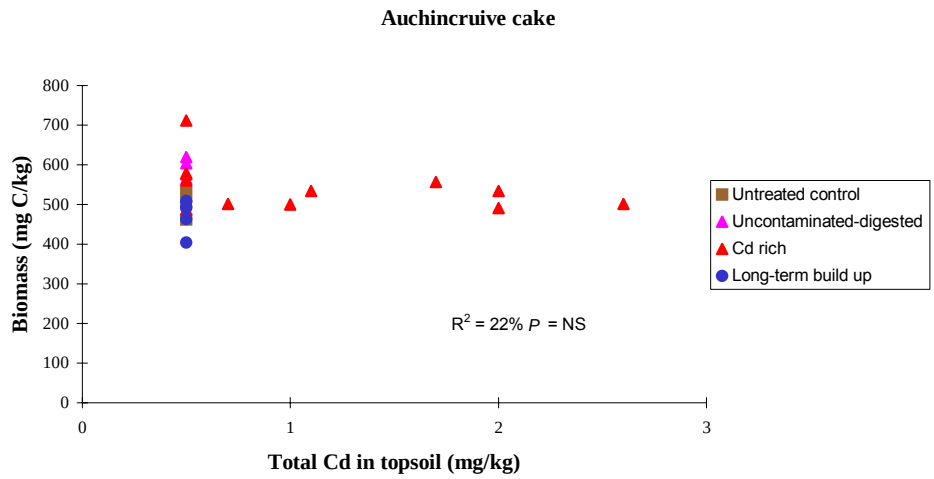


Figure 36. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Shirburn (spring 2005)

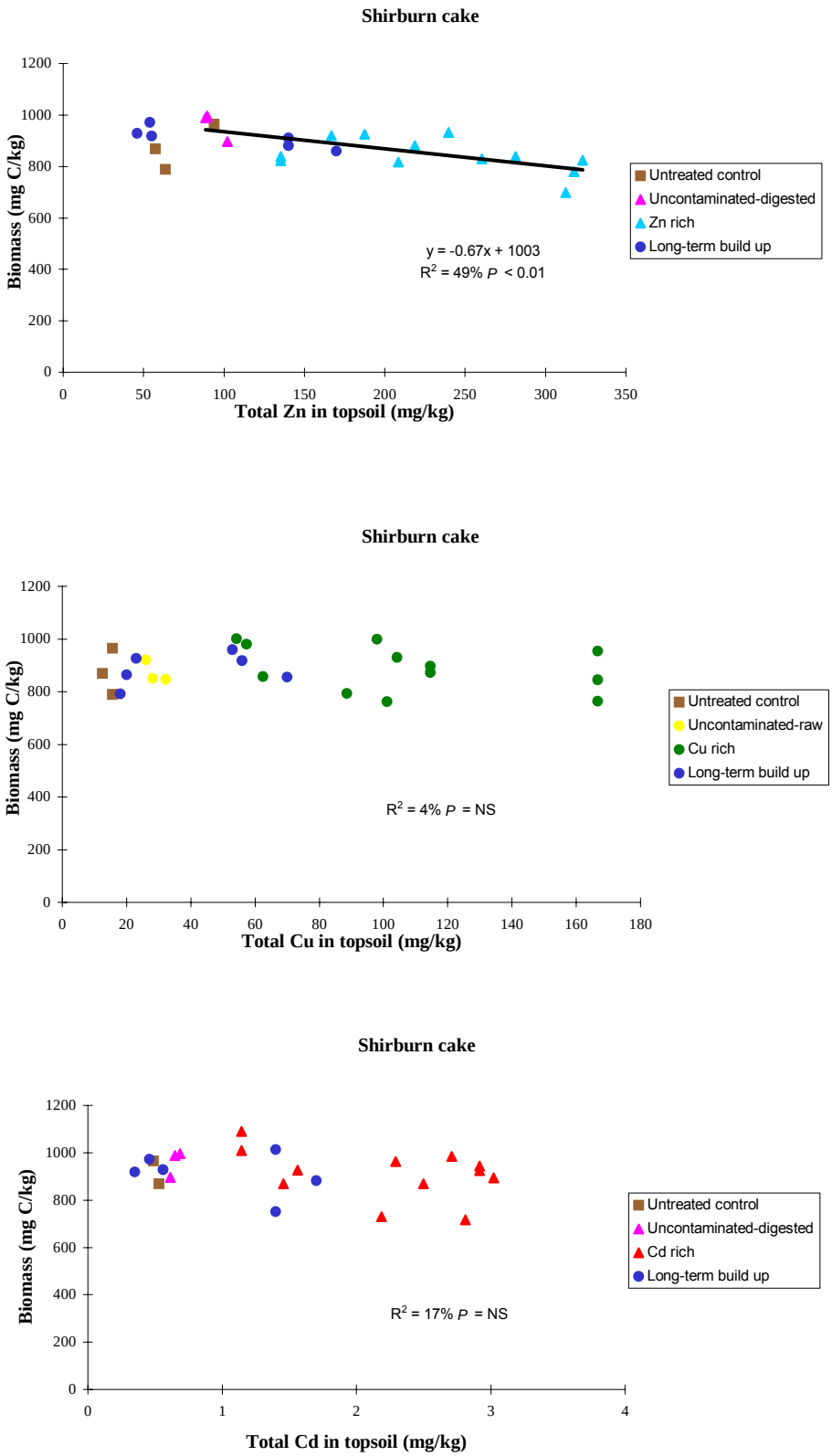


Table 16. Summary of relationships between soil biomass C concentration (mg/kg) and total topsoil metals - sludge cake sites (spring 2005 samples)

	Zn	Cu (r^2 ; *P*)	Cd
GLE	NS	14%; $P < 0.05$	NS
WOB	36%; $P < 0.01$	19%; $P < 0.01$	NS
WAT	19%; $P < 0.05$	22%; $P < 0.05$	NS
PWL	NS	NS	NS
ROS	30%; $P < 0.01$	20%; $P < 0.05$	NS
BRI	19%; $P < 0.01$	NS	NS
HAR	NS	24%; $P < 0.01$	NS
AUC	NS	NS	NS
SHI	15%; $P < 0.01$	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd.. Red = inverse relationship. Model parameters for significant responses are given in Figures 28-36.

Significant inverse relationships (P < 0.05) between soil biomass C and total topsoil Zn concentrations were evident at four sites in both 2003 and 2005 (Woburn, Watlington, Rosemaund and Bridgets), at Hartwood and Auchincruive in 2003, and Shirburn in 2005. For Cu, significant inverse relationships were evident at three sites in both 2003 and 2005 (Woburn, Watlington and Hartwood), and at Gleadthorpe and Rosemaund in 2005. For Cd, there was no consistent effect on soil biomass C concentrations.

Soil organic carbon

Soil organic carbon (C) levels were measured on soil samples taken from all the treatment plots in spring 2003 and 2005 (see Appendices C and D).

2003 sampling: Soil organic C levels were generally higher on the treatments receiving sludge cakes compared with the untreated controls.

On the metal dose-response treatments, regression of the soil organic C levels against total topsoil metal concentrations identified two significant positive relationship ($P < 0.05$, Table 17) (*i.e.* increasing soil C with increasing total topsoil metal concentration) on the Cu treatments at Watlington and Auchincruive *i.e.* two out of 27 dose-response treatments.

Table 17. Relationship between soil organic carbon contents and total topsoil metal concentrations – spring 2003

	Zn	Cu (r^2 ; *P*)	Cd
GLE	NS	NS	NS
WOB	NS	NS	NS
WAT	NS	7%; $P < 0.05$	NS
PWL	NS	NS	NS
ROS	NS	NS	NS
BRI	NS	NS	NS
HAR	NS	NS	NS
AUC	NS	10%; $P < 0.05$	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship

2005 sampling: Over all the sites, soil organic C levels were generally higher on the treatments receiving sludge cakes compared with the untreated controls, with the smallest (and most variable changes) on some of the long-term build up plots which had received the lowest annual additions of organic matter.

On the metal dose-response treatments, regression of the soil organic C levels against total topsoil metal concentrations identified a single significant positive relationship ($P < 0.05$, Table 18) (*i.e.* increasing soil C with increasing total topsoil metal concentration) on the Cd treatment at Woburn *i.e.* one out of 27 dose-response treatments.

Table 18. Relationship between soil organic carbon contents and total topsoil metal concentrations – spring 2005

	Zn	Cu (r^2 ; *P*)	Cd
GLE	NS	NS	NS
WOB	NS	NS	23%; $P < 0.05$
WAT	NS	NS	NS
PWL	NS	NS	NS
ROS	NS	NS	NS
BRI	NS	NS	NS
HAR	NS	NS	NS
AUC	NS	NS	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship

Respiration Quotients

In order to compare results from individual plots having different soil microbial biomass concentrations and soil respiration rates, the data were normalised using specific microbial quotients. Respiration quotients are widely used in the literature to compare soil microbial properties (e.g. Giller *et al*., 1998). The Respiration Quotient – RQ (see Tables 19 and 20 for 2003 and 2005 samples, respectively) is:

$$RQ = \frac{Soil\ respiration\ rate\ (mgCO_2 - C / kg\ oven\ dry\ soil / h)}{Soil\ microbial\ biomass\ C\ \ (mgC / kg\ oven\ dry\ soil)} \times 100$$

A high RQ is a common characteristic of the soil biomass in long-term metal contaminated soils (Brookes and McGrath, 1984; Witter *et al.,* 1993; Bardgett *et al.,* 1994; Fliessbach *et al*., 1994; Yeates *et al*.,1994; Valsecchi *et al*., 1995; Dahlin *et al*., 1997), and has been suggested as a useful indicator of metal stress in soils (Brookes, 1993; Wardle and Ghani, 1995). An increase in RQ is often considered as an indication of metal toxicity and stress (Giller *et al.,* 1999). However, caution is required when interpreting RQ as this is affected by several factors as well as metal stress, particularly the nutritional state of the microbes and the source and type of available growth substrates.

Table 19. Soil respiration rate as a proportion of soil microbial biomass C (spring 2003 samples): *Respiration Quotient*

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHR
Control	0.27	0.23	0.08	0.12	0.06	0.07	1.15	0.06	0.06
Unc. Digested	0.24	0.29	0.14	0.09	0.08	0.10	1.00	0.08	0.06
Unc. Raw	0.25	0.24	0.05	0.12	0.08	0.08	1.27	0.07	0.06
Zn rate 1	0.29	0.18	0.09	0.09	0.08	0.09	0.89	0.07	0.06
Zn rate 2	0.28	0.20	0.10	0.17	0.10	0.09	0.54	0.09	0.07
Zn rate 3	0.35	0.23	0.15	0.10	0.10	0.11	0.67	0.09	0.08
Zn rate 4	0.31	0.26	0.16	0.09	0.09	0.12	0.50	0.09	0.07
Cu rate 1	0.22	0.17	0.07	0.09	0.09	0.07	1.06	0.06	0.07
Cu rate 2	0.28	0.18	0.08	0.12	0.12	0.11	0.89	0.07	0.07
Cu rate 3	0.27	0.21	0.11	0.10	0.10	0.10	0.75	0.08	0.08
Cu rate 4	0.41	0.22	0.16	0.12	0.14	0.11	0.34	0.10	0.07
Cd rate 1	0.23	0.18	0.10	0.10	0.09	0.11	1.09	0.07	0.07
Cd rate 2	0.28	0.18	0.11	0.13	0.10	0.13	0.91	0.07	0.09
Cd rate 3	0.31	0.16	0.12	0.11	0.09	0.10	0.93	0.10	0.08
Cd rate 4	0.26	0.15	0.11	0.14	0.10	0.12	0.82	0.09	0.08

The respiration quotients for each plot were regressed against total topsoil metal concentrations for treatments receiving the same organic matter inputs during Phase I (*i.e.* the moderate rate build up treatments). A summary of the regression analyses is given in Tables 21 and 22 for the 2003 and 2005 samplings, respectively.

2003 sampling: There was no relationship between RQ and total topsoil Zn at any of the sites. However, there were significant ($P < 0.05$) positive relationships between RQ and total topsoil Cu at five of the sites (Watlington, Rosemaund, Bridgets, Hartwood and Auchincruive). There were significant ($P < 0.05$) relationships between RQ and total topsoil Cd at two sites, inverse at Woburn and positive at Pwllpeiran. On six of the seven metal-dose response treatments where a relationship between RQ and total topsoil metals was found, RQ *increased* with increasing total topsoil metal concentrations.

Table 20. Soil respiration rate as a proportion of soil microbial biomass C (spring 2005 samples): *Respiration Quotient*

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHR
Control	0.12	0.19	0.09	0.11	0.13	0.08	0.15	0.08	0.05
Unc. Digested	0.12	0.18	0.11	0.11	0.10	0.07	0.19	0.10	0.05
Unc. Raw	0.08	0.13	0.07	0.12	0.06	0.07	0.16	0.09	0.05
Zn rate 1	0.12	0.23	0.11	0.09	0.09	0.08	0.29	0.11	0.07
Zn rate 2	0.15	0.19	0.13	0.11	0.14	0.08	0.19	0.10	0.07
Zn rate 3	0.17	0.35	0.13	0.11	0.11	0.09	0.21	0.10	0.07
Zn rate 4	0.15	0.40	0.15	0.10	0.16	0.10	0.24	0.12	0.07
Cu rate 1	0.13	0.15	0.09	0.10	0.09	0.08	0.19	0.08	0.06
Cu rate 2	0.13	0.17	0.09	0.11	0.07	0.08	0.19	0.09	0.07
Cu rate 3	0.13	0.19	0.10	0.13	0.08	0.07	0.21	0.11	0.08
Cu rate 4	0.14	0.25	0.13	0.13	0.09	0.08	0.29	0.11	0.07
Cd rate 1	0.15	0.19	0.11	0.13	0.10	0.06	0.23	0.10	0.06
Cd rate 2	0.14	0.20	0.10	0.12	0.10	0.09	0.21	0.10	0.07
Cd rate 3	0.16	0.22	0.10	0.10	0.09	0.08	0.19	0.11	0.06
Cd rate 4	0.15	0.20	0.09	0.15	0.11	0.08	0.26	0.10	0.07

Table 21. Summary of relationships between RQ and total topsoil metals - sludge cake sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
GLE	NS	NS	NS
WOB	NS	NS	59%; $P < 0.05$
WAT	NS	101%; $P < 0.001$	NS
PWL	NS	NS	29%; $P < 0.01$
ROS	NS	51%; $P < 0.01$	NS
BRI	NS	26%; $P < 0.05$	NS
HAR	NS	44%; $P < 0.05$	NS
AUC	NS	28%; $P < 0.001$	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship Blue = positive relationship

2005 sampling: There were significant ($P < 0.05$) relationships between RQ and total topsoil Zn and Cu at five of the sites. At Gleadthorpe and Shirburn, there were significant ($P < 0.05$) relationships between RQ and total topsoil Cd. On all 12 metal dose-response treatments where a relationship between RQ and total topsoil metals was found, RQ *increased* with increasing total topsoil metal concentrations.

Table 22. Summary of relationships between RQ and total topsoil metals - sludge cake sites (spring 2005 samples)

	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
GLE	NS	NS	17%; $P < 0.05$
WOB	98%; $P < 0.01$	52%; $P < 0.001$	NS
WAT	33%; $P < 0.01$	51%; $P < 0.001$	NS
PWL	NS	NS	NS
ROS	62%; $P < 0.05$	NS	NS
BRI	35%; $P < 0.01$	NS	NS
HAR	NS	47%; $P < 0.01$	NS
AUC	NS	19%; $P < 0.01$	NS
SHI	24%; $P < 0.05$	39%; $P < 0.05$	26%; $P < 0.05$

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship

Biomass Quotient

Significant positive relationships (P < 0.05) between RQ and total topsoil Zn concentrations were not evident at any of the sites in 2003, but were evident at five sites in 2005 (Woburn, Watlington, Rosemaund, Bridgets and Shirburn). For Cu, significant positive relationships were evident at three sites in both 2003 and 2005 (Watlington Hartwood and Auchincruive), at Rosemaund and Bridgets in 2003, and Shirburn in 2005. For Cd, there were significant positive relationships at Pwllpeiran (2003), Gleadthorpe and Shirburn (2005), and a significant inverse relationship at Woburn (2003).

In order to compare results from individual plots having different soil organic C contents and microbial biomass C concentrations the data were normalised using a specific microbial quotient (Tables 23 and 24 for the 2003 and 2005 samples, respectively). This quotient is widely used in the literature to compare soil microbial properties (Giller *et al.*, 1998).

The Biomass Quotient - BQ is:

$$BQ = \frac{Soil\ microbial\ biomass\ (mgC/kg\,oven\,dry\,soil)}{Soil\ \ organic\ \ C\ \ (mg\ C/kg\ oven\ dry\ soil)} \times 100$$

Table 23. Soil microbial biomass C as a proportion of soil organic C (spring 2003 samples): *Biomass Quotient*

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHR
Control	1.52	1.37	2.14	1.43	2.15	2.03	0.84	1.98	3.04
Unc. Digested	1.22	1.14	1.54	1.13	1.73	1.48	1.19	1.71	2.42
Unc. Raw	1.34	1.48	2.14	1.21	1.95	1.66	0.96	1.68	2.48
Zn rate 1	1.12	1.22	1.63	1.23	1.60	1.30	1.15	1.63	2.58
Zn rate 2	0.94	0.91	1.49	0.97	1.55	1.26	1.06	1.45	2.19
Zn rate 3	0.90	0.99	1.37	1.18	1.39	1.13	1.03	1.36	2.48
Zn rate 4	0.95	1.09	1.10	1.12	1.43	1.46	0.93	1.46	2.22
Cu rate 1	1.42	1.41	2.03	1.28	2.03	1.91	0.93	1.53	2.66
Cu rate 2	1.27	1.37	1.85	1.21	1.77	1.42	0.98	1.69	2.54
Cu rate 3	1.21	1.16	1.67	1.21	1.85	1.55	0.94	1.42	2.31
Cu rate 4	0.85	1.06	1.38	1.37	1.50	1.36	0.77	1.15	2.38
Cd rate 1	0.95	1.26	1.62	1.17	1.72	1.40	1.13	1.53	2.60
Cd rate 2	1.04	1.01	1.53	1.15	1.59	1.59	0.98	1.59	2.20
Cd rate 3	0.88	1.17	1.61	1.08	1.83	1.39	0.96	1.53	2.55
Cd rate 4	0.87	1.08	1.35	1.08	1.62	1.26	0.79	1.37	2.30

Table 24. Soil microbial biomass C as a proportion of soil organic C (spring 2005 samples): *Biomass Quotient*

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHR
Control	1.56	1.72	2.22	1.05	1.58	1.78	1.20	1.52	3.91
Unc. Digested	1.42	2.05	1.63	1.02	1.60	1.55	1.18	1.70	3.16
Unc. Raw	1.36	1.98	2.26	0.96	2.11	1.70	1.46	1.64	3.32
Zn rate 1	1.25	1.43	1.36	0.99	1.53	1.28	0.83	1.51	2.78
Zn rate 2	1.12	1.52	1.31	0.90	1.27	1.36	0.78	1.22	2.84
Zn rate 3	1.20	0.80	1.43	0.91	1.51	1.03	0.91	1.54	2.91
Zn rate 4	1.14	0.88	1.25	1.04	1.14	1.18	0.69	1.25	2.51
Cu rate 1	1.57	1.85	2.07	1.08	1.64	1.54	1.11	1.76	3.30
Cu rate 2	1.17	1.78	1.57	0.97	1.94	1.70	1.06	1.43	3.08
Cu rate 3	1.35	1.67	1.44	0.99	1.58	1.56	0.86	1.30	2.94
Cu rate 4	1.21	1.18	1.37	0.91	1.63	1.38	0.49	1.73	2.94
Cd rate 1	1.15	1.58	1.41	0.98	1.40	1.45	1.05	1.64	3.10
Cd rate 2	1.09	1.70	1.58	0.98	1.41	1.26	0.96	1.42	2.94
Cd rate 3	1.24	1.40	1.66	1.02	1.86	1.30	1.27	1.42	3.05
Cd rate 4	1.35	1.48	1.39	0.87	1.59	1.29	0.83	1.26	2.95

The biomass quotients for each plot were regressed against total topsoil metal concentrations for treatments that received the same organic matter inputs during Phase I (*i.e.* the moderate rate build up treatments). A summary of the regression analyses are given in Tables 25 and 26 for the 2003 and 2005 samplings, respectively.

2003 sampling: At Watlington, Rosemaund, Hartwood and Auchincruive, there were significant ($P < 0.05$) inverse relationships between BQ and total topsoil Zn. At six of the sites, there were significant ($P < 0.05$) inverse relationships between BQ and total topsoil Cu. There were no significant ($P > 0.05$) relationships between BQ and total topsoil Cd at any of the sites. On all ten metal dose-response treatments where a relationship between BQ and total topsoil metals was found, BQ *decreased* with increasing metal concentrations (Table 25).

Table 25. Summary of relationships between BQ and total topsoil metals - sludge cake sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
GLE	NS	24%; $P < 0.05$	NS
WOB	NS	22%; $P < 0.01$	NS
WAT	21%; $P < 0.001$	18%; $P < 0.001$	NS
PWL	NS	NS	NS
ROS	14%; $P < 0.05$	18%; $P < 0.05$	NS
BRI	NS	NS	NS
HAR	23%; $P < 0.05$	36%; $P < 0.01$	NS
AUC	13%; $P < 0.05$	14%; $P < 0.05$	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship

2005 sampling: At Woburn, Bridgets, Hartwood and Shirburn, there were significant ($P < 0.05$) inverse relationships between BQ and total topsoil Zn. Also, at Woburn, Watlington, Rosemaund and Shirburn there were significant ($P < 0.05$) inverse relationships between BQ and total topsoil Cu concentrations. There were no significant ($P > 0.05$) relationships between BQ and total topsoil Cd at any of the nine sites. On the eight metal dose-response treatments where a relationship between BQ and total topsoil metals was found, BQ *decreased* with increasing metal concentrations (Table 26).

Table 26. Summary of relationships between BQ and total topsoil metals - sludge cake sites (spring 2005 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
GLE	NS	NS	NS
WOB	55%; P < 0.05	23%; P < 0.01	NS
WAT	NS	32%; *P* < 0.01	NS
PWL	NS	NS	NS
ROS	NS	21%; *P* < 0.05	NS
BRI	22%; P < 0.001	NS	NS
HAR	17%; P < 0.05	NS	NS
AUC	NS	NS	NS
SHI	14%; P < 0.05	11%; *P* < 0.05	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship

Significant inverse relationships (P < 0.05) between BQ and total topsoil Zn concentrations were evident at Hartwood in both sampling years, at Watlington, Rosemaund and Auchincruive in 2003, and Woburn, Bridgets and Shirburn in 2005. For Cu, significant (P < 0.05) inverse relationships were evident at three sites in both 2003 and 2005 (Woburn, Watlington and Rosemaund), at three sites in 2003 (Gleadthorpe, Hartwood and Auchincruive) and Shirburn in 2005. For Cd, no significant (P > 0.05) relationships were evident at any of the nine sites in either sampling year.

Rhizobium numbers

Most probable numbers (MPNs) of rhizobia were measured in soil samples taken from all the treatment plots in spring 2003 (with the exception of the long-term build-up plots) and 2005. Treatment mean *Rhizobium* numbers for all the soil samples taken in spring 2003 and 2005 are presented in Tables 27 and 29.

Table 27. Mean topsoil (0-25 cm) *Rhizobium leguminosarum* biovar *trifolii* numbers (log_{10} cells/g soil) on treatments sampled in spring 2003

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR
Control	4.96	4.75	5.06	2.92	5.18	4.23	4.54	4.95	4.23
Unc. Digested	4.80	4.55	4.48	2.27	5.18	4.42	5.26	4.14	4.45
Unc. Raw	5.06	4.18	4.76	2.77	5.06	4.32	4.97	4.41	4.49
Unc. High Dig.	-	-	-	-	-	-	-	-	4.07
Unc. High Raw	-	-	-	-	-	-	-	-	4.47
Zn rate 1	4.95	3.64	5.02	2.90	4.31	4.24	2.88	4.00	4.14
Zn rate 2	3.45	2.58	4.31	2.56	3.03	3.65	0.81	3.19	3.96
Zn rate 3	3.30	2.48	3.61	2.04	2.72	2.62	0.00	2.91	4.06
Zn rate 4	2.82	2.45	3.50	2.15	3.99	3.85	0.00	3.90	4.14
Zn rate 5	-	-	-	-	-	-	-	-	3.15
ANOVA (1) p	*< 0.001*	*< 0.001*	*0.002*	*NS*	*< 0.001*	*0.008*	*< 0.001*	*0.011*	*NS*
$LSD_{0.05}$	0.787	1.656	1.114	-	1.364	1.319	2.173	1.521	-
CV%	7	17	9	34	12	12	34	14	11
Cu rate 1	5.06	4.18	4.76	2.77	5.06	4.32	4.97	4.41	4.40
Cu rate 2	4.96	4.46	5.12	2.92	4.75	3.89	4.76	4.59	4.47
Cu rate 3	4.81	4.05	4.80	3.10	5.12	4.86	4.77	4.32	3.88
Cu rate 4	4.39	3.74	4.65	2.20	4.86	4.01	4.64	3.23	4.00
Cu rate 5	-	-	-	-	-	-	-	-	3.74
ANOVA (2) p	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*< 0.001*	*NS*
$LSD_{0.05}$	-	-	-	-	-	-	-	0.913	-
CV%	9	13	8	25	5	13	17	7	9
Cd rate 1	4.71	3.85	4.81	1.99	5.06	4.22	3.63	3.55	3.80
Cd rate 2	4.73	3.87	4.60	2.92	4.71	4.21	3.57	3.26	3.43
Cd rate 3	4.57	4.00	4.65	2.80	4.55	4.48	3.89	4.02	3.54
Cd rate 4	4.18	3.81	4.35	2.92	3.88	4.28	4.22	3.79	3.12
Cd rate 5	-	-	-	-	-	-	-	-	3.57
ANOVA (3) p	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.005*	*0.020*
$LSD_{0.05}$	-	-	-	-	-	-	-	1.100	1.186
CV%	8	14	10	34	10	16	17	10	11

Three ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05). As the soil MPN results examined on a site by site basis did not approximate to normal distributions, the data were transformed to log $base_{10}$ before statistical analysis.

2003 sampling: On the Zn-rich treatments, there were smaller (> 1.5 log_{10}) *Rhizobium* populations (*P* <0.05) on the rate 2 to 4 treatments at Gleadthorpe and Woburn, the rate 3 and 4 treatments at Watlington, the rate 2 and 3 treatments at Rosemaund, the rate 3 treatment at Bridgets, the rate 1 to 4 treatments at Hartwood and the rate 2 and 3 treatments at Auchincruive compared with the untreated controls. At only two sites

(Pwllpeiran and Shirburn) were there no significant reductions ($P > 0.05$) in Rhizobium populations on the Zn-rich treatments.

On the Cu-rich treatments, there was a smaller (> 1.0 $\log_{10}$) *Rhizobium* population ($P < 0.05$) on the Cu-rich rate 4 treatment at Auchincruive compared with the uncontaminated raw sludge cake and untreated control treatments.

On the Cd-rich treatments, there were smaller (> 1.0 $\log_{10}$) *Rhizobium* populations ($P < 0.05$) on the rate 1, 2 and 4 treatments at Auchincruive compared with the untreated control treatment, and on the Cd-rich rate 4 treatment at Shirburn compared with the uncontaminated digested sludge cake treatment.

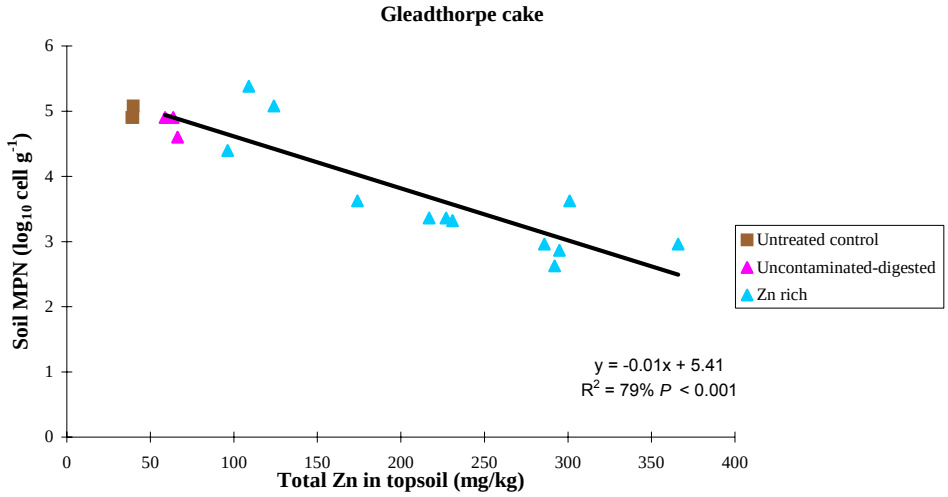
On the metal dose-response treatments, regression of soil rhizobia MPNs against total topsoil Zn concentrations identified significant inverse relationships ($P < 0.05$) at six sites (Gleadthorpe, Woburn, Watlington, Rosemaund, Bridgets and Hartwood), for Cu at Auchincruive, and for Cd at Rosemaund and Shirburn. On all of the nine metal-dose response treatments where there was a relationship between rhizobia MPNs and total topsoil metals, MPNs *decreased* with increasing total topsoil metal concentrations (Figures 37 to 45). A summary of the regression analyses is given in Table 28.

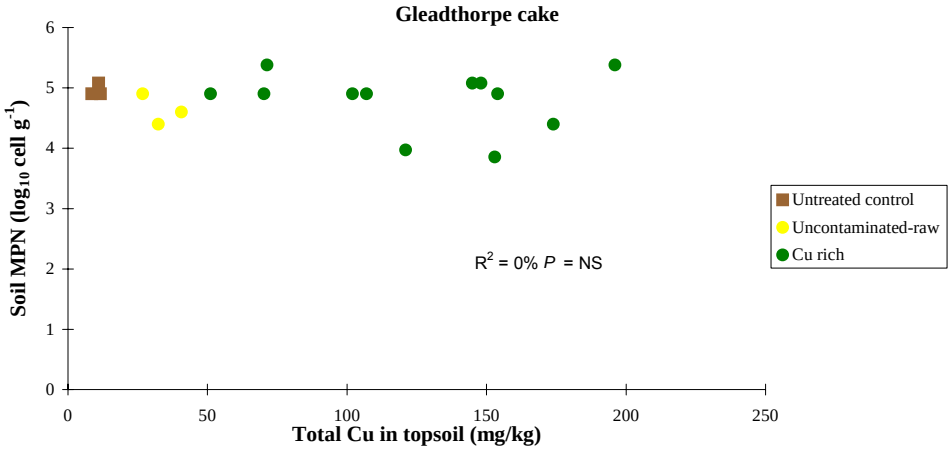
Table 28. Summary of relationships between rhizobia MPNs ($\log_{10}$) and total topsoil metals - sludge cake sites (spring 2003 samples)

	Zn	Cu (r^2 ; P)	Cd
GLE	39%; $P < 0.001$	NS	NS
WOB	37%; $P < 0.01$	NS	NS
WAT	19%; $P < 0.01$	NS	NS
PWL	NS	NS	NS
ROS	28%; $P < 0.05$	NS	27%; $P < 0.001$
BRI	22%; $P < 0.05$	NS	NS
HAR	78%; $P < 0.001$	NS	NS
AUC	NS	14%; $P < 0.001$	NS
SHI	NS	NS	25%; $P < 0.01$

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship. Model parameters for significant responses are given in Figures 37-45.

Figure 37. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Gleadthorpe (spring 2003)





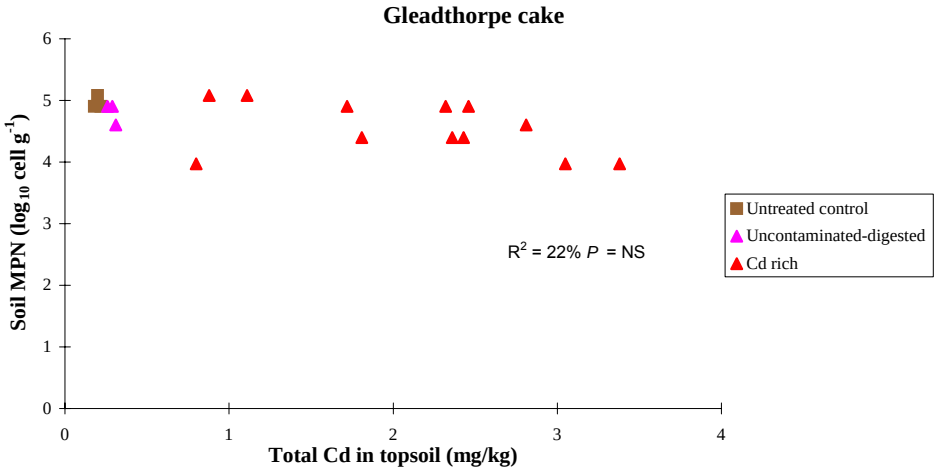


Figure 38. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Woburn (spring 2003)

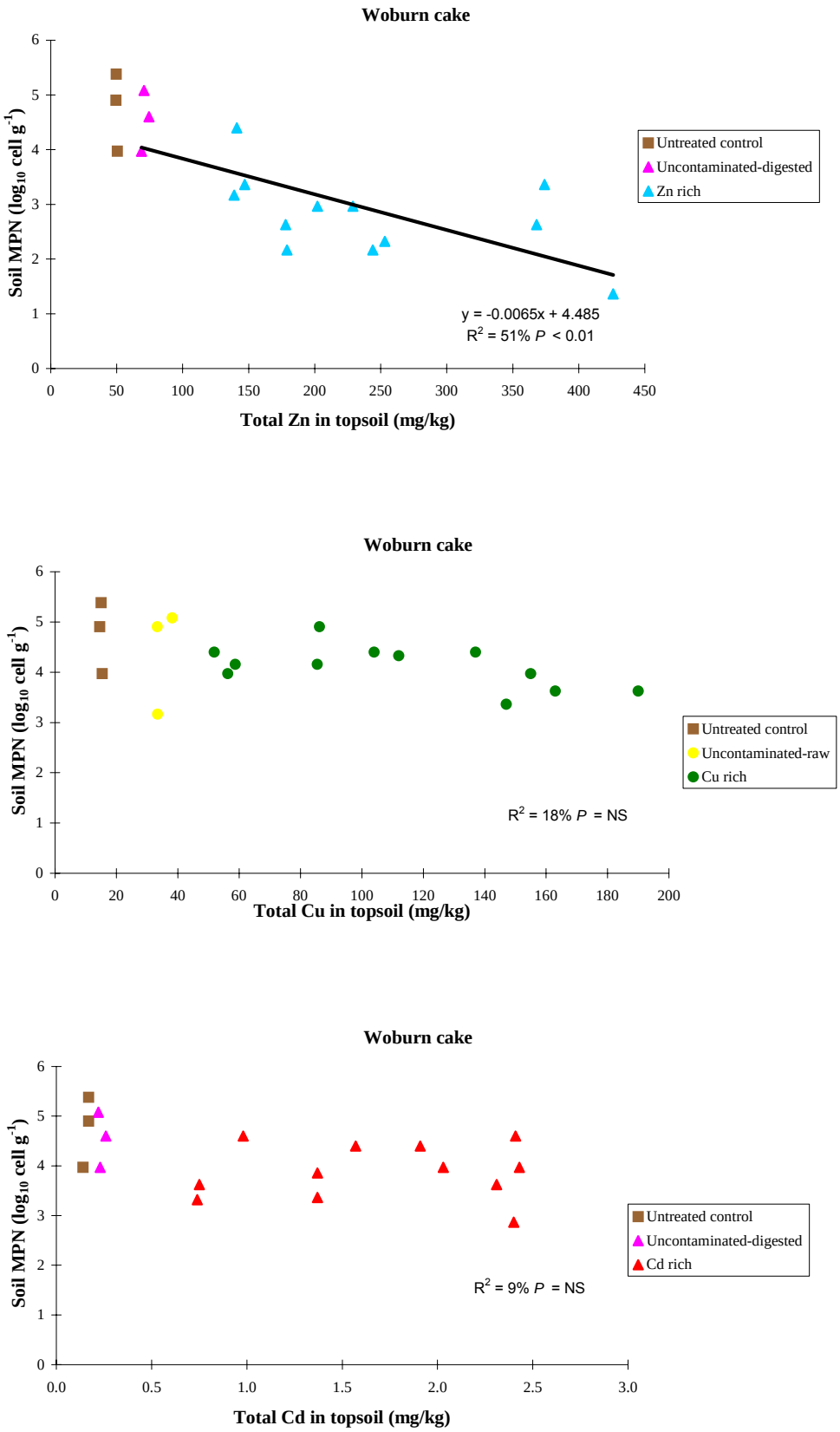
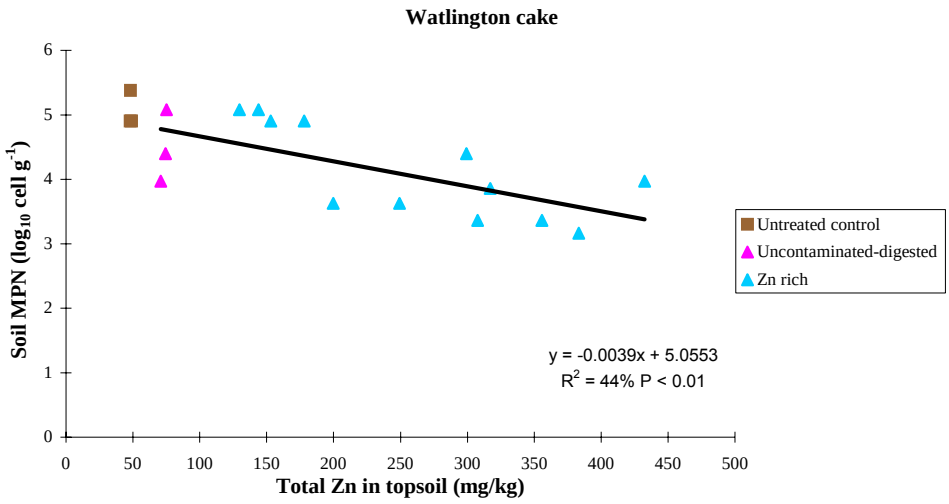
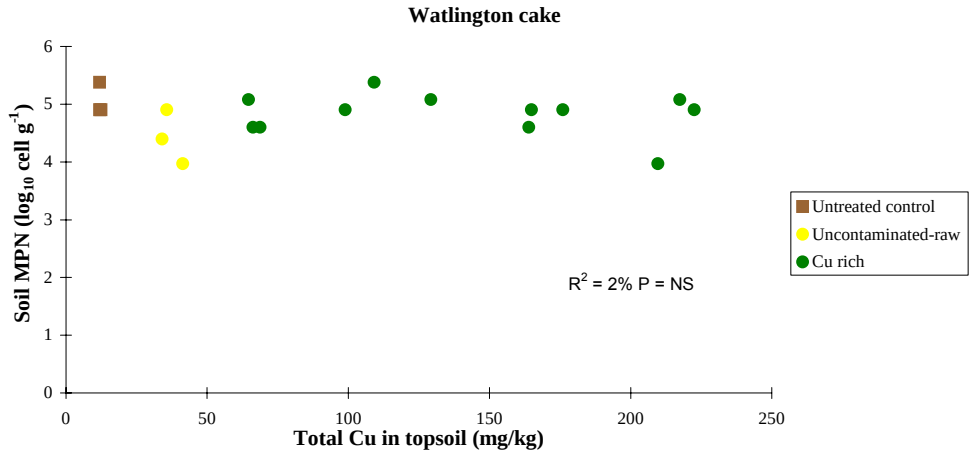


Figure 39. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Watlington (spring 2003)





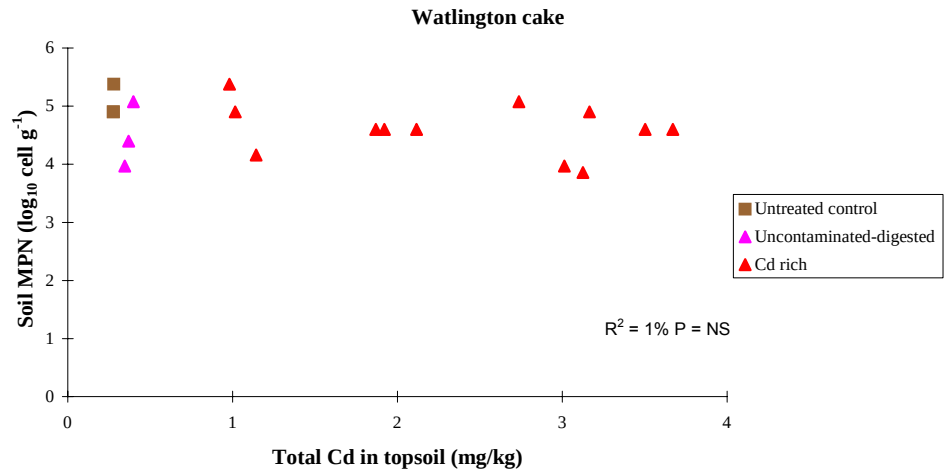


Figure 40. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Pwllpeiran (spring 2003)

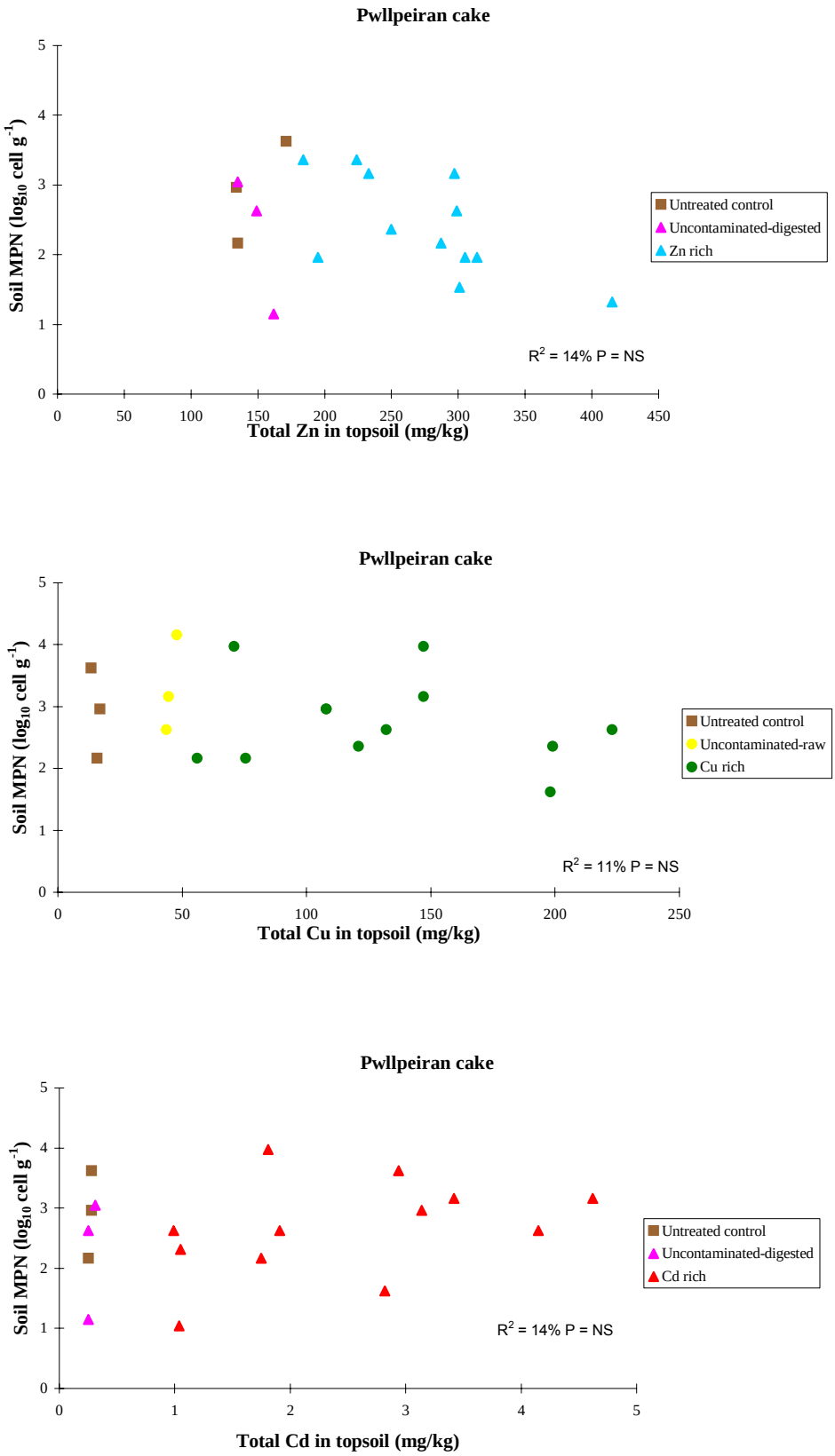


Figure 41. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Rosemaund (spring 2003)

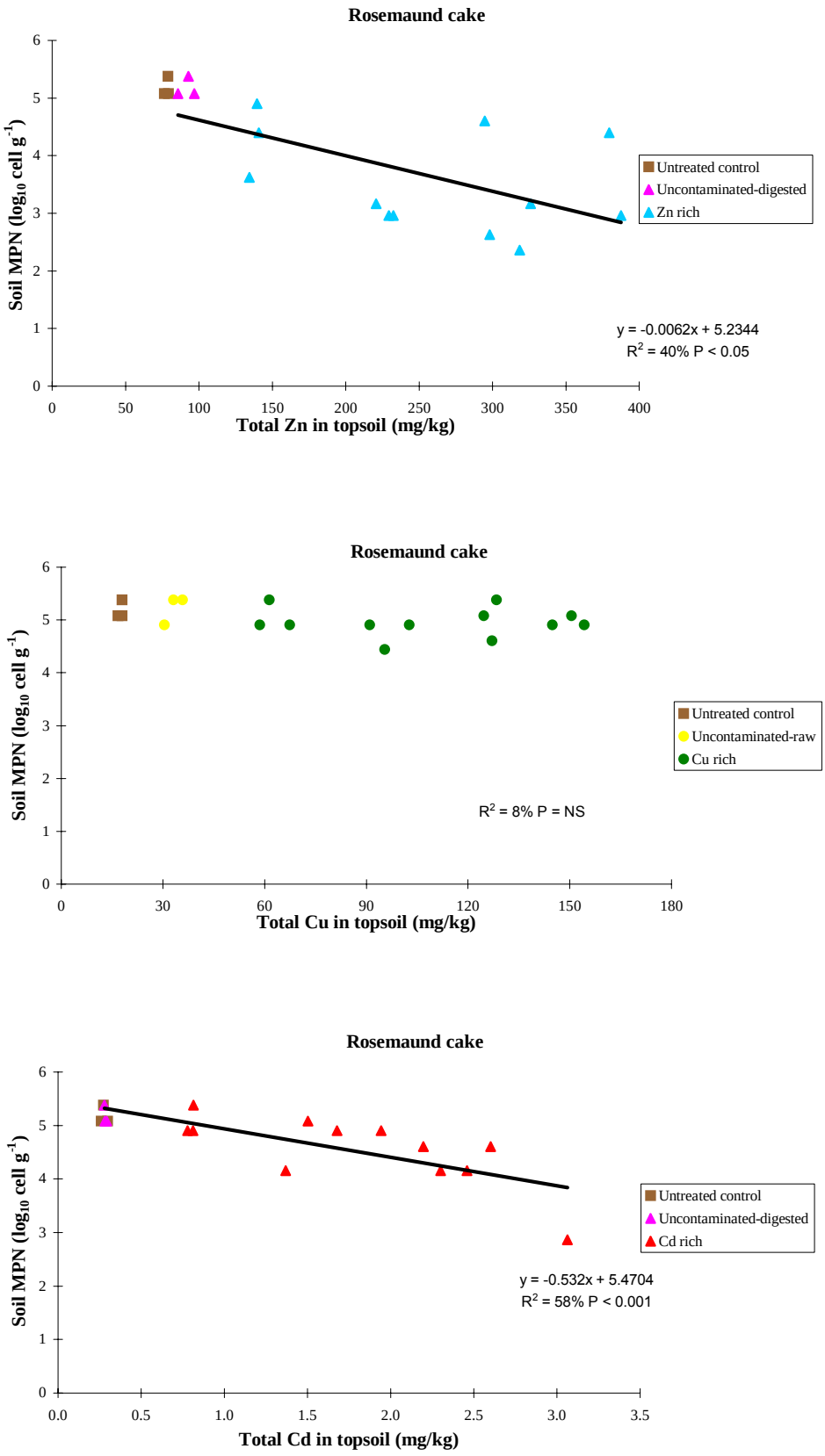


Figure 42. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Bridgets (spring 2003)

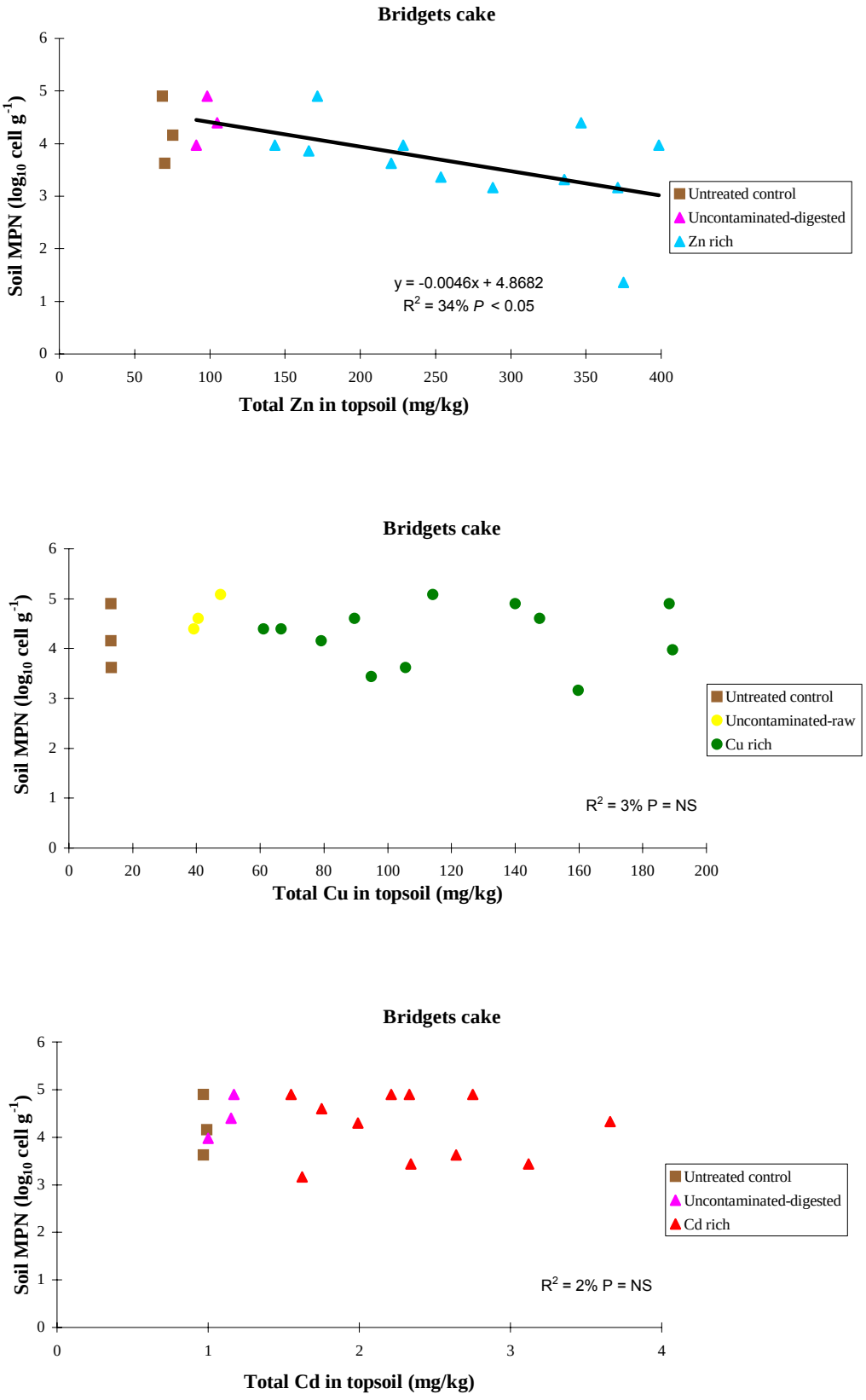


Figure 43. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Hartwood (spring 2003)

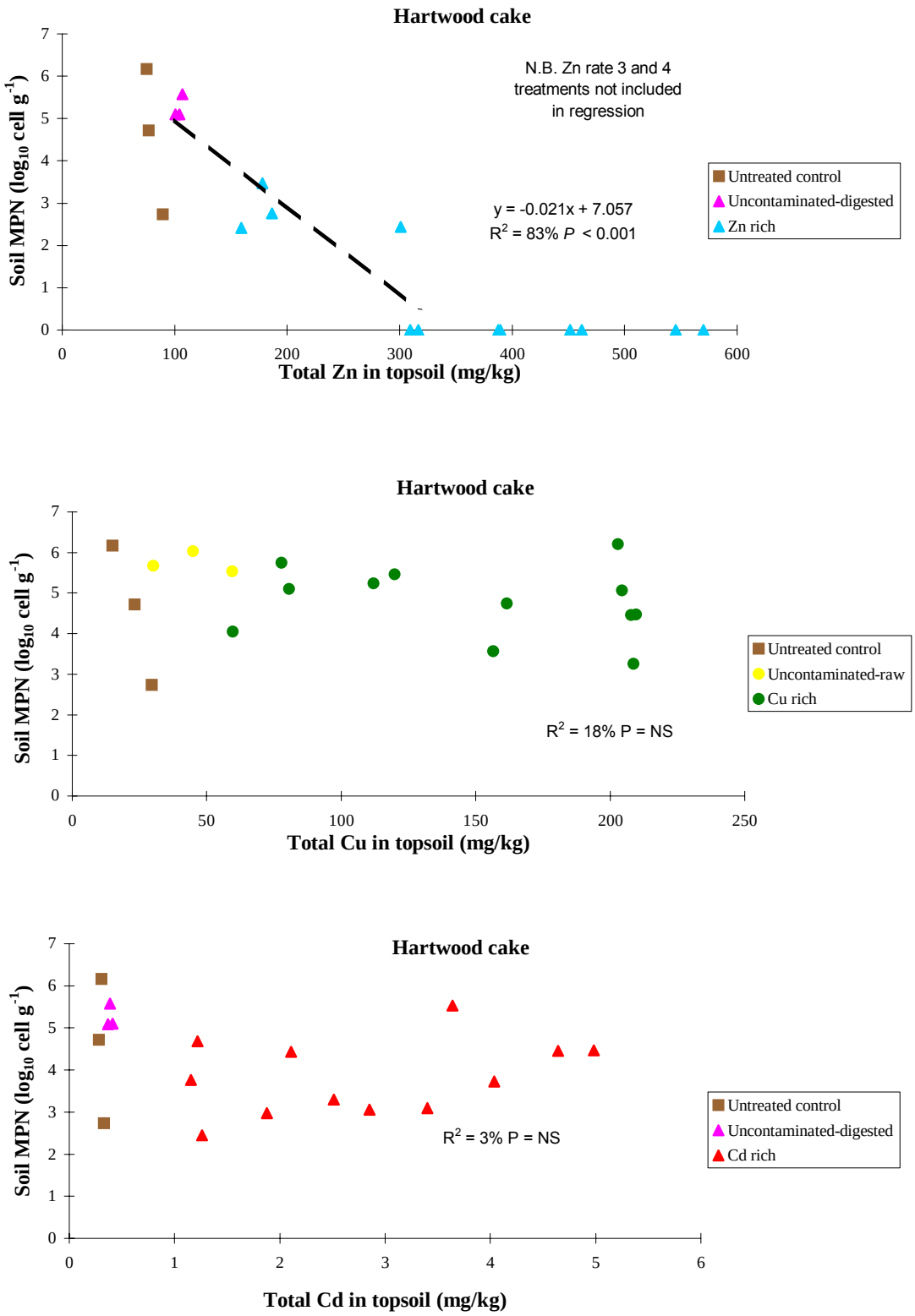


Figure 44. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs-Auchincruive (spring 2003)

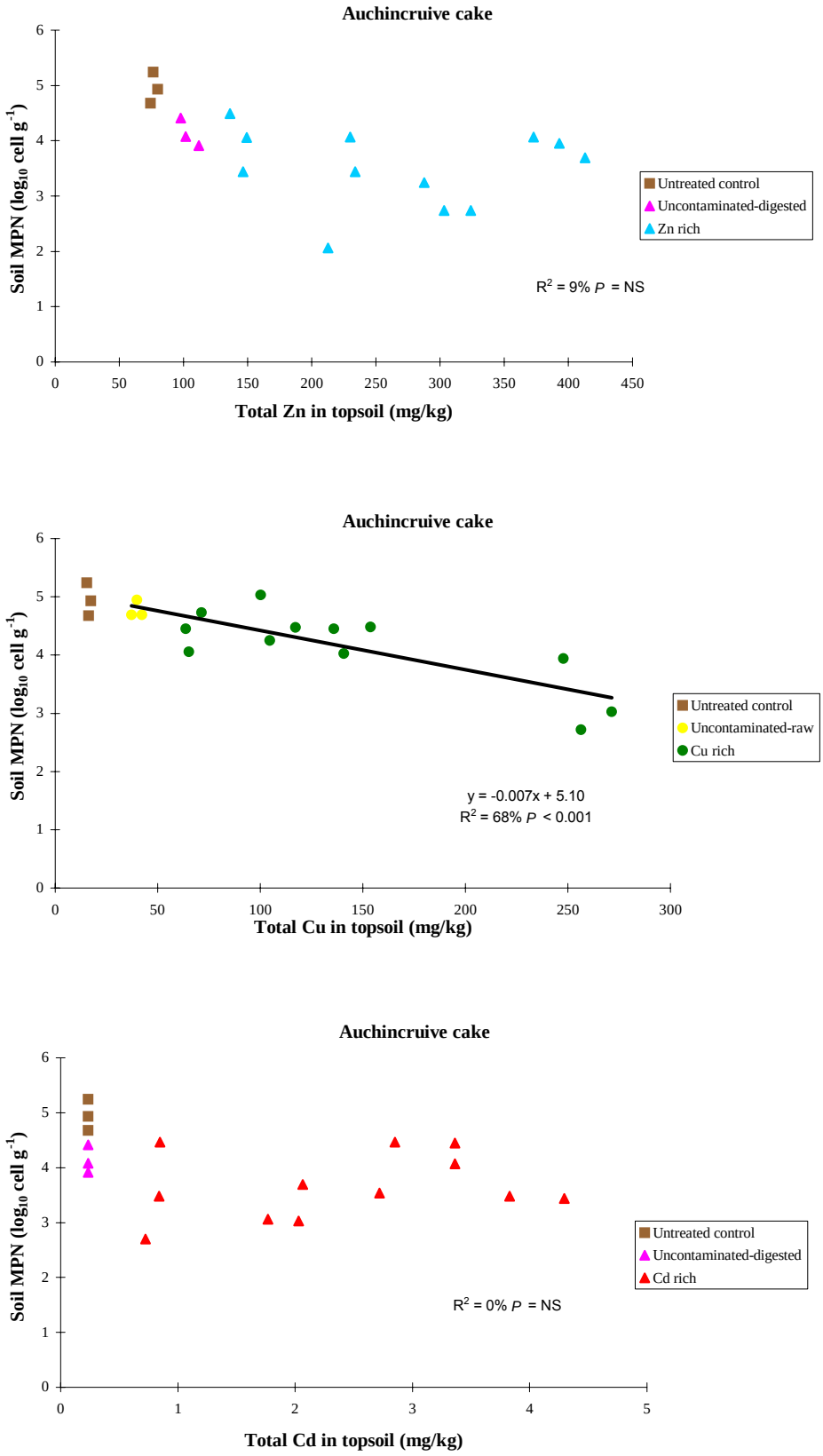


Figure 45. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Shirburn (spring 2003)

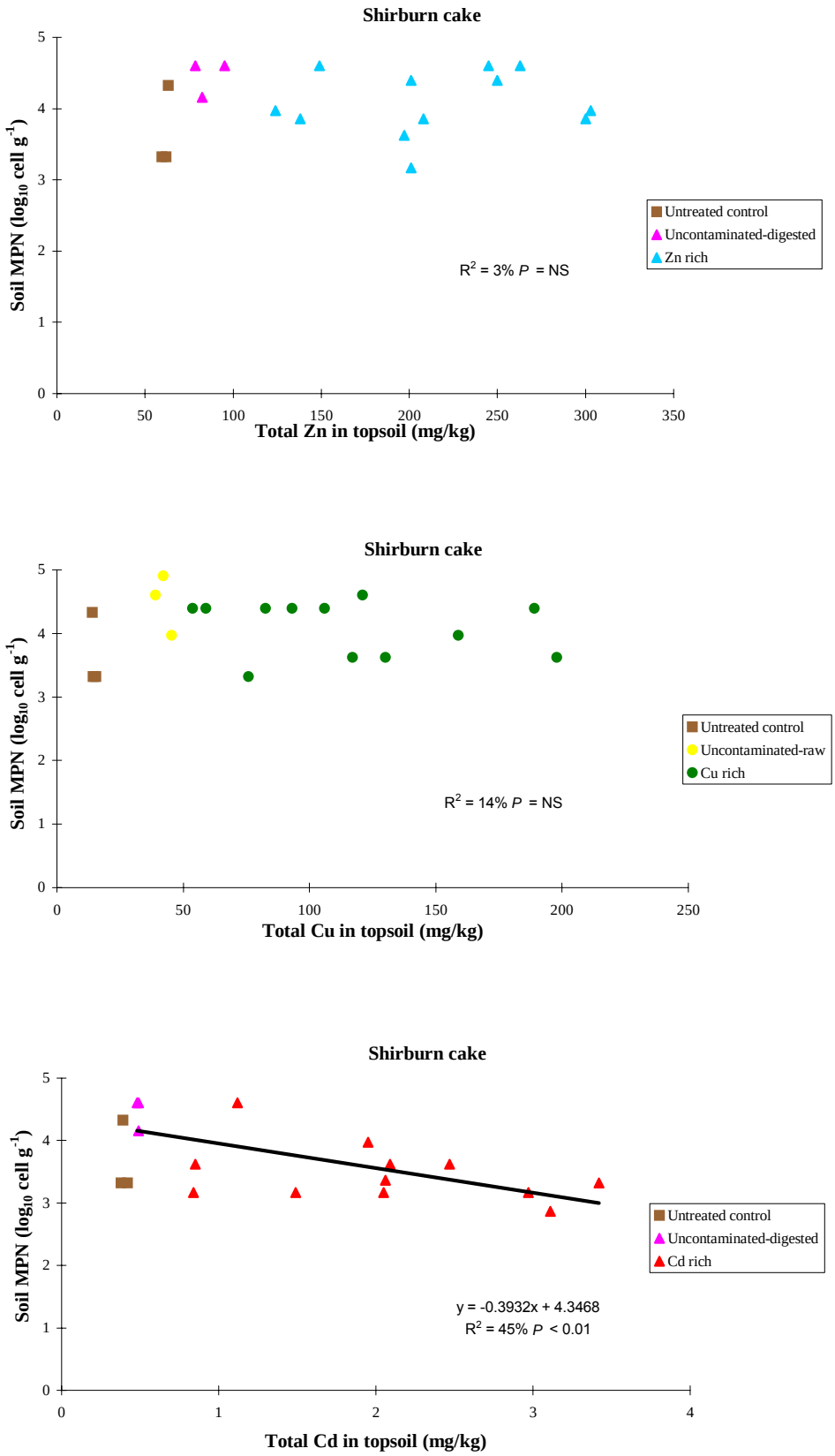


Table 29. Mean topsoil (0-25 cm) *Rhizobium leguminosarum* biovar *trifolii* numbers (log_{10} cells/g soil) on treatments sampled in spring 2005

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR
Control	4.86	4.48	5.22	3.92	5.28	5.12	3.05	4.45	4.40
Unc. Digested	5.02	4.18	4.57	3.78	4.76	5.06	4.16	4.06	3.91
Unc Raw	5.12	4.03	4.80	3.80	5.12	4.96	3.75	4.43	4.00
Unc. High Dig.	-	-	-	-	-	-	-	-	4.12
Unc. High Raw	-	-	-	-	-	-	-	-	4.47
Zn rate 1	3.92	3.46	4.34	3.46	4.79	4.92	1.22	3.46	3.96
Zn rate 2	2.81	2.00	4.32	3.07	3.10	3.96	0.00	3.06	4.13
Zn rate 3	3.21	2.32	3.32	2.83	2.78	4.00	0.00	2.75	3.55
Zn rate 4	1.96	0.60	3.05	2.03	2.29	4.46	0.00	2.08	4.12
Zn rate 5	-	-	-	-	-	-	-	-	3.65
ANOVA (1) p	*< 0.001*	*< 0.001*	*< 0.001*	*0.022*	*< 0.001*	*0.002*	*< 0.001*	*0.007*	*NS*
LSD	1.285	1.021	1.139	1.632	1.809	.849	1.441	1.705	-
CV%	13	13	10	18	17	7	36	18	11
Cu rate 1	5.06	4.48	4.80	3.60	5.18	4.80	4.10	4.13	4.57
Cu rate 2	4.55	3.84	4.65	3.45	5.18	4.63	3.23	4.21	4.32
Cu rate 3	4.70	3.65	4.63	3.25	5.12	4.50	2.91	4.55	4.14
Cu rate 4	4.86	4.14	4.19	2.88	4.63	4.70	2.36	3.73	4.32
Cu rate 5	-	-	-	-	-	-	-	-	4.18
ANOVA (2) p	*NS*	*NS*	*NS*	*NS*	*0.015*	*0.017*	*0.019*	*NS*	*NS*
LSD	-	-	-	-	0.502	0.505	1.413	-	-
CV%	6	16	10	12	4	4	15	7	12
Cd rate 1	5.02	3.91	4.80	3.44	4.80	5.02	3.15	3.18	3.87
Cd rate 2	4.07	3.72	4.29	3.08	5.18	4.96	2.21	3.60	3.86
Cd rate 3	4.17	3.38	4.55	3.45	4.32	4.86	1.89	3.52	4.06
Cd rate 4	3.49	3.29	4.54	2.52	4.55	4.96	1.08	3.05	3.57
Cd rate 5	-	-	-	-	-	-	-	-	3.92
ANOVA (3) p	*0.007*	*0.015*	*NS*	*0.005*	*0.021*	*NS*	*0.032*	*0.008*	*NS*
LSD	1.200	1.009	-	0.961	0.845	-	2.708	1.100	-
CV%	10	9	8	10	6	5	37	10	10
LT Unc. Dig.	4.96	4.40	4.96	3.86	5.38	4.81	3.73	4.06	4.26
LT Unc. Raw	5.12	4.18	5.08	3.55	5.28	5.12	3.43	4.59	4.18
LT Zn	3.68	4.11	4.52	3.22	4.54	4.77	1.28	3.67	4.28
LT Cu	3.69	4.18	5.02	3.86	5.18	5.00	3.21	4.26	4.00
LT Cd	4.25	4.42	5.08	3.22	5.22	5.18	3.72	4.07	3.97
ANOVA (4) p	*0.010*	*NS*	*NS*	*NS*	*0.042*	*NS*	*0.013*	*NS*	*NS*
$LSD_{0.05}$	1.333	-	-	-	0.791	-	1.892	-	-
CV%	11	9	6	12	5	7	22	12	8

Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. The ANOVA fitted was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05). As the soil MPN results examined on a site by site basis did not approximate to normal distributions, the data were transformed to log $base_{10}$ before statistical analysis.

2005 sampling: At Gleadthorpe, on the Zn-rich rate 2-4 treatments, there were smaller > 2.0 $\log_{10}$ *Rhizobium* populations ($P < 0.001$) compared with the uncontaminated digested sludge cake and untreated control treatments. At Woburn, on the Zn-rich rate 1 treatment the *Rhizobium* population size was > 1 $\log_{10}$ smaller ($P < 0.001$) than the untreated control, and the Zn-rich rate 2-4 treatments > 2 $\log_{10}$ smaller ($P < 0.001$) than the uncontaminated digested sludge cake and untreated control treatments. At Watlington, *Rhizobium* population sizes on the Zn-rich rate 3 and 4 treatments were > 1 $\log_{10}$ smaller ($P < 0.001$) than the uncontaminated digested sludge cake and untreated control treatments. At Pwllpeiran, the *Rhizobium* population size on the Zn-rich rate 4 treatment was > 1.7 $\log_{10}$ smaller than the uncontaminated digested sludge cake and untreated control treatments. At Rosemaund, on the Zn-rich rate 2 treatment the *Rhizobium* population size was > 2 $\log_{10}$ smaller ($P < 0.001$) than the untreated control, and the Zn-rich rate 3 and 4 treatments > 2 $\log_{10}$ smaller ($P < 0.001$) than the uncontaminated digested sludge cake and untreated control treatments. At Bridgets, *Rhizobium* population sizes on the Zn-rich rate 2 and 3 treatments were > 1 $\log_{10}$ smaller than the uncontaminated digested sludge cake and untreated control treatments. At Hartwood, *Rhizobium* population sizes on the Zn-rich rate 1-4 treatments were > 3 $\log_{10}$ smaller than the uncontaminated digested sludge cake treatment. Moreover at Hartwood, on the Zn-rich rate 2-4 treatments *Rhizobium* cells were not detected. At Auchincruive, *Rhizobium* population sizes on the Zn-rich rate 3 and 4 treatments were > 1 $\log_{10}$ smaller than the untreated control treatment. At Shirburn, there were no differences ($P > 0.05$) in *Rhizobium* population sizes between any of the Zn-rich, uncontaminated digested sludge cake and untreated control treatments.

At Rosemaund on the Cu-rich rate 4 treatment and at Bridgets on the Cu-rich 3 treatment, *Rhizobium* population sizes were *c.*0.6 $\log_{10}$ smaller ($P < 0.05$) than the untreated control treatments. At Hartwood, on the Cu-rich rate 4 treatment the *Rhizobium* population size was *c.*0.8 $\log_{10}$ smaller ($P < 0.05$) than the uncontaminated raw sludge cake treatment. There were no differences in *Rhizobium* population sizes ($P > 0.05$) between any of the Cu rich, uncontaminated raw sludge cake and untreated control treatments and any of the other six sites.

At Gleadthorpe and Pwllpeiran, on the Cd-rich rate 4 treatment, there were smaller (> 1.0 $\log_{10}$) *Rhizobium* populations ($P < 0.01$) than the uncontaminated digested sludge cake and untreated control treatments. At Woburn, on the Cd-rich rate 1-4 treatments there were smaller (> 0.6 $\log_{10}$) *Rhizobium* populations ($P < 0.05$) than the untreated control treatment. At Rosemaund, on the Cd-rich rate 3 treatment there were smaller (> 0.9 $\log_{10}$) *Rhizobium* populations ($P < 0.05$) than the untreated control treatment. At Hartwood, on the Cd-rich rate 4 treatment, there were smaller (> 3.0 $\log_{10}$) *Rhizobium* populations ($P < 0.05$) than the uncontaminated digested sludge cake treatment. At Auchincruive, on the Cd-rich rate 1 and 4 treatments there were smaller (> 1.0 $\log_{10}$) *Rhizobium* populations ($P < 0.01$) than the untreated control treatment. There were no differences in *Rhizobium* population sizes ($P >$

0.05) between any of the Cd rich, uncontaminated digested and untreated control treatments at the other three sites.

At Gleadthorpe, Rosemaund and Hartwood on the long-term Zn build-up treatment, *Rhizobium* population sizes were 0.7-2.4 $\log_{10}$ smaller (P > 0.05) than the long-term uncontaminated digested build-up treatment at each site. At Gleadthorpe, on the long-term Cu build-up treatment, *Rhizobium* population sizes were > 1.0 $\log_{10}$ smaller (P > 0.05) than the long-term uncontaminated raw build-up treatment. There were no differences in *Rhizobium* population sizes (P > 0.05) between the long-term Cd build-up treatment and the untreated control at any of the nine sites.

On the metal dose-response treatments, regression of soil rhizobia MPNs against total topsoil Zn concentrations identified significant inverse relationships (P < 0.05) at eight of the nine sites, there was no relationship at Shirburn. There were inverse relationships (P < 0.05) between rhizobia MPNs and total topsoil Cu concentrations at four sites (Pwllpeiran, Rosemaund, Bridgets and Hartwood). On the Cd dose-response treatments, there were significant inverse relationships (P < 0.05) at four sites (Gleadthorpe, Woburn, Pwllpeiran and Hartwood). On all of the metal-dose response treatments where there was a relationship between rhizobia MPNs and total topsoil metals, rhizobia MPNs *decreased* with increasing total topsoil metal concentrations (Figures 46 to 54). A summary of the regression analyses is given in Table 30.

Figure 46. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Gleadthorpe (spring 2005)

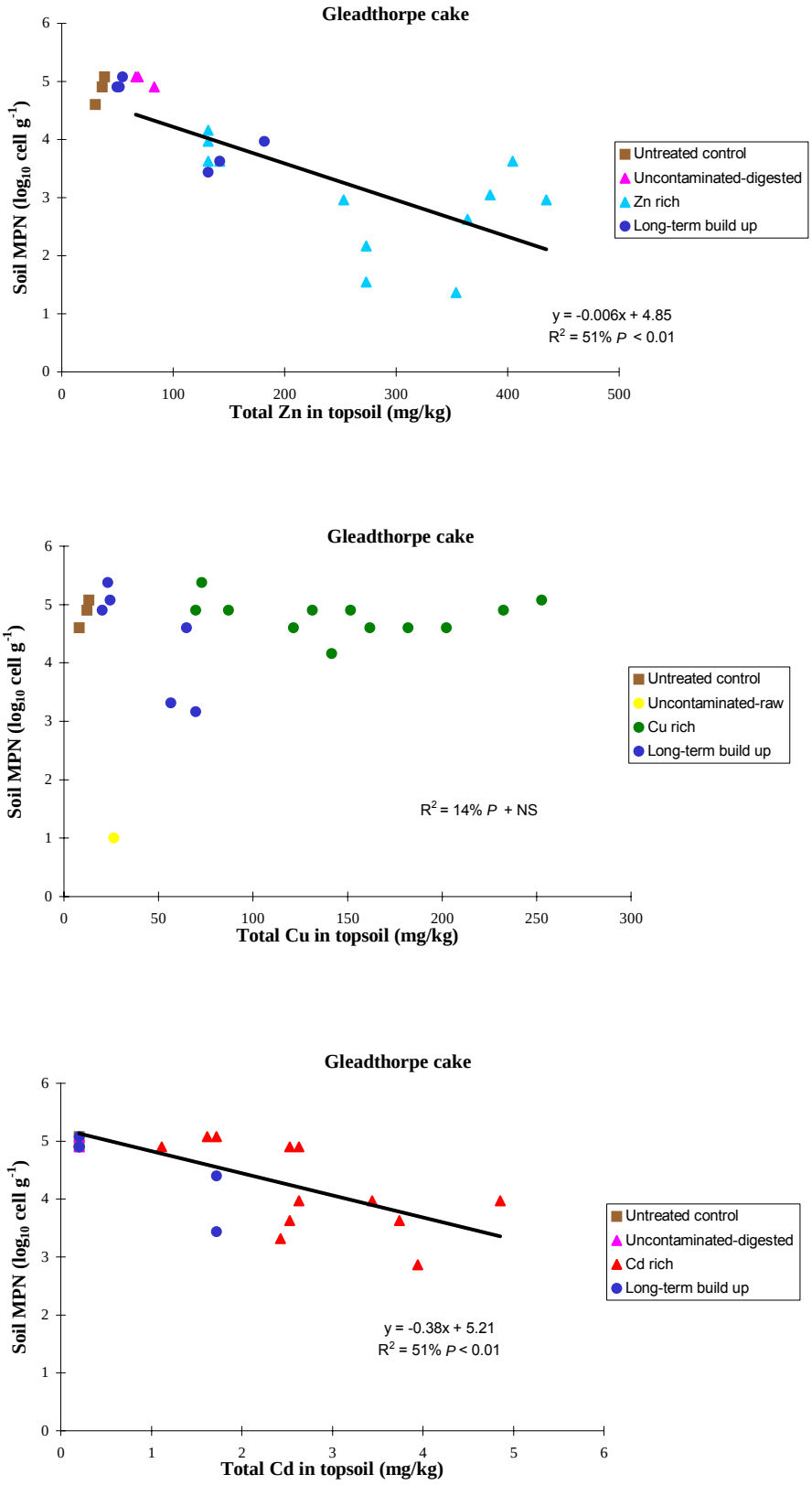
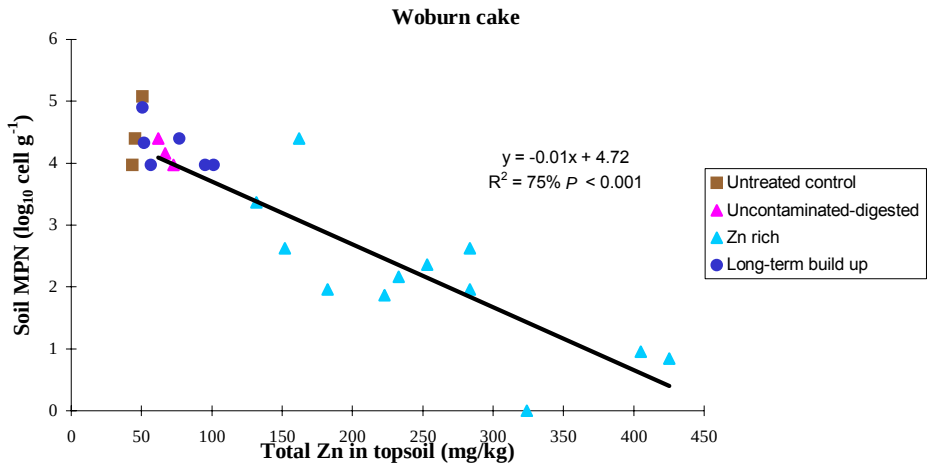
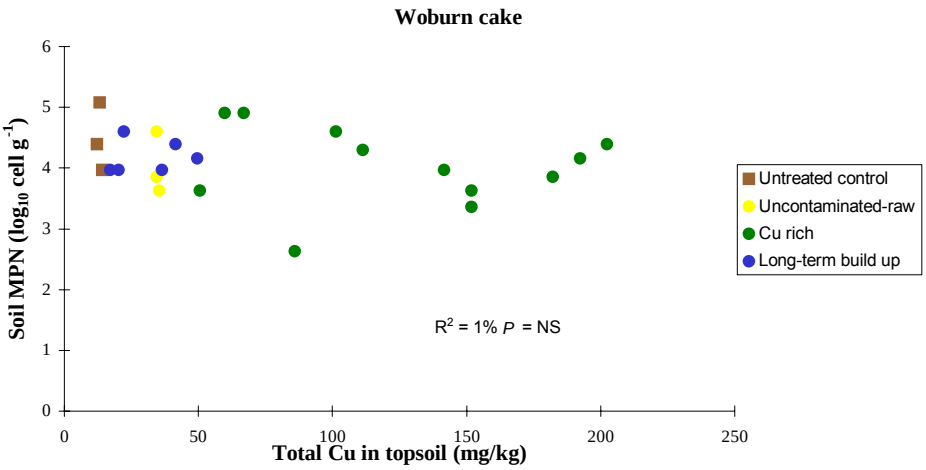


Figure 47. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Woburn (spring 2005)





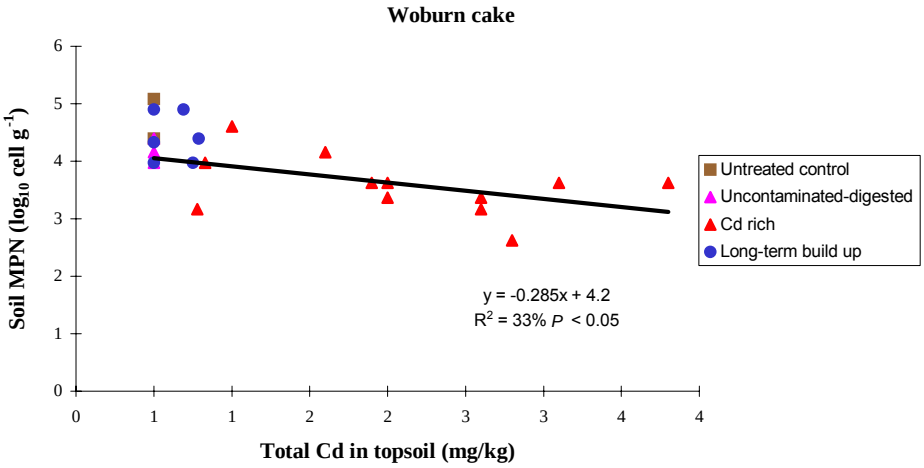


Figure 48. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Watlington (spring 2005)

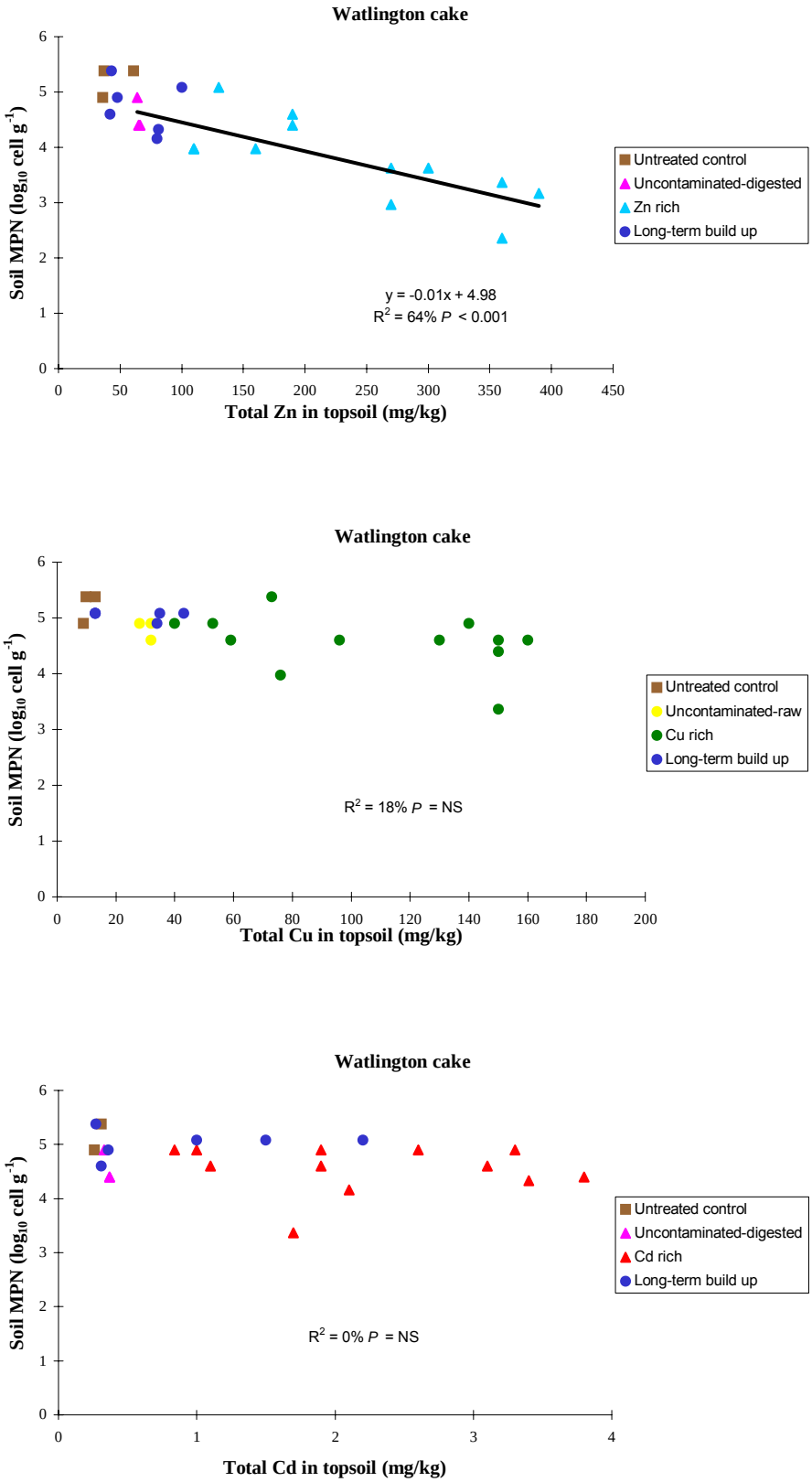


Figure 49. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Pwllpeiran (spring 2005)

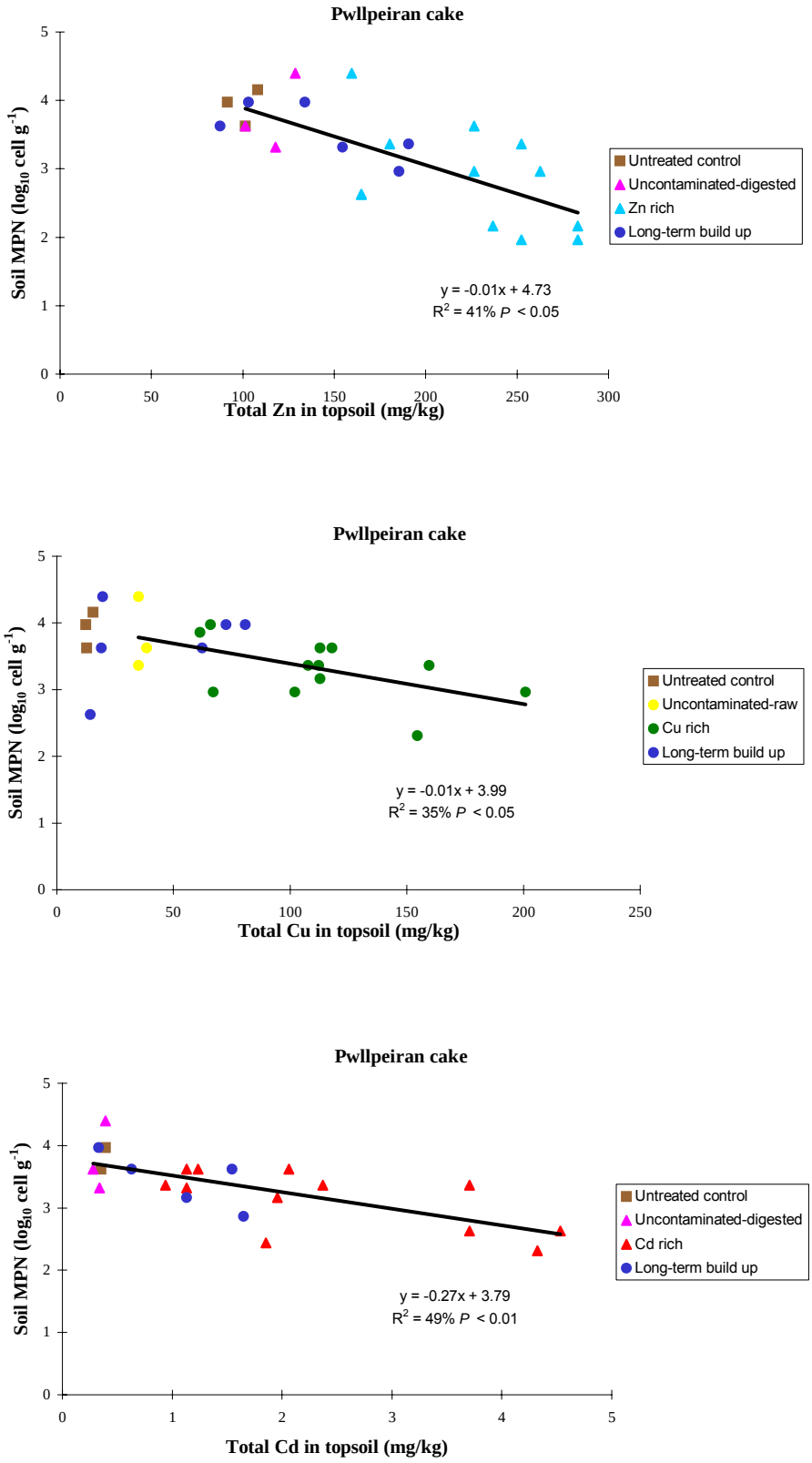


Figure 50. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Rosemaund (spring 2005)

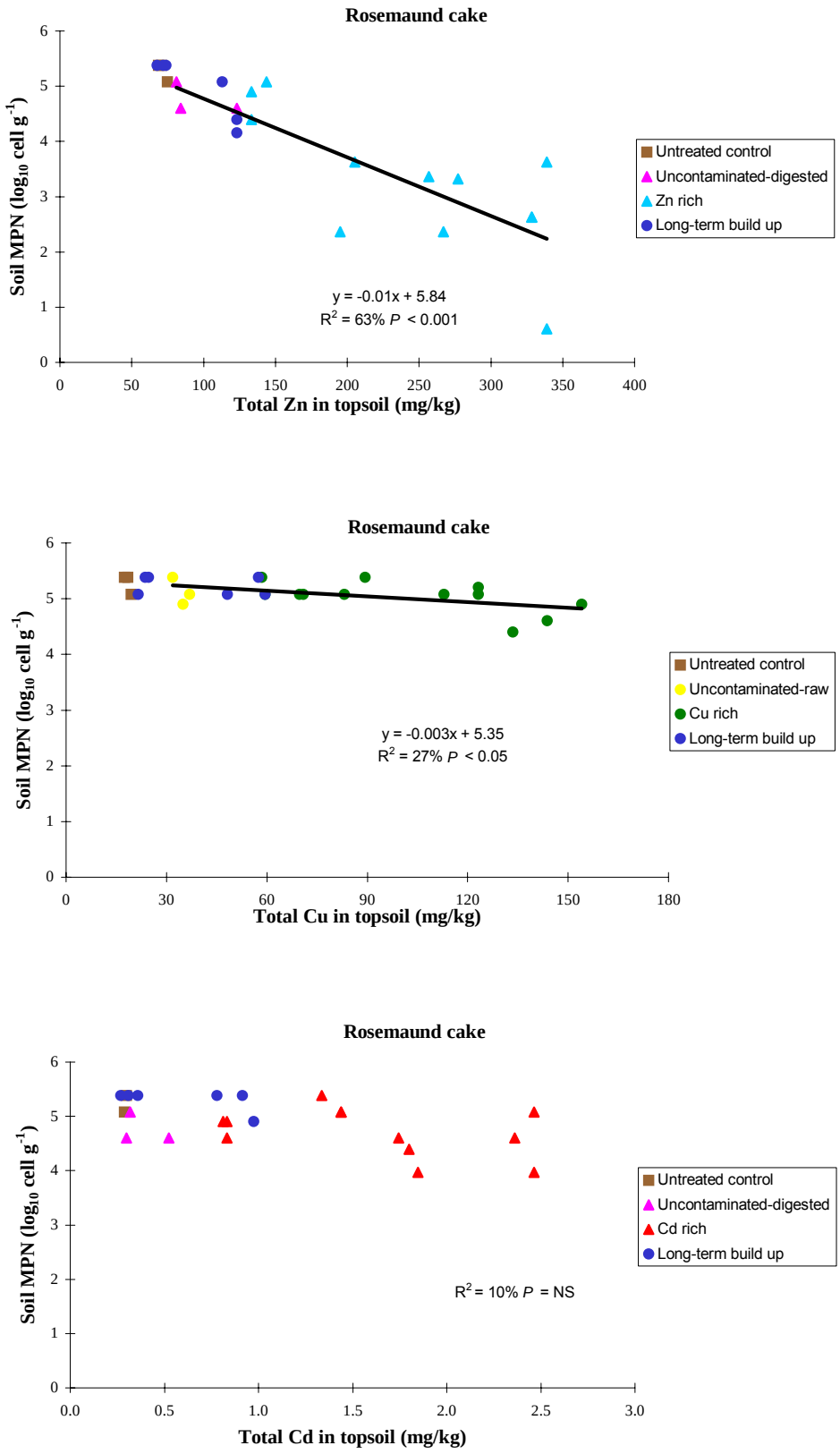
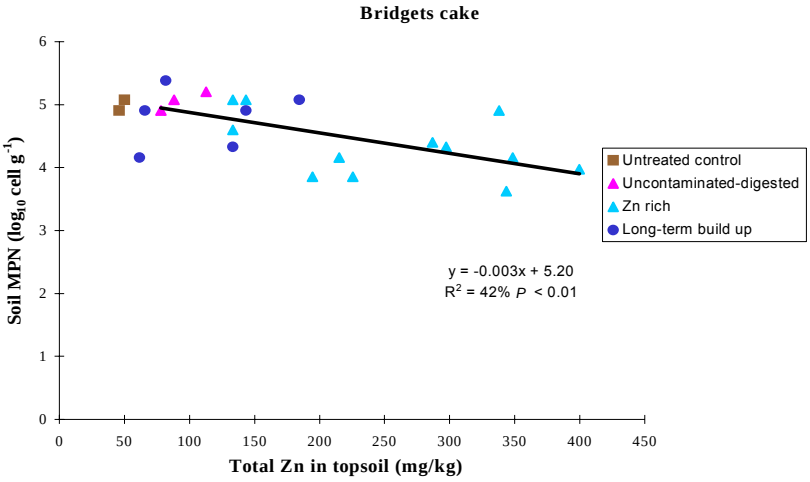
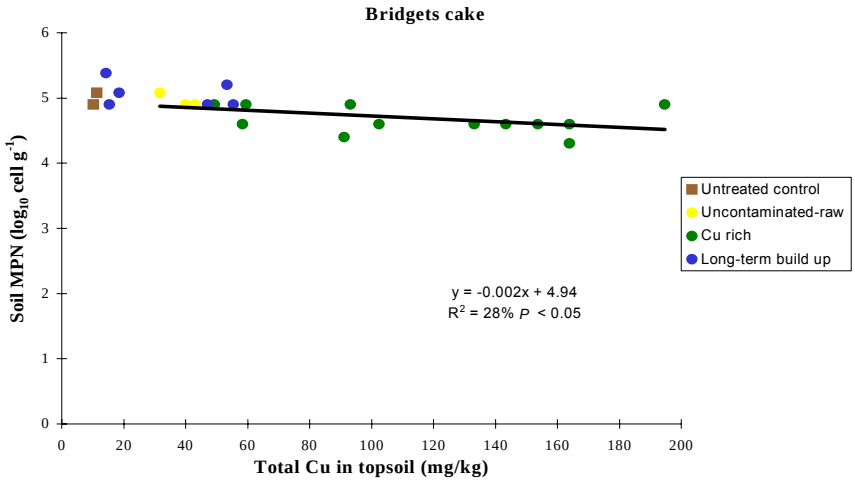


Figure 51. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Bridgets (spring 2005)





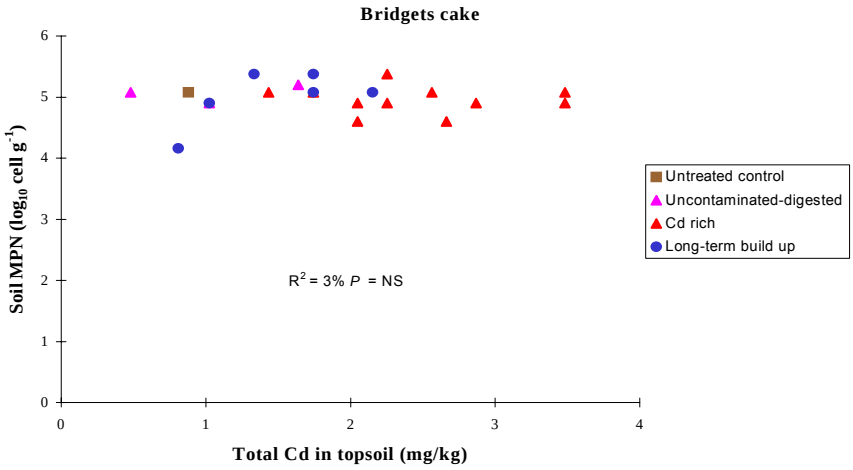
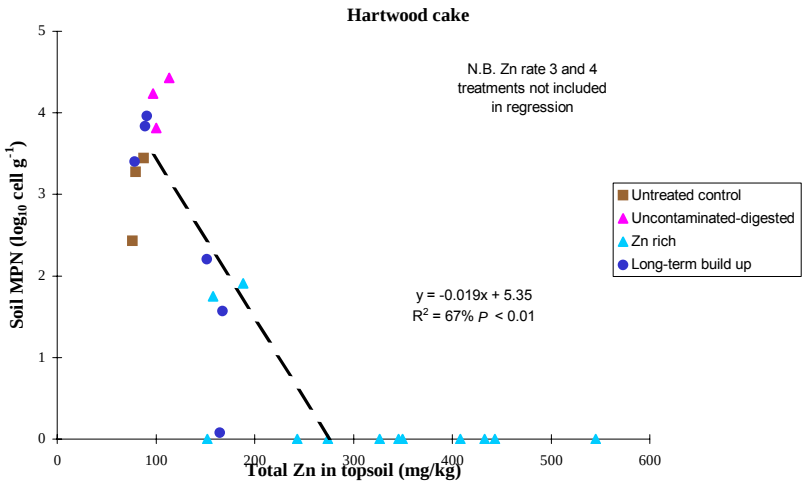
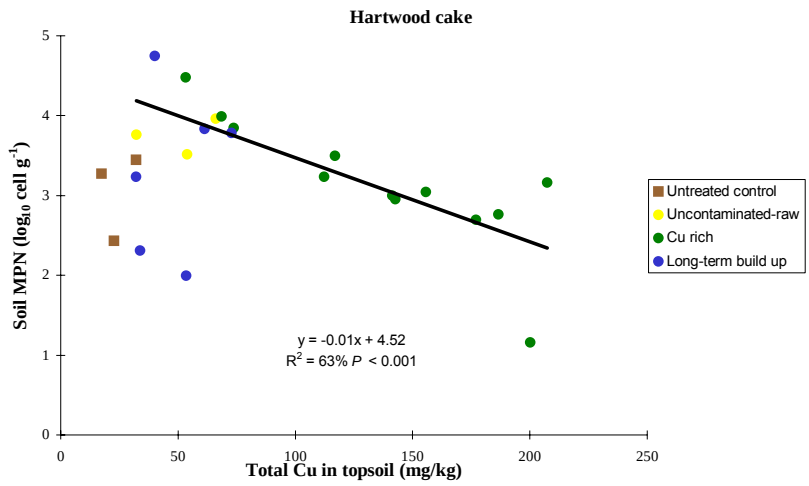


Figure 52. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Hartwood (spring 2005)





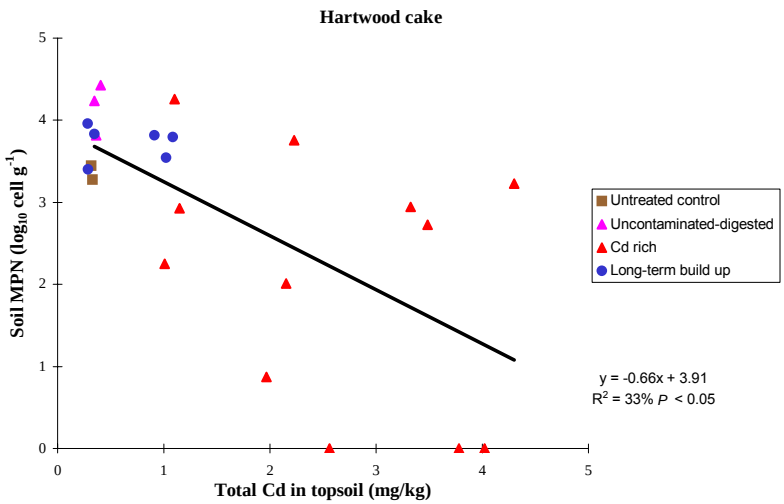


Figure 53. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs-Auchincruive (spring 2005)

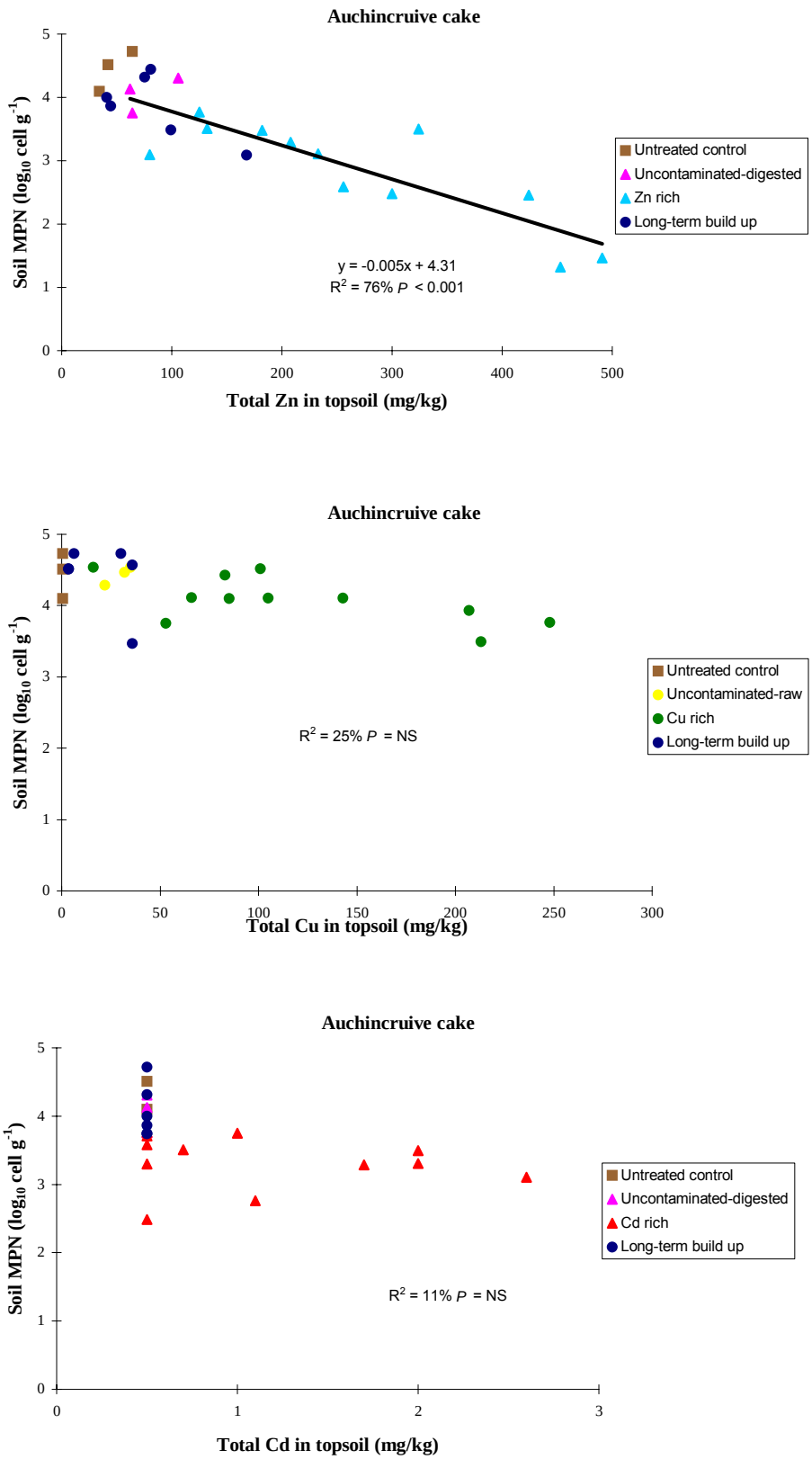


Figure 54. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Shirburn (spring 2005)

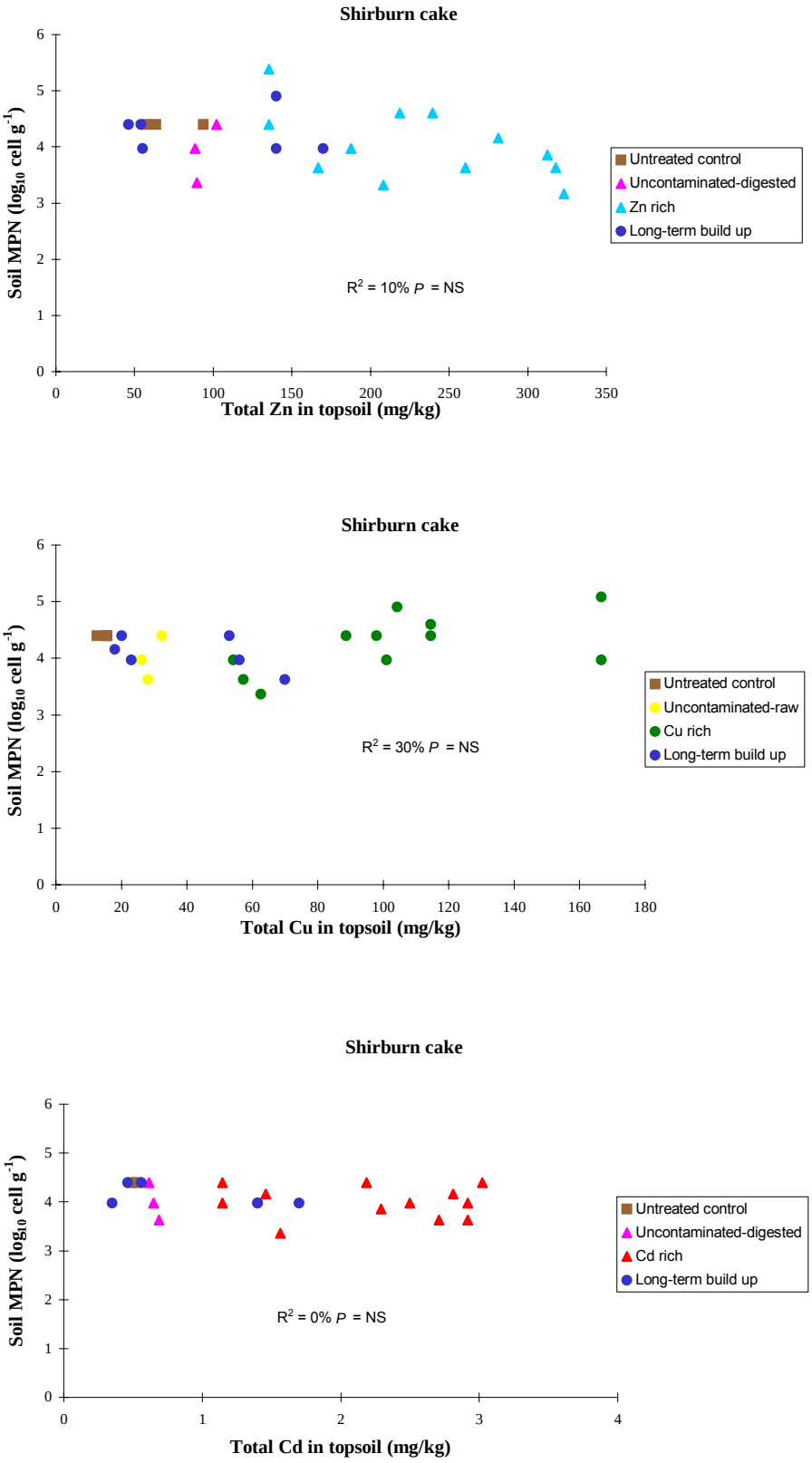


Table 30. Summary of relationships between rhizobia MPNs (log_{10}) and total topsoil metals - sludge cake sites (spring 2005 samples)

	Zn	Cu (r^2 ; *P*)	Cd
GLE	33%; *P* < 0.01	NS	21%; *P* < 0.01
WOB	59%; *P* < 0.001	NS	18%; *P* < 0.05
WAT	27%; *P* < 0.001	NS	NS
PWL	43%; *P* < 0.05	16%; *P* < 0.05	20%; *P* < 0.01
ROS	47%; *P* < 0.001	7%; *P* < 0.05	NS
BRI	13%; *P* < 0.01	5%; *P* < 0.05	NS
HAR	100%; *P* < 0.01	26%; *P* < 0.001	47%; *P* < 0.05
AUC	32%; *P* < 0.001	NS	NS
SHI	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship Blue = positive relationship. Model parameters for significant responses are given in Figures 46-54.

Significant inverse relationships (P < 0.05) between rhizobia MPNs and total topsoil Zn concentrations were evident at six sites in both 2003 and 2005 (Gleadthorpe. Woburn, Watlington, Rosemaund, Bridgets and Hartwood) and two further sites (Pwllpeiran and Auchincruive) in 2005. For Cu, a significant (P < 0.05) inverse relationship was evident at Auchincruive in 2003 and at four other sites in 2005 (Pwllpeiran, Rosemaund, Bridgets and Hartwood). For Cd, significant (P > 0.05) inverse relationships were evident at two sites in 2003 (Rosemaund and Shirburn) and at four other sites in 2005 (Gleadthorpe, Woburn, Pwllpeiran and Hartwood).

4.1.2 MPN Quality Assurance (Defra project SP0134)

Three laboratories conduct the determination of most probable numbers (MPNs) of *Rhizobium leguminosarum* bv. *trifolii* (clover rhizobia), *viz.*: Rothamsted Research for the seven sites in England and Wales, the Macaulay Institute for the Hartwood field site and SAC for the Auchincruive field site. In order to underpin confidence in the results generated from the *R. leguminosarum* bv. *trifolii* measurements, a QA exercise was conducted between the three laboratories undertaking this assay (Defra project SP0134). A summary of Defra project SP0134 is given below, the final report can be found in Appendix E.

Topsoil samples (0-25 cm) were taken from three replicate plots on each of four treatments, to provide a range in likely rhizobia MPN levels based on the assays undertaken in spring 2003 (2004 at Auchincruive), viz.:

Field site	Treatment
Gleadthorpe	Cu rate 1 (*c.* 115,000 cells g^{-1})
Auchincruive	Untreated control (*c.* 11,000 cells g^{-1})
Pwllpeiran	Cd rate 3 (*c.* 630 cells g^{-1})
Hartwood	Zn rate 4 (< 1 cell g^{-1})

The three replicate plots at each site were sub-sampled individually (*c.*20 cores/plot) and bulked in the field to give one sample per site. The bulked soil samples were sieved moist, and each of the participating laboratories (Rothamsted Research, the Macaulay Institute and SAC) received three sub-samples from each site (*c.*100g each) for determination of *R. leguminosarum* bv. *trifolii*, *to* enable inter laboratory precision, as well as between laboratory variability to be assessed.

There were no significant differences (ANOVA, $P > 0.05$) in *R. leguminosarum* bv. *trifolii* most probable numbers between the three laboratories participating in this QA scheme. The three laboratories analysed the Hartwood sample independently and none found rhizobia, thereby confirming the Hartwood data. Moreover, there was no evidence to suggest that rhizobia numbers should differ based on the laboratory conducting the determination *i.e.* soil samples from England and Wales analysed by Rothamsted Research are not likely to differ in rhizobia MPNs from soil samples taken and analysed in Scotland by the Macaulay Institute or SAC.

4.1.3 Topsoil sampling depth and microbial properties (Defra projects SP0135 and SP0137)

Metal concentrations in soil samples (0-25cm depth) taken at some sites during Phase II of the main project (Defra project SP0125, 1999 and 2001) and the first sampling during Phase III (SP0130, 2003), showed a general trend of decreasing concentrations with time.

An extensive soil sampling programme was undertaken as part of a subsidiary contract (Defra project SP0135) that focussed on the high Zn treatments (as these would provide the most sensitive indicator of metal distribution with soil depth) at three of the sludge cake experimental sites. The sludge cake sites selected represented two sites (Watlington and Rosemaund) that had shown a decrease in topsoil metal concentrations with time and a third site (Gleadthorpe) that had shown no apparent decrease in metal concentrations. A summary of SP0135 is given below, a copy of the Defra final report can be found in Appendix F.

In order to measure the distribution of Zn and organic carbon (as an additional indicator of topsoil depth and sludge cake distribution) six prescribed sampling depths were used (0-15, 15-20, 20-25, 25-30, 30-35, 35-40 cm), with the greatest intensity of sampling focussed around the likely interface depth between the topsoil and subsoil.

Soil total Zn concentrations were higher ($P < 0.05$) on the Zn rate-4 treatments in the 0-15, 15-20 and 20-25cm depth horizons at all three

sites, and the 25-30cm depth at two of the sites, compared with the corresponding untreated control samples. The differences between the Zn rate-4 treatments and the untreated control samples were not unexpected, as once metals are added to topsoil they accumulate, as they are not easily leached, and the amounts removed by crops are small. The corresponding soil organic C concentrations were also higher ($P < 0.05$) on the Zn rate-4 treatments in the 0-15cm horizon at all three sites, compared with the untreated control samples.

There was no evidence from the data to support a hypothesis of metals leaching to depth within the soil profile. Also, the lack of an increase in organic C at depth (> 25cm) supports the fact that sludge has not been diluted by deep cultivation below the 'standard' 0-25cm sampling depth. The data, from all three sites sampled in this study, show clear decreases in Zn (and organic C) concentrations with depth, even within the standard 0-25cm sampling horizon. It is most likely that the metal concentration decrease over time (at some of the sites) are due to soil 'settlement', as the added sludge organic matter has oxidised and bulk density has increased, which has progressively resulted in the cultivated horizon becoming shallower and the 'standard' 0-25cm depth sampling being diluted by relatively uncontaminated subsoil. This was supported by visual evidence of the plots being lower than the surrounding discard areas and increased resistance noted in the field by samplers when pushing the sampling auger down in to the soil to achieve the full 25cm sampling depth *i.e.* at some sites the 'standard' 0-25cm sampling depth is lower than the depth of soil cultivation.

As a result of the clear changes in metal concentrations within the 'standard' 0-25cm sampling depth, there was a need to assess potential metal effects on soil microbial properties (biomass carbon, respiration rates and rhizobia MPNs) within the most concentrated metal horizon (*i.e*. 0-15cm), and any associated impacts on soil metal concentration and microbial property relationships (Defra project SP0137).

Within SP0137, the soil sampling programme focused on the Zn treatments at two sites for rhizobia MPNs (*i.e*. Gleadthorpe and Watlington where there had been relationships between Zn and rhizobia MPNs in 3 consecutive study years) and on the Cu treatments at two sites for biomass carbon and respiration rate (*i.e*. Woburn and Watlington where there had been consistent relationships with these parameters). A summary of the results obtained from project SP0137 are described below, a copy of the full Defra report can be found in Appendix G.

At both Gleadthorpe and Watlington for rhizobia MPNs, and Woburn and Watlington for biomass C and respiration rate, there was little evidence of any marked differences in soil microbial properties between soil samples taken from either the 0-15 or 0-25cm depth horizons. The lack of difference in soil microbial properties was somewhat surprising considering the significant differences ($P < 0.05$) in soil organic carbon between the two soil depth horizons at all the sites sampled, with the exception of the Cu plots at Watlington. There were significant

differences ($P < 0.05$) in total metal concentrations between the two soil depth horizons at all the sites sampled, with the exception of the Cu plots at Woburn.

4.1.4 Soil chemical properties

Soil metals

Total and NH_4NO_3 extractable topsoil metal (Zn, Cu and Cd) concentrations in samples taken from all the treatment plots in spring 2003 and 2005 at all the sites (England, Wales and Scotland) are presented in Appendices C and D.

2003 sampling: Topsoil NH_4NO_3 extractable Zn concentrations were consistently higher at Hartwood, Rosemaund and Auchincruive than at the other non-calcareous sites, and were the lowest at Shirburn (the calcareous site). Extractable Zn concentrations on the Zn rate 4 treatment at Hartwood were *c.*100 fold higher than at Shirburn (54 mg/kg compared with 0.5 mg/kg, respectively).

Topsoil NH_4NO_3 extractable Cu concentrations were consistently higher at Shirburn (the calcareous site), and lower at Woburn, Bridgets and Hartwood than at the other sites. Extractable Cu concentrations on the rate 4 treatment at Shirburn were *c.*4 to 5-fold higher than at Woburn, Bridgets and Hartwood (3.0 mg/kg compared with 0.60 to 0.75 mg/kg, respectively) and *c.*1.5 to 3-fold higher than at the other sites (0.9 to 1.8 mg/kg).

Topsoil NH_4NO_3 extractable Cd concentrations were consistently higher at Hartwood and Auchincruive than at the other sites, and were the lowest at Shirburn. Extractable Cd concentrations on the rate 4 treatment at Hartwood and Auchincruive were *c.*7-fold higher than at Shirburn (0.101 mg/kg compared with 0.014 mg/kg, respectively).

At all of the nine sites, there were moderate to strong relationships ($P < 0.001$) between total topsoil Zn, Cu and Cd concentrations and corresponding topsoil NH_4NO_3 extractable concentrations (r^2 = 44-95% for Zn, 60-97% for Cu and 31-81% for Cd).

2005 sampling: Topsoil NH_4NO_3 extractable Zn concentrations were consistently higher at Hartwood and Auchincruive than at the other non-calcareous sites, and were the lowest at Shirburn (the calcareous site). Extractable Zn concentrations on the Zn-rich rate 4 treatment at Hartwood were *c.*130-fold higher than at Shirburn (44 mg/kg compared with 0.3 mg/kg, respectively), and 2 to 20-fold higher than at the other six non-calcareous sites (2 to 22 mg/kg).

Topsoil NH_4NO_3 extractable Cu concentrations were consistently higher at Watlington and Pwllpeiran than at the other sites, and were the lowest at Hartwood. Extractable Cu concentrations on the rate 4 treatment at Watlington were *c.*4-fold higher than at Hartwood (2.1 mg/kg compared with 0.54 mg/kg, respectively) and *c.*2-fold higher than at the other sites (*c.*1 mg/kg).

Topsoil NH_4NO_3 extractable Cd concentrations were consistently higher at Rosemaund than at the other sites, and were the lowest at Bridgets

and Shirburn. Extractable Cd concentrations on the rate 4 treatment at Rosemaund were *c*.14-fold higher than at Shirburn (0.20 mg/kg compared with 0.014 mg/kg, respectively), and 1.5 to 3-fold higher than at the other sites (0.07-0.13 mg/kg).

At all of the nine sites (six for Cd), there were moderate to strong relationships ($P < 0.01$) between total topsoil Zn, Cu and Cd concentrations and corresponding topsoil NH_4NO_3 extractable concentrations (r^2 = 50-78% for Zn, 43-89% for Cu and 14-76% for Cd). Note: At Gleadthorpe, Woburn and Shirburn analytical problems were encountered in the determination of low Cd concentrations in the NH_4NO_3 extracts.

Metal speciation

Total soil pore water Zn, Cu and Cd concentrations, and free ion Zn^{2+}, Cu^{2+} and Cd^{2+} concentrations were measured in soil samples taken from selected treatments (1, 2, 3, 6, 10, 14 at sites 1 to 6, and 1, 2, 3, 8, 13 and 18 at site 9) in autumn 2004 (Tables 31 - 36).

Total Zn concentrations in soil solution were numerically higher on the Zn rate 3 treatments than the uncontaminated digested sludge cake and untreated control treatments at all sites. However, these differences were only significant ($P < 0.05$) at Gleadthorpe, Woburn, Pwllpeiran and Rosemaund. Indeed, at Shirburn the Zn concentration in solution was highest ($P < 0.001$) (surprisingly) on the Cu rate 3 treatment (Table 31). The order of decreasing total soil solution Zn concentrations was: Gleadthorpe > Watlington > Woburn > Rosemaund > Bridgets > Pwllpeiran > Shirburn.

Table 31. Total Zn in soil solution (mg/l) extracted from soils sampled in autumn 2004.

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	SHIR
Control	0.070	0.121	0.024	0.022	0.022	0.021	0.022
Unc. Digested	0.104	0.090	0.036	0.024	0.044	0.039	0.028
Unc. Raw	0.101	0.158	0.245	0.026	0.036	0.097	0.025
Zn rate 3	0.939	0.463	0.706	0.050	0.360	0.088	0.034
Cu rate 3	0.081	0.100	0.002	0.026	0.071	0.070	0.236
Cd rate 3	0.085	0.102	0.002	0.038	0.093	0.067	0.002
ANOVA p	< 0.001	< 0.001	NA	0.013	0.041	NS	< 0.001
$LSD_{0.05}$	0.181	0.162	-	0.023	0.331	-	0.181
CV%	28	33	-	27	112	52	25
n =	18	18	18	18	18	18	18
LoDe*	0	0	6	0	0	0	3

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out). NA = not assessed

Free Zn^{2+} soil solution concentrations were higher ($P < 0.05$) on the Zn rate 3 treatments than the other treatments at three of the sites (Gleadthorpe, Woburn and Rosemaund). At Pwllpeiran and Bridgets there were no differences ($P > 0.05$) between the rate 3 and other treatments, and at Shirburn (surprisingly) free Zn^{2+} concentrations were

higher ($P < 0.05$) on the Cu rate 3 treatments (Table 32). Soil pore water free Zn^{2+} decreased in order: Gleadthorpe > Woburn = Watlington > Rosemaund> Shirburn = Bridgets = Pwllpeiran.

Table 32. Free Zn^{2+} in soil solution (mg/l) extracted from soils sampled in autumn 2004

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	SHIR
Control	0.005	0.111	0.001	0.003	0.005	0.008	0.019
Unc. Digested	0.005	0.038	0.011	0.004	0.005	0.037	0.021
Unc. Raw	0.032	0.130	0.050	0.006	0.009	0.031	0.020
Zn rate 3	0.689	0.275	0.282	0.019	0.165	0.021	0.024
Cu rate 3	0.027	0.026	0.001	0.012	0.014	0.058	0.073
Cd rate 3	0.022	0.051	0.001	0.018	0.008	0.018	0.001
ANOVA p	< 0.001	0.002	NA	NS	0.015	NS	< 0.001
$LSD_{0.05}$	0.316	0.149	-	-	0.141	-	0.029
CV%	86	50	-	96	144	76	39
n =	18	18	18	18	18	18	18
LoDe*	0	0	11	0	0	0	3

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out). NA = not assessed

Total soil solution Cu concentrations were higher ($P < 0.001$) on the Cu rate 3 treatments than the other treatments at all sites (Table 33). The order of decreasing total soil solution Cu concentrations was Gleadthorpe> Woburn= Watlington> Rosemaund> Bridgets> Shirburn> Pwllpeiran.

Table 33. Total Cu in soil solution (mg/l) extracted from soils sampled in autumn 2004

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	SHIR
Control	0.022	0.012	0.012	0.002	0.011	0.007	0.007
Unc. Digested	0.130	0.080	0.090	0.013	0.072	0.050	0.029
Unc. Raw	0.066	0.053	0.054	0.014	0.043	0.041	0.024
Zn rate 3	0.158	0.128	0.166	0.018	0.107	0.096	0.060
Cu rate 3	0.306	0.235	0.255	0.050	0.137	0.105	0.087
Cd rate 3	0.119	0.103	0.119	0.023	0.074	0.057	0.051
ANOVA p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	0.066	0.051	0.062	0.024	0.061	0.024	0.010
CV%	17	18	19	42	29	14	8
n =	18	18	18	18	18	18	18
LoDe*	0	0	0	3	0	0	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

Free Cu^{2+} soil solution concentrations were higher ($P < 0.001$) on the Cu rate 3 treatments than the other treatments at all sites. (Table 34). Less than 0.001% of the soil pore water total soluble Cu was present as soil pore water free Cu^{2+}. The order of decreasing soil pore water free Cu^{2+}

was Woburn> Gleadthorpe> Pwllpeiran= Watlington> Rosemaund> Bridgets> Shirburn.

Table 34. Free Cu^{2+} in soil solution (ng/l) extracted from soils sampled in autumn 2004

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	SHIR
Control	0.0045	0.0377	0.0060	0.0031	0.0064	0.0049	0.0014
Unc. Digested	0.1929	0.2207	0.0593	0.0685	0.0310	0.0201	0.0085
Unc. Raw	0.1032	0.5084	0.0333	0.2252	0.0227	0.0487	0.0101
Zn rate 3	1.3294	1.5904	0.3815	0.1687	0.3610	0.0835	0.0447
Cu rate 3	4.0756	16.411	1.0970	1.1931	0.6194	0.3556	0.0893
Cd rate 3	0.4527	0.5525	0.4166	0.3717	0.0289	0.0401	0.0373
ANOVA p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	0.389	0.798	0.753	0.900	0.888	0.813	0.270
CV%	2	5	4	5	4	4	1
N =	18	18	18	18	18	18	18
LoDe*	0	0	0	0	0	0	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

Total soil solution Cd concentrations were numerically higher on the Cd rate 3 treatments compared with the other treatments at six of the sites, and were statistically different ($P < 0.05$) at four of the sites (Gleadthorpe, Woburn, Pwllpeiran and Rosemaund). At Bridgets (surprisingly) the Cd concentration in solution was highest ($P < 0.05$) on the Cu rate 3 treatment (Table 35). Soil total pore water soluble Cd decreased in the order: Gleadthorpe > Woburn > Watlington = Rosemaund = Pwllpeiran > Shirburn > Bridgets.

Table 35. Total Cd in soil solution (µg/l) extracted from soils sampled in autumn 2004

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	SHIR
Control	0.146	0.056	0.014	0.022	0.065	0.008	0.0003
Unc. Digested	0.224	0.145	0.003	0.048	0.188	0.050	0.0003
Unc. Raw	0.346	0.079	0.0003	0.022	0.086	0.042	0.0003
Zn rate 3	1.709	0.504	0.119	0.184	0.597	0.096	0.070
Cu rate 3	0.363	0.145	0.0003	0.049	0.077	0.105	0.0003
Cd rate 3	1.782	1.235	1.049	0.889	1.023	0.057	0.228
ANOVA p	< 0.001	< 0.001	NA	< 0.001	0.019	< 0.001	NA
$LSD_{0.05}$	0.239	0.317	-	0.377	0.894	0.025	-
CV%	11	31	-	67	93	15	-
N =	18	18	18	18	18	18	18
LoDe*	0	0	9	2	0	0	12

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out). NA = not assessed

Free Cd^{2+} soil solution concentrations were numerically higher on the Cd rate 3 treatments compared with the other treatments at five of the sites

and were statistically different ($P < 0.05$) at three of the sites. At Gleadthorpe, the free Cd^{2+} soil solution concentration was higher ($P < 0.05$) on the Zn rate 3 treatment (Table 36) than the other treatments. Soil pore water free Cd^{2+} decreased in the order Pwllpeiran > Gleadthorpe = Woburn > Watlington > Rosemaund > Shirburn > Bridgets.

Table 36. Free Cd^{2+} in soil solution (µg/l) extracted from soils sampled in autumn 2004

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	SHIR
Control	0.042	0.049	0.014	0.001	0.023	0.001	0.001
Unc. Digested	0.076	0.083	0.003	0.009	0.062	0.001	0.001
Unc. Raw	0.164	0.078	0.001	0.002	0.044	0.001	0.001
Zn rate 3	0.952	0.273	0.068	0.133	0.268	0.002	0.030
Cu rate 3	0.194	0.123	0.001	0.025	0.023	0.003	0.001
Cd rate 3	0.543	0.522	0.441	0.655	0.329	0.001	0.099
ANOVA p	< 0.001	< 0.001	NA	< 0.001	NS	NA	NA
$LSD_{0.05}$	0.484	0.190	-	0.318	-	-	-
CV%	52	36	-	82	130	-	-
N =	18	18	18	18	18	18	18
LoDe	0	0	9	5	0	14	12

LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

Across the seven sites in England and Wales there were strong relationships ($P < 0.001$) between soil pore water total soluble Zn, Cu and Cd, and soil pore water free Zn^{2+} (r^2 = 85%), Cu^{2+} (r^2 = 58%) and Cd^{2+} (r^2 = 83%) concentrations, respectively (data not shown). Approximately 60% of the soil pore water total soluble Zn was in the form of Zn^{2+} and 45% of the soil pore water total soluble Cd was in the form of Cd^{2+}, but less than 0.1% of the total soluble soil Cu was in the form of Cu^{2+}. In the metal speciation studies undertaken in 2001, soil pore water free Zn^{2+} and Cd^{2+} represented 80% and 75% of soil pore water total soluble Zn and Cd concentrations, respectively. The reductions in the proportion of soil pore water total soluble Zn and Cd found as free Zn^{2+} and Cd^{2+} in 2004 compared with 2001 is most likely to be due to the soil pH in 2004 being higher and closer to the target pH of 6.5 for all non-calcareous field experiments.

Soil nutrients

The nutrient status (*i.e.* extractable P, K and Mg) and pH of the soil samples taken in spring 2005 is presented in full in Appendix D. Changes in soil nutrient levels (mean across all sites) as a result of the sludge cake additions are summarised in Table 37.

Table 37. Mean change in topsoil nutrient status as a result of the sludge cake additions relative to the untreated control treatments across all sites - spring 2005

Treatment	Ext. P (mg/l)	Ext. K (mg/l)	Ext. Mg (mg/l)	Total N (%)	Organic C (%)
Unc. Digested	58	- 16	3	0.10	0.66
Unc. Raw	57	- 6	- 26	0.07	0.57
Zn rich	59	- 25	- 2	0.09	0.73
Cu rich	41	- 23	- 13	0.08	0.63
Cd rich	72	- 19	- 3	0.12	0.87
Long-term build up	22	0.1	2	0.03	0.14

Soil extractable (Olsen) P concentrations on the moderate rate sludge cake treatments were higher (41 to 72 mg P/l) than the untreated controls. Soil extractable K and Mg concentrations were lower (-6 to -25 mg K/l and -3 to -26 mg Mg/l) as a result of crop offtakes, than the untreated controls.

Soil organic C levels on the 'digested' sludge cake treatments (uncontaminated digested, Zn rich, Cd rich) were on average *c.*0.8% higher than the untreated controls. On the undigested sludge cake treatments (uncontaminated raw and Cu rich) soil organic C contents were on average *c.*0.6% higher than the untreated controls.

Soil total N was on average 0.07 to 0.12% higher on the moderate rate build up treatments; an increase equivalent to 1750 – 3000 kg N/ha (assuming a bulk density of 1.0 [average bulk density across sludge cake treatments] and a topsoil depth of 25 cm) compared with the untreated controls.

As expected, the successive sludge cake applications to the long-term build-up treatments had only a small effect on soil nutrient status compared with the untreated controls, the most notable being the 22 mg/l increase in soil extractable P status and 0.14% increase in organic carbon content.

4.1.5 Soil physical properties

The soil bulk density measurements taken in spring 2005 are presented in Table 38. Across all the sites, the soil bulk density was *c*. 0.12 g/cm^3 lower on the treatments that had received sludge cakes compared with the untreated controls. The largest mean decreases were on the lighter textured soils at Woburn and Watlington (0.17 and 0.23 g/cm^3), and smallest (0.06 g/cm^3) on the high organic matter soil at Pwllpeiran.

Table 38. Soil bulk density determinations made at each site in spring 2005

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHIR*
1. Control	1.34	1.59	1.41	0.98	1.19	1.19	0.92	1.18	1.07
SE	0.054	0.095	0.028	0.034	0.030	0.020	0.005	0.012	0.048
2. Unc. digested	1.24	1.44	1.23	0.88	1.04	1.06	0.78	1.06	0.94
SE	0.043	0.002	0.044	0.013	0.020	0.039	0.033	0.025	0.019
3. Unc. raw	1.24	1.48	1.22	0.93	1.13	1.15	0.84	1.07	0.99
SE	0.003	0.032	0.046	0.021	0.019	0.009	0.083	0.016	0.022
6. Zn-rich	1.27	1.38	1.15	0.93	1.05	1.05	0.78	1.05	0.98
SE	0.028	0.019	0.014	0.014	0.022	0.026	0.035	0.026	0.013
10. Cu-rich	1.26	1.35	1.17	0.92	1.11	1.11	0.86	1.12	0.92
SE	0.005	0.037	0.010	0.015	0.010	0.029	0.032	0.013	0.023
14. Cd-rich	1.24	1.43	1.17	0.95	1.09	1.08	0.81	1.05	0.94
SE	0.044	0.024	0.036	0.010	0.012	0.036	0.014	0.006	0.029
Mean change†	-0.09	-0.17	-0.23	-0.06	-0.11	-0.10	-0.11	-0.11	-0.12

† Mean change = mean of sludge treatments minus control. SE = standard error

* Bulk densities measured on 'rate 3' metal-rich treatments (treatments 8, 13 and 18 at Shirburn).

4.1.6 Grain and grass yields and metal concentrations

Wheat grain yields (six sites in 2003 and 2005) and grass yields (2002 and 2004 at all sites, and 2003 and 2005 at Pwllpeiran, Hartwood and Auchincruive), and metal concentrations in the harvested dry matter were measured at the nine sites. The mean crop yields (Tables 39-42) and metal concentrations in the grain and grass dry matter are presented in Appendices H - K.

Table 39. Mean first cut grass yields (t dm/ha) at each site (May-June 2002)

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHI
Control	5.12	3.90	4.83	6.50	10.79	3.57	1.56	2.61	0.61
Unc. Dig.	4.98	6.32	5.49	6.99	10.97	5.05	2.89	3.55	1.23
Unc. Raw	5.22	6.07	5.17	7.03	9.93	5.07	2.76	3.65	1.26
Zn rate 1	5.51	6.02	4.96	6.07	11.61	5.37	2.32	4.11	0.96
Zn rate 2	5.16	6.47	5.76	6.12	10.12	5.78	3.05	4.36	0.84
Zn rate 3	5.25	6.90	4.93	6.45	10.58	5.29	2.48	4.14	1.00
Zn rate 4	5.74	6.80	5.22	6.78	10.80	5.67	2.60	4.45	0.80
ANOVA (1)	0.042	0.003	NS	NS	NS	0.010	NS	0.002	0.017
$LSD_{0.05}$	0.733	1.910	-	-	-	1.663	-	1.139	0.480
CV%	5	11	10	11	11	12	27	10	18
Cu rate 1	5.96	6.60	5.67	6.43	10.35	4.78	2.95	3.85	1.03
Cu rate 2	5.85	5.80	5.65	6.34	11.38	5.67	2.30	3.75	1.22
Cu rate 3	5.34	6.40	5.64	7.55	12.36	4.87	2.90	4.06	1.07
Cu rate 4	5.69	6.73	5.13	7.30	11.71	6.33	2.54	4.33	1.14
ANOVA (2)	NS	<0.001	NS	NS	NS	0.026	NS	<0.001	NS
$LSD_{0.05}$	-	1.545	-	-	-	2.243	-	0.719	-
CV%	10	9	9	14	11	16	29	7	25
Cd rate 1	4.50	6.77	5.53	6.28	10.48	5.29	2.58	3.99	1.22
Cd rate 2	5.42	6.57	5.22	7.20	10.41	4.85	2.49	3.82	1.39
Cd rate 3	5.55	6.57	5.18	6.24	10.87	5.58	2.31	3.68	1.31
Cd rate 4	6.10	6.80	5.89	7.54	11.69	5.10	2.51	4.21	0.97
ANOVA (3)	NS	<0.001	NS	NS	NS	NS	NS	0.022	0.036
$LSD_{0.05}$	-	1.274	-	-	-	-	-	1.305	0.748
CV%	19	7	7	12	17	14	23	13	24
LT Unc. Dig.	5.12	5.55	5.27	6.32	12.46	4.15	2.60	3.02	0.97
LT Unc. Raw	5.51	5.53	5.46	6.83	11.60	4.02	2.59	2.83	0.82
LT Zn	5.40	5.83	5.48	5.92	10.75	3.97	3.19	3.44	0.81
LT Cu	5.77	4.93	5.77	6.43	10.52	4.10	1.69	3.06	1.28
LT Cd	4.99	5.17	4.92	6.90	10.31	3.84	1.92	2.68	1.15
ANOVA (4)	NS	NS	0.022	NS	NS	NS	NS	0.024	NS
$LSD_{0.05}$	-	-	0.840	-	-	-	-	0.720	-
CV%	9	15	6	9	12	17	28	9	30

Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. The ANOVA fitted was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P <0.05)

2002 harvest: At Gleadthorpe, grass dry matter yield was 0.76 t/ha lower ($P < 0.05$) on the uncontaminated digested sludge cake treatment compared with the Zn-rich rate 4 treatment. At Woburn, grass dry matter yield was 2.1-3.0 t/ha lower ($P < 0.01$) on the untreated control compared with the uncontaminated digested sludge cake and Zn-rich rate 1-4 treatments. At Bridgets and Auchincruive, grass dry matter yields on the untreated control treatments were 1.7-2.2 and 1.5-1.8 t/ha lower ($P < 0.001$), respectively, than the Zn-rich rate 1-4 treatments. At Shirburn, grass dry matter yield on the uncontaminated digested sludge cake treatment was 0.6 t/ha higher ($P < 0.05$) than the untreated control (Table 39).

At Woburn, grass dry matter yield was 1.9-2.8 t/ha lower ($P < 0.001$) on the untreated control compared with the uncontaminated raw and Cu-rich rate 1-4 sludge cake treatments. At Bridgets, grass dry matter yield on the Cu-rich rate 4 treatment was 2.8 t/ha higher ($P < 0.05$) than the untreated control. At Auchincruive, grass dry matter yield on the untreated control was 1.0-1.7 t/ha lower ($P < 0.05$) than the uncontaminated raw and Cu-rich rate 1-4 sludge cake treatments.

At Woburn, grass dry matter yield on the untreated control was 2.4-2.9 t/ha lower ($P < 0.001$) than the uncontaminated digested and Cd-rich rate 1-4 sludge cake treatments. At Auchincruive, grass dry matter yields on the Cd-rich rate 1 and 4 treatments were 1.4 and 1.6 t/ha higher ($P < 0.05$), respectively, than the untreated control treatment. At Shirburn, grass dry matter yield on the Cd-rich rate 2 treatment was 0.8 t/ha higher ($P < 0.05$) than the untreated control.

On the long-term build-up treatments, grass dry matter yields on the Cu-rich treatment at Watlington and the Zn-rich treatment at Auchincruive were 0.9 and 0.8 t/ha, respectively, higher than the untreated control treatments.

On the metal dose-response treatments, regression of grass yields against total topsoil metal concentrations (2001 data) identified significant ($P < 0.05$) positive relationships only at Auchincruive for Zn and Cu (*i.e*. increasing grass yields with increasing total topsoil metal concentrations).

2003 harvest: There were no grain/grass yield differences between any of the Zn-rich treatments and the uncontaminated digested sludge cake or untreated control treatments (Table 40).

At Auchincruive, grass dry matter yield on the Cu-rich rate 1 treatment was 1.2 t/ha lower ($P < 0.05$) than the untreated control.

At Gleadthorpe, grain yield was 0.8 t/ha lower ($P < 0.05$) on the untreated control compared with the Cd-rich rate 3 sludge cake treatment. At Hartwood, grass yield was 1.3 t/ha lower ($P < 0.05$) on the untreated control compared with the Cd-rich rate 1 treatment.

At Auchincruive, grass dry matter yield on the long-term Zn treatment was 1.0 t/ha lower ($P < 0.05$) than the untreated control.

At Woburn, grain yield was 1.1 t/ha lower ($P < 0.05$) on the long-term Cu build-up treatment compared with the long-term Cd build up treatment and the long-term uncontaminated digested build-up treatment.

Table 40. Mean grain (t/ha, 85% dm basis) and grass yields (t/ha, 100% dm basis) at each site in 2003

Treatment	GLE	WOB	WAT	PWL*	ROS	BRG	HAR*	AUC*	SHI
Control	7.70	4.24	2.90	4.40	10.97	3.63	1.32	7.49	1.87
Unc. Dig.	7.73	4.33	2.78	4.67	10.96	4.09	1.78	6.86	1.78
Unc. Raw	7.58	3.07	3.12	5.26	10.75	4.01	1.55	6.88	1.61
Zn rate 1	8.05	3.34	2.69	5.12	10.87	3.97	1.63	6.31	1.43
Zn rate 2	7.36	4.12	2.65	5.33	9.70	4.15	1.97	6.82	1.45
Zn rate 3	7.16	4.07	2.80	4.95	10.01	3.91	1.65	6.48	1.73
Zn rate 4	8.25	3.51	2.69	4.90	10.66	4.14	1.45	6.04	1.76
ANOVA (1)	NS	NS	NS	NS	NS	NS	NS	NS	NS
$LSD_{0.05}$	-	-	-	-	-	-	-	-	-
CV %	6	10	13	12	9	7	21	8	24
Cu rate 1	8.10	3.25	3.01	4.45	10.67	4.38	2.24	6.27	1.62
Cu rate 2	7.69	3.10	3.17	5.64	11.40	4.23	1.52	7.42	1.26
Cu rate 3	7.71	3.73	3.11	5.83	10.55	3.82	1.46	6.44	1.66
Cu rate 4	6.70	3.81	2.57	5.07	10.11	4.21	1.74	6.51	2.00
ANOVA (2)	NS	NS	NS	NS	NS	NS	NS	0.019	NS
$LSD_{0.05}$	-	-	-	-	-	-	-	1.058	-
CV %	8	13	10	14	21	9	34	6	29
Cd rate 1	7.85	3.69	2.52	4.47	11.08	3.85	2.64	6.55	1.82
Cd rate 2	7.71	4.19	2.91	4.73	9.73	4.45	2.21	6.09	1.45
Cd rate 3	8.67	3.46	3.17	5.15	10.41	3.89	1.50	6.31	2.05
Cd rate 4	8.45	4.09	3.19	4.67	10.34	4.49	2.19	6.45	1.45
ANOVA (3)	0039	NS	NS	NS	NS	NS	0.024	NS	NS
$LSD_{0.05}$	0.937	-	-	-	-	-	1.182	-	-
CV%	5	18	12	12	13	8	22	7	22
LT Unc. Dig.	7.90	4.44	3.36	4.74	12.42	4.23	1.66	6.98	1.92
LT Unc. Raw	7.85	3.96	3.31	4.97	12.45	3.85	1.72	6.71	2.37
LT Zn	7.10	3.71	2.99	4.39	10.04	4.40	1.73	6.46	2.35
LT Cu	7.56	3.04	3.24	4.93	10.05	3.83	1.06	6.86	2.14
LT Cd	8.09	4.31	3.48	4.42	11.44	3.75	1.61	7.21	1.92
ANOVA (4)	NS	0.044	NS	NS	NS	NS	NS	0.029	NS
$LSD_{0.05}$	-	1.246	-	-	-	-	-	0.895	-
CV%	8	14	8	8	15	9	25	6	17

* At Pwllpeiran, Hartwood and Auchincruive ryegrass was grown. Results expressed on a dm basis. Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. The ANOVA fitted was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P <0.05)

On the metal dose-response treatments, regression of grain yields against total topsoil metal concentrations identified a significant ($P < 0.05$) positive relationship only at Woburn for Cu.

The relationships between mean grain Zn, Cu and Cd concentrations and ryegrass concentrations at Pwllpeiran, Hartwood and Auchincruive (harvest year 2003) and total topsoil metal concentrations at each of the sites are presented in Figures 55 to 63.

At all of the sites, there were strong relationships ($P < 0.001$) between Zn (r^2 = 40-84%) concentrations in the grain and total topsoil metal concentrations. Grain zinc concentrations on the Zn-rich rate 4 treatments ranged between 52 and 113 mg/kg across the seven sites. At the non-calcareous sites, the increase in grain Zn concentrations over the untreated controls was between 3 and 4-fold (50-80 mg/kg), whereas at Shirburn (the calcareous site) the increase was much smaller *c.*6% (3 mg/kg).

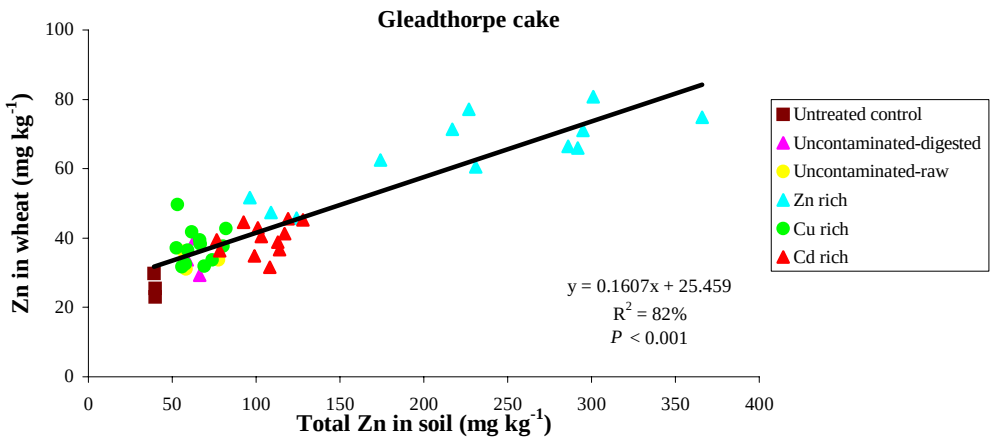
There were moderate to strong relationships ($P < 0.001$) between grain Cu concentrations and total topsoil Cu concentrations at five of the sites (r^2 = 32-79%). At Shirburn, the relationship was only weak ($P < 0.05$; r^2 = 7%). Grain Cu copper concentrations on the Cu-rich rate 4 treatments ranged between 6.0 and 8.3 mg/kg across the six non-calcareous sites. The mean increase in grain Cu concentrations over the untreated control was *c.*50% (2 mg/kg).

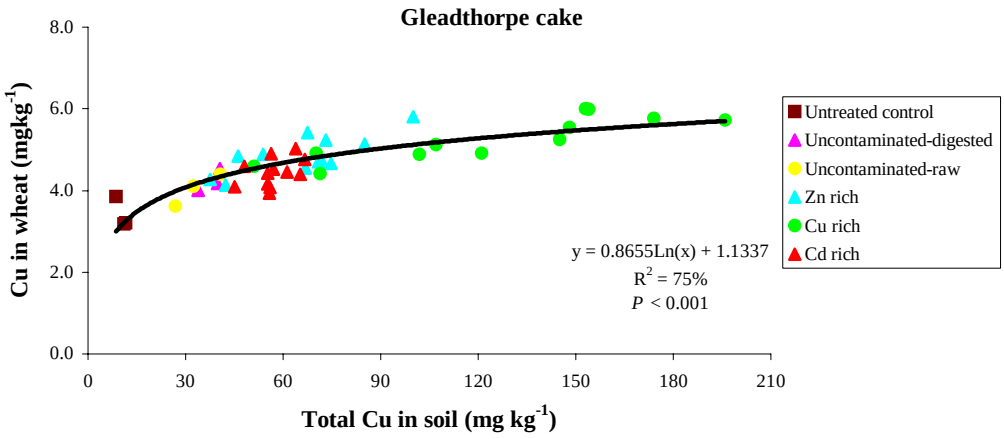
There were strong relationships ($P < 0.001$) between grain Cd (r^2 = 45-94%) concentrations and total topsoil Cd concentrations at all of the sites. Grain Cd concentrations on the Cd-rich rate 4 treatments ranged between 0.13 and 0.43 mg/kg at the non-calcareous sites, and at Shirburn concentrations were *c.*0.07 mg/kg. The increases in grain Cd concentrations ranged from *c.*2-fold (Bridgets) up to *c.*8-fold (Woburn).

At Pwllpeiran, Hartwood and Auchincruive, there were strong relationships ($P < 0.001$; r^2 = 39-93%) between ryegrass Zn and total topsoil Zn concentrations. For Cu, there were moderate relationships ($P < 0.001$; r^2 = 28-54%) between ryegrass Cu and total topsoil Cu concentrations at Hartwood and Auchincruive, but there was no relationship ($P > 0.05$) at Pwllpeiran. At Pwllpeiran and Hartwood, there were moderately strong relationships ($P < 0.001$; r^2 = 33-40%) between ryegrass Cd and total topsoil Cd concentrations, but there was no relationship ($P > 0.05$) at Auchincruive (the latter most probably due to analytical problems at low plant concentrations).

Similarly, there were strong relationships between grain/grass metal concentrations and topsoil NH_4NO_3 extractable metal concentrations (data not shown).

Figure 55. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Gleadthorpe (harvest 2003)





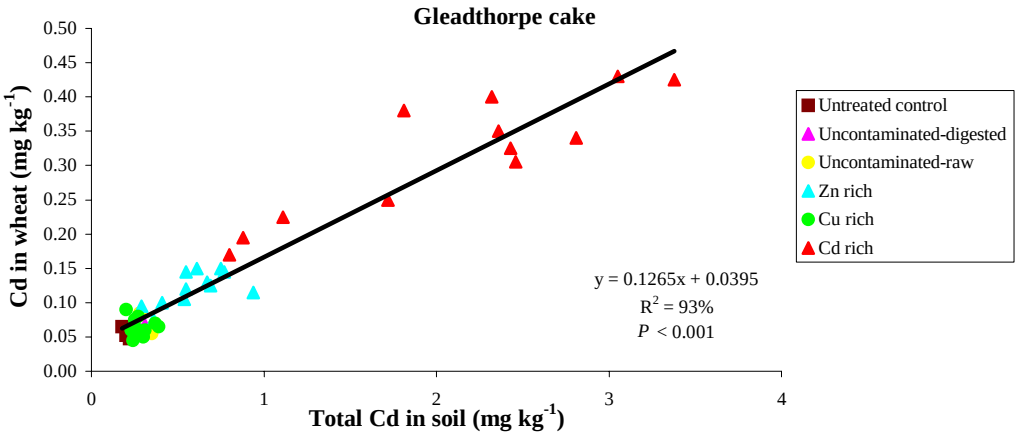
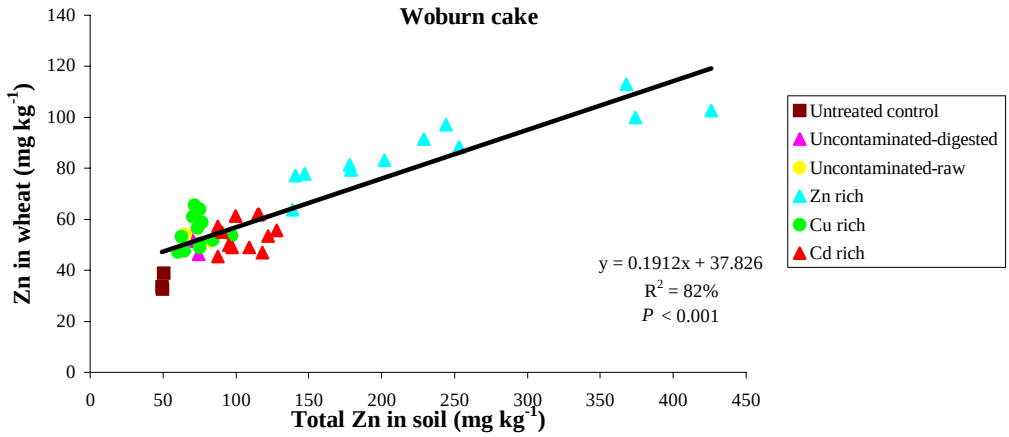
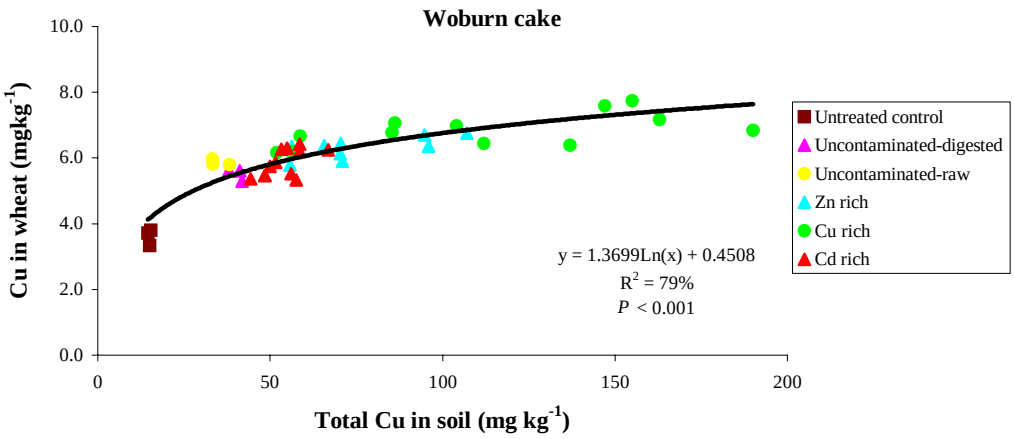


Figure 56. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Woburn (harvest 2003)





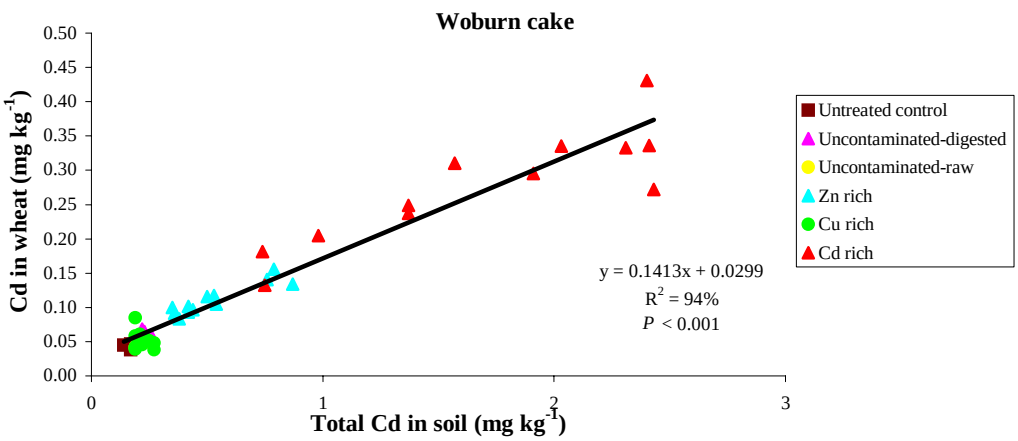


Figure 57. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Watlington (harvest 2003)

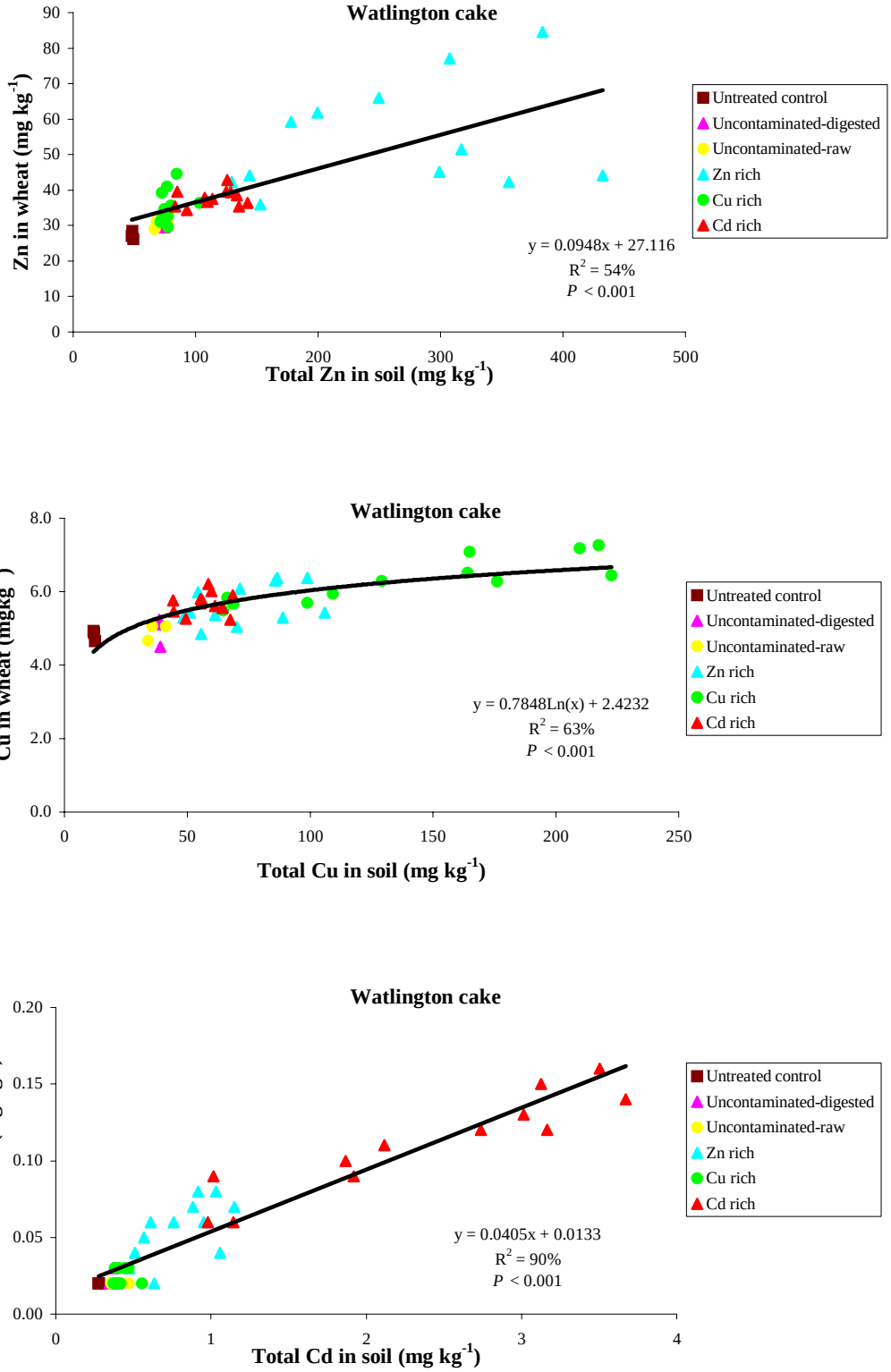
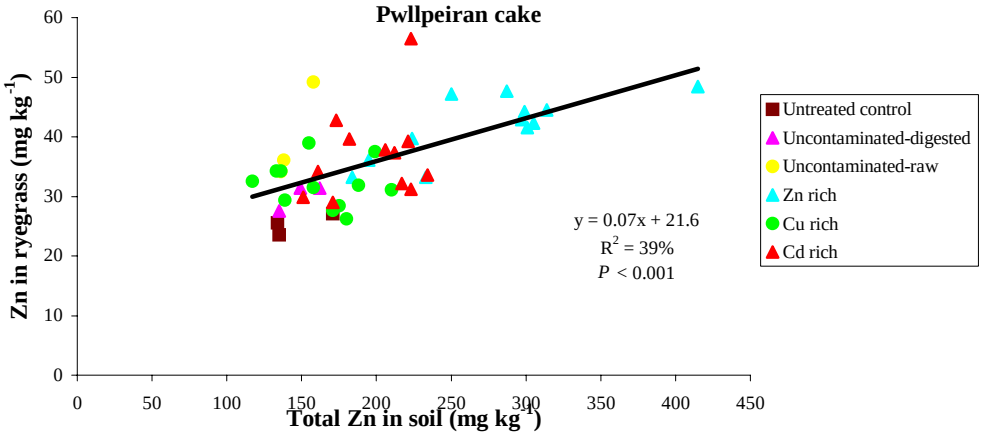
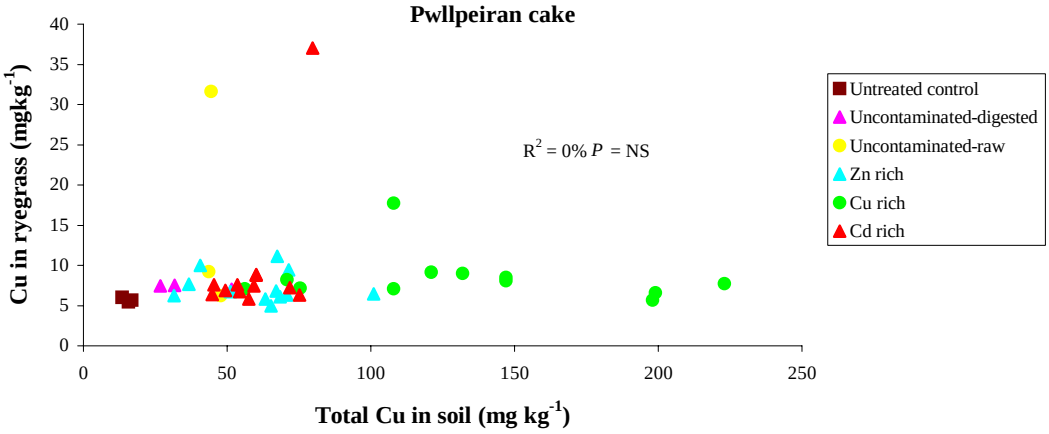


Figure 58. Relationships between ryegrass Zn, Cu and Cd concentrations and total topsoil metal concentrations - Pwllpeiran (harvest 2003)





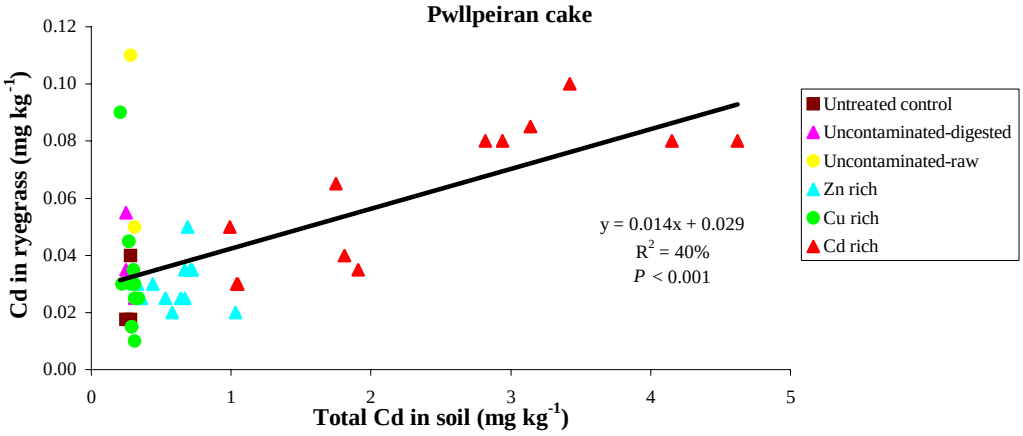


Figure 59. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Rosemaund (harvest 2003)

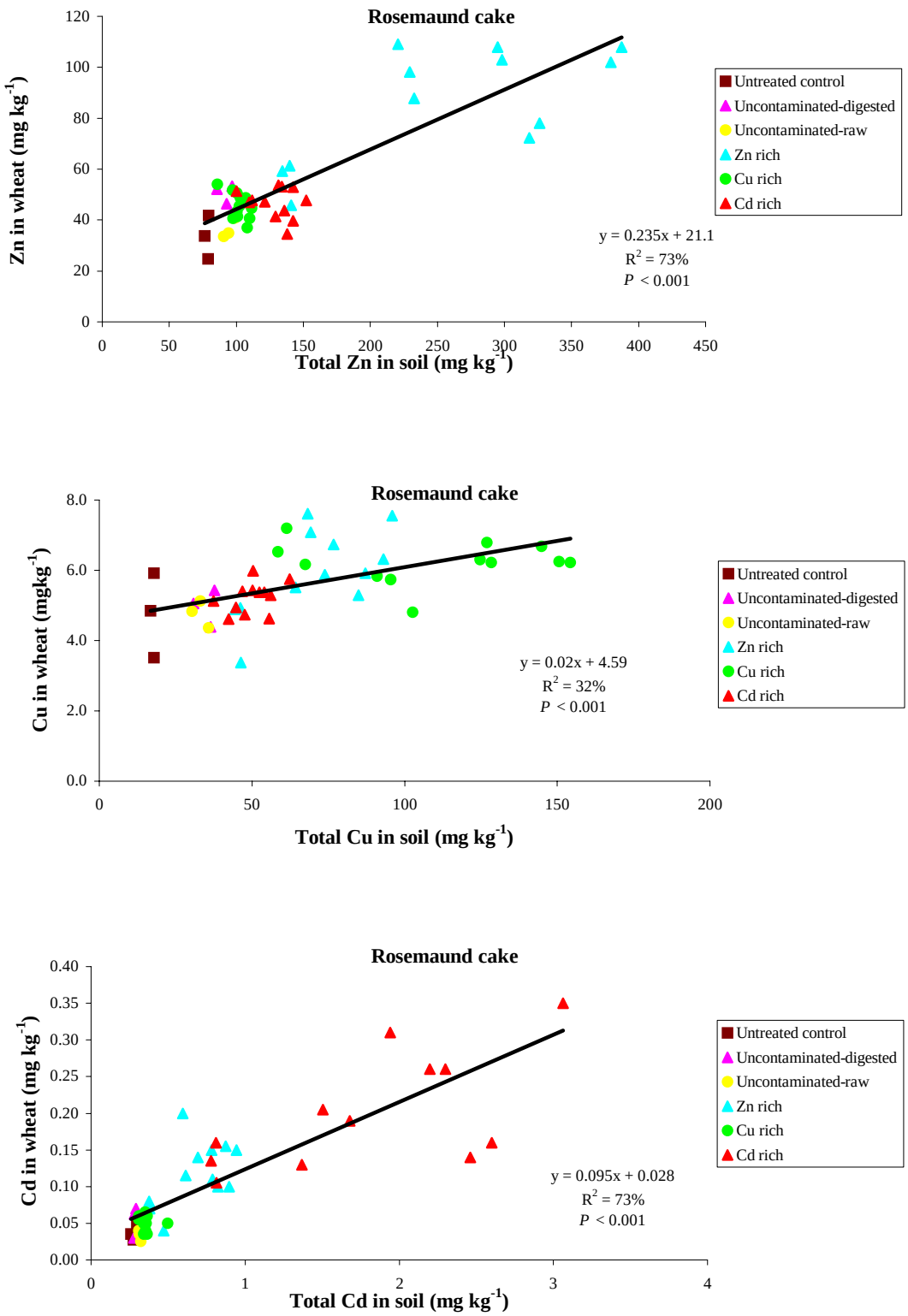
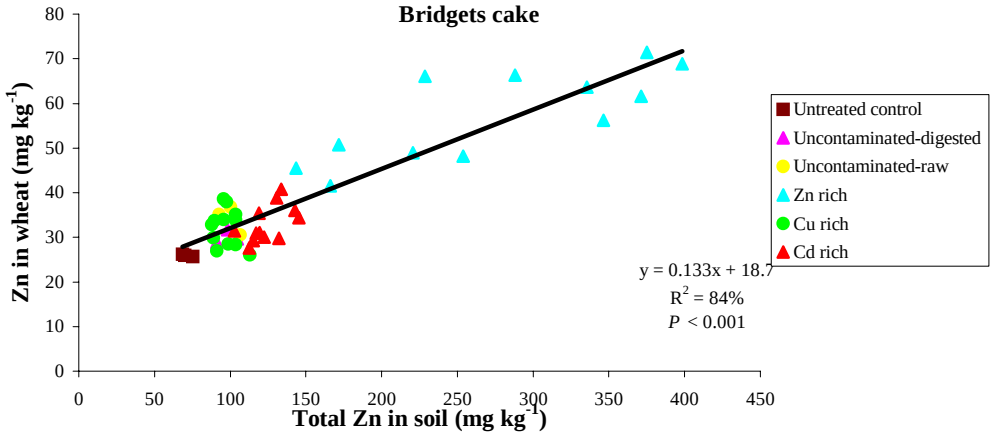
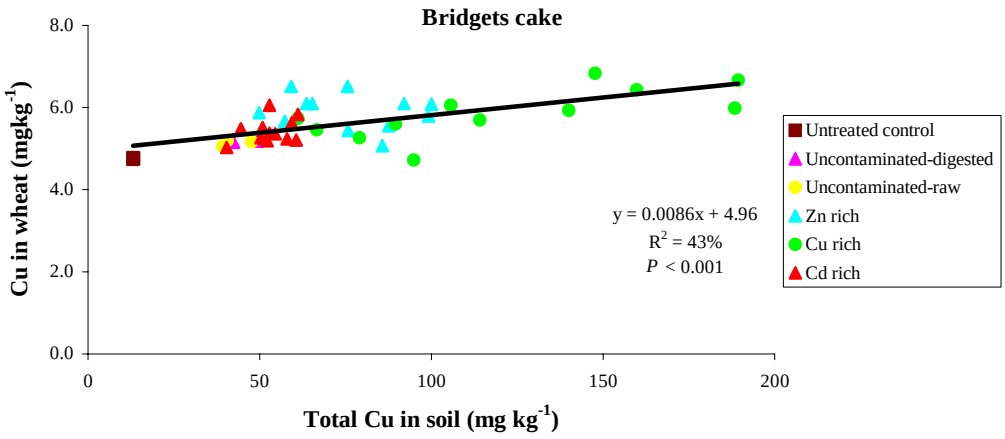


Figure 60. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Bridgets (harvest 2003)





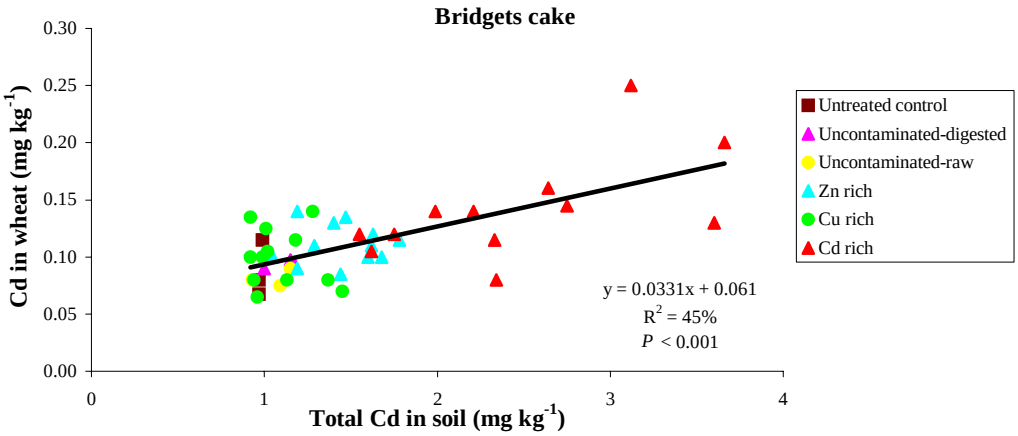
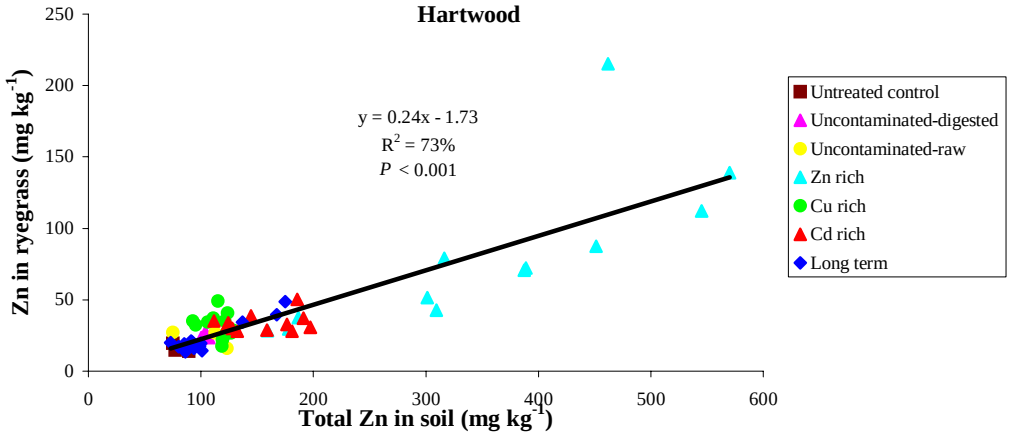
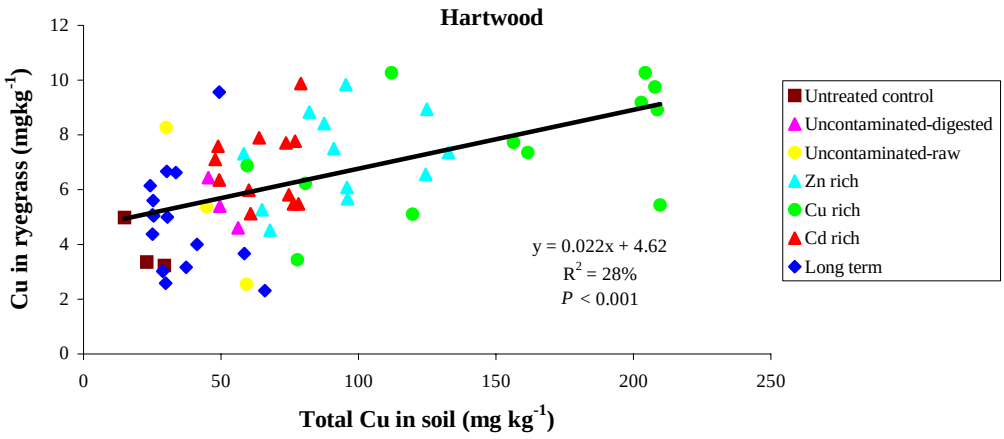


Figure 61. Relationships between ryegrass Zn, Cu and Cd concentrations and total topsoil metal concentrations - Hartwood (harvest 2003)





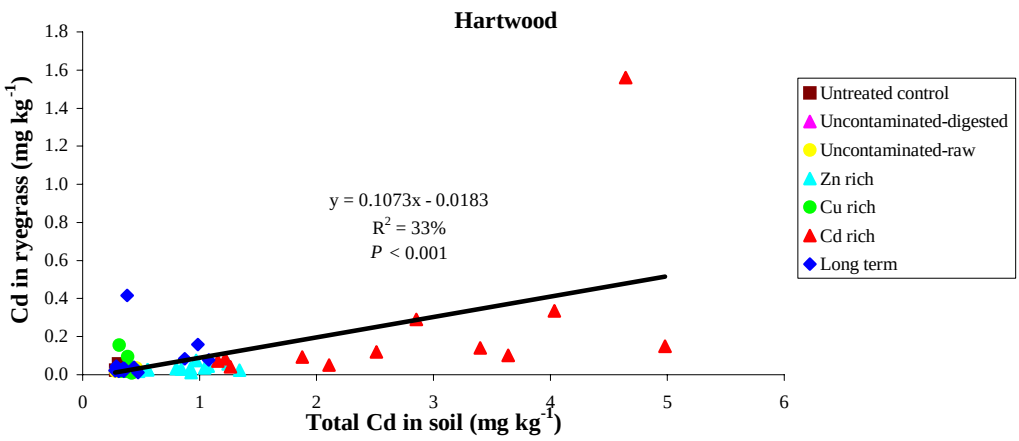
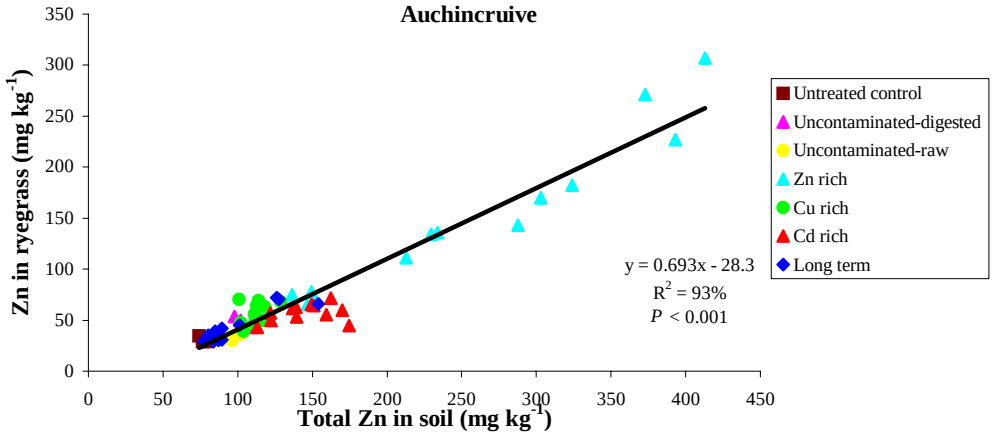
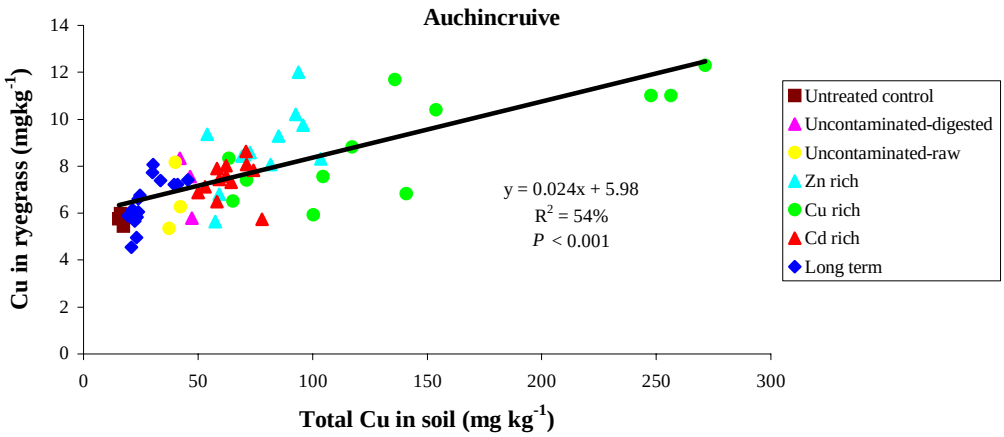


Figure 62. Relationships between ryegrass Zn, Cu and Cd concentrations and total topsoil metal concentrations - Auchincruive (harvest 2003)





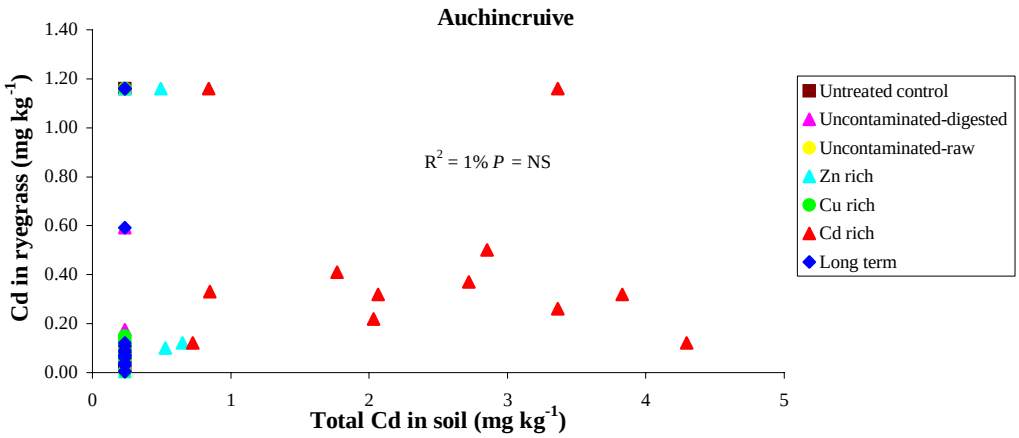
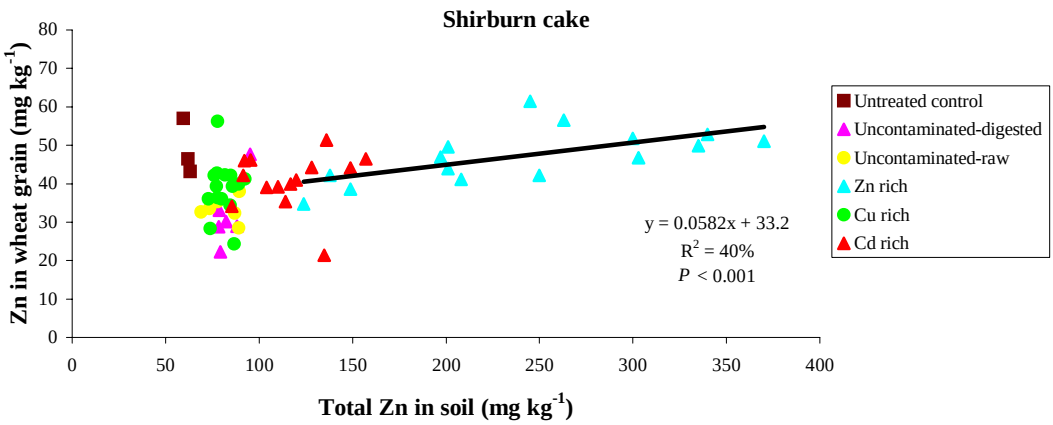
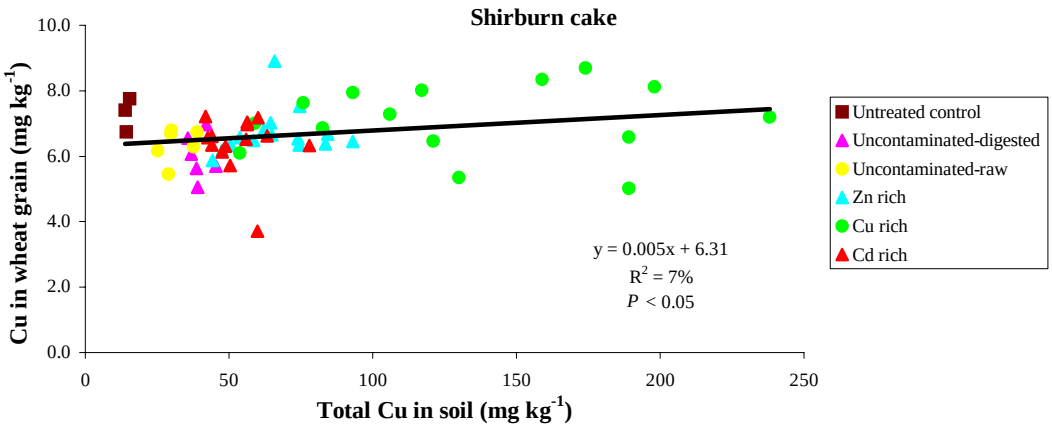
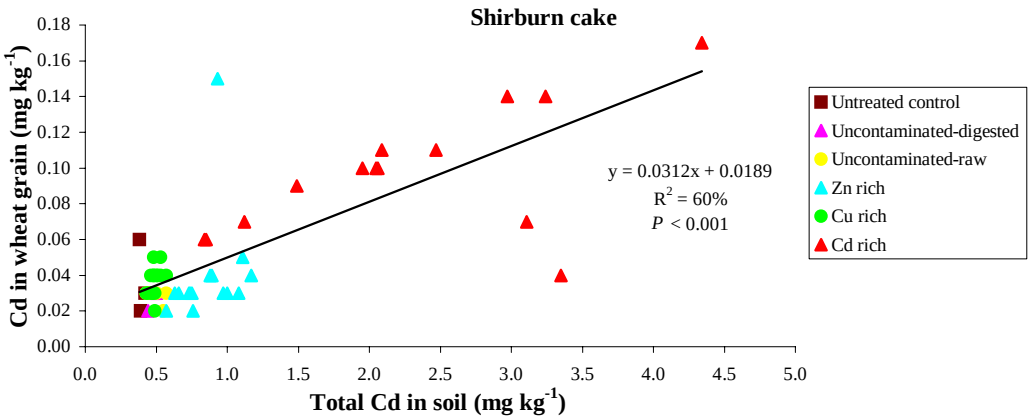


Figure 63. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Shirburn (harvest 2003)







2004 harvest: At Gleadthorpe, ryegrass yields on all the Zn-rich treatments were 1.86-2.86 t/ha higher ($P < 0.05$) than the untreated control. At Woburn, ryegrass yield on the Zn-rich rate 3 treatment was 2.36 t/ha higher ($P < 0.05$) than the untreated control. At Bridgets, ryegrass yields on the Zn-rich rate 2-4 treatments were 1.77-1.90 t/ha higher ($P < 0.05$) than the untreated control and uncontaminated digested sludge cake treatments (Table 41).

There were no grass yield differences between any of the Cu-rich treatments and the uncontaminated raw sludge cake and untreated control treatments.

On the Cd-rich rate 2 treatment at Gleadthorpe, ryegrass yield was 2.77 t/ha higher ($P < 0.05$) than the untreated control.

On the long-term build-up treatments at Watlington, ryegrass yields on the long-term Zn and Cu treatments were both 0.85 t/ha higher than the untreated control.

At Bridgets, there was a significant ($P < 0.05$) positive relationship between ryegrass yields and total topsoil Zn concentrations (2003 data), and at Hartwood there was a significant ($P < 0.001$) positive relationship between ryegrass yields and total topsoil Cu concentrations (2003 data).

Table 41. Mean first cut grass yields (t dm/ha) at each site (May-June 2004)

Treatment	GLE	WOB	WAT	PWL	ROS	BRG	HAR	AUC	SHI
Control	7.72	4.78	4.56	3.77	5.54	4.28	1.83	9.28	7.84
Unc. Dig.	9.72	5.78	6.20	3.39	6.36	4.26	2.27	8.57	7.38
Unc. Raw	9.79	6.06	5.54	4.32	4.96	4.08	1.58	7.93	7.65
Zn rate 1	10.00	6.41	5.98	3.75	5.91	4.98	1.60	8.19	8.26
Zn rate 2	9.58	6.07	6.04	4.29	5.95	6.16	2.76	8.33	7.63
Zn rate 3	9.88	7.14	6.22	4.97	5.43	6.03	2.00	8.44	7.98
Zn rate 4	10.60	6.51	5.36	4.14	4.68	6.13	2.71	8.31	8.49
ANOVA (1)	0.005	0.044	NS	NS	NS	0.005	NS	NS	NS
$LSD_{0.05}$	1.83	2.10	-	-	-	1.71	-	-	-
CV%	7	12	12	27	33	11	30	9	8
Cu rate 1	9.52	5.93	5.04	4.41	5.83	5.52	1.69	8.70	7.84
Cu rate 2	9.51	5.39	5.89	4.30	5.55	4.05	1.82	8.20	7.03
Cu rate 3	9.28	6.11	5.22	4.18	7.11	4.54	2.21	8.86	7.06
Cu rate 4	8.92	6.96	5.39	4.20	5.42	4.29	2.45	7.71	7.26
ANOVA (2)	NS	NS	NS	NS	NS	NS	NS	NS	NS
$LSD_{0.05}$	-	-	-	-	-	-	-	-	-
CV%	15	14	13	15	32	19	18	10	7
Cd rate 1	9.27	5.86	6.04	3.74	6.58	4.52	2.08	9.09	7.80
Cd rate 2	10.50	5.89	5.38	4.29	6.50	5.11	2.20	8.46	7.05
Cd rate 3	10.20	5.89	5.77	3.76	8.57	4.97	2.15	8.28	8.10
Cd rate 4	9.66	6.23	5.70	4.28	7.69	5.01	1.84	8.39	7.57
ANOVA (3)	0.047	NS	NS	NS	NS	NS	NS	NS	NS
$LSD_{0.05}$	2.61	-	-	-	-	-	-	-	-
CV%	10	10	14	18	27	16	28	7	7
LT Unc. Dig.	10.10	nd	5.33	4.17	6.50	4.77	1.53	9.14	7.53
LT Unc. Raw	9.15	nd	4.94	3.37	7.37	3.41	1.85	8.59	7.21
LT Zn	9.01	nd	5.41	3.93	6.02	3.98	2.11	8.80	7.66
LT Cu	9.19	nd	5.41	4.28	5.98	4.60	1.92	8.85	7.23
LT Cd	9.76	nd	5.04	3.81	5.78	4.41	1.78	8.79	7.73
ANOVA (4)	NS	-	0.022	NS	NS	0.038	NS	NS	NS
$LSD_{0.05}$	-	-	0.84	-	-	1.25	-	-	-
CV%	9	-	6	19	16	10	33	6	12

Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. The ANOVA fitted was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P <0.05)

2005 harvest: At Hartwood, grass yields were 1.4-1.7 t/ha lower ($P < 0.01$) on the untreated control compared with the uncontaminated digested sludge cake and Zn-rich rate 1 treatments. Also, grass yield on the Zn-rich rate 4 treatment was 1.5 t/ha lower ($P < 0.01$) than the Zn-rich rate 1 treatment. At Auchincruive, grass yields were 0.6 t/ha lower ($P < 0.05$) on the Zn-rich rate 3 and 4 treatments than the untreated control (Table 42).

At Auchincruive, grass dry matter yield was *c.*0.6 t/ha higher ($P < 0.05$) on the Cu-rich rate 2 treatment compared with the untreated control.

At Hartwood, grass dry matter yields were 1.4-2.2 t/ha higher ($P< 0.05$) on the Cd-rich rate 1-4 treatments than the untreated control.

On the long-term build up treatments at Hartwood, ryegrass yields on the uncontaminated digested and Zn-rich sludge cake treatments were 1.5-1.7 t/ha higher ($P < 0.05$) than the untreated control.

On the metal dose-response treatments, regression of grain yields against total topsoil metal concentrations identified no significant ($P > 0.05$) relationships.

The relationships between grain Zn, Cu and Cd concentrations and ryegrass concentrations at Pwllpeiran, Hartwood and Auchincruive (harvest year 2005), and total topsoil metal concentrations at each of the sites are presented in Figures 64 to 72.

At five of the sites, there were moderate to strong relationships ($P < 0.001$) between grain Zn (r^2 = 34-66%) concentrations and total topsoil Zn concentrations, with no relationship ($P > 0.05$) evident at Shirburn. Grain Zn concentrations on the Zn rate 4 treatments ranged between 43 and 94 mg/kg at the non-calcareous sites, compared with 46 mg/kg at the calcareous Shirburn site. At the five non-calcareous sites, the increase in grain Zn concentrations over the untreated controls was between 1.15 and 2-fold (7-59 mg/kg), whereas at Shirburn no increase was measurable.

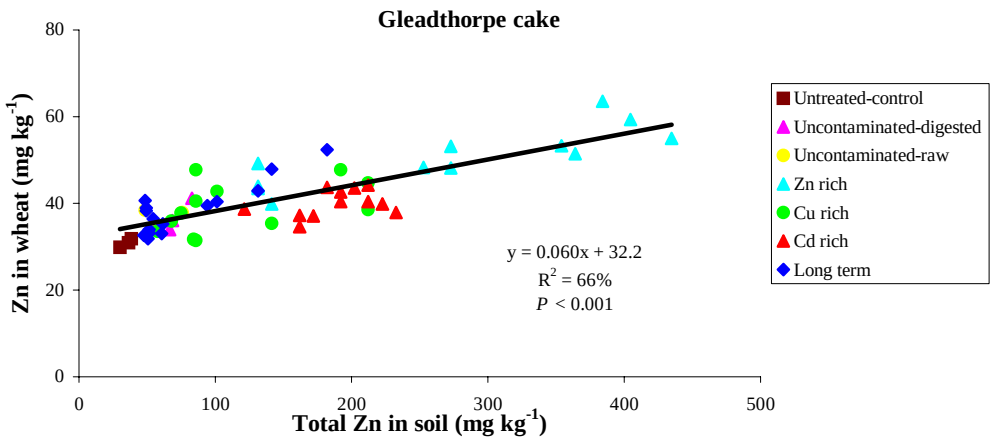
There were moderately strong relationships ($P < 0.001$) between grain Cu concentrations and total topsoil Cu concentrations at Gleadthorpe and Woburn (r^2 = 57 and 46%, respectively). Grain Cu concentrations on the Cu-rich rate 4 treatments at Gleadthorpe and Woburn were *c.*6.5 mg/kg, which represented *c.*1.5-fold increases over the untreated control. At the other four sites, there were no relationships between grain Cu concentrations and total topsoil Cu concentrations ($P > 0.05$).

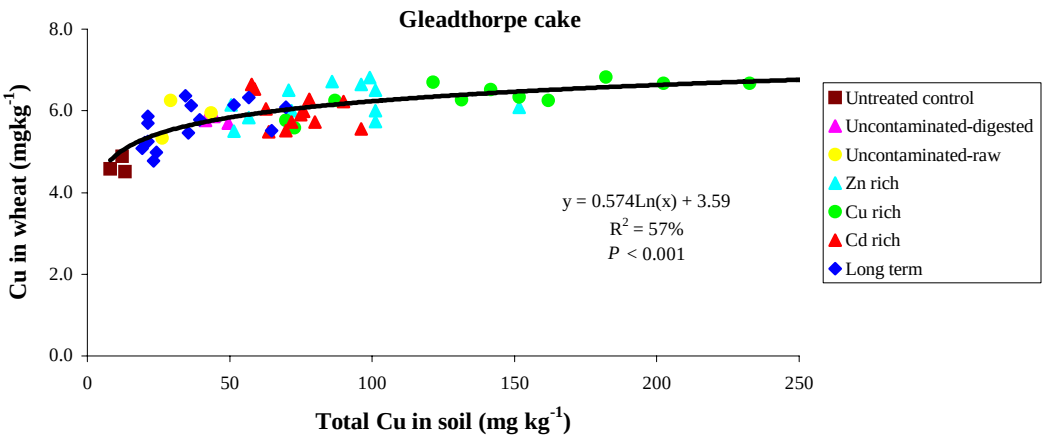
Table 42. Mean grain (t/ha, 85% dm basis) and grass yields (t/ha, 100% dm basis) at each site in 2005

Treatment	GLE	WOB	WAT	PWL*	ROS	BRG	HAR*	AUC*	SHI
Control	3.13	nd	nd	6.11	4.04	1.57	1.86	6.17	2.71
Unc. Dig.	3.19	nd	nd	5.78	3.71	2.05	3.25	6.49	2.18
Unc. Raw	3.14	nd	nd	5.86	4.39	2.03	3.08	6.16	2.60
Zn rate 1	4.29	nd	nd	5.14	3.58	2.11	3.55	6.32	2.53
Zn rate 2	3.92	nd	nd	5.13	3.44	2.28	3.00	6.40	1.87
Zn rate 3	3.10	nd	nd	5.42	3.80	2.28	2.79	5.59	2.32
Zn rate 4	4.60	nd	nd	5.33	3.07	2.02	2.01	5.61	1.51
ANOVA (1)	NS	-	-	NS	NS	NS	0.008	0.011	NA
$LSD_{0.05}$	-	-	-	-	-	-	1.364	0.834	-
CV %	20	-	-	19	18	20	18	5	21
Cu rate 1	3.52	nd	nd	5.99	4.00	2.26	2.92	6.39	1.28
Cu rate 2	3.72	nd	nd	5.25	4.05	2.18	2.90	6.80	1.87
Cu rate 3	3.64	nd	nd	5.75	4.69	1.94	2.75	6.70	0.61
Cu rate 4	2.62	nd	nd	5.34	4.08	2.25	2.85	6.33	0.77
ANOVA (2)	NS	-	-	NS	NS	NS	NS	0.013	NA
$LSD_{0.05}$	-	-	-	-	-	-	-	0.579	-
CV %	28	-	-	13	15	23	23	3	56
Cd rate 1	3.02	nd	nd	4.44	4.04	1.56	3.57	6.63	0.56
Cd rate 2	2.91	nd	nd	6.00	3.81	1.94	3.27	7.02	0.87
Cd rate 3	4.42	nd	nd	5.47	5.16	2.07	3.44	6.45	1.07
Cd rate 4	3.29	nd	nd	6.12	4.80	1.99	4.07	6.43	0.78
ANOVA (3)	NS	-	-	NS	NS	NS	<0.001	NS	NA
$LSD_{0.05}$	-	-	-	-	-	-	0.969	-	-
CV%	16	-	-	17	22	28	11	8	42
LT Unc. Dig.	3.51	nd	nd	5.08	4.49	1.96	3.39	6.16	nd
LT Unc. Raw	3.56	nd	nd	5.49	4.76	1.49	2.84	6.36	nd
LT Zn	3.59	nd	nd	5.18	3.90	1.83	3.52	6.20	nd
LT Cu	3.31	nd	nd	6.40	3.41	1.54	2.61	6.24	nd
LT Cd	3.62	nd	nd	5.32	3.54	1.86	2.73	6.51	nd
ANOVA (4)	NS	-	-	NS	NS	NS	0.019	NS	-
$LSD_{0.05}$	-	-	-	-	-	-	1.364	-	-
CV%	32	-	-	17	22	31	17	6	-

* At Pwllpeiran, Hartwood and Auchincruive ryegrass was grown. Results expressed on a dm basis. Four ANOVA's were carried out; the three metal rich groups (Zn, Cu and Cd) were each tested with the untreated control treatment and the appropriate uncontaminated sludge control, and the long-term treatments with the untreated control treatment. Except at Shirburn, where no tests were performed owing to the very large CV%'s. The ANOVA fitted was a one-way randomised block design. Specific treatment differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance ($P < 0.05$). NA = not assessed. Nd = not determined due to crop failure.

Figure 64. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Gleadthorpe (harvest 2005)





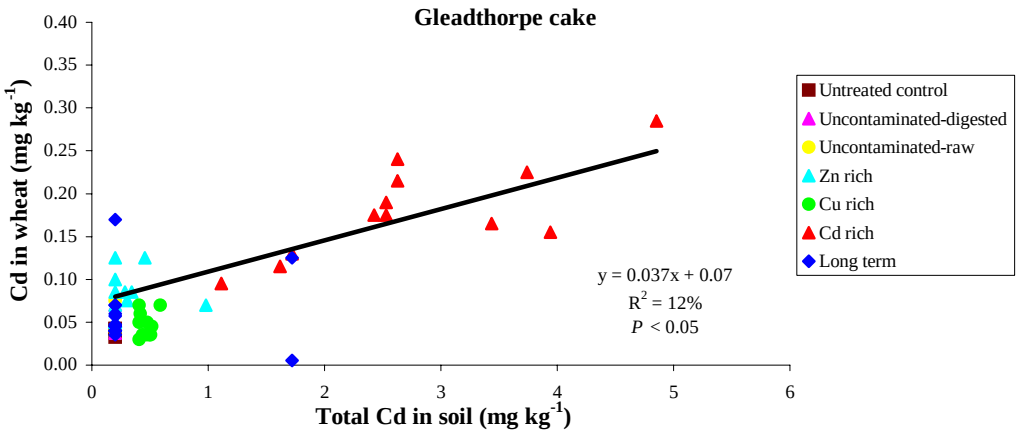
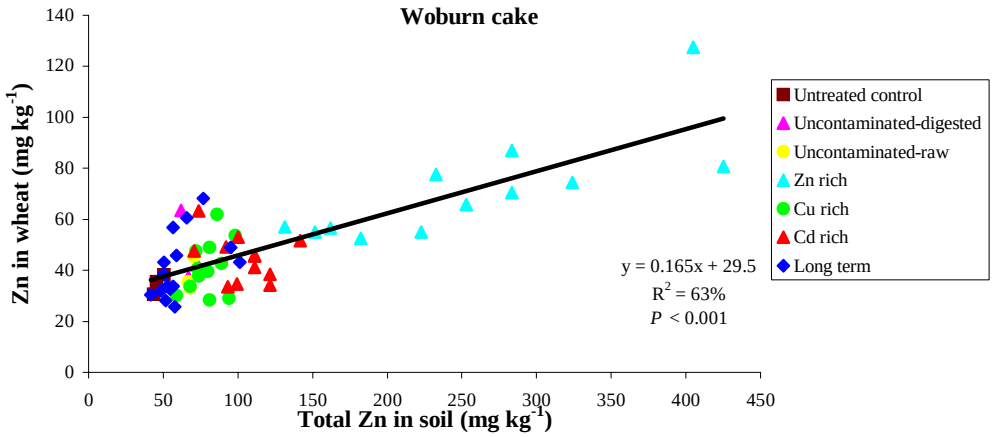
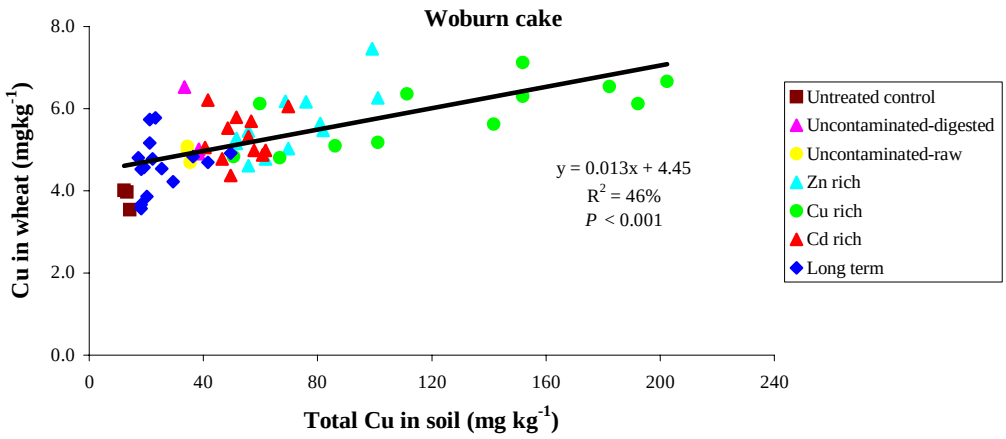


Figure 65. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Woburn (harvest 2005)





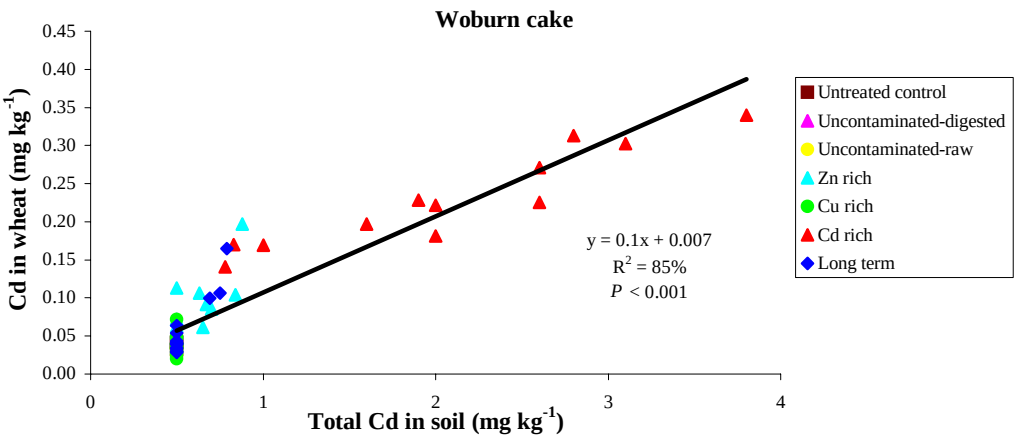
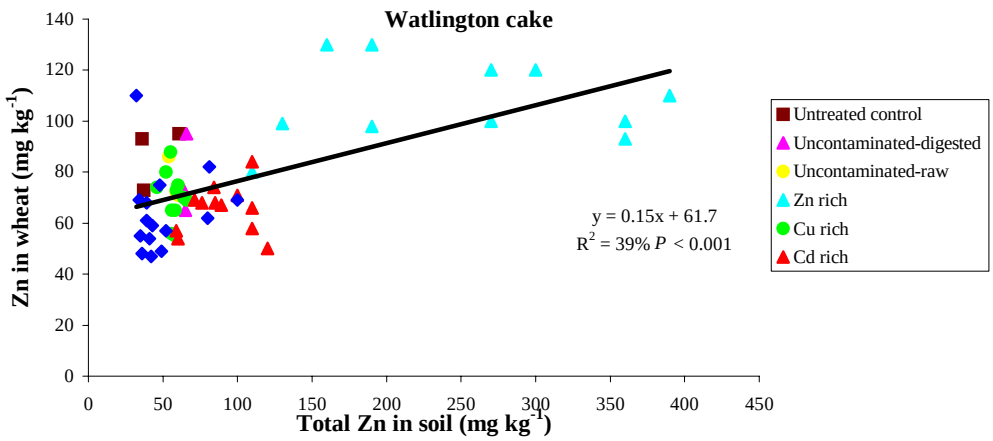
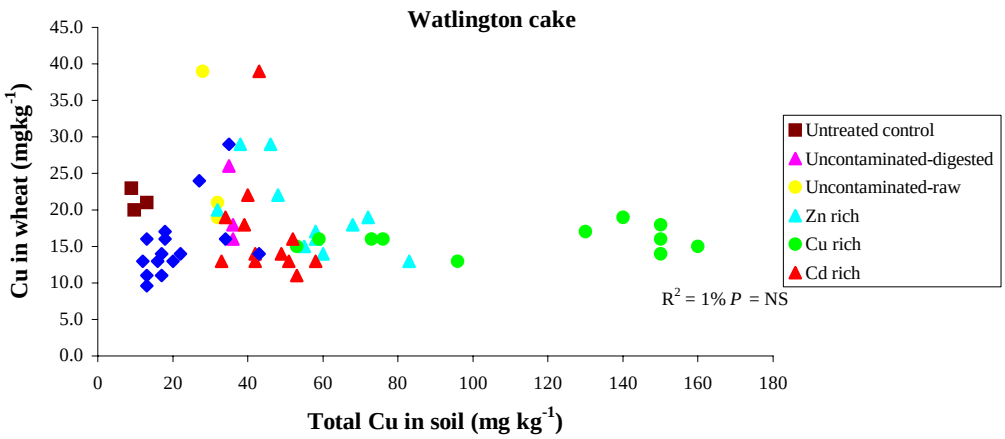


Figure 66. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Watlington (harvest 2005)





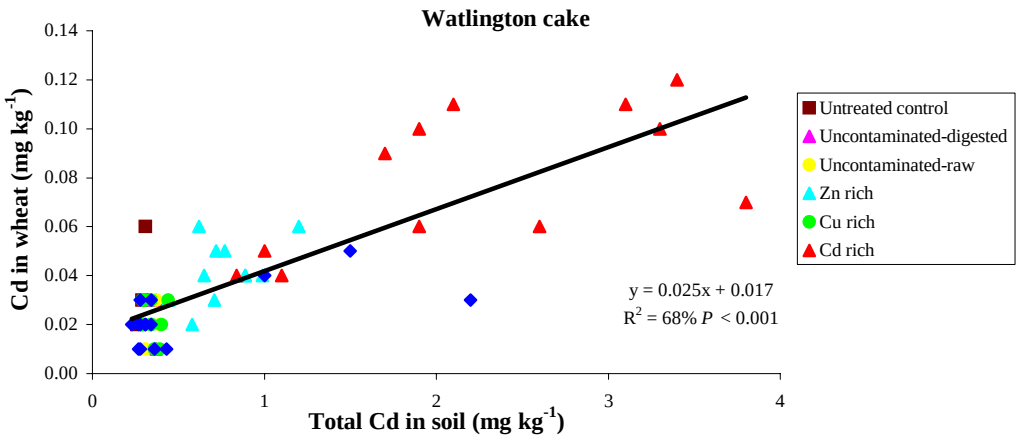


Figure 67. Relationships between ryegrass Zn, Cu and Cd concentrations and total topsoil metal concentrations - Pwllpeiran (harvest 2005)

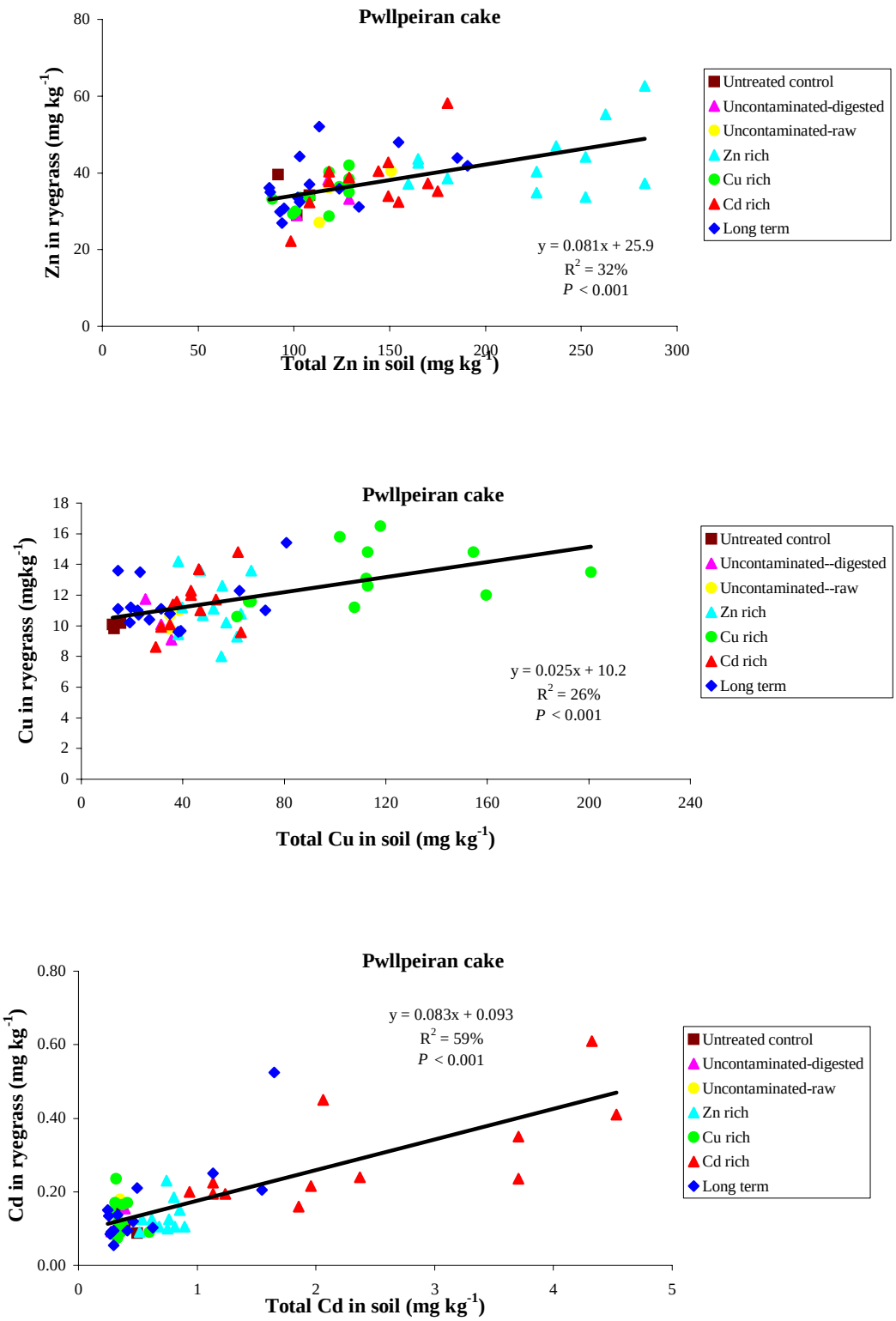
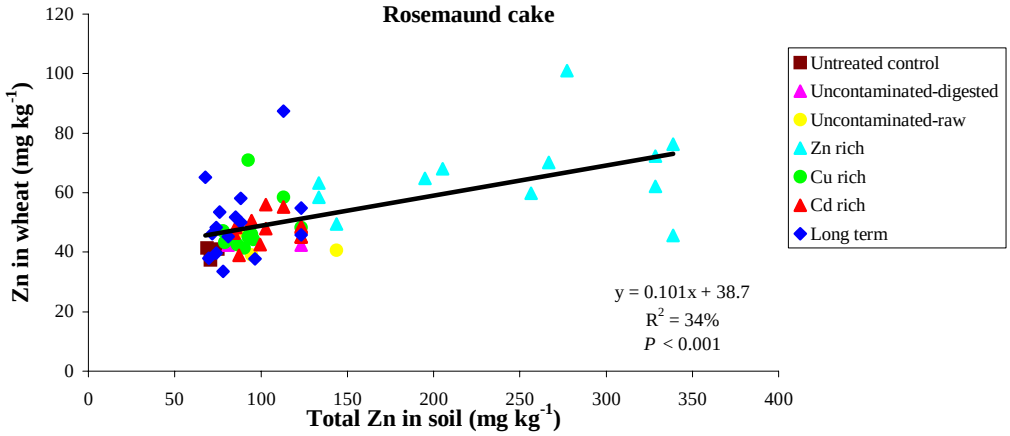
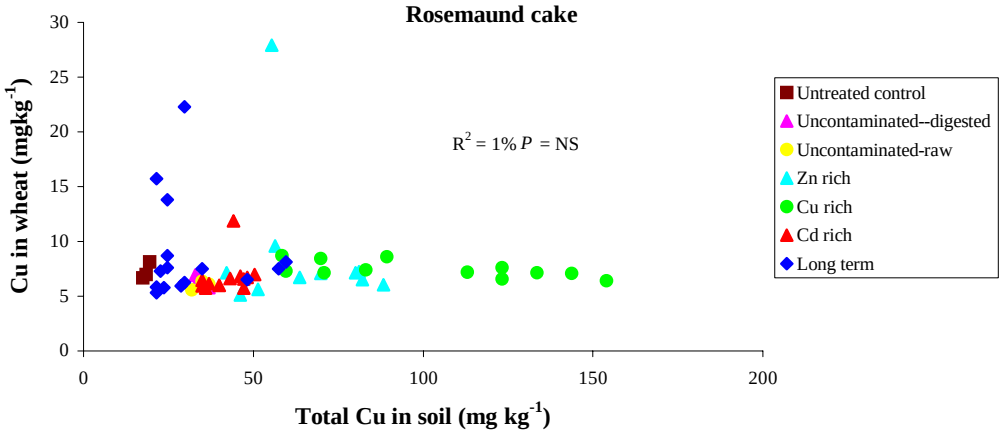


Figure 68. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Rosemaund (harvest 2005)





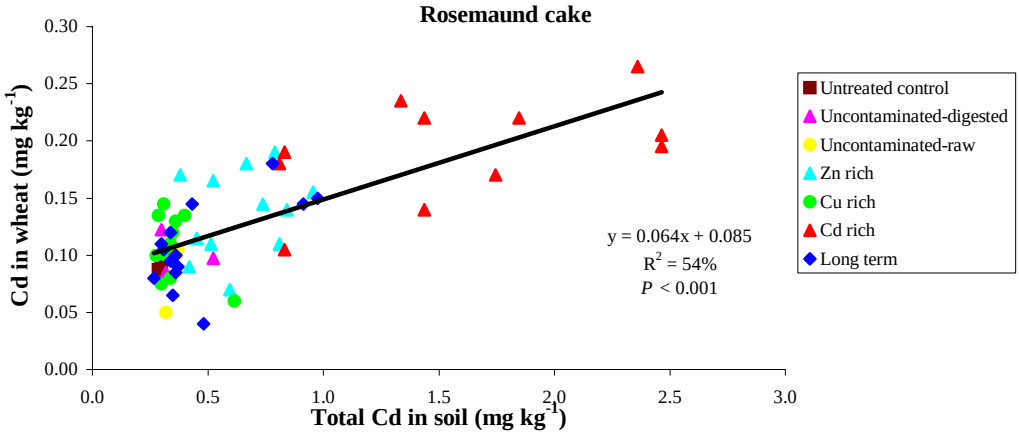
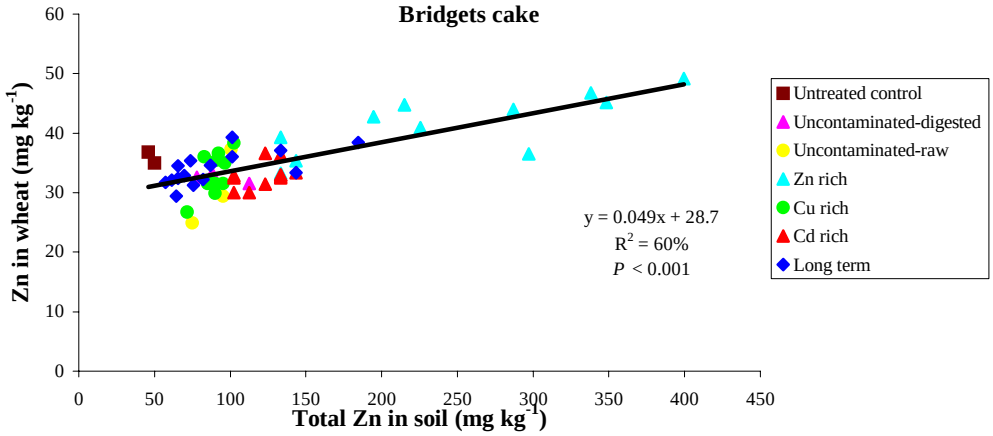
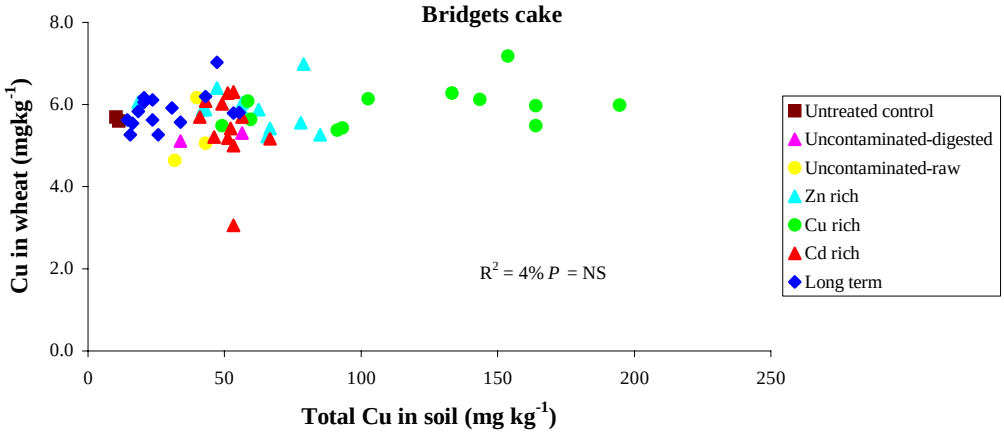


Figure 69. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Bridgets (harvest 2005)





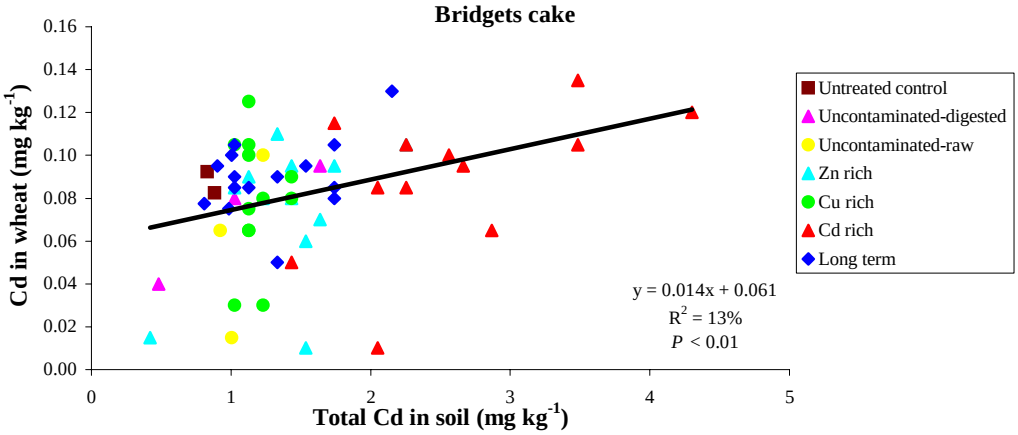
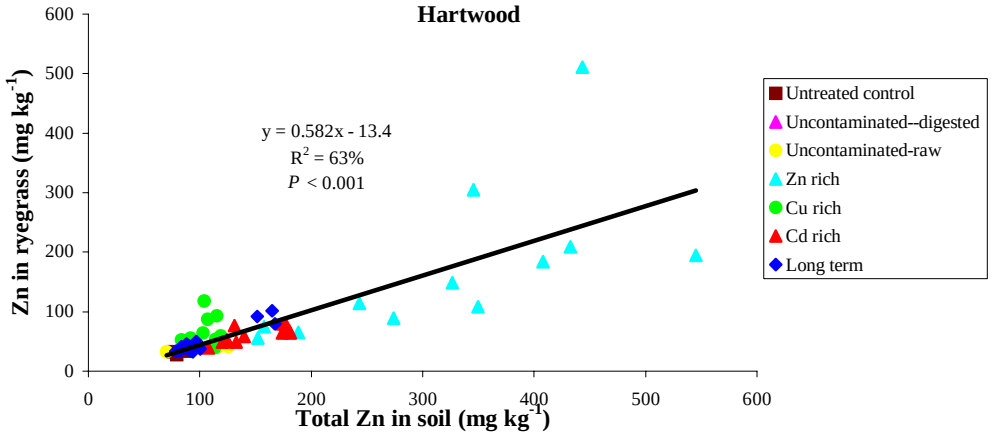
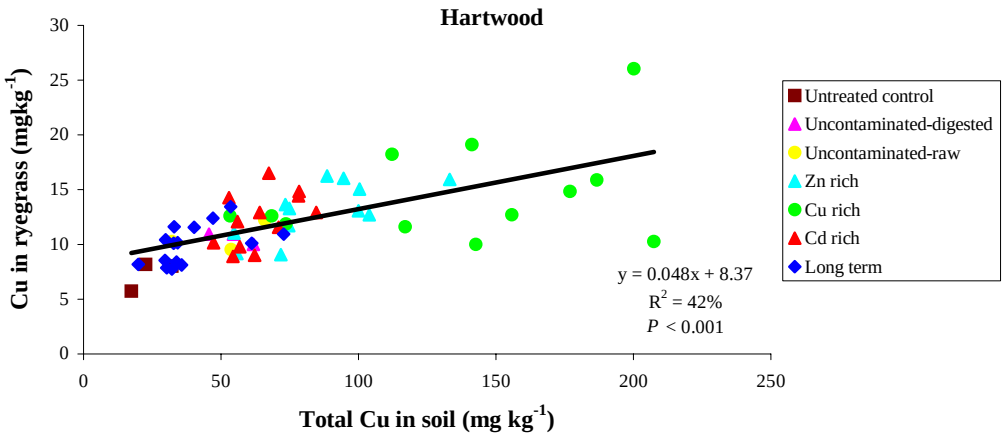


Figure 70. Relationships between ryegrass Zn, Cu and Cd concentrations and total topsoil metal concentrations - Hartwood (harvest 2005)





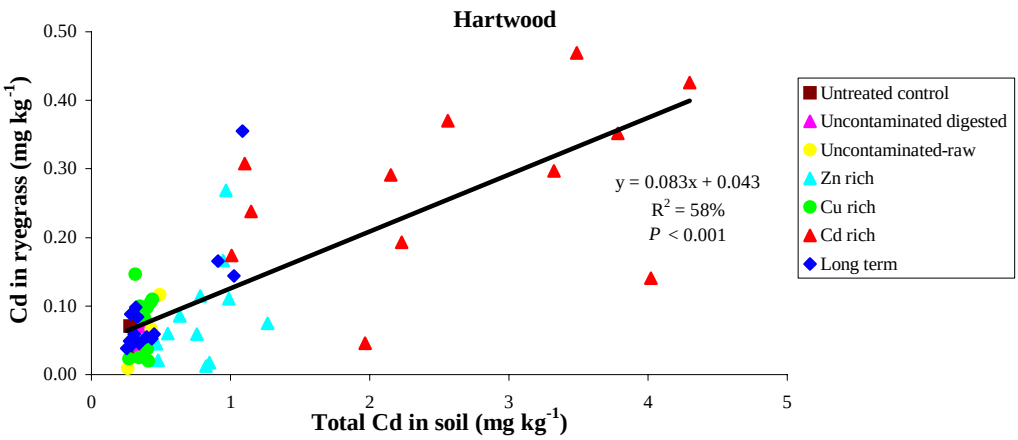
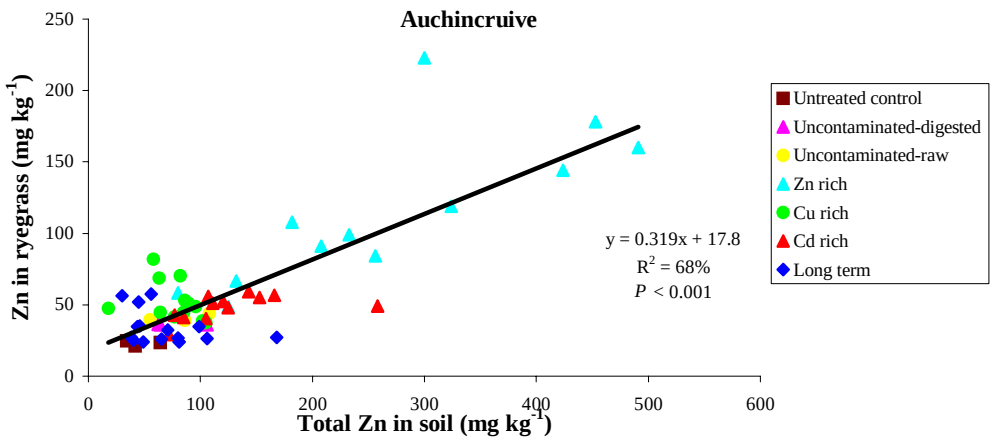
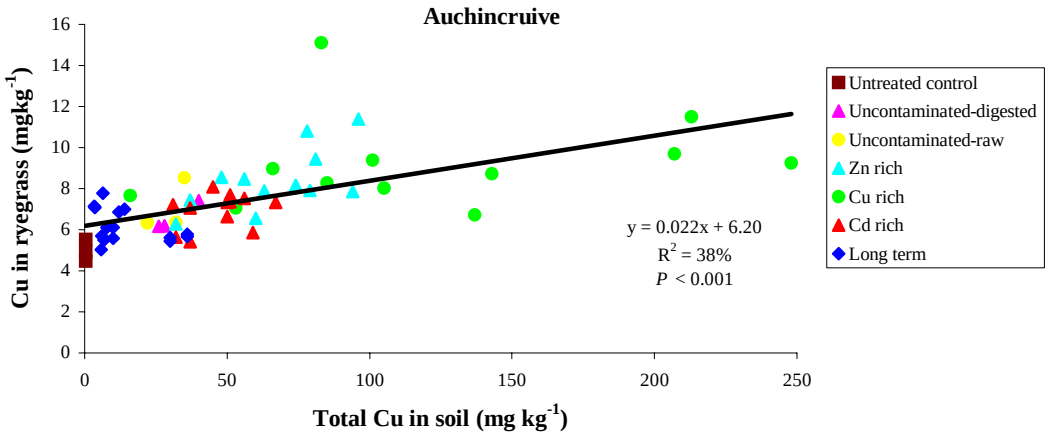


Figure 71. Relationships between ryegrass Zn, Cu and Cd concentrations and total topsoil metal concentrations - Auchincruive (harvest 2005)





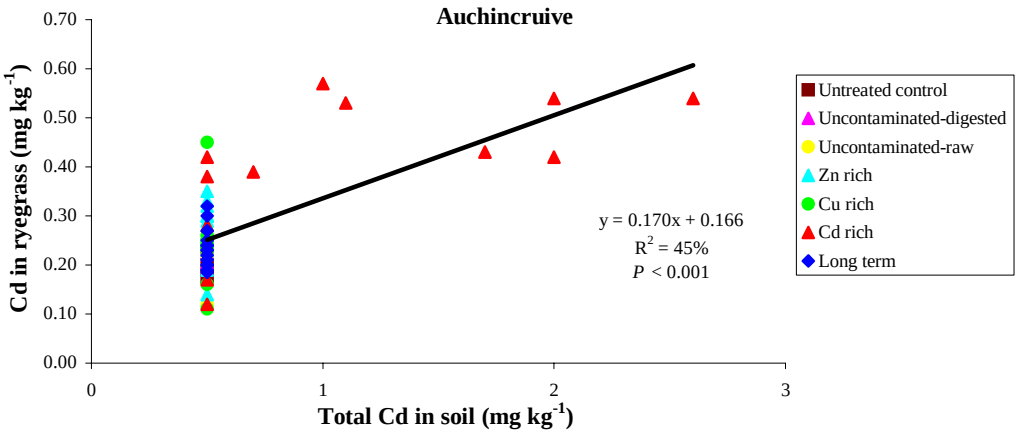
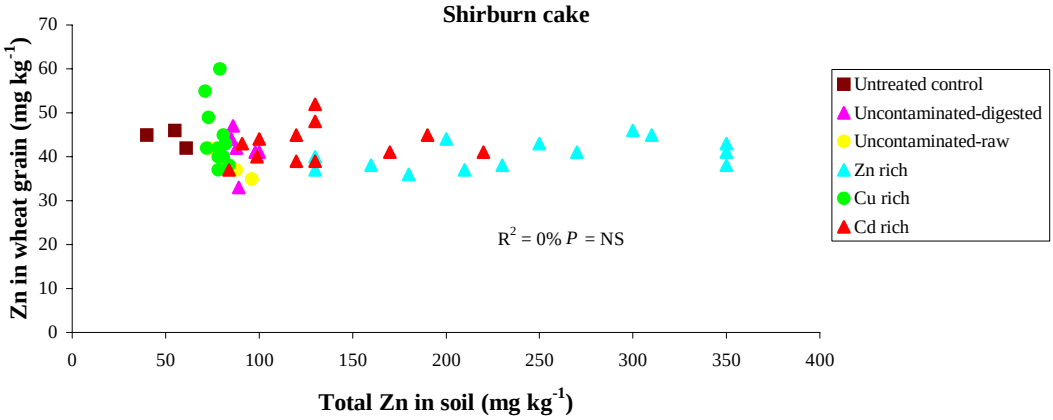
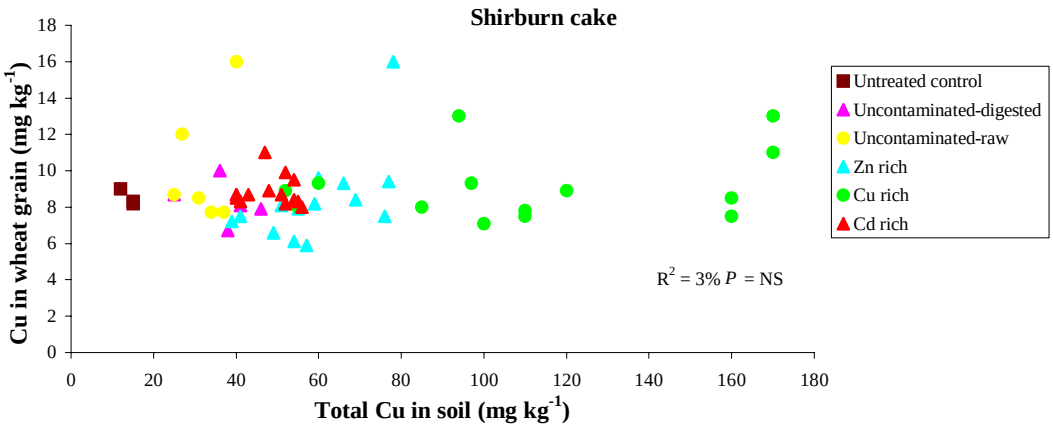
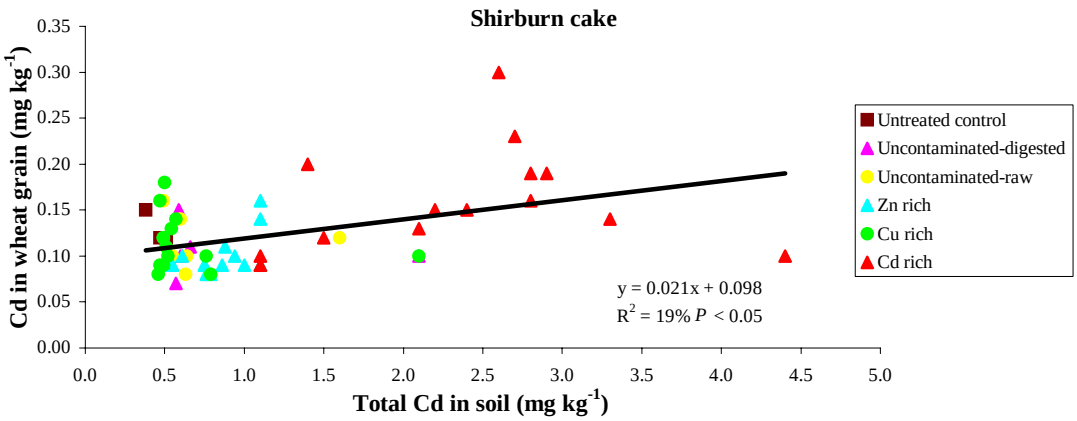


Figure 72. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Shirburn (harvest 2005)







At Woburn, Watlington and Rosemaund, there were moderate to strong relationships ($P < 0.001$) between grain Cd (r^2 = 54-85%) concentrations and total topsoil Cd concentrations. At the other three sites, there were weak relationships ($P < 0.05$) between grain Cd (r^2 = 12-19%) concentrations and total topsoil Cd concentrations. Cadmium concentrations in grain on the Cd-rich rate 4 treatments ranged between 0.12 and 0.32 mg/kg at the non-calcareous sites, compared with 0.19 mg/kg at Shirburn. The increases in grain Cd concentrations over the untreated controls ranged from *c.*1-fold (Bridgets) up to *c.*8-fold (Woburn).

At Pwllpeiran, Hartwood and Auchincruive, there were moderate to strong relationships ($P < 0.001$) between ryegrass Zn (r^2 = 39-68%), Cu (r^2 = 26-42%) and Cd (r^2 = 45-59%) concentrations and total topsoil metal concentrations.

Similarly, there were moderate to strong relationships ($P < 0.001$) between grain/grass metal concentrations and topsoil NH_4NO_3 extractable metal concentrations (data not shown).

4.2 Sludge cake analysis

Sludge cakes from the ADAS Gleadthorpe storage facility were analysed for heavy metal and nutrient concentrations prior to application on the long-term build up treatments (numbers 16-20 at sites 1 to 8; numbers 21-25 at Shirburn). Mean heavy metal concentrations in the sludge cakes applied to the long-term build-up plots during Phase III are summarised in Table 43. The Zn, Cu and Cd-rich sludge cakes had mean concentrations of *c.*4600 mg Zn/kg dry solids (ds), *c.*4250 mg Cu/kg ds and *c.*44 mg Cd/kg ds, respectively. The uncontaminated digested and raw sludge cakes contained the desired low metal concentrations. The concentrations were similar to those in the sludge cakes applied in Phase I (Table 4).

Table 43. Mean heavy metal concentrations in the sludge cakes applied to the long-term build up treatments during Phase III (mg/kg dry solids)

	Uncontaminated Digested	Uncontaminated raw	Zinc-rich	Copper-rich	Cadmium-rich
Zn	574	672	**4623**	949	955
Cu	609	624	1436	**4249**	462
Cd	1.43	1.55	5.88	1.34	**43.5**
Ni	43.7	30.7	623	43.9	129
Pb	103	148	469	1199	401
Cr	56.3	78.6	1121	565	411
Al	28685	8548	17161	8806	9144
Fe	11194	5721	23115	14049	20736
Mn	556	276	732	332	598

Dry matter, organic carbon and nutrient analysis results for the applied sludge cakes are presented in Table 44. Total nitrogen (N) loadings on

the long-term build-up treatments (*i.e*. treatments 16-20 at sites 1 to 8, and treatments 21-25 at Shirburn) were *c.*80, 35, 90, 50, and 85 kg N/ha for the uncontaminated digested and raw sludge, and the Zn, Cu and Cd-rich sludge cake treatments, respectively. Total phosphorus loadings on the same treatments were *c.*100, 35, 115, 25 and 120 kg P/ha, respectively.

Mean total carbon loadings on the long-term build-up treatments were in the range 0.50 and 1.0 t/ha, and dry solids loadings in the range 1.3 and 7.5 t/ha.

Table 44. Mean dry matter, organic carbon and nutrient contents of the sludge cakes applied to the long-term build up treatments during Phase III

	Uncontaminated digested	Uncontaminated raw	Zinc-rich	Copper-rich	Cadmium-rich
DM (%)	53.8	77.8	53.2	74.0	88.8
Org. C (% ds)	33.1	37.1	29.7	28.6	10.3
N (% ds)	2.69	2.58	2.80	3.05	1.12
P (% ds)	3.25	2.52	3.61	1.34	1.63
K (% ds)	0.15	0.16	0.20	0.26	0.12

4.3 England and Wales - Metal amended liquid sludge experiments

4.3.1 Soil microbial properties

Soil samples taken from all the treatment plots in spring 2003 and 2005 were analysed for soil respiration rates, microbial biomass carbon concentrations and *Rhizobium leguminosarum* biovar *trifolii* most probable numbers (MPNs).

Soil respiration rates

Soil respiration rates were measured on soil samples taken from all the treatment plots in spring 2003 and 2005, with mean treatment respiration rates summarised in Tables 45 and 47. Soil respiration rates in 2003 and 2005 were generally higher on the treatments receiving liquid sludge compared with the untreated controls.

2003 sampling: At Watlington, soil respiration rates on the Zn-rich rate 1 and Cd-rich rate 1 and 3 treatments, and at Rosemaund on the Cd-rich rate 1 and 3 treatments were higher ($P < 0.01$) than on the Cu-rich rate 1 treatment at each site. Although there were respiration rate differences ($P < 0.01$) between the metal-rich treatments at both Watlington and Rosemaund, there were no differences ($P > 0.05$) between any of the metal-rich treatments and either the uncontaminated digested liquid sludge or untreated control treatments at any of the three sites (Table 45).

Soil respiration rates were regressed against total topsoil metal concentrations (Figures 73-75). On the metal dose-response treatments, regression of the soil respiration rate measurements against total topsoil metal concentrations identified significant positive relationships ($P < 0.05$) at Rosemaund for Zn, Woburn for Cu and

Watlington for Cd (3 positive dose-response treatments out of 9). A summary of the regression analyses is given in Table 46.

Table 45. Mean topsoil (0-25 cm) respiration rates (mg CO_2-C/kg oven dry soil/hour) on treatments sampled in spring 2003.

Treatment	WOB	WAT	ROS
Control	0.28	0.31	0.25
Unc. Digested	0.30	0.30	0.26
Zn rate 1	0.42	0.43	0.25
Zn rate 2	0.39	0.40	0.32
Zn rate 3	0.36	0.34	0.32
Cu rate 1	0.35	0.29	0.20
Cu rate 2	0.33	0.30	0.28
Cu rate 3	0.40	0.32	0.32
Cd rate 1	0.25	0.43	0.39
Cd rate 2	0.39	0.42	0.33
Cd rate 3	0.31	0.43	0.36
ANOVA *p*	NS	0.003	0.004
$LSD_{0.05}$	-	0.152	0.146
CV%	21	14	17

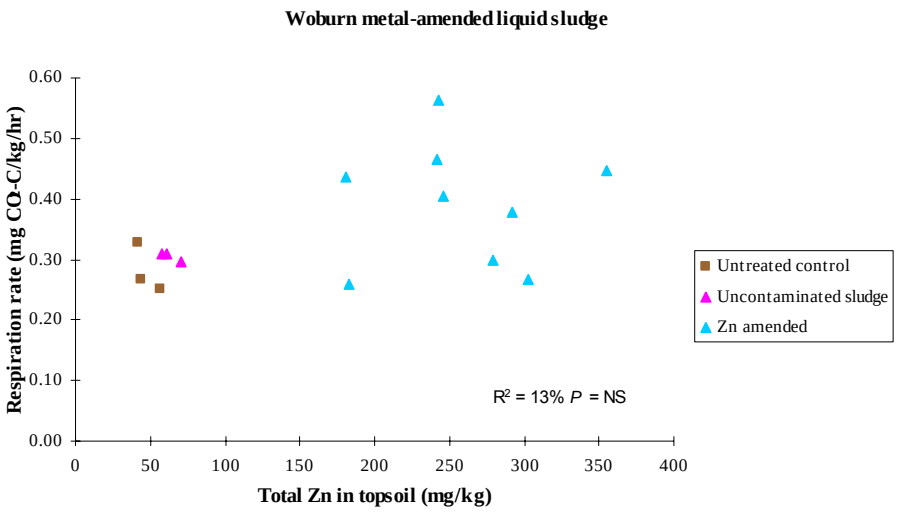
A single ANOVA was carried out. The three metal rich groups (Zn, Cu and Cd) were compared with the untreated control treatment and the uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment mean differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance ($P = 0.05$).

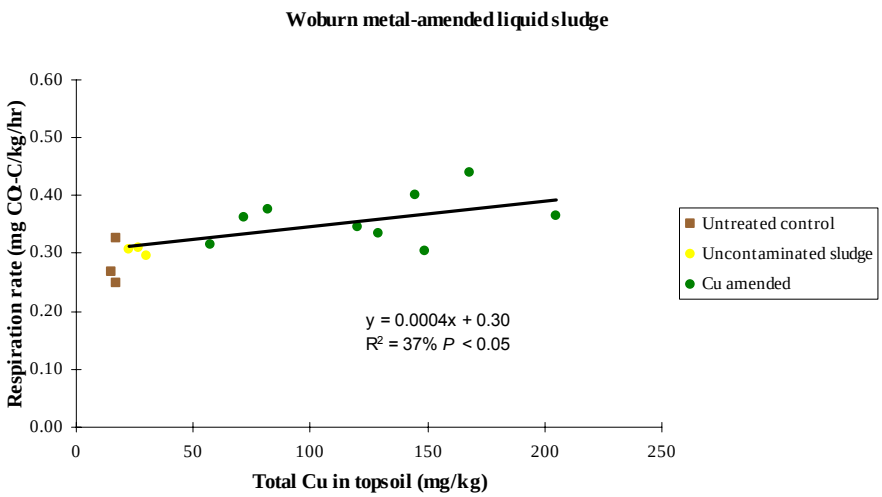
Table 46. Summary of relationships between respiration rates and total topsoil metals – metal amended liquid sludge sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	26%; $P < 0.05$	NS
WAT	NS	NS	52%; $P < 0.01$
ROS	37%; $P < 0.01$	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship

Figure 73. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) – Woburn liquid sludge (spring 2003)





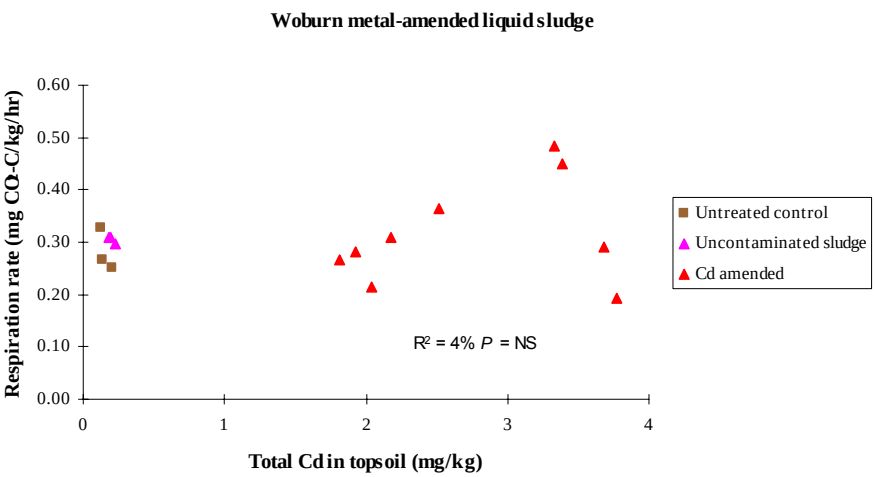


Figure 74. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) – Watlington liquid sludge (spring 2003)

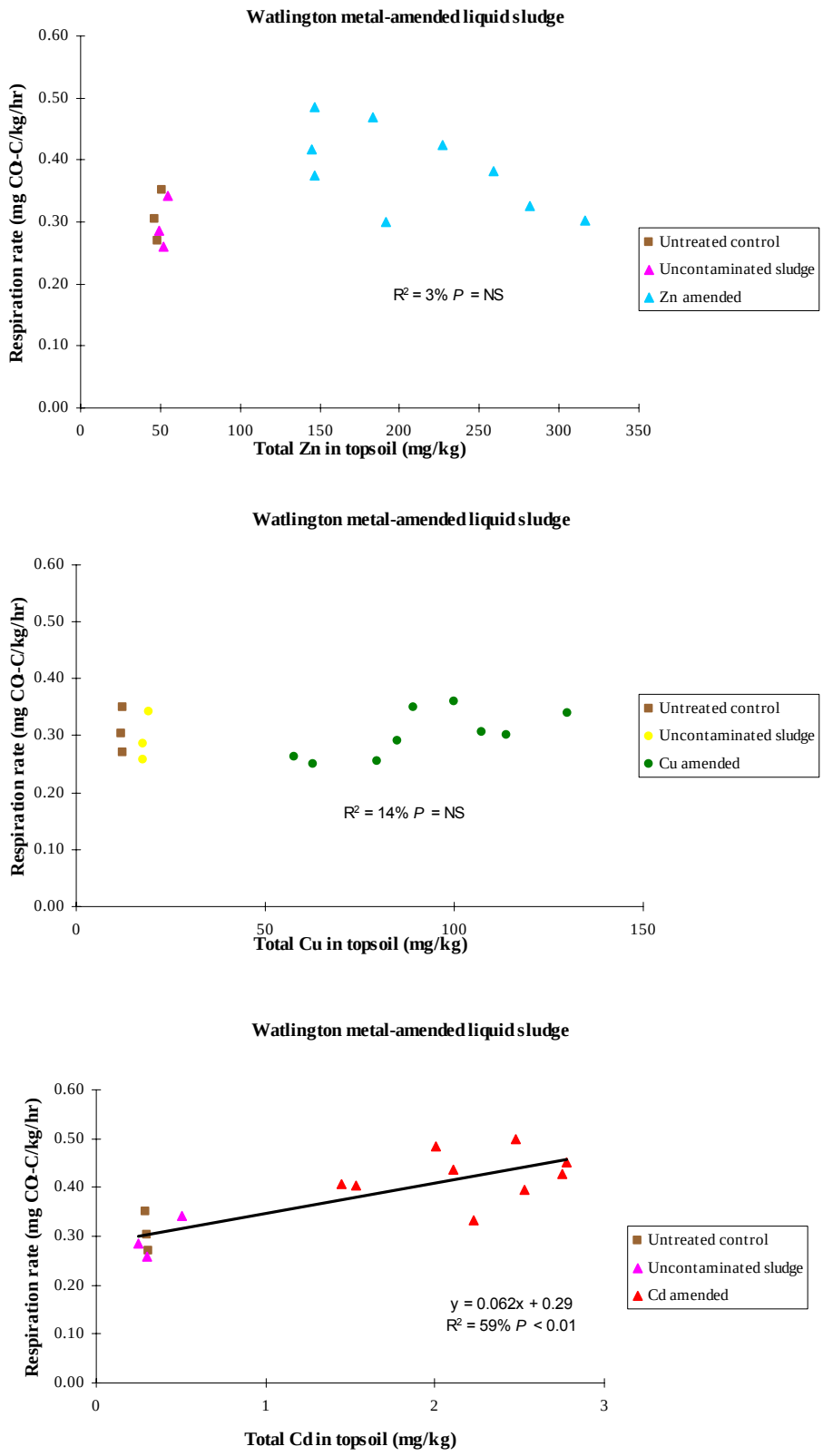
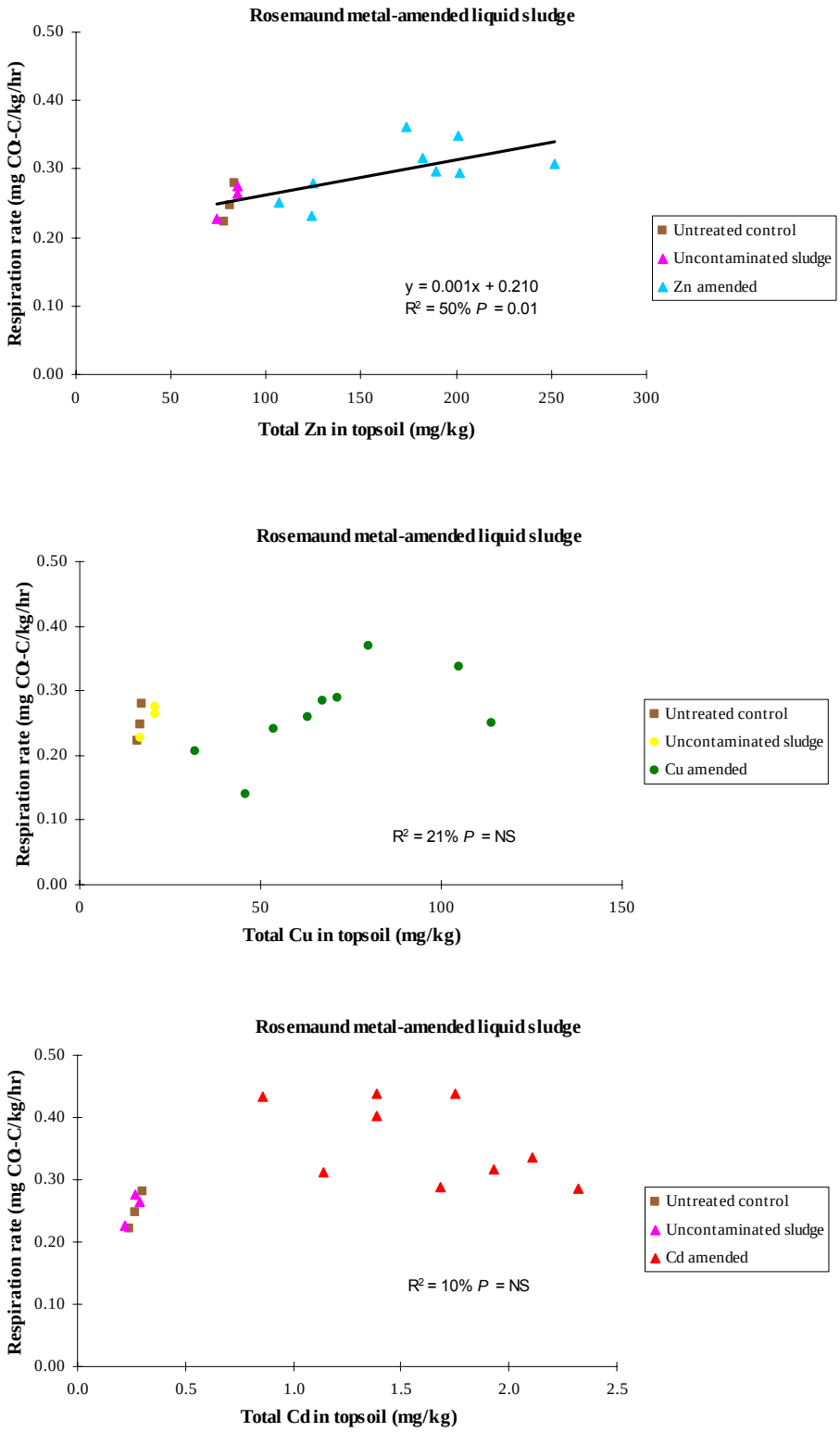


Figure 75. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) – Rosemaund liquid sludge (spring 2003)



2005 sampling: At Woburn, soil respiration rates on the untreated control treatment were lower ($P < 0.05$) than the Cu-rich rate 2 and 3 treatments.

Soil respiration rates were regressed against total topsoil metal concentrations (Figures 76-78). On the metal dose-response treatments, regression of the soil respiration rate measurements against total topsoil Cu identified a significant positive relationship ($P < 0.05$) at Woburn. A summary of the regression analyses is given in Table 48.

Table 47. Mean topsoil (0-25 cm) respiration rates (mg CO_2-C/kg oven dry soil/hour) on treatments sampled in spring 2005.

Treatment	WOB	WAT	ROS
Control	0.21	0.28	0.29
Unc. Digested	0.28	0.26	0.28
Zn rate 1	0.25	0.25	0.32
Zn rate 2	0.27	0.28	0.33
Zn rate 3	0.28	0.25	0.28
Cu rate 1	0.32	0.34	0.27
Cu rate 2	0.33	0.35	0.29
Cu rate 3	0.35	0.30	0.27
Cd rate 1	0.24	0.25	0.27
Cd rate 2	0.25	0.26	0.26
Cd rate 3	0.27	0.30	0.28
ANOVA *p*	0.016	NS	NS
$LSD_{0.05}$	0.117	-	-
CV%	14	23	11

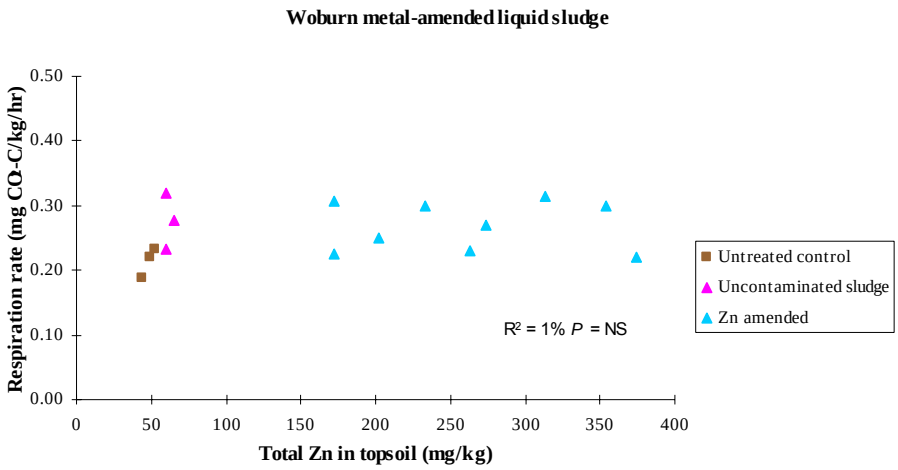
A single ANOVA was carried out. The three metal rich groups (Zn, Cu and Cd) were compared with the untreated control treatment and the uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment mean differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance ($P = 0.05$).

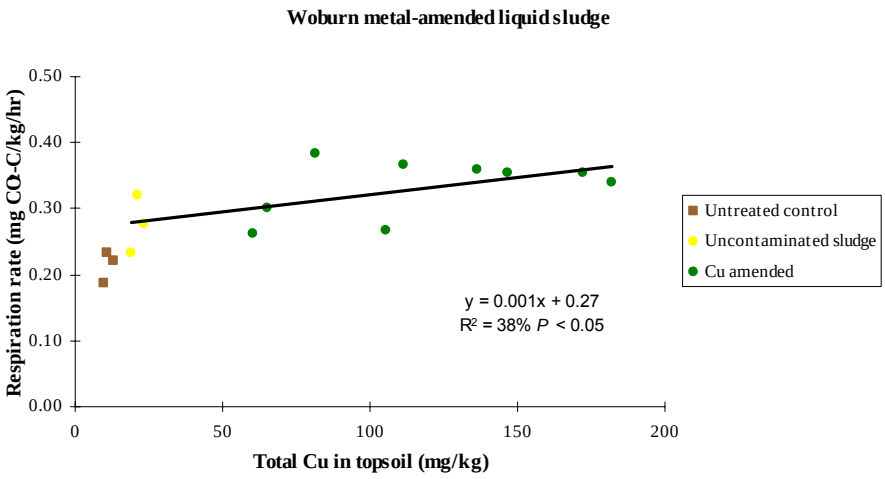
Table 48. Summary of relationships between Respiration rates and total topsoil metals – metal amended liquid sludge sites (spring 2005 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	22%; $P < 0.05$	NS
WAT	NS	NS	NS
ROS	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship

Figure 76. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) – Woburn liquid sludge (spring 2005)





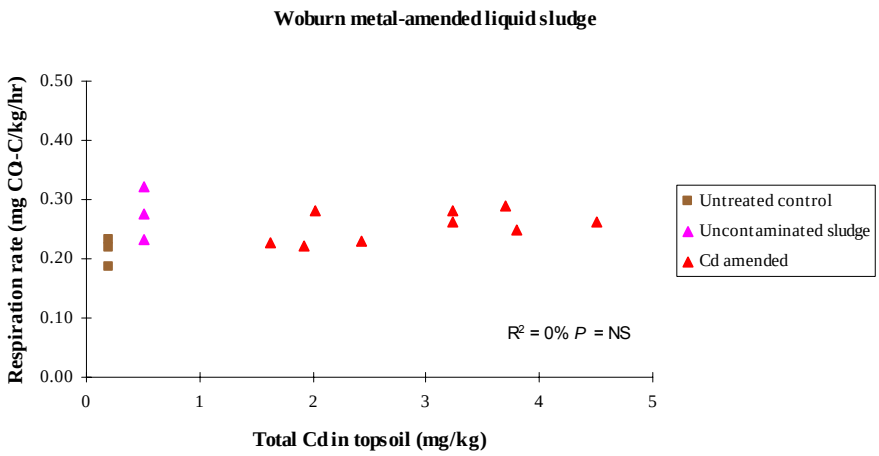


Figure 77. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) – Watlington liquid sludge (spring 2005)

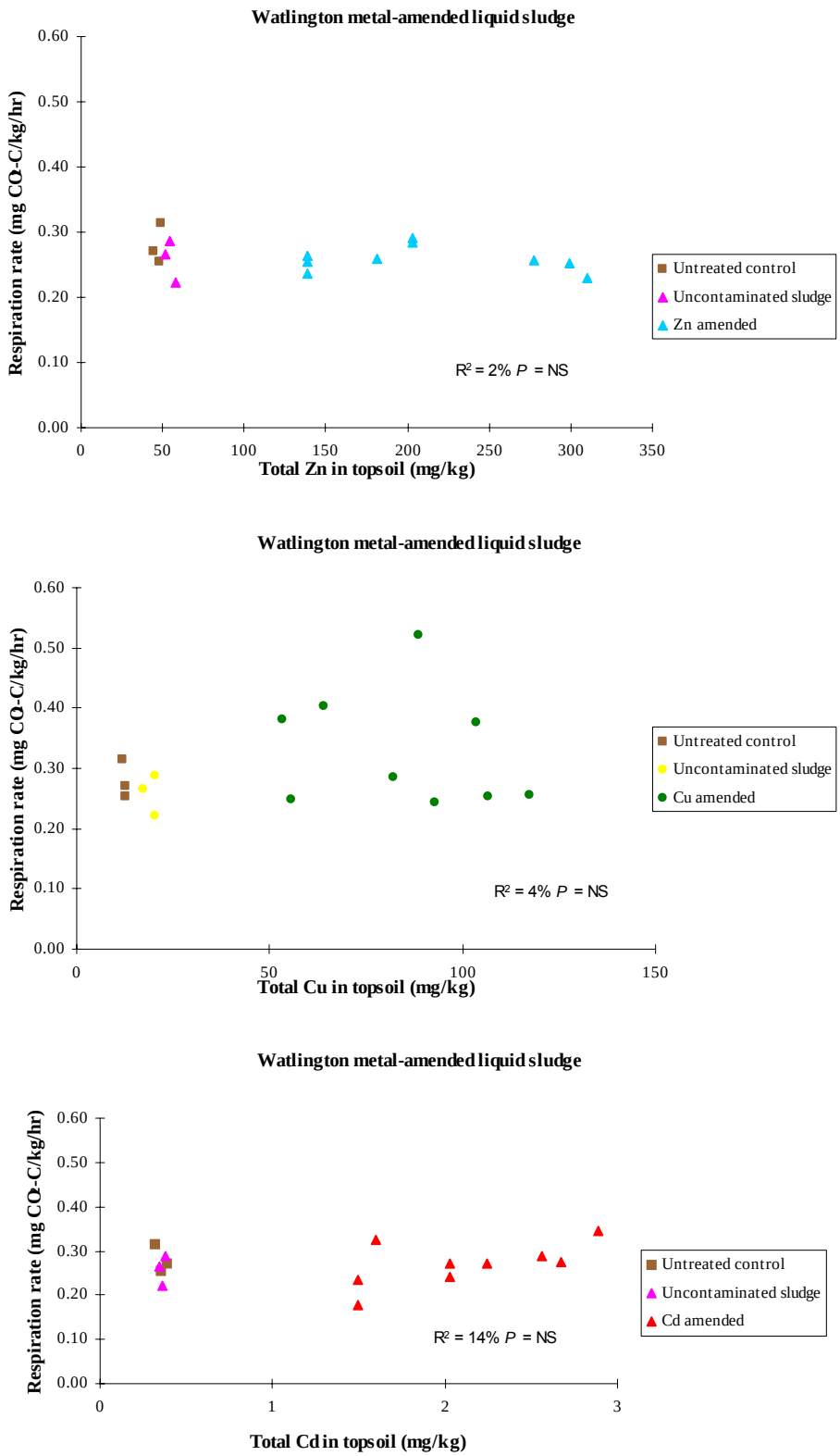
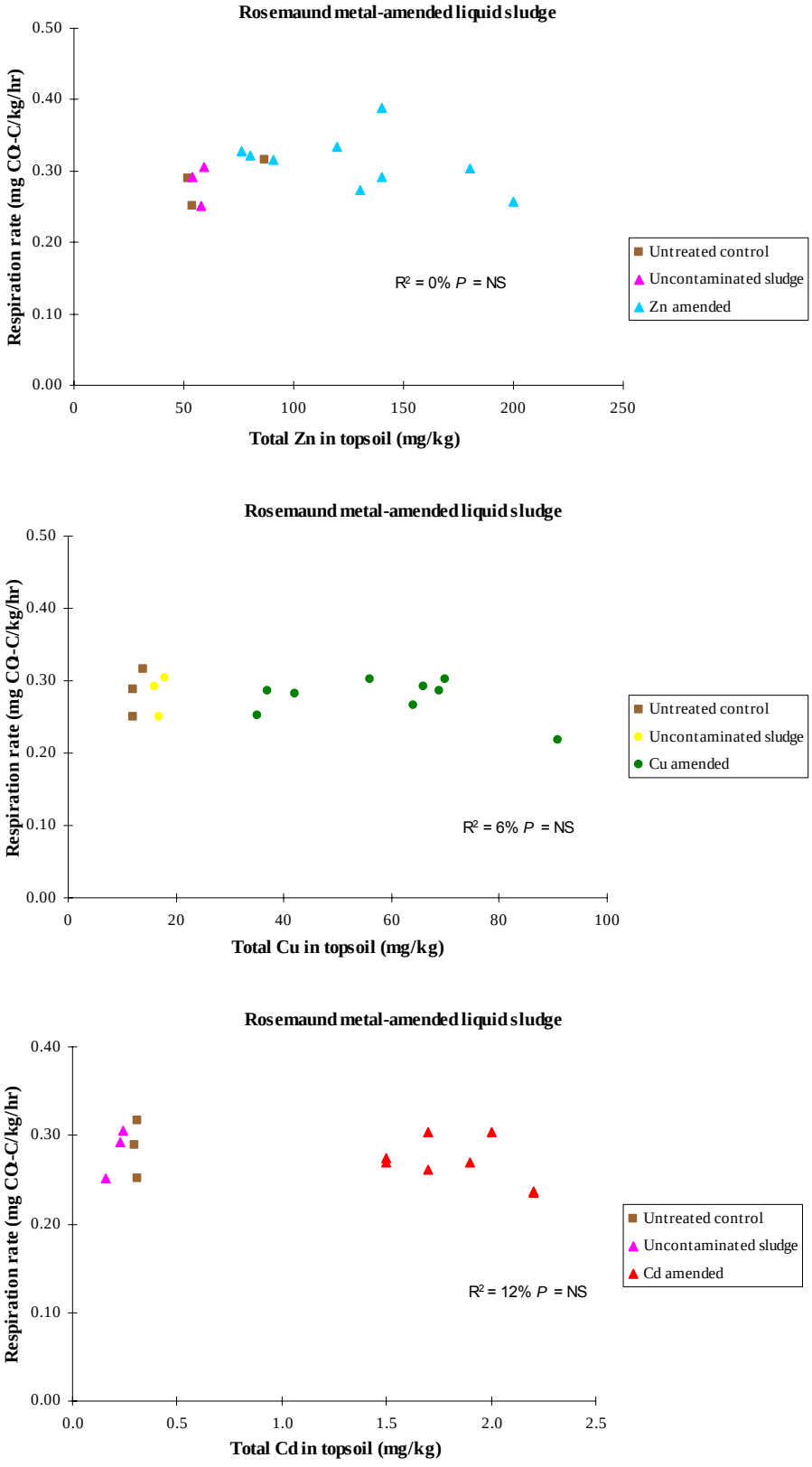


Figure 78. Relationships between total topsoil Zn, Cu and Cd concentrations and soil respiration rates (mg CO_2-C/kg/hr) – Rosemaund liquid sludge (spring 2005)



Microbial biomass carbon concentrations

Soil microbial biomass carbon concentrations were measured on soil samples taken from all the treatment plots in spring 2003 and 2005, with mean treatment microbial biomass concentrations summarised in Tables 49 and 51. Soil microbial biomass carbon concentrations were generally higher in both 2003 and 2005 on the treatments receiving liquid sludge compared with the untreated controls.

Table 49. Mean topsoil (0-25 cm) microbial biomass carbon (mg C/kg oven dry soil) concentrations on treatments sampled in spring 2003.

Treatment	WOB	WAT	ROS
Control	162	278	380
Unc. Digested	230	346	403
Zn rate 1	201	340	389
Zn rate 2	232	356	410
Zn rate 3	199	292	408
Cu rate 1	180	310	391
Cu rate 2	189	292	350
Cu rate 3	192	270	378
Cd rate 1	214	370	416
Cd rate 2	216	343	409
Cd rate 3	243	348	391
ANOVA p	0.027	0.002	NS
$LSD_{0.05}$	63	70	-
CV%	12	9	7

A single ANOVA was carried out. The three metal rich groups (Zn, Cu and Cd) were compared with the untreated control treatment and the uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment mean differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance ($P = 0.05$).

2003 sampling: At Woburn, soil microbial biomass carbon concentrations were higher ($P < 0.05$) on the uncontaminated digested, Zn-rich rate 2 and Cd-rich rate 3 treatments than the untreated control treatment. At Watlington, soil microbial biomass carbon concentrations were higher ($P < 0.01$) on the Zn-rich rate 2 and Cd-rich rate 1 and 3 treatments than the untreated control treatment. In contrast, the biomass carbon concentration on the Cu-rich rate 3 treatment at Watlington was lower ($P < 0.01$) than the uncontaminated digested liquid sludge treatment.

Soil microbial biomass carbon concentrations were regressed against total topsoil metal concentrations (Figures 79-81; Table 50). On the metal dose-response treatments, a significant inverse relationship ($P < 0.001$) was measured at Watlington for Cu.

Figure 79. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Woburn metal amended liquid sludge (spring 2003)

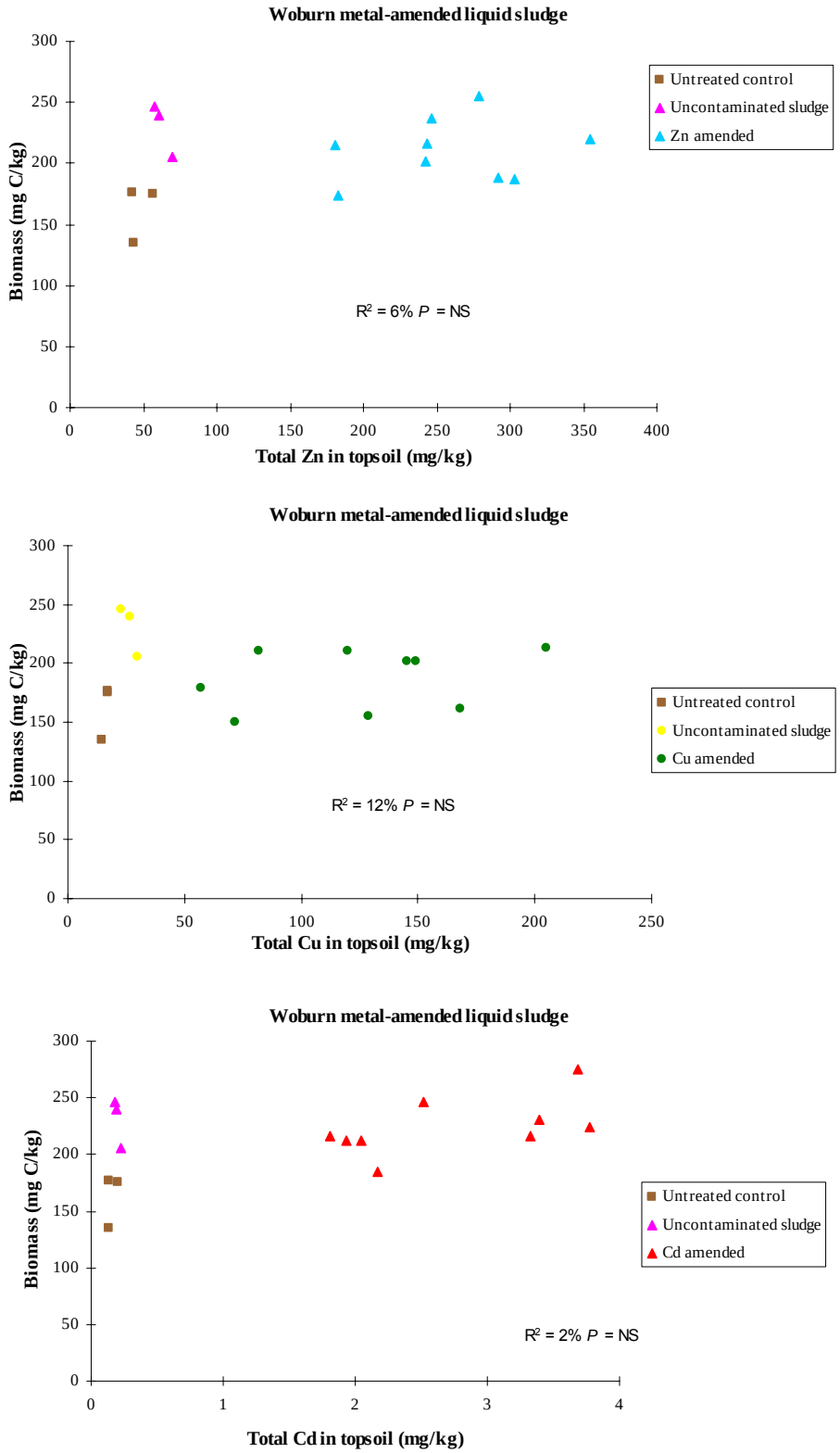


Figure 80. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Watlington metal amended liquid sludge (spring 2003)

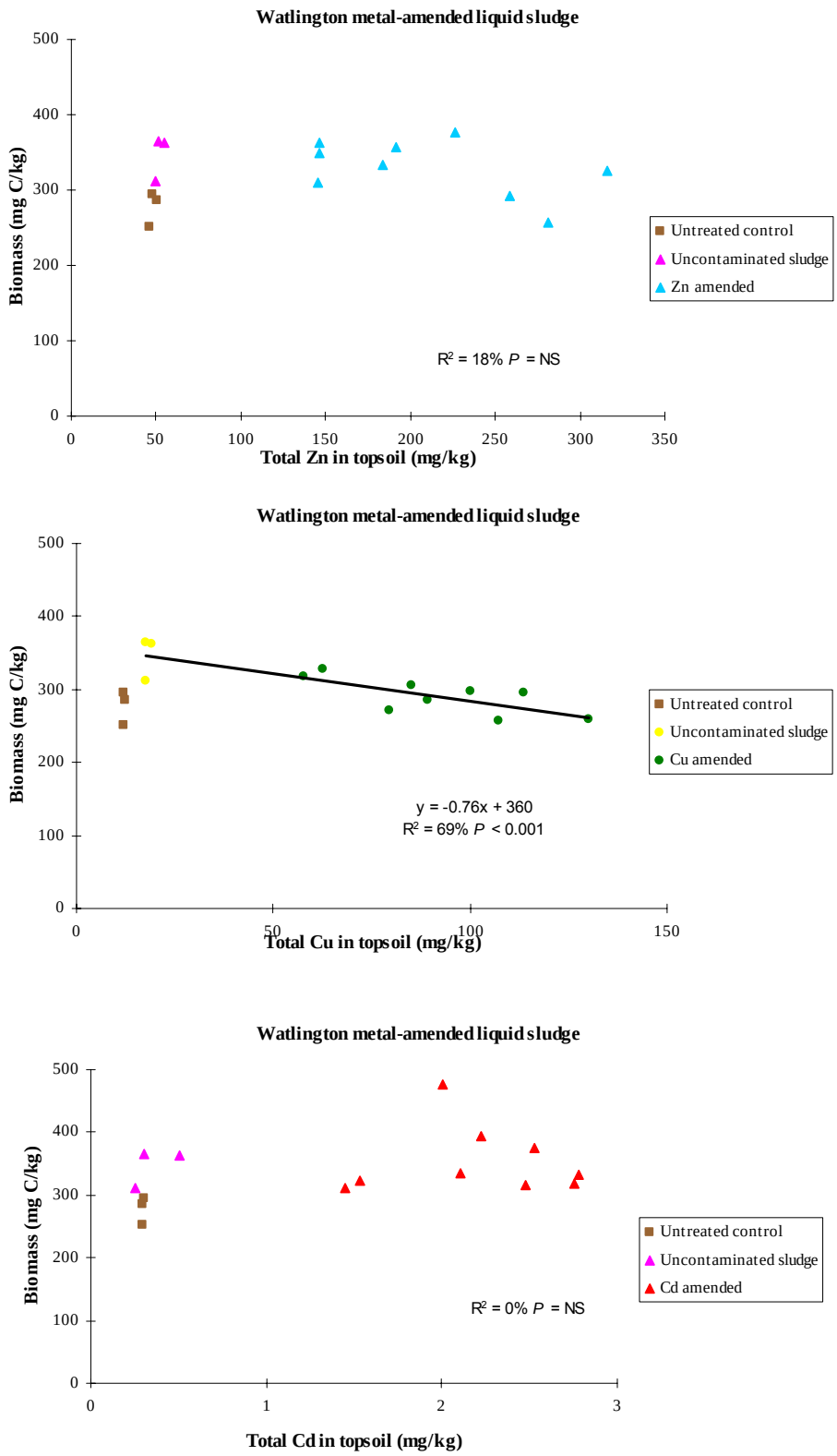


Figure 81. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Rosemaund metal amended liquid sludge (spring 2003)

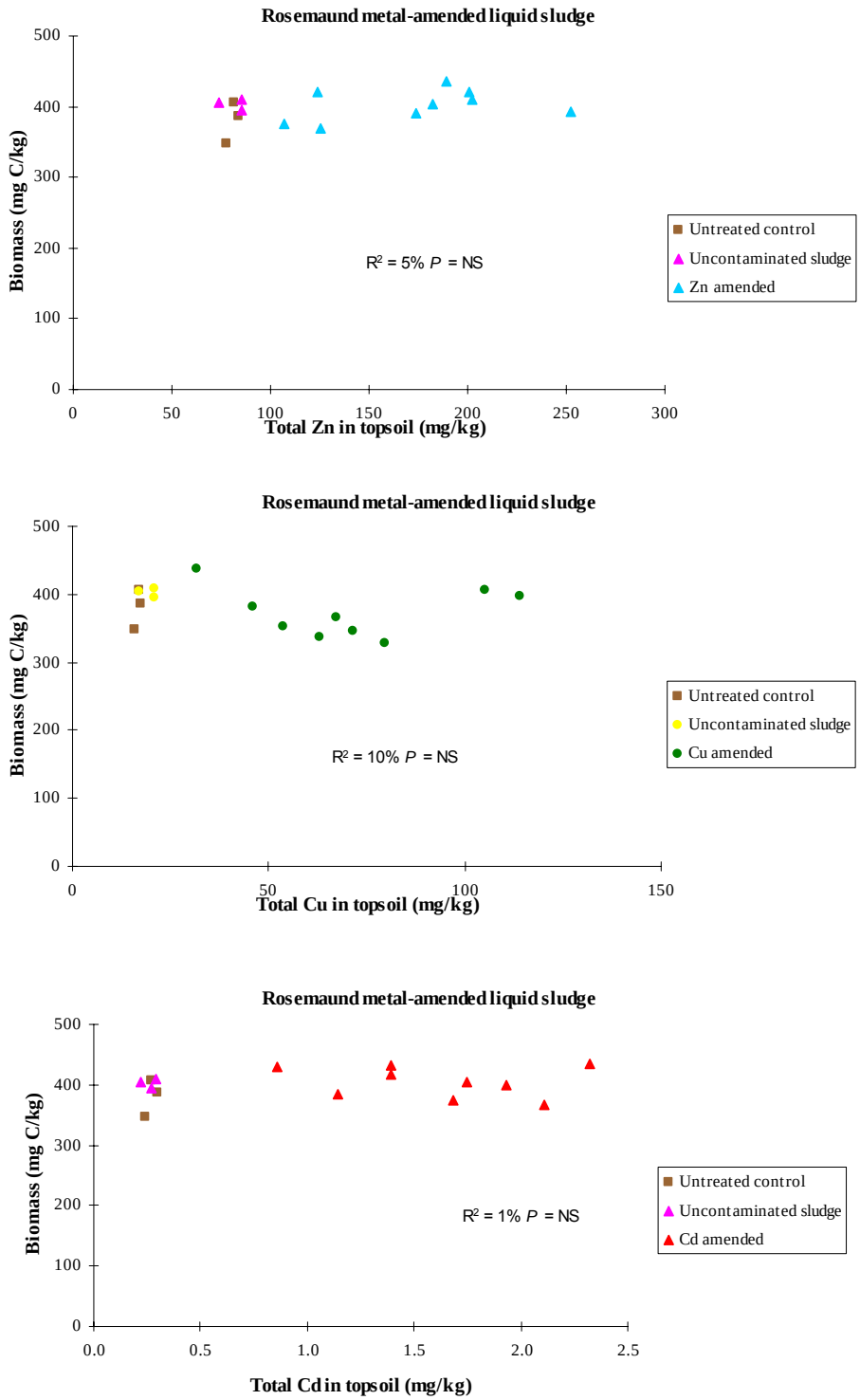


Table 50. Summary of relationships between biomass carbon and total topsoil metals – metal amended liquid sludge sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	NS	NS
WAT	NS	24%; $P < 0.001$	NS
ROS	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship

Table 51. Mean topsoil (0-25 cm) microbial biomass carbon (mg C/kg oven dry soil) concentrations on treatments sampled in spring 2005.

Treatment	WOB	WAT	ROS
Control	167	332	431
Unc. Digested	217	374	410
Zn rate 1	136	346	425
Zn rate 2	153	374	447
Zn rate 3	163	338	456
Cu rate 1	191	374	483
Cu rate 2	151	318	421
Cu rate 3	140	296	416
Cd rate 1	172	369	449
Cd rate 2	164	373	456
Cd rate 3	207	336	458
ANOVA p	0.029	0.015	NS
$LSD_{0.05}$	81	76	-
CV%	16	8	8

A single ANOVA was carried out. The three metal rich groups (Zn, Cu and Cd) were compared with the untreated control treatment and the uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment mean differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance ($P = 0.05$).

2005 sampling: At Woburn and Watlington, soil microbial biomass carbon concentrations were lower ($P < 0.05$) on the Zn-rich rate 1 and Cu-rich rate 3 treatments, respectively, than the uncontaminated digested liquid sludge treatments.

Soil microbial biomass carbon concentrations were regressed against total topsoil metal concentrations (Figures 82-84; Table 52). On the

metal dose-response treatments, significant inverse relationships ($P < 0.05$) were measured at Woburn for Zn, and at Woburn and Watlington for Cu. For Cd, a significant ($P < 0.05$) positive relationship was measured at Rosemaund.

Table 52. Summary of relationships between biomass carbon and total topsoil metals – metal amended liquid sludge sites (spring 2005 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	14%; $P < 0.05$	29%; $P < 0.05$	NS
WAT	NS	28%; $P < 0.05$	NS
ROS	NS	NS	21%; $P < 0.05$

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship Blue = positive relationship.

Figure 82. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Woburn metal amended liquid sludge (spring 2005)

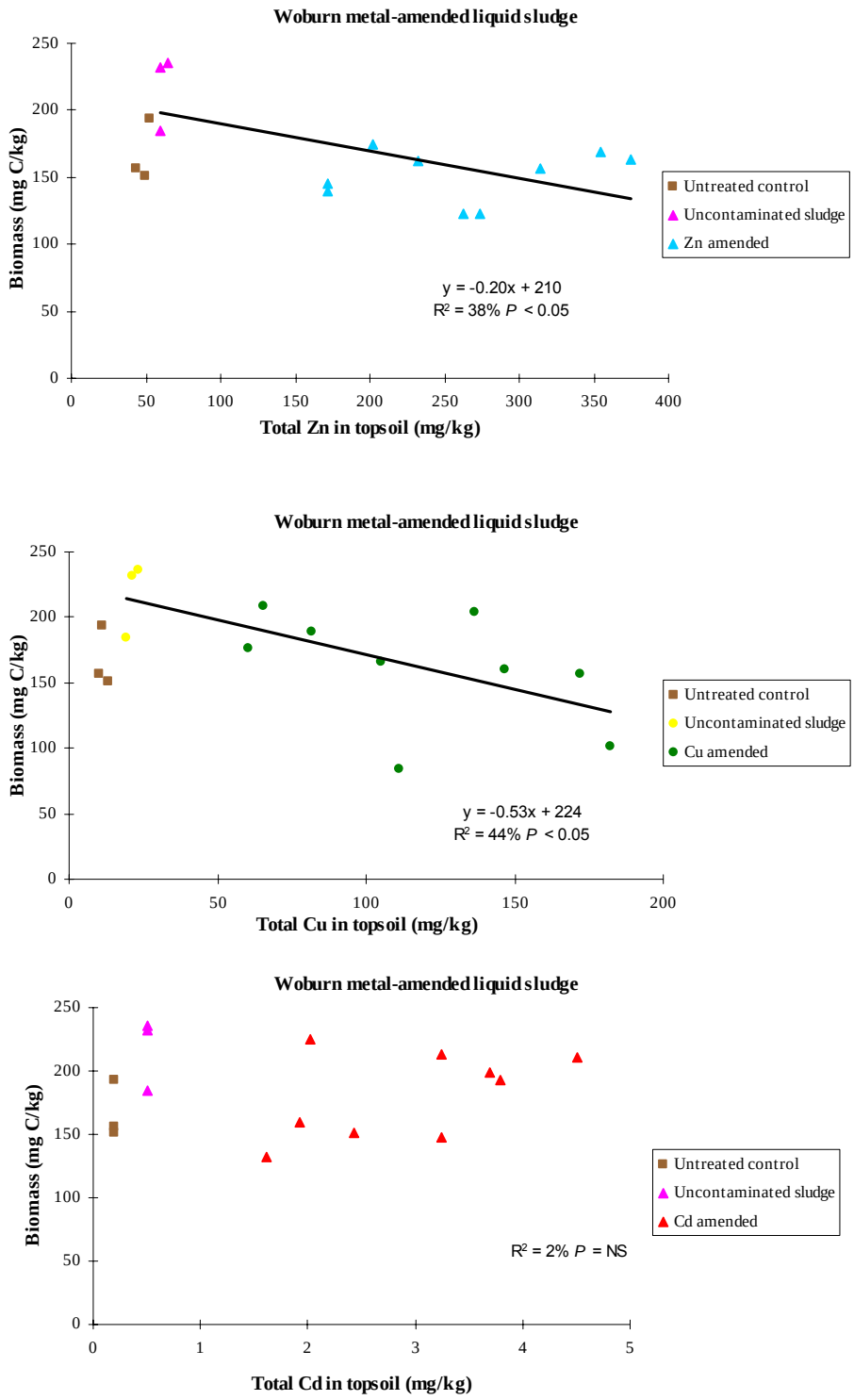


Figure 83. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Watlington metal amended liquid sludge (spring 2005)

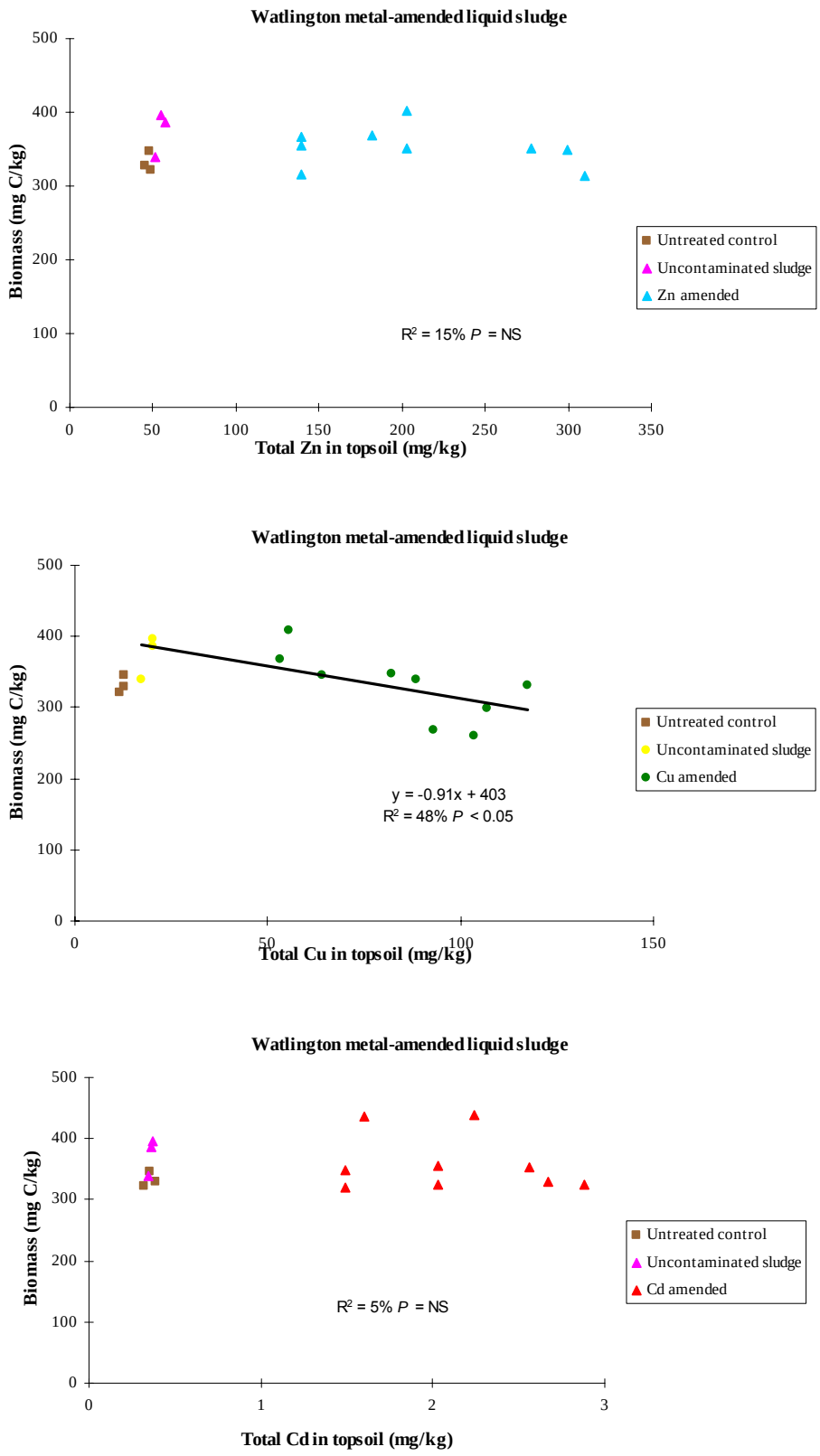
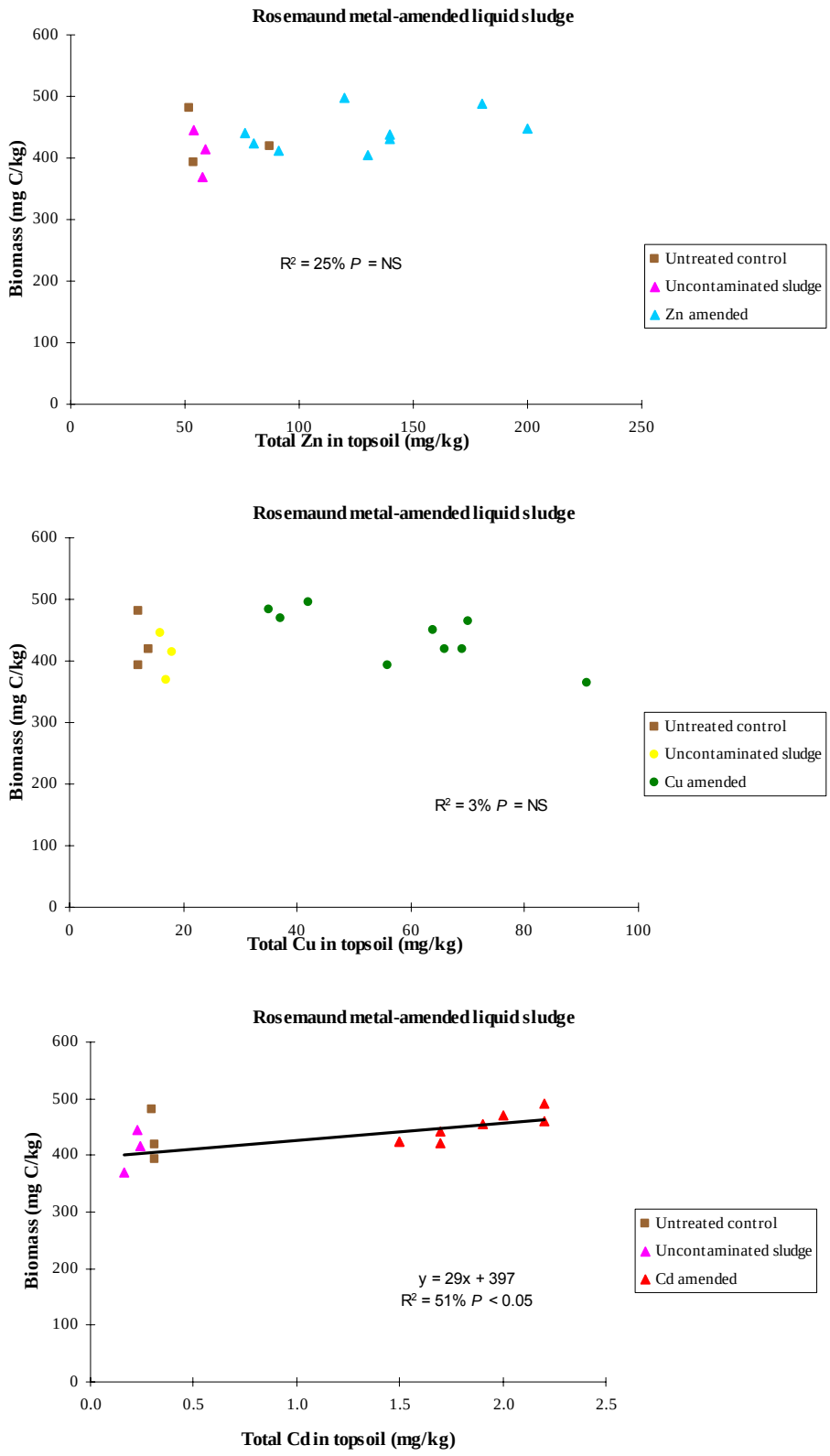


Figure 84. Relationships between total topsoil Zn, Cu and Cd and soil biomass C concentrations (mg/kg) - Rosemaund metal amended liquid sludge (spring 2005)



Respiration Quotient

In order to compare the results from individual plots having different soil microbial biomass concentrations and soil respiration rates, the data were normalised using specific microbial quotients, Tables 53 and 54 (for the methodology used, see earlier Section 4.1.1 – Respiration Quotient).

Table 53. Soil respiration rate as a proportion of soil microbial biomass C (spring 2003 samples): *Respiration Quotient*

Treatment	WOB	WAT	ROS
Control	0.18	0.11	0.07
Unc. Digested	0.13	0.09	0.06
Zn rate 1	0.20	0.13	0.07
Zn rate 2	0.17	0.11	0.08
Zn rate 3	0.18	0.12	0.08
Cu rate 1	0.20	0.09	0.05
Cu rate 2	0.18	0.10	0.08
Cu rate 3	0.21	0.12	0.09
Cd rate 1	0.12	0.12	0.09
Cd rate 2	0.18	0.13	0.08
Cd rate 3	0.13	0.12	0.09

Table 54. Soil respiration rate as a proportion of soil microbial biomass C (spring 2005 samples): *Respiration Quotient*

Treatment	WOB	WAT	ROS
Control	0.13	0.08	0.07
Unc. Digested	0.13	0.07	0.07
Zn rate 1	0.19	0.07	0.08
Zn rate 2	0.18	0.07	0.07
Zn rate 3	0.17	0.07	0.06
Cu rate 1	0.17	0.09	0.06
Cu rate 2	0.26	0.11	0.07
Cu rate 3	0.26	0.10	0.06
Cd rate 1	0.15	0.07	0.06
Cd rate 2	0.16	0.07	0.05
Cd rate 3	0.13	0.09	0.06

The respiration quotients for each plot were regressed against total topsoil metal concentrations for treatments receiving the same organic matter inputs during Phase I. A summary of the regression analyses is

given in Tables 55 and 56 for the 2003 and 2005 samplings, respectively.

2003 sampling: At Rosemaund, there was a significant ($P < 0.05$) positive relationship between RQ and total topsoil Zn. At Watlington, there were significant ($P < 0.05$) positive relationships between RQ and total topsoil Cu and Cd. On all three of the metal-dose response treatments where a relationship between RQ and total topsoil metals was found, RQ *increased* with increasing total topsoil metal concentrations.

Table 55. Summary of relationships between RQ and total topsoil metals – metal amended liquid sludge sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	NS	NS
WAT	NS	55%; $P < 0.01$	55%; $P < 0.05$
ROS	41%; $P < 0.05$	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Blue = positive relationship

2005 sampling: At Woburn, there was a significant ($P < 0.05$) positive relationship between RQ and total topsoil Cu. At Rosemaund, there was a significant ($P < 0.05$) inverse relationship between RQ and total topsoil Cd.

Table 56. Summary of relationships between RQ and total topsoil metals – metal amended liquid sludge sites (spring 2005 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	90% < 0.05	NS
WAT	NS	NS	NS
ROS	NS	NS	26% < 0.05

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd.. Blue = positive relationship. Red = inverse relationship

A significant positive relationship ($P < 0.05$) between RQ and total topsoil Zn concentration was evident at one site in 2003 (Rosemaund). For Cu, significant ($P < 0.05$) positive relationships were evident at Watlington in 2003 and Woburn in 2005. For Cd, a significant ($P < 0.05$) positive relationship was evident at Watlington in 2003 and an inverse relationship at Rosemaund in 2005.

Biomass Quotient

In order to compare results from individual plots having different soil organic C contents and microbial biomass C concentrations the data were normalised using a specific microbial quotient, Tables 57 and 58 (for the methodology used see earlier Section 4.1.1 – Biomass Quotient).

Table 57. Soil microbial biomass C as a proportion of soil organic C (spring 2003 samples): *Biomass Quotient*

Treatment	WOB	WAT	ROS
Control	1.50	1.92	2.41
Unc. Digested	1.64	2.01	2.18
Zn rate 1	1.47	1.81	2.09
Zn rate 2	1.60	1.98	2.20
Zn rate 3	1.54	1.58	2.26
Cu rate 1	1.37	1.64	2.06
Cu rate 2	1.32	1.65	1.84
Cu rate 3	1.37	1.42	1.90
Cd rate 1	1.56	1.95	2.25
Cd rate 2	1.58	1.76	2.10
Cd rate 3	1.67	1.89	2.09

Table 58. Soil microbial biomass C as a proportion of soil organic C (spring 2005 samples): *Biomass Quotient*

Treatment	WOB	WAT	ROS
Control	1.53	2.38	3.04
Unc. Digested	1.56	2.32	2.72
Zn rate 1	2.21	2.22	2.54
Zn rate 2	1.67	2.18	2.80
Zn rate 3	1.27	1.93	2.70
Cu rate 1	2.43	2.18	2.59
Cu rate 2	1.27	1.84	2.33
Cu rate 3	1.30	1.85	2.52
Cd rate 1	1.78	2.22	2.69
Cd rate 2	1.99	2.63	3.17
Cd rate 3	1.55	2.00	2.54

The biomass quotients for each plot were regressed against total topsoil metal concentrations for treatments receiving the same organic matter inputs during Phase I. A summary of the regression analyses is given in Tables 59 and 60 for the 2003 and 2005 samplings, respectively.

2003 sampling: At Watlington, there was a significant ($P < 0.001$) inverse relationship between BQ and total topsoil Cu (Table 59).

Table 59. Summary of relationships between BQ and total topsoil metals – metal amended liquid sludge sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	NS	NS
WAT	NS	34% < 0.001	NS
ROS	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship.

2005 sampling: At Watlington, there were significant ($P < 0.05$) inverse relationships between BQ and total topsoil Zn and Cu (Table 60).

Table 60. Summary of relationships between BQ and total topsoil metals – metal amended liquid sludge sites (spring 2005 samples)

	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	NS	NS
WAT	18% < 0.05	29% < 0.01	NS
ROS	NS	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship

A significant inverse relationship ($P < 0.05$) between BQ and total topsoil Zn concentrations was evident at Watlington in 2005. For Cu, significant ($P < 0.01$) inverse relationships were evident at Watlington in both 2003 and 2005.

Rhizobium numbers

Most probable numbers of rhizobia were measured in soil samples taken from all the treatment plots in spring 2003 and 2005. Treatment mean rhizobia numbers for all the soil samples taken in spring 2003 and 2005 are presented in Tables 61 and 63, respectively.

2003 sampling: There were no significant differences ($P > 0.05$) in rhizobia population sizes between the metal-amended liquid sludge treatments and the untreated controls at any of the three sites (Table 61).

Table 61. Mean topsoil (0-25 cm) *Rhizobium leguminosarum* biovar *trifolii* numbers (log_{10} cells/g soil) on treatments sampled in spring 2003

Treatment	WOB	WAT	ROS
Control	4.41	4.86	5.28
Unc. Digested	4.53	5.12	5.02
Zn rate 1	4.63	5.02	5.02
Zn rate 2	4.32	4.92	5.02
Zn rate 3	4.32	4.90	4.28
Cu rate 1	4.73	4.67	5.12
Cu rate 2	4.21	4.86	4.86
Cu rate 3	4.53	5.08	4.89
Cd rate 1	4.63	5.02	5.22
Cd rate 2	4.49	4.79	5.08
Cd rate 3	4.31	5.18	4.96
ANOVA	NS	NS	NS
$LSD_{0.05}$	-	-	-
CV%	8	6	6

A single ANOVA was carried out. The three metal rich groups (Zn, Cu and Cd) were compared with the untreated control treatment and the uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment mean differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance (P = 0.05). As the soil MPN results examined on a site by site basis did not approximate to normal distributions, the data were transformed to Log $base_{10}$ before statistical analysis; therefore the values in the table are geometric means.

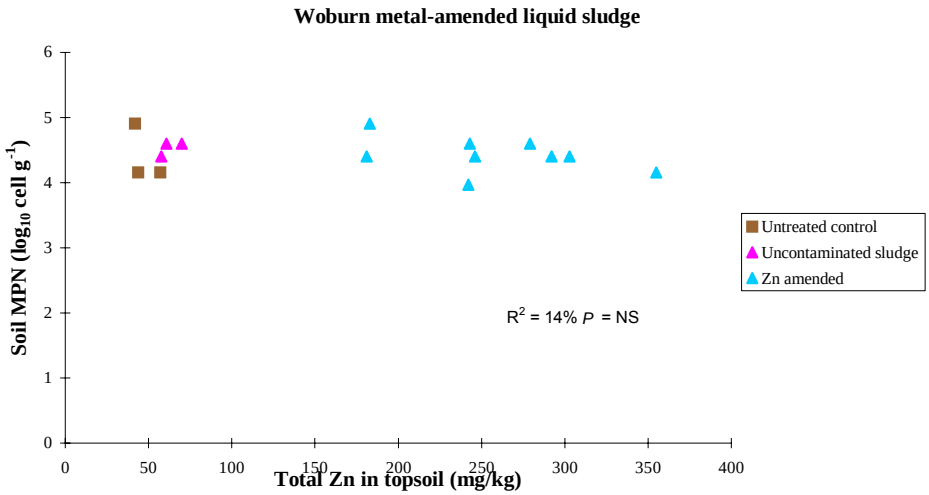
On the metal dose-response treatments, regression of the soil rhizobia MPN values against total topsoil metal concentrations (Figures 85-87) showed one significant (P < 0.05) inverse relationship for Zn at Rosemaund (Table 62).

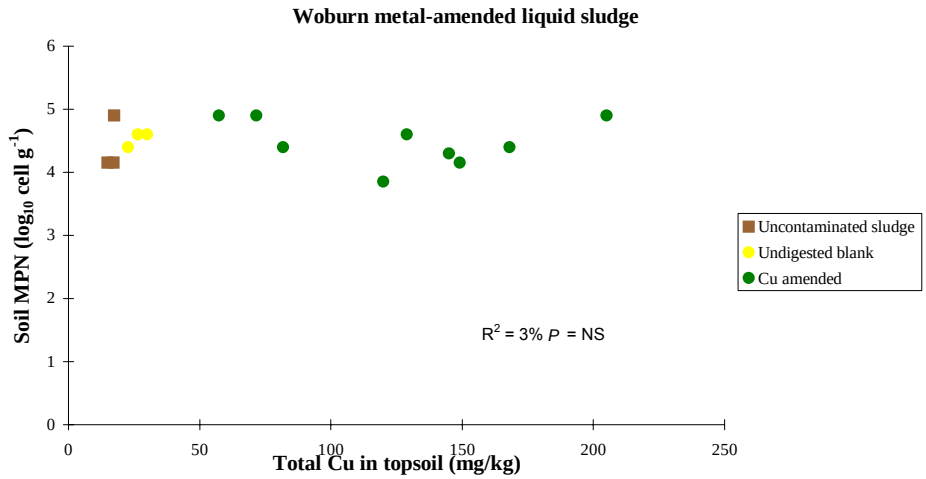
Table 62. Summary of relationships between rhizobia MPNs and total topsoil metals – metal amended liquid sludge sites (spring 2003 samples)

Site	Zn	Cu	Cd
	(% change to current metal limit ; *P*)		
WOB	NS	NS	NS
WAT	NS	NS	NS
ROS	17%; $P < 0.05$	NS	NS

% values give the size of the response up to the current statutory heavy metal limits *i.e.* 300 mg/kg Zn, 135 mg/kg Cu and 3 mg/kg Cd. Red = inverse relationship

Figure 85. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Woburn (spring 2003)





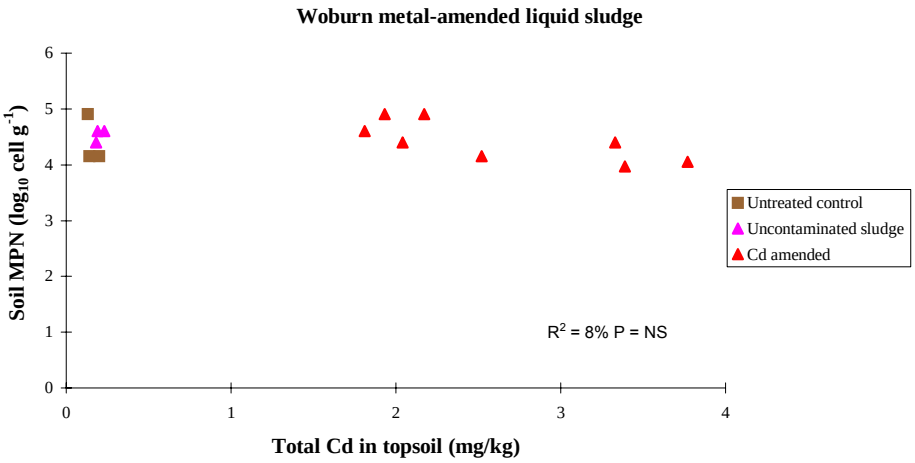


Figure 86. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Watlington (spring 2003)

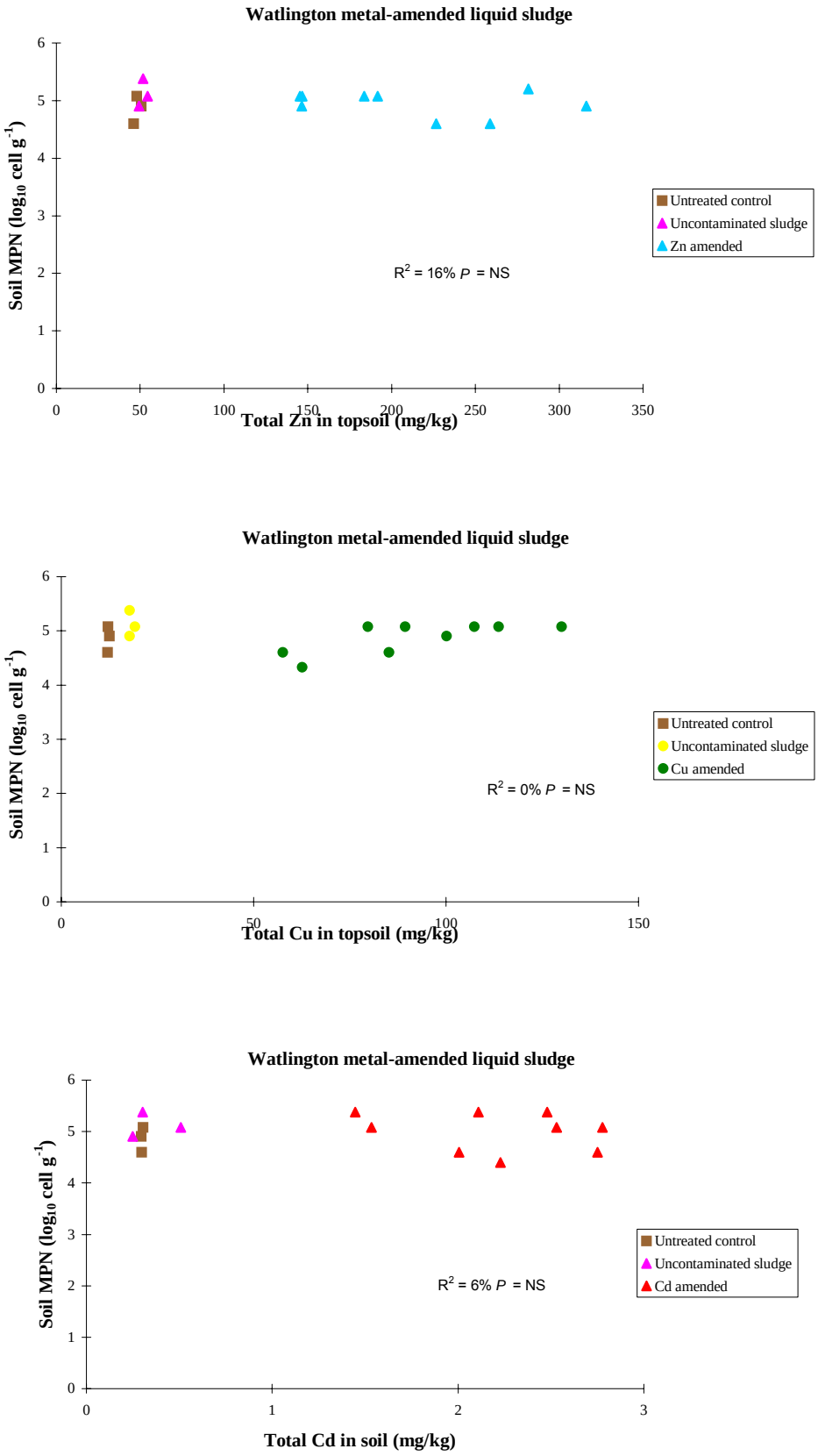
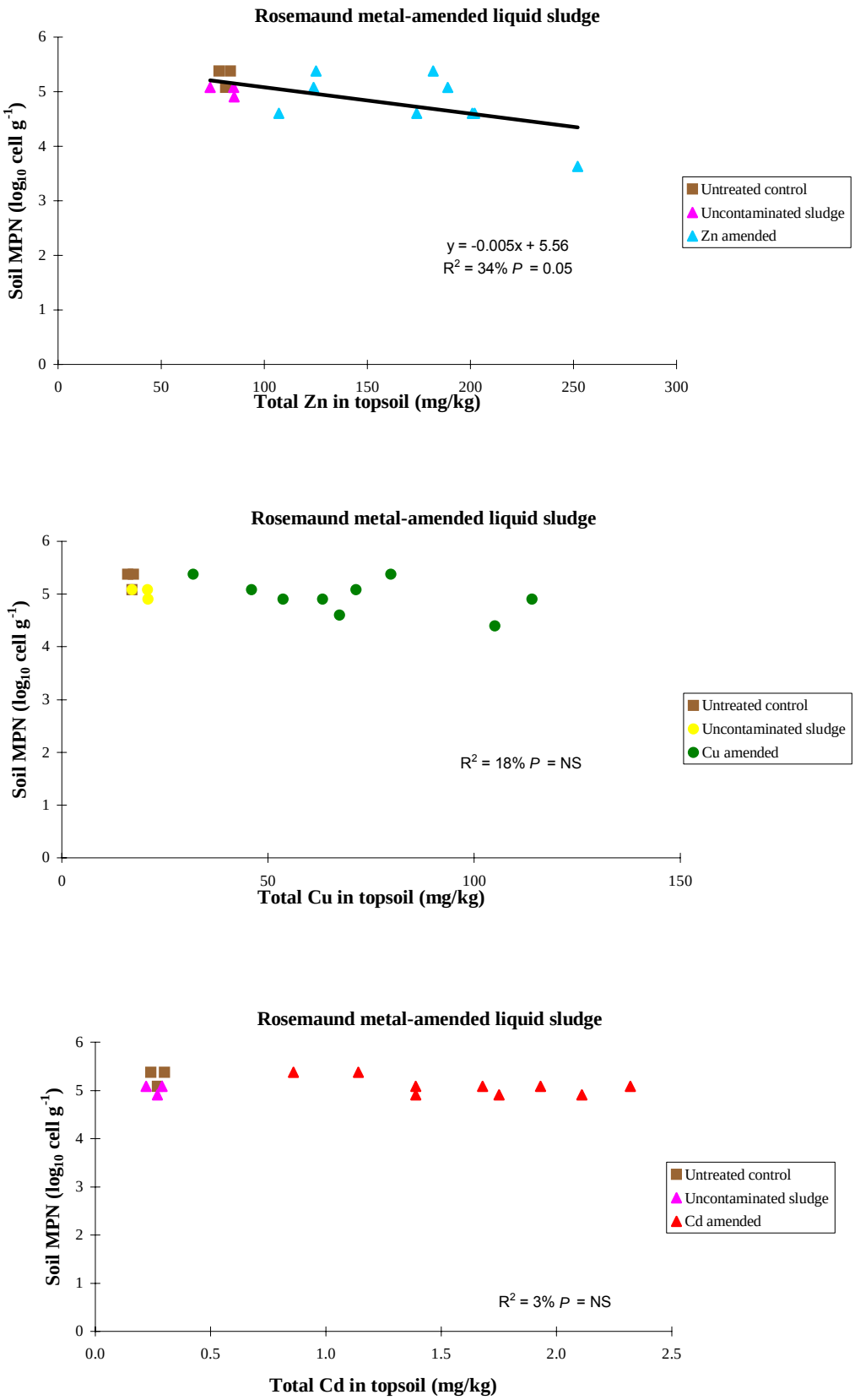


Figure 87. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Rosemaund (spring 2003)



2005 sampling: The only significant ($P < 0.05$) difference between treatments was at Woburn, where the *Rhizobium* population size on the Zn-rich rate 1 treatment was smaller than uncontaminated digested liquid sludge treatment (Table 63).

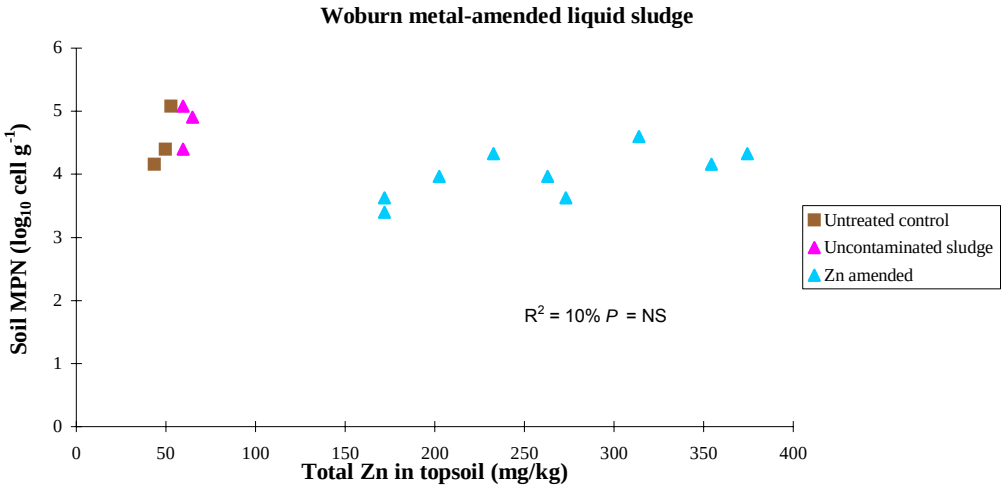
Table 63. Mean topsoil (0-25 cm) *Rhizobium leguminosarum* biovar *trifolii* numbers ($\log_{10}$ cells/g soil) on treatments sampled in spring 2005

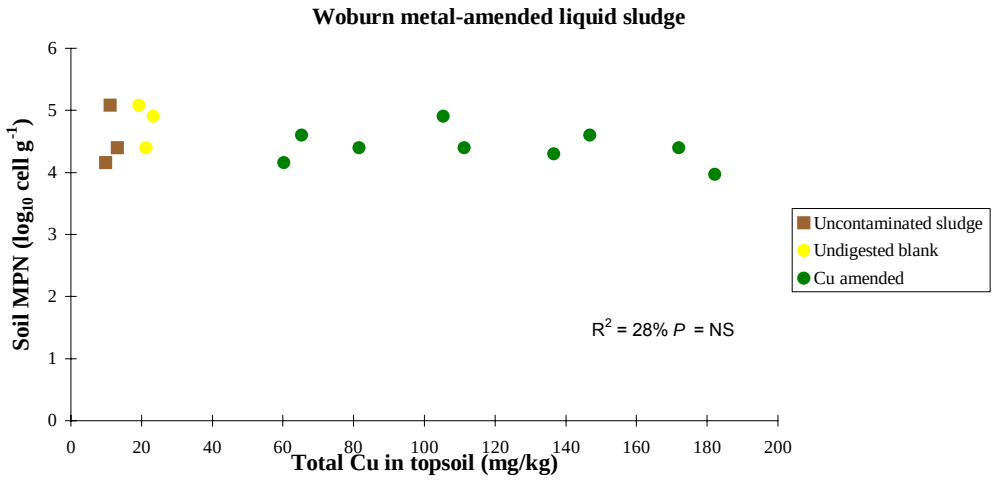
Treatment	WOB	WAT	ROS
Control	4.54	5.22	5.08
Unc. Digested	4.79	4.80	5.12
Zn rate 1	3.67	5.28	4.75
Zn rate 2	3.97	4.83	5.12
Zn rate 3	4.36	5.08	5.22
Cu rate 1	4.39	5.06	5.22
Cu rate 2	4.53	4.92	4.70
Cu rate 3	4.32	4.71	4.96
Cd rate 1	4.18	5.28	5.12
Cd rate 2	4.54	5.02	5.12
Cd rate 3	4.55	4.87	5.18
ANOVA	0.030	*NS*	*NS*
$LSD_{0.05}$	0.974	-	-
CV%	8	7	8

A single ANOVA was carried out. The three metal rich groups (Zn, Cu and Cd) were compared with the untreated control treatment and the uncontaminated sludge control. The ANOVA fitted was a one-way randomised block design. Specific treatment mean differences have been judged using Tukey's least significant difference estimate ($LSD_{0.05}$) at the 5% level of significance ($P = 0.05$). As the soil MPN results examined on a site by site basis did not approximate to normal distributions, the data were transformed to log $base_{10}$ before statistical analysis; therefore the values in the tables are geometric means.

On the metal dose-response treatments, regression of the soil rhizobia MPN values against total topsoil metal concentrations showed no significant ($P > 0.05$) relationships at any of the three sites (Figures 88-90).

Figure 88. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Woburn (spring 2005)





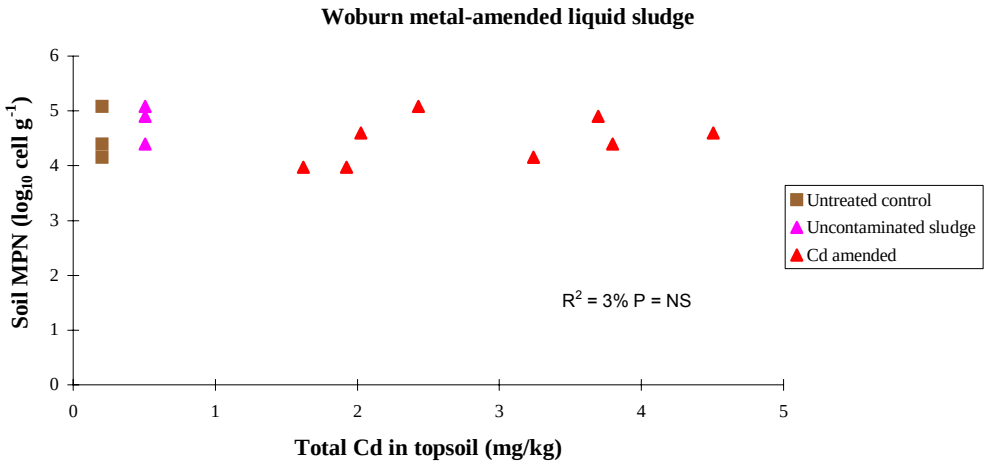
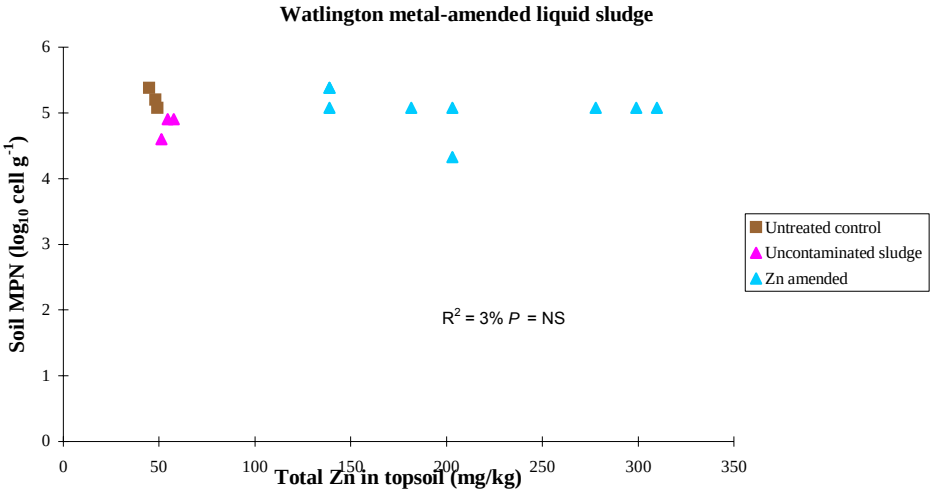
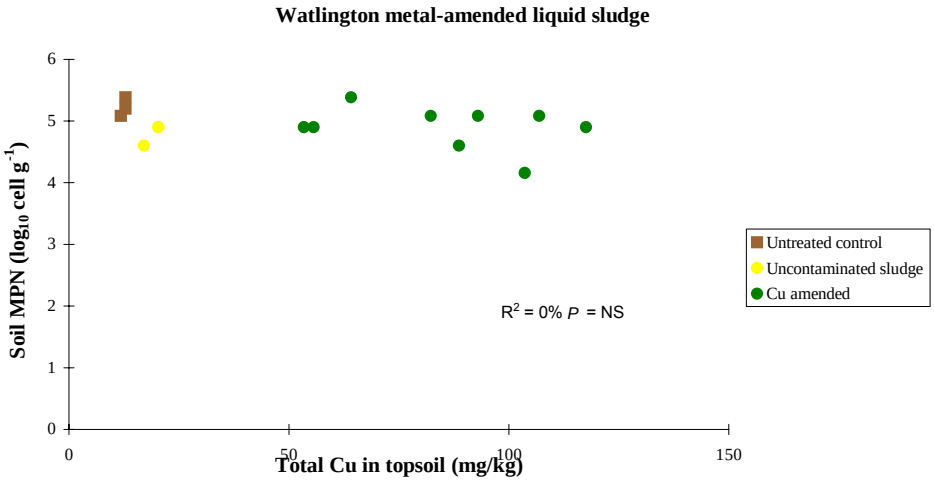


Figure 89. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Watlington (spring 2005)





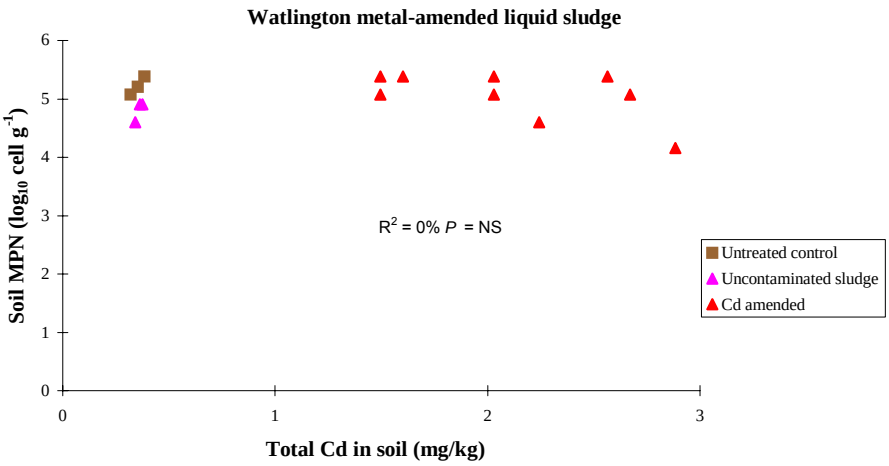
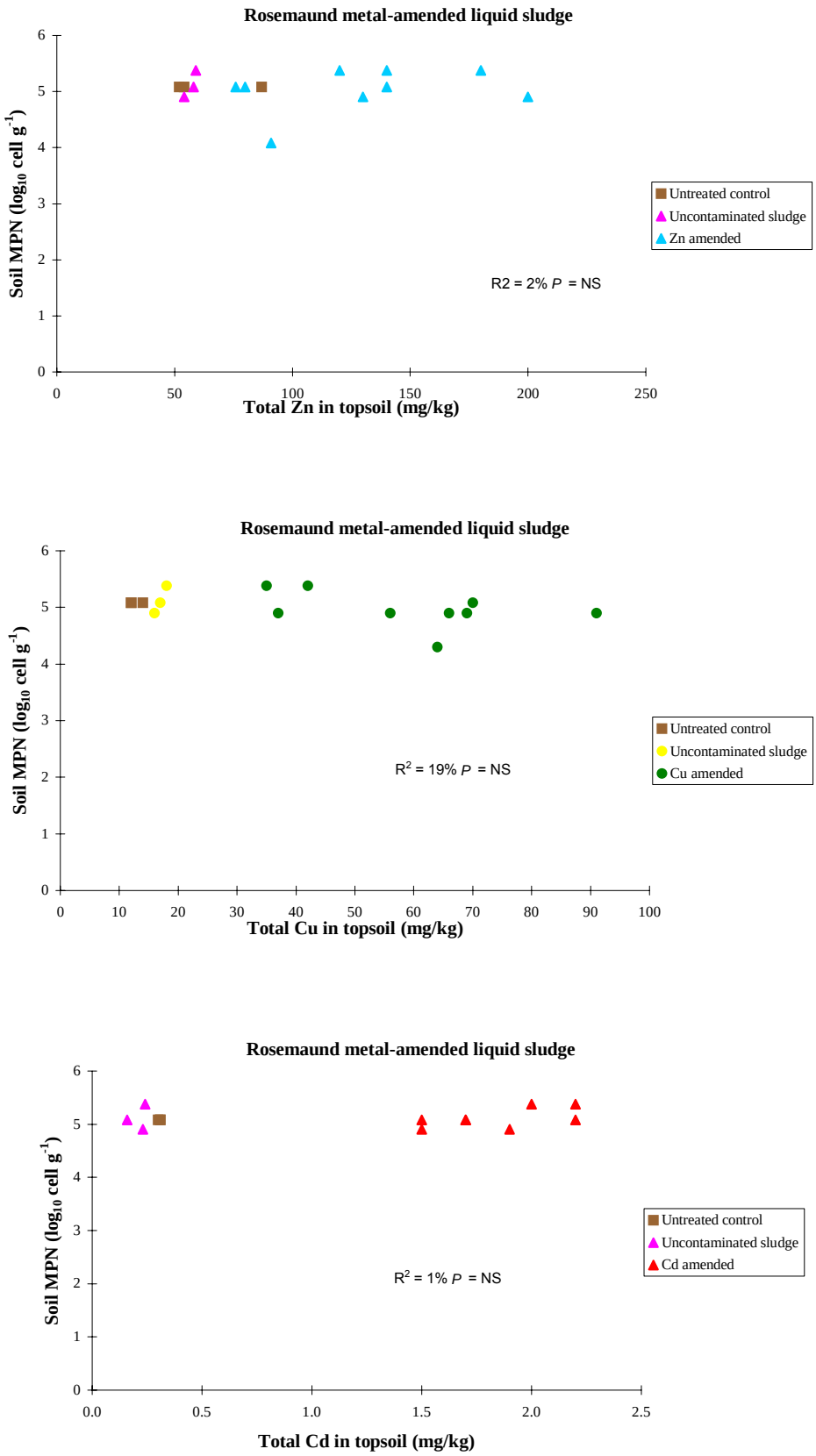


Figure 90. Relationships between total topsoil Zn, Cu and Cd concentrations and rhizobia MPNs - Rosemaund (spring 2005)



4.3.2 Soil chemical properties

Soil metals

Total and NH_4NO_3 extractable topsoil metal (Zn, Cu and Cd) concentrations on samples taken from all the treatment plots in spring 2003 and 2005 at the three sites are presented in Appendices L and M.

2003 sampling: At Rosemaund, topsoil NH_4NO_3 extractable Zn concentrations were approximately 8-fold higher than at Watlington and 4-fold higher than at Woburn (23.1 mg/kg, 2.8 mg/kg and 6.2 mg/kg, on the rate 3 treatments, respectively). At Woburn, topsoil NH_4NO_3 extractable Cu concentrations were approximately double those at Watlington and Rosemaund (*c.*1.2 mg/kg at Woburn and 0.7 mg/kg at Watlington and Rosemaund on the rate 3 treatments, respectively). Topsoil NH_4NO_3 extractable Cd concentrations at Rosemaund were approximately 6-fold higher than at Watlington and nearly double the concentrations at Woburn (*c.*0.13 mg/kg, 0.02 mg/kg and 0.08 mg/kg on the rate 3 treatments, respectively).

At all three sites, there were strong relationships ($P < 0.001$) between total topsoil Zn, Cu and Cd concentrations and corresponding topsoil NH_4NO_3 extractable concentrations (r^2 = 77-92% for Zn, 88-98% for Cu and 31-93 for Cd).

2005 sampling: Topsoil NH_4NO_3 extractable Zn concentrations were higher at Woburn than the other two sites. At Woburn, the Zn-rich rate 3 treatment had NH_4NO_3 extractable Zn concentrations (16 mg/kg) which were *c.*2-fold higher than the concentrations at Rosemaund and 6-fold higher than the concentrations at Watlington (7.6 and 2.8 mg/kg, respectively). NH_4NO_3 extractable Cu concentrations on the rate 3 treatments at Woburn and Watlington (1.01 and 1.60 mg/kg, respectively) were *c.*2-fold higher than the concentration at Rosemaund (0.63 mg/kg). At Woburn, the Cd-rich rate 3 treatment had a NH_4NO_3 extractable Cd concentration (0.10 mg/kg) that was 1.5 to 3- fold higher than the concentrations at Watlington and Rosemaund (0.07 and 0.03 mg/kg, respectively).

At all three sites, there were strong relationships ($P < 0.001$) between total topsoil Zn, Cu and Cd concentrations and corresponding topsoil NH_4NO_3 extractable concentrations (r^2 = 73-76% for Zn, 74-93% for Cu and 70% for Cd). Note: For Zn at Watlington and Cd at Woburn and Watlington analytical problems were encountered with the NH_4NO_3 extracts.

Metal speciation

Total soil pore water Zn, Cu and Cd concentrations, and free ion Zn^{2+}, Cu^{2+} and Cd^{2+} concentrations were measured on soil samples taken from selected treatment plots (1, 2, 4, 7 and 10) in autumn 2004 (Tables 64-69).

Total soil solution Zn concentrations were greater ($P < 0.05$) on the Zn-rich rate 2 treatments at all sites than the untreated control and other liquid sludge amended treatments, with the highest concentration at

Woburn (*c.*0.45 mg/l). There were no differences ($P > 0.05$) in total solution Zn concentrations between the other metal-rich liquid sludge treatments and the untreated control or uncontaminated liquid sludge treatments at any of the sites (Table 64).

Table 64. Total Zn in soil solution (mg/l) extracted from soils sampled in autumn 2004

Treatment	WOB	WAT	ROS
Control	0.152	0.051	0.021
Unc. Digested.	0.130	0.053	0.019
Zn rate 2	0.451	0.126	0.102
Cu rate 2	0.055	0.055	0.017
Cd rate 2	0.108	0.053	0.019
ANOVA	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	0.101	0.013	0.020
CV%	20	7	20
n =	15	15	15
LoDe*	0	0	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

There were higher ($P < 0.05$) free solution Zn^{2+} concentrations on the Zn-rich rate 2 treatments at all sites (Table 65) compared with the untreated control and other liquid sludge treatments, with the highest concentration at Woburn (*c.*0.21 mg/l). There were no differences ($P > 0.05$) in free solution Zn^{2+} concentrations between the other metal-rich liquid sludge treatments and the untreated control or uncontaminated liquid sludge treatments at any of the sites.

Table 65. Free Zn^{2+} in soil solution (mg/l) extracted from soils sampled in autumn 2004

Treatment	WOB	WAT	ROS
Control	0.040	0.002	0.002
Unc. Digested	0.035	0.003	0.003
Zn rate 2	0.209	0.016	0.036
Cu rate 2	0.024	0.004	0.001
Cd rate 2	0.031	0.003	0.003
ANOVA	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	0.076	0.006	0.015
CV%	40	39	58
n =	15	15	15
LoDe*	0	0	1

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

Total soil solution Cu concentrations were higher ($P < 0.05$) on the Cu-rich rate 2 treatments at all three sites (Table 66) compared with the

untreated control and other liquid sludge treatments, with the highest concentration at Woburn (*c.*290 µg/l). There were no differences (P > 0.05) in total solution Cu concentrations between the other metal-amended liquid sludge treatments and the untreated control or uncontaminated liquid sludge treatments at any of the sites (Table 66).

Table 66. Total Cu in soil solution (µg/l) extracted from soils sampled in autumn 2004

Treatment	WOB	WAT	ROS
Control	22	10	11
Unc. Digested.	45	24	20
Zn rate 2	52	21	28
Cu rate 2	294	114	136
Cd rate 2	52	24	25
ANOVA	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	77	16	11
CV%	30	15	9
n =	15	15	15
LoDe*	0	0	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

There were higher (P < 0.05) free solution Cu^{2+} concentrations on the Cu-rich rate 2 treatments at all sites (Table 67) compared with the untreated control and other liquid sludge treatments, with the highest concentration at Woburn (*c.*0.0024 µg/l). There were no differences (P > 0.05) in free solution Cu^{2+} concentrations between the other metal-rich liquid sludge treatments and the untreated control or uncontaminated liquid sludge treatments at any of the sites.

Table 67. Free Cu^{2+} in soil solution (µg/l) extracted from soils sampled in autumn 2004

Treatment	WOB	WAT	ROS
Control	0.0001	< 0.0001	< 0.0001
Unc. Digested.	0.0001	< 0.0001	< 0.0001
Zn rate 2	0.0001	< 0.0001	< 0.0001
Cu rate 2	0.0024	0.0004	0.0008
Cd rate 2	0.0001	< 0.0001	< 0.0001
ANOVA	0.003	< 0.001	< 0.001
$LSD_{0.05}$	0.0010	0.0003	0.0005
CV%	5	1	2
n =	15	15	15
LoDe*	0	0	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

Total soil solution Cd concentrations were higher ($P < 0.05$) on the Cd-rich rate 2 treatments (Table 68) compared with the untreated control and other liquid sludge treatments, with the highest concentration at Woburn (*c.*1.8 µg/l). There were no differences ($P > 0.05$) in total solution Cd concentrations between the other metal-rich liquid sludge treatments and the untreated control or uncontaminated liquid sludge treatments at any of the sites.

Table 68. Total Cd in soil solution (µg/l) extracted from soils sampled in autumn 2004

Treatment	WOB	WAT	ROS
Control	0.037	0.016	0.042
Unc. Digested.	0.055	0.026	0.077
Zn rate 2	0.133	0.057	0.125
Cu rate 2	0.127	0.024	0.070
Cd rate 2	1.840	0.232	0.830
ANOVA	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	0.604	0.065	0.296
CV%	49	33	46
n =	15	15	15
LoDe*	0	0	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

There were higher ($P < 0.05$) free solution Cd^{2+} concentrations on the Cd-rich rate 2 treatments (Table 69) compared with the untreated control and other liquid sludge treatments, with the highest concentration at Woburn (*c.*0.48 µg/l). There were no differences ($P > 0.05$) in free solution Cd^{2+} concentrations between the other metal-rich liquid sludge treatments and the untreated control or uncontaminated liquid sludge treatments at any of the sites.

Table 69. Free Cd^{2+} in soil solution (µg/l) extracted from soils sampled in autumn 2004

Treatment	WOB	WAT	ROS
Control	0.004	0.001	0.005
Unc. Digested.	0.020	0.004	0.033
Zn rate 2	0.078	0.003	0.048
Cu rate 2	0.049	0.004	0.042
Cd rate 2	0.481	0.071	0.222
ANOVA	< 0.001	< 0.001	< 0.001
$LSD_{0.05}$	0.201	0.015	0.069
CV%	56	32	35
n =	15	15	15
LoDe*	2	4	0

*LoDe = number of results at/or below detection limit (if > 5 ANOVA test not carried out)

Soil nutrients

The nutrient status (*i.e.* total N, extractable P, K, Mg) and pH of soil samples taken in spring 2005 are presented in full in Appendix M. Changes in soil nutrient levels (mean across all sites) as a result of the liquid sludge additions are summarised in Table 70.

Table 70. Mean change in topsoil nutrient status as a result of liquid sludge additions relative to the untreated control treatments at the three sites - spring 2005

Treatment	Ext. P (mg/l)	Ext. K (mg/l)	Ext. Mg (mg/l)	Organic C (%)	Total N (%)
Unc. Digested	20	- 33	- 18	0.19	0.05
Zn-rich	17	- 25	- 27	0.16	0.04
Cu-rich	20	- 22	- 21	0.18	0.04
Cd-rich	21	- 22	- 18	0.14	0.04

Soil extractable (Olsen) P concentrations were overall higher (17 to 21 mg P/l) and extractable K and Mg concentrations were lower (-22 to -33 mg K/l and -18 to -27 mg Mg/l) than the untreated controls.

Soil organic C levels in the liquid sludge treatments were on average *c.*0.17% higher than the untreated controls. The difference of 0.17% (between the untreated control and liquid sludge treatments) was lower than in the sludge cake experiments (*c.*0.8%), which reflects the lower organic matter additions (*c.*17 t C/ha) in the liquid sludge experiments compared with the sludge cake experiments (*c.*60 t C/ha).

Soil total N was on average 0.04% higher on the metal-amended liquid sludge treatments – an increase equivalent to 1100 kg N/ha (assuming a bulk density of 1.1 [average bulk density across liquid sludge treatments] and a topsoil depth of 25 cm), compared with the untreated controls.

4.3.3 Soil physical properties

The soil bulk density measurements taken in spring 2005 are presented in Table 71. At the three sites, soil bulk density on the liquid sludge treatments was *c.*0.08 g/cm^3 lower (range 0.02 to 0.15 g/cm^3) than the untreated controls. The differences in soil bulk density between the untreated control and liquid sludge treatments were smaller than the 'paired' sludge cake sites (mean = 0.17 g/cm^3), which is a reflection of the lower organic matter additions to the liquid sludge experiments.

Table 71. Soil bulk density determinations made at each site in spring 2005

Treatment	WOB	WAT	ROS
Control	1.39	1.34	1.18
SE	0.048	0.029	0.018
Unc. Digested	1.24	1.35	1.11
SE	0.020	0.015	0.018
Zinc-rich	1.26	1.30	1.14
SE	0.027	0.029	0.009
Copper-rich	1.27	1.33	1.12
SE	0.010	0.017	0.013
Cadmium-rich	1.22	1.29	1.12
SE	0.022	0.032	0.031
Mean change†	- 0.15	- 0.02	- 0.06

† Mean change = mean of sludge treatments [22,24,27,30] minus untreated control [21]. SE = standard error

4.3.4 Grain and grass yields and metal concentrations

Ryegrass (2002 and 2004) and grain yields (2003 and 2005) plus metal concentrations in the harvested grass/grain dry matter were measured at the three sites. The mean crop yields (Tables 72-75) and metal concentrations in the harvested dry matter are presented in Appendices Q - T.

2002 harvest: At Woburn, grass dry matter yields were lower ($P < 0.05$; 1.0-2.9 t/ha) on the untreated control than all the liquid sludge treatments. There were no differences ($P > 0.05$) in grass dry matter yields between the untreated control and the liquid sludge treatments at Watlington and Rosemaund in 2002 (Table 72). On the metal dose-response treatments, regression of grass yields against total topsoil metal concentrations showed no significant relationships ($P > 0.05$) at any of the three sites.

Table 72. Mean first cut grass yields (t dm/ha) at each site (May-June 2002)

Treatment	WOB	WAT	ROS
Control	3.96	5.30	7.58
Unc. Digested	5.25	5.42	8.48
Zn rate 1	5.36	4.77	8.19
Zn rate 2	5.97	4.79	7.00
Zn rate 3	4.62	4.74	7.83
Cu rate 1	5.57	5.62	8.18
Cu rate 2	5.56	5.90	8.12
Cu rate 3	6.86	5.49	8.55
Cd rate 1	5.28	4.60	7.87
Cd rate 2	4.98	5.09	8.12
Cd rate 3	5.04	4.97	8.45
ANOVA (p)	< 0.001	NS	NS
$LSD_{0.05}$	1.337	-	-
CV%	9	10	11

2003 harvest: At Watlington, grain yields were lower ($P < 0.05$) on the Zn-rich rate 3 treatment than the uncontaminated digested liquid sludge treatment (*c.*1.8 t/ha). At Woburn, grain yields on the Cu-rich rate 3 treatment were higher ($P < 0.05$) than the untreated control (*c.*0.8 t/ha). At Rosemaund, there were no grain yield differences ($P > 0.05$) between any of the treatments (Table 73). On the metal dose-response treatments, regression of grain yields against total topsoil Zn and Cu concentrations identified significant ($P < 0.05$) inverse relationships at Watlington, and a significant positive relationship for Cu at Woburn.

Relationships between grain metal concentrations and total topsoil metal concentrations at each of the sites are presented in Figures 91 to 93. Generally, there were moderate to strong relationships ($P < 0.01$) between grain Zn (r^2 = 62 to 93%), Cu (r^2 = 26 to 83%) and Cd (r^2 = 87 to 98%) concentrations and total topsoil metal concentrations at the three sites. Although, at Watlington there was no relationship ($P > 0.05$) between grain and total topsoil Cu concentrations.

There were similar relationships between grain metal concentrations and topsoil NH_4NO_3 extractable metal concentrations (data not shown).

Table 73. Mean grain yields (t/ha, 85% dm basis) at each site in 2003

Treatment	WOB	WAT	ROS
Control	0.54	1.52	1.12
Unc. Digested	0.70	2.81	1.59
Zn rate 1	0.95	1.67	1.06
Zn rate 2	1.24	1.44	0.58
Zn rate 3	0.97	1.06	0.68
Cu rate 1	0.86	2.39	1.13
Cu rate 2	0.95	2.24	0.67
Cu rate 3	1.30	1.60	1.79
Cd rate 1	0.96	2.48	1.19
Cd rate 2	0.92	2.62	0.91
Cd rate 3	0.70	2.12	2.15
ANOVA (p)	0.041	0.013	NS
$LSD_{0.05}$	0.611	1.378	-
CV%	27	28	53

2004 harvest: There were no differences ($P > 0.05$) in grass yields between any of the liquid sludge treatments and the untreated controls at any of the three sites (Table 74). On the metal dose-response treatments, regression of grass yields against total topsoil metal concentrations showed no significant relationships ($P > 0.05$) at any of the three sites.

Figure 91. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Woburn metal-amended liquid sludge (harvest 2003)

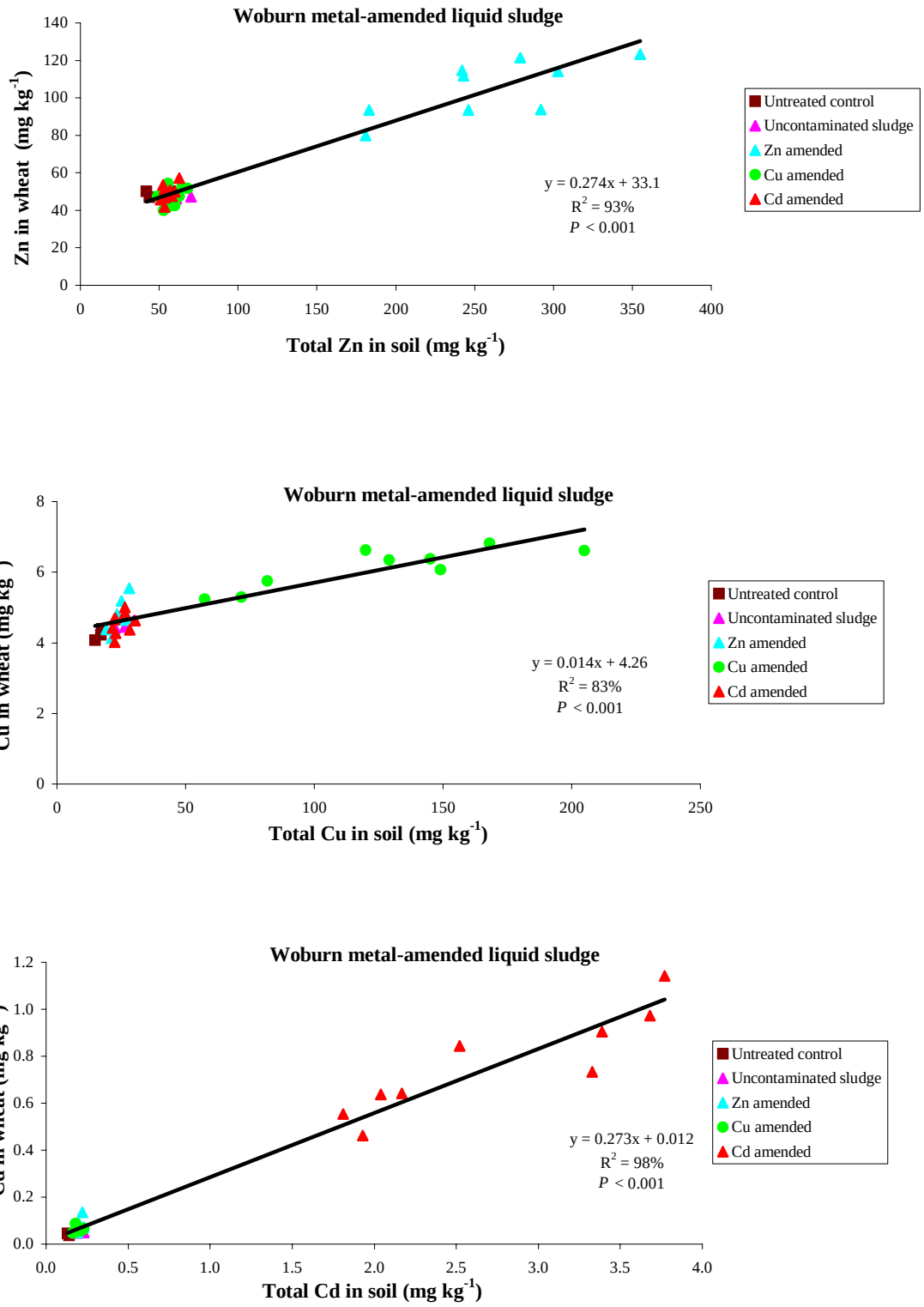
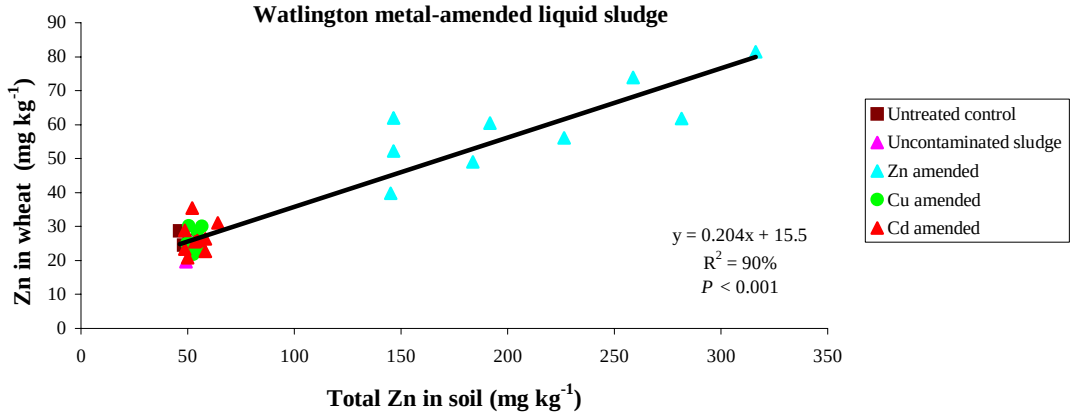
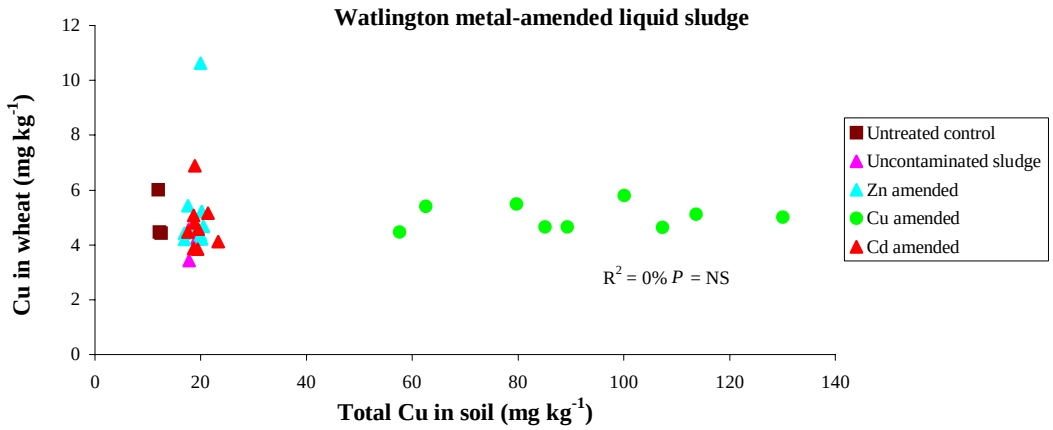


Figure 92. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Watlington metal-amended liquid sludge (harvest 2003)





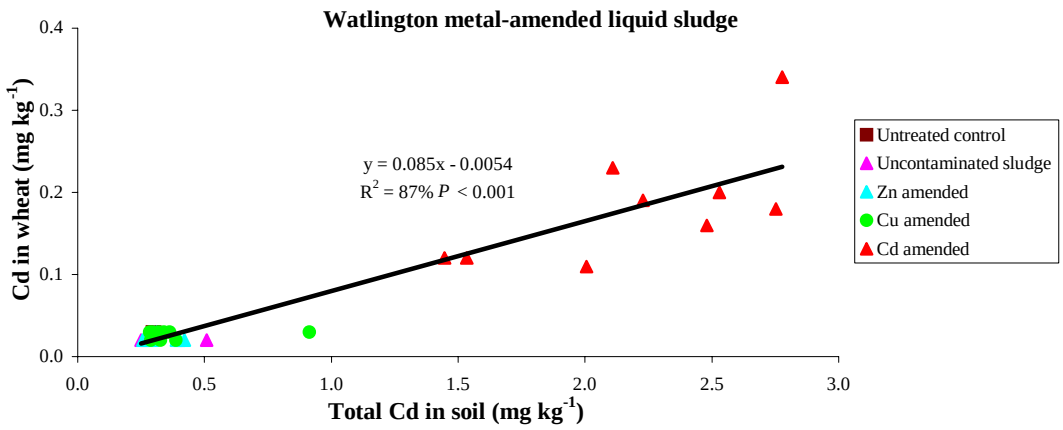
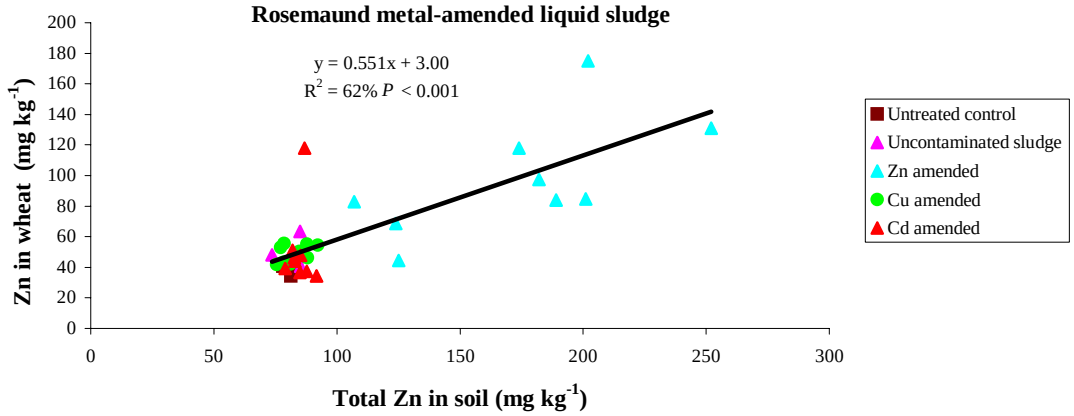
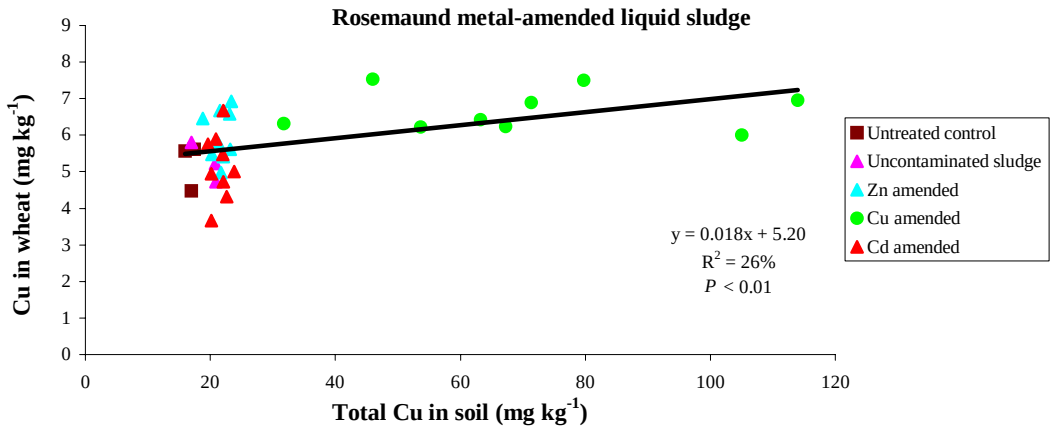


Figure 93. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Rosemaund metal-amended liquid sludge (harvest 2003)





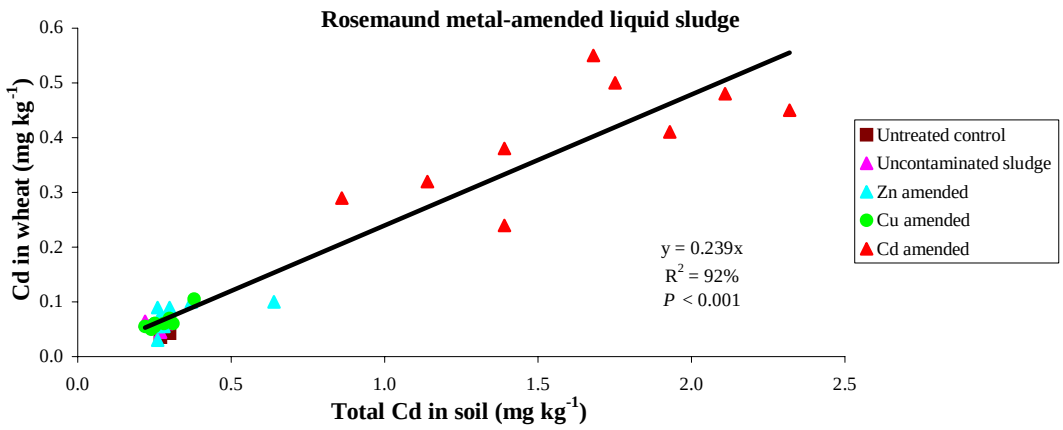


Table 74. Mean first cut grass yields (t dm/ha) at each site (May-June 2004)

Treatment	WOB	WAT	ROS
Control	3.41	1.58	3.41
Unc. Digested	2.99	2.15	2.99
Zn rate 1	4.19	2.10	4.19
Zn rate 2	2.79	2.06	2.79
Zn rate 3	3.83	1.92	3.83
Cu rate 1	3.71	1.75	3.71
Cu rate 2	4.18	1.66	4.18
Cu rate 3	3.37	2.24	3.37
Cd rate 1	2.97	2.11	2.97
Cd rate 2	3.74	2.21	3.74
Cd rate 3	4.40	2.44	4.40
ANOVA (p)	NS	*NS*	*NS*
$LSD_{0.05}$	-	-	-
CV%	24	17	24

2005 harvest: At Rosemaund, there were no yield differences ($P > 0.05$) between any of the treatments (Table 75). At Woburn and Watlington, because of crop establishment difficulties there was insufficient grain in order to determine an accurate crop yield. At Rosemaund on the liquid sludge metal dose-response treatments, regression of grain yields against total topsoil metals concentrations identified no significant ($P > 0.05$) relationships.

Table 75. Mean grain yields (t/ha, 85% dm basis) at each site in 2005

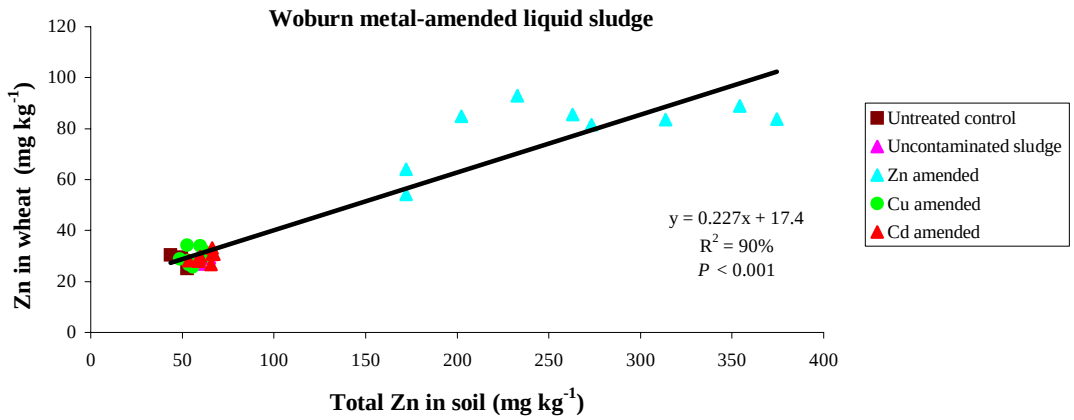
Treatment	WOB*	WAT *	ROS
Control	-	-	1.74
Unc. Digested	-	-	2.06
Zn rate 1	-	-	1.52
Zn rate 2	-	-	1.16
Zn rate 3	-	-	1.21
Cu rate 1	-	-	2.15
Cu rate 2	-	-	1.57
Cu rate 3	-	-	1.55
Cd rate 1	-	-	1.85
Cd rate 2	-	-	2.23
Cd rate 3	-	-	1.97
ANOVA (p)	-	-	NS
$LSD_{0.05}$	-	-	-
CV%	-	-	39

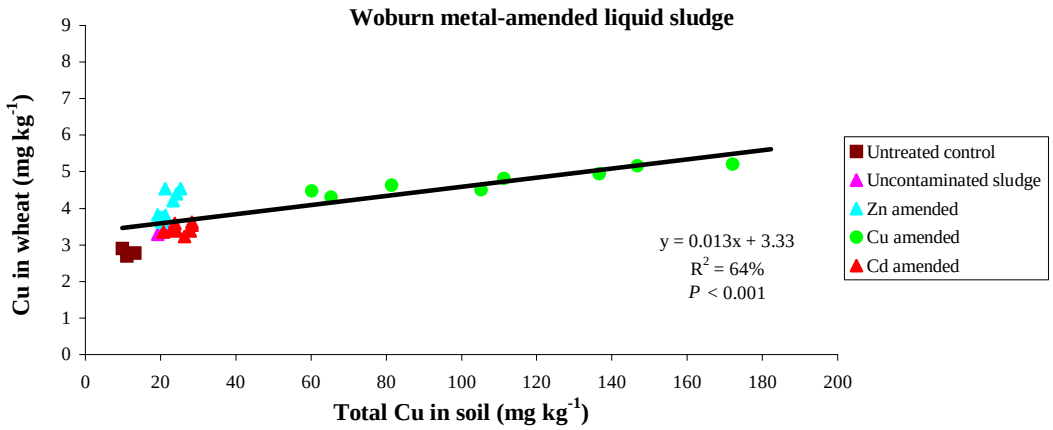
* Grain yields were too low to make a robust measurement, although at Woburn there was a sufficient sample size to measure metal concentrations in the grain.

The relationships between grain metal concentrations and total topsoil metal concentrations at Woburn and Rosemaund are presented in Figures 94 and 95. There were strong relationships ($P < 0.001$) between grain Zn (r^2 = 82 to 90%), Cu (r^2 = 64%) and Cd (r^2 = 89 to 96%) concentrations and total topsoil metal concentrations at both sites. Although, at Rosemaund there was no relationship ($P > 0.05$) between grain and soil Cu concentrations.

There were similar relationships between grain metal concentrations and topsoil NH_4NO_3 extractable metal concentrations (data not shown).

Figure 94. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Woburn metal-amended liquid sludge (harvest 2005)





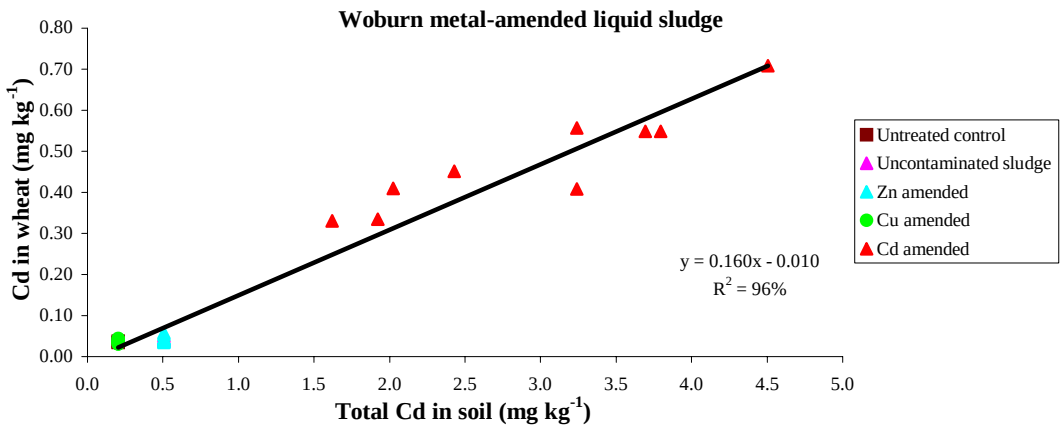
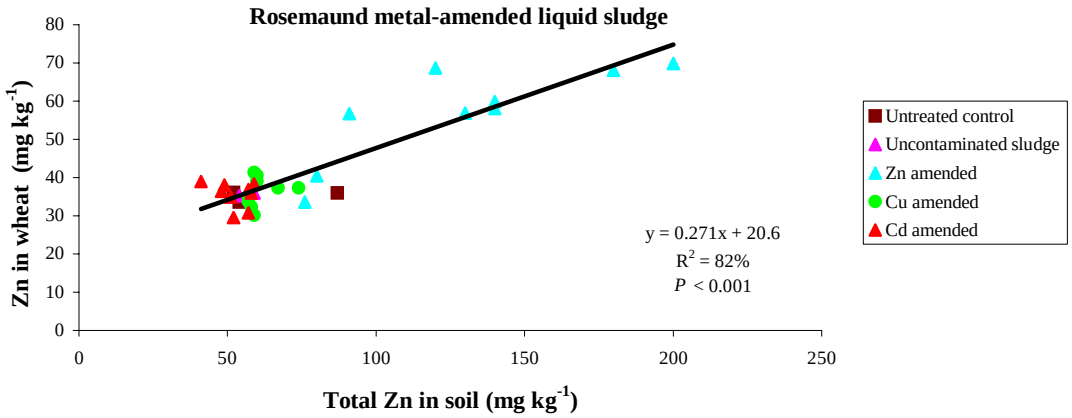
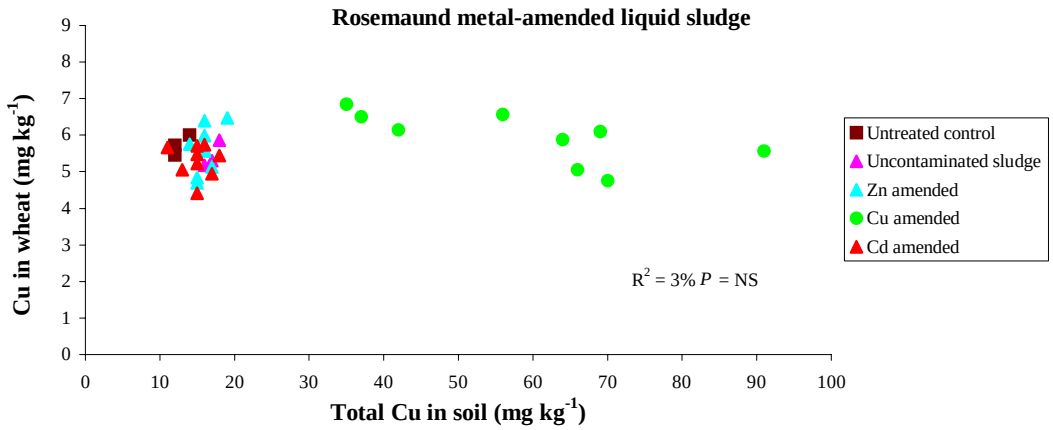
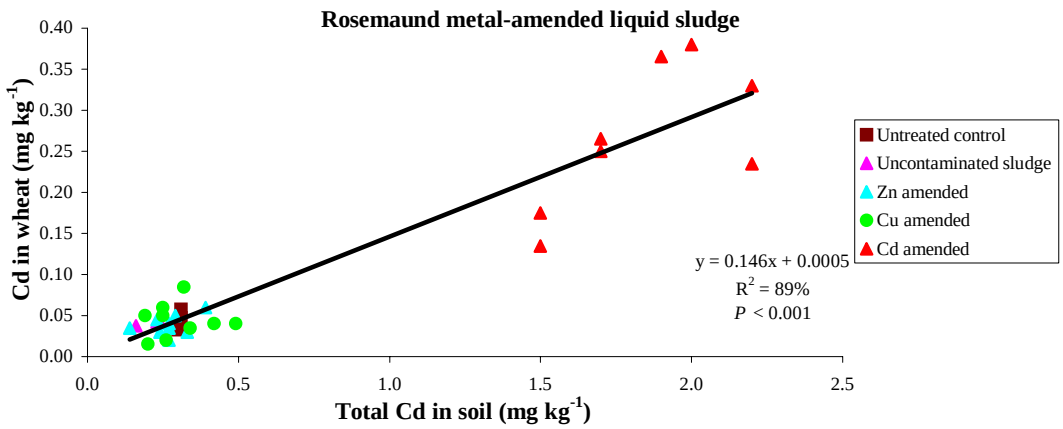


Figure 95. Relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations - Rosemaund metal-amended liquid sludge (harvest 2005)







5. DISCUSSION

5.1 Sludge cake experiments

Soil respiration rates

Soil respiration rates measured at all nine sites in spring 2003 and 2005 were generally higher on the treatments receiving sludge cakes compared with the untreated controls. Higher respiration rates in the sludge amended plots indicate that the applied sludge cakes had stimulated soil respiration. As increased soil respiration rates were measured in both sampling years (2003 and 2005), this indicates that the sludge cake added had continued to slowly degrade throughout Phase III.

On the metal dose-response treatments, there were significant ($P < 0.05$) inverse relationships between soil respiration rates and total topsoil Zn concentrations at Hartwood in both 2003 and 2005, and for Cd at Woburn and Hartwood in 2003 *i.e.* soil respiration rates decreased with increasing Zn and Cd concentrations. For Cu, there were significant ($P < 0.05$) positive relationships between soil respiration rates and total topsoil Cu at Watlington and Auchincruive in both 2003 and 2005, and at Rosemaund in 2003 *i.e.* soil respiration rates increased with increasing Cu concentrations.

During Phase II (measurements made on soil samples in 1999 and 2001) there were significant positive relationships between soil respiration rates and total topsoil Zn at Watlington and Rosemaund in 1999, for Cu at four sites (Watlington and Rosemaund in 1999 and Pwllpeiran and Auchincruive in 2001) and for Cd at Shirburn in 1999. Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) it was difficult to see a clear and consistent effect of the metal additions on soil respiration rates.

Soil microbial biomass carbon

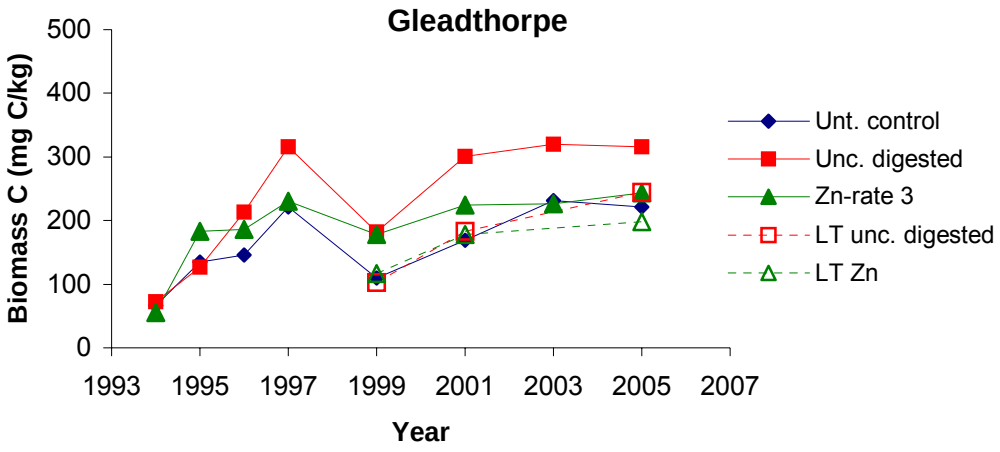
Soil microbial biomass C concentrations measured at all nine sites in spring 2003 and 2005 were generally higher on the treatments receiving sludge cakes compared with the untreated controls. The increases in soil microbial biomass carbon concentrations were related to the organic matter additions made via the sludge cake applications during Phase I, as cultivation practices and crop yields (and associated carbon returns to the soil) were similar on all treatment plots.

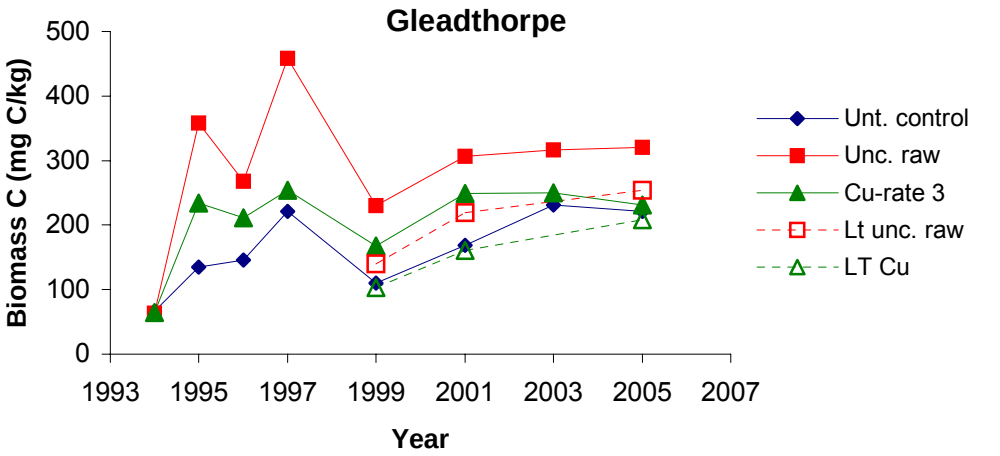
On the metal dose-response treatments, there were significant ($P < 0.05$) inverse relationships between soil biomass C concentrations and total topsoil Zn concentrations at four sites in both 2003 and 2005 (Woburn, Watlington, Rosemaund and Bridgets), at Hartwood and Auchincruive in 2003, and Shirburn in 2005. Also, there were significant ($P < 0.05$) inverse relationships between soil biomass C concentrations and total topsoil Cu concentrations at three sites in both 2003 and 2005 (Woburn, Watlington and Hartwood), and at Gleadthorpe and Rosemaund in 2005. For Cd, there were no consistent effects on biomass concentrations.

During Phase II there were significant inverse relationships ($P < 0.05$) between soil biomass C concentrations and total Zn at Bridgets in 1999 and at Watlington and Rosemaund in 2001; for Cu at Gleadthorpe, Woburn and Rosemaund in 1999 and Woburn, Watlington, Rosemaund and Bridgets in 2001; and for Cd at Gleadthorpe in 2001. At all sites where there was a relationship between soil biomass C and total topsoil metals, the relationship was *inverse i.e.* biomass levels decreased with increasing metal concentrations.

Soil microbial biomass C concentrations were measured on selected treatments (untreated control, uncontaminated digested and raw sludge cake treatments, Zn-rate 3, Cu-rate 3 and Cd-rate 3) at each sampling event, since the start of "The Long-term Sludge Experiments" in 1994 (8 sampling events). The rate 3 metal-rich treatments were selected as these represented the treatments closest to the current statutory soil heavy metal limits. Additionally, on the long-term build-up treatments, biomass C concentrations were measured on three occasions during Phases II and III of the project (1999, 2001 and 2005). Biomass C concentrations over time on the selected Zn, Cu and Cd dose-response treatments are illustrated in Figures 96 – 104.

Figure 96. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Gleadthorpe sludge cake





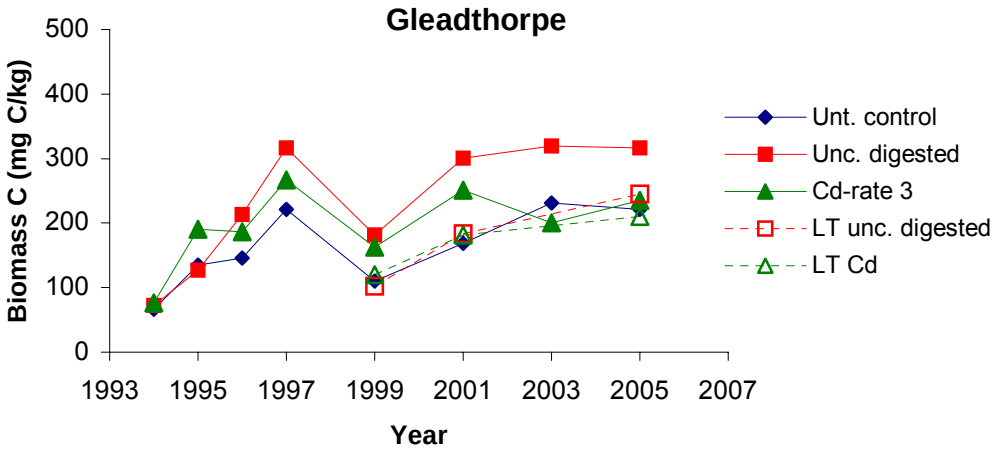
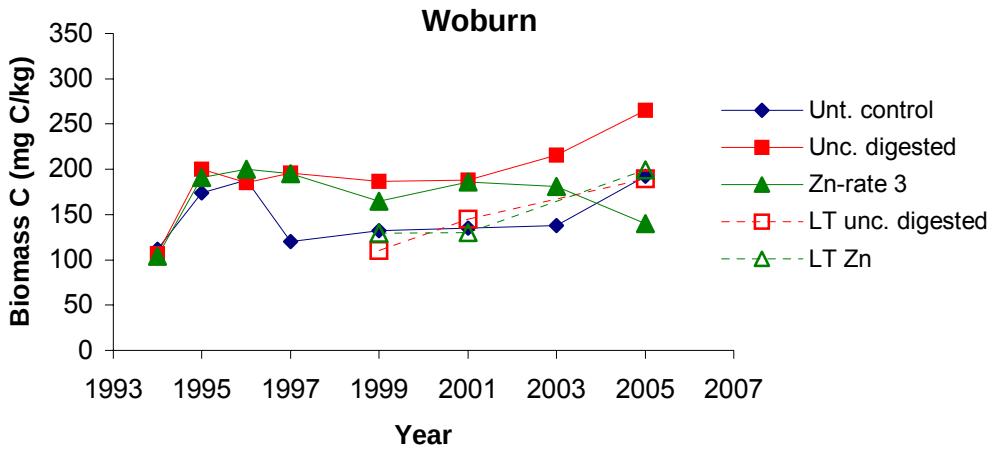
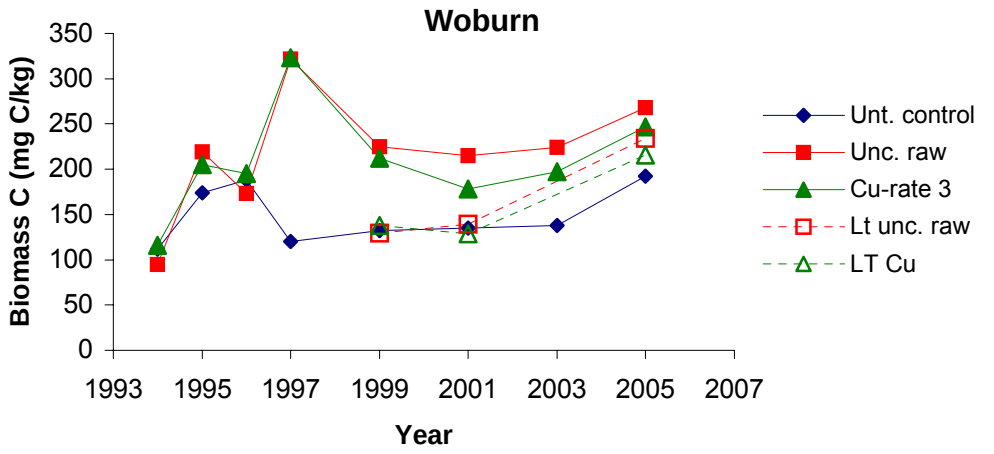


Figure 97. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Woburn sludge cake





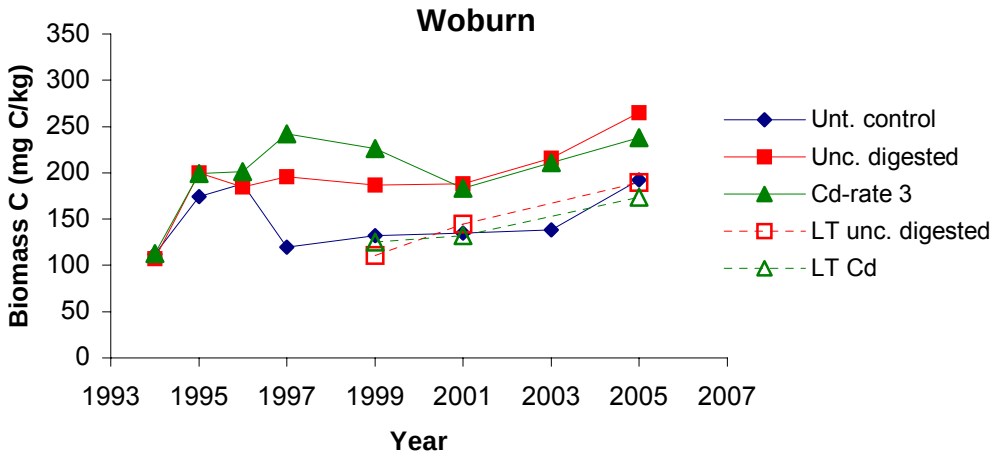


Figure 98. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Watlington sludge cake

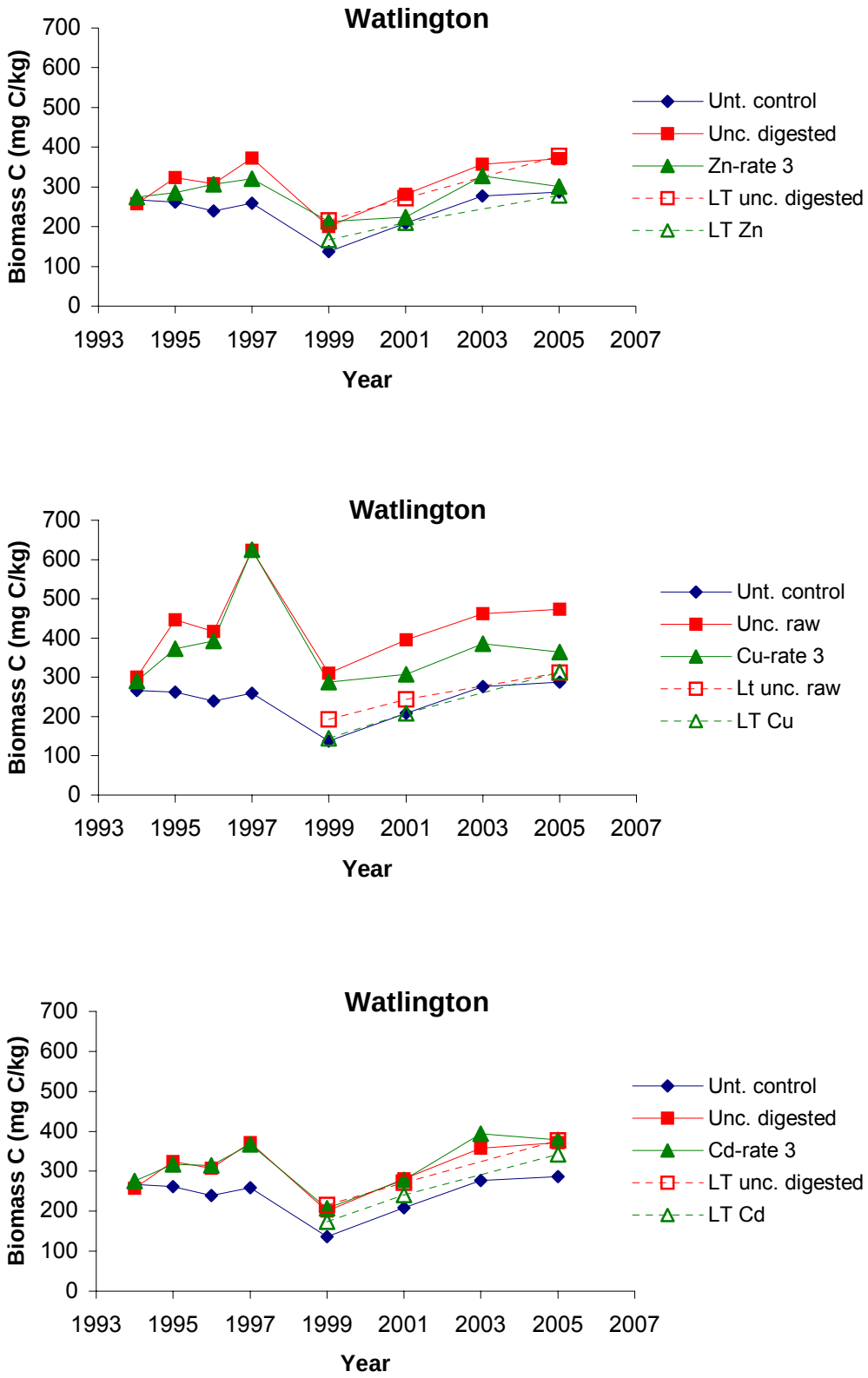
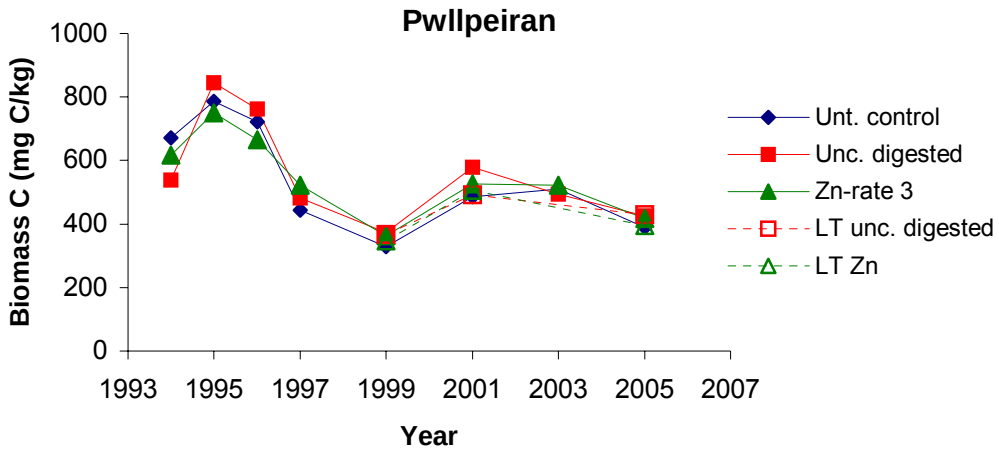
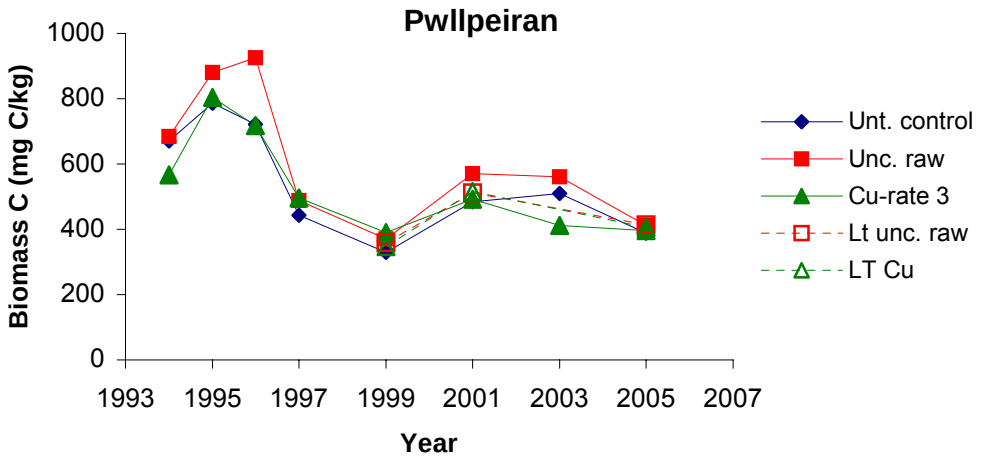


Figure 99. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Pwllpeiran sludge cake





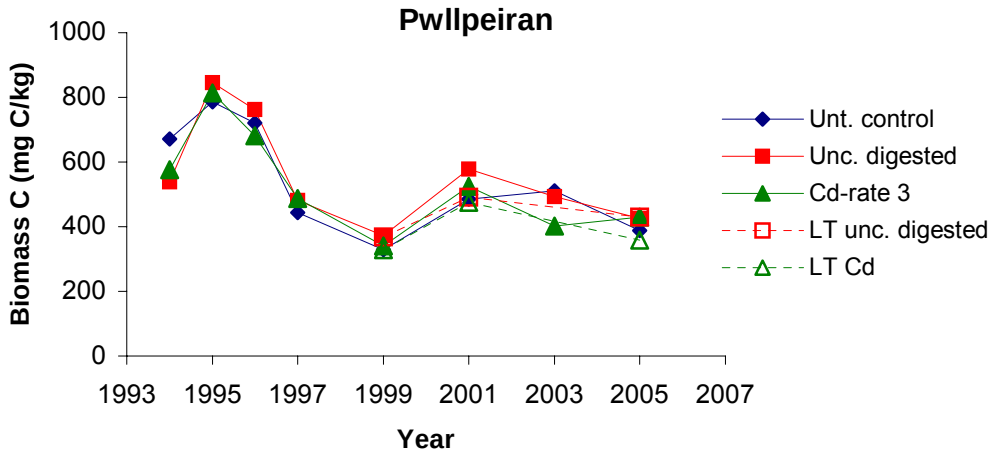
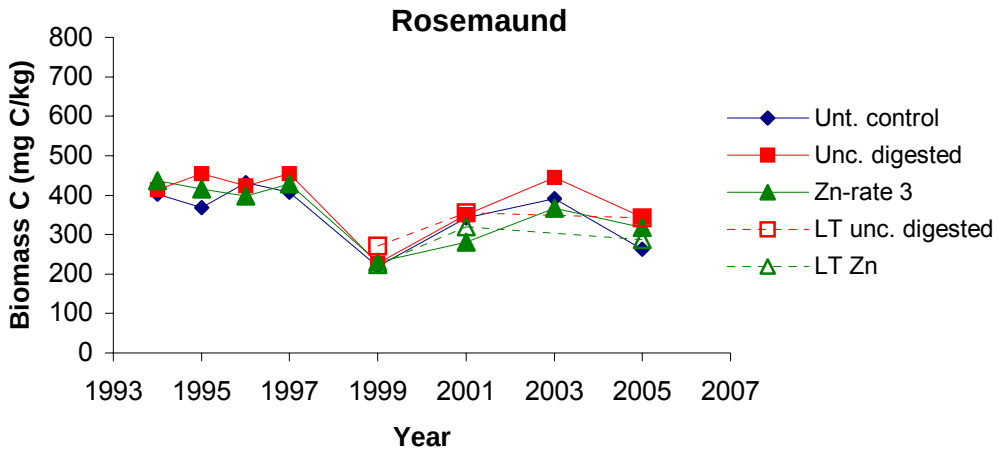
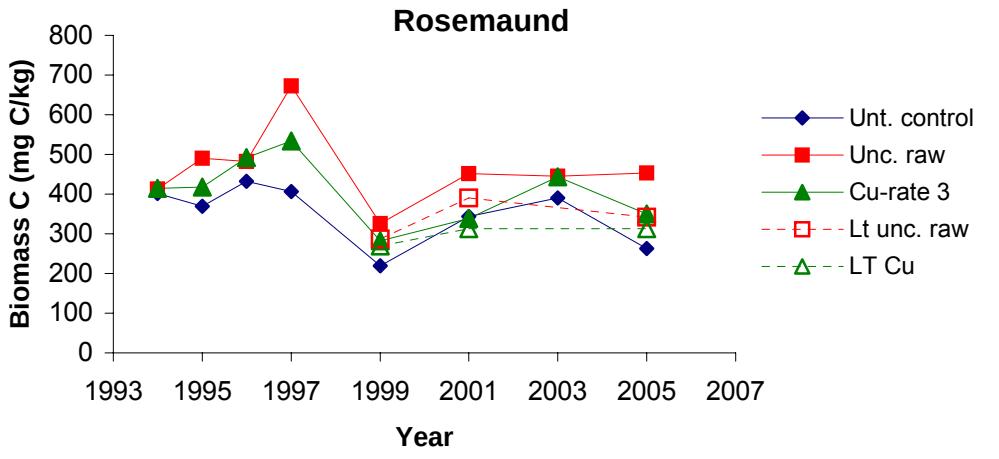


Figure 100. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Rosemaund sludge cake





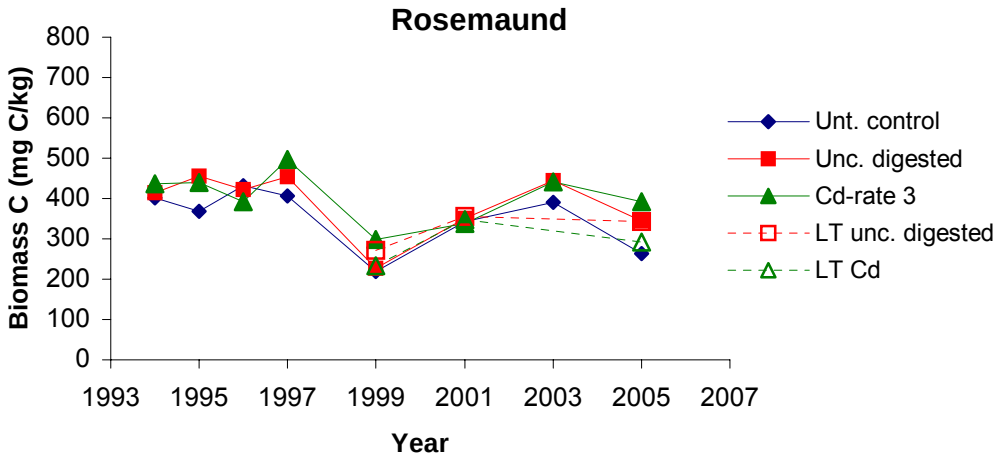
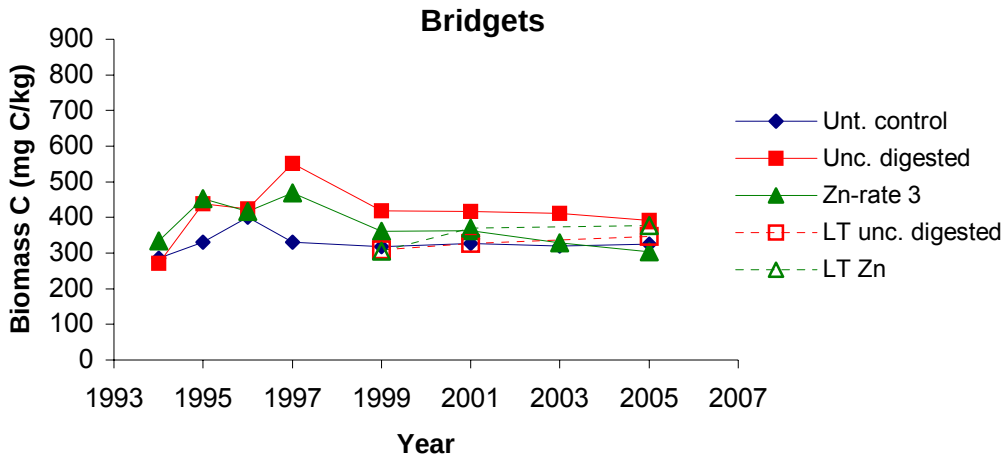
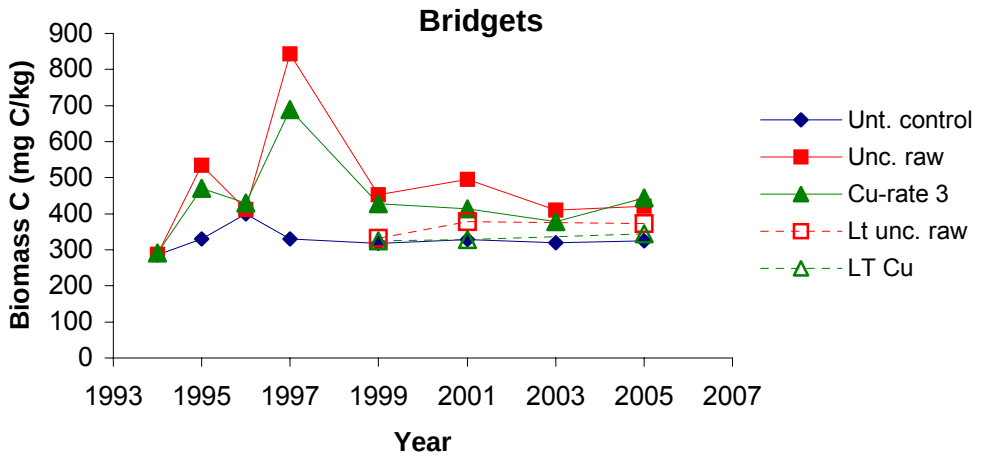


Figure 101. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Bridgets sludge cake





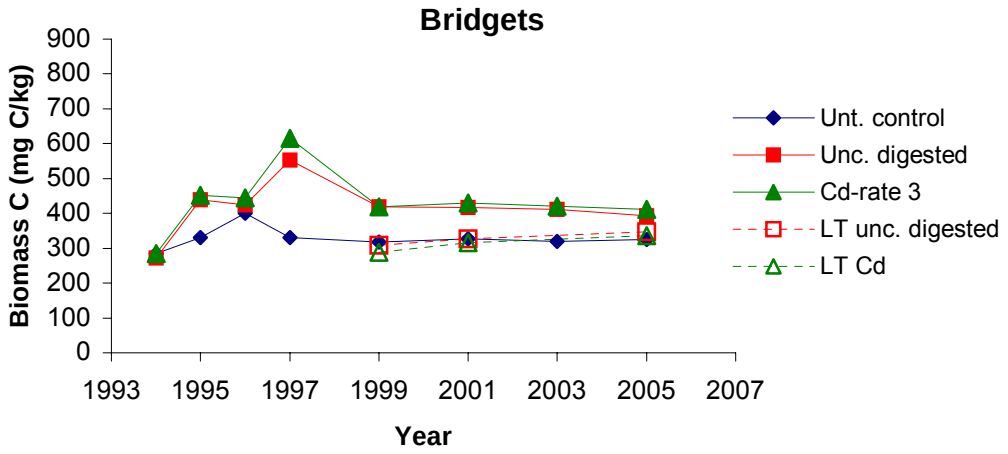


Figure 102. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Hartwood sludge cake

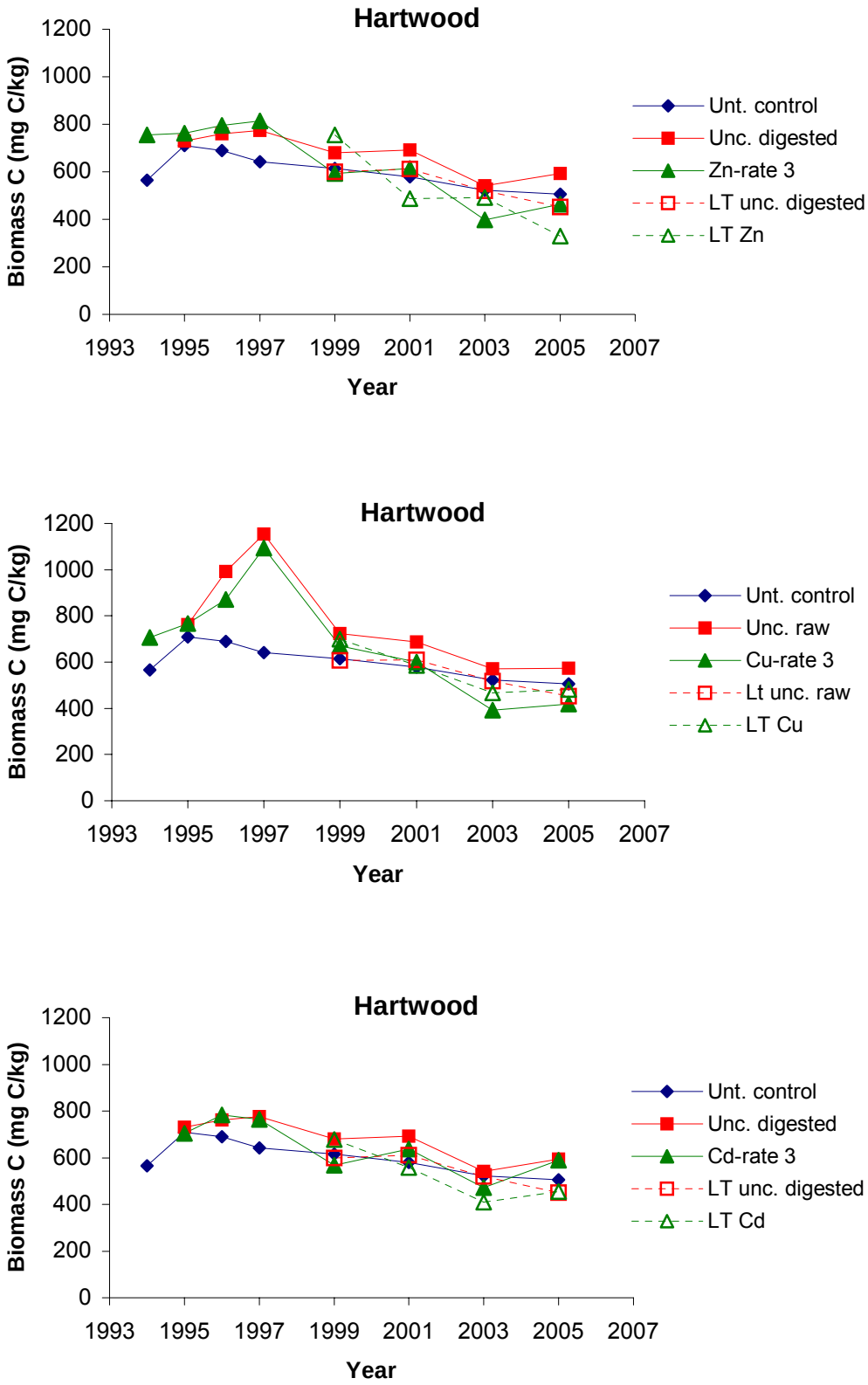
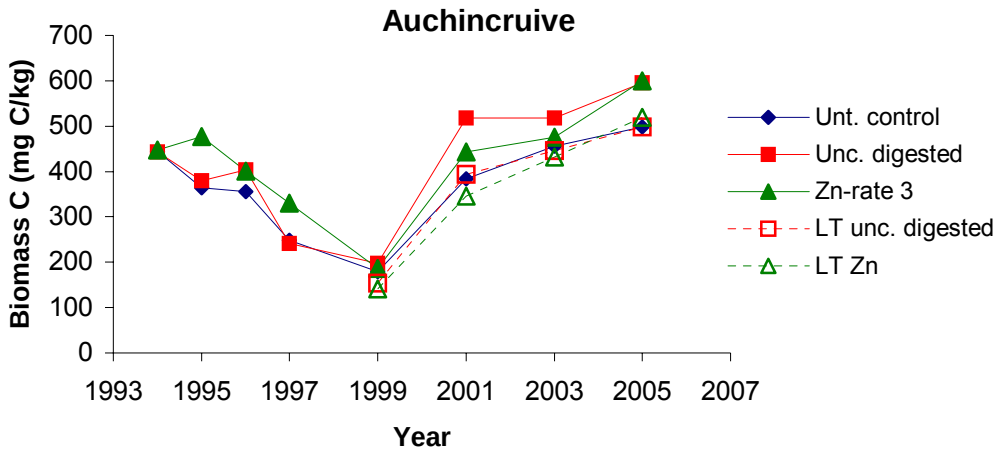
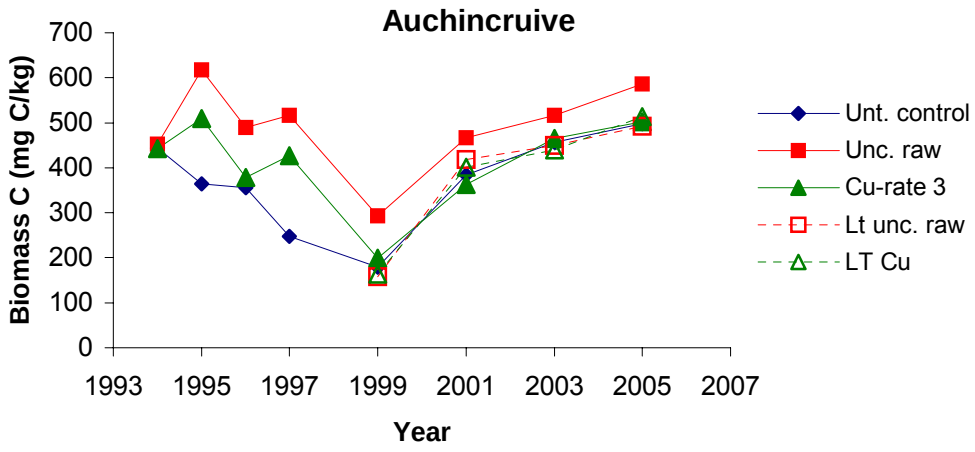


Figure 103. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Auchincruive sludge cake





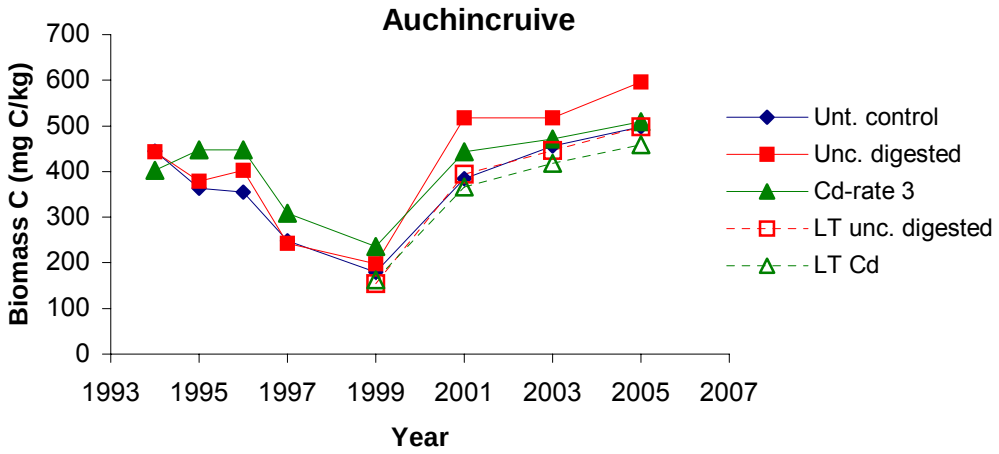
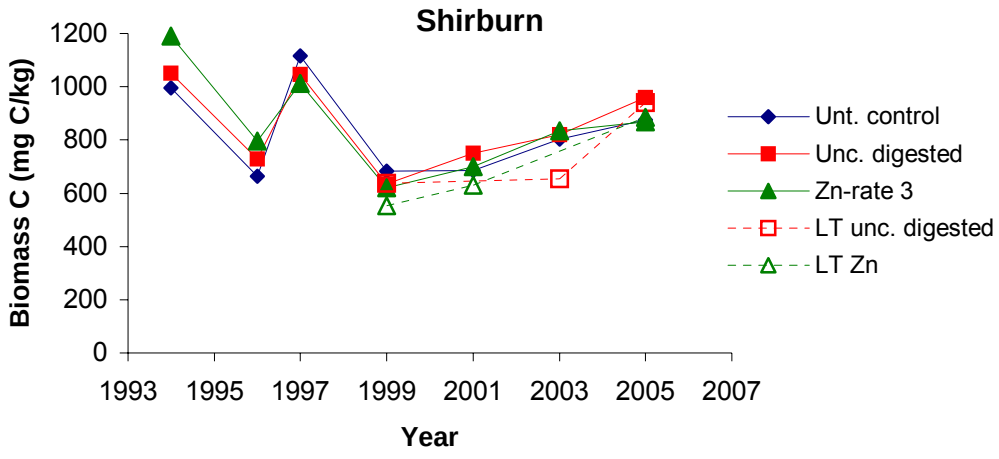
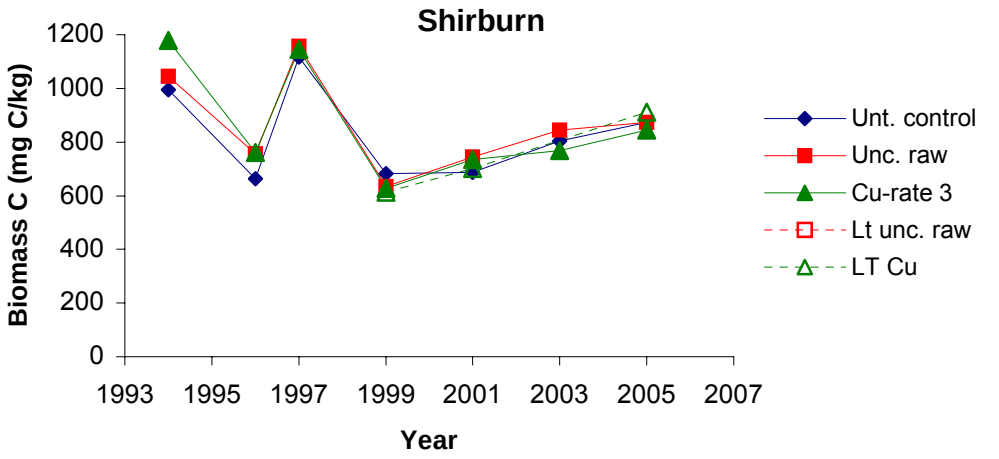
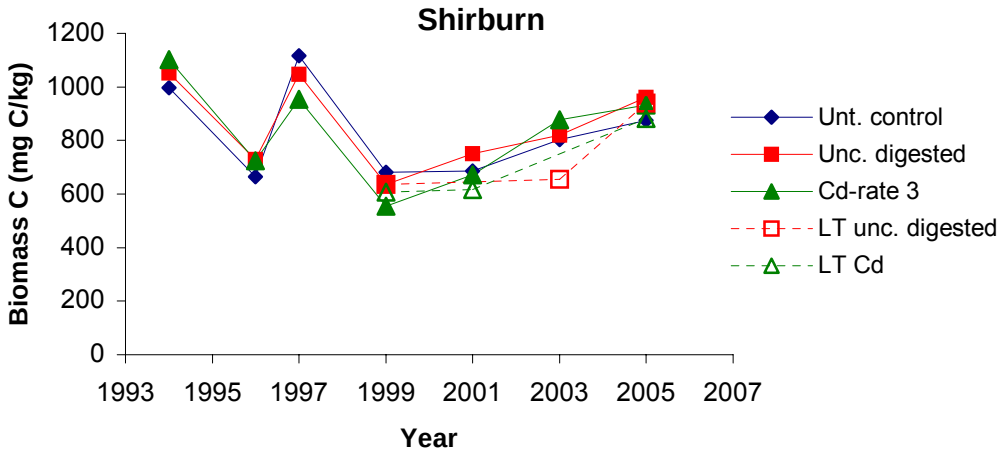


Figure 104. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Shirburn sludge cake







At all nine sites, there was variation over time in biomass C concentrations, as demonstrated by the microbial biomass size on the untreated control treatments. However, at Gleadthorpe, Woburn, Watlington, Rosemaund, Bridgets and Hartwood biomass C concentrations on the Zn and Cu rich sludge cake treatments showed consistent trends to be smaller than on the 'paired' uncontaminated sludge cake treatment. Also, there was some indication of a trend for smaller microbial biomass C concentrations on the long-term metal rich sludge cake treatments compared with the uncontaminated sludge cake treatments at Gleadthorpe, Rosemaund and Hartwood, although clear and consistent differences are difficult to discern to date (Figures 96-104).

The residual sludge organic matter at the end of Phase III is largely composed of slowly degradable carbon, with its degradation during subsequent years likely to have a smaller effect on soil microbial biomass C concentrations. Microbial assimilation of C during subsequent seasons, in the absence of readily degradable sludge cake organic matter additions, will largely be dependent upon the inputs of organic matter from plant growth (*e.g.* plant debris, root turnover and root exudates). Hence, during subsequent seasons sludge degradation effects should have a smaller influence on soil microbial biomass C concentrations.

Soil organic carbon

Soil organic C levels in spring 2003 and 2005 were *higher* on all the treatments receiving sludge cakes compared with the untreated controls, reflecting the sludge cake organic matter additions during Phase I.

On the metal dose-response treatments, regression of the plot soil organic C contents against total topsoil metal concentrations identified significant positive relationships ($P < 0.05$) on two Cu dose-response treatments in 2003 (Watlington and Auchincruive), and one Cd dose-response treatment at Woburn (in 2005). No relationships were found on the other 51 metal dose-response treatments over both sampling years.

These results indicate that there have not been any major impacts on overall soil organic C degradation rates on the metal dose-response treatments. Therefore, differences in C degradation rates between the different sludge types that were used to create the metal dose-response treatments can largely be discounted as having a major impact on the measured soil microbial properties *i.e.* respiration rates, biomass C concentrations and rhizobia MPNs. However, it is recognised that the measurement of total soil organic C can be a relatively insensitive indicator of changes in soil organic C degradation rates.

Respiration Quotient

In order to compare results from individual plots having different soil microbial biomass concentrations and soil respiration rates, the plot data were expressed as a Respiration Quotient – RQ:

$$RQ = \frac{Soil\ respiration\ rate\ (mgCO_2 - C / kg\ oven\ dry\ soil / h)}{Soil\ microbial\ biomass\ C\ \ (mgC / kg\ oven\ dry\ soil)} \times 100$$

On the metal dose-response treatments, there were no significant (P > 0.05) relationships between RQ and total topsoil Zn at any of the sites in 2003, but there were significant (P < 0.05) positive relationships at five sites (Woburn, Watlington, Rosemaund, Bridgets and Shirburn) in 2005. During Phase II, there were significant (P < 0.05) positive relationships between RQ and total topsoil Zn at both Watlington and Rosemaund in 1999 and 2001, and at Gleadthorpe, Woburn and Bridgets in 2001. Therefore, over the course of four sampling events, there have been significant *positive* relationships between RQ and total topsoil Zn at two sites (Watlington and Rosemaund) in three (1999, 2001 and 2005) out of the four sampling years.

For Cu, there were significant (P < 0.05) positive relationships between RQ and total topsoil Cu at three of the sites in both 2003 and 2005 (Watlington, Hartwood and Auchincruive), at Rosemaund and Bridgets in 2003, and Woburn and Shirburn in 2005. During Phase II, there were significant positive relationships (P < 0.05) between RQ and total topsoil Cu at all sites in 1999 and 2001 (except Pwllpeiran and Hartwood in 1999 and 2001, and Shirburn in 2001). Therefore, over the course of four sampling events, there have been significant *positive* relationships between RQ and total topsoil Cu at two sites (Watlington and Auchincruive) in all four of the sampling years, and significant relationship at three sites (Woburn, Rosemaund and Bridgets) in three out of the four sampling years.

For Cd, there was a significant (P < 0.05) inverse relationship between RQ and total topsoil Cd at Woburn and a positive relationship at Pwllpeiran in 2003. In 2005, there were significant (P < 0.05) positive relationships between RQ and total topsoil Cd at Gleadthorpe and Shirburn. During Phase II, there was a significant positive relationship (P < 0.05) between RQ and total topsoil Cd at Gleadthorpe in 2001. Therefore, at Gleadthorpe over the course of four sampling events, there were significant positive relationships between RQ and total topsoil Cd on two occasions.

It is noteworthy that the regression line slopes (between RQ and total topsoil metal concentrations) were generally lower in 2003 and 2005, than they were in 1999 and 2001, which maybe due to seasonal effects or an indication that microbial respiration rates are becoming more stable as carbon degradation slows.

The significant relationships (P < 0.05) between RQ and total topsoil metal concentrations were all *positive* (with one exception for Cd at Woburn) *i.e.* respiration rate per unit of biomass increased with increasing soil metal concentrations throughout Phases II and III.

Biomass Quotient

In order to compare results from individual plots having slightly differing soil organic C contents and microbial biomass C concentrations, the plot data were expressed as a Biomass Quotient - BQ:

$$BQ = \frac{Soil\ microbial\ biomass\ (mgC/kg\ oven\ dry\ soil)}{Soil\ organic\ C\ (mg\ C/kg\ oven\ dry\ soil)} \times 100$$

On the metal dose-response treatments, there were significant (P < 0.05) inverse relationships between BQ and total topsoil Zn at Hartwood in both sampling years, at Watlington, Rosemaund and Auchincruive in 2003, and Woburn, Bridgets and Shirburn in 2005. During Phase II, there were significant (P < 0.05) inverse relationships between BQ and total topsoil Zn at Pwllpeiran and Bridgets in 1999, and at Gleadthorpe, Watlington and Hartwood in 2001. Therefore, over the course of the four sampling events, there have been significant inverse relationships between BQ and total topsoil Zn at Hartwood in three (2001, 2003 and 2005) out of the four sampling years.

For Cu, there were significant inverse relationships (P < 0.05) between BQ and total topsoil Cu at three sites in both 2003 and 2005 (Woburn, Watlington and Rosemaund), at Gleadthorpe, Hartwood and Auchincruive in 2003, and Shirburn in 2005. During Phase II, there were significant (P < 0.05) inverse relationships between BQ and total topsoil Cu at Gleadthorpe and Rosemaund in both 1999 and 2001, at Woburn and Auchincruive in 1999, and at Watlington, Bridgets, Hartwood and Shirburn in 2001. Therefore, over the course of the four sampling events, there have been significant *inverse* relationships between BQ and total topsoil Cu at Rosemaund in all four sampling years, and at Gleadthorpe (1999, 2001 and 2003), Woburn (1999, 2003 and 2005) and Watlington (2001, 2003 and 2005) in three out of the four sampling years.

For Cd, there were no significant relationships (P > 0.05) between BQ and total topsoil Cd at any of the nine sites in either sampling year (2003 and 2005) during Phase III. During Phase II, there were significant inverse relationships between BQ and total topsoil Cd at Gleadthorpe in 1999 and Pwllpeiran in 2001. Overall, there have been no consistent effects of Cd on BQ.

Where significant relationships (P < 0.05) between BQ and total topsoil metals were present, they were all *inverse*, *i.e.* biomass carbon as a proportion of total soil organic C decreased with increasing total topsoil metal concentrations. The mechanisms of metal tolerance invariably carry an additional energy cost to the soil microbial population (Silver *et al.,* 1989; Metre and Winge, 1991; Ross, 1993; Cervantes, 1994). Such an extra energy demand can result in a decrease in the amount of substrate available for growth. The diversion of energy from growth to cell maintenance is therefore a plausible explanation for a decrease in BQ with increasing topsoil metal concentrations.

Rhizobium most probable numbers

Rhizobium populations measured in soil samples taken in spring 2003 and 2005 were generally similar on the treatments that had received uncontaminated sludge cakes compared with untreated controls.

On the metal dose-response treatments, there were significant inverse relationships (P < 0.05) between rhizobia MPNs and total topsoil Zn

concentrations at six sites in both 2003 and 2005 (Gleadthorpe, Woburn, Watlington, Rosemaund, Bridgets and Hartwood), and at Pwllpeiran and Auchincruive in 2005.

For Cu, there were significant ($P < 0.05$) inverse relationships between rhizobia MPNs and total topsoil Cu concentrations at Auchincruive in 2003, and at Pwllpeiran, Rosemaund, Bridgets and Hartwood in 2005, and a positive relationship at Shirburn in 2005.

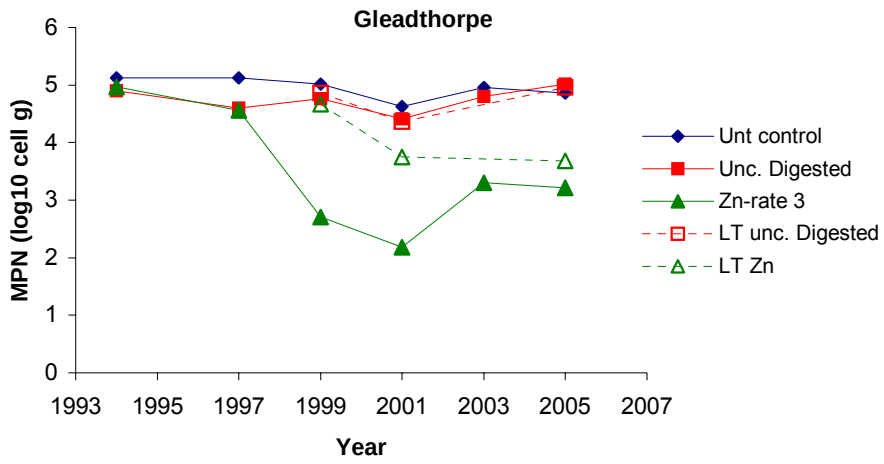
For Cd, there were significant ($P < 0.05$) inverse relationships between rhizobia MPNs and total topsoil Cd at two sites (Rosemaund and Shirburn) in 2003, and at four sites (Gleadthorpe, Woburn, Pwllpeiran and Hartwood) in 2005.

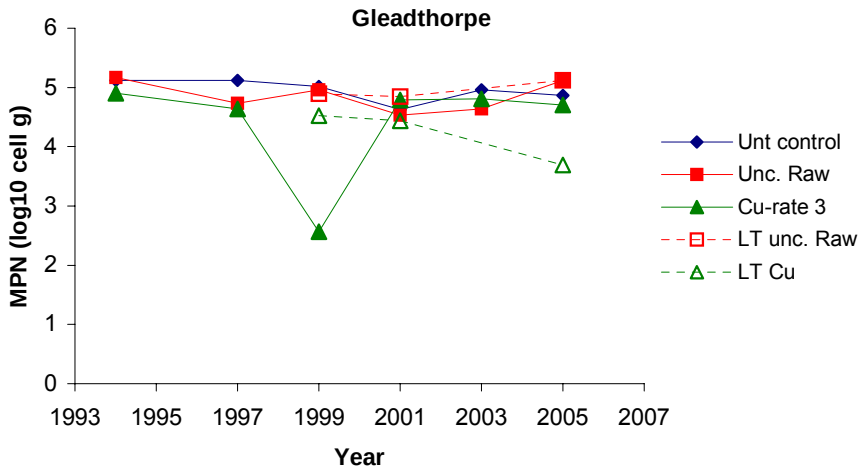
The significant relationships ($P < 0.05$) between rhizobia MPNs and total topsoil metal concentrations were all *inverse* (with the exception of Cu at Shirburn in 2005) *i.e.* rhizobia MPNs decreased with increasing total topsoil metal concentrations.

Rhizobium population sizes in soil samples taken from all treatment plots in 1999, 2001, 2003 and 2005 were generally *similar* on the uncontaminated sludge cake, Cu dose-response, Cd dose-response and untreated control treatments. However, on the Zn dose-response treatments, there were significant decreases ($P < 0.05$) in rhizobia MPNs with increasing total topsoil Zn concentrations at three sites (Gleadthorpe, Watlington and Hartwood) in all four measurement years, and at eight of the sites in 2005 (Table 76).

On the Zn dose-response treatments at eight of the sludge cake experimental sites, there was a trend of decreasing rhizobia MPNs with time. These trends are illustrated in Figures 105 – 113. At the Hartwood site, rhizobia MPNs were not detectable on the Zn dose-response treatment above a total topsoil Zn concentration of 301 mg/kg in 2003 and above a concentration of 188 mg/kg in 2005. In addition, at both Gleadthorpe and Hartwood on the long-term (LT) treatments, there appear to be possible emerging downward trends in rhizobia population sizes.

Figure 105. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Gleadthorpe sludge cake





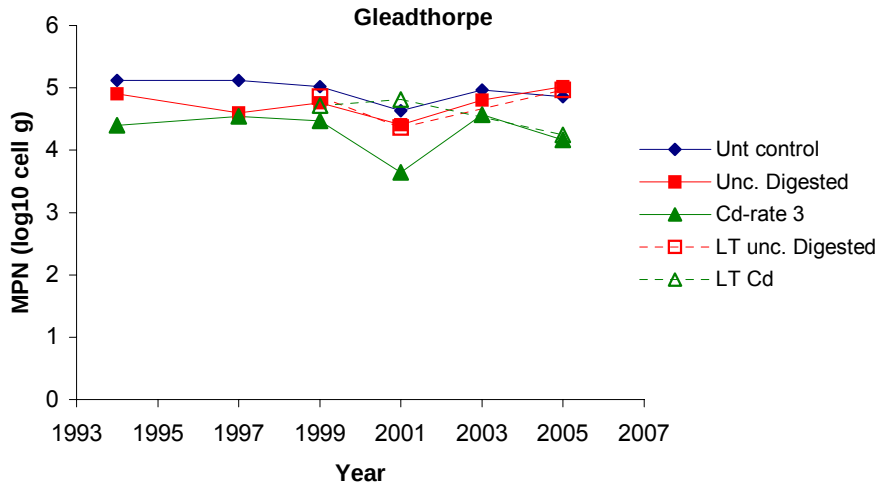
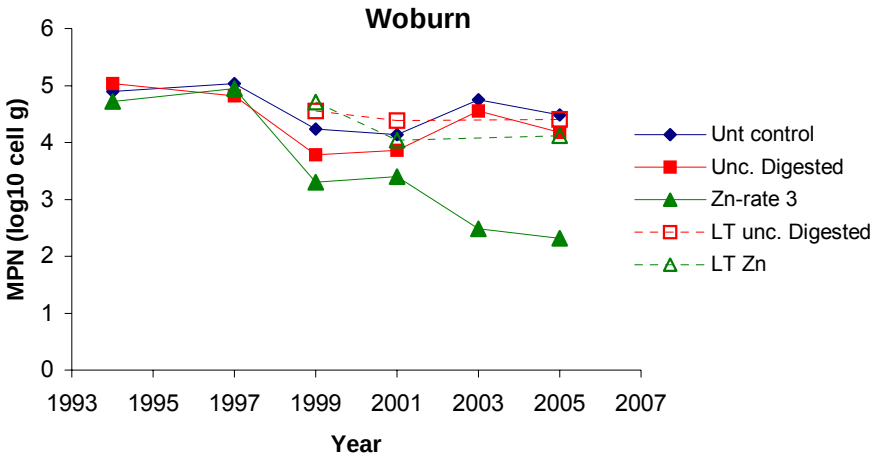
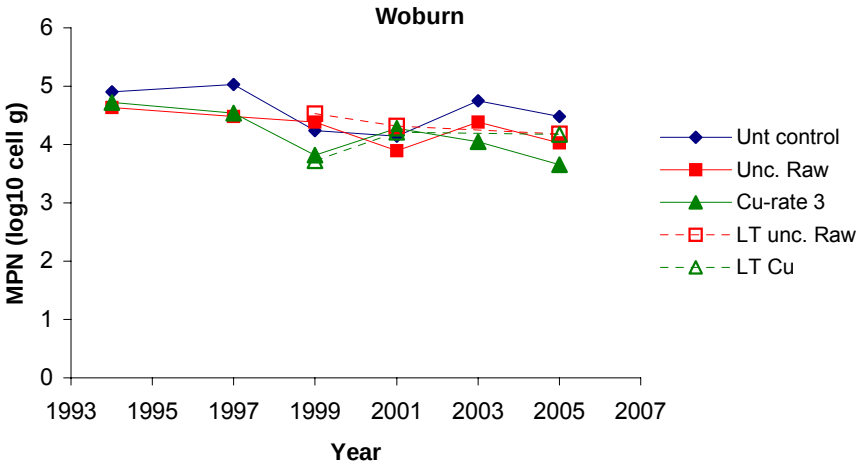


Figure 106. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Woburn sludge cake





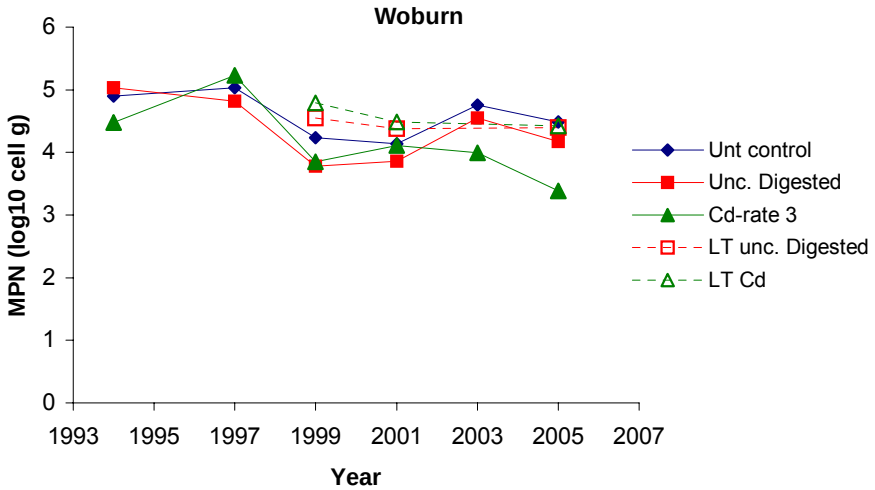
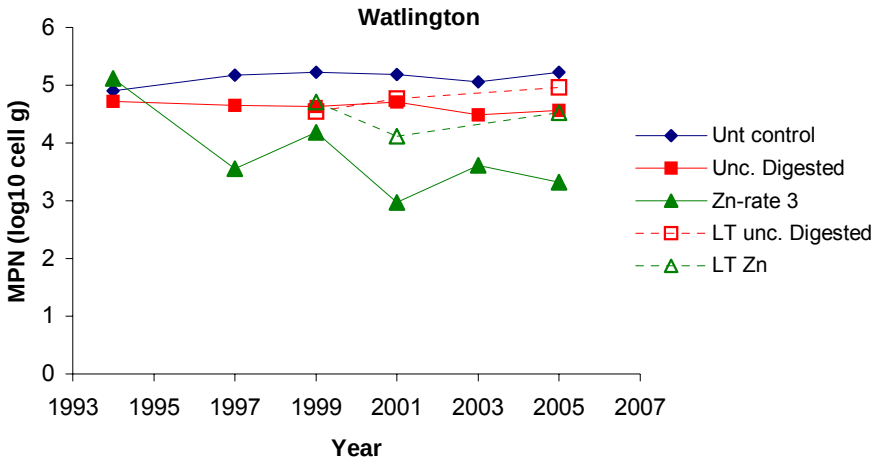
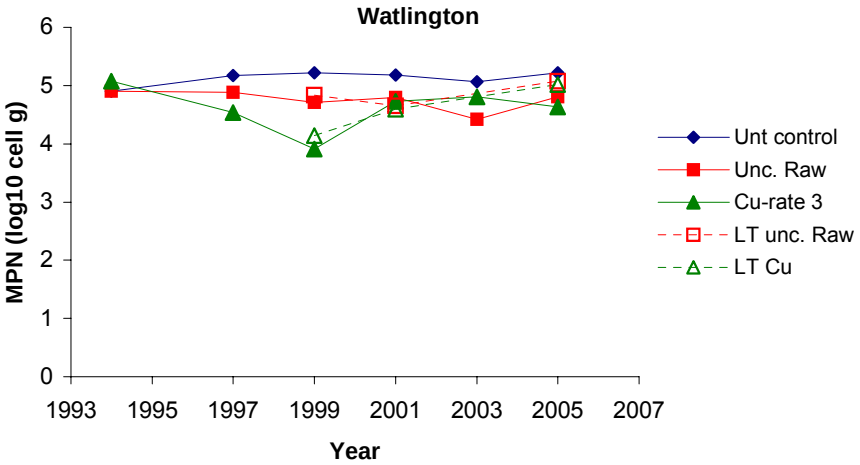


Figure 107. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Watlington sludge cake





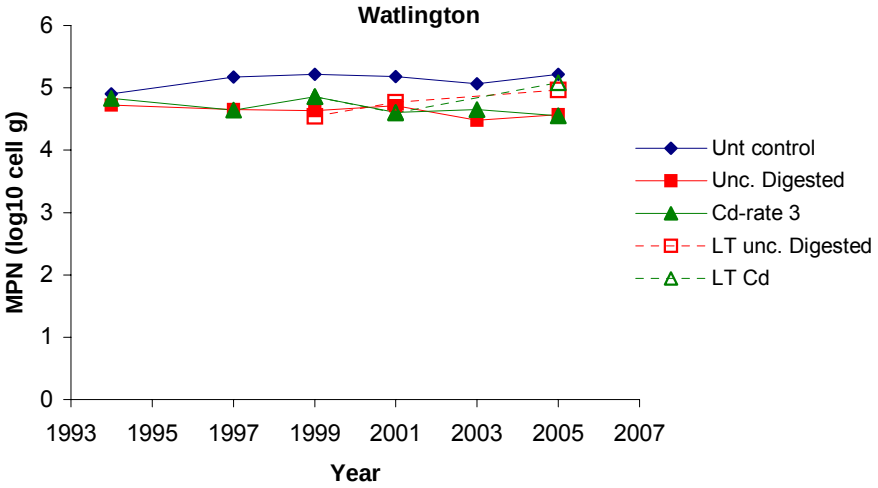
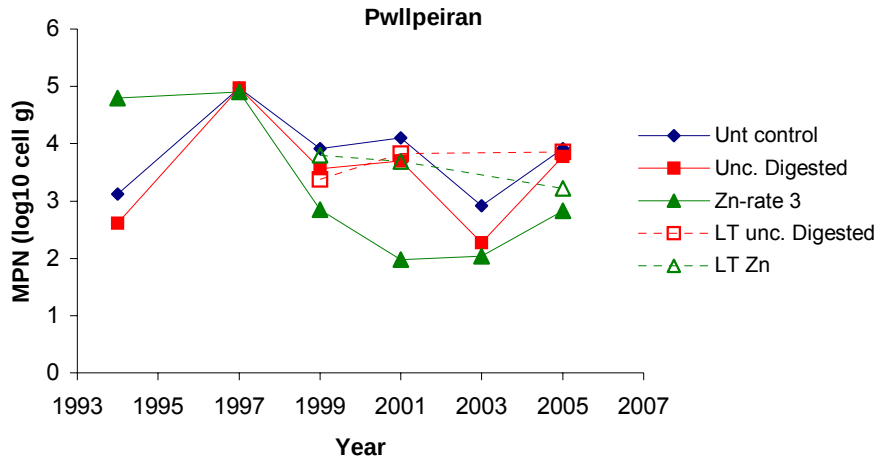
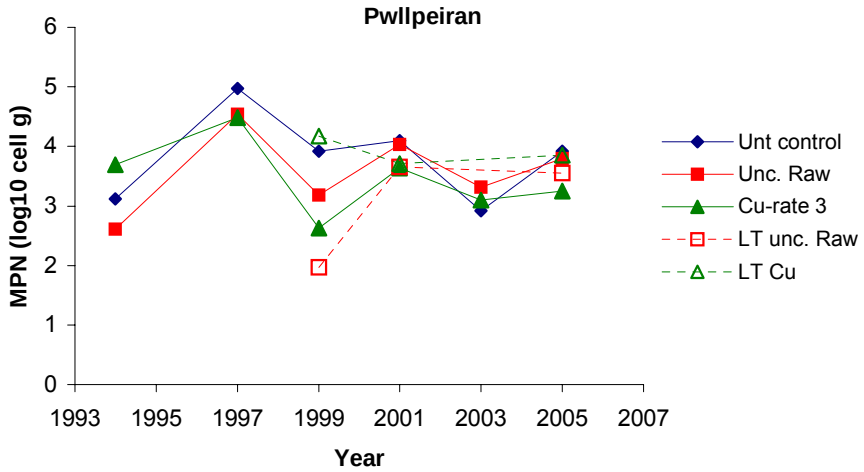


Figure 108. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Pwllpeiran sludge cake





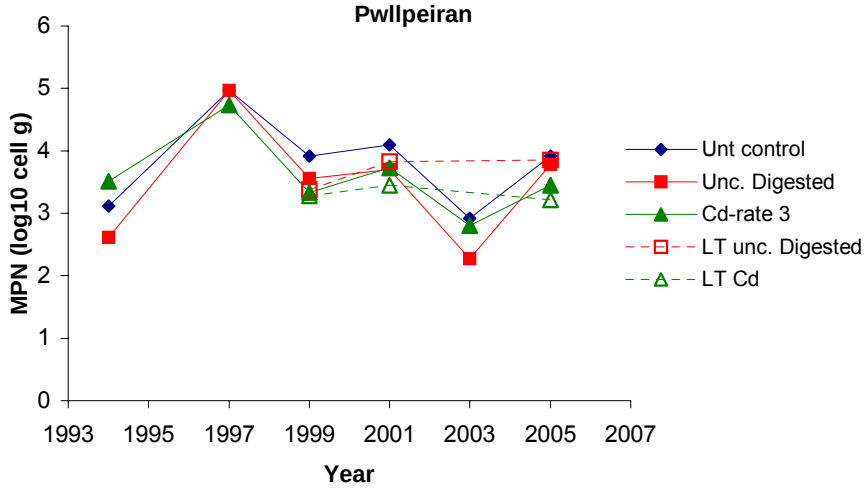
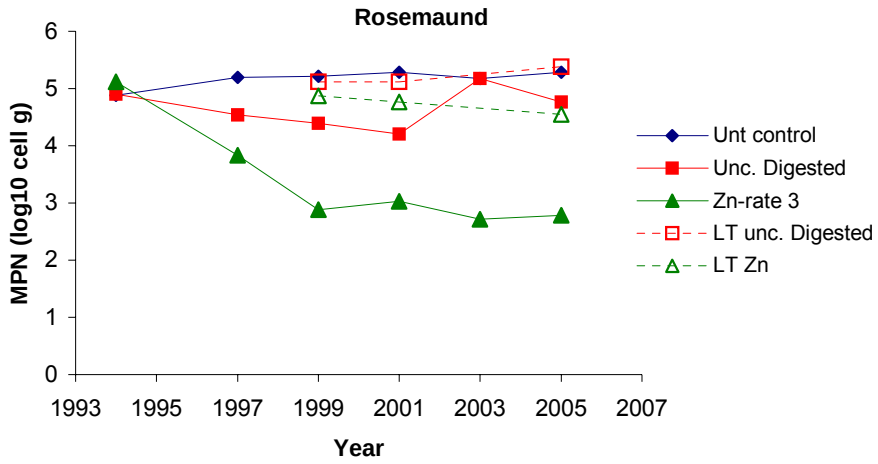
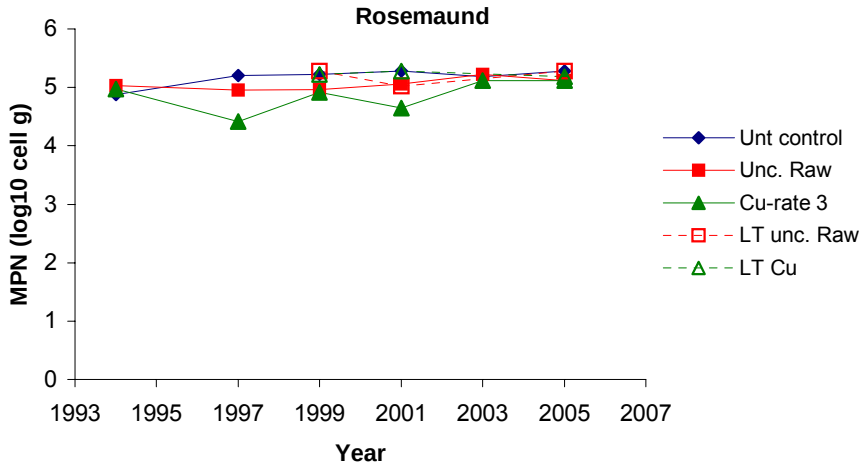


Figure 109. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Rosemaund sludge cake





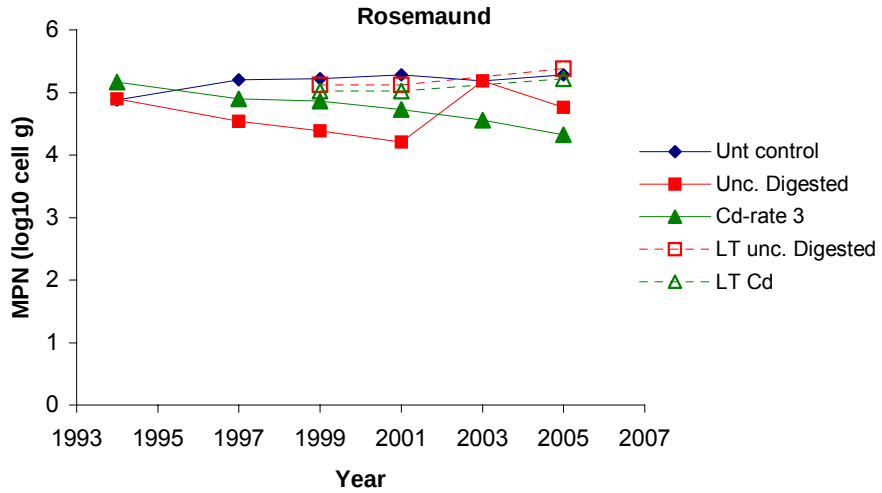
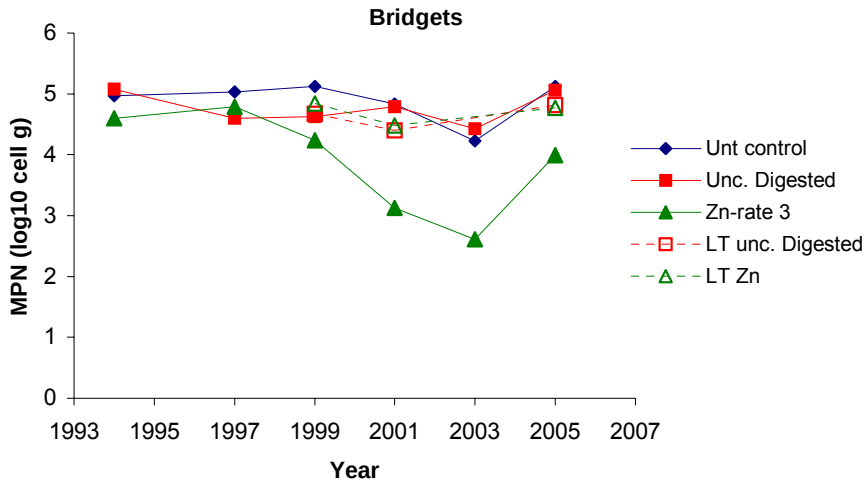
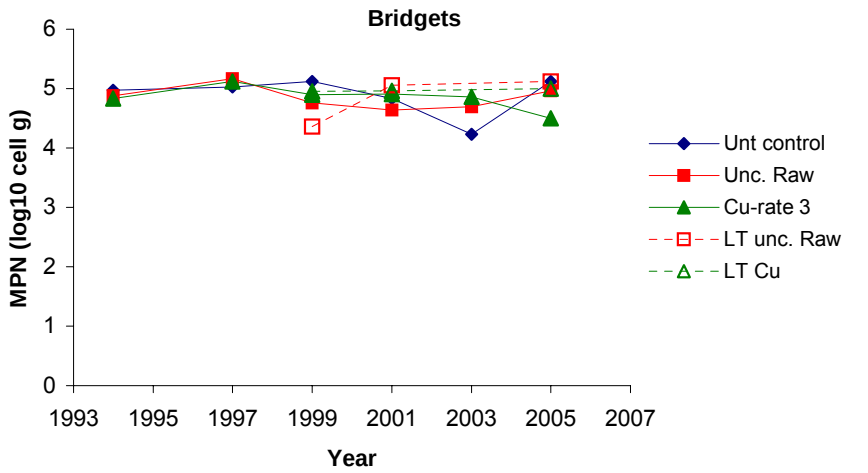


Figure 110. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Bridgets sludge cake





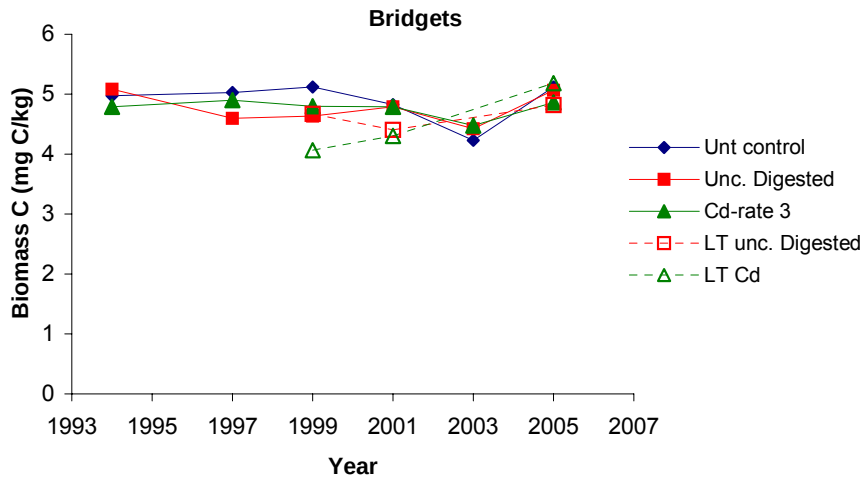
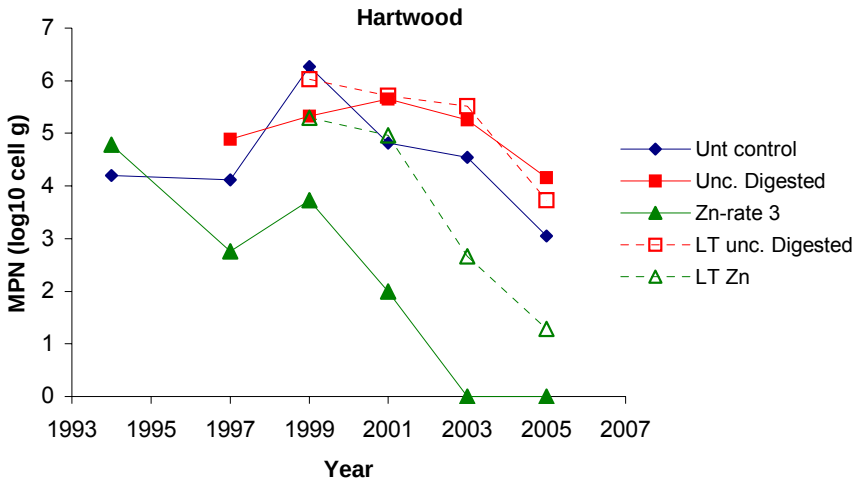
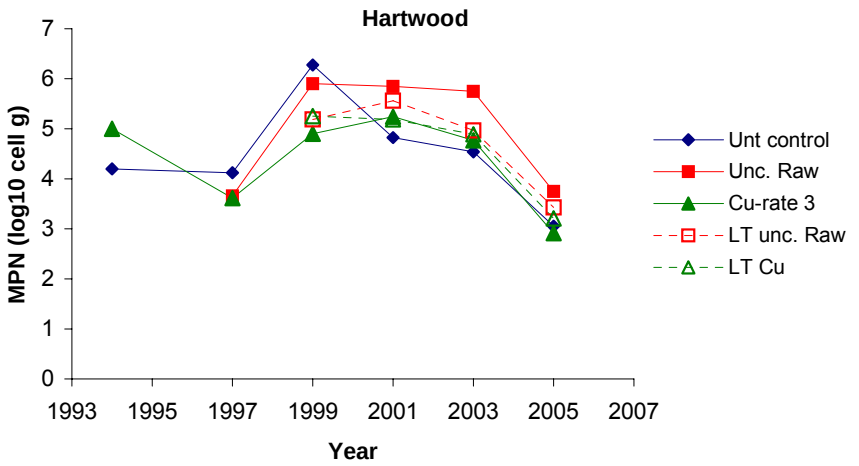


Figure 111. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Hartwood sludge cake





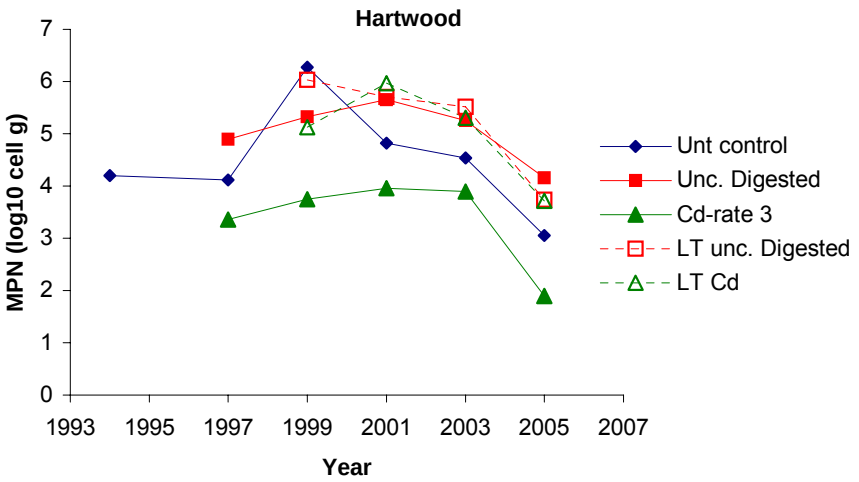
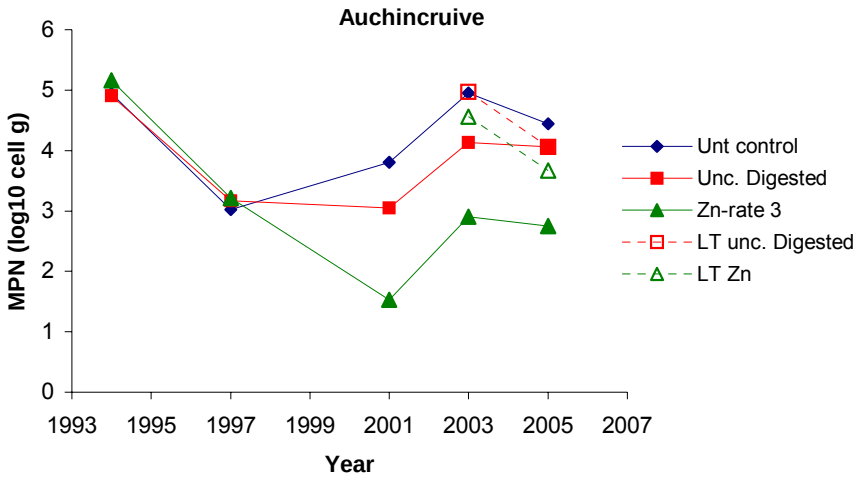
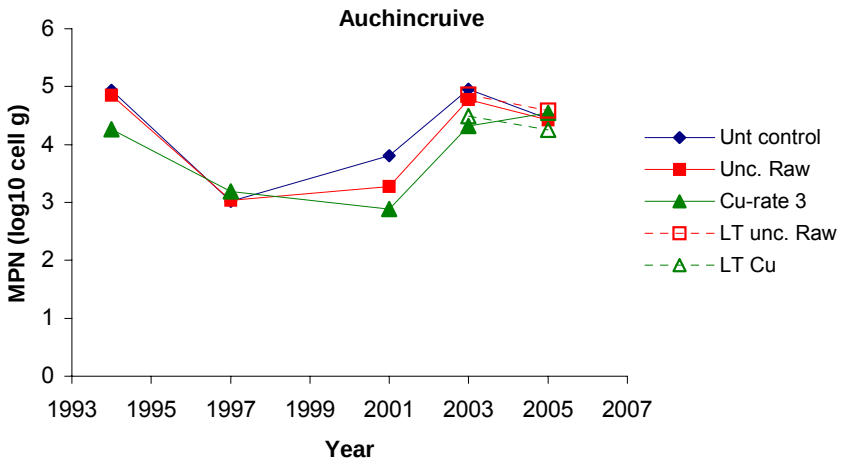


Figure 112. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Auchincruive sludge cake





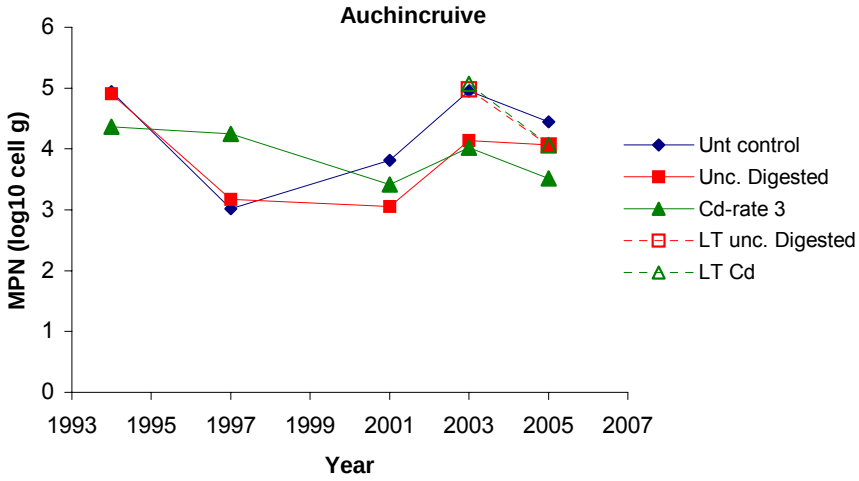
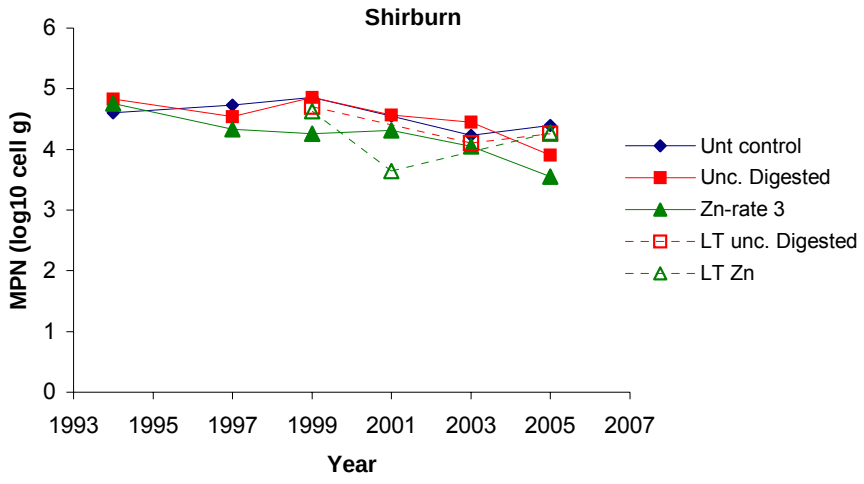
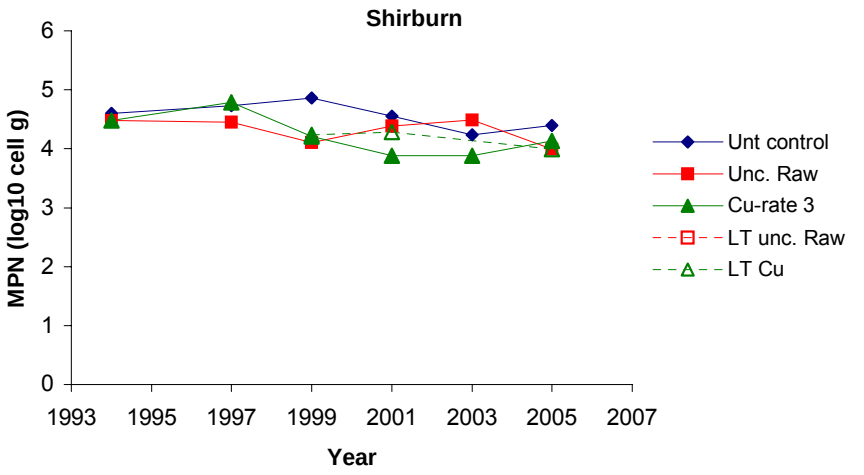


Figure 113. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Shirburn sludge cake





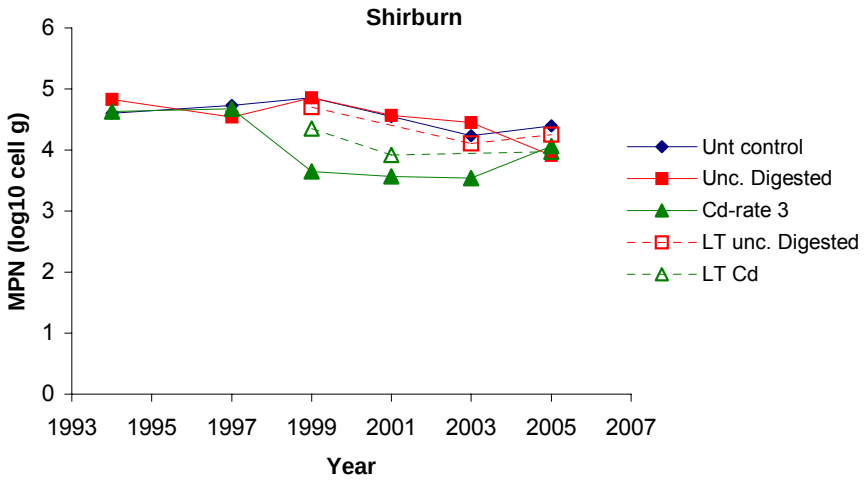


Table 76. Relationship between total topsoil metals and Rhizobia MPNs – Sludge cake experiments (% change across dose-response treatments up to statutory soil metal limits)

Site	Zn (300 mg/kg)				Cu (135 mg/kg)				Cd (3 mg/kg)			
	99	01	03	05	99	01	03	05	99	01	03	05
GLE	38%***	45%***	39%***	33%**	31%***	NS	NS	NS	NS	(36%**)	NS	21%**
WOB	NS	NS	37%**	59%***	16%*	NS	NS	NS	NS	NS	NS	18%*
WAT	11%*	25%**	19%**	27%***	13%**	NS	NS	NS	NS	NS	NS	NS
PWL	NS	52%***	NS	43%*	NS	NS	NS	16%*	NS	NS	NS	(20%**)
ROS	NS	20%***	28%**	47%***	5%*	NS	NS	7%*	NS	NS	(27%***)	NS
BRI	NS	21%**	22%*	13%**	NS	NS	NS	5%*	NS	NS	NS	NS
HAR	> 2log	39%**	78%***	100%**	5%**	NS	NS	26%***	NS	NS	NS	47%*
AUC	-	NS	NS	32%***	-	NS	14%***	NS	NS	NS	NS	NS
SHI	NS	NS	NS	NS	NS	NS	NS	17%*	NS	21%*	(25%**)	NS

$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Red = decrease Blue = increase.
N.B. % Change calculated on $\log_{10}$ cells/g values.
( ) brackets indicate correlation also with soil total Zn.

Adverse effects on free-living clover rhizobia population sizes were observed in soils from eight of the nine sludge cake field experiments in 2005 (Table 76), and a downward trend in population sizes was observed on the same treatments over the time (Figures 105-113). Broos *et al*. (2004) made similar observations and postulated that factors other than metals, such as other contaminants or excess N-supply, may be responsible for these effects. Mineralisation of added sludge organic matter does produce appreciable amounts of N in soil. Also, it is known that high concentrations of soil N can inhibit nodulation, which may be mistakenly interpreted as reductions in, or elimination of, rhizobia from a soil. However, in these experiments, to avoid these possible confounding effects, low metal content sludge cakes were applied to act as organic matter control plots and all the plots received metal-rich sludge cakes at the same time (Gibbs *et al.,* 2006a). This achieved as near as practically possible constant organic matter inputs and mineral N in all treatments, which did not independently reduce rhizobia numbers, as shown in the low metal sludge cake control treatments. Furthermore, Italian rye grass was grown on all the field experiments every year during Phase I to remove excess mineralised N (Gibbs *et al*., 2006a). During Phases II and III, inorganic N fertilisers were applied to all plots according to standard farm practice (Anon., 2000). In addition, it is notable that the effects of Zn became stronger with time, when mineralisation of sludge N would have been decreasing and at its lowest in 2005. Therefore, the comparison between the low-metal sludge cake control plots and their respective metal-rich sludge cake treatment plots in these experiments disproves the conjecture of Broos *et al*. (2004) concerning N.

Soil pH, organic carbon and total N were not significantly correlated with the number of clover rhizobia at any of the sites. The survival of

Rhizobium leguminosarum biovar *trifolii* can be affected at soil pH < 5.5, with a severe reduction in numbers at pH < 4.5 (Richardson and Simpson, 1988), although others have reported survival in soils of pH 4.7 (Hirsch, 1996). Soil pH was > 5.3 in all of the field experimental plots over the 11 year period and, therefore, is unlikely to have markedly affected the survival of clover rhizobia. For example, at Hartwood in 2005, although soil pH in one of the uncontaminated sludge cake plots was 5.3, it still had 4.24 $\log_{10}$ rhizobia cells g^{-1} soil. Broos *et al*. (2005) also found no significant correlation between the number of rhizobia and soil pH in survival experiments, using soils from field experiments previously treated with either metal contaminated sewage sludges or metal salts.

It is also unlikely that the effects can be attributed to organic contaminants in the applied sludges. Chaudri *et al*. (1996) added ten organic compounds frequently occurring in sewage sludge to soil (without the sludge) at 'worst case' concentrations and reported an adverse effect of only pentachlorophenol, which significantly reduced the population of indigenous *Rhizobium leguminosarum* biovar *trifolii*. Furthermore, in our study no reductions in rhizobia numbers occurred in plots receiving the uncontaminated sludge cakes. Therefore, the reductions in clover rhizobia numbers on the metal-rich sludge cake treatments cannot be attributed to soil pH, organic carbon or total nitrogen, and they are unlikely to be due to organic contaminants.

We considered the potential for other factors to be responsible for the rhizobia numbers (and biomass) decreases on the Zn dose-response treatments *e.g.* redox potential, ammonia toxicity, salt effects, volatile acids, organic matter type differences. However, we consider that such effects are unlikely to be responsible for the rhizobia decreases measured, as the organic matter types (*i.e.* both digested cakes) were 'matched' on the Zn dose-response treatments, both sludges had similar NH_4-N contents (0.55 and 0.82% on a dry solids basis for the low-metal and Zn-rich cakes, respectively) and similar $CaCO_3$ contents (2.3 and 3.0% on a dry solids basis for the low-metal and Zn-rich sludge cakes, respectively). Also, there were no consistent differences in soil pH (which was maintained at a target of pH 6.5 at the non-calcareous sites) or conductivity between any of the Zn dose-response treatments. Moreover, it is important to note that the decreases in rhizobial numbers (and biomass) have generally been progressive with time, whereas most of the potential factors highlighted above are likely to have influenced rhizobia/biomass numbers in the short-term following application. We do not believe that co-contaminants present in the Zn sludge (*e.g.* other associated metals and organic contaminants) are likely to have been responsible for the rhizobia number responses measured to date, but to rule out this possibility, we recommend that further analyses are undertaken on the soils and sludge cake applied to establish the Zn metal-dose response treatments.

As noted above, the reductions for Zn have been progressive over time, with no recovery in numbers to date (Figures 105-113). It appears from Figures 37-45 that there is no clear threshold for the effect of Zn across

all sites. It is not possible, therefore, to set a limit based on no effects. It is perhaps more acceptable to base a limit on a given percentage reduction, remembering that loss of function (in this case lack of symbiotic nitrogen fixation with clover) occurs when very few cells remain (likely when population size is <1 on a log scale). Any chosen percentage (on a log scale) can be read from the linear relationships shown on Figures 37-45.

For Cu and Cd, the effects with time were inconsistent (Figures 37-45). The effects seen in the Cu-rich sludge cake treatments in 1999 at Gleadthorpe, Woburn, Watlington, Rosemaund and Hartwood were not strong, and in the 2001 and 2003 sampling years were not significant ($P > 0.05$: Table 76). The effects of Cu occurred again in 2005 at Pwllpeiran, Rosemaund, Bridgets and Hartwood, and although significant, were weak, except at Hartwood. The effects seen in the Cd-rich sludge cake treatments at Gleadthorpe in 2001, at Rosemaund and Shirburn in 2003, and at Pwllpeiran in 2005 also gave significant negative correlation between rhizobia numbers and soil total Zn concentrations in these plots. Soil total Zn and Cd were also found to be significantly ($P < 0.05$) correlated with each other on the Cd-rich sludge cake treatments at these sites ($r^2 = 0.61$ to 0.98), making it difficult to unequivocally identify the metal causing toxicity. However, the consistent evidence from the Zn-rich sludge cake treatments at these sites suggests that Zn in the Cd-rich sludge cake treatments is most likely to be responsible for the reduction in rhizobia numbers.

In two long-term field experiments at Braunschweig, in Germany, to which metal contaminated liquid sludges had been added, reductions in clover rhizobia numbers were attributed to increasing soil total Zn concentrations, even though the treatments applied contained a mixture of metals (Chaudri *et al*., 1993). Soil total metal concentrations in these experiments were below their respective UK statutory soil limits, except for Zn. Similarly, in a metal-contaminated sludge cake experiment at Gleadthorpe, in England, significant reductions in numbers of clover rhizobia and pea rhizobia with increasing soil total Zn, and to some extent Cu, were reported (Chaudri et al., 2000). In that study, Cu concentrations where effects were seen were above the UK statutory soil limit of 135 mg/kg, whereas Cd was below the limit of 3 mg/kg, and generally had no effect. Contrary to this, some workers have suggested that Cd may be more toxic to rhizobia than other metals (Smith and Giller, 1992; Smith, 1997). This study, to date, points to Zn as the metal consistently causing toxicity to rhizobia, and not Cu or Cd at the concentrations in these experiments. In a recent study, Broos *et al*. (2005) also concluded that Zn and not Cd was the metal most toxic to free-living rhizobia in soils in the absence of the host plant. They assessed the survival of *R. leguminosarum* bv. *trifolii* that was inoculated into soils from field experiments previously receiving either sewage sludge or Cd salts in the laboratory, whereas these experiments have looked at the effects of metals added in either sludge cake or metal-amended liquid sludge on the indigenous populations of *R. leguminosarum* bv. *trifolii* under field conditions.

In the sludge cake experiments, topsoil total Zn concentrations were found to account for 55% of the variance in the clover rhizobia MPNs data (excluding Bridgets and Shirburn where the effects were not strong), whilst NH_4NO_3 extractable soil Zn only accounted for 28% of the variance. This is surprising as NH_4NO_3 extractable Zn would be expected to be a more sensitive indicator of Zn bioavailablity than total metal concentrations. The inclusion of either soil pH, organic carbon or total N, or all three, with soil total Zn or NH_4NO_3 extractable soil Zn did not significantly improve the variance accounted for in the MPN data. The fact that 55% of the variance in clover rhizobia MPN data from the seven sludge cake experiments is accounted for by total soil Zn is remarkable. These field experiments are geographically and climatically different, and represent a range of soil physical and chemical properties, although the experimental design and treatments applied were the same, and all received the same uncontaminated and metal-rich sludge cakes.

The presence of the host plant is known to confer protection on rhizobia against metal toxicity (Giller *et al*., 1998; Broos *et al*., 2004). In this study, the host clover plant, *Trifolium repens*, was not present on any of the plots at any field site. The exclusion of clover was deliberate, since the aim of the study was to identify which metal, if any, was responsible for toxicity to the free-living rhizobia in the soil. The presence of the host plant would have offered protection to the *Rhizobium*, therefore, confounding this aim.

The current UK statutory limit for Zn in soils of pH 5.5-6.0 and 6.0-7.0 receiving sewage sludge is 250 mg/kg and 300 mg/kg, respectively, and in Germany it is 150 mg/kg and 200 mg/kg, respectively (SI, 1989; Bundesministerium für Umwelt, 1992). In the US the implied limit, as calculated by McGrath *et al*. (1994) is 1400 mg/kg for Zn, 750 mg/kg for Cu and 20 mg/kg for Cd. In these experiments, to date, no consistent adverse effects have been seen on clover rhizobia at the UK statutory soil limits for Cu and Cd of 135 mg/kg and 3 mg/kg, respectively. The German Zn limits would appear to offer better protection to rhizobia in these soils; they are based not only on protecting plants and the food chain, but also soil micro-organisms, which are vital for sustaining soil fertility and agricultural productivity. Presently, neither the UK statutory and US metal limits explicitly take into consideration effects on soil micro-organisms. The data from this study suggests that the current UK statutory limits may not be adequately protective for Zn. In the UK, the Independent Scientific Committee reviewing the implications of using sewage sludges in agriculture recommended that the limit for Zn in soils of pH 5-7 be changed on an advisory basis to 200 mg/kg (MAFF/DoE, 1993) and the UK Government adopted this recommendation (DoE, 1996). The European Commission draft 'Working Document on Sludge' proposes maximum soil total Zn concentrations in soils receiving sludge of 60 mg/kg for soils of pH 5-6, 150 mg/kg for soil of pH 6-7, and 200 mg/kg for soils of pH ≥ 7 (European Commission, 2000). These proposed limits have been drafted to protect plants, the food chain, and soil biota.

Sludges currently being recycled to agricultural land in the UK are generally much lower in metal content (Water UK, 2006), than those applied in this experiment during Phase I. Indeed, the sludges were sourced specifically for their high metal content. Similarly, the metal-rich sludges used in this study would not meet the criterion for 'clean' sludge under the USEPA-503 regulations, which are based on maximum cumulative loading rates rather than soil metal concentrations (USEPA, 1993). Under USEPA-503, 'clean' sludges can be applied to land over longer periods of time until the US maximum loading rates are met. Indeed, in this study we are also investigating the impact of gradually building-up soil metal concentrations over a longer time period, using the current UK maximum permissible sludge application rates (*i.e.* 15 kg Zn/ha/yr., 7.5 kg Cu/ha/yr. and 1.5 kg Cd/ha/yr.). At Gleadthorpe and Hartwood, after 11 years of repeated sludge cake application, there is some evidence of declining rhizobia population sizes on the long-term Zn-rich sludge cake treatment at both sites (Figures 105 and 111).

It is well established that micro-organisms in soils are largely associated with organic matter, which they use as a substrate for energy and reproduction. Numerous studies have documented increases in microbial biomass with organic matter additions (e.g. Jenkinson and Ladd, 1981). On a microscopic scale, the incorporated sludge organic matter particles are likely to have attracted most of the soil microbes, as a substrate. These particles in the Zn-rich sludge cake treated soils will have high concentrations of Zn (approx. 6000 mg/kg) reflecting Zn concentrations in the applied sludge cake (Gibbs *et al*., 2006a). Therefore, we propose that Zn toxicity to the free-living rhizobia and microbial biomass seen at a number of the sludge cake sites is due to direct toxicity through exposure to very high concentrations of Zn in and around the sludge cake particle 'hot spots' in the soil, and 'poisoning' at a cellular level when mineralising this organic matter for cell maintenance and reproduction, leading to cell disruption. In our opinion, this is a plausible hypothesis for reductions in the size of the soil microbial biomass and rhizobia population. However, this explanation discounts any 'sludge protection' hypothesis. De Brouwere and Smolders (2006) drew a similar conclusion when looking at the effect of sludge metals on crop yields, they concluded that "in the initial years, sludge and soil were not homogeneously mixed and plant roots might have been exposed to locally elevated Zn and Cd sludge concentrations that were in toxic ranges".

A possible reason for seeing inconsistent effects on indigenous rhizobia in either the Cu-rich or Cd-rich sludge cake treatments may be that these metals were not toxic at the concentrations achieved in the sludge cake treated soils.

Ammonium nitrate extractable metal concentrations

Zinc: NH_4NO_3 extractable Zn concentrations in topsoil were consistently higher at Hartwood than the other non-calcareous sites, and lower at the calcareous Shirburn site. At Hartwood, this was partly due to the higher total topsoil metal concentrations, and the greater extractability of Zn at

this site, which represented *c.*7% of total topsoil Zn concentrations in 2005 compared with *c.*4.6% at Gleadthorpe, *c.*2.7% at Pwllpeiran and Auchincruive, *c.*1.7% at Woburn and Watlington and *c.*0.7% at Rosemaund and Bridgets and 0.1% at Shirburn (Table 77). The higher extractability of Zn at Hartwood was also likely to be a reflection of the lower pH at this site (pH range 5.7-6.0 on the Zn dose-response treatments). The low Zn availability at Shirburn (< 0.1% of the total topsoil Zn concentrations in 2005) was related to the high pH and presence of free $CaCO_3$. At Rosemaund and Auchincruive, the decreases in Zn availability between 2001 and 2005 can be related to lime additions which resulted in topsoil pH increases (soil pHs in 2001: 5.4-5.6; and in 2005: 6.0-6.7 on the Zn dose-response treatments).

Copper: NH_4NO_3 extractable Cu concentrations in topsoil were consistently higher at Watlington (*c.*1.5% of total topsoil Cu) than the other sites and lower at Hartwood (*c.*0.25% of total topsoil Cu) (Table 77).

Cadmium: NH_4NO_3 extractable Cd concentrations in topsoil were consistently higher at Auchincruive and Rosemaund (*c.*7.9% of total topsoil Cd) than the other sites, and lower at Bridgets and Shirburn (*c.*0.6%) (Table 77).

Table 77. Mean ammonium nitrate extractable soil metal concentrations as a percentage of mean total topsoil metal concentrations at all of the sludge cake sites – 1997, 2001 and 2005

Site		Zinc			Copper			Cadmium		
		1997	2001	2005	1997	2001	2005	1997	2001	2005
GLE	**Rate 1**	0.86	3.70	2.07	1.35	0.57	0.61	1.22	2.10	7.00
	Rate 2	1.45	5.54	6.24	1.03	0.56	0.61	1.11	1.65	3.96
	Rate 3	1.95	6.72	4.39	1.19	0.69	0.68	0.75	1.57	3.56
	Rate 4	2.55	6.65	5.90	0.87	0.64	0.62	0.89	1.32	2.42
Mean		*1.70*	*5.65*	*4.65*	*1.19*	*0.62*	*0.63*	*0.99*	*1.66*	*4.24*
WOB	**Rate 1**	1.58	2.08	1.20	1.00	0.63	0.93	1.63	1.97	11.62
	Rate 2	2.43	3.12	1.89	0.86	0.35	0.34	1.17	1.32	6.46
	Rate 3	2.15	2.39	2.30	0.84	0.41	0.49	0.90	1.21	4.32
	Rate 4	3.78	2.22	1.31	0.75	0.38	0.56	0.79	1.29	3.14
Mean		*2.49*	*2.45*	*1.68*	*0.86*	*0.44*	*0.58*	*1.12*	*1.45*	*6.39*
WAT	**Rate 1**	0.81	0.72	1.31	0.75	0.52	1.68	1.05	1.24	1.43
	Rate 2	1.40	0.82	2.42	0.61	0.53	1.55	0.69	0.88	1.44
	Rate 3	2.17	1.39	1.17	1.06	0.56	1.32	0.74	0.86	2.00
	Rate 4	2.76	2.18	1.60	0.75	0.47	1.37	0.69	0.88	1.90
Mean		*1.79*	*1.28*	*1.63*	*0.79*	*0.52*	*1.48*	*0.79*	*0.97*	*1.69*
PWL	**Rate 1**	0.81	1.07	1.97	0.54	0.23	1.26	1.21	4.14	3.13
	Rate 2	1.64	1.40	1.53	0.36	0.24	0.93	1.36	2.94	2.21
	Rate 3	1.69	2.36	4.78	0.40	0.34	1.36	0.70	2.35	2.59
	Rate 4	2.06	2.86	2.29	0.46	0.33	1.12	0.93	1.81	2.85
Mean		*1.55*	*1.92*	*2.64*	*0.44*	*0.29*	*1.17*	*1.05*	*2.81*	*2.70*
ROS	**Rate 1**	2.97	8.88	0.21	0.68	0.47	0.53	3.40	6.87	5.65
	Rate 2	5.80	14.78	1.02	0.66	0.56	0.60	0.78	2.45	7.53
	Rate 3	5.56	16.59	0.64	0.48	0.60	0.61	1.95	5.05	10.14
	Rate 4	5.91	15.98	1.68	0.63	0.80	0.69	1.44	3.49	8.12
Mean		*5.06*	*14.06*	*0.89*	*0.61*	*0.61*	*0.61*	*1.89*	*4.47*	*7.86*
BRI	**Rate 1**	3.02	1.74	0.45	0.57	0.33	0.47	1.79	1.20	0.60
	Rate 2	2.98	1.55	0.10	0.50	0.23	0.45	1.16	0.80	0.47
	Rate 3	2.96	2.45	0.71	0.51	0.33	0.43	0.97	1.32	0.50
	Rate 4	4.02	4.30	0.63	0.36	0.44	0.41	0.63	1.11	0.55
Mean		*3.25*	*2.51*	*0.47*	*0.49*	*0.33*	*0.44*	*1.14*	*1.11*	*0.53*
HAR	**Rate 1**	4.70	5.51	4.36	0.88	0.23	0.26	5.78	3.70	3.22
	Rate 2	7.38	9.20	4.20	0.92	0.22	0.22	5.51	3.07	3.58
	Rate 3	9.00	11.14	9.15	1.11	0.25	0.25	3.55	2.93	3.56
	Rate 4	11.58	13.18	9.59	1.11	0.35	0.28	2.59	1.75	3.15
Mean		*8.17*	*9.76*	*6.83*	*1.01*	*0.26*	*0.25*	*4.36*	*2.84*	*3.38*
AUC	**Rate 1**	3.60	8.22	1.40	0.46	0.33	0.45	3.00	6.45	3.83
	Rate 2	6.02	13.50	2.94	0.42	0.35	0.23	2.52	5.06	14.32
	Rate 3	7.13	14.29	2.95	0.44	0.41	0.51	1.77	2.82	7.77
	Rate 4	11.14	16.61	3.92	0.51	0.59	0.28	1.88	2.83	5.66
Mean		*6.97*	*13.04*	*2.80*	*0.46*	*0.42*	*0.37*	*2.29*	*4.35*	*7.90*
SHI	**Rate 1**	0.05	0.06	0.14	0.80	0.63	0.77	0.39	0.28	0.84
	Rate 2	0.07	0.06	0.05	0.78	0.78	0.73	0.37	0.28	0.53
	Rate 3	0.07	0.07	0.11	0.86	0.80	0.75	0.24	0.29	0.39
	Rate 4	0.11	0.09	0.17	0.88	0.86	1.68	0.44	0.31	0.48
	Rate 5	0.13	0.10	0.12	1.06	0.84	1.20	0.38	0.30	0.85
Mean		*0.09*	*0.08*	*0.12*	*0.88*	*0.79*	*1.03*	*0.36*	*0.29*	*0.62*

Across the nine sites, there were strong relationships ($P < 0.01$) between total topsoil Zn, Cu and Cd concentrations and the corresponding topsoil NH_4NO_3 extractable soil concentrations (r^2 = 44-95 and 50-78% for Zn; 61-97 and 43-89% for Cu; and 31-81 and 14-76% for Cd in 2003 and 2005, respectively). At Gleadthorpe and Woburn (for Cd), and Shirburn (for Zn and Cd) in 2005, NH_4NO_3 extractable metal levels were below/close to the limits of analytical detection and thus correlations could not be determined with total topsoil metal concentrations.

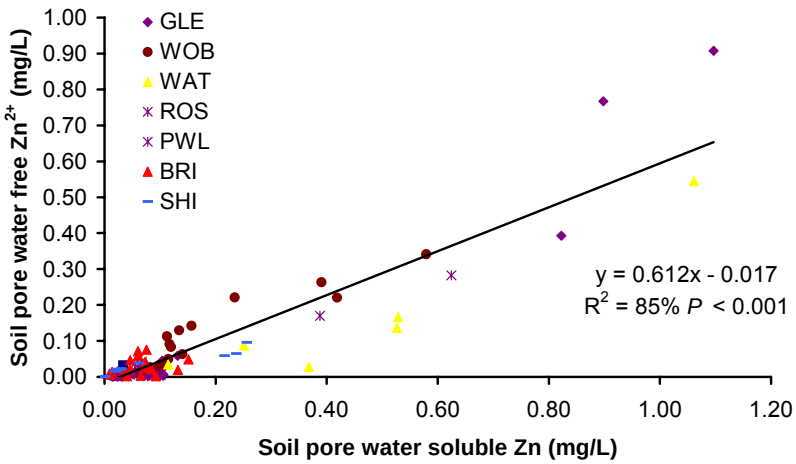
Metal speciation

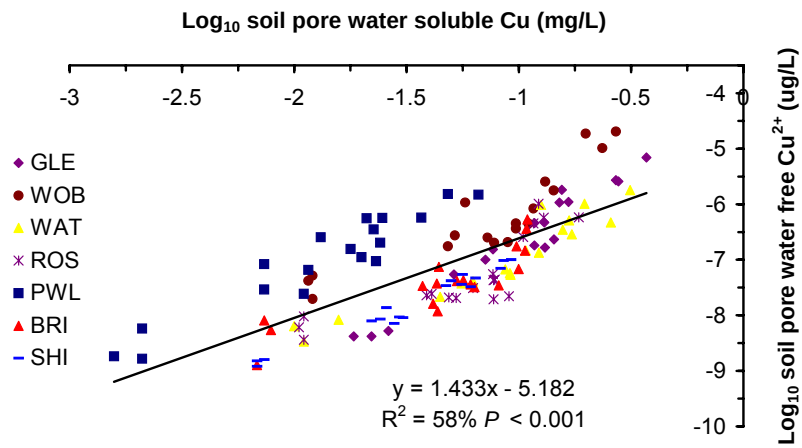
Across the seven sites in England and Wales, there were strong relationships ($P < 0.001$) between soil pore water total soluble Zn, Cu and Cd and soil pore water free Zn^{2+} (r^2 = 0.85), Cu^{2+} (r^2 = 0.58) and Cd^{2+} (r^2 = 0.83), respectively (Figure 114). Approximately 60% of the soil pore water total soluble Zn was in the form of free Zn^{2+} and *c.*45% of the soil pore water total soluble Cd was in the form of free Cd^{2+}. In the metal speciation measurements undertaken in 2001, soil pore water free Zn^{2+} and Cd^{2+} represented *c.*80% and 75% of soil pore water total soluble Zn and Cd concentrations, respectively. The reductions in proportions of soil pore water total soluble Zn and Cd present as free Zn^{2+} and Cd^{2+} in 2004 compared with 2001 is most likely due to the soil pH being closer to the target pH of 6.5 in all the field experiments, (excepting the calcareous site Shirburn). Soil pore water free Zn^{2+} decreased in the order Gleadthorpe > Woburn = Watlington > Rosemaund> Bridgets ≥ Pwllpeiran ≥ Shirburn, and free Cd^{2+} Gleadthorpe > Woburn > Rosemaund > Pwllpeiran > Watlington > Bridgets > Shirburn.

Less than 0.1% of soil pore water total soluble Cu was present as soil pore water free Cu^{2+}. The order of decreasing soil pore water free Cu^{2+} was Gleadthorpe > Woburn > Watlington > Rosemaund > Pwllpeiran > Bridgets > Shirburn.

The relationships between total soil pore water soluble Zn, Cu and Cd concentrations, and topsoil NH_4NO_3 extractable metal concentrations across the seven sites are shown in Figure 115. The relationship for Zn accounted for 53% of the variation, for Cu 37% and for Cd 30% (note at a number of sites analytical problems were encountered at low NH_4NO_3 extractale Cd concentrations). Water soluble Zn, Cu and Cd accounted for *c.*5%, 10% and 0.5% of NH_4NO_3 extractable Zn, Cu and Cd concentrations, respectively.

Figure 114. Relationship between total soil pore water soluble Zn, Cu and Cd and free Zn^{2+}, Cu^{2+} and Cd^{2+} - 2004





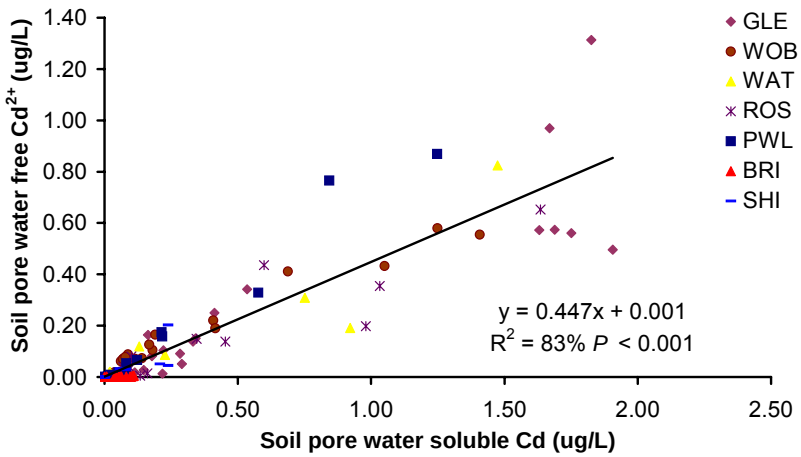
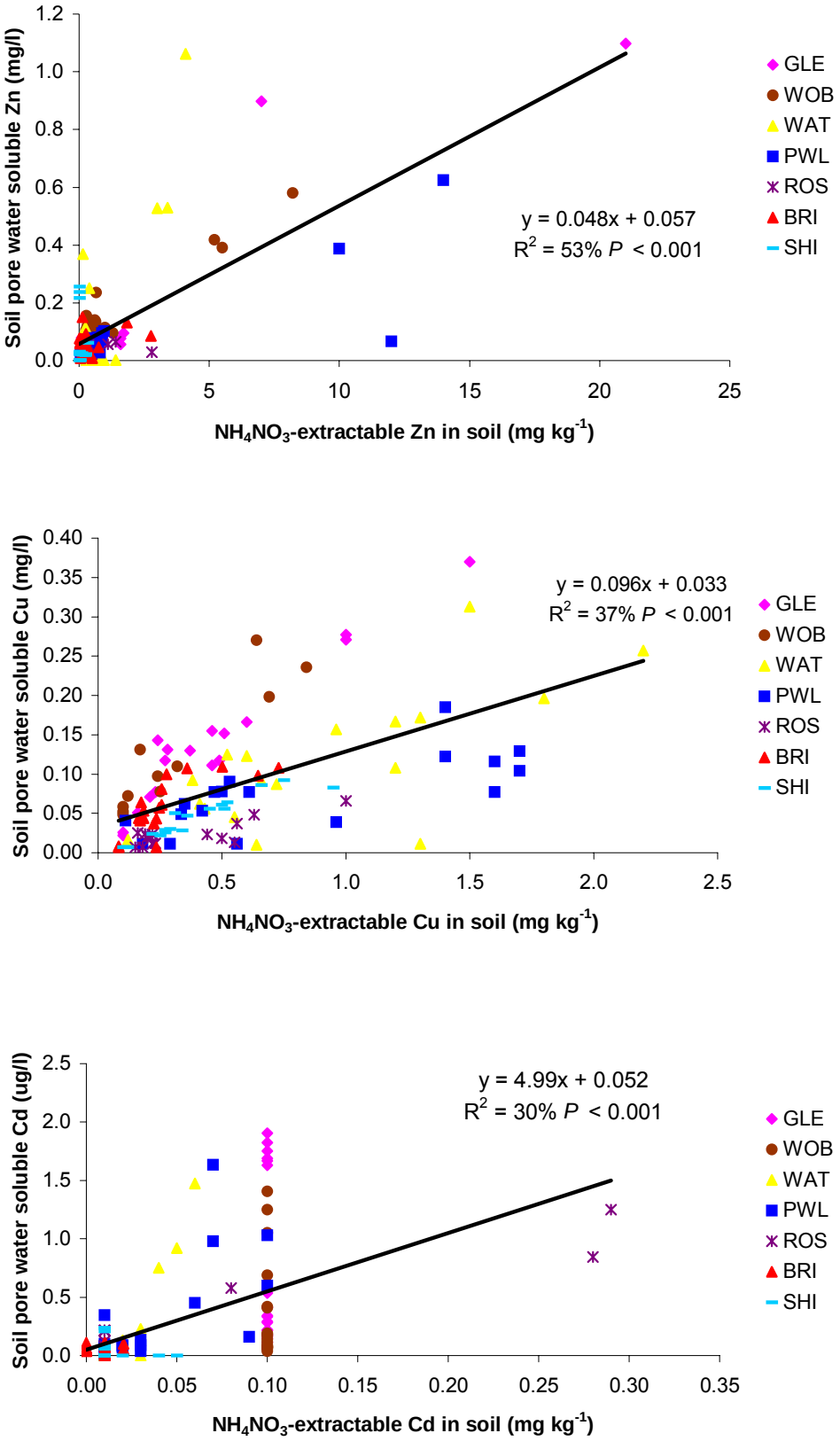


Figure 115. Relationship between total soil pore water soluble Zn, Cu and Cd topsoil NH_4NO_3 extractable Zn, Cu and Cd concentrations - 2004



Crop yields and crop metal concentrations

First cut grass yields (2002 and 2004 at all sites, plus 2003 and 2005 at Pwllpeiran, Hartwood and Auchincruive) and grain yields (2003 and 2005 at the six sites in England) were measured to assess the impact of the heavy metal-additions in Phase I on crop metal bioavailability and productivity. Generally, the sludge cake treatments produced similar or higher yields compared with the untreated controls, which was probably a reflection of the beneficial effects of adding large amounts of organic matter.

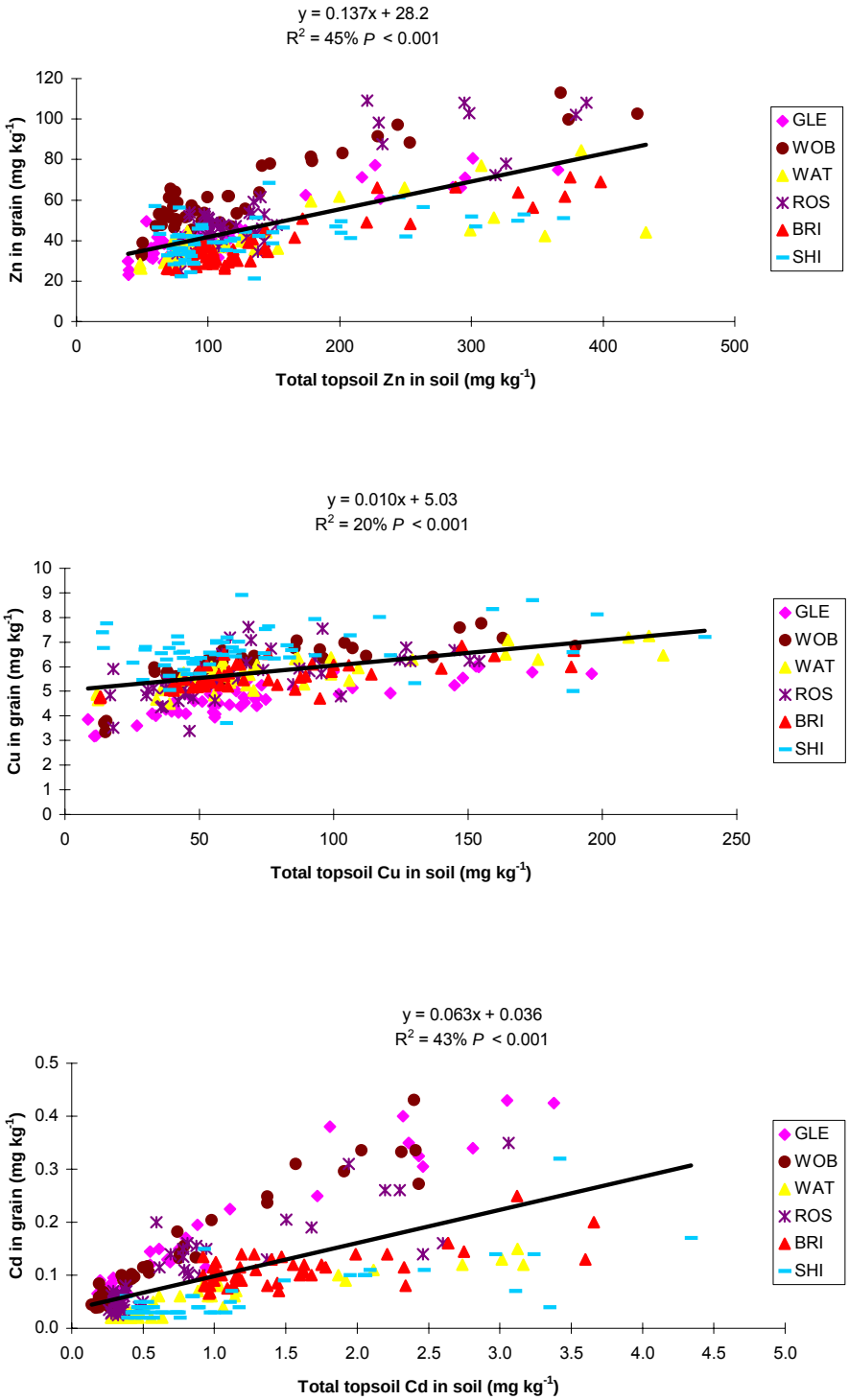
There were significant ($P < 0.05$) positive relationships (*i.e.* increasing grass yields with increasing total topsoil metal concentrations) between grass yields and total topsoil Zn and Cu concentrations at Auchincruive in 2002, and at Bridgets (total Zn) and Hartwood (total Cu) in 2004.

At Woburn in 2003, there was a significant ($P < 0.05$) positive relationship between grain yields and total topsoil Cu concentrations. However, in 2005, no significant relationships ($P > 0.05$) were found between grain yields and total topsoil metal concentrations at any of the sites.

There were moderate to strong relationships ($P < 0.001$) between grain Zn (r^2 = 59-84%), Cu (r^2 = 32-79%) and Cd (r^2 = 45-94%) concentrations, and corresponding total topsoil metal concentrations in 2003. However, in 2005, relationships ($P < 0.05$) between grain metals and total topsoil Zn and Cd concentrations were not as strong (r^2 = 34-66% for Zn and 12-85% for Cd, respectively). For Cu, there were relationships ($P < 0.05$) between grain Cu and total topsoil Cu concentrations only at Gleadthorpe and Woburn (r^2 = 57% and 46%, respectively).

The relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations across all sites in 2003 are shown in Figure 116. The data from 2005 are not presented owing to the lack of sufficient crop biomass for yields to be reliably measured at three sites (Woburn, Watlington and Shirburn). In 2003, the relationships between grain metals and total topsoil metal concentrations accounted for 45% of the variation for Zn, 20% of the variation for Cu and 43% of the variation for Cd concentrations.

Figure 116. Relationship between grain Zn, Cu and Cd concentrations (harvest 2003) and total topsoil Zn, Cu and Cd concentrations – spring 2003



Grain cadmium

The potential risk to the human food chain from Cd contamination, via crop uptake or accumulation in animal products, is the principal reason for EU legislation on soil Cd concentrations (European Commission, 2001). In addition to soil Cd limits, legislation has been put in place to limit wheat grain Cd concentrations to less than 0.2 mg Cd/kg fresh weight (0.235 mg Cd/kg dry matter-dm) and barley grain Cd concentrations to less than 0.1 mg Cd/kg fresh weight (0.118 mg Cd/kg dm). Chaudri *et al.* (2007) combined soil physio-chemical and wheat grain Cd concentration data from the sludge cake, and associated metal-amended liquid sludge and metal salt studies, to derive a single algorithm that could reliably predict wheat grain Cd concentrations under field conditions. Chaudri *et al.* (2007) used stepwise multiple linear regression to identify significant variables that could be used to fit a model to predict wheat grain Cd concentrations. The soil variables used initially included clay content, total Cd, total Zn, total Cu, pH, organic carbon and oxides of Fe, Al, Mn and P, along with grain yield data. Only soil total Cd, pH and organic carbon were found to be significant ($P < 0.001$) terms, accounting for 78% of the variance in the data set. The significant influence of soil organic carbon was most probably a reflection of Cd bioavailability differences between the sludge cake and metal-amended liquid sludge (and metal salt) experiments. Further stepwise inclusion of clay content, soil total Zn and Cu, the soil Zn:Cd ratio, soil oxides (*i.e.* Fe, Al, Mn and P) and grain yields produced little further improvement in the percentage variance accounted for. Therefore, in the final regression only soil total Cd, pH and organic carbon were included to obtain a model to predict grain Cd. The model obtained was:

$$\log_{10} \text{wheat grain Cd (mg kg}^{-1}) = 3.9\ (\pm 0.158) + 0.86\ (\pm 0.013) \log_{10} \text{soil total Cd (mg kg}^{-1}) - 0.25\ (\pm 0.009) \text{ soil pH} - 0.75\ (\pm 0.036) \log_{10} \text{soil organic carbon (mg kg}^{-1}) \quad (1)$$

Data from 1408 plots, $r^2_{adj} = 0.78$, SE = 0.19

This equation (1) indicates that increasing soil total Cd increased wheat grain Cd, whilst increasing soil pH and organic carbon levels decreased grain Cd. Therefore, high soil pH and organic matter content will reduce plant Cd uptake in to grain.

Chaudri *et al.* (2007) used this regression model to predict wheat grain Cd, with 95% confidence, for different combinations of soil total Cd and pH, with the mean organic carbon content across the sites (2.22 %) held constant. The predicted wheat grain Cd concentration increased with increasing soil total Cd and decreasing soil pH. The model predicted, with 95% confidence, that the EU wheat grain Cd maximum permissible concentration 0.235 mg/kg dry weight would be reached at soil total Cd concentrations of 0.85, 1.21, 1.71, 2.39, 3.30 and 4.53 mg/kg for soils of pH 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, respectively. At present, the UK statutory total Cd limit for soils receiving sewage sludge is 3 mg/kg

regardless of soil pH (SI, 1989). Based on the these data, with 3 mg/kg Cd in soil, the soil pH would have to be maintained at 6.8 or above to avoid exceeding the EU grain Cd maximum permissible concentration, with 95% confidence. The model of Chaudri *et al.* (2007) and that of Adams *et al*. (2004) using an independent data set, showed reasonable agreement in predicting that the soil pH required to ensure compliance with the EU maximum permissible grain Cd concentration in soil containing 3 mg Cd/kg was above 6.8 and 6.3, respectively.

The European Commission draft 'Working Document on Sludge' proposed variations in soil metal limits with pH (European Commission, 2000). The proposed maximum permitted total Cd concentrations for soils receiving sewage sludge were 0.5 mg/kg in the pH range 5 - 6, 1.0 mg/kg in the pH range 6 - 7, and 1.5 mg/kg for soils of pH ≥ 7. Based on the modelled data reported by Chaudri *et al.* (2007) the proposed draft EU soil total Cd limits would protect against wheat grain Cd exceeding EU maximum permissible grain Cd concentrations.

5.2 England and Wales – Metal-amended liquid sludge experiments

Soil respiration rates

Soil respiration rates measured on samples from all the treatment plots at the three sites in spring 2003 and 2005 were generally higher on the metal-amended liquid sludge treatments compared with the untreated controls. The higher soil respiration rates ($P < 0.05$) on a number of the liquid sludge amended treatments at the three sites, indicate that the liquid sludges applied during Phase I had continued to stimulate soil microbial activity, as measured by the respiration rate. As increased soil respiration rates were measured in both sampling years, it would appear that the liquid sludge additions had continued to degrade throughout Phase III.

On the metal dose-response treatments, there were significant ($P < 0.05$) relationships between soil respiration rates and total topsoil Zn at Rosemaund in 2003, total topsoil Cu at Woburn in both 2003 and 2005, and total topsoil Cd at Watlington in 2003. During Phase II, there were significant positive relationships between soil respiration rates and total topsoil Zn at Rosemaund and for Cd at Watlington in 1999. At all three sites, the relationships between soil respiration rates and total topsoil metal concentrations were positive *i.e.* soil respiration rates increased with increasing topsoil metal concentrations.

The liquid sludge additions made during Phase I still appear to be stimulating soil respiration rates above background levels during Phase III. Based on the measurements made to date, it is difficult to draw firm conclusions regarding the impact of metals *per se* on soil respiration rates in the long-term.

Comparing soil respiration rate responses in the sludge cake and metal-amended liquid sludge experiments indicated only limited agreement between the two experiments (Table 78), with only one sampling event measuring a response on both the sludge cake and liquid sludge experiments at the same site on the same treatment (*i.e.* at Rosemaund

in 1999 for total Zn). However, where a respiration response was evident this was invariably *positive* (8 out of 9 responses at the cake sites and 6 out of 7 at the liquid sludge sites).

Table 78. Summary of relationships between total topsoil Zn, Cu and Cd and soil respiration rates – sludge cake and metal amended liquid sludge experiments (% change up to statutory soil metal limits)

Zinc	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	NS	NS	NS	20% *	NS	NS
WAT	24% *	NS	NS	NS	NS	NS	NS	NS
ROS	17% **	NS	NS	NS	32% **	NS	47% **	NS

Copper	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	54% *	NS	NS	NS	NS	NS	16% *	22% *
WAT	29% **	NS	62% **	15% *	NS	NS	NS	NS
ROS	19% *	NS	24% *	NS	NS	NS	NS	NS

Cadmium	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	61% **	NS	NS	NS	NS	NS
WAT	NS	NS	NS	NS	42% ***	NS	56% **	NS
ROS	NS	NS	NS	NS	NS	NS	NS	NS

Red = inverse relationship Blue = positive relationship

Soil microbial biomass

Soil microbial biomass C concentrations measured at all three sites in spring 2003 and 2005 were generally higher on the metal-amended liquid sludge treatments compared with the untreated controls. The increases in soil microbial biomass carbon concentrations on the liquid sludge treatments were due to the organic matter additions made via the liquid sludge applications during Phase I.

On the metal dose-response treatments, there were significant ($P < 0.05$) inverse (*i.e.* soil biomass C concentrations decreased with increasing metal concentrations) relationships between soil biomass C concentrations and total topsoil Zn concentrations at Woburn in 2005, and total topsoil Cu at Watlington in 2003 and at both Woburn and Watlington in 2005. At Rosemaund in 2005, there was a significant ($P < 0.05$) positive relationship between soil biomass C concentrations and

total topsoil Cd concentrations (*i.e.* soil biomass C concentrations increased with increasing total topsoil Cd concentrations).

Soil microbial biomass C concentrations have been measured on selected treatments (*i.e.* untreated control, uncontaminated liquid sludge, Zn-rate 2, Cu-rate 2 and Cd-rate 2) at each sampling event since the start of this long-term study (1995). The rate 2 metal treatments were selected as these represented the target treatments closest to the current statutory soil heavy metal limits. Trends in biomass C concentrations on the untreated controls, uncontaminated liquid sludge and the rate 2 Zn, Cu and Cd treatments are illustrated in Figures 117 – 119.

At the three sites, there were variations in biomass C concentrations between sampling events on the untreated control treatments, in response to seasonal changes/variation in inherent biomass C concentrations. However, across the three sites and metal treatments, biomass C concentrations on the Zn, Cu and Cd amended treatments were generally similar to or slightly smaller than the 'paired' uncontaminated liquid sludge treatment. Differences in soil biomass C concentrations between the metal amended and uncontaminated liquid sludge treatments were most evident on the sandy textured soil at Woburn (Figure 117).

The residual liquid sludge organic matter at the end of Phase III will be largely composed of slowly degradable carbon, with its degradation during subsequent years likely to have a smaller effect on soil microbial biomass C concentrations. Microbial assimilation of C during subsequent seasons, in the absence of readily degradable sludge organic matter, will be dependent on the input of organic matter from plant growth (*e.g.* plant debris and root exudates). Hence, during subsequent seasons sludge degradation effects would be expected to have less influence on soil microbial biomass C concentrations, and it is under these circumstances that any effects from metals *per se* are likely to be most evident.

Figure 117. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Woburn liquid sludge

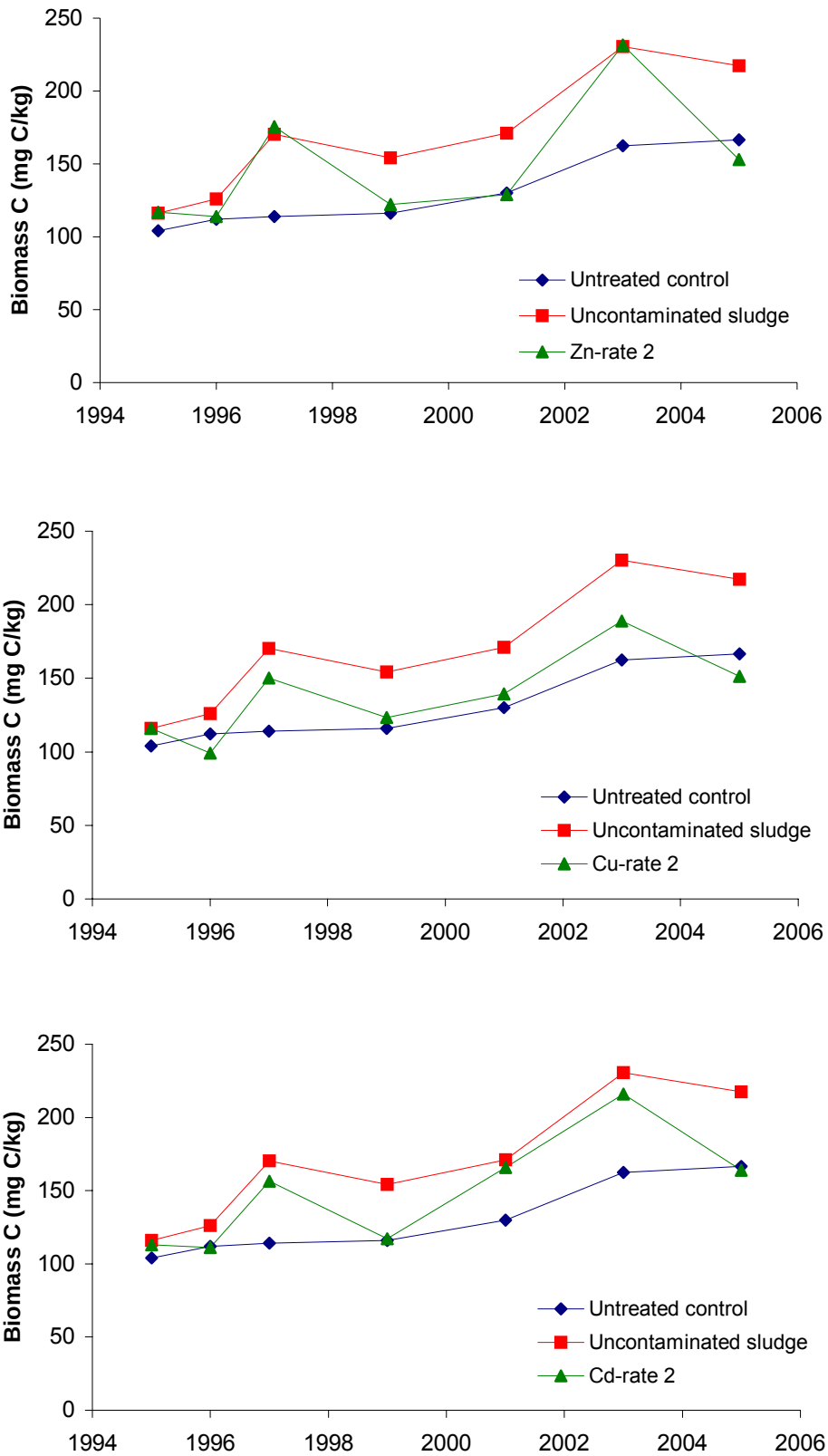


Figure 118. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Watlington liquid sludge

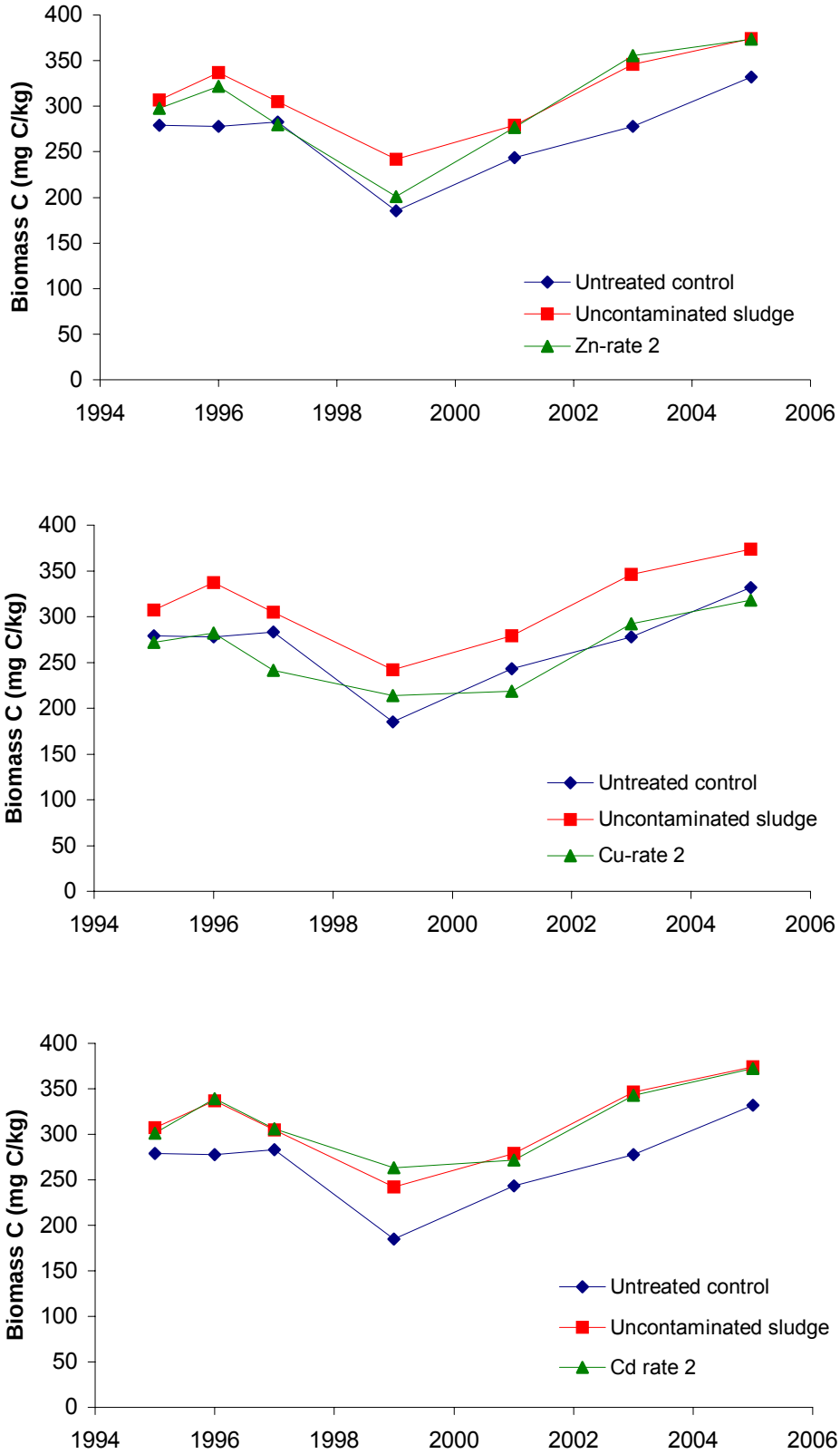
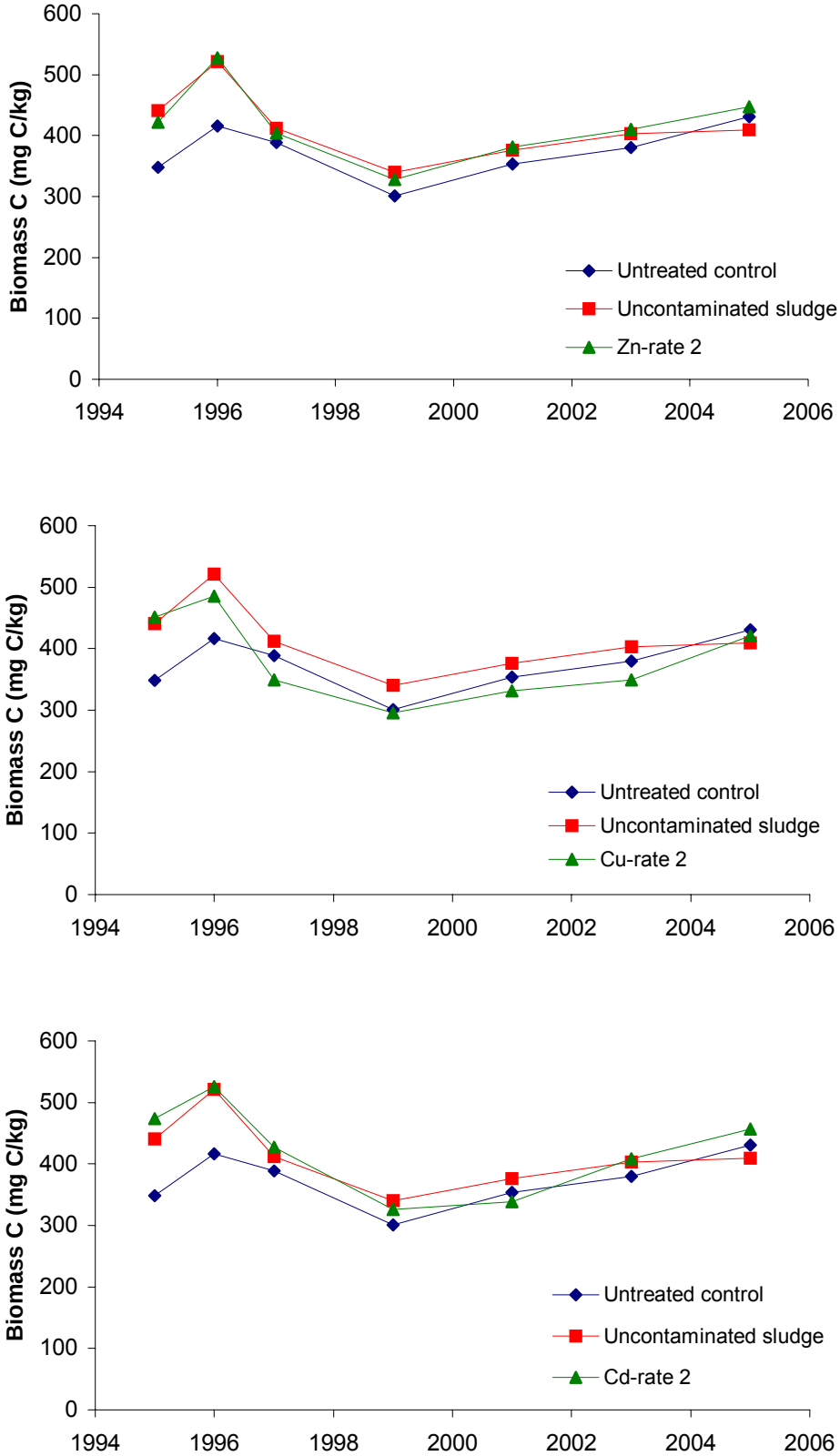


Figure 119. Mean soil biomass C concentrations on the Zn, Cu and Cd treatments over time – Rosemaund liquid sludge



Comparing soil biomass C concentration responses on the sludge cake and metal-amended liquid sludge experiments indicated strong agreement between the two studies for Cu and Cd (Table 79). For Cu, the size and number of responses were similar on both experiments and these were all inverse. For Cd in both experiments, there were no consistent responses. In the case of Zn, there was little agreement between the two studies with eight responses (out of 12) on the cake experiments and only two on the liquid sludge experiments, although where responses were present these were always inverse.

Table 79. Summary of relationship between total topsoil Zn, Cu and Cd and soil biomass C – sludge cake and metal amended liquid sludge experiments (% change up to statutory soil metal limits)

Zinc	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	16% **	36% ***	NS	NS	NS	24% *
WAT	NS	22% *	19% **	19% *	22% *	NS	NS	NS
ROS	NS	14% *	14% **	30% **	NS	NS	NS	NS

Copper	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	18% *	12% **	16% **	19% **	26% *	13% *	NS	29% *
WAT	NS	17% **	13% *	22% *	NS	33% **	26% ***	28% *
ROS	10% **	30% ***	NS	20% *	NS	24% **	NS	NS

Cadmium	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	31% **	NS	NS	NS	NS	NS	NS	NS
WAT	NS	NS	NS	NS	NS	NS	NS	NS
ROS	NS	NS	NS	NS	NS	NS	NS	21% *

Red = inverse relationship Blue = positive relationship

The presence of a greater number of inverse relationships on the Zn sludge cake compared with the Zn-amended liquid sludge treatment was unexpected. Conventional wisdom would suggest that metals added in metal-amended liquid sludge might be more toxic to micro-organisms than sludge cake amendments. Furthermore, it was thought that the added organic matter in the sludge cake experiments would ameliorate metal toxicity to micro-organisms by binding metals, thus reducing bioavailability, and also through 'physical' protection (Babich and Stotzky, 1980). Certainly, NH_4NO_3 extractable soil Zn concentrations

were initially higher in the metal-amended liquid sludge treatments (Gibbs *et al.,* 2006b), thus supporting the above line of thought. However, the Zn added as sludge cake appears to be more detrimental to the soil microbial biomass, compared to little or no measurable effects of adding Zn to soil in liquid sludge. In contrast, the effects of Cu on the soil biomass size were consistent between the two studies, and Cd had little effect.

It is possible that no detrimental effects from Zn were seen in the liquid sludge experiments, because the metals were not associated with organic matter. The Zn would be much more evenly incorporated throughout the soil and not be found as 'hot spots' of high concentrations associated with organic matter like in the sludge cake experiments (see Section 5.1). We suggest that in the liquid sludge experiment micro-organisms are mostly associated with uncontaminated organic matter from root exudates, dead roots and small amounts of straw or grass residues left after harvest, and incorporated into the soil during cultivation. Therefore, in the liquid sludge experiment the micro-organisms are not exposed to 'hot spots' of high Zn concentrations as they would be in the sludge cake experiments.

Therefore, it would appear that sludge chemistry and the presence of Zn in association with organic matter is controlling metal bioavailability to the microbial biomass and ultimately toxicity. This hypothesis is supported by several studies (*e.g.* Lake *et al.,* 1984; Merrington *et al.,* 2003) which indicate that metal availability in sludge-amended soils is initially more related to sludge chemistry than to soil chemistry. Moreover, the probable interaction between organic matter and Zn observed in this study may have implications beyond the recycling of sludge to agricultural land, as there are large amounts of other organic materials (*e.g.* farm manures, green waste compost, paper mill sludges *etc.*) recycled to land that also contain Zn.

For Cu, consistent effects on biomass C were present in both the sludge cake and liquid sludge experiments, indicating that in this case, Cu bioavailability to the microbial biomass was not being influenced through association with added organic matter. Indeed, the extractability of the Cu in the sludge cake experiments was higher than the Cu-amended liquid sludge (as measured by NH_4NO_3 extraction) experiments. Hence, the form of Cu application appears to be secondary to total topsoil Cu concentrations *per se*. This hypothesis is further supported by data from the metal salt sites (Defra Project SP0133), where inverse relationships between soil Cu and biomass C were also evident (Table 80). Again, this could have important implications for the application of a wide range of materials to agricultural land (*e.g.* copper sulphate fungicides, farm manures, green waste compost, paper mill crumble *etc.*).

Table 80. Summary of relationship between total topsoil Zn and Cu and soil biomass C – sludge cake, metal-amended liquid sludge and metal salt experiments (% change up to statutory soil metal limits)

Sludge cake	Zinc				Copper			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	16% **	36% ***	18% *	12% **	16% **	19% **
WAT	NS	22% *	19% **	19% *	NS	17% **	13% *	22% *
ROS	NS	14% *	14% **	30% **	10% **	30% ***	NS	20% *
Liquid sludge								
WOB	NS	NS	NS	24% *	26% *	13% *	NS	29% *
WAT	22% *	NS	NS	NS	NS	33% **	26% ***	28% *
ROS	NS	NS	NS	NS	NS	24% **	NS	NS
Metal salts								
WOB	9% *	NS	NS	NS	16% **	12% ***	16% *	19% *
WAT	NS	NS	NS	NS	NS	12% *	16% **	29% ***
ROS	NS	NS	NS	NS	NS	10% *	NS	21% ***

Red = inverse relationship

Soil organic carbon

Soil organic C levels in spring 2003 and 2005 were generally *higher* on the treatments receiving metal-amended liquid sludges compared with the untreated controls, reflecting the liquid sludge organic matter additions during Phase I.

At Watlington on the metal dose-response treatments, regression of the plot soil organic C contents against total topsoil metal concentrations identified significant positive relationships ($P < 0.05$) on the Zn dose-response treatment in 2005, and the Cu dose-response treatment in 2003. However, there were no relationships on the Cd treatment at Watlington or at the other two sites.

These results indicate that there have not been any major effects on soil organic C degradation rates on the metal dose-response treatments, an effect which might have been expected if metal stress was occurring. However, the measurement of soil organic C can be a relatively insensitive indicator of changes in soil organic C degradation rates.

Respiration Quotient

In order to compare results from individual plots having different soil microbial biomass concentrations and soil respiration rates, the plot data were expressed as a Respiration Quotient – RQ:

On the metal dose-response treatments, there was a significant ($P < 0.05$) positive relationship between RQ and total topsoil Zn at Rosemaund in 2003, but no significant ($P < 0.05$) relationships at any of the three sites in 2005. During Phase II, there was a significant ($P < 0.05$) positive relationship between RQ and total topsoil Zn at Watlington in 1999 only.

For Cu, there were significant ($P < 0.05$) positive relationships between RQ and total topsoil Cu at Watlington in 2003 and Woburn in 2005. During Phase II, there were significant positive relationships ($P < 0.05$) between RQ and total topsoil Cu at Watlington and Rosemaund in 2001.

For Cd, there was a significant ($P < 0.05$) positive relationship between RQ and total topsoil Cd at Watlington, and an inverse relationship ($P < 0.05$) at Rosemaund in 2005. During Phase II, there were significant positive relationships ($P < 0.05$) between RQ and total topsoil Cd at Woburn and Rosemaund in 2001.

It is noteworthy that the regression line slopes were generally lower in 2003 and 2005, than they were in 1999 and 2001, which maybe due to seasonal effects or an indication of microbial respiration rates becoming more stable as carbon degradation slows.

The significant relationships ($P < 0.05$) between RQ and total topsoil metal concentrations have all been *positive* (excepting at Woburn in 2001 and Rosemaund in 2005) *i.e.* respiration rate per unit of biomass increased with increasing soil metal concentrations throughout Phases II and III.

Comparing RQ responses on the sludge cake and metal-amended liquid sludge experiments indicated only limited consistency between the two experiments (Table 81). For Zn, there were seven positive relationships ($P < 0.05$) between RQ and total topsoil Zn concentrations in the sludge cake experiments, but only two in the 'paired' liquid sludge experiments. On the Cu treatments, there were 10 positive relationships ($P < 0.05$) between RQ and total topsoil Cu concentrations in the sludge cake experiments, and four in the 'paired' liquid sludge experiments. For Cd, there was a single positive relationship ($P < 0.05$) between RQ and total topsoil Cd in the sludge cake experiments and four responses (two inverse and two positive) in the 'paired' liquid sludge experiments.

Table 81. Summary of relationship between total topsoil Zn, Cu and Cd and RQ – sludge cake and metal amended liquid sludge experiments (% change up to statutory soil metal limits)

Zinc	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	23% *	NS	98% **	NS	NS	NS	NS
WAT	19% *	38% **	NS	33% **	48% *	NS	NS	NS
ROS	29% ***	22% *	NS	62% *	NS	NS	41% *	NS

Copper	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	96% *	28% **	NS	52% ***	NS	NS	NS	90% *
WAT	72% **	52% ***	101% ***	51% ***	NS	51% **	55% **	NS
ROS	38% **	89% **	51% **	NS	NS	40% *	NS	NS

Cadmium	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	59% *	NS	NS	11% *	NS	NS
WAT	NS	NS	NS	NS	NS	NS	55% *	NS
ROS	NS	NS	NS	NS	NS	39% *	NS	26% *

Red = inverse relationship Blue = positive relationship

Biomass Quotient

In order to compare results from individual plots having different soil organic C contents and microbial biomass C concentrations the plot data were expressed as a Biomass Quotient - BQ:

On the metal dose-response treatments, there was a significant ($P < 0.05$) inverse relationship between BQ and total topsoil Zn at Watlington in 2005. During Phase II, there were significant inverse relationships ($P < 0.05$) between BQ and total topsoil Zn at Woburn in 1999 and Watlington in 2001.

For Cu, there were significant inverse relationships ($P < 0.05$) between BQ and total topsoil Cu at Watlington in both 2003 and 2005. During Phase II, there were significant inverse relationships ($P < 0.05$) between BQ and total topsoil Cu at Woburn and Watlington in 1999 and 2001, and at Rosemaund in 1999.

For Cd, there were no significant relationships ($P > 0.05$) between BQ and total topsoil Cd at any of the three sites in either sampling year. Also, during Phase II there were no significant relationships ($P > 0.05$) between BQ and total topsoil Cd at any of the three sites.

Where significant relationships ($P < 0.05$) between BQ and topsoil metals were present, they were all *inverse*, *i.e.* biomass carbon as a proportion of total soil organic C decreased with increasing total topsoil metal concentrations.

Comparing BQ responses on the sludge cake and metal-amended liquid sludge experiments indicated good agreement between the two experiments (Table 82). For Zn, there were four inverse relationships ($P < 0.05$) between BQ and total topsoil Zn on the sludge cake experiments, and three on the 'paired' liquid sludge experiments. On the Cu treatments, there were 10 inverse relationships ($P < 0.05$) between BQ and total topsoil Cu on the sludge cake experiments, and seven on the paired liquid sludge experiments. For Cd, there were no relationships ($P > 0.05$) between BQ and total topsoil Cd on either the sludge cake or the 'paired' liquid sludge experiments.

Table 82. Summary of relationships between total topsoil Zn, Cu and Cd and BQ – sludge cake and metal amended liquid sludge experiments (% change up to statutory soil metal limits)

Zinc	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	NS	45% *	NS	15% *	NS	NS
WAT	NS	29% *	21% ***	NS	22% *	NS	NS	17% *
ROS	NS	NS	14% *	NS	NS	NS	NS	NS

Copper	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	20% *	NS	22% ***	23% **	23% *	13% *	NS	NS
WAT	NS	21% **	18% ***	32% **	17% **	34% *	34% ***	29% **
ROS	17% ***	25% **	18% *	21% *	NS	33% **	NS	NS

Cadmium	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	NS	NS	NS	NS	NS	NS
WAT	NS	NS	NS	NS	NS	NS	NS	NS
ROS	NS	NS	NS	NS	NS	NS	NS	NS

Red = inverse relationship.

Rhizobium most probable numbers

Rhizobium populations measured in soil samples taken in 2003 and 2005 were generally similar on the treatments that had received uncontaminated metal-amended liquid sludges compared with the untreated controls.

On the metal dose-response treatments, there was a single significant relationship ($P < 0.05$) between rhizobia MPNs and total topsoil Zn at Rosemaund in 2003, but no relationships ($P > 0.05$) for Cu or Cd. During Phase II, there were no significant relationships ($P > 0.05$) between rhizobia MPNs and total topsoil Zn, Cu or Cd at any of the three sites in either sampling year.

Comparing rhizobia MPN responses on the sludge cake and metal-amended liquid sludge experiments indicated little (or no) consistency between the two experiments (Table 83). For Zn, there were nine inverse relationships ($P < 0.05$) between rhizobia MPNs and total topsoil Zn on the sludge cake experiments, and only one on the 'paired' liquid sludge experiment, with responses on the Watlington sludge cake experiment in all four years, at Rosemaund in the three most recent years and Woburn in the two most recent years. On the Cu treatments, there were four inverse relationships ($P < 0.05$) between rhizobia MPNs and total topsoil Cu on the sludge cake experiments and none on the 'paired' liquid sludge experiments, although the responses on the sludge cake experiments themselves were not consistent. For Cd, there were two inverse relationships ($P < 0.05$) between rhizobia MPNs and total topsoil Cd on the sludge cake experiments and none on the 'paired' liquid sludge experiments, although the responses on the sludge cake experiment were not consistent between sites and years.

Table 83. Summary of relationship between total topsoil Zn, Cu and Cd and rhizobia MPNs – sludge cake and metal-amended liquid sludge experiments (% change up to statutory soil metal limits)

Zinc	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	36% **	59% ***	NS	NS	NS	NS
WAT	11% *	25% **	19% **	27% ***	NS	NS	NS	NS
ROS	NS	20% ***	28% **	47% ***	NS	NS	21% *	NS

Copper	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	16% *	NS	NS	NS	NS	NS	NS	NS
WAT	13% **	NS	NS	NS	NS	NS	NS	NS
ROS	5% *	NS	NS	7% *	NS	NS	NS	NS

Cadmium	Sludge cake				Liquid sludge			
	1999	2001	2003	2005	1999	2001	2003	2005
WOB	NS	NS	NS	18% **	NS	NS	NS	NS
WAT	NS	NS	NS	NS	NS	NS	NS	NS
ROS	NS	NS	27% ***	NS	NS	NS	NS	NS

Red = inverse relationship

On the metal-amended liquid sludge experiments, there was no evidence of decreasing rhizobia MPNs over time (Figures 120 - 122).

Figure 120. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Woburn liquid sludge

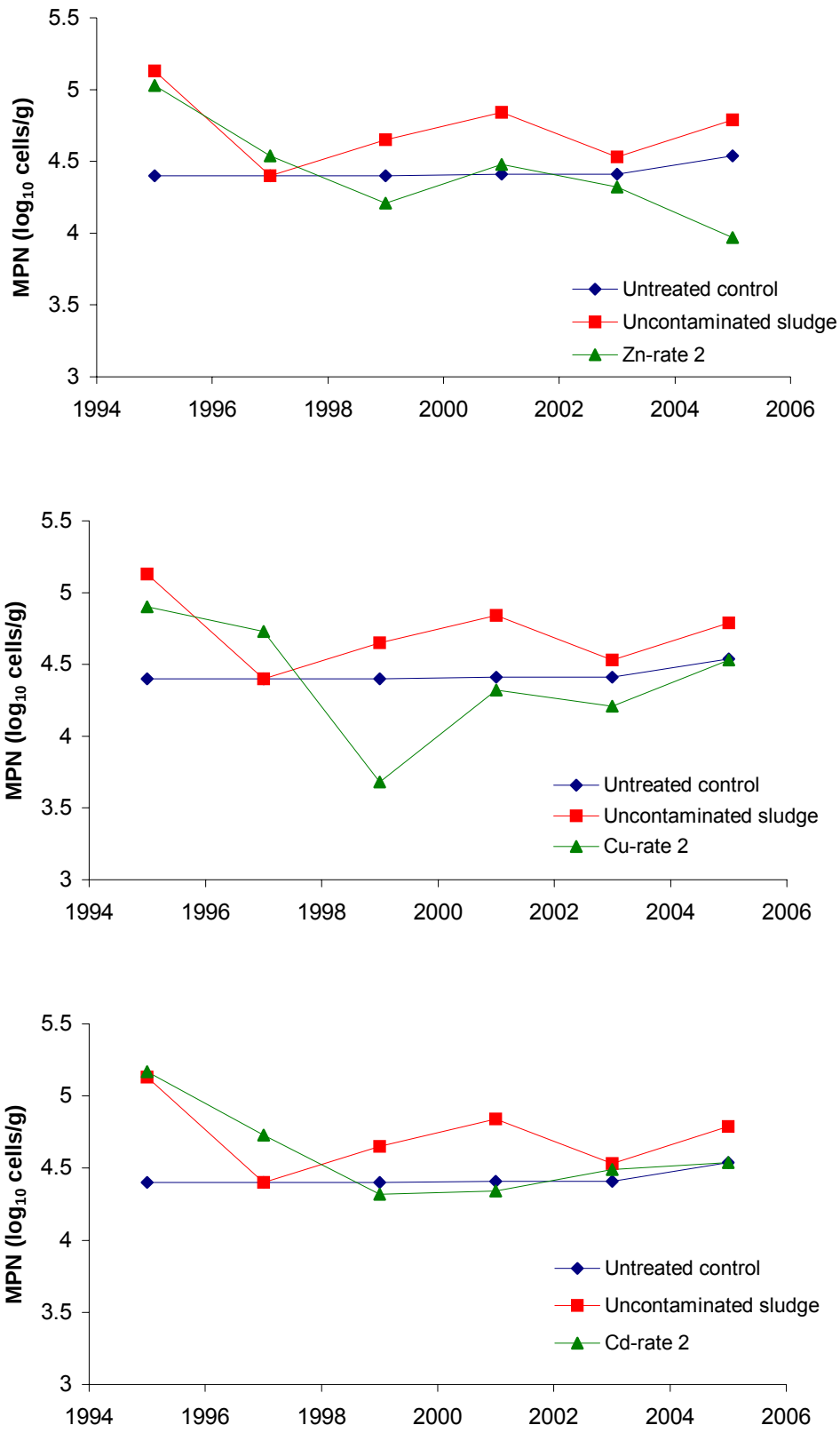


Figure 121. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Watlington liquid sludge

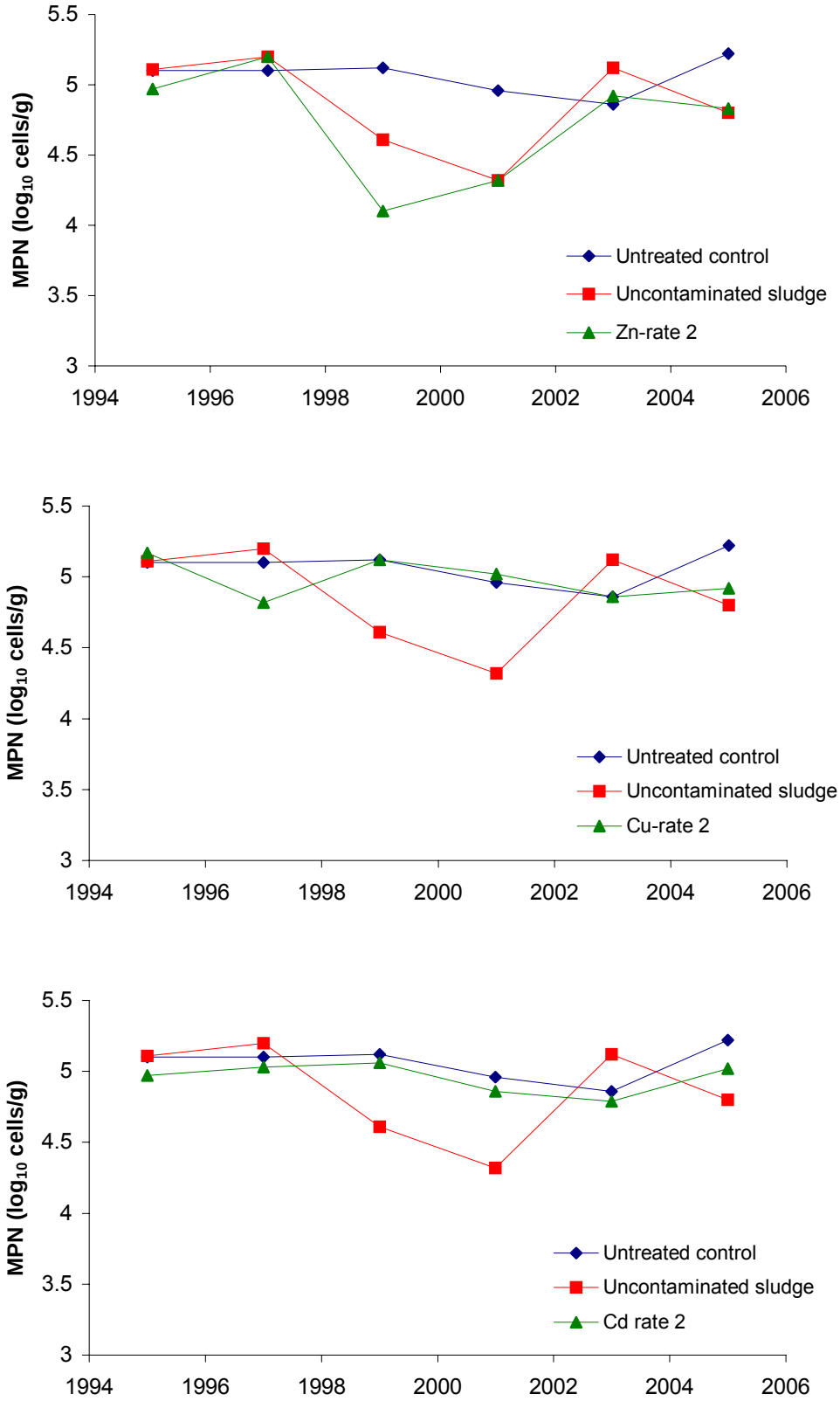
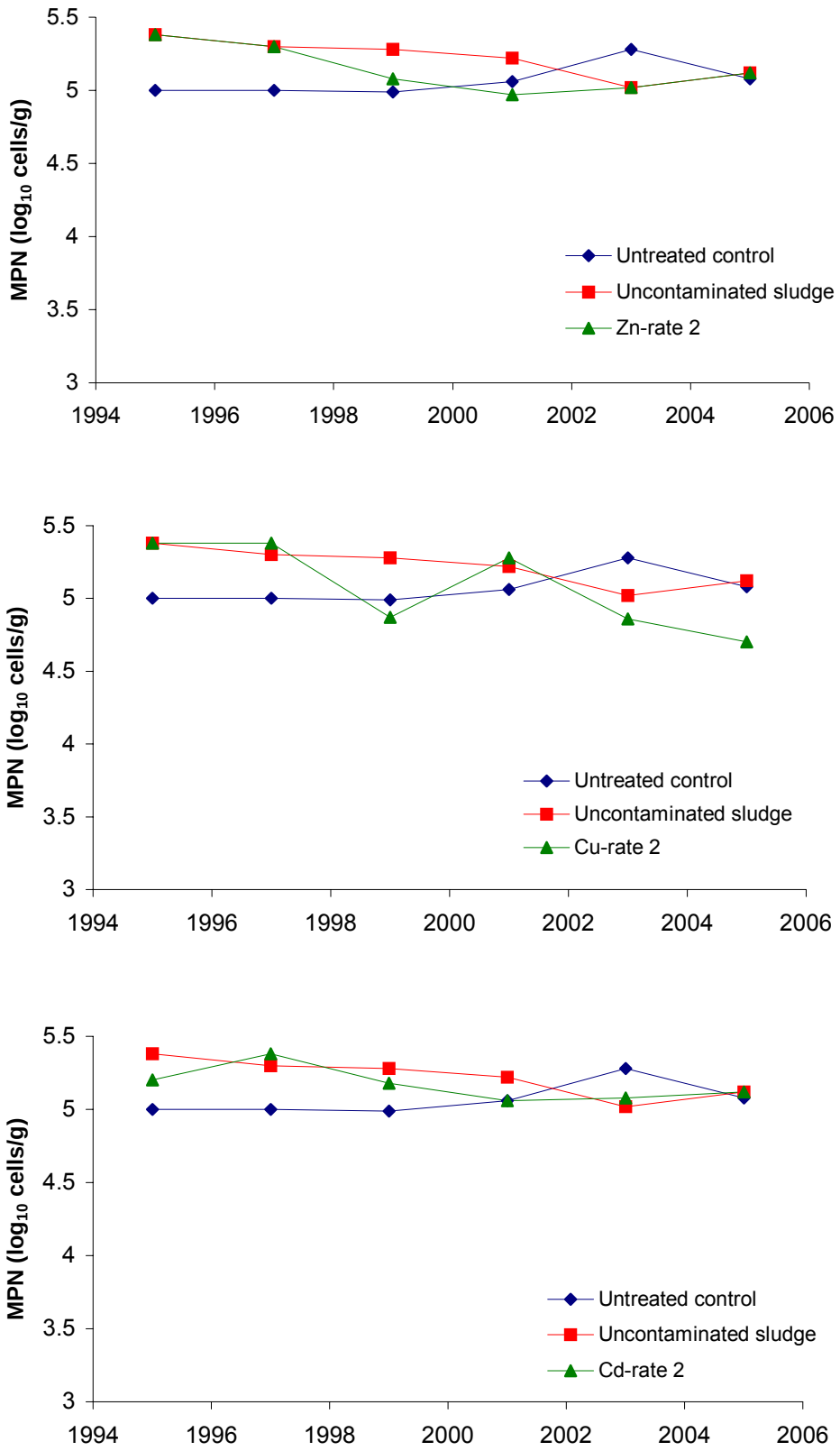


Figure 122. Mean rhizobia MPNs on the Zn, Cu and Cd treatments over time – Rosemaund liquid sludge



The fact that no consistent effects were seen on rhizobia MPNs in the metal-amended liquid sludge experiments, compared with the sludge cake experiments, was again unexpected and surprising. Conventional wisdom would suggest that metals added in the metal-amended liquid sludges would be more detrimental to micro-organisms than the sludge cake amendments, because the added organic matter in the sludge cake experiments would ameliorate metal toxicity to micro-organisms by binding metals, thus reducing bioavailability, and also through 'physical' protection (Babich and Stotzky, 1980). Certainly, NH_4NO_3 extractable soil Zn and Cd concentrations were initially higher in the liquid sludge treatments (Gibbs *et al.,* 2006b), thus supporting the above line of thought. However, Zn added to soil in the sludge cake had more adverse effects on rhizobia MPNs compared to little or no measurable effects of adding Zn to soil in the metal-amended liquid sludge.

It is possible that no detrimental effects of Zn were seen on the liquid sludge experiments because the metals were <u>not</u> associated with organic matter. In the liquid sludge experiment, the applied Zn would be more evenly incorporated throughout the soil and not as 'hot spots' of high concentrations associated with organic matter like in the sludge cake experiments (see Section 5.1). We suggest that in the metal-amended liquid sludge experiment rhizobia are mostly associated with uncontaminated organic matter from root exudates, dead roots and small amounts of straw or grass residues left after harvest, and incorporated into the soil during cultivation. Therefore, in the metal-amended liquid sludge experiment the rhizobia are not exposed to the 'hot spots' of high Zn concentrations and associated organic matter, as they would be in the sludge cake experiments.

Ammonium nitrate extractable metal concentrations

Topsoil NH_4NO_3 extractable Zn and Cd concentrations in 2003 were highest at Rosemaund, where Zn concentrations on the rate 3 treatment (23mg/kg) were 4-fold higher than at Woburn (6 mg/kg) and 8-fold higher than at Watlington (3 mg/kg), and Cd concentrations on the rate 3 treatment (0.13 mg/kg) were double those at Woburn (0.08 mg/kg) and 8-fold higher than at Watlington (0.017 mg/kg). However, NH_4NO_3 extractable Cu concentrations were higher at Woburn (1.2 mg/kg) than at Watlington (0.64 mg/kg) or Rosemaund (0.72 mg/kg). In 2005, NH_4NO_3 extractable Zn and Cd concentrations were highest at Woburn, where Zn concentrations on the rate 3 treatment (16 mg/kg) were double those at Rosemaund (7.6 mg/kg) and 6-fold higher than at Watlington (2.8 mg/kg). For Cd, concentrations (0.10 mg/kg) were 50% higher than at Watlington (0.07 mg/kg) and 3-fold higher than at Rosemaund (0.03 mg/kg). Ammonium nitrate extractable Cu concentrations in 2005, were higher at Watlington (1.60 mg/kg) than at Woburn (1.01 mg/kg) or Rosemaund (0.63 mg/kg).

At all three sites, there were strong relationships ($P < 0.001$) between total topsoil Zn, Cu and Cd concentrations, and corresponding topsoil NH_4NO_3 extractable concentrations (r^2 = 73-92% for Zn, 74-98% for Cu and 31-88% for Cd). At Watlington (for Zn and Cd) and Woburn (for Cd)

in 2003, extractable metal concentrations were below the limits of analytical detection.

Liquid sludge and cake sites: Ammonium nitrate extractable Zn concentrations were generally higher in the metal-amended liquid sludge experiment than the sludge cake experiment at Woburn, with concentrations similar at Watlington and Rosemaund (Figures 123 and 124). Extractable Cu concentrations tended to be higher in the sludge cake experiment at Watlington compared with the metal-amended liquid sludge experiment, with similar concentrations at Woburn and Rosemaund (Figures 123 and 124). There were no consistent differences with time (2003 and 2005) in extractable Cd concentrations between the metal-amended liquid sludge and sludge cake experiments, with extractable Cd concentrations most strongly influenced by pH differences between sites.

Figure 123. Comparison of mean metal extractability between sludge cake and metal-amended liquid sludge treatments – spring 2003 (3 sites in England)

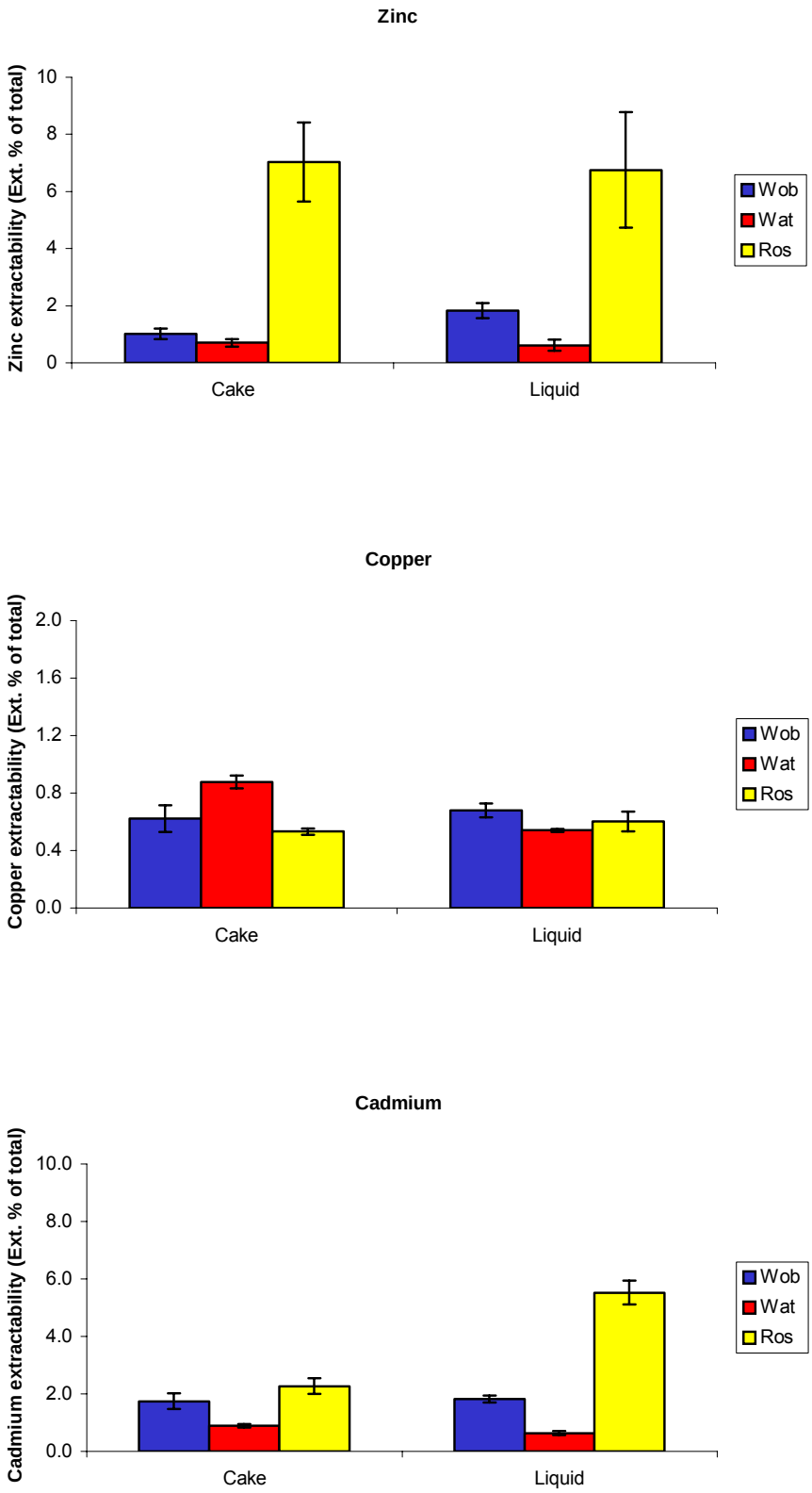
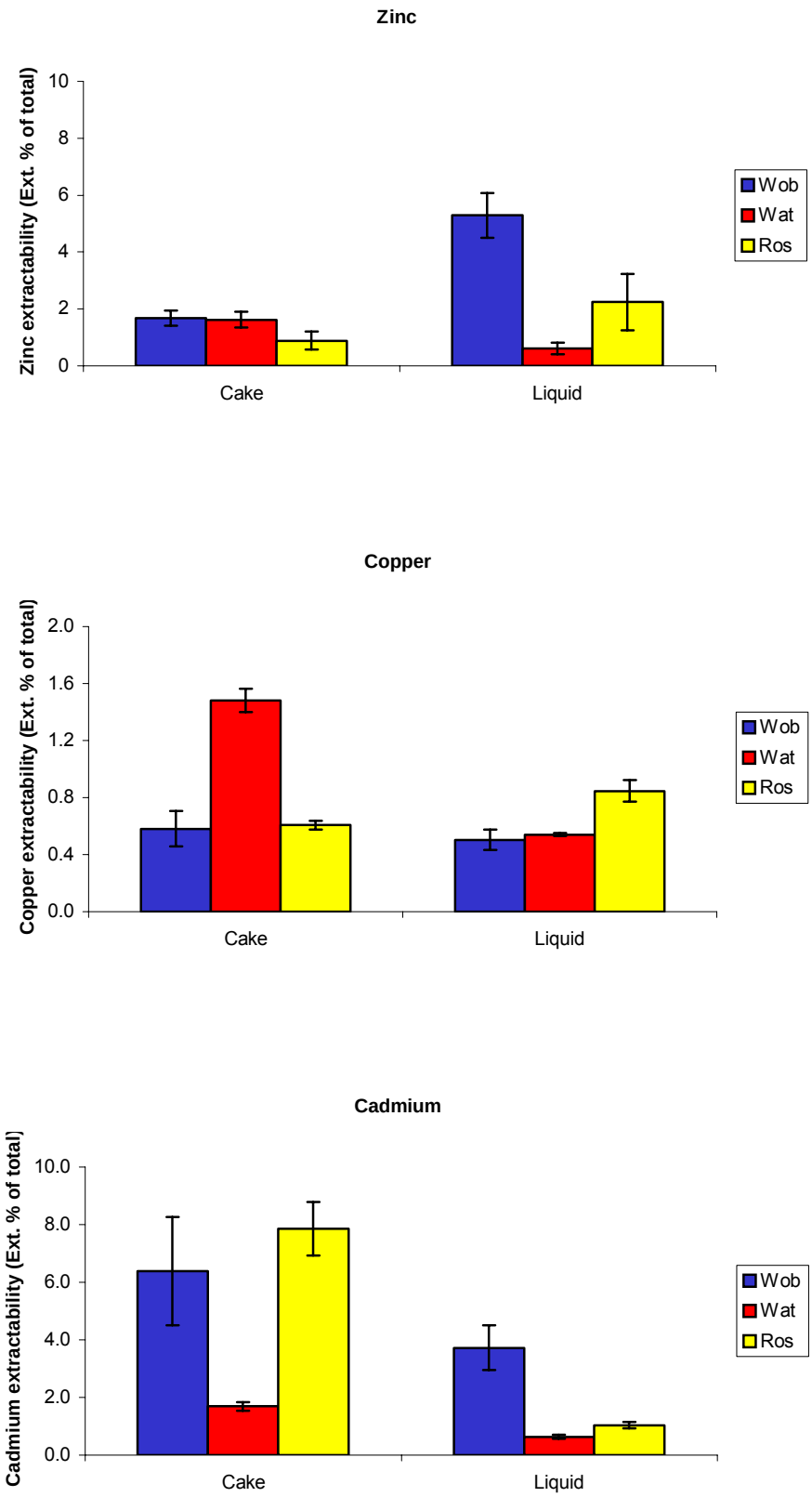


Figure 124. Comparison of mean metal extractability between sludge cake and metal-amended liquid sludge treatments – spring 2005 (3 sites in England)



Metal speciation

Across the three sites in England, there were strong relationships ($P < 0.001$) between soil pore water total soluble Zn, Cu and Cd and soil pore water free Zn^{2+} (r^2 = 92%), Cu^{2+} (r^2 = 88%) and Cd^{2+} (r^2 = 98%) concentrations, respectively (Figure 125). Approximately 50% of the soil pore water total soluble Zn was in the form of free Zn^{2+}, and 30% of soil pore water total soluble Cd in the form of free Cd^{2+}. In the metal speciation measurements carried out in 2001, soil pore water free Zn^{2+} and Cd^{2+} represented 85% and 98% of soil pore water total soluble Zn and Cd concentrations, respectively. The reduction in the proportion of soil pore water total soluble Zn and Cd present as free Zn^{2+} and Cd^{2+} in 2004 compared with 2001, was most probably due to the soil pH being closer to the target pH of 6.5 in the field experiments. Soil pore water free Zn^{2+} and free Cd^{2+} both decreased in the order Woburn > Rosemaund > Watlington.

Less than 0.1% of the soil pore water total soluble Cu was present as soil pore water free Cu^{2+}. The order of decreasing soil pore water free Cu^{2+} was Woburn > Rosemaund > Watlington.

The relationships between soil pore water total soluble Zn, Cu and Cd concentrations, and topsoil NH_4NO_3 extractable metal concentrations across the three sites are shown in Figure 126. The relationship for Zn accounted for 77%, for Cu 52% and for Cd 82% of the variation. Water soluble Zn, Cu and Cd on average accounted for 4, 20 and 2% of the NH_4NO_3 extractable Zn, Cu and Cd concentrations, respectively.

Figure 125. Relationship between soil pore water total soluble Zn, Cu and Cd and free Zn^{2+}, Cu^{2+} and Cd^{2+} - 2004

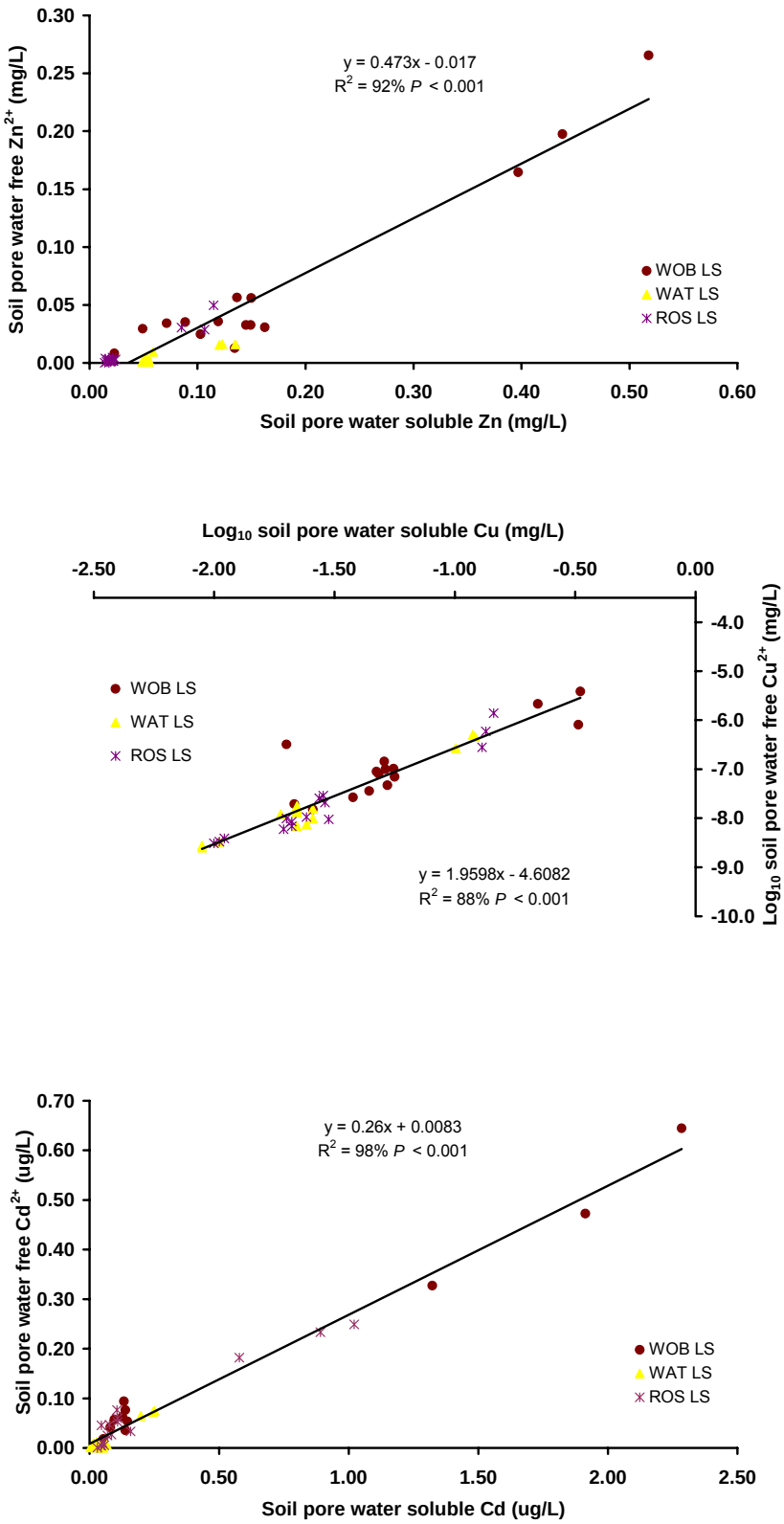
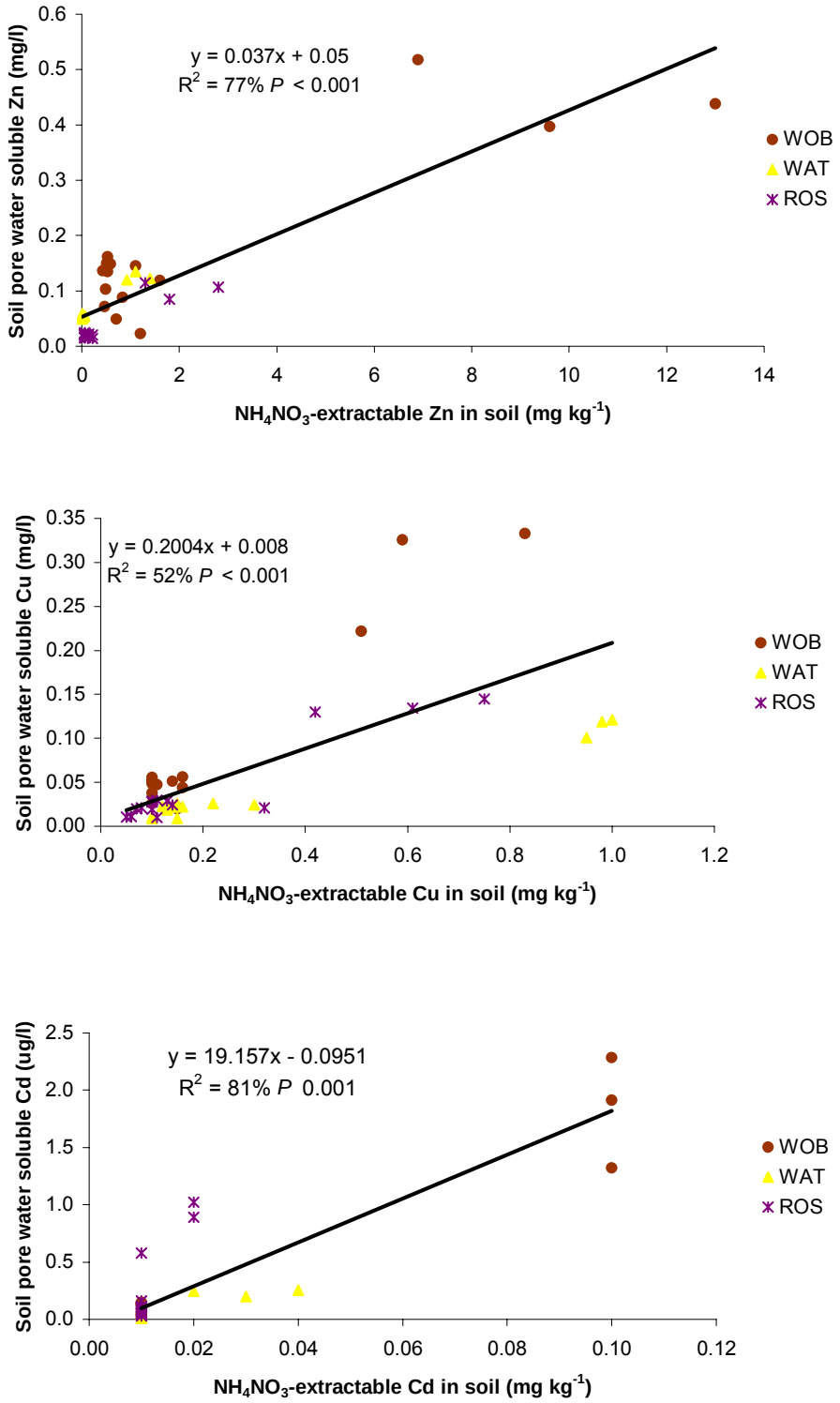


Figure 126. Relationship between soil pore water total soluble Zn, Cu and topsoil NH_4NO_3 extractable Zn, Cu and Cd concentrations



Crop yields and crop metal concentrations

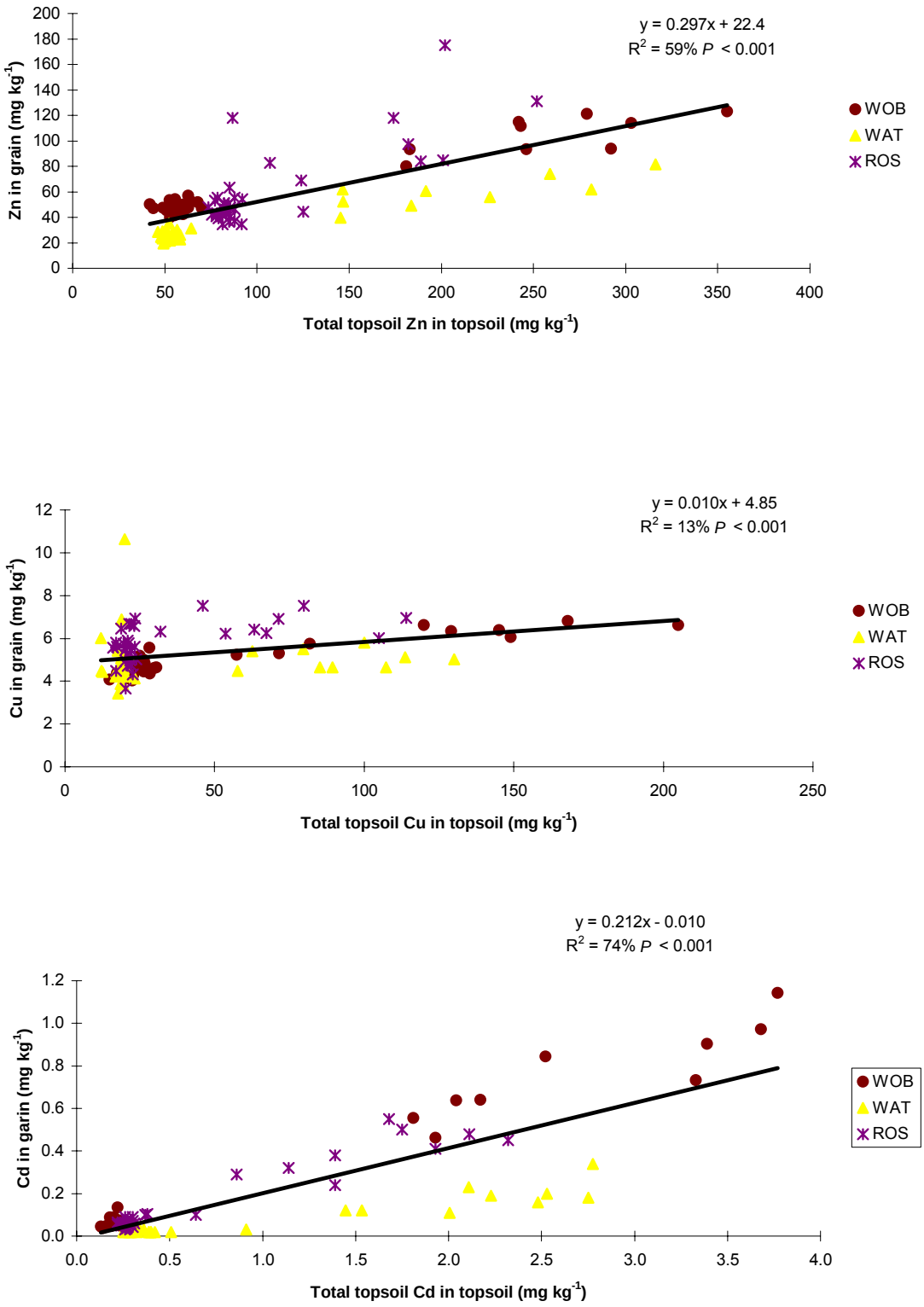
First cut grass yields (2002 and 2004) and grain yields (2003 and 2005) were measured to assess any impacts from the metal-amended liquid sludge applications in Phase I on crop metal bioavailability and productivity. Generally, the metal-amended liquid sludge treatments produced similar or higher yields than the untreated controls, which was probably a reflection of the beneficial effects of adding organic matter to establish the metal dose-response treatments.

On the metal-dose response treatments, there were no significant ($P > 0.05$) relationships between grass yields and total topsoil metal concentrations in either 2002 or 2004. However, there were significant ($P < 0.05$) inverse relationships between grain yields and total topsoil Zn and Cu concentrations at Watlington in 2003, and a significant positive relationship for total topsoil Cu at Woburn in 2003.

Over all there were strong positive relationships ($P < 0.001$) between grain Zn (r^2 = 62-93% and 82-90% in 2003 and 2005, respectively) and Cd (r^2 = 87-98% and 89-96% in 2003 and 2005, respectively) concentrations and corresponding total topsoil metal concentrations at all three sites, with the exception of Watlington in 2005 where grain yields were insufficient to provide a sufficient sample size for grain analysis. At Woburn, in both 2003 and 2005, there were strong relationships ($P < 0.001$) between grain Cu and total topsoil Cu concentrations, however, at Rosemaund in 2003 the relationship was weak ($P < 0.01$: r^2 = 26%) and at Watlington in 2003 and Rosemaund in 2005 there were no relationships ($P > 0.05$).

The relationships between grain Zn, Cu and Cd concentrations and total topsoil metal concentrations across the three sites in 2003 are shown in Figure 127. In 2003, the relationships ($P < 0.001$) between grain metals and total topsoil Zn concentrations accounted for 59% of the variation, for Cu 13% and for Cd 74%. The data from 2005 have been excluded owing to the lack of sufficient yields at Woburn and Watlington.

Figure 127. Relationship between grain Zn, Cu and Cd concentrations (harvest 2003) and total topsoil Zn, Cu and Cd concentrations – 2003



6. CONCLUSIONS

6.1 Sludge cake experiments

Soil microbial properties

- Soil respiration rates measured in soil samples taken from all the treatment plots in spring 2003 and 2005 tended to be *higher* (*i.e.* increased CO_2 evolution) on the treatments receiving low metal sludge cakes compared with the untreated controls, due to the sludge cake organic matter additions providing additional organic C for microbial respiration.

  On the metal dose-response treatments, there were significant ($P < 0.05$) inverse (*i.e.* soil respiration rate decreased with increasing metal concentration) relationships between soil respiration rates and total topsoil Zn concentrations at Hartwood in both 2003 and 2005, and for Cd at Woburn and Hartwood in 2003. For Cu, there were significant ($P < 0.05$) positive (*i.e.* soil respiration rate increased with increasing metal concentration) relationships between soil respiration rates and total topsoil Cu concentrations at Watlington and Auchincruive in both 2003 and 2005, and Rosemaund in 2003.

  Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) it is difficult to see any clear and consistent effect of metal additions on soil respiration rates.

- Soil microbial biomass carbon concentrations in samples taken from all the treatment plots in 2003 and 2005 were generally higher on the treatments receiving low metal sludge cakes compared with the untreated controls, again due to the sludge cake organic matter additions which provide additional organic C for microbial assimilation.

  On the metal dose-response treatments, there were significant ($P < 0.05$) inverse relationships between soil biomass C concentrations and total topsoil Zn concentrations at four sites in both 2003 and 2005 (Woburn, Watlington, Rosemaund and Bridgets), at Hartwood and Auchincruive in 2003, and Shirburn in 2005. For Cu, there were significant relationships at three sites in both 2003 and 2005 (Woburn, Watlington and Hartwood), and at Gleadthorpe and Rosemaund in 2005. For Cd, there was no consistent effect on biomass concentrations.

  Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) at all the sites where there was a relationship between soil biomass C and total topsoil Zn or Cu, the relationship was inverse i.e. biomass levels decreased with increasing metal concentrations. These significant responses were on average c.20% lower at the UK statutory soil metal limit values than the low metal sludge cake controls.

- There were significant relationships ($P < 0.05$) between the *Respiration Quotient-RQ* and total topsoil metal concentrations. For Zn, there were significant positive relationships ($P < 0.05$) between RQ and total topsoil Zn at five sites (Woburn, Watlington,

Rosemaund, Bridgets and Shirburn) in 2005. For Cu, at three sites in both 2003 and 2005 (Watlington, Hartwood and Auchincruive), at Rosemaund and Bridgets in 2003, and Woburn and Shirburn in 2005. For Cd, at Pwllpeiran in 2003 and Gleadthorpe and Shirburn in 2005, there were significant positive relationships, and a significant inverse relationship at Woburn in 2003.

Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) at all the sites (except Cd at Woburn in 2003) where there was a statistically significant relationship between RQ and total topsoil metal concentrations the relationship was positive, i.e. RQ increased with increasing topsoil metal concentrations.

- There were significant inverse relationships ($P < 0.05$) between the *Biomass Quotient-BQ* and total topsoil metal concentrations. For Zn, there were significant relationships ($P < 0.05$) between BQ and total topsoil Zn at Hartwood in both 2003 and 2005, at Watlington, Rosemaund and Auchincruive in 2003, and at Woburn, Bridgets and Shirburn in 2005. For Cu, there were significant relationships at three sites in both 2003 and 2005 (Woburn, Watlington and Rosemaund), at Gleadthorpe, Hartwood and Auchincruive in 2003, and Shirburn in 2005. For Cd, there were no significant ($P > 0.05$) relationships between BQ and total topsoil Cd concentrations in either 2003 or 2005.

  Over the course of the four sampling events to date (1999, 2001, 2003 and 2005) at all the sites where there was a relationship between the BQ and total topsoil Zn or Cu, the relationship was inverse i.e. BQ decreased with increasing metal concentrations.

- There were significant relationships ($P < 0.001$) between clover rhizobia MPNs and total topsoil metal concentrations. For Zn, on the metal dose-response treatments, there were inverse relationships ($P < 0.05$) between rhizobia MPNs and Zn at six sites in both 2003 and 2005 (Gleadthorpe, Woburn, Watlington, Rosemaund, Bridgets and Hartwood), and at Pwllpeiran and Auchincruive in 2005. For Cu, there were significant relationships at Auchincruive in 2003 and at Pwllpeiran, Rosemaund, Bridgets and Hartwood in 2005. For Cd, there were significant relationships at Rosemaund and Shirburn in 2003, and at Gleadthorpe, Woburn, Pwllpeiran and Hartwood in 2005. (although three of these relationships were also significant for Zn) At all the sites (except for Cu at Shirburn in 2005) where a statistically significant relationship between rhizobia MPNs and total topsoil metals was evident rhizobia MPNs *decreased* with increasing topsoil metal concentrations.

  Soil rhizobia MPNs decreased with increasing total topsoil Zn concentrations at three of the sludge cake sites in all four measurement years (1999, 2001, 2003 and 2005), and at eight of the sites in 2005. Moreover, at the Hartwood site rhizobia MPN levels were not detectable above 301 mg/kg in 2003 and above 188 mg/kg Zn in 2005. Additionally, on the Zn rate 3 treatments over time (i.e. 1994 – 2005) there was a trend of decreasing rhizobia MPNs at eight

of the sludge cake sites compared with the low metal sludge cake control treatment. The body of evidence from the results to date indicates that Zn, rather than Cd, is likely to have been responsible for decreases in Rhizobium population sizes measured in previous studies.

Soil chemical properties

- Ammonium nitrate (NH_4NO_3) extractable topsoil Zn concentrations were consistently higher at Hartwood (7% of total) than at the other non-calcareous sites, and lowest at the calcareous Shirburn site (0.1% of total). At Hartwood, this was partly due to higher total topsoil Zn concentrations, but also the greater extractability of Zn at this site, which was probably related to the lower soil pH (5.7-6.0) than the other sites (the non-calcareous sites in England and Wales are limed to a target pH of 6.5). The low extractable Zn concentrations at Shirburn were principally related to the high soil pH value (*c.*8.4) and associated free calcium carbonate (lime) content of the soil. Extractable topsoil Cu concentrations were consistently higher at Watlington (1.5% of total) than at the other sites, and lowest at Hartwood (0.25% of total). Extractable Cd concentrations were consistently higher at Auchincruive and Rosemaund (*c.*7.9% of total) than at the other sites, and lowest at Bridgets and Shirburn (*c.*0.55% of total).

  Across the nine sites, there were strong relationships ($P < 0.01$) between total topsoil Zn, Cu and Cd concentrations and the corresponding topsoil NH_4NO_3 extractable metal concentrations (r^2 = 44-95% for Zn, 43-97% for Cu and 14-76% for Cd); where extractable Zn and Cd concentrations were above analytical limits of detection.

- Across the seven sites in England and Wales, there were strong relationships ($P < 0.001$) between soil pore water total soluble Zn (r^2 = 85%), Cu (r^2 = 58%) and Cd (r^2 = 83%) and soil pore water free Zn^{2+}, Cu^{2+} and Cd^{2+}, respectively. Approximately 60% of the soil pore water total soluble Zn was in the form of free Zn^{2+} and 45% of the soil pore water total soluble Cd was in the form of free Cd^{2+}. In the case of Cu, < 0.1% of porewater total soluble Cu was in the form of free Cu^{2+}.

  There were strong relationships ($P < 0.001$) between soil pore water total soluble Zn (r^2 = 53%), Cu (r^2 = 37%) and Cd (r^2 = 30%) concentrations, and corresponding topsoil NH_4NO_3 extractable Zn, Cu and Cd concentrations.

Crop yields and metal concentrations

- First cut grass yields (2002 and 2004) were similar or higher on the sludge cake treatments compared with the untreated controls. There were significant ($P < 0.05$) *positive* relationship between ryegrass yields and total topsoil Zn and Cu concentrations at Auchincruive in 2002, and at Bridgets (total Zn) and Hartwood (total Cu) on 2004.

- Grain yields (2003 and 2005) at the 6 sites in England were similar or higher on the sludge cake treatments compared with the untreated controls. There was a significant ($P < 0.05$) *positive* relationship between grain yields and total topsoil Cu at Woburn in 2003. However, there were no significant relationships ($P > 0.05$) between total topsoil metal concentrations and grain yields on any of the other treatments at the six sites in 2003 and 2005.

  There were moderate to strong relationships ($P < 0.001$) between grain Zn (r^2 = 59-84%), Cu (r^2 = 32-79%) and Cd (r^2 = 45-94%) concentrations, and corresponding total topsoil metal concentrations in 2003. However, in 2005, relationships between grain metals and total topsoil metals were not as strong.

6.2 Metal-amended liquid sludge experiments

Soil microbial properties

- Soil respiration rates measured in samples taken from all the treatment plots at the three sites in spring 2003 and 2005 were generally *higher* on the treatments receiving low metal-amended liquid sludges compared with untreated controls, as a result of the sludge organic matter additions made during Phase I which provided organic C for microbial respiration.

  On the metal dose-response treatments, there were significant ($P < 0.05$) *positive* relationships between soil respiration rates and total topsoil Zn concentrations at Rosemaund in 2003, for Cu at Woburn in both 2003 and 2005, and Cd at Watlington in 2003.

  Over the course of the four measurements to date (1999, 2001, 2003 and 2005) where there were relationships between soil respiration rates and total topsoil metal concentrations, these were all positive i.e. soil respiration rates increased with increasing topsoil metal concentrations. Although, overall the sites it was difficult to see any clear and consistent effects of the metal additions on soil respiration rates.

- Soil microbial biomass C concentrations measured on samples from the three sites were generally *higher* on the treatments receiving low metal liquid sludges compared with the untreated controls. These increases can be related to the sludge organic matter additions made during Phase I providing organic C for microbial assimilation.

  On the metal dose-response treatments, there were significant ($P < 0.05$) *inverse* relationships between soil biomass C concentrations and total topsoil Zn concentrations at Woburn in 2005, and for Cu at Watlington in both 2003 and 2005 and at Woburn in 2005.

  Over the course of the four measurements to date (1999, 2001, 2003 and 2005) where there were significant relationships between total soil Zn and Cu concentrations these were always inverse i.e. biomass C concentrations decreased with increasing metal concentrations.

- On the metal dose-response treatments, there were significant ($P < 0.05$) *positive* relationships between the Respiration Quotient-RQ and

total topsoil Zn concentrations at Rosemaund in 2003, for Cu at Watlington in 2003 and Woburn in 2005, and for Cd at Watlington in 2003.

Over the course of the four measurements to date (1999, 2001, 2003 and 2005) where there were significant relationships between RQ and total topsoil Zn and Cu concentrations and these were all positive i.e. RQ increased with increasing metal concentrations.

- On the metal dose-response treatments, there were significant ($P < 0.05$) *inverse* relationships between the Biomass Quotient-BQ and total topsoil Zn concentrations at Watlington in 2005, and for Cu at Watlington in both 2003 and 2005. There were no relationships between BQ and total topsoil Cd.

  Over the course of the four measurements to date (1999, 2001, 2003 and 2005) where there were significant relationships between BQ and total topsoil Zn and Cu these were all inverse i.e. BQ decreased with increasing metal concentrations.

- On the metal dose-response treatments, there was only one significant ($P < 0.05$) inverse relationship between *rhizobia* MPNs and total topsoil Zn at Rosemaund in 2003, but no relationships ($P > 0.05$) for either Cu or Cd.

  Over the course of the four measurements to date (1999, 2001, 2003 and 2005) there was a single significant ($P < 0.05$) inverse relationship between rhizobia MPNs and total topsoil Zn (Rosemaund in 2003) and no relationships for Cu or Cd.

Soil chemical properties

- At all three sites, there were strong relationships between topsoil total Zn, Cu and Cd concentrations (r^2 = 73-92% for Zn, 74-98% for Cu and 31-93% for Cd) and corresponding topsoil NH_4NO_3 extractable metal concentrations.

- Across the three sites, there were strong relationships ($P < 0.001$) between soil pore water total soluble Zn (r^2 = 92%), Cu (r^2 = 88%) and Cd (r^2 = 98%), and soil pore water free Zn^{2+} Cu^{2+} and Cd^{2+} concentrations, respectively. Additionally there were strong relationships ($P < 0.001$) between soil pore water total soluble Zn (r^2 = 77%), Cu (r^2 = 52%) and Cd (r^2 = 82%) concentrations, and corresponding topsoil NH_4NO_3 extractable metal concentrations.

Crop yields and metal concentrations

- First cut grass yields (2002 and 2004) and grain yields (2003 and 2005) at the three sites were similar or higher on the metal-amended liquid sludge treatments compared with the untreated controls.

- On the metal dose-response treatments, there were no relationships ($P > 0.05$) between grass yields and total topsoil metal concentrations. However, there were significant ($P < 0.05$) inverse relationships between grain yields and total topsoil Zn and Cu at

Watlington in 2003, and a significant positive relationship between grain yield and total topsoil Cu at Woburn in 2003.

There were strong positive relationships ($P < 0.05$) between grain Zn (r^2 = 62-93%) and Cd (r^2 = 87-98%) concentrations and corresponding total topsoil metal concentrations at the three sites. At Woburn in both 2003 and 2005 there were strong relationships ($P < 0.001$) between grain Cu and total topsoil Cu concentrations. However, at Rosemaund in 2003 the relationship was weak (r^2 = 26%) and for the other site years there were no relationships.

Comparison of sludge cake and liquid sludge sites

- Soil respiration rate responses on the sludge cake and metal-amended liquid sludge experiments showed *limited agreement* (only on the Zn treatments at Rosemaund in 2003 were there similar responses). *To date in both experiments, it is difficult to see any clear and consistent respiration rate responses to metal additions*.

- Soil biomass C responses in the sludge cake and metal-amended liquid sludge experiments showed 'good' agreement for Cu and Cd. For Cu, the size and number of responses were similar in both experiments, and for Cd there were no consistent responses in either experiment. However, there was little agreement for Zn, with eight responses (out of 12) in the sludge cake experiments and only two in the 'paired' Zn-amended liquid sludge experiment.

  For Cu, there appear to be consistent effects on the microbial biomass in both the sludge cake and metal-amended liquid sludge experiments. This indicates, in the case of Cu, that bioavailability to the microbial biomass is not being influenced through association with added organic matter. Indeed, the soil extractability of Cu in the sludge cake experiments was higher than the Cu-amended liquid sludge experiments (as measured by NH_4NO_3 extraction). Hence, the form of Cu application appears to be secondary to total topsoil Cu concentrations *per se*. This could have major implications for the application of other materials to agricultural land (*e.g.* copper sulphate fungicides, farm manures, green waste compost, paper mill crumble *etc).*

- Respiration Quotient-RQ responses in the sludge cake and metal-amended liquid sludge experiments showed only *limited agreement*. For Zn, there were seven positive relationships ($P < 0.05$) between RQ and total topsoil Zn concentrations in the sludge cake experiments, but only two (out of 12) in the 'paired' liquid sludge experiments. On the Cu treatments, there were 10 positive relationships ($P < 0.05$) between RQ and total topsoil Cu concentrations in the sludge cake experiments and four (out of 12) in the 'paired' liquid sludge experiments. For Cd, there was a single inverse relationship ($P < 0.05$) between RQ and total topsoil Cd in the sludge cake experiments, and in the liquid sludge experiments it was difficult to see any consistent pattern (two inverse and two positive relationships).

- Biomass Quotient-BQ responses on the sludge cake and metal-amended liquid sludge experiments showed good agreement. For Zn, there were four inverse relationships ($P < 0.05$) between BQ and total topsoil Zn in the sludge cake experiments and three (out of 12) in the 'paired' liquid sludge experiments. On the Cu treatments, there were 10 inverse relationships ($P < 0.05$) between BQ and total topsoil Cu in the sludge cake experiments and seven (out of 12) in the 'paired' liquid sludge experiments. For Cd, there were no relationships ($P > 0.05$) between BQ and total topsoil Cd in either the sludge cake or 'paired' liquid sludge experiments.
- Rhizobia MPN responses on the sludge cake and metal-amended liquid sludge experiments showed little agreement. For Zn, there were nine inverse relationships ($P < 0.05$) between rhizobia MPNs and total topsoil Zn in the sludge cake experiments and only one (out of 12) in the liquid sludge experiments. For Cu, there were four inverse relationships ($P < 0.05$) in the sludge cake experiments and none in the liquid sludge experiments And for Cd, there were two inverse relationships ($P < 0.05$) in the sludge cake experiments and none in the liquid sludge experiments.

Conventional wisdom would suggest that metals added in metal-amended liquid sludges might be more detrimental to micro-organisms than sludge cake amendments, because the added organic matter in the sludge cake would ameliorate metal toxicity to micro-organisms by binding metals, thus reducing bioavailability, and also through 'physical' protection. However, in this study, Zn added to soil in metal-rich sludge cake (6,000 mg Zn/kg dry solids) had a much more adverse effect on rhizobia MPNs and the soil microbial biomass, compared with little or no measurable effects of adding Zn to soil in the metal-amended liquid sludge.

We propose that Zn toxicity to microbial cells in the sludge cake experiments was due to direct toxicity through exposure to high concentrations of Zn in and around the sludge cake particles, and poisoning at a cellular level when mineralising this organic matter for cell maintenance and reproduction, leading to cell disruption. The results to date in this study discount the 'sludge organic matter protection' hypothesis proposed by some workers, but support the conclusions of other workers that in the early years after sludge application it is the properties of the sludge that are important rather than the bulk soil chemistry (*e.g.* Lake *et al.,*1984; Merrington *et al.,* 2003).

We believe that no detrimental effects were seen from Zn in the metal-amended liquid sludge experiments, because the metals were <u>not</u> intimately associated with the added organic matter. Thus, the Zn would be much more evenly incorporated throughout the soil and not in association with organic matter 'hot spots' as in the sludge cake experiments. We hypothesise that in the liquid sludge experiments, microbial cells are mostly associated with uncontaminated organic matter from root exudates, dead roots and the small amounts of straw

and grass residues incorporated in to the soil during post harvest cultivations.

- There were no clear differences in topsoil NH_4NO_3 extractable metal concentrations between the sludge cake and metal-amended liquid sludge experiments, with soil pH probably the most important factor controlling Zn and Cd extractability between sites.
- Combining grain Cd data from the sludge cake and metal-amended liquid sludge experiments (and associated inorganic metal salt studies; Defra project SP0133) indicated that the current soil total Cd limit of 3 mg/kg was not sufficiently protective to produce grain below the EU maximum permissible grain cadmium concentration of 0.235 mg/kg dry matter, unless the soil pH was maintained above 6.8.

7. FUTURE WORK

1. The field experiments in this study have been running for 12 years, and it is possible that the effects of either Cu or Cd in the sludge cake experiments, or Zn and Cd in the metal-amended liquid sludge experiments, may occur in the future. Therefore, further monitoring of soil microbial biomass, respiration rate and rhizobia MPNs in all plots of these experiments (sludge cake, metal-amended liquid sludge and associated metal salt experiments) needs to be undertaken.

2. Subsequent Phases of this programme (2007 onwards) should principally be designed to test in the medium to long-term, whether the established Zn and Cu dose-response treatments have *consistent and permanent* effects on soil microbial properties, and the influence of soil Cd on grain cadmium concentrations.

3. In particular, it will be important to determine whether:

   (i) detrimental effects to the *soil microbial biomass* in the Zn-rich sludge cake treatments and the Cu-rich treatments on both the sludge cake and liquid sludge (plus associated metal salts) experiments increase with time and/or occur at lower soil Zn and Cu concentrations;

   (ii) detrimental effects seen on *rhizobia MPNs* in the Zn-rich sludge cake treatments recover to those in the untreated control and uncontaminated sludge cake control treatments, and/or are seen in the liquid sludge experiment;

   (iii) Cu becomes detrimental with time to rhizobia MPNs in the sludge cake and/or liquid sludge experiments;

   (iv) effects of Cd are seen on any microbial property.

4. Additionally, the effects of the established sludge cake metal concentrations should be compared against the effects of the *long-term annual treatments* (applied at maximum permissible rates) and those of the associated metal-amended liquid sludge and metal salt experiments.

5. Future research needs to examine and understand the intimate relationship between uncontaminated and metal-contaminated sludge organic matter and micro-organisms, via a combination of detailed laboratory and field investigations. For example, determining the *spatial distribution of Zn and micro-organisms* in relation to sludge organic matter in soils treated with low metal sludge and Zn-rich sludge cakes may lead to a better understanding of the mechanisms of effects observed to date in this study.

6. There would be merit in undertaking a more comprehensive analysis of the low- and Zn-rich sludge cakes, to assess whether potential co-contaminants (*e.g.* other metals and organic contaminants *etc.*) are likely to be responsible for rhizobia and microbial biomass decreases measured on the Zn metal dose-response treatments.

7. Consideration should be given to using additional (*e.g.* earthworm counts, pea/bean rhizobia numbers) and newer (*e.g.* phospholipid fatty acid profiles) assay techniques to further assess the effects of Zn and Cu additions on the soil ecosystem, with perhaps some of the existing assays (*e.g.* soil respiration rate, microbial biomass and clover rhizobia numbers) undertaken on fewer treatments/less frequently.

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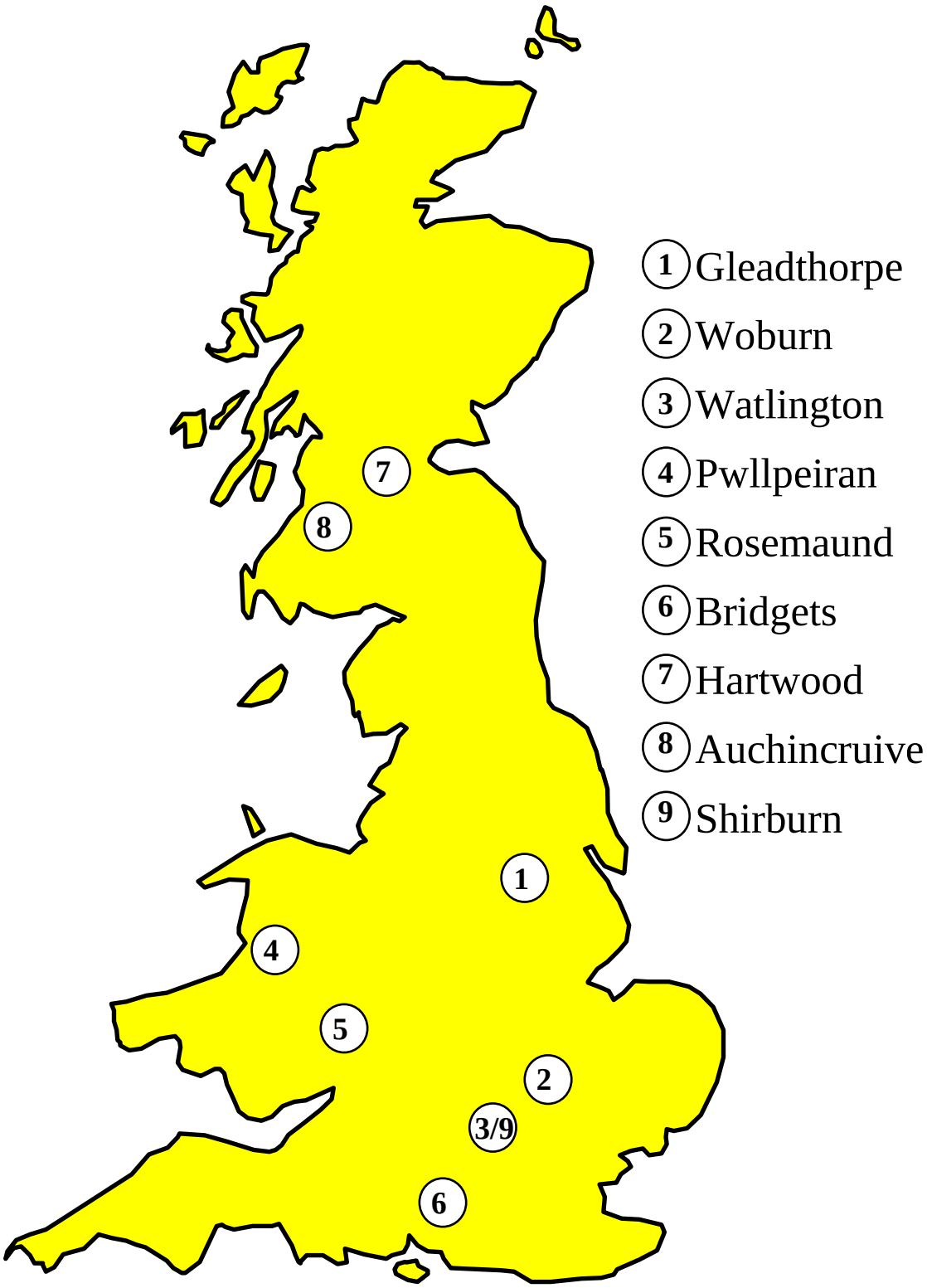
APPENDIX A Location of Long-term Sludge Experiments (LtSEs)



APPENDIX B The analytical protocol agreed between collaborators for all soil and sludge analyses

Rothamsted, MLURI and SAC will keep dried samples for future reference purposes. *MLURI and SAC will undertake the same analytical protocol for the Scottish sites (sites 7 and 8), with the exception of the metal speciation analyses.*

i) 2003:

WRc - total metals, extractable metals in spring 2003 [330 samples: treatments 1-15 at sites one to six – 45 samples per site; 60 samples for Shirburn- treatments 1-20][ENV511 – WRc, ING91, ING50, ING74, ING113 – WRc-NSF].

ADAS - soil pH and Tinsley organic C in spring 2003 [330 samples: treatments 1-15 at sites one to six – 45 samples per site; 60 samples for Shirburn- treatments 1-20][B/003, PHYS/002 – Direct Laboratory Services]

Macaulay - total metals, extractable metals, soil pH and soil organic C in spring 2003 [45 samples: treatments 1-15 at site seven][F007, F008, F009, ESG\SOILS\001, ESG\SOILS\009 - Macaulay]

SAC - total metals, extractable metals, soil pH and soil organic C in spring 2003 [45 samples: treatments 1-15 at site eight][SAC Environmental Lab Manual Methods 2, 3, 4, 5, 6]

ii) 2004:

ADAS - soil pH in spring 2004 [435 samples: all treatments – 360 samples for sites one to six; 75 samples for Shirburn][B/003 – Direct Laboratory Services]

Macaulay - soil pH in spring 2004 [60 samples: all treatments] [F007, ESG\SOILS\001 - Macaulay]

SAC - soil pH in spring 2004 [60 samples: all treatments] [SAC Environmental Lab Manual Methods 2, 5]

iii) 2005:

WRc - total metals, extractable metals in spring 2005 [435 samples: 360 samples for sites one to six; 75 samples for Shirburn][ENV511- WRc, ING91, ING50, ING74, ING113 – WRc-NSF].

Rothamsted - metal speciation in spring 2005 [126 samples: 108 samples for sites one to six - treatments 1, 2, 3, 6, 10, 14; 18 samples - treatments 1, 2, 3, 8, 13 and 18 at Shirburn] [Rothamsted Research Lab Manual Method 1].

ADAS – Tinsley organic carbon, total N, pH, extractable P, K, Mg and conductivity in spring 2005 [435 samples: all treatments], cation exchange capacity in spring 2005 [126 samples: 108 samples for sites one to six - treatments 1, 2, 3, 6, 10, 14; 18 samples - treatments 1, 2, 3, 8, 13 and 18 at Shirburn][PHYS/002, Z/001, B/003, B/046, B/018, ICP/006, B/009, B/042, AQUA/007 – Direct Laboratory Services]

Macaulay - total metals, extractable metals, organic carbon, total N, pH, extractable P, K, Mg and conductivity in April 2005 [60 samples: treatments 1-20 at site seven], cation exchange capacity in April 2005[18 samples for site seven – treatments 1, 2, 3, 6, 10, 14] [F005, F006, F007, F008, F009, A019, ESG\SOILS\001, ESG\SOILS\009 - Macaulay]

SAC - total metals, extractable metals, organic carbon, total N, pH, extractable P, K, Mg and conductivity in spring 2005 [60 samples: treatments 1-20 at site eight] cation exchange capacity in spring 2005 [18 samples for site eight – treatments 1, 2, 3, 6, 10, 14] [SAC Environmental Lab Manual Methods 2, 3, 4, 5, 6, 7, 8, 9, 10].

iv) 2006:

ADAS - soil pH in spring 2006 [435 samples: all treatments – 360 samples for sites one to six; 75 samples for Shirburn][B/003 – Direct Laboratory Services]

Macaulay - soil pH in spring 2006 [60 samples: all treatments][F007, ESG\SOILS\001 - Macaulay]

SAC - soil pH in spring 2006 [60 samples: all treatments] [SAC Environmental Lab Manual Methods 2, 5]

Soil microbial properties

i) 2003:

WRc - soil respiration in spring 2003 [330 samples: treatments 1-15 at sites one to six – 45 samples per site; 60 samples for Shirburn- treatments 1-20][ENV512, ENV513, ENV514, ENV515, ENV516, ENV517, ENV518 – WRc].

Rothamsted - biomass carbon, rhizobia MPNs in spring 2003 [330 samples: treatments 1-15 at sites one to six – 45 samples per site; 60 samples for Shirburn- treatments 1-20] [Rothamsted Research Lab Manual Method 2, 3, 4, 5, 6, 7, 8]

Macaulay - biomass carbon, rhizobia MPNs, soil respiration in spring 2003 [45 samples: treatments 1-15 at site seven][ESG\SOILS\001, ESG\SOILS\004, ESG\SOILS\005, ESG\SOILS\007 - Macaulay]

SAC - biomass carbon, rhizobia MPNs, soil respiration in spring 2003 [45 samples: treatments 1-15 at site eight][SAC Environmental Lab Manual Methods 2, 11, 12, 13].

ii) 2005:

WRc - soil respiration in spring 2005 [435 samples: all treatments - 360 samples for sites one to six; 75 samples for Shirburn][ENV512, ENV513, ENV514, ENV515, ENV516, ENV517, ENV518 – WRc].

Rothamsted - biomass carbon, rhizobia MPNs in spring 2005 [435 samples: all treatments - 360 samples for sites one to six; 75 samples for Shirburn] [Rothamsted Research Lab Manual Method 2, 3, 4, 5, 6, 7, 8]

Macaulay - biomass carbon, rhizobia MPNs, soil respiration in spring 2005 [60 samples: treatments 1-20 at site

seven][ESG\SOILS\001, ESG\SOILS\004, ESG\SOILS\005, ESG\SOILS\007 - Macaulay]
SAC - biomass carbon, rhizobia MPNs, soil respiration in spring 2005 [60 samples: treatments 1-20 at site eight][SAC Environmental Lab Manual Methods 2, 11, 12, 13].

Soil physical properties

i) 2005:

ADAS - bulk density in spring 2005 [126 samples: 108 samples for sites one to six - treatments 1, 2, 3, 6, 10, 14; 18 samples - treatments 1, 2, 3, 9, 14 and 19 at Shirburn][SOILS/029 – ADAS].
Macaulay - bulk density in spring 2005 [18 samples for site seven - treatments 1, 2, 3, 6, 10, 14][SOILS/029 - ADAS]
SAC - bulk density in spring 2005 [18 samples for site eight - treatments 1, 2, 3, 6, 10, 14][SAC Environmental Lab Manual Method 14]

Plant metal analysis

i) Harvest 2002 (grass first cut)

ADAS - Total Zn, Cu, Cd, Ni, Pb [240 samples: from 4 ADAS sites][I/046, ICP/003 – Direct Laboratory Services] [N.B. Use lab suite code *PGTM01* on LA1 form]
Rothamsted - Total Zn, Cu, Cd [60 samples: from Woburn] [Rothamsted Research Lab Manual Method 9, 10, 11, 12, 13]
WRc - Total Zn, Cu, Cd, Ni, Pb [135 samples: 60 from Watlington; 75 from Shirburn][ENV571-WRc, ING34, ING113 – WRc-NSF)
Macaulay - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Hartwood] [ESG\HERB\002 - Macaulay]

SAC - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Auchincruive][SAC Environmental Lab Manual Methods 16, 17]

ii) Harvest 2003(grain at harvest/grass first cut at Pwllpeiran, Hartwood and Auchincruive):

ADAS - Total Zn, Cu, Cd, Ni, Pb [240 samples: from 4 ADAS sites][I/046, ICP/003 – Direct Laboratory Services] [N.B. Use lab suite code *PGTM01* on LA1 form]
Rothamsted - Total Zn, Cu, Cd [60 samples: from Woburn] [Rothamsted Research Lab Manual Method 9, 10, 11, 12, 13]
WRc - Total Zn, Cu, Cd, Ni, Pb [135 samples: 60 from Watlington; 75 from Shirburn][ENV571-WRc, ING34, ING113 – WRc-NSF)
Macaulay - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Hartwood] [ESG\HERB\002 - Macaulay]

SAC - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Auchincruive][SAC Environmental Lab Manual Methods 16, 17]

iii) Harvest 2004 (grass first cut):

ADAS - Total Zn, Cu, Cd, Ni, Pb [240 samples: from 4 ADAS sites][I/046, ICP/003 – Direct Laboratory Services] [N.B. Use lab suite code *PGTM01* on LA1 form]
Rothamsted - Total Zn, Cu, Cd [60 samples: from Woburn] [Rothamsted Research Lab Manual Method 9, 10, 11, 12, 13]

WRc - Total Zn, Cu, Cd, Ni, Pb [135 samples: 60 from Watlington; 75 from Shirburn] [ENV571-WRc, ING34, ING113 – WRc-NSF)
Macaulay - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Hartwood] [ESG\HERB\002 - Macaulay]

SAC - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Auchincruive][SAC Environmental Lab Manual Methods 16, 17]

iv) Harvest 2005 (grain at harvest/grass first cut at Pwllpeiran, Hartwood and Auchincruive):
ADAS - Total Zn, Cu, Cd, Ni, Pb [240 samples: from 4 ADAS sites][I/046, ICP/003 – Direct Laboratory Services] [N.B. Use lab suite code *PGTM01* on LA1 form]
Rothamsted - Total Zn, Cu, Cd [60 samples: from Woburn] [Rothamsted Research Lab Manual Method 9, 10, 11, 12, 13]
WRc - Total Zn, Cu, Cd, Ni, Pb [135 samples: 60 from Watlington; 75 from Shirburn] [ENV571-WRc, ING34, ING113 – WRc-NSF]
Macaulay - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Hartwood] [ESG\HERB\002 - Macaulay]

SAC - Total Zn, Cu, Cd, Ni, Pb [60 samples: from Auchincruive][SAC Environmental Lab Manual Methods 16, 17]

Sludge cake analysis

i) 2002*:*

WRc - total Zn, Cu, Cd, Ni, Cr, Hg, Pb, Al, Fe, Mn (all aqua - regia digestion), N, P, K, NH_4-N and organic matter [15 samples][ENV511, ENV513 – WRc, ING91, ING50, ING113 – WRc-NSF].

APPENDIX C Soil analyses on all treatments in England, Wales and Scotland: cake sites - spring 2003

Site 1. Gleadthorpe
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	39.50	10.40	0.20	0.157	0.050	0.010
s.e.m.	0.25	0.92	0.01	0.013	0.012	0.000
Unc. Digested	62.97	38.03	0.29	0.693	0.283	0.010
s.e.m.	2.17	2.13	0.01	0.023	0.003	0.000
Unc. Raw	64.13	33.30	0.30	0.507	0.223	0.010
s.e.m.	6.69	4.04	0.02	0.016	0.023	0.000
Zn rate 1	**109.80**	41.93	0.35	**3.307**	0.327	0.011
s.e.m.	**7.98**	2.49	0.03	**0.167**	0.015	0.001
Zn rate 2	**206.00**	64.87	0.57	**9.953**	0.447	0.020
s.e.m.	**16.26**	5.71	0.02	**1.193**	0.035	0.001
Zn rate 3	**299.33**	83.97	0.75	**17.733**	0.523	0.028
s.e.m.	**38.98**	9.57	0.12	**2.105**	0.012	0.002
Zn rate 4	**291.00**	72.37	0.70	**23.267**	0.570	0.033
s.e.m.	**2.65**	1.19	0.02	**1.049**	0.035	0.002
Cu rate 1	66.80	**64.23**	0.28	0.570	**0.477**	0.010
s.e.m.	4.75	**6.58**	0.02	0.044	**0.038**	0.000
Cu rate 2	68.17	**119.00**	0.29	0.923	**0.930**	0.010
s.e.m.	7.61	**14.57**	0.04	0.069	**0.025**	0.000
Cu rate 3	66.30	**154.00**	0.29	1.090	**1.313**	0.010
s.e.m.	8.08	**22.11**	0.05	0.135	**0.234**	0.000
Cu rate 4	57.93	**160.33**	0.24	1.290	**1.417**	0.010
s.e.m.	2.58	**6.84**	0.02	0.137	**0.064**	0.000
Cd rate 1	82.43	49.83	**0.93**	1.407	0.370	**0.021**
s.e.m.	5.01	3.40	**0.09**	0.167	0.031	**0.002**
Cd rate 2	106.30	59.00	**1.95**	1.153	0.357	**0.032**
s.e.m.	6.38	3.80	**0.19**	0.067	0.022	**0.002**
Cd rate 3	108.00	58.07	**2.42**	1.157	0.380	**0.040**
s.e.m.	2.89	1.66	**0.03**	0.029	0.000	**< 0.001**
Cd rate 4	119.67	61.70	**3.08**	1.180	0.417	**0.049**
s.e.m.	4.26	2.88	**0.17**	0.116	0.027	**0.004**

Microbial and chemical properties (nutrients)

Treatment		Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g	pH	Organic C %
Control		231.29	0.60	4.96	6.40	1.52
	s.e.m.	37.94	0.08	0.06	0.00	0.16
Unc. Digested		319.59	0.71	4.80	6.07	2.58
	s.e.m.	63.23	0.09	0.10	0.03	0.14
Unc. Raw		316.63	0.76	4.63	6.23	2.39
	s.e.m.	27.42	0.07	0.15	0.07	0.14
Zn rate 1		266.46	0.66	4.95	6.07	2.35
	s.e.m.	56.27	0.14	0.29	0.07	0.08
Zn rate 2		243.61	0.65	3.45	6.00	2.56
	s.e.m.	41.23	0.10	0.09	0.00	0.24
Zn rate 3		226.47	0.75	3.30	6.03	2.50
	s.e.m.	32.42	0.06	0.19	0.09	0.22
Zn rate 4		191.96	0.59	2.82	6.00	2.03
	s.e.m.	18.58	0.13	0.10	0.00	0.03
Cu rate 1		324.50	0.65	5.06	6.23	2.25
	s.e.m.	61.00	0.02	0.16	0.03	0.12
Cu rate 2		267.70	0.72	4.96	6.30	2.13
	s.e.m.	33.12	0.03	0.06	0.00	0.20
Cu rate 3		249.65	0.66	4.81	6.27	2.17
	s.e.m.	33.11	0.02	0.43	0.03	0.34
Cu rate 4		196.51	0.73	4.39	6.20	2.26
	s.e.m.	43.13	0.04	0.30	0.06	0.23
Cd rate 1		308.43	0.71	4.71	6.03	3.35
	s.e.m.	20.84	0.09	0.37	0.09	0.59
Cd rate 2		270.34	0.70	4.73	6.13	2.78
	s.e.m.	51.20	0.05	0.17	0.03	0.34
Cd rate 3		200.48	0.51	4.57	6.17	2.30
	s.e.m.	56.36	0.07	0.17	0.03	0.04
Cd rate 4		220.52	0.56	4.18	6.27	2.56
	s.e.m.	24.10	0.01	0.21	0.07	0.20

Site 2. Woburn
Chemical properties (metals)

Treatment		Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu Mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control		49.90	15.00	0.16	0.170	0.027	0.010
	s.e.m.	0.31	0.23	0.01	0.027	0.003	0.000
Unc. Digested		71.30	40.33	0.24	0.470	0.207	0.010
	s.e.m.	1.64	1.18	0.01	0.106	0.032	0.000
Unc. Raw		70.93	34.97	0.21	0.187	0.180	0.010
	s.e.m.	5.89	1.62	0.02	0.062	0.031	0.000
Zn rate 1		**142.33**	56.67	0.36	**0.763**	0.397	0.010
	s.e.m.	**2.40**	0.73	0.01	**0.058**	0.019	0.000
Zn rate 2		**186.33**	61.33	0.43	**1.727**	0.523	0.010
	s.e.m.	**7.84**	4.78	0.01	**0.427**	0.059	0.000
Zn rate 3		**242.00**	68.80	0.52	**3.137**	0.463	0.011
	s.e.m.	**7.00**	1.55	0.01	**1.234**	0.064	0.001
Zn rate 4		**389.33**	99.27	0.81	**4.980**	0.637	0.013
	s.e.m.	**18.41**	3.88	0.03	**1.625**	0.081	0.002
Cu rate 1		72.20	**55.63**	0.23	0.250	**0.313**	0.010
	s.e.m.	3.87	**1.99**	0.02	0.046	**0.023**	0.000
Cu rate 2		76.67	**94.53**	0.23	0.243	**0.653**	0.010
	s.e.m.	10.35	**8.74**	0.02	0.084	**0.106**	0.000
Cu rate 3		70.20	**129.33**	0.21	0.287	**1.030**	0.010
	s.e.m.	5.00	**12.99**	0.01	0.080	**0.038**	0.000
Cu rate 4		76.43	**169.33**	0.21	0.620	**0.650**	0.004
	s.e.m.	3.98	**10.59**	0.02	0.071	**0.032**	< 0.001
Cd rate 1		88.53	50.03	**0.82**	1.353	0.200	**0.021**
	s.e.m.	0.93	0.90	**0.08**	0.329	0.027	**0.006**
Cd rate 2		97.33	48.67	**1.44**	0.833	0.190	**0.020**
	s.e.m.	1.33	2.57	**0.07**	0.107	0.021	**0.002**
Cd rate 3		117.67	61.00	**2.08**	0.997	0.307	**0.028**
	s.e.m.	5.55	2.91	**0.12**	0.282	0.082	**0.004**
Cd rate 4		118.33	56.50	**2.41**	1.177	0.210	**0.039**
	s.e.m.	2.03	1.10	**0.01**	0.292	0.020	**0.006**

Microbial and chemical properties (nutrients)

Treatment		Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g	pH	Organic C %
Control		138.41	0.32	4.75	6.73	1.02
	s.e.m.	3.93	0.03	0.41	0.09	0.07
Unc. Digested		216.35	0.62	4.55	6.30	1.91
	s.e.m.	9.07	0.06	0.32	0.06	0.14
Unc. Raw		224.37	0.52	4.38	6.70	1.53
	s.e.m.	13.75	0.06	0.61	0.15	0.14
Zn rate 1		214.96	0.38	3.64	6.50	1.78
	s.e.m.	10.99	0.07	0.38	0.12	0.15
Zn rate 2		175.76	0.36	2.58	6.50	1.94
	s.e.m.	1.27	0.05	0.23	0.15	0.11
Zn rate 3		180.50	0.41	2.48	6.43	1.83
	s.e.m.	11.13	0.06	0.24	0.12	0.09
Zn rate 4		173.10	0.45	2.45	6.50	1.60
	s.e.m.	1.11	0.06	0.58	0.12	0.09
Cu rate 1		224.16	0.38	4.18	6.67	1.60
	s.e.m.	4.42	0.03	0.12	0.12	0.11
Cu rate 2		210.19	0.38	4.46	6.77	1.55
	s.e.m.	9.01	0.04	0.23	0.13	0.09
Cu rate 3		196.75	0.42	4.05	6.77	1.70
	s.e.m.	13.71	0.08	0.35	0.17	0.14
Cu rate 4		172.78	0.36	3.74	6.43	1.63
	s.e.m.	12.36	0.06	0.12	0.03	0.02
Cd rate 1		217.44	0.39	3.85	6.47	1.76
	s.e.m.	6.03	0.05	0.39	0.07	0.20
Cd rate 2		199.80	0.36	3.87	6.50	1.98
	s.e.m.	7.70	0.08	0.30	0.12	0.06
Cd rate 3		210.75	0.34	4.00	6.57	1.83
	s.e.m.	20.65	0.08	0.22	0.15	0.10
Cd rate 4		216.76	0.32	3.81	6.70	2.02
	s.e.m.	7.44	0.06	0.51	0.00	0.11

Site 3. Watlington
Chemical properties (metals)

Treatment		Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control		48.37	12.10	0.28	0.057	0.023	0.010
	s.e.m.	0.30	0.16	0.00	0.003	0.003	0.000
Unc. Digested		73.46	38.84	0.37	0.107	0.240	0.010
	s.e.m.	1.30	0.11	0.02	0.022	0.023	0.000
Unc. Raw		70.82	37.06	0.40	0.040	0.273	0.010
	s.e.m.	3.42	2.19	0.04	0.012	0.018	0.000
Zn rate 1		**142.22**	51.68	0.54	**0.460**	0.390	0.010
	s.e.m.	**6.81**	2.12	0.05	**0.163**	0.031	0.000
Zn rate 2		**225.68**	62.02	0.75	**1.523**	0.513	0.010
	s.e.m.	**37.34**	4.56	0.16	**0.341**	0.034	0.000
Zn rate 3		**291.39**	81.22	0.85	**2.623**	0.593	0.010
	s.e.m.	**21.18**	4.95	0.05	**1.020**	0.087	0.000
Zn rate 4		**390.51**	97.91	1.05	**3.173**	0.820	0.013
	s.e.m.	**22.45**	4.92	0.06	**0.491**	0.095	0.003
Cu rate 1		73.94	**66.54**	0.38	0.060	**0.610**	0.010
	s.e.m.	1.78	**1.17**	0.01	0.006	**0.030**	0.000
Cu rate 2		75.10	**112.46**	0.40	0.077	**1.067**	0.010
	s.e.m.	1.28	**8.89**	0.01	0.015	**0.139**	0.000
Cu rate 3		86.92	**187.50**	0.46	0.166	**1.399**	0.010
	s.e.m.	8.16	**17.87**	0.05	0.058	**0.146**	0.000
Cu rate 4		77.86	**197.33**	0.44	0.223	**1.780**	0.010
	s.e.m.	3.47	**16.36**	0.02	0.058	**0.416**	0.000
Cd rate 1		87.09	46.00	**1.05**	0.093	0.320	**0.010**
	s.e.m.	2.92	1.70	**0.05**	0.007	0.021	**0.000**
Cd rate 2		110.34	55.63	**1.97**	0.247	0.313	**0.020**
	s.e.m.	1.85	0.22	**0.08**	0.087	0.033	**0.000**
Cd rate 3		130.14	62.81	**2.97**	0.247	0.353	**0.023**
	s.e.m.	2.83	2.38	**0.13**	0.097	0.043	**0.003**
Cd rate 4		133.86	63.79	**3.43**	0.220	0.357	**0.027**
	s.e.m.	5.07	2.83	**0.16**	0.059	0.027	**0.003**

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g	pH	Organic C %
Control	276.61	0.23	5.06	6.77	1.30
s.e.m.	5.22	0.04	0.16	0.09	0.04
Unc. Digested	357.42	0.51	4.48	6.70	2.33
s.e.m.	12.08	0.06	0.32	0.12	0.08
Unc. Raw	461.97	0.25	4.42	7.10	2.17
s.e.m.	28.08	0.04	0.27	0.10	0.16
Zn rate 1	413.57	0.38	5.02	6.80	2.55
s.e.m.	14.51	0.05	0.06	0.12	0.15
Zn rate 2	367.25	0.37	4.31	6.63	2.47
s.e.m.	21.14	0.07	0.37	0.07	0.11
Zn rate 3	327.67	0.49	3.61	6.53	2.41
s.e.m.	7.35	0.08	0.14	0.09	0.15
Zn rate 4	270.69	0.43	3.50	6.60	2.45
s.e.m.	28.71	0.05	0.24	0.06	0.12
Cu rate 1	429.02	0.30	4.76	6.93	2.11
s.e.m.	35.64	0.00	0.16	0.09	0.10
Cu rate 2	410.29	0.34	5.12	6.83	2.21
s.e.m.	34.81	0.05	0.14	0.15	0.10
Cu rate 3	385.87	0.45	4.80	6.70	2.31
s.e.m.	29.76	0.08	0.10	0.20	0.06
Cu rate 4	318.91	0.52	4.65	6.67	2.32
s.e.m.	1.37	0.04	0.34	0.03	0.12
Cd rate 1	399.22	0.41	4.81	6.67	2.47
s.e.m.	24.75	0.09	0.36	0.03	0.02
Cd rate 2	405.02	0.44	4.60	6.60	2.64
s.e.m.	25.78	0.05	0.00	0.15	0.17
Cd rate 3	394.30	0.48	4.65	6.73	2.46
s.e.m.	22.61	0.02	0.34	0.09	0.17
Cd rate 4	336.46	0.38	4.35	6.63	2.50
s.e.m.	29.55	0.04	0.25	0.07	0.05

Site 4. Pwllpeiran
Chemical properties (metals)

Treatment		Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control		146.67	15.30	0.27	0.050	0.078	0.010
	s.e.m.	12.17	1.00	0.01	0.000	0.008	0.000
Unc. Digested		148.67	36.73	0.27	0.064	0.247	0.010
	s.e.m.	7.80	7.57	0.02	0.011	0.079	0.000
Unc. Raw		144.00	45.33	0.31	0.054	0.307	0.010
	s.e.m.	7.02	1.31	0.01	0.004	0.017	0.000
Zn rate 1		**201.00**	36.33	0.38	**0.276**	0.224	0.010
	s.e.m.	**11.93**	2.61	0.03	**0.101**	0.078	0.000
Zn rate 2		**256.67**	67.33	0.61	**0.145**	0.567	0.010
	s.e.m.	**15.94**	2.37	0.04	**0.029**	0.049	0.000
Zn rate 3		**304.67**	68.77	0.69	**0.717**	0.433	0.010
	s.e.m.	**4.70**	1.14	0.01	**0.105**	0.061	0.000
Zn rate 4		**339.00**	72.50	0.77	**0.951**	0.452	0.011
	s.e.m.	**38.07**	14.82	0.13	**0.395**	0.037	0.001
Cu rate 1		175.33	**67.43**	0.30	0.120	**0.393**	0.010
	s.e.m.	29.34	**5.82**	0.01	0.058	**0.103**	0.000
Cu rate 2		149.67	**129.00**	0.32	0.050	**0.778**	0.010
	s.e.m.	6.89	**11.36**	0.01	0.000	**0.121**	0.000
Cu rate 3		166.00	**125.33**	0.25	0.058	**0.698**	0.010
	s.e.m.	14.36	**11.46**	0.03	0.005	**0.052**	0.000
Cu rate 4		162.67	**206.67**	0.27	0.072	**1.600**	0.010
	s.e.m.	14.90	**8.17**	0.00	0.022	**0.269**	0.000
Cd rate 1		179.67	49.47	**1.03**	0.150	0.356	**0.013**
	s.e.m.	19.54	2.34	**0.02**	0.100	0.076	**0.003**
Cd rate 2		185.67	53.17	**1.82**	0.065	0.472	**0.014**
	s.e.m.	18.99	4.46	**0.05**	0.001	0.028	**0.001**
Cd rate 3		205.00	64.27	**3.06**	0.142	0.471	**0.026**
	s.e.m.	11.79	5.46	**0.18**	0.030	0.054	**0.002**
Cd rate 4		221.00	70.30	**3.97**	0.221	0.533	**0.035**
	s.e.m.	8.14	5.94	**0.44**	0.086	0.124	**0.004**

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g	pH	Organic C %
Control	510.13	0.60	2.92	6.83	3.56
s.e.m.	16.34	0.08	0.42	0.03	0.09
Unc. Digested	493.25	0.44	2.27	6.77	4.39
s.e.m.	39.23	0.11	0.58	0.09	0.31
Unc. Raw	560.74	0.64	3.32	6.87	4.64
s.e.m.	43.89	0.12	0.45	0.03	0.04
Zn rate 1	483.66	0.44	2.90	6.80	3.93
s.e.m.	20.26	0.15	0.47	0.10	0.16
Zn rate 2	476.50	0.84	2.56	6.93	4.93
s.e.m.	26.36	0.16	0.31	0.07	0.05
Zn rate 3	522.11	0.50	2.04	6.83	4.47
s.e.m.	5.11	0.02	0.32	0.09	0.30
Zn rate 4	456.91	0.40	2.15	6.80	4.12
s.e.m.	43.24	0.06	0.54	0.00	0.49
Cu rate 1	489.50	0.46	2.77	6.67	3.82
s.e.m.	31.58	0.09	0.60	0.03	0.22
Cu rate 2	520.55	0.62	2.92	6.80	4.32
s.e.m.	30.29	0.05	0.16	0.06	0.17
Cu rate 3	412.45	0.40	3.10	6.63	3.52
s.e.m.	7.12	0.07	0.47	0.09	0.45
Cu rate 4	521.02	0.62	2.20	6.87	3.82
s.e.m.	21.59	0.04	0.30	0.07	0.20
Cd rate 1	546.80	0.57	1.99	6.83	4.70
s.e.m.	8.15	0.04	0.48	0.09	0.26
Cd rate 2	512.67	0.68	2.92	6.90	4.53
s.e.m.	17.08	0.04	0.54	0.10	0.33
Cd rate 3	501.45	0.58	2.80	6.80	4.68
s.e.m.	16.62	0.09	0.61	0.10	0.40
Cd rate 4	488.43	0.67	2.92	6.87	4.55
s.e.m.	34.12	0.03	0.16	0.15	0.34

Site 5. Rosemaund
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	78.25	17.55	0.28	0.100	0.027	0.010
s.e.m.	0.85	0.39	0.01	0.021	0.003	0.000
Unc. Digested	91.81	35.05	0.28	0.737	0.163	0.010
s.e.m.	3.27	2.10	0.00	0.174	0.015	0.000
Unc. Raw	94.74	33.11	0.32	0.347	0.147	0.010
s.e.m.	2.49	1.57	0.00	0.117	0.024	0.000
Zn rate 1	**138.31**	45.69	0.41	**4.373**	0.200	0.012
s.e.m.	**1.95**	0.59	0.03	**1.013**	0.023	0.002
Zn rate 2	**227.56**	67.31	0.68	**17.177**	0.350	0.027
s.e.m.	**3.53**	1.52	0.07	**1.977**	0.025	0.005
Zn rate 3	**314.26**	82.93	0.82	**24.097**	0.360	0.033
s.e.m.	**8.32**	3.16	0.04	**1.989**	0.015	0.003
Zn rate 4	**353.95**	87.56	0.84	**34.440**	0.443	0.042
s.e.m.	**29.68**	6.93	0.08	**3.225**	0.023	0.005
Cu rate 1	103.14	**62.49**	0.35	0.820	**0.323**	0.010
s.e.m.	4.29	**2.62**	0.01	0.279	**0.023**	0.000
Cu rate 2	103.76	**96.39**	0.33	1.010	**0.473**	0.010
s.e.m.	2.15	**3.40**	0.01	0.095	**0.003**	0.000
Cu rate 3	103.66	**132.67**	0.41	1.397	**0.683**	0.010
s.e.m.	3.59	**6.21**	0.05	0.150	**0.079**	0.000
Cu rate 4	97.81	**143.92**	0.33	2.097	**0.853**	0.011
s.e.m.	6.20	**8.53**	0.01	0.175	**0.024**	0.001
Cd rate 1	107.10	41.48	**0.80**	1.307	0.157	**0.022**
s.e.m.	3.64	2.19	**0.01**	0.488	0.009	**0.005**
Cd rate 2	129.44	49.83	**1.52**	0.960	0.190	**0.022**
s.e.m.	4.35	1.60	**0.09**	0.542	0.020	**0.009**
Cd rate 3	138.07	53.34	**2.20**	2.477	0.217	**0.054**
s.e.m.	2.48	1.60	**0.15**	0.200	0.009	**0.003**
Cd rate 4	141.33	55.43	**2.65**	2.523	0.210	**0.063**
s.e.m.	6.70	4.28	**0.22**	0.519	0.010	**0.011**

Microbial and chemical properties (nutrients)

Treatment		Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g	pH	Organic C %
Control		391.43	0.22	5.18	6.43	1.82
	s.e.m.	32.63	0.02	0.10	0.07	0.03
Unc. Digested		443.88	0.37	5.18	6.10	2.58
	s.e.m.	34.33	0.08	0.10	0.10	0.07
Unc. Raw		444.50	0.38	5.22	6.43	2.28
	s.e.m.	25.30	0.07	0.16	0.15	0.08
Zn rate 1		445.63	0.37	4.31	5.90	2.79
	s.e.m.	11.18	0.03	0.37	0.12	0.13
Zn rate 2		401.08	0.42	3.03	5.90	2.59
	s.e.m.	11.73	0.01	0.07	0.06	0.09
Zn rate 3		366.71	0.36	2.72	5.90	2.66
	s.e.m.	3.09	0.05	0.24	0.10	0.20
Zn rate 4		370.98	0.32	3.99	5.93	2.60
	s.e.m.	21.78	0.02	0.52	0.07	0.12
Cu rate 1		474.90	0.41	5.06	6.20	2.34
	s.e.m.	36.48	0.03	0.16	0.06	0.05
Cu rate 2		411.65	0.47	4.75	6.20	2.33
	s.e.m.	25.64	0.05	0.15	0.00	0.08
Cu rate 3		443.23	0.46	5.12	6.13	2.41
	s.e.m.	21.88	0.03	0.14	0.03	0.18
Cu rate 4		345.64	0.48	4.86	6.07	2.33
	s.e.m.	21.37	0.02	0.14	0.03	0.25
Cd rate 1		467.98	0.43	5.06	5.97	2.74
	s.e.m.	16.52	0.03	0.16	0.09	0.18
Cd rate 2		432.78	0.43	4.71	6.33	2.73
	s.e.m.	30.26	0.01	0.28	0.18	0.17
Cd rate 3		441.01	0.38	4.55	6.00	2.42
	s.e.m.	32.24	0.01	0.22	0.00	0.21
Cd rate 4		410.26	0.39	3.88	6.07	2.53
	s.e.m.	17.74	0.03	0.52	0.07	0.15

Site 6. Bridgets
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	71.27	13.20	0.98	0.050	0.027	0.012
s.e.m.	2.11	0.06	0.01	0.000	0.007	0.002
Unc. Digested	98.00	43.90	1.11	0.243	0.180	0.010
s.e.m.	3.98	2.83	0.05	0.066	0.070	0.000
Unc. Raw	99.73	42.47	1.06	0.303	0.117	0.010
s.e.m.	4.02	2.60	0.07	0.115	0.007	0.000
Zn rate 1	**160.27**	55.47	1.18	**1.843**	0.173	0.017
s.e.m.	**8.64**	2.84	0.07	**0.332**	0.030	0.002
Zn rate 2	**234.20**	68.20	1.34	**4.473**	0.210	0.022
s.e.m.	**10.01**	3.77	0.08	**1.328**	0.032	0.006
Zn rate 3	**332.87**	87.43	1.57	**9.383**	0.290	0.035
s.e.m.	**25.12**	6.84	0.05	**1.184**	0.040	0.003
Zn rate 4	**372.03**	92.57	1.69	**8.473**	0.337	0.038
s.e.m.	**14.93**	4.14	0.05	**3.064**	0.032	0.008
Cu rate 1	105.03	**68.93**	1.15	0.177	**0.213**	0.010
s.e.m.	4.13	**5.33**	0.15	0.072	**0.019**	0.000
Cu rate 2	92.10	**96.70**	0.96	0.460	**0.313**	0.014
s.e.m.	1.84	**4.71**	0.03	0.149	**0.022**	0.002
Cu rate 3	96.03	**133.93**	1.15	0.490	**0.397**	0.018
s.e.m.	4.28	**10.16**	0.12	0.047	**0.041**	0.000
Cu rate 4	96.37	**179.20**	1.13	0.120	**0.750**	0.010
s.e.m.	4.67	**9.70**	0.11	0.021	**0.081**	0.000
Cd rate 1	110.33	46.50	**1.64**	0.277	0.120	**0.014**
s.e.m.	3.80	4.18	**0.06**	0.063	0.006	**0.002**
Cd rate 2	120.27	51.93	**2.18**	0.277	0.150	**0.017**
s.e.m.	1.07	0.55	**0.10**	0.110	0.021	**0.004**
Cd rate 3	126.53	55.90	**2.57**	0.670	0.140	**0.030**
s.e.m.	4.78	2.78	**0.13**	0.215	0.006	**0.006**
Cd rate 4	140.60	58.20	**3.46**	0.577	0.157	**0.035**
s.e.m.	3.57	2.70	**0.17**	0.222	0.023	**0.009**

Microbial and chemical properties (nutrients)

Treatment		Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g	pH	Organic C %
Control		319.41	0.23	4.23	6.90	1.58
	s.e.m.	17.54	0.01	0.37	0.17	0.05
Unc. Digested		412.03	0.40	4.42	6.37	2.78
	s.e.m.	12.94	0.09	0.27	0.12	0.09
Unc. Raw		410.26	0.33	4.69	6.33	2.48
	s.e.m.	15.72	0.04	0.20	0.07	0.07
Zn rate 1		359.43	0.33	4.24	6.27	2.77
	s.e.m.	30.98	0.04	0.33	0.09	0.13
Zn rate 2		353.42	0.32	3.65	6.10	2.82
	s.e.m.	9.79	0.06	0.18	0.10	0.17
Zn rate 3		329.24	0.35	2.62	6.20	2.91
	s.e.m.	12.46	0.06	0.63	0.10	0.07
Zn rate 4		338.51	0.39	3.85	6.33	2.36
	s.e.m.	18.93	0.04	0.36	0.12	0.18
Cu rate 1		412.30	0.31	4.32	6.37	2.26
	s.e.m.	36.28	0.07	0.08	0.13	0.42
Cu rate 2		338.09	0.38	3.89	6.30	2.38
	s.e.m.	11.36	0.03	0.36	0.10	0.05
Cu rate 3		377.31	0.38	4.86	6.40	2.43
	s.e.m.	34.38	0.02	0.14	0.06	0.07
Cu rate 4		337.32	0.36	4.01	6.60	2.48
	s.e.m.	30.68	0.03	0.50	0.06	0.15
Cd rate 1		414.07	0.47	4.22	6.33	2.96
	s.e.m.	30.19	0.05	0.54	0.07	0.19
Cd rate 2		453.06	0.57	4.21	6.37	2.89
	s.e.m.	46.27	0.02	0.42	0.12	0.18
Cd rate 3		420.41	0.40	4.48	6.30	3.09
	s.e.m.	37.55	0.05	0.43	0.00	0.24
Cd rate 4		367.93	0.41	4.28	6.30	2.93
	s.e.m.	47.58	0.04	0.47	0.06	0.04

Site 7. Hartwood
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	80.46	22.53	0.31	0.480	0.033	0.008
s.e.m.	4.48	4.21	0.01	0.148	0.005	0.001
Unc. Digested	103.79	50.37	0.39	3.038	0.105	0.016
s.e.m.	1.88	3.16	0.01	0.214	0.006	0.000
Unc. Raw	103.48	44.74	0.39	0.635	0.107	0.007
s.e.m.	14.63	8.47	0.05	0.083	0.013	0.000
Zn rate 1	**174.53**	63.67	0.53	**6.564**	0.140	0.019
s.e.m.	**8.12**	2.84	0.03	**0.530**	0.020	0.001
Zn rate 2	**308.95**	89.78	0.86	**18.179**	0.173	0.027
s.e.m.	**4.41**	4.06	0.04	**2.887**	0.027	0.003
Zn rate 3	**409.39**	102.67	0.98	**27.891**	0.270	0.043
s.e.m.	**21.02**	11.16	0.03	**7.053**	0.014	0.007
Zn rate 4	**525.81**	117.78	1.22	**53.775**	0.329	0.066
s.e.m.	**32.73**	11.37	0.08	**4.859**	0.026	0.007
Cu rate 1	122.66	**72.70**	0.44	0.877	**0.147**	0.008
s.e.m.	2.33	**6.59**	0.01	0.152	**0.005**	0.001
Cu rate 2	111.87	**129.46**	0.39	1.603	**0.318**	0.011
s.e.m.	8.32	**13.72**	0.01	0.301	**0.039**	0.001
Cu rate 3	122.44	**191.90**	0.39	3.396	**0.514**	0.018
s.e.m.	3.87	**15.21**	0.00	0.214	**0.048**	0.000
Cu rate 4	103.38	**206.49**	0.35	3.273	**0.609**	0.015
s.e.m.	5.38	**1.80**	0.03	0.827	**0.191**	0.004
Cd rate 1	128.07	56.27	**1.21**	2.831	0.105	**0.039**
s.e.m.	2.29	4.20	**0.03**	0.390	0.006	**0.005**
Cd rate 2	131.34	58.85	**2.16**	4.085	0.130	**0.071**
s.e.m.	14.16	9.69	**0.19**	0.588	0.014	**0.010**
Cd rate 3	167.28	71.63	**3.30**	3.902	0.160	**0.098**
s.e.m.	11.40	3.91	**0.23**	0.855	0.009	**0.014**
Cd rate 4	191.45	76.55	**4.55**	4.259	0.197	**0.101**
s.e.m.	3.39	1.54	**0.28**	0.902	0.015	**0.020**
LT Unc. Digested	82.43	26.61	0.30	1.060	0.042	0.012
s.e.m.	4.80	1.99	0.02	0.183	0.004	0.001
LT Unc. Raw	94.93	30.98	0.32	0.782	0.036	0.012
s.e.m.	3.12	3.49	0.02	0.125	0.005	0.001
LT Zn	159.80	36.16	0.43	6.060	0.075	0.023
s.e.m.	11.58	6.97	0.03	1.545	0.015	0.004
LT Cu	88.43	55.23	0.31	1.287	0.076	0.015
s.e.m.	5.57	7.35	0.02	0.164	0.008	0.002
LT Cd	93.24	27.93	0.98	1.180	0.043	0.039
s.e.m.	3.98	1.49	0.06	0.198	0.001	0.006

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg/hr	pH	Organic C %
Control	522.3	4.54	0.84	5.80	4.62
s.e.m.	161.9	1.00	0.12	0.07	0.15
Unc. Digested	542.1	5.26	1.19	5.64	5.43
s.e.m.	43.1	0.16	0.04	0.07	0.11
Unc. Raw	571.9	5.75	0.96	6.00	4.60
s.e.m.	71.8	0.15	0.17	0.05	0.77
Zn rate 1	503.2	2.88	1.15	5.77	5.85
s.e.m.	55.4	0.31	0.04	0.06	0.67
Zn rate 2	402.8	0.81	1.06	5.70	7.97
s.e.m.	93.4	0.81	0.05	0.04	0.80
Zn rate 3	397.3	< 1.00	1.03	5.86	5.98
s.e.m.	54.0	-	0.03	0.03	0.61
Zn rate 4	310.1	< 1.00	0.93	5.66	6.04
s.e.m.	78.3	-	0.12	0.09	0.65
Cu rate 1	540.9	4.97	0.93	6.03	5.22
s.e.m.	88.4	0.49	0.06	0.07	0.62
Cu rate 2	428.6	4.76	0.98	6.02	5.08
s.e.m.	70.1	0.60	0.11	0.05	0.55
Cu rate 3	392.4	4.77	0.94	5.77	5.23
s.e.m.	77.2	0.17	0.07	0.07	0.32
Cu rate 4	273.2	4.64	0.77	5.82	5.92
s.e.m.	50.2	0.85	0.01	0.08	1.61
Cd rate 1	661.8	3.63	1.13	5.72	5.96
s.e.m.	158.3	0.65	0.11	0.02	0.29
Cd rate 2	509.0	3.57	0.98	5.79	5.65
s.e.m.	125.9	0.44	0.10	0.05	0.47
Cd rate 3	472.8	3.89	0.96	5.93	5.15
s.e.m.	26.6	0.82	0.13	0.11	0.44
Cd rate 4	403.0	4.22	0.79	5.91	5.31
s.e.m.	97.9	0.25	0.05	0.03	0.65
LT Unc. Digested	520.1	5.52	0.89	5.73	4.39
s.e.m.	37.8	0.27	0.03	0.01	0.33
LT Unc. Raw	517.1	4.96	0.85	5.80	4.32
s.e.m.	29.6	0.38	0.12	0.03	0.46
LT Zn	491.1	2.66	0.76	5.73	3.67
s.e.m.	98.7	1.36	0.08	0.08	0.04
LT Cu	467.1	4.89	0.86	5.60	5.05
s.e.m.	104.0	0.87	0.13	0.07	0.42
LT Cd	410.3	5.31	0.82	5.80	3.91
s.e.m.	85.0	0.29	0.07	0.02	0.28

Site 8. Auchincruive
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	76.81	16.35	< 0.47	0.674	0.232	< 0.035
s.e.m.	1.66	0.57	-	0.407	0.151	-
Unc. Digested	103.90	45.29	< 0.47	0.953	0.139	< 0.035
s.e.m.	4.16	1.68	-	0.373	0.033	-
Unc. Raw	100.23	39.85	< 0.47	0.342	0.149	< 0.035
s.e.m.	2.00	1.44	-	0.075	0.015	-
Zn rate 1	**143.97**	56.94	< 0.47	**3.574**	0.159	< 0.035
s.e.m.	**3.96**	1.57	-	**1.689**	0.047	-
Zn rate 2	**225.44**	70.71	< 0.47	**24.333**	0.369	0.066
s.e.m.	**6.43**	0.99	-	**15.966**	0.075	-
Zn rate 3	**304.92**	86.47	< 0.47	**16.192**	0.393	< 0.035
s.e.m.	**10.52**	3.23	-	**5.006**	0.018	-
Zn rate 4	**392.94**	97.79	0.56	**24.458**	0.328	0.050
s.e.m.	**11.54**	2.94	0.05	**9.855**	0.028	-
Cu rate 1	110.78	**66.71**	< 0.47	0.529	**0.208**	< 0.035
s.e.m.	3.85	**2.33**	-	0.149	**0.008**	-
Cu rate 2	106.12	**107.34**	< 0.47	0.685	**0.199**	< 0.035
s.e.m.	2.99	**5.10**	-	0.525	**0.126**	-
Cu rate 3	108.67	**143.52**	< 0.47	1.384	**0.363**	< 0.035
s.e.m.	3.96	**5.36**	-	0.562	**0.151**	-
Cu rate 4	119.77	**258.52**	< 0.47	3.533	**1.053**	< 0.035
s.e.m.	4.80	**6.91**	-	0.106	**0.046**	-
Cd rate 1	118.99	53.72	**0.80**	1.666	0.220	**0.035**
s.e.m.	3.05	2.43	**0.04**	0.137	0.005	**-**
Cd rate 2	138.42	59.94	**1.95**	3.022	0.254	**0.075**
s.e.m.	0.80	1.20	**0.09**	0.492	0.030	**0.009**
Cd rate 3	153.07	66.05	**2.98**	2.622	0.151	**0.079**
s.e.m.	3.09	2.69	**0.20**	1.281	0.069	**-**
Cd rate 4	168.94	74.37	**3.83**	2.247	0.219	**0.103**
s.e.m.	3.49	2.02	**0.27**	1.038	0.083	**0.006**
LT Unc. Digested	90.19	22.83	< 0.47	1.24	0.20	0.08
s.e.m.	5.54	0.75	-	0.52	0.06	-
LT Unc. Raw	82.25	21.40	< 0.47	0.552	0.193	< 0.035
s.e.m.	2.79	1.17	-	0.149	0.097	-
LT Zn	135.75	31.37	< 0.47	2.830	0.161	0.068
s.e.m.	9.06	1.13	-	0.666	0.035	-
LT Cu	84.92	42.07	< 0.47	0.297	0.122	< 0.035
s.e.m.	2.69	1.82	-	0.030	0.011	-
LT Cd	87.14	23.38	< 0.47	0.714	0.114	< 0.035
s.e.m.	1.22	0.69	-	0.427	0.051	-

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg/hr	pH	Organic C %
Control	456.0	4.95	0.27	5.78	2.31
s.e.m.	38.8	0.16	0.03	0.03	0.05
Unc. Digested	517.7	4.14	0.42	5.56	3.04
s.e.m.	5.5	0.15	0.01	0.03	0.15
Unc. Raw	516.7	4.77	0.34	6.16	3.09
s.e.m.	71.1	0.09	0.03	0.05	0.10
Zn rate 1	508.0	4.00	0.38	5.54	3.12
s.e.m.	26.8	0.30	0.02	0.09	0.01
Zn rate 2	486.0	3.19	0.43	5.70	3.35
s.e.m.	22.7	0.59	0.05	0.08	0.09
Zn rate 3	476.0	2.91	0.42	5.61	3.53
s.e.m.	17.7	0.17	0.01	0.08	0.30
Zn rate 4	435.3	3.90	0.39	5.59	2.98
s.e.m.	44.2	0.11	0.03	0.06	0.06
Cu rate 1	502.0	4.41	0.32	6.03	3.27
s.e.m.	45.6	0.19	0.02	0.09	0.20
Cu rate 2	520.0	4.59	0.36	5.87	3.09
s.e.m.	15.4	0.23	0.01	0.10	0.23
Cu rate 3	464.7	4.32	0.39	5.75	3.31
s.e.m.	17.3	0.15	0.01	0.06	0.28
Cu rate 4	429.0	3.23	0.44	5.56	3.81
s.e.m.	59.3	0.37	0.05	0.06	0.19
Cd rate 1	512.0	3.55	0.38	5.65	3.34
s.e.m.	43.0	0.51	0.02	0.05	0.14
Cd rate 2	532.3	3.26	0.39	5.66	3.36
s.e.m.	33.8	0.22	0.02	0.04	0.20
Cd rate 3	471.7	4.02	0.39	5.83	3.07
s.e.m.	98.7	0.27	0.05	0.05	0.10
Cd rate 4	485.7	3.79	0.43	5.80	3.52
s.e.m.	37.7	0.33	0.01	0.02	0.13
LT Unc. Digested	446.0	4.98	0.29	5.93	2.75
s.e.m.	24.1	0.14	0.01	0.04	0.21
LT Unc. Raw	449.0	4.86	0.29	6.03	2.75
s.e.m.	21.5	0.07	0.04	0.09	0.10
LT Zn	431.7	4.56	0.32	5.73	2.58
s.e.m.	25.6	0.33	0.05	0.04	0.01
LT Cu	440.0	4.49	0.28	5.80	2.41
s.e.m.	9.5	0.22	0.02	0.11	0.14
LT Cd	418.7	5.07	0.26	5.89	2.47
s.e.m.	10.2	0.22	0.02	0.06	0.16

Site 9. Shirburn
Chemical properties (metals)

Treatment		Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control		61.5	14.53	0.40	0.050	0.130	0.010
	s.e.m.	1.0	0.45	0.01	0.000	0.006	0.000
Unc. Digested		85.4	37.13	0.49	0.050	0.400	0.010
	s.e.m.	5.0	0.85	0.00	0.000	0.044	0.000
Unc. Raw		78.7	27.93	0.46	0.050	0.303	0.010
	s.e.m.	6.0	1.39	0.02	0.000	0.007	0.000
High Unc. Digested		82.0	42.17	0.48	0.050	0.483	0.010
	s.e.m.	3.2	1.85	0.02	0.000	0.037	0.000
High Unc. Raw		83.2	35.47	0.52	0.050	0.397	0.010
	s.e.m.	4.9	2.80	0.04	0.000	0.009	0.000
Zn rate 1		**137.0**	47.43	0.62	**0.097**	0.523	0.010
	s.e.m.	**7.2**	1.82	0.03	**0.003**	0.032	0.000
Zn rate 2		**218.3**	65.07	0.81	**0.210**	0.763	0.010
	s.e.m.	**16.1**	4.78	0.06	**0.017**	0.050	0.000
Zn rate 3		**215.7**	61.50	0.79	**0.223**	0.793	0.010
	s.e.m.	**14.7**	3.86	0.05	**0.003**	0.064	0.000
Zn rate 4		**288.7**	71.07	0.95	**0.337**	0.943	0.010
	s.e.m.	**12.9**	3.29	0.04	**0.003**	0.038	0.000
Zn rate 5		**348.3**	87.00	1.12	**0.483**	0.993	0.010
	s.e.m.	**10.9**	3.06	0.03	**0.009**	0.045	0.000
Cu rate 1		77.5	**62.80**	0.47	0.050	**0.833**	0.010
	s.e.m.	4.1	**6.67**	0.01	0.000	**0.048**	0.000
Cu rate 2		81.0	**98.83**	0.49	0.050	**1.233**	0.010
	s.e.m.	4.1	**11.49**	0.02	0.000	**0.188**	0.000
Cu rate 3		78.4	**117.67**	0.46	0.050	**1.557**	0.010
	s.e.m.	0.8	**6.94**	0.02	0.000	**0.130**	0.000
Cu rate 4		83.7	**182.00**	0.50	0.050	**2.273**	0.010
	s.e.m.	1.0	**11.79**	0.01	0.000	**0.199**	0.000
Cu rate 5		85.5	**200.33**	0.52	0.050	**3.020**	0.010
	s.e.m.	4.3	**19.32**	0.02	0.000	**0.306**	0.000
Cd rate 1		90.9	42.87	**0.94**	0.050	0.417	**0.010**
	s.e.m.	2.9	0.65	**0.09**	0.000	0.026	**0.000**
Cd rate 2		105.4	49.73	**1.83**	0.060	0.540	**0.010**
	s.e.m.	8.1	3.61	**0.17**	0.010	0.017	**0.000**
Cd rate 3		113.7	51.33	**2.21**	0.060	0.537	**0.010**
	s.e.m.	2.0	2.45	**0.13**	0.010	0.028	**0.000**
Cd rate 4		143.3	59.93	**3.17**	0.070	0.647	**0.014**
	s.e.m.	4.3	2.02	**0.13**	0.012	0.007	**0.000**
Cd rate 5		140.3	67.10	**3.64**	0.063	0.693	**0.016**
	s.e.m.	8.6	5.47	**0.35**	0.003	0.022	**0.001**

Microbial and chemical properties (nutrients)

Treatment		Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg/hr	pH	Organic C %
Control		803.2	4.23	0.44	7.87	2.64
	s.e.m.	40.5	0.13	0.01	0.03	0.06
Unc. Digested		820.1	4.45	0.52	7.53	3.39
	s.e.m.	45.3	0.15	0.09	0.03	0.05
Unc. Raw		844.3	4.49	0.55	7.63	3.07
	s.e.m.	72.1	0.27	0.07	0.03	0.17
High Unc. Digested		771.1	4.07	0.48	7.60	3.41
	s.e.m.	28.2	0.29	0.03	0.06	0.05
High Unc. Raw		883.1	4.47	0.55	7.57	3.16
	s.e.m.	31.2	0.07	0.04	0.03	0.06
Zn rate 1		904.1	4.14	0.52	7.50	3.50
	s.e.m.	24.5	0.23	0.06	0.00	0.12
Zn rate 2		784.0	3.96	0.52	7.40	3.60
	s.e.m.	25.6	0.23	0.07	0.06	0.19
Zn rate 3		834.9	4.06	0.65	7.57	3.37
	s.e.m.	22.5	0.45	0.08	0.09	0.11
Zn rate 4		731.8	4.14	0.50	7.33	3.29
	s.e.m.	35.9	0.23	0.10	0.03	0.12
Zn rate 5		680.8	3.15	0.48	7.37	3.48
	s.e.m.	29.7	0.48	0.08	0.09	0.06
Cu rate 1		845.6	4.40	0.56	7.60	3.18
	s.e.m.	35.1	0.00	0.07	0.10	0.15
Cu rate 2		846.1	4.47	0.58	7.60	3.34
	s.e.m.	59.7	0.07	0.03	0.00	0.25
Cu rate 3		767.6	3.88	0.65	7.57	3.33
	s.e.m.	22.4	0.26	0.03	0.07	0.10
Cu rate 4		808.0	4.00	0.57	7.63	3.40
	s.e.m.	25.3	0.22	0.06	0.03	0.00
Cu rate 5		679.8	3.74	0.61	7.67	3.28
	s.e.m.	30.0	0.37	0.07	0.07	0.18
Cd rate 1		892.7	3.80	0.60	7.53	3.43
	s.e.m.	40.4	0.42	0.08	0.03	0.04
Cd rate 2		769.9	3.43	0.67	7.47	3.51
	s.e.m.	26.2	0.27	0.08	0.07	0.23
Cd rate 3		876.6	3.54	0.65	7.53	3.44
	s.e.m.	51.7	0.09	0.09	0.03	0.16
Cd rate 4		777.0	3.12	0.60	7.53	3.38
	s.e.m.	7.5	0.13	0.03	0.03	0.07
Cd rate 5		724.2	3.57	0.58	7.50	3.79
	s.e.m.	42.1	0.20	0.02	0.06	0.33

APPENDIX D Soil analyses on all treatments in England, Wales and Scotland: cake sites - spring 2005

Site 1. Gleadthorpe
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	35.05	11.12	0.20	0.113	0.100	0.100
s.e.m.	2.43	1.54	0.00	0.013	0.000	0.000
Unc. Digested	72.80	45.16	0.20	0.787	0.263	0.100
s.e.m.	5.09	2.36	0.00	0.030	0.012	0.000
Unc. Raw	59.99	33.03	0.20	0.683	0.200	0.100
s.e.m.	8.18	5.30	0.00	0.079	0.021	0.000
Zn rate 1	**134.82**	52.92	0.20	**2.767**	0.403	0.100
s.e.m.	**3.37**	1.88	0.00	**0.689**	0.034	0.000
Zn rate 2	**256.15**	112.91	0.24	**14.333**	0.457	0.100
s.e.m.	**67.66**	19.87	0.03	**1.764**	0.007	0.000
Zn rate 3	**347.15**	90.66	0.33	**16.000**	0.523	0.100
s.e.m.	**47.55**	9.46	0.07	**4.509**	0.041	0.000
Zn rate 4	**353.89**	89.32	0.49	**21.667**	0.497	0.100
s.e.m.	**46.70**	9.38	0.25	**5.783**	0.019	0.000
Cu rate 1	81.56	**76.51**	0.46	1.103	**0.460**	0.100
s.e.m.	3.42	**5.30**	0.03	0.419	**0.030**	0.000
Cu rate 2	79.88	**138.19**	0.50	1.080	**0.833**	0.100
s.e.m.	6.07	**8.92**	0.05	0.172	**0.019**	0.000
Cu rate 3	123.69	**171.89**	0.46	0.997	**1.167**	0.100
s.e.m.	46.06	**21.05**	0.01	0.310	**0.167**	0.000
Cu rate 4	182.00	**215.71**	0.44	1.433	**1.300**	0.100
s.e.m.	21.05	**27.59**	0.03	0.088	**0.058**	0.000
Cd rate 1	195.48	75.16	**1.48**	1.667	0.403	**0.100**
s.e.m.	14.69	11.22	**0.19**	0.219	0.019	**0.000**
Cd rate 2	208.97	69.43	**2.53**	1.360	0.377	**0.100**
s.e.m.	3.37	3.80	**0.06**	0.209	0.007	**0.000**
Cd rate 3	158.41	67.41	**2.86**	1.400	0.440	**0.100**
s.e.m.	20.50	6.40	**0.29**	0.252	0.036	**0.000**
Cd rate 4	192.11	80.89	**4.18**	2.000	0.427	**0.100**
s.e.m.	21.05	4.63	**0.34**	0.404	0.012	**0.000**
LT Unc. Digested	51.90	21.23	0.20	0.207	0.107	0.100
s.e.m.	1.47	0.00	0.00	0.024	0.007	0.000
LT Unc. Raw	52.92	22.58	0.20	0.657	0.137	0.100
s.e.m.	3.97	1.22	0.00	0.223	0.020	0.000
LT Zn	151.67	42.47	0.20	4.800	0.250	0.100
s.e.m.	15.45	4.63	0.00	1.735	0.026	0.000
LT Cu	53.25	63.70	0.20	0.313	0.410	0.100
s.e.m.	4.22	3.83	0.00	0.145	0.070	0.000
LT Cd	82.24	29.66	1.21	0.720	0.197	0.100
s.e.m.	15.47	5.23	0.51	0.412	0.032	0.000

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	5.97	0.18	31.33	140.00	167.33	1.42
s.e.m.	0.03	0.00	2.60	6.11	9.49	0.08
Unc. Digested	5.87	0.30	94.00	126.33	206.33	2.22
s.e.m.	0.03	0.01	1.53	15.06	15.96	0.17
Unc. Raw	5.87	0.26	78.67	141.67	115.00	2.35
s.e.m.	0.03	0.01	9.53	7.54	4.58	0.10
Zn rate 1	5.87	0.27	92.00	117.67	194.00	1.89
s.e.m.	0.03	0.01	5.20	3.84	8.50	0.12
Zn rate 2	5.87	0.28	95.67	143.33	183.33	2.23
s.e.m.	0.07	0.02	7.88	14.38	7.54	0.25
Zn rate 3	5.87	0.29	92.67	124.33	177.67	2.01
s.e.m.	0.03	0.01	8.88	4.63	12.99	0.12
Zn rate 4	5.83	0.23	78.00	112.67	149.00	1.67
s.e.m.	0.07	0.01	3.51	6.33	6.56	0.06
Cu rate 1	5.80	0.26	76.33	130.00	127.00	1.79
s.e.m.	0.00	0.01	3.84	5.77	6.81	0.17
Cu rate 2	5.93	0.26	67.33	112.33	155.33	2.08
s.e.m.	0.03	0.02	8.35	7.88	6.17	0.25
Cu rate 3	5.87	0.25	59.67	116.67	170.00	1.72
s.e.m.	0.03	0.03	8.17	5.21	7.51	0.13
Cu rate 4	5.93	0.26	51.67	111.00	185.67	1.91
s.e.m.	0.09	0.01	2.40	13.00	17.03	0.12
Cd rate 1	5.83	0.33	110.33	135.00	219.00	2.69
s.e.m.	0.07	0.01	1.33	16.80	12.01	0.22
Cd rate 2	5.83	0.31	74.00	127.00	186.33	2.74
s.e.m.	0.03	0.02	31.97	4.58	2.40	0.17
Cd rate 3	5.97	0.26	97.67	111.33	146.00	1.89
s.e.m.	0.03	0.00	5.55	8.95	3.06	0.06
Cd rate 4	5.87	0.29	104.00	123.33	144.33	1.99
s.e.m.	0.09	0.02	3.61	8.88	7.36	0.17
LT Unc. Digested	6.03	0.20	53.00	141.00	179.67	1.28
s.e.m.	0.09	0.01	3.21	5.86	11.35	0.12
LT Unc. Raw	5.93	0.23	61.00	157.00	117.00	1.57
s.e.m.	0.09	0.04	10.44	11.37	6.08	0.32
LT Zn	5.90	0.21	60.67	120.67	160.33	1.61
s.e.m.	0.06	0.00	7.67	10.11	9.56	0.18
LT Cu	6.23	0.18	31.00	122.67	158.67	1.37
s.e.m.	0.03	0.01	1.53	6.64	10.49	0.07
LT Cd	6.03	0.21	57.33	133.33	117.00	1.64
s.e.m.	0.12	0.00	4.37	6.64	4.16	0.12

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN Log_{10} cells/g
Control	9.64	2226.67	221.19	0.303	4.86
s.e.m.	0.62	14.53	21.86	0.232	0.14
Unc. Digested	11.5	2220.00	316.13	0.219	5.02
s.e.m.	0.50	60.28	42.62	0.327	0.06
Unc. Raw	10.4	1580.67	320.03	0.390	5.12
s.e.m.	0.79	681.78	14.96	0.331	0.14
Zn rate 1		2113.33	236.50	0.240	3.92
s.e.m.		43.72	25.16	0.262	0.16
Zn rate 2		2176.67	249.40	0.297	2.81
s.e.m.		74.46	30.97	0.246	0.43
Zn rate 3	12.3	2136.67	243.63	0.294	3.21
s.e.m.	1.23	68.88	37.81	0.331	0.21
Zn rate 4		2110.00	189.94	0.319	1.96
s.e.m.		75.50	11.19	0.389	0.51
Cu rate 1		2196.67	281.12	0.365	5.06
s.e.m.		137.40	22.68	0.378	0.16
Cu rate 2		2126.67	238.34	0.374	4.55
s.e.m.		51.75	12.73	0.430	0.22
Cu rate 3	10.3	2276.67	230.96	0.296	4.70
s.e.m.	0.94	58.40	9.24	0.315	0.10
Cu rate 4		1973.33	232.66	0.233	4.86
s.e.m.		93.33	26.76	0.418	0.14
Cd rate 1		2280.00	312.63	0.300	5.02
s.e.m.		101.49	45.96	0.362	0.06
Cd rate 2		2176.67	296.35	0.270	4.07
s.e.m.		97.01	6.53	0.240	0.46
Cd rate 3	12.0	1940.00	235.47	0.440	4.17
s.e.m.	0.83	47.26	10.76	0.321	0.38
Cd rate 4		2266.67	263.73	0.254	3.49
s.e.m.		29.06	18.33	0.325	0.33
LT Unc. Digested		2263.33	244.90	0.258	4.96
s.e.m.		109.90	9.62	0.390	0.06
LT Unc. Raw		2123.33	254.13	0.318	5.12
s.e.m.		28.48	32.15	0.435	0.14
LT Zn		2176.67	198.00	0.500	3.68
s.e.m.		171.69	16.92	0.405	0.16
LT Cu		2170.00	208.69	0.433	3.69
s.e.m.		46.19	10.85	0.384	0.46
LT Cd		2233.33	210.35	0.458	4.25
s.e.m.		65.66	13.20	0.374	0.43

Site 2. Woburn
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd Mg/kg
Control	46.56	13.16	0.50	0.530	0.123	0.100
s.e.m.	2.11	0.58	0.00	0.058	0.009	0.000
Unc. Digested	67.14	36.77	0.50	0.573	0.203	0.100
s.e.m.	3.22	1.69	0.00	0.105	0.042	0.000
Unc. Raw	68.49	34.75	0.50	0.427	0.100	0.100
s.e.m.	1.22	0.34	0.00	0.117	0.000	0.000
Zn rate 1	**148.45**	52.97	0.50	**1.733**	0.320	0.100
s.e.m.	**8.93**	1.35	0.00	**0.296**	0.181	0.000
Zn rate 2	**212.55**	62.08	0.50	**3.967**	0.560	0.100
s.e.m.	**15.46**	3.80	0.00	**0.882**	0.184	0.000
Zn rate 3	**273.28**	75.57	0.65	**6.300**	0.200	0.100
s.e.m.	**10.12**	3.22	0.01	**0.954**	0.062	0.000
Zn rate 4	**384.62**	94.13	0.81	**4.867**	0.317	0.100
s.e.m.	**30.92**	6.10	0.05	**0.689**	0.053	0.000
Cu rate 1	75.24	**59.04**	0.50	0.843	**0.570**	0.100
s.e.m.	10.29	**4.69**	0.00	0.329	**0.231**	0.000
Cu rate 2	79.28	**99.53**	0.50	0.537	**0.327**	0.100
s.e.m.	6.19	**7.35**	0.00	0.103	**0.097**	0.000
Cu rate 3	77.26	**148.45**	0.50	0.770	**0.723**	0.100
s.e.m.	4.42	**3.37**	0.00	0.298	**0.060**	0.000
Cu rate 4	86.37	**192.31**	0.50	0.803	**1.083**	0.100
s.e.m.	5.91	**5.84**	0.00	0.349	**0.158**	0.000
Cd rate 1	78.95	43.52	**0.87**	0.797	0.270	**0.100**
s.e.m.	6.64	2.55	**0.07**	0.307	0.031	**0.000**
Cd rate 2	103.58	53.31	**1.87**	0.810	0.267	**0.117**
s.e.m.	3.89	4.46	**0.13**	0.162	0.013	**0.017**
Cd rate 3	108.64	55.67	**2.37**	0.677	0.270	**0.100**
s.e.m.	8.29	3.25	**0.23**	0.198	0.046	**0.000**
Cd rate 4	124.83	61.07	**3.23**	1.037	0.220	**0.100**
s.e.m.	8.93	4.42	**0.30**	0.141	0.038	**0.000**
LT Unc. Digested	52.97	19.57	0.50	0.293	0.100	0.100
s.e.m.	1.88	0.89	0.00	0.085	0.000	0.000
LT Unc. Raw	55.67	19.91	0.50	0.203	0.100	0.100
s.e.m.	2.55	1.47	0.00	0.067	0.000	0.000
LT Zn	91.09	25.30	0.50	2.500	0.100	0.100
s.e.m.	7.30	2.34	0.00	0.755	0.000	0.000
LT Cu	47.57	42.51	0.50	0.330	0.100	0.100
s.e.m.	3.25	3.83	0.00	0.099	0.000	0.000
LT Cd	59.04	19.91	0.74	0.650	0.100	0.100
s.e.m.	3.42	1.69	0.03	0.125	0.000	0.000

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.90	0.14	47.67	163.67	128.67	1.12
s.e.m.	0.06	0.02	3.71	10.41	14.44	0.00
Unc. Digested	6.70	0.25	96.33	154.00	74.67	1.43
s.e.m.	0.06	0.02	1.33	8.50	13.22	0.27
Unc. Raw	6.90	0.18	88.00	143.00	60.00	1.36
s.e.m.	0.12	0.03	1.53	13.65	9.07	0.10
Zn rate 1	6.83	0.22	86.33	142.00	57.00	1.68
s.e.m.	0.07	0.02	1.33	12.17	7.51	0.14
Zn rate 2	6.87	0.23	86.67	149.67	54.00	1.67
s.e.m.	0.03	0.01	6.74	4.37	5.03	0.12
Zn rate 3	6.80	0.22	88.33	158.00	66.33	1.78
s.e.m.	0.00	0.01	0.33	4.58	0.33	0.08
Zn rate 4	6.73	0.21	81.67	138.00	55.67	1.64
s.e.m.	0.03	0.02	2.85	6.35	6.33	0.11
Cu rate 1	6.87	0.16	90.67	145.67	57.00	1.62
s.e.m.	0.07	0.03	4.41	9.53	5.57	0.11
Cu rate 2	6.93	0.22	81.33	147.00	59.67	1.56
s.e.m.	0.09	0.01	3.38	10.39	11.05	0.06
Cu rate 3	6.73	0.22	77.67	136.67	59.00	1.47
s.e.m.	0.07	0.02	0.67	5.84	3.00	0.04
Cu rate 4	6.63	0.24	66.67	150.67	63.33	1.64
s.e.m.	0.07	0.05	1.76	3.53	1.76	0.10
Cd rate 1	6.70	0.25	101.33	135.33	58.67	1.60
s.e.m.	0.00	0.01	3.38	10.90	14.52	0.06
Cd rate 2	6.90	0.26	103.67	160.00	63.67	1.57
s.e.m.	0.06	0.02	2.33	13.45	8.19	0.10
Cd rate 3	6.83	0.21	97.33	142.67	64.00	1.69
s.e.m.	0.09	0.05	3.71	2.67	6.43	0.08
Cd rate 4	6.80	0.28	103.33	148.33	66.33	1.87
s.e.m.	0.06	0.02	2.73	4.33	8.21	0.16
LT Unc. Digested	6.77	0.18	60.67	165.00	144.67	0.95
s.e.m.	0.03	0.00	0.33	3.06	4.18	0.11
LT Unc. Raw	7.00	0.16	69.00	168.67	98.67	1.12
s.e.m.	0.15	0.01	6.03	11.84	18.37	0.07
LT Zn	6.77	0.16	65.00	165.33	112.67	1.06
s.e.m.	0.07	0.00	2.52	9.26	8.76	0.09
LT Cu	7.10	0.18	52.00	150.33	108.33	0.98
s.e.m.	0.15	0.03	2.00	6.33	13.38	0.10
LT Cd	6.93	0.14	54.00	165.33	118.33	1.05
s.e.m.	0.09	0.01	4.73	4.37	10.71	0.07

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g
Control	8.12	1973.33	192.12	0.36	4.48
s.e.m.	0.87	29.06	19.75	0.01	0.32
Unc. Digested	10.7	2023.33	265.05	0.47	4.18
s.e.m.	0.76	64.89	14.21	0.03	0.12
Unc. Raw	9.11	2010.00	268.11	0.36	4.03
s.e.m.	0.91	35.12	10.85	0.01	0.29
Zn rate 1		1976.67	229.27	0.48	3.46
s.e.m.		54.87	45.38	0.04	0.51
Zn rate 2		2013.33	250.95	0.48	2.00
s.e.m.		35.28	7.64	0.03	0.09
Zn rate 3	10.3	1986.67	139.76	0.48	2.32
s.e.m.	0.52	84.13	15.98	0.06	0.19
Zn rate 4		2023.33	142.68	0.56	0.60
s.e.m.		8.82	7.16	0.05	0.30
Cu rate 1		1870.00	292.41	0.44	4.48
s.e.m.		37.86	25.58	0.05	0.43
Cu rate 2		1926.67	277.85	0.47	3.84
s.e.m.		81.72	13.93	0.02	0.61
Cu rate 3	8.91	2033.33	246.97	0.47	3.65
s.e.m.	0.91	75.35	13.60	0.03	0.18
Cu rate 4		1953.33	188.90	0.46	4.14
s.e.m.		71.72	23.90	0.02	0.16
Cd rate 1		2003.33	252.33	0.47	3.91
s.e.m.		32.83	2.97	0.04	0.42
Cd rate 2		1950.00	265.51	0.53	3.72
s.e.m.		70.95	19.39	0.07	0.23
Cd rate 3	10.7	2036.67	237.80	0.51	3.38
s.e.m.	0.70	26.03	35.13	0.04	0.13
Cd rate 4		2013.33	266.80	0.53	3.29
s.e.m.		39.30	25.18	0.03	0.33
LT Unc. Digested		2030.00	189.78	0.49	4.40
s.e.m.		23.09	29.66	0.06	0.27
LT Unc. Raw		1963.33	233.84	0.46	4.18
s.e.m.		23.33	19.67	0.03	0.21
LT Zn		1930.00	199.58	0.52	4.11
s.e.m.		63.51	7.38	0.01	0.14
LT Cu		1996.67	216.00	0.56	4.18
s.e.m.		58.97	22.02	0.08	0.12
LT Cd		1980.00	173.75	0.45	4.42
s.e.m.		61.10	20.02	0.05	0.27

Site 3. Watlington
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	44.67	10.60	0.29	0.520	0.687	0.010
s.e.m.	8.17	1.22	0.01	0.096	0.341	0.000
Unc. Digested	65.00	35.67	0.36	0.530	0.587	0.010
s.e.m.	0.58	0.33	0.01	0.144	0.105	0.000
Unc. Raw	57.67	30.67	0.36	0.260	0.463	0.010
s.e.m.	1.86	1.33	0.02	0.070	0.044	0.000
Zn rate 1	**116.67**	38.67	0.50	**1.500**	0.797	0.017
s.e.m.	**6.67**	4.06	0.04	**0.208**	0.116	0.003
Zn rate 2	**180.00**	53.67	0.66	**4.500**	0.857	0.033
s.e.m.	**10.00**	2.96	0.03	**1.570**	0.034	0.012
Zn rate 3	**300.00**	61.67	0.79	**3.500**	1.153	0.027
s.e.m.	**30.00**	2.73	0.05	**0.321**	0.101	0.003
Zn rate 4	**350.00**	74.33	1.03	**5.500**	1.117	0.037
s.e.m.	**26.46**	4.48	0.09	**0.306**	0.083	0.003
Cu rate 1	55.33	**50.67**	0.35	0.433	**0.830**	0.010
s.e.m.	2.91	**5.61**	0.03	0.097	**0.020**	0.000
Cu rate 2	52.00	**81.67**	0.33	0.350	**1.270**	0.010
s.e.m.	3.46	**7.22**	0.03	0.071	**0.237**	0.000
Cu rate 3	61.67	**140.00**	0.36	0.567	**1.833**	0.010
s.e.m.	3.38	**5.77**	0.04	0.153	**0.203**	0.000
Cu rate 4	57.67	**153.33**	0.33	0.577	**2.100**	0.010
s.e.m.	0.88	**3.33**	0.01	0.182	**0.153**	0.000
Cd rate 1	63.33	35.33	**0.98**	0.413	0.510	**0.013**
s.e.m.	3.84	1.86	**0.08**	0.038	0.056	**0.003**
Cd rate 2	83.00	41.67	**1.83**	2.123	0.913	**0.027**
s.e.m.	3.79	0.88	**0.07**	1.689	0.235	**0.007**
Cd rate 3	98.33	48.00	**2.60**	0.943	0.773	**0.050**
s.e.m.	7.26	3.21	**0.29**	0.266	0.215	**0.006**
Cd rate 4	113.33	53.67	**3.50**	1.023	0.637	**0.067**
s.e.m.	3.33	2.19	**0.15**	0.090	0.048	**0.007**
LT Unc. Digested	44.33	17.00	0.31	0.350	0.250	0.010
s.e.m.	1.86	0.58	0.03	0.053	0.045	0.000
LT Unc. Raw	39.67	13.00	0.28	0.327	0.503	0.010
s.e.m.	0.67	0.00	0.00	0.050	0.071	0.000
LT Zn	87.00	23.00	0.37	1.133	0.197	0.010
s.e.m.	6.51	2.08	0.03	0.463	0.072	0.000
LT Cu	33.67	37.33	0.27	0.300	0.123	0.010
s.e.m.	0.88	2.85	0.02	0.000	0.020	0.000
LT Cd	45.67	15.67	1.57	0.450	0.440	0.023
s.e.m.	4.91	1.86	0.35	0.081	0.227	0.007

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.77	0.17	58.67	155.67	98.33	1.29
s.e.m.	0.07	0.01	0.88	7.88	7.06	0.02
Unc. Digested	6.57	0.31	140.00	122.67	74.33	2.28
s.e.m.	0.03	0.00	6.66	4.33	4.84	0.14
Unc. Raw	7.03	0.29	152.33	129.00	68.33	2.08
s.e.m.	0.20	0.01	6.33	11.27	3.48	0.12
Zn rate 1	6.67	0.30	137.00	140.33	83.67	2.58
s.e.m.	0.07	0.03	13.53	16.33	9.28	0.19
Zn rate 2	6.53	0.30	128.00	127.00	75.67	2.38
s.e.m.	0.07	0.02	4.36	11.06	6.17	0.04
Zn rate 3	6.77	0.29	125.00	127.33	76.67	2.14
s.e.m.	0.03	0.01	6.00	4.06	7.84	0.17
Zn rate 4	6.63	0.27	117.00	126.00	79.67	2.24
s.e.m.	0.03	0.01	6.24	8.33	5.49	0.08
Cu rate 1	6.93	0.29	136.00	129.33	68.33	2.02
s.e.m.	0.09	0.01	2.89	7.75	3.33	0.07
Cu rate 2	6.80	0.29	124.67	131.67	67.33	2.65
s.e.m.	0.12	0.02	2.73	7.75	5.81	0.62
Cu rate 3	6.83	0.29	116.00	134.00	79.33	2.62
s.e.m.	0.15	0.01	8.89	6.03	6.57	0.33
Cu rate 4	6.63	0.30	104.33	123.00	76.00	2.34
s.e.m.	0.09	0.01	4.41	6.11	11.15	0.02
Cd rate 1	6.67	0.30	132.00	126.00	73.67	2.36
s.e.m.	0.07	0.01	3.00	2.65	6.12	0.02
Cd rate 2	6.73	0.32	147.33	129.00	83.67	2.48
s.e.m.	0.03	0.01	11.46	20.01	10.73	0.20
Cd rate 3	6.63	0.42	144.33	138.67	86.67	2.29
s.e.m.	0.09	0.09	2.91	3.28	2.19	0.18
Cd rate 4	6.67	0.32	147.33	125.00	91.00	2.78
s.e.m.	0.03	0.00	0.88	9.00	5.13	0.27
LT Unc. Digested	6.73	0.23	97.00	148.00	136.67	1.69
s.e.m.	0.09	0.01	5.69	17.24	10.84	0.04
LT Unc. Raw	6.90	0.21	87.33	148.00	79.33	1.41
s.e.m.	0.06	0.01	3.28	14.73	7.97	0.06
LT Zn	6.70	0.22	101.33	149.33	97.33	1.68
s.e.m.	0.06	0.01	5.36	11.46	5.36	0.10
LT Cu	6.73	0.20	61.33	146.00	93.67	1.29
s.e.m.	0.09	0.01	2.40	9.61	3.38	0.17
LT Cd	6.77	0.20	89.00	173.00	93.33	1.50
s.e.m.	0.03	0.01	6.00	27.61	6.74	0.18

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g
Control	10.4	1993.33	287.2	0.27	5.22
s.e.m.	0.91	8.82	20.5	0.02	0.16
Unc. Digested	12.1	2056.67	371.5	0.39	4.57
s.e.m.	0.23	12.02	31.4	0.01	0.17
Unc. Raw	12.2	2040.00	473.3	0.33	4.80
s.e.m.	1.01	5.77	62.1	0.04	0.10
Zn rate 1		2053.33	358.5	0.38	4.34
s.e.m.		12.02	72.9	0.04	0.37
Zn rate 2		2036.67	311.9	0.40	4.32
s.e.m.		14.53	14.1	0.02	0.19
Zn rate 3	11.6	2043.33	301.1	0.39	3.32
s.e.m.	1.11	16.67	14.5	0.02	0.19
Zn rate 4		2016.67	279.0	0.41	3.05
s.e.m.		8.82	25.3	0.01	0.37
Cu rate 1		2023.33	417.1	0.36	4.80
s.e.m.		3.33	7.6	0.01	0.10
Cu rate 2		2040.00	387.9	0.36	4.65
s.e.m.		15.28	36.4	0.01	0.41
Cu rate 3	11.5	2036.67	364.2	0.37	4.63
s.e.m.	0.16	13.33	39.7	0.02	0.15
Cu rate 4		2043.33	319.3	0.40	4.19
s.e.m.		14.53	11.1	0.03	0.41
Cd rate 1		2043.33	332.8	0.35	4.80
s.e.m.		8.82	44.5	0.02	0.10
Cd rate 2		2046.67	395.8	0.38	4.29
s.e.m.		18.56	56.9	0.01	0.47
Cd rate 3	11.5	2050.00	378.1	0.38	4.55
s.e.m.	0.82	15.28	22.3	0.04	0.22
Cd rate 4		2030.00	382.0	0.36	4.54
s.e.m.		10.00	19.4	0.02	0.18
LT Unc. Digested		2036.67	377.8	0.25	4.96
s.e.m.		14.53	41.1	0.03	0.23
LT Unc. Raw		2026.67	311.2	0.25	5.08
s.e.m.		14.53	26.6	0.01	0.00
LT Zn		2030.00	278.0	0.30	4.52
s.e.m.		15.28	13.7	0.03	0.28
LT Cu		1990.00	313.5	0.24	5.02
s.e.m.		15.28	16.4	0.02	0.06
LT Cd		2030.00	341.7	0.23	5.08
s.e.m.		17.32	49.8	0.03	0.00

Site 4. Pwllpeiran
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	100.4	13.56	0.41	0.377	0.343	0.023
s.e.m.	4.8	0.96	0.04	0.059	0.113	0.007
Unc. Digested	116.0	30.72	0.34	0.560	0.477	0.020
s.e.m.	7.9	2.99	0.03	0.127	0.075	0.006
Unc. Raw	127.5	36.22	0.35	0.230	0.468	0.013
s.e.m.	11.8	1.20	0.01	0.095	0.254	0.003
Zn rate 1	**168.2**	38.62	0.60	**3.220**	0.537	0.040
s.e.m.	**6.2**	0.51	0.08	**1.404**	0.052	0.012
Zn rate 2	**206.0**	50.64	0.71	**2.750**	0.843	0.043
s.e.m.	**20.6**	3.27	0.09	**1.506**	0.329	0.015
Zn rate 3	**250.6**	56.81	0.74	**12.000**	1.633	0.097
s.e.m.	**7.5**	3.18	0.04	**1.155**	0.033	0.003
Zn rate 4	**272.9**	61.11	0.80	**6.333**	0.867	0.053
s.e.m.	**10.3**	3.42	0.03	**2.848**	0.120	0.013
Cu rate 1	118.9	**64.71**	0.35	1.753	**0.817**	0.033
s.e.m.	9.8	**1.74**	0.01	0.754	**0.045**	0.009
Cu rate 2	113.3	**110.88**	0.38	0.537	**1.033**	0.013
s.e.m.	7.9	**1.64**	0.02	0.083	**0.088**	0.003
Cu rate 3	113.6	**110.88**	0.30	0.690	**1.500**	0.017
s.e.m.	12.6	**4.70**	0.01	0.140	**0.100**	0.003
Cu rate 4	112.6	**171.64**	0.42	0.523	**1.933**	0.020
s.e.m.	5.8	**14.67**	0.09	0.192	**0.186**	0.006
Cd rate 1	118.4	35.02	**1.07**	0.493	0.463	**0.033**
s.e.m.	5.9	1.86	**0.07**	0.193	0.056	**0.003**
Cd rate 2	122.0	35.87	**1.68**	0.413	0.460	**0.037**
s.e.m.	14.8	4.04	**0.23**	0.038	0.026	**0.003**
Cd rate 3	149.3	45.49	**2.71**	0.863	0.483	**0.067**
s.e.m.	3.0	1.13	**0.51**	0.065	0.033	**0.003**
Cd rate 4	175.1	59.22	**4.19**	1.097	0.783	**0.120**
s.e.m.	3.0	3.10	**0.25**	0.363	0.095	**0.020**
LT Unc. Digested	108.1	25.06	0.48	0.400	0.267	0.017
s.e.m.	13.6	6.94	0.12	0.129	0.007	0.003
LT Unc. Raw	101.1	17.68	0.28	0.287	0.277	0.010
s.e.m.	11.4	1.64	0.01	0.075	0.043	0.000
LT Zn	176.8	35.19	0.46	3.000	0.563	0.023
s.e.m.	11.3	2.23	0.02	0.764	0.055	0.003
LT Cu	99.9	71.92	0.27	0.463	0.827	0.017
s.e.m.	2.6	5.36	0.01	0.191	0.140	0.003
LT Cd	105.0	24.03	1.44	0.790	0.290	0.050
s.e.m.	5.9	1.40	0.16	0.505	0.021	0.008

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.37	0.41	19.33	94.67	35.00	3.71
s.e.m.	0.09	0.02	0.88	4.67	1.73	0.13
Unc. Digested	6.40	0.49	49.33	76.33	34.33	4.26
s.e.m.	0.17	0.03	2.85	10.53	1.76	0.31
Unc. Raw	6.37	0.51	51.00	105.67	36.67	4.30
s.e.m.	0.12	0.01	4.62	3.71	3.18	0.20
Zn rate 1	6.23	0.47	51.33	92.67	37.33	4.28
s.e.m.	0.15	0.01	0.67	4.91	3.67	0.12
Zn rate 2	6.37	0.52	53.67	93.00	36.33	4.57
s.e.m.	0.03	0.03	3.93	6.43	0.33	0.03
Zn rate 3	6.20	0.49	61.00	101.67	39.33	4.61
s.e.m.	0.06	0.03	5.03	7.84	4.41	0.29
Zn rate 4	6.37	0.44	59.33	83.33	38.00	4.09
s.e.m.	0.12	0.02	4.33	6.39	5.57	0.30
Cu rate 1	6.10	0.47	42.67	80.67	44.67	3.86
s.e.m.	0.10	0.02	0.33	3.84	6.77	0.21
Cu rate 2	6.30	0.50	46.00	104.00	37.67	4.59
s.e.m.	0.06	0.02	4.73	7.23	0.88	0.08
Cu rate 3	6.40	0.44	33.33	68.00	32.67	4.05
s.e.m.	0.10	0.03	0.88	8.08	3.28	0.45
Cu rate 4	6.43	0.47	34.33	85.67	33.33	4.40
s.e.m.	0.09	0.01	3.84	15.76	1.33	0.26
Cd rate 1	6.40	0.49	65.67	80.67	45.67	4.45
s.e.m.	0.10	0.03	7.22	6.67	12.44	0.20
Cd rate 2	6.53	0.48	66.33	84.67	33.00	4.34
s.e.m.	0.09	0.01	8.95	11.85	4.36	0.32
Cd rate 3	6.23	0.50	86.67	77.00	44.67	4.24
s.e.m.	0.13	0.04	6.89	10.60	7.75	0.45
Cd rate 4	6.40	0.55	103.33	92.00	46.00	4.67
s.e.m.	0.12	0.03	7.54	9.02	5.13	0.26
LT Unc. Digested	6.23	0.43	41.33	79.33	55.67	3.80
s.e.m.	0.09	0.00	0.67	4.10	0.67	0.11
LT Unc. Raw	6.50	0.42	36.67	90.67	32.33	4.09
s.e.m.	0.17	0.02	2.91	17.89	3.48	0.34
LT Zn	6.33	0.45	53.67	397.67	42.00	4.19
s.e.m.	0.07	0.01	4.26	299.69	3.51	0.13
LT Cu	6.27	0.44	25.67	110.33	33.00	4.08
s.e.m.	0.18	0.02	1.76	2.60	4.04	0.12
LT Cd	6.33	0.40	34.67	77.00	38.67	3.60
s.e.m.	0.03	0.01	5.46	15.89	2.60	0.20

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g
Control	20.5	2120.00	388.7	0.42	3.92
s.e.m.	0.59	111.50	17.9	0.02	0.16
Unc. Digested	22.7	2006.67	426.3	0.47	3.78
s.e.m.	3.24	38.44	32.8	0.02	0.32
Unc. Raw	24.4	2035.00	412.3	0.46	3.80
s.e.m.	1.02	4.08	37.0	0.05	0.31
Zn rate 1		2143.33	424.9	0.39	3.46
s.e.m.		152.13	11.6	0.02	0.51
Zn rate 2		2063.33	410.7	0.43	3.07
s.e.m.		136.42	31.3	0.02	0.29
Zn rate 3	23.1	2133.33	417.0	0.44	2.83
s.e.m.	2.67	120.19	8.8	0.03	0.35
Zn rate 4		2090.00	419.5	0.42	2.03
s.e.m.		36.06	32.2	0.03	0.07
Cu rate 1		2146.67	412.6	0.43	3.60
s.e.m.		158.57	17.9	0.04	0.32
Cu rate 2		2136.67	443.6	0.48	3.45
s.e.m.		137.76	23.3	0.04	0.09
Cu rate 3	20.3	2115.00	396.5	0.50	3.25
s.e.m.	3.28	61.24	27.0	0.04	0.20
Cu rate 4		2003.33	397.2	0.50	2.88
s.e.m.		124.54	14.6	0.03	0.31
Cd rate 1		1943.33	432.5	0.55	3.44
s.e.m.		77.96	24.8	0.09	0.10
Cd rate 2		2170.00	419.7	0.50	3.08
s.e.m.		60.83	15.3	0.07	0.34
Cd rate 3	23.5	2176.67	429.7	0.44	3.45
s.e.m.	3.31	119.21	29.7	0.02	0.09
Cd rate 4		2133.33	407.9	0.60	2.52
s.e.m.		90.25	28.6	0.05	0.11
LT Unc. Digested		2156.67	429.6	0.48	3.86
s.e.m.		37.12	10.7	0.03	0.12
LT Unc. Raw		2040.00	414.1	0.54	3.55
s.e.m.		102.63	26.9	0.03	0.51
LT Zn		2216.67	394.2	0.51	3.22
s.e.m.		72.65	4.5	0.01	0.13
LT Cu		2036.67	406.4	0.49	3.86
s.e.m.		99.39	12.7	0.03	0.12
LT Cd		1993.33	357.8	0.46	3.22
s.e.m.		31.80	25.5	0.02	0.22

Site 5. Rosemaund
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	71.53	18.48	0.30	-	-	-
s.e.m.	1.81	0.59	0.01	-	-	-
Unc. Digested	96.17	34.57	0.38	0.130	0.193	0.010
s.e.m.	13.55	1.23	0.07	0.044	0.023	0.000
Unc. Raw	108.15	34.57	0.34	0.137	0.187	0.010
s.e.m.	17.95	1.49	0.02	0.023	0.018	0.000
Zn rate 1	**136.89**	46.54	0.42	**0.283**	0.270	0.010
s.e.m.	**3.42**	2.67	0.02	**0.115**	0.012	0.000
Zn rate 2	**225.87**	56.81	0.54	**2.200**	0.373	0.010
s.e.m.	**25.84**	0.91	0.03	**0.802**	0.043	0.000
Zn rate 3	**284.05**	71.18	0.75	**1.767**	0.497	0.010
s.e.m.	**22.44**	4.79	0.05	**0.524**	0.032	0.000
Zn rate 4	**335.39**	83.85	0.85	**5.667**	0.447	0.017
s.e.m.	**3.42**	2.24	0.05	**1.550**	0.023	0.003
Cu rate 1	110.20	**62.63**	0.35	0.213	**0.330**	0.010
s.e.m.	8.41	**3.61**	0.03	0.100	**0.015**	0.000
Cu rate 2	88.64	**81.11**	0.30	0.297	**0.487**	0.010
s.e.m.	5.38	**5.43**	0.02	0.137	**0.029**	0.000
Cu rate 3	91.38	**119.78**	0.34	0.237	**0.730**	0.010
s.e.m.	0.84	**3.42**	0.01	0.129	**0.137**	0.000
Cu rate 4	82.82	**143.74**	0.42	0.343	**0.967**	0.030
s.e.m.	2.08	**5.93**	0.10	0.144	**0.226**	0.006
Cd rate 1	85.56	35.25	**0.82**	0.107	0.183	**0.047**
s.e.m.	0.91	0.34	**0.01**	0.048	0.028	**0.012**
Cd rate 2	99.93	41.41	**1.40**	0.417	0.147	**0.107**
s.e.m.	2.74	2.24	**0.03**	0.141	0.012	**0.037**
Cd rate 3	111.91	44.83	**1.80**	0.237	0.207	**0.217**
s.e.m.	6.84	2.40	**0.04**	0.078	0.009	**0.068**
Cd rate 4	123.20	48.25	**2.43**	0.490	0.190	**0.197**
s.e.m.	0.00	1.19	**0.03**	0.110	0.025	**0.024**
LT Unc. Digested	71.18	21.90	0.31	0.197	0.037	0.075
s.e.m.	1.81	0.34	0.03	0.024	0.007	0.020
LT Unc. Raw	74.61	23.27	0.40	0.360	0.047	0.050
s.e.m.	2.47	0.91	0.04	0.040	0.012	0.006
LT Zn	119.78	31.49	0.34	1.933	0.110	0.063
s.e.m.	3.42	1.71	0.02	0.809	0.020	0.003
LT Cu	81.11	55.10	0.37	1.897	0.160	0.095
s.e.m.	4.15	3.47	0.03	0.810	0.000	0.004
LT Cd	90.01	26.01	0.89	0.270	0.090	0.105
s.e.m.	3.37	1.37	0.06	0.098	0.035	0.004

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.23	0.25	20.33	226.33	158.00	1.69
s.e.m.	0.09	0.01	1.86	15.06	16.62	0.05
Unc. Digested	6.63	0.34	68.67	195.33	202.67	2.23
s.e.m.	0.15	0.02	3.53	15.38	17.42	0.28
Unc. Raw	6.43	0.31	62.33	223.33	117.00	2.16
s.e.m.	0.13	0.01	4.10	23.75	14.29	0.08
Zn rate 1	6.73	0.37	78.00	210.67	216.67	2.56
s.e.m.	0.18	0.02	2.65	15.65	28.36	0.09
Zn rate 2	6.70	0.34	69.00	132.33	200.33	2.27
s.e.m.	0.10	0.01	6.00	49.83	23.18	0.30
Zn rate 3	6.50	0.35	71.00	132.00	182.67	2.14
s.e.m.	0.06	0.01	4.00	31.66	13.38	0.20
Zn rate 4	6.67	0.36	68.00	172.33	174.67	2.07
s.e.m.	0.07	0.01	2.65	35.69	18.76	0.10
Cu rate 1	6.63	0.32	57.00	168.67	149.67	2.11
s.e.m.	0.07	0.02	3.06	48.56	13.37	0.13
Cu rate 2	6.57	0.31	57.67	229.33	149.33	2.20
s.e.m.	0.12	0.01	3.28	32.03	8.41	0.11
Cu rate 3	6.57	0.33	48.00	149.67	158.00	2.25
s.e.m.	0.15	0.02	5.51	60.45	13.01	0.18
Cu rate 4	6.63	0.30	49.67	195.33	154.33	2.13
s.e.m.	0.09	0.03	4.33	42.83	14.31	0.07
Cd rate 1	6.83	0.37	71.67	192.67	189.33	2.75
s.e.m.	0.15	0.01	6.36	18.85	17.37	0.06
Cd rate 2	6.53	0.32	76.00	294.00	166.67	2.41
s.e.m.	0.15	0.01	6.11	56.80	16.48	0.19
Cd rate 3	6.73	0.34	76.33	169.00	158.33	2.16
s.e.m.	0.09	0.02	8.41	46.05	18.28	0.31
Cd rate 4	6.53	0.33	76.00	129.00	147.33	2.15
s.e.m.	0.09	0.01	2.00	28.92	11.57	0.10
LT Unc. Digested	6.47	0.29	38.00	225.67	182.33	1.78
s.e.m.	0.09	0.02	4.73	63.31	24.36	0.06
LT Unc. Raw	6.27	0.28	33.33	130.67	121.67	1.71
s.e.m.	0.09	0.01	4.48	44.49	16.76	0.10
LT Zn	6.40	0.28	46.67	244.33	171.67	1.89
s.e.m.	0.10	0.02	7.06	120.24	13.04	0.10
LT Cu	6.13	0.29	23.67	214.67	174.00	1.73
s.e.m.	0.32	0.01	2.73	30.37	31.56	0.21
LT Cd	6.47	0.26	29.67	187.33	166.33	1.66
s.e.m.	0.09	0.01	1.45	36.04	16.76	0.13

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g
Control	15.4	2023.33	262.9	0.30	5.28
s.e.m.	0.62	31.80	60.4	0.01	0.10
Unc. Digested	18.0	1996.67	344.7	0.34	4.76
s.e.m.	0.45	63.60	30.8	0.01	0.16
Unc. Raw	17.6	2023.33	453.5	0.28	5.12
s.e.m.	0.90	51.75	36.7	0.01	0.14
Zn rate 1		2140.00	393.3	0.36	4.79
s.e.m.		10.00	23.0	0.01	0.20
Zn rate 2		1910.00	280.2	0.37	3.10
s.e.m.		100.00	33.7	0.02	0.38
Zn rate 3	18.1	2050.00	318.3	0.32	2.78
s.e.m.	1.17	81.65	47.8	0.03	0.30
Zn rate 4		2120.00	234.3	0.35	2.29
s.e.m.		48.99	27.4	0.03	0.89
Cu rate 1		2113.33	345.4	0.29	5.18
s.e.m.		32.83	26.8	0.00	0.10
Cu rate 2		2086.67	422.8	0.29	5.18
s.e.m.		98.21	34.4	0.03	0.10
Cu rate 3	17.8	2000.00	349.9	0.28	5.12
s.e.m.	0.71	20.00	41.5	0.03	0.04
Cu rate 4		2010.00	348.4	0.31	4.63
s.e.m.		35.12	22.7	0.05	0.15
Cd rate 1		2106.67	382.4	0.39	4.80
s.e.m.		35.28	53.1	0.03	0.10
Cd rate 2		2003.33	338.0	0.34	5.18
s.e.m.		61.73	32.4	0.03	0.10
Cd rate 3	17.7	2040.00	392.3	0.35	4.32
s.e.m.	1.50	132.29	31.3	0.03	0.19
Cd rate 4		1966.67	335.2	0.36	4.55
s.e.m.		42.56	55.0	0.03	0.32
LT Unc. Digested		2090.00	343.0	0.30	5.38
s.e.m.		41.63	47.6	0.02	0.00
LT Unc. Raw		2143.33	341.3	0.33	5.28
s.e.m.		40.96	45.6	0.04	0.10
LT Zn		2056.67	287.8	0.31	4.54
s.e.m.		86.47	64.3	0.01	0.28
LT Cu		2100.00	312.1	0.30	5.18
s.e.m.		41.63	38.0	0.03	0.10
LT Cd		2103.33	292.7	0.32	5.22
s.e.m.		52.39	23.8	0.02	0.16

Site 6. Bridgets
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	48.16	10.76	0.86	0.331	0.133	0.010
s.e.m.	1.67	0.42	0.02	0.107	0.051	0.000
Unc. Digested	92.90	45.08	1.05	0.161	0.176	0.000
s.e.m.	10.34	9.20	0.33	0.089	0.004	0.000
Unc. Raw	90.16	38.25	1.05	0.082	0.208	0.000
s.e.m.	7.83	3.36	0.09	0.020	0.022	0.000
Zn rate 1	**136.61**	44.06	1.40	**0.601**	0.219	0.007
s.e.m.	**3.42**	1.57	0.15	**0.377**	0.015	0.003
Zn rate 2	**211.75**	58.74	1.26	**0.198**	0.352	0.003
s.e.m.	**9.04**	1.90	0.15	**0.029**	0.025	0.003
Zn rate 3	**343.24**	56.69	1.47	**1.786**	0.297	0.014
s.e.m.	**46.01**	19.85	0.55	**0.583**	0.031	0.003
Zn rate 4	**327.87**	74.11	1.43	**2.083**	0.342	0.020
s.e.m.	**15.65**	4.28	0.06	**0.415**	0.028	0.000
Cu rate 1	84.70	**55.67**	1.09	0.085	**0.263**	0.007
s.e.m.	6.52	**3.26**	0.03	0.024	**0.012**	0.003
Cu rate 2	86.75	**95.63**	1.13	0.161	**0.427**	0.007
s.e.m.	1.71	**3.47**	0.00	0.067	**0.061**	0.003
Cu rate 3	98.02	**146.86**	1.37	0.048	**0.625**	0.003
s.e.m.	2.24	**9.04**	0.07	0.022	**0.066**	0.003
Cu rate 4	87.09	**170.77**	1.13	0.184	**0.693**	0.010
s.e.m.	2.37	**12.31**	0.06	0.073	**0.048**	0.000
Cd rate 1	122.95	50.20	**1.74**	0.072	0.195	**0.010**
s.e.m.	10.25	3.88	**0.18**	0.006	0.010	**0.000**
Cd rate 2	112.70	52.25	**2.19**	0.048	0.219	**0.010**
s.e.m.	10.25	7.55	**0.07**	0.012	0.024	**0.000**
Cd rate 3	119.54	50.55	**2.70**	0.287	0.229	**0.014**
s.e.m.	3.42	2.24	**0.09**	0.021	0.022	**0.003**
Cd rate 4	136.61	52.60	**3.76**	0.225	0.307	**0.020**
s.e.m.	3.42	0.68	**0.27**	0.031	0.068	**0.000**
LT Unc. Digested	69.67	20.15	1.06	0.396	0.184	0.020
s.e.m.	6.26	2.08	0.15	0.149	0.024	0.006
LT Unc. Raw	65.23	16.05	1.00	0.174	0.106	0.007
s.e.m.	4.74	1.23	0.07	0.062	0.024	0.003
LT Zn	153.69	35.86	1.37	0.478	0.202	0.010
s.e.m.	15.65	3.69	0.21	0.125	0.017	0.000
LT Cu	70.36	51.91	1.19	0.191	0.212	0.014
s.e.m.	2.98	2.46	0.17	0.151	0.009	0.003
LT Cd	96.65	23.22	1.88	0.342	0.171	0.020
s.e.m.	4.78	1.49	0.14	0.148	0.039	0.006

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.33	0.23	20.33	146.67	62.00	1.91
s.e.m.	0.09	0.01	2.91	5.67	2.52	0.30
Unc. Digested	6.53	0.34	87.33	137.33	65.00	2.56
s.e.m.	0.07	0.00	2.60	11.61	5.29	0.18
Unc. Raw	6.57	0.32	107.33	144.00	66.00	2.47
s.e.m.	0.09	0.01	3.48	6.66	6.11	0.03
Zn rate 1	6.43	0.36	110.67	126.00	72.67	2.85
s.e.m.	0.09	0.03	10.67	19.86	8.69	0.22
Zn rate 2	6.70	0.35	114.00	134.33	72.33	2.86
s.e.m.	0.06	0.00	5.57	7.69	2.33	0.09
Zn rate 3	6.40	0.34	105.00	133.33	71.67	2.94
s.e.m.	0.00	0.02	2.08	12.68	4.37	0.14
Zn rate 4	6.57	0.31	92.00	130.33	81.67	2.68
s.e.m.	0.09	0.01	3.21	8.97	5.46	0.07
Cu rate 1	6.63	0.35	100.00	120.00	69.00	2.51
s.e.m.	0.09	0.03	8.62	18.72	4.73	0.16
Cu rate 2	6.63	0.34	88.00	133.00	67.00	2.45
s.e.m.	0.22	0.01	2.08	9.45	6.51	0.06
Cu rate 3	6.53	0.34	79.00	137.33	74.00	2.85
s.e.m.	0.20	0.01	3.79	12.41	5.29	0.09
Cu rate 4	6.43	0.32	70.67	122.00	69.67	2.59
s.e.m.	0.09	0.01	4.67	18.50	6.64	0.05
Cd rate 1	6.73	0.37	112.33	122.00	83.67	3.08
s.e.m.	0.07	0.03	6.23	7.81	3.53	0.15
Cd rate 2	6.57	0.41	128.67	146.67	87.67	3.31
s.e.m.	0.12	0.01	4.91	2.73	3.28	0.06
Cd rate 3	6.47	0.39	136.67	142.33	90.67	3.17
s.e.m.	0.03	0.01	9.24	20.12	7.06	0.10
Cd rate 4	6.53	0.37	135.67	158.33	93.67	3.14
s.e.m.	0.09	0.01	1.86	14.19	8.67	0.08
LT Unc. Digested	6.07	0.27	52.00	131.33	91.67	2.04
s.e.m.	0.15	0.02	5.03	8.65	3.71	0.07
LT Unc. Raw	6.53	0.25	36.67	135.00	69.00	1.81
s.e.m.	0.15	0.01	2.19	8.66	6.51	0.11
LT Zn	6.60	0.29	70.33	151.67	85.67	2.16
s.e.m.	0.12	0.01	4.48	16.41	7.22	0.11
LT Cu	6.63	0.27	28.33	137.00	72.00	1.86
s.e.m.	0.32	0.01	1.45	18.52	2.08	0.10
LT Cd	6.37	0.26	43.33	137.33	75.67	1.87
s.e.m.	0.19	0.01	1.86	3.38	3.93	0.09

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g
Control	13.8	1860.00	325.4	0.27	5.12
s.e.m.	0.26	8.16	6.9	0.02	0.14
Unc. Digested	16.4	1950.00	392.4	0.28	5.06
s.e.m.	0.80	-	4.9	0.01	0.09
Unc. Raw	17.3	1936.67	420.4	0.29	4.96
s.e.m.	0.78	91.35	46.3	0.03	0.06
Zn rate 1		1890.00	366.9	0.28	4.92
s.e.m.		16.33	36.8	0.04	0.16
Zn rate 2		2075.00	388.6	0.32	3.96
s.e.m.		36.74	20.3	0.02	0.10
Zn rate 3	17.5	1950.00	304.1	0.28	4.00
s.e.m.	0.59	115.04	25.5	0.01	0.22
Zn rate 4		1920.00	315.9	0.31	4.46
s.e.m.		97.98	12.8	0.01	0.23
Cu rate 1		1993.33	383.7	0.31	4.80
s.e.m.		44.10	7.8	0.01	0.10
Cu rate 2		1973.33	416.3	0.34	4.63
s.e.m.		36.67	12.9	0.01	0.15
Cu rate 3	18.1	1930.00	444.2	0.30	4.50
s.e.m.	0.29	51.32	22.2	0.03	0.10
Cu rate 4		2000.00	356.5	0.29	4.70
s.e.m.		25.17	26.4	0.02	0.10
Cd rate 1		2003.33	448.3	0.29	5.02
s.e.m.		52.07	34.0	0.02	0.06
Cd rate 2		2026.67	414.7	0.38	4.96
s.e.m.		33.83	25.6	0.01	0.23
Cd rate 3	19.2	1990.00	410.7	0.33	4.86
s.e.m.	0.58	55.08	17.4	0.02	0.14
Cd rate 4		1966.67	404.4	0.32	4.96
s.e.m.		59.25	8.3	0.01	0.06
LT Unc. Digested		2020.00	347.3	0.27	4.81
s.e.m.		37.86	19.7	0.02	0.36
LT Unc. Raw		1973.33	372.4	0.28	5.12
s.e.m.		66.42	37.6	0.03	0.14
LT Zn		2076.67	376.6	0.32	4.77
s.e.m.		16.67	38.2	0.01	0.23
LT Cu		2020.00	344.1	0.32	5.00
s.e.m.		15.28	30.9	0.05	0.10
LT Cd		1896.67	336.7	0.26	5.18
s.e.m.		61.73	4.4	0.03	0.10

Site 7. Hartwood
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	81.0	24.09	0.31	0.475	0.024	0.008
s.e.m.	3.4	4.28	0.02	0.020	0.005	0.001
Unc. Digested	103.6	53.94	0.37	1.962	0.108	0.011
s.e.m.	4.9	4.69	0.02	0.588	0.005	0.002
Unc. Raw	103.9	50.69	0.39	0.652	0.100	0.006
s.e.m.	17.2	9.89	0.07	0.206	0.009	0.001
Zn rate 1	**166.0**	61.76	0.50	**6.986**	0.145	0.019
s.e.m.	**11.3**	6.50	0.03	**2.068**	0.026	0.003
Zn rate 2	**281.1**	82.27	0.75	**12.598**	0.173	0.019
s.e.m.	**24.4**	8.94	0.06	**5.820**	0.025	0.005
Zn rate 3	**367.8**	87.47	0.86	**33.141**	0.240	0.050
s.e.m.	**20.2**	7.80	0.06	**3.589**	0.013	0.003
Zn rate 4	**473.6**	110.58	1.06	**43.700**	0.274	0.055
s.e.m.	**35.9**	11.62	0.10	**9.454**	0.020	0.012
Cu rate 1	112.7	**65.21**	0.41	0.723	**0.169**	0.006
s.e.m.	0.8	**6.11**	0.01	0.393	**0.009**	0.002
Cu rate 2	110.4	**123.98**	0.38	1.896	**0.271**	0.011
s.e.m.	9.4	**9.47**	0.02	0.486	**0.047**	0.002
Cu rate 3	113.8	**157.99**	0.37	2.452	**0.384**	0.014
s.e.m.	3.6	**10.38**	0.01	0.426	**0.019**	0.002
Cu rate 4	96.7	**198.04**	0.34	3.037	**0.544**	0.013
s.e.m.	6.6	**6.07**	0.05	0.851	**0.170**	0.004
Cd rate 1	121.5	55.47	**1.08**	2.480	0.108	**0.035**
s.e.m.	7.3	4.41	**0.04**	1.025	0.010	**0.009**
Cd rate 2	127.8	58.18	**2.12**	4.330	0.143	**0.074**
s.e.m.	5.9	2.98	**0.08**	1.208	0.016	**0.015**
Cd rate 3	162.1	71.98	**3.12**	4.290	0.198	**0.105**
s.e.m.	15.6	9.74	**0.29**	1.342	0.028	**0.023**
Cd rate 4	177.1	72.24	**4.03**	5.719	0.209	**0.125**
s.e.m.	1.8	3.17	**0.15**	0.837	0.023	**0.021**
LT Unc. Digested	85.9	31.02	0.31	0.633	0.046	0.007
s.e.m.	3.8	0.89	0.02	0.144	0.005	0.002
LT Unc. Raw	96.4	35.38	0.30	0.535	0.041	0.008
s.e.m.	2.3	2.45	0.02	0.179	0.000	0.002
LT Zn	161.2	38.12	0.43	6.983	0.084	0.020
s.e.m.	5.0	4.52	0.02	1.854	0.011	0.003
LT Cu	84.2	62.51	0.29	0.710	0.088	0.008
s.e.m.	2.0	5.62	0.02	0.093	0.008	0.001
LT Cd	90.3	28.44	1.01	0.476	0.049	0.022
s.e.m.	3.6	4.58	0.05	0.145	0.012	0.003

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	5.90	0.25	250.5	5.4	132.4	4.32
s.e.m.	0.08	0.00	29.3	0.5	21.8	0.32
Unc. Digested	5.71	0.36	207.3	41.5	116.5	5.04
s.e.m.	0.20	0.01	15.9	3.3	12.3	0.05
Unc. Raw	5.86	0.26	149.0	43.8	109.0	4.16
s.e.m.	0.18	0.04	26.4	5.3	26.3	0.72
Zn rate 1	5.75	0.34	187.6	50.9	113.1	5.02
s.e.m.	0.17	0.05	7.6	9.4	19.3	0.56
Zn rate 2	5.94	0.44	273.3	53.8	128.8	6.83
s.e.m.	0.29	0.02	36.3	0.4	12.1	0.80
Zn rate 3	5.66	0.30	169.2	52.2	109.9	5.12
s.e.m.	0.10	0.04	9.9	3.2	18.9	0.41
Zn rate 4	5.69	0.35	193.6	57.9	125.8	5.33
s.e.m.	0.14	0.05	53.7	6.5	21.2	0.65
Cu rate 1	6.06	0.33	193.1	54.0	102.7	4.71
s.e.m.	0.17	0.03	22.7	13.4	20.6	0.52
Cu rate 2	5.79	0.32	137.0	47.3	100.4	4.52
s.e.m.	0.16	0.04	17.3	11.5	1.5	0.54
Cu rate 3	5.91	0.32	145.5	40.2	101.1	4.63
s.e.m.	0.10	0.03	8.8	1.0	11.2	0.40
Cu rate 4	5.72	0.32	198.6	37.9	107.9	5.21
s.e.m.	0.09	0.06	34.9	6.9	24.2	1.47
Cd rate 1	5.80	0.40	221.9	52.3	127.0	5.36
s.e.m.	0.15	0.01	14.7	4.9	9.4	0.24
Cd rate 2	5.69	0.36	165.1	94.0	99.1	5.16
s.e.m.	0.08	0.04	13.6	4.4	11.9	0.46
Cd rate 3	5.68	0.35	163.7	110.0	110.6	4.65
s.e.m.	0.21	0.05	23.0	11.4	6.6	0.44
Cd rate 4	5.60	0.34	161.9	256.5	86.6	4.68
s.e.m.	0.16	0.01	11.7	34.0	7.5	0.48
LT Unc. Digested	5.88	0.25	250.5	18.3	97.6	4.14
s.e.m.	0.13	0.01	12.9	2.2	5.8	0.20
LT Unc. Raw	5.99	0.26	218.1	9.8	124.5	3.83
s.e.m.	0.10	0.03	36.9	0.7	17.7	0.35
LT Zn	5.70	0.23	205.7	19.7	93.3	3.39
s.e.m.	0.02	0.02	8.6	2.1	6.2	0.08
LT Cu	5.88	0.25	230.2	9.7	124.4	4.52
s.e.m.	0.03	0.01	23.0	1.2	1.9	0.27
LT Cd	6.07	0.24	231.2	12.8	119.8	3.56
s.e.m.	0.11	0.03	30.9	2.0	13.0	0.34

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg/hr
Control	11.2	505.7	3.05	0.73
s.e.m.	1.12	94.0	0.31	0.04
Unc. Digested	12.6	593.0	4.16	1.11
s.e.m.	0.66	59.8	0.18	0.10
Unc. Raw	13.3	572.2	3.75	0.91
s.e.m.	1.58	29.5	0.13	0.12
Zn rate 1		423.8	1.22	1.19
s.e.m.		77.7	0.61	0.16
Zn rate 2		517.5	< 1.00	0.98
s.e.m.		24.4	-	0.03
Zn rate 3	11.5	463.9	< 1.00	0.99
s.e.m.	0.29	27.7	-	0.06
Zn rate 4		364.4	< 1.00	0.88
s.e.m.		36.9	-	0.02
Cu rate 1		525.1	4.10	0.99
s.e.m.		65.3	0.19	0.19
Cu rate 2		470.4	3.23	0.92
s.e.m.		25.9	0.16	0.13
Cu rate 3	11.6	418.0	2.91	0.89
s.e.m.	0.72	25.1	0.11	0.08
Cu rate 4		307.8	2.36	0.85
s.e.m.		65.6	0.61	0.11
Cd rate 1		560.5	3.15	1.28
s.e.m.		20.5	0.59	0.27
Cd rate 2		493.9	2.21	1.05
s.e.m.		36.8	0.84	0.06
Cd rate 3	13.3	588.1	1.89	1.14
s.e.m.	1.47	42.8	0.95	0.25
Cd rate 4		393.7	1.08	0.91
s.e.m.		81.8	1.08	0.02
LT Unc. Digested		451.1	3.73	0.90
s.e.m.		34.7	0.17	0.12
LT Unc. Raw		452.3	3.43	0.79
s.e.m.		47.7	0.71	0.11
LT Zn		328.9	1.28	0.65
s.e.m.		33.4	0.63	0.04
LT Cu		480.3	3.21	0.81
s.e.m.		11.4	0.60	0.07
LT Cd		454.7	3.72	0.88
s.e.m.		36.4	0.09	0.05

Site 8. Auchincruive
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd Mg/kg
Control	46.67	< 1.00	< 1.00	0.566	0.085	< 0.0135
s.e.m.	8.97	-	-	0.017	0.033	
Unc. Digested	77.33	31.33	< 1.00	0.861	0.245	0.014
s.e.m.	14.34	4.37	-	0.215	0.009	0.002
Unc. Raw	83.00	29.67	< 1.00	0.980	0.165	0.015
s.e.m.	15.37	3.93	-	0.288	0.014	-
Zn rate 1	**112.33**	39.00	< 1.00	**1.788**	0.320	0.023
s.e.m.	**16.29**	4.73	-	**0.882**	0.027	-
Zn rate 2	**223.67**	64.33	< 1.00	**8.691**	0.345	0.034
s.e.m.	**21.87**	5.24	-	**3.361**	0.024	0.007
Zn rate 3	**341.00**	73.33	< 1.00	**16.365**	0.379	0.049
s.e.m.	**82.14**	6.69	-	**3.474**	0.021	0.005
Zn rate 4	**392.33**	89.33	< 1.00	**25.613**	0.438	0.070
s.e.m.	**46.92**	5.70	-	**2.807**	0.041	-
Cu rate 1	65.67	**45.00**	< 1.00	1.435	**0.215**	0.020
s.e.m.	24.90	**14.98**	-	-	**-**	-
Cu rate 2	75.67	**91.00**	< 1.00	0.513	**0.286**	0.015
s.e.m.	14.77	**7.02**	-	0.392	**0.111**	-
Cu rate 3	79.00	**127.00**	< 1.00	1.363	**0.653**	0.016
s.e.m.	8.08	**13.11**	-	0.175	**0.073**	0.001
Cu rate 4	88.00	**222.67**	< 1.00	0.886	**0.939**	0.018
s.e.m.	4.16	**12.78**	-	0.560	**0.003**	0.004
Cd rate 1	84.67	33.33	**< 1.00**	1.761	0.215	**0.045**
s.e.m.	10.27	1.86	**-**	0.091	0.004	**-**
Cd rate 2	116.00	46.00	**0.70**	2.498	0.255	**0.130**
s.e.m.	16.86	4.51	**-**	0.414	0.014	**0.041**
Cd rate 3	172.67	50.67	**1.67**	3.004	0.307	**0.144**
s.e.m.	44.69	3.18	**0.33**	1.228	0.017	**0.023**
Cd rate 4	134.00	58.67	**1.80**	2.531	0.320	**0.128**
s.e.m.	16.50	4.91	**0.44**	0.026	0.031	**0.002**
LT Unc. Digested	53.67	8.10	< 1.00	0.51	0.07	0.01
s.e.m.	7.17	1.00	-	0.18	0.02	-
LT Unc. Raw	43.67	4.47	< 1.00	0.742	0.182	0.019
s.e.m.	7.54	0.97	-	0.205	0.103	0.003
LT Zn	116.00	18.00	< 1.00	5.216	0.171	0.035
s.e.m.	26.51	6.11	-	2.789	0.001	-
LT Cu	72.33	34.00	< 1.00	0.834	0.146	0.013
s.e.m.	17.70	2.00	-	0.193	0.021	-
LT Cd	55.67	7.87	< 1.00	0.800	0.095	0.036
s.e.m.	10.37	2.07	-	0.109	0.012	0.005

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.23	0.20	36.33	54.67	124.00	3.39
s.e.m.	0.03	0.01	2.73	1.86	11.14	0.53
Unc. Digested	6.17	0.28	117.33	50.33	37.00	3.53
s.e.m.	0.03	0.01	0.67	3.53	3.61	0.22
Unc. Raw	6.13	0.27	98.67	58.67	66.33	3.63
s.e.m.	0.03	0.00	6.39	2.40	3.76	0.33
Zn rate 1	6.13	0.28	115.00	52.33	37.33	3.53
s.e.m.	0.03	0.01	4.58	4.41	2.91	0.12
Zn rate 2	6.07	0.29	108.33	55.00	45.00	4.25
s.e.m.	0.03	0.00	6.39	2.89	6.66	0.17
Zn rate 3	6.03	0.27	108.67	57.67	41.67	3.98
s.e.m.	0.03	0.00	2.60	4.06	5.55	0.35
Zn rate 4	6.00	0.24	102.00	54.00	41.00	3.98
s.e.m.	0.00	0.02	1.53	6.11	2.52	0.30
Cu rate 1	6.10	0.28	94.00	59.67	53.33	3.55
s.e.m.	0.00	0.01	2.08	5.36	8.35	0.28
Cu rate 2	6.13	0.27	85.33	54.33	45.33	3.99
s.e.m.	0.03	0.01	3.48	4.33	7.42	0.16
Cu rate 3	6.17	0.27	79.33	53.67	47.33	3.87
s.e.m.	0.03	0.02	1.20	5.21	7.88	0.05
Cu rate 4	6.17	0.30	73.00	56.00	36.67	3.45
s.e.m.	0.03	0.03	2.08	4.04	2.73	0.10
Cd rate 1	6.17	0.29	107.33	57.33	43.33	3.51
s.e.m.	0.07	0.01	1.76	2.85	2.73	0.17
Cd rate 2	6.17	0.29	118.33	53.00	44.00	4.14
s.e.m.	0.03	0.01	3.76	2.52	4.51	0.34
Cd rate 3	6.07	0.29	129.33	49.67	60.00	3.66
s.e.m.	0.03	0.01	0.33	2.19	6.35	0.35
Cd rate 4	6.17	0.31	135.67	53.67	49.67	4.25
s.e.m.	0.03	0.01	0.88	3.53	0.67	0.24
LT Unc. Digested	6.27	0.22	49.67	50.33	123.67	3.33
s.e.m.	0.03	0.01	1.63	2.25	15.80	0.18
LT Unc. Raw	6.17	0.21	51.33	48.00	102.67	3.52
s.e.m.	0.03	0.01	3.84	4.62	21.74	0.35
LT Zn	6.27	0.20	56.33	52.67	89.33	3.04
s.e.m.	0.03	0.01	1.33	4.33	4.67	0.30
LT Cu	6.37	0.21	36.00	52.67	116.33	3.19
s.e.m.	0.09	0.01	2.08	2.60	21.09	0.12
LT Cd	6.27	0.21	52.33	48.33	112.00	3.20
s.e.m.	0.03	0.01	2.40	4.26	20.53	0.39

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg/hr
Control	8.77	2113.3	498.2	4.45	0.42
s.e.m.	1.09	8.8	19.6	0.18	0.01
Unc. Digested	11.63	2046.7	595.9	4.06	0.62
s.e.m.	0.43	3.3	16.5	0.16	0.01
Unc. Raw	11.25	2066.7	585.6	4.43	0.53
s.e.m.	0.92	3.3	8.4	0.08	0.01
Zn rate 1		2036.7	530.6	3.46	0.57
s.e.m.		3.3	6.3	0.20	0.01
Zn rate 2		2046.7	517.0	3.06	0.53
s.e.m.		6.7	9.5	0.26	0.01
Zn rate 3	11.87	2046.7	599.6	2.75	0.62
s.e.m.	0.85	14.5	22.6	0.65	0.03
Zn rate 4		2046.7	489.6	2.08	0.60
s.e.m.		3.3	13.6	0.38	0.02
Cu rate 1		2056.7	610.1	4.13	0.50
s.e.m.		12.0	43.6	0.23	0.03
Cu rate 2		2053.3	568.8	4.21	0.50
s.e.m.		3.3	13.2	0.11	0.04
Cu rate 3	9.67	2060.0	502.8	4.55	0.55
s.e.m.	1.14	5.8	7.0	0.27	0.01
Cu rate 4		2033.3	597.4	3.73	0.67
s.e.m.		8.8	39.4	0.13	0.04
Cd rate 1		2040.0	572.0	3.18	0.57
s.e.m.		5.8	5.7	0.37	0.02
Cd rate 2		2043.3	566.6	3.60	0.56
s.e.m.		6.7	72.7	0.06	0.03
Cd rate 3	12.13	2053.3	508.8	3.52	0.56
s.e.m.	0.80	6.7	13.1	0.13	0.04
Cd rate 4		2046.7	531.1	3.05	0.52
s.e.m.		3.3	15.9	0.15	0.04
LT Unc. Digested		2118.3	498.0	4.06	0.43
s.e.m.		10.5	4.4	0.20	0.02
LT Unc. Raw		2103.3	491.8	4.59	0.41
s.e.m.		24.0	41.0	0.07	0.03
LT Zn		2083.3	518.7	3.67	0.42
s.e.m.		6.7	25.8	0.40	0.02
LT Cu		2106.7	514.3	4.26	0.50
s.e.m.		18.6	23.0	0.40	0.04
LT Cd		2096.7	458.1	4.07	0.43
s.e.m.		16.7	29.6	0.32	0.01

Site 9. Shirburn
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	71.53	14.58	0.47	0.052	0.115	0.014
s.e.m.	11.26	1.04	0.04	0.042	0.006	0.003
Unc. Digested	93.40	34.38	1.16	0.045	0.313	0.021
s.e.m.	4.35	4.21	0.51	0.035	0.022	0.010
Unc. Raw	142.36	28.82	0.92	0.035	0.257	0.010
s.e.m.	64.25	1.84	0.38	0.019	0.015	0.000
High Unc. Digested	96.18	44.10	0.62	0.056	0.552	0.014
s.e.m.	4.00	1.93	0.01	0.021	0.205	0.003
High Unc. Raw	97.22	38.54	0.65	0.031	0.281	0.010
s.e.m.	2.78	1.80	0.01	0.012	0.016	0.000
Zn rate 1	**145.83**	46.53	0.66	**0.188**	0.424	0.014
s.e.m.	**10.42**	4.90	0.06	**0.104**	0.039	0.003
Zn rate 2	**204.86**	53.82	0.80	**0.097**	0.413	0.010
s.e.m.	**9.19**	1.84	0.01	**0.013**	0.018	0.000
Zn rate 3	**260.42**	61.11	0.93	**0.295**	0.531	0.010
s.e.m.	**12.03**	0.92	0.03	**0.025**	0.006	0.000
Zn rate 4	**246.53**	112.85	0.88	**0.333**	0.479	0.010
s.e.m.	**71.24**	42.54	0.21	**0.028**	0.026	0.000
Zn rate 5	**364.58**	80.21	1.15	**0.455**	0.628	0.010
s.e.m.	**0.00**	0.60	0.00	**0.088**	0.093	0.000
Cu rate 1	79.17	**57.99**	0.55	0.010	**0.448**	0.010
s.e.m.	2.08	**2.43**	0.03	0.000	**0.043**	0.000
Cu rate 2	80.56	**96.88**	1.08	0.031	**0.708**	0.028
s.e.m.	2.43	**4.54**	0.55	0.021	**0.006**	0.017
Cu rate 3	85.76	**110.07**	0.50	0.014	**0.819**	0.024
s.e.m.	0.92	**4.51**	0.01	0.003	**0.089**	0.014
Cu rate 4	75.69	**120.14**	0.60	0.063	**1.153**	0.028
s.e.m.	9.21	**46.53**	0.09	0.052	**0.097**	0.017
Cu rate 5	125.00	**136.11**	0.62	0.014	**1.250**	0.010
s.e.m.	46.94	**40.97**	0.10	0.003	**0.060**	0.000
Cd rate 1	95.14	42.71	**1.25**	0.056	0.465	**0.010**
s.e.m.	4.51	1.04	**0.10**	0.019	0.137	**0.000**
Cd rate 2	118.06	48.61	**2.01**	0.014	0.337	**0.010**
s.e.m.	6.94	3.31	**0.23**	0.003	0.030	**0.000**
Cd rate 3	135.42	55.90	**2.71**	0.031	0.389	**0.010**
s.e.m.	0.00	1.51	**0.12**	0.016	0.043	**0.000**
Cd rate 4	357.64	52.08	**2.92**	0.042	0.420	**0.014**
s.e.m.	144.38	1.20	**0.06**	0.012	0.018	**0.003**
Cd rate 5	288.19	55.21	**3.54**	0.101	0.493	**0.028**
s.e.m.	121.68	1.59	**0.57**	0.031	0.037	**0.003**
LT Unc. Digested	392.36	24.65	0.48	0.066	0.285	0.010
s.e.m.	163.31	3.67	0.06	0.041	0.082	0.000
LT Unc. Raw	274.31	21.18	0.51	0.038	0.153	0.010
s.e.m.	81.21	1.51	0.01	0.018	0.003	0.000
LT Zn	208.33	40.28	0.67	0.201	0.358	0.059
s.e.m.	26.21	0.69	0.03	0.035	0.023	0.049
LT Cu	208.33	71.53	0.52	0.101	0.458	0.010
s.e.m.	57.37	2.50	0.02	0.039	0.012	0.000
LT Cd	298.61	29.51	1.56	0.135	0.292	0.014
s.e.m.	163.31	0.35	0.10	0.051	0.038	0.003

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	8.07	0.35	12.33	104.00	33.00	2.23
s.e.m.	0.03	0.01	1.20	20.66	0.58	0.04
Unc. Digested	7.83	0.45	82.67	103.67	44.00	3.05
s.e.m.	0.03	0.02	0.88	24.18	0.00	0.08
Unc. Raw	8.00	0.38	67.67	103.33	38.00	2.63
s.e.m.	0.00	0.02	3.33	42.37	1.53	0.07
High Unc. Digested	7.77	0.45	108.33	83.67	46.00	3.11
s.e.m.	0.03	0.01	2.91	8.69	2.65	0.09
High Unc. Raw	7.77	0.43	88.67	73.00	39.67	2.78
s.e.m.	0.13	0.01	5.33	5.03	1.86	0.10
Zn rate 1	7.90	0.44	79.67	64.00	42.67	3.11
s.e.m.	0.06	0.03	1.67	6.24	1.33	0.12
Zn rate 2	7.83	0.47	87.67	67.33	46.33	3.09
s.e.m.	0.03	0.01	4.06	2.19	3.18	0.13
Zn rate 3	7.80	0.44	89.67	74.33	49.33	2.98
s.e.m.	0.00	0.02	9.87	1.33	1.45	0.05
Zn rate 4	7.63	0.43	81.67	65.00	52.67	3.06
s.e.m.	0.12	0.01	4.98	3.46	1.20	0.06
Zn rate 5	7.60	0.45	82.33	67.67	54.00	3.14
s.e.m.	0.06	0.01	6.89	4.33	1.15	0.03
Cu rate 1	7.90	0.44	81.33	73.33	39.00	2.87
s.e.m.	0.06	0.02	10.17	9.74	0.00	0.09
Cu rate 2	7.77	0.43	65.00	98.33	42.33	2.95
s.e.m.	0.09	0.03	4.16	20.85	1.86	0.21
Cu rate 3	8.00	0.40	49.00	67.33	38.00	2.88
s.e.m.	0.06	0.02	6.00	6.96	3.06	0.08
Cu rate 4	7.93	0.42	38.67	65.67	38.00	2.92
s.e.m.	0.12	0.02	5.04	7.06	3.21	0.17
Cu rate 5	7.90	0.42	41.00	68.67	38.33	2.90
s.e.m.	0.10	0.00	2.31	3.18	1.76	0.10
Cd rate 1	7.67	0.49	88.00	79.00	43.67	3.19
s.e.m.	0.09	0.03	3.61	5.57	1.33	0.01
Cd rate 2	7.80	0.46	82.67	77.33	44.33	2.98
s.e.m.	0.06	0.03	8.09	13.33	4.91	0.15
Cd rate 3	7.67	0.46	90.00	70.67	50.33	3.07
s.e.m.	0.09	0.02	11.59	11.89	2.85	0.18
Cd rate 4	7.83	0.46	83.33	64.00	48.00	2.86
s.e.m.	0.03	0.02	2.03	0.58	1.15	0.06
Cd rate 5	7.80	0.47	99.67	76.33	52.00	3.10
s.e.m.	0.15	0.04	13.17	11.46	3.51	0.19
LT Unc. Digested	7.90	0.42	48.33	87.00	64.67	2.67
s.e.m.	0.06	0.01	3.53	2.65	2.19	0.05
LT Unc. Raw	7.80	0.40	41.67	87.00	38.33	2.69
s.e.m.	0.12	0.02	0.33	9.81	1.76	0.10
LT Zn	7.80	0.42	66.00	110.67	53.67	2.75
s.e.m.	0.06	0.01	5.20	21.42	1.45	0.12
LT Cu	7.90	0.41	23.00	89.33	42.00	2.60
s.e.m.	0.06	0.01	2.00	9.39	3.21	0.03
LT Cd	7.83	0.39	38.33	79.00	40.67	2.49
s.e.m.	0.17	0.02	1.45	6.66	0.88	0.08

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Soil respiration mg CO_2-C/kg/hr	Rhizobia MPN log_{10} cells/g
Control	14.4	1933.33	874.3	0.42	4.40
s.e.m.	0.44	44.10	51.0	0.01	0.00
Unc. Digested	16.2	2113.33	960.5	0.51	3.91
s.e.m.	0.56	33.83	32.2	0.01	0.30
Unc. Raw	14.8	1953.33	872.7	0.46	4.00
s.e.m.	0.10	69.60	24.5	0.01	0.22
High Unc.		2040.00	1006.1	0.50	4.12
s.e.m.		100.66	55.3	0.03	0.38
High Unc. Raw		2016.67	974.0	0.51	4.47
s.e.m.		23.33	52.2	0.02	0.51
Zn rate 1		2000.00	860.2	0.59	3.96
s.e.m.		75.06	30.0	0.02	0.37
Zn rate 2		1913.33	874.9	0.61	4.13
s.e.m.		85.11	31.5	0.05	0.28
Zn rate 3	16.2	1973.33	867.0	0.59	3.55
s.e.m.	0.46	66.92	33.0	0.02	0.20
Zn rate 4		2080.00	767.2	0.57	4.12
s.e.m.		32.15	36.7	0.03	0.25
Zn rate 5		1966.67	807.8	0.61	3.65
s.e.m.		99.39	28.3	0.03	0.18
Cu rate 1		1993.33	945.8	0.59	4.57
s.e.m.		44.85	45.1	0.04	0.17
Cu rate 2		2155.00	907.1	0.67	4.32
s.e.m.		53.07	60.8	0.05	0.19
Cu rate 3	14.4	2053.33	843.8	0.71	4.14
s.e.m.	0.10	92.44	41.7	0.11	0.50
Cu rate 4		1976.67	854.7	0.63	4.32
s.e.m.		82.53	55.1	0.05	0.19
Cu rate 5		2033.33	736.2	0.66	4.18
s.e.m.		99.55	25.1	0.06	0.12
Cd rate 1		2020.00	989.6	0.63	3.87
s.e.m.		147.31	64.3	0.04	0.30
Cd rate 2		2116.67	873.3	0.63	3.86
s.e.m.		37.12	72.2	0.05	0.12
Cd rate 3	17.3	2390.00	931.9	0.58	4.06
s.e.m.	0.39	410.73	33.9	0.03	0.23
Cd rate 4		2040.00	844.4	0.61	3.57
s.e.m.		55.08	65.0	0.01	0.20
Cd rate 5		2116.67	851.1	0.68	3.92
s.e.m.		89.50	61.4	0.05	0.16
LT Unc. Digested		1923.33	939.8	0.64	4.26
s.e.m.		34.80	16.4	0.02	0.14
LT Unc. Raw		2073.33	860.5	0.61	4.18
s.e.m.		78.81	38.8	0.04	0.12
LT Zn		2133.33	884.0	0.65	4.28
s.e.m.		82.93	14.8	0.05	0.31
LT Cu		2180.00	910.7	0.67	4.00
s.e.m.		81.65	30.4	0.05	0.22
LT Cd		1920.00	882.5	0.54	3.97
s.e.m.		73.71	75.8	0.01	0.00

APPENDIX E FINAL REPORT FOR DEFRA PROJECT SP0134

General enquiries on this form should be made to:

Defra, Science Directorate, Management Support and Finance Team,
Telephone No. 020 7238 1612
E-mail: research.competitions@defra.gsi.gov.uk

defra
Department for Environment Food and Rural Affairs

- **Note**
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  A SID 5A form must be completed where a project is paid on a monthly basis or against quarterly invoices. No SID 5A is required where payments are made at milestone points. When a SID 5A is required, no SID 5 form will be accepted without the accompanying SID 5A.

- This form is in Word format and the boxes may be expanded or reduced, as appropriate.

- **ACCESS TO INFORMATION**
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Project identification

1. Defra Project code: SP0134

2. **Project title**
   INTER-LABORATORY RHIZOBIUM LEGUMINOSARUM BIOVAR TRIFOLII QA EXERCISE

3. Contractor organisation(s)
   ADAS Gleadthorpe Research Centre
   Meden Vale
   Mansfield
   Notts.
   NG20 9PF

4. Total Defra project costs: £ 15,833

5. Project: start date..... 01 January 2005

   end date..... 31 March 2005

6. It is Defra's intention to publish this form.

Please confirm your agreement to do so. ..... YES ☐ NO ☒

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

Any results produced under the umbrella of the main DEFRA project (SP0130) cannot be published without the formal approval of the DEFRA/UKWIR/EA/WAG/SEERAD Management Group.

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The objective of this project was to undertake an inter-laboratory Quality Assurance (QA) exercise for the determination of *Rhizobium leguminosarum* biovar *trifolii*.

At nine sites with contrasting clay contents throughout Britain, selected metal-rich sludge cakes were applied between 1994 and 1997 (Phase I) to establish individual zinc, copper and cadmium metal dose-response treatments. During Phases II and III of the project (SP0125 and SP0130), effects of the established soil metal concentrations on selected soil microbial (biomass, respiration and rhizobia), chemical (total and extractable metals, speciation of metals, organic carbon, cation exchange capacity and soil pH) and physical properties (bulk density) are being measured.

Within the main programme, three laboratories conduct the determination of most probable numbers (MPNs) of *Rhizobium leguminosarum* bv. *trifolii* (clover rhizobia), *viz.*: Rothamsted Research for the sites in England and Wales (seven field sites), the Macaulay Institute for the 'Hartwood' field site and SAC for the 'Auchincruive' field site. In order to maintain confidence in the results generated from the *R. leguminosarum* bv. *trifolii* measurements, a QA exercise was conducted between the three laboratories undertaking this assay.

Topsoil samples (0-25 cm) were taken from three replicate plots on each of four treatments, to provide a range in likely rhizobia MPN levels based on the assays undertaken in spring 2003 (2004 at Auchincruive), viz.:

Field site	Treatment
Gleadthorpe	Cu rate 1 (*c.* 115,000 cells g^{-1})
Auchincruive	Untreated control (*c.* 11,000 cells g^{-1})
Pwllpeiran	Cd rate 3 (*c.* 630 cells g^{-1})
Hartwood	Zn rate 4 (< 1 cell g^{-1})

The three replicate plots at each site were sub-sampled individually (*c.*20 cores/plot) and bulked in the field to give one sample per site. The bulked soil samples were sieved moist, and each of the participating laboratories (Rothamsted Research, the Macaulay Institute and SAC) received three sub-samples from each site (*c.*100g each) for determination of *R. leguminosarum* bv. *trifolii*, *to* enable inter laboratory precision, as well as between laboratory variability to be assessed.

There was no significant difference (ANOVA, $P > 0.05$) in *R. leguminosarum* bv. *trifolii* most probable numbers between the three laboratories participating in this QA scheme. Moreover, there was no evidence to suggest that rhizobia numbers determined as part of the main programme (SP0130) should differ based on the laboratory conducting the determination *i.e.* soil samples from England and Wales analysed by Rothamsted Research are not likely to differ in rhizobia MPNs from soil samples taken and analysed in Scotland by the Macaulay Institute or SAC.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

OBJECTIVE

- To undertake an inter-laboratory Quality Assurance exercise for the determination of *Rhizobium leguminosarum* biovar *trifolii.*

The work undertaken has fully met the objectives set out in the contract specification and confirmed that there was very good agreement between the three laboratories undertaking the determination of most probable numbers (MPNs) of *R.leguminosarum* bv. *trifolii* in the main experimental programme (SP0130).

1. INTRODUCTION

There is a recognised need to determine whether present maximum permitted soil metal concentrations where sludge is applied to agricultural land are safe for crops, soil microbes and their activity. Present maximum permissible soil metal concentrations were developed using coarse textured soil types and sensitive crop species, representing likely ‘worst-case’ conditions for metal impacts on crops and human dietary intakes (CEC, 1986; SI, 1989; DOE, 1996). However, no account is explicitly taken in the present regulations of potential impacts from metals on soil micro-organisms in sludge treated soils (McGrath *et al*. 1995).

At nine sites with contrasting clay contents throughout Britain, selected metal-rich sludge cakes were applied between 1994 and 1997 (Phase I) to establish individual zinc (Zn), copper (Cu) and cadmium (Cd) metal dose-response treatments. During Phases II and III of the project (SP0125 and SP0130), effects of the established topsoil metal concentrations on selected soil microbial (biomass, respiration, rhizobia), chemical (total and extractable metals, speciation of metals, organic carbon, cation exchange capacity and soil pH) and physical properties (bulk density) are being measured.

Metal-amended liquid sludges were also applied at three sites in England during Phase I of the project to establish individual Zn, Cu and Cd metal dose-response treatments, and to provide a contrast in likely metal availability to the metal-rich sludge cake additions.

The main experimental programme (Defra project SP0130) is intended to provide a sound scientific base for the continued development of policies on soil protection where sludge is applied to agricultural land, and to identify the effects of sludge derived metals on soil (microbial, chemical and physical) properties and agricultural productivity.

The determination of most probable numbers (MPNs) of *R.leguminosarum* bv. *trifolii* (clover rhizobia) is conducted by three laboratories, *viz.*: Rothamsted Research for the sites in England and Wales (seven field sites), the Macaulay Institute for the 'Hartwood' field site and SAC for the 'Auchincruive' field site. In order to maintain confidence in the results generated from the *R.leguminosarum* bv. *trifolii* measurements, a QA exercise was conducted between the three laboratories undertaking this assay. This repeats a similar QA exercise conducted during Phase I (1994 – 1998) of the project, which confirmed that there was good agreement (P>0.05) between the three laboratories undertaking the assay (Chambers, 1999).

2. METHOD

Topsoil samples (0-25 cm) were taken from three replicate plots on each of four selected treatments at four field sites in the main experimental programme (SP0130), to provide a range in likely rhizobia MPN levels based on the assays undertaken in spring 2003 (2004 at Auchincruive), viz.:

Field site	**Treatment**
Gleadthorpe	Cu rate 1 (*c.* 115,000 cells g^{-1})
Auchincruive	Untreated control (*c.* 11,000 cells g^{-1})
Pwllpeiran	Cd rate 3 (*c.* 630 cells g^{-1})
Hartwood	Zn rate 4 (< 1 cell g^{-1})

The three replicates plots at each site were sub-sampled individually (*c.*20 cores/plot) and bulked in the field to give one sample per site. Approximately 1.5 kg of fresh soil was taken from each site. The soil samples taken from each site (ADAS undertook the sampling at Gleadthorpe and Pwllpeiran; the Macaulay Institute at Hartwood and SAC at Auchincruive) in January 2005, were sent to ADAS Boxworth where they were sieved moist and stored at 4 ºC prior to further sub-sampling. Each of the participating laboratories (Rothamsted Research, the Macaulay Institute and SAC) received three separate sub-samples from each site (*c.*100g each) for determination of *R. leguminosarum* bv. *trifolii* most probable numbers (*i.e.* each laboratory received 12 samples) which enabled inter laboratory precision, as well as between laboratory variability to be assessed. Each laboratory used the standard methodologies agreed between the contractors for the determination of *Rhizobium* most probable numbers (Vincent, 1970; Woomer *et al*., 1990).

Enumeration of indigenous populations of Rhizobium leguminosarum biovar trifolii

The most probable number (MPN) method was used to estimate the number of indigenous clover rhizobia in the selected soils, using a 10-fold dilution series. *Trifolium repens* (white clover) was used as the trap host for *R. leguminosarum* bv. *trifolii*. Rothamsted Research supplied clover seeds to all participating laboratories. For each of the topsoil samples, plant infection tubes were inoculated with 1 ml aliquots of each of eight dilution steps, each replicated three times and the tubes placed in a controlled environment growth cabinet with 14 hour days at 20 ^{o}C, 2.5x10^{3} lux and 16 ^{o}C nights. All tubes were examined for nodulation after 3 weeks and the most probable number of rhizobia calculated using the MPNES computer program (Woomer *et al.,* 1990).

3. RESULTS

Topsoil samples taken in January 2005 from selected treatments at Gleadthorpe (Gle), Auchincruive (Auc), Pwllpeiran (Pwl) and Hartwood (Har) were analysed for *R. leguminosarum* bv. *Trifolii* most probable numbers by the three laboratories participating in the main sludge experimental programme, *viz*: Rothamsted Research, the Macaulay Institute and SAC (Table 1).

Table 1. Mean topsoil (0-25 cm) *Rhizobium leguminosarum* biovar *trifolli* numbers ($\log_{10}$ cells g^{-1} soil) on treatments sampled (winter 2005)

	Laboratory			**Coefficient of variation**
	Rothamsted	**Macaulay**	**SAC**	**(%)**
Gle – Cu rate 1	4.7	4.6	4.1	5.2
Auc – Unt. Control	4.6	4.4	4.5	1.7
Pwl – Cd rate 3	2.3	3.0	1.7	10.6
Har – Zn rate 4	< 0.3*	< 0.3*	< 0.3*	-

N.B: As the soil MPN results examined on a site-by-site basis did not approximate to normal distributions, the data were transformed to a log base$_{10}$ before statistical analysis.
* No cells were counted by any of the three laboratories for the Hartwood sample (0.3 = 1 cell to $\log_{10}$)

There was good inter laboratory precision and between laboratory agreement (ANOVA, $P > 0.05$) in the number of rhizobial cells reported by the three laboratories participating in the QA exercise (Figure 1).

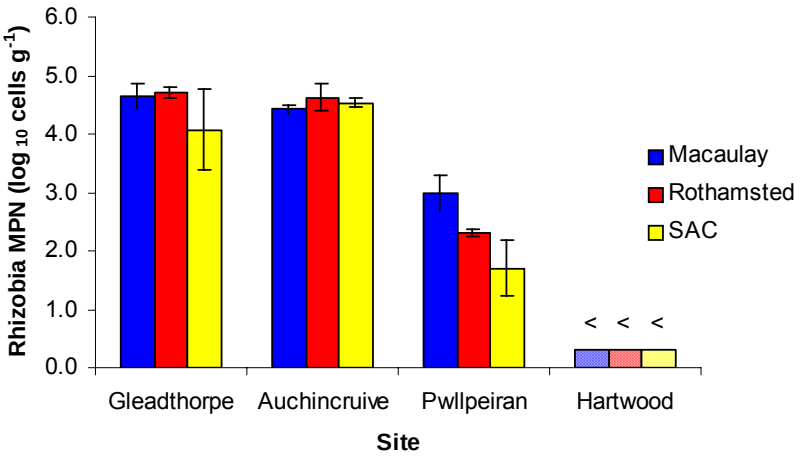

Figure 1. *Rhizobium leguminosarum* biovar *trifolii* most probable numbers.

Bars represent standard errors of individual means (n = 3).

The lack of any rhizobial cells in the Zn-rate 4 sample from the Hartwood field site (reported by all three laboratories) is shown in Figure 2, where clover was grown in soil samples from Gleadthorpe and Hartwood. The clover plants grown in the Hartwood soil showed acute signs of N deficiency and stunted growth, indicating a lack of N fixation, and provide further supporting evidence of no rhizobia being present in the soil.

Figure 2. White clover plants grown from surface sterilised seed in 10g soil for 58 days. Treatments: Gleadthorpe sludge cake copper rate 1 and Hartwood sludge cake zinc rate 4 (photo – Rothamsted Research).

4. DISCUSSION

There was no significant difference (ANOVA, $P > 0.05$) in *R. leguminosarum* bv. *trifolii* most probable numbers between the three laboratories participating in this QA scheme. These results support those generated as part of a similar QA exercise conducted during Phase I (1994-1998) of the project, where there was also good agreement ($P > 0.05$) between the three laboratories. Hence, there is no evidence to suggest that rhizobia numbers determined as part of the main programme (SP0130) should differ based on the laboratory conducting the determination *i.e.* soil samples from England and Wales analysed by Rothamsted Research are not likely to differ in rhizobia MPNs from soil samples taken and analysed in Scotland by the Macaulay Institute or SAC.

5. CONCLUSION

- The three laboratories participating in the QA exercise were in good agreement ($P > 0.05$) in their determination of *Rhizobium leguminosarum* biovar *trifolii* most probable numbers.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

CEC. (1986) Commission of the European Communities. Council Directive 12 June 1986 on the protection of the environment, and in particular the soil, when sewage sludge is used in agriculture. Official *Journal of the European Communities*, No L.181 (86/278/EEC), p. 6-12.

Chambers, B.J. (1999) *Effects of Sewage Sludge Applications to Agricultural Soils on Soil Microbial Activity and the Implications for Agricultural Productivity and Long-term Soil Fertility.* ADAS/Rothamsted/WRC/Macaulay Institute/SAC Final Contract Report to Ministry of Agriculture, Fisheries and Food, project SP0109.

DoE (1996) UK Department of the Environment. *Code of Practice for the Agricultural Use of Sewage Sludge*. HMSO: London.

McGrath, S.P., Chaudri, A.M. and Giller, K.E. (1995) Long-term effects of metal in sewage sludge on soils, micro-organisms and plants. *Journal of Industrial Microbiology*, 14, 94-104.

SI (1989) United Kingdom Statutory Instrument No 1263 *The Sludge (Use in Agriculture) Regulations* 1989. HMSO: London.

Vincent, J.M. (1970) *A Manual for the Practical Study of Root-nodule Bacteria*. Blackwell, Oxford.

Woomer, P., Bennet, J. and Yost, R. (1990) Overcoming the inflexibility of the most-probable number procedures. *Agronomy Journal,* 82, 349-353.

APPENDIX F FINAL REPORT FOR DEFRA PROJECT SP0135

General enquiries on this form should be made to:
Defra, Science Directorate, Management Support and Finance Team
Telephone No. 020 7238 1612
E-mail: research.competitions@defra.gsi.gov.uk

defra
Department for Environment Food and Rural Affairs

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Project identification

1. Defra Project code: SP0135

2. **Project title**
   SOIL SAMPLING TO DETERMINE TOPSOIL HEAVY METAL DISTRIBUTION

3. Contractor organisation(s)
   ADAS Gleadthorpe Research Centre
   Meden Vale
   Mansfield
   Notts.
   NG20 9PF

4. Total Defra project costs: £ 14,796

5. Project: start date..... 01 January 2005
   end date..... 31 March 2005

6. It is Defra's intention to publish this form.

Please confirm your agreement to do so. ..... YES ☐ NO ☒

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

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(b) If you have answered NO, please explain why the Final report should not be released into public domain

Any results produced under the umbrella of the main DEFRA project (SP0130) cannot be published without the formal approval of the DEFRA/UKWIR/EA/WAG/SEERAD Management Group.

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The objective of this project was to undertake a soil sampling exercise at selected long-term study sites to determine the depth wise distribution of sludge cake heavy metals applied during Phase I of the project.

At nine sites with contrasting clay contents throughout Britain, selected metal-rich sludge cakes were applied between 1994 and 1997 (Phase I) to establish individual zinc, copper and cadmium dose-response treatments. During Phases II and III of the project (SP0125 and SP0130), effects of the established soil metal concentrations on selected soil microbial (biomass, respiration rate and rhizobia most probable numbers), chemical (total and extractable metals, speciation of metals, organic carbon, cation exchange capacity and soil pH) and physical properties (bulk density) are being measured.

Metal concentrations in soil samples (0-25cm) taken at some sites during Phase II of the main project (SP0125, 1999 and 2001), and the first sampling during Phase III (SP0130, 2003) have shown a general trend of decreasing levels with time. In particular, these concentration decreases were most evident at Watlington (sludge cake and liquid sludge), Rosemaund (sludge cake and liquid sludge), Bridgets (sludge cake) and Shirburn (sludge cake).

An extensive soil sampling programme was undertaken at three of the sludge cake sites, that focussed on the high Zn treatments, as these would provide the most sensitive indicator of metal distribution with soil depth. The sludge cake sites selected represented two sites (Watlington and Rosemaund) that had shown a decrease in soil metal concentrations with time, and a third site (Gleadthorpe) that had shown no apparent decrease in metal concentrations.

In order to determine the distribution of Zn and organic carbon (as an additional indicator of 'topsoil' depth and sludge cake distribution), six prescribed sampling depths were used: 0-15, 15-20, 20-25, 25-30, 30-35, 35-40cm. The greatest intensity of sampling was around the likely interface depth between the 'topsoil' and 'subsoil'.

Soil total Zn concentrations were higher ($P < 0.05$) on the Zn rate-4 treatments in the 0-15, 15-20 and 20-25cm horizons at all three sites, and the 25-30cm depth at two of the sites (Watlington and Rosemaund) compared with the corresponding untreated control samples. The differences between the Zn rate-4 treatments and the untreated control samples were not unexpected, as once metals are added to topsoils they accumulate, as they are not easily leached and the amounts removed by crops are small. The corresponding soil organic C concentrations were also higher ($P < 0.05$) on the Zn rate-4 treatments in the 0-15cm horizon at all three sites, compared with the untreated control samples.

There was no evidence from the data of metals leaching to depth within the soil profile. Also, the lack of an increase in organic C at depth (> 25cm) provides further evidence that the added sludge had not been diluted by cultivation below the standard 0-25cm sampling depth. However, the data from all three sites sampled in this study, show clear decreases in Zn (and organic C) concentrations with depth, even within the standard 0-25cm sampling horizon. The most likely reason for metal concentration decreases over time (at some of the sites) is due to soil 'settlement', as the added sludge organic matter has oxidised and bulk density has increased, which has progressively resulted in the cultivated horizon becoming shallower and the standard 0-25cm depth samples being diluted by relatively uncontaminated subsoil. This is supported by visual evidence of the plots being lower than the surrounding discard areas and the increased auger resistance noted by samplers at the base of the standard 0-25cm sampling depth.

As a result of the clear changes in metal concentrations within the standard 0-25cm sampling depth, there is a need to assess potential metal effects on soil microbial properties (biomass carbon, respiration rates and rhizobia most probable numbers-MPNs) within the most concentrated metal horizon (*i.e.* 0-15cm), and any associated impacts on soil metal concentrations and microbial property relationships.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

1. OBJECTIVE

- To determine at selected long-term study sites the depth wise distribution of sludge cake heavy metals added during Phase I of the project .

The work undertaken has fully met the objectives set out in the contract specification and has shown that there are clear differences in soil total Zn (and organic carbon) concentrations between the most concentrated (*i.e.* 0-15cm sample depth) and lower depth horizons (*e.g.* 15-20 and 20-25cm depths), which would be contained in the composite 0-25cm depth sample taken to date as standard in the project.

2. INTRODUCTION

There is a recognised need to determine that the present maximum permitted soil metal concentrations where sewage sludge is applied to agricultural land are safe for crops, soil microbes and their activity. Present maximum permissible soil metal concentrations were developed using coarse textured soil types and sensitive crop species, representing 'worse-case' conditions for metal impacts on crops and human dietary intake (CEC, 1986; SI, 1989; DOE, 1996). However, no account is taken in the present regulations of potential impacts from metals on soil microorganisms in sludge treated soils (McGrath *et al.,* 1995).

At nine sites with contrasting clay contents throughout Britain, selected metal-rich sludge cakes were applied between 1994 and 1997 (Phase I) to establish individual zinc (Zn), copper (Cu) and cadmium (Cd) metal dose-response treatments. During Phases II and III of the project (SP0125 and SP0130), effects of the established topsoil metal concentrations on selected soil microbial (biomass, respiration, rhizobia), chemical (total and extractable metals, speciation of metals, organic carbon, cation exchange capacity and soil pH) and physical properties (bulk density) are being measured.

Metal-amended liquid sludges were also applied at three sites in England during Phase I of the project to establish individual Zn, Cu and Cd metal dose-response treatments, and to provide a contrast in likely metal availability to the metal-rich sludge cake additions.

The main experimental programme (SP0125 and SP0130) is intended to provide a sound scientific base for the continued development of policies on soil protection where sewage sludge is applied to agricultural land, and to identify the effects of sludge derived metals on soil (microbial, chemical and physical) properties and agricultural productivity.

Metal concentrations in soil samples (0-25cm) taken at some sites during Phase II of this project (1999 and 2001), and the first sampling during Phase III (2003), have shown a general trend of decreasing levels with time. In particular, these concentration decreases were most evident at Watlington (sludge cake and liquid sludge), Rosemaund (sludge cake and liquid sludge), Bridgets (sludge cake) and Shirburn (sludge cake). This downward trend needed to be investigated to determine the reasons for these decreases.

There were four likely hypotheses, that could explain the downward trends, viz:

- The metals were being lost from the topsoil horizon via leaching to lower layers.
- The metals were being lost from the topsoil horizon via lateral movement of the soil on to the surrounding discards during cultivation.
- The 'topsoil' was being diluted by uncontaminated 'subsoil' (*i.e.* cultivation depth is too deep)
- Annual soil cultivation was progressively increasing bulk density (*i.e.* soil 'settlement' during Phases II/III as a result of organic matter oxidisation had resulted in the cultivated topsoil horizon becoming shallower, so that the soil samples to 25cm depth were being diluted by increasing amounts of 'uncontaminated' subsoil with time).

3. METHODS

An extensive soil sampling programme was undertaken at three of the sludge cake experimental sites, that focussed on the high Zn treatments, as these would provide the most sensitive indicator of metal distribution with soil depth. The sludge cake sites selected represented two sites (Watlington and Rosemaund) that had shown a decrease in soil metal concentrations with time and a third site (Gleadthorpe) that had shown no apparent decrease in metal concentrations.

In order to assess the depth wise distribution of metals within the selected treatments, eight years after the cessation of sludge cake additions, soil samples were taken as follows:

- Twenty random sampling points within each replicate of the Zn rate 4 treatments at each site.
- Twenty random sampling points within each replicate of the untreated control at each site (ten samples per A and B plot).

At each sampling point within each replicate plot on the Zn rate 4 treatments, a core was taken to a depth of 40cm. In order to determine the distribution of Zn and organic carbon (as an additional indicator of topsoil depth and sludge cake distribution) each core was subdivided into six prescribed sampling depths: 0-15, 15-20, 20-25, 25-30, 30-35, 35-40cm. The greatest intensity of sampling was around the likely interface depth between the 'topsoil' and 'subsoil'. The twenty sub-samples from each depth formed a composite sample from that plot, resulting in 6 soil depth horizons for analysis per Zn rate 4 plot (*i.e.* 18 samples per site). The untreated control plots at each site were also sampled using the same method as the Zn rate 4 treatments, in order to determine the 'background' distribution of Zn (*i.e.* 18 samples per site).

Chemical analysis of soil

The soil samples were acid digested using aqua-regia and total metal concentrations determined by Inductively Coupled Mass Spectrometry (ICP-MS). A separate sub-sample was analysed for Tinsley soil organic C (MAFF, 1986), as an additional indicator of sludge cake distribution with soil depth.

4. RESULTS

Soil samples, taken in winter 2005, from the untreated control and Zn rate 4 treatments at Gleadthorpe, Watlington and Rosemaund were analysed for soil total Zn concentrations and soil organic C levels (Tables 1 and 2).

Table 1. Mean treatment soil total Zn (mg/kg) concentrations and organic carbon (%) contents – *untreated control plots* (winter 2005 samples)

Depth (cm)	Gleadthorpe		Watlington		Rosemaund	
	Total Zn (mg/kg)	Organic C (%)	Total Zn (mg/kg)	Organic C (%)	Total Zn (mg/kg)	Organic C (%)
0-15	48.8	1.37	51.5	1.35	92.7	1.80
15-20	54.1	1.24	55.9	1.12	91.9	1.62
20-25	45.3	1.03	55.8	0.90	90.8	1.50
25-30	47.7	0.98	56.6	0.70	85.7	1.02
30-35	39.9	0.90	65.1	0.60	84.1	0.67
35-40	34.0	0.83	69.4	0.58	85.9	0.46

Table 2. Mean treatment soil total Zn (mg/kg) concentrations and organic carbon (%) contents – *Zinc rate 4 plots* (winter 2005 samples)

Depth (cm)	Gleadthorpe		Watlington		Rosemaund	
	Total Zn (mg/kg)	Organic C (%)	Total Zn (mg/kg)	Organic C (%)	Total Zn (mg/kg)	Organic C (%)
0-15	364	1.88	464	2.57	416	2.87
15-20	267	1.54	412	2.24	391	2.70
20-25	153	1.16	160	1.38	232	1.83
25-30	73.5	1.06	95.7	0.94	156	1.31
30-35	41.8	0.83	67.7	0.75	105	0.78
35-40	32.3	0.76	65.5	0.60	96.1	0.44

At all three sites, soil total Zn concentrations were higher (ANOVA, $P < 0.05$) on the Zn rate 4 treatments in the 0-15, 15-20 and 20-25cm horizons, plus the 25-30cm horizon at Watlington and Rosemaund, compared with the corresponding untreated controls (Figures 1-3). No significant ($P > 0.05$) differences in soil total Zn concentrations were found between the Zn rate 4 treatments and the untreated control samples in the 30-35 and 35-40cm horizons, at any of the three sites.

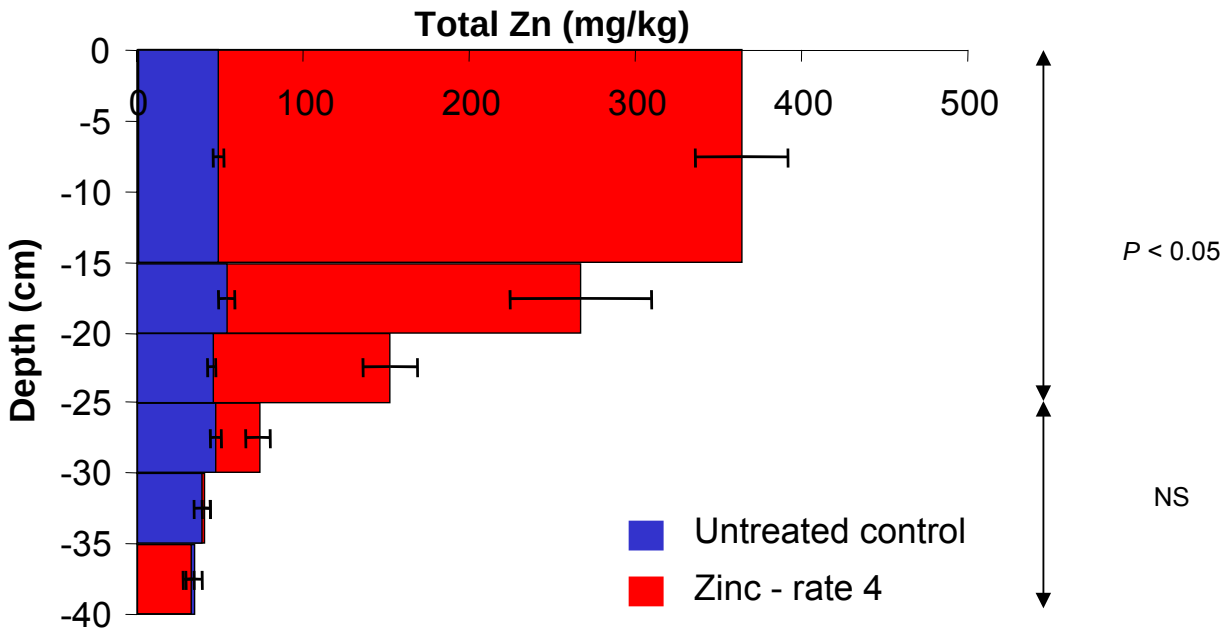

Figure 1. Total soil Zn concentrations (mg/kg) – Gleadthorpe

Bars = Standard errors (n=3). The higher treatment mean at each depth is shown in the background.

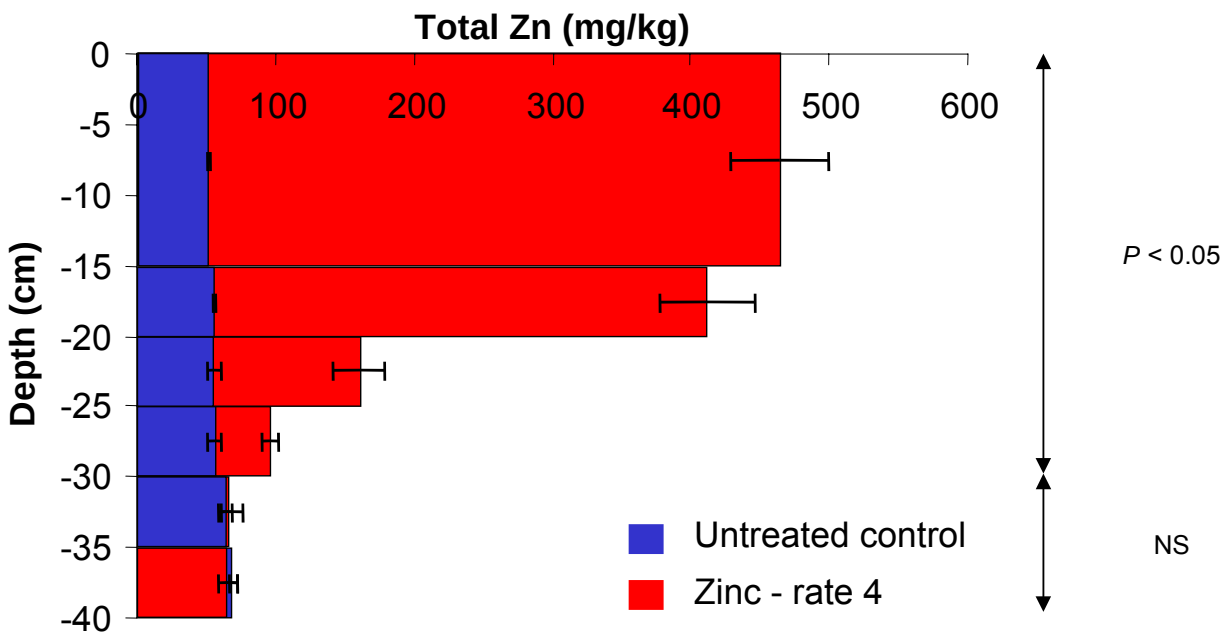

Figure 2. Total soil Zn concentrations (mg/kg) – Watlington

Bars = Standard errors (n=3). The higher treatment mean at each depth is shown in the background.

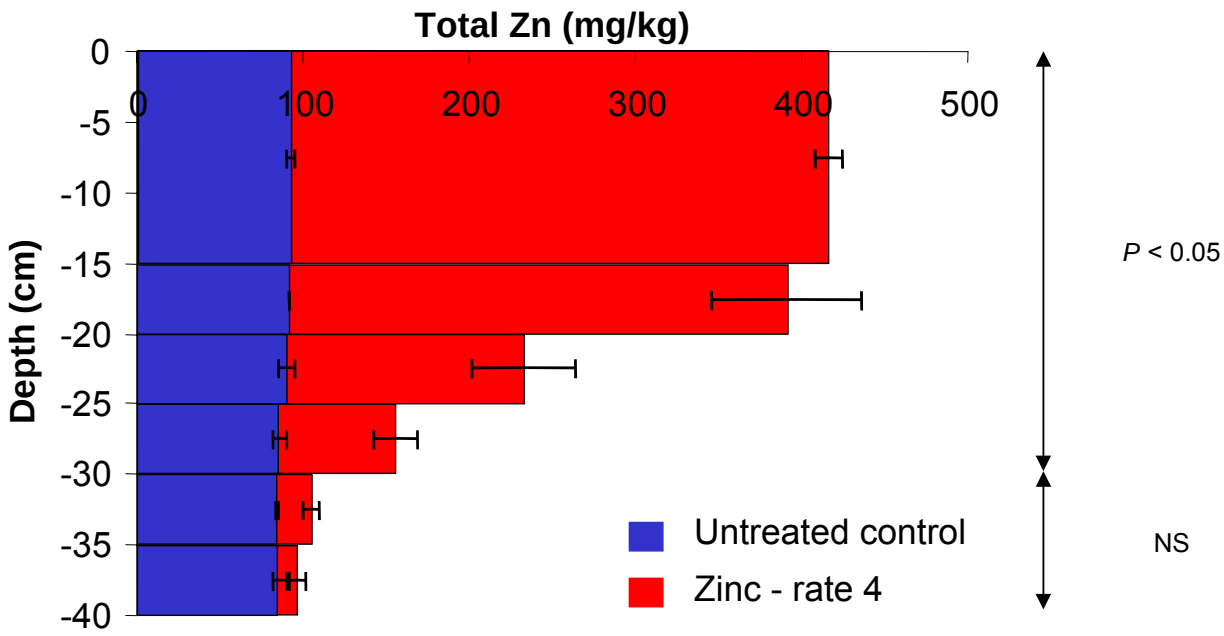

Figure 3. Total soil Zn concentrations (mg/kg) – Rosemaund

Bars = Standard errors (n=3). The higher treatment mean at each depth is shown in the background.

At all three sites, soil organic C levels were higher (ANOVA, $P < 0.05$) on the Zn rate 4 treatments in the 0-15cm horizon, plus the 15-20cm horizon at Rosemaund and the 15-20 and 20-25cm horizons at Watlington, compared with the corresponding untreated controls (Figures 4-6). No significant ($P > 0.05$) differences in soil organic C concentrations were found between the Zn rate 4 treatments and the untreated control samples in the 25-30, 30-35 and 35-40cm horizons at any of the three sites.

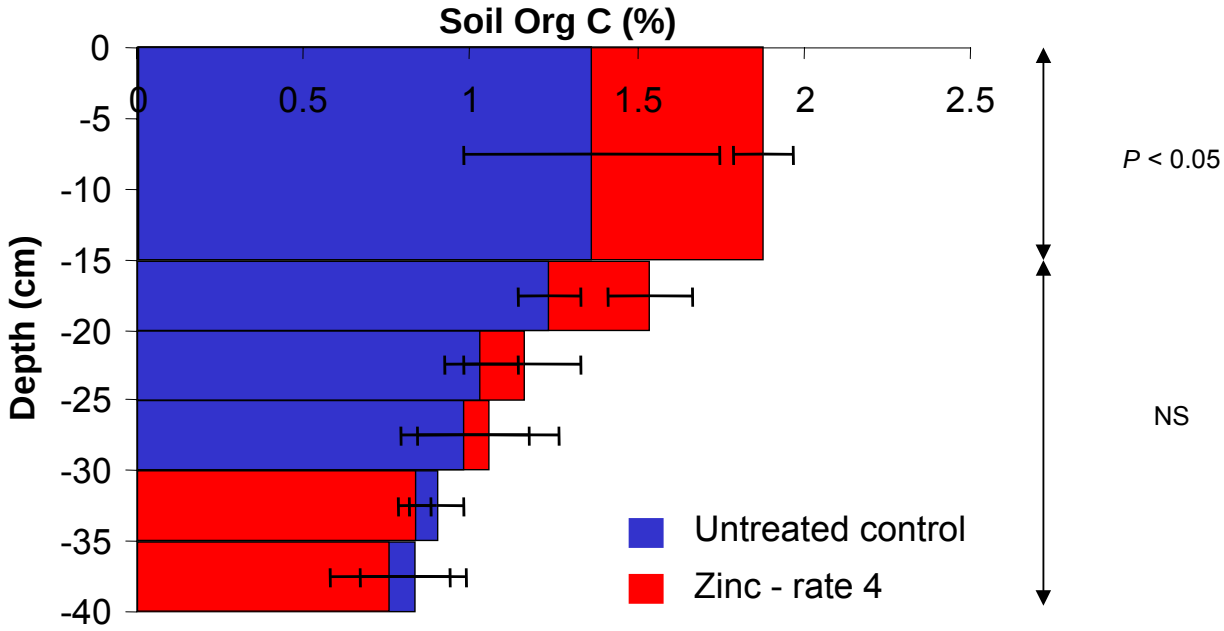

Figure 4. Soil organic C (%) – Gleadthorpe

Bars = Standard errors (n=3). The higher treatment mean at each depth is shown in the background.

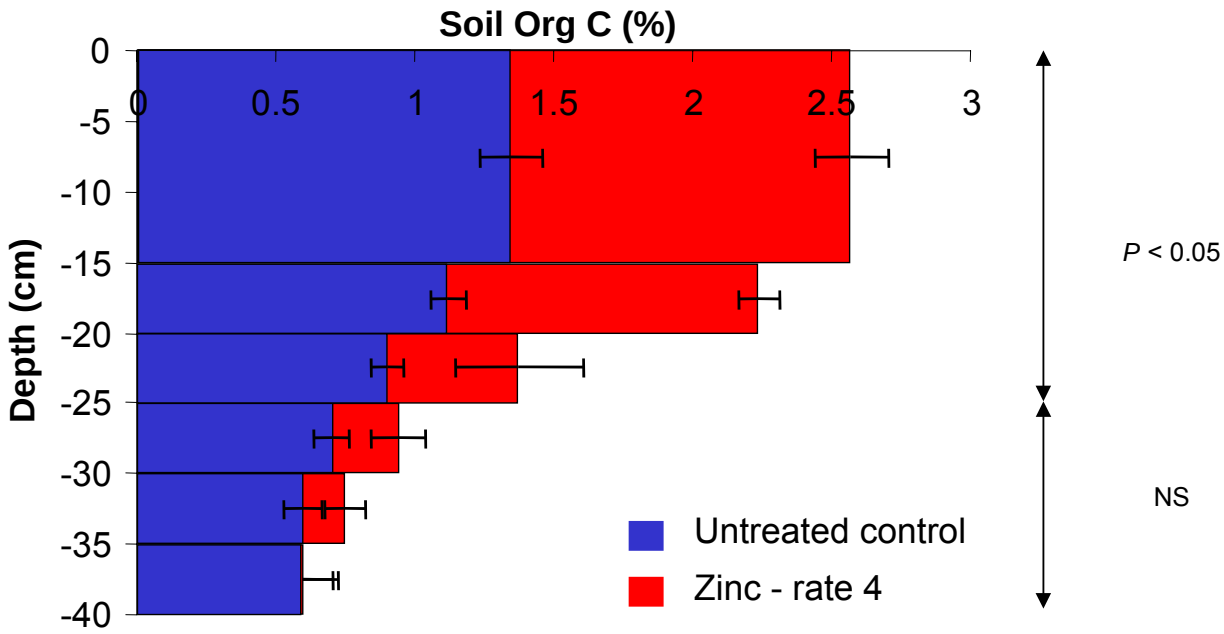

Figure 5. Soil organic C (%) – Watlington

Bars = Standard errors (n=3). The higher treatment mean at each depth is shown in the background.

The most likely reason for the metal concentration decreases over time (at some sites) is due to soil ‘settlement’, as the added sludge organic matter has oxidised and bulk density has increased, which has progressively resulted in the cultivated horizon becoming shallower and the ‘standard’ 0-25cm depth samples being diluted by relatively uncontaminated subsoil (hypothesis 4). This is supported by visual evidence of the cultivated surface of the plots being lower at some sites than the uncultivated plot discard areas and the increased auger resistance noted by samplers at the base of the standard 0-25cm sampling depth.

6. CONCLUSIONS

- At all three sites in this study, soil total Zn concentrations were higher on the Zn rate- 4 treatments than the corresponding untreated control samples to a depth of 25cm.
- Soil organic C concentrations were higher on the Zn rate - 4 treatments than the corresponding untreated control samples to a depth of 15cm at all three sites investigated in this study.
- The apparent soil total metal concentration decreases over time (at some sites) are most likely due to soil ‘settlement’, as the added sludge organic matter has oxidised and bulk density has increased, which has progressively resulted in the cultivated horizon becoming shallower and the ‘standard’ 0-25cm depth samples being diluted by relatively uncontaminated subsoil (hypothesis 4). This is supported by visual evidence of the cultivated surface of the plots being lower at some sites than the uncultivated plot discard areas and the increased auger resistance noted by samplers at the base of the standard 0-25cm sampling depth.

7. FUTURE WORK

As a result of the clear changes in metal concentrations within the ‘standard’ 0-25cm sampling depth, there is a need to assess potential metal effects on soil microbial properties (biomass carbon, respiration rate and rhizobia most probable numbers-MPNs) within the most concentrated metal horizon (0-15cm) and any associated impacts on soil metal concentration and microbial property relationships.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

CEC. (1986) Commission of the European Communities. Council Directive 12 June 1986 on the protection of the environment, and in particular the soil, when sewage sludge is used in agriculture. Official *Journal of the European Communities*, No L.181 (86/278/EEC), p. 6-12.

DoE (1996) UK Department of the Environment. *Code of Practice for the Agricultural Use of Sewage Sludge*. HMSO: London.

MAFF 1986. Analysis of Agricultural Materials. Ministry of Agriculture, Fisheries and Food Reference Book 427 (3rd Edition). HMSO London.

McGrath, S.P., Chaudri, A.M. and Giller, K.E. (1995) Long-term effects of metal in sewage sludge on soils, micro-organisms and plants. *Journal of Industrial Microbiology*, 14, 94-104.

SI (1989) United Kingdom Statutory Instrument No 1263 *The Sludge (Use in Agriculture) Regulations* 1989. HMSO: London.

APPENDIX G FINAL REPORT FOR DEFRA PROJECT SP0137

General enquiries on this form should be made to:

Defra, Science Directorate, Management Support and Finance Team,
Telephone No. 020 7238 1612
E-mail: research.competitions@defra.gsi.gov.uk

- **Note**

  In line with the Freedom of Information Act 2000, Defra aims to place the results of its completed research projects in the public domain wherever possible. The SID 5 (Research Project Final Report) is designed to capture the information on the results and outputs of Defra-funded research in a format that is easily publishable through the Defra website. A SID 5 must be completed for all projects.

  A SID 5A form must be completed where a project is paid on a monthly basis or against quarterly invoices. No SID 5A is required where payments are made at milestone points. When a SID 5A is required, no SID 5 form will be accepted without the accompanying SID 5A.

- This form is in Word format and the boxes may be expanded or reduced, as appropriate.

- **ACCESS TO INFORMATION**

  The information collected on this form will be stored electronically and may be sent to any part of Defra, or to individual researchers or organisations outside Defra for the purposes of reviewing the project. Defra may also disclose the information to any outside organisation acting as an agent authorised by Defra to process final research reports on its behalf. Defra intends to publish this form on its website, unless there are strong reasons not to, which fully comply with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

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Project identification

1. Defra Project code: SP0137

2. **Project title**: Soil Sampling to Measure Topsoil (0-15cm) Microbial Properties

3. Contractor organisation(s): ADAS Gleadthorpe Research Centre, Meden Vale, Mansfield, Notts. NG20 9PF

4. Total Defra project costs: £ 36,987

5. Project: start date..... 1st March 2006

   end date..... 31st March 2006

6. It is Defra's intention to publish this form.

Please confirm your agreement to do so. ..... YES ☐ NO ☒

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

Any results produced under the umbrella of the main Defra project (SP0130) cannot be published without the formal approval of the Defra/UKWIR/EA/WAG/SEERAD Management Group.

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The objective of this project was to undertake a soil sampling exercise to determine microbial properties (*i.e.* biomass carbon, respiration rate and rhizobia most probable numbers - MPNs) in the 0-15cm topsoil depth compared with the 'standard' 0-25cm topsoil depth samples at selected long-term study sites.

At nine sites with contrasting clay contents throughout Britain, selected metal-rich sludge cakes were applied between 1994 and 1997 and metal-amended liquid sludges at three sites between 1995 and 1997 (Phase I) to establish individual zinc (Zn), copper (Cu) and cadmium (Cd) metal dose-response treatments (Gibbs *et al*., 2006a; Gibbs *et al.,* 2006b). During Phases II and III of the project (SP0125 and SP0130), effects of the established soil metal concentrations on selected microbial (biomass, respiration rate and rhizobia MPNs), chemical (total and extractable metals, speciation of metals, organic carbon, cation exchange capacity and pH) and physical properties (bulk density) are being measured.

Metal concentrations in soil samples (0-25cm depth) taken at some sites during Phase II of the main project (Defra project SP0125, 1999 and 2001) and the first sampling during Phase III (SP0130, 2003), showed a general trend of decreasing concentrations with time. In particular, these concentration decreases were most strongly evident at Watlington (sludge cake and liquid sludge), Rosemaund (sludge cake and liquid sludge), Bridgets (sludge cake) and Shirburn (sludge cake). A soil sampling programme was undertaken in spring 2005 (Defra project SP0135) at three of the sludge cake sites, that focussed on the high Zn treatments, as these would provide the most sensitive indicator of metal distribution with soil depth. The sludge cake sites selected represented two sites (Watlington and Rosemaund) that had shown a decrease in soil metal concentrations with time, and a third site (Gleadthorpe) that had shown no apparent decrease in metal concentrations in the 0-25 cm samples. However, previous results from all three of the sludge cake sites studied showed significant ($P<0.05$) decreases in total topsoil Zn and soil organic carbon concentrations between the 0-15cm sample depth and lower depth horizons (e.g. 15-20cm and 20-25cm depths).

As a result of the clear changes in metal concentrations within the 'standard' 0-25cm

sampling depth, there was a need to assess potential metal effects on soil microbial properties (biomass carbon, respiration rates and rhizobia MPNs) within the most concentrated metal horizon (i.e. 0-15cm), and any associated impacts on soil metal concentration and microbial property relationships.

In this project, the soil sampling programme focused on the Zn treatments at two sites for rhizobia MPNs (i.e. Gleadthorpe and Watlington where there had been relationships between Zn and rhizobia MPNs in all 3 study years) and on the Cu treatments at two sites for biomass carbon and respiration rate (i.e. Woburn and Watlington where there had been consistent relationships with these parameters).

RESULTS and DISCUSSION

At both Gleadthorpe and Watlington for rhizobia MPNs, and Woburn and Watlington for biomass C and respiration rate, there was little evidence of any marked differences in soil microbial properties between soil samples taken from either the 0-15 or 0-25cm depth horizons. The lack of differences in soil microbial properties was somewhat surprising considering that there were significant differences ($P < 0.05$) in soil organic carbon between the two soil depth horizons at all the sites sampled (with the exception of the Cu plots at Watlington), and significant differences ($P < 0.05$) in total metal concentrations between the two soil depth horizons at all the sites sampled (with the exception of the Cu plots at Woburn).

CONCLUSIONS

- Significant ($P < 0.05$) differences between the 0-15 and 0-25cm soil depth horizons were measured in soil organic C and total topsoil Zn and Cu concentrations on three out of the four dose-response treatments sampled. However, somewhat surprisingly there was no evidence of any significant differences in soil biomass C, respiration rate and rhizobia MPNs between the 0-15 and 0-25cm soil depth horizons.
- In terms of soil microbial property relationships, there was no strong evidence to suggest that there was a need to alter the 'standard' soil sampling depth from 0-25cm to 0-15cm in "The Long-term Sludge Experiments" (Defra projects SP0130 and SP0133).

Project Report to Defra

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   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

OBJECTIVE

- To determine microbial properties (*i.e.* biomass carbon, respiration rate and rhizobia most probable numbers - MPNs) in the 0-15cm topsoil depth compared with the 'standard' 0-25cm topsoil depth samples.

The work undertaken has fully met the objectives set out in the contract specification and

has shown that there are no significant differences ($P > 0.05$) in soil biomass carbon contents, respiration rates or rhizobia MPNs between the most concentrated metal horizon (*i.e.* the 0-15cm depth) and the 'standard' soil sampling depth (*i.e.* 0-25cm) in the experimental programme.

INTRODUCTION

There is a recognised need to determine that the present maximum permitted soil metal concentrations where sewage sludge is applied to agricultural land are safe for crops, soil microbes and their activity. Present maximum permissible soil metal concentrations were developed using coarse textured soil types and sensitive crop species, representing 'worse-case' conditions for metal impacts on crops and human dietary intake (CEC, 1986; SI, 1989; DOE, 1996). However, no account is explicitly taken in the present regulations of potential impacts from metals on soil microorganisms in sludge treated soils (McGrath *et al.,* 1995).

At nine sites with contrasting clay contents throughout Britain, selected metal-rich sludge cakes were applied between 1994 and 1997 and metal-amended liquid sludges applied at three sites between 1995 and 1997 (Phase I) to establish individual zinc (Zn), copper (Cu) and cadmium (Cd) metal dose-response treatments (Gibbs *et al*. 2006a; Gibbs *et al.* 2006b). During Phases II and III of the project (SP0125 and SP0130), effects of the established topsoil metal concentrations on selected soil microbial (biomass, respiration, rhizobia), chemical (total and extractable metals, speciation of metals, organic carbon, cation exchange capacity and soil pH) and physical properties (bulk density) are being measured.

The main experimental programme (Defra projects SP0125 and SP0130) is intended to provide a sound scientific base for the continued development of policies on soil protection where sewage sludge is applied to agricultural land, and to identify the effects of sludge derived metals on soil microbial, chemical and physical properties, and agricultural productivity.

Metal concentrations in topsoil (0-25cm depth) samples taken at some sites during Phase II of this project (1999 and 2001) and the first sampling during Phase III (2003) showed a general trend of decreasing concentrations with time. In particular, these concentration decreases were most evident at Watlington (sludge cake and liquid sludge), Rosemaund (sludge cake and liquid sludge), Bridgets (sludge cake) and Shirburn (sludge cake).

As a result of the clear changes in metal concentrations within the 'standard' 0-25cm sampling depth, there was a need to assess potential metal effects on soil microbial properties (biomass carbon, respiration rates and rhizobia MPNs) within the most concentrated metal horizon (i.e. 0-15cm), and any associated impacts on soil metal concentration and microbial property relationships.

METHOD

An extensive soil sampling programme was undertaken that focused on the Zn treatments at two sites for rhizobia MPN's (i.e. Gleadthorpe and Watlington where there had been relationships between Zn and rhizobia MPNs in all 3 study years) and on the Cu treatments at two sites for biomass carbon and respiration rate (i.e. Woburn and Watlington where there had been consistent relationships with these parameters). In order to assess the impact of soil sampling depth (i.e. 0-15cm vs. 0-25cm depth samples) on microbial parameters, samples were taken as follows:

- Twenty random points within each replicate plot of the untreated control, uncontaminated sludge cake control and metal rate 1, 2, 3 and 4 treatments at each site (i.e. 18 plots at each site) to a sampling depth of 0-15cm (ADAS). The twenty sub-samples from each plot formed a composite sample for analysis.

- Topsoil (0-15cm depth) samples from the Zn treatments were analysed for rhizobia MPNs (by Rothamsted Research) (Vincent, 1970; Woomer *et al.*, 1990), and from the Cu treatments for biomass carbon (by Rothamsted Research) (Vance *et al.*, 1987) and respiration rate (by WRc)(Smith and Hadley, 1990), using the same assay procedures as the main programme (Defra projects SP0125, SP0130 and SP0133).
- Topsoil (0-15cm depth) samples were analysed for total (perchloric nitric acid digestion – MAFF, 1986) Zn and Cu concentrations determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (by WRc). Also, a separate sub-sample was analysed for soil organic carbon (potassium dichromate oxidation – MAFF, 1986) as an additional barometer for microbial location/activity, and soil pH (by ADAS)(MAFF, 1986).

The data from the microbial determinations on the 0-15cm depth samples in this study were directly compared with those from the 'standard' 0-25cm depth samples taken as part of the main sampling programme (at the same time) in spring 2005.

RESULTS

Rhizobia MPNs

Soil samples (0-15cm), taken in spring 2005, from the untreated control, uncontaminated sludge cake and Zn sludge cake rate 1-4 treatments at Gleadthorpe and Watlington were analysed for total Zn concentrations, rhizobia MPNs, organic C and pH (Tables 1 and 2).

Table 1. Mean treatment soil total Zn (mg/kg) concentrations, rhizobia MPNs (log_{10} cells/g), organic carbon (%) contents and pH – *Gleadthorpe*

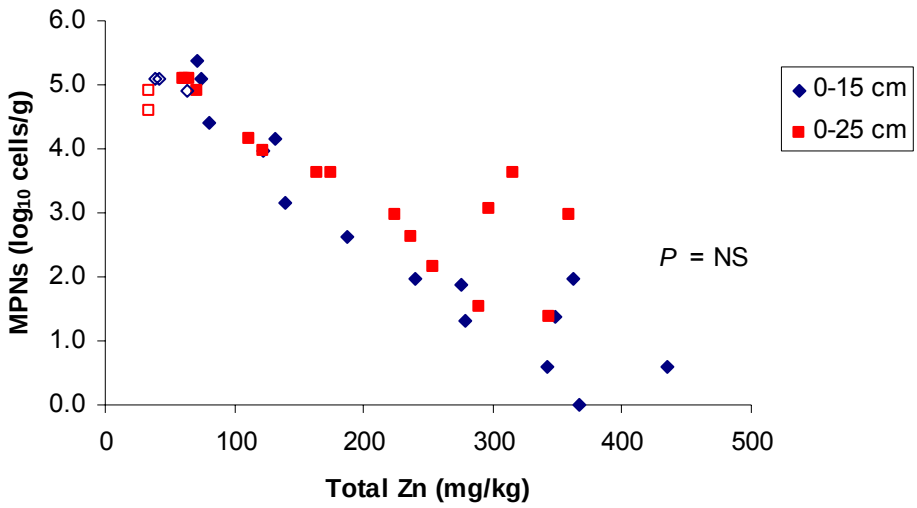
Treatment	Total Zn (mg/kg)	Rhizobia MPNs (log_{10} cells/g)	Organic C (% w/w)	pH
Unt. Control	47.7	5.02	1.57	6.0
Unc. cake	75.4	4.95	2.36	5.9
Zn cake rate 1	131	3.77	2.17	5.9
Zn cake rate 2	248	1.94	2.28	5.9
Zn cake rate 3	310	1.31	2.46	5.8
Zn cake rate 4	388	0.86	2.02	5.8

Table 2. Mean treatment soil total Zn (mg/kg) concentrations, rhizobia MPNs (log_{10} cells/g), organic carbon (%) contents and pH – *Watlington*

Treatment	Total Zn (mg/kg)	Rhizobia MPNs (log_{10} cells/g)	Organic C (% w/w)	pH
Unt. Control	43.7	5.12	2.14	6.8
Unc. cake	77.3	5.18	2.61	6.7
Zn cake rate 1	140	4.31	2.71	6.8
Zn cake rate 2	260	2.61	2.65	6.6
Zn cake rate 3	330	2.59	2.60	6.7
Zn cake rate 4	400	2.56	2.67	6.8

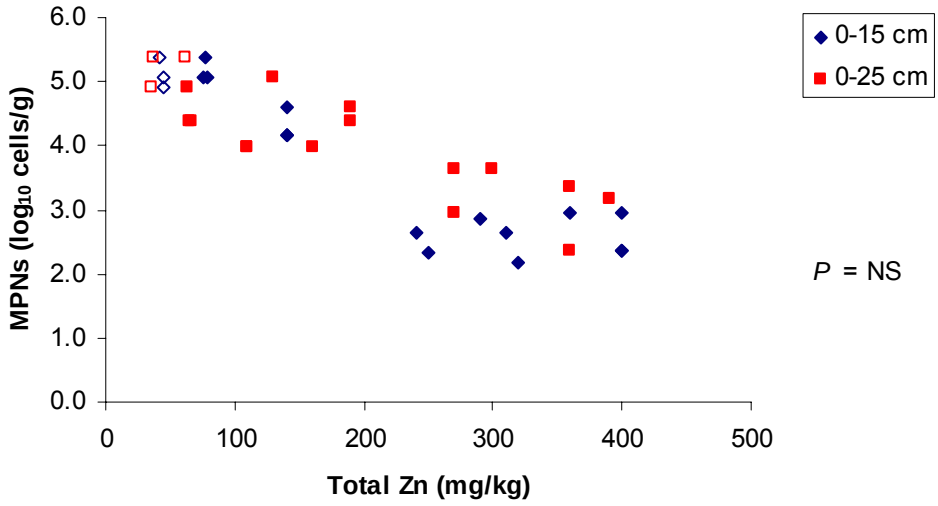
At both Gleadthorpe and Watlington, there were no significant ($P > 0.05$) differences (based on the parallel fitting of regression lines) in rhizobia MPNs (Figures 1 and 2, respectively) and soil pH (data not shown) between the soil samples taken from the 0-15cm and 0-25cm sampling depths. However, at both Gleadthorpe and Watlington, soil organic C contents were higher ($P < 0.05$) in the 0-15cm depth samples compared with the 0-25cm depth samples (Figures 3 and 4).

Figure 1. Relationship between total soil Zn concentrations and rhizobia MPNs – *Gleadthorpe*



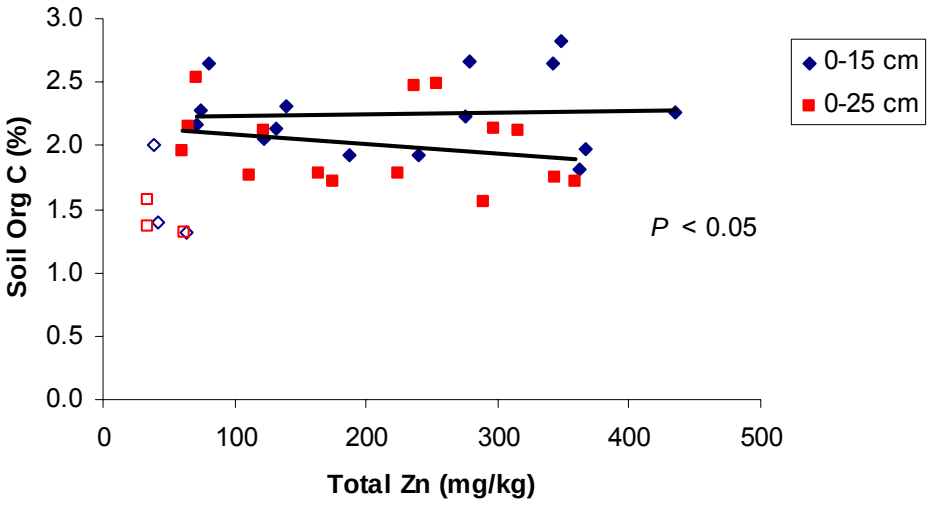
N.B. hollow symbols represent untreated controls

Figure 2. Relationship between total soil Zn concentrations and rhizobia MPNs – *Watlington*



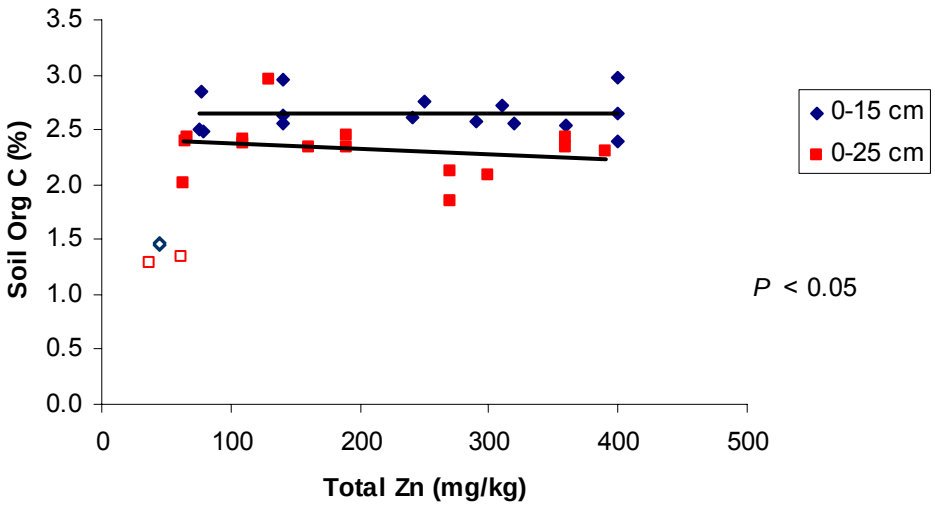
N.B. hollow symbols represent untreated controls

Figure 3. Relationship between total soil Zn concentrations and organic C content – *Gleadthorpe*



N.B. hollow symbols represent untreated controls

Figure 4. Relationship between total soil Zn concentrations and organic C content – *Watlington*



N.B. hollow symbols represent untreated controls

Biomass carbon and respiration rate

Soil samples (0-15cm), taken in spring 2005, from the untreated control, uncontaminated sludge cake and Cu sludge cake rate 1-4 treatments at Woburn and Watlington were analysed for total Cu concentrations, biomass C, respiration rate, organic C and pH (Tables 3 and 4).

Table 3. Mean treatment soil total Cu (mg/kg) concentrations, biomass carbon (mg/kg), organic carbon (%) and pH – *Woburn*

Treatment	Total Cu (mg/kg)	Biomass C (mg/kg)	Respiration rate (mg CO_2-C/kg/hr)	Organic C (% w/w)	pH
Unt. Control	14.3	206	0.33	0.79	6.7
Unc. cake	32.7	292	0.43	1.01	6.6
Cu cake rate 1	55.0	293	0.44	1.21	6.5
Cu cake rate 2	108	275	0.40	1.32	6.5
Cu cake rate 3	150	239	0.43	1.25	6.3
Cu cake rate 4	193	233	0.39	1.08	6.3

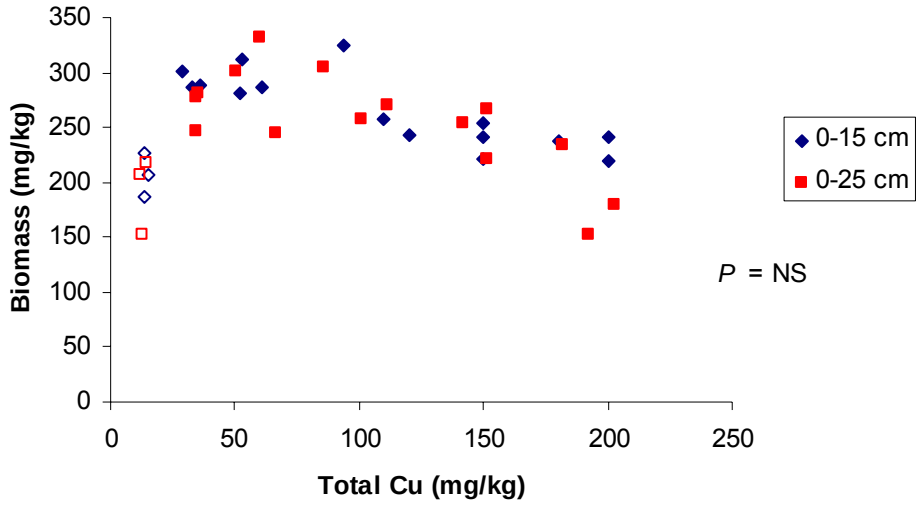
Table 4. Mean treatment soil total Cu (mg/kg) concentrations, biomass carbon (mg/kg), organic carbon (%) and pH – *Watlington*

Treatment	Total Cu (mg/kg)	Biomass C (mg/kg)	Respiration rate (mg CO_2-C/kg/hr)	Organic C (% w/w)	pH
Unt. Control	11.2	291	0.30	2.14	6.8
Unc. cake	35.3	481	0.36	2.37	7.0
Cu cake rate 1	68.0	409	0.36	2.26	7.0
Cu cake rate 2	98.0	373	0.29	2.37	6.8
Cu cake rate 3	173	364	0.43	2.58	6.7
Cu cake rate 4	217	314	0.36	2.67	6.5

At both Woburn and Watlington, there were no significant ($P > 0.05$) differences (based on the parallel fitting of regression lines) in biomass C (Figures 5 and 6, respectively), respiration rates (Figures 7 and 8, respectively) and soil pH (data not shown) between soil samples taken from the 0-15cm and 0-25cm sampling depths.

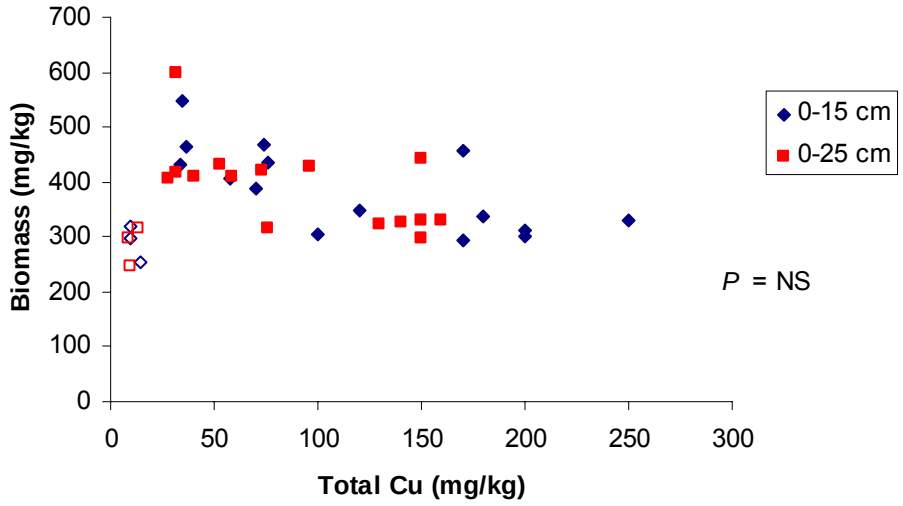
At Woburn, soil organic C contents were higher ($P < 0.05$) in the 0-15cm samples compared with the 0-25cm depth samples (Figure 9). However, at Watlington there were no differences ($P > 0.05$) in soil organic C contents between the different depth samples (Figure 10).

Figure 5. Relationship between total soil Cu concentrations and biomass C – *Woburn*



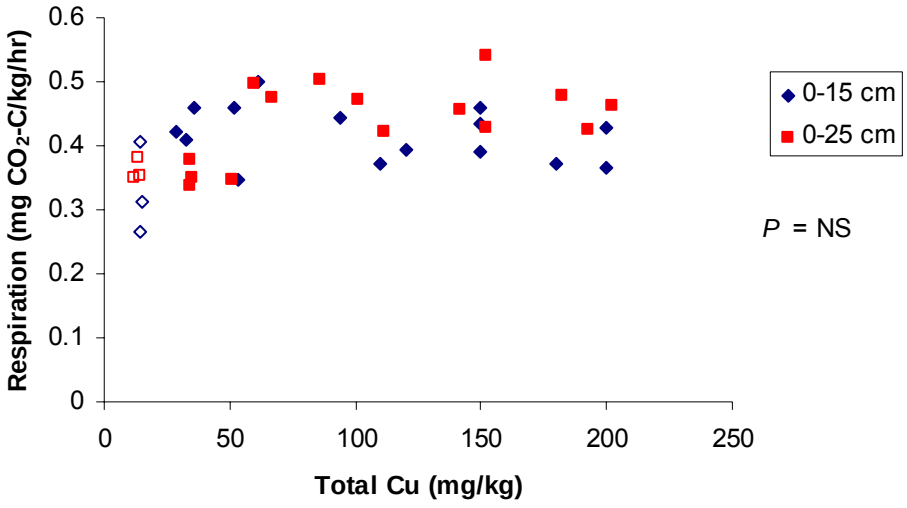
N.B. hollow symbols represent untreated controls

Figure 6. Relationship between total soil Cu concentrations and biomass C – *Watlington*



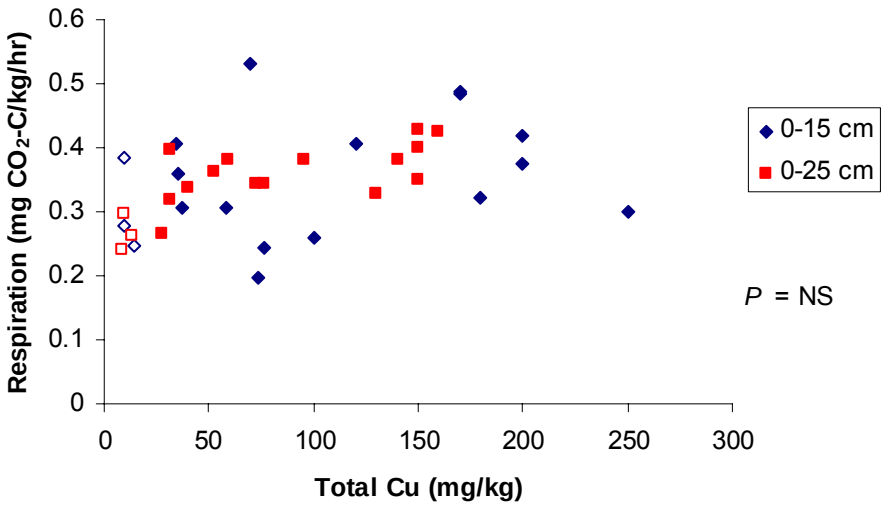
N.B. hollow symbols represent untreated controls

Figure 7. Relationship between total soil Cu concentrations and respiration rate – *Woburn*



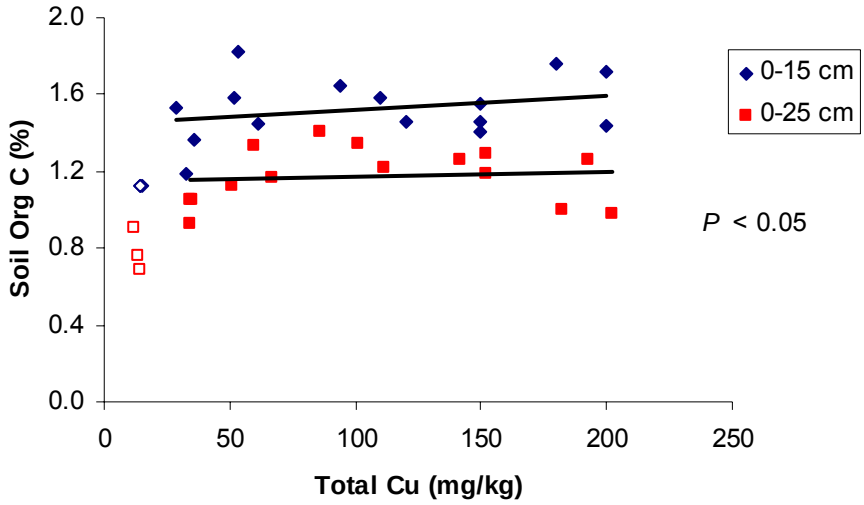
N.B. hollow symbols represent untreated controls

Figure 8. Relationship between total soil Cu concentrations and respiration rate – *Watlington*



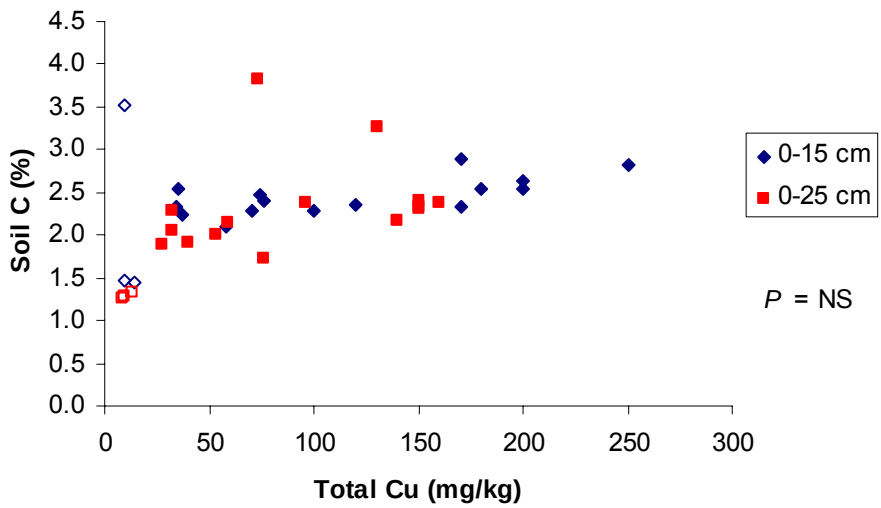
N.B. hollow symbols represent untreated controls

Figure 9. Relationship between total soil Cu concentrations and organic C content – *Woburn*



N.B. hollow symbols represent untreated controls

Figure 10. Relationship between total soil Cu concentrations and organic C content – *Watlington*

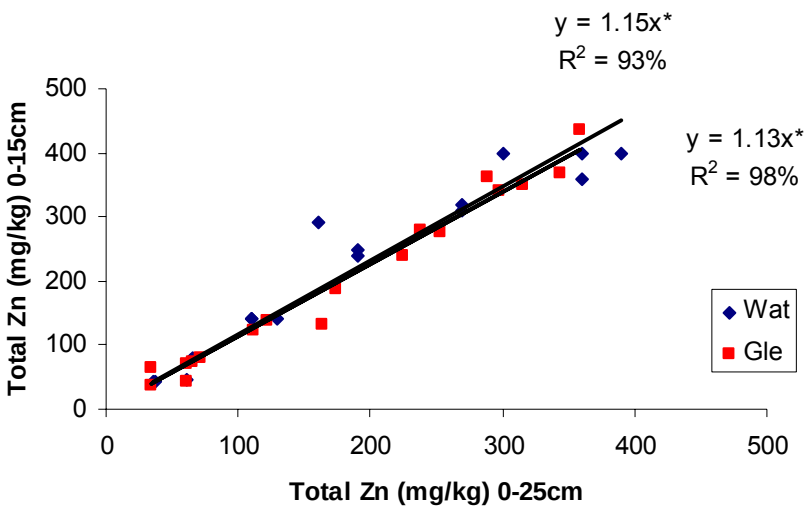


N.B. hollow symbols represent untreated controls

DISCUSSION

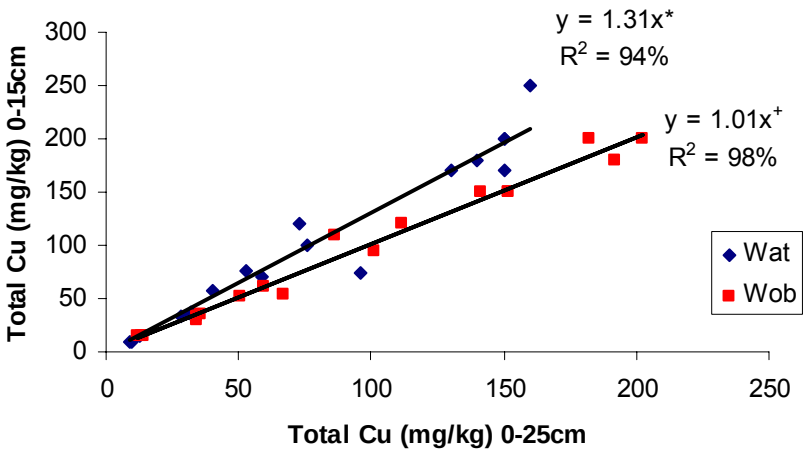
At both Gleadthorpe and Watlington for rhizobia MPNs, and Woburn and Watlington for biomass C and respiration rate, there were no marked differences ($P > 0.05$) in soil microbial property relationships between soil samples taken from either the 0-15cm or 0-25cm sampling depths. The lack of difference in soil microbial property relationships between the two depth samples was somewhat surprising given that soil organic carbon contents (Figures 3, 4 and 9) and metal concentrations (Figures 11 and 12) on three of the four dose-response treatments were significantly ($P < 0.05$) higher in the 0-15cm compared with the 0-25cm sampling depths.

Figure 11. Relationship between 0-15cm and 0-25 cm soil horizons – *total Zn*



* Slope of line significantly ($P < 0.05$) different from 1:1 line

Figure 12. Relationship between 0-15cm and 0-25 cm soil horizons – *total Cu*



*Slope of line significantly ($P < 0.05$) different from 1:1 line. $^{+}$ = NS

CONCLUSIONS

- Significant ($P < 0.05$) differences between the 0-15 and 0-25cm soil depth horizons were measured in soil organic C and total topsoil Zn and Cu concentrations on three out of the four dose-response treatments sampled. However, somewhat surprisingly there was little evidence of any marked ($P > 0.05$) differences in soil biomass C, respiration rate and rhizobia MPNs between the 0-15 and 0-25cm soil depth horizons. It appears that the biomass C and respiration rate measurements were not sensitive enough indicators to pick up the magnitude of soil property differences at the study sites. However, for rhizobia MPNs there was an indication that the number of rhizobia were lower at higher Zn concentrations at the Gleadthorpe site, which has potential implications for the results obtained at the different sites in the main project.
- In terms of soil microbial property relationships, further information would be needed at more sites in order to determine whether there is a need to alter the 'standard' soil sampling depth from 0-25cm to 0-15cm in "The Long-term Sludge Experiments" (Defra projects SP0130 and SP0133).

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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APPENDIX H Crop yield and metal concentrations: Sludge cake sites - 2002

Site 1. Gleadthorpe

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	5.12	21.42	5.19	0.085
s.e.m.	0.15	0.78	0.30	0.008
Unc. Digested	4.98	35.42	8.71	0.109
s.e.m.	0.11	0.67	0.40	0.005
Unc. Raw	5.22	30.30	7.69	0.098
s.e.m.	0.31	2.06	0.17	0.028
Zn rate 1	5.51	60.27	10.64	0.127
s.e.m.	0.03	2.37	0.54	0.014
Zn rate 2	5.16	81.53	11.07	0.137
s.e.m.	0.24	5.65	0.67	0.009
Zn rate 3	5.25	98.23	11.57	0.125
s.e.m.	0.22	6.64	0.42	-
Zn rate 4	5.74	95.67	10.11	0.125
s.e.m.	0.07	10.17	0.49	0.015
Cu rate 1	5.96	32.50	8.22	0.075
s.e.m.	0.56	2.16	0.72	0.013
Cu rate 2	5.85	39.07	9.31	0.092
s.e.m.	0.17	1.68	0.76	0.007
Cu rate 3	5.34	41.70	11.46	0.098
s.e.m.	0.43	1.65	0.88	0.019
Cu rate 4	5.69	46.63	11.74	0.087
s.e.m.	0.37	6.11	1.49	0.012
Cd rate 1	4.50	52.60	10.80	0.313
s.e.m.	0.23	6.47	0.91	0.061
Cd rate 2	5.42	42.33	8.46	0.407
s.e.m.	1.07	5.89	1.23	0.082
Cd rate 3	5.55	41.10	9.26	0.523
s.e.m.	0.21	2.64	0.25	0.034
Cd rate 4	6.10	38.70	8.05	0.428
s.e.m.	0.69	1.67	0.29	0.028
LT Unc. Digested	5.12	25.15	6.00	0.084
s.e.m.	0.33	1.70	0.14	0.018
LT Unc. Raw	5.51	22.63	5.82	0.083
s.e.m.	0.16	0.39	0.50	0.010
LT Zn	5.40	48.70	7.40	0.112
s.e.m.	0.13	6.13	0.27	0.027
LT Cu	5.77	33.90	8.11	0.118
s.e.m.	0.51	1.99	0.74	0.019
LT Cd	4.99	28.43	6.19	0.290
s.e.m.	0.10	2.06	0.32	0.035

Site 2. Woburn

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	3.90	27.66	3.37	0.047
s.e.m.	0.13	1.07	0.28	0.006
Unc. Digested	6.32	40.51	5.07	0.06
s.e.m.	0.14	2.26	0.37	0.00
Unc. Raw	6.07	32.89	3.93	0.035
s.e.m.	0.24	1.99	0.05	0.004
Zn rate 1	6.02	73.55	5.49	0.074
s.e.m.	0.51	6.92	0.24	0.002
Zn rate 2	6.47	94.86	5.94	0.074
s.e.m.	0.41	7.35	0.62	0.008
Zn rate 3	6.90	106.93	6.19	0.082
s.e.m.	0.36	7.32	0.52	0.011
Zn rate 4	6.80	146.73	6.72	0.116
s.e.m.	0.46	4.71	0.23	0.005
Cu rate 1	6.60	36.17	5.57	0.036
s.e.m.	0.42	2.34	0.62	0.007
Cu rate 2	5.80	43.53	5.55	0.042
s.e.m.	0.06	3.14	0.33	0.006
Cu rate 3	6.40	48.97	6.29	0.040
s.e.m.	0.44	3.02	0.23	0.006
Cu rate 4	6.73	50.58	7.04	0.078
s.e.m.	0.37	4.41	0.83	0.032
Cd rate 1	6.77	47.76	5.85	0.143
s.e.m.	0.27	4.33	0.79	0.025
Cd rate 2	6.57	45.26	4.75	0.203
s.e.m.	0.44	1.21	0.74	0.032
Cd rate 3	6.57	43.13	5.13	0.211
s.e.m.	0.39	0.69	0.34	0.010
Cd rate 4	6.80	44.52	4.49	0.282
s.e.m.	0.12	3.87	0.20	0.007
LT Unc. Digested	5.55	28.76	3.27	0.050
s.e.m.	0.38	1.22	0.35	0.004
LT Unc. Raw	5.53	25.34	3.60	0.036
s.e.m.	0.27	3.30	0.30	0.006
LT Zn	5.83	49.65	4.39	0.059
s.e.m.	0.57	3.22	0.20	0.011
LT Cu	4.93	28.66	3.79	0.035
s.e.m.	0.63	2.00	0.11	0.003
LT Cd	5.17	25.19	3.06	0.139
s.e.m.	0.27	2.31	0.09	0.011

Site 3. Watlington

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	4.83	13.73	3.07	0.036
s.e.m.	0.15	0.12	0.02	0.003
Unc. Digested	5.49	20.90	4.31	0.030
s.e.m.	0.47	1.16	0.28	0.002
Unc. Raw	5.17	18.07	4.05	0.018
s.e.m.	0.30	0.19	0.13	0.003
Zn rate 1	4.96	28.27	3.99	0.037
s.e.m.	0.79	1.71	0.35	0.004
Zn rate 2	5.76	47.00	4.54	0.052
s.e.m.	0.21	2.98	0.13	0.002
Zn rate 3	4.93	57.13	5.36	0.056
s.e.m.	0.14	4.87	0.22	0.003
Zn rate 4	5.22	66.50	5.58	0.064
s.e.m.	0.40	3.46	0.36	0.002
Cu rate 1	5.67	21.97	4.60	0.020
s.e.m.	0.20	1.59	0.37	0.003
Cu rate 2	5.65	24.03	5.00	0.026
s.e.m.	0.51	3.28	0.40	0.008
Cu rate 3	5.64	24.97	5.35	0.020
s.e.m.	0.53	1.87	0.48	0.002
Cu rate 4	5.13	28.20	5.62	0.147
s.e.m.	0.20	1.46	0.12	0.121
Cd rate 1	5.53	23.00	3.96	0.069
s.e.m.	0.44	1.40	0.25	0.016
Cd rate 2	5.22	24.60	4.18	0.090
s.e.m.	0.43	1.29	0.38	0.020
Cd rate 3	5.18	27.47	4.43	0.176
s.e.m.	0.38	0.29	0.31	0.027
Cd rate 4	5.89	25.93	3.89	0.220
s.e.m.	0.29	1.52	0.35	0.072
LT Unc. Digested	5.27	17.30	3.59	0.026
s.e.m.	0.14	0.40	0.12	0.000
LT Unc. Raw	5.46	17.17	3.75	0.026
s.e.m.	0.11	0.46	0.18	0.004
LT Zn	5.48	29.80	3.47	0.032
s.e.m.	0.05	0.87	0.07	0.002
LT Cu	5.77	20.40	4.38	0.021
s.e.m.	0.36	1.46	0.31	0.002
LT Cd	4.92	17.20	3.31	0.072
s.e.m.	0.03	0.80	0.15	0.002

Site 4. Pwllpeiran

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	6.50	21.12	4.22	0.037
s.e.m.	0.48	1.28	0.40	0.011
Unc. Digested	6.99	28.82	4.58	0.024
s.e.m.	0.44	2.33	0.36	0.002
Unc. Raw	7.03	26.47	4.34	0.043
s.e.m.	0.32	1.93	0.32	0.002
Zn rate 1	6.07	49.00	5.54	0.038
s.e.m.	0.25	6.92	0.17	0.014
Zn rate 2	6.12	81.03	5.97	0.067
s.e.m.	0.54	9.87	0.27	0.010
Zn rate 3	6.45	77.80	6.08	0.043
s.e.m.	0.55	14.53	0.43	0.019
Zn rate 4	6.78	108.43	6.49	0.070
s.e.m.	0.15	11.34	0.46	0.010
Cu rate 1	6.43	29.27	5.09	0.052
s.e.m.	0.29	5.57	1.15	0.009
Cu rate 2	6.34	36.43	5.27	0.020
s.e.m.	0.72	4.66	0.06	0.005
Cu rate 3	7.55	39.47	6.43	0.033
s.e.m.	0.24	4.09	0.48	0.007
Cu rate 4	7.30	34.80	7.54	0.032
s.e.m.	0.92	3.23	0.78	0.004
Cd rate 1	6.28	38.07	5.67	0.088
s.e.m.	0.16	9.87	0.39	0.036
Cd rate 2	7.20	38.37	5.00	0.183
s.e.m.	0.67	2.85	0.53	0.052
Cd rate 3	6.24	49.27	6.39	0.268
s.e.m.	0.23	9.79	0.59	0.012
Cd rate 4	7.54	51.77	5.66	0.242
s.e.m.	0.66	6.94	0.17	0.019
LT Unc. Digested	6.32	22.75	4.91	0.035
s.e.m.	0.34	2.56	0.11	0.005
LT Unc. Raw	6.83	20.50	4.62	0.032
s.e.m.	0.43	3.13	0.78	0.002
LT Zn	5.92	63.87	5.61	0.040
s.e.m.	0.14	5.87	0.10	0.015
LT Cu	6.43	32.00	5.41	0.022
s.e.m.	0.13	1.78	0.89	0.003
LT Cd	6.90	25.60	4.03	0.175
s.e.m.	0.50	3.42	0.45	0.032

Site 5. Rosemaund

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	10.79	21.57	3.27	0.037
s.e.m.	0.20	1.91	0.17	0.008
Unc. Digested	10.97	49.05	4.80	0.085
s.e.m.	0.17	5.48	0.25	0.015
Unc. Raw	9.93	29.30	4.33	0.080
s.e.m.	0.99	0.25	0.25	0.043
Zn rate 1	11.61	78.57	5.10	0.107
s.e.m.	0.63	18.48	0.24	0.040
Zn rate 2	10.12	141.67	5.87	0.115
s.e.m.	1.27	8.21	0.40	0.018
Zn rate 3	10.58	169.00	7.33	0.205
s.e.m.	0.32	19.70	2.06	0.076
Zn rate 4	10.80	252.33	6.18	0.152
s.e.m.	0.67	32.77	0.59	0.037
Cu rate 1	10.35	39.97	4.68	0.038
s.e.m.	0.62	9.09	0.60	0.006
Cu rate 2	11.38	52.30	5.66	0.057
s.e.m.	0.99	7.64	0.01	0.016
Cu rate 3	12.36	58.10	7.33	0.130
s.e.m.	0.86	5.69	0.51	0.065
Cu rate 4	11.71	77.10	7.21	0.168
s.e.m.	0.61	4.79	0.28	0.098
Cd rate 1	10.48	64.20	5.12	0.105
s.e.m.	0.77	13.60	1.04	0.040
Cd rate 2	10.41	40.50	4.62	0.135
s.e.m.	0.59	7.71	0.48	0.056
Cd rate 3	10.87	54.60	4.42	0.182
s.e.m.	1.91	10.05	0.17	0.025
Cd rate 4	11.69	52.80	4.66	0.185
s.e.m.	1.16	4.10	0.40	0.009
LT Unc. Digested	12.46	24.72	4.16	0.073
s.e.m.	0.66	1.66	0.15	0.032
LT Unc. Raw	11.60	23.60	3.93	0.090
s.e.m.	0.51	2.75	0.29	0.053
LT Zn	10.75	59.03	4.14	0.085
s.e.m.	1.00	15.34	0.47	0.044
LT Cu	10.52	42.37	5.35	0.070
s.e.m.	1.27	0.55	0.74	0.018
LT Cd	10.31	30.87	3.68	0.113
s.e.m.	1.01	5.64	0.20	0.050

Site 6. Bridgets

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	3.57	14.47	4.72	1.101
s.e.m.	0.27	1.29	0.24	0.391
Unc. Digested	5.05	17.97	5.72	0.674
s.e.m.	0.11	0.82	0.10	0.111
Unc. Raw	5.07	17.80	5.36	0.323
s.e.m.	0.21	0.64	0.28	0.015
Zn rate 1	5.37	42.57	6.46	0.677
s.e.m.	0.21	3.54	0.94	0.194
Zn rate 2	5.78	45.77	5.69	0.570
s.e.m.	0.62	1.89	0.44	0.136
Zn rate 3	5.29	67.33	5.90	0.525
s.e.m.	0.41	17.42	0.69	0.118
Zn rate 4	5.67	84.50	4.82	0.545
s.e.m.	0.42	7.34	0.36	0.108
Cu rate 1	4.78	20.30	6.33	0.815
s.e.m.	0.65	1.27	0.24	0.220
Cu rate 2	5.67	21.57	5.96	0.385
s.e.m.	0.67	1.81	0.70	0.108
Cu rate 3	4.87	23.97	6.00	0.402
s.e.m.	0.47	2.74	0.46	0.037
Cu rate 4	6.33	29.10	7.53	0.610
s.e.m.	0.45	0.81	0.32	0.095
Cd rate 1	5.29	22.80	5.70	0.515
s.e.m.	0.11	1.05	0.67	0.118
Cd rate 2	4.85	20.77	5.10	0.422
s.e.m.	0.03	2.18	0.52	0.105
Cd rate 3	5.58	22.50	4.56	0.372
s.e.m.	0.60	2.10	0.29	0.082
Cd rate 4	5.10	21.57	4.71	0.550
s.e.m.	0.59	1.17	0.25	0.125
LT Unc. Digested	4.15	16.12	5.34	0.794
s.e.m.	0.07	0.53	0.42	0.190
LT Unc. Raw	4.02	15.37	4.66	0.765
s.e.m.	0.36	0.27	0.46	0.127
LT Zn	3.97	28.57	4.87	0.507
s.e.m.	0.11	1.01	0.60	0.092
LT Cu	4.10	16.37	4.82	0.537
s.e.m.	0.17	0.22	0.28	0.147
LT Cd	3.84	18.93	5.16	0.648
s.e.m.	0.86	2.15	0.55	0.110

Site 7. Hartwood

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	1.56	37.29	6.47	0.100
s.e.m.	0.28	2.76	0.30	0.022
Unc. Digested	2.89	53.70	8.04	0.062
s.e.m.	0.22	2.42	0.18	0.008
Unc. Raw	2.76	39.87	8.13	0.281
s.e.m.	0.38	1.73	0.61	0.242
Zn rate 1	2.32	84.22	9.24	0.081
s.e.m.	0.60	11.42	0.25	0.017
Zn rate 2	3.05	94.60	7.63	0.075
s.e.m.	0.48	6.86	0.47	0.001
Zn rate 3	2.48	136.75	9.02	0.094
s.e.m.	0.58	25.07	0.48	0.020
Zn rate 4	2.60	235.36	10.19	0.132
s.e.m.	0.52	28.65	0.78	0.020
Cu rate 1	2.95	47.15	8.72	0.065
s.e.m.	0.51	6.36	0.43	0.019
Cu rate 2	2.30	63.02	9.27	0.076
s.e.m.	0.52	12.91	1.68	0.024
Cu rate 3	2.90	68.51	9.54	0.078
s.e.m.	0.55	6.84	1.12	0.014
Cu rate 4	2.54	70.50	11.82	0.049
s.e.m.	0.12	9.61	0.57	0.014
Cd rate 1	2.58	63.95	7.67	0.212
s.e.m.	0.23	8.52	0.46	0.038
Cd rate 2	2.49	63.13	7.95	0.292
s.e.m.	0.36	0.84	1.15	0.027
Cd rate 3	2.31	69.40	7.80	0.462
s.e.m.	0.23	3.52	0.41	0.093
Cd rate 4	2.51	63.49	9.30	0.327
s.e.m.	0.41	11.97	0.30	0.096
LT Unc. Digested	2.60	34.86	6.71	0.060
s.e.m.	0.13	2.31	0.26	0.008
LT Unc. Raw	2.59	38.16	6.80	0.072
s.e.m.	0.59	3.16	0.52	0.017
LT Zn	3.19	65.43	7.63	0.075
s.e.m.	0.50	7.34	0.43	0.013
LT Cu	1.69	47.21	7.48	0.095
s.e.m.	0.29	7.75	0.43	0.021
LT Cd	1.92	50.06	7.01	0.327
s.e.m.	0.39	3.41	0.40	0.057

Site 8. Auchincruive

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	2.61	16.83	2.64	0.032
s.e.m.	0.16	0.17	0.09	0.002
Unc. Digested	3.55	41.42	3.72	0.052
s.e.m.	0.36	1.68	0.13	0.004
Unc. Raw	3.65	23.33	3.25	0.023
s.e.m.	0.06	2.37	0.23	0.003
Zn rate 1	4.11	79.50	4.51	0.080
s.e.m.	0.24	8.25	0.49	0.015
Zn rate 2	4.36	91.60	3.93	0.047
s.e.m.	0.11	8.32	0.05	0.003
Zn rate 3	4.14	140.33	4.89	0.073
s.e.m.	0.11	13.48	0.17	0.017
Zn rate 4	4.45	237.33	5.91	0.110
s.e.m.	0.23	67.59	1.00	0.040
Cu rate 1	3.85	32.80	4.24	0.023
s.e.m.	0.18	4.50	0.34	0.003
Cu rate 2	3.75	40.40	4.28	0.033
s.e.m.	0.21	3.00	0.44	0.003
Cu rate 3	4.06	54.50	5.08	0.050
s.e.m.	0.07	1.31	0.44	0.006
Cu rate 4	4.33	47.83	5.56	0.050
s.e.m.	0.07	6.88	0.52	0.012
Cd rate 1	3.99	47.07	4.17	0.177
s.e.m.	0.15	1.90	0.31	0.018
Cd rate 2	3.82	51.33	3.89	0.200
s.e.m.	0.35	2.09	0.21	0.045
Cd rate 3	3.68	54.43	4.34	0.260
s.e.m.	0.14	5.57	0.61	0.065
Cd rate 4	4.21	59.30	4.22	0.270
s.e.m.	0.20	1.56	0.02	0.020
LT Unc. Digested	3.02	18.87	2.91	0.040
s.e.m.	0.15	1.31	0.18	0.005
LT Unc. Raw	2.83	17.87	2.69	0.027
s.e.m.	0.12	0.90	0.06	0.003
LT Zn	3.44	60.43	3.17	0.057
s.e.m.	0.10	3.85	0.21	0.009
LT Cu	3.06	25.40	3.08	0.037
s.e.m.	0.18	0.97	0.14	0.009
LT Cd	2.68	22.70	2.73	0.137
s.e.m.	0.06	0.59	0.04	0.018

Site 9. Shirburn

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	0.61	20.57	6.74	0.059
s.e.m.	0.07	0.30	0.12	0.006
Unc. Digested	1.23	22.33	8.13	0.065
s.e.m.	0.25	0.73	0.32	0.003
Unc. Raw	1.26	20.37	7.50	0.056
s.e.m.	0.18	0.69	0.48	0.004
High Unc. Digested	0.85	24.27	8.52	0.071
s.e.m.	0.10	3.07	0.72	0.002
High Unc. Raw	1.02	23.03	8.33	0.065
s.e.m.	0.23	0.72	0.55	0.009
Zn rate 1	0.96	27.23	8.43	0.076
s.e.m.	0.05	2.58	0.76	0.012
Zn rate 2	0.84	34.77	10.26	0.089
s.e.m.	0.13	1.90	0.64	0.009
Zn rate 3	1.00	37.90	11.18	0.098
s.e.m.	0.21	2.46	0.63	0.012
Zn rate 4	0.80	35.40	10.49	0.080
s.e.m.	0.27	3.04	0.79	0.004
Zn rate 5	1.05	38.17	10.63	0.085
s.e.m.	0.10	3.57	0.15	0.013
Cu rate 1	1.03	24.20	9.33	0.070
s.e.m.	0.19	0.68	0.22	0.010
Cu rate 2	1.22	26.50	10.34	0.079
s.e.m.	0.35	3.24	0.60	0.021
Cu rate 3	1.07	24.87	10.70	0.059
s.e.m.	0.07	0.23	0.20	0.005
Cu rate 4	1.14	26.40	11.40	0.059
s.e.m.	0.19	0.81	0.21	0.004
Cu rate 5	0.93	27.80	13.87	0.065
s.e.m.	0.04	0.40	0.83	0.005
Cd rate 1	1.22	26.70	9.61	0.127
s.e.m.	0.17	1.21	0.64	0.003
Cd rate 2	1.39	27.37	9.70	0.223
s.e.m.	0.14	2.51	0.92	0.048
Cd rate 3	1.31	25.67	8.60	0.213
s.e.m.	0.39	0.47	0.37	0.026
Cd rate 4	0.97	27.90	9.82	0.263
s.e.m.	0.18	1.21	0.44	0.027
Cd rate 5	1.04	28.13	9.71	0.263
s.e.m.	0.12	1.13	0.75	0.038
LT Unc. Digested	0.97	21.83	7.69	0.06
s.e.m.	0.19	1.24	0.44	0.01
LT Unc. Raw	0.82	20.97	6.83	0.05
s.e.m.	0.22	1.19	0.28	0.00
LT Zn	0.81	37.63	11.01	0.08
s.e.m.	0.34	4.74	1.53	0.02
LT Cu	1.28	23.17	9.29	0.04
s.e.m.	0.19	0.22	0.11	0.00
LT Cd	1.15	24.10	7.96	0.13
s.e.m.	0.20	1.63	0.17	0.01

APPENDIX I Crop yield and metal concentrations: Sludge cake sites - 2003

Site 1. Gleadthorpe

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	7.70	26.12	3.42	0.055
s.e.m.	0.14	1.96	0.22	0.005
Unc. Digested	7.73	34.20	4.24	0.063
s.e.m.	0.14	3.06	0.16	0.002
Unc. Raw	7.58	33.67	4.04	0.065
s.e.m.	0.32	1.47	0.23	0.010
Zn rate 1	8.05	48.27	4.42	0.090
s.e.m.	0.08	1.77	0.22	0.008
Zn rate 2	7.36	70.33	5.18	0.138
s.e.m.	0.38	4.27	0.16	0.009
Zn rate 3	7.16	72.03	5.17	0.122
s.e.m.	0.55	5.96	0.36	0.012
Zn rate 4	8.25	67.80	4.71	0.135
s.e.m.	0.05	1.60	0.04	0.008
Cu rate 1	8.10	32.73	4.64	0.052
s.e.m.	0.15	0.52	0.15	0.004
Cu rate 2	7.69	37.63	5.18	0.065
s.e.m.	0.50	3.17	0.19	0.003
Cu rate 3	7.71	38.10	5.30	0.067
s.e.m.	0.25	0.72	0.23	0.004
Cu rate 4	6.70	42.63	5.92	0.077
s.e.m.	0.52	3.80	0.08	0.009
Cd rate 1	7.85	40.17	4.53	0.197
s.e.m.	0.22	2.39	0.24	0.016
Cd rate 2	7.71	41.10	4.45	0.343
s.e.m.	0.37	3.24	0.17	0.047
Cd rate 3	8.67	36.97	4.31	0.327
s.e.m.	0.12	2.73	0.19	0.013
Cd rate 4	8.45	41.03	4.50	0.398
s.e.m.	0.12	2.49	0.28	0.029
LT Unc. Digested	7.90	26.73	3.44	0.058
s.e.m.	0.23	0.33	0.06	0.001
LT Unc. Raw	7.85	29.27	3.47	0.060
s.e.m.	0.42	0.79	0.13	0.005
LT Zn	7.10	58.80	4.30	0.097
s.e.m.	0.56	5.69	0.34	0.009
LT Cu	7.56	32.50	4.73	0.068
s.e.m.	0.30	3.54	0.11	0.009
LT Cd	8.09	36.40	3.79	0.323
s.e.m.	0.04	3.90	0.27	0.045

Site 2. Woburn

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	4.24	35.02	3.62	0.043
s.e.m.	0.15	1.97	0.14	0.002
Unc. Digested	4.33	50.27	5.51	0.064
s.e.m.	0.23	1.98	0.10	0.003
Unc. Raw	3.07	52.48	5.86	0.046
s.e.m.	0.26	0.84	0.05	0.002
Zn rate 1	3.34	72.95	6.11	0.092
s.e.m.	0.11	4.56	0.17	0.005
Zn rate 2	4.12	81.35	6.13	0.097
s.e.m.	0.36	1.12	0.12	0.002
Zn rate 3	4.07	92.28	6.31	0.112
s.e.m.	0.38	2.60	0.10	0.004
Zn rate 4	3.51	105.17	6.60	0.143
s.e.m.	0.14	4.01	0.13	0.006
Cu rate 1	3.25	52.38	6.34	0.044
s.e.m.	0.06	3.36	0.16	0.005
Cu rate 2	3.10	55.98	6.76	0.051
s.e.m.	0.24	2.57	0.18	0.005
Cu rate 3	3.73	53.38	6.98	0.048
s.e.m.	0.38	5.35	0.34	0.005
Cu rate 4	3.81	57.97	7.25	0.065
s.e.m.	0.38	4.01	0.27	0.010
Cd rate 1	3.69	52.60	5.69	0.173
s.e.m.	0.36	3.62	0.12	0.021
Cd rate 2	4.19	53.34	5.70	0.265
s.e.m.	0.59	4.01	0.28	0.023
Cd rate 3	3.46	55.55	6.00	0.321
s.e.m.	0.38	3.75	0.34	0.013
Cd rate 4	4.09	54.12	6.01	0.346
s.e.m.	0.44	4.36	0.24	0.046
LT Unc. Digested	4.44	39.02	4.19	0.038
s.e.m.	0.18	1.05	0.10	0.007
LT Unc. Raw	3.96	35.27	4.18	0.036
s.e.m.	0.08	0.57	0.11	0.001
LT Zn	3.71	57.00	5.25	0.052
s.e.m.	0.39	3.87	0.23	0.005
LT Cu	3.04	43.54	5.71	0.040
s.e.m.	0.18	2.87	0.20	0.002
LT Cd	4.31	39.39	4.24	0.114
s.e.m.	0.49	2.97	0.26	0.003

Site 3. Watlington

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	2.90	27.20	4.82	0.020
s.e.m.	0.11	0.70	0.09	0.000
Unc. Digested	2.78	30.77	4.95	0.023
s.e.m.	0.35	1.03	0.23	0.003
Unc. Raw	3.12	30.13	4.93	0.020
s.e.m.	0.26	0.59	0.13	0.000
Zn rate 1	2.69	40.77	5.19	0.030
s.e.m.	0.30	2.45	0.17	0.006
Zn rate 2	2.65	55.40	5.46	0.050
s.e.m.	0.23	5.20	0.28	0.006
Zn rate 3	2.80	64.80	6.25	0.070
s.e.m.	0.20	7.41	0.09	0.006
Zn rate 4	2.69	56.93	5.70	0.070
s.e.m.	0.30	13.79	0.34	0.006
Cu rate 1	3.01	30.90	5.66	0.020
s.e.m.	0.22	0.70	0.10	0.000
Cu rate 2	3.17	32.87	5.98	0.023
s.e.m.	0.32	0.98	0.17	0.003
Cu rate 3	3.11	35.47	6.41	0.020
s.e.m.	0.12	0.58	0.07	0.000
Cu rate 4	2.57	41.60	7.17	0.030
s.e.m.	0.31	1.53	0.05	0.000
Cd rate 1	2.52	36.43	5.49	0.070
s.e.m.	0.28	1.61	0.15	0.010
Cd rate 2	2.91	37.33	5.75	0.100
s.e.m.	0.21	0.33	0.04	0.006
Cd rate 3	3.17	39.20	5.62	0.123
s.e.m.	0.21	2.14	0.22	0.003
Cd rate 4	3.19	38.17	5.89	0.150
s.e.m.	0.05	0.94	0.19	0.006
LT Unc. Digested	3.36	26.77	4.52	0.020
s.e.m.	0.15	1.05	0.15	0.000
LT Unc. Raw	3.31	27.27	4.65	0.020
s.e.m.	0.29	0.62	0.23	0.000
LT Zn	2.99	38.83	4.96	0.017
s.e.m.	0.29	1.85	0.13	0.003
LT Cu	3.24	32.40	5.82	0.020
s.e.m.	0.18	0.06	0.15	0.000
LT Cd	3.48	29.53	4.82	0.067
s.e.m.	0.40	0.97	0.10	0.009

Site 4. Pwllpeiran

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	4.40	25.40	5.71	0.025
s.e.m.	0.34	1.03	0.16	0.007
Unc. Digested	4.67	30.12	7.33	0.038
s.e.m.	0.14	1.26	0.15	0.009
Unc. Raw	5.26	39.83	15.70	0.062
s.e.m.	0.36	4.72	8.00	0.025
Zn rate 1	5.12	36.34	7.97	0.028
s.e.m.	0.54	1.88	1.10	0.002
Zn rate 2	5.33	42.70	8.79	0.025
s.e.m.	0.69	4.75	1.55	0.000
Zn rate 3	4.95	43.40	6.41	0.040
s.e.m.	0.36	0.95	0.22	0.005
Zn rate 4	4.90	44.53	6.04	0.025
s.e.m.	0.11	1.94	0.56	0.005
Cu rate 1	4.45	33.70	7.50	0.020
s.e.m.	0.56	1.94	0.37	0.005
Cu rate 2	5.64	34.90	11.75	0.028
s.e.m.	0.20	2.16	2.98	0.003
Cu rate 3	5.83	29.67	8.09	0.043
s.e.m.	0.18	1.22	0.59	0.024
Cu rate 4	5.07	29.63	6.68	0.040
s.e.m.	0.54	2.42	0.60	0.005
Cd rate 1	4.47	30.37	7.34	0.037
s.e.m.	0.35	0.95	0.24	0.007
Cd rate 2	4.73	36.07	7.33	0.047
s.e.m.	0.22	3.48	0.78	0.009
Cd rate 3	5.15	38.70	6.99	0.087
s.e.m.	0.14	0.71	0.92	0.007
Cd rate 4	4.67	42.63	17.22	0.082
s.e.m.	0.46	7.04	9.89	0.002
LT Unc. Digested	4.74	28.90	6.39	0.031
s.e.m.	0.35	0.46	0.41	0.004
LT Unc. Raw	4.97	29.43	6.63	0.040
s.e.m.	0.13	1.96	0.31	0.003
LT Zn	4.39	39.50	8.75	0.045
s.e.m.	0.11	1.40	1.10	0.014
LT Cu	4.93	30.43	11.82	0.017
s.e.m.	0.15	6.11	5.44	0.004
LT Cd	4.42	26.67	5.60	0.087
s.e.m.	0.21	1.55	0.26	0.018

Site 5. Rosemaund

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	10.97	33.42	4.76	0.038
s.e.m.	0.27	4.88	0.69	0.007
Unc. Digested	10.96	50.58	4.96	0.054
s.e.m.	0.24	2.09	0.30	0.012
Unc. Raw	10.75	36.40	4.77	0.033
s.e.m.	1.79	2.23	0.22	0.004
Zn rate 1	10.87	55.47	4.40	0.063
s.e.m.	0.74	4.82	0.51	0.012
Zn rate 2	9.70	98.27	6.74	0.138
s.e.m.	0.74	6.15	0.63	0.031
Zn rate 3	10.01	84.43	5.98	0.120
s.e.m.	0.42	9.43	0.42	0.015
Zn rate 4	10.66	106.00	6.58	0.148
s.e.m.	0.19	2.00	0.50	0.004
Cu rate 1	10.67	46.60	6.63	0.050
s.e.m.	0.33	2.62	0.30	0.006
Cu rate 2	11.40	41.30	5.45	0.050
s.e.m.	0.43	2.43	0.33	0.008
Cu rate 3	10.55	43.27	6.40	0.045
s.e.m.	1.22	2.67	0.14	0.005
Cu rate 4	10.11	51.10	6.42	0.060
s.e.m.	2.02	1.55	0.19	0.003
Cd rate 1	11.08	48.73	4.89	0.133
s.e.m.	0.56	1.35	0.15	0.016
Cd rate 2	9.73	48.23	5.40	0.175
s.e.m.	0.29	2.90	0.02	0.023
Cd rate 3	10.41	46.87	5.32	0.237
s.e.m.	1.36	6.13	0.39	0.050
Cd rate 4	10.34	42.97	5.26	0.257
s.e.m.	0.99	2.48	0.29	0.055
LT Unc. Digested	12.42	40.18	4.89	0.049
s.e.m.	0.24	0.83	0.17	0.004
LT Unc. Raw	12.45	34.10	4.89	0.038
s.e.m.	0.42	0.95	0.31	0.003
LT Zn	10.04	65.40	5.85	0.058
s.e.m.	0.91	7.77	0.65	0.016
LT Cu	10.05	43.17	5.85	0.048
s.e.m.	0.28	3.03	0.42	0.004
LT Cd	11.44	38.27	4.77	0.117
s.e.m.	0.85	4.70	0.61	0.019

Site 6. Bridgets

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	3.63	25.97	4.76	0.088
s.e.m.	0.09	0.15	0.01	0.014
Unc. Digested	4.09	30.08	5.16	0.094
s.e.m.	0.16	0.86	0.01	0.002
Unc. Raw	4.01	34.13	5.14	0.082
s.e.m.	0.42	1.88	0.04	0.004
Zn rate 1	3.97	45.90	6.02	0.117
s.e.m.	0.29	2.66	0.25	0.012
Zn rate 2	4.15	54.43	5.88	0.102
s.e.m.	0.12	5.84	0.22	0.014
Zn rate 3	3.91	67.13	5.96	0.122
s.e.m.	0.08	2.26	0.29	0.007
Zn rate 4	4.14	62.23	5.75	0.105
s.e.m.	0.16	3.68	0.34	0.005
Cu rate 1	4.38	27.67	5.48	0.098
s.e.m.	0.48	0.78	0.14	0.016
Cu rate 2	4.23	31.57	5.46	0.107
s.e.m.	0.19	2.28	0.39	0.016
Cu rate 3	3.82	34.53	6.16	0.075
s.e.m.	0.04	2.53	0.35	0.005
Cu rate 4	4.21	34.87	6.36	0.118
s.e.m.	0.12	1.55	0.20	0.012
Cd rate 1	3.85	29.43	5.29	0.115
s.e.m.	0.23	1.10	0.14	0.005
Cd rate 2	4.45	32.13	5.37	0.120
s.e.m.	0.22	1.66	0.09	0.020
Cd rate 3	3.89	33.20	5.38	0.140
s.e.m.	0.07	2.87	0.13	0.013
Cd rate 4	4.49	37.10	5.70	0.193
s.e.m.	0.39	1.91	0.25	0.035
LT Unc. Digested	4.23	24.82	5.15	0.090
s.e.m.	0.15	1.29	0.14	0.008
LT Unc. Raw	3.85	28.43	5.09	0.080
s.e.m.	0.24	1.27	0.16	0.000
LT Zn	4.40	40.40	5.40	0.100
s.e.m.	0.43	2.51	0.24	0.006
LT Cu	3.83	31.17	5.95	0.085
s.e.m.	0.10	2.31	0.20	0.003
LT Cd	3.75	28.93	4.79	0.100
s.e.m.	0.12	3.37	0.40	0.015

Site 7. Hartwood

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	1.32	16.21	3.86	0.042
s.e.m.	0.10	1.73	0.56	0.009
Unc. Digested	1.78	24.70	5.49	0.028
s.e.m.	0.23	1.28	0.53	0.003
Unc. Raw	1.55	23.17	5.39	0.031
s.e.m.	0.33	3.61	1.65	0.007
Zn rate 1	1.63	31.88	5.71	0.024
s.e.m.	0.44	2.87	0.83	0.004
Zn rate 2	1.97	57.84	7.33	0.031
s.e.m.	0.28	10.90	0.92	0.002
Zn rate 3	1.65	76.86	7.01	0.041
s.e.m.	0.34	5.36	0.71	0.019
Zn rate 4	1.45	155.57	8.71	0.042
s.e.m.	0.03	30.83	0.73	0.010
Cu rate 1	2.24	23.57	5.51	0.019
s.e.m.	0.56	3.01	1.05	0.002
Cu rate 2	1.52	29.75	7.70	0.025
s.e.m.	0.21	4.00	1.49	0.006
Cu rate 3	1.46	39.61	7.69	0.055
s.e.m.	0.35	5.90	1.41	0.021
Cu rate 4	1.74	35.60	9.29	0.062
s.e.m.	0.34	0.88	0.24	0.048
Cd rate 1	2.64	30.57	6.08	0.066
s.e.m.	0.31	1.69	0.57	0.012
Cd rate 2	2.21	31.99	6.47	0.088
s.e.m.	0.28	1.92	0.61	0.020
Cd rate 3	1.50	33.12	6.40	0.178
s.e.m.	0.34	3.11	0.76	0.058
Cd rate 4	2.19	39.44	8.46	0.682
s.e.m.	0.33	5.69	0.71	0.442
LT Unc. Digested	1.66	19.32	5.41	0.021
s.e.m.	0.16	0.35	0.37	0.001
LT Unc. Raw	1.72	18.60	5.14	0.031
s.e.m.	0.33	2.21	1.04	0.005
LT Zn	1.73	40.98	7.08	0.156
s.e.m.	0.18	4.24	1.32	0.130
LT Cu	1.06	16.08	3.32	0.032
s.e.m.	0.05	1.20	0.52	0.008
LT Cd	1.61	17.26	3.33	0.107
s.e.m.	0.29	1.14	0.53	0.026

Site 8. Auchincruive

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	7.49	30.93	5.73	0.426
s.e.m.	0.07	1.98	0.16	0.367
Unc. Digested	6.86	48.78	7.23	0.268
s.e.m.	0.49	2.98	0.75	0.167
Unc. Raw	6.88	37.40	6.59	0.418
s.e.m.	0.28	4.50	0.83	0.372
Zn rate 1	6.31	72.63	7.27	0.418
s.e.m.	0.49	3.77	1.10	0.372
Zn rate 2	6.82	127.00	8.50	0.120
s.e.m.	0.43	8.02	0.04	0.015
Zn rate 3	6.48	165.00	9.19	0.103
s.e.m.	0.58	11.53	0.62	0.015
Zn rate 4	6.04	268.33	10.02	0.460
s.e.m.	0.11	23.13	1.07	0.350
Cu rate 1	6.27	45.13	7.43	0.117
s.e.m.	0.08	3.13	0.53	0.028
Cu rate 2	7.42	47.70	7.44	0.062
s.e.m.	0.22	3.09	0.84	0.031
Cu rate 3	6.44	65.23	9.65	0.080
s.e.m.	0.48	4.74	1.45	0.017
Cu rate 4	6.51	65.30	11.43	0.453
s.e.m.	0.22	1.56	0.43	0.354
Cd rate 1	6.55	50.00	7.30	0.537
s.e.m.	0.41	4.16	0.31	0.318
Cd rate 2	6.09	59.13	7.31	0.317
s.e.m.	0.30	2.99	0.45	0.055
Cd rate 3	6.31	61.70	7.66	0.677
s.e.m.	0.73	3.17	0.22	0.245
Cd rate 4	6.45	58.63	7.40	0.233
s.e.m.	0.12	7.80	0.86	0.059
LT Unc. Digested	6.98	36.10	5.73	0.228
s.e.m.	0.27	4.86	0.62	0.184
LT Unc. Raw	6.71	30.60	5.96	0.067
s.e.m.	0.33	1.25	0.10	0.003
LT Zn	6.46	69.67	7.73	0.427
s.e.m.	0.18	1.87	0.19	0.367
LT Cu	6.86	38.93	7.29	0.402
s.e.m.	0.20	1.92	0.07	0.379
LT Cd	7.21	33.50	5.79	0.463
s.e.m.	0.44	1.34	0.52	0.348

Site 9. Shirburn

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	1.87	48.87	7.30	0.037
s.e.m..	0.09	4.17	0.30	0.012
Unc. Digested	1.78	37.00	6.08	0.030
s.e.m..	0.22	5.42	0.27	0.000
Unc. Raw	1.61	35.30	6.10	0.030
s.e.m..	0.46	1.56	0.36	0.000
High Unc. Digested	1.54	26.67	5.91	0.027
s.e.m..	0.24	2.18	0.56	0.003
High Unc. Raw	1.91	31.47	6.61	0.027
s.e.m..	0.27	1.53	0.16	0.003
Zn rate 1	1.43	38.53	6.14	0.027
s.e.m..	0.47	2.14	0.14	0.003
Zn rate 2	1.45	43.37	6.54	0.067
s.e.m..	0.18	1.79	0.13	0.042
Zn rate 3	1.73	51.60	7.38	0.033
s.e.m..	0.16	5.16	0.77	0.003
Zn rate 4	1.76	51.70	7.03	0.033
s.e.m..	0.30	2.80	0.29	0.003
Zn rate 5	1.89	51.27	6.50	0.040
s.e.m..	0.01	0.90	0.09	0.006
Cu rate 1	1.62	34.60	6.91	0.030
s.e.m.	0.32	3.29	0.44	0.000
Cu rate 2	1.26	39.43	7.09	0.043
s.e.m.	0.32	1.69	0.44	0.003
Cu rate 3	1.66	43.90	6.88	0.037
s.e.m.	0.30	6.22	0.80	0.007
Cu rate 4	2.00	39.60	7.68	0.040
s.e.m.	0.21	2.60	0.55	0.000
Cu rate 5	1.31	36.07	6.97	0.033
s.e.m.	0.15	5.90	1.07	0.007
Cd rate 1	1.82	40.77	6.71	0.063
s.e.m.	0.19	3.53	0.26	0.003
Cd rate 2	1.45	42.03	6.65	0.097
s.e.m.	0.36	2.06	0.21	0.003
Cd rate 3	2.05	38.17	6.12	0.107
s.e.m.	0.22	1.45	0.23	0.003
Cd rate 4	1.45	44.57	5.70	0.177
s.e.m.	0.39	13.57	1.01	0.074
Cd rate 5	1.79	47.33	6.71	0.117
s.e.m.	0.13	2.13	0.25	0.039
LT Unc. Digested	1.92	40.37	5.71	0.040
s.e.m.	0.22	1.73	0.15	0.000
LT Unc. Raw	2.37	53.43	6.81	0.047
s.e.m.	0.60	8.98	1.11	0.012
LT Zn	2.35	48.93	6.02	0.067
s.e.m.	0.11	5.56	0.23	0.032
LT Cu	2.14	44.23	7.19	0.027
s.e.m.	0.19	2.18	0.15	0.003
LT Cd	1.92	40.10	6.55	0.080
s.e.m.	0.28	0.21	0.26	0.006

APPENDIX J Crop yield and metal concentrations: Sludge cake sites - 2004

Site 1. Gleadthorpe

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	7.72	36.52	4.40	0.300
s.e.m.	0.41	8.11	0.41	0.057
Unc. Digested	9.72	86.80	5.84	0.327
s.e.m.	0.29	35.35	0.23	0.037
Unc. Raw	9.79	67.20	5.06	0.387
s.e.m.	0.44	30.45	0.74	0.049
Zn rate 1	10.02	74.73	6.31	0.347
s.e.m.	0.24	13.36	0.45	0.029
Zn rate 2	9.58	128.97	6.46	0.235
s.e.m.	0.68	23.54	0.12	0.063
Zn rate 3	9.88	103.33	6.91	0.392
s.e.m.	0.97	26.73	0.59	0.074
Zn rate 4	10.58	108.00	6.04	0.403
s.e.m.	0.42	24.68	0.42	0.020
Cu rate 1	9.52	41.97	5.28	0.292
s.e.m.	0.89	5.30	0.51	0.019
Cu rate 2	9.51	56.43	6.23	0.360
s.e.m.	0.88	8.69	0.07	0.078
Cu rate 3	9.28	60.80	6.62	0.422
s.e.m.	0.52	14.63	1.07	0.105
Cu rate 4	8.92	84.77	7.34	0.375
s.e.m.	1.29	14.57	1.17	0.003
Cd rate 1	9.27	45.33	4.94	0.387
s.e.m.	0.94	5.04	0.40	0.097
Cd rate 2	10.49	80.43	6.19	0.535
s.e.m.	0.37	18.35	0.16	0.162
Cd rate 3	10.22	58.27	6.14	0.347
s.e.m.	0.93	5.59	0.64	0.027
Cd rate 4	9.66	59.67	5.72	0.407
s.e.m.	0.33	6.48	0.10	0.024
LT Unc. Digested	10.07	38.68	4.43	0.368
s.e.m.	0.22	4.09	0.27	0.067
LT Unc. Raw	9.15	39.53	4.88	0.355
s.e.m.	0.06	0.09	0.55	0.063
LT Zn	9.01	70.93	5.60	0.328
s.e.m.	0.17	30.86	1.21	0.072
LT Cu	9.19	56.60	6.22	0.353
s.e.m.	1.20	7.16	0.57	0.028
LT Cd	9.76	52.20	6.05	0.530
s.e.m.	0.43	2.08	0.29	0.124

Site 2. Woburn

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	4.78	41.88	4.89	0.12
s.e.m.	0.288	2.829	0.258	0.028
Unc. Digested	5.78	51.55	6.12	0.14
s.e.m.	0.178	2.458	0.241	0.012
Unc. Raw	6.06	44.47	6.33	0.07
s.e.m.	0.785	0.597	0.079	0.006
Zn rate 1	6.41	76.02	6.73	0.11
s.e.m.	0.676	7.226	0.378	0.014
Zn rate 2	6.07	117.61	7.22	0.10
s.e.m.	0.269	4.606	0.635	0.012
Zn rate 3	7.14	100.29	6.81	0.08
s.e.m.	0.225	4.704	0.253	0.007
Zn rate 4	6.51	134.38	7.40	0.13
s.e.m.	0.917	7.803	0.921	0.014
Cu rate 1	5.93	52.07	7.12	0.09
s.e.m.	0.507	1.929	0.15	0.012
Cu rate 2	5.39	55.30	7.44	0.08
s.e.m.	0.369	2.673	0.454	0.003
Cu rate 3	6.11	58.18	8.58	0.08
s.e.m.	0.575	3.100	0.659	0.009
Cu rate 4	6.96	63.56	7.92	0.09
s.e.m.	0.418	3.091	0.255	0.025
Cd rate 1	5.86	51.28	6.60	0.25
s.e.m.	0.314	4.272	0.668	0.101
Cd rate 2	5.89	57.98	6.37	0.38
s.e.m.	0.205	2.832	0.683	0.062
Cd rate 3	5.89	62.89	6.93	0.39
s.e.m.	0.515	4.834	0.142	0.112
Cd rate 4	6.23	56.19	6.33	0.53
s.e.m.	0.624	0.854	0.405	0.09
LT Unc. Digested	6.61	27.40	4.71	0.041
s.e.m.	0.33	0.57	0.07	0.004
LT Unc. Raw	6.29	25.92	4.25	0.053
s.e.m.	0.33	1.55	0.26	0.022
LT Zn	6.49	41.18	4.92	0.046
s.e.m.	0.25	3.09	0.29	0.004
LT Cu	5.54	31.52	5.79	0.045
s.e.m.	1.01	3.13	1.08	0.011
LT Cd	6.62	27.03	4.40	0.046
s.e.m.	0.21	1.17	0.43	0.004

Site 3. Watlington

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	4.56	16.83	3.98	0.050
s.e.m.	0.21	0.35	0.12	0.000
Unc. Digested	6.20	28.03	6.06	0.057
s.e.m.	0.94	2.29	0.60	0.003
Unc. Raw	5.54	23.83	5.54	0.047
s.e.m.	0.57	1.90	0.29	0.007
Zn rate 1	5.98	41.20	5.91	0.080
s.e.m.	0.82	1.83	0.16	0.006
Zn rate 2	6.04	63.40	6.31	0.120
s.e.m.	0.49	0.76	0.05	0.010
Zn rate 3	6.22	77.03	6.97	0.140
s.e.m.	0.69	8.01	0.15	0.031
Zn rate 4	5.36	86.83	7.29	0.127
s.e.m.	0.60	5.08	0.30	0.009
Cu rate 1	5.04	30.80	6.91	0.060
s.e.m.	0.47	1.67	0.31	0.006
Cu rate 2	5.89	28.70	6.53	0.053
s.e.m.	0.88	2.20	0.13	0.009
Cu rate 3	5.22	32.13	6.49	0.110
s.e.m.	0.30	1.82	0.69	0.065
Cu rate 4	5.39	35.00	7.28	0.063
s.e.m.	0.42	1.08	0.17	0.013
Cd rate 1	6.04	27.20	5.18	0.103
s.e.m.	0.66	1.80	0.41	0.007
Cd rate 2	5.38	29.07	5.52	0.127
s.e.m.	1.08	3.80	0.44	0.067
Cd rate 3	5.77	35.23	5.59	0.270
s.e.m.	0.69	1.23	0.17	0.032
Cd rate 4	5.70	36.53	5.76	0.330
s.e.m.	0.27	1.62	0.16	0.038
LT Unc. Digested	5.33	21.97	4.82	0.040
s.e.m.	0.31	0.82	0.05	0.000
LT Unc. Raw	4.94	18.57	4.47	0.030
s.e.m.	0.12	1.22	0.11	0.000
LT Zn	5.41	48.20	5.12	0.080
s.e.m.	0.36	2.95	0.26	0.015
LT Cu	5.41	24.63	5.15	0.097
s.e.m.	0.42	1.83	0.09	0.057
LT Cd	5.04	25.50	4.59	0.177
s.e.m.	0.28	1.03	0.23	0.012

Site 4. Pwllpeiran

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	3.77	28.65	7.32	0.055
s.e.m.	0.38	1.57	0.15	0.001
Unc. Digested	3.39	31.70	8.51	0.103
s.e.m.	0.41	4.61	0.23	0.048
Unc. Raw	4.32	28.97	9.28	0.052
s.e.m.	0.17	1.05	1.39	0.013
Zn rate 1	3.75	44.50	8.75	0.105
s.e.m.	1.11	5.96	0.53	0.023
Zn rate 2	4.29	51.23	8.27	0.120
s.e.m.	0.33	8.82	0.59	0.038
Zn rate 3	4.97	88.57	9.31	0.198
s.e.m.	0.51	12.88	0.08	0.030
Zn rate 4	4.14	63.97	9.62	0.147
s.e.m.	0.81	9.37	0.96	0.032
Cu rate 1	4.41	37.00	8.84	0.058
s.e.m.	0.80	2.22	0.51	0.012
Cu rate 2	4.30	40.67	11.60	0.082
s.e.m.	0.61	2.69	0.89	0.014
Cu rate 3	4.18	43.37	8.77	0.095
s.e.m.	0.06	5.02	0.91	0.013
Cu rate 4	4.20	35.83	10.83	0.062
s.e.m.	0.81	1.53	0.80	0.019
Cd rate 1	3.74	43.17	7.84	0.152
s.e.m.	0.72	5.53	0.15	0.028
Cd rate 2	4.29	33.93	7.81	0.158
s.e.m.	0.28	3.93	1.16	0.069
Cd rate 3	3.76	46.00	9.36	0.297
s.e.m.	0.30	4.80	0.69	0.058
Cd rate 4	4.28	47.00	8.41	0.245
s.e.m.	0.39	6.07	0.30	0.118
LT Unc. Digested	4.17	25.30	6.92	0.041
s.e.m.	0.13	2.27	0.15	0.003
LT Unc. Raw	3.37	30.10	7.87	0.073
s.e.m.	0.26	3.23	0.39	0.022
LT Zn	3.93	48.70	8.13	0.110
s.e.m.	0.42	5.14	0.36	0.053
LT Cu	4.28	37.37	7.90	0.045
s.e.m.	0.66	6.65	1.00	0.018
LT Cd	3.81	38.50	7.69	0.157
s.e.m.	0.52	5.25	0.47	0.016

Site 5. Rosemaund

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	5.54	33.65	4.01	0.046
s.e.m.	0.68	5.65	0.17	0.003
Unc. Digested	6.36	43.42	4.97	0.064
s.e.m.	1.11	5.42	0.49	0.009
Unc. Raw	4.96	32.23	4.67	0.075
s.e.m.	0.73	4.20	1.22	0.025
Zn rate 1	5.91	57.50	4.43	0.067
s.e.m.	1.50	1.05	0.40	0.037
Zn rate 2	5.95	76.80	5.05	0.084
s.e.m.	1.11	8.77	0.75	0.041
Zn rate 3	5.43	105.20	5.28	0.075
s.e.m.	1.47	10.62	0.11	0.016
Zn rate 4	4.68	140.67	4.64	0.080
s.e.m.	1.45	14.67	0.48	0.013
Cu rate 1	5.83	37.47	4.15	0.052
s.e.m.	1.84	6.31	0.41	0.022
Cu rate 2	5.55	58.77	5.62	0.067
s.e.m.	0.45	9.92	0.34	0.020
Cu rate 3	7.11	43.80	5.49	0.050
s.e.m.	2.14	4.47	0.06	0.008
Cu rate 4	5.42	48.60	5.59	0.043
s.e.m.	0.98	0.74	0.19	0.002
Cd rate 1	6.58	37.30	4.35	0.169
s.e.m.	0.08	5.80	0.39	0.041
Cd rate 2	6.50	42.47	4.99	0.158
s.e.m.	0.14	2.31	0.27	0.007
Cd rate 3	8.57	46.20	4.95	0.200
s.e.m.	1.54	3.77	0.33	0.015
Cd rate 4	7.69	48.90	4.71	0.175
s.e.m.	2.53	10.57	0.41	0.084
LT Unc. Digested	6.50	31.22	4.63	0.043
s.e.m.	0.65	0.39	0.11	0.008
LT Unc. Raw	7.37	30.03	4.65	0.058
s.e.m.	1.78	3.44	0.34	0.013
LT Zn	6.02	54.77	5.10	0.048
s.e.m.	0.73	2.27	0.38	0.009
LT Cu	5.98	48.53	4.34	0.127
s.e.m.	0.64	7.08	0.30	0.050
LT Cd	5.78	35.47	4.33	0.105
s.e.m.	0.37	2.27	0.16	0.053

Site 6. Bridgets

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	4.28	21.38	6.21	0.196
s.e.m.	0.29	0.51	0.11	0.002
Unc. Digested	4.26	24.70	8.12	0.181
s.e.m.	0.39	0.97	0.28	0.014
Unc. Raw	4.08	25.00	8.57	0.115
s.e.m.	0.63	2.14	0.19	0.040
Zn rate 1	4.98	38.17	8.65	0.208
s.e.m.	0.67	0.28	0.47	0.038
Zn rate 2	6.16	52.93	8.79	0.171
s.e.m.	0.74	1.71	0.21	0.016
Zn rate 3	6.03	64.07	8.41	0.213
s.e.m.	0.68	4.46	0.53	0.032
Zn rate 4	6.13	85.17	8.91	0.285
s.e.m.	0.78	9.73	0.22	0.026
Cu rate 1	5.52	25.83	8.97	0.175
s.e.m.	0.33	0.38	0.17	0.028
Cu rate 2	4.05	30.83	9.40	0.232
s.e.m.	0.50	0.77	0.76	0.074
Cu rate 3	4.54	31.37	10.58	0.187
s.e.m.	0.93	1.51	0.61	0.017
Cu rate 4	4.29	32.30	10.53	0.170
s.e.m.	0.51	0.89	0.47	0.021
Cd rate 1	4.52	25.00	8.87	0.279
s.e.m.	0.30	1.25	0.39	0.035
Cd rate 2	5.11	26.60	8.56	0.243
s.e.m.	0.93	1.06	0.79	0.040
Cd rate 3	4.97	28.40	8.36	0.240
s.e.m.	0.38	1.10	0.85	0.029
Cd rate 4	5.01	30.33	7.98	0.268
s.e.m.	1.06	1.79	0.33	0.017
LT Unc. Digested	4.77	23.00	7.40	0.199
s.e.m.	0.33	1.62	0.33	0.026
LT Unc. Raw	3.41	23.20	7.14	0.211
s.e.m.	0.03	2.40	0.41	0.015
LT Zn	3.98	41.70	7.98	0.264
s.e.m.	0.25	6.72	0.20	0.079
LT Cu	4.60	24.53	8.42	0.220
s.e.m.	0.32	1.73	0.36	0.023
LT Cd	4.41	26.30	6.90	0.285
s.e.m.	0.13	1.61	0.07	0.045

Site 7. Hartwood

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	1.83	23.17	4.18	0.034
s.e.m.	0.22	0.91	0.18	0.007
Unc. Digested	2.27	32.28	5.76	0.034
s.e.m.	0.21	4.10	0.73	0.009
Unc. Raw	1.58	25.54	5.34	0.029
s.e.m.	0.05	2.56	0.12	0.010
Zn rate 1	1.60	44.44	4.78	0.030
s.e.m.	0.15	3.16	0.56	0.005
Zn rate 2	2.76	52.37	5.49	0.038
s.e.m.	0.55	8.64	0.09	0.013
Zn rate 3	2.00	77.99	6.54	0.047
s.e.m.	0.49	10.88	0.34	0.020
Zn rate 4	2.71	107.55	5.94	0.066
s.e.m.	0.69	14.36	0.46	0.039
Cu rate 1	1.69	31.41	5.62	0.028
s.e.m.	0.01	2.49	0.68	0.006
Cu rate 2	1.82	33.91	6.14	0.041
s.e.m.	0.23	0.74	0.54	0.005
Cu rate 3	2.21	38.31	6.81	0.042
s.e.m.	0.30	1.32	0.51	0.006
Cu rate 4	2.45	37.12	6.93	0.028
s.e.m.	0.19	3.64	0.94	0.007
Cd rate 1	2.08	31.19	5.03	0.066
s.e.m.	0.05	3.01	0.07	0.014
Cd rate 2	2.20	34.24	5.52	0.083
s.e.m.	0.20	3.17	0.11	0.029
Cd rate 3	2.15	39.62	5.67	0.164
s.e.m.	0.27	4.26	0.31	0.018
Cd rate 4	1.84	40.31	7.09	0.163
s.e.m.	0.92	4.93	0.67	0.053
LT Unc. Digested	1.53	22.25	4.55	0.031
s.e.m.	0.08	0.71	0.20	0.009
LT Unc. Raw	1.85	23.94	4.90	0.036
s.e.m.	0.52	0.79	0.19	0.011
LT Zn	2.11	38.07	5.02	0.040
s.e.m.	0.45	1.28	0.54	0.003
LT Cu	1.92	26.19	4.67	0.035
s.e.m.	0.61	2.05	0.24	0.016
LT Cd	1.78	26.91	4.35	0.083
s.e.m.	0.25	1.12	0.15	0.006

Site 8. Auchincruive

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	9.28	25.27	4.53	0.050
s.e.m.	0.17	4.27	0.01	0.000
Unc. Digested	8.57	43.88	6.20	0.050
s.e.m.	0.31	2.14	0.51	0.000
Unc. Raw	7.93	34.63	6.26	0.050
s.e.m.	0.40	4.78	0.48	0.000
Zn rate 1	8.19	79.63	6.98	0.050
s.e.m.	0.57	4.75	0.20	0.000
Zn rate 2	8.33	124.67	7.10	0.077
s.e.m.	0.60	5.61	0.42	0.027
Zn rate 3	8.44	185.33	8.14	0.050
s.e.m.	0.59	26.85	0.14	0.000
Zn rate 4	8.31	290.67	9.29	0.107
s.e.m.	0.44	49.97	0.70	0.057
Cu rate 1	8.70	38.37	6.55	0.050
s.e.m.	0.40	1.27	0.17	0.000
Cu rate 2	8.20	43.83	7.01	0.050
s.e.m.	0.61	3.55	0.55	0.000
Cu rate 3	8.86	74.20	9.07	0.050
s.e.m.	0.16	1.08	0.21	0.000
Cu rate 4	7.71	75.73	10.45	0.050
s.e.m.	0.44	10.31	0.63	0.000
Cd rate 1	9.09	42.40	6.03	0.090
s.e.m.	0.38	2.40	0.26	0.040
Cd rate 2	8.46	56.33	6.62	0.120
s.e.m.	0.25	4.44	0.23	0.012
Cd rate 3	8.28	56.77	6.61	0.313
s.e.m.	0.41	5.39	0.49	0.047
Cd rate 4	8.39	51.67	6.55	0.193
s.e.m.	0.01	3.18	0.27	0.048
LT Unc. Digested	9.14	26.85	5.12	0.050
s.e.m.	0.21	4.15	0.54	0.000
LT Unc. Raw	8.59	25.07	5.53	0.050
s.e.m.	0.36	2.07	0.94	0.000
LT Zn	8.80	66.77	6.36	0.050
s.e.m.	0.36	4.91	0.52	0.000
LT Cu	8.85	28.47	6.18	0.050
s.e.m.	0.29	1.85	0.51	0.000
LT Cd	8.79	28.70	4.35	0.073
s.e.m.	0.48	3.14	0.41	0.023

Site 9. Shirburn

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	7.84	11.83	2.65	0.030
s.e.m.	0.21	0.37	0.24	0.000
Unc. Digested	7.38	17.00	3.91	0.033
s.e.m.	0.47	4.06	0.87	0.003
Unc. Raw	7.65	15.53	3.27	0.030
s.e.m.	0.34	1.19	0.32	0.000
High Unc. Digested	7.95	15.77	3.90	0.033
s.e.m.	0.43	1.79	0.27	0.003
High Unc. Raw	6.78	16.50	3.46	0.033
s.e.m.	0.35	0.44	0.20	0.009
Zn rate 1	8.26	23.13	3.97	0.037
s.e.m.	0.54	0.54	0.33	0.003
Zn rate 2	7.63	25.00	3.82	0.050
s.e.m.	0.04	0.55	0.19	0.006
Zn rate 3	7.98	27.20	3.89	0.053
s.e.m.	0.24	3.79	0.51	0.009
Zn rate 4	8.49	28.83	3.90	0.053
s.e.m.	0.13	0.52	0.26	0.003
Zn rate 5	6.95	35.90	4.48	0.067
s.e.m.	0.50	0.50	0.26	0.003
Cu rate 1	7.84	14.70	3.51	0.030
s.e.m.	0.20	0.25	0.09	0.006
Cu rate 2	7.03	18.83	4.98	0.040
s.e.m.	0.26	2.37	0.36	0.000
Cu rate 3	7.06	16.43	4.54	0.043
s.e.m.	0.27	0.55	0.39	0.013
Cu rate 4	7.26	14.73	4.18	0.033
s.e.m.	0.28	3.34	0.98	0.009
Cu rate 5	7.53	10.22	2.71	0.020
s.e.m.	0.02	0.73	0.17	0.000
Cd rate 1	7.80	11.37	2.20	0.047
s.e.m.	0.37	1.52	0.21	0.003
Cd rate 2	7.05	13.14	2.63	0.083
s.e.m.	0.05	1.85	0.50	0.015
Cd rate 3	8.10	10.04	1.84	0.077
s.e.m.	0.32	0.41	0.09	0.007
Cd rate 4	7.57	10.26	2.07	0.090
s.e.m.	0.31	0.85	0.14	0.006
Cd rate 5	7.50	13.38	2.43	0.097
s.e.m.	0.45	3.54	0.63	0.027
LT Unc. Digested	7.53	9.21	2.05	0.02
s.e.m.	0.33	0.86	0.11	0.00
LT Unc. Raw	7.21	7.32	1.62	0.02
s.e.m.	0.62	0.65	0.06	0.00
LT Zn	7.66	14.27	2.02	0.02
s.e.m.	0.34	0.35	0.18	0.00
LT Cu	7.23	9.61	2.10	0.02
s.e.m.	0.68	1.05	0.17	0.00
LT Cd	7.73	10.09	2.09	0.08
s.e.m.	0.75	1.39	0.24	0.00

APPENDIX K Crop yield and metal concentrations: Sludge cake sites - 2005

Site 1. Gleadthorpe

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	3.13	30.88	4.66	0.039
s.e.m.	0.20	0.58	0.11	0.003
Unc. Digested	3.19	37.05	5.77	0.053
s.e.m.	0.23	2.13	0.05	0.009
Unc. Raw	3.14	37.03	5.84	0.288
s.e.m.	0.15	1.13	0.27	0.231
Zn rate 1	4.29	42.43	5.83	0.062
s.e.m.	0.22	1.28	0.19	0.012
Zn rate 2	3.92	49.57	6.27	0.075
s.e.m.	0.81	0.97	0.22	0.014
Zn rate 3	3.10	57.07	6.19	0.093
s.e.m.	0.66	4.54	0.32	0.016
Zn rate 4	4.60	53.80	6.55	0.093
s.e.m.	0.29	0.60	0.05	0.016
Cu rate 1	3.52	33.63	5.87	0.040
s.e.m.	0.58	2.03	0.20	0.005
Cu rate 2	3.72	41.37	6.52	0.048
s.e.m.	0.67	3.43	0.10	0.012
Cu rate 3	3.64	40.30	6.59	0.195
s.e.m.	0.87	3.45	0.17	0.153
Cu rate 4	2.62	40.60	6.46	0.055
s.e.m.	0.42	3.72	0.12	0.010
Cd rate 1	3.02	39.13	5.98	0.113
s.e.m.	0.29	1.03	0.34	0.010
Cd rate 2	2.91	42.73	5.85	0.202
s.e.m.	0.37	1.18	0.17	0.020
Cd rate 3	4.42	38.63	5.92	0.185
s.e.m.	0.17	2.31	0.32	0.015
Cd rate 4	3.29	39.60	6.14	0.222
s.e.m.	0.42	2.06	0.12	0.038
LT Unc. Digested	3.51	36.18	5.60	0.055
s.e.m.	0.43	1.26	0.18	0.004
LT Unc. Raw	3.56	32.47	4.99	0.042
s.e.m.	0.14	0.35	0.13	0.002
LT Zn	3.59	47.73	6.02	0.058
s.e.m.	1.17	2.74	0.12	0.012
LT Cu	3.31	38.30	5.98	0.042
s.e.m.	0.33	1.59	0.24	0.002
LT Cd	3.62	37.70	5.64	0.100
s.e.m.	0.61	2.26	0.38	0.049

Site 2. Woburn

Treatment	Grain yield* t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	-	34.84	3.84	0.038
s.e.m.	-	2.24	0.15	0.003
Unc. Digested	-	47.61	5.48	0.060
s.e.m.	-	8.02	0.52	0.008
Unc. Raw	-	38.06	4.92	0.031
s.e.m.	-	3.72	0.12	0.003
Zn rate 1	-	56.22	5.29	0.066
s.e.m.	-	0.67	0.09	0.005
Zn rate 2	-	61.70	5.19	0.083
s.e.m.	-	8.01	0.50	0.015
Zn rate 3	-	74.33	5.61	0.086
s.e.m.	-	6.46	0.33	0.013
Zn rate 4	-	94.25	6.40	0.129
s.e.m.	-	16.76	0.58	0.035
Cu rate 1	-	33.30	5.25	0.033
s.e.m.	-	3.72	0.44	0.004
Cu rate 2	-	34.96	5.55	0.029
s.e.m.	-	4.20	0.41	0.005
Cu rate 3	-	49.13	6.35	0.048
s.e.m.	-	7.05	0.43	0.012
Cu rate 4	-	47.46	6.45	0.044
s.e.m.	-	4.13	0.17	0.004
Cd rate 1	-	53.42	5.60	0.160
s.e.m.	-	4.94	0.33	0.010
Cd rate 2	-	42.94	5.19	0.200
s.e.m.	-	5.39	0.31	0.012
Cd rate 3	-	37.78	4.98	0.242
s.e.m.	-	3.88	0.38	0.015
Cd rate 4	-	45.32	5.45	0.319
s.e.m.	-	3.81	0.31	0.011
LT Unc. Digested	-	41.17	4.47	0.042
s.e.m.	-	8.34	0.44	0.006
LT Unc. Raw	-	38.19	4.48	0.037
s.e.m.	-	6.34	0.31	0.004
LT Zn	-	53.46	4.84	0.049
s.e.m.	-	7.55	0.46	0.008
LT Cu	-	32.05	4.82	0.031
s.e.m.	-	0.99	0.07	0.002
LT Cd	-	42.39	4.62	0.124
s.e.m.	-	9.10	0.64	0.021

*Insufficient grain to determine yield measurement

Site 3. Watlington

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	-	87.00	21.33	0.037
s.e.m.	-	7.02	0.88	0.012
Unc. Digested	-	77.67	20.00	0.023
s.e.m.	-	8.97	3.06	0.007
Unc. Raw	-	76.33	26.33	0.020
s.e.m.	-	4.84	6.36	0.006
Zn rate 1	-	89.50	26.00	0.025
s.e.m.	-	7.76	3.00	0.004
Zn rate 2	-	119.33	17.67	0.043
s.e.m.	-	10.67	2.19	0.009
Zn rate 3	-	106.67	15.50	0.047
s.e.m.	-	6.67	1.22	0.003
Zn rate 4	-	107.67	16.67	0.047
s.e.m.	-	7.88	1.86	0.007
Cu rate 1	-	70.00	15.50	0.027
s.e.m.	-	4.08	0.41	0.003
Cu rate 2	-	69.67	15.00	0.020
s.e.m.	-	7.54	1.00	0.006
Cu rate 3	-	75.67	18.00	0.023
s.e.m.	-	6.17	0.58	0.007
Cu rate 4	-	64.67	15.00	0.020
s.e.m.	-	4.91	0.58	0.000
Cd rate 1	-	60.00	16.67	0.043
s.e.m.	-	4.58	1.86	0.003
Cd rate 2	-	69.67	25.00	0.083
s.e.m.	-	2.19	7.37	0.012
Cd rate 3	-	68.33	12.67	0.093
s.e.m.	-	1.45	0.88	0.017
Cd rate 4	-	64.00	14.00	0.097
s.e.m.	-	10.26	1.00	0.015
LT Unc. Digested	-	60.33	14.33	0.017
s.e.m.	-	8.11	0.88	0.003
LT Unc. Raw	-	61.00	12.20	0.020
s.e.m.	-	4.04	1.94	0.006
LT Zn	-	71.00	17.00	0.020
s.e.m.	-	5.86	3.51	0.006
LT Cu	-	78.00	19.67	0.017
s.e.m.	-	16.50	4.70	0.003
LT Cd	-	51.33	13.67	0.040
s.e.m.	-	2.85	1.76	0.006

*Insufficient grain to determine yield measurement

Site 4. Pwllpeiran

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	6.11	34.23	10.04	0.118
s.e.m.	0.10	3.07	0.11	0.022
Unc. Digested	5.78	33.42	10.31	0.121
s.e.m.	0.58	2.66	0.77	0.018
Unc. Raw	5.86	34.57	10.50	0.125
s.e.m.	0.57	3.94	0.36	0.028
Zn rate 1	5.14	39.73	11.62	0.113
s.e.m.	0.33	1.98	1.39	0.012
Zn rate 2	5.13	39.23	11.50	0.113
s.e.m.	0.72	2.31	1.06	0.006
Zn rate 3	5.42	48.73	11.50	0.147
s.e.m.	0.33	3.33	0.56	0.042
Zn rate 4	5.33	44.53	10.30	0.145
s.e.m.	0.94	9.09	1.69	0.025
Cu rate 1	5.99	34.20	11.27	0.110
s.e.m.	0.25	2.65	0.33	0.028
Cu rate 2	5.25	34.83	12.30	0.123
s.e.m.	0.39	1.28	0.57	0.024
Cu rate 3	5.75	37.17	15.70	0.155
s.e.m.	0.58	2.58	0.49	0.044
Cu rate 4	5.34	32.97	13.43	0.127
s.e.m.	0.30	3.64	0.81	0.023
Cd rate 1	4.44	37.13	10.98	0.207
s.e.m.	0.67	2.46	0.52	0.009
Cd rate 2	6.00	31.33	10.34	0.190
s.e.m.	1.05	4.70	1.07	0.016
Cd rate 3	5.47	38.50	12.23	0.308
s.e.m.	0.35	3.12	0.79	0.071
Cd rate 4	6.12	43.57	12.03	0.457
s.e.m.	0.17	7.34	1.51	0.079
LT Unc. Digested	5.08	32.83	10.47	0.113
s.e.m.	0.63	1.15	0.45	0.013
LT Unc. Raw	5.49	33.97	11.67	0.107
s.e.m.	0.75	2.03	1.01	0.014
LT Zn	5.18	44.60	10.53	0.142
s.e.m.	0.50	1.80	0.43	0.035
LT Cu	6.40	36.17	12.90	0.097
s.e.m.	0.61	4.15	1.31	0.028
LT Cd	5.32	38.67	11.63	0.327
s.e.m.	0.38	7.32	0.95	0.100

Site 5. Rosemaund

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	4.04	39.93	7.27	0.095
s.e.m.	0.18	1.30	0.43	0.006
Unc. Digested	3.71	43.73	6.43	0.102
s.e.m.	0.81	1.38	0.34	0.011
Unc. Raw	4.39	42.17	6.00	0.088
s.e.m.	0.59	2.30	0.23	0.019
Zn rate 1	3.58	57.03	5.95	0.125
s.e.m.	0.58	4.01	0.61	0.024
Zn rate 2	3.44	77.97	15.07	0.115
s.e.m.	0.58	11.56	6.44	0.028
Zn rate 3	3.80	64.03	6.98	0.155
s.e.m.	0.32	3.15	0.12	0.013
Zn rate 4	3.07	64.67	6.59	0.152
s.e.m.	0.09	9.60	0.35	0.023
Cu rate 1	4.00	50.93	8.15	0.110
s.e.m.	0.37	3.84	0.44	0.018
Cu rate 2	4.05	45.57	7.70	0.105
s.e.m.	0.63	0.86	0.46	0.016
Cu rate 3	4.69	51.73	7.14	0.128
s.e.m.	0.36	9.59	0.30	0.010
Cu rate 4	4.08	43.73	6.87	0.087
s.e.m.	0.26	0.81	0.24	0.013
Cd rate 1	4.04	44.60	6.06	0.158
s.e.m.	0.45	2.86	0.22	0.027
Cd rate 2	3.81	51.53	8.22	0.198
s.e.m.	0.39	2.32	1.85	0.029
Cd rate 3	5.16	48.77	6.11	0.210
s.e.m.	0.08	3.67	0.25	0.021
Cd rate 4	4.80	46.17	6.85	0.222
s.e.m.	0.82	1.07	0.08	0.022
LT Unc. Digested	4.49	50.35	9.62	0.095
s.e.m.	0.69	7.63	3.09	0.008
LT Unc. Raw	4.76	41.70	8.29	0.075
s.e.m.	0.73	6.04	2.76	0.018
LT Zn	3.90	62.77	12.01	0.088
s.e.m.	0.56	12.64	5.16	0.013
LT Cu	3.41	50.50	7.37	0.120
s.e.m.	0.63	3.86	0.47	0.014
LT Cd	3.54	46.53	7.41	0.158
s.e.m.	0.40	4.39	0.79	0.011

Site 6. Bridgets

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	1.57	36.53	6.13	0.069
s.e.m.	0.60	0.84	0.49	0.019
Unc. Digested	2.05	32.45	5.92	0.072
s.e.m.	0.23	0.52	0.71	0.016
Unc. Raw	2.03	30.53	5.29	0.060
s.e.m.	0.16	3.62	0.46	0.025
Zn rate 1	2.11	36.00	6.05	0.085
s.e.m.	0.19	1.76	0.17	0.008
Zn rate 2	2.28	42.80	5.88	0.075
s.e.m.	0.22	1.13	0.09	0.008
Zn rate 3	2.28	44.83	5.58	0.072
s.e.m.	0.11	2.32	0.25	0.028
Zn rate 4	2.02	42.80	5.92	0.067
s.e.m.	0.29	3.19	0.54	0.030
Cu rate 1	2.26	31.07	5.74	0.092
s.e.m.	0.06	2.92	0.18	0.013
Cu rate 2	2.18	31.53	5.65	0.100
s.e.m.	0.30	0.03	0.25	0.014
Cu rate 3	1.94	34.93	5.96	0.067
s.e.m.	0.15	1.96	0.24	0.019
Cu rate 4	2.25	34.43	6.38	0.058
s.e.m.	0.15	1.32	0.40	0.015
Cd rate 1	1.56	32.70	6.02	0.058
s.e.m.	0.18	0.12	0.17	0.031
Cd rate 2	1.94	31.83	5.62	0.092
s.e.m.	0.23	0.93	0.25	0.007
Cd rate 3	2.07	32.67	5.65	0.087
s.e.m.	0.17	2.01	0.34	0.011
Cd rate 4	1.99	34.23	4.41	0.120
s.e.m.	0.10	1.04	0.68	0.009
LT Unc. Digested	1.96	32.27	5.90	0.073
s.e.m.	0.29	0.07	0.18	0.012
LT Unc. Raw	1.49	32.17	5.57	0.085
s.e.m.	0.10	1.75	0.16	0.006
LT Zn	1.83	36.30	5.89	0.093
s.e.m.	0.14	1.50	0.18	0.006
LT Cu	1.54	32.87	6.21	0.093
s.e.m.	0.45	0.95	0.41	0.004
LT Cd	1.86	36.63	5.69	0.105
s.e.m.	0.41	1.39	0.26	0.014

Yield too low on two replicates of the Cu rate 4 treatments at Bridgets to obtain a robust measure in t/ha

Site 7. Hartwood

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	1.86	31.30	7.31	0.055
s.e.m.	0.17	1.84	0.79	0.010
Unc. Digested	3.25	44.85	10.62	0.062
s.e.m.	0.09	0.87	0.31	0.011
Unc. Raw	3.08	37.99	10.68	0.064
s.e.m.	0.56	2.72	0.82	0.031
Zn rate 1	3.55	65.32	10.63	0.042
s.e.m.	0.48	5.69	0.77	0.011
Zn rate 2	3.00	117.19	11.79	0.054
s.e.m.	0.17	17.31	1.37	0.020
Zn rate 3	2.79	199.09	14.98	0.079
s.e.m.	0.34	57.09	0.76	0.033
Zn rate 4	2.01	304.84	14.88	0.170
s.e.m.	0.13	102.86	1.10	0.056
Cu rate 1	2.92	44.42	12.37	0.059
s.e.m.	0.29	4.66	0.24	0.025
Cu rate 2	2.90	51.64	13.29	0.053
s.e.m.	0.38	2.13	2.52	0.023
Cu rate 3	2.75	79.74	15.55	0.077
s.e.m.	0.08	10.26	1.89	0.014
Cu rate 4	2.85	78.06	17.39	0.093
s.e.m.	0.61	20.06	4.62	0.037
Cd rate 1	3.57	47.17	9.67	0.240
s.e.m.	0.12	4.07	0.34	0.039
Cd rate 2	3.27	52.79	11.31	0.177
s.e.m.	0.23	3.05	1.22	0.071
Cd rate 3	3.44	73.61	14.01	0.379
s.e.m.	0.39	4.65	0.57	0.050
Cd rate 4	4.07	70.13	14.16	0.306
s.e.m.	0.13	4.34	1.43	0.085
LT Unc. Digested	3.39	37.35	9.46	0.059
s.e.m.	0.08	2.62	0.80	0.015
LT Unc. Raw	2.84	40.37	9.24	0.075
s.e.m.	0.12	4.89	1.17	0.013
LT Zn	3.52	91.08	11.39	0.055
s.e.m.	0.15	6.30	0.66	0.002
LT Cu	2.61	40.94	11.49	0.065
s.e.m.	0.66	3.32	1.00	0.018
LT Cd	2.73	37.24	8.29	0.222
s.e.m.	0.27	3.68	0.13	0.067

Site 8. Auchincruive

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	6.17	23.20	4.98	0.202
s.e.m.	0.20	1.07	0.30	0.016
Unc. Digested	6.49	36.23	6.60	0.225
s.e.m.	0.08	0.13	0.41	0.012
Unc. Raw	6.16	40.83	7.07	0.190
s.e.m.	0.08	1.54	0.73	0.035
Zn rate 1	6.32	57.93	7.43	0.240
s.e.m.	0.09	5.09	0.65	0.032
Zn rate 2	6.40	97.10	8.17	0.280
s.e.m.	0.23	6.98	0.16	0.006
Zn rate 3	5.59	123.40	7.97	0.270
s.e.m.	0.29	19.98	0.83	0.066
Zn rate 4	5.61	181.67	10.02	0.310
s.e.m.	0.26	22.88	1.09	0.010
Cu rate 1	6.39	42.60	7.89	0.200
s.e.m.	0.14	2.53	0.56	0.046
Cu rate 2	6.80	54.67	10.47	0.307
s.e.m.	0.12	13.72	2.32	0.072
Cu rate 3	6.70	54.90	8.28	0.247
s.e.m.	0.23	7.20	0.80	0.003
Cu rate 4	6.33	57.47	10.15	0.217
s.e.m.	0.07	6.54	0.69	0.028
Cd rate 1	6.63	37.30	6.65	0.260
s.e.m.	0.58	4.16	0.50	0.076
Cd rate 2	7.02	50.83	6.59	0.327
s.e.m.	0.10	5.27	0.66	0.079
Cd rate 3	6.45	53.37	7.66	0.510
s.e.m.	0.06	2.21	0.22	0.046
Cd rate 4	6.43	52.00	6.84	0.500
s.e.m.	0.23	2.51	0.49	0.035
LT Unc. Digested	6.16	30.38	5.82	0.198
s.e.m.	0.24	3.28	0.54	0.007
LT Unc. Raw	6.36	26.23	5.76	0.223
s.e.m.	0.42	0.62	0.18	0.018
LT Zn	6.20	55.30	7.33	0.233
s.e.m.	0.05	1.71	0.22	0.007
LT Cu	6.24	28.67	6.19	0.207
s.e.m.	0.20	3.19	0.45	0.012
LT Cd	6.51	29.03	5.69	0.297
s.e.m.	0.20	2.99	0.05	0.015

Site 9. Shirburn

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	-	-	-	-
s.e.m.	-	-	-	-
Unc. Digested	-	-	-	-
s.e.m.	-	-	-	-
Unc. Raw	-	-	-	-
s.e.m.	-	-	-	-
High Unc. Digested	-	-	-	-
s.e.m.	-	-	-	-
High Unc. Raw	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 1	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 2	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 3	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 4	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 5	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 1	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 2	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 3	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 4	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 5	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 1	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 2	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 3	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 4	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 5	-	-	-	-
s.e.m.	-	-	-	-
LT Unc. Digested	-	-	-	-
s.e.m.	-	-	-	-
LT Unc. Raw	-	-	-	-
s.e.m.	-	-	-	-
LT Zn	-	-	-	-
s.e.m.	-	-	-	-
LT Cu	-	-	-	-
s.e.m.	-	-	-	-
LT Cd	-	-	-	-
s.e.m.	-	-	-	-

*Insufficient grain to determine yield and grain metal concentrations

APPENDIX L Soil analyses on all treatments: metal-amended liquid sludge sites - spring 2003

Site 2. Woburn – metal-amended liquid sludge
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	47.6	16.50	0.16	0.123	0.033	0.010
s.e.m.	4.7	0.80	0.02	0.022	0.003	0.000
Unc. Digested	62.9	26.30	0.20	0.133	0.130	0.010
s.e.m.	3.7	2.11	0.02	0.019	0.006	0.000
Zn rate 1	**202.3**	27.23	0.20	**4.933**	0.137	0.010
s.e.m.	**20.3**	0.50	0.01	**2.853**	0.027	0.000
Zn rate 2	**255.7**	26.10	0.20	**3.470**	0.127	0.010
s.e.m.	**11.7**	0.55	0.01	**0.395**	0.007	0.000
Zn rate 3	**316.7**	21.27	0.20	**6.157**	0.117	0.010
s.e.m.	**19.4**	1.18	0.02	**0.972**	0.018	0.000
Cu rate 1	56.0	**70.27**	0.19	0.127	**0.420**	0.010
s.e.m.	2.1	**7.08**	0.01	0.035	**0.057**	0.000
Cu rate 2	57.5	**132.67**	0.19	0.177	**1.003**	0.010
s.e.m.	5.5	**8.57**	0.02	0.052	**0.070**	0.000
Cu rate 3	59.7	**172.67**	0.20	0.487	**1.173**	0.010
s.e.m.	3.9	**17.48**	0.01	0.135	**0.127**	0.000
Cd rate 1	56.4	26.53	**1.93**	0.150	0.130	**0.033**
s.e.m.	2.1	2.25	**0.07**	0.010	0.015	**0.003**
Cd rate 2	54.1	24.13	**2.67**	0.117	0.123	**0.043**
s.e.m.	2.0	2.09	**0.34**	0.044	0.020	**0.009**
Cd rate 3	56.6	16.40	**3.61**	0.307	0.093	**0.080**
s.e.m.	3.0	8.27	**0.11**	0.054	0.013	**0.006**

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg	pH	Organic C %
Control	162.3	4.41	0.28	6.87	1.10
s.e.m.	13.4	0.25	0.02	0.03	0.16
Unc. Digested	230.4	4.53	0.30	6.87	1.42
s.e.m.	12.8	0.07	0.00	0.12	0.11
Zn rate 1	201.4	4.63	0.42	6.70	1.37
s.e.m.	13.8	0.15	0.09	0.06	0.09
Zn rate 2	231.5	4.32	0.39	6.63	1.44
s.e.m.	15.6	0.19	0.05	0.09	0.02
Zn rate 3	198.6	4.32	0.36	6.80	1.29
s.e.m.	10.8	0.08	0.05	0.10	0.04
Cu rate 1	180.1	4.73	0.35	6.83	1.34
s.e.m.	17.5	0.17	0.02	0.12	0.10
Cu rate 2	189.0	4.21	0.33	6.77	1.47
s.e.m.	17.0	0.22	0.01	0.12	0.24
Cu rate 3	192.0	4.53	0.40	6.47	1.40
s.e.m.	15.5	0.19	0.02	0.07	0.03
Cd rate 1	213.5	4.63	0.25	6.70	1.38
s.e.m.	1.5	0.15	0.02	0.06	0.08
Cd rate 2	215.8	4.49	0.39	6.87	1.36
s.e.m.	17.8	0.22	0.05	0.19	0.02
Cd rate 3	242.9	4.31	0.31	6.60	1.46
s.e.m.	16.3	0.30	0.07	0.06	0.01

Site 3. Watlington – metal-amended liquid sludge
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	48.3	12.21	0.30	0.050	0.033	0.010
s.e.m.	1.3	0.15	0.00	0.000	0.009	0.000
Unc. Digested	51.8	18.23	0.35	0.050	0.080	0.010
s.e.m.	1.5	0.47	0.08	0.000	0.021	0.000
Zn rate 1	**146.1**	20.20	0.35	**0.473**	0.100	0.010
s.e.m.	**0.4**	0.17	0.05	**0.109**	0.021	0.000
Zn rate 2	**200.6**	18.23	0.36	**1.060**	0.093	0.010
s.e.m.	**13.2**	1.01	0.04	**0.291**	0.012	0.000
Zn rate 3	**285.5**	18.52	0.31	**2.803**	0.090	0.010
s.e.m.	**16.7**	0.85	0.01	**0.405**	0.010	0.000
Cu rate 1	54.5	**69.85**	0.30	0.050	**0.400**	0.010
s.e.m.	0.9	**9.84**	0.01	0.000	**0.091**	0.000
Cu rate 2	52.6	**88.28**	0.33	0.050	**0.463**	0.010
s.e.m.	2.1	**6.10**	0.02	0.000	**0.026**	0.000
Cu rate 3	51.1	**117.02**	0.55	0.067	**0.637**	0.010
s.e.m.	0.9	**6.76**	0.18	0.009	**0.193**	0.000
Cd rate 1	52.3	20.48	**1.66**	0.057	0.087	**0.010**
s.e.m.	3.0	1.45	**0.17**	0.007	0.009	**0.000**
Cd rate 2	57.4	19.86	**2.49**	0.050	0.080	**0.012**
s.e.m.	3.4	0.78	**0.15**	0.000	0.010	**0.002**
Cd rate 3	53.1	18.36	**2.47**	0.053	0.087	**0.017**
s.e.m.	2.8	0.38	**0.19**	0.003	0.019	**0.004**

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg	pH	Organic C %
Control	277.6	4.86	0.31	6.90	1.45
s.e.m.	13.2	0.14	0.02	0.31	0.10
Unc. Digested	346.1	5.12	0.30	6.93	1.73
s.e.m.	17.5	0.14	0.02	0.15	0.06
Zn rate 1	340.1	5.02	0.43	6.83	1.88
s.e.m.	16.0	0.06	0.03	0.07	0.02
Zn rate 2	355.6	4.92	0.40	6.93	1.81
s.e.m.	12.7	0.16	0.05	0.07	0.06
Zn rate 3	291.7	4.90	0.34	6.70	1.84
s.e.m.	20.3	0.17	0.02	0.06	0.02
Cu rate 1	310.4	4.67	0.29	6.83	1.89
s.e.m.	12.7	0.22	0.03	0.18	0.02
Cu rate 2	292.1	4.86	0.30	6.93	1.78
s.e.m.	10.7	0.14	0.03	0.09	0.06
Cu rate 3	270.2	5.08	0.32	6.90	1.91
s.e.m.	12.3	0.00	0.01	0.10	0.07
Cd rate 1	370.4	5.02	0.43	7.03	1.89
s.e.m.	53.4	0.23	0.03	0.03	0.04
Cd rate 2	342.7	4.79	0.42	7.00	1.94
s.e.m.	25.4	0.30	0.05	0.06	0.05
Cd rate 3	347.5	5.18	0.43	7.10	1.83
s.e.m.	14.1	0.10	0.02	0.25	0.03

Site 5. Rosemaund – metal-amended liquid sludge
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	80.9	16.80	0.27	0.323	0.023	0.012
s.e.m.	1.6	0.42	0.02	0.026	0.003	0.001
Unc. Digested	81.4	19.57	0.26	0.420	0.037	0.010
s.e.m.	3.8	1.28	0.02	0.056	0.003	0.000
Zn rate 1	**118.7**	20.13	0.26	**3.973**	0.040	0.012
s.e.m.	**5.8**	0.73	0.00	**0.754**	0.000	0.001
Zn rate 2	**181.7**	22.27	0.31	**12.100**	0.057	0.017
s.e.m.	**4.3**	0.50	0.03	**0.764**	0.003	0.003
Zn rate 3	**218.3**	22.73	0.39	**23.133**	0.053	0.017
s.e.m.	**16.8**	0.52	0.12	**5.983**	0.003	0.003
Cu rate 1	81.6	**43.83**	0.26	0.473	**0.203**	0.011
s.e.m.	3.2	**6.41**	0.01	0.226	**0.018**	0.001
Cu rate 2	79.2	**67.30**	0.26	0.627	**0.420**	0.012
s.e.m.	2.8	**2.37**	0.02	0.115	**0.026**	0.001
Cu rate 3	87.0	**99.60**	0.31	0.690	**0.723**	0.014
s.e.m.	3.2	**10.24**	0.04	0.091	**0.146**	0.003
Cd rate 1	84.1	20.87	**1.13**	0.463	0.043	**0.054**
s.e.m.	1.0	0.72	**0.15**	0.111	0.003	**0.013**
Cd rate 2	85.8	22.03	**1.80**	0.577	0.050	**0.098**
s.e.m.	1.6	1.04	**0.27**	0.262	0.006	**0.025**
Cd rate 3	84.5	21.60	**1.93**	0.577	0.043	**0.130**
s.e.m.	3.8	0.72	**0.10**	0.003	0.003	**0.009**

Microbial and chemical properties (nutrients)

Treatment	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg	pH	Organic C %
Control	380.1	5.28	0.25	5.90	1.59
s.e.m.	17.2	0.10	0.02	0.10	0.15
Unc. Digested	402.9	5.02	0.26	5.90	1.85
s.e.m.	4.4	0.06	0.01	0.10	0.07
Zn rate 1	388.6	5.02	0.25	5.87	1.86
s.e.m.	16.2	0.23	0.01	0.03	0.04
Zn rate 2	409.9	5.02	0.32	5.80	1.87
s.e.m.	13.2	0.23	0.02	0.00	0.04
Zn rate 3	408.0	4.28	0.32	5.73	1.81
s.e.m.	8.4	0.33	0.02	0.09	0.06
Cu rate 1	391.2	5.12	0.20	5.93	1.92
s.e.m.	24.6	0.14	0.03	0.18	0.09
Cu rate 2	349.8	4.86	0.28	5.77	1.90
s.e.m.	8.8	0.14	0.01	0.12	0.07
Cu rate 3	377.7	4.89	0.32	5.83	1.98
s.e.m.	24.7	0.28	0.04	0.03	0.08
Cd rate 1	416.0	5.22	0.39	5.97	1.86
s.e.m.	15.5	0.16	0.04	0.19	0.06
Cd rate 2	408.5	5.08	0.33	5.80	1.94
s.e.m.	18.3	0.00	0.04	0.12	0.10
Cd rate 3	390.5	4.96	0.36	5.80	1.87
s.e.m.	11.8	0.06	0.04	0.12	0.03

APPENDIX M Soil analyses on all treatments: metal-amended liquid sludge sites - spring 2005

Site 2. Woburn
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	48.6	11.40	0.20	0.713	0.133	0.100
s.e.m.	2.7	0.94	0.00	0.193	0.017	0.000
Unc. Digested	61.4	21.26	0.51	0.853	0.120	0.100
s.e.m.	1.7	1.17	0.00	0.374	0.020	0.000
Zn rate 1	**202.4**	22.27	0.51	**14.500**	0.103	0.100
s.e.m.	**30.3**	1.55	0.00	**4.805**	0.003	0.000
Zn rate 2	**236.2**	22.94	0.51	**9.833**	0.113	0.100
s.e.m.	**20.5**	0.89	0.00	**1.765**	0.013	0.000
Zn rate 3	**347.5**	20.24	0.51	**16.033**	0.100	0.100
s.e.m.	**17.9**	0.58	0.00	**4.550**	0.000	0.000
Cu rate 1	56.2	**68.99**	0.20	1.037	**0.253**	0.100
s.e.m.	2.8	**6.41**	0.00	0.347	**0.027**	0.000
Cu rate 2	56.3	**117.75**	0.20	0.913	**0.643**	0.100
s.e.m.	4.7	**9.61**	0.00	0.147	**0.096**	0.000
Cu rate 3	57.4	**167.00**	0.20	0.727	**1.013**	0.100
s.e.m.	2.4	**10.53**	0.00	0.290	**0.146**	0.000
Cd rate 1	61.6	24.12	**1.86**	0.670	0.163	**0.100**
s.e.m.	2.9	2.23	**0.12**	0.121	0.045	**0.000**
Cd rate 2	59.7	24.97	**3.15**	0.517	0.123	**0.100**
s.e.m.	3.7	1.44	**0.40**	0.037	0.019	**0.000**
Cd rate 3	63.9	26.32	**3.81**	0.577	0.150	**0.100**
s.e.m.	2.1	1.17	**0.37**	0.023	0.010	**0.000**

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.90	0.12	50.0	158.3	143.0	1.15
s.e.m.	0.06	0.01	3.0	10.9	3.0	0.17
Unc. Digested	7.00	0.20	77.3	127.3	90.3	1.39
s.e.m.	0.06	0.01	3.8	7.0	3.2	0.07
Zn rate 1	6.77	0.19	72.0	150.0	74.3	1.03
s.e.m.	0.09	0.01	0.6	3.0	2.9	0.38
Zn rate 2	6.83	0.18	70.0	138.0	68.7	0.99
s.e.m.	0.09	0.01	2.6	2.5	2.4	0.22
Zn rate 3	6.83	0.19	66.0	134.0	72.0	1.28
s.e.m.	0.03	0.00	2.1	6.0	3.6	0.02
Cu rate 1	6.93	0.17	71.3	141.3	89.7	0.82
s.e.m.	0.07	0.01	1.3	8.5	9.8	0.12
Cu rate 2	6.80	0.18	69.3	149.0	87.0	1.17
s.e.m.	0.06	0.01	1.5	13.7	7.9	0.11
Cu rate 3	6.67	0.19	69.0	142.0	69.7	1.13
s.e.m.	0.12	0.01	0.6	4.0	0.3	0.12
Cd rate 1	6.80	0.18	69.3	151.7	92.7	0.95
s.e.m.	0.06	0.00	2.0	12.5	9.2	0.10
Cd rate 2	6.93	0.16	71.7	149.3	91.0	0.96
s.e.m.	0.09	0.01	2.0	14.5	5.9	0.24
Cd rate 3	6.87	0.18	74.0	145.7	99.0	1.34
s.e.m.	0.03	0.01	2.9	6.9	3.6	0.04

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg
Control	5.85	2093.33	166.7	4.54	0.21
s.e.m.	0.62	33.83	13.3	0.28	0.01
Unc. Digested	7.23	2126.67	217.3	4.79	0.28
s.e.m.	0.16	8.82	16.4	0.20	0.03
Zn rate 1		2066.67	135.9	3.67	0.25
s.e.m.		12.02	6.6	0.17	0.03
Zn rate 2	7.79	2043.33	153.0	3.97	0.27
s.e.m.	0.29	49.10	15.4	0.20	0.01
Zn rate 3		2063.33	162.7	4.36	0.28
s.e.m.		27.28	3.5	0.13	0.03
Cu rate 1		2060.00	191.2	4.39	0.32
s.e.m.		50.00	9.3	0.13	0.04
Cu rate 2	6.88	2093.33	151.1	4.53	0.33
s.e.m.	0.43	24.04	35.2	0.19	0.03
Cu rate 3		2110.00	139.5	4.32	0.35
s.e.m.		32.15	19.3	0.19	0.01
Cd rate 1		1996.67	172.0	4.18	0.24
s.e.m.		46.67	27.4	0.21	0.02
Cd rate 2	7.34	2110.00	163.8	4.54	0.25
s.e.m.	0.31	15.28	14.8	0.28	0.01
Cd rate 3		2103.33	207.3	4.55	0.27
s.e.m.		50.44	4.5	0.22	0.01

Site 3. Watlington
Chemical properties (metals)

Treatment		Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control		47.4	12.46	0.35	0.010	0.117	0.010
	s.e.m.	1.3	0.36	0.02	< 0.001	0.017	0.000
Unc. Digested		54.5	19.23	0.36	0.037	0.160	0.010
	s.e.m.	1.9	1.07	0.01	0.015	0.031	0.000
Zn rate 1		**122.3**	18.68	0.35	**0.213**	0.143	0.010
	s.e.m.	**3.6**	0.78	0.02	**0.079**	0.020	0.000
Zn rate 2		**166.3**	21.47	0.36	**1.140**	0.133	0.010
	s.e.m.	**5.2**	4.12	0.02	**0.140**	0.003	0.000
Zn rate 3		**249.5**	16.78	0.33	**2.767**	0.150	0.010
	s.e.m.	**5.3**	0.17	0.02	**0.841**	0.010	0.000
Cu rate 1		47.1	**50.51**	0.33	0.010	**0.603**	0.010
	s.e.m.	1.6	**2.52**	0.02	< 0.001	**0.043**	0.000
Cu rate 2		47.2	**69.81**	0.33	0.010	**0.977**	0.010
	s.e.m.	0.8	**5.66**	0.01	< 0.001	**0.015**	0.000
Cu rate 3		48.8	**99.50**	0.38	0.010	**1.600**	0.010
	s.e.m.	2.7	**2.85**	0.03	< 0.001	**0.153**	0.000
Cd rate 1		56.6	30.27	**1.53**	0.010	0.190	**0.020**
	s.e.m.	6.4	11.04	**0.04**	< 0.001	0.015	**0.006**
Cd rate 2		56.3	19.94	**2.10**	0.010	0.203	**0.030**
	s.e.m.	3.7	0.94	**0.07**	< 0.001	0.048	**0.006**
Cd rate 3		50.6	19.23	**2.71**	0.010	0.223	**0.067**
	s.e.m.	0.7	0.62	**0.09**	< 0.001	0.020	**0.023**

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	7.23	0.19	46.3	160.7	83.7	1.40
s.e.m.	0.12	0.00	1.8	9.4	8.9	0.01
Unc. Digested	6.97	0.24	71.7	129.7	86.7	1.61
s.e.m.	0.03	0.01	3.3	4.3	5.4	0.06
Zn rate 1	7.00	0.22	69.0	135.7	83.0	1.56
s.e.m.	0.06	0.01	2.6	12.6	3.1	0.07
Zn rate 2	7.03	0.21	67.3	134.0	86.7	1.71
s.e.m.	0.09	0.01	1.7	12.7	4.2	0.03
Zn rate 3	6.97	0.23	62.0	144.0	83.7	1.76
s.e.m.	0.09	0.01	1.5	8.1	1.7	0.04
Cu rate 1	7.07	0.23	74.3	127.0	83.7	1.71
s.e.m.	0.03	0.01	2.4	4.0	6.3	0.11
Cu rate 2	6.97	0.23	71.3	130.7	83.3	1.74
s.e.m.	0.07	0.01	1.8	9.7	6.6	0.04
Cu rate 3	6.90	0.22	69.0	131.7	79.7	1.61
s.e.m.	0.00	0.01	2.5	7.8	3.5	0.10
Cd rate 1	7.03	0.23	71.7	117.0	76.3	1.68
s.e.m.	0.09	0.01	2.4	7.8	2.0	0.08
Cd rate 2	7.10	0.23	76.3	140.7	82.0	1.51
s.e.m.	0.00	0.00	5.9	13.9	0.6	0.20
Cd rate 3	7.10	0.22	70.7	124.0	82.7	1.69
s.e.m.	0.26	0.00	2.3	9.8	4.2	0.05

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg
Control	13.6	2006.7	331.9	5.22	0.28
s.e.m.	0.64	27.3	7.4	0.09	0.02
Unc. Digested	15.0	1936.7	373.9	4.80	0.26
s.e.m.	0.57	37.6	17.7	0.10	0.02
Zn rate 1		1990.0	345.6	5.28	0.25
s.e.m.		5.8	15.3	0.10	0.01
Zn rate 2	13.9	1895.0	373.6	4.83	0.28
s.e.m.	0.32	69.4	14.9	0.25	0.01
Zn rate 3		1950.0	337.5	5.08	0.25
s.e.m.		81.6	12.3	0.00	0.01
Cu rate 1		1930.0	373.7	5.06	0.34
s.e.m.		60.3	18.2	0.16	0.05
Cu rate 2	14.0	1956.7	318.2	4.92	0.35
s.e.m.	0.40	69.8	25.1	0.16	0.09
Cu rate 3		1876.7	296.2	4.71	0.30
s.e.m.		66.9	20.5	0.28	0.04
Cd rate 1		1983.3	368.7	5.28	0.25
s.e.m.		64.9	35.1	0.10	0.04
Cd rate 2	14.4	1946.7	372.5	5.02	0.26
s.e.m.	0.30	74.2	34.4	0.23	0.01
Cd rate 3		1856.7	336.4	4.87	0.30
s.e.m.		31.8	8.9	0.37	0.02

Site 5. Rosemaund
Chemical properties (metals)

Treatment	Total Zn mg/kg	Total Cu mg/kg	Total Cd mg/kg	NH_4NO_3 Extractable Zn mg/kg	NH_4NO_3 Extractable Cu mg/kg	NH_4NO_3 Extractable Cd mg/kg
Control	64.3	12.67	0.31	0.083	0.073	0.010
s.e.m.	11.3	0.67	0.00	0.024	0.019	0.000
Unc. Digested	57.0	17.00	0.21	0.130	0.083	0.010
s.e.m.	1.5	0.58	0.03	0.010	0.009	0.000
Zn rate 1	**82.3**	16.00	0.29	**0.870**	0.093	0.010
s.e.m.	**4.5**	1.53	0.03	**0.166**	0.003	0.000
Zn rate 2	**130.0**	16.00	0.27	**1.967**	0.110	0.010
s.e.m.	**5.8**	0.00	0.07	**0.441**	0.010	0.000
Zn rate 3	**173.3**	16.00	0.26	**7.633**	0.110	0.010
s.e.m.	**17.6**	0.58	0.01	**2.764**	0.017	0.000
Cu rate 1	59.0	**38.00**	0.24	0.310	**0.277**	0.010
s.e.m.	0.6	**2.08**	0.02	0.110	**0.050**	0.000
Cu rate 2	58.0	**62.00**	0.25	0.167	**0.593**	0.010
s.e.m.	1.0	**3.06**	0.04	0.053	**0.096**	0.000
Cu rate 3	66.7	**76.67**	0.42	0.223	**0.630**	0.010
s.e.m.	4.3	**7.17**	0.04	0.095	**0.047**	0.000
Cd rate 1	47.0	13.67	**1.50**	0.253	0.220	**0.020**
s.e.m.	3.1	1.33	**0.00**	0.022	0.120	**0.006**
Cd rate 2	52.3	15.33	**1.93**	0.060	0.190	**0.017**
s.e.m.	2.6	1.45	**0.15**	0.000	0.066	**0.003**
Cd rate 3	58.0	16.00	**1.97**	0.123	0.083	**0.027**
s.e.m.	0.6	0.58	**0.15**	0.013	0.009	**0.003**

Chemical properties (nutrients)

Treatment	pH	Total N %	Extractable P mg/l	Extractable K mg/l	Extractable Mg mg/l	Organic C %
Control	6.73	0.25	21.0	242.7	237.0	1.42
s.e.m.	0.03	0.01	0.6	11.8	15.0	0.07
Unc. Digested	6.83	0.26	29.3	206.7	232.7	1.52
s.e.m.	0.03	0.01	3.3	28.4	2.7	0.08
Zn rate 1	6.70	0.26	32.0	193.3	222.0	1.71
s.e.m.	0.15	0.01	2.5	23.5	10.2	0.20
Zn rate 2	6.67	0.26	34.3	221.7	228.3	1.60
s.e.m.	0.09	0.01	2.4	18.5	13.6	0.13
Zn rate 3	6.60	0.27	32.3	205.3	231.3	1.70
s.e.m.	0.10	0.00	3.4	22.7	5.9	0.07
Cu rate 1	6.60	0.27	34.0	216.7	218.0	1.87
s.e.m.	0.12	0.00	3.0	36.4	25.2	0.05
Cu rate 2	6.73	0.29	37.3	234.3	246.0	1.81
s.e.m.	0.12	0.00	0.9	10.8	16.1	0.06
Cu rate 3	6.67	0.27	36.0	217.7	247.0	1.68
s.e.m.	0.12	0.01	2.5	37.9	14.7	0.12
Cd rate 1	6.53	0.27	36.0	206.3	216.7	1.67
s.e.m.	0.09	0.01	4.4	40.4	19.3	0.19
Cd rate 2	6.70	0.27	37.7	245.0	250.3	1.48
s.e.m.	0.06	0.01	2.7	44.2	28.7	0.11
Cd rate 3	6.63	0.26	31.7	208.7	237.3	1.85
s.e.m.	0.09	0.01	3.0	51.3	19.8	0.26

Chemical and microbial properties

Treatment	CEC $cmol_c$ /kg	Conductivity µs/cm	Biomass C mg C/kg	Rhizobia MPN log_{10} cells/g	Soil respiration mg CO_2-C/kg
Control	16.5	2050.0	431.0	5.08	0.29
s.e.m.	1.22	40.4	26.0	0.00	0.02
Unc. Digested	18.7	2076.7	409.5	5.12	0.28
s.e.m.	0.57	43.7	22.0	0.14	0.02
Zn rate 1		2090.0	425.4	4.75	0.32
s.e.m.		10.0	8.5	0.33	0.00
Zn rate 2	18.83	2110.0	447.2	5.12	0.33
s.e.m.	0.11	63.5	27.7	0.14	0.03
Zn rate 3		2056.7	456.1	5.22	0.28
s.e.m.		43.3	17.2	0.16	0.01
Cu rate 1		2086.7	482.7	5.22	0.27
s.e.m.		8.8	7.5	0.16	0.01
Cu rate 2	17.8	2043.3	421.0	4.70	0.29
s.e.m.	1.19	53.6	16.6	0.20	0.01
Cu rate 3		2093.3	415.8	4.96	0.27
s.e.m.		43.3	29.4	0.06	0.03
Cd rate 1		2063.3	449.2	5.12	0.27
s.e.m.		35.3	25.2	0.14	0.00
Cd rate 2	18.0	1990.0	456.3	5.12	0.26
s.e.m.	0.87	73.7	20.7	0.14	0.01
Cd rate 3		2080.0	457.7	5.18	0.28
s.e.m.		15.3	8.9	0.10	0.02

APPENDIX N Crop yield and metal concentrations: metal-amended liquid sludge sites - 2002

Site 2. Woburn

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	3.96	28.81	3.22	0.04
s.e.m.	0.05	4.39	0.28	0.01
Unc. Digested	5.25	50.24	3.71	0.04
s.e.m.	0.14	2.34	0.06	0.00
Zn rate 1	5.36	149.40	3.77	0.04
s.e.m.	0.27	15.19	0.32	0.01
Zn rate 2	5.97	195.52	3.84	0.03
s.e.m.	0.39	3.08	0.33	0.00
Zn rate 3	4.62	307.47	3.68	0.03
s.e.m.	0.11	44.17	0.10	0.00
Cu rate 1	5.57	51.63	4.48	0.03
s.e.m.	0.28	5.56	0.46	0.00
Cu rate 2	5.56	63.92	5.86	0.04
s.e.m.	0.07	6.03	0.34	0.00
Cu rate 3	6.86	72.06	6.05	0.03
s.e.m.	0.36	7.48	0.37	0.00
Cd rate 1	5.28	43.52	3.79	0.36
s.e.m.	0.55	3.36	0.45	0.05
Cd rate 2	4.98	45.19	2.97	0.60
s.e.m.	0.23	4.82	0.30	0.01
Cd rate 3	5.04	46.30	3.57	0.64
s.e.m.	0.21	1.75	0.42	0.06

Site 3. Watlington

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	5.30	10.23	3.39	0.034
s.e.m.	0.38	0.43	0.31	0.005
Unc. Digested	5.42	13.30	4.04	0.038
s.e.m.	0.20	0.30	0.10	0.003
Zn rate 1	4.77	26.53	4.36	0.033
s.e.m.	0.30	2.75	0.14	0.004
Zn rate 2	4.79	34.07	4.29	0.033
s.e.m.	0.39	0.73	0.16	0.001
Zn rate 3	4.74	56.97	4.08	0.031
s.e.m.	0.14	5.73	0.14	0.003
Cu rate 1	5.62	17.57	5.02	0.037
s.e.m.	0.04	0.75	0.12	0.003
Cu rate 2	5.90	16.27	4.96	0.039
s.e.m.	0.30	0.84	0.08	0.007
Cu rate 3	5.49	16.47	5.35	0.065
s.e.m.	0.35	0.52	0.14	0.013
Cd rate 1	4.60	13.20	3.91	0.259
s.e.m.	0.52	0.40	0.12	0.067
Cd rate 2	5.09	12.37	4.58	0.245
s.e.m.	0.20	1.07	0.22	0.018
Cd rate 3	4.97	13.30	3.94	0.223
s.e.m.	0.34	0.46	0.08	0.002

Site 5. Rosemaund

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	7.58	19.70	6.04	0.117
s.e.m.	0.23	1.59	0.47	0.034
Unc. Digested	8.48	27.85	7.45	0.066
s.e.m.	0.25	3.46	1.26	0.009
Zn rate 1	8.19	52.73	5.96	0.090
s.e.m.	0.13	1.74	0.54	0.026
Zn rate 2	7.00	98.03	6.69	0.082
s.e.m.	0.41	15.28	0.89	0.027
Zn rate 3	7.83	170.33	5.99	0.078
s.e.m.	0.41	15.62	0.09	0.006
Cu rate 1	8.18	30.10	6.75	0.058
s.e.m.	0.95	2.80	1.21	0.007
Cu rate 2	8.12	35.13	7.86	0.070
s.e.m.	0.22	1.78	0.42	0.015
Cu rate 3	8.55	36.77	7.91	0.100
s.e.m.	0.76	1.65	0.39	0.023
Cd rate 1	7.87	26.13	6.08	0.207
s.e.m.	0.18	0.38	0.38	0.038
Cd rate 2	8.12	41.80	7.44	0.268
s.e.m.	0.39	5.15	1.01	0.030
Cd rate 3	8.45	26.17	6.32	0.352
s.e.m.	0.61	2.43	0.27	0.053

APPENDIX O Crop yield and metal concentrations: metal-amended liquid sludge sites - 2003

Site 2. Woburn

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	0.54	49.11	4.23	0.045
s.e.m.	0.15	1.03	0.09	0.004
Unc. Digested	0.70	46.30	4.50	0.050
s.e.m.	0.08	0.53	0.06	0.001
Zn rate 1	0.95	95.05	4.95	0.085
s.e.m.	0.16	9.23	0.30	0.025
Zn rate 2	1.24	109.83	4.90	0.074
s.e.m.	0.19	8.43	0.15	0.006
Zn rate 3	0.97	110.36	4.44	0.059
s.e.m.	0.03	8.76	0.20	0.007
Cu rate 1	0.86	41.39	5.43	0.056
s.e.m.	0.30	0.72	0.17	0.004
Cu rate 2	0.95	51.13	6.35	0.059
s.e.m.	0.18	1.97	0.16	0.006
Cu rate 3	1.30	48.48	6.61	0.068
s.e.m.	0.08	1.92	0.13	0.010
Cd rate 1	0.96	51.43	4.78	0.551
s.e.m.	0.14	1.11	0.11	0.051
Cd rate 2	0.92	44.94	4.27	0.740
s.e.m.	0.10	1.66	0.12	0.059
Cd rate 3	0.70	51.74	4.59	1.006
s.e.m.	0.10	2.99	0.17	0.071

Site 3. Watlington

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	1.52	26.37	4.98	0.030
s.e.m.	0.24	1.23	0.52	0.000
Unc. Digested	2.81	22.77	4.07	0.023
s.e.m.	0.29	1.74	0.33	0.003
Zn rate 1	1.67	51.33	4.42	0.020
s.e.m.	0.10	6.40	0.14	0.000
Zn rate 2	1.44	55.13	4.96	0.020
s.e.m.	0.30	3.32	0.38	0.000
Zn rate 3	1.06	72.38	6.59	0.025
s.e.m.	0.34	5.72	2.02	0.003
Cu rate 1	2.39	25.33	4.84	0.023
s.e.m.	0.24	1.58	0.29	0.003
Cu rate 2	2.24	27.77	5.32	0.030
s.e.m.	0.46	2.38	0.35	0.000
Cu rate 3	1.60	24.00	4.92	0.027
s.e.m.	0.24	1.10	0.14	0.003
Cd rate 1	2.48	24.17	4.35	0.117
s.e.m.	0.17	2.42	0.37	0.003
Cd rate 2	2.62	27.47	4.84	0.177
s.e.m.	0.41	1.82	0.17	0.009
Cd rate 3	2.12	28.37	5.07	0.257
s.e.m.	0.58	3.61	0.93	0.043

Site 5. Rosemaund

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	1.12	39.38	5.23	0.044
s.e.m.	0.08	2.51	0.37	0.006
Unc. Digested	1.59	50.63	5.25	0.057
s.e.m.	0.16	6.76	0.31	0.006
Zn rate 1	1.06	65.37	5.90	0.050
s.e.m.	0.31	11.19	0.29	0.010
Zn rate 2	0.58	99.87	5.90	0.088
s.e.m.	0.20	9.86	0.39	0.007
Zn rate 3	0.68	130.20	6.16	0.082
s.e.m.	0.48	26.10	0.60	0.014
Cu rate 1	1.13	48.33	6.69	0.057
s.e.m.	0.09	3.75	0.42	0.003
Cu rate 2	0.67	48.43	6.52	0.060
s.e.m.	0.32	3.34	0.20	0.005
Cu rate 3	1.79	50.57	6.82	0.075
s.e.m.	0.47	4.29	0.44	0.015
Cd rate 1	1.19	45.10	5.46	0.283
s.e.m.	0.40	4.36	0.37	0.023
Cd rate 2	0.91	67.23	5.54	0.460
s.e.m.	0.26	25.52	0.56	0.049
Cd rate 3	2.15	39.20	4.49	0.463
s.e.m.	0.61	2.83	0.53	0.027

APPENDIX P Crop yield and metal concentrations: metal-amended liquid sludge sites - 2004

Site 2. Woburn

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	3.96	36.66	5.56	0.14
s.e.m.	0.05	2.74	0.22	0.03
Unc. Digested	5.25	44.50	6.60	0.14
s.e.m.	0.14	1.81	0.06	0.02
Zn rate 1	5.36	119.25	6.45	0.09
s.e.m.	0.46	22.09	0.85	0.01
Zn rate 2	5.97	144.35	7.20	0.10
s.e.m.	0.68	11.53	0.42	0.04
Zn rate 3	4.62	213.44	6.27	0.08
s.e.m.	0.19	26.00	0.50	0.02
Cu rate 1	5.57	37.00	7.97	0.12
s.e.m.	0.49	2.69	0.88	0.01
Cu rate 2	5.56	48.37	9.88	0.13
s.e.m.	0.12	2.05	1.29	0.04
Cu rate 3	6.86	46.43	12.40	0.18
s.e.m.	0.62	7.39	1.09	0.02
Cd rate 1	5.28	43.68	6.90	1.21
s.e.m.	0.95	1.61	0.95	0.25
Cd rate 2	4.98	40.99	6.76	1.50
s.e.m.	0.40	5.90	1.36	0.18
Cd rate 3	5.04	43.11	6.33	1.99
s.e.m.	0.36	0.06	0.42	0.36

Site 3. Watlington

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	1.58	20.93	5.37	0.083
s.e.m.	0.22	0.88	0.27	0.003
Unc. Digested	2.15	22.93	6.12	0.073
s.e.m.	0.15	1.10	0.18	0.003
Zn rate 1	2.10	37.70	5.80	0.053
s.e.m.	0.38	2.41	0.36	0.009
Zn rate 2	2.06	47.57	6.26	0.053
s.e.m.	0.53	4.97	0.26	0.009
Zn rate 3	1.92	69.00	6.63	0.060
s.e.m.	0.45	8.15	0.28	0.006
Cu rate 1	1.75	25.90	7.68	0.077
s.e.m.	0.34	0.65	0.43	0.009
Cu rate 2	1.66	23.40	7.25	0.067
s.e.m.	0.13	0.56	0.07	0.003
Cu rate 3	2.24	25.23	7.88	0.073
s.e.m.	0.03	1.99	0.24	0.007
Cd rate 1	2.11	21.90	6.18	0.257
s.e.m.	0.45	0.49	0.13	0.020
Cd rate 2	2.21	19.53	5.28	0.300
s.e.m.	0.23	1.84	0.60	0.015
Cd rate 3	2.44	20.30	5.60	0.450
s.e.m.	0.35	0.47	0.13	0.056

Site 5. Rosemaund

Treatment	Grass yield t DM/ha	Grass Zn mg/kg	Grass Cu mg/kg	Grass Cd mg/kg
Control	3.41	21.85	3.93	0.050
s.e.m.	0.21	0.83	0.14	0.009
Unc. Digested	2.99	27.33	4.15	0.039
s.e.m.	0.34	2.99	0.16	0.002
Zn rate 1	4.19	37.70	3.95	0.047
s.e.m.	0.73	3.91	0.18	0.004
Zn rate 2	2.79	64.73	4.69	0.038
s.e.m.	0.41	9.20	0.20	0.007
Zn rate 3	3.83	73.17	3.96	0.035
s.e.m.	0.24	19.26	0.45	0.003
Cu rate 1	3.71	31.07	4.03	0.032
s.e.m.	0.39	6.25	0.05	0.004
Cu rate 2	4.18	24.23	4.46	0.043
s.e.m.	0.28	1.34	0.22	0.007
Cu rate 3	3.37	30.70	4.30	0.063
s.e.m.	0.20	4.34	0.62	0.014
Cd rate 1	2.97	25.77	4.23	0.055
s.e.m.	0.57	0.33	0.28	0.025
Cd rate 2	3.74	28.23	3.59	0.108
s.e.m.	0.20	5.08	0.12	0.021
Cd rate 3	4.40	26.67	3.90	0.187
s.e.m.	1.00	0.97	0.07	0.044

APPENDIX Q Crop yield and metal concentrations: metal-amended liquid sludge sites - 2005

Site 2. Woburn

Treatment	Grain yield* t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	-	28.27	2.79	0.036
s.e.m.	-	1.66	0.06	0.001
Unc. Digested	-	28.43	3.42	0.043
s.e.m.	-	0.81	0.06	0.004
Zn rate 1	-	67.92	3.99	0.043
s.e.m.	-	9.22	0.28	0.008
Zn rate 2	-	86.40	4.38	0.043
s.e.m.	-	3.37	0.10	0.002
Zn rate 3	-	85.40	3.77	0.046
s.e.m.	-	1.78	0.03	0.005
Cu rate 1	-	28.95	4.47	0.034
s.e.m.	-	1.62	0.09	0.002
Cu rate 2	-	28.19	4.76	0.037
s.e.m.	-	1.25	0.13	0.003
Cu rate 3	-	22.71	3.46	0.028
s.e.m.	-	11.36	1.73	0.014
Cd rate 1	-	29.31	3.49	0.358
s.e.m.	-	0.77	0.08	0.026
Cd rate 2	-	29.17	3.47	0.469
s.e.m.	-	0.77	0.06	0.041
Cd rate 3	-	29.92	3.38	0.605
s.e.m.	-	1.89	0.09	0.052

*Insufficient grain to determine yield measurement

Site 3. Watlington

Treatment	Grain yield* t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	-	-	-	-
s.e.m.	-	-	-	-
Unc. Digested	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 1	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 2	-	-	-	-
s.e.m.	-	-	-	-
Zn rate 3	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 1	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 2	-	-	-	-
s.e.m.	-	-	-	-
Cu rate 3	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 1	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 2	-	-	-	-
s.e.m.	-	-	-	-
Cd rate 3	-	-	-	-
s.e.m.	-	-	-	-

*Insufficient grain to determine yield or grain metal concentrations

Site 5. Rosemaund

Treatment	Grain yield t 85% DM/ha	Grain Zn mg/kg	Grain Cu mg/kg	Grain Cd mg/kg
Control	1.74	35.23	5.73	0.046
s.e.m.	0.41	0.84	0.16	0.007
Unc. Digested	2.06	34.92	5.45	0.041
s.e.m.	0.66	0.69	0.21	0.002
Zn rate 1	1.52	43.60	5.68	0.037
s.e.m.	0.83	6.85	0.47	0.007
Zn rate 2	1.16	61.73	5.98	0.043
s.e.m.	0.46	3.54	0.24	0.008
Zn rate 3	1.21	65.33	5.14	0.035
s.e.m.	0.35	3.65	0.27	0.008
Cu rate 1	2.15	38.13	6.50	0.028
s.e.m.	0.88	2.88	0.20	0.011
Cu rate 2	1.57	36.00	5.83	0.065
s.e.m.	0.89	1.61	0.43	0.010
Cu rate 3	1.55	34.93	5.48	0.038
s.e.m.	0.63	2.37	0.39	0.002
Cd rate 1	1.85	37.33	5.53	0.155
s.e.m.	0.90	1.24	0.15	0.012
Cd rate 2	2.23	34.27	4.97	0.283
s.e.m.	0.24	2.39	0.30	0.041
Cd rate 3	1.97	35.00	5.39	0.325
s.e.m.	0.25	2.21	0.23	0.033