THE TRANSFER AND FATE OF CADMIUM AND ZINC FROM SEWAGE SLUDGE AMENDED AGRICULTURAL SOIL IN AN ARTHROPOD FOOD CHAIN

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ABSTRACT

The transfer and fate of cadmium and zinc from a sewage sludge amended agricultural soil in an arthropod food chain

Ian David Green

The re-cycling of sewage sludge is the most significant entry point for trace metals in to the agroecosystem. However, the investigation of the transfer and fate of trace metals in soil-plant-arthropod systems in an agricultural context has received little attention, despite the potential threat that secondary toxicity to predatory arthropods poses to the biological control of crop pests. In this study, an agricultural soil was amended with sewage sludge at rates up to an equivalent of 100 t (dry solids) ha⁻¹. The subsequent transfer of Zn and Cd through an ecologically relevant soil-crop-aphid-arthropod predator system was investigated in a series of pot trials. Results show that Zn was transferred to a greater extent than Cd between all components of the system, except between the roots and shoots. Cadmium was only biomagnified in roots and was biominimised in shoots, aphids and ladybirds. Zinc was biomagnified in roots, shoots, and aphids compared to the soil, but concentrations in ladybirds were similar to those in the aphids they consumed. Differences between winter and spring wheat were found to have a larger influence on the transfer of Cd and Zn in the system than differences between winter wheat and spring barley. It was also shown that the rose grain aphid (Metopolophium dirhodum) accumulates higher concentrations of Cd than the grain aphid (Sitobion avenae). Whilst concentrations of Zn did not differ between the two species of aphid, concentrations in M. dirhodum appeared to be more closely regulated than in S. avenae. Consumption of S. avenae by the fourth instar larvae of the seven-spotted ladybird (Coccinella septempunctata) did not result in significant differences between treatments in newly emerged adults. This was partly the result of the sequestration of the two metals in the pupal exuviae. This mechanism had a greater effect on the Cd concentrations in newly emerged adult ladybirds than on Zn concentrations. In a further experiment, there appeared to be no pathway for the transfer of Cd from aphids to adult ladybirds, but a pathway was indicated for Zn. The implications of the results are discussed in relation to the agroecosystem and the wider environment.
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1.0 INTRODUCTION

Figure 1.1. The potential consequences of trace metal contamination of agricultural soils. The concentrations of Zn in the soil of this field have been raised to ca 1200 mg kg\(^{-1}\) by additions of sewage sludge. The phytotoxicity of the trace metals in the soil is such that crop production was uneconomical and the field has been left fallow.

1.1 Introduction

Biomagnification describes a process whereby the concentration of a substance is greater in an organism than in the food on which it feeds (Janssen, 1993). During the 1960s it was realised that certain chemicals were biomagnified during transfer through food chains. This led to the hypothesis that the biomagnification of contaminants is a general property of food chains (Moriaty and Walker, 1985; Janssen et al., 1993). However, subsequent investigations into the extent of trace metal biomagnification in arthropod food chains reported mixed results (van Straalen and Wensem, 1986; Laskowski and Maryanski, 1993). For instance, Pb was shown to be biomagnified in invertebrate food chains in roadside ecosystems contaminated by vehicle exhausts (Price et al., 1974). Cadmium, derived from metalliferous mine tailings, was shown to be biomagnified by both herbivore and predatory arthropods, with concentrations in predators 3-4 times higher than in herbivores (Roberts and Johnson, 1978). Furthermore, Hunter and Johnson (1982) found biomagnification of both Cu
and Cd in both herbivorous and predatory arthropods in a grassland ecosystem polluted by emissions from a metal smelting factory. However, other reports conflict with these results. For example, Williamson and Evans (1972) found no biomagnification of Pb in a vehicle-contaminated roadside, Beyer et al. (1985) found no evidence of Cd, Cu, Pb or Zn biomagnification in an ecosystem contaminated by emissions from a metal smelter and Kratz et al. (1985) found no evidence of Cd biomagnification in a ruderal ecosystem deliberately contaminated with Cd salts. The widely differing results of these trials has been attributed to inadequacies in the methods employed, which failed to analyse organisms individually and instead grouped diverse kinds of organisms into the broad categories of predator and carnivore (van Straalen and van Wensem, 1986; Beyer, 1986).

Van Straalen and van Wensem (1986) surveyed the metal body burdens of 13 invertebrates living in the litter of a forest contaminated by Zn smelter emissions. Eleven of the 13 species were analysed individually to preclude the criticisms of earlier methods. The invertebrates contained varied concentrations of Cd and Zn that were not accounted for by their trophic level or body size, but by their specific physiology. A larger survey of 23 invertebrate species in the same ecosystem also found no clear relationship between Cd body concentration and trophic level or body weight (Janssen, 1988). The conclusion was, again, that the physiology of the species, assessed by their ability to store and excrete metals, was more important in determining Cd concentrations than trophic level (Janssen, 1988). The same conclusion was reached in a study of soil dwelling invertebrates in 4 Polish forests with differing levels of trace metal contamination (Laskowski and Maryanski, 1993).

The nature and efficacy of metal regulating physiology in arthropods can vary widely, even among closely related species feeding on similar diets (Janssen, 1991; Rabitsch, 1995; Glowacka et al., 1997). Species exist with physiology that results in a poor ability to regulate trace metals (Janssen et al, 1991; Kramarz, 1999b). Consumption of contaminated food leads to the accumulation of trace metals within these species (Janssen et al, 1991; Kramarz, 1999b). If a group of such organisms forms a food chain, a 'critical pathway' may
become established along which the biomagnification of trace metals occurs, potentially endangering carnivores (van Straalen and Ernst, 1991).

Agroecosystems receive trace metal inputs from a variety of natural and anthropogenic sources. Nevertheless, the transfer of trace metals in soil-plant-arthropod food chains in agroecosystems has received little attention in the published literature. This is despite the economic and ecological importance of arthropod populations. For example, agricultural land can support a diverse arthropod fauna, which may exceed 900 species in the UK alone (Potts, 1990). Few of these species are considered to be of direct conservation value (Potts, 1990), but many form an important component of the diet of some species of farmland birds that are declining in number (Wilson et al., 1999) or are economically important as game (Borg and Toft, 2000). Furthermore, some arthropods are agricultural pests that can reduce crop yields (George and Gair, 1978; Soffe, 1997). Carnivorous arthropods can contribute to keeping pest densities below economically damaging densities and can thus be important biological control agents (Chambers et al., 1986; Sutherland et al., 1987; Wratten and Powell, 1990; Ekbom et al., 1992; Lang et al., 1999). For example, Östman et al. (2003) attributed a yield increase of 303 kg ha\(^{-1}\) in wheat to effects of ground living arthropod predators on the abundance of aphids.

Detrimental effects caused by biomagnification of trace metals in critical pathways may therefore have significant ecological and/or economic impacts. However, the current knowledge of trace metal transfer in agroecosystems is insufficient to determine the level of contamination at which significant impacts occur to arthropod predators (Merrington et al., 1997a; Merrington et al., 1997b; Winder et al., 1999).

1.2 Literature Review

1.2.1 Introduction

Much research has been conducted on the behaviour of trace metals in the environment because some are essential nutrients and all of them can cause toxicity above a certain
threshold concentration. Consequently, there is a vast body of published literature regarding their transfer in the soil-plant system and accumulation in invertebrates. In the latter case this is primarily concerned with soil dwelling fauna. Some selection of the literature included in this review was therefore required. This was done on the basis that selected work made a contribution to the current understanding of the mechanisms determining the transfer of trace metals from sewage sludge amended soil through the soil-crop-aphid system.

1.2.2 Sewage sludge as a source of trace metal in agricultural soils

Trace metals can enter agricultural soils from many sources such as the weathering of soil parent material, atmospheric deposition (Critchly and Agg, 1986), or from the use of common agricultural materials (Table 1.1). However, sewage sludge is the principle source of trace metal additions where it is applied to agricultural soils (Nriagu and Pacyna, 1988).

Table 1.1 Reported mean concentrations (mg kg⁻¹) of selected trace metals in various agricultural materials commonly applied to soils (Smith, 1996; Raven and Loeppert, 1997) and in sewage sludge used agriculturally in the UK in 1996/7 (Gendebien et al., 1999).

<table>
<thead>
<tr>
<th>Material</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial fertiliser</td>
<td>&lt;0.2</td>
<td>2.14</td>
<td>&lt;0.6</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>35.5</td>
<td>88.9</td>
<td>41.8</td>
<td>48.3</td>
<td>13.2</td>
<td>386</td>
</tr>
<tr>
<td>Rock phosphate</td>
<td>1.3</td>
<td>33.2</td>
<td>9.6</td>
<td>16.8</td>
<td>4.6</td>
<td>78.8</td>
</tr>
<tr>
<td></td>
<td>48.8</td>
<td>140</td>
<td>50.4</td>
<td>29.2</td>
<td>78.2</td>
<td>382</td>
</tr>
<tr>
<td>Liming agents</td>
<td>&lt;0.2</td>
<td>32.3</td>
<td>2.3</td>
<td>1.4</td>
<td>0.7</td>
<td>8.01</td>
</tr>
<tr>
<td>Cattle manure</td>
<td>0.4</td>
<td>14.4</td>
<td>17.5</td>
<td>6.2</td>
<td>2.6</td>
<td>164</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>3.3</td>
<td>157</td>
<td>568</td>
<td>57</td>
<td>221</td>
<td>792</td>
</tr>
</tbody>
</table>

The concentrations of trace metals in sewage sludges typically exceed those of the soil (Tables 1.2). Consequently, its application may lead to increased trace metal concentrations in soil. Furthermore, it is widely believed that on reaching the plough layer of soils, many trace metals remain indefinitely, and therefore repeated additions may lead to long-term accumulation (McLaughlin et al., 2000; Smith 2001). To prevent accumulation having an
adverse effect on agricultural practices (e.g. Figure 1.1), controls on the agricultural use of sewage sludge, in the form of legislative frameworks and codes of practice, have been introduced in many developed countries (Smith, 1996; Renner, 2000).

Table 1.2 Trace metal concentrations (mg kg\(^{-1}\)) found in sewage sludges produced in the UK in 1996/1997 (Gendebien et al., 1999) and in the soils of England and Wales (McGrath and Loveland, 1992).

<table>
<thead>
<tr>
<th>Trace metal</th>
<th>10 percentile</th>
<th>Median</th>
<th>90 percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil</td>
<td>Sludge</td>
<td>Soil</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.2</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Chromium</td>
<td>15</td>
<td>12</td>
<td>39</td>
</tr>
<tr>
<td>Copper</td>
<td>9</td>
<td>192</td>
<td>18</td>
</tr>
<tr>
<td>Lead</td>
<td>20</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Nickel</td>
<td>7</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Zinc</td>
<td>38</td>
<td>347</td>
<td>82</td>
</tr>
</tbody>
</table>

Large quantities of sewage sludge are produced during the treatment of wastewater (Gendebien et al., 1999). This poses a disposal problem requiring an economical and environmentally safe solution (Pimentel and Warneke, 1989). In the UK, approximately 50% of sewage sludge production is applied to agricultural land as organic fertiliser (Gendebien et al., 1999). This beneficially recycles the nitrogen and phosphorous in sewage sludge and can reduce the consumption of limited mineral phosphate sources (Hall, 1992; Steen and Agro, 1998). Sewage sludge production is predicted to increase (Gendebien et al., 1999) and combined with the banning of ocean dumping in 1998, it is likely that there will be increasing pressure to apply sewage sludge to agricultural soils.

Beneficial re-cycling of sewage sludge is restricted by the presence of potentially toxic elements, including trace metals, in sewage sludges (Smith, 1996). Trace metals enter wastewater from permitted industrial discharges (Cd, Cr, Pb and Ni), domestic plumbing (Cu, Pb), road runoff (Pb, Zn) and from the use of baby creams, shampoos and cosmetics (Zn) (McGrath et al. 1994; Smith, 1996). The bulk of trace metals are associated with the solid
phase of wastewater (McGrath, 1994). This phase is removed during treatment (summarised in Figures 1.2a to 1.2f) forming a sewage sludge highly enriched in trace metals as a by-product (McGrath, 1994).

Figure 1.2a. Wastewater entering a treatment plant.

Figure 1.2b. Wastewater in a settlement Tank; the first stage of treatment.

Figure 1.2c. The solid phase settles to the bottom of the tank and is pumped to a sludge digestion plant.

Figure 1.2d. An anaerobic sludge digestion plant looking across the drying lagoons. The volume of sludge is reduced by 70% in the digestion process.

Figure 1.2e. Sewage sludge entering a drying lagoon. The digested sludge leaves the digester with a dry matter content of *ca* 5 %. This may be injected directly into agricultural soil or the dry matter content can be increased to *ca* 20 % in lagoons to form a sludge 'cake'.

Figure 1.2f. Sludge cake ready for incorporation into an agricultural soil
1.2.3 Controls governing the agricultural use of sewage sludge

In the member states of the European Union, the agricultural use of sewage sludge is controlled by European Community Directive 86/278/EEC (CEC, 1986). Three types of trace metal limits are set out (i) the maximum concentration allowed in sludge for agricultural use, (ii) the maximum concentrations allowable in sludge amended soils and (iii) the 10 year average rate of metal addition (McGrath et al., 1994). The aim of the regulations is to prevent the input of trace metals reaching levels that may cause phytotoxicity to crops, or that may cause harm to human and animal health (McGrath et al., 1994). The metal limits set by the directive represent maximum values allowable and countries within the EU are free to set metal limits below these figures. The metal levels set by directive 86/278/EEC and the relevant UK legislation (UK, 1989) are given in Table 1.3.

In the United States a risk assessment using the transfer of 9 trace metals in 14 potential transfer pathways was used to develop a legislative frame work, 40 CFR Part 503 (US EPA 503, 1993). For each of the 14 pathways the maximum metal concentration to which the terminal organism could be exposed without causing an unacceptable risk was calculated. The pathway that gave the lowest acceptable concentration for each metal was then used to set a maximum cumulative pollutant loading for each metal (Harrison et al., 1999). The regulations also define the annual loading rate of metals and the level of metals permissible in 'Exceptional Quality' sludges, which may be applied to soil without restrictions on the amount or duration of application (Harrison et al., 1999).

Table 1.3. Comparison of the maximum concentration of trace metals (mg kg⁻¹) allowed to accumulate in agricultural soils as a result of sewage sludge application in the EU, US and UK (McGrath et al., 1994).

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>1-3</td>
<td>50-140</td>
<td>30-75</td>
<td>50-300</td>
<td>150-300</td>
<td>1-1.5</td>
</tr>
<tr>
<td>UKᵃ</td>
<td>3</td>
<td>135</td>
<td>75</td>
<td>300</td>
<td>300</td>
<td>1</td>
</tr>
<tr>
<td>USAᵇ</td>
<td>20</td>
<td>750</td>
<td>210</td>
<td>150</td>
<td>1400</td>
<td>8</td>
</tr>
</tbody>
</table>

ᵃ Values for soils in the pH range 6-7. Other values apply for soils of a pH of 5-6 and above pH7.
ᵇ Calculated from the cumulative pollutant loading without taking into account the native metal concentration.
A comparison of values in Table 1.3 demonstrates that the US regulations are far less stringent than those adopted by the EU. The US regulations allow larger concentrations of metals in soil, allow metals to be applied at larger annual rates and allow sludge with a greater degree of metal contamination to be applied to soil (McGrath et al., 1994). Consequently, the effectiveness of the US regulations has been questioned (McBride, 1995; Schmidt, 1997; McBride, 1998; Harrison et al., 1999; McBride, 2003a; McBride, 2003b). Harrison et al. (1999) particularly point to fundamental errors in the assessment structure on which the US regulations are based. For instance, they point to the use of a number of untenable assumptions and serious omissions in the assessment, such as the disregard of synergistic effects, the assumption that organisms are only exposed through single pathways and the inadequate assessment of ecological effects (Harrison et al., 1999).

The philosophy behind the controls in the EU and USA appears to regard agricultural land as an industrial site for the production of food. This has led to little consideration of the effects trace metals may have on organisms present in agroecosystems other than those that have, or may have, an effect on soil fertility i.e. earthworms, *Rhizobium* spp. and arbuscular mycorrhizal fungi (MAFF/DoE, 1993). Despite the ecological and economic importance of arthropods, this component of the agroecosystem has been insufficiently considered in the drafting of controls for the use of sewage sludge in the European Union and the United States. Currently, little is known about the possible detrimental effects that trace metals may have on arthropods. It remains to be determined if the present soil trace metal limits are sufficiently restrictive to protect arthropods in agroecosystems. This has led to the questioning of the efficacy of the current controls (Merrington et al., 1997a; Merrington et al., 1997b; Winder et al., 1999).

### 1.2.4 Phytoavailability of trace metals

Trace metal uptake by plants is dependent on the way in which the metal is associated with the soil. These associations can be generalised to occur with five operationally defined
fractions in the soil (Kabata-Pendias and Pendias, 2000). These are in the soil solution (either as a free cation or in soluble organic or inorganic complexes) or sorbed to cation exchange sites, carbonates and hydrous or amorphous oxides of Fe/Mn, particulate organic matter and as sulphides or within the crystal structure of minerals (Markert, 1994; Rieuwerts et al., 1998). Distribution among the five fractions is related to the strength of trace metal sorption to each fraction. This varies among trace metals (Table 1.4) and can result in large differences in speciation amongst trace metals (Kabata-Pendias and Pendias, 2000; Hammer and Keller, 2002; Yoo and James, 2002; Holm et al., 2003).

Table 1.4 The relative binding strength of selected trace metals to three soil constituents with the largest influence on trace metal adsorption in soil (Kabata-Pendias and Pendias, 2000; Blume and Brummer, 1991).

<table>
<thead>
<tr>
<th>Soil component</th>
<th>Strength of sorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
</tr>
<tr>
<td>Clay minerals</td>
<td>Fe$^{3+}$</td>
</tr>
<tr>
<td>Fe/Mn oxides</td>
<td>Cr$^{3+}$, Hg, Pb</td>
</tr>
<tr>
<td>Organic matter</td>
<td>Cr$^{3+}$, Fe$^{3+}$, Pb, Hg</td>
</tr>
</tbody>
</table>

The concentration and distribution of trace metals in the five fractions strongly influences the supply of trace metals to the plant (Grant et al., 1997; Rieuwerts et al., 1998). Trace metals present in the soil solution and adsorbed to cation exchange sites on clay and humus are considered to be the most phytoavailable (Kabata-Pendias and Pendias, 2000). However, trace metals in these two fractions represent only a small proportion of the total in soil (Kabata-Pendias and Pendias, 2000). Plants can also access trace metals bound to carbonates, insoluble organic matter and Mn oxides, but not Fe oxides (Hammer and Keller, 2002). Trace metals sorbed to organic matter, sulphides or within the crystal structure of minerals, are also unavailable directly to the plant (Hammer and Keller, 2002). The soil fractions from which a plant species can take up trace metals depend on the plant species and soil chemistry (particularly carbonate content and pH; Hammer and Keller, 2002).
Trace metal speciation is strongly influenced by the physio-chemical parameters of the soil (Kabata-Pendias and Pendias, 2000). The amount of organic material, silt and clay content, solute behaviour, redox conditions, content of electrolytes, pH, and the composition of ions in the soil solution are reported to influence speciation (Punz and Seighardt, 1993; Rieuwerts et al., 1998; Yoo and James, 2002). Interactions can occur between some of these properties (Rieuwerts et al., 1998;). For example, cation exchange capacity increases with increasing pH (Yoo and James, 2002). The most important parameter in determining phytoavailability is pH (Sauerbeck, 1991; Smith, 1996; Puschenreiter and Horak, 2000). However, the effect of changing soil pH on the availability of trace metals differs among elements (Sauerbeck, 1991; Planquart et al., 1999; Smith, 2001). A pH decrease significantly increases the phytoavailability of Cd, Ni and Zn, slightly increases Cu phytoavailability and causes no change in the already low phytoavailability of Pb (Sauerbeck, 1991; Puschenreiter and Horak, 2000).

Plants can influence some chemical parameters of the soil surrounding their roots (the rhizosphere) to increase or decrease their uptake of trace metals (Markert, 1994). For example, plant roots can change rhizosphere pH by up to 2 units through the excretion of H⁺, H₃O⁺ or HCO₃⁻ (Marschner, 1995; Punz and Sieghardt, 1993; Hammer and Keller, 2002; Jauert, et al., 2002). Roots can also change rhizosphere redox potential by discharging H₃O⁺ and O₂ (Markert, 1994). These strategies also increase the availability of non-essential and potentially toxic metals.

Roots can also mobilise trace metals in to extractable forms through chelation to exudates such as low molecular weight organic acids (Krishnamurti et al., 1997) and Phytosiderophores (von Wiren et al., 1996; Shenker et al., 1998). For example, Mench and Martin (1991) demonstrated that unidentified root exudates of Nicotiana spp. could increase the extraction of Cu and Cd from soil. The extraction efficiency of the exudates was correlated with phytoavailability of the two metals, indicating that they are instrumental in
facilitating trace metal uptake. Furthermore, the concentration of low-molecular weight organic acids excreted in to the rhizosphere by durum wheat roots appeared to be positively related to the uptake of Cd by the plant (Cieslinski et al., 1998).

Although the matrix of sewage sludges contain between 20 and 75 % organic matter (Albiachi et al. 2001; Jakubus and Czekala, 2001), inorganic as well as organic binding components are also present in the sludge matrix (Alloway and Jackson, 1991; Legret, 1993; Jakubus and Czekala, 2001; Li et al., 2001). The physio-chemical properties of the sewage sludge can be highly variable, as can the speciation of trace metals (Legret, 1993; Jakubus and Czekala, 2001). However, sewage sludge amendments can significantly elevate the concentration of Cd, Cu, Cr, Ni and Zn in the three most phytoavailable soil fractions (Sloan et al., 1997). Consequently, the agricultural use of sewage sludge may result in the elevation of trace metals in a wide variety of crop plants (Sauerbeck, 1991; McGrath et al., 2000).

Phytoavailability of trace metals from sewage sludge amended soils varies not only with the soil physio-chemical parameters, but also with plant species and element (Sauerbeck, 1991). Cadmium and Zn are consistently found to be the most labile trace metals, with typical transfer co-efficients (the ratio of plant to soil trace metal concentration) between 1 and 10 (Sauerbeck, 1991). Copper and Ni are less labile with typical transfer co-efficients of between 0.1 and 1. The lowest transfer co-efficients (between 0.01 and 0.1) are reported for Pb and Cr$^{3+}$ (Sauerbeck, 1991). The relatively high mobility of Cd and Zn in the sewage sludge amended soil-plant system suggests that these trace metals will have the greatest potential to be transferred further along the food chain. This mobility is combined with the relatively high concentrations of Zn in sludges (Table 1.2a) and the high toxicity of Cd (Stoeppler, 1989). Consequently, these two metals have the greatest potential to cause harm to the wider agro-environment when amendments of sewage sludge have been made to soil (MAFF/DoE, 1993).
1.2.5 Uptake and translocation of trace metals by plants

To endanger the above-soil arthropod herbivore fauna, trace metals must be transported from the soil to plant shoots. Hart et al. (2002) demonstrated that in bread- and durum- wheat, this process begins by the absorption of Cd\(^{2+}\) and Zn\(^{2+}\) from the soil over the plasma membrane of roots by a shared high affinity trans-membrane transporter. This process is metabolically dependent (Hart et al., 1998a; Hart et al., 1998b; Hacisalihoglu et al., 2001; Hart et al., 2002) and probably driven by an H\(^{+}\)-ATPase system that pumps protons through the plasma membrane of the root cell into the soil solution (Mengel and Kirby, 1990; Grant et al., 1998).

This results in a negative charge in the cytoplasm compared to the exterior of the cell. The trans-membrane carrier protein shuttles cations over the plasma membrane down the electrochemical gradient created by the proton pump (Mengel and Kirby, 1990). The typical differences in charge between the cytoplasm and the exterior of the cell are reported to be in the order of 100 - 150mV (Grant et al., 1998). This is a sufficiently large electrical potential gradient for root cells to accumulate considerable quantities of cations against a concentration gradient (Mengel and Kirby, 1990; Kochain, 1991; Marschner, 1995).

It has been demonstrated that the presence of Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\), Mn\(^{2+}\) and Zn\(^{2+}\) has an antagonistic effect on cadmium uptake (Jarvis et al., 1976; Cataldo et al., 1983; Hardiman and Jacoby, 1984; Smilde et al., 1992). This suggests that the same trans-membrane carrier system is involved in the uptake of all these cations (Cataldo et al., 1983). The trans-membrane transport protein has a tendency to bind with cations with lower hydration energies (Mengel and Kirby, 1990). Divalent cations with lower hydration energies will be more readily transported across the plasma membrane as a consequence and this results in the system having some selectivity (Mengel and Kirby, 1990). This would suggest that the carrier system would have a greater affinity for Cd than Zn (enthalpy of hydration 1807 KJ mol\(^{-1}\) for Cd, 2046 KJ mol\(^{-1}\) for Zn) and this is supported by experimental evidence (Hart et al., 2002).
Uptake by roots is expected to deplete the phytoavailable fraction of trace metals in the rhizosphere (Hinsinger, 2001), potentially limiting plant uptake. Consequently, the rate of trace metal uptake by plants is determined by the supply of trace metals in phytoavailable forms to plant roots (Mullins et al., 1986). Three processes are involved in this supply. The first is diffusion down the concentration gradient between the depleted rhizosphere and the bulk soil (Marschner, 1995). The second process is mass flow of trace metals in the soil solution. This is driven by the water potential gradient that develops because of the transpiration driven uptake of water by roots (Marschner, 1995; Jungk, 1996). These two processes are interdependent (Jungk, 1996). The results of Mullins et al. (1986) indicated that mass flow supplied 25% of Zn taken up by maize seedlings grown in sewage sludge amended soil. Mass flow also supplied a significant proportion of the Cd taken up by the seedlings. The third process involves the uptake of trace metals by roots growing into previously untapped soil (root interception; Marschner, 1995). The last process is thought to be responsible for a relatively small proportion of trace metals supplied to roots (Marschner, 1995; Jungk, 1996). Consequently, diffusion appears to be the most important process in supplying trace metals to plant roots (Mullins et al., 1986; Marschner, 1995).

From the cells on the surface of the root, ions are transported through the root cortex in both the symplast and apoplast to the endodermis (Punz and Sieghardt, 1993; Figure 1.3). The impervious casparian strip surrounding the endodermis cell walls prevents apoplastic transport and forces trace metals to move through the cytoplasm of the endodermal cells. Consequently, it is believed that the endodermis acts as a semi-permeable membrane across which ions can be regulated and actively transported into the pericycle (Punz and Sieghardt, 1993). From the pericycle, trace metals must be loaded into the xylem sap for transportation to the shoot. The mechanisms involved in the loading process therefore have an important influence on the exposure of herbivores to trace metals. However, these mechanisms remain unknown (Welch, 1995).
Figure 1.3. A simplified diagram of the symplastic and apoplastic transfer of trace metal from the soil to xylem sap (adapted from Kochain, 1991). Tm⁺ - Trace metal ion.

When in the xylem sap, trace metals may be transported as either free metal ions (Petit and van de Geijn, 1978; Hardiman and Jacoby, 1984; Cataldo et al., 1988) or bound to ligands (Cataldo et al., 1988; Salt et al., 1995b). These are reported to be organic acids and amino acids/peptides (anionic and cationic; Cataldo et al. 1988). The type of ligand is metal-specific and each metal may be bound to more than one type (Cataldo et al. 1988). Transport of trace metals in the xylem vessels may be by simple bulk transport or by a chromatographic exchange process (Petit and van de Geijn, 1978; Hardiman and Jacoby, 1984). The exact process appears to be dependent on the metal and the charge of the metal-ligand complex (Petit and van de Geijn, 1978). In both processes, trace metal translocation in the xylem is proportional to the rate of transpiration (Hardiman and Jacoby, 1984; Salt et al., 1995b; Grifferty and Berrington, 2000).
As well as the xylem system, higher plants possess a second vascular transport system, phloem. This system transports various metabolites around the plant (Welch, 1995). It has been demonstrated that translocation in the phloem sap can result in the redistribution of Cd, Co, Ni and Zn from the stem, flag leaf and peduncle to the ears of bread wheat (Herren and Feller, 1997; Zeller and Feller, 1999) and a similar redistribution of Cd also occurs in durum wheat (Cieslinski et al., 1996; Harris and Taylor, 2001). In addition, re-translocation of trace metals to the roots of wheat plants also occurs by phloem transport (Cakmak et al., 2000b). The high levels of carbohydrate in phloem sap make it a useful food source that may be exploited by liquid-feeding arthropods (Glowacka et al., 1997). Consequently, transport of trace metals in phloem sap has a significant influence on the exposure of liquid feeding herbivorous arthropods.

Trace metals can enter the phloem sap by selective transport from the xylem vessels within the stem or by re-mobilisation from other plant tissues (Pearson et al., 1995; Cakmak et al., 2000a; Cakmak et al., 2000b; Erenoglu et al., 2002), especially in the nodes (van Bel, 1990) and leaf vein network (Pate, 1975). In wheat plants, the flag leaf and peduncle appear to be important sites for the transfer of trace metals between xylem and phloem (Herran and Feller, 1994; Pearson et al., 1995; Herren and Feller, 1997; Zeller and Feller, 1999). Two distinct processes, with different control mechanisms, seem to be involved in this transfer; (i) removal of metal from the xylem; (ii) loading of metal into the phloem sap (Herran and Feller, 1994). At high concentrations Zn is removed from the xylem in the stem of wheat, where it is retained and not loaded in to the phloem (Herran and Feller, 1994). Factors affecting the loading and unloading of trace elements in phloem are thought to be pH, E_h, ionic strength and organic constituents within the phloem sap (Welch, 1995). As in xylem fluid, the majority of trace metals in phloem sap are generally in the form of complexes rather than ions (van Goor and Wiersma, 1976; Welch, 1995).
Transport of trace elements within the phloem is by mass transport, driven by the up-loading of phloem within source tissues and the down-loading of sucrose at sink tissues (Welch, 1995). The mobility of metals within the phloem varies with the metal and is also influenced by competition between metals and, at high concentrations, the toxic effects of metals on phloem transport (Welch, 1995; Herren and Feller, 1997; Zeller and Feller, 1999). Phloem vessels are metabolically active and may regulate the levels of trace metals (Welch, 1995; Herran and Feller, 1996; Zeller and Feller, 1999) and the form in which they are present in phloem sap (Welch, 1995).

The distribution of a trace metal between root and shoot will partially reflect the balance between xylem transport from the root to shoot and phloem transport from shoot to root. Overall, the translocation of trace metals typically results in a pattern of distribution within plants where metal concentration falls in the order root-shoot-reproductive organs (Petterson, 1976; Lübben and Sauerbeck, 1991; Cieslinski et al., 1996; Merrington et al., 1997b). Typically, over 50% of the trace metal content of a plant is in the roots (Petterson, 1976). However, there are large differences in the extent of root to shoot trace metal translocation between species (Jarvis et al., 1976; Petterson, 1976; Sauerbeck, 1991; Guo and Marschner, 1995; Hart et al., 1998a). Cadmium, particularly, stands out as an exception to the normal pattern as it is found in higher concentrations in shoots of some species, e.g. flax and leafy vegetables such as spinach (Sauerbeck, 1991; Cieslinski et al., 1996). Consequently, crop species may influence the transfer of trace metals from the soil to herbivores.

1.2.6 Trace metals in arthropods of the agroecosystems

The only arthropod group for which trace metal accumulation has been studied at the species level in an agricultural context is the Aphididae. Laboratory, glasshouse and field trials have demonstrated that compared to their host plant, aphids can biomagnify Cd (Crawford et al., 1995; Merrington et al., 1997b) and Zn (Merrington et al., 1997a;
Merrington et al., 1997b), but not Cu (Crawford et al., 1995). Reported biomagnification factors (Trace metal concentration in the aphid/concentration in the plant) are reported in the order of 7-10 for both metals (Crawford et al., 1995; Merrington et al., 1997a; Merrington et al., 1997a). Aphids are soft bodied, colonial, immobile for long periods and give off enticing odours, which make them attractive prey (Rotheray, 1989). The data of Crawford et al. (1995) and Merrington et al. (1997a; 1997b) demonstrate that in contaminated environments there is the potential for secondary poisoning of aphid predators.

There is the potential for the biomagnification of trace metals in the soil-plant-aphid system to form part of a critical pathway. Trace metal uptake kinetics are currently known for only four species of arthropod predator. Of these Neobisium muscorum showed a negligible ability to regulate its Cd body burden (Janssen et al. 1991), whilst Lithobius mutalis demonstrated little ability to regulate Cd and Zn (Kramarz, 1999b). There are an estimated 300 species of polyphagous predators that feed on aphids in the agroecosystems of the UK (Burn, 1987). Together with obligate aphid predators such as hover fly larvae, ladybirds, aphid midges, flower bugs, and lacewing larvae, this represents a large number of predatory arthropods that are potentially vulnerable to trace metal contamination.

Although invertebrates dwelling in and on the surface of soil have been studied thoroughly in many environments (e.g. van Straalen and van Wensem, 1986; Janssen, 1988; Laskowski and Maryanski, 1993), only one large-scale field study of trace metal transfer in arthropods from an agroecosystem is evident in the literature (Butovsky and van Straalen, 1995). This study investigated oat/clover and ryegrass/clover ecosystems contaminated by Cu and Zn from a major road system. However, the flawed methods of earlier studies of grouping diverse ranges of arthropods into arbitrary units were repeated, making it difficult to draw conclusions from this source. To predict those species at risk from biomagnification in the soil-plant–arthropod system requires a detailed knowledge of the accumulation of trace metals by the component species. But not enough is presently known about the transfer of trace metals in arthropod
food chains in agroecosystems to make such predictions. Research is needed to supplement the existing data on the transfer of trace metals in the soil-plant–arthropod system and to establish if a critical pathway or pathways exist in agroecosystems. This is important given the widespread application of trace metal contaminated sewage sludge to agricultural land and the ecological and economic importance of arthropods.

1.3 The present study

Cadmium and Zn possess similar chemical properties (Chesworth, 1991) and are known to be amongst the most labile trace metals in the soil-plant system (Sauerbeck, 1991). However, they differ in that while Zn is an essential element for plants (Römheld and Marschner, 1991) and animals (Miller et al., 1991), Cd has no known essential function in metazoan organisms. It has been suggested that concentrations of non-essential trace metals are less tightly regulated by arthropods (Laskowski, 1991; Kramarz, 1999b). Consequently, the two metals may exhibit contrasting behaviour in the soil-plant-arthropod system. The intention of this study was to add to the knowledge of the transfer and fate of sewage sludge derived Cd and Zn in arthropod food chains in agroecosystems.

The philosophical basis for this investigation is represented diagrammatically in Figure 1-4. The soil-plant-arthropod system is considered as a series of ‘black boxes’ between which Cd and Zn are transferred. Transfer is only considered in one direction (solid black arrows in Figure 1.4). Pathways exist that transport trace metals in the opposite direction, but these transfer a negligible quantity of metal (e.g. shoot to root transfer; Florijn et al., 1993 b; Guo and Marschner, 1995; Cakmak et al., 2000b), or are difficult to quantify (e.g. arthropod faeces ‘rain’ on to soil, decomposition of dead arthropod bodies in the soil).

The aim of this study is to investigate the transfer of Cd and Zn between these black boxes and to determine between which boxes the transfer of the metals is restricted or results in biomagnification. In order to do so, the soil-plant-arthropod system was modelled in four pot
based experimental systems. The experimental systems were designed to answer three main objectives (highlighted on Figure 1.4).

Figure 1.4 A simplified diagram of the transfer of trace metals through a soil-plant-arthropod food chain (solid black arrows represent pathways studied in this investigation, while dashed lines represent pathways not considered).
2.0 MATERIALS AND METHODS

Figure 2.1. An atomic absorption spectrometer fitted with a slotted atom trap. The trap is a glass tube with a slot cut in the top and bottom that the flame flows through. The upper slot is 10 mm shorter at the top, restricting the flow of atoms. Consequently, the atoms spend longer in the beam of light, resulting in higher light absorbance, and therefore sensitivity, for a given concentration in the analyte.

2.1 Introduction

In this investigation, the transfer of Cd and Zn through an agriculturally relevant soil-plant-arthropod system was modelled in a series of four pot trials. Trace metal uptake by plants in pot trials is known to be generally higher than in field trials (de Vries and Tiller, 1978; Logan and Chaney, 1983; Mullins et al., 1986; McBride, 1995). This is thought to be due to higher transpiration rates, inability of roots to penetrate below the contaminated zone and optimal light and soil moisture conditions (de Vries and Tiller, 1978; Mullins et al., 1986). However, field trials have been criticised because of the high degree of unexplained variability caused by the heterogeneity of field conditions (McBride, 1995). In addition, a
high level of aphid predation complicates experimentation with aphids in small-scale field trials. This often results in only a small mass of aphid material, which can affect precision during analysis (Merrington et al., 1997b). As a consequence and considering the time requirements, field trials were not considered appropriate to meet the aims of this study.

The same general method was used in all experiments and is described in this chapter. More specific methods appear in each experimental chapter. In addition, this chapter also describes the methods used to collect, process and analyse samples taken from the trials. Finally, the approach taken to analyse statistically the data gained from the analysis is described.

2.2 Pot trial materials and methods

One soil type, a freely draining sandy loam of the Fyfield series (Ruderford, et al., 1984) was used as a substrate for pot-based experiments. It was taken as a bulk soil sample removed from the Ap horizon from an agricultural field in East Lulworth, Dorset, UK. The soil sample was homogenised by repeatedly mixing with a shovel ready for use in subsequent pot trials. A supply of ‘caked’ anaerobically digested sewage sludge was obtained from a municipal source. Selected physio-chemical parameters of the sludge and soil are given in Table 2.1.

A sub-sample of sewage sludge of approximately 20 kg was air dried, crushed in an agate pestle and mortar and passed through a 2.0 mm plastic sieve before being stored ready to amend the soil. Approximately 40 kg of the soil substrate was amended with sewage sludge at a treatment rate equivalent to 0, 10, 30 or 100 t ha⁻¹. In the first pot trial, the highest amendment used was 30 t ha⁻¹, resulting in total Cd and Zn concentrations in the soil below the median values reported for soils in England and Wales (McGrath and Loveland, 1992). Subsequently, the highest amendment rate was increased to 100 t ha⁻¹ to raise soil concentrations. The amended soil was used to fill six replicate 7.5 litre pots. Each pot was adjusted to field capacity with distilled water and then seeded with spring wheat, winter wheat or spring barley at a rate equivalent to 400 kg ha⁻¹. The pots were then fertilised at a rate
equivalent to 187 kg ha$^{-1}$ of N, P$_2$O$_4$ and K$_2$O. The freely draining pots were then randomly placed in a block design in a glasshouse. Randomisation of pots was achieved by drawing of lots (Samuels, 1989).

The four pot trials were conducted separately. Consequently, conditions in the greenhouse were maintained as close as possible to a day: night ratio of 16: 8 h using high pressure sodium lamps (light intensity ca 9,000 lux) and a temperature range from 20 to 25 °C using electric heaters and automatic roof vents, in all experiments. The development of the plants was recorded according to the decimal growth system of Zadoks et al. (1974).

Table 2.1 Selected physiochemical parameters of the unamended soil and the sewage sludge used in its amendment (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Physio-chemical parameter</th>
<th>Linden’s soil</th>
<th>Sewage sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.33 ± 0.03$^a$</td>
<td>5.9 ± 0.03</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>3.1 ± 0.1$^c$</td>
<td>28.74 ± 0.529</td>
</tr>
<tr>
<td>Loss on ignition (%)</td>
<td>5.93 ± 0.34</td>
<td>59.93 ± 0.306</td>
</tr>
<tr>
<td>Bulk Density (g cm$^{-3}$)</td>
<td>1.12 ± 0.02$^c$</td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>61$^a$</td>
<td></td>
</tr>
<tr>
<td>Silt (%)</td>
<td>18$^a$</td>
<td></td>
</tr>
<tr>
<td>Clay (%)</td>
<td>21$^a$</td>
<td></td>
</tr>
<tr>
<td>Total P (mg kg$^{-1}$)</td>
<td>308.8 ± 21.5$^c$</td>
<td></td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.15 ± 0.02$^b$</td>
<td>3.38 ± 0.061</td>
</tr>
<tr>
<td>Total Cd (mg kg$^{-1}$)</td>
<td>0.140 ± 0.007</td>
<td>2.4</td>
</tr>
<tr>
<td>Extractable Cd (mg kg$^{-1}$)</td>
<td>0.078 ± 0.003</td>
<td>0.090 ± 0.001</td>
</tr>
<tr>
<td>Total Zn (mg kg$^{-1}$)</td>
<td>38.2 ± 1.1</td>
<td>724.8 ± 14.1$^b$</td>
</tr>
<tr>
<td>Extractable Zn (mg kg$^{-1}$)</td>
<td>3.62 ± 0.30</td>
<td>68.71 ± 3.88</td>
</tr>
</tbody>
</table>

$^a$ Merrington et al., 1997a  
$^b$ Merrington et al., 1997b  
$^c$ Winder et al., 1999

In three of the pot trials, three different crops were sown (spring wheat, winter wheat and spring barley) and the plants infested with grain aphids (Sitobion avenae). The effect of differing crop type on the transfer of Cd and Zn from the soil to the aphids was determined in
these trials. In the fourth pot trial, winter wheat was sown as before, but plants were infested with the rose grain aphid (Metopolophium dirhodum). The results of this trial were compared to those obtained from the winter wheat/ S. avenae trial to determine the effect of aphid species on the transfer of Cd and Zn from soil to aphids. Grain aphids from two of the pot trials were subsequently fed to larval and adult seven spotted ladybirds (Coccinella septempunctata).

**Arthropod cultures**

All arthropods used in the present study were taken from laboratory cultures. Aphid cultures were established from specimens held at the Institute of Arable Crops Research, Rothampstead. Coccinella septempunctata cultures were established from specimens collected from domestic gardens. In all cases, several generations (> 5) elapsed before specimens from the cultures were used in the experiments.

**2.3 Chemical analysis**

**2.3.1 Cleaning of laboratory equipment**

All glassware, plastic bottles, volumetric flask stoppers and funnels used in this investigation were acid washed in 25 % nitric acid (Aristar grade, BDH, Poole) for at least 2 hr. After acid washing, glassware was washed twice in distilled water and once in deionised water (Analar grade, BDH, Poole) before being dried in a warming cabinet at 30 °C.

**2.3.2 Sample preparation and analysis**

The soil substrate in each pot was sampled by five random cores taken from the upper 15 cm, and were bulked together, mixed and then air-dried. The substrate was then ground gently before being passed through a 2 mm plastic sieve. All subsequent analysis of the soil was carried out in triplicate on this fraction unless otherwise stated. Metals were extracted from 0.5 g sub-samples by refluxing in 69 % nitric acid (Aristar/Primar grade) for two days at 90 °C. Digests were then evaporated almost to dryness and then re-suspended in 10 ml of
10% nitric acid before filtration through a Whatman No. 42 filter paper (Tyler, 1981, as modified by Merrington et al., 1997a). Filtrate was collected in a 50 ml volumetric flask and made up to volume with Analar grade water. Reported results suggest that, in general, the efficiency of extractants commonly used in the analysis of trace metals in soil reduce in the order 3:1 mixture of nitric acid: perchloric acid > aqua regia (4:1 hydrochloric: nitric acid) > nitric acid (Agemian and Chau, 1976; McGrath and Cunliffe, 1985; McBride, 2000). However, nitric acid was used in this study as it is reported to offer slightly superior extraction of Cd and Zn compared to aqua regia in sewage sludge amended soils (Berrow and Stein, 1983). It is also relatively safe to use compared to nitric acid: perchloric acid mixtures and does not contain chlorine, which can interfere with the determination of Cd by Atomic Absorption Spectroscopy (S. Singh, Thermo Electron Corporation, Cambridge, pers. Comm.).

The bioavailability of Cd and Zn in the soil was estimated by an extraction from 10 g of soil substrate with 50 ml of 0.1 M CaCl₂ shaken for 16 hr (Sauerbeck and Stypereck, 1984). The extract was then filtered through a Whatman No. 42 filter paper. The first few millilitres of filtrate were discarded. Despite the presence of chlorine in the extract, CaCl₂ is reported to provide a reliable estimation of the bioavailable fraction of trace metals (Sauerbeck and Stypereck, 1984; Lebourg et al., 1998). Soil pH was determined in 2.5:1 water: soil suspension (Rowell, 1994) with an ISFET pH meter.

There are three methods commonly used for the determinations of organic carbon or organic matter in soils: loss on ignition; the Walkley-Black Chromium oxidisable C method and dry combustion in an elemental analyser (Sollins et al., 1999; Hanson et al., 1998; Jolivet and Arrouays, 1997). Inaccuracies are associated with all three. Loss on ignition measures the weight loss from oxidation of organic matter in oven-dried soil heated to around 450 °C. The results of this method tend to be over estimates of the organic matter content of soil because structural water retained in clays is also lost (Rowell, 1994; Sollins et al., 1999). The Walkley-Black method uses dichromate to oxidise carbon in the soil, but can underestimate
soil C by 20-30 % (Nelson and Sommers, 1982) and suffers from interference in the presence of chloride ions. The dry combustion method relies on the removal of inorganic C by pre-treating the soil with nitric acid (Agnelli et al., 2002), but this process can lead to the volatilisation of organic matter. Because each of these analysis methods have associated errors, organic matter content was estimated by loss on ignition, the simplest and most rapid method. Therefore, ca 2g of weighed oven dried soil substrate was heated to 450 °C for 12 hours. Ignited soil substrate was cooled in a dissector, before re-weighing to determine the loss in mass (Rowell, 1994).

Plant samples were washed once in 0.1 % Teepol® solution and twice in distilled water before being dried to a constant weight at 70 °C. Dried plant samples were digested in 10 ml of 69 % nitric acid (Aristar grade) at 90 °C for 15 hours (Merrington et al. 1997a). Digests were then filtered through Whatman No. 541 filter paper into 25 ml volumetric flask and made up to volume with Analar grade water. Nitric acid was used as an extractant on the basis of the work of Zarcinas et al. (1987), which demonstrated the efficacy of this extractant in analysis of plant material for trace metals.

Arthropod samples were washed once in 0.1 % teepol solution and twice in distilled water before being dried to a constant weight at 70 °C. Individual ladybirds and 20-40 mg subsamples of aphids were digested in 2 ml of nitric acid in sealed glass vessels at a temperature of 80 °C. The clear residue was then diluted to volume (5 ml) using de-ionised water. Whole pupal exuviae were digested in a similar way, but using 1 ml of nitric acid and diluted to a final volume of 2 ml with Analar grade water. Total nitrogen content of finely ground plants and whole aphids (ca 10 individuals) was determined using a Carlo Erba EMASyst 1160 Elemental analyser with a certified rye grass material as a standard (European Commission BCR 281).
Relevant certified reference materials (European Commission BCR 143R sewage sludge amended soil, BCR 60 Largarosiphon major and BCR 281 Lolium perenne) and reagent blanks were digested and analysed with each batch of soils, wheat plants aphids and ladybirds. Mean extraction efficiencies for Cd were 99, 108, 70 % for BCR CRM 143R, BCR 60 and BCR 281 respectively. The corresponding efficiencies for Zn were 83, 100.8 and 132.5 %.

2.3.3 Determination of Cd and Zn concentration

Cadmium and Zn content in extracts and digests were determined by an ATI Unicam Solar 939 atomic absorption spectrometer (AAS). Electro-thermal AAS (GFAAS) was utilised in the analysis of Cd in all samples and Zn in pupal exuviae, whilst flame AAS (both with and without STAT trap) was utilised in the analysis of Zn in soils, wheat, aphids and adult ladybirds. In all instances, deuterium background correction was used. Calibration of the instrument was carried out using a blank and three standard solutions of known concentration, matrix-matched to the concentration of the extractant in the samples.

**Flame AAS**

The absorption over 4 seconds was measured for three re-samples taken from each standard and sample analysed. Because of the small volume of analyte, metal absorption in arthropod samples was measured over 3 seconds in 2 re-samples. In all cases the mean value of the re-samples was taken as the concentration in the sample. If the residual standard deviation (RSD) of the re-samples was greater than 15 % then the mean was rejected and the sample re-analysed. A solution of known concentration (usually equal to that of the middle standard) was analysed every 6 -10 samples as a quality control (QC) check. If the result of the QC check deviated by a value of more than ± 10 % from the known value, the QC check was deemed to have failed. In this event, the results of all samples analysed since the last successful QC check were rejected and the samples reanalysed.
The matrix of Cd standards or samples was modified with a 1% ammonium di-hydrogen orthophosphate solution. This modifier was chosen because, in contrast to other matrix modifiers, it has a two-fold interaction with the sample matrix (S. Singh, Thermo Electron Corporation, Cambridge, pers. Comm.). Firstly, it combines with chloride in the sample matrix to form ammonium chloride. This compound has a boiling point of 520 °C and is therefore easily volatised and removed during the ashing phase. Secondly, the modifier reacts with Cd to form cadmium phosphate. This compound is stable to relatively higher temperatures, allowing a higher atomisation temperature. Consequently, a higher ashing temperature may be used, which results in a cleaner sample at atomisation. A 10 µl sub-sample was mixed with an equal volume of matrix modifier in preparation for injection into the graphite cuvette. For Zn analysis a 20 µl sub-sample was taken and mixed with 5 µl of 0.28% magnesium nitrate matrix modifier. For both metals the transient height of absorption was measured in two re-samples from each sample. As in flame analysis, the mean result of the re-samples was taken as the concentration in the sample if the RSD was below 15%. Quality control checks were carried out after every three samples and the preceding results rejected if the result was greater than ± 15% of the correct value. The GFAAS parameters used for both metals are given in table 2.4a and b.

Table 2.4a Furnace programme parameters for the analysis of cadmium. Phase 1 - sample drying; Phase 2 - sample cleaning, volatilisation of nitric acid; Phase 3 - sample ashing; Phase 4 - atomisation of cadmium and reading of absorption; Phase 5 - cuvette cleaning.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Temperature °C</th>
<th>Time (Sec)</th>
<th>Ramp (°C S⁻¹)</th>
<th>Gas</th>
<th>Gas Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105</td>
<td>30.0</td>
<td>10</td>
<td>Argon</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>20.0</td>
<td>5</td>
<td>Argon</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>20.0</td>
<td>50</td>
<td>Argon</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1500</td>
<td>3.0</td>
<td>Off</td>
<td>Argon</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2650</td>
<td>2.0</td>
<td>Off</td>
<td>Argon</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2.4b Furnace programme parameters for the analysis of zinc. Phase 1 - sample drying; Phase 2 - sample cleaning, volatilisation of nitric acid; Phase 3 - sample ashing; Phase 4 - atomisation of cadmium and reading of absorption; Phase 5 - cuvette cleaning.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Temperature (°C)</th>
<th>Time (Sec)</th>
<th>Ramp (°C S⁻¹)</th>
<th>Gas</th>
<th>Gas Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>10.0</td>
<td>Off</td>
<td>Argon</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>30.0</td>
<td>10</td>
<td>Argon</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>700</td>
<td>20.0</td>
<td>50</td>
<td>Argon</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1700</td>
<td>3.0</td>
<td>Off</td>
<td>Argon</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2500</td>
<td>2.0</td>
<td>Off</td>
<td>Argon</td>
<td>3</td>
</tr>
</tbody>
</table>

Detection limits and working ranges

The limit of detection of the AAS was calculated from 3 times the standard deviation of ten measurements of the concentration of distilled water. The optimum working range of the AAS was determined by multiplying the sensitivity by 20 for the lower working range and 200 for the upper range. The resultant limit of detection for Cd using GFAAS was 0.048 µg kg⁻¹ with an optimum working range of 0.8 – 8.0 µg kg⁻¹. If the concentration of Cd in samples was low, the sensitivity of the instrument was improved by increasing the volume of sample analysed to 15 µl. This lowered the optimum working range to 0.53-5.3 µg kg⁻¹. For Zn the optimum working range in GFAAS operations was determined as 0.5 – 5.0 µg kg⁻¹, whilst for AAS the optimum working range with a 100 mm length burner was 0.5 – 5.0 mg kg⁻¹, and with a slotted atom trap 0.26 – 2.6 mg kg⁻¹.

2.4 Statistical analysis

Statistical analysis was conducted with SPSS (versions 10 and 11). Differences among ratios (transfer co-efficients, partitioning co-efficient and biomagnification factors) were compared non-parametrically by the Kruskal-Wallis test. Data sets were analysed for homogeneity of variance and for normality with Levene’s test and the Shapiro-Wilk test respectively. Subsequently, data were subjected to analysis of variance and Student’s t-test, or when assumptions were not met, data were log₁₀ or inversely transformed as appropriate. Additional
tests for linearity and homogeneity of regression slopes were made prior to analysis of data by analysis of co-variance.

Data sets analysed by multiple regression were tested for multi-collinearity and singularity by comparing the correlation between variables (Pearson’s product-moment) and by the collinearity diagnostics function of the SPSS statistical package. Normality, linearity, homoscedasticity and independence of residuals were tested by examination of residuals scatter plots and normal probability plots. Outliers identified from residuals scatter plots were removed from the multiple regression analysis.
3.0 CADMIUM AND ZINC TRANSFER IN THE SOIL-PLANT SYSTEM

3.1 Introduction

For trace metals in the soil to enter the food chain of herbivorous arthropods living above the soil, they must first enter the plant. Uptake of trace metals by plants is strongly influenced by the physio-chemical properties of the soil (Kabata-Pendias and Pendias, 2000), of which total soil trace metal concentration is thought to be the most important (Hooda et al., 1997). Recent industrial trade effluent controls have resulted in a significant decrease in the concentration of trace metals in sewage sludges (Smith, 2001). However, in the majority of sewage sludges trace metal concentrations are still higher than those in agricultural soils (Gendebien et al. 1999). Repeated applications of sewage sludge may therefore lead to an increase in trace metal concentration in the plough layer of the soil (e.g. Krebs et al., 1998; Barbarick et al., 1998) and, consequently, in crop plants (Sauerbeck, 1991; McGrath et al., 2000).
Apart from the concentration in the soil, uptake of trace metals by plants is strongly influenced by soil pH (Sauerbeck, 1991; Hooda et al., 1997; Evans et al., 1995). Sewage sludge amendment can change soil pH (Tadesse et al., 1991; Hooda and Alloway, 1993; Merrington et al., 1997b). The effect is generally reported to be small (Tadesse et al., 1991; Merrington et al., 1997b; Sastre et al., 2001), but may lead to a significant reduction in pH over the long term (McBride, 1995; Krebs et al., 1998). Organic matter content may also be an important factor influencing the phytoavailability of trace metals in sewage sludge amended soils (Hooda and Alloway, 1994; McBride, 1995; Merrington et al, 2003).

After uptake into plant roots, the trace metals translocation to the aerial parts of the plant will be paramount in determining the exposure of above ground herbivores. However, the greater proportion of trace metals taken up by plants tends to remain in the roots (Sauerbeck, 1991; Guo and Marschner, 1995). For example, Jarvis et al. (1976) found that in 20 of 23 species growing in a solution culture, over 50 % of the Cd accumulated by the plants was retained in the roots. Only a relatively small quantity of metal is translocated to shoot tissues and even less to the reproductive tissues (Lübben and Sauerbeck, 1991; Sauerbeck, 1991; Cieslinski et al., 1996; Hart et al., 1997).

The partitioning between root and shoot is dependent on the mechanisms governing the uptake by the root and/or those controlling translocation from root to shoot (Florijn et al., 1993b; Guo and Marschner, 1995; Hart et al., 1998a; Hart et al., 1998b). These mechanisms can vary significantly among crop species and cultivars (Florijn et al., 1993a; Florijn et al., 1993 b; Guo and Marschner, 1995; Hart et al., 1998a; Hart et al., 1998b) and can result in large differences in shoot concentrations among different species (Sauerbeck, 1991) and cultivars of crops (Florijn and Beusichem, 1993; Cieslinski et al., 1996). It is not presently clear how difference in shoot concentration between crop species and cultivars affect the accumulation of trace metals by sap feeding herbivores such as aphids.
There are conflicting reports regarding the accumulation of trace metals in plant shoots in response to increasing soil concentrations resulting from sewage sludge application. Some workers have found a linear relationship between total concentration in the soil and in crop shoots (Logan et al., 1997; McGrath et al., 2000), whilst others report a curvilinear relationship through which shoot concentrations may reach a plateau (Chang et al., 1997; Logan et al., 1997; Smith 1997; Hamon et al., 1999). Both soil (Corey et al., 1987; Chaney and Ryan, 1993) and plant factors (Hamon et al., 1999) have been suggested to explain the latter plateaued response in shoots.

In addition to the above, the effects of aphid infestation can affect the concentration of trace metals in plant shoots. For example, aphid infestations have been shown to result in reduced concentrations of Cd, Cu and Mn in plant shoots (Ernst, 1987; Crawford et al., 1995). This may act as a negative feedback mechanism, reducing the accumulation of trace metals in the shoot and therefore restricting the exposure of herbivores to trace metals (Crawford et al., 1995). However, is not presently known how aphid infestation affects the concentration of trace metals in the shoots of cereal crops.

The aim of this Chapter is to determine the effects of sewage sludge amendment on the partitioning of Cd and Zn in components of the sewage sludge amended soil-plant system. The objectives of work presented in this Chapter are;

i) to investigate the partitioning Cd and Zn in the soil-plant system to determine if shoot accumulation follows a plateau response with increasing sewage sludge amendment;

ii) to determine the effect of aphid infestation on the partitioning Cd and Zn in the soil-plant system;

iii) to determine the effect of wheat variety on the concentration of Cd and Zn in the plant trophic level;

iv) to determine the effect of crop species on the concentration of Cd and Zn in the plant trophic level.
3.2 Materials and Methods

3.2.1 Effect of sewage sludge amendment and aphid infestation on the partitioning of Cd and Zn in the soil-plant system.

A bulk soil sample was taken from the Ap horizon of an agricultural field. Five sub-samples of ca 40 kg were removed from the bulk sample. Two of these were left unamended and the remaining parts were amended with sewage sludge at treatment rates equivalent to 10, 30 or 100 t ha\(^{-1}\) (dry solids). After thorough mixing of the soil and sewage sludge, the five soils were used to fill six replicate 7.5 l pots each. All 30 pots were seeded with winter wheat (*Triticum aestivum* L. cv. Challenger) at a rate equivalent to 400 kg ha\(^{-1}\). Seeded pots were covered in netting to prevent unintentional infestation of the plants by aphids, before being placed in a glasshouse in a randomised block. Aphid cultures were initiated on the plants of six of the pots containing unamended soil and all pots with amended soil, when they reached decimal growth stages between 37 and 51. No aphid cultures were initiated on the remaining 6 pots containing unamended soil to assess the effect of aphid feeding on trace metal uptake and partitioning within wheat plants.

The experiment was terminated when the plants had developed beyond flowering (decimal growth stages between 71 and 87). Soil samples and wheat plants, including roots to the third order, were sampled from each pot. The aerial parts of the plants were separated from the roots and washed in 0.1 % teepol solution and twice in distilled water. Root samples were washed free of soil on the surface of a 500 μm sieve, before being further washed in 0.1 % teepol solution and three times in distilled water. Three individual shoots and ears from each pot were digested whole for the determination of metal concentration. Root samples were cut into ca 0.5 cm strips and duplicate 0.1 g sub-samples were taken from this material and digested. The analysis of soils, roots, shoots and ears for Cd and Zn are described in Chapter 2.5.
3.2.2 Effect of wheat variety on the concentration of Cd and Zn in the plant trophic level

Two sets of three ca 40 kg sub-samples of a bulk soil sample were taken from the same location as in the previously described above. Each set of sub-samples was amended with municipal sewage sludge at a treatment rates equivalent to 0, 10, 30 t (dry solids) ha\(^{-1}\). The sewage sludge and soil was thoroughly mixed and used to fill six replicate 7.5 l pots. This gave two sets of six pots for each treatment rate. One set of pots was seeded with spring wheat (\textit{Triticum aestivum} L. cv. Alexander) and the other with winter wheat (\textit{Triticum aestivum} cv. Challenger). Both crops were seeded at a rate equivalent to 400 kg ha\(^{-1}\). The freely draining pots were placed in a fully randomised block in a glasshouse and watered with distilled water. When the plants were between growth stages 37 and 51, cultures were established on the plants by placing 200 grain aphids (\textit{Sitobion avenae}) from laboratory cultures in each pot. Individual pots were covered with netting to prevent the transfer of aphids between treatments. Aphid cultures were left until plants began to senesce before all aphids from each pot were collected from the plants by careful brushing with a fine paintbrush. Aphids from different pot cultures were kept separately from each other at -18\(^\circ\) C until used in the feeding trial (see Chapter 5). Soil and plants were also sampled for analysis at this time. The analysis of soils, roots, shoots and ears for Cd and Zn are described in Chapter 2.5.

3.2.3 Effect of crop species on the concentration of Cd and Zn in the plant trophic level

This experiment used the same basic method as described in the previous pot trial. However, the following exceptions should be noted. The two sets of 4 sub-samples of the bulk soil were used. Each part was amended with sewage sludge at rates equivalent to 0, 10, 30 and 100 t ha\(^{-1}\). One set of pots was seeded with winter wheat (\textit{Triticum aestivum} cv Challenger), the other with spring barley (\textit{Hordeum distichon} cv. Optic).
3.3 Results

3.3.1 Partitioning of Cd and Zn in the soil-plant system

Selected physio-chemical parameters of the unamended soil sewage sludge are given in Chapter 2 (Table 2.1.). The amendment of soil with this sludge resulted in statistically significant increases in both the estimated organic matter content and pH of the soil (Table 3.1). The increase in pH was only 0.12 units, reflecting the higher pH of the sewage sludge compared to the unamended soil. Both the ‘total’ and CaCl₂ extractable Cd and Zn concentrations in the soil increased with sewage sludge amendment rate (Table 3.2 and 3.3). After log₁₀ transformation of the total concentrations of both metals and extractable concentration of Zn to meet the assumption of homogeneity of variance, a one-way analysis of variance indicated that the observed difference between treatments were significant for the two metals in both soil fractions (Table 3.2 and 3.3).

Table 3.1 The pH and loss on ignition (LOI) of unamended soil and soil amended with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>pH</th>
<th>LOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>4.63 ± 0.02</td>
<td>5.33 ± 0.09</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>4.73 ± 0.03</td>
<td>6.27 ± 0.11</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>4.75 ± 0.00</td>
<td>6.58 ± 0.04</td>
</tr>
<tr>
<td>100 t ha⁻¹</td>
<td>4.75 ± 0.00</td>
<td>8.44 ± 0.13</td>
</tr>
</tbody>
</table>

The two metals showed distinctly different behaviour in their partitioning between the extractable fraction (i.e. metal in the soil solution or weakly held to cation exchange sites) and other soil fractions. The percentage of Cd in extractable fractions fell with increasing sewage sludge amendment rate from 43% in the control to 24% in the 100 t ha⁻¹ amendment (Table 3.2). This decline was statistically significant (Table 3.2). In all treatments a far higher proportion of Cd was in the extractable fraction compared to Zn. The partitioning of Zn
between the total and extractable fraction increased in line with sewage sludge until the 30 t ha$^{-1}$ amendment before falling to the lowest value in the 100 t ha$^{-1}$ amendment. However, differences between treatments were not significantly different (Table 3.3).

Table 3.2 The concentration of total, extractable cadmium (mg kg$^{-1}$) and partitioning co-efficient between the extractable soil fraction and the remaining fractions after the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Soil 'total'</th>
<th>Soil 'extractable'</th>
<th>Total: Extractable</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha$^{-1}$</td>
<td>0.209 ± 0.009</td>
<td>0.089 ± 0.003</td>
<td>0.43</td>
</tr>
<tr>
<td>10 t ha$^{-1}$</td>
<td>0.220 ± 0.008</td>
<td>0.087 ± 0.007</td>
<td>0.40</td>
</tr>
<tr>
<td>30 t ha$^{-1}$</td>
<td>0.315 ± 0.005</td>
<td>0.115 ± 0.003</td>
<td>0.37</td>
</tr>
<tr>
<td>100 t ha$^{-1}$</td>
<td>0.514 ± 0.018</td>
<td>0.124 ± 0.003</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Test $F(3, 20) = 148.5$ $F(3, 20) = 16.0$ $\chi^2(3) = 15.39$
Significance level $p < 0.001$ $p < 0.001$ $p = 0.002$

Table 3.3 The concentration of total extractable zinc (mg kg$^{-1}$) and partitioning co-efficient between the extractable soil fraction and the remaining fractions after the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Soil 'total'</th>
<th>Soil 'extractable'</th>
<th>Total: Extractable</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha$^{-1}$</td>
<td>38.2 ± 1.1</td>
<td>3.62 ± 0.30</td>
<td>0.096</td>
</tr>
<tr>
<td>10 t ha$^{-1}$</td>
<td>43.0 ± 1.3</td>
<td>4.63 ± 0.22</td>
<td>0.109</td>
</tr>
<tr>
<td>30 t ha$^{-1}$</td>
<td>63.2 ± 1.5</td>
<td>7.38 ± 0.30</td>
<td>0.117</td>
</tr>
<tr>
<td>100 t ha$^{-1}$</td>
<td>101.9 ± 4.3</td>
<td>9.81 ± 0.82</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Test $F(3, 20) = 194$ $F(3, 20) = 42.9$ $\chi^2(3) = 4.05$
Significance level $p < 0.001$ $p < 0.001$ $p = 0.256$

Cadmium concentration in plant roots increased with the size of sewage sludge amendment (Table 3.4). Root concentrations were inversely transformed to meet the assumption of homogeneity of variance prior to comparison of treatment means by one-way ANOVA, which indicated that observed differences among treatments were statically significant (Table 3.4).
Transfer co-efficients between the ‘total’ cadmium in the soil and the roots ranged from 1.33 to 1.97 and decreased with sewage sludge amendment except in the 30 t ha\(^{-1}\) amendment (Table 3.4). Transfer co-efficients between the extractable fraction and roots increased with sewage sludge amendment and ranged from 4.49 to 5.48 (Table 3.4). Transfer co-efficients among treatments were significantly different between total Cd in soil and root, but not in the case of the extractable fraction (Table 3.4). This reflected the decreased partitioning of Cd in the extractable fraction at high sludge amendments.

**Table 3.4** Cadmium concentration (mg kg\(^{-1}\)) in winter wheat roots and transfer co-efficients between Cd and extractable soil Cd following the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Root</th>
<th>Total - Root</th>
<th>Extractable - root</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha(^{-1})</td>
<td>0.401 ± 0.023</td>
<td>1.92</td>
<td>4.49</td>
</tr>
<tr>
<td>10 t ha(^{-1})</td>
<td>0.381 ± 0.025</td>
<td>1.74</td>
<td>4.52</td>
</tr>
<tr>
<td>30 t ha(^{-1})</td>
<td>0.625 ± 0.067</td>
<td>1.97</td>
<td>5.49</td>
</tr>
<tr>
<td>100 t ha(^{-1})</td>
<td>0.681 ± 0.042</td>
<td>1.33</td>
<td>5.48</td>
</tr>
</tbody>
</table>

Significance level: \(F(3, 20) = 15.1\) \(\chi^2(3) = 10.9\) \(\chi^2(3) = 4.93\) \(p < 0.001\) \(p = 0.012\) \(p = 0.18\)

**Table 3.5** Zinc concentration (mg kg\(^{-1}\)) in winter wheat roots and transfer co-efficients between Cd and extractable soil Cd following the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Root</th>
<th>Total - Root</th>
<th>Extractable - root</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha(^{-1})</td>
<td>183.8 ± 15.2</td>
<td>4.86</td>
<td>51.4</td>
</tr>
<tr>
<td>10 t ha(^{-1})</td>
<td>230.6 ± 19.8</td>
<td>5.39</td>
<td>50.4</td>
</tr>
<tr>
<td>30 t ha(^{-1})</td>
<td>309.2 ± 25.6</td>
<td>4.94</td>
<td>41.8</td>
</tr>
<tr>
<td>100 t ha(^{-1})</td>
<td>343.9 ± 20.4</td>
<td>3.42</td>
<td>36.8</td>
</tr>
</tbody>
</table>

Significance level: \(F(3, 20) = 12.5\) \(\chi^2(3) = 8.25\) \(\chi^2(3) = 6.61\) \(p < 0.001\) \(p = 0.041\) \(p = 0.086\)

Zinc concentrations in roots increased significantly in line with sewage sludge amendment rate (Table 3.5). Zinc transfer co-efficients between the total soil and the roots ranging from
3.4 to 5.4 coefficients were greater than the control, but were lowest at the highest amendment rate (Table 3.5). Observed differences among treatments were statistically significant (Table 3.5). A post hoc Nemenyi’s test indicated that only the 10 and 100 t ha⁻¹ amendment treatments were significantly different from each other at the α = 0.05 level. Transfer co-efficients between the extractable Zn concentration and roots were high, ranging from 37 to 51 (Table 3.5) and decreased with increasing sewage sludge amendment. Differences among treatments were found not to be statistically significant (Table 3.5). Consequently, Zn accumulation by the roots did not appear to be regulated.

Cadmium shoot concentration increased with sewage sludge amendment levels until the 30 t ha⁻¹, but fell in the 100 t ha⁻¹ amendment. Differences among treatments were compared by one-way ANOVA, which showed that observed differences among treatments were significant (Table 3.6). Trend analysis was conducted by a planned polynomial comparison to determine if shoot accumulation followed a curvilinear pattern. This showed a significant quadratic trend in the increase in shoot concentration with sewage sludge amendment ($F_{(1, 20)} = 7.9, p = 0.01$). This suggests the accumulation of Cd in shoots followed a plateau type response.

Cadmium partitioning co-efficients between roots and shoots were < 0.5 in all treatments, demonstrating that root to shoot transfer was restricted. Partitioning co-efficients increased in line with sewage sludge until the 30 t ha⁻¹ amendment. The lowest co-efficient was for the 100 t ha⁻¹ treatment. A comparison of means by the Kruskal-Wallis test revealed that, overall, there was no significant difference in root-shoot partitioning of Cd among the treatments (Table 3.6). However, there was a 9 % increase in the Cd concentration in the roots, but a 23 % decrease in shoot concentration between the 30 and 100 t ha⁻¹ amendments. This resulted in a fall in root-shoot partitioning co-efficient from 0.48 in the 30 t ha⁻¹ amendment to 0.34 in the 100 t ha⁻¹ amendment. Consequently, there was a strong indication that restricted root to shoot translocation contributed to the plateau response in shoot accumulation.
Cadmium concentrations in wheat ears increased with sewage sludge in the order 0<10<100<30 t ha⁻¹ (Table 3.6). Concentrations in the ears were lower than in the shoots, and partitioning co-efficients between shoots and ears were in the order of 0.7. Partitioning co-efficients decreased with the size of sewage sludge amendment, but differences among treatments were not found to statistically significant (Table 3.6).

Table 3.6 Cadmium concentrations (mg kg⁻¹) in wheat shoots and ears and partitioning co-efficients among wheat roots and shoots, and shoots and ears following the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Shoot</th>
<th>Ear</th>
<th>Root - Shoot</th>
<th>Shoot - Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>0.182 ± 0.040</td>
<td>0.128 ± 0.024</td>
<td>0.45</td>
<td>0.82</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>0.171 ± 0.017</td>
<td>0.131 ± 0.014</td>
<td>0.45</td>
<td>0.78</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>0.300 ± 0.028</td>
<td>0.212 ± 0.152</td>
<td>0.48</td>
<td>0.71</td>
</tr>
<tr>
<td>100 t ha⁻¹</td>
<td>0.232 ± 0.026</td>
<td>0.168 ± 0.104</td>
<td>0.34</td>
<td>0.71</td>
</tr>
</tbody>
</table>

\[ F(3,20) = 4.13 \hspace{0.5cm} \chi^2(3) = 6.19 \hspace{0.5cm} \chi^2(3) = 0.18 \]

Significance level \( p = 0.020 \) \hspace{0.5cm} \( p = 0.045 \) \hspace{0.5cm} \( p = 0.10 \) \hspace{0.5cm} \( p = 0.98 \)

Table 3.7 Zinc concentrations (mg kg⁻¹) in wheat shoots and ears and wheat roots and the partitioning co-efficients among wheat roots, and, shoots and shoots and ears following the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Shoot</th>
<th>Ear</th>
<th>Root - Shoot</th>
<th>Shoot - Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>35.4 ± 5.7</td>
<td>150.1 ± 15.4</td>
<td>0.18</td>
<td>4.71</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>63.4 ± 5.2</td>
<td>121.0 ± 7.7</td>
<td>0.28</td>
<td>1.95</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>102.2 ± 11.9</td>
<td>208.5 ± 24.8</td>
<td>0.34</td>
<td>2.08</td>
</tr>
<tr>
<td>100 t ha⁻¹</td>
<td>106.4 ± 9.7</td>
<td>261.7 ± 67.0</td>
<td>0.31</td>
<td>2.57</td>
</tr>
</tbody>
</table>

\[ F(3,20) = 14.0 \hspace{0.5cm} F(3,20) = 5.6 \hspace{0.5cm} \chi^2(3) = 9.88 \hspace{0.5cm} \chi^2(3) = 9.56 \]

Significance level \( p < 0.001 \) \hspace{0.5cm} \( p = 0.006 \) \hspace{0.5cm} \( p = 0.02 \) \hspace{0.5cm} \( p = 0.02 \)

Zinc concentrations in shoots increased with the rate of the sewage sludge amendment (Table 3.7). Differences among treatments were compared by one-way ANOVA, which confirmed that observed differences among treatments were statistically significant (Table 3.7). Trend analysis was conducted by a planned polynomial comparison to determine
if shoot Zn accumulation followed a similar pattern to Cd. This showed the increase in shoot Zn concentration with sewage sludge amendment was not curvilinear, but followed a statistically significant linear trend ($F_{(1, 19)} = 25.2, p < 0.001$). Zinc partitioning co-efficients between roots and shoots were between 0.18 and 0.34 and were higher than the control in all sewage sludge amendments, but differences among treatments were not significant (Table 3.7).

Zinc concentrations in ears increased with sewage sludge amendment in the order $10<0<30<100$ t ha$^{-1}$. Observed differences among treatments were statistically significant (Table 3.7). Zinc partitioning co-efficients between shoots and ears ranged from 1.9 to 4. Co-efficients were lower than the control at all sludge amendments, but increased with the size of the amendment. Observed differences in co-efficients among treatments were statistically significant (Table 3.7).

3.3.2 The effect of aphid infestation on the partitioning of Cd and Zn in winter wheat plants

![Graph of Cd concentrations in roots, shoots, and ears with and without aphid infestation](image)

**Figure 3.2** Cadmium concentrations (mg kg$^{-1}$) in roots, shoots and ears of winter wheat plants (mean ± 1 SE) with and without aphid infestation.
Cadmium concentrations were higher in the roots and shoots of wheat plants infested with aphids compared to uninfested plants (Figure 3.2), whilst Zn concentrations were lower in the roots and higher in the shoots of infested plants (Figure 3.3). Differences between treatments were not found to be significant for either metal in the roots ($t_{(9)} = -0.24$, $p = 0.82$, $t_{(10)} = 0.38$, $p = 0.71$ for Cd and Zn respectively) or shoots ($t_{(10)} = -1.13$, $p = 0.30$, $t_{(9)} = -0.72$, $p = 0.51$ for Cd and Zn respectively). Cadmium concentrations in the ears of infested plants were approximately twice those of uninfested plants, whilst Zn concentrations were approximately 1.5 times higher. Differences between treatments were found to be statistically significant for both metals ($t_{(10)} = -2.84$, $p = 0.03$, $t_{(10)} = -2.67$, $p = 0.01$ for Cd and Zn respectively).

![Figure 3.3 Zinc concentrations (mg kg$^{-1}$) in roots, shoots and ears of winter wheat plants (mean ± 1 SE) with and without aphid infestation.](image)

**Figure 3.3** Zinc concentrations (mg kg$^{-1}$) in roots, shoots and ears of winter wheat plants (mean ± 1 SE) with and without aphid infestation.

The mean dry mass of ears from the infested plants was $0.124 \text{ g} \pm 0.044$ (mean ± 1 SE) whereas that of the uninfested plants was $0.280 \pm 0.019$ (mean ± 1 SE), which were statistically significantly different ($t_{(10)} = 3.27$, $p = 0.008$). Consequently, the lower concentrations of Cd and Zn in the uninfested plants may have been caused by dilution in a
greater mass of the ear. The mean mass of Cd per ear was $1.61 \times 10^{-5}$ mg and $1.42 \times 10^{-5}$ mg for the uninfested and infested plants respectively. This is not a statistically significant difference ($t(10) = 0.66$, $p = 0.52$) and suggests the lower Cd concentrations in the ears of the uninfested plants were due to the diluting effect of the larger ears. For Zn, the mean mass of metal per ear was lower in the infested plants ($1.74 \times 10^{-2}$ mg compared to $2.61 \times 10^{-2}$ mg) and this was significant ($t(10) = 2.86$, $p = 0.017$). Consequently, less Zn reached the ears of infested plants and the higher concentrations of Zn in the ears of infested plants therefore appeared to result from the concentrating effect of the smaller size of the ears.

### 3.3.3 The effect of wheat variety on the concentration of Cd and Zn in the plant trophic level

![Figure 3.4](image)

**Figure 3.4** The concentration of cadmium (mg kg$^{-1}$) in soils and spring and winter wheat shoots (mean ± 1 SE) after the amendment of soil with sewage sludge (t ha$^{-1}$).

Cadmium and Zn concentrations in soil increased with sludge application rate (Figures 3.4 and 3.5). Cadmium concentrations in the soil were higher for winter wheat than for spring wheat, but Zn concentrations were similar in the soil of both crops. The significance of the
differences in soil concentration between the two wheat varieties was determined by two-way ANOVA. This indicated that sewage sludge treatment had a statistically significant effect on the concentration of Cd in the soil \(F(2, 30) = 95.6, p < 0.001\); the observed difference in concentration between varieties was statistically significant \(F(1, 30) = 37.1, p < 0.001\). The interaction term between sludge amendment rate and crop variety was also significant \(F(2, 30) = 3.61, p = 0.04\), indicating that the increase in soil Cd concentration with sewage sludge amendment rate differed between the two varieties. Zinc was also significantly increased by sewage sludge amendments \(F(2, 30) = 58.2, p < 0.001\), but there was no significant difference in soil Zn concentration between the two crop varieties \(F(1, 30) = 4.0, p = 0.06\). The interaction term between sewage sludge amendment and crop variety was not significant \(F(2, 30) = 1.7, p = 0.2\), indicating that the Zn concentration in the soil of both varieties responded in a similar way to sewage sludge amendments.

![Graph showing the concentration of zinc (mg kg\(^{-1}\)) in soils and spring and winter wheat shoots (mean ± 1 SE) after the amendment with sewage sludge (t ha\(^{-1}\)).](image)

**Figure 3.5** The concentration of zinc (mg kg\(^{-1}\)) in soils and spring and winter wheat shoots (mean ± 1 SE) after the amendment with sewage sludge (t ha\(^{-1}\)).
Cadmium concentrations in both spring and winter wheat shoots did not increase in line with the sewage sludge application rate. In spring wheat shoots, Cd concentration increased with sewage application in the order $0 < 30 < 10$ t ha$^{-1}$, whilst for winter wheat the order of increase was $10 < 0 < 30$ t ha$^{-1}$ (Figure 3.4). Zinc concentrations in the shoots of both crops increased with sewage sludge amendment (Figure 3.5). Concentrations of both metals were highest in spring wheat. However, Zn concentrations rose faster with increasing sewage sludge amendment in winter wheat, so that in the largest amendment, concentrations were similar in both crops.

The effect of sewage sludge amendment and wheat type on the concentration of Cd in shoots was initially investigated by a two-by-two analysis of co-variance (ANCOVA). Independent variables consisted of size of sewage sludge amendment and crop type. Soil Cd was included in the analysis as the co-variant to control for the differences in this variable between the two crops. However, the co-variant was not found to be significant ($F_{(1, 29)} = 0.48, p = 0.49$) and was discounted in subsequent analysis. Therefore, a two-way among groups ANOVA was conducted to explore the effect of sewage sludge amendment and wheat type on the concentration of both metals in shoots. This indicated that sewage sludge amendment had no significant effect on the Cd concentration of shoots ($F_{(2, 30)} = 2.65, p = 0.087$), but did for the Zn concentration of shoots ($F_{(2, 29)} = 38.9, p < 0.001$). For both metals wheat type had a significant effect on shoot concentration ($F_{(1, 30)} = 11.17, p = 0.002; F_{(1, 29)} = 28.3, p < 0.001$ for Cd and Zn respectively). The interaction between sludge amendment and wheat type was statistically significant for Cd, but not Zn ($F_{(2, 30)} = 4.71, p = 0.017; F_{(2, 29)} = 2.27, p = 0.12$ for Cd and Zn respectively). The two types of wheat therefore differed in the way shoot concentration responded to sludge amendment for Cd but not for Zn.

3.3.4 The effect of cereal species on the concentration of Cd and Zn in the plant trophic level

As with the other study systems, Cd and Zn concentrations in the soil increased with the addition of sewage sludge (Figures 3.6 and 3.7). Cadmium concentrations in the soil were
similar for both crop species at all amendment rates, whilst Zn concentrations were slightly higher in the two largest amendments for winter wheat. Treatment means for both metals were compared by two-way analysis of variance to determine if concentrations were significantly different among treatments. To meet the assumption of homogeneity of variance, Zn concentrations were log_{10} transformed. Both Zn and Cd concentrations were found to be significantly different among treatments ($F(3, 39) = 67.5 \ p < 0.001$; $F(3, 39) = 335.8, \ p < 0.001$, for Cd and Zn respectively). Cd concentrations were not significantly different between crop species ($F(1, 39) = 0.31, \ p = 0.58$), but Zn concentrations were ($F(1, 39) = 6.0, \ p = 0.02$). The interaction term between sewage sludge amendment and crop type was significant for Zn ($F(3, 39) = 12.29, \ p < 0.001$), but was not for Cd ($F(3, 39) = 0.51, \ p = 0.68$), showing that the higher Zn concentrations observed at the two highest sludge amendments in the winter wheat were statistically significant.

Figure 3.6 The concentration of cadmium (mg kg$^{-1}$) in soil, spring barley and winter wheat shoots (mean ± 1 SE) after the amendment of soil with sewage sludge (t ha$^{-1}$).

The accumulation pattern of Cd in the shoots of barley and wheat showed a similar pattern. Shoot concentration increased in line with sewage sludge amendment up to the 30 t ha$^{-1}$
amendment, but was lower in the 100 t ha\(^{-1}\) amendment than in 30 t ha\(^{-1}\) amendment (Figure 3.6). With the exception of the 10 t ha\(^{-1}\) treatment, concentrations of Cd were higher in winter wheat. Zinc concentration in shoots increased in line with sewage sludge amendment in both crops (Figure 3.7).

![Graph showing the concentration of zinc (mg kg\(^{-1}\)) in soils and spring and winter wheat shoots (mean ± 1 SE) after the amendment of soil with sewage sludge (t ha\(^{-1}\)).](image)

**Figure 3.7** The concentration of zinc (mg kg\(^{-1}\)) in soils and spring and winter wheat shoots (mean ± 1 SE) after the amendment of soil with sewage sludge (t ha\(^{-1}\)).

An initial two-by-two analysis of co-variance was conducted on the Zn data to determine the effect of the different Zn concentrations in the soils between the two crop species on shoot concentrations. This revealed that soil Zn was not a significant co-variant \(F(1, 39) = 0.30, p = 0.59\). Consequently, soil Zn concentration was not considered further and the effect of sewage sludge amendment and wheat type on the concentration of both Cd and Zn in shoots was explored by a two-way between groups analysis of variance. This showed that sewage sludge amendment had a significant effect on shoot concentration for both metals \(F(3, 39) = 6.95, p = 0.001; F(3, 39) = 51.2, p < 0.001\), for Cd and Zn respectively). Crop type had no significant effect on the concentration of either metal in shoots \(F(1, 39) = 1.20, p = 0.28;\)
The interaction between sludge amendment and crop species was not statistically significant for Cd ($F_{(3, 39)} = 1.33, p = 0.28$) but was for Zn ($F_{(3, 39)} = 3.28, p = 0.03$), showing that there was a difference in the pattern of Zn accumulation in shoots between the crop species as sewage sludge amendment increased.

### 3.4 Discussion

The concentration of both Cd and Zn in the sewage sludge used to amend the soil was between the fifty and ninety percentile values for sludges used agriculturally in England and Wales (Gendebien et al., 1999). Application of this sewage sludge resulted in concentrations of Cd and Zn in the soil of 0.53 mg kg$^{-1}$ and 102 mg kg$^{-1}$ respectively at the 100 t ha$^{-1}$ amendment rate. These values are well within the current UK limits for Cd and Zn in soils with a pH range of 5.5-5.0, which are 3 mg kg$^{-1}$ and 200 mg kg$^{-1}$ respectively (MAFF/DoE, 1993). However, it has been proposed to reduce the permitted level of trace metal concentrations in sludge-amended soils within the EU. If these proposals were to be adopted, the maximum permitted concentrations of Cd and Zn would be reduced to 0.5 mg kg$^{-1}$ and 60 mg kg$^{-1}$, respectively, for soils in the pH range 5-6 (Towers et al., 2001). These values are reached at the 30 t ha$^{-1}$ amendment for Zn and 100 t ha$^{-1}$ for Cd in the present study.

**Partitioning of Cd and Zn in the soil-plant system**

The concentrations of Cd and Zn in the sludge were 11.5 and 19 times higher, respectively, in the sludge than in the soil. As a consequence, the concentrations of both metals in the soil increased with sewage sludge amendment. This corresponds with the finding of other reports (e.g. Sauerbeck, 1991; Hooda and Alloway, 1994, Merrington et al. 1997b). The concentration in the CaCl$_2$ extractable fraction of the soil also increased with sewage sludge amendment, reflecting the total concentration. This is in agreement with the reported positive linear relationship between total and extractable concentration in soils exhibiting a wide range of total: extractable partitioning (Lehoczky and Zsanett, 2002).
The two metals exhibited markedly differently partitioning between the extractable fraction and the remaining soil fractions. The percentage of Cd in the extractable fraction ranged from 24 to 43 %, whilst for Zn the range was between 9.6 and 11.7 %. For both metals these figures fall in, or are very close to, the reported ranges (6.8–41.4 % and 5–35 % for Cd and Zn, respectively; Lehoczky and Zsanett, 2002). A lower proportion of Zn in the extractable fraction compared to Cd was also found by Bell et al. (1991) for an acidic (pH 5.8) sandy loam soil amended with sewage sludge. In this study 51% of the Cd was in the extractable form compared to 3.5 % of the Zn. The partitioning co-efficients between the total soil and the extractable soil fraction were lowest in the 100 t ha⁻¹ amendment for Cd. This reflects the lower partitioning co-efficient in the sewage sludge and suggests that the binding sites in the sludge are resulting in an increased soil affinity for Cd (Hooda and Alloway, 1994; Li et al., 2001).

The amendment of soil with sewage sludge led to the significant accumulation of Cd and Zn in the roots of winter wheat plants. Concentrations in the roots were between 0.40 and 0.68 mg kg⁻¹ for Cd and 184 and 344 mg kg⁻¹ for Zn. These values were within the reported range for cereal crops (0.34 – 0.82 and 61 – 190 mg kg⁻¹ for Cd and Zn respectively; Cieslinski et al., 1996; Frost and Ketchum, 2000). The highest concentrations in the soil-plant system were found in the root tissue. This is again consistent with published data (Jarvis et al., 1976; Sauerbeck, 1991, Green et al., 2003).

Transfer co-efficients for the transfer of Cd from the total soil fraction to the root decreased at the highest sewage sludge amendment, whilst there was no significant difference in the transfer co-efficients between the extractable fraction and the roots. The decreased total soil to root transfer of Cd therefore appears to be due to the lower partitioning of Cd between the total soil and the extractable fraction. Consequently, the concentration of Cd in the extractable fraction appears to be a more accurate reflection of the concentration to which plant roots are exposed (Bujtas et al., 1987; McBride, 1995).
The transfer co-efficients for Zn from the extractable fraction in the soil and the roots were greater than for Cd (35-50 for Zn compared to 4.5 – 5.5 for Cd). Transfer co-efficients showed no decrease with increasing sewage sludge amendment for either the total soil or extractable concentration, suggesting that there was no restriction to Zn uptake over root membranes. This agrees with the results of Green et al. (2003), which indicate that there is no regulation of Zn in the roots of wheat plants up to a concentration of 1200 mg kg\(^{-1}\) in the root.

The transfer co-efficients between the extractable soil fraction and roots, show that Cd uptake by the roots is restricted compared to Zn. Cadmium and Zn are transported across the membranes of root cells by the same facilitated diffusion carrier system (Lasat et al., 1996; Hart et al., 2002). This carrier system has a greater affinity for Cd than Zn (Hart et al., 2002), which should result in greater transport of Cd across the plasma membrane (Mengel and Kirby, 1990). However, root absorption of Cd is reduced by competition for the carrier protein by numerous other metallic cations (Jarvis et al., 1976; Cataldo et al., 1983; Hardiman and Jacoby, 1984; Smilde et al., 1992), especially Zn (Hart et al., 2002). Consequently, competition for uptake by the trans-membrane carrier protein may explain the reduced uptake of Cd by the roots.

Cadmium concentrations in the winter wheat shoots were increased by sewage sludge amendments. Cadmium concentrations in the shoots were within the reported background concentrations for Poaceae of 0.07 - 0.27 mg kg\(^{-1}\) (Kabata–Pendias and Pendias, 1992), except in the 30 t ha\(^{-1}\) amendment. Concentrations were lower in shoots than in roots with partitioning co-efficients between the roots and shoots falling between 0.34 and 0.45, showing that the translocation of Cd from roots to shoots was restricted. This is consistent with published data for cereal crops (Jarvis et al., 1976; Petterson, 1976; Sauerbeck, 1991).

Cadmium accumulation in wheat shoots showed a curvilinear trend, which appeared to result in a plateau in shoot concentrations being reached between the 30 and 100 t ha\(^{-1}\) treatments. A similar plateau in shoot concentrations was reported to occur in Indian mustard.
Both soil and plant factors have been suggested as an explanation for the plateau in shoot concentrations observed in plants grown in sludge-amended soil. In the former, it is postulated that as the size of sewage sludge amendment rises, the properties of the amended soil increasingly reflect those of the sewage sludge matrix (Corey et al., 1987; Chaney and Ryan, 1993). When the properties of the sludge predominate, the availability of trace metals in the soil will reflect those of the sludge. Consequently, further amendments of sludge lead to a diminishing increase in shoot concentrations until the soil becomes, in effect, pure sludge.

Logan et al. (1997) suggest that for maize plants, the plateau response became evident when the sludge matrix represented 13% of the amended soil. In the present trial the sludge matrix represented ca 7.7% of the amended soil in the 100 t ha\(^{-1}\) treatment. Nevertheless, the results of the present trial show that the properties of the sewage sludge affected the partitioning of Cd in the extractable fraction of the soil. As Cd concentrations in the roots appeared to reflect the concentration in the extractable fraction of the soil, this may have contributed to the plateau in wheat shoot concentrations.

Hamon et al. (1999), observed a plateau in radish shoots that was the result of plant physiology and not the chemistry of the amended soil. They concluded from this experiment, and a review of the literature, that mechanisms within the plant are responsible for the plateau response in shoot concentration. The work of Salt et al. (1995b) and Guo and Marschner (1995) suggest that these mechanisms control the root to shoot translocation of trace metals in the xylem sap. The results of the present trial confirm that restricted root to shoot translocation of Cd is an important mechanism contributing to the plateau response seen in wheat shoots. However, as mentioned above, soil factors may also have an influence.

Zinc was also accumulated in the shoots in response to sewage sludge amendments. However, the concentrations observed were within the range normally reported for wheat.
(27-150 mg kg\(^{-1}\); Kabata-Pendias and Pendias, 1992) in all treatments. As with Cd, Zn concentrations in the shoots were lower than in roots and partitioning co-efficients were between 0.41-0.67. This is in agreement with published data on Zn partitioning between roots and shoots in crop plant (Petterson, 1976; Sauerbeck, 1991). Unlike Cd, Zn accumulation in the shoots showed a linear trend with increasing sewage sludge amendment. This is consistent with published data (Smilde et al., 1992; McGrath et al., 2000). Consequently, Zn uptake from the soil and transfer through wheat plants showed no signs of regulation by the plants.

Various mechanisms may be responsible for sequestration of Cd and Zn in roots and the regulation of their translocation to the shoots (Figure 3.8). These are reported to include binding to cell wall components, binding to short chain peptides containing relatively high number of sulphur hydryl groups (phytochelatins/metallothioneins), compartmentalisation in the vacuole and binding to cell organelles and nuclei (Lane et al., 1987; Puntz and Sieghardt, 1993; Hall, 2002; Ramos et al., 2002). How these mechanisms contribute to the regulation of trace metal translocation from roots to shoots is currently unclear (Clemens, 2001).

![Figure 3.8 Mechanism of Trace metal Storage and detoxification in plant cells](image-url)

**Figure 3.8** Mechanism of Trace metal Storage and detoxification in plant cells (Puntz and Sieghardt, 1993; Brune et al., 1994; Salt et al., 1993a; Salt et al., 1995a and b; Ramos et al., 2002). Tm – trace metal, Pc:Tm – Phytochelatin bound trace metal, pptt – precipitated trace metal.
Root-shoot partitioning co-efficients for Cd were higher than for Zn, indicating that relatively more Cd was translocated to the shoots than Zn. This contrasts with the greater root to shoot translocation of Zn noted in Zea mays after sewage sludge amendment of 100 t ha\(^{-1}\) (Mullins et al., 1986). However, in the present trial root-shoot partitioning co-efficients increased with sewage sludge amendment so that in the 100 t ha\(^{-1}\) amendment values for both metals were similar (0.34 and 0.31 for Cd and Zn respectively). It has been suggested that phytochelatins are important in the de-toxification of trace metals in plants (Grill et al., 1987; Clemens, 2001; Hall, 2002) and they may sequester trace metals in roots (Rauser, 1986; Welch et al., 1999). Phytochelatins have a higher affinity for binding Cd than Zn (Maitani et al., 1996; Leopold and Günther, 1997). Consequently, if phytochelatins play an important role in restricting trace metal translocation from root to shoot, it would be expected that relatively more Zn would be translocated than Cd (Welch et al., 1999). However, Salt et al. (1995b) found that binding to phytochelatins had no affect on the translocation of Cd in the xylem sap of Indian mustard. The proportionally higher translocation of Cd to the shoot found in the present study provides evidence that this may also be the case in wheat. Zinc may be bound to phytic acid in a variety of plant roots, including wheat, whilst Cd is not (van Steveninck et al., 1994). It has been suggested that binding to phytic acid plays a role in regulating the translocation of Zn from the roots to the shoots (van Steveninck et al., 1994). Thus, a difference in binding mechanism(s) between the two metals in the root could account for the greater translocation of Cd to the shoots.

Studies on a number of diverse plant species have demonstrated that shoot Cd concentrations are significantly correlated to the concentration in the xylem sap, suggesting that the mechanisms controlling the loading of Cd into the xylem regulate the root to shoot transport of Cd (Florijn and Beusichem, 1993; Guo and Marschner, 1995; Salt et al., 1995b). Experimental evidence indicates that the same trans-membrane carrier protein transports Cd and Zn in root cells (Hart et al., 2002). There is a competitive interaction between Cd and Zn for this carrier system and this can result in restricted uptake of either metal, depending on
their relative concentrations (Smilde et al., 1992; Hart et al., 2002). It is hypothesised that similar mechanisms are involved in the translocation of Cd and Zn to the shoots (Hart et al., 1998a). The finding of McKenna et al. (1993) that Zn can interfere with Cd translocation from roots to shoots supports this hypothesis. Accordingly, the observed decrease in Cd translocation to the shoot in the 100 t ha\(^{-1}\) amendment could also be the result of a competitive interaction between Cd and Zn for loading into xylem sap as Zn concentrations in the root rise.

There was no significant accumulation of Cd in the ears of the wheat plants and partitioning co-efficients between shoots and ears were ca 0.7, which suggests that ear concentration is related to that in the shoot. A similar relationship was found by Mench et al. (1997) between the concentration in the shoot and grain of wheat plants. Zinc accumulated in ears with increasing sewage sludge application and was concentrated there, with partitioning co-efficients between the shoot and ear ranging between 2 and 4.7. The concentration of Zn in the ears compared to shoots was also reported in spring wheat (Lübben and Sauerbeck, 1991) and for wheat and rye (Puschenreiter and Horak, 2000). The comparatively low translocation of Cd probably results from a combination of the lower phloem mobility of Cd than Zn and the greater re-translocation of Zn from the flag leaf to the developing grain (Herran and Feller, 1997; Harris and Taylor, 2001). This would suggest that a sap feeding herbivore feeding on the ear has a relatively higher exposure to Zn and lower exposure to Cd than one feeding on the shoot.

**The effect of aphid infestation on the partitioning of Cd and Zn in winter wheat plants**

Aphid infestation had no effect on the concentration of Cd or Zn in wheat roots or shoots. This confirms the findings of Ernst (1987) that aphid infestation does not decrease shoot Zn concentrations. However, this is at variance with the findings of Crawford et al., (1990), who found that infestation by the black bean aphid (*Aphis fabae*) significantly reduced the Cd concentration in bean plants, regardless of the concentration to which the plants were
exposed. Aphid infestation increased the concentration of both metals in the ears of wheat plants. Infestation resulted in a significant reduction in the mass of ears. This was probably due to the decreased energy reserves available to the plant caused by aphid feeding (Ernst, 1987). The mass of Cd the ears contained did not differ between treatments, whilst the mass of Zn in the ears was lower in the plants infested with aphids. This indicates that the supply of both metals to the ear is fairly constant and that difference in concentration between treatments was due to the diluting effect of greater ear mass. Thus, the lower mass of Zn in infested ears reflects a proportionally greater removal by aphids from the phloem stream. Cadmium is less phloem mobile than Zn and reaches the ear more by xylem transport (Herren and Feller, 1997). The proportion of Cd removed by the ingestion of phloem sap by aphids would be less than for Zn, which probably explains the lack of a reduction in the mass of Cd in aphid infested ears.

The effect of wheat variety on the concentration of Cd and Zn in the plant trophic level

Cadmium concentrations in shoots did not increase with soil concentration as is generally reported (Kabata-Pendias and Pendias, 2000). Cadmium concentrations in the shoots were within the reported typical range (0.07 –0.27 mg kg\(^{-1}\); Kabata-Pendias and Pendias, 1992) for members of the Poaceae in all treatments. Although the amendment of soil with sewage sludge did not result in increased accumulation of Cd in either crop variety, there were significantly higher concentrations of Cd in spring wheat in the control and 10 t ha\(^{-1}\) treatments. In the highest amendment, concentrations were similar in both crop types. Cadmium concentration reached a plateau at this amendment in the winter wheat and the similarity in shoot concentration may reflect a plateau at a similar concentration in spring wheat.

The observed differences in the Cd shoot concentration between the two wheat varieties are probably related to difference in the control of Cd translocation from root to shoot rather than root uptake characteristics (Florijn and van Beusichem, 1993; Guo and Marschner, 1995;
Salt et al., 1995b). For example, Hart et al. (1998a) found no difference in the uptake of Cd by the roots of *Triticum turgidum* (durum wheat) and *Triticum aestivum* (bread wheat). However, *T. aestivum* had Cd root/shoot partitioning co-efficients 3 times higher than *T. turgidum*. It therefore appears that the mechanism(s) regulating shoot Cd concentration in Triticales are either controlling the loading of Cd into the xylem sap, or the storage/detoxification mechanisms operating in the root or a combination of both (Hart et al., 1998a).

In contrast, sewage sludge amendment of the soil led to increased Zn concentrations in shoots of both types of wheat. However, concentrations did not exceed the typical range (27-150 mg kg\(^{-1}\)) reported for wheat plants (Kabata-Pendias and Pendias, 1992). The pattern of Zn accumulation in the shoots was similar to Cd in that spring wheat exhibited higher concentrations, but concentrations in winter wheat were similar in the 30 t ha\(^{-1}\) treatment. No data is evident in the literature on difference in shoot accumulation of Zn between these two wheat varieties with which to compare this finding.

**The effect of cereal species on the concentration of Cd and Zn in the plant trophic level**

The amendment of soil with sewage sludge led to a significant increase in the concentration of Cd and Zn in the shoots of both crop plants. Zinc concentrations were all within the range of concentrations considered typical, but Cd concentrations in winter wheat shoots exceed this range at an amendment of 30 t ha\(^{-1}\) (Kabata-Pendias and Pendias, 1992). Crop species had no effect on the concentration of Cd in the shoots and both crops appeared to reach a plateau in shoot accumulation between the 30 and 100 t ha\(^{-1}\) amendments. This suggests that similar mechanisms are involved in the uptake and translocation of Cd in both species. However, Jarvis et al. (1976) reported lower Cd concentrations in barley shoots than in wheat when grown in hydroponic solutions. Differences between these findings and those of the present study probably reflect the radically different experimental conditions, especially the very high Cd concentrations in the hydroponic solution.
Concentrations of Zn in the shoots did not differ significantly between the two crops. However, the two crop species differed in the pattern of accumulation; there was a greater transfer of Zn to barley shoots than wheat shoots at the 100 t ha\(^{-1}\) amendment. This suggests that wheat may be more efficient at regulating shoot Zn concentrations at higher soil concentrations. Consequently, there may be significantly greater transfer of Zn in the soil-barley system than in the soil-wheat system at soil concentrations in excess of those in the present study. There is no evident work in the literature comparing Zn concentrations in winter wheat and spring barley to confirm this hypothesis.

3.5 Conclusion

The concentrations of both Cd and Zn were increased in winter wheat shoots with sewage sludge amendment. However, the trends in shoot accumulation with increasing sewage sludge amendment differed. Cadmium showed a curvilinear trend, which appeared to result from a combination of decreasing partition in the extractable fraction of the soil and reduced root-shoot translocation as sewage sludge amendment increased. In contrast, the increase in Zn concentration with sewage sludge amendment showed a linear trend, which suggests that Zn uptake and translocation to the shoots was not regulated.

Aphid infestation had no effect on the concentration of Cd or Zn in the roots and shoots of plants. However, the concentration of both metals in the ears was higher in infested plants. This appeared to be the result of the concentration of the metals in a smaller mass of ear. Therefore the supply of Cd to the ear did not seem to be affected by aphid infestation, whilst the supply of Zn may have been reduced by a small extent due to infestation.

Like winter wheat, spring wheat and barley freely accumulated Zn in their shoots, whilst Cd accumulation appeared to be restricted at higher amendment rates. Differences between varieties of the same crop were found to be greater than between different crop species for both metals. Although statistically significant differences were found for Cd and Zn
concentrations between spring and winter wheat, differences in concentration at the highest amendment rate were small for both metals.

The results of this chapter show that soil and plant factors are involved in determining the shoot concentrations of both metals. These factors result in a non-linear increase in shoot concentrations with sewage sludge amendment for Cd. Consequently, the exposure of herbivorous arthropods will not increase linearly with sewage sludge amendment. In addition, exposure of aphids to both metals was not affected by negative feedback resulting from the effects of infestation. The relatively small differences in concentration of both metals among the crop types suggest that crop type is unlikely to have a significant biological effect on the exposure of arthropod herbivores. However, the higher concentrations of Zn and lower concentrations of Cd in the wheat ears may result in a differential exposure to arthropods feeding here compared to the shoots.
4.0 CADMIUM AND ZINC TRANSFER BETWEEN CEREALS AND APHIDS

Figure 4.1 Sitobion avenae feeding on an ear of winter wheat

4.1 Introduction

Crop species and cultivars of the same crop can differ in the concentration of trace metals in their shoots (Jarvis et al., 1976; Sauerbeck, 1991; Cieslinski et al., 1996; Chapter 3). However, there is no evident literature on how differences in shoot concentration effect accumulation and biomagnification by sap feeding arthropods. Amongst the most common sap feeding arthropods in agroecosystems are aphids. In Britain there are three main species found on cereal crops, Sitobion avenae F., Metopolophium dirhodum Walk. and Rhopalosiphum padi L. (Carter et al., 1980). The most abundant of the three species is S. avenae (Carter et al., 1980). Metopolophium dirhodum is the prime aphid cereal pest in South America, but in Britain it is generally less abundant than S. avenae. Rhopalosiphum padi tends to be a problem only in the west and north of Britain and then only as a vector for the transmission of the barley yellow dwarf virus (Carter et al., 1980; Burn, 1987).
In contaminated systems, aphids are reported to biomagnify Cd (Crawford et al., 1995; Merrington et al., 1997b) and Zn (Merrington et al., 1997a; Merrington et al., 1997b) compared to the plants on which they feed. This implies that aphids may be important vectors for the transport of trace metals from plants to higher trophic levels. However, the biomagnification of trace metals in arthropods depends on the physiology of individual species and can vary greatly among even closely related species (van Straalen and van Wensem, 1986; Janssen, 1988; Hopkin, 1990a; Rabitsch, 1995). For example, Glowacka et al., (1997) found the accumulation of trace metals was species and metal-specific in sap feeding Psyllids, even for closely related species feeding on the same species of plant. Consequently, the exposure of a predator to trace metals can be strongly influenced by the species on which it feeds. For instance, predators feeding on the isopod Oniscus asellus may consume four times the Cd and nine times the Cu than if they were feeding on the related isopod Porcelio scaber (Hopkin, 1990a). Therefore, the species of aphid in the soil-plant-arthropod system may have a significant effect on the transfer and fate of trace metals in that system.

Sewage sludge amendment may alter the biomass of aphid populations and affect the quantity of trace metals available for transfer to their predators. For example, the application of organic wastes, including sewage sludge, to soil can be beneficial for some arthropods (Pimentel and Warneke, 1989). For instance, Larsen et al. (1996) reported a higher diversity of carabid beetles in field plots fertilised with sewage sludge than in plots fertilised with inorganic fertiliser. This increase in diversity may result from i) the additional food resources in the environment, either from the direct consumption of the waste or increased plant biomass resulting from the nutrient benefit provided by the waste material; ii) for carnivorous arthropods, increased number of prey; iii) improved micro-climate, i.e. increased soil moisture due to water retention by sewage sludge organic matter (Pimentel and Warneke, 1989). In addition, the nitrogen fertiliser value of the sludge may beneficially affect aphid populations by increasing plant nitrogen levels (Honek, 1991; Duffield et al., 1997). However, the presence of toxic contaminants in organic wastes,
especially in sewage sludges, could be detrimental to arthropods (Culliney et al., 1986; Eigenbrode and Pimentel, 1988; Pimentel and Warneke, 1989; Larsen et al., 1994b), including aphids (Culliney and Pimentel, 1986).

The aim of this study is to investigate the effect of the species of plant and aphid in the plant-aphid system on the concentration of Cd and Zn in the trophic level of the herbivore. How this affects the biomagnification of the two metals in aphids was also examined. An additional aim of this chapter was to determine if sewage sludge amendment has an effect on the mass of the aphid population and therefore on the amount of Cd and Zn that may be transferred to aphid predators.

The objectives of the work presented in this Chapter are;

(i) to compare the concentration of Cd and Zn in grain aphids (*Sitobion avenae*) feeding on different cereal species;

(ii) to compare the concentration of Cd and Zn in the rose grain aphid (*Metopolophium dirhodum*) and the grain aphid (*Sitobion avenae*) feeding on wheat plants;

(iii) to compare the magnitude and relative transport of Cd and Zn between crop plant and aphid in the experimental systems.

(iv) To determine how the amendment of soil with sewage sludge affects the mass of Cd and Zn contained within aphid populations available for transfer to their predators.

4.2 Materials and methods

4.2.1 The effect of crop species on the concentration of Cd and Zn in grain aphids (*Sitobion avenae*)

Two sub-samples of a bulk soil sample taken from an Ap horizon of an agricultural field were further sub-divided into equal parts. These were then amended with municipal sewage sludge at treatment rates equivalent to 0, 10, 30 t (dry solids) ha⁻¹. The sewage sludge amended soils were thoroughly mixed and used to fill six replicate 7.5 l pots. This gave two sets of six pots
for each treatment rate. One set of pots was seeded with spring wheat (*Triticum aestivum* L. cv. Alexander) and the other with spring barley (*Hordeum distichon* cv. Optic). Both crops were seeded at a rate equivalent to 400 kg ha\(^{-1}\). The freely draining pots were placed in a fully randomised block in a glasshouse and were watered with distilled water. When the plants were between growth stages 37 and 51, cultures were established on the plants by placing 200 grain aphids (*Sitobion avenae*; Figure 4.1) from laboratory cultures in each pot. Individual pots were covered with netting to prevent the transfer of aphids between treatments. Aphid cultures were left to establish for 3 weeks before all aphids, soil and plants were sampled for analysis.

### 4.2.2 The effect of aphid species on the concentration of Cd and Zn in herbivore trophic level

![Figure 4.2 Metopolophium dirhodum feeding on a wheat leaf](image)

The same method was used as described above, with the following exceptions. The top amendment rate was increased to 100 t ha\(^{-1}\) and both sets of pots were seeded with winter
wheat (*Triticum aestivum* cv. Challenger). In one set of pots, cultures of the rose-grain aphids (*Metopolophium dirhodum*; Figure 4.2) were established, whilst in the other set grain aphids (*Sitobion avenae*) were used. After 28 days, all aphids were carefully removed from the shoots by gently brushing with a small paintbrush and collected.

### 4.2.3 The effect of amending of soil with sewage sludge on the mass of Cd and Zn within aphid populations

Samples taken from the winter wheat pots infested with *S. avenae* in the above trial were used to test the hypothesis that the amendment of soil with sewage sludge can affect the mass of Cd and Zn contained within the aphid populations. Additional analysis was conducted to determine the nitrogen content of wheat shoots and aphids by a dry cumbustion method (See Chapter 2.3.2). Samples of *S. avenae* were needed for use in a subsequent feeding trial (see Chapter 5); therefore the aphids were kept in a form as palatable as possible to aphid predators. This precluded drying to determine dry mass and therefore the fresh mass of aphids harvested from each pot was determined. A factor was determined to convert fresh mass to dry mass by recording the change in mass of aphid samples taken from laboratory cultures. This factor was then used to convert the fresh mass into dry mass.

### 4.3 Results

#### 4.3.1 The effect of crop species on the concentration of Cd and Zn in grain aphids (*Sitobion avenae*)

Increasing sewage sludge application resulted in no discernible pattern of Cd accumulation in *S. avenae* in either crop species (Figure 4.3). However, concentrations in *S. avenae* feeding on spring wheat were higher at all sewage sludge amendment rates, reflecting the significantly higher concentrations in spring wheat shoots (*F*{sub:1, 30} = 22.5, *p* < 0.001). A two-way between groups analysis of variance was conducted to explore the effect of sewage sludge amendment and crop species on the concentration of Cd in *S. avenae*. The results of the analysis confirmed that the Cd body burdens of aphids feeding on both crops were not significantly
increased by sewage sludge amendment \( (F(2, 29) = 0.36, p = 0.71) \). However, aphids feeding on the spring wheat shoots had significantly higher concentrations of Cd than those feeding on spring barley did \( (F(1, 29) = 4.5, p = 0.04) \). The interaction between sludge amendment and crop type was not significant \( (F(2, 29) = 0.69, p = 0.51) \), showing that there was no discernible difference in the way body burdens of aphids differed in response to increasing sludge application rate between the two crops.

![Graph showing concentrations of Cd in spring wheat and barley and in grain aphids (Sitobion avenae) feeding on them, following the amendment of soil with sewage sludge (t ha\(^{-1}\)).](image)

**Figure 4.3** Concentrations of Cd (mg kg\(^{-1}\); mean ± 1 SE) in spring wheat and barley and in grain aphids (*Sitobion avenae*) feeding on them, following the amendment of soil with sewage sludge (t ha\(^{-1}\)).

A two-by-two ANCOVA was conducted to investigate the effect of shoot Cd concentration in the two crops on the body burden of *S. avenae*. The dependent variable was aphid Cd concentration (which was inversely transformed to meet the assumption of homogeneity of variance), the independent variables were sludge amendment and crop type and shoot Cd concentration was the co-variate. The results of the analysis indicated that shoot Cd concentration was a significant co-variate \( (F(1, 27) = 9.7, p = 0.004) \). After adjusting the means
for shoot concentration, crop type had no significant effect on aphid body burden ($F_{(1, 27)} = 0.48, p = 0.49$). The interaction between sewage sludge amendment and crop species remained not significant ($F_{(2, 27)} = 0.18, p = 0.84$). This suggests that the concentration of Cd in aphids is related to the concentration in the shoot.

![Figure 4.4 Concentrations of Zn (mg kg$^{-1}$; mean ± 1 SE) in spring wheat and barley and in grain aphids (sitobion avenae) feeding on them, following the amendment of soil with sewage sludge (t ha$^{-1}$).](image)

The concentration of Zn in aphids was higher than the control in both sewage sludge amendments in both crops. However, whilst concentrations in aphids feeding on spring wheat increased with the size of sewage sludge amendment, the concentration in aphids feeding on barley peaked at the 10 t ha$^{-1}$ amendment (Figure 4.4). Like Cd, concentrations of Zn were higher in aphids feeding on spring wheat. This again reflected the significantly higher concentrations of these metals in the shoots of this crop (Table 4.2; $F_{(2, 29)} = 26.48, p < 0.001$). A two-way between groups analysis of variance was conducted to explore the effect of sewage sludge amendment and crop type on the concentration of Zn in S. avenae. The results
showed that Zn concentrations in aphids were significantly increased by sewage sludge amendment \((F(2, 29) = 16.58, p < 0.001)\) and that aphids feeding on the wheat plants had significantly higher concentrations of Zn in their bodies than those feeding on barley \((F(1, 29) = 92.89, p < 0.001)\). The interaction between sludge amendment and crop species was statistically significant \((F(2, 29) = 3.91, p = 0.03)\), showing that there was a difference in the pattern of Zn accumulation in aphids between the two crops.

To determine the influence of shoot concentration on Zn accumulation in aphids, a two-by-two ANCOVA was conducted. In this analysis the dependent variable was aphid Zn concentration, the independent variables were sludge amendment and crop species and shoot Zn concentration was the co-variate. The results of the analysis indicated that crop Zn concentration was not a significant co-variate \((F(1, 27) = 0.22, p = 0.64)\). After adjusting the means for shoot concentration, crop species still had a significant effect on the concentration of Zn in aphids \((F(1, 27) = 25.1, p < 0.001)\). This suggests that the differences in Zn concentrations between aphid populations feeding on the two crop species are the result of differences in physiological mechanisms other than those controlling the concentration in the shoots.

4.3.2 The effect of aphid species on the concentration of Cd and Zn in herbivore trophic level

Cadmium concentrations in \(S. avenae\) were higher than in the control only at the highest amendment rate, whilst concentrations in \(M. dirhodum\) showed no increase as a result of sewage sludge amendment (Figure 4.5). Despite the significantly higher concentrations in the shoots on which they were feeding, concentrations is \(S. avenae\) were lower than in \(M. dirhodum\). These observations were tested for statistical significance by a two-by-two ANCOVA in which the dependent variable was aphid Cd concentration and the independent variables were sewage sludge amendment rate and aphid species. Owing to the wide difference in shoot concentration between the two experiments, shoot concentration was included as a co-variate. The results of this analysis demonstrated that shoot concentration
was not a significant co-variate ($F_{(1,27)} = 2.13, p = 0.16$). Sewage sludge amendment had no significant effect on the concentration of Cd in aphids ($F_{(2,27)} = 0.48, p = 0.63$), but aphid species did ($F_{(1,27)} = 8.73, p = 0.006$). The interaction term between sewage sludge amendment and aphid species was also not significant ($F = 0.17, p = 0.84$), indicating that there was no difference in the way the two aphid species responded to increasing sewage sludge amendment.

![Figure 4.5](image)

**Figure 4.5** Concentrations of Cd (mg kg$^{-1}$; mean ± 1 SE) in grain aphids (*Sitobion avenae*) and rose-grain aphids (*Metopolophium dirhodum*) and in the winter wheat and barley on which they were feeding following the amendment of soil with sewage sludge (t ha$^{-1}$).

Zinc concentrations in both species of aphid increased with the size of sewage sludge amendment and there was no apparent difference in the pattern of accumulation between the two species (Figure 4.6). The concentration of Zn in the wheat tissues differed appreciably between the two experimental systems (Figure 4.6). Consequently, differences between treatments were assessed for statistical significance by a two-by-two ANCOVA. Shoot Zn concentration was included in the analysis as the co-variate and aphid Zn concentration was log$_{10}$ transformed to meet the assumption of homogeneity of variance. The result of this
analysis showed that shoot Zn concentration was not a significant co-variate ($F_{(1, 27)} = 0.019$, $p = 0.89$). The observed increase in aphid Zn concentration with sewage sludge amendment was significant ($F_{(2, 27)} = 3.41$, $p = 0.048$), but differences between the two aphid species were not statistically significant ($F_{(12, 27)} = 5.21$, $p = 0.48$). The interaction term between sewage sludge application rate and aphid species was not significant ($F_{(2, 27)} = 0.70$, $p = 0.51$), showing that there was no difference in the way the two species accumulated Zn, in response to increasing sewage sludge applications.

![Graph showing Zn concentration in different treatments](image)

**Figure 4.6** Concentrations of Zn (mg kg$^{-1}$) in grain aphids (*Sitobion avenae*) and rose-grain aphids (*Melopolophium dirhodum*) and in the winter wheat and barley on which they were feeding following the amendment of soil with sewage sludge (t.d.s. ha$^{-1}$).

### 4.3.3 Transport of Cd and Zn between crop shoot and aphid

Cadmium biomagnification factors were above 1 in the control treatment of every experimental system except *S. avenae* feeding on winter wheat. There was also a slight biomagnification in the 10 t ha$^{-1}$ treatment for *S. avenae* feeding on spring wheat. In all other treatments there was no biomagnification of Cd in aphids (Table 4.1). In all experimental
systems there was a trend for biomagnification factors to decrease with increasing sewage sludge amendment. However, this was found to be significant only for *S. avenae* and *M. dirhodum* feeding on winter wheat (Table 4.1).

**Table 4.1** Biomagnification factors for the transfer of Cd from cereal shoots to the aphids feeding on them.

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th><em>S. Avenae</em> (Spring wheat)</th>
<th><em>S. Avenae</em> (Spring barley)</th>
<th><em>S. Avenae</em> (Winter wheat)</th>
<th><em>M. dirhodum</em> (Winter wheat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>1.19</td>
<td>1.21</td>
<td>0.49</td>
<td>1.40</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>1.11</td>
<td>0.86</td>
<td>0.33</td>
<td>1.04</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>0.82</td>
<td>0.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100 t ha⁻¹</td>
<td>-</td>
<td>-</td>
<td>0.13</td>
<td>0.72</td>
</tr>
</tbody>
</table>

χ²(α) = 2.24
Signif. \( p = 0.33 \) \( p = 0.25 \) \( p = 0.004 \) \( p = 0.046 \)

**Table 4.2** Biomagnification factors for the transfer of Zn from cereal shoots to the aphids feeding on them.

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th><em>S. avenae</em> (Spring wheat)</th>
<th><em>S. avenae</em> (Spring barley)</th>
<th><em>S. avenae</em> (Winter wheat)</th>
<th><em>M. dirhodum</em> (Winter wheat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>2.40</td>
<td>2.67</td>
<td>1.90</td>
<td>2.80</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>2.16</td>
<td>2.26</td>
<td>2.22</td>
<td>1.51</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>2.16</td>
<td>1.39</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100 t ha⁻¹</td>
<td>-</td>
<td>-</td>
<td>1.90</td>
<td>1.17</td>
</tr>
</tbody>
</table>

χ²(α) = 0.67
Signif. \( p = 0.72 \) \( p = 0.012 \) \( p = 0.36 \) \( p = 0.031 \)

Biomagnification factors for the transfer of Zn from shoots to aphids were over 1, demonstrating that Zn was biomagnified by aphids in all four crop-aphid systems (Table 4.2). The transfer of Zn from the shoots of both wheat varieties to *S. avenae* resulted in biomagnification factors in the order of 2 and this was not significantly affected by the size of the sewage sludge amendment (Table 4.2). In contrast, biomagnification factors for the transfer between spring barley shoots and *S. avenae* and winter wheat shoots and *M. dirhodum* were greater than 2.5 in the control, but were to close to 1 at the highest sewage
sludge amendment rates (Table 4.2). Observed differences between treatments in both cases were found to be significant (Table 4.2).

4.3.4 The effect of sewage sludge on the quantity of Cd and Zn within the herbivore trophic level

Table 4.3 Cadmium concentrations (mg kg⁻¹) in soil, wheat roots and shoots and aphids after the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Soil</th>
<th>Root</th>
<th>Shoot</th>
<th>Aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t. d. s. ha⁻¹</td>
<td>0.147 ± 0.007</td>
<td>0.569 ± 0.024</td>
<td>0.244 ± 0.023</td>
<td>0.109 ± 0.031</td>
</tr>
<tr>
<td>10 t. d. s. ha⁻¹</td>
<td>0.198 ± 0.009</td>
<td>0.794 ± 0.046</td>
<td>0.282 ± 0.012</td>
<td>0.093 ± 0.006</td>
</tr>
<tr>
<td>100 t. d. s. ha⁻¹</td>
<td>0.691 ± 0.047</td>
<td>1.339 ± 0.036</td>
<td>1.117 ± 0.122</td>
<td>0.147 ± 0.012</td>
</tr>
<tr>
<td>F(2,15) =</td>
<td>215.31</td>
<td>6.91</td>
<td>123.25</td>
<td>1.95</td>
</tr>
<tr>
<td>Signif.</td>
<td>p&lt; 0.001</td>
<td>p = 0.011</td>
<td>p = 0.002</td>
<td>p = 0.177</td>
</tr>
</tbody>
</table>

Table 4.4 Zinc concentrations (mg kg⁻¹) in soil, wheat roots and shoots and aphids after the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Soil</th>
<th>Root</th>
<th>Shoot</th>
<th>Aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t. d. s. ha⁻¹</td>
<td>40.0 ± 1.2</td>
<td>343.89 ± 29.75</td>
<td>37.9 ± 1.9</td>
<td>70.6 ± 3.6</td>
</tr>
<tr>
<td>10 t. d. s. ha⁻¹</td>
<td>48.0 ± 1.4</td>
<td>399.77 ± 42.75</td>
<td>36.0 ± 1.3</td>
<td>79.3 ± 5.2</td>
</tr>
<tr>
<td>100 t. d. s. ha⁻¹</td>
<td>111.1 ± 2.2</td>
<td>648.95 ± 31.11</td>
<td>66.7 ± 1.3</td>
<td>125.8 ± 12.7</td>
</tr>
<tr>
<td>F(2,15) =</td>
<td>548.72</td>
<td>21.50</td>
<td>55.19</td>
<td>13.96</td>
</tr>
<tr>
<td>Signif.</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Concentrations of Cd fell in the order root>shoot>soil>aphid, whilst Zn concentrations fell in the order root>aphid>soil>shoot (Tables 4.3 and 4.4). Cadmium concentrations increased in line with sewage sludge amendment in the soil, roots and shoots, but not aphids (Table 4.3), whilst for Zn, concentrations increased in line in the soil, roots and aphids (Table 4.4). Zinc concentrations in the plant shoots were slightly lower in the 10 t ha⁻¹ amendment than in the control, but shoot concentrations at the largest amendment were higher. For both metals, the differences in concentration between treatments were significant in all components of the system, except Cd in aphids (Tables 4.3 and 4.4).
The concentration of N in both the shoots and aphids increased with sludge amendment and differences between treatments were found to be statistically significant for both (Table 4.5). Tukey’s HSD indicated that the N concentration in the shoots was significantly different from the control in the 100 t ha\(^{-1}\) amendment (\(p = 0.041\)), whilst aphid N concentration differed significantly between the control and both sludge amendments (\(p = 0.046\) and \(P < 0.001\) for the 10 and 100 t ha\(^{-1}\) amendments respectively.

Table 4.5 The dry weight (g) of harvested roots, shoots and aphids and the N concentration (%) of shoots and aphids following the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Root Weight</th>
<th>Shoot Weight</th>
<th>Aphid Weight</th>
<th>Shoot N</th>
<th>Aphid N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t.d.s. ha(^{-1})</td>
<td>0.354 ± 0.049</td>
<td>0.207 ± 0.003</td>
<td>1.083 ± 0.084</td>
<td>4.61 ± 0.15</td>
<td>5.74 ± 0.19</td>
</tr>
<tr>
<td>10 t.d.s. ha(^{-1})</td>
<td>0.460 ± 0.063</td>
<td>0.201 ± 0.006</td>
<td>1.095 ± 0.069</td>
<td>5.02 ± 0.23</td>
<td>6.56 ± 0.20</td>
</tr>
<tr>
<td>100 t.d.s. ha(^{-1})</td>
<td>0.273 ± 0.025</td>
<td>0.196 ± 0.006</td>
<td>0.056 ± 0.126</td>
<td>5.38 ± 0.22</td>
<td>7.40 ± 0.29</td>
</tr>
</tbody>
</table>

\(F(2,15) = 3.59\) \(F(2,15) = 1.38\) \(F(2,15) = 10.16\) \(F(2,15) = 3.65\) \(F(2,14) = 13.07\)

Signif. \(p = 0.053\) \(p = 0.282\) \(p = 0.002\) \(p = 0.05\) \(p = 0.001\)

Table 4.6 The mass of cadmium (\(\mu g\)) contained in the soil, wheat roots and shoots and aphids after the amendment of soil with sewage sludge (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment rate</th>
<th>Soil</th>
<th>Root</th>
<th>Shoot</th>
<th>Aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t.d.s. ha(^{-1})</td>
<td>954± 44.1</td>
<td>0.203 ± 0.0300</td>
<td>0.855 ± 0.1711</td>
<td>0.027 ± 0.0070</td>
</tr>
<tr>
<td>10 t.d.s. ha(^{-1})</td>
<td>1286 ± 61.0</td>
<td>0.352 ± 0.0416</td>
<td>1.143 ± 0.0660</td>
<td>0.024 ± 0.0018</td>
</tr>
<tr>
<td>100 t.d.s. ha(^{-1})</td>
<td>4491 ± 309.0</td>
<td>0.367 ± 0.0373</td>
<td>8.872 ± 0.4754</td>
<td>0.020 ± 0.0048</td>
</tr>
</tbody>
</table>

\(F(2,15) = 215.58\) \(6.19\) \(34.02\) \(0.58\)

Signif. \(p<0.001\) \(p=0.011\) \(p < 0.001\) \(p= 0.578\)

There was no significant difference in the weight of roots or shoots harvested from each treatment (Table 4.5). The fresh mass of aphids was reduced at the largest amendment rate and the differences between treatments were significant. The mass of Cd and Zn contained in the soil, roots and shoots increased with sewage sludge amendment (Tables 4.6 and 4.7).
Differences among treatments were significant for all three system components for both metals (Tables 4.6 and 4.7). In contrast to the soil-plant system, there was no discernible pattern in the mass of Cd or Zn in contained in the aphid populations and differences among treatments were not significant (Tables 4.6 and 4.7).

Table 4.7 The mass of zinc (mg) contained in soil, wheat roots and shoots and aphids after the amendment of soil with sewage sludge (mean ± 1 SE).

| Amendment rate | Soil         | Root         | Shoot        | Aphids       |
|               | 0 t.d.s. ha⁻¹ | 260.2 ± 4.40 | 0.101 ± 0.0148 | 0.129 ± 0.0192 | 0.018 ± 0.0020 |
|               | 10 t.d.s. ha⁻¹ | 312.0 ± 9.27 | 0.176 ± 0.0208 | 0.149 ± 0.0135 | 0.021 ± 0.0019 |
|               | 100 t.d.s. ha⁻¹ | 722.3 ± 14.44 | 0.183 ± 0.0186 | 0.222 ± 0.0292 | 0.015 ± 0.0023 |

\[ F(2, 15) = 548.72 \]
\[ \text{Signif.} \quad p < 0.001 \quad p = 0.011 \quad p = 0.011 \quad p = 0.19 \]

4.4 Discussion

The effect of crop species on the concentration of Cd and Zn in grain aphids

Cadmium concentrations in the wheat shoots were high compared to the reported range of between 0.016 to 0.386 mg kg⁻¹ for unamended soils and soils amended with moderate additions of sludge (Merrington et al. 1997a; Merrington et al. 1997b; Winder et al., 1999). Cadmium concentrations in barley shoots were within the reported range of 0.03 to 2.79 mg kg⁻¹ for sewage sludge amended soils (Sommers et al., 1991). This is in agreement with the findings of Jarvis et al. (1976) that Cd concentrations are higher in wheat than barley shoots. However, the results of Chapter 3 in the present study indicate that the difference between crops can be less than between cultivars of the same crop.

Cadmium concentrations in aphids were not affected by the amendment of soil with sewage sludge. This was a reflection of the statistically significant relationship between the Cd concentration in S. avenae and the spring wheat and barley shoots on which they were feeding. This is in agreement with the results of Merrington et al., (1997b) that concentrations
of Cd in *S. avenae* are related to the concentrations in the shoots of the wheat plants on which they feed. As a consequence of this relationship, the lower concentration of Cd in barley shoots resulted in lower concentrations in the aphids. Restricted root to shoot transport in wheat and barley (Chapter 3; Jarvis et al., 1976) therefore has an important influence on the concentration of Cd in *S. avenae*.

After adjusting for Cd concentration in the shoot, there was no significant difference in the Cd body burdens of aphids feeding on the two crops. Crawford et al. (1995) found that aphids have only a slight control over Cd accumulation. This suggests that concentrations of Cd in aphids are a reflection of the concentration in the phloem sap of the plants on which they feed. Thus, the relationship between aphid and shoot concentration suggests that Cd loading into the phloem sap is related to the accumulation of this metal in the shoot.

Sewage sludge amendment of soil resulted in significant accumulation of Zn in *S. avenae* regardless of crop species. Accumulation was in line with reported results, which have found that *S. avenae* readily accumulated Zn from wheat plants grown in sludge amended soils (Merrington et al., 1997a; Merrington et al. 1997b; Winder et al., 1999). Concentrations of Zn in the aphid populations reached 249 and 133 mg kg⁻¹ for wheat and barley systems respectively. These values are within the reported range for *S. avenae* in field trials where the soil has been amended with sludge (20 to 354 mg kg⁻¹; Merrington et al., 1997b; Winder et al., 1999).

The significantly higher concentrations in *S. avenae* feeding on the wheat plants demonstrate that crop species can have an important influence on the transport of Zn from the soil to the herbivore trophic level. This effect remained significant when differences in shoot concentration were adjusted for. This suggests that the two crops differ in the physiological mechanisms regulating Zn concentration in the phloem sap and, unlike Cd, Zn loading in the phloem sap was not related to the concentration in the shoot. The lower concentrations of Zn in aphids feeding on barley shoots and the reduction in the biomagnification factors between
barley shoots and *S. avenae* as sewage sludge amendment increases, show that barley may have a more precise control over Zn concentrations in the phloem sap. This could be related to restricted loading of Zn in to the phloem sap. However, differences in the availability of Zn in the phloem sap (i.e. the relative proportion of free metal, the type of ligand to which the metal is bound or a mixture of both) may also account for the lower concentration in aphids. There is currently no work evident in the literature regarding differences in the concentrations or forms of Zn in the phloem sap among cereal crops from which to draw conclusions.

The effect of aphid species on the concentration of Cd and Zn in herbivore trophic level

Neither aphid species accumulated Cd as a result of amending soil with sewage sludge. This contrasts with some published data, which has demonstrated significant accumulation of Cd in *S. avenae* following sewage sludge amendment (Merrington *et al.*, 1997b). However, Cd was not accumulated by the bird-cherry oat aphid (*Rhopalosiphum padi*; Merrington *et al.*, 1997a) and the concentration of Cd in *S. avenae* was very low compared to the values reported by Merrington *et al.* (1997b; 0.026 to 0.354 mg kg⁻¹), even though the Cd concentrations in the shoot were much higher in the current study (1.12 mg kg⁻¹ compared to 0.035 mg kg⁻¹; Merrington *et al.*, 1997b). Given that aphids have a very limited ability to excrete Cd (Crawford *et al.*, 1995), the concentrations in aphids should reflect the concentration in the phloem sap. This suggests that the cultivar of winter wheat used in this experiment effectively regulates the concentration of Cd in phloem sap at low levels compared to the other varieties. There appears to be no published data for Cd concentrations in *M. dirhodum* with which to compare the values observed in this trial. However, the higher concentrations of Cd in *M. dirhodum* demonstrate that this species is a higher net assimilator of Cd than *S. avenae*.

In contrast to Cd, both species of aphid accumulated Zn as a result of increasing sewage sludge amendment. Furthermore, the concentration in the populations of both species showed the same pattern of response to sludge amendment. This suggests that similar physiological mechanisms control Zn regulation in both species of aphid. Concentrations of Zn in the shoots
were higher than in other reported investigations, but concentrations in *S. avenae* were lower. For instance Merrington *et al.* (1997b) found that shoot concentrations in winter wheat of 31 mg kg\(^{-1}\) resulted in concentrations in *S. Avenae* of 179 mg kg\(^{-1}\). Whilst in this trial shoot concentrations of 67 mg kg\(^{-1}\) resulted in a concentration of 126 mg kg\(^{-1}\). This suggests that Zn is regulated at relatively low concentrations in the phloem sap of this cultivar in a similar way to Cd. There are currently no evident data regarding the concentration of Zn, or any other trace metal in *M. dirhodum* with which to compare the results of this study. However, concentrations were within the range reported for other cereal aphids (Merrington *et al.*, 1997a; Merrington *et al.*, 1997b; Merrington *et al.* 2001).

**Transport of Cd and Zn between crop shoot and aphid**

The accumulation of Zn in aphids resulted in similar biomagnification factors in all four experimental systems (Tables 4.7 and 4.8). Comparable biomagnification of Zn derived from sewage sludge amended soils has been reported in a winter wheat - bird-cherry oat aphid (*Rhopalosiphon padi*) system (Merrington *et al.*, 1997a), and in *S. avenae* feeding on winter wheat in a field trial (Merrington *et al.*, 1997b). In the former, Zn was biomagnified by a factor of 6.8, whilst in the latter the factor was 12.8. The consistent biomagnification factors of about 2 found for Zn in aphids in this study has also been found in the pea - pea aphid (*Acyrthosiphon pisum*) system (Green and Tibbett, 2002). This raises the possibility that the concentrations of Zn in aphids are a reflection of the concentration in the phloem sap and there is no biomagnification. There are no published data on the concentration of Zn in the phloem sap of cereal plants against which to test this. However, the reported concentrations for other plants generally fall in the range of 30 to 90 mg kg\(^{-1}\) (Stephan and Scholz, 1993), although concentrations of up to 245 mg kg\(^{-1}\) have been reported (Hocking, 1980). Consequently, whilst it is possible that the concentrations of Zn in aphids are similar to those in the phloem sap, it appears more likely that aphids biomagnify Zn compared to their diet.
In none of the four experiments was Cd biomagnified. Other studies have reported varying Cd biomagnification in aphids. Crawford et al. (1995) found that *Aphis fabae* biomagnified Cd by a factor of 8 compared to the broad beans on which they were fed. Similarly, Merrington et al. (1997b) found *S. avenae* biomagnified Cd by a factor of 9.3 compared to the ears of wheat on which it was feeding when the soil had modest (> 20 t ha\(^{-1}\)) additions of sewage sludge. In contrast, Merrington et al. (1997a) found no biomagnification of Cd in *Rhopalosiphon padi* feeding on wheat shoots grown in soil with modest (> 20 t ha\(^{-1}\)) additions of sewage sludge. Crawford et al. (1995) found that *Aphis fabae* efficiently absorbed Cd from phloem sap and only a small quantity was excreted. This finding suggests that aphids have little control over the concentration of Cd in their bodies and that Cd biomagnification is dependent on the physiology of the plant, not the aphid. Given the variation in the behaviour of Cd in different plant species (Jarvis, 1976; Guo and Marschner, 1995) and cultivars (Cieslinski et al., 1996; Florijn and van Beusichem, 1993), this emphasises the important role played by the plant species and cultivar in determining the extent of Cd transfer from soil to herbivore.

It has been suggested that the biomagnification of Cd and Zn by aphids is linked to a deficiency of amino acids in their diet (Merrington et al., 1997a). To meet their physiological requirements for amino acids, aphids must ingest large quantities of phloem sap (Dixon, 1973). As a result, even a modest increase in the concentration of trace metals in phloem sap will lead to a significant increase in the quantity of metal ingested by the aphid. It is hypothesised that insects exert relatively little control on the uptake of trace metals once they are in an available form in the mid-gut lumen (Hopkin and Martin, 1984; Dallenger, 1993). Consequently, increased ingestion of trace metals may result in increased uptake into the aphid’s body.

Arthropods avoid toxicity from assimilated trace metals by either storing the metal in an inactive state (storage-detoxification) or by excreting excessive metal out of the body in the faeces (Hopkin, 1989; Dallenger 1993). The Arthropod alimentary canal consists of three
regions, the foregut, midgut and hindgut. Both the foregut and hindgut are lined with cuticle, which leaves the midgut as the major site of food absorption (Kershaw, 1983; Chapman, 1998). On entering the midgut epithelia cells, trace metals appear to be rendered metabolically inactive through binding to metallothionein (Maroni and Watson, 1985; Hopkin, 1989; Hopkin 1990b). These are short chain ploy-peptides with a large proportion (up to 30% w/w) of cysteine residues, which contain SH groups that bind metal ions to the metallothioein through thiolate ligands (Schäffer and Kägi, 1991).

![Gut Lumen Diagram](image)

**Figure 4.7** Detoxification pathways of selected trace metals and Fe in the mid-gut epithelium cells of insects (adapted from Hopkin, 1990b).

The ultimate fate of the metallothionein-trace metal complex is believed to be incorporation into intracellular granules formed by the lysosome system of the cell (Figure 4.7;
Hopkins, 1989; Hopkins, 1990b). Metals incorporated into granules are effectively insoluble and therefore unavailable to take part in, or disrupt, the metabolic activity of the cell (Hopkins, 1989). This mechanism results in the accumulation of trace metals in the mid-gut, efficiently preventing trace metals entering the soma of the organism (Maroni and Watson, 1985; Hopkin and Martin, 1984; Lindqvist et al., 1995; Vandenbulcke et al., 1998). For example, 92% of the Cd in the body of the collembolan Orchesella cincta was found in the gut, 15% of this was bound to metallothionein (Hensbergen et al., 2000).

After formation, granules may be excreted from the animal or be retained, perhaps for life (Hare, 1992). Species that retain these granules will accumulate, and potentially biomagnifying, trace metals (Dallenger, 1993). Species that can excrete granules lower their body burden and should not, therefore, accumulate trace metals to such an extent. For instance, Collembola renew the mid-gut epithelium on moulting, eliminating the granules stored within (Posthuma et al., 1992). Hensbergen et al. (2000) found that O. cincta was able to remove 35% of its Cd body burden in this way. There is also evidence to suggest that some arthropods can eject granules into the gut lumen (Sohal et al., 1977; Vandenbulcke et al., 1998), where they are presumably excreted with the faeces.

In some insects, Zn does not appear to induce metallothionein synthesis (Maroni and Watson, 1985; Sterenborg et al., 2003). Instead, it appears to be bound to smaller peptides (Sterenborg et al., 2003). Moreover, although Zn is localised in insect gut tissue, it is also found evenly distributed through the other tissues of the body (Lindqvist et al., 1995). The Zn detoxification pathway for Zn may, therefore, differ from Cd, perhaps reflecting Zn's status as an essential element. The accumulation of Zn in the malpighian tubules suggests that they may have a function in excreting excess Zn passing through the mid-gut (Lindqvist et al., 1995). Storage in ommochrome pigments in the granules of the proximal segment of the malpighian tubules may be important in the long-term accumulation of Zn as a result (Sohal et al., 1976; Smit and van Gestal, 1996).
The biominification of Zn by aphids found in the present study strongly suggests that they use a storage-detoxification mechanism(s). The biominification of Cd reported by other workers (Crawford et al., 1995: Merrington et al., 1997b), suggests similar mechanisms operate in aphids for this metal. The advantage of storage-detoxification is that it may avoid an assumed additional energetic cost involved in the process of excretion (Kramarz and Laskowski, 1997; Maryanski et al., 2002). The disadvantage of this mechanism is that the organism has only a limited capacity to store trace metals. When this capacity is exceeded, the organism is unable to detoxify adequately any further assimilation of trace metal (Hopkin, 1990b). Toxic effects then become evident, and rapidly become lethal within a relatively short increase in the internal concentration (Crommentuijn et al., 1995).

**The effect of sewage sludge on the mass of Cd and Zn within the herbivore trophic level**

The amendment of soil with sewage sludge resulted in a significant increase in the concentration of both Cd and Zn in the soil, wheat roots, and shoots. Zinc concentrations in aphids increased with sludge amendment, whilst Cd concentrations did not. This confirms the results found previously in the present study and other published investigations (Merrington et al., 1997b; Merrington et al., 2000). The mass of roots and shoots did not differ significantly among the treatments. This agrees with the findings of Mullins et al. (1986) that sewage sludge amendments of 100 t ha⁻¹ have no significant effect on the mass of crop roots or shoots. The net result of sewage sludge amendment was to increase significantly the mass of both Cd and Zn contained in the soil and in the roots and shoots of plants. Consequently, there was no restriction on the amount of either metal potentially available for transfer to aphids.

There was a significant decrease in the mass of aphids associated with the amendment of soil with sewage sludge at a rate of 100 t ha⁻¹. This shows that sewage sludge amendment of soil can have a detrimental affect on aphids. A reduction in the fecundity of the rose-grain aphid (*Metopolophium dirhodum*) has also been found at a sewage sludge amendment of 100 t ha⁻¹.
Reduced fecundity was also observed by Culliney and Pimentel (1986) in green peach aphids (*Myzus persicae*) feeding on collards grown in soil amended with contaminated sewage sludge at a rate of 224 t ha\(^{-1}\). Sewage sludge with a low level of contamination applied at the same rate had no effect on green peach aphid fecundity, suggesting that contaminants in the sludge were responsible. However, it was not apparent from their work which contaminant of the sewage sludge was responsible for this.

The reduction in the fecundity of *M. dirhodum* occurred only after the aphids had been feeding on the wheat plants for 21 days (Green, unpublished). A similar chronic toxic reaction was observed by Laskowski (2001) in pea aphids (*Acyrthosiphon pisum*) feeding on broad beans contaminated with 75 mg kg\(^{-1}\) of Cd. This suggests that an accumulation of a substance or substances of low acute toxicity is responsible for the toxic effects in aphids (Laskowski, 2001). In the present study, this substance is unlikely to be Cd as concentrations in the aphid populations were below the 30 mg kg\(^{-1}\) concentration found to cause minor sub-lethal effects in black bean aphids (*Aphis fabae*; Crawford *et al.*, 1995). However, the concentrations of a particular trace metal that cause toxic effects can differ markedly between species with a close taxonomic relationship (Crommentuijn *et al.*, 1995). Cadmium toxicity therefore remains a possibility, although this appears unlikely.

Zinc has been reported to have negative effects on arthropods at the population level when internal concentrations are as low as 60 mg kg\(^{-1}\) (van Gestal and Hensbergen, unpublished, cited by Smit and van Gestal, 1996). Aphids form an obligate symbiotic relationship with bacteria of the genus *Buchnera* (Douglas, 1998). The bacteria synthesise essential amino acids that are lacking in the aphids' diet (Wilkinson and Ishikawa, 2000). Without *Buchnera* aphids grow poorly and produce few offspring (Douglas, 1998). The sensitivity of Microorganisms to Zn (McGrath *et al.*, 1995), may make aphids more vulnerable to Zn toxicity, than arthropod groups not reliant on microbial symbionts. However, no reduction in the fresh mass of *S. avenae* was found at concentrations of Zn up to...
250 mg kg\(^{-1}\) (Green and Tibbett, 2001), a much higher value than was found in the affected aphid populations in the present study. It therefore seems unlikely that Zn is responsible for the observed reduction in the mass of the aphid populations.

Aphid population densities are reported to be positively related to the nitrogen content of their host plant (Hanisch, 1980; Honek, 1991; Duffield et al., 1997). However, in the present study aphid fresh mass was negatively related to N levels in the shoot. A similar relationship was reported by Gange et al. (1999), who found high mortality in aphids feeding on plants grown in a nutrient solution with high N concentrations. This was attributed to increased production of toxic alleochemicals (TAs) by the plant (Gange et al., 1999). Many of these chemicals (such as Alkaloids, cyanogenic glycosides, glucosinalates, some protein and peptides and non-protein amino acids) are N based (Mattson, 1980). The concentrations of TAs can increase with increased availability of N to the plant (Mattson, 1980; Fragoyiannis, 1999). Aphids accumulate TAs from their host plant (Wink and Witte, 1985; Witte et al., 1990; Szentesi and Wink, 1991; Wink and Witte, 1991), sometimes to very high levels. For example, Wink and Witte (1985) found that the *Aphis cytisorum* accumulated quinolizidine alkaloids to a level of 0.4 % of fresh weight. It has been suggested by Wink and Witte (1991) that TAs are probably toxic to aphids and their symbionts and that storage/detoxification mechanisms for TAs must exist in aphids. Toxic alleochemicals can be associated with a decreased fecundity and relative growth rates and increased mortality in aphid populations (Thackray, et al., 1990; Berlandier, 1996; Fragoyiannis et al., 1998). This suggests that there is a point where storage/detoxification mechanisms are overwhelmed and further accumulation of TAs results in toxicity to the aphid (Wink and Witte, 1991).

Although it can not be certain which of the many toxic elements and compounds found in sewage sludges are responsible for the reduction in aphid mass, TAs provide a plausible explanation. The accumulation of these compounds to the extent that storage/detoxification mechanisms are overloaded fits with the observed time-dependent reduction in fecundity.
found for *M. dirhodum*. In addition, the reduction in aphid mass is associated with a substantial increase in the N content of the aphids. This may partially reflect the accumulation of N based TAs within the aphids.

The effect of the reduction in aphid mass was that there was no difference in the mass of Zn contained in the aphid populations among the treatments, despite a significant elevation in concentration as sewage sludge amendment increased. Consequently, whilst predators feeding on these aphids would be exposed to increased Zn concentrations, they would not consume any more Zn than predators feeding on aphids from other treatments. The reduction in aphid mass may, therefore, form a negative feedback mechanism. This may effectively restrict the transport of Cd and Zn from aphids to their predators, if the latter switch to feeding on less contaminated prey species or move to feeding on aphids in uncontaminated fields when contaminated aphid populations have been exhausted. In case of less mobile aphid specific predators, such as the larvae of Coccinellidae, Syrphidae and Neuoptera, which would be confined to feeding on aphids in the contaminated field, there would be no such negative feedback. Instead, the lower fresh mass of aphids in the field would result in lower numbers of these predators.

4.5 Conclusion

Neither species of aphid accumulated Cd and the transfer between shoot and aphid resulted in the biominimisation of Cd. This appeared to be a consequence of restricted transport in the soil-plant system, in particular the mechanisms regulating root-shoot translocation and the loading of the metal into the phloem sap. In contrast, Zn was accumulated and biomagnified by both species of aphid, regardless of crop type. The significantly higher concentrations in spring wheat shoots were reflected in the concentrations of *S. avenae* for both metals. Crop type also had an affect on the biomagnification of Zn by *S. avenae*. Crop type and cultivar can therefore have an important influence on the transfer of Cd and Zn to aphids. In addition, *M. dirhodum* accumulated more Cd than *S. avenae*, showing that the species of
aphid also has an important influence on the transport of Cd, but not Zn, in the soil-plant-
arthropod system.

The concentration of Cd and Zn to which aphid predators will be exposed to as a result of
sewage sludge amendment of soil will, therefore, depend on the species of crop and aphid in
the soil-crop-aphid system. Furthermore, the likely accumulation of both metals by predators
may be restricted by the lack of an increased mass of metal in the aphid population resulting
from the reduction in the population fresh mass at an amendment rate of 100 t ha⁻¹.
5.0 CADMIUM AND ZINC TRANSFER BETWEEN APHIDS AND APHID PREDATORS

Figure 5.1 An adult seven-spotted ladybird (*Coccinella septempunctata*) searching an ear of wheat for grain aphids (*Sitobion avenae*).

5.1 Introduction

Aphids feeding on wheat and barley grown in sludge amended soil may biomagnify both Cd and Zn (Merrington *et al.* 1997a; Merrington *et al.* 1997b; Chapter 4). This can result in concentrations in aphids of up to 8 (Cd) and 10 (Zn) times greater than in the wheat ears on which they were feeding (Merrington *et al.*, 1997b). This suggests that the soil-wheat-grain aphid system might form part of a 'critical pathway', along which Cd and Zn may be biomagnified, endangering aphids (van Straalen and Ernst, 1991).

The biomagnification of trace metals in herbivores can be continued in predatory arthropods (Roberts and Johnson, 1978; Hunter and Johnson, 1982; Hunter *et al.*, 1987). The largest biomagnification of Cd has been reported in this trophic level (Roberts and Johnson, 1978).
However, biomagnification of trace metals in predatory arthropods is not a general behaviour of food chains (Bayer et al., 1985; Laskowski, 1991; Laskowski and Maryanski, 1993). The body burden of predatory arthropods is related to the physiological mechanisms responsible for the assimilation and excretion of trace metals (Janssen et al., 1991; Hendrickx et al., 2003), not their trophic level (van Straalen and van Wensem, 1986; Laskowski, 1991; Laskowski and Maryanski, 1993).

Whilst some species possess a physiology efficient in regulating trace metal body burden (Janssen et al., 1991; Kamaraz, 1999a), others do not and trace metals can be biomagnified in these species (Hendrickx et al., 2003). For example, Janssen et al. (1991) found the Cd body burden of the pseudo-scorpion Neobisium muscorum increased linearly with time on a contaminated diet. Kamaraz (1999b) found a similar relationship for the Cd body burden of the centipede Lithobium mutabilis. Thus, some aphid predators may further biomagnify Cd and/or Zn to levels that may be potentially toxic to them.

Both adults and larvae of Coccinella septempunctata are monophagous aphid predators and can consume large quantities of cereal aphids (Carter et al. 1980; Ferran and Dixon, 1993). This may expose them to elevated, and potentially damaging, levels of trace metals in areas of repeated sewage sludge application. Trace metals accumulated in the larval stage can be concentrated by weight loss during pupation (Gintenreiter et al., 1993; Ortel, 1995), potentially compounding accumulation by the adult life stage. However, studies examining the influence of metamorphosis on the trace metal concentration of adult insects have not shown any particular pattern, and trace metal transfer from food consumed in the larval stage to adult insects is not well understood (Lindqvist, 1992). As both adult and larval C. septempunctata may be important in the biological control of aphids in cereal crops (Carter et al. 1980; Chambers et al., 1986), the biomagnification of trace metals within them may have an important impact on the integrated pest management of aphids.
The aim of this chapter is to establish if a pathway exists for the transfer of Cd and Zn from aphids to the monophagous aphid predator *Coccinella Septempunctata*. An assessment is also made of the potential for *C. septempunctata* to regulate the body burden of newly emerged adults through the sequestration of Cd and Zn in the pupal exuviae.

The objectives of the work presented in this chapter are;

(i) to determine the extent to which sludge derived Cd and Zn are transferred to newly emerged adults seven ladybirds;

(ii) to determine the contribution made by three independent variables (size of sewage sludge amendment, time on diet and fresh mass of aphids consumed) to the prediction of Cd and Zn concentrations in adult ladybirds.

5.2 Materials and methods

5.2.1 The transfer of sludge derived Cd and Zn to the newly emerged adults seven spotted ladybirds

A sample of soil sample taken from an Ap horizon of an agricultural field was further subdivided into equal parts. These were then amended with municipal sewage sludge at treatment rates equivalent to 0, 10, 30 t (dry solids) ha\(^{-1}\). The sewage sludge and soil was thoroughly mixed and used to fill six replicate 7.5 l pots for each treatment. Pots were seeded with spring wheat (*Triticum aestivum* L. cv. Alexander) at a rate equivalent to 400 kg ha\(^{-1}\). The freely draining pots were placed in a fully randomised block in a glasshouse and were watered with distilled water. When the plants were between growth stages 37 and 51, cultures were established on the plants by placing 200 grain aphids (*Sitobion avenae*) from laboratory cultures in each pot. Individual pots were covered with netting to prevent the transfer of aphids between treatments. Aphid cultures were left to establish for 3 weeks before all aphids from each pot were collected from the plants by gentle brushing with a fine paintbrush. Aphids from different pot cultures were kept separately from each other at \(-18^\circ\) C until used in the feeding trial. Soil and plants were also sampled for analysis at this time.
A preliminary trial was conducted under the same experimental conditions used in the main trial to establish the fresh weight of *S. avenae* consumed by *C. septempunctata* larvae at different instar stages. The results of this trial demonstrated that first and second instar larvae would not eat aphids that had been frozen and then thawed. Third and fourth instar larvae consumed thawed aphids and appeared to develop normally on this diet. The results of the preliminary trial also showed that insufficient aphid mass had been collected from the pot cultures to feed sufficient replicates of third instar larvae to pupation. The experiment therefore proceeded with the fourth instar only.

![Experimental set up used to investigate transfer of Cd and Zn to newly emerged adult seven spotted ladybirds.](image)

**Figure 5.2.** Experimental set up used to investigate transfer of Cd and Zn to newly emerged adult seven spotted ladybirds.

Newly laid ladybird eggs from a single female were removed from a laboratory culture and placed in a 9 cm diameter petri dish containing filter paper moistened with distilled water (in order to maintain a high humidity). For the first twenty-four hours after hatching *C. septempunctata* larvae fed on the egg case. After this period larvae were fed by placing
cuttings of winter barley colonised by aphids in to the petri dish. Fresh cuttings were placed in the petri dishes each day until the larvae developed to the third instar stage. Larvae were then isolated individually in petri dishes to prevent cannibalism (Figure 5.2). Feeding was continued as previously described.

Fifteen larvae from these cultures that had developed in to the fourth instar overnight were selected at random and were transferred to new 9 cm petri dishes, again containing a moistened filter paper. Selected larvae were then placed in a controlled environment cabinet set to a 16 hr, 24°C day: 8hr, 24°C night regime. Larvae were divided into three equal treatment groups and each individual larva was randomly assigned frozen aphids harvested from one of the pot cultures on which to be fed. Larvae were fed a known weight of defrosted aphids in excess of the normal daily requirement (calculated from the preliminary trial). After 24 hrs, uneaten aphids were re-weighed to determine the weight of aphids consumed, and weighed, freshly thawed aphids were added to the petri dish in their place (Winder et al., 1999). Feeding continued in this way until the larvae pupated. On emergence from the pupa, adult ladybirds were weighed and frozen at -18 °C until analysis for Cd and Zn was undertaken.

5.2.2 The contribution of size of sewage sludge amendment, time on diet and fresh mass of aphids consumed to the prediction of the variability of Cd and Zn concentrations in adult ladybirds

A second trial was conducted to determine the contribution made by the size of sewage sludge amendment, time on diet and fresh mass of aphids consumed to the Cd and Zn concentrations in adult ladybirds. A sub-sample of a bulk soil sample was taken from an Ap horizon of an agricultural field. This was treated as described above with the exception that the treatment rates used were equivalent to 0, 10, 100 t (dry solids) ha⁻¹ and the pots were seeded with winter wheat (Triticum aestivum L. cv. Challenger). When the plants were between growth stages 37 and 51, cultures were established on the plants by placing 200 grain aphids
(Sitobion avenae) from laboratory cultures in each pot. Individual pots were covered with netting to prevent the transfer of aphids between treatments. After 28 days, all aphids were removed from the shoots and were frozen prior to use in the feeding trial.

Forty five ladybirds were selected at random from a laboratory culture and were isolated individually in 9 cm petri dish lined with damp filter papers to maintain a high humidity. Ladybirds were placed in a controlled environment cabinet set to a 16 hr, 24°C day: 8hr, 24°C night regime. The ladybirds were allowed to acclimatise for 72 hrs, during which no food was given. Ladybirds were divided into three equal treatment groups and each group was assigned aphid samples, bulked together, from either the 0, 10 or 100 t ha\(^{-1}\) treatments, on which to be fed. Ladybirds were fed a known weight of defrosted aphids in excess of their normal daily requirement (calculated from a preliminary trial). After 24 hrs, uneaten aphids were removed, weighed to determine the mass of aphids eaten and were replaced by weighed, freshly thawed aphids (Winder et al., 1999). Every 24 hours one ladybird was sampled at random from each of the three treatment groups. Sampled ladybirds were starved for 48 hours to allow the expulsion of gut contents before being frozen prior to the determination of Cd and Zn. This process was repeated for 15 days, by which time all the ladybirds in each treatment had been sampled.

5.3 Results

5.3.1. The transfer of sludge derived Cd and Zn to the newly emerged adults seven spotted ladybirds

Cadmium concentrations in aphids were highest in the 10 t ha\(^{-1}\) amendment and lowest in the 30 t ha\(^{-1}\) amendment (Table 5.1). Concentrations of Zn in aphids increased with the size of sewage sludge amendment, the largest amendment resulting in an increase of 50 % over the concentration in the control (Table 5.2). A one-way ANOVA indicated that no statistically significant differences existed in the aphid populations among treatments for Cd
Table 5.1 Cadmium concentrations (mg kg⁻¹) transferred from a soil amended with sewage sludge in wheat, aphids, pupal exuviae and newly emerged adult ladybirds (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment Rate</th>
<th>Aphids</th>
<th>Pupal Exuviae</th>
<th>Adult Ladybirds</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>0.373 ± 0.051</td>
<td>0.137 ± 0.035</td>
<td>0.162 ± 0.35</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>0.419 ± 0.115</td>
<td>0.086 ± 0.004</td>
<td>0.223 ± 0.67</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>0.244 ± 0.085</td>
<td>0.196 ± 0.069</td>
<td>0.115 ± 0.027</td>
</tr>
</tbody>
</table>

\[ F(2,12) = 0.54 \quad F(2, 10) = 2.23 \quad F(2, 12) = 1.29 \]

Signif. \( p = 0.59 \) \( p = 0.16 \) \( p = 0.31 \)

Table 5.2 Zinc concentrations (mg kg⁻¹) transferred from a soil amended with sewage sludge in wheat, aphids, pupal exuviae and newly emerged adult ladybirds (mean ± 1 SE).

<table>
<thead>
<tr>
<th>Amendment Rate</th>
<th>Aphids</th>
<th>Pupal Exuviae</th>
<th>Adult Ladybirds</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 t ha⁻¹</td>
<td>162.9 ± 11.2</td>
<td>230.5 ± 18.9</td>
<td>184.2 ± 15.3</td>
</tr>
<tr>
<td>10 t ha⁻¹</td>
<td>215.9 ± 12.4</td>
<td>220.4 ± 13.5</td>
<td>204.5 ± 13.5</td>
</tr>
<tr>
<td>30 t ha⁻¹</td>
<td>248.5 ± 11.5</td>
<td>229.4 ± 16.7</td>
<td>217.1 ± 21.4</td>
</tr>
</tbody>
</table>

\[ F(2, 15) = 13.59 \quad F(2, 12) = 0.11 \quad F(2, 12) = 0.95 \]

Signif. \( p < 0.001 \) \( p = 0.90 \) \( p = 0.42 \)

Sewage sludge amendment of soil had no statistically significant effect on the weight of aphids consumed by larvae \( (F(2, 12) = 1.70, p = 0.23) \) or on the dry weight of newly emerged adults \( (F(2, 12) = 3.92, p = 0.14) \). Cadmium concentrations in the newly emerged adult ladybirds reflected concentrations in the aphids. After log transformation of the data to meet the assumption of homogeneity of variance, no statistically significant difference among treatments was found (Table 5.1). Zinc body burdens of adult \( C. septempunctata \) increased with sewage sludge application rate, although not statistically significantly (Table 5.2). Zinc concentrations in exuviae were similar for all treatments, whereas Cd concentrations varied with no trend apparent (Tables 5.1 and 5.2). After log transformation of the Cd data to meet
the assumption of homogeneity of variance, one-way ANOVA showed that differences in exuviae concentrations among treatments were not statistically significant for both metals (Tables 5.1 and 5.2).

To determine the influence of Cd and Zn sequestration in the exuviae on the body burden of the newly emerged adults, a one-way analysis of co-variance (ANCOVA) was conducted where the independent variable was sludge application rate, the dependent variable was metal concentration in ladybirds. For the assumption of linearity to be met, the percentage of Cd sequestered in the exuviae was inversely transformed. The percentage of Zn sequestered in the exuviae and transformed cadmium data were included as the co-variates. Effect size was calculated as part of the ANCOVA by the partial eta squared method ($\eta^2_p$).

After adjusting for the percentage of Zn sequestered in the exuviae, there was no statistically significant difference among treatments in the concentration of Zn in newly emerged adults ($F(2, 11) = 0.11, p = 0.90, \eta^2_p = 0.06$). There was a significant relationship between the percentage of zinc sequestered in the exuviae and the body burden of the newly emerged adults ($F(1, 11) = 5.48, p = 0.036, \eta^2_p = 0.30$). For Cd there was no statistically significant difference among treatments in the concentration of Cd in the newly emerged adults ($F(2, 9) = 0.05, p = 0.95, \eta^2_p = 0.01$) after adjusting for the percentage sequestered in the exuviae. There was a statistically significant relationship between the percentage of Cd sequestered in the exuviae and the body burden of the newly emerged adults ($F(1, 9) = 22.0, p = 0.001, \eta^2_p = 0.71$).

5.3.2 The contribution of size of sewage sludge amendment, time on diet and fresh mass of aphids consumed to the prediction of the variability of Cd and Zn concentrations in adult ladybirds

Concentrations of Cd in the aphids fed to the ladybirds were 0.109, 0.093 and 0.147 mg kg$^{-1}$ for the 0, 10, 100 t ha$^{-1}$ treatments, respectively. Differences among treatments were not found to be statistically significant ($F(2, 15) = 1.95, p = 0.18$). Zinc concentrations in the aphids were
70.6, 79.3, 122.8 mg kg\(^{-1}\) for the 0, 10, 100 t ha\(^{-1}\) treatments, respectively. In contrast to Cd, differences among treatments were found to be statistically significant \((F_{(2, 15)} = 13.96, p < 0.001)\). The concentrations of Cd in adult ladybirds feeding on these aphids showed no apparent trend (Figure 5.3).

![Figure 5.3](image)

**Figure 5.3** Changes in Cd concentration in adult ladybirds (mg kg\(^{-1}\)) with time on a diet of aphids that had been feeding on wheat plants grown in sewage sludge amended soil at three treatment rates (t ha\(^{-1}\)).

The contribution made by three independent variables (size of sewage sludge amendment, time on diet and fresh mass of aphids consumed) to the prediction of the Cd body burden of adult ladybirds was determined by standard multiple regression analysis. The assumptions of multi-collinearity, linearity, normality, homoscedasticity and independence of residuals was tested prior to the commencement of the analysis. The correlations between the variables, the unstandardised regression co-efficients \((B)\) and intercept, the standardised regression co-efficients \((\beta)\), the semi-partial correlations \((sp^2)\) and \(R, R^2\), and adjusted \(R^2\) are given in Table 5.3. None of the independent variables made a statistically significant unique contribution to the prediction of Cd concentration in the adult ladybirds and the R-value for
the regression did not differ significantly from zero ($F(3, 42) = 0.94, p = 0.43$). A combination of all three independent variables predicted only 6% of the variability in Cd concentration in the adult ladybirds.

Table 5.3 Standard multiple regression of time spent on diet (days), sewage amendment rate ($t$ ha$^{-1}$) and aphid consumption (mg) on the Cd concentration (mg kg$^{-1}$) of adult ladybirds.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adult Cd conc.</th>
<th>Sludge amendment</th>
<th>Aphid consumpt.</th>
<th>Time on diet</th>
<th>B</th>
<th>β</th>
<th>$sr^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sludge amendment</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td>0.239</td>
<td>0.111</td>
<td>0.01</td>
</tr>
<tr>
<td>Aphid consumpt.</td>
<td>-0.18</td>
<td>-0.29 *</td>
<td></td>
<td></td>
<td>-0.109</td>
<td>-0.036</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time on diet</td>
<td>-0.22</td>
<td>0.00</td>
<td>0.59 **</td>
<td></td>
<td>-4.116</td>
<td>-1.033</td>
<td>0.002</td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.151</td>
<td>36.67</td>
<td>32.40</td>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D.</td>
<td>0.098</td>
<td>45.45</td>
<td>32.45</td>
<td>4.66</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $p < 0.05$, ** $p < 0.01$

Zinc concentration in the adult ladybirds showed a declining trend with time spent feeding on aphids in all three treatments (Figure 5.4). Concentrations were generally higher in the treatments receiving sewage sludge amendments compared to the control and were highest in the 100 t ha$^{-1}$ amendment. Standard multiple regression was repeated as above for the Zn data. The correlations between the variables, the unstandardised regression co-efficients (B) and intercept, the standardised regression co-efficients (β), the semi-partial correlations ($sr^2$) and $R$, $R^2$, and adjusted $R^2$ are given in Table 5.4.

The overall R for the regression was significantly different from zero ($F(3, 42) = 5.86, p = 0.002$). The results of the multiple regression analysis showed that both time on diet and the size of sewage sludge amendment made a significant contribution to the prediction of adult Zn concentration (Table 5.4). Time spent on diet made a slightly greater contribution ($sr^2 = 0.15$) than the size of sewage sludge amendment ($sr^2 = 0.14$). The fresh mass of aphids consumed did not make a significant contribution to the prediction of adult ladybird Zn

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concentration and could only explain 1% of the variance observed in adult concentration (Table 5.4). All three variables together predicted 30% of the variability in the Zn concentration of adult ladybirds.

Figure 5.4 Changes in Zn concentration in adult ladybirds (mg kg\(^{-1}\)) with time spent on a diet of aphids that had previously fed on wheat plants grown in sewage sludge amended soil at three treatment rates (t ha\(^{-1}\)).

Table 5.4 Standard multiple regression of time spent on diet (days), sewage amendment rate (t ha\(^{-1}\)) and aphid consumption (mg) on the Zn concentration (mg kg\(^{-1}\)) of adult ladybirds.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adult Zn conc.</th>
<th>Sludge amendment</th>
<th>Aphid consumpt.</th>
<th>Time on diet</th>
<th>B</th>
<th>β</th>
<th>(\text{sr}^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sludge amendment</td>
<td>0.37**</td>
<td></td>
<td></td>
<td></td>
<td>0.245</td>
<td>0.402**</td>
<td>0.15</td>
</tr>
<tr>
<td>Aphid consumpt.</td>
<td>-0.27*</td>
<td>-0.29*</td>
<td></td>
<td></td>
<td>0.10</td>
<td>0.116</td>
<td>0.01</td>
</tr>
<tr>
<td>Time on diet</td>
<td>-0.39*</td>
<td>0.00</td>
<td>0.59**</td>
<td></td>
<td>-2.73</td>
<td>-0.458**</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean</td>
<td>90.06</td>
<td>36.67</td>
<td>32.40</td>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D.</td>
<td>27.72</td>
<td>45.45</td>
<td>32.45</td>
<td>4.66</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ R^2 = 0.30 \]
\[ \text{Adj. } R^2 = 0.25 \]
\[ R = 0.54 \]

* \(p < 0.05\), ** \(p < 0.01\)
The results of the correlation analysis suggested that a significant negative relationship existed between size of sewage sludge amendment and the fresh mass of aphids consumed by adult ladybirds (Table 5.4). To confirm that sewage sludge amendment had a negative effect on the consumption of aphids by ladybirds, the consumption of aphids by 10 ladybirds over the first 6 days of the experiment was investigated. This showed that the fresh mass of aphids consumed was 46.9 ± 8.02, 35.4 ± 7.20 and 18.9 ± 4.03 (mean ± 1 SE) for the 0, 10 and 100 t ha⁻¹ treatments respectively. To determine the statistical significance of the decrease in consumption, a one-way ANOVA was conducted. This showed that the difference among treatments were significant ($F_{(2, 27)} = 4.20, p = 0.026$).

5.4 Discussion

The transfer of sludge derived Cd and Zn to the newly emerged adults seven spotted ladybirds

The fourth instar *C. septempunctata* consumes some 65% of the total food needed for the development from egg to pupa (Carter *et al.*, 1980). Consequently, the majority of trace metals accumulated during development may be expected to occur in the fourth instar. Accumulation may be compounded by weight loss during pupation (Gintenreiter *et al.*, 1993; Ortel, 1995). However, insects may exclude accumulated trace metals from the adult life stage via sequestration in the pupal exuviae (Andrzejewska, *et al.*, 1990). Autoradiography experiments demonstrate that most Cd in insects is bound in the midgut epithelium, but is also localised in the integument (Lindqvist *et al.*, 1995; Inza *et al.*, 2001). The sequestration of Cd in the pupal exuviae, which are formed from larval integument, may therefore be likely. In this investigation, the mean percentage of cadmium sequestered in the exuviae was 10.5 %. The calculated effect size indicated that 71 % of the observed variance in the cadmium body burden of the adult could be explained by the percentage of Cd sequestered in the exuviae. This therefore appears to be an effective mechanism for excluding Cd from the adult.
In contrast to Cd, Zn is evenly distributed throughout the soma of Coleoptera (Lindqvist, et al., 1995). Zinc could therefore be more readily transferred to the adult during metamorphosis. In the current study the mean percentage of zinc sequestered in the exuviae is higher than for cadmium at 11.5%. However, the calculated effect size indicated that 30% of the observed variance in the Zn concentration in the adult was explained by the percentage of zinc sequestered in the exuviae. Thus, sequestration in the pupal exuviae appears to be a less effective mechanism for excluding Zn from the adult than Cd.

After adjusting for the percentage of metal sequestered in the exuviae, there was no significant difference between treatments in the body burden of adults for either metal. This would be expected for Cd, as there was no significant increase in concentration in the aphids on which the larvae fed. However, Zn concentrations were increased in the aphids. This suggests that larval ladybirds were able to regulate their Zn body burden, probably by excreting excess Zn in the faeces (Janssen et al., 1991; Ortel, 1995).

The contribution of size of sewage sludge amendment, time on diet and fresh mass of aphids consumed to the prediction of the variability of Cd and Zn concentrations in adult ladybirds

The concentrations of both Cd and Zn in adult ladybirds showed considerable heterogeneity in each treatment. This is not unusual for arthropod populations (Merrington et al., 2001; Sterenborg et al., 2003) and could result from differences in the age (Hunter et al., 1987; Rabitsch, 1995) and/or sex (Rabitsch, 1995; Lindqvist and Block, 1998) among individual ladybirds. None of the independent variables measured made a significant contribution to the prediction of the Cd concentration in adult ladybirds. This may be due to the efficient regulation of Cd concentration, which has been demonstrated for Carabid beetles (Janssen et al., 1991; Kramarz, 1999a; Scheifler et al., 2002). In contrast, Zn concentration in the ladybirds showed a positive relationship with the size of the sewage sludge amendment.
A significant relationship between sewage sludge amendment and Zn concentration was also found for the Carabid beetle *Bembidion lampros* feeding on aphids (Winder, *et al*., 1999).

Although the current study provides evidence for the transfer of Zn to adult ladybirds, it is difficult to interpret the biological significance of these findings as the assimilation of Zn was exceeded by excretion. The concentration of Zn in the aphids fed to the ladybirds prior to the commencement of the experiment was 43.1 ± 3.0 mg kg⁻¹. Consequently, ladybirds in all treatments were exposed to higher concentrations of Zn during the trial and, therefore, the results are not due to decontamination of the ladybirds. Zinc would therefore appear to be efficiently excreted by *C. septempunctata*. This is in agreement with the efficient elimination of Zn reported for other Coleoptera (Mason *et al*., 1983; Kramarz, 1999a).

Whilst it is known that aphids have a low nutritional value for polyphagous predators (Bilde and Toft, 1994; Toft, 1995), *S. avenae* is reported to be a relatively high quality diet for *C. septempunctata* (Nielsen *et al*., 2002). The diet of frozen aphids did not appear to unpalatable to the ladybirds, as consumption of aphids did not decline with time on diet. However, the decline in Zn concentration shows that the ladybirds' diet was unsuitable in some respects, perhaps due to changes in the smell or taste of the aphids after freezing.

The transfer of both metals to the adult ladybirds would have been restricted by the reduction in the fresh mass of aphids consumed by the ladybirds at the 100 t ha⁻¹ amendment rate. The reduction in consumption does not appear to be due to the accumulation of Cd or Zn in the aphids, as the concentrations were not excessive (Merrington *et al*., 1997b; Merrington *et al*., 2001). It is possible that the reduction in consumption is linked to the hypothesised accumulation of toxic allelochemicals (TAs) in the aphids on which the ladybirds were fed (see Chapter 4). The accumulation of TAs has been shown to provide partial protection to the aphid against predation (Wink and Römer, 1986). Furthermore, in a choice experiment, a species of aphid that did not accumulate alkaloids was subject to
significantly greater predation by adult *C. septempunctata* than a species that accumulated alkaloids (Gruppe and Römer, 1988). Consequently, the accumulation of TAs by aphids may be responsible for a reduction in the palatability, and hence consumption, of the aphids to ladybirds in the present study.

**5.5 Conclusions**

Newly emerged adult ladybirds did not accumulate significant concentrations of Cd or Zn after feeding on contaminated aphids as fourth instar larvae. This was partially due to the sequestration in the pupal exuviae of 10.5 % and 11.5 % of the accumulated Cd and Zn respectively. Despite the higher percentage of Zn lost in the exuviae, this mechanism had a greater affect on the Cd concentration in the adults.

No significant relationship was found between the Cd concentration in adult ladybirds and the fresh mass of aphids consumed, the size of sewage sludge amendment or time spent on the diet. This suggests that *C. septempunctata* is able to efficiently regulate the concentration of Cd in the adult life stage. In contrast, both the fresh mass of aphids consumed and the size of sewage sludge amendment made a significant contribution to the prediction of the concentration of Zn in ladybirds. The regression model containing both the size of sewage sludge amendment and time on diet explained 30 % of the observed variance in adult ladybird Zn concentration. However, the predictive properties of the regression model are limited by the decrease in the internal concentration of Zn in the ladybirds with time.
Figure 6.1 The safe re-cycling of sewage sludge? The soil in this field has received repeated amendments of sewage sludge, which have raised the Zn and Cd concentration in the soil to 600 and 3.3 mg kg\(^{-1}\) respectively. The crop of oil seed rape is healthy, but what about the arthropod community?

6.1 Introduction

The majority of studies investigating the transfer of trace metals in soil-plant-arthropod systems group organisms in the broad assemblages of plant, herbivore and predator or taxonomic order. However, the concentration of trace metals in these assemblages is a function of the variation in metal regulation in the component species rather than trophic level (van Straalen and van Wensem, 1986; Beyer, 1986). Consequently, species in critical pathways where trace metals are biomagnified may be lost in the ‘noise’ of species more efficient in maintaining homeostatic body burdens. A full understanding of the transfer of trace metals between trophic levels requires a detailed knowledge of the composition of the diet of the component species of the food chain. However, this is difficult information to obtain for arthropods (Janssen, 1993).
To overcome these fundamental problems, this study used monophagous invertebrates whose diet in the agroecosystem is known and which was replicated in model pot based systems. Research focused on the winter wheat (the commonest crop grown in the UK) and the grain aphid (*Sitobion avenae*, the most common aphid pest of wheat). As a consequence of the reported variation in trace metal accumulation among crop species and cultivars and among closely related arthropod species, the crop, cultivar and aphid species were changed in the studied system. The effects of these changes on the transfer of Cd and Zn in the system were assessed and the transfer of the two metals from aphids to the larvae and adults of monophagous aphid predators was also investigated.

Discussions in each of the experimental chapters focus on the detail of the experimental work presented in that chapter. This chapter presents an overview of the findings of the work as a whole. Firstly, the transfer of Cd and Zn through the study system is summarised. This includes where in the system transfer is enhanced or reduced (potential biomagnification or biominimisation). The present study is also placed in the context of other research and the contribution made to the knowledge in this field identified. Secondly, the limitations of the study are discussed. This is followed by a discussion of the implications of findings of this study to agroecosystems and beyond. Finally, the overall conclusions of the study are drawn and recommendations for further work made.

### 6.2 Transfer of Cd and Zn in the soil-plant-arthropod system

*Cadmium and zinc transfer in the soil-plant system*

The concentrations of Cd and Zn found in the various components of the system are summarised in Figures 6.2 and 6.3. The change in the percentage of Cd and Zn partitioned in the extractable fraction of the soil as the sewage sludge amendment increased, reflected the partitioning in the sludge. Consequently, the properties of the sewage sludge have an influence, at least initially, on the availability of the two metals to crop plants. The close
Figure 6.2. The range of cadmium concentrations (mg kg\(^{-1}\)) found in the components of the soil-plant-arthropod system after the amendment of soil with sewage at rates between 0 and 100 t ha\(^{-1}\).
'typical' sewage sludge. Therefore, the accumulation by the crop plants may have been uncharacteristically low for Cd and high for Zn.

The partitioning of trace metals in the extractable fraction of the sewage sludge amended soil is unlikely to remain constant; both short- and long-term changes may occur. For example, Chang et al., (1987), McBride (1995; 2000b) and Merrington et al. (2003) conclude from the literature that the availability of trace metals is highest shortly after sewage sludge application. However, Logan et al. (1997) reported an increase in Cd and Zn availability one year after application. Therefore, atypical changes in availability are possible.

![Diagram](image)

Figure 6.3. The range of zinc concentrations (mg kg\(^{-1}\)) found in the components of the soil-plant-arthropod system after the amendment of soil with sewage at rates between 0 and 100 t ha\(^{-1}\).
Long-term changes in trace metal availability in sewage sludge amended soils have been postulated to follow two differing scenarios. Firstly, it has been hypothesised that the availability of trace metals in sewage sludge amended soil may increase as the adsorption capacity of the organic material from the sludge matrix is lost as it degrades (McBride, 1995). However, there is a counter-argument suggesting that the sorption properties of the inorganic components of the sludge (e.g. Fe, Al and Mn oxides, phosphates and silicates) will have sufficient capacity to bind trace metals in non-extractable forms almost indefinitely (Corey et al., 1987; Chaney and Ryan, 1993).

The work of Li et al (2001), using a range of soils and sludges, has demonstrated that both the inorganic and organic matter in sludges contribute to the increased adsorption of trace metals in sewage sludge amended soil. However, there is conflicting evidence on the relative roles of organic and inorganic binding sites from the sludge matrix, in the adsorption of trace metals in amended soils (Merrington et al., 2003). For instance, some studies indicate that increased adsorption is primarily related to the inorganic binding sites that are added to the soil with the sludge (Brown et al., 1998; Hyun et al., 1998). In contrast, other workers stress the importance of organic matter, present in the sludge, in increasing soil adsorption capacity (Hooda and Alloway, 1994; Hooda and Alloway, 1998; Sastre et al., 2001). The degradation of sludge organic matter could, therefore, result in a decrease in soil sorption capacity (Hooda and Alloway, 1994).

Sewage sludge organic matter degradation is initially rapid (Hooda and Alloway, 1994), but 15% of the sludge organic matter may still remain in the soil 23 years after the amendment (McGrath et al., 2000). Evidence from field trials suggests that organic matter breakdown affects trace metal availability only in the first two years after application and thereafter has little effect (Logan et al., 1997; Brown et al., 1998; Hyun et al., 1998; McGrath et al., 2000). After the initial changes in availability, the findings of McGrath et al. (2000) indicate no further change in availability of Cd and Zn for over 20 years. However, it remains to be
determined whether the remaining organic matter in the soil has sufficient capacity to bind the trace metals added with the sludge, or if the inorganic binding sites are retaining metals in the solid phase (Hyun et al., 1998; McGrath et al., 2000).

In the present study, soil was sampled for assessment of the extractable concentration of Cd and Zn ca 200 days after the sewage sludge was incorporated into the soil. Thus, the processes associated with the short-term changes in metal adsorption will have affected the concentration of the extractable fraction of Cd and Zn in the soil. However, this will not have reached completion and it is likely that the availability of Cd and Zn would decrease over a further 2 to 3 years (McBride, 2003b). Changes in the adsorption of Cd and Zn over the long-term are difficult to predict with the knowledge currently available, but will be determined by the physio-chemical properties of the sewage sludge, the method of application and the physio-chemical and biological properties of the soil (McBride 2000; McBride, 2003b; Merrington et al., 2003).

Winter wheat roots accumulated both Cd and Zn to levels in excess of the total and extractable concentration in the soil. This resulted in the highest concentrations in the plant, which confirms previous finding for wheat plants (Jarvis et al., 1976; Lübben and Sauerbeck, 1991; Sauerbeck, 1991). Root concentration of both metals more closely reflected the extractable concentration than the total concentration. Consequently, the first restriction in the transfer of Cd through the soil-plant system resulted from a reduced partitioning in the extractable fraction of the soil as sewage sludge amendment increased. Root accumulation from the extractable fraction of the soil appeared to be unrestricted. Similar unrestricted root uptake of Cd has been reported for maize (Florijn and van Beusichem, 1993).

The translocation of both metals from the roots to the shoots of winter wheat plants restricted the transfer of both metals in the soil-plant system. This had a smaller effect on Cd transfer than Zn, but the difference between the two metals lessened with increasing sewage sludge amendment. The concentrations of both metals were lower in the shoots than in the roots as a
result of the restricted translocation. This is in agreement with other reported findings for wheat (Jarvis et al., 1976; Petterson, 1976; Lübben and Sauerbeck, 1991; Benitez et al. 2001).

The accumulation of Cd in wheat shoots did not increase linearly with sewage sludge amendment; accumulation showed a tailing off in the 100 t ha$^{-1}$ treatment. The same response was also observed in barley shoots. This appeared to result from a combination of decreasing partitioning of Cd in the extractable fraction of the soil and restricted root to shoot translocation. In contrast, accumulation of Zn, in wheat and barley, was linear. Linear relationships between plant shoots and total metal concentration in soil have been reported (Smilde et al., 1993; Kabata-Pendias and Pendias, 2000), including barley grown in sewage sludge amended soil (McGrath et al., 2000). However, other studies report shoot accumulation showing a plateau response to increasing sewage application (Corey et al., 1987; Chang et al., 1997; Logan et al., 1997; Hamon et al., 1999).

The plateau response observed in the accumulation of Cd in wheat and barley shoots might breakdown at higher soil concentrations (Hamon et al., 1999). For example, Salt et al. (1995b) observed bi-phasic root-shoot translocation of Cd in Indian mustard (Brassica juncea) grown in nutrient solutions. Cadmium concentration in the xylem sap increased with increasing concentration in the nutrient solution until reaching saturation when Cd levels reached 0.3 $\mu$g ml$^{-1}$. Cadmium concentration then remained constant until concentrations in the nutrient solution reached 0.8 $\mu$g ml$^{-1}$, after which concentrations in the xylem sap increased linearly. A similar bi-phasic pattern was observed in the concentration of Cd in the mustard shoots. This suggests that when the plant is exposed to a certain level of Cd, toxicity results in a breakdown of the physiological mechanisms regulating root-shoot translocation (Salt et al., 1995b). A bi-phasic accumulation of Zn in shoots has also been reported for Zn (Hamon et al., 1999).
That the plateau response does not persist at higher soil concentrations is suggested by a comparison of Cd transfer in the two pot trials in the present study in which winter wheat was grown. In the first trial, the concentrations in the soil, roots and shoots in the 100 t ha\(^{-1}\) treatment were 0.51, 0.68 and 0.232 mg kg\(^{-1}\) respectively. In the second trial, the corresponding concentrations were 0.69, 1.35 and 1.12 mg kg\(^{-1}\). The higher concentrations in the second trials resulted in a higher root: shoot partitioning co-efficient (0.83 compared to 0.34 in the first trial). Consequently, the mechanisms restricting the translocation of Cd from root to shoot in winter wheat may have broken down between a root concentration of 0.68 and 1.35 mg kg\(^{-1}\).

The net effect of reduced partitioning in the extractable fraction of the soil, biomagnification in the root and restricted root-shoot translocation, was that shoot Cd concentrations were lower than in the soil. This is at variance with reported findings for a range of plants, including wheat grown in sludge-amended soils (Sauerbeck, 1991; Merrington et al., 1997a). For example, Merrington et al. (2001) found that a single application of triple phosphate fertiliser resulted in transfer co-efficients between the soil and wheat shoots of 12 and 15 for the control and fertiliser treatments respectively. However, Merrington et al., (1997a) found lower Cd concentrations in the winter wheat shoots than in soil amended with sewage sludge at a rate of 20 t ha\(^{-1}\). Furthermore, Mench et al. (1997) found transfer co-efficients were less than 1, and often below 0.5, in the shoots of field grown wheat plants grown in a variety of soils. In contrast to Cd, Zn concentrations in the shoot were higher than in the soil in which they were grown at all sewage sludge amendments. This is in agreement with the transfer co-efficients found for Zn in the sludge amended soil-wheat system by other workers (Sauerbeck, 1991; Lübben and Sauerbeck, 1991). However, the findings of Bentitez et al., (2001) are at variance with this finding.
Cadmium and Zinc transfer in the plant-aphid system

The transfer of Cd through the food chain was further limited by the lack of biomagnification of Cd in *S. avenae* in all sewage sludge amendments. This is in disagreement with the findings of Merrington *et al.* (1997b) and Merrington *et al.* (2001), who found substantial biomagnification of Cd in *S. avenae* and the bird cherry-oat aphid (*Rhopalosiphum padi*) respectively. However, this is in close agreement with the level of biominimisation noted by Merrington *et al.* (1997a) in *R. padi* in a sludge amended soil-wheat-aphid system. The biominimisation of Cd in the aphids in the present study is hypothesised to be the result of restricted loading of Cd into the phloem sap. This agrees with the conclusion reached Merrington *et al.* (2001) for the wheat - *R. padi* system.

*Sitobion avenae* accumulated Zn as sewage sludge amendment increased, confirming previous work that *S. avenae* accumulates Zn from wheat shoots (Merrington *et al.*, 1997b). Zinc was biomagnified by *S. avenae* compared to the shoots on which they fed by factors ranging from 1.4 to 2.7. This is toward the lower end of the range reported for wheat shoot - *S. avenae* biomagnification under field conditions of 1 to 12 (Merrington *et al.*, 1997b; Winder *et al.*, 1999). Generally, biomagnification factors for *S. avenae* feeding on wheat were ca 2 in the present trial and did not fall with increasing sewage sludge amendment. However, the pattern of biomagnification differed in *S. avenae* feeding on barley shoots; biomagnification was initially high compared to wheat, but fell with increasing sewage sludge amendment. Thus, the effect of crop species on the transfer of Zn to aphids cannot be attributed only to regulation of shoot concentration, but must also be determined by the mechanisms controlling the loading of Zn into the phloem sap.

The concentrations of Cd and Zn in *Sitobion avenae* populations were significantly correlated with the concentration in the wheat and barley shoots on which they were feeding. This demonstrates that the type of crop has an important influence on the transfer of the two metals in the soil-crop-aphid system. Cadmium concentrations in shoots are reported to increase in
the order barley – oat – wheat, and Zn concentrations are reported to be higher in wheat than oats (Jarvis et al., 1976; Pettersson, 1976; Sauerbeck, 1991). In addition, bread wheat shoots were found to accumulate more Cd and Zn than durum wheat (Hart et al., 1998a; Hart et al., 1998b). Consequently, selecting a crop with a lower shoot accumulation could reduce the transfer of metal in the soil-crop-aphid system. This could be of use where Cd and/or Zn concentrations in the soil are near to the legal limits. However, the present study suggests that differences among cultivars of the same crop could be greater than difference between crops. The potential variation in shoot Cd concentration within a species was demonstrated by Florijn and Beusichem (1993) in 19 inbred lines of maize, which showed a range of Cd shoot concentrations from 0.9 to 9.9 mg kg⁻¹. Thus, the cultivar as well as the crop needs to be selected in order to ensure effective reduction in metal transfer.

The relationship between shoot and *S. avenae* concentration emphasises the importance of the plateau response of shoot concentration in determining the transfer of both metals, but especially Cd, from the soil to aphids. However, in one of the pot trials, winter wheat grown in soil amended with sludge at a rate of 100 t ha⁻¹ showed no signs of a plateau in shoot concentration. Consequently, the regulation of root to shoot translocation may have broken down. Despite accumulating Cd to 1.12 mg kg⁻¹, Cd was substantially biominimised in the aphid (biomagnification factor = 0.13). Herran and Feller, (1994) hypothesise that two processes, under different control mechanisms, are required for trace metals to enter phloem sap. The first process is unloading from the xylem sap, the second is loading into the phloem sap. Therefore, a breakdown in the restriction of root-shoot translocation of Cd does not necessary imply a breakdown in the regulation of phloem loading. It is not clear at present at what shoot concentration the breakdown in the regulation in phloem loading may occur. However, Merrington *et al.* (2001) found a biomagnification factor of 0.49 between wheat shoots and aphids at the control shoot concentration of 0.85 mg kg⁻¹. After contamination of the soil by a phosphate fertiliser application had raised the shoot concentration to 1.45 mg kg⁻¹, the biomagnification factor increased to 2. Aphids exert little control over their
accumulation of Cd (Crawford et al., 1995), which would suggest that the increase in biomagnification was the result of an increase in the concentration of Cd in the phloem sap. Thus, it may be hypothesised that the regulation of Cd loading into the phloem sap of wheat may have failed when shoot concentrations have reached 1.45 mg kg⁻¹.

The present study also demonstrated, for the first time, that Metopolophium dirhodum also accumulates Zn as a result of sewage sludge amendment of soil. In contrast to Cd, the two species of aphid did not differ in the concentration of Zn accumulated. This shows that the species and metal specific nature of trace metal accumulation found in another sap feeding Homoptera (Psyllids; Glowacka et al., 1997) and isopods (Hopkins, 1990a) is also true of the Aphididae. Moreover, Zn biomagnification in M. dirhodum showed variance with that found in S. avenae. The highest and lowest biomagnification factors in the present study were found in M. dirhodum in the 0 and 100 t ha⁻¹ amendments respectively. This may indicate that M. dirhodum regulates the internal concentration of Zn within a narrower concentration range than S. avenae. This could be the result of different physiological mechanisms governing the Zn regulation, which would be consistent with the findings of Hopkin (1990a) for Zn in two species of isopods (Hopkin, 1990a).

The stress caused to the plant because of aphid infestation did not result in a negative feedback that reduced the accumulation of either Cd or Zn in wheat shoots. This accords with the results of Ernst (1987) that aphid infestation does not affect Zn concentrations in plant shoots. However, this is at variance with the finding reported by Crawford et al. (1990) that infestation decreases the Cd concentrations in shoots. Aphid infestation did result in increased concentrations of Cd and Zn in wheat ears. However, Merrington et al. (1997b) found no significant relationship between the concentration of either Cd or Zn in S. avenae and the ears of wheat on which they were feeding. Moreover, the supply of Cd and Zn to the ears of wheat plants did not appear to be significantly affected by infestation. Aphid infestation did not, therefore, appear to affect Cd and Zn transfer from soil to aphids.
Cadmium and zinc transfer in the aphid-predator system

Cadmium concentrations in newly emerged adults of *C. septempunctata* reflected the concentrations in the aphids on which the preceding larvae fed. However, transfer of Cd was again restricted, to the extent that concentrations in the newly emerged *C. septempunctata* were about half the value in aphids. In contrast, Cd concentrations in the pupa of the green lacewing (*Chrysoperla carnea*) were found to be between 3-5 times higher than in the aphids on which the preceding larvae fed (Green and Jeffries, 2003). The difference in Cd transfer from aphids to predator between *C. septempunctata* and *C. carnea* could be due to different feeding mechanism. Cadmium is primarily accumulated within the soft proteinaceous tissues in arthropods (Hughes *et al.*, 1980). *Chrysoperla carnea* feeds by injecting digestive enzymes into its prey and sucking out the resultant partially digested tissues, whilst fourth instar *C. septempunctata* larvae consume the whole aphid (Rotheray, 1989). Therefore, lacewing larvae consume the tissues in which Cd is accumulated and are therefore likely to be exposed to higher concentrations. However, Merrington *et al.* (2001) found Cd concentrations in larvae of the lacewing *Mallada signata* feeding on Cd contaminated aphids were below the limit of detection. This suggests inter-specific differences in Cd regulating physiology may therefore cause greater differences in Cd accumulation than the feeding mechanism. However, *M. signata* was fed on *R. padi*, a species of aphid shown to have a low food value to arthropod predators (Toft, 1995). Chen and Liu (2001) demonstrated that consumption of aphids by lacewing larvae is reduced when feeding on aphid species with a low food value. Cd concentrations in *M. signata* may, therefore, reflect restricted feeding and subsequent malnutrition.

Feeding on aphids with elevated Zn concentrations during the fourth instar failed to increase the concentrations in newly emerged adult *C. septempunctata*. Concentrations in the adult were slightly lower than in the aphids on which the larvae were feeding in the sludge amended treatments, but were higher than in the aphids in the control. A similar pattern of
accumulation was observed in the carabid beetle *Bembidion lampros* (Winder, *et al.*, 1999) and in *C. carnea* pupae feeding on aphids from sludge amended soil-wheat-aphid systems (Green and Jeffries, 2003). The results of the current study are indicative of effective regulation of Zn body burden around 200 mg kg\(^{-1}\) in newly emerged adult *C. septempunctata*. This supports the hypothesis of Butovsky and van Straalen (1995) that monophagous predators may become adapted to high levels of trace metals when their prey accumulates them.

A partial explanation of the restricted transfer of Cd between aphids and newly emerged *C. septempunctata* was sequestration in the pupal exuviae. This excluded 10.5 % of the Cd accumulated by the larvae at pupation from the adult. Statistical analysis showed that this had a large effect on the concentration of Cd in the adult ladybird, accounting for 70% of the observed variance in Cd concentration. Sequestering Zn in the pupal exuviae accounted for a higher percentage (11.5 %) of the Zn accumulated in the pupa. However, this mechanism accounted for only 30% of the observed variance in adult Zn concentration, suggesting that this mechanism is more effective at excluding Cd from the adult. This confirms the finding of Andrezjewsla *et al.* (1990) that sequestration of trace metals in the pupal exuviae can be an important mechanism for excluding trace metals from the adult. However, the principle regulatory mechanism responsible for excluding Cd and Zn from the larvae, and consequently the adult at exclosure, is suggested to be excretion of the metals in the faeces of the larvae (cf. Janssen *et al*., 1991).

There was no evidence of Cd accumulation in adult *C. septempunctata* fed on a diet of frozen aphids and none of the measured independent variables made a significant prediction to Cd concentration. This suggests that *C. septempunctata* can efficiently regulate Cd concentrations in its body. This has also been shown for carabid beetles (Janssen *et al*., 1991; Kamaraz, 1999a; Scheifler *et al*., 2002). In contrast to Cd, Zn body burden of adult ladybirds showed a significant positive relationship with sewage sludge amendment. This indicates that
sludge amendments can increase Zn concentration in the ladybirds. A similar relationship was found by Winder et al. (1999) for the carabid beetle *Bembidion lampros*. However, in the present study Zn concentration decreased with time on diet regardless of treatment. This suggests that a diet of thawed aphids is unsuitable for adult ladybirds. No conclusions could therefore be reached on the transfer of Cd or Zn from aphids to adult *C. septempunctata*. However, the results demonstrate that Zn can be efficiently excreted by adult *C. septempunctata*. Accumulated Zn could, therefore, be effectively removed from the body when the ladybirds switch from feeding on patches of contaminated to uncontaminated aphids.

It has been suggested that internal concentrations of essential trace metals are more tightly regulated in arthropods than non-essential trace metals (Laskowski, 1991; Kramarz, 1999b). This is supported by published data, which generally shows that internal Zn concentrations are regulated in arthropods, whilst Cd concentrations are not (Hopkin, 1990a; Wilczek and Migula, 1996; van Gestal and Hensbergen, 1997; Heikens et al., 2001; Maryanski et al., 2002). The accumulation and biomagnification of Zn in aphids, especially *S. avenae*, provides evidence against this hypothesis. The significant contribution made by the size of the sewage sludge to the prediction of the concentration of Zn, but not Cd in adult ladybirds provides further negative evidence. However, larval ladybirds appeared to efficiently regulate Zn concentration. Cadmium concentration also appeared to be regulated, but this could be due to the lack of a significant difference in the concentration of Cd in the aphids consumed by the larvae. The lack of accumulation in aphids did not appear to be due to the regulation of Cd concentration, but rather to factors in the crop plants.

Other workers have also reported results varying in compliance with this hypothesis. For example, Janssen et al. (1991) found evidence of Cd regulation in the carabid beetle *Notiophilus biguttatus* and the springtail *Orchesella cincta*, but not in the pseudoscorpion *Neobisium muscorum* or the orbited mite *Platynothrus peltifer*, whilst Larsen et al. (1994a)
found the accumulation and biomagnification of both Cd and Zn in spiders. Consequently, the above hypothesis cannot be generalised to all arthropod food chains, especially those that contain cereal aphids.

The present study contributes to the body of evidence that shows that the pattern of trace metal transfers in arthropod food chains is complex and species and metal dependent. Generalisation about the behaviour of trace metals in arthropod food chains is therefore inappropriate. Predictions of the fate of trace metal contamination must therefore be based on knowledge of the accumulation characteristics of individual species and their food.

Comparison of pot trials to field conditions.

The size of sewage sludge amendments used in the present trial would not be used in an agricultural context in the UK (the reasons for this are discussed below under implications within agriculture on page 127). Consequently, the soil metal loadings in the present trial would only be reached after repeated additions of small quantities of sludge over many years. Over this time, a number of reactions will occur in the amended soil such as the breakdown of sludge organic matter, the re-equilibrium of trace metals between the different soil fractions and the possible acidification of the soil due the oxidation of reduced N and S present in the sludge (Chaney and Ryan, 1993; McBride 1995). Thus, the phytoavailability of trace metals from repeated small additions may differ from single large amendments giving the same metal loading in the soil (Chaney and Ryan, 1993). However, evidence from field trials indicates that the concentration of trace metals within plant shoots is dependant on the soil loading and is independent of whether the sludge is applied as several single or as one large application (Berrow and Burridge, 1983; Sommers et al., 1991).

Soil pH is consistently found to be the soil property that has the greatest effect on the availability of trace metals (Sauerbeck, 1991; Hooda et al., 1997; Evans et al., 1995). Previous work conducted on the soil used in the present trial reported the soil pH as ranging
from 4.6 to 5.33 (Merrington et al., 1997b; Winder et al., 1999). In the present trial the pH was found to be at the lower end of this range. If this soil was used agriculturally to grow cereal crops, it would almost certainly have been limed to increase the pH to above pH 6. This would have resulted in a reduction of the extractable concentration of Cd and Zn in the soil. For example, the same soil taken from a field trial that had received two applications of sewage from the same source as the present trial was limed to pH 5.9. This caused a reduction in the percentage of metal in the extractable fraction from 27.7 % to 10.2 % for Cd and 10.4 % to 0.8 % for Zn (Ridings, 2003).

In addition to the effect of a relatively low pH in the pot trials reported here, there is in general a higher trace metal uptake by plants in pot trials compared to field trials (Logan and Chaney, 1983; McBride, 1995; Smith, 1996). This is thought to be caused by i) the inability of roots to penetrate below the zone of soil contamination ii) increased transpiration rate caused by the glasshouse micro-climate and ideal moisture regime, which increases root to shoot translocation of trace metals and the supply to the root by mass flow (de Vries and Tiller, 1978; Mullins et al, 1986; Chaney and Ryan, 1993; Salt et al., 1995b). Consequently, the transfer of Cd and Zn from the soil to plants in the present study is likely to be greater than would be found in the same soil-plant system in an agricultural field. Considering the soil pH, the nature of metal uptake by plants in pot trials and the recent incorporation of sewage sludge into the soil, the present study represents a ‘worst case’ scenario. The transfer of Cd and Zn in the soil-plant system is likely to be far lower under typical agricultural conditions.

The observed concentrations of Cd and Zn in wheat shoots were high compared to the concentrations observed in wheat shoots reported by Merrington et al. (1997b) and Winder et al. (1999) using soil and sewage sludge from the same sources as the present trial. This provides support to the hypothesis that there is a greater uptake of Cd and Zn in pot trials. However, the Cd concentrations in the crop shoots in the present trial were within the
range reported for barley leaves growing in a wide variety of soils amended with 100 t ha\textsuperscript{-1} of
sewage sludge (0.03 to 2.79 mg kg\textsuperscript{-1} for Cd and 20 to 112 mg kg\textsuperscript{-1} for Zn; Sommers \textit{et al.}, 1991) and Zn in wheat (27- 150 mg kg\textsuperscript{-1}; Kabata–Pendias & Pendias, 1992).
Consequently, both the transfer co-efficients and concentrations of Cd and Zn in the shoots in
the present trial did not exceed those found under field conditions.

Despite the relatively high concentration of Cd and Zn in the shoots of wheat, the extent to
which the two metals were transferred to \textit{S. avenae} was low when compared to data from the
field conditions. For example, in the study of Merrington \textit{et al.} (1997b), using soil and sewage
sludge from the same source as the present study, found that concentrations of Cd and Zn in
winter shoots were 0.063 and 27.7 mg kg\textsuperscript{-1} respectively, after the amendment of the soil with
10 t ha\textsuperscript{-1} of sludge. The populations of \textit{S. avenae} feeding on these plants accumulated
0.320 mg Cd kg\textsuperscript{-1} and 160.7 mg Zn kg\textsuperscript{-1}. In the present study, at the same amendment rate,
the concentrations in the wheat shoots were 0.282 and 36 mg kg\textsuperscript{-1} for Cd and Zn respectively,
which resulted in concentrations in \textit{S. avenae} populations of 0.092 mg Cd kg\textsuperscript{-1} and
79 mg Zn kg\textsuperscript{-1}. In all treatments in the present study, both Cd and Zn concentrations were
within the range found by Merrington \textit{et al.} (1997b) even though the maximum amendment in
their study was only 20 t ha\textsuperscript{-1}. Consequently, the extent of the transfer of both metals from
shoot to \textit{S. avenae} in the present study was much lower than in field conditions.

The low transfer of metal to aphids detailed in Chapter 4 may be a reflection of the
differences in the sites from which aphid samples were taken. In the present study aphid
population were taken from the stem and leaves of plants, whilst Merrington \textit{et al.} (1997b)
sampled aphids from the ears. The re-translocation of trace metals from the shoot to the ears
of wheat plants may have result in higher concentrations of both metals in the phloem sap
translocated to the ear, which would account for the variance in findings between the two
studies. However, no evident data are present in the literature regarding the concentrations of
Cd and Zn in the phloem sap in different parts of wheat plants to confirm this hypothesis. If
there is a greater concentration of the two metals in the phloem sap supplying the ears, then the present study may have underestimated the transfer of the metals from shoots to S. avenae at the observed plant concentrations under field conditions. This is because S. avenae preferentially feeds on the developing ear of wheat, but it may also be found feeding on leaves, especially the flag leaf (Soffe, 1997). In contrast, M. dirhodum preferentially feeds on the upper leaves of cereal crops (Soffe, 1997). Consequently, the metal transfer between shoot and M. dirhodum in the present study should accurately reflect transfer under field conditions.

_Coccinella septempunctata_ kept in petri dishes are exposed to conditions radically different to those in the field environment. The foraging behaviour of adult and larval ladybirds for aphids is influenced by both biotic and abiotic factors in the field (Ferran and Dixon, 1993). It is therefore probable that the feeding behaviour of the ladybirds in the petri dishes will not be normal. This may have been compound by the feeding of the ladybirds ad libitum. Consequently, the consumption of aphids, and therefore ingestion and accumulation of trace metals, may be significantly different in the present study compared to the field environment.

In conclusion, the magnitude of the change in concentration of Cd and Zn as they are transported through the system in the present study may differ from that which would occur for the same system in the field. However, the direction of the concentration change, i.e. the biomagnification or biominimisation of metal, during transfer between the components of the system should be conserved under field conditions. Moreover, the differences in Cd and Zn accumulation between crop varieties and aphid species observed in the present study would also be expected to occur in the field. Consequently, the present study has contributed to the knowledge of which transfer steps in the system are important in restricting or facilitating trace metal transfer in agroecosystems and how this may vary when the components of the system change.
Cadmium/Zinc interactions

The chemical similarity of Cd and Zn can lead to antagonistic and synergistic interactions in plants (Smilde et al., 1992). However, the nature of the interaction is inconsistent. For example, Hart et al. (2002) observed mutual inhibition of uptake from a nutrient solution by bread and durum wheat roots. In contrast, Chaoui et al. (1997) reported Cd had a synergistic effect on Zn uptake from nutrient solutions by the roots of the bean Phaseolus vulgaris. In the latter study it was also observed that Cd reduced the concentration of Zn in the shoot, suggesting that interaction also occurs in root-shoot translocation (Chaoui et al., 1997). However, Zn was found to have a synergistic effect on Cd uptake in durum wheat seedling, but there was no effect on root-shoot translocation of Cd (Welch et al., 1999). Moreover, Green et al., (2003) observed no significant effect on the uptake of Cd by hard red spring wheat roots, but that Zn reduced the translocation of Cd to the shoot.

Interactions between the two metals are also reported in arthropods, but the nature of the interaction is again inconsistent. For example, Martinez et al. (1999) found no interaction in accumulation in the crustacean Artemia parthogenica at background concentrations. However, pre-exposure to Cd reduced subsequent accumulation of Zn (Martinez et al., 1999). In terrestrial arthropods, an antagonistic effect of Zn on Cd uptake was found in the house cricket (Acheta domesticus; Migula et al., 1989 a and b) and the springtail Orchesella cincta (Sterenborg et al., 2003). However, no interaction between the two metals was found in either assimilation or elimination for the beetle Poecilus cupreus (Kramarz, 1999a) and no interaction was found for uptake by the springtail Folsomia candida (van Gestal and Hensbergen, 1997).

Interactions between Cd and Zn have not been investigated in the present study. Given the inconsistent interaction between Cd and Zn in all components of the soil-plant-arthropod system, it is impossible to draw conclusions about how any interaction may affect the transfer
of the two metals in the system studied. Moreover, it appears that any interaction has a small
effect on the transfer of the two metals in the soil-wheat system under field conditions
(Mench et al., 1997) and on their toxicity to arthropods (Hopkin and Spurgeon, 2001).
Consequently, interactions between the two metals may not be ecologically significant in
natural environments.

6.3 Implications of results

Implications within agriculture

Owing to its high mobility in the soil-plant system (Sauerbeck, 1991) and its high toxicity to
invertebrates (Hopkins and Hames, 1994), Cd would seem to be the metal with the greatest
potential to cause damage to agroecosystems. However, this study demonstrates that the plant
effectively regulates the transfer of Cd from the soil to herbivores. Cadmium may therefore
cause little damage in agroecosystems, at least up to the shoot concentrations of 1.1 mg kg\textsuperscript{-1}
found in the present study. However, regulation by the plant may not function so effectively
at higher soil concentrations (Hamon et al., 1999). This may possibly occur within the current
soil limits in the UK.

Zinc is typically found at much higher concentrations in sewage sludges than any other trace
element (CIWEM, 1995; Gendebien et al., 1999) and it is the accumulation of this metal in
soil that most frequently limits the amount of sewage sludge that can be recycled to an
agricultural soil over the long-term (MAFF/DoE, 1993). The relatively rapid accumulation of
Zn in sewage sludge amended soil, and the ready transfer of this metal in the soil-plant-aphid
system demonstrated in the present study, suggests that this metal poses a greater danger than
Cd to arthropods in agroecosystems.

In the UK, considerable effort has been aimed at the control of S. avenae in cereal fields
(Greenwood, 2000). Increased abundance of S. avenae (Woiwod, 1990) points to the failure
of the widespread chemical based methods of aphid control (Greenwood, 2000). Together
with increasing public pressure on farming to produce crops using more environmentally friendly techniques, this is likely to lead to greater use of biological agents to control aphids as part of integrated crop management.

There are ca 300 species of invertebrate aphid predators found in UK agroecosystems (Burn, 1987). The proportion of these species that may be vulnerable to trace metal contamination at concentrations permitted by the current regulations in the EU and USA is unknown. The results of the present study show that *C. septempunctata* is not part of a critical pathway when soil concentrations are within the suggested reduced limits for Zn and Cd in the EU. However, this does not rule out the possibility of deleterious effects occurring within the current EU limits and serious concern should exist over the far less stringent controls in place in the USA. These could potentially lead to Cd and Zn concentrations in agricultural soils of more than 20 mg kg\(^{-1}\) and 1400 mg kg\(^{-1}\) respectively (McGrath *et al.*, 1994). Furthermore, trace metal contaminants can interact synergistically with insecticide can increase negative impacts on aphid predators (Kramarz and Stark, 2003).

The present study provides evidence that sewage sludges may have a significant negative affect on herbivorous arthropods. This is may reflect the accumulation of N-based plant defence chemicals by aphids (Szentessi and Wink, 1991; Wink and Witte, 1985; Witte *et al.*, 1990; Wink and Witte, 1991; Berlandier, 1996). These compounds can be toxic to aphids (Corcuera, 1984; Berlandier, 1996), but accumulation appears to be a defence against predators (Gruppe and Römer, 1988; Wink and Witte, 1991). At a sewage sludge application rate of 100 t.d.s ha\(^{-1}\) there is a significant reduction in the fresh mass of aphids, perhaps due to these defences. In addition, there was a significant negative correlation between sewage sludge application and consumption of aphids by adult ladybirds. The guidelines controlling the application of N from organic fertilisers, including sludges, to agricultural land restricts the input of N to 250 kg N ha\(^{-1}\) yr\(^{-1}\) in nitrate vulnerable zones (MAFF, 2002). This means that sewage sludge application rates will be restricted to no
greater than about 8 t ha\(^{-1}\) yr\(^{-1}\) for an average sludge (MAFF, 2000) and no greater than 7.4 t ha\(^{-1}\) yr\(^{-1}\) for the sludge used in the present study. It follows that in an agricultural context there will never be a situation where single sewage sludge amendments are large enough for the added N to result in detrimental effects on arthropods. However, only about 70% of the area of the UK falls within nitrate vulnerable zones. If excessive nitrogen fertilisation is responsible for the results found in the present study, then in the remaining 30% of farmland, levels of available N may need to be carefully controlled in agricultural systems employing integrated pest management or wholly organic practices. Otherwise, there is the possibility that the aphid-predator relationship may be disrupted in a way that may reduce the effectiveness of predatory arthropods in aphid control.

**Implications beyond agriculture**

There are over 500 species of aphid in the UK (DGS, 2003), which feed on a wide variety of plants species (Rotheray, 1989). Consequently, aphids may be found in almost all ecosystems in the UK at some time during the year. Drawing general conclusions about trace metal accumulation in invertebrate groups is difficult owing to the potentially wide variation in accumulation amongst closely related species (Glowacka *et al.*, 1999). However, the results of this study, and others such as Crawford *et al.* (1995), Merrington *et al.* (1997a and b) and Merrington *et al.* (2001), imply that the accumulation and biomagnification of Cd and Zn by aphids is common in agroecosystems. Biomagnification occurs over a wide range of concentrations and, in the case of Cd, does not appear to reach a plateau until very high plant concentrations (>30 mg kg\(^{-1}\); Crawford *et al.*, 1995). Consequently, in areas of trace metal contamination, aphids pose a threat of secondary toxicity to their predators.

Sewage sludge has applications other than agriculture in which large quantities of sewage sludge are applied. Such applications included the reclamation of landfill sites and mine spoils and in forestry, including biomass crops such as fast rotation coppice willow (CIWEM, 1995). For both of these applications, an amendment rate of 50 t ha\(^{-1}\) is common,
but rates of up to 100 t ha\(^{-1}\) and 200 t ha\(^{-1}\) may be used in forestry and land reclamation respectively (CIWEM, 1995). Large quantities of sewage sludge are also disposed of on sites dedicated for this purpose (Gendebien et al., 1999). In these situations, the results of this study indicate that there may be a reduction in herbivore biomass and a subsequent reduction in the quantity of metal held within that trophic level. This negative feedback may reduce the quantity of trace metals available for transfer from the soil to the secondary consumer level and beyond. This may be an important mechanism in restricting the transfer of trace metals from these sites to the wider environment.

6.4 Overall conclusions

Cadmium and Zn contrasted in their transport and fate in the system. Cadmium transfer was initially restricted by a reduced partitioning in the extractable soil fraction as sewage sludge amendment increased. Transfer from the soil to wheat roots increased Cd concentrations, but restricted translocation to the shoots resulted in lower concentrations than in the soil. In contrast to some other studies, transfer was further restricted by biominimisation in grain aphids, probably due to the regulation of Cd concentration in the phloem sap. There was no apparent transfer of Cd from aphids to either larval or adult ladybirds. Concentrations in newly emerged adults were approximately half those in the aphids consumed by the preceding larvae, whilst concentrations in the adults were similar to the aphids that they consumed.

Zinc transfer was not affected by changes to the partitioning in the extractable fraction as sewage sludge amendment increased. Transfer to the roots resulted in a much greater increase in the concentration of Zn than Cd. However, proportionally less Zn was translocated from the roots to the shoots. Nevertheless, Zn showed a greater overall transfer from the soil to the shoots than Cd. Transfer of Zn in the system was further enhanced by biomagnification in aphids. There was no evidence of Zn transfer from aphids to newly emerged adult ladybirds, but concentrations were similar in both. Interpretation of the transfer of Zn from aphids to adult ladybirds was complicated by deficiencies in the diet, which indicated that food quality
is an important factor in determining trace metal transfer from herbivore to predator. However, results suggest that Zn concentrations in the adult ladybirds increased with sewage sludge amendment.

The variety of crop grown in the system influenced the transfer of both metals. Consequently, by growing crop varieties that show low accumulation of Cd and Zn, transfer along the food chain could be restricted. This may be of particular use when the concentrations of the two metals in the soil are close to the legal limit or in the first few years after sewage sludge is applied when the phytoavailability of trace metals is likely to be greatest. Cadmium transfer was also influenced by the species of aphids, but Zn was not. However, differences in Cd concentration between the two aphid species were small and unlikely to be of biological significance. Therefore, aphid predators should not be exposed to significantly higher concentrations of either metal in the exceptional years when outbreaks of Metopolophium dirhodum occur.

The non-accumulation of Cd or Zn in the larvae and Cd in the adults of C. septempunctata is postulated to result from the efficient excretion of these two metals, which was demonstrated for Zn in adults. Furthermore, Cd and Zn accumulated by larval ladybirds were partially excluded from newly emerged adults by sequestration of the metals in the pupal exuviae adults. This mechanism appeared to be more important for the regulation of Cd concentration in the adult than for Zn. As a consequence of these mechanisms, there was no critical pathway to C. septempunctata, and this species did not appear to be endangered at the concentrations of Cd and Zn found in the present trial. However, as the tendency to accumulate trace metals, and their subsequent toxicity is species specific, this finding cannot be generalised to all arthropod predators of aphids. Nor can the findings of the present study be extrapolated situations where concentrations in aphids exceed those of the present study. Predators with biting and sucking mouthparts may be at particular risk due to the accumulation of Cd in soft proteinaceous tissues that form a greater proportion of their diet.
In summary, Zn was much more labile in the soil-plant-arthropod food chain than Cd. This may have been due to the restriction of Cd translocation from root to shoot at the highest sludge amendments and the restriction of Cd loading into the phloem sap. The mechanisms governing these restrictions may breakdown at higher concentrations. Consequently, Cd transfer through the system may be much greater at soil concentrations exceeding those in the present study.

6.5 Recommendations for further work

The variation in metal accumulation among crop species and aphids demonstrated in the present study suggests that the transfer of Cd and Zn needs to be investigated in a wider range of crop plants and their associated aphid specie(s). This may indicate crop-aphid systems that result in particularly high or low transfer of trace metal. In addition, the transfer of Cd and Zn should be investigated at higher soil concentrations, i.e. up to the concentrations that could be reached under US EPA part 503. This could include an investigation of the hypothesis that the accumulation of Cd, and probably Zn, in cereal shoots follows a plateau response. This hypothesis requires further investigation to determine if this phenomenon occurs and if so, the range of concentrations over which the plateau is maintained. This information could be useful in managing the agricultural use of sewage sludge in ways that minimise the transfer of trace metals from the soil. This may be important in not only reducing the exposure of herbivorous arthropods, but also minimising the concentrations of potentially harmful trace metals in the edible parts of crops. The effect of a breakdown in the plateau response on the transfer of Cd and Zn to aphids also needs to be investigated.

Work on the understanding of how physiological mechanisms controlling the detoxification of trace metals in arthropods differ between and within taxonomic groups is still in its infancy. This needs to be developed, together with an understanding of how different
detoxification physiologies are related to ecotoxicology of trace metals. This information will
give a better understanding of which predatory arthropods are at most risk from the
biomagnification of trace metals by aphids.

Sewage sludges contain trace metal contaminants other than Cd and Zn. Data on the transfer
of these metals in the soil-plant arthropod system should be obtained. After Cd and Zn, the
most labile trace metals in the sewage sludge amended soil-plant system are reported to be Cu
and Ni (Sauerbeck, 1991). One of the few generalisations that can be made about the transfer
of trace metals in arthropod food chains is that Cu is not biomagnified by arthropods
(Crawford et al., 1996; Rabitsch, 1995), including aphids (Crawford et al., 1995). However,
there are still some exceptions (Hunter et al., 1982; Hopkin and Martin, 1983). Little is
known about the transfer of Ni through arthropod food chains, and consequently, the study of
Ni in arthropod food chains is particularly important.
7.0 GLOSSARY

Apoplast A continuous, non-metabolically active pathway through the cell walls of plants.

ANCOVA Analysis of co-variance

ANOVA Analysis of variance

Biomagnification An increase in the concentration of a substance in an organism compared to its food.

Biomagnification factor The concentration of a substance in an organism divided by the concentration in its food.

Biominimisation A decrease in the concentration of a substance in an organism compared to its food.

Extractable soil Trace metals in the soil solution or easily exchange between fraction adsorption sites on the solid phase of soil and the soil solution.

Electro-Thermal Atomic Absorption Spectroscopy Similar to flame AAS, but atoms are produced by electrically heating graphite tube (cuvette). This allows much greater control of the atomisation processes as well as the removal, by volatilisation, of some parts of the sample matrix. This results in a cleaner sample and subsequently detection limits in the low part per million to parts per trillion level.

Exclusion Emergence of an adult insect from the pupa

FAAS See Flame Absorption Spectroscopy

Flame Absorption Spectroscopy An analytical technique in which the absorption of radiation at a particular wavelength by gaseous atoms of element created by flame is measured. Detection limits are in the high part per billion to parts per million range.

GFAAS See Electro-Thermal Atomic Absorption Spectroscopy
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Partition Co-efficient</td>
<td>The change in concentration between fractions of the soil or parts of a plant</td>
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<tr>
<td>Phloem</td>
<td>Living part of plant vascular systems, which consists of tube like cells. Transports phloem sap around the plant.</td>
</tr>
<tr>
<td>Phloem Sap</td>
<td>A fluid containing sugars and other plant metabolites found in phloem vessels.</td>
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<tr>
<td>Pupal Exuviae</td>
<td>The shell of the pupa left after exclosure</td>
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<tr>
<td>QC</td>
<td>Quality control</td>
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<tr>
<td>Rhizosphere</td>
<td>The area of soil influenced by plant roots. Typically the soil extending up to 5mm from the root surface.</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>Slotted Atom Trap</td>
<td>A slot cut in the top and bottom that the flame flows through. The upper slot is 10 mm shorter at the top, restricting the flow of atoms. Consequently, the atoms spend longer in the beam of light, resulting in higher light absorbance, and therefore sensitivity, for a given concentration in the analyte.</td>
</tr>
<tr>
<td>STAT Trap</td>
<td>See slotted atom trap</td>
</tr>
<tr>
<td>Symplast</td>
<td>A continuous pathway through the cytoplasm and plasmodesmata of plant cells</td>
</tr>
<tr>
<td>TAs</td>
<td>Toxic alleochemicals. Secondary plant metabolites that may function in the chemical defence of plants.</td>
</tr>
<tr>
<td>Trace Metal</td>
<td>Metallic and metalloid elements that typically occur in soil at concentrations less than 1000 mg kg⁻¹</td>
</tr>
<tr>
<td>Transfer Co-efficient</td>
<td>The change in concentration between the soil and plant.</td>
</tr>
</tbody>
</table>
**Xylem** Part of plant vascular systems, which consist of dead, tube like cells. Transports xylem sap from the roots to the rest of the plant.

**Xylem sap** A fluid found in xylem vessels consisting of mainly water, but also dissolved and complexed minerals take up by plant roots.
8.0 REFERENCES


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