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Transfer of cadmium and zinc from sewage sludge amended soil through a plant–aphid system to newly emerged adult ladybirds (*Coccinella septempunctata*)

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Abstract

An agricultural soil was amended with sewage sludge at rates equivalent to 0, 10 and 30 t (dry solids) ha^{-1} and the subsequent transfer of zinc and cadmium through a soil–plant–arthropod system was investigated. Zinc concentration in soil, wheat and aphids increased significantly with sludge amendment rate. Zinc was biomagnified during transfer along the pathway, resulting in concentrations in the aphids four times greater than in the soil. Cadmium concentration in the soil was also significantly elevated by the addition of sludge, but there was no significant difference in cadmium concentration in the shoots of wheat plants. Cadmium concentration in aphids followed the pattern found in plants, but again, differences between treatments were not significant. Aphids collected from the plants were subsequently fed to fourth instar *Coccinella septempunctata*. Consumption of these aphids did not result in significant differences between treatments in the body burden of newly emerged adult *C. septempunctata* for either metal. Sequestration of cadmium in the pupal exuviae had a greater effect on the body burden of newly emerged adult ladybirds than for zinc. Results are discussed in relation to possible risks posed by the transfer of trace metals via the soil–plant–arthropod system to predatory arthropods.

Keywords: Biomagnification; Cadmium; Zinc; Sewage sludge; *Coccinella septempunctata*; *Sitobion avenae*

1. Introduction

The treatment of waste water produces large quantities of sewage sludge, which must be disposed of in a safe and economic manner (Smith, 1996). In the UK, this results in the application to agricultural land of 47% of the 1.12 million t (dry solids) of sewage sludge produced annually (Gendebien et al., 1999). Increased sewage sludge production, combined with the banning of ocean dumping is likely to raise pressure on agricultural land to receive sewage sludges. The agricultural use of sewage sludge as a fertiliser recycles significant

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quantities of nitrogen and phosphorous (Hall, 1992). However, it is constrained by the wide range of potentially toxic elements, including cadmium and zinc, present in sewage sludges (Smith, 1996). Although trace metals can enter the agroecosystem from other sources, such as atmospheric deposition (Critchley and Agg, 1986) and the use of agrochemicals (Raven and Loeppert, 1997), sewage sludge is the principal source of trace metal addition to the agricultural soils to which it is applied (Nriagu and Pacyna, 1988). As the concentrations of trace metals in sewage sludges exceed those of the soil, applications of sewage sludge may increase trace metal concentration in the soil. Furthermore, it is 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). To prevent such accumulation reaching damaging levels, legislative frameworks and codes of practice have been introduced in many developed countries (Smith, 1996).

Agricultural land is an ecologically important habitat that supports an arthropod fauna that may exceed 900 species in the UK (Potts, 1990). Whilst only a few of these species are considered to be of direct conservation value (Potts, 1990), many form an important component of the diet of a number of declining farmland birds (Wilson et al., 1999). In addition, some arthropods are herbivorous crop pests that can cause economic damage (George and Gair, 1978), but predatory arthropods can help prevent populations reaching damaging densities (Chambers et al., 1986; Wratten and Powell, 1990). However, this component of the agroecosystem has been poorly 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 how trace metals are transferred through arthropod food chains in agroecosystems, or about possible detrimental effects this may have on arthropods. Consequently, it remains to be determined if the present soil trace metal limits are sufficiently restrictive to protect agroecosystems (Merrington et al., 1997a,b; Winder et al., 1999).

The assumption that trace metals are biomagnified by the transfer through arthropod food chains is no longer considered valid (Van Straalen and Van Wensem, 1986; Laskowski and Maryansk, 1993). It is generally accepted that the physiology of an arthropod determines the concentration of trace metals within its body and not its trophic level (Janssen, 1988; Laskowski and Maryansk, 1993). The nature and efficacy of metal regulating physiology may vary widely, even between closely related species feeding on similar diets (Janssen et al., 1991; Glowacka et al., 1997; Kramarz, 1999a,b). Some species have a poor ability to regulate trace metals and consumption of contaminated food results in the accumulation of trace metals in these species (Janssen et al., 1991; Kramarz, 1999b). In food chains including these species, ‘critical pathways’ may become established that potentially endanger carnivores (Van Straalen and Ernst, 1991).

Cadmium and zinc have similar chemical properties (Chesworth, 1991) and are known to be amongst the most labile trace metals in the soil–plant system (Sauerbeck, 1991). While zinc is an essential element for plants (Römheld and Marschner, 1991) and animals (Miller et al., 1991), cadmium has no known essential function in metazoan organisms and is much more toxic to arthropods than zinc (Hopkins and Hames, 1994). Aphids feeding on winter wheat grown in sludge amended soil may biomagnify both of these trace metals (Merrington et al., 1997a,b). This can result in levels of cadmium and zinc in aphids that are between 8 and 10 times greater than in the ears of wheat 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.

The fourth instar of *Coccinella septempunctata* L. is a voracious consumer of cereal aphids (Carter et al., 1980; Ferran and Dixon, 1993), which may expose them to elevated, potentially damaging, levels of trace metals in areas of repeated sewage sludge application. Trace metals accumulated in the larval stage can be transferred to the adult (Lindqvist, 1992). Studies examining the influence of metamorphosis on trace metal concentration in adult insects have not shown any particular pattern and trace metal transfer from food consumed in the larval stage to the adult insect is not well understood (Lindqvist, 1992).

The aim of this investigation was to assess the magnitude of cadmium and zinc transport in a soil–plant–arthropod system. An assessment was also made of the potential for *C. septempunctata* to regulate the body burden of newly emerged adults through the sequestration of cadmium and zinc in the pupal exuviae. Results are discussed in the light of the possible risks posed by the transfer of trace metals in the soil–plant–arthropod system to predatory arthropods.

2. Materials and methods

2.1. Experimental design

A sample of a freely draining sandy loam soil of the Fyfield series (Rudeforth et al., 1984) was taken from the top 20 cm of an agricultural field situated in East Lulworth, Dorset (UK). The homogenised soil sample was divided into three equal parts and each part was then amended with a dried, anaerobically digested municipal sewage sludge at three treatment rates equivalent to 0, 10 or 30 t (dry solids) ha^{-1}. The soil and sewage sludge was thoroughly mixed and each treatment used to fill six replicate 7.5 l pots.

The pots were subsequently 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 (Zadoks et al., 1974), cultures were established on the plants by placing 200 grain aphids (*Sitobion avenae* F.) from laboratory cultures per 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 were collected from each pot. Aphids from different pot cultures were kept separately from each other at −18 °C until used in the feeding trial. Soil and plants were also sampled for analysis at this time.

Newly laid *C. septempunctata* 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. After 24 h, newly hatched larvae were fed by placing cuttings of winter barley colonised by aphids in the Petri dish. Fresh cuttings were placed in the Petri dish each day until the larvae developed to the third instar, when they were isolated to prevent cannibalism. Feeding was continued as previously described. Fifteen larvae that had developed to the fourth instar overnight were selected at random, transferred to new 9 cm Petri dishes with a moistened filter paper and placed in a controlled environment cabinet at 24 °C, 16–8 h day–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. Larvae were fed a known weight of thawed aphids in excess of the normal daily requirement. After 24 h, the weight of aphids consumed was determined and freshly thawed aphids added to the Petri dish (Winder et al., 1999). Feeding continued in this way until the larvae pupated. Adult ladybirds were weighed on emergence and frozen at −18 °C until analysis for cadmium and zinc.

2.2. Chemical analysis

Five soil samples were taken from the top 15 cm of each pot, bulked, air dried and ground gently before being passed through a 2 mm plastic sieve. Subsequent soil analysis was carried out in triplicate on this fraction. Soil organic matter content was estimated by loss on ignition (LOI) and soil pH was determined in a 2.5:1 water:soil suspension (Rowell, 1994). Cadmium and zinc were extracted from 0.5 g sub-samples by refluxing in 69% nitric acid for 2 days, before dilution to a volume of 50 ml with deionised water (Merrington et al., 1997a). Three wheat plants were randomly sampled from each pot, washed once in a 0.1% detergent solution and twice in distilled water before being dried to a constant weight at 70 °C. Dried plants were individually digested in 10 ml of 69% nitric acid (Aristar grade) and diluted to 25 ml with deionised water (Merrington et al., 1997a). Aphid sub-samples and ladybirds were washed and dried as described for wheat. Individual ladybirds and 40 mg sub-samples of aphids were digested in 2 ml of nitric acid in sealed glass vessels at 80 °C. The clear residue was then diluted to 5 ml using deionised water. Pupal exuviae were digested in a similar way, using 1 ml nitric acid and diluted to 2 ml. Two replicates of exuviae were lost from the 30 t ha^{-1} treatment during the analytical procedure to determine cadmium concentration.

Cadmium and zinc concentration in samples was determined by an ATI Unicam Solaar 939 atomic absorption spectrometer (AAS). Electro-thermal AAS was used in the analysis of cadmium in all samples and zinc in pupal exuviae, whilst flame AAS (both with and without STAT trap) was used to analyse zinc in soils, wheat, aphids and adult ladybirds. In all instances, deuterium background correction was used. Relevant certified reference materials (GBW 07407 & BCR 60) and reagent blanks were digested and analysed with each batch of soils, wheat plants, aphids and adult ladybirds.

2.3. Data analysis

Statistical analysis was conducted using SPSS (version 10). The homogeneity of variance between treatment groups was assessed with Levene's test. This assumption was not met for some parameters and group means were, therefore, compared using the Kruskal–Wallis test (χ^2) for all parameters. Where a one-way analysis of covariance (ANCOVA) was used, preliminary checks were conducted to ensure that the assumptions of normality, linearity, homogeneity of variances and homogeneity of regression slopes were met. Effect size was calculated as part of the ANCOVA procedure by the partial eta squared method (hp^2). Biomagnification factors for the transfer of trace metals between trophic levels were calculated by dividing the concentration of metal in the higher trophic level by the concentration of the metal in the lower trophic level.

3. Results

The amendment of the soil with sewage sludge (organic matter 60%, total cadmium 2.4 mg kg^{-1} and total zinc 724.6 mg kg^{-1}) resulted in a significant increase in the loss on ignition values, zinc and cadmium concentrations in the soil compared to the control (Table 1). The largest sludge amendment rate resulted in a 50 and 120% increase in soil zinc and cadmium, respectively. Transfer of zinc from the soil to spring wheat plants reflected sewage sludge application with significant differences in wheat zinc concentration between treatments (Table 2). Aphid zinc burdens also differed significantly between treatments, the largest amendment of sludge resulting in a 50% increase in body burden over the control. Zinc was biomagnified from soil to plant and plant to aphid, the largest biomagnification occurred in the latter (Table 3). Concentrations of cadmium were higher in wheat grown in amended soils when compared to the control, but differences between treatments were not significant (Table 4). Cadmium concentration in aphids was highest in the 10 t ha^{-1} and lowest in the 30 t ha^{-1} amendment, but differences between treatments were not significant (Table 4). Wheat shoots and aphids exhibited

Table 1
Concentration of cadmium and zinc, loss on ignition (LOI) and pH of the experimental soil with and without sewage sludge amendment (mean ± 1S.E.)

Amendment rate	Cd (mg kg^{-1})	Zn (mg kg^{-1})	LOI (%)	pH
0 t ha^{-1}	0.130 ± 0.004	42.1 ± 2.0	4.71 ± 0.13	4.73 ± 0.03
10 t ha^{-1}	0.169 ± 0.008	49.0 ± 2.1	5.33 ± 0.07	4.80 ± 0.03
30 t ha^{-1}	0.284 ± 0.002	62.1 ± 3.6	6.05 ± 0.08	4.81 ± 0.05
χ^2-test	15.2	13.2	15.16	0.91
Significance (P)	0.001	0.001	0.001	n.s.

Table 2
Zinc concentrations (mg kg^{-1} ± 1S.E.) transferred from soil amended with sewage sludge in wheat, aphids, pupal exuviae and newly emerged adult ladybirds

Amendment rate	Wheat	Aphids	Pupal exuviae	Adult ladybirds
0 t ha^{-1}	68.9 ± 2.9	162.9 ± 11.2	230.5 ± 18.9	184.2 ± 15.3
10 t ha^{-1}	100.6 ± 5.1	215.9 ± 12.4	220.4 ± 13.5	204.5 ± 13.5
30 t ha^{-1}	115.0 ± 1.8	248.5 ± 11.5	229.4 ± 16.7	217.1 ± 21.4
χ^2-test	13.1	11.1	0.380	2.2
Significance (P)	0.001	0.04	n.s.	n.s

Table 3
Zinc and cadmium biomagnification factors at three trophic levels as a result of the amendment of soil with sewage sludge

Amendment rate ($t\,ha^{-1}$)	Metal	Trophic level		
		Wheat	Aphid	Ladybird
0	Zn	1.64	2.36	1.13
	Cd	2.03	1.42	−0.43
10	Zn	2.05	2.15	0.95
	Cd	1.86	1.33	−0.53
30	Zn	1.85	2.16	0.87
	Cd	0.99	0.87	0.47

a similar pattern of cadmium accumulation. This suggests that the wheat plant may influence the transfer of cadmium from the soil to aphids. This hypothesis was tested with a one-way analysis of covariance where sludge application rate was the independent variable, aphid cadmium concentration the dependent variable and wheat cadmium concentration the covariate. After adjusting the concentration of cadmium in the wheat plants, there was no significant difference between the sludge application rates in the concentration of cadmium in aphids ($F = 0.42$, $P = 0.67$, $hp^2 = 0.06$). There was a significant relationship between the concentration of cadmium in the wheat and the concentration of cadmium in aphids ($F = 5.48$, $P = 0.036$, $hp^2 = 0.30$).

Sewage sludge amendment of soil had no significant effect on the weight of aphids consumed by larvae ($\chi^2 = 3.12$, $P = 0.21$) or on the dry weight of newly emerged adults ($\chi^2 = 3.92$, $P = 0.14$). Zinc body burdens of adult *C. septempunctata* increased with sewage sludge application rate, though not significantly (Table 2). Cadmium concentrations in adult ladybirds reflected concentrations in the aphids, but without significant difference between treatments (Table 4). Zinc concentrations in exuviae were similar for all treatments, whereas cadmium concentrations varied with no trend apparent (Tables 2 and 4). For both metals, differences between treatments were not significant.

To determine the influence of cadmium and zinc sequestration in the exuviae on the body burden of the newly emerged adults, a one-way analysis of covariance was conducted where the independent variable was sludge application rate and the dependant variable was metal concentration in ladybirds. For the assumption of linearity to be met, the percentage of cadmium sequestered in the exuviae was inversely transformed. The percentage of zinc sequestered in the exuviae and transformed cadmium data were included as the covariate. After adjusting for the percentage of zinc sequestered in the exuviae, there was no significant difference between the sludge application rates in the body burden of newly emerged adults ($F = 0.11$, $P = 0.90$, $hp^2 = 0.06$). There was a significant relationship between the percentage of zinc sequestered in the exuviae and the body burden of the newly emerged adults ($F = 5.3$, $P = 0.042$, $hp^2 = 0.30$). For cadmium there was also no significant difference between sludge application rates in the body burden of newly emerged adults ($F = 0.05$, $P = 0.95$, $hp^2 = 0.01$) after adjusting for the percentage sequestered in the exuviae. There was a relationship between the percentage of cadmium sequestered in the exuviae and the body burden of the newly emerged adults ($F = 22.0$, $P = 0.001$, $hp^2 = 0.71$).

4. Discussion and Conclusion

The concentration of both cadmium and zinc in the sewage sludge fell between the 50 and 90 percentile

Table 4
Cadmium concentrations ($mg\,kg^{-1} \pm$ 1S.E.) transferred from soil amended with sewage sludge in wheat, aphids, pupal exuviae and newly emerged adult ladybirds

Amendment rate	Wheat	Aphids	Pupal exuviae	Adult ladybirds
$0\,t\,ha^{-1}$	0.262 ± 0.034	0.373 ± 0.051	0.137 ± 0.035	0.162 ± 0.35
$10\,t\,ha^{-1}$	0.314 ± 0.033	0.419 ± 0.115	0.086 ± 0.004	0.223 ± 0.67
$30\,t\,ha^{-1}$	0.281 ± 0.013	0.244 ± 0.085	0.196 ± 0.069	0.115 ± 0.027
χ^2-test	1.14	2.53	5.495	2.94
Significance	n.s.	n.s.	n.s.	n.s

values for sewage sludges used agriculturally in England and Wales (Gendebien et al., 1999). At the highest application rate, sewage sludge resulted in concentrations of cadmium and zinc in the soil of 0.284 and 62.1 mg kg^{-1}, respectively, which are within the current UK limits for soils with a pH of 5.5–5.0 (3 and 200 mg kg^{-1}, respectively; MAFF/DoE, 1993). There is a proposal to reduce the permitted levels in sludge amended soils within the EU for cadmium and zinc to 0.5 and 60 mg kg^{-1}, respectively, for soils with a pH of 5–6 (Towers et al., 2001).

The transfer of zinc from the soil to the wheat plants resulted in concentrations at the higher end of reported values, but levels were still within the range for spring wheat (27–150 mg kg^{-1}; Kabata-Pendias and Pendias, 1992). Biomagnification in wheat plants was repeated in grain aphids, with concentrations reaching 250 mg kg^{-1}. Similar biomagnification of zinc derived from sewage sludge amended soils has been reported in winter wheat–aphid system for the bird–cherry oat aphid (*Rhopalosiphum padi*; Merrington et al., 1997a) and *S. avenae*. In the latter case, aphids accumulated zinc to >300 mg kg^{-1} from sludge amendment rates of up to 20 t ha^{-1} (Merrington et al., 1997b). There was no significant difference in the fresh mass of aphids harvested in the current study. This suggests that there was little, if any negative impact on grain aphids for concentrations of zinc in the soil within the proposed EU limit.

Cadmium concentrations in wheat plants exceeded the background range for Poaceae (0.07–0.27 mg kg^{-1}; Kabata-Pendias and Pendias, 1992). This may reflect the greater uptake of trace metals in pot systems compared to field conditions (Smith, 1996) and the pH of the soil. Cadmium concentration in aphids was also high compared to the 0.016–0.386 mg kg^{-1} range for soils unamended or amended with moderate additions of sludge (Merrington et al., 1997a,b; Winder et al., 1999). Despite relatively higher concentrations in wheat and aphids, cadmium was found to be less labile than zinc in the food chain. Soil–wheat biomagnification fell with increasing cadmium soil concentration (Table 3) and was lower between wheat and aphids than for zinc. This probably reflects lower mobility of cadmium in the phloem sap of wheat plants (Herren and Feller, 1997; Cakmak et al., 2000). The calculated effect size indicated that 30% of the observed variation in the cadmium concentration in aphids was explained by the cadmium concentration in wheat. The regulation of cadmium in wheat plants may, therefore, have an important influence on the transfer of cadmium from soil to higher trophic levels.

Fourth instar *C. septempunctata* consume some 65% of the total food needed for the development from egg to pupa (Carter et al., 1980). Therefore, the majority of trace metals accumulation may be expected to occur in the fourth instar. Accumulation may be compounded by weight loss during pupation (Gintenreiter et al., 1993). However, insects may exclude accumulated trace metals via sequestration in the pupal exuviae (Andrzejewska et al., 1990). Autoradiography experiments demonstrate that most cadmium in insects is bound in the mid gut epithelium, but it is also localised in the integument (Lindqvist et al., 1995; Inza et al., 2001). The sequestration of cadmium 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 variation in the cadmium body burden of the adult could be explained by the percentage of cadmium sequestered in the exuviae. This appears to be an effective mechanism for excluding cadmium from the adult.

In contrast to cadmium, zinc is evenly distributed thoughout 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 was higher than for cadmium at 11.5%. However, the calculated effect size indicated that 30% of the observed variation in the zinc concentration in the adult was explained by the percentage of zinc sequestered in the exuviae. Sequestration in the pupal exuviae appears to be less effective in excluding zinc from the adult than for cadmium.

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 cadmium, as there was no significant increase in concentration in the aphids on which the larvae fed. However, zinc concentrations were increased in the aphids. This suggests that larval ladybirds were able to regulate their zinc body burden, probably by excreting excess zinc in the faeces (Janssen et al., 1991).

From an economic and an ecological perspective, soil trace metal concentrations have to remain below levels detrimental to aphid predators. The efficient regulation of cadmium and zinc by the plant and larval *C. septempunctata* suggests that predators consuming aphids will be protected within the current EU cadmium and zinc limits. However, less stringent controls are in place in the USA, which may lead to cadmium and zinc concentrations in agricultural soils of over 20 and 1400 mg kg^{-1}, respectively (McGrath et al., 1994). Despite the evidence for efficient regulation/sequestration of trace metals, a precautionary approach needs to be taken when using metal transfer data alone to determine the detrimental effects of elevated trace metal concentrations in food chains. It has been demonstrated that increased trace metal concentrations in the diet of Coleoptera larvae can cause changes in adult locomotive behaviour without significantly affecting the body burden of the adult (Bayley et al., 1995). A low adult body burden can, therefore, be a poor predictor of the ecological effects of trace metal contamination.

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