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The transfer and fate of Pb from sewage sludge amended soil in a multi-trophic food chain: a comparison with the labile elements Cd and Zn

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ABSTRACT

The contamination of agroecosystems due to the presence of trace elements in commonly used agricultural materials is a serious issue. The most contaminated material is usually sewage sludge and the sustainable use of this material within agriculture is a major concern. This study address a key issue in this respect, the fate of trace metals applied to soil in food chains. The work particularly addresses the transfer of Pb, which is an understudied element in this respect and compares the transfer of Pb with two of the most labile metals, Cd and Zn. The transfer of these elements was determined from sludge amended soils in a food chain consisting of Indian mustard (*Brassica juncea*), the mustard aphid (*Lipaphis erysimi*) and a predatory beetle (*Coccinella septempunctata*). The soil was amended with sludge at rates of 0, 5, 10 and 20% (w/w). Results showed that Cd was readily transferred through the food chain until the predator trophic level. Zn was the most readily transferred element in the lower trophic levels, but transfer to aphids was effectively restricted by the plant regulating shoot concentration. Pb had the lowest level of transfer from soil to shoot and exhibited particular retention in the roots. Nevertheless, Pb concentrations were significantly increased by sludge amendment in aphids and Pb was increasingly transferred to ladybirds as levels increased. The potential for Pb to cause secondary toxicity to organisms in higher trophic levels may have therefore been underestimated.

Keywords Sewage sludge; Trace metal; Food chain; Plant; Aphid; Ladybird

Introduction

The treatment of waste-water is a global problem that is exacerbated by the presence of potentially toxic trace metals in the treatment stream. During treatment, metals become concentrated within the solids phase, resulting in large volumes of contaminated sludge, which requires disposal in an environmentally safe yet economic way (Smith 1996). The beneficial use of sewage sludge in agriculture is the most commonly recommended disposal technique (Singh and Agrawal 2008) and has the benefit of recycling the organic matter, nitrogen, phosphorous and essential trace element (Torri and Lavado 2008) content of the sludge. However, the benefit to crop production (Qasim et al. 2001; Singh and Agrawal 2010) must be balanced by the presence of trace metals and this constrains the use of sludge in agriculture (Singh and Agrawal 2008). Failure to achieve the correct balance can result in the build up of trace metals in the soil, potentially affecting soil fertility and increasing concentrations within plants, which then poses a risk to the human and animal food chains (Winder et al. 1999; Prince et al. 2001; Zhuang et al. 2009; Green and Walmsley. 2013).

Phytophagous arthropods have an important functional role in the terrestrial ecosystems transferring energy from plants within food webs (Lindqvist and Block 1997). In trace metal contaminated ecosystems, this trophic position also results in a fundamental role in the accumulation and subsequent transfer of trace metals to higher trophic levels (Devkota and Schimidt 2000). Predatory arthropods consuming prey that have accumulated trace metals by feeding on contaminated plants may experience adversely affected fitness. Within agroecosystems, these predators have a valuable function by contributing to the control of pests (Merrington et al. 1997a, b; Winder et al. 1999; Green et al. 2003). Thus, soil contamination can potentially result in secondary poisoning in higher trophic levels, which may limit the beneficial role played by predatory arthropods in agroecosystem (Green et al. 2010).

Currently, investigations into the transfer and fate of trace metals in the food chains have been primarily conducted in temperate agricultural systems. Consequently, there is a dearth of understanding about the fate and consequences of trace metal contamination in tropical and sub-tropical agroecosystems. The large populations in these areas combine the pressures of sludge disposal with food production. Moreover, biological control of predators is of particularly economic importance due to the high costs of pesticides. Thus, protection of species required for biocontrol is prerequisite for safe and sustainable recycling of sewage sludge in these regions.

Both Cd and Pb are highly zootoxic, non-essential elements. They are reported to contrast in their transfer between sewage sludge amended soil and plants; Cd is labile, whilst Pb is strongly retained in the soil (Sauerbeck 1991). Zn is an important essential element and shows similar properties to Cd in the soil-plant system (Sauerbeck 1991). Due to their labile nature, both Cd and Zn can be readily transferred through multi-trophic food chains, potentially threatening biocontrol of pests (Merrington et al. 1997a, b; Green et al. 2006). The behaviour of Pb in multi-trophic systems is far less understood.

The aim of the present investigation was to address the current gap in understanding by comparing the extent to which Cd, Pb and Zn from sewage sludge amended soil are biotransferred in the plant-aphid-ladybird food chain. Indian mustard (*Brassica juncea*) was selected as a model plant because of its wide spread geographic exploitation, fast growth, large biomass, and high tolerance to the accumulation of number of toxic metals. The aphid *Lipaphis erysimi* is a common and serious insect pest of Indian mustard (Rehman et al. 2014), whilst *Coccinella septempunctata* L. has been widely introduced and used as a biological control agent. The specific objectives of the work were to:

(i) Compare the mobility of Cd, Pb and Zn in the food chain

(ii) Determine fate by identifying steps where in the food chain metal transfer may be enhanced or constrained

Materials and Methods

Experimental Design

A bulk soil sample was obtained from an agricultural field of the Aligarh Muslim University (AMU), which was then air- dried and then divided into 4 equal parts. The sewage sludge was collected from the AMU sewage treatment plant and then air-dried, finely powdered, and sieved to < 2 mm before use.

One part of the soil was used as an unamended control, whereas the remaining 3 parts were amended with sewage sludge at rates of 5%, 10%, and 20% (w/w). Treatments were replicated 4 times and designated as T_0 for the control, T_1 for 5%, T_2 for 10% and T_3 for 20% sewage sludge amendments, respectively. The soil and sludge was thoroughly mixed before filling 25 cm diameter pots. The soils were brought to field capacity and incubated for 20 days in order to allow the sludge to reach equilibrium with the soil (Epstein 2003). Soil samples were taken from each pot for physico-chemical analyses at this point.

Ten mustard seeds were sown in each pot to a depth of 0.5 cm. The pots were then placed in a fully randomised block in a glasshouse (about 16 h d, 20- 25^{o}C temperature regime) and were irrigated with de-ionized water at regular intervals. To prevent leaching from pots, water retaining plates were placed below each pot in order to trap percolated water. The seedlings were thinned at the 5- 6 leaves stage to retain 3 uniform seedlings per pot. When the remaining plants reached the flowering growth stage (40 d), aphid cultures were established on the plants by placing 200 mustard aphids (*L. erysimi*), taken from laboratory cultures, in each pot. Individual pots were subsequently covered with fine nets (sleeve cages) to prevent the transfer of aphids between treatments. Aphid cultures were left to establish for 21 days before all aphids were collected from each pot (Green et al. 2003). Collected aphid samples were divided into 2 sub-samples. One sub-sample was used for trace metal analysis and other was kept at -18 ^{o}C until used for feeding ladybirds. Plants were also sampled for analysis at this time. Trace metal content was also analysed in honeydew of aphids by following the method of Crawford et al. (1995).

The feeding trial was conducted as described by Green et al. (2003). Briefly, 16 *C. septempunctata* (seven-spotted ladybirds) 4th instar larvae were isolated in a controlled environment cabinet set to 25 ^{o}C and a 16:8 h day–night regime. Larvae were divided into four equal treatment groups and each individual larva was fed frozen aphids collected from one of the pot cultures. Consumption of aphids by individual larva was measured daily using the method described by Winder et al. (1999). Feeding continued until the larvae pupated. After pupation, adult ladybirds were weighed and frozen at -18 ^{o}C until analysis for Cd, Pb and Zn was conducted.

Trace metal analysis

Soil samples, collected in triplicate from each pot, were air dried, crushed, passed through a 2 mm mesh sieve. Exactly 1 g of soil was digested in 20 ml of triacid mixture (HNO_3:H_2SO_4:$HClO_4$; 5:1:1) at 80°C (Allen et al. 1986). After complete digestion, the solution was allowed to cool and filtered through Whatman No. 42 filter paper and made up to a final of 50 ml with DDW. The extractable fraction of metal in the soil was obtained by mechanical shaking of 15 g of sample with 40 ml of DTPA extractant (0.005 DTPA, 0.01M $CaCl_2$ and 0.1M TEA buffered at pH 7.3) for 2 hours (Lindsay and Norvell 1978).

The pH of the soil at different treatments was measured in a soil: water suspension (1:2.5 w/v) with a pH meter (Digital pH, conductivity and temperature meter 181). Organic carbon and total nitrogen contents of the soil samples were determined by Walkley and Black's rapid titration method (Allison 1973) and Gerhardt automatic analyzer, respectively.

Mustard plants from each pot were harvested and washed with tap water to remove adhering soil particles and finally with DDW. Root and shoots were separated using scissors and were dried to a constant weight at 70°C. Plant samples of 0.3 g were digested in 10ml of triacid mixture (HNO_3:H_2SO_4:$HClO_4$; 5:1:1) at 80°C until black fumes turned white and solution became completely clear (Allen et al. 1986). The digest was allowed to cool, diluted with DDW and then filtered through whatman's No. 42 filter paper. The filtrate was then made up to 50 ml using DDW.

Aphid sub-samples and ladybirds were washed and dried as described for plant samples. Individual ladybirds and 20 mg sub-samples of aphids were digested in 2ml of triacid mixture (HNO_3:H_2SO_4:$HClO_4$; 5:1:1) at 80 ^oC. The clear residue was then diluted to 5ml using DDW. Pupal exuviae (~ 0.91-0.97 mg) were digested in a similar way, using 2ml acid mixture, which was made back up to 2 ml with DDW.

Concentrations of Cd, Pb and Zn in samples was determined by an atomic absorption spectroscopy (GBC SensAA, Dandenong, Australia). All chemicals used were analytical grade and supplied by Sigma-Aldrich. Analytical quality was ensured by the analysis of certified reference materials (GBW 07402 for soil, NIM-GBW10048 for plants and GBW 8552 for insects). Mean recoveries from these materials were 96.76%, 98.42% and

94.7% for Cd, Pb and Zn respectively. Reagent process blanks were also digested and run in triplicate to check for process contamination.

Data analysis

Transfer coefficients were calculated as a ratio of the concentration of a metal in a component compared to the concentration in the component preceding it in the transfer pathway. Concentrations in the pupa before emergence were calculated by the addition of the metal content of the newly emerged adult to that of the exuviae. The significance of differences among treatments was established by Duncan's Multiple Range Test (DMRT) using SPSS vs. 17.

Results

Effect of sludge amendment on soil

The soil amended with different ratios of sewage sludge had low pH, high organic carbon and high nitrogen content in comparison to the unamended soil. However, only organic carbon was found to be significantly affected by all sewage sludge amendment ratios compared to the unamended soil ($p<0.05$; Table 1).

The largest sludge amendment resulted in 275%, 40% and 170% increase in total soil Cd, Pb and Zn respectively (Table 1). Overall, the amendment of the soil with sewage sludge significantly elevated the total concentrations of Cd, Pb and Zn in the soil. Similarly, the concentration of DTPA- extractable Cd, Pb and Zn in the soil was also elevated compared to the control at all sewage sludge amendment ratios. In the case of Zn, the increase compared to the control was statistically significant at all the sewage sludge amendment rates ($p<0.05$). In the case of Cd, the increase was significant at the T_2 and T_3 amendment rates, whilst for Pb, the significant increase was found only at highest rate (T_3; Table 1).

Transfer of trace metals between soil and plants

Cd concentration in the mustard roots showed a significant increase with sludge amendment ($P<0.05$) except at T_1, reflecting the total and available concentrations in the soil and reached maximum of 7.14 mg kg^{-1} dry matter in T_3 (Fig 1A). Transfer coefficients between the total and DTPA Cd concentration in the soil and the roots showed an increasing trend with sludge amendment and ranged between 2.88 and 4.22 for total to root and 9.14-16.23 for DTPA to root. Transfer coefficients of Cd between the root and shoot increased with amendment rate , but showed a lesser increase with sludge amendment levels and were always below one (Table 2). Except in T_1, the concentration of Cd in shoots was significantly increased ($P<0.05$) by sludge amendment and reached 5.38 mg kg^{-1} dry matter at highest amendment level (T_3), 520% greater than in the control (Figure 1A).

Concentration of Pb in roots also increased significantly ($P<0.05$) with sludge amendment except in T_1, where the difference was non-significant when compared to control. The maximum concentration was found in T_3, in which the concentration reached 34.12 mg kg^{-1} (Fig. 1B). Like Cd, transfer coefficients between the total Pb concentration in the soil and roots increased with amendment rate and ranged between 1.56 and 2.24. A similar trend of increase was found in transfer coefficients between DTPA extractable Pb in soil and roots, which ranged between 8.5 and 11.8. Concentration of Pb in shoots also increased in plants grown in sludge amended soils, but only shoots from T_2 and T_3 differed significantly from the control. The highest concentration (18.94 mg kg^{-1} dry mass) was found in T_3, but this was not significantly higher than the concentration in T_2. In marked contrast to Cd, transfer coefficients fell as amendment rate increased.

Zn concentrations in roots increased significantly in the T_2 and T_3 amendment rates, reaching a maximum of 264.7 mg kg^{-1} in T_3 (Fig. 1C), which was significantly higher than in T_2. Zn in the roots was 1.55–2.7 times higher than the total concentration in the soil and 18-

24 times higher than the extractable fraction of the soil, which were by far the highest coefficients found in the system. Zn concentrations in shoots were also increased significantly by the T_2 and T_3 amendments, but not by T_1. Zn concentrations in the shoots of T_3 were significantly higher than in T_2 at a concentration of 205.7 mg kg^{-1}. Transfer coefficients between the roots and shoots were always below one and were higher than the control for all sludge amendments, but unlike Cd, there was no rise in the value of the coefficient with amendment rate.

Transfer from shoots to aphids

The Cd body burden in aphids increased with sludge amendment rate, reaching a maximum of 4.27 mg kg^{-1} dry matter in T_3 (Figure 1A). All sludge amendments caused a significant increase in aphid Cd concentration, but T_1 and T_2 did not differ significantly from each other, whilst T_3 was significantly higher than all other treatments. Cd concentrations in aphids were 1.6 and 1.7 times higher than the mustard shoots grown in T_0 and T_1 respectively, but in T_2 and T_3, transfer coefficients between the aphids and the shoots were less than 1 and were lowest in T_3 (Table 2).

Pb body burden of aphids also increased with increase in sewage sludge ratios (Figure 1B). The significance of difference among treatments was complex, but T_2 and T_3 resulted in significantly higher Pb concentrations in the aphids, T_1 and T_2 did not differ significantly, nor did T_2 and T_3. Transfer coefficients for Pb transfer between mustard shoot and aphid were the lowest of the three metals and were always less than one (Table 2).

Zn body burden of aphids also increased with the size of sewage sludge amendment and all sludge amendments resulted in significantly higher aphid Zn concentrations than in the control. Whilst the maximum concentration was found in T_3 (253.07 mg kg^{-1}, an increase of 160%; Fig. 1C), there was no significant difference in Zn concentration between the aphids in T_2 and T_3, although both these treatments differed significantly from T_1. As was the case for Cd and Pb, transfer coefficients between shoots and aphids fell with increasing sludge amendment. However, the coefficients for Zn were much higher than for the other two metals in all treatments (Table 2).

Estimation of metal levels in aphid honeydew showed that metal concentration in honeydew increased with sludge amendment for each metal, indicating that aphids excreted the metals (Table 3). However, the ratio of metal in honeydew to metal in aphid exhibited marked differences among the three elements. Cd elimination via the honeydew appeared to be most efficient as the ratio of metal in honeydew to aphid rose with increasing exposure. Elimination of Pb exhibited a similar, but smaller trend, whilst Zn elimination with honeydew was 2-3 times lower than for the other two metals and did not vary with exposure.

Transfer between aphids and ladybirds

Cd body burden in *C. septempunctata* increased with sludge amendment, reaching 2.29 mg kg^{-1} in T_3. Cd concentrations were significantly elevated in T_2 and T_3 only. T_2 did not differ significantly from T_1, but was significantly lower than T_3. Transfer coefficients between aphids and ladybirds was always lower than one (Table 2), but showed little variation among treatments.

Pb burdens in adult ladybirds reflected the concentration of Pb in aphids, but significant difference was found for T_2 and T_3 (Fig. 1B). Concentrations also differed significantly between T_2 and T_3. The transfer coefficient of Pb between aphid and ladybirds showed an increasing pattern with the increase in amendment ratios and ranged from 0.56 to 0.79. Although transfer coefficients were lower than one in all amendments, they were higher than the corresponding coefficients for Cd. Indeed, in T_3, the transfer of Pb to ladybirds was proportionally 55% greater for Pb than Cd.

Zn burdens in adult ladybirds increased with sewage sludge amendment ratio, reaching highest 226.28 mg kg^{-1} (dry matter). The significance of differences among treatment followed the same pattern as for aphids, all amendments significantly increased Zn concentration in ladybirds, but differences between T_2 and T_3 were not significant. Aphid to ladybird transfer coefficients decreased with sludge amendment and a low level of biomagnification only occurred in T_0 and $T_{1.}$

Concentrations of metal in the exuviae increased with amendment rate for all three metals. However, significant elevation was only noted in T_3 for Cd, T_2 and T_3 for Pb and Zn (Table 4). Typical losses via the exuviae were ca. 10%. Cd was most readily eliminated via the exuviae, whilst Zn most strongly retained by the adult ladybirds (Table 4). However, the percentage of the pupal body burden lost on emergence via the exuviae tended to decline with increasing amendment, and therefore body burden, in the case of all three elements. This was most pronounced in the cases of Cd and least in the case of Zn.

Sewage sludge amendment of soil had no significant effect on the dry weight of aphids or on the dry weight of newly emerged adults (Table 5)

Discussion

Soil-plant transfer

In the present study, the amendment of soil with sewage sludge had little impact on the total nitrogen content of the soil, which agrees with the results of Singh and Agrawal (2010). Amendment of sewage sludge reduced the soil pH as compared to the unamended control soil. This may be due to the lower pH of sewage sludge as compared to unamended soil. Both organic carbon and both total and phytoavailable (DTPA extractable) concentrations of all three elements in the soil were significantly elevated by sludge amendment, but total concentrations of all three metals in the soil were below the Indian permissible limits (Cd: 3-6 mg kg^{-1}; Pb: 250-500 mg kg^{-1}; Zn: 300-600 mg kg^{-1}) in all amendments (Awashthi 2000).

The increased phytoavailability of Zn was reflected in increasing accumulation of this element in roots as sewage sludge amendment increased. As root concentration increased, an increasingly greater translocation of Zn from the root to the shoot took place. As a result, Zn concentration in both the roots and shoots of *B. juncea* were found to be relatively high when plants were grown in sewage sludge amended soils. Normal range of Zn concentration in plant tissues is 27-150 mg kg^{-1} (Kabata-Pendias and Pendias 2011) compared to 42-265mg kg^{-1} found in the present study.

It is evident that multiple transport systems are involved in Zn uptake by roots (Verbruggen et al., 2009). ZRT1/IRT1-like proteins are well characterised transporters that play a role in the trans-membrane uptake of Zn^{2+} by root cells (Kramer et al. 2007). Further movement from root to shoot of essential metals like Zn is facilitated by proteins of ZIP (SLC39) and CDF/ZnT (SLC30) families (Verbruggen et al. 2009). Thus, the higher transfer coefficients for Zn from soil to root and from root to shoot can be explained by the presence of specific uptake mechanisms for this essential metal, which are lacking for non-essential elements.

The interaction between Zn and Cd in the biological system is likely to be similar (Singh and Fulekar 2012), which results in Cd transport by the members of the ZIP family transporters (Kramer et al., 2007). Moreover, there is also indirect evidence that Cd^{2+} makes entry into plant cells via Ca^{2+} uptake channels, again at lower affinity (Perfus-Barbeoch et al. 2002).

The biochemical similarity of Cd and Zn was reflected in a similar pattern of accumulation in the roots and translocation to the shoots; Cd was increasingly accumulated in the roots as sludge amendment increased and translocation to the shoot similarly increased as root concentration rose. The resulting concentrations in roots and shoots of *B. juncea* did not exceed the critical limit of Cd in plants, which is 5-10 mg kg^{-1} (Kabata-Pendias and Pendias 2011) except at highest amendment where Cd concentration in root was observed 7.14 mg kg^{-1} dry matter.

The present study found that Pb had the lowest soil to root transfer coefficient. As a non-essential element, plants do not possess specific transport mechanisms to take up Pb. Monferan and Wunderlin (2013) have suggested that Pb present in the soil solution is adsorbed on root surface and penetrates the root system passively, but Pourrut et al. (2013) have postulated that ZIP and CDF transporters may play role in the active transport of Pb. Whatever the mechanism, plants clearly have a restricted ability to take up Pb compared to both Cd and Zn.

The subsequent translocation of Pb from root to shoot was also more restricted than Cd and Zn. Nevertheless, the transfer coefficients for total soil to shoot ranged from 1-1.25, exceeding the typical range (0.01-0.1) reported for a series of crops grown on sewage sludge amended soils (Sauerbeck 1991). Despite this relatively extensive transfer from soil to shoot, the transfer of Pb contrasted to Cd and Zn in that coefficients for Pb decreased as sludge amendment increased, whilst for both Cd and Zn coefficients increased.

The results confirm the findings of Karak et al. (2013) that the majority of Pb accumulation is in the roots of *B. Juncea* and demonstrates that an effective root-shoot barrier restricts Pb transfer within the plant. Retention of more Pb in the roots can be explained by its particular affinity for the carboxyl groups and pectins within the cell wall (Qiao et al. 2015), but a range of other processes such as accumulation in plasma membranes (Islam et al. 2007), precipitation of insoluble Pb salts in intercellular spaces (Malecka et al. 2008) and sequestration in the vacuoles of rhizodermal and cortical cells (Pourrut et al. 2013) will also contribute to Pb sequestration.

Shoot to aphid transfer

Insects have two main excretory routes; firstly via processes involving secretion from or loss of cells of the midgut and associated tissues and the second is via the malpighian tubules. Aphids lack this second route, which is the only apparent route for the excretion of metal passing through the gut into the wider soma. They may, therefore, be more likely to store/detoxify metals rather than excrete them when compared to other inverts. The literature reflects this possibility with high levels of biomagnification in aphids reported for Cd and Zn in particular (Merrington et al. 1997b), although excretion in the honeydew is still an effective excretion mechanism for some trace metals, for instance Cu (Crawford et al. 1995).

In the present study, Zn was the only element biomagnified by the aphid *L. erysimi* in all treatments. Even so, as with the other two elements, transfer coefficients fell with increasing sludge amendment. The relatively high transfer of Zn reflects the reported ready transportation of this element in the phloem (Riesen and Feller 2005). Because aphids feed directly on the phloem sap (Dixon 2005), they are exposed to and therefore accumulate high concentration of Zn. Nevertheless, Zn transfer from shoot to aphid was regulated as there was no significant change in aphid Zn concentration in T_2 compared to T_3, despite a significant increase in shoot concentration. As previously stated, the main mechanism available for aphids to excrete trace metals is via the honeydew. The Zn concentration in honeydew did not alter in proportion to the concentration within aphids, suggesting that increased secretion was not the regulatory mechanism. This implies that mechanisms within the plant were responsible for limiting Zn transfer to the aphids, i.e. the plants restricted the loading of Zn into the phloem sap. This contrasts to work investigating the transfer of Zn from the shoots of cereal plants to aphids, which found a linear relationship between shoot and aphid Zn concentration (Green et al. 2006).

Cd burden in the bodies of *L. erysimi* were high compared to the previous reported values in other aphid species from food chains contaminated with sewage sludge (Merrington et al. 1997a, b; Winder et al. 1999; Green et al. 2003, 2010). In the present study, transfer coefficients of Cd between shoot and *L. erysimi* ranged between 0.8 and 1.7, which are in accordance with the results of Green et al. (2003), who reported that transfer coefficients of Cd from wheat shoot to aphid *Sitobion avenae* were between 0.85 and 1.6. Cd biomagnification in aphids has also been reported by other workers (Merrington et al. 1997b; Alonso et al. 2009).

Whilst Cd in *L. erysimi* was biomagnified at lower levels of sewage sludge amendment, it was biominimized at higher levels. A similar declining pattern in transfer coefficients between shoot and the aphid *Sitobion avenae* was found by Green et al. (2010). The decrease in transfer coefficients for Cd was similar, although slightly smaller, than that exhibited by Zn, but Cd was less affected at the highest sludge amendment. Consequently, Cd was the only element that was significantly higher in the aphids of the T_3 treatment compared to the T_2. In a further contrast to Zn, Cd concentrations in honeydew increased in comparison to the concentration in the aphids as the aphid concentration increased. Thus, aphids appeared to be able to partially regulate Cd accumulation via this mechanism.

It has been suggested that aphids exert little control over their uptake of Cd (Crawford et al. 1995) and that Cd concentration in the shoot is the predominant factor determining Cd concentration in aphids (Green et al. 2006). It is apparent that if the plant restricts transfer of Cd to the shoot, then transfer through the higher trophic levels of the food chain must also be restricted, as seen in the case of Zn in the present study. However, the present study demonstrated the potential for aphids respond to elevated concentration of Cd in shoots by increasing the concentration of Cd excreted in their honeydew. Consequently, they can exert a modicum of control over the accumulation of Cd. Nevertheless, the contrast in results between the present study and that of Crawford et al. (1995) and Green et al. (2006) suggests that regulation of Cd by this mechanism may not apply to all aphid species.

Very few studies have been published that describe the transport of Pb in plant-arthropod system. To the best of our knowledge, Pb detection in aphids is reported first time in a terrestrial system, although Cowgill (1973) reported concentrations of Pb in the leaves of the water lilly *Nymphaea odorata* and aphid *Rhopalosiphum nymphaea* feeding on them. In the present investigation, Pb was the only trace metal which did not show biomagnification in aphids at any amendment rate. Similar results for Pb accumulation have been previously reported for various species of grasshoppers (Devkota and Schimidt 2000; Zhang et al. 2012), *Bombyx mori* (Zhou et al. 2015) and *R. nymphaea* (Cowgill 1973), suggesting similar accumulation processes within and between taxa.

Despite a lack of biomagnification, Pb exhibited the smallest decrease in transfer coefficient between shoot and aphid as sludge amendment increased. However, this reflects the magnitude of increase in shoot concentration, i.e. Pb is apparently less affected because it reflects a lower increase in shoot concentration. Still, at high sludge amendments (T_2 and T_3) Pb and Cd had similar transfer coefficients between shoot and aphids. Like Cd, Pb was increasingly excreted in the honeydew as sludge amendment increased, but the increase in excretion was not as large as for Cd, contributing the observed smaller decline in transfer to aphids. Although comparison between the two non-essential elements is complicated by the lower relative increase in Pb shoot concentration, it appears that Cd and Pb do not differ particularly in respect to their transfer and regulation in aphids in contaminated systems.

Aphid to ladybird transfer

Zn accumulation in newly emerged adult ladybirds followed a similar pattern to the aphids population on which they had fed as larvae. Thus, there was no significant increase in concentration between T_2 and T_3. The regulation of Zn transfer between shoot and aphid therefore had a direct effect on the concentration in ladybirds, further demonstrating the importance of the plant, determining the transfer of trace metals in food chains. Zn in adult ladybirds was biomagnified at lower levels of sludge amendment, but at higher levels (T_2 and T_3), the transfer coefficient fell below one. In contrast to Zn, Cd and Pb transfer coefficient never exceed the lowest value recorded for Zn. This relatively high transfer of Zn reflects the essential metabolic requirement for Zn and this has been suggested to result in a greater retention of Zn within organisms to maintain homeostatic supply in case of scarcity arises (Calhoa et al. 2011; Green and Walmsley 2013). Nevertheless, the decreasing transfer coefficients suggest that there was partial regulation of Zn by the ladybirds.

Sequestration in the pupal exuviae appeared to be an ineffective mechanism to exclude Zn from adult ladybirds as the proportion of Zn sequestered in the exuviae did not differ among treatments. Green et al. (2003) reached the same conclusion after establishing that Zn sequestration in pupal exuviae of *C. septempunctata* had no statistical effect on the concentration in newly emerged adult. Consequently, regulation of Zn most probably occurred in the larval stage, which unlike some insect larvae, do not have blind ending gut and are therefore capable to excreting metals via the faeces.

As with Zn, Cd concentration in newly emerged ladybirds followed the pattern in aphids and increased significantly with sludge amendment. However, Cd body burdens in adult ladybirds were between 0.49 and 0.55 times lower than in aphids on which they fed. This represented a considerable bio-minimisation of Cd from aphids to adult ladybirds. This agrees with the findings of Green et al. (2003), who reported biominimisation of Cd in same species in almost same range.

The present study found the proportion of the larval body burden lost in the exuviae was 10.8-14.8%, a little higher than the 10.5 % reported by Green et al. (2003). The present study also demonstrated that this excretion mechanism becomes less effective as the level of Cd exposure increases, i.e. the proportion of the larval body burden lost decreases with increased body burden. This did not seem to affect the proportion of larval body burden transferred to the adult as transfer coefficients between aphids and adult ladybirds did not increase. As a consequence, there must have been a mechanism that reduced uptake or increased excretion of Cd in the larval stage to account for the decreasing proportion of Cd lost in the exuviae.

Transfer from aphids to ladybirds is the only point in the multi-trophic system where the extent of Pb transfer exceeds that of Cd. Moreover, the extent of Pb transfer to adult ladybirds increased with sludge amendment and thus the concentration in the aphids. Significantly higher concentrations of Pb were found in the adults of T_3 than in the other treatments as a result. Less of the larval body burden was lost via sequestration in the exuviae than for Cd, with 10-10.5% lost via this mechanism. The proportion of Pb sequestered into the exuviae decreased as treatment and concentration in the ladybirds increased. However, the decrease in excretion via the exuviae appears insufficient on its own to explain the increase in concentration in the newly emerged adult ladybirds. It therefore appears that Pb was in an increasingly available form in the aphids and/or Pb was increasingly accumulated in tissues not lost during metamorphosis, i.e. tissues within the soma. These tissues are possibly more vulnerable to Pb toxicity than tissues with a more defined detoxification role, such as the midgut epithelium. As a consequence, the risk of Pb toxicity to ladybirds appeared to increase much more rapidly with the level of contamination than for Cd.

Conclusion

This study has shown that points in the soil-plant-herbivore-predator pathway where the transfer of trace metals can be enhanced or restricted differ between elements. Of the three elements studied, Cd was the most mobile through the food chain at high sludge amendments. Transfer of Pb through the food chain was the most restricted of the three elements, especially in the soil-plant system. The decrease in root-shoot transfer coefficients in contrast to increasing coefficients for Cd and Zn was particularly noticeable and was a major restriction on Pb transfer. However, Pb became increasingly mobile in the higher trophic levels, particularly to the predatory ladybirds. Indeed, Pb exceeded Cd in the extent to which it was transferred to the adult ladybirds and Pb was increasingly transferred to the ladybirds as sludge amendment increased. The result was a marked difference in the transfer of Cd and Pb from aphids to ladybirds at the highest sludge amendment rate.

The present study also highlighted that the fate of an element differs with the nature of the food chain as the present study found that both Cd and Pb were more readily transferred through the food chain and Zn less so than has been reported for other systems. The potential consequences of Pb mobility in the consumer trophic levels and the potential ecotoxicological consequences are particularly concerning. Thus, a precautionary approach should be taken when setting soil metal limits, especially to ensure environmentally safe use of organic by-products, until a fuller understanding of the complex contaminant transfer process in food chains is understood.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Tables

Table 1. Selected physico-chemical properties of soil, sewage sludge and soil after amendment with different ratios of sewage sludge (mean ± 1 SE, *n*=4). Values with different superscript letters in each group are significantly different from each other at $p < 0.05$.

Parameters	Sewage sludge	T_0	T_1	T_2	T_3
pH	7.12 ± 0.04	7.82^{a} ± 0.04	7.80^{a} ± 0.03	7.76^{a} ± 0.05	7.69^{a} ± 0.04
Org C (%)	5.75 ± 0.12	0.67^{a} ± 0.04	0.85^{b} ± 0.05	1.19^{c} ± 0.03	1.48^{d} ± 0.08
Total N (%)	1.18 ± 0.06	0.16^{a} ± 0.02	0.18^{a} ± 0.02	0.21^{ab} ± 0.02	0.26^{b} ± 0.02
Total heavy metals (mg kg^{-1})					
Cd	7.34±0.32	0.45^{a}±0.05	0.62^{a}±0.07	1.05^{b}±0.07	1.69^{c}±0.11
Pb	36.61±1.80	11.17^{a}±0.47	11.97^{a}±1.11	13.3^{ab}±1.16	15.24^{b}±1.04
Zn	374.18±14.72	36.46^{a}±2.17	49.97^{b}±2.05	65.8^{c}±3.53	98.02^{d}±6.20
DTPA extractable heavy metals (Plant available heavy metals; mg kg^{-1})					
Cd	-	0.14^{a} ±0.01	0.18^{a}±0.02	0.29^{b}±0.02	0.44^{c}±0.04
Pb	-	2.24^{a} ±0.19	2.45^{ab} ±0.22	2.69^{ab} ±0.17	2.92^{b} ±0.20
Zn	-	3.13^{a} ±0.22	4.57^{b} ±0.21	7.62^{c} ±0.18	11.18^{d} ±0.19

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Table 2. Transfer coefficients for the transfer of Cd, Pb and Zn contents between various components of the soil-plant-aphid-ladybird system after the amendment of soil with sewage sludge.

Heavy metal	Amendments	Total soil-root	Extractable soil-root	Root-Shoot	Shoot-Aphid	Aphid-Adult ladybird
Cd	T_0	2.84	9.14	0.68	1.63	0.53
	T_1	3.08	10.61	0.65	1.73	0.49
	T_2	3.21	11.62	0.72	0.93	0.55
	T_3	4.22	16.23	0.75	0.79	0.51
Pb	T_0	1.56	7.75	0.65	0.87	0.56
	T_1	1.57	7.69	0.61	0.93	0.64
	T_2	1.93	9.55	0.65	0.79	0.66
	T_3	2.24	11.68	0.56	0.75	0.79
Zn	T_0	1.55	18.09	0.74	2.29	1.22
	T_1	1.69	18.49	0.80	2.16	1.13
	T_2	2.57	22.19	0.81	1.72	0.93
	T_3	2.70	23.68	0.78	1.23	0.89

Table 3. Heavy metal contents in honeydewed and honeydew-free (washed) plants of *B. juncea* grown in different amendments of sewage sludge and the ratio of metal levels in honeydew against metal contents in aphids (mean ± 1 SE, *n* = 4). Values with different superscript letters in each group are significantly different from each other at $p < 0.05$.

Metals	Concentration (mg kg^{-1})			
	Honeydewed	Washed	Contribution by honeydew	Honeydew/aphid
Cd				
T_0	1.20^{a} ± 0.11	0.89 ± 0.12	0.31	0.21
T_1	1.86^{a} ± 0.10	1.33 ± 0.12	0.53	0.24
T_2	3.01^{b} ± 0.16	2.37 ± 0.16	0.64	0.28
T_3	6.84^{c} ± 0.52	5.44 ± 0.46	1.45	0.33
Pb				
T_0	14.07^{a} ±1.45	12.34 ±1.38	1.73	0.18
T_1	15.90^{a} ±0.71	13.93 ± 0.81	1.97	0.18
T_2	23.35^{b} ±1.57	20.20 ± 1.60	3.15	0.24
T_3	25.42^{b} ±1.84	21.80 ± 2.07	3.62	0.25
Zn				
T_0	48.33^{a} ± 1.60	38.59 ± 2.40	9.74	0.10
T_1	85.28^{b} ± 3.97	71.69 ± 4.78	13.59	0.09
T_2	164.3^{c} ± 12.56	137.9 ± 10.92	26.41	0.11
T_3	240.4^{d} ± 14.43	211.8 ± 13.77	28.54	0.11

Table 4. Mean (± 1SE, *n* = 4) heavy metal content (mg kg^{-1} dry weight) in pupal exuviae of *C. septempunctata*. Values with different superscript letters in each group are significantly different from each other at $p < 0.05$.

Metals	Amendments	Pupal exuviae	% lost in exuviae
Cd			
	T_0	$0.86^a \pm 0.09$	14.79
	T_1	$0.95^a \pm 0.12$	12.02
	T_2	$1.12^a \pm 0.08$	11.95
	T_3	$1.87^b \pm 0.19$	10.82
Pb			
	T_0	$4.37^a \pm 0.33$	10.55
	T_1	$5.31^a \pm 0.31$	10.25
	T_2	$6.68^b \pm 0.30$	10.32
	T_3	$8.25^c \pm 0.48$	9.97
Zn			
	T_0	$74.75^a \pm 6.24$	8.81
	T_1	$109.54^{ab} \pm 12.10$	9.02
	T_2	$140.52^b \pm 15.42$	8.69
	T_3	$131.83^b \pm 12.14$	8.02

Table 5. Mean (± 1SE, $n = 4$) dry weight (mg individual^{-1}) of roots and shoots of *Brassica juncea*, aphids (*L. erysimi*) and newly emerged adult ladybirds (*C. septempunctata*). Values with different superscript letters in each group are significantly different from each other at $p < 0.05$

Amendments	Root	Shoot	Aphid	Adult Ladybird
T_0	$0.372^{a} \pm 0.03$	$1.74^{ab} \pm 0.24$	$0.032^{a} \pm 0.003$	$6.14^{a} \pm 0.31$
T_1	$0.380^{a} \pm 0.04$	$1.54^{a} \pm 0.22$	$0.037^{a} \pm 0.002$	$6.48^{a} \pm 0.35$
T_2	$0.430^{a} \pm 0.02$	$2.48^{b} \pm 0.17$	$0.028^{a} \pm 0.003$	$6.25^{a} \pm 0.41$
T_3	$0.458^{a} \pm 0.05$	$2.39^{b} \pm 0.11$	$0.030^{a} \pm 0.005$	$6.07^{a} \pm 0.27$

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Figures

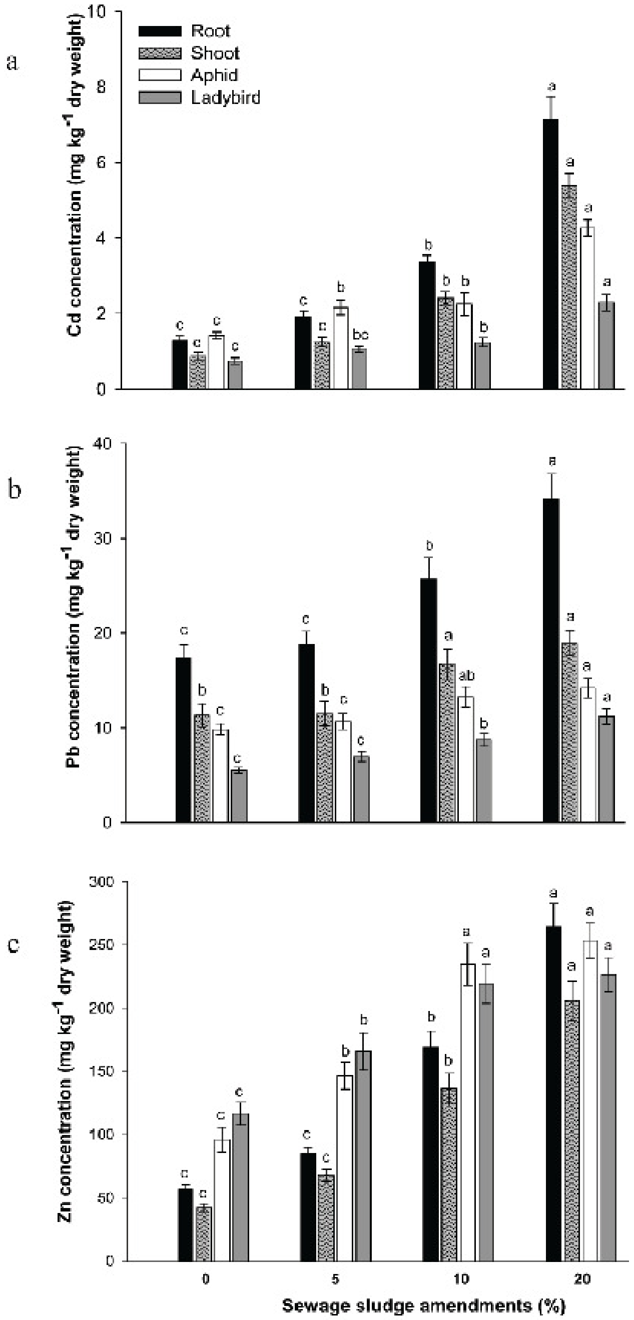

Fig. 1 Trace metal concentration (mg kg^{-1} dry weight) transferred from sewage sludge amended soil in mustard, aphid and newly emerged adult ladybird. (a) Cd, (b) Pb and (c) Zn. Each value is mean of four replicates ± SE. Bars with different letters in each group are significantly different from each other at $p < 0.05$