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Accumulation and detoxification of cadmium by larvae of *Prodenia litura* (Lepidoptera: Noctuidae) feeding on Cd-enriched amaranth leaves

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HIGHLIGHTS

- ▶ This is the first study on the subcellular distribution of Cd in insect food chain.
- ► Cadmium accumulation in *Prodeina litura* larvae increased, but no biomagnification.
- ► Cadmium was predominantly bound with the heat-stable protein fraction in larvae.
- ▶ Subcellular study shed lights on the Cd detoxification and its transfer in food chain.

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ABSTRACT

Cadmium is a potentially toxic and carcinogenic nonessential heavy metal. This study investigated Cd accumulation along the soil–plant (*Amaranthus hypochondriacus* L.)–insect (*Prodenia litura*) food chain and the detoxification strategies at different trophic levels. *A. hypochondriacus* leaves could accumulate high levels of Cd from polluted soil. The Cd concentration in *P. litura* larvae increased with increasing Cd concentrations in the food plant. Transfer coefficients of Cd were high from soil to leaf and from larvae to feces. The leaves of *A. hypochondriacus* had the highest value of Cd accumulation in pectates and protein-integrated forms (extracted by 1 M NaCl). Among all the subcellular fractions in larvae of *P. litura*, the heat-stable protein fraction was the dominant metal-binding compartment for Cd. The Cd subcellular level played an important role in Cd sequestration and excretion by *P. liura* larva feeding on Cd contaminated amaranth leaves. This is the first attempt to account for subcellular distribution associated with Cd in *P. litura* when interpreting Cd detoxification and transfer along insect food chain.

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1. Introduction

Cadmium is a widespread heavy metal pollutant with great potential toxicity and high mobility in the environment. Cd contamination has been a worldwide public health concern, because Cd has the potential to bioaccumulate in plants (McLaughlin et al., 2006) and invertebrates (Peijnenburg, 2002) and a greater potential for trophic transfer along some food chains (Croteau et al., 2005; Bechard et al., 2008). Metal biotransformation patterns among plants and animals are dependent on both the metal availability and physiological constraints on uptake into an organism, and both of these aspects are in turn dependent on chemical speciation, i.e. the chemical form in which the metal is presented to the consumer (Calhoa et al., 2011). There is an important and urgent need to identify the underlying principles governing Cd trophic transfer.

Chemical speciation of heavy metals in plants has much to do with its biological functions; there is some evidence that the different chemical forms of Cd provide control over Cd toxicity to plant tissues and Cd migration to primary consumer (Wang et al., 2009; Zhang et al., 2009a). For example, it has been documented that inorganic and organic water-soluble Cd (extracted by 80% ethanol and d-H₂O, respectively) has higher potential for migration than other extracted forms (Fu et al., 2011). In Chinese flowering cabbage (Brassica parachinensis), the inorganic salt forms of Cd could more readily transferred from root to shoot than other forms (Qiu et al., 2011). The pattern of Cd chemical forms in plants is also considered as important factors influencing the characteristics of Cd migration and degree of accumulation and phytotoxicity in different species or genotypes. For instance, Wu et al. (2005) found Cd-resistant barley genotypes had a larger concentration of pectates and protein-integrated Cd than that in Cd-sensitive barley genotypes.

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Subcellular distribution of metals in organisms reflects internal processing that occurs during metal accumulation and can provide valuable information about metal toxicity and tolerance (Wallace et al., 1998). Subcellular metals are partitioned into fractions including cellular debris, organelles, metal-rich granules (MRGs), heat-denatured proteins (HDPs) and heat-stable proteins (HSPs) (Wallace and Lopez, 1996). Many studies on metal subcellular distribution have been mainly concerned with metal toxicity and tolerance and trophic transfer processes of metals in aquatic animals (Wallace and Luoma, 2003; Rainbow, 2007; Dubois and Hare, 2009), but trophic transfer of metals from plants through terrestrial food chain has received negligible attention. Wallace and Lopez (1997) found that Cd associated with HDP and HSP in Limnodrilu hoffmeisteri was 100% trophically available to Palaemonetes pugio and Cd bound to organelles was 70% trophically available while Cd bound to MRG was unavailable. A recent study investigating the insect larvae of Chaoborus punctipennis showed that the HSP fraction was the dominant metal-binding compartment for Cd, Ni and Cu (Rosabal et al., 2012). Previous work suggests that trophically available metal (TAM) [i.e. metal associated with HSP (e.g., metallothionein), HDP (e.g., enzymes) and organelles] could potentially result in a bioenhancement of Cd transfer along aquatic food chains (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Mechanistic understanding of trophic metal transfer could benefit from the measure of metal bioavailability in each subcellular fraction, but this has never been accomplished in previous studies.

This study describes the accumulation and transfer of Cd in a soil-plant (Amaranthus hypochondriacus L.)-insect larvae (Prodeina litura) terrestrial food chain. A. hypochondriacus, amaranth plant, is a potential Cd hyperaccumulator with high biomass and high tolerance to Cd toxicity (Li et al., 2012). P. litura is one of the most common herbivorous pests distributed widely in Asia and is abundantly found in soybean, tobacco, and vegetable plants. This herbivorous insect has long been known as a highly harmful pest to crops and forage plants. As such, P. litura is an important part of the agroecosystem and provides a significant source of protein for wildlife such as arthropods and birds, and therefore has the potential to transfer metals to higher trophic levels. Several studies had reported that some phytophagus insects have the ability to bioaccumulate heavy metals in their bodies through food and may transfer them to higher trophic levels (Devkota and Schmidt, 2000; Merrington et al., 2001; Prince et al., 2001). The specific objectives of this study were to: (1) determine the extent to which Cd is transferred from the soil through the plant to the insect. And (2) determine how the chemical form of Cd in the plant and the subcellular distribution of Cd in the insect can elucidate an understanding of Cd accumulation and detoxification along the soilplant (A. hypochondriacus) -insect (P. litura) food chain.

2. Materials and methods

2.1. Pot experiment and soil characterization

The pot experiment was conducted in the greenhouse at South China Botanical Garden using a soil with N content of 1.47 g kg^{-1} ; P of 0.34 g kg^{-1} , Cd concentration of 0.22 mg kg^{-1} and available Cd of 0.07 mg kg^{-1} . After being air-dried and thoroughly mixed, the soil was sieved through 1 cm mesh and 4 kg of the processed soil was placed in each pot. The pot experiment was designed in a factorial completely randomized design, including control, 2, 5, 10 and 20 mg Cd kg⁻¹ soil treatment. Each treatment was conducted with four replicates, Cd was added into each pot at the required concentration using aqueous solutions of CdCl₂·2.5H₂O. The diethylenetetramine-pentaacetate acid (DTPA) extractable Cd was 41-91%

of total Cd concentration in the treated soil (Table 1). A. hypochondriacus L. seeds were sown in each pot and watered every day to keep proper soil moisture (70–80%). 20 d after seeding, every pot was thinned to 5 uniform-sized seedlings and plants were grown for 40 d. The soils were sampled, air-dried and ground for Cd analysis. Plant samples were harvested and separated into roots, stems and leaves, and immediately frozen in liquid N₂ and kept frozen until use.

2.2. Feeding study

Larvae of *P. litura* (on the 3rd instar, about 1 cm length) were obtained from South China Agricultural University. The larvae were divided into five equal treatment groups with each group (75 larvae) feeding on different Cd treated amaranth leaves (control, 5, 10 and 20 mg kg⁻¹ Cd treatments). The feeding experiment was conducted in a cultivation chamber, with a 12 h/12 h light/dark illumination regime at constant temperature of 26 °C and relative humidity of 50%. The larvae were fed twice every day for 7 d, and the feces and unconsumed leaves were collected every day and oven-dried and weighed to calculate the total feeding mass.

2.3. Chemical forms extraction

The chemical forms of Cd in A. hypochondriacus leaves were extracted by designated solutions in the following order (Yang et al., 1995): (i) 80% ethanol, extracting inorganic Cd including nitrate/nitrite, chloride, and aminophenol cadmium; (ii) deionized water (d-H₂O), extracting water soluble Cd-organic acid complexes and Cd (H₂PO₄)₂; (iii) 1 M NaCl, extracting pectates and protein-integrated Cd; (iv) 2% HAc, extracting Cd-phosphate complexes; and (v) 0.6 M HCl, extracting cadmium oxalate. Frozen leaf tissues (about 2 g) were homogenized in extraction solution (1:75, w/v) using a glass tissue homogenizer. Then the homogenate was shaken at 30 °C for 18 h, centrifuged at 5000g for 10 min, and obtained the first supernatant solution in a glass beaker. The sedimentation was re-suspended twice in the same extraction solution and shaken 2 h at 30 °C, centrifuged at 5000g for 10 min, then pooled the supernatant of the three suspending and centrifuge steps for each of the five extraction solution. Each of the pooled supernatant solution was evaporated to constant weight, and digested with HNO₃:HClO₃ (3:1, v/v).

2.4. Subcellular distribution of Cd

At the end of the 7 d feeding study, subcellular Cd distribution in larvae of *P. litura* was investigated using a methodology described by Wallace et al. (1998) with minor modifications. Frozen larval bodies were homogenized in 5 mL (1:2, m/v) 20 mM Tris buffer (pH 7.6) using a glass tissue homogenizer. The homogenate was centrifuged at 1950g for 15 min at 4 °C. The resulting pellet was re-suspended in 1 mL distilled water and heated at 100 °C for 2 min, then an equal volume of NaOH was added followed by heated at 70 °C for 1 h. The remaining material was centrifuged

Table I

Concentrations of total and DTPA-extractable Cd in the different treated soil (means \pm SE, mg kg⁻¹, n = 4).

Total	DTPA-extractable	Proportion (%) ^a
0.24 ± 0.03	0.10 ± 0.01	41
1.8 ± 0.05	1.7 ± 0.03	91
4.2 ± 0.11	3.7 ± 0.07	88
8.2 ± 0.14	7.3 ± 0.14	89
19 ± 0.12	15 ± 0.32	77
	Total 0.24 ± 0.03 1.8 ± 0.05 4.2 ± 0.11 8.2 ± 0.14 19 ± 0.12	Total DTPA-extractable 0.24±0.03 0.10±0.01 1.8±0.05 1.7±0.03 4.2±0.11 3.7±0.07 8.2±0.14 7.3±0.14 19±0.12 15±0.32

^a Indicated the percentage of DTPA-extractable Cd to total Cd concentration.

at 5000g for 10 min at 20 °C. The pellet formed contained MRG, and the resulting supernatant was designated cellular debris, containing metal associated with dissolved tissues. The supernatant of the first centrifugation step, containing the cytosol, was centrifuged at 100,000g for 1 h at 4 °C to produce organelles. Then the supernatant containing the cytosol was heat-denatured at 80 °C for 10 min, cooled on ice for 10 min. HDP were separated from the HSP by centrifugation at 50,000g for 10 min at 4 °C. All fractions were collected and evaporated on an electric-plate at 70 °C, then digested at 145 °C with 5 mL concentrated HNO₃ until tissue samples were dissolved.

2.5. Cadmium concentrations analysis and quality control

Soil samples were digested with an oxidative concentrated acid mixture of HNO_3 :HF:HClO₄ (4:2:1, v/v/v) to determine total Cd, and available Cd in soils was extracted with DTPA. Plant samples were digested in HNO_3 :HClO₄ (3:1, v/v) and the insect larvae were digested with 5 mL HNO₃. Cd concentrations in the soil, plant and insect larvae were determined by atomic absorption spectrophotometry (AAS). Certified reference materials of GBW 08303 for soil, GBW 07604 for total Cd concentrations of plant and feces and GBW(E) 080193 for total Cd contents of larva were used for quality control, and reagent blank and analytical duplicates were also used.

2.6. Data analysis

The transfer coefficient was calculated by the following formula:

Transfer coefficients = concentration of metal (mg kg⁻¹)in the

receiving level/concentration of metal (mgkg⁻¹)

in the source level.

The accumulation factor (AF) is the ratio of metal concentration measured in plant to the concentration measured in the soil (Prince et al., 2001).

One-way analysis of variance was carried out using the SPSS 18.0 program and least significant difference was used for multiple comparisons at the 0.01 or 0.05 significance level.

3. Results

3.1. Concentration of Cd in A. hypochondriacus and P. litura

The average biomass, Cd concentration and uptake in different parts of *A. hypochondriacus* with different treatments are presented in Fig. 1. The leaf and root biomass of amaranth decreased with increasing Cd concentration in the soil (Fig. 1a). The trend of biomass in the three tissues was in the descending order of leaf > - stem > root. The root and leaf biomass of amaranth was reduced 29 and 49% at the 20 mg kg⁻¹ Cd soil contamination level, respectively, compared to the control group. As illustrated in Fig. 1b, the concentrations of Cd in different parts of the amaranth plant



Fig. 1. Total biomasses (a), Cd concentrations (b) and Cd uptakes (c) in the different parts of *Amaranthus hypochondriacus* grown in the control, 2, 5, 10 and 20 mg kg⁻¹ Cd treated soils for 40 d (means \pm SE, n = 4).

increased significantly with increasing Cd concentration in the soil. The concentrations of Cd in the different tissues of amaranth were in the order: leaf > stem > root, with the highest Cd concentration (371 mg kg^{-1}) in the leaves when the soils were contaminated at 20 mg kg⁻¹ Cd. Cadmium total uptake of amaranth increased significantly with increasing Cd application (Fig. 1c). A high percentage (>90% of the total uptake) of Cd accumulated was transferred from the soil to aerial part of *A. hypochondriacus*.

The consumption of plant leaves fed to the larvae of *P. litura*, and the concentrations of Cd in both larval body and feces with different Cd treatments are shown in Table 2. The Cd accumulation in the insect larvae showed a dramatic increase with increasing Cd concentrations in their food plants, though the consumption of plant leaves was not significantly different among treatments. The concentrations of Cd in the larval feces were about 5 times as high as in the larvae for all treatments, with the highest concentration (606 mg kg⁻¹) found at the 20 mg kg⁻¹ soil Cd treatment.

3.2. Transfer coefficients of Cd in soil-plant-insect

The transfer coefficient of Cd between the soil and the roots for the *A. hypochondriacus* in the control treatment was approximately half those of the 2, 5 and 10 mg kg⁻¹ Cd treatments (Table 3).

Table 2

Consumption of leaves of *Amaranthus hypochondriacus* (75 larvae gourp⁻¹), dry biomass and Cd concentrations in the larval body and feces of *Prodenia litura* feeding on Cd-enriched amaranth plants for 7 d (means ± SE, n = 4).

Treatment	Larval body			Larval feces		
	Consumption (g group ⁻¹ , dw)	Biomass (g group $^{-1}$)	Concentration (mg kg ⁻¹ , dw)	Biomass (g group ⁻¹)	Concentration (mg kg ⁻¹ , dw)	
СК	11 ± 1.10	2.2 ± 0.14	1.5 ± 0.11	6.4 ± 0.27	7.0 ± 0.36	
Cd 5	9.4 ± 0.86	2.3 ± 0.16	63 ± 2.68	6.5 ± 0.34	305 ± 5.44	
Cd 10	11 ± 0.35	2.6 ± 0.17	105 ± 14.3	6.6 ± 0.34	518 ± 20.0	
Cd 20	11 ± 0.44	3.5 ± 0.09	187 ± 10.2	7.5 ± 0.19	606 ± 19.2	

Table 3

Transfer coefficients for the transfer of Cd between different level of the soil-plant (*Amaranthus hypochondriacus*)—insect (*Prodenia litura*) food chain.

Treatment	Transfer o	coefficients	Accumulation		
	Soil to root	Root to leaf	Leaf to larva	Larva to feces	factor
СК	13	1.4	0.32	4.8	16
Cd 2	26	1.8	-	-	39
Cd 5	27	2.1	0.31	4.9	47
Cd 10	32	1.2	0.35	5.1	42
Cd 20	13	1.6	0.50	3.3	19
CK Cd 2 Cd 5 Cd 10 Cd 20	13 26 27 32 13	1.4 1.8 2.1 1.2 1.6	0.32 - 0.31 0.35 0.50	4.8 - 4.9 5.1 3.3	16 39 47 42 19

Transfer coefficients = concentration of metal $(mg kg^{-1})$ in the receiving level/ concentration of metal $(mg kg^{-1})$ in the source level.

Accumulation factor = mean plant concentration $(mg kg^{-1})$ (root + stem + leaf)/ mean soil total $(mg kg^{-1})$ concentration.

- Referred to no data in the feeding experiment for 2 mg kg⁻¹ treatment.

Transfer coefficient values between the roots and the leaves of amaranth were all above one, ranging from 1.4 to 2.1. Cadmium transfer coefficients between the leaves to the larval body of *P. litura* were below one in all treatments, which was explained by the fact that most of the ingested Cd was excreted through feces. The transfer coefficients between the larval body and feces were above one, and were 6–15 times higher than those between the leaves and the larvae. The overall accumulation factor of Cd from the soil to leaf showed the highest values (47) at the 5 mg kg⁻¹ soil Cd treatment.

3.3. Chemical forms of Cd in A. hypochondriacus

The predominant form of Cd was that bound in pectates and protein-integrated forms (extracted by 1 M NaCl), amounting to 56–75% of the total Cd for all treatments, followed by undissolved Cd phosphate (extracted by 2% HAc) and organic forms (extracted by d-H₂O), with the residual fraction of Cd being the lowest (Table 4). There was a significant difference among the six different chemical forms of Cd depending on treatment. The proportions of Cd in inorganic form (nitrite, chloride and aminophenol cadmium), and pectates and protein-integrated form declined with increasing Cd concentrations in the soil, while those of other forms showed a tendency to increase, especially for the Cd phosphate fraction which increased from 8% to 30%.

3.4. Subcellular distribution of Cd in P. litura

Subcellular distribution of Cd (MRG, cellular debris, organelles, HDP and HSP) and the reconstruction of different subcellular compartments (insoluble, soluble and TAM) in *P. litura* exposed for 7 d are shown in Table 5. Partitioning of Cd among all fractions

in *P. litura* followed the pattern of: HSP (49–58%) > MRG (19–25%) > cellular debris (19–22%) > organelles (2.5–3.1%) > HDP (1.3–1.6%). The partitioning among all five fractions in insect larvae was generally responsive to increasing Cd treatment. As shown in Table 5, the percentages of the TAM fraction (53–62%) and the soluble fraction (51–59%) in the *P. litura* larvae showed a tendency to increase with increasing Cd treatments. In contrast, the percentage of insoluble fraction (41–49%) decreased with the increase of Cd treatments.

4. Discussion

4.1. Accumulation and transfer of Cd in the soil-plant-insect food chain

This study presented original data on the transfer of Cd in the soil-plant-insect food chain, showing a deconcentration of Cd from contaminated plant (A. hypochondriacus) to insect larvae (P. litura) accompanied by excessive excretion of Cd from insect feces. Although no biomagnification of Cd was observed, mechanisms of Cd detoxification and migration existed in this food chain. The exceptionally high Cd concentration and soil-plant transfer coefficients of amaranth plant confirmed our previous finding in which A. hypochondriacus was reported to be a potential Cd hyperaccumulator (Li et al., 2012). The peak value of Cd concentration in the leaves of amaranth (371 mg kg^{-1}) was higher than the proposed "tolerated" Cd leaf concentration for plants of 200 mg kg⁻¹ (Dickinson et al., 2007), yet no apparent toxic response was observed, suggesting that this plant species may provide an important pathway for transfer of metals to primary plant consumers. The overall AF of Cd from the soil to aboveground tissues showed remarkably high values (19-47) in all treatments, which were higher than those (2.6-10.2) found in silkworm Bombyx mori L. for Cd (Prince et al., 2001). This pattern of high transfer of Cd from the soil to the leaf could be probably explained by the high percentage of DTPA-extractable Cd in the soil (Table 1) permitting Cd uptake by A. hypochondriacus from contaminated soil (Kabata-Pendias, 2004) and the inherent potential for Cd accumulation by A. hypochondriacus.

The present results indicate a decrease of Cd concentrations in transfer from contaminated food plants to insect larvae, demonstrating no biomagnification of Cd in this food chain. Cadmium transfer coefficients between the leaves and the insect larvae were below one in all treatments. This result was in agreement with the soil-plant (*Rumex* K-1)-insect (*Spodoptera litura*)-chicken food chain found in our previous work (Zhuang et al., 2009). A similar trend was reported for Cd in slugs *Deroceras reticulatum* feeding on contaminated lettuce and carrot (Gräff et al., 1997) and was also found in snails *Helix pomatia* feeding on Cd-enriched lettuce (Dallinger and Wieser, 1984). In contrast, several studies have

Table 4

Chemical forms of Cd in the leaves of Amaranthus hypochondriacus grown in the 2, 5, 10 and 20 mg kg⁻¹ Cd treated soils for 40 d. Values are means ± SE.

Treatment	F(i)	F(ii)	F(iii)	F(iv)	F(v)	F(vi)	Total
Cd 2	0.47 ± 0.03 (4.7) ^a	1.2 ± 0.09 (12)	7.5 ± 0.03 (75)	0.79 ± 0.07 (7.9)	0.06 ± 0.001 (0.57)	0.02 ± 0.001 (0.16)	10
Cd 5	1.1 ± 0.15 (3.3)	1.7 ± 0.35 (4.9)	26±0.9 (75)	5.8 ± 0.24 (17)	0.15 ± 0.03 (0.43)	0.02 ± 0.001 (0.06)	35
Cd 10	1.3 ± 0.18 (2.7)	3.4 ± 0.77 (7.1)	32 ± 1.86 (67)	11 ± 1.19 (23)	0.29 ± 0.03 (0.61)	0.04 ± 0.01 (0.08)	48
Cd 20	0.93 ± 0.08 (1.8)	4.9 ± 1.06 (9.2)	30 ± 1.78 (56)	16 ± 1.31 (30)	1.3 ± 0.23 (2.5)	0.07 ± 0.01 (0.13)	53

F(i), extracted by 80% ethanol; F(ii), extracted by d-H₂O; F(iii), extracted by 1 M NaCl; F(iv), extracted by 2% acetic acid; F(v), extracted by 0.6 M HCl; F(vi), Cd in residual form. ^a The number in parentheses indicated the percentages of Cd (%) associated with different chemical forms.

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Treatment	MRG	CD	ORG	HDP	HSP	Insoluble	Soluble	TAM
Cd5	1.3 ± 0.1 (25) ^a	1.1 ± 0.09 (21)	0.13 ± 0.01 (2.4)	0.07 ± 0.001 (1.4)	2.6 ± 0.26 (49)	(49)	(51)	(53)
Cd10	1.5 ± 0.05 (19)	1.7 ± 0.37 (22)	0.24 ± 0.01 (3.2)	0.11 ± 0.01 (1.4)	4.1 ± 0.24 (54)	(45)	(55)	(58)
Cd20	3.1 ± 0.11 (19)	3.2 ± 0.26 (19)	0.52 ± 0.12 (3.1)	0.26 ± 0.02 (1.5)	9.6 ± 0.25 (58)	(41)	(59)	(62)

Concentrations (means ± SE, mg kg⁻¹, *n* = 4, fw) and percentage distributions (%) of Cd in five different fractions (MRG, cellular debris, organelles, HDP and HSP) and combinations of these fractions (insoluble, soluble and TAM) in *Prodenia litura* feeding on Cd-enriched amaranth plants for 7 d.

MRG, metal-rich granules; CD, cell debris; ORG, organelle components; HDP, heat-denatured proteins; HSP, heat-stable protein; insoluble, MRG + CD + ORG; Soluble, HDP + HSP; TAM, trophically available metal, including ORG, HDP and HSP.

^a The number in parentheses indicated the percentage distributions (%).

Table 5

reported that some insects have the ability to bioaccumulate Cd in their bodies through food (Heliovaara, 1990; Devkota and Schmidt, 2000; Prince et al., 2001). For example, the transfer coefficients of Cd were 2.4 and 10.5 in *Eligma narcissus* and *Holotrichia* fed on plants, respectively (Zhang et al., 2009b). It was reported that housefly larvae (*Musca domestica*) fed on 50 mg Cd kg⁻¹ in artificial medium could accumulate Cd to concentrations as much as three times the dietary level (Kramarz, 1999).

Our results revealed that Cd accumulation in larvae increased substantially and significantly with increasing Cd treatment, reaching the highest Cd level (187 mg kg^{-1}) for the 20 mg kg⁻¹ Cd soil treatment (Table 2), yet no toxic effect was displayed in the larvae. These tissue Cd levels are lower than that of a previous study which showed the primary consumer, snail (Helix aspersa), when fed Cd-enriched plants at 100 mg kg⁻¹, could survive and accumulate tissue Cd to levels as high as 282 mg kg⁻¹ (Scheifler et al., 2002). Notably, Cd concentrations in feces of insect larvae were significantly higher than those in insect larvae or plant leaves (Table 2). The transfer coefficients between the larvae and feces were above one, and were 6-15 times higher than those between the leaves and the larvae. In a soil-plant (Urtica dioica)-snail (Cepaea nemoralis) food chain, it was observed that the Pb concentrations in the feces of snail exceeded those in the food plants (Notten et al., 2005). Such a result might be partly explained by excretion of excess metal as a major strategy for limiting toxic effects of nonessential metals (Vijver et al., 2004).

4.2. Detoxification of Cd in the plant and insect

Metal bioavailability and detoxification in plants is controlled by biological manipulation of the chemical forms of heavy metals (Wang et al., 2008). It has been recognized that Cd in inorganic and water soluble forms exhibits higher capacity to migrate and hence cause more deleterious effects on plant cells in comparison to insoluble Cd bound into pectates and integrated in proteins (Wang et al., 2008; Xu et al., 2011). A. hypochondriacus leaves can alleviate Cd toxicity by bonding large amounts of accumulated Cd into pectins and proteins (extracted by NaCl), forms which are less toxic than forms extracted by ethanol and d-H₂O (Table 4). This result is in accordance with studies of Cd-resistant barley genotypes (Wu et al., 2005) and the Cd hyperaccumulator ramie Bechmeria nivea L. (Wang et al., 2008). In the present study, Cd in stable forms (extracted by 2% HAC and 0.6 M HCl) was enhanced significantly with increasing soil Cd application, suggesting a decline in toxic Cd stress to larvae. If the metal is in excess, other internal protective mechanisms can become active, and the detoxified metal can be either stored or actively excreted.

Metal subcellular distribution could reflect metal tolerance tactics and the potential trophic transfer to the organism (Wallace and Luoma, 2003). It is widely accepted that metal sequestration and detoxification in organisms involve the synthesis of HSP and

MRG (Brown, 1982). In the present results, the increasing partitioning of Cd to the HSP fraction (from 49% to 58%) in *P. litura* with increased exposed to dietary Cd has implications for the great detoxification ability of P. litura to Cd, and the result is in agreement with studies by Seebaugh and Wallace (2004) and Rosabal et al. (2012). Cheung et al. (2006) have also found a significant positive correlation between the Cd subcellular distribution in HSP and Cd body concentration in the welk Thais clavigera fed the snail Monodonta labio. The large proportions of Cd in MRG (19-25%) and cellular debris (19-22%) in P. litura larvae were similar to those reported for Cd in Gammarus fossarum by Geffard et al. (2010). In our study, this proportion decreased from 25% to 18% with increasing soil Cd concentrations, indicating that an effective detoxification strategy for P. litura larvae may not be to transfer assimilated Cd into MRG. The percentage of Cd associated with the organelles fraction was constant over the range of Cd treatment (about 3%), suggesting that toxicity in P. litura feeding on Cd-contaminated leaves may not be relevant to organelles. The cellular debris fraction contained a substantial and fairly constant (19-22%) portion of Cd in insect larvae. Wallace and Luoma (2003) proposed the TAM fraction (a combination of organelles, HDP and HSP) as a predictor of trophic transfer. The percentage of TAM-[Cd] in P. litura larvae increased with increasing Cd treatments and was due to increased partitioning of Cd to HSP.

Excretion patterns of animals give useful information on probable mechanisms of metal detoxification (Kozlova et al., 2000). The total Cd concentrations in feces were 7-13 times as high as those in *P. litura* larvae in all treatments, implying that large amounts of assimilated-Cd were eliminated through fecal matter, which suggested that P. litura larvae could survive when fed on Cd-enriched amaranth leaves utilizing an evolved efficient detoxification mechanism. In a previous study we also reported that S. litura larvae in the food chain (soil-plant-insect-chicken) could detoxify metals through excretion of feces (Zhuang et al., 2009). The majority of nickel accumulated in S. litura larvae could be excreted through feces (Sun et al., 2008). It was noted that the interaction among metals at the subcellular level often had significant impacts on excretion of individual metals, suggesting that MRG not only plays a role in Cd sequestration but may also contribute to excretion (Galay Burgos and Rainbow, 1998; Wallace and Luoma, 2003; Vijver et al., 2004). In the present study, the large proportion (41-49%) of Cd in the insoluble fraction (i.e. MRG, cellular debris and organelle components) was not available for assimilation by insect larvae (Table 5), resulting in an increased release of metals via fecal pellets to balance uptake when contamination exceeds a certain level (Galay Burgos and Rainbow, 1998). This is one indication that *P. litura* could handle high Cd stress, showing no toxic response to a severely contaminated food source.

A Cd mass-balance calculation yielded contradictory results: in the higher Cd treatments, the mass of Cd found in the larvae plus larval feces was greater than the estimated amount of Cd in the

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consumed part of the leaf parenchyma. Conversely, in the control treatment at low Cd, the mass of Cd consumed was greater than the mass of Cd in the larvae plus larval feces. This discrepancy could probably be explained by the fact that insect larvae only ingested leaf laminae, which may have accumulated a higher Cd concentration than the whole leaf (lamina + leafstalk), resulting in an underestimation of the mass of Cd consumed from leaves in the present study. Although an accumulation of metals has been reported for several insect herbivores (Crawford et al., 1995; Devkota and Schmidt, 2000; Prince et al., 2001), some species demonstrated an opposite pattern, i.e. excretion of most of the Cd and Ni (Kozlova et al., 2000; Notten et al., 2005). In the present results, only a part of the Cd (7–13%) consumed is retained within the larval body, and the ability of larvae to excrete most of the consumed Cd provides a physiological basis for the high resistance of P. litura larvae to severe Cd contamination in food plants. This pattern was in confirmation of previous findings of Kozlova et al. (2000). Although we found that insect larvae achieved a remarkable detoxification process in a highly Cd polluted environment, further experimental evidence will be required to determine if this system can prevail under field conditions and whether Cd stress under less controlled conditions could affect the lifecycle of P. litura.

5. Conclusion

To summarize, the transfer of Cd through the soil–plant system was enhanced at higher soil Cd levels due to both increased soil to root and root to leaf transfer. However, Cd transfer was restricted in the leaf-insect larvae system, and most of the ingested Cd was excreted via larval feces. Analysis of the chemical form of Cd in plant foliar tissue demonstrated that a large proportion of Cd was associated with pectates and protein-integrated forms. Subcellular forms of Cd played an important role in Cd sequestration and excretion by *P. liura* larvae feeding on Cd-contaminated food plants. The use of subcellular fractionation has the potential to reduce the uncertainties associated with interpreting Cd accumulation in relation to detoxification in insect larvae, and could provide a valuable tool for assessing potential effects of Cd on the insect food chain.

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