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Internal distribution of Cd in lettuce and resulting effects on Cd trophic transfer to the snail: *Achatina fulica*

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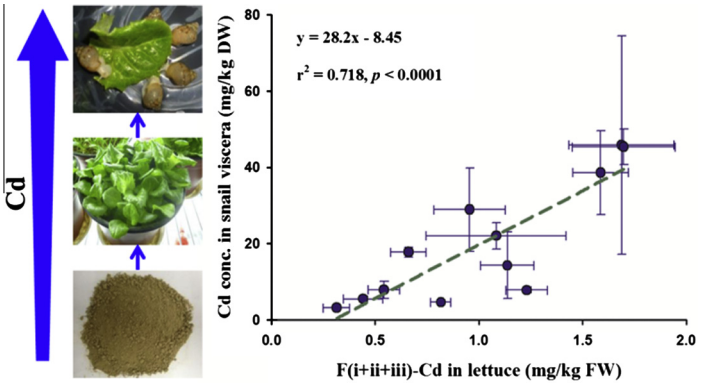
HIGHLIGHTS

- This is the first study on the chemical forms of Cd in a lettuce–snail food chain.
- Chemical forms of Cd (F(i + ii + iii)-Cd) in lettuce best explain Cd trophic transfer.
- Subcellular study of TAM-Cd failed to enlighten Cd transfer from lettuce to snail.

GRAPHICAL ABSTRACT



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ABSTRACT

The mechanisms underlying Cd trophic transfer along the soil–lettuce–snail food chain were investigated. The fate of Cd within cells, revealed by assessment of Cd chemical forms and of subcellular partitioning, differed between the two examined lettuce species that we examined (*L. longifolia* and *L. crispa*). The species-specific internal Cd fate not only influenced Cd burdens in lettuce, with higher Cd levels in *L. crispa*, but also affected Cd transfer efficiency to the consumer snail (*Achatina fulica*). Especially, the incorporation of Cd chemical forms (Cd in the inorganic, water-soluble and pectates and protein-integrated forms) in lettuce could best explain Cd trophic transfer, when compared to dietary Cd levels alone and/or subcellular Cd partitioning. Trophically available metal on the subcellular partitioning base failed to shed light on Cd transfer in this study. After 28-d of exposure, most Cd was trapped in the viscera of *Achatina fulica*, and cadmium bio-magnification was noted in the snails, as the transfer factor of lettuce-to-snail soft tissue was larger than one. This study provides a first step to apply a chemical speciation approach to dictate the trophic bioavailability of Cd through the soil–plant–snail system, which might be an important pre-requisite for mechanistic understanding of metal trophic transfer.

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1. Introduction

Cadmium is readily accumulated and can be toxic to organisms (Berger and Dallinger, 1989; Vijver et al., 2006; De Jonge et al., 2012). Invertebrates such as snails serve as a link between plants and their predators; Cd contamination in plants may thus pose a potential threat to snails and consequently to wildlife. For instance, Cd transferred from plants to the snail (*Helix aspersa*), and induced evident mortality to the predatory carabid beetle *Chrysocarabus splendens* (Scheifler et al., 2002a). Rats accumulated subsequently more Cd in their kidney when fed with contaminated snail-based rat food than the inorganic food ($CdCl_2$ dosed rat food) (Hispard et al., 2008a). Recent studies have shown that accumulated Cd in aquatic animals was largely derived from food (Wang and Ke, 2002) and in some cases Cd biomagnified along the coastal food chain (Blackmore and Wang, 2004). However, compared to the aquatic food chains (Ruangsomboon and Wongrat, 2006; Guo et al., 2013), Cd trophic transfer through terrestrial food chains remains largely un-explored (Scheifler et al., 2002b, 2006; Notten et al., 2006; Monteiro et al., 2008; Sinnett et al., 2009; Ding et al., 2013). Especially, most previous research concerning metal trophic transfer along the terrestrial food chains employed artificial food types, such as agar (Berger and Dallinger, 1989), gelatine substrate (Calhôa et al., 2011) and vegetable flour (Scheifler et al., 2002b), which exhibited different bioavailability from natural food contaminated under environmentally realistic conditions (Notten et al., 2006).

Animals assimilate metal from their prey dependent in part on the form in which the metal is bound within the prey cells. Over the past years, the subcellular partitioning model (SPM) has been extensively used for predicting metal bioavailability during trophic transfer. In its conceptual framework, metals in cells were operationally divided into five fractions (Wallace and Luoma, 2003; Lavoie et al., 2009), (i) cellular debris, (ii) metal-rich granules (MRG), (iii) organelles, (iv) heat-denatured fractions (HDF) and (v) heat-stable fractions (HSF). Thereafter, a large number of studies evidenced that the combination of different fractions in prey could best explain its trophic availability to predators (Cheung and Wang, 2005; Rainbow et al., 2006). Especially, the trophically available metal fraction (TAM, combination of organelles + HSF + HDF) in the prey, proposed by Wallace and Luoma (2003), has been widely tested in various organisms (Rainbow et al., 2011).

In addition to metal subcellular partitioning, metal chemical forms have been developed to predict metal toxicity to terrestrial plants (Wu et al., 2005). This approach quantifies the fate of metal within cells by sequentially extracting metals with designated solutions (Farago and Pitt, 1977; Wu et al., 2005). The total amount of metal accumulated was separated into F(i): inorganic forms, F(ii): water soluble forms, F(iii): pectate- and protein- integrated forms, F(iv): metal phosphates forms, F(v): metal oxalate forms and F(vi): residual forms. Previous research showed that F(i)-Cd of *Brassica parachinensis* was more readily transferred upward from root to shoot (Qiu et al., 2011). However, the utilization of chemical forms to predict metal trophic transfer was concluded to remain a key challenge.

In this study we investigated the internal fate of Cd (i.e., Cd chemical forms and subcellular partitioning) within the cells of two lettuce species, *L. longifolia* (*Lactuca Sativa* L. var. *longifolia*) and *L. crispa* (*Lactuca Sativa* L. var. *crispa*), which were exposed to different levels of soil Cd for various lengths of time. We hypothesized that the internal Cd fate differed either between the lettuce species or with exposure duration, and subsequently the transfer efficiency of Cd to the consumer snail (*Achatina fulica*) was also expected to differ. This would provide a mechanistic understanding of the factors that influence Cd transfer between a plant and a consumer snail along the terrestrial food chain.

2. Materials and methods

2.1. Test organisms

The Chinese white jade snail (*A. fulica*) was selected because it is one of the most popular edible terrestrial invertebrates and widely distributed in South China. It mainly feeds on lettuce, a Cd-accumulating leaf vegetable. Juvenile snails were obtained from Fuliang farms, Zhejiang Province, China, and acclimated in the laboratory at 25 °C and 85% relative humidity under a 16:8 h artificial light:dark photoperiod for one week.

2.2. Food labeling

The seedlings of *L. longifolia* (*Lactuca Sativa* L. var. *longifolia*) and *L. crispa* (*Lactuca Sativa* L. var. *crispa*) were exposed for 76 d in Cd-contaminated soils. Detailed information on the exposure conditions is given elsewhere (Li et al., 2014). Lettuce was harvested at days 55, 62, 69 and 76, the shoots were sampled, and rinsed with 10 mM EDTA and deionized water to remove any adsorbed Cd. All shoots samples were dried on filter papers, weighed and stored at 4 °C for further processing.

One Cd-contaminated soil and one background soil were collected near Yingtan, Jiangxi Province, China. The soils, which were taken from the 0–20 cm, were both red soils. After air-drying, the soils were ground and sieved through a 4.0 mm mesh. Then they were mixed thoroughly at various ratios to produce Cd concentrations of 0.20 mg kg^{-1} (Control), 0.61 mg kg^{-1} (Low-Cd) and 1.1 mg kg^{-1} (High-Cd). These target levels were chosen based on the Soil Environmental Quality Guideline Levels in China (GB15618-1995). Experimental soils were analyzed for pH (1:10 soil to water) and total Cd concentrations.

2.3. Feeding experiment

For each treatment, one acclimated snail (2.06 ± 0.19 g, ww) was transferred to a 750-mL acid-washed polypropylene vial (10.5-cm diameter × 7.5-cm height). The shoots were cut into small pieces (about 1 cm^2), mixed thoroughly, and offered as diet for snails. Note that shoots were fed to snails immediately after harvesting (no longer than 7 d). Therefore, Cd concentrations in diet did not show significant variation during exposure (see text). Shoots were supplied ad libitum (3.0 ± 0.1 g for each vial) to the snails. To avoid fungi infection, which poses a risk to snails and alters Cd bioavailability (Monteiro et al., 2008), shoots were replaced every other day. Snails were exposed to Cd-accumulated lettuce for 4 weeks under the laboratory conditions as those described above. During this feeding period, snails did not exhibit a preference for one particular lettuce species. Each week three snails were removed, rinsed with ultrapure water, and depurated individually for 48 h in petri dishes. After rinsing by ultrapure water, snails were weighted, sacrificed by freezing and dissected for viscera (i.e., the visceral complex containing the posterior gut, digestive gland, kidney, mantle, and part of the reproductive tract), foot (containing the foot sensu stricto, anterior gut, and rest of the genital tract) and shell. All tissues were frozen at −70 °C until further analysis. During the 4-week exposure, snail mortality was below 5% for all treatments.

2.4. Metal analysis and quality control

Plant and snail samples were oven-dried at 80 °C and digested in concentrated HNO_3 at 300 °C. After air-drying and sieving ⩽ 0.149 mm, soils were subjected to HF: HNO_3: $HClO_4$ (4:2:1, v/v/v) digestion. Certified reference materials (TORT-2, lobster

hepatopancreas from National Research Council Canada, Canada; GBW 10015, spinach from the Institute of Geophysical and Geochemical Exploration and GBW 07408, soil from the National Research Center, Chinese Academy of Geological Sciences, China) were concurrently digested. All digests were analyzed for Cd by flame atomic absorption spectrophotometer (Hitachi Z2000). The recoveries were 94 ± 2%, 107 ± 4% and 112 ± 5% in TORT-2, GBW 10015 and GBW 07408, respectively.

2.5. Chemical form extraction

Chemical forms of Cd in lettuce shoots were determined following Farago and Pitt (1977) and Wu et al. (2005): F(i) 80% ethanol to extract Cd in some amino acids, chlorophyll, low weight compounds (inorganic forms); F(ii) deionized water to extract water-soluble Cd bound with organic acids and metaphosphate (water soluble forms); F(iii) 1 M NaCl to extract Cd combined to pectate and protein (pectate- and protein-integrated forms); F(iv) 2% HAc to extract Cd binding to phosphate (metal phosphates forms); F(v) 0.6 M HCl to extract Cd associated with oxalate (metal oxalate forms); F(vi) Cd in the residues (residual forms).

In brief, frozen lettuce shoots (about 2 g) were homogenized in 30.0 mL of extraction solution and shaken at 120 rpm for 22 h at 25 °C. The homogenate was centrifuged at 5000*g* for 10 min to obtain the supernatant. The residue was re-suspended twice with the same extraction solution and shaken for another 2 h as described above. After centrifugation at 5000*g*, 90.0 mL supernatant was pooled and digested in concentrated HNO_3 for Cd determination.

2.6. Subcellular Cd partitioning

Cadmium subcellular partitioning in the lettuce shoots was performed according to the phytoplankton-based method (Lavoie et al., 2009). Five subcellular fractions were separated: (i) a debris fraction comprising nuclei, cell membranes and cell walls (cellular debris); (ii) NaOH resistant or phosphorus/sulfur-containing compounds (MRG: metal-rich granules); (iii) a fraction combining organelles, e.g. mitochondria, microsomes (organelles); (iv) heat-stable fractions, including metallothioneins and phytochelatins (HSF); (v) heat denatured fractions, e.g. enzymes (HDF).

Briefly, frozen tissues (0.2 g lettuce shoots) were homogenized in 10.0 mL Tris–HCl solution (pH 7.5, 50 mM Tris–HCl with 1.0 mM dithioerythritol and 250 mM sucrose (Weigel and Jäger, 1980)). After microscopic examination of unbroken cells, the lettuce homogenate was centrifuged at 2500*g* for 15 min. The pellet was first suspended in 2.0 mL ultra-pure water and kept in a 100 °C water bath for 2 min, digested with 2.0 mL 1.0 M NaOH at 70 °C for 60 min, and finally centrifuged at 10,000*g* for 15 min to obtain the pools of MRG (pellet) and cellular debris (supernatant). The first centrifugal supernatant was centrifuged at 100,000*g* for 60 min to sediment the organelles fraction. The supernatant was placed in an 80 °C water bath for 10 min and subsequently cooled on ice for 60 min, and then centrifuged again at 50,000*g* for 30 min to separate the pools of heat-denatured fractions (HDF, pellet) and the heat-stable fractions (HSF, supernatant). Unless otherwise specified, all operations were conducted at 4 °C.

2.7. Data analysis

One-way ANOVA (Student–Newman–Keuls (S–N–K)) was applied to assess the significant differences among groups. Linear regression analysis was performed to test for correlations. All statistical analyses were carried out by SPSS 19.0 software. Data are presented as the mean ± SD.

3. Results and discussion

3.1. Cadmium in lettuce

Cadmium in soil was highly bioavailable to lettuce, as shown here and in earlier studies (Kuboi et al., 1986). Cadmium accumulation in lettuce showed intra-species and dose-specific variations (Table 1). *L. crispa* typically accumulated more Cd than *L. longifolia.* Within each species, Cd was increasingly accumulated in the plants with increasing soil Cd levels, with internal Cd concentrations in the shoots ranging from 0.51 to 30.8 mg kg^{-1} (dw). No statistical differences in Cd accumulation over time were observed except for the controls of *L. longifolia* and Low-Cd of *L. crispa*, implying steady-state Cd accumulation in lettuce after 55 d of exposure (Peijnenburg et al., 2000). Thus, snails were exposed to a large range of dietary Cd levels, which were generally constant over the 4 week exposure period.

Differences in Cd accumulation patterns between the two lettuce species could be explained by the internal Cd fate within the cells (cadmium chemical forms and subcellular partitioning) (Supporting Information Figs. S1 and S2). The majority of Cd (mean proportions of Cd during exposure time) was present in F(iii) (pectate- and protein-integrated forms: *L. longifolia*: 43.3 ± 3.69%, *L. crispa*: 55.4 ± 3.18%) and F(ii) (water soluble forms: *L. longifolia*: 40.8 ± 6.17%, *L. crispa*: 31.9 ± 4.43%), followed by F(i) (inorganic forms: *L. longifolia*: 8.63 ± 3.15%, *L. crispa*: 8.00 ± 3.28%). This suggests that related plant species have a similar Cd chemical form ranking, but the proportions differed between the plant species. Collectively, a larger proportion of Cd was distributed in F(iii) of *L. crispa*, where Cd was considered to be detoxified (Li et al., 2014).

The mean proportions of Cd in the various subcellular fractions of *L. longifolia* decreased as: cellular debris (34.7 ± 7.62%) > MRG (metal-rich granules, 27.3 ± 9.53%) > organelles (23.3 ± 4.48%) > HSF (heat-stable fractions, 15.8 ± 8.27%) > HDF (heat-denatured fractions, 2.47 ± 2.09%); however, a slightly different pattern was observed in *L. crispa*, which contained higher Cd levels: cellular debris (35.7 ± 3.50%) > MRG (27.9 ± 4.49%) > HSF (18.4 ± 5.84%) > organelles (15.3 ± 4.18%) > HDF (2.73 ± 4.05%). Cadmium in HSF played an important role in Cd sequestration of *L. crispa*, which resulted in 3.5, 1.9 and 2.0 times more Cd in *L. crispa* in the Control, Low-Cd and High-Cd treatment, respectively than in *L. longifolia* exposed to the same treatments.

Table 1
Cadmium concentrations and pH in the different treated soils and Cd concentrations in lettuce shoots during 76 d exposure to Cd contaminated soils.

Treatments	Soil		Cd conc. in *L. longifolia* (mg kg^{-1} dw)				Cd conc. in *L. crispa* (mg kg^{-1} dw)			
	pH(H_2O)	Cd conc. (mg kg^{-1})	55 d	62 d	69 d	76 d	55 d	62 d	69d	76 d
Control	4.88 ± 0.03	0.197 ± 0.01	1.30 ± 0.12	0.514 ± 0.06	1.75 ± 0.50	2.41 ± 0.56	8.18 ± 3.42	6.74 ± 5.93	2.76 ± 0.50	3.47 ± 1.11
Low-Cd	4.99 ± 0.02	0.610 ± 0.03	6.04 ± 0.91	11.5 ± 3.73	7.15 ± 4.60	6.10 ± 2.69	12.9 ± 1.25	18.1 ± 2.29	18.7 ± 1.59	8.88 ± 1.61
High-Cd	5.11 ± 0.01	1.12 ± 0.04	9.99 ± 0.57	19.5 ± 9.76	12.0 ± 1.14	15.2 ± 4.66	21.6 ± 5.28	31.4 ± 4.16	30.5 ± 0.72	30.8 ± 6.53

Values are presented as the mean ± SD (n = 3).

3.2. Cadmium in the consumer snail

Total Cd concentrations in the soft tissues of snails, ranged from 2.10 to 53.7 mg kg^{-1} dw, and increased readily over the 4 week exposure (Fig. 1). Especially, Cd concentrations in snail tissues correlated linearly with Cd concentrations in lettuce (Table 2, Column 1). Snails fed on *L. crispa* typically accumulated more Cd than those fed on *L. longifolia*. This difference could be explained in part by the fact that *L. crispa* had about 1.9–3.5 times higher Cd concentrations than those of *L. longifolia*. The difference can, however, also be a consequence of the differences in internal Cd fate between the two examined lettuce species (i.e., Cd chemical forms and subcellular distribution, see Section 3.3).

For a given food, the snail viscera contained the highest Cd levels, which accounted for more than 83% of the total Cd content in the snail, followed by the foot (17-fold lower than in the viscera) and shells (180-fold lower than in case of the viscera). High Cd accumulation in viscera was not surprising given that viscera was the uptake organ of dietary Cd (Dallinger and Wieser, 1984; Dallinger et al., 1997). Moreover, it appeared that most Cd was trapped by the viscera, when comparing viscera Cd levels to those in foot and shell, which was consistent with previous studies (Gimbert et al., 2008b; Hispard et al., 2008b)

The trapped Cd in viscera was reported to be either excreted via the mucus (Notten et al., 2006) or sequestrated within cells (e.g., metallothionein induction (Dang and Wang, 2009) and mineral granules (Vijver et al., 2004)), and the latter was considered to be important in Cd detoxification in invertebrates (Dallinger et al., 1989). After viscera uptake, dietary Cd was transferred into the foot, as suggested by the progressive Cd accumulation in the foot. Transportation of Cd across the viscera cell to internal tissues, however, appeared to be a limiting step of Cd uptake in snails.

3.3. Cadmium trophic transfer

Transfer factors (TF, e.g., defined as the lettuce-to-snail tissue Cd concentration ratio) were used to assess Cd trophic availability along the soil–lettuce–snail food chain. Interestingly, Cd biomagnified along the lettuce–snail food chain, where Cd levels were environmentally realistic, as suggested by the high TFs of lettuce-to-snail soft tissue (>1, Table 3). Likewise, *H. asperses* were reported to concentrate Cd with TFs ranging from 2.45 to 4.79 (Scheifler et al., 2002b). A lower TF of *H. aspersa* was also documented (0.58–3.39 (Scheifler et al., 2002a)) when Cd (as fine powder of anhydrous $CdCl_2$, Aldrich) spiked plant-based food was offered as food for two weeks. Typically, differences in Cd concentrations and species in prey (Cheung and Wang, 2005), along with different digestive process of predators (Cheung et al., 2007; Dubois and Hare, 2009) could contribute to differences in metal bioavailability. The Cd TF depended on lettuce metal

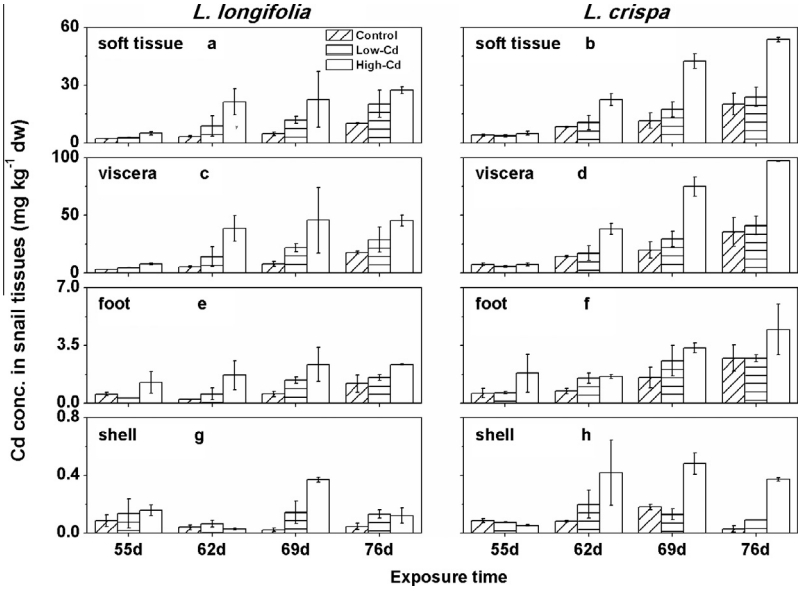

Fig. 1. Cadmium accumulation in different tissues of the snails fed on Cd-contaminated *L. longifolia* (a, c, e, g) and *L. crispa* (b, d, f, h). Values are mean ± SD ($n = 3$).

Table 2
Relationships between Cd accumulation in snails and Cd bioavailability in lettuce corrected by internal Cd fate.

Lettuce	Snail tissue	Snail-Cd and lettuce-Cd	Snail-Cd and F(i + ii + iii)-Cd	Snail-Cd and subcellular fraction-Cd	
				Snail-Cd and TAM-Cd	Snail-Cd and cellular debris-Cd
L. longifolia	Soft tissues	$r^2 = 0.52^{**}$	$r^2 = 0.64^{**}$	$r^2 = 0.35^{*}$	$r^2 = 0.60^{**}$
	Viscera	$r^2 = 0.50^{**}$	$r^2 = 0.72^{****}$	$r^2 = 0.34^{*}$	$r^2 = 0.61^{**}$
	Foot	$r^2 = 0.46^{*}$	$r^2 = 0.70^{***}$	$r^2 = 0.42^{*}$	$r^2 = 0.57^{**}$
	Shell	$r^2 = 0.06$	$r^2 = 0.27$	$r^2 = 0.16$	$r^2 = 0.29$
L. crispa	Soft tissues	$r^2 = 0.38^{*}$	$r^2 = 0.73^{****}$	$r^2 = 0.06$	$r^2 = 0.29$
	Viscera	$r^2 = 0.36^{*}$	$r^2 = 0.39^{*}$	$r^2 = 0.05$	$r^2 = 0.29$
	Foot	$r^2 = 0.24$	$r^2 = 0.34^{*}$	$r^2 = 0.05$	$r^2 = 0.22$
	Shell	$r^2 = 0.65^{**}$	$r^2 = 0.40^{*}$	$r^2 = 0.22$	$r^2 = 0.32$

*, **,*** and **** suggest linear relationships at significant levels at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. Those without star indicated no significant correlations.

Table 3
Cadmium transfer factors (defined as the mean Cd concentrations ratio, see the text) along the soil–plant (*Lactuca sativa*)-snail (*Achatina fulica*) food chain.

Treatments		Soil to plant	Plant to snail				Soil to snail soft tissues
Soil	Plant		Plant to soft tissues	Plant to viscera	Plant to foot	Plant to shell	
Control	*L. longifolia*	7.58	6.82	11.9	0.793	0.031	51.7
Low-Cd		12.6	2.63	3.76	0.202	0.017	33.2
High-Cd		12.7	1.93	3.19	0.165	0.009	24. 4
Control	*L. crispa*	26.8	3.82	6.76	0.518	0.005	102
Low-Cd		24.0	1.63	2.81	0.186	0.006	39.1
High-Cd		25.5	1.88	3.40	0.157	0.013	47.9

concentrations, i.e., the higher the Cd levels in lettuce, the lower the TF by the consumer except for the chain *L. crispa*-to-snail viscera in the High-Cd treatment (Table 3). This was in agreement with earlier observations in snails (Scheifler et al., 2002b) and aphids (Green et al., 2010). Likewise, snails fed on *L. longifolia* showed higher TFs than that of *L. crispa* (Table 3), partly due to the lower Cd levels in *L. longifolia*.

The Cd TF was also influenced by the availability of Cd in prey, because not all Cd in prey was equally bioavailable to predators (Wallace and Lopez, 1996). Even though Cd levels in snail tissues correlated linearly with the dietary Cd levels of the snails in this study, the significance of the correlation of Cd in snails and Cd in lettuces was further improved by the involvement of Cd chemical forms (F(i + ii + iii)) in lettuce as variables, e.g., the corresponding r^2 value was 0.718 and $p < 0.0001$ for the viscera of snail fed on *L. longifolia* (Table 2). This suggested that Cd in F(i + ii + iii) was highly bioavailable when compared to other chemical forms within lettuce. Indeed, Cd in F(i + ii + iii) represents Cd bound to some amino acids, chlorophyll, low weight compounds and proteins, which are more bioavailable than other Cd species in the plant (Wallace and Lopez, 1997; Wang et al., 2008). If Cd in the organelles, HDP and HSP fractions (so-called trophically available metal fractions: TAM) in lettuce was assumed to be highly available to the snail, as proposed by Wallace and Luoma (2003), then the inclusion of TAM-Cd should significantly affect the regression equations, as shown by Cd in F(i + ii + iii). However, this was not the case in our study (Table 2). Rainbow et al. (2011) addressed that the fractions available for accumulation across trophic levels varied with the predators of different digestive processes. Therefore, the TAM-Cd proposed in the aquatic grass shrimp (Wallace and Luoma, 2003) might be different from the TAM-Cd in terrestrial snails. Furthermore, significant relationships between TAM-Cd and assimilation efficiency were quantified by pulse-chase feeding previously (Wallace and Luoma, 2003; Rainbow et al., 2011). Whilst in our experiment TFs were calculated after 28-d of dietary Cd exposure, during which physiological disruptions (e.g., Cd elimination, differences in Cd subcellular partitioning in lettuce) were inevitable. Thus, feeding regime differed from those pulse-chase feeding, which maybe another reason for TAM-Cd in lettuce failed to predict the Cd bioavailabilty to snails. Of particular interest, cellular debirs Cd in *L. longifolia* appeared to be more bioavailable to snails (Table 2). The high bioavailability of cellular debris Cd in *Lactuca sativa* (to *Porcellio dilatatus*) was also reported by Monteiro et al. (2008). Wallace and Lopez (1997) have demonstrated that Cd bound to the debris fraction (cellular debris + MRG) of *Limnodrilus hoffmeisteri* is bioavailable to *Palaemonetes pugio* with an assimilation efficiency of 48.6%. Some pectates and proteins integrated Cd in F(iii) which exhibit high trophic transfer ability are probably belong to the cellular debris (mainly composed of cell wall (Lavoie et al., 2009)) Cd as well. Because pectins, proteins and polyoses (e.g., cellulose, hemicellulose, lignin and mucilage glue) are dominant components of plant cell wall (Haynes, 1980). Bulk Cd K-edge extended X-ray absorption fine structure spectroscopy showed that Cd was predominantly bound to COOH/OH groups belonging to organic acids and/or cell wall components (Huguet et al., 2012).

We previously demonstrated that both Cd chemical forms and subcellular partitioning could advance our understanding of plant responses to metal exposure (Li et al., 2014). In this study we further showed that internal Cd chemical forms, rather than subcellular partitioning, is a better predictor of Cd trophic availability in lettuce.

3.4. Environmental implications of Cd biomagnification in snails

Dietary Cd-exposure may increase the tolerance of snails to metals, because the protective mechanisms (e.g., MT induction or subcellular distribution) developed during dietary Cd exposure provide more binding sites for subsequent metal exposure (Michaud et al., 2005). Furthermore, snails serve as an important link between plant litter and carnivores (e.g. small mammals, birds and other invertebrate predators (Laskowski and Hopkin, 1996; Hispard et al., 2008a)). Thus, Cd biomagnification in snails was found to pose a potential risk to the predators. Cadmium levels in snails in this study (2.10–53.7 mg kg^{-1} dw) were similar to those reported in the field experiment (Gimbert et al., 2008a), and close to the No Observed Effect Concentrations (NOECs) of birds and mammals (2–75 mg kg^{-1}, (Laskowski and Hopkin, 1996)). Additionally, Cd biomagnification in *A. fulica* (the Chinese white jade snail) is an amounting concern for the public because this species of snail is widely consumed in South China. Cadmium levels in the edible foot were as high as 0.8 mg kg^{-1} fw, which is in line with the guideline limit for seafood recommended by the Ministry of Health of the People's Republic of China (0.1–2 mg kg^{-1}, GB 2762-2012).

4. Conclusion

This study suggested that Cd trophic transfer efficiency was likely to be influenced by both Cd burdens and the internal fate of Cd within cells at the lower trophic level. To our knowledge, this study is the first to reveal that metal chemical forms (F(i + ii + iii)-Cd: inorganic Cd, water-soluble Cd and pectates and protein-integrated Cd) in lettuce are better predictors of the trophic bioavailability of Cd than subcellular Cd partitioning. Whatever the food source was, Cd biomagnified along the food chain was examined.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.03.096.

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