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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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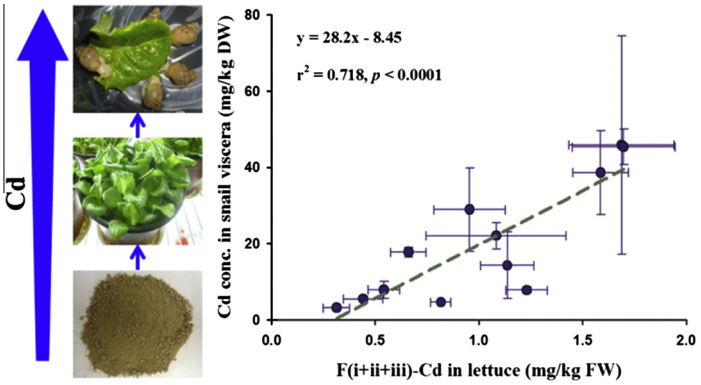
HIGHLIGHTS

- 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