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Accumulation of cadmium and copper by female *Oxya chinensis* (Orthopera: Acridoidea) in soil-plant-insect system

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Abstract: One purpose of this research is to present accumulation of cadmium (Cd) and copper (Cu) by female *Oxya chinensis* (Orthopera: Acridoidea) in a simulated soil-plant-insect ecosystem treated with Cd. Fourth-instar nymphs of *O. chinensis* had been fed on wheat (*Triticum aestivum*) seedlings contaminated with Cd and Cu for one month. In the ecosystem, the Cd concentration in wheat seedlings rose greatly with the increasing of Cd in the soil, but the Cu concentration in wheat seedlings was not found elevated. There was a highly significant difference($P<0.05$) in Cd concentrations of wheat seedlings and not any significant difference($P>0.05$) in Cu concentrations of wheat seedlings. The Cd and Cu concentration in different body part-head, thorax, abdomen, and hind femur, varied under different Cd concentrations in soil. There were significant differences ($P<0.05$) in the four parts of Cd and Cu accumulations with all treatments. The order of Cd accumulation was thorax > abdomen > head > hind femur and the Cu was abdomen > thorax > head > hind femur. The results indicated that Cd and Cu were accumulated from the soil to grasshoppers through the plant; that is to say, Cd and Cu in environment could be transported to animal or human via food chain.

Keywords: accumulation; cadmium(Cd); copper(Cu); grasshopper(*Oxya chinensis*); soil-plant-insect ecosystem

Introduction

The occurrence of toxic heavy metals in the soil is of geogenic or anthropogenic origins. The natural concentration of heavy metals in the soil is dependent on geochemical and geophysical processes. Heavy metals from the point and other sources of emission can be transported to distant environments. Transport of heavy metals from the atmosphere to the soil and vegetation takes place through dust fall, bulk precipitation and gas or aerosol adsorption processes and so on. Heavy metals are integrated components of the biosphere and thus occur naturally on soils and plants. Soils can be naturally or anthropogenically (from metal-smelting industries, coal combustion, auto emissions and application of commercial fertilizers, liming materials, sewage sludge, animal wastes, irrigation waters and agrochemicals) enriched with heavy metals. Heavy metals from the point and other sources of emissions can be transported to distant environments (Devkota and Schmidt, 2000; Belimov *et al.*, 2003). The heavy metals, such as Cd and Cu, are among the widespread pollutants in the surface soil layer. Cadmium is not an essential nutrient for plant and it is normally toxic, with the clear exception of certain hyperaccumulating plant species. Cadmium may be not only increasingly taken up by the crop, inhibiting plant growth and mineral nutrition, but also transferred further to the food chain, causing diseases in animals and human beings as well as accumulating in different human tissues and organs (Devkota and Schmidt, 2000; Fornazier *et al.*, 2002; Gallego *et al.*, 2002; Pereira *et al.*, 2002; Belimov *et al.*, 2003; Wang and Stuanes, 2003). A low quantity of Cu is known to be an essential micronutrient for many living creatures, but it can also be a toxic element at a tissue concentration only slightly higher than optimal. Copper excess induces a wide range of biochemical effects and metabolic disturbances that are responsible for a strong inhibition of plant growth and plant yield decreases. The accumulation of Cu was found in some animals and it produced biochemical effects and metabolic disturbances (Chiou *et al.*, 1997; Fry *et al.*, 2002; Lwanga *et al.*, 2003; Wójcik and Tukiendorf, 2003).

A risk for contamination of the food chain may arise when heavy metals accumulate in plant tissues to concentrations above the admitted threshold concentration, which is considered to represent a threat to human or animals feeding on the same crops. Plants can tolerate high metal concentrations in substrates by excluding or controlling transport from the roots to the vegetative aerial parts, or by precipitating metals in the rhizosphere (avoidance), or by concentrating them at high concentrations in the tissues without negative effects (tolerance). The capacity of certain plant species to concentrate heavy metals within their tissues enhances the risk for contamination of food chain. Health risks for animals and humans derive from the fact that whilst they are less sensitive to heavy metal toxicity than plants, they are capable of concentrating heavy metals in certain

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Oxya chinensis (Orthopera: Acridoidae) belonging to Catantepinae, is one of the most common and widespread insects in Asia and is abundantly found in rice paddies, in sugar cane and other gramineous plants has long been known as a highly harmful pest to crops and forage plants. Therefore, this insect has been the subjects of numerous studies throughout the world. Because this organism causes extensive damage in an agriculturally important sector, it has received much more attention at different concentrations. In recent years, grasshoppers continue to be a major economic pest of breaks crops in China (Ren *et al.*, 2002; Han *et al.*, 2002). Organisms, like grasshoppers, occurring in such environments are also exposed to gradually increasing toxic metals and contribute to the accumulation and biotransfer of heavy metals (Devkota and Schmidt, 2000). Effects of heavy metals during the embryonic development of acridid grasshopper and the accumulation in wings, gut and ovaries had been reported (Schmidt and Ibrahim, 1994; Devkota and Schmid, 1999), but less information is known about the bioaccumulation in different part-tested in the experiment—grasshopper (*O. chinensis*).

In environmental point of view, extractable metal concentrations are vital since the assessment and forecast of heavy metals contamination in agriculturally harvested goods related to bioavailable portions of trace metals. It is more seriously that they can accumulate in animals and human beings through food chain. The present study is a part of a comprehensive program planned for environmental studies and assessments of trace and toxic elements on the ecosystem of the soil-plant-insect.

1 Materials and methods

1.1 Insect model, wheat seed and soil

O. chinensis, fourth-instar nymphs, was collected on July 2004 from Yuanping (113°4′ E, 38°40′ N), Shanxi Province, China, and kept in iron-wire cages (620 mm× 510 mm×400 mm) at room temperature.

Soil was obtained at a site from 0 to 30 cm depth from Taiyuan (112°36′ E, 37°46′ N), Shanxi Province, China. Some chemical and physical characteristics of the soil are as follows: pH: 8.15, organic matter: 3.54%, N: 0.088%, available P: 8.72 mg/kg and available K: 171 mg/kg. The soil was amended with different amount of $CdCl_2 \cdot 2.5H_2O$ and the concentrations of Cd in soils were expected to be 0, 20, 40, 60, 80, 100, 120 mg/kg, respectively. The concentration of Cu was 46.86 ±1.46 mg/kg (background value of the soil).

The wheat (*Triticum aestivum*) seeds were Jin-Tai-170, cultivated by Shanxi Academy of Agriculture Sciences.

1.2 Chronic experiment

Every 4.5 kg soil of each concentration was placed in a hollowware (355 mm × 275 mm ×70 mm). Eighty wheat seeds were planted in each soil hollowware at 4 rows with a row distance of 90 mm. All hollowwares were watered with tap-water(Cd and Cu concentration were not detectable). After 7 d, the two hollowwares of the same Cd treatment and 26 females and 26 males of grasshoppers were removed into per iron-wire cage(620 mm×510 mm×400 mm) covered with window screening at natural temperature. There was a plastic bottle in each cage to cycle some wheat seedlings as samples for heavy metals analysis. Each treatment was replicated 4 times. When the wheat seedlings were eaten out, another two hollowwares of the same concentration were put into each cage. During the period of the experiment, there were 6 times to add the wheat seedlings. After 30 d, the number of lively insects were recorded and the grasshoppers were stored at -80℃ refrigerator for further chemical analyses.

1.3 Sample collection

After each batch wheat seedlings were eaten out, the soil of each cage was mixed and about 1 kg was put aside for metal analyses. The soil samples were dried at room temperature. When the experiment finished, each cage soil of six batches was mixed and about 1 kg was the soil sample for subsequent heavy metal analyses.

The wheat seedling samples were washed with distilled water to remove the heavy metals attached on the surface and were dried in an oven at 70℃ for 24 h and stored in airer for subsequent heavy metal analyses.

1.4 Heavy metal analyses

When insects were analyzed, female grasshoppers were divided into four parts: head, thorax, abdomen, and hind femur. Grasshopper and plant were digested with HNO_3 and $HClO_4$, and the soil was digested with HCl-HNO_3 (3 ∶ 1) and $HClO_4$ until clearly dried. After this step, they were solved with certain amount of thrice distilled water and the soil samples were also filtered through filter paper into a 50-ml volumetric flask. The total metal concentrations were then determined by an atomic absorption spectrometer (Shimadzu AA-6300 Japan). Concentrations of heavy metals were calculated according to the standard curve, and they were expressed as mg/kg dw for the soil and plant, wet weight for grasshopper.

1.5 Statistical analysis

Data were statistically analyzed within experiment and the results expressed as mean ±*SE* of three and four independent replicates for Cd and Cu concentrations. All analyses were carried out with SPSS (11.5) or Excel 2003. One way ANOVA was used to compare the differences of different parts of insect at same treatment and student's *t*-test was used to compare the differences of the same part at the different treatment.

2 Results and discussion

2.1 Cadmium concentration in soil and Cu concentration in wheat seedling

The Cd of soil and Cd and Cu of wheat seedling were analyzed and shown in Table 1. The accumulation of Cd in plant increased as the Cd of soil increased. The maximum value was 50.70 mg/kg at treatment 7. One way ANOVA showed that there was a highly significant difference ($P<0.05$) in Cd concentrations for wheat seedlings. The accumulation of Cu in plant was not increased as the Cd of soil increased. One way ANOVA revealed that there was not any significant difference ($P>0.05$) in Cu concentrations of wheat seedlings.

Table 1 predicted that Cd and Cu could be accumulated in wheat seedlings(Mantovi *et al.*, 2003). The Cd concentration in wheat seedlings deepened on the Cd concentration in soil. It suggested that the increasing Cd concentration in soil induced Cd accumulation in wheat seedlings (Ren *et al.*, 2003). The wheat seedlings grew for about 10 d, but the Cd concentrations were greatly higher from treatment 2 to 7 than the control value, which showed that Cd was accumulated so fast in wheat seedlings that the Cd concentration arrived at a high amount in wheat seedlings in such a short period. So Cd was very dangerous to wheat seedlings (Song *et al.*, 2001, 2002). The similar trend had been found in other plants (Lagriffoul *et al.*, 1998). The Cu concentration in wheat seedling was about a constant value at Cd series, because the Cu concentration in soil was only a background concentration, which suggested that Cu concentration in wheat seedlings was not affected by increasing Cd concentration in soil.

2.2 Effects on Cd accumulation

Concentrations of Cd in different parts of grasshopper are shown in Table 2. It can be seen from Table 2: (1) Cd concentration in the head increased with the increasing Cd concentration both in soil and in plant at low Cd concentrations and decreased at high Cd concentrations, but all of them were higher compared with the control value. The highest concentration was 11.70 mg/kg at treatment 6. There was a significant difference ($P<0.05$) of Cd concentration in the head. (2) The accumulation of Cd in the thorax elevated firstly and then fell as Cd concentration in soil and in plant increased and they were all higher than that of the control value. The highest concentration was 18.59 mg/kg at treatment 5. There was a significant difference ($P<0.05$) of Cd concentration in the thorax. (3) The Cd concentration in the abdomen increased with the increasing of Cd in soil and in plant, but it was lower at treatment 6 than that at treatment 5 and 7. The Cd concentrations were higher compared with the control value with all treatments. The maximum accumulation was 9.61 mg/kg at treatment 7. There was a significant difference ($P<0.05$) in Cd concentration in the abdomen. (4) The Cd concentration in the hind femur increased at first six treatments and decreased at the last treatment with the increasing of Cd concentration and all of them were higher compared with the control value. The highest concentration was 0.76 mg/kg at treatment 6. There was a significant difference ($P<0.05$) of Cd concentration in the hind femur.

Table 1 Concentrations of Cd in soil and Cd and Cu in wheat seedling, mg/kg

Treatment	Cd(soil)	Cd(wheat seedling)	Cu(wheat seedling)
1	1.06±0.20	0.25±0.06^{a}	14.91±0.74^{ab}
2	23.69±0.08	24.57±1.46^{b}	12.61±0.51^{a}
3	43.82±0.86	36.67±1.94^{c}	14.22±0.73^{ab}
4	69.50±2.11	43.50±2.20^{d}	13.10±0.78^{ab}
5	81.19±1.32	43.63±1.71^{d}	12.78±0.71^{a}
6	110.50±0.70	48.71±2.19^{de}	13.07±0.73^{ab}
7	126.94±0.55	50.70±2.54^{e}	15.33±1.12^{b}

Notes: Each value of wheat seedling is the mean of three replicate±*SE* for Cd(soil), of twenty-four replicate ±*SE* for Cd(wheat seedling) and of twenty-three replicate±*SE* for Cu (wheat seedling); the same letter after data within a column represents no significant difference at 0.05 level

The effect of different Cd concentrations of soil on the Cd accumulation of grasshopper (Table 2) and one way ANOVA demonstrated that the accumulation of Cd in different part was great difference ($P<0.05$) at the same treatment. The order of the Cd accumulation in different part was thorax > abdomen > head > hind femur with all treatments (except for treatment 6), and at treatment 6 was thorax>head>abdomen>hind femur.

Cadmium concentrations in insect body in Table 2 demonstrate that increased concentrations of Cd in soil generally resulted in higher Cd concentration in grasshoppers, but the degree of increase in different parts varied. The Cd accumulation in the head varied with the increasing Cd both in soil and in plant and it was lower at treatment 7 than at treatment 6. The trend of the thorax was similar to the head and the Cd accumulations at treatments 6 and 7 were lower than that at treatment 5. The trend of Cd accumulation in the hind femur was similar to the head. The Cd accumulation was lower at treatment 7 than at treatment 6. The phenomena that Cd accumulation in the head, thorax and hind femur were decreased at higher Cd concentration in soil and in plant predicted that there were the largest capacity of Cd accumulation in head, thorax and hind femur, and after being exposed to Cd at high concentration for a l

Table 2 Concentrations of Cd in female insect body, mg/kg

Treatment	Head	Thorax	Abdomen	Hind femur
1	0.01 ± 0.01^{a}	0.05 ± 0.01^{b}	0.02 ± 0.00^{a}	0.00 ± 0.00^{a}
2	$1.74 \pm 0.12^{a***}$	$3.57 \pm 0.22^{b***}$	$2.51 \pm 0.29^{c**}$	$0.12 \pm 0.02^{d**}$
3	$2.54 \pm 0.76^{a*}$	$6.00 \pm 0.22^{b***}$	$3.31 \pm 0.19^{a***}$	$0.12 \pm 0.01^{c***}$
4	$4.01 \pm 0.38^{a***}$	$11.22 \pm 0.79^{b***}$	$4.19 \pm 0.13^{a***}$	0.32 ± 0.03^{c}
5	$6.75 \pm 0.79^{a**}$	$18.59 \pm 1.17^{b***}$	$9.43 \pm 1.08^{a**}$	$0.66 \pm 0.35^{c***}$
6	$11.70 \pm 1.72^{a**}$	$14.17 \pm 1.27^{b***}$	$6.48 \pm 1.05^{a**}$	$0.76 \pm 0.11^{c**}$
7	$8.96 \pm 0.85^{a***}$	$15.45 \pm 1.25^{a***}$	$9.61 \pm 1.56^{b**}$	$0.43 \pm 0.10^{c*}$

Notes: Each value of wheat seedling is the mean of three replicate ± *SE* for Cd; the same letter after data within a row represents no significant difference at 0.05 level; $*P<0.05$, $**P<0.01$, $***P<0.001$ within a column

Table 3 Concentrations of Cu in female insect body, mg/kg

Treatment	Head	Thorax	Abdomen	Hind femur
1	4.94 ± 0.41^{a}	14.13 ± 0.16^{b}	31.65 ± 0.78^{c}	2.07 ± 0.10^{d}
2	5.44 ± 0.05^{a}	$12.19 \pm 0.35^{b**}$	$28.49 \pm 0.94^{c*}$	2.01 ± 0.13^{d}
3	4.25 ± 0.27^{a}	13.47 ± 1.21^{b}	$25.42 \pm 1.35^{c**}$	$1.28 \pm 0.09^{d**}$
4	5.60 ± 0.43^{a}	$15.08 \pm 0.33^{b*}$	$26.85 \pm 1.12^{c*}$	2.05 ± 0.07^{d}
5	5.61 ± 0.50^{ac}	$15.71 \pm 0.42^{ab*}$	20.34 ± 6.94^{b}	$1.28 \pm 0.07^{c***}$
6	$7.68 \pm 0.59^{a**}$	12.61 ± 3.17^{a}	$27.76 \pm 0.42^{b**}$	$1.28 \pm 0.10^{c**}$
7	4.83 ± 0.41^{a}	15.78 ± 0.98^{b}	30.11 ± 2.01^{c}	$1.24 \pm 0.08^{d***}$

Notes: Each value of different part is the mean of four replicate ± *SE* for Cu; the same letter after data within a row represents no significant difference at 0.05 level; $* P<0.05$, $**P<0.01$, $***P<0.001$ within a column

ong time, the insects took some measures to protect themselves from the Cd harm (Cheng *et al.*, 2001). The trend of abdomen was not totally similar to other parts of grasshoppers. It was that Cd concentration in the abdomen was lower at treatment 6 than at treatment 7, which suggested that when the Cd concentration in soil and in wheat seedling increased, the defensive mechanism of the abdomen (as a barrier) might have been exhausted and more Cd were found at treatment 7. This phenomenon was similar to the report in other organism (Tam and Wong, 1997). The highest concentration of Cd in different parts was different at the same treatment suggested that Cd had various influences on different parts of insect at the same treatment. The order of Cd accumulation in different parts was thorax > abdomen > head > hind femur with all treatments (except for treatment 6). There are some important organs in thorax and abdomen, so the accumulation of Cd was higher than in other parts (Liu *et al.*, 2001a, b; Ren *et al.*, 2003; Zhou *et al.*, 2002).

2.3 Effects on Cu accumulation

The effects of Cd concentration on the Cu accumulation in the head, thorax, abdomen, and hind femur are demonstrated in Table 3. The Cu concentration in the head increased slightly as the increasing of Cd in soil and in plant increased, and the highest accumulation of Cu was 7.68 mg/kg at treatment 6. There was a significant difference ($P<0.05$) in Cu concentration in the head. The Cu concentration in the thorax increased as Cd concentration in soil and in plant increased, and the maximum value was 15.78 mg/kg at treatment 7. There was no significant difference ($P<0.05$) in Cu concentration in the thorax. The Cu concentration in the abdomen decreased slightly as the Cd concentration in soil and in plant increased and the highest accumulation was 31.65 mg/kg at the control. There was a significant difference ($P < 0.05$) in Cu concentration in the abdomen. The accumulation of Cu in the hind femur decreased greatly with the Cd concentration in soil and in plant increased and the maximum concentration was 2.07 mg/kg at the control. There was a significant difference ($P<0.05$) in Cu concentration in the hind femur.

Table 3 presents there were different Cu concentrations in different parts of insect body at the same Cd concentration in soil and in wheat seedling, which indicated that the different Cd concentration effects on the Cu accumulation of insects in different part. At each treatment, the accumulation of Cu in different parts was great significant difference ($P<0.01$), and the order of the intake Cu was abdomen > thorax > head > hind femur in Cd series. Result of one-way

ANOVA indicated that there were significant differences ($P<0.01$) of Cu concentration at the same Cd concentration with all treatments.

From the results shown in Table 3, it was not observed that there was a relationship between Cu accumulation in the four parts of grasshoppers and Cd concentration in soil and in plant. This reflected that Cu in insect body was not influenced markedly by the increasing Cd concentration in both soil and plant. The tendency of Cu accumulation in head and hind femur increased at lower Cd concentrations and decreased at higher Cd concentrations, which might be that the higher Cd concentration in plant inhibited the Cu accumulation in both head and hind femur. The Cu concentration in abdomen and hind femur decreased with increasing Cd in soil and in plant revealed that abdomen and hind femur were sensitive to Cu. Although Cu concentration kept a rough constant in each part of insect body at all cases, there was an interesting phenomena that the order of Cu accumulation was abdomen > thorax > head > hind femur. This predicated that the Cu binding capacity of abdomen exceeded other parts, and the Cu concentration in hind femur was the lowest among the four parts. There was gut in abdomen, which could accumulate more Cu than the other body parts, and this had been demonstrated in other species (Ruan *et al.*, 2001). The accumulation of Cu in different parts of insect body was lower than in soil, it resulted in (1) Cu accumulation in insects might be inhibited with the increased Cd in the plant; (2) Cu in the body might be lost from the insect via excreta or another unmeasured pathway (Chiou *et al.*, 1997); (3) the feeding time was not enough to accumulation more Cu in the body (Ge *et al.*, 2002); (4) Cu concentration in plant was lower and this was reflected in low Cu concentration in grasshopper.

The highest accumulation of Cd in insect body was the thorax, while the highest Cu accumulation was the abdomen. This revealed that Cd was easier taken up in the thorax and Cu was easier absorbed in the abdomen, because the thorax and abdomen are the center of body metabolism (Liu *et al.*, 2001a; Zhou *et al.*, 2002). The lowest concentration of both Cd and Cu was the hind femur in which muscle is the major component, and it demonstrated that the muscle was more difficult to accumulate Cd and Cu than other parts. Some researches have indicated that the muscle accumulated heavy metals less than other organs (Liu *et al.*, 2001a, b).

3 Conclusions

In this study, high concentrations of Cd used in the experiment are probably higher than those occurring naturally in or on vegetation within polluted areas. In this way, we treated *O. chinensis* as a model of animal to research the accumulation tendency of heavy metals (Cd and Cu) in the simulated soil-plant-insect ecosystem. The results predicted that Cd and Cu were accumulated in wheat seedlings and grasshoppers. Changed concentrations of Cd in soil and in wheat seedling and of Cu in wheat seedling resulted in various Cd and Cu concentration in different parts of grasshoppers in the ecosystem. From the results, it is suggested that the insect body can accumulate pollutants via food-chain from plant.

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