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RESEARCH ARTICLE

Influences of chromium and cadmium on the development of black soldier fly larvae

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Abstract The black soldier fly *Hermetia illucens* is a good candidate for waste management. The harvested insects are rich in protein and have the potential to be used in animal feed. However, people are wary about heavy metals in waste. Therefore, it is necessary to understand how the uptake of heavy metals could affect *H. illucens* and where and to what extent metals are accumulated by the black soldier fly. Based on these considerations, developmental parameters were investigated in the different life stages of *H. illucens* fed an increasing concentration gradient of cadmium (Cd) and chromium (Cr); additionally, Cd and Cr distribution in the body parts of *H. illucens* at the different life stages was monitored. We found that Cd and Cr have no effects on larvae survival and eclosion rate, but they do have effects on larvae duration and pupation rate. Both Cd and Cr were transferred into larvae, prepupae, and pupae. While the concentrations of Cd in larvae and prepupae were much higher than that in their diets, the opposite case was observed with Cr. The concentrations of Cd and Cr in *H. illucens* decreased in later development stages. In individual larva and prepupa, Cd and Cr were mainly included in the body and not in the integument. In the pupa, the puparium contained higher Cd and Cr concentrations than the pupa body. The distribution of Cd and Cr in the different life stages and body parts may present a potential strategy for how *H. illucens* tolerate and remove heavy metal stress.

Keywords *Hermetia illucens* · Cadmium · Chromium · Growth parameters · Metal distribution

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Introduction

Saprophagous insects can consume a wide range of organic materials and have the potential to be used in waste management (Nguyen et al. 2015a; Zhang et al. 2012b). The larva of saprophagous insects can reduce environmental contamination and transform waste into high-quality fertilizer (Green and Popa 2012). The harvested insects can be used for animal feed, which is a potential avenue to improve the sustainability of animal diets and meet the growing global demand for livestock products (Khusro et al. 2012; Van Huis 2013; Verbeke et al. 2015). A survey in Belgium showed that two thirds of the study participants were willing to accept the use of insects in animal feed, most notably for fish and poultry feed (Verbeke et al. 2015). Strikingly, *H. illucens* performs well as a candidate in manure management and biomass production. *H. illucens* are large and have larvae that can consume a wide range of organic material, ranging from fruits and vegetables to animal remains and manures (Nguyen et al. 2015b).

It was reported that *H. illucens* reduced poultry manure mass by 50% (Newton et al. 2005). A highly efficient conversion, it is possible to produce more than 180 kg of live weight of *H. illucens* larvae in 42 days from 1 m^2 manure, whereas only 30 kg of adult crickets can be produced under optimum rearing conditions, which demonstrates great potential for commercial production (Jozefiak et al. 2016). Prepupae of the black soldier fly contain 44% protein and 33% fat, which makes it a good additive and substitute of fishmeal, whose price ranges from USD 1600 to USD 1650 per ton (market value in 2015 for fishmeal, www.globefish.org) (Diener et al. 2009; Newton et al. 2005; St-Hilaire et al. 2007). In addition, it would not disturb people's lives with the spread of insect-borne diseases as black soldier flies do not feed as adults (Tomberlin et al. 2002). Moreover, Lalander et al. (2013) found a reduction in the concentration of some pathogenic microorganisms, such as *Salmonella* spp., in human feces treated by black soldier fly larvae.

With the rapid development of the poultry and livestock industry all over the world, the average production of manure is approximately 587 billion tons per year (Afazeli et al. 2014). Therefore, manure disposal is an urgent issue that needs to be solved. As introduced above, it is an opportunity to farm insects using animal manure. However, it is also a challenge in regard to the safety of insect feed and food production. Copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), and nickel (Ni) have been detected in poultry and livestock manure (Moral et al. 2008; Wang et al. 2013a; Xia et al. 2013), and some of them even exceed the China national heavy metal limitation in animal feed (GB13078-2001) and the EU threshold on undesirable substances in animal feed (EC 2002). For example, an investigation of manure samples from farms in northeast China estimated that the typical content of Cd in pig manure was 15.1 mg/kg dry manure. This is much higher than the allowed limits in China and the EU, which is 2 mg/kg dry material. The highest content of Cr in chicken manure was 2402.95 mg/kg dry manure, while the national limitation in China is 200 mg/kg dry material (Zhang et al. 2012a). Another study in an intensive animal production region of China reported that from 1990 to 2010, Cu, Zn, As, Cd, and Cr contents in manure increased considerably, especially in pig and poultry manure (Wang et al. 2014; Wang et al. 2013b). Cr and Cd can be easily found in animal manure because they are generally present in the animal diet. It is reported that chromium methionine is an important animal feed additive to improve animal immunity, while the overuse of the feed additive could cause mutagenic and genotoxic effects (Eastmond et al. 2008; Tian et al. 2014). Although Cd is not an animal feed additive, it is reported that Cd is often present in mineral supplements such as phosphates, zinc sulfate, and zinc oxide, which were used to supplement growth-necessary nutrient elements (Li et al. 2010). Moreover, Cd and Cr are usually poisonous to human and animals even at low concentrations, especially Cd (Li-na et al. 2013; Saha et al. 2011).

There are studies that indicate that heavy metal contents may affect insect development and growth. For example, elevated zinc concentrations in the diet caused high larvae mortality of the black soldier fly (Diener et al. 2011); in addition, 500 ppm Cd in a larvae diet caused a significant reduction in pupae and adults of *Drosophila melanogaster*, and when the count of Cd was increased to 1000 ppm, the survival percentage of adults declined to 15 and 0% (Al-Momani and Massadeh 2005). Cr in the diet significantly reduced the survival rate and increased the development time of *Megaselia scalaris* larvae (Trumble and Jensen 2004).

Using black soldier fly larvae is a novel management strategy with great potential in organic waste treatment where the harvested insects could use as animal feed. The sustainable regeneration of the insect population is the precondition to farm insects with animal manure, while the increasing content of heavy metals in animal manure brings a potential risk in farming insects with manure. The aim of this study was to investigate to what extent Cd and Cr could affect black soldier fly larvae and to evaluate the risk of excess heavy metal accumulated by harvested insects at the different life stages.

Materials and methods

Heavy metals

A cadmium-containing compound ($CdCl_2 \cdot 2.5H_2O$) and a chromium-containing compound (K_2CrO_4) were purchased from a reagent company (Sinopharm Chemical Reagent Co., Ltd). These compounds were dissolved in deionized water to selected concentrations for subsequent experiments.

Insects

Black soldier flies were obtained from a colony maintained year-round in the lab of Hubei International Cooperation Base for Waste Conversion by Insects, Huazhong Agricultural University. This colony was established in spring 2015 from the eggs of a colony at the Huadou Black Soldier Fly Farm, Guangdong Province, which is maintained year-round in an outdoor greenhouse. Wheat bran was used as insect feed for experiments to create a relatively standard feed condition as it is a standard feed in the lab. In accordance with Tomberlin et al. 2009, neonate larvae were fed with wheat bran moistened with deionized water (every 10 g of wheat bran was mixed with 20 ml water), were held at 27 ± 2 °C for 4 days to reduce mortality from handling, and then distributed to experimental treatments.

Experiments

Growth parameters of H. illucens fed with the dose gradient of heavy metals

The selected concentrations for the Cd study were 20, 40, and 80 mg/kg dry wheat bran, and for the Cr study, the concentrations were 100, 200, and 300 mg/kg dry wheat bran. The concentrations are within the range of metal concentrations that can be found in contaminated manure and commercial organic fertilizer. The larvae were kept in a cylindrical plastic container (10-cm diameter, 5 cm high) with a cloth covering fixed by a rubber band. The prepared diet for the larvae was wheat bran mixed with deionized water or heavy metal solution. Every 10 g of wheat bran was mixed with 20-ml solution. One hundred 4-day-old larvae were placed in a container described above as one replicate; four containers, each containing 100 larvae, were fed each particular concentration of Cd and Cr. Wheat bran mixed with deionized water without added Cd and Cr was fed to the control larvae. At the start, larvae were fed 20 g prepared diets. The addition of 20 g of new food contaminated with the selected concentration of metals was done every other day. Feeding stopped when over 40% of the larvae reached prepupal stage (as indicated by a darkening of the integument). Daily observations continued until all pupae entered the adult stage or died. The number of prepupae, pupae, and adults were recorded every day. All the prepupae were kept in the container until they reached the pupation stage and were then moved to a clear container without food for eclosion.

Heavy metal content and distribution in the different immature stages

A constant concentration of Cd and Cr were chosen to treat *H. illucens.* The final milligram quantities of Cd and Cr in 1 kg of dry wheat bran were 4.5 and 300, respectively. The experimental setup was as follows. Both metal treatments began with 500 4-day-old larvae in one container as a replicate; three containers containing 500 larvae each were used for each particular concentration of Cd and Cr to repeat the experiment three times. Initially, larvae were fed with 100 g prepared diets inoculated with Cd or Cr, and the addition of 100 g of new food inoculated with Cd or Cr was done every other day and terminated for each container when over 40% of the larvae in that container had reached the prepupal stage. Sixty larvae at 12 days old, 60 prepupae, and 60 pupae were randomly sampled for subsequent experiments.

All harvested insects were washed with deionized water, followed by 12 h of starvation. In half of the collected samples, the integument and leftover parts were dissected, collected separately, and dried at 105 °C. The other half of the collected samples were not dissected and were dried as whole insects at 105 °C. The samples were digested according to the China National Standard (Cd:GB/T 17141-1997; Cr:HJ-491-2009). Two-hundred milligrams dry samples were digested with 5 ml of nitric acid and 2 ml of perchloric acid using reflux cooler digestion equipment heating with an electric heating board at 200 °C. After the sample completely digested, the residue acid was evaporated at 100 °C. Finally, the clear solutions were diluted with deionized water to 50 ml, filtered with qualitative filter paper, and then analyzed with an atomic absorption spectrophotometer (240FS AA; Agilent Technologies, USA). The standard solution was received from Goubiao (Beijing) Testing and Certification Co., Ltd. (Cd: GSB 04-1721-2004; Cr: GBS 04-1723-2004(a)) and diluted by deionized water as the following concentrations: 0.01, 0.1, 1, 10, 100, and 1000 mg/l.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 19.0 software. Data regarding the development parameters (four replicates) were analyzed by ANOVA (confidence interval 0.05). The homogeneity of variance was tested using Levene's test before ANOVA, and the result was $p < 0.01$. Data regarding the distribution of Cd or Cr in the different stages and different parts (three replicates) were analyzed using *t* test (confidence interval 0.05).

Results

Growth parameters of *H. illucens* fed with the dose gradient of heavy metals

We investigated the effects of Cd and Cr contaminated diets on the growth and development of black soldier flies (Fig. 1). Most larvae developed successfully to prepupal stage (Fig. 1a, d). Cd- and Cr-contaminated diets showed an impact on pupation (Fig. 1b, e). The percentage of pupation was the lowest when the Cr concentration was 300 mg/kg (Fig. 1e). The emergence of pupae was not affected (Fig. 1c, f).

In the Cd treatments and the control, 20% of the larvae reached pupae stage at the 21st day of hatching and 50% of the larvae reached pupae stage at the 24th day of hatching (Fig. 2a). In the Cr treatments, 10% of the larvae reached pupae stage at the 21st day of hatching and 50% of the larvae reached pupae stage at the 26th day of hatching (Fig. 2b). The accumulated number of pupae in the Cd or Cr treatments was lower than that in the control (Fig. 2a, b). There was no significant difference among the different concentrations of the Cd treatment on the accumulated number of pupae (Fig. 2a, b). The concentrations of Cr affected the speed of the increase of accumulated number of pupae (Fig. 2b); higher

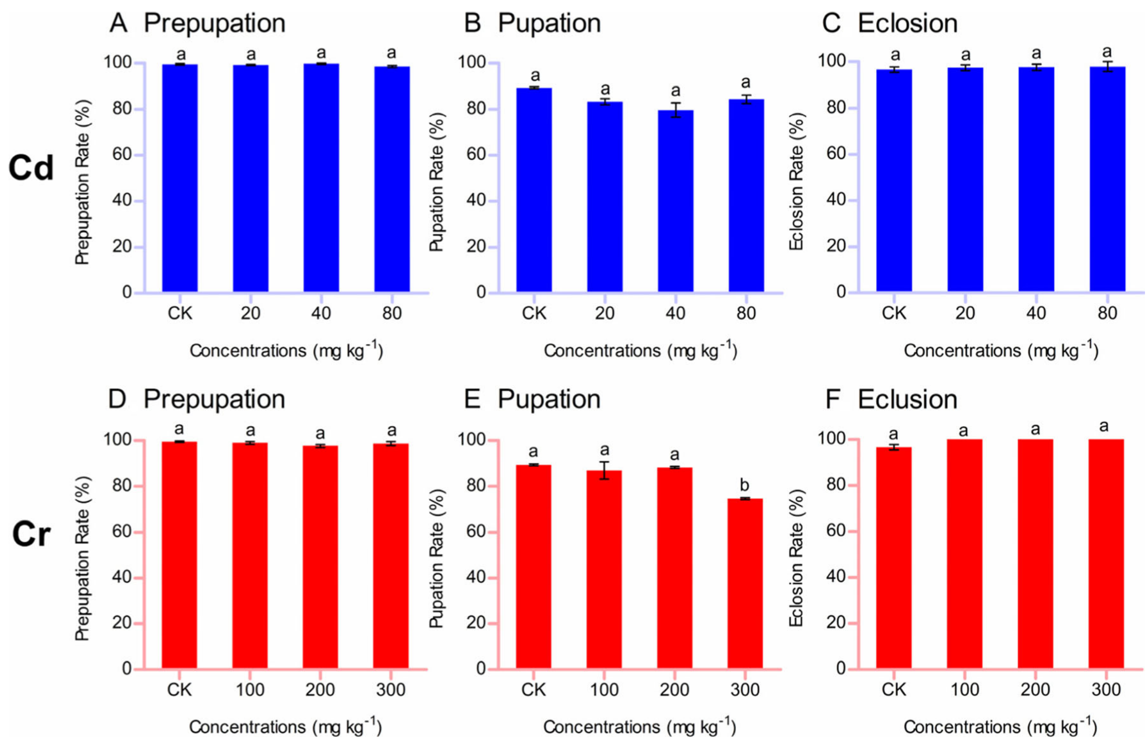

Fig. 1 Prepupation rate, pupation rate, and eclosion rate of *H. illucens* fed with dose gradient of heavy metals. Prepupation rate, pupation rate, and eclosion rate when fed on various concentration of Cd (**a**–**c**). Prepupation rate, pupation rate, and eclosion rate when fed on various concentration of Cr (**d**–**f**). *Columns marked by the same small letter* do not vary significantly ($P > 0.05$)

concentrations of Cr caused a slower increase of the accumulated number of pupae (Fig. 2b).

Heavy metal content and distribution in the different immature stages

The Cd and Cr contents in the 12-day-old larvae, prepupae, and pupae were tested. Cd and Cr in the diet could be transferred into the bodies of black soldier flies (Fig. 3). The concentrations of Cd in larvae and prepupae were 20.857 mg/kg (SE = 0.848) and 18.892 mg/kg (SE = 1.210), respectively, which are not significantly different from each other while significant higher than that in pupae (Fig. 3a). The concentrations of Cr in the three life stages demonstrated significant differences. The concentration in larvae was 191.886 mg/kg (SE = 16.284), which was much higher than that in prepupae (19.525 mg/kg, SE = 1.775) and pupa (1.196 mg/kg, SE = 0.069) (Fig. 3b). Both Cd and Cr show that the concentrations of the metals decreased with insect development (Fig. 3).

The bioaccumulation factor (BAF) which means the concentration of a pollutant in organisms divided by its concentration in the diet (Diener et al. 2015). The BAF of Cd in larvae (4.635) and prepupae (4.198) were greater than 1, while that of pupae (0.507) was much lower (Fig. 3a). All three life stages maintained lower Cr concentrations than that of the initially added concentration (Fig. 3b). The BAF of Cr in the three life stages range from 0.002 to 0.640 (Fig. 3b).

In an individual larva and prepupa, Cd or Cr was mainly found in the body rather than in the integument (Fig. 4a, b). No Cd was detected in the larval integument, and the pupa body did not contain Cr (Fig. 4a, b). In the pupa, the puparium contained higher Cd and Cr than the pupa body (Fig. 4a, b). Using Student's *t* test, the distribution of Cd in the integument and the other parts of a prepupa were significantly different ($P < 0.05$) (Fig. 4a). The content of Cd in the puparium was significantly higher than that in the body of a pupa ($P < 0.05$) (Fig. 4a). A significant higher content of Cr was found in the body rather than in the integument of larva or prepupa ($P < 0.01$) (Fig. 4b).

Discussion

Our results indicate that *H. illucens* shows tolerance to heavy metals at the examined levels. Under the given concentrations of Cd or Cr, most *H. illucens* larvae developed successfully to the prepupal stage (Fig. 3). Although Cd- and Cr-contaminated diets showed an impact on pupation rate, there were no significant effects on eclosion (Figs. 1 and 2). *H. illucens* shows a consistent trend in the delay of pupation, as has been seen with many other insects. Other insects show higher sensitivity to Cd and Cr, and the evidence comes from

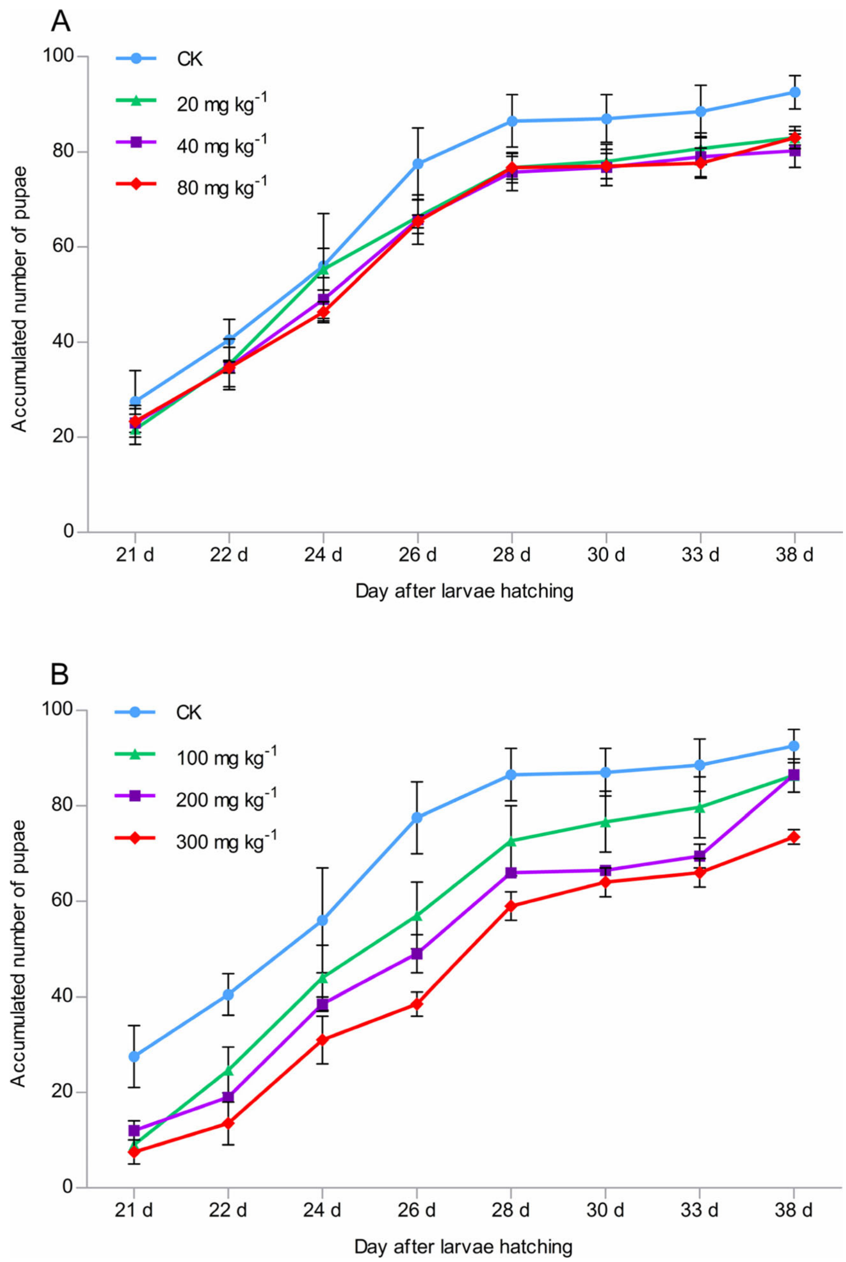

Fig. 2 Accumulated numbers of pupae change with the day after hatching. **a** Accumulated numbers of pupae when *H. illucens* treated with various concentrations of Cd. **b** Accumulated numbers of pupae when *H. illucens* treated with various concentrations of Cr

various studies. The increased concentration of Cd added in the diet led to a higher mortality and a longer duration of housefly larvae (Niu et al. 2000). At 500 ppm, Cd in a larvae diet caused a significant reduction in the pupae and adults of *D. melanogaster* (Al-Momani and Massadeh 2005). Additionally, Cr in the diet significantly reduced the survival rate and increased the development time of *Megaselia scalaris* larvae (Trumble and Jensen 2004). Exposure to a cadmium-contaminated diet (44 mg/kg dry diet) caused a higher mortality and a longer duration of *Spodoptera exigua* larvae (Kafel et al. 2012). A high concentration (100 mg/kg dry diet) of Cd in the diet caused 100% death of *Aiolopus thalassinus* adults (Schmidt et al. 1992). A possible reason for the delay of pupation is that heavy metals in the diet could affect the metabolism of carbohydrates, lipids, and proteins and cause a shortage of energy for development and growth (Tylko et al. 2005). In addition, the decrease of pupation may be a survival cost to alleviate the effects in the adults and the next generation. The effects of Cd and Cr on the pupation of the black soldier fly reminds people who want to use manure to farm insects that heavy metals in manure, such as Cd and Cr, need to be monitored.

Metals in insect food and the living environment can transfer into an insect. In this study, we found that Cd and Cr in the diet were transferred into the different life stages of *H. illucens* (Fig. 3). Many other studies provide consistent evidence of heavy metals transferring from food. For example, Diener et al. (2015) reported that Cd, Pb, and Cr could transfer from

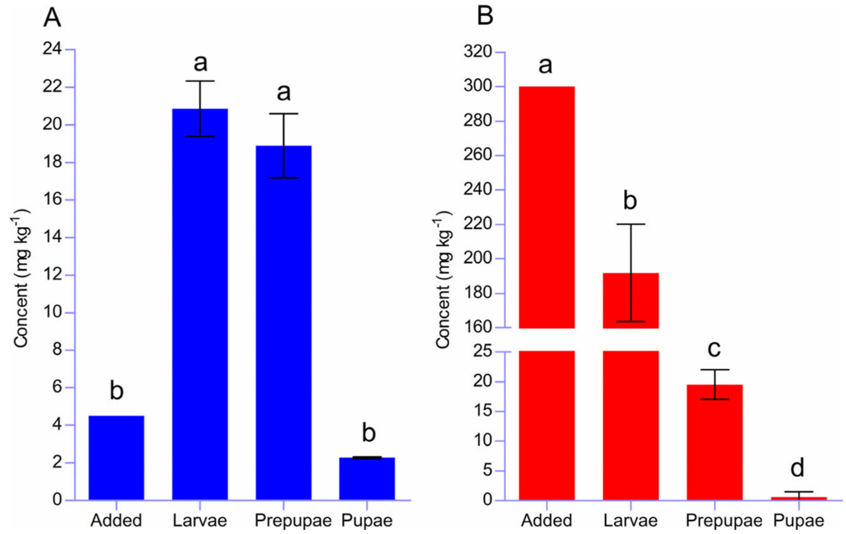

Fig. 3 Cd and Cr content in different stages of *H. illucens* when fed with the diet containing Cd (**a**) or Cr (**b**). *Columns with the same color marked by the same small letter* do not vary significantly ($P > 0.05$)

food into the black soldier fly; Cd and Pb could transfer from contaminated soil into detritivorous animals (Adeniyi et al. 2003); and increased concentrations of Zn in the host shoots could transfer to aphids and their predators, seven-spotted ladybirds (Green et al. 2010). The content of heavy metals in the larvae of the phantom midge (*Chaoborus punctipennis*), which lives in lakes contaminated by Cd and Se, increased significantly (Rosabal et al. 2014).

The black soldier fly showed different Cd and Cr accumulation abilities. Larvae and prepupae contained higher Cd in the body compared to the added concentration in the feed. The concentrations of Cr in larvae, prepupae, and pupae were lower than that in the added concentration in the feed. Our results were consistent with the Cd and Cr accumulation patterns of the housefly (Adeniyi et al. 2003). In addition, the Cd accumulation patterns coincided with the study of Diener et al. (2015), who reported that the BAF of Cd uptake by detritivorous insects averaged at 2.86. The high BAF may because Cd^{2+} can be easily absorbed by the cell through Ca^{2+} channels because it is similar to Ca^{2+} (Braeckman et al. 1999; Diener et al. 2015).

Based on the mass conservation law, the strong capability of black soldier larvae in accumulating Cd may give us a possibility to use black soldier fly larvae to remove Cd contained in its feed. According to our results, 1 kg of 12-day-old dry larvae (almost 16,666 individuals (unpublished date)) could contain 20.857 mg Cd; that means 1 kg of Cd contaminated (4.5 mg/kg dry feed) dry feed could be "purified" by 400 black soldier larvae in 12 days. Figure 4 shows that in the pupa stage, most of the Cd had been distributed in the puparium. This result was consistent with that reported by Diener et al. (2015). It is probable that the black

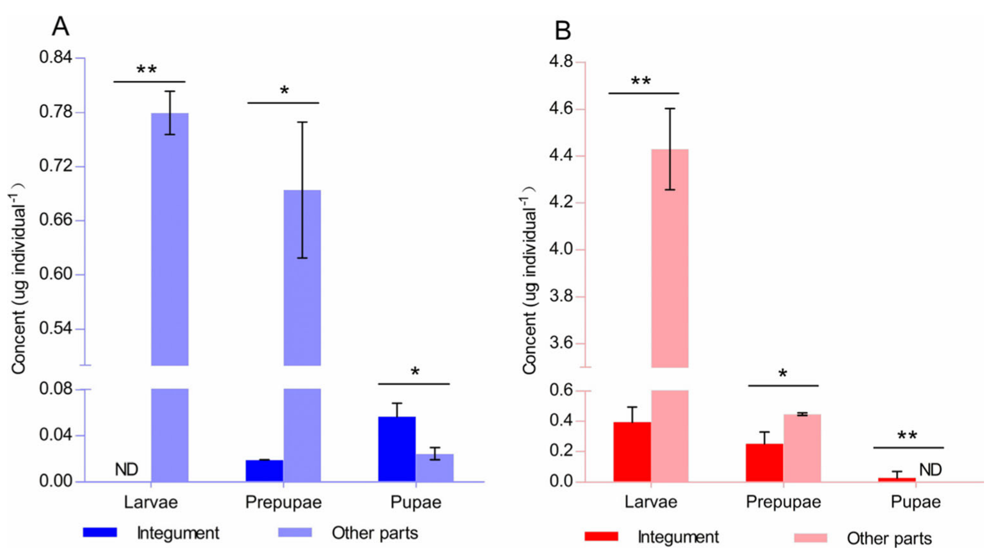

Fig. 4 Content of Cd (**a**) and Cr (**b**) in integument and other parts of *H. illucens* at different stages when fed with the diet containing Cd (4.5 mg/kg dry diet) or Cr (300 mg/kg dry diet). *Columns marked by single asterisk and double asterisk* represent the significance level of *t* test; $p < 0.05$ and $p < 0.01$, respectively

soldier fly could excrete the absorbed Cd by molting and metamorphosis, so we could potentially collect the pupae exuviae for Cd recycling.

Although heavy metals could be accumulated by the black soldier fly, they may have removal strategies. From our results, the amount of Cd in the pupae was significantly lower than that in the larvae and prepupae. The content of Cr in the prepupae and pupae was significantly lower than that in the larvae (Fig. 3). The decrease of heavy metals in the later stages of insects may be attributed to excretion by molting and metamorphosis, which may be an important strategy in insect tolerance to heavy metals. We also find similar evidence in other insects. In the fleshfly *Sarcophaga peregrina*, 53% of Cd accumulated in the pupae was excreted from the adults immediately after emergence (Aoki and Suzuki 1984). Cd was mainly accumulated in the larvae than in the pupae and adults of beet armyworm *S. exigua*, and the beet armyworm could decrease Cd content by means of prepupae exuviate and puparium (Su et al. 2014). High Pb concentrations were detected from the larvae exuviate and the puparium of *S. exigua* (Hu et al. 2014).

To evaluate whether the harvest insect which accumulated metals could be used as animal fed, we compared our results with national (China, EU) regulations for heavy metal in food and feed. The EU threshold for Cd is 2 mg/kg, and the China threshold for Cr is 200 mg/kg. We evaluated the metal risk of harvested insects that fed on the contaminated feed (Cd, 4.5 mg/kg dry feed; Cr, 300 mg/kg dry feed). The concentrations of Cd in larvae and prepupae exceeded the threshold; the concentration of Cd in pupa was under the threshold; and the concentrations of Cr in larvae, prepupae, and pupae were under the threshold. Based on these results, we suggested the later development stage for harvest.

Conclusion

In conclusion, Cd and Cr in the feed could affect the pupation rate but not larvae survival and the eclosion of *H. illucens.* Both Cd and Cr could be transferred into different immature stages. The concentration of examined metals in *H. illucens* decreased at later development stages. Cd and Cr were mainly accumulated in the body but not in the integument of an individual larva and prepupa, while pupa showed the opposite results. The distribution of Cd and Cr in the different life stages and body parts may show a potential strategy of *H. illucens* to tolerate and remove heavy metal stress. According to the results, we recommend later life stages for harvest, which have a lower metal risk.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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