Journal of Insects as Food and Feed, 2015; 1(4): 261-270

Wageningen Academic
Publishers

Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle

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Received: 25 December 2014 / Accepted: 22 May 2015

RESEARCH ARTICLE

Abstract

In developing countries, effective waste management strategies are constrained by high collection costs and lack of adequate treatment and disposal options. The organic fraction in particular, which accounts for more than 50% of the waste production, constitutes a great, yet mostly neglected, reuse potential. Concomitantly, the demand for alternative protein sources by the livestock feed industry is sharply increasing. A technology that effectively transforms organic waste into valuable feed is therefore a timely option. Larvae of the non-pest black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), may be used to reduce the mass of organic waste significantly. Concurrently, larval feeding converts organic waste into prepupae (last larval stage) which is high in protein. In combination with a viable market, this potential animal feed may cover the waste collection costs and thus promote innovative, small-scale entrepreneurs to establish a profitable business niche. Organic waste, however, often contains persistent pollutants, such as heavy metals, that may accumulate in the larvae and prepupae of black soldier flies and consequently in the food chain. In this study, we fed black soldier fly larvae chicken feed spiked with heavy metals (cadmium, lead and zinc at three concentrations each) to examine the extent of metal accumulation in the different life stages and the effect of heavy metal concentration in the feed on the life cycle determinants of the flies. The cadmium accumulation factor in prepupae (metal concentration in the body divided by metal concentration in the food) ranged between 2.32 and 2.94; however, the lead concentration remained well below its initial concentration in the feed. The bioaccumulation factor of zinc in prepupae decreased with increasing zinc concentration in the feed (from 0.97 to 0.39). None of the three heavy metal elements had significant effects on the life cycle determinants (prepupal weight, development time, sex ratio).

Keywords: bioaccumulation, developing countries, food security, organic waste management, protein

1. Introduction

Urban poverty is a fundamental challenge in low and middle-income countries associated with rapid urban sprawl (Moore *et al.*, 2003). The urban poor suffer most from inadequate sanitary services and deficient municipal solid waste management leading to increased health risks and impaired household resilience. While informal collection and recycling systems of inorganic material with a market value are currently available, the organic waste fraction often remains uncollected and untreated. Indiscriminately dumped organic waste accumulates along streets, clogs stormwater drains, pollutes water bodies, rots, and attracts disease-transmitting vectors (e.g. flies, rodents), thus posing serious direct or indirect health risks to local residents. Local authorities, community-based organisations, non-governmental organisations and research institutions have recognised this deficiency and identified the need for simple, environmentally and economically sustainable organic waste treatment solutions in urban areas (Fluitman, 2000; Zurbrügg *et al.*, 2007).

In many low and middle-income countries, the mass of organic waste may be substantially reduced using larvae of the non-pest black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) (Diener *et al.*, 2009). *H. illucens* larvae feed voraciously on decaying organic leftovers from markets and restaurants, animal droppings and on human faeces. Myers *et al.* (2008) and Sheppard *et al.* (1994) reported a 33-58% reduction in organic matter from cow manure and 50% from chicken manure respectively and Diener *et al.* (2011) reached a dry matter reduction of 70% in municipal organic waste.

The final larval instar of *H. illucens* is called the prepupa and consists of 32-44% raw protein and 33-35% crude fat (Booram *et al.*, 1977; Diener *et al.*, 2009; St-Hilaire *et al.*, 2007). Hence, larval feeding converts organic waste into a highly valuable protein that may be used as a substitute for fishmeal. The increase in aquaculture led to a growing demand for feed for aquatic organisms and therefore to increasing prices (Riddick, 2014). Fishmeal is becoming less available and the production and the sale of insect protein can thus contribute to cover the waste collection costs as well as allow innovative, small-scale entrepreneurs to establish a profitable business niche.

However, organic waste often contains persistent pollutants such as heavy metals that may accumulate in larvae and prepupae and therefore enter the food chain. The heavy metals enter the waste stream in various ways, be it through atmospheric emissions or inappropriate disposal of heavy metal containing refuse. While terrestrial organisms ingest contaminants orally (biomagnification), aquatic organisms also enrich pollutants in their biomass through diffusion (bioconcentration). Bioaccumulation refers to both bioconcentration and biomagnification (Walker, 1990). The bioaccumulation factor (BAF) thus is the concentration of a pollutant in organisms divided by its concentration in the diet.

A stable black soldier fly population generating viable eggs and producing healthy offspring are prerequisites for running a sustainable organic waste treatment facility using black soldier flies. However, heavy metals in organic waste may influence life history traits. For example copper- and lead-contaminated host plants negatively affected fecundity and intrinsic rate of natural increase (r_m) in the cabbage aphid, *Brevicoryne brassicae* L. (Görür, 2006). Reduced bodyweight in the offspring of the carabid beetle, *Pterostichus oblongopunctatus*, inhabiting a metal-polluted environment has been observed by Lagisz and Laskowski (2008) and Moroń *et al.* (2014) found a clear relation between increasing metal concentrations in the soil layer and the increased negative impact on life cycle determinants (e.g. population growth rate, number of brood cells, survival rate) for wild bees, *Osmia rufa*.

In this study, the larvae of the black soldier fly, *H. illucens*, were fed with chicken feed contaminated by different levels of cadmium, lead and zinc to investigate the following research questions:

- To what extent do cadmium, lead and zinc – fed at different concentrations – accumulate in the prepupae of the black soldier fly?
- Does heavy metal in the food influence the life cycle determinants of the flies? (i.e. development time, body weight, sex ratio)?

2. Materials and methods

Animals

Black soldier flies, *H. illucens* L. (Diptera: Stratiomyidae), were obtained from a laboratory colony grown in an indoor cage (1.5 m × 1.5 m × 2.0 m) at constant temperature (26.5±0.05 °C, 60.8±0.8% RH). The room was fitted with two windows as direct sunlight is crucial for successful mating (Tomberlin and Sheppard, 2002).

The newly hatched larvae used for the experiments were reared on chicken feed (UFA 625, digestible energy: 11.7 MJ/kg, 60% moisture). A detailed description of the rearing and hatching facility is given in Diener *et al.* (2009).

The experiments conducted in Switzerland did not violate Swiss law (e.g. Animal Protection Law, Animal Husbandry Act) or any of the provisions or regulations stipulated in these laws. The experiments also met the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences (CIOMS, 1985).

Experimental setup

Larvae were fed with chicken feed pellets moistened (final moisture level: 60%) with either pure deionised water (control) or a solution of deionised water containing heavy metal ions (three concentration levels for each metal). The 2% HNO_3 solutions used for feedstock preparation contained cadmium (1000 mg/kg), lead (1000 mg/kg) or zinc (10,000 mg/kg). The nominal concentrations in the food were: 0.0 μg/g (control), 2.0, 10.0, and 50.0 μg/g Cd; 0.0 μg/g (control), 5.0, 25.0, and 125.0 μg/g Pb; 0.0 μg/g (control), 100, 500, and 2,000 μg/g Zn. Low concentrations corresponded to the metal concentrations typical for market vegetables in India or Bangladesh (Alam *et al.*, 2003; Marshall *et al.*, 2003; Sharma *et al.*, 2007). Middle concentrations for cadmium and lead corresponded to concentrations typical for organic waste in Bangladesh or Sweden (Eklind *et al.*, 1997; Rytz, 2001) (Table 1).

Metal concentrations in the control groups were derived from the chicken feed itself. Unfortunately, the experiment series with the low concentrations of zinc were contaminated and the results had to be discarded. However, as the chicken feed itself had similar concentrations of zinc (145.3 mg/kg, standard error (SE) = 10.1) as what was used

Table 1. Heavy metal concentration in municipal solid waste and vegetables (based on dry weight) compared to the legal maximum threshold level allowed in animal feed, human food and compost.[1]

	Cadmium (mg/kg)	Lead (mg/kg)	Zinc (mg/kg)	Reference
Heavy metal concentration in municipal solid waste				
Sweden	0.16-0.6	2.4-26	49-165	Eklind *et al.*, 1997
Dhaka, Bangladesh	5.0	n/a	226	Rytz, 2001
Heavy metal concentration in vegetables				
Garden vegetables, rural village, Bangladesh	0.05-0.4	0.2-1.7	11-54	Alam *et al.*, 2003
Market vegetables, Delhi, India	1.0-5.5	0.3-2.2	41-150	Marshall, 2003
Field vegetables, Varanasi, India	0.5-4.3	3-16	3-41	Sharma *et al.*, 2007
Heavy metal limits in animal feed				
European Union	2	10	n/a	EC, 2002
Heavy metal limits in human food				
European Union	0.05-1.0	0.02-1.0	n/a	EC, 2001
India	0.1-1.5	0.2-10	5-100	Government of India, 1954
Heavy metal limits in compost from household waste				
European Union	0.7	45	200	EC, 1991
Proposed standard in LMIC	3	150	300	Hoornweg *et al.*, 1999

[1] LMIC = low and middle-income countries; n/a = not applicable.

in the experiment with low concentrations (177.4 mg/kg, SE=3.8) we were able to utilise the control series (only chicken feed and its respective zinc concentration) as indication of results for the low zinc concentration series. Thus the control series could be considered as the low zinc concentration series and was compared to the medium and high zinc series.

Each replicate (three replicates per treatment) contained 200 7-day old larvae placed in plastic containers (14.0 × 7.5 × 7.0 cm) and covered with nylon tulle held in place by the lid of the box. The lids with nine holes (ø 15 mm) allowed air circulation. The food and larvae were covered with a re-sealable polyethylene bag containing a piece of black cardboard to shield larvae from light. The pre-prepared meal portions were packed into separate polyethylene bags and kept frozen until use. The quantity of the diet was calculated based on 100 mg food (wet weight) per larva and day. The larvae were fed three times a week. Feeding stopped when 50% of the larvae in the box metamorphosed into prepupae to avoid overfeeding the remaining larvae.

Sampling and analysis

The samples (larvae, larval exuviae, prepupae, pupal exuviae, and adults) were washed with deionised water, weighed, lyophilised to measure dry weight, and ground in an agate mortar for heavy metal analysis. The food samples and the remains at the end of the experiments, the so-called residue, were treated the same way except for the washing. Larvae and prepupae were killed by freezing (-10 °C), while adults were killed with ethyl acetate. To prepare the samples for the analyses, ~50 mg of the ground material was digested in polytetrafluoroethylene beakers (HPR-300/10; MLS GmbH, Leutkirch im Allgäu, Germany). The material was moistened with deionised water. Approximately 4 ml HNO_3 and 1 ml H_2O_2 were added before the sample was heated in a laboratory microwave digester (MLS 1200 MEGA; MLS GmbH). The clear solution was diluted with deionised water (10× for Cd and 100× for Pb and Zn) and analysed with the high resolution inductively coupled plasma-mass spectrometer (HR-ICP-MS, Element II; Thermo Fisher Scientific, Waltham, MA, USA). The standard solutions were made using Merck ICP multi-element standard solution IV (Merck Millipore, Darmstadt, Germany): 10, 100, 1000, 5,000 and 10,000 ng/l. The natural river water standard SLRS-4 and the TM-28.3 trace elements fortified calibration standard (National Research Council Canada, Ottawa, Canada) were used as a control. The detection limit for these elements was 10 ng/l.

BAF was calculated according to Walker (1990) as:

$$\text{BAF} = \frac{\text{concentration in organism } (C_i)}{\text{concentration in food and/or water ingested } (C_o)} \quad (1)$$

In the present case, C_o consisted solely of the heavy metal concentration in the food.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL, USA). For some data, a violation of the Levene homogeneity of variances was calculated. However, as the groups are equal in size, ANOVA is very robust to this violation.

3. Results

Heavy metal accumulation

In all development stages (larvae, prepupae, and adults), the metal concentration generally increased significantly with increasing metal concentration in the food (Table 2-5). However, BAF, i.e. the ratio of the amount of metal in the body compared to that in the food varied among the different metal elements and development stages (Table 6). In prepupae, the BAF ranged from 2.32 to 2.94 for cadmium, independent of the concentration in the food, while the BAF remained <1 (0.25-0.74) for lead. In adults, the BAF was very low for both cadmium and lead concentrations (BAF=0.12-0.21). For zinc, the BAF decreased with increasing concentration in the food (prepupae: from 0.97 to 0.39; adults: from 0.98 to 0.19). The EU threshold value for cadmium (2 mg/kg) in animal feed was exceeded in prepupae even at low cadmium concentration (7.9 mg/kg, SE=0.6). Only prepupae from the low lead concentration group (1.5 mg/kg, SE=0.7) met the EU concentration limit for lead (10 mg/kg) in animal feed (EC, 2002).

Table 2. Cadmium concentration in soldier flies, *Hermetia illucens*, at different life stages based on dry weight, in digested material (residue) and in food source. Larvae fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with cadmium (three different concentrations).[1,2]

	Control		Low cadmium		Medium cadmium		High cadmium	
	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE
Food	0.2 a	0.02	2.7 b	0.2	13.3 b	0.7	61.5 b	2.3
Residue	0.2 a	0.01	2.9 b	0.1	16.0 bc	0.4	89.8 c	2.1
Larvae	0.2 a	0.02	7.0 d	0.3	32.5 d	0.6	170.5 d	8.5
Prepupae	n.d.	–	7.9 d	0.6	36.2 d	1.9	142.9 e	8.3
Adults	n.d.	–	0.6 a	0.04	1.9 a	0.2	7.8 a	0.4
Larval exuviae	0.1 a	0.03	2.2 ab	0.3	18.8 bc	4.1	54.2 b	3.7
Pupal exuviae	0.5 b	0.1	5.2 c	0.7	22.9 c	2.6	94.1 c	12.1

[1] Mean values followed by the same small letter in the same column do not vary significantly ($P>0.05$).

[2] SE = standard error; n.d. = not detected.

Table 3. Lead concentration in soldier flies, *Hermetia illucens*, at different life stages based on dry weight, in the digested material (residue) and in food source. Larvae fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with lead at three different concentrations.[1,2]

	Control		Low lead		Medium lead		High lead	
	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE
Food	1.1 ab	0.4	5.9 bc	0.3	34.3 c	1.8	142.9 b	2.9
Residue	0.1 a	0.01	7.8 cd	0.6	53.2 d	3.3	267.9 c	12.8
Larvae	n.d.	–	3.8 ab	0.4	22.8 b	1.6	141.7 b	17.2
Prepupae	n.d.	–	1.5 a	0.7	25.3 bc	1.9	40.1 ab	3.7
Adults	n.d.	–	n.d.	–	5.9 a	0.57	17.3 a	1.36
Larval exuviae	5.9 c	1.3	11.3 e	0.01	87.7 e	4.8	312.9 c	74.1
Pupal exuviae	3.7 bc	0.1	9.3 de	1.0	24.2 bc	2.1	66.7 ab	3.6

[1] Mean values followed by the same small letter in the same column do not vary significantly ($P>0.05$).

[2] SE = standard error; n.d. = not detected.

Table 4. Zinc concentration in soldier flies, *Hermetia illucens*, at different life stages based on dry weight, in the digested material (residue) and in food source. Larvae fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with zinc at three different concentrations. Samples from the series 'Low zinc' were contaminated during the experiment and could not be used for interpretation.[1,2]

	Control		Low zinc		Medium zinc		High zinc	
	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE
Food	145.3 b	10.1	177.4	3.8	616 b	37.8	2,044 c	16.2
Residue	192.3 b	13.1	n/a	–	1,196 c	66.7	3,313 d	240.9
Larvae	165.8 b	19.9	n/a	–	596 b	88.0	866 b	141.9
Prepupae	138.9 b	24.3	n/a	–	513 ab	44.5	801 ab	32.1
Adults	141.4 b	9.2	n/a	–	272 ab	22.8	389 ab	37.6
Larval exuviae	275.5 c	13.5	n/a	–	1,514 c	240.2	1,883 c	104.8
Pupal exuviae	35.1 a	4.1	n/a	–	145 a	39.2	334 a	56.5

[1] Mean values followed by the same small letter in the same column do not vary significantly ($P>0.05$).

[2] SE = standard error; n/a = not applicable.

Table 5. Kendall's tau rank correlation (r) between the heavy metal concentration in food and the concentration values in larvae, prepupae, larval exuviae, and adults of the black soldier fly, *Hermetia illucens*.

	Larvae			Prepupae			Larval exuviae			Adults		
	r	P	N	r	P	N	r	P	N	r	P	N
Cadmium	0.778*	0.004	12	0.833*	0.002	9	0.722*	0.007	9	0.741*	0.000	18
Lead	0.778*	0.004	9	0.833*	0.002	9	0.778*	0.004	9	0.671*	0.001	17
Zinc	0.667*	0.012	9	0.611*	0.022	9	0.833*	0.002	9	0.647*	0.000	18

* $P<0.05$

Effects of heavy metals on life cycle determinants

Prepupae treated with cadmium were significantly heavier than the control group. No significant effects were found in prepupae treated with lead and zinc (Table 7). Development time from hatching of the larva to the prepupal stage generally increased with heavy metal concentration although the increase was statistically insignificant (Table 8). Average development time until pupation amounted to 15.2 days (SE=0.1) and did not differ significantly between treatments or sexes. Heavy metals had no influence on the sex ratio of adults (average males/females ratio: 0.98, SE=0.02).

4. Discussion

The black soldier fly, *H. illucens*, fed with cadmium, lead and zinc, exhibits different accumulation patterns. Larvae and prepupae accumulated cadmium, yet the incorporation of lead and zinc was suppressed as concentrations found in the body were lower than in the food. These findings are consistent with literature data (Figure 1). In the literature reports, the BAF of cadmium uptake by detritivorous insects averages 2.86 (SE=0.30, range 0.46-6.09) (Gintenreiter *et al.*, 1993; Kazimirova and Ortel, 2000; Kramarz, 1999; Lindqvist, 1992; Maryanski *et al.*, 2002; Ortel, 1995). Cellular cadmium uptake probably occurs through Ca^{2+} channels. Due to their very similar ionic radii, Cd^{2+} ions can easily enter the cell via Ca^{2+} channels, independent of endocytosis or an ATP requiring ion pump (Braeckman *et al.*, 1999). Moreover, Braeckman *et al.* (1999) found a protein of the HSP70-family induced by elevated cadmium concentrations in the environment of *Aedes albopictus* (Diptera: Culicidae) cells. Production of this protein, which protects other proteins from denaturation, may also explain the low effect of contaminated food on life-cycle parameters such as development time or fluctuating asymmetry despite the observed bioaccumulation of cadmium (cf. present study).

Table 6. Bioaccumulation factor (BAF) for larvae, prepupae and adults of the black soldier fly, *Hermetia illucens*, fed heavy metal contaminated food at three different concentrations (low, medium and high; Table 2, Table 3 and Table 4). BAF for 'low concentration, zinc' was calculated using data from the control group (see explanation in text). Because the concentrations of cadmium and lead in the control group were so low, the analytical error had the effect of providing inaccurate BAFs, and are therefore not shown here.[1]

	Low		**Medium**		**High**	
	BAF	**SE**	**BAF**	**SE**	**BAF**	**SE**
Cadmium						
Larvae	2.65	0.10	2.46	0.11	2.79	0.24
Larval exuviae	0.86	0.19	1.41	0.31	0.88	0.06
Prepupae	2.94	0.09	2.75	0.25	2.32	0.09
Adults	0.21	0.01	0.15	0.01	0.13	0.01
Lead						
Larvae	0.66	0.09	0.67	0.07	0.99	0.10
Larval exuviae	1.9	0.11	2.56	0.01	2.21	0.56
Prepupae	0.25	0.12	0.74	0.03	0.28	0.03
Adults	n/a	–	0.17	0.01	0.12	0.01
Zinc						
Larvae	1.14	0.09	0.97	0.14	0.42	0.07
Larval exuviae	1.92	0.19	2.45	0.32	0.92	0.04
Prepupae	0.97	0.20	0.84	0.09	0.39	0.01
Adults	0.98	0.08	0.45	0.04	0.19	0.02

[1] SE = standard error; n/a = not applicable.

Table 7. Prepupal dry weight of *Hermetia illucens* fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with three different heavy metals at different concentrations (low, medium and high).[1,2]

	Control		**Low**		**Medium**		**High**	
	Mean (mg)	**SE**	**Mean (mg)**	**SE**	**Mean (mg)**	**SE**	**Mean (mg)**	**SE**
Cadmium	55.9a	2.3	96.2c	10.0	75.3b	2.0	83.6bc	3.3
Lead	55.9ab	2.3	61.5b	6.8	51.3a	2.1	59.1ab	0.6
Zinc	55.9a	2.3	n/a	–	64.8a	5.7	59.2a	4.8

[1] Mean values followed by the same small letter in the same row do not vary significantly ($P>0.05$).

[2] SE = standard error; n/a = not applicable.

Table 8. Effects of heavy metal concentration (low, medium and high) in food on development time (eclosion from egg to prepupa) of *Hermetia illucens* larvae.[1,2]

	Control		**Low**		**Medium**		**High**	
	Mean (days)	**SE**	**Mean (days)**	**SE**	**Mean (days)**	**SE**	**Mean (days)**	**SE**
Cadmium	18.4ab	0.5	18.0a	0.5	18.8ab	0.4	19.3b	0.6
Lead	18.4a	0.5	18.8ab	0.3	19.4b	0.4	20.7c	0.2
Zinc	18.4a	0.5	n/a	–	18.9a	0.5	20.1b	0.6

[1] Mean values followed by the same small letter in the same row do not vary significantly ($P>0.05$).

[2] SE = standard error; n/a = not applicable.

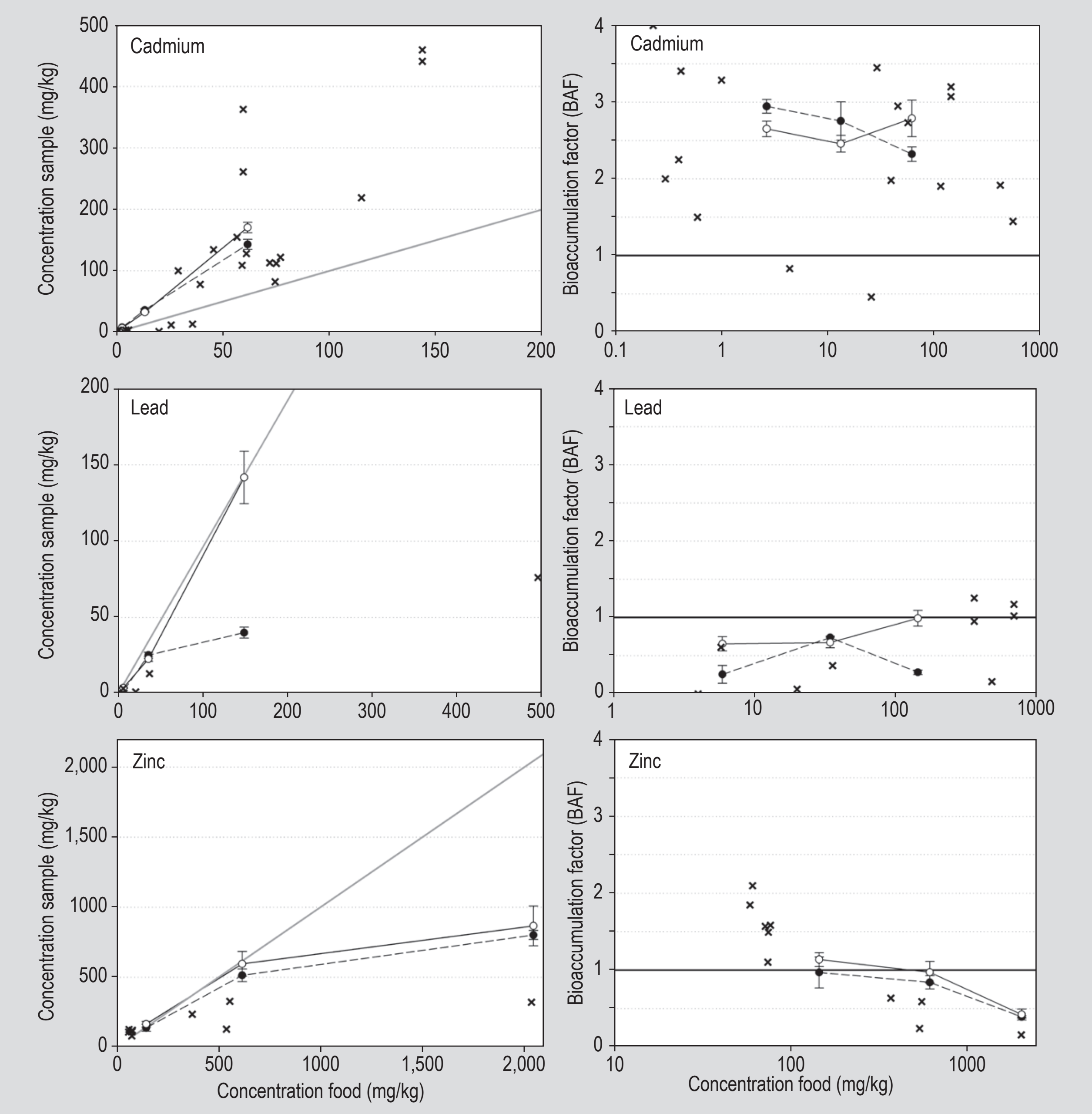

Figure 1. Concentration and bioaccumulation factor (BAF) of heavy metals in black soldier fly larvae (○) and prepupae (●), which were fed with heavy metal spiked food (current study). Crosses show data from literature which originates from similar studies about heavy metal concentrations in various insect larvae (Cd: Kazimirova and Ortel, 2000; Kramarz, 1999; Lindqvist, 1992; Maryanski *et al.*, 2002; Ortel, 1995; Pb: Gintenreiter *et al.*, 1993; Kazimirova and Ortel, 2000; Ortel, 1995; Zn: Kramarz, 1999; Maryanski *et al.*, 2002). 1:1 line shown for reference. Missing BAF values are attributed to undetectable concentrations in control groups.

In contrast to cadmium, the BAF for zinc decreased with increasing zinc concentration in the food, which suggests active regulation of zinc within the body (Table 6). Similarly, larvae of the house fly, fed with zinc-contaminated food (from 61 to >7,000 mg/kg) accumulated zinc only up to a maximum level of 216 mg/kg (Kramarz, 1999; Maryanski *et al.*, 2002). Even though the mean zinc concentration in the literature data for *Musca domestica* is lower than that found in prepupae of *H. illucens* of the present study (484 mg/kg, SE=97.5), it is possible that the two organisms possess a similar regulation mechanism.

Active regulation of zinc in insects has been described previously (Lindqvist, 1995; Mason *et al.*, 1983). Zinc is an essential, yet potentially toxic element. Therefore, it is not surprising that its intracellular uptake is actively regulated. Especially the metal-responsive-element-binding transcription factor-1 (MTF-1) is a key regulator in higher eukaryotic cells. It is responsible for the activation of several genes involved in intracellular zinc sequestration and transport (Laity and Andrews, 2007).

Though larvae and prepupae contained low lead concentrations in the present study, the larval exuviae accumulated lead. Lead tends to be stored in granular, metal-containing structures of the cells before it is transported to and immobilised in the exoskeleton (Hare, 1992). Similar to terrestrial insects, lead is most likely disposed of during moulting (Roberts and Johnson, 1978).

Heavy metal concentration in adults was significantly lower than in prepupae. We assume that this phenomenon occurs mainly because animals defecate before pupation or shortly after adult emergence. Yet Sheppard *et al.* (1994) reported without supporting data that prepupae had an empty gut when migrating. Conversely, Aoki and Suzuki (1984) describe an over 50% loss of the larva's cadmium content due to defecation in newly emerged flesh flies within the first two days following emergence. In the present study, prepupae were collected 1-3 days after transformation. We assume that defecation had not occurred during this period, and cadmium was removed during the later prepupal phase, i.e. during pupation, or after emergence. Therefore, toxic substances and pathogens present in the waste may remain in the gut of the harvested prepupae and in this way may be taken up by fishes or poultry fed with the prepupae. Future studies have to determine the period between initiation of the last larval instar (prepupa) and defecation, including the potential loss of feedstuff energy due to such a protraction.

The effective elimination of heavy metals by defecation has been described for larvae of the social paper wasp *Polistes dominulus* (Hymenoptera: Vespidae) (Urbini *et al.*, 2006). However, even if heavy metals accumulate in the cells lining the alimentary canal, they may be rejected after a short time. For example, *Tenebrio molitor* (Coleoptera: Tenebrionidae) discards cells of the midgut epithelium after four days (Lindqvist and Block, 1995; Thomas and Gouranto, 1973). Heavy metal accumulated in these cells will therefore be rejected with defecation.

5. Conclusions

Our studies reveal that the concentrations of lead and zinc in larvae or prepupae remain below the initial amounts in the food. Furthermore, the three heavy metal elements examined had only minor effects on the development of the black soldier fly even at very high concentrations. Yet, since cadmium accumulated in the prepupae, it could potentially limit the use of prepupae in the production of animal feed. In the case of lead and zinc, concerns about the use of prepupae in animal feed are less critical. The waste treatment technology using black soldier flies may contribute to reducing the burden of an animal protein shortage in the animal feed market and provide new income opportunities for small entrepreneurs in low and middle-income countries.

Acknowledgements

We wish to express our thanks to Simone Blaser and Mauro Esposito for assisting during the experiments, David Kistler for supporting us in lab analysis and Alejandra Teresita Arroyo for her statistical advice. We appreciate the comments by Janet Hering on an early version of the manuscript, and are grateful to Sylvie Peter for linguistic editing support. The authors would like to acknowledge the financial support by the Velux Foundation, Eawag: the Swiss Federal Institute of Aquatic Science and Technology, the Swiss National Centre of Competence in Research (NCCR North South) and the Swiss Agency for Development and Cooperation (SDC).

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