Absence of cadmium excretion and high assimilation result in cadmium biomagnification in a wolf spider

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Abstract

Cd biomagnification in the terrestrial food chain appears to be dependent on the physiological properties of the organisms rather than on their trophic level. Although high Cd body burdens in spiders from the field have been reported many times, experimental verification of the key factors that determine the rate of cadmium accumulation is lacking. We investigated the cadmium assimilation rate in the common wolf spider Pirata piraticus fed with contaminated fruit flies. Spiders were fed for 42 days with contaminated flies, followed by a detoxification period of 28 days. Every 14 days, a subsample of spiders and flies was taken for Cd determination. It was demonstrated that a high cadmium assimilation (69.5%) and an excretion rate approaching zero resulted in high Cd concentration factors. The results indicate the importance of spiders in cadmium biomagnification along critical pathways.

Keywords: Wolf spiders; Assimilation; Accumulation; Cd; Terrestrial food chain; Biomagnification

1. Introduction

Biomagnification of heavy metals, being the increase in the concentration of a metal in an organism compared to its food, is not a widespread phenomenon in terrestrial food chains (reviewed in Janssen et al., 1993). Comparison of metal concentrations among different trophic levels often revealed concentration factors of 1 when different taxonomic groups were pooled (Laskowski, 1991). This is mainly caused by the high variability in metal concentration between the taxonomic groups within a trophic level. An important mechanism, explaining this variation in biomagnification within a trophic level, is the difference in physiology among the taxonomic groups (Van Straalen and Van Wensem, 1986; Janssen et al., 1991; Gräff et al., 1997). Some organisms, such as isopods and snails, accumulate heavy metals in their hepatopancreas (e.g., Wieser, 1961; Berger and Dallinger, 1989; Hopkin, 1989; Dallinger, 1993), while other taxonomic groups, like insects (Van Straalen et al., 1987; Lindqvist and Block, 1994, 1995; Lindqvist et al. 1995), are able to excrete the toxic substances, resulting in intermediate to low metal body burdens. Van Straalen and Ernst (1991) noted that on a species-to-species basis, biomagnification might however occur along specific trophic chains. The identification of those critical pathways is therefore important in the determination of which species might be endangered.

The main physiological processes explaining the variation in metal body burden are the rate of excretion and to a lesser extent the assimilation efficiency (Janssen et al., 1993). If the rate of metal excretion in an organism is very low, high equilibrium concentrations might be attained that might even reflect the amount of metals assimilated during the entire lifetime of the organism.

Ecotoxicological field research has revealed that spiders can attain very high Cd concentrations near contaminated sites (e.g., Carter, 1983; Hunter et al., 1987; Larsen, 1994; Rabitsch, 1995; Maelfait, 1996; Wilczek and Migula, 1996; Duffey, 1997; Maelfait and Hendrickx, 1998) and in unpolluted areas as well (Knutti et al., 1988). Comparison of bioaccumulation data for different terrestrial invertebrates revealed that for a given soil concentration, spiders were among the highest Cd accumulators of all investigated taxonomic
groups (Heikens et al., 2001). As spiders might account for up to 75% of the prey for small mammals and birds (Hunter et al., 1987; Naef-Daenzer et al., 2000), their role in Cd transfer along the food chain could be important.

Despite these high metal body burdens in spiders, the experimental quantification of Cd assimilation and excretion in spiders has been investigated in only a few cases. While Cd elimination was extremely low for the excretion in spiders has been investigated in only a few experimental quantification of Cd assimilation and important.

This article reports assimilation and excretion rates of Cd in the wolf spider Pirata piraticus. Like all other members of the wolf spider family, this species is a generalist predator that is active on the bottom surface (Bristowe, 1939; Wise, 1993), and therefore it comes in close contact with (contaminated) soil. Former studies have demonstrated that this species is very abundant along the contaminated banks of the river Schelde (Hendrickx et al., 1998), and high metal body burdens have been detected (Maelfait and Hendrickx, 1998; Tojal et al., 2002).

2. Material and methods

2.1. Origin of the animals and rearing conditions

Ten large juvenile individuals of P. piraticus were sampled in November 1998 from a tidal marsh near the river Schelde (Belgium). As these spiders were already contaminated with Cd from the field (22.5 μg g⁻¹; N = 5; SD 2.63), they could not be used for the experiment. To obtain uncontaminated spiders to investigate the accumulation pattern their offspring were used. Spiders sampled from the field were therefore fed uncontaminated fruit flies until adulthood. Feeding was carried out individually in Petri dishes (⌀ 8.5 cm) with a layer of plaster of Paris on the bottom. The dishes were maintained at 100% humidity. All spiders were kept in an incubator (L:D 16:8) at 22°C. Ten days after reaching adulthood, each female was mated with a different male. After a few days, females produced an egg cocoon from which juvenile spiders were released after a few days. From each mother (N = 4), between 15 and 34 juvenile spiders were placed separately in Petri dishes, resulting in a total of 82 juveniles. During the first two immature instars, juveniles were fed Isotoma viridis collembolans that were collected with an aspirator from an uncontaminated old grass lawn in the botanical garden of the University of Gent (Belgium). Afterward, they were fed uncontaminated fruit flies until they were 12 weeks old.

At the start of the accumulation experiment, at day 1, 15 spiders were killed and stored in a freezer to determine the initial Cd concentrations present in the animals. The remaining spiders were fed with fruit flies, reared on a medium containing 10 μg g⁻¹ Cd, which was added as CdCl₂. After 14, 28, and 42 days, respectively, 14, 15, and 14 spiders were used for heavy metal determination. The remaining 24 individuals were fed uncontaminated food. At Days 56 and 70, respectively, 9 and 15 spiders were removed and tested for heavy metal determination. All spiders received seven flies every 3 days, and dead but unconsumed flies were counted and removed from the dish. This enabled determination of the number of flies consumed per spider. During the course of the experiment, spiders moulted several times and most of them reached adulthood after Day 28.

2.2. Metal analysis

All spiders were analyzed individually. Before digestion, spiders were dried for 48 h at 70°C and weighed on a Mettler Toledo AT 21 Comparator analytical balance to the nearest 0.01 mg. They were then transferred to porcelain crucibles that had been washed with an ultrapure 65% HNO₃ solution and rinsed with deionized water. The dry ashing method was used as a destruction method in which the samples were first preashed for 15 min at 200°C and for 20 min at 300°C, and subsequently ashed for 3 h at 450°C. The crucibles were then transferred to a hot plate (150°C), 5 mL of 6 mol L⁻¹ HNO₃ was added and the mixture was evaporated to 1 mL. After adding another 5 mL of 3 mol L⁻¹ HNO₃, the solution was filtered (S&S, blue ribbon) and diluted to 10 mL with a 1 mol L⁻¹ HNO₃ solution. Cd was analyzed by graphite furnace atomic absorption (SpectrAA-100, Varian, Paco-Alto, CA), equipped with Zeeman background correction.

At the end of each 7 days the spiders were fed contaminated fruit flies and two pooled samples of 15 flies were taken from the culture, resulting in a total of 10 samples, to analyze the Cd content of the fruit flies. The method of analysis was identical to that for the spiders.

3. Results

3.1. Cd content in fruit flies

The dry weight of the contaminated fruit flies averaged 0.34 mg (N = 10; SD 0.0367). Their Cd body burden averaged 0.0186 μg (N = 10; SD 0.00527), which corresponds to an average Cd concentration of
3.2. Spider Cd content

Cd content and Cd concentration of the spiders at the start of the experiment (0.03 µg, SD 0.018; 6.2 µg g⁻¹, SD 2.92, respectively) was negligibly low compared to the amounts of Cd for the rest of the experiment. The low contamination probably comes from the collembolans sampled at the botanical garden. The Cd content of *P. piraticus* increased significantly during the first 42 days of the experiment \((r = 0.836; P < 0.0001)\), indicating strong accumulation in the spiders (Fig. 1). From Day 42 until the end of the experiment, no significant decrease in Cd could be noted \((r = 0.266; P = 0.11)\) thus Cd elimination was not detected after 1 month of feeding with uncontaminated flies (Fig. 1).

The differences in total Cd body burden within each group that was removed from the experiment were dependent on the number of contaminated fruit flies eaten \((r = 0.426; P = 0.0001)\). Differences between the groups that were removed from the experiment disappeared when the number of contaminated flies consumed by each spider was used as a covariate \((\text{ANCOVA}; \text{covariate} = \text{number of flies ingested}; F_{(5,75)} = 2.18; P > 0.05)\), indicating an absence in Cd elimination during the experiment, even when they were fed uncontaminated food for 28 days.

To estimate the amount of Cd assimilated by the spider, spider Cd content was regressed on the total Cd content to which they were exposed, obtained by multiplying the Cd content of the fruit flies by the total amount of Cd for the rest of the experiment. The slope of the regression indicates that 69.5% \((\text{SE} \ 4.44)\) of the Cd was assimilated by the spiders (Fig. 2).

Regarding the total amount of Cd, Cd concentration also increased significantly with the number of fruit flies ingested \((r = 0.798; P < 0.0001)\) \((\text{Fig. 3})\). As expected, the biomagnification factor, being the ratio between the concentration(160,858),(807,915)

4. Discussion

The present study demonstrates that for the wolf spider *P. piraticus*, no Cd excretion could be detected after being fed with uncontaminated flies for 28 days. After 14, 28, and 42 days of feeding with contaminated fruit flies, spider Cd content was dependent only on the number of fruit flies consumed and not on the duration of the period after which the spiders were removed from the experiment. This was confirmed by the absence of a significant difference in Cd content between the spiders removed after the different time intervals when the number of flies consumed was taken into account. Results thus indicate that Cd excretion could not be detected during the entire time course of the experiment, i.e., 70 days. The rate of excretion, expressed as the amount of Cd excreted per day, therefore approaches zero.

As calculated from the total amount of metal exposed to the spiders, 69.5% of the Cd content of the fruit flies was present in the spiders. In the absence of excretion, this fraction is an unbiased estimate of the assimilation efficiency \((\text{Janssen et al., 1991}; \text{Dallinger, 1993})\).

This large amount of assimilated Cd is probably the result of the typical feeding behavior of spiders. Spiders are liquid feeders that suck out the inner part of their prey. For Diptera, it has been demonstrated that most metals are present in the soft tissue \((\text{Hopkin, 1989})\), so that spiders are exposed to higher metal concentrations than expected from whole body concentrations. A second mechanism explaining the high assimilation rate is the pronounced assimilation efficiency of the midgut diverticulae \((\text{Foelix, 1996})\), which is also the most important storage organ of metals in spiders \((\text{Ludwig and Alberti, 1988}; \text{Hopkin et al., 1989})\) For *D. crocata*, Cd is stored in the digestive cells of the midgut diverticulae in type B granulae \((\text{Hopkin et al., 1989})\). The excretion of Cd observed in this spider due to breakdown of the digestive cells, with release of metals into the lumen of the midgut diverticulae \((\text{Hopkin et al., 1989})\), does not seem to occur in *P. piraticus*. Van Hook
and Yates (1975) observed a similar pattern of extremely slow Cd elimination in wolf spiders. Other metals such as zinc (Breymeyer and Odum, 1969), iron (Lee et al., 1978), and cesium (Nabholz and Crossley, 1978) are also excreted very slowly by spiders.

The very low rate of Cd excretion observed in this study ranks this spider, together with snails, isopods, earthworms, and pseudoscorpions (Hopkin, 1989; Janssen et al., 1991; Dallinger, 1993; Gräff et al., 1997) as one of the most profound Cd accumulators in the terrestrial invertebrate fauna. Combined with this is high assimilation efficiency, as revealed in the literature, summarized by Janssen et al. (1991). Both processes are responsible for the observed degree of biomagnification in the artificial food chain. The results obtained in the current experiment confirm physiological hypotheses explaining the high Cd concentrations in spiders compared to insect predators in field studies (Van

![Fig. 2. The relationship between the cumulative Cd content in fruit flies (µg) and the Cd content in P. piraticus (µg). Solid line represents the observed assimilation for P. piraticus; dashed line represents the expected amount of Cd in P. piraticus if all Cd had been assimilated. Standard error of the mean value of each point is 0.09 times its average value.](image1)

![Fig. 3. Relationship between Cd concentration in P. piraticus (µg g⁻¹) and number of flies consumed. Arrow indicates average Cd concentration in fruit flies.](image2)
References


