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Dynamics of Accumulation and Decontamination of Cadmium and Zinc in Carnivorous Invertebrates. 2. The Centipede *Lithobius mutabilis* Koch

P. Kramarz

Institute of Environmental Sciences, Department of Ecotoxicology, Jagiellonian University, Ingardena 6, PL-30-060, Kraków, Poland

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Metals, being non-degradable, tend to accumulate in animals and hence long-term exposure even to low concentrations of metals may lead to harmful effects. From the physiological point of view, invertebrates may avoid metal poisoning either by retaining metals in inactivate forms, intracellular compartmentalisation or by discharging assimilated metals (Hopkin, 1989). Animals which represent the first strategy are able to concentrate elevated amounts of metals and are regarded as "macroconcentrators" (Dallinger, 1993). The second strategy is exhibited by animals possessing low concentrations of heavy metals, and these are termed “deconcentrators”. Consequently, numerous authors have reported that soil invertebrates belonging to the same trophic level exposed to the same environmental concentrations of metals often display different internal concentrations of metals and strategies for decontamination (e.g., Van Straalen and Van Wensem, 1986; Grodziska et al., 1987; Laskowski and Maryanski, 1993). In the trophic level containing predators, “macroconcentrators” are represented by spiders, “deconcentrators” by carabid beetles, whilst centipedes are regarded as being intermediate between these two extremes. Thus, an investigation comparing these three taxonomic groups in terms of kinetics of metals would be especially interesting. In the previous paper I have investigated the accumulation and decontamination dynamics of Zn and Cd in a carabid beetle *Poecilus cupreus* L. and showed that carabids exposed to elevated concentrations of Zn in food were able to maintain internal Zn concentrations at a constant level (Kramarz 1999). Although Cd concentrations increased in beetles fed Cd-contaminated food, its excess was quickly and efficiently excreted when switched to uncontaminated food. Additionally, zinc and cadmium did not affect each others uptake by *P. cupreus* (Kramarz, 1999). In this paper I report the results of the experiment on kinetics of the same metals, Zn and Cd in a centipede *Lithobius mutabilis* Koch.

The metals were chosen on the basis their different physiological function. Cadmium is regarded as xenobiotic, whilst zinc plays an important role in physiological processes (Nieboer and Richardson, 1980). Both Cd and Zn are members of the group IIB metals and together with copper, mercury and silver are bound by metal-binding proteins (metallothioneins) (Peraza et al., 1998). Cadmium has a higher affinity for metallothioneins and may displace zinc. At the same time, Zn (and Cu) addition are supposed to induce metallothionein synthesis, which allow an organism to combat the

Cd injury (Peraza et al., 1998). On that base, cadmium interactions with zinc are known for several organisms (Hemelraad et al., 1987; Migula et al., 1989; Wicklund et al., 1990). Furthermore, in a centipede, *Lithobius variegatus*, it was shown that both cadmium and zinc were stored mainly in the same tissue, the midgut (Hopkin and Martin, 1984) and zinc also in sub-cuticular tissues.

MATERIALS AND METHODS

Centipedes were captured in a beech-pine forest of the Ratanica catchment, located approximately 40 km south of Krakow, Poland. Samples of the whole organic layer were sieved through a l-cm mesh sieve and the centipedes *(Lithobius mutabilis* Koch.) were collected from the sieved material manually after transportation to laboratory. Prior to the experiment, the centipedes were acclimated for at least 2 weeks to laboratory conditions. During this time, the centipedes were held in 30x50x20 cm transparent plastic containers, approximately 100-200 individuals in each, kept at the temperature 20°C±0.5°C, relative humidity ≥80%, and a 1ight:dark regime of 16:8 hours. The bottoms of the container-was covered with a layer of moist natural soil 2-3 cm thick covered by a 2-3 cm layer of leaf-litter on the surface. The animals were fed with frozen housefly larvae *(Musca domestica* L.), taken from laboratory cultures

For studies, adult centipedes of 15-45 mg fresh mass (10-l5 mm length) were selected from the culture. Single animals were kept in 8.5 cm diameter plastic boxes. A piece of filter paper (ca. 25 cm^2) was put into each box as a shelter and to maintain humidity. The centipedes were fed with three frozen housefly larvae per individual every third day.

During the accumulation period, which lasted 90 days, the centipedes were fed with housefly larvae reared on one of four different kinds of artificial medium (metal concentrations given on a dry-weight basis): (1) contaminated with 500 mg Zn kg^{-1} (Zn treatment), (2) 50 mg Cd kg^{-1} (Cd treatment), (3) mixture of 500 mg Zn kg^{-1} with 50 mg Cd kg^{-1} (ZnCd treatment) and (4) uncontaminated (K control). During the decontamination period, which lasted 31 days the centipedes were fed with uncontaminated larvae. The medium for the housefly larvae was made of 515.6 g rabbit chow, 20 g powdered milk, 10 g sugar, 0.02 g baker's yeast and 11 of distilled water or experimental solution. Three randomly chosen centipedes were sacrificed at 2, 4, 6 and 13 days and weekly afterwards counting from the beginning of the accumulation and the decontamination period. Before killing and refrigerating, each animal was kept in a clean box for one day to empty of the gut contents.

Individual centipedes and samples of the housefly larvae and their diet were digested in boiling HNO_3. Zinc concentrations were measured by flame atomic absorption spectrometry (AAS), cadmium concentration by a graphite furnace AAS.

Table 1. Parameters estimated (±asymptotic SE) for the one-compartment model fitted to the data obtained for Cd accumulation and decontamination dynamics in *Lithobius mutabilis.* Significance levels, p, were assessed on the basis of asymptotic confidence intervals for the estimated parameters. The differences between the parameters obtained for Cd and ZnCd treatments were assessed using a t-test. The models are given in the previous paper (Kramarz 1999).

Parameter	Cd treatment		ZnCd treatment		Differences between treatments
C_0 (mg kg^{-1})	1.73±0.584	$p<0.001$	0.92±0.210	$p<0.001$	n.s.
a (mg day^{-1})	0.25±0.043	$p<0.001$	0.19±0.017	$p<0.001$	n.s.
k_a (day^{-1})	0.01±0.005	n.s.	0.01±0.003	$p<0.001$	n.s.
C_{90} (mg kg^{-1})	16.88±0.727	$p<0.001$	10.64±0.364	$p<0.001$	$p<0.0001$
k_e (day^{-1})	0.17±0.017	$p<0.001$	0.04±0.003	$p<0.001$	$p<0.0001$

The kinetics of metals in the centipedes metamatically were descibed and statistically analyses performed as detailed in the previous paper (Kramarz 1999).

RESULTS AND DISCUSSION

In the case of Cd treatments, the models used fitted well to the data (Fig. 1) and the parameters estimated for both phases were statistically significant except k_a in the Cd treatment (Tab. 1). During the accumulation phase the concentrations of Cd in centipedes treated only with cadmium exhibited an almost linear increase with time (Fig. 1). This, together with the nonsignificant k_a in the centipedes from Cd the treatment, suggest that no excretion of cadmium during the accumulation period. Similar patterns of cadmium dynamics were found in a pseudoscorpion *Neobiscum muscorum* and in oribatid mite *Platynothurus peltifer* by Janssen (1991) and for an earthworm *Eisenia fetida* (Spurgeon and Hopkin, 1999). The increase in cadmium concentration during the accumulation period was almost linear in animals treated with the mixture of Cd and Zn (Fig. I), but at the same time the excretion rate (k_a) was significant (Tab. 1).

Furthermore, the body concentration of cadmium at the end of the accumulation period was significantly lower for ZnCd than for the Cd treatment in terms of the estimated regression parameter (Tab. 1) as well as the measured concentration ($p<0.001$, Tab. 2). These differences can be explained by differences in concentrations of cadmium in the food of centipedes, housefly larvae. Cadmium concentration was significantly lower (p<0.00l, Tab. 2) in larvae reared on diet contaminated with the mixture of Cd and Zn than with Cd alone. On the other hand, these results may indicate an antagonistic influence of zinc addition on cadmium uptake. A similar effect of zinc on cadmium assimilation has been detected also in the fresh water clam, *Anodonata cygnea* (Hemelraad et al., 1987),

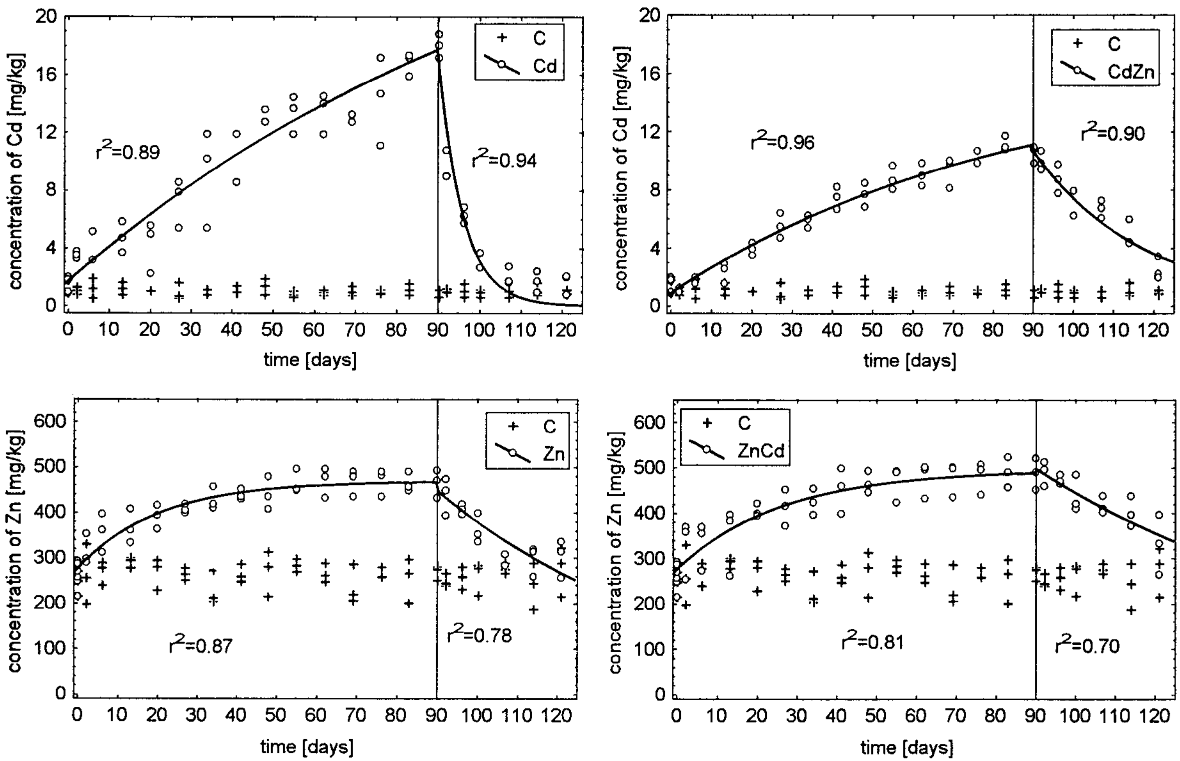

Figure 1. Kinetics of cadmium (upper plots) and zinc (lower plots) in the centipede *Lithobius mutabilis*. Thick lines indicate the models fitted. Thin lines indicated the time of switching to uncontaminated food. Symbols: C - control animals, Zn - centipedes treated with zinc, Cd - centipedes treated with cadmium, ZnCd - centipedes treated with a mixture of zinc and cadmium.

Table 2. Concentrations of Cd in mg kg^{-1} dry weight (m&SE) in housefly larvae diet (n=21), larvae (n=21) and centipedes at the beginning of the experiment (day 0) (n= 10), at the end of the accumulation period (day 90) (n=3) and at the end of the experiment (day 121) (n=3).

Treatment	Diet	Larvae	Centipedes		
			day 0	day 90	day 121
K	0.13±0.02	<0.001	1.38±0.465	0.79±0.322	0.93±0.169
Zn	0.37±0.09	0.76±0.14	1.38±0.465	1.20 ±0.410	1.45±0.576
Cd	56.58±3.94	154.6±2.19	1.38±0.465	18.0±0.790	1.59±0.587
ZnCd	45.59±5.68	134.4±1.36	1.38±0.465	10.50±0.620	2.54±0.610

the cricket, *Acheta domesticus* (Migula et al., 1989), and the zebrafish, *Brachydyanio rerio* (Wicklund et al., 1990). It should also be stressed that cadmium concentrations in centipedes from both treatments did not reach an equilibrium level during the accumulation period. Contrary to my study, Descamps et al. (1996) reported that in centipede *L. forficatus* fed with Cd-contaminated, food cadmium concentration increased linearly but showed a tendency to stabilise afterwards and even to decrease. But his study concerns centipedes from Cd-polluted sites, and as it was show by Hopkin and Martin (1984) centipedes from polluted environments exhibit lower rates of Cd assimilation that those from unpolluted sites.

During the decontamination period, the excretion rate (k_e) of cadmium was significantly lower in the centipedes treated with the mixture of metals than in those treated with cadmium alone (Tab. 1). Despite the higher C_{90} for the Cd than the ZnCd treatment, concentrations of cadmium at the end of decontamination period in both

Cd treatments were comparable and approached the control level (Tab. 2). On the other hand, in the case of the Cd treatment initial concentration was achieved much faster - about two weeks after switching to unpolluted food, whilst in the ZnCd treatment -just at the end of decontamination period (Fig. 1). This may suggest that in *L. mutabilis* cadmium was removed more effectively when the food is not contaminated with zinc.

As in the case of Cd, the kinetics of Zn in the centipedes was well described by the model used (Fig. 1) and all parameters were statistically significant (Tab. 3). During the accumulation period zinc concentration increased slowly and after about 50 days reached an equilibrium concentration in both treatments. This fact may suggest that in centipedes from sites contaminated with Zn, its concentrations should be similar to the *C90* volume obtained in this study , that is c.a. 450 - 490mg kg^{-1}. This is in contrast to previous report. Hopkin and Martin (1984) and Rabitsch (1995) reported concentrations of zinc in centipedes approximately two times higher than C_{90}. There are some possible explanations of these differences between

Table 3. Parameters estimated (±asymptotic SE) for the one-compartment model fitted to the data obtained for Zn accumulation and decontamination dynamics in *Lithobius mutabilis.* Significance levels, p, were assessed on the basis of asymptotic confidence intervals for the estimated parameters. The differences between the parameters obtained for Zn and ZnCd treatments were assessed using a t-test. The models are given in the previous paper (Kramarz 1999).

Parameter	Zn treatment		ZnCd treatment		Differences between treatment
C_0 (mg kg^{-1})	277.5±9.47	$p<0.001$	277.2±12.25	$p<0.001$	n.s.
a (mg day^{-1})	9.5±1.67	$p<0.001$	8.4±1.78	$p<0.001$	n.s.
k_a (day^{-1})	0.05±0.01	$p<0.001$	0.04±0.009	$p<0.001$	n.s.
C_{90} (mg kg^{-1})	449.0±12.81	$p<0.001$	498.2±12.20	$p<0.001$	$p<0.001$
k_e (day^{-1})	0.02±0.002	$p<0.001$	0.01±0.002	$p<0.001$	n.s.

my study and others authors. Firstly, they studied other centipede species: *Lithobius forficatus* and *L. tricuspis* (Rabitsch, 1995) or *L. variegatus* (Hopkin and Martin, 1984) which are larger than *L. mutabilis* interspecies differences in metal concentrations are well known. Another explanation could be an additive influence of the other metals (like Cd for example) on Zn uptake. In my studies estimated zinc concentration at the end of the accumulation *(C90* period was significantly higher in centipedes treated with a mixture of Zn and Cd than in those treated with Zn alone. But this latter finding may be a simple reflection of the fact that the zinc concentration in larvae was two times higher in the ZnCd than in the Cd treatment (Tab. 4).

At the same time there were no significant differences in estimated regression parameters or in measured concentrations, between the Zn and ZnCd treatments during the decontamination period (Fig. 1, Tab. 3 and 4). Zinc was removed slowly but efficiently and independently of internal concentration. At the end of the experiment, zinc concentrations in both Zn treatments almost reached their initial levels (Fig. 1).

In summary my study confirms the hypothesis that *L. mutabilis* can regulate concentrations of essential metal such as zinc more efficiently than the xenobiotic metal, cadmium. While Cd concentrations in centipedes exposed to Cd-contaminated food seems to increase indefinitely (k_a=0), zinc concentrations in animals fed Zn-contaminated food increased at the beginning and stabilised later at the elevated level. At the same time I observed that of zinc and cadmium influence each others kinetics in centipedes.

Table 4. Concentrations of Zn in mg kg^{-1} dry weight (mean±SE) in housefly larvae diet (n=21), larvae (n=21) and centipedes at the beginning of the experiment (day 0) (n=10), at the end of the accumulation period (day 90) (n=3) and at the end of the experiment (day 121) (n=3).

Treatment	Diet	Larvae	Centipedes		
			day 0	day 90	day 121
K	83.3±7.55	69.9±7.62	261.6±26.05	269.7±15.41	269.0±77.02
Zn	555.3±52.05	328.3±7.5	261.6±26.05	466.1±31.22	301.8±33.37
Cd	74.5±1.02	82.2±2.04	261.6±26.05	317.6±32.18	335.2±47.66
ZnCd	539.4±14.69	130.3±9.06	261.6±26.05	487.8±34.42	332.0±54.05

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