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Dynamics of Accumulation and Decontamination of Cadmium and Zinc in Carnivorous Invertebrates. 1. The Ground Beetle, *Poecilus cupreus* L.

P. Kramarz

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

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Although the concept of biomagnification, that is, an increase in the concentration of anthropogenic pollutants along trophic chains, has been applied to many chemicals (e.g., Gilles et al., 1973; Price et al, 1974) more recent studies have shown that it does not hold true as a general rule at least for metals (e.g., Beyer, 1986; van Straalen and van Wensem, 1986; Laskowski, 1991). A number of authors have suggested that for metals species-specific differences in assimilation and excretion efficiencies are more important than trophic position (Beyer, 1986; van Straalen and van Wensem, 1986; Grodziñska et al., 1987; Laskowski and Maryanski, 1993). Among terrestrial invertebrates, ground beetles are a particularly good example. Being in most cases second-order consumers they are at least potentially exposed to increased concentrations of metals in their diet, yet at the same time as a group of invertebrates with the lowest metal concentrations (Butovsky, 1997). This may be due to efficient decontamination processes such as removal of whole degenerated cells, exocytosis, and extrusion of metal-containing vesicles into the lumen of the digestive tract (Hopkin, 1989). In fact, Janssen (1991) showed that a carabid, *Notiophilus biguttatus* exhibits low accumulation rate and high excretion rate of cadmium.

Some authors have pointed out that concentrations of nutritional metals (e.g., Cu or Zn) can be regulated more efficiently than those of xenobiotic metals (e.g., Pb or Cd) (Grodziñska et al., 1987; Hunter and Johnson, 1982; Laskowski and Maryanski, 1991). On the other hand, chemically related metals such as zinc and cadmium are assumed to react in a similar way in biological systems (Nieboer and Richardson, 1980).In terms of availability of metals, invertebrates living in industrial regions are exposed to environments much altered from those in which the regulatory processes for metals evolved (Hopkin, 1989). Thus, despite the lower toxicity of zinc in comparison with cadmium, in industrially polluted areas zinc concentrations are usually many times higher and more harmful than cadmium concentrations (Hopkin and Hames, 1994).

The aim of this study was to investigate whether the differences in biological function between Cd and Zn are followed by differences in the dynamics of accumulation and decontamination of these metals in a carabid beetle, *Poecilus cupreus* L.

MATERIALS AND METHODS

Adult specimens of *P. cupreus* were taken from a clean stock laboratory culture kept at the Jagiellonian University. Individual beetles were put into 8.5 cm diameter plastic boxes. A piece of filter paper (ca. 25 cm^2) was put into each box as shelter and to maintain humidity. The beetles each were offered three frozen housefly larvae *(Musca domestica* L.) every third day. During the accumulation period, which lasted 90 days, the carabids 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} of (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 beetles 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 1 l of distilled water or experimental solution.

Six randomly chosen beetles (three of each sex) were sacrificed at 2, 4, 6 and 13 days and weekly thereafter, counting from the beginning of the accumulation and the decontamination periods. To empty the gut contents the beetles were starved for 24 hours, then killed and refrigerated for further analyses.

Individual carabids and samples of the housefly larvae and their diets were digested in boiling $HN0_3$. Zinc concentrations were measured by flame atomic absorption spectrometry (AAS), and cadmium concentrations by a graphite furnace AAS.

The kinetics of metals in the beetles during the accumulation period were described with the one-compartment model (Janssen, 1991). It was assumed that the metal is assimilated at a constant rate *a* (mg day^{-1}) and eliminated at constant rate k_a (day^{-1}). The following equation was used to estimate the parameters:

$$C_t = C_0 + \frac{a}{k_a}[1 - e^{-k_a t}],$$

where C_t is the total amount of a metal in the beetle (mg kg^{-1}), C_0 (mg kg^{-1}) is the average concentration of the metal before the start of the experiment (t_0), *t* is time in days, and *e* is the base of the natural logarithm.

For the decontamination period only the excretion rate *(k_e)* was studied and the simplified exponential model was used:

$$C_t = C_{90} * e^{-k_e(t-t_c)},$$

where C_{90} (mg kg^{-1}) is the metal concentration at *t*= 90 days (end of accumulation period), k_e, is the excretion rate for the decontamination phase, and t_c, (days) is the time of switching to uncontaminated food.

Table 1. Parameters estimated ±asymptotic SE) for the one-compartment model fitted to the data obtained for Cd accumulation and decontamination dynamics in *Poecilus cupreus* L. Significance levels (p) were assessed on the basis of asymptotic confidence intervals for the estimated parameters. The models are given in the text.

Parameter	Cd treatment		ZnCd treatment	
a (mg day^{-1})	0.28±0.020	$p<0.001$	0.28±0.015	$p<0.001$
k_a (day^{-1})	0.04±0.004	$p<0.001$	0.04±0.003	$p<0.001$
C_{90} (mg kg^{-1})	7.16±0.179	$p<0.001$	6.54±0.222	$p<0.001$
k_e (day^{-1})	0.30±0.019	$p<0.001$	0.16±0.012	$p<0.001$

The parameters were estimated from the untransformed data using the Marquardt method as implemented in the Statgraphics (Manugistics Inc.) software package. The statistical significance of the models used was assessed on the basis of the confidence intervals of the estimated parameters. To check for interaction between cadmium and zinc, the parameters obtained and metal concentrations at the end of the accumulation period were compared between the Zn and ZnCd treatments and Cd and ZnCd treatments using the t-test (Zar, 1974).

RESULT AND DISCUSSION

The patterns of uptake and excretion differed considerably between Zn and Cd. In the case of cadmium the fitted model provided a good description of the data obtained for carabids fed with cadmium or with a mixture of zinc and cadmium (Fig. 1). Both parts of the regression model for accumulation and decontamination periods were statistically significant (Tab. 1). During the accumulation period cadmium showed at first a very fast increase in concentration followed by a plateau (Fig. 1). During the decontamination period, Cd concentrations in the beetles rapidly decreased in both treatments, reaching almost their initial values after two weeks. Hence, even if the concentrations of Cd in the beetles fed with Cd-contaminated food increased about thirty times, it seems that carabids are able to eliminate excess metal quickly after switching to clean food. Similar rapid decreases in Cd concentration in other carabid species *(Notiophilus biguttatus* and *Pterostichus niger)* have been reported by Janssen (1991) and Linquist et al. (1995).

No effect of added zinc on cadmium assimilation or excretion was found: there were no significant differences in the average cadmium concentrations at the end of the accumulation and decontamination periods or in the estimated regression parameters between the Cd-treated groups (Tab. 1 and Tab. 2).

An in all treatments the beetles were able to maintain the Zn concentration at an almost constant level, that is about 58-69 mg kg^{-1} for the whole time of the experiment (Fig. 1). Neither part of the regression model was significant for the Zn

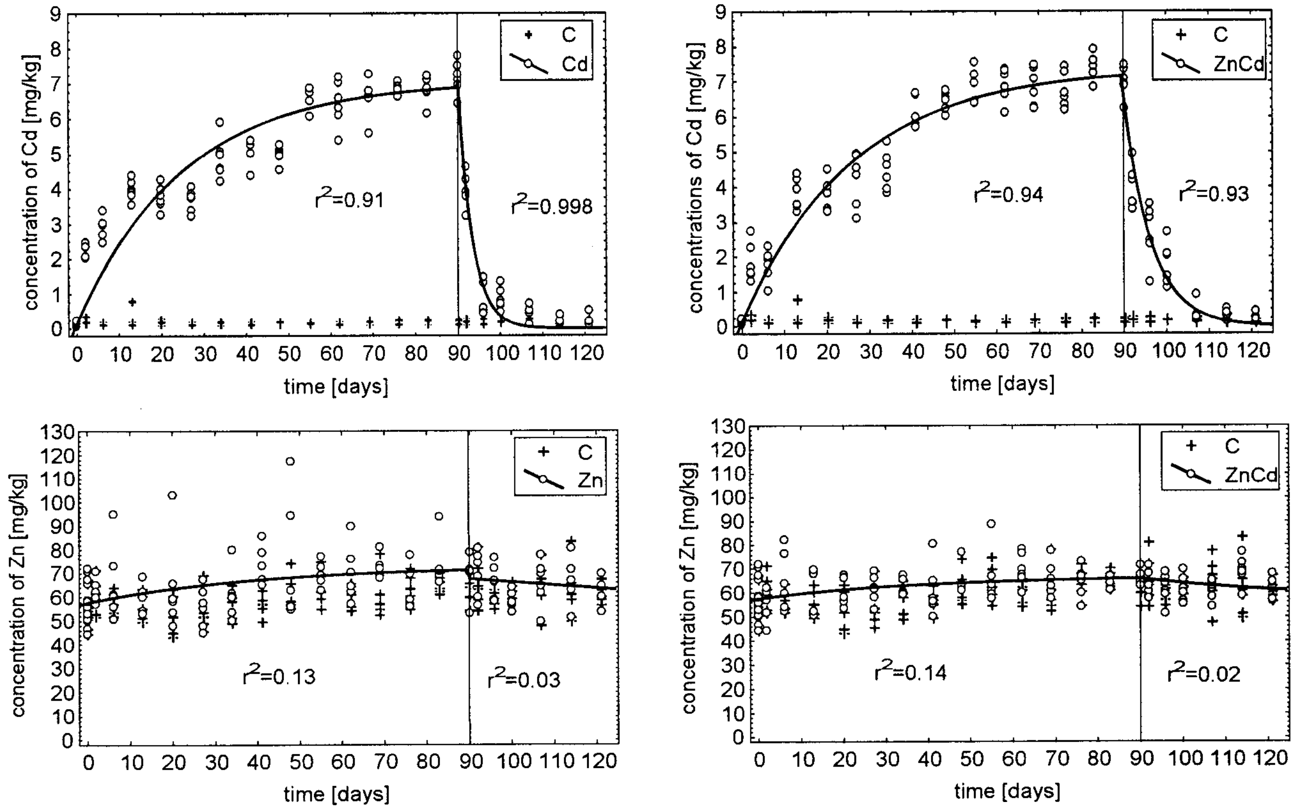

Figure 1. Kinetics of cadmium (upper plots) and zinc (lower plots) in the carabid beetle *Poecilus cupreus*. Thick lines indicate the models fitted. Thin lines indicate the time of switching to uncontaminated food. Symbols: C - control animals, Zn - carabids treated with zinc, Cd - carabids treated with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

Table 2 Concentrations of Cd in mg kg^{-1} dry weight (mean±SE) in housefly larvae diet (n=21), larvae (n=21) and beetles at the beginning of the experiment (day 0) (n=20), at the end of the accumulation period (day 90) (n=6) and at the end of the experiment (day 121) (n=6).

Treatment	Diet	Larvae	Beetles		
			day 0	day 90	day 121
K	0.13±0.022	<0.001	0.15±0.048	0.17±0.066	0.17±0.066
Zn	0.37±0.091	0.76±0.143	0.15±0.048	0.21±0.049	0.13±0.032
Cd	56.58±3.94	154.6±2.19	0.15±0.048	7.18±0.437	0.24±0.143
ZnCd	45.59±5.68	134.4±1.36	0.15±0.048	6.99±0.408	0.28±0.087

and the ZnCd treatments (Tab. 3), implying a lack of Zn accumulation in the species studied. Zn concentrations in the beetles at the end of the accumulation period and at the end of the experiment were the same for all treatments ($p>0.05$, Tab. 4). These results suggest that the beetles efficiently and rapidly remove practically all the excess of nutritional metals such as zinc. Corresponding field data for *P. cupreus* reported by Gongalski and Butovsky (1998) showed almost no differences in Zn concentrations between individuals from a non-polluted site and those from sites in the vicinity of an iron smelter.

Interaction of zinc and cadmium has been reported by many authors. An additive effect of zinc on cadmium uptake was shown, for example, in the shrimp, *Calliniasa australiensis* (Ahsanullah et al., 198l), and an antagonistic effect in the cricket, *Acheta domesticus* (Migula et al., 1989) and the zebrafish, *Brachydyanio rerio* (Wicklund et al., 1990).

In my study the differences between the kinetics of metals in carabids treated with single metals or with a mixture of zinc and cadmium were nonsignificant. This indicates that zinc and cadmium did not affect each other's uptake. A similar lack of interaction was found in the snail, *Helix pomatia* (Berger et al., 1993), and the springtail, *Folsomia candida* Willem (van Gestel and Hensbergen, 1997).

Table 3 Parameters estimated (±asymptotic SE) for the one-compartment model fitted to the data obtained for Zn accumulation and decontamination dynamics in *Poecilus cupreus* L. Significance levels (p) were assessed on the basis of asymptotic confidence intervals for the estimated parameters, n.s. stands for $p>0.05$ (statistically non-significant). Negative excretion constants (k_e) for the decontamination period in the beetles from ZnCd treatment has no biological sense. The models are given in the text.

Parameter	Zn treatment		ZnCd treatment	
a (mg day^{-1})	0.37±0.203	n.s.	0.31±0.169	n.s.
k_a (day^{-1})	0.02±0.023	n.s.	0.04±0.028	n.s.
C_{90} (mg kg^{-1})	67.35±2.024	p<0.001	63.49±1.325	p<0.001
k_e (day^{-1})	0.002±0.002	n.s.	-0.0001±0.001	n.s.

Table 4. Concentrations of Zn in mg kg^{-1} dry weight (mean±SE)) in housefly larvae diet (n=21), larvae (n=21) and beetles at the beginning of the experiment (day 0) (n=20), at the end of the accumulation period (day 90) (n=6) and at the end of the experiment (day 121) (n=6).

Treatment	Diet	Larvae	Beetles		
			day 0	day 90	day 121
K	83.3±7.55	69.9±7.62	57.6±7.97	62.1±4.96	61.4±3.51
Zn	555.3±52.1	328.3±7.50	57.6±7.97	69.2±8.59	63.8±5.77
Cd	74.5±1.02	82.2±2.04	57.6±7.97	68.5±5.43	66.0±9.28
ZnCd	539.4±14.7	130.3±9.06	57.6±7.97	65.8±3.29	61.9±4.04

My studies showed that carabids avoid metal poisoning by rapid discharge of assimilated metals. This was especially obvious in the case of Zn. As predators, carabids share the same trophic level with spiders who exhibit high concentrations of almost all metals, and with centipedes, showing intermediate concentrations of metals (Grodzinska et al., 1987; Hunter and Johnson, 1982; Laskowski and Maryanski, 1991). These three groups constitute the majority of carnivorous invertebrate fauna of soil ecosystems. In the following papers, I investigate the kinetics of metals in centipedes.

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REFERENCES

Ahsanullah M, Negilski DS, Mobley MC (198 1) Toxicity of zinc cadmium and copper to the shrimp *Callianass australiensis,* III: Accumulation of metals, Mar Biol 64: 311-316

Berger B, Dallinger R, Felder E, Moser J (1993) Budgeting the flow of cadmium and zinc through the terrestrial gastropod, *Helix pomanatia.* In: Dallinger R, Rainbow PS (eds) Ecotoxicology of Metals in Invertebrates. Lewis Publisher p 291

Beyer W.N. (1986) A re-examination of biomagnitication of metals in terrestrial food chains. Environ Toxicol Chem 5: 863-864

Butovsky RO (1997) Heavy metals and carabids (Coleoptera, Carabidea), Agrihimija 11: 78-86

Gilles FE, Middleton SG, Grau JG (1973) Evidence for the accumulation of atmospheric lead by insects in areas of high traffic density. Environ Entomol 2: 299-300

Gongalski KB and Butovsky RO (1998) Heavy metal pollution and carabid beetles (Coleoptera, Carabidea) in the vicinity of the Kosogorski metallurgic plant at

Kosaya Gora. In: Butovsky RO and van Straalen NM (eds) Pollution-induced changes in soil invertebrate food-webs, Amsterdam and Moscow, p 55

Grodzinska K, Godzik B, Darowska E, Pawlowska B (1987) Concentrations of heavy metals in trophic chains of Niepolomice Forest, s. Poland. Ekol Pol 35: 327-344

Hopkin SP (1989) Ecophysiology of metals in invertebrates. Elsevier Applied Science, London - New York

Hopkin SP, Hames CAC (1994) Zinc, among a 'cocktail' of metal pollutants, is responsible for the absence of terrestrial isopod *Porcellio scaber* from vicinity of a primary smelting works. Ecotoxicology 2: 68-78

Hunter BA, Johnson MS (1982) Food chain relationships of copper and cadmium in contaminated grassland ecosystems. Oikos 38: 108-l17.

Janssen MPM (1991) Comparison on cadmium kinetics in four soil arthropods species. Ph.D. Thesis, Vrije Univeriteit, Amsterdam 75-89

Laskowski R (199 1) Are the top carnivores endangered by biomagnification? Oikos 60: 387-390

Laskowski R, Maryanski M (1993) Heavy metals in epigeic fauna: trophic-chain and physiological hypotheses. Bull Environ Contam Toxicol 59: 232-240

Lindqvist L, Block M, Tjalve H (1995) Distribution and excretion of Cd, Hg, methyl-Hg and Zn in the predatory beetle *Pterostichus niger* (Coleoptera: Carabidea). Environ Toxicol Chem 14: 1195-1201

Migula P, Kedziorski M, Nakonieczny M, Kafel A (1989) Combined and separate effects of heavy metals on energy budget an metal balances in *Acheta domesticus.* Uttar Pradesh J Zool 9: 140-149

Nieboer E, Richardson DHS (1980) The replacement of the nondescript term "heavy metals" by a biologically and chemically significant classification of metal ions. Environ Pollut (Ser.B) 1: 3-26

Price PW, Rathcke BJ, Gentry D A (1974) Lead in terrestrial arthropods: evidence for biological concentration, Environ Entomol 3 : 370-372

Van Gestel CAM, Hensbergen PJ (1997) Interaction of Cd and Zn toxicity for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. Environ Toxicol Chem 16: 1177-1186

Van Straalen NM, van Wensem J (1986). Heavy metal content of forest litter arthropods as related to body-size and trophic level. Environmental Pollution 42: 209-221.

Wicklund A, Norrgrenn L, Runn P (1990) The influence of cadmium and zinc on cadmium turnover in zebrafish *Brahydario rerio.* Arch Environ Contam Toxicol 19: 348-353

Zar JH (1974) Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J.