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Kinetics of Zn and Cd accumulation in the isopod *Porcellio scaber* exposed to contaminated soil and/or food

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Abstract

To derive accumulation kinetics from different exposure matrices and account for the contribution of different exposure routes, the isopod *Porcellio scaber* was exposed to Cd and Zn, either in soil or in food, and a combination of both. Accurate uptake and elimination kinetics of Zn and Cd were determined using radioisotopes, allowing non-destructive time measurements. To describe the data, a simple kinetic model was used accounting for the internal distribution of the metals. A strong influence of the elimination kinetics, together with the metal fraction that is compartmentalized in a storage fraction, was found explaining the Cd steady-state level reached in the isopods. Zn turnover kinetics were relatively fast within the first days of exposure and elimination, followed by slow Zn kinetics from the storage compartment. The storage fraction appeared to be inert for elimination of both Zn and Cd within the experimental duration of 32 days and the fraction of metal taken up that was stored in this fraction was influenced by the uptake source. The quantity of metals taken up from soil or food depended on the concentration. For both Zn and Cd, the uptake rate constant from soil equalled the uptake rate constant from food, moreover, the uptake from both routes was shown to be additive. Therefore, it is concluded that the relative contribution of both routes (via soil or food) is explained by the partitioning of metals between soil and food.

Keywords: Metal kinetics; Soil; Food; Isopoda; Radioisotopes

1. Introduction

In a metal-contaminated ecosystem, soil-dwelling organisms are not only exposed to metals in the soil, but also litter-layer in which metals are most effectively accumulated (Martin et al., 1982) adds to the risk. Detritivorous organisms such as isopods in particular face high metal exposure, because both their food source (organic matter) and their microhabitat coincide with the places—upper layer of the soil—where metal accumulation takes place. Metal accumulation in terrestrial isopods has been studied intensively (Dallinger and Prosi, 1988; Witzel, 1998; Hopkin and Martin, 1982) and these organisms are, although controversially discussed, recognized as suitable bioindicators of metal pollution (Paoletti and Hassall, 1999). Their capacity to accumulate high metal concentrations can be ascribed to an efficient storage organ, the hepatopancreas, which contains up to 90% of the metals in the isopod's body (Hopkin, 1990). The internal sequestration in metabolically active metal pools and storage compartments is likely to have an impact on elimination abilities of the animal. Although there is strong evidence for these kinds of physiological effects (Rainbow, 2002), they are hardly ever accounted for in bioaccumulation studies.

Metals may be taken up via different routes and traffic through the body until the targets are reached. Metals taken up orally enter the digestive tract from where they go directly from the gut fluid or via the typhlosole channels to

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the hepatopancreas (Hames and Hopkin, 1991a). They may also diffuse into the haemolymph, from where they are partly osmoregulated via the maxillary glands (Donker et al., 1996). Another route of metal uptake can be via the pleopods under the abdomen of the isopod, which absorb water from the environment by capillary action (Sutton, 1972). Metals taken up via this route will circulate in the haemolymph through the entire body until a target organ is reached.

Although metal uptake is the net result of uptake from different routes of exposure, uptake kinetics usually are derived under laboratory conditions in which organisms are exposed to either contaminated soil or food. In that case, accumulation kinetics are relatively easy to describe as only one single source is considered. Single exposure, however, rarely occurs in polluted ecosystems, as elevated levels occur in both soil and litter layers.

In this study, different exposure regimes were used, focusing on either soil or food exposure or a combination. The main aim was to investigate the relative contribution of each uptake route to Zn and Cd accumulation and to quantify the impact of the effective storage capacity on metal elimination kinetics in the isopod *Porcellio scaber*. Since litter is the main food source and the predominant microhabitat of isopods compared to soil, it was hypothesized that metal uptake from food predominates over uptake from soil and pore water.

Kinetics were determined for the non-essential metal Cd and the essential metal Zn by using radioisotopes. Radioisotopes with an atomic mass greater than 35 act as stable metals and animals do not discriminate between the isotopes (e.g. Croal et al., 2004). The radioisotope technique is especially useful to provide insight into the turnover and specific accumulation kinetics of essential metals that often occur at highly regulated concentrations in animals. Furthermore, the technique allowed measurement of individual organisms over time.

2. Materials and methods

2.1. Test animals

Adult isopods *P. scaber* Latreille, 1804, were collected from an uncontaminated garden in Bilthoven, The Netherlands. Before the start of the experiment, isopods were kept in a clayey soil with litter of poplar leaves *Populus x canadensis* on top, under constant laboratory conditions for at least 3 weeks at 15 °C with constant light. Shelter for the isopods was provided by placing some stone fragments on the soil surface. The same conditions were applied during the experiments.

2.2. Experimental design

Organisms, females and males, were taken together because gender of the organisms was shown not to cause metal accumulation differences (Donker and Bogert, 1992). A 2-week uptake experiment was carried out, in which the test-animals were exposed to soil or food labelled with radioisotopes. To quantify the impact of more than one uptake route on metal accumulation by soil organisms, different exposure regimes were applied: treatment A, labelled soil and no food, treatment B labelled soil and unlabelled food, treatment C both labelled soil and food and treatment D was both unlabelled soil and food. After 14 days, isopods were transferred to unlabelled soil and unlabelled food and the decrease of internal concentrations was determined up to 32 days.

All experimental treatments were performed in triplicate. In each test jar (750 ml) one isopod was placed. For pragmatic and logistic reasons, uptake and elimination experiments had to be executed separately. Isopods kept in the same soil although unlabelled and with unlabelled food were weekly monitored as a check for 'normal' behaviour and for the analysis of background metal levels in the isopod's body.

2.3. Soil and food labelling

The soil used in the experiment was collected from the freshwater estuary floodplain "Ruitersplaat" in the Biesbosch, The Netherlands, having 17–19% organic matter, 22–28% clay, and a pH (0.01 M $CaCl_2$) of 7.1–7.4. The soil was kept at a moisture content of 80% w/w (= 72% of maximum water holding capacity). Food was supplied ad libitum to the isopods in pieces sized 4–8 mm on stone fragments placed on the soil surface. The food consisted of soaked litter of poplar leaves *P. x canadensis*. Soil and food were labelled with radioisotopes as duo-label with ^{109}Cd and ^{65}Zn. Radioisotopes were certified and supplied as $^{109}CdCl_2$ (Amersham Biosciences, Buckinghamshire, UK) and $^{65}ZnCl_2$ solution (Perkin Elmer, Boston, USA). Specific activity of the isotopes was $1\,Bq = 54 \times 10^{-9}$ ng Cd and $1\,Bq = 81.6 \times 10^{-6}$ μg Zn. Of both isotopes, 1.25 MBq was added to 150 g dry soil and 120 ml water. Food of 0.06 g fresh weight was soaked in a 5 ml solution containing 1.25 MBq of both isotopes, after which it was dried for two days. The isotopes added to soil and food was allowed to equilibrate for 14 days in the laboratory before starting exposure of the isopods.

2.4. Measurements and analyses

Daily, the isopods were removed from the jars, rinsed with tap water, and ^{109}Cd and ^{65}Zn concentrations were determined using a Wallac gamma counter (Model 1480 3, EG&G company, Finland) while the animal was held in a measuring vessel. To minimize stress, these measurements were performed within 15 min. The metal concentrations in the gut of the isopod were determined in the start of the elimination experiment. After 4 h of exposure within the unlabelled soil with unlabelled food, all isopods were measured on their ^{109}Cd and ^{65}Zn concentrations.