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The influence of metal speciation on the bioavailability and sub-cellular distribution of cadmium to the terrestrial isopod, *Porcellio dilatatus*

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ABSTRACT

Cadmium is a non-essential toxic metal that is able to bioaccumulate in both flora fauna and has the potential to biomagnify in some food chains. However, the form in which cadmium is presented to consumers can alter the bioavailability and possibly the internal distribution of assimilated Cd. Previous studies in our laboratory highlighted differences in Cd assimilation among isopods when they were provided with a plant-based food with either Cd biologically incorporated into plant tissue or superficially amended with ionic Cd^{2+}. Cd is known for its high affinity for sulphur ligands in cysteine residues which form the basis for metal-binding proteins such as metallothionein. This study compares Cd assimilation efficiency (AE) in *Porcellio dilatatus* fed with food amended with either cadmium cysteinate or cadmium nitrate in an examination of the influence of Cd speciation on metal bioavailability followed by an examination of the sub-cellular distribution using a centrifugal fractionation protocol. As hypothesized the AE of Cd among isopods fed with $Cd(NO_3)_2$ (64%, SE = 5%) was higher than AE for isopods fed with $Cd(Cys)_2$ (20%, SE = 3%). The sub-cellular distribution also depended on the Cd species provided. Those isopods fed $Cd(Cys)_2$ allocated significantly more Cd to the cell debris and organelles fractions at the expense of allocation to metal-rich granules (MRG). The significance of the difference in sub-cellular distribution with regard to toxicity is discussed. This paper demonstrates that the assimilation and internal detoxification of Cd is dependent on the chemical form of Cd presented to the isopod.

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1. Introduction

Cadmium (Cd) is a non-essential metal that is considered a priority pollutant in Europe in light of the risk it presents to the environment and human health (ECB, 2007). Although Cd occurs naturally in soils and waters at low concentrations, deposition within the biosphere has increased dramatically over the last century as a consequence of anthropogenic activities. Concern arises because like many other non-essential metals, Cd has the potential to bioaccumulate in plants (McLaughlin, 2002; McLaughlin et al., 2006) and invertebrates (Peijnenburg, 2002), but unlike many other metals, Cd has a greater potential for trophic movement and biomagnification along some food chains (Croteau et al., 2005; Mann, 2010). Bioaccumulation patterns among flora and fauna are dependent on both the environmental availability of Cd and physiological constraints on uptake into an organism, and both these aspects are in turn dependent on chemical speciation, i.e. the chemical form in which the metal is presented to the consumer.

Metals that are distributed within the biosphere, seldom occur as free metal ions. Free metal ions are highly reactive chemicals that have the capacity to disrupt biological systems. Therefore, when metal ions (even essential ions) enter biological organisms, numerous detoxification and sequestration pathways are initiated to either, deliver essential metals to the place where they are required or stored until they are required, or isolate and eliminate toxic metals and prevent damage. Among vascular plants, mechanisms of tolerance include the induction of metal-binding proteins such as phytochelatins or metallothionein-like proteins (Prasad, 1995). Phytochelatins and metallothioneins (MTs) are small proteins with a significant concentration of cysteine (30%) (Klaassen et al., 1999; Ndayibagira et al., 2007). Cadmium ions have a high affinity for the sulfydryl group in cysteine residues and this fact accounts for the induction of the metallothionein genes by Cd (Zalups and Ahmad, 2003; Roosens et al., 2005). In plants, as a consequence of these detoxification pathways, Cd may reach high concentrations before phytotoxicity is manifested (Nolan et al., 2003), thereby providing a pool of Cd which may or may not be available to herbivores.

A previous dietary study on the assimilation of Cd in the terrestrial isopod *Porcellio dilatatus* (Calhôa et al., 2006) indicated that Cd speciation dictated the assimilation efficiency (AE) of Cd. Cadmium

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AE was higher among isopods provided with food (lettuce) superficially amended with $Cd(NO_3)_2$ than among isopods provided with lettuce grown in Cd-contaminated media. These results were consistent with the free ion activity model (FIAM) that dictates that metals which are complexed with organic molecules are less bioavailable than free metal ions (Nolan et al., 2003). However, previous studies in vertebrates have demonstrated that Cd bound to complex molecules is also, at least to some degree, bioavailable (Groten et al., 1991; Sugawara and Sugawara, 1991; Harrison and Curtis, 1992), and that Cd bound to metallothionein was not only taken up in the rat gut, but also preferentially distributed to the kidney (Groten et al., 1991; Sugawara and Sugawara, 1991). Assuming a significant proportion of Cd that accumulates in plants (i.e. lettuce) is bound to sulphur ligands (Maier et al., 2003; Monteiro et al., 2008), we set out to specifically examine the bioavailability (measured as AE) of Cd when bound to cysteine, and tested the hypothesis that the internal compartmentalization of Cd among isopods provided with a diet amended with Cd-cysteinate would not be the same as seen in isopods fed more bioavailable Cd species (i.e. $Cd(NO_3)_2$). Internal compartmentalization was assessed using a centrifugal fractionation procedure (Wallace et al., 2003; Wallace and Luoma, 2003; Monteiro et al., 2008) which has been adopted by numerous researchers as a simple and pragmatic approach in the prediction of trophic transfer of metals to higher trophic levels, and is a first step forward in the development of practical tools that could explain most of the variability observed in metals accumulation and toxicity in organisms (Vijver et al., 2004; Mann, 2010).

2. Materials and methods

2.1. Test organisms and culture conditions

Isopods were selected from laboratory cultures of *P. dilatatus* that were derived from individuals collected in a secondary coastal dune system in central Portugal. They had been maintained for more than 3 years on a substrate of sand in plastic containers at 20 °C with a 16:8 h (light:dark) photoperiod. Alder leaves were provided *ad libitum* as a food source (Caseiro et al., 2000; Kautz et al., 2000) and distilled water was added to maintain moisture.

2.2. Cadmium–cysteine conjugate (Cys–S–Cd–S–Cys) (1:2)

Cadmium acetate (90 mM) including 462 μCi mL^{-1} of ^{109}Cd (Perkin–Elmer, Boston, MA, USA) was added to L-cysteine (180 mM) in water while stirring. Sodium acetate (0.3 M) was added until a white amorphous precipitate formed (Russell Bell, pers. comm.; Barrie et al., 1993). The precipitate was filtered off, washed with deionised water, and dried in the oven at 50 °C. The dried $Cd(Cys)_2$ was kept at 4 °C until required. Cd content was analysed by inductively coupled plasma spectroscopy (ICPS) in a Jobin Ivon JY70 with a Meinard C001 nebuliser; confirming the molar ratio (1:2).

2.3. Lettuce and gelatine substrate

A mixture of lettuce leaves and gelatine was selected as a suitable food substrate to be used as the exposure vehicle (Mann et al., 2005; Monteiro et al., 2008). The gelatine discs provided a homogenous vehicle for the delivery of Cd, and therefore reduced variability of Cd absorption among isopods within treatments (Wallace and Lopez, 1996). Also, because gelatine is derived from animal protein, its inclusion effectively decreased the C/N ratio, thereby improving palatability and nutritional value of the food (Zimmer, 2002; Zimmer et al., 2003). Non-contaminated dried leaves of lettuce (*Lactuca sativa*) were reduced to powder using a mortar and pestle, and 1.25 g of lettuce powder was mixed into a gelatine solution prepared from 2.5 g gelatine powder (VWR Prolabo, Fontenay Sous Bois, France) and 12.5 mL deionised water (Milli-Q®) and then mixed by vortexing (Wallace and Lopez, 1996). Depending on treatment, either $Cd(Cys)_2$ or $Cd(NO_3)_2$ were dissolved in the deionised water prior to mixing with gelatine and lettuce powder to produce nominal concentrations of 500 μg Cd g^{-1} wet weight for $Cd(Cys)_2$, and 300 μg Cd g^{-1} wet weight for $Cd(NO_3)_2$. Aliquots of 7 μL of the gelatine/lettuce mixture were pipetted onto Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA), forming gelatine discs that were stored frozen at −20 °C until required (Wallace and Lopez, 1997). A sub-sample of gelatine discs (15) that had been contaminated with $Cd(Cys)_2$ and $Cd(NO_3)_2$ were assayed for Cd by radiospectrometry to obtain mean concentrations of Cd in each food treatment.

Three treatments (diets) were established to evaluate the influence of metal speciation on the bioavailability and compartmentalization of Cd to the terrestrial isopod *P. dilatatus*:

1. $Cd(Cys)_2$ contaminated food – gelatine/lettuce contaminated with Cd-cysteinate (incorporating ^{109}Cd as a tracer – see Section 2.2. *Cadmium–cysteine conjugate*).
2. $Cd(NO_3)_2$ contaminated food – gelatine/lettuce contaminated with $Cd(NO_3)_2$ (including 23.1 μCi mL^{-1} ^{109}Cd as a tracer).
3. Control food – gelatine/lettuce with no contamination.

2.4. Feeding study

Before the start of the test, a total of 120 juvenile isopods were selected by weight (mean = 42 mg, ranging from 23 to 65 mg) and isolated individually in test boxes for 24 h without food to purge their gut. No distinction was made between sexes. Polyethylene terephthalate (PET) test boxes were used (85 mm × 43 mm; Termoformagen, Leiria, Portugal) containing in the bottom a thin layer of plaster of Paris mixed with activated charcoal (8:1 v/v) for the retention of moisture.

Forty individuals were randomly allocated to each treatment. Animals were fed for a period of 28 d exclusively on gelatine discs according to treatment. Gelatine discs were replaced every week to prevent the consumption of food which had become inoculated with fungi – the growth of fungi may have altered the speciation and bioavailability of Cd. The remains of food were also weighed and Cd content assayed by radiospectrometry (see below). Faecal pellets were collected every 2 d to prevent coprophagy and dried (2 d at 60 °C).

After 28 d, isopods were left for 24 h without food to purge their guts and subsequently weighed and analysed for Cd burdens by radiospectrometry (see *Cadmium analysis*). Data on isopod, faecal pellet and gelatine mass were used to determine indices of isopod growth, food consumption and food assimilation efficiency. The Cd content in isopods and in the gelatine discs were used to determine Cd assimilation efficiency (Cd AE).

2.5. Sub-cellular distribution of Cd in isopods

At the end of the 28-d feeding study, differences in sub-cellular Cd distribution in isopods was investigated using a methodology described by Wallace and co-workers (Wallace et al., 1998, 2003; Wallace and Luoma, 2003) with minor modifications. Briefly, replicates ($n = 6$) of 3 isopods each were homogenized in 2 mL of Tris buffer at pH 7.6 (20 mM; 1:10 (m/v) tissue to buffer ratio). The homogenate was centrifuged at 1450*g* for 15 min at 4 °C. The resulting pellet was re-suspended in 0.5 mL distilled water and heated at 100 °C for 2 min. An equal volume of NaOH (1 N) was then added followed by heating at 70 °C for 1 h. The digest was then centrifuged at 5000*g* for 10 min at 20 °C. The pellet formed

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