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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.

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

contained the metal-rich granules (MRG) and the supernatant was designated cell debris, containing mainly exoskeleton, tissue fragments, and other cellular debris. The supernatant of the first centrifugation step, containing the cytosol, was centrifuged at 100,000*g* for 1 h at 4 °C to sediment organelle components. The 100,000*g* supernatant containing the soluble fraction of the cytosol was then heat denatured at 80 °C for 10 min and cooled on ice for 10 min. Heat-denatured proteins were separated from the heat stable proteins (HSPs) by centrifugation at 50,000*g* for 10 min at 4 °C. All fractions were assayed for Cd by radiospectrometry and metal contents were used to calculate distributions of Cd within isopods based on summation of Cd content of the five sub-cellular fractions. Each fraction was subsequently assayed for Cd content.

In total, five sub-cellular fractions were obtained: cellular debris (exoskeleton, cell membranes and unbroken cells); metal-rich granules (MRG); organelles (mitochondria, microsomes, and lysosomes); heat-denatured proteins (HDP) and heat stable proteins (HSP) like metallothionein (Wallace et al., 2003). We also applied the predictive model proposed by Wallace et al. (2003) that allocates the organelles, HDP and HSP as a trophically available metal (TAM) (Wallace and Luoma, 2003), the organelles and HDP as a metal-sensitive fraction (MSF), and HSP and MRG as biologically detoxified metal (BDM) (Wallace et al., 2003).

2.6. Cadmium analysis

Dry gelatine discs (before and after feeding), whole isopods and sub-cellular fractions were placed in 3.5-mL Röhren tubes (Sarstedt, Newtown, NC, USA) and were analysed for Cd by radiospectrometry in a Genesis Gamma1 bench-top gamma counter (Laboratory Technologies, USA). The $Cd(NO_3)_2$ and $Cd(Cys)_2$ contamination solutions were analysed by inductively coupled plasma spectroscopy (ICPS) in a Jobin Ivon JY70 with a Meinard C001 nebuliser. Specific activities of the two contamination solutions were assessed by comparing gamma counts with measurements obtained by ICPS.

2.7. Data analysis

Food assimilation efficiency was calculated as:

$$AE_{food} = (C_{food} - F)/C_{food} * 100$$

where C_{food} is mass of gelatine discs (dry weight) consumed, and F is the mass of faecal material (dry weight) produced (Calhôa et al., 2006).

Radiospectrometry data obtained from the isopods and food were used to determine indices of Cd AE. Cadmium AE was calculated as:

$$AE_{Cd} = I_{Cd}/C_{Cd} * 100$$

where I_{Cd} is the amount of Cd within the isopod at the end of the feeding trial, and C_{Cd} is the amount of Cd consumed.

All values presented in the Section 3 are mean values ± standard error.

SigmaStat (version 3.01, SPSS, Chicago, IL, USA) was used to perform all statistical tests. One-way analyses of variance (ANOVA) were performed to determine differences ($\alpha = 0.05$) in changes in isopod mass, indices of food consumption and food assimilation efficiency among treatments. When necessary, data were transformed to achieve normality and equality of variance and when these criteria where not satisfied, the nonparametric Kruskal–Wallis one-way ANOVA was performed, followed by Dunn's method post hoc test when differences were attained. Student's *t*-tests were performed to determine differences ($\alpha = 0.05$) in indices of Cd consumption, assimilation and assimilation efficiency among treatment groups and a Mann–Whitney rank sums test was employed, when data failed to fit a normal distribution. Cd assimilation and fractionation by isopods was analysed using a two-way ANOVA – with interaction between factors (i) Cd species, i.e. $Cd(Cys)_2$ and $Cd(NO_3)_2$ (ii) and fractions (Cellular debris, MRG, Organelles, HSP and HDP) and compartments (BDM, MSF and TAM) – and pair-wise multiple comparison procedures with the Student–Newman–Keuls method whenever significant differences ($\alpha = 0.05$) between treatments were found. All proportional data for the sub-cellular compartmentalization were arcsine transformed to ensure normality and homoscedascity of data.

3. Results

3.1. Concentrations of Cd in food treatments

Gelatin discs amended with $Cd(NO_3)_2$ contained (mean ± standard deviation) 296 ± 11 μg Cd g wet food^{-1} (1.41 ± 0.06 μg Cd mg dry food^{-1}). Gelatin discs amended with $Cd(Cys)_2$ contained (mean ± standard deviation) 575 ± 1.18 μg Cd g wet food^{-1} (2.75 ± 0.53 μg Cd mg dry food^{-1}).

3.2. Isopod growth, food consumption and assimilation efficiency

During the 28-d feeding experiment, only control isopods increased in weight (Fig. 1A). Growth among isopods provided with gelatine contaminated with $Cd(NO_3)_2$ was significantly lower than the control and $Cd(Cys)_2$ treatments (Kruskal–Wallis with Dunn's post hoc test; $p < 0.05$). No significant difference was found in isopod growth when comparing control and $Cd(Cys)_2$ treatments. Mortality was below 10% in all treatments (4, 1 and 1 animals died in Control, $Cd(Cys)_2$ and $Cd(NO_3)_2$, respectively).

Isopods ate approximately 0.2–0.3 mg mg animal^{-1} over the 28 d (Fig. 1B). Food consumption was significantly different between the $Cd(Cys)_2$ and the other two treatments (ANOVA with Holm-Sidak post hoc; $p < 0.001$). Food AE was significantly higher in isopods fed with $Cd(Cys)_2$ when compared with the other treatments (Kruskal–Wallis with Dunn's post hoc test; $p < 0.05$). Isopods fed with control lettuce displayed AEs of 63 ± 4.3%, and treatments with $Cd(Cys)_2$ and $Cd(NO_3)_2$ food showed AEs of 81 ± 3.1% and 61 ± 4.3%, respectively (Fig. 1C).

3.3. Cadmium consumption, assimilation, and AE

Cd consumption (Fig. 2A) was significantly higher (Mann–Whitney rank sum test; $p < 0.001$) in isopods fed with $Cd(Cys)_2$ gelatine, although Cd assimilation was higher in isopods fed with $Cd(NO_3)_2$ gelatine (Fig. 2B). There was a significant difference in Cd assimilation between treatments (t-test$_{(\ln \text{ transformed data})}$; $p = 0.002$). The AE of Cd by isopods fed with $Cd(NO_3)_2$ (64.3 ± 4.47%) was higher than Cd AE of isopods fed with $Cd(Cys)_2$ (20.15 ± 2.82%) (Mann–Whitney rank sum test; $p < 0.001$) (Fig. 2C).

3.4. Cadmium sub-cellular compartmentalization

Comparing sub-cellular fractionation of Cd between $Cd(Cys)_2$ and $Cd(NO_3)_2$ treatments in relation to the total assimilated Cd revealed significant differences (ANOVA $F_{4,50} = 9.337$; $p < 0.001$) (Fig. 3). There was a statistically significant interaction between Cd speciation and respective fractionation according to the two-way ANOVA calculations ($p < 0.001$). The cell debris fraction is significantly different from the other fractions ($p < 0.05$) and represents the largest storage of the total accumulated Cd (59–64%) in both treatments. Within the cells of *P. dilatatus*, storage of Cd (Fig. 3) for (i) $Cd(Cys)_2$ was located in: cellular debris (64%) > HDP (12.1%) > MRG (11.8%) > organelles (9.2%) > HSP

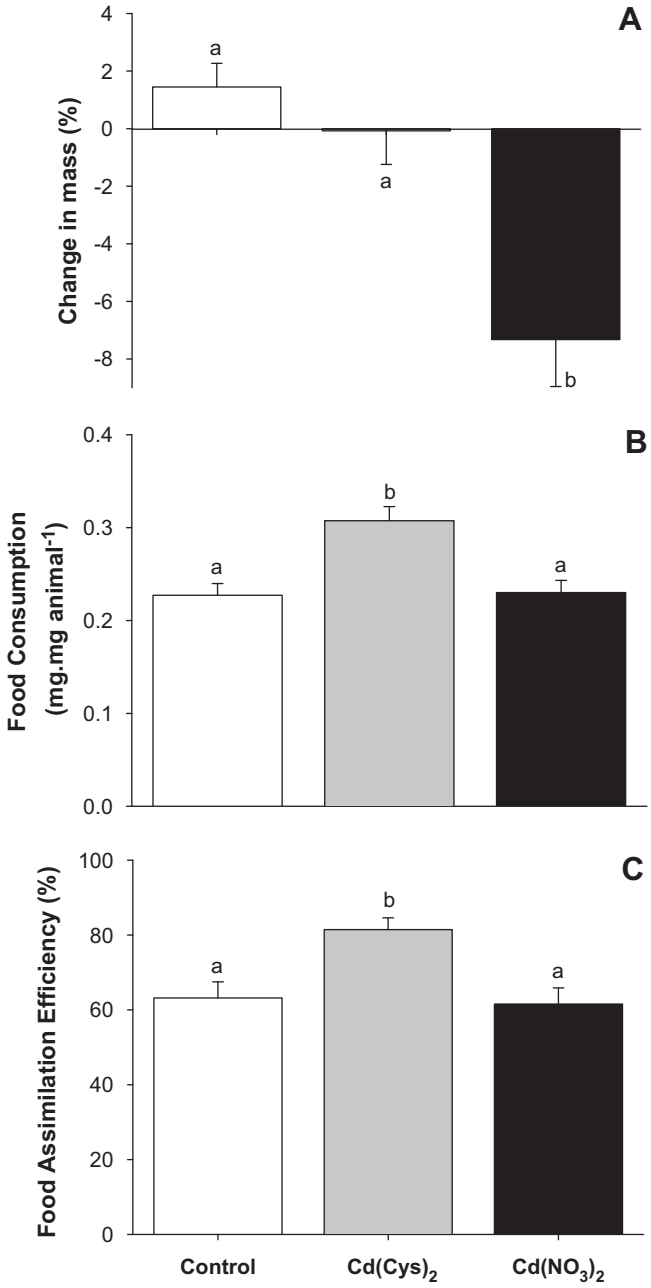

Fig. 1. Food related traits of *Porcellio dilatatus* exposed to control, $Cd(Cys)_2$ and $Cd(NO_3)_2$ lettuce gelatines for 28 d. (A) Changes in isopod biomass (%); (B) food consumption (wet weight); (C) food assimilation efficiency. All error bars represent standard error ($n = 36–39$). Lower case letters denote significant differences among groups following ANOVA with Dunn's method post hoc test. Control – control food; $Cd(Cys)_2$ = $Cd(Cys)_2$ contaminated food; $Cd(NO_3)_2$ = $Cd(NO_3)_2$ contaminated food.

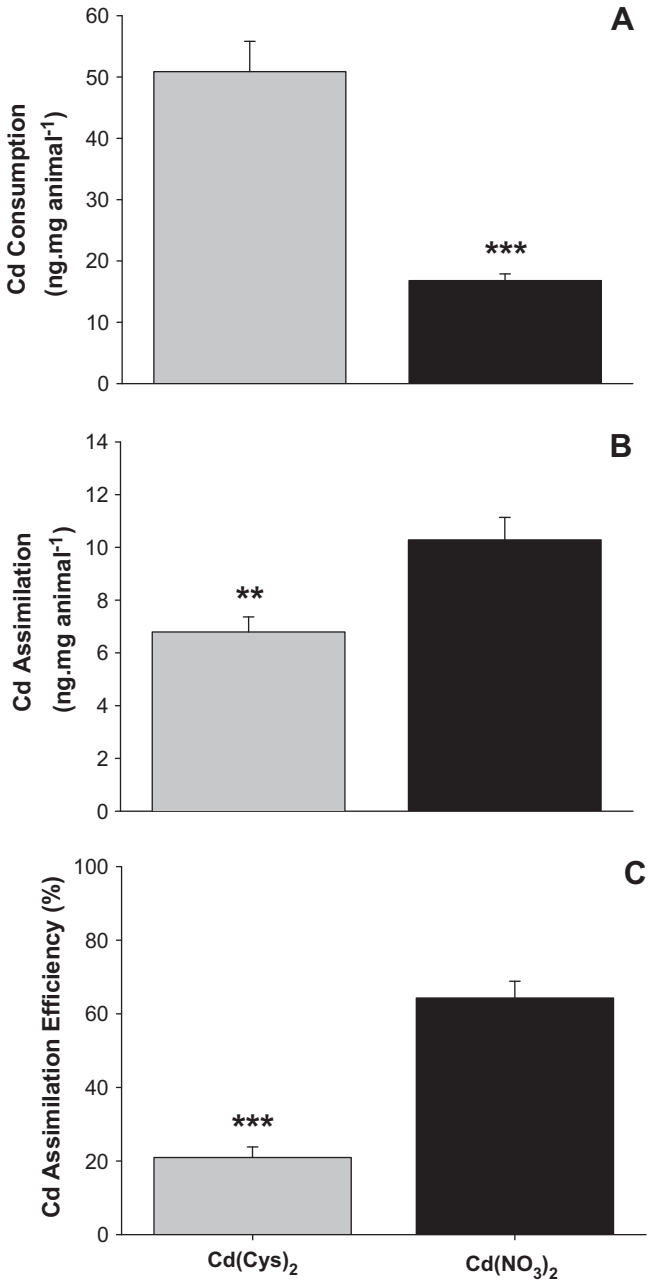

Fig. 2. Cadmium related traits for *Porcellio dilatatus* exposed to control food, and $Cd(Cys)_2$ and $Cd(NO_3)_2$ contaminated food for 28 d. (A) Cd consumption (wet weight); (B) Cd assimilation (ng mg isopod^{-1} (wet weight)). (C) Cd assimilation efficiency. All error bars represent SE ($n = 39$). The asterisks denote significant differences ($^{**}p < 0.01$; $^{***}p < 0.001$). $Cd(NO_3)_2$ = $Cd(NO_3)_2$ contaminated food; $Cd(Cys)_2$ = $Cd(Cys)_2$ contaminated food.

(2.9%); and for (ii) $Cd(NO_3)_2$ located in: cellular debris (59.1%) > MRG (19%) > HDP (12.5%) > organelles (6.2%) > HSP (3.3%).

4. Discussion

The use of Cd-cysteinate in this study provides an experimental device to explore the bioavailability of Cd that is complexed within biological tissue. Cysteine is the primary source of sulfydryl ligands in metal-binding proteins such as MTs or phytochelatins, and a proportion of the Cd that has been assimilated into biological tissues is likely to be associated with cysteine residues incorporated into MT or MT-like proteins (Monteiro et al., 2008). Therefore Cd-cysteinate represents the most elementary form (species) of thiol-bound Cd in biological systems.

The results in the present study are in accordance with findings from a previous study by Calhôa et al. (2006). In that study, *P. dilatatus* were provided with lettuce contaminated superficially with either $Cd(NO_3)_2$ (SCL – superficially contaminated lettuce) or lettuce that had been grown hydroponically in Cd-contaminated media (BCL – biologically contaminated lettuce). That study relied on an assumption that a high proportion of Cd in the BCL treatment group would be bound to Cd–S-conjugates. This assumption was borne out by a subsequent sub-cellular fractionation study (Monteiro et al., 2008) that indicated that 22.4% of Cd was bound

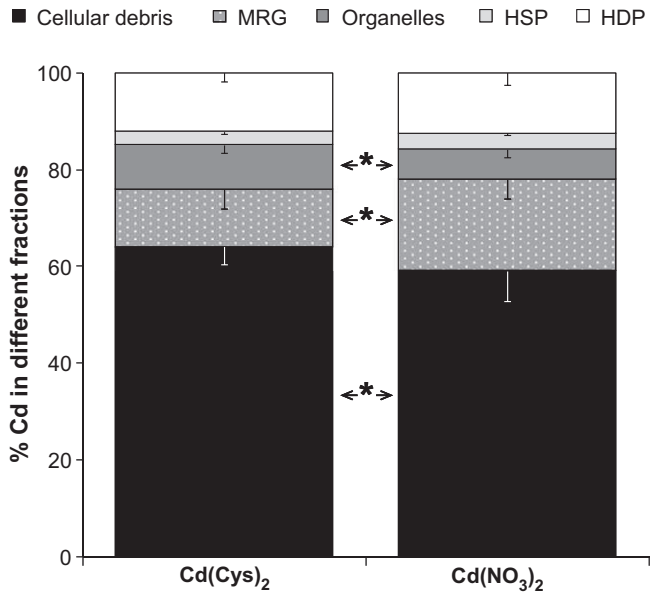

Fig. 3. Percentage distribution of Cd in sub-cellular fractions of *Porcellio dilatatus* (means ± S.D., $n = 6$) fed $Cd(Cys)_2$ or $Cd(NO_3)_2$. Significant differences between $Cd(Cys)_2$ and $Cd(NO_3)_2$ fractions ($p < 0.05$) are indicated with asterisks.

to the heat stable protein fraction (MT-like proteins). Therefore, in several aspects, the present study parallels that of Calhôa et al. (2006), with numerous similarities, but also some distinct differences.

Firstly, food consumption and assimilation indices are similar to those reported by Calhôa et al. (2006). As was the case with the BCL treatment group (Calhôa et al., 2006), food assimilation among isopods in the $Cd(Cys)_2$ treatment group was higher than other treatment groups, and likely reflects an increase in palatability conferred by cysteine residues.

Secondly, in both studies (present study and Calhôa et al., 2006) growth was poor, although the inclusion of gelatine as a source of nitrogen in the present study did improve growth indices in control and $Cd(Cys)_2$ treatment groups. The negative growth rates among isopods in the $Cd(NO_3)_2$, treatment remain similar to that observed in Calhôa et al. (2006), and is therefore indicative of a distinct difference between the $Cd(NO_3)_2$ and $Cd(Cys)_2$ treatment groups. Growth inhibition among isopods is a commonly reported consequence of Cd exposure (Odendaal and Reinecke, 2004), and the fact that food assimilation in the $Cd(NO_3)_2$ treatment group was similar to controls indicates that the poor growth indices are not simply a reflection of an avoidance behaviour (Odendaal and Reinecke, 1999), but rather, may reflect the increased cost of detoxification of Cd^{2+} (Rowe, 1998; Rowe et al., 1998; Hopkins et al., 1999). The absence of a similar growth inhibition among isopods in the $Cd(Cys)_2$ treatment group is notable, and suggests that Cd, when presented as a Cd–S-conjugate, does not elicit the same metabolic response as Cd^{2+}. This inference requires verification because Cd assimilation was slightly (but significantly) lower in the $Cd(Cys)_2$ treatment group and the growth inhibition among isopods fed $Cd(NO_3)_2$ may occur simply as a consequence of higher Cd assimilation.

In the present study, the assimilation efficiency of $Cd(Cys)_2$ is relatively low (20%) compared to $Cd(NO_3)_2$, and much lower than the Cd AE in the BCL group (~50%) reported previously (Calhôa et al., 2006), and confirms the relatively low bioavailability of Cd associated with Cd–S-conjugates (Groten et al., 1991; Mann et al., 2006). Monteiro et al. (2008) examined the sub-cellular distribution of Cd in lettuce following hydroponic contamination, and demonstrated that only a small proportion of metal (22.4%) was bound to a sub-cellular fraction (HSP) synonymous with phytochelatins or MT-like proteins. In the same study, Monteiro et al. (2008) provided isopods with isolated sub-cellular fractions, and similarly demonstrated that the Cd in the fraction containing Cd–S-conjugates (HSP) had a low AE (22.8%), which is close to the AE for Cd in $Cd(Cys)_2$ in the present study, suggesting that the ability of isopods to assimilate $Cd(Cys)_2$ is the same as their ability to assimilate Cd associated with HSP. Another interesting similarity with Monteiro et al. (2008) is that the estimated Cd AE in all fractions in the isopod *P. dilatatus* fed with Cd-contaminated lettuce was 44.1%; this is similar to the AE obtained by Calhôa et al. (2006) for BCL of 52%.

The concentration of Cd within food is known to affect AE of Cd (Zidar et al., 2003), and the small disparity in Cd concentrations in the food might be expected to explain the differences in Cd AE. However, the overall concentrations used in this study are high, and the effect of food-Cd-concentration is reduced when Cd intake exceeds the uptake capacity of the gut (Zidar et al., 2003). Therefore, the differences in Cd AE in the present study are unlikely to be as a consequence of differences in the concentrations of Cd in the food, although the effect of food-Cd-concentration on uptake of Cd–S-conjugates is not known, and requires clarification.

The manner in which Cd–S-conjugates are metabolised or detoxified by organisms may be quite different to the manner in which they handle Cd^{2+}. Although bioavailability of Cd bound within Cd–S-conjugates has been demonstrated in the present study and elsewhere to be low, some Cd is assimilated, and it is likely that $Cd(Cys)_2$ and other Cd–S-conjugates cross the gut epithelium intact (Groten et al., 1991; Sugawara and Sugawara, 1991; Harrison and Curtis, 1992). Cd-cysteinates are known to behave as molecular mimics at the sites of specific transport proteins that normally serve to absorb amino acids or oligopeptides (Zalups and Ahmad, 2003). In rats provided with a diet rich in Cd-MT, Cd was preferentially distributed to the kidney (Groten et al., 1991; Sugawara and Sugawara, 1991). In the present study, there was a significant ($p < 0.001$) difference in the sub-cellular distribution of Cd between the two Cd treatment groups. Isopods fed $Cd(Cys)_2$ had significantly more Cd distributed in the cell debris and organelles than isopods fed with $Cd(NO_3)_2$, at the expense of Cd compartmentalization in the MRG (Fig. 3). Although somewhat speculative, the negative growth among isopods fed $Cd(NO_3)_2$ may reflect the increased metabolic cost of Cd^{2+} detoxification along the metabolic pathway that results in sequestration in MRG. If Cd is assimilated as a Cd–S-conjugate, then active detoxification is possibly not required.

Exoskeleton is expected to be a major component of the Cell Debris fraction (Van Hattum et al., 1996), which is a distinct difference between the present study and those conducted with, for example, molluscs (e.g. Wallace et al., 2003; Wallace and Luoma, 2003). In other crustaceans, deposition of cadmium in the exoskeleton has been demonstrated (e.g. Bjerregaard, 1991). However, among terrestrial isopods the exoskeleton does not appear to be an important sink for Cd (Hopkin and Martin, 1982; Hames and Hopkin, 1991).

Apart from the implications for isopod growth under conditions of high Cd intake (as was the case in the present study), the overall consequences for isopod health with regard to accumulation of Cd^{2+} or Cd–S-conjugates remains unclear. Wallace et al. (2003) proposed a model for predicting which sub-cellular fractions might present a toxicological risk to those organisms possessing a metal burden (MSF), which fraction represent detoxified metal (BDM) and which fractions were available for transfer to predators (TAM). Fig. 4 represents the magnitude of these categories for the isopods in the present study, and it is clear that the MSF is similar for both treatment groups. However, just as application of this predictive template has proved unreliable for predicting trophic

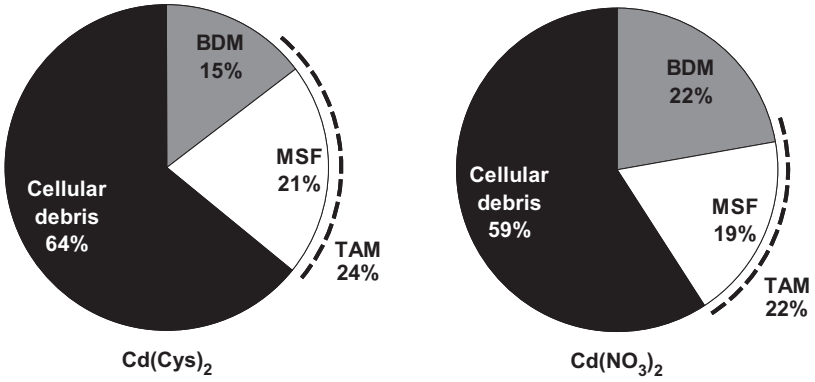

Fig. 4. Sub-cellular compartments in *Porcellio dilatatus* fed with $Cd(Cys)_2$ and $Cd(NO_3)_2$ based on the biological significance of the various sub-cellular fractions as proposed by Wallace et al. (2003). MSF (metal-sensitive fraction) = organelles + HDP; BDM (biologically detoxified fraction) = HSP + MRG, cellular debris and TAM (trophically available metal)(arc) = HSP + HDP + organelles.

transfer of metals to predators (e.g. Cheung and Wang, 2005; Steen Redeker et al., 2007; Monteiro et al., 2008; Rainbow and Smith, 2010), it remains unclear whether the MSF-Cd (Organelles and HDP) represents a greater toxic risk to the isopods in this study than any other fraction. Furthermore, there was a significant allocation of Cd to the cell debris fraction, and the toxicological significance of metal bound to cell debris remains undefined. Indeed the cell debris is likely the most poorly defined fraction in this technique. Experimental evidence will be required to determine if the form in which Cd is assimilated or the sub-cellular fraction to which it is allocated, can be correlated with toxic risk.

5. Conclusion

Results from this study shed more light on how the mechanisms by which plants and animals sequester and detoxify accumulated metals can determine metal speciation and subsequent bioavailability to consumers. This study presented a comparison of the bioavailability of Cd-cysteinate compared to a simple Cd salt ($Cd(NO_3)_2$) to terrestrial isopods, and demonstrated that Cd-speciation influences the degree of Cd assimilation by terrestrial isopods. $Cd(Cys)_2$ was significantly less bioavailable than the Cd provided as $Cd(NO_3)_2$. Perhaps more importantly, Cd speciation also dictated the internal compartmentalization, which may have implications for both toxicity and subsequent bioavailability to secondary predators. Treatment dependant differences in growth among isopods may be related to metabolic trade-offs associated with detoxification pathways that lead to the formation of metal-rich granules. Future studies that examine the trophic movement of metals in food chains and subsequent toxicity need to consider speciation and the manner in which different metal species are handled internally.

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