Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Soil Biology 41 (2005) 153–161
EUROPEAN JOURNAL OF SOIL BIOLOGY
http://france.elsevier.com/direct/ejsobi

Original article

Foundation studies for cadmium accumulation studies in terrestrial isopods—diet selection and diet contamination

Reinier M. Mann *, Paula Matos, Susana Loureiro, Amadeu M.V.M. Soares

Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Available online 17 October 2005

Abstract

Food palatability studies were carried out with the terrestrial isopods *Porcellio dilatatus* and *Porcellinoides pruinosus*. They were provided with leaves of lettuce (*Lactuca sativa*), pea (*Pisum sativum*), *Elodea* and alder (*Alnus glutinosa*) to determine their suitability as food substrates for metal accumulation tests with these isopods. Lettuce, pea and *Elodea* were also assessed for their ability to take up cadmium in hydroponic culture. In the feeding trials, growth, consumption and assimilation indices indicated that lettuce may be a suitable food, while the plant contamination trials indicated that lettuce and *Elodea* could take up large quantities of cadmium into the leaves. These studies form part of a project to develop a system for delivering metal contaminants to terrestrial isopods for metal accumulation studies that incorporate the principles of trophic transfer.

Keywords: *Porcellio dilatatus*; *Porcellinoides pruinosus*; Heavy metal; Cadmium; Bioavailability; Trophic transfer; Terrestrial ecotoxicology; *Lactuca sativa*; *Pisum sativum*; *Alnus glutinosa*; *Elodea*

1. Introduction

Various food sources have been utilised for dietary studies with terrestrial isopods, including, pet foods [9,14,20], carrot [45], and more importantly, the leaves of various deciduous trees [3] such as poplar [1,22,36], maple [10,20,44], hazelnut [11,21], hornbeam [47], or birch [9]. The leaves of the above listed trees are used because they are important components of the natural litters where isopods live. Recent dietary exposure studies in Portugal with *Porcellio dilatatus* and *Porcellinoides pruinosus* have relied on alder (*Alnus glutinosa*) leaves [24,38,40,42]. However, from the perspective of metal-toxicity/accumulation studies, there are two overriding problems with the use of tree leaves. Firstly, tree leaves are not always palatable to isopods at the outset, either because the nutrients within the leaves are not immediately available to isopods without substantial microbial preconditioning, or because the presence of phenolic compounds deter consumption prior to microbial preconditioning (for review see [50]). Second, tree species such as alder do not allow for the biological incorporation of metal contaminants into the food source (i.e. assimilation of metals by living organisms).

In recent years, a growing number of researchers have recognised the need to incorporate the principles of trophic transfer within their experimental design [2,13,15,19,23,28,29,32,33,41,46]. These authors provided their test species with prey items that had accumulated metallic contaminants while still alive. In this way, they attempt to simulate the movement of metallic contaminants through the food chain, and thereby incorporate within their tests, the complexities of metal

* Corresponding author. Tel.: +351 234 370 779; fax: +351 234 426 408.
E-mail address: rmann@bio.ua.pt (R.M. Mann).

1164-5563/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejsobi.2005.09.013

speciation and bioavailability in biological systems. The results of such studies are not easily predictable, because both the metal binding properties of the prey species and subsequent bioavailability to the predator are likely to be highly variable. For example, Harrison and Curtis [18] found the assimilation of Cd by trout to be much higher from biologically Cd-contaminated amphipods than Cd from an artificially contaminated diet. Conversely, lacewings feeding on aphids growing on Cd-contaminated media did not assimilate the Cd pool that had been accumulated by the aphids [32].

Isopods are saprophytic detritivores. If the food they consume is contaminated with a metallic compound, it is not likely to be present as an inorganic salt. It is more likely to be present in a form that has resulted from biological transformation by either the micro-organisms growing on the decaying vegetable matter or by the organic matter itself while it was part of a living system. Previous examinations of metal toxicity in terrestrial isopods have relied exclusively on addition of inorganic metal salts to organic food substrates. The degree to which the metals in those studies were transformed into 'species' of greater or lesser bioavailability is dependant on the degree of microbial activity within the experimental systems. The aim of this study was to develop a system of delivery of biologically transformed metallic contaminants to terrestrial isopods that minimises the confounding effects of microbial activity; such a delivery system should provide consistency of metal-speciation and bioavailability for the purpose of metal accumulation studies. The system requires a nutritious plant food that can be easily grown in contaminated media within the laboratory. Several easily grown vegetables such as lettuce [5], rape [12], alfalfa [35], pea [48] and some aquatic macrophytes [34,37] presented themselves as worthy candidates for metal accumulation. This study asked two questions:

1. Which of the candidate feeds promotes growth in juvenile *P. dilatatus* Brandt, 1833 and *P. pruinosus* Brandt, 1833?
2. Which of the candidate feeds most effectively accumulates Cd within the leaf tissue?

2. Materials and methods

2.1. Test organisms

Isopods were selected from in-house cultures. Cultures of *P. pruinosis* were derived from individuals collected from an unpolluted site in Coimbra, Portugal [24]. They were maintained within plastic containers at 25 °C, with a 16:8 h (light:dark) photoperiod, on a substrate of commercial potting soil (with at least 10% organic matter). Cultures of *P. dilatatus* were derived from individuals collected from a secondary coastal dune system in central Portugal. They were maintained at 20 °C with a 16:8 h (light:dark) photoperiod on a substrate of sand within plastic containers. Alder leaves were provided as food for both species.

2.2. Candidate feeds

Lettuce (*Lactuca sativa* cv.Reine de Mai de Pleine Terre) and pea plants (*Pisum sativum*) were grown from seeds obtained from commercial suppliers (see below). The lettuce provided as food for *P. pruinosus* was bought commercially from an organic market garden and the lettuce provided to *P. dilatatus* was grown hydroponically. *Elodea canadensis* was purchased commercially as adult stems (25 cm) and maintained at 25 °C in 30 l of nutrient medium. Alder leaves were harvested from local trees. The leaves of each food type were dried for 2 days at 60 °C before the feeding studies.

2.3. Feeding study

Individual juvenile isopods were isolated within polyethylene terephthalate (PET) boxes (ø 85 mm × 43 mm; Termoformagen, Leiria, Portugal). The bottom of each box was replaced with a 2 mm nylon screen Each of these boxes was inserted within a second box containing a thin layer of plaster of Paris mixed with activated charcoal (8:1 vol/vol) for the retention of added moisture. The distance between the nylon screen and the plaster of Paris was ~5 mm.

Individuals of both species were fed exclusively on one of each of the candidate feeds for a period of 4 weeks. Ten individuals were allocated to each food type. One day before the beginning of each trial, isopods were isolated within their boxes without food to purge their guts. At the beginning of each trial the average mass (± standard error (S.E.)) of isopods was 5.2 ± 0.26 mg for *P. pruinosis* and 17.5 ± 0.70 mg for *P. dilatatus*. The food was cut into individual portions weighing between 14 and 20 mg (dry weight) and moistened before placing it within each box. Faecal material was removed from the surface of the plaster of Paris every 2 days and dried (2 days at 60 °C). At the end of each week, the food was replaced with fresh leaves of a known mass, and the remains of the old

food were dried (2 days at 60 °C) and weighed. The isopods were also weighed weekly. After 4 weeks, the isopods were starved for 1 day to empty the gut before a final weight was determined. Data on isopod, and leaf mass were used to determine indices of food consumption, and isopod growth. An analysis of variance with Games–Howell post-hoc test for samples with unequal variance was used to determine if significant ($\alpha = 0.05$) differences in these indices existed among different diets.

2.4. Lettuce, pea and Elodea contamination

Lettuce seeds were germinated on a bed of perlite moistened with distilled water and subsequently grown hydroponically at 25 °C on a ~6 mm column of perlite within polystyrene seedling trays (24 mm; Polisur 2000, Huelva, Spain) floating on nutrient media within plastic boxes. Pea was germinated by immersion in running, oxygenated tap water for 3 days and subsequently grown in a similar manner as lettuce, but without the perlite. The nutrient media used for growth of lettuce, pea and maintenance of *Elodea* were based on Hoagland's media (Table 1). For all plants a 16:8 (light: dark) photoperiod was established with an array of fluorescent tubes (Mazdafluor Prestilflux TFP 36W/CFT) suspended ~30 cm above the seedlings.

Lettuce seedlings were grown for a period of 5 weeks in six identical hydroponic systems, each with seven lettuce seedlings. After 5 weeks the nutrient media (Table 1) was altered to include Cd as $Cd(NO_3)_2$. Five treatments and a control were established; 0, 18, 32, 56, 100, 178 μM Cd. The Cd was added from a 197.5 mM $Cd(NO_3)_2$ stock solution that also contained 21 μCi ml^{-1} ^{109}Cd. The lettuce plants were grown within the contaminated media for a further 7 days, and then harvested. Pea plants were grown in nutrient media (Table 1) and exposed to Cd in a similar manner to lettuce but without the addition of radio-tracer. At the time of harvest, the roots of lettuce and pea plants were soaked for 5 min in 20 mM EDTA (titriplex, Merck) and then washed twice in distilled water to remove externally bound Cd [17,48]. The leaves were individually separated from each plant and weighed. Leaves, roots and stems (pea) were dried (2 days at 60 °C) before analysis for Cd.

Table 1
Chemical composition of growth media for lettuce, pea and *Elodea*

	Lettuce	Pea	*Elodea*
Macronutrients	mM		
KNO_3	6	12	2
$Ca(NO_3)_2$	4	8	1.33
$NH_4H_2PO_4$	2	4	2
$MgSO_4$	2	2	0.66
Micronutrients	μM		
H_3BO_3	50	46	15
$MnCl_2$	10	2.4	3.3
$ZnSO_4$	0.77	4.0	0.24
$CuSO_4$	0.36	1.0	0.12
Na_2MoO_4	0.37	1.0	0.12
Fe^{3+}–EDTA	4.5	4.6	1.5

Elodea was exposed to Cd (also without the radio-tracer) in flow-through systems. The system employed 1.5-l PET bottles with an inlet at the bottom of the bottle and an outlet near the top. Cadmium amended nutrient media (Table 1) was introduced at a rate of 8 ml h^{-1}. Four treatments and a control were established; 0, 32, 56, 100, 178 μM Cd. At the end of the exposure the plants were soaked for 5 min in 20 mM EDTA to remove externally bound Cd. The plants were separated into stems and leaves, and dried (2 days at 60 °C) before analysis for Cd.

All growth media were analysed at the beginning of the exposure period. Additional samples of growth media were analysed for Cd after 3 and 7 days for lettuce and after 7 days for *Elodea*. For lettuce, a second set of samples of growth media taken at 7 days were filtered through 0.45 μm syringe filters to discriminate between solid phase and dissolved phase Cd [27]. The samples were analysed for Cd by inductively coupled plasma spectroscopy (ICPS) in a Jobin Ivon JY70 with a Meinard C001 nebuliser. All lettuce leaves that were greater than 1 g (wet wt) were divided in half along the midline. One half of each leaf (midvein excluded) was crushed in a mortar and pestle and digested for 48 h at 75 °C in a mixture of 1 ml 2 M HNO_3 (GR for analysis, Merck, Darmstadt, Germany) and 0.5 ml 35% H_2O_2 (Purum, Fluka, Buchs, Switzerland). The digests were made up to 10 ml, centrifuged at 3.2 × *g* (4500 rpm) in a desktop centrifuge (MPW, Warsaw, Poland) and the supernatants were analysed for Cd by ICPS. A subsample of leaf extracts were analysed by radiospectrometry to evaluate the applicability of this kind of non-destructive analysis.

Uniformity of contamination across each lettuce leaf was determined for a sub sample of leaves from plants exposed to 100 μM Cd by radiospectrometry. Sections of dry leaf representing different regions of the leaf (midvein excluded) with an average mass (± S.E.) of 4.6 ± 0.1 mg were counted in a 1470 Wallac WIZARD automated gamma counter (Perkin Elmer, Wellesley, MA, USA) for up to 5 min (i.e. long enough to maintain counting errors below 1%). Specific activity was assessed by comparing gamma counts and ICP analysis

of the 100 μM Cd contaminated growth media. An analysis of variance with Games–Howell post-hoc test for samples with unequal variance was used to determine if significant ($\alpha = 0.05$) differences existed among Cd concentrations of different leaf regions.

In a second contamination trial, lettuce seeds were germinated and grown as described above for a period of 5 weeks in hydroponic systems. After 5 weeks the nutrient media was altered to include 100 μM Cd as $Cd(NO_3)_2$. The Cd was added from a 200 mM $Cd(NO_3)_2$ stock solution that also contained 21 μCi ml^{-1} ^{109}Cd. The plants were grown within this amended media for a further 14 days with replacement of growth media every 2 days to avoid large changes in Cd concentration as a consequence of evaporation or exclusion from the plants. Three plants were harvested at each sampling time, after exposure for 1, 3, 7 and 14 days. The leaves were individually separated from each plant, weighed and dried (2 days at 60 °C). Those leaves that represented leaf numbers 3 and 4 (i.e. the third and fourth youngest leaves) after 1 day of exposure to 100 μM Cd (i.e. after 3, 7 and 14 days, these same leaves were represented by leaf numbers 5 and 6, 7 and 8, and 9 and 10, respectively) were analysed for Cd by radiospectrometry as described above. Only leaves 3 and 4 were analysed because older leaves failed to survive the full 14 days.

3. Results

3.1. Feeding trial

Soil grown lettuce was the better of the three candidate feeds for growth in juvenile *P. pruinosis* over a period of 28 days (Fig. 1). Growth in *P. dilatatus* was also higher in those animals fed hydroponic lettuce than those fed leaves of pea, *Elodea*, or alder, although not significantly better than those provided with pea and *Elodea*. Both species of isopods feeding on lettuce leaves displayed significantly higher levels of food consumption than isopods feeding on either alder or *Elodea* leaves (Table 2). Isopods feeding on pea leaves (*P. dilatatus* only) also displayed relatively high levels of food consumption (Table 2). The moderate growth rates amongst *P. dilatatus* feeding on *Elodea* leaves occurred despite the relatively low rates of consumption. Also, the lowest rates of mortality were seen among isopods feeding on *Elodea* (Table 2). Growth was poor in isopods feeding on alder leaves with high levels of mortality (Table 2 and Fig. 1).

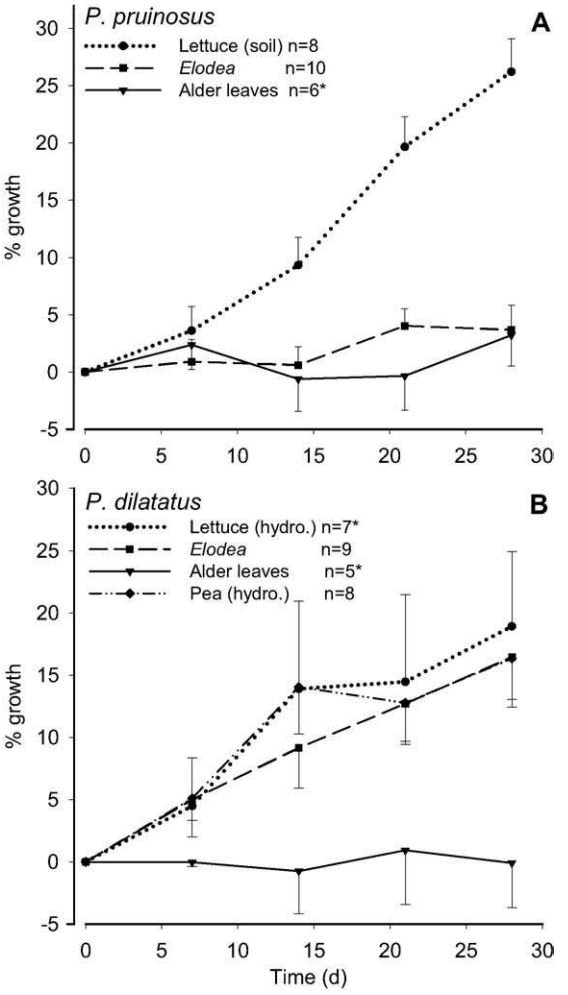

Fig. 1. Percentage growth over successive weeks in (A) *P. pruinosus* and (B) *P. dilitatus*. Low *N* values (*) are indicative of mortality (alder) or the presence of females that produced young during the trial (lettuce).

Table 2
Isopod feeding parameters for *P. pruinosus* and *P. dilatatus*. Superscript letters denote statistically significant ($P < 0.05$) groupings in analysis of variance with Games–Howell post-hoc test

Feed	Consumption (mg day^{-1})	Growth (μg day^{-1})	Growth over 28 days (%)
P. pruinosus			
Lettuce ($N = 8$)	1.45 ± 0.02^a	57.6 ± 5.5^a	26.2 ± 2.4^a
Elodea ($N = 10$)	0.97 ± 0.04^b	10.0 ± 3.3^b	4.5 ± 1.6^b
Alder ($N = 6$)	0.55 ± 0.02^c	3.9 ± 2.5^c	3.2 ± 1.9^b
P. dilatatus			
Lettuce ($N = 7$)	1.75 ± 0.06^a	115 ± 36^a	18.9 ± 6.0^a
Elodea ($N = 9$)	0.93 ± 0.06^b	82 ± 15^a	16.5 ± 3.4^a
Pea ($N = 8$)	1.96 ± 0.05^a	82 ± 17^a	16.3 ± 3.9^a
Alder ($N = 5$)	0.52 ± 0.05^c	-50 ± 29^b	-5.78 ± 4.4^b

3.2. Lettuce, pea, and Elodea contamination

Measured Cd concentrations at the beginning of each exposure were close to the predicted nominal concentrations (Table 3). Also, Cd concentrations determined by radiospectrometry were within ± 10% of those obtained by ICP. For lettuce the concentration within each nutrient solution changed little over the first 3 days of exposure, but after 7 days, the concentration of Cd in the 18, 32, 56, 100 μM exposure solutions increased by 137%, 51%, 17%, and 74%, respectively. Filtration of these 7-day samples indicated that 19.7%, 2.9%, 8.8% and 7.6% of the Cd that remained in each of the solutions was not available for uptake (Table 3). Nutrient media for pea plants were not analysed for Cd after the beginning of the test. After 7 days the Cd concentration within the 32, 56, and 100 μM exposure solutions for *Elodea* had decreased by 8.7%, 18.6% and 16.6%, respectively.

Lettuce, pea and *Elodea* plants exposed to 178 μM Cd either died or deteriorated over the 7 days, and no further analysis was performed on these plants. The leaves of *Elodea* plants exposed to 56 and 100 μM Cd turned chlorotic within 4 days but there was also new growth on each of the stems and the stems remained green. All plants exposed to Cd between 18 and 100 μM displayed dose dependant accumulation of Cd within roots, leaves, and stems (Fig. 2).

No Cd was detected in any of the control plants. In both pea and lettuce, the roots accumulated higher tissue burdens than leaves or stems. Similarly, the stems of the *Elodea* and pea plants accumulated higher tissue burdens than the leaves. The leaves of lettuce accumulated much higher Cd burdens than pea (Fig. 2). *Elodea* leaves accumulated very high Cd burdens (Fig. 2). In lettuce leaves there was a large degree of variability in the concentration of Cd in different leaf regions. Regions closest to the midvein had higher concentrations of Cd than marginal regions (Fig. 3). The analysis of variance did not highlight formal differences ($\alpha = 0.05$) between central and marginal regions because of the large variation between individual leaves that is reflected in the large S.E.s (Fig. 3). However, for individual leaves, the central regions of the leaf always had average Cd concentrations that were 18–50% higher than the average Cd concentration for marginal regions.

In the time course study with lettuce, plants that were exposed for 2 weeks to 100 μM Cd did not accumulate more Cd than they did after 7 days (Fig. 4).

4. Discussion

4.1. Food preference

Dietary toxicity or metal accumulation studies with isopods require a substrate that can be used as a high quality and consistent source of nutrition for the animals over the test-period because poor nutrition during the test may affect the outcome of the test [14,31]. Alder leaves have formed the principal diet of isopods within our laboratory cultures, and have proved to be a better source of nutrition than other hard-leaved plants [43], and a better source of nutrition when offered exclusively, than when offered with other dietary supplements [4]. However, within the laboratory cultures, microbial colonisation of the leaves is encouraged. Microorganisms are known to improve palatability of hard leaves for saprophytic detritivores, and make nutrients more available to them (for review see [49,50]). The poor performance of the isopods provided with alder leaves in this study is likely a consequence of weekly replacement of the food, which impeded widespread colonisation of the leaves by fungi. The use of easily digestible food substrates that can be replaced frequently within a dietary study goes some way to avoiding the additional and unpredictable influence of microbial action.

Table 3
Cadmium concentrations in exposure media for lettuce, pea and *Elodea*. ND, Not detected above ICP detection limit of 1.5 μg l^{-1}

Nominal [Cd] (μM)	Measured [Cd] (μM)						
	$t = 0$ days			$t = 3$ days	$t = 7$ days		$t = 7$ days (filtered)
	Lettuce	Pea	*Elodea*	Lettuce	Lettuce	*Elodea*	Lettuce
0	ND	ND	ND	ND	ND	ND	ND
18	17.7	17.4	–	18.4	42.1	–	33.7
32	36.2	34.7	36.7	36.6	54.7	33.5	53.1
56	55.2	52.9	62.5	61.3	64.6	50.8	59.0
100	100.3	91.4	105.3	97.0	175.4	87.9	162.1
178	172.5	160.2	172.5	182.9	198.69	–	196.0

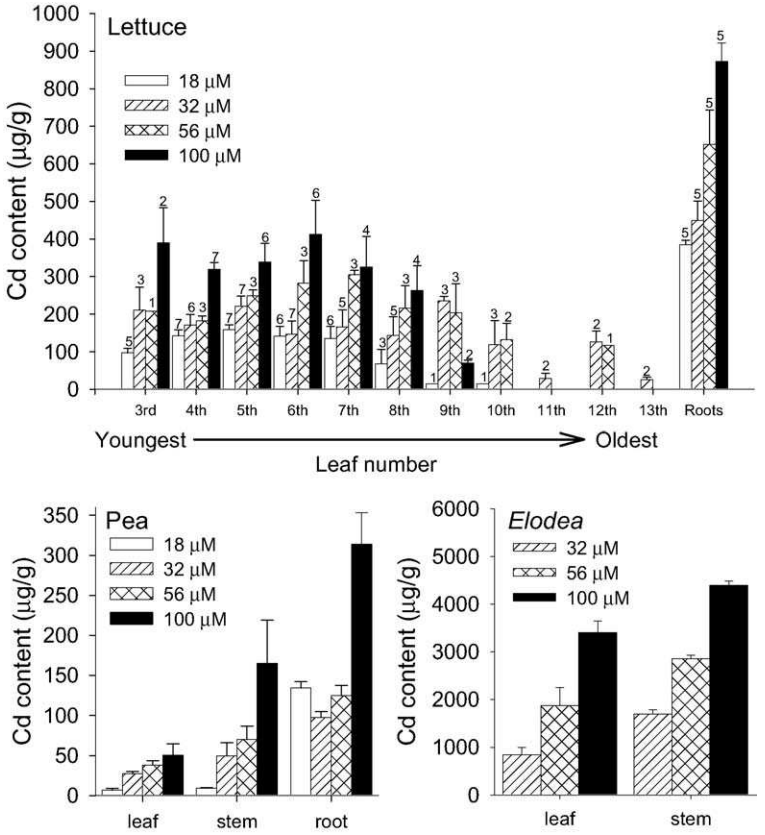

Fig. 2. Cadmium accumulation within leaves, roots and stems of lettuce, pea and *Elodea* plants grown for 7 days in nutrient solution amended with 18–100 µM $Cd(NO_3)_2$. The error bars represent standard error. For lettuce, the numbers of leaves (N) are indicated above each bar. For pea and *Elodea*, $N = 3$.

On the basis of the results presented here, lettuce is likely to provide the necessary level of nutrition over the course of a dietary study. It must be stressed though, that the data presented here do not provide information on the long-term suitability of any of the candidate feeds. The high levels of consumption, but relatively low levels of growth for *P. pruinosus* feeding on *Elodea*, and *P. dilatatus* feeding on pea leaves are consistent with a compensatory strategy to obtain maximal nutrition from a relatively 'low-quality' food (for review see [49]). Such a strategy may also involve coprophagy or increased gut residence time. For the purpose of dietary toxicity studies, food consumption rates, gut residence and even coprophagy may be important factors in assimilation of added contaminants.

The palatability data presented here also provide few insights into the palatability of metal contaminated leaves. However, preliminary data indicate that consumption of lettuce leaves previously contaminated with an average of 375 µg Cd g^{-1} (contaminated through hydroponic culture) is also readily consumed by isopods, though at a significantly lower rate than uncontaminated leaves (unpublished data).

4.2. Food contamination

An essential aspect of this study was to determine whether one or more of the candidate feeds could accumulate Cd to levels that would make it useful for a trophic transfer protocol to examine Cd accumulation in a terrestrial model. It is conceivable that Cd bound to strong organic ligands or metallothionein (MT)-like proteins (i.e. cysteine rich phytochelatins) within plant leaf tissue is more bioavailable than Cd added superficially to the diet of an isopod. Such a scenario assumes that the consumer possesses uptake mechanisms for Cd bound to cysteine and cysteine rich peptides, as was the case for Cd in biologically contaminated amphipods in the study by Harrison and Curtis [18]. The two terrestrial plants used in this study (lettuce and pea)

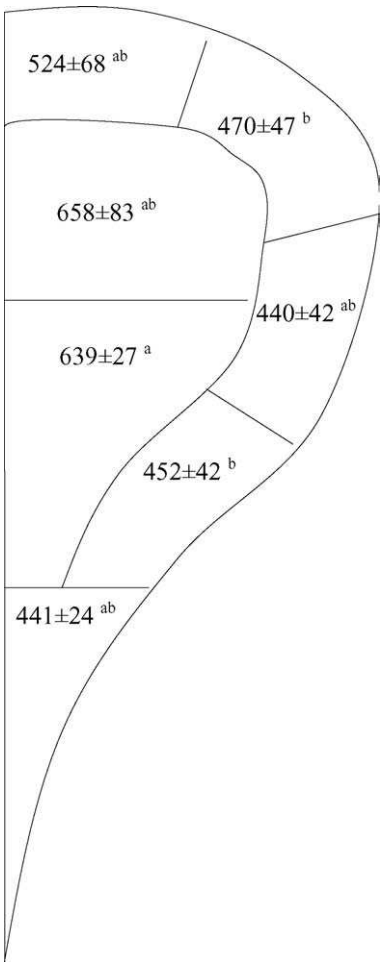

Fig. 3. Accumulation of Cd (μg g^{-1}) in different lettuce leaf regions. The numbers represent mean ± standard error ($N = 5$–9). Only half of each leaf (without midvein) was analysed. Superscript letters denote statistically significant ($P < 0.05$) groupings in analysis of variance with Games–Howell post-hoc test.

are known to actively take up divalent metal ions through there roots [6,7], and produce MT-like proteins in response to Cd uptake [25,26].

All three plant species accumulated Cd within their root, stem and leaf tissues. The accumulation and retention of larger amounts of Cd within roots than in leaves of both lettuce and pea likely occurs as a consequence of passive apoplasmic transport and binding to apoplastic tissue. In this scenario, further transport to the above-ground components via the xylem is restricted by the physical barrier of the casparian strip, a long diffusive distance, and numerous binding sites for metals within the apoplasm [30]. Movement into the shoots and leaves requires active transport across the plasma membrane, either as the free ion or a metal complex [30].

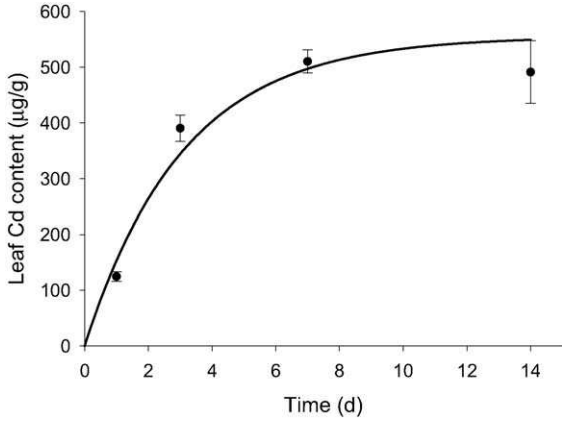

Fig. 4. Time–course of Cd accumulation within lettuce leaves over 14 days. The error bars represent standard error $N = 3$. At each time point the Cd concentration for each of the three plants is an average of the concentration in leaves 3 and 4.

Pea plants accumulated Cd in roots and aerial shoots to similar concentrations and in similar proportions as described by Wong et al. [48] who used similar exposure conditions as were used in this study. However, MT-like protein production in pea is restricted to the roots [25] which may explain the lower levels of Cd transport into leaf tissue compared to lettuce. Costa and Morel [7] suggested that up to 80% of Cd bound to lettuce roots was taken into cells through an active process with transport of Cd bound to an MT-like protein [26] into the leaves facilitated by transpirational flow [39]. The non-uniform accumulation within the leaves is consistent with the movement of protein bound Cd moving up into the leaves through the xylem as the plant transpires. The absence of further accumulation after 7 days likely occurred as a consequence of stomatal closure [8].

The aquatic macrophyte, *Elodea*, was by far the most efficient at accumulating Cd. Aquatic macrophytes are known to accumulate metals more efficiently than terrestrial plants, because there is direct uptake through the leaves [16]. Although it is likely that the soluble nature of Cd bound to MT-like proteins or other ligands within lettuce leaves confers a degree of bioavailability to the Cd [18], it remains to be seen what proportion of the high levels of Cd accumulated within the *Elodea* tissue is bioavailable to an isopod. It seems likely that much of the Cd was bound to apoplastic components in much the same way as Cd is bound to the apoplasm of roots in terrestrial plants [30], although the large accumulation seen within the stem, suggests uptake into the plants internal transport systems and possible binding to MT-like proteins.

5. Conclusion

This study has provided preparatory data for further studies in terrestrial isopods involving the trophic transfer of Cd and other metals. Soft leaved, easily grown vegetables such as lettuce provide a good nutritional substrate for dietary accumulation studies. Lettuce was shown to be particularly useful because isopods feeding on lettuce displayed high levels of consumption, assimilation and growth, and because high levels of Cd could be quantitatively accumulated within the leaves.

Acknowledgments

Thanks are extended to Armando Costa and Conceição Santos, of the Department of Biology, University of Aveiro, for advice on plant culture, Thanks also to Serafim Guimarães and Helder Pinheiro of the Institute of Pharmacology and Therapeutics, University of Porto, for access to their gamma counter. This work was supported through a grant from the Portuguese Fundação para Ciência e Tecnologia (FCT) and a post-doctoral fellowship awarded to R. Mann, also from FCT.

References

[1] H.M. Abdel-Lateif, M.H. Donker, N.M. Van Straalen, Interaction between temperature and cadmium toxicity in the isopod *Porcellio scaber*, Funct. Ecol. 12 (1998) 521–527.

[2] G. Allinson, L.J.B. Laurenson, G. Pistone, F. Stagnitti, P. L. Jones, Effects of dietary copper on the Australian freshwater crayfish *Cherax destructor*, Ecotoxicol. Environ. Saf. 46 (2000) 117–123.

[3] L. Beck, E. Brestowsky, Auswahl und verwertung verschiedener fallaubarten durch *Oniscus asellus* (Isopoda), Pedobiologia (Jena) 20 (1980) 428–441.

[4] I. Caseiro, S. Santos, J.P. Sousa, A.J. Nogueira, A.M.V. M. Soares, Optimization of culture conditions of *Porcellio dilatatus* (Crustacea: Isopoda) for laboratory test development, Ecotoxicol. Environ. Saf. 47 (2000) 285–291.

[5] G.P. Cobb, K. Sands, M. Waters, B.G. Wixson, E. Dorward-King, Accumulation of heavy metals by vegetables grown in mine wastes, Environ. Toxicol. Chem. 19 (2000) 600–607.

[6] C.K. Cohen, T.C. Fox, D.F. Garvin, L.V. Kochian, The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants, Plant Physiol. 116 (1998) 1063–1072.

[7] G. Costa, J.L. Morel, Efficiency of H^+-ATPase activity on cadmium uptake by 4 cultivars of lettuce, J. Plant Nutr. 17 (1994) 627–637.

[8] G. Costa, J.L. Morel, Water relations, gas-exchange and amino-acid content in Cd-treated lettuce, Plant Physiol. Biochem. 32 (1994) 561–570.

[9] T. Crommentuijn, C.J.A.M. Doodeman, J.J.C. Van Der Pol, A. Doornekamp, M.C.J. Rademaker, C.A.M. Van Gestel, Sublethal sensitivity index as an ecotoxicity parameter measuring energy allocation under toxicant stress: application to cadmium in soil arthropods, Ecotoxicol. Environ. Saf. 31 (1995) 192–200.

[10] D. Drobne, S.P. Hopkin, The toxicity of zinc to terrestrial isopods in a "standard" laboratory test, Ecotoxicol. Environ. Saf. 31 (1995) 1–6.

[11] D. Drobne, M. Rupnik, A. Lapanje, J. Štrus, M. Janc, Isopod gut microflora parameters as endpoints in toxicity studies, Environ. Toxicol. Chem. 21 (2002) 604–609.

[12] S.D. Ebbs, M.M. Lasat, D.J. Brady, J. Cornish, R. Gordon, L. V. Kochian, Phytoextraction of cadmium and zinc from a contaminated soil, J. Environ. Qual. 26 (1997) 1424–1430.

[13] H. Ettajani, B. Berthet, J.C. Amiard, L. Chevolot, Determination of cadmium partitioning in microalgae and oysters: Contribution to the assessment of trophic transfer, Arch. Environ. Contam. Toxicol. 40 (2001) 209–221.

[14] S. Farkas, E. Hornung, E. Fischer, Toxicity of copper to *Porcellio scaber* Latr. (Isopoda) under different nutritional status, Bull. Environ. Contam. Toxicol. 57 (1996) 582–588.

[15] I.D. Green, G. Merrington, M. Tibbett, Transfer of cadmium and zinc from sewage sludge amended soil through a plant-aphid system to newly emerged adult ladybirds (*Coccinella septempunctata*), Agric. Ecosyst. Environ. 99 (2003) 171–178.

[16] M. Greger, Metal availability and bioconcentration in plants, in: M.N.V. Prasad, J. Hagemeyer (Eds.), Heavy Metal Stress in Plants—From Molecules to Ecosystems, Springer-Verlag, Berlin, 1999, pp. 1–27.

[17] M. Greger, M. Johansson, A. Stihl, K. Hamza, Foliar uptake of Cd by Pea (*Pisum sativum*) and Sugar-Beet (*Beta vulgaris*), Physiol. Plant. 88 (1993) 563–570.

[18] S.E. Harrison, P.J. Curtis, Comparative accumulation efficiency of ^{109}cadmium from natural food (*Hyalella azteca*) and artificial diet by rainbow trout (*Oncorhynchus mykiss*), Bull. Environ. Contam. Toxicol. 49 (1992) 757–764.

[19] S.E. Hook, N.S. Fisher, Sublethal effects of silver in zooplankton: importance of exposure pathways and implications for toxicity testing, Environ. Toxicol. Chem. 20 (2001) 568–574.

[20] E. Hornung, E. Fischer, S. Farkas, Isopod reproduction as a tool for sublethal-toxicity tests, Isr. J. Zool. 44 (1998) 445–450.

[21] V. Jereb, M. Horvat, D. Drobne, B. Pihlar, Transformations of mercury in the terrestrial isopod *Porcellio scaber* (Crustacea), Sci. Total Environ. 304 (2003) 269–284.

[22] D. Lavy, M.J. Van Rijn, H.R. Zoomer, H.A. Verhoef, Dietary effects on growth, reproduction, body composition and stress resistance in the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*, Physiol. Entomol. 26 (2001) 18–25.

[23] E.M. Lores, J.R. Pennock, Bioavailability and trophic transfer of humic-bound copper from bacteria to zooplankton, Mar. Ecol. Prog. Ser. 187 (1999) 67–75.

[24] S. Loureiro, J.P. Sousa, A.J. Nogueira, A.M. Soares, Assimilation efficiency and toxicokinetics of ^{14}C-lindane in the terrestrial Isopod *Porcellionides pruinosus*: the role of isopods in degradation of persistent soil pollutants, Ecotoxicology 11 (2002) 481–490.

[25] E. Lozano-Rodriguez, L.E. Hernandez, P. Bonay, R.O. Carpena-Ruiz, Distribution of cadmium in shoot and root tissues of maize and pea plants: physiological disturbances, J. Exp. Bot. 48 (1997) 123–128.

[26] E.A. Maier, R.D. Matthews, J.A. McDowell, R.R. Walden, B. A. Ahner, Environmental cadmium levels increase phytochelatin and glutathione in lettuce grown in a chelator-buffered nutrient solution, J. Environ. Qual. 32 (2003) 1356–1364.

[27] R.M. Mann, M.J. Ernste, R.A. Bell, J.R. Kramer, C.M. Wood, Evaluation of the protective effect of reactive sulfide on the

acute toxicity of silver to rainbow trout (*Oncorhynchus mykiss*), Environ. Toxicol. Chem. 23 (2004) 1204–1210.

[28] R.M. Mann, M. Grosell, A. Bianchini, C.M. Wood, Biologically incorporated dietary silver has no ion regulatory effects in American red crayfish (*Procambarus clarkii*), Environ. Toxicol. Chem. 23 (2004) 388–395.

[29] M. Maryanski, P. Kramarz, R. Laskowski, M. Niklinska, Decreased energetic reserves, morphological changes and accumulation of metals in carabid beetles (*Poecilus cupreus* L.) exposed to zinc- or cadmium-contaminated food, Ecotoxicology 11 (2002) 127–139.

[30] M.J. McLaughlin, Bioavailability of metals to terrestrial plants, in: H.E. Allen (Ed.), Bioavailability of Metals in Terrestrial Ecosystems: Importance of Partitioning for Bioavailability to Invertebrates, Microbes and Plants, Society of Environmental Toxicology and Chemistry, Pensacola, FL, USA, 2002, pp. 39–68.

[31] E.W. McNulty, F.J. Dwyer, M.R. Ellersieck, E.I. Greer, C. G. Ingersoll, C.F. Rabeni, Evaluation of ability of reference toxicity tests to identify stress in laboratory populations of the amphipod *Hyalella azteca*, Environ. Toxicol. Chem. 18 (1999) 544–548.

[32] G. Merrington, D. Miller, M.J. McLaughlin, M.A. Keller, Trophic barriers to fertilizer Cd bioaccumulation through the food chain: a case study using a plant–insect predator pathway, Arch. Environ. Contam. Toxicol. 41 (2001) 151–156.

[33] I.H. Ni, W.X. Wang, Y.K. Tam, Transfer of Cd, Cr and Zn from zooplankton prey to mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* fishes, Mar. Ecol. Prog. Ser. 194 (2000) 203–210.

[34] P.M. Outridge, B.N. Noller, Accumulation of toxic trace-elements by fresh-water vascular plants, Rev. Environ. Contam. Toxicol. 121 (1991) 1–63.

[35] J.R. Peralta-Videa, J.L. Gardea-Torresdey, J. Walton, W. P. Mackay, M. Duarte-Gardea, Effects of zinc upon tolerance and heavy metal uptake in alfalfa plants (*Medicago sativa*), Bull. Environ. Contam. Toxicol. 70 (2003) 1036–1044.

[36] A.D. Pokarzhevskii, N.M. van Straalen, The influence of dietary nitrogen and phosphorus on Cd accumulation in the woodlouse *Porcellio scaber* Latr, Appl. Soil Ecol. 16 (2001) 97–107.

[37] M.N.V. Prasad, P. Malec, A. Waloszek, M. Bojko, K. Strzalka, Physiological responses of *Lemna trisulca* L. (duckweed) to cadmium and copper bioaccumulation, Plant Sci. 161 (2001) 881–889.

[38] S. Ribeiro, J.P. Sousa, A.J. Nogueira, A.M. Soares, Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*, Ecotoxicol. Environ. Saf. 49 (2001) 131–138.

[39] D.E. Salt, R.C. Prince, I.J. Pickering, I. Raskin, Mechanisms of cadmium mobility and accumulation in Indian mustard, Plant Physiol. 109 (1995) 1427–1433.

[40] S.A.P. Santos, J.P. Sousa, M. Frost, A.M.V.M. Soares, Time-dependent toxicokinetics of ^{14}C-lindane in the terrestrial isopod *Porcellionides pruinosus*, Environ. Toxicol. Chem. 22 (2003) 2219–2227.

[41] O. Simon, A. Boudou, Use of the biological model, the crayfish as a biomarker of pollution. Consequences in term of cadmium trophic transfer rates, Bull. Fr. Pech. Piscic. (2002) 795–803.

[42] J.P. Sousa, S. Loureiro, S. Pieper, M. Frost, W. Kratz, A. J. Nogueira, A.M. Soares, Soil and plant diet exposure routes and toxicokinetics of lindane in a terrestrial isopod, Environ. Toxicol. Chem. 19 (2000) 2557–2563.

[43] J.P. Sousa, J.V. Vingada, S. Loureiro, M.M. da Gama, A.M.V. M. Soares, Effects of introduced exotic tree species on growth, consumption and assimilation rates of the soil detritivore *Porcellio dilatatus* (Crustacea: Isopoda), Appl. Soil Ecol. 9 (1998) 399–403.

[44] K. Szlávecz, V.C. Maiorana, Supplementary food in the diet of the terrestrial isopod *Porcellio scaber* Latr (Isopoda: Oniscidea), Isr. J. Zool. 44 (1998) 413–421.

[45] B. Ullrich, V. Storch, H. Schairer, Bacteria on the food, in the intestine and on the feces of the woodlouse *Oniscus asellus* (Crustacea, Isopoda)—species composition and nutritive-value, Pedobiologia (Jena) 35 (1991) 41–51.

[46] W.G. Wallace, S.N. Luoma, Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM), Mar. Ecol. Prog. Ser. 257 (2003) 125–137.

[47] B. Witzel, The influence of zinc on the uptake and loss of cadmium and lead in the woodlouse, *Porcellio scaber* (Isopoda, Oniscidea), Ecotoxicol. Environ. Saf. 47 (2000) 43–53.

[48] Y.S. Wong, H.M. Lam, E. Dhillon, N.F.Y. Tam, W.N. Leung, Physiological effects and uptake of cadmium in *Pisum sativum*, Environ. Int. 14 (1989) 535–543.

[49] M. Zimmer, Nutrition in terrestrial isopods (Isopoda: Oniscidea): an evolutionary-ecological approach, Biol. Rev. 77 (2002) 455–493.

[50] M. Zimmer, G. Kautz, W. Topp, Leaf litter-colonizing microbiota: supplementary food source or indicator of food quality for *Porcellio scaber* (Isopoda: Oniscidea)? Eur. J. Soil Biol. 39 (2003) 209–216.