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Single and joint effects of Zn and Cd on *Porcellio scaber* (Crustacea, Isopoda) exposed to artificially contaminated food

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ABSTRACT

This study aimed at determining effects of Zn, Cd and their equitoxic mixtures on metal assimilation and food consumption of the terrestrial isopod *Porcellio scaber*, in relation to metal availability in the food. Cd was four times less water-extractable than Zn. Cd or Zn extractability was affected neither by metal concentration nor by the presence of the other metal. In single metal exposures, assimilation efficiency (AE) was up to five times higher for Cd than for Zn. In a mixture, AE of Cd significantly increased at low mixture concentrations and decreased at high mixture concentrations. AE of Zn significantly increased at intermediate mixture concentrations. Effects of the Zn and Cd mixture on food consumption were additive (28-day $EC_{50,total} = 1.10$ TU; $EC_{50,water\text{-}extractable} = 1.18$ TU) when based on total and water-extractable concentrations but antagonistic when related to internal metal concentrations in the isopods ($EC_{50,internal} = 1.40$ TU).

1. Introduction

In the natural environment, animals are usually exposed simultaneously to various contaminants via media (soil, water) and food. Joined effects of contaminants in a mixture may be stronger or weaker than expected from separate exposure due to interactions between mixture constituents (Calamari and Alabaster, 1980; Groten et al., 2001). Interactions outside the organism, together with physicochemical conditions of a substratum (pH, concentration of inorganic and organic ligands, etc.), might affect the availability of contaminants, and thus assimilation potential (Lock and Janssen, 2001; Van Straalen et al., 2005; Spurgeon et al., 2006). At the toxicokinetic phase, interactions between contaminants might affect uptake, distribution, metabolism or excretion of a single contaminant from the organism, while at the toxicodynamic phase interactions might affect chemical actions at target sites (Groten et al., 2001).

In this paper, single and joint effects of the essential metal Zn and the non-essential metal Cd were studied in terrestrial isopods *Porcellio scaber* Latr., exposed for 28 days to artificially contaminated food. *P. scaber* is worldwide distributed and one of the most investigated isopods in terrestrial ecotoxicology. It is a potential candidate for a standard test organism (Drobne and Hopkin, 1995; Drobne, 1997; Hornung et al., 1998) and for monitoring the bioavailability of metals to soil and leaf litter invertebrates (Hopkin et al., 1986; Dallinger et al., 1992; Hopkin et al., 1993). Toxic effects of Zn and Cd on *P. scaber* through single metal exposure were subject of numerous investigations (e.g., Beyer et al., 1984; Donker and Bogert, 1991; Drobne and Hopkin, 1995; Khalil et al., 1995; Drobne and Štrus, 1996; Farkas et al., 1996; Köhler et al., 1996; Bibič et al., 1997; Donker et al., 1998; Zidar et al., 2003). However, only little is known about joint effects of these metals, although they are commonly found associated in polluted environments (Hopkin and Hames, 1994; Witzel, 2000; Zidar, 2005). For risk assessment purposes and for proper interpretation of bio-monitoring data brought by terrestrial isopods, additional investigations of the joint action of metals are crucial.

The aim of this study was to quantify the joint effects of Zn and Cd in a mixture on metal assimilation and feeding, in relation to metal availability. The digestive system is the main route for the uptake of metals in terrestrial isopods. Uptake takes place in the gut, covered with permeable cuticle, and in the hepatopancreas, which is also the main storage site for metals (reviewed in Hopkin, 1989). Metal uptake from food depends mainly on metal concentration, food consumption rate, and availability of metals. Decreased consumption rate is a prime sub-lethal response at an organism level, followed by, e.g. growth rate reduction and

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extension of moult cycle (Drobne and Štrus, 1996; Zidar et al., 2005). By rejecting food contaminated with metals, isopods might also regulate metal uptake to a certain degree (Kaschl et al., 2002; Zidar et al., 2004, 2005). Availability of metals can be assessed by different extractions, presented and compared by Martin et al. (1976). In this study, only water-extractability of Zn and Cd was followed. Bioavailability was determined by measuring Cd and Zn uptake in the isopods from the homogenised complex food used. The effects on food consumption after single Zn and single Cd exposure were compared with effects of Zn and Cd mixtures by recalculation of metal concentrations in toxic units (Sprague, 1970). Possible mechanisms of Zn–Cd interactions are discussed.

2. Materials and methods

2.1. Origin of animals and experimental design

We used laboratory-raised animals of both sexes with live weights between 18 and 25 mg. The parent population was collected in an unpolluted environment in the vicinity of Ljubljana, Slovenia. Animals were separated (one per Petri dish) and fed for 28 days with complex food consisting of hazel leaves, gelatine and fish food in a 63:34:3 ratio (for details see Kaschl et al., 2002; Zidar, 2005; Zidar et al., 2005). Food was spiked with Zn or Cd or Zn and Cd mixtures using aqueous solutions of $Zn(NO_3)_2 \cdot 6H_2O$ ($\geq$99 pure, Scharlau, Barcelona, Spain) and $Cd(NO_3)_2 \cdot 4H_2O$ ($\geq$99 pure, Merck, Dermstad, Germany). Control animals were fed with food where no metals were added. Food pellets were offered in small plastic dishes ($\varnothing = 2$ cm, height of rim 3 mm) that separated food from moist filter paper placed on the bottom of Petri dishes. During the 28-day experiment food was renewed after 14 days. For each metal concentration or combination, 25 animals were used. Animals were checked for mortality twice a week. Animals that died were excluded from further calculations. The experiment was conducted in a climate chamber at 21 $\pm$1 °C, with 16/8 h light/dark.

Food consumption was measured as a difference in the dry weight of food pellets before and after feeding, divided by the dry weight of animals.

The experimental animals, *P. scaber*, used in this study were treated in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

2.2. Data analyses

To reduce the influence of food consumption rate on metal assimilation, assimilation efficiencies (AEs) of Zn and Cd were calculated as the increase of a single metal concentration in isopods per amount of metal consumed (adapted from Dallinger and Wieser, 1977) using the equation: $AE = ((C_a - BG)/(C_f FC)) \times 100$, with C_a = metal concentration in animals after the experiment (mg kg^{-1} dry body weight); BG = background metal concentration in animals (mean concentration in control animals in mg kg^{-1} dry body weight); C_f = measured metal concentration in food (mg kg^{-1} dry weight); FC = amount of food consumed (kg dry weight).

It was assumed that Cd and Zn had a similar mode of action at the level of food consumption of the isopods. For that reason, the toxic unit (TU) approach (Sprague, 1970) was used to evaluate the mixture effects of Zn and Cd on food consumption. Concentrations of Zn and Cd in a mixture were selected as equitoxic concentrations based on the results of previous studies (Zidar et al., 2003). Zn was tested at 650, 1300, 2600, 5200, 7800 mg Zn kg^{-1} dry food, Cd at 90, 180, 360, 720, 1080 mg Cd kg^{-1} dry food and the Zn and Cd mixture at 325+45, 650+90, 1300+180, 2600+360, 5200+720 mg Zn+mg Cd kg^{-1} dry food. These concentrations presented toxic strengths of approximately 0.125, 0.25, 0.5, 1 and 2 TU, respectively. After the experiment TU were recalculated based on the actual median effective concentrations (EC_{50}) for reduction of food consumption: $TU_i = C_i/EC_{50i}$. For total and water-extractable food concentrations and concentrations of Zn, Cd, and their mixtures in the isopods, EC_{50} was determined by the logistic model of Haanstra et al. (1985). Calculations were done with the computer programmes S-PLUS 4.5 and Systat 10. Dose–response curves for the single metals and the mixtures were compared applying a generalised-likelihood ratio test as described by Van Gestel and Hensbergen (1997) and using Systat 10.

Statistical analyses (ANOVA with Tukey's post hoc test) were performed by a computer programme SPSS 14.0 for Windows.

2.3. Metals analyses

After exposure to the metal-treated food, animals were food deprived for 24 h to empty their guts. Then they were lyophilised, weighed and completely digested in a concentrated nitric/perchloric acid mixture (7:1). After evaporation of the acid, the residue was dissolved in 0.1% HNO_3. Zn and Cd concentrations in whole animals were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100). Verification of the analytical procedure was performed using certified reference material for trace metals, TORT-2 (National Research Council of Canada), made of lobster hepatopancreas. The reference material was dried in a vacuum desiccator, weighted (10–20 mg/sample) and digested and analysed in the same way as the rest of the samples. Measured Zn and Cd concentrations in the reference material did not differ more than 10% from the certified concentrations.

Actual concentrations of Zn and Cd in food pellets were measured by AAS and compared to nominal concentrations. The concentration of water-extractable Zn and Cd in the food pellets was defined by soaking food pellets in 4 ml of demineralised water for 24 h at rising temperatures up to 40 °C, centrifuging the solution and analysing the supernatant for Zn and Cd concentrations by flame AAS. For this purpose freshly prepared food pellets with an average dry weight of 50.9 mg were selected randomly ($n = 5$). Relationship between extractable (C_{H_2O} in mg l^{-1}) and total (C_s in mg kg^{-1}) concentrations was analysed applying a Freundlich sorption isotherm, reading $C_s = K_f(C_{H_2O})^n$, in which K_f = Freundlich sorption constant (1 kg^{-1}) and n = constant indicative of the influence of concentration on metal desorption. To study the possible impact of the metals on each other's sorption, isotherms of the single metals and the mixtures were compared applying a generalised-likelihood ratio test in the programme Systat 10.

3. Results

3.1. Water-extractability of Zn and Cd in food

Total concentrations of Zn and Cd in control food were 25.4 and 0.6 mg kg^{-1}, respectively (Tables 1 and 2). Water-extractable concentrations of Zn and Cd in control food were below the detection limit.

Measured total concentrations of both metals in treated food on average did not differ more that 11% from nominal concentrations (Tables 1 and 2). Water-extractable concentrations of Zn ranged from 35% up to 50% of total Zn concentration in food samples. No statistically significant changes in water-extractability of Zn were found due to the addition of Cd (ANOVA, $p > 0.05$). Water-extractable Cd concentrations in Cd-enriched food ranged

Table 1
Nominal, total and water-extractable concentration of Zn in food, used in the toxicity experiments with isopods (*Porcellio scaber*).

	C	Zn 650	Zn 1300	Zn 2600	Zn 5200	Zn 7800	Zn 325 +Cd45	Zn 650 +Cd90	Zn 1300 +Cd 180	Zn 2600 +Cd 360	Zn 5200 +Cd 720
Nominal	0	650	1300	2600	5200	7800	325	650	1300	2600	5200
Total	25.4	720	1305	2683	5272	8224	346	697	1322	2627	5177
SE	0.97	6.27	2.80	22.70	45.17	23.59	0.83	9.31	7.01	14.19	33.44
Water-extractable	<0.001*	251	467	1041	2643	3254	120	271	540	1035	2409
SE		5.42	7.62	12.02	30.96	14.72	4.36	2.93	5.49	11.58	23.01
% WE (estimation)		35	36	39	50	40	35	39	41	39	47

Average and SE values, all in mg kg^{-1} dry weight ($n = 5$). *Under detection limit. %WE—water-extractable metal concentration in % of total metal concentration.

Table 2
Nominal, total and water-extractable concentration of Cd in food, used in the toxicity experiments with isopods (*Porcellio scaber*).

	C	Cd 90	Cd 180	Cd 360	Cd 720	Cd 1080	Zn 325 +Cd 45	Zn 650 +Cd 90	Zn 1300 +Cd 180	Zn 2600 +Cd 360	Zn 5200 +Cd 720
Nominal	0	90	180	360	720	1080	45	90	180	360	720
Total	0.6	95.4	188	394	766	1158	49.1	94.3	197	395	801
SE	0.19	0.25	1.07	2.33	3.72	9.66	0.14	1.03	0.96	1.66	4.73
Water-extractable	<0.002*	7.3	14.5	28.0	62.9	82.8	4.1	8.4	13.8	29.7	85.2
SE		0.19	0.46	0.41	1.32	1.70	0.19	0.35	0.56	0.79	1.72
% WE (estimation)		8	8	7	8	7	8	9	7	8	11

Average and SE values, all in mg kg^{-1} dry weight ($n = 5$). *Under detection limit. %WE—water-extractable metal concentration in % of total metal concentration.

between 7% and 8% of total concentrations and were not significantly affected by the addition of Zn (ANOVA, $p > 0.05$).

Freundlich sorption isotherms indicated that Cd was bound approximately four times stronger to the food than Zn, with K_f values of 1048 and 257 l kg^{-1}, respectively. For Cd, sorption isotherm was perfectly linear with $n = 1.007$, for Zn sorption decreased with increasing concentration: $n = 0.900$. Desorption of Cd and Zn was slightly but not significantly ($\chi^2 = 3.51$ and 2.23, respectively) lower in the mixture with $K_f = 853$ l kg^{-1}, $n = 0.941$ and $K_f = 231$ l kg^{-1}, $n = 0.917$, respectively.

To avoid confusion, nominal concentrations were used throughout the text and tables, but all calculations were done using measured values.

3.2. Assimilation of Zn and Cd from food

The average concentration of Zn in control animals was 140 mg kg^{-1} dry body weight (Fig. 1A). In animals exposed to Zn-contaminated food, internal Zn concentration increased with increasing exposure concentration in the food and reached 530 mg kg^{-1} dry body weight in the group exposed to 5200 mg Zn kg^{-1} food. The differences in internal Zn concentrations between groups fed with 2600–7800 mg Zn kg^{-1} food were not significant. The addition of Cd had no influence on Zn accumulation in the isopods except for the animals exposed to the mixture of 1300 mg Zn and 180 mg Cd kg^{-1} dry food, which contained significantly higher Zn levels (Tukey, $p < 0.05$).

Assimilation efficiency of Zn was around 5% in all groups exposed to single Zn, irrespective of Zn concentration (Fig. 2A). The addition of Cd significantly increased Zn AE in the groups Zn1300+Cd180 and Zn2600+Cd360 (Tukey, $p < 0.01$). The highest AE of Zn (over 10%) was recorded in the group Zn325+Cd45 but we have no comparable data for single Zn exposure.

Concentrations of Cd in the control animals did not exceed 10 mg Cd kg^{-1} dry body weight (Fig. 1B). In animals exposed to single Cd-contaminated food, internal Cd concentrations increased with increasing Cd concentrations in the food up to nearly 500 mg Cd kg^{-1} dry body weight in the group exposed to 720 mg Cd kg^{-1} food. No significant differences in Cd concentration were recorded in animals fed with 360, 720 and 1080 mg Cd kg^{-1} food (Tukey, $p > 0.05$). In animals exposed to a mixture of Zn and Cd, the pattern for Cd accumulation changed compared to single exposures. At the concentration of 90 mg Cd kg^{-1} food combined with 650 mg kg^{-1} food of Zn accumulation of Cd was increased (Tukey, $p < 0.01$), whereas at combinations of 360 mg Cd+2600 mg Zn kg^{-1} food and 720 mg Cd+5200 mg Zn kg^{-1} food, accumulation of Cd was significantly lower (Tukey, $p < 0.001$).

AE of Cd was around 25% in all groups exposed to single Cd and was not significantly lower in the group of animals with the highest Cd concentration in their food (Fig. 2B). The addition of Zn to Cd-contaminated food increased the AE of Cd in the lower treatments, but decreased AE in the higher Zn treatments. This effect was statistically significant (Tukey, $p < 0.05$) in the groups Zn650+Cd90 (increase) and Zn5200+Cd720 (decrease). The highest AE of Cd (over 35%) was recorded in the group Zn325+Cd45 but we have no comparable data for single Cd exposure.

3.3. Adverse effects of elevated concentrations of Zn and Cd in food

In the control group seven out of the 25 animals died during the 28-day exposure period. Mortality among the metal-exposed animals was in general higher, but inconsistent with metal concentrations in the food. The highest mortality (68%) was recorded in the animals exposed to 5200 mg Zn kg^{-1} dry food.

Food consumption rate was dose-relatedly decreased (ANOVA, $p < 0.05$; Fig. 3). Animals from the control group consumed around 5 mg dry food per mg dry body weight in 4 weeks. Compared to the control group, 5200 mg Zn kg^{-1} food was the lowest Zn concentration used where food consumption decreased significantly (Tukey, $p < 0.001$). In Cd-treated animals, the lowest concentration that significantly (Tukey, $p < 0.001$) reduced food consumption was 720 mg Cd kg^{-1} dry food. In case of Zn and Cd mixtures, food consumption decreased already at 2600 mg Zn+360 mg Cd kg^{-1} dry food.

EC_{50} values for the effect on food consumption rate, related to total Zn and Cd concentrations, were 5032 (95% confidence interval: 3678–6386) and 1000 (698–1302) mg kg^{-1} dry food, respectively. EC_{50} values based on water-extractable Zn and Cd concentrations were 2175 (68.0–2783) and 74.8 (53.0–96.6) mg kg^{-1} dry food, respectively. These EC_{50} values were considered as 1 TU for Zn and Cd in further calculations. EC_{50} values for a mixture of Zn and Cd were 1.10 (0.75–1.45) and 1.18 (0.73–1.62) TU for total and water-extractable concentrations, respectively. When analysed by a generalised-likelihood ratio test, these EC_{50} values were found not to be significantly different from 1.0 ($\chi^2 = 0.32$ and 0.76 for total measured and water-extractable concentrations, respectively), suggesting an additive effect of the Zn and Cd mixtures on food consumption.

When expressed on the basis of internal concentrations in the test animals, EC_{50} values for the effect of Cd, Zn and the mixture were 571 (366–777) and 443 (338–547) mg kg^{-1} dry body weight and 1.40 (1.32–1.49) TU, respectively. The EC_{50} for the mixture was significantly different from 1.0 TU ($X_1^2 = 7.07$; $p < 0.01$), suggesting an antagonistic effect.

4. Discussion

4.1. Interactions between Zn and Cd outside the organism

Water-extractability of Zn was not significantly affected by the presence of Cd at any concentration, and vice versa. In single

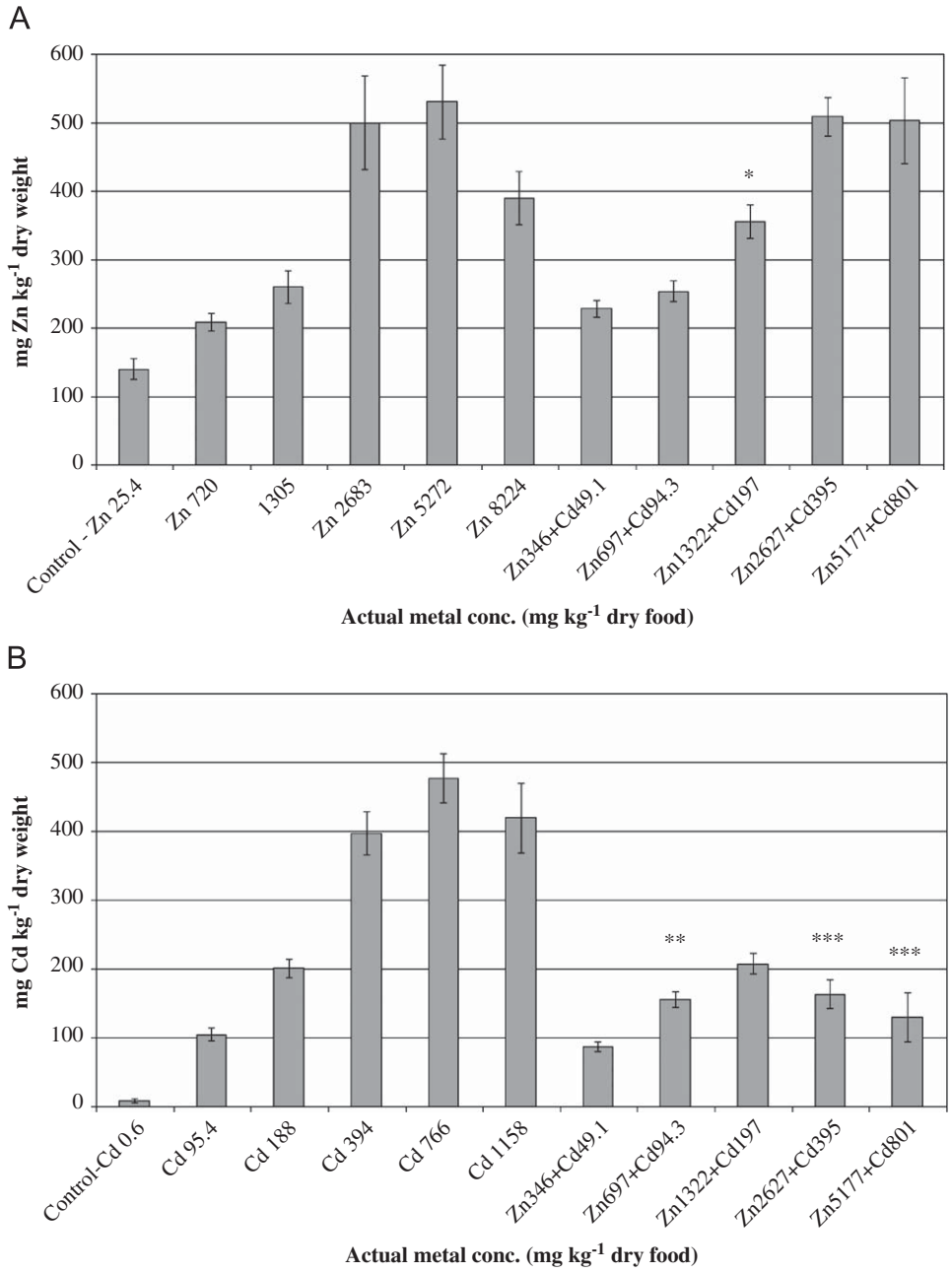

Fig. 1. Concentrations of Zn (A) and Cd (B) in *Porcellio scaber* after feeding with Cd, Zn, and Zn+Cd contaminated food for 28 days (Average and SE values; $n \geq 8$). Statistically significant differences between single and mixture exposure are marked with asterisks (ANOVA; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

metal additions, the ratio between total and water-extractable Cd remained constant at all concentrations, while in the case of Zn, the ratio increased insignificantly with concentration. Water-extractability of Cd was four times less than water-extractability of Zn.

Zn and Cd bind preferentially to nitrogen- and sulphur-bearing ligands, but the affinity is greater for Cd (reviewed in Brzóska and Maniuszko-Jakoniuk, 2001). Van Gestel and Hensbergen (1997) found a lower water-extractability for Cd than for Zn in soil as well, but in contrast to our study, extractability of both metals increased with increasing concentration of added metals. Besides, water-extractability of Cd significantly increased in the presence of Zn, which was explained by chloride complexation (Van Gestel and Hensbergen, 1997). In our study food was rich in organic matter compared to the artificial soil used by Van Gestel and Hensbergen (1997), which probably maintained a more constant ratio between water-extractable and non-extractable fractions of both metals. In mixtures of Zn and Cd, where increased extractability of Zn was expected, the surplus of Zn most likely neutralised the higher binding potential of Cd. Wilkins et al. (1998) found that the organic matter content in sandy soil increased the sorption potential of Cd, but the increased solute concentration of Zn decreased Cd sorption by competing for the sorption sites.

We conclude that in our study interactions between Zn and Cd in the high organic food source used probably did not affect availability of the single metals and their mixtures.

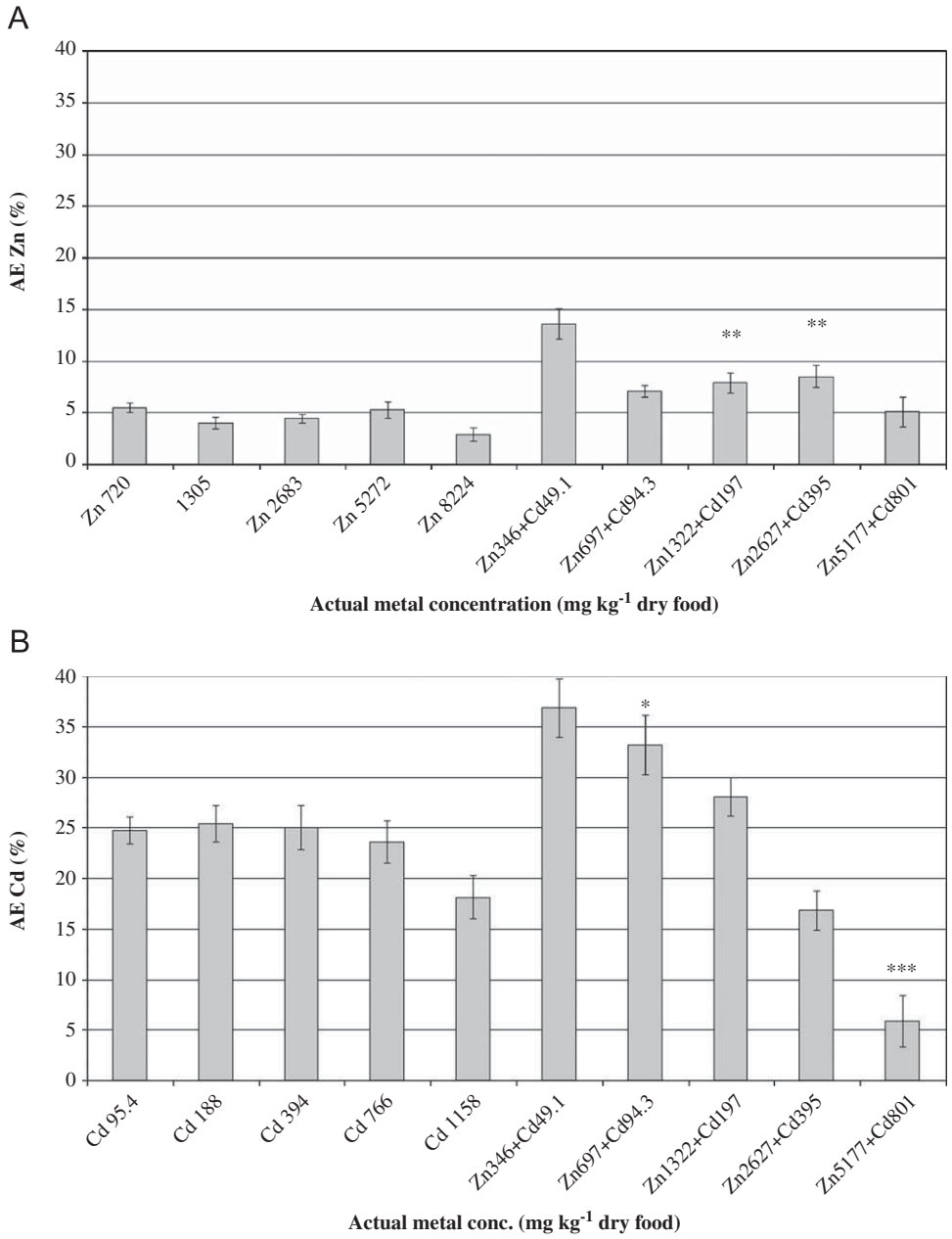

Fig. 2. Assimilation efficiency (AE) of Zn (A) and Cd (B) in *Porcellio scaber* after feeding with Zn and Cd contaminated food for 28 days (Average and SE values; $n \geq 8$). Statistically significant differences between single and mixture exposure are marked with asterisks (ANOVA; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

4.2. The effects of interactions on Zn and Cd assimilation

In animals exposed to food contaminated with a mixture of Zn and Cd, lower concentrations stimulated assimilation of both metals while higher concentrations (>1300 mg Zn kg^{-1}, >180 mg Cd kg^{-1}) significantly reduced Cd but not Zn assimilation.

Literature data on the interactions between Zn and Cd during assimilation are inconsistent. In the freshwater fish *Tilapia nilotica* Zn reduced Cd accumulation while Zn accumulation was increased in the presence of Cd (Kargin and Çogun, 1999). In the zebrafish *Danio rerio* Zn had an antagonistic effect on Cd uptake in the intestine but not through body surface or gills (Wicklund et al., 1988). In the freshwater clam *Anodonta cygnea* the presence of Zn reduced the uptake of Cd and changed its internal distribution (Hemelraad et al., 1987). In the marine mussel *Mytilus edulis* (Vercauteren and Blust, 1999) and the house cricket *Acheta domesticus* (Migula et al., 1989a) reciprocal antagonistic effects of Zn and Cd were described. In the springtails *Orchesella cincta*, exposed via diet (Sterenborg et al., 2003), and *Folsomia candida*, exposed via soil (Van Gestel and Hensbergen, 1997), an antagonistic effects of Zn on Cd accumulation or no effect on single metal accumulation were recorded, respectively. In the marine isopod *Idotea baltica*, Cd reduced Zn accumulation in the hepatopancreas (De Nicola and De Benedictis, 1996). In the terrestrial isopod *P. laevis* Cd assimilation in the hepatopancreas increased at lower Cd and Zn concentrations, while assimilation of both metals decreased at higher exposure concentrations (Odendaal and Reinecke, 2004a). Witzel (2000) revealed a significantly higher uptake of Cd and Zn in the isopod *P. scaber* when exposed to both metals simultaneously for a longer period.

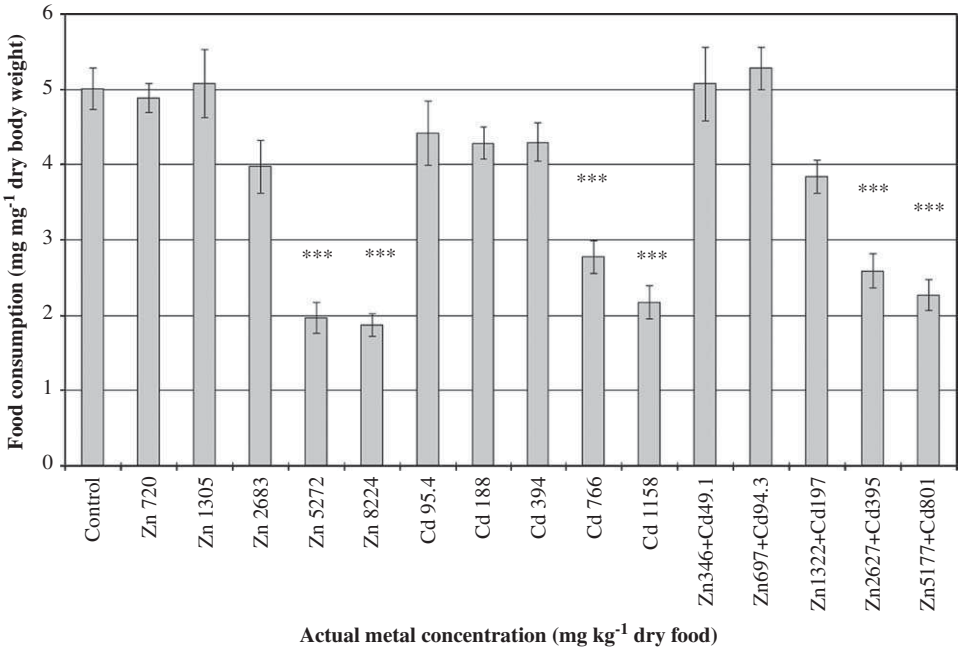

Fig. 3. Food consumption of *Porcelio scaber* 28 days exposed to Zn, Cd or Zn+Cd contaminated food (Average and SE values; $n \geq 8$). Statistically significant differences between control and metal treated groups of animals are marked with asterisks (ANOVA, Tukey test; $^{***}p < 0.001$).

Considering different exposure times and metal concentrations, our results are very similar to those of Witzel (2000) and Odendaal and Reinecke (2004a). The accumulation of Cd was enhanced by Zn at concentrations that could be found in moderately polluted environments (Hopkin, 1989), in spite of different ratios between Zn and Cd in the mixtures tested: 66.7:1 in the study of Witzel (2000), 50:1 in Odendaal and Reinecke (2004a) and 7.2:1 in this study. At higher concentrations Zn in excess significantly reduced the assimilation of Cd as described also for *P. laevis* (Odendaal and Reinecke, 2004a). In contrast to Odendaal and Reinecke (2004a), we found no decrease in Zn assimilation by the addition of Cd. These results confirm that Zn interferes with the Cd transport system and reversely, resulting in different net assimilations of the single metals when exposed to metal mixtures.

The transport of Cd and Zn through the cell membrane is primarily by facilitated transport that can be inhibited by sulphydryl blockers (Wang and Dei, 1999; Bobilya et al., 1992). No data on active transport of Zn are available, while in marine crustaceans Cd might enter cells by active uptake of Ca (reviewed in Rainbow, 1997). In facilitated transport the assimilation rate is regulated by concentration gradients, e.g. by binding of metals to proteins and by lysosomal storage as described for isopods (reviewed in Hopkin, 1989). In isopods, already a small dosage of Zn ($793\,mg\,kg^{-1}$ dry food, 6 days) stimulates synthesis of metallothionein-like proteins (Žnidaršič et al., 2005). As described for some decapod crustaceans (Brouwer et al., 1992), the same proteins are probably responsible for Cd and Zn binding. Probably also in isopods Cd induces the synthesis of proteins that also bind Zn, which explains an increased AE of Cd and Zn at lower concentrations offered simultaneously in our study. AE of Cd dropped at higher concentrations of Zn and Cd in food, because of lower uptake due to saturation of the transport system or a higher excretion via faeces or elsewhere. AE of Zn was not affected at higher concentrations, indicating different kinetics of Zn.

4.3. Toxicity of Zn and Cd

The lowest concentrations of Zn and Cd that caused a significant reduction in food consumption were higher than expected from previous results (Zidar et al., 2003). In view of this, EC_{50} values for the effect of single Zn and single Cd were also higher. This discrepancy can be explained by the complex food type used in this study. Farkas et al. (1996) reported lower effects of Cu on food consumption rate when a mixture of leaves, potato powder and rabbit food was used, compared to leaves only. So, food source may affect metal availability, and as a consequence also toxicity.

When both metals were added to food, consumption decreased at lower concentrations of Zn and Cd compared to single metal exposure. Similar results were published by Van Capelleveen (1987). However, recalculation of exposure concentrations in toxic units revealed that the effects of Zn and Cd in a mixture were additive, with a tendency to antagonism. EC_{50} values calculated from concentration–response curves were not significantly higher than one toxic unit, neither for total and nor for water-extractable concentrations. Hensbergen and Van Gestel (1995, reviewed in Weltje, 1998) found a similar discrepancy in the interpretation of results after recalculation of the data of Van Capelleveen (1987) in toxic units. When expressed on the basis of internal concentrations in the animals, the effect of the Cd–Zn mixture on food consumption of *P. scaber* was significantly antagonistic. This could be explained from the influence of Zn on the assimilation of Cd (Figs. 1 and 2). In the case of the isopod *P. laevis* (Odendaal and Reinecke, 2004b), the antagonistic effect of Zn and Cd in a mixture on body weight is unequivocal. In general, data about metal mixture toxicity for terrestrial animals are limited. Most studies performed on earthworms and insects revealed mainly additive or antagonistic effects of Zn and Cd mixtures in food or soil (Migula, 1989; Migula et al., 1989; Medici and Taylor, 1967; Khalil et al., 1996a,b; Van Gestel and Hensbergen, 1997; Weltje, 1998; Lock and Janssen, 2002).

5. Conclusions

It may be concluded that the simultaneous presence of Zn and Cd in food affects the assimilation potential of both metals in *P. scaber*, although they do not affect each other's water-extractability. As a consequence, the effect of the mixture of Zn and Cd on the food consumption of *P. scaber* is additive when based on external exposure concentrations, but antagonistic when related to internal concentrations.

Acknowledgments

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