ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2005 Vol. 48, Št. 1: 3-12

Sprejeto (accepted): 2005-09-27

The influence of zinc on the accumulation of cadmium and copper in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda)

Vpliv cinka na akumulacijo kadmija in bakra pri kopenskem enakonožcu *Porcellio scaber* (Crustacea, Isopoda)

Primož ZIDAR

Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia; e-mail: primoz.zidar@bf.uni-lj.si

Abstract. Relative to data derived from single-metal exposure not much is known about metal kinetics in terrestrial isopods exposed to a mixture of metals. In the work presented the accumulation pattern of Zn, Cd and Cu were studied in *Porcellio scaber*, one of the most investigated isopods. Animals were fed with hazel leaves dosed with single metals or their binary mixtures. After twenty-one days of exposure, food consumption and metal accumulation were measured. Results revealed that the accumulation of Zn is not affected by a decreased food consumption rate or by possible interactions between the mixture constituents. In contrast, the accumulation of Cd and Cu is significantly reduced when food is also contaminated with Zn. The lower accumulation of Cd and Cu can be ascribed to interactions with Zn that affect uptake and/or loss of metals.

Keywords: Isopods, *Porcellio scaber*, metal mixtures, zinc, copper, cadmium, food consumption, accumulation

Izvleček. Kinetika kovinskih ionov pri kopenskih rakih enakonožcih, ki so izpostavljeni zmesi kovin je, v nasprotju z izpostavitvijo le eni kovini, malo poznana. V predstavljenem delu smo spremljali akumulacijo cinka, kadmija in bakra pri kopenskem raku enakonožcu vrste *Porcellio scaber.* Poskusne živali smo hranili z listi leske, ki smo jim dodali raztopino ene ali pa zmes dveh kovin. Po enaindvajsetih dneh izpostavitve smo izmerili količine zaužite hrane in količino akumuliranih kovinskih ionov. Rezultati so pokazali, da na akumulacijo cinka ne vpliva upad prehranjevanja kakor tudi ne interakcije med kovinami v zmesi. Nasprotno je akumulacij kadmija in bakra značilno nižja ob sočasni prisotnosti cinka v hrani. Nižja akumulacija kadmija in bakra je posledica njune interakcije s cinkom, kar vpliva na manjši privzem ali pa večje izločanje kadmija in bakra iz telesa.

Ključne besede: raki enakonožci, *Porcellio scaber*, zmesi kovin, cink, baker, kadmij, prehranjevanje, akumulacija kovin

Introduction

Terrestrial isopods have been successfully adopted as monitor organisms in assessing the bioavailability of metals (Wieser & al. 1976, Coughtrey & al. 1977, Hopkin & al.1986, Dallinger & al. 1992, Hopkin & al.1993, Cortet & al. 1999, Paoletti & Hassall 1999). Many investigations have proved that they accumulate the highest tissue concentrations of zinc, copper, lead, and cadmium known for any invertebrate (Hopkin 1989, Cortet & al. 1999, Heikens & al. 2001). Their main metal storage organ is the hepatopancreas (Wieser 1961, Coughtrey & al. 1980, Hopkin & Martin 1982) where metals are bound to specific low-molecular-weight peptides (Donker & al 1990, Žnidaršič 2003), or stored as undissolved metal granules (Wieser & Klima 1969, Prosi & Dallinger 1988, Hopkin 1989). The hepatopancreas of terrestrial isopods consists of two cell types in which two types of intracellular granules have been observed; B granules in S cells and C granules in B cells (Wieser & Klima 1969, Hopkin & Martin 1982, Prosi & Dallinger 1988, Hopkin 1989, Hopkin & al. 1989). In isopods collected from contaminated sites both types of granules contain Zn whereas only B granules contain Cu and Cd (Hopkin 1989). Zn stored in B cells can be excreted as these cells show a daily cycle of apocrine secretion whereas Cd and Cu stored in S cells cannot be excreted because S cells have primarily a storage function and were never observed to secrete material into the gland lumen (Hopkin 1990, Hames & Hopkin 1991a).

The digestive system is the main route for metal intake in terrestrial isopods. Metal uptake from food depends on the availability of metals (Martin & al. 1976), the number of micro-organisms in the gut and food (Coughtrey & al. 1980), the nutritional status (Farcas & al. 1996), the rate of food consumption (Drobne & Hopkin 1995, Bibič & al. 1997), the temperature (Donker & al. 1998), pH inside the gut (Hopkin 1989), and some other factors.

Relative to data derived from single-metal exposures not much is known about the metal uptake in terrestrial isopods exposed to mixtures of metals. Interactions between metals in a mixture may affect the bioavailability of a single metal (Van Gestel & Hensbergen 1997) and the accumulation and excretion kinetics may differ when animals are exposed to various metals simultaneously (Witzel 2000, Odendaal & Reinecke 2004). As isopods in an industrially polluted environment are usually exposed to several metals at the same time (Beyer & al. 1984, Hopkin 1989), some further investigations with metal mixtures are needed.

In the present work, we studied the accumulation of Zn, Cd and Cu in one of the most investigated isopods, *Porcellio scaber*, after exposure to single metals or their mixtures. We aimed to show the importance of such investigations for better understanding of metal accumulation and excretion kinetics in isopods and for proper interpretation of biomonitoring data.

Materials and methods

Experimental animals and procedure

Specimens of *Porcellio scaber* raised in the laboratory were used in the experiments. The parental population was collected in an unpolluted environment in the vicinity of Ljubljana, Slovenia. Juvenile isopods of 15-25 mg fresh weight were placed in plastic Petri dishes (diameter 9 cm), three animals per dish (N=36 for each concentration). A filter paper moistened with water was added to each Petri dish to assure constant humidity. Animals were fed with hazel leaves (*Corylus avellana*) dosed with a single metal (Zn, Cd, Cu) or binary mixtures (Zn+Cd, or Zn+Cu) for 21 days. The leaves were collected from an uncontaminated area in autumn. Solutions of $ZnCl_2$ (>98 % pure Merck, Darmstad, Germany), $CuCl_2*2H_2O$ (≥99 % pure Merck, Darmstad, Germany) and $CdCl_2*H_2O$ (>98 % pure Merck, Darmstad, Germany) were applied to the leaves by spraying. The amount of solution applied to each leaf was adjusted to give the following nominal concentrations: Zn – 1000 and 3500 mg kg^{-1} dry weight; Cu – 500 and 2000 mg kg^{-1} dry weight; Cd – 250 and 500 mg kg^{-1} dry weight. Actual concentrations were analysed and did not differ of those desired by more than 5%. The base concentrations of Zn, Cu and Cd in the control leaves from the collection site were 40 mg Zn kg^{-1} dry weight, 20 mg Cu kg^{-1} dry weight and 0.15 mg Cd kg^{-1} dry weight. Control animals were fed with untreated food. The experiment was performed in a climate chamber at a relative humidity of 100% and at 16 hours light and 8 hours dark regime. Temperature was kept constant at 21°C (±1°C).

Food consumption was measured as the difference in the weight of leaves at the beginning and at the end of the experiment. Food consumption rates (CR) were calculated as the absolute consumption of food per week divided by the dry weight of the animals.

Metals concentration analyses

After exposure to the metal treated food, animals were food deprived for 24 hours to empty their guts. Then they were lyophilised, weighed and completely digested in a nitric/ perchloric acid mixture (7:1). After evaporation of the acid, the residue was taken up in 0.1% HNO_3. Total Zn, Cu and Cd concentrations in whole animals were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100). As certified reference material a Dogfish Liver (DOLT-2, National Research Council Canada) was used. The leaves were analysed for metals in the same way.

Statistical analyses of data

Data on metal accumulation and food consumption rates were analysed using two-way ANOVA with the factors Zn concentration in food (3 levels), Cd concentration in food (3 levels), Cu concentration in food (3 levels), and the food consumption rate as the covariate (the criteria for significance: $p<0.05$). For each metal concentration or combination used accumulation of a single metal was correlated with food consumption rate by using Pearson's correlation coefficient (the criteria for significance: $p<0.05$). For all statistical analyses the SPSS 12.0 for Windows software was used.

Results

Accumulation of cadmium and zinc

The concentration of Cd in control animals and animals fed with only Zn-dosed food did not exceed 2 mg kg^{-1} dry body weight. Animals exposed to food contaminated with 250 and 500 mg Cd kg^{-1} dry food weight accumulated up to 400 mg Cd kg^{-1} dry body weight (Fig. 1). Cadmium accumu-

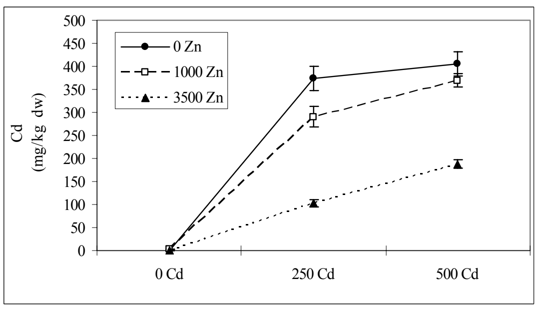

Figure 1: Concentration of Cd in *Porcellio scaber* after three weeks exposure to food contaminated with Zn or Cd or their mixture (AVR±SE).

lation from food was significantly lower where Cd in food was combined with Zn (Tab. 1). Cadmium accumulation was in general correlated with the food consumption rate which decreased with the increased concentration of both Cd and Zn in food (Fig. 3). No correlation between accumulated Cd and accumulated Zn was found.

Table 1: ANOVA (p values) for Zn, Cd and Cu accumulation in *Porcellio scaber* after 21 days of exposure to food dosed with a single metal (Zn, Cd, or Cu) or binary metal mixtures (Zn+Cd or Zn+Cu).

	Dependent variable			
	Zn accumulation		*Cd accumulation*	*Cu accumulation*
Factors:	*(Zn+Cd)*	*(Zn+Cu)*	*(Zn+Cd)*	*(Zn+Cu)*
Zn in food	*0.000*	*0.000*	*0.007*	*0.001*
Cd in food	*0.007*		*0.000*	
Cu in food		*0.897*		*0.000*
*Two way interaction Zn*Cd*	*0.136*		*0.000*	
*Two way interaction Zn*Cu*		*0.614*		*0.000*
Covariate: *Food cons. rate*	*0.098*	*0.602*	*0.000*	*0.136*

Accumulation of Zn (Fig. 2) was not dependent on food consumption rate (Tab. 1 and Fig. 3). In contrast to Cd, the accumulation of Zn from food even slightly increased when offered in a mixture with Cd (Fig. 2).

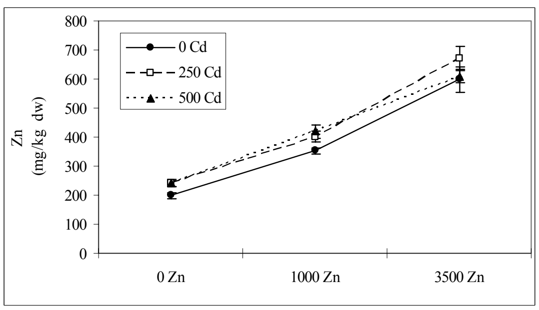

Figure 2: Concentration of Zn in *Porcellio scaber* after three weeks exposure to food contaminated with Zn, Cd, or their mixture (AVR±SE).

Accumulation of copper and zinc

The concentration of Cu in control animals and animals fed with Zn-dosed food was around 200 mg kg^{-1} dry body weight (Fig. 4). The copper concentration in Cu treated animals increased with increasing Cu concentration in food. Animals exposed to food contaminated with 3500 mg Zn kg^{-1} food in a mixture with 500 or 2000 mg Cu kg^{-1} food accumulated significantly less Cu compared to animals fed with only Cu-contaminated food. 1000 mg Zn kg^{-1} food did not affect the accumulation

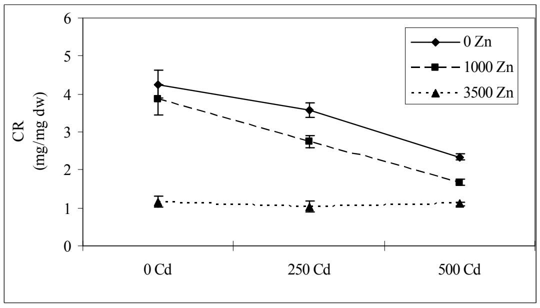

Figure 3: Food consumption rate (CR) in *Porcellio scaber* after three weeks exposure to food contaminated with Zn, Cd, or their mixture (AVR±SE).

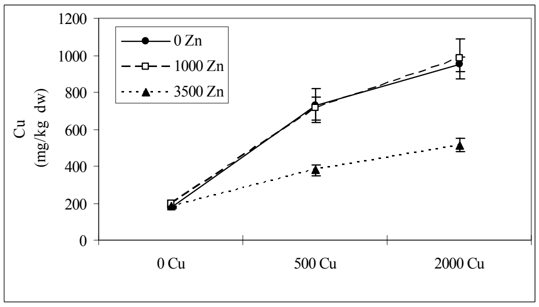

Figure 4: Concentration of Cu in *Porcellio scaber* after three weeks exposure to food contaminated with Zn, Cu, or their mixture (AVR±SE).

of Cu. In general, the food consumption rate had no significant effect on the accumulation of Cu, although the consumption rate decreased with increasing concentration of Zn and Cu in food (Fig. 6, Tab. 1). On the other hand, in animals exposed to 500 mg Cu kg^{-1} + 3500 mg Zn kg^{-1} food, Cu accumulation significantly correlated with food consumption rate.

The accumulation of Zn was affected neither by the Cu concentration in food nor by the food consumption rate (Tab. 1) and increased with increasing concentration of Zn in food (Fig. 5).

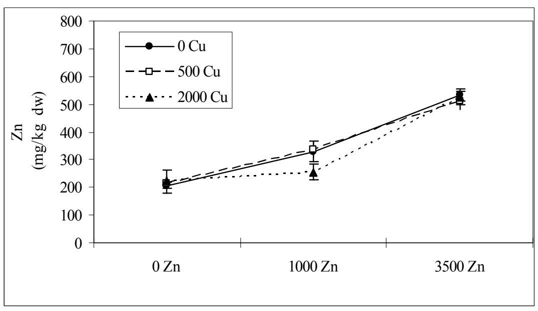

Figure 5: Concentration of Zn in *Porcellio scaber* after three weeks exposure to food contaminated with Zn, Cu, or their mixture (AVR±SE).

stevilka 1_05.qxp 13.12.2005 12:32 Page 8

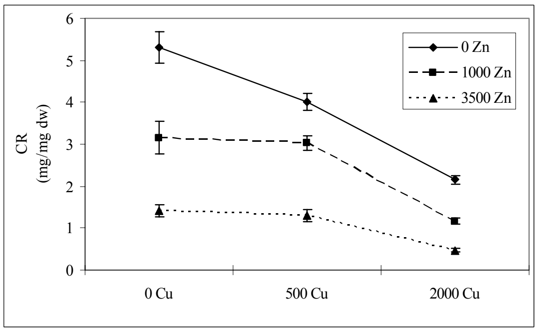

Figure 6: Food consumption rate (CR) in *Porcellio scaber* after three weeks exposure to food contaminated with Zn, Cu, or their mixture (AVR±SE).

Discussion

Our results demonstrated lower accumulation of Cd and Cu in *Porcellio scaber* after exposure to food dosed with Zn+Cd or Zn+Cu mixtures compared to single metal exposure. In contrast, the accumulation of Zn was nearly unaffected by the presence of Cd or Cu. The changed accumulation pattern for Cd and Cu can be attributed to a changed food consumption rate or to interactions between the mixture constituents.

Metal intake may be avoided by regulation of the food consumption rate (DROBNE & HOPKIN 1995, ZIDAR & AL. 2003b) or by food selection (KASCHL & AL. 2002, ZIDAR & AL. 2003a, 2004, 2005). The two selected concentrations of Zn, Cd, and Cu used in our experiment were previously determined as the concentrations with no major impact on food consumption rate and as concentrations that decrease the food consumption rate in short term exposure to a single metal (ZIDAR 2000). In contrast to our previous finding, 1000 mg Zn /kg and 500 mg Cu /kg were recognised as concentrations that already affect food consumption rate. In a mixture, all metal concentrations decreased food consumption. At all concentrations used the food consumption rate was correlated with accumulation of Cd, but not with Cu or Zn accumulation, although the latter had the strongest effect on food consumption rate. This proved that Cd accumulation could be reduced by the regulation of food consumption rate, as found in our previous studies (KASCHL & AL. 2002, ZIDAR & AL. 2003ab, ZIDAR & AL. 2005).

Beside food consumption rate, chemical and physiological interactions between food constituents might also affect metal bioavailability. It is known that Cd has a higher affinity for chloride ions than Zn (reviewed in RAINBOW 1997) which affects the uptake of Cd from the substrate in collembolans (VAN GESTEL & HENSBERGEN 1997). In isopods Cd complexation with chloride most probably does not affect the uptake of Cd. HAMES & HOPKIN (1991b) found an even higher assimilation rate for Cd compared to Zn when both metals were offered in a mixture as chloride salts (100 mg Zn/ kg + 100 mg Cd/ kg dry food weight). In *Porcellio laevis* exposed to food contaminated with a Zn and Cd sulphate mixture, lower accumulation of Cd was observed (ODENDAAL & REINECKE 2004). Copper is not known to complex with chloride but it has stronger affinity for organic ligands than Zn (HERTZ & AL. 1990). POSTHUMA & AL. (1997) reported that Cu reduced the sorption of Zn to soil whereas Cu sorption was inert toward Zn addition. In contrast to our findings, Cu also stimulates the uptake of Zn in *Enchytraeus crypticus* (POSTHUMA & AL.1997).

Elevated concentrations of Zn might either reduce Cd and Cu uptake or increase their loss from storage sites in *P. scaber*. The only significant route by which metals can be assimilated or excreted in terrestrial isopods is via the digestive system: via the cells of the hepatopancreas and papilate region of the gut (HOPKIN & MARTIN 1984, HAMES & HOPKIN 1989). There are two potential major mechanisms for the uptake of dissolved trace metals in crustaceans; binding to metal-specific membrane carriers, or entry via routes used in the uptake of major ions like Ca (RAINBOW 1997). The later route is known for Cd (WRIGHT 1977), whereas Zn uptake does not appear to follow any route for major ion uptake (RAINBOW & DALLINGER 1993). Evidence from studies on invertebrates suggests that assimilation of Zn into cells is regulated metabolically whereas Cu transport is strictly passive, dependent solely on the extracellular-intracellular gradient (summarised in HOPKIN 1993). Once in the cell, Cu and Cd are bound to a sulphur-containing ligand whereas Zn is bound to phosphate (HOPKIN 1993). DONKER (1992) found that the more Zn is stored in the hepatopancreas of *P. scaber* the lower is the proportion of Cd accumulated in this organ, while the concentration of Cd in the remainder of the body increased. DONKER (1992) assumed a limited storage capacity for metals in the hepatopancreas. The study of WITZEL (2000) revealed a significant increase in uptake and excretion of Cd and Zn when exposed to Zn and Cd simultaneously. WITZEL (2000) assumed that combined contamination with Cd and Zn inhibits the complete translocation of the assimilated metals into the hepatopancreas. Probably a fraction of Zn and/or Cd remains in the haemolymph or in other tissues, which can be excreted more easily (WITZEL 2000). This means that Zn might compete with Cu or Cd for metal-specific membrane carriers or for binding sites in metal binding proteins when entering the cell.

Conclusions

In *Porcellio scaber* the accumulation of Zn is not affected by decreased food consumption rate or by possible interactions between mixture constituents. In contrast, the accumulation of Cd and Cu is significantly reduced when food is also contaminated with Zn. The lower accumulation of Cd and Cu can be ascribed to metal interactions that affect uptake and/or loss of metals and which should be taken into account in biomonitoring data evaluation.

Acknowledgements

This work was financially supported by the Slovenian Ministry of Education, Science and Sport (projects No. Z1-3189 and PO-0525).

Povzetek

Kopenski raki enakonožci so pomembni pokazatelji obremenjenosti okolja s kovinami, saj kovine v telesu kopičijo. Kovine sprejemajo pretežno s hrano, skozi prebavilo, skladiščijo pa zlasti v celicah prebavnih žlez, vezane na nizko molekularne beljakovine ali pa v netopnih granulah. Količina privzetih kovin je odvisna od količine zaužite hrane, količine mikroorganizmov v hrani in prebavilu, dostopnosti kovin v okolju, pH prebavnih sokov, temperature v okolju in drugih dejavnikov. Interakcije med sestavinami hrane in kovinami lahko vplivajo na dostopnost, akumulacijo in izločanje kovin. Podatkov o medsebojnem vplivu različnih kovin v zmesi na privzem posamezne kovine je malo. Kopenski enakonožci so v industrijsko onesnaženem okolju običajno izpostavljeni visokim koncentracijam več kovin hkrati, zato so raziskave o medsebojnem vplivu kovin potrebne.

V predstavljenem delu smo spremljali akumulacijo cinka, kadmija in bakra pri kopenskem raku

enakonožcu vrste *Porcellio scaber* po izpostavitvi eni kovini ali pa dvem kovinam hkrati. Tovrstne raziskave so pomembne za razumevanje kinetike kovin pri rakih enakonožcih ter za pravilno vrednotenje okoljskih podatkov.

V poskusih smo uporabili mladiče kopenskih enakonožnih rakov vrste *Porcellio scaber*, ki so bili vzgojeni v laboratoriju. Živali smo 21 dni hranili z listi leske (*Corylus avellana*), ki smo jim dodali vodno raztopino cinka, bakra ali kadmija (Zn – 1000 in 3500 mg kg^{-1} suhe teže hrane; Cu – 500 in 2000 mg kg^{-1} suhe teže hrane; Cd – 250 in 500 mg kg^{-1} suhe teže hrane) ali pa zmesi cinka in kadmija oziroma cinka in bakra. Primerjali smo količino akumuliranih kovin v odvisnosti od koncentracije kovin v hrani in količine zaužite hrane v času poskusa.

Pri živalih, ki so bile izpostavljene le eni od kovin je telesna vsebnost kovin naraščala s koncentracijo kovine v hrani. Z naraščajočo koncentracijo kovin je upadala količina zaužite hrane, kar je vplivalo na privzem kadmija, na privzem cinka in bakra pa ni imelo značilnega vpliva. Akumulacija bakra in kadmija je bila značilno nižja ob prisotnosti cinka v hrani, medtem ko na akumulacijo cinka prisotnost bakra ali kadmija ni vplivala. Rezultati so tako pokazali, da med cinkom in kadmijem ter cinkom in bakrom verjetno prihaja do interakcij, zaradi česar se privzem kadmija in bakra zmanjša. Prav tako interakcije med cinkom in kadmijem ter cinkom in bakrom lahko vplivajo na kinetiko asimiliranega kadmija in bakra zaradi česar je izločanje presežnih koncentracij slednjih uspešnejše. Omenjene ugotovitve je potrebno upoštevati pri vrednotenju okoljskih podatkov.

Literature:

BEYER W.N., G.W. MILLER & E.J. CROMARTIE 1984: Contamination of the O2 soil horizon by zinc smelting and its effect on woodlouse survival. J. Environ. Qual. 13 (2): 247-251.

BIBIČ A, D. DROBNE, J. ŠTRUS & A.R. BYRNE 1997: Assimilation of zinc by *Porcellio scaber* (Isopoda, Crustacea) exposed to zinc. Bull. Environ. Contam. Toxicol. 58: 814-821.

CORTET J., A. GOMOT-DE VAUFLERY, N. POINSOT-BALAGUER, L. GOMOT, C. TEXIER & D. CLUZEAU 1999: The use of invertebrate soil fauna in monitoring pollutant effects. Eur. J. Soil. Biol. 35 (3): 115-134.

COUGHTREY P.J., M.H. MARTIN & E.W. YOUNG 1977: The woodlouse, *Oniscus asellus*, as a monitor of environmental cadmium levels. Chemosphere 12: 827-832.

COUGHTREY P.J., M.H. MARTIN, J. CHARD & S.W. SHALES 1980: Micro-organisms and metal retention in the woodlouse *Oniscus asellus*. Soil. Biol. Biochem. 12: 23-27.

DALLINGER R., B. BERGER & S. BIRKEL 1992: Terrestrial isopods: Useful biological indicators of urban pollution. Oecologia 89: 32-41.

DONKER M.H 1992: Energy reserves and distribution of metals in populations of the isopod *Porcellio scaber* from metal-contaminated sites. Funct. Ecol. 6: 445-454.

DONKER, M.H., KOEVOETS, P., VERKLEIJ, J.C.C., VAN STRAALEN, N. 1990. Metal binding compounds in hepatopancreas and haemolymph of *Porcellio scaber* (Isopoda) from contaminated and reference areas. Comp. Biochem. Physiol. 97: 119-126.

DONKER M.H., H.M ABDEL-LATEIF, M.A. KHALIL, B.M. BAYOUMI & N.M. VAN STRAALEN 1998: Temperature, physiological time, and zinc toxicity in the isopod *Porcellio scaber*. Environ. Tox. Chem. 17/8: 1558-1563.

DROBNE D. & S.P. HOPKIN 1995: The toxicity of zinc to terrestrial isopods in a standard laboratory test. Ecotox. Environ. Safe. 31: 1-6.

FARKAS S., E. HORNUNG & E.FISCHER 1996: Toxicity of copper to *Porcellio scaber* Latr. (Isopoda) under different nutritional status. Bull. Environ. Contam. Toxicol. 57: 582-588.

Hames C.A.C. & S.P. Hopkin 1989: The structure and function of the digestive system of terrestrial isopods. J. Zool. 217: 599-627.

Hames C.A.C. & S.P. Hopkin 1991a: A daily cycle of apocrine secretion by the B cells in the hepatopancreas of terrestrial isopods. Can. J. Zool. 69: 1931-1937.

Hames C.A.C. & S.P. Hopkin 1991b: Assimilation and loss of ^{109}Cd and ^{65}Zn by the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Bull. Environ. Contam. Toxicol. 47: 440-447.

Heikens A., W.J.G.M. Peijnenburg & A.J. Hendriks 2001: Bioaccumulation of heavy metals in terrestrial invertebrates. Environ. Pollut. 113: 385-393.

Hertz J., C. Angehrn-Bettinazzi & H. Stöckli 1990: Distribution of heavy metals in various litter horizons and forest soil. Inter. J. Environ. Anal. Chem. 39: 91-99.

Hopkin S.P, G.N. Hardisty & M.H. Martin 1986: The woodlouse *Porcellio scaber* as a žbiological indicator'of zinc, cadmium, lead and copper pollution. Environ. Pollut. 11: 271-290.

Hopkin S.P. & M.H. Martin 1982: The distribution of zinc, cadmium, lead and copper within the hepatopancreas of a woodlouse. Tiss. Cell 14: 703-715.

Hopkin S.P. & M.H. Martin 1984: Heavy metals in woodlice. Symp. zool. Soc. Lond. 53: 143-166.

Hopkin S.P. 1989: Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Science, Barking, U.K..

Hopkin S.P. 1990: Critical concentrations, pathways of detoxification and cellular ecotoxicology of metals in terrestrial arthropods. Funct. Ecol. 4: 321-327.

Hopkin S.P. 1993: Deficiency and excess of copper in terrestrial isopods. In: Dallinger R. & P.S. Rainbow (Eds.): Ecotoxicology of Metals in Invertebrates. SETAC Spec. Publ. Ser., Lewis Publishers, pp. 359-382.

Hopkin S.P., C.A.C. Hames & A. Dray 1989: X-ray microanalytical mapping of the intracellular distribution of pollutent metals. Microsc. Anal. 23-27.

Hopkin S.P., D.T. Jones & D. Dietrich 1993: The isopod *Porcellio scaber* as a monitor of the bioavailability of metals in terrestrial ecosystems: towards a global žwoodlouse watch' scheme. The Science of the Total Environment, Supplement 1993, Elsevier Science Publishers B.V., Amsterdam, 357-365.

Kaschl U.I., P. Zidar, J. Štrus & V. Storch 2002: Food choice experiments with cadmium nitrate dosed food in terrestrial isopod *Oniscus asellus* (Crustacea). Acta Biol. Slov. 45 (2), 3 -14.

Martin M.H, P.J. Coughtrey & E.W. Young 1976: Observations on the availability of lead, zinc and cadmium by the woodlouse, *Oniscus asellus*. Chemosphere 5: 313-318.

Odendaal J.P. & A.J. Reinecke 2004: Evidence of metal interaction in the bioaccumulation of cadmium and zinc in *Porcellio laevis* (Isopoda) after exposure to individual and mixed metals. Water Air Soil Poll. 156: 145-161.

Paoletti M.G. & M. Hassall 1999: Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agr. Ecosyst. Environ. 74: 157-165.

Posthuma L., R. Baerselman, R.P.M. Van Veen & E.M. Dirven-Van Breemen 1997: Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soil. Ecotox. Environ. Safe. 38: 108-121.

Prosi F. & R. Dallinger 1988: Heavy metals in the terrestrial isopod *Porcellio scaber* Latreille. I. Histochemical and ultrasructural characterisation of metal-containing lysosomes. Cell. Biol. Toxicol. 4: 81-96.

Rainbow P.S. & R. Dallinger 1993: Metal uptake, regulation, and excretion in freshwater invertebrates. In: Dallinger R. & P.S. Rainbow (Eds.): Ecotoxicology of Metals in Invertebrates. SETAC Spec. Publ. Ser., Lewis Publishers, pp. 119-131.

RAINBOW P.S. 1997: Ecophysiology of trace metals uptake in Crustaceans. Estuar. Coast. Shelf. S. 44: 169-175.

VAN GESTEL C.A.M. & P.J. HENSBERGEN 1997: Interaction of Cd and Zn toxicity for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. Envir. Tox. Chem. 16: 1177-1186.

WIESER W. & J. KLIMA 1969: Compartmentalization of copper in the hepatopancreas of isopods. Microscopie 24: 1-9.

WIESER W. 1961: Copper in isopods. Nature 191:1020.

WIESER W., G. BUSCH & L. BÜCHEL 1976: Isopods as indicators of copper content of soil and litter. Oecol. 23: 107-114.

WITZEL B: 2000: The influence of zinc on the uptake and loss of cadmium and lead in the woodlouse, *Porcellio scaber* (Isopoda, Oniscidea). Ecotox. Environ. Safety. 47: 43-53.

WRIGHT D.A. 1977: The effect of calcium on cadmium uptake by the shore crab Carcinus maenas. J. Exp. Biol. 67: 163-173.

ZIDAR P. 2000: Effects of metal ions on food consumption of terrestrial isopod *Porcellio scaber* (Crustacea: Isopoda). Ph.D thesis. Ljubljana

ZIDAR P., D. DROBNE, J. ŠTRUS & A. BLEJEC 2003b: Intake and assimilation of Zn, Cu and Cd in the terrestrial isopod *Porcellio scaber* Latr. (Crustacea, Isopoda). Bull. Environ. Contam. Toxicol. 70: 1028 – 1035.

ZIDAR P., D. DROBNE, J. ŠTRUS, C.A.M. VAN GESTEL & M. DONKER 2004: Food selection as a means of Cu intake reduction in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda). Appl. Soil Ecol. 25 (3): 257-265.

ZIDAR P., I.U. KASCHL, J. ŠTRUS, D. DROBNE & J. BOŽIČ 2003a: Avoidance behaviour in *Oniscus asellus* (Crustacea, Isopoda) as an indicator of food quality. Arch. Ind. Hyg. Toxicol. 54, 177-181.

ZIDAR P., J. BOŽIČ & J. ŠTRUS 2005: Behavioural response in the terrestrial isopod *Porcellio scraber* (Crustacea) offered a choice of uncontaminated and cadmium-contaminated food. Ecotox. (Lond.) 14: 1-10.

ŽNIDARŠIČ N. 2003: Copper and zinc dynamics and related cellular mechanisms in the digestive system of isopod crustaceans *Porcellio scaber* (Crustacea: Isopoda). Ph.D thesis, Ljubljana.