Food choice experiments with cadmium nitrate dosed food in terrestrial isopod *Oniscus asellus* (Crustacea)

Poskusi izbire hrane z različno vsebnostjo kadmijevega nitrata na kopenskem enakonožcu *Oniscus asellus* (Crustacea)

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Abstract. The influence of different concentrations of cadmium nitrate on food choice behaviour was studied in the terrestrial isopod *Oniscus asellus*. In paired food choice tests, consumption rates were compared in relation to cadmium nitrate concentrations and duration of feeding. The consumption of contaminated food was reduced at even the lowest cadmium concentration already in the first week of feeding. In the third week the consumption rates for uncontaminated and contaminated food reached a ratio of 6:4 in all animal groups. Consumption of contaminated food during the experiment resulted in increased cadmium content in the animals. It is presumed that *O. asellus* cannot distinguish food according to cadmium concentration. The difference in consumption rates between uncontaminated and cadmium-contaminated food could be based on integration of feeding behaviour and the adverse metabolic effects of cadmium.

Keywords: Isopods, *Oniscus asellus*, food-choice, cadmium nitrate, contamination, consumption rate, accumulation

Izvleček. Proučevali smo vpliv različnih koncentracij hrani dodanega kadmijevega nitrata na izbiro hrane pri kopenskem enakonožcu *Oniscus asellus*. V poskusih izbire smo primerjali stopnjo hranjenja z neonesnaženo in onesnaženo hrano, v odvisnosti od koncentracije kadmijevega nitrata in trajanja hranjenja. Živali so zaužile manj onesnažene hrane že v prvem tednu poskusa izbire tudi pri najnižji koncentraciji kadmijevega nitrata dodanega hrani. V tretjem tednu poskusa je bilo pri vseh skupinah živali razmerje med stopnjo hranjenja z neonesnaženo in onesnaženo hrano 6:4. Pri živalih, ki so med poskusom jedle hrano z dodanim kadmijevim nitratom je bila telesna vsebnost kadmija povečana. Iz rezultatov sklepamo, da raki enakonožci verjetno ne razlikujejo med hrano z različno vsebnostjo kadmijevega nitrata. Razlike v stopnji

hranjenja z neonesnaženo in onesnaženo hrano so verjetno posledica prehranjevalnega vedenja živali povezanega s presnovnimi učinki kadmija.

Ključne besede: enakonožci, *Oniscus asellus*, izbira hrane, kadmijev nitrat, onesnaževanje, stopnja hranjenja, akumulacija

Introduction

Faced with an ever growing input of waste substances to the chemical cycles of the biosphere due to anthropogenic activities, the importance of assessing levels of pollution by biomonitoring programmes in various ecosystems has gained increasing recognition over the last decades (BAYNE 1979, HOPKIN 1989, GOLDBERG & BERTINE 2000). The success of the 'Mussel Watch Program' in marine ecosystems has led to considerable efforts to establish similar programmes for terrestrial ecosystems. Terrestrial isopods are amongst the most promising animals for global terrestrial biomonitoring, and the species *Porcellio scaber* has been proposed as the terrestrial biomonitoring equivalent of *Mytilus edulis* (CORTET & al. 1999, COUGHTREY & al. 1997, DROBNE 1997, HOPKIN & al. 1986, HOPKIN 1990, HOPKIN & al. 1993, PAOLETTI & HASSALL 1999). However, in recent years some data on possible pollutant-dependent food selection behaviour have been presented (VAN CAPELLEVEEN & al. 1986, DALLINGER 1977, ODENDAAL & REINECKE 1999, DROBNE & al. 1995). If terrestrial isopods are able to discriminate between differently contaminated food, their value as biomonitoring organisms would diminish.

Terrestrial isopods play an important role in decomposition of organic material and fulfill most of the criteria required of a good biomonitor (HOPKIN 1989, HOPKIN & al. 1993, COUGHTREY & al. 1997, PAOLETTI & HASSALL 1999). Many aspects of the ecology of *Porcellio scaber* are well known, and it is one of the most studied isopods (CORTET & al. 1999, DROBNE 1997, GUNNARSON 1987, ODENDAAL & REINECKE 1999, PAOLETTI & HASSALL 1999). Field studies and monitoring programmes have used the species successfully in assessing the bioavailability of pollutants (HAMES & HOPKIN 1989, HOPKIN 1990, RABITSCH 1995). Terrestrial isopods accumulate the highest tissue concentrations of cadmium, copper, lead, and zinc known for any invertebrate (HOPKIN 1989, HOPKIN & MARTIN 1984). The robustness against pollution with metals in isopods apparently stems from a compartmentalization mechanism dependent on metal-containing granules in small cells of the hepatopancreas and possibly a detoxification mechanism with binding metallothioneins, which yet remains to be verified (CROMENTUJIN & al. 1994, HAMES & HOPKIN 1991, HOPKIN 1989, PROSI & al. 1983). The shiny woodlouse *Oniscus asellus* has been proposed as a substitute for *P. scaber* in areas where the latter is scarce or missing from the fauna (DROBNE 1997). *O. asellus* shows higher accumulation rates for cadmium and other metals than *P. scaber*, and has likewise a wide distribution, thus constituting a suitable alternative to *P. scaber* (HAMES & HOPKIN 1991, HOPKIN 1990).

In recent years, much information has been gathered about the sensory equipment of terrestrial isopods. They are able to distinguish between and show preferences for different qualities of food, e.g. different levels of fungal permeation of their food (ZIMMER & al 1996, GUNNARSON 1987, SZLAVECZ & MAIORANA 1990). In addition, some results of food choice experiments have suggested that terrestrial isopods may also sense different levels of contaminants in their food, e.g. *P. laevis* has been shown to discriminate against cadmium sulfate added to leaves and *P. scaber* can apparently discriminate and avoid lead and copper in its food (VAN CAPELLEVEEN & AL. 1986, DALLINGER 1977, ODENDAAL & REINECKE 1999). If terrestrial isopods have evolved mechanisms to avoid uptake of biotoxic substances from their food, then the concentrations of these substances in their tissues do not represent levels of habitat pollution, but are the result of an integrated behavioural response to pollution (DROBNE & al 1995, DONKER & BOGERT 1991). However, one of the criteria for the use of an organism as a biomonitor

is that accumulation of a pollutant should reflect exposure. Results from biomonitoring experiments using terrestrial isopods could therefore be unreliable and should be treated with caution (DROBNE & al. 1995).

In the present work we investigated the possibility of an avoidance mechanism for the consumption of cadmium-contaminated food by the terrestrial isopod *O. asellus* in paired food choice experiments. We aimed to show that the results from our experiments, which document preference for uncontaminated food, in correlation with previous publications, can be explained without assuming the existence of a sensory detection mechanism for cadmium in the diet, thus rendering the species an accurate biomonitor of bioavailable cadmium in its habitat. We assumed that the observed preference for uncontaminated food could be explained by the combined influence of feeding behaviour and the adverse metabolic effects of cadmium in the isopods.

Materials and Methods

Sampling of animals from the field

About 250 specimens of *Oniscus asellus* were collected in October 2001 from the litter layer of a woodland area near a former smelter site in the vicinity of Nussloch near Heidelberg, Germany. For three weeks the animals were kept in a glass container on moist plaster of Paris and fed on partly decomposed unpolluted leaves of various tree species. The temperature in the glass container was kept steady at about 17°C, and exposure to direct light was prevented. To avoid desiccation, the leaves and the plaster of Paris were lightly sprayed with commercial bottled water every two days. After three weeks, ten animals were selected at random, lyophilised and weighed, and digested in a hot acid mixture (HNO_3:$HClO_4$ = 7:1; final temperature 185°C) until dryness. The residue was suspended in 1.5 ml HNO_3 (0.2%) and analysed for cadmium content by flame atomic absorption spectrophotometry (AAS), using a Perkin Elmer AA Analyst 100 atomic absorption spectrophotometer. All further animal samples were digested and analysed according to this method.

Sampling of soil

From the same site, five soil samples of the top 10 cm of the soil horizon were collected, cleaned of all visible organic components, and dried for two hours at 110° C, then homogenised in a mortar and digested in hot acid mixture (HNO_3:HCl = 1:3) until dryness. As the samples were not digested completely by this procedure, it was repeated once. The samples were then diluted with weak nitric acid (0.2 %), and filtered. The filtrate was subsequently analysed for cadmium content by flame AAS analysis.

Experimental set-up

The animals were separated, sexed and weighed, and kept individually on moist filter paper in plastic petri dishes ($\varnothing$ = 9 cm). Only males and non-gravid females were selected for the experiment. The petri dishes were kept in a climate chamber at a relative humidity of 100% and under a 16 hours light and 8 hours dark regime. Temperature was kept constant at 21°C (±1°C). Animals were checked every two days, and the filter paper was moistened with commercial bottled water if necessary. Any dead animals were removed immediately and their data excluded from the results. Moulting animals or animals that did not produce faecal pellets were marked and counted.

The animals were fed exclusively with complex food pellets designed especially for this experiment. For the production of food pellets, partly decomposed hazel leaves (*Corylus avellana*) were collected in an unpolluted woodland area in the vicinity of Cerknica near Ljubljana, Slovenia. After drying the leaves at room temperature for several days, leaf stems were removed and the leaves pulverised with a coffee mill and subsequently sieved through a 0.25 mm mesh net. The leaf powder was dried for 2

hours at 60°C. Other components of the complex food pellets were commercial Dr. Oetker's gelatine, which was dried at 40°C, and fish food for aquarium fish (JBL Novobel; JBL GmbH& Co. KG), which was homogenised by hand in a glass mortar and dried for two hours at 60°C.

Dry leaf powder, gelatine, and fish food were mixed in a 63:34:3 ratio and turned into a paste with demineralized water (15 ml per gram gelatine). For the preparation of contaminated food, four different amounts of cadmium nitrate ($Cd(NO_3)_2$) solution (2712.5 mg Cd/l) were added to the food paste to give nominal concentrations of 0, 20, 45, 200 and 450 mg Cd kg^{-1} dry weight of food. In order to exclude food choice due to nitrate content (Hopkin 1989), corresponding amounts of potassium nitrate (KNO_3) were added to the respective control food. Food pellets were formed out of the paste by depositing equal amounts in plastic blisters (V = 0.3 ml). The food pellets were allowed to solidify for 24 h at 5°C, dried at room temperature for 24 h and then dried at 70°C for the next 48 h. The actual concentrations of cadmium in the food pellets were measured by AAS analysis and compared to the nominal cadmium contents (Tab. 1). The amount of water-soluble cadmium in the food pellets was measured by soaking food pellets in demineralized water for 24 h at increasing temperatures up to 40°C, centrifuging the solution and analysing the supernatant for Cd content by flame AAS (Tab. 1). The water soluble concentration of cadmium in food pellets was observed to be less than 10 % of the actual cadmium concentration.

Table 1: Concentration of cadmium in food pellets as measured by flame AAS (* = concentration below detection limit)

Preglednica 1: Koncentracije kadmija v hrani izmerjene s plamensko AAS (* = koncentracija pod zaznavno mejo)

Nominal Conc. (mg/kg)	Measured Conc. (mg/kg) n=20		Water Soluble Conc. (mg/kg) n=10	
	Mean	SE	Mean	SE
0	0.06	0.02	*	
20	20.55	0.30	1.14	0.13
45	45.34	0.51	3.78	0.07
200	194.55	2.69	14.05	0.20
450	445.26	1.58	36.58	0.40

Food pellets were offered in small plastic dishes ($\varnothing$ = 2 cm, height of rim ca. 3 mm). Prior to food choice experiments, the animals were acquainted with the form and taste of the food pellets by offering them uncontaminated food for one week. After seven days, the animals were starved for 24 h to empty their guts (HAMES & HOPKIN 1991). Ten animals were selected randomly and analysed for tissue cadmium content by flame AAS.

Food choice experiments

The isopods were assigned to five groups of 35 individuals each, giving a total number of 175 animals. Each animal was offered a choice between an uncontaminated and a Cd-contaminated food pellet; in the group "0-20" the nominal cadmium concentration in the contaminated pellet was 20 mg kg^{-1} dry food weight, in the group "0-45" 45 mg kg^{-1} dry food weight, in the group "0-200" 200 mg kg^{-1} dry food weight, and in the group "0-450" 450 mg kg^{-1} dry food weight. Animals in the control group "0-0" were offered a choice of two uncontaminated, but differently marked food pellets. The dry weight of food pellets was determined before and after exposure to animals using a micro-balance.

After seven days of the food choice experiment, the animals were starved for 24 h to empty their guts (HAMES & HOPKIN 1991). Eight animals of each group were randomly selected and analysed for tissue cadmium content by AAS analysis. The food pellets were collected, dried for two days at room

temperature, cleaned from faeces and dried at 70 °C for 48 h. Consumption of food was calculated according to the differences in weight before and after the seven day period of exposure to the animals.

The remaining animals were offered a new choice between new food pellets with the same Cd-concentrations as before. The whole cycle of feeding, removal of food, starvation for 24 h, and random selection of eight animals for analysis of cadmium content by AAS analysis was repeated twice, resulting in a total exposure period of twenty-one days for the last cohort of animals.

Analysis of data

To compare food consumption rates (CR), the absolute consumption of food per week was divided by the dry weight of animals. The data of the experiment were analysed using Microsoft Excel 97 computer software. Ratios of consumption for uncontaminated food and contaminated food were calculated from consumption rates for each individual. The percentage of uncontaminated food consumed was calculated and compared to a null-hypothesis of 50 % with a two-tailed Student's t-test. Standard errors (SE) and 95 % confidence intervals were calculated where appropriate. Weekly consumption rates within groups and weekly consumption rates between groups were analysed for significant differences with a one-way ANOVA test and a Tukey test, using SPSS for Windows statistics software.

Results

Concentration of cadmium in soil samples and animals

Analysis of soil samples by flame Atomic Absorption Spectrometry yielded a mean concentration of 11.42 (SE=1.63) mg Cd per kg dry weight of soil. Analysed animal samples from the field contained a mean of 64.54 (SE=5.26) mg Cd per kg dry weight of animals. Analysed animal samples from food choice experiment yielded increased levels of cadmium correlated to the duration of the experiment and the concentration of cadmium in the offered food (Fig. 1).
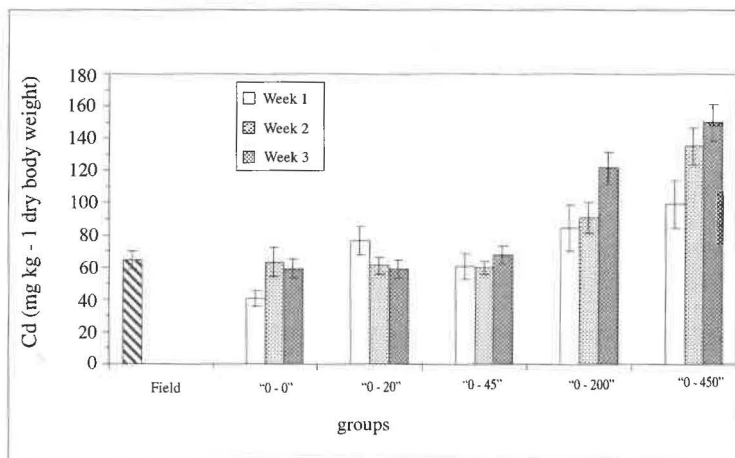

Figure 1: Concentration of cadmium in whole *Oniscus asellus* measured by flame AAS followed over three weeks of the food choice experiment. Concentration of cadmium in field animals is represented on the far left (mean ± SE).

Slika 1: Koncentracije kadmija v telesu raka enakonožca *Oniscus asellus*, izmerjene s plamensko AAS v treh tednih poskusa izbire hrane. Povprečna koncentracija kadmija v živalih iz okolja je prikazana skrajno levo (povp. ± stand. napaka).

Consumption rates

During the preliminary experiment, the mean food consumption rate was 0.52 (SE=0.03) mg dry food weight per kg dry weight of animals. In the third week of the food choice experiment, the mean food consumption rate increased to 1.24 (SE=0.05).

Combined consumption rates of contaminated and uncontaminated food increased significantly between the first and third week in group "0-0" (Tukey test: $p < 0.05$) (Fig. 2). In group "0-20", combined consumption rates between the first and second week are significantly different (ANOVA: $p<0.05$); however, with the Tukey test no statistically significant increase between weekly consumption rates could be shown. Combined consumption rates in group "0-45" were significantly higher in the second and third week (Tukey test: $p < 0.005$) than in the first week. In group "0-200", combined consumption rates in the third week were significantly higher than in the first week (Tukey test: $p < 0.05$); between the first and second and the second and third week there was no statistically significant difference. In group "0-450", no statistically significant increase in weekly consumption rates could be shown.
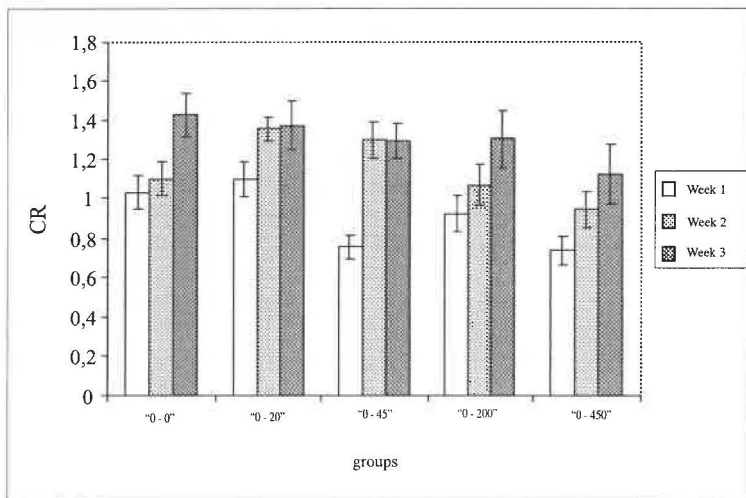

Figure 2: Combined consumption rates (CR) of uncontaminated and contaminated food in different groups of animals for each of the three weeks of the food choice experiment (mean ± SE).
Slika 2: Stopnja hranjenja (CR) z neonesnaženo in onesnaženo hrano skupaj v posameznih tednih poskusa izbire, pri različnih skupinah živali (povp. ± stand. napaka).

Consumption rates of contaminated and uncontaminated food in different groups

Consumption rates show that animals within group "0-0" did not significantly discriminate between food pellets during the three weeks (Fig. 3, Tab. 2). Animals within group "0-20" and "0-45" significantly preferred uncontaminated food to contaminated food in the first and third week but not in the second week. Animals of the group "0-200" consumed significantly more uncontaminated food in all three weeks. In group "0-450", there was no significant preference for uncontaminated over contaminated food in the first week; however, in the second and third week, animals consumed significantly less contaminated food.
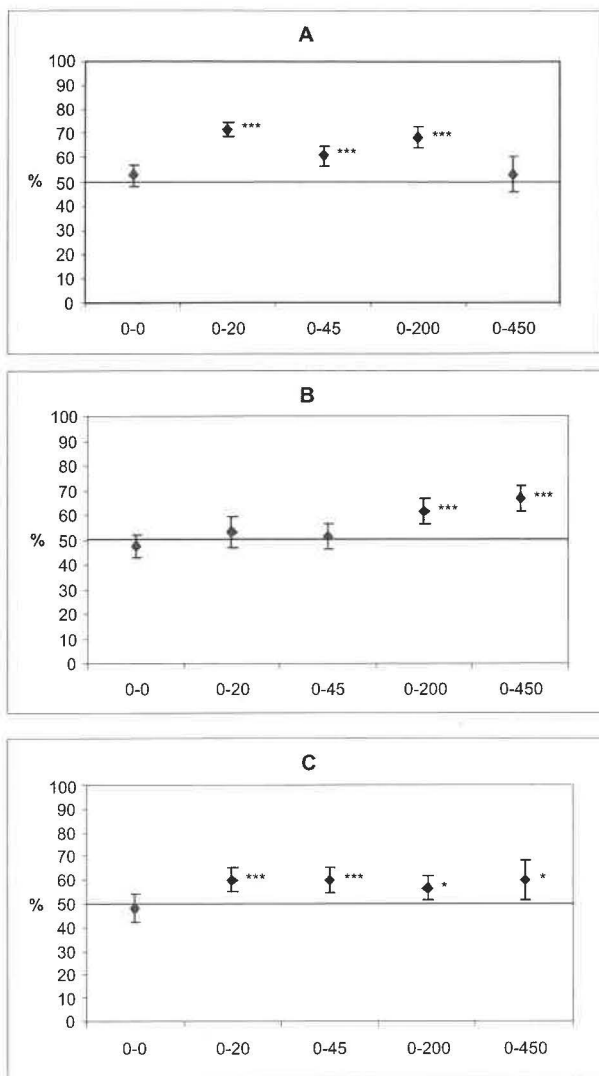
Figure 3: Consumption rate (CR) of uncontaminated food shown as percentage values (♦) of combined CR of uncontaminated and Cd-contaminated food (means and 95% conf. int.): A = 1st week, B = 2nd week, C = 3rd week of food choice experiments. Stars above bars represent significant differences between CR of uncontaminated food and 50 % (horizontal line) (t-test: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.005$).

Slika 3: Stopnja hranjenja (CR) z neonesnaženo hrano prikazana kot odstotek (♦) skupne stopnje hranjenja z neonesnaženo in s kadmijem onesnaženo hrano (povp. in 95% interval zaupanja): A = 1. teden, B = 2. teden, C = 3. teden poskusov izbire hrane. Statistična značilnost razlik med stopnjo hranjenja z neonesnaženo hrano in 50% (vodoravna črta) je prikazana z zvezdicami (t-test: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.005$).

Differences in combined consumption rates between the groups in the first week were mainly due to differences in consumption of uncontaminated food (Tab. 2). In the second week, the differences between combined CRs of the groups were mainly connected with consumption of contaminated food, whereas consumption levels of uncontaminated food were roughly similar in all groups (Tab. 2). During the third week, CRs between uncontaminated and contaminated food reached almost identical ratios of about 6:4 in all groups (Tab. 2, Fig. 3).

Table 2: Feeding and moulting behaviour of *Oniscus asellus* in different groups over three weeks of food choice experiment. (0 = uncontaminated food, Cd = food contaminated with cadmium; CR = Consumption Rate)
Preglednica 2: Prehranjevanje in levitev v treh tednih poskusa izbire pri *Oniscus asellus* iz različnih skupin. (0 = neonesnažena hrana, Cd = hrana onesnažena s kadmijem; CR = stopnja hranjenja)

		"0-0"		"0-20"		"0-45"		"0-200"		"0-450"	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Week 1	CR 0	0.53	0.05	0.79	0.07	0.47	0.05	0.64	0.07	0.41	0.05
	CR Cd	0.50	0.06	0.31	0.03	0.29	0.02	0.29	0.03	0.36	0.05
	% of moulting animals	33.33		21.43		42.86		24.14		38.46	
	Number of animals	26		27		27		28		24	
Week 2	CR 0	0.54	0.06	0.71	0.05	0.67	0.07	0.67	0.07	0.64	0.07
	CR Cd	0.56	0.04	0.65	0.06	0.63	0.05	0.40	0.05	0.30	0.04
	% of moulting animals	5.26		10.00		10.00		33.33		41.18	
	Number of animals	18		19		19		20		16	
Week 3	CR 0	0.66	0.04	0.81	0.06	0.78	0.06	0.75	0.10	0.70	0.10
	CR Cd	0.77	0.10	0.56	0.07	0.51	0.05	0.56	0.06	0.43	0.05
	% of moulting animals	18.18		8.33		0		0		11.11	
	Number of animals	11		12		12		12		9	

Discussion

Compared with data from HOPKIN (1989), the soil samples from Nussloch show intermediate to slight pollution levels with cadmium. Variability in body concentrations of cadmium in field animals was shown. It has been demonstrated that there may be variability of metal concentrations in sampled field populations, and that levels of metals in the hepatopancreas of individual woodlice may be more than twice the mean value for the whole population within a site (HOPKIN & MARTIN 1984, HOPKIN & al. 1986). Furthermore, the amount of pollutants in soil and litter are not always predictive of bioavailability and actual accumulation of pollutants, especially in environments that are polluted due to anthropogenic activity, such as former smelters, mines, and roadsides (HOPKIN & al. 1986). The variable cadmium concentrations in the sampled animals may be further explained by the variable age of the sample animals (reviewed in DROBNE 1997).

Cadmium concentrations in experimental animals increased with increasing concentrations of cadmium in the food. This is in accordance with laboratory experiments on the accumulation of pollutants from food according to consumption and dry weight (DROBNE 1996, HOPKIN & MARTIN 1984)

Food consumption rates in all groups increased with the course of the experiment. This may be explained by the great numbers of moulting animals in the first week, and the fact that animals were not adapted to laboratory conditions. It is recommended to accustom isopods to laboratory conditions for some weeks before the start of an experiment, as moulting is stimulated by changed conditions and

thus interferes with normal behavioural responses to a stimulus (STEEL 1980; DROBNE 1996; ZIDAR & al. 1998). An interval of 28 days between two ecdyses has been reported for *O. asellus* (STEEL 1982), so the impact of moulting behaviour on food consumption rate cannot be excluded in long-term experiments. Almost 80% of animals moulted during the experiments which resulted in decreased food consumption rates. Another factor influencing the food consumption rate might be the phenomenon of hyperphagia which describes the observation that isopods in the laboratory tend to consume more food than a comparable group of field animals (BECK & BRESTOWSKY 1980, HOPKIN 1990).

The combined consumption rates for the whole duration of the food choice experiment show that low levels of cadmium (20 and 45 mg kg^{-1} dry food weight) seem to have only little or no adverse effects on consumption rate. This is in accordance with data from the literature, which reports the low observed effective concentration (LOEC) for Cd in *P. scaber* at about 20 mg Cd kg^{-1} (DONKER & BOGERT 1991). For intermediate and high levels of Cd-pollution in the food a decrease of food consumption was observed.

After three weeks of the food choice experiment, the amounts of uncontaminated food and contaminated food consumed reached an apparently stable ratio of 6:4 in all groups, thus suggesting some kind of avoidance reaction to cadmium independent of cadmium concentration. If *O. asellus* was indeed able to taste and discriminate against cadmium contaminated food, we would expect a reaction in relation to cadmium concentration.

However, some interesting patterns of discrimination against contaminated food related to the concentration of cadmium and the duration of exposure can be observed. In groups "0-20" and "0-45" we found a similar pattern of rejection over three weeks. In the first week, there was a strong preference for uncontaminated food, whereas in the second week, the avoidance reaction against cadmium contaminated food was barely observable. In the third week, animals again discriminated significantly against contaminated food. In group "0-200", preference for uncontaminated food dropped from a ratio of 7:3 in the first week to a final 6:4 in the third week. In group "0-450", discrimination against contaminated food in the first week was not very pronounced, whereas in the second week, animals strongly discriminated against contaminated food, reaching a consumption ratio of about 6:4 in the third week.

The differences and time-dependent pattern of the avoidance reactions to cadmium contaminated food observed in the food-choice experiment might be explained by the assumption of two combined physiological effects: Firstly, an animal that starts to feed on cadmium contaminated food will suffer adverse metabolic effects from cadmium which will cause a premature cessation of feeding. Secondly, the intake of cadmium initiates a detoxification mechanism.

As described in the literature, isopods show a regular cycle of feeding behaviour (HAMES & HOPKIN 1990). After a phase of resting, animals void their guts, search for food, and, upon finding suitable food, start feeding. It may be assumed that once an animal starts feeding on suitable food, it will continue to feed on the same food if not disturbed. If animals start to feed on contaminated food, they will ingest this food until the ingested cadmium produces the assumed adverse metabolic effect, which will result in cessation of feeding. The higher the concentrations of ingested cadmium, however, the more pronounced the toxic reaction will be, thus reducing uptake even of uncontaminated food. The effect observed in group "0-450", where consumption levels of contaminated and uncontaminated food are equally low, might therefore be explained by assuming that the ingested levels of Cd are already so toxic that it also reduces the consumption of uncontaminated food.

Comparing the course of food consumption in the respective groups over time supports the second assumption. If animals that are exposed to small concentrations of cadmium acclimatise to these levels of contaminants in their food, after some time they will not be subject to the negative metabolic effects of low doses of cadmium in their food. Consumption rates of uncontaminated and contaminated food will be more equal, as was observed in the second week for groups exposed to low cadmium concentrations (groups "0-20" and "0-45"), and to a lesser degree in the group exposed to intermediate

cadmium concentrations (group "0-200"). In the group exposed to high cadmium concentrations (group "0-450"), this acclimatisation process will take longer. In the third week, the acclimatisation to cadmium in the food reached a steady state, which allows consumption of contaminated food, but still favours consumption of uncontaminated food. This state is represented by similar consumption ratios for uncontaminated and contaminated food in all groups.

Conclusions

In paired food choice tests, the consumption of cadmium contaminated food by terestrial isopods is reduced. This has formerly been interpreted as the ability of isopods to detect and discriminate against pollutants in their food, rendering results from biomonitoring experiments less significant. In our experiment, we could show that *O. asellus* does eat less contaminated food than uncontaminated if offered a choice. However, this effect is not related to the concentrations of the pollutant or the duration of exposure. This leads to the presumption that *O. asellus* (and perhaps other terrestrial isopods) cannot distinguish food according to cadmium concentration. The difference in consumption rates between uncontaminated and contaminated food is based on integration of feeding behaviour and the adverse metabolic effects of cadmium.

If this presumption is proven to be correct, results from experiments where *O. asellus* is employed as biomonitoring organism are accurate. The findings of this paper strongly support the generally held concept that the maximum amount of knowledge has to be accumulated about a species prior to its potential use as a biomonitor.

Acknowledgements

Povzetek

Kopenski raki enakonožci v telesu kopičijo kovine, kar jih uvršča med možne pokazatelje dostopnosti kovin v okolju. Nekatere raziskave so pokazale, da enakonožci izbirajo hrano z nižjo vsebnostjo kovin. Izbira hrane z manj kovin lahko pomeni, da vsebnost kovin v enakonožcih ne kaže količine dostopnih kovin v okolju. V tem primeru bi bila vprašljiva uporaba enakonožcev kot pokazateljev obremenjenosti okolja s kovinami.

V predstavljenem delu smo proučevali vpliv različnih koncentracij hrani dodanega kadmijevega nitrata na izbiro hrane pri kopenskem enakonožcu *Oniscus asellus*. Živalim smo hkrati ponudili hrano brez dodanega kadmija in hrano z dodanim kadmijevim nitratom (20, 45, 200 in 400 mg Cd kg^{-1} suhe teže hrane). Hrano smo pripravili kot mešanico mletih listov leske, ribje hrane in želatine v razmerju 63:3:34. Količino nitratnih ionov v ponujeni hrani smo uravnotežili z dodajanjem raztopine kalijevega nitrata (KNO_3). Primerjali smo stopnjo hranjenja z neonesnaženo in onesnaženo hrano, v odvisnosti od koncentracije kadmijevega nitrata v hrani in trajanja hranjenja.

Živali so zaužile manj s kadmijem onesnažene hrane že v prvem tednu poskusa izbire tudi pri najnižji koncentraciji kadmijevega nitrata dodanega hrani. V tretjem tednu poskusa je bilo pri vseh skupinah živali razmerje med stopnjo hranjenja z neonesnaženo in onesnaženo hrano 6:4. Pri živalih,

ki so med poskusom jedle hrano z dodanim kadmijevim nitratom je telesna vsebnost kadmija naraščala s koncentracijo kadmija v hrani in časom hranjenja.

Rezultati poskusov izbire hrane so pokazali, da *O. asellus* zaužije manj s kadmijem onesnažene hrane, če ima na voljo neonesnaženo. Vendar razmerje med zaužitjem neonesnažene in onesnažene hrane ne narašča s koncentracijo kadmija v hrani kakor tudi ne s časom hranjenja. Tako sklepamo, da *O. asellus* verjetno ne razlikujejo med hrano z različno vsebnostjo kadmijevega nitrata. Razlike v stopnji hranjenja z neonesnaženo in onesnaženo hrano so verjetno le posledica prehranjevalnega vedenja živali povezanega s presnovnimi učinki kadmija. Če je naša domneva pravilna, uporaba *O. asellus* kot pokazatelja dostopnosti kovin v okolju ni vprašljiva.

Literature:

BAYNE B.L. 1979: Assessing effects of marine pollution. Nature **280**: 14-15.

BECK L. & E. BRESTOWSKY 1980: Auswahl und Verwertung verschiedener Fallaubarten durch *Oniscus asellus* (Isopoda). Pedobiologia **20**: 428-441.

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 1997: The woodlouse, *Oniscus asellus*, as a monitor of environmental cadmium levels. Chemosphere **12**: 827-832.

CROMMENTUIJN T., J. A. M. DOODEMAN & A. DOORNEKAMP 1994: Lethal body concentrations and accumulation patterns determine time-dependent toxicity of Cadmium in soil arthropods. Environmental Toxicology and Chemistry **13** (11): 1781-1789.

DONKER M.H. & C.G. BOGERT 1991: Adaptation to cadmium in three populations of the isopod *Porcellio scaber*. Comp. Biochem. Physiol. **12**: 143-146.

DALLINGER R. 1977: The flow of copper through a terrestrial food chain: III. Selection of an optimum copper diet by isopods. Oecologia **30**: 273-276.

DROBNE D., P. ZIDAR & P. BJERREGAARD 1995: Could proposed bio-monitoring organisms select differently contaminated food? Abstracts, Sixth International Symposium, Metal Compounds in Environment and Life, Jülich, Germany, May 9-12, p. 35.

DROBNE D. 1996: Zinc toxicity to terrestrial isopods (Isopoda, Crustacea). Dissertation Thesis. Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo, Ljubljana, Slovenia.

DROBNE D. 1997: Terrestrial isopods – a good choice for toxicity testing of pollutants in the terrestrial environment. Environmental Toxicology and Chemistry 16: 1159-1164.

GOLDBERG E. D. & K. K. BERTINE 2000: Beyond the mussel watch – new directions for monitoring marine pollution. The Science of the Total Environment **247**: 165-174.

GUNNARSSON T. 1987: Soil arthropods and their food: choice, use and consequences. Doctoral thesis. Department of Ecology, University of Lund, Sweden.

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 1990: 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 1991: 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.

HOPKIN S. P. & M. H. MARTIN 1984: Heavy metals in woodlice. Symp. Zool. Soc. (London) **53**: 143-166.

HOPKIN S. P, G. N. HARDISTY & M. H. MARTIN 1986: The woodlouse *Porcellio scaber* as a 'biological Indicator' of zinc, cadmium, lead and copper pollution. Environmental Pollution **11**: 271-290.

HOPKIN S. P. 1989: Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Sciences, London and New York.

HOPKIN S. P. 1990: Species-specific differences in the net assimilation of zinc, cadmium, lead, copper and iron by the terrestrial isopod *Oniscus asellus* and *Porcellio scaber*. Journal of Applied Ecology **27**: 460-474.

HOPKIN S. P., DT. JONES & D. DIETRICH 1993: The isopod *Porcellio scaber* as a monitor of the bioavailability of metals in terrestrial ecosystems: towards a global 'woodlouse watch' scheme. The Science of the Total Environment, Supplement 1993, Elsevier Science Publishers B.V., Amsterdam, 357-365.

ODENDAAL J. P. & A . J. REINECKE 1999: The sublethal effects and accumulation of cadmium in the terrestrial isopod *Porcellio laevis* Latr. (Crustacea, Isopoda). Arch. Environ. Contam. Toxicol. **36**: 64-69.

PAOLETTI M. G. & M. HASSALL 1999: Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems and Environment **74**: 157-165.

PROSI F., V. STORCH & H. H. JANSSEN (1983): Small cells in the midgut glands of terrestrial Isopoda: Sites of heavy metal accumulation. Zoomorphology **102**: 53-64.

RABITSCH W. B. 1995: Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. I. Environmental Pollution **90**, No. 2: 221-237.

STEEL C. G. H. 1980: Mechanisms of coordination between moulting and reproduction in terrestrial isopod (Crustacea). Biol. Bull. **159**, 206-218.

STEEL C. G. H. 1982: Stages of the intermoult cycle in the terrestrial isopod *Oniscus asellus* and their relation to biphasic cuticle secretion. Can. J. Zool. **60**: 429-437.

SZLAVECZ K. & V. C. MAIORANA 1990: Food selection by isopods in pared choice tests. In: Biology of Terrestrial Isopods. Third International Symposium. Poitiers, July 10-12, 1990.

VAN CAPELLEVEEN H. E., N. M. VAN STRAALEN, M. VAN DEN BERG & E. VAN WACHEM 1986: Avoidance as a mechanism of tolerance for lead in terrestrial arthropods. In: Proc 3rd Eur. Congress. Entom. Part 2 (H.H.W. Veldhuis, Ed.), 251-255. N. Ev., Amsterdam.

ZIDAR P., D. DROBNE & J. ŠTRUS 1998: Determination of moult stages of *Porcellio scaber* (Isopoda) for routine use. Crustaceana **71**, 6: 646-654.

ZIMMER M., G. KAUTZ & W. TOPP 1996: Olfaction in terrestrial isopods (Crustacea: Oniscidea): responses of *Porcellio scaber* to the odour of litter. Eur. J. Soil. Biol. **32** (3): 141-147.