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Invertebrates Minimize Accumulation of Metals and Metalloids in Contaminated Environments

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Abstract Many studies were conducted measuring the lethal concentration of pollutants by using a contaminated solution or polluted sediments. Considering the impact of polluted food on mortality and uptake quantity of invertebrate shredders in batch cultures, little is known about, e.g. uranium and cadmium. Consequently, we investigated in situ the impact of metal and metalloid polluted food and water on *Gammarus pulex* L. under nature-like conditions. In contrast to other publications, a very low mortality rate of the invertebrates was found. Furthermore, fixation of elements by *G. pulex* was shown to be low compared to initial concentrations. Fixation of non essential metals and metalloids is shown to take place mainly on the surface of the invertebrates. This is deduced from easy desorption of a relevant amount of fixed metals and metalloids. It is concluded that the accumulation of metals and metalloids in situ under nature-like conditions within the food web via invertebrate shredders is very low. The invertebrates seem to minimize the uptake of non essential elements in the presence of nutrient-rich food even in habitats with higher contamination levels. Hence, invertebrates seem to be adapted to higher contamination levels in their favourable habitats.

Keywords Fixation · Avoidance · Invertebrates · Leaf litter · Chemical speciation · Desorption kinetics

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1 Introduction

High metal and metalloid concentrations in ground and surface waters are a global problem, enhanced by mining activities (Jakubick and Kahnt 2002). These high concentrations may be responsible for shifting food webs and hence changing ecosystem processes. A reason for these changes probably is the toxicity of metals and metalloids on water organisms and the possible enrichment in the food web (Alves et al. 2009; Borgmann et al. 2005; Gopalakrishnan et al. 2007). *Gammarus pulex* L., used in this investigation as sample species, is an important food base for fish (Winkelmann et al. 2007) and a key species for processing of leaf litter in freshwater ecosystems (Robinson et al. 1998). Most of the species in these ecosystems are described as very sensitive to inorganic pollutants (e.g. *Hyalella azteca* and *G. pulex*) (Milani et al. 2003; Wang and Zauke 2004) when tested with contaminated water and sediments with a relevant inorganic share. Little is known using highly

contaminated food. Metals and metalloids may adsorb on organic matter in high amounts (Guo et al. 2006; Maltby and Crane 1994; Sridhar et al. 2008). At the same time, organic matter is the main food source for the abundant animals (Li and Dudgeon 2008; Tiegs et al. 2008). So far, the effect of polluted food on *G. pulex* has only been shown for a few elements like iron, manganese, uranium and zinc (Maltby and Crane 1994). Low sensitivity of *G. pulex* to uranium but high sensitivity for zinc in food is described (Abel and Barlocher 1988; Schaller et al. 2009). However, the effect of inorganic pollutants on the sampled invertebrates and the resulting toxicity are not yet clarified in detail. For a pronounced understanding of toxicity effects, uptake and avoidance mechanisms of invertebrates have to be elucidated. In other publications, the uptake of metals by invertebrates is attributed to total metal concentration on and in the invertebrates, not differentiating between adsorbed (surface) and incorporated metals (Santoro et al. 2009; Woelfl et al. 2006). Considering a much higher toxicity effect the incorporated fraction is first of all of importance. On the other hand, both surface adsorbed and incorporated pollutants may be passed on in the food web leading to biomagnification.

We tested the effect of metal- and metalloid-rich food (leaves from *Alnus glutinosa* Gaertn.) in early stages of decay on *G. pulex* in situ in a polluted stream. The amount of elements fixed in/on the invertebrates were investigated including a desorption experiment to quantify more stable bonds of elements on invertebrate surfaces.

2 Materials and Methods

2.1 Test Organism

Specimens of *G. pulex* were collected from the stream Prieβnitz near Dresden. *G. pulex* specimens were tested for background concentrations of selected metals and radionuclides ascertaining that the stream is almost uncontaminated. The following metal and metalloid concentrations in *G. pulex* were measured (median in milligrammes per kilogramme per dry matter): phosphorus (7,693), manganese (26.9), iron (25.8), copper (75.4), zinc (75.2), arsenic (2.53), lead (1.42) and uranium (lower than the limit of detection (LOD)= 0.04). The specimens were kept for 5 days in the laboratory (in 80 L of tap water, 10–12°C and ~100 lx for 12 h a day) before starting the experiments. During the 5-day laboratory pre-culture, the *G. pulex* specimens were supplied with degraded alder leaves (*A. glutinosa*).

2.2 Fixation Experiment of Metals and Metalloids on *G. pulex* In Situ

Fresh-fallen leaf litter (*A. glutinosa* L.), collected from an uncontaminated site in the Tharandt forest near Dresden (Germany), was air dried. Before using the leaves in the experiment, the leafstalks were removed because *G. pulex* do not feed on leafstalks. Ten leaves weighing about 5 g of DM were filled into each of several litter bags made of nylon gauze with a mesh size of 250 μm and sawed with polyethylene thread. The litter bags were placed in running water of a contaminated stream in a former uranium mining site (Mechelgrün-Neuensalz, Saxony, Germany) for 4 weeks as pre-treatment procedure to allow accumulation of heavy metals, metalloids and radionuclides onto the leaf litter and to allow the development of a decomposer community (fungi and bacteria) without the influence of invertebrates. The resulting metal and metalloid concentrations in the pre-treated leaf litter were as follows (median in milligrammes per kilogramme per DM): phosphorus (2,528), manganese (5,812), iron (1,290), copper (122), zinc (249), arsenic (54), lead (2.0) and uranium (794). In the stream water, the element concentrations during the experiment were in median (microgrammes per litre): phosphorus (486), manganese (375), iron (199), copper (4.62), zinc (5.84), arsenic (29.6), lead (0.40) and uranium (154). The main chemical speciation of metals and metalloids in the water (Table 1) were calculated using PhreeqC (version 2.15.1) Geochemical Modeling Software with a modified Minteq thermodynamic database. Also measured were conductivity (800–1,000 μS cm^{-1}), pH (7.5 and 7.9) (Ross and Dudel 2008) and Eh (219–287 mV). The water temperature was relatively stable between 12.7°C and 15.0°C.

After 4 weeks of pre-treatment in the stream water, leaves were removed from the litter bags, washed with the stream water carefully before putting them into a drop-like cage containing 30 specimens of *G. pulex*. The drop-like shaped cages (replication of 15) were made from chemical inert material (nylon).

Table 1 Chemical speciation for metals and metalloids in the water at study site

Element	Species	Distribution (%)
Phosphorus	HPO_4^{2-}	56.0
	H_2PO_4	15.7
	$MgHPO_4$	12.9
	$CaHPO_4$	5.1
	$UO_2(HPO_4)_2$	3.8
	$NaHPO_4^-$	0.9
Manganese	Mn^{2+}	65.2
	$MnSO_4$	33.9
	$MnHCO_3^+$	0.8
Iron	$Fe(OH)^{2+}$	93.8
	$Fe(OH)_3$	5.3
	$Fe(OH)_4^-$	0.9
Copper	$CuCO_3$	68.6
	Cu^{2+}	13.2
	$CuSO_4$	12.2
	$CuOH^+$	4.1
	$Cu(CO_3)_2^{2-}$	1.2
	$CuHCO_3^+$	0.7
Zinc	Zn^{2+}	43.3
	$ZnSO_4$	39.8
	$Zn(SO_4)_2^{2-}$	13.1
	$ZnCO_3$	2.2
	$ZnHCO_3^+$	1.1
Arsenic	$HAsO_4^{2-}$	88.9
	$H_2AsO_4^-$	11.1
Lead	$PbCO_3$	35.0
	$PbSO_4$	30.2
	Pb^{2+}	13.3
	$PbHCO_3^+$	7.8
	$Pb(SO_4)_2^{2-}$	6.3
	$PbOH^+$	6.1
	$Pb(CO_3)_2^{2-}$	0.7
	$PbCl^+$	0.5
Uranium	$UO_2(HPO_4)_2$	96.4
	$UO_2(CO_3)_3^{4-}$	2.1
	$UO_2(CO_3)_2^{2-}$	1.4

The speciation was calculated using PhreeqC under field physicochemical conditions: pH of 7.5, pe of 8.45 and temperature of 14.0°C

Supporting sticks covered with nylon gauze of 250-μm mesh size were used (Schaller et al. 2010). The experiment was run for 2 weeks in the stream at a former uranium mining site.

2.3 Desorption Experiment

The desorption experiment was started with 5 L of tap water, an adjusted pH of 7.5 by the addition of $NaHCO_3$ and metal concentrations below the detection limit. Ten specimens of *G. pulex* (from the food experiment) were placed into each test vessel. Samples of five individuals were taken after 30 and 60 min, respectively.

2.4 Sampling, Sample Preparation and Analysis

After 14 days of experiment, the specimens of *G. pulex* were separated from the cages as single samples by manual picking. They were dried, wet digested and analysed. Specimens of *G. pulex* and leaf litter samples were digested in a closed vessel microwave system (MARS5 CEM Corp., Matthews, USA) according to DIN (DIN-EN-13805 2002) using nitric acid and hydrogen peroxide. All water samples were acidified and kept at room temperature before metal and metalloid analysis with inductively coupled plasma mass spectrometry (ICP-MS) measurement (PQ2+ Instrument, VG Elemental, Winsford, UK) according to DIN (DIN 2004). The ICP-MS was calibrated using standards prepared from single-element and multi-element solutions (Bernd Kraft, Duisburg, Germany). Calibration validity was confirmed with standard reference material GBW7604, popular leaves (Office of CRM, China), digested in the same manner as the litter samples. LOD was calculated as the threefold standard deviation of instrument blank (acidified water). Carbon and nitrogen contents in the leaf litter samples were measured with Elementar vario el III (Hanau, Germany) in accordance with (DIN-ISO-10694 1995). To determine dissolved organic carbon in the water samples a TOC Analyzer 5000 (Shimadzu, Japan) was used according to (DIN 1997).

3 Results and Discussion

3.1 Characteristics of Metal and Metalloid Fixation by *G. pulex*

During the 14-day in situ food experiment, a significant enrichment ($p<0.05$, Wilcoxon test) in/on *G. pulex* for phosphorus, manganese, iron, copper, zinc, arsenic, lead and uranium was observed (Fig. 1). Comparing leaf litter concentrations with concentrations

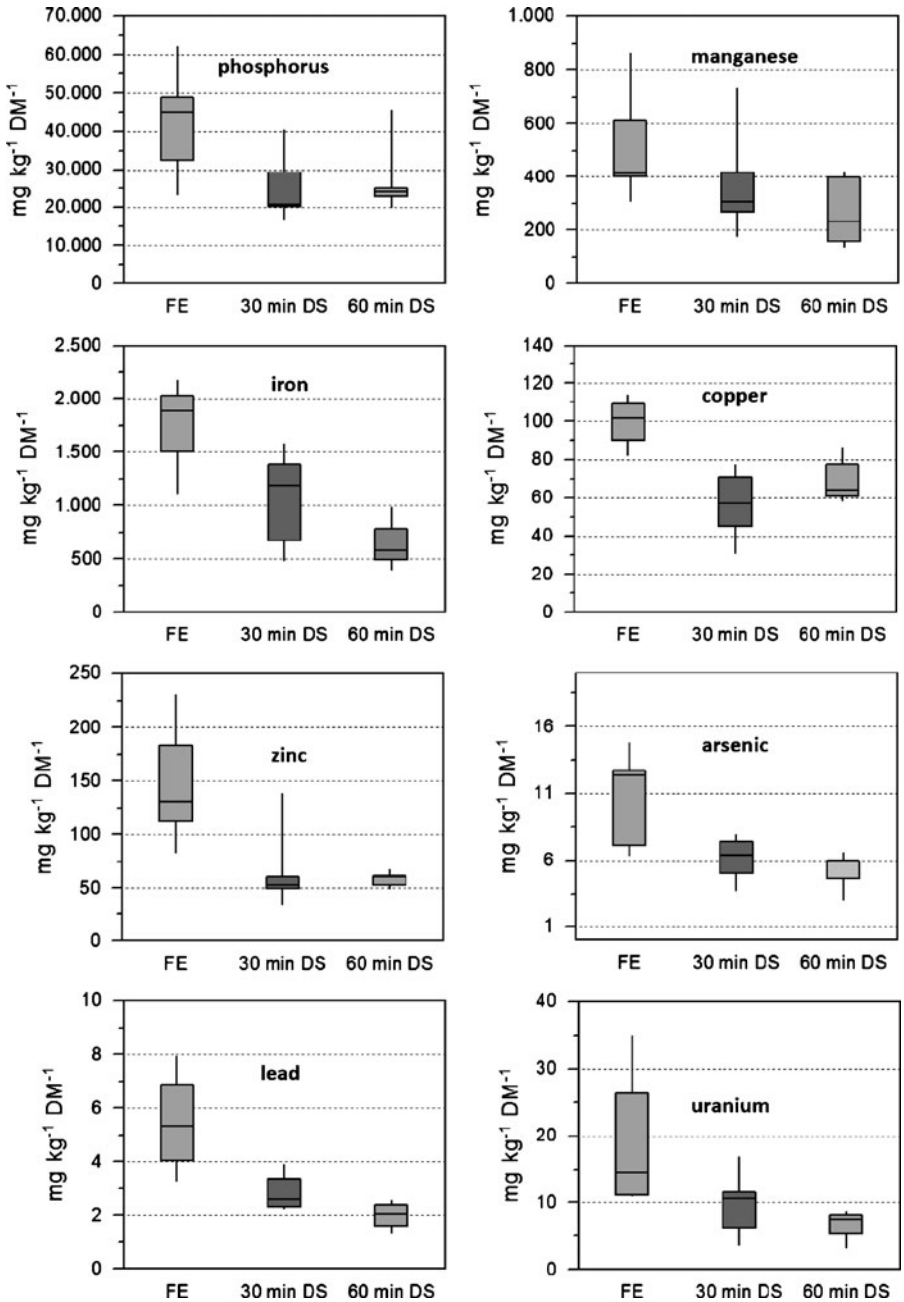

Fig. 1 Metals and metalloids concentrations in/on *G. pulex* after 14 days of the in situ food experiment (*FE*) and after desorption in uncontaminated water after 30 (*30 min DS*) and 60 min (*60 min DS*). $P<0.05$, significant differences in metal concentration of *G. pulex* between animals sampled after food experiment (*FE*) and after further 60 min of DS in non-contaminated water (*60 min DS*) were proven for iron, copper, zinc, lead and uranium

in *G. pulex* at the end of the experiment, the following concentration factors were found: phosphorus (17.8), manganese (0.07), iron (1.57), copper (0.86), zinc (0.52), arsenic (0.23), lead (2.93) and uranium (0.01). Furthermore, an accumulation of the elements into *G. pulex* (in milligrammes per kilogramme) during the experiment with contaminated water and food were proven (calculated as subtraction of concentration of elements in *G. pulex* at the end of experiment minus concentration at start): phosphorus (37.300), manganese (384), iron (1860), copper (29.3), zinc (55.5), arsenic (9.77), lead (3.89) and uranium (14.49). A major fraction of the elements is organically bound on/in the leaf litter with the attached heterotrophic biofilm (D'Souza et al. 2006; Purchase et al. 2009). The leaf litter in this experiment is probably of high food quality (C/N ratio of 15±3) for *G. pulex*. For other element concentrations in the leaf litter, see

(Schaller et al. 2010). With this kind of food, the selective uptake and/or specific detoxification (removal) mechanisms seem to be able to avoid non essential elements resulting in the observed low enrichment of metals and metalloids by *G. pulex*. As essential for invertebrates, phosphorus, manganese, iron, copper and zinc are known (Hopkin 1989). However, we found that enrichment of metals, metalloids and radionuclides in the presence of nutrient-rich food (high quality and no food limitation) is very low.

The uptake of essential elements can take place via different pathways: (1) via surface and/or (2) via ingestion and uptake in the alimentary channel (Dallinger 1995; De Schamphelaere et al. 2004; Pagenkopf 1983). The fixation of metals and metalloids in/on invertebrates is mainly dependent on body size (Alves et al. 2009; Wang and Zauke 2004). The desorption experiment shows that a relevant amount of the fixed metals and metalloids can easily be desorbed (Fig. 1). This can partially be explained with biofilm growth, including microbes and their exudates like EPS on the surface of the invertebrates (Bulgheresi et al. 2006), and the desorption properties for metals and metalloids of this biofilm (Zhou et al. 1998).

The desorption experiment then shows which part of the metals and metalloids is fixed on the body surface. Desorption is known to be very fast (Shin et al. 2006). In other studies, desorption time was allowed to be much longer (gut clearance) (Alves et al. 2009), but with longer desorption time, the risk is included that element concentrations of the invertebrates decrease also by excretion and loss via gills. A significant ($p<0.05$ tested with Wilcoxon test) desorption during the first 60 min in non-contaminated water is demonstrated for iron, copper, zinc, lead and uranium. For nutrient elements like phosphorus and the non essential element arsenic, no significant difference can be shown. In opposite to other findings that invertebrates exhibit homogeneous concentrations (actively steered) of essential elements (Karimi and Folt 2006), we found an easy desorption of essential elements like zinc and copper. The reason for this could be a high uptake of these elements in environments with high metal load (via special uptake channels) and a permanent excretion of excess amounts.

3.2 Tolerance of *G. pulex* to Metals and Metalloids

The high survival rate of *G. pulex* (median of 29 (26–30) individuals from inoculated 30 specimens at start in the 14-day food experiment) with polluted food in a polluted stream at a former uranium mining site is in contrast to other findings that invertebrates are sensitive for inorganic pollutants (Bartlett et al. 2004; Robertson and Liber 2007). This is of special interest considering enhanced toxicity of organically bound metals compared to inorganically bound metals (Calamari et al. 1980) (Moriarty et al. 2009).

The used invertebrates were not adapted to high inorganic pollution and had nevertheless a very low mortality rate (median of one of the 30 specimens). This can be explained with active avoidance in the presence of nutrient-rich food by strong chemical bond of metals to nitrogen (Crawford et al. 1996) in food and the invertebrate faeces which had high nitrogen content (Wright 1995) enhanced by nitrogen-rich food. Furthermore, the avoidance can also be explained with selective uptake of essential elements, excretion of elements that had been taken up (Watanabe et al. 2008) and moulting with attached pollutants (Bergey and Weis 2007).

4 Conclusions

Our results reveal that mortality rate of *G. pulex* may not affected by high concentrations of metals and metalloids in water and especially food. The fixation of metals and metalloids is very low. A significant part of the metals and metalloids fixed by these invertebrates is not strongly bound but can easily be desorbed. Consequently, biomagnification of metals and metalloids when including the trophic level represented by *G. pulex* may be low. This can be explained also by an accumulation of metals and metalloids in the habitats of these invertebrates. Hence, invertebrates seem to be adapted to higher levels of metals and metalloids by avoiding the uptake.

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