Chemosphere 87 (2012) 84–90

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Single versus combined exposure of *Hyalella azteca* to zinc contaminated sediment and food

Lien T.H. Nguyen *, Brita T.A. Muyssen, Colin R. Janssen

Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Jozef Plateaustraat 22, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 7 July 2011
Received in revised form 22 November 2011
Accepted 25 November 2011
Available online 22 December 2011

Keywords:
Hyalella azteca
Zinc
Sediment toxicity
Dietary exposure
Selective feeding

ABSTRACT

The amphipod *Hyalella azteca* was exposed for 28 d to different combinations of Zn contaminated sediment and food. *Sediment exposure* (+clean food) resulted in increased Zn body burdens, increased mortality and decreased body mass when the molar concentrations of simultaneously extracted Zn were greater than the molar concentration of Acid Volatile Sulfide (SEM_{Zn}–AVS > 0), suggesting that dissolved Zn was a dominant route of exposure. No adverse effect was noted in the *food exposure* (+clean sediment), suggesting selective feeding or regulation. *Combined exposure* (sediment + food) significantly increased adverse effects in comparison with *sediment exposure*, indicating contribution of dietary Zn to toxicity and bioaccumulation. The observed enhanced toxicity also supports the assumption on the presence of an avoidance/selective feeding reaction of the amphipods in the single sediment or food exposures. During 14 d post-exposure in clean medium, the organisms from the same *combined exposure* history received two feeding regimes, i.e. clean food and Zn spiked food. Elevated Zn bioaccumulation and reduced reproduction were noted in amphipods that were offered Zn spiked food compared to the respective organisms that were fed clean food. This was explained by the failure of avoidance/selective feeding behavior in the absence of an alternative food source (sediment), forcing the amphipods to take up Zn while feeding. Increasing Zn body burdens rejected the assumption that Zn uptake from food was regulated by *H. azteca*. Our results show that the selective feeding behavior should be accounted for when assessing ecological effects of Zn or other contaminants, especially when contaminated food is a potential exposure route.

1. Introduction

Benthic invertebrates take up metals from both the surrounding aquatic medium (pore water and overlying water) and ingestion of particles (sediment and food) (Rainbow, 2002; Hare et al., 2003). The significance of the dietary exposure pathway in contributing to metal accumulation and toxicity has been reported in numerous studies (e.g., Wiklund and Sundelin, 2002; Griscom and Fisher, 2004; Besser et al., 2005). Yet, in many laboratory and experimental protocols, sediment toxicity assays are performed with contaminated sediment and clean food. It is either presumed that animals do not take up metal from food or food is not considered as metal source.

Several geochemical approaches have been used to study the bioavailability of metals in sediment. A common notion is that the association of metals with AVS influences toxicity (and bioaccumulation) in sediments by controlling pore water soluble metal concentrations (Ankley et al., 1996; Di Toro et al., 2005). Bioavailability of metals from food is metal and species-specific but also depends on feeding behavior, e.g. an ingestion rates and assimilation efficiency. Additionally, feeding selectivity and avoidance behavior might play a role in sediment metal toxicity (Simpson and King, 2005; Simpson and Batley, 2007; Micevska and Simpson, 2008). Earlier experiments have shown that amphipods can avoid contaminated sediment (De Lange et al., 2006) and similar behavior for contaminated food was demonstrated for terrestrial invertebrates (Zidar et al., 2005; Wiklund et al., 2006).

The amphipod *Hyalella azteca* is a commonly used organism for sediment toxicity testing. According to standard guidelines (e.g., EC, 1997; US EPA, 2000), feeding is requested during the tests and the amphipods do not need to rely on food particles in the sediment. As mentioned above, these standard assays are performed with contaminated sediment and clean food. The contribution of diet-borne metals to metal accumulation and toxicity to *H. azteca* has been observed in Besser et al. (2005) and Borgmann et al. (2005). However, in these studies, the organisms were exposed to diet-borne metal in water (with no sediment present) and the potential of sediment particles as dietary source was not taken into

Abbreviations: AVS, Acid Volatile Sulfide; SEM, Simultaneously Extracted Metal; TOC, Total Organic Carbon.

* Corresponding author. Tel.: +32 (0)9 264 3993; fax: +32 (0)9 264 3766.
E-mail addresses: lien.nguyenhong@UGent.be (L.T.H. Nguyen), Brita.Muyssen@UGent.be (B.T.A. Muyssen), Colin.Janssen@UGent.be (C.R. Janssen).

0045-6535/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2011.11.066

account. It has been reported that *H. azteca* has the ability to select food quality according to its metabolic requirements (Wang et al., 2004). Thus, adding clean food might discourage ingestion of metal contaminated sediment particles. In this way, the potential diet-borne metal toxicity, especially when the presence of contaminated food is a real scenario of contaminant exposure, could be underestimated and the ecological relevance of the sediment laboratory tests with *H. azteca* may be questioned.

The objectives of the present study were to investigate the relative contribution of dietary exposure routes to Zn accumulation and toxicity in *H. azteca* and to gain insights in the behavioral changes related to the presence of contaminated food. We performed 42 d standard sediment toxicity tests with *H. azteca*. During the 28 d sediment exposure, the amphipods were exposed to different combinations of Zn in sediment and diet (i.e. single exposure to sediment or food, and simultaneous exposure to sediment and food). Mortality, body mass and Zn accumulation were measured. During the 14 d post-exposure (water-only) the contribution of diet-borne Zn in the absence of sediment was monitored with reproduction as an additional endpoint. Bioavailability of Zn in the sediment was assessed in the context of the equilibrium partitioning model based on AVS and SEM (Ankley et al., 1996; Di Toro et al., 2005).

2. Materials and methods

2.1. Experimental design

H. azteca was exposed to sediment spiked with 0, 100, 320 and 1000 μg Zn g^{-1} dry weight (d.w.) for 28 d. The food was Rabbit chow and was added uncontaminated or spiked with the same three Zn concentrations [Zn]. Experiments were set up by exposing the amphipods to one of the following exposure scenarios: (1) Zn spiked sediment and clean food (*sediment exposure*), (2) Zn spiked food and uncontaminated sediment (*food exposure*), and (3) Zn spiked sediment and Zn spiked food (*combined exposure*). A 28 d exposure period to sediment was immediately followed by a 14 d post-exposure in clean medium (water-only). During the post-exposure period, *H. azteca* from the *combined exposure* were divided into two groups. One group was continued to be fed Zn spiked food as received during the 28 d exposure and the other group was fed clean food.

2.2. Sediment preparation

The test sediment was collected from an uncontaminated area near Brakel, Belgium (50°45′N, 3°46′E). The main characteristics of the sediment are presented in Table 1. For each of the Zn treatments, an appropriate amount of sediment was thoroughly mixed with a desired amount of $ZnCl_2$ (Merck, Belgium) by means of rolling in a sealed plastic bag (control sediment was mixed with an equal volume of deionized water) for 15 min. Aliquots of 250 g spiked sediment were dispensed into each of the test jars and 250 mL of the test medium (84.1 mg L^{-1} $NaHCO_3$, 1.03 mg L^{-1} NaBr, 3.73 mg L^{-1} KCl, 147 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 61.7 mg L^{-1} $MgSO_4{\cdot}7H_2O$) (Borgmann, 1996) was added. A 35 d equilibration period (with regular renewal of the overlying water) under the same conditions as used during the exposure was allowed prior to the introduction of the test organisms. It has been shown in our earlier studies that the artifacts introduced by spiking Zn into sediment, e.g., low pH, precipitated Fe(OOH), were eliminated and the sediment–water system reached equilibration after 35 d (Nguyen et al., 2005).

Table 1
Characteristics of sediment used in the study.

Particle size (%)	
Clay (0–2 μm)	8
Silt (2–50 μm)	36
Sand (50–2000 μm)	56
TOC (% C d.w.)	1.5–2.5
Co (μg g^{-1} d.w.)	5
Cd (μg g^{-1} d.w.)	0.6
Cu (μg g^{-1} d.w.)	7.6
Pb (μg g^{-1} d.w.)	20
Ni (μg g^{-1} d.w.)	17.5
Zn (μg g^{-1} d.w.)	55
Fe (μg g^{-1} d.w.)	20950
SEM (μmol g^{-1} d.w.)	0.5
AVS (μmol g^{-1} d.w.)	3–10

2.3. Food preparation

Zn contaminated food particles were prepared by mixing ground Rabbit chow (<350 μm) with the desired amount of $ZnCl_2$. The Zn–food mixture was equilibrated for 28 d at 4 °C. At the end of the equilibration period, the Zn–food mixture was centrifuged for 25 min at 2800 rpm (2500*g*) and the supernatant was removed. The food particles (with Zn adsorbed) were then re-suspended in deionized water (rinsing process) and were allowed to settle for 7 d at 4 °C. Prior to the start of the tests, deionized water was decanted and settled food particles were used in the experiments.

2.4. Toxicity tests

Sediment toxicity tests were conducted according to US EPA standard guidelines (US EPA, 2000). *H. azteca* used in the present study was obtained from the stock cultures maintained at the Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium. For the experiments, 7–8 d old *H. azteca* were collected by a sieving technique (US EPA, 2000) and were randomly introduced into each of the test jars (20 individuals per jar). Each treatment had eight replicates for biological samples and two replicates for chemical analysis. Replicates used for chemical analysis also contained test organisms and received identical treatment (e.g. feeding, overlying water renewal). During the test period, amphipods were fed daily (0.6 mg d.w. per individual) with previously prepared Rabbit chow. The tests were performed at 23 ± 1 °C and a light–dark cycle of 16–8 h (light intensity of 100–300 lux). Overlying water was renewed two times per week and was sampled (1 cm above sediment surface) at 0, 7, 14, 21 and 28 d after the initiation of the exposure. At the same interval, overlying water quality including dissolved oxygen (DO), temperature, pH, hardness, conductivity and NH_4^+ concentration were checked. Prior to the start and at day 28, one replicate of each treatment was taken for sediment Zn analysis. Sediments were collected at 0.5–1 cm depth. Total Zn, AVS and SEM concentrations were measured. Sediment pore water was extracted by centrifugation for 15 min at 2800 rpm (2500*g*). Overlying water and pore water samples were filtered (0.45 μm) and preserved with HNO_3 for Zn analysis. At day 28, the sediment in the test jars was sieved to collect the amphipods and mortality was assessed. Surviving *H. azteca* from each replicate were pooled and placed in clean medium for 24 h for gut clearance prior to determination of body mass and Zn analysis of the tissue.

2.4.1. Post-exposure

After 28 d sediment exposure, *H. azteca* was transferred into test jars containing clean medium (250 mL) with cotton gauze as a substrate. Over the 14 d post-exposure period, the organisms were fed daily (following feeding regimes mentioned in Section 2.1) and the medium was renewed two times per week. Surviving