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Influences of sediment geochemistry on metal accumulation rates and toxicity in the aquatic oligochaete *Tubifex tubifex*

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ABSTRACT

Metal bioaccumulation and toxicity in the aquatic oligochaete *Tubifex tubifex* exposed to three metal-contaminated field-sediments was studied in order to assess whether sediment-geochemistry (AVS, TOC) plays a major role in influencing these parameters, and to assess if the biodynamic concept can be used to explain observed effects in *T. tubifex* tissue residues and/or toxicity. An active autotomy promotion was observed in three studied sediments at different time points and reproduction impairment could be inferred in *T. tubifex* exposed to two of the tested sites after 28 days. The present study showed that sediment metal concentration and tissue residues followed significant regression models for four essential metals (Cu, Co, Ni and Zn) and one non-essential metal (Pb). Organic content normalization for As also showed a significant relationship with As tissue residue. Porewater was also revealed to be an important source of metal uptake for essential metals (e.g. Cu, Ni and Zn) and for As, but AVS content was not relevant for metal uptake in *T. tubifex* in studied sediments. Under the biodynamic concept, it was shown that influx rate from food (I_F, sediment ingestion) in *T. tubifex*, in a range of sediment geochemistry, was able to predict metal bioaccumulation, especially of the essential metals Cu, Ni and Zn, and for the non-essential metal Pb. Additionally, I_F appeared to be a better predictor for metal bioaccumulation in *T. tubifex* compared to sediment geochemistry normalization.

1. Introduction

Sediments are essential, integral and dynamic components of aquatic ecosystems (Salomons and Brils, 2004). Contaminants are mainly associated with the fine particulate material (both organic and mineral) and sediments can act as a reservoir and source of contamination to the water column and aquatic biota (Burton, 2002). Metals can occur in different chemical forms, and their bioavailability in sediments is difficult to determine because it may depend on several factors: such as their speciation (e.g., dissolved, sulfide/organic carbon-bound metals); the sediment–water partitioning relationships; the organism physiology (e.g., uptake rates and assimilation efficiency, excretion); feeding behaviour (e.g., particle-size selection) and other behaviour (e.g., burrowing activity) (Luoma and Rainbow, 2005; Simpson, 2005; Simpson and Batley, 2007). Thus, metal toxicity depends on the relative contribution of each exposure route, on the metal concentration in each compartment (i.e., overlying water, porewater, and sediment), the relative importance of each compartment for the individual organism biology, and upon duration of exposure (Adams et al., 2011; Simpson and Batley, 2007).

A factor that has been proposed to be important in controlling metal bioavailability in anoxic sediments is the amount of acid volatile sulfides (AVS). Metals associated with AVS are called simultaneously extracted metals (SEM). SEM is generally defined as the sum of molar concentrations of toxicologically important, cationic metals (Cu, Pb, Cd, Zn, Ni, and also Cr and Ag) which are extracted together with AVS. From this, Di Toro et al. (1990) formulated the SEM–AVS model for estimating metal toxicity from contaminated sediments. This model predicts that when AVS concentrations in sediments, on a molar basis, exceed SEM concentrations ($SEM_{Me} - AVS < 0$), all metals will be bound to sulfides and the sediment porewater is considered to be nontoxic. In contrast, when the sediment contains an excess of SEM ($SEM_{Me} - AVS > 0$), metals will be released into the porewater and become potentially toxic to the aquatic life. Organic matter can also bind non-sulfide bound trace metals, thus preventing them from entering the dissolved phase (Mahony et al., 1996). Based on this, [SEM − AVS]/TOC has been proposed as a measure of bioavailable metal (Di Toro et al., 2005). The [SEM − AVS]/TOC concept assumes that there is no metal

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toxicity caused by transformations of the sulfide and organic matter bound metal in the gut of sediment-ingesting organisms or via exposure to contaminated food (Meyer et al., 2005). Although the SEM–AVS model has been validated for both acute and chronic toxicity (Di Toro et al., 1990; Casas and Crecelius, 1994; Hare et al., 1994; Pesch et al., 1995), significant evidence has been gained that benthic invertebrates can accumulate metals in large amounts, even when $SEM_{Me} - AVS < 0$ (Lee et al., 2000a,b, 2001; Hare et al., 2001; De Jonge et al., 2009, 2010).

But the presence of accumulation of a metal in an organism is not an adverse biological effect in and of itself; only the biological responses induced by the presence of the metal(s) are potential adverse effects (Rand, 1995). A key factor in understanding observed bioaccumulated metals in aquatic organisms is based on understanding biodynamics of metal bioaccumulation processes; the biodynamic concept provides a framework to explain how and why trace elements bioaccumulation differs among metals, species, and environments (Luoma and Rainbow, 2005). Toxicity is then expected to occur when the rate of metal uptake summed across all sources (solution and diet) exceeds the combined rates of efflux and detoxification of metal into metabolically inert forms (Rainbow, 2002; Luoma and Rainbow, 2005, 2008).

To our knowledge, the influences of sediment geochemistry (e.g. AVS, organic matter) on accumulation rates and resulting chronic toxicity from field-collected sediments are poorly documented. For that purpose, we used the model organisms *Tubifex tubifex* Müller, (1774), an aquatic oligochaete commonly used in sediment toxicity assessment (ASTM, 2005). Therefore, the general aim of this study is (1) to evaluate metal bioaccumulation and toxicity in *T. tubifex* exposed to three metal-contaminated field-sediments; (2) to assess whether sediment-geochemistry (AVS, TOC) plays a major role in influencing these parameters in *T. tubifex*; and (3) to assesses if the biodynamic concept can be used to explain observed effects in *T. tubifex* tissue residues and/or toxicity.

2. Material and methods

2.1. Study area and sediment sampling

The study area included three sites from the Nete/Scheldt basin (Flanders, Belgium): Scheppelijke nete (208591UTMX, 210830UTMY), Kneutersloop (185353UTMX, 208963UTMY), and Molse nete (201657UTMX, 207709UTMY). These sites were selected based on previously conducted studies (Bervoets et al., 1997, 2004; Bervoets and Blust, 2003; De Jonge et al., 2009, 2010), in which high sediment metal concentrations have been measured. Historic metal pollution dating back to the nineteenth century has left numerous areas in Flanders, the northern part of Belgium, contaminated with high concentrations of zinc, cadmium, copper, nickel, lead and arsenic. One of these areas is situated in the north-eastern part (i.e. Noorderkempen). The contamination in this area originated from three zinc smelters that discharged metals to the environment using very polluting pyrometallurgic zinc refining procedures (Groenendijk et al., 1999). Despite a stop of direct emission to the environment since the early nineties of last century, soils and sediments still contain high metal concentrations and can act as possible sources of metal pollution for the aquatic environment in this area (De Jonge et al., 2008).

Sediment samples were taken in the field, from the upper 10 cm layer with a spade, for the realization of the sediment bioassays and for metal concentrations analysis. A subsample was taken for measurements of sediment AVS concentrations, as well as total organic content (TOC) and particle size distribution estimation. Sediments were stored in a cool chamber at 4 °C for maximum period of 3 weeks before the experiments were conducted.

2.2. Experimental design

Sediment used for the worm cultures and as control in the toxicity test was obtained from a large pool, filled with groundwater, at Iturbatz (556682X, 4740877Y, 30T) in Entzia Mountains (Álava, Spain), named Special Area of Conservation by Natura 2000 network (Habitat Directive 92/43/EEC). Sediment was also collected with a spade from the upper 10 cm layer. The aquatic oligochaete worm *T. tubifex* was used as study organism. A healthy stock of the aquatic oligochaete *T. tubifex* was provided by the Animal Ecotoxicity and Biodiversity laboratory from the University of Basque Country (UPV/EHU). Individual culture batches were initiated with a cohort of 100–150 young worms, and reached sexual maturity in 6–7 weeks. A supplement of finely ground 1 g Tetramin® fish food was added at the beginning of each culture as a nutritional complement.

T. tubifex toxicity experiments were conducted following the methods proposed by Reynoldson et al. (1991) and ASTM (2005). Sediment used for the bioassay was sieved through a 500-μm mesh to remove indigenous species so they do not interfere in experiment results (Reynoldson et al., 1995). *T. tubifex* worms were exposed for 3, 10 and 28 days. Four sexually mature worms with a well developed clitellum were included in each replicate. These worms were in their first reproductive cycle and had similar age (7–8 weeks). Four replicates were prepared per tested sediment and were used for biological determinations at the different exposure times (metal bioaccumulation and toxicity endpoints). For the 28 days experiment, one extra replicate per tested sediment was included for sediment chemical measurements at the end of the experiment. Each replicate contained 100 ml of sediment, 80 mg of supplementary food (Tetramin®) to minimize inter-sediment differences due to nutritional quality and quantity, and 100 ml of overlying reconstituted water (ISO 6341-1982), in a 250 ml glass beaker. These beakers were placed into an incubator at 22 ± 1 °C, in the dark and gently aerated. Beakers were prepared 24 h prior to the beginning of the experiment to allow the sediment to settle.

Studied endpoints at 3, 10 and 28 days were: survival (% SUR), autotomy (% AUT) and worm biomass (WB, mg). Autotomy is the loss of segments in the hind part of the body in oligochaetes due to a local constriction of the circular muscles, which can be seen macroscopically (Kaster, 1979). In this study, autotomy was calculated as proposed by Meller et al. (1998), who calculated autotomy as the ratio of dead plus autotomized to the total exposed animals (constrictions were not considered autotomy). After 28 days exposure, it was considered as a chronic bioassay, so reproduction endpoints were also measured (No. of Total Cocoons: TCC; No. of Total Cocoons per Adult: CCAD; No. of Empty Cocoons: ECC; percentage of Hatched cocoons: % HATCH; No. of Total Young: TYG; and number of Total Young per Adult: YGAD). Working procedures are detailed in a previous publication (ASTM, 2005; Maestre et al., 2007) and test conditions are summarized in Appendix A, Table A.1.

At the end of the experiment, worms were allowed to empty their guts by placing them for 5 h in clean reconstituted freshwater, and then worms were stored at −20 °C (for tissue residue) until the samples could be prepared. Worm dry weight was determined in an electrobalance (Sartorius M3P, DL = 1 μg) after drying in an oven at 60 °C for at least 48 h. For tissue residue analysis, worms were digested with ultrapure 69% HNO_3 and 30% H_2O_2 in a Hotblock and diluted to a final volume of 7 ml with Mili-Q water. Samples were stored at −20 °C until analysis.

During the experiments, dissolved oxygen, pH and T (Hach HQ30d Multi-Parameter kit) were measured twice per week in the overlying water (while aeration was visually checked every working day).