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Toxicity assessment of sediments from three European river basins using a sediment contact test battery

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ABSTRACT

The toxicity of four polluted sediments and their corresponding reference sediments from three European river basins were investigated using a battery of six sediment contact tests representing three different trophic levels. The tests included were chronic tests with the oligochaete *Lumbriculus variegatus*, the nematode *Caenorhabditis elegans* and the mudsnail *Potamopyrgus antipodarum*, a sub-chronic test with the midge *Chironomus riparius*, an early life stage test with the zebra fish *Danio rerio*, and an acute test with the luminescent bacterium *Vibrio fischeri*. The endpoints, namely survival, growth, reproduction, embryo development and light inhibition, differed between tests. The measured effects were compared to sediment contamination translated into toxic units (TU) on the basis of acute toxicity to *Daphnia magna* and *Pimephales promelas*, and multi-substance Potentially Affected Fractions of species (msPAF) as an estimate for expected community effects. The test battery could clearly detect toxicity of the polluted sediments with test-specific responses to the different sediments. The msPAF and TU-based toxicity estimations confirmed the results of the biotests by predicting a higher toxic risk for the polluted sediments compared to the corresponding reference sediments, but partly having a different emphasis from the biotests. The results demonstrate differences in the sensitivities of species and emphasize the need for data on multiple species, when estimating the effects of sediment pollution on the benthic community.

1. Introduction

A wide variety of contaminants, including heavy metals, pesticides and industrial chemicals have been discharged into European rivers and have accumulated in sediments. The concentrations of contaminants in sediments may be several orders of magnitude higher than in the overlying water, and although certain chemicals are strongly sorbed to sediment, they may still be available for biota, thus causing a risk to benthic organisms (e.g. Chapman et al., 1998; Lamoureux and Brownawell, 1999). It is generally recommended that sediment toxicity is estimated by both chemical analysis and bioassays, as toxicity has to be defined as a biological response to a particular test exposure (Chapman et al., 2002). Exposing organisms to contaminated, whole sediment allows the integrated effects of sediment-bound chemicals to be measured. However, the effects can vary among the species due to different exposures. The uptake route and the amount of accumulated chemicals are determined by sediment and chemical characteristics (e.g. Chapman et al., 1998; Ehlers and Loibner, 2006), but also by the characteristics of the organism, such as its size or feeding behavior (Leppänen and Kukkonen, 2006). In addition, the sensitivities of species to different chemical types may vary (Vaal et al., 2000), and the organism may have different sensitivities at different life stages (Hutchinson et al., 1998). This variability means that no single species is optimal for all assessments of sediment toxicity. It is therefore recommended

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to use toxicity test batteries with species from different trophic levels, habitats and test endpoints in order to cover different exposure routes and sensitivities to toxicants, and to represent the natural population as extensively as possible (Giesy and Hoke, 1989; Chapman et al., 2002; Davoren et al., 2005).

In this study, the toxicity of four contaminated sediments from three European river basins, the Elbe, the Scheldt and the Llobregat was compared to the toxicity observed at their respective reference sites. Within the EU project MODELKEY (511237-GOCE; Brack et al., 2005), these river basins were chosen as case study rivers because they represent different ecological regions of Europe and are heavily modified by anthropogenic influences (Von der Ohe et al., 2009). Whole-sediment contact tests were conducted with six different organisms and test types: survival and growth was determined for (i) midge larvae (*Chironomus riparius*), and survival, growth, and reproduction were determined for (ii) mudsnail (*Potamopyrgus antipodarum*), (iii) nematode (*Caenorhabditis elegans*), and (iv) oligochaete (*Lumbriculus variegatus*). Embryo development was tested with (v) fish eggs (*Danio rerio*), and light production with (vi) a luminescent bacterium (*Vibrio fischeri*). Furthermore, sediment toxicity was estimated on the basis of measured contaminant concentrations using two mixture-toxicity estimation models: calculations of toxic unit for invertebrate and fish (TU; Sprague, 1970) and multi-substance Potentially Affected Fraction of species (msPAF; De Zwart and Posthuma, 2005). Finally, it was studied whether the effects that were measured in toxicity tests could be explained by the sediment mixture-toxicity estimates msPAF and TU, or by natural characteristics, which were analyzed for the sediments (Sormunen et al., 2010).

2. Materials and methods

2.1. Sediments

The sediment samples used in the experiments were collected from sites in the river basins of the Elbe in the Czech Republic, the Llobregat in Spain and the Scheldt in Belgium in late May and early June 2006 (Table 1). The sampling sites were selected on the basis of their expected toxicities, which were estimated on the basis of their chemical concentration data, which were available for several sites in these river basins from regular monitoring programs. Sites that showed high toxicity were then selected as polluted sites. Reference sites were selected so that they had environmental conditions similar to those at the polluted sites, but were expected to have a lower level of contamination.

All the sediment samples were subjected to physical and chemical analyses in order to determine their natural characteristics. The sediments were analyzed for dry weight and weight loss on ignition, and contents of organic carbon, black carbon, nitrogen, hydrogen, and lipid-like compounds. Furthermore, humic acid content and aromaticity of humic acid were determined, and the particle size distribution and organic carbon content in different size fractions were measured. All of the analyses were performed in triplicate after sieving the sediments through a 2-mm sieve to remove large particles and debris. The analysis methods are detailed in Sormunen et al. (2010). In addition, a wide variety of contaminant concentrations (polychlorinated dibenzodioxins and furans, polychlorinated biphenyls and -naphthalenes, polybrominated diphenylethers, organochlorine pesticides, other pesticides and herbicides, perfluorinated carboxylates and -sulfonates, methyl mercury, organotins, estrogenic steroids, polyaromatic hydrocarbons, alkylphenol and nonylphenolpolyethoxylates and heavy metals) from the sites interest were analyzed in the MODELKEY project. The exception was the Llobregat LL4 site, where only methyl mercury, organotins, estrogenic steroids, alkylphenols and nonylphenolpolyethoxylates, (non-organochlorine) pesticides and herbicides were included into concentration analysis. Only the sum concentrations of different chemical groups are reported here.

In addition to the reference river sediments, clean natural or artificial sediments were used as negative controls in biotests. In the experiments with *C. elegans*, the artificial sediment was prepared according to the ISO 10872-standard (ISO, 2010). The control sediment was quartz sand in the experiments with *D. rerio* embryos, and quartz sand containing 5% fine-ground beech leaves (*Fagus sylvatica*) as a carbon source (Duft et al., 2007) in the experiments with *P. antipodarum*. For *C. riparius* natural sediment from the uncontaminated Finnish Lake Höytiäinen (Ristola et al., 1996) was used as a control. This sediment was sieved with a 1-mm sieve to remove large debris and animals, and stored at 4 °C for 12 months prior to the experiment.

2.2. Experimental setting

2.2.1. Caenorhabditis elegans

C. elegans var. Bristol, strain N2, was maintained as stocks of dauer larvae (an alternative juvenile phase that occurs on starvation) on NG agar (Brenner, 1974) according to standard procedures (Sulston and Hodgkin, 1988; Lewis and Fleming, 1995). The nematode bioassay with *C. elegans* was carried out according to standard methods (ISO, 2010). Briefly, 0.5 g of sediment (wet weight) was transferred into test vials (12-well polystyrene multidishes, Nunc, Wiesbaden, Germany). The sediment was then mixed with 0.5 mL of a bacterial suspension (*Escherichia coli* OP50, approximately 10^{10} cells/mL) in M9-medium as a food supply Brenner, 1974). At the start of the test, five first-stage (J1) juvenile worms were transferred to each test vial. Four replicates were set up for each treatment. After 96 h of incubation at 20 °C, the test was stopped by heat-killing the worms. The samples were then mixed with 0.5 mL of an aqueous solution of rose bengal (0.5 g/L) (to stain the worms for easier recovery) and stored at 4 °C until further use. To determine nematode reproduction and growth, the worms were separated from the sediment, the juvenile offspring were counted under a dissecting microscope at 25-fold magnification, and the body lengths of the test organisms were measured by microscopy at 100-fold magnification, respectively. Growth was calculated by subtracting the mean initial length of the test organisms from the mean length after incubation.

2.2.2. Chironomus riparius

C. riparius were reared in aquaria, containing natural clean lake sediment and artificial freshwater, under constant laboratory conditions (Ristola et al., 1999). First-instar larvae hatched from the egg ropes were used at the age of $\leq$96 h in the toxicity test. These larvae were not fed prior to the experiments. For each sediment 21 replicate beakers each containing one larva, were used. The experiment was conducted in seven time sections, in order to stagger the laborious sampling at the end of the experiment. For each experimental section a new hatching egg rope was used, and three beakers from each of the sediments were prepared, in order to share the larvae of different egg ropes equally among the sediments. The Lake Höytiäinen control sediment was included in the experiment during the last two time sections. The experimental units were prepared by adding 10 g of wet sediment to a 50 mL glass beaker, which was filled with aerated artificial freshwater, (pH 7, hardness 1.5 mmol/L as Ca+Mg, SFS standard, 1984). The Llobregat A3 and LL4 sediments were exceptions, as due to the lack of sufficient sediment, only 5 g of wet sediment was added to each beaker. On the next day, 1.2 mg of water-diluted TetraMin® fish food and one first-instar larva was added into each beaker in a random order (Ristola et al., 1999). The experiment was conducted at room temperature (20 ± 1 °C) and with a 16:8 h light–dark cycle under a yellow fluorescent lamp (>500 nm). During the experiment, the temperature and oxygen level of the water were monitored regularly, the water was aerated continuously, and aerated Millipore® water was added to compensate for evaporation. After 10 days, the larvae were sieved from the sediments, survivals were counted, and the larvae were placed in empty plastic tubes and kept at -20 °C until determination of the developmental stage and dry weight. The developmental stage was determined by measuring the width of the head capsule with a Nikon SMZ 800 microscope, using 50-fold magnification. The dry weight was measured with an electronic microbalance (Sartorius 4503MP 6, Sartorius GmbH, Germany), after drying at 60 °C for at least 24 h.

2.2.3. Lumbriculus variegatus

L. variegatus were reared under the same conditions as *C. riparius*, with the only exception that instead of sediment, shredded and pre-soaked paper towels were used as a substrate. The experiment with *L. variegatus* was conducted in seven time sections in order to stagger the laborious sampling at the end of the experiment, each of the sediments at a time. To ensure that the masses of oligochaetes were similar for different sediments, the wet weights of the 16 sets of 50 oligochaetes of the culture aquaria were measured along with the beginning of the sections. The test sediment (1000 g, ww) was placed in a 3-L beaker, which was filled with 2.5 L of aerated, artificial freshwater (pH 7, hardness 1.5 mmol/L as Ca+Mg; SFS standard, 1984). For each of the sediments, there were three replicate beakers. On the following day, 150 oligochaetes were added to each beaker. The experiment was conducted under the same conditions, and water was aerated as in the experiment with *C. riparius*. After 28 days, the organisms were sieved from the sediment and their guts purged in clean water for 6 h. The number of individuals was counted, and the total biomass was determined using an analytical scale (Scaltec SBC 31, Brenier Oy, Finland).

2.2.4. Potamopyrgus antipodarum

P. antipodarum were bred in 10 L aquaria filled with reconstituted water (OECD, 2006) at 15 ± 1 °C and with a 16:8 h light:dark cycle (Duft et al., 2007). The experiment was conducted in a static system in 1 L glass beakers under the same