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Baseline

Toxicity identification evaluation of sediments in Liaohe River

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ABSTRACT

Liaohe River has received significant attention in the northeast region and even in the entire country. As part of a recently completed water quality assessment, a series of water column and sediment toxicity tests was performed throughout the watershed. In the current study, we subjected sediments from the Liaohe River to toxicity identification evaluation manipulations and tests for chronic toxicity with midge (*Chironomus riparius*), with survival as the end point. In Phase I, the sediments were treated with zeolite, cation-exchange resin, and powdered coconut charcoal. Results confirmed that ammonia compounds were the major contaminants in terms of toxicity, although toxic effects from metals were also a concern in at least three sites. In Phase II identification, chemical analysis provided a strong evidence that the metals As and Cd are the probable causes of toxicity in the sediments, without the influence of ammonia. Temporally, ammonia is responsible for the toxicity of the selected sediments.

Anthropogenic chemical substances are known to promote the accumulation of contaminated sediments in watercourses (Kwok et al., 2005). Contaminated sediments are fairly common because overlying water disturbances release pollutants back to the water to form secondary pollution (Araujo et al., 2013). Thus, sediments can function as a sink and as a source of contaminants in the water column (Buruaem et al., 2013; Kwok et al., 2005). The Liaohe River running approximately 538 km, with a delta extending over an area of 1869.2 km^2 and annual flow of 302 $km^3\ yr^{-1}$. As the largest river in the northeast China, Liaohe River has occupied an important position in China's economy, and is the cradle of Chinese civilization. The metallurgy, chemical industry, pulp and paper industry, pharmaceutical, mining, petrochemical and other industries with the agriculture on the both sides of river have contribute to the pollutants of the populated and industrialised regions. In industrialized areas, such as Liaohe River area, where human activities are prevalent, contaminated sediments have become ubiquitous; each complex mixture of chemical contaminants can cause toxicity to aquatic organisms in distinct manners if it reaches sufficient levels (Goodsir et al., 2013; Perron et al., 2010). Given that sediments represent a substrate for a wide range of species and a habitat for flora and fauna (Araujo et al., 2013), identifying contaminants responsible for sediment toxicity can provide significant insights into sediment quality assessment (Ankley et al., 1996). Some guidelines are provided to manage pollutants in harbors and rivers in China. The decision-making process is based on a set of procedures. Recommended tests have also been included in these guidelines (Montero et al., 2013). In addition, risk assessment procedures in Europe rely solely on the correlation between toxicity and contaminant concentrations to suggest causes of observed effects. However, such correlation has certain limitations, including: (1) compounds causing the observed toxicity may not be included in the survey of chemicals, (2) concentration of toxic chemicals may vary, (3) difficulty in assessing the bioavailability of contaminants measured in the sediments may not be considered, and (4) possible interactions (e.g., synergistic, antagonistic, or additive effects) may not be taken into account (Wang et al., 2010).

Bioassays may prove to be more advantageous than correlations because they assess toxicity based on biological parameters and are indicative of potential adverse effects to aquatic organisms; however, bioassays do not elucidate which particular substance is causing the observed toxicity (Montero et al., 2013; Stringer et al., 2014). As an alternative, chemical analysis of the environmental matrix is the most direct approach to reveal the pollution status of an environment (Pignata et al., 2013). However, bioassays and chemical analysis of the environmental matrix also have their respective weaknesses. Both techniques have inherent drawbacks when dealing with variable and complex sediments (Mehler et al., 2010). Consequently, short-term organism chronic toxicity tests and chemical analysis are required to assess sediment quality and to test the toxicity and bioavailability of chemical compounds in sediments that can affect freshwater organisms (Serafim et al.,

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2013). Bioassays and chemical analysis have been effectively integrated to characterize and identify many suspended hazardous compounds present in sediments (Kwok et al., 2005). The findings of these two methods can be clarified by toxicity identification evaluation (TIE) approaches (EPA, 2007), the ultimate objective of which is to identify contaminants that are exerting toxicity to establish cause-effect relationships (Montero et al., 2013; Wang et al., 2010). TIE is a bioassay-guided fractionation protocol that was first developed by the United States Environmental Protection Agency (1991) to determine what fractions of chemicals cause observed adverse effects in bioassays and to enable the isolation of compounds in complex mixtures. This method has been used to characterize and identify toxicants in samples of freshwater and marine effluents, receiving waters, interstitial waters, sediment pore waters, and whole sediments.

At present, TIE procedures are routinely used in the US, Canada, and Australia to perform environmental risk assessments. In China, however, few studies have applied TIE techniques to identify contaminants responsible for observed toxicity (Montero et al., 2013). Furthermore, some areas in China have no ecotoxicological information (Buruaem et al., 2013) for identifying the bioavailable fractions of chemicals. Techniques related to TIE, such as addressing multiple toxicant interactions and establishing direct relationships between toxicity and analytical outputs, are not widely conducted for risk assessment in China (Macken et al., 2009).

The TIE process is divided into three phases. The physical and chemical manipulations of the samples to reduce the bioavailability of specific compounds generally make up Phase I (Montero et al., 2013). In Phase II, individual toxicants can be isolated and tentatively identified through analytical techniques and toxicity evaluation (Wang et al., 2010). Phase III involves validating if the toxicants identified in Phase II are indeed responsible for the sample toxicity observed in Phase I (Brack, 2003; Macken et al., 2009).

Selecting a suitable testing matrix is important in assessing toxicity in a biota. Previous studies have shown that the extraction procedure seems to increase bioavailability, and subsequently, toxicity, compared with observing intact sediments in situ. Consequently, we chose whole sediments (Wiklund and Dag Broman, 2005) for the current study. When examining sediment toxicity, aquatic species in exposed whole sediments are considered to be the best indicators because of their direct contact with the sediments. Among many species, *Chironomidae* larvae or midge larvae are considered to be particularly sensitive because they have been extensively used as a model organism in research on toxicity assessments in freshwater and in developmental biology. Midge larva (*Chironimus riparius*) is sensitive to some test toxicants, easy to culture and manage in a laboratory, and available throughout the year. This larva has been developed for ecological risk assessment in sediments and water because of the aforementioned properties. Moreover, several end points, such as mortality, life span, reproduction, behavior, and body length, have been used to test for acute toxicity in *C. riparius* (*Chironimus riparius*). The TIE method, coupled with using midge as a bioindicator, has been successfully employed in the toxicity evaluation of stream sediments (Wang et al., 2010). Thus, we consider midge larvae as a relevant test species in bioassays for sediment toxicity in Liaohe River (Wiklund and Dag Broman, 2005).

A total of 21 sediment samples were collected from streams ranging from undeveloped to highly urbanized. The current study differs from previous studies because the former samples larger wadable streams, avoids point sources (such as storm drains), and evaluates other inflows. In aquatic toxicity tests, sensitive test organisms have an important role in TIE procedures; therefore, midge (American Society for Testing and Materials (ASTM) International, 2012) was selected. The advantages of performing TIE on *Chironomidae* larvae are as follows. (1) *C. riparius* has a broad geographical distribution. (2) This species has a toxicological database that demonstrates relative sensitivity. (3) It comes in contact with the sediments being studied. (4) *C. riparius* is easy to culture in a laboratory. (5) It is compatible with the selected exposure methods and end points (ASTM International, 2012).

The current study evaluated the relationships between sediment chemistry and sediment toxicity in a 10-day whole sediment exposure experiment conducted on midge *C. riparius* (EPA, 2007). The toxicity end points evaluated through midge exposure included the effect of field-collected sediments on the survival of the test organisms.

The samples were collected from Liaohe River over a four-day period in October 2014. Sampling stations were selected based on the historical chemical analyses of sediments (Fig. 1).

The study areas are represented by the following symbols: St. 1 = Chai River, St. 2 = Sha He Estuary, St. 3 = Qing He Estuary, St. 4 = Liang Zi Estuary, St. 5 = Zhao Su Tai River, St. 6 = Fu De Dian River, St. 7 = Wang He, St. 8 = Fan He, St. 9 = Chang Gou Zi River, St. 10 = La Ma River, St. 11 = Fu Jia Wo Bao Pai Gan, St. 12 = Liu River, St. 13 = Yanfeili Paigan, St. 14 = before Qi Zing wetland, St. 15 = Zuo Xiao River, St. 16 = after Qi Xing wetland, St. 17 = small Liu River, St. 18 = Yi Tong River, St. 19 = Tai Ping He, St. 20 = Pang Xie Gou, St. 21 = Rao Yang He.

Each sample was a composite of multiple grab samples of recently deposited materials collected by skimming approximately the top 2 cm of sediments with a Teflon™ sheeting or a clean small scoop from multiple depositional zones within the stream reach during low-flow conditions (Chapman et al., 2013; Ho et al., 2004).

Interstitial water was extracted from whole sediments collected at each station via high-speed centrifugation using a temperature-controlled centrifuge at 9000*g* and 4 °C for 30 min. After centrifugation, interstitial water was decanted from each centrifuge tube into a common glass container and kept in a walk-in cooler at 4 °C prior to sample collection for chemical analysis and toxicity testing.

Sediment samples were analyzed for trace elements, organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), total organic carbon (TOC), moisture content, and grain size. Sediments were sieved in the field to <2 mm for organic contaminant analyses and toxicity testing and to <63 μm for trace element analyses. Pore water was analyzed for ammonia.

Initial toxicity tests were conducted to verify that the sediment sample is toxic and determine whether diluting it before Phase I testing is necessary. Sediment toxicity tests were conducted with midge (10-day exposure) according to the methods outlined by the USEPA (EPA, 2007). Based on previous studies, if an undiluted sediment sample does not cause 100% mortality, then it should be tested at full strength.

This test was chosen because of its simplicity. Ten midges were exposed to 100 mL sediment sample with 175 mL overlying water in 300 mL beakers, with a total of three replicates per treatment per species according to the USEPA (2007), the Guidelines for the Methods of Freshwater Test. The midges in each beaker were fed daily with 1.5 mL TetraFin goldfish food (6 mg of dry solids) in water suspension (EPA, 2007).

The organisms were tested at 23 °C under a 16 h:8 h light: dark photoperiod of ambient laboratory light (cool white fluorescent) at approximately 100 lux. Temperature was measured daily; dissolved oxygen, pH, and conductivity thrice per week; and ammonia, alkalinity, and hardness twice during the experiment. Toxicity end points for the midge included 10-day chronic toxicity tests (e.g., control survival was typically > 90% during the 10-day exposure).

In this experiment, the USEPA TIE method, with additional optimization and improvement, was adopted. Cation-exchange resin, zeolite, and coconut charcoal were used as additives to remove