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Use, development and improvements in the protocol of whole-sediment toxicity identification evaluation using benthic copepods

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ABSTRACT

The whole-sediment Toxicity Identification Evaluation (TIE) approach is a useful technique that allows for the identification of the contaminants responsible for the toxicity of complex sediment samples. This study aimed to compare the effectiveness of this technique in identifying the causes of toxicity when the test organism used in the toxicity test is capable of ingesting sediment particles. Two forms of exposure were compared: whole-sediment (WS), which integrates dermic and dietary exposures; and sediment–water interface (SWI), which involves dermic exposure only. The combined analysis of the TIE experiments revealed that metals, ammonia and, at one station, organic compounds, were responsible for sediment toxicity. The integrated use of WS and SWI TIE manipulations provided a more complete overview of the causes of toxicity, and thus enabled a better comprehension of complex contamination situations and, consequently, a better ecological assessment.

1. Introduction

Ecotoxicology is a multidisciplinary field of science that was emerged from the need to study the effects of substances on organisms and ecosystems using knowledge from chemistry, pharmacology, biochemistry, physiology, biology, genetics, economics, and other disciplines (Zakrewski, 1991). Ecotoxicological methods have been applied with varied objectives, such as (1) to estimate the quality of water, sediments, soils and the atmosphere; (2) for regulation purposes and to define maximum permitted limits for the discharge of effluents and chemical substances into the environment; (3) to estimate the effects of contaminant discharges on natural populations; (4) to define critical areas; (5) to take part in ecological risk assessments and environmental monitoring programs; (6) to detect the early signals of impacts of chemicals (early warning); and (7) to provide biological meaning for chemical information, among other purposes (Abessa et al., 2008).

Historically, the use of ecotoxicological approaches in field studies has placed more importance on evaluating sediment and water quality, and, more specifically, on considering the potential effects of contaminant discharges on natural populations. Sediments are therefore of great concern (de Magalhães and da Ferrão-Filho, 2008; Power and Chapman, 1995), because chemical and physical processes cause the precipitation of contaminants into the bottom sediments (Pereira and Soares-Gomes, 2002; Seriani et al., 2006), eventually reaching concentrations that are several orders of magnitude greater than the adjacent water column (Ingersoll, 2003; Schubauer-Berigan et al., 1993; Wenholz and Crunkilton, 1995). Contaminated sediments can lead to the transfer of contamination along the food chain. Hence, sediments have been used as an important indicator of the health of aquatic ecosystems.

The Toxicity Identification Evaluation (TIE) approach consists of a useful technique that may be applied to ecotoxicological studies. It was developed to determine the active substances responsible for the toxicity of complex mixtures (Burgess et al., 2013), and it involves a series of physical and chemical manipulations of samples that may cause a decrease, an increase or transformations in the bioavailability of different toxic groups (Anderson et al., 2007). Biological tests (toxicity tests) must also be applied to these samples in order to allow for the detection of the chemicals responsible for the toxicity (Schubauer-Berigan et al., 1993). TIE manipulations are conceptually used in a three-phase approach, in which Phase I characterizes the toxicants into main classes (characterization), Phase II identifies the specific toxicants (identification), and Phase III confirms the findings of Phases I and II (confirmation) (Ho and Burgess, 2013; USEPA, 2007). TIE techniques have been used worldwide to assess effluents and liquid samples (Burgess et al., 1995; Burkhard and Jensen, 1993; Norberg-King et al., 1991; Schubauer-Berigan et al., 1993). However, this

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technique is still being developed for its use with sediments, and there are few studies available that applied TIE to assess whole-sediment toxicity (Amweg and Weston, 2007; Ankley and Schubauer-Berigan, 1995; Besser et al., 1998; Burgess et al., 2000; Chapman et al., 2002; Ho and Burgess, 2009; Kosian et al., 1999; Lebo et al., 2000, 1999).

A whole-sediment TIE study employed chronic toxicity tests with copepods (Araujo et al., 2013) and discussed some issues regarding the effectiveness of sediment manipulations in removing toxicity from the sediment sample. In that case, the authors commented that the treatments for organic compounds and metals were based on the removal of contaminants from the dissolved phase and by their immobilization through adsorption onto particles or precipitated salts, but if the test organisms feed on sediment particles (as benthic copepods do), there would be relevant exposure through dietary route, and this would hinder the evaluation. Ingestion of contaminated sediment particles may be the dominant uptake pathway to the deposit-feeding invertebrates in sediments, according to Forbes et al. (1998). Casado-Martinez et al. (2009) and Rainbow and Luoma (2011) showed that the dietary exposure may represent more than 50% of absorbed contaminants in some benthic invertebrates. This finding represents the importance of properly considering such form of exposure. Thus, understanding how TIE manipulations work under different exposure conditions becomes an important aspect that should be explored in order to improve the use of this technique as a tool for environmental assessment.

The present study sought to compare the results of two forms of conducting sediment TIE: the whole-sediment test (dermic and dietary exposures) and sediment–water interface test (dermic exposure only) both of them using an infaunal copepod, which is capable of ingesting sediment particles.

2. Material and methods

2.1. Study area

The study area includes the marine portion of Xixová-Japuí State Park (XJSP), which is located on the central coast of the state of São Paulo (SP), Brazil. Its sediments were previously reported to be contaminated (hydrocarbons, and detergents) and toxic to sea-urchin embryos (*Lytechinus variegatus*), to the burrowing amphipod *Tiburonella viscana* and to the benthic copepod *Nitocra* sp. (Abessa et al., 2008; Araujo et al., 2013; Hortellani et al., 2008). The park is 901 ha in size, and is divided between the municipalities of São Vicente and Praia Grande. It has a 600-ha terrestrial portion, while its marine portion comprises the entire area surrounding the coastline and continues 200 m offshore (São Paulo, 2010).

XJSP was created through state Decree No. 37536 (São Paulo, 1993), and it exists within a region that is severely affected by environmental impacts, such as unplanned urban settlement, industrialization, and port activities (São Paulo, 2010). The marine portion of XJSP is of great ecological importance for the protection of marine species due to its location in one of the major estuarine complexes of the state of São Paulo (São Paulo, 2010). However, its marine ecosystems are under intense pressure, including direct and indirect effects from activities at the port of Santos, submarine sewage outfalls, and industrial waste (Abessa et al., 2012; Araujo et al., 2013; São Paulo, 2010).

The Santos Estuarine System is located in the Baixada Santista metropolitan region (BSMR), São Paulo state, Brazil, and is distinguished by three main economic sectors: tourism, which is responsible for much of the economy of some local cities; the industrial plants, which are located inside the estuary, and which include chemical, petrochemical, fertilizers and major steel plants; and the port of Santos, which occupies a section of land at the shores of the estuarine area (Lamparelli et al., 2001; Rachid, 2002). This region experiences severe environmental degradation due to the disposal of hazardous compounds into the rivers, estuarine and marine areas, including nutrients, heavy metals, organic compounds, petroleum hydrocarbons (Braga et al., 2000), pharmaceuticals, organotins and other contaminants (Lamparelli et al., 2001).

2.2. Sediment sampling

Sediment samples were collected at two sampling stations (Fig. 1), in August 8th 2014, with a stainless steel Van Veen grab sampler, and aliquots were separated for ecotoxicological and sedimentological analyses. These samples were firstly characterized for geochemistry and toxicity by Araujo et al. (2013), as samples P1 and P4, respectively. The sediments were considered contaminated by Cd, Cu and Zn and produced toxicity to amphipods (*T. viscana*) and copepods (*Nitocra* sp.). Araujo et al. (2013) also applied a preliminary whole-sediment TIE which suggested that ammonia could be a contaminant of concern. Immediately after the mentioned characterization, the TIE experiments were repeated for the present study. The station known as S1 was located close to the city of Praia Grande (24.0245S; 46.4020W), whereas S2 was situated inside the Santos Bay (24.0059S; 46.3842W) (Fig. 1). The control sediment was collected at Engenho d'Água Beach, in the city of Ilhabela, Sao Paulo (23.8000S; 45.3667W). After their collection, samples were identified, conditioned in plastic bags, and immediately cooled on ice. The entire time interval for sediment sampling, ecotoxicological analysis made by Araujo et al. (2013) and conduction of the TIE experiments of this study was within 4 months. As a set of sequential analysis were made with the collected samples, the recommended holding time (10 weeks) was exceeded (USEPA/ACOE, 1998). According to USEPA (2001), exceeding the 10-week period may be acceptable in some cases.

2.3. Sediment characterization

As mentioned, this investigation re-tested the sediment samples that already were analyzed by Araujo et al. (2013) for geochemistry. The analysis of sediment grain size distribution consisted of a two-step sieving process (Mudroch and MacKnight, 1994): in the first step, wet sieving was used to separate fine particles (silt and clay, <0.062 mm); and the second step consisted of dry sieving in order to separate different classes of sands, the classification of which was based on the scale established by Wentworth (1922). The determination of calcium carbonate ($CaCO_3$) contents followed the protocol established by Grant-Gross (1971), which describes the sample attack with hydrochloric acid (HCl). The estimation of organic matter (OM) contents in the sediment samples followed the loss by ignition method (Grant-Gross, 1971).

2.4. Chronic sediment toxicity tests

The chronic toxicity assay with the copepod *Nitocra* sp. was performed with whole-sediment (WS) following the protocol developed by Lotufo and Abessa (2002); sediment–water interface (SWI) according to the method described by Anderson et al. (1996). This experimental design considered the fact that the WS bioassays integrate exposure that occurs through ingestion and dermic absorption of dissolved chemicals, whereas the SWI bioassays consider only the dermic absorption of dissolved contaminants.

For both of WS and SWI, aliquots of collected samples were introduced into 30 mL high-density polyethylene flasks containing