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Porewater as a matrix in toxicity bioassays with sea urchins and bivalves: Evaluation of applicability to the Venice lagoon (Italy)

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ABSTRACT

Porewater plays an important role in sediment toxicity assessment using bioassays, but the most reliable extracting method and the potential contribution of confounding factors to the real toxicity need to be studied. The applicability of bioassays with the early life stages of *Paracentrotus lividus*, *Mytilus galloprovincialis* and *Crassostrea gigas* on porewaters extracted by centrifugation from the Venice Lagoon (Italy) is evaluated and demonstrated: toxicity tests can discriminate the toxicity of porewaters from sites with different kinds and levels of pollution and, using toxicity scores, data are classified in five toxicity classes. Sulphides do not represent a confounding factor in porewater toxicity; in contrast ammonia exhibited some concentrations above the toxicity threshold for sea urchin embryos.

1. Introduction

Sediment porewater is a key exposure route to contaminants for organisms associated with sediments (Chapman et al., 2002), particularly for non-polar organic ones (Adams et al., 1985). Chemical analysis provides an estimate of the level of pollution but the bioavailable fraction affecting organisms can only be determined by using toxicity bioassays (Beiras, 2002), which have been shown to be promising tools for assessing the quality of contaminated sediments (Lopes and Ribeiro, 2005).

Porewater is used to evaluate marine and estuarine sediment toxicity in several works (Carr and Chapman, 1992; Burgess et al., 1993; Carr et al., 1996a,b; Ho et al., 1997; Fairey et al., 1998; Beiras, 2002). Porewater is often one of the test matrices in a battery of toxicity bioassays; for example, it is used jointly with organic extract using the Microtox® test (Carr et al., 1996b), with whole sediment using amphipods (Carr and Chapman, 1992; Carr et al., 1996a,b; Ho et al., 1997; Fairey et al., 1998; Nipper et al., 2002) and with elutriate using sea urchin embryos (Beiras, 2002). Most bioassays on porewater regard the exposure of gametes and embryos of sea urchins such as *Arbacia punctulata* (Carr and Chapman, 1992; Burgess et al., 1993; Carr et al., 1996b; Ho et al., 1997; Nipper et al., 2002), *Strongylocentrotus purpuratus* (Fairey et al., 1998; Greenstein et al., 1996) and *Paracentrotus lividus* (Beiras, 2002). Other organisms used for testing porewater are molluscs (Carr and Nipper, 2003), polychaetes (Carr and Chapman, 1992; Nipper et al., 2002), bacteria (Ho et al., 1997) and macroalgae, particularly *Ulva* sp. (Hooten and Carr, 1998; Nipper et al., 2002).

Toxicity tests on gametes and embryos of sea urchins and bivalves, even if these organisms are not naturally exposed to porewater, are generally accepted and utilised, since larval stages are a good instrument for detecting otherwise not measurable sub-lethal effects (Nipper, 2000; Carr et al., 2001; Nipper et al., 2002). These bioassays have also recently been suggested for monitoring transitional environments (USEPA, 2000).

The use of porewater as test matrix is widely discussed in Carr and Nipper (2003): the literature review demonstrated that porewater tests detect toxicity much more frequently than solid-phase tests do; the main problems in porewater use are the choice of the most suitable methodology for porewater extraction, sample storage time and conditions and the presence of potentially confounding factors.

Several methods are proposed for porewater extraction: in-situ methods reduce sediment manipulation and sources of error (oxidation, sediment sampling, metal contamination, temperature artefacts, filtration), but are generally difficult to use and produce small porewater volumes; the widely-used ex-situ methods produce large volumes but have to operate in an inert atmosphere to reduce sediment oxidation and require collection of large amounts of sediment.

Among the ex-situ procedures, we choose centrifugation because this extraction method meets our needs: a) the sediment sampling, performed by the procedure set up according to Quality Assurance/Quality Control (QA/QC) criteria (Volpi Ghirardini et al., 2005a), provides an integrated sample for a battery of bioassays, so that it is easy to homogenise the sediment sample and then prepare all test matrices; b) the centrifugation permits the extraction of porewater in most of our study site, the Venice Lagoon (Italy), where the surficial

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sediments are mainly clayey silt (except near the three inlets where sediments are more sandy) (Apitz et al., 2007); c) a previous study conducted in the Lagoon revealed that the toxicity of porewater extracted by an in-situ method is strongly influenced by sulphides using the sperm cell test with *P. lividus* (Losso et al., 2004).

This work investigates the applicability to the Venice Lagoon of porewater extracted by centrifugation as test matrix for bioassays using the early life stages of sea urchin and bivalves. The idea is to evaluate the possibility of using porewater jointly with elutriate and whole sediment in order to develop a multimetric toxicity index for assessing lagoonal sediment toxicity. This paper reports the toxicity data on porewaters extracted from the Lagoon from 1998 to 2005. The results are classified into five toxicity classes, evaluating both the discriminatory ability of the bioassays used and the possible influence of confounding factors in the test results. Porewater and elutriate toxicities are lastly compared.

2. Materials and methods

2.1. Sediment sampling and porewater preparation

Twenty shallow sampling sites in the Venice Lagoon were investigated from 1998 to 2005, covering different levels of pollution from the industrial zone to areas close to the sea inlets (Fig. 1).

Two sites are typically estuarine (Dese=DE, Osellino=OS) and influenced by pollutants deriving from the mainland; Osellino is a very polluted canal affected by multifactorial pollution due to treated and untreated wastewaters from the town of Mestre and leaching from a recently reclaimed but uncontrolled landfill. Four sites were selected inside the Porto Marghera industrial area: BR=Brentelle Industrial Canal, SA=South Industrial Canal, BA=Brentella Industrial Canal; in the Brentella Industrial Canal two nearby stations were investigated, named BA1 and BA2. Three sites are located around the industrial area: PI=Pili, TR and TE (just in front of the island of Tresse). Three sites (SG=S.Giuliano, CEL=Celestia, FN=Fondamenta Nove) were chosen in points mainly affected by mixed urban–industrial pollution. Three sites were selected for their vicinity to the sea inlets: OT=Ottagono close to the Malamocco sea inlet; SE=S. Erasmo and SN=San Nicolò close to the Lido sea inlet. In the Northern Lagoon three sites were investigated far from the pollution point source: an inter-tidal mudflat CE=Centrega (two nearby stations named CEa and CEb) and the sub-tidal sites PM=Palude Maggiore and RO=Palude della Rosa. Finally, SS=Sacca Sessola is a site located in the open lagoon, far from sources of pollution. Most of these sites were chosen because they had been investigated in previous studies so that chemico-physical information is available.

The 15 cm depth sediments were collected by a corer in an area represented by a circle with a diameter of 30 m, with a central point fixed by geographical co-ordinates, following the integrated sampling design previously evaluated according to a Quality Assurance/Quality Control (QA/QC) procedure (Volpi Ghirardini et al., 2005a). Sediment samples were homogenised at first in air; samples from 2001 were homogenised in a glove-box at nitrogen atmosphere. Centrifugation conditions were determined examining methods available in the literature (Table 1): although the lack of an unambiguous protocol is evident, the suggestions of the standard methods from ASTM, Environment Canada and USEPA were taken into consideration. Homogenised sediments were centrifuged at 13,000 rpm (>10,000 g) for 30 min at 4 °C in a Beckman Ultracentrifuge using polycarbonate bottles. Surnatant was carefully collected avoiding the resuspension of particulate matter.

Measures of pH and salinity were made for each extracted porewater. The pH range was 7.7–8.3, in accordance with bioassays requirements. Salinity ranged from 25 to 35 ppt: for bivalve bioassays no adjustments were necessary, while for sea urchin bioassays, salinity was adjusted adding NaCl.

Extracted porewaters were frozen until use according to Carr et al. (2001). Only subsamples for ammonia and sulphides analyses were filtered at 0.45 µm. Sulphide and total ammonia concentrations in porewaters were measured with a spectrophotometer (DR/2010, HACH) using the methylene blue method (USEPA SM 4500-S2 D) for sulphides and the salicylate method (Reardon et al., 1966) for total ammonia.

2.2. Toxicity tests

The sperm cell and embryo toxicity tests with *P. lividus* were performed using the procedure reported in detail by Volpi Ghirardini and Arizzi Novelli (2001) and Arizzi Novelli et al. (2002), respectively. Adults, collected in the northern Adriatic and Tyrrhenian seas, stored for at least one week at a temperature of 18±1 °C and salinity of 35‰±1, with a natural photoperiod (Volpi Ghirardini and Arizzi Novelli, 2001), were induced to spawn by injecting 1 ml of 0.5–1 M KCl solutions. Pools of male and female gametes (minimum 3 males and 3 females) were prepared. For the sperm cell toxicity test, a volume of 0.1 ml of adjusted suspension of 4×10^7 sperm was added to test solutions. After 60 min of exposure, 1 ml of standardized egg suspension (thermostated at 18 °C) was added to test vessels, and a period of 20 min allowed to pass. Samples for counting were preserved in 1 ml of concentrated buffered formalin, and the percentage fertilization in each treatment was determined by counting 200 eggs. For the embryo toxicity test, fertilization was performed at a sperm:egg ratio of 20,000:1. A volume of 1 ml of fertilized egg suspension was added to 10-ml aliquots of test solution and incubated in the dark at 18 °C for 72 h. At the end of the experiment, samples for counting were preserved by 1 ml of concentrated buffered formalin, and the percentage of plutei with normal development in each treatment determined by observing 100 larvae, distinguishing abnormalities in the following categories: 1) embryos unable to undergo larval differentiation, including all phases blocked before differentiation into plutei (i.e., gastrula and pre-gastrula stages); 2) prisms; 3) retarded plutei, corresponding to early plutei; 4) malformed plutei, including larvae which had developed but showed some malformations (e.g., defects of skeleton and/or digestive apparatus) (Arizzi Novelli et al., 2002).

The acceptability of test results is fixed at: a) percentage of normal plutei ≥70% in control tests; b) EC_{50} using the reference toxicant (copper) falling within previously defined acceptability ranges for both tests (Volpi Ghirardini and Arizzi Novelli, 2001; Arizzi Novelli et al., 2002).

Bivalve bioassays were performed according to the method proposed by His et al. (1997), modified for using gamete pools. Adults of *M. galloprovincialis* were sampled from a natural population in the Venice Lagoon, at a site far from pollution point sources, near the Malamocco sea inlet (Volpi Ghirardini et al., 2005b). Conditioned adults of *C. gigas* were purchased from the Guernsey Sea Farm Limited hatchery (Guernsey, UK). Bivalves were induced to spawn by thermal stimulation (temperature cycles at 18 °C and 28 °C). Good quality gametes from the best males and females were selected and filtered at 32 µm (sperm) and 100 µm (eggs) to remove impurities. A pool of eggs from at least three females (1000 mL) was fertilized by injecting 10 mL of sperm; fecundation was verified by microscopy. Egg density was determined by counting four subsamples of known volume. Fertilized eggs, added to test solutions in order to obtain a density of 60–70 eggs ml^{-1}, were incubated for 48 h at 18 °C for *M. galloprovincialis* and 24 h at 24 °C for *C. gigas*, then fixed with buffered formalin. 100 larvae were counted, distinguishing between normal larvae (D-shape) and abnormalities (malformed larvae and pre-larval stages). Test results acceptability was based on: a) negative control for a percentage of normal D-shape larvae ≥80% (His et al., 1999); b) for *M. galloprovincialis*, EC_{50} using the reference toxicant (copper) falling within the previously defined acceptability range (Volpi Ghirardini et al., 2005b).