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Exposure–dose–response of *Tellina deltoidalis* to metal contaminated estuarine sediments 2. Lead spiked sediments

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ABSTRACT

Lead accumulation in estuarine sediments, as a result of activities such as mining and ore smelting, and through urban runoff is a continuing problem in the increasingly developed world. Marine organisms accumulate lead, which is known to be highly toxic to biological processes and to degrade organism and ecosystem health. Here the relationship between lead exposure, dose and response was investigated in the sediment dwelling, deposit feeding, marine bivalve *Tellina deltoidalis*. Bivalves were exposed in the laboratory to individual lead spiked sediments at < 0.01, 100 and 300 μg/g dry mass, for 28 days and accumulated total tissue lead concentrations of 4, 96 and 430 μg/g, respectively. Subcellular fractionation indicated that around 70% of the total accumulated tissue lead was detoxified, three quarters of the detoxified lead fraction was converted into metal rich granules, with the remainder in the metallothionein like protein fraction. The majority of biologically active lead was associated with the mitochondrial fraction with up to a 128 fold increase in lead burden in exposed organisms compared to controls. This indicates lead detoxification was occurring but the organism was unable to prevent lead interacting with sensitive organelles. With increased lead exposure *T. deltoidalis* showed a suppression in glutathione peroxidase activity, total glutathione concentration and reduced GSH:GSSG ratios, however, these differences were not significant. Lead exposed *T. deltoidalis* had a significantly reduced total antioxidant capacity which corresponded with increased lipid peroxidation, lysosomal destabilisation and micronuclei frequency. The exposure–dose–response relationships demonstrated for lead exposed *T. deltoidalis* supports its potential for the development of sublethal endpoints in lead toxicity assessment.

1. Introduction

Lead is released into the environment through a variety of human activities including lead ore mining and smelting, alkyl-lead petroleum combustion, coal burning, waste incineration, production and storage of lead-acid batteries, leaded glass, lead oxide pigments and ferroalloys, metal fabricating industries, cement manufacture and urban runoff (Chiaradia et al., 1997; Renberg et al., 2002; Cook and Gale, 2005; ARCARP, 2006; Genaidy et al., 2009; Ãlvarez-Iglesias et al., 2012). In Australia the majority of the population is located along coastlines, the bays and estuaries of which are repositories for sediment bound lead (ABS, 1996; Carroll, 1996; Chiaradia et al., 1997; ANZECC and ARMCANZ, 2000).

The accumulation of lead by marine organisms has implications for both ecological and human health at both individual and population levels (Gnassia-Barelli and Romeo, 1993; Clark, 2001). Although it has no known biological function, lead is accumulated by marine organisms through similar pathways as essential elements such as calcium, iron and zinc, making it one of the more toxic metals in these environments (Company et al., 2008, 2011). The metal exposure route, through either food or water, affects the organism's metal handling and potentially its toxicity (Rainbow, 2007). Deposit feeding bivalves are of particular interest as biomonitors as they are exposed to contaminants through water, food, and directly through ingested fine grain sediment particles, containing organic material (Lee et al., 1998, 2000; Griscom and Fisher, 2004).

Whole sediment toxicity tests are used worldwide as an integral part of toxicity assessment as they assess contaminant exposure from several exposure routes; including those bound to sediments, from pore waters and in overlying waters (Ingersoll et al., 2000; Adams et al., 2005; ASTM, 2010). In Australia, aquatic toxicity tests have been established (ANZECC and ARMCANZ, 2000) but the use of whole sediment toxicity tests has been hampered by the limited established test procedures and knowledge of species sensitivity to contaminants. Early investigations of suitable Australian organisms' sensitivity to metal contamination in whole sediment toxicity tests have included polychaetes, amphipods and copepods (Mann et al., 2009; Perez-Landa and Simpson, 2011; Campana et al., 2012) and bivalves, of which, the bivalve *Tellina deltoidalis* was highly sensitive to zinc and copper (King et al., 2004, 2005, 2010). *T. deltoidalis* is a deposit feeding bivalve which satisfies

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most of the basic requirements to be an effective biomonitor, they are: hardy, accumulate metal and have sufficient tissue for analysis (King et al., 2004, 2005, 2010; Taylor and Maher, 2013); and are widely distributed around Australian coastal estuaries, living in sediments at a depth several times their 15–25 mm shell length with siphons extended to the surface to feed (Beesley et al., 1998).

The development of Australian guidelines for sediment quality assessment, which includes contaminant exposure, uptake and ecotoxicological effects, identified *T. deltoidalis* as a suitable species for whole sediment toxicity testing and a protocol for its use is included in the Australian Handbook for Sediment Quality Assessment (Simpson et al., 2005). The assessment of ecotoxicological effects of contaminants is moving from the use of mortality as an endpoint to the use of biomarkers of sublethal effects and there is a need, in understanding organism sensitivity to contaminants, to develop suitable biomarkers that can be included in routine testing. The use of biomarkers enables links to be made between external exposure and an internal toxicant dose, which has exceeded the organism's detoxification and repair capacity, allowing ecologically relevant effect concentrations to be determined (Adams et al., 2011; Depledge et al., 1992). Biomarkers of exposure and effect for use in aquatic toxicological assessment have been progressively developed and refined for a range of species and toxicants worldwide (Cajaraville et al., 2000; Lam and Gray, 2003; Domouhtsidou et al., 2004; Galloway et al., 2004; Damiens et al., 2007; Durou et al., 2007; Viarengo et al., 2007; Bocchetti et al., 2008; Bergayou et al., 2009; Lam, 2009; Taylor and Maher, 2010).

Lead has a high affinity for sulfhydryl, forming mercaptides with the sulfhydryl group of cysteine and inhibiting several enzymes having functional sulfhydryl groups, thereby initiating oxidative stress responses (Gurer and Ercal, 2000; Ercal et al., 2001; Dafre et al., 2004). Changes in the oxidative pathway including increased reactive oxygen by-products and suppression or increased expression of oxygen reduction enzymes have been shown for a number of lead exposed molluscs (Alcutt and Pinto, 1994; Cossu et al., 2000; Dafre et al., 2004; Jing et al., 2007; Taylor and Maher, 2012). Lead is also thought to interact with a variety of cellular lipids altering the lipid composition of cellular membranes and increased susceptibility to lipid peroxidation (Campana et al., 2003; Mateo et al., 2003). This can result in perturbations in cell membrane integrity, permeability and function increasing lysosomal destabilisation (Gurer and Ercal, 2000; Ercal et al., 2001; Einsporn and Koehler, 2008). Lead has been shown to interact directly with DNA by covalent binding of Pb^{2+} to DNA (Hong et al., 2007) producing micronuclei, through either the breakage of chromosomes or dysfunction of the mitotic spindle apparatus (Winter et al., 2007; Monteiro et al., 2011). The micronuclei induction assay which can detect these small masses of cytoplasmic chromatin, present outside the main nucleus, has proven useful in assessing the genotoxic effects of a range of compounds in fish and other aquatic organisms (Bolognesi et al., 2004; Kalpaxis et al., 2004; Udroiu, 2006).

The purpose of this study was to examine the exposure–dose–response relationship of lead in *T. deltoidalis* using a 28 day sediment bioaccumulation test (Ingersoll et al., 2000; ASTM, 2010), to develop useful biomarkers of effect, with a view to determining their suitability for laboratory sediment metal toxicity tests using sublethal endpoints. Three lead sediment exposure concentrations were used, < 0.01 (control) reference sediment with no lead added, 100 μg/g based on the high value for lead sediment concentrations from the ANZECC and ARMCANZ (2000) Interim Sediment Quality Guidelines and 300 μg/g based on concentrations previously measured in lead contaminated Australian estuarine sediments (Roach, 2005). The study examines lead accumulation and subcellular tissue lead distribution. Biomarkers of lead exposure effects were total antioxidant capacity, the associated oxidative enzymes; total, reduced and oxidised glutathione and glutathione peroxidase and the extent of lipid peroxidation. The cellular effects measure lysosomal destabilisation and a genotoxic measure, micronuclei occurrence were also measured in a weight of evidence approach to establishing lead sublethal toxic effects.

2. Materials and methods

2.1. Organism and sediment collection

Sediments were collected from a NSW Environment Protection Authority reference site in Durras Lake, NSW, and stored at 4 °C. *T. deltoidalis* of 15–20 mm in size were collected from Durras Lake and Lake Tabourie, NSW in July 2005 and January 2006 and placed in coolers with sediment and water from the collection sites for transportation. Organisms were maintained for a maximum of two weeks at 22 °C in Durras Lake reference sediments, depth 15 cm, in glass aquaria with filtration and aeration, to allow acclimation before experimentation. Overlying water used in aquaria was collected from coastal waters near Murramurrang National Park, NSW and adjusted from 35‰ to 28‰ with deionised water to match the salinity of the estuarine water from which organisms were collected.

2.2. Sediment lead spiking

Sediments were sieved through a 2 mm stainless steel sieve to remove large pieces of organic matter and organisms prior to the addition of lead. Subsamples of the collected sediments were measured for moisture content and grain size. Sediment with normalised properties, suitable for organism burrowing and feeding, based on the grain size properties of the sediments where organisms were collected, was created. To ensure added lead was rapidly adsorbed and bound to the sediment particles the procedure developed by Simpson et al., (2004) for producing metal spiked marine sediments, was followed. Wet sediment was added to mixing containers. $PbCl_2$ salt, (AR grade Sigma-Aldrich) in deoxygenated sea water, was added to concentrations of 100 μg/g and 300 dry mass of sediment. All containers were topped up with clean deoxygenated sea water and the final mixture was completely deoxygenated by bubbling with nitrogen for 2 h. Head spaces of containers were filled with nitrogen prior to sealing. Any pH adjustments were made immediately after the addition of lead using 1 M NaOH (AR grade BDH), prepared in seawater. pH was checked weekly and maintained at pH 7–8.2. Sediments were maintained at room temperature 22–25 °C and mixed on a Cell-production Roller Apparatus (Belco, USA) for several hours each day. The time required for equilibration of added metals is affected by the sediment properties, equilibration pH and the concentration and properties of the metal (Simpson et al., 2004). To determine when the added lead was completely bound to sediment particles, pore waters were collected and acidified to 1% v/v with nitric acid (AristaR, BDH, Australia) and lead measured using an ELAN® 6000 ICP-MS (PerkinElmer SCIEX, USA). Once pore water lead concentrations had fallen below instrument detection limit of 0.01 μg/L, the sediment was ready for use. Time to full adsorption was 4–6 weeks. Unspiked sediments were treated in the same way and used for control treatments. Sediment lead concentrations were measured by ICP-MS after digestion of 0.2 g of lyophilised sediment in 3 mL of nitric acid (AristaR, BDH, Australia) in polyethylene 50 mL centrifuge tubes for 60 min at 115 °C (Maher et al., 2003). Lead in NRCC Certified Reference Materials, BCSS-1 marine sediment measured along with samples was 21 ± 4 μg/g ($n = 10$) and in agreement with certified values 22.7 ± 3.4 μg/g. Pre exposure sediment lead concentrations were <0.01, 100 ± 5 and 300 ± 8 μg/g and post exposure were <0.01, 101 ± 5 and 298 ± 10 μg/g.

2.3. Microcosm experiment design

Procedures for conducting the exposures were adapted from the test method for conducting 28 day sediment bioaccumulation tests