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Effects of enhanced bioturbation intensities on the toxicity assessment of legacy-contaminated sediments☆

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Many benthic communities within estuarine ecosystems are highly degraded due to the close proximity of urban and industrial contamination sources. The maintenance of recolonised, healthy ecosystems following remediation is a challenge, and better techniques are required for monitoring their progressive recovery. Rates of ecosystem recovery are influenced by the changes in the concentrations and forms of contaminants, the sensitivity of recolonising organisms to bioavailable contaminants, and a range of abiotic and biotic factors influencing the exposure of organisms to the contamination. Here we investigate the influence of bioturbation by an active amphipod (*Victoriopisa australiensis*) on the bioavailability of metals and hydrocarbons in highly contaminated sediments. Changes in contaminant bioavailability were evaluated by assessing sublethal effects to a smaller cohabiting amphipod (*Melita plumulosa*). For predominantly metal-contaminated sediments, the presence of *V. australiensis* generally increased survival and reproduction of *M. plumulosa* when compared to treatments with only *M. plumulosa* present (from 42 to 93% survival and from 3 to 61% reproduction). The decrease in toxic effects to *M. plumulosa* corresponded with lower dissolved copper and zinc concentrations in the overlying waters (14 to 9 μg Cu L^{-1}, and 14 to 6 μg Zn L^{-1} for absence to presence of *V. australiensis*). For sediments contaminated with both hydrocarbons and metals, the increased bioturbation intensity by *V. australiensis* resulted in decreased reproduction of *M. plumulosa*, despite lower dissolved metal exposure, and indicated increased bioavailability of the hydrocarbon contaminants. Thus, the presence of a secondary active bioturbator can enhance or suppress toxicity to co-inhabiting organisms, and may depend on the contaminant class and form. The results highlight the need to consider both abiotic and biotic interactions when using laboratory studies to evaluate the ability of organisms to recolonise and reproduce within benthic environments degraded by contamination, or for more general extrapolation for sediment quality assessment purposes.

1. Introduction

The magnitude of land use and industrialisation within coastal regions is increasing globally, putting aquatic ecosystems under threat from a range of stressors (Borja et al., 2016; Gunderson et al., 2016; Wenger et al., 2016). Sediments act as a sink for a wide range of contaminants, and those located within estuaries represent some of the most degraded ecosystems (Chariton et al., 2010; Clark et al., 2015; Dafforn et al., 2013; Lindgren et al., 2016; Lotze et al., 2006; Perelo, 2010).

The remediation of contaminated sites is often an expensive exercise, leading to monitored natural recovery (MNR) being favoured as a remediation option (Lotze et al., 2006; Reible and Algar, 2014; Pedersen et al., 2016). Natural recovery relies on natural physical, chemical, and biological processes to isolate, destroy, or otherwise reduce the exposure to and bioavailability and toxicity of contaminants (Magar and Wenning, 2006; Perelo, 2010; Reible and Algar, 2014). Consequently, there are both abiotic and biotic processes that will influence whether contaminated sediments will recover naturally, and the rate of recovery. Better understanding of factors influencing ecosystem recovery may enable resource managers to invest in moderate remediation actions that significantly increase recovery rates.

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Although the abundance of some benthic organisms, such as polychaetes, may increase in response to contamination (Dafforn et al., 2013), many systems become deprived of other larger bioturbating organisms. The burrowing and feeding activities of these larger benthic organisms are responsible for physically moving considerable amounts of sediments, e.g. bringing deeper sediments to the surface, and facilitating a range of biogeochemical processes, including the degradation of organic matter (Aller et al., 2001; Cadée, 2001; Levinton, 1995). Thus the absence of larger bioturbators will change the dynamics of the ecosystem, influencing the bioavailability and degradation of sediment-bound contaminants directly (Simpson et al., 2002; Ciutat and Boudou, 2003; Josefsson et al., 2010), and indirectly through changes to the behaviour and function of other organisms, including benthic microorganisms and hard substrata organisms immediately above sediments (Branch and Pringle, 1987; Reichardt, 1988; Goñi-Urriza et al., 1999; Hill et al., 2013). The disturbance of sediments by bioturbation displaces porewater and modifies fluxes of both dissolved and particulate substances into the overlying waters (Ciutat and Boudou, 2003; Ciutat et al., 2006; Granberg et al., 2008; Josefsson et al., 2010; Amato et al., 2016). This disrupts redox equilibria that influence the sediment-water partitioning of contaminants that has established in the absence of disturbance (Pischedda et al., 2008; Simpson et al., 2012). Therefore, in highly-contaminated sediments that are depauperate of larger invertebrates, the assessment of contaminant bioavailability will be influenced by the sensitivity, size and behaviour of the organisms used in bioassays or observed in the field, and the interactions between organisms. Failure to consider such factors is likely to result in misinterpretation of the rate of natural recovery of benthic ecosystems that have been degraded due to high concentrations of contaminants.

Several studies have reported that the bioturbation activities of secondary organisms alter the exposure of test organisms to contaminants. Ciutat and Boudou (2003) reported increased exposure and accumulation of cadmium and zinc in the bivalve *Corbicula fluminea* when it was exposed to sediments bioturbated by the nymph *Hexagenia rigda*. Colombo et al. (2016) reported decreased zinc toxicity in the chironomid *Chironomus tepperi* in the presence of bioturbation by the oligochaete *Lumbriculus variegatus*. Changes to dietary behaviour, growth and survival of the bivalve *Eumarcia paupercula l* were observed after bioturbation by the sand prawn *Callianassa kraussi* (Pillay et al., 2007); and of fish *Mugil cephalus* when exposed to bioturbation by *Sipunculus nudus* (unsegmented marine worm) (Li et al., 2015). Both Granberg et al. (2008) and Josefsson et al. (2010) have reported significant bioturbation-induced release of sediment-bound organic contaminants (polychlorinated biphenyls (PCBs) and polybrominated biphenylethers (PBDEs)) with the polychaete *Marenzelleria* spp, but only Josefsson et al. (2010) observed an apparent relationship between bioturbation and accumulation in this species.

When assessing the potential for sediment contamination to cause toxicity to benthic organisms, standard bioassays are used which typically comprise single species within exposure chambers that isolate them from disturbances by other organisms, and also from abiotic processes such as water currents that might cause sediment to be resuspended intermittently in their natural setting (ASTM, 2014; Simpson and Kumar, 2016). It has been clearly demonstrated that sediment disturbance will alter sediment-metal partitioning and overlying water chemistry (Atkinson et al., 2007). Remaili et al. (2016) showed in metal-contaminated sediments, the survival of the bivalve *Tellina deltoidalis* was higher when cohabiting with the actively bioturbating amphipod *Victoriopisa australiensis*, compared to bivalve-only communities in the same contaminated sediments. This was attributed to the increased intensity of bioturbation resulting in increased scavenging of dissolved copper by resuspended particulate phases resulting in lower exposure of the bivalve to copper.

The importance of designing tests to adequately mimic exposures and conditions encountered in the field is not a new consideration for sediment quality assessments (Burton et al., 2005, 2012; Mann et al., 2010; Belzunce-Segarra et al., 2015; Costello et al., 2015). However, generally such considerations only extend to the abiotic nature of field conditions (e.g. dissolved oxygen and light conditions, food availability etc.). Few studies consider the importance of organism-organism interactions when assessing contaminant bioavailability or, potentially more importantly, when applying bioassay results to predicting the trajectory of the natural recovery of contaminated sediments.

The present study tested the hypothesis that bioturbation activities of a secondary organism will change contaminant bioavailability to an extent sufficient to alter the outcomes of a chronic sediment toxicity test. The specific objective of this work was to investigate the reproduction of a test amphipod exposed to contaminated sediments in the presence and absence of an active secondary bioturbator. The test species used was the small epibenthic amphipod *Melita plumulosa*, and the secondary bioturbator was the larger more tolerant endobenthic amphipod *V. australiensis*. Two sediment types were tested, one primarily contaminated with metals and the second had both metals and petroleum hydrocarbons. The results are discussed in relation to the adequacy in which standardised test procedures replicate the assessment of the environment, and also our understanding of the recovery processes for natural sediments.

2. Materials and methods

2.1. Test media and organisms

Clean seawater was obtained from the southeast coast of New South Wales (NSW), Australia, filtered (1 μm) and analysed prior to use to ensure that the metals of interest were below 1 μg L^{-1}. Sediments (0–15 cm depth) were collected from Lake Illawarra (control, S1), and five contaminated sites: one in Port Kembla, NSW (S2), and four along the south-western foreshore of the Parramatta River, NSW (S3, S4, S5 and S6). Sediment collection, homogenisation and storage (4 °C) were conducted as per Belzunce-Segarra et al. (2015).

The epibenthic amphipod *M. plumulosa*, endemic to the estuarine tidal mudflats of south-eastern Australia, was used to assess the effects on reproduction. *M. plumulosa* individuals were obtained from previously established laboratory cultures, maintained as described per Spadaro and Simpson (2016). The bioturbator used in this experiment was the endobenthic, deposit-feeding amphipod *V. australiensis* (Chilton, 1923; 2–3 cm body length) collected from Lake Illawarra.

2.2. Reproduction bioassay

The 10–day renewed whole-sediment bioassay was conducted according to Spadaro and Simpson (2016), with modifications to incorporate bioturbation intensity as a variable. The toxicity tests were conducted at constant temperature (21 ± 1 °C) within an environmental chamber (Labec Refrigerated Cycling Incubator, Laboratory Equipment) on a 12-h light/12-h dark rotational cycle (light intensity = 3.5 μmol photons s^{-1} m^{-2}) and aeration provided. Each test comprised of three conditions: (i) with no added organisms (No bioturbation), (ii) with *M. plumulosa* added (Low bioturbation), and (iii) with both *M. plumulosa* and the larger bioturbating amphipod *V. australiensis* added (High bioturbation). The No bioturbation

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