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Effects of *Lumbriculus variegatus* (Annelida, Oligochaete) bioturbation on zinc sediment chemistry and toxicity to the epi-benthic invertebrate *Chironomus tepperi* (Diptera: Chironomidae)☆

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ABSTRACT

Classical laboratory-based single-species sediment bioassays do not account for modifications to toxicity from bioturbation by benthic organisms which may impact predictions of contaminated sediment risk to biota in the field. This study aims to determine the effects of bioturbation on the toxicity of zinc measured in a standard laboratory bioassay conducted with chironomid larvae (*Chironomus tepperi*). The epi-benthic chironomid larvae were exposed to two different levels of sediment contamination (1600 and 1980 mg/kg of dry weight zinc) in the presence or absence of annelid worms (*Lumbriculus variegatus*) which are known to be tolerant to metal and to have a large impact on sediment properties through bioturbation.

Chironomids had 5–6x higher survival in the presence of *L. variegatus* which shows that bioturbation had a beneficial effect on the chironomid larvae. Chemical analyses showed that bioturbation induced a flux of zinc from the pore water into the water column, thereby reducing the bioavailability of zinc in pore water to the chironomid larvae. This also suggested that pore water was the major exposure path for the chironomids to metals in sediment. During the study, annelid worms (*Oligochaetes*) produced a thin layer of faecal pellets at the sediment surface, a process known to: (i) create additional adsorption sites for zinc, thus reducing its availability, (ii) increase the microbial abundance that in turn could represent an additional food source for opportunistic *C. tepperi* larvae, and (iii) modify the microbial community's structure and alter the biogeochemical processes it governs thus indirectly impact zinc toxicity.

This study represents a contribution in recognising bioturbating organisms as "ecological engineers" as they directly and indirectly influence metal bioavailability and impact other sediment-inhabiting species. This is significant and should be considered in risk assessment of zinc levels (and other metals) in contaminated sediment when extrapolating from laboratory studies to the field.

1. Introduction

Zinc is one of the most abundant metals; it is a component of various enzymes and as such essential to all living organisms at low concentrations, but toxic if in excess (Lushchak, 2011). Zinc contamination of freshwater sediments occurs predominantly by anthropogenic activities such as mining and metallurgic operations (electroplating, smelting), followed by discharges of municipal wastewater effluents and urban stormwater runoff predominantly from galvanised metal roofing and vehicular sources (Naito et al., 2010). Some field surveys have shown that chironomid species richness was reduced in zinc contaminated streams at concentrations >770 μg/L (Wright and Burgin, 2009), a value quite high, however, compared to the impact on the whole macro-invertebrate community (e.g. taxon richness decreased at 84 μg/L in the study of Iwasaki et al., 2011). Exposure of benthic and epi-benthic organisms to sediment-bound zinc can occur through all the main routes of uptake, including ingestion of sediment particles and associated

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food such as algae and macroinvertebrates (Eggleton and Thomas, 2004) and uptake of dissolved metals from the pore water (Han et al., 2005). Most of the studies that focus on the impact of zinc load on freshwater invertebrates do not include sediment and aim to mimic exposure through the aquatic phase. For example, during a one week exposure, *Daphnia magna* (Crustacea, Branchiopoda) survival decreased to 40% at a zinc concentration of 250 μg/L (Muyssen et al., 2006), while the growth of the mayfly *Epeorus latifolium* was reduced at a 30 μg/L zinc concentration.

To appreciate these values in a general context, it is crucial to take into account the speciation of zinc in determining its bioavailability and toxicity. In surface waters, zinc can occur in various speciation forms, of which the free dissolved divalent ion (Zn^{2+}) is the most labile and readily bioavailable. In aerobic sediment zinc binds to iron and manganese oxides, clay, silt, and colloids, and becomes stored in particulate form. Under anaerobic conditions, acid volatile sulphide (AVS) such as iron sulphides (FeS) are the dominant binding phase for zinc (Luoma, 1989). Organic carbon represents a significant secondary binding phase in both anaerobic and aerobic sediments. The column of overlying water works as a barrier to oxygen (diffusion of oxygen into sediment is much slower from water than from air) and thus zinc and other metals could remain buried in this form for hundreds of years, be less bioavailable, and pose a low environmental risk (Coulthard and Macklin, 2003). However, several re-oxidation processes can remobilise sediment-bound metals by exposing the sediment to oxygen; such processes can be abiotic and slow (flood and drought episodes) or biotic and fast (Hutchins et al., 2007; Schaller et al., 2011).

One major biotic process that can remobilise sediment-bound zinc is bioturbation. Bioturbation refers to all activities carried out by benthic organisms that directly and indirectly modify their environment through activities such as particle transport and bioirrigation (Kristensen et al., 2012; Mermillod-Blondin, 2011). Bioturbating organisms may alter the structure and chemistry of sediments, including causing changes in the particle size distribution (McLachlan, 1978), higher water content through an increase of sediment porosity (Mermillod-Blondin et al., 2005), and oxygen penetration (Svensson and Leonardson, 1996). By ingesting anoxic sediment from the depth and bringing it to the surface in the form of cylindrical faecal pellets (Robbins et al., 1979), certain bioturbating organisms such as annelid worms cause alterations in the sediment biogeochemistry and consequently can influence the fate and bioavailability of contaminants (Petersen et al., 1998) and/or metal partitioning (Matisoff, 1995). Furthermore, bioturbation indirectly affects the structure and density of the microbial community which in turn is known to modify sediment chemistry (Mermillod-Blondin, 2011) and ultimately the biogeochemical cycling of many metals (Nelson and Campbell, 1995); an increase in microbial density may also act as an additional food source for sediment-inhabiting invertebrates.

Bioturbation is an ecologically-relevant process that directly and indirectly has the potential to influence the type and magnitude of toxicity of a pollutant to aquatic organisms (Foit et al., 2012; Gutiérrez and Jones, 2006; Pang et al., 2012). However, only a few studies have focused on the interaction between bioturbation and the resulting changes in the fate and toxicity of metals in freshwater systems compared to marine systems: for example, the presence of tubificids increased the molecular diffusion from sediment into the water column of particulate-bound cadmium (Ciutat et al., 2007), uranium (Lagauzere et al., 2009) and pollutants from stormwater (Nogaro and Mermillod-Blondin, 2009). Our general understanding of the mechanisms by which bioturbation influences the transfer of metals from sediment to other abiotic compartments as well as the mechanisms responsible for the transfer between abiotic and biotic components is still limited. Such connections are necessary to understand whether predictions of toxicity based on standard laboratory-based bioassays could be influenced by ecological interactions occurring in the field.

This study was designed to better understand the role bioturbation plays on sediment contaminated with zinc and its consequent increased or decreased bioavailability. This was investigated by conducting chronic sediment bioassays with two species, the bioturbating *Oligochaete* worm *Lumbriculus variegatus* and the epi-benthic insect midge larva *Chironomus tepperi,* as single species and co-habiting species. Chironomids are also known to be bioturbators and bioirrigators and influence the sediment chemistry around their tubes (Schaller et al., 2011). Chironomid-mediated bioturbation happens in the initial larval stage when the larvae dig into the sediment in order to build their tube. For *C. tepperi* this stage is very short; larvae start building their tube in the 1st larval stage that lasts ca. 2 days at 21 °C (V. Colombo personal observations). Once the tube is built, the contribution of the larvae to bioturbation is minor (Schaller et al., 2011; Hölker et al., 2015). In comparison, *L. variegatus* live in the sediment, forming burrows mainly in the upper 10 cm; they are conveyor-belt deposit-feeders that bring reduced sediment from the depth to the surface (Ciutat et al., 2005; Lagauzere et al., 2009; Rodriguez and Reynoldson, 2011).

Through the ecological interaction of bioturbation by the *Oligochaete* and the accompanying formation of burrows in the sediment, we hypothesized that zinc would be increasingly released from the sediment pore water to the overlying water, and potentially be more toxic to the chironomid larvae than in the absence of bioturbation. We measured zinc in sediment, pore water, and overlying water as well as trace metals such as iron and manganese, and acid volatile sulfides to determine how bioturbation influenced the geochemistry of zinc. At the same time, we measured the 21-d survival of the chironomid larvae exposed to low and high zinc in sediment with and without bioturbation, i.e. the chironomid larvae do not contribute significantly to bioturbation, therefore it is mainly the presence/absence of the *Oligochaetes* that controls the effect of bioturbation on sediment geochemistry. By linking bioturbation with geochemistry changes and a biological response in standard laboratory bioassays, we consider the implications for such bioassays as predictive tools in contaminated sediment risk assessments. This study therefore provided a test of the extent to which an ecologically-relevant interaction (bioturbation) will modify the bioavailability of zinc-contaminated sediment and influence zinc toxicity to the epibenthic *C. tepperi*, as well as providing information on the influence of zinc on the survival and burrowing behaviour of *L. variegatus*.

2. Materials and methods

2.1. Spiking of sediment with zinc

Sediment with low background metal concentrations (see Supplemental Table 1) was collected from an uncontaminated wetland at Glynns, Warrandyte (VIC, Australia) (Pettigrove and Hoffmann, 2005), sieved (<63 μm), and allowed to settle for 1 week in the dark at 4 °C, under a layer of site water. The sediment was sieved for multiple reasons: (1) to achieve homogenised samples, with a very similar grain size; (2) to remove other organisms that could confound the results (e.g. naturally occurring *Chironomus* species); and (3) to represent an overly protective scenario as metals bind easily to clay and silt. When diluted with coarser sediment, the sediment was expected to have a lower impact on biota (ANZECC, 2000) than seen here, which is