Chemosphere 167 (2017) 19–27

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The effect of lead contamination on bioturbation by *Lumbriculus variegatus* in a freshwater microcosm

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HIGHLIGHTS

- This study investigated the effect of Pb on bioturbation for two laboratory populations of *Lumbriculus variegatus*.
- The result showed that bioturbation by *L. variegatus* was negatively affected by Pb in the sediment.
- The two *L. variegatus* populations differed in Pb sensitivity (survival), but not in bioturbation or its sensitivity to Pb.
- The effect of Pb on worm bioturbation may indirectly influence other sediment organisms and biogeochemical processes.

ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form
22 September 2016
Accepted 26 September 2016

Handling Editor: Martine Leermakers

Keywords:
Lumbriculus variegatus
Lead
Populations
Bioturbation
Modeling

ABSTRACT

The present study investigated the effect of lead (Pb) on bioturbation by the oligochaete worm *Lumbriculus variegatus*, using freshwater microcosms. The experiment used lead at “0”, 140, 700, and 3500 μg/g in sediment, and used two different laboratory populations of *L. variegatus*. A molecular genetic analysis and bioassays were conducted to determine if the two populations differed genetically and whether they differed in Pb-sensitivity. The bioturbation of *L. variegatus* was estimated using luminophores placed at the sediment-water interface at the beginning of the experiment. After the 14 d experiment the luminophore profiles in sediment were used to estimate the biodiffusion and bioadvection coefficients, using the diffusion-advection model. The results showed that the biodiffusion and bioadvection coefficients were generally negatively related to the Pb concentrations in the sediment. Lead at 700 and 3500 μg/g reduced both coefficients, while Pb at 140 μg/g did not. Luminophore profiles in the “0” and 140 μg/g treatments were indicative of a non-local transport, while a diffusive transport was observed at the higher Pb levels. The two laboratory populations of *L. variegatus* used in the experiment differed in their sensitivity to Pb when mortality was used as the endpoint, but they did not differ in sediment bioturbation or the Pb-sensitivity of this process. Moreover, the genetic analysis did not detect any genetic differences between the populations. This study demonstrated that elevated levels of Pb can impact ecosystem functioning by decreasing the bioturbation activity of benthic organisms such as *L. variegatus*.

1. Introduction

Bioturbation by benthic organisms results from activities such as feeding and burrowing that redistribute sediment and thereby modify the physical, chemical and biological characteristics of sediments (Ouellette et al., 2004; Gerino et al., 2007; Gilbert et al., 2007). In the process of bioturbation, benthic organisms enhance the exchange of solutes and solids across the sediment-water interface (Allen, 1995; Ciutat et al., 2005). The exchanged materials include oxygen from the overlying water, which diffuses to depths that are subject to bioturbation (Bianchi, 2007). This will cause reduced environments in the sediments to become oxidized and this affects sediment chemistry and biological conditions (Lohrer et al., 2004; Bianchi, 2007). This in turn can influence the fate of contaminants in aquatic environments. For example, metals bound to sulfides in reduced sediments are remobilized upon oxidation and released to the overlying water (Bianchi, 2007). However the opposite effect, where an increase in oxygen concentration results in higher bioavailability of metals, has also been reported (Chuan et al., 1996). Bioturbation can also bring about very

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http://dx.doi.org/10.1016/j.chemosphere.2016.09.128
0045-6535/

localized differences in pH (Zhu et al., 2006) and such pH changes are also known to affect metal bioavailability (Chuan et al., 1996). Bioturbation by benthic organisms also influences nutrient cycling and organic matter mineralization in the sediment (Lohrer et al., 2004; Bianchi, 2007; Klerks et al., 2007). The magnitude of the effects of bioturbation on sediment characteristics is influenced by environmental conditions in the sediment (such as temperature and contaminant levels), and the type, size, density and activity of the benthos involved in bioturbation (Sun et al., 1999; Ouellette et al., 2004; De Backer et al., 2011). The present study investigated whether contamination by lead has an effect on bioturbation. The biogeochemical changes associated with bioturbation and the toxicological effects of contaminants on benthic organisms are both well studied, but less attention has been paid to the potential for contamination to affect bioturbation. Yet it is important to understand these effects because of the importance of bioturbation in influencing sediment biogeochemistry and the environmental fate of contaminants (Kure and Forbes, 1997; Blankson and Klerks, 2016). Results of previous studies generally suggest a decline in bioturbation and associated biogeochemical processes with an increase in sediment contamination (Fernandes et al., 2006; Mermillod-Blondin et al., 2013). The decrease in bioturbation has been reported for both field and laboratory studies (Keilty et al., 1988; Mazik and Elliott, 2000; Mulsow et al., 2002; Fernandes et al., 2006). In the field, the reduction in bioturbation was associated with reduced abundance and diversity of the benthic infauna (Mazik and Elliott, 2000). The laboratory studies demonstrated that the reduction in bioturbation also occurs at a fixed abundance for a single bioturbator species, and that the effect on bioturbation may change over time (Keilty et al., 1988; Fernandes et al., 2006).

In the present study we are using Pb as our model contaminant. Lead is a toxic metal with an affinity for sediments that is relatively high among metals (Horowitz, 1991). The toxic effects of lead on benthic organisms are well known, and some of the adverse biological effects associated with lead toxicity include mortality, decreased reproduction and abnormal development (Pattee and Pain, 2003; Aisemberg et al., 2005). However, it is not known if bioturbation is affected at sublethal Pb levels. We hypothesize that Pb will affect bioturbation negatively.

The present study used *Lumbriculus variegatus* as a model organism for determining the effect of Pb on bioturbation. This species is a freshwater oligochaete with a high tolerance for some contaminants and is commonly-used to assess sediment toxicity (Phipps et al., 1993). *Lumbriculus variegatus* is widely distributed and is an important sediment bioturbator (Brinkhurst et al., 1971; Phipps et al., 1993). Benthic fauna are generally categorized into five functional groups on the basis of their feeding behavior and sediment reworking (Bianchi, 2007). This study's model species falls in the “upward conveyor” category, as it ingests sediment from deeper depths and egests it at the sediment-water interface (Bianchi, 2007; Kristensen et al., 2012). Bioturbation by benthic organisms is usually measured using tracers such as luminophores, pollen grains, radionuclides and glass beads (Gerino et al., 1998; Maire et al., 2008). The present study estimated bioturbation with the use of luminophores, and was carried out using laboratory microcosms.

It is has been demonstrated that laboratory culturing can result in genetic divergence and changes in contaminant tolerance (Athrey et al., 2007). Moreover, populations from different sources can differ in their tolerance (and thus in the contaminant-sensitivity of an ecological process such as bioturbation) as a consequence of adaptation to locally elevated levels of contaminants and other environmental stressors. Local adaptation to metals has been reported for both laboratory and natural populations of freshwater oligochaetes (Klerks and Levinton, 1989; Vidal and Horne, 2003). Consequently, results on contaminant-sensitivity for a single population may not be representative for the species as a whole. The present study therefore looked at the effects of Pb on bioturbation for two different laboratory populations. In order to be able to put any among-population differences in bioturbation and the Pb-sensitivity of this bioturbation in a broader context, differences in survival upon Pb exposure and population genetic differences between these populations were also assessed.

2. Materials and methods

2.1. Study organism

The experiment used two laboratory populations of *L. variegatus*; both had been acclimated to laboratory conditions. One population (further referred to as population 1) came from Ward's Science (Rochester, NY, USA), while population 2 was obtained from Flinn Scientific (Batavia, IL, USA).

2.2. Design of bioturbation experiment

The bioturbation experiment was conducted with sediments obtained from the Vermillion River (30°12′38.92″N, 92° 0′8.94″W), which is relatively fine-grained with high organic content (see section 2.3). The sediment was sieved (<1 mm fraction retained) and frozen for 48 h at −20 °C to eliminate most fauna present in the sediment. The sediment's organic content, silt/clay content, and lead concentration were measured using methodologies described below. Lead in the form of $Pb(NO_3)_2$ was added to sediment (except the control) to obtain sediment lead concentrations of “0” (background levels), 140, 750 and 3500 μg/g Pb. These concentrations were chosen based on the results of preliminary 14 d toxicity test with the two populations – in which no mortality was recorded at concentrations below 6000 μg/g Pb. The present study's concentrations are within the range observed in freshwater aquatic sediments (Dennis et al., 2009). After homogenizing the lead-spiked sediments and subsampling for metal analysis (see below), microcosms were set up. A total of 508 g wet weight of sediment, uncontaminated sediment for the control and lead-spiked sediment for the other groups, was placed in each microcosm – using three replicate microcosms for each of the four lead concentrations and each of the two worm populations. Microcosms were made using a 20-cm long piece of 7.8-cm diameter, transparent, PVC pipe, with the end closed off by a plastic cap glued to the pipe bottom. Overlying water, consisting of 400 ml of soft reconstituted freshwater (pH 7.2–7.6, $CaCO_3$ hardness 40–48 mg/L), was added to each microcosm (APHA et al., 1995). The sediment and overlying water in each microcosm had depths of 6 and 10 cm respectively. Luminophores were added to each microcosm in order to be able later to quantify the bioturbation by *L. variegatus*. The microcosms were left undisturbed for 14 d to allow most of the suspended sediments to settle. Gentle aeration was then provided 24 h prior to the addition of *L. variegatus*. Worms from the two laboratory populations of *L. variegatus* were added to the microcosms at densities of 30 ind./microcosm. This density (6279 ind./m^2) is within the range observed in freshwater aquatic sediments (Cook, 1969).

2.3. Quantification of sediment organic content and silt/clay content

The loss-on-ignition method was used to estimate the sediment organic content (De Jonge et al., 2010). The organic content of the sediment used in these experiments was 5.90 ± 0.068% (mean ± S.E., $n = 6$). The silt/clay content was determined by first