Environmental Pollution xxx (2017) 1–7

Contents lists available at ScienceDirect

Environmental Pollution

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

Effects of red earthworms (*Eisenia fetida*) on leachability of lead minerals in soil

Armin Kavehei [a, *], Grant C. Hose [b], Damian B. Gore [a]

[a] Department of Environmental Sciences, Macquarie University, Sydney 2109, Australia
[b] Department of Biological Sciences, Macquarie University, Sydney 2109, Australia

ARTICLE INFO

Article history:
Received 13 August 2017
Received in revised form
31 October 2017
Accepted 6 November 2017
Available online xxx

Keywords:
Metal
Vermiremediation
Eisenia fetida
Aqueous extraction

ABSTRACT

Contamination of soils by metals and metalloids is an important environmental problem in many residential and industrial sites around the world. Lead is a common contaminant, which enters the soil through mining, industrial activities and waste disposal. A range of technologies can be used to remediate soil lead, however most remediation technologies adversely affect the environment and particularly soil biota. We have assessed the efficacy of vermiremediation (the use of earthworms for remediation) to reduce water extractable lead concentrations in soil. Earthworms were introduced to a sandy soil spiked with the common lead minerals cotunnite ($PbCl_2$), cerussite ($PbCO_3$), massicot (PbO) or galena (PbS) at 1000 mg (Pb) kg^{-1}. Lead concentrations in pore water extracted during the experiment were not significantly different in contaminated soil with and without worms. However, concentrations of lead in water from a deionised water extraction (washing) of contaminated soil were significantly lower in soil with earthworms than in soil without. Earthworms accumulated on average (±1 standard deviation) 276 ± 118, 235 ± 66, 241 ± 58 and 40 ± 30 mg kg^{-1} (dry weight of earthworms) of lead in their bodies, in $PbCl_2$, $PbCO_3$, PbO and PbS-dosed soils, respectively. During the experiment, earthworms lost weight in all contaminated soils, except those containing PbS.

1. Introduction

Lead (Pb) is a toxic metal that can create environmental problems, such as reduced biological activity (Jusselme et al., 2012), in contaminated soil. Pb contamination of soil occurs through industrial activity, mining, ore processing, use of some pesticides, fertilizers and wastewater irrigation, and the past use of leaded paint and gasoline (Chen et al., 2016; Zhang et al., 2015). Pb compounds are used widely in industrial processes and are common contaminants of soils in these areas. Lead acetate is used in cotton dye, water repellent and mildew protection products. The production of automobile clutch or brake linings, and flame retardants requires lead chloride. Ceramic industries use lead sulfide and lead oxide, and lead carbonate is used in electronics, optical industries and coatings for thermographic copying (NTP, 2004). Pb is one of the priority contaminants for remediation in the USA (Chen et al., 2015) but the remediation of Pb in soil can be costly and adversely affects the environment, particularly soil biota (Jusselme et al., 2012).

Vermiremediation is the use of earthworms for the remediation of soils. It is cost-effective, and can be carried out *in situ* and in tandem with other remediation technologies such as phytoremediation or soil flushing (Sinha et al., 2010a). Earthworms can remediate contaminated soil, simultaneously improving soil structure, pH, aeration and increasing available carbon, nitrogen and other nutrients for plants (Ruiz et al., 2009; Nannoni et al., 2011). Earthworm species that are metal tolerant and are used for vermiremediation, include *Allobophora chlorotica, Aporrectodea tuberculata, Dendrobaena rubida, Dendrobaena tetraedra, Eisenia fetida, Lumbricus rubella* and *Lumbricus terrestris*. *Eisenia fetida* in particular are effective bioaccumulators of metalliferous contaminants (Sinha et al., 2010b), their biology and ecology are well described, they are commercially available and easy to keep in laboratory environments, and as a consequence they are broadly used in studies of vermiremediation (Sizmur and Hodson, 2009).

Earthworms accumulate contaminants in their bodies or change the bioavailability of the contaminants in soil (Nannoni et al., 2011; Pattnaik and Reddy, 2011). As soil is ingested and passes through the earthworm gut, the soil is adjusted to ~ pH 7, and coupled with changes in dissolved organic carbon, the mobility of metals can

* Corresponding author. Fax: +61 2 9850 8420.
E-mail address: armin.kavehei@students.mq.edu.au (A. Kavehei).

https://doi.org/10.1016/j.envpol.2017.11.021
0269-7491/

change (Sherameti and Varma, 2015). Earthworms can also adjust soil pH via excretion of calcium carbonate (Wen et al., 2004). Calciferous glands in the worms, including *Eisenia fetida* (Karaca, 2010), secrete amorphous calcium carbonate, which crystallizes in the gut to calcite and in some cases aragonite or the intermediary phase vaterite, although some amorphous material may also be occluded in the calcite crystal or its pores (Fraser et al., 2011; Gago-Duport et al., 2008; Hodson et al., 2015; Lee et al., 2008; Nardi, 2009; Wen et al., 2004). These minerals are then excreted into the soil. Pb and other metals may sorb to these mineral surfaces (Brinza et al., 2014) or form metal-bearing minerals such as the lead carbonate mineral cerussite (Fraser et al., 2011). Metals also react with metallothioneins and other proteins or sugars produced by the worms to create organometallic compounds (Alloway, 2013; Gago-Duport et al., 2008; Sahariah et al., 2015). The chemical bond between metallothioneins and metals allows them to be stored in different organs of worm bodies (Alloway, 2013; Gonick, 2011).

Worms have been reported to increase (Stephens et al., 1994; Sizmur and Hodson, 2009; Sizmur et al., 2011a) or decrease metal extractability (Lukkari et al., 2006; Zorn et al., 2005), and while reasons for this variability remain unclear, it is likely that mineral composition and solubility are important controls on metal availability (Davies et al., 2003).

The overall aim of this study was to further explore the role of earthworms in influencing availability of Pb in soil. We aimed to 1) determine the effects of *E. fetida* on leachability of Pb and soil pore water composition in soils spiked with the Pb minerals cotunnite (lead chloride; $PbCl_2$), cerussite (lead carbonate; $PbCO_3$), massicot (lead oxide; PbO) or galena (lead sulfide; PbS), and 2) investigate how these minerals affect the mass of *E. fetida* and the concentration of Pb accumulated in their tissue.

2. Materials and methods

Red worms (*Eisenia fetida* Savigny 1826) were used in this experiment. The worms were purchased from Wormlovers Pty Ltd (Australia), and kept and cultured in a Pb free environment prior to the experiment.

Soil from Australian Native Landscapes Pty Ltd (Terrey Hills, Sydney), was a mixture of 50:50 w/w organic potting mix:sand. Gravels and large organic particles were first removed using a 2.0 mm mesh sieve (Du et al., 2014; Nirola et al., 2016). The soil, with a D_{30} of 75 μm, D_{50} of 480 μm and D_{90} of 1430 μm, and with 96% sand and 4% clay, is classified as sand in the USDA textural classification (Das, 2010). Loss on Ignition (LOI) was performed according to BS EN 15169:2007 (BSI, 2009) and soil organic matter content was 21.2%. Soil pH of 4.8 was measured following USEPA method 9045D (USEPA, 2004).

Worms were introduced to soils contaminated with one of the Pb minerals cotunnite ($PbCl_2$), cerussite ($PbCO_3$), massicot (PbO) or galena (PbS). These minerals were used because they are common and represent a wide range of solubilities in water (Abadin et al., 2007; Lide and Haynes, 2009). The four minerals were reagent-grade (>98% purity) powders purchased from Chem-Supply Pty Ltd (Australia), and were added to the soil at 1000 mg (Pb) kg^{-1}. Each dry, spiked soil was mixed for 3 h on a rolling shaker at 60 rpm and 3 h on a Ratek RM4 rotary end over end shaker at 60 rpm.

200 g of dry spiked soil was placed in 300 mL glass jars (diameter 85 mm, height 75 mm) which had one 8.0 mm diameter hole drilled in the side and one in the bottom. The bottom hole allowed the soil to drain freely and the side hole was used for installing micro-porous sampling tubes (micro-rhizons; Rhizosphere Research Products B.V., The Netherlands) through which soil pore water was extracted at various times. Micro-rhizons are samplers consisting of an 8.0 mm long porous tip attached to a 1.0 mm outside diameter polyether ether ketone tube. Each jar had one micro-rhizon installed into the centre of the soil, and the hole through the jar was then sealed using silicone sealant.

The experiment was conducted with four mineral spikes (cotunnite ($PbCl_2$), cerussite ($PbCO_3$), massicot (PbO) or galena (PbS)) and unspiked soil as a control. For each Pb mineral and control there were six replicate jars containing worms and six jars without worms, with 96 containers in total. Ten mature worms (fully clitellate) were placed in each jar. This number and the resulting density of worms was chosen to ensure thorough mixing and processing of the soil by the worms during the 70 d experimental period. 2 g of oats and 2 mL of 16 MΩ deionised water were added to the soil every week. 120 mL of 16 MΩ grade deionised water was added to the soil to start the experiment. Pore water samples were extracted every two weeks by applying vacuum using a 5 mL syringe connected to the micro-rhizons. The Pb content of water samples was measured immediately after sampling. At the end of the experiment, leaching was undertaken to determine changes in extractability of Pb after worm activity. Deionised water was slowly added to each jar until 200 mL leached water was collected from the bottom hole.

The experiment was conducted at 14 ± 1 °C to be representative of soil temperatures at mid-latitude sites. A data logger (EasyLog, EL-USB-2-LCD) was used to record temperature and relative humidity every 30 min during the experiment. The relative humidity in the room was 92 ± 6% during the experiment.

Pore water and final leached water were analysed using a Bruker S2 Picofox Total Reflection X-ray Fluorescence (TXRF) spectrometer with molybdenum anode X-ray tube, operated at 50 kV and 750 μA, with no filter, for 600 s. 1.3 mL of sample was filtered with a cellulose acetate Sartorius MiniSart syringe filter, then acidified with 10 μL of analytical grade concentrated nitric acid. A spike of gallium was added to the sample to attain 1.0 mg L^{-1} for quantification. 10 μL of prepared sample was placed on an acid-washed quartz disc, and evaporated on a heating block at 60 °C prior to analysis. Repeated measurement of a Merck XVI multi-element standard showed analytical inaccuracy to be better than 10% for the analyte concentrations measured here. Also, measurement of replicate preparations had an average of 5.4% relative standard deviation, showing that sample preparation was acceptable for this analysis.

Pb in worm tissue was measured to quantify bioaccumulated Pb. All of the worms were removed from the soil at the end of the experiment and rinsed with 16 MΩ deionised water to remove soil adhered to their bodies. In order to remove soil and faecal casts in their guts, the worms were placed in a box with damp tissue and kept in the dark for three days. Worms were then killed by freezing at −30 °C and dried at 65 °C for 48 h (Li et al., 2010). Finally, microwave assisted acid digestion (10 mL HNO_3 (70%), Ultra-pure reagents) of the dried worms and casts were completed following USEPA method 3051A (USEPA, 2007) and analysed using TXRF.

The Bioaccumulation Factor (BAF) is a metric that reflects how much of an analyte, in this case Pb, has accumulated in an organism's tissue (Pattnaik and Reddy, 2011). The BAF is calculated using the equation;

$$BAF = C_{biota}/C_{substrate}$$

where C_{biota} is the total concentration of Pb in the worm tissue (mg kg^{-1} dry worm) and $C_{substrate}$ is the concentration of Pb in substrate materials (mg kg^{-1} dry weight).

Worms were first washed in water to remove soil particles, then they were dried on tissue paper and weighed. Worms were reweighed at the end of the experiment to determine the effects of Pb minerals on body mass.

Concentrations of Pb in pore water were compared between

Please cite this article in press as: Kavehei, A., et al., Effects of red earthworms (*Eisenia fetida*) on leachability of lead minerals in soil, Environmental Pollution (2017), https://doi.org/10.1016/j.envpol.2017.11.021