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Earthworm bioturbation influences the phytoavailability of metals released by particles in cultivated soils

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ABSTRACT

The influence of earthworm activity on soil-to-plant metal transfer was studied by carrying out six weeks mesocosms experiments with or without lettuce and/or earthworms in soil with a gradient of metal concentrations due to particles fallouts. Soil characteristics, metal concentrations in lettuce and earthworms were measured and soil porosity in the mesocosms was determined. Earthworms increased the soil pH, macroporosity and soil organic matter content due to the burying of wheat straw provided as food. Earthworm activities increased the metals concentrations in lettuce leaves. Pb and Cd concentrations in lettuce leaves can increase up to 46% with earthworm activities … These results and the low correlation between estimated by $CaCl_2$ and EDTA and measured pollutant phytoavailability suggest that earthworm bioturbation was the main cause of the increase. Bioturbation could affect the proximity of pollutants to the roots and soil organic matter.

1. Introduction

Today, soil pollution by persistent metals concerns large areas at the global scale, particularly in industrial and mining environments (Newhook et al., 2003; Schreck et al., 2011). Although the sustainability of industrial processes has been progressively improved unfortunately numerous cases of past contamination exist (Uzu et al., 2010; Shahid et al., 2013a) and metals are still released into the environment (Nair et al., 2010). In particular, the different stages of lead-recycling processes (crushing, fusion, reduction and refining) emit atmospheric particles (PM) enriched in metal(loid)s: Pb, Cd, As, Cu, Zn, Sb and Sn (Cecchi et al., 2008).

Urban areas have expanded worldwide (Szolnoki et al., 2013), and due to numerous anthropogenic activities, the amount of metals released in soils has generally increased (Wong et al., 2006), with Pb and Zn being the most commonly observed pollutants (Szolnoki et al., 2013). Originally located on the outskirts of cities, numerous industrial or mining sites, often abandoned, are now in urban areas and are therefore likely to cause environmental and health risks to surrounding populations (Van Hees et al., 2008; Foucault et al., 2013b; Szolnoki et al., 2013). These sites are sources of fine particles enriched with pollutants leading to contamination of soils (Lee et al., 2008; Stampoulis et al., 2009; Schreck et al., 2011) and plants (Uzu et al., 2009; Schreck et al., 2013).

Recently, pollutant bioavailability in soils has become a crucial scientific question (Foucault et al., 2013a), with ecotoxicity (Hedde et al., 2012; Leveque et al., 2013) and human health risk assessments (Pelfrêne et al., 2012; Uzu et al., 2011). Soil to plant transfer has been widely studied and it is nowadays well-documented. It is well-known that soil to plant transfer of metals strongly depends on pollutant compartmentalization and speciation that are directly in relation to soil edaphic properties such as soil organic matters (SOM) or pH (Shahid et al., 2013b). According to Chenot et al. (2013) and Dumat et al. (2013), various environmental or human factors can strongly modify soil pH, texture and soil organic matters (SOM: content and type) in urban areas. Actually, even if these soils are polluted with metal(loid)s, they still provide a habitat for plants and soil organisms. Earthworms, the dominant macrofauna in soils,

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can also modify the soil characteristics through their bioturbation activities such as burrow creation, production of casts and thus the mixing of litter and soil (Nahmani et al., 2007). Moreover, earthworms preferentially ingest SOM (Lee et al., 1985) and several interactions (indirect and feedback effects) have been reported between metals, soil living organisms and SOM (Dumat et al., 2006; Quenea et al., 2009). In consequence, earthworms can also modify the mobility of metals (Sizmur and Hodson, 2009) and their bioavailability (Wen et al., 2006; Capowiez et al., 2011).

Further studies are therefore needed in the case of PM polluted soils, to investigate the influence of earthworm activities on metals uptake by vegetables, the mechanisms involved (Sizmur and Hodson, 2009) and later their implication in terms of metals phytoavailability and consequences for human health after plant consumption. Food is the main source of human exposure to pollutants and vegetables are a major component in the diet of the world's population (Mansour et al., 2009). Thus, determining how metals are taken up by plants, accumulate and eventually enter the human food chain is becoming a key issue for assessing the health risks associated with the consumption of polluted vegetables (Polichetti et al., 2009; Shahid et al., 2013b). The present study had two scientifics aims: (i) In the context of health risk assessments, does earthworm activity in soil modify metal concentrations in the edible parts of vegetables? (ii) What are the biogeochemicals and physicals mechanisms involved in the soil-plant-earthworm-metal systems? In order to answer these questions, lettuces were cultivated on historically polluted soils with a range of increasing concentrations of metals (Pb, Cd, Zn and Cu) carried by process particles (Uzu et al., 2009; Schreck et al., 2011), for six-weeks in mesocosms experiment with or without earthworms. Total, exchangeable (EDTA) and phytoavailable ($CaCl_2$) metal concentrations in soils were studied in relation to soil pH, organic matter and porosity. Thus, this article provides new insights to improve the understanding of the role of earthworm activities on metals phytoavailability in polluted soils and health risks incurred by vegetables consumers.

2. Materials and methods

2.1. Soil preparation

The polluted soil used in this study was collected from a lead recycling factory, the chemical metal treatment company (Société de Traitements Chimiques des Métaux, STCM), located in the urban area of Toulouse, southwest France (43°38′12″ N, 01°25′34″ E), which currently recycles batteries. For several decades, atmospheric fallout of metal-enriched particles from the industrial activities at the STCM site have resulted in high concentrations of Pb and other metals such as Cd, Cu and Zn in nearby top-soils (Uzu et al., 2009; Schreck et al., 2011). Thus, the sampled soil is contaminated with a complex mixture of metals: Pb, Zn, Cu and Cd at concentrations of 39,800, 294, 286 and 18.36 mg kg^{-1} of dry weight, respectively. This historically polluted soil was sampled from the 0–25 cm top-soil layer, air-dried at room temperature for a week, disaggregated and, finally, sieved to retain aggregates smaller than 2 mm. This highly contaminated soil was used to establish a pollution concentration gradient to which earthworms were exposed in mesocosms. To prepare the soil, a specific amount of the highly contaminated soil (pH = 6, OM content = 5.2%, 12% clay, 47% silt and 41% sand) was mixed with non-contaminated soil (pH = 5.7, OM content = 4.5%, 13% clay, 48% silt and 38% sand) to obtain a range of contamination with 30, 780, 2800 and 3750 mg kg^{-1} of dry weight for Pb, as described by Leveque et al. (2013). The properties of each soil were determined by INRA Arras. Metal concentrations in the soils used for the mesocosm experiments are reported in Table 1. These metal concentrations were chosen to minimize ecotoxicological effects and optimize earthworm bioturbation activities, as assessed by earthworm cast production in a previous experiment (Leveque et al., 2013).

Table 1
Mean values and standard deviations ($n = 5$) of metals concentrations in soils (mg kg^{-1}).

	Pb	Cd	Cu	Zn
C_0	19.2 ± 2.5	0.3 ± 0.02	21.6 ± 1.4	75.7 ± 0.6
C_1	800.5 ± 36.8	0.6 ± 0.04	26.8 ± 0.9	80 ± 2.8
C_2	2822.8 ± 127.2	1.5 ± 0.03	40.2 ± 0.8	91.1 ± 5.1
C_3	3730.7 ± 583.5	2 ± 0.056	46.3 ± 7.2	96.1 ± 6.1

2.2. Earthworms

Two earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*) were chosen from two different ecological types (anecic and endogeic respectively) and thus are expected to have different behaviours. *L. terrestris* is an anecic species which dominates earthworm biomass in various temperate ecosystems and strongly affects organic matter transformation and soil development (Maleri et al., 2008). *A. caliginosa* is a common endogeic species well known for its strong bioturbation activities (Dittbrenner et al., 2011). Adult *L. terrestris* earthworms were purchased from a local supplier (Decathlon®, France). Adult *A. caliginosa* earthworms were found at an uncontaminated fallow site near Valence (France). Prior to the start of the experiment, the earthworms were allowed to acclimatize for one week in the experimental conditions.

2.3. Experimental set-up

Rectangular plastic pots (50 × 20 × 15 cm) containing 1 mm diameter holes to allow water drainage were filled with 6 kg of soil sieved to 2 mm. Four different experimental series were carried out simultaneously. The four experimental series were: (i) control soil without earthworms and plants (denoted S); (ii) soil with earthworms but no plants (denoted E); (iii) soil with lettuce but no earthworms (denoted L) and (iv) soil with lettuce and earthworms (denoted LE). For each experimental condition, four concentrations denoted C_0, C_1, C_2 and C_3 were studied with five true replicates per series.

Lettuce (*Lactuca sativa capitata*) was purchased at the 2-leaf stage from a local supplier (Botanic®, France). Three lettuces were planted per pot in the experimental series L and LE. At the beginning of the experiment, the soil moisture was measured and adjusted to 19% (corresponding to 65% of its water holding capacity, as previously measured *in situ* by filter paper press method). All the pots were weighed and, every two days the weight lost was replaced with deionized water. The E and LE experimental series were inoculated with 2 *L. terrestris* and 9 *A. caliginosa* per pot. All individuals were adult worms, with a mean fresh individual weight of 0.66 ± 0.1 g ($n = 360$) and 4.3 ± 0.7 g ($n = 80$) respectively for *A. caliginosa* and *L. terrestris*, (the errors are standard deviations). The experiment was carried out for six weeks at an average temperature of 20 °C ± 2 °C.

The earthworms (especially *L. terrestris*) were fed seven grams of dry wheat straw cut into small pieces and spread on the soil surface.

At the end of the experiment, i) the lettuce was sampled and washed prior to acidic digestion, ii) some mesocosms were chosen to assess internal macroporosity (see below), iii) straw was recovered, air-dried and weighed, iv) in all mesocosms, living earthworms were recovered, counted, weighed again and then placed on filter paper for five days to empty their gut before acidic digestion, v) soils from each mesocosm were then mixed and sampled for chemical extractions.

The intensity of straw burial by the earthworms was calculated as the ratio of the mass of straw that had disappeared from the surface at the end of the experiment (i.e. that was buried, in g of dry weight) to the initial mass of the straw (in g of dry weight). This intensity was used to evaluate the effect of metal contamination on earthworm foraging and burial behaviour.

2.4. Phytoavailability measurements

2.4.1. Total metal content in lettuce shoots

After harvest, lettuce shoot tissues were weighed (fresh biomasses) and then washed twice with deionized water (Uzu et al., 2010). Plant tissues were oven-dried at 40 °C for 72 h. After mineralization of plant samples in aqua regia (1:3 mixture of HNO_3 and HCl) with a Digiprep® instrument (from SCP Science producer) at 80 °C for 4 h, the Pb, Cd, Cu and Zn concentrations were measured by inductively coupled plasma-optical emission spectrometry ICP-OES (IRIS Intrepid II XXDL) or inductively coupled plasma-mass spectrometry ICP-MS (X Series II, Thermo Electron). Ten blanks were submitted to the same treatment (mineralization and assay) for method control. Each sample was analysed in triplicate. The detection limits of Pb, Zn, Cu and Cd were 0.3, 2.2, 1.3 and 0.2 μg l^{-1}, respectively, whereas the limits of quantification were approximately 0.4, 3, 2 and 0.3 μg l^{-1} respectively. The accuracy of measurements was checked using reference materials: Virginia tobacco leaves, CTA-VTL-2, ICHTJ and TM-26.3 certified reference material from the National Water Research Institute, Canada. The concentrations found were within 95–102% of the certified values for all measured elements.

2.4.2. $CaCl_2$ and EDTA extractions

$CaCl_2$ extractions were performed on soil samples from experimental series (S) and (E). Neutral salt extraction on polluted soils is the current standard method used to determine metal phytoavailability (Menzies et al., 2007). According to Houba et al. (1996) and Schreck et al. (2011), 10 ml of $CaCl_2$ 10^{-2} M were mixed with 1.0 g of soil in a 20 ml centrifuge tube placed on an end-over-end shaker at 5 rpm for 2 h at 20 °C. After extraction, samples were centrifuged at 10,000 *g* for 30 min. The supernatant was then filtered at 0.22 μm, acidified to 2% with distilled HNO_3 (15 M, suprapur 99.9%), and then stored at 4 °C before analysis. A Standard Reference Material