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Effects of historic metal(loid) pollution on earthworm communities

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HIGHLIGHTS

- The impact of industrial metal(loid) pollution was studied on earthworm community.
- Earthworm's density increases progressively with the distance to the factory.
- A greater proportion of adults is observed in the most polluted zones.
- Metal(loid)s are more exchangeable in surface casts than in bulk soil.

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ABSTRACT

The effects of metal(loid)s (Pb, Cd, Cu, Zn, As and Sb) from atmospheric fallout on earthworm communities were investigated in a fallow meadow located close to a 60-year-old lead recycling factory. We examined abundance and species diversity as well as the ratio of adult-to-juvenile earthworms, along five 140 m parallel transects. The influence of soil pollution on the earthworm community at the plot scale was put in context by measuring some physico-chemical soil characteristics (OM content, N content, pH), as well as total and bioavailable metal(loid) concentrations. Earthworms were absent in the highly polluted area (concentration from 30,000 to 5000 mg Pb·kg^{-1} of dried soil), just near the factory (0–30 m area). A clear and almost linear relationship was observed between the proportion of juvenile versus mature earthworms and the pollution gradient, with a greater proportion of adults in the most polluted zones (only adult earthworms were observed from 30 to 50 m). *Apporectodea longa* was the main species present just near the smelter (80% of the earthworms were *A. longa* from 30 to 50 m). The earthworm density was found to increase progressively from five individuals·m^{-2} at 30 m to 135 individuals·m^{-2} at 140 m from the factory. On average, metal(loid) accumulation in earthworm tissues decreased linearly with distance from the factory. The concentration of exchangeable metal(loid)s in earthworm surface casts was higher than that of the overall soil. Finally, our field study clearly demonstrated that metal(loid) pollution has a direct impact on earthworm communities (abundance, diversity and proportion of juveniles) especially when Pb concentrations in soil were higher than 2050 mg·kg^{-1}.

1. Introduction

Over several decades the activities of industrial sites around the world can significantly pollute their surrounding ecosystems (Kříbek et al., 2014; Schreck et al., 2011). Activities related to metal(loid) processing have a strong impact on ecosystems (Doshi et al., 2008; Shahid et al., 2014) and human health (Pascaud et al., 2014; Uzu et al., 2011; Xiong et al., 2014). Particles enriched with metal(loid)s are gradually emitted into the atmosphere and deposited in the environment which eventually leads to ecosystem pollution. Sectors of the metal industry release fine lead (Pb)-enriched particles into the atmosphere by canalized (chimney) and diffuse emissions (Schreck et al., 2012). As reported by Cecchi et al. (2008), the different stages of the metal(loid)

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recycling process (crushing, fusion, reduction and refining) in these facilities generate undesirable by-products enriched with metal(loid)s. These elements have been, for the most part, classified as Substances of Very High Concern (SVHC) in the European REACH law (Regulation EC 1907/2006 of the European Parliament and of the Council of 18 December 2006). European regulations have thus imposed management measures for these industrial sites to determine and reduce the potential impacts on the environment and human health (Foucault et al., 2013).

Earthworms are important organisms in soil functionality (Nahmani et al., 2007a; Brown et al., 2000) and consequently play a key role in terrestrial ecotoxicological risk assessments (Hankard et al., 2004). However, few field studies examined the impact of pollution on earthworms in industrial sites (Spurgeon and Hopkin, 1996a). In general short-term microcosm experiments in controlled conditions were performed with artificially spiked soils (Nahmani et al., 2007a) and with a single metal element. However in reality, field pollution often concerns a mixture of contaminants (Dumat et al., 2006; Pascaud et al., 2014). The main studies on the effects of metal(loid)s on earthworms measured mortality, weight loss and fertility (Nahmani et al., 2007b) or were focused on ecotoxicity tests (Reinecke et al., 2001; Scheffczyk et al., 2014; van Coller-Myburgh et al., 2014). Furthermore, as previously reported (Leveque et al., 2014), it is now well-known that metal(loid) transfer in soils strongly depends on pollutant compartmentalization and speciation which are influenced by soil edaphic properties such as soil organic matter (SOM) and pH (Shahid et al., 2013). According to Chenot et al. (2013) and Dumat et al. (2013), various environmental and human factors can strongly modify soil pH, texture and SOM content and type. Some species of earthworms preferentially ingest SOM (Leveque et al., 2013) and are thus responsible for indirect and feedback effects between metals, soil living organisms and SOM (Quenea et al., 2009). As a consequence, earthworms can also modify metal mobility (Sizmur and Hodson, 2009) and bioavailability (Ruiz et al., 2011; Wen et al., 2006).

In this context, we carried out a field study at an industrial site established in the 1960s consisting of a parcel of land surrounding a secondary lead smelter that recycles car batteries. This factory has emitted metal(loid)s for nearly 50 years, since it began operating in 1967. Our aim was to investigate the consequences of historically polluted soil on earthworm communities at the field scale. Thus, to better understand the mechanisms involved, earthworm communities (abundance, young/adult ratio, and species diversity) were studied under field conditions in soil polluted with a mixture of metal(loid)s (mainly Pb and secondary Cd, Cu, Zn, As and Sb). Soil physico-chemical characteristics (pH, total N and organic matter contents) as well as the total and exchangeable metal(loid) quantities in the soil were determined. Additionally, metal(loid) concentrations in earthworm tissues and surface casts were measured at selected locations along five 140 m transects established at the site.

2. Materials and methods

2.1. Description of the study site and sampling strategy

The study site is located in Bazoches-les-Gallérandes in the Loiret department (45, France), 45 km from Orleans and 80 km south of Paris. The study was carried out in a fallow grassland, on a calcic cambisol, adjoining a lead recycling factory: the Chemical Treatment Company (Société de Traitements Chimiques des Métaux, STCM) which has been in operation since 1967. This plant currently recycles lead batteries and the recycling process is carried out in several stages (crushing, fusion, reduction and refining), each generating, in addition to Pb, undesirable by-products such as Cu, Zn, As and Sb. Metal(loid)s are emitted from the factory through a chimney (furnace emissions) and also diffuse emissions due to the recycling processes. The prevailing winds blow from the South-West. Thus, soils close to this kind of plant can contain multi-metallic contamination due to atmospheric fallout (Schreck et al., 2011; Shahid et al., 2014). At the field scale, the vegetation is well-developed, even in the strongly polluted area (0–30 m from the factory). *Silene vulgaris*, a metal tolerant pseudometallophyte species, is the most abundant species. Metallophyte grassland is evidence of adaptation to an ecosystem disrupted due to anthropogenic activities (Austruy et al., 2013).

Five linear transects along the field site were defined as shown in Fig. 1, all beginning close to the factory walls (distance = 0 m) and ending in the nearest cultivated plot (distance = 140 m) (Fig. 1). These transects followed the direction of the prevailing wind (Fig. 1). Each transect consisted of 12 sampling points at 0, 10, 20, 30, 40, 50, 70, 80, 95, 110, 130 and 140 m from the factory wall.

2.2. Soil characteristics

Soil samples were collected from the upper soil horizon at all locations along the transects (topsoil: 0–20 cm) and air dried for a week. The soils were then ground and sieved through a 2 mm stainless steel sieve and a 250 μm nylon sieve. Several soil characteristics were determined, approximately every 10 m, along the meadow. The soil pH was measured in water using a 1/5 (v/v) soil/solution ratio according to the standard ISO 10390 procedure. The soil organic carbon and total nitrogen contents were determined by heating the samples at 1000 °C in the presence of oxygen according to standard procedures ISO 10694 and ISO 13878 respectively. Those soil characteristics were determined at an independent analytical laboratory in France (INRA, Arras).

2.3. Determination of total and exchangeable metal(loid) concentrations in soils and earthworm casts

At each location on each transect, 100 g of soil was sampled from the upper soil horizon (topsoil: 0–20 cm) and, whenever possible, surface casts were collected within 2 m from the sampling point.

To determine total metal(loid) concentrations, soil samples (0.5 g per sample) were acid mineralized with a mixture of 1 mL HNO_3 70% and 0.5 mL of 51% fluorhydric acid in Teflon bombs placed on heating plates at 90 °C for 2 days. After evaporation, 1 mL 30% H_2O_2 and 1 mL of 70% HNO_3 were added and the Teflon bombs replaced on heating plates at 90 °C for 2 days. The samples were then evaporated at 50 °C. Ultra-high purity water was used for dilutions. Solutions were stored at 4 °C in polyethylene tubes before analysis. All reagents were purchased from Sigma Aldrich®.

To assess the exchangeable fraction and the role of earthworms in metal(loid) fate, soil and earthworm cast samples collected in the plot were extracted with EDTA. 1 g of soil sample was mixed with 10 mL of 0.05 M EDTA in a centrifuge tube (Zhang et al., 2014). The tubes were agitated for 2 h using an end-over-end shaker at 5 rpm 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.

After acidic mineralization or chemical extraction, metal(loid) concentrations (total and exchangeable fractions) were then 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 control blank samples were subjected to the same treatment (mineralization and assay) for method control. Controls were performed to verify that samples were not contaminated during acidic digestion. Each sample was analyzed in triplicate. The detection limits for Pb, Cd, Cu, Zn, As and Sb were 0.3, 0.2, 1.3, 2.2, 0.2 and 0.2 μg L^{-1} respectively, whereas, the limits of quantification were approximately 0.4, 0.3, 2, 3, 0.3, and 0.4 μg L^{-1} respectively for the same inorganic elements. The accuracy of the acid digestion or chemical extraction and the analytical procedures was verified using reference materials: the standard reference soils 'Montana