



# Ecological changes in historically polluted soils: Metal(loid) bioaccumulation in microarthropods and their impact on community structure

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## ABSTRACT

Soil pollution by persistent metal(loid)s present environmental and sanitary risks. While the effects of metal(loid)s on vegetation and macrofauna have been widely studied, their impact on microarthropods (millimetre scale) and their bioaccumulation capacity have been less investigated. However, microarthropods provide important ecosystem services, contributing in particular to soil organic matter dynamics.

This study focussed on the impact of metal(loid) pollution on the structure and distribution of microarthropod communities and their potential to bioaccumulate lead (Pb). Soil samples were collected from a contaminated historical site with a strong horizontal and vertical gradient of Pb concentrations. Microarthropods were extracted using the Berlese method.

The field experiments showed that microarthropods were present even in extremely polluted soils (30,000 mg Pb kg<sup>-1</sup>). However, while microarthropod abundance increased with increasing soil C/N content ( $R^2 = 0.79$ ), richness decreased with increasing pollution. A shift in the community structure from an oribatid-to a springtail-dominated community was observed in less polluted soils ( $R^2 = 0.68$ ). In addition, Pb bioamplification occurred in microarthropods, with higher Pb concentrations in predators than in detritivorous microarthropods. Finally, the importance of feeding and reproductive ecological traits as potentially relevant descriptors of springtail community structures was highlighted. This study demonstrates the interest of microarthropod communities with different trophic levels and ecological features for evaluating the global environmental impact of metal(loid) pollution on soil biological quality.

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## 1. Introduction

At the global scale, historical soil pollution by persistent metal(loid)s presents environmental and sanitary risks (Schreck et al., 2011; Levêque et al., 2013; Dumat and Austruy, 2014; Kpan et al., 2014; Xiong et al., 2014; Levêque et al., 2015). While the harmful effects of metal(loid)s on vegetation and soil macrofauna have been widely studied (chapter 10 of Hopkin, 1997; Reddy et al., 2005; Sharma and Dubey, 2005; Gichner et al., 2008; Austruy et al., 2013), their impact on microarthropod communities (millimetre scale) and their bioaccumulation capacity have been less investigated. However, microarthropods contribute significantly to soil organic matter dynamics, for example by improving leaf

litter decomposition and organic matter recycling (Chagnon et al., 2000; Gobat et al., 2010; Van Eekeren et al., 2009). Indeed, soil microarthropods contribute directly to decomposition processes of 5 to 10% of fresh organic matter (Sechi et al., 2014). Feeding directly on decaying materials and soil fungi, microarthropods provide an early indication of ecosystem health and therefore have an important role in functional ecology (Coleman et al., 2004; Van Eekeren et al., 2009) and associated ecosystem services (Lavelle et al., 2006; section 1.3 of Wall et al., 2013). Moreover, a significant proportion of the carbon consumed by microarthropods originates from the rhizosphere (Hishi and Takeda, 2008). Springtails (Collembola) have been used as an ecotoxicological model species due to their high sensitivity to various environmental changes (Ardestani et al., 2014). Metal(loid) bioaccumulation in springtails can be an efficient indicator of the exposure level in polluted areas. Moreover, the ecological traits are dependent on ecosystem characteristics and could be used to interpret the distribution of the springtail community as a function of the physico-chemical parameters and metal pollution of soil. Indeed,

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springtail diet is dependent on the nutritional quality of the soil. The breeding patterns might allow a better understanding of the mechanisms of distribution and development of these species (Salmon et al., 2014). This is particularly useful in the case of taxa with substantial functional redundancy like Collembola.

According to Van Gestel and Koolhaas (2004), the main route for metal(loid) uptake by microarthropods is the soil solution, which contains fungi and which is in contact with the soil organic matter. Soil solution is actually considered as a major exposure pathway for the majority of pedofauna. Metal(loid) bioaccumulation is a complex process which includes absorption, intrinsic distribution, storage, and excretion (Wang and Rainbow, 2008). Indeed, the type of metal(loid) and soil physico-chemical properties as well as organism physiology can affect metal(loid) bioaccumulation (Lanno et al., 2004; Ardestani and Van Gestel, 2013). Likewise, metal bioavailability is mainly influenced by soil pH and the amount of soil organic matter (Levêque et al., 2013) or by the presence of various ligands in the soil solution (Shahid et al., 2014). Metal bioaccumulation in microarthropods could therefore be a relevant measure of metal bioavailability and thus of the overall soil ecotoxicity.

In a global scientific context, our research hypothesis was that metal(loid) pollution could modify microarthropod ecology. The two main objectives of this study were therefore to: (1) evaluate the influence of historical metal(loid) soil pollution on microarthropod community structure, and notably springtail population, and (2) compare bioconcentration factors across microarthropod trophic levels. The study was carried out in a fallow meadow located near a metal treatment factory. A previous study from Levêque et al. (2015) documented the existence of a pollution gradient in this area due to the airborne emissions from the factory, thus providing an interesting site to investigate the potential effects of metal(loid)s on soil fauna biodiversity and bioaccumulation. We demonstrate the relevance of microarthropod communities and bioaccumulation as bioindicators of soil pollution and ecotoxicity.

## 2. Materials and methods

### 2.1. Field sampling

Soils were sampled in a fallow meadow located close to a metal treatment factory (Bazoches-les-Gallerandes, Region Centre, France). The study area is 4.5 ha. The level of metal contamination steeply decreases with increasing distance from the factory (Levêque et al., 2015). Soil samples were taken along a linear transect similar to that in the previous study (distances to the factory: 10, 30, 50, 70, 95, 130, and 140 m) (Fig. 1). In the present study, 10 soil samples were taken at each distance for further microarthropod analyses.

For the analysis of physico-chemical parameters and metal concentrations, three soil samples were sampled at each distance. For each distance, the three samples were then pooled and homogenised to constitute a composite sample used for the analysis. The results were published by Levêque et al. (2015). Physico-chemical soil parameters (pH, total organic carbon, total nitrogen, carbon nitrogen ratio (C/N), organic matter) are summarised in Table 1. Soil pH is alkaline. Organic matter, total nitrogen, and C/N decreased with increasing distance to the factory (Levêque et al., 2015). Table 2 shows metal(loid) contents (Zn, Cu, As, Cd, Sb, and Pb) in soil. Close to the factory (10 m), the total metal concentration was extremely high notably due to high Pb and Cd concentrations (29,600 and 314 mg kg<sup>-1</sup>, respectively) and to a lesser extent Sb, Cu, As, and Zn. Concentrations decreased with increasing distance to the factory; Pb was the pollutant found at the highest concentrations at the study site, ranging from 29,600 mg Pb kg<sup>-1</sup> (10 m from the factory) to 468 mg Pb kg<sup>-1</sup> (140 m). Observed changes in agronomic parameters were induced by bio-physicochemical modifications.

### 2.2. Microarthropod extraction and identification

Ten samples were taken at each distance in the organic horizon including the litter, using a corer of 7 cm in diameter with a volume of 500 cm<sup>3</sup> and at a depth of 6.5 cm (Gobat et al., 2010; Fountain and Hopkin, 2005). Soil samples were put in Berlese Tullgren funnels (Berlese, 1905; Edward and Fletcher, 1971) for 10 days for microarthropod extraction. Soil samples were weighed before and after desiccation on the Berlese funnels to estimate soil moisture. Soil moisture varied between 10 and 20%. Mesofauna was collected in a 70% ethanol (96% Fisher chemical diluted with milliQ water). Microarthropods were identified under a binocular microscope, at the species scale for springtails (Massoud, 1967; Rusek, 1971; Arbea and Jordana, 1997; Bretfeld, 1999; Potapov, 2001; Thibaud et al., 2004) and at a lower scale for mites (oribatid or gamasid) and other microarthropods (Coineau et al., 1997). Springtails and oribatid mites are detritivorous and gamasid mites are predators. For each springtail species, species code, feeding traits (sucker/shredder), reproduction traits (standard, standard-to-explosive, explosive, parthenogenesis), vertical distribution according to Gisin (1943), and total count in the soil samples are shown in Table 3 (Hopkin, 1997). The determining of springtail reproduction modes was performed from work of Czarnetzki and Tebbe (2004) and Tully and Ferriere (2008). The diet of springtails (sucker/shredder) was defined from the shape of the mouthparts notably maxillar (Chen et al., 1997; Santorufo et al., 2014a, 2014b; Hoskins et al., 2015). In this study, the species having 'suctorial' mouthparts were called sucker and the ones having big 'grinding' mouthparts were called grinder.

### 2.3. Analysis of metal(loid) content in microarthropods

Intracorporeal metal(loid) concentrations were measured for each of the 3 microarthropod groups (springtails, oribatid mites, and gamasid mites). Microarthropod samples (10 for each distance and group) were pooled (into 3 samples) in order to obtain a sufficient amount of biological material for metal analysis. As a summary, 63 samples were gathered for metal content analysis (7 distances, 3 microarthropod groups, 3 replicates). The dry weight of microarthropod samples was measured with a micro analytical balance (Mettler Toledo AT21 Comparator). Acid digestion with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> was used to mineralise microarthropod samples, which were then diluted with milliQ water for analysis by ICP-MS (Bur et al., 2010, 2012). The quality of the dissolution procedure was verified for each sample series using international reference materials (TORT-2, Lobster Hepatopancreas) and blanks. Measured values for reference materials did not exceed 10% of the certified value.

### 2.4. Calculation of soil toxic units and bioaccumulation factors in microarthropods

Metal concentrations were compared to those found in other soils as well as to standard values for unpolluted soils (Table 2). On the one hand, we used the values representing the average metal contents measured in the agricultural soils of the Midi-Pyrénées region for calcareous and non-calcareous soils (Réseau de Mesures de la Qualité des Sols, Redon et al., 2013). On the other hand, we used the impact statement values for sensitive areas (VCI) determined by the BRGM (2002), which is a threshold value for proven pollution.

To determine the origin of metals in soil surface horizons, the Enrichment Factor (EF) was calculated for each metal. Scandium (Sc) was chosen as the reference element (Eq. (1)), according to several criteria developed in Sterckeman et al. (2006) and N'Guessan et al. (2009). The deep soil horizon was used as the reference material. An EF value exceeding 1 theoretically indicates anthropogenic input. In order to account for uncertainties in the comparison process,

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