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http://dx.doi.org/10.1016/j.geoderma.2016.02.011

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Keywords:
Microarthropod communities
Springtails
Mites
Metal(loid) pollution
Bioaccumulation

A B S T R A C T

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^{-1}). 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.

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^{-1}, 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^{-1} (10 m from the factory) to 468 mg Pb kg^{-1} (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^3 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_3 and H_2O_2 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 microarthopods

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,

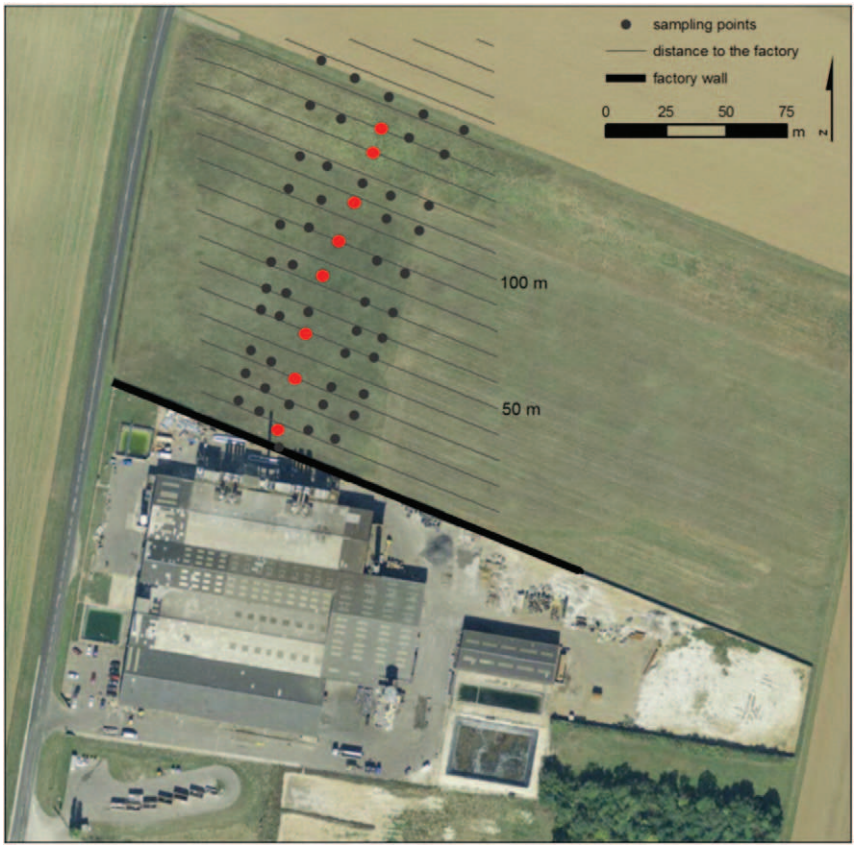

Fig. 1. Map of the study site: lead recycling smelter in Bazoches-les-Gallérandes and the experimental plot. The sampling is performed on the transects indicated with red points from 10 to 140 m to the smelter courtyard.

a value greater than 2 was considered here to indicate significant soil contamination (Hernandez et al., 2003).

The EF was calculated as follows:

$$EF = ([metal]/[Sc])_{surface\ horizon}/([metal]/[Sc])_{deep\ horizon} \quad (1)$$

The transfer of metals from soil to microarthropods is evaluated via the bioaccumulation factor (BCF). BCF is the ratio between metal concentration in microarthropods and concentration in soil (Eq. (2)).

$$BCF = [metal]_{microarthropods}/[metal]_{soil} \quad (2)$$

2.5. Statistical analyses

Microarthropod counts for each soil sample were distributed into four groups (springtails, oribatid mites, gamasid mites, and other microarthropods) and springtail counts were then split into 18 species. For comparative purposes, microarthropod counts were normalised with respect to soil dry weight resulting in microarthropod densities (number of individuals per kg DM of soil).

Variations of microarthropod density and springtail Pb BCF as a function of distance and/or group were investigated through one-way and two-way analyses of variance (ANOVA). The normality and homoscedasticity of ANOVA residuals were verified using Shapiro–Wilk (SW) and Brown–Forsythe (BF) tests, respectively. Appropriate transformations (power, log) were used to satisfy the normality and homoscedasticity requirements of the ANOVA testing procedure. Pairwise differences were later investigated through Tukey multiple comparison tests.

Prospective dissimilarities in the springtail community structure as a function of the distance to the factory were investigated by correspondence analyses (CA). CA is an ordination method, such as principal component analysis, which preserves the chi-squared distance between rows and columns of frequency tables. In our case, the springtail density data table was turned into a proportion table by dividing the density of

Table 1
Physico-chemical parameters in upper horizon of soil in function of distance to factory.

Distance to factory	Organic matter	Total organic carbon	Total nitrogen	C/N	pH
m	g kg^{-1}				
10	59.0	34.1	2.71	12.6	7.90
30	53.0	30.7	2.59	11.9	8.26
50	55.1	31.8	2.52	12.6	8.36
70	34.8	20.1	1.72	11.7	8.34
95	43.7	25.3	2.07	12.2	8.21
130	34.1	19.7	1.70	11.6	8.21
140	24.8	14.3	1.35	10.6	8.34

Table 2
Metal concentrations in upper horizon of soil in function of distance to factory.

Distance to factory (m)	Pb	Zn	Cu	As	Cd	Sb	TU[a]
	mg kg^{-1}						
10	29,600.0	527.1	160.0	114.3	314.5	959.3	3211.7
30	3700.0	127.0	46.5	21.5	20.2	118.6	331.6
50	4530.0	98.8	39.7	19.5	16.3	85.7	370.9
70	1590.0	86.4	31.8	16.6	9.0	58.2	145.7
95	1290.0	73.0	22.6	18.0	5.9	36.1	112.9
130	1030.0	46.4	17.2	13.5	2.5	18.9	81.4
140	468.0	51.0	14.8	12.5	1.8	11.5	40.9
Geochemical background[b]	<30	75–150	1–20	1–25	0.02–0.50	–	

[a] Toxic units (TU) for all metals except Sb.
[b] Gis Sol, 2011

Table 3
Name, reference, code, feeding trait (percentage of individuals in each class; sucker: 31.6%; grinder: 68.4%), reproduction trait (standard: 55.1%; standard to explosive: 35.7%; explosive: 7.1%; parthenogenesis: 2.1%), vertical distribution (epiedaphic: 32.1%; hemiedaphic: 58.2%; euedaphic: 9.7%), and counts for all springtail species. *Rare species (Fse, Opa, and Sni).

Name	Reference	Code	Feeding	Reproduction	Distribution	Count
Brachystomella parvula	Schaffer (1896)	Bpa	Sucker	Standard	Hemiedaphic	176
Ceratophysella armata	Nicolet (1842)	Car	Grinder	Explosive	Hemiedaphic	72
Desoria olivacea	Tullberg (1871)	Dol	Grinder	Standard to explosive	Hemiedaphic	100
Entomobrya lanuginosa	Nicolet (1842)	Ela	Grinder	Standard	Epiedaphic	52
Entomobrya multifasciata	Tullberg (1871)	Emu	Grinder	Standard to explosive	Epiedaphic	73
Folsomia sensibilis	Kseneman (1934)	Fse	Grinder	Standard	Euedaphic	2*
Orogastrura parva	Gisin (1949)	Opa	Grinder	Explosive	Hemiedaphic	2*
Isotoma viridis	Bourlet (1839)	Ivi	Grinder	Standard to explosive	Epiedaphic	34
Lepidocyrtus cyaneus	Folsom (1932)	Lcy	Grinder	Standard	Hemiedaphic	16
Lepidocyrtus lanuginosus	Gmelin (1788)	Lla	Grinder	Standard to explosive	Hemiedaphic	86
Mesaphorura krausbaueri	Boerner (1901)	Mkr	Grinder	Parthenogenesis	Euedaphic	22
Pseudosinella alba	Packard (1873)	Pal	Grinder	Standard to explosive	Euedaphic	32
Protaphorura armata	Tullberg (1869)	Par	Grinder	Standard to explosive	Euedaphic	45
Pseudachorutes palmiensis	Boerner (1903)	Ppa	Sucker	Standard	Hemiedaphic	153
Sminthurinus elegans	Fitch (1862)	Sel	Grinder	Standard	Epiedaphic	16
Sminthurinus niger	Lubbock (1870)	Sni	Grinder	Standard to explosive	Epiedaphic	1*
Sphaeridia pumilis	Krausbauer (1898)	Spu	Grinder	Standard	Epiedaphic	123
Stenacidia violacea	Reuter (1881)	Svi	Grinder	Standard	Epiedaphic	35

each cell by overall density. The chi-squared distance between two sites (rows) quantifies the dissimilarity between the community structures found at these sites. Such a measure excludes double-zeros from the calculations – that is to say the absence of some species at two sites is not accounted for when computing the similarity between these sites – and is insensitive to the presence of over-abundant species. Conversely, the chi-squared distance between two species (columns) quantifies the dissimilarity between the spatial distributions of these two species. Statistical computations were carried out with R software version 3.0.2 (R Core Team, 2014).

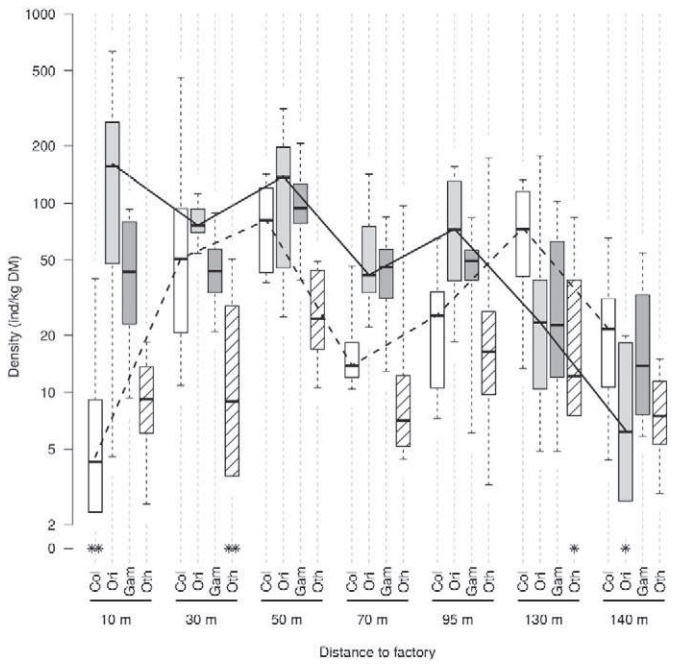

Fig. 2. Microarthropod density in soil with respect to distance to factory: springtail (Col; in white), oribatid mites (Ori; light grey), gamasid mites (Gam; dark grey), and other microarthropods (Oth; white, hatched). Microarthropod densities – microarthropod counts normalised against soil dry weight – are represented as box-and-whisker plots (median, upper and lower quartiles, and maximum values; 10 data samples per box). The detritivorous community shifts from an oribatid mite (median density highlighted with a solid line) dominated to a springtail-dominated (dashed line) community with increasing distance from the factory. Some groups were not found in six soil samples, leading to null densities, highlighted by stars.

3. Results

3.1. Microarthropod community structure

Microarthropod densities are illustrated in Fig. 2. There was a significant change in total microarthropod density – sum of springtails, oribatid and gamasid mites, and other microarthropods densities – associated with the distance to the factory (1-way ANOVA on log transformed total densities; distance: $p < 10^{-5}$; SW: $p = 0.95$; BF: $p = 0.09$) due to a higher density at 50 m and a lower density at 140 m (Tukey; significant differences at the 1% level: 140–10, 140–30, 140–50, 140–95; additional significant differences at the 5% level: 70–50, 130–50). The total microarthropod density appears to be related to the C/N ratio in soil, as suggested by the significant linear increase in density with C/N (linear regression on average densities; slope: $p = 0.0077$; $R^2 = 0.79$; Fig. S1).

The structure of microarthropod groups with respect to the distance to the factory is not trivial with a strong distance x group interaction (2-way ANOVA on log transformed densities; distance: $p < 10^{-15}$; group: $p < 10^{-15}$; distance × group: $p < 10^{-8}$; SW: $p = 0.07$; BF: $p = 0.015$; Fig. 2). There was a shift in the detritivorous community structure from an oribatid mite-dominated community at a short distance (10 m) to a springtail-dominated community at larger distances (130 and 140 m) as indicated by a significant negative relationship between the proportions of oribatid mites and springtails (linear regression on 0.7 power transformed proportions; slope: $p < 10^{-12}$, $R^2 = 0.68$, Fig. 3A). The overall proportion of detritivorous microarthropods was equal for all distances (2-way ANOVA on 0.7 power transformed proportions; distance: $p = 0.14$; SW: $p = 0.38$; BF: $p = 0.068$; samples with null densities were discarded) with significant differences between the proportion of oribatid mites and springtails (group: $p < 10^{-5}$; distance x group: $p < 10^{-15}$) due to a higher proportion of oribatid mites at 10 m (Tukey; $p < 10^{-12}$), 70 m ($p = 0.00017$), 95 m ($p = 0.000020$), and a higher proportion of springtails at 130 m ($p = 0.000036$) and 140 m ($p = 0.00046$). The gamasid mite density was proportional to the detritivorous densities on a log–log scale (linear regression on log transformed densities; intercept: $p = 0.09$; slope: $p < 10^{-12}$; $R^2 = 0.54$; Fig. 3B).

In summary, microarthropod density increased linearly with the C/N ratio of soil. The total proportion of detritivorous microarthropods (springtails and oribatid mites) was identical in high- and low-polluted soils with a shift from an oribatid mite dominated community in high-polluted soils to a springtail-dominated community in low-polluted soils. The density of predator microarthropods (gamasid mites) was proportional to the available stock of detritivorous microarthropods.

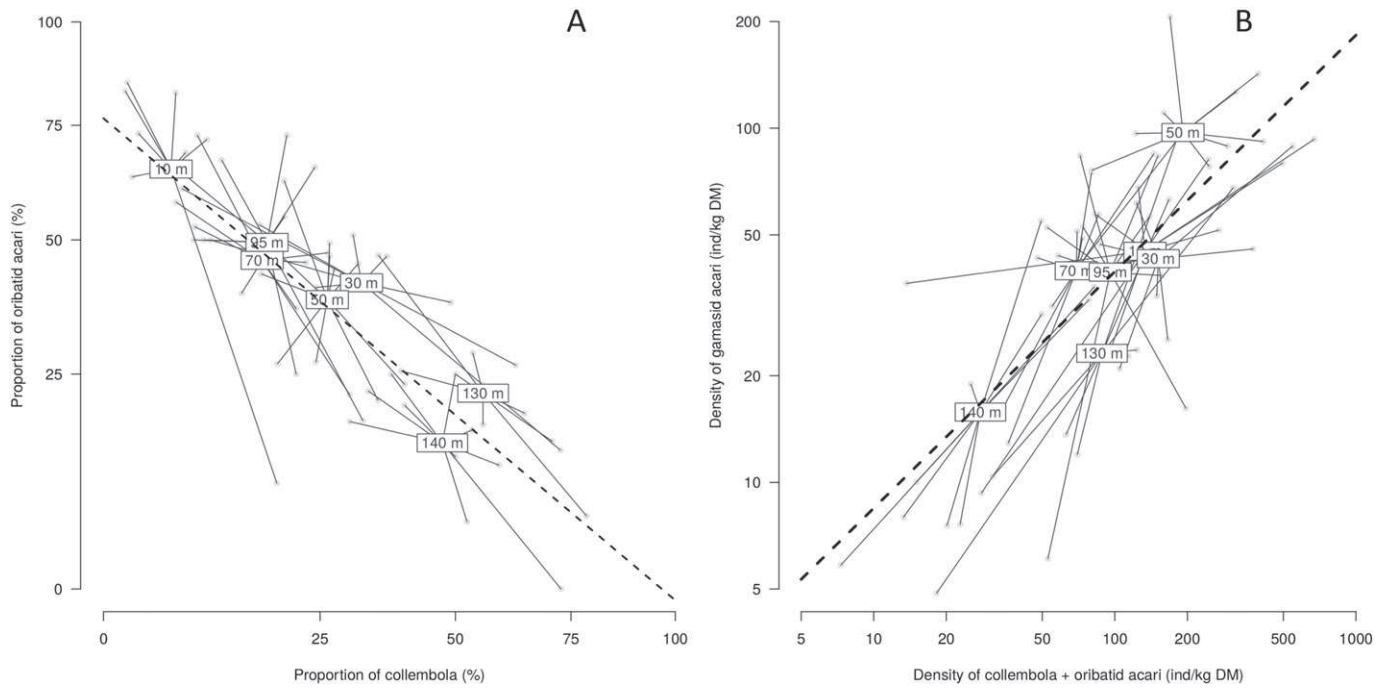

Fig. 3. (A) Proportion of oribatid mites compared to the proportion of springtails and (B) predator density (gamasid mites) compared to detritivorous density (oribatid mites and springtails). The proportion of oribatid mites decreases with the increasing proportion of springtails (power transform: 0.7; $R^2 = 0.68$). Predator density is proportional to the detritivorous density (log-transform; $R^2 = 0.54$).

3.2. Springtail community structure

The distribution of springtail densities across species with respect to the distance to the factory is shown in Fig. 4. In total, 329 individuals of 18 different springtail species were identified at the site, with two species of suckers (Table 3). Four species were found in highly contaminated soils (10 m) and increased richness in diversity was found at larger distances (total of 11, 15, 13, 11, 13, 10 species at 30, 50, 70, 95, 130, 140 m respectively). Beta-diversity, here expressed as the number of species that differed between pairs of distances, decreased monotonically with increasing distance (10–30: 9; 30–50: 6; 50–70: 6; 70–95: 4; 95–130: 4; 130–140: 3; see Fig. 4). Juvenile springtails were underrepresented in highly contaminated soils (10 m).

3.3. Metal accumulation in microarthropods

Pb and Cd concentrations in springtails, oribatid and gamasid mites with respect to the distance to the factory are illustrated in Fig. S4. Bioaccumulation factors (BCF) for Pb and Cd are shown in Fig. 6. Eight BCF values at 70 and 95 m were discarded due to missing concentration data samples (3 out of 8) and extreme concentration values leading to higher BCF values (0.65, 4.17, 0.99, 1.91, 1.14) than for the observed range. Pb BCF differed across groups and were constant across distances (2-way ANOVA on square root transformed BCF; group: $p = 10^{-6}$; distance: 0.065; group × distance: $p = 0.88$; SW: $p = 0.18$; BF: $p = 0.87$). Average BCF for springtails, oribatid and gamasid mites were (pooled over samples and distances) 0.13, 0.24, and 0.34 respectively. As illustrated in Fig. 6, Pb BCF for gamasid mites was approximately equal to that for springtails plus twice the difference of the BCF between oribatid mites and springtails. Cd BCF were higher than those for Pb regardless of the distance from the factory, highlighting that Cd is more bioavailable than Pb in soil. The Cd BCF was always greater than 1 for the gamasid mites except at 10 m from the factory. Likewise, the Cd BCF was greater than 1 for oribatid mites and springtails at 70 m from the factory.

4. Discussion

4.1. Effect of soil pollution on the functional traits of microarthropod communities

4.1.1. Distribution of microarthropod groups with respect to soil pollution

The total density, as well as community structure, of microarthropods changed with respect to soil metal pollution and the C/N ratio. Similar results were observed in previous studies (Gillet and Ponge, 2003; Cluzeau et al., 2012; Calugar, 2013). On the one hand, the total density of microarthropods is affected by the soil toxicity illustrated by the high toxic units decreasing with the distance to the factory (UT = 3212 and 41 respectively 10 and 140 m from the factory). On the other hand, this result highlights the importance of organic matter content in soil for the development of microarthropod communities. As revealed by Neher et al. (2012), soil microfauna depends on the decomposition of organic residues and on the available nitrogen. Our results also showed a dominance of mites in the most contaminated areas, up to 100 m around the industrial site, where this group represented between 52 and 93% of microarthropod density. In contrast, in areas of lower contamination ([Pb] < 1000 mg kg^{-1}), springtails were more abundant than mites with a distribution of microarthropod groups close to that observed in an open environment, e.g. in farming fields and grasslands (Cluzeau et al., 2012; Winkler and Toth, 2012). The mite community was essentially represented by oribatid mites, which suggests that detritivorous microarthropods (oribatid mites and springtails) were always more abundant than predators regardless of soil pollution levels. However, contrary to the observations of Calugar (2013), soil pollution does not seem to have an impact on gamasid mite density, which is largely present in the most polluted areas. Detritivorous microarthropods are the major group in the invertebrate biomass pyramid in most environments (Paoletti et al., 2007) and play a leading role in organic matter turnover. This is certainly why soil metal pollution influences both the C/N and microarthropod distribution.

Detritivorous microarthropods also provide food for predator mites (Cluzeau et al., 2012; Paoletti et al., 2007; Caruso and Migliorini, 2006). Indeed, the positive correlation ($R^2 = 0.54$) which was observed

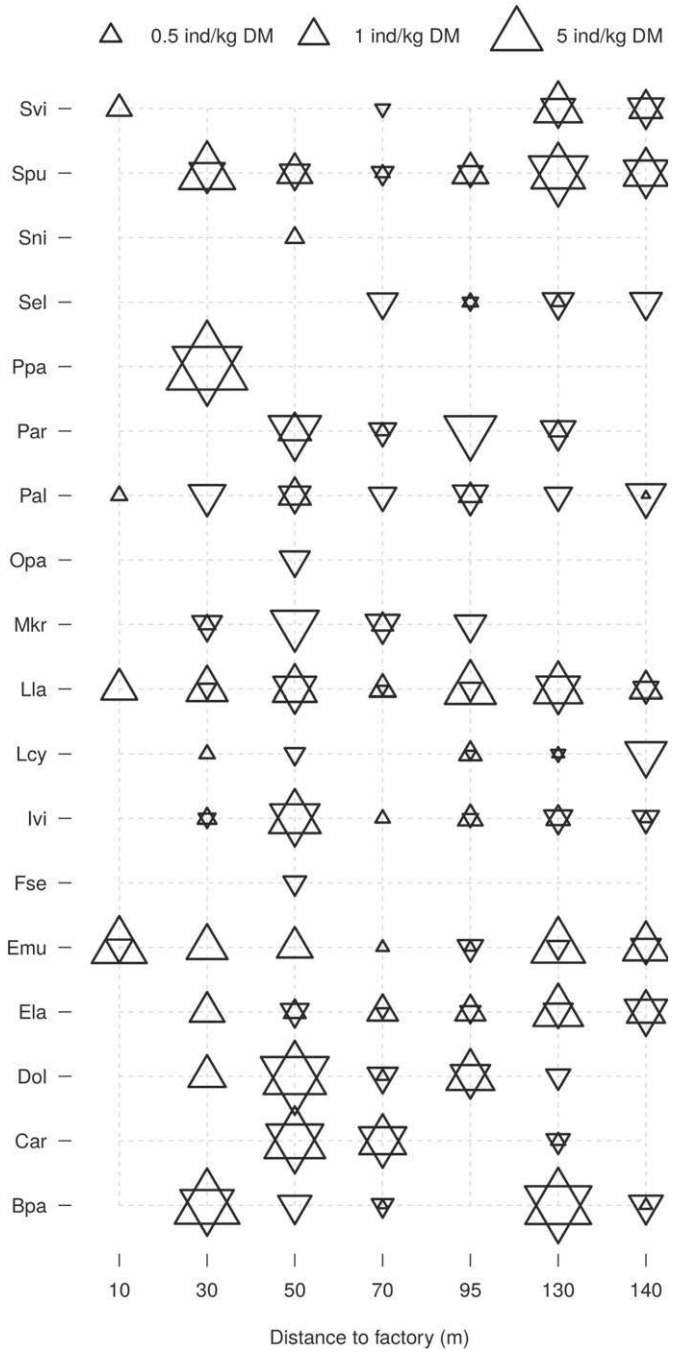

Fig. 4. Springtail densities in soil as a function of distance to the factory. Springtail densities – counts normalised to soil dry weight – are averaged over soil samples (n = 10) and are represented as symbols (triangle up: adult; down: juvenile) the size of which is proportional to the log-density. Unabbreviated species names are shown in Table 3.

between the number of predator (gamasid mites) and detritivorous (oribatid mites and springtails) highlights the influence of the trophic level on the distribution of microarthropod communities. Detritivorous microarthropods, notably springtails, are the main prey of gamasid mites, so there is a balance between the number of detritivorous microarthropods and predators in the upper soil horizon. Similarly, the negative correlation ($R^2 = 0.68$) between oribatid mite and springtail densities illustrates the concept of ecological niche (Lavelle et al., 2006). Such variations in microarthropod densities also appear to be dependent on soil macro-invertebrates, such as earthworms, whose diversity and abundance were measured at the site in a previous study, with a lower abundance and diversity in the most polluted areas (Levêque et al., 2015). In contrast, micraorthropod density is greater in heavily polluted soils than low polluted soils (located more than 100 m from the industrial site) and thus inversely proportional to soil macrofauna measured densities. Such a trend was previously observed in several studies (Salmon et al., 2005; Caruso et al., 2009; Hedde et al., 2012; Santorufo et al., 2014a, 2014b). Indeed, microarthropod abundance tends to be higher at polluted sites. This is at least partly due to the decrease of earthworm activity that induces superficial non-decomposed litter accumulation which then provides increased resource availability for litter mesofauna (Gutierrez-Lopez et al., 2010).

4.1.2. Effect of metal(loid) pollution on springtail community structure

Focussing on springtail community highlights an ecological response to environmental perturbations. Indeed, specific responses were observed in terms of species diversity. On the one hand, the number of encountered species was negatively correlated to metal pollution: with only 4 species in highly contaminated areas and more than 10 species found on low contaminated areas. This observation can be directly linked with the soil toxicity. At 10 m, the toxic unit of soil was above 3200 and springtail richness was 4; at 140 m, the toxic unit was 40 and 10 springtail species were listed. A study conducted near a former mining site in Poland (Syrek et al., 2006; Fiera, 2009) showed a similar trend. On the other hand, a decrease in sensitive species was observed in the contaminated area in the case of the genus *Sminthurinus* sp. and to a lesser extent *Protaphorura armata* and *Ceratophysella armata* (Winkler, 2014). Such a phenomenon promotes the development of ubiquitous species such as *Lepidocyrtus lanuginosus*, *Mesaphorura krausbaueri*, *Entomobrya multifasciata* and *Pseudosinella alba* (Fountain and Hopkin, 2004; Fiera, 2009; Santamaria et al., 2012; Winkler, 2014). These four species seem tolerant to metal soil pollution, as they were found throughout the entire study area. *E. multifasciata* and *L. lanuginosus* did not show significant changes in their numbers (except at 70 m from the industrial site) and represented close to 20 and 70% respectively of springtail communities in the most polluted soils. According to Syrek et al. (2006), the increase in the abundance of some springtail species subjected to environmental stress can be the result of released ecological niches. Springtails have the ability to make use of a wide spectrum of resources under competitive conditions which enable them to exploit resources more efficiently than competitors (Fountain and Hopkin, 2005, Winkler and Toth, 2012), and therefore to survive in polluted environments. A study by Gillet and Ponge (2003) of food contents in the guts of springtail species occurring in soils with different degrees of metal pollution showed that some species were able to select the less polluted food. For example, epigeic species, which were the most represented in the most polluted soils in our study, stopped feeding on fungi once they started accumulating trace metals and shifted their diet to hemi-organic humus (Syrek et al., 2006). Thus, the change in springtail ecological characteristics, observed here with respect to the distance to the industrial site, illustrates the behavioural adaptation of springtail populations to soil disturbance (Chagnon et al., 2000). As illustrated in Fig. S3C, the majority of springtail species at the site are of 'standard' and 'standard-to-explosive' reproductive types, with strong variations between both types. Species of 'explosive' reproductive type were found almost exclusively at 50 m and 70 m and species of 'parthenogenesis' type were found between 30 m and 95 m. As illustrated in Fig. S3D, almost exclusively adult springtails were sampled at 10 m while farther away juveniles and adults were present with average proportions oscillating between 35% and 65%. Several authors (Skubała and Zaleski, 2012; Santamaria et al., 2012) have found that low metal concentrations are positively correlated with the development of microarthropod communities, and notably detritivorous, and with the stimulation on the rate of reproduction.

The CA of the springtail density data highlights the structure of the springtail community with respect to the distance to the factory

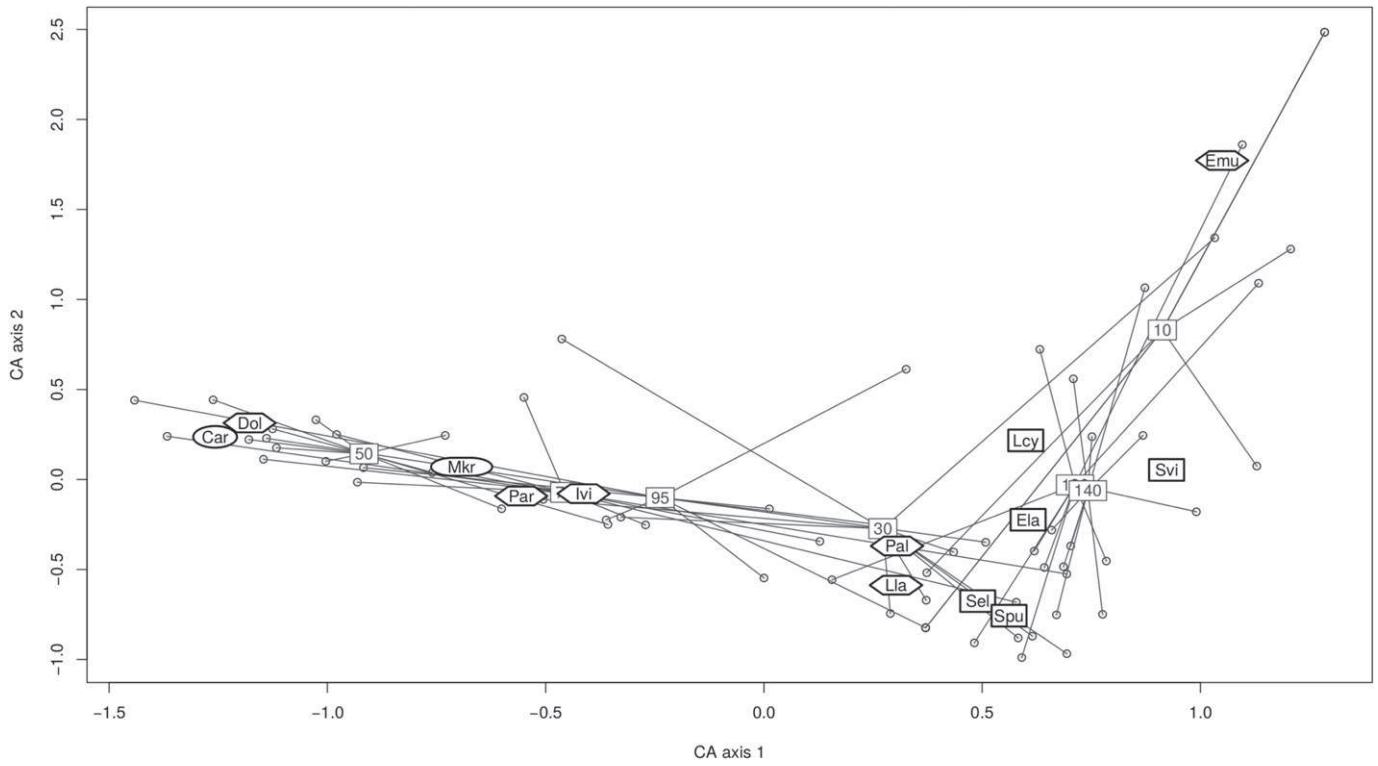

Fig. 5. CA joint plot (scaling type 1) of densities for grinder springtail species. Distance among soil samples in reduced space approximates their chi-squared distance (in this case, similarity of springtail community composition), soil samples found near a springtail species centroid has a high contribution to that species (in this case, soil samples contain that species), and species of close centroids are found in similar soil samples. Distance to factory of soil samples as well as species reproduction traits (rectangle: standard; hexagon: standard-to-explosive; ellipse: explosive; rounded rectangle: parthenogenesis) are highlighted in the plot. The first CA axis (21.1% of the total inertia) discriminates reproduction traits from standard (Ela, Lcy, Sel, Spu, Svi; right side), standard-to-explosive (Dol, Emu, Ivi, Lla, Par, Pal; center), to explosive and parthenogenesis (Car, Mkr; left side).

(Fig. 5). The first two CA axes explained 19.0% and 15.3% of the total inertia, respectively, the joint plot of which is shown in Fig. S2. This plot shows that the first axis discriminates the species having 'suctorial' mouthparts called suckers (*Brachystomella parvula* and *Pseudachorutes palmiensis*: mostly found at 30 m) from grinders (remaining species). These two species present the most important headcounts with respect

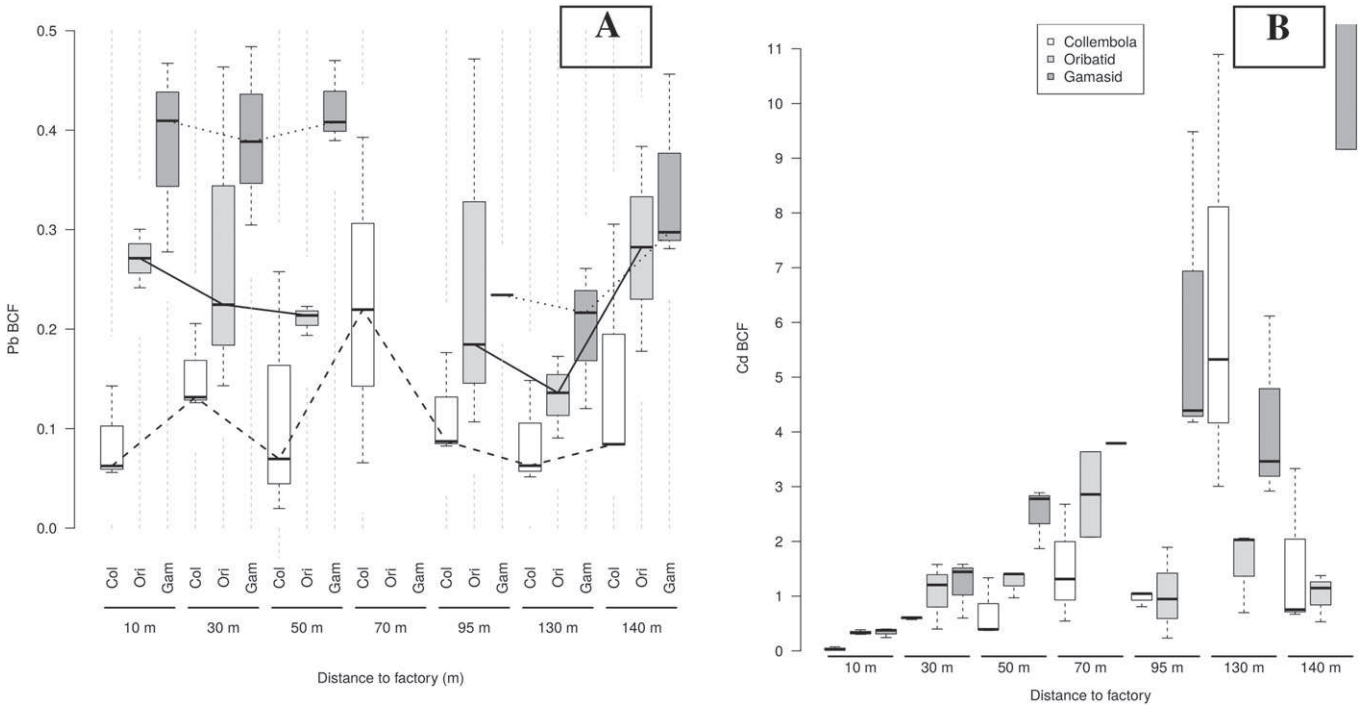

Fig. 6. Lead (A) and cadmium (B) bioaccumulation factors in microarthropods (Col: springtails; Gam: gamasid mites; Ori: oribatid mites) with respect to the distance to factory as box-and-whisker plots (median, upper and lower quartiles, and maximum values; 3 data samples per box). Outliers were discarded (see text). Medians are highlighted with lines (Springtail: dashed; Oribatid: solid; Gamasid: dotted).

to all species identified in the site (Table 3). These results seem to indicate the importance of diet in the tolerance of metal pollution and changes of physico-chemical parameters of soil, notably nitrogen, highlighted in differents works (Chahartaghi et al., 2005; Larsen et al., 2008; Jorgensen et al., 2008; Hoskins et al., 2015). Otherwise, the presence of juvenile springtails 30 m from the factory seems to indicate the existence of mycorrhizal fungi essential to the springtail reproduction (Jorgensen et al., 2008; Hedenec et al., 2013; Hoskins et al., 2015) and principal feeding resource of sucker species, *Brachystomella parvula* and *Pseudachorutes palmiensis*. A second CA on springtail density data highlighted the structure of the remainder of the springtail community by excluding sucker species (*B. parvula* and *P. palmiensis*) as well as rare species (*Folsomia sensibilis*, *Orogastrura parva*, and *Sminthurinus niger*; See Table 3 for a definition of rare species). The first two axes explained 21.1% and 16.1% of the total inertia, respectively, and a joint plot is shown in Fig. 5. The first CA axis discriminates reproduction traits from standard (*E. lanuginosa*, *Lepidocyrtus cyaneus*, *P. palmiensis*, *Sminthurinus elegans*, *Sphaeridia pumilis*, *Stenacidia violacea*) to standard-to-explosive (*Desoria olivacea*, *Isotoma viridis*, *L. lanuginosus*, *Protaphorura armata*, *Pseudosinella alba*), explosive and parthenogenesis (*Ceratophysella armata*, *M. krausbaueri*). The ordination results are similar to those found above (Fig. S2), that is rare species as well as sucker species did not affect the ordination of more abundant, grinder species. All species with standard reproductive traits were found in similar samples (positive value for CA Axis 1). And, with the exception of *E. multifasciata*, all species with non-standard reproductive traits were also found in similar samples (negative value for CA Axis 1). These results show that reproduction strategies of springtails play a role in the springtail community distribution. Moreover, the very low presence of asexual reproduction, only one species *M. krausbaueri*, highlights the importance of sexual reproduction in the polluted environment and confirms the results of previous works (Santorufo et al., 2014a, 2014b; Salmon et al., 2014). Indeed, the asexual reproduction is a short term response in contrast to sexual reproduction which favours polymorphism and is thus an advantage to colonise an ecosystem disrupted like the polluted soils. This highlights the concept of balanced dynamics of ecosystems. Finally, the low ratio of juvenile/adult in very contaminated areas, up to 30 m of the factory, demonstrates the impact of soil toxicity on the reproductive capacity of springtails (Xu et al., 2009). Therefore, this study allowed for significant progress in understanding the diversity of environmental responses.

4.2. Metal bioaccumulation across microarthropod groups

We found a low level of lead bioaccumulation in microarthropods. Similar results were observed in *Folsomia candida* exposed to various polymetallic contaminated soils (Santorufo et al., 2012). Previous studies (Nursita et al., 2009; Ardestani et al., 2014) showed that the Pb and Cd concentrations in springtails reflect the soluble and easily exchangeable soil fraction. Moreover, the metal fraction bound to the soil organic matter, presenting an important percentage in the study soil, can affect the non-essential metal uptake rate by soil microarthropods (Nursita et al., 2009; Bur et al., 2012; Ardestani et al., 2014). In the studied polluted soil, high soil pH would decrease the solid-solution transfer of Cd and Pb and reduce their bioavailability as observed in the work carried out by Bur et al. (2012).

The Pb absorption rate of oribatid mites is significantly higher than for springtails. In addition, observing low variations in BCF in oribatids suggests a capacity to regulate Pb absorption. Skubała and Zaleski (2012) described this type of regulation for other toxic metals such as Cd. However, these results show a high bioaccumulation capacity of Cd for oribatid and gamasid mites with BCF superior to 1 for the distance to factory more than 30 m. Springtails seem to control the Pb and Cd absorption with BCF always less than 1 except at the distance of 130 m from the factory for Cd. It should be noted that springtails are able to regulate their metal body content by eliminating absorbed metals (Ardestani and Van Gestel, 2014). Regulation does not concern essential metals such as Cu which some oribatid species have the ability to bioaccumulate with BCF > 10 (Skubala and Kafel, 2004).

BCF comparison across the three groups of microarthropods showed that gamasid mites have the highest capacity for Pb and Cd bioaccumulation. One possible explanation is that gamasid mites are predators (Calugar, 2013) and so they are at a higher trophic level than detritivorous oribatid mites.

5. Conclusions and perspectives

Metal(loid) pollution had several impacts on microarthropod communities. Oribatid mite abundance increased while springtail richness and abundance decreased with increasing soil pollution. The abundance of gamasid mite communities, which were relatively stable with metal soil concentrations, suggested that there was an ecological equilibrium between populations, and certainly an ecological niche phenomenon.

Pb and Cd bioaccumulation depends on the total metal soil concentration and microarthropod characteristics such as maturity and species. In this study the influence of trophic level on Pb and Cd accumulation was also highlighted. For Pb, bioaccumulation factors were low (BF < 1) in highly polluted soils certainly due to exclusion/avoidance mechanisms and to low Pb bioavailability in soil.

Three springtail species, *E. multifasciata*, *L. lanuginosus* and *P. alba*, were always present regardless of the soil contamination level. These species may therefore be interesting "tools" as biotests to characterize soil ecotoxicity. This approach leads to a better understanding of the adaptation of springtail populations to metal soil pollution with a majority of epigeic species presenting 'standard' and 'standard-to-explosive' reproduction. In this way, our study demonstrated the interest of characterising the environmental impact of metal(loid) pollution in the field at the community scale. Examining both ecological and ecotoxicological changes in the entire microarthropod community is a global approach for environmental impact assessment of soil pollution.

Acknowledgements

The authors thank Leigh Gebbie for English review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2016.02.011.

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