Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils

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Abstract

The concentrations of Zn, Cd, Pb and Cu in earthworm tissues were compared with the total and DTPA-extractable contents of these heavy metals in contaminated soils. Samples were taken from a pasture polluted by waste from a metallurgic industry over 70 y ago. Three individuals of *Aporrectodea caliginosa* and *Lumbricus rubellus* and soil samples were collected at six points along a gradient of increasing pollution. Total metal contents of earthworms, soil, and metals extracted by DTPA from the soil were measured. Total heavy metal contents of the soils ranged from 165.7 to 1231.7 mg Zn kg^{-1}, 2.7 to 5.2 mg Cd kg^{-1}, 45.8 to 465.5 mg Pb kg^{-1} and 30.0 to 107.5 mg Cu kg^{-1}. Their correlations with metals extracted by DTPA were highly significant. Contents of the metals in earthworm tissues were higher in *A. caliginosa* than in *L. rubellus*, with values ranging from 556 to 3381 mg Zn kg^{-1}, 11.6 to 102.9 mg Cd kg^{-1}, 1.9 to 182.8 mg Pb kg^{-1} and 17.9 to 35.9 mg Cu kg^{-1} in *A. caliginosa*, and from 667.9 to 2645 mg Zn kg^{-1}, 7.7 to 26.3 mg Cd kg^{-1}, 0.5 to 37.9 mg Pb kg^{-1} and 16.0 to 37.6 mg Cu kg^{-1} in *L. rubellus*, respectively. Correlations between body loads in earthworms with either total or DTPA-extractable contents of soil metals were significant, except for Cd in *L. rubellus* and Cu in *A. caliginosa*. Considering its simple analytical procedure, DTPA-extractable fraction may be preferable to total metal content as a predictor of bio-concentrations of heavy metals in earthworms. Biota-to-Soil Accumulation Factor (BSAF) of these four metals are Cd > Zn > Cu > Pb, with range of mean values between: Cd (6.18–17.02), Zn (1.95–7.91), Cu (0.27–0.89) and Pb (0.08–0.38) in *A. caliginosa*, and Cd (3.64–6.34), Zn (1.5–6.35), Cu (0.29–0.87) and Pb (0.04–0.13) in *L. rubellus*. The BSAF of Ca, Fe and Mn are Ca > Mn > Fe, with mean values of: Ca (0.46–1.31), Mn (0.041–0.111), Fe (0.017–0.07) in *A. caliginosa* and Ca (0.98–2.13), Mn (0.14–0.23), Fe (0.019–0.048) in *L. rubellus*, respectively. Results of principal component analysis showed that the two earthworm species differ in the pattern of metal bioaccumulation which is related to their ecological roles in contaminated soils.
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1. Introduction

Earthworms constitute a major component in soil functioning, and they play an important role in chemical element transformations (Lee, 1985). They utilise a significant amount of soil organic matter for feeding, produce huge amounts of biogenic structures, and determine the activities of micro-organisms and other smaller invertebrates included in their 'functional domains' defined as the sum of biogenic structures that they have created in soil and the organisms that inhabit them (Lavelle, 1997).

Contamination by heavy metal can change the functioning of soil ecosystems qualitatively and quantitatively by disturbing the activities of soil fauna (Cortet et al., 1999) and can lead to contamination of the terrestrial food chain by, for example, the transfer of heavy metals to predators of the soil fauna (Abdul Rida and Bouché, 1994). The concentrations of heavy metals in soils, based on the determination of the total metal contents of soil samples, are

widely used in many countries as a criterion for soil pollution (Sheppard et al., 1992). However, this does not provide precise information on the ability of the elements to be absorbed by plants, and it does not predict the transfer of toxic elements in the food chain (Morel, 1997). A range of empirically derived extraction procedures has been developed to simulate the availability of essential elements, and some toxic elements, such as Cd, Pb, Cr, Ni to plants. Among them, the diethylenetriaminepentaacetic acid (DTPA) test is widely used as a method for multi-element extraction of micronutrients (Sims, 2000) and has been used for assessing the bioavailability of non-essential trace metals (Lebourg et al., 1996).

The influence of heavy metals in soils on earthworms and their bioaccumulation has been the subject of many studies for a long time (e.g. Bouché, 1984; Morgan and Morgan, 1999; Kennette et al., 2002). Van Hook (1974) claimed that earthworms could serve as useful biological indicators of contamination because of the fairly consistent relationships between the concentrations of certain contaminants in earthworms and soils. Metal bioavailability to earthworms can be evaluated both in terms of relative toxicity and through bioaccumulation determinations, yielding a Biota-to-Soil Accumulation Factor (BSAF) (Cortet et al., 1999). Our aims were to study the relationships between contents of some heavy metal (Zn, Cd, Pb and Cu) in earthworms with the total and DTPA-extractable contents of these heavy metals in soils; and also to compare the characteristics of bioaccumulation of the heavy metals in two ecologically different species of earthworms: the soil feeding endogeic *Aporrectodea caliginosa* and the mostly litter feeding epigeic *Lumbricus rubellus* (Bouché, 1972).

2. Material and methods

2.1. Site description

The site studied is located in Nord-Pas-de-Calais, northern France. In this area, there are two of the most important Pb and Zn smelters in Europe (Sterckeman et al., 2000; Buatier et al., 2001). The soils have been contaminated by wastes and dust emissions from the metallurgical industry since the middle of the 19th century. The emission of dust in the atmosphere leads to diffuse pollution of soils surrounding the factories. This type of pollution decreased after 1975 due to a change in the extraction process in the Zn smelter and to the use of a filtration system in the Pb smelter (Denaix et al., 2001). Although the environmental effects of the smelters are relatively limited today, the fields surrounding the factories are highly contaminated. Very high metal pollution results from the deposition of such waste in the vicinity of the industrial sites or by the use of this waste as materials of country road construction in these rural areas (Thiry and van Oort, 1999).

The study site is a field of about 0.5 ha, currently used as pasture, contaminated by Zn, Cd, Pb and Cu. It is partly contaminated by diffuse pollution. However, the high levels of pollution are related to the presence of an ancient country road that was constructed 70 y ago to connect up to a World War II bunker using wastes from the metallurgic industry. Thus, the amounts of pollution decrease sharply with the distance from the road (Nahmani and Lavelle, 2002).

2.2. Sample collection

Soil samples and earthworms were taken in May 2001 from a pasture polluted by metallic wastes at six points along a gradient of pollution. These were numbered from 1 to 6, the first being the closest to (2.5 m), and the sixth furthest from (40 m) the contamination source. The sample points were about 4 m^2 (2 × 2 m) each. Three soil samples of the surface horizons (0–20 cm) were collected at each point. The main characteristics of the soil samples are presented in Table 1. The clay content of the soils varied from 11.8 to 27%. They were weakly acidic with pH ranging from 5.8 to 6.4. The total organic C, and N and the C-to-N ratio varied considerably from 1.86 to 9.39%, 0.12 to 0.65% and 13.50 to 16.65, respectively.

Three mature individuals (clitellates) of *A. caliginosa*, and *L. rubellus*, the most abundant species at this site, were manually collected at each point. They belong to two ecological categories, epigeic for *L. rubellus* and endogeic for *A. caliginosa* (Bouché, 1972). The earthworms were placed immediately into Petri dishes with a small amount of soil (one animal per dish). In the laboratory, they were thoroughly rinsed with distilled water as soon as possible and placed in Petri dishes with one Whatman No. 1 filter paper and a few drops of distilled water to maintain them

Table 1
Main physio-chemical characteristics of the soil samples (mean ± standard deviation, $n = 3$)

Sampling site	Distance to pollution origin (m)	Organic C (g kg^{-1})	Total N (g kg^{-1})	C-to-N ratio	Clay (g kg^{-1})	pH (H$_2$O)
1	2.5	46.2 ± 1.7	3.3 ± 0.2	13.9 ± 0.6	185 ± 45	5.8 ± 0.1
2	5.0	72.3 ± 9.6	5.4 ± 0.9	13.5 ± 0.7	270 ± 23	5.8 ± 0.3
3	7.5	93.9 ± 13.0	6.5 ± 1.2	14.4 ± 0.8	200 ± 37	6.2 ± 0.0
4	30	25.6 ± 2.6	1.6 ± 0.1	16.3 ± 0.7	156 ± 75	6.1 ± 0.1
5	35	18.6 ± 1.3	1.2 ± 0.1	15.2 ± 0.3	132 ± 40	6.3 ± 0.1
6	40	20.0 ± 1.5	1.2 ± 0.1	16.7 ± 0.8	118 ± 9	6.4 ± 0.2

moist. They were kept at 14 °C for 7 d and the filter paper was changed daily to allow complete evacuation of the gut contents. The earthworms were killed by freezing and then oven dried (48 h at 70 °C) to constant weight. Their dry weight was measured with a Mettler AE200 balance after cooling the samples in a desiccator. The dry weights of the earthworms ranged from 108 to 171 mg for *A. caliginosa* and from 103 to 174 mg for *L. rubellus*.

2.3. Analysis of earthworms

The dry earthworms were crushed (<0.2 mm), then dissolved as follows: 100 mg of sample was mixed with 4 ml conc. $HNO_3 + 2$ ml conc. HCl for 12 h, heated progressively to 150 °C at 800 kPa in a closed Teflon bottle in a micro-wave oven for 2 h. After cooling at ambient temperature, the solution was made up to 50 ml with ultra-pure distilled water. The metal contents were determined by inductively coupled plasma-atomic emission spectroscopy (ICP AES) (Jobin-Yvon 238).

2.4. Analysis of soils

The soil samples were air-dried at ambient temperature, and sieved (<2 mm). For the total metal contents, the samples were crushed to 0.2 mm. A wet mineralisation of the sample was performed by mixing 100 mg of the crushed soil with 4 ml conc. HNO_3 for 12 h at ambient temperature. The mixture was then heated progressively for 3 h in a micro-wave oven at 150 °C at 800 kPa in closed Teflon bottles. After cooling, 2 ml of conc. HCl were added and the heating process was repeated for 3 h. After cooling, the solution was made up to 50 ml with ultra-pure distilled water. The metal contents were determined by ICP AES (Jobin-Yvon 238). The diethylenetriaminepentaacetic acid (DTPA) extraction of the metals was made by shaking 5.0 g of soil with 25 ml of a solution of 5 mM DTPA and 10 mM $CaCl_2$ in a end-over-end shaker for 1 h (Lindsay and Norvell, 1978). Then, the suspension was centrifuged for 30 min at 5100g, and filtered on Whatman No. 1 filter paper. The metal concentrations were determined as described above. The total organic C and

N of the samples were measured by dry combustion with a CHN 1108 Carlo Erba chromatograph. The pH was determined in water suspension (1:2.5 (w:v) soil:solution ratio) by an HANNA HL8424 pH meter. The particle size was measured by sieving in water for the fractions >50 μm after elimination of the organic matter by H_2O_2, and with a Sedigraph for the fractions <50 μm.

2.5. Biota-to-soil accumulation factor

The BSAF of the metals in the earthworms were calculated by the following formula: BSAF = metal content for in earthworm/total metal content in soil (Cortet et al., 1999), Zn, Cd, Pb, Cu, Ca, Fe and Mn measured in the tissues of earthworms and in the soils.

2.6. Statistics and data analysis

Statistical analyses were made with StatView 4.0 Software (Abacus Concepts, Inc.). Linear regressions were used to test correlation between variables. The effects of the sampling site on the total and DTPA-extractable metal contents of the soil and the BSAF of the metals in the two earthworm species were evaluated by analysis of variance (ANOVA). Means were compared using the Mann-Whitney test. Principal Component Analysis (PCA) was performed with ADE-4 Software (Thioulouse et al., 1997) to compare accumulation of metals and Ca by the two earthworm species. A permutation test was used to test the significance of the groupings suggested by PCA. Values were considered as different when probability to reject hypothesis nil was lower than 0.05.

3. Results

3.1. Total and DTPA-extractable metal contents of the soils

The mean total heavy metal contents of the soil samples are shown in Table 2 and these decreased in amounts with distance from sample points 1 to 6. Samples 1–3 also contained more clay and organic matter than samples 4–6 (Table 1).

Table 2
Mean total and DTPA-extractable metal contents of the soil samples (mg kg^{-1}) collected along a gradient of increasing contamination, and results of ANOVA tests applied on these data

Sampling site	Zn-total	Zn-DTPA	Cd-total	Cd-DTPA	Pb-total	Pb-DTPA	Cu-total	Cu-DTPA
1	1231[a]	455[a]	4.5[a]	1.4[a]	466[a]	150[a]	108[a]	23.2[a]
2	1198[a]	400[a]	5.2[a]	1.6[a,b]	453[a]	169[a]	88.8[a,b]	15.4[a,b]
3	1030[a]	396[a]	5.0[a]	1.9[b]	335[a]	135[a]	74.5[b]	10.9[b,c]
4	271[b]	93.3[b]	3.2[b]	0.6[c]	135[b]	25.8[b]	38.5[c]	3.9[c]
5	176[b]	54.5[b]	3.0[b]	0.4[c]	52.2[b]	10.8[b]	30.0[c]	2.7[c]
6	166[b]	40.8[b]	2.7[b]	0.4[c]	45.8[b]	9.3[b]	36.2[c]	2.4[c]
F	10.23	35.84	21.75	39.22	11.05	27.63	9.72	8.73
P	0.0005	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	0.0007	0.001

The different letters behind the figures relate to the different levels of the PLSD of Fisher comparisons.

Likewise, the amount of metal extracted by DTPA also decreased from sampling point 1 to 6 (Table 2). The correlations between total and DTPA-extractable contents of Zn, Cd, Pb and Cu have a coefficient of determination (r^2) ranging between 0.86 and 0.94 with corresponding $P < 0.0001$ in all cases. The proportion of metal contained in the DTPA-extract was greater for Cd (49%) than for Zn and Pb (33–34%) and Cu (22%).

3.2. Heavy metal contents of the earthworms in relation to metal contents of the soils

The results of Mann-Whitney tests (Fig. 1) show that Cd and Pb differed significantly between the species in most
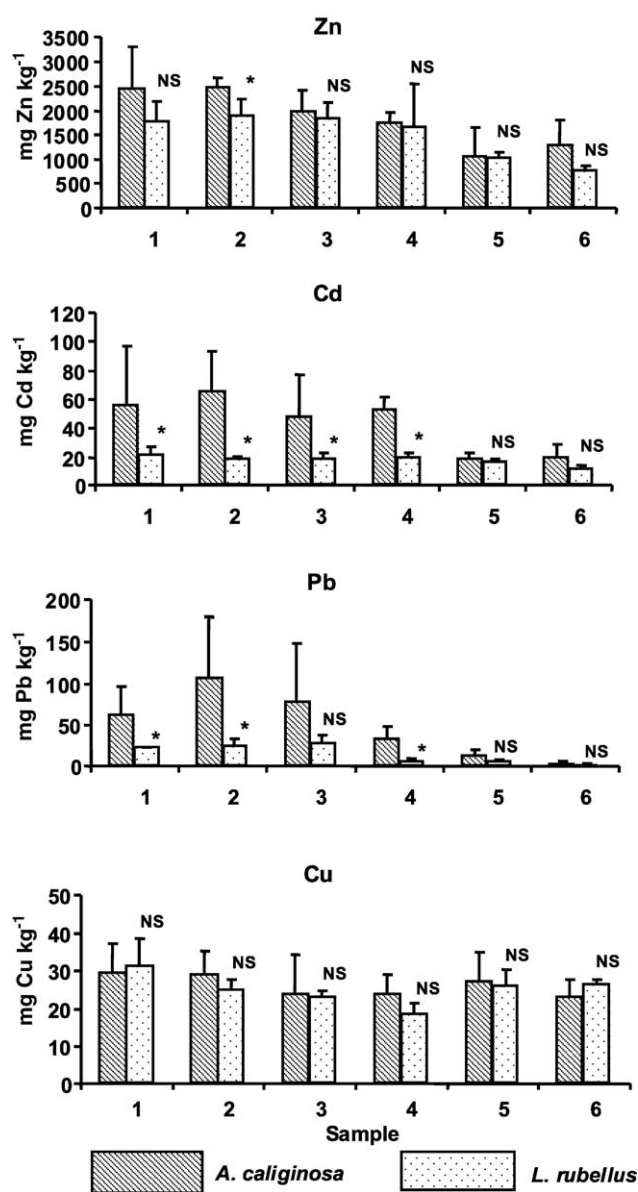

Fig. 1. Comparaison of the bioconcentrations of heavy metals (mg kg^{-1}) in two earthworm species (A. caliginosa and L. rubellus) (* indicates significant differences between species of Mann-Whitney test, NS indicates: non-significant).

Table 3
Correlations between bioconcentration of the heavy metals in A. caliginosa and L. rubellus earthworm tissues and total or DTPA-extractable metals contents in the soil samples

	A. caliginosa		L. rubellus	
	r	P	r	P
Zn-total	0.74	0.0004	0.59	0.01
Zn-DTPA	0.76	0.0003	0.62	0.006
Cd-total	0.49	0.04	0.36	0.15
Cd-DTPA	0.48	0.05	0.35	0.16
Pb-total	0.47	0.05	0.62	0.007
Pb-DTPA	0.57	0.01	0.73	0.0005
Cu-total	0.42	0.08	0.44	0.07
Cu-DTPA	0.42	0.09	0.54	0.02

cases, especially in the most heavily contaminated soils. The differences in Zn content between species were not significant in any case, except at point 2 (2485 mg Zn kg^{-1} for A. caliginosa and 1890 mg Zn kg^{-1} for L. rubellus), although the Zn contents were higher in A. caliginosa than in L. rubellus in all the samples. There were no difference in Cu contents in any of the samples for either earthworm species.

The concentrations of heavy metals in earthworms showed significant correlations with both the total and DTPA-extractable contents of soil metals with exceptions for Cd in L. rubellus and Cu in A. caliginosa (Table 3).

3.3. Biota-to-Soil Accumulation Factors of the earthworms

The BSAFs of the four heavy metals were ranked as Cd > Zn > Cu > Pb. The BSAF of Cu and Pb were less than 1 in all cases. As regards Ca, Fe and Mn, the BSAF were Ca > Mn > Fe (Table 4). The results of ANOVA (Table 4) indicated that the BSAF for Zn, Cu, Ca and Fe of the two species were significantly higher for samples 4, 5 and 6 than for samples 1, 2 and 3. However, there were no significant difference for the BSAF of Cd, Pb and Mn of the two species between the sampling points.

The PCA was performed with the BSAF of the metals (Zn, Cd, Pb, Cu, Ca, Fe and Mn) for the two species of earthworm to analyse the relationships of these indices. The results (Fig. 2a and b) showed that the first principal component was positively determined by the BSAF of Pb and Cd and negatively determined by the BSAF of Ca, Mn, Fe, Cu and Zn, respectively, and its relative inertia was 42.7%. The second principal component was positively determined by the BSAF of Mn and Ca and negatively determined by the BSAF of Cd, Pb and Zn, respectively, and its relative inertia was 29.3%. The two species of earthworms were clearly distinguished by their BSAF with different metals (Fig. 2b): A. caliginosa had higher BSAFs for Cd, Pb and Zn than L. rubellus. By contrast, their BSAFs for Ca and Mn were considerably lower than those of

Table 4
Biota-to-Soil Accumulation Factors (BSAF) of the metals in *A. caliginosa* and *L. rubellus* earthworm species (mean ± standard deviation, $n = 3$) and results of the ANOVA tests on BSAF responses of earthworms exposed to increasing metal contamination

| Species	Sampling site	Zn	Cd	Pb	Cu	Ca	Fe	Mn
A.caliginosa	1	1.95 ± 0.25	12.08 ± 7.36	0.13 ± 0.04	0.27 ± 0.03	0.83 ± 0.03	0.02 ± 0.01	0.08 ± 0.01
	2	2.46 ± 1.22	13.45 ± 7.17	0.31 ± 0.29	0.34 ± 0.10	0.76 ± 0.08	0.02 ± 0.01	0.07 ± 0.03
	3	1.95 ± 0.51	9.51 ± 5.91	0.24 ± 0.22	0.31 ± 0.05	0.46 ± 0.09	0.02 ± 0.00	0.04 ± 0.02
	4	6.60 ± 0.94	17.02 ± 4.08	0.38 ± 0.32	0.63 ± 0.18	1.22 ± 0.21	0.04 ± 0.01	0.11 ± 0.02
	5	6.31 ± 3.58	6.18 ± 1.41	0.22 ± 0.14	0.89 ± 0.18	1.31 ± 0.11	0.07 ± 0.03	0.09 ± 0.02
	6	7.91 ± 2.29	7.75 ± 2.72	0.08 ± 0.05	0.73 ± 0.36	1.07 ± 0.19	0.05 ± 0.00	0.07 ± 0.01
ANOVA	F	9.85	1.89	1.07	6.79	23.09	8.58	4.97
	P	0.0006	0.2	0.4	0.003	<0.0001	0.001	0.011
L. rubellus	1	1.50 ± 0.54	5.11 ± 1.40	0.04 ± 0.03	0.29 ± 0.04	1.74 ± 0.06	0.02 ± 0.00	0.17 ± 0.01
	2	1.82 ± 0.71	3.64 ± 0.91	0.06 ± 0.02	0.30 ± 0.09	1.35 ± 0.24	0.02 ± 0.01	0.20 ± 0.03
	3	1.80 ± 0.39	3.68 ± 0.99	0.09 ± 0.03	0.33 ± 0.09	0.98 ± 0.12	0.02 ± 0.01	0.14 ± 0.01
	4	6.15 ± 2.69	6.34 ± 1.22	0.07 ± 0.06	0.49 ± 0.11	1.98 ± 0.72	0.03 ± 0.01	0.18 ± 0.05
	5	5.99 ± 0.64	5.56 ± 0.78	0.13 ± 0.03	0.87 ± 0.06	2.13 ± 0.09	0.04 ± 0.02	0.23 ± 0.07
	6	4.85 ± 1.44	4.12 ± 1.43	0.03 ± 0.02	0.81 ± 0.30	1.94 ± 0.16	0.05 ± 0.01	0.20 ± 0.03
ANOVA	F	10.91	2.36	2.58	9.57	7.12	6.82	2.09
	P	0.0004	0.1	0.08	0.0007	0.003	0.003	0.14
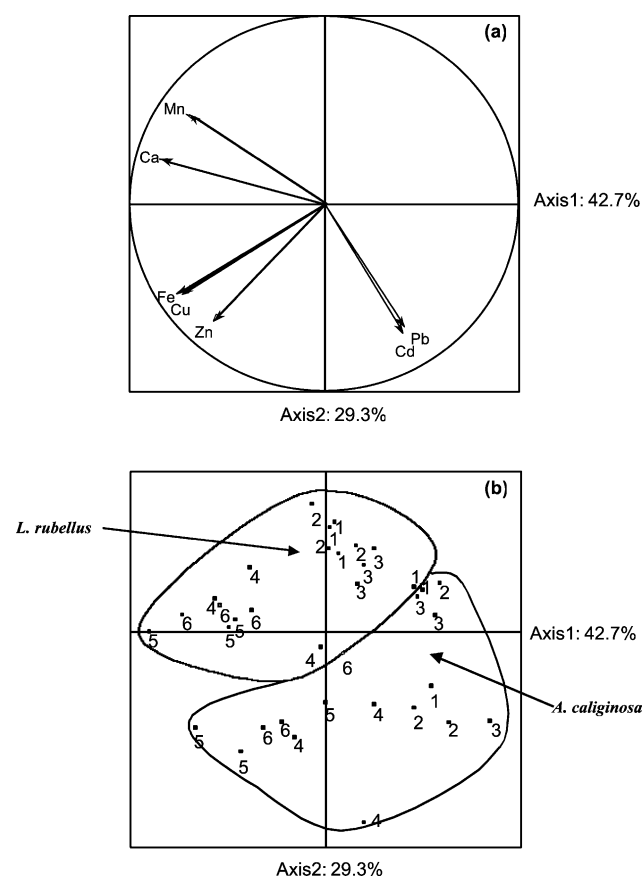
Fig. 2. Ordination of sites and earthworm species based on BASF values for Ca and metals by Principal Component Analysis: (a) correlations circle of the BASF of Zn, Cd, Pb, Cu, Ca, Fe and Mn in the tissues of the two earthworm species (*A. caliginosa* and *L. rubellus*) and (b) differentiation of the two species on the basis of their BSAF of Zn, Cd, Pb, Cu, Ca, Fe and Mn.

L. rubellus. The permutation test of PCA showed that the differences of BASF between species were significant ($P < 0.00001$).

4. Discussion

4.1. Bioavailability of heavy metals in soils

The bioavailability of heavy metals is related to their chemical forms in the soils. Several fractions or compartments of the soil act as reservoirs of available metals. The literature shows that tests based on determination of most available metal pools, while not perfect, give a better answer for evaluating toxicity of the soils than total metal concentrations (McLaughlin et al., 2000) and could be used as indices of the bioavailable fraction of metals in soils. DTPA extraction, which is a widely used soil testing method (Lebourg et al., 1996; Sims, 2000), provides an operationally defined soil-compartment that is characterised by its solubility. DTPA can release the soluble, exchangeable, adsorbed and organically bound metals, and possibly some of the metals fixed by oxides (Lindsay and Norvell, 1969). In some cases, the relationship between total and DTPA-extractable metal contents of the soils are poor (McLaughlin et al., 2000).

In our study, the total amounts of metals are of the same magnitude of those studied by Sterckeman et al. (2000) and Buatier et al. (2001), but lower than those of Denaix et al. (2001) in the same region. The good relationship between total and DTPA-extractable contents of metals indicate that the available metals should come from the same mineralogical sources and that pedological processes occurring in

the soil sampling points should be similar. Various studies have been made in this contaminated area on the metal bearing phases (Sobanska et al., 1999; Buatier et al., 2001) and on the behaviour of metals in the soils and in soil solutions (Sterckeman et al., 2000; Denaix et al., 2001). Whereas the major Pb and Zn bearing solid phase of the dust emissions are Pb and Zn sulfide and Pb sulfate and oxysulfate (Sobanska et al., 1999), the metal speciation in the contaminated soils is substantially different. Transmission electron microscopy associated with energy dispersive X-ray spectrometry investigations performed by Buatier et al. (2001) showed that the major phases retaining Zn were Fe-oxyhydroxides and smectites; Pb was mainly present as a pyromorphite-like mineral. Sterckeman et al. (2000) and Denaix et al. (2001) showed that high amounts of metals were present in soil solutions. Cambier et al. (1993) (cited by Sterckeman et al. (2000)) have shown that the organo-metallic forms represent 50% of Pb in solution in the surface horizon.

4.2. Heavy metals accumulation by earthworms

The total metal contents and the DTPA-extractable fractions of soils are positively and significantly related to the concentrations of metals in the earthworms. Similar trends for the ratios of the total metal contents in soils to earthworm contents have been observed by Morgan and Morgan (1993, 1999), Abdul Rida and Bouché (1997), and Kennette et al. (2002). The concentrations of Cu and Pb in the earthworm tissues were of the same order than those reported from the literature for other contaminated soils by Kennette et al. (2002). However, Zn concentrations of earthworms in the most polluted sites of our study are substantially higher than those recorded in other studies (Fig. 1). The fractions of metals extracted by DTPA presented similar correlations with the concentrations of the metals in earthworm tissues than those of the total metal content in soils. Considering its simple analytical procedure, our results suggest that the DTPA extraction method can be used as a predictor of bio-concentrations of heavy metals in earthworms rather than total content which involves a much more expensive analysis.

4.3. Factors affecting the patterns of metal accumulation between earthworm species

Earthworms are well known to be selective consumers (Edwards and Bohlen, 1996). The differences between species in metal bio-concentration partly reflect the differences in their food selectivity and niche separation (Morgan and Morgan, 1992). As a litter dwelling species, *L. rubellus* has generally lower concentrations of Cd, Pb and Zn than *A. caliginosa* (Morgan and Morgan, 1992, 1993, 1999), which are endogeic earthworms, feeding on soil variously contaminated with metal-bearing mineral phases. Similar differences have also been demonstrated between

endogeic and epigeic earthworms from uncontaminated soils (Beyer et al., 1987).

It is important to be able to identify the forms of metals in the soil, in order to better understand the dynamics of metals in agricultural and natural ecosystems (Alloway, 1990). Different factors interact to determine the amounts of pollutants accumulated by earthworms. The affinity of metals for soil constituents is the primary element to take into account. The distribution of metals among the soil phases is important for the bioaccumulation by earthworms as the main pathways for chemical absorption are the skin for soluble elements, gut transit and digestion (Weltje, 1998). Cadmium tends to be more mobile in soils and therefore, more available to plants than many other heavy metals, including Pb and Cu (Alloway, 1990). Morera et al. (2001) showed for various soils that the relative affinity of metals is consistent with the value of the first hydrolysis constant of the cations proposed by Basta and Tabatabai (1992): Cu $(pK = 7.7) = $ Pb $(pK = 7.7) > >$ Zn $(pK = 9.0) > $ Cd $(pK = 10.1)$. These data are also fairly consistent with the BSAF values obtained here that where: Pb $<$ Cu $\ll$ Zn $\ll$ Cd.

The solubility of metal-bearing minerals is also of great importance. As observed by Weltje (1998), the earthworm gut could modify the mobility of metals due to pH change and favour their assimilation. Lavelle et al. (1995) had observed that earthworm gut had neutralization effects on the digestive contents. The major metal-bearing phases in the soils surrounding Pb- and Zn-smelters are the pyromorphite for Pb, Fe-oxyhydroxydes and smectites for Zn (Buatier et al., 2001). The very low solubility of pyromorphite and the high concentrations of exchangeable Zn in Zn-smectites could explain the higher bioaccumulation of Zn over Pb in the earthworms.

Another important point about the bioaccumulation of metals in earthworms is their ability to eliminate the excess of metals. Spurgeon and Hopkin (1999) showed that for essential metals, such as Cu and Zn, a fast initial uptake was followed by equilibrium after a few days of exposure, highlighting a physiological control and a possible excretion of these elements. For the xenobiotic metals, such as Cd and Pb, the excretion was slow or absent. The same bioconcentrations of Cu in earthworms for all our sampling points, leading to the decrease of the BSAF for polluted soils, could be attributed to this mechanism. For Zn, even if the concentrations were high, the BSAF were also lower in the most contaminated soils. Excretion of a part of Zn could contribute to the regulation of the metal concentrations in earthworms.

Our results confirmed and extended the results of Beyer et al. (1987) and Morgan and Morgan (1999) that *A. caliginosa* has a higher BSAF of Cd, Pb and Zn and lower BSAF of Ca and Mn than *L. rubellus*, while there is no difference for BASF of Cu and Fe between the two species. The BSAF of the metals are useful indices to distinguish between the two ecologically different species.

5. Conclusions

Our study demonstrated firstly, the highly significant correlations between the total contents of Zn, Cd, Pb and Cu and their fractions extracted by DTPA in the metal contaminated soils under study. Subsequently, the results showed similar correlations between total and DTPA-extractable contents of the heavy metals in soils and the bioconcentrations of these metals in earthworms. This suggests that the DTPA extraction method can probably be used as a predictor of bioconcentrations of heavy metals in earthworms rather than total content which requires much more expensive analysis. Finally, *A. caliginosa* and *L. rubellus* presented different characteristics of bioaccumulation of the metals, showing that soil feeding earthworms (endogeic) which ingest large amounts of soil may be much more affected by metallic pollution than epigecis which ingest large amounts of leaf litter.

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