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Fate of airborne metal pollution in soils as related to agricultural management: 2. Assessing the role of biological activity in micro-scale Zn and Pb distributions in A, B and C horizons

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Summary

This work assesses relationships between characteristic aggregate microstructures related to biological activity in soils under different long-term land use and the distribution and extractability of metal pollutants. We selected two neighbouring soils contaminated with comparable metal loads by past atmospheric deposition. Currently, these soils contain similar stocks, but different distributions of zinc (Zn) and lead (Pb) concentrations with depth. One century of continuous land use as permanent pasture (PP) and conventional arable (CA) land, has led to the development of two soils with different macro- and micro-morphological characteristics. We studied distributions of organic matter, characteristic micro-structures and earthworm-worked soil by optical microscopy in thin sections from A, B and C horizons. Concentrations and amounts of total and EDTA-extractable Zn and Pb were determined on bulk samples from soil horizons and on size-fractions obtained by physical fractionation in water. Large amounts of Zn and Pb were found in 2–20- μ m fractions, ascribed to stable organo-mineral micro-aggregates influenced by root and microbial activity, present in both soils. Unimodal distribution patterns of Zn, Pb and organic C in size-fractions were found in horizons of the CA soil. In contrast, bimodal patterns were observed in the PP soil, because large amounts of Zn and Pb were also demonstrated in stable larger micro-aggregates (50–100- μ m fractions). Such differing distribution patterns characterized all those horizons markedly influenced by earthworm activity. Larger earthworm activity coincided with larger metal EDTA-extractability, particularly of Pb. Hence, land use-related biological activity leads to specific soil microstructures affecting metal distribution and extractability, both in surface and subsurface horizons.

Introduction

In metal-polluted soils, the incorporation and distribution dynamics of heavy metals in the soil profile are closely related to environmental risks. In particular, mobility and bioavailability of heavy metals, and exposure of soil living organisms to these toxic compounds, depend to a large extent on their location in the soil matrix and notably on the nature of chemical association with organic and mineral constituents (Peijnenburg *et al.*, 2007; Degryse *et al.*,

2009). Soil characteristics and land use are known to influence the biogeochemical cycling of metals (Adriano, 2001) and hence the distribution of metal pollutants in soils. However, relationships between land use and the fate of metal pollutants in soils are poorly documented. In particular, detailed investigations in real field situations regarding fine-scale distribution patterns of metal pollutants in relation to long-term agricultural land management are scarce.

In a previous paper, we studied distributions of metal pollutants in horizons of sandy-textured paired soils at a location with comparable site conditions, but under different long-term agricultural land use that is permanent grassland and conventional arable land (Fernandez *et al.*, 2007). We showed how the same airborne zinc (Zn) and lead (Pb) load was variably incorporated

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in relation to soil depth. Such variable metal incorporation patterns were mainly ascribed to differences in bioturbation resulting from different long-term agricultural land use. For the same soil site, a significantly larger earthworm activity in the grassland soil in comparison with the soil under arable land was reported by Nahmani *et al.* (2003). From these studies, performed at the scale of soil horizons, we hypothesized that different biological activities induced by such continuous long-term different land uses, leads to a different metal speciation at a micro-scale. The present study was therefore conducted to study relations between diverging metal incorporation in the soil profile and micro-scale distribution patterns of metals in soil micro-aggregates.

The aims of this work were to (i) quantify characteristic aggregate micro-structures related to biological activity in surface and subsurface horizons of soils under different agricultural management and (ii) assess relations between soil aggregation, metal distribution and extractability. For this, we studied characteristic soil micro-structures in thin sections from A, B and C horizons and we quantified the groundmass 'worked' by earthworms. Physical fractionation of bulk samples in water was performed to obtain size fractions including mineral, organic and water-stable organomineral (micro)-aggregates. Total organic carbon contents and concentrations of Zn and Pb were determined on all size-fractions. The extractability of Zn and Pb in soil horizons and selected size-fractions of the surface horizons was assessed using ethylenediaminetetraacetic acid (EDTA), a commonly used extractant.

Materials and methods

Site conditions

The study area is located in the vicinity of an old, major metallurgical plant in northern France, which has led to large atmospheric metal deposition on surrounding agricultural land

(van Oort *et al.*, 2001, 2009). On the basis of a detailed soil survey, two soils were selected, at a comparable distance (approximately 3 km) and in a windward location with respect to the former industrial site, but managed for one century under different agricultural land uses. The selected soils were characteristic of those used for agriculture land on aeolian cover sand deposits in northern France. Soil formation and characteristics were extensively reported by Fernandez *et al.* (2007). Some essential data, to provide background to the present work, are given below.

Soil characteristics

The two soils were located approximately 300 m apart, developed under similar topographical and drainage conditions and presumably under comparable agricultural management at the end of the 19th century. From the beginning of the 20th century, coinciding with the start of the metal industry in this area, the soils were continuously managed under different land uses, one permanent pasture (PP), unploughed since 1900, and the other conventional arable (CA) land. Such long-term management has led to the development of two soils with distinctive morphological characteristics: an A11, A12, A(B), AC, C horizon sequence for the PP soil, and an Ap, (B), C sequence for the CA soil (Table 1). Both soils showed close similarities in particle size distribution, clay mineralogy, iron content and cation exchange properties (Fernandez *et al.*, 2007), illustrating their common pedological background. The organic matter (OM) content in the PP soil, however, showed a more progressive decrease with depth than in the CA soil, where it was mainly restricted to the Ap horizon (Table 2). This OM distribution was also demonstrated with a general darker soil colour of horizons at an equivalent soil depth in the PP soil compared with the CA soil (Table 1). In the PP soil, the bulk density was markedly less and the root abundance (Table 1) greater than in the CA soil. The pH was less acidic in the upper horizons of

Table 1 Root abundance and soil colour of horizons of the soils under permanent pasture (PP) and conventional arable (CA) land

Horizon	Depth / cm	Roots ^a	Soil colour ^b	Org. C / Zn _{Tot} × 10 ⁻³	Org. C / Pb _{Tot}
PP soil					
A11	0–6	Many	10 YR 4/2	0.28	0.49
A12 ^c	6–26	Common	10 YR 4/3	0.18	0.26
A(B) ^c	26–53	Few	10 YR 5/2	0.08	0.25
AC(g) ^c	53–76	Few	10 YR 6/2	0.02	0.17
Cg2	>76	Very few	5 YR 6/2	0.05	0.50
CA soil					
Ap ^c	0–26	Common to few	10 YR 4/4	0.09	0.15
(B)1 ^c	26–44	Very few	10 YR 4/6	0.04	0.19
(B)2	44–72	Very few	10 YR 5/6	0.05	0.16
Cg1 ^c	72–87	None	10 YR 6/4	0.05	0.12
Cg2	>87	None	7.5 YR 5/8	0.05	0.16

^aAbundance classes after FAO (2006).

^bMunsell Soil Colour Charts.

^cSelected horizons for detailed study on size-fractions.

Organic C-to-Zn and C-to-Pb ratios in horizons were calculated from Fernandez *et al.* (2007) (see Table 2).

Table 2 Total organic carbon contents, total and EDTA-extractable concentrations of Zn and Pb

Horizon	Organic C _{Tot}		Zn _{Tot}		Zn _{EDTA}		Zn _{EDTA} /Tot / %	Pb _{Tot}		Pb _{EDTA}		Pb _{EDTA} /Tot / %
	Bulk ^a	∑ fr.	Bulk ^a	∑ fr.	Bulk	∑ fr. ^b		Bulk ^a	∑ fr.	Bulk	∑ fr. ^b	
	— / g kg ⁻¹ —		/ mg kg ⁻¹		/ mg kg ⁻¹			/ mg kg ⁻¹		/ mg kg ⁻¹		
PP soil												
A11	22.31		79.3 ± 3.8		36.3 ± 2.5		46	45.7 ± 1.5		32.2 ± 1.8		70
A12 ^c	14.28	14.30	81.1 ± 1.2	74.6	29.3 ± 0.7	30.9	36	54.9 ± 4.0	49.3	35.1 ± 0.8	35.8	62
A(B) ^c	5.54	4.45	54.8 ± 3.0	53.5	26.6 ± 0.6	nd	40	17.7 ± 2.1	15.6	7.8 ± 0.8	nd	43
AC(g) ^c	1.40	1.41	57.5 ± 4.1	54.6	26.5 ± 2.6	nd	46	8.2 ± 1.2	8.9	3.0 ± 0.2	nd	37
Cg2	2.20		42.0 ± 1.2		7.9 ± 0.1		19	4.4 ± 0.8		1.1 ± 0.1		25
CA soil												
Ap ^c	9.45	9.51	108.7 ± 1.8	105.8	45.9 ± 0.6	43.3	42	61.5 ± 2.4	59.5	30.2 ± 0.1	33.5	49
(B)1 ^c	2.60	2.63	60.4 ± 3.0	59.6	29.2 ± 1.4	nd	48	13.7 ± 0.9	13.6	4.1 ± 0.3	nd	30
(B)2	1.70		36.0 ± 1.9		11.9 ± 0.1		33	10.6 ± 0.8		3.0 ± 0.1		31
Cg1 ^c	1.18	1.22	26.5 ± 1.7	27.0	6.5 ± 0.2	nd	24	9.7 ± 1.3	9.9	2.5 ± 0.2	nd	25
Cg2	1.60		33.0 ± 3.1		0.8 ± 0.01		2	10.1 ± 0.7		0.9 ± 0.01		9

^aAfter Fernandez *et al.* (2007)^bBecause of practical problems regarding the workability of these fractionation/extractability studies, only surface samples A12 and Ap were analysed for EDTA extractions on size-fractions, and not done for the other soil horizons (nd).Summing the elemental stocks in size-fractions (∑ fr.) should correspond to the total element concentrations determined on the bulk samples. Zn, Pb(EDTA/Tot) shows the proportion of EDTA extracted metals as the percentage of total concentrations; after Fernandez *et al.* (2007).^cSelected horizons for fractionation study.

the CA soil, because it had been periodically limed (Fernandez *et al.*, 2007).

Distribution of Zn and Pb concentrations versus depth

Concentrations of Zn and Pb were smaller in the upper 26 cm of PP (Table 2) soil in comparison with CA soil. In the PP soil the total Zn and Pb concentrations below the A horizons decreased progressively with depth, whereas this decrease was much more abrupt for subsurface horizons in the CA soil. Relationships between the distribution patterns of metal and organic matter were inferred from C-to-Zn and C-to-Pb concentration ratios (Table 1). In the CA soil, this ratio was fairly constant, both for Zn and for Pb, whereas in the PP soil it was more variable, because of the large carbon content in the dense root horizon (A11). In spite of such clear and different Zn and Pb concentration profiles, the total metal stocks to 1-m soil depth were comparable in both soils (Fernandez *et al.*, 2007, 2008). Estimates of proportions of exogenous metal amounts indicated, however, that both Zn and Pb migrated to depth, to approximately 60–80 cm in both soils, but that Zn migration was more marked than Pb.

Methods

Thin section preparation. Undisturbed samples were taken in cardboard boxes (7 × 7 × 5 cm) in two replicates in all characteristic horizons to 1-m depth, in the side walls of large soil pits at each site. These samples were air-dried and impregnated under vacuum with polyester resin (synolith 544, Euroresins Benelux BV, Budel, the Netherlands). After polymerization, 25-μm thick soil thin sections were prepared (FitzPatrick, 1970) and

studied with a polarizing light microscope (E400 POL, Nikon, Tokyo, Japan) using the terminology of Stoops (2003). Surface percentages of recognizable worm-worked groundmass (worm casts, infillings) were quantified by counting 800 points (ignoring voids) on each thin section (van der Plas & Tobi, 1965).

Physical fractionation. Particle-size fractionations of samples from different soil horizons were performed in water (modified after Balesdent *et al.*, 1991; Ducaroir & Lamy, 1995). No chemical dispersants were used in order to limit relocation of metals during fractionation experiments and to enhance the separation of water-stable aggregates and micro-structures. For fractionation, 50 g of air-dried soil <2 mm was stirred in water (125 ml) with 10 glass beads for 16 hours in polypropylene bottles on a rotating shaker. Fractions >50 μm were separated by wet sieving. The <50-μm suspension was fractionated by centrifugation, at 180 g for the <20 μm, and 18600 g for the <2-μm fraction. During all steps of the fractionation procedure, the supernatants were recycled by high-speed centrifugation in order to limit the total amount of used water (Lamy *et al.*, 1999). Final size-fractions were 200–2000, 100–200, 50–100, 20–50, 2–20, and <2 μm. Fractionations were made in three replicates for the A12, A(B) and AC(g) horizons of the PP soil, and for the Ap, (B)1 and Cg1 horizons of the CA soil. The composition of size-classes was checked under binocular microscopy. Aggregation phenomena in size-fractions were confirmed by other experimental work using moderate ultrasonic treatment (Fernandez-Cornudet, 2006).

Chemical analyses. All size-fractions were analysed for total Zn, Pb and organic C contents. Total metal concentrations were

determined after a combined HF (3 ml) and HNO₃ (9 ml) digestion (Suprapur grade, Merck, Darmstadt, Germany) of approximately 500 mg soil in pressured Teflon bombs using microwave (Mars-Xpress, CEM, Matthews, NC, USA) heating, with settings of 15 minutes at 1200 W, 2.41 MPa, 170°C, and 5 minutes at 1200 W, 2.41 MPa, 180°C (Fernandez *et al.*, 2008). The solutions were evaporated to dryness, redissolved in HNO₃ and then diluted with 50 ml of milliQ water. Concentrations of Zn and Pb were determined by either flame or graphite furnace atomic absorption spectrophotometry (AAS 220, GTA 110, VARIAN, Mulgrave, Victoria, Australia). Replicate analyses of metal concentrations were performed on a certified reference soil sample (CRM 7001, CMI, Prague, Czech Republic), yielding a recovery of 90–102% with respect to certified values. Total C content was determined by dry combustion in an CHN autoanalyzer (NC2500, CE Instruments, Milan, Italy). Absence of inorganic C allowed us to assume that total C was equal to organic C. Amounts of Zn, Pb and organic C were calculated for each size-fraction: mass ratio of fraction $\times$ element concentration, and either expressed in mg (Zn, Pb) or in g (organic C), per kg of bulk sample. Quality control was carried out by comparing the sum of element stocks in size-fractions with the total element concentration in the bulk horizon (Table 2). The recovery rates were satisfactory: 92–102% for Zn, 89–109% for Pb and 98–104% for organic C.

EDTA extractions of Zn and Pb. EDTA is a strong chelating agent, currently used in agronomy to assess metal bioavailability, which was recently reported as one of the best choices of extractant for estimating the labile pool of several metals in soils under certain experimental conditions (Degryse *et al.*, 2009). In the present study, we used EDTA extraction to obtain an indication of the proportion of labile metal elements in soil horizons and in size-fractions. EDTA extractions were carried out on bulk samples from all soil horizons, as well as on all size-fractions from the surface horizons of the two soils. After preliminary studies conducted to optimize the experimental conditions of extraction (pH, concentration of EDTA and soil/solution ratio, results not shown here), the final operating conditions were: 1 g of soil or size-fraction was shaken with 10 ml of 0.05 M EDTA for 24 hours, and then filtered through a 0.45- μ m cellulose acetate (Millipore) membrane (Labanowski *et al.*, 2008). Extractions were conducted at a constant pH of 6.6. Such conditions have been shown to minimize pH variation during extraction and prevent competitive extraction of the EDTA complex by H⁺ ions (Fangueiro *et al.*, 2002). The solutions obtained were analysed for Zn and Pb by flame AAS.

Results

Optical microscopy

Characteristic micro-structures related to biological activity and distribution patterns of OM in A, B and C horizons of the soils are presented in Figure 1. Observations were made at low magnification and focused on the quantification of aggregation

and bio-porosity related to earthworm activity in the three selected horizons of each soil, also studied for fractionation. In the A12 horizon of the PP soil, recognizable worm-worked groundmass was dominant (56% of the analysed surface of thin sections, Table 3). This soil material consisted of single, aggregated and strongly aggregated earthworm faecal pellets ranging in size from 0.5 to 3 mm, indicating intensive earthworm activity (Figure 1a,b). Within such pellets, but predominantly at the outer skins, fine textured OM-aggregates occurred (Figure 1c, arrows) without a recognizable cell structure; the OM was intensively mixed with clay and fine silt. Such aggregates, with sizes ranging from about 20 to 100 μ m, reflect the presence of mull-humus (van Delft *et al.*, 2006). Similar micro-structures were observed in soils markedly influenced by earthworms, in the Ivory Coast (Blanchard *et al.*, 1993) and in the Netherlands (Jongmans *et al.*, 2003). Many (inter-connected) cavities were observed, resulting from earthworm activity as well. The amount of recognizable worm-worked groundmass decreased with depth, to approximately 25% in the A(B) horizon and to <10% in the AC(g) horizon (Table 3). Partly decomposed plant remnants were rare, probably because earthworms consumed all fresh OM quickly. The A12 horizon displayed the darkest groundmass colour because of the presence of mull-OM, but at 60-cm depth, soil colour was still influenced by the presence of OM. These findings are consistent with little variation of soil colour in the PP soil (Table 1) and the progressively decreasing trends of OM contents (Table 2).

In contrast, in the Ap horizon of the CA soil, the amount of recognizable worm-worked soil material was less than 10% (Table 3). Below this horizon, this percentage increased to approximately 15%, but at 60-cm depth such groundmass was no longer observed. The small amount in the Ap horizon suggested that original biological micro-structures were fragmented and destroyed by annual ploughing. OM-aggregates were absent in the CA soil (Figure 1e). Individual OM particles of <50 μ m, locally demonstrating recognizable cell structures (Figure 1f), were distributed randomly through the scarce earthworm pellets. Such observations suggest that conditions were less favourable for mull-humus formation than in the PP soil. Frequently, partly decomposed and fresh plant remnants occurred (Figure 1g) and few voids of biological origin were present. Because of the different OM incorporation and distribution, the groundmass colours of the CA soil had higher chroma values than those in corresponding horizons of the PP soil (Table 1). The groundmass of the B horizon was also denser than in the PP soil. Occasionally, thin outer skins were observed, showing aggregates that were ascribed to former biological activity (Figure 1h, arrows).

Distribution of size-fractions

Results of physical fractionation in water of bulk samples of the selected horizons of the PP and the CA soils are presented in Table 4. For comparison, we also included the results of the total sand (50–2000 μ m) and the <2- μ m fractions determined by particle size distribution (PSD) analyses using chemical dispersive

Figure 1 Micromorphology of characteristic microstructures in horizons of the soils studied. (a–d): PP soil. Worm casts (a), faecal pellets (b), fine textured OM-aggregates in outer skins of faecal pellets (c, arrows) in the A12 horizon; worm-worked groundmass (d) in the A(B) horizon. (e–f): CA soil. Overview of the groundmass of the AP horizon (e), with occurrence of small individual OM particles in earthworm cast (f) and partly decomposed plant residues (g). In the (B)1 horizon, thin parts of outer skins of former bio-aggregates (arrows) are sporadically observed (h). All photographs are in plain-polarized light.

agents. Total mass recovery after fractionation in all samples and replicates reached 98.5–101.0% ($\pm 1.5\%$) of the initial sample mass. The fine sand (50–200 μm) fraction was dominant, representing approximately 60% and 50% in the samples from PP and CA soils, respectively. The 100–200- μm fractions in horizons of the PP soil were about 10% larger than in the CA soil. In contrast, the 200–2000- μm fractions were about 10% larger in the CA soil than in the PP soil, in agreement with field observations. The <2- μm fraction represented only between 2 and 5%, smaller than that determined in the PSD analyses with chemical agents for dispersion (ranging between 5.2 and 8.6%,

Table 4). These differences were ascribed to the presence and the role as aggregating agents of clay in >2- μm size-fractions obtained by physical fractionation in water, as confirmed by X-ray diffraction performed on powder samples.

Distribution of Zn and Pb in size-fractions

Total Zn, Pb and organic C concentrations in size-fractions are presented in Table 5. Concentrations in comparable size-fractions of surface A horizons were generally larger than those of B and C horizons, for each element and for both soils. Within each soil,

Table 3 Worm-worked groundmass (WWG, surface percentage) in selected horizons of the PP and CA soils ($\pm$ standard deviation), determined by point-counting on thin sections

Horizon	WWG / %
PP soil	
A12	56 $\pm$ 2
A(B)	26 $\pm$ 2
AC(g)	7 $\pm$ 1
CA soil	
Ap	8 $\pm$ 1
(B)1	16 $\pm$ 2
Cg1	—

concentrations of Zn, Pb and organic C increased with decreasing size of fractions and were largest in the $<2\text{-}\mu\text{m}$ fraction. The Zn and Pb concentrations of the $<2\text{-}\mu\text{m}$ and $2\text{--}20\text{-}\mu\text{m}$ fractions were from 1.5 to 2 times larger in horizons of the CA soil than in corresponding horizons of the PP soil. In the $50\text{--}100\text{-}\mu\text{m}$ fractions of A and B horizons in the PP soil, Zn and Pb concentrations were slightly larger than in fractions of corresponding horizons of the CA soil.

There were markedly different patterns in the distributions of the amounts of Zn and Pb in size-fractions in the two soils (Figure 2). For the CA soil, in the Ap horizon (Figure 2d), the largest amounts of Zn and Pb were in the finest size-fractions: $2\text{--}20$ and $<2\text{-}\mu\text{m}$. In the underlying (B) and Cg1 horizons, the largest amount of metals was found in the $<2\text{-}\mu\text{m}$ fraction. Distribution of organic C among size-fractions showed a fairly similar pattern (Figure 2d–f). In contrast, in the PP soil no such single distribution patterns were observed, because the $2\text{--}20\text{-}\mu\text{m}$ and the $50\text{--}100\text{-}\mu\text{m}$ fractions contributed most to the total amounts of Zn, Pb and organic C in the A12, A(B) and ACg horizons (Figure 2a–c).

Distribution of EDTA-extractable Zn and Pb concentrations

The concentrations of Zn and Pb extracted by 0.05 M EDTA in bulk samples of horizons and their percentage of the total Zn and Pb concentrations are presented in Table 2. In the PP soil, about 35–45% of Zn was extracted by EDTA until 80-cm depth (AC(g)) and then decreased to approximately 20% in the Cg2 horizon. For Pb, this percentage decreased progressively from 60 to 70% in the A12 horizon to about 35 to 40% in the AC(g) horizon, and then to about 25% in the Cg2 horizon. In the CA soil, the amounts of extractable Zn and Pb represented some 40–50% of the total metal concentrations in the AP horizon (Table 2). EDTA-extractability of Zn represented about 50% in the underlying (B)1 horizon, decreasing to 30% in the (B)2 and Cg1 horizons and to $<2\%$ in the Cg2 horizon. For Pb, the extractability was about 25–30% in the B and Cg1 horizons, decreasing also to $<10\%$ in the Cg2 horizon.

Results of EDTA extractions of Zn and Pb from size-fractions of the surface horizons are presented in Figure 3. They are expressed as the amounts of EDTA-extracted Zn and Pb in each size-fraction, and their sum is theoretically equal to the total concentration of EDTA-extracted Zn and Pb in the bulk samples of each horizon (Table 2). For the A12 horizon of the PP soil (Figure 3a), a bimodal distribution pattern was revealed, with largest contributions from the $50\text{--}100$ and $2\text{--}20\text{-}\mu\text{m}$ fractions. These distribution patterns were similar to the distributions of the total amounts of Zn and Pb in the fractions shown in Figure 2. In contrast, for size-fractions of the Ap horizon of the CA soil (Figure 3b), the $2\text{--}20$ and $<2\text{-}\mu\text{m}$ fractions contributed most to the total amount of EDTA-extractable Zn and Pb in the bulk sample and this was two- or threefold greater than the coarser size-fractions. Consequently, a unimodal distribution pattern was observed, similar to the distribution of total metal amounts in size-fractions (Figure 2).

Table 4 Size-fraction distribution obtained by physical fractionation in water performed on bulk samples of characteristic horizons in PP and CA soils (mean values of three replicates, $\pm$ standard deviation)

	Physical fractionation in water / μm						Particle size distribution / μm	
	200–2000	100–200	50–100	20–50	2–20	<2	50–2000	<2
	/ g kg ⁻¹							
PP soil								
A12	154 $\pm$ 12	361 $\pm$ 32	247 $\pm$ 11	126 $\pm$ 3	85 $\pm$ 4	24 $\pm$ 4	701	86
A(B)	141 $\pm$ 24	344 $\pm$ 2	251 $\pm$ 11	122 $\pm$ 14	83 $\pm$ 5	39 $\pm$ 1	719	67
AC(g)	120 $\pm$ 16	369 $\pm$ 10	255 $\pm$ 11	123 $\pm$ 1	78 $\pm$ 5	47 $\pm$ 1	739	64
CA soil								
Ap	262 $\pm$ 5	246 $\pm$ 10	262 $\pm$ 5	126 $\pm$ 5	75 $\pm$ 4	25 $\pm$ 2	762	59
(B)1	286 $\pm$ 5	233 $\pm$ 10	252 $\pm$ 7	145 $\pm$ 1	58 $\pm$ 2	24 $\pm$ 2	737	57
Cg1	288 $\pm$ 8	231 $\pm$ 5	242 $\pm$ 5	133 $\pm$ 9	62 $\pm$ 2	29 $\pm$ 2	747	52

Data on particle size distribution (after Fernandez *et al.*, 2007) were obtained after destruction of organic carbon and chemical dispersion of soil samples (NFX 31-107, Afnor, 2004).

Table 5 Total Zn, Pb and Org. C concentrations of size-fractions of selected A, B and C horizons under permanent pasture (PP) and conventional arable (CA) land

		Size-fractions / μm					
		200–2000	100–200	50–100	20–50	2–20	<2
PP soil							
Zn	A12	25.3	23.6	70.7	97.5	264	475
/ mg kg ⁻¹	A(B)	14.9	26.9	39.9	53.6	137	550
	AC(g)	6.4	17.4	39.3	60.8	61.5	120
Pb	A12	20.0	11.3	51.0	67.7	163	341
/ mg kg ⁻¹	A(B)	4.8	8.2	14.1	15.7	35.2	146
	AC(g)	12.0	3.0	7.0	8.0	19.5	68.4
Org. C	A12	5.2	6.2	13.3	25.9	39.5	82.2
/ g kg ⁻¹	A(B)	1.3	1.8	3.8	9.5	14.9	19.6
	AC(g)	0.5	0.4	1.2	1.5	6.7	15.7
CA soil							
Zn	Ap	31.9	40.7	57.5	119	385	984
/ mg kg ⁻¹	(B)1	13.6	20.5	30.0	51.0	216	871
	Cg1	4.0	9.0	13.2	24.5	109	336
Pb	Ap	15.2	23.6	32.6	67.8	210	587
/ mg kg ⁻¹	(B)1	2.0	3.2	3.9	7.8	60.6	250
	Cg1	2.8	4.1	4.5	19.6	42.8	158
Org. C	Ap	3.6	4.9	5.3	12.1	32.7	65.0
/ g kg ⁻¹	(B)1	0.9	1.2	1.3	2.9	7.3	18.0
	Cg1	0.6	0.9	1.1	2.3	5.2	14.5

Discussion

Relations between land use, microstructures and biological activity

The impacts of changing long-term land use primarily affect the amounts, nature and distribution of organic matter restitution in the soil, influencing biological activity, biodegradation of organic matter and organo-mineral soil aggregation (van Breemen, 1998; Six *et al.*, 2002; Pulleman *et al.*, 2005). In particular, when comparing soils under permanent pasture with arable soils, major impacts on the composition and the importance of the earthworm community have been highlighted (Lavelle & Spain, 2001). Our work demonstrated a different uni- or bimodal distribution pattern for the organic C contents in size-fractions obtained by physical fractionation in water, attributed to the presence of water-stable micro-aggregates in the two soils.

The first mode concerned the 2–20- μm fraction, which is often strongly connected to microbial activity and root exudates, which produce reactive organic compounds and favour the formation of stable, predominantly silt-sized micro-aggregates (Jocteur Monrozier *et al.*, 1991; Chotte *et al.*, 1993, 1998). Together with polyvalent cations, such OM is a major stabilizing agent of 2–20- μm micro-aggregates (Christensen, 2001), leading to strong aggregation that is stable over a long time and not significantly influenced by changes in land management (Christensen, 2001). The 2–20- μm fraction was present in all the horizons of the permanent grassland soil with significant root activity (A12, A(B), Ac(g)), as well as in the Ap horizon of the CA soil, where roots

were noted (Table 1). In B and C horizons of the CA soil, with very few or no roots, such micro-aggregates were not observed, and organic C was mainly stored in the <2- μm fraction.

The occurrence of organic C in a second mode (the 50–100- μm fraction) was ascribed to earthworm activity. Notable contributions of this fraction for organic C occurred only in the PP soil, and in all horizons affected by earthworm activity (Table 2). Earthworms determine soil macro- and micro-structures by incorporating OM into the fine-textured mineral soil (Christensen, 2001). In the digestive system of earthworms, mineral compounds are intimately mixed with OM and bound by aggregating agents, such as clay, iron compounds and polysaccharides produced by microbial decay of OM. Such aggregates were reported to be more water-stable than surrounding bulk-soil aggregates (Shipitalo & Protz, 1988; Pulleman *et al.*, 2004, 2005), and soil organic matter included in such aggregates will be protected (Blanchard *et al.*, 1993). Moreover, our conclusions on different earthworm activities are consistent with the work of Nahmani *et al.* (2003), who examined the faunal composition of the same soils from our study site: they quantified a total earthworm density of 392 (± 12) individuals including endogeic species per m² in the PP soil, but only 52 (± 5) individuals per m² for the CA soil, which were exclusively epigeic species.

In our experiments on physical fractionation in water, soil samples were mechanically dispersed by the combined action of water and glass beads. Large worm casts and faecal pellets disintegrate into mineral constituents and organo-mineral particles occurring in different size-fractions and explain the importance

Figure 2 Distribution of the amounts of Zn, Pb and organic C in size-fractions (1–6) of the A12 (a), A(B) (b) and AC(g) (c) horizons of the PP soil, and the Ap (d), (B)1 (e) and C1g (f) horizons of the CA soil. Results are presented as total amounts of Zn, Pb (mg) and organic C ($\text{g} \times 10$) expressed per kg of bulk sample. The sum of stocks in size-fractions is the total element concentration determined in the soil horizon (see Table 2). 1 = 200–2000 μm ; 2 = 100–200 μm ; 3 = 50–100 μm ; 4 = 20–50 μm ; 5 = 2–20 μm ; 6 = <2 μm .

Figure 3 Distribution of the amounts of EDTA-extractable Zn and Pb in size-fractions (1–6) of the A12 horizon of the PP soil (a) and the Ap horizon of the CA soil (b). Results are presented as the amounts of EDTA-extracted Zn and Pb (mg) expressed per kg of bulk sample. The sum of stocks in size-fractions is the EDTA-extractable metal concentrations of Zn and Pb determined in the soil horizon (see Table 2). 1 = 200–2000 µm; 2 = 100–200 µm; 3 = 50–100 µm; 4 = 20–50 µm; 5 = 2–20 µm; 6 = <2 µm.

of organic C contents in >50-µm fractions. In the PP soil, macro and large micro-structures with intimate mixing of OM and fine-textured mineral material were demonstrated by optical microscopy in the worm-worked soil (Figure 1, Table 3), in the surface horizons and as deep as the AC(g) horizon. In contrast, in the CA soil, repetitive soil tillage favouring macro-aggregate disruption (Balesdent *et al.*, 2000), and a much reduced earthworm activity (Nahmani *et al.*, 2003), explain the smaller amounts of organic C in the 50–100-µm fraction compared with the 2–20-µm fraction (Figure 2d).

Impact of land use on metal distribution and extractability

Effects of land use-related biological activity were also demonstrated on Zn and Pb distributions in size-fractions in the two soils, with remarkably similar distribution patterns compared with those observed for organic C contents (Figure 2). In particular, the bimodal distribution of the amounts of Zn and Pb included in the 50–100 and the 2–20-µm fraction was systematic in the PP soils for the A12, A(B) and AC(g) horizons. These three horizons were significantly re-worked by earthworms, as demonstrated by optical microscopy and quantification of worm-worked soil in thin sections. In the Ap horizon of the CA soil, predominantly explored by plant roots and microorganisms, the 2–20-µm fraction was the main fraction retaining Zn and Pb, and in the B and C horizons with small biological aggregation activity, most Zn and Pb occurred in the <2-µm fraction. Our findings suggest that, once incorporated into the soils, Zn and Pb derived from atmospheric deposition are rapidly and preferentially redistributed on the clay–iron–humus complex in the finest fraction. This statement is corroborated by the observation of the largest Zn and Pb concentrations in the <2-µm fractions, in all horizons and for both soils

(Table 5). Because of the different biological activity induced by continuous long-term land use, this clay–humus complex occurs either as an easily dispersible <2-µm fraction, or as an aggregating agent in coarser fractions. The effects of such aggregation on metal distribution patterns are mostly visible in the 2–20 µm and 50–100-µm fractions after physical fractionation in water.

Land use-related biological activity also influenced the extractability of metals in the two soil profiles. Significant changes in the availability of nutrients were reported after digestion of soil by earthworms, but also in the distribution of heavy metals in soil fractions (Devliegher & Verstraete, 1996; Lavelle & Spain, 2001; Sizmur & Hodson, 2009). Earthworm activity was noted as favouring increased availability of Zn, Pb, Cu and Cd (Cheng & Wong, 2002; Ma *et al.*, 2002; Wen *et al.*, 2004; Sizmur & Hodson, 2009). Such findings, often derived from laboratory experiments in micro- or mesocosms, were confirmed in our work on samples collected from field situations. Equal or larger proportions of EDTA-extractable Zn, but clearly larger proportions of Pb (with respect to the total Zn and Pb concentrations), were found in horizons at comparable depths of the PP soil, compared with the CA soil. These differences in metal extractability concerned all horizons that had been markedly influenced by earthworm activity. In the underlying Cg2 horizons, with much larger clay and iron contents, the extractability of Zn and Pb was clearly less, for both soils, indicating a stronger binding of Zn and Pb with soil constituents in this horizon. Differences in Zn and Pb extractability were also demonstrated at a micro-scale, in size-fractions. In Table 6, the concentrations of EDTA-extractable Zn and Pb in three size-fractions (50–100, 2–20 and <2 µm) are presented, as well as the contribution of each size-fraction to the total amounts of Zn and Pb extracted by EDTA in bulk samples of

Table 6 EDTA Zn and Pb concentrations in selected size-fractions of the A12 and Ap horizons of the PP and CA soils, and contribution (percentage) of the amounts of Zn and Pb extracted by EDTA in the size-fractions to the total EDTA extracted amount of Zn and Pb in the bulk soil

Size-fractions	EDTA concentrations		Amounts EDTA _{fr} / EDTA _{bulk}	
	Zn	Pb	Zn	Pb
	/ mg kg ⁻¹		/ %	
PP, A12 horizon				
50–100 µm	26.4	30.4	23.4	23.2
2–20 µm	106	99.2	29.1	23.6
<2 µm	170	272	13.8	19.0
CA, Ap horizon				
50–100 µm	21.7	9.9	13.4	7.9
2–20 µm	204	167	36.9	39.0
<2 µm	433	402	27.1	32.4

the surface horizons. In the Ap horizon of the CA soil, 60–65% of extractable Zn and Pb are included in the <2 µm and 2–20-µm fractions, whereas in the A12 horizon of the PP soil about 50% of total extracted amounts of Zn and Pb were located in the 2–20 and 50–100-µm fractions. The main differences concern the <2 µm and the 50–100-µm fractions, in agreement with a hypothesis that soil aggregation is promoted by faunal activity, with the <2-µm fraction acting as a cohesion agent.

Conclusion

This work showed that different long-term land use and related biological activity (abundance of roots, activity of microorganisms and earthworms) led to different distribution patterns of organic C, Zn and Pb in size-fractions, particularly the 2–20 and 50–100-µm fractions. Such differences were quantified by optical microscopy and physical fractionation in water. Within the soils, element distribution patterns were remarkably similar: under permanent grassland, a clear bimodal distribution pattern was demonstrated for the amounts of C, Zn and Pb in all horizons affected by significant earthworm activity. In contrast, under arable land, only unimodal distribution patterns were found. Such different bimodal and unimodal distributions were also found for Zn and Pb EDTA-extractable amounts in the fractions from the PP and CA soil, respectively, indicating that the localization and lability of metal pollutants at a micro-scale was governed by different land use-related biological activity. Finally, the reliability of information from real metal-polluted fields on the relationships between long-term land use, soil aggregation and micro-scale metal distributions and extractability requires a sound sampling strategy, based on good pedo-geochemical survey principles. For such studies, soils located in the proximity of metallurgical plants offer a unique opportunity because factors affecting metal pollutant dynamics in soils (land use, metal loads and vegetation) consistently vary over short distances, whereas those related to the soil background characteristics remain unchanged.

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