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HEAVY METAL LINKAGES WITH MINERAL, ORGANIC AND LIVING SOIL COMPARTMENTS

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Summary—For soil ecotoxicological assessment, we can observe lethal effects (on organism as presence or absence) or sublethal effects due to bioconcentrations of contaminants in organisms. This paper deals with the analysis of seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb and Zn) and Ca: (i) in soils, by three chemical extraction techniques; (ii) in earthworm tissues; (iii) the relationships between earthworm bioconcentrations and soil heavy metal contents; and (iv) the linkage of these metals with different soil components. Sixty soil sites were examined. Soil samples were analyzed by three metal extraction techniques: total, acetic acid and dethylene triamine pentacetic acid. The results of soil metal extractions have been reported in relation to total soil or to various soil fractions (organic matter, clay, silt, sand). Data were interpreted using a principal component analysis (PCA) to observe a relationship depending on soil and earthworm heavy metal contents and soil properties. The correlations between earthworm metal body burdens and soil total contents were positively significant for all metals except Fe and Ni. These correlations varied with the other soil extraction methods. The observed correlations could not be interpreted in term of simple mechanisms due, to the complexity of systems, and could not be used as a tool to predict soil biohazard. The relationships between earthworm metal bioconcentrations and the various soil metal estimations depend on many mechanisms which are discussed. The direct measurement of heavy metal concentrations in earthworm tissues is safer for ecological assessments.

INTRODUCTION

The term ecotoxicological soil assessment refers to ecology, toxicology and assessment. Ecology needs to make such assessments in relation to true ecosystems, i.e. bio-physico-chemical spontaneous systems on which man acts. Toxicology focuses on the deleterious biological effects of chemicals on living organisms. Ecotoxicology evaluates deleterious biological effects mediated by the (eco-)system. Assessment refers to an appraisal of chemical effects through the various man-made evaluations (price, security, amenity, etc.) (Bouché, 1990).

Heavy metals are present practically everywhere (air, soil, water, organism, etc.). Natural heavy metal contents of soils occur from erosion and weathering of parent rocks (Sillanpaa, 1972; Bohn *et al.*, 1985). Some, such as Cu, Zn and Fe, are necessary, in low concentration, for all living organisms while, most of them present toxicity hazard at high concentrations (Allaway, 1968; Lisk, 1972; Kabata-Pendias and Pendias, 1986; Abdul Rida and Bouché, 1994, 1995). For many years, earthworms have been considered interesting biological indicators of many heavy metals in soil (Gish and Christensen, 1973; Van Hook, 1974; Edwards and Lofty, 1977; Ireland, 1983; Lee, 1985; Abdul Rida, 1992). Earthworms have been used to evaluate the chemical lethal and sublethal effects and to assess the contaminant fractions acting on organisms. This paper deals with this last point which is critical for environmental assessment. A high soil heavy metal content may not be toxic and conversely a low concentration could be harmful, depending on biotic and abiotic soil conditions. Earthworms are omnipresent in almost all soils and are easy to catch and analyse. Earthworms ingest the various biological soil fractions: dead plants, microorganisms, humus, possibly fine living roots (Cortez and Bouché, 1992) and most mineral fractions (clay, silt, fine and coarse sands). By digestion an assimilable fraction is taken up and partly excreted, so their body content reflects the true bioavailability of soil contaminants. Compared with other organisms, earthworms are a most interesting living tool for soil biomonitoring, whereas plants reflect both soil contents and atmospheric fallouts. Microorganisms are not easily extractable from soils and other biological compartments are scarce or irregularly dispatched.

For ecotoxicological assessments, a traditional practice is to compare concentrations of contaminant, for example between sea water and fish. Both compartments have a density of nearly one, so the comparison is logical for a wide range. This practice has been expanded to soils, without care for the density and chemical dispersion heterogeneities.

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This leads to misleading ecological conclusions. We should bear in mind that:

(1) A variation of soil density leads to a variation of the concentration with the same amount of contaminant (Sillanpaa, 1972).
(2) Soil–organism exchanges depend on the contact surface between the two compartments. This depends on surface area, but not on weight.
(3) Chemicals are more or less linked with soil components (Aubert and Pinta, 1971; Kabata-Pendias and Pendias, 1986). The soil is not a solution: it is structurally heterogenous.
(4) Soil is structured not only by its heterogenous components, but also in space and time. Soil–organism exchanges observed in the top soil in spring do not occur in deep soil in autumn.

Consequently, concentration comparisons are far more difficult in soils than in water systems. This difficulty also occurs with the "bioavailable" or "assimilable" fractions measured by physico-chemical extraction techniques. These extraction procedures, applied to soils, are assumed to be "similar" to biological assimilation processes. This unfalsifiable assumption could be avoided if we use soil organisms like earthworms.

To contribute to an improvement in ecotoxicological soil assessments, this work has been done in soils with seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, Zn) and Ca, and earthworms as the biological compartment. The goal of this paper is to show: (1) the benefit of earthworms as reference compartment to observe soil contaminant bioavailabilities; (2) the limits of the direct soil heavy metal contents for ecological assessments; (3) the effects of soil physical and chemical properties on earthworm heavy metal bioconcentrations; and (4) the relationships between earthworm metal body burdens and the various soil components which could be "carriers" of the heavy metal bioavailable fractions.

MATERIALS AND METHODS

Soil characteristics

Sixty soil sites were sampled in six different areas in southern France with various concentrations of soil Ca, Cd, Cu, Fe, Mn, Ni, Pb and Zn (Abdul Rida, 1992). The soils have a broad diversity of physico-chemical properties (Table 1).

Earthworm species

The following species and ecological categories have been found in the studied soils: endogeic (*Allolobophora rosea, A. chlorotica, Octolasion cyaneum, Nicodrilus caliginosus*), epianecic (*Lumbricus terrestris*) and euanecic (*Nicodrilus meridionalis, N. nocturnus, N. giardi, N. longus ripicola, Scherotheca monspessulensis, S. gigas dinoscolex, S. dugesi sanaryensis, S. gigas rhodana, S. gigas gigas*) species. No epigeic species were observed, as is often the case in the mediterranean climate (Bouché, 1985).

Analyses of heavy metals

The heavy metal extraction methods from earthworms and soils have been described in Abdul Rida (1992). Earthworms were dissected, eliminating their digestive tract content, then dissolved by nitric acid and analyzed. Soil heavy metals were extracted by three types of mineralization: total extraction (quoted T) with a mixture of HNO_3 and HCl, "assimilable" or "bioavailable" extraction with acetic acid (quoted A) and diethylene triamine pentaacetic acid-DTPA (quoted D).

The soil and earthworm heavy metal concentrations were measured with an atomic absorption spectrophotometer, type Varian AA-SPECTRA, using an air–acetylene flame.

Expression of results

Soil concentrations of heavy metals were expressed in relation to the sum of all soil fractions (TS) or to a single fraction. In this last case, we considered that the metal was linked with this fraction (organic matter = O, Clay = C, silt = Si, sand = Sa). To this extent, the content of each metal for each analysis is reported for each fraction and to the total mass of soil. For the earthworms, the symbol E is used to express heavy metal body burdens (each value is the mean of individual measures of the earthworms sampled from each soil site; at least, four individuals).

Table 1. Minimum, maximum, mean and standard deviation (SD) values of soil physico-chemical characteristics

	pH	O.M.	Clay	Silt	Sand	CEC	Ca	Mg	K	Na
			(%)					(meg 100 g^{-1})		
Minimum	4.68	0.11	3.80	5.80	7.50	2.10	1.70	0.21	0.08	0.01
Maximum	8.31	8.14	44.00	59.40	90.40	23.80	55.90	2.55	1.77	0.37
Mean	7.14	2.77	19.60	28.30	51.60	10.70	21.70	1.18	0.63	0.05
SD	0.90	1.58	8.60	10.50	17.80	4.40	16.20	0.49	0.36	0.06

O.M. = organic matter, CEC = cation exchange capacity.

RESULTS

Table 2 presents the overall results from which a general trend can be noticed; i.e. higher metal concentrations in earthworms for Cd and Zn, a similar level for Cu and lower level for Fe, Ca, Mn, Ni and Pb than the same metal extracted by soil total technique. Uptake by earthworms was far more efficient than the assimilable extractions (A and D) for Cd, Cu, Fe, Mn, Ni, Pb and Zn. Acetic acid extracts soil Ca more efficiently than what earthworms concentrate, with a great variability of the data due to the great diversity of studied soils (Table 1). Earthworm Ca is generally greater than Ca extracted from soils by DTPA. This general trends shows that the amount of heavy metals extracted from soils and earthworms varies widely. So, soil analyses do not give us suitable information to appraise ecological risks.

In order to demonstrate relationships between earthworm heavy metal concentrations and the large number of soil numerical values obtained (eight metals × three extraction techniques × six fractions), we applied a Principal Component Analysis (PCA). This statistical analysis allows treatment of large amounts of data and to assess the relationships between them. As an example, Fig. 1 shows the two principal components of a PCA made from some Cu and Pb data vs soil properties. Lead was correlated closely with sand and low pH, and the opposite was true for Cu. Low concentrations of Cu in "heavy" soil (high clay, CEC and organic matter content) were combined with the relatively poor extraction of Cu from such soils by earthworms and acetic acid. However, such a figure does not summarize all the information, since only acetic acid extraction and two metals are included. Table 3 shows the correlation matrix between earthworm tissue bioconcentrations and soil physico-chemical properties. There were no correlations for Cd and Ni. Earthworm Ca and Cu were linked mostly with silt and high pH, and the opposite was true for Pb and Zn.

For a total comparison and to evaluate the "best" extraction technique and the "best" expression of results, Table 4 gives a correlations matrix between earthworm heavy metal body burdens and soil heavy metal contents in various fractions. All significant correlations were positive. There was no correlation between Fe and to some extent between Ni. In general, the "assimilable" or "bioavailable" fractions (A, D) do not give better estimates than the total extraction (T) with the exception of CuA/O and CdD/O. Thus, the classical relationship of results according to the total dry weight of soil (TS) is no better than the other expressions. The relations between earthworm Ni body burdens and soil Ni contents vs sand is slightly better than the other fractions. Zinc has a better expression of results according to clay or silt.

DISCUSSION

From Table 4, some general conclusions could be made with a few exceptions discussed below: (i) Neither, DTPA nor acetic acid heavy metal extractions gave us better correlations than the soil total extraction; (ii) the expression of soil heavy metal contents in relation to soil fractions is not more significant than the expression related to the total soil; (iii) the soil total heavy metal contents generally showed good correlations with the earthworm tissue bioconcentrations. These correlations were positively significant for Ca, Cd, Cu, Mn, Pb and Zn, but not significant for Fe and Ni. In general, the amount of Fe was high in soils and low in earthworms (Table 2). This means that earthworm Fe uptake was very low. For Ni, only a few studies have tried to correlate soil content of Ni with earth-

Table 2. Minimum, maximum, mean and standard deviation values of concentrations (mg kg^{-1}) in earthworms and soils, with total, acetic acid and DTPA extraction techniques

	Earthworm (E)			Soil total(T)		
Metal	Min	Max	M ± SD	Min	Max	M ± SD
Cd	2.48	176	35.8 ± 40.7	0.35	7.5	2.5 ± 1.4
Cu	11.8	189	54.6 ± 42.5	14.9	237	57.9 ± 43
Pb	0.56	3645	251 ± 570	19.5	8415	945 ± 1813
Zn	209	2650	779 ± 541	39.6	1682	416 ± 418
Ni	2.2	42.1	9.96 ± 6.9	3.3	49	24.2 ± 9.9
Mn	19.5	317	140 ± 65.8	33.5	2325	642 ± 561
Fe	475	6183	1593 ± 897	6161	64930	26965 ± 13569
Ca	1178	23421	4138 ± 4544	234	230800	42389 ± 66169
	Soil acetic acid (A)			Soil DTPA (D)		
Metal	Min	Max	M ± SD	Min	Max	M ± SD
Cd	0.25	4.47	1.37 ± 0.87	0.1	5.82	1.26 ± 1.27
Cu	0.29	30.6	4.3 ± 5.7	0.9	86.5	17.1 ± 18.6
Pb	0.27	507	56.4 ± 98.9	0.2	897	108 ± 163
Zn	3.06	509	52.3 ± 78.5	0.63	330	42.4 ± 56.8
Ni	0.2	10.9	3.8 ± 3.3	0.04	5.1	1.1 ± 1
Mn	5.6	290	94.9 ± 51.7	1.4	97	31 ± 19.7
Fe	0.2	245	23.1 ± 44	12.6	475	68.9 ± 70.8
Ca	4	196050	36908 ± 60062	2	4312	960 ± 888

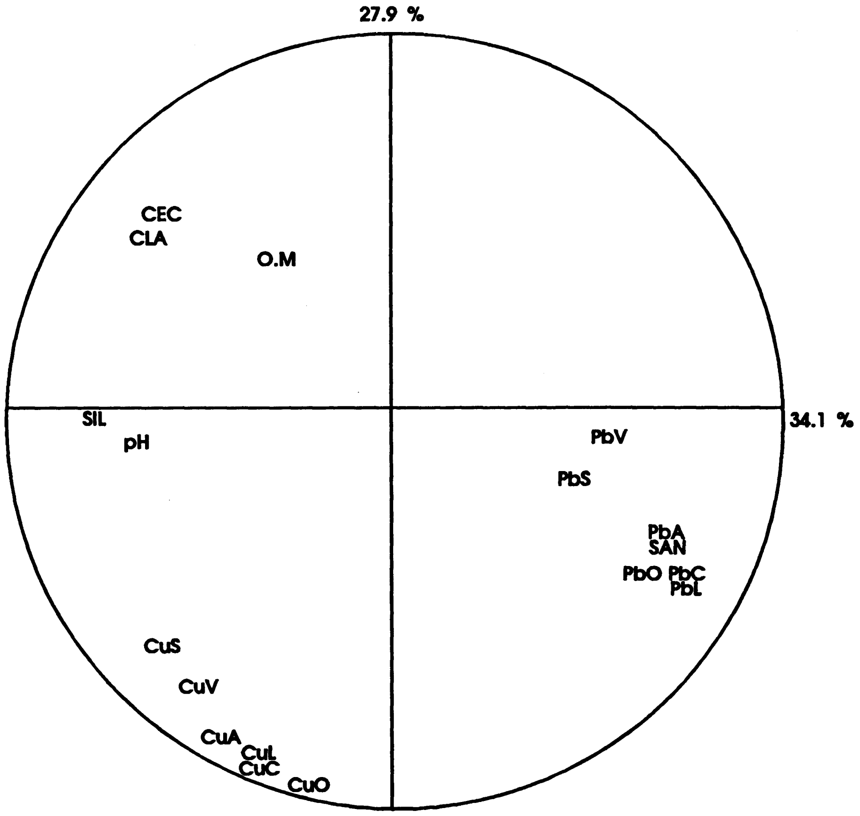

Fig. 1. Principal component analysis of lead and copper analysed by acetic acid (A) and estimates in earthworms and soil fractions vs soil properties. (E = earthworm, C = clay, O = organic matter, Si = silt, Sa = sand, TS = all fractions).

worm bioconcentrations. As Gish and Christensen (1973) found, we did not find such a correlation with total soil content. Nevertheless, we did observe a correlation if this content was related to the sand fractions (Table 4). This correlation was not predictive and related to few soil samples (Fig. 2).

For the other metals, many studies have been made, but with few earthworm species (Edwards and Lofty, 1977; Lee, 1985; Morgan and Morgan, 1992; Abdul Rida, 1992; Marino *et al.*, 1992). Among three species, found at a highly zinc-polluted site, Ireland (1979) noted that *Lumbricus rubellus* contained the highest concentrations of Zn and Mn, and the lowest concentration of Pb. *Dendrobaena veneta* contained the highest concentration of Cd and *Eiseniella tetraedra* the highest concentration of Pb. Ireland (1983) stated that the difference in earthworm heavy metal contents was probably due to various uptake mechanisms. Piearce (1972) reported that the intestinal uptake of Ca was much higher by *L. rubellus* than by *Allolobophora caliginosa*.

Table 3. Correlation matrix between earthworm heavy metal concentrations and soil physico-chemical properties

	Cd	Cu	Ni	Pb	Zn	Mn	Fe	Ca
O.M.	NS	NS	NS	NS	NS	NS	-**	NS
Clay	NS	NS	NS	-*	-**	***	-*	NS
Silt	NS	***	NS	-*	-**	NS	NS	***
Sand	NS	-*	NS	*	***	-**	NS	-***
CEC	NS	NS	NS	NS	NS	**	-*	NS
pH	NS	*	NS	-***	-***	NS	NS	***

(*) = $P < 0.05$, (**) = $P < 0.01$, (***) = $P < 0.001$, NS = not significant, (-) = negative correlation.

In a polluted soil, Morgan and Morris (1982) noted that *L. rubellus* had highly concentrated Ca and Zn levels and lower Pb and Cd than *D. rubida*. The authors explained this difference by an antagonism competition between these metals. The antagonism between Pb and Ca was advocated by Andersen and Laursen (1982) who noted that in a Ca high level site, earthworm Pb uptake was very low, even from Pb high level soils. In contrast, they observed that earthworms concentrate more Pb in calcium-deficient soil, even when the soil Pb content is moderate.

Studies of the bioavailable or assimilable fractions by physico-chemical means are very numerous. They assume that the extracted fractions represent the bioavailable parts which can be taken up by organisms (Lindsay, 1972; Lindsay and Norvell, 1978; Juste, 1983 and Juste, 1988). This assumption is very difficult to prove. This can be illustrated by the many techniques, methods and re-

Table 4. Correlation matrix of the earthworm concentrations (E) with soil metal contents measured by three extractions techniques (T, A, D) and reported to total soil (TS) or only to organic matter (O), clay (C), silt (Si) or sand (Sa)

Soil extractions vs earthworms	Total (T)					Acetic acid (A)					DTPA (D)				
	TS	O	C	Si	Sa	TS	O	C	Si	Sa	TS	O	C	Si	Sa
Cd E	**	NS	NS	*	**	**	NS	**	**	**	***	***	***	***	***
Cu E	***	*	***	***	***	***	***	***	***	***	***	***	***	***	***
Ni E	NS	NS	NS	NS	**	NS	NS	NS	NS	**	NS	NS	NS	NS	***
Pb E	***	**	***	***	***	***	*	***	***	***	***	NS	**	**	***
Zn E	***	NS	***	***	**	**	**	***	***	*	**	**	***	***	*
Mn E	***	***	***	***	***	**	NS	NS	**	**	***	**	**	***	***
Fe E	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ca E	***	***	***	***	***	***	***	***	***	***	***	**	***	NS	*

(*) = $P < 0.05$, (**) = $P < 0.01$, (***) = $P < 0.001$, (NS) = not significant.

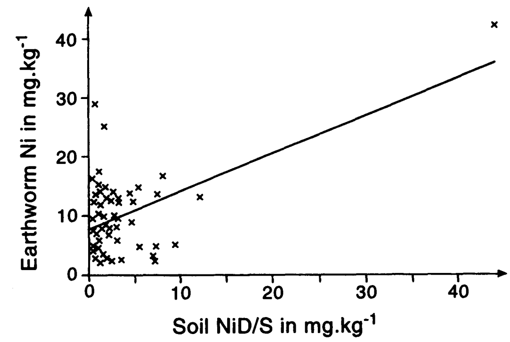

Fig. 2. Illustration of the best correlation observed for Ni between earthworm body burdens and soil sand fractions extracted by DTPA.

agents proposed to make these types of extraction (Scott and Thomas, 1977; Abdul Rida, 1992). As given in Table 4, the correlations between earthworm heavy metal body burdens and soil total contents were significant. These correlations varied with the other soil extraction methods. Looking at the mathematical values, we noted that some correlation values of assimilable extractions (A and D) were better than the correlation values of soil total extraction. This exception is observed, for example, for Cu (Fig. 3) where the acetic acid extraction gave a better correlation than the total soil extraction related to total soil (r 0.782 > 0.656) or to the silt fraction (r 0.782 > 0.386). However, biological concentration in living organisms is very different to chemical extractions from soils. It reflects many physiological mechanisms, such as assimilation and excretion, while chemical extractions reflect the different efficiencies of chemical reagents to extract heavy metals from soils (Juste, 1983 and Juste, 1988; Abdul Rida, 1996).

Soil physico-chemical properties also act on earthworm heavy metal concentrations (Ma, 1982; Ma *et al.*, 1983; Beyer *et al.*, 1982 and Beyer *et al.*, 1987; Abdul Rida, 1992; Van Gestel, 1992). Our study confirm the results of these authors concerning a negative correlation between soil pH and

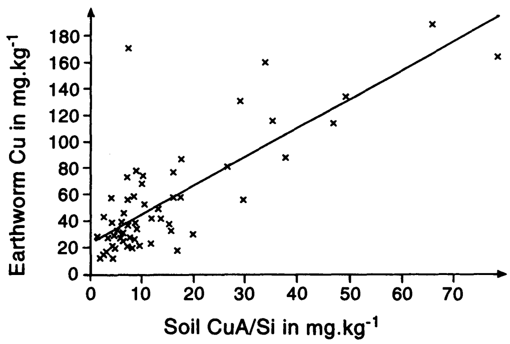

Fig. 3. Illustration of one of the highest correlation observed for Cu between earthworm body burdens and soil silt fractions extracted by acetic acid.

earthworm Pb body burdens (Table 3). It also shows a negative correlation for Zn and a positive correlation for Ca between soil pH and earthworm body burdens. Beyer *et al.* (1987) noted the role of organic matter on the earthworm Pb bioconcentration. However, our study did not show this, not only for Pb but also for all heavy metals studied except Fe.

The selectivity of ingested materials by earthworms depends on their different ecological behaviour or ecological categories. Earthworms select their food in soils carefully (Lee, 1995). It is also well known that heavy metals are linked to soil fine fractions, especially clay and organic matter (Sillanpaa, 1962; Duchaufour, 1965; Mengel and Kirkby, 1978; Kabata-Pendias and Pendias, 1986). The observed earthworm bioconcentrations consequently depend on their feeding behaviour and whether they select these soil fine fractions or not.

The complexity of the soil ecosystem and its variability in space and time is such that it is very difficult to assume that soil pollutant analyses can be used as a tool to predict biohazards. The earthworm heavy metal uptake is correlated with many mechanisms (species diversity, metal antagonisms, assimilability, soil physicochemical properties, feeding behaviours), and the best way to made an ecotoxicological assessment is to observe the effects of pollutants directly on the organisms.

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