Acta Zool. Fennica 196:307–310 ISBN 951-9481-41-9 ISSN 0001-7299
Helsinki 28 February 1995

XI International Colloquium on Soil Zoology, Jyväskylä, Finland, 10–14 August, 1992

Earthworm contribution to ecotoxicological assessments

A. M. M. Abdul Rida & M. B. Bouché

Abdul Rida, A. M. M. & Bouché, M. B., Laboratoire de zooécologie du sol (INRA/CNRS), CEFE, BP 5051, F-34033 Montpellier Cedex, France

A study of heavy metals in soil (total, acetic acid and DTPA extractions) and in earthworms was made on 186 sites (more or less polluted by heavy metals) to evaluate earthworms both as a tool to assess biorisks (60 sites) and as contaminated prey for wildlife (126 sites). This paper demonstrates the poor assessment of risks given by soil analysis. In spite of some correlation between soil analyses and contents in earthworms, the total pattern of the contamination of earthworms is not predictable from soil analyses, including CEC, organic matter content, C/N ratio, etc. The genus *Scherotheca* could not survive in soil having more than 3.3 mg/kg of Cd and 410 mg/kg of Pb, while *Nicodrilus* spp. survived to 7.5 mg/kg of Cd and 8415 mg/kg of Pb. This susceptibility could be the result of a weak adaptation of *Scherotheca* (gigantic earthworms, *K*-selected) to environmental perturbation in contrast to the *Nicodrilus* group, today widespread in Europe. Such earthworm elimination could be used as a bioindication of contamination but limits the possibility to assess biorisks by bioaccumulation studies.

1. Introduction

Toxicological assessments aim to measure the possible harmful effects of chemical substances. Ecotoxicology effectuates this assessment within ecosystems, i.e. in biophysicochemical systems which operate spontaneously, although under partial human control by chemical substances. Ecotoxicology is therefore both biological (harmful effects on living organisms) and ecological (biophysicochemical). In terrestrial ecosystems chemical substances are sooner or later deposited and drawn into the soil where they remain for different lengths of time.

In the soil, earthworms are suitable for monitoring on toxicity, because:

1) They occupy a key place in ecosystem exchanges. They ingest mineral particles, micro-organisms and dead matter of vegetable and animal origin;
2) They constitute the most important animal mass. They are a key link in food webs: more than 190 vertebrates (Granval & Aliaga 1988) and numerous invertebrates (Lee 1985) feed on them.
3) They occupy an almost constant place in time and space, so the relation to each site is clear.
4) Compared to plants and other animals, they have a global body composition (proteins, lipids, ash, etc.) which is practically constant and allows one to relate contamination to a homogeneous reference (in contrast with the soil compartment).
5) They are linked only to soil contaminants, unlike plants, which can be contaminated by contact with the air around.
6) Whilst being one of the three main biomasses (along with plants and micro-organisms), they can be relatively easily separated from soil, unlike roots and micro-organisms.

For these reasons, earthworms form an important and privileged study object in terrestrial ecotoxicology. They have been studied as bioindicators with respect to mortality under the influence of toxic substances, and bioaccumulation at levels of individuals and food chains (Lee 1985, Greigh-Smith et al. 1992, etc.).

2. Material and methods

In order to link as closely as possible the contents of trace elements in earthworms with that of the soils, the "punctual procedure" was applied (Bouché 1990). This method consists of sampling earthworms and soil at single study points, without taking averages of several points. Soils and earthworms were sampled in seven zones of South-East France. The choice of the zones was made not necessarily to cover an area uniformly, but rather to cover diverse ecotoxicological situations: zones of industrial, urban and mining contamination, and zones expected to be uncontaminated, and soils with varying pedological and physicochemical characteristics. The different ecological categories of earthworm present in the study areas are as follows: endogeics (*Allolobophora rosea*, *A. chlorotica*, *Octolasion cyaneum* and *Nicodrilus caliginosus*), epianecics (only *Lumbricus terrestris*) and anecics belonging to two genera (*Nicodrilus* and *Scherotheca*), respectively *N. longus.*, *N. caliginosus meridionalis*, *N. nocturnus*, *N. longus ripicola*, *N. giardi* and *S. monspessulensis*, *S. gigas dinoscolex*, *S. dugesi sanaryensis*, *S. gigas rhodana*, *S. gigas gigas*.

Five heavy metals (Cd, Cu, Ni, Pb, Zn) were analysed in soils and earthworms. The earthworms were digested in concentrated nitric acid. For the soils, three techniques were used: 1) a mixture of two concentrated acids (HNO_3 and HCl) to get the "total" content, 2) acetic acid (2.5%), i.e. partial extraction, 3) DTPA (0.005 M), i.e. partial DTPA extraction. The partial extractions were used comparatively as they are often considered as means of measuring potentially available contents for plants (Follett & Lindsay 1971, Haq & Miller 1972, Lindsay & Norvell 1978).

3. Results

3.1. Bio-availability versus methods of extraction

Table 1 shows that the "bio-available" amounts vary according to the method of extraction and the metal concerned. Acetic acid extracts more Cd, Ni and Zn than DTPA and the opposite is true for Cu and Pb. The partial content extracted varies significantly depending on the physicochemical properties of the soils. For example, depending on the pH, the soluble quantities of the five metals vary immensely; this variation is shown in Fig. 1 for Cd. Table 2 shows the correlation between the two partial extractions on the one hand, and their correlation with total soil content on the other. The links are numerous and varied; however, the correlations are highly significant for Cu, Pb and Zn, whatever the technique used.

In order to understand the biological meaning of these total or partial extractions, Table 3 shows the correlation between soil contents on the one hand, and that in the earthworms on the other. The correlations vary greatly, depending on element and extraction method. However, Cu and Pb are highly correlated, whatever the technique used. But is it possible to predict the bioconcentration from soil contents in the form of a mathematical equation? Fig. 2 shows the graphs linking total soil contents of three elements (Cd, Pb and Ni) and

Table 1. Mean concentrations of trace elements (mg/kg) extracted from 186 soils according to the type of extraction used: total and partial (CH_3COOH or DTPA).

Element	Total	CH_3COOH	DTPA
Cd	4 ± 13.8	2.5 ± 12	0.8 ± 1.1
Cu	61 ± 51.0	4 ± 5.7	20 ± 27.5
Ni	25 ± 16.4	4.6 ± 3.6	1.5 ± 1.1
Pb	1034 ± 2060	74 ± 163.9	143 ± 319.6
Zn	358 ± 666	56 ± 173	38 ± 83.4

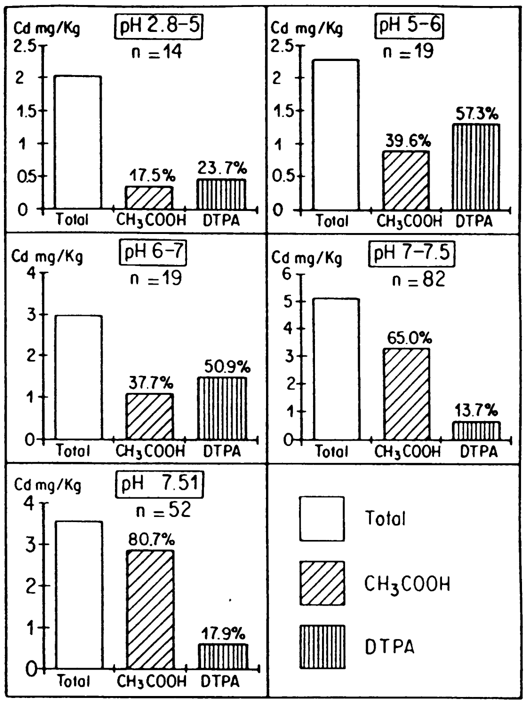

Fig. 1. Quantity of Cd extracted by total and partial extraction according to the initial pH-H_2O of soils.

Table 2. Significant correlations (*** $P < 0.001$, * $P < 0.05$) between the concentrations measured by the three different methods of extraction from 186 soils.

Element	Total versus CH_3COOH	Total versus DTPA	CH_3COOH versus DTPA
Cd	***	–	–
Cu	***	***	***
Ni	–	*	*
Pb	***	***	***
Zn	***	***	***

Table 3. Significant correlations (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) between concentration in earthworms and the three soil content estimates (total and partial) from 60 sites.

Element	Earthworms versus Total	Earthworms versus CH3COOH	Earthworms versus DTPA
Cd	**	**	***
Cu	***	***	***
Ni	–	–	–
Pb	***	***	***
Zn	**	*	*

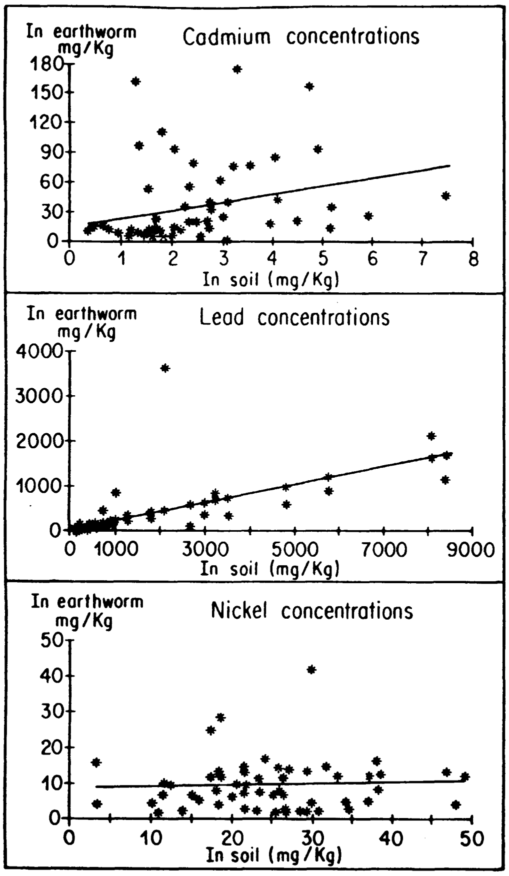

Fig. 2. Correlations between total soil content and earthworm bioconcentrations (60 sites each). Cd = $P < 0.001$, Pb = $P < 0.01$, Ni not significant.

bioconcentrations in earthworms. These elements differ in their levels of correlation: lead ($P < 0.001$), cadmium ($P < 0.01$), nickel (unsignificant). It was noticed that for Pb and Cd the linear model only translates a general positive relationship between bioconcentration in earthworms and total soil contents. However, these correlations do not allow us to predict that in the organisms. For example, in Cd, with concentration in the soil at around 1.2 mg/kg, the concentration in earthworms can vary from 7 to about 164 mg/kg! This does not mean that an analysis is completely meaningless: it is in fact the only information when absence of a species is noted as a result of mortal intoxication (see sect. 3.4.).

3.2. Earthworms as physiological bio-indicators

Physiological bio-indication refers to a change in an organism's functioning or its state resulting from contamination of its environment. These changes affect, for example, its growth, reproduction, respiration, and bioconcentration of trace elements. Depending on their ecological category, earthworms do not accumulate trace elements in the same way (Abdul Rida 1992); this must therefore be taken into account. Table 4 shows the mean contents in the earthworms studied, belonging to different ecological groups. These groups show great differences in contents and therefore in ability to adjust to trace elements. This has already been observed in certain earthworms by Piearce (1972), Morgan & Morris (1982) and Ireland (1983). But what is the biological significance of these values?

3.3. Accumulation in food chains

Earthworms represent an important part in the diet of many vertebrates. Therefore we calculated the daily quantities of trace elements consumed through earthworm ingestion for four predators (pig, badger, black-headed gull and woodcock). For these four animals there was sufficient bibliographical information available concerning the amounts of earthworms eaten daily (Cuendet 1979, 1983, Granval 1988, Kruuk 1978, Neal 1977 and Rose 1981a, b). The daily amounts ingested were compared with norms adopted in food toxicology. These levels are based on the harmful effects in animals (mammals or birds) in laboratory conditions, and enable us to establish a maximum daily dose acceptable for Man (FAO/OMS 1984). The amount of metals ingested daily by wildlife was divided by the maximum acceptable dose. This ratio, presented in Fig. 3, is always much higher than 1 (the maximum acceptable) except for copper in pigs (for 126 points of study). This study includes all zones whether contaminated or not. Even if locally the conditions for wildlife may be better, sites with more than 1000 times the acceptable level of contamination have been discovered (Abdul Rida 1992).

3.4. Earthworms as "existential bio-indicators"

Different species of earthworms accumulate trace elements in variable ways. Besides, they react differently according to their environment. It has been noted that *Scherotheca* spp.

Table 4. Mean concentrations (mg/kg) in earthworms belonging to endogeic, epianecic (*L. terrestris*) and anecic (*Nicodrilus*, *Scherotheca*) groups, as well as mean values of all of them, sampled at 60 sites in South-East France. (Modified from Abdul Rida & Bouché 1994.)

	Cd	Cu	Ni	Pb	Zn
Endogeic	46	113	9	475	502
L. terrestris	31	71	13	374	837
Nicodrilus	31	48	9	286	770
Scherotheca	39	38	7	65	967
Whole	35	69	9	283	770

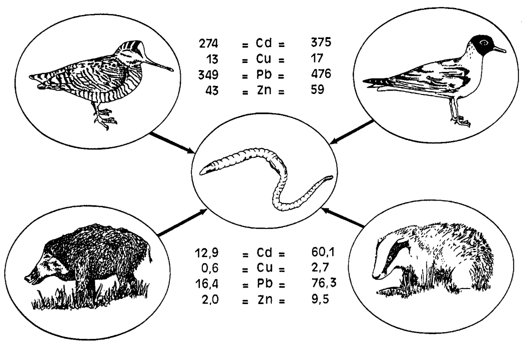

Fig. 3. Ratio of the calculated doses of trace elements consumed by four predators from 126 samples taken in South-East France, and the acceptable doses in human diet.

colonize soils least contaminated by trace elements compared to other species of earthworms (Table 5). Various hypotheses to explain such an observation have been discussed in another study (Abdul Rida & Bouché 1994). *Scherotheca* could be absent in certain soils for other reasons than pollution: soil property, climate, soil calcium content, sampling weakness or historical reasons. After dismissing opposing hypotheses, there only remains one: the contamination of soils by trace elements leading to eradication of an entire genus in all soils exceeding critical levels in metal content. To our knowledge, this is the first time when such an eradication has been brought to light.

4. Conclusion

Earthworms are a valuable source of ecotoxicological information concerning the state of the environment. It has been seen that observation of the earthworm-soil system: 1) does not allow us, by soil analysis alone, to predict bioavailability and thus the risks connected to contaminants, 2) enables us to assess bioavailability directly in a living compartment, 3) enables us to assess the risks in food chains, 4) enables us, thanks to certain sensitive species, at least in certain critical cases, to bring to light faunal eradication, which has gone unnoticed when conventional methods of observation have been used. Furthermore, when one realises the important part played by earthworms in nature, the ecotoxicological assessment of soils should and must imperatively take these organisms into consideration.

Table 5. Maximum concentrations (mg/kg) in the soils observed in the presence of *Scherotheca* and other earthworms at 186 sites. (Modified from Abdul Rida & Bouché 1994.)

	Cd	Cu	Ni	Pb	Zn
Scherotheca	3.3	134	121.5	410	1248
Others	19.4	237	88	10400	5808

References

Abdul Rida, A. M. M. 1992: Biosurveillance de la contamination du sol: apport de l'étude des lombriciens à l'évaluation des risques liés aux éléments traces. — Doc. Pedozool. 1(4):1–234.

Abdul Rida, A. M. M. & Bouché, M. B. 1994: The eradication of an earthworm genus by heavy metals in southern France. — Appl. Soil Ecol. (in press).

Bouché, M. B. 1990: Ecologie opérationnelle assistée par ordinateur. — Masson, Paris. 572 pp.

Cuendet, G. 1979: Etude du comportement alimentaire de la Mouette rieuse (Larus ridibundus L.) et de son influence sur les peuplements lombriciens. — Doc. Thesis. Conservation de la faune, Section protection de la nature et des sites du Canton de Vaud, Suisse. 111 pp.

— 1983: Predation on earthworms by the black-headed Gull (Larus ridibundus L.). — In: Satchell, J. E. (ed.), Earthworms ecology: from Darwin to vermiculture: 415–424. Chapman and Hall, London.

FAO/OMS, 1984: Codex alimentarius. Volume XVII. Contaminants. — Commission du Codex alimentarius. Rome.

Follett, R. H. & Lindsay, W. L. 1971: Changes in DTPA-extractable zinc, iron, manganese and copper in soils following fertilization. — Soil Sci. Soc. Amer. Proc. 35:600–602.

Granval, Ph. 1988: Approche écologique de la gestion de l'espace rural: des besoins de la Bécasse (Scolopax rusticola L.) à la qualité des milieux. — Doc. Thesis Univ. Rennes 1. 179 pp.

Granval, Ph. & Aliaga, R. 1988: Analyse critique des connaissances sur les prédateurs de lombriciens. — Gibier et Faune Sauvage 5:71–94.

Greigh-Smith, P. W., Becker H., Edwards P. J. & Heimbach F. 1992: Ecotoxicology of earthworms. — Intercept, Andover, Hants, U. K. 269 pp.

Haq, A. U. & Miller, M. H. 1972: Prediction of available soil Zn, Cu and Mn using chemical extractions. — Agron. J. 64:779–782.

Ireland, M. P. 1983: Heavy metal uptake and tissue distribution in earthworms. — In: Satchell, J. E. (ed.), Earthworm ecology: from Darwin to vermiculture: 247–265. Chapman and Hall, London.

Kruuk, H. 1978: Spatial organization and territorial behaviour of the European badger Meles meles. — J. Zool. London 184:1–19.

Lee, K. E. 1985: Earthworms: their ecology and relationships with soils and land use. — Academic press, 411 pp.

Lindsay, W. L. & Norvell, W. A. 1978: Development of a DTPA soil test for zinc, iron, manganese, and cooper. — Soil Sci. Soc. Amer. J. 42:421–428.

Morgan, A. J. & Morris, B. 1982: The accumulation and intracellular compartmentation of cadmium, lead, zinc and calcium in two earthworm species (Dendrobaena rubida and Lumbricus rubellus) living in highly contaminated soil. — Histochemistry 75(2):269–285.

Neal, E. G. 1977: Badgers. — Blandford Press, Poole, Dorset. 321 pp.

Piearce, T. G. 1972: The calcium relations of selected lumbricidae. — J. Anim. Ecol. 2(1):167–188.

Rose, C. J. 1981a: Preliminary observations on the performance of village pigs (Sus scrofa papuesis) under intensive outdoor management. Part I. Dietary intake and liveweight gain. — Science in New Guinea 8(2):132–140.

— 1981b: Preliminary observations on the performance of village pigs (Sus scrofa papuensis) under intensive outdoor management. Part II. Feed conversion efficiency, carcase composition and gastro-intestinal parasites. — Science in New Guinea 8(3):156–163.