PU238

ELSEVIER

Applied Soil Ecology 2 (1995) 45-52

Applied Soil Ecology

The eradication of an earthworm genus by heavy metals in southern France

A.M.M. Abdul Rida*, M.B. Bouché

Laboratoire de Zooécologie du Sol (INRA), 1919 Route de Mende, CEFE/CNRS, BP 5051, 34033 Montpellier, France

Accepted 11 July 1994

Abstract

During the course of a broad study of polluted and non-polluted soils in southeastern France, we noticed that in contrast to other earthworms, the genus *Scherotheca* was only present in certain soils. Given the omnipresence of these invertebrates in the economy of nature, notably serving as food for a great number of birds and mammals, contributing to soil fertility, and maintaining high levels of water infiltration (thus reducing surface erosion in various soil profiles), we sought to determine what was responsible for the unusual absence of these animals in certain parts of our study area. For this purpose, we tested alternative hypotheses dealing with biogeographic, pedological and climatic factors before coming to the conclusion that it was a result of heavy metal contamination. We then proceeded to test all the potentially toxic heavy metals present in the soil in order to determine which one was responsible for the absence of *Scherotheca*. The results show that *Scherotheca* is highly susceptible to lead and copper.

Keywords: Earthworm; Heavy metal; *Scherotheca*; Soil pollution; Toxic hazard

1. Introduction

As early as 1837 and then again in 1881, Darwin called attention to the important ecological role that earthworms play in many terrestrial ecosystems. In fact, they constitute a major component in soil functioning (Edwards and Lofty, 1977; Lee, 1985), as a physical tool (burrowing, aeration, infiltration, ...), as one of the principal sources of animal protein for many predators (Lee, 1985; Granval and Aliaga, 1988) and as a major compartment in chemical element cycles (Lee, 1985; Ferrière, 1986) including heavy metal transfer to their predators (Abdul Rida, 1992; Abdul Rida and Bouché, 1994a). They reach on average, 1 ton F.Wt. ha^{-1} in soils and up to 3 tons F.Wt. ha^{-1} in permanent pastures.

Earthworm species can be classified into ecological categories on the basis of morphological characteristics which have functional significance (Bouché, 1971): epigeics, endogeics, anecics, and the epianecics which are intermediate between epigeics and anecics.

The epigeics live in the organic matter such as litter or dung patches above the mineral soil. Exposed to both predators and drought, they are homochromic (i.e. generally red brown like dead leaves), small (10–30 mm) and very prolific (r-strategists). They have no capacity to regenerate

* Corresponding author: Tel. (33)67.61.32.59, Fax. (33)67 41 21 38.

0929-1393/95/$09.50
SSDI 0929-1393(94)00032-8

lost segments nor do they enter diapause.

Endogeic earthworms live at a moderate depth (60 cm), in sub-horizontal burrows, feeding on soil more or less enriched with organic matter. These animals are non-pigmented, of medium size (often 20–50 mm) and do not have a diapause. Size, regeneration capacities and reproduction strategies are variable.

Anecic earthworms are of large size (50–1100 mm), living in sub-vertical burrows which reach a depth of at least 1 m. They feed at night on the organic matter which has accumulated on the soil surface, mixing it with mineral layers. These animals are darkly pigmented and have a true diapause. They are often dominant (80% of earthworm biomass) and consequently play a major role in functions describes above, especially in air and water soil circulation. In our study the anecic category was clearly sampled by two groups: (1) a gigantic earthworm group (adults greater than 250 mm) represented exclusively by *Scherotheca* spp., (2) a normal sized anecic group (230 mm >adults >80 mm) where all earthworms belong in the *Nicodrilus* genus. In this study *Lumbricus terrestris* are the sole member of the epianecics. It has the same way of life as the anecics. However, it has brown–purple pigmentation and has no diapause.

In a general survey made in southeast France we observed the frequent absence of *Scherotheca*, in contrast with the regular presence of other earthworm genera. This abnormal distribution can be explained by various hypotheses; specific soil or climatic requirements or differentiated historical establishment (paleogeographic event) or high susceptibility to pollutants. The purpose of this paper is to test each of the hypotheses.

2. Materials and methods

2.1. Study areas

Soils and earthworms were sampled in 186 points belonging in six areas of southeast France (Fig. 1). The choice of study areas took into account diverse pedological and physiochemical characteristics, as well as various levels of heavy metal contaminations. Each study point was sampled by the 'punctual process' (Bouché, 1990), i.e. samples were taken from each local geographical point for soil and earthworms living closely associated (in practice, few square decimeters of soil were hand sorted for earthworms and the same soil was sampled for chemical analyses).

Earthworms were chemically analyzed for two reasons

(1) To assess the true body burden concentration, i.e. without the gut content, this gut content which was too small to be individually analyzed was eliminated. This elimination was made on earthworms from 60 sites (labelled A sites) to estimate the bioconcentration of heavy metals.

(2) To measure the prey content, for earthworm predators, we analyzed the 'full' (body+gut) content ingested by these predators in the remaining 126 sites (labelled B sites).

2.2. Species sampled and their grouping

The species observed in sites could be divided in four groups: (1) The endogeics. *Nicodrilus caligino. ıs caliginosus*, *Allolobophora rosea*,*Allolobophora chlorotica*, *Octolasion cyaneum*; (2) *Scherotheca*. The gigantic anecics (*Scherotheca gigas*, *Scherotheca monspessulensis*, *Scherotheca dugesi*, *Scherotheca dinoscolex*, *Scherotheca rhodana*); (3) *Nicodrilus*. The genus of 'normal sized anecics' (*Nicodrilus giardi*, *Nicodrilus caliginosus meridionalis*, *Nicodrilus longus*, *Nicodrilus nocturnus*); (4) *Lumbricus terrestris*. The sole epianecic. The epigeics are not represented, because they are very scarce in these dry regions.

2.3. Chemical analyses

Soil physico-chemical properties were analysed (Table 1). In addition, eight metals (Ca, Cd, Cu, Fe, Mn, Ni, Pb, Zn) were analysed in soils and earthworms (Abdul Rida and Bouché, 1994a).

Earthworms were dissected to eliminate the gut content, then oven-dried in glass flasks at 105°C.

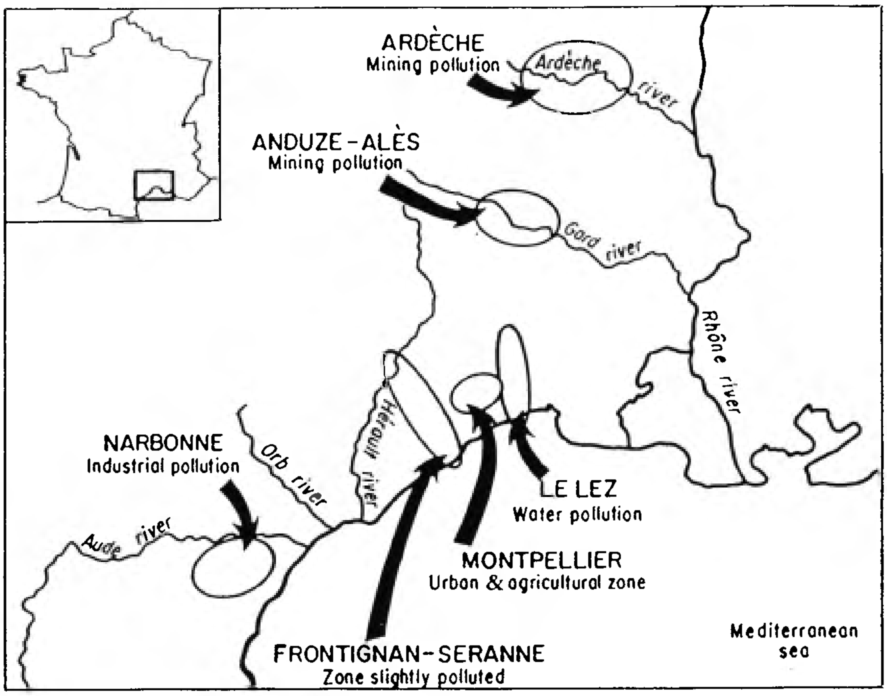

Fig. 1. Location of study areas in southeast France and their pollution sources.

Table 1
Soil physico-chemical properties where each earthworm group is present among the 60 A sites (mean values)

	pH	O.M (%)	N (%)	C/N	Clay (%)	Loam (%)	Sand (%)	CEC (meq/100 g)
Scherotheca spp.	7.3	2.4	0.1	10.7	19.3	27.2	53.5	10.3
Nicodrilus anec.	7.0	2.8	0.2	10.5	19.6	26.0	53.5	10.5
L. terrestris	6.8	2.2	0.1	10.2	15.0	23.2	59.2	8.1
Endogeics	7.4	3.3	0.2	10.9	18.2	31.8	50.0	11.1

The dried earthworms were digested in nitric acid at a rate of 5 ml HNO_3 per 100 mg dry weight of earthworm. After digestion, samples were diluted with deionized water in the same flasks to get an acid concentration of 10% HNO_3 according to the acid quantity used for mineralization.

'Total' heavy metals were extracted from the soils with HNO_3 and HCl acids. One gram of oven-dried soil at 105°C was digested in 5 ml HNO_3 for 5 h at 160°C. The mixture was cooled before the addition of 5 ml HNO_3 and 5 ml HCl and the mixture was heated again for 5 h at 160°C. After cooling, the samples were diluted with deionized water to achieve a volume of 100 ml.

The concentrations of elements were determined with an atomic absorption spectrophotometer, using an air-acetylene flame.

3. Results and interpretations

Among the large amount of data collected we noticed that only 39 sites (out of 186) were occupied by representatives of the *Schrotheca* genus compared with endogeics (88), *Nicodrilus*

(62), epigeics (26) and *Lumbricus terrestris* (19). As quoted above epigeics are rare in the localities that we sampled because of drought. The cause of the scarcity of *Lumbricus terrestris* group was clear and well known; this species lives only in permanently wet soils (Bouché, 1984) which are rare in this region. The *Scherotheca* were sampled into the central part of their biogeographic area and were observed only in rather clean soils. The presence or absence of *Scherotheca* could not be explained by a single factor alone, many causes could be advocated: historical establishment of the genus and/or a pedo-climatic selection and/or the need of particular soil properties, and finally toxic hazards from pollutants. These hypotheses were tested.

3.1. Hypothesis one: historical effect

The members of the genus *Scherotheca* found in southeast France are of relatively large size and are poor colonizers of new sites. Their distribution in southern France and northern Spain is the result of paleogeographic events of the Tertiary, followed by the Quaternary glaciation episodes (Bouché, 1983). In this sample, detailed observations in the Ligne river valley (Ardèche, France), in the centre of their distribution area, revealed only seven sites in which the genus was present (on soils with relatively low contamination) as compared with 23 contaminated sites from which the genus was absent (Fig. 2). This distribution pattern can not be explained by the poor colonizing ability of members of this genus because the sites are very close together and all of them could be colonized in a short time span.

3.2. Hypothesis two: climatic effect

In spite of the climatic differences between the six areas studied (from 'hot' mediterranean climate to rather 'cold' submountainous climate), the *Scherotheca* are generally present in all these areas (Fig. 1). In addition, the climatic differences between the sites in the same valley (Fig. 2) are very weak. Thus, the climate could not be the key factor.

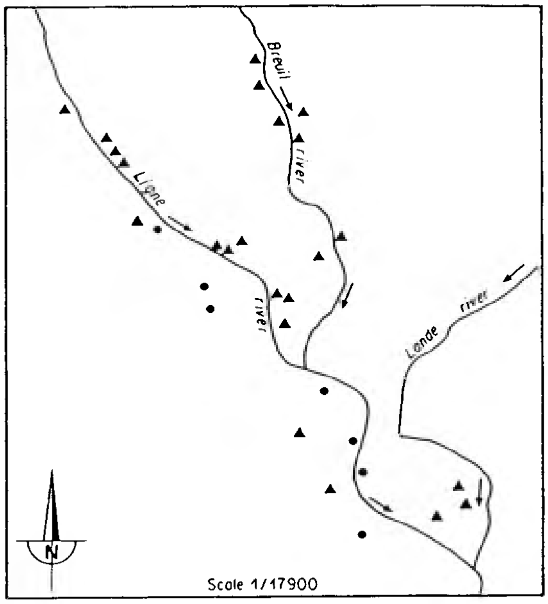

Fig. 2. Studied sites in the Ligne river valley area (Ardèche, France). With *Scherotheca* (●), without *Scherotheca* (▲).

3.3. Hypothesis three: soil properties effects

The study area, which includes a large portion of southeastern France (Fig. 1), includes both calcareous and acidic (granitic substrate) soils. However, in spite of these variations, *Scherotheca* are generally present throughout. Table 1 shows that there are no important chemical or physical differences between the soil types of the sites in which the four different earthworm categories were observed. Clearly, the edaphic characteristics do not account for the unusual distribution of *Scherotheca* in our study area.

The concentration of calcium in soils and earthworm bodies was also compared because of the importance of this element in the Morren's gland functioning in earthworm physiology. Looking at Table 2 for *Scherotheca*, a relatively high calcium mean and maximum soil content was observed. In fact, this genus inhabits soils with a wide range of calcium concentration, down to 383 p.p.m. (Abdul Rida, 1992). However, their calcium body burden is low and clearly lower than *Lumbricus terrestris* and endogeics groups (Table 3). Thus, it can be concluded that *Scherotheca* occupied a wide soil calcium spec-

Table 2
Mean and (maximum) levels of studied elements (in mg kg^{-1}) in soil where each earthworm group is present among the 60 A sites

	Cd	Cu	Zn	Pb	Ni	Mn	Fe	Ca
Scherotheca spp.	2.4(3.3)	43(108)	360(1248)	145(364)	24(34.6)	353(897)	19965(30390)	72676(230800)
Nicodrilus anec.	2.6(7.5)	50(174)	490(1682)	1086(8415)	24(49.0)	777(2325)	30347(64930)	15430(198950)
L. terrestris	2.2(7.5)	61(156)	451(1507)	1437(8100)	18(37.2)	536(1457)	23774(50115)	16550(140200)
Endogeics	2.8(7.5)	84(237)	489(1507)	1380(4797)	27(49.0)	666(1457)	30075(55235)	49470(203850)

Table 3
Mean and (maximum) loads of studied elements (in mg kg^{-1}) in the four earthworm groups studied in the 60 A sites

	Cd	Cu	Ni	Pb	Zn	Mn	Fe	Ca
Scherotheca spp.	39(236)	38(193)	7(17)	65(260)	~~970~~ 987(3267)	~~10~~ 100(178)	1326(3196)	5645(14273)
Nicodrilus anec.	31(234)	48(215)	9(51)	286(6339)	770(7142)	145(392)	1487(6283)	1941(7582)
L. terrestris	31(102)	71(379)	13(37)	374(1640)	837(4757)	215(1286)	2124(9821)	7013(34810)
Endogeics	46(271)	113(697)	9(30)	475(4685)	502(1541)	183(493)	2555(13925)	6192(38306)

trum and the calcium content of soil is not a limiting factor. So no soil properties explain the abnormal distribution of *Scherotheca*.

3.4. Hypothesis four: soil disturbance

As cited in hypothesis one, *Scherotheca* earthworms are relatively poor colonizers. They might be eliminated from disturbed soils especially b_ mining activities, and unable to recolonize such sites. Therefore, samples were taken from soil not previously disturbed with the exception of abandoned vineyards which had been ploughed (ploughing does not eliminate earthworms). Most soil had been contaminated by aerial dispersion of aerosols, dusts or copper sulfid sprayings and some by alluvial deposits from spoil heaps. Soil disturbance alone cannot explain the selective absence of *Scherotheca*.

3.5. Hypothesis five: toxic hazard effects

Mean and maximum values of Cu, Zn, Pb, Mn and Fe, in the soils where *Scherotheca* were present, differ markedly from those observed in sites where the other three earthworm groups were found (Table 2). Maximum values of Cd also differed significantly. Observations showed that *Scherotheca* contained low levels of heavy metals, even when considering individual maximum values (Table 3). This is very clear for Cu and Pb, and also, to some extent, for Mn and Fe. However, Cd and Zn do not show the same pattern. This low body content reflects their presence in low contaminated soils, but it is relatively higher than the other genus found because the *Scherotheca* bioaccumulation is higher in Cd, Pb, and Zn (Table 4).

To interpret such observations, they must be considered in their ecological context as no analytical characteristic is totally independent.

Thus, in soil samples, Pb, Zn and Ca are highly intercorrelated at $P<0.001$ (Pb and Zn positively and Ca negatively). Moreover, Cd and Cu are highly intercorrelated, as are Mn and Fe (both pairs at $P<0.001$). In other words we have three groups of non-independent chemical elements; (Pb-Zn-Ca, Cd-Cu and Mn-Fe).

In comparison with other earthworm groups, *Scherotheca* Pb and Cu are the only two elements investigated that show distinct limiting concentrations, both in the surrounding soil (Table 2), and in the earthworm bodies (Table 3). Clearly these two elements are toxic and *Scherotheca* could not settle in contaminated soils with Pb and/or Cu (Fig. 3) and could not accumulate

Table 4
Bio-accumulation ratios of the four earthworm groups in the 60 A sites

	Cd	Cu	Ni	Pb	Zn	Mn	Fe	Ca
Scherotheca spp.	16.2	0.89	0.28	0.45	2.69	0.28	0.07	0.08
Nicodrilus anec.	11.8	0.97	0.39	0.26	1.57	0.19	0.05	0.13
L. terrestris	14.2	1.16	0.72	0.26	1.86	0.40	0.09	0.42
Endogeics	16.5	1.33	0.34	0.34	1.03	0.27	0.08	0.13

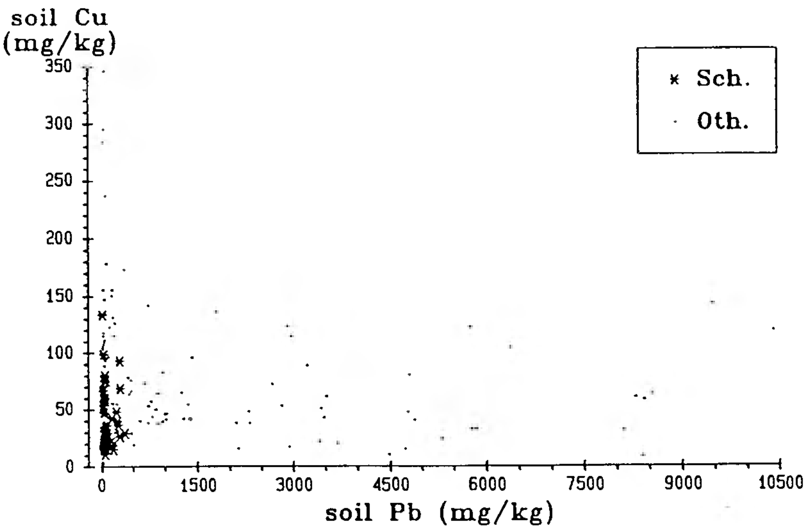

Fig. 3. Distribution of *Scherotheca* (*) and others genus (·) according to soil copper and lead concentrations.

Table 5
Mean and (maximum) levels of studied elements in soil where each earthworm group is present among the 60 A+126 B sites

	Cd	Cu	Zn	Pb	Ni	Mn	Fe	Ca
Scherotheca spp.	1.9(3.3)	44(134)	168(1248)	101(410)	29(122)	384(2336)	20473(67675)	110173(298000)
Nicodrilus anec.	2.6(19.4)	53(174)	455(4922)	1121(10400)	24(57)	678(3013)	29264(67660)	53252(294000)
L. terrestris	2.6(19.4)	59(143)	493(5808)	2359(9463)	18(88)	498(1457)	23860(58025)	31424(234000)
Endogeics	2.8(19.4)	68(237)	303(5808)	664(10400)	24(88)	418(3013)	22542(67660)	116727(298000)
Epigeics	3.7(19.4)	65(174)	819(5808)	3675(10400)	20(88)	384(1447)	27919(67660)	39598(270000)

$n=39$ with *Scherotheca* spp., 62 with *Nicodrilus* spp., 19 with *L. terrestris*, 88 with endogeics and 26 soils with epigeics.

them. So, in the group of intercorrelated elements Pb-Zn-Ca clearly the toxic factor is Pb. The same for Cu-Cd; the first one is the toxic element. The intercorrelated couple Mn-Fe (Tables 2 and 3) appears also to be toxic, but this observation is not so clear. It is somewhat difficult to ascertain the toxicity of the couple Mn-Fe because it is strongly and positively correlated with Pb ($P<0.01$). To extend our sample we add to our study the 126 soil analysis of B sites where a prey accumulation was done. In this sample we have no bioaccumulation estimates but data related to epigeics (absent in A sites). Table 5 presents this global results as in Table 2.

This extension confirmed that the pairs Cu-Cd and Pb-Zn are responsible for the absence of *Scherotheca* from the most contaminated soils while suspected Ca, Mn and Fe do not limit this genus in a wider sample. Notice also that epigeics are not especially susceptible to heavy metals.

Among all hypothesis and observed facts we can conclude that the eradication (or non-settlement for natural high levels) of *Scherotheca* results only from the high susceptibility of this genus for lead and copper.

4. Discussion

To demonstrate an ecotoxicological event on the presence/absence of organisms, there is a need to eliminate other factors playing a role in their distribution, including the historical one (establishment). To isolate 'the' factors explaining the absence, it is necessary to sort among a multivariate surrounding description. Correlations between variables increase the difficulty of such an interpretation.

It should be noted that it is only through field studies that ecotoxicologists can demonstrate correlations between local absences and real-world chronic toxicities. In these studies, the widely used bio-accumulation ratio (concentration of a toxic substance in the earthworms divided by the concentration of the same toxic substance in the soil) was avoided. This approach is misleading as it compares soil physical processes with the metabolic regulatory activities of earthworms, i.e. two very different processes (Abdul Rida, 1992). To illustrate this point, a comparison of the bioaccumulation ratios between the four groups of earthworms described above is made (Table 4). Thus, despite the important differences in soil and earthworm heavy metal concentrations (Table 2 and 3), the differences between bio-accumulation ratios were negligible or erratic for the four earthworm groups.

The absence of *Scherotheca* in slightly Pb and/or Cu contaminated soils is mostly an eradication. Some soils in the studied areas are naturally rich in Pb but the contamination observed in this study is due primarily to human activities (Pb, largely through mining activities and from automobile exhausts; and Cu, mainly from pesticides). This eradication has gone unnoticed until now because it concerns subterranean and poorly studied animals (the earthworms), and because it has a patchy distribution in contaminated soils which are also too poorly studied. This may be the first reported case of an eradication of an entire earthworm genus, as the result of heavy metal contamination.

It is noteworthy that the other four groups of earthworms in this study area tolerate soil toxicity much better than *Scherotheca*. Why should this genus be so much more sensitive, even than *Nicodrilus*, with which it shares the same ecological category, i.e. way of life? As mentioned above, the major difference between the two genera is in their respective sizes: in our sample, adult *Nicodrilus* weigh on average 1922 mg while adult *Scherotheca* weigh on average 8017 mg. Accordingly, the two groups have contrasting demographic strategies. *Nicodrilus* reach their adult stage more rapidly than *Scherotheca*, and reproduce more prolifically. Thus, it is possible that the lower generation turnover of *Scherotheca* results in a slower adaptation to changing environmental conditions.

However, even if the other earthworms are able to survive with high levels of heavy metals in their bodies, there may still be severe ecological consequences as they constitute one of the primary sources of protein for many animals in terrestrial ecosystems. For example, approximately 200 vertebrate species feed on them in western Europe alone. Some, such as badger, woodcock, and, to a lesser extent, fox and wild boar, depend heavily upon them. As a result, animals eating contaminated earthworms will far exceed the recommended daily admissible dose of such metals (FAO/OMS, 1984; Abdul Rida, 1992).

Scherotheca are large animals among earthworms and they play an important role in the ecosystems of southern France. Through their continual activity in the soil they are able to maintain water infiltration levels of up to 50 cm h^{-1} (Al Addan et al., 1991). Their eradication may thus increase the likelihood of surface erosion and the severe flooding that periodically occurs in this region (as in the dramatic overflow at Vaisons-la-Romaine, France, in 1992). This is particularly important in agricultural areas such as vineyards where Cu is widely used in fungicides.

This study illustrates the need to extend ecotoxicological studies into the field. By doing so, we were able to document the extermination of an entire earthworm genus due to heavy metal pollution. This knowledge is needed to evaluate direct toxic hazards but also the indirect consequences of the effect of pollutants.

Acknowledgements

The authors would like to thank J. Aronson, M. Gould and K.O. McTiernan for translating the original manuscript and for helpful comments. Also Régine Aliaga for the secretarial assistance.

References

Abdul Rida, A.M.M., 1992. Biosurveillance de la contamination du sol: apport de l'étude des lombriciens è l'évaluation des risques liés aux éléments traces, Doc. Pédozool., vol. 1, Fasc. 4, France, 1-234.

Abdul Rida A.M.M. and Bouché, M.B., 1994a. Earthworm contribution to ecotoxicological assessments. Acta Zool. Fenn. (in press).

Abdul Rida, A.M.M. and Bouché, M.B., 1994b. A method to assess chemical biorisks in terrestrial ecosystems. In: M. Donker, H. Eijsakers and F. Heimbach (Editors), Ecotoxicology of Soil Organisms. Lewis Publishers, Boca Raton, FL, pp. 383-394.

Al Addan, F., Aliaga, R., and Bouché, M.B., 1991. Relations entre peuplements lombriciens et propriétés des sols méditerranéens. In: G.K. Veeresh, D. Rajagopal and C.A. Viraktamath (Editors), Advances in Management and Conservation of Soil Fauna. Oxford & IBH Publishing, New-Delhi, pp. 525-537.

Bouché, M.B., 1971. Relations entre les structures spatiales et fonctionnelles des écosystèmes illustrées par le rôle pédologique des vers de terre. In: P. Pesson (Editor), La Vie Dans les Sols. Gauthier-Villars, Paris, pp. 187-209.

Bouché, M.B., 1983. The establishment of earthworm communities. In: J.E. Satchell (Editor), Earthworm Ecology from Darwin to Vermiculture. Chapman and Hall, London, pp. 431-448.

Bouché, M.B., 1984. Les modalités d'adaptation des lombriciens à la sécheresse. Bull. Soc. Bot. Fr., 131 Actual Bot., 2/3/4, pp. 319-327.

Bouché, M.B., 1990. Ecologie opérationnelle assistée par ordinateur. Masson, Paris, pp. 1-572.

Darwin, C., 1837. On the formation of mould. Trans. Geol. Soc., 5(2): 505-509.

Darwin, C., 1881. The formation of vegetable mould through the action of worms, with observations on their habits. Murray, London, pp. 1-298.

Edwards, C.A. and Lofty, J.R., 1977. Biology of Earthworms. Chapman and Hall, London, pp. 1-333.

FAO/OMS, 1984. Codex alimentarius, XVII Contamination, Commission du Codex Alimentarius, Rome.

Ferrière, G., 1986. Mouvements naturels des éléments dans une prairie: quantification des échanges d'azote entre lombriciens, sol et plantes. Thesis Doctorat, Univ. Lyon I, France, pp. 1-148.

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

Lee, K.L., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press, Australia pp. 1-411.