ECOTOXICOLOGY OF SOIL ORGANISMS

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CHAPTER 28

A Method to Assess Chemical Biorisks in Terrestrial Ecosystems

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I. INTRODUCTION

The aim of an ecotoxicological appraisal is to assess chemical effects on living components of ecosystems.[1] In short, ecotoxicology is toxicology in ecological conditions, i.e., falsifiable in fields. Analytical studies, particularly in microcosms, are too restricted to assess biorisks directly. We must get our information from ecosystems. However, it is difficult to select in ecosystems key variables among the great number of ecosystem characteristics.

There are two kinds of chemical biorisks:

1. Risks suffered by organisms from a chemical
2. Risks coming from an organism having a toxic burden and contaminating its predators

The first one may be observed from the presence (= survival) or the absence (= intoxication) of an organism assumed as a bioindicator in an ecosystem. The second one results from the transfer of chemical from an apparently healthy (but burdened) "prey" to a poisoned "predator" in a food chain.

The assessment of these biorisks in terrestrial ecosystems are a very difficult task:

- because most of the pollutants reach soils (i.e., a great chemical and origin diversity)
- because soil is a very complex three-phase system (air/water/solid)
- because soils are very variable in space and time from a minute crumble to a landscape
- because most organisms (plants, animals, and microorganisms) are dependent on abiotic solid fractions (various minerals and dead organic matter)
- because the concept of food chain involves a clear relationship between a "prey" and a "predator" while *in fact* ecosystem compartment relations are both a succession of organic/inorganic transfers[2] and a food web
- because the soil concentration is a ratio depending on the pollutant amount *and* on the density of the matrix which could vary 15-fold on a volume basis[3]
- because the true availability of the contaminant depends of numerous soil properties and could not be measured by a chemical partial extraction

It seems easier to assess chemical biorisk directly on biotic compartments than on abiotic fractions which are questionable. We need an organism:

1. which plays a central role in ecosystems, closely connected with plant and microorganism biomass,
2. present in space and time the greatest variety of terrestrial ecosystems independent of local and seasonal conditions,
3. with a rather constant dry weight density,
4. which is not too susceptible to contaminants (to get it alive in food chain studies),
5. easy to sample and analyze.

Due to the great diversity of plant relations with their milieus (atmosphere, soil, rain) and plant composition, and the great difficulty to sample directly the microorganism biomass, earthworms as a whole seem to be the sole almost ubiquitous biological group able to fulfill the conditions described above.

Bioindications are of two basic types. The *existential bioindication* describes in ecosystems, by the presence or absence of the observed organism, the upper limit below which this organism could survive and the *physiological bioindications* observed on biological properties of surviving organisms: as, for example, respiration, reproduction, bioconcentrations, enzymatic inductions, etc. Notice that the comparison of ecosystems by physiological characters are limited by the organism survival. Among physiological bioindications, bioconcentrations have the advantage of relating the chemical directly with the organisms.

We choose to study the existential bioindications (survival) and heavy metal bioconcentrations in earthworms to estimate their potential risk in food chains. Earthworms are eaten in West Palearctic[4] by about 200 vertebrate species and an unestimated number of invertebrate predators.[5] They are the main terrestrial animal biomass available to predators (about 1 t fresh weight per hectare [average] in France).

II. MATERIALS AND METHODS

186 sites have been sampled using the "punctual" approach,[6] i.e., in each "point" a sample with closely related biotic and abiotic soil characteristics. These points were selected in six geographical areas with various levels of urban, industrial, or agricultural pollution and very different soil types and human influences (Figure 1). Research was limited to easy-to-analyze and nondegradable substances, i.e., heavy metals as models of chemical pollutants.

Existential bioindication was studied by the presence or absence of earthworms. To study earthworms as a prey compartment, earthworms and their gut contents were analyzed in 126 points. To understand the metabolic accumulation of heavy metals (= *physiological bioindication*), earthworms from 60 points were dissected, eliminating their soil gut content, and analyzed thereafter. In total 790 earthworms, belonging to different taxa and ecological categories, were analyzed. To relate soil concentrations and properties to earthworm concentrations, one total and two partial analyses were performed. Some soil properties (pH, texture, organic matter, C/N, cation exchange capacity, exchangeable cations) were also measured. All analytical data of these 186 points are managed and available in the Relational Data Base ECORDRE.[7] These data were analyzed by a great variety of different statistics.[8]

A. Analyses of Earthworms

The animals were weighed and oven dried in glass flasks at 105°C for 24 h. After reweighting nitric acid was added (5 ml HNO_3 for 100 mg dry earthworm

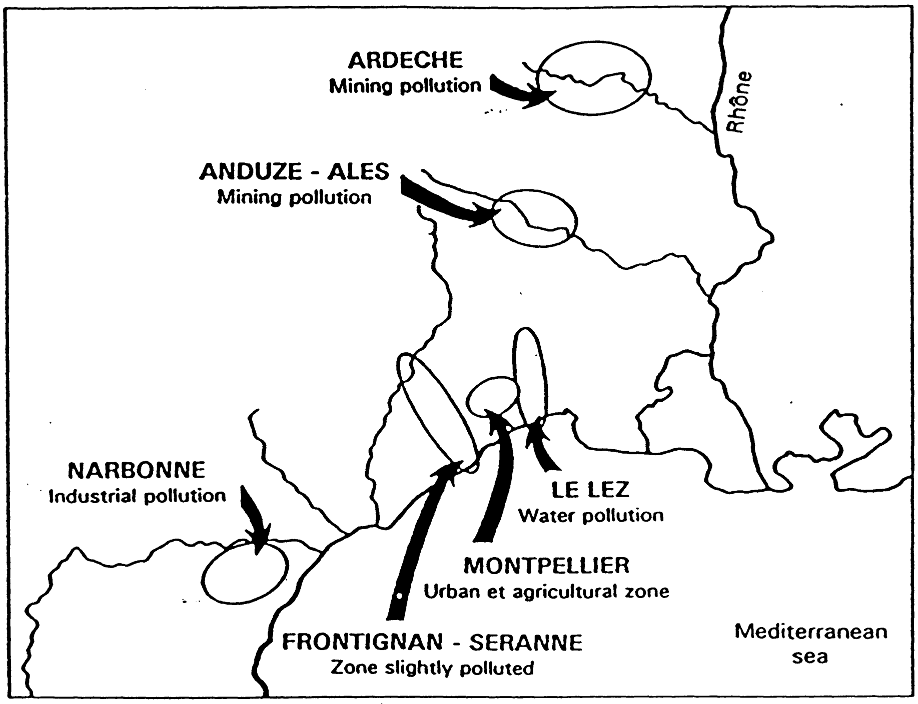

FIGURE 1. Localization of studied areas (South France).

weight), heated at 70°C for 24 h and then diluted with deionized water to 10% acid.

B. Analyses of Soils

The total extraction of trace elements was carried out by mineralizing 1 g of air-dried soil (sieved fraction <0.5 mm) with 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. The mixture was heated again for 5 h at 160°C, thereafter cooled and diluted with deionized water to 100 ml.

The partial extractions of trace elements were made from soils by two extractants: acetic acid and diethylene triamine pentaacetic acid (DTPA). (1) Acetic acid: 3 g air-dried soil (sieved fraction <2 mm) and 60 ml of an aqueous acetic acid solution (2.5%) were shaken for 1 h. Then, the suspension was filtered before titration. (2) DTPA: a water solution was prepared with 0.005 *M* DTPA, 0.01 M $CaCl_2$ and 0.1 *M* triethanolamine (TEA)[9] and adjusted to pH 7.3 with HCl, then 30 g air-dried soil (sieved fractions <2 mm) and 60 ml of this solution were shaken for 2 h. Then the suspension was filtered before titration.

Table 1. Comparison Between Mean and Maximum Values of Total Heavy Metal Content of Soils Where *Scherotheca* and Other Genera Are Present

	Cd mg/kg	Cu mg/kg	Ni mg/kg	Pb mg/kg	Zn mg/kg
Scherotheca					
Mean	1.9	44	29	101	168
Maximum	3.3	134	122	410	1248
Other genera					
Mean	2.8	62	23	1376	439
Maximum	19.4	237	88	10400	5808

The concentrations of elements were determined with a Varian® AA-SPECTRA atomic absorption spectrophotometer (using an air-acetylene flame) and appropriate standards.

III. RESULTS

A. Existential Bioindication and a Bias in the Sampling of Bioconcentrations

It is classical to consider the presence or absence of organisms to interpret some environmental factors, as for example the use of calcifuge plants as an indicator of soil pH. Looking at our general results we were surprised to show such an indication for heavy metal pollutions. In the general sample of 186 points of the Mediterranean South France with diverse levels of soil contamination we observed that the genus *Scherotheca* was particularly sensitive to high soil levels of Cd, Cu, Pb, and Zn in contrast to other earthworms (Table 1).

A more careful study of their distribution demonstrates that competing hypotheses (historical bias on population settlements; need of high level of Ca) does not explain such an eradication of this genus in all polluted sites.[10] From a methodological point of view, we can use such a demonstration to use this genus as a bioindicator. We need also to take into account the bias introduced by the absence of *Scherotheca* in the most polluted soils. Their susceptibility seems generally higher than others earthworms if we compare their bioconcentration and bioaccumulation (bioconcentration: ratio of metal content weight to total dry tissue weight; bioaccumulation: ratio of bioconcentration to total soil concentration) (Table 2). They concentrate particularly more Cd and Zn than other earthworms.

B. Concentrations of Metals in Soil and Earthworms

We focused our study on 60 points having a great variety of heavy metal burdens for analysis of soils (total, acetic acid and DTPA extraction) and the

Table 2. Comparison Between Mean Bioconcentration (mg/kg) and Bioaccumulation for *Scherotheca* and Other Genera

	Cd	Cu	Ni	Pb	Zn
Scherotheca					
Bioconcentration	38.9	37.8	6.6	65.2	967.0
Bioaccumulation	16.1	1.1	0.3	0.3	7.9
Other genera					
Bioconcentration	34.3	66.1	9.7	342.0	717.0
Bioaccumulation	16.0	1.1	0.6	0.4	3.1

concentration of metals in 279 dissected earthworms which were analyzed in two groups:

1. 30 points concerning the Ardèche, where soil characteristics and trace element sources are almost similar,
2. 30 points concerning five different areas labeled as "others" where soil characteristics and the trace element sources are variable (Table 3).

The comparison between levels of soil trace elements and earthworm burden (Table 4) shows discrepancies between quantities of trace elements extracted by total analysis methods and partial analysis methods. This comparison shows also a considerable difference between trace element patterns of soils and earthworms.

The concentrations in earthworms are higher for Zn and in particular for Cd, but are lower in Ni and Pb than in soil. Cu concentration is the same in both soils and earthworms. Concentrations of Pb and Zn in earthworms increase

Table 3. Mean Values of Physicochemical Characteristics for Soils From Different Areas

	Ardèche	Others
Water pH	6.8	7.4
KCl pH	6.3	7.0
Clay %	19.3	19.8
Fine silt %	15.6	21.1
Gross silt %	7.7	12.1
Fine sand %	19.5	23.7
Gross sand %	36.7	23.3
Organic matter %	2.2	3.3
Carbon %	1.3	1.9
Nitrogen %	0.1	0.2
Ratio C/N	9.9	11.5
Cation exchange Capacity meq/100 g	10.0	11.3
Calcium meq/100 g	14.2	29.1
Magnesium meq/100 g	1.1	1.3
Potassium meq/100 g	0.7	0.5
Sodium meq/100 g	0.03	0.07

Table 4. Mean Concentrations of Trace Elements in Earthworms and Total and Partial Soil Contents of Studied Points

	Cd mg/kg	Cu mg/kg	Ni mg/kg	Pb md/kg	Zn mg/kg
		60 points			
Earthworms	35.3	60.1	9.1	283	770
Total	2.5	57.9	24.2	945	416
CH_3COOH	1.4	4.3	3.8	56	52
DTPA	1.3	17.1	1.1	108	42
		Ardèche			
Earthworms	38.4	50.6	9.4	132	832
Total	2.4	42.3	21.8	349	521
CH_3COOH	1.2	2.3	1.8	26	70
DTPA	1.7	10.7	0.8	64	53
		Others			
Earthworms	32.5	68.5	8.8	418	715
Total	2.6	73.5	26.5	1541	312
CH_3COOH	1.6	6.2	5.7	87	35
DTPA	0.8	23.5	1.5	151	32

with soil concentration while the Cd, Cu, and Ni earthworm concentrations do so only slightly, probably because of the rather constant soil contents. These differences are due to:

1. Different extraction powers of chemicals reagents vs. the different species of chemical elements. For example, the mixture HNO_3 and HCl extracts almost all trace element contents of soils,[11-13] while acid acetic extracts them from carbonates or oxides, and DTPA extracts elements from oxides and breaks bounds with the organic matter.[14]
2. Differences between chemical mineralization and physiological earthworm metabolic control. Physiological mechanisms are very complex and depend both on the control of organisms and of the soil bioavailability of trace elements. It should be noted that the species of earthworms have different sensitivities towards trace elements.
3. Soil physicochemical characteristics, in particular pH and redox potential, affect the bioavailability of elements.

In Table 5 metal concentrations in earthworms and the three types of soil analysis are compared.

The relationships vary according to elements, soil properties, and chemical analysis methods. In general, the correlations between earthworm concentrations and total and DTPA estimates are more significant than with the acid acetic extraction. The Cd, Cu, and Pb earthworm concentrations seem tightly bound

Table 5. Differences Significant at $p <0.05$ (*), $p <0.01$ (**), and $p <0.001$ (***) Between Trace Element Concentrations in Earthworms and Soils of Ardèche (Ar), the Other Areas (Ot), and Both Ar and Ot (All)

	HNO_3 + HCl			CH_3COOH			DTPA		
Zone	Ar	Ot	All	Ar	Ot	All	Ar	Ot	All
Cd	***	**	**	***	**	**	***	***	***
Cu	**	***	***	***	***	***	***	***	***
Ni	*	—	—	—	—	—	—	—	—
Pb	***	***	***	***	***	***	***	***	***
Zn	***	—	**	*	—	*	**	—	*

to soil contents, whatever the soil characteristics and analysis techniques are. In contrast, the relations of Zn and in particular Ni are very different according to analysis methods and soil characteristics.

C. Earthworms as a Biorisk in Food Chains

The heavy metal burden in earthworms from the study areas may pose a risk to earthworm predators.

Laboratory toxicological studies on vertebrates (mouse, rat, or others) provide the basis of assessments of human risks. Most of these risks are expressed as a limit: the maximum daily admissible amount in food. This is expressed as a ratio: weight of ingested toxicant in daily diet/live weight of the consumer. In man[15] for Cd, Cu, Pb, and Zn ratios are 0.0075, 0.275, 0.05, and 0.65 (in mg/kg), respectively.

As toxicologists using rat and mouse as a "human" representative, these results can also be used as bird or mammal representatives in general.

Granval and Aliaga[4] listed 200 vertebrate earthworm predators in Western Europe. They ordered them as using earthworms as occasional, important, or major food. In fact, all the literature gives an underestimated figure both qualitatively and quantitatively because of the erratic and scarce studies. About invertebrate predators we have only scattered observations for vertebrates: only four valid diet estimates are available. Mean live weight of predators and daily ingested amount of earthworms for woodcock are 330 g and 100 g/d;[16] for black headed gull: 290 g and 120 g/d;[17,18] for badger: 10.85 kg and 720 g/d;[19,20] and for pig 29.34 kg and 419 g/d,[21,22] respectively.

Using these two values and assuming that earthworms ingested by such predators have the same heavy metal body burden as observed in our sampling (Table 6), we have calculated the ratio of estimated daily food content/daily admissible diet limit. This ratio gives us a level of risks for the wild fauna in comparison with the admissible limits for men. Table 7 presents these values.

For simplicity, we have assumed that predators consume only earthworms. This may not be the case, especially for omnivores such as badgers and pigs.

Our method to directly observe field relationships between toxicants and earthworms as survivors, earthworms as "biocontainers", and earthworms as a prey, leads to three conclusions:

1. The direct observation of toxicant burden in a biological compartment could not be substituted by a chemical analysis of soil, and especially by the so-called available fraction estimates.
2. The observation of the total eradication of an earthworm genus in contaminated soil reveals a serious ecological disaster.
3. The level of observed earthworm contamination reveals a nonadmissible burden of the food for wildlife, including game consumed by man.

Obviously, the described method needs to be expanded to more applications and controls to extend our biomonitoring, but it seems that today this method is enough settle to be practically used.

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