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# The use of invertebrate soil fauna in monitoring pollutant effects

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**Abstract** – A critical review of biological parameters used to indicate pollutant impact on soil quality was conducted. These parameters mention some soil invertebrates. The value of an indicative organism depends on its life expectancy, life style and specific importance. Nematodes, mites, collembolans, enchytraeids, earthworms, isopods and molluscs are good potential biological indicators. Biological indicators of bioaccumulation and biological indicators of effects (toxicological and ecological) can be distinguished. Bioaccumulation studies are difficult to interpret, as wide variations could be found, depending on taxonomic group, habitat, organ studied, soil type or even pollutant type. Some groups, such as Collembola, require in depth bioaccumulation studies. It is suggested to use a pool of macro-concentrators, including at least some earthworm, isopod and gastropod species. Toxicological indicators have been well studied and their lethal and sublethal pollutant effects are well known. However, studies have focused on only a few species, such as the earthworm *Eisenia foetida* or the collembolan *Folsomia candida*. These studies should be extended to other zoological groups, as well as to several species from the same group, to generate a representative test battery. Exposure biomarkers and physiological change studies should be emphasised, as they act as very early warning systems of contamination. Data are currently lacking on how soil biological processes malfunction due to pollution. We need to explore the links between pollutant effects on soil fauna and pollutant effects on soil functioning. Concerning ecological indicators, studies should develop sampling techniques and parameters, which are specific to ecotoxicological goals. Before-after impact control procedures should be carried out, to eliminate the background noise of the study site and only evaluate the influence of pollutants. On the other hand, ecological indices, such as taxonomic diversity or richness, should be used carefully especially concerning chronic pollution. Effects of pollutants on biological cycle studies seem very promising, but need further information on the life history strategies of many species. Furthermore, the pollutant tolerance of rare species should be considered. The different types of biological indicators yield complementary information on pollutant effects. They all need standard procedures. In this context, studies should be extended and diversified, and associate bioaccumulation, toxicological and ecological indicators to provide better information on soil quality.

**Ecotoxicology / bioindicators of effects / bioaccumulation / soil ecology / soil quality / soil invertebrates / soil fauna / earthworms / microarthropods / mites / collembolans / isopods / enchytraeids / snails / soil pollution**

**Résumé** – **Utilisation de la faune invertébrée du sol comme bio-indicateur des effets des polluants.** Une liste critique des paramètres biologiques utilisés dans certains travaux pour indiquer l'impact des polluants sur la qualité des sols a été établie. Ces paramètres font référence à un ou plusieurs invertébrés du sol. Le rôle d'organisme indicateur dépend de leurs caractéristiques biodémographiques, de leur mode de vie, de leur taille spécifique. Les nématodes, acariens, collembolans, enchytréides, vers de terre, isopodes et gastéropodes sont potentiellement des indicateurs biologiques. Indicateurs biologiques de bioaccumulation et indicateurs biologiques d'effets (toxicologiques et écologiques) peuvent être distingués. Les études de bioaccumulation sont difficiles à interpréter, car de fortes variations sont observées. Ces variations dépendent du groupe taxonomique étudié, de l'habitat, de l'organe étudié, du type de sol ou bien encore du type de pollution. Certains groupes, comme les collembolans demandent des études plus poussées à ce

sujet. Il est suggéré d'utiliser un pool de macro-concentrateurs, avec au moins des espèces de vers de terres, isopodes et gastéropodes. Concernant les indicateurs toxicologiques d'effets, les études les plus nombreuses concernent les effets létaux et sublétaux. Toutefois ces études concernent peu de groupes taxonomiques (essentiellement le vers *Eisenia foetida* et le collembole *Folsomia candida*), et devraient être étendues à d'autres groupes zoologiques, ainsi qu'à différentes espèces du même groupe, afin de créer une batterie de tests représentatifs. Les travaux concernant les biomarqueurs et les changements physiologiques devraient être amplifiés, car ils permettent d'obtenir des systèmes d'alarme très précoces concernant l'impact des contaminants. Un manque persistant de données concerne les conséquences de pollutions sur le fonctionnement des écosystèmes. Il est en effet important désormais d'explorer les liens entre les effets des polluants sur la faune du sol et les effets réels de ces mêmes polluants sur le fonctionnement des sols. Concernant les indicateurs écologiques, les travaux entrepris devraient développer des méthodes d'échantillonnage et des paramètres spécifiques au domaine écotoxicologique. Ainsi, il est préconisé d'entreprendre des études combinant les approches synchroniques et diachroniques, afin d'éliminer le bruit de fond induit par le site d'étude et évaluer uniquement l'influence du polluant. En outre, les indices écologiques, comme la diversité ou la richesse taxonomique, doivent être utilisés avec précautions, particulièrement lors des études de pollution chronique. Par ailleurs, les études concernant les effets de polluants sur les cycles biologiques semblent prometteuses, mais demandent de meilleures connaissances concernant les stratégies de vies des différentes espèces étudiées. De plus, la tolérance aux pollutions des espèces rares devrait être fortement prise en considération. Les différents types d'indicateurs biologiques fournissent des informations complémentaires. Ils demandent tous une standardisation des protocoles. Dans ce contexte, les études devraient être développées et diversifiées et associer les indicateurs de bioaccumulation avec les indicateurs biologiques d'effets pour améliorer l'information sur la qualité des sols.

**Écotoxicologie / bio-indicateurs d'effets / bioaccumulation / écologie du sol / qualité du sol / invertébrés du sol / faune du sol / vers de terre / microarthropodes / acariens / collemboles / isopodes / enchytraeides / mollusques / pollution du sol**

## 1. INTRODUCTION

Fauna is an important part of soil environments. It is involved in many aspects of organic matter decomposition, partial regulation of microbial activities, nutrient cycles and crumbly structure. Depending on their diet (saprophagous, phytophagous or predation), soil fauna are closely linked both to each other and to microorganisms, plants and soil [167]. They are a vital key to understanding the soil ecosystem.

Disturbances caused by pollutants in the soil results in both qualitative and quantitative changes in fauna, which affect soil functioning. Some of soil fauna groups can act as bioindicators [12, 136].

A bioindicator must fulfil the following requirements [83]:

- play an important role in the functioning of the soil ecosystem;
- be widely distributed, common and easy to sample;
- be robust: should not be killed by very low levels of pollutants;
- have measurable responses (pollutant concentration in tissues, characteristic biological disturbances in growth and fertility or genetic resistance);
- have reproducible responses: should produce similar responses to the same levels of pollutant exposure in different sites.

In addition, the selected zoological groups should be practical for use in both laboratory and in situ studies. Laboratory tests provide a simplified, analytical approach to obtain information on how an individual reacts and how its organs or cellular and molecular functions are affected. In situ ecotoxicological studies are more complicated than laboratory tests, but are better adap-

ted to environmental management as they examine the effects and consequences of pollutants in the ecosystem.

Several zoological groups can be used:

– Soil microfauna (smaller than 0.02 mm). These are primarily Protozoa and Nematoda living in the water free soil compartments. They can be used to study soil ecotoxicology. Tests using these organisms are similar to those used in aquatic toxicology [194]. These organisms have a high bioaccumulation potential [11]. Microcosm tests are currently being designed for several Protozoa (*Cercomonas*, *Acanthamoeba* and *Colpoda genera*) [57]. Toxicity tests in artificial soils using the nematode *Plectus acuminatus* have provided additional perspectives for studies of soil contaminants [98]. In situ studies using nematodes have yielded several maturity indices which provide data on the soil function. These indices could be used to monitor the soil ecology as they reflect changes in soil condition [130, 131].

– Soil mesofauna (from 0.02 to 4 mm). Enchytraeids (oligochaete annelids) are common in most soils (primarily in the first 5 cm of soil) and play an important role in decomposition and humification processes. They are generally microphagous and capable of reusing other animals (earthworms or microarthropods) excrements [4]. Few ecotoxicological studies have been conducted on them, but initial reports, particularly in situ studies have been promising [121, 150]. Springtails (apterygote insects) and acarids (Arachnida) are the most common soil arthropods [25] and play a key role in soil functioning. They are vital indicators for soil ecotoxicology.

– Soil macrofauna (larger than 4 mm). Snails and slugs (terrestrial gastropod molluscs) move along the soil surface eating plants and dirt, but part of their bio-

## Invertebrate soil fauna as pollution indicators

logical cycle (rest, shelter, egg-laying, embryo development, hibernation) takes place in the soil, from which they absorb nutrients and contaminants. They have a wide range, are easy to sample, have a high bioaccumulation capacity and a phytophagous or saprophagous diet which make them very good bioindicators [8]. Earthworms (oligochaete annelids) are also good indicators, as almost every soil contains at least one earthworm species [14]. Their range varies depending on depth: epigeous, endogeous and anecic worms can be found in temperate zones [116]. In addition, they migrate over short distances [82]. They are both resistant and sensitive. Isopods (crustacean arthropods) are particularly interesting due to their capacity to accumulate metals and their abundance in some soils [82]. They are detritivore or microphytophagous but strongly linked to humid environments, which could limit their in situ utilisation. Holometabolous insects are often only present in the soil during a particular phase in their development and are generally considered to be poor accumulators. However, some studies have been undertaken on the beetle *Oxythyrea funesta* (Cetoniidae) for laboratory use [128] and xylophagous beetles could be used as bioindicators of forest quality [12, 72, 17]. In addition, a laboratory test has been standardised using staphylinid adults (*Aleochara bilineata*) in agriculture [155, 156].

Several works on the use of soil fauna in soil ecotoxicology have already been published [75, 110, 193, 195, 200] and are published still.

The goal of the present work is to identify the most significant results obtained for each faunal group in terms of soil monitoring, and evaluate how suitable a group might be used as bioindicator, with a particular focus on expected measurable responses.

We also suggest new requirements and new study directions, that should be carried out.

For this purpose, we will follow Echaubard [50], who makes a distinction between:

- biological indicators of bioaccumulation;
- biological indicators of effects, including toxicological and ecological bioindicators.

Biomarkers reflect either an individual biological reaction or lower level reactions (cellular, molecular, genetic) and are thus toxicological indicators of effects. However, they cannot always accurately indicate a genuinely toxic effect, at the individual or the upper levels [103, 202].

## 2. BIOINDICATORS OF BIOACCUMULATION

Bioaccumulation occurs when a substance present in the biotope penetrates into an organism where it is stored in various forms. Bioaccumulation of heavy metals by terrestrial organisms has been well studied. It is a very important phenomenon which provides information on the bioavailability of trace elements

which often can lead to biomagnification of pollutants during transfers along the food chain. The same process occurs for some organic compounds, particularly pesticides. However, no standard procedure exists to measure bioaccumulation [137].

It should be noted that there is no direct relationship between bioconcentration of a pollutant and intoxication [194]. It is thus necessary to study not only the internal concentration but also the effects of pollutants on organisms.

Concentrations of pollutants are measured using various chemical extraction techniques followed by gas chromatography [122] or atomic absorption spectrophotometry analysis [173, 196]. Pollutant fluxes can also be calculated by kinetics studies (ingestion and excretion) of pollutants [94].

Although often associated with toxicological tests [163, 182], few bioaccumulation studies have been made on Collembola [53]. However, Collembola feed partly on fungal mycelia, which are known to concentrate both metals and organic pollutants and radionucleotides [84]. In addition, they are often preyed upon by other arthropods [58].

For copper accumulation, Collembola can be ranked second among detritivorous invertebrate right after isopods [85]. Hunter et al. [90] found that copper concentration was 45 times higher in the same species pool at a polluted industrial site than that at an unpolluted site.

However, due to the microarthropods small size, a large number of organisms must be sampled for testing, which increases the margin of error. In addition, although interclonal variation in some species (particularly *Folsomia candida*) does not influence their usefulness in ecotoxicological tests [33], a wide range of reactions exists between Collembola species. Lupetti et al. [122] found considerable differences between DDT and PCB concentrations in seven species sampled at the same site. *Orchesella cincta* was found to be highly vulnerable to DDT concentrations in its tissues. These differences can be explained by the excretion/assimilation ratio and the capacity to eliminate substances from some tissues, which varies between species [83].

In vivo metal contents in earthworms and isopods vary depending on species or metal type. Differences in concentrations have been observed:

- between species from the same taxa (earthworms: [91, 3, 173], isopods: [205, 97, 87]);
- between different aged individuals from the same species (earthworms: [21], isopods: [86]);
- between seasons (earthworms: [92, 2], isopods: [204]).

Bioaccumulation of metals depends on:

- the element (isopods: [88]);
- its concentration in the environment [67, 73];
- the physical and chemical soil conditions (earthworm: [123]).

**Table I.** Lead concentration in earthworms ( $\text{mg}\cdot\text{kg}^{-1}$  dry weight) and concentration factor (ratio of Pb concentration in worms and in soil) for three common earthworms species at Tikkurila (soil heavily polluted by Pb) and Pornainen (control site) [173]. \* and \*\*\*, significant differences; ns, non-significant differences.

Species	Tikkurila			Pornainen			Mann-Whitney
	Mean $\pm$ SD	<i>n</i>	Median	Mean $\pm$ SD	<i>n</i>	Median	
<b>Pb concentrations in worms</b>							
<i>Aporrectodea caliginosa</i>	1 058.0 $\pm$ 480.4	10	904.5	61.7 $\pm$ 42.7	7	50.0	***
<i>Lumbricus rubellus</i>	133.6 $\pm$ 116.5	9	129.1	56.2 $\pm$ 36.7	5	61.1	ns
<i>L. castaneus</i>	75.6 $\pm$ 109.4	6	11.9	88.3 $\pm$ 59.8	3	97.2	ns
Kruskall-Wallis between species		***			***		
<b>Concentration factor</b>							
<i>A. caliginosa</i>	0.98 $\pm$ 0.38	10	0.92	3.8 $\pm$ 3.1	7	3.1	*
<i>L. rubellus</i>	0.07 $\pm$ 0.05	9	0.08	3.2 $\pm$ 2.1	5	3.8	0
<i>L. castaneus</i>	0.05 $\pm$ 0.06	6	0.01	5.3 $\pm$ 5.0	3	4.3	*
Kruskall-Wallis between species		***			***		

Terhivuo et al. [173] noted different bioaccumulation rates among different earthworm species at a site damaged by lead pollution (table I).

Most studies of terrestrial gastropods (table II) have focused on analysing bioaccumulation among some experimentally contaminated animals or animals sampled at a polluted site. Gastropods survive in sites polluted by metals. This resistance stems from their ability to retain and inactivate toxic metals, either through intracellular compartmentalisation (confinement within granules, vesicles, etc.) and excretion, or

through protein binding, including metallothioneins, which can scavenge metals (primarily cadmium) and store them for long periods [38].

By evaluating the metal contents in different gastropod species from different environments, Berger and Dallinger [8] were able to reveal three pollution levels. In a similar study on edible snail quality (in farms and natural environments), Pihan et al. [138] suggested a classification according to metal contents (Cu, Zn and Pb) and studied the relationship between metal concentrations in snail tissues and their food supply [71].

**Table II.** Pulmonated land gastropods as contamination indicators.

Reference	Pollutant	Contamination source	Species	Test duration	Parameters
[129]	Fe, Pb, Zn		<i>Helix pomatia</i>	4 weeks (Aug.–Sept. 1971)	Temperature effect on Fe, Pb, Zn in different organs
[29]	Cd, Cu, Pb, Zn	Contaminated and uncontaminated sites	<i>H. aspersa</i>	Natural snails (Sept. 1975)	Metal concentration in organs
[206]	Cd, Pb, Zn	Roadsides	<i>Cepaea hortensis</i>	(Jul.–Sept. 1975)	
[37]	Cd, Cu, Pb, Zn	Contaminated food (lettuce)	<i>H. pomatia</i>	30–35 d (March 1981)	Bioaccumulation, cellular and molecular distribution of metals
[6]	Pb	Agar gelose +Pb or +Pb and Ca	<i>H. aspersa</i>	4 weeks	Bioaccumulation; Pb assimilation and excretion
[76]	Ca, Cd, Cu, Pb, Zn	Abandoned mine Pb/Zn	Six slug species	Natural slugs (Sept. 1986)	Metal concentration in tissues
[36]	diverse	Diverse	Gastropods and land invertebrates	Variable	Contamination, cellular compartment, detoxification
[38]	Cd, Zn	Agar plates with Cd et Zn	<i>H. pomatia</i>	30 d	Subcellular distribution in digestive glands
[8]	Cd, Cu, Pb, Zn	Urban areas	<i>Arianta arbustorum</i>	Seasonal sampling	Metal concentration in tissues (+ influence of individual size)
[138]	Cu, Zn	Natural and through food	<i>H. aspersa aspersa</i> and <i>H. aspersa maxima</i> <i>H. pomatia</i>	Natural and raised adult snails	Metal concentration in tissues, Bioaccumulation and pollution factors
[139]	Pb	Contaminated sites and breeding	<i>H. aspersa</i> , <i>H. pomatia</i>	Natural and raised adult snails	Pb concentration in foot and viscera
[73]	Cd, Pb, Zn	Food and substrate	<i>Deroceras reticulatum</i>	3 weeks	Metal concentrations in the body
[71]	Cu, Zn	Food	<i>H. aspersa aspersa</i> and <i>H. aspersa maxima</i>	10 weeks	Bioaccumulation

## Invertebrate soil fauna as pollution indicators

In addition, gastropods can be used to study metal transfers into the ecosystem through the food chain [6]. As they are eaten by many different mammals, they are probably involved in the biomagnification of metals [36, 206].

Bioaccumulation of pesticides by terrestrial gastropods has not yet been well studied, but atrazine and azinphosmethyl do not appear to be accumulated through the food chain [157].

Current studies on bioconcentration and bioaccumulation have provided information about the bioavailability of contaminants found in the surrounding environment and contained in food. To compare results from different analyses performed on animals sampled in nature or on animals which have been used for contamination experiments, bioconcentration (BCF) or bioaccumulation (BAF) factors can be calculated using the following ratio:

$$\frac{\text{concentration of pollutant in animal} \text{ (}\mu\text{g}\cdot\text{g}^{-1} \text{ dry weight)}}{\text{concentration of pollutant in the environment} \text{ or in food (}\mu\text{g}\cdot\text{g}^{-1} \text{ dry weight)}}$$

Concentration factors are often used for aquatic organisms which are bioindicators of accumulation, but they are slightly more difficult to use with terrestrial animals where the environment is more heterogeneous. Furthermore, such ratios are only useful for non-regulated metals and persistent pesticides. Comparing metal concentrations (Cd, Pb, Zn) in substrates and food to study metal accumulation in isopods, collembolans, diplopods (leaf litter) and slugs (soil) yielded highly different results (from 14 to 750 mg·kg<sup>-1</sup> of Cd and from 490 to 22 000 mg·kg<sup>-1</sup> of Zn) when they were experimentally soaked in the same solutions [73]. This experiment illustrates the problems inherent in between species and between habitat comparisons. Nevertheless, the overall data indicate that invertebrates behave as either macro-concentrators, micro-concentrators or de-concentrators [13, 36]. Macro-concentrator species are the ones that provide the most

direct information about environmental contamination. It thus seems wise to use a pool of macro-concentrators: for example, isopods (e.g. *Oniscus asellus*) and pulmonate gastropods (e.g. *Helix aspersa*) have provided information about the degree of metal contamination (Cu, Cd, Pb, Zn) in sites located near English foundries (table III, [82]). This author also noted that bioaccumulation varies depending on the organ and is particularly high in the digestive gland for Cd, Zn and Pb. Earthworms can have very high and significant BCFs for organic pollutants, such as chlorophenols (particularly, pentachlorophenol), which vary depending on soil type (476 and 956 in two agricultural soils for *Lumbricus rubellus* [181]).

Until recently, the indications provided by contaminant concentration analysis in animals sampled in the field have not yielded any information about exposure time. To compensate for this, sentinel animals (from standardised cultures) are used either in microcosms, mesocosms or on site to evaluate specific exposure times. Copper accumulation was thus studied in *Lumbricus rubellus* [126] and in *Eisenia andrei* [171, 172]. Exposing cultured snails (*Helix aspersa*) for 30 d at distances from 1 to 200 m away from a busy motorway (Nancy-Metz: 40 000 vehicles per day) demonstrated that lead pollution remains high at distances up to 40 m away and causes lead accumulation in the snail viscera (16–22 mg·kg<sup>-1</sup> dry weight; Pihan and Gomot, unpubl.).

Controlling breeding of some main macro-concentrator soil fauna species should make it possible to study pollution over time and define its fluctuations.

### 3. BIOINDICATORS OF EFFECTS

Data based on the biological responses of organisms to pollutant is used to link pollutant toxicity with ecological consequences. Several biological levels exist, from the molecule to the community level, which affect the degree of toxicological or ecological reactions.

**Table III.** Heavy metal concentrations in the bodies of *Helix aspersa* and *Oniscus asellus* from an uncontaminated site and a contaminated (smelting work) in England (from [82]).

Species	Metals	Metal body concentrations (mg·kg <sup>-1</sup> dry weight)		Increase coefficients
		Uncontaminated site (Kynance Cove Cornwall)	Contaminated site (Avonmouth)	
<i>Helix aspersa</i> (garden snail)	Cu	104 ± 28	228 ± 43	2.2
	Zn	103 ± 14	418 ± 71	4
	Cd	4.88 ± 0.62	49.6 ± 10.7	10.1
	Pb	5.79 ± 1.06	121 ± 24	21.8
<i>Oniscus asellus</i> (wood lice)	Cu	115 ± 42	567 ± 138	4.9
	Zn	108 ± 25	488 ± 103	4.5
	Cd	5.93 ± 1.01	152 ± 37	2.5
	Pb	4.15 ± 0.91	413 ± 150	99.5

Among the biological indicators used (which provide data on reaction to pollutant exposure), two main categories can be found:

- toxicological indicators (molecules, cells, organisms, groups of organisms). A close relationship exists between intoxication and an observed reaction. This data helps reveal phenomena occurring in the upper levels but has little ecological value. These indicators can act as early warning systems of contamination whose effects are possibly reversible [50];

- ecological indicators. Possible changes in population, community or population group structure as shown by ecological analysis indicate changes occurring but provide no information on causes of disturbances.

Concerning methodologies, except for mathematical models, two approaches can be used [147]:

- experimental models, with single species and multispecies tests and bioassays;

- in situ indicators, based on field studies and biota which live or are introduced in these sites.

However, the difference between these two approaches is often arbitrary. For example, field observations need an experimental part; and although bioassays are linked to laboratory tests because they are often standardised, they could be considered to be a particular form of in situ indicator as they use polluted substrates rather than pure substances. These considerations concur with Forbes and Forbes [63], who distinguish between field indicators and experimental tests, but prefer the more general concept of ecotoxicity tests, where observations or experimental manipulations are used to evaluate the fate or effects of chemical pollutants, without any reference to the biological organisation level.

## 3.1. Toxicological indicators

### 3.1.1. Testing lethal and sublethal effects

The first descriptor used to evaluate the impact of a pollutant is acute toxicity. Mortality in a population exposed to xenobiotics is a severe reaction. Extreme toxicity is expressed as a proportion or a concentration which leads to the death of 50 % of the tested individuals. Lethal dose or  $LD_{50}$ , expressed in  $mg \cdot kg^{-1}$  of body weight, depends on the animal.  $LC_{50}$  indicates a lethal concentration expressed in  $mg \cdot kg^{-1}$  of substrate. It is probably more reasonable to speak of  $LC_{50}$  for soil fauna.

Studies on sublethal effects generally calculate  $EC_{50}$  or the effective concentration for a biological process which leads to a 50 % inhibition or decrease [143]. LOEC (lowest observed effect concentration) is the lowest concentration yielding a significant difference for the studied parameter compared to the control. Occasionally the term NOEC (no observed effect concentration) is used in toxicity testing.

NOEC and LOEC are not always considered to be good criteria as they require using a large range of concentrations to obtain a value close to reality.  $LC_{50}$  and  $EC_{50}$  are more accurate and have a better ecological value as they directly reflect fundamental changes in stages of an organisms biological processes (growth, reproduction).

In addition, some species appear to be more sensitive when lethal concentration is tested, while others are more sensitive when sublethal concentration is tested. Crommentuijn et al. [32] have suggested using a new index to harmonise results, which they called the sublethal sensitivity index (SSI). It is calculated using the ratio  $LC_{50}/EC_{10}$  or  $LC_{50}/NOEC$  when sublethal concentration cannot be measured.

Recent studies used the ILL (incipient lethal level), which is the concentration of toxicant below which 50 % of the exposed organisms can be expected to survive for an indefinite period [164]. It normalises the toxic response so that the assumption can be made that all comparisons of toxicity within and between chemicals and species of organisms are made at a steady-state with respect to the dose response curve [114].

Until now, toxicity tests for terrestrial organisms have been based on single species to measure the impact of toxic products on survival, growth and reproduction. Although three tests have been standardised (two for earthworms, one for Collembola), experimental data are available for most soil fauna groups.

#### 3.1.1.1. Nematodes

These are the most common soil invertebrates. Tests have been performed with *Caenorhabditis elegans* [42, 141, 187], but toxic exposure conditions (mainly to Cd) differed too widely to yield correct comparisons of results.

Recently, experiments have been performed with the nematode *Plectus acuminatus*, a bacterivorous species which lives freely in the soil but is not associated with plant roots or fungal hyphae. Kammenga et al. [98] used the juveniles to adults ratio as the test parameter in an artificial soil (OECD) under optimal conditions for *P. acuminatus* population growth in the soil. Tests were performed using Cd, Cu, and PCP [98].

#### 3.1.1.2. Enchytraeids

Few studies have currently been performed (table IV). However, they have illustrated the complexity involved in ecotoxicological studies in soils and the need for method standardisation: Rundgren and Augustsson [150] noted a decrease in fragmentation rate for *Cognettia sphagnetorum* individuals exposed to increasing concentrations of copper in an artificial soil (LUFÄ), and Sjögren et al. [161] noted an increase in fragmentation rate in the same species in a forest soil.

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**Table IV.** Examples of evaluation tests with enchytraeids.

Reference	Pollutant	Contamination source	Species	Test duration	Parameters
Weistheide et al. (1991), in [194]	benomyl	Contaminated gelose	<i>Enchytraeus crypticus</i> , <i>E. minutus</i>	30 d	Number of hatched juveniles cocoons
[148]	parathion amitrole diuron	Substrate	<i>E. albidus</i>	56 d	LC <sub>50</sub> on cocoons
[22]	dimethoate pirimicarb fenpropirimorph	Food (oatmeal)	<i>E. bigeminus</i>	7 d	LC <sub>50</sub> ; growth
[150]	dimethoate	Substrate, food (spores)	<i>Cognettia sphagnetorum</i>	70 d	NOEC; LOEC; EC <sub>x</sub> (fragmentation rate and number of formed segments)
[161]	Cu	Substrate	<i>C. sphagnetorum</i>	15–50 d	Fragmentation rate, model of dispersal, minimum population size
[102]	Cu + Ni	Forest soil	<i>C. sphagnetorum</i> (add to other organisms)	138 d	Litter mass loss, soil respiration, change in nitrates
[127]	dimethoate	Artificial soil (OECD, humic, sandy and clayey)	<i>E. crypticus / variatus</i>	32 d	NOEC on juveniles

### 3.1.1.3. Oligochaetes (earthworms)

This group has undergone considerable lethal and sublethal testing [52, 135].

The species *Eisenia fetida andrei* from the *E. fetida* complex is generally used for lethal testing, as it is genetically relatively uniform [15]. Some results are shown in table V. However, other studies have shown that *E. fetida* worms with a long-term history of exposure to cadmium developed resistance to the metal [146]. The species *Lumbricus terrestris* and *Allolobophora caliginosa*, although more sensitive, are less frequently used as they are harder to raise [39, 56, 79, 80, 127, 168].

Sublethal testing is generally performed on cocoon production [24, 119, 132, 145, 185, 188–191, 199]. Juvenile growth studies are more difficult to perform

as they involve several parameters: test duration, fate of pollutant, interaction between pollutant and substrate [101]. Maturation of secondary sexual characteristics is often combined with earthworm growth rate [119, 145, 165, 199].

On the other hand, several studies propose to use toxicant kinetics and critical body residues (CBR) in relation to toxicity endpoints, which may provide a better estimate of toxic dose than soil concentration of contaminants, especially for hydrophobic organic chemicals [60, 61, 114].

### 3.1.1.4. Gastropods

Experimental studies on metal toxicity have been performed on snails and slugs from the stylommat-

**Table V.** Using the *Eisenia fetida* group as a toxicological bioindicator of effect.

Reference	Pollutant	Contamination source	Test duration	Parameters
[80]	Nineteen pollutants fungicides and insecticides	contact (filter paper), artificial soil, artisol [1]	2 and 14 d	LC <sub>50</sub>
[133]	Cd, Cu, Ni, Pb, Zn; ten organic pollutants	contact (filter paper), artificial soil	2 and 14 d	LC <sub>50</sub>
[145, 199]	Dieldrin	organic culture medium	90 d	Growth, sexual maturity and reproduction
[140]	Five herbicides	contact (filter paper), brown soil	2 and 7 d	LC <sub>50</sub> ; growth (biomass)
[184]	Cu, pentachlorophenol, 2,4-dichloroaniline	artificial soil	21 and 35 d	Growth, reproduction
[132]	Ten pollutants	horse manure + sand	28 and 42 d	Growth, reproduction
[185]	Cd, Cu, PCP	artificial soil + cow dung	6, 8, 10 and 12 weeks	EC <sub>50</sub> ; NOEC, growth, reproduction
[109]	Parathion, propuxur	artificial soil	14, 42 and 56 d	Growth, reproduction
[186]	Cd, Cr, insecticides, fungicides and herbicides	artificial soil	14, 21 and 35 d	LC <sub>50</sub> ; growth, reproduction
[16]	Soils and waste	artisol	14 d	Non-toxic threshold
[60]	Pentachlorophenol	artificial soil	28 d	LC <sub>50</sub> ; ILL
[201]	Terbutylazine, carbofuran	food	14, 42 and 56 d	Reproduction, respiration (CO <sub>2</sub> emissions), excretion (nitrogen)

**Table VI.** Examples of experimental studies for determination of lethal and sublethal effects of chemicals in land gastropods pulmonates.

Reference	Species	Test parameter	Pollutant	Exposure	
				Duration	Feeding tests
[151]	<i>Helix aspersa</i>	Growth; feeding activity; reproduction	Cd	30 d	Rat food + CaCO <sub>3</sub> + CdCl <sub>2</sub>
[124]	<i>Arion ater</i>	Growth; food consumption	Cu, Hg, Pb, Zn	27 d	Natural diet with 1.5 % agar + different dosages of metals
[157]	<i>H. aspersa</i>	NOAEL, LOAEL; sublethal EC <sub>50</sub> ; lethality; LC <sub>50</sub>	Twelve pesticides	12, 14 d	rat food + agar + pesticides
[125]	<i>A. ater</i>	Quantitative structure of digestive tubules	Hg	30 d	Natural diet with 1.5 % agar + different Hg concentrations
[115]	<i>H. aspersa</i>	Growth rate; food consumption; fecundity	Cd, Cu, Pb, Zn	90 d (juveniles); 120 d (adults)	Artificial diet + different metal concentrations
[69]	<i>H. aspersa aspersa</i>	Growth	Cd, Cr, Pb, Zn	21, 28 d	Food Helixal R <sup>®</sup> + Cd or contaminated soil (Cd, Cr, Pb, Zn)
[70]	<i>H. aspersa aspersa</i> ; <i>H. aspersa maxima</i>	Growth, NOEC, EC <sub>50</sub> , EC <sub>75</sub> ...; feeding activity	Cd	28 d	Diet for snails (Helixal R <sup>®</sup> ) + CdCl <sub>2</sub>

phorous pulmonate gastropod group (table VI). These organisms can survive high levels of heavy metals in their food. However, growth and reproduction are inhibited differently depending on the metal and species.

Schuytema et al. [157] studied toxicity of pesticides on snails and identified four substance groups according to their toxicity through ingestion mixed with food. In group 1, acephate, atrazine, glyphosate, hexazinone and picloram were not lethal for snails at 5 000 mg·kg<sup>-1</sup>. The death rate was 13 % for group 2 (carbaryl) and 75 % for group 3 (fenitrothion and methyl parathion). These high relative differences in pesticide toxicity indicate the need for additional studies to evaluate the risks involved in pesticide treatments.

### 3.1.1.5. Collembola

The Collembola *Folsomia candida* is the most frequently used microarthropod both for sublethal and lethal testing. Testing can be performed for organic or metallic pollutants. Some examples are shown in table VII. *F. candida* is currently used as a model for comparative studies of new molecules behaviour. Data obtained on this species can also be used for improving testing techniques [106].

Although one test using *F. candida* has been standardised, most publications on this species indicate wide differences between test results depending on strain, individual age [33] and soil moisture content [182]. In addition, this parthenogenic species is easily raised, but does not provide an accurate picture of natural environments. This is why other species, such as *Isoptoma viridis* are also studied [95]. Tests have also been performed on *Orchesella cincta* [5, 142, 197], *Onychiurus armatus* [7, 177] and *I. notabilis* [177]. Other species should also be studied to generate a complementary test pool.

According to Krogh and Petersen [105], reproduction is a better parameter than mortality and provides more accurate information. This parameter integrates the possible long-term effects of a pollutant by demonstrating the possible changes which may occur in the species future reproduction capacities.

### 3.1.1.6. Isopods

The most frequently used species are *Porcellio scaber* [31, 32, 35, 59, 194, 198], *Oniscus asellus* [31, 194], *Trichoniscus pusillus* [194] and *P. laevis* [134].

According to Crommentuijn et al. [31], care must be taken in studies involving isopods, particularly *P. scaber* and *O. asellus*. These species can accumulate high concentrations of pollutants and lethal effects occur only when their accumulation capacities are fully saturated. Exposure time to a pollutant plays a key role.

Isopods generally show low sensitivity in sublethal tests which increases test duration. Drobne [48] estimates that growth, reproduction and life cycle studies are not usually the best for isopods. In fact, growth rates can vary over a period of several weeks, even for one individual, and females may retain sperm for a long time before reproducing. Furthermore, life cycles are relatively long, often more than 6 to 8 months. However, sublethal tests performed with copper on *P. scaber* revealed negative effects on food intake and individual growth at high concentrations (1 000 mg·kg<sup>-1</sup>) while low amounts stimulated juvenile growth rates [59]. In addition, these animals are easy to raise.

### 3.1.1.7. Arachnids

Lethal and sublethal tests were performed on *Platynothrus peltifer* for copper and dimethoate [179]. The results have been encouraging and it may be possible to use this acarid, although current study methods

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**Table VII.** Using *Folsomia candida* as a toxicological bioindicator of effects.

Reference	Pollutant	Contamination	Duration	Variable	Parameter
[18]	DDT	Food (yeast)		Temperature; concentration	Life span, mortality, fecundity, egg viability
[176]	Seventeen insecticides	Artificial soil (sand)	1 d	Concentration	LD <sub>50</sub>
[170]	Paraquat, atrazine	Food (yeast)	154 d	Concentration	Mortality, moulting frequency, fecundity, egg viability, incubation
[30]	Cd	Artificial soil (OECD)	63 d	Concentration	LC <sub>50</sub> ; EC <sub>50</sub> on growth and egg-laying
[103]	Dimethoate	Methylated soil	28 d	Spatial distribution of food and pollutant	LC <sub>50</sub> ; EC <sub>50</sub> ; NOAEC, LOAEC on reproduction
[33]	Cd, chlorpyrifos, triphenyltin hydroxide	Artificial soil (OECD)	35 d	Genetic variability	LC <sub>50</sub> ; EC <sub>50</sub> on growth, juveniles and population
[32]	Cd	Food (yeast)	63 d (egg-laying); 392 d (growth)	Specific variability	LC <sub>50</sub> ; EC <sub>10</sub> ; EC <sub>50</sub> on egg-laying and growth; SSI
[127]	Dimethoate	Artificial soil (OECD, humic, sandy and clayey)	32 d	Soil type	NOEC on juveniles
[162]	Zn	Artificial soil (LUFA); natural soil	42 d	Soil type	LC <sub>50</sub> ; EC <sub>50</sub> on growth and reproduction
[149]	Dimethoate, parathion	Artificial soil, water	14 d	Comparison of measurement methods	EC <sub>50</sub> on mobility in water
[89]	Parathion, dimethoate, carbofuran, oxamyl	Water	14 d	Differences between pesticides	EC <sub>50</sub> on mobility in water
[180]	Cd, Zn	Artificial soil	42 d	Interaction between Cd and Zn	LC <sub>50</sub> ; EC <sub>50</sub> on growth and juveniles
[182]	Cd	Artificial soil (OECD)	42 d	Soil moisture	LC <sub>50</sub> ; EC <sub>50</sub> ; EC <sub>10</sub> on biomass juveniles
[163]	Zn	Artificial soil	42 d	Temperature; concentration	LC <sub>50</sub> ; EC <sub>50</sub> ; EC <sub>10</sub> on biomass, juveniles

are time-consuming and difficult (high juvenile mortality, lack of biological synchronisation).

Work has also been performed on the effect of dimethoate on the predator gamasid *Hypoaspis aculeifer* [62, 78, 103, 104]. These tests have demonstrated the importance of examining between species relationships, particularly predator-prey interactions, as test results indicated that dimethoate essentially affects the gamasid indirectly due to a decrease in number of available prey (i.e. the Collembola *Folsomia fimetaria*).

### 3.1.1.8. In summary

Irrespective of the chosen organism, the tests must be replicable and standardised according to:

- species (genetic stability and growth stage);
- breeding techniques and contamination method;
- experiment duration;
- measurement of effects (NOEC, LOEC, EC<sub>50</sub>, etc.).

Substrate is a vital element, as an organism's reaction is influenced by substrate composition, which can interact with pollutants. Every test made with natural, artificial (ISO defined soil) or inert (silica, glass beads) substrate must be double checked by tests using the substrate alone as a standard.

To overcome the disadvantages inherent in each type of test, several assays should be run using different zoological groups as well as several species from the same group to create a test battery and an evaluation grid for toxic product hazards. In addition, the most sensitive variables for a given species should not be the only ones examined [99]. Several studies have already been performed using the cadmium model (*table VIII*): these test results should be numerically evaluated.

Dutch scientists have attempted to design an index which integrates toxicological data for several organisms: Van Straalen and Denneman [192] suggested the HC<sub>5</sub> index (hazardous concentration for 5% of the species) to harmonise and correlate toxicological data based on NOEC for different organisms for a given pollutant. However, as Hopkin [85] has pointed out, this index was designed using a very small number of organisms and suffers from a lack of toxicological data for different organism types. In addition, it fluctuates widely depending on the species analysed [5].

### 3.1.2. Exposure biomarkers and physiological changes

The search for exposure biomarkers which react to pollutants is currently under way, for both plants and animals. The goal is to define the measurable molecular and biochemical changes which occur after exposure to toxic substances [41, 50, 111–113].

**Table VIII.** EC<sub>50</sub> values and NOEC for the effect of cadmium, one of the most toxic among the heavy metals polluting the soil, on the growth, the reproduction and the survival of soil invertebrates.

References	Species	Test parameters	Exposure		Results (mg·kg <sup>-1</sup> dry wt)	
			Duration	Routes	NOEC	EC <sub>50</sub>
[98]	Nematodes <i>Plectus acuminatus</i>	ratio juvenile/adult	3 weeks	artificial soil (OECD)	< 10	321 ± 1.7
[123]	Annelids Oligochaeta <i>Lumbricus rubellus</i>	survival	6 weeks	loamy sand	10	500
[183]	<i>Eisenia andrei</i>	cocoon production	12 weeks			
[185]	<i>E. andrei</i>	survival	3 weeks	artificial soil		> 1 000
		somatic growth	42, 56, 70, 84 d	artificial soil + cow dung	18–32	33–96
		sexual development			< 10	27–108
[33]	Apterygota - Collembola <i>Folsomia candida</i>	survival	19–35 d	artificial soil		778–977
		growth	23–35 d			376–807
		reproduction			34.8 and 326	159 and > 326
[32]	<i>F. candida</i>	growth	63 d	food		1 687
		eggs				1 357
[32]	<i>Orchesella cincta</i>	growth and eggs	63 d	food		> 85.5
[32]	Crustacea Isopoda <i>Porcellio scaber</i>	growth	63 d	food		195
[32]	Arachnidea Oribatida <i>Platynothrus peltifer</i>	eggs	63 d	food	2.9	310
[69]	Molluscs Gastropoda <i>Helix aspersa aspersa</i>	growth	28 d	food	50	140
	<i>H. aspersa maxima</i>	growth	28 d	food	50	120

Current works are examining:

- induction of proteins in biotransformation and detoxification systems (cytochrome P450, conjugation enzymes, etc.) or more or less specific stress proteins (metallothioneins, catalases, etc.);

- specific target molecules (acetylcholinesterase, DNA, etc.).

Tranvik et al. [178] focused their study on allozyme polymorphisms in the springtail *Orchesella bifasciata* and found no significant differences between several populations subjected to different degrees of metallic stress. However, Frati et al. [66] observed different reactions to heavy metals in several loci in tolerant and sensitive *O. cincta* populations.

Due to their induction by metals, metallothioneins can be simultaneously responsible for detoxification of trace elements, such as cadmium, and regulation of essential trace elements. They could be used as biomarkers for environmental pollution. Berger et al. [9] isolated metallothioneins in land snails (*Arianta arbustorum*) which had been fed Cd contaminated food (lettuce) and sequenced two isoforms (Mta and Mtb) which they compared to the structure of other invertebrates. Other metallothioneins have been identified for the Collembola *O. cincta* [81] and the earthworm *Lumbricus rubellus* [169].

Walsh et al. [203] studied early, sensitive markers for pollution in earthworms. They verified the feasibility

of measuring DNA adducts by <sup>32</sup>P post-labelling in *L. terrestris* exposed to more or less strongly polluted soils. Determining DNA adducts was considered to be a good way of detecting genotoxic substances in soils.

Cytological, physiological and behavioural modifications can also be considered to be valuable biomarkers. In a study of snails whose food was contaminated by Cd for 30 d, Russel et al. [151] observed hyperplasia and necrosis of the epithelium and a disorganisation of the spermatozoid flagella in the ovotestis, although the ovocytes did not appear to be affected. In a study of *Arion ater* slugs, Marigomez et al. [125] observed significant quantitative modifications in the digestive tube structure and blood vessel walls depending on Hg concentration in food and exposure time. Studies on isopods have examined the effects of metals on the digestive gland epithelium and hepatopancreatic reactions of *Porcellio scaber* [49, 100].

A good example of a physiological effect is the neurotoxic effect (disturbances in nerve impulse speed) of pollutants on earthworms [43–47]. Nevertheless, this in vivo method is rather costly [101].

Behavioural studies are essentially ways of quantitatively evaluating the effect of pollutants: e.g. measuring the effect on burrowing behaviour in earthworms exposed to carbamate in microcosms [174]. On the other hand, Collembola dispersal can be affected by metallic pollution in soils [160] and some isopods,

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such as *P. laevis*, can identify and avoid leaves contaminated with high cadmium sulphate levels [134].

### 3.1.3. Malfunction of soil biological processes

Few studies have compared the interruption of biological process in contaminated soils and soil fauna biological reactions. These changes are difficult to evaluate in the field as soil biological processes are quite complex and involve many different factors, including soil fauna. However, their activity is essential for the whole ecosystem and must be maintained for ecosystem survival.

Laboratory studies have attempted to analyse the different biological processes, particularly changes caused by a pollutant on soil fauna.

Salminen et al. [154] have demonstrated that the herbicide terbuthylazine can provoke changes in microarthropods introduced into forest soils by effecting trophic interactions and thus indirectly disturbing soil functioning. However, the same experiments were performed with PCP and did not yield any significant results [153]. Komulainen and Mikola [102] observed a clear reduction in respiration and nitrification in soils polluted by heavy metals, although the role of soil mesofauna could not be defined. The effects of different pesticides on litter-bag microarthropod colonisation and maize decomposition in a maize field yield complementary information, as some pesticides strongly effect microarthropods but had no visible effect on litter decomposition [28].

Kula and Römbke [107] have compared methods for testing organic matter breakdown in the field (bait lamina tests, litterbags and litterboxes, minicontainers, cotton strip). Different criteria have been used: ecological and ecotoxicological (in terms of functional importance for ecosystems, realistic exposure or sensitivity), practicability (in terms of possibilities of standardisation or cost effective) and evaluation (in terms of reproducibility or variation coefficient). Bait lamina test was recommended to study biological activity parameters. The authors pointed out the urgent need for further studies on the litter decomposition parameter, especially to improve test reproductiveness.

Earthworm activity, which is usually responsible for fragmentation and incorporation of organic matter into the soil, is modified by pollutants [26]. These changes have been measured for *Lumbricus terrestris* by evaluating the disappearance of litter or other bait in a Daniel funnel [10].

### 3.1.4. Conclusion

The lethal effects of severe changes (disappearance of certain species) and particularly the sublethal effects on the most sensitive and important phases (growth and reproduction) in the animal life cycle should be

closely studied as they affect species survival. Biomarker studies will help explain how pollutants interact in organisms, both at cellular and subcellular levels, to understand how toxic substances affect genomes. Studies on how soil processes malfunction should be extended and diversified to involve multi-disciplinary research teams to examine a wide number of parameters and thus better analyse the different factors involved.

## 3.2. Ecological indicators

Several studies have attempted to extrapolate laboratory results to the field. Diekkrüger and Röske [40] tried to simulate collembolan population dynamics using *Isotoma notabilis* in agricultural fields based on data obtained in the laboratory on this species ecophysiology. Løkke [120] also tried to link laboratory experiments on the dimethoate model with experiments using mesocosms with different collembolan species (*Folsomia candida*, *F. fimetaria*, *I. notabilis*).

Although the first results have been encouraging, results obtained in laboratory experiments cannot yet be extrapolated to the field. Spurgeon [166] noted that a particular earthworm, *Eisenia fetida*, could live in soils containing much higher concentrations of metals than the  $LC_{50}$  and  $EC_{50}$  values found in the laboratory.

Since it is impossible to control all the different factors affecting soil fauna, bioindicators of ecological effects are used.

Studies to evaluate the in situ impact of different types of pollution on animal groups have been performed for soil microarthropods and earthworms.

Both synchronic (two similar polluted or unpolluted sites) and diachronic (measurements performed before and after pollution) comparisons must be made. In general, these two approaches are combined: this procedure is well known as before-after impact control (BACI) design [93]. In fact, different populations often reveal natural fluctuations which can be due either to standard (climate, photoperiod) or exceptional (severe cold, drought) abiotic factors or to biotic factors (between and within species competition, individual species life cycle). These different factors are like background noise for a study site and can hide the effects of a pollutant. They must be quantified as accurately as possible.

Comparisons are generally based on two types of criteria:

- those involving population density, i.e. species, population or community abundance or biomass;
- those involving population structure, i.e. specific richness or diversity.

Sampling techniques for microarthropods can vary: boring cores, litter-bags [34] or pitfall traps [74, 96]. Results obtained using these techniques are often quite different.

In fact, different studied species often react differently to a given pollutant. Frampton et al. [65] demonstrated the different reactions of Collembola species subjected to different culture pressures: while *Folsomia quadrioculata* was shown to benefit from integrated culture systems, the genus *Lepidocyrtus* reacted better using standard systems.

In addition, apart from the pollutant itself, the pollutant and the site's history can affect the reactions of a microarthropod community. Sabatini et al. [152] found no significant differences between microarthropod populations in sites treated with atrazine and untreated sites when the study site had been exposed to the pollutant for several years, as pollution-sensitive species had probably vanished prior to the study.

Diversity indices should be used carefully: as Ramade [143] pointed out for the Shannon index, at sublethal doses, the dominant pollution-sensitive species can decrease, without disappearing, which paradoxically may increase the index. These indices should only be used in extremely polluted sites.

Taxonomic diversity alone is not enough to evaluate a site's biodiversity. Coenotic diversity, which expresses the distribution of taxa within a species, should also be considered [19].

Cortet [27] has demonstrated significant differences between two culture systems (standard and integrated) for microarthropod biodiversity in Normandy (France) using an index which evaluated taxonomic diversity, specific diversity, abundance, taxonomic richness and spatial distribution of taxa [20].

Normality, in the mathematical sense of the word, is not a reliable criteria either: highly disturbed communities can have a log normal distribution, whose dominant species or groups are not the same as those found for stressed communities. Hågvar [77] insists that rare species should also be considered, as they evolve towards the lower dominance classes if they are sensitive and towards higher dominance classes if they are tolerant.

Frampton [64] also suggests using an indicative species pool with a known sensitivity to certain pollutants for field studies. Using a single species could obscure inter-species relationships, while considering all the species in a system will not reveal effects on rare species, which can be more affected than abundant species. These conclusions should be treated with caution, however, as different statistical analysis techniques, such as correspondence analysis, now make it possible to evaluate the influence of highly abundant species compared to rarer species.

The functional aspect must also be considered, particularly in terms of the influence of pollutants on species biological cycles. However, these biological cycles must first be characterised and the different taxa must be ranked. Siepel [159] designed an evaluation grid for microarthropod life cycles which differentiated between twelve different life cycle strategies on

either a trophic or a sexual basis. This is particularly interesting in the field of ecotoxicology. However, it is difficult to rank species whose biological cycle is not totally understood. This is where laboratory diversity studies are vital, particularly for studies of a large number of taxa.

Results analysing the role of mesofauna activity on changes in organic matter are both highly varied and irregular, reflecting the complexity of decomposition processes which are only partly dependent on soil fauna [158, 175]. In addition, no studies using the litter bag method have been able to significantly correlate decomposition of organic matter and microarthropod density.

Currently, there is no standardised method for in situ studies of microarthropods.

Different extraction techniques have been used for earthworms: hand sorting [23], formaldehyde [144] and the ethophysical method.

Most earthworm studies have only examined lethal toxicity of pollutants [54, 55, 117]. This is an illustration of the difficulty of seriously estimating the real toxicity of different pollutants, as a lot of differences occur between study protocols.

During the Sheffield ecotoxicology workshop, several proposals were made to standardise and co-ordinate certain research topics [51, 109, 118] (*table IX*).

It should be noted that field studies primarily use methods derived from ecology. However, it is vital to develop specific methods for ecotoxicology, whose goals are different. Studies must be made on different types of organisms to describe species or groups which could be used as bioindicators in the field. These studies should lead to the standardisation of research procedures. In the long run, an evaluation grid should be designed which includes many different types of organisms and would help create a biotic index of soil quality to objectively quantify pollution.

#### 4. CONCLUSION

This paper has reviewed the main data available about how pollution affects soil fauna invertebrates used as indicator organisms. There is no ideal indicator [83]; however, enchytraeids, earthworms, Collembola, acarids, isopods and pulmonate gastropods have provided additional data on the three bioindicator categories (bioaccumulation, toxicological and ecological consequences).

Taxa which could be used as bioindicators, in terms of measurable responses, are shown in *table X*.

Bioaccumulation measurements provide data on the bioavailability of pollutants in the environment and make it possible to evaluate species sensitivity to xenobiotics [31]. The current lack of standardised measurement procedures for bioaccumulation will hopefully

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**Table IX.** Summary of suggestions for field study standard protocols [174]. \*\*\* Undefined characteristics.

	Edwards [51]	Lofs [118]	Kula [108]
Site	grass, arable land	***	grass, arable land, artificial site
Soil description	texture, OM, homogeneity	texture	at least texture, OM, pH, pF
History	> 5 years without pesticides	***	***
Additional parameters			temperature, rainfall
Density	> 100 ind·m <sup>2</sup>	> 100 ind·m <sup>2</sup>	> 100 ind·m <sup>2</sup>
Representative species	at least the six most common species	one endogeous + one anecic	<i>Lumbricus terrestris</i> ; <i>Aporrectodea caliginosa</i>
Initial plot size	5 to 10 m <sup>2</sup>	***	100 m <sup>2</sup>
Number of replicates	> 4 per treatment	> 4 per treatment	> 4 per treatment
Pesticide	standard pesticide + physical and chemical toxicity parameters	physical and chemical toxicity parameters	physical and chemical toxicity parameters
Dosages used	the most important recommended	***	***
Treatment dates	spring	earthworms activity period	spring
Estimated criteria	abundance and biomass	abundance and biomass; growth stages	abundance: biomass with reserves
Sampling methods (sample size)	hand extraction (0.25–1 m <sup>2</sup> ) *30–60 cm; formol extraction (0.25–1 m <sup>2</sup> ); mixed extraction (0.25–1 m <sup>2</sup> ) *5–10 cm	non-destructive extraction; ***	formaldehyde extraction; electric extraction (> 0.25 m <sup>2</sup> )
Number/duration of sampling	> 4/***	***/ 1 d	> 2/***
Experiment duration	> 6 months	4–6 months	12 months
Sampling date	Monthly and > 2 years for remnants of pesticides (1, 2, 4, 8, 12, 24 months after treatment)		1, 4, 6, 12 months after treatment

**Table X.** The use of different taxonomic groups as bioindicators. (–), No available studies; –, use unlikely; +, possible use under certain conditions; ++, interesting for use.

		Nematodes	Enchytraeids	Earthworms	Gastropods	Collembola	Acarida	Isopods
Bioaccumulation		(–)	–	++	++	+	(–)	++
Toxicological bioindicators of effects	Exposure biomakers	(–)	(–)	++	+	+	(–)	++
	Neurological disturbance	(–)	(–)	++	(–)	(–)	(–)	(–)
	Mortality	+	+	++	–	+	+	–
	Reproduction	+	++	++	++	++	++	+
	Function	(–)	(–)	++	(–)	+	+	(–)
Ecological bioindicators of effects								
	Abundance							
	Biomass richness							
	Diversity							
All groups can be used								

lead to the development of an appropriate methodology [137].

Indicators of toxicological or ecological effects provide information which should help develop a series of tests which can be used not only with earthworms [16], but also with other invertebrate species to localise pollutant effects at different levels in the life cycle (figure 1).

In the near future, the various existing tests and those soon-to-be standardised should provide a means of evaluating the environmental quality of different soils. Microcosm and mesocosm experiments should

be continued and expanded to species living in different soil layers to design field study methods.

Toxicological data from laboratory and field studies should be used to evaluate environmental hazards linked to pollutants. Organism species of all sizes (microfauna, mesofauna and macrofauna) and diversity living in the soil should be used to design microcosm and mesocosm models reflecting the diversity and the vital trophic relationships which characterise a living soil. Single species tests are a step towards environmental risk evaluation, but progressively more complicated systems should be designed to analyse interactions between the environment and the creatures which live there.

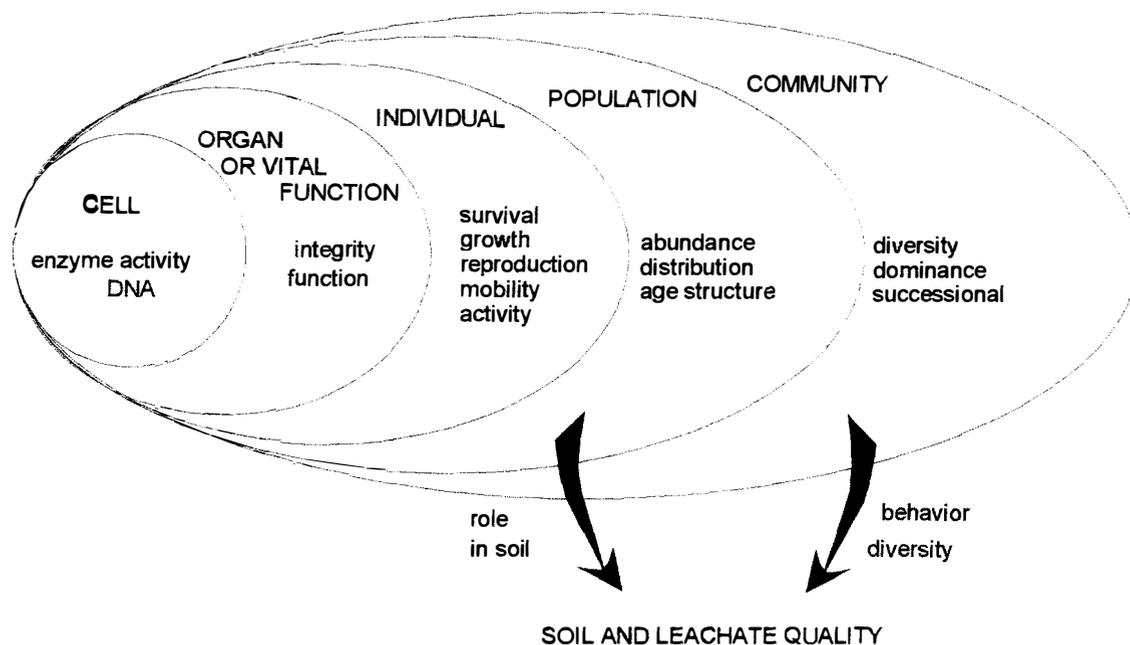


Figure 1. The different levels of pollutant activity.

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