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## Soil Ecotoxicology

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### 1. Introduction

Expanding human activities have generated a mounting stream of waste, which is sometimes released into ecosystems at concentrations considered toxic for living organisms. As a result, populations of wild plants and animals are frequently exposed to toxic risks derived from the industrial wastes, pesticides, heavy metals, and other compounds that are released into the environment on a daily basis (Kappeler, 1979). These pollutants typically end up in soils, where potential toxic compounds come into direct contact with clays and organic material, which have a high capacity for binding to chemical compounds and substances (Bollag et al., 1992). Many organisms that live in soils, including beneficial soil fauna, are thus routinely exposed to high levels of pollution.

In these terrestrial ecosystems invertebrates and microorganisms drive a diverse array of biological and biochemical processes and play important roles in the carbon, nitrogen, phosphorus, and sulfur cycles by breaking down organic matter. Their transformation (mainly the mineralization) of organic material is broadly important for ecosystems and specifically important for agriculture, since the cycling of chemical elements provides much of plant's nutritional needs. Anthropogenic impacts, like the use of agricultural pesticides, can contaminate soils and thereby lead to an ecological imbalance in the soil community that may subsequently compromise the sustainability of the system (Cortet et al., 1999).

The study of the toxic effects that chemical substances have on living organisms, especially in the populations and communities of particular ecosystems, is the aim of the multidisciplinary field of ecotoxicology—a science that incorporates elements of ecology, toxicology, and chemistry, and explores the links between them (Römbke & Moltmann, 1996). Ecotoxicological tests aim to retrace the routes that pollutants take through the environment and to understand their interactions with it (Holloway et al., 1997).

In countries around the world, various such tests on soil organisms have been carried out for at least three decades (Spahr, 1981), in many cases using standardized soils and organisms in a laboratory setting (Organization of Economic Cooperation and Development [OECD], 1984a). Most ecotoxicological studies of soils are based on invertebrates and focus on worms, collembolans, or enchytraeids as bioindicators. The use of these groups has become standard because they are widely distributed, play important ecological roles, live in permanent contact with soils, reproduce quickly, and are easily maintained in laboratories (Edwards, 1989; Edwards et al., 1995; Römbke et al., 1996). The resulting body of research has succeeded in documenting a variety of negative effects that pesticides have

on arthropods, microarthropods, and oligochaetes (Cortet et al., 2002; Jänsch et al., 2005b; Moser & Obrycki, 2009), as well as on soil microorganisms (Zhang et al., 2010).

## 2. The development of soil ecotoxicology

The growth of the human population, allied with technological advances and increasing consumption worldwide, have had adverse effects on the environment. The most worrisome repercussions are those that interfere with natural processes, and these are becoming more common. The impacts of human activities have mounted steadily since the industrial revolution in the eighteenth century, and by the twentieth century some irreversible and uncontrollable consequences for global environmental change and their destabilizing impacts on ecosystems had become apparent (Twardowska, 2004). Environmental disturbances are now capable of threatening the global environment, as shown by climatic changes and atmospheric pollution (Ramanathan et al., 2001), the qualitative and quantitative degradation of water and soil resources, and the severe impoverishment of biodiversity worldwide.

Given these developments, there is a mounting awareness that the very survival of humanity is dependent on current and future influences on environmental sustainability. Likewise, there is growing interest in understanding how the environment responds to sustained anthropogenic pressures, where man-made and natural pollutants end up in ecosystems, and what impacts they have there. Scientifically rigorous answers to these questions are urgently needed to develop the tools necessary to preserve a viable environment in the context of a growing human population (Twardowska, 2004).

The term “ecotoxicology” was coined in 1969 by R. Truhaut, who defined it as the scientific discipline that describes the toxic effects of various chemical agents on living organisms, and especially on populations and communities in ecosystems (Truhaut, 1977). Ecotoxicology is thus a blend of two different kinds of research: research on the natural environment (ecology), and research on the interactions of toxic chemical substances with individual living organisms (toxicology). Its links to chemistry, pharmacology, and epidemiology make it a truly multidisciplinary science that aims at understanding the origins and endpoints of chemical products in the environment (Connell et al., 1999). Present-day ecotoxicology encompasses a variety of scientific principles and methods capable of identifying and assessing the effects of substances released into the environment by mankind (Markert et al., 2003). It has evolved into a predictive science that aims to forecast the effects of potentially toxic agents on natural ecosystems and non-target organisms (Hoffman et al., 2003).

Ecotoxicology's obvious importance in environmental safety at both regional and global scales spurred the creation of a large number of organizations dedicated to environmental safety around the world. These include the International Academy of Environmental Safety (IAES) created in 1971, the International Society of Ecotoxicology and Environmental Safety (SECO-TOX) in 1972 and, in North America, the Society of Environmental Toxicology and Chemistry (SETAC) in 1979. In turn, the creation of these organizations called attention to the serious vacuum of useful scientific tools to guide decision-making regarding how to effectively regulate the release of pollutants into ecosystems (Twardowska, 2004).

In its early years, ecotoxicology developed more quickly for aquatic ecosystems than for terrestrial ecosystems. Water quality criteria were first defined by the United States

Environmental Protection Agency (EPA), which proposed benchmarks for analyzing leading pollutants and water quality attributes with the goal of broadening the use of hydrologic resources (for consumption, bathing, fishing, agriculture, and industrial uses) (Vighi et al., 2006). The European Inland Fisheries Advisory Commission (EIFAC), however, proposed that water quality criteria should focus on preventing impacts on any portion of the life cycle of fishes, preventing leaks from contaminated waterbodies, and, in addition, preventing the bioaccumulation of dangerous substances at levels dangerous to fish (Alabaster & Lloyd, 1978). As ecotoxicology matured for aquatic environments, the rise of internationally standardized methods and bioassays with invertebrates, fish, and algae generated a large database of toxicity for aquatic organisms long before such information existed for soil organisms (Van Straalen, 2002a).

In terrestrial ecosystems, despite a widespread recognition that soil invertebrates were functionally essential and useful as bioindicators of ecological disturbance (Schüürmann & Markert, 1997), until 1995 only two internationally accepted ecotoxicological methods were developed for soil organisms: a test using worms in artificial soils (OECD, 1984a) and an assessment method using plants (OECD, 1984b). Since then a broad assortment of new methods have appeared and the soil ecotoxicology database has grown impressively (Gomez-Eyles et al., 2009; Løkke & Van Gestel, 1998). Even so, the number of standardized tests available for the soil component of terrestrial systems is still lower than that available for aquatic systems.

Terrestrial ecotoxicology has been defined as the subfield of ecotoxicology which uses tests to study, evaluate and quantify the effects of toxic substances on the diversity and function in soil-based plants and animals (Garcia, 2004). Apart from measuring the relevant parameters and meeting environmental requirements, an effective toxicity test should be quick, simple, and replicable. A standard test should reveal a toxic response given variation in environmental conditions such as pH, solubility, exposure time, antagonism, and synergy. In this way, ecotoxicity tests can be classified in terms of exposure time (acute or chronic), observed effect (mortality, reduced growth, or compromised reproduction) or effective response (lethal or sublethal) (Kapanen & Itavaara, 2001).

The first stages of ecotoxicity analyses are carried out in the laboratory, where varying concentrations of the chemical products under study are added to an artificial substrate (International Organization for Standardization [ISO], 1999a, 1999b) or to natural soil (ISO, 2003), and acute toxicity, chronic toxicity, and behavioral effects are measured using soil quality bioindicators (Jänsch et al., 2005a).

Many bioassay methods have been developed to test acute toxicity (mortality) of chemical compounds, including local application, force-feeding, and immersion (Kula & Larink, 1997). However, standard international methods (OECD, 1984a; ISO, 1993, 1999a, 2004) are more broadly accepted. Most such tests are carried out using standard indicator organisms, but some authors have extrapolated and adapted the methods for other bioindicators (Förster et al., 2006; Jänsch et al., 2005b). While short-term tests of acute toxicity are useful for identifying highly toxic chemical compounds, they cannot determine whether organisms are more sensitive to those compounds during particular stages of their life cycles (Rida & Bouché, 1997). By contrast, medium-term tests of chronic toxicity measure the sublethal effects of harmful compounds on reproduction and growth, caused by biochemical and physiological disturbances (Hoffman et al., 2003). Standard tests of this kind have been

established for some of the most commonly used non-target invertebrates in soil ecotoxicology, including collembola (ISO, 1999a), enchytraeids (ISO, 2004), and worms (OECD, 2004; ISO, 1998).

Since the duration of tests and the labor they require determine the costs of ecological risk assessments, it is sometimes preferable to obtain faster results using higher thresholds of sensitivity (ISO, 2008). For this reason avoidance tests, which provide a preliminary evaluation of contaminated soils in a short time, have increased in popularity (Natal-da-Luz et al., 2008) compared to toxicity tests. These tests are also ecologically relevant due to the high sensitivity thresholds, especially in assessments of remediated soils (Shugart, 2009), and are commonly used as a first triage tool to assess the habitation function of soils (Hund-Rinke et al., 2003), as they often provide results that other toxicity tests do not (Yearcley et al., 1996). Carrying out these tests in addition to acute and chronic toxicity tests can provide more detailed information on pollutants' impacts on organisms (Heupel, 2002), since changes in animal behavior can help quantify the effects of stress on individuals and populations (Markert et al., 2003).

The results of acute toxicity tests are expressed in values of  $LC_{50}$ ,  $LC_x$  (lethal concentration), while those of chronic toxicity and avoidance tests are expressed in  $EC_{50}$ ,  $EC_x$  (effective concentration), which indicates the concentration at which half of the study organisms die ( $LC_{50}$ ) or the concentration at which a specific change in normal life parameters or behavior occurs ( $EC_{50}$ ).

Ecotoxicological laboratory tests are a fundamental first tool for evaluating the ecological risks posed by polluted areas and remain commonly used (Bartlett et al., 2010; Correia & Moreira, 2010). However, exposure under laboratory conditions typically represents a worst-case scenario under which the effects on the organism are more severe than those observed in field conditions. This reflects the fact that the soil ecosystem is an extremely complex network of physical, chemical, and biological interactions, while laboratory tests are carried out in optimal conditions for growth and reproduction and thus ignore several variables that may play an important role in interactions between organisms and their environment. As this makes it difficult to extrapolate results from laboratory tests to field conditions (Van Gestel & Van Straalen, 1994), assessments of ecological risk should always seek to reproduce conditions in the field as closely as possible.

The first stages of ecotoxicological tests focus on individual species of the most important functional groups in the soil ecosystem (invertebrates and plants) (Garcia, 2004). The next stage aims to examine effects at the community level, using experiments in microcosms and mesocosms that incorporate multiple species and can provide more concrete results (Alonso et al., 2009). Microcosm tests (Figure 1) are generally carried out in the laboratory, where a few species of animals and/or plants are placed in natural soil. Mesocosm tests are large, multispecies systems that offer a high degree of environmental realism, since they are often carried out outdoors under natural light and rainfall conditions (Isomaa & Lilius, 1995). In a mesocosm test it is possible to study effects not only on individual species, but also at the population and community levels within an ecosystem (Crossland, 1994). These tests can identify cases in which impacts on a single species, whether a microorganism or a soil invertebrate, affect not just that species but the rest of the biota as well, potentially compromising entire ecosystems (Landis & Yu, 2004).



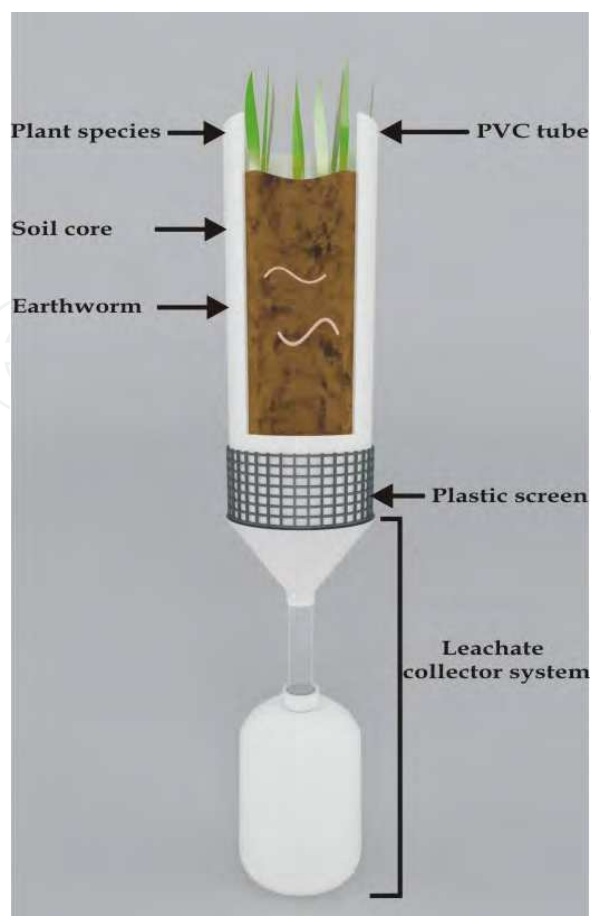


Fig. 1. Terrestrial Model Ecosystem (TME) used in the microcosms tests. On right side we represent the components of the TME, and on the left side, the ecological components. Adopted from Santos et al. (2011) (Virtual representation: Spisla, L. F.).

One drawback of the mesocosm model is the more challenging interpretation of results; in more complex systems it is harder to establish cause and effect between pollutant levels and degrees of community change, since many components of the system are dynamic and interdependent (Clements & Kiffney, 1994). Mesocosms assessments are also expensive and typically have few replicates, making them unviable for routine ecotoxicological triage (Crossland, 1994).

A third stage in environmental risk evaluations, considered the final step of this kind of analysis, involves measuring impacts in the field, under totally natural ecosystem and climate conditions. In this case, the observed responses reflect the effects of pollutants on soils with greater precision. Such tests may include analyses of bioaccumulation in animal tissues (Hoffman et al., 2003); the use of traps (pitfalls) (Querner & Bruckner, 2010) or soil samples (TSBF and Berlese funnels) to quantify the diversity and abundance of macro-, meso-, and microfaunal species (Anderson & Ingram, 1993; Araújo et al., 2010); litter bags (Förster et al., 2006) and bait-lamina tests (André et al., 2009) to measure the decomposition of organic material, in addition, direct (microbial biomass) and indirect (respirometry and enzyme tests) microbiological analyses may be used (Kapanen & Itävaara, 2001). Armed with this broad array of research tools for studying field conditions, it is possible to describe contaminated areas more precisely and to make more informed decisions, whether in the remediation or the prevention of environmental impacts.

In addition to the tests applied in the various stages of ecological risk assessments, new techniques and perspectives continue to enrich terrestrial ecotoxicology. One example are the genotoxicity tests with specific microorganisms, used to assess the impacts of waste products (Brown et al, 1991; Donnelly et al, 1991). Other studies have documented important adaptive responses (genetics) following the exposure of living organisms to chemical products (European Centre for the Ecotoxicology & Toxicology of Chemicals [ECETOC], 2001; Lovett, 2000; Nota et al., 2010; Snape et al., 2004). This new subfield offers one more tool (molecular biology) for improving our understanding of environmental responses to toxic pollutants (Bradley & Theodorakis, 2002).

As noted previously, ecotoxicological tests are typically classified by their duration, the number of species involved (Landis & Yu 2004; Römbke et al., 1996), and further, subdivided along a gradient ranging from basic laboratory tests to complex field experiments (Figure 2). But while more complex tests offer more reliable information in ecotoxicological risk assessments, they have been used sparingly due to their complexity, cost, and long duration (Römbke & Notenboom, 2002). In general, terrestrial ecotoxicology has evolved towards ever more precise quantitative or qualitative assessments of the effects of pollutants in soils, as called for by numerous regulatory agencies worldwide as part of their efforts to determine acceptable concentrations of pollutants in soils, to limit their exposure, and to protect the terrestrial biota (Shugart, 2009).

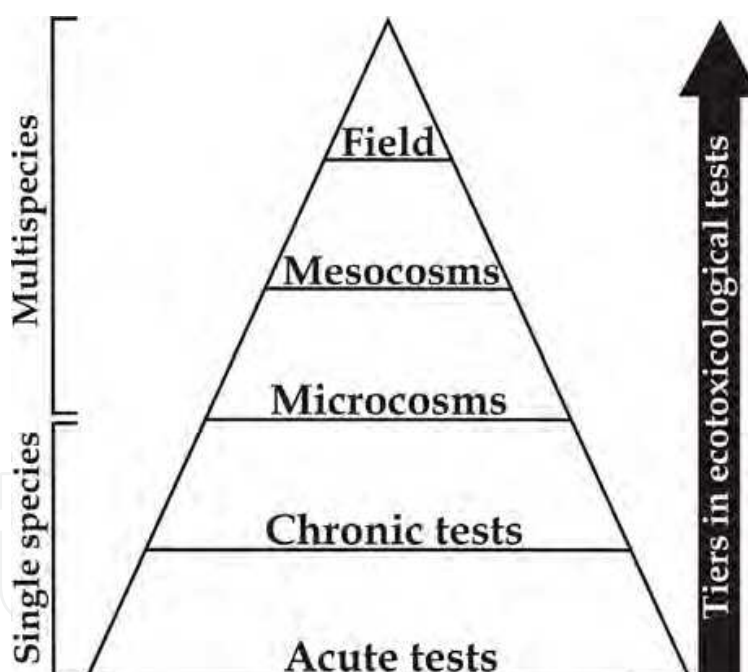


Fig. 2. Sequence of tiers used in Environmental Risk Assessment (ERA). From basic tests (acute) to most complex systems (field) of evaluation in soil ecotoxicology. According to the increases of tiers levels there is an increase of the bioassays complexity and costs. Adapted from Landis & Yu (2004).

### 3. Bioindicators of soil quality

The term "soil health" has been defined by Pankhurst et al. (1997) as "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to

sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health". However, quantifying soil health with tools that track measurable properties of the system remains a steep challenge.

When measuring the health of a person or animal, one typically relies on parameters that describe the function of various organs, tissues, body fluids, physical structures (bones), and other components of the subject's body. In the same way, the living soil ecosystem is also composed of physical structures, chemical solutions, and biological communities in constant interactions that maintain vitality. Soils are a heterogeneous mix of biotic and abiotic components inhabited by a very complex community of organisms. The basic functions of the system depend on its structural and functional integrity, and this functionality is directly impacted by disturbances, needing some parameters for a better analysis (Edwards, 2004).

One appropriate way to quantify soil health is by measuring the parameters that make it a living system. Most attention in this regard has focused on indicators of the chemical and physical properties of the edaphic system, since biological properties are typically considered more difficult to predict or even measure. While a vast array of such indicators of soil quality or vitality have been proposed to date, it has become increasingly clear that biological indicators are needed to describe these dynamic systems (Blair et al., 1996). Biological processes may be more sensitive to soil changes than are indicators based on physical and chemical properties, which suggests that biological indicators could potentially offer early warnings of risks to ecosystems (Pankhurst et al., 1997).

Among the various definitions of the term "bioindication" (Heink & Kowarik, 2010), one that is widely accepted in terrestrial ecotoxicology describes it as a scientific analysis of field-collected ecological data with the aim of characterizing the environmental quality of a given area or region (Van Straalen, 1998). The same author emphasizes that bioindicator organisms should be directly or indirectly associated with the particular factor or suite of factors that they are intended to monitor. The utility of such biological indicators, also known as bioindicators or biomarkers, is that they describe the responses of living organisms exposed to or harmed by pollutants and thus provide information that can help prevent future damage to ecosystems (Hoffman et al., 2003). Biological indicators may thus be thought of as a barometer; just as barometers measure air pressure, the soil biota reflect the health of soils by revealing the cause of a particular effect or condition of the ecosystem (Van Straalen, 1998).

More than four centuries ago, long before the agricultural revolution, Vincenzo Tanara observed that soils are fertile when "birds such as ravens are attracted to a recently plowed field and scratch at the earth to eat the small invertebrates exposed by plowing". Much later, in the nineteenth century, Darwin argued that worms play an important role by building chambers in the soil profile and producing humus—which suggested that animals contributed directly to soil function (Paoletti et al., 1991). Since then, several studies have documented the importance of the soil biota for soil quality and vitality (Lavelle, 1996; Lavelle et al., 2006), and its potential for reflecting anthropogenic disturbances (Cortet et al., 1999; Paoletti et al., 1991; Van Straalen, 1998). By this measure, the first studies of bioindicators date to the beginning of the twentieth century. It is only more recently, amid mounting concern about the management and conservation of natural resources, that they have come to be regarded as important for assessing degraded environments (Paoletti et al., 1991).



In order to be considered promising biological indicators, organisms should occur in a variety of ecosystems, so that between-ecosystem comparisons are possible; should also be in contact with various stress factors, and thus interact with physical, chemical, and biological processes in soils; and should have direct functions and ecological importance in the edaphic system. Finally, a biological indicator should be easy and inexpensive to measure in order to facilitate sampling and experimentation, and be sensitive to a range of different management impacts, but not so sensitive that it vanishes altogether (Edwards et al., 1995; Stenberg, 1999).

Nowadays, bioindicators can be useful if a certain impact factor is not easily measurable. For example, in agricultural ecosystems the effects of pesticides such as the synthetic pyrethroid deltamethrin are easily observed in the epigeal invertebrate fauna, but the chemical determination of the residue is more difficult (Everts et al., 1989; Krogh, 1994). As toxicity tests are developed to help prevent immediate impacts of pollutants released into the environment, biomonitoring methods assess impacts over longer periods and track changing conditions in addition to the stressors or pollutants already present in the environment (Office of Environmental Health Hazard Assessment [OEHHA], 2008).

Invertebrate species that have shown potential as soil quality indicators include beetles (Kromp, 1999), ants (Lobry de Bruyn, 1999), spiders (Marc et al., 1999), mites (Koehler, 1999), collembolans (Cortet et al., 1999), enchytraeids (Didden & Römbke, 2001), nematodes (Yeates & Bongers, 1999), and worms (Blair et al., 1996; Paoletti et al., 1991; Paoletti, 1999). These invertebrates are used as inexpensive bioindicators, due to their ease of monitoring and the lack of restrictions on their use (Pankhurst et al., 1997). However, soil microorganisms can also offer valuable insights that can help interpret effects attributed to disturbance in terrestrial ecosystems (Bossio et al., 2005; Kapanen & Itävaara, 2001; Staley et al., 2010).

In an ideal world, all chemical products would be tested on every animal species before being released into the environment. As this is an unobtainable goal, representative species are used as triage tools in order to identify substances that are especially toxic. For soils, worms (*Eisenia* sp.), enchytraeids (*Enchytraeus* sp. and *Cognettia* sp.) and collembolans (*Folsomia* sp.) are the most widely used groups (Figure 3), due to their easy maintenance in laboratories and their relatively short generation times (Achazi et al., 1997; Fountain & Hopkin, 2005; Ronday & Houx, 1996).

There is a growing consensus among soil ecologists and farmers that worms may be one of the best indicators of soil quality (Doube & Schmidt, 1997). They are relatively easy to sample and identify, present in a broad variety of soils, regions, and climates, and indisputably important to food webs. Worms are important indicators of both soil quality and vitality, given that worm communities and their behavior are strongly affected by many leading agricultural practices such as crop plantation and rotation, fertilization, and the application of pesticides (Edwards, 2004).

Another important but less studied group in the subclass Oligochaeta are enchytraeids (Bardgett, 2005). While enchytraeids account for a small fraction of living biomass in soils, they are extremely important for nutrient cycling, feeding on fungal mycelium, rotting organic material, and associated microorganisms (Brussaard et al., 1990; Didden, 1993). They are considered "Key-species" for bioindication (Didden & Römbke, 2001; Römbke & Moser,

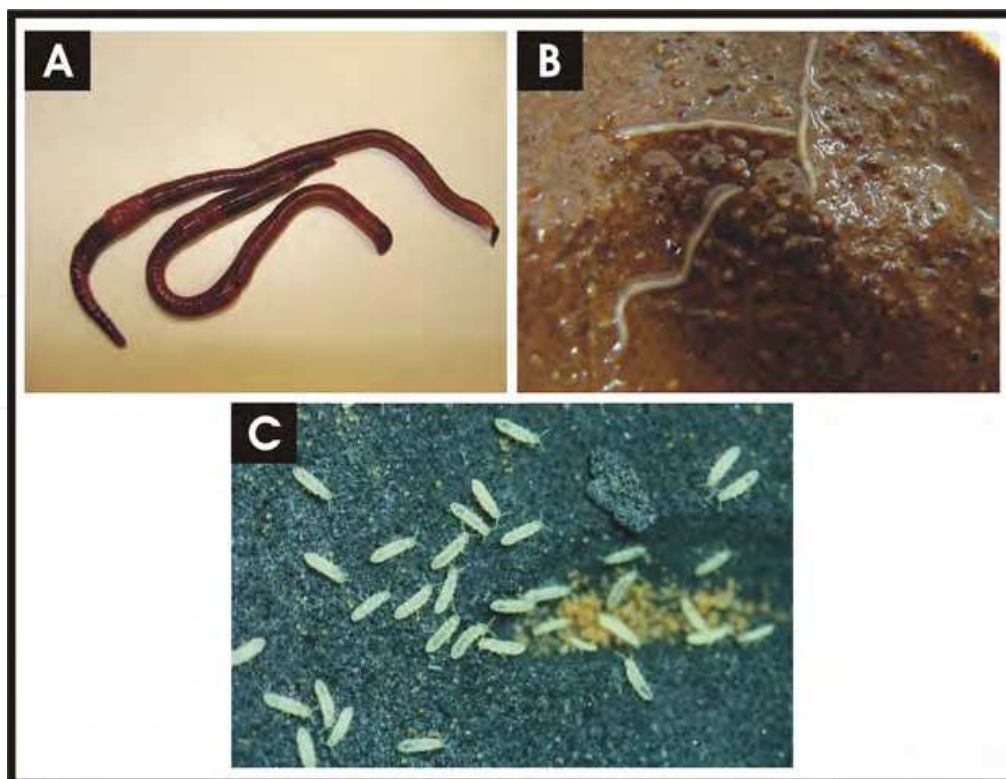


Fig. 3. Principal bioindicators of soil quality used in laboratory tests: *Eisenia andrei* (A); *Enchytraeus* sp. (B); *Folsomia candida* (C). (Photo: Ribeiro, C. M. & Santos, C. A.).

2002), especially in acidic soils (Didden, 1993), where they can replace worms as the functionally dominant soil fauna and where their abundance makes them good indicators under restricted conditions (Cole et al. 2002; Didden, 1993). Even so, while enchytraeids sometimes play an irreplaceable role in decomposition, they are less appropriate indicators than worms in more conventional soil conditions (Pankhurst et al., 1997).

The order Collembola is one of the most diverse and abundant groups of terrestrial arthropods on Earth, where they occur across all biomes (Coleman et al., 2004; Resh & Cardé, 2003;). A single square meter of dirt in temperate forest may contain more than 100,000 individuals (Anderson, 1978). While the direct effects of collembolans on ecosystem processes (like energy flows) may appear small due to their modest contribution to soil biomass and respiration (Coleman et al., 2004; Jänsch et al., 2005a), these organisms exercise significant influence on microbial ecology and soil fertility, since by feeding on dead organic matter and soil microorganisms they regulate decomposition and nutrient cycling processes (Culik & Zeppelini, 2003). The responses of these organisms to chemical products in the laboratory can thus be used to assess stress and inform legislative action concerning the ecological risks of certain substances (Van Straalen, 2002b).

Based on these foundations, the use of biological indicators of soil health and the process of assessing soil impacts have made considerable advances in recent years, as a large number of tests have used these model invertebrates to examine pesticide substances in soils and assess the risks they pose to the environment (Didden & Römbke, 2001; Gomez-Eyles et al., 2009; San Miguel et al., 2008).

#### 4. Pesticides in soils

The term "pesticides" encompasses all active ingredients (a.i.) produced to control agricultural pests, independent of composition, formulation, and concentration. While the term is typically associated with substances that are used to control or kill pests (e.g., insecticides, fungicides, herbicides), it also applies to chemical compounds that alter pest behavior or physiology (e.g., insect repellents and growth regulators) (World Health Organization [WHO], 2010).

Synthetic insecticides and fungicides were developed after the Second World War and their application to crops spread quickly. Because insecticides were initially inexpensive and effective, farmers became dependent on the new tools, which soon replaced other chemical, cultural, and biological methods of pest control. The use of insecticides and fungicides in agriculture grew massively in the 1950s and 1960s and has continued to grow since; applying pesticides remains the leading method of pest and pathogen control today (Gulan & Cranston, 1994; Plimmer et al., 2003). Indeed, some studies on the benefits of pesticides have warned of the harm that could be caused by banning them, due to fears of a drop in agricultural productivity caused by pests (Delaplane, 2000; Walters, 2009).

Synthetic insecticides can be classified according to the presence or absence of carbon atoms, in its main molecule, as inorganic insecticides (e.g. boric acid) (Habes et al., 2006) and organo-synthetic insecticides. However, the organo-synthetic insecticides are considered the most important class of insecticides. They include the organochlorines, organophosphates, and carbamates, as well as a number of other classes like pyrethroids and neonicotinoids (Munkvold et al., 2006; Paulsrud et al., 2001). The first classes of fungicides included benzimidazoles, carboximides, morpholines, amino-pyrimidines, and organophosphorus compounds. In the 1970s there emerged a second generation of synthetic fungicides that included dicarboximides, phenylimides, triazoles, and fosetyl-aluminum. More recently developed fungicides include anilinopyrimidines, phenylpyrrole, and strobilurins, which show broad-spectrum action on the most common classes of pathogenic fungi (Plimmer et al., 2003).

In recent years the use of pesticides has increased in agriculture. Whether as a gas, a liquid, a powder, or in granulated form, these products remain commonly used to prevent or control pest outbreaks. One way in which pesticides are used to prevent pest outbreaks is by treating seeds with insecticides and fungicides. All commercially available corn and sorghum seeds are treated with fungicides, and some are also treated with insecticides (Lipps et al., 1988; Munkvold et al., 2006; Paulsrud et al., 2001). The practice is highly effective, offering significant reductions in pest damage (Giesler & Ziemis, 2008; Lenz et al., 2008; Vernon et al., 2009). Treating seeds with insecticides has also become more common worldwide; in fact, a large proportion of the global increase in insecticides use is due to their increasing use on seeds (Hicks, 2000).

While the use of pesticides in agriculture provides benefits, it also has negative impacts, as evidenced by the increasing amounts of these chemical substances required year after year. It is well known that when pollutants come into contact with ecosystems they cause repercussions that can be traced from the molecular level up through tissues, organs, individuals, populations, and even entire communities (Schüürmann & Markert, 1997). Examples of the damage caused by pesticide wastes released into the environment include

accidental poisoning of children and animals, intoxication of the people applying them, trace amounts in foods, phytotoxicity in plants, as well as soil, water, and air pollution (Dhingra, 1985; Paulsruud et al., 2001; Van Straalen, 2002b).

We have already discussed how the quality of terrestrial environments is compromised when non-target soil organisms are negatively impacted. Disturbances caused by pesticides in soils lead to qualitative and quantitative changes in the functioning of soils (Cortet et al., 1999), via two pathways. The first is via the direct contact of organisms with the substance, as is the case with pollinators, parasitoids, and beneficial predators, among others. The second way that organisms are negatively impacted is via the residues of these substances left on plants and elsewhere in the environment, which can harm the local fauna over longer time scales (Waxman, 1998).

Many insecticides kill target insects by acting on their nervous system. This specificity for the binding site of the substance also kills non-target organisms in the same way (Marrs & Ballantyne, 2004). Fungicides can harm non-pathogenic microorganisms (important in nutrient cycling) and antagonistic microorganisms (pathogen suppressors), thereby exercising a direct effect on disease control (Walters, 2009). In addition to insecticides and fungicides, herbicides are another class of pesticides with worrisome effects on the soil fauna. Developed to control weeds, they have shown harmful effects on macrofauna (Correia & Moreira, 2010), mesofauna (Heupel, 2002), and microorganisms (Widenfalk et al., 2008; Zhang et al., 2010).

Several studies to date have attempted to detect and assess the sublethal effects of pesticides on organisms exposed to contaminated soils. Bioassays have documented acute toxicity in the species *Eisenia fetida* exposed to fungicides in artificial soils (Anton et al., 1990), and under a variety of conditions (temperate and tropical climates). In tropical climates fungicides affect worms less severely than in temperate climates, perhaps due to the faster breakdown of pesticides in warm temperatures (Römbke et al., 2007). In addition to fungicides, studies have documented the impacts of insecticides (Gomez-Eyles et al., 2009) on the survival (Mostert et al., 2000), behavior (Capowiez & Berard, 2006) and even physiological processes (inhibition of cellulase activity) of worms in contact with contaminated soils (Luo et al., 1999). Severe population reductions of the collembolan *Folsomia candida* have also been documented in soils contaminated with insecticides (Cortet et al., 2002), but this effect is less apparent in field conditions, due to collembolans' propensity to avoid from contaminated areas. Collembola have also shown some degree of tolerance to some insecticides (San Miguel et al., 2008).

Herbicides used in agriculture also have impacts on worms (Heupel, 2002; Pereira et al., 2009). In tropical conditions, glyphosate and oxadiazon do not cause mortality in *E. fetida*. However, sublethal concentrations of these herbicides hamper the development of juvenile worms (Garcia et al., 2008). Other effects on worms in the presence of herbicides, such as population reductions and loss of body biomass, have also been documented (Stojanović et al., 2007).

In addition to the standard invertebrates typically used in terrestrial ecotoxicology tests, several other ecologically important organisms have been shown to suffer impacts from pesticides in soils (Kilpatrick et al., 2005). Examples include natural predators (Danfa et al., 2003; Moser & Obrycki, 2009) and invertebrate decomposers (Drobne et al., 2008;



Kreutzweiser et al., 2009; Niemeyer et al., 2006). There are also reports of significant reductions of microbial activity in soil (Malkomes, 1993), and it is known that bacteria numbers and fungal biomass are altered in pesticide-treated soils, perturbing decomposition processes and harming other organisms in the trophic chain (e.g., reducing the number of nematodes that feed on fungi) (Colinas et al., 1994; Ingham et al., 1991).

Thanks to the frequent use of these bioindicators in monitoring programs, it is now possible to determine which environmental changes occur when pesticides are exposed to terrestrial systems, with important implications for managing the early stages of pollution and for assessing the effectiveness of preventive or remedial measures taken to improve environmental quality (Van Straalen, 1998).

## 5. Case study: an ecotoxicological assessment of the pesticides used in chemical seed treatments

Treating seeds with chemicals is a common strategy for controlling pests and pathogens during the establishment of agricultural crops, since pesticides applied to the seed surface can repel and reduce the populations of insects and other organisms that attack seeds and seedlings (Baudet & Peske, 2006). In spite of these advantages, treating seeds with pesticides (e.g., fungicides, insecticides, and nematicides) can also have negative impacts, since it puts the a.i. of these substances in direct contact with soils, where soil organisms are exposed to them (Garcia, 2004).

Early studies of these xenobiotics' effects on soil organisms relied on standard tests with worms (*E. andrei*) and collembolans (*F. candida*), and demonstrated the potential of certain insecticides and fungicides for causing acute toxicity (OECD, 1984a; ISO, 1999a). In turn, the responses of these organisms in laboratory tests can be used to forecast, in a first tier, the environmental stresses that pesticides generate in the field (Van Straalen, 2002b).

Among the pesticides commonly used in chemical seed treatments, insecticides with the a.i. imidacloprid and fipronil have been studied via ecotoxicity tests on *F. candida* (San Miguel et al., 2008) and other collembolan species (Cortet & Poinso-Balaguer, 2000; Heijbroek & Huijbregts, 1995; Peck, 2009). Reproductive impacts on the worm species *E. fetida* (Gomez-Eyles et al., 2009) and mortality on other oligochaete species (Mostert et al., 2002) have also been documented in the presence of these insecticides. Among the fungicides used to treat seeds, carboxin + thiram are known to have negative impacts on collembola (Larink & Sommer, 2002) and captan is known to affect worms (Anton et al., 1990).

The object of this study was to assess if pesticides (both fungicides and insecticides) used in chemical seed treatments affect the survival (acute toxicity) of *E. andrei* and *F. candida* in artificial soils.

### 5.1 Materials and methods

Methods for raising collembolans and worms were adapted from the ISO 11268-2 and ISO 11268-2 standards (ISO, 1998, 1999a). The organisms were raised and the bioassays carried out in a climate-controlled room where temperature measured  $20 \pm 2^\circ\text{C}$  and illumination was set to a 12-hour cycle of light and darkness. The substrate typically used in terrestrial ecotoxicological tests is OECD artificial soil (OECD, 1984a); in this study we used a version



of that substrate modified by Garcia (2004) and known as Tropical Artificial Soil (TAS), which includes powdered coconut fiber in its organic fraction.

We applied an aqueous solution containing the fungicides Captan® (captan - 480g of a.i. L<sup>-1</sup>) and Vitavax® (38.7% carboxin + 37.5% thiram - 200g of a.i. L<sup>-1</sup>), in addition to the insecticides Gaucho® (imidacloprid - 600g of a.i. L<sup>-1</sup>) and Standak® (fipronil - 250g of a.i. L<sup>-1</sup>), to the TAS immediately before starting the tests. Pesticide concentrations were established based on preliminary tests, and the control treatment was deionized water.

To assess mortality effects we carried out acute toxicity tests with *E. andrei* and *F. candida* (ISO, 1998, 1999a). For these tests, containers were filled with artificial soil treated (different volumes of soil for each organism) with the pesticide solution or with deionized water (control). At the start of the bioassay, 10 organisms (worms or collembolans) were added to each test container. Mortality was recorded by counting the number of adult organisms still alive after 14 days.

## 5.2 Results and discussion

Collembola (*F. candida*) suffered significantly higher mortality in TAS treated with the insecticides imidacloprid and fipronil compared to the control treatment (Figure 4). Mortality has previously been documented in this species following exposure to a low-concentration aqueous solution of fipronil for 96 hours (San Miguel et al., 2008). Likewise, imidacloprid, which is used in seed treatments, has been shown to reduce the abundance of the collembolan species *Onychiurus armatus* (Heijbroek & Huijbregts, 1995). It thus makes sense that soils where imidacloprid is present have reduced numbers of collembolans, possibly due to mortality (Peck, 2009).

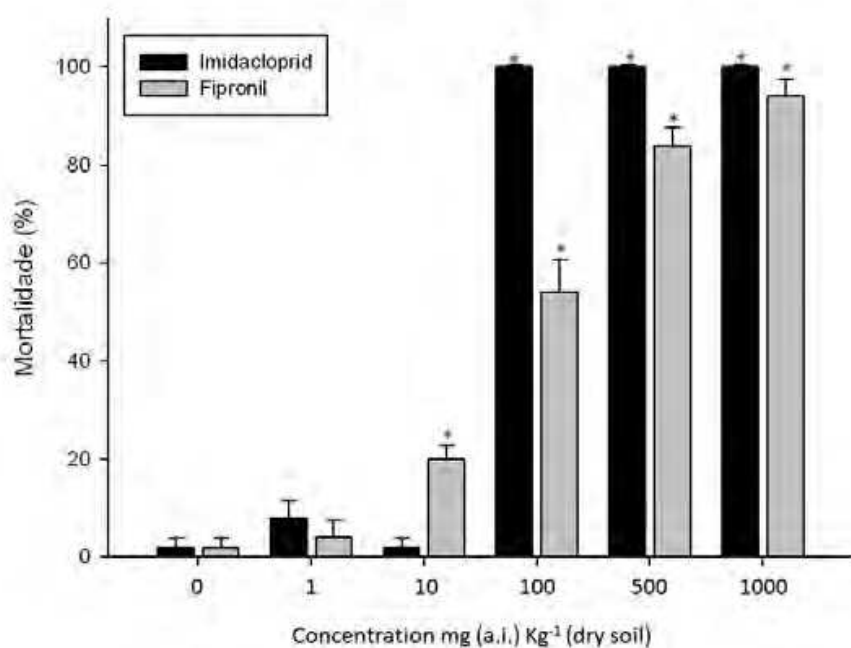


Fig. 4. Mortality (%) of *F. candida* in artificial soil treated with increasing concentrations of imidacloprid and fipronil ( $P < 0,05$  on Dunnett's test)

Fipronil acts on the insects' central nervous systems, where it inhibits a neurotransmitter responsible for regulating neuronal arousal. This neurotransmitter prevents the overstimulation of nerves, and its inhibition results in death. This mechanism of the insecticide's toxicity is mostly activated via ingestion, and is followed by spastic paralysis, death, and the elimination of sensitive insects (Coutinho et al., 2005). Since both the organisms and their food were in contact with contaminated soil in our study, it was very likely that the collembolans died due to inhibition of the neurotransmitter.

Imidacloprid also caused significant mortality in the worm species *E. andrei*, compared to the control treatment. Morphological alterations were also observed in these oligochaetes, which later died (Figure 5). Similar effects of mortality in *E. andrei/fetida* have been documented in studies of OECD artificial soil treated with imidacloprid (Buffin, 2003; Luo et al., 1999). While mortality of this worm species has been documented in polluted natural soils (Gomez-Eyles et al., 2009), effects in natural soils are typically weaker than those recorded in artificial soils, according to Kula and Larink (1997).



Fig. 5. Morphological alterations and mortality of worms (*E. andrei*) exposed to soils contaminated with the insecticide imidacloprid in an acute toxicity test. (Photo: Ribeiro, C. M.).

The lethal effect of imidacloprid on worms and collembolans may also be linked to blocked receptors in the nervous system. While insects are generally more susceptible to such blockage, which leads to the accumulation of acetylcholine (Buffin, 2003), this important neurotransmitter is present in many organisms and its blockage results in paralysis and sometimes death (Kidd & James, 1991). The worms that were in full contact with contaminated substrate for 14 days may have been affected in this way and subsequently died.

In addition to the insecticides, the fungicide captan also caused mortality in *F. candida*. In this case, however, the effect was only observed at high concentrations of the a.i. in the TAS. Mortality was not observed for worms exposed to captan, even at high concentrations. This result is corroborated by other studies that have shown that captan does not limit survival in these oligochaetes (Anton et al., 1990). Collembolan mortalities may be attributed to the compound tetrahydrophthalimide, a metabolite that is more common in captan (Reigart & Roberts, 1999) and which is responsible for the interaction of this fungicide, within cells, with the sulfhydryl, amino and hydroxyl groups of enzymes. These interactions are important because they may inhibit certain metabolic processes in the cells, leading to death (Waxman, 1998).

As in the test with the fungicide captan, we did not observe mortality of *E. andrei* exposed for 14 days to substrates treated with the pesticide carboxin + thiram. Worm mortality in the presence of fungicides has been previously reported in LUFA natural soil (ISO, 2003), in much lower doses than those in artificial soils (Röembke et al., 2007), which suggests that in our study the use of Artificial Tropical Soil may have partially buffered the negative effects of fungicides on worms.

In collembolans, significant mortality occurred in the presence of carboxin + thiram. In long-term studies, Frampton (2002) likewise documented declining collembolan abundance (and the total disappearance of some species) in agricultural soils treated with fungicides. This may be related to the mortality caused by the two fungicides examined in our study, and could furthermore indicate that collembolans are relatively sensitive to fungicides. There are few reports in the literature of the acute effects of carboxin + thiram on soil invertebrates. However, carboxin showed some impacts on and thiram was highly toxic to the aquatic organism *Daphnia magna* (EPA, 2004a, 2004b). It is possible that just one of the two substances in this product has negative impacts on collembolans. However, the concentration at which toxicity was observed was much higher than the calculated values for exposure to the fungicide in soils (data not shown). A similar conclusion was reached by Coja et al. (2006), who assessed the lethal effects of pesticides on *F. candida* and determined that the concentrations at which negative impacts were observed were higher than those found in soils, suggesting that they did not pose a threat in agricultural field conditions.

### 5.3 Conclusions

Artificial soils treated with the insecticides imidacloprid and fipronil, as well as the fungicides captan and carboxim + thiram, caused significant mortality in the collembola *Folsomia candida*. Only imidacloprid caused significant mortality in the worm *Eisenia andrei*.

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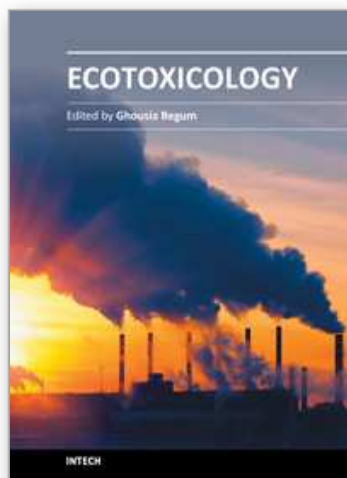


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## **Ecotoxicology**

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This is a good book on upcoming areas of Ecotoxicology. The first chapter describes genotoxicity of heavy metals in plants. The second chapter offer views on chromatographic methodologies for the estimation of mycotoxin. Chapter three is on effects of xenobiotics on benthic assemblages in different habitats of Australia. Laboratory findings of genotoxins on small mammals are presented in chapter four. The fifth chapter describes bioindicators of soil quality and assessment of pesticides used in chemical seed treatments. European regulation REACH in marine ecotoxicology is described in chapter six. X-ray spectroscopic analysis for trace metal in invertebrates is presented in chapter seven. The last chapter is on alternative animal model for toxicity testing. In conclusion, this book is an excellent and well organized collection of up dated information on Ecotoxicology. The data presented in it might be a good starting point to develop research in the field of ECOTOXICOLOGY.

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