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RESEARCH AND EDUCATION HIGHLIGHTS

Soil ecotoxicology in Brazil is taking its course

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Abstract Soil ecotoxicology has been motivated by the increasing global awareness on environmental issues. Northern Hemisphere has been the main driver of this science branch; however, the number and quality of contributions from the Southern Hemisphere are increasing quickly. In this case study, Brazil is taken as an example of how soil ecotoxicology has developed over the last 30 years. It starts with a brief historical overview depicting the main events on soil ecotoxicology in the country. Following, an overview on the Brazilian legislation related to soil ecotoxicology is given, covering regulations with prospective focus, mainly on the registration of pesticides. Regulations with retrospective focus in contaminated areas are also given. Then, an outline of the actors in soil ecotoxicology and examples of prospective ecotoxicological studies performed with soil organisms and plants are given by stressor groups: pesticides, pharmaceuticals, metals, and residues. Experiences from retrospective studies, mainly looking at the assessment of industrial sites, are also covered. Emphasis is given on methodological aspects, pointing to needed actions, mainly regarding the different biotic and abiotic conditions of a tropical country. Finally, the last session discusses how soil ecotoxicology could be improved in methodological adaptations as well as legal requirements.

Keywords Soil ecotoxicology · Tropics · Legislation · Soil organisms · Pesticides · Residues · Risk assessment

Introduction

Currently, the science of soil ecotoxicology is becoming more and more complex, both in terms of basic science and of legal requirements. For many years, Organization for Economic

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Cooperation and Development (OECD) member countries, mainly from the Northern Hemisphere, were the main drivers of soil ecotoxicology, both not only in terms of methodology (e.g., the standardization of test guidelines) but also in terms of regulatory requirements (e.g., the environmental risk assessment of pesticides). However, things are changing, i.e., the number and quality of contributions from the Southern Hemisphere are increasing quickly. Therefore, it is justified to step back for a moment in order to analyze the status of soil ecotoxicology in these countries. Brazil has been chosen as an example, because of its very active scientific community which does not only transfer experiences from the north but also develops own approaches and methods. Right now, these activities are (slowly) transformed into legal requirements. As in temperate regions, this development in soil ecotoxicology has been motivated by the increasing global awareness on environmental issues.

In this contribution, we will start with a brief historical overview, highlighting that this branch of ecotoxicology is just over 30 years old, taking the publication date of the first OECD test guideline with soil organisms (earthworms and plants (OECD - Organization for Economic Co-operation and Development 1984a, b) as a starting point in time. Afterwards, an overview on the Brazilian legislation related to soil ecotoxicology will be given, covering two approaches:

- Regulations with a prospective focus, i.e., laws or ordinances dedicated to prevent soil contamination. The best example is the registration of pesticides—a process in which (among others) environmental risks of a new pesticide have to be studied and assessed before such a chemical is allowed to be marketed.
- Regulations related to the "quality" of soil, i.e., the focus is retrospective. This means that soils have to be evaluated taking into consideration which are (or may be) the contaminations caused by anthropogenic activities. Typical examples are soils containing often highly complex mixtures of organic chemicals or metals, frequently for many years.

Following a short overview on who (and where) is active in soil ecotoxicology in Brazil, we will give an overview on prospective ecotoxicological studies performed with soil organisms and plants, organized by main stressor groups: pesticides, pharmaceuticals, and metals. Afterwards, we will present experiences from retrospective studies, mainly looking at the assessment of industrial sites. In both cases, our focus will be on methodological aspects, i.e., the performance of these studies (especially when different from studies in temperate regions), including the way how an environmental risk assessment has been performed.

Tropical soil ecotoxicology does—and should—follow the same principles and approaches as ecotoxicology in general. However, it is clear that tropical ecotoxicology has to accept the fact that both the abiotic (e.g., soil properties) as well as the biotic (e.g., species composition) conditions differ from those in temperate regions of the world. Therefore, in a final chapter, we will discuss how soil ecotoxicology in Brazil could be improved, covering both methodological adaptations as well as legal requirements—in both cases with the aim to provide ideas and proposals for a better protection of Brazilian (and other tropical) soils.

This paper aims to show an outline of the actors and their activities in soil ecotoxicology in Brazil. It is not supposed to be an extensive review but a snapshot of what has been done and what should be the next steps to ensure a sound and effective advancement in soil ecotoxicology and environmental conservation.

Historical overview on soil ecotoxicology in Brazil

The first publications on soil ecotoxicological tests using soil organisms in Brazil can be dated from the end of twentieth to the beginning of the twenty-first century. The papers, as well as national legislation, reflect well the flourishing concern on the effects of pesticides on the environment, partly influenced by the increasing worldwide awareness on environmental issues at that time. During this early phase of soil ecotoxicology in the country (Table 1), the first published paper was on the acute effect of pesticides on the earthworm *Eisenia fetida* in natural soil, sand, and also artisol, a substrate of an amorphous silica gel mixed with glass balls (Ichinose et al. 1996). At that time, the Brazilian National Environmental Agency (IBAMA Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis 1990) and the São Paulo State Environmental Agency (CETESB Companhia de Tecnologica de Saneamento Ambiental 1990) recommended acute tests based on Association Française de Normalisation (1984) and OECD - Organization for Economic Co-operation and Development (1984a) for pesticide registration in the country.

Later on, the acute test with earthworms became one of the tests required for the registration of pesticides in the environmental risk assessment as recommended by the normative ordinance IBAMA No. 84 (Brazil 1996) which is linked to Law 7.802 (Brazil 1989) (see details below). From year 2000 onwards, several independent working groups started mainly prospective studies with pesticides, using edaphic organisms (e.g., Andrea and Papini 2005; Jänsch et al. 2005; Römbke et al. 2007; Chelinho et al. 2011; Nunes and Espíndola 2012; Alves et al. 2013; Nunes et al. 2016). During this phase, which we called awakening phase (Table 1), the second and third papers on soil ecotoxicology in Brazil had been published. They reported the effect of pesticides on soil microbiota (Andrea et al. 2000) and bioaccumulation of pesticides in *E. fetida/andrei* (Papini and Andréa 2001; Andréa and Papini 2005). The acute test with the earthworm *E. fetida/andrei*, using artificial or

Table 1 List of publications and events of the last 32 years which illustrate the diversity of topics and evolution of soil ecotoxicology in Brazil in three phases

Phases	Publication/events	Content	Reference (year)
1984–2001 Early	Legislation/normative	First international guideline of acute and chronic tests with earthworms	OECD - Organization for Economic Co-operation and Development (1984a, 1984b)
	Legislation/normative	Pesticides law	IBAMA n. 84 (Brazil 1996)—linked to Law 7802 (Brazil 1989)
	Prospective (pesticide)	Earthworm in different substrates	Ichinose et al. (1996)
	Prospective/retrospective (pesticide)	Effect of pesticides on soil oxidative and anaerobic processes and microbial biomass	Andrea et al. (2000)
	Prospective (pesticide)	Bioaccumulation in earthworms	Papini and Andréa (2001)
2002–2012 Awakening	Prospective	Tropical artificial soil (TAS)	Garcia (2004)
	Prospective (pesticides)	Tropical isopod as test organism	Jänsch et al. (2005)
	Prospective/retrospective	First papers about soil ecotoxicology in the Journal of the Brazilian Society of Ecotoxicology (abstracts presented at Ecotox 2004-VIII Brazilian Congress of Ecotoxicology)	Sisinno et al. (2006a), Niemeyer et al. (2006a), Niemeyer et al. (2006b)
	Prospective (pesticides)	Comparison of acute and chronic tests with earthworms in tropical and temperate conditions	Römbke et al. (2007)
	Legislation/normative	First guideline in Portuguese—acute test with earthworms	ABNT - Associa ão Brasileira de Normas Técnicas (Associa ão Brasileira de Normas Técnicas 2007)
	Prospective (pesticides)	Comparison of avoidance tests with earthworms in tropical and temperate conditions	Garcia et al. (2008)
	Legislation/normative	Resolution for soil protection	CONAMA 420 (2009b)
	Retrospective (metal)	ERA—tier I: screening phase	Niemeyer et al. (2010)
	Prospective (dredged sediments)	Ecotoxicological effects of metal-contaminated dredged sediments on earthworms	Cesar et al. (2014b)
	Prospective (pesticides)	Integrated ERA: case sudy on runoff effect	Chelinho et al. (2012)
	Event	Founding of the Brazilian Network of Terrestrial Ecotoxicology—TerrEcotox	at Ecotox 2012- XII Brazilian Congress of Ecotoxicology
	Prospective (pesticides)	Aternative earthworm species—*P. corethrurus* in TAS	Buch et al. (2013)
	Prospective (oil)	Biomarkers in wild rodents exposed to crude oil on soil	Da Silva-Júnior et al. (2013)
	Prospective (pesticides)	Acute, chronic, and avoidance tests with seed-coating pesticides in TAS	Alves et al. (2013)
2013—now Consolidating	Prospective (sludge)	Ecotoxicological assessment of the coal-contaminated soil remediation using sludge as an amendment	Chiochetta et al. (2013)
	Event	Special Session on Terrestrial Ecotoxicology in the Ecotox 2014	Niva et al. (2014)
	Prospective (swine manure)	Acute, chronic, and avoidance tests with earthworms in three natural soils	Segat et al. (2015)
	Retrospective (metal)	ERA—tier II: detailed assessment	Niemeyer et al. (2015)
	Prospective (vinasses)	Chronic and avoidance tests with earthworms, mites, enchytraeids, springtails in TAS, and natural soil	Alves et al. (2015)
	Prospective (pesticides)	Acute, chronic tests, and effect on morphology with earthworms in natural soils and TAS	Nunes et al. (2016)
	Prospective (metal)	Acute, chronic, and avoidance tests *F. candida* and an alternative springtail species—*P. minuta* in TAS and natural soil	Buch et al. (2016)

natural soil, had been the most popular test for assessing effects on non-target soil invertebrates during this phase and is still popular today (Fig. 2). This method turned out to be the first guideline on soil ecotoxicology published in Portuguese in 2007 and has been reviewed recently (ABNT Associa ão Brasileira de Normas Técnicas 2014) based on a method originally published in English (ISO International Organization for Standardization 1993a).

The Associação Brasileira de Normas Técnicas (ABNT) is a non-profit organization engaged in the preparation of national standards in Brazil. ABNT is the national partner of the International Organization for Standardization (ISO), meaning that methods standardized by ISO can be transferred to Brazil relatively easily. The first guideline published by ABNT was an important step for soil ecotoxicology in Brazil, but it happened only 20 years after the publication of the first standard method of aquatic tests in the country. Several studies focused on the reuse of residues during the last half decade characterize what we called here "consolidating phase," because many of the acting groups started to interact and to make efforts in turning soil ecotoxicology a sound science in the country (Table 1). The ecotoxicity of different materials other than pesticides increased gradually in the last 10 years such as oil hydrocarbons and different kinds of residues of mining activities and sewage, swine manure, and vinasses (e.g., Sisinno et al. 2006; Cesar et al. 2013; Segat et al. 2015; Alves et al. 2015).

One of the early turning points in soil ecotoxicology was the development of tropical artificial soil (TAS in English; SAT in Portuguese) by Garcia (2004), who modified the artificial soil originally described in the first ecotoxicological soil test method (OECD - Organization for Economic Co-operation and Development 1984a). This new composition was one of the results of a Brazilian-German co-operation project which aim was to set up the first soil ecotoxicological laboratory in Brazil at the Embrapa Amazônia Ocidental Institute (Römbke et al. 2007; Garcia et al. 2011). Indeed, partly because of the studies developed by this group (Jänsch et al. 2005; Römbke et al. 2006; Garcia et al. 2008), several standard methods were either modified (e.g., ISO 11268-1 (International Organization for Standardization 2011)) or newly developed (e.g., ISO 18311 (International Organization for Standardization 2015)) in order to cover tropical conditions. Their implementation in Brazil is still ongoing. However, the use of several other methods have gained interest and practicability as a result of training courses and workshops promoted by groups in universities and/or research institutes and also by exchange programs of graduate students with European universities (especially Portugal and Germany). With the publication of the Resolution No. 420 (Brazil 2009b), which deals with contaminated areas, an interest for environmental risk assessment (ERA) has arisen. Therefore, a better understanding of methods for the evaluation and monitoring of soil contamination is emergent.

An effort for adapting ecotoxicological tests with naturally occurring oligochaetes in ecotoxicological tests has arisen in the beginning of the millennium (Garcia 2004) and later from a group specialized in Oligochaeta ecology and taxonomy from 2008 onwards (Brown et al. 2013). These studies mainly dealt with pesticide effects on *Pontoscolex corethrurus*, a widespread peregrine species in Brazil (Garcia 2004; Buch et al. 2013). As an initiative of this group, a book with detailed methods and adaptations for ecotoxicological tests in Brazil with oligochaetes has been written with the collaboration of 26 researchers and will be published in 2016 (Niva and Brown 2016 in press). Another book describing methods for ecotoxicological tests with Collembolla is to be published in the same year by a group of 16 authors (Oliveira Filho et al. 2016). Both books were written in Portuguese in an attempt to make the methods more popular in the country and to fulfill the needs of beginners in the field. Other two books highlighting soil ecotoxicology have also been published in Portuguese: one on metal soil ecotoxicology (Cesar et al. 2014a) and another on environmental safety and chemical control (Papini et al. 2015).

Another important landmark for Brazilian ecotoxicology is, probably, a study carried out in a former metal smelter site and its surroundings, with soils heavily contaminated by metals, called Santo Amaro (Bahia State, Brazil) (Niemeyer et al. 2010, 2015). As we will show later in this paper, this pioneer study became a reference in the country for ERA approach specially because of the publication of CONAMA 420 (Brazil 2009b), a legislation related to the management of contaminated areas with the establishment of criteria and guiding values of soil quality.

The Brazilian Society of Ecotoxicology, Ecotox-Brasil (officially linked to SETAC-Latin America), has played an important role in promoting and supporting soil ecotoxicology in Brazil by giving opportunities for talks and sessions on the topic during the biennial Brazilian congresses since the year 2000, although sporadic at the beginning. A more effective support started in 2012, when a technical course on Terrestrial Ecotoxicology was held with participants from academy and government during the congress. During the course, the 21 participants decided to start a network through a virtual group to exchange information. That was an important step because it allowed to know who were the people/institutions working or interested in the topic and also helped to identify the needs of this community. At the occasion, 48 abstracts (5 % of the total) on the topic "soil" were accepted. In the following congress, in 2014, the number of presentations increased to 87 (12 % of the total) and a special session with ten talks counted with more than 60 participants (Niva et al. 2014). Currently, this network called "Rede Brasileira de Ecotoxicologia Terrestre" (Brazilian Network of Terrestrial Ecotoxicology) consists of more than 90 members. Since then, several courses on soil ecotoxicology and ecological risk assessment have been held mainly in Santa Catarina, São Paulo, Rio Grande do Sul, and Paraná states, all of them as isolated initiatives generally linked to universities but supported by Ecotox-Brasil.

Overview on the Brazilian legislation related to soil ecotoxicology

Legislation on risk assessment of chemicals in soil

Ecotoxicological studies with soil organisms are only required in the legislation that concerns pesticide and wood preservative product registration, in which environmental preservation is supported by a broad legal framework. In this context, Law No. 7.802 of July 11, 1989 (Brazil 1989), known as the "Pesticides Law," has special importance. This law deals with the research, experimentation, production, packaging and labeling, transportation, storage, marketing, advertising, use, import, export, waste and packaging disposal, registration, classification, control, inspection, and surveillance of pesticide products, their components, and related products. This law includes the assessment of technical grade active ingredients, formulations, and components. The control of these products is divided between three governmental institutions, each responsible for different aspects of controlling these products, namely the Ministry of Agriculture, Livestock and Supply—MAPA (agriculture agency), the National Health Surveillance Agency—ANVISA (health agency), and the Brazilian Institute of Environment and Renewable Natural Resources—IBAMA (environment agency). Thus, during the registration or the reevaluation processes, these federal agencies must make sure that their respective directives are observed by the pesticide-producing industry (Sundfeld and Câmara 2011).

Several additional documents are available, describing how Law No. 7.802/89 is to be used in practice. For instance, Decree No. 4.074/02 (Brazil 2002) established a collaboration system among these federal agencies. The assessment of all products under registration is performed by all three agencies, according to their competences. However, the certificate of registration will be issued by one of these three agencies, depending on the use of the pesticide. For pesticide products destined to be used in the sectors of production, storage, and processing of agricultural products, in forests and pastures, the registering agency will be MAPA. For pesticide products of urban, industrial, residential, and public use, as well as for water treatment and public health campaigns, ANVISA will be the registering agency. IBAMA will assume this role when the pesticide products is intended to be used in aquatic environments and in protecting native forests and other natural ecosystems (Sundfeld and Câmara 2011).

Specifically for the environmental assessment of a pesticide product, IBAMA Normative Ordinance No. 84, of October 15, 1996, established procedures regarding the registration and environmental hazard assessment of pesticide products, their components, and related products. This ordinance also established the "Permanent System of Assessment and Control of Pesticides," as well as other procedures, including the general instructions for environmental risk assessment. In detail, this ordinance requests physico-chemical studies, environmental fate studies, as well as ecotoxicological studies. The latter should be conducted with representative species from different trophic levels. Thus, from the assessment reports of these requested studies, it is possible to understand the pesticide product's behavior and its environmental fate, as well as its toxicity to different organisms. It is also noteworthy that, during the environmental assessment of a pesticide product, IBAMA may request additional studies depending on the test results, the mode of application and use, and its intrinsic characteristics. Regarding ecotoxicological studies performed with soil organisms, this normative ordinance also requests the presentation of the results of acute toxicity studies with earthworms, toxicity tests with soil microorganisms involved in carbon and nitrogen cycles, and, if the pesticide product is persistent in soil, the study of its phytotoxicity to non-target plants (Brazil 1996).

Moreover, still concerning IBAMA competence, there are other specific rules controlling processes such as reevaluation of registered pesticide products (Brazil 2006c) (Brasil 2009a), amendments on formulations of pesticide products (Brasil 2013), registration of biochemical (Brazil 2005), semiochemical (Brazil 2006a), and microbiological control agent pesticide products (Brazil 2006b), as well as registration of wood preservative products (Brazil 1992). In all of these cases, the same earthworms, microorganisms, and plant tests listed previously may be requested; however, various other ecotoxicological tests could also be required by the authorities.

Regarding other types of chemical products, such as industrial and pharmaceuticals, Brazilian legislation does not request any terrestrial ecotoxicology tests. Moreover, aiming on a more efficient environmental protection, the establishment of legislation requiring more terrestrial ecotoxicological studies for chemical contaminant activities is extremely necessary.

An important point regarding ecotoxicological studies is the use of a natural standard soil. According to soil classification of Embrapa (Santos et al. 2006), Brazil has 13 soil classes where 31 % is latosol, 27 % is acrisol, and 13 % is neosol. In the normative (IBAMA number 84/1996) for pesticide behavior tests, latosol and gleysol with specific characteristics are recommended. These could be considered as standard natural soils in Brazil and proved to be useful in tests with *E. andrei* by Ferreira et al. (2015). However, Cantelli (2011) found problems in the adequability of a latosol with alternative species of earthworms. Segat (2012) and Segat et al. (2015) successfully tested three types of Brazilian soils of Santa Catarina State, entisol, latosol, and ultisol with pH correction, to study the toxicity of swine manure. Generally speaking, the knowledge of the Brazilian natural soil adequability to ecotoxicological tests is still scarce, and more studies to determine which soil(s) is(are) more representative and appropriate should be promoted in Brazil.

Legislation on (retrospective) assessment of soil quality

The most recent important step toward soil protection in Brazilian legislation was the Resolution No. 420/2009 performed by the National Environmental Council—

CONAMA, published in 2009 by the Brazilian Ministry of the Environment, which is a specific legislation related to the management of contaminated areas, establishing criteria and guiding values for soil quality, as well as instruction for its preservation (Brazil 2009b). This resolution presents "prevention values" and "intervention values," equivalent to the Dutch target values and intervention values, respectively. Prevention values indicate the level at which there is a sustainable soil quality, ensuring the recovery of the functional properties of the soil for humans, plant, and animal life, while intervention values indicate serious contamination level, with risk of compromising soil quality and functioning. According to the resolution, for a contaminated site with significant impacts to environmental resources, risk management should be based on the results of an ERA. However, the proposed values adopted were not based in tests with Brazilian natural soils; neither the resolution indicates how the ecological risk assessment should be carried out. The requirement of ecotoxicity tests, as well, is not explicit in the resolution. These issues have been discussed by environmental agencies of São Paulo and Minas Gerais.

Ecotoxicological test guidelines

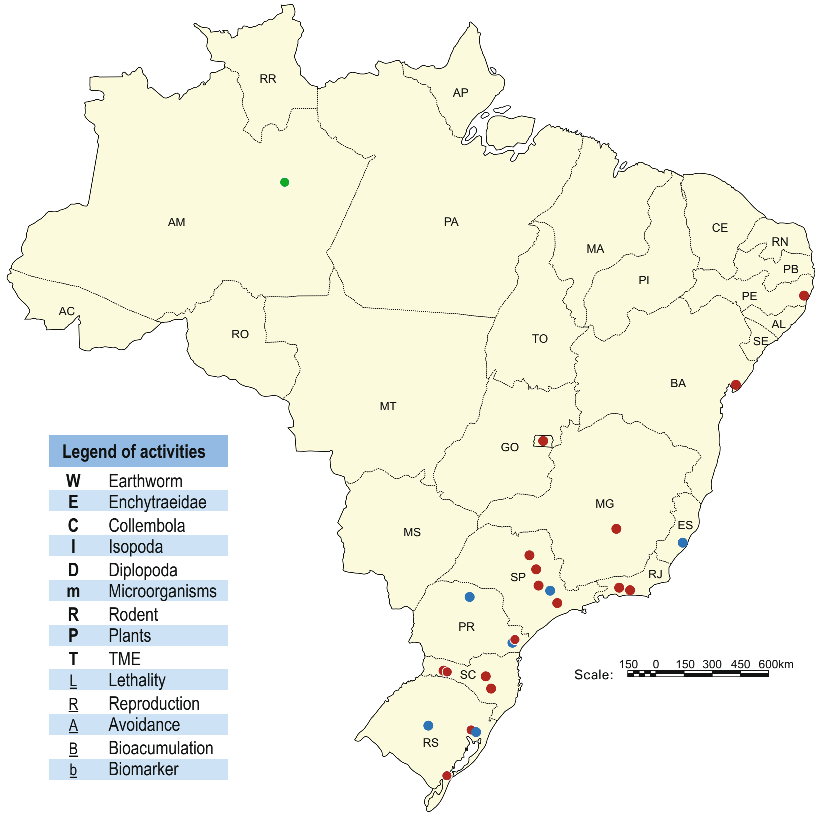
Currently, seven standard guidelines concerning soil quality were published in Portuguese in Brazil by ABNT. With the exception of the guideline on acute toxicity with earthworms (ABNT Associa ão Brasileira de Normas Técnicas 2014—first published in 2007), all others are translations of ISO guidelines but with a note recommending the use of the tropical artificial soil (ABNT - Associa ão Brasileira de Normas Técnicas 2010; ABNT - Associa ão Brasileira de Normas Técnicas 2011a; ABNT - Associa ão Brasileira de Normas Técnicas 2011b; ABNT Associa ão Brasileira de Normas Técnicas 2011; ABNT Associa ão Brasileira de Normas Técnicas 2012). These translations were the result of some volunteering professionals working on soil ecotoxicology in the Special Committee on Ecotoxicological Analyses, organized by ABNT. Since 2014, another ABNT Special Committee on Solid Residues has discussed methodologies for ERA for the implementation of a guideline to be applied on contaminated sites. This has been discussed as a reflex of CONAMA Resolution 420 (Brasil 2009a).

Soil ecotoxicology: who is active and where in Brazil?

Since the year 2000, a significant increase of people involved in soil ecotoxicology and their activities can be noticed in Brazil. However, it was difficult to identify those people, since a conventional survey in the web query interface of Brazilian researchers (Lattes Platform) was not effective in detecting actors in soil ecotoxicology. That was because many of them do not include the term "soil ecotoxicology" or "terrestrial ecotoxicology" in their profiles, instead they are hidden under "soil contamination" (860 hits), "residues" (182 hits), "pesticides" (138 hits), "oil" (137 hits), "mining" (134 hits), and other keywords, making it difficult to localize them. Thus, in 2014, the Brazilian Network of Terrestrial Ecotoxicology distributed a questionnaire among its members, inquiring about their affiliation, types of methods and test organisms used, as well as contaminants studied. This questionnaire was responded by 63 people of 44 different institutions. The respondents included people from academy (73 %), research (16 %), government (6 %), and business (5 %). Among them, 34 % were professionals (e.g., professors, researchers, analysts, consultants) and 66 % were students, mostly at graduate level. It is noteworthy that the most active groups in soil ecotoxicology often consisted of scientists from governmental research stations or universities with a strong background in soil biology, e.g., using invertebrates or microorganisms as bioindicators of land use, but at least two groups have strong aquatic ecotoxicology background. As can be noticed in Fig. 1, most of the active groups are located in the south and southeastern regions of Brazil, most of them in the state of Santa Catarina. These regions are the most populated and economically strong areas of Brazil and also those with the highest density of educational and scientific institutions.

As shown in Fig. 2, the earthworm *E. fetida/andrei* is the most popular test species (65 % of the respondents) and in most cases in acute tests (59 %). However, the use of reproduction and avoidance tests (29 and 39 %) was also frequent among the respondents. The latter is performed mostly with earthworms and in a couple of cases, with Isopoda. Residues and effluents in Fig. 2 include sewage sludge, swine manure, biochar, residues, and wastes derived from mining activities, most materials showing some potential as soil conditioners to improve agricultural production because of their high content of organic matter/nutrients or which disposal is done on soil. These contaminants, together with pesticides, were the most frequent ones assessed (ca. 40 %), showing a great adherence of soil ecotoxicology with agricultural activities. On the other hand, studies with contaminants related to urban activities did also appear in the survey. In the topic "contaminated area," mainly ERA studies related to oil or mining areas are included. The great majority of the studies performed in Brazil are single species tests, partly because of a lack of capacity in multi-species tests or semifield tests such as terrestrial model ecosystems (TMEs). However, there are several initiatives (Rio de Janeiro, Santa Catarina, São Paulo) trying to set up staff and equipment in order to perform ecologically more relevant studies as part of

Institutions	Type of activity
Aplysia (ES)	WL P
Bioensaios (RS)	WL m
CENA-USP (SP)	m
CETEM (RJ)	WLB m
EESC-USP (SP)	WLRA CLRF ELR
Embrapa Agrobiologia (RJ)	WLRA ELR
Embrapa Amazônia Ocidental (AM)	WLRA ILR
Embrapa Florestas (PR)	WLA
Embrapa Cerrados (DF)	ELR
ESALQ (SP)	WLRA CLRA m
FEPAM (RS)	Rb P
FURG (RS)	RLAb I P
IAC (SP)	PB
Instituto Biológico (SP)	WLRAB m
Solotox (RJ)	WLRA ELR
UDESC (SC)	WLRA CLRA ELR m T
UEL (PR)	WLA
UFBA (BA)	WLRA CLRA ELR
UFES (ES)	WL
UFF (RJ)	WLRA CLRA ELR
UFFS (SC)	WLRA CLRA ELR T
UFMG (MG)	WLRA
UFPE (PE)	m
UFPR (PR)	WLA Eb
UFSC (SC)	WLRA CLRA ELR
UFSM (RS)	WL
UNESP (SP)	Db
UNICAMP (SP)	W
Universidade Positivo (PR)	WLA
UTFPR (PR)	ELR



Legend of activities	
W	Earthworm
E	Enchytraeidae
C	Collembola
I	Isopoda
D	Diplopoda
m	Microorganisms
R	Rodent
P	Plants
T	TME
L	Lethality
R	Reproduction
A	Avoidance
B	Bioacumulation
b	Biomarker

Fig. 1 Map of Brazil showing institutions actively (*red*) or sporadically (*blue*) being active in soil ecotoxicology and their research activities. Institute with discontinued activities (*green*). Research institutes: CETEM, Embrapa, IAC, Instituto Biológico; universities: CENA-USP, EESC-USP, ESALQ, FURG, UDESC, UEL, UFBA, UFES, UFF, UFFS, UFMG, UFPE, UFPR, UFSC, UFSM, UNESP, UNICAMP, Universidade Positivo, UTFPR; service providers/consultancies: Aplysia, Bioensaios, Solotox; regulatory agencies: FEPAM. States: Acre (*AC*), Alagoas (*AL*), Amazonas (*AM*), Amapá (*AP*), Bahia (*BA*), Ceará (*CE*), Distrito Federal (*DF*), Espírito Santo (*ES*), Goiás (*GO*), Maranhão (*MA*), Minas Ferais (*MG*), Mato Grosso do Sul (*MS*), Mato Grosso (*MT*), Paraíba (*PB*), Piauí (*PI*), Pernambuco (*PE*), Paraná (*PR*), Rio de Janeiro (*RJ*), Rondonia (*RO*), Roraima (*RR*), Rio Grande do Sul (*RS*), Rio Grande do Norte (*RN*), Santa Catarina (*SC*), São Paulo (*SP*). (Drawing by Wellington Cavalcanti, Embrapa Cerrados)

higher tiers of environmental risk assessments. Especially in these activities, often students and their supervisors which had returned from exchange programs in soil ecotoxicology abroad are involved.

Studies in the area of prospective risk assessment in Brazil

In this chapter, an overview of important soil ecotoxicological studies performed in Brazil is given. Special focus was put on methodological aspects, meaning to address the specific requirements of tropical (and in particular Brazilian) soil ecotoxicology in comparison to those studies performed under temperate conditions. Therefore, only in rare cases, test results of these studies are reported here. Finally, it has to be mentioned that all studies discussed here were performed as part of scientific projects—no regulatory data are available from Brazilian authorities regarding the environmental risk assessment of chemicals or other stressors being potentially harmful to soils.

Ecotoxicology of pesticides including adaptations of tests to tropical conditions

The pesticide use in agricultural systems has increased considerably over the last decades, especially in tropical regions (Silva and van Gestel 2009). In Brazil, this increase came along with the intensification of agriculture, and as a result, it is considered the world's largest pesticide consumer since 2008 (Ferreira et al. 2010; IBAMA Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis 2012). Knowledge on pesticide impact in the tropical environment, however, is still incipient compared to temperate systems.

It is difficult to talk about ecotoxicological studies with pesticides in Brazil without discussing adaptations of the current available guidelines which were designed for temperate conditions. Taking as an example the acute test with earthworms, guidelines such as those from OECD - Organization for Economic Co-operation and Development (1984a), ISO 11268-1 (International Organization for Standardization 1998), or Brazilian authorities (IBAMA

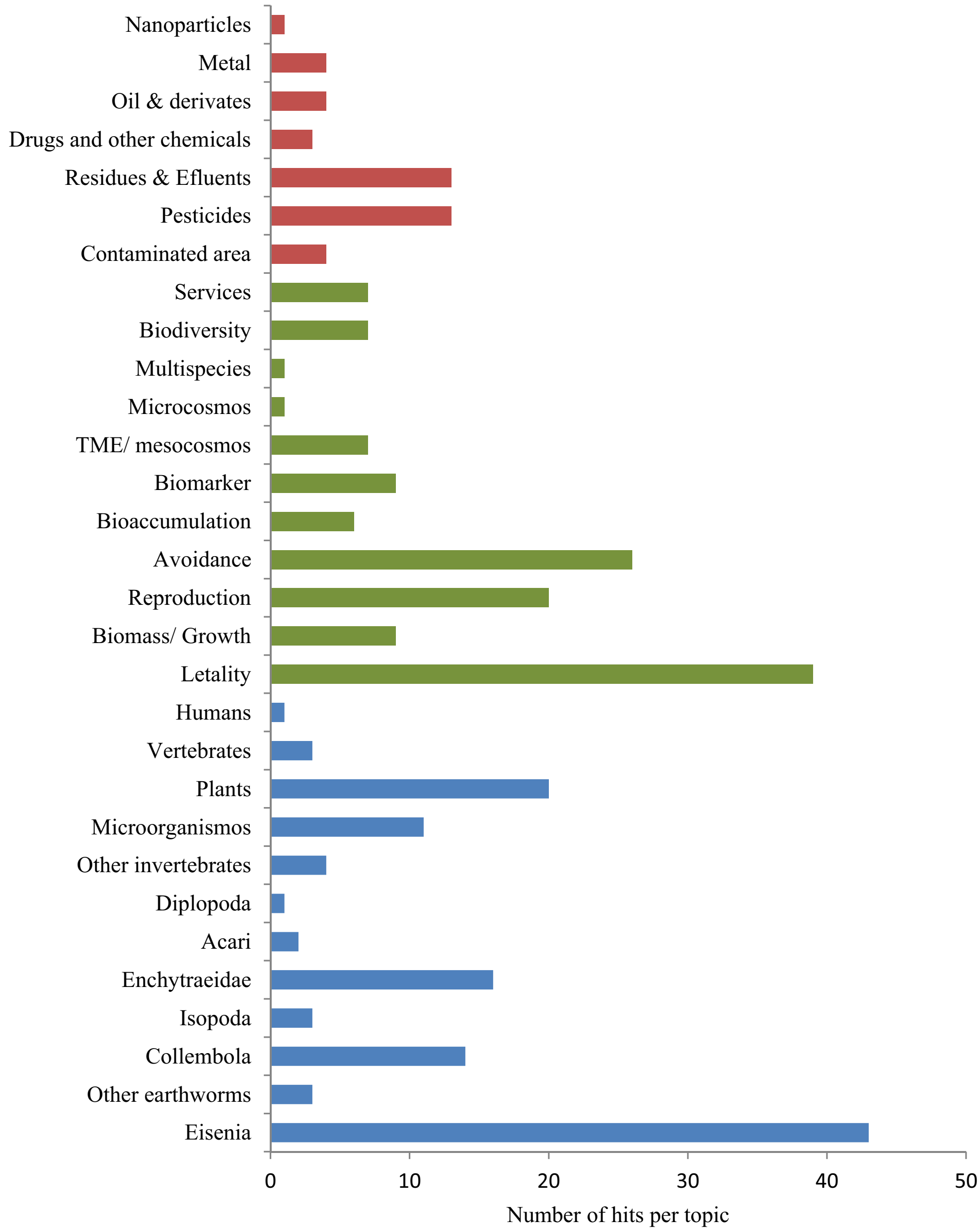


Fig. 2 Number of hits per topic studied by 63 respondents of the questionnaire about their activities carried out in soil ecotoxicology in Brazil. *Blue bars* represent the test organism used; *green bars* represent the methods; *orange bars* represent the contaminants

Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis 1990) recommend the earthworm *E. andrei*/*fetida* as test species, a temperature of 20 ± 2 °C, and the OECD artificial soil consisting of sand, kaolin, and sphagnum peat. The test species *E. andrei*/*fetida* is an epigeic litter-dweller, feeds on fresh organic matter on the soil surface, and does not ingest soil (Lee 1985). So, and despite the fact that has been found in compost heaps all over Brazil (even in Manaus, Amazonas (Garcia 2004)), they have a suboptimal ecological relevance for assessing effects of chemicals in natural soils. Concerning temperature, Brazil is a continental country with—mainly—tropical and subtropical regions. Therefore, when generating ecologically relevant data, the temperature of a test should be regionalized. Finally, regarding the artificial soil, sphagnum peat had to be substituted for coir peat (powdered fiber of coconut mesocarp) in the composition of the artificial soil, as proposed by Garcia (2004). Sphagnum peat moss is very difficult to get in Brazil since it has a very restricted occurrence in the tropics. This alternative organic matter was quickly adopted among researchers. Actually, it has been incorporated in standard guidelines such as in the ISO earthworm test guidelines (e.g., ISO International Organization for Standardization 2011) or the Brazilian ABNT NBR 15537 (ABNT Associa ão Brasileira de Normas Técnicas 2014). The tropical artificial soil has been largely used for ecotoxicity tests with pesticides in Brazil, either in acute and avoidance tests with earthworms (Garcia et al. 2008; De Sousa and de Andréa 2011; Nunes and

Espíndola 2012; Buch et al. 2013) as well as in reproduction tests (Alves et al. 2013; Ferreira et al. 2015).

In addition, some efforts have been made to identify alternative species for conducting ecotoxicological tests in Brazil. The most common earthworm species all over the moist tropics (Christoffersen 2008), originating probably from Northern Brazil, is *P. corethrurus* (Glossoscolecidae), a mineral-dwelling species (Brown et al. 2006). Thus, Garcia (2004) studied the sensitivity of this species, commonly found in disturbed agricultural and peri-urban soils, to the fungicide carbendazim, a well-studied reference substance in earthworm ecotoxicology. His results indicated a higher sensitivity of the native species in comparison to *E. fetida*, but no general recommendations can be based on just one test series with one chemical and two soils. Later, Buch et al. (2013) performed avoidance and mortality tests with the same two species, exposed in TAS to three pesticides commonly used in Brazilian agriculture: carbendazim, carbofuran, and glyphosate. The authors concluded that the sensitivity of *P. corethrurus* appears to be similar to the standard species (*E. andrei*). However, they pointed out that there are limitations associated with the widespread use of this species and other mineral-dwelling earthworm species in ecotoxicological tests: *P. corethrurus* has long life cycle and low fecundity, meaning that mass rearing, although possible, is more time-consuming (Buch et al. 2011) in comparison to the work needed for compost worms such as *E. fetida/andrei*. In addition, the performance of chronic reproduction tests would be very time-consuming. However, shorter life cycles were obtained by other authors in different soils and higher temperatures (Lavelle et al. 1987), suggesting that more studies with *P. corethrurus* are needed to evaluate its potential as a test species for ecotoxicological studies. Another litter-dwelling tropical species (*Perionyx excavatus*), introduced from Asia, might be an alternative, since its life cycle and culturing conditions is similar to those of *E. fetida/andrei* (Silva and van Gestel 2009). So far, no experience in testing this species is available in Brazil. Cantelli (2011) used a latosol and a TAS in acute with three pesticides and found *E. andrei* accepted well both soils, while *Dichogaster annae* did not show satisfactory survival in latosol and neither *Amynthas gracillis* in TAS.

Predictive risk assessment of pesticides in a regulatory context should be improved in Brazil by including further tests with soil invertebrates besides the earthworm mortality test. Among the test organisms other than earthworms, *Folsomia candida* (Collembola) (Carniel 2015), *Enchytraeus crypticus* (Enchytraeidae) (Chelinho et al. 2012), and isopods (Jänsch et al. 2005; Niemeyer et al. 2006a; Niemeyer et al. 2006b) have also been used in ecotoxicity tests to evaluate pesticide effects on soil organisms in Brazil. The potworm *E. crypticus*, for example, has been used by some research groups in ecotoxicological tests with SAT and natural soils (e.g., Chelinho et al. 2012; Alves et al 2014; Benazzi 2015; Niemeyer et al. 2015). This species has not been found in Brazil or other natural or cultivated area around the world (Castro-Ferreira et al. 2012). However, it has the advantage of being tolerant to a wide range of temperature and to have a short life cycle (Castro-Ferreira et al. 2012), characteristics desirable for a test organism in tropical conditions. Three new Brazilian species of *Enchytraeus* with still unpublished descriptions have been successfully reared and maintained in laboratory, among which, one has been tested in reproduction assays to test pesticides and other contaminants in SAT and latosol (Assis 2015; Niva C.C., unpublished). Fragmenting species, such as *Enchytraeus bigeminus*, are also a good option as a test organism (Bandow et al. 2013), since they are found in Brazilian soils and can be easily reared in laboratory conditions even in TAS (Niva et al. 2010; Schmelz et al. 2013).

There are also some few examples of naturally occurring Isopoda being used as test species, such as *Porcellionides pruinosus* in acute tests (Jänsch et al. 2005), *Cubaris murina* in avoidance, lethality and biomass loss tests with glyphosate (Niemeyer et al. 2006a; Niemeyer et al. 2006b; Niemeyer et al. 2009), and *Armadillidium vulgare* and *Porcellio dilatatus* in acute and avoidance tests in contaminated area (Da Silva-Júnior et al. 2013). Given the few examples of tropical organisms used in ecotoxicology, it is clear that more studies on soil biodiversity and their ecology are necessary to develop more ecologically relevant methods for environmental risk assessment.

Aiming to increase the ecological relevance, studies have included natural soils in ecotoxicological tests with earthworms such as with abamectin (Nunes and Espíndola 2012; Nunes et al. 2016) and carbofuran (Ferreira et al. 2015). Studies on the effects of pesticides on other soil organisms have also been assessed in natural soils in Brazil. For nematodes, a trait-based approach was used in order to obtain new perspectives on the evaluation of the toxic potential of chemical substances (Chelinho et al. 2012). Microbial parameters (i.e., soil respiration, soil microbial biomass, symbiotic processes, and fingerprint data) have also been studied in order to assess their suitability in determining the environmental risk of pesticides in natural soil (Andrea et al. 2000; Ferreira et al. 2009; Stefani et al. 2012).

The different environmental conditions and, consequently, differences in exposure should be considered in estimating the risk of pesticides in tropical versus temperate regions. For example, differences in toxicity can be expected among different temperatures and soil types (Garcia et al. 2011; Ferreira et al. 2015). It seems reasonable that temperature used in Brazil should be higher than the 20 °C normally recommended in international guidelines. In fact, Garcia et al. (2008) recommended using 28 °C for tropical conditions; however, because of the continental size of Brazil, this temperature is realistic to northern but not to southern regions; therefore, it is difficult to standardize that temperature to Brazil. For reasons of practicability, comparability, and, most importantly, general acceptance, one standard temperature should be fixed, at least for lower tier laboratory tests. Actually, the test temperature has been modified to 26–28 °C (ISO International Organization for Standardization 2011) and 25 ± 2 °C (ABNT Associa ão Brasileira de Normas Técnicas 2014). It has to be highlighted that the latter recommendation was a result of intensive discussions among the members of the ABNT Special Committee because fixing one temperature in such a huge country as Brazil is a difficult task.

And yet, the environmental risk assessment strategies of pesticides in tropical areas rely mostly on the extrapolation of data from temperate regions, which can induce errors (Garcia 2004; Garcia et al. 2008; Silva and van Gestel 2009). That has been already criticized by Römbke et al. (2008) who pointed out that fate and effect data to be used in the environmental risk assessment of pesticides in tropical soils are extremely scarce. Therefore, the generation of ecotoxicological data on pesticides in tropical regions is imperative for performing a more realistic risk analysis. Attempting to change this situation, methods delivering ecotoxicological data sets to tropical and, more specifically, to Brazilian conditions have been developed (Garcia et al. 2008; De Sousa and de Andréa 2011; Chelinho et al. 2011, 2012; Nunes and Espíndola 2012; Buch et al. 2013; Alves et al. 2014; Ferreira et al. 2015; Nunes et al. 2016), but many more are necessary in order to perform a reliable environmental risk assessment for pesticides in tropical soils.

Metals

The metal contamination in Brazil is a matter of concern, since the mining activity is intense in some regions and the safe disposal of residues and its reuse is a constant preoccupation. The recent tailing dam disaster of an iron ore mine in Minas Gerais State illustrates the problematic in the country (http://www.economist.com/news/americas/21679299-embattled-government-not-helping-much-it-should-growing-environmental-costs).

In the Doce and São Francisco river basins, State of Minas Gerais, Alves and Rietzler (2015) evaluated the toxicity of arsenic to *E. andrei* in soils around gold mining operations. Tests were also conducted with spiked arsenic in TAS and natural soil, though no effects were detected in survival or biomass. In spiked soils, arsenic LC_{50} in artificial tropical soil was six times lower than in natural soil; such lower bioavailability of As in natural soils was attributed to its association with iron ore in the samples. These results demonstrate the potential damage of mining activities on the fauna in the surrounding soil. In another study, laboratory studies were performed by Buch et al. (2016) to evaluate the ecotoxicity of Hg(II) to collembola species collected in Brazil, *Proisotoma minuta* (autochthonous) and *F. candida*, as a tool to predict effects in ecological risk assessment of tropical regions. Filamentous fungi assembly in semiarid soils has also been used to assess the impact of metallurgical industry wastes with lead (Da Silva-Júnior et al. 2014). Other studies on metal contamination will be discussed in Studies about residue toxicity and reuse section.

Pharmaceuticals

Pharmaceutical substances may be toxic to soil invertebrates and affect edaphic microbiota (e.g., Thiele-Bruhn 2003), because of the antibiotic, antimicrobial, and bacteriostatic action of many pharmaceutical substances dumped in the environment. An important part of the Brazilian economy is based on livestock, which, in turn, may affect the environment through the influence of drugs used in cattle breeding and their residues in manure and sewage sludge. This situation may cause adverse effects on living organisms (especially dung and soil invertebrates involved in dung degradation) (e.g., Boxall et al. 2004). Leal et al. (2012) studied the occurrence and sorption of four fluoroquinolones (norfloxacin, ciprofloxacin, danofloxacin, and enrofloxacin) in poultry litter and soil samples. The sorption potential in soil was higher than in poultry litter, and the main conclusion of the study was that the continuous use of antibiotics in poultry production may represent an important source of soil contamination, a scenario neglected in Brazil. So far, some research groups mainly derived from aquatic ecotoxicology have started studies on the effect on soil organisms in this area (Fig. 2), but studies on the behavior of these substances can be found. For example, Peruchi et al. (2015) investigated the sorption of the antibiotics norfloxacin in four different natural soils of agricultural sites in São Paulo State showing a high affinity and immobility in all soil samples, and Leal et al. (2012) studied the occurrence and sorption of four fluoroquinolones (norfloxacin, ciprofloxacin, danofloxacin, and enrofloxacin) in poultry litter and soil samples.

Studies about residue toxicity and reuse

The use of sewage sludge in agriculture is an alternative of residue disposal which can be beneficial to plants due to the gain in nutrients and organic matter by the soil. This alternative has gained popularity in Brazil because of the environment-friendly appeal. However, this practice may cause problems through the contamination by metal, meaning there is a risk over time at these sites. Furthermore, sludges contain a mixture of complex substances, showing specific toxicities depending of their components, such as ammonium or other water-soluble compounds (Domene et al. 2010) may affect soil biota. Nogarol and Fontanetti (2010) analyzed the ultrastructural alterations in the midgut of the diplopod *Rhinocricus padbergi* exposed to substrate containing sewage mud from a sewage treatment station of São Paulo State, Brazil. This study indicates cytotoxic and genotoxic effects even at concentrations of 1 % of sewage mud in soil. Several studies were carried out with different organisms and endpoints to evaluate dredged sediment deposition in chernosol and ferralsol soils (Cesar et al. 2014b) and to propose an ecological risk index based on these data (Cesar et al. 2015a).

The effect of different sources of sludges on soil biota has been studied. Chiochetta et al. (2013) applied ecotoxicological tests to determine the application rates of organic industrial sludge required as a soil amendment to restore soil production in abandoned coal mining sites, while avoiding environmental impact. Chemical analysis of the solids (industrial sludge and soil) and their leachates was carried out as well as a battery of ecotoxicity tests on enzymes (hydrolytic activity) and organisms of several trophic levels such as bacteria, algae, daphnids, earthworms, and higher plants, according to standardized methodologies. Segat (2012) evaluated the effects of the application of swine manure on natural tropical soils and TAS by using several bioassays with *E. andrei* and *F. candida*. Based on their results, the authors suggest that the application of swine manure on tropical soils should be done carefully because of the potential environmental risk of this material. In another example, the dosage of tannery sludge was found to modify the spore community of arbuscular mycorrhizal fungi (Nakatani et al. 2011).

The use and exploration of fossil fuel and other energetic matrixes can potentially harm the environment, and yet, their residues, when inadequately discharged, or in case of accidents or local catastrophes, can impair an ecosystem irreversibly. In the soil, the deposition of these fuels affects organisms of different trophic levels. Ecotoxicity tests have been used in Brazil in studies of residue toxicity and in monitoring of biodegradation processes. A preliminary study of Sisinno et al. (2006a) analyzed samples contaminated by hydrocarbons, collected in a bus garage, in a gas service station and in an area with oil spill in Brazil. Then, Sisinno et al. (2007) applied avoidance behavior tests with earthworms together with aquatic tests using eluate to monitoring a bioreactor efficiency to treat soil contaminated by crude oil. Tamada et al. (2012) observed a high toxicity of three lubricant oils to *E. andrei* and seeds of *Lactuca sativa* e *Eruca sativa* after 180 days of biodegradation. Phytotoxic effects of oil exposition have been identified by Maranho et al. (2009) who showed a reduction of the length, diameter, and tracheid wall thickness of *Podocarpus lambertii* exposed to oil. In addition, Cruz et al. (2013) found root and hypocotyl elongation inhibition in three species of plants exposed to oil and derivatives. Lopes et al. (2010) showed the toxicity of lubricating oil on *E. sativa* and the earthworm *Eisenia andrei* even after 90 days of biodegradation.

Mining and industry are important economic activities in Brazil and potential sources of environmental contamination. The concern with these potential problems can be noticed in academic studies of a research group from Rio de Janeiro in which ERA and functional biodiversity of soil organisms has been used to assess problems with bauxite residues and polychlorinated biphenyls (PCBs) (Bianchi 2015).

Coal and oil and derivative residues may have innumerous substances that are toxic to soil organisms. However, Cesar et al. (2013) found toxicity to *E. andrei* and *Lactuca sativa* only in higher concentrations of mining residues mixed to soil and, in the elutriate, to the microcrustacean *Daphnia similis*, although the authors admit that these short-term tests do not consider the effect of acid leaching and long-term effects, which should be included in ecotoxicological studies of coal mining residues. De Souza et al. (2015) used the snail *Helix aspersa* to evaluate the genotoxic potential of soil contaminated with mineral and recommended this animal as a sensitive instrument for risk assessment of environmental pollution.

Cesar et al. (2015a) proposed an ecological risk index for numerical estimation of toxic effects associated with disposal of dredged sediments. The authors ranked the ecological indicators and bioassays according to their ecological relevance. Effects that compromise the existence of the specie (mortality, germination, and immobility) received weight 3, while effects associated with perpetuation of the specie (reproduction) received weight 2, and other effects (avoidance behavior, feeding inhibition, and biomass loss) received weight 1. Thus, acute and chronic assays received weights 1 and 2, respectively. Classes for safe ecological doses of sediment to be applied to the studied soils were defined. Similar approaches could be applied in Brazil to determine safe residue disposition in soils.

Studies in retrospective risk assessment in Brazil

Retrospective risk assessment or site-specific ERA is a process of collecting and analyzing data, aiming to support site management decisions in contaminated sites (Suter et al. 2000). There is an increasing interest in ERA in Brazil evoked mainly by the legal requirement in the Resolution 420/2009 (CONAMA; Brasil 2009a) which establishes general guidelines for environmental management of contaminated sites, but no detail is presented on how to conduct the ERA process in that document. The main schemes of ERA known worldwide recognize the unique value of including biological assessments to evaluate contaminated sites (USEPA 1998; Weeks et al. 2004; Rutgers et al. 2008).

Few studies with ERA have been carried out in Brazil so far. The screening phase (tier 1) of a site-specific ERA in a former smelter area heavily contaminated with metals (Santo Amaro, Bahia, Brazil) was carried out by Niemeyer et al. (2010). The TRIAD approach was applied (Jensen and Mesman 2006) joining information from three lines of evidence (LoE): chemical, ecotoxicological, and ecological. Risk values for the chemical LoE were derived from calculating the toxic pressure based on total metal concentrations. Those for the ecotoxicological LoE were based on avoidance behavior tests (*F. candida* and *E. andrei*) and aquatic tests with eluates (*Daphnia magna* acute test and Microtox), whereas for the ecological LoE, the bait lamina test, soil basal respiration, and vegetation cover were used to derive risk values. Very high-risk levels, associated with sandy soils and residue deposits, suggested the need to proceed with remediation actions. However, the uncertainties associated with the contradictory information given by certain LoEs for certain sampling points showed the need to confirm potential risks in a tier 2 analysis. In this second phase (Niemeyer et al. 2015), risk values were calculated for the habitat and for the retention functions for each sampling point. Habitat function included the chemical LoE calculated from total metal concentrations: ecotoxicological LoE based on reproduction tests with terrestrial invertebrates (*F. candida*, *E. crypticus*, *E. andrei*), shoot length, and plant biomass (*Avena sativa*, *Brassica rapa*) and ecological LoE based on microbial parameters, soil invertebrate community, and litter breakdown. Retention function included the chemical LoE, calculated from extractable metal concentrations, and ecotoxicological LoE based on eluate tests with aquatic organisms (*D. magna* reproduction and *Pseudokirchneriella subcapitata* growth). Results related to the habitat function indicated that the metal residues are sufficient to cause risk to biota, while the low metal levels in extracts and the general lack of toxicity in aquatic tests indicated a high soil retention capacity in most sampling points. Integrated risk of tier 2 showed the same trend of tier 1, suggesting the need to proceed with remediation actions. Metal contamination affected the ecological status of the site, leading to a risk for ecosystem functioning and provisioning of ecosystem services like organic matter decomposition and nutrient cycling, again 17 years after the end of the smelting activities. Regarding the sensitivity of the ecological parameters assessed, most were able to distinguish contaminated sites within the smelter area boundaries from those outside (control sites). Bait lamina, basal respiration, and microbial biomass carbon presented a high capacity to distinguish the level of soil contamination, since they were significantly correlated with metal loadings and thus are promising candidates to be integrated in the ecological line of evidence of a site-specific ERA (Niemeyer et al. 2012a). However, usual ecological indices (diversity, species richness, evenness, species abundance) using surface-dwelling invertebrates were not able to distinguish the ecological status among sites, but a significant change in community composition was observed between inside and outside the smelter area (Niemeyer et al. 2012b). In conclusion, the performance of a TRIAD approach is recommendable when a complex situation and its long-term consequences have to be assessed. This work highlights the importance of including biological tests and *in situ* evaluations to a better understanding of the bioavailability and effects of contaminants, especially in contaminated sites where mixture of contaminants is common, reducing uncertainties in environmental decision making.

Biomarkers

We cannot deny that the use of biomarkers have gained attention of some groups in academy as tools for the monitoring of soil contamination. Although they may not be classically used in soil ecotoxicology, they may be interesting early warning indicators. For example, Da Silva-Junior & Vargas (2009) evaluated the mutagenic potential of soil samples adjacent to a coal-fired power plant and of a coal bottom ash deposit in a recovery process, through *Salmonella*/microsome assay, to estimate ecological and human health impacts. In another study, alterations of hematological profile were observed in the wild mouse *Calomys laucha* when exposed for 14 days to a concentration of 8 % (*v*/*v*) of crude oil (Da Silva-Júnior et al. 2013).

Tendences and required actions

Although soil ecotoxicology in Brazil has advanced and has been taking its course as a scientific discipline, some issues should be highlighted in order to improve this field of research in the country.

Soil ecotoxicology is becoming global, meaning that test and assessment methodologies no longer focus only on the specific needs and conditions of parts of the Northern Hemisphere. However, legal requirements (and especially their implementation) still differ very much in different parts of the world. While in North America, Europe, and some countries like Japan and Australia, a well-established legal framework requiring ecotoxicological studies does exist, such requirements are either not available at all or they exist but are not relevant in daily reality in countries like Brazil. In Brazil, legal requirements for soil conservation move in a very slow pace as well as their implementation.

Soil ecotoxicology is becoming soil stress ecology, meaning that the focus is shifting from pure chemical stressors toward the inclusion of non-chemical stressors in the assessments, e.g., how climate change events interact with pesticides and alter their environmental risk. Moreover, other types of contaminants (e.g., veterinary pharmaceuticals) and potentially toxic matrices used as soil amendments or as soil fertilizers, namely all types of organic residues, have gained strength in Brazil with the increase of interest from academia.

Ecotoxicologists are becoming more and more aware that our basic knowledge on the diversity of soil organism communities, their ecological functions, and the services provided by them is very limited, mainly in non-temperate regions. The number of test species in Brazil, as well as in other tropical countries, has to be extended, since the currently used species do not cover the huge biodiversity and exposure scenarios found in soil. Therefore, a much more intensive co-operation between ecology and ecotoxicology, also including socio-economic aspects, is urgently needed—an obvious plea in the context of ecosystem service approach. Furthermore, in order to increase the ecological relevance of ecotoxicological tests, moving to other types of tests are necessary, especially chronic tests and higher tier studies, such as semifield and field tests.

Finally, it must be assured that consequences of legal actions (both for chemicals and other contaminants) are implemented in reality. This should be done in combination with, e.g., training of regulators and farmers or on imposing quality assurance measures in laboratories. The government should support the development of soil ecotoxicology in their scientific institutions by financially supporting programs in this area. This is relevant in areas as the environmental risk assessment of plant protection products, where more research is needed in order to fully implement a true ERA in Brazil. Furthermore, environmental agencies in Brazil (at federal or state level) need funds to conduct studies needed to support decision making about contaminated sites or even to set soil guidelines more realistic to exposure scenarios in Brazil, taking into account the existing diversity of soil types and climatic scenarios. Only with these types of initiatives will be possible to improve existing legislation and to enforce existing legal demands in different areas.

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