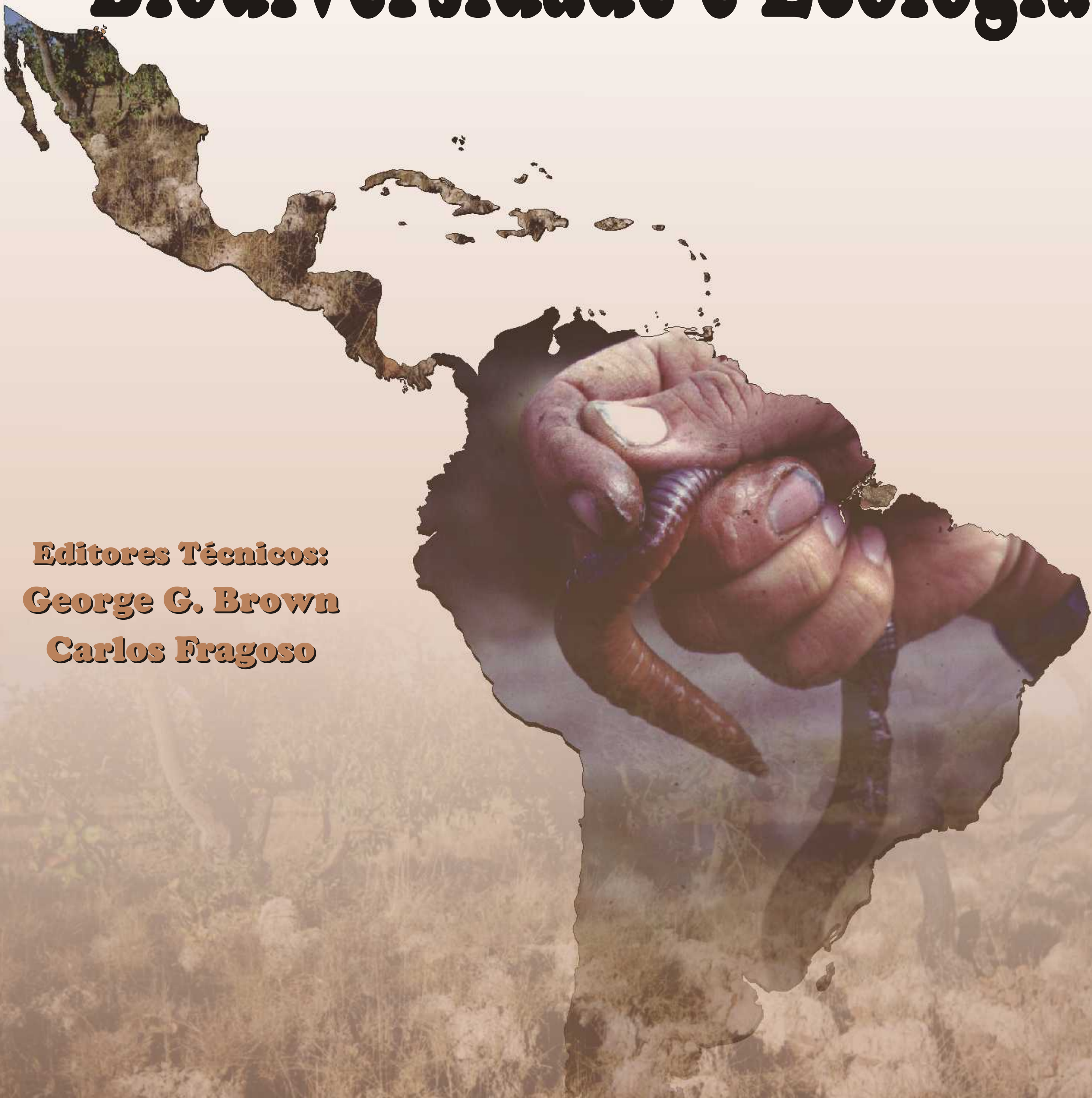
Minhocas na América Latina: Biodiversidade e Ecologia



Editores Técnicos:
George G. Brown
Carlos Fragoso

Embrapa

Empresa Brasileira de Pesquisa Agropecuária
Embrapa Soja
Ministério da Agricultura, Pecuária e Abastecimento

Minhocas na América Latina: Biodiversidade e Ecologia

Editores:
George G. Brown e *Carlos Fragoso*

Londrina, PR
2007

Exemplares desta publicação podem ser adquiridos na:

Embrapa Soja
Rodovia Carlos João Strass - Acesso Orlando Amaral
Caixa Postal 231
86001-970 - Londrina, PR
Fone: (43) 3371-6000 - Fax: 3371-6100
www.cnpso.embrapa.br
sac@cnpso.embrapa.br

Ministério do Meio Ambiente - MMA
Centro de Informação e Documentação Luiz Eduardo Magalhães / CID Ambiental
Esplanada dos Ministérios - Bloco B - térreo
70068-900 - Brasília, DF
Fone: (61) 4009-1235 – Fax (61) 4009-1980
www.mma.gov.br
cid@mma.gov.br

Coordenação editorial:
George G. Brown / Carlos Fragoso

Revisão de texto:
George G. Brown / Carlos Fragoso

Normalização bibliográfica:
Ademir Benedito Alves de Lima

Projeto gráfico:
Neide Makiko Furukawa

Capa:
Neide Makiko Furukawa / George G. Brown

Editoração eletrônica:
Neide Makiko Furukawa

Fotos da capa:
George G. Brown / András Zicsi

1ª Edição
1ª impressão (2007): 2050 exemplares

Dados Internacionais de Catalogação na Publicação (CIP)
Embrapa Soja

Minhocas na América Latina: biodiversidade e ecologia / editores técnicos: George G. Brown, Carlos Fragoso. Londrina: Embrapa Soja, 2007.
545 p. : il. ; 29,7 cm.

ISBN 978-85-7033-019-2

1.Minhoca-biodiversidade. 2.Minhoca-ecologia. I.Brown, G.G. II.Fragoso, C. III.Título.

CDD 592.64098

Earthworms as bioindicators (in particular for the influence of land use)

Jörg Römbke; Stephan Jänsch; Marcos Garcia

Abstract

The biological quality of soil can be assessed using various methods, but there is no universally accepted procedure. Actually, depending on the problem to be addressed very different approaches as well as soil organisms can be used. For the assessment of the presence and consequences of single chemicals like pesticides or heavy metals, standardised laboratory and field tests are regularly used. Contaminated soil, often containing mixtures of chemicals, is investigated with laboratory tests or field monitoring methods. Finally, non-chemical stresses like land use systems (e.g. agroecosystems) are evaluated by field monitoring. Due to their wide distribution, high regional but low local diversity, high biomass and important functional role in soil ecosystems, earthworms of the family Lumbricidae (Oligochaeta) have often been used for all these tests or monitoring activities, while the use of other families like tropical Glossoscolecidae or Enchytraeidae is much rarer. Besides a short overview on standardised ecotoxicological laboratory and field test methods, concepts for the study of the influence of land-use on the abundance, biomass and species composition of earthworms are described in this chapter. The latter issue is discussed in detail, using two case studies from Germany and Brazil (earthworm communities in agriculture and agroforestry, respectively) as examples. Oligochaeta, and in particular earthworms are good indicators for the effects of chemicals as well as different land use forms on the soil biocoenosis. In order to improve the scientific basis for the latter approach, the performance of large-scale and regionally differentiated monitoring programs as well as the improvement of taxonomic tools for soil organisms is necessary.

Resumo

A qualidade do solo pode ser verificada usando vários métodos, mas não existe procedimento universalmente aceito para tal finalidade. Dependendo do problema a ser abordado, diferentes táticas e organismos do solo podem ser usados. Para avaliar a presença e conseqüências de contaminantes químicos como pesticidas ou metais pesados, testes padronizados de laboratório e de campo são usados regularmente. Solo contaminado, freqüentemente contendo uma mistura de diferentes produtos químicos é normalmente avaliado com testes de laboratório ou monitoramento no campo. Estresses não-químicos como sistemas de uso do (agroecossistemas) são avaliados com monitoramento no campo. Devido à sua ampla distribuição por ex., alta diversidade regional mas baixa diversidade local, alta biomassa e importantes efeitos sobre a função dos solos, as minhocas da família Lumbricidae (Oligochaeta) têm sido frequentemente usadas para todos os tipos de testes laboratoriais e monitoramento no campo. Porém, minhocas de outras famílias como a Glossoscolecidae (típica dos neotrópicos) ou os enquitreídeos (Enchytraeidae) são raramente usados para estes fins. Além de uma breve revisão das metodologias padronizadas para testes laboratoriais ecotoxicológicos e avaliações no campo, este capítulo também descreve diversos conceitos para o uso das minhocas (abundância, biomassa e composição de espécies) como indicadores do efeito do uso do solo sobre sua qualidade. Este assunto é discutido em maior detalhe, usando dois estudos de caso, da Alemanha (comunidades de minhocas em agroecossistemas) e do Brasil (minhocas em sistemas agroflorestais). Os oligoquetas, e, em particular, as minhocas são bons indicadores dos efeitos de produtos químicos, assim como de diferentes formas de uso da terra, sobre a biocenose edáfica. Para melhorar a base científica de seu uso como indicadores, programas de monitoramento de comunidades de minhocas em larga escala e em diferentes regiões devem ser realizados, além de uma melhora significativa na capacidade de identificação taxonômica destes animais.

Introduction

In our modern society, soil has to meet several functions (EU, 2002), e.g., to buffer pesticides, nutrients, and metals, to enable agricultural production, and to support houses, streets, and railroads. In addition to these functions, which are directly useful to man, soil also has to perform natural functions like being the substrate for natural vegetation and the habitat for soil organisms, i.e. it has to accommodate microbes, animals and plants (German Federal Soil Protection Act of 17 March 1998 - BBodSchG). However, the biological assessment of soil quality is still in a preliminary stage and there is no commonly accepted procedure (Graefe, 1993; Spurgeon et al., 1996, Dunger, 1998). From an ecological point of view, the assessment of the quality of soil as a habitat for soil organisms (i.e. the biodiversity of soil organisms) must integrate all possible stress factors. In addition, a soil biological classification and assessment concept must also be able to differentiate between the myriad different "natural" soil types and those which have been adversely affected.

Different methods representing different approaches are available for the classification and the assessment of soil quality. They often differ according to the predicted or actual level of anthropogenic stress. For example, for the assessment of single chemicals like pesticides or heavy metals standardised laboratory and field tests are regularly used. Contaminated soil, often containing mixtures of chemicals, may be investigated using laboratory tests or field monitoring methods. Finally, non-chemical stress like land use (e.g. agriculture) is generally evaluated by field monitoring.

In this contribution, biological classification and assessment approaches are described. In particular, the use of earthworms as indicators of land use in these approaches is discussed. Examples from temperate (Germany) as well as tropical (Brazil) regions are presented. Since another oligochaete family, Enchytraeidae, is also intensively used for the assessment of land use, some recent literature is compiled in an annex.

Selection of indicator organisms

Many groups of soil organisms are potentially suited to be used in biological classification and assessment of soils. These include nematodes, enchytraeids, earthworms, springtails, oribatid and gamasid mites, isopods, diplopods, chilopods and micro-organisms, among others (Höper, 1999). One single organism group will certainly not be sufficient for assessing a site, but the use of all groups is usually not possible due to limited resources; therefore a battery approach is suggested (Ruf et al., 2000). Qualitative parameters like species composition are usually better suited than quantitative parameters like abundance. Whenever possible, the species level should be studied, but when this is not practical, higher taxonomic levels can be used.

In order to identify the groups that should be included in such a battery of tests, certain criteria can be used (Römbke et al., 1996). For instance, indicators organisms should:

- live in or close to (e.g. litter) the soil
- be ecologically important
- occur in "medium" species numbers
- be taxonomically "easy" (e.g. identification keys are available)
- be sampled by standardised guidelines
- be sensitive indicators.

Earthworms are widely used as indicator species because they fulfil these criteria:

- Nearly all earthworms are true soil inhabitants and many of them are ecologically important. Several species like *Lumbricus terrestris* (Lumbricidae) are considered ecosystem engineers (Lavelle et al., 1997).
- Earthworms are globally distributed, but at one site less than 20 species occur, i.e. such species numbers are practical. In Central Europe, usually up to 10 earthworm species are found at one site (Römbke et al., 1997).
- Identification keys are available, mainly for temperate regions. In many parts of Europe (in particular Central and Northern regions), identification keys are available (e.g. Sims & Gerard, 1985).
- Breeding and handling of some species is easy
- Standardised guidelines were developed by

OECD and ISO for several investigation levels (e.g. OECD, 1984; ISO, 1998; ISO, 1999).

Earthworms are sensitive indicators for anthropogenic stress factors (in particular chemicals). For example, they have been successfully used as bio-indicators for (at least): chemicals (e.g. pesticides, biocides, drugs; Edwards et al., 1996), mixed soil contaminations (e.g. heavy metals, PAHs; Stephenson et al., 1998; Hund-Rinke et al., 2003), physical factors (e.g. compaction, hydrology; Pizl, 1992; Lowe & Butt, 1999) and land use (e.g., agriculture, forestry, orchards; Lee, 1985). Therefore, earthworms are clearly suitable for soil quality classification and assessment approaches.

Standardised Methods

Laboratory and field tests for chemicals and contaminated soil

Among terrestrial oligochaetes, earthworms of the family Lumbricidae are the most widely used test organisms. World-wide tests standardised by OECD or ISO and adapted for temperate regions are generally used, but methods especially suitable for the tropics have also been developed (Garcia, 2004). The acute toxicity test with the compost worm *Eisenia fetida* in artificial soil (OECD, 1984) is the most important test with soil organisms up-to-date (Photo 25.1). In a slightly modified version published by the ASTM (No. E 1676; 1995), testing of single chemicals and soil quality assessment are put together, resulting in a very general test. Interestingly, in Brazil an even more artificial acute test based on a French guideline is used (AFNOR, 1984). In this test the chemical is mixed into a plastic substrate with glass balls instead of soil.

The chronic earthworm reproduction test, despite being based on the OECD acute test, was standardized first by ISO (No. 11268-2; 1998) and only five years later by OECD (No. 222; 2003). Slightly modified versions of the acute and chronic earthworm tests have been used for the assessment of remediated soil substrate in Canada, e.g., by defining reference values for field soils (Stephenson, personal communication, 2004). In Brazil, this is not yet required. A few years ago, the parameter avoidance behaviour was used to evaluate the effect of contaminated substrate on earthworms (Table 25.1; Photo 25.2; Stephenson et al., 1998; Hund-Rinke & Wiechering, 2001). Based on the Canadian and German experiences, the method is being standardised by ISO (ISO, 2004a). The sensitivity of this screening method is comparable to the much longer lasting (but ecologically more relevant) reproduction test.

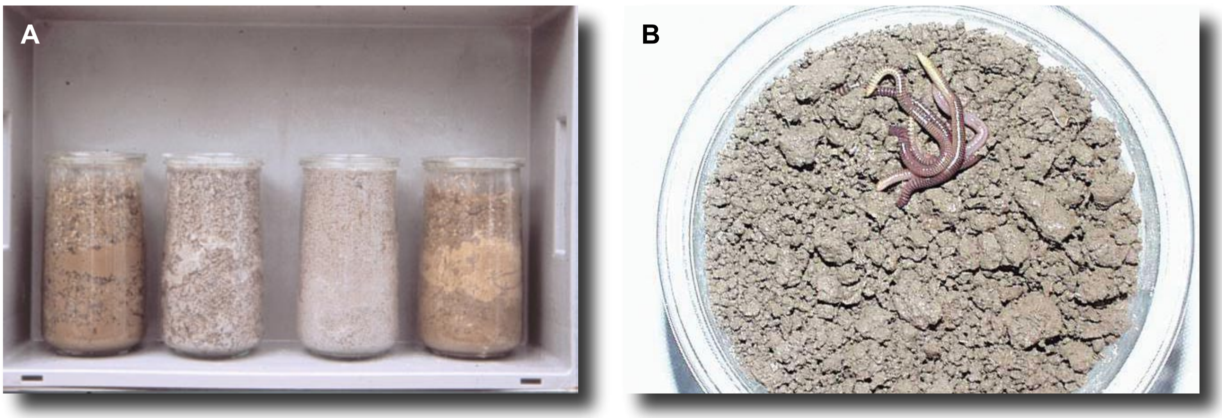


Photo 25.1. Test vessels of the Acute Test (A). Grey: OECD artificial soil; Brown: field soil. *Eisenia fetida* on the surface of a test vessel (B). (Photos J. Römbke)

Table 25.1. Short characterisation of the earthworm avoidance test (ISO, 2004a).

Species	*Eisenia fetida*, *E. andrei*
Parameter	Behaviour
Substrate	Artificial soil or field soils (e.g., LUFA)
Duration	2 days
Design	NOEC, ECx (either with two or six sections)
Experience	Many tests with different contaminated soils have been performed

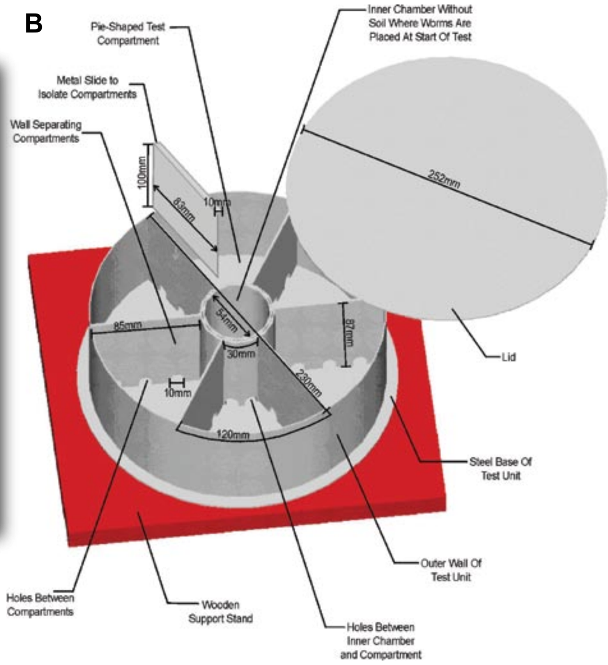


Photo 25.2. Design of the ISO Earthworm Avoidance Test. Two section design (A) and six section design (B). (Photo J. Römbke)

Even for the assessment of chemicals there are nearly no standardized test methods in the field. One exception is a field test with earthworms in which the long-term effect of a pesticide on the natural lumbricid coenosis in temperate regions are evaluated (Table 25.2; Photo 25.3; ISO, 1999). Only a few studies very roughly comparable to the ISO test have been performed for the assessment of contaminated soils (e.g., Callahan et al., 1991).

Photo 25.3. Three earthworm species representative of different ecological groups: *Lumbricus terrestris* (largest individual), *Aporrectodea caliginosa* (intermediate-sized individual), *Eisenia fetida* (smallest individual). (Photo J. Römbke)

Field monitoring methods for the assessment of contaminated soil samples

Sampling and processing of samples, including determination of the earthworms, can be performed using standardised methods widely accepted in soil ecology (Dunger & Fiedler, 1997; Coleman et al., 1999), but standardisation has just begun at ISO TC 190 (ISO, 2004b; see Römbke, 2007; chapter 29 for more details). Earthworms are collected using a combination of hand-sorting and formalin extraction methods before being stored in alcohol and/or formalin (ISO ,2004b). For tropical conditions special modifications may be included (e.g. the TSBF method or large scale formalin application). The latter case is used when giant species (length > 50 cm) are present at the collection site (see Photo

Table 25.2. Short characterisation of ISO test No. 11268-3 (ISO, 1999).

Species	Natural community; e.g., *Aporrectodea caliginosa*, *Lumbricus terrestris*
Substrate	Field sites (meadows, crop sites)
Duration	Up to 12 months
Parameter	Abundance, biomass, species composition
Design	Limit test
Experience	Very few, if any in Europe.

29.7). A detailed description and discussion of the standardised method is provided by Römbke (2007; see chapter 29).

The influence of land use on earthworm communities

Land use integrates a number of management factors which can influence earthworm communities in different ways: while for instance chemicals or tillage act negatively, fertilisation with organic materials or crop rotation can benefit the worms (see Figure 30.3, Brown et al., 2007; chapter 30).

Examples from temperate regions

In a first attempt to evaluate the influence of land use on earthworm communities the average abundance, biomass and number of species in various land use systems (LUS) can be compiled as published in the literature (Table 25.3; Römbke et al., 1997). Three conclusions can be drawn from such a compilation, which is in this case is based on lumbricid data from Central and Northern Europe:

1. Earthworm abundance, and in particular biomass tend to be lower at crop sites and in acid forests than in grasslands or orchards. The number of species per LUS varies considerably less than the two other parameters;
2. All parameters show a broad overlap which simply implies that other factors besides land use are influencing the earthworm communities;
3. In addition, the data indicate an intrinsic problem of literature compilations; different methods (e.g., sampling, biomass determination, data assessment, site description) have often been used, making comparisons difficult and impractical in some cases.

Since literature reviews can only act as a first, very rough approximation of the effedt of LUS, a more precise approach is necessary. Therefore, a study performed in Bavaria (Southern Germany) can be used as an example (Bauchhenss, 1997). Using the same sampling method (a combination of hand-sorting and formalin extraction), Bauchhenss (1997) sampled 116 crop sites and 20 grasslands in the same region and time. A wide range of earthworm abundance was encountered (Figure 25.1): at crop sites it ranged from 0 to nearly 300 ind/m^2 (median = 9 ind/m^2) and at the grasslands from 25 to > 350 ind/m^2 (median = 163 ind/m^2).

However, when all available information was combined, it became clear that the lumbricid communities of both land uses were different (Table 25.4). Despite a small overlap in abundance at some sites (Figure 25.1), total number of species, mean abundance, biomass, number of species per

Table 25.3. Earthworm abundance, biomass and number of species in various sites and land use systems in the temperate region. Average data from various literature sources (Römbke et al., 1997).

Land use form	Abundance (Ind./m^2)	Biomass (dry weight g/m^2)	No. species
Cropping systems	6 – 453	0.5 – 15.2	0 – 11
Grasslands	94 – 646	3.5 – 32.0	4 – 12
Acid Forests	14 – 167	<1 – 5.6	3 – 5
Mull Forests	28 – 220	0.9 – 12.1	ca. 10
Orchards	218 – 848	12.0 – 38.1	ca. 10

Table 25.4. Comparison of the earthworm communities at 116 crop sites and 20 grassland sites in Bavaria (Bauchhenss, 1997).

Parameter	Cropping systems Mean ± SD	Grasslands Mean ± SD
Abundance (Ind./m^2)	77.4 ± 6.5	185.1 ± 29.3
Biomass (g DW/m^2)	6.2 ± 0.5	45.5 ± 7.2
Total number of species	5	8
No. species per sample	2.1 ± 0.1	4.1 ± 0.6
Juvenile : adult ratio (%)	82 : 18	62 : 38

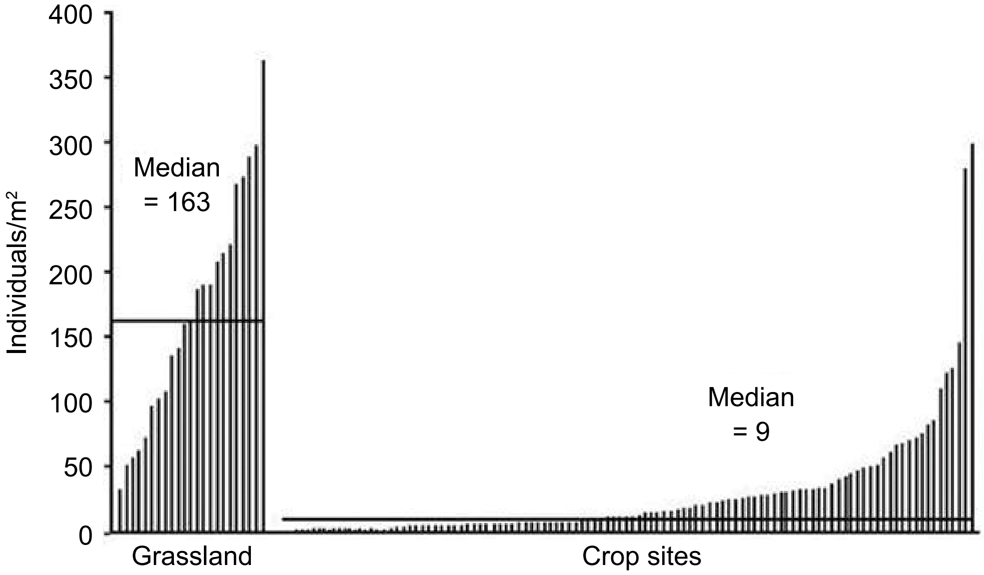


Figure 25.1. Earthworm abundance (ind./m^2) at 116 crop sites and 20 grassland sites in Bavaria (Bauchhenss, 1997)

sample and the ratio of juveniles to adult worms was different in the two LUS.

In addition, Bauchhenss (1997) found that differences in earthworm biomass were not only due to differences in abundance: individuals of most species were smaller in the grassland sites than at the crop sites (Figure 25.2). Although apparently contradictory, this result can be explained by the limited food resources at the crop sites which hinder earthworm reproduction. Earthworms at these sites are larger and heavier but reproduce less, while worms in the grassland are smaller but produce more cocoons.

Examples from tropical regions

The average data for earthworm communities in different tropical LUS have been compiled from various sources (Table 25.5). However, the number of studies is much lower compared to those in temperate regions, and the individual sites are distributed over a much larger (and diverse) area of the world. In addition, the number of earthworm species and families is considerably higher in the tropics (in the Holarctic, often less than 20, mainly Lumbricidae species are involved). For these reasons, the data shown in Table 25.5 can only

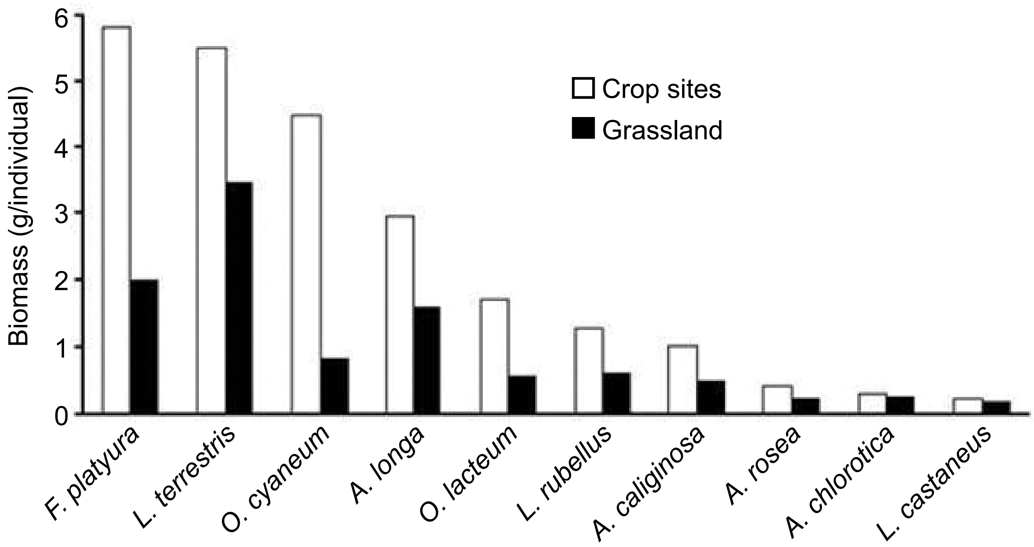


Figure 25.2. Biomass of individual earthworm species in various grasslands and cropping systems in Bavaria (Bauchhenss, 1997).

Table 25.5. Earthworm abundance, biomass and number of species in various LUS in Latin America. Average data from various literature sources.

Land use	Abundance (Ind./m^2)	Biomass (g DW/m^2)	No. species
Rainforest	68 ± 32	12.9 ± 6.2	6.5 ± 1.3
Secondary forest	171 - 189	52 - 61	?
Plantation	91 - 150	29 - 59	?
Savanna	188 - 582	17 - 49	4 - 14
Cropping systems	?	?	?

be seen as a preliminary estimate. However, the conclusions drawn previously can also be arrived at here: there is a considerable overlap between the different LUS but a tendency for smaller earthworm populations in primary rainforests compared to disturbed forests, plantations and savannas. Differentiated data from crop sites are not available but the tendency is obvious: fewer worms and species than at “wooded” sites (see Lavelle et al., 1994). On a very general level, the numbers seem to be in the same order of magnitude in both temperate and tropical regions (Fragoso et al., 1999).

As in the temperate region, the influence of land use on earthworm populations and diversity in the tropics becomes clearer when looking at individual sites in one region sampled with the same methods. Since no extensive data set like the one from Bavaria is available for a particular site in the tropics, an example from Amazonas (Brazil) is presented here (Höfer et al., 2001). At an Embrapa research station near Manaus, the earthworm community of three different forest types (primary rain forest, secondary forest, and two agroforestry plantations) was sampled using formalin extraction for three years (Römbke et al., 1999). Abundance, biomass and species composition, plus organic matter decomposition were measured to assess the functional role of soil fauna communities in this process (Table 25.6).

While abundance and species number did not differ clearly at the four sites, biomass and juvenile to adult ratio indicate a distinction between secondary forest and agroforestry plantation-A on one side and the primary forest plus the agroforestry plantation-C on the other side. The percentage of peregrine species shows a continuum: only natives in the primary forest but up to 12% peregrines were present in the agroforestry plantation-A.

Considering the species level, the picture becomes more complex (Table 25.7). Some species (like *Tuiba dianeae*) were evenly distributed at all four sites, while others like *Rhinodrilus contortus* clearly preferred the primary forest. The presence of the pantropical peregrine species *Pontoscolex corethrurus* was highly correlated with disturbance. The distribution pattern of the giant species *R. priollii*, previously known only from primary forest around Manaus, showed that, in fact, this species is able to adapt to various different abiotic conditions, being found at at all four study sites in a range of 5-12%.

OM breakdown rates (measured via the litterbag method in two runs; Höfer et al., 2001) in agroforestry systems and the secondary forest reached only between 25 and 60% of the primary forest values (Table 25.8). Depending on the site and duration of the evaluation (which differed according to the season of measurement), plant litter decomposition half-life (PLD) was two-to-

Table 25.6. Earthworm communities in primary and secondary forests and agroforestry systems near Manaus, Central Amazonia (Römbke et al., 1999; Höfer et al., 2001).

	Primary Forest	Secondary Forest	Agroforestry plantation-A	Agroforestry plantation-C
Abundance (Ind./m^2)	16.2	11.7	12.0	14.5
Biomass (fresh weight g/m^2)	15.6	2.6	4.0	9.6
No. species	7	8	5	8
Juvenile/adult ratio (%)	81 : 19	64 : 36	67 : 33	77 : 23
Native : peregrine species ratio (%)	100 : 0	98 : 2	88 : 12	95 : 5

Table 25.7. Species composition of earthworms in primary and secondary forests and in agroforestry systems near Manaus, Brazil (Römbke et al., 1999; Höfer et al., 2001). Numbers represent percentages based on abundance (No. ind./m^2).

Species	Primary Forest	Secondary Forest	Agroforestry plantation-A	Agroforestry plantation-C
Andiorrhinus amazonicus	11%	10%	12%	22%
Pontoscolex corethrurus	0%	2%	12%	5%
Rhinodrilus contortus	31%	17%	0%	14%
Rhinodrilus priollii	6%	5%	12%	5%
Urobenus brasiliensis	20%	34%	33%	10%
Tuiba dianae	25%	28%	30%	33%
Other spp.	7%	6%	1%	11%

Table 25.8. Decomposition rates at the primary and secondary forests and in agroforestry systems measured using coarse mesh litter bags near Manaus (Höfer et al., 2001). Plant litter decomposition (PLD) is represented by the number of days after which 50% of the OM is decomposed during the two different measurement periods (PLD50).

Study site	PLD50 [d] 1st study	PLD50 [d] 2nd study
Primary forest	108	82
Secondary forest	289	232
Agroforestry plantation-A	433	258
Agroforestry plantation-C	182	239

four times longer in these systems than in the primary forest. The measured differences in PLD50 values were caused mainly by the different abundance, biomass and species composition of the macrofauna (besides earthworms, isopods and diplopods were also important). A direct relationship was observed between the structure and the function of the soil biocenosis. However, even at the site with the strongest decomposition inhibition (plantation A), the litter standing stock was not significantly enhanced compared to the primary forest.

Biological approaches for the classification and assessment of soils

Biological assessment concepts have been used successfully for many years in aquatic ecotoxicology and vegetation sociology. These ideas have been recently transferred to terrestrial ecosystems, assuming that similar soils in the same region should have a similar soil fauna. Comparable concepts have been proposed for the UK (SOILPACS; Weeks, 1997) and are being used in several Dutch investigations (Sinnige et al., 1992; Schouten et al., 2000). In Germany, mainly two approaches have been used: the decomposer communities and the Soil Biological Site Classification (BBSK) approaches.

In the **"Decomposer communities"** approach (Graefe, 1993), frequently used in several states of Germany, the classification of soils is performed using oligochaetes (mainly earthworms and enchytraeids) as indicators for a typical community of saprophagous animals. First of all, the ecological profiles of oligochaete species in relation to soil moisture, pH and salinity (each divided into 9 classes) are determined. When classifying a certain site, the species composition, abundance, frequency and the occurrence of characteristic species are used as measurement endpoints. According to these parameters, each site is classified into one of nine hierarchical groups, named according to the rules originally proposed for vegetation sociology.

The **"Soil Biological Site Classification"** approach is the result of several studies investigating ecotypes and their respective biocoenosis (Römbke et al., 1997; Ruf et al., 2003). In this approach, the use of biological parameters for assessing soil quality is divided into three steps (for a schematic overview see Figure 25.3):

- Firstly, soils are classified in ecotypes. Ecotypes are units of sites with similar natural properties (= factors like soil organic content and pH, but also climate and other environmental parameters) and therefore the same preconditions for the establishment of a certain soil biocoenosis. Different forms of land use (forests, meadows, arable land) must be distinguished when defining ecotypes. Based on investigations of unaffected sites, the natural biocenosis (e.g. mainly the species composition) of the various ecotypes in a certain region is determined.
- Secondly, using the "expected" (baseline or reference) species composition any other soil with the same properties can be assessed by comparing the biocoenosis actually found in this soil. For example, a clear deviation can indicate an anthropogenic impact. A comparison can be done using either multivariate statistical methods (e.g. CANOCO, TWINSPAN), indices (e.g. maturity index; Bongers, 1990), or by qualitative evaluation using indicator species ("expert knowledge"). However, evaluation methods and assessment criteria have not yet been fixed.
- Thirdly, based on the degree of similarity between the expected and sampled biocoenosis, further studies are required or management activities can be performed.

The BBSK approach was evaluated several times (e.g. Ruf et al., 2003). The results can be summarised as follows:

- No methodological problems occurred, since ISO draft guidelines were used.
- Different sites could be grouped according to LUS and ecotypes.
- In some cases, the species composition was conspicuous, indicating anthropogenic stress.
- Increasing the number of organism groups evaluated improved the classification and assessment scheme.
- Oligochaetes alone were not sufficient for the classification and assessment of soils, but their exclusion also invalidated the use of the approach.

However, some open questions must still be answered to improve the use of the BBSK approach. For example, in the literature pedological and biological data are rarely described from the same site, which means that in order to develop a suitable set of reference data new studies are necessary (Römbke et al., 2005). While in Europe the earthworm communities of different land-use types are relatively well known, the situation is more complex in tropical regions; primarily due to lack of knowledge (in particular concerning taxonomy of these organisms). Unfortunately, no automatic, computerized taxonomic key is available for earthworms (a recently published key for aquatic oligochaetes is not useful for this purpose).

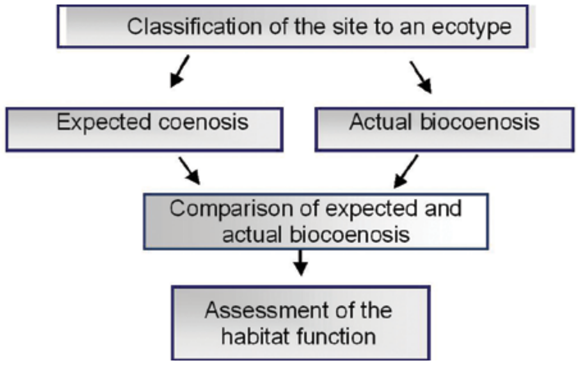


Figure 25.3. Overview on the different steps of the BBSK approach.

Outlook

Oligochaeta, and in particular earthworms, are good indicators for the effects of different land use forms on the soil biocoenosis. This statement is true for several investigation levels, e.g. starting from tests in the laboratory up to field monitoring methods. Standardised guidelines for the use of Oligochaeta as indicator organisms have been developed, but concepts like the BBSK approach for the biological classification and assessment of soils must still be improved, gathering data on earthworm communities in large-scale and regionally differentiated monitoring programs. Better taxonomic tools for soil organisms are also necessary. Only with these actions will a practical concept for the protection of the habitat function of soils and its biodiversity be possible.

References

AFNOR (ASSOCIATION FRANCAISE DE NORMALISATION). **Determination de la toxicité d'une substance vis-a-vis des lombriciens (espèce *Eisenia fetida*). Methode «Artisol». Norme expérimentale**. Paris, 1984. X 31-250.

ASTM (American Society for Testing and Materials). Standard guide for conducting a laboratory soil toxicity test with the Lumbricid earthworm *Eisenia foetida*. **Annual Book of ASTM**, n. 1676, p. 1123-1139, 1995.

BAUCHHENSS, J. Bodenzoologie. Boden-Dauerbeobachtungsflächen (BDF). Bericht nach zehnjähriger Laufzeit 1985 - 1995. Teil III. Boden: gefüge, organische substanz, bodenorganismen, vegetation. Bodenkultur und Pflanzenbau. **Schriftenreihe der bayrischen Landesanstalt für Bodenkultur und Pflanzenbau**, 6/97, p. 219-234, 1997.

BBodSchG (Bundes-Bodenschutzgesetz). **Gesetz zum Schutz vor schädlichen Bodenveränderungen und zur Sanierung von Altlasten**. Berlin: Ministry of the Environment, 1998. BGBl I 502 (17.03.1998).

BONGERS, T. The maturity index: an ecological measure of environmental disturbance based on nematodes species composition. **Oecologia**, v. 83, p. 14-19, 1990.

BROWN, G. G. SENAPATI, B. K.; PASHANASI, B.; VILLENAVE, C.; PATRÓN, J. C.; LAVELLE, P.; BAROIS, I.; BLAKEMORE, R. J. In: BROWN, G. G.; FRAGOSO, C. (Ed.). **Minhocas na América Latina**: biodiversidade e ecologia. Londrina: Embrapa Soja, 2007. Chapter 30.

CALLAHAN, C. A.; MENZIE, C. A.; BURMASTER, D. E.; WILBORN, D. C.; ERNST, T. On-site methods for assessing chemical impact on the soil environment using earthworms: a case study at the Baird and McGuire Superfund Site, Holbrook, Massachusetts. **Environmental Toxicology Chemistry**, v. 10, p. 817-826, 1991.

COLEMAN, D. C.; BLAIR, J. M.; ELLIOTT, E. T.; WALL, D. H. Soil invertebrates. In: ROBERTSON, G. P.; COLEMAN, D. C.; BLEDSOE, C. S.; SOLLINS, P. (Ed.). **Standard Soil Methods for Long-term Ecological Research**. New York: Oxford University Press, 1999. (LTER Network Series).

DUNGER, W.; FIEDLER, H. J. **Methoden der bodenbiologie**. Stuttgart: Fischer Verlag, 1997. 539 p.

DUNGER, W. Die Bindung zwischen Bodenorganismen und Böden und die biologische Beurteilung von Böden. **Bodenschutz**, v. 3, p. 62-68, 1998.

EDWARDS, C. A.; BOHLEN, P. J. **Biology and ecology of earthworms**. 3.ed. London: Chapman and Hall, 1996.

EDWARDS, C. A.; SUBLER, S.; CHEN, S. K.; BOGOMOLOV, D. M.; VAN STRAALEN, N. M.; KRIVOLUTSKY, D. A. Essential criteria for selecting bioindicator species, processes, or systems to assess the environmental impact of chemicals on soil ecosystems. In: VAN STRAALEN, N. M.; KRIOVOLUTSKY, D. A. (Ed.). **Bioindicator systems for soil pollution**. Dordrecht: Kluwer Academic Publishers, 1996. p. 67-84.

EU (European Union). **Towards a thematic strategy for soil protection**. Brussels: Commision of the European Communities, 2002. 35 p. COM(2002) 179.

FRAGOSO, C.; KANYONYO, J.; MORENO, A.; SENAPATI, B. K.; BLANCHART, E.; RODRÍGUEZ, C. A survey of tropical earthworms: taxonomy, biogeography and environmental plasticity. In: LAVELLE, P.; BRUSSAARD, L.; HENDRIX, P. (Ed.). **Earthworm management in tropical agroecosystems**. Wallingford: CAB International, 1999. p. 1-25.

GARCIA, M. V. **Effects of pesticides on soil fauna**: development of ecotoxicological test methods for tropical regions. 2004. Thesis (Ph. D.) - University of Bonn, Bonn.

GRAEFE, U. Die Gliederung von Zersetzergesellschaften für die Standortökologische Ansprache. **Mitteilungen Deutsche Bodenkundliche Gesellschaft**, v. 69, p. 95-98, 1993.

HÖFER, H.; HANAGARTH, W.; GARCIA, M.; MARTIUS, C.; FRANKLIN, E.; RÖMBKE, J.; BECK, L. Structure and function of soil fauna communities in Amazonian anthropogenic and natural ecosystems. **European Journal of Soil Biology**, v. 37, p. 229-235, 2001.

HÖPER, H. Die Bedeutung abiotischer Bodeneigenschaften für bodenmikrobiologische Kennwerte Ergebnisse aus der Bodendauerbeobachtung in Niedersachsen. **Mitteilungen Deutsche Bodenkundliche Gesellschaft**, v. 89, p. 253-256, 1999.

HUND-RINKE, K.; WIECHERING, H. Earthworm avoidance test for soil assessments. An alternative for acute and reproduction tests. **Journal of Soils and Sediments**, v. 1, p. 15-20, 2001.

HUND-RINKE, K.; RÖMBKE, J.; ACHAZI, R.; WARNEKE, D. Avoidance test with *E. fetida* as indicator for the habitat function of soils – results of a laboratory comparison test. **Journal of Soils and Sediments**, v. 3, p. 7-12, 2003.

ISO (International Organization for Standardization). **Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*)**. Part 2: Determination of effects on reproduction. ISO 11268-2. Geneve, 1998.

ISO (International Organization for Standardization). **Soil quality - Effects of pollutants on earthworms**. Part 3: Guidance on the determination of effects in field situations. ISO 11268-3. Geneve, 1999.

ISO International Organization for Standardization. **Soil quality - Avoidance test for evaluating the quality of soils and the toxicity of chemicals. Test with Earthworms (*Eisenia fetida/andrei*)**. ISO 17512. Geneve, 2004a.

ISO (International Organization for Standardization). **Soil quality - Sampling of soil invertebrates**. Part 1: Hand-sorting and formalin extraction of earthworms. ISO 23611-1. Geneve, 2004b.

LAVELLE, P.; DANGERFIELD, M.; FRAGOSO, C.; ESCHENBRENNER, V.; LÓPEZ-HERNÁNDEZ, D.; PASHANASI, B.; BRUSSAARD, L. The relationship between soil macrofauna and tropical soil fertility. In: WOOMER, P. L.; SWIFT, M. J. (Ed.). **The biological management of tropical soil fertility**. Chichester: Wiley, 1994. p.137-169.

LAVELLE, P.; BIGNELL, D.; LEPAGE, M. Soil function in a changing world: the role of invertebrate ecosystem engineers. **European Journal of Soil Biology**, v. 33, p. 159-193, 1997.

LEE, K. E. **Earthworms**: their ecology and relationships with soils and land use. Sydney: Academic Press, 1985. 411 p.

LOWE, C. N.; BUTT, K. R. Interspecific interactions between earthworms: a laboratory-based investigation. **Pedobiologia**, v. 43, p. 808-817, 1999.

OECD (Organisation for Economic Co-Operation and Development). **Earthworm acute toxicity test**. Paris, 1984. (Guideline for testing of chemicals No. 207).

OECD (Organisation for Economic Co-Operation and Development). **Earthworm reproduction test**. Paris, 2003. (Guideline for Testing of Chemicals No. 222).

PIZL, V. Effect of soil compaction on earthworms (Lumbricidae) in apple orchard soil. **Soil Biology and Biochemistry**, v. 24, p. 1573-1576, 1992.

RÖMBKE, J. Quantitative earthworm sampling methods: In search of standardization: The ISO methodology. In: BROWN, G. G.; FRAGOSO, C. (Ed.). **Minhocas na América Latina**: biodiversidade e ecologia. Londrina: Embrapa Soja, 2007. Chapter 29.

RÖMBKE, J.; BAUER, C.; MARSCHNER, A. Hazard assessment of chemicals in soil. Proposed Ecotoxicological Test Strategy. **Environmental Science Pollution Research**, v. 3, p. 78-82, 1996.

RÖMBKE, J.; BECK, L.; FÖRSTER, B.; FRÜND, H. C.; HORAK, F.; RUF, A.; ROSCICZEWSKI, K.; SCHEURIG, M.; WOAS, S. **Boden als lebensraum für bodenorganismen und die bodenbiologische standortklassifikation**: eine literaturstudie. Karlsruhe: Landesanstalt für Umweltschutz Baden-Württemberg, 1997. (Texte und Berichte zum Bodenschutz, 4/97).

RÖMBKE, J.; JÄNSCH, S.; DIDDEN, W. The use of earthworms in ecological soil classification and assessment concepts. **Ecotoxicology and Environmental Safety**, v. 62, p. 249-265, 2005.

RÖMBKE, J.; MELLER, M. ; GARCIA, M. Earthworm densities in central Amazonian primary and secondary forests and a polyculture forestry plantation. **Pedobiologia**, v. 43, p. 518-522, 1999.

RUF, A.; BECK, L.; RÖMBKE, J.; SPELDA, J. Standortspezifische Erwartungswerte für die Gemeinschaftsstruktur ausgewählter Taxa der Bodenfauna als Bodenqualitätskriterium. **Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck**, v. 87, p. 361-380, 2000.

RUF, A.; BECK, L.; DREHER, P.; HUND-RINKE, K.; RÖMBKE, J. ; SPELDA, J. A biological classification concept for the assessment of soil quality: “Biological soil classification scheme” (BBSK). **Agriculture, Ecosystems and Environment**, v. 98, p. 263-271, 2003.

SCHOUTEN, T.; BLOEM, J.; DIDDEN, W. A. M.; RUTGERS, M.; SIEPEL, H.; POSTHUMA, L.; BREURE, A. M. Development of a biological indicator for soil quality. **Society of Environmental Toxicology and Chemistry**, v. 4, p. 30-32, 2000.

SIMS, R. W.; GERARD, B. M. **Earthworms**. Keys and notes for the identification and study of the species. London: E. J. Brill/Dr. W. Backhuys, 1985. (New Series) Synopses of the British Fauna. No. 31. 171 p.

SINNIGE, N.; TAMIS, W.; KLIJN, F. **Indeling van Bodenfauna in oecologische Soortgroupen**. Leiden: Centrum voor Milieukunde, Rijksuniversität Leiden, 1992.

SPURGEON, D. J.; SANDIFER, R. D.; HOPKIN, S. P. The use of macro-invertebrates for population and community monitoring of metal contamination - indicator taxa, effect parameters and the need for a soil invertebrate prediction and classification scheme (SIVPACS). In: VAN STRAALEN, N. M.; KRIVOLUTSKY, D. A. **Bioindicator systems for soil pollution**. Dordrecht: Kluwer Academic, 1996. p. 95-109.

STEPHENSON, G. L.; KAUSHIK, A.; KAUSHIK, N. K.; SOLOMON, K. R.; STEELE, T.; SCROGGINS, R. Use of an avoidance-response test to assess the toxicity of contaminated soils to earthworms. In: SHEPPARD, S. C.; BEMBRIDGE, J. D.; HOLMSTRUP, M. ; POSTHUMA, L. (Ed.). **Advances in earthworm ecotoxicology**. Boca Raton: SETAC Press, 1998. p. 67-81

WEEKS, J. M. (Ed.). **A demonstration of the feasibility of SOILPACS**. London: Environment Agency, 1997.

Annex 1. Important references concerning the use of enchytraeids as test and indicator organisms.

DIDDEN, W. Ecology of terrestrial Enchytraeidae. **Pedobiologia**, v. 37, p. 2-29, 1993.

DIDDEN, W. A. M.; RÖMBKE, J. Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. **Ecotoxicology and Environmental Safety**, v. 50, p. 25-43, 2001.

GRAEFE, U.; SCHMELZ, R. Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. **Newsletter on Enchytraeidae**, v. 6, p. 59-67, 1999.

ISO (International Organization for Standardization). **Soil quality - Effects of pollutants on Enchytraeidae (*Enchytraeus* sp.)**. Determination of effects on reproduction and survival. ISO 16387. Geneve, 2003.

ISO (International Organisation for Standardisation). **Soil quality - Sampling of soil invertebrates working draft**. Part 3: Sampling and soil extraction of enchytraeids. ISO 23611-3. Geneve, 2007.

JÄNSCH, S.; RÖMBKE, J. Ecological characterisation of selected enchytraeid species (Enchytraeidae, Oligochaeta) - a literature research. **Newsletter on Enchytraeidae**, v. 8, p. 57-68, 2003.

JÄNSCH, S.; RÖMBKE, J.; DIDDEN, W. The use of enchytraeids in ecological soil classification and assessment concepts. **Ecotoxicology and Environmental Safety**, v. 62, p. 266-277, 2005a.

JÄNSCH, S.; AMORIM, M.J.B.; RÖMBKE, J. Identification of the ecological requirements of important terrestrial ecotoxicological test species. **Environmental Reviews**, v. 13, p. 51-83, 2005b.

LAAKSO, J.; SETÄLÄ, H. Sensitivity of primary production to changes in the architecture of belowground food webs. **Oikos**, v. 87, p. 57-64, 1999.

NIELSEN, C. O.; CHRISTENSEN, B. The Enchytraeidae, critical revision and taxonomy of European species. **Natura Jutlandica**, v. 8-9, p. 1-160, 1959.

O'CONNOR, F. B. Extraction of enchytraeid worms from a coniferous forest soil. **Nature**, v. 175, p. 815-816, 1955.

OECD (Organisation for Economic Co-Operation and Development). **Enchytraeidae reproduction test**. Paris, 2003. (Guideline for Testing of Chemicals No. 220).

RÖMBKE, J. Enchytraeen (Oligochaeta) als bioindikator. UWSF - **Zeitschrift Zeitschrift Umweltchemikalien & Schadstoff-Forschung**, v. 7, p. 246-249, 1995.

RÖMBKE, J. Ecotoxicological laboratory tests with enchytraeids: a review. **Pedobiologia**, v. 47, p. 607-616, 2003.