Soil ecotoxicology: soil bioassays

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What is soil?

Soil Taxonomy, second edition.

- Soil is a natural body comprised of solids (minerals and organic matter), liquid, and gases that occurs on the land surface, occupies space, and is characterized by one or both of the following: horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment.

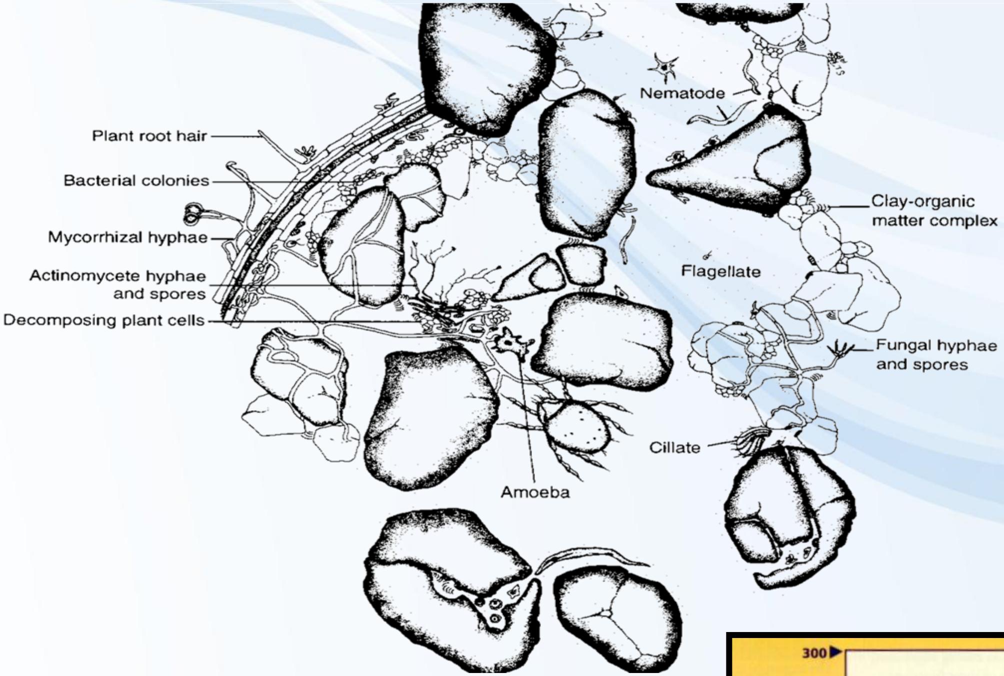
Why soil does matter?

- important part of nature
- non-renewable source
- non-replaceable functions of ter. ecosystems
- base for plant growth
- nutrient storage - fertility – production
- start and end of food chains
- biogeochemical cycles
- decomposition of organic matter, humification
- filtration, immobilization and degradation of pollutants
- water cycling
- biodiversity treasure

WE MUST PROTECT SOIL QUALITY

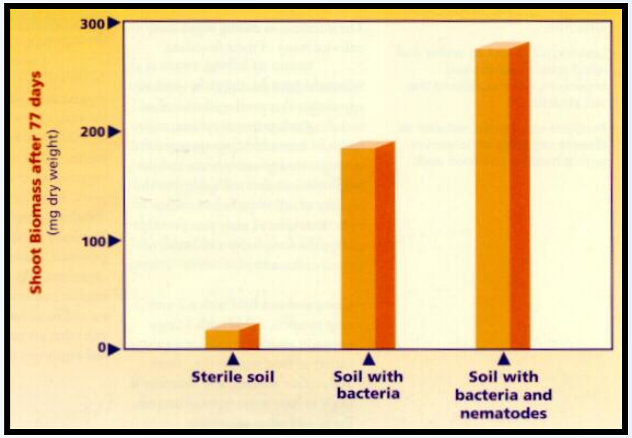
soil = biotic + abiotic = complex

bacteria
protozoa
fungi
algae
nematoda
rotifera
annelida
arthropodes
collembola
mollusca
…..



biota is important for

- formation of soil, for soil structure
- soil fertility
- organic residues decomposition, release nutrients
- element cycles
- air and water regime



Soil biota

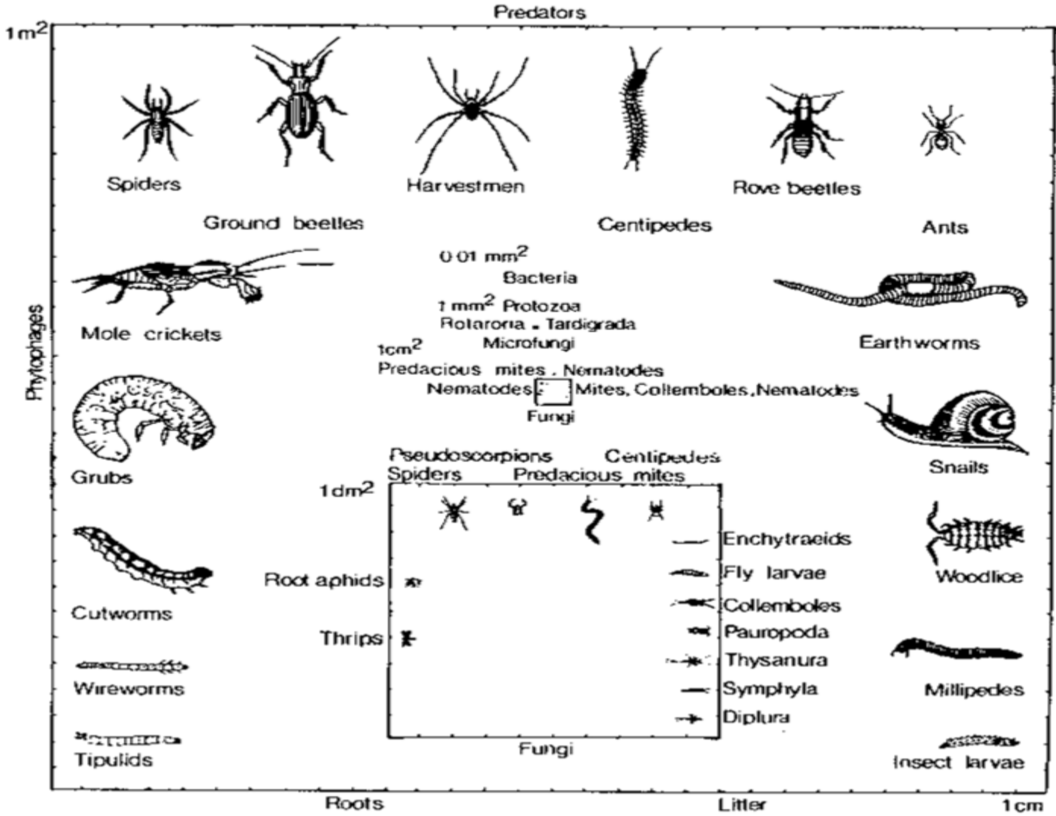


FIGURE 4. Schematic survey of the soil fauna community. Squares indicate habitat size and relevant sampling area. Species are arranged according to feeding type. Important species are drawn at scale. (From Eijsackers, H. and Van de Bund, C.F., in *Interactions Between Herbicides and the Soil*, Hance, R.J., Ed., Academic Press, London, 1980, 255. With permission.)

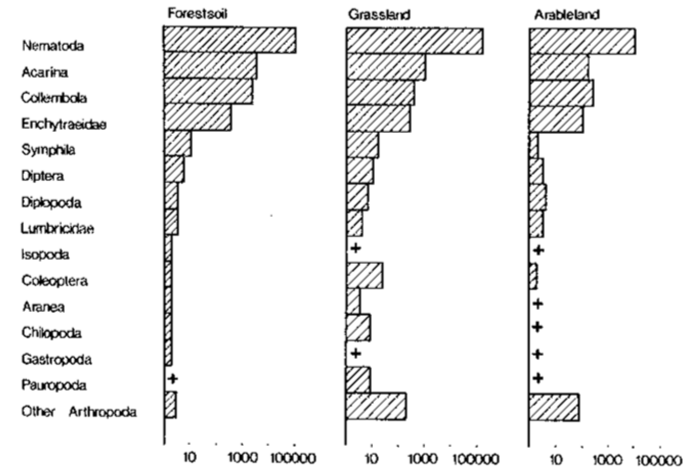
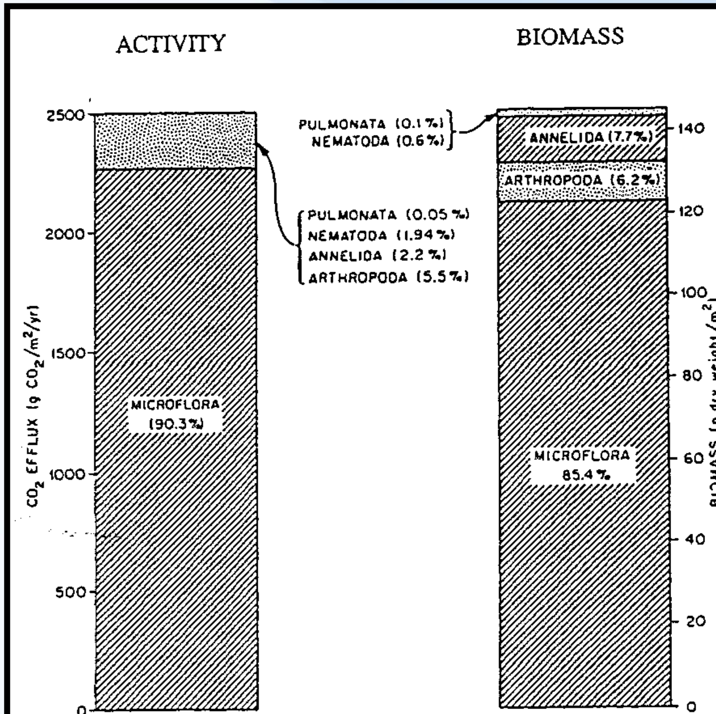


FIGURE 3. Mean composition of the soil fauna in a forest, meadow, and arable soil (mean numbers per dm² for the 0- to 30-cm layer; + means present). (From Eijsackers, H. and Van de Bund, C.F., in *Interactions Between Herbicides and the Soil*, Hance, R.J., Ed., Academic Press, London, 180, 255. With permission.)



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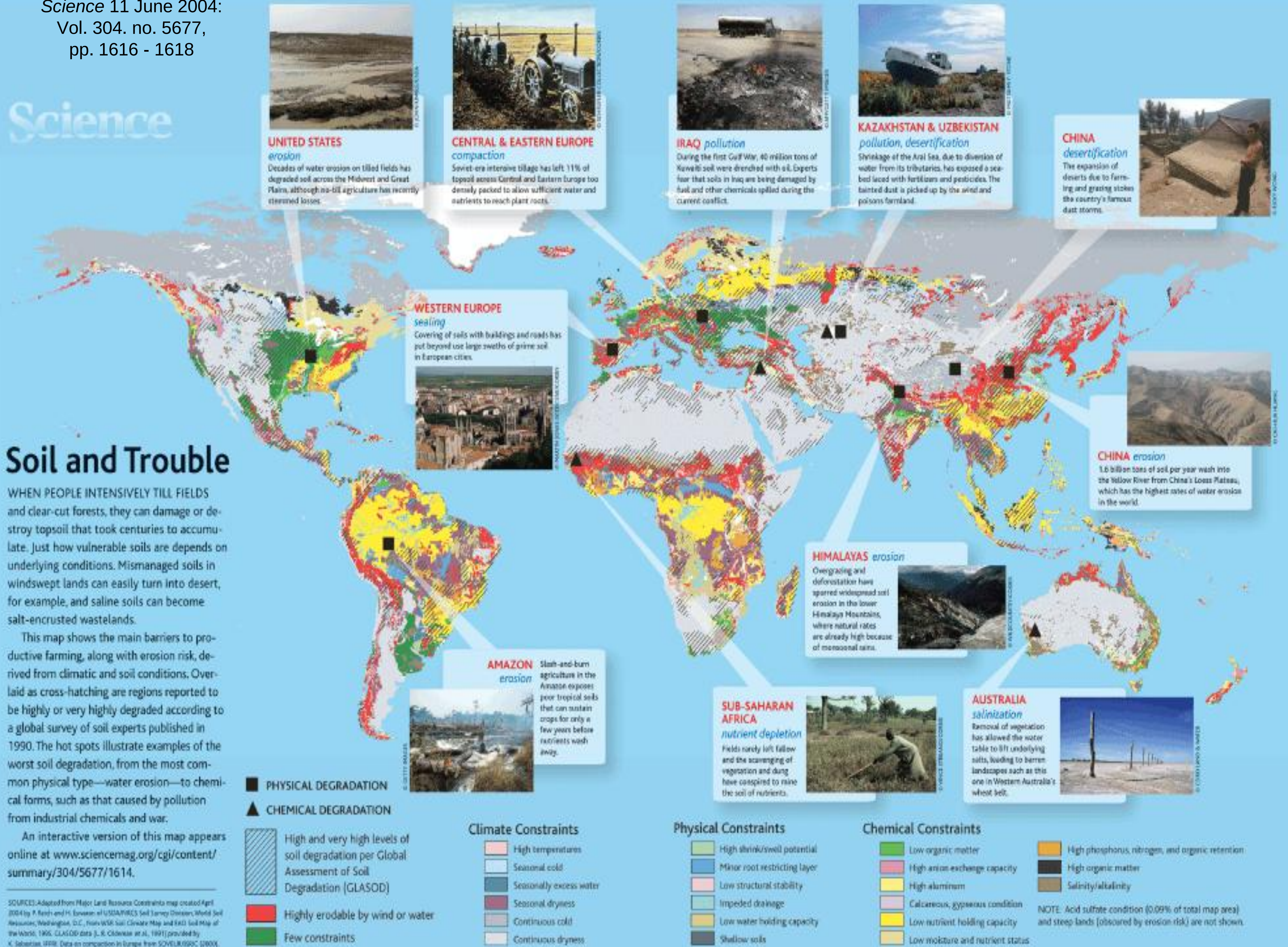
pp. 1616 - 1618



Soil and Trouble

WHEN PEOPLE INTENSIVELY TILL FIELDS and clear-cut forests, they can damage or destroy topsoil that took centuries to accumulate. Just how vulnerable soils are depends on underlying conditions. Mismanaged soils in windswept lands can easily turn into desert, for example, and saline soils can become salt-encrusted wastelands.

This map shows the main barriers to productive farming, along with erosion risk, derived from climatic and soil conditions. Overlaid as cross-hatching are regions reported to be highly or very highly degraded according to a global survey of soil experts published in 1990. The hot spots illustrate examples of the worst soil degradation, from the most common physical type—water erosion—to chemical forms, such as that caused by pollution from industrial chemicals and war.

An interactive version of this map appears online at www.sciencemag.org/cgi/content/summary/304/5677/1614.

SOURCES: Adapted from Major Land Resource Constraints map created April 2004 by P. Reich and H. Eswaran of USDA/NRCS Soil Survey Division, World Soil Resources, Washington, D.C., from WSR Soil Climate Map and FAO Soil Map of the World, 1995. GLASOD data (L. R. Oldeman et al., 1991) provided by K. Sebastian, IFPRI. Data on compaction in Europe from SOVEUR/ISRIC (2000).

Soils have problems

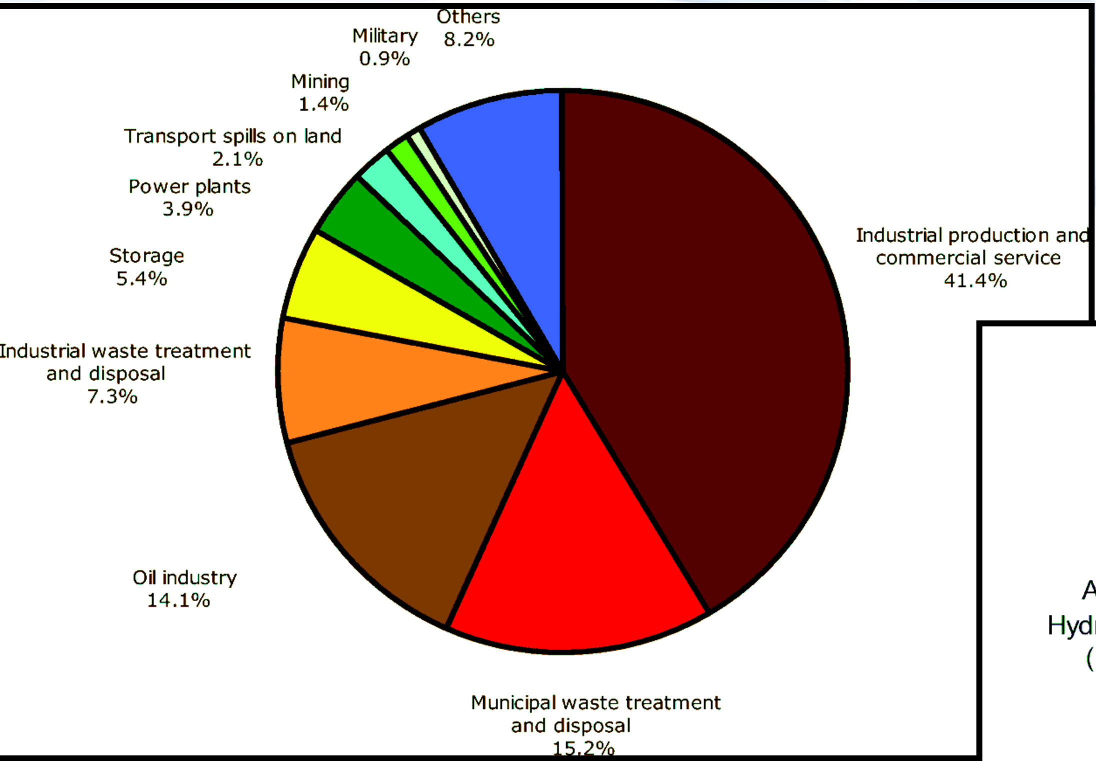
- EU Thematic Strategy for Soil Protection (COM/2006/231) defines main threats for soils (sealing, erosion, compaction, salinisation, OM loss, contamination …)
- 3,5 mil. contaminated sites in EU
- 0.5 mil. are seriously contaminated and need remediation
- Costs related to contaminated sites in EU: 2-17 bil. € (Impact assessment (SEC/2006/620))

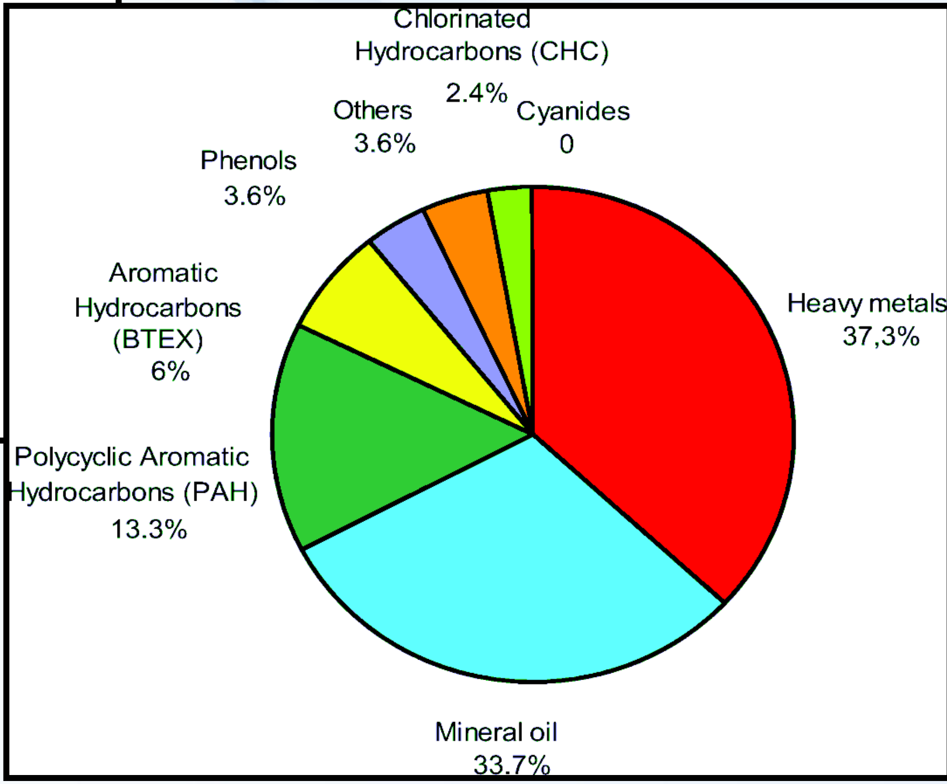
Soil protection

- we must protect soil quality
  - policy
  - legislation
  - science and education

- European Commission (2006a): Communication from the commission to the council, the european parliament, the european economic and social committee and the committee of the regions. Thematic strategy for soil protection. COM(2006)231.
- European Commission (2006b): Proposal for a directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC. COM (2006) 232.

Contamination of soils

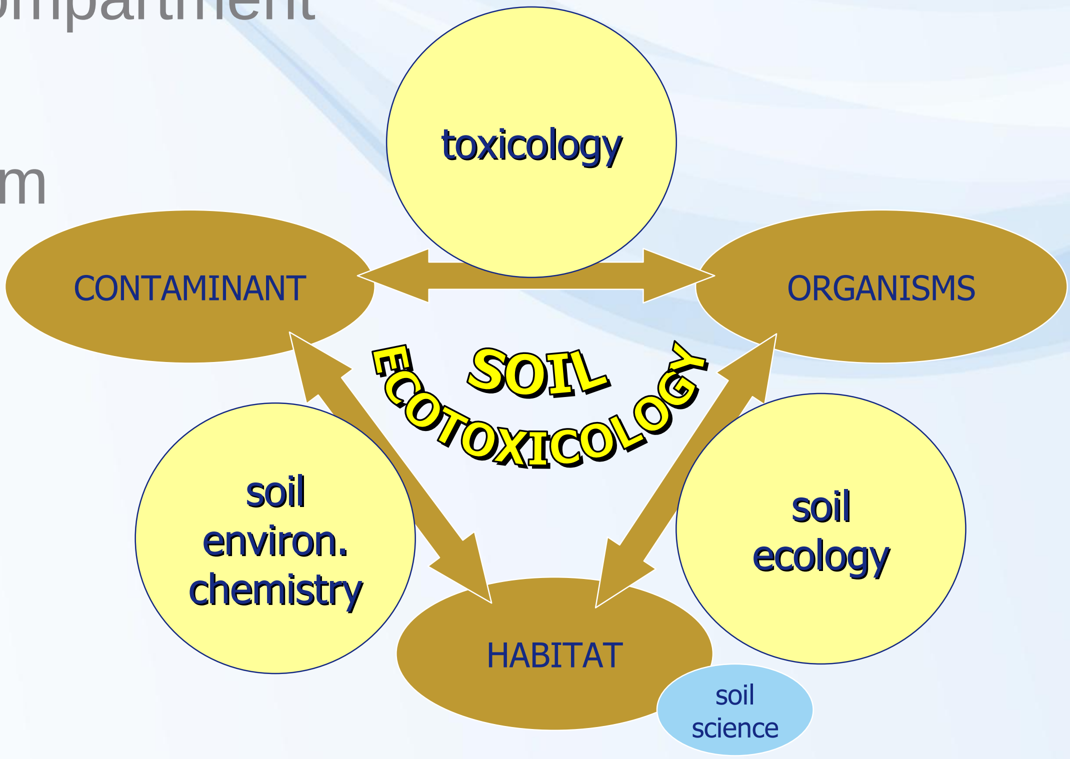




Soil ecotoxicology

FOCUS:

- soil as environmental compartment
- soil organisms
- exposure in solid medium



THE ENVIRONMENT (SOIL), WHERE RELATIONSHIP BETWEEN ORGANISM AND THE CHEMICAL EXISTS, MUST BE STUDIED TOO !!

Role of soil ecotoxicology in soil protection

FOCUS: Investigate relationships between soil organisms and contaminants

ROLE: Scientific basis of soil protection

Activities:

Provide **tools** - **bioassays** for routine praxis:

- Chemical and pesticides testing
- Testing wastes, sludge, contaminated sites
- Soil quality assessment

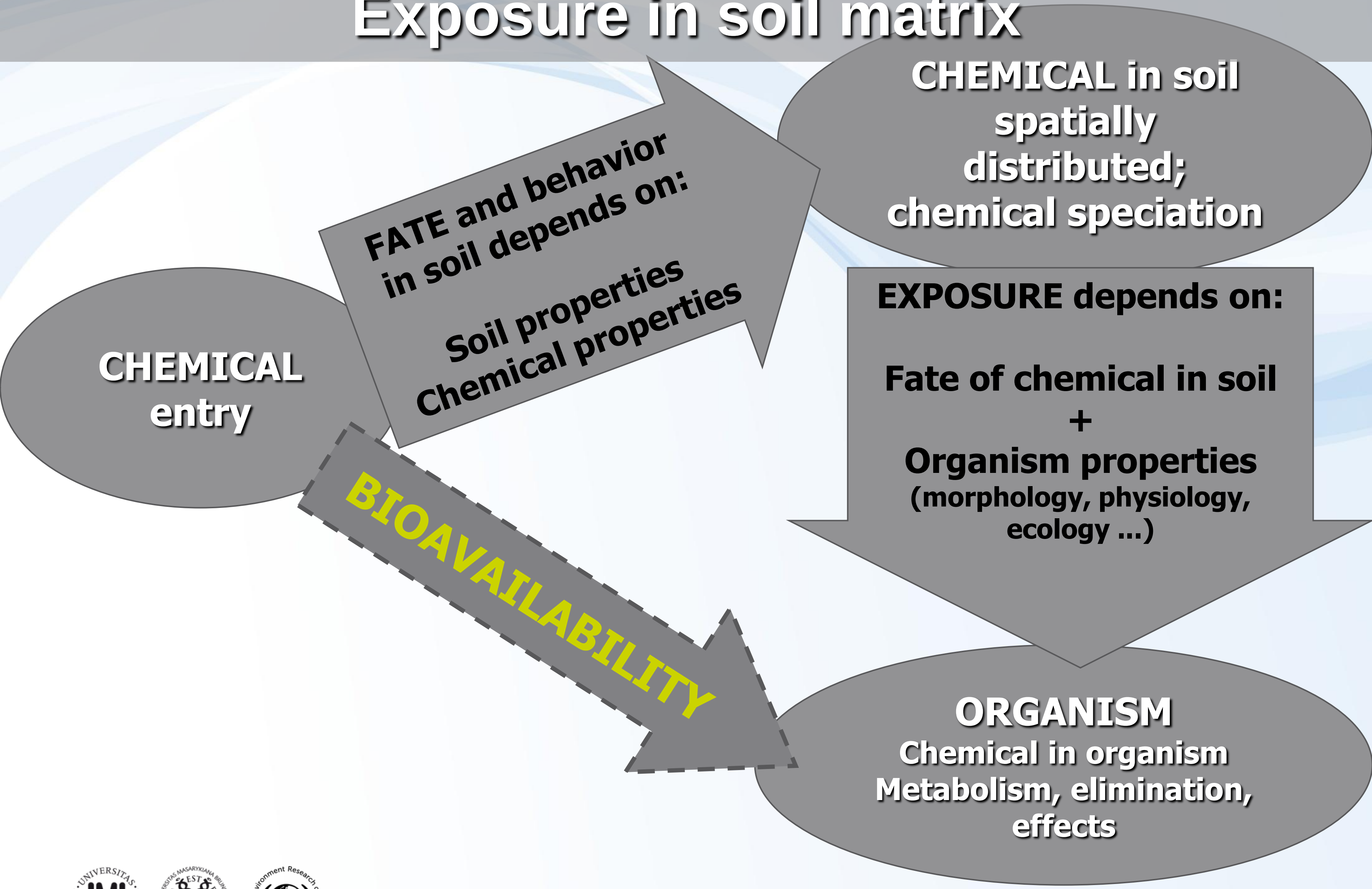
Research of:

- Fate and bioavailability
- Mixture toxicity
- Biodiversity …

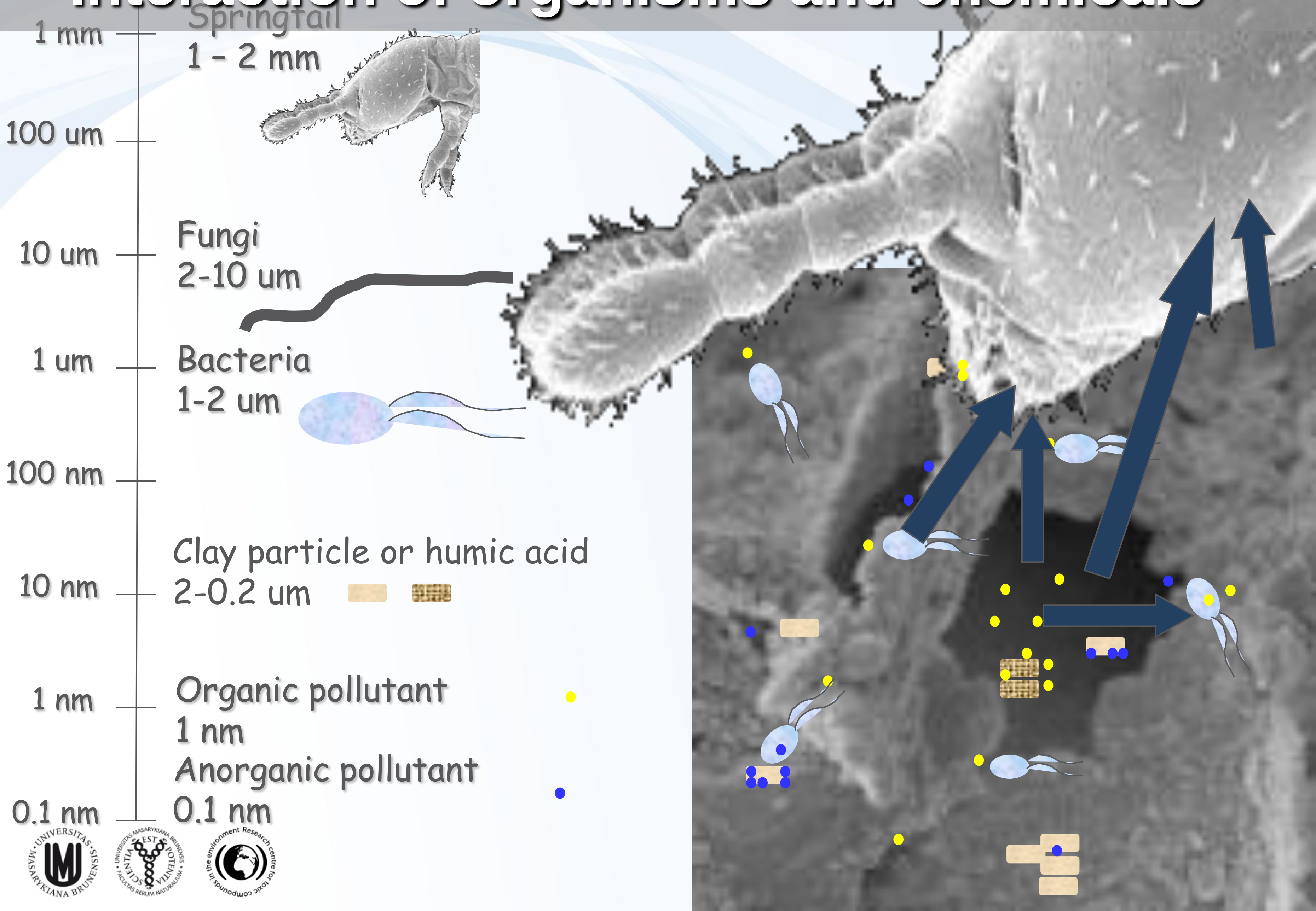
Soil environment is very different from aquatic

- Very different from aquatic ecotoxicology
- Solid matrices are heterogenous
- Soil contains all three phases SOLID, LIQUID (pore water) and GAS (soil air)
- Solid phase especially influences strongly FATE and BEHAVIOUR of chemicals
- Depending on soil and chemical properties and depending on TIME, chemical is DISTRIBUTED in soil, chemical SPECIATION occurs
- SORPTION is the crucial process and leads to changes of BIOAVAILABILITY – the key factor of soil ecotoxicology
- All this changes final TOXICITY and RISKS
- All this hampers EXTRAPOLATION possibilities

Exposure in soil matrix



Interaction of organisms and chemicals



Why to bother with bioavailability ?

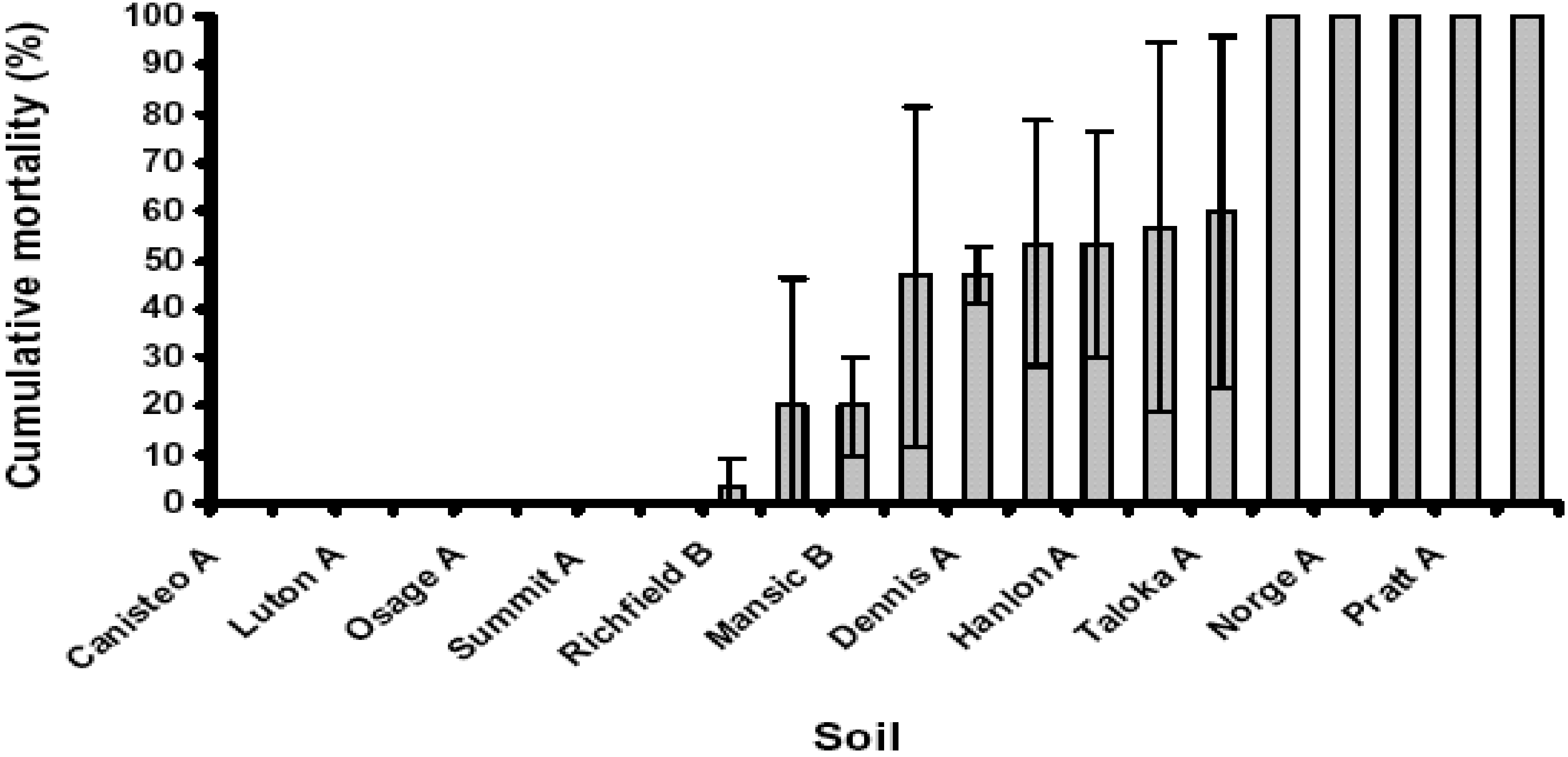
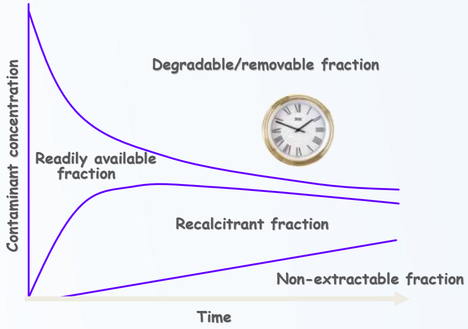


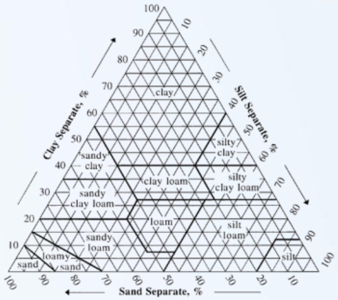
Fig. 1. Cumulative mortality (mean of three replicates, ± 95% CI) of Eisenia andrei exposed to 2,000 mg Pb/kg spiked soils for 28 days.

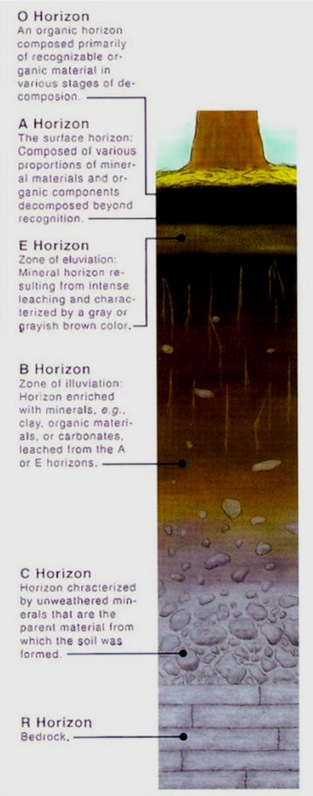
From Bradham et al. (2003)

Factors affecting bioavailability

- **Soil properties**
  - Soil composition, organic matter, texture, pH, CEC, moisture, temperature, structure - pores
- **Chemical properties**
  - Chemical structure, Kow, Sw, Koc, pKa, MW, H, pv
- **Organisms properties**
  - physiology (uptake, metabolism, elimination), morphology, ecology
- **Time effects**
  - Aging, sequestration
- **Other chemicals** (např. NAPL) and interactions







Why to bother with bioavailability ?

- For correct risk assessment:
  - Soil animals (individuals, communities)
  - Organisms eating soil (e.g. children)
  - Plants
- Prediction of biodegradation and remediation efficiencies
- Legislative framework
  - Not the total concentrations for limits!
- Extrapolation possibilities:
  - Between different soils
  - From aquatic to soil tests
  - From lab experiments to field situation

How to measure bioavailability ?

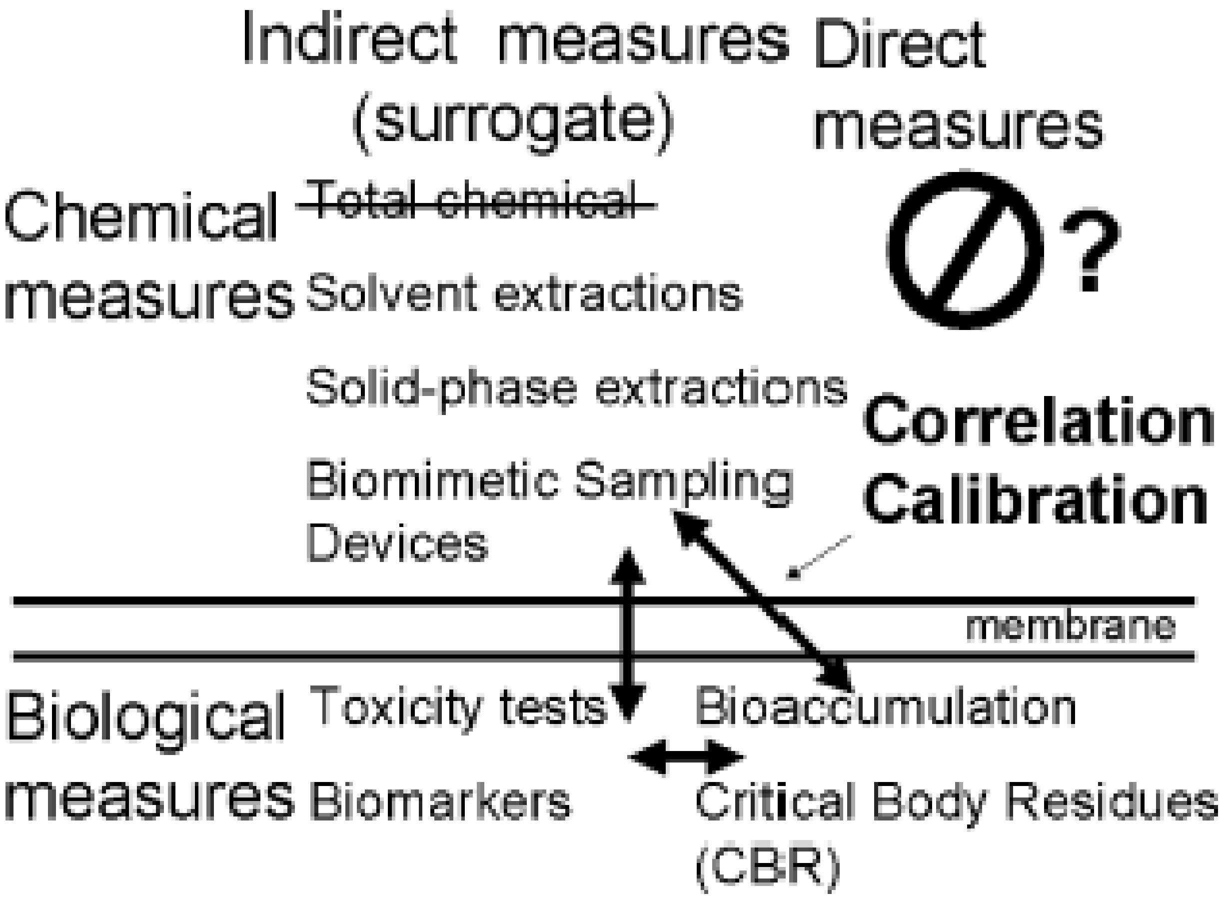


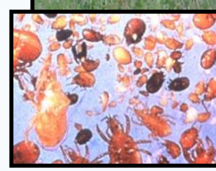
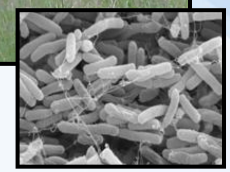
Fig. 3. Methods for measuring bioavailability.

Approaches of soil ecotoxicology

Bio-indication – (bio)monitoring



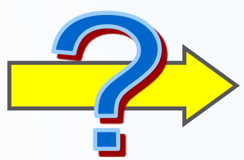
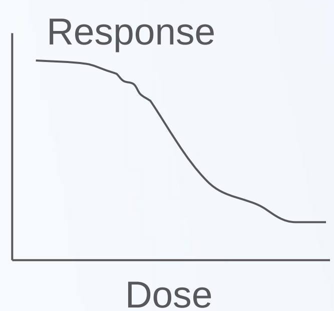


Goal

Define and describe relationship between biota conditions and contamination

Ecotoxicity bioassays





Goal

Define safe concentration, describe risks

Bioindication in soil ecotoxicology

Microbes

Soil ecosystem parameter		Microbial indicator
Function	C-cycling	Soil respiration
		Metabolic quotient (qCO_2)
		Decomposition of organic matter
		Soil enzyme activity
	N-cycling	N-mineralization
		Nitrification
		Denitrification
		N-fixation
	General activities	Bacterial DNA synthesis
		RNA measurements
		Bacterial protein synthesis
		Community growth physiology
	Root-activity	Mycorrhiza
Biodiversity	General biomass	Microbial biomass: direct methods
		Microbial biomass: indirect methods
		Microbial quotient
		Fungi
		Fungi-bacteria ratio
		Protozoa
	Biodiversity	Structural diversity
		Functional diversity
		Marker lipids
		Suppressiveness to pathogens
	Bioavailability of contaminants	Biosensor bacteria
		Plasmid-containing bacteria
		Biomarker species
		Incidence and expression of catabolic genes

Doelman, P. and Eijsackers, H.J.P. (2004): Vital Soil - Function, Value and Properties. Elsevier. 358 p. ISBN: 0-444-51772-3.

Bioindication in soil ecotoxicology

Invertebrates

Indicator system	Principle	Application	Reference
Nematode maturity index	Nematodes classified on a "colonizer" - "persister" scale	Can be applied to all soils; measures general response to stress (metals, acidification, eutrophication)	Bongers (1990), Yeates and Bongers (1999)
Predatory mite maturity index	Mesostigmatid mites classified according to an r-K score	Mostly limited to forest soils; measures soil properties related to mull/mor humus	Ruf (1998)
Earthworm life-history strategies	Earthworms classified according to position in the soil profile and burrowing behaviour	Can be applied to all soils with sufficient number of species; measures aspects of humus type, pH and cultivation (ploughing)	Bouché (1977), Paoletti (1999a)
REAL model for earthworms	Integrated data base of various aspects related to the ecological and agronomical role of earthworms	Very wide application	Bouché (1996)
Enchytraeid Reaktionzahl	Scores related to responses to acidity and humidity assigned to enchytraeids	Applicable to situations where effects on soil pH are manifested, for example cement factories	Graefe (1993), Beylich et al. (1995)
SIVPACS	Pollution responses of earthworms, isopods and spiders, comparable to RIVPACS	Data base on species-specific responses not yet operational; at the moment only applied to heavy-metal pollution	Spurgeon et al. (1996)
Woodlice life-forms	Classification of woodlice according to body shape and movement pattern	Composition of isopod fauna indicates effects of soil cultivation in agricultural landscapes	Paoletti and Hassell (1999)

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Bioindication in soil ecotoxicology

Invertebrates

Indicator system	Principle	Application	Reference
Macro invertebrate biodiversity	Enumeration of species richness of earthworms, beetles, isopods, spiders, ants, millipedes, centipedes, etc.	Applied in orchards and other agricultural ecosystems to indicate land use and copper pollution	Paoletti and Somaggio (1996), Paoletti (1999b)
Ant functional groups	Classification of ants according to groups reflecting susceptibility to stress	Wide application; used in evaluation of nature restoration and effects of mining	Andersen (1995)
Diptera feeding groups	Classification of dipteran larvae in five feeding groups	Reflects type of organic materials in soil; applicable to organic soils	Frouz (1999)
Arthropod acidity index	Classification of arthropods (Collembola, oribatids, isopods) according to pH preference	Allows quantitative estimation of soil pH from invertebrate community structure	Van Straalen and Verhoef (1997), Van Straalen (1998)
Oribatid mite life-history strategies	Classification of mites according to reproductive and dispersal strategies	Indicates intensity of anthropogenic influence and successional stage of forests and grassland ecosystems	Siepel (1994), Siepel (1996)
Life-forms of Collembola	Classification of Collembola according to morphological types reflecting position in the soil profile	Indicates profile build-up and ecological processes stratified according to the profile; mostly applicable to forest soils	Van Straalen et al. (1985), Faber (1991)
Dominance distribution of micro arthropods	Lognormal distribution of numbers over species	General impression of disturbance; applied to effects of heavy metals and acid rain in forest and grassland soils	Hågvar (1994)
Biological Index of Soil Quality (BSQ)	System of scores assigned to groups of soil micro arthropods	Provides indication of biodiversity; wide applicability	Parisi (2001), Gardi et al. (2002)

Doelman, P. and Eijsackers, H.J.P. (2004): Vital Soil - Function, Value and Properties. Elsevier. 358 p. ISBN: 0-444-51772-3.

Bioindication in soil ecotoxicology

Pollutant group	Vulnerable animal groups	Remarks
Polycylic aromatic hydrocarbons, azaarenes and derivates	Isopods, Collembola	Little knowledge available. Large inter-species differences in metabolism. Metabolizers expected to be more sensitive than accumulators.
Persistent organochlorines (PCBs, dioxins)	Vertebrates (Rodentia and Insectivora)	Low toxicity to invertebrates. Effects appear higher up in the food-chain Earthworms are important in transfer.
Chlorinated ethylenes, phenoles and benzenes	Earthworms	Toxicity due to general narcotic effects, probably small inter-species differences.
Oil, BTEX	Earthworms	Toxicity partly due to changes in soil structure.
Alkyl benzene sulfonates and other detergents	Enchytraeids, nematodes, earthworms	Field data scanty. Laboratory data suggest highest toxicity to pore water-dependent species.
Veterinary drugs, antibiotics, hormones	No data available	Interactions in decomposer-micro-organism interactions expected, but not documented.
Copper	Earthworms, slugs, snails, oribatid mites	Copper toxicity to earthworms well documented.
Zinc	Enchytraeids, nematodes, earthworms, isopods, soft-bodied springtails	Toxicity of zinc does not follow the main taxonomic groups of soil invertebrates. Many groups contain sensitive as well as tolerant species.
Cadmium	Oribatid mites, spiders, some springtails, vertebrates (shrews, mole)	Cadmium seems to be most toxic to invertebrates that take up the metal with the food. Due to food-chain accumulation effects appear in predators and vertebrates.
Lead	Oribatid mites, shrews, mole	Differences between invertebrate species relatively small. Main hazard of lead is higher up in the food chain.
Herbicides	No group in particular	Low toxicity of modern herbicides to animals. Effects are mostly secondary (avoidance of sprayed leaves, loss of food, increase of litter cover).
Fungicides	Earthworms, enchytraeids, isopods	Benzimidazoles, carbamates and organotins are known for their considerable side-effects on animals

Invertebrates

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Pollutant group	Vulnerable animal groups	Remarks
Insecticides	Many arthropod groups, in particular beetles, spiders, mesostigmatid mites and springtails	Animals with high surface activity are particularly vulnerable. Large differences between species due to species-specific exposures and metabolic capacities. Many secondary effects among detritivores due to suppression of predators.
Acidic precipitation	Snails, dipteran larvae, earthworms, some oribatid mites, some Collembola, some isopods	Large differences between species within each group. Earthworms generally avoid acid soils. Many Collembola and mites are acid tolerant, but some are very alkalophilic and suffer from acid precipitation.
Radiation	Earthworms, oribatid mites	Species-specific vulnerability due to exposure, rather than inherent differences in sensitivity. Permanent soil dwellers and soil ingesters receive high doses.

Note: this table only describes the general trends and ignores the many species-specific sensitivities related to metabolism, microhabitat choice and life-cycle

Use of soil bioassays in soil protection

- Sofar, mostly for **assessment of hazard of chemicals and pesticides**

- Increase of use for **evaluation of hazard of complex mixtures** like wastes, sewage sludge, sediments, composts, fertilizers …

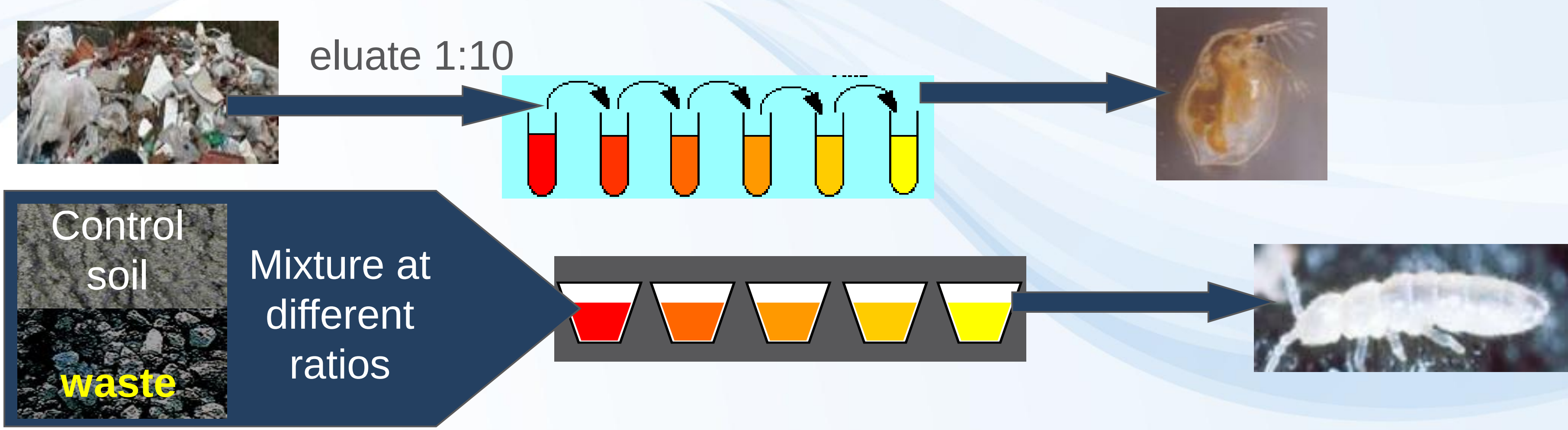
- Great potential in the future for **assessment of soil quality** e.g. Before and after the remediation, contaminated sites assessment etc.

Why bioassays?

Chemical analyses are not able to identify risks properly because:

1) Real exposure is different – bioavailability in particular situation
2) Pollutant mixture - always in real ecosystems
3) Matrix itself has effects or interacts with effects of contaminants
4) Anylytical methods are limited vs. Wide spectrum of possibly toxic chemicals

Why soil bioassays?

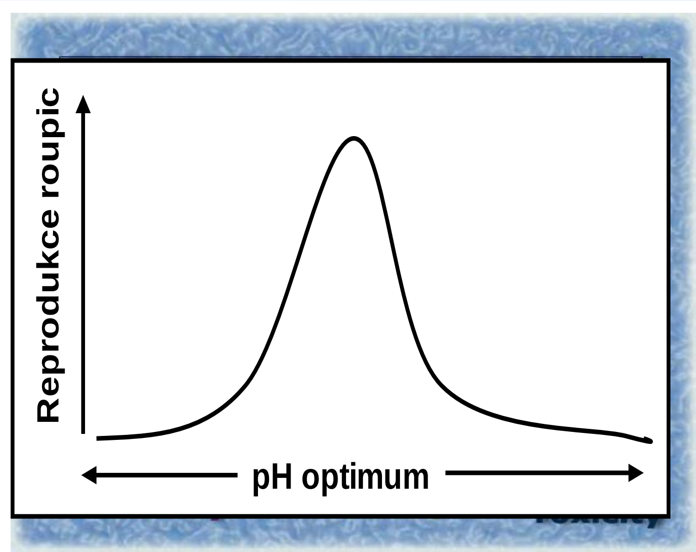


Eluate tests are not able to predict solid phase exposure

WHY ?

→ real bioavailability

→ effect of matrix involved



Are soil bioassays used now ?

Chemicals

EU: COMMISSION DIRECTIVE 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances; Annex V. (earthworms, plants) **+ REACH!!!**

USA: TSCA; OPPTS (The Office of Prevention, Pesticides and Toxic Substances) (earthworms, microbes, plants)

OECD: Guidelines for the testing of chemicals (many ……)

ISO: many ……..

Plant protection products

EU: COUNCIL DIRECTIVE 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market; Annex II.

USA: TSCA - OPPTS (The Office of Prevention, Pesticides and Toxic Substances)

OECD: Guidelines for the testing of chemicals

ISO + EPPO, IOBC, SETAC, BBA

Bioassay standards - OECD

Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	**2006**
Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test	2006
Test No. 207: Earthworm, Acute Toxicity Tests	**1984**
Test No. 220: Enchytraeid Reproduction Test	**2004**
Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)	**2004**
Test No. 228: Determination of Developmental Toxicity of a Test Chemical to Dipteran Dung Flies(Scathophaga stercoraria L. (Scathophagidae), Musca autumnalis De Geer (Muscidae))	2008
Test No. 232: Collembolan Reproduction Test in Soil	**2009**
Test No. 226: Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil	2008
Test No. 216: Soil Microorganisms: Nitrogen Transformation Test	**2000**
Test No. 217: Soil Microorganisms: Carbon Transformation Test	**2000**
Test No. 317: Bioaccumulation in Terrestrial Oligochaetes	**2010**
Test No. 304A: Inherent Biodegradability in Soil	1981

http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html

Bioassay standards - ISO

ISO 10381-6:2009	Soil quality -- Sampling -- Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory
ISO 14240-1:1997	Soil quality -- Determination of soil microbial biomass -- Part 1: Substrate-induced respiration method
ISO 14240-2:1997	Soil quality -- Determination of soil microbial biomass -- Part 2: Fumigation-extraction method
ISO 16072:2002	Soil quality -- Laboratory methods for determination of microbial soil respiration
ISO 17155:2002	Soil quality -- Determination of abundance and activity of soil microflora using respiration curves
ISO 15685:2004	Soil quality -- Determination of potential nitrification and inhibition of nitrification -- Rapid test by ammonium oxidation
ISO 14238:1997	Soil quality -- Biological methods -- Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes
ISO 23753-1:2005	Soil quality -- Determination of dehydrogenase activity in soils -- Part 1: Method using triphenyltetrazolium chloride (TTC)
ISO 23753-2:2005	Soil quality -- Determination of dehydrogenase activity in soils -- Part 2: Method using iodotetrazolium chloride (INT)
ISO/DIS 11063	Soil quality -- Method to directly extract DNA from soil samples
ISO/TS 29843-1:2010	Soil quality -- Determination of soil microbial diversity -- Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis
ISO/PRF TS 29843-2	Soil quality -- Determination of soil microbial diversity -- Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method
ISO/TS 10832:2009	Soil quality -- Effects of pollutants on mycorrhizal fungi -- Spore germination test
ISO/TS 22939:2010	Soil quality -- Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates
ISO 11266:1994	Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions
ISO 15473:2002	Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions
ISO 14239:1997	Soil quality -- Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions

Bioassay standards - ISO

ISO 11269-1:1993	Soil quality -- Determination of the effects of pollutants on soil flora -- Part 1: Method for the measurement of inhibition of root growth
ISO 11269-2:2005	Soil quality -- Determination of the effects of pollutants on soil flora -- Part 2: Effects of chemicals on the emergence and growth of higher plants
ISO 17126:2005	Soil quality -- Determination of the effects of pollutants on soil flora -- Screening test for emergence of lettuce seedlings (Lactuca sativa L.)
ISO 22030:2005	Soil quality -- Biological methods -- Chronic toxicity in higher plants
ISO/CD 29200	Soil quality -- Assessment of genotoxic effects on higher plants -- Micronucleus test on Vicia faba

Bioassay standards - ISO

ISO 11268-1:1993	Soil quality -- Effects of pollutants on earthworms (Eisenia fetida) -- Part 1: Determination of acute toxicity using artificial soil substrate
ISO 11268-2:1998	Soil quality -- Effects of pollutants on earthworms (Eisenia fetida) -- Part 2: Determination of effects on reproduction
ISO 11268-3:1999	Soil quality -- Effects of pollutants on earthworms -- Part 3: Guidance on the determination of effects in field situations
ISO 11267:1999	Soil quality -- Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants
ISO 16387:2004	Soil quality -- Effects of pollutants on Enchytraeidae (Enchytraeus sp.) -- Determination of effects on reproduction and survival
ISO 15952:2006	Soil quality -- Effects of pollutants on juvenile land snails (Helicidae) -- Determination of the effects on growth by soil contamination
ISO 20963:2005	Soil quality -- Effects of pollutants on insect larvae (Oxythyrea funesta) -- Determination of acute toxicity
ISO 17512-1:2008	Soil quality -- Avoidance test for determining the quality of soils and effects of chemicals on behaviour -- Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei)
ISO/DIS 17512-2	Soil quality -- Avoidance test for determining the quality of soils and effects of chemicals on behaviour -- Part 2: Test with collembolans (Folsomia candida)
ISO 23611-1:2006	Soil quality -- Sampling of soil invertebrates -- Part 1: Hand-sorting and formalin extraction of earthworms
ISO 23611-2:2006	Soil quality -- Sampling of soil invertebrates -- Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)
ISO 23611-3:2007	Soil quality -- Sampling of soil invertebrates -- Part 3: Sampling and soil extraction of enchytraeids
ISO 23611-4:2007	Soil quality -- Sampling of soil invertebrates -- Part 4: Sampling, extraction and identification of soil-inhabiting nematodes
ISO/DIS 23611-5	Soil quality -- Sampling of soil invertebrates -- Part 5: Sampling and extraction of soil macro-invertebrates
ISO/DIS 23611-6	Soil quality -- Sampling of soil invertebrates -- Part 6: Guidance for the design of sampling programmes with soil invertebrates

Bioassay standards – US EPA

850.2450 - Terrestrial (Soil-Core) Microcosm Test (PDF) (19 pp, 123K)
850.4000 - Background-Nontarget Plant Testing (PDF) (15 pp, 50K)
850.4025 - Target Area Phytotoxicity (PDF) (15 pp, 51K)
850.4100 - Terrestrial Plant Toxicity, Tier I (Seedling Emergence) (PDF) (8 pp, 29K)
850.4150 - Terrestrial Plant Toxicity, Tier I (Vegetative Vigor) (PDF) (8 pp, 28K)
850.4200 - Seed Germination/Root Elongation Toxicity Test (PDF) (8 pp, 29K)
850.4225 - Seedling Emergence, Tier II (PDF) (10 pp, 36K)
850.4230 - Early Seedling Growth Toxicity Test (PDF) (9 pp, 33K)
850.4250 - Vegetative Vigor, Tier II (PDF) (10 pp, 35K)
850.4300 - Terrestrial Plants Field Study, Tier III (PDF) (8 pp, 27K)
850.4600 - Rhizobium-Legume Toxicity (PDF) (14 pp, 73K)
850.4800 - Plant Uptake and Translocation Test (PDF) (13 pp, 35K)
850.5100 - Soil Microbial Community Toxicity Test (PDF) (11 pp, 46K)
850.6200 - Earthworm Subchronic Toxicity Test (PDF) (13 pp, 43K)

http://www.epa.gov/ocspp/pubs/frs/home/draftguidelines.htm

Solid material toxicity testing

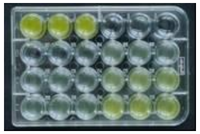
ISO 15799 (2003): Guidance on the ecotoxicological characterization of soils and soil materials
ISO 17616 (2008): Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials

Bioassays for soil like matrices

Retention function – Biotests with eluates

Ecotoxic contents

Luminescent bacteria test



Algal inhibition test

Genotoxic contents

Umu-test

Habitat function - Biotests with solids

Site inherent test organisms

Respiration test

Nitrification test

Added test organisms

Bacteria contact test

Earthworm avoidance test

Plant test

Earthworm test

Collembolan test

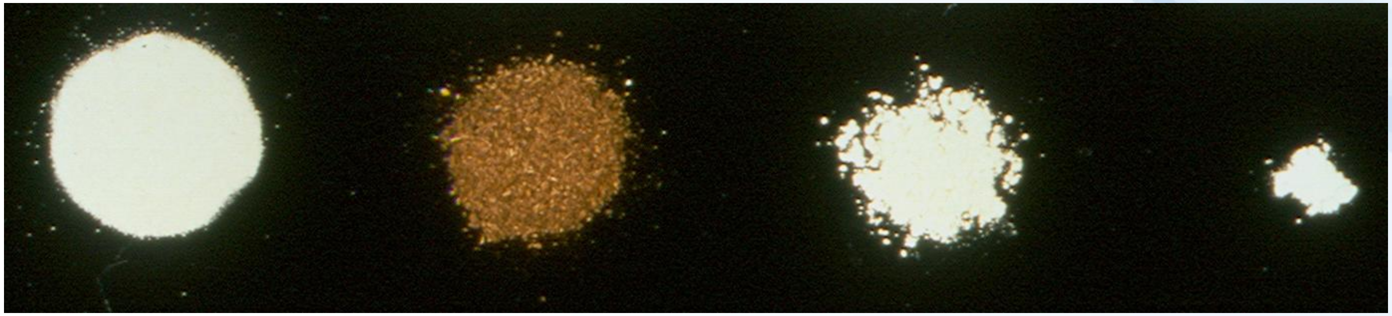
EU – test battery for wastes

	ISO 11268-1 (1997): Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*). Part 1: Determination of acute toxicity using artificial soil substrate.
	ISO 11269-2 (2004): Soil quality – Determination of the effects of pollutants on soil flora. Part II: Effects of chemicals on the emergence and growth of higher plants.

	ISO 16387 (2004): Soil quality - Effects of pollutants on *Enchytraeidae* - Determination of effects on reproduction and survival.
	ISO 11267 (1999): Soil quality - Inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil pollutants
	ISO 11268-2 (1998): Soil Quality - Effects of pollutants on earthworms (*Eisenia fetida*). Part 2: Determination of effects on reproduction
	ISO 17512-1 (2008): Soil Quality – Avoidance test for evaluating the quality of soils and the toxicity of chemicals. Test with Earthworms (*Eisenia fetida/andrei*).

Exposure methods

- **Tested chemical mixed with soil**
  - **Artificial soil (OECD, ISO)**
  - **Real soil (LUFA 2.2 …)**



- Topic applications, injections, forced feeding … not so relevant

What is artificial soil ?

Soil component	Content expressed on % dry mass basis
• Sphagnum peat (air dried), finely ground and with no visible plant remains	10
• Kaolinite clay (air dried), containing not less than 30 % kaolinite	20
• Industrial quartz sand (air dried), predominantly fine sand with more than 50 % by mass of particle size 0,05-0,2 mm (amount dependent on calcium carbonate required)	70
• Calcium carbonate ($CaCO_3$, pulverised, analytical grade) to obtain an initial pH of 6.0 ± 0.5	0.3-1.0

OECD 1984. Guideline for testing chemicals 207. Earthworm acute toxicity test.

- **Is standard medium for many soil bioassays …**
- **Is much more relevant than solution, agar, filter paper …**
- **Should solve problem of high variability of natural soils …**
- **Should resemble natural loamy soil …**
- **Should enable the toxicity extrapolation to natural soils …**

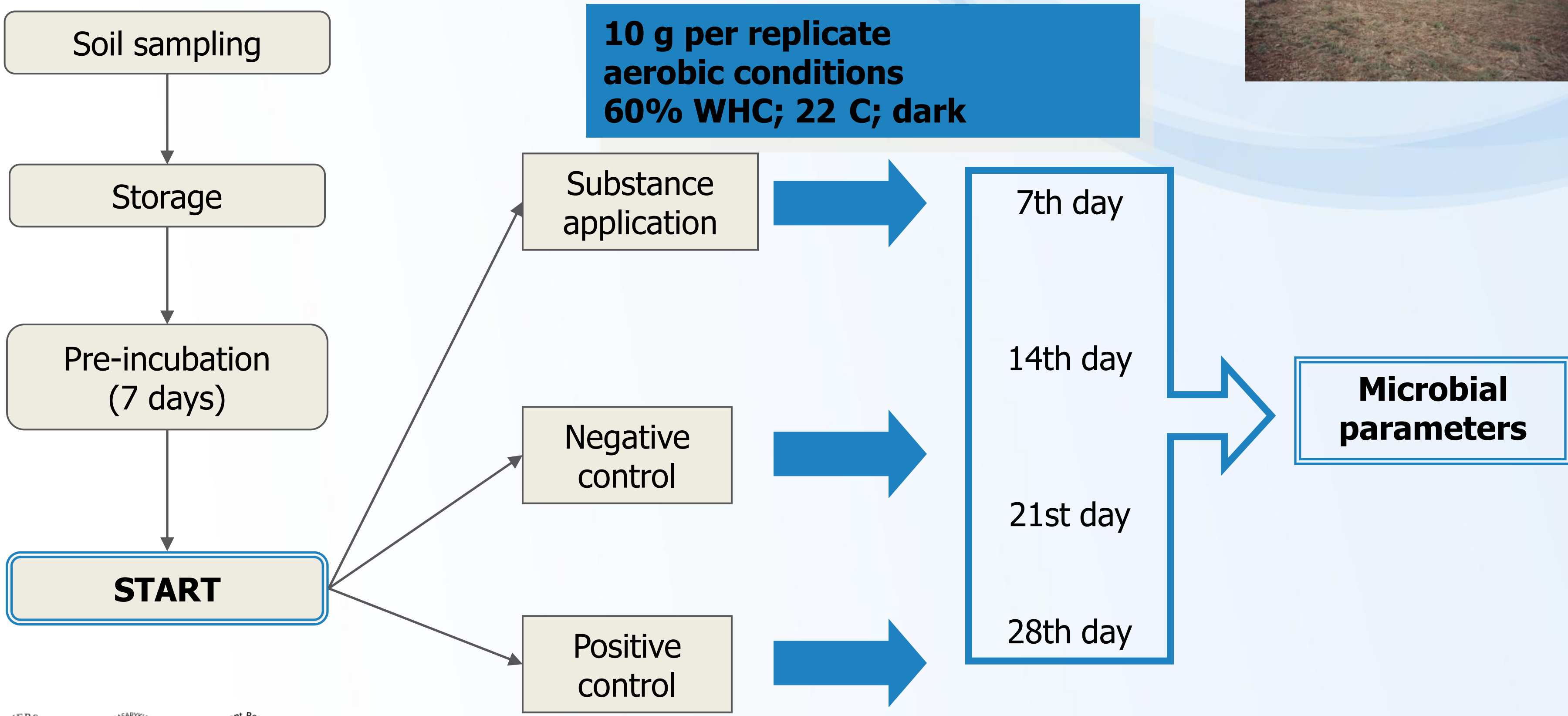
Soil microbial assays

- EPA (1996): OPPTS 850.5100 Soil microbial community toxicity test. Ecological effects test guidelines. United States Environmental Agency.
- EPPO (1994): Decision making scheme for the environmental risk assessment of plant protection products. EPPO Bulletin 24, Chapter 7, Soil Microflora.
- Lynch, M.R. (1995): Procedures for assessing the environmental fate and ecotoxicity of pesticides. SETAC, Brussels, Belgium.
- OECD (1999): Proposal for a new guideline 217. Soil microorganisms: Carbon transformation test. OECD guideline for the testing of chemicals. OECD.
- OECD (1999): Proposal for a new guideline 216. Soil microorganisms: Nitrogen transformation test. OECD guideline for the testing of chemicals. OECD.
- ISO 14238 (1997): Soil quality - Determination of nitrogen mineralization and nitrification in soil and the influence of chemicals on these processes. International Organization for Standardization. Geneve, Switzerland.

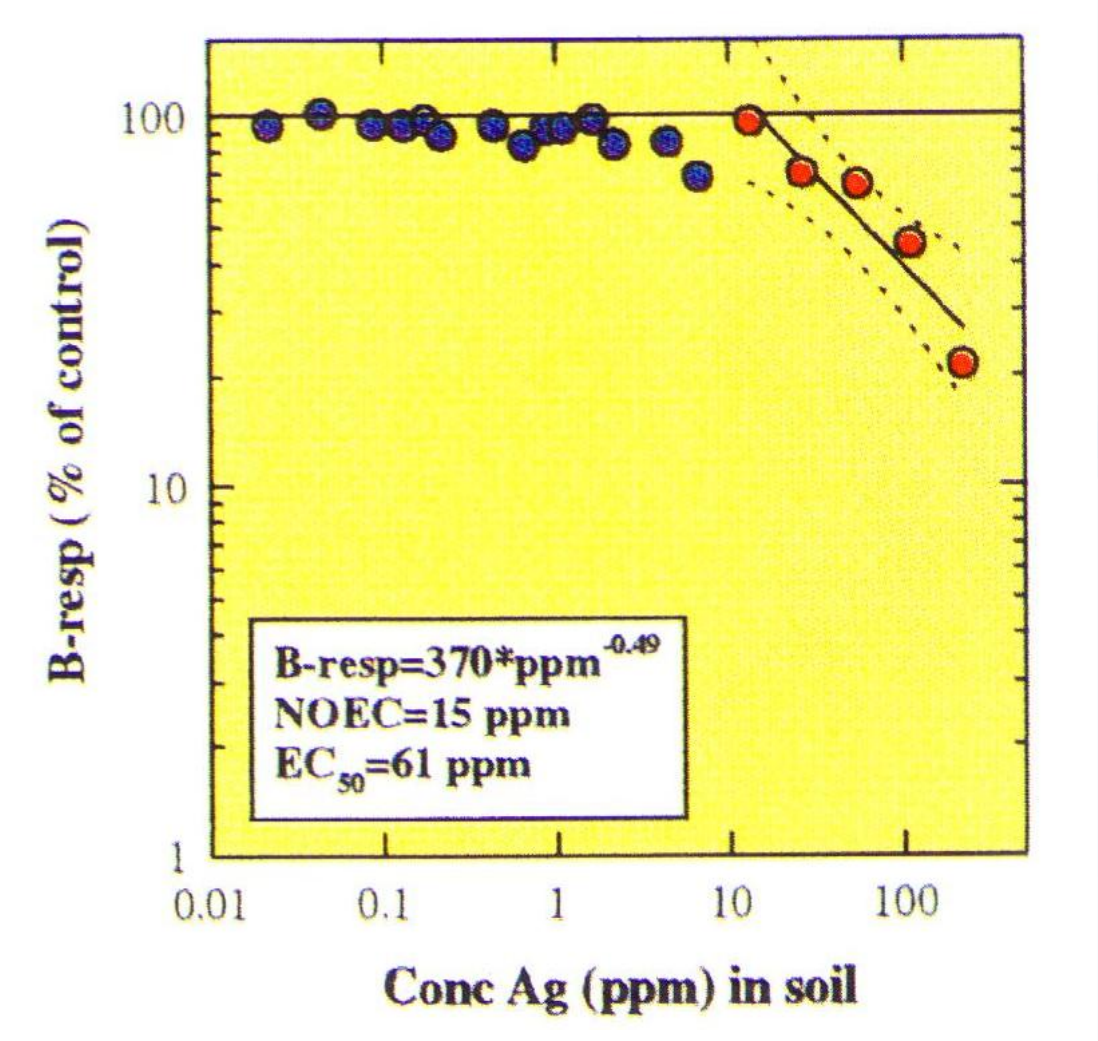
Soil microbial assay according to OECD, ISO

Real uncontaminated agricultural soil with indigenous microflora:

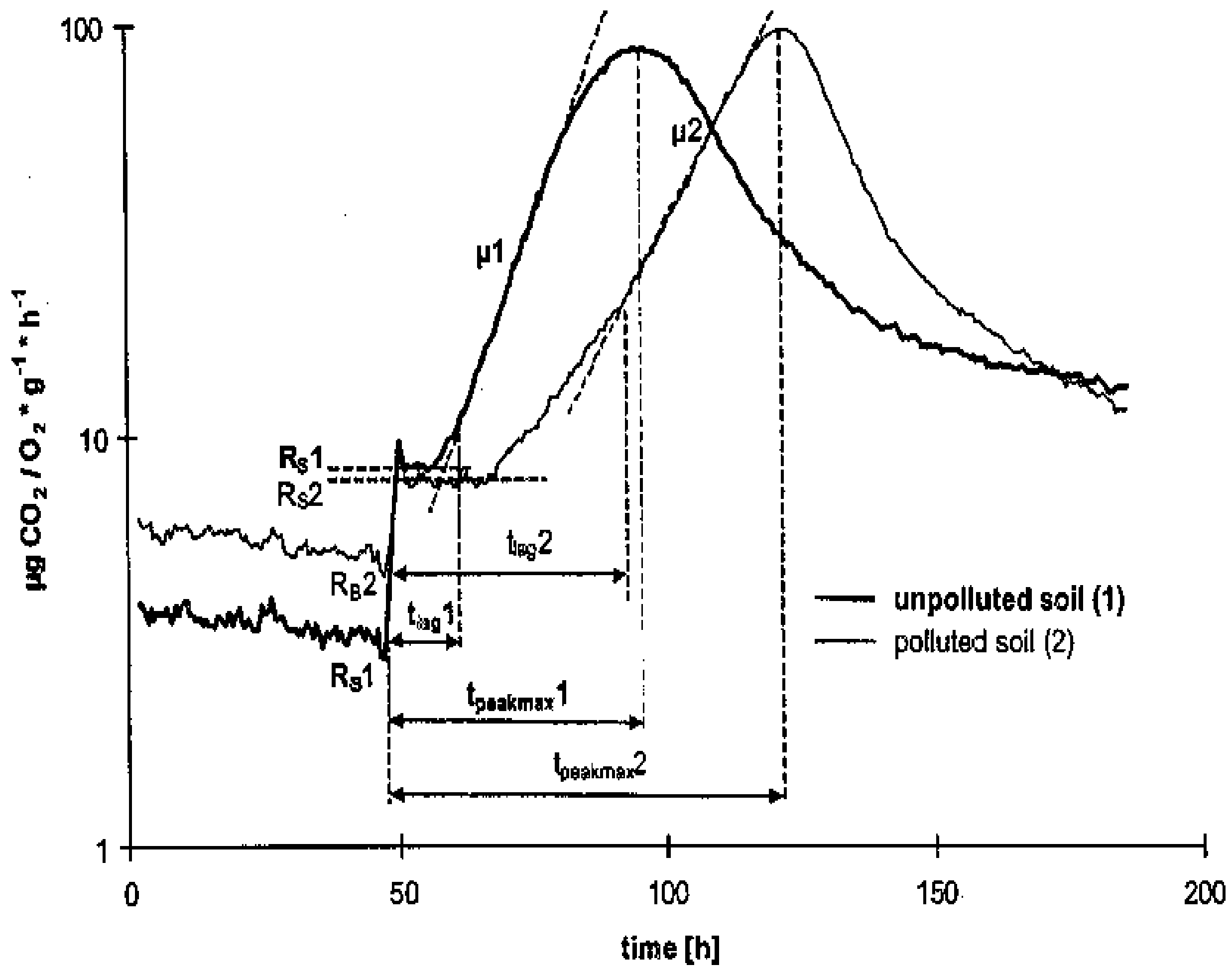
pH_{KCl} = 7 – 7.5 C_{bio} = 400 – 700 μg.$g_{d.w.}^{-1}$
C_{org} = 1.5%   BR = 0.5 – 0.7 μg CO_2-C.h^{-1}.$g_{d.w.}^{-1}$
sand = 70%



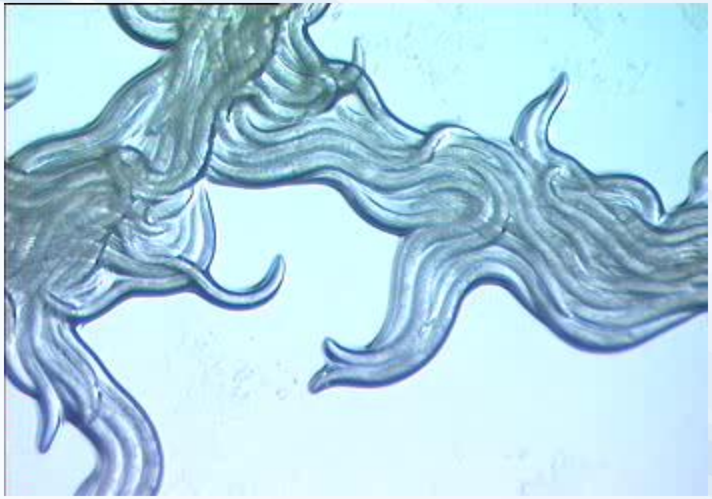
Effects on microbial respiration

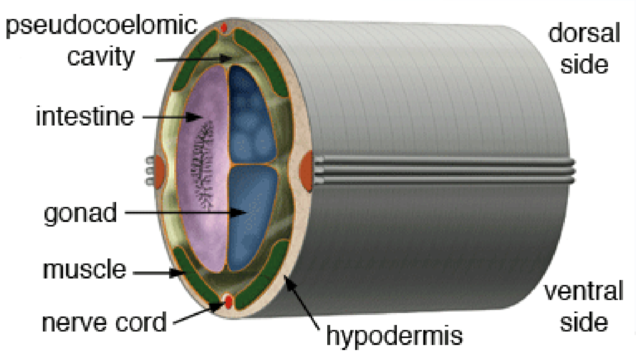


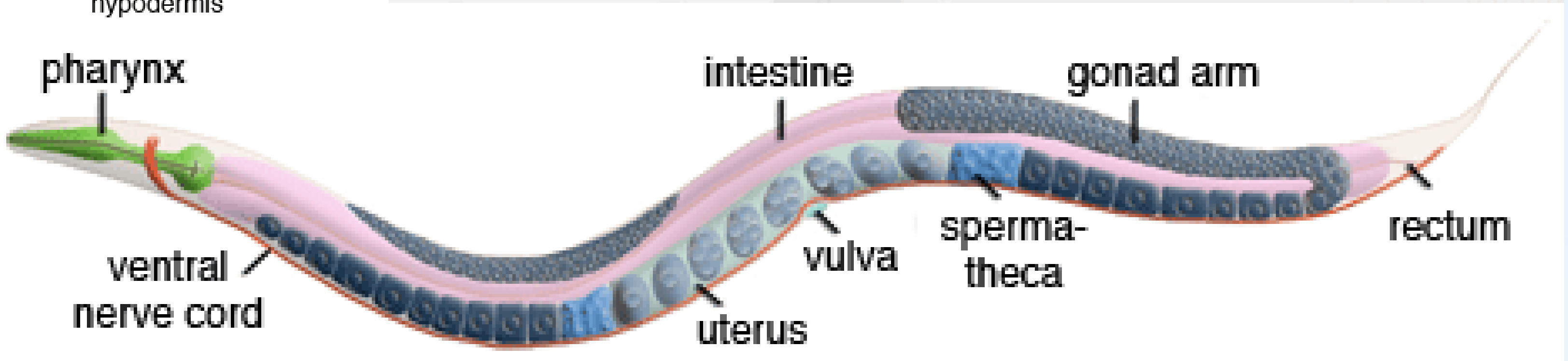
Respirometry



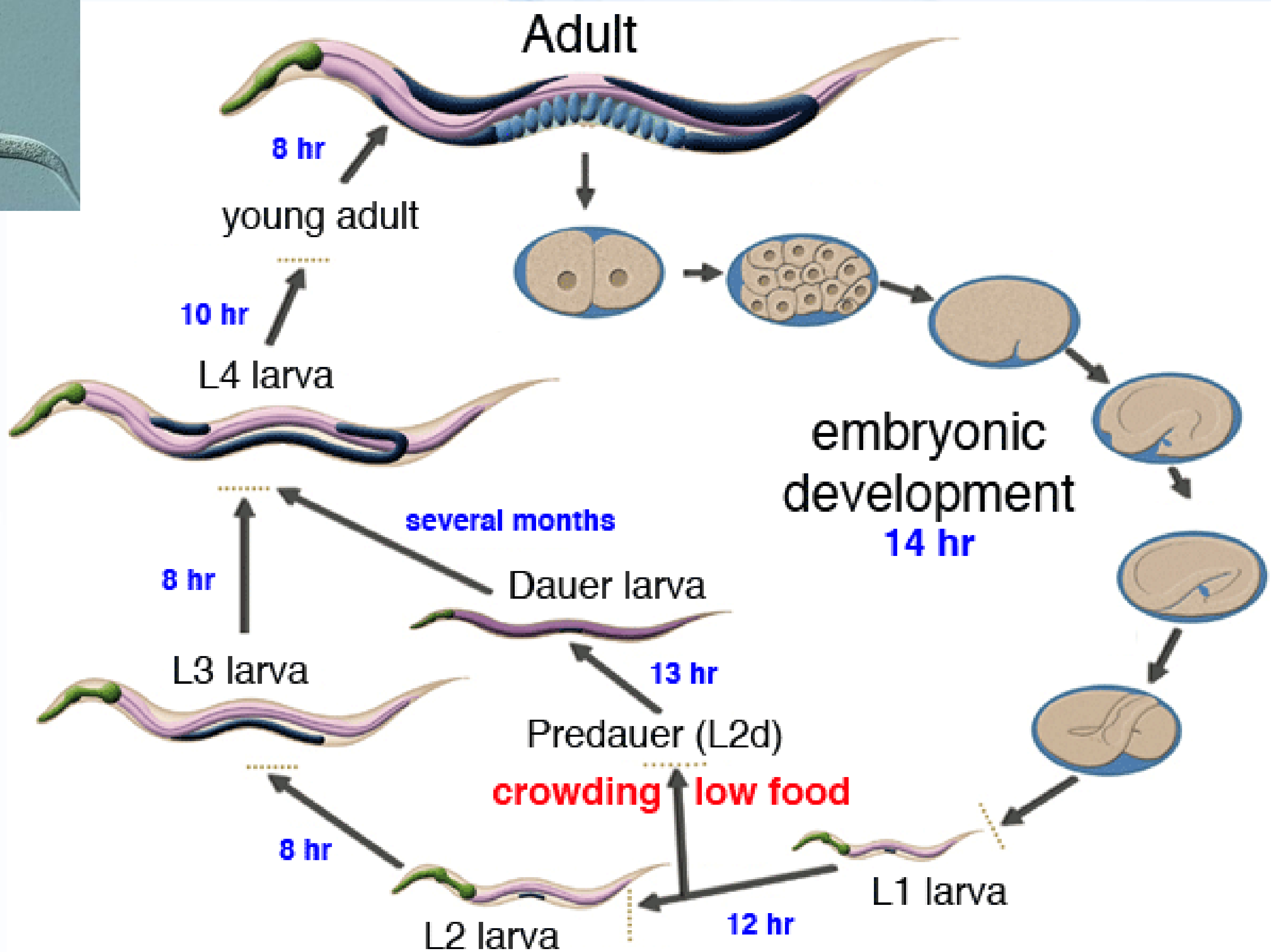
Nematodes




pseudocoelomic cavity
intestine
gonad
muscle
nerve cord
hypodermis
dorsal side
ventral side

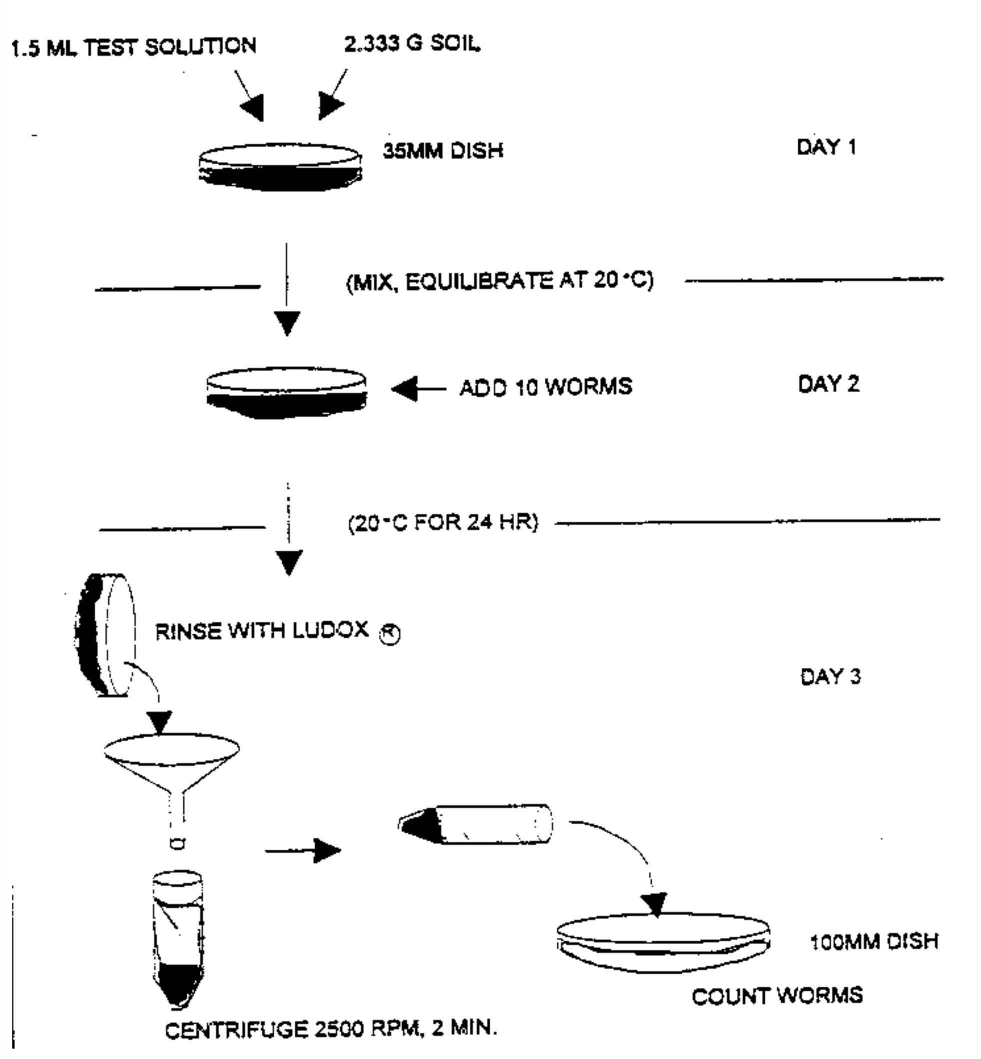

pharynx
intestine
gonad arm
ventral nerve cord
uterus
vulva
sperma-theca
rectum

Caenorhabditis elegans



Caenorhabditis elegans

ASTM: E2172-01 Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode *Caenorhabditis elegans*



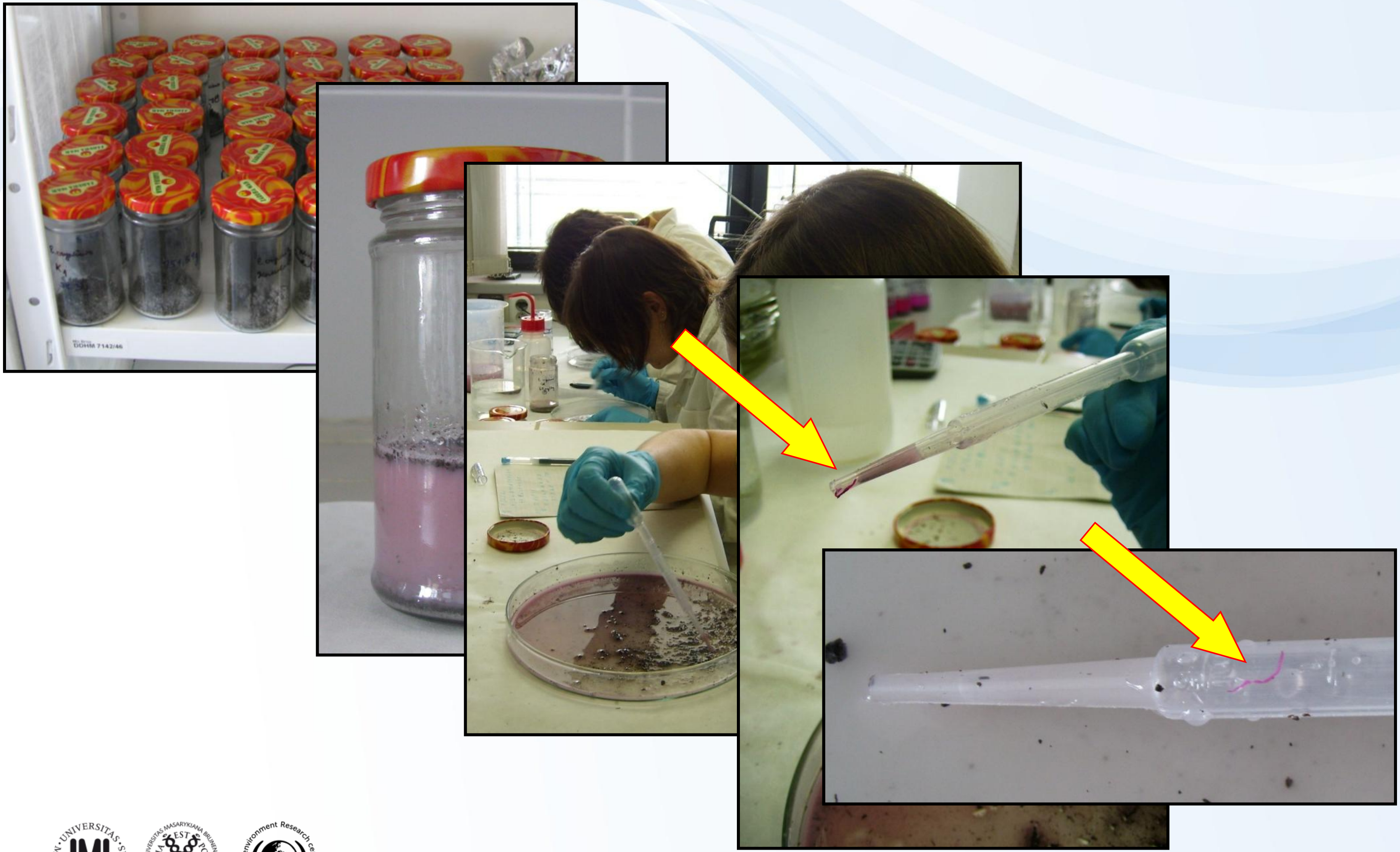
Enchytraeidae



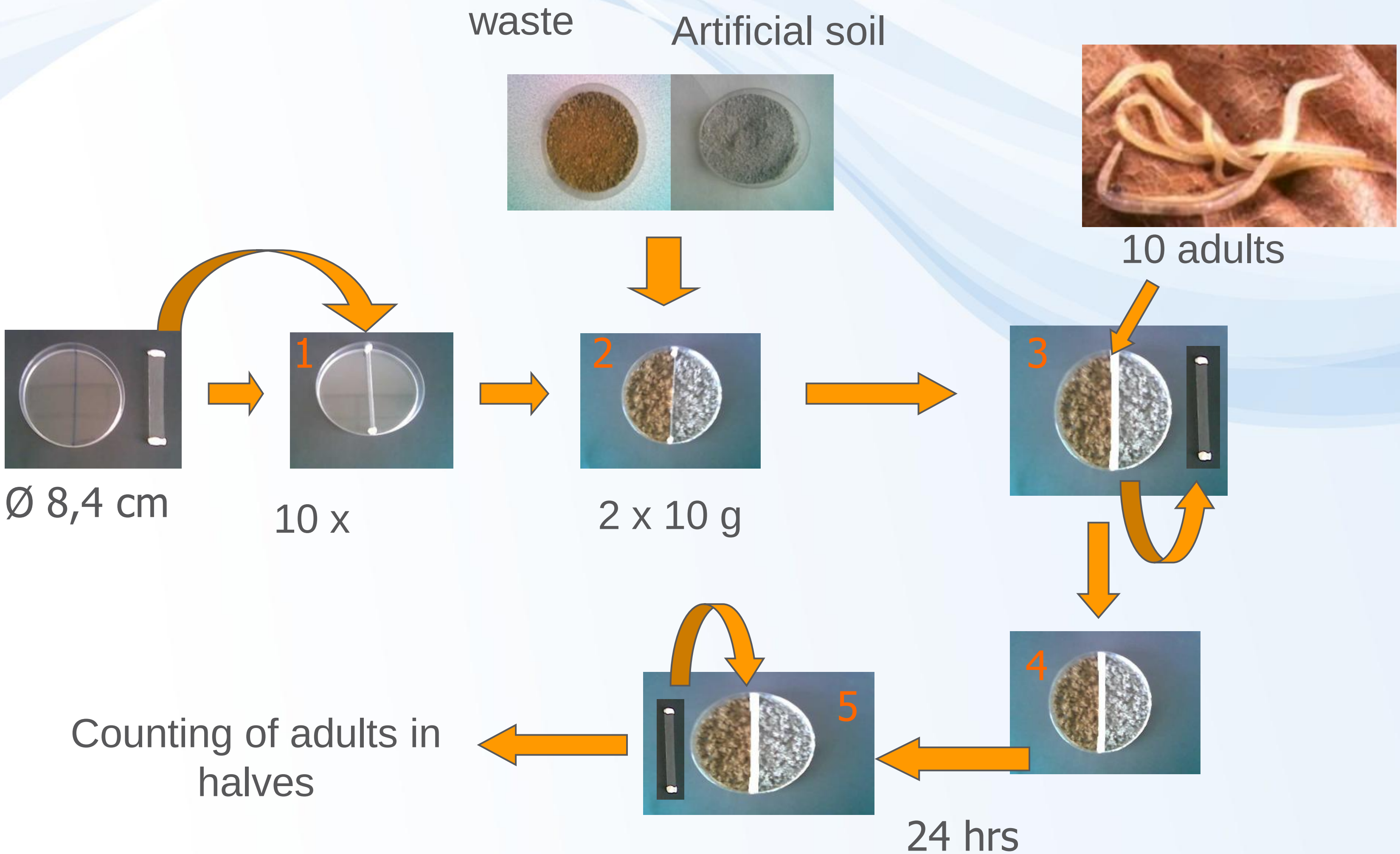




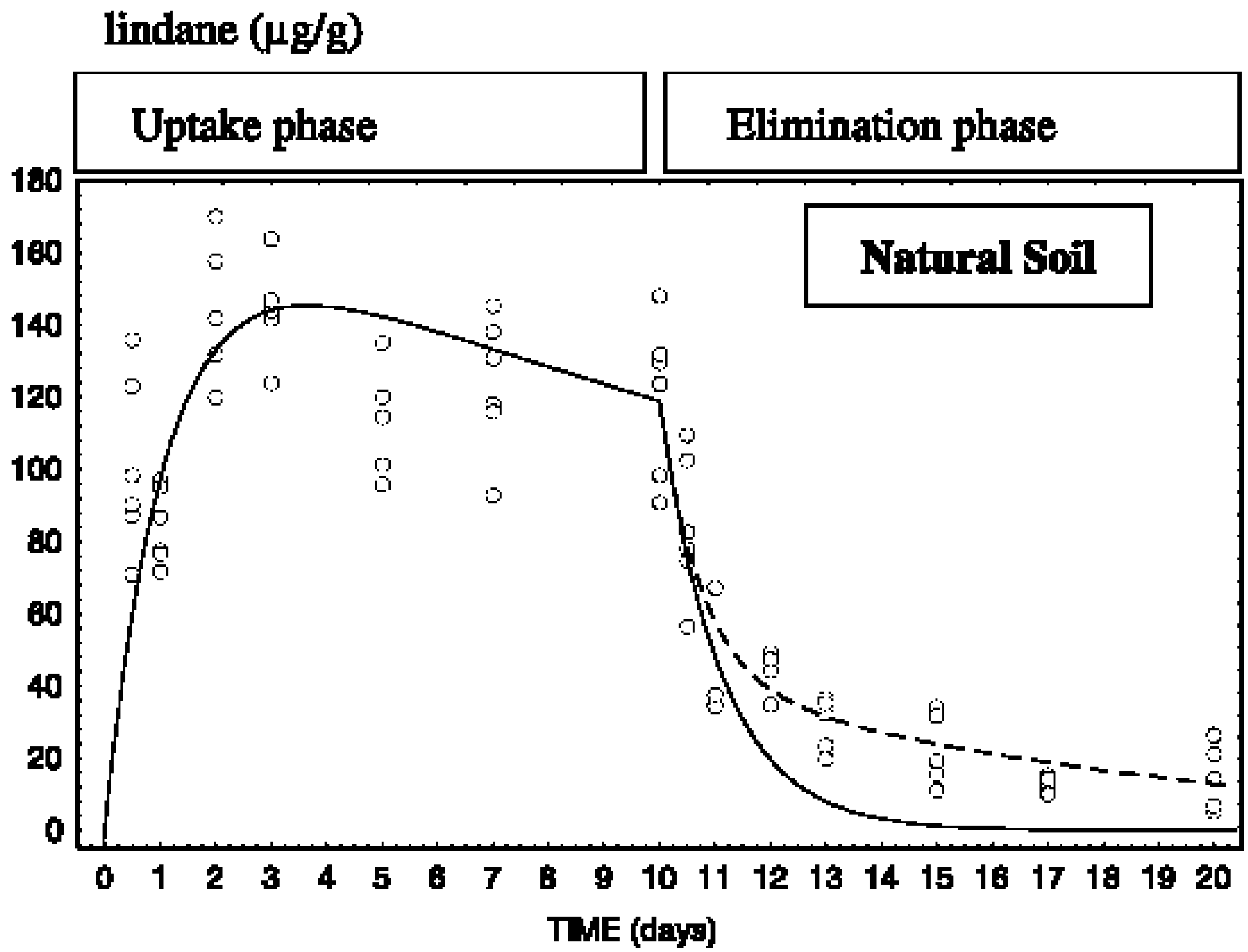
Enchytraeidae



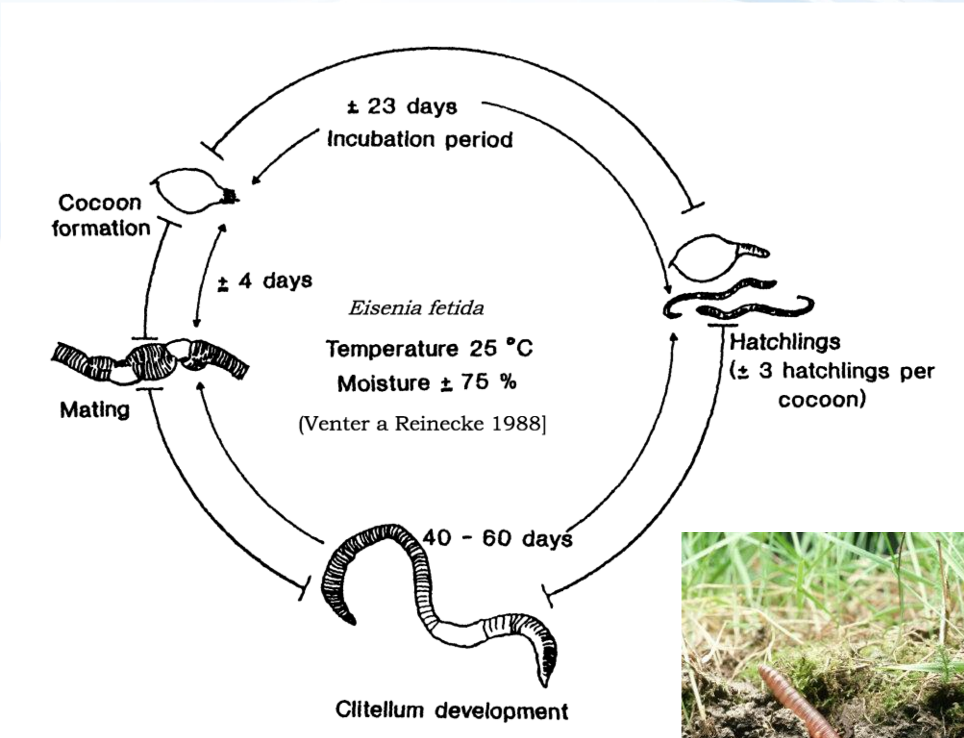
Avoidance test with *E. albidus*



Bioaccumulation experiments with enchytraeids

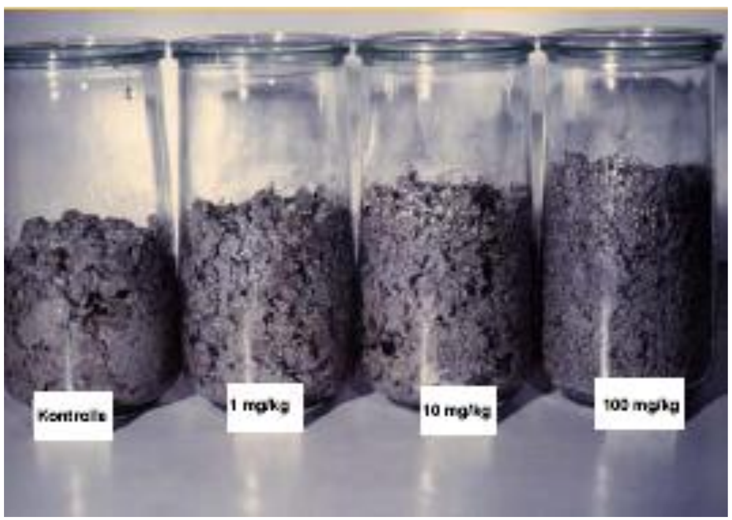


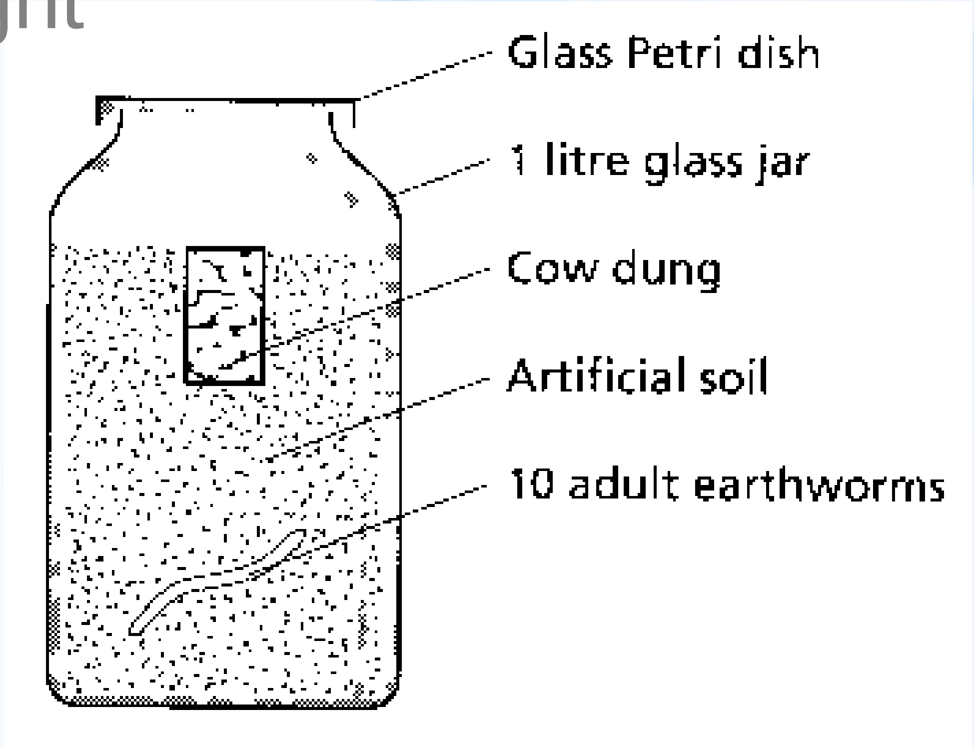
Earthworms


± 23 days
Incubation period
Cocoon formation
± 4 days
Eisenia fetida
Temperature 25 °C
Moisture ± 75 %
[Venter a Reinecke 1988]
Mating
Hatchlings
(± 3 hatchlings per cocoon)
40 - 60 days
Clitellum development

Earthworm acute toxicity test

- 14 days
- 500 g soil + 10 adult *Eisenia fetida*
- mortality and weight





Earthworm reproduction test

- 56 days
- 500 g soil + 10 adult *Eisenia fetida*
- horse manure as food
- juveniles extracted using water bath

Eisenia fetida reproduction test

Soil preparation

WHC measurement

Water added

Soil weighted to jars

10 adults to 1 jar

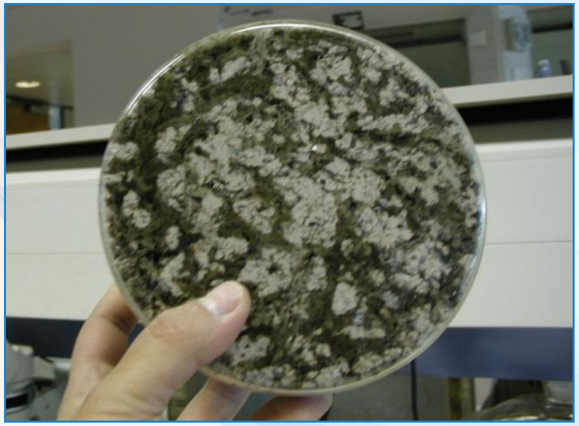
Weighting worms

10 adults from culture

Washed

E. fetida test – after 28 days

Temperated room



Control of the jars, activity markers

Mortality assessment

Weighting the worms

E. fetida – 8 weeks

Water bath, increasing temperature 40°C - 60°C

After 20 min juveniles appear

Collecting and counting juveniles

Sieving the soil

Hand sorting of cocoons

Counting

Avoidance test

Guideline: ISO/DIS 17512 (draft)

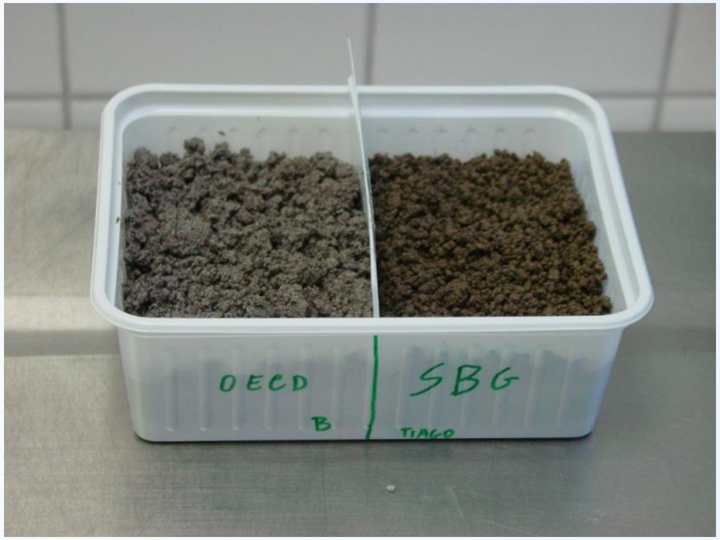
Species: ***E. andrei***

Substrate: LUFA St. 2.2 standard soil

Duration: 1 - 2 days

Parameter: Behaviour of the worms

Test vessels: Dual chamber



Risk assessment with earthworms

Prüfung der Auswirkungen auf Regenwürmer

Labortest mit Kompostwurm

1. Akute Toxizität (2 Wochen)

Bewertung:
Mortalität,
Körpergewicht

$$TER = \frac{LC50}{PEC} < 10$$

Kokons des Kompostwurms

2. Einfluss auf die Fortpflanzung (8 Wochen)

Bewertung:
Anzahl der Jungtiere,
Körpergewicht

$$TER = \frac{NOEC}{PEC} < 5$$

einheimische Regenwurmart

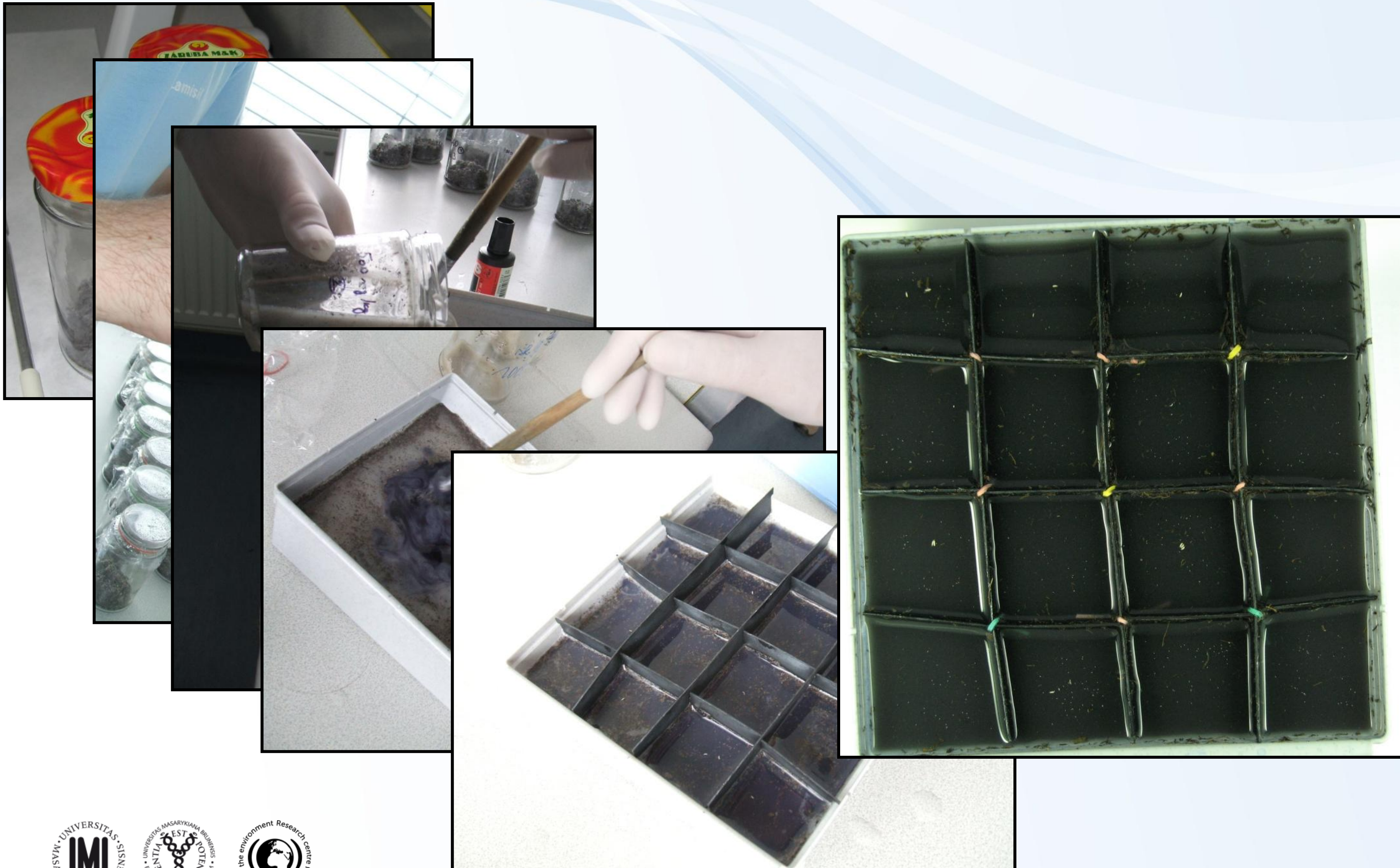
3. Auswirkungen im Freiland (1 Jahr)

Bewertung: Individuenzahlen, Risiken für Populationen und Lebensgemeinschaften

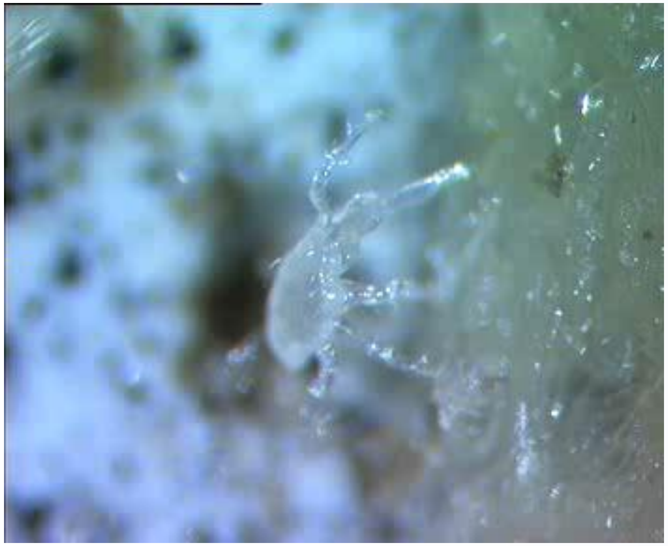
Fachbeirat Naturhaushalt

Folsomia candida

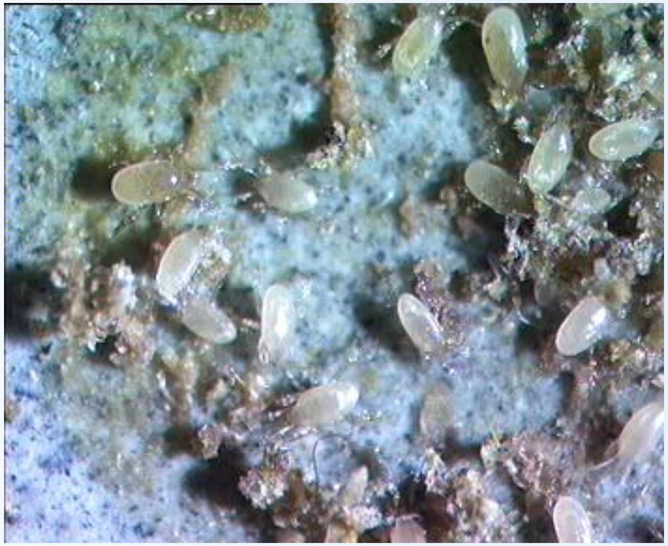
Folsomia candida



Mites



Hypoaspis aculeifer



Mites

predator

cont. soil

prey

Plants

Lactuca sativa root growth

Plant protection products risk assessment

Auswirkungen auf andere Pflanzen

Verschiedene Konzentrationsstufen im:

Auflauftest

Lein

Wachstumstest

Erbse

Prüfpflanzen: 6 Pflanzenarten aus unterschiedlichen Familien

1. Stufe: Prüfungen im Gewächshaus

- **Auflauftest: Auswirkungen auf Keimung und Auflauf**
- **Wachstumstest: Auswirkungen auf den Biomassezuwachs**

TER < 10

2. Stufe: Weiterführende Versuche

- **Verlängerte Gewächshausversuche**
- **Mehr Arten**
- **Freilandversuche**

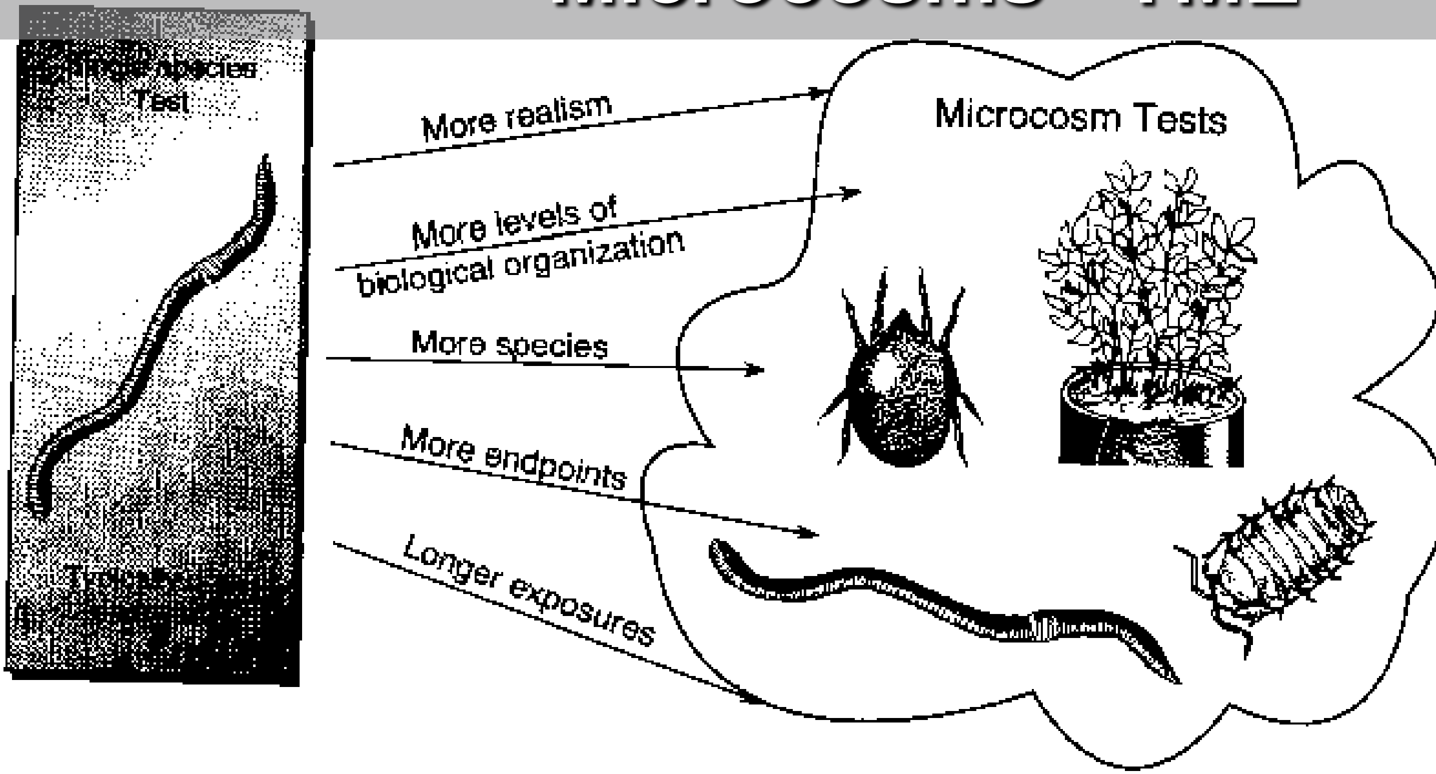
Fachbeirat Naturhaushalt

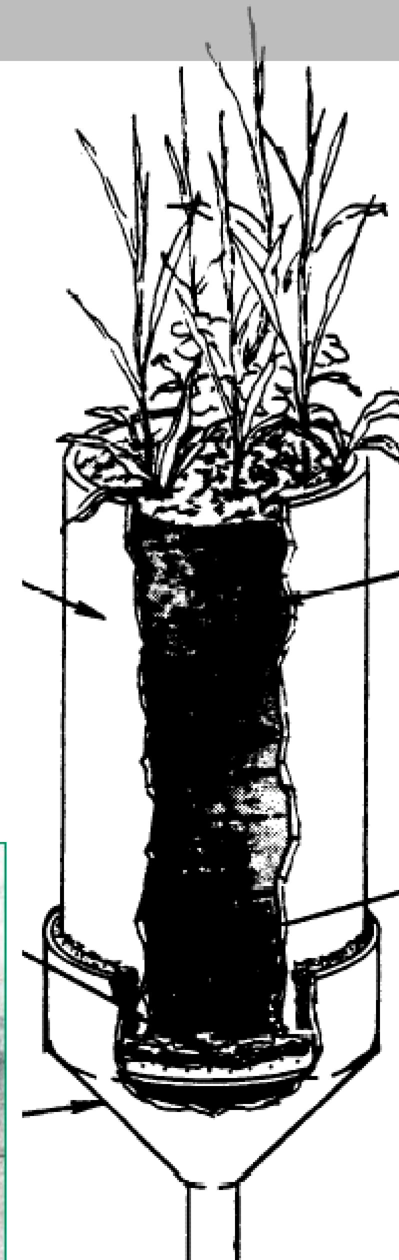
Microcosms, mesocosms

Table 9.1 Classification of Various Semi-Field Tests

1. Model ecosystem segments (= "microcosms") *Natural or artificially assembled units; a few centimeters in size — up to approx. 1 m³ (contents up to a few hundred liters); closed and open systems are both possible.*	
Specialized techniques:	e.g. the plant metabolism box of the NATEC (FIGGE, 1992) or small "artificial streams" (CLEMENTS et al., 1989).
Integrated techniques:	e.g. the Terrestrial Model Ecosystem (TME) (VAN VORIS et al., 1984; KNACKER et al., 1990, 1991) or the Standardized Aquatic Microcosm (SAM) (TAUB et al., 1986; EPA, 1987).
2. Ecosystem segments in the field (= "mesocosms") *Field segments which remain exposed to normal environmental conditions; various sizes ranging between 1 m³ and several hundred m³.*	
Specialized techniques:	Partial enclosures in lakes or rivers, e.g. plastic bags with algae coenoses (EIDE et al., 1979). Lysimeter (usually about 1 m3 in size), e.g. tests on the mobility of pesticides in natural soil cores (e.g. BBA, 1990a). Semi-field tests (usually tests with beneficial organisms), e.g. effects of pesticides on ground beetles (carabids) in cultivated soil system segments (ABEL & HEIMBACH, 1992).
Integrated techniques (very rarely in the terrestrial medium):	Artificial testing systems, e.g. "artificial streams" — reconstructions of real streams including sediment (EATON et al., 1985). Natural enclosures, e.g. "Bremerhaven-Caissons" in wadden seas (FARKE et al., 1984).

Microcosms - TME





Microcosms - TME

Literature

- van Straalen, N.M. and van Gestel, C.A.M. (1993): Soil invertebrates and Micro-organisms. In Calow, P. (Ed.): Handbook of ecotoxicology. Oxford : Blackwell scientific publications. p. 251-277. ISBN 0-632-03573-0.
- Donker, M.H., Eijsackers, H., and Heimbach, F. (1994): Ecotoxicology of soil organisms. CRC Press, Inc., Boca Ranton. ISBN 0-87371-530-6.
- Tarradellas, J., Bitton, G. and Rossel, D. (1996): Soil Ecotoxicology. Lewis Publishers, Boca Raton, FL. ISBN: 1-56670-134-1.
- Lokke, H. and van Gestel, C.A.M. (1998): Handbook of soil invertebrate toxicity tests. John Wiley & Sons, Chichester. ISBN 0-471-97103-0. 281 pp.
- Römbke, J. and Moltmann, J.F. (1996): Applied ecotoxicology. CRC Press LLC, New York. ISBN 0-56670-070-1.
- Hoffman, D.J. , Rattner, B.A. (1994): Handbook of Ecotoxicology. Boca Raton, FL, USA : CRC Press.
- Suter, G.W. II, Efroymson, R.A., Sample, B.E., and Jones, D.S. (2000): Ecological risk assessment for contaminated sites. Lewis Publishers, Boca Ranton. ISBN 1-56670-525-8. 437p.
- Doelman, P. and Eijsackers, H.J.P. (2004): Vital Soil - Function, Value and Properties. Elsevier. 358 p. ISBN: 0-444-51772-3.

Literature

- Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A. (1994): Defining soil quality for a sustainable environment. SSSA Special Publication Number 35. Soil Science Society of America, Inc.
- Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (1997): Biological indicators of soil health. CAB International, Wallingford. ISBN 0851991580.
- Doran, J.W. and Parkin, T.B. (1996): Quantitative indicators of soil quality: A minimum data set. In Doran, J.W. and Jones, A.J. (Eds.): Methods for assessing soil quality, p. 25-38. SSSA Special Publication Number 49. Soil Science Society of America, Inc., Madison.
- Doran, J.W. and Jones, A.J. (Eds.): Methods for assessing soil quality. SSSA Special Publication Number 49. Soil Science Society of America, Inc., Madison.
- Doran, J. W., Parkin, T. B. (1994): Defining and assessing soil quality. In: Defining soil quality for a sustainable environment. SSSA special publication number 35. SSSA, Inc., American Society of Agronomy, Inc. Madison, Wisconsin, USA, 1994, pp. 3 – 21.
- European Commission (2002): Communication from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions - Towards a Thematic Strategy for Soil Protection. COM (2002) 179.
- European Commission (2006a): Communication from the commission to the council, the european parliament, the european economic and social committee and the committee of the regions. Thematic strategy for soil protection. COM(2006)231.
- European Commission (2006b): Proposal for a directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC. COM (2006) 232.
- European Commission (2006c): Commission staff working document accompanying the communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the regions. Thematic strategy for soil protection. Impact assessment of the thematic strategy on soil protection. SEC (2006) 260.

Literature

- Van-Camp, L., Bujarrabal, B., Gentile, A-R., Jones, R.J.A., Montanarella, L., Olazabal, C., Selvaradjou, S.K. (2004): Reports of the Technical Working Groups Established under the Thematic Strategy for Soil Protection. Volume V – Monitoring. EUR 21319 EN/5, 872 pp. Office for Official Publications of the European Communities, Luxembourg.
- EEA (2007): CSI 015 - Progress in management of contaminated sites - Assessment published Aug 2007. European Environmental Agency. www.eea.eu
- Carlon, C. (2007): Derivation methods of soil screening values in Europe. A review and evaluation of national procedures towards harmonization. European Commission, Joint Research Centre, Ispra, EUR 22805-EN, 306 pp.
- De Bruijn, J., Crommentuijn, T., van Leeuwen, K., van der Plassche, E., Sijm, D., van der Weiden, M. (1999): Environmental Risk Limits in The Netherlands. RIVM Rapport 601640001. pp. 900.
- De Vries, W. and Bakker, D.J. (1998): Manual for Calculating Critical Loads of Heavy Metals for Terrestrial Ecosystems. Guidelines for Critical Limits, Calculation Methods and Input Data. Report 166. DLO Winand Staring Centre, Wageningen, The Netherlands, 144 pp.
- http://ec.europa.eu/environment/waste/index.htm
- http://www.eea.europa.eu/themes/waste