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Soil ecotoxicology: soil bioassays

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What is soil?

Soil Science Glossary (Soil Science Society of America).

- The unconsolidated mineral or organic matter on the surface of the earth that has been subjected to and shows effects of genetic and environmental factors of: climate (including water and temperature effects), and macro- and microorganisms, conditioned by relief, acting on parent material over a period of time. A product-soil differs from the material from which it is derived in many physical, chemical, biological, and morphological properties and characteristics.

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

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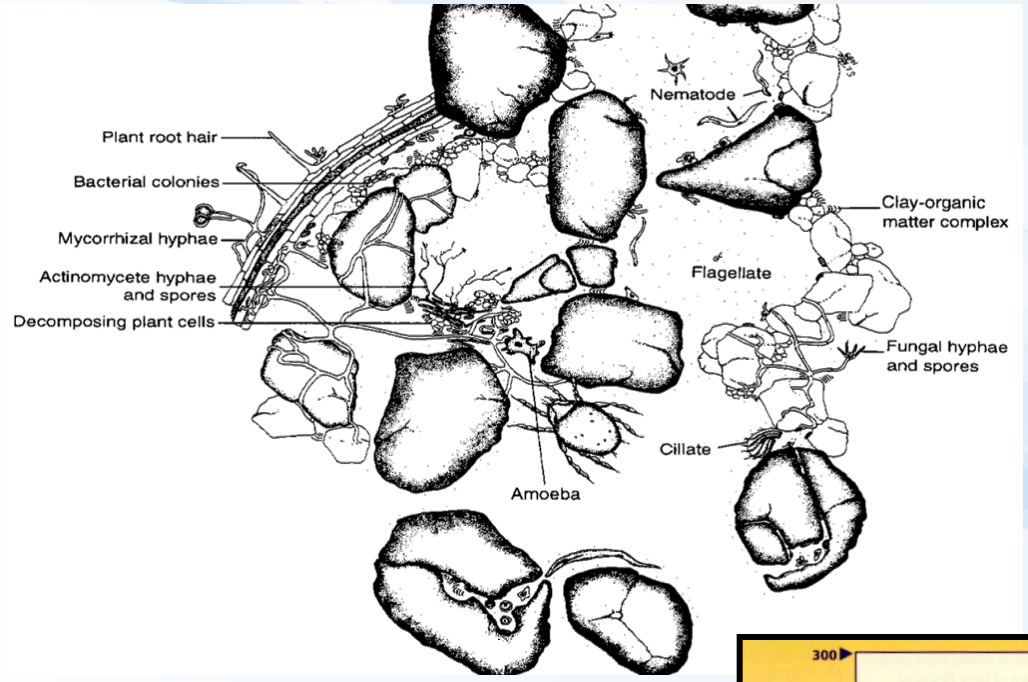
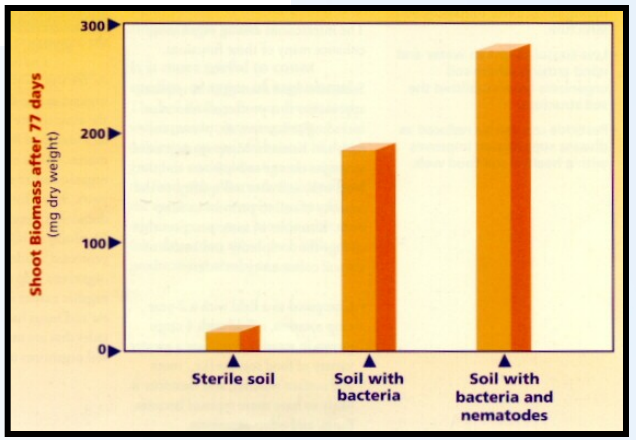
soil = biotic + abiotic = complex

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



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Soil biota

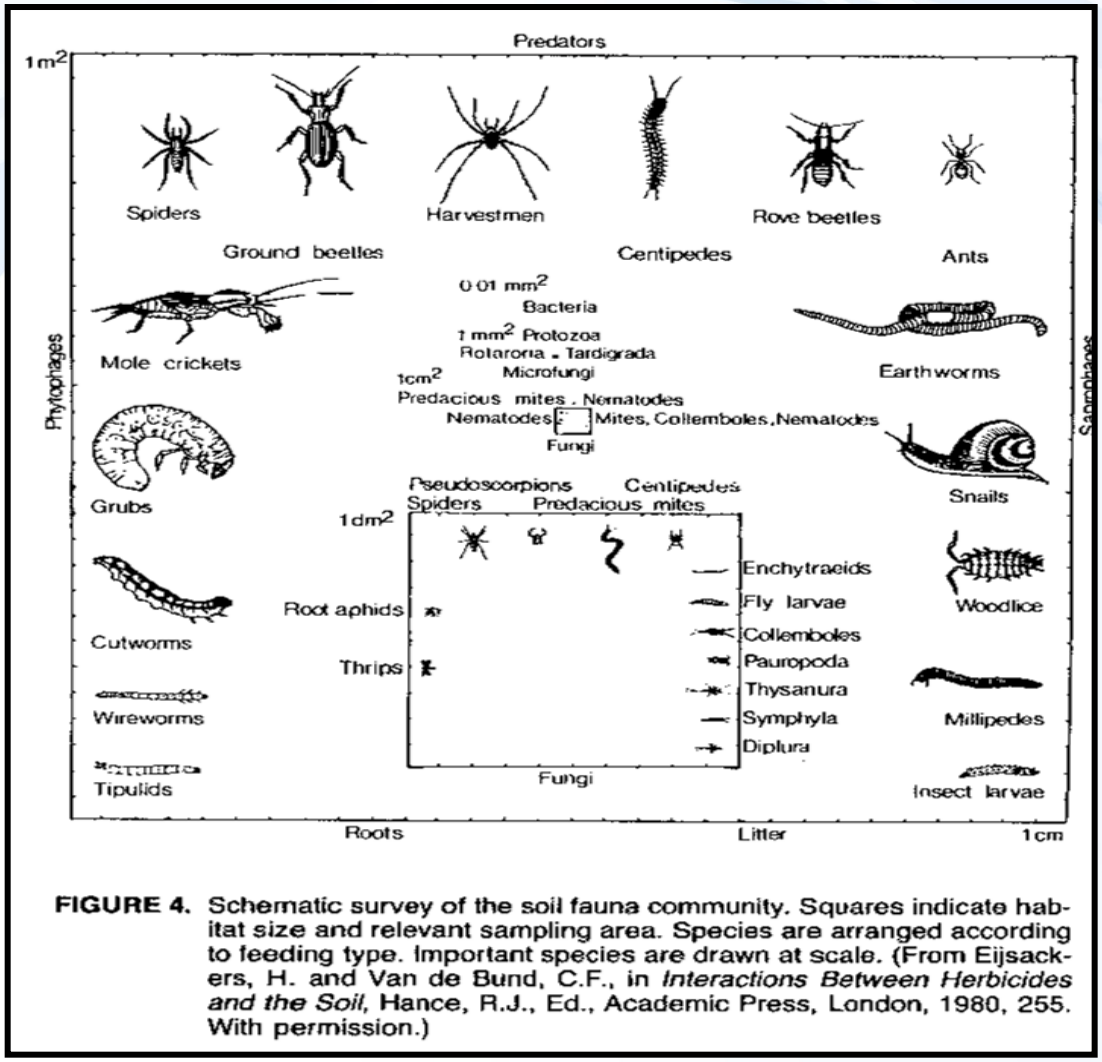


FIGURE 4. Schematic survey of the soil fauna community. Squares indicate habitat size and relevant sampling area. Species are arranged according to feeding type. Important species are drawn at scale. (From Eijsackers, H. and Van de Bund, C.F., in *Interactions Between Herbicides and the Soil*, Hance,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

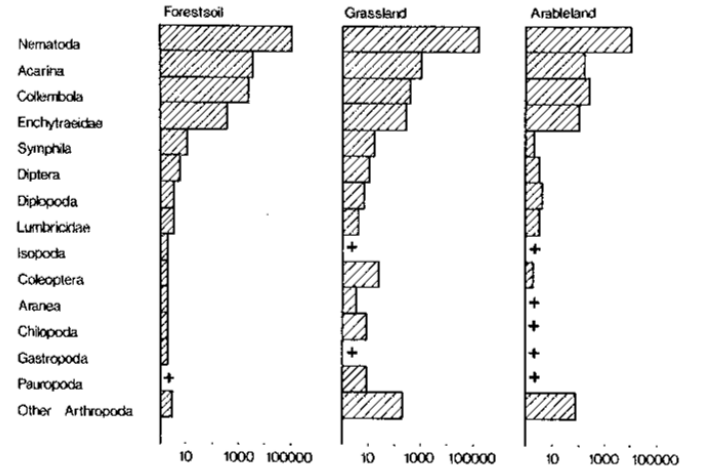
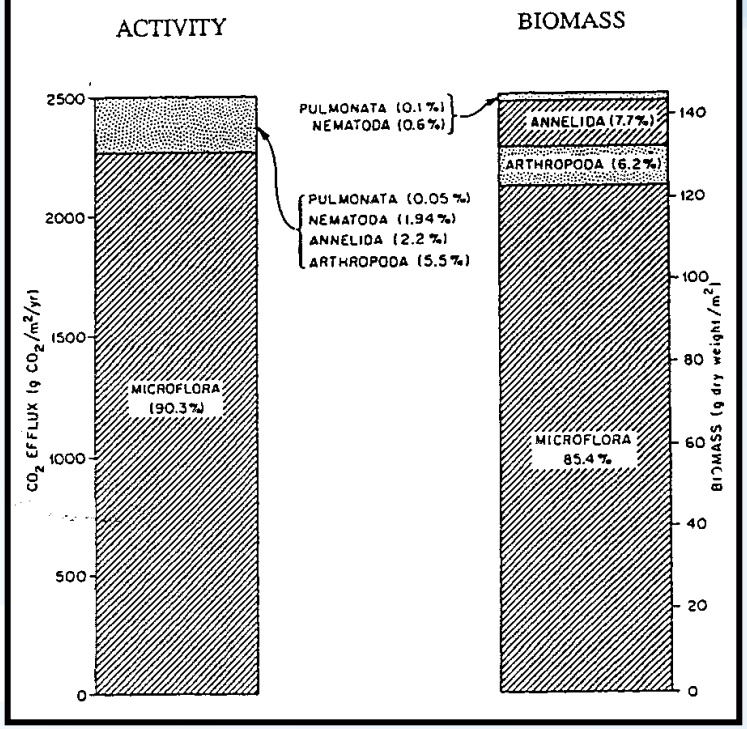


FIGURE 3. Mean composition of the soil fauna in a forest, meadow, and arable soil (mean numbers per dm² for the 0- to 30-cm layer; + means present). (From Eijsackers, H. and Van de Bund, C.F., in *Interactions Between Herbicides and the Soil*, Hance, R.J., Ed., Academic Press, London, 180, 255. With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



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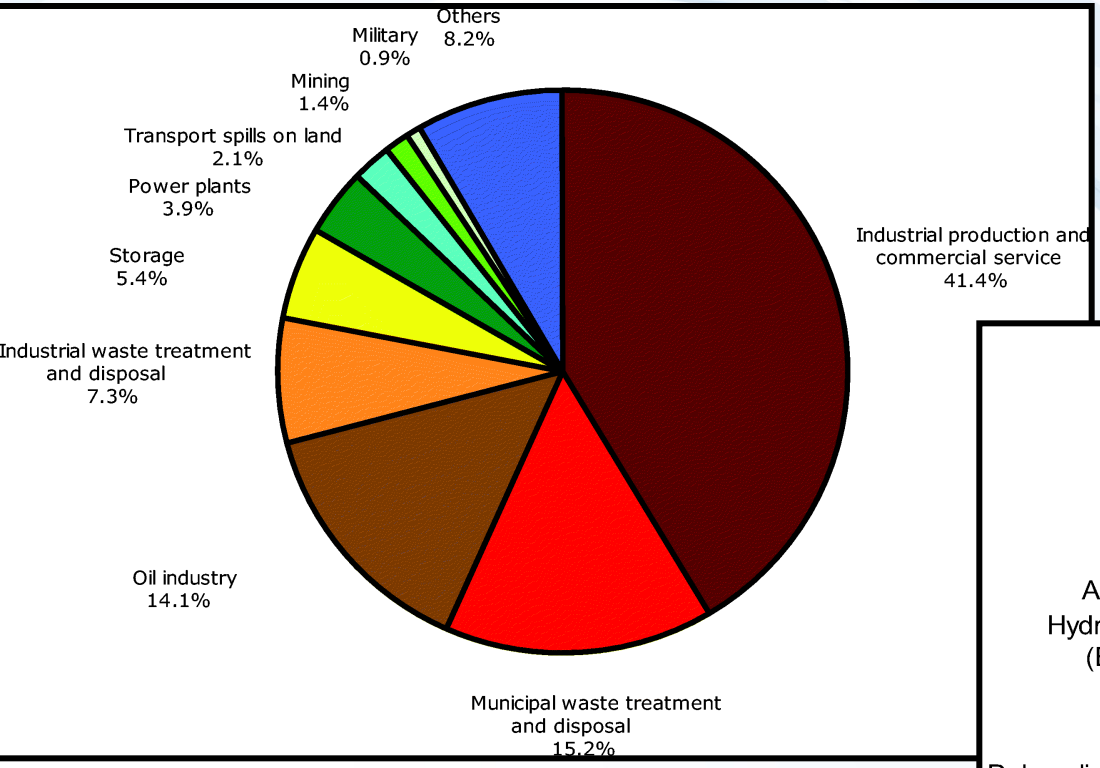
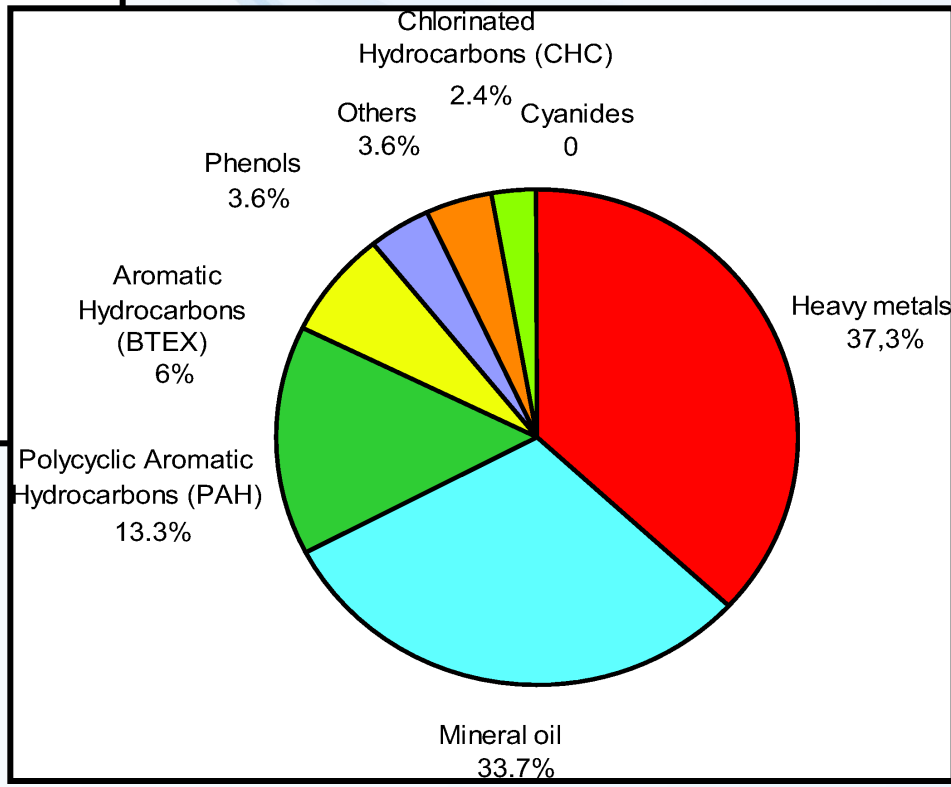
Soils have problems

- 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))

Contamination of soils





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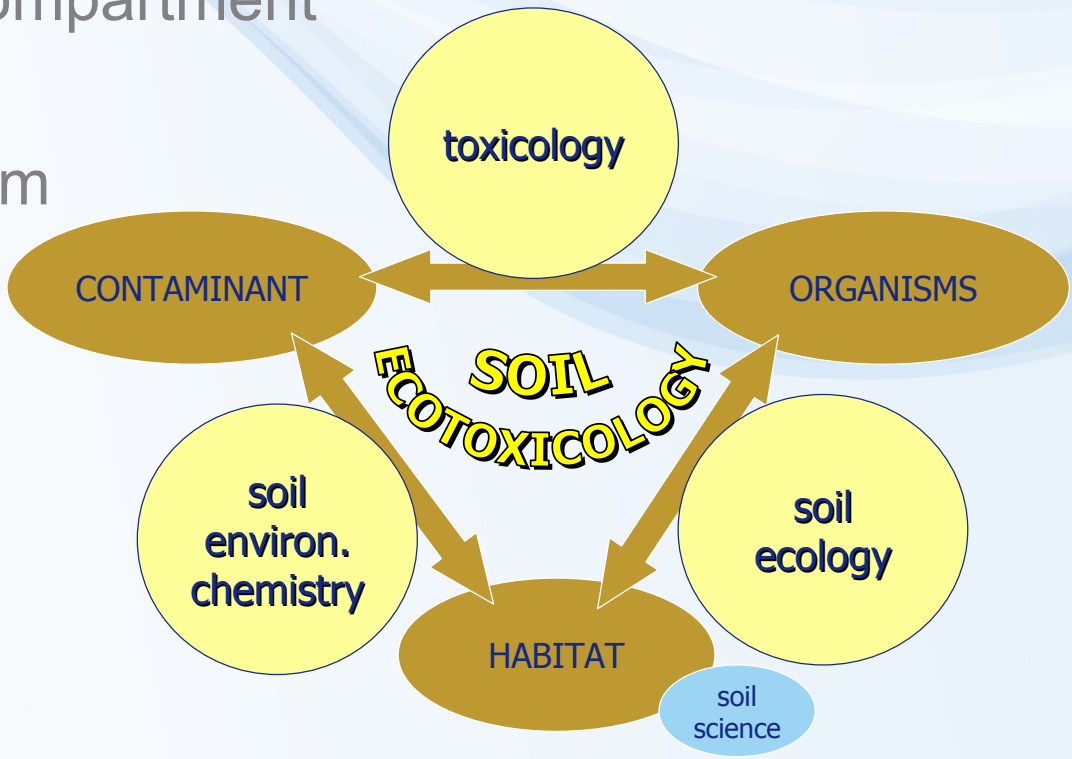
Soil ecotoxicology

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 !!

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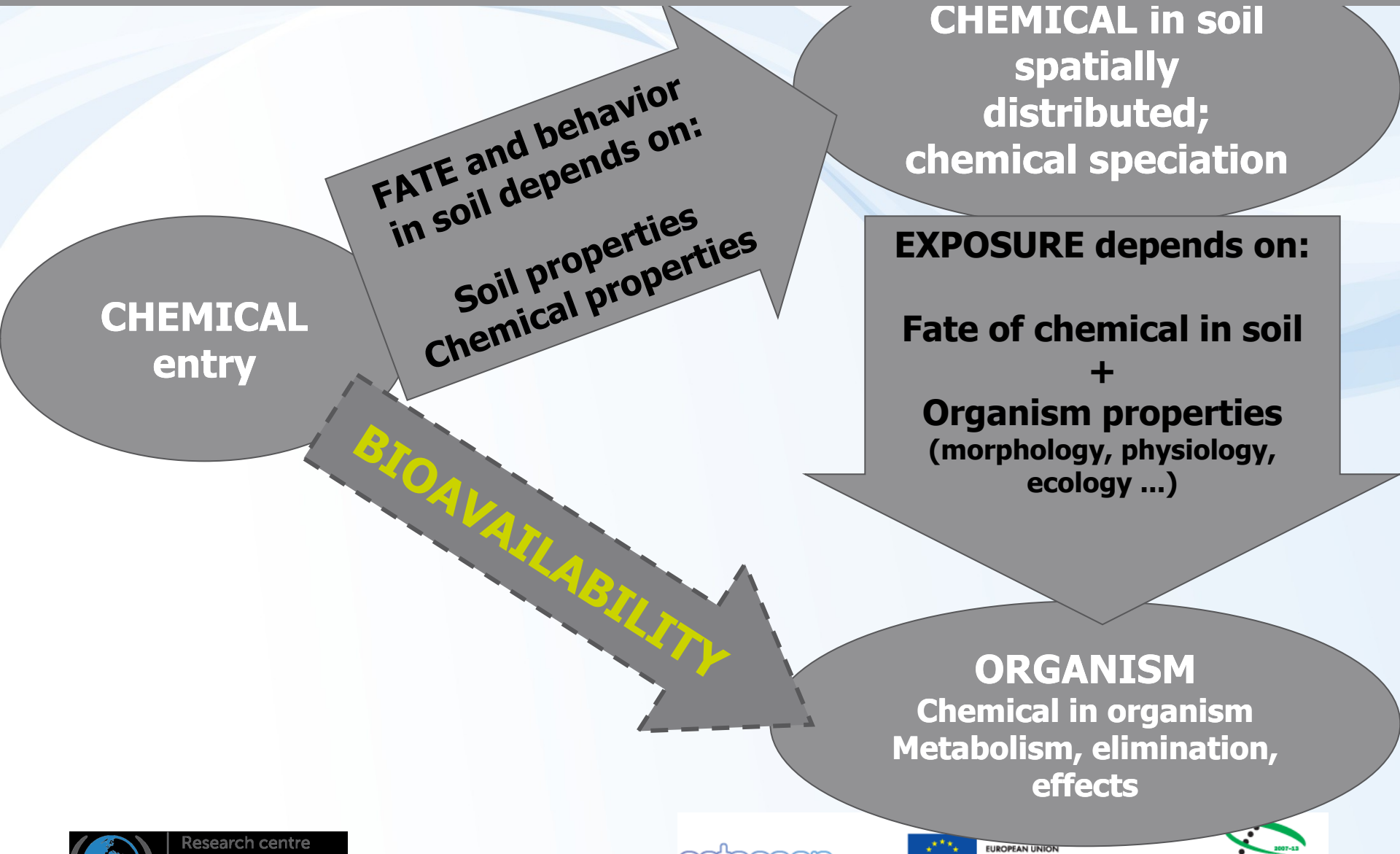
Soil environment is very different from aquatic

- 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



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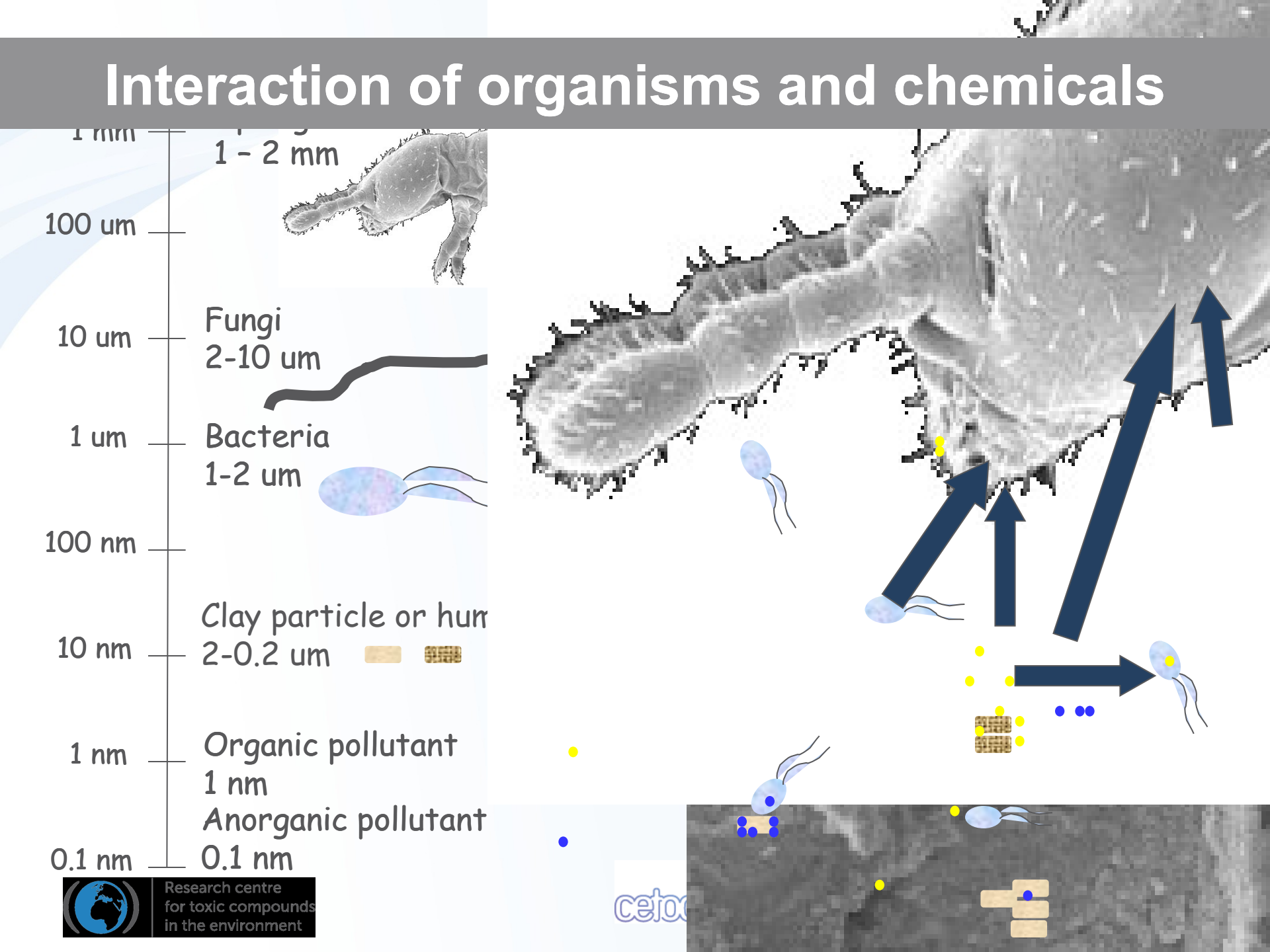
Interaction of organisms and chemicals



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Why to bother with bioavailability ?

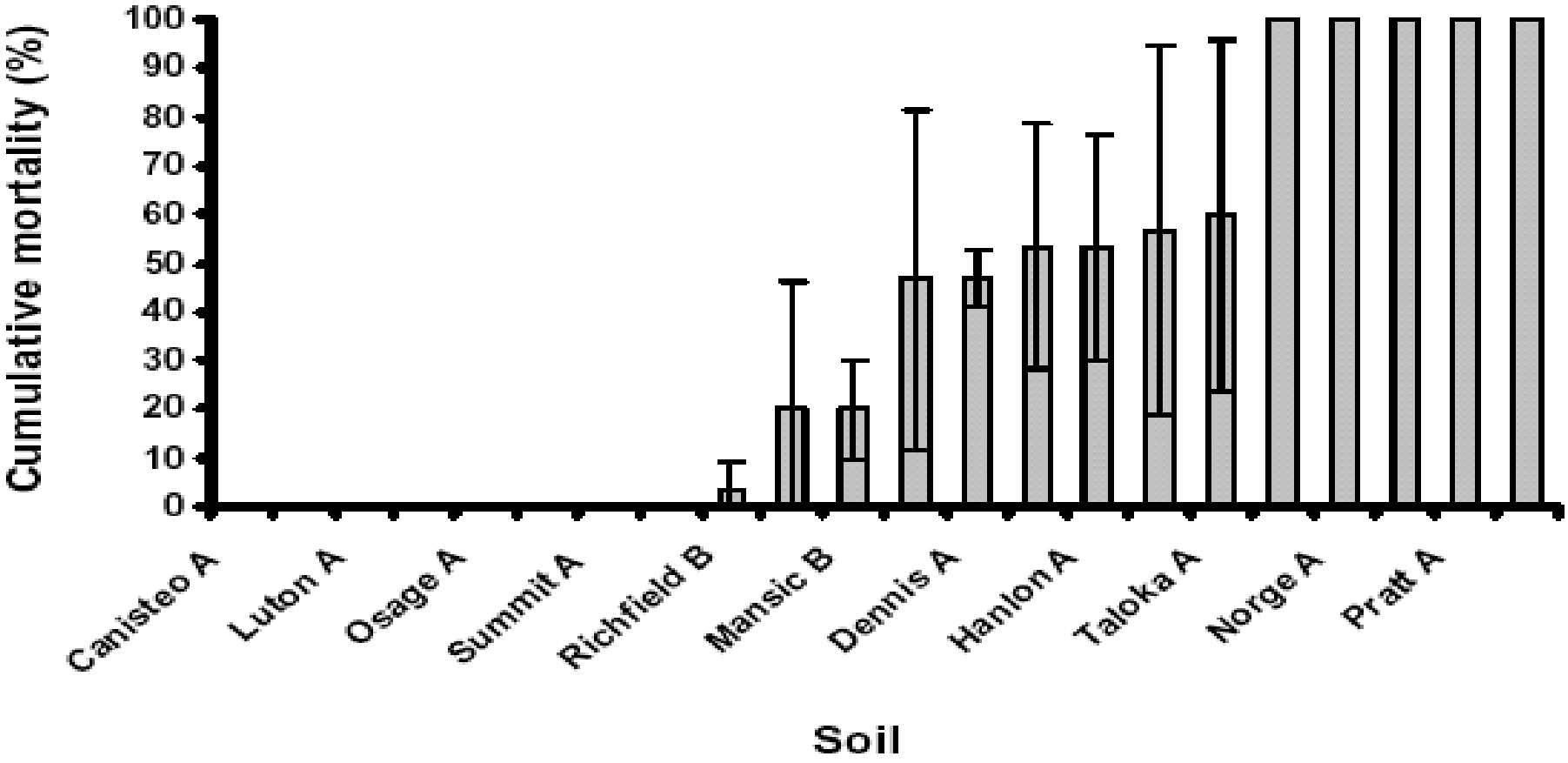
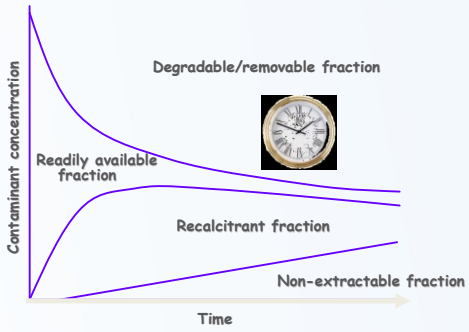


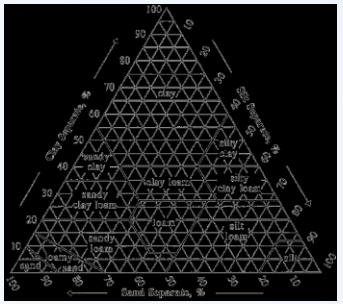
Fig. 1. Cumulative mortality (mean of three replicates, ± 95% CI) of Eisenia andrei exposed to 2,000 mg Pb/kg spiked soils for 28 days.

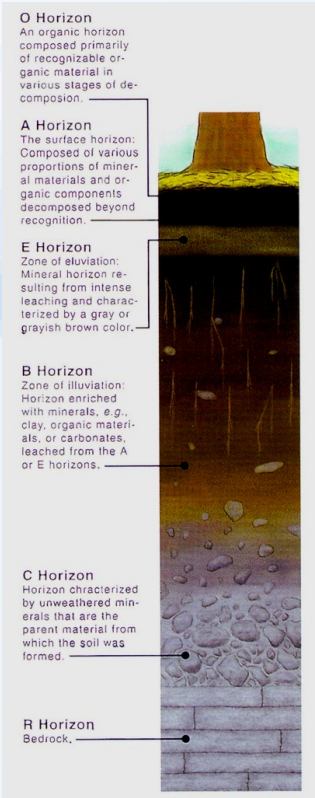
From Bradham et al. (2003)

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







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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 ?

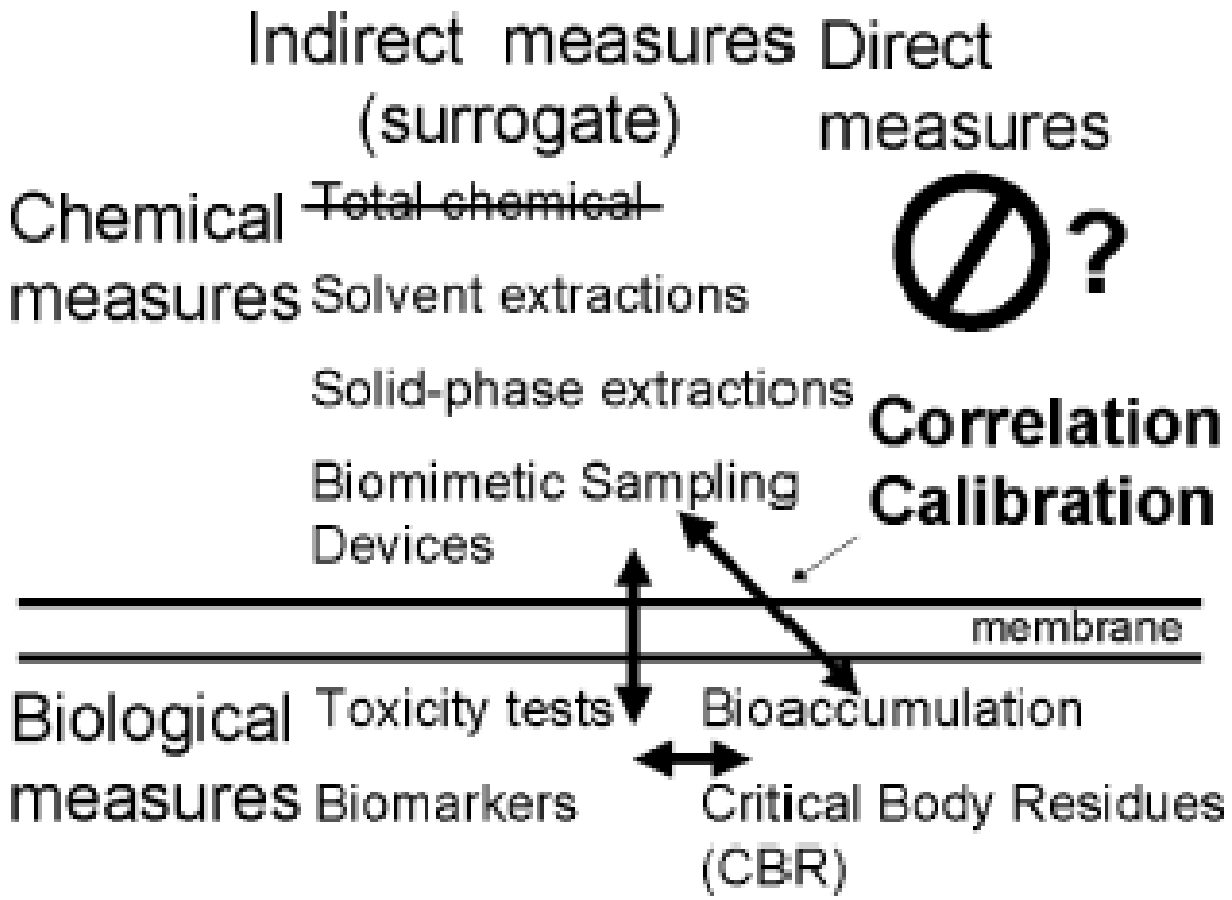
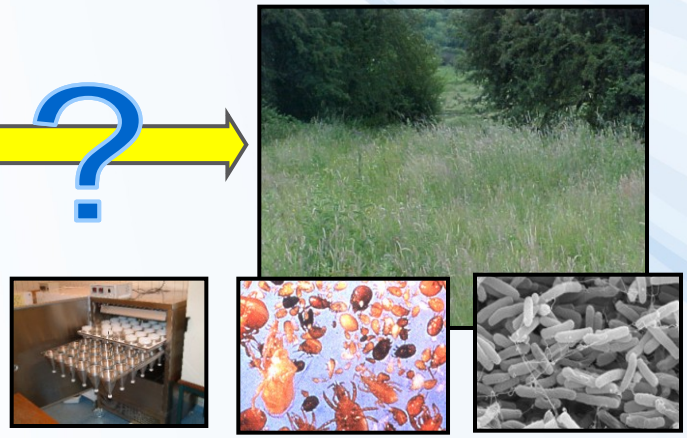


Fig. 3. Methods for measuring bioavailability.

Approaches of soil ecotoxicology

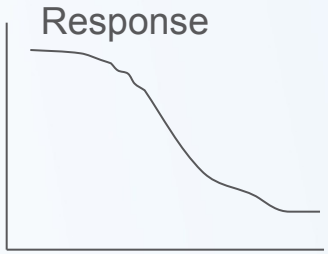
Bio-indication – (bio)monitoring



Goal

Define and describe relationship between biota conditions and contamination

Ecotoxicity bioassays



Goal

Define safe concentration, describe risks

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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 …

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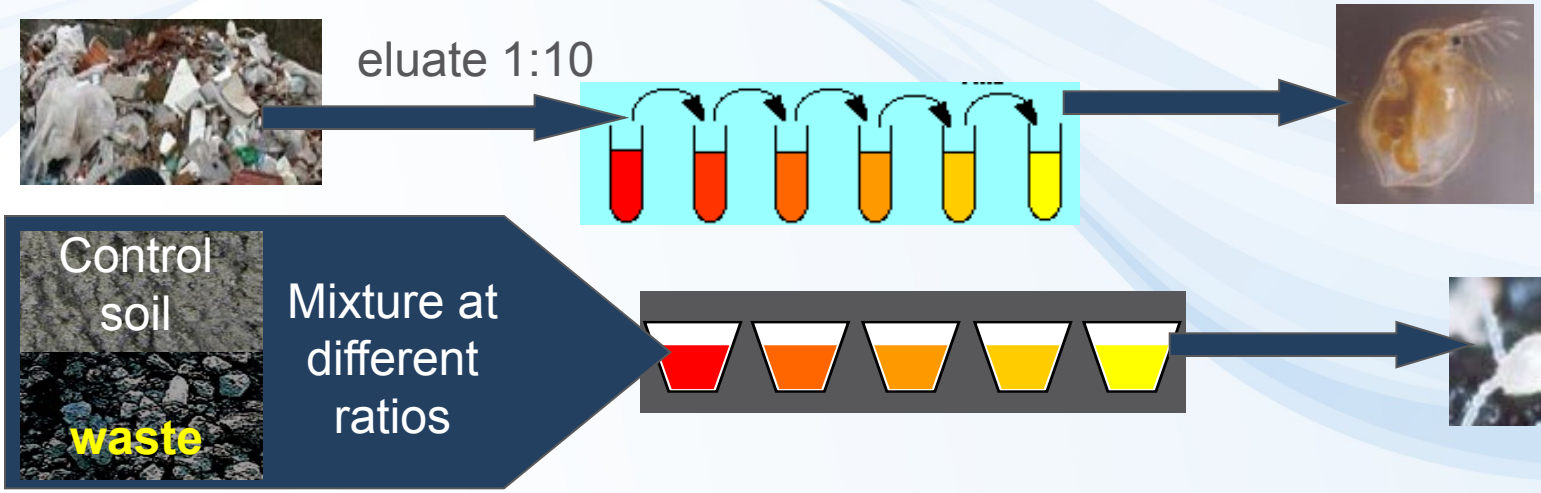
Why bioassays?

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?

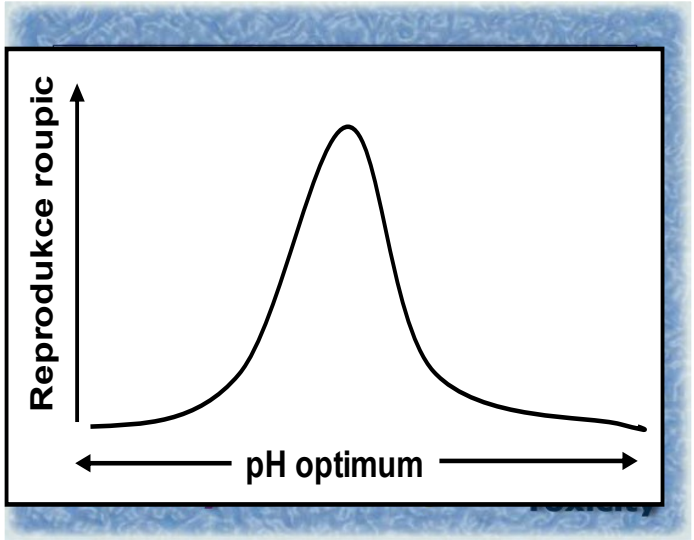


Eluate tests are not able to predict solid phase exposure

WHY ?

→ real bioavailability

→ effect of matrix involved



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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)

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

Valid standards
207 Earthworm, Acute Toxicity Tests (4th April 1984)
208 Terrestrial Plants, Growth Test (19th July 2006)
216 Soil Microorganisms, Nitrogen Transformation Test (21st January 2000)
217 Soil Microorganisms, Carbon Transformation Test (21st January 2000)
220 Enchytraeid Reproduction Test (13th April 2004)
222 Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei) (13th April 2004)
227 Terrestrial Plant Test: Vegetitive Vigour Test (19th July 2006)
Drafts
Predatory Mite Reproduction Test in Soil (*Hypoaspis (Geolaelaps) Aculeifer*)
Determination of Developmental Toxicity of a Test Chemical to Dipteran Dung Flies (*Scathophaga stercoraria* L. (*Scathophagidae*), *Musca autumnalis* De Geer (*Muscidae*))

In preparation: *Folsomia* sp. test a bioaccumulation test

Bioassay standards - ISO

ISO 15799	2003	Guidance on the ecotoxicological characterization of soils and soil materials
ISO 11268-1	1993	Effects of pollutants on earthworms (*Eisenia fetida*) - Part 1: Determination of acute toxicity using artificial soil substrate
ISO 11268-2	1998	Effects of pollutants on earthworms (*Eisenia fetida*) - Part 2: Determination of effects on reproduction
ISO 11267	1999	Inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil pollutants
ISO 16387	2004	Effects of pollutants on *Enchytraeidae* (*Enchytraeus* sp.) - Determination of effects on reproduction and survival
ISO 20963	2005	Effects of pollutants on insect larvae (*Oxythyrea funesta*) - Determination of acute toxicity
ISO 15952	2006	Effects of pollutants on juvenile land snails (*Helicidae*) - Determination of the effects on growth by soil contamination
ISO/DIS 17512-1		Avoidance test for testing the quality of soils and effects of chemicals on behaviour - Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*)
ISO 11269-1	1993	Determination of the effects of pollutants on soil flora - Part 1: Method for the measurement of inhibition of root growth
ISO 11269-2	2005	Determination of the effects of pollutants on soil flora - Part 2: Effects of chemicals on the emergence and growth of higher plants
ISO 22030	2005	Chronic toxicity in higher plants
ISO 14238	1997	Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes
ISO 14240-1	1997	Determination of soil microbial biomass - Part 1: Substrate-induced respiration method
ISO 14240-2	1997	Determination of soil microbial biomass - Part 2: Fumigation-extraction method
ISO 16072	2002	Laboratory methods for determination of microbial soil respiration
ISO 17155	2002	Determination of abundance and activity of soil microflora using respiration curves
ISO 15685	2004	Determination of potential nitrification and inhibition of nitrification - Rapid test by ammonium oxidation
ISO 23753-1	2005	Determination of dehydrogenase activity in soils - Part 1: Method using triphenyltetrazolium chloride (TTC)

Bioassay standards – US EPA

850.2450 Terrestrial (soil-core) microcosm test
850.4000 Background-Nontarget plant testing
850.4100 Terrestrial plant toxicity, Tier I (seedling emergence)
850.4150 Terrestrial plant toxicity, Tier I (vegetative vigor)
850.4200 Seed germination/root elongation toxicity test
850.4225 Seedling emergence, Tier II
850.4230 Early seedling growth toxicity test
850.4250 Vegetative vigor, Tier II
850.4300 Terrestrial plants field study, Tier III
850.4600 Rhizobium-legume toxicity
850.4800 Plant uptake and translocation test
850.5100 Soil microbial community toxicity test
850.6200 Earthworm subchronic toxicity test

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.

Solid material toxicity testing

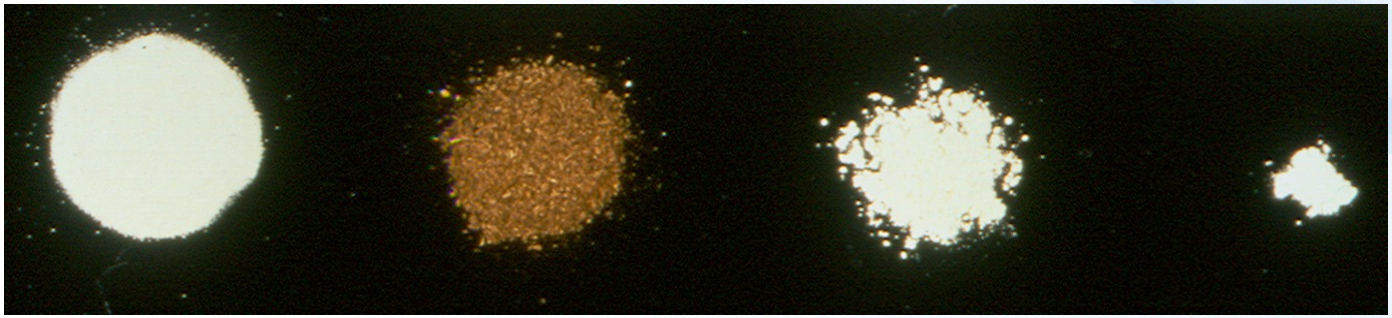
ISO 15799 (2003): Guidance on the ecotoxicological characterization of soils and soil materials
ISO 17616 (2008): Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials

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%

Soil sampling

Storage

Pre-incubation (7 days)

START

10 g per replicate
aerobic conditions
60% WHC; 22 C; dark

Substance application

Negative control

Positive control

7th day

14th day

21st day

28th day

Microbial parameters

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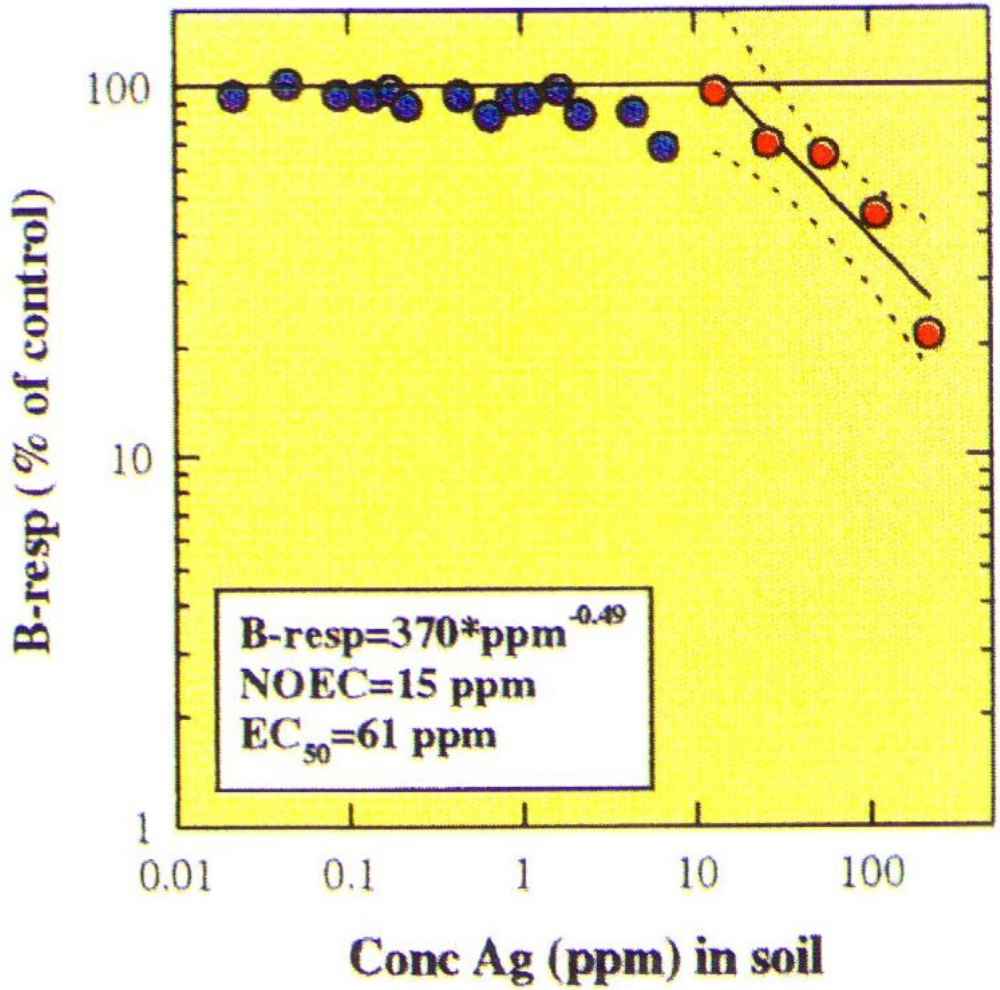
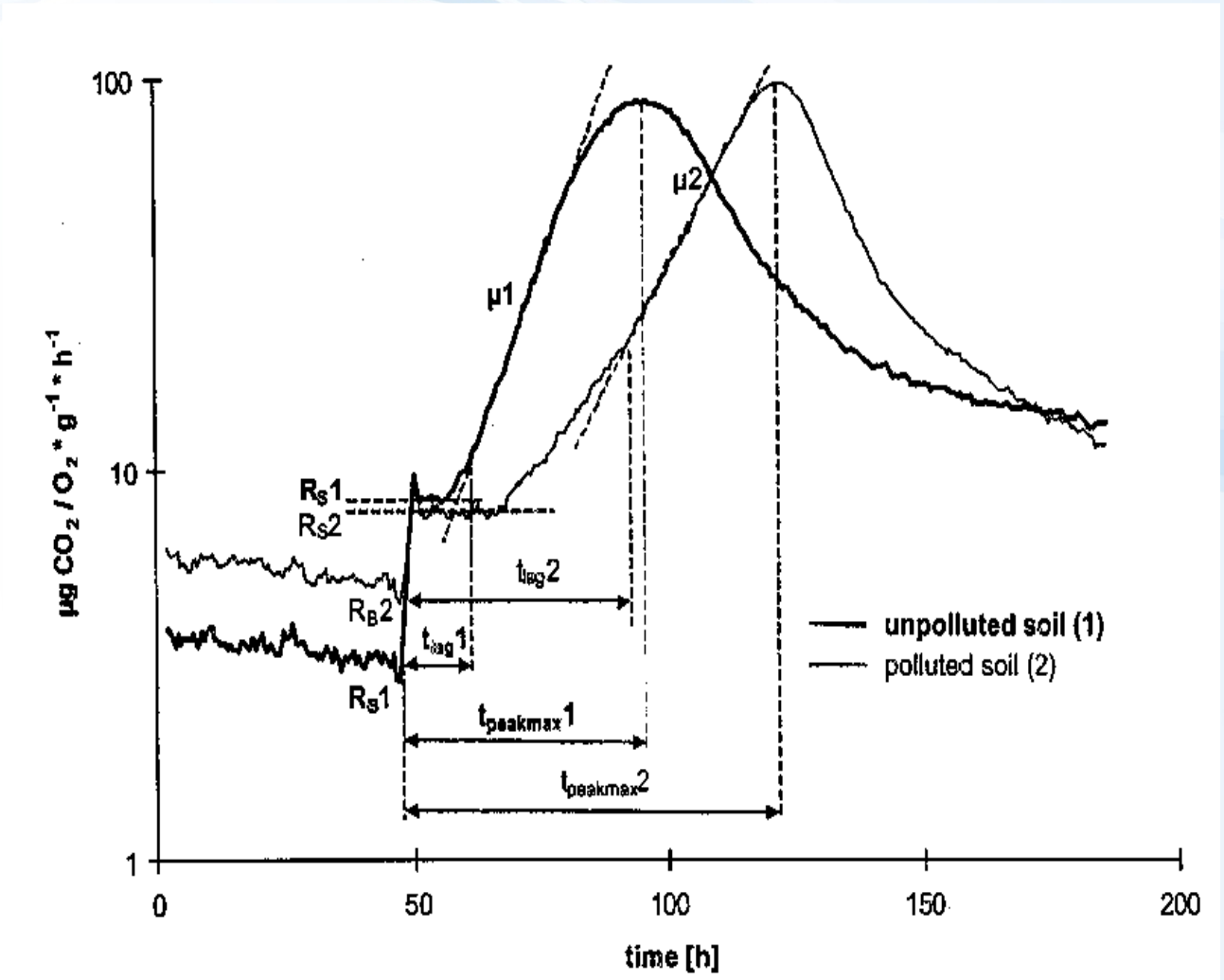
Effects on microbial respiration



Respirometry



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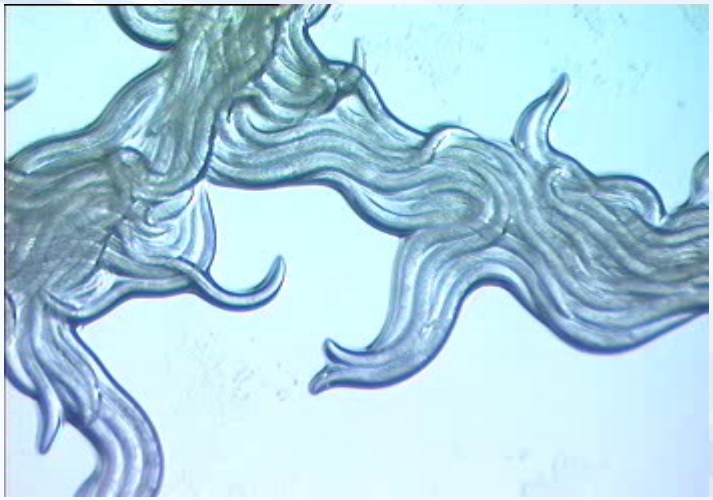
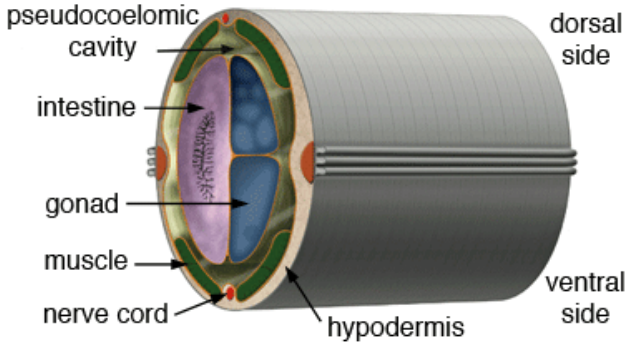
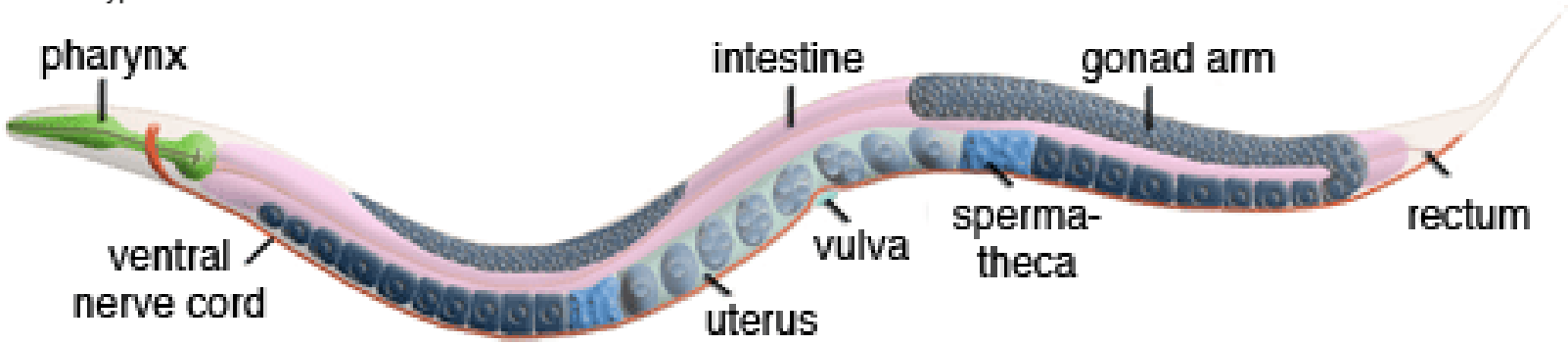
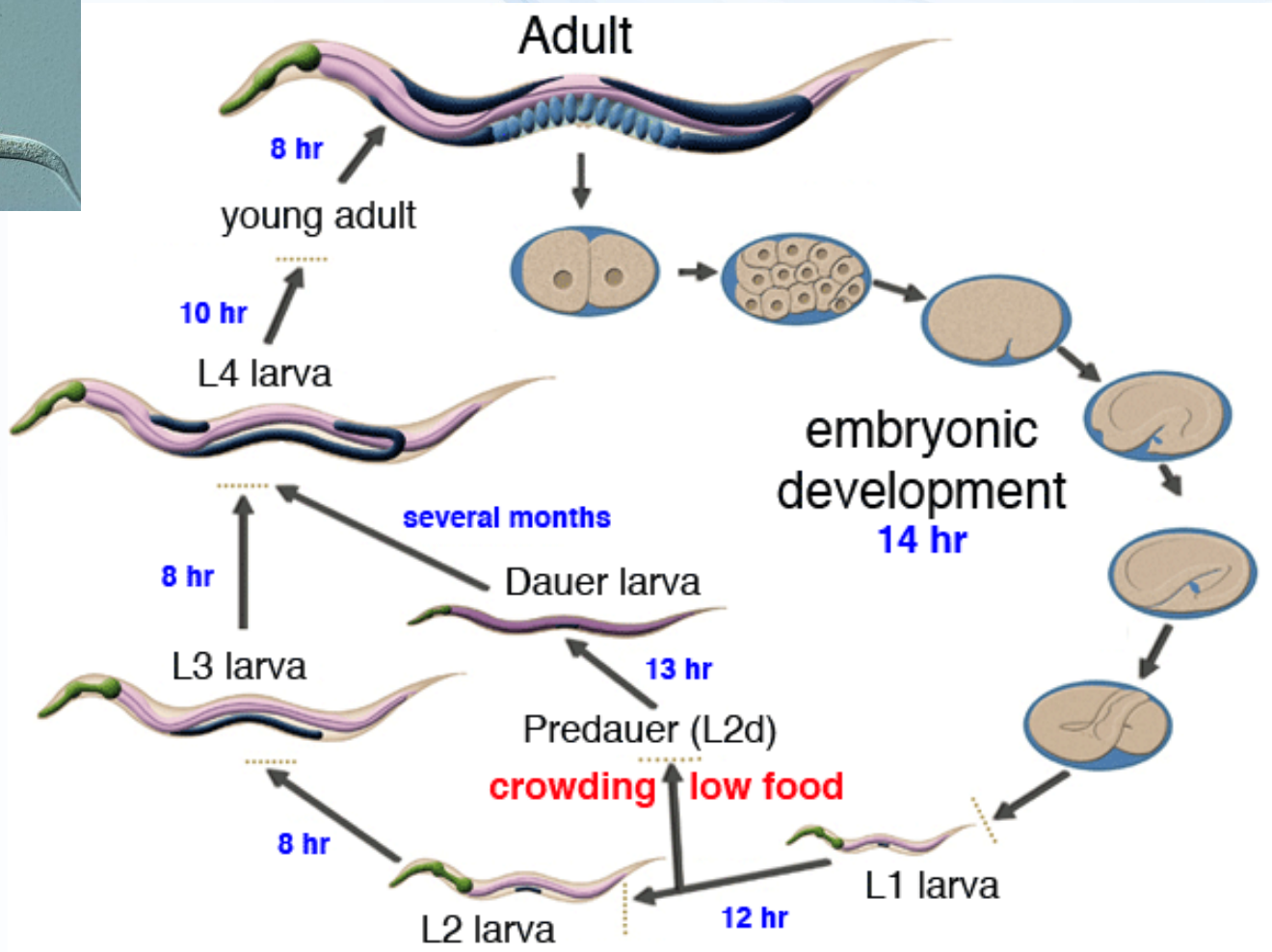
Nematodes




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


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

Caenorhabditis elegans



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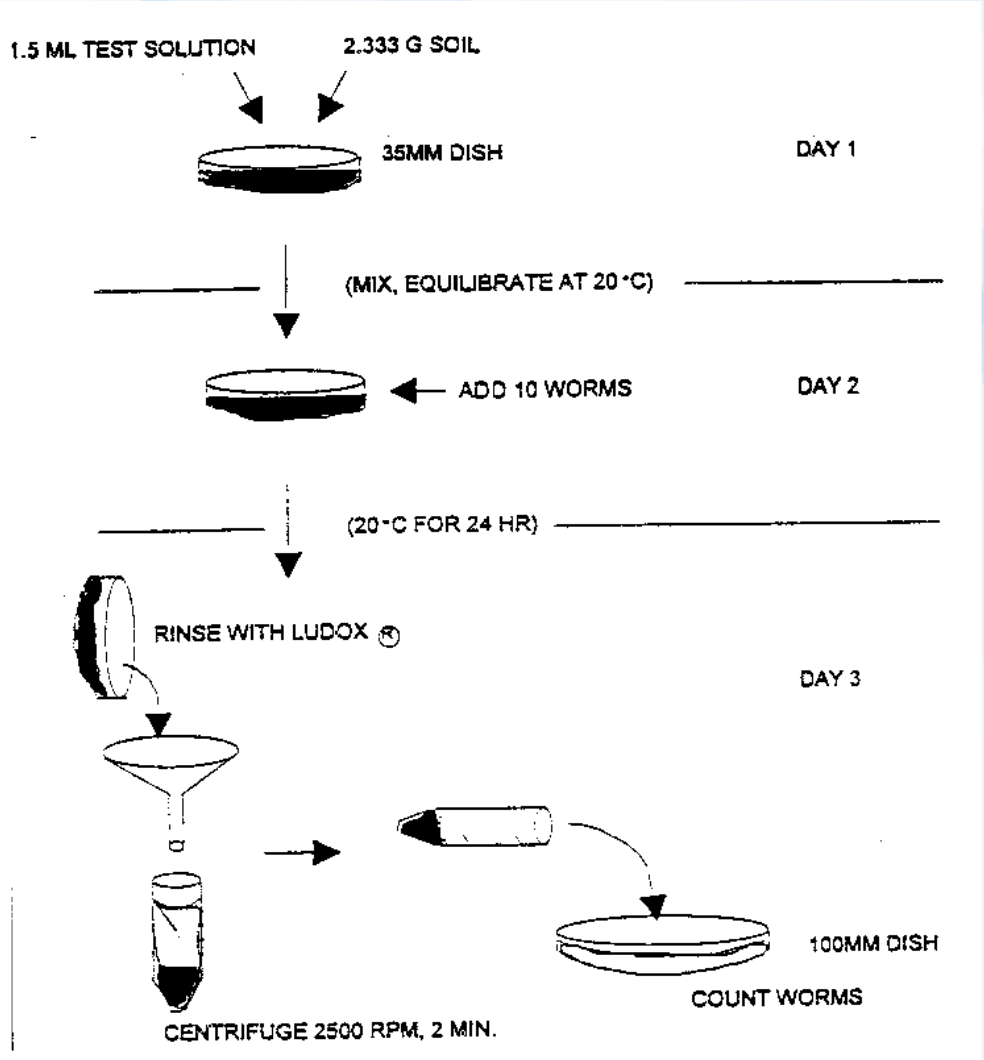
Caenorhabditis elegans

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



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Enchytraeidae

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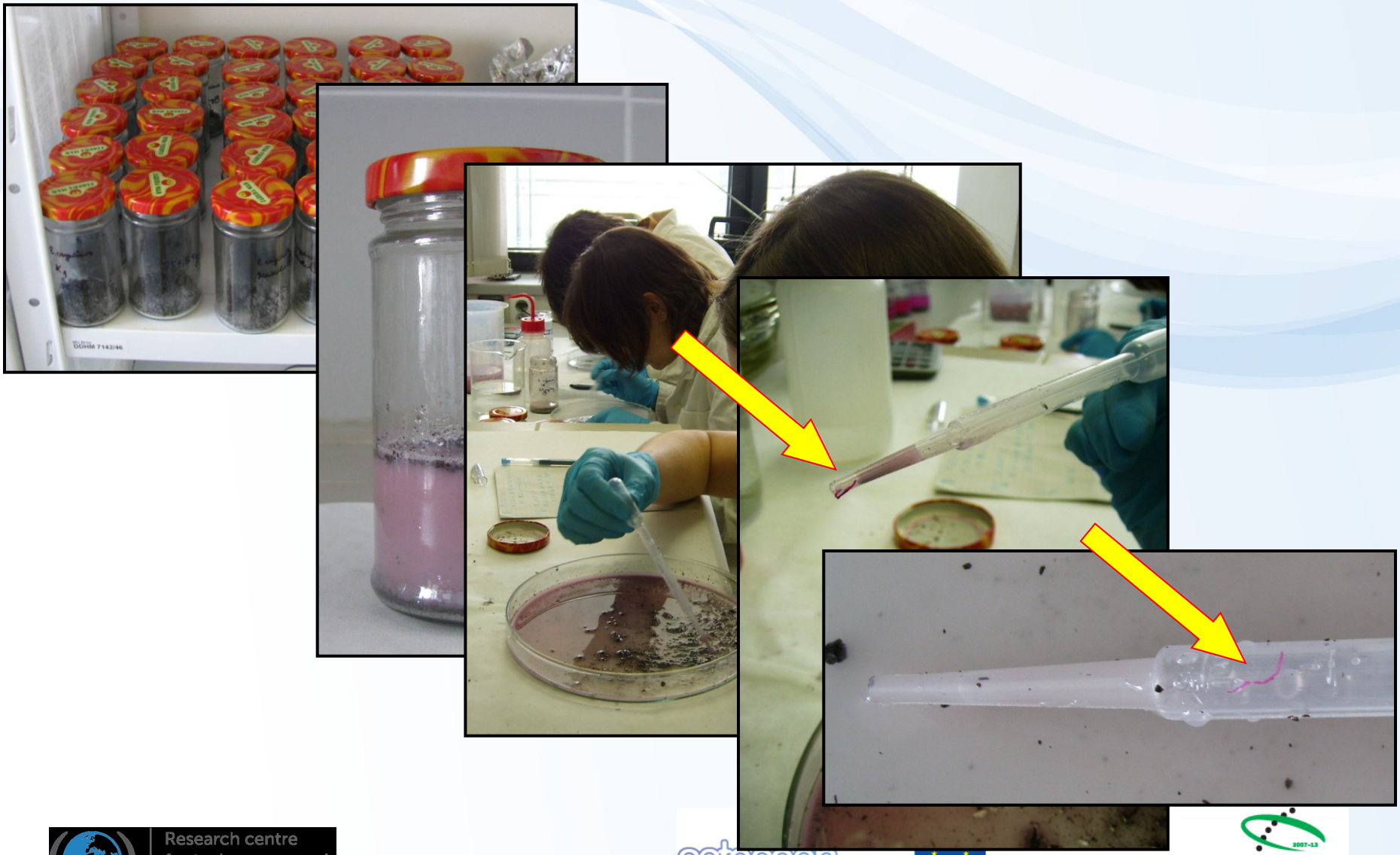
Enchytraeidae



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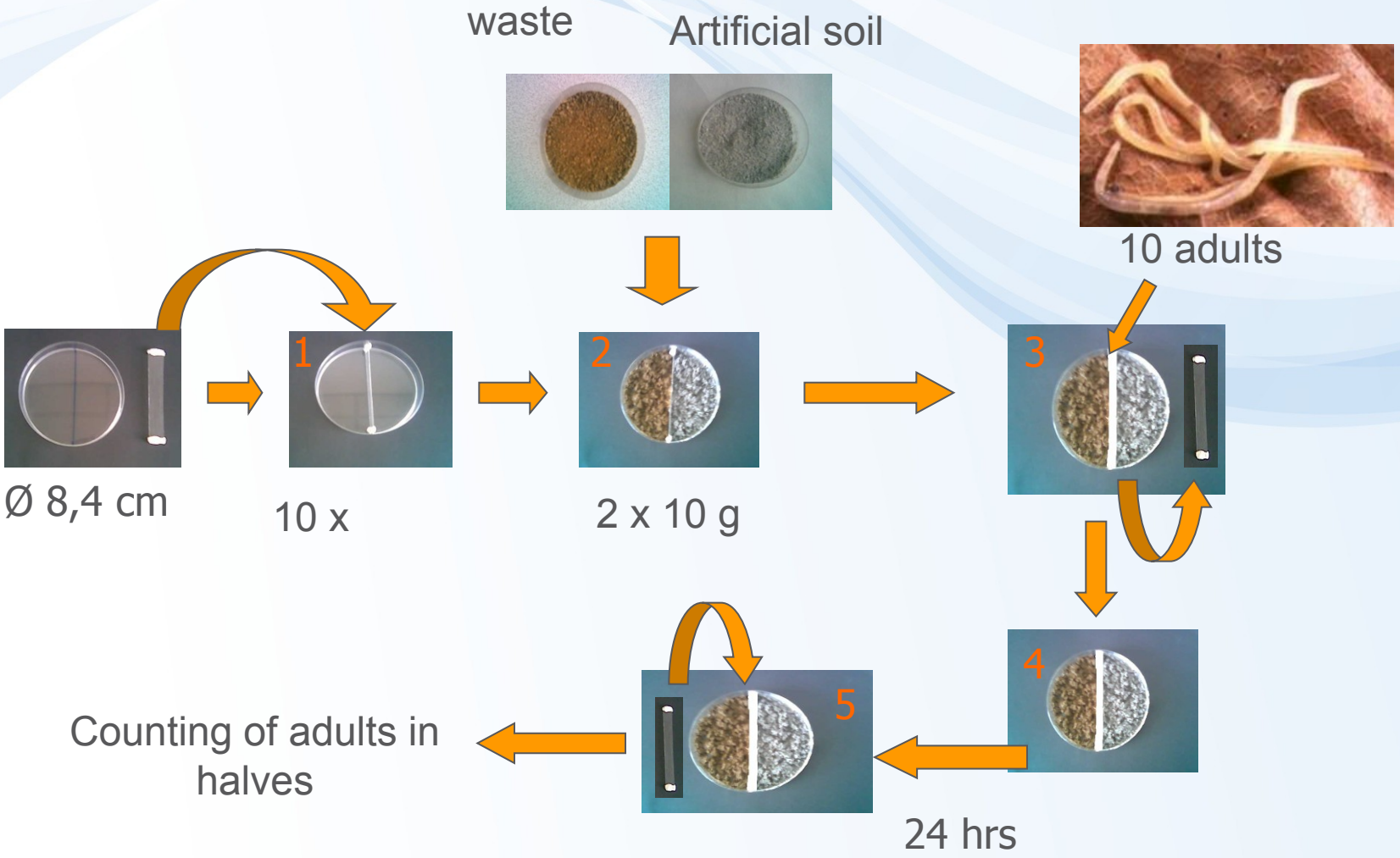
Avoidance test with *E. albidus*



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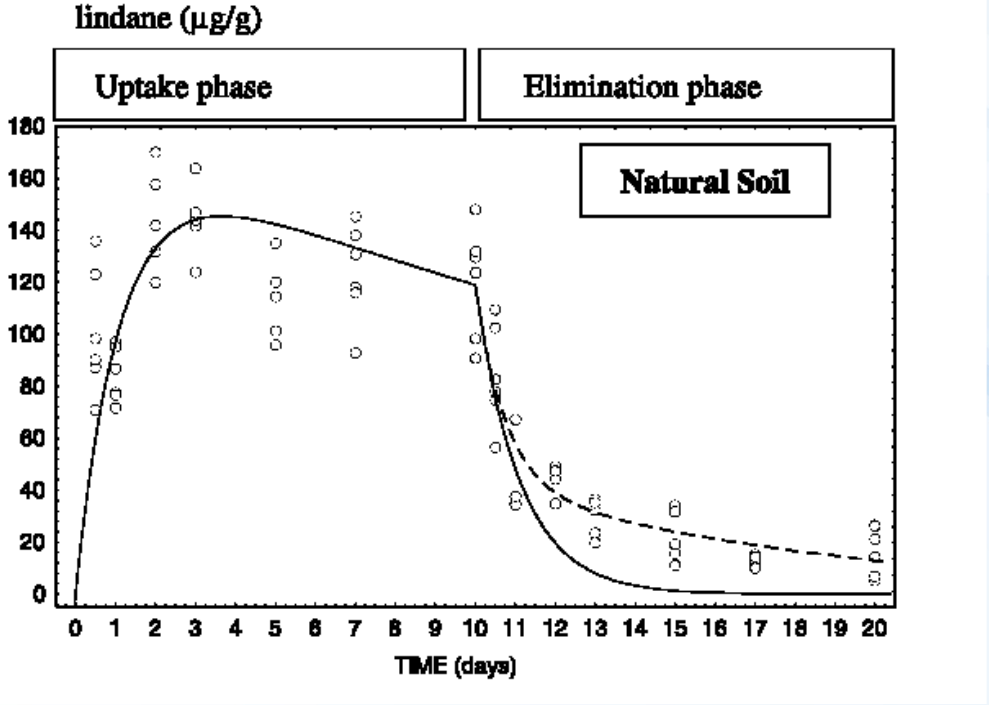
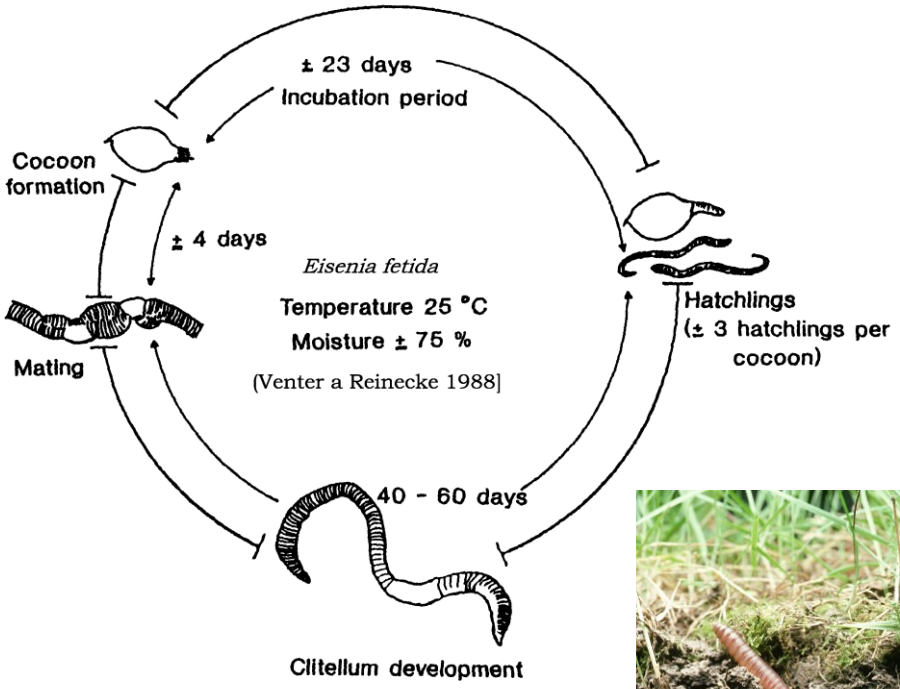
Bioaccumulation experiments with enchytraeids

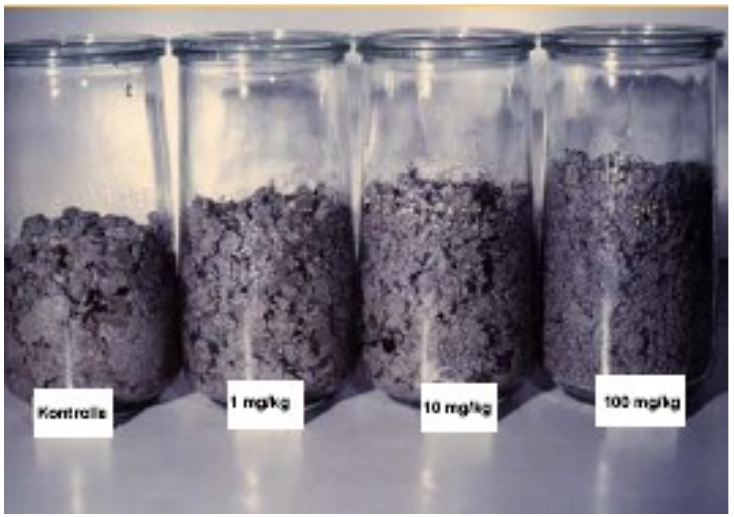


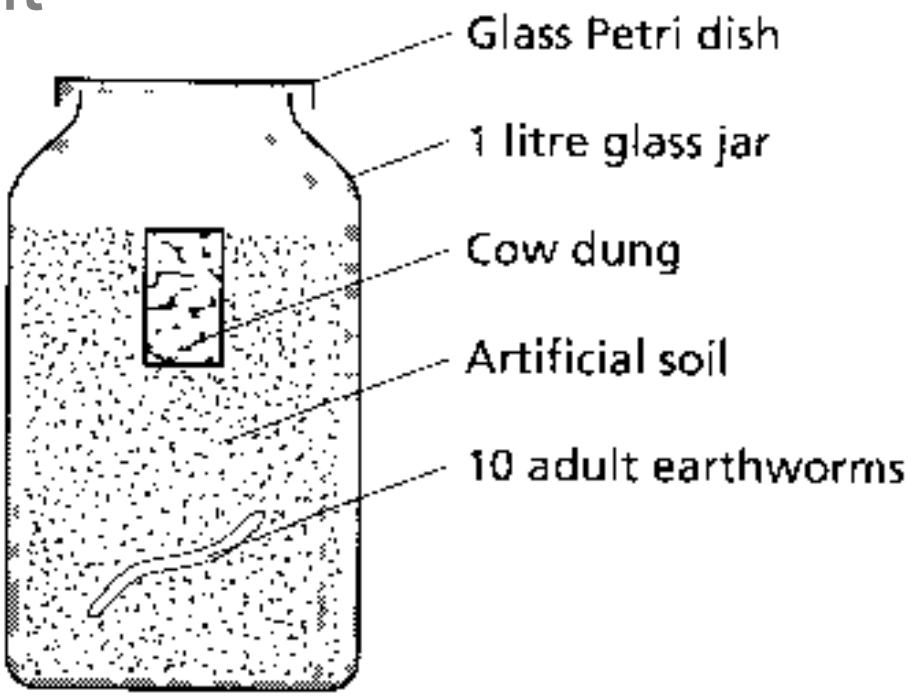
Earthworms


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

Earthworm acute toxicity test

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





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Earthworm reproduction test

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

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Eisenia fetida reproduction test

Příprava půd

Měření WHC půd

Ovlhčená AS rozvážená do testovacích nádob

Výběr 10 reprezentativních adultů z chovu a jejich omytí dH_20

Zvážení jedinců

Přídavek 10 adultů do nádoby na test

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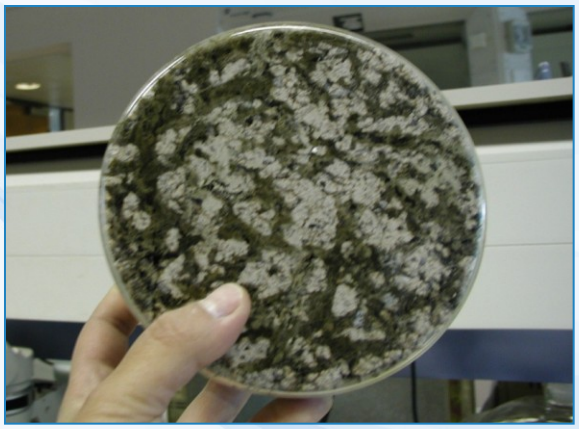
E. fetida test – po 28 dnech

Nádoby během testu v kontrolované místnosti



Prohlídka nádob (známky aktivity)

Zhodnocení mortality

Zvážení žížal

E. fetida – po 8 týdnech

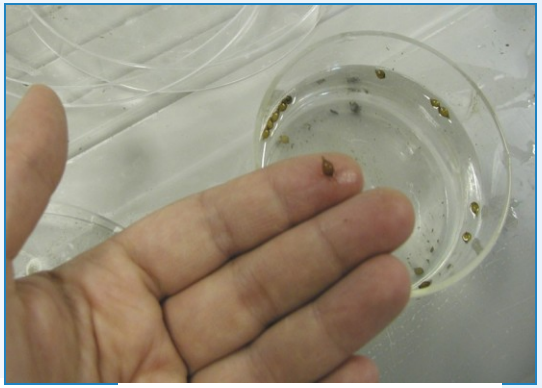
Vodní lázeň s narůstající teplotou 40°C až 60°C

Po cca 20 min juvenilové na povrchu

Sbírání a počítání

Přesátí půdy

Ruční třídění kokonů



Počítání

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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

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Folsomia candida

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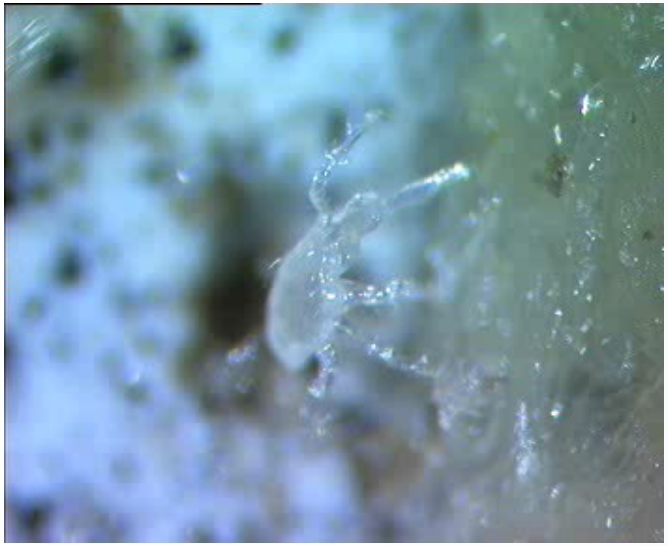
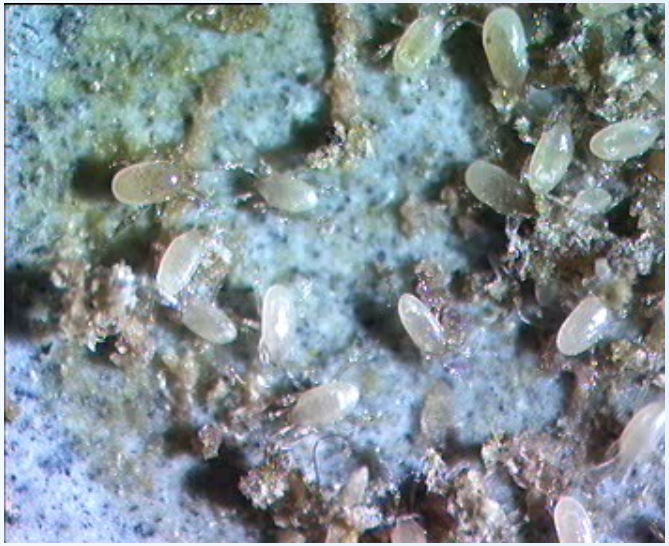
Mites



Hypoaspis aculeifer



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Mites

predator

cont. soil

prey

Plants

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Lactuca sativa root growth

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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

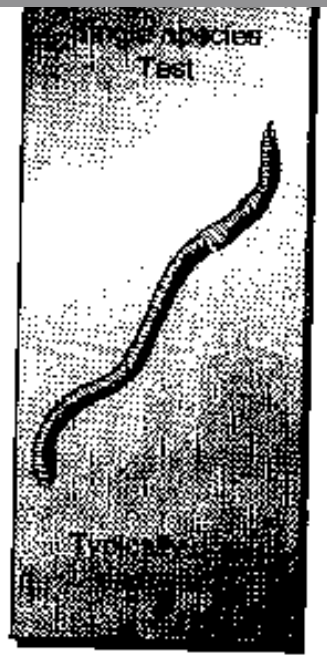
- **Auflauftest**: Auswirkungen auf Keimung und Auflauf
- **Wachstumstest**: Auswirkungen auf den Biomassezuwachs

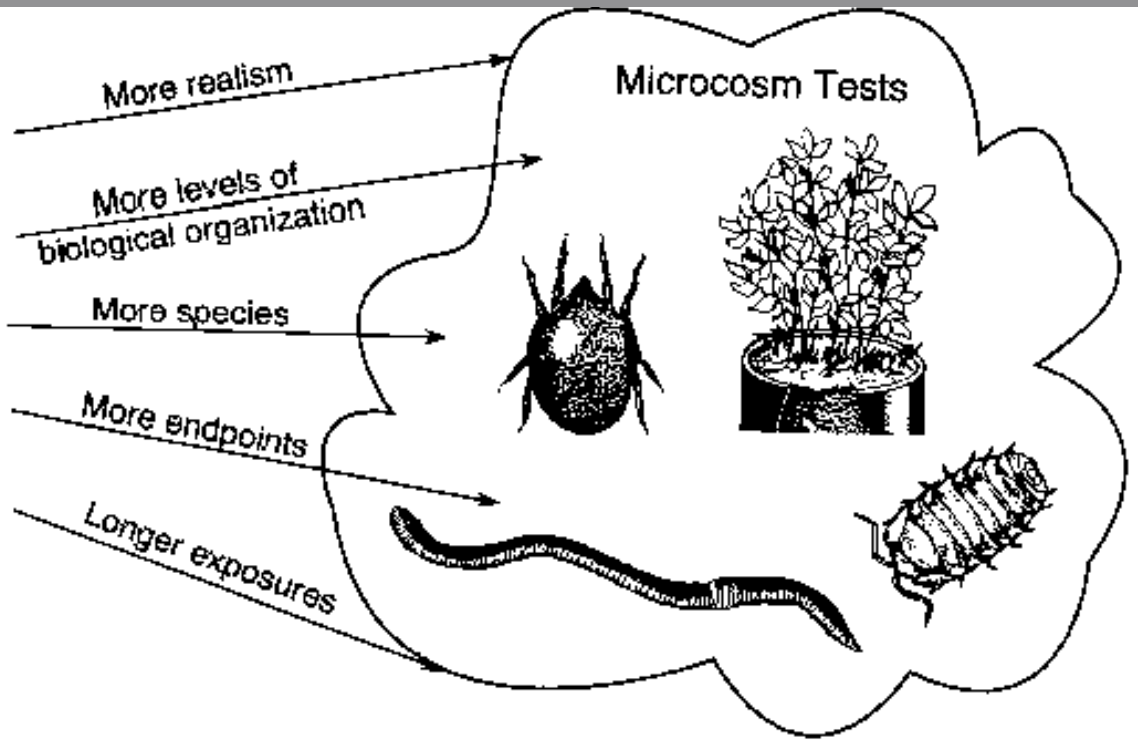
TER < 10

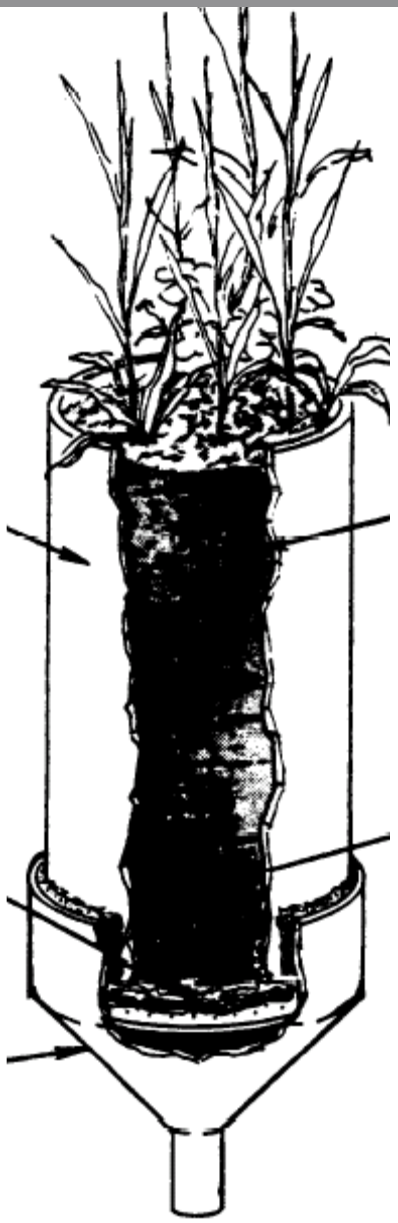
2. Stufe: Weiterführende Versuche

- Verlängerte Gewächshausversuche
- Mehr Arten
- Freilandversuche

Microcosms - TME







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Microcosms - TME