Innovative Decision Support Tools for Risk Based Management of the Environment in Hungary

MOKKA CONFERENCE

INTERACTIVE SOIL TOXICITY TESTS

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Introduction

- During the last ten years it has been recognised, that traditionally applied physico-chemical methods do not provide enough information and a sufficient basis for the environmental risk assessment of pollutants.
- The conventional analytical concept of risk assessment does not consider:
  - the mobility of contaminants in the soil matrix,
  - the partition of chemicals among various phases of environmental elements,
  - their biodegradability,
  - their bioaccumulation potential and
  - their biological effects.

Biological and toxicity testing may close this gap

Toxicity testing of soil

Characterisation of the actual risk of the contaminant on the soil ecosystem

Soil-extract or whole soil?

- The partition of the contaminant in the phases of the soil (sediment) is not in equilibrium and depends on the effect of the biota.
- Soil-elutriate testing can lead to an underestimation or overestimation of total soil toxicity.
- The interactions between the soil and the test-organism, and the contaminant and the test-organism can not be realised in case of elutriate testing.

To model the real situation

Direct contact test of whole soil – interactive soil toxicity tests

Interactive soil toxicity test

Direct contact toxicity testing of soil samples

- Measures chemically not measurable toxicants by their effect
- Integrates interactions between toxicants in case of mixed pollutant
- Integrates interactions between toxic contaminants and matrix, contaminants and biota
- Is able to measure the effects of whole soil
- Characterises the binding capacity of the soil
- Measures bioavailable ratio of the contaminant
- Measures the effects of chemicals not included into the analytical programme

Methodological developments

1. *Laboratory bioassays with single-species testorganisms*

   These are self-developed direct contact soil toxicity tests with microbes, plants and animals, based on similar Hungarian and European standard methods for wastewaters or hazardous waste materials.

2. *Whole soil tests*

   Whole soil as a complex test-organism is applied for toxicity testing, similarly to single organisms. In our laboratory we used nitrification and respiration of good quality healthy soil as an endpoint for ecotoxicity testing of contaminated soil.

3. *Soil microcosms*

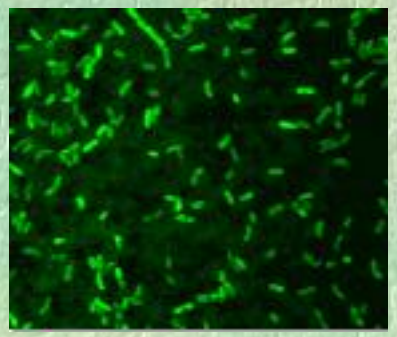
   Microcosms are multi-species systems and the testing in them are generally multiparameter measurements (respiration, enzyme activities etc.).

Vibrio fischeri bioluminescence inhibition test

- Testorganism: *Vibrio fisheri* NRRLB – 11177

  Vibrio fischeri is a common marine organism and can routinely be isolated from fresh fish. Under optimum growth conditions, it is a very brightly glowing species.

- Principle of the method: detection of the luminescence light emitted by *Vibrio fischeri;* when toxic substances are present, the emitted light decreases.



Micrograph: fluorescently stained cells

Liquid culture in well lit place

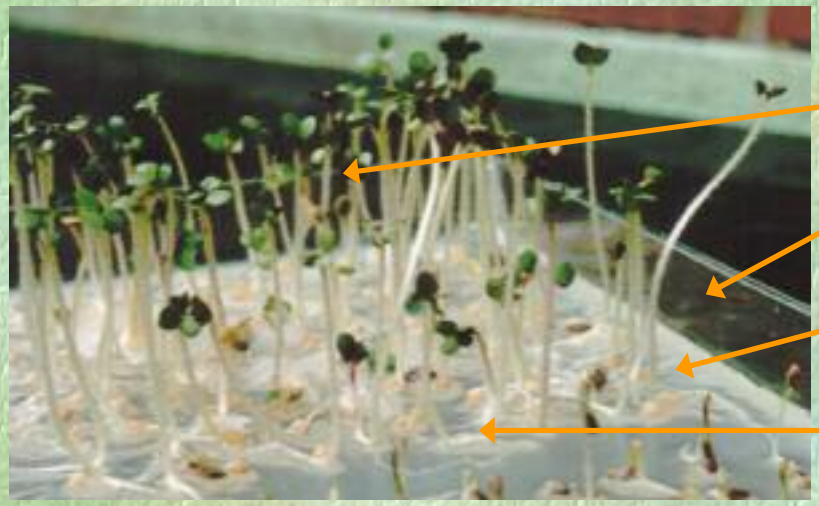
Liquid culture in darkness

On Petri plates with solid medium

Vibrio fischeri bioluminescence inhibition test

- Type of the test: bacterial, single-species acute toxicity test
- Testorganism: *Vibrio fischeri* in freshly prepared inoculum
- Area of application: investigation of water, wastewater, porewater, soil extract, soil and sediment
- Sensitivity of the testorganism: *Vibrio f.* is sensitive to heavy metals and also organic contaminants
- Guideline: DIN 38412, US EPA microtox (modified by BUTE for whole soil, direct contact biotest)
- Apparatus: Luminometer
- Test medium: solid phase soil and sediment
- Test parameter: inhibition of light emission
- Endpoint: ED_{20} and ED_{50}; Cu-equivalent (ΣCu_{20} and ΣCu_{50} (mg Cu/kg soil)
- Duration of the test: 30 min

Plant tests



- A large number of plants were examined as testplant: white mustard *(Sinapis alba)*, pea (*Pisum sativum*), garden cress (*Lepidum sativum),* radish *(Raphynus sativus),* wheat (*Triticum sativum*), oat *(Avena sativa)* and maize *(Zea mays)*.
- They were seeded directly into the soil (or jellyfied soil: stabilising soil by agar-agar gel) to ensure the interaction between root and soil.
- Plant growth in acute tests is measuring by root- and shoot-elongation.

Sinapis alba root and shoot elongation test

- **Type of the test**: plant, single-species acute toxicity test
- **Testorganism**: *Sinapis alba (*white mustard*)*
- **Area of application**: investigation of water, wastewater, porewater, soil extract, soil and sediment
- **Sensitivity of the testorganism**: *Sinapis alba* is sensitive to heavy metals and also organic contaminants
- **Guideline**: HS 21976-17, 1994 (modified by BUTE for whole soil, direct contact biotest)
- **Apparatus**: ruler (visual evaluation)
- **Test medium**: solid phase soil and sediment
- **Test parameter**: inhibition of root- and shoot elongation
- **Endpoint**: ED_{20} and ED_{50}
- **Duration of the test**: 3 days

Folsomia candida mortality test

The *Collembolan*s, commonly known as springtails, are the most numerous and widely occurring insects in terrestrial ecosystems.

- Microarthropods: have an important function regarding the maintenance of soil functions
- Short life cycles, high number of species and high density (indicator organisms)

Test method requires a sufficient number of sub-adult animals at the same age (12-14 days old)

Adult female (1.8 mm) with eggs

Adult females (2 mm in length) with juveniles

Adult female (2.0 mm).

Folsomia candida mortality test

- Type of the test: animal, single-species **acute** (mortality) and/or chronic (reproduction) test
- Area of application: investigation of soil and sediment
- Sensitivity of the testorganism: *Collembola* is more sensitive for organic contaminants than heavy metals
- Guideline: ISO/TC 190 SC4, WG2 ISO, Riepert and Kula, 1996
- Test medium: solid phase soil or sediment
- Test parameter: **acute test – mortality**, chronic test - reproduction
- Endpoint: acute test – LD20, LD50; chronic test – NOEC
- Duration of the test: **2 weeks (acute),** 4 weeks (reproduction)

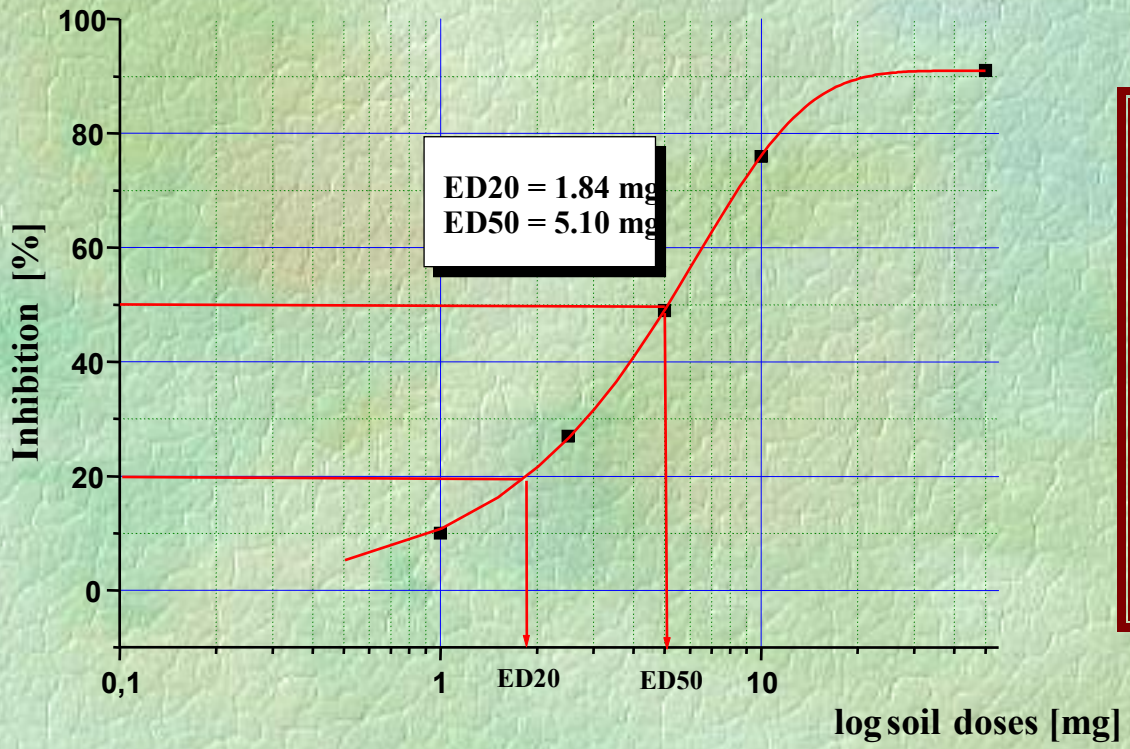
Evaluation and interpretation of toxicity tests

- Inhibition percent values are plotted at the given mass of contaminated soils by the use of Origin 6.0 software.
- ED_{20} and ED_{50} values are determined from dose-response curve (inhibition percent values of different dilutions) after sigmoidal fitting of data by ORIGIN 6.0 software. ED_{20} and ED_{50} = soil doses, which caused 20 % and 50 % of inhibition.
- In case of *Vibrio fischeri* test, the inhibition of samples is given in Cu-equivalent by comparing the measured results to a Cu-calibration curve: ΣCu_{20} and ΣCu_{50} (mg Cu/kg soil).
- These Cu-equivalent values can be compared with the effect based soil quality guidelines.
- $\Sigma Cu20 = ED_{20\ Cu} / ED_{20\ sample} * 10^6$; toxicity given in Cu-equivalent: the Cu-concentration, which would cause the same toxicity.

Evaluation and interpretation of toxicity tests

Vibrio fischeri bioluminescence inhibition test

Dose-response curve of a contaminated soil sample



Characterisation of the contaminated soil on the bases of the ΣCu_{20} / ΣCu_{50}

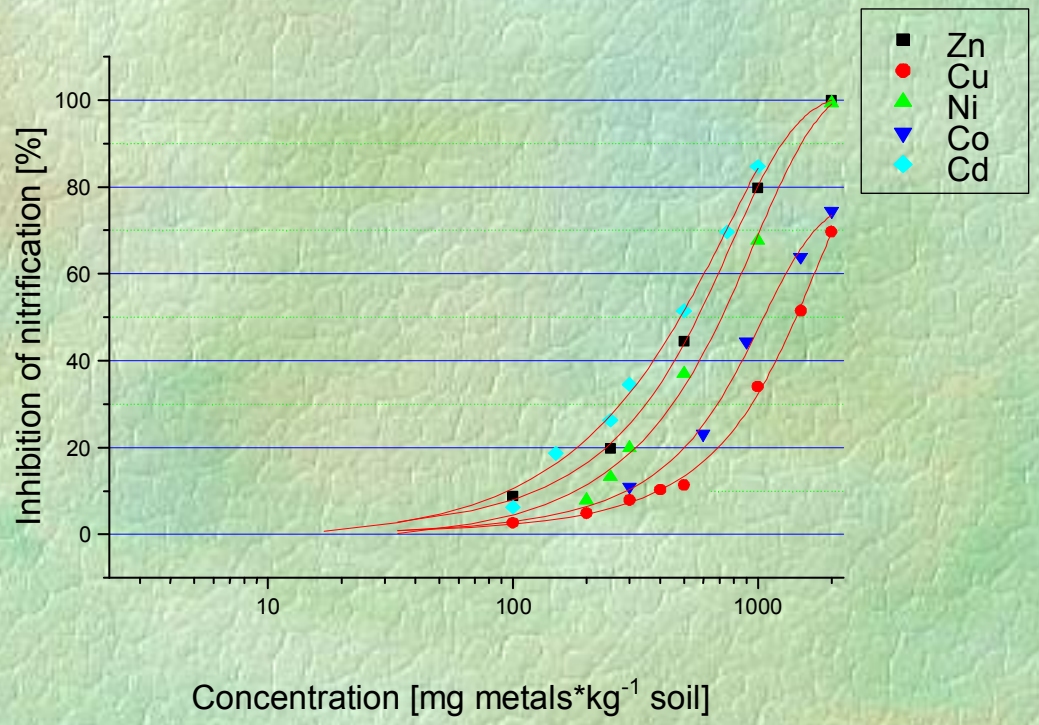
ΣCu_{20} [mg Cu / kg soil]	ΣCu_{50} [mg Cu / kg soil]	Characterization
< 80	< 120	Non toxic
80-250	120-300	Slightly toxic
250-400	300-500	Toxic
> 400	> 500	Very toxic

„Whole soil tests”

- **Whole soil as a complex test-organism** can also be useful, similarly to single organisms in bioassays for ecotoxicity testing.
- A good quality, healthy reference soil is needed.
- The two end of the series are the contaminated soil and the clean soil, in between the dilutions in form of soil mixture.
- The activity of the clean soil will be inhibited by the tested soil.
- In our laboratory we used nitrification and respiration of a good quality healthy soil as endpoint for ecotoxicity testing of contaminated soil and wastes.
- ED_{20}, ED_{50} or NOEL can be determined, which means the amount of the contaminated soil, which causes 20 or 50 % decrease or no observable effect in the measured endpoint (nitrification, respiration).

„Whole soil tests" – inhibition of nitrification

- The nitrification activity inhibition of heavy metals contaminated soils was tested .
- The good quality, uncontaminated garden soil with high nitrification activity was used as "test organism" for the nitrification activity inhibition test.

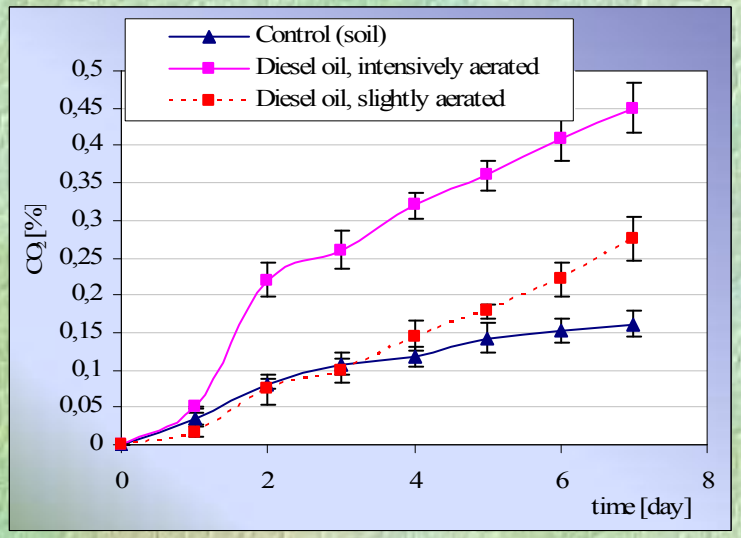


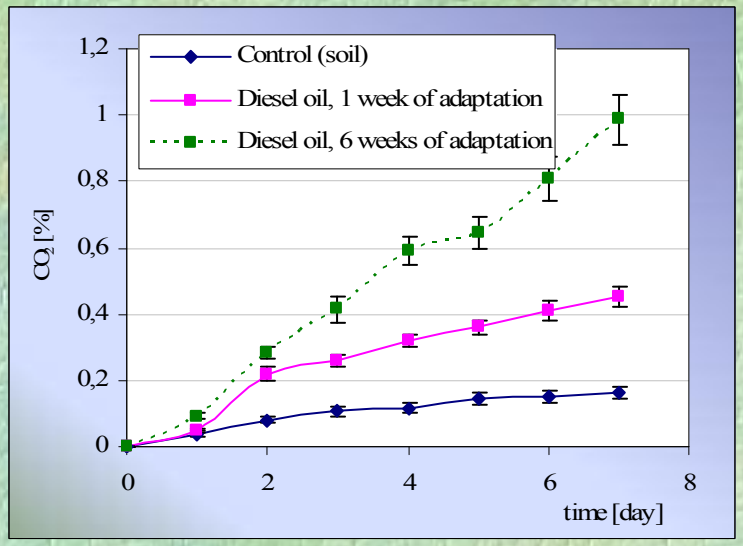
Soil microcosms

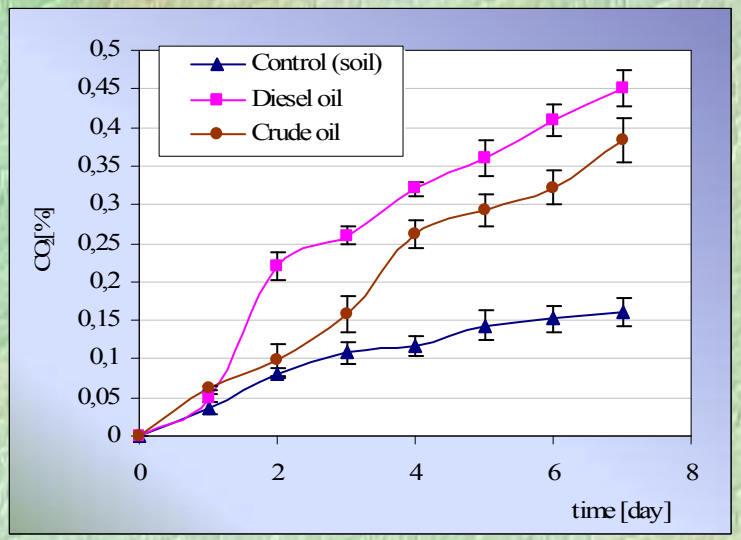
- Microcosms are multispecies systems and the testing in them are generally multiparameter measurements.
- Complex procedures and interactions can be investigated; the soil can be sampled and the history of the microcosm can be followed.
- Depending of the aim and strategy of the testing we can measure:
  - the absolute values of soil respiration or enzyme activities,
  - the changes of the activities during natural or experimental processes,
  - response on the effect of added contaminants or contaminated materials,
  - behaviour of the soil during natural attenuation and enhanced attenuation,
  - effect of technological parameters on bioremediation.

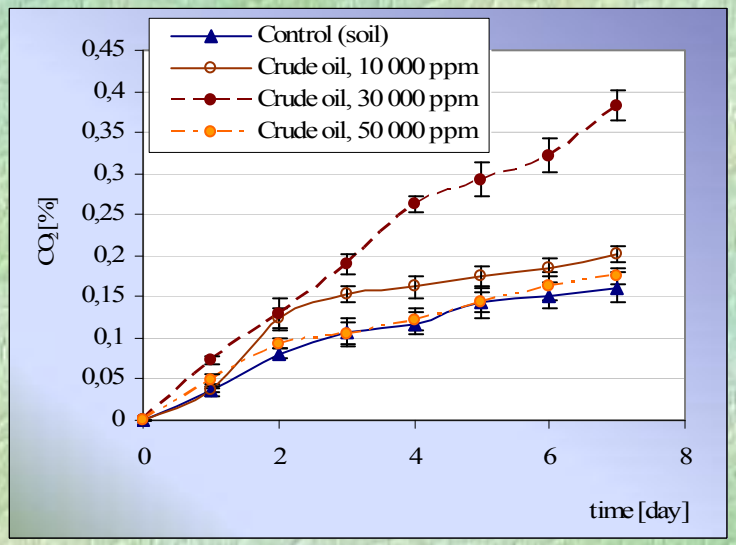
Soil microcosms – respiration of the soil

The effect of aeration, adaptation and types and concentrations of contaminants were tested in a self-developed soil-respirometer.









Characteristics of developed bioassays

- Acute toxicity and chronic toxicity tests
- *Endpoints*: survival, growth, immobilisation, respiration, enzyme activities, reproducibility, etc.
- *Testorganisms*: single species used from three different trophic levels (bacterial, plant, animal); whole soil (good quality healthy reference soil)
- *Test-methods*: single species or multispecies (microcosms) test-sytems
- *Evaluation:* ED_{20} (LD_{20}) and ED_{50} ($LD5_0$) values are determined from dose-response curve after sigmoidal fitting of data by ORIGIN 6.0 software
- *Interpretation*: The inhibition of samples (in case of *Vibrio fischeri* bioluminescence test) is given in Cu-equivalent by comparing the measured results to a Cu-calibration curve: ΣCu_{20} and ΣCu_{50} (mg Cu/kg soil).

  The Cu-equivalent value means the Cu concentration that would causes the same toxicity as the actual pollution in the soil → "Copper Equivalent Method"
- *Application*: determination of toxicity, biodegradability, bioavailability, bioaccumulation potential etc.

Thanks for your attention!