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Integrated methodology and interactive ecotoxicity tests for contaminated soil

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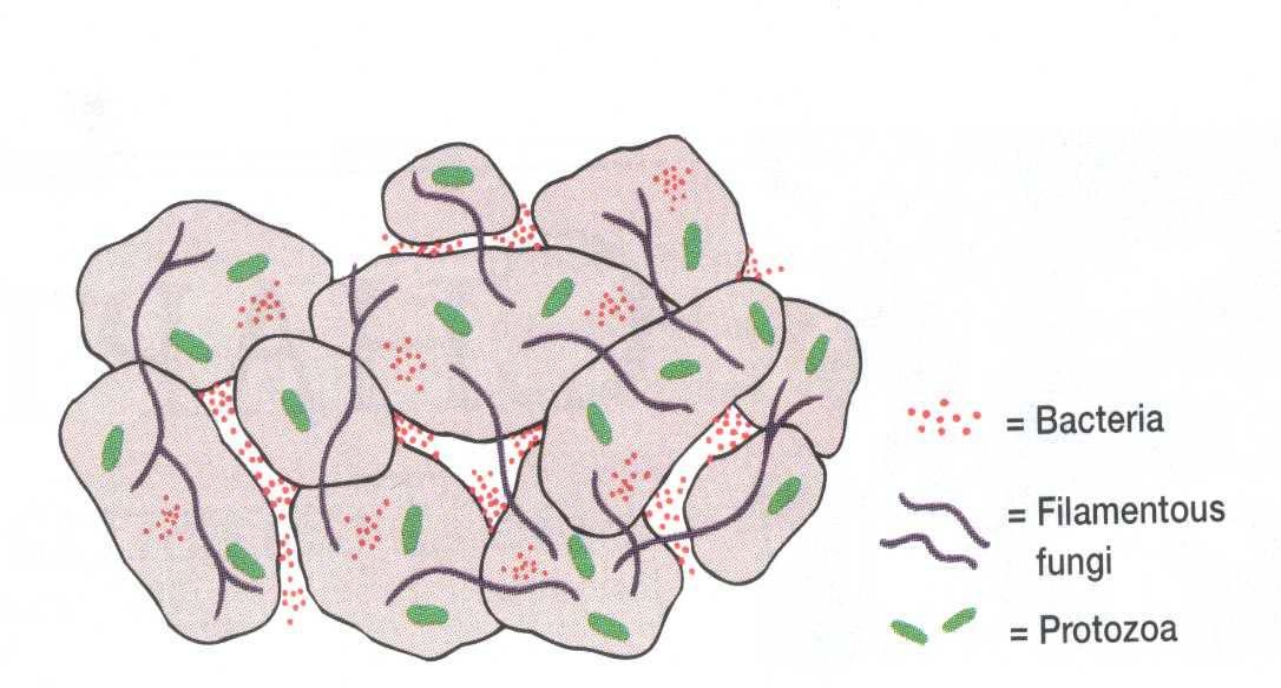
2008

Soil is an organism



10^6-10^9 organisms are living in 1 cm^3 of soil.

Health conditions and the adaptive behaviour of the soil are responsible for the actual effects of the pollutants.

Results of chemical analyses alone are not able to characterise the risk of contaminants for soil and its users.

Position and role of environmental toxicology

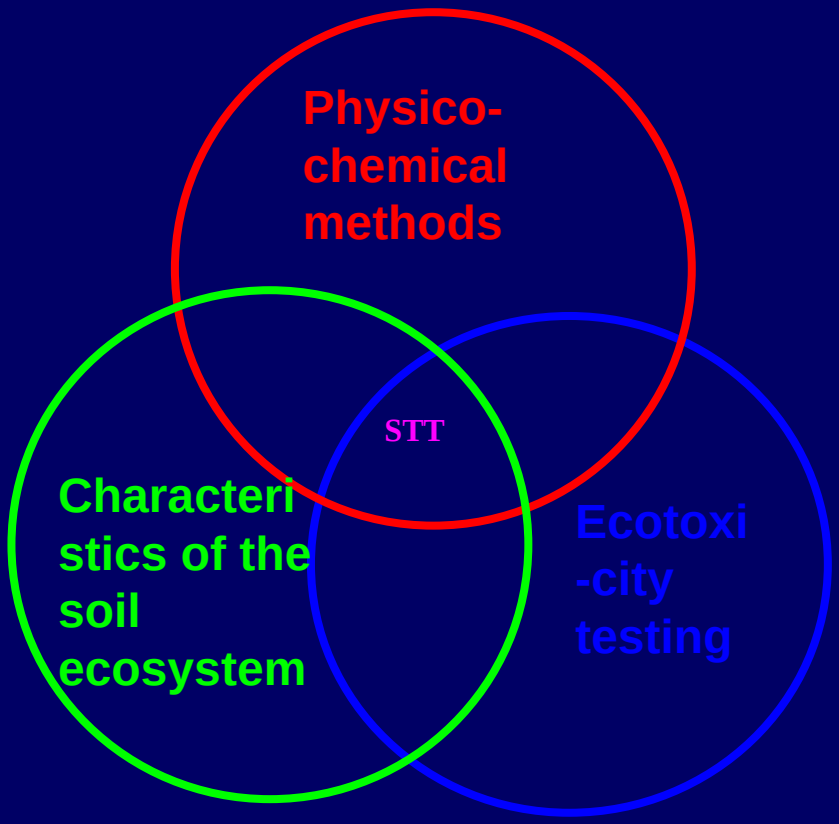
Identification of hazard
Assessment of risk
RQ=PEC/PNEC

categorization and priorisation
generic and site specific RA

Prevention
Remediation
Restricted use

legislation
monitoring
wwtp
limitation in production and use

STT, the SoilTestingTriad



STT, the SoilTestingTriad

Importance of physico-chemical, biological methods and toxicity testing is the same. They are complementary.

STT gives information

- on the quality and quantity of contaminant,
- on the characteristics of the soil: the biological status, the activity, vitality and adaptive behaviour
- about the effects, mobility, bio-availability, iodegradability of the contaminant.

STT measures the response of the soil for external effects.

STT application

Screening / mapping of contaminated sites

Environmental monitoring

Detailed site assessment

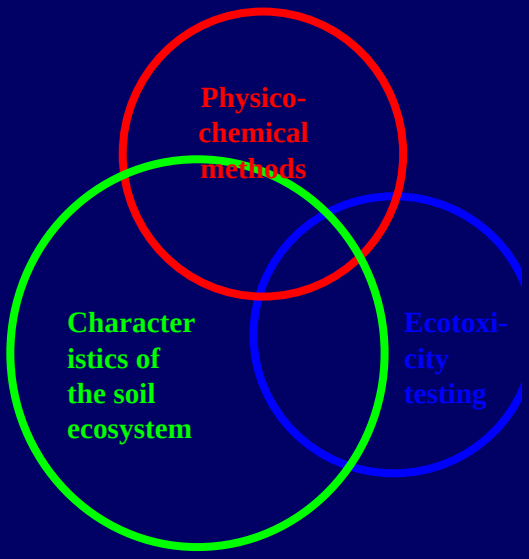
Planning remediation technology

Planning environmental biotechnology

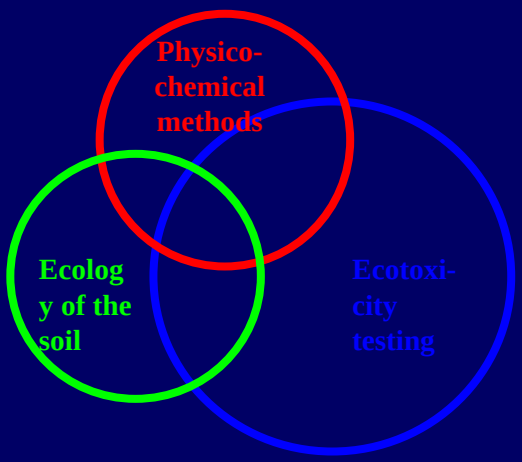
Monitoring of soil remediation

Qualification of the remedied soil

Screening and mapping contaminated sites



In the assessment of a contaminated natural site the ecological assessment may have dominance. The visually assessable characteristics,like the lack of the vegetation, or presence or absence of certain animal species is characteristic in case of an old, long term contamination. Typical endpoints: lethality, yellowish vegetation, limited growth, special changes in diversity, resistant species, escape of animals from the site, etc.



SoilTestingTriad has high importance in the assessment of inherited, long-term contaminated industrial or military sites.

Ecotoxicity testing has dominate importance in this case, because the applicability of physico-chemical analytical methods are limited by the presence of not identified chemicals, mixtures of chemicals and metabolic products. On industrial and military sites generally no visual endpoints are to be found. Applicable bioassays: *Vibrio fisheri* bioluminescence-inhibition or *Bacillus subtilis* growth-inhibition tests.

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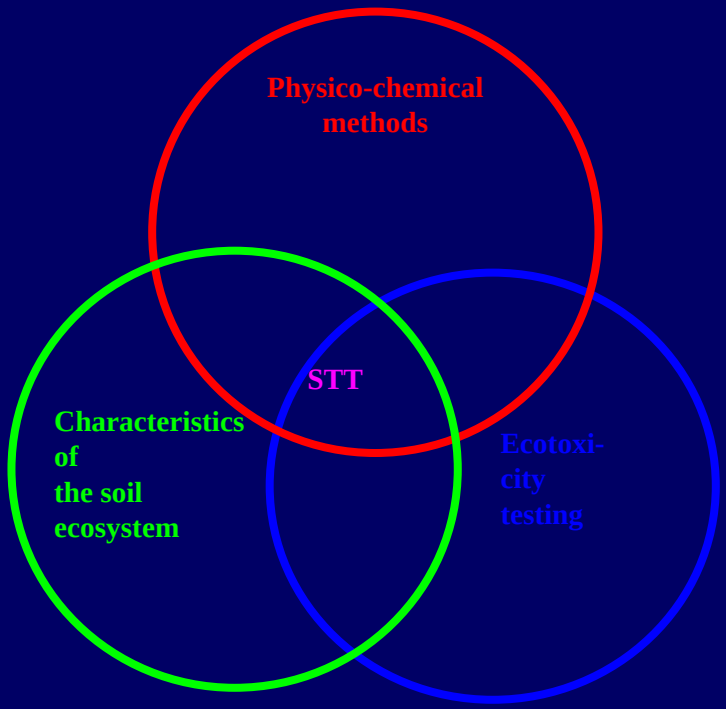
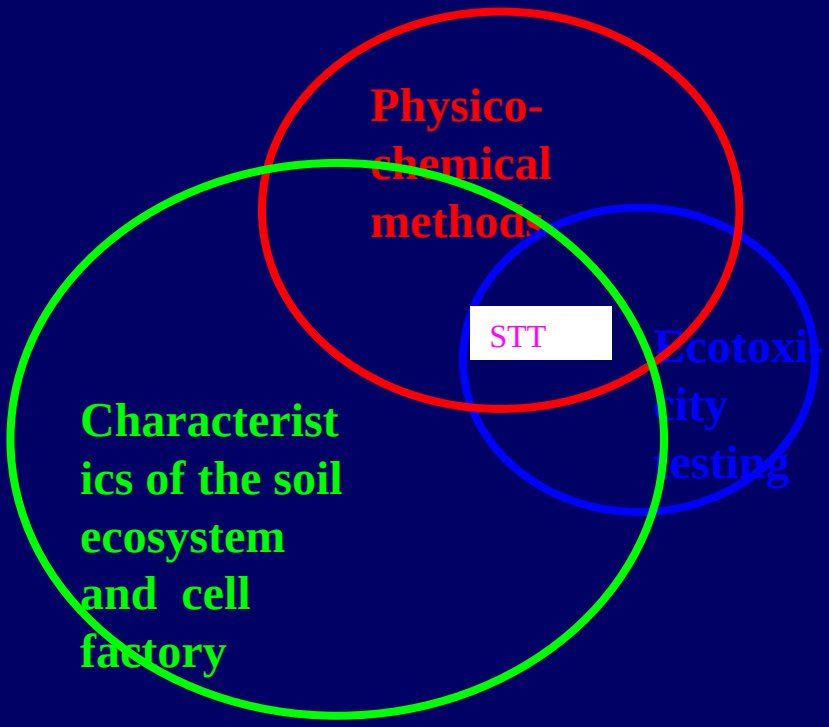
Detailed site assessment, planning an *in situ* remediation technology



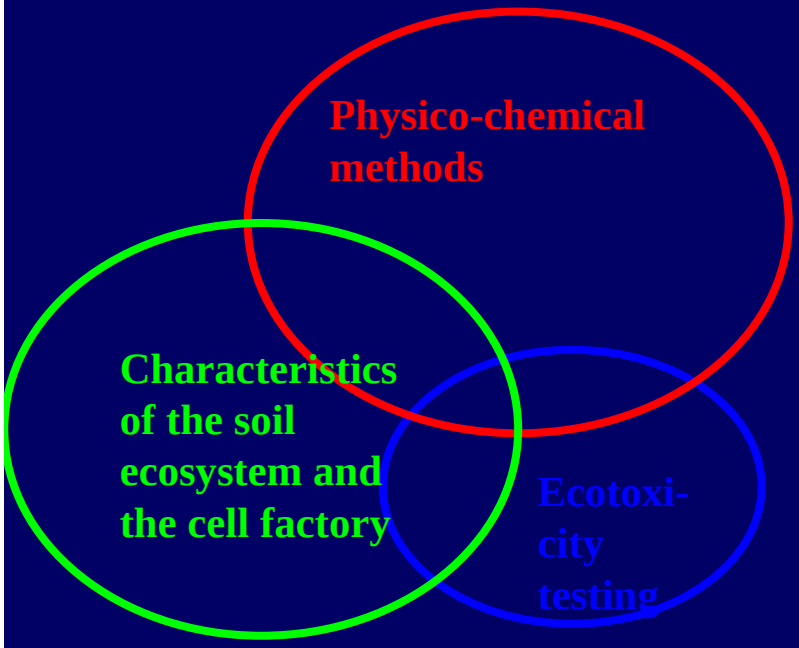
STT: SoilTestingTriad: during detailed site assessment and planning an *in situ* remediation technology the three elements of the Triad have equal importance. They give complementary information on the quality and quantity of the contaminant, the state of the soil, its viability and activity, on the effect of the contaminant, its mobility and availability, biodegradability, etc.

Planning of an environmental biotechnology



The utilisation of the STT in the planning of a bioremediation technology means the dominance of the biological testing of the soil microflora, the central core of the biotechnology. The quantity and quality of the cells, the enzyme activities and the respiration directly show the bioremedial potential and activity. Completed with the chemical-analytical data on the decrease of the contaminant concentration, we can prove the effective bioremediation.
Ecotoxicity gives information on bioavailability and serves to control soil quality.

Monitoring of the bioremediation



The technology monitoring makes possible:

1. To run the technology on the optimum
2. To control and regulate the technology
3. To control the emission
4. To control and qualify the final treated soil

STT: SoilTestingTriad

During the monitoring of the bioremediation both the physico-chemical characteristics and the information about the state of the cell factory are important. Part of the physico-chemical analyses serves the characterisation of the biological activity: respiration, metabolites. Ecotoxicity testing has less importance during the remediation, it serves the safety or gives information on bioavailability of the contaminant.

In case of *in situ* remediation, ecotoxicity testing plays role in the monitoring of emission from the technology. In the final phase of remediation it serves the qualification of the remedied soil.

Ecotoxicity testing of contaminated soil

Problems of testing soil samples from contaminated land

- mixture of contaminants: sinergism, antagonism
- interactions between contaminants, matrix and biota
- medium: extract, whole sample
- biotransformation: effect of products, biodegrdation
- availability: physico-chemical and biological availability differs
- analytical programme includes only part of the contaminants
- biotic and abiotic composition of the environmental sample

Ecotoxicity testing

- integrates interactions between toxicants
- integrates interactions between toxicant and matrix
- measures bioavailable ratio of the contamination
- measures chemically not measurable toxicants by their effect
- measures the effects of chemicals not included into the analytical programme

Expectations:

- ecological relevance
- reproducibility
- reliability
- robustness
- rensitivity

Direct contact

The **actual toxicity**: when measuring the effects of solid state samples and absorbed contaminants bioavailability is an important parameter.

Interaction: the test-results integrate mutual interactions between all participants: contaminant, contaminated media and test organism.

Environmental nature and fate of the contaminant: mobility, availability, biodegradability and partition is continuously changing in non-equilibrium systems, highly influencing the actual toxicity and the risk.

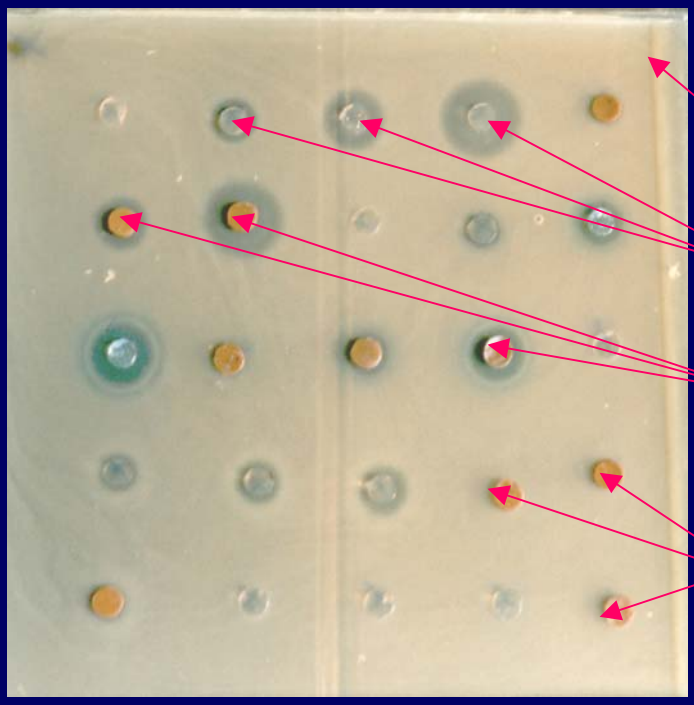
Integrated approach: physico-chemical analyses complemented by biological and ecotoxicity testing is used for assessing the **site specific** environmental risk of pollutants.

Ecotoxicity testing of soil extracts – except of modelling the risk for ground water by leaching, percolation, etc. – has two main disadvantages:

1. Chemical availability differs from the biological
2. Dilution of the sample results in a decrease in the sensitivity of the test.

Toxicity mapping of contaminated sites

***Bacillus subtilis* soil-diskette method: direct contact between the soil and the testorganism ensures mutual interaction.**



A non expensive screening method for contaminated sites, where the contaminants are not identified and their distribution is heterogeneous and unknown.

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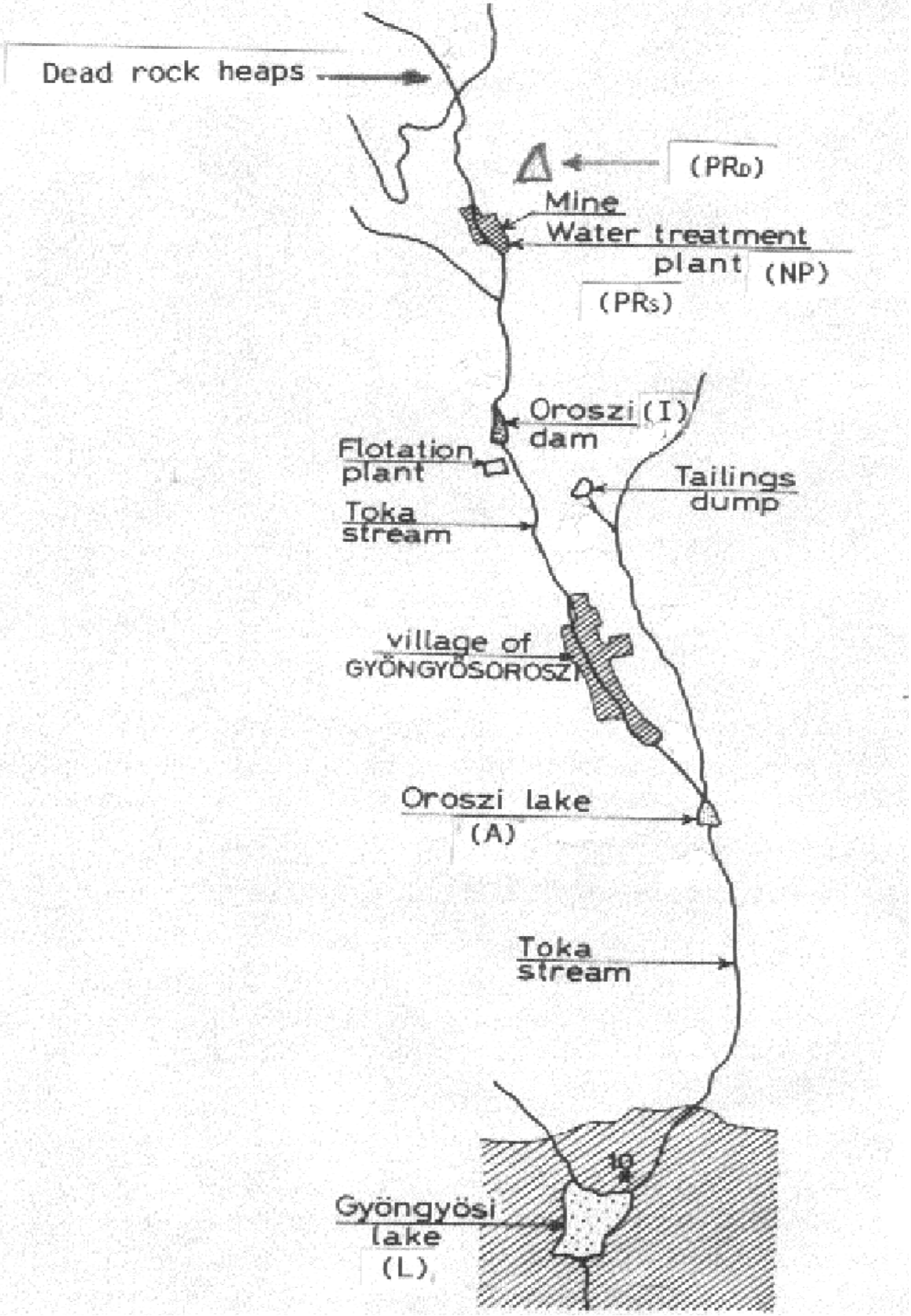
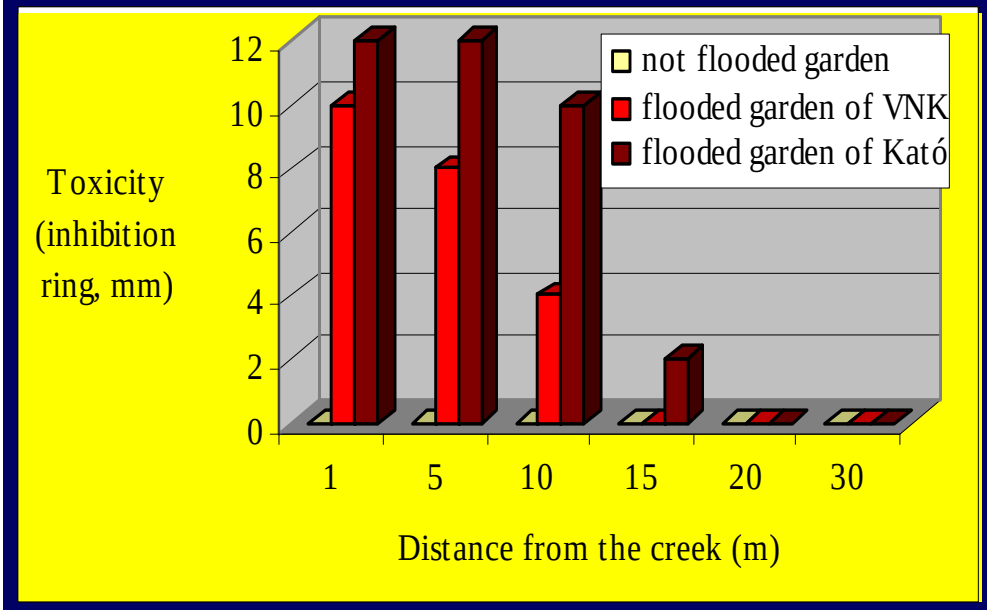
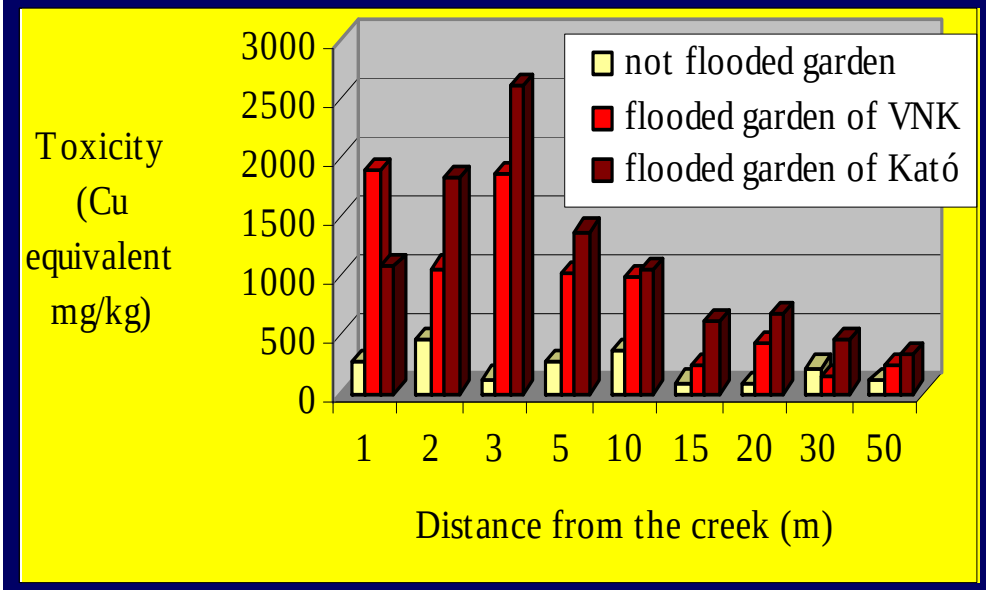
Demonstration site: Toka valley



Toxicity of the soil of the regularly flooded gardens



Toxicity of garden soils measured by the *Bacillus subtilis* "soil diskette" method



Toxicity of garden soils measured by *Vibrio fisheri* luminescence inhibition

Good correlation with the analytical data of the mobile fraction of the metals, extracted by EDTA, but not good correlation with the total metal content, extracted by king`s water.

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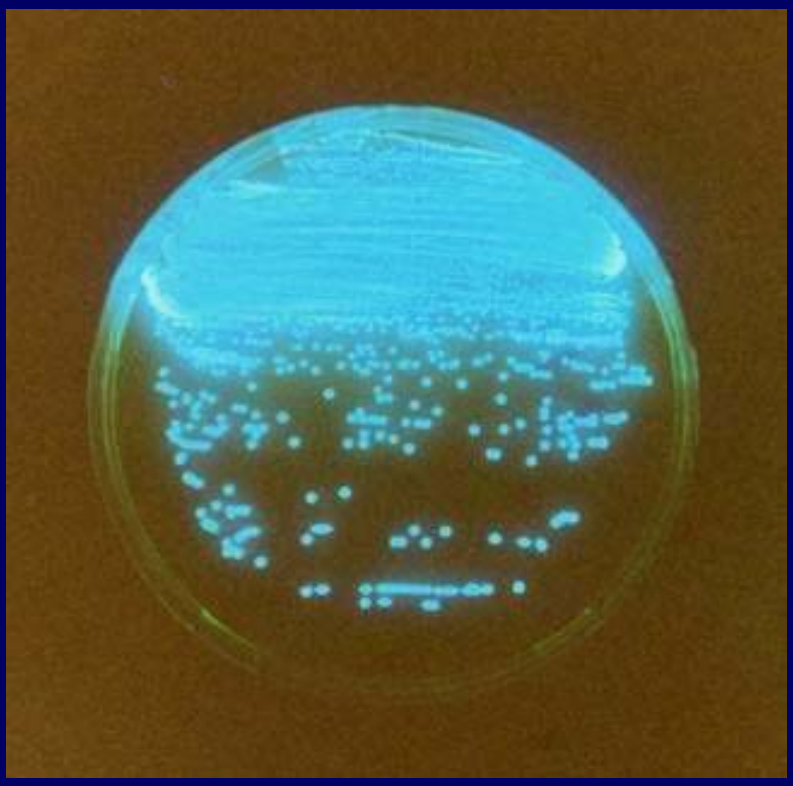
Culture of the luminobacterium *Vibrio fischeri*



$$FMNH_2 + O_2 + RCHO \xrightarrow{\text{luciferase enzyme}} h\nu\ (490\ nm) + FMN + H_2O + RCOOH$$

$FMNH_2$: reduced flavine-mononucleotide,
RCHO: luciferine: long chain aldehyde: light emitter

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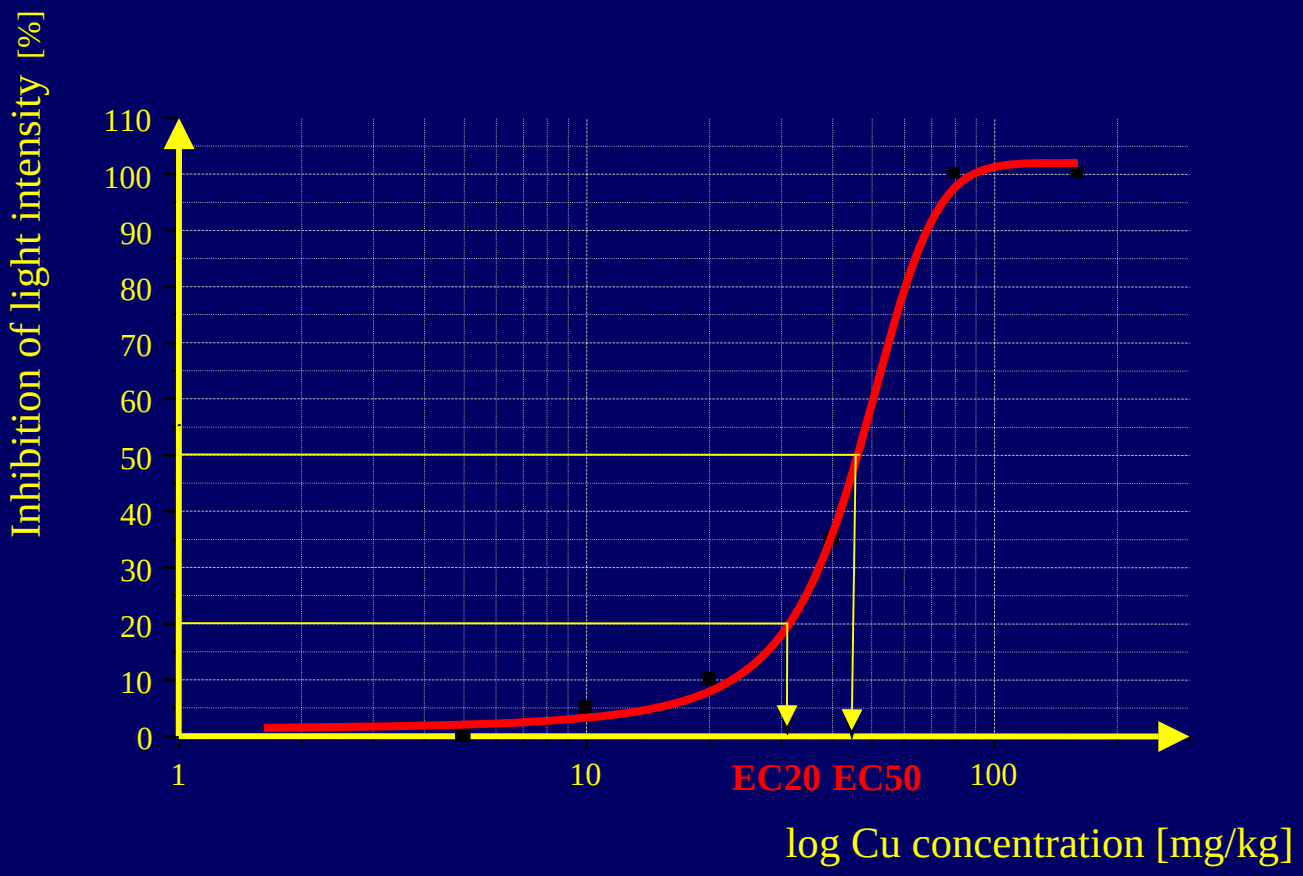
Luminescence inhibition expressed in copper equivalent



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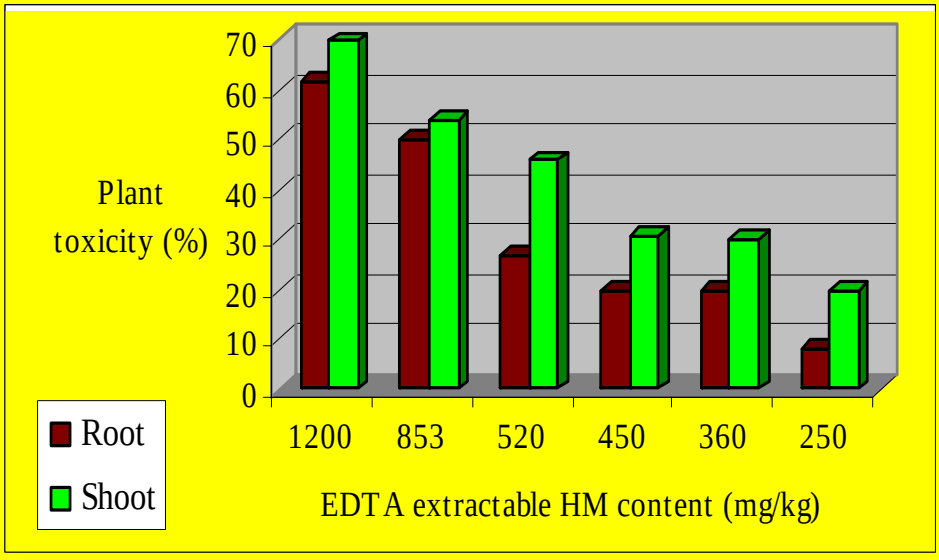
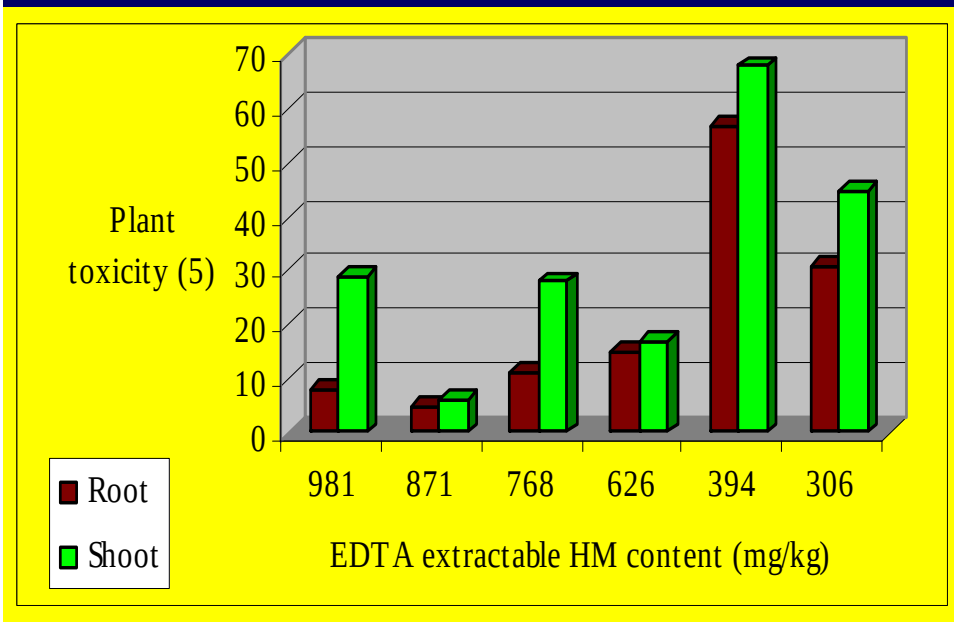
Biological and chemical availability differ from e.a.



Plant toxicity and the concentration of the measured mobile HM correlates well on soil samples from a homogeneous, regularly flooded garden.



No association was found between plant toxicity and the chemical analytical results of pollutants of different age and morphology from an other garden.

HM = As + Cd + Cu + Hg + Pb + Zn (mg/kg)

Metal content of samples from the tailing dump

Tailing	Zn (%)	Pb (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Co (mg/kg)
3M	9.1	7 041	6	5 940	1 680	606
4M	11.3	21 120	1	6 140	1 450	0
8M	1.9	2 970	46	1 010	90	175

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Ecotoxicity of tailing samples

	Azotobacter agile **dehydrogenase enzyme activity**	***Sinapis alba*** **seed germination and root elongation**	***Photobacterium phosphoreum*** **bioluminescence test**
Upper layer of the tailing material (mixed with soil) [M2]	Very toxic	Toxic	Very toxic
Inner layer of the tailing material (inert) [M6]	Non toxic	Slightly toxic	Non toxic

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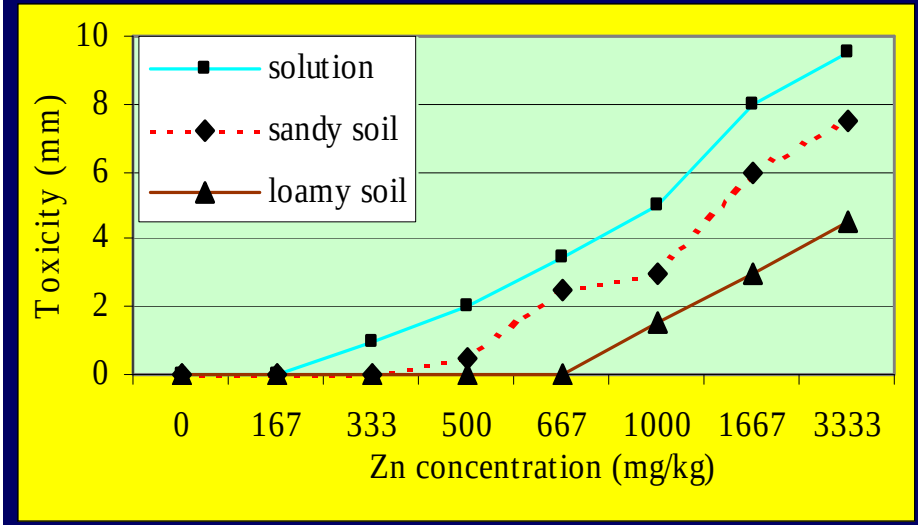
Total and soluble Zn, Pb and Cu content of tailing and soil samples

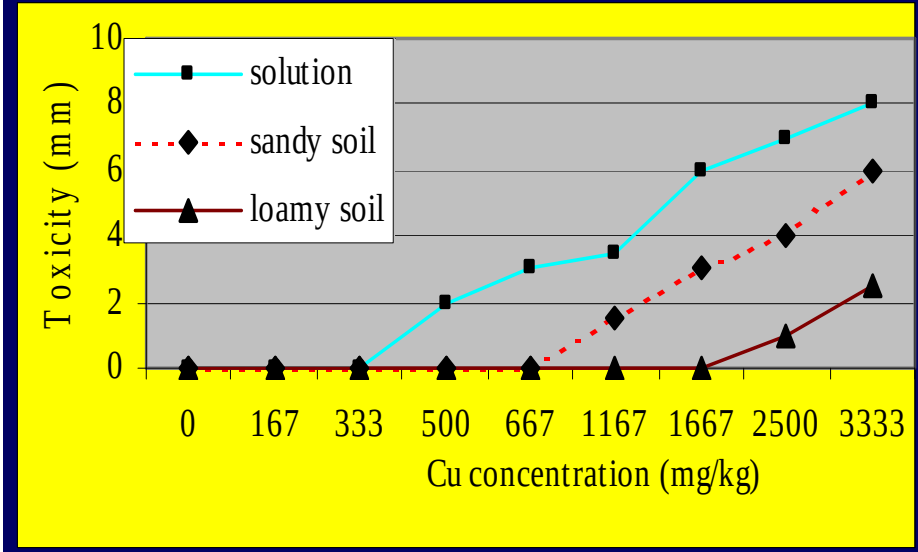
Sample	pH	Total metal content (mg/kg)			Mobile metal content (mg/kg)		
		Zn	Pb	Cu	Zn	Pb	Cu
Deeper layer, grey	7.0	31 858	4 971	2 450	3.4	1.2	0.6
Deeper layer, red	7.1	2 248	481	114	4.3	0.1	0.0
Deeper layer, yellow	7.3	7 571	2 766	984	3.9	1.7	0.6
Cover layer soil like	4.7	603	186	72	42.2	1.9	0.5

Sample	pH	Total metal content (%)	Mobile metal content (% of the total)		
			Zn	Pb	Cu
Soils of cont. gardens	4.0	100	78	50	58
Mine wastes	7.5		<0.1	<0.1	<0.1

Toxicity buffering capacity of the soil shown by ecotoxicity



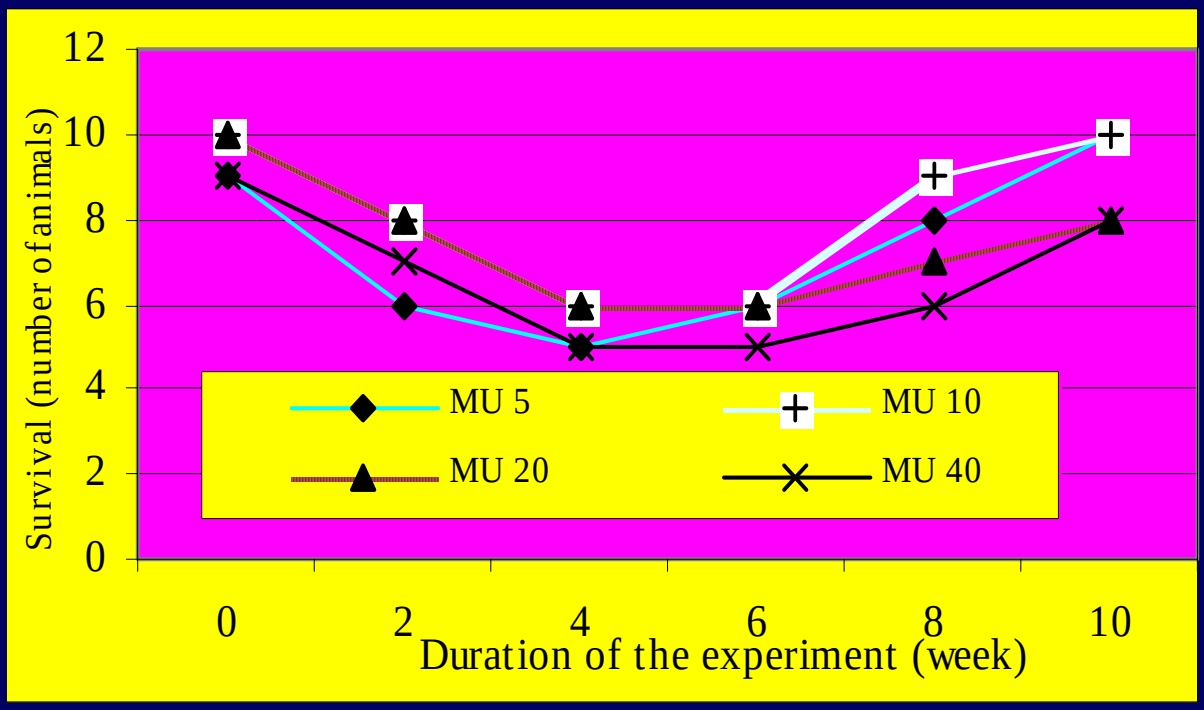
Pseudomonas fluorescence growth inhibition test with the same concentration of zinc in water, in sandy and in loamy soil



Pseudomonas fluorescence growth inhibition test with the same concentration of copper in water, in sandy and in loamy soil

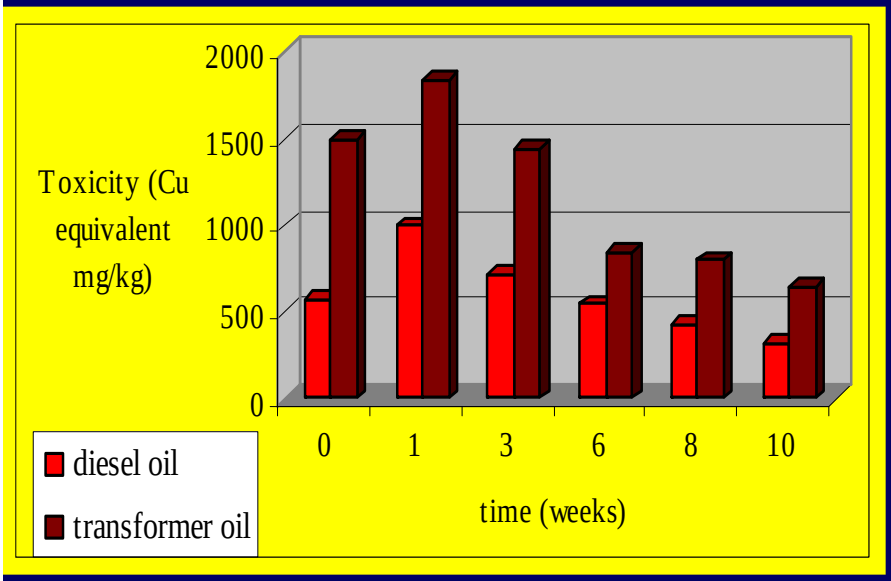
The minimal effective level is two times more in the sandy, 4–5 times more in the loamy soil compared to the water solution.

Microcosm test for modeling soil pollution by flood

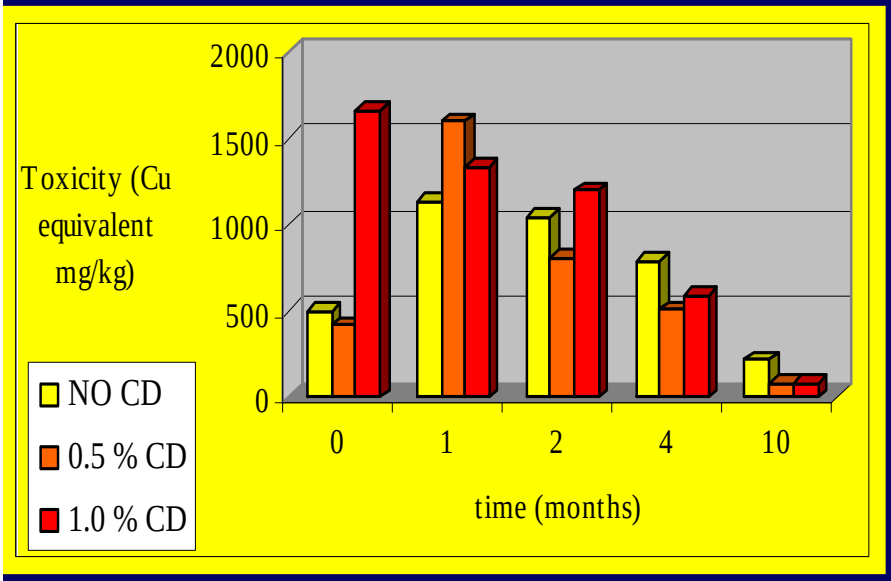


Changes of the toxicity in microcosm after polluting the soil with the mine-waste containing creek sediment in 5, 10, 20 and 40 %. Results of the earthworm (*Eisenia foetida*) acute toxicity test of samples taken from the microcosm in every two weeks are shown here. Faster mobilisation was measured in case of lower contaminant concentration (MU 5%), due to faster weathering and lower pH. After a while the toxicity buffering effect of the soil has been arised.

Direct contact tests for the monitoring of bioremediation

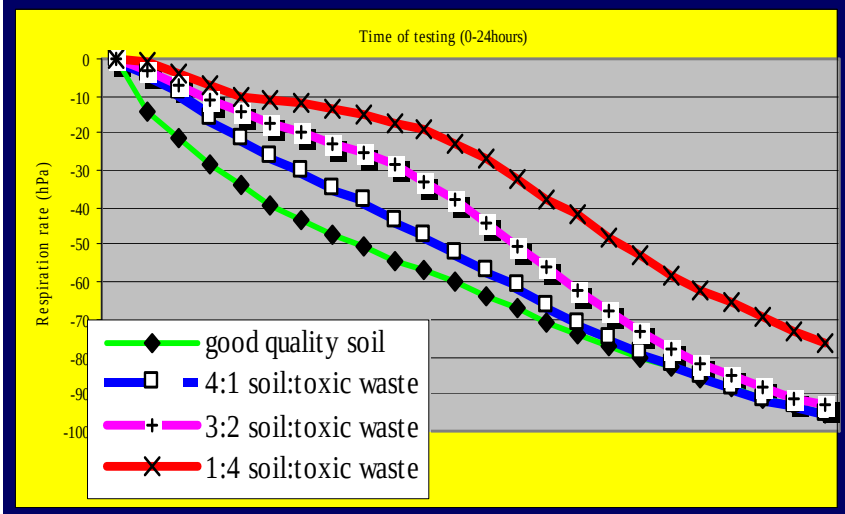


Typical toxicity curve of readily biodegradable diesel oil and moderately biodegradable (PCB-free) transformer oil.
First step: mobilization as indicated by growing toxicity. Second step: biodegradation.

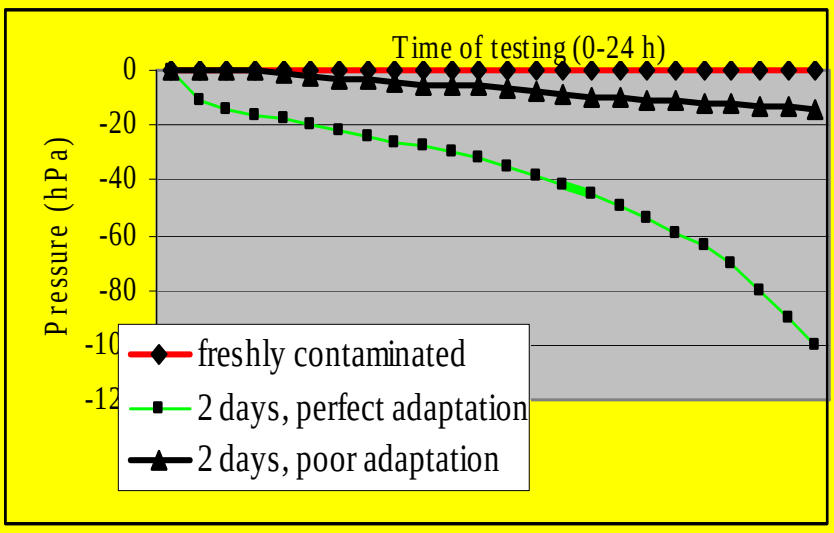


The changes in toxicity during the bioreme-diation of a coal-tar polluted soil.
Bioavailability has been increased by an availability enhancing amendment , the cyclodextrin.

Response of the indigenous soil microflora in soil microcosms



Typical respiration curve of a good quality soil. Adding toxic waste to the soil in 4:1 and 3:2 ratio, the respiration is inhibited temporary, but after a while recovers and makes up for lost time. 1:4 waste ratio caused irreversible inhibition.



A mixture of diesel and engine oil was added to the soil. Immediately after the contamination the soil respiration was completely inhibited. After 2 days the good quality soil shows normal respiration, plus an increase from the 20th hour due to the oil biodegradation. The bad quality soil was not able to adapt within 2 days.

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Evaluation of the results of the integrated assessment

Relation between chemical and biological results

1. C = B: The chemical and biological results agree

1.1. Both of them are ++: high contaminant concentration with strong negative effect, high risk

1.2. Both of them are - -: no contaminant, or low concentration, no measurable effect, low risk

2. C > B: High concentration measured by chemical analysis, but no effect on the test organisms

2.1. Contaminant is present, but not toxic: latent risk

2.2. Contaminant is present, not bioavailable: chemical time bomb, high latent risk

3. C < B: Chemically not measurable/not measured, but strong ecotoxicological effect

3.1. Very toxic even in low concentration: high risk

3.2. Toxic substance is present, but was not included into the analytical programme: high risk

3.3. No analytical method is available: high risk, due to unknown compounds

Direct contact ecotoxicity testing of contaminated soil
Summary

Direct contact ecotoxicity testing gives additional information on soil contamination:

- •integrates interactions between toxicants
- •integrates interactions between toxicant and matrix
- •integrates interaction between testorganism and toxicant
- •integrates interaction between testorganism and matrix
- •measures bioavailable ratio of the contamination
- •measures chemically not measurable toxicants by their effect
- •measures the effects of chemicals not included into the analytical programme.

Direct contact tests are useful for:

- •site assessment and direct risk estimation
- •technology monitoring
- •soil qualification
- •testing the behaviour and fate of contaminants
- •testing bioavailability
- •dynamic testing of adaptation and the response of soil