Environmental toxicology: a tool for risk management

III.

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Multispecies toxicity tests

Aquatic microcosms

- Benthic-pelagic microcosms
- Compartmentalised lake
- Mixed flask culture mesocosm
- Pond microcosm
- Wcocore microcosm
- Standard aquatic microcosm
- Stream microcosm
- Waste treatment microcosm
- FIFRA microcosm

Terrestrial microcosms

- Root microcosm system
- Soil core microcosm
- Soil in jar
- Terrestrial microcosm chamber
- Terrestrial microcosm system
- Versacore

Multispecies toxicity tests

Multispecies methods: microcosm & mesocosm tests

Size: from 0.1 liter to thousands of liters (18 000 000)

Main characteristics

H**istorical:** like the ecosystem itself they are irreversible in time.

Trophic levels: they have a trophic structure, sometimes very simple, sometimes close to real env.

Evolutionary events occur in the micro- and mesocosms: strains, resistant for the xenobiotic arise.

Evolution of new metabolic pathways for biodegradation (pesticides, xenobiotica) is possible. It can be enforced too.

Reduced complexity: comparing with natural systems the number of species is smaller.

Dynamics of the ecosystem: the enforced isolation into a small scale makes changes in the dynamics. These changes should be distinguished from the effect of the toxicant.

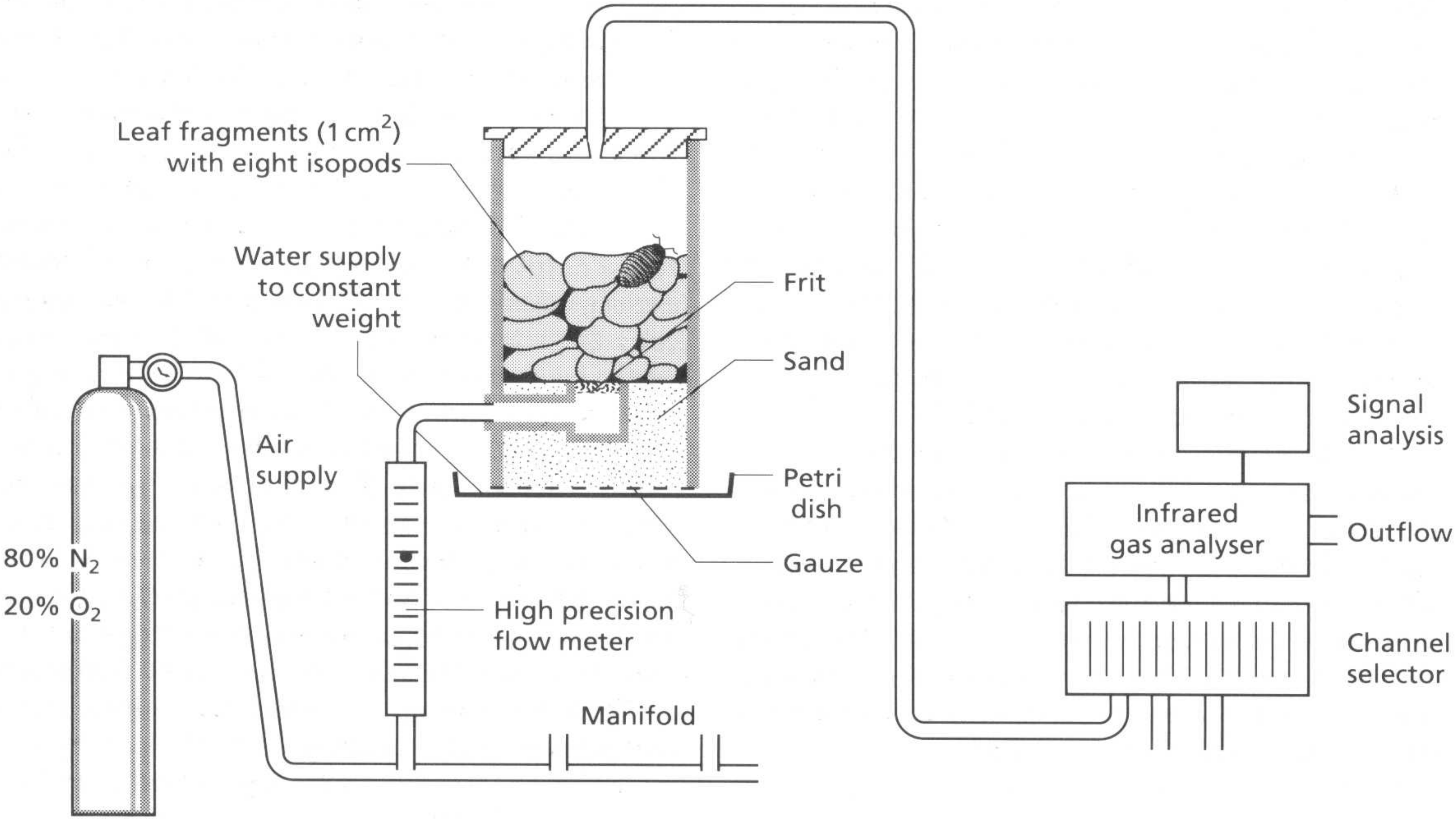
Heterogeneity: in natural ecosystems spatial and temporal heterogeneity is the key to species richness. Artificial test systems should not be heterogeneous or unique, not to lose their statistical power.

Multispecies tests are complex systems, with dynamics and history, so they are not repeatable like the simple species tests or biochemical assay, as the past is conserved in population dynamics down to the DNA sequence. All these information should be considered when we design and evaluate those tests.

FIFRA mesocosm for pesticide registrations

Organisms	bluegill sunfish, fathead minnow, channel catfish, phytoplankton, periphyton, zooplankton, emergent insects, benthic macroinvertebrates
Size of organism	biomass of fish added, should not exceed 2 g/m^3 of water
Test vessel size and type	tanks with a surface area of 5 m^2, a depth of at least 1.25 m, volume at least 6 m^3, made of inert material. Smaller tanks can be used without fish.
Addition of test material	after 6-8 weeks ageing by spraying on across water surface, by applying into a water-soil slurry or tin a water based stock solution
Sampling	begins after 2 weeks of the construction of the microcosm, comtinues for 2-3 months after the last treatment with the test-material. Frequency of testing depends upon characteristics of the test substance and on treatment regime
Dosage of pesticide	level, frequency and number of replicates are determined based on objectives of the study
Temperature	partially burying tanks in the ground or immersing in a flat bottomed pond
Sediment	obtained from existing pond containing natural benthic community, placed onto the bottom of the mesocosm, in trays, in a 5 cm thick layer
Water	from healthy, ecologically active pond, water level should be controlled and regulated, +/- 10 %, by adding or releasing of water
Weather	should be recorded ant take into consideration by the evaluation of the test

Soil mesocosm



Soil in jar: simulation of contamination of soil in mesocosmos

Three, typical mine wastes (lime precipitate, tailing material, lake sediment) were mixed into garden soil in different concentrations, from 5 % to 40%. An integrated, chemical-ecotoxicological monitoring was applied to follow the process: total metal content, extractable with LE, microbiological characterisation, *Vibrio fischeri* luminescence inhibition test, *Azotobacter agile* dehydrogenae activity and *Sinapis alba* plant growth test. Some results are summarised in the followings:

1. Number of soil microorganisms did not show significant changes.
2. **Lime precipitate:** the smallest concentration of the waste in soil (5%) showed the highest toxicity in the beginning, when high toxicity was associated with low pH and high am. of available metals. In higher concentrations (10-20 %) the high pH of the waste increased the pH of the garden soil and lowered metal availability until a certain duration.
3. **Flotation tailing** material: the toxic metal content of the higher waste concentration needed longer time to become available by weathering, the toxicity was never proportional with the total toxic metal content, but similar in the different conc. Jars.
4. **Sediment of the lake** on the tailing dump went through weathering and mixing with humus material. It`s toxic metal content became available easily, the toxic effect was proportional with the amount of the waste in the soil.

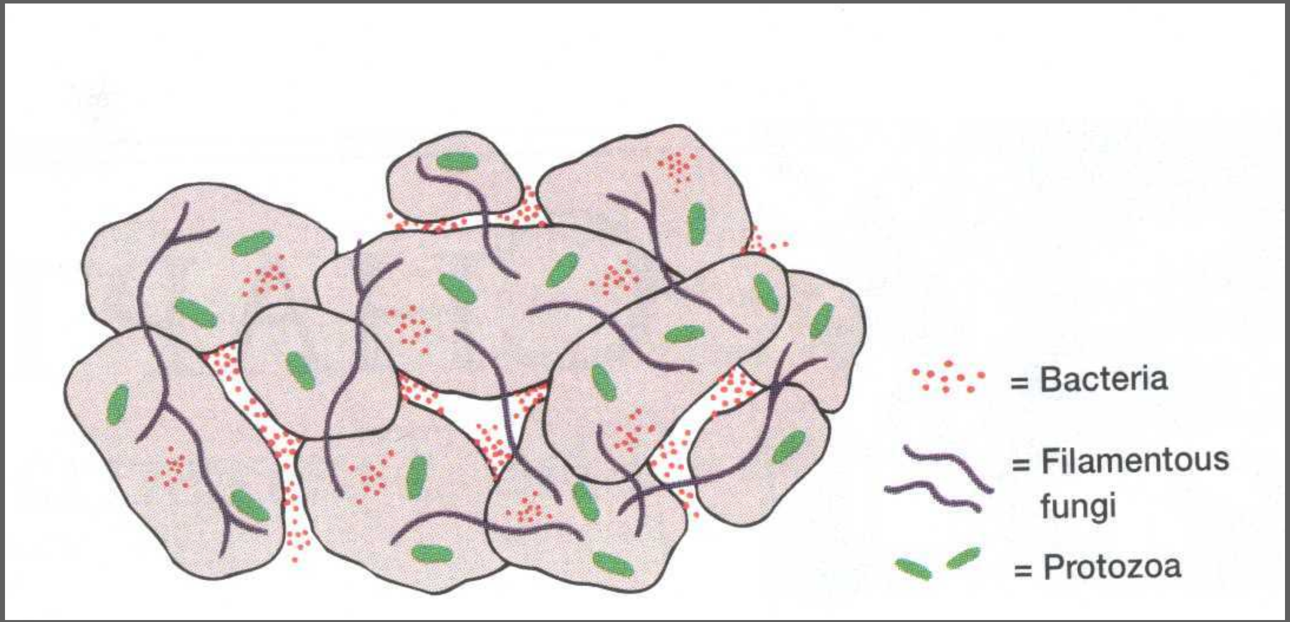
Use of ecotoxicity data for Environmental Risk Assessment

Testing method	SAFETY FACTOR
Three trophic level, at least 1-1 acute toxicity tests (3 x LC_{50} Daphnia, algae, fish)	1000
Three trophic level, at least 1 chronic toxicity test (2 x LC_{50} + 1 x NOEC)	100
Three trophic level, at least 2 chronic toxicity tests (1 x LC_{50} + 2 x NOEC)	50
Three trophic level, 3 chronic toxicity tests (3 x NOEC)	10
Mesocosm or field data	1

Use of PNEC: for hazard assessment, legislation, licensing chemicals, establishing effect based quality criteria for legislation, intervention and target values for risk management

risk assessment, risk characterization: land use, spatial planning etc.

Soil is a complex system



Fate and behaviour of chemical substances in the environment

Environmental nature and fate, mobility, availability, biodegradability of the contaminant or more often the mixture of contaminants in the environment highly influence the actual toxicity, the hazard and the risk.

Integrated approach: the physical, chemical and hydrogeological data should be accomplished by biological and ecotoxicological data to assess the **site specific** environmental risk of pollutants.

Mobility and bioavailability of the contaminant depends on its nature and on the characteristics of the environment. The ecotoxicological results depend not only on the sensitivity of the testorganisms but on their interaction with both of the contaminant(s) and the matrix. Transport and availability of contaminants may be characterised by the integration of chemical analytical and biological/ecotoxicological data.

Absorption capacity of the solid phase of environmental compartments like soil and sediment and the **partition** of the contaminant between physical phases specifies leaching, desorption and volatilisation and highly influences transport and availability.

The partition of toxicity between the solid, the water and the gaseous fractions of the soil results in a risk on ground water or on air. Partition between solid phase and pore water in sediments determines water quality. Strong binding and low biodegradability leads to the evolution of a chemical time bomb.

The **actual toxicity of solid samples**: effects of the solid state sample and the absorbed contaminant can be better characterised by direct contact tests, where mutual interactions may appear. The results of interactive bioassays include the results of the possible interactions between all participants: contaminant, contaminated media and test organism.

Environmental toxicity testing of contaminated soil

Problems of testing of environmental (soil) samples:

- mixture of contaminants
- interactions between contaminants, matrix and biota
- medium: extract, whole sample

Problems of testing soil samples from contaminated land

- mixture of contaminants: sinergism, antagonism
- biotransformation: effect of products, biodegrdation
- availability: physico-chemical and biological availability differs
- analytical programme includes only part of the really occurring chemicals
- biotic and abiotic composition of the environmental sample

Ecotoxicity testing gives solution for

- 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 effects of chemicals not included into the analytical programme

Expectations:

- Ecological relevance
- Reproducibility
- Reliability
- Robustness
- Sensitivity

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

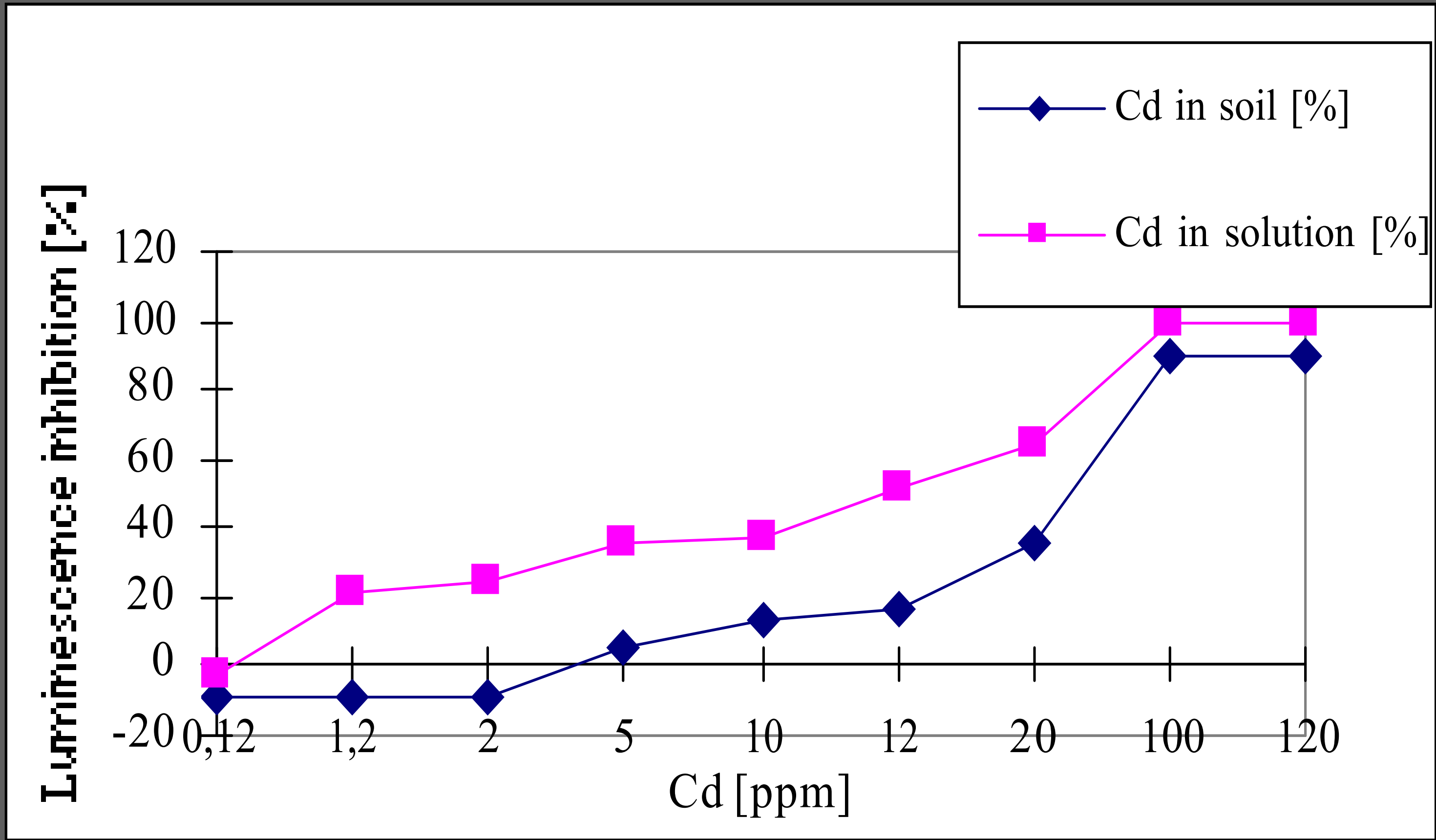
Integrated assessment of environmental phases

The comparison of the toxicity **of dissolved and adsorbed** contaminants**, the parallel testing of whole soil/sediment and pore water** (water extract) may give further details about the nature of risk.

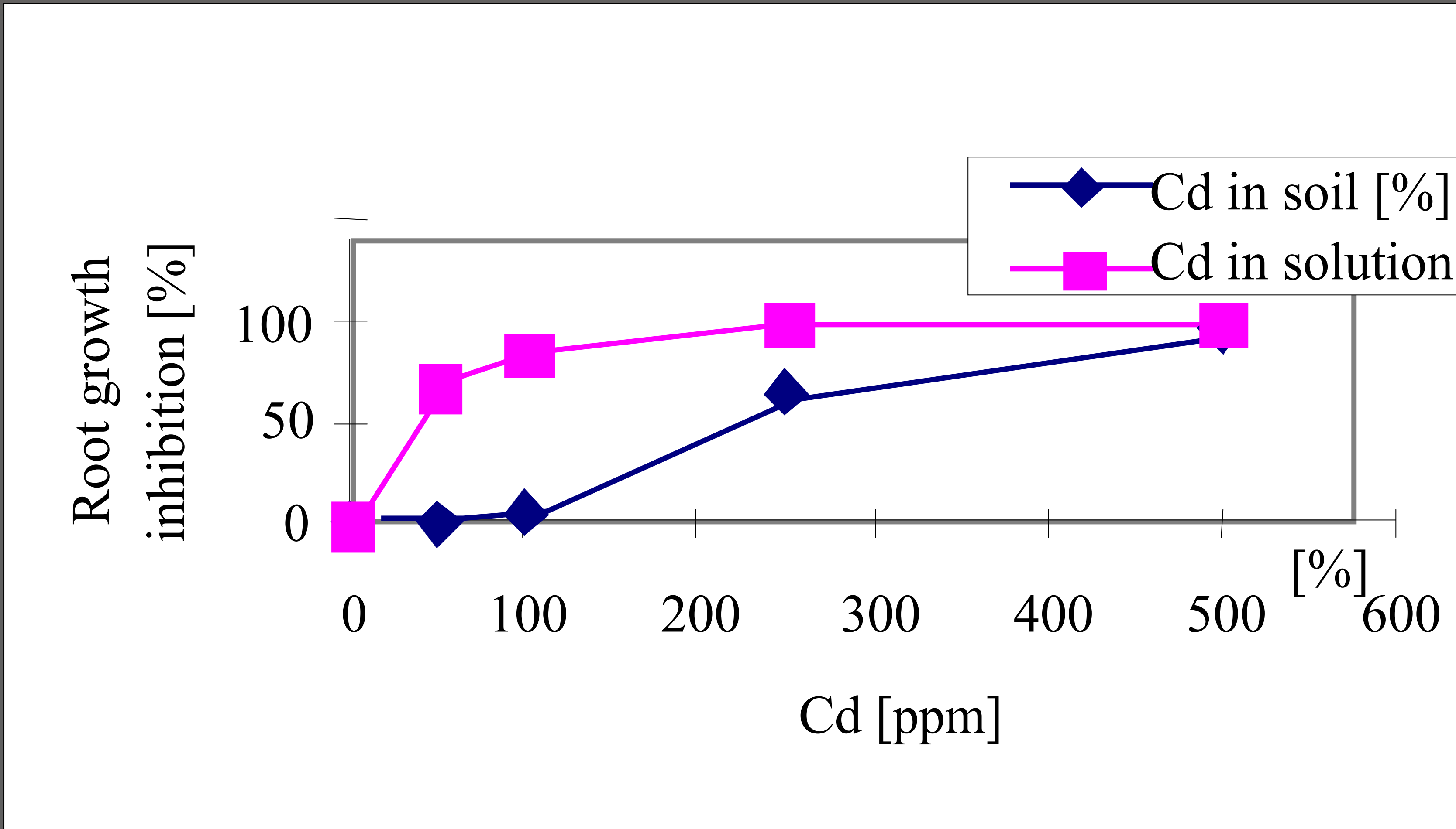
Toxicity buffering capacity of soils

It can be characterised by comparing the toxicity of the contaminants in dissolved and in absorbed form. Toxic effect - concentration curves are different for dissolved and adsorbed heavy metals; the area between the two curves was proportional with the absorption capacity of the soil. Toxicity of toxic metals adsorbed onto a soil with a high clay content was only 1-20% of their toxicity in dissolved form.

Bioavailability: comparision between dissolved and absorbed metal content



Bioavailability: comparision between dissolved and absorbed metal content



Integrated assessment of environmental phases

Partition of toxicity

Ecotoxicity of the pore water (P) and the whole sediment (S) was compared in case of Danube sediment. From the measured data the nature and fate of the pollution and its environmental risk maybe characterised:

1. P + and S +	Toxic, bioavailable, partition between solid and pore water is equal or similar
2. P + and S -	Toxic, bioavailable, mobile (water soluble)
3. P - and S +	Toxic, bioavailable, immobile (mostly absorbed on the solid phase)
4. P - and S-	Non toxic or not bioavailable, necessary to compare with chemical results!

Anvantages of interactive bioassays

Interactive bioassays are able to characterise the binding capacity of the soil, the availability and the actual effect of the contaminant and the interactions between the soil (sediment), the contaminant and the test organism.

Site specific environmental risk of contaminated sites can be characterised by ecotoxicological tests. From the result of **bioassays** with test organisms of three different trophic level (e.g. microorganisms, plants, animals) an extrapolation for the terrestrial or benthic ecosystem is possible.

Biological characterisation should accomplish the chemical results. Chemical analysis gives the concentration of the compounds. Ecotoxicological tests measures the effect characterised by the Effective Concentration or the No Effect Concentration of contaminant (EC_{50} or NOEC).

The **comparative evaluation** of chemical (C) and ecotoxicological (B) results makes possible a more detailed risk characterisation, gives important information about the fate and nature of the contaminant about the interactions between the contaminant, the matrix and the ecosystem.