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E5080 / E0323

Ecotoxicology

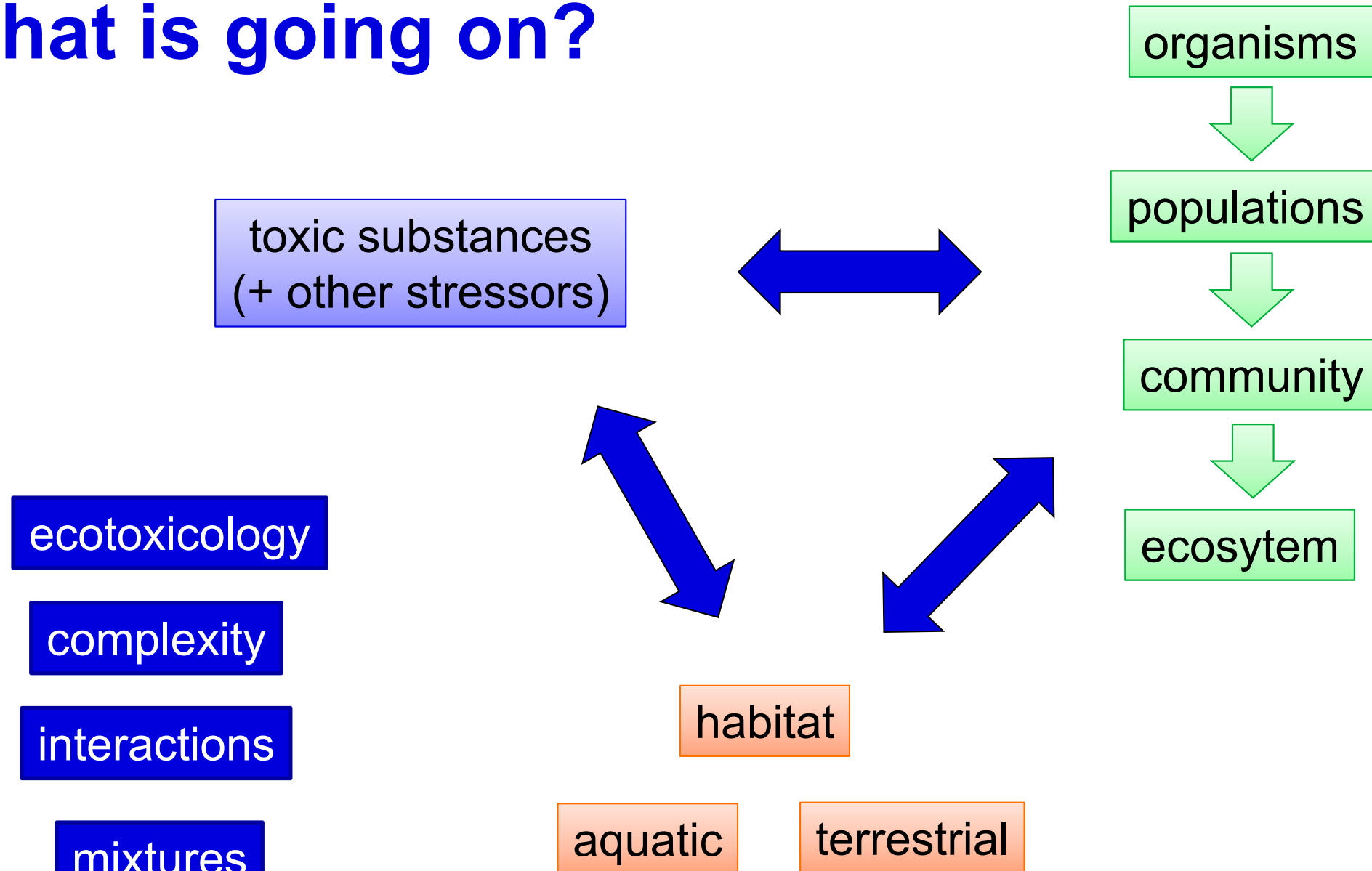
Ecotoxicology in Field Studies

Jakub Hofman

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Introduction

What is going on?



Why?

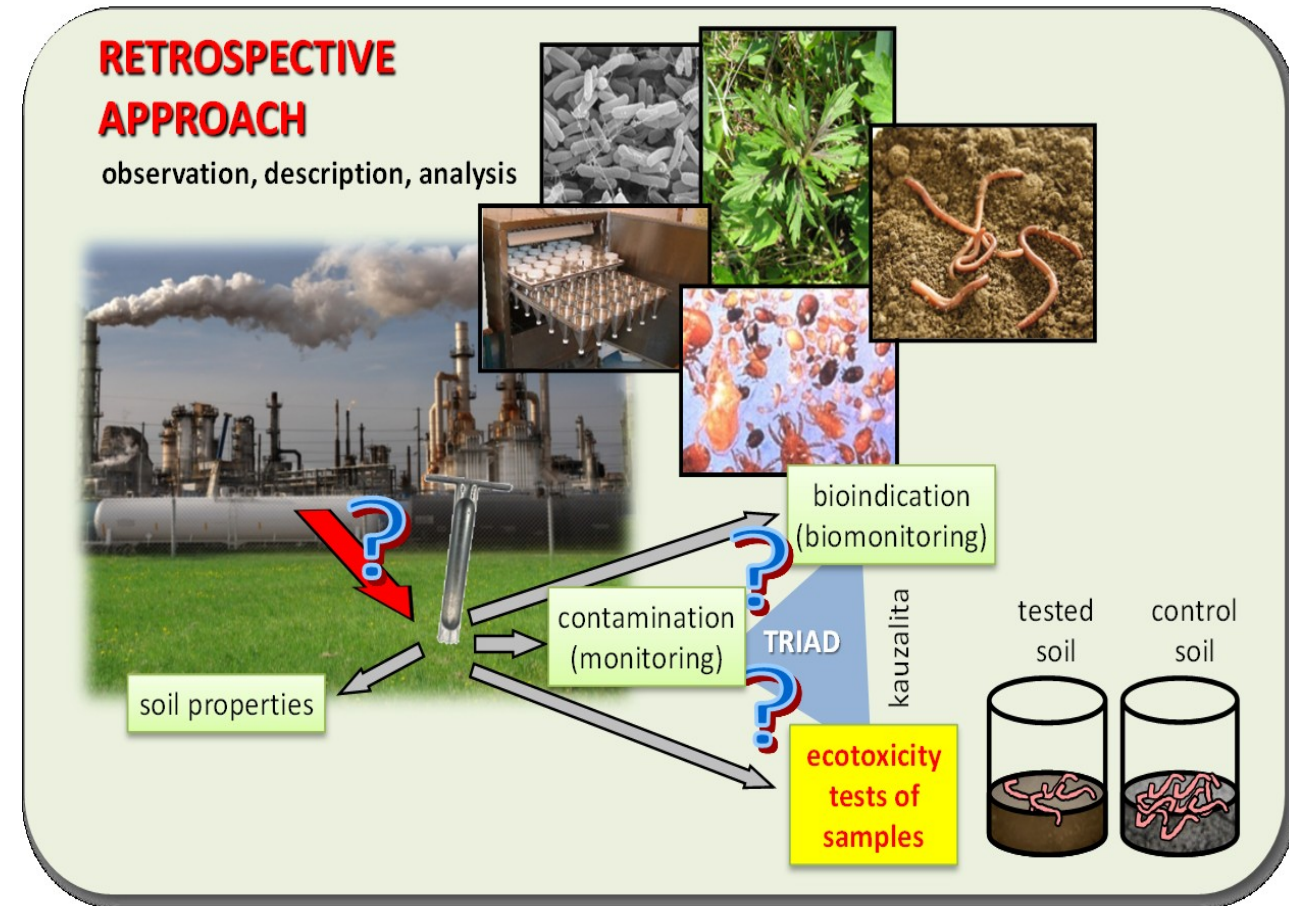
- real problems are in **real** ecosystems !
- lot of problems already **happened** !

Challenges

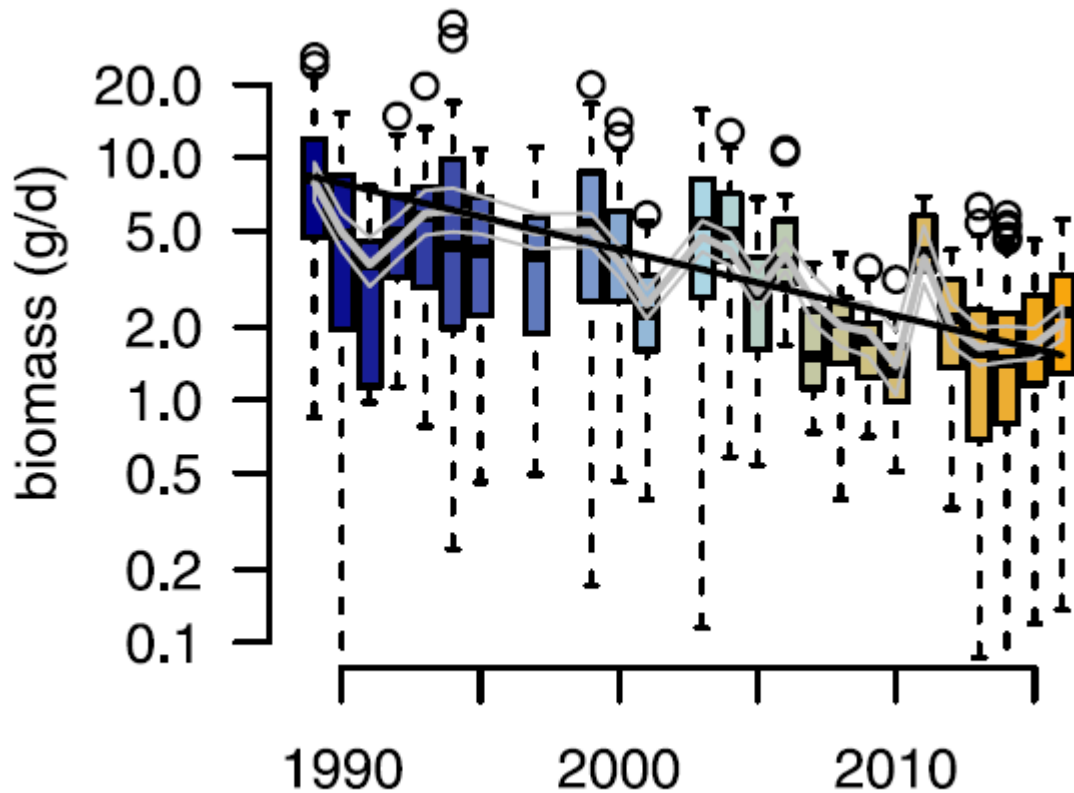
- how to address **ecotoxicity in real** situation?
- how to find **causality** between degradation and ekosystem state?

How?

- measurements (observations) directly in the field
- sampling + analyses
- bioindication, biomonitoring
- causality, correlations, weight of evidence, TRIAD approach



Bioidication – example of alarming results



RESEARCH ARTICLE

More than 75 percent decline over 27 years in total flying insect biomass in protected areas

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Abstract

Global declines in insects have sparked wide interest among scientists, politicians, and the general public. Loss of insect diversity and abundance is expected to provoke cascading effects on food webs and to jeopardize ecosystem services. Our understanding of the extent and underlying causes of this decline is based on the abundance of single species or taxonomic groups only, rather than changes in insect biomass which is more relevant for ecological functioning. Here, we used a standardized protocol to measure total insect biomass using Malaise traps, deployed over 27 years in 63 nature protection areas in Germany (96 unique location-year combinations) to infer on the status and trend of local entomofauna. Our analysis estimates a seasonal decline of 76%, and mid-summer decline of 82% in flying insect biomass over the 27 years of study. We show that this decline is apparent regardless of habitat type, while changes in weather, land use, and habitat characteristics cannot explain this overall decline. This yet unrecognized loss of insect biomass must be taken into account in evaluating declines in abundance of species depending on insects as a food source, and ecosystem functioning in the European landscape.

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0185809>

Neonicotinoid use & pollinator decline: a 17 year correlation

Oilseed rape cover (+ve)



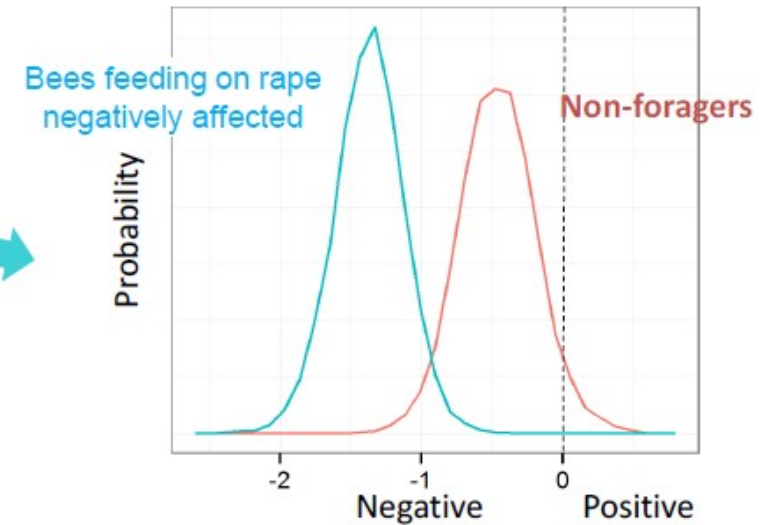
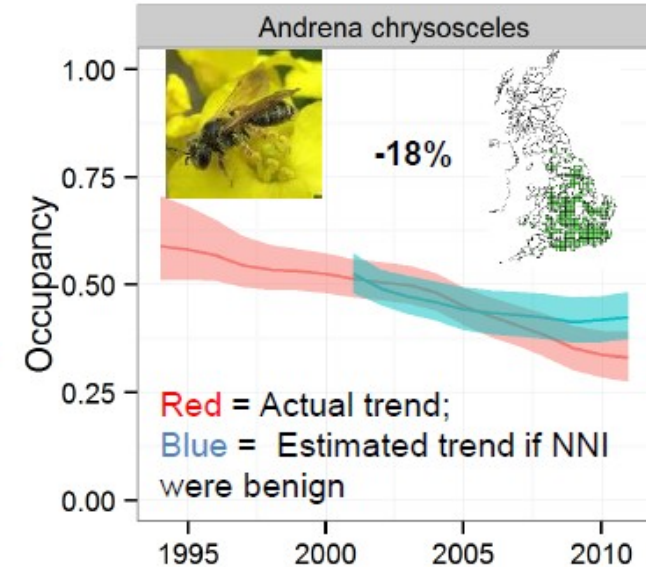
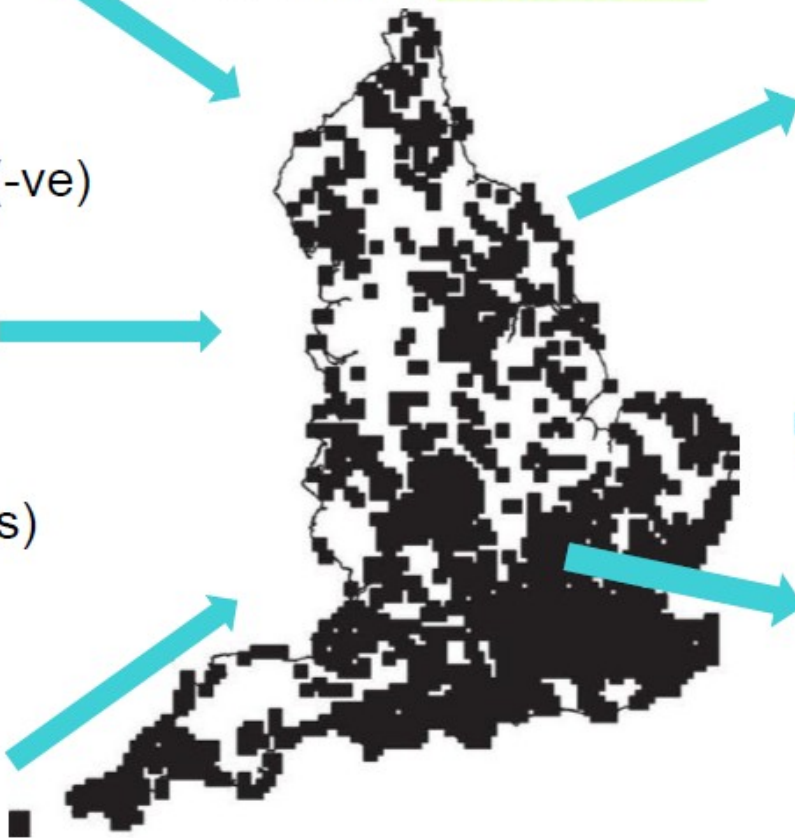
Volunteer surveys of bees



Neonicotinoid use (-ve)



Other pesticides (ns)



B.A. Woodcock *et al.* (2016) Impacts of neonicotinoid use on long-term population changes in wild bees in England. *Nature Communications* 7, 12459. doi:10.1038/ncomms12459

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How to?

General scheme

- 1. site characterization**, survey directly in the field
- 2. assessment parameters selection** for the given ecosystem in relation to the stress impact
 - abiotic components
 - biotic components
 - structure parameters (eg species composition – diversity, abundances ...)
 - functional parameters (eg flows of energy / materials, processes, balances, resilience/resistance ...)
- 3. sampling plan** (sampling frequency, numbers ...)
 - abiotic components (water, sediments, soil air)
 - biotic components (producers – consumers – destruent)
- 4. sampling campaign + analyses**
- 5. assessment and interpretation**, comparison of exposure vs control (!), conclusions

1) Site characterization

- depending on:
 - terrestrial ecosystem: terrain influences – slopes, vegetation ...
 - aquatic ecosystem: flowing – static (lentic / lotic), depth, size, flow speed, fragmentation (macrophyta, benthos ...)
- other properties needed to be recorded:
 - main weather conditions, wind directions, light intensity ...
 - specific parameters (any anthropogenic activities nearby?, sources of pollution? ...)
 - map records ...
 - what else ?
 - ...

2) Parameters selection

abiotic components

- where (water, sediment, soil, air) the stressor does occur / act ?
- where the residues are expected ?

biotic components

- which organisms will be evaluated to see the impacts of stressors:
 - relation to stressor's influence (eg planktonic – substances dissolved in the water column, ie hydrophilic versus sediments - hydrophobic)
 - evaluated groups (eg producers – algae, consumers – zooplankton, fish; destruent – planktonic bacteria)
 - key species, bioindicators ...
 - parameters evaluated
 - structural (taxonomic parameters, biomass, abundance ...)
 - functional (production / respiration, food chains ...)

3) Sampling and analyses

A: sampling and analyses of abiotic components

- plan and design of sampling plots / sites
 - areal, vertical – depth, air sampling
- merging and creating mixed samples („average" sample from the site)
- assessment of the fundamental chemical and physical parameters (organic carbon, pH, particle sizes)
- characterization and determination of the contamination
 - analytical chemistry and environmental chemistry
- ecotoxicological bioassays of the real matrices special use of bioassays

3) Sampling and analyses

A: sampling and analyses of abiotic components

Water



Sediment



Eckmans sampler

3) Sampling and analyses

A: sampling and analyses of abiotic components



Soil



Soil core



Air



Photo © CERHA/TE/EN

3) Sampling and analyses

B: sampling and analyses of biota

- plan and distribution of the sampling plots / sites
- sampling – variable according to organisms...
- characterization of defined biotic parameters
 - techniques of botanical, zoological, microbiological and ecological disciplines
- characterization and determination of contamination of biota
 - techniques of analytical chemistry and environmental chemistry

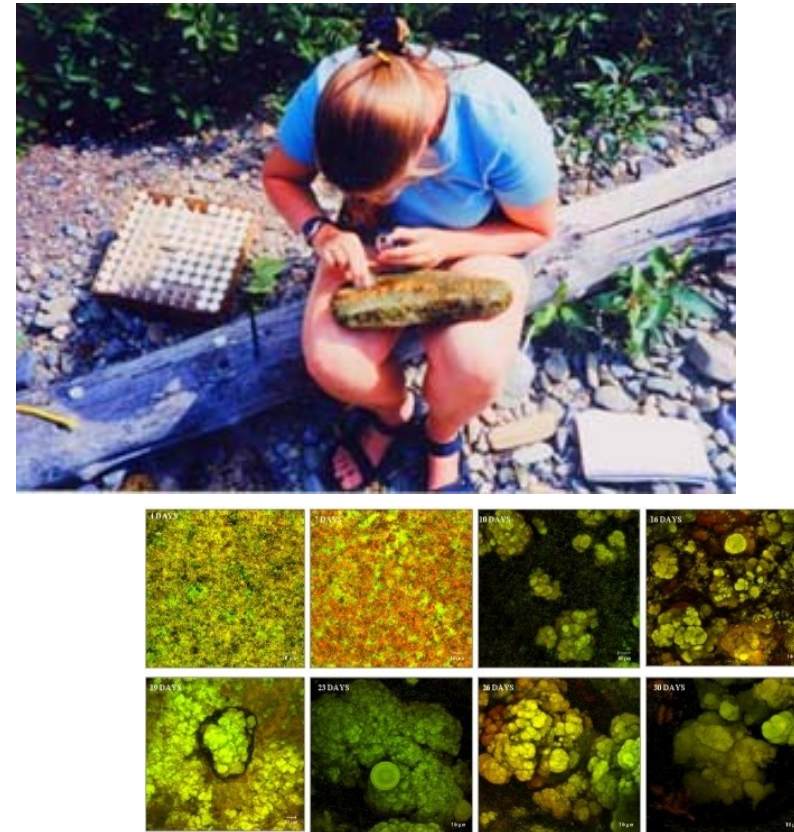
3B) Sampling - biota

- water

Planctonic nets



Periphyton – biofilm



3B) Sampling - biota

- water

Benthic invertebrates

Fish



CETOX

3B) Sampling – biota – soil biota

- different according to type and especially the size of organisms
- manual sorting, picking
- pitfall traps
- extracting methods: Tulgren's extraction, O'Connor's extraction ...



3B) Sampling – biota – soil biota



3B) Sampling – biota – soil biota

| | |
|------------------|--|
| 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 <i>design of sampling programmes</i> with soil invertebrates |

3B) Sampling – biota – soil biota

- earthworms



3B) Sampling – biota – soil biota

- earthworms



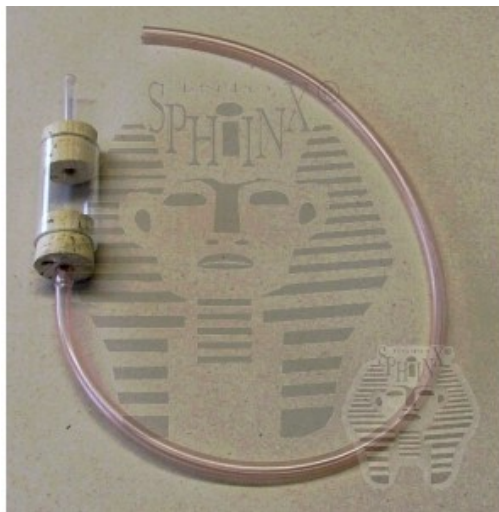
3B) Sampling – biota – soil biota

- earthworms



3B) Sampling – biota – insects

- capture into pitfall traps - those living on the surface of the soil
- capture using exhaustor
- by sweeping with an entomological net - from vegetation or from air
- collection or falling from vegetation
- Malaise trap
- impact traps (without or with attractants, pheromones)
- ... and many other methods



E I O X

3B) Sampling – biota – insects



3B) Sampling – biota – terrestrial plants

Phytocoenological snapshot

- defining area, square or rectangle
- units or hundreds of m²
- plants are divided according to height into several vegetation floors:
 - bryophytes and lichens
 - herbs, seedlings of trees
 - shrubs and trees with possible epiphytes
- estimation of the coverage of individual floors
- on each floor, all species, including an estimate of the area they cover (in percent or special scale – 7-point Braun-Blanquet or 11-point Domino)
- other information is recorded, of course the exact location and date, but also the slope and its orientation
- soil samples can also be taken for later analyzes (eg pH and other chemical analyzes)



3B) Sampling – biota – terrestrial plants

Quadrat method

How many weeds are in this field?



Total area of field: (200m x 50m) = 10,000m²

Area sampled: (10 quadrats x 0.25m²) = 2.5m²

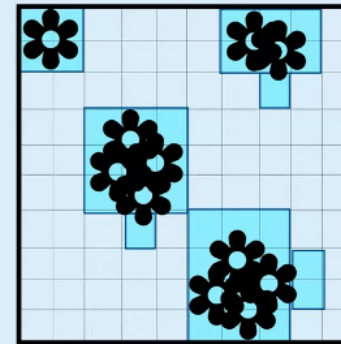
Number of weeds in sampled area: 56

Total weeds in field: (10,000/2.5) x 56 = 224,000

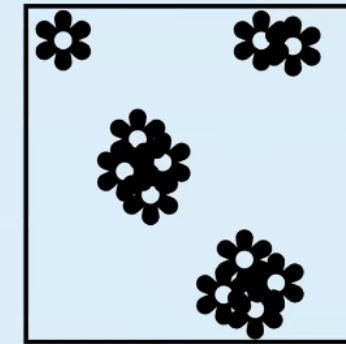


METHODS TO ESTIMATE THE ABUNDANCE OF A SPECIES

1. **Local frequency** (% of squares in the quadrat with the species present)
2. **Density** (The number of one species in a given area)
3. **Percentage cover** (proportion of the ground occupied by the species)

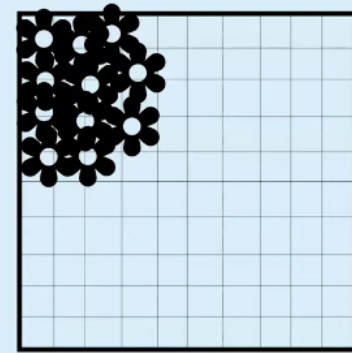


35 squares contain the species = 35% local frequency



11 in 0.5m x 0.5m (0.25m²) The whole field is 280m²
(280/0.25) x 11

=12,320 of species in entire field



18 full squares covered = 18% percentage cover

3B) Sampling – biota – mammals

Direct methods

- **sampling** - capture the representative part of the population
- **dead-traps (animal is killed)** – clap-traps, wire eyes, “pitfall traps” with water and other traps, shooting
- **alive traps** - corridors, fall-doors, baits; Sherman's or Longworth trap; tagging (rings, ears, color ...), release and re -capture (CMR - Catch, Mark, Release)



3B) Sampling – biota – mammals

Direct methods

- **observation** – big animals or cameras or phototraps
- **labelling** – bands, collars, telemetry (GPS)



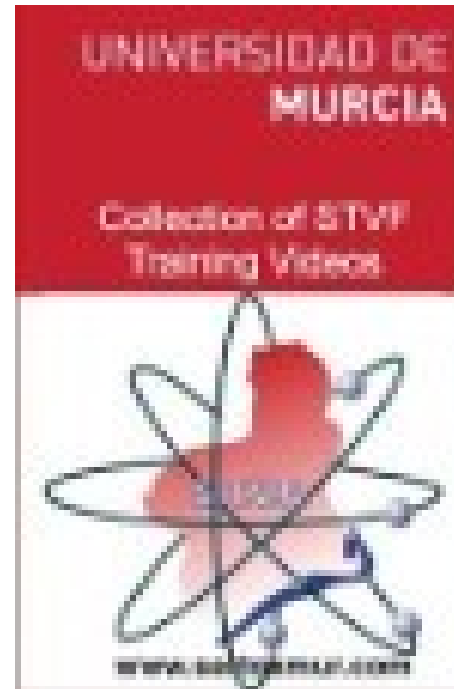
Handcrafted GPS Devices
Starting at 5 grams

Building small, lightweight products
incorporating the best technology
and battery life is our specialty.



3B) Sampling – biota – birds

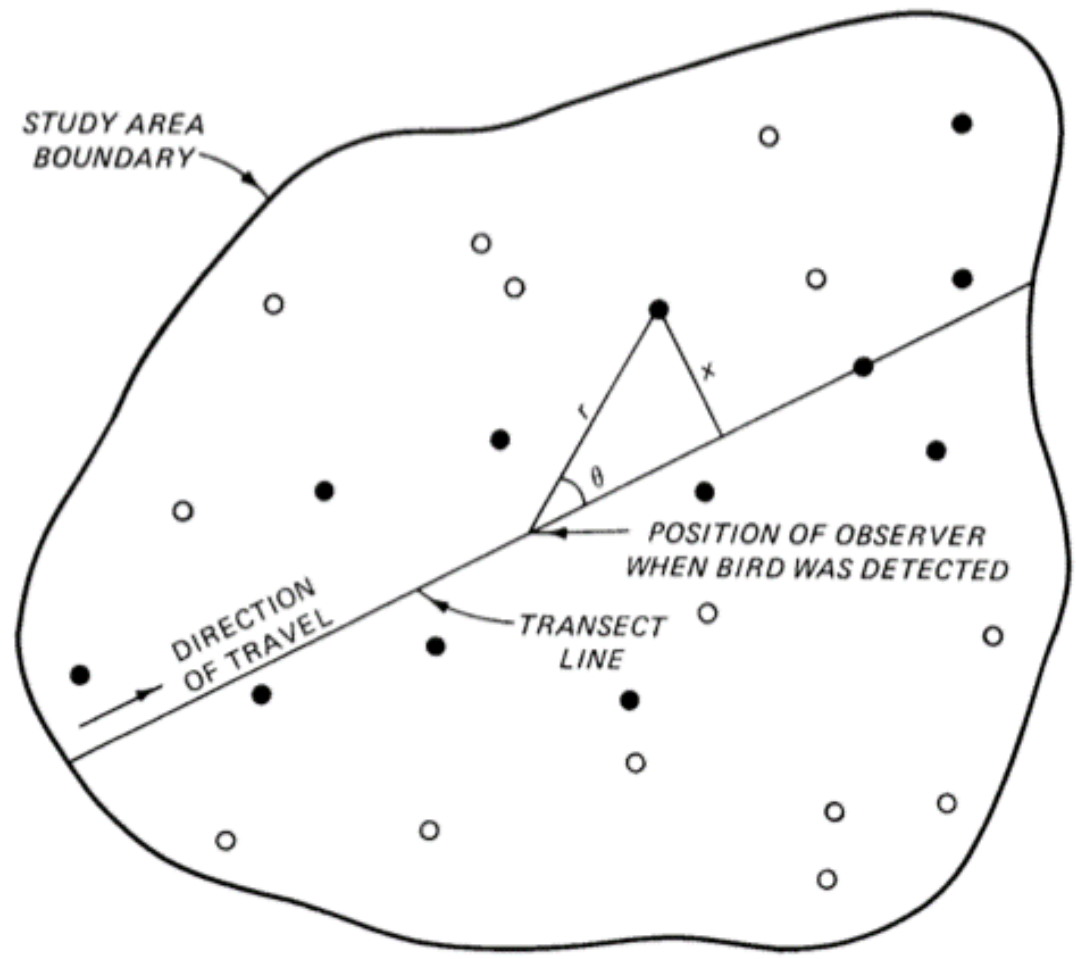
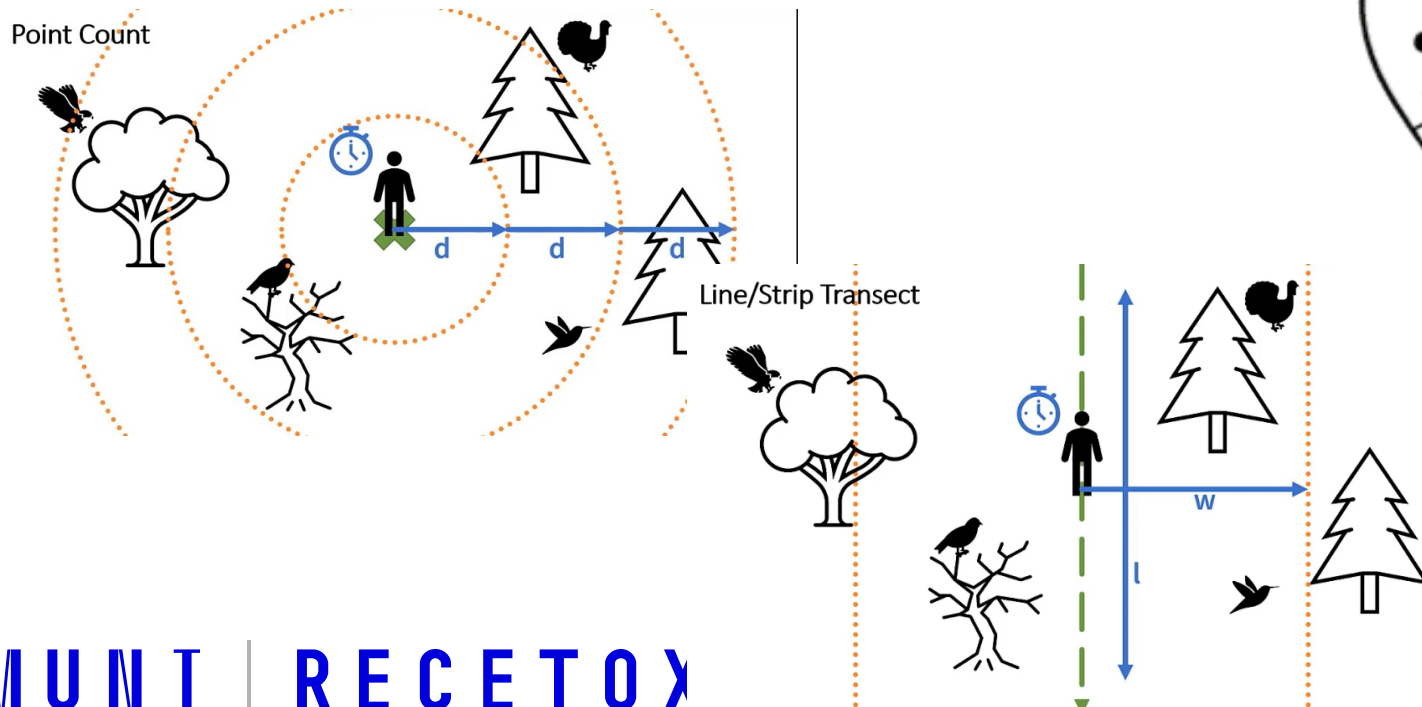
- catching – nets, rings, blood sampling, feathers sampling etc.



Procedures for collection of blood samples of raptors.

3B) Sampling – biota – k

- **observation** (individuals, nests, singing



• General representation of line-transect sampling of a bird population. An observer walking along the transect line detects some birds (solid circles) and fails to detect others (hollow circles). For each bird observed, either the perpendicular distance (x), or both the sighting distance (r) and the sighting angle (θ) are measured. Most line-transect methods use the distribution of right-angle distances to estimate population density

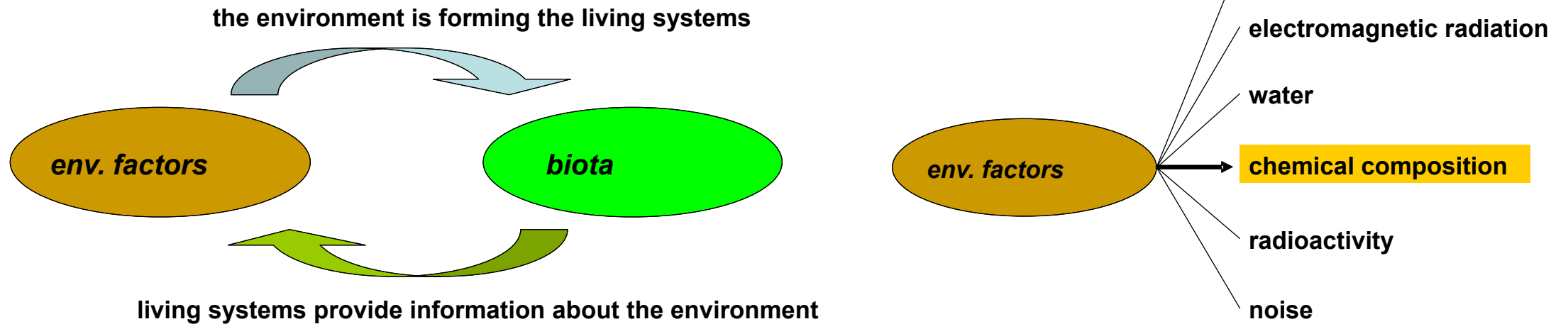
4) Assessment and interpretation

- **comparision of the exposed and control ecosystem**
- fundamental parameters of the compared ecosystems should be SIMILAR / COMPARABLE (eg pH values, water hardness, similar geochemical parameters – subsurface ...)
- chemical contamination of the environmental compartments **versus** biota in the compared ecosystems
 - are there differences in the concentrations of the toxic compounds?
 - is there any relationship between concentrations in the environment and in biota?
- comparing biotic parameters in both compared ecosystems
 - are there differences in the taxonomic composition of the communities?
 - are there differences in the coverage – abundance – biomass?
 - are the food relationships different?
 - what about rezistence and resilience (how long the stress has acted and how long it does not act any more?)
- **correlation is NOT equal to causality !**

Bioindication, biomonitoring

Bioindication

method, when the Environmental status is assessed on the basis of the properties of biological systems



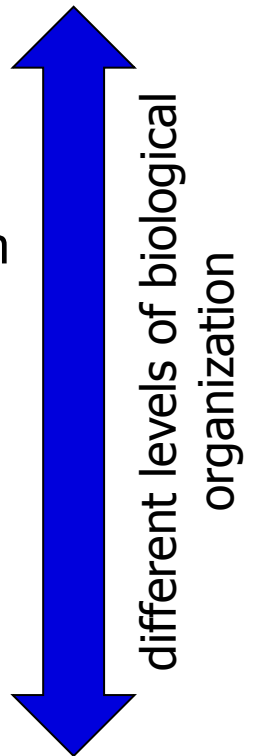
in broader context, we mean all methods when we observe reactions of organisms present in the environment (from individuals to communities) on stress

Bioindication versus biomonitoring

- bio + monitoring
- bioindication is an approach
- biomonitoring is the use of this approach in the field studies, especially at number of sites and repeatedly in time

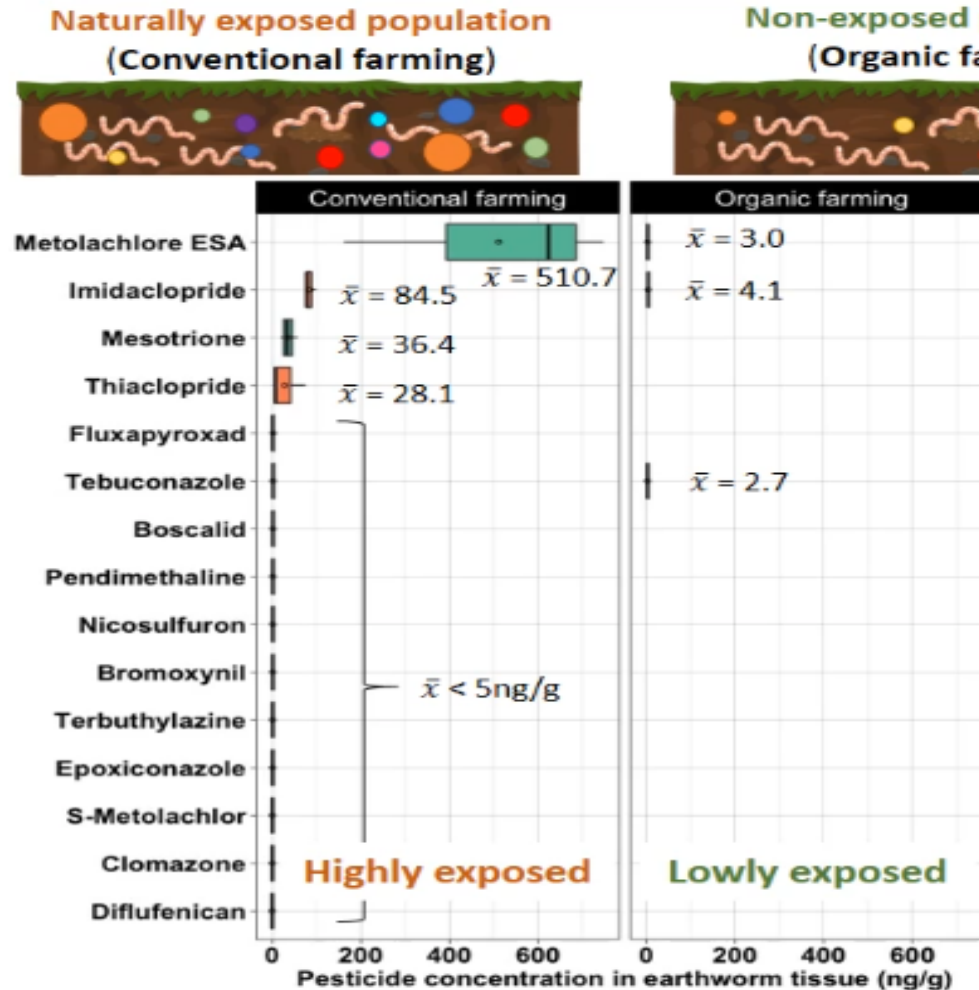
Bioindication

- monitoring of chemicals in the collected biota samples
 - in anything, preferentially so -called bioaccumulators or bioindicator species / samples (eg needles)
- tracking biota and its response to the environmental factors
 - biochemical markers
 - of effects (stress proteins - HSP - Heat Shock Proteins, chromosome aberations ...)
 - of exposure (Methalothioneins, EROD – Ethoxyresorufin-O-Deethylase ...)
 - indicator species - presence/absence indicates a certain feature of the ekosystém
 - sensitive species (eg stoneflies, mountain Tubellaria, lichens)
 - oportunist species (eg chironomids, leeches ...)
 - the condition and function of organisms
 - population - numbers of organisms, distribution, age composition ...
 - community - species composition and representation, biodiversity
 - state of ecosystem or landscape - structure, dynamics, function



Accumulation bioindicators - example

Residual pesticide contamination in naturally exposed and non-exposed earthworms



Up to 11 of the 73 selected pesticides were detected in earthworms from conventional farming against only 3 in organic farming

Earthworms cope with high concentrations of several chemicals in their natural environment

SETAC 2020: 1.04.8 Deciphering the molecular mechanisms of pesticide tolerance of the soil engineer biodiversity

Indicator species – example: Saprobity index

- sapos = rot, blight, decomposition ...
- organic "non-toxic" substances (fecal pollution, „nutrients“ for microbes)
- many organic chemicals → nutrients for bacteria → degradation of organic substances and consumption of oxygen → impacts on aquatic biota

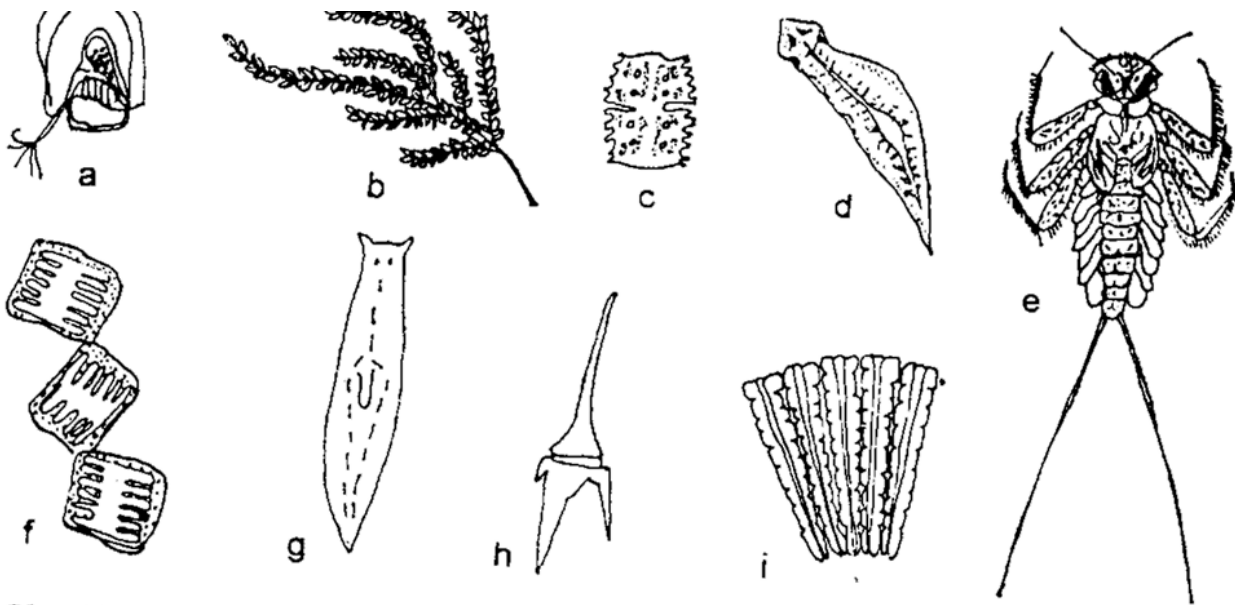
Increased saprobity

- one of the major threats for water quality (and indicator of water pollution / purity) in Europe
- not the direct toxicity, rather oxygen depletion (!)
- assessment = categorization
- polysaprobity / mesosaprobity (alfa-, beta-) / oligosaprobity
- (new: catarobity / limnosaprobity / eusaprobity / transsaprobity)

Indicator species – example: Saprobity index

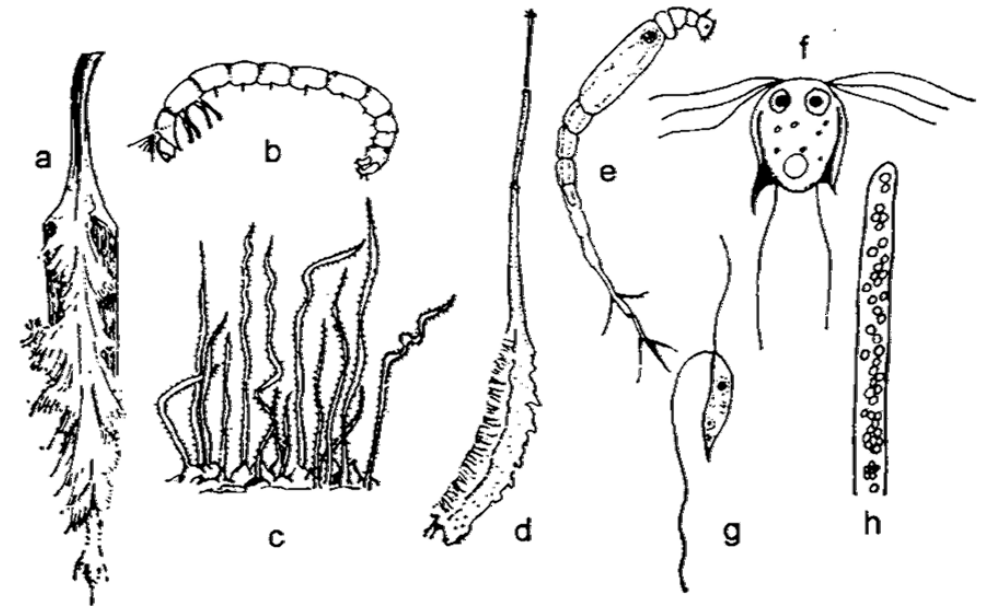
Indicator species for saprobity - examples

Xeno & oligosaprobity



Obr. 132. Příklad xenosaprobních a oligosaprobních organismů
a - perloočka *Holopedium gibberum*, b - vodní mech *Fontinalis*, c - dvojčatkovitá řasa *Micrasterias truncata*, d - ploštěnka *Dugesia gonocephala*, e - jepice *Epeorus asimilis*, f - rozsivka *Tabellaria flocculosa*, g - ploštěnka *Crenobia alpina*, h - obrněnka *Ceratium hirundinella*, i - rozsivka *Meridion circulare*

Polysaprobity



Obr. 135. Příklad polysaprobních organismů
a - bakterie *Sphaerotilus natans*, b - pakomár *Chironomus thummi*, c - nitěnky *Tubifex tubifex*, d - pestřenka r. *Eristalis*, e - vířník *Rotaria neptunia*, f - bičíkovec *Hexamitus inflatus*, g - bičíkovec *Bodo putrinum*, h - bakterie *Beggiatoa alba*

Indicator species – example: Saprobity index

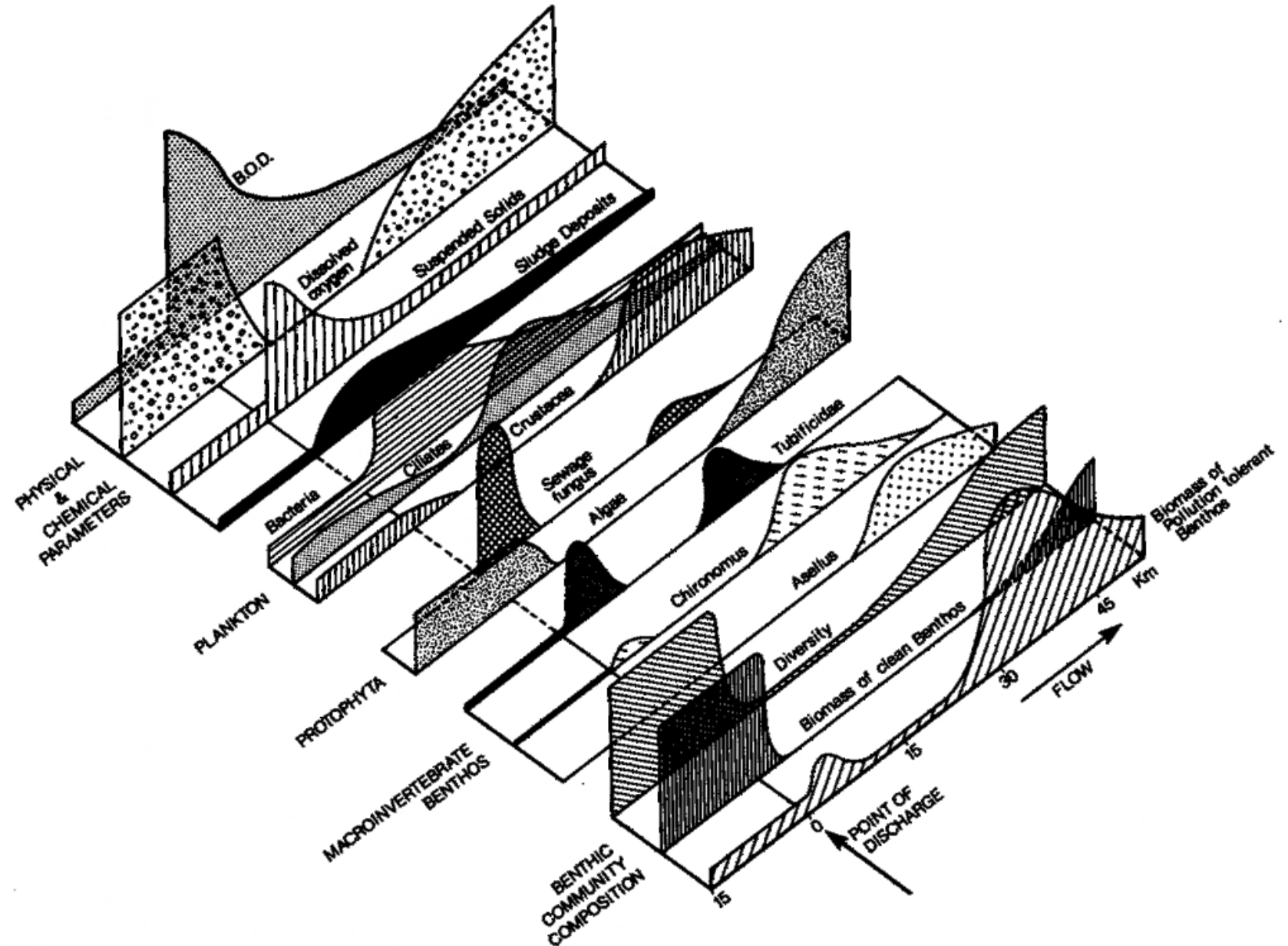
- Community shift

$$S = \frac{\sum_{i=1}^n A_i \cdot s_i \cdot g_i}{\sum_{i=1}^n A_i \cdot g_i}$$

A_i – abundance of species i

s_i – individual saprobity value of species i

g_i – indicative value of species i



How to choose the bioindicator ?

Procedures used to monitor biological endpoints in real ecosystems should ideally be:

1. virtually applicable
2. easily interpreted by the executive body
3. ecologically relevant to multiple ecosystems
4. the resulting parameter should be separable from natural fluctuations
5. should give a causal relationship between substance and effect
6. fast and cheap
7. standardizable

How to choose the bioindicator ?

Intrinsic importance

Key: indicator is the endpoint

Economic species

Endangered species

Other aspects of direct importance to humans

Early warning indicator

Key: rapid indication of effect

Use when endpoint is slow or delayed in response or too variable in time or space

Minimal time lag in response to stress (rapid response rate)

Signal-to-noise ratio low; discrimination low

Screening tool; accept false positives

Sensitive indicator

Key: reliability in predicting

Use when endpoint is relatively insensitive

Stress specificity

Signal-to-noise ratio high

Minimize false positives

Process indicator

Key: endpoint is process

Monitoring other than biota, e.g., decomposition rates

Complement structural indicators

Indicator of ecosystem sensitivity/vulnerability

Key: system attributes

Abiotic indicators such as flushing rates; neutralization capacity; nearby seed sources

Signal-to-noise ratio

Sensitivity to stress

Intrinsic stochasticity

Rapid response

Early exposure

Quick dynamics

Stress-specific sensitivity

Reliability of response

Specificity to stress

Ease/economy of monitoring

Field sampling

Laboratory expertise

Preexisting data base and history

Easy test for process

Relevance to endpoint

Intrinsic

String of ecological connections

Feedback to regulation or management

Adaptive management potential

Hierarchical suites of indicators

Relevance to recovery processes

Short-term and long-term processes

Refugia, colonizing capacity

Adaptation to new physical constraints

Selection of parameters - pros and contras

Table 5-1: Simple indicators of soil biodiversity. Meas.= measurability

| Functional group | Organisms | Indicator | Method | Standard | Sensitivity to soil type | Sensitivity to land use | Meas.. |
|--------------------------|--------------------------------------|-------------------------|--|------------------------------|--|--|------------------------------|
| Microbial Decomposers | Microorganisms | Biomass / activity | SIR, fumigation-extraction ATP concentration, initial rate of mineralisation of glucose | Yes Yes | Good | Good | Good |
| | | Activity | Respiration rate/quotient/ratio, Nitrification, N mineralisation, C mineralisation Denitrification N-fixation Mycorrhizae (% of root colonised) | Yes Yes No No No | Good Medium Medium Good Good | Medium Medium Medium Medium Good | Good |
| | | Enzymatic activity | Dehydrogenase activity Other enzymatic activity tests: phosphatase, sulphatase, etc. Enzyme index | Yes No No | Good Good Very good | Good Good Very good | Medium Good |
| | | Diversity | Culture-dependent methods: direct count, community-level physiological profiles Culture independent methods: fatty acids analysis, nucleic acid analysis | No No | Poor Poor | Poor Very good | Good Good (technical) |
| Biological regulators | Protists, nematodes | Abundance and Diversity | Culture-dependent methods: direct count (diversity index, functional or trophic diversity) Culture independent methods: fatty acids analysis, nucleic acid analysis | Yes | Good | Very good | Low (time, expertise) |
| | Microarthropods (springtails, mites) | Counting | Litter-bag technique (colonisation capacity) Soil coring | No | Good | Good | Low (time, expertise) |
| | | Abundance and Diversity | Community composition, ecological groupings | Yes | Very good | Very good | Low (time, expertise) |
| Soil ecosystem engineers | Earthworms, isopods | Abundance Diversity | Species richness, diversity, evenness | Yes (ongoing) | Very good | Good | Good (low expertise, simple) |

Bioindication

- example of soil quality bioindicators – are there related to soil ecosystem services?

Doelman & Eijsackers (2004)

| 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 | |

| Ecosystem service | Important ecological parameters |
|-------------------------------------|---|
| Supply of nutrients | Food web including earthworms Primary production Ratio of bacteria/fungi (De)nitrification |
| Water regulation | Earthworms Abundance and ratio bacteria/fungi pH, content of soil organic matter, groundwater level |
| Soil Structure | Earthworms Abundance and ratio of bacteria/fungi pH, content of soil organic matter Nematode Channel Ratio |
| Supply of clean shallow groundwater | Specific activity of bacteria and fungi Clean soil (concentration of pollutants lower than a maximum concentration) Extent of leaching of nitrogen, phosphate, and halogenated pollutants (EOX) Activity of the nitrogen cycle |
| Supply of clean deep groundwater | Amount and biodiversity of bacteria and fungi Clean soil Extent of washout of nitrogen and phosphate |
| Pest control in agriculture | Plant Parasitic Index of nematodes Amount and ratio of bacteria and fungi Mycorrhiza fungi |
| Changeability of soil use | Diversity of soil organisms Concentration of nitrogen and phosphate in the soil |
| Resilience and resistance | Diversity (within functional groups) |

Jensen J. & Mesman M. (2006). Ecological risk assessment of contaminated land. Decision support for site specific investigations. Report 711701047. RIVM, Netherlands

Bioindication

Example of available methods to measure soil microbial properties

| | | |
|--|-------------------------------------|---|
| | ISO 14238:2012 | Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes |
| | ISO 15685:2012 | Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation |
| | ISO 18187:2016 | Soil quality — Contact test for solid samples using the dehydrogenase activity of <i>Arthrobacter globiformis</i> |
| | ISO 17155:2012 | Soil quality — Determination of abundance and activity of soil microflora using respiration curves |
| | ISO/TS 10832:2009 | Soil quality — Effects of pollutants on mycorrhizal fungi — Spore germination test |
| | ISO/CD 23265 | Soil quality — Test for estimating organic matter decomposition in contaminated soil |
| | ISO 16072:2002 | Soil quality — Laboratory methods for determination of microbial soil respiration |
| biomass | 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 |
| enzyme activity | ISO 23753-1:2019 | Soil quality — Determination of dehydrogenases activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC) |
| | ISO 23753-2:2019 | Soil quality — Determination of dehydrogenases activity in soils — Part 2: Method using iodotetrazolium chloride (INT) |
| diversity • structural • genetic • functional | 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/TS 29843-2:2011 | Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method |
| | ISO 11063:2020 | Soil quality — Direct extraction of soil DNA |
| | ISO 17601:2016 | Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil |
| denitrification | ISO 20130:2018 | Soil quality — Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates |
| | ISO/TS 20131-1:2018 | Soil quality — Easy laboratory assessments of soil denitrification, a process source of N ₂ O emissions — Part 1: Soil denitrifying enzymes activities |
| | ISO/TS 20131-2:2018 | Soil quality — Easy laboratory assessments of soil denitrification, a process source of N ₂ O emissions — Part 2: Assessment of the capacity of soils to reduce N ₂ O |
| | 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 14239:2017 | Soil quality — Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions |

Bioidication

Example of available methods to measure soil 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) |

| Indicator system | Principle | Application | Reference |
|---------------------------|--|--|--------------------------------------|
| 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) |

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

Bioidication

Example of available methods to measure soil 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) |

| Indicator system | Principle | Application | Reference |
|--|---|--|--|
| 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. & Eijsackers H.J.P. (2004): Vital Soil - Function, Value and Properties. Elsevier. 358 p. ISBN: 0-444-51772-3

Bioidication

Example of approaches for plants

- composition of plant communities – phytocenology
- function and condition of plants
 - measurement of photosynthesis (oxygen production, fluorescence of photosynthetic pigments)
 - biochemical markers
 - genotoxicity (micronuclei, chromosome aberations)
 - functioning of nitrogen fixation, mycorrhiza
- leaf coverage
- monitoring the occurrence of indicator organisms
 - mycorrhitic fungi
 - lichens
 - diseases



Bioidication

Example of approaches for mammals

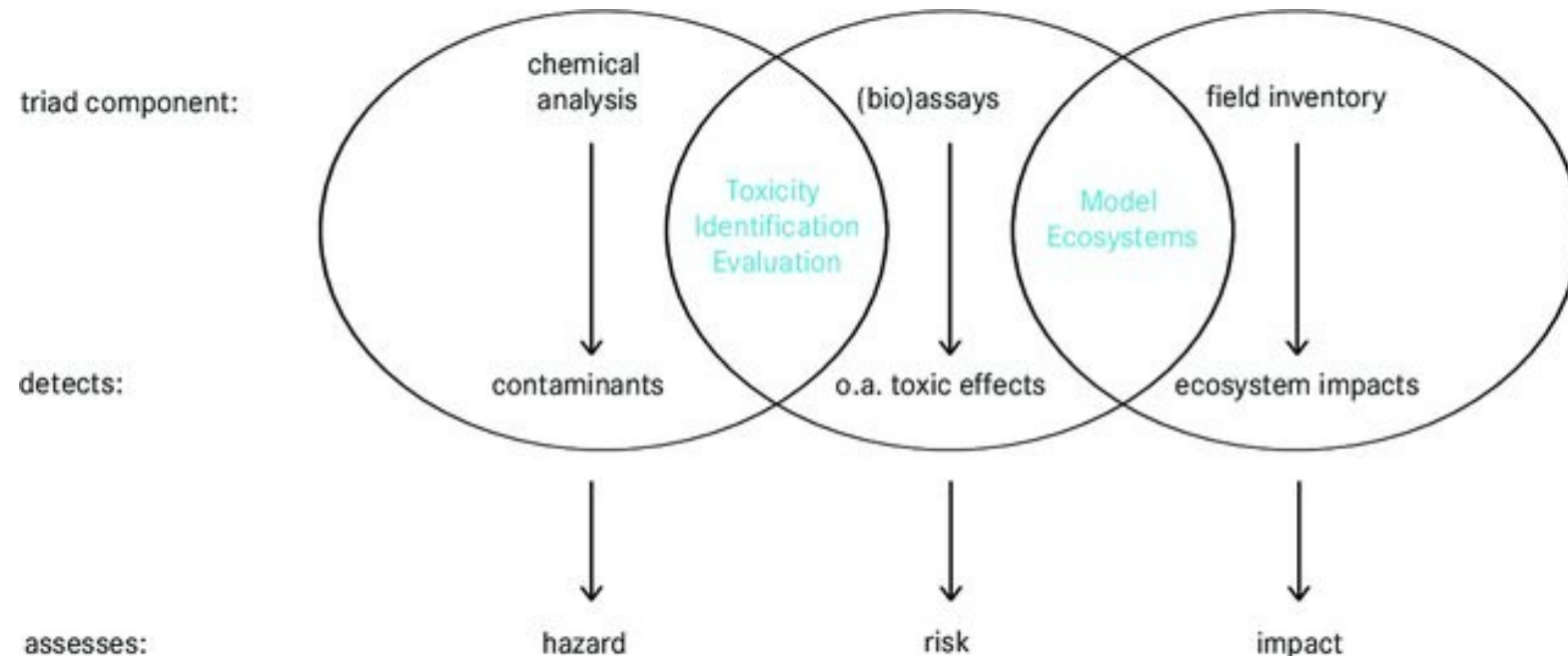
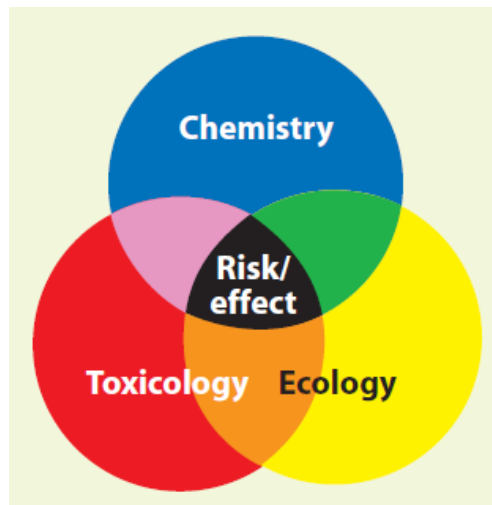
- from practical reasons often focused on „small mammals“
 - presence / absence
 - repeated catch
 - activity
 - abundance
 - density
 - richness
 - diversity
 - dynamics of the population / community...

MUNI | RECETOX

TRIAD approach

TRIAD

- long tradition
- ISO 19204 (2017): Soil quality - Procedure for site-specific ecological risk assessment of soil contamination (soil quality TRIAD approach)
- site-specific risk assessment with 3 lines of evidence (LoE)
- their evaluation = „weight of evidence“ - WoE



TRIAD

- there is scaling step
- and finally integration of all results

Text Box 1. Examples on how to scale the results from two types of toxicity tests.

Scaling. Example 1. Results in percentages.

This method can be used as default when the results from the test are expressed as percentages (%), e.g. mortality (negative effect) or survival (positive effect). Note: the results have to lie between 0 and 100%.

Scaling method 1A. Negative response in reference/control sample

Test Example: Algae light inhibition

| Data: | Reference | Site A | Site B |
|-------------------|-----------|--------|--------|
| Test results (%): | 4.0 | 46 | 71 |

Step 1. Divide data by 100. $R1 = X / 100$

| Result (R1) | Reference | Site A | Site B |
|-------------|-----------|--------|--------|
| | 0.04 | 0.46 | 0.71 |

Step 2: Scale difference between X and reference. $R2 = (X - Ref) / (1 - Ref)$

| Result (R2) | Reference | Site A | Site B |
|-------------|-----------|--------|--------|
| | 0.0 | 0.44 | 0.70 |

Scaling method 1B. Positive response in reference/control sample

Test Example: Survival of earthworms

| Data: | Reference | Site A | Site B |
|-------------------|-----------|--------|--------|
| Test results (%): | 98 | 40 | 10 |

Step 1. Subtract from 100 and then divide by 100. $R1 = (100 - X) / 100$

| Result (R1) | Reference | Site A | Site B |
|-------------|-----------|--------|--------|
| | 0.02 | 0.60 | 0.90 |

Step 2. Scale difference between X and reference. $R2 = (X - Ref) / (1 - Ref)$

| Result (R2) | Reference | Site A | Site B |
|-------------|-----------|--------|--------|
| | 0.0 | 0.59 | 0.90 |

Jensen J. & Mesman M. (2006). Ecological risk assessment of contaminated land. Decision support for site specific investigations. Report 711701047. RIVM, Netherlands

Integrated risk.

| | Reference | Site A | Site B |
|-------------------|-----------|--------|--------|
| LoE – Chemistry: | 0.00 | 0.77 | 0.84 |
| LoE – Toxicology: | 0.00 | 0.23 | 0.34 |
| LoE – Ecology: | 0.00 | 0.21 | 0.29 |

Step 1. Calculate log to (1-scaled result). $R1 = \log(1-X)$

| | Reference | Site A | Site B |
|-------------------|-----------|--------|--------|
| LoE – Chemistry: | 0.00 | -0.64 | -0.80 |
| LoE – Toxicology: | 0.00 | -0.11 | -0.18 |
| LoE – Ecology: | 0.00 | -0.10 | -0.15 |

Step 2. Average all log-values to one integrated log value. $R2 = \text{Average}(X_1 \dots X_n)$

| Result (R2) | Reference | Site A | Site B |
|-------------|-----------|--------|--------|
| | 0.00 | -0.29 | -0.38 |

Step 3. Transform log-values into integrated risk (IR) values. $R3 = 1 - (10^{R2})$

| Result (R3 = Integrated Risk) | Reference | Site A | Site B |
|-------------------------------|-----------|--------|--------|
| | 0.00 | 0.48 | 0.58 |

Step 4. Calculate standard deviation (Std) of the integrated results for each site, i.e. three LoE

| Result (R4 = Std) | Reference | Site A | Site B |
|-------------------|-----------|--------|--------|
| | 0.00 | 0.55 | 0.53 |

TRIAD příklad

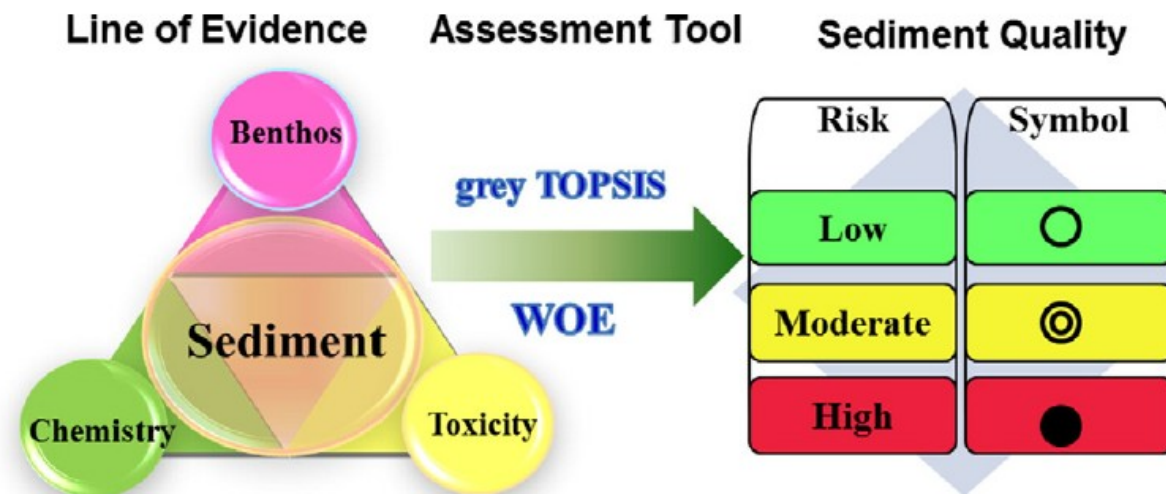
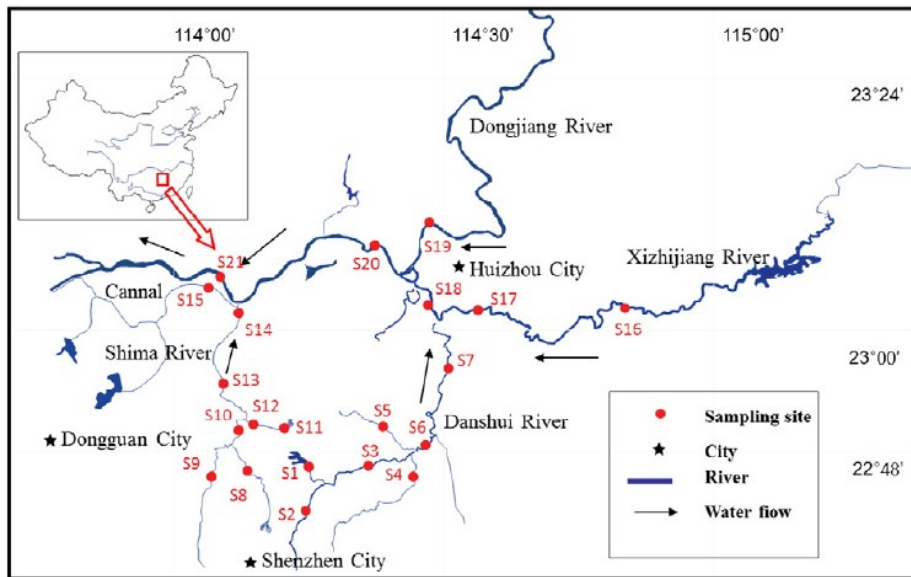


Table 1
Selection method of metric values of the better site and worse site used in the case study.

| | Chemical metric | Toxicological metric | Ecological metric |
|-----------------|----------------------|---|--|
| The better site | CB-TECs ^a | $0.2 \times (100\% \text{ inhibition rate or the maximum FTI index } ^c)$ | $0.2 \times \text{the 95th percentile of cost metric values or } 0.8 \times \text{the 95th percentile of benefit metric values } ^d$ |
| The worse site | CB-PECs ^b | $0.5 \times (100\% \text{ inhibition rate or the maximum FTI index})$ | $0.5 \times \text{the 95th percentile of cost metric values or } 0.5 \times \text{the 95th percentile of benefit metric values}$ |

^a CB-TECs = threshold effect concentration of Consensus-Based Sediment Quality Guidelines (MacDonald et al., 2000).

^b CB-PECs = probable effect concentration of Consensus-Based Sediment Quality Guidelines (MacDonald et al., 2000).

^c FTI index is the fish teratogenic index of zebrafish embryo, whose range is 0–3.

^d Cost metric is the metric that smaller is better, while benefit metric is the metric that bigger is better.

Table 2
Ecological risk ranking and final management decision.

| Ecological risk | Corresponding symbol | Sequence | Definitive final decision of overall evaluation |
|-----------------|----------------------|--|---|
| Low | ○ | In front of the better site | No further actions needed |
| Moderate | ⊙ | Between the better site and worse site | Additional assessment required |
| High | ● | Behind the worse site | Management actions required |

Jiang et al. (2015)

TRIAD - příklad

| Sites | Chemical LOE | | | Toxicological LOE | | | Ecological LOE | | |
|-------------|-----------------|-----------------------|---------------------|-------------------|----------|--------|----------------|----------|--------|
| | C+ ^a | Sequence ^b | Symbol ^c | C+ | Sequence | Symbol | C+ | Sequence | Symbol |
| Better site | 0.743 | 3 | ○ | 0.704 | 3 | ○ | 0.766 | 1 | ○ |
| Worse site | 0.378 | 22 | ⊙ | 0.522 | 11 | ⊙ | 0.682 | 5 | ⊙ |
| S1 | 0.721 | 5 | ⊙ | 0.553 | 8 | ⊙ | 0.666 | 6 | ● |
| S2 | 0.627 | 17 | ⊙ | 0.405 | 20 | ● | 0.405 | 16 | ● |
| S3 | 0.446 | 20 | ⊙ | 0.488 | 14 | ● | 0.314 | 18 | ● |
| S4 | 0.443 | 21 | ⊙ | 0.777 | 1 | ○ | 0.494 | 11 | ● |
| S5 | 0.700 | 10 | ⊙ | 0.468 | 18 | ● | 0.720 | ? | ⊙ |
| S6 | 0.690 | 12 | ⊙ | 0.472 | 16 | ● | 0.604 | | |
| S7 | 0.690 | 11 | ⊙ | 0.580 | 6 | ⊙ | 0.499 | | |
| S8 | 0.558 | 18 | ⊙ | 0.471 | 17 | ● | 0.167 | | |
| S9 | 0.748 | 2 | ○ | 0.269 | 22 | ● | 0.491 | | |
| S10 | 0.711 | 8 | ⊙ | 0.542 | 9 | ⊙ | 0.322 | | |
| S12 | 0.667 | 15 | ⊙ | 0.384 | 21 | ● | 0.238 | | |
| S13 | 0.718 | 6 | ⊙ | 0.425 | 19 | ● | 0.167 | | |
| S14 | 0.650 | 16 | ⊙ | 0.681 | 4 | ⊙ | 0.414 | | |
| S15 | 0.722 | 4 | ⊙ | 0.506 | 13 | ● | 0.302 | | |
| S16 | 0.753 | 1 | ○ | 0.754 | 2 | ○ | 0.596 | | |
| S17 | 0.713 | 7 | ⊙ | 0.478 | 15 | ● | 0.689 | | |
| S18 | 0.503 | 19 | ⊙ | 0.666 | 5 | ⊙ | 0.443 | | |
| S19 | 0.707 | 9 | ⊙ | 0.520 | 12 | ● | 0.607 | | |
| S20 | 0.678 | 13 | ⊙ | 0.570 | 7 | ⊙ | 0.710 | | |
| S21 | 0.675 | 14 | ⊙ | 0.540 | 10 | ⊙ | 0.454 | | |

| Sites | Relax effect | | | Strict effect | | |
|-------------|-----------------|-----------------------|---------------------|---------------|----------|--------|
| | C+ ^a | Sequence ^b | Symbol ^c | C+ | Sequence | Symbol |
| Better site | 0.711 | 1 | ○ | 0.765 | 1 | ○ |
| Worse site | 0.499 | 14 | ⊙ | 0.537 | 9 | ⊙ |
| S1 | 0.623 | 4 | ⊙ | 0.613 | 5 | ⊙ |
| S2 | 0.451 | 18 | ● | 0.445 | 18 | ● |
| S3 | 0.412 | 19 | ● | 0.399 | 19 | ● |
| S4 | 0.538 | 10 | ⊙ | 0.523 | 11 | ● |
| S5 | 0.615 | 5 | ⊙ | 0.615 | 4 | ⊙ |
| S6 | 0.563 | 8 | ⊙ | 0.561 | 8 | ⊙ |
| S7 | 0.543 | 9 | ⊙ | 0.537 | 10 | ● |
| S8 | 0.390 | 22 | ● | 0.377 | 22 | ● |
| S9 | 0.473 | 16 | ● | 0.464 | 16 | ● |
| S10 | 0.482 | 15 | ● | 0.473 | 15 | ● |
| S12 | 0.391 | 21 | ● | 0.388 | 21 | ● |
| S13 | 0.399 | 20 | ● | 0.393 | 20 | ● |
| S14 | 0.526 | 11 | ⊙ | 0.520 | 12 | ● |
| S15 | 0.464 | 17 | ● | 0.455 | 17 | ● |
| S16 | 0.637 | 3 | ⊙ | 0.626 | 3 | ⊙ |
| S17 | 0.609 | 6 | ⊙ | 0.600 | 6 | ⊙ |
| S18 | 0.508 | 13 | ⊙ | 0.500 | 14 | ● |
| S19 | 0.589 | 7 | ⊙ | 0.593 | 7 | ⊙ |
| S20 | 0.639 | 2 | ⊙ | 0.629 | 2 | ⊙ |
| S21 | 0.518 | 12 | ⊙ | 0.506 | 13 | ● |

Jiang et al. (2015)

Problems in field ecotoxicology

Problems in the field studies

- **natural fluctuations, large influence of environmental factors**
- Contamination data in most cases focus on total content
 - biota, however, reacts only to **bioavailable** fraction that depends on many factors (cannot be well modeled)
 - as a result, we often do not see the causality between pollution and the condition of biota, except of very high concentrations
- The observed phenomena have a stochastic character
 - There is a natural scattering in space and time!
 - Do we have a sufficiently representative sample? What do we really sample and measure?
- Contamination often acts as a selection pressure
 - Long -term load can lead to creating adaptations and tolerances or even stimulation (especially in microorganisms)
 - Do we know the history of the locality contamination well?

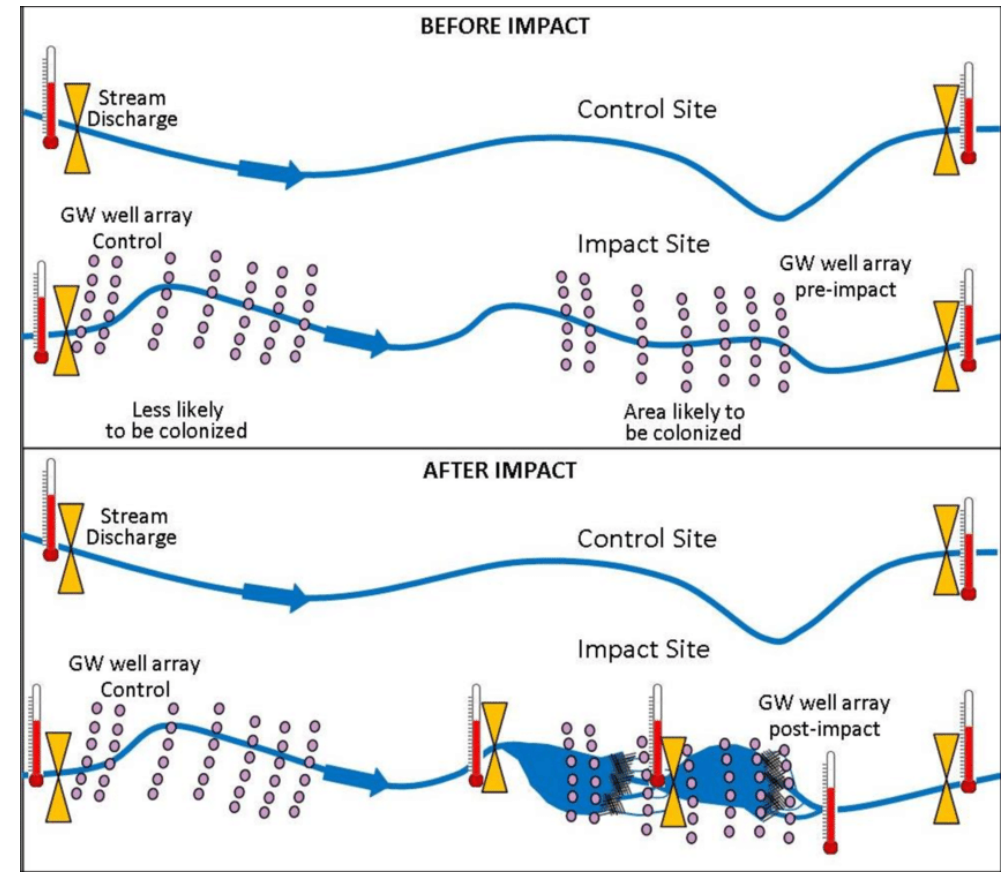
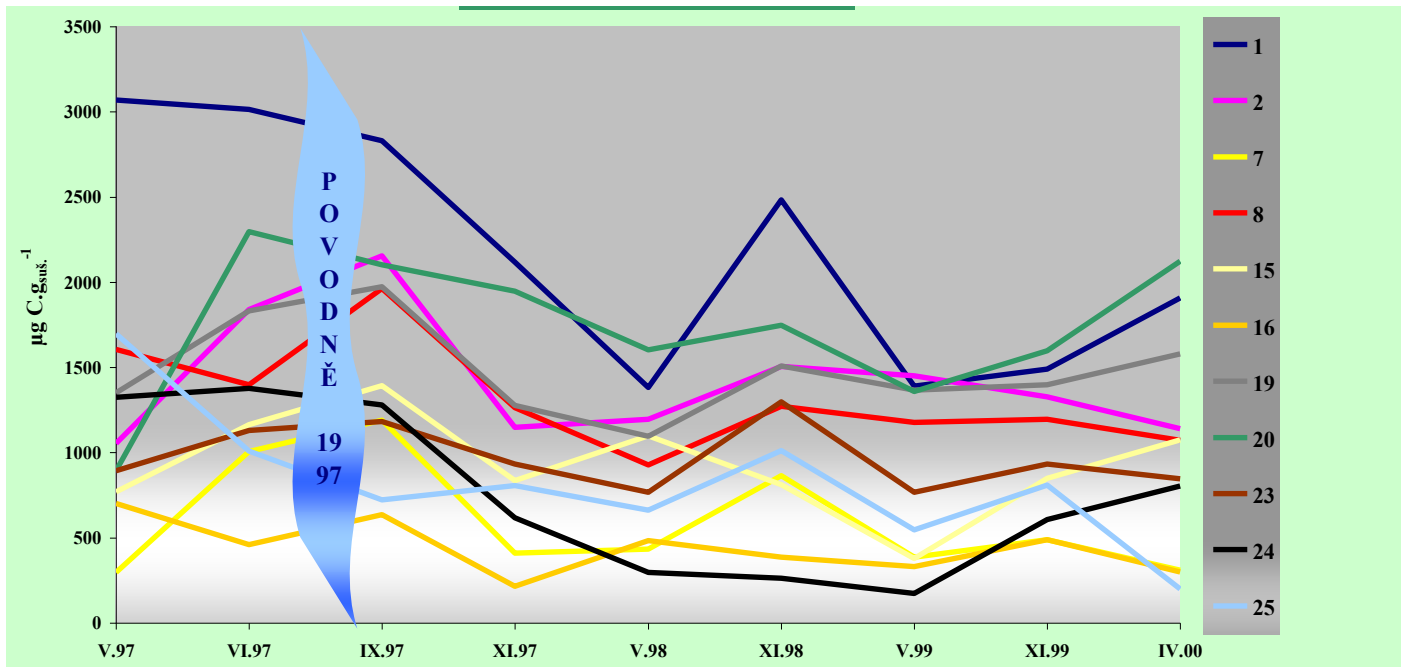
Problems in the field studies

- Total interconnection by food and ecological links, continuity of processes
 - Changes in the activity of one community or population in relation to other communities and functions that are linked
 - Inhibition of one ecosystem component can stimulate another component
- Organisms themselves can affect chemical forms of pollutants
 - For example, sorbed forms of substances may be mobilized again, or microbial degradation may come
- The problem of optimal field study design (biomonitoring)
 - Need of a reference state – non-contaminated / non-impacted site (comparison with control)
 - or a large dataset (correlation, causality)
 - or time trends (BACI)

A reference state is needed

BACI = comparing Before and After Control Impact

- a control = state of ekosystém before the impact
- it needs a monitoring before the impact happens (both biotic and abiotic components must be observed)
- ie background values and „natural“ state



A reference state is needed

Comparison of an exposed ecosystem with another ("control,, – un-impacted) ecosystem

- The key is the choice of a control ecosystem:
 - Both ecosystems have comparable abiotic properties (terrain, geology, altitude ...)
 - Similar biological properties are expected in normal state (ie the same communities, food relations ...)
- The derivation of the conclusions in this case is always complicated (there are no two same / equally evolving ecosystems)



„Normal“ state in the ecosystems

stationary state

- long term state, no disturbances
- this is often not „normal“: ecosystems are naturally „variable“ and „changing“

stable state

- surrounding conditions / factors do not change the major features (functions, overall performance ...), but inside there might be changes and fluctuations

dynamic stability / ekvilibrrium = homeostasis

- using action/reaction, positive and negative feedback it keeps long-term stable state

succession

- ecosystems are never „stationary“ – they go through development in time: so, the Protection should not simply aim on „conservation of the current state“

„Normal“ state in the ecosystems

- regulatory approach – **example: water framework directive EU (WFD)**
- EU WFD aims at good status of all surface waters in EU till 2020
- 2 components of quality assessment (“good state”) - „ecological“ and „chemical“

Chemical component

- 3 lists of defined substances
 - Priority substances list
 - good quality = concentration of each individual chemical < EQS (Environmental Quality Standards), AA-EQS – annual average concentration, MAC-EQS – maximum acceptable concentration
 - watch list – these should be measured for the future assessment, they may become Priority substances
 - specific pollutants – according to the plans of the river basins („river basin specific pollutants)

„Normal“ state in the ecosystems

- regulatory approach – **example: water framework directive EU (WFD)**
- EU WFD aims at good status of all surface waters in EU till 2020

- 2 components of quality assessment (“good state”) - „ecological“ and „chemical“

Ecological component

