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E5080 / E0323

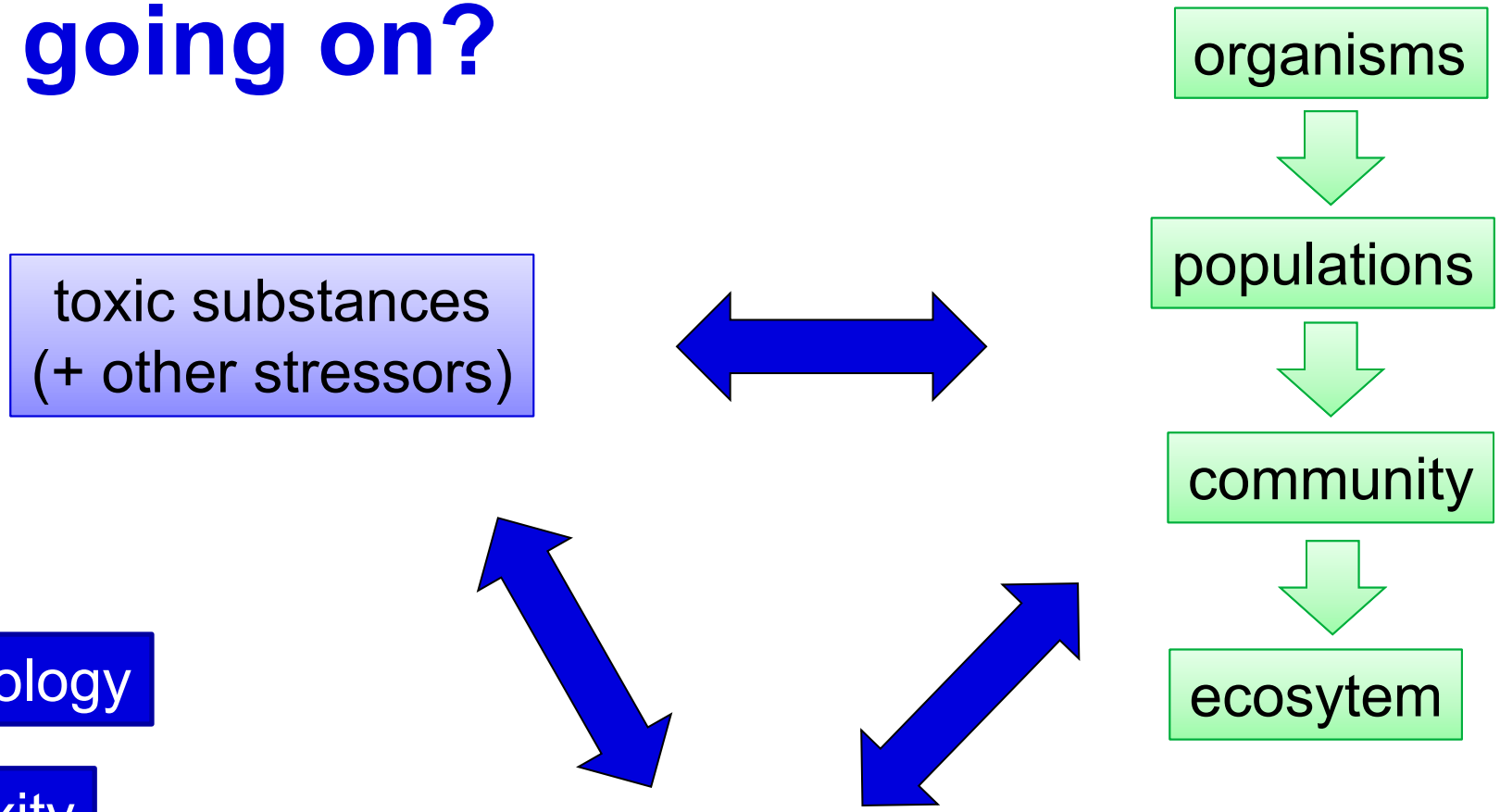
Ecotoxicology

Ecotoxicology in Field Studies

Jakub Hofman

Introduction

What is going on?



ecotoxicology

complexity

interactions

mixtures

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Why?

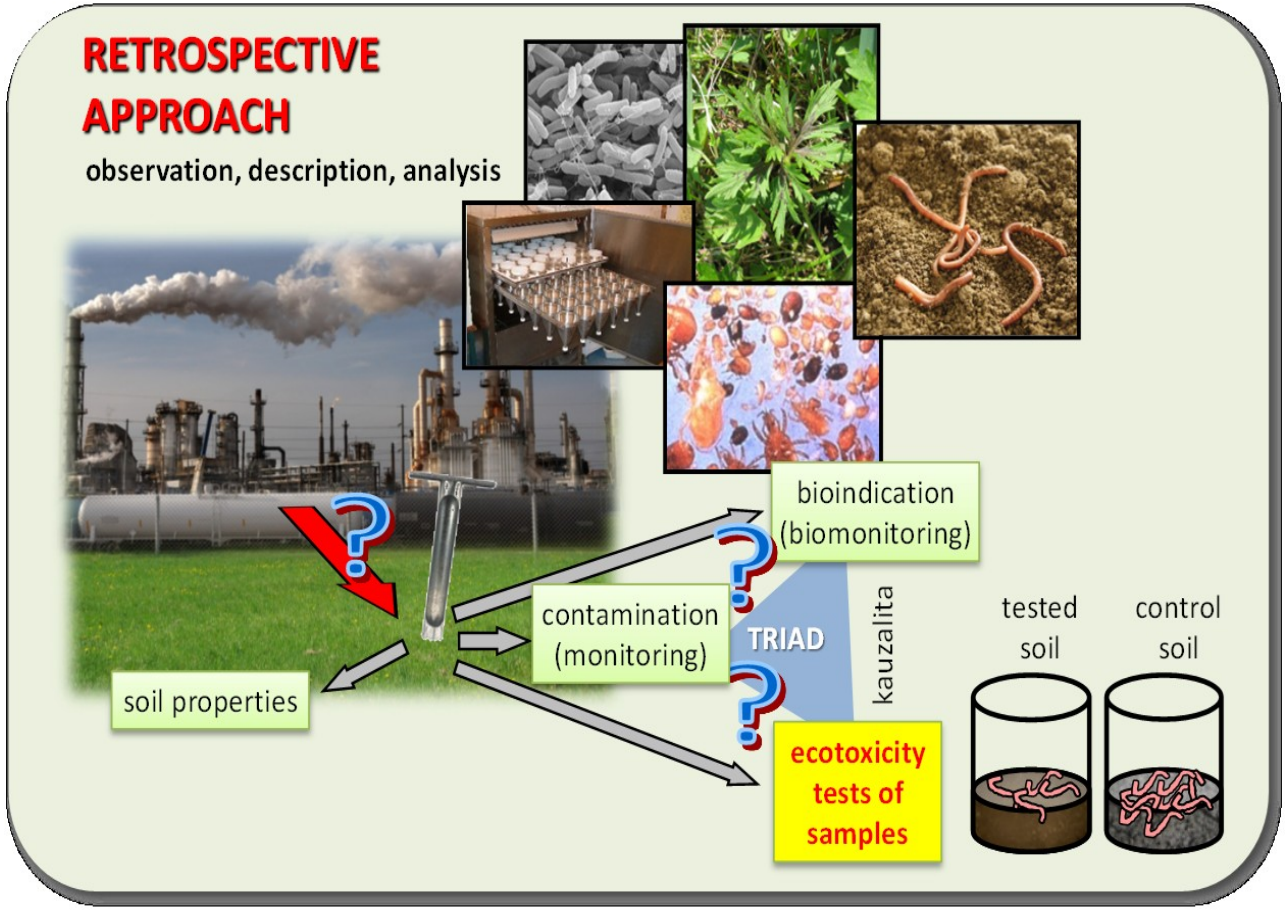
- real problems are in **real** ecosystems !
- lot of problems already **happened** !

Challenges

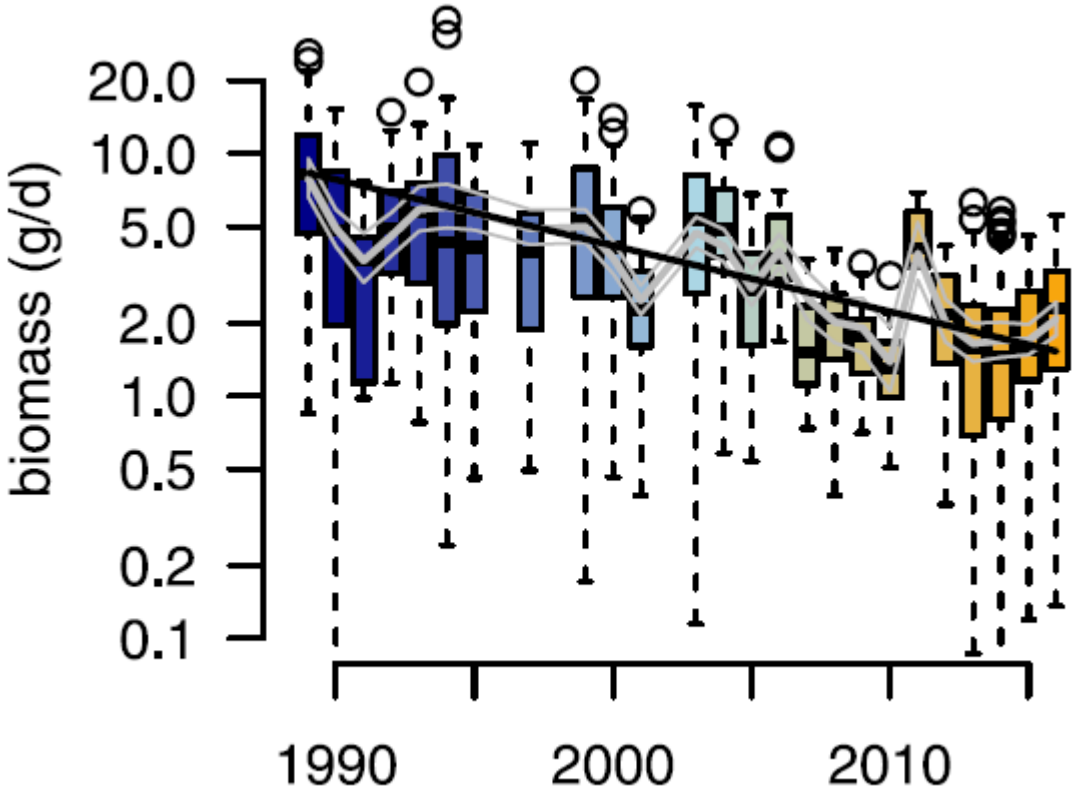
- how to address **ecotoxicity in real** situation?
- how to find **causality** between degradation and ekosystem state?

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)

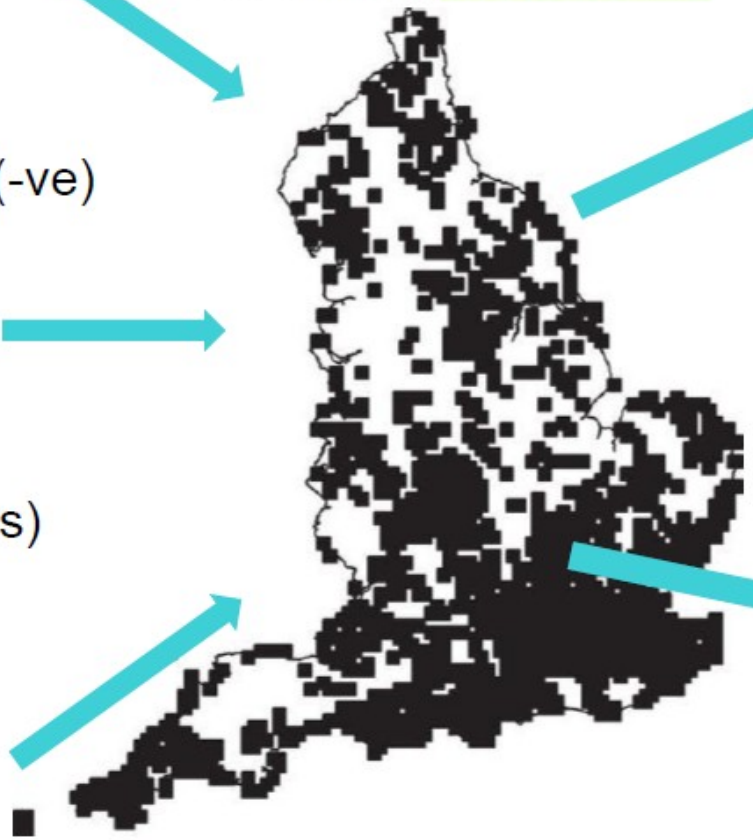
Neonicotinoid use (-ve)

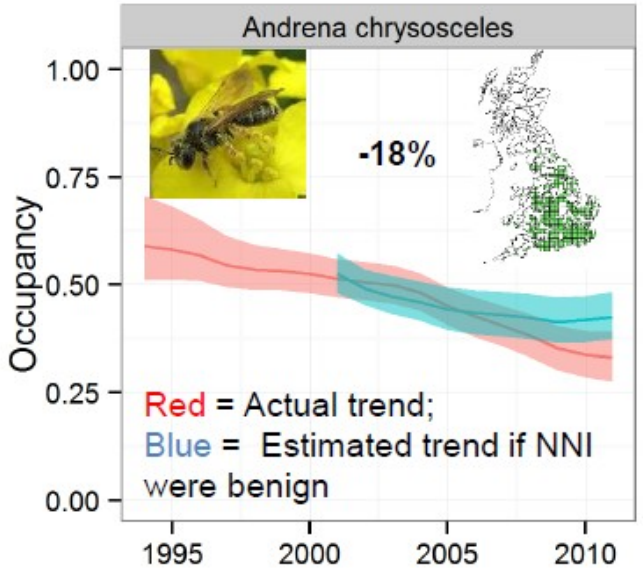
Other pesticides (ns)

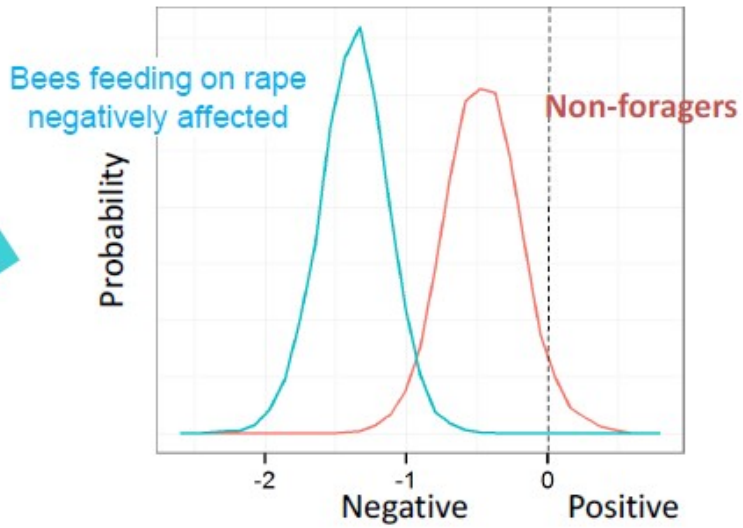
Volunteer surveys of bees

BWARS Bees, Wasps & Ants Recording Society

BRC Biological Records Centre







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

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, bilances, resilience/resistence ...)
3. **sampling plan** (sampling frequency, numbers ...)
   - abiotic components (water, sediments, soil air)
   - biotic components (producers – consumers – destruents)
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 antrhopogenic 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 – zooplancton, fish; destruents – 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

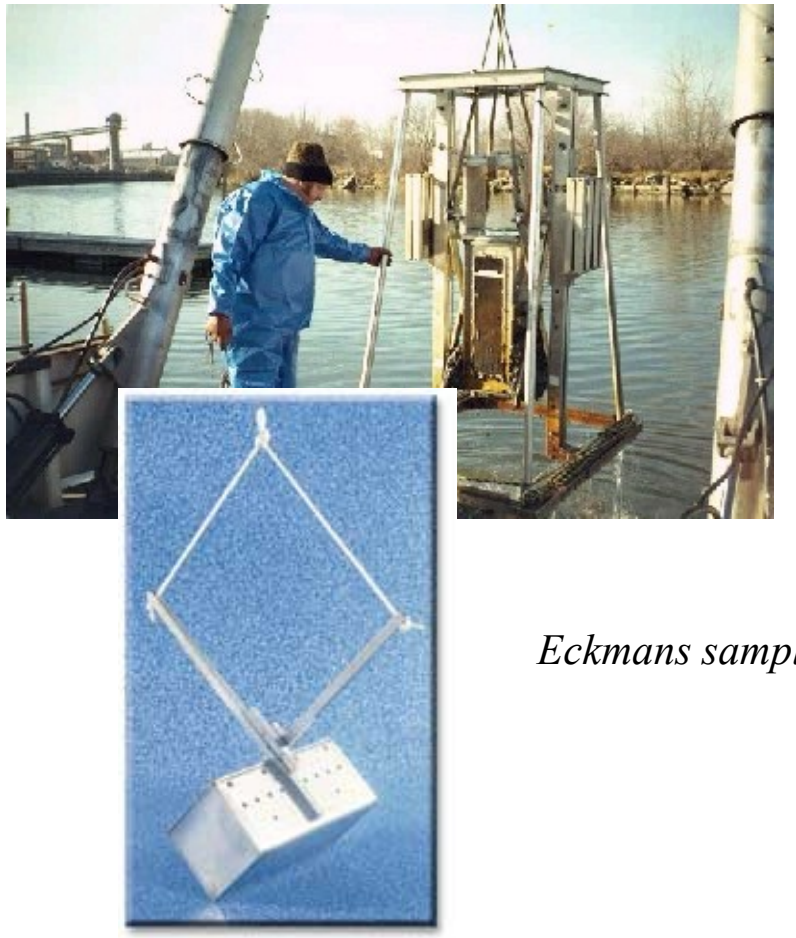
- plan and design of sampling plots / sites
  - areal, vertical – depth, air sampling
- merging and creating mixed samples („average" sample from the site)
- assessment of the fundamental chemical and physical parameters (organic carbon, pH, particle sizes ....)
- characterization and determination of 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

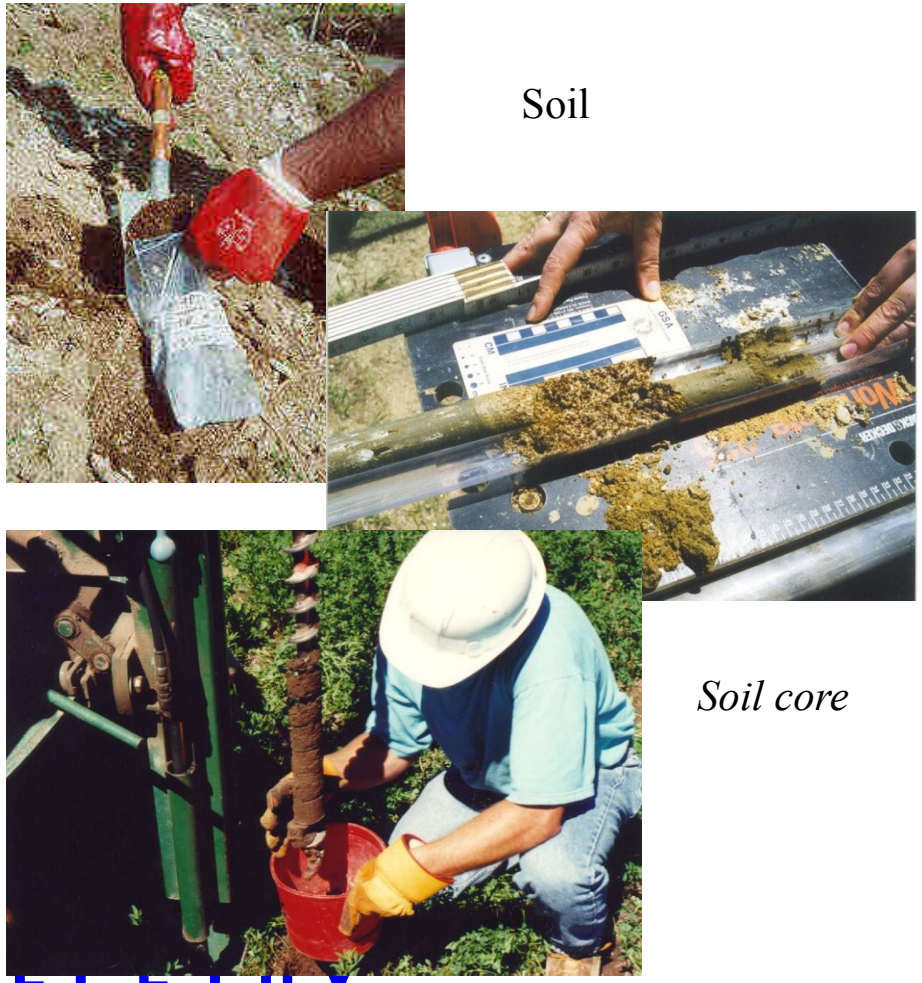
Sediment



Eckmans sampler

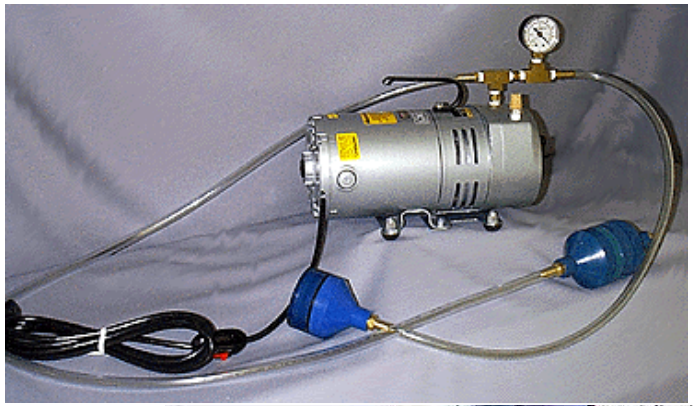
3) Sampling and analyses

A: sampling and analyses of abiotic components



Soil

Soil core



Air

3) Sampling and analyses

B: sampling and analyses of biota

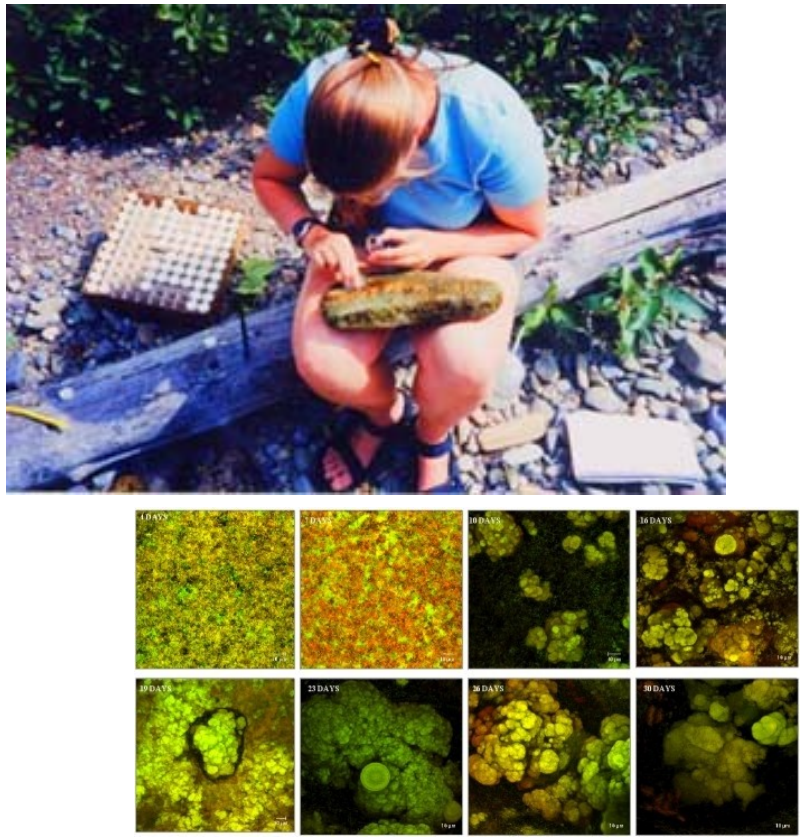
- plan and distribution of the sampling plots / sites
- sampling – variable according to organisms...
- characterization of defined biotic parameters
  - techniques of botanical, zoological, microbiological and ecological disciplines
- characterization and determination of contamination of biota
  -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

3B) Sampling - biota

- water

Planctonic nets

Periphyton – biofilm



3B) Sampling - biota

- water

Benthic invertebrates

Fish

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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

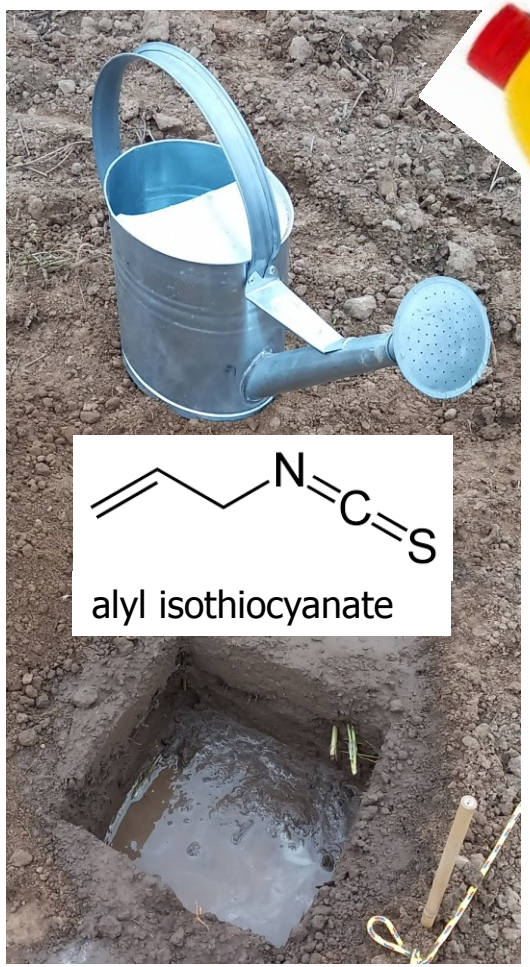
ISO 23611-1:2006	Soil quality -- Sampling of soil invertebrates – Part 1: Hand-sorting and formalin extraction of **earthworms**
ISO 23611-2:2006	Soil quality -- Sampling of soil invertebrates – Part 2: Sampling and extraction of **micro-arthropods** (Collembola and Acarina)
ISO 23611-3:2007	Soil quality -- 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 ***design of sampling programmes*** with soil invertebrates

3B) Sampling – biota – soil biota

- earthworms

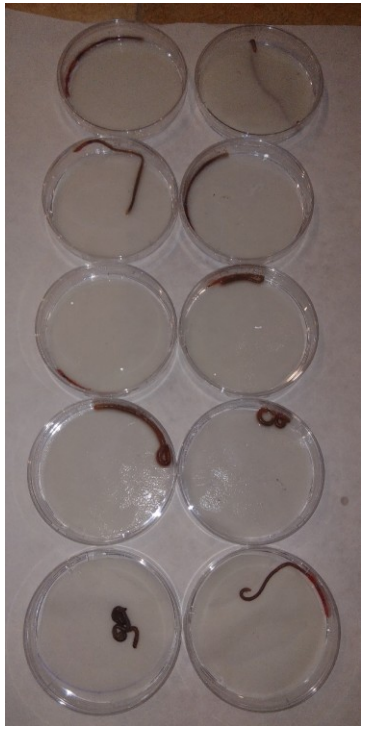
3B) Sampling – biota – soil biota

- earthworms



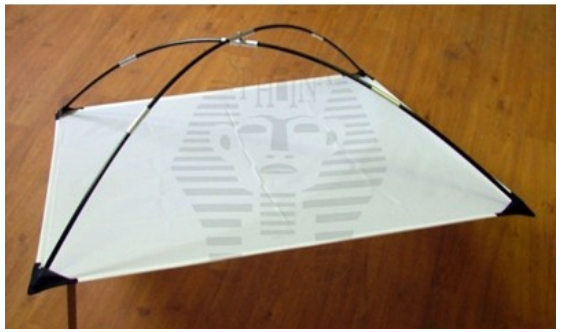
3B) Sampling – biota – soil biota

- 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



3B) Sampling – biota – insects

3B) Sampling – biota – terrestrial plants

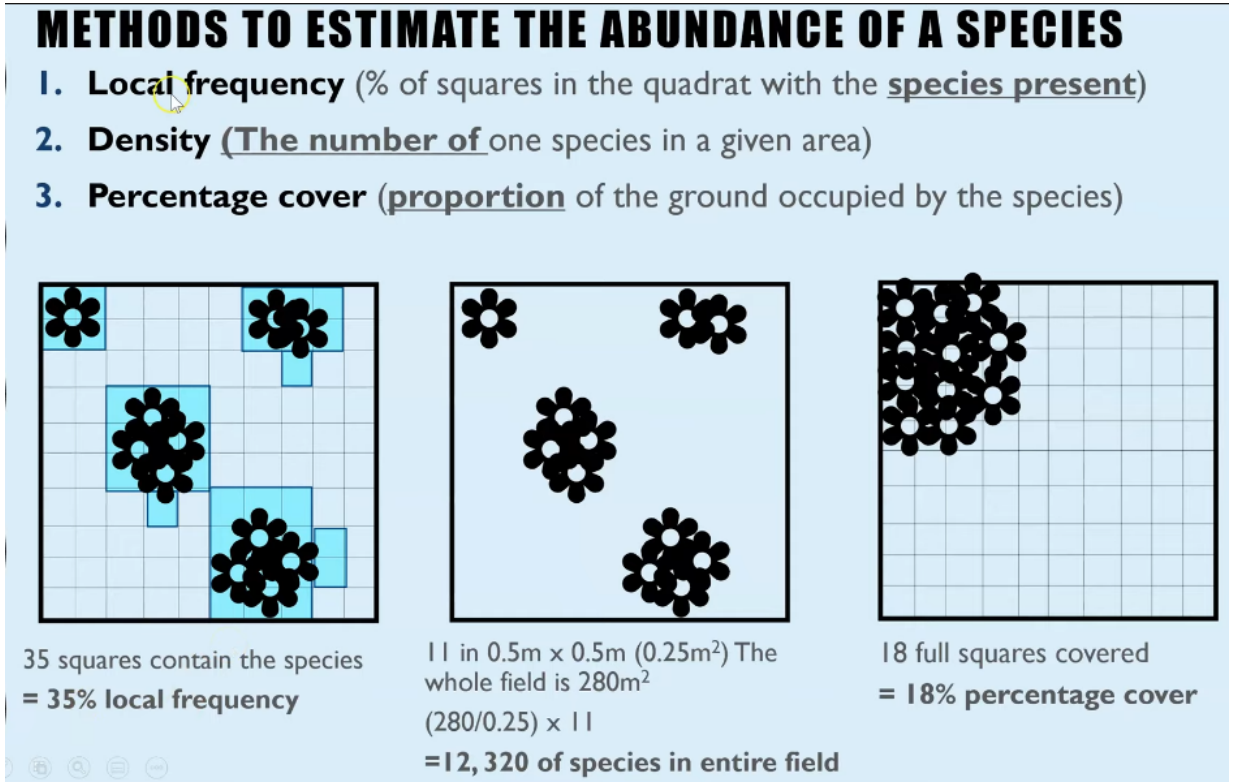
Phytocoenological snapshot

- defining area, square or rectangle
- units or hundreds of m^2
- 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



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)

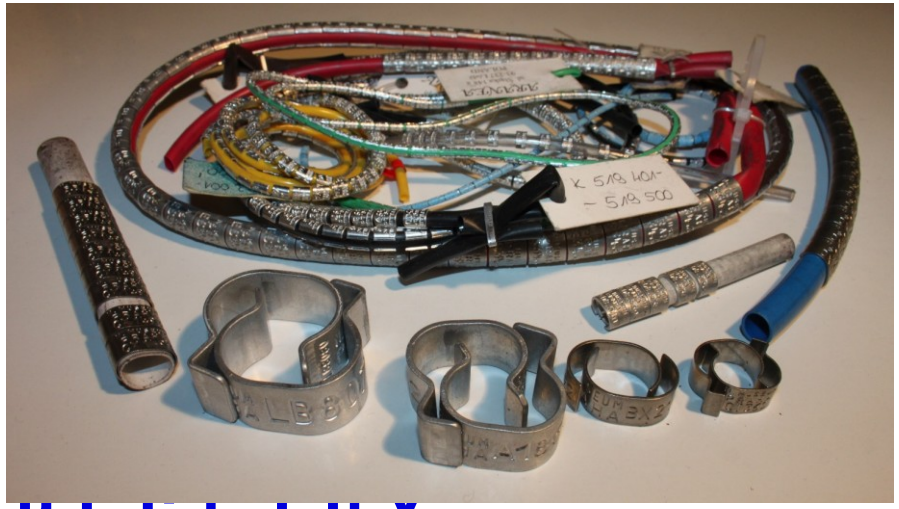
3B) Sampling – biota – mammals

Direct methods

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

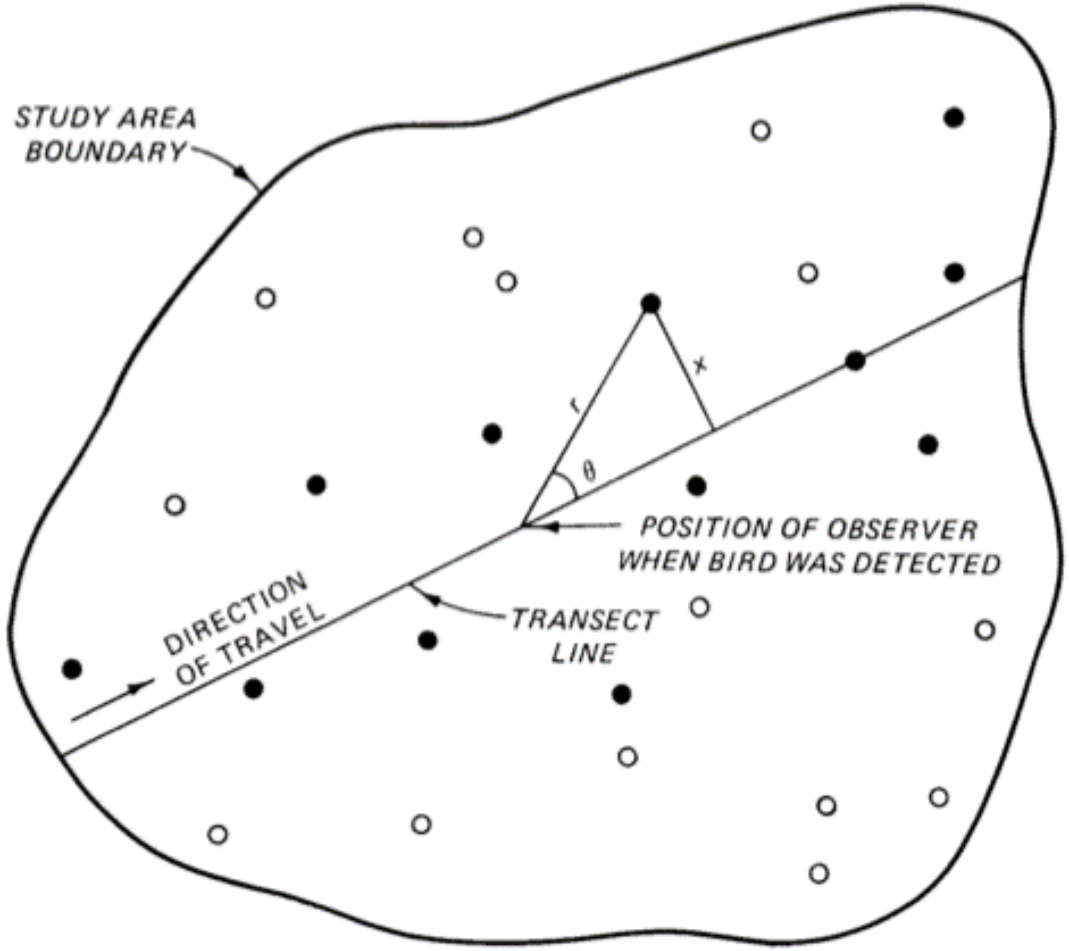
3B) Sampling – biota – birds

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

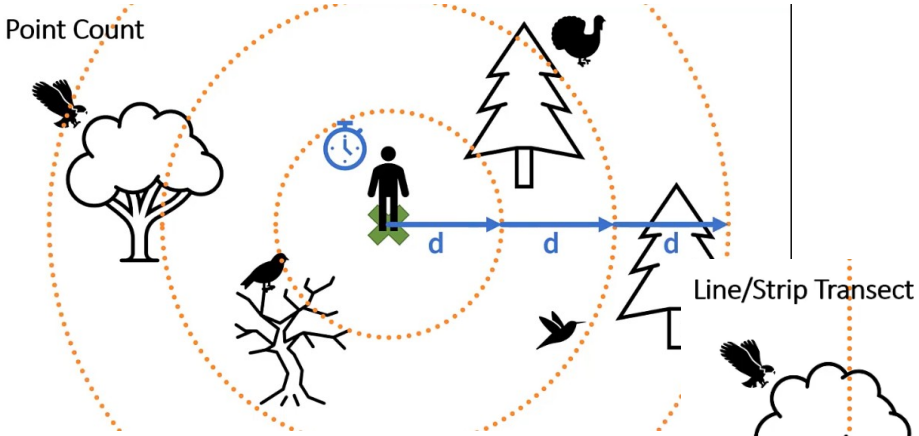


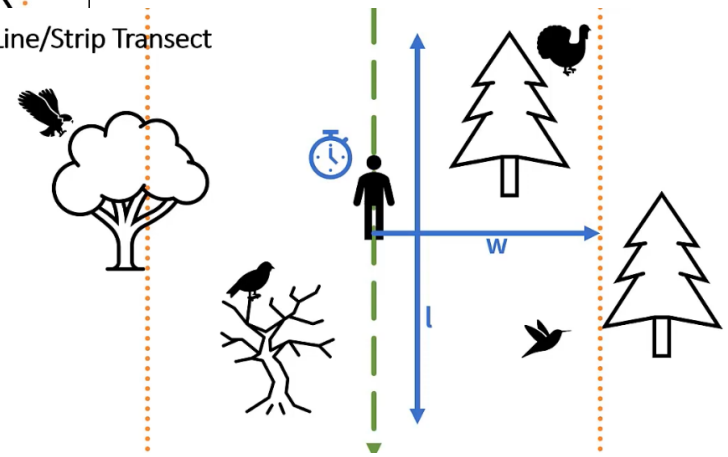
3B) Sampling – biota – b

- **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





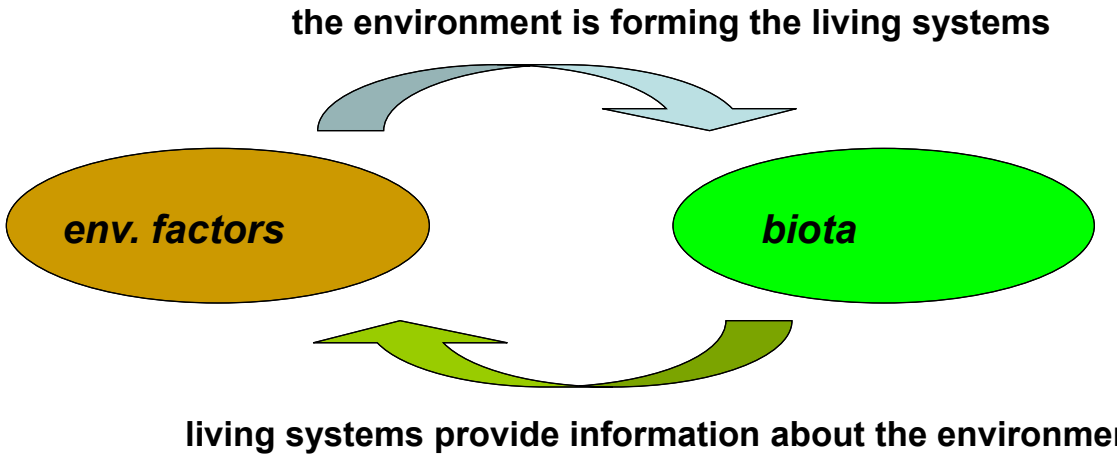
4) Assessment and interpretation

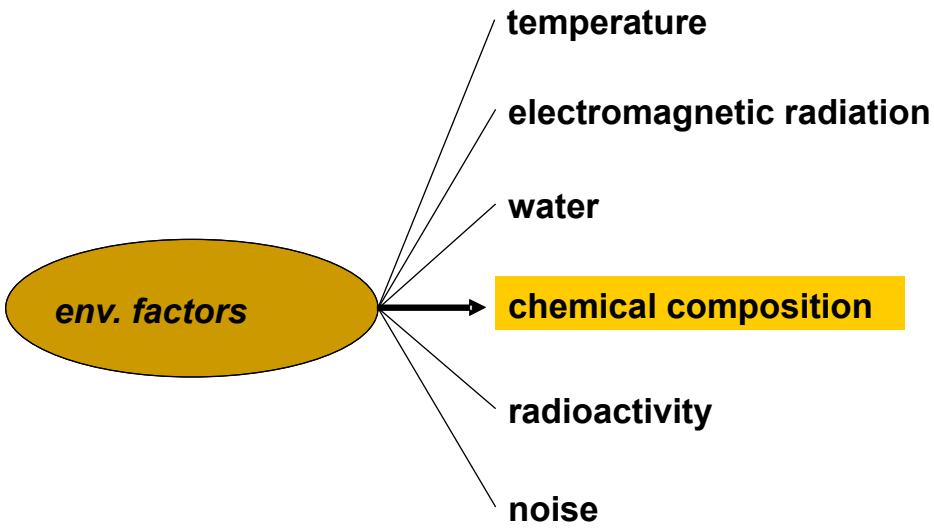
- **comparision of the exposed and control ecosystem**
- fundamental parameters of the compared ecosystems should be SIMILAR / COMPARABLE (eg pH values, water hardness, similar geochemical parameters – subsurface ...)
- chemical contamination of the environmental compartments **versus** biota in the 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





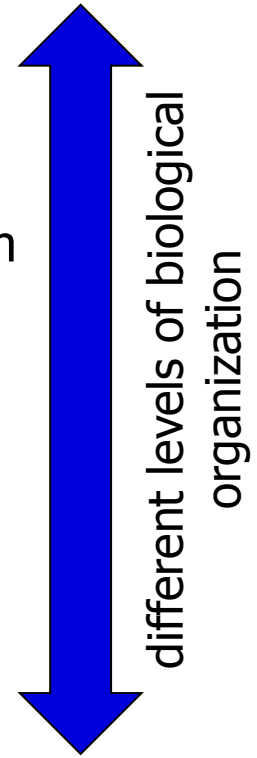
in broader context, we mean all methods when we observe reactions of organisms present in the environment (from individuals to communities) on stress

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

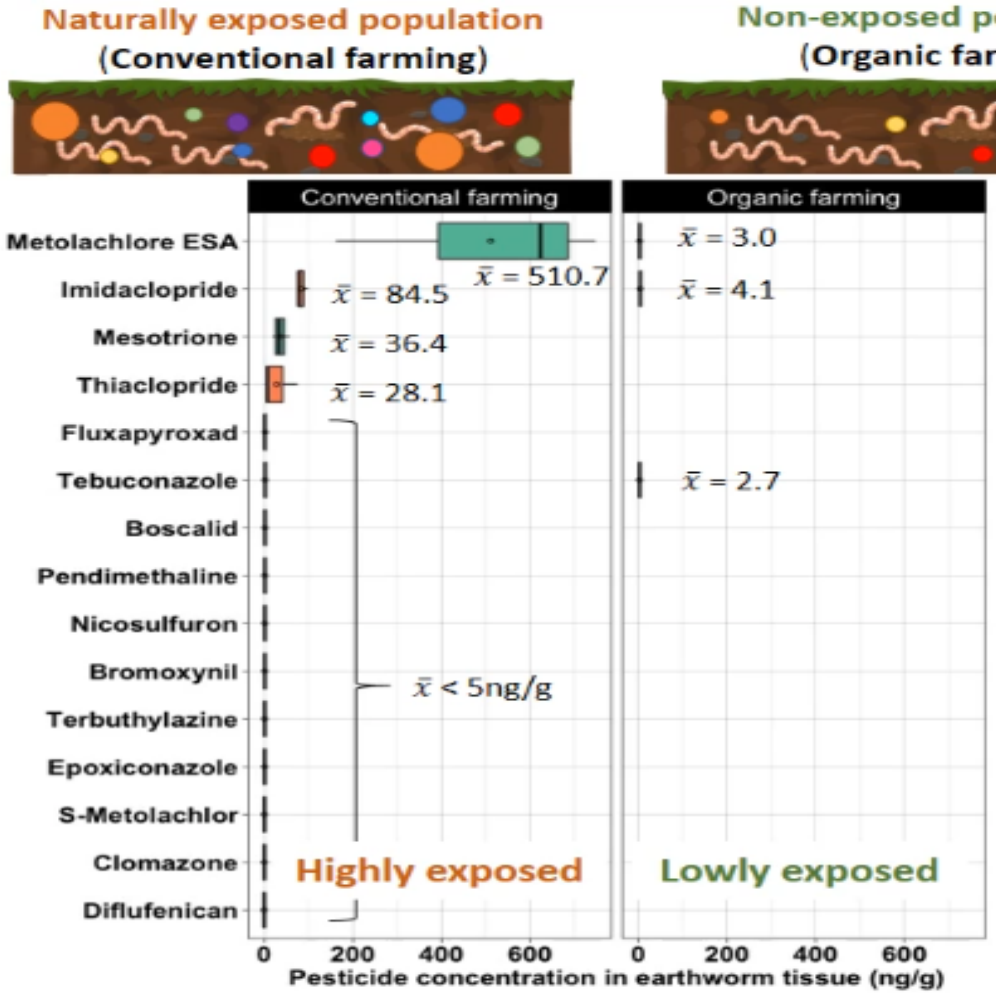
Bioindication

- monitoring of chemicals in the collected biota samples
  - in anything, preferentially so -called bioacumulators 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

Class
- Fungicide
- Herbicide
- Insecticide

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

- sapros = 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

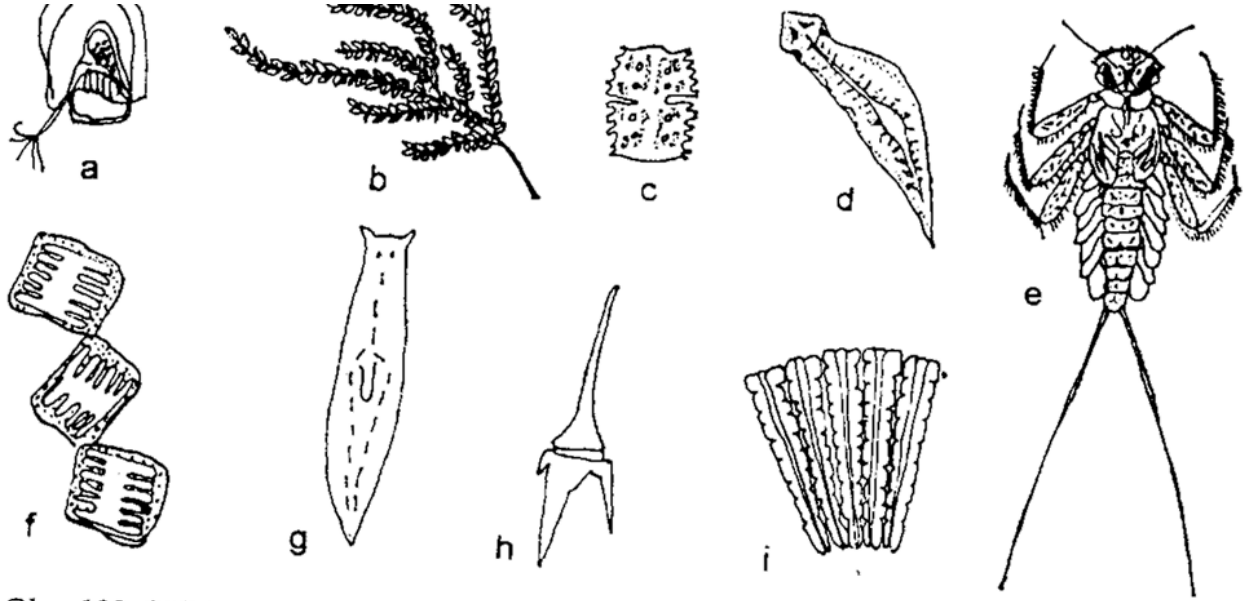
Increased saprobity

- 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

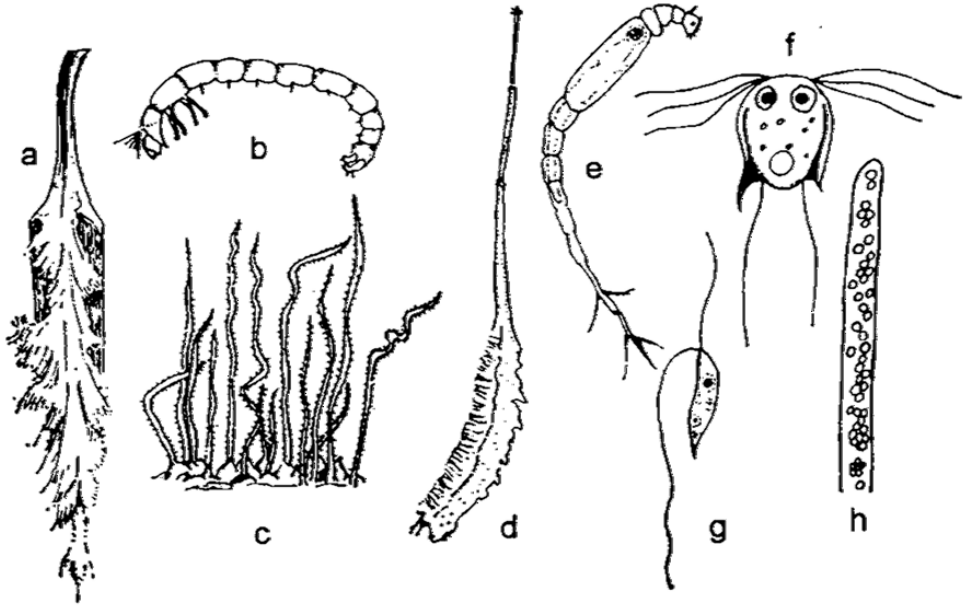
Indicator species for saprobity - examples

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



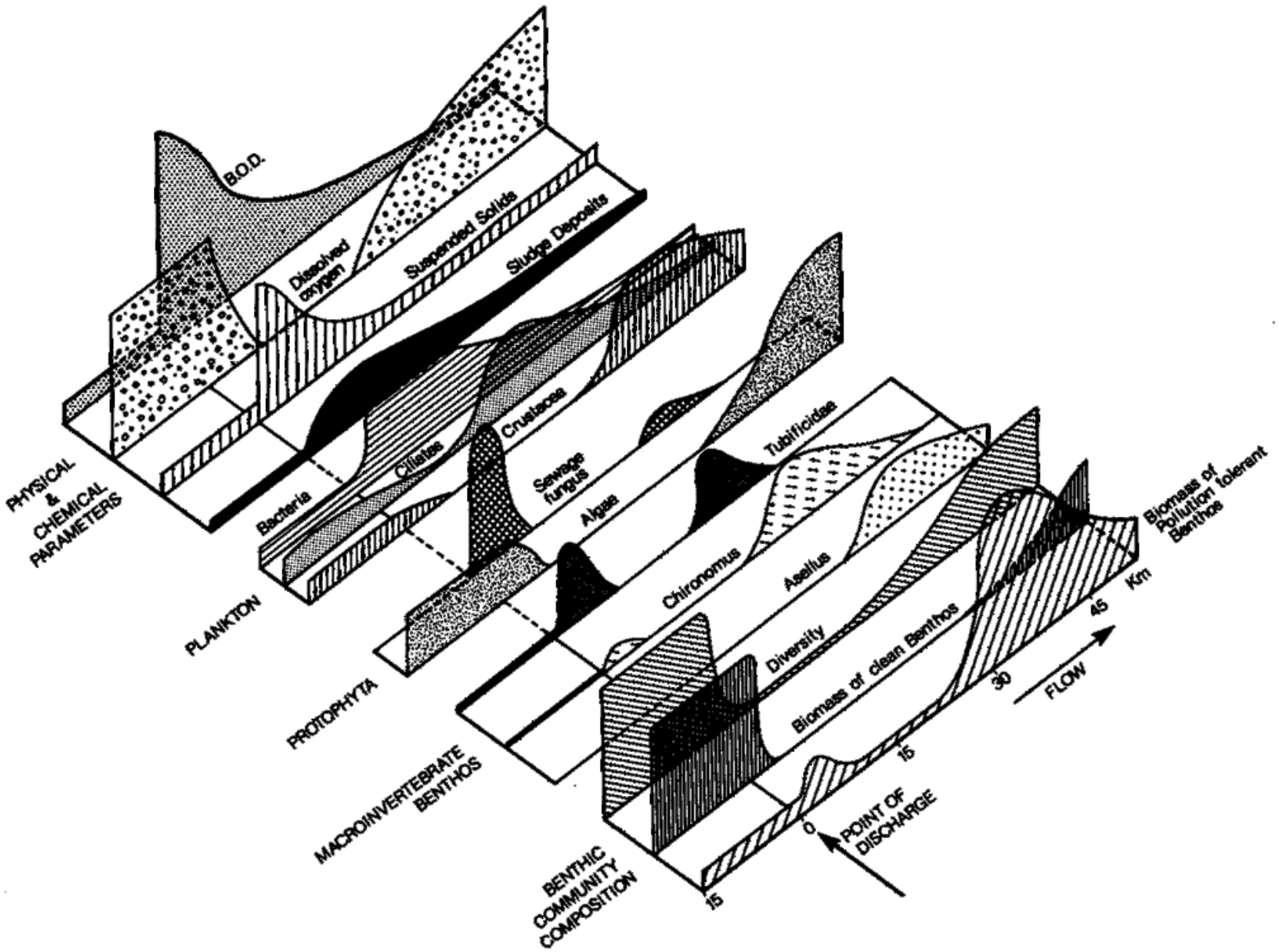
Obr. 135. Příklad polysaprobních organismů
a – bakterie *Sphaerotilus natans*, b – pakomár *Chironomus thummi*, c – nitěnky *Tubifex tubifex*, d – pestřenka r. *Eristalis*, e – vířník *Rotaria neptunia*, f – bičíkovec *Hexamitus inflatus*, g – bičíkovec *Bodo putrinum*, h – bakterie *Beggiatoa alba*

Indicator species – example: Saprobity index

- Community shift

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

Ai – abundance of species i
Si – individual saprobity value of species i
gi – indikative 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
- Instrinsic
- 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

 Kelly J. & Harwell M. (1990).

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	Dehydgenase 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)

EC (2010): Soil biodiversity: functions, threats and tools for policy makers. https://core.ac.uk/display/29245351

Bioindication

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

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

Doelman & Eijsackers (2004)

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 Arthrobacter globiformis
	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
	ISO 20130:2018	Soil quality — Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates
denitrification	ISO/TS 20131-1:2018	Soil quality — Easy laboratory assessments of soil denitrification, a process source of N2O emissions — Part 1: Soil denitrifying enzymes activities
	ISO/TS 20131-2:2018	Soil quality — Easy laboratory assessments of soil denitrification, a process source of N2O emissions — Part 2: Assessment of the capacity of soils to reduce N2O
	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
  - pollutants in plants

Bioidication

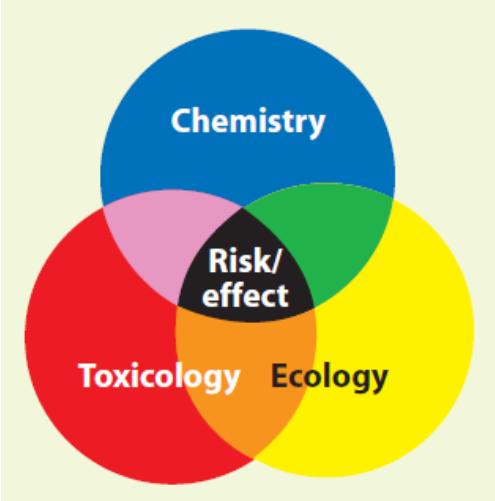
Example of approaches for mammals

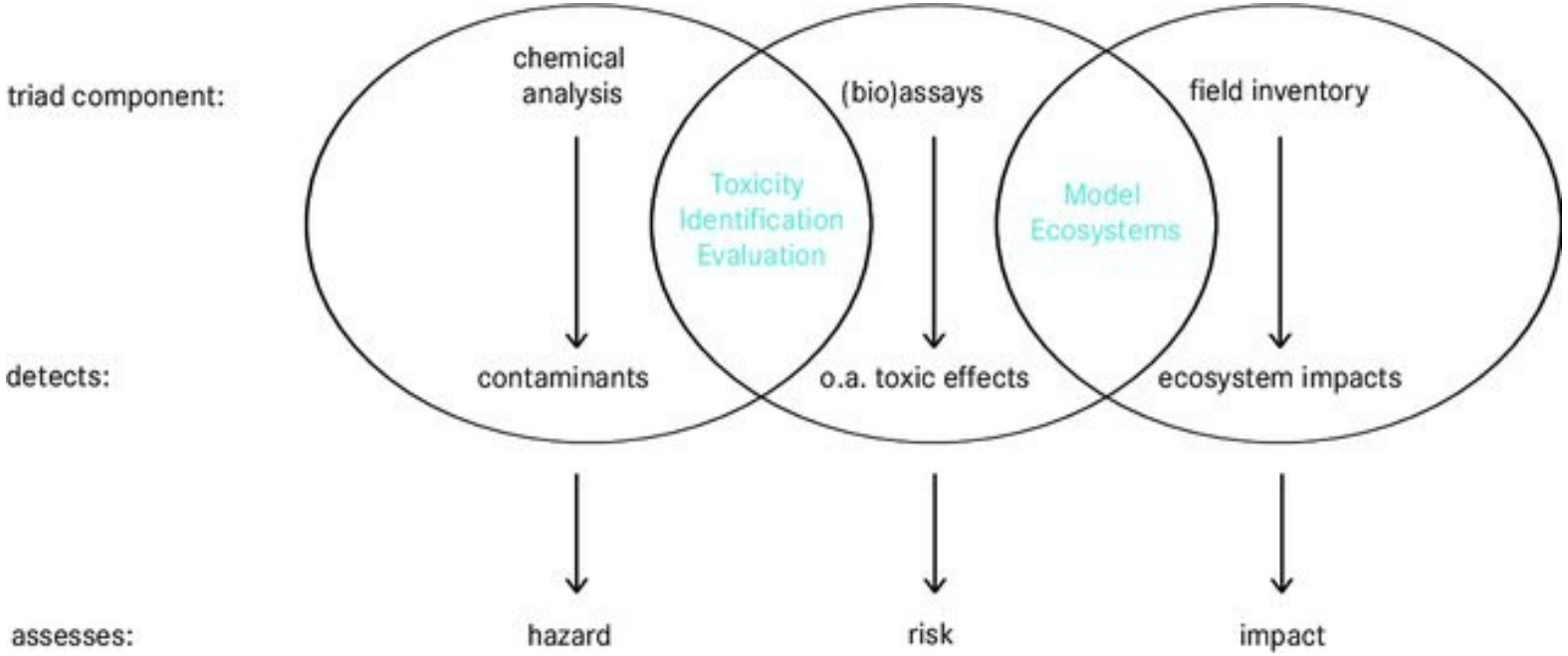
- from practical reasons often focused on „small mammals“
  - presence / absence
  - repeated catch
  - activity
  - abundance
  - density
  - richness
  - diversity
  - dynamics of the population / community...

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

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

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

	Reference	Site A	Site B
Result (R2)	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

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

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

	Reference	Site A	Site B
Result (R2)	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 = Average ($X_1 \ldots X_n$)

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

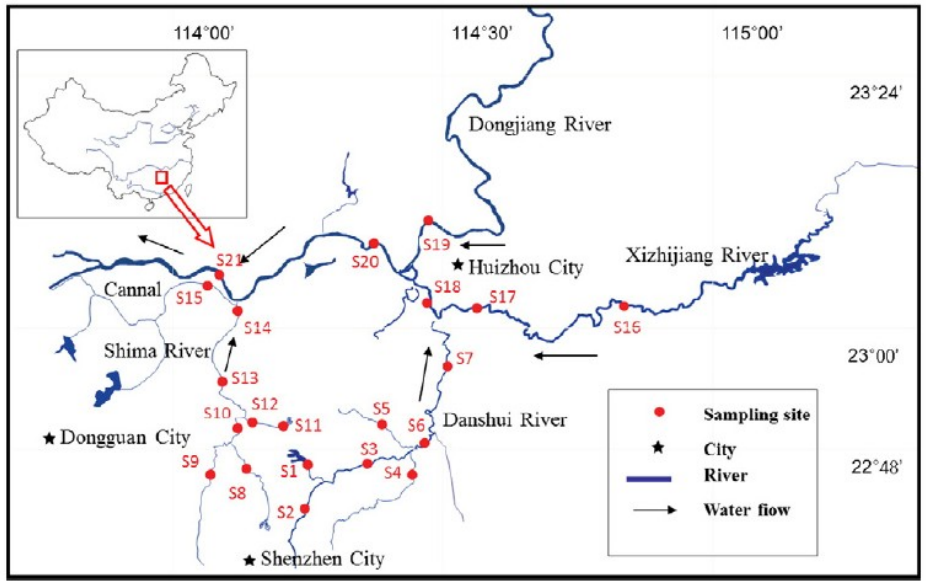
Step 3. Transform log-values into integrated risk (IR) values. R3 = 1-(10^R2)

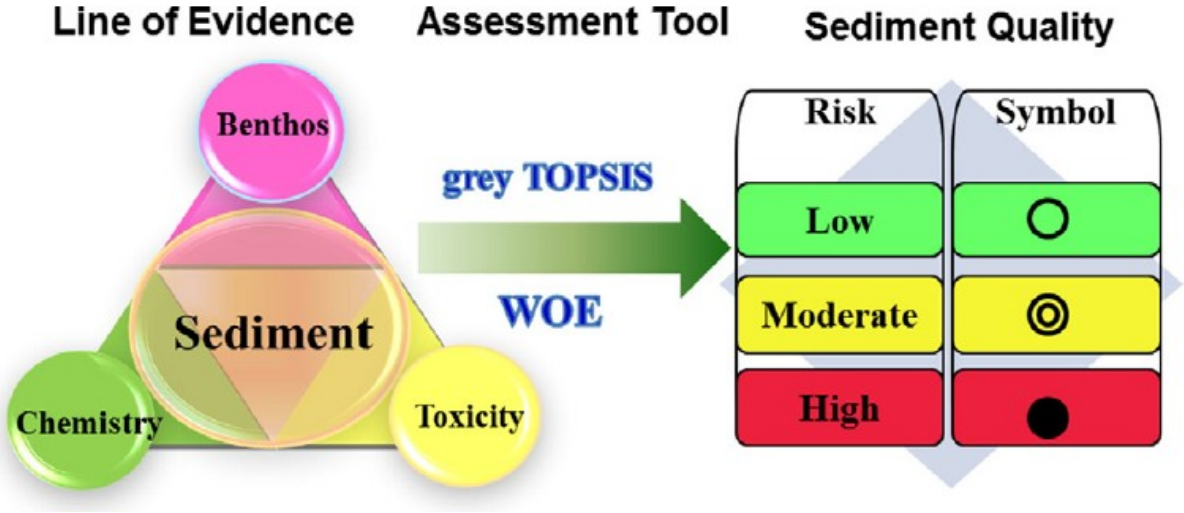
	Reference	Site A	Site B
Result (R3 = Integrated Risk)	**0.00**	**0.48**	**0.58**

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

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

TRIAD - příklad





Jiang et al. (2015)

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 × (100% inhibition rate or the maximum FTI index [c])	0.2 × the 95th percentile of cost metric values or 0.8 × the 95th percentile of benefit metric values [d]
The worse site	CB-PECs [b]	0.5 × (100% inhibition rate or the maximum FTI index)	0.5 × the 95th percentile of cost metric values or 0.5 × 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

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?

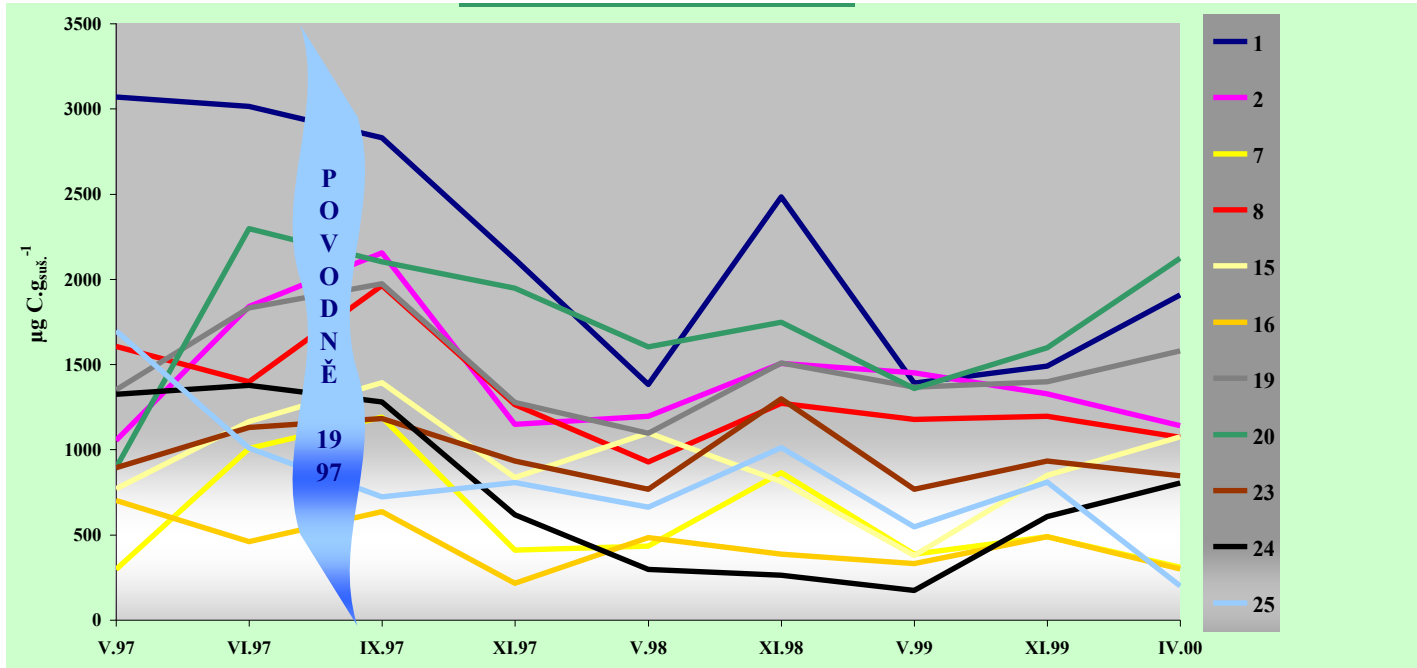
Problems in the field studies

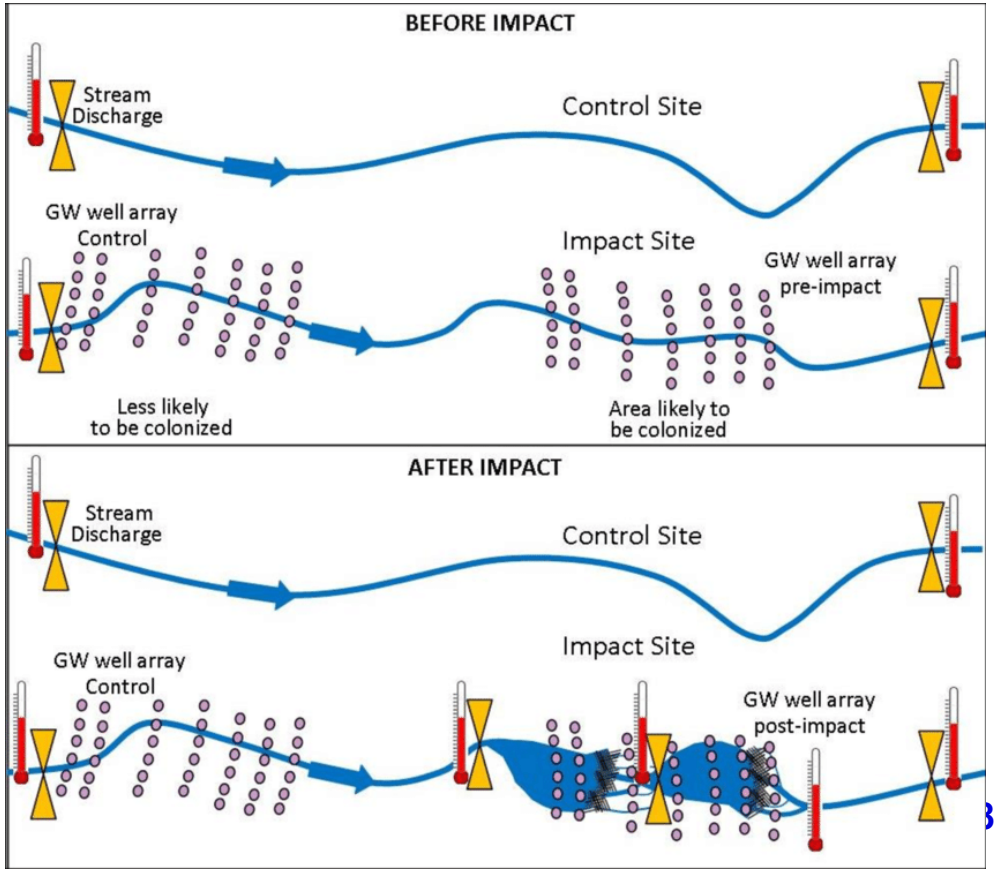
- Total interconnection by food and ecological links, continuity of processes
  - Changes in the activity of one community or population in relation to other communities and functions that are linked
  - Inhibition of one ecosystem component can stimulate another component
- Organisms themselves can 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, overal performance ...), but inside there might be changes and fluctuations

dynamic stability / ekvilibrium = homeostasis

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

succession

- ecosystems are never „stationary“ – the 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)**
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- 2 components of quality assessment (“good state”) - „ecological“ and „chemical“

Ecological component