Ecotoxicology & ERA

Ecotoxicological assays (tests)

Genotypic plasticity

Community ecotoxicology

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Ecotoxicological assays (tests)

- Assume that the test species represents reactions of a broad range of organisms
- Usefulness criteria:
  1. results must be interpretable in terms of ecological risks
  2. must allow extrapolation to other species, communities and whole ecosystems
  3. test species must be sensitive to toxicants
  4. must offer good precision
  5. must be simple and possible to standardize
  6. must be repeatable

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Types of ecotoxicological assays

- **Acute:** exposure time short enough for control organisms not to show negative reactions if not fed during the test; endpoints: mortality
- **Sub-chronic:** longer than acute, but not above 1/3 of the pre-reproductive time; organisms fed during the test; endpoints: survival, other (e.g., enzyme activity, AEC, respiration, etc.)
- **Chronic:** longer than sub-chronic (frequently required that the test covers at least whole pre-reproductive period and part of the reproductive period; better – whole individual lifespan); organisms fed during the test; endpoints: survival and reproduction, other (as above)

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Mirobial tests: advantages and examples

- **Advantages**: functionally meaningful, many microorganisma are easy to culture, fast response
- **Examples** :
  - growth rate (biomass or cell numbers)
  - enzymatic activity (e.g., nitrate reductase, ATPase, dehydrogenase)
  - ATP concentration (unsatisfactory sensitivity!)
  - **luminescence – Microtox assay: *Vibrio fisheri* (*Photobacterium phosphoreum*)**
  - **respiration and microcalorimetry**

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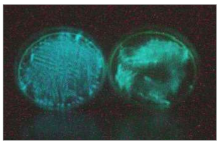
Microtox assay

Based on *Vibrio fisheri* bacteria (NRRL B-11177)

Acute assay (45 min) and chronic (22 h)

Decrease in luminescence (EC_{50}, IC_{50})

Compliant with ISO i EPA regulations



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Assays on plants

- **Algae**: standard US EPA – AAP *Algal Assay Procedure Bottle test* → acute (days)
  - *Selenastrum, Chlorella, Anabaena, Microcystis*
- **Duckweed** (*Lemna* sp.) → acute (4-7 days), chronic also possible
- **Terrestrial plants** → sub-chronic assays (>10 days)
  - seed germination (grass – *Lolium* sp., cereals, lettuce, Chinese cabbage, turnips and others)
  - root growth (various species, as above)
  - life cycle (*Arabidopsis* sp., *Brassica* sp.)
  - photosynthesis (and respiration)

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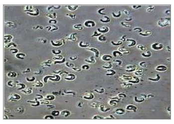
ALGALTOXKIT F

Based on the algae *Selenastrum capricornutum*

Commercial kits containing *Selenastrum capricornutum* contained in the substrate (each algae ball contains approx. 1 million cells)

72 hour assay:

- determining population growth rate by measuring the optical density (EC_{50})
- compliant with OECD 201 recommendations, easy to adapt to ISO or US-EPA standards



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Sea water ALGALTOXKIT F

Based on diatoms *Phaeodactylum tricornutum*

Commercial kits containing *Phaeodactylum tricornutum*

72 h assay:

- determining population growth rate by measuring the optical density (EC_{50})
- compliant with ISO regulations

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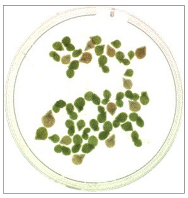
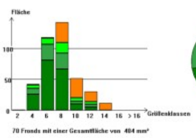
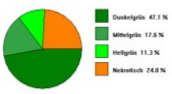
Duckweed assay (*Lemna* sp.)

LemnaTec







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PHYTOTOXKIT

Based on *Sorghum saccharatum*, watercress (*Lepidium sativum*) and mustard (*Sinapis alba*)

Commercial kits containing seeds of the above-mentioned plants

3-day test:

- determination of germination efficiency and growth rate of the root and shoot (EC50)
- compliant with ISO recommendations

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Assays using aquatic animals

- brine shrimp (*Artemia salina*) and several species of rotifera – standard commercial assays (ToxKit) → acute test
- *Daphnia* sp.
  - acute test (OECD – 24 h; EPA – 48 h)
  - chronic test – reproduction (21 days)
- mussels and snails → acute and chronic tests
- fish → acute tests (4 – 7 d.) and chronic (30 - >250 d., depending on the species)

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ARTOXKIT M

Based on the brine shrimp *Artemia salina*

Commercial kits containing eggs (cysts) of *Artemia salina*

24 h assay:

- mortality (LC_{50})
- in many countries used as the standard test for salt water pollution research

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CERIODAPHTOXKIT F

Based on the shellfish *Ceriodaphnia dubia*

Commercial kits containing eggs (cysts) of *Ceriodaphnia dubia*

24-godzinny test:

- mortality (LC_{50})
- compliant with US-EPA regulations

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DAPHTOXKIT F

Based on the shellfish *Daphnia magna i D. pulex*

Commercial kits containing eggs (cysts) of *Daphnia magna* or *Daphnia pulex*

48 h assay:

- effects on immobilization (EC_{50}) and mortality (LC_{50})
- compliant with OECD i ISO regulations

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Assays using terrestrial animals

- Invertebrates → acute and chronic assays:
  - soil and epigeic animals: earthworms (*Eisenia foetida*), snails (*Helix aspersa*), mites, springtails (*Folsomia candida*)
  - predatory invertebrates (beneficial): carabids, ladybugs, spiders, bugs, spiders
  - parasitoids (beneficial): wasps, e.g. Aphidiidae, Ichneumonidae and others
  - other beneficials: honey bee (*Apis mellifera*)
- birds: quail, mallard duck
- rodents: mouse, rat

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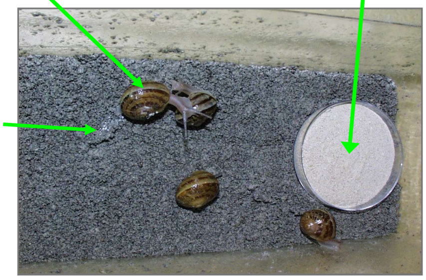
Assay on the snail *Helix aspersa*

 A. De Vaufleury, M. Coeurdassier, P. Pandard, Université de Franche Comté, France

Helix aspersa 3 - 5 weeks old
Body mass 1 ± 0.3 g
Shell diameter 15.5 ± 1 mm

Standard food

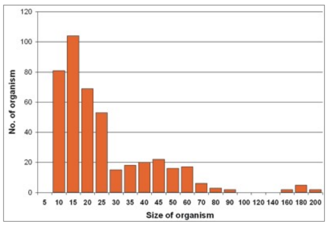
Substrate:
artificial ISO soil,
ca. 50% WHC



20 ± 2 °C, photoperiod 18 h L : 6 h D

 A. De Vaufleury, M. Coeurdassier, P. Pandard, Université de Franche Comté, France

Assay on the springtail *Folsomia candida*



Other terrestrial invertebrate species used in ecotoxicological tests

Enchytreaus sp.

Eisenia foetida andrei

Pterostichus oblongopunctatus (Carabidae)

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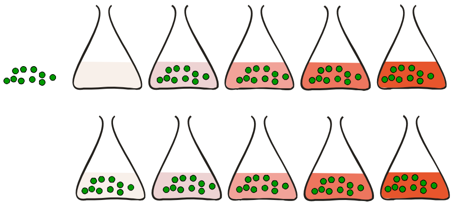
Performing the test



Control geometric series of substance concentrations

- Minimum 4 concentrations + control (no toxicant, everything else identical)
- Minimum 3 replicates; usually 5 – 10
- Observe the measured effect (e.g. number of dead) after a set time

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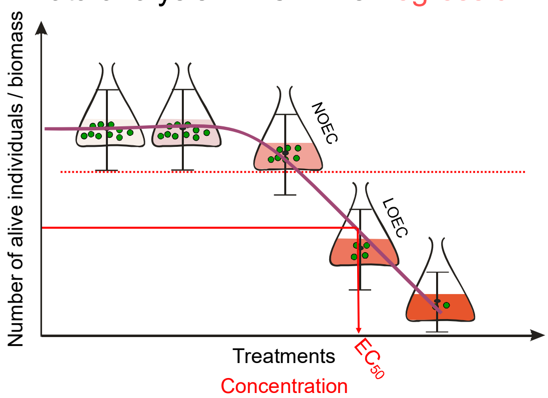
Data analysis: ANOVA vs. regression



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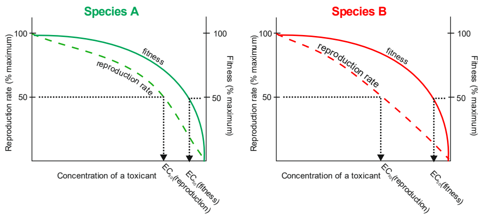
The influence of toxic substances on the phenotype and genotype of an individual

1. Phenotypic plasticity → reversible acclimation
   – *the ability of an organism to react differently to environmental factors within one genetic program*



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The influence of toxic substances on the phenotype and genotype of an individual

2. Development conversion
   – *launching an alternative genetic program during individual development*

   1 + 2 = *genotypic plasticity*

3. **Adaptation**
   – *directional selection (elimination of more sensitive individuals)*
   – *hard selection*

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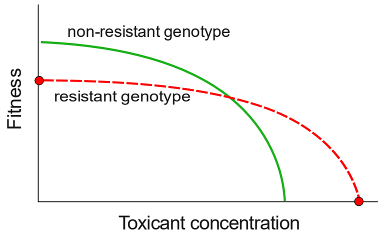
Genotype fitness

The duration of exposure to a toxic substance and the frequency of exposure will determine what will be preferred by selection



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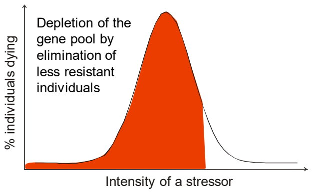
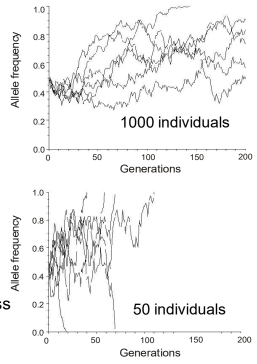
The influence of toxic substances on the population gene pool



Depletion of the gene pool by elimination of less resistant individuals



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Ecotoxicology of communities

- Transfer of pollutants in trophic chains
  - Bioconcentration Factor (BCF):

    BCF = C_n/C_0

  - Biomagnification Factor (BMF):

    BMF = C_{n+1}/C_n

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Bioconcentration and biomagnification of pesticides

DDD concentrations at successive trophic levels in the ecosystem of Lake Clear in California, USA

Trophic chain element	Trophic chain level	DDD concentration (mg kg^{-1})
Water	0	0.014
Phytoplankton	I	5
Plankton-eating fish	II & III	7 - 9
Predatory fish	III & IV	22 - 221
Fish-eating birds	V	2500 (in fat)

Hunt & Bischoff, 1960

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Bioconcentration and biomagnification of metals

Cadmium (Cd) concentrations in successive trophic levels in the area contaminated with cadmium

Tropic level	Cd concentrations (mg kg^{-1})
Producers (plants)	6 - 25
Herbivores (isopods, snails)	29 - 171
Predators (thrush kidneys, *Turdus philomelos*)	387

Martin & Coughtrey, 1975

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Biomagnification – a general phenomenon?

Concentrations and biomagnification factors (BMF) of lead, zinc and cadmium for herbivores and predators of the contaminated terrestrial ecosystem

Metal	Herbivores (mg kg^{-1})	Predators (mg kg^{-1})	BMF
Pb	122 - 685	66 - 551	**0.54 – 0.80**
Zn	260 - 2395	382 - 1299	**1.50 – 0.54**
Cd	2 – 25	29 - 377	**14.50 – 15.1**

Martin & Coughtrey, 1976

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Ecotoxicology of communities: the influence of toxic substances on biodiversity

- Number of species (**S**)
- Species richness – Margalef index: $R = \frac{S-1}{\ln N}$
- Species diversity – Shannon-Wiener index: $H' = -\sum_{i=1}^{S} p_i \log p_i$
- Species evenness – Pielou index: $J = \frac{H'}{H'_{max}}$ $H'_{max} = \log S$
- Dominance structure – Simpson index: $D = \sum_{i=1}^{S} p_i^2$
- **Hierarchical biodiversity index**: $HRI = \sum_{i=1}^{S} (n_i \times i)$

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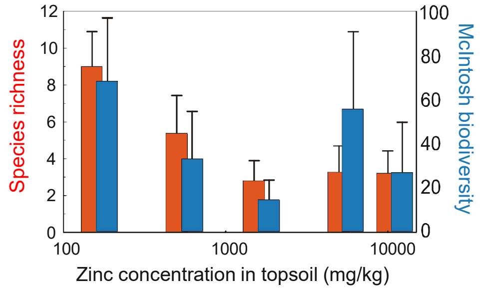
Be careful with biodiversity indicators!



Changes in **species richness** and the **McIntosh biodiversity index** of carabid beetles along the gradient of metal contamination in the vicinity of the zinc and lead smelter "Bolesław" near Olkusz

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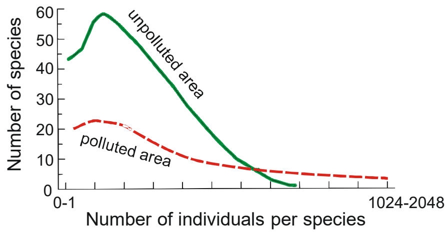
Change in the structure of the community in the polluted area compared to the control area



Unpolluted area: many rare species, few very numerous species

Polluted area: fewer rare species, community highly dominated by few species

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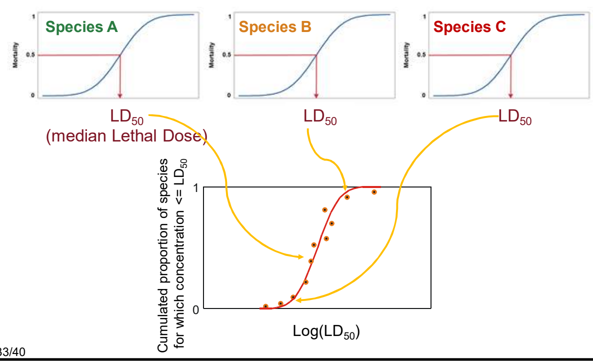
Clements & Newman, 2002

Species Sensitivity Distribution (**SSD**) as a tool in Ecological Risk Assessment



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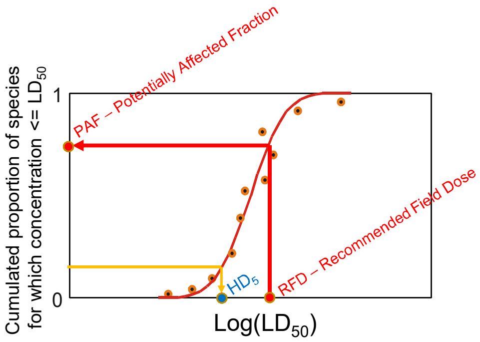
Using SSD for Ecological Risk Assessment



HD$_5$ – Hazardous Dose for 5% species

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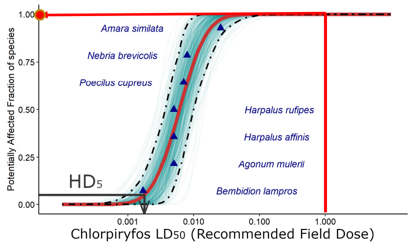
SSD curve for sensitivity of carabids from oilseed rape fields towards chlorpyrifos



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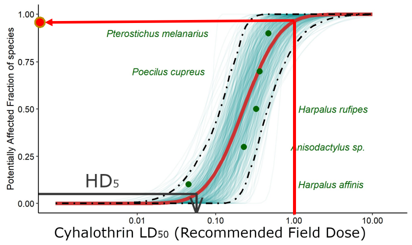
SSD curve for sensitivity of species collected from wheat fields towards λ-cyhalothrin



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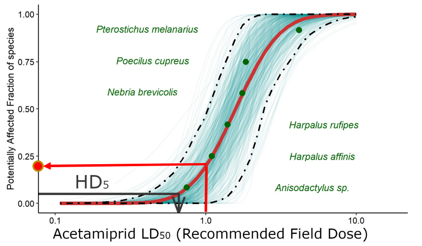
SSD curve for sensitivity of species collected from wheat fields towards acetamiprid



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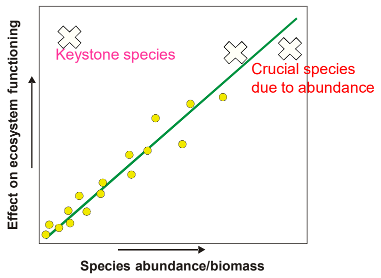
Be careful with HC_5-type estimates!



➔ Taxonomy and taxonomists are not dead yet!

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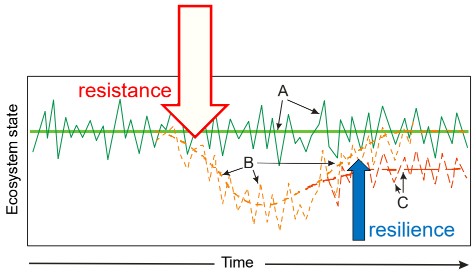
Ecosystem resistance and resilience to disturbances



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Unfortunately, resilience is not always enough...

In 1978 acid rains which occurred in Western Sudety Mts caused ecological disaster and deforestation of Izerskie and Karkonosze Mts

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