MICROCALORIMETRY: A SENSITIVE METHOD FOR SOIL TOXICITY TESTING

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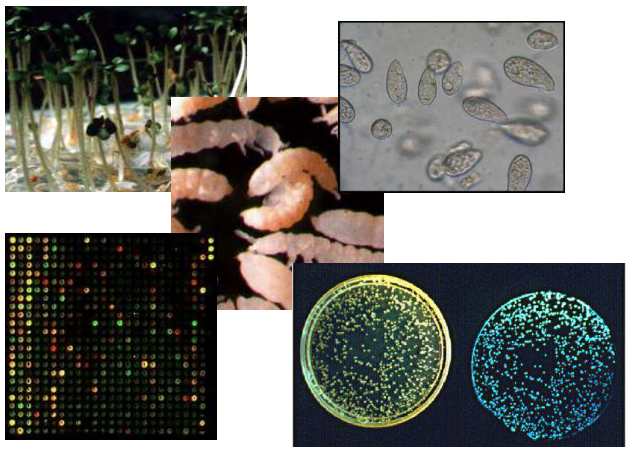
Content

- Efficient environmental management and the necessity of innovative tools
- Heat production as an endpoint
- Toxicity testing of liquid and solid samples
- Aims of our development
- Microcalorimetry: a new tool for environmental toxicology
- Testing in microcalorimeter: some examples
- Advantages and limitations
- Perspectives

Efficient environmental management

- Need for innovative tools,
- new test types
- with easy-to-measure sensitive endpoints
- supporting decision making.



Heat production as an endpoint

- All chemical, physical and biological processes are accompanied by net flow of heat.
- The response of a testorganism on adverse effects is also accompanied by increased (defense) or decreased (inhibition, death) heat production.
- Microcalorimeter: Measures very small heat flows (±50 nW with TAM – $0.5 \cdot 10^{-6}$ ℃).
- Heat production can be a sensitive endpoint of bioassay in microcalorimeter.

Problems of soil ecotoxicity testing in general

- Extraction from contaminated soil:
  chemical accesibility ≠ with biological availability

  ⇨ the results have low environmental reality
- Direct contact of the testorganism with the soil:
  real interactions, realistic results

  BUT: selective endpoint detection can be a problem (e.g. visualization, counting)
- NEED FOR selective and easy-to-measure endpoints

  ⇨ make direct contact soil and sediment tests more widespread

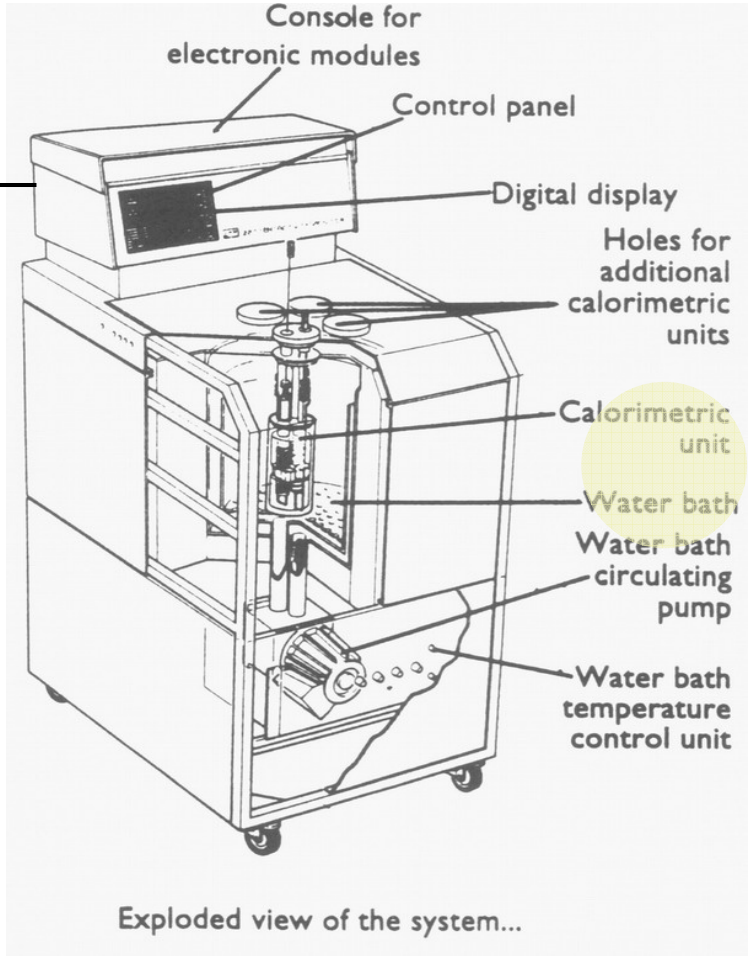
Aims

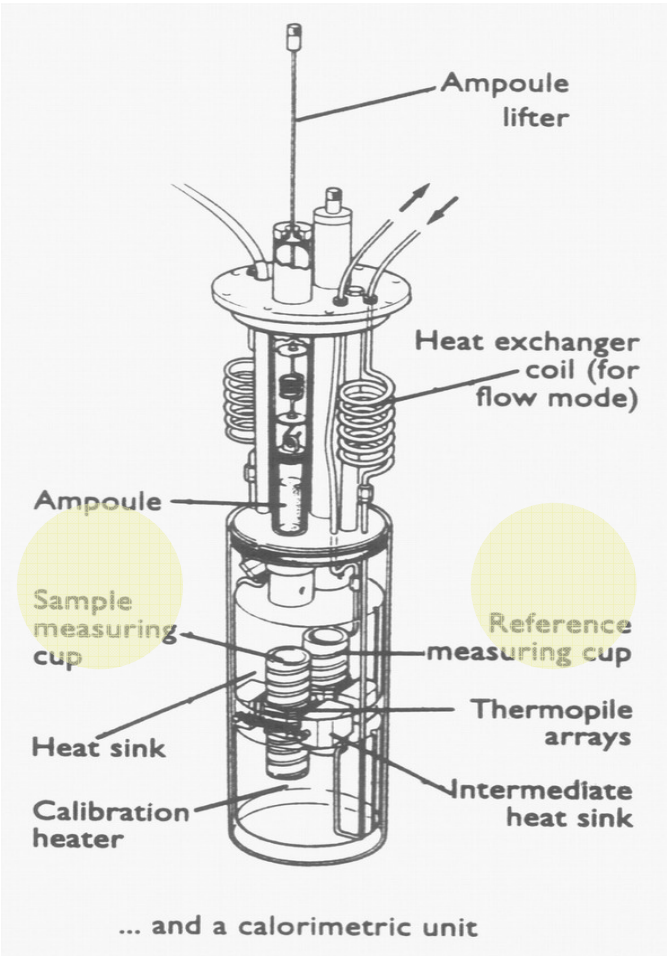
- To measure a selective endpoint in solid matrix
- To find an-easy-to-measure endpoint for known testorganisms
- To increase the selection of test-methods
- To investigate relation between dose – heat production

TAM – Thermal Activity Monitor (LKB Bromma)

Biofilm Center, University of Duisburg-Essen, Germany

PC (Digitam™ software)



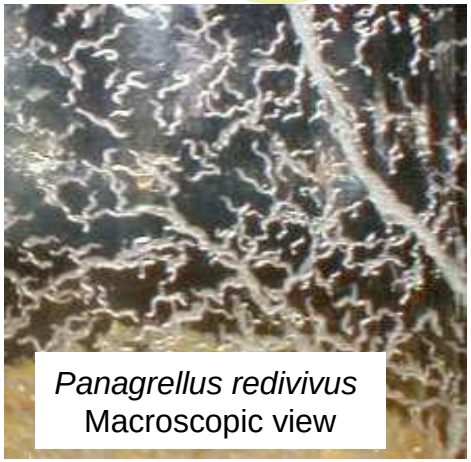


Thermostated water bath: <±0.0001 ℃/24 h

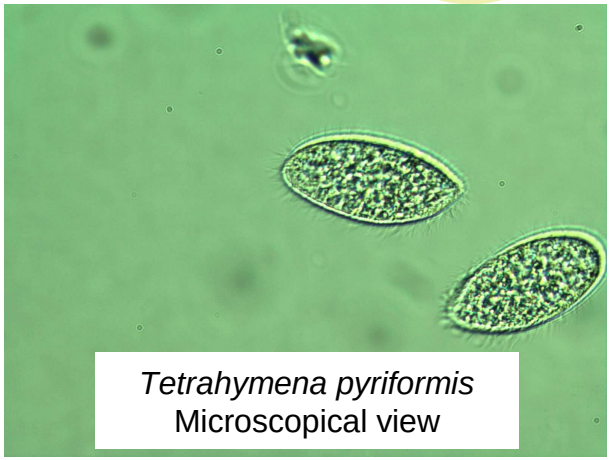
Tested soils and contaminants

- Brown forest soil (from Hungary) spiked with:
  - Metals
    - Mercury
    - Zinc
    - Copper
  - Organic pollutants
    - Diesel oil
    - Transformer oil
    - Phenantrene
    - Cypermetrine
    - PCP (Pentachlorophenol)
    - DBNPA (2,2-dibromo-3-nitril-propionamide)

Testorganisms used in microcalorimeter

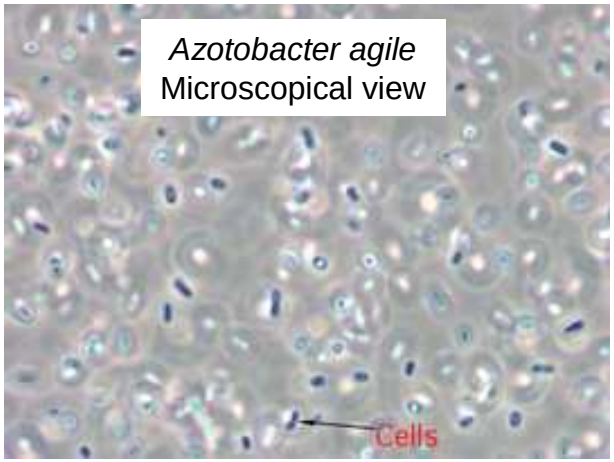


Panagrellus redivivus
Macroscopic view

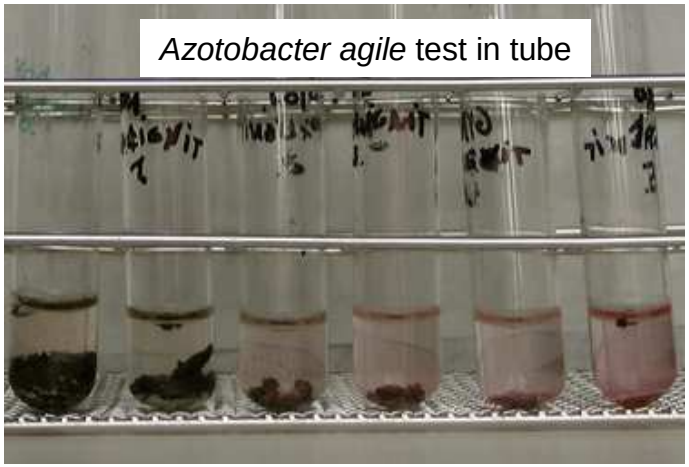


Tetrahymena pyriformis
Microscopical view

Folsomia candida
Macroscopic view



Azotobacter agile
Microscopical view



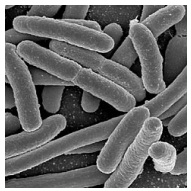
Azotobacter agile test in tube

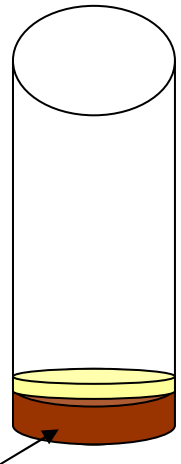
Sinapis alba
length measurement

Experimental design – direct contact

Bacteria

0.75 µl Fjodorov media
100 µl *Azotobacter agile* suspension

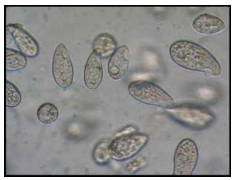




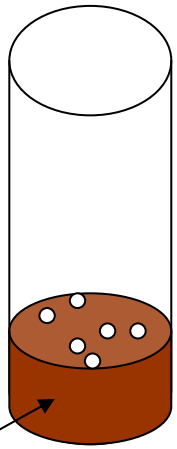
0.5 g sterile soil

Small animals

250 µl TP media
250 µl *Tetrahymena pyriformis* (Protozoa) suspension



500 µl *Panagrellus redivivus* (Nematoda) suspension



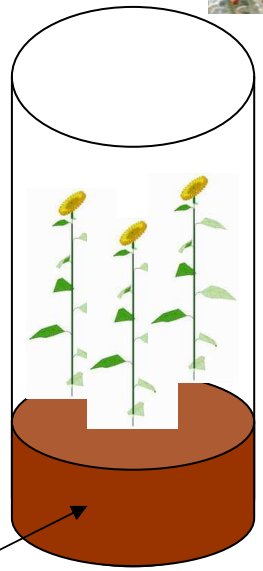
1 g sterile soil

50 *Folsomia candida or Collembola (Insect)*

5 ml glass ampoules

Plants

10 *Sinapis alba* (white mustard) seeds

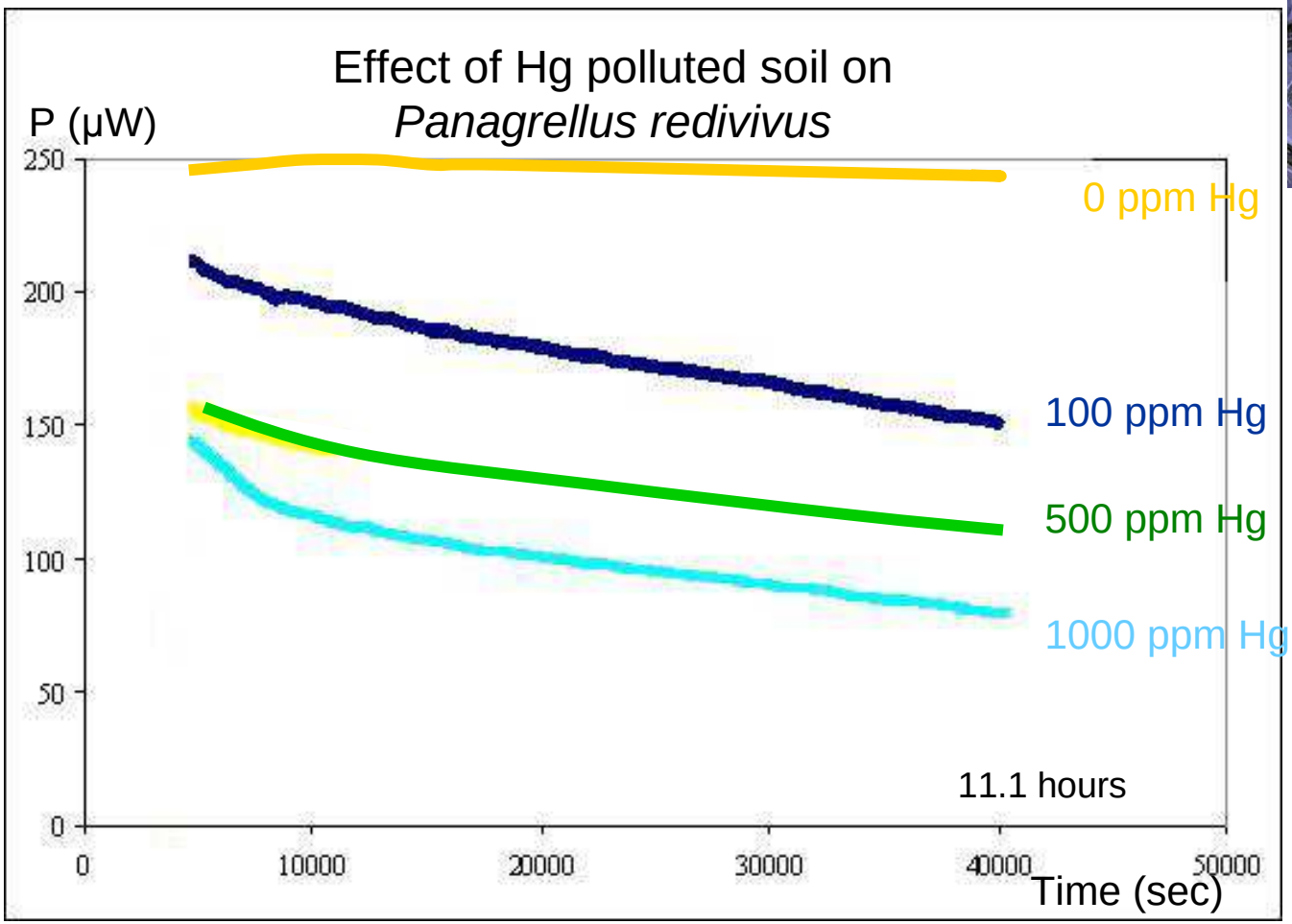


2.5 g sterile soil

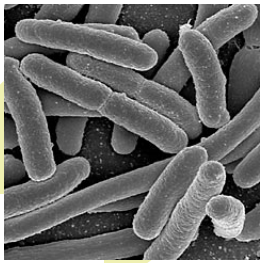
20 ml glass ampoule

Results

Effect of polluted soil on test organisms

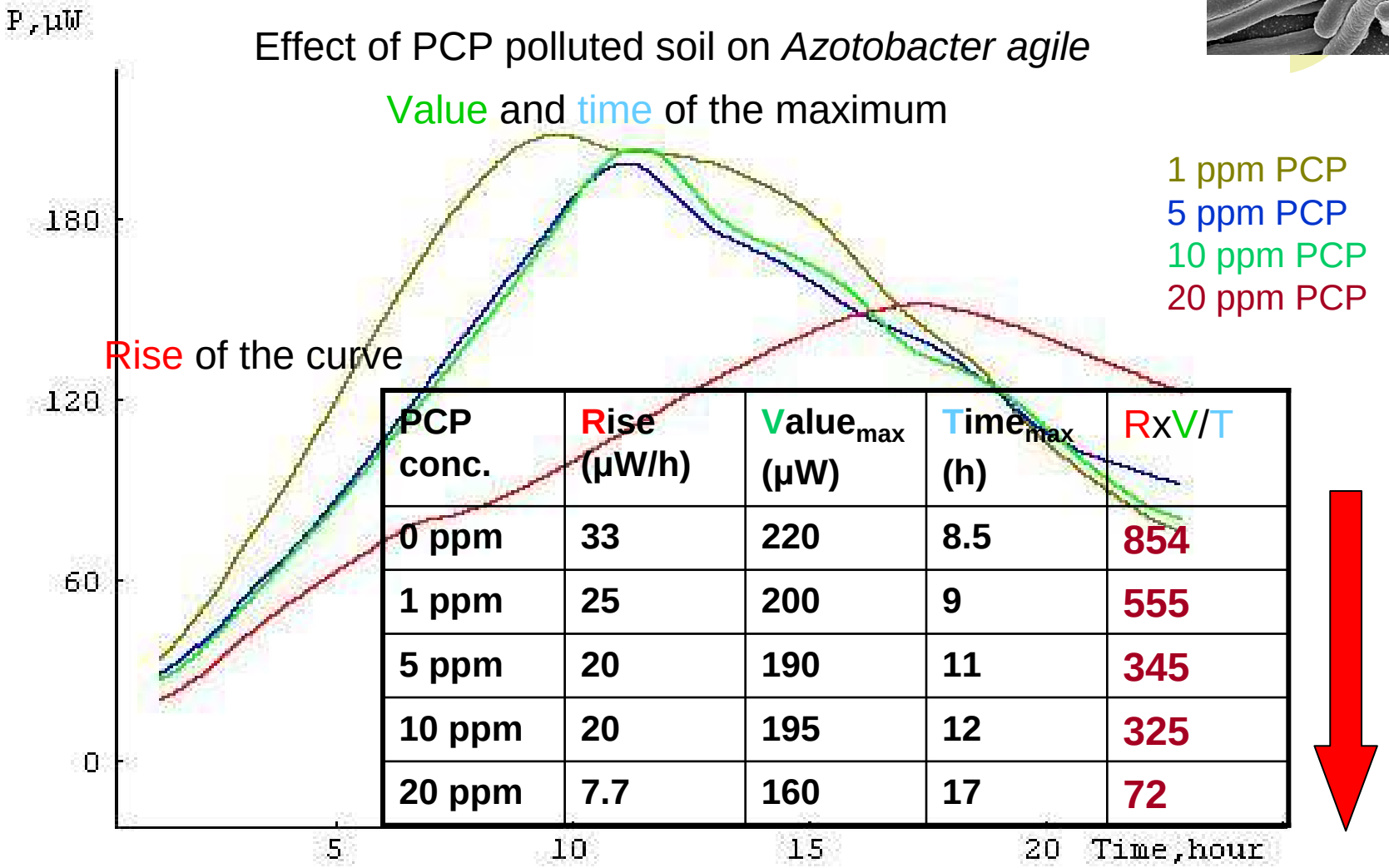


Data evaluation



Effect of PCP polluted soil on *Azotobacter agile*

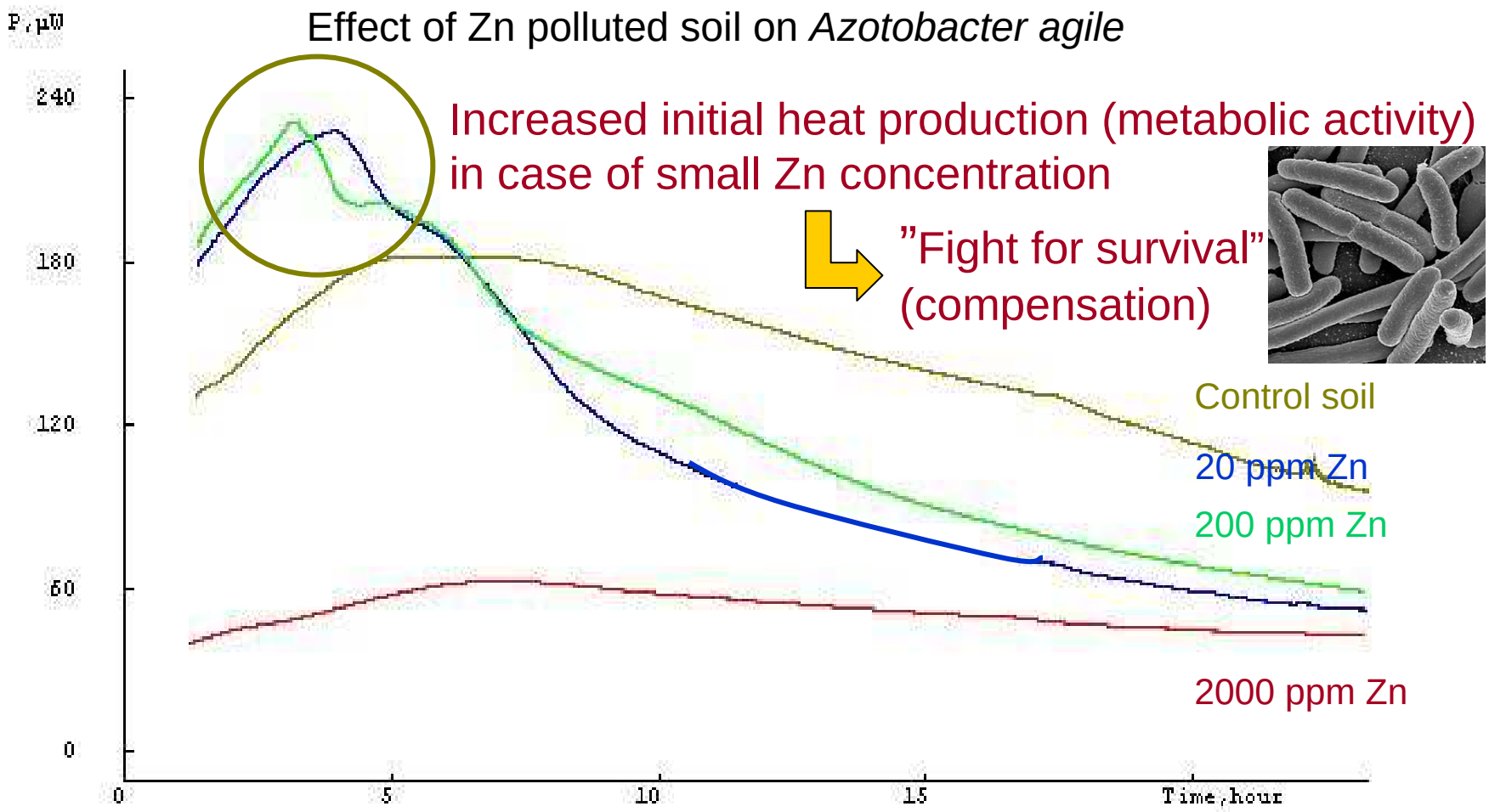
Value and time of the maximum



Rise of the curve

PCP conc.	Rise (µW/h)	$Value_{max}$ (µW)	$Time_{max}$ (h)	RxV/T
0 ppm	33	220	8.5	**854**
1 ppm	25	200	9	**555**
5 ppm	20	190	11	**345**
10 ppm	20	195	12	**325**
20 ppm	7.7	160	17	**72**

Effect of polluted soil on test organisms

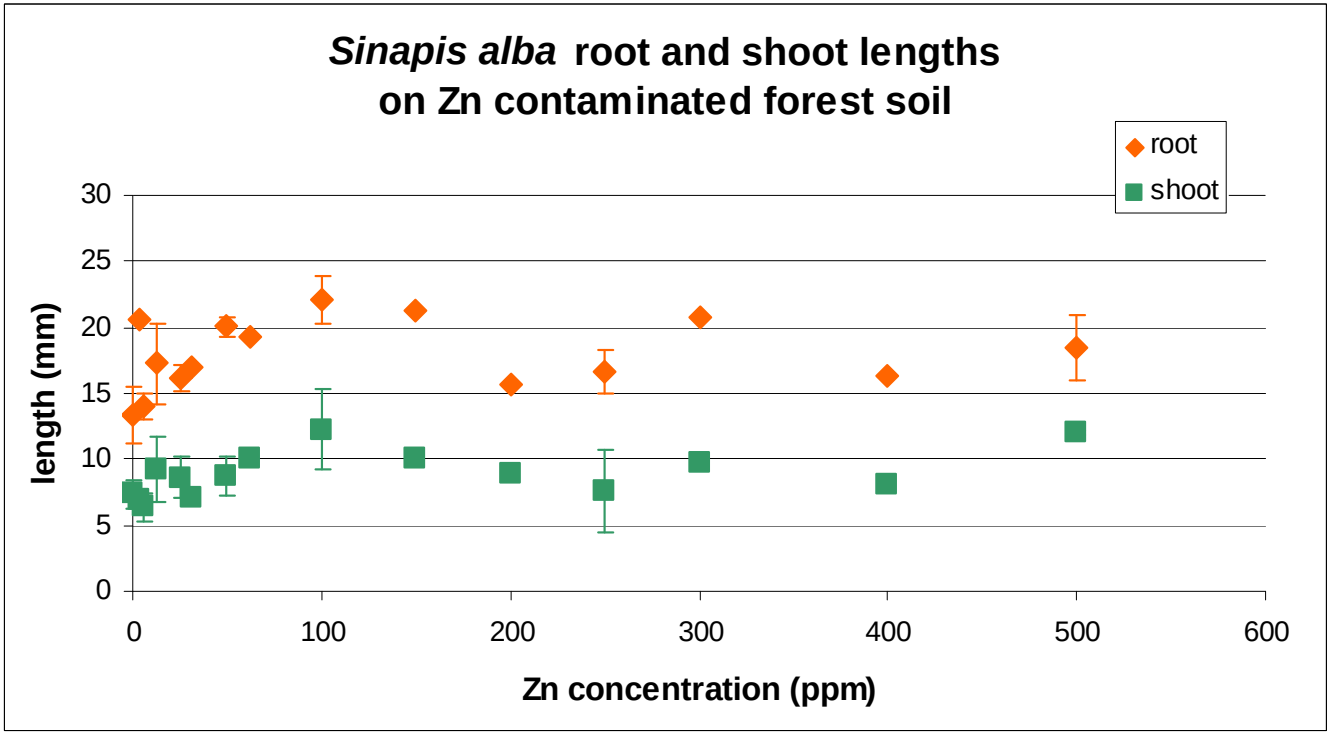


Effect of polluted soil on plant

Effect of Zn polluted soil on *Sinapis alba*

Zn	Rise (P/h)	Value$_{max}$ (µW)	Time$_{max}$ (h)	RxV/T
0 ppm	13	600	35	223
50 ppm	12	500	44	136
100 ppm	12	450	46	117
500 ppm	5	350	58	30

Measurement of plant growth as end point in the traditional plant test

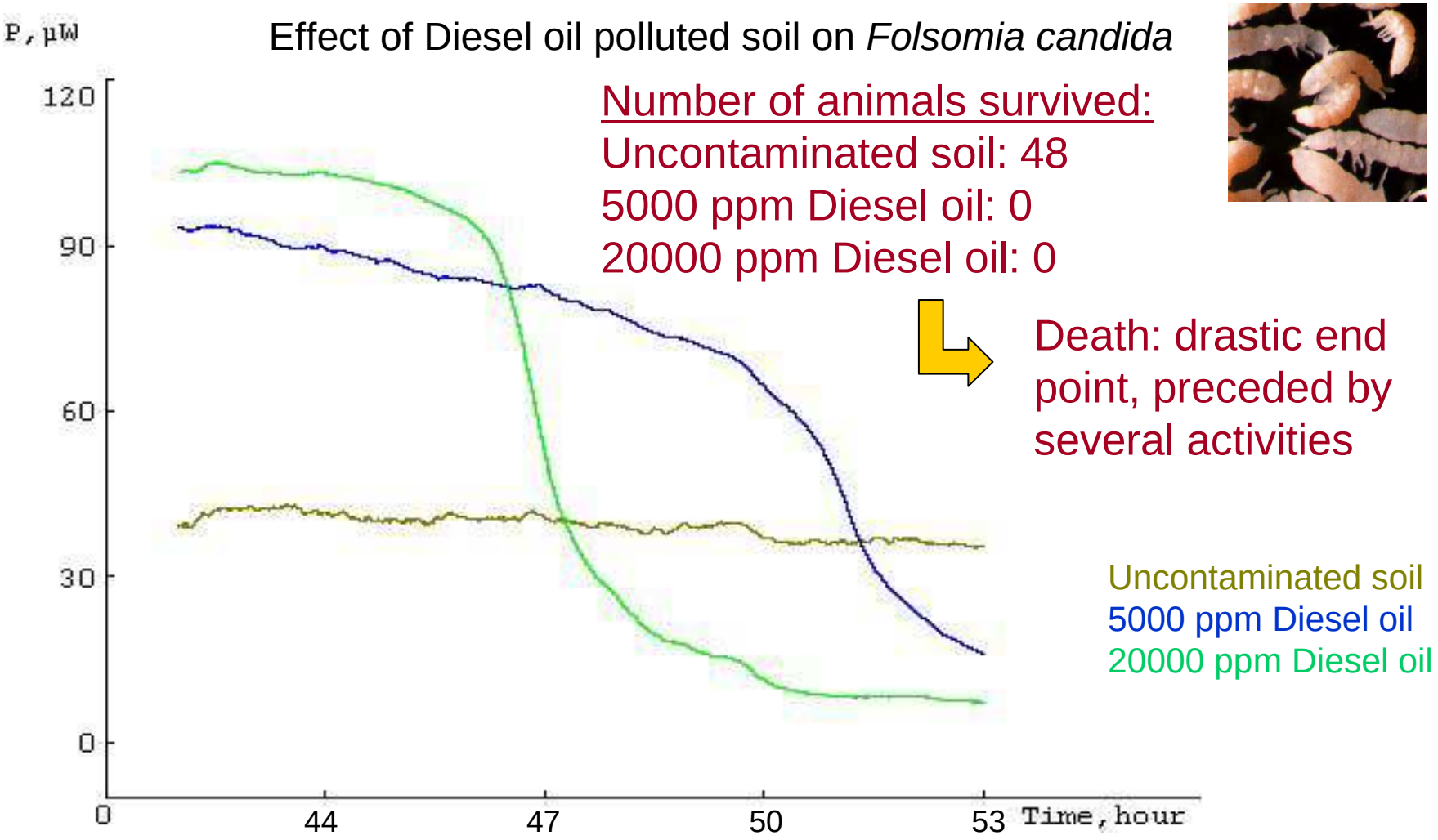


No significant difference in root and shoot lengths

Heat measurement is more sensitive!

Traditional plant test in Petri-dish

Effect of polluted soil on Collembola



<u>Number of animals survived:</u>
Uncontaminated soil: 48
5000 ppm Diesel oil: 0
20000 ppm Diesel oil: 0

Death: drastic end point, preceded by several activities

Uncontaminated soil
5000 ppm Diesel oil
20000 ppm Diesel oil

Advantages of microcalorimetry in ecotoxicity testing

- Measured heat production is proportional with adverse effects
- Higher sensitivity compared to traditional methods
- Non-destructive – further analysis of samples is possible
- Allows direct contact with solid samples (no extraction needed)
- Real-time quantitative data
- No microscopical counting or subjective evaluation is needed
- Other (then toxic) effects and mechanisms can be researched
- Traditional testorganisms can be used
- Soil’s own heat-production and its activity can be measured
- Complex ecosystem response can be measured

Limitations

- Time duration – can be shortened, after we know when to measure
- Low sample number in simple MC – new TAM with 48 measurement chambers solves this problem
- Soil own heat-production may interfere – control
- Closed atmosphere – oxygen can be limiting factor
- Ampoule size – max. 20 ml (max. 5 ml in TAM 48)

Prospects

TAM 24 or 48 with more measurement units

- increased replicability
- measuring dilution series
- testing with more, than one testorganism at a time
- flow through/ flow mix modes
- perfusion titration mode
- etc.

Conclusions

- Microcalorimetry may increase the selection of bioassays, can be one of the choices in environmental monitoring and risk assessment.
- Our research proved, that heat production and its measurement can serve as basis of ecotoxicity testing, producing a selective signal measurable also in solid matrix.
- Both the total amount of heat transmitted by the organisms and the shape of the power-time curve are suitable for evaluation and interpretation.
- In certain cases at low pollutant concentrations an increase in heat production (metabolic activity) was measured compared to control. It can be interpreted as “fight for survival” (compensation) → interesting for “omics”.
- Nowadays high capacity equipments are available with 48 independent cells – new options.

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