



David Spurgeon  
Centre for Ecology and Hydrology  
Wallingford

Extrapolating laboratory toxicity data to  
predict toxic effects in the field –  
challenges for trace metals.

# Standard toxicity tests

- Developed as part of the pesticide registration process.
- Aim to use surrogate species to estimate toxicity
- Need to be standardised - same chemical = same result.
- Use for wider chemical risk assessment (not just pesticides)

# Standard soil toxicity tests

- Carbon mineralisation (OECD, ISO)
- Nitrogen mineralisation (OECD, ISO)
- Non-target plant toxicity (OECD, ISO)
- Earthworm toxicity (OECD, ISO)
- Enchytraeid toxicity (ISO)
- Springtail toxicity (ISO)
- Nematode toxicity (ASTM)

# Standard toxicity tests - earthworm

## *Eisenia fetida*

Mean adult weight 0.4 g

Live in organic rich environments such as compost and manure heaps

Can tolerate high density

Produce over 2 cocoons from each worm per week

More than one juvenile can hatch from each cocoon.





# Standard toxicity tests - soil

Something consistent was needed



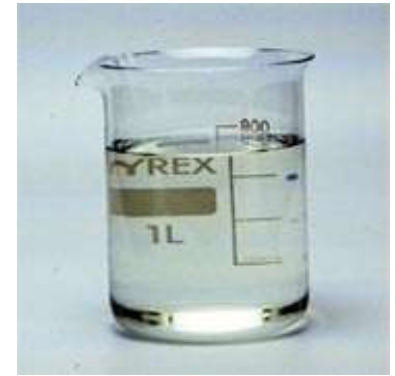
70% sand



20% kaolin clay



10% peat



33% w/w water



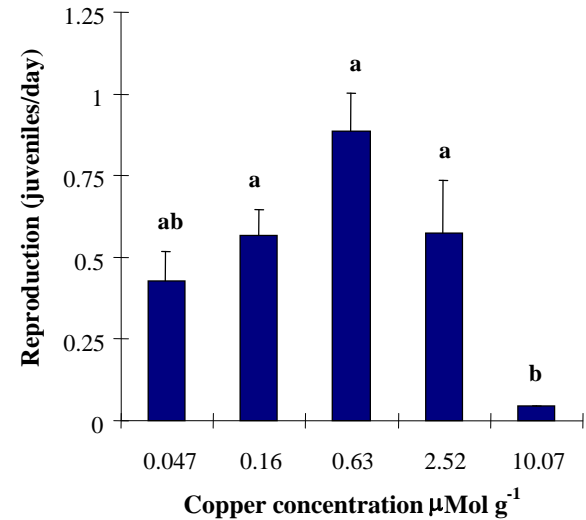


# Example data-set

## Copper - cocoon production

"Hormesis", but significantly lower at highest exposure

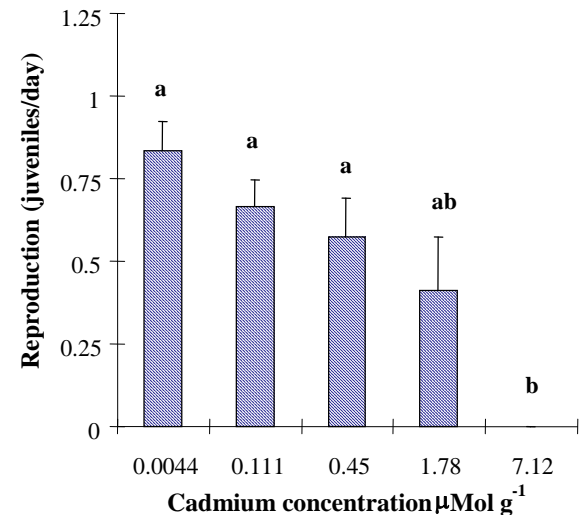
$$EC_{50} = 5.17 (2.7 - 5.87) \mu\text{M Cu g}^{-1}$$



## Cadmium - cocoon production

Simple dose dependence. Lower at highest exposure

$$EC_{50} = 1.78 (0.79 - 2.93) \mu\text{M Cd g}^{-1}$$



# Factors influencing toxicity in the field (Van Straalen and Denneman, 1989)

## Increase toxicity in the field

- In the laboratory, organisms are tested under optimal conditions

- In the field, organisms are exposed to mixtures of stressors

- Adaptation often entails cost in ecological performance

- In the field, exposure is long term compared to short term in lab tests

## Reduce toxicity in the field

- In the field, biological availability is lower than in laboratory tests

- In the field ecological compensation and regulation mechanisms are operating

- Evolutionary change may allow populations to adapt to high concentrations

- Contamination is heterogeneous in the field, homogenous in the lab



# Factors influencing toxicity in the field (Van Straalen and Denneman, 1989)

## Increase toxicity in the field

---

- In the laboratory, organisms are tested under optimal conditions
- In the field, organisms are exposed to mixtures of many stressors
- Adaptation often entails cost in ecological performance
- In the field, exposure is long term compared to short term in lab tests

## Reduce toxicity in the field

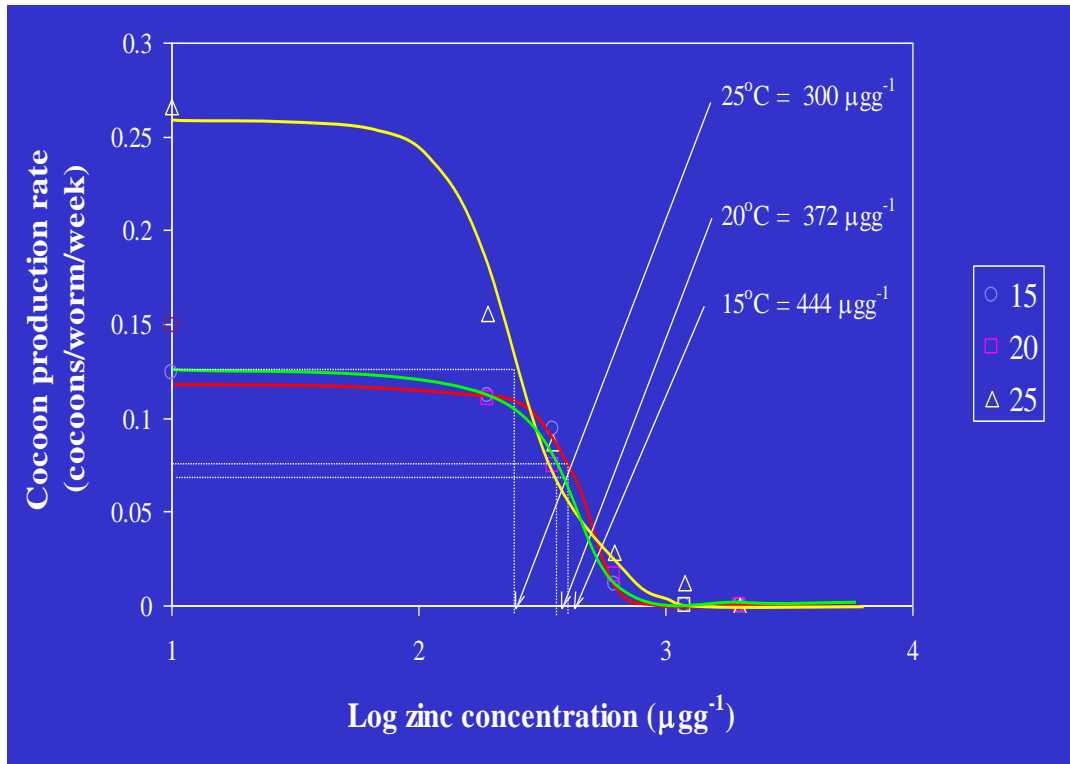
---

- In the field, biological availability is lower than in laboratory tests
- In the field ecological compensation and regulation mechanisms are operating
- Evolutionary change may allow populations to adapt to high concentrations
- Contamination is heterogeneous in the field, homogenous in the lab

# Sub-optimal temperature effects

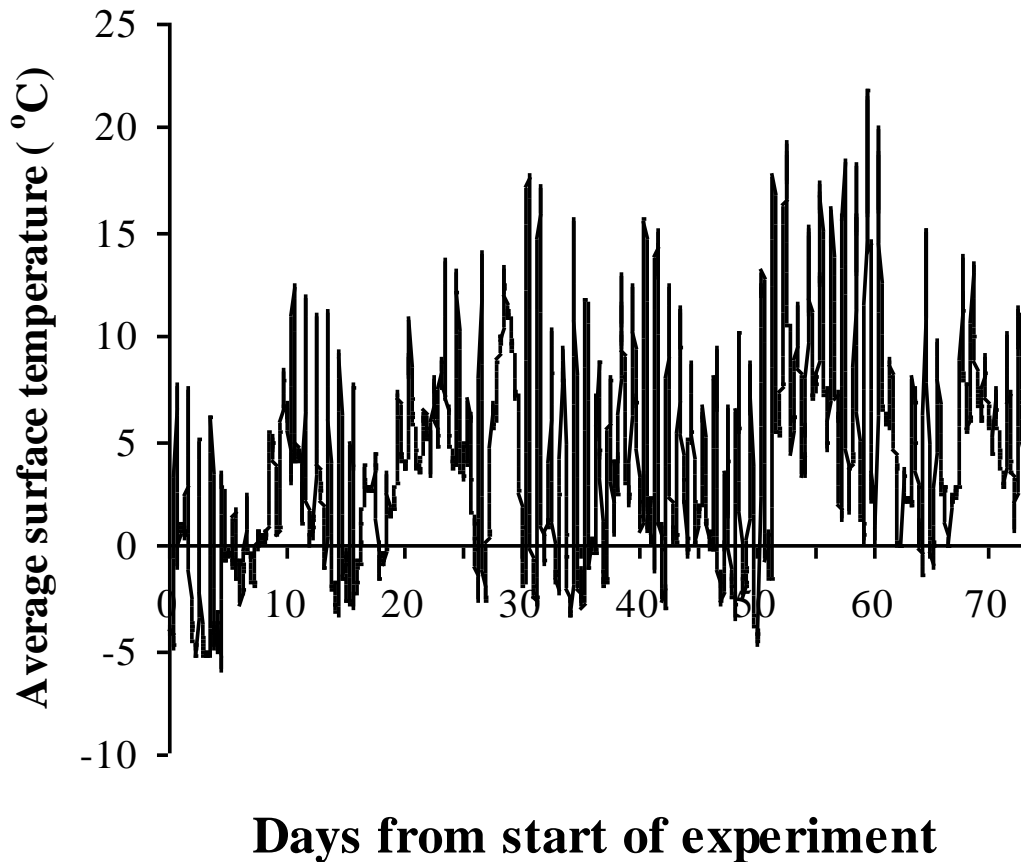
- Effects of zinc on the earthworm *Eisenia fetida*
- Toxicity at three temperature. One above optimal (25°C), one at optimal (20°C) (standard temperature used in laboratory tests), one below optimal (15°C)

# Sub-optimal temperature effects



- Cocoon production rate is temperature dependent
- Toxicity (expressed as  $\text{EC}_{50}$ ) increases (lower values) as temperature increases
- So in the field, toxicity is greater than in the laboratory when temperature exceeds  $20^\circ\text{C}$

# Soil temp at 10 cm depth SE England



- Almost all year temperature at 10cm is less than 20°C, so toxicity in field usually lower than optimal used in laboratory tests.
- Toxicity only greater than predicted in the laboratory (in summer in tropics)



# Lots of studies of this type

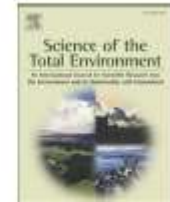
Science of the Total Environment 408 (2010) 3746–3762



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)



## Review

### Interactions between effects of environmental chemicals and natural stressors: A review

Martin Holmstrup<sup>a,\*</sup>, Anne-Mette Bindesbøl<sup>a</sup>, Gertie Janneke Oostingh<sup>b</sup>, Albert Duschl<sup>b</sup>, Volker Scheil<sup>c</sup>, Heinz-R. Köhler<sup>c</sup>, Susana Loureiro<sup>d</sup>, Amadeu M.V.M. Soares<sup>d</sup>, Abel L.G. Ferreira<sup>d</sup>, Cornelia Kienle<sup>c,e</sup>, Almut Gerhardt<sup>e</sup>, Ryszard Laskowski<sup>f</sup>, Paulina E. Kramarz<sup>f</sup>, Mark Bayley<sup>g</sup>, Claus Svendsen<sup>h</sup>, David J. Spurgeon<sup>h</sup>

<sup>a</sup> National Environmental Research Institute, Aarhus University, Department of Terrestrial Ecology, Vejløvej 25, DK-8600 Silkeborg, Denmark

<sup>b</sup> Department of Molecular Biology, University of Salzburg, Hellbrunner Strasse 34, A-5020 Salzburg, Austria

<sup>c</sup> Animal Physiological Ecology, Institute of Evolution and Ecology, University of Tübingen, Konrad-Adenauer-Str. 20, D-72072 Tübingen, Germany

<sup>d</sup> Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

<sup>e</sup> LimCo International, Oststrasse 24, 49477 Ibbenbüren, Germany

<sup>f</sup> Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

<sup>g</sup> Zoophysiology, Department of Biological Sciences, Aarhus University, Building 131, DK-8000 Aarhus C, Denmark

<sup>h</sup> Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxon, OX10 8BB, United Kingdom

## ARTICLE INFO

### Article history:

Received 31 July 2009

Received in revised form 19 October 2009

Accepted 26 October 2009

Available online 17 November 2009

### Keywords:

Natural stressors

Contamination

Ecotoxicology

Cumulative risk assessment

Interaction

Combined stressors

## ABSTRACT

Ecotoxicological effect studies often expose test organisms under optimal environmental conditions. However, organisms in their natural settings rarely experience optimal conditions. On the contrary, during most of their lifetime they are forced to cope with sub-optimal conditions and occasionally with severe environmental stress. Interactions between the effects of a natural stressor and a toxicant can sometimes result in greater effects than expected from either of the stress types alone. The aim of the present review is to provide a synthesis of existing knowledge on the interactions between effects of “natural” and chemical (anthropogenic) stressors. More than 150 studies were evaluated covering stressors including heat, cold, desiccation, oxygen depletion, pathogens and immunomodulatory factors combined with a variety of environmental pollutants. This evaluation revealed that synergistic interactions between the effects of various natural stressors and toxicants are not uncommon phenomena. Thus, synergistic interactions were reported in more than 50% of the available studies on these interactions. Antagonistic interactions were also detected, but in fewer cases. Interestingly, about 70% of the tested chemicals were found to compromise the immune system of humans as





# Factors influencing toxicity in the field (Van Straalen and Denneman, 1989)

## Increase toxicity in the field

---

- In the laboratory, organisms are tested under optimal conditions
- In the field, organisms are exposed to mixtures of many chemicals
- Adaptation often entails cost in ecological performance
- In the field, exposure is long term compared to short term in lab tests

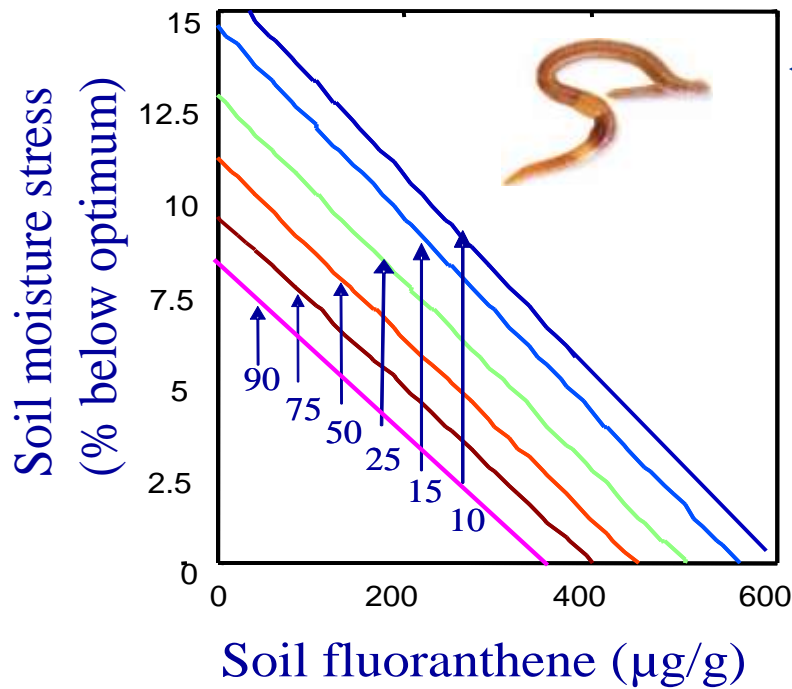
## Reduce toxicity in the field

---

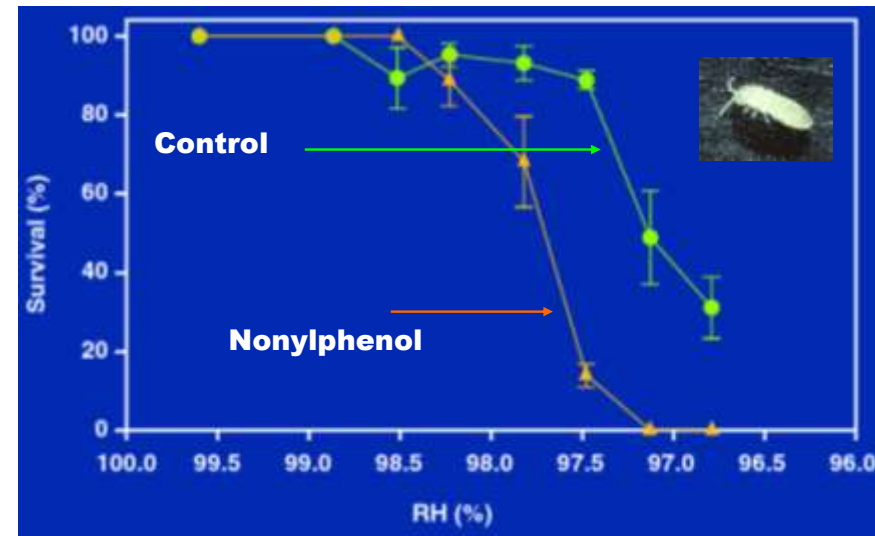
- In the field, biological availability is lower than in laboratory tests
- In the field ecological compensation and regulation mechanisms are operating
- Evolutionary change may allow populations to adapt to high concentrations
- Contamination is heterogeneous in the field, homogenous in the lab

# Results from combined stressor studies

Studies have shown that these are often additive according to the principle of response addition but can deviate



Additive or synergistic?



# Longer exposure greater toxicity?

## Summary

Do multiple stressor effects increase  
toxic effects in the field?

Often additive (when both in effect range)  
Can be more than additive

# Factors influencing toxicity in the field (Van Straalen and Denneman, 1989)

## Increase toxicity in the field

---

- In the laboratory, organisms are tested under optimal conditions
- In the field, organisms are exposed to mixtures of many chemicals
- Adaptation often entails cost in ecological performance
- In the field, exposure is long term compared to short term in lab tests

## Reduce toxicity in the field

---

- In the field, biological availability is lower than in laboratory tests
- In the field ecological compensation and regulation mechanisms are operating
- Evolutionary change may allow populations to adapt to high concentrations
- Contamination is heterogeneous in the field, homogenous in the lab

# Longer exposure greater toxicity?

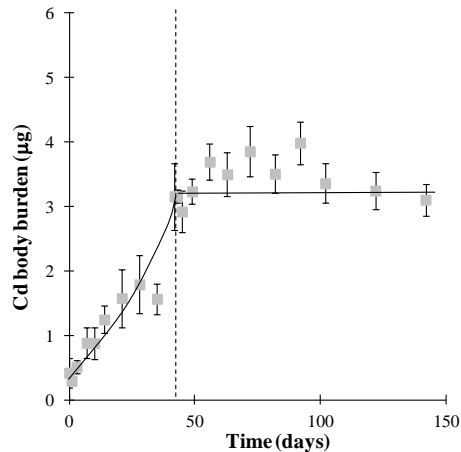
- Based on the assumption that toxicity is time dependent
- Time dependence based on assumption that body concentrations increases with time
- So time dependent patterns in body concentration give insight into effects of exposure duration



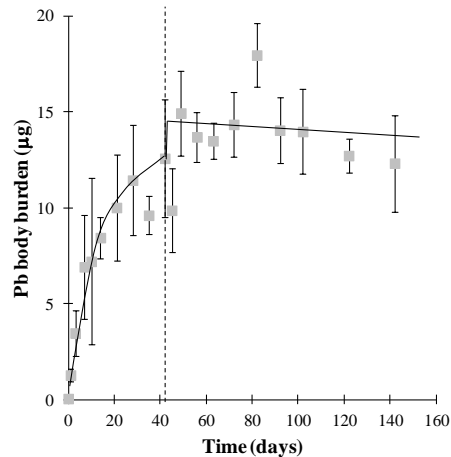
# Metal uptake *Eisenia fetida* in field soil

## Non-essential metals

### Cadmium



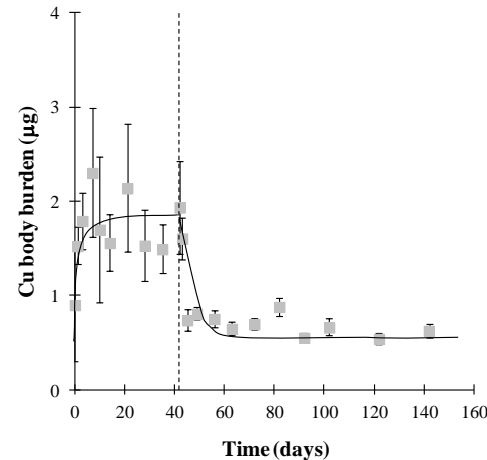
### Lead



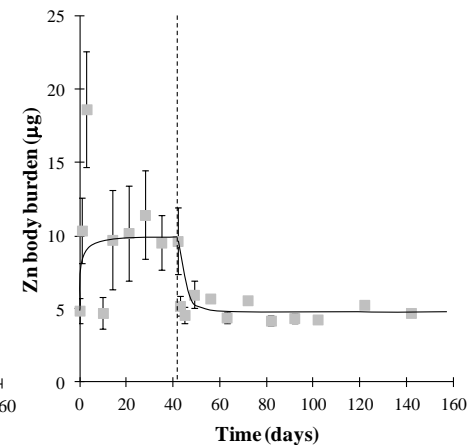
- Low rates of elimination
- Body burden is time dependent

## Essential metals

### Copper



### Zinc



- High rates of elimination
- Body burden only time dependent over 7 days

# Longer exposure greater toxicity?

## Summary

Does long-term increase toxic effects in the field

Chemical dependent

Need kinetic data

# Factors influencing toxicity in the field (Van Straalen and Denneman, 1989)

## Increase toxicity in the field

---

- In the laboratory, organisms are tested under optimal conditions
- In the field, organisms are exposed to mixtures of many chemicals
- Adaptation often entails cost in ecological performance
- In the field, exposure is long term compared to short term in lab tests

## Reduce toxicity in the field

---

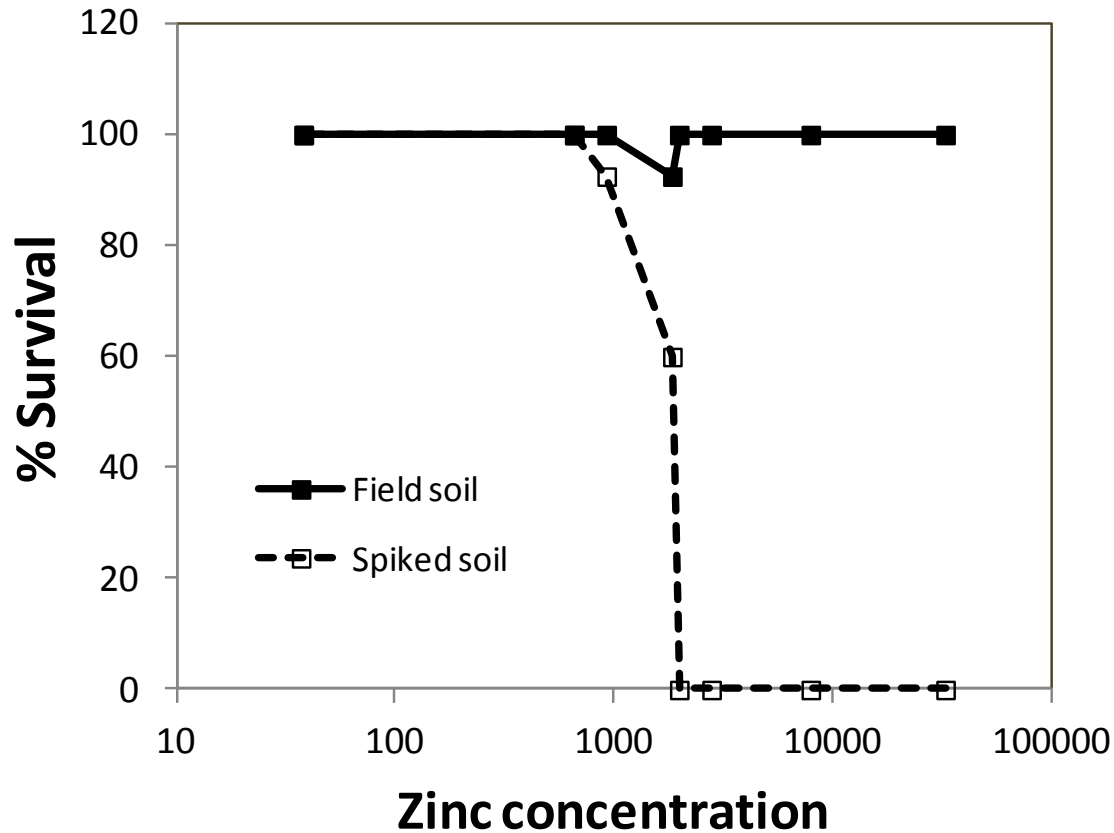
- In the field, biological availability is lower than in laboratory tests
- In the field ecological compensation and regulation mechanisms are operating
- Evolutionary change may allow populations to adapt to high concentrations
- Contamination is heterogeneous in the field, homogenous in the lab

# Field soil versus spiked exposures?

## Exposed worms to

1. A field contaminated by smelter emissions over many decades
2. A laboratory soil containing the same concentrations of metals added as a solution of the nitrate salt as in a standard lab test.

# Field vs spike bioavailability





# Field vs spike bioavailability

## True

At least for metals in terrestrial systems. See the papers/reports of :

Spurgeon

Posthuma

Smit

Vjiver

Van Gestel

Janssen(s)

Smolders

McLaughlin

# Field vs spike bioavailability

Now part of EU policy

3 fold lab - field

extrapolation factor in

metals risk assessment

# Factors influencing toxicity in the field (Van Straalen and Denneman, 1989)

## Increase toxicity in the field

---

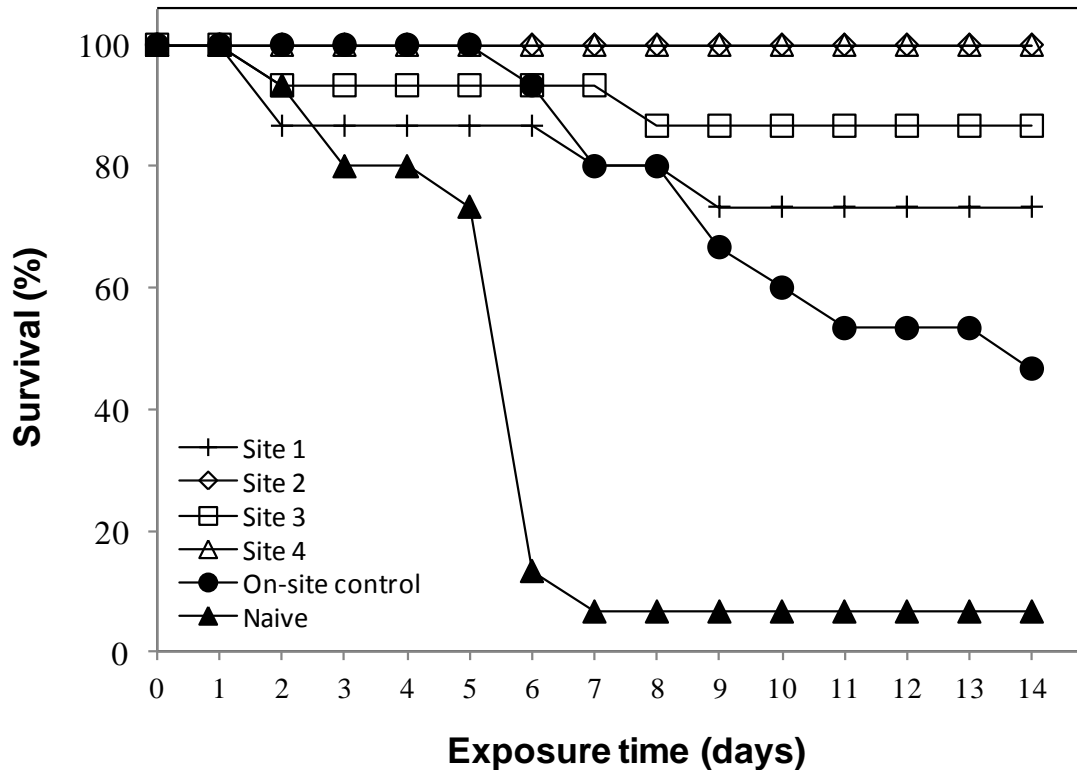
- In the laboratory, organisms are tested under optimal conditions
- In the field, organisms are exposed to mixtures of many chemicals
- Adaptation often entails cost in ecological performance
- In the field, exposure is long term compared to short term in lab tests

## Reduce toxicity in the field

---

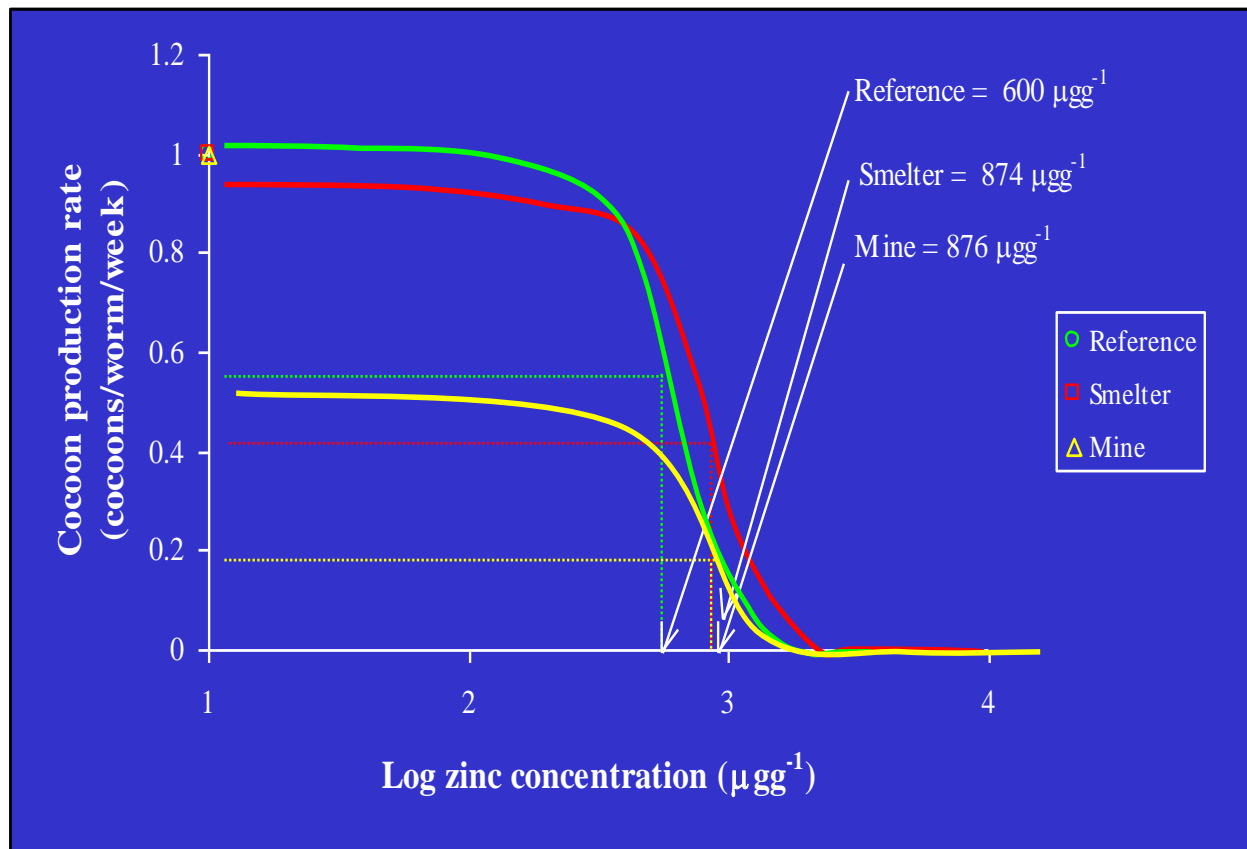
- In the field, biological availability is lower than in laboratory tests
- In the field ecological compensation and regulation mechanisms are operating
- Evolutionary change may allow populations to adapt to high concentrations
- Contamination is heterogeneous in the field, homogenous in the lab

# *Lumbricus rubellus* exposed to As - 6 populations



- Rapid mortality of the naive population and partial mortality of on-site controls
- High survival of most polluted population.
- Tolerance is conserved over 2 generation - genetic basis.

# *Lumbricus rubellus* exposed to Zn - 3 populations



Significant differences in the shapes of the dose response curves

Toxicity (expressed as  $\text{EC}_{50}$ ) lowest in reference population

Considering the different exposure histories, difference in small



# ADAPTATION AS AN EFFECT MITIGATION

## For Arsenic

Evidence of development of genetic adaptation.

## For Zinc

No clear evidence of substantial adaptation for polluted site populations even after 400 years exposure.  $EC_{50}$ s similar for the three populations.

# WHY NO ZN TOLERANCE IN THE FIELD?

- Selection pressure at the polluted sites is insufficient to promote resistance - UNLIKELY
- Meta-population effects prevent the development of resistance - POSSIBLE BUT WORMS SEDENTARY
- Physiological constraints limit resistance - zinc is essential, so the phenotypic variability of some species may be limited. The fact that the field species differ from that in the laboratory may explain the anomalous results - POSSIBLE

# ADAPTATION AS AN EFFECT MITIGATION

In the field adaptation to chemical stress may occur

Reduces toxic effects in field?

Not necessarily. Evidence of adaptation for some pollutants but not all.

# EXTRAPOLATION FACTORS - CONCLUSIONS

## Increases toxicity in field

Exposure to non-optimal conditions increases field toxicity - depends on factor and the extent of change

Long-term exposure in the field increases toxicity in the field - chemical dependent

Mixed stressor increase effects - additive and can be synergistic

## Reduces toxicity in field

Lower availability reduces toxicity in the field - lab to field comparative work indicates this is important

Adaptation reduces effect - not necessarily the case

# OVERALL CONCLUSION

There are few simple relationships.

Need to think in the context of the  
biology of the stressor being  
considered

Mechanistic info valuable.





It's Over