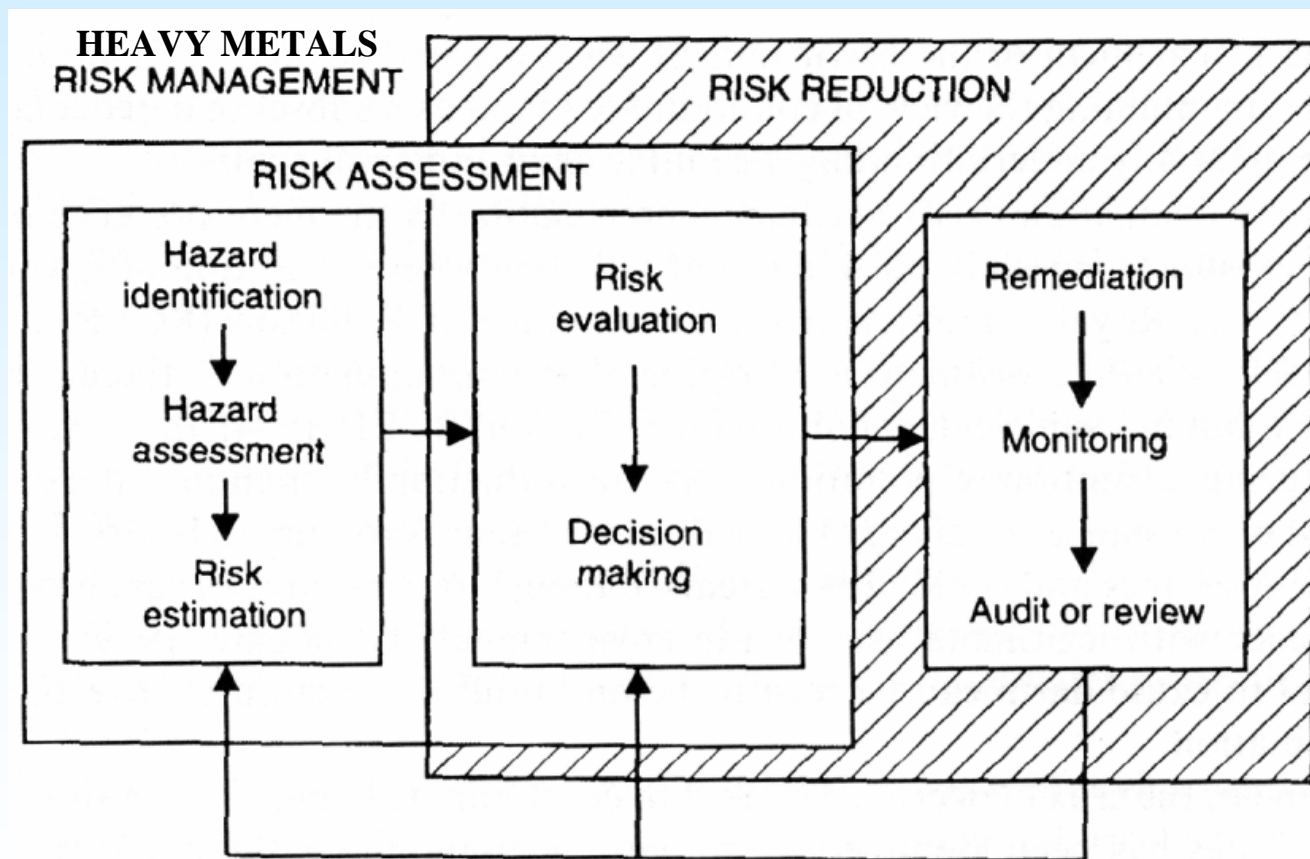


Hazard and Risk Assessment of Inorganic Pollutants in Soils

Julian J.C. Dawson and Graeme I. Paton,

School of Biological Sciences, University of Aberdeen, UK

Contaminated Land in the Regulatory Framework





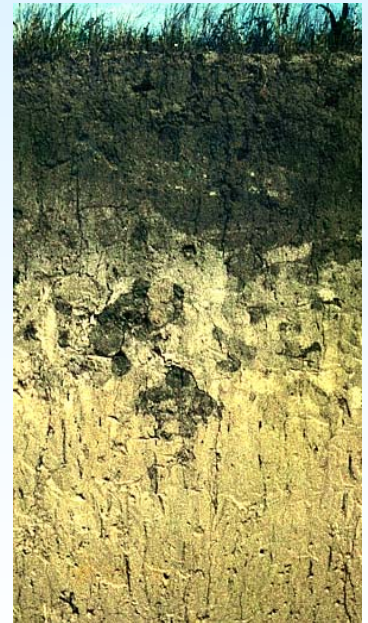


Background

- Land application of sewage sludge may have adverse effects on ecological health due to heavy metal (HM) loads.
- Quality standards for HM in soils are an important component of the environmental risk assessment process.
- Despite evidence that total HM in soil is a poor indicator of environmental risk, regulatory policy is still reliant on this.
- There is a need to understand HM mobility and bioavailability in the range of soils to evaluate the hazard posed.

Ecotoxicity and Soils

- How does soil-type relate to binding affinity?
- Can we relate soil-type to potential ecological vulnerability?
- Can observations be related to modelled systems?



Binding Affinity

- The key soil parameters that describe HM binding are:
 - pH
 - Organic Matter
 - Texture (clay content)
 - Iron oxide content

Procedure

- A physico-chemical weighted classification using the soil parameters predicts the relative binding.

(Blume & Brümmer, 1991)

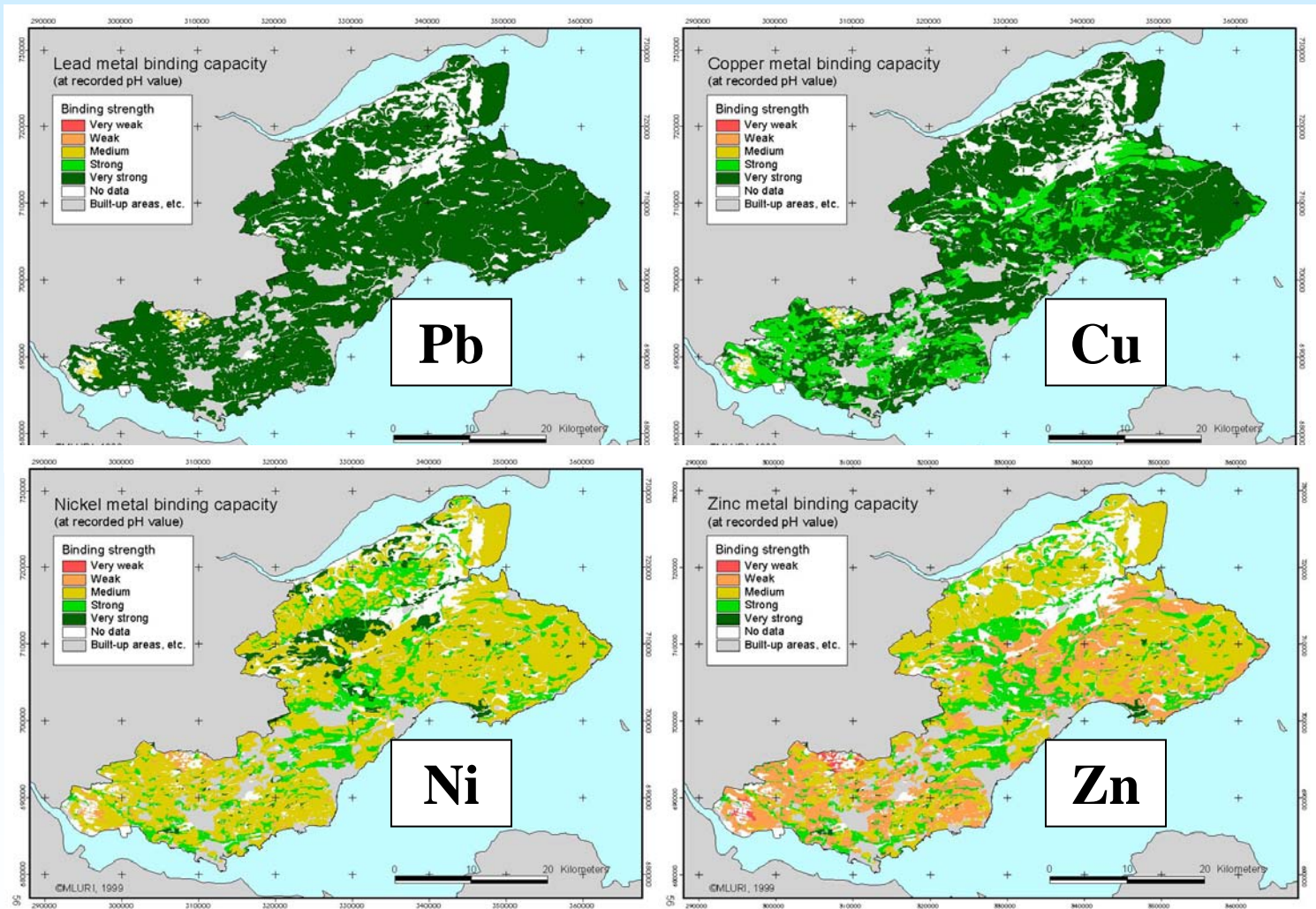
- Using soil database from a well defined geographical area,



e.g. Fife, Eastern Scotland. 1320 km²,
1.7% of total land area.

- Apply a HM binding map - assess soil vulnerability?

Heavy Metal Binding Maps



So what does this mean?

- A valuable and effective mechanism.
- Applicable, protective but ultimately conservative – offers little ecological justification.
- Determination of total soil HM contents is indiscriminate to quantifying potential ecological and human risks,
- What about species selection or adaptability - urban soils and industrial?

Ecotoxicological Assessment

- Parallel experiment using amended soils with HM
 - –Cd, Co, Cu, Ni, Pb & Zn.
- Soils used had the full range of HM binding capacities.
- Characterised soil and pore-water chemistry.
- Microbial tests – determine bioavailable effects.
 - - Biolog-AWCD, Respiration, Biomass: *bulk soil*
 - - Bacterial-based biosensors: *soil pore-waters*

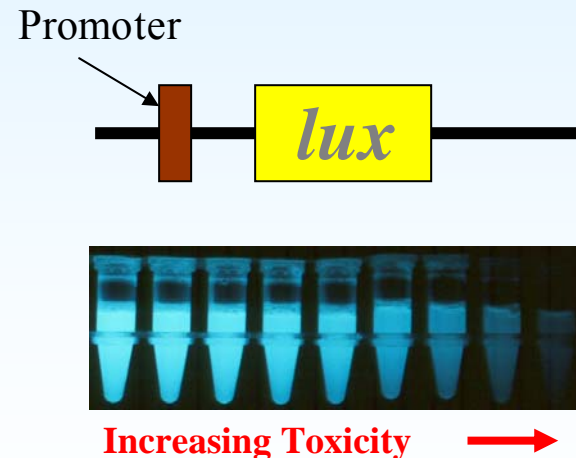
Method

- Soil preparation: wet sieved to 5 mm, amended with individual HM doses at 0, 0.5, 1, 2 & 4x UK sewage sludge regulatory values.
- Pore-water extracted following 1 month incubation at 15 °C.
- Ecotoxicity tests using 3 separate *lux*-based biosensors.

Metabolic biosensor:

GM soil organisms
Constitutive luminescence
General indicators of toxicity

Aqueous, sediment & soil extracts



Pore-water extraction

Rhizon soil moisture sampler

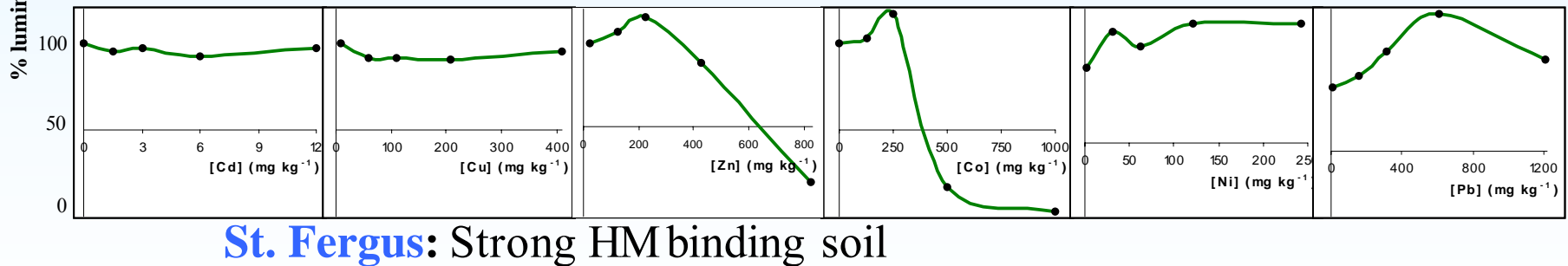
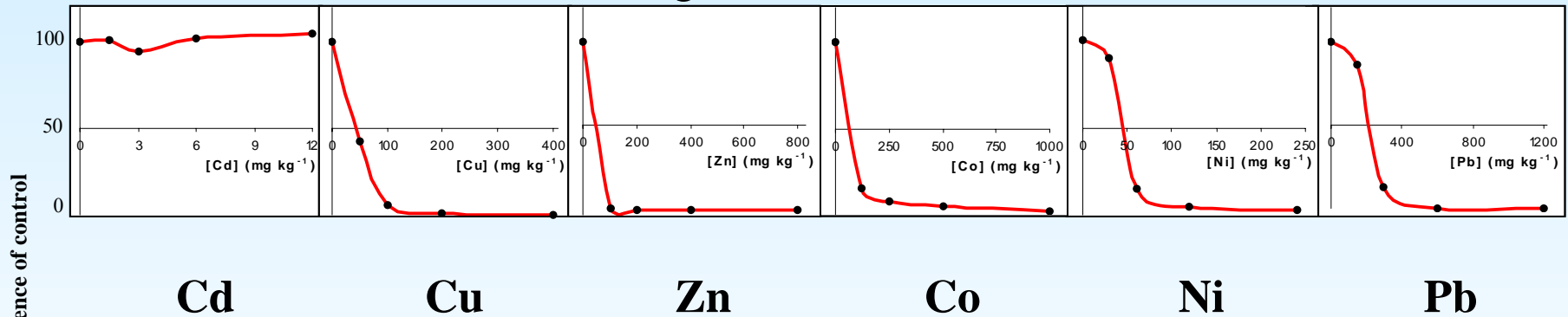
- Suction applied over night through a 10 cm inert porous (0.2 μm) fibre tube and collected in syringe.
- Non-destructive technique.



Dose Response Curves

2 soils, 6 HM - *E. coli* HB101 pUCD607 biosensor.

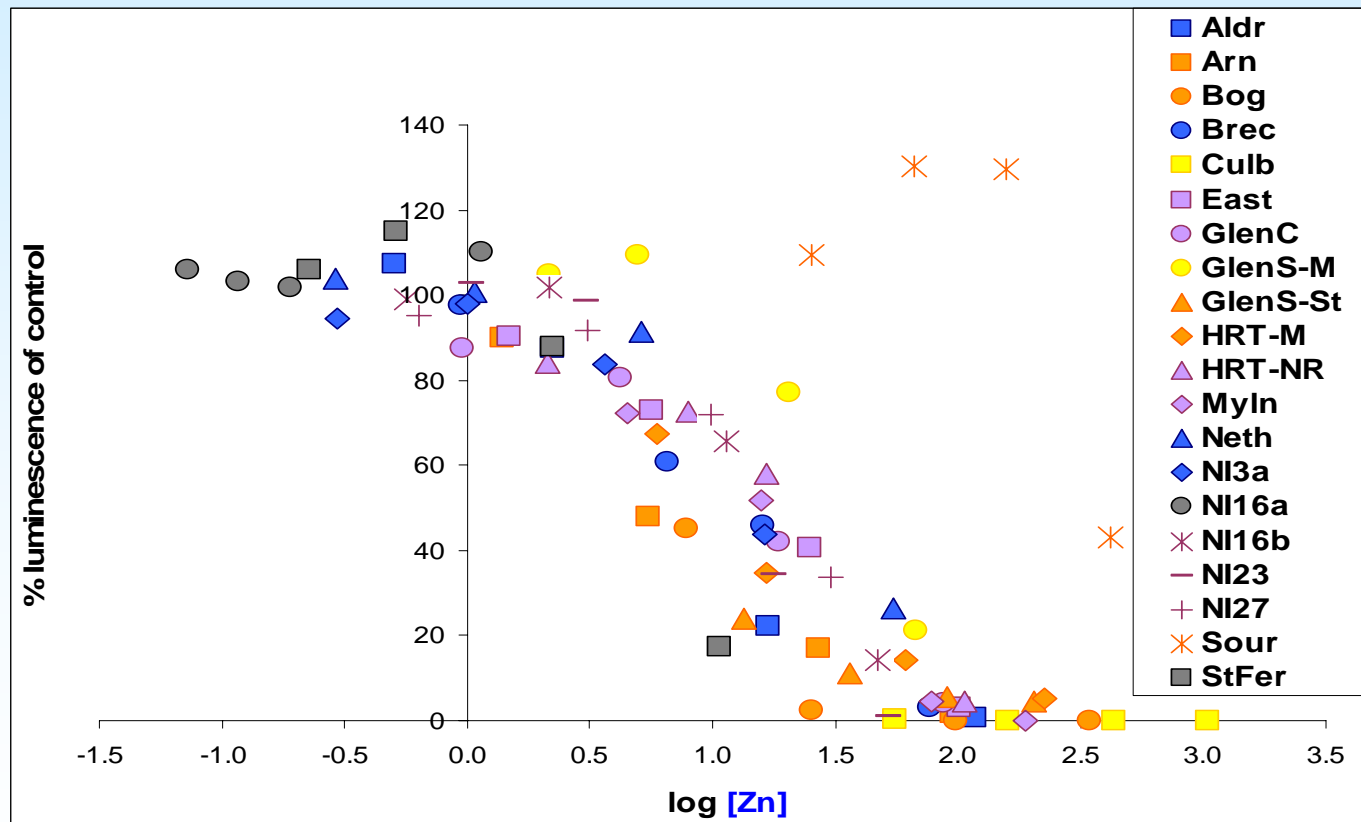
Culbin: Weak HM binding soil



St. Fergus: Strong HM binding soil

Combined Data

(Zn - *E. coli* HB101 pUCD607)



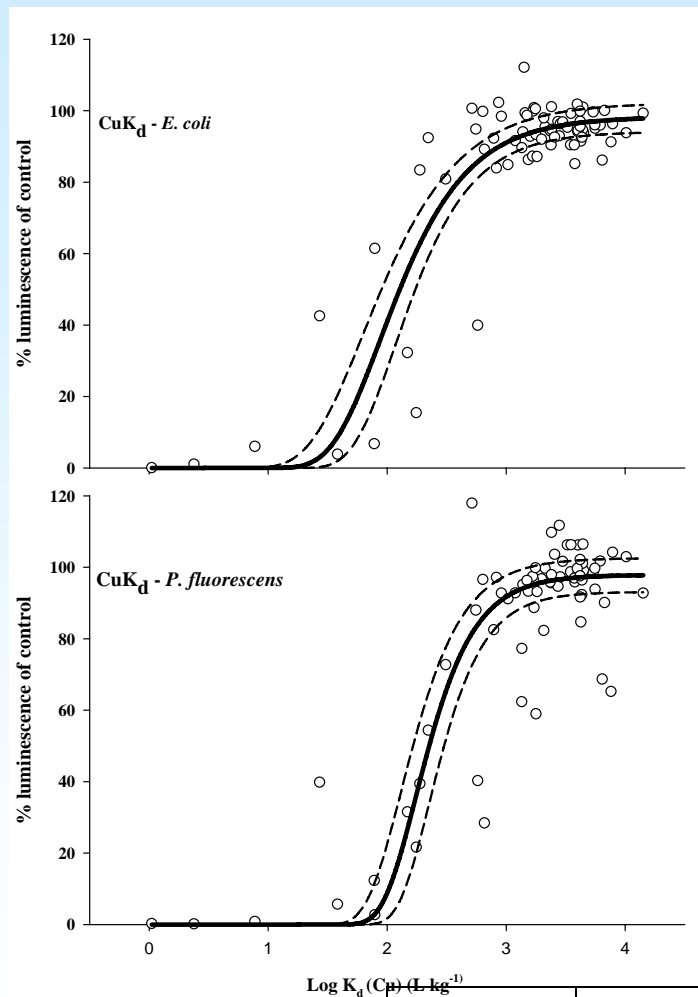
Linkage to HM Partitioning

- Soil processes that influence mobilisation or transformation can be evaluated by determination of solid-liquid partitioning coefficients – K_d .
- **High** values indicate that greater proportions of HM are adsorbed to the solid phase.
- **Low** values demonstrate that an elevated proportion of the HM is soluble.
- Link values to ecotoxicological responses – indicator of HM bioavailability.

K_d values ($L\ kg^{-1}$, $n = 19$ soils) at each Cu and Zn amendment.

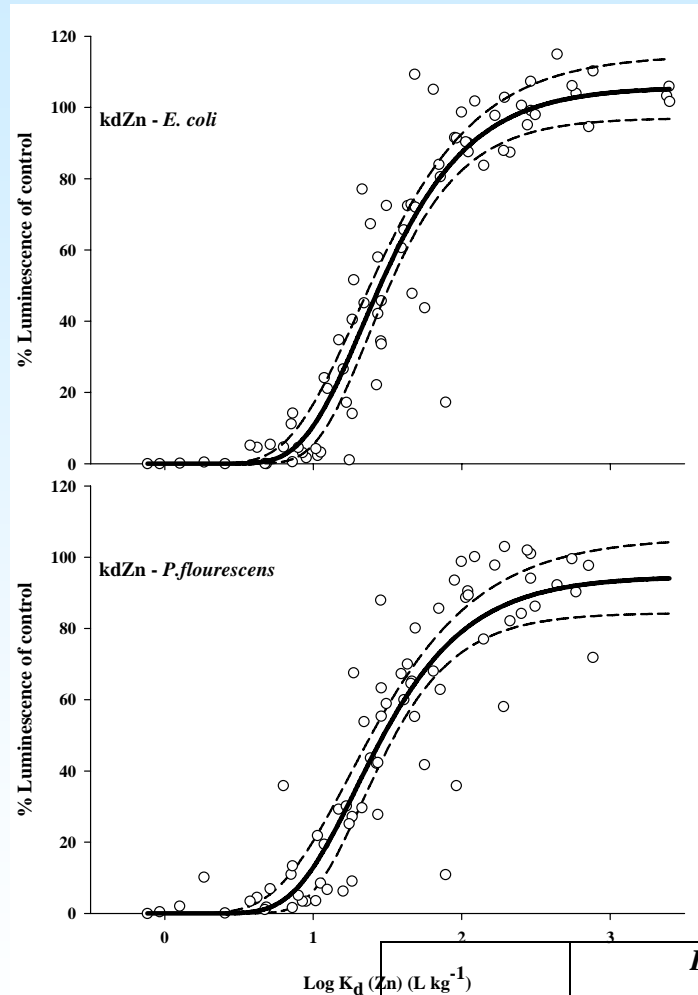
Copper dose ($mg\ kg^{-1}$)	0^a	50^{ab}	100^{ab}	200^{ab}	400^b
Min	102	27	7.7	2.4	1.1
Max	67135	14151	7602	5548	4338
Mean	8866	3825	3076	2290	1613
St. err	3490	844	513	380	348
Median	4278	2383	2843	1688	1355
Zinc dose ($mg\ kg^{-1}$)	0^a	100^b	200^{bc}	400^c	800^{cd}
Min	20	1.8	1.3	0.9	0.8
Max	3501	2483	2393	2507	764
Mean	960	327	218	170	56
St. err	210	129	124	130	40
Median	715	167	48	27	10

K_d Cu - Biosensors



	<i>E. coli</i>	<i>P. fluorescens</i>
Cu EC ₅	1995 (871, >10,000)	1698 (692, >10,000)
Cu EC ₂₀	355 (257, 525)	457 (302, 660)

K_d Zn - Biosensors



Log K _d (Zn) (L kg ⁻¹)	<i>E. coli</i>	<i>P. fluorescens</i>
Zn EC ₅	174 (112, 468)	2455 (204, >10,000)
Zn EC ₂₀	69.2 (57.5, 87.1)	97.7 (70.8, 195)

Value of K_d

- Binding affinity for HM in soils - quantified by calculating K_d values,
- Combining with other ecological responses, it is possible to assess critical K_d values for soils at which deleterious effects occur.
- Species specific.
- Extend to other ecologically relevant soil receptors.
- Could be incorporated into environmental fate and risk assessment models.

Linkage to Regulatory Values

Toxicity data derived from *E. coli* bioassays.

Soil-type	EC ₅₀ Value (mg kg ⁻¹)					
	Cd	Cu	Zn	*Co	Ni	Pb
<i>Aldroughty</i>	>12	>400	297	87	120	550
<i>Brechin</i>	>12	>400	346	470	120	535
<i>Culbin</i>	>12	44	49	66	45	215
<i>Glensaugh –Moor</i>	>12	>400	590	77	-	409
<i>Glensaugh-Steep</i>	>12	75	58	754	195	96
<i>Nethermains</i>	>12	>400	656	701	>240	>1200
<i>St. Fergus</i>	>12	>400	614	388	>240	>1200
Regulatory Value (pH 5.5)	3	100	200	30-240	60	300

* No UK Guidelines for Cobalt. Swedish/Dutch intervention values for soil remediation

Linkage to Ecological Assays

➤ **HM amended soils** :

microbial community assays i.e. Biolog-AWCD, respiration, biomass – ecotoxicological classification of soils.

➤ **Naturally elevated soils** Ni & Cr (Serpentinite) :

earthworm and plant assays.

➤ **Historically contaminated soils** Ni & Cu (Kola Peninsula) :

microbial community assays, nematodes, MIC, fungal assays.

➤ **Hydrocarbon contaminated soils.**

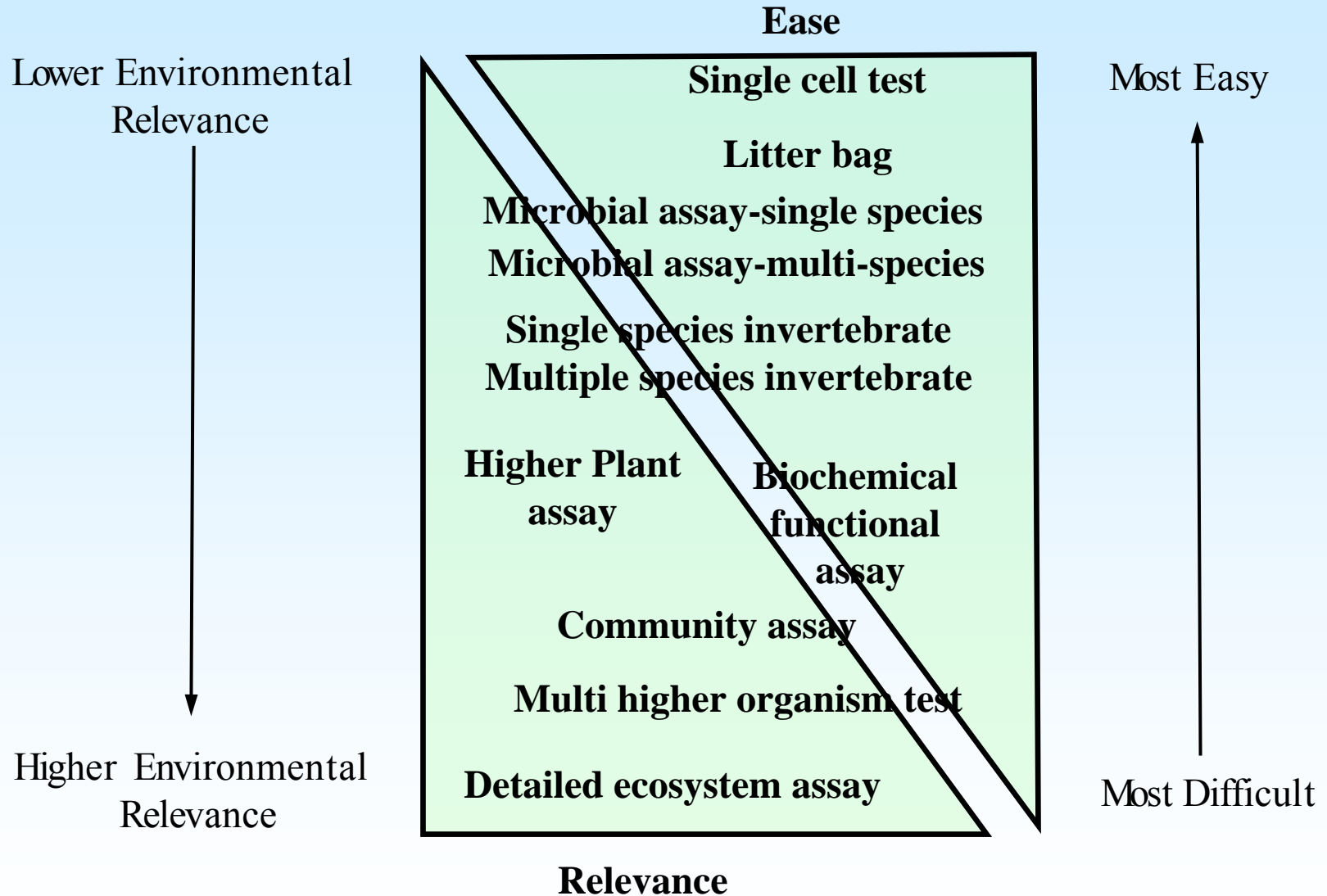
Conclusions

- Chemical analysis coupled to biological responses is key to understanding the impact of HM in soils.
- With additional ecological data, an ecotoxicological map can be produced, using soil databases and GIS technology.
- Production of ecologically derived critical K_d values.
- Some EC_{50} values below regulatory limits providing evidence for individual soil vulnerability to HM loadings.

Case Study: forest soils from the Kola Peninsula

Soil Ecotoxicological Analysis

- Toxicity assays that use living organisms can complement conventional chemical quantification.
- Many techniques, which are derived from tests in agricultural soils, may not respond to the generally more conserved nutrient balance of forest soils.
- Important that selected bioassays reflect the ecology of the particular soil ecosystem under study.



Study Area

- Kola Peninsula, North - west Russia



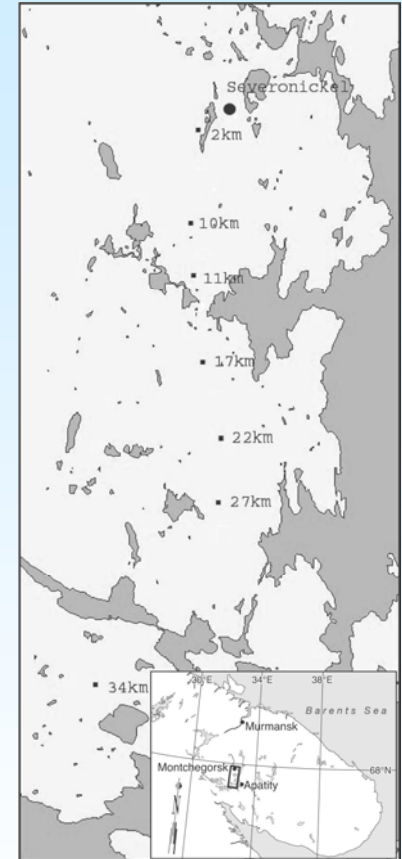
Study Area

- Extensive ecosystem part of northern taiga zone.
 - Vegetation - spruce (*Picea abies*) and pine (*Pinus sylvestris*) forest with *Vaccinium* species (*V. vitis-idaea* and *V. myrtillus*) and lichens.
 - Soil - podzolic distinct profile with a *ca.* 5-10 cm deep organic horizon.

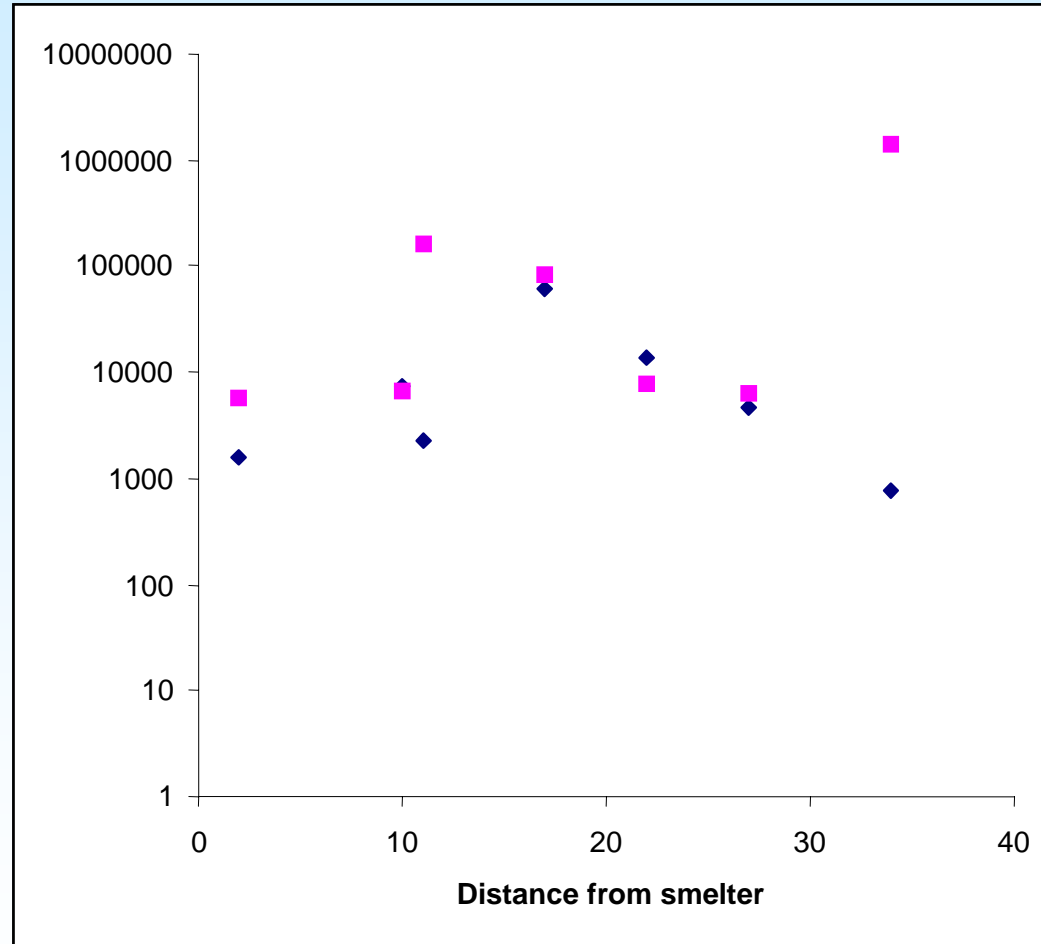


Study Area

- South of Monchegorsk City, the ‘Severonickel’ nickel-smelting complex opened in 1937.
- Source of environmental pollution with annual emissions of SO₂ and HM (Cu & Ni), amongst the highest in Europe.
- Mid 1960’s, effects of contamination studied.
 - HM concentrations in soils, berries, mushrooms and snow
 - ecological effects on forests, small mammals and birds
- Linkage between HM concentration and its effects on soil biology?
- Sampling taken along a southerly transect at 2, 10, 11, 17, 22, 27 and 34 km from the smelting complex.



Kd Summary of Results



Chemical analysis

Bulk soil &

Water-soluble fraction

Distance (km)	pH	Total Cu (mg kg ⁻¹)	Total Ni (mg kg ⁻¹)	Water-soluble Cu (mg l ⁻¹)	Water-soluble Ni (mg l ⁻¹)	Total OM (%)
34	5.28	66.3	138	<0.01	<0.01	85.91
34	3.51	97.8	174	0.12	<0.01	62.23
34	3.55	66.7	142	0.14	<0.01	73.70
27	3.79	271	390	0.13	0.12	68.29
27	4.10	576	669	<0.01	0.21	60.62
27	3.73	488	743	0.09	0.09	68.63
22	4.04	1380	1570	0.09	0.01	67.57
22	4.03	2080	1490	0.30	0.61	67.80
22	3.92	1360	1570	0.09	0.70	83.24
17	3.79	1170	1040	0.01	0.02	74.46
17	4.14	265	400	0.02	0.01	74.66
17	4.09	308	413	0.01	0.01	43.98
11	4.08	1920	1979	0.08	0.03	59.63
11	3.78	1240	1450	0.67	0.41	35.27
11	3.79	3040	2210	0.81	0.10	83.20
10	3.97	519	761	<0.01	0.07	16.19
10	4.05	725	981	1.04	0.87	15.38
10	3.92	1360	1819	0.28	0.02	23.84
2	4.44	845	1979	1.20	0.20	0.60
2	4.55	1330	4589	0.89	0.19	38.04
2	4.25	1739	4227	1.01	1.10	46.09

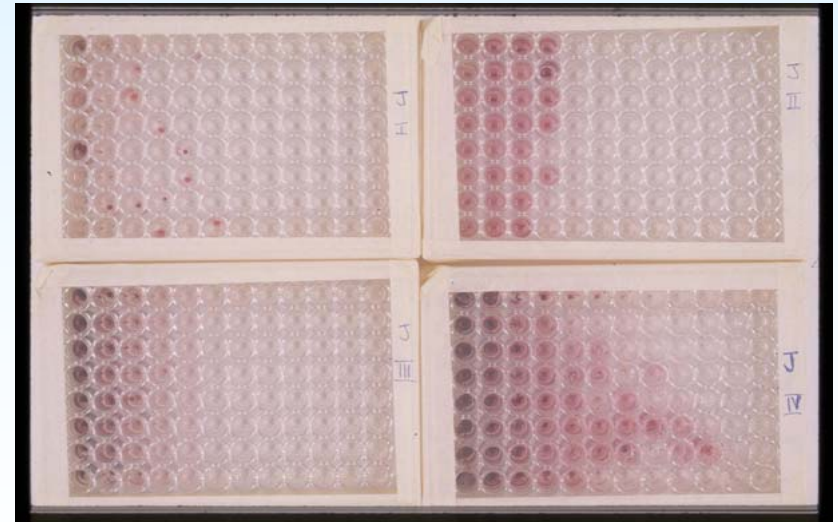
Microbial Respiration

- Measure of total community activity in bulk soil.
- Soil samples incubated for 24 h in a closed vial at 15 °C.
- CO₂ in headspace determined by GC-FID with an attached methanizer.
- Respiration calculated in mg CO₂ g soil⁻¹ day⁻¹ following subtraction of a blank vial containing atmospheric CO₂.

MPN - Dehydrogenase

- Simple technique to determine microbial numbers
- Dehydrogenase-based methods have been identified as ecologically appropriate for the testing of forest soils after pollution incidents.

Following serial dilutions and incubation, dehydrogenase positive wells (purple) were used to determine cells g soil^{-1}



Paton *et al.* 2003

Minimum Inhibitory Concentration

- Many bacterial and fungal species have evolved different mechanisms to survive in HM contaminated soils.
- Measurement of the minimum inhibitory concentration (MIC) of a community can indicate the presence and extent of this population
- Same MPN-dehydrogenase was applied but the wells with 10^6 cells were supplemented with a gradient of either Cu or Ni amendments.

Nematodes

- Earthworms, commonly used in soil ecotoxicology studies, not present in the higher latitude podzolic soils of this study.
- Thus, nematodes - a more appropriate meiofaunal group.
- Indirect effects on both number and type of nematodes can be a response to HM mediated microbial community or biomass changes.

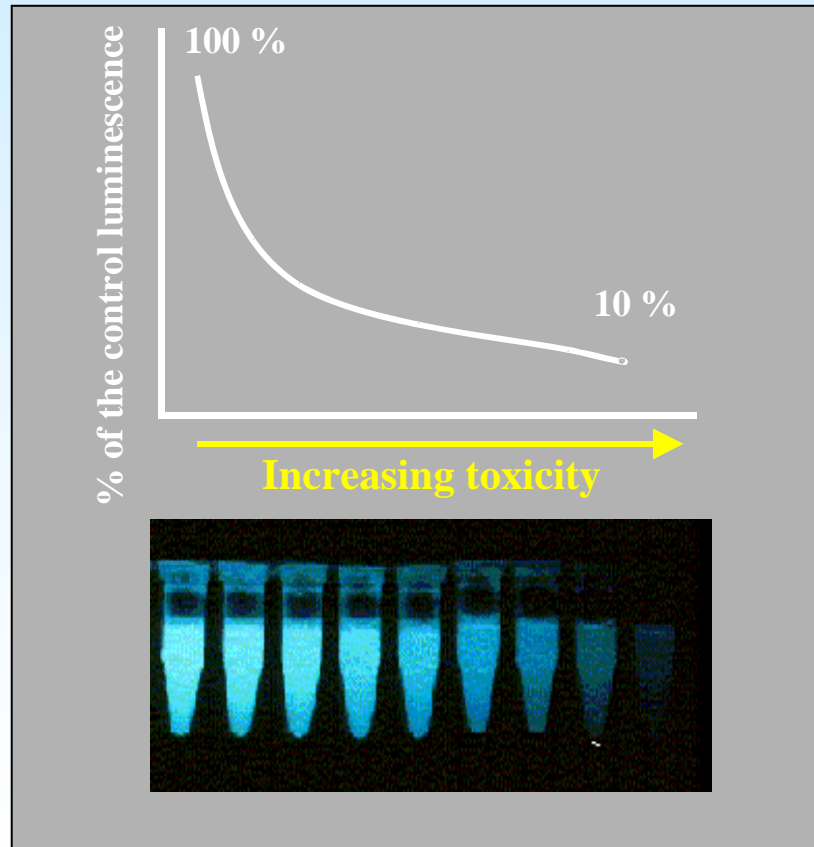


Water-soluble fraction

- Soil pore water was extracted in a 1:2 soil:water mixture.
- Shaken for two hours on an end-over-end shaker.
- Centrifugation
- Supernatant used for biosensor toxicity assays

Bacterial Bioassay

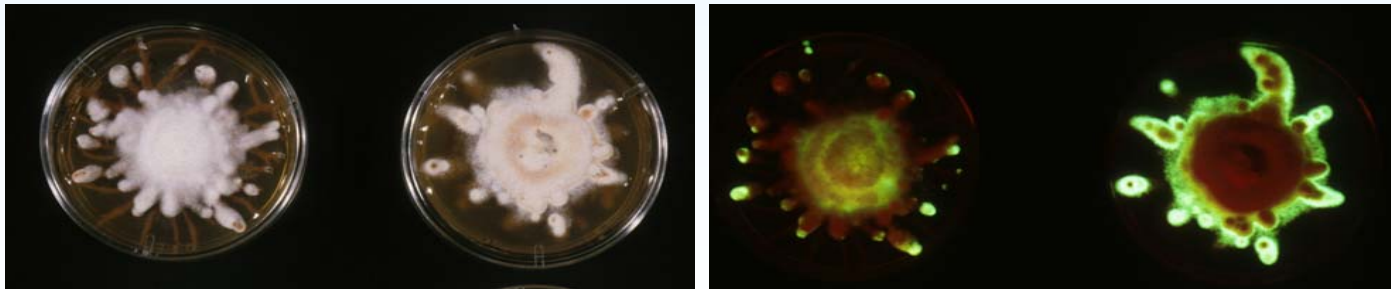
Concept of bioluminescence-based assay



Fungal Bioassay

Armillaria mellea (ATCC 11113)

- Honey or bootlace fungus, soil-borne fungus, tree pathogen.
- Relevant for forest soil studies, important for eukaryotic vs. prokaryotic biosensors.
- Naturally luminescent mycelium and rhizomorphs,
- Suitable for both organic and inorganic pollutant sensing, compatible with existing biosensor technology.



Weitz *et al.* 2002

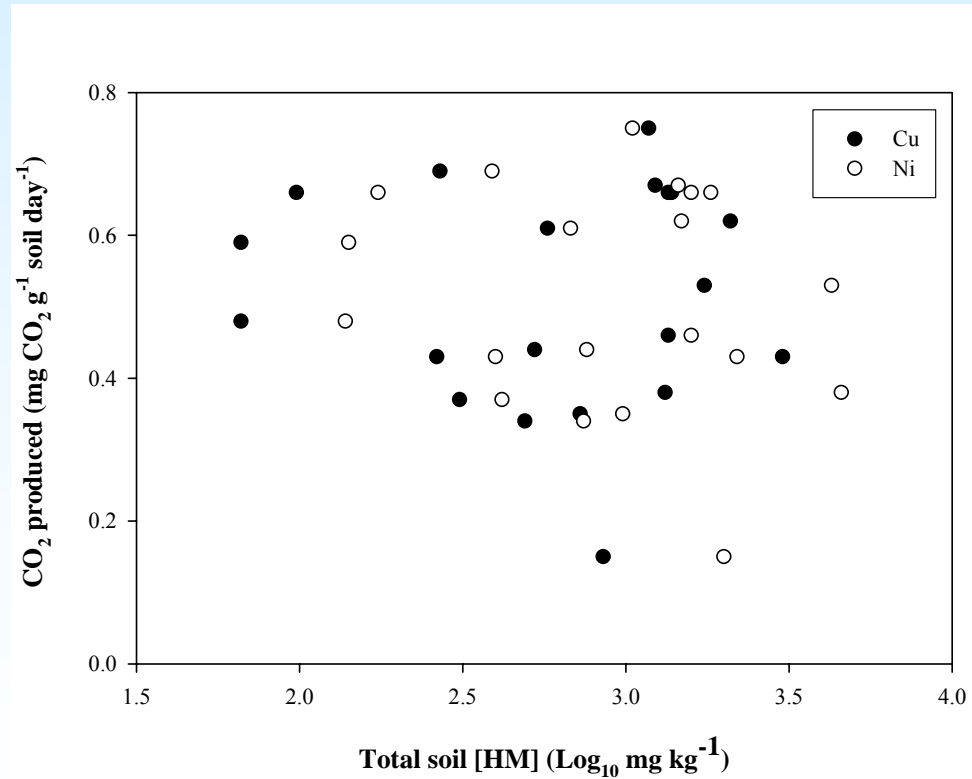
Bioluminescent globular mycelia - *A. mellea*



Ecotoxicological Results

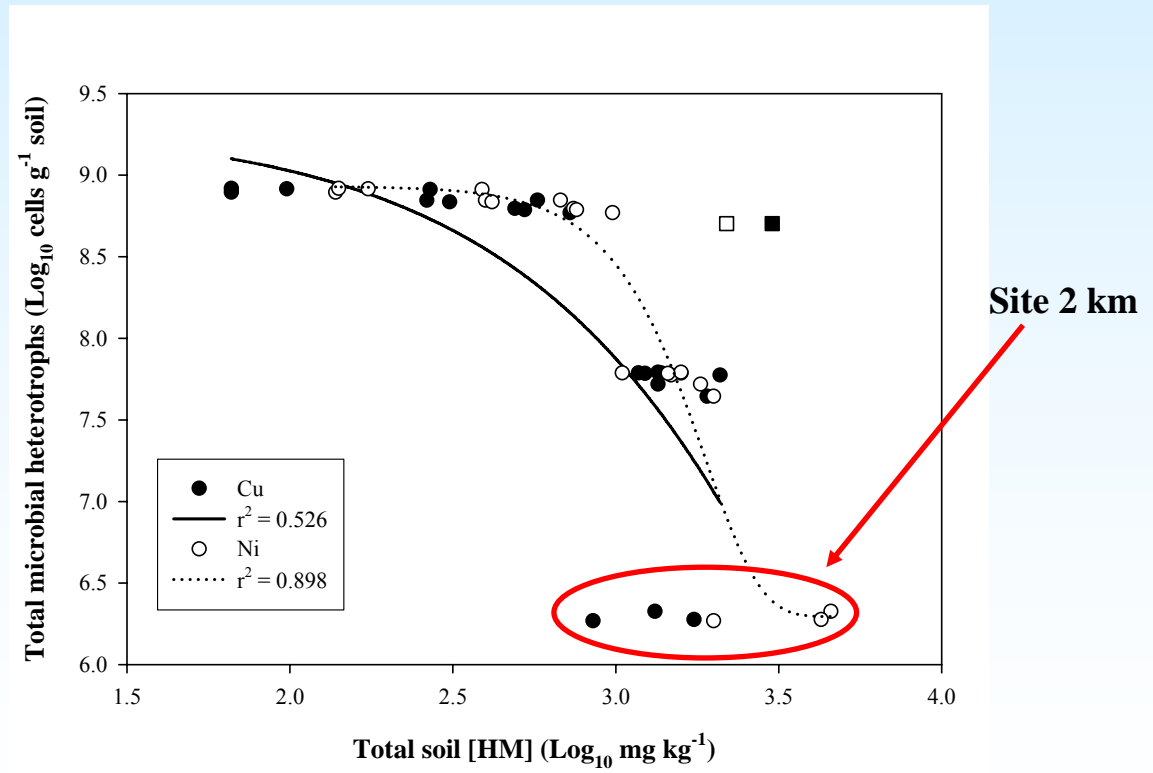
Microbial Respiration

No clear relationship
between respiration and
concentration of either of
the HM

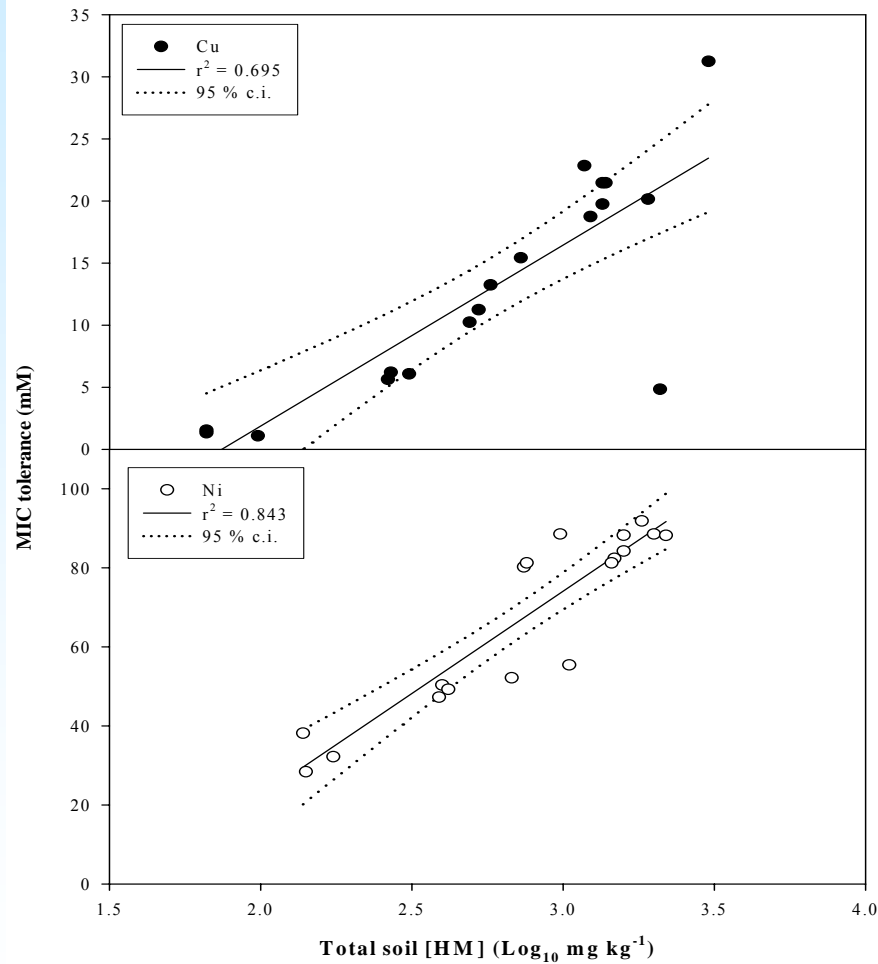


Microbial Numbers

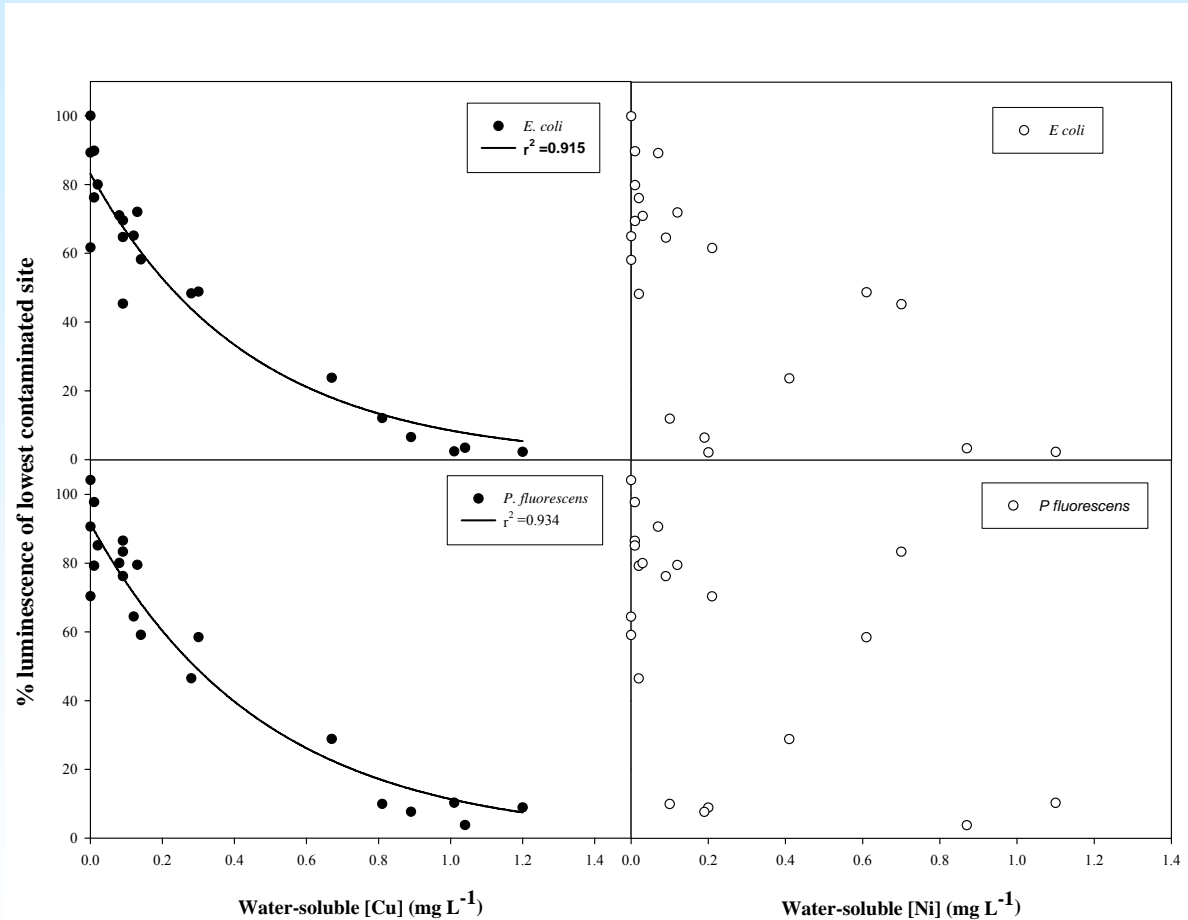
At $\sim 1000 \text{ mg HM kg}^{-1}$ of soil, there was a major decrease in the total number heterotrophs.



Metal Tolerance

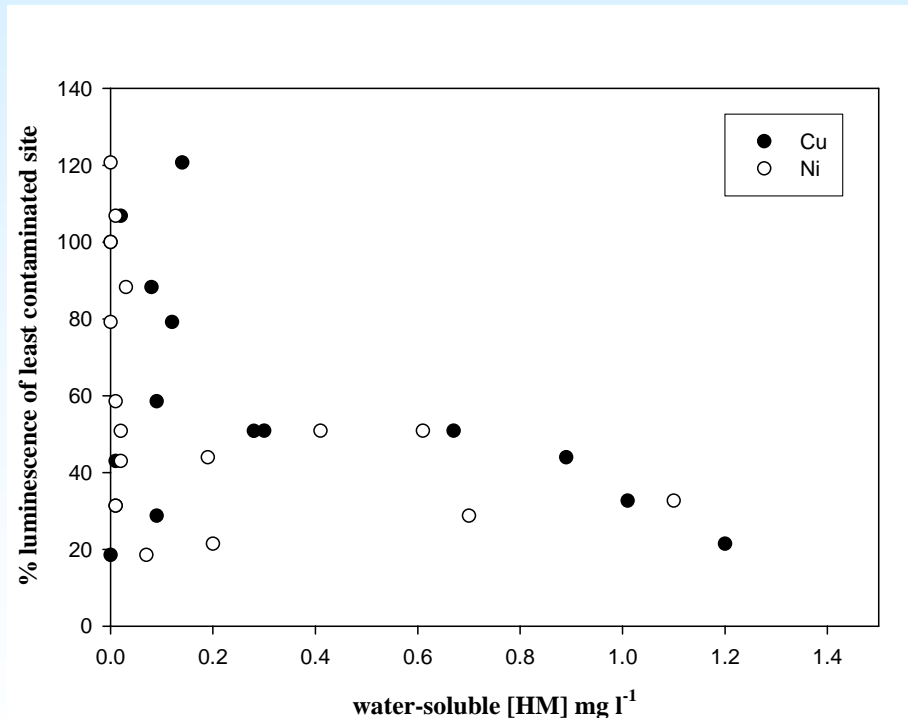


E. coli & *P. fluorescens*



A. mellea

Overall 0.15 mg l^{-1} of each HM presented a threshold level at which *A. mellea* function was impacted.



Kola Findings

- Chemical analysis of bulk soil alone lacks sensitivity.
- The soil biota have become conditioned to decades of pollution burden and are quite dynamic. The exception being at the site 2 km from the smelter.
- The biosensor assays show acute responses to the water-soluble fraction.
- Although the HM is partially complexed or immobilised in the soil, a component of each HM is bioavailable and long-term exposure has revealed a response.

General Conclusions

- There are excellent tools for environmental modelling and interpretation but we often lack the imagination for application.
- Relating the micro to the macro is an essential demand.
- Empirical data gives genuine value to modelling data.
- Ecological appraisal demands battery screening by assays appropriate to the ecosystem under stress.

Hazard and Risk Assessment of Hydrocarbons and Persistent Organic and Organometallic Pollutants in Soils

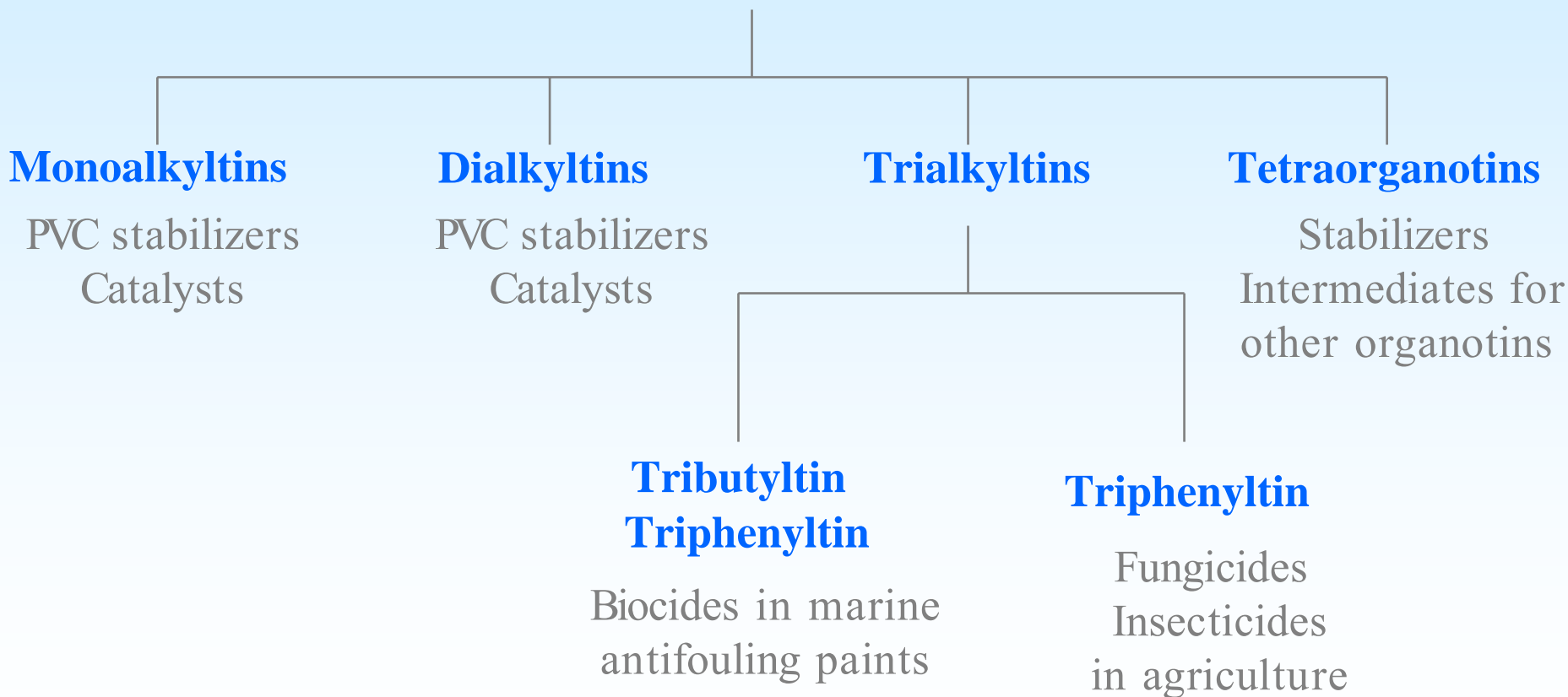
Julian J.C. Dawson and Graeme I. Paton

School of Biological Sciences, University of Aberdeen, UK

Fate of phenyltin compounds in soil

Introduction

Organotin compounds (R_nSnX_{4-n})



Marine Environment (TBT)

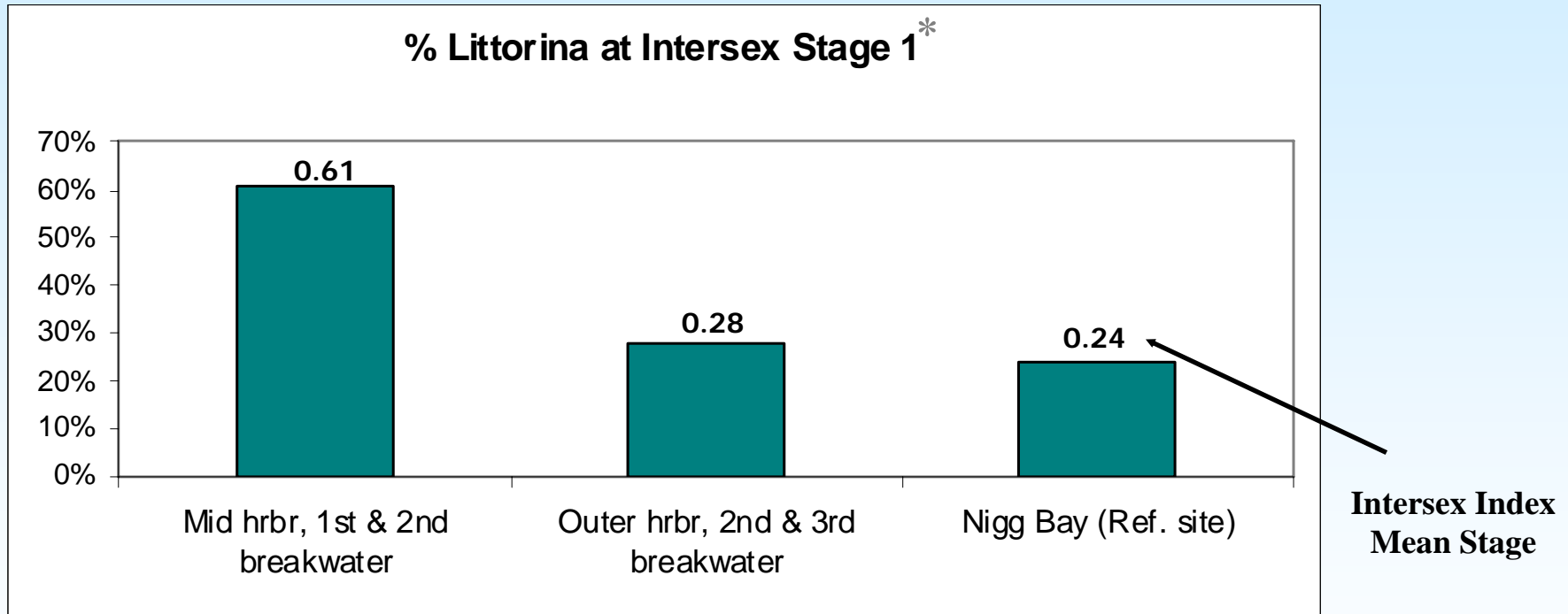
Endocrine disruption in Periwinkle

- *Littorina littorea*

- TBT causes female organs to turn into male organs.
- Mode of action: stops conversion of male to female hormones.
- Sex changes classified in stages visually with a dissecting microscope.
- TBT has a half-life ~5 yrs in sediment.
- Likely to also be an acute and chronic toxin.

Intersex Measurements

Aberdeen Harbour - Jan. 2003



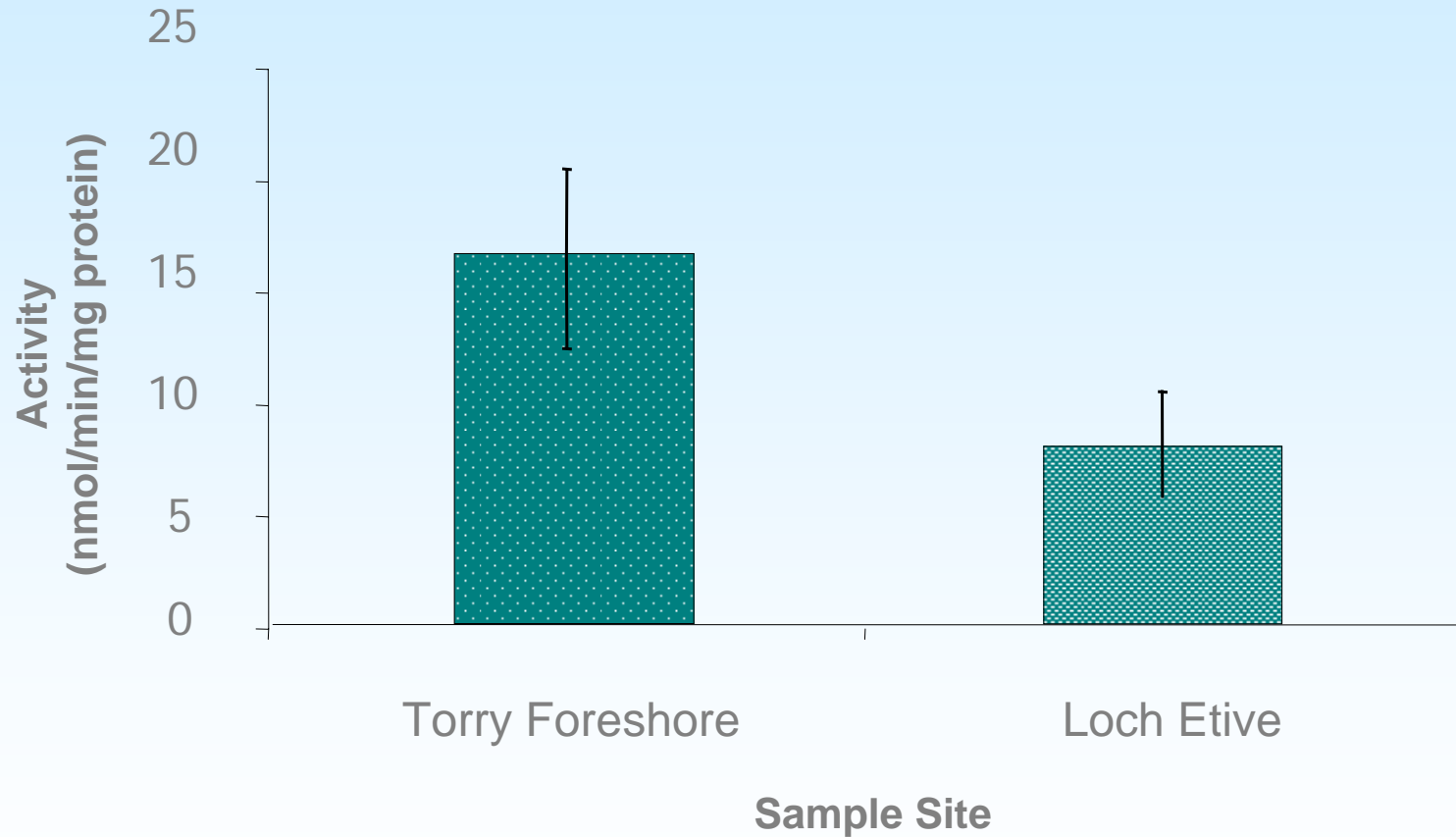
***Stage 1** = distortion of vaginal opening, organism still fertile.

GST Enzyme Assay

Glutathione S Transferase in mussels

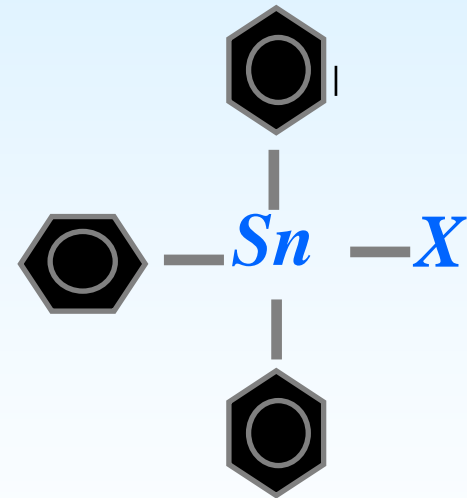
- A phase II enzyme that adds conjugates to primary metabolites, increasing polarity, i.e. water solubility.
- Mussel digestive tissue (hepatopancreas) is used, known to have high concentration of metabolic enzymes.
- Increased GST enzyme activity shows exposure to pollutants - non-specific, Cd, PCBs, PAHs.

Mean GST Activity



Triphenyltin (TPT)

- **A contact fungicide (fentin)**
 - blight* : potatoes, sugar beets, celery, peanuts, pecans, carrots, soybeans
 - algae* : rice field
 - fresh water snail & mites* : orchards
- **An antifoulant** in paints but has been regulated as tributyltin (TBT)



Triphenyltin compound

Triphenyltins in Soil

(162- 420 g ha⁻¹)

(210-240 g ha⁻¹)

TPT.OAc **TPT.OH**

Air

Soil

3 h

Application
5-15 times yr⁻¹

Phytophthora infestans

TPT

4-5 days

DPT

Time ?

MPT

Time ?

Sn

organotin residues ~ **30%** of annual dose

Aims

- Assess the toxicity of phenyltin compounds (TPT, DPT &MPT) in aqueous solution by using microbial biosensors.
- Study the **degradation** of triphenyltin compound & assess the **toxicity** of TPT and its degradation products in soil.

**Aqueous
Solution**

**Pore-water extracts
from Soil**

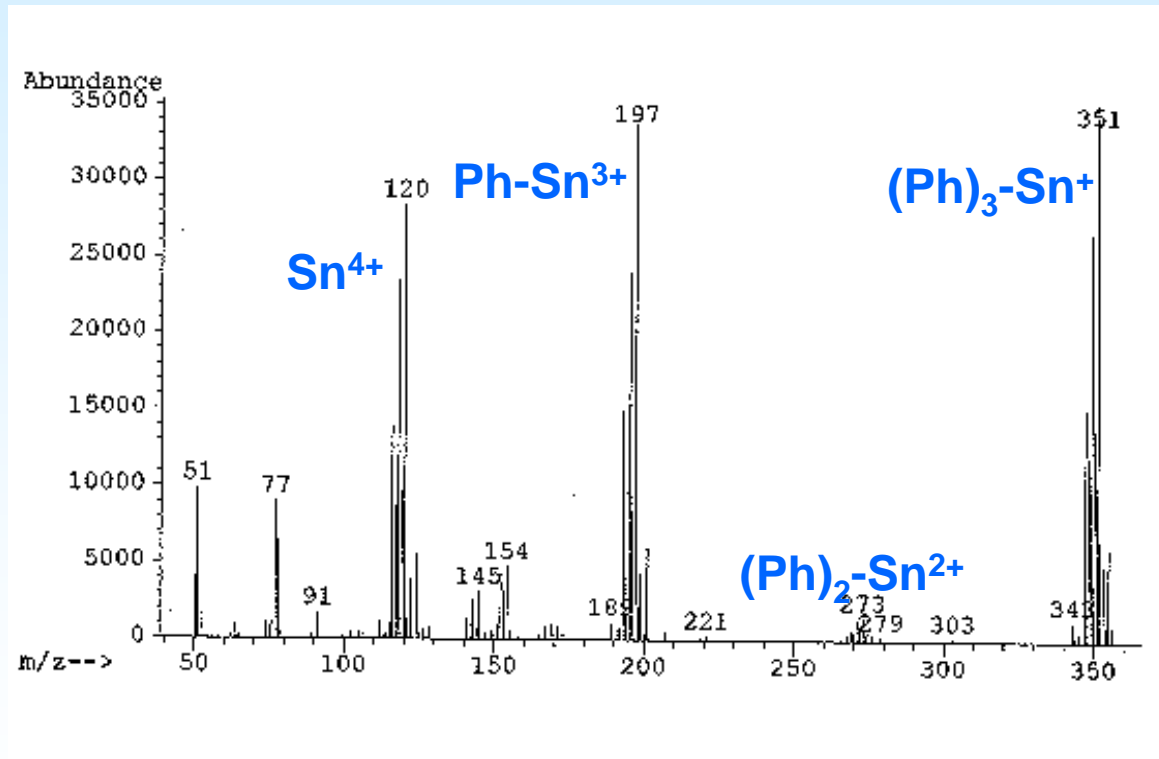
PHENYLTINS

**Microbial
toxicity**

**Chemical
determination**

Chemical Analysis

Organotins in aqueous/pore water solutions/soil extracts were analysed by GC-EI-MS under SIM mode, following derivitisation.

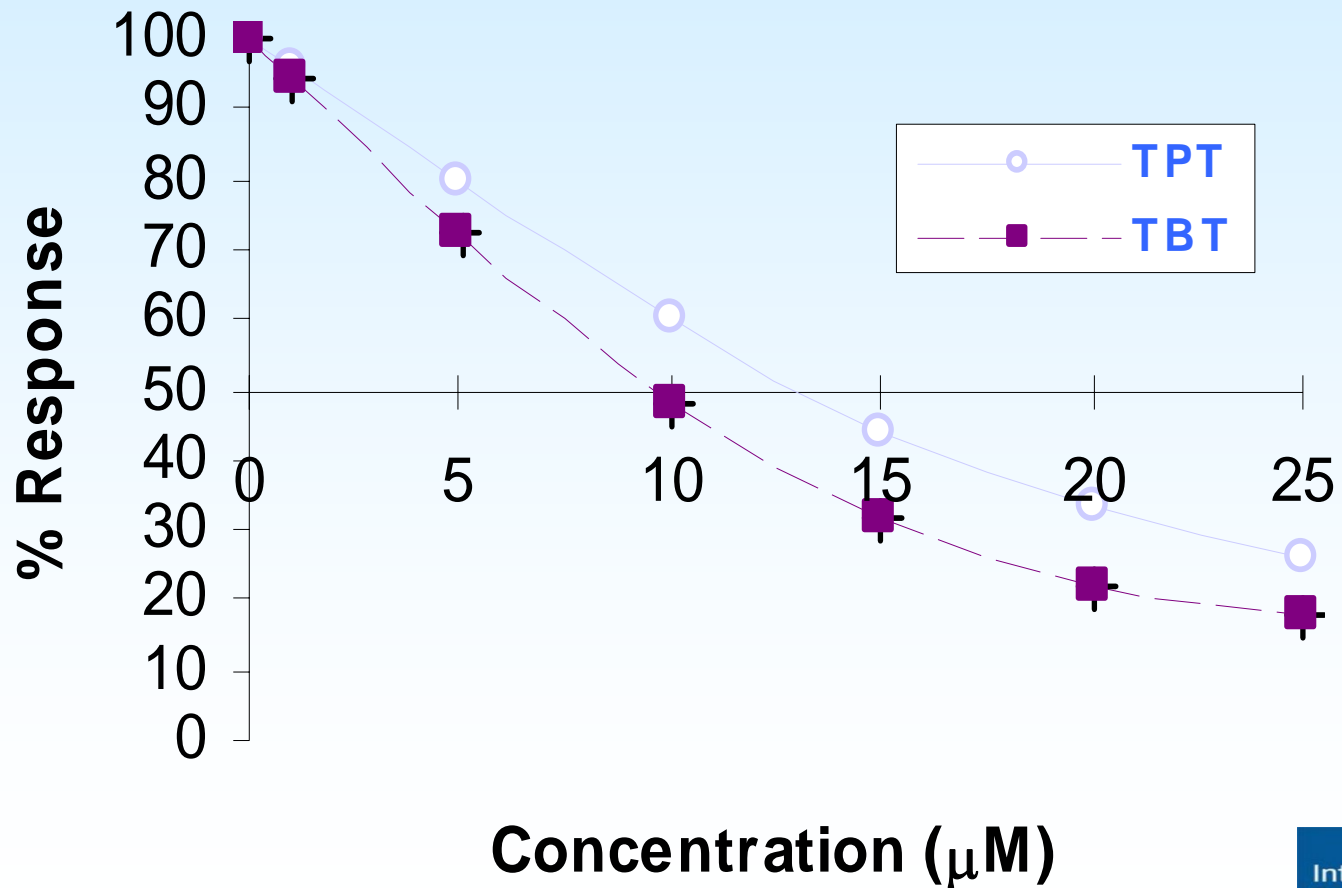


Mass spectrum of ethylated TPT

Microbial Toxicity

Dose-Response to aqueous solutions of TPT/TBT

E. coli



Aqueous Solutions

The EC₅₀ (μM) values for a range of TPT compounds and TBT in aqueous solutions using three different biosensors.

	<i>E. coli</i>	<i>P. fluorescens</i>	Microtox
TPT.CI	12.51	4.95	0.41
TPT.OAc	7.63	3.03	0.36
TPT.OH	8.42	4.60	0.41
TBT.CI	9.18	3.84	0.42

Lsd = 0.64, $n = 3$

Pore - Water Extracts

Dose-Response to spiked pore-water extracts

Control soil – (*Sandy loam, pH 4.5, 5.5, 7.1*)

↓
3 g soil + 12 ml distilled water

↓
Control soil slurry : **Spiked soil slurry (μM)**

100 : 0

75 : 25

50 : 50

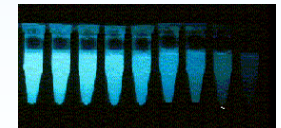
25 : 75

0 : 100

→ Centrifuge →

Supernatant
(0%, 25%, 50%, 75%, 100%
of spiked soil extracts)

Test toxicity - biosensors



↓
Derive EC₅₀

Pore - Water Extracts

The EC₅₀ (μM) values for a range of phenyltins amended into pore-water extracts using *E. coli* & *P. fluorescens*.

	MPT.Cl₃		DPT.Cl₂		TPT.Cl		TPT.OAc	
	<i>E. coli</i>	<i>P. fluor.</i>	<i>E. coli</i>	<i>P. fluor.</i>	<i>E. coli</i>	<i>P. fluor.</i>	<i>E. coli</i>	<i>P. fluor.</i>
Deionised Water:								
	12.6	4.94	14.5	15.4	12.5	4.95	7.63	3.03
Soil Extracts:								
pH 4.5	30.5	18.7	15.2	7.98	15.0	8.47	24.4	9.70
pH 5.5	30.0	17.4	45.5	8.76	21.4	12.7	66.3	24.6
pH 7.1	9.70	15.8	29.8	11.2	54.0	21.4	41.6	26.8

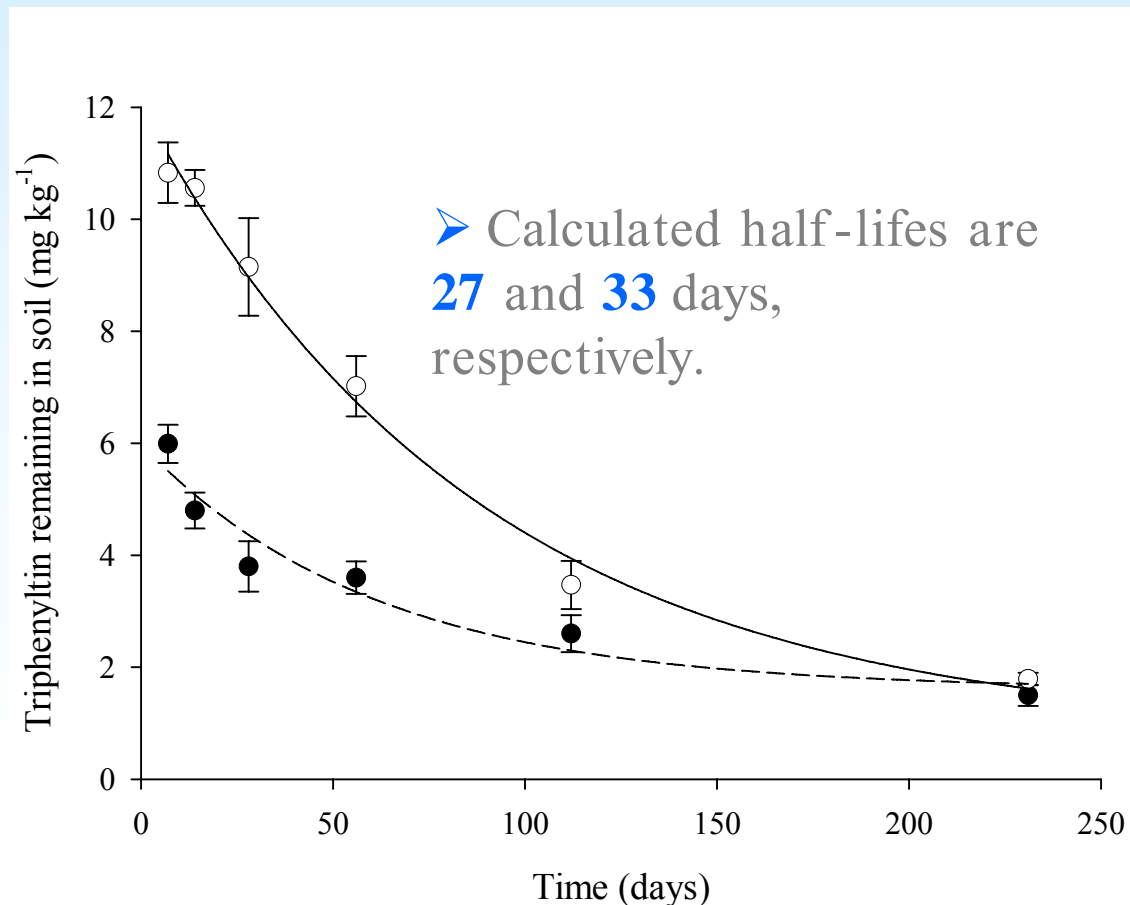
Lsd = 2.10, n = 3

Degradation/ Toxicity Experiment

- Non-amended control (pH 5.5) and 3 treated soils:
 - (i) sterile soil by autoclaving
 - (ii) autoclaved-reinoculated soil
 - (iii) non-sterilised soil
- TPT.OAc solution was added to 300 g of each soil treatment ($n = 3$).
Final concentrations of 10 mg Sn kg⁻¹ and 20 mg Sn kg⁻¹
- Soil samples were incubated at 20 °C under a standard 12 hour day/night regime.
- Destructive sampling after 7, 14, 28, 56, 112 and 231 days.
- Chemical analysis – bulk soils
- Biosensor toxicity tests - soil pore-water extracts.

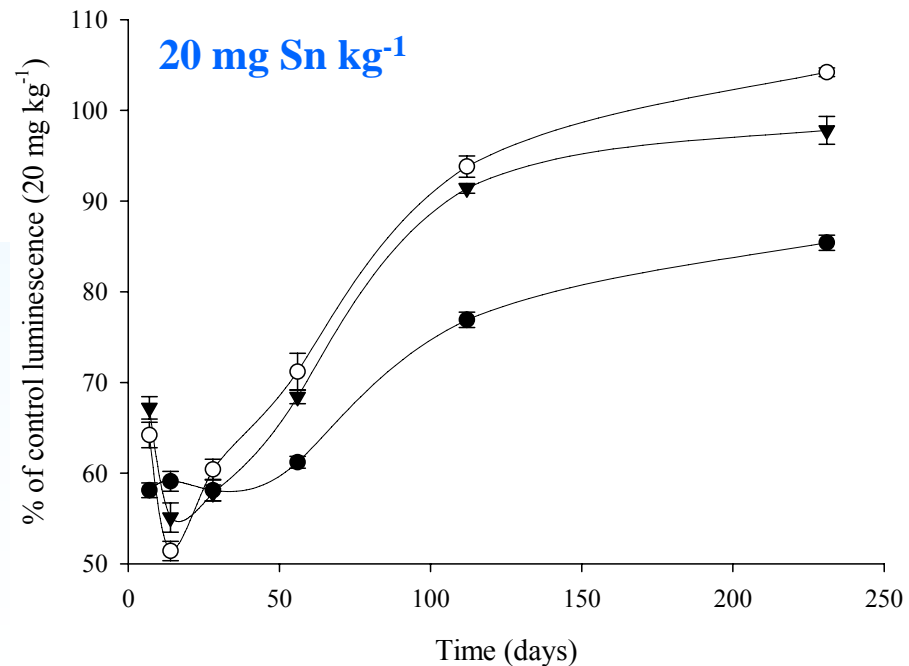
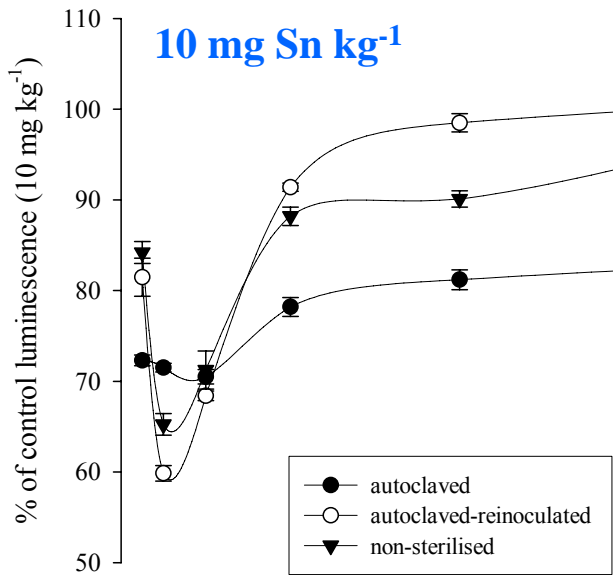
Degradation

- Temporal determination of extractable concentrations of TPT.
- Soil amended with 10 mg Sn kg⁻¹ (●) and 20 mg Sn kg⁻¹ (○).



Toxicity

Toxicity: (*P. fluorescens*) of soil pore-water extracts from following amendment with **TPT.OAc**.



Toxicity Results

- For both non-sterile soils, where increased degradation of TPT occurred, there was an increase in toxicity followed by a recovery phase.
- Initial increase associated with the production of more bioavailable and toxic metabolites than the parent molecule.
- Net toxicity subsequently diminishes because of either:
 - reduced concentration of the parent molecule
 - metabolites are being aged or further degraded
- Pore-waters from the non-sterile soils showed no toxicity by the end of the incubation period.

Conclusions

- Toxicity of organotins is determined by its form and the biosensor selected for testing.
- Biodegradation was significantly higher than abiotic degradation.
- Both amended concentrations reached the same end-point reflecting organotin unavailable for further biodegradation.
- The persistence of the TPT in soil will be affected by soil parameters that influence bioavailability.
- Microbial responses may be indicative of higher organism and process-level stresses.

Biological indicators

Assessment of remediation success in HC-impacted soils

Background

- Remediation programmes are considered complete when human risk-based criteria are met.
- These targets are unrelated to the ecological parameters that may be important with regard to future soil uses.
- There is a move towards using biological indicators for hazard assessment in conjunction with remediation of contaminated soils.

Aims

- This study uses a range of biological assays to assess the ecological health of soils from a former gas works site undergoing various remediation treatments.
- Soil, that had been initially biopiled, was incubated in triplicate 20 kg mesocosms for 400 days (mean temp. 15 °C; 50 % WHC) with a range of remediative treatments.

Mesocosm Set-up

Biopile



Mesocosms



Mixing



Incubation

Bioremediation treatments

- **UN** – *untreated* soil from the original biopile, *no turning*
- **UN+T** – *untreated but turned* every 2 weeks
- **NA+T** – *nutrient addition* (100C:15N:1P ratio),
turned every 2 weeks
- **SA+T** – *surfactant addition* (1 %v/w, sorbitan monooleate),
every 2 weeks turned
- **MIX+T** – *equal mixtures* of UN+T, NA+T and SA+T,
turned every 2 weeks
- **Boyndie** –texturally similar *non-contaminated control* soil,
every 2 weeks turned

Chemical Extraction

Mean hydrocarbon concentration (S.E.)
(mg g⁻¹)

Treatment	DCM-Acetone	Methanol
UN	^a 0.64 (0.01)	^a 0.38 (0.00)
UN+T	^{ab} 0.31 (0.11)	^a 0.26 (0.04)
NA+T	^{bc} 0.21 (0.05)	^b 0.12 (0.00)
SA+T	^{bc} 0.17 (0.08)	^b 0.09 (0.01)
MIX+T	^{bc} 0.19 (0.07)	^b 0.09 (0.02)
Boyndie	^c 0.02 (0.01)	^c <0.01 (0.00)

Significance at $p \leq 0.05$ are shown for comparisons within each column

Bioassays

Microbial



Biomass

Respiration

Nitrification

Enzyme assays

lux-based bacteria

Earthworms



Lethal and Sub-lethal

Eisenia fetida

Lumbricus terrestris

Plants



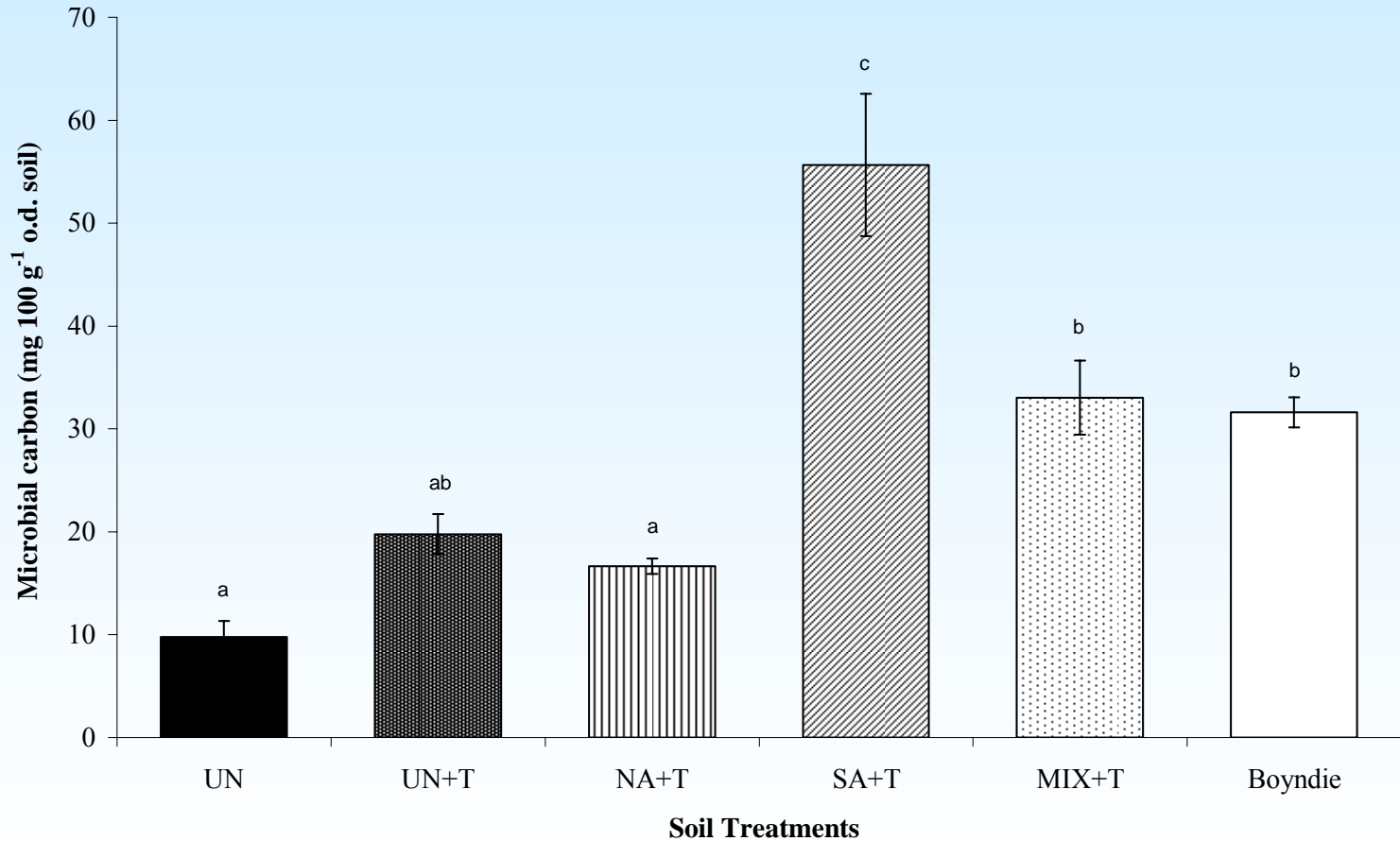
Seed Germination

Mustard

Rye grass

Pea

Microbial Assays



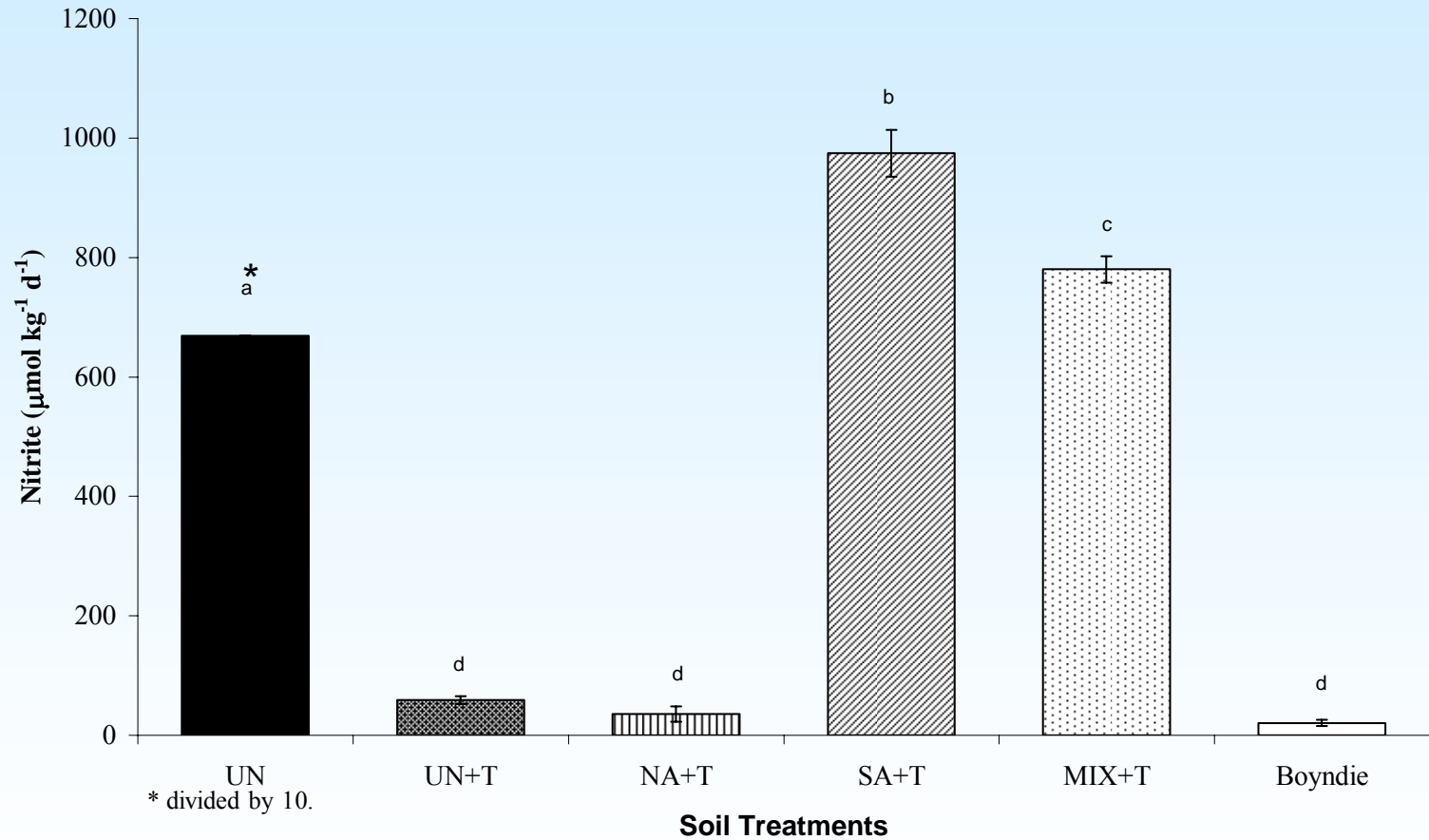
Microbial Biomass Carbon - Vance *et al.* 1987

Microbial Assays

Substrate Induced Respiration

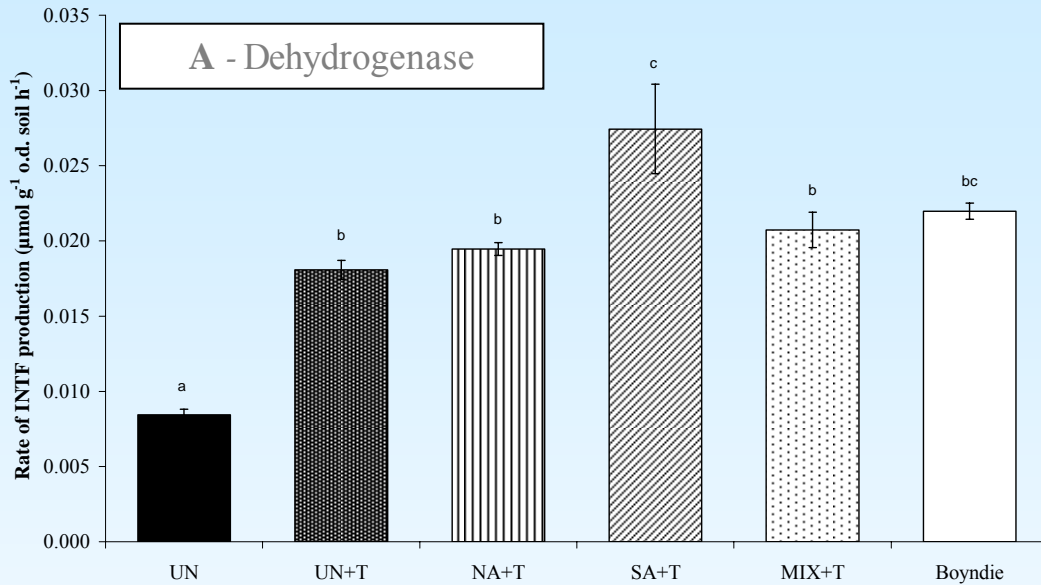
Treatment	Basal	Glucose	Glutamine	Maleic Acid	Mannose	Phenol
UN	^a 0.013 (0.001)	^a 0.015 (0.000)	^b 0.027 (0.001)	^b 0.521 (0.006)	^a 0.011 (0.003)	^a 0.011 (0.001)
UN+T	^a 0.019 (0.005)	^{ab} 0.030 (0.001)	^c 0.039 (0.002)	^c 0.651 (0.010)	^b 0.038 (0.006)	^{ab} 0.027 (0.002)
NA+T	^a 0.017 (0.001)	^{ab} 0.031 (0.000)	^b 0.033 (0.002)	^c 0.648 (0.025)	^{ab} 0.035 (0.004)	^{ab} 0.030 (0.001)
SA+T	^a 0.028 (0.002)	^b 0.047 (0.001)	^c 0.044 (0.001)	^c 0.669 (0.048)	^{bc} 0.051 (0.001)	^c 0.062 (0.008)
MIX+T	^a 0.021 (0.004)	^b 0.050 (0.006)	^c 0.043 (0.001)	^{bc} 0.605 (0.036)	^c 0.061 (0.006)	^b 0.039 (0.003)
Boyndie	^b 0.049 (0.006)	^{ab} 0.031 (0.009)	^a 0.017 (0.000)	^a 0.016 (0.003)	^{ab} 0.035 (0.005)	^b 0.037 (0.001)

Microbial Assays

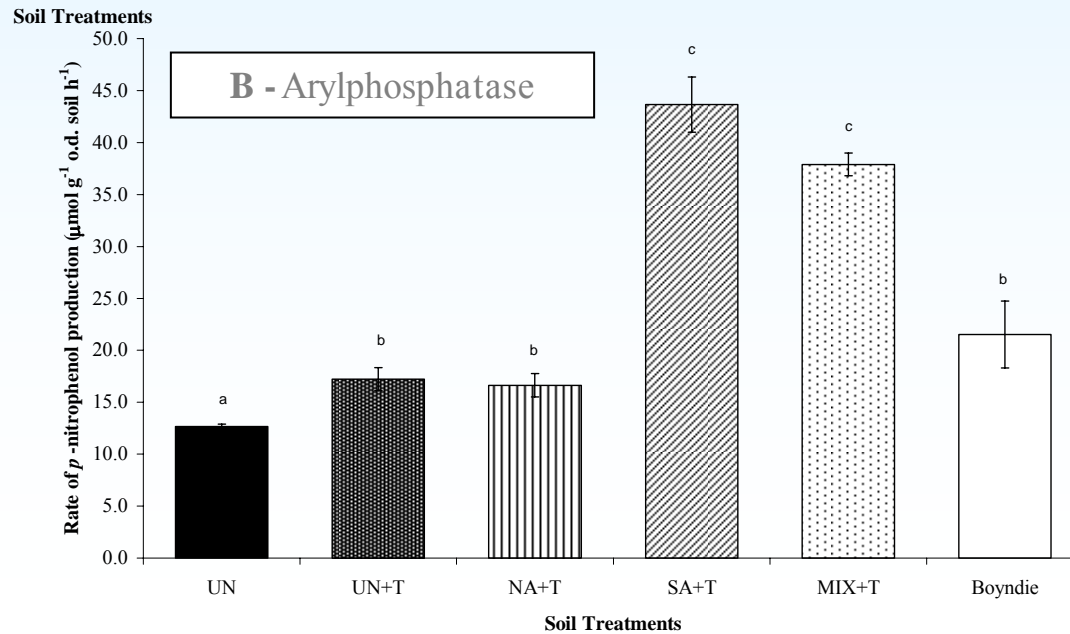


Potential Nitrification Rate - Sauv  et al. 1999

Enzyme Assays

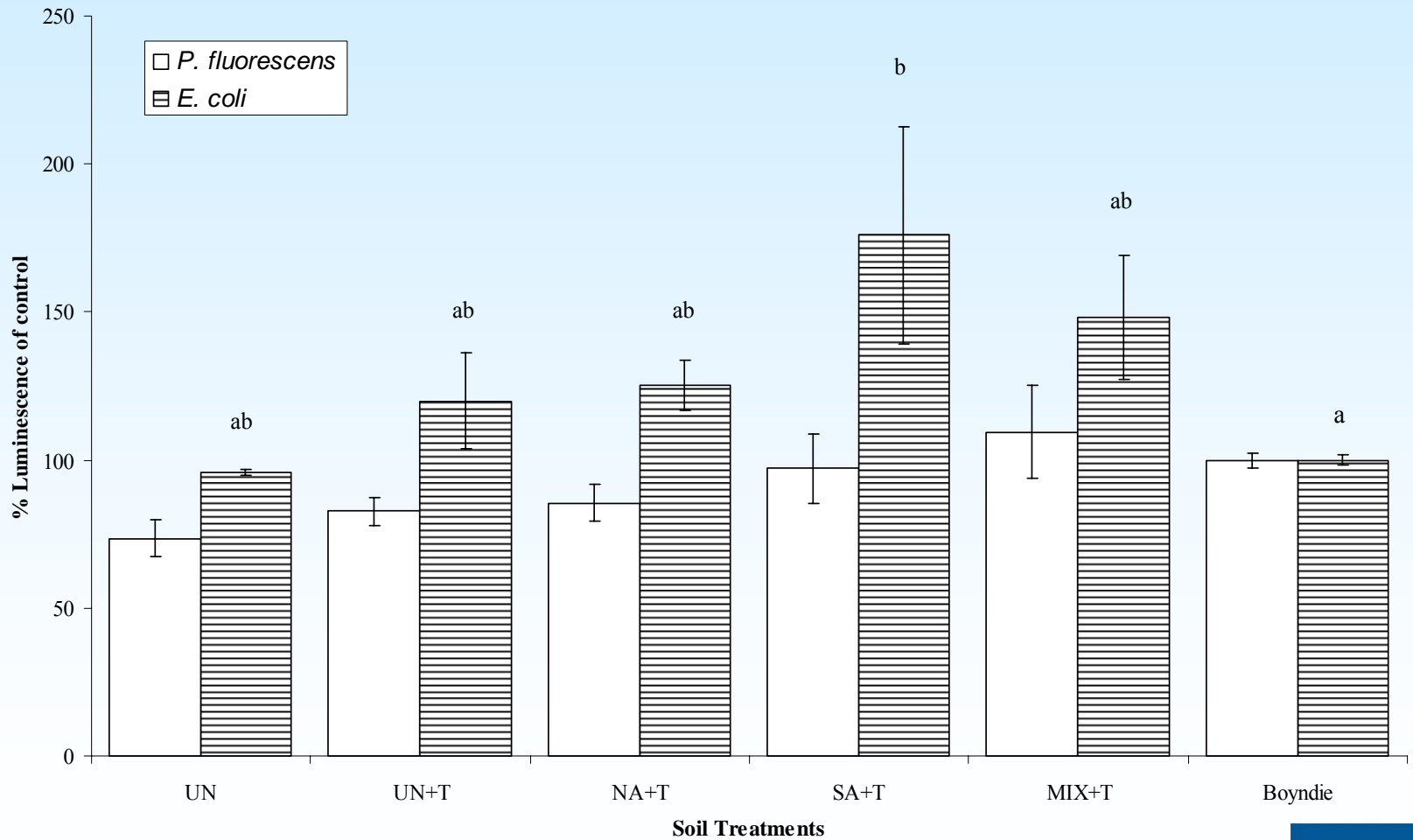


Casida *et al.*, 1964
Trasar-Cepeda *et al.*, 2000



Tabatabai and Bremner, 1969
Saã, *et al.*, 1993;

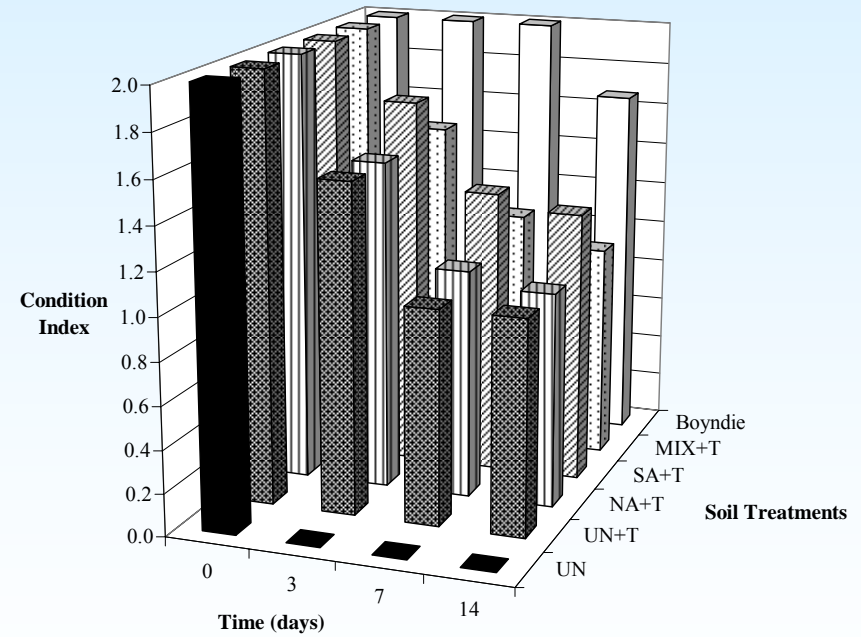
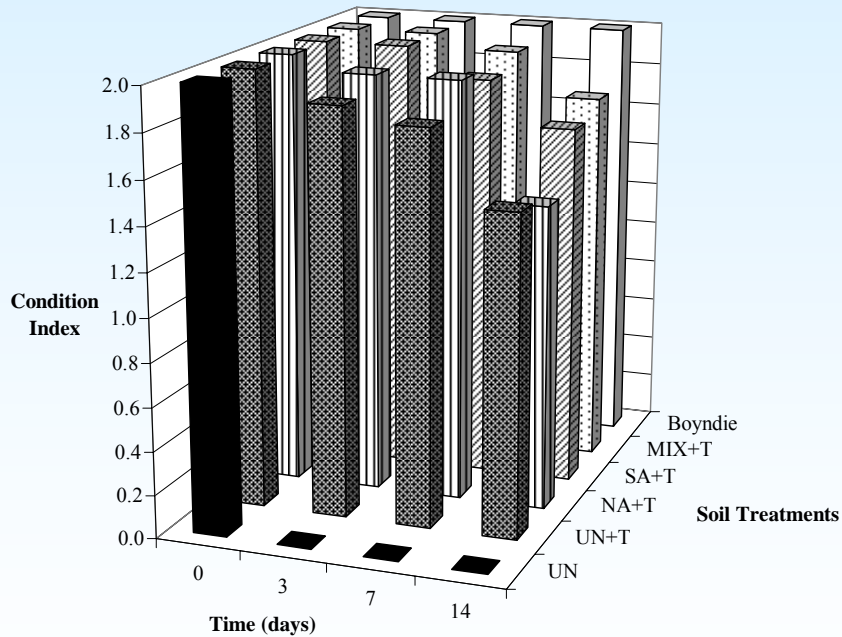
Biosensors



Earthworms

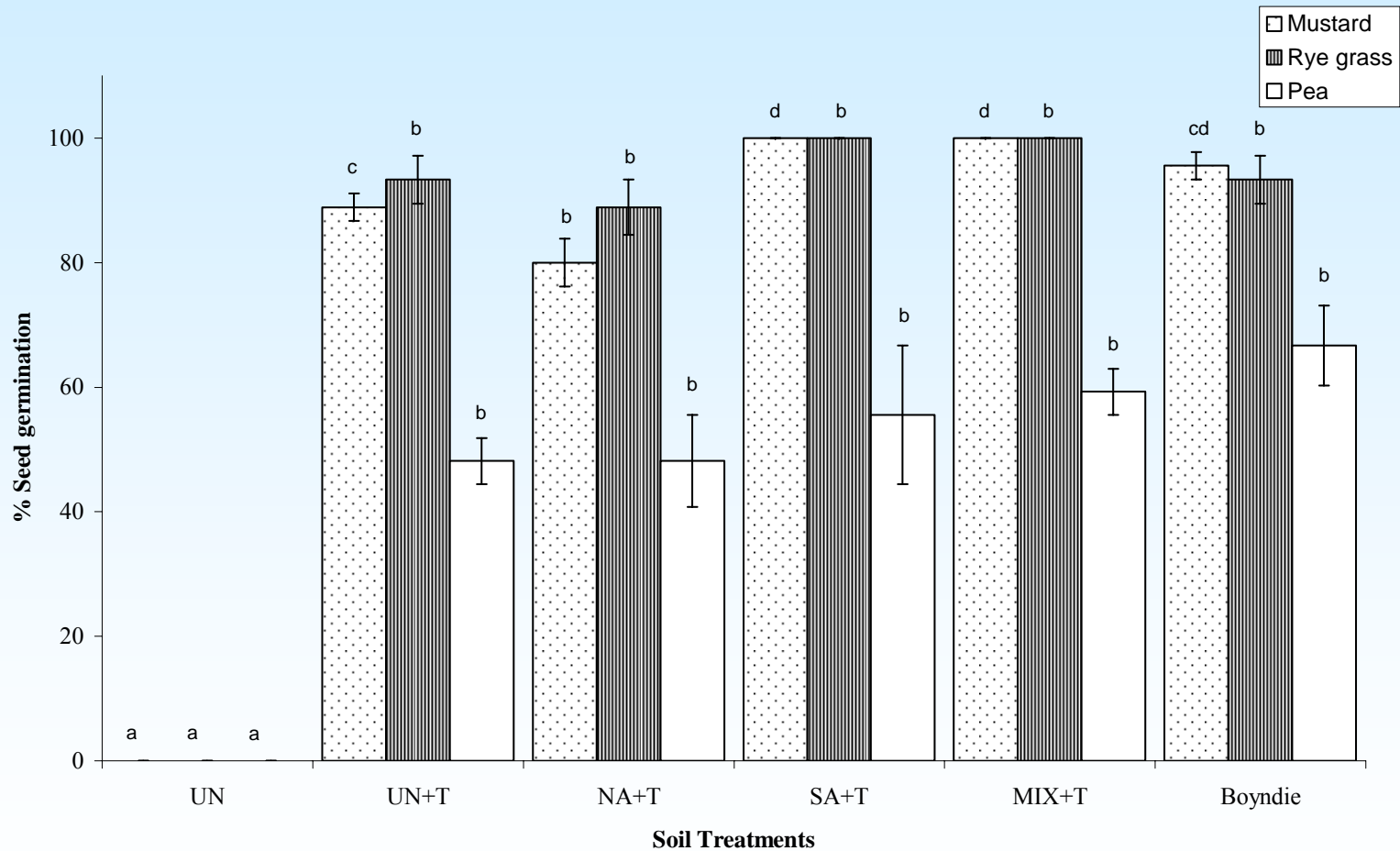
A - *E. fetida*

B - *L. terrestris*



Condition Index – Langdon *et al.*, 1999

Plants



Soil Quality Index

From results of the bioassays, there is a need to integrate the findings to quantify and rank the effectiveness of remediation treatments, the biological indicator data were transformed into a **Soil Quality Index**.

Suitability of individual assays:

Sensitivity was the ability of an indicator to determine significant adverse end-points between control and contaminated soils. **Robustness** reflected consistency of an end-point in the control soil replicates.

Andrews et al. 2002; Broos et al. 2005

Sensitivity and Robustness
values to determine
suitability

Pass Criteria:

Sensitivity >1 or
Robustness >20

(5 % coefficient of variation).

Biological Indicator	Sensitivity	Robustness	Use in Soil Quality Index
*Dichloromethane extraction	2	2.2	
*Methanol extraction	5	0.6	
Microbial Biomass Carbon	2	12.5	✓
Basal Respiration	5	4.5	✓
SIR Glucose	0	2.0	x
SIR Glutamine	5	120.8	✓
SIR Maleic Acid	5	3.1	✓
SIR Mannose	0	4.3	x
SIR Phenol	1	19.0	x
Dehydrogenase activity	1	23.4	✓
Arylphosphatase activity	1	3.9	x
Potential Nitrification Rate	1	2.2	x
<i>P. fluorescens</i> biosensor	0	25.1	x
<i>E. coli</i> biosensor	0	38.2	x
<i>E. fetida</i> CI - Day 14	4	nd	✓
<i>E. fetida</i> weight change	1	0.2	x
<i>L. terrestris</i> CI - Day 14	5	14.4	✓
<i>L. terrestris</i> weight change	4	3.5	✓
Mustard seed germination	2	24.8	✓
Rye Grass seed germination	1	14.0	x
Pea seed germination	1	6.8	x

Using only biological indicators that passed

Within each bioassay, soil treatment observations were proportionally scored to 1.0 depending if values were '+ve' or '-ve' in terms of ecological health.

The scores for each soil treatment were combined from the **9** bioassays used and a mean value taken to obtain an overall ecological health

Soil Rankings

Soil	Soil Quality Index	Ecological Health
UN	0.172 ± 0.07	6 ^c
UN+T	0.474 ± 0.09	5 ^{bc}
NA+T	0.484 ± 0.08	4 ^{bc}
SA+T	0.703 ± 0.10	2 ^{ab}
MIX+T	0.578 ± 0.09	3 ^{ab}
Boyndie	0.925 ± 0.05	1 ^a

Differences in ecological health of the contaminated soils can be attributed to the relative efficiency of each remediation treatment

Conclusions

- The complexity of the biological indicators and the lack of collectively defined end-points were overcome by transforming data into a quantitative index of soil health.
- Results correlated with the H-C data derived from chemical analysis, indicating that the biological indicators chosen for the index accurately represent these soils.
- SA+T and MIX+T remediation treatments were most successful.
- The choice of particular biological indicators is a function of soil characteristics, extent and type of contaminant and the remedial strategy adopted.
- This work shows the potential of biological indicators in hazard assessment and in the development of remediation clean-up criteria.

Acknowledgments

An aerial photograph of a port city, likely Aberdeen, showing a large harbor filled with numerous ships and industrial buildings. A river flows through the city, and the surrounding area is densely packed with urban and industrial structures.

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