Terrestrial Toxicity Testing in Support of Site-specific Risk Assessments:

An Integrative Tool for Contaminated Site Management

Gladys Stephenson[1], **Natalie Feisthauer[1]**, Kathryn Bessie[2] and Rick Scroggins[3]

[1]Stantec Consulting, Guelph, ON
[2]EBA Engineering Consultants, Calgary, AB
[3]Environment Canada, Ottawa, ON

Contaminated Sites: A Priority

- Contaminated sites are hazardous to human health and the environment
- Risks and liabilities associated with contamination not acceptable
- Land values have increased: value of land > costs of remediation
- Loss of useable space (e.g., land) can no longer be tolerated
- Governments have an expectation of responsible corporate citizenship and stewardship

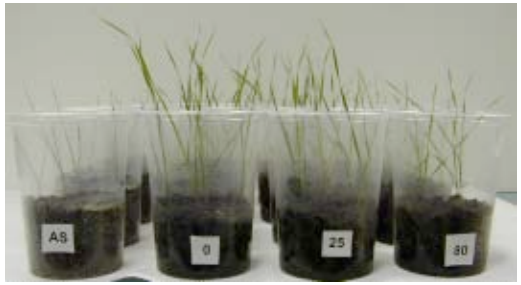
- Recognition of this priority by all levels of government has resulted in legislation mandating assessment, remediation and management of contaminated land

Risk Assessment of Contaminated Land

- Assesses the risk of contaminants to humans and ecological receptors by evaluating:
- Exposure of the receptor to the contaminant
- Toxicity of the contaminant to the receptor
  - Toxicity is the potential of a substance to cause adverse effects to living organisms

- Effective strategy for management of contaminated land
- Relies on scientific knowledge and information
- Frequently used to guide management decisions

Ecotoxicity Testing



- **Toxicity tests are tools to determine the toxicity of a substance to organisms under defined exposure conditions**

Site-specific toxicity testing of soils conducted to:

1. Assess soil quality
2. Generate data for derivation of soil quality standards
3. Measure the efficacy of remediation technologies
4. Assess relative toxicity of contaminated soil to organisms directly through the soil contact exposure pathway
5. Determine bioaccumulation, bioaccessibility, bioconcentration and potential for trophic transfer of soil contaminants

Site-specific Ecotoxicity Testing

- **One line of evidence for higher tier risk assessments conducted using a weight-of-evidence approach**
  - (e.g., toxicity tests conducted in combination with soil chemistry data and site surveys)

- **Satisfy regulatory requirements for site closure**
  - Alberta Environment Tier 2 remediation guideline development for sites contaminated with petroleum hydrocarbons (PHCs) using three options:
  - Modify generic Tier 1 values based on site conditions
  - Eliminate exposure pathways
  - Conduct a prescribed set of toxicity tests to demonstrate that the soil is not toxic to ecological receptors through the soil contact exposure pathway (e.g., plants, soil invertebrates)

Application of Terrestrial Toxicity Tests in Contaminated Land Assessment

Terrestrial Toxicity Test Results Used to:

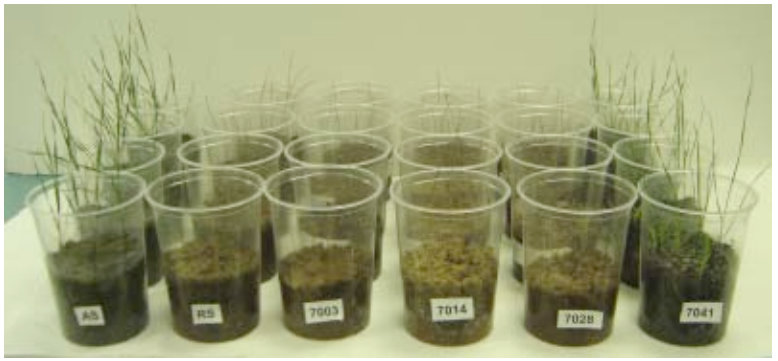
- **Derive national and provincial soil quality criteria**
  - Petroleum hydrocarbons, BTEX, barium, glycols, methanol
- **Characterize the magnitude and extent of soil toxicity to ecological receptors**
  - Quantify concentrations of contaminants toxic to soil organisms
  - Ecological receptors represented by a suite of test organisms (plant and invertebrate) following acute and chronic exposure
- **Establish site-specific, risk-based clean-up standards**
  - Identify concentrations of contaminants not toxic to soil organisms
- **Assess the efficacy of remediation technologies**
  - Confirm that soil is no longer toxic to soil organisms

Overview of Environment Canada Soil Test Methods

- Environment Canada (EC) has standardized a number of terrestrial toxicity test methods (acute and chronic) that can be used to assess contaminated soils
- EC is currently expanding test methods to include soils from other major Canadian ecozones (e.g., boreal forest, taiga, tundra, wetlands) so standardized tests will be available to assess contaminated lands across Canada using ecologically relevant test systems
- New methods will also include new species that are relevant to these ecozones and include mites, herbaceous and woody plants, wetland plants, and earthworms

Plant Test Method – EPS 1/RM/45 (2005)

- 12 test species: alfalfa, barley, blue gramma grass, carrot, cucumber, durum wheat, lettuce, northern wheatgrass, radish, red clover, red fescue and tomato
- 14- or 21-day test
- 5 replicates per concentration (single concentration tests)
- 500 mL soil per test unit
- 5-10 seeds
- Emergence, shoot and root length, wet (optional) and dry mass
- Plant performance in contaminated soil vs. that in a control soil
- Control soil can be field-collected reference soil or artificial soil
- Multi-concentration tests use regression design to generate point estimates of toxicity (e.g., ICp, ECx)



Earthworm Test Method – EPS 1/RM/43 (2004)

- **3 test species:**

Eisenia andrei

Eisenia fetida

Lumbricus terrestris

- Acute lethality test (14-d) - *Eisenia* sp. and *L. terrestris*
- Acute avoidance test (48-h) – *Eisenia* sp. and *L. terrestris*
- Chronic reproduction test (56- or 63-d) – *Eisenia* sp. only

Earthworm Test Method – EPS 1/RM/43 (2004)

Acute Lethality Test

- 350 mL soil/test unit
- 3 to 5 individuals/test unit
- 3 to 5 replicates
- Survival D 7 (optional)
- **Survival D 14**
- Compared to survival in control soil (single conc.)
- LC50 if multi-conc. test

Chronic Reproduction Test

- 350 mL soil/test unit
- 2 mature adults/test unit
- 10 replicates
- Remove adults D 28 or 35
- **Adult survival D 28 or 35**
- **No. progeny, progeny wet and dry mass D 56 or 63**
- Compare reproduction to control soil (single conc.)
- ICp if multi-conc. test

Earthworm Test Method – EPS 1/RM/43 (2004)

Acute Sublethal Avoidance Test

- Evaluates the avoidance behaviour of earthworms in the presence of contaminated and clean (reference) soil
- Ten earthworms/test unit
- 300 mL per compartment (900 mL per test unit)
- 48-h *Eisenia* sp., 72-h *L. terrestris*
- % avoidance of contaminated soil
- Multi-concentration test data can be used to generate ECx by regression analyses

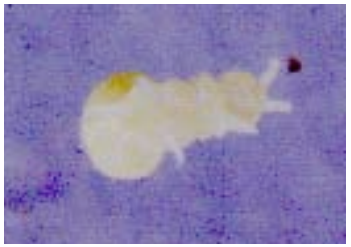
Collembola Method – EPS 1/RM/47 (2007)

Chronic Reproduction Test

- 3 species

Folsomia fimetaria

Folsomia candida



Orthonychiurus folsomi

- 21-d for *F. fimetaria*, 28-d *O. folsomi* and *F. candida*
- 30 mL soil/test unit
- 20 adults (*F. fimetaria*), 15 adults (*O. folsomi*), 10 subadults (*F. candida*)
- 5 replicates (control) and 3 replicates (site soil treatments)
- 21- or 28-d adult survival and number of progeny produced
- Compared to control if single concentration test
- LCx and ICp generated through regression if multi-concentration test

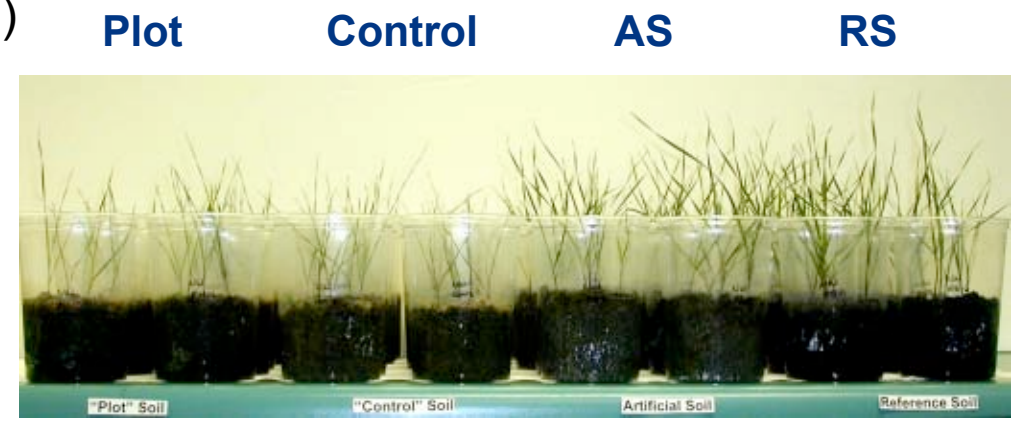
Case Study 1 - Introduction

- Preliminary quantitative risk assessment was conducted on biotreated surface soils from a site at a former land treatment facility[1]
- Barium, salinity and PHCs (Fraction 3, C_{16}-C_{34}) in exceedance
- Ecotoxicity data needed to:
  - derive site-specific, risk-based remediation objectives
  - determine risk to ecological receptors via soil contact exposure pathway
- Ecotoxicity tests conducted and used as one of five lines of evidence in a weight-of-evidence approach
- Uncertainty analysis conducted to assess degree of confidence in characterization of risk and to determine if detailed quantitative risk assessment was required

[1]Bullinger, A, Buckles, S, Bessie, K, Stephenson, G and Feisthauer, N. 2002. A risk-based approach to closure of a former land treatment facility, pp 100-105. In: Proceedings form the 39th Annual Alberta Soil Science Workshop, Edmonton, AB, February 19-20, 2002.

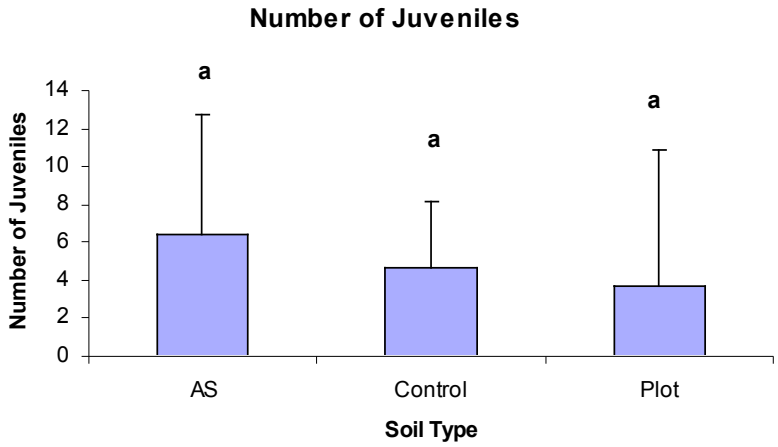
Case Study 1 – Materials and Methods

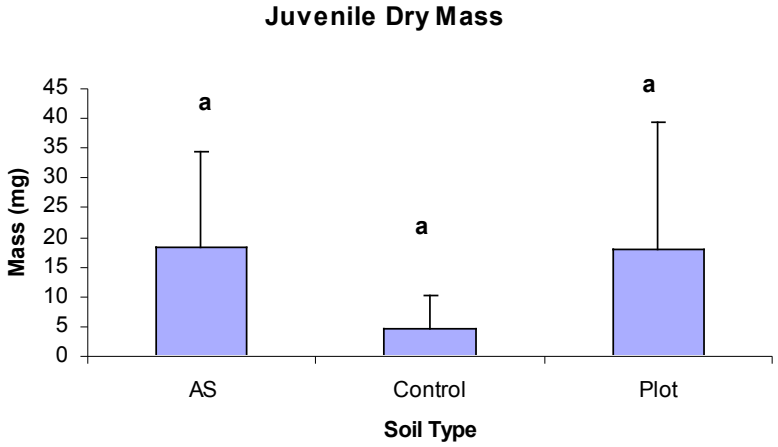
- EC (draft) methods were used to conduct tests
- Plants - barley, red clover, northern wheatgrass
  - Acute (7-d) and definitive (14-d barley, 21- d RC, NW) tests
- Earthworm - *Eisenia andrei*
  - Acute lethality (14-d) and chronic reproduction (56 and 63-d) tests
- Experimental Design (4 treatments)
  - **QA/QC treatments**
    - AS (artificial soil)
    - RS (generic reference soil)
  - **Control** (site-specific reference soil)
  - **Plot** (biotreated site soil)

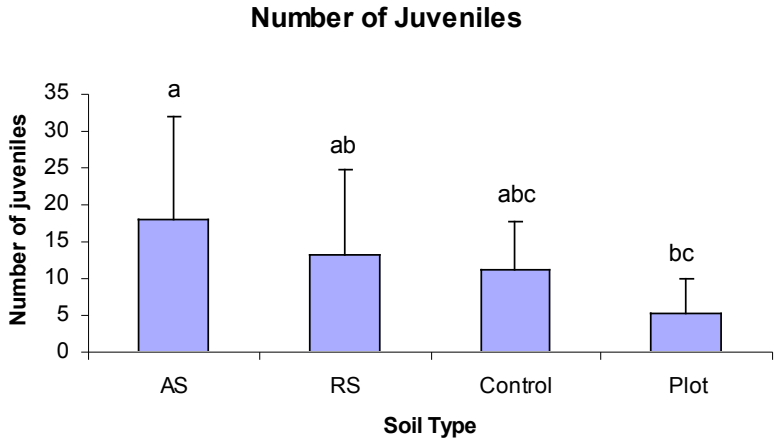


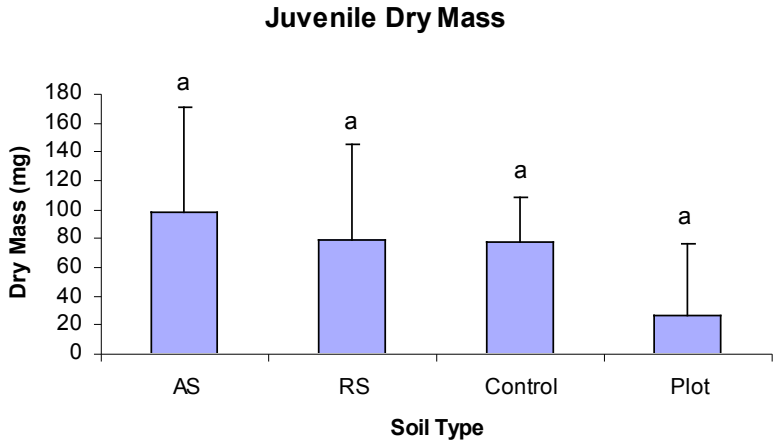
Case Study 1 – Earthworm Results

- No acute or chronic adult mortality
- No adverse effect on reproduction (56- or 63-d test)



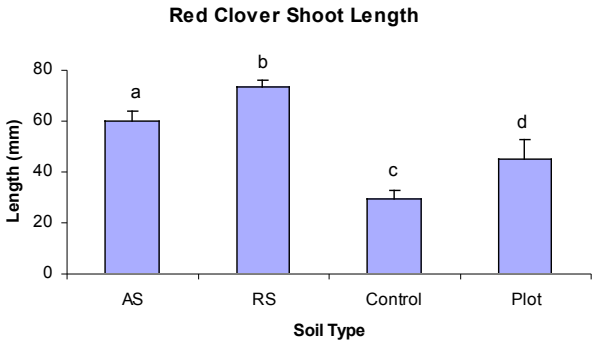


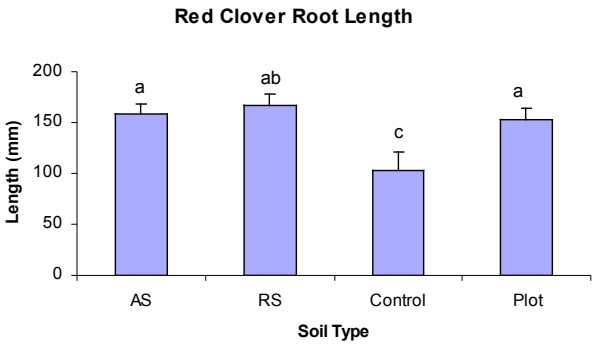


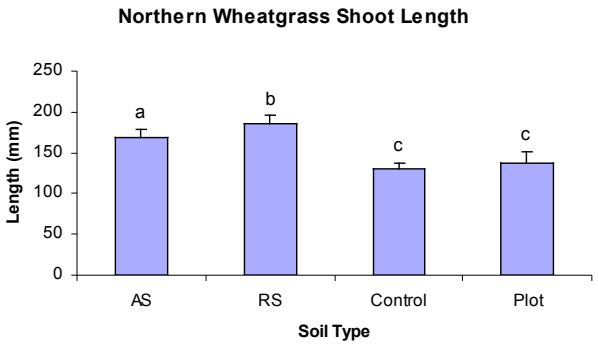


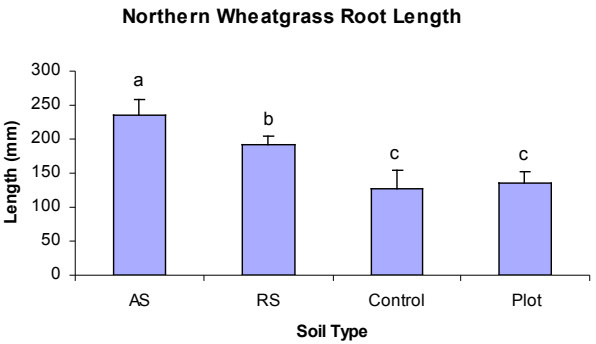
Case Study 1 – Plant Results

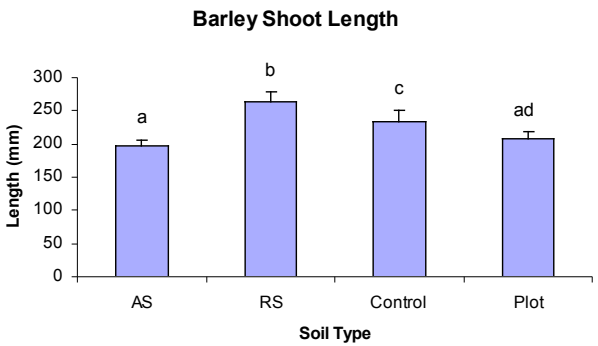
- NW and RC: no effect on acute or chronic emergence or growth
- Barley chronic emergence and both acute and chronic growth affected

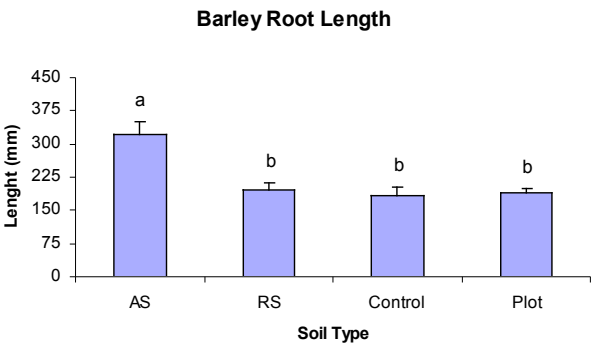












Case Study 1 - Discussion

- Toxicity testing program generated a large number of endpoints
  - 42 plant endpoints and 14 earthworm endpoints
  - Some endpoints autocorrelated (e.g., wet and dry mass)
- Statistically analyzed data and evaluated uncertainty
- No observable adverse effects on acute or chronic survival or reproduction following exposure to earthworms to biotreated soil
- No toxicity to red clover or northern wheatgrass
- Barley was adversely affected
  - Site-specific reference and biotreated soil suboptimal for growth
  - Inconsistency among endpoints
- Barley results indicate that the physicochemical properties of the soils influenced the test results

Case Study 1 – Conclusion

- Toxicity test results integrated with the other lines of evidence:
  - Literature data
  - Soil leachate test data
  - Total and available contaminant concentrations
  - Derivation of human health soil quality objective for barium

- The integrated data revealed that the highest potential for adverse effects was through direct soil contact; however,
- There was a high degree of confidence that site soil was not toxic to receptors, and that it represented a negligible risk to potential receptors at the site

Case Study 2 - Introduction

- An assessment was conducted on a site contaminated with petroleum hydrocarbons
- The topsoil at the site had undergone bioremediation; however, residual PHC Fraction 3 levels still exceeded Tier 1 levels for coarse soil (550 mg/kg)
- The site was a natural area with a grazing lease; in order to re-spread the bioremediated topsoil, a Pass was required under the new Alberta Environment (AE) Tier 2 Pass/Fail Option for PHC-contaminated soils
- The draft (2007) Tier 2 Eco-contact Guideline Derivation Protocol[2] for PHC-contaminated soil provides two options:
  - Tier 2 Pass/Fail Option (must be used for regulatory closure)
  - Tier 2 Provisional Site-specific Risk-based Objective (SSRO)
- Both options require site-specific ecotoxicity testing

[2]Alberta Environment. 2007. Tier 2 Eco-contact Guideline Derivation Protocol. July 13 2007 Draft. ISBN: 978-0-7785-6753-0

Case Study 2 – Materials and Methods

- Environment Canada test methods are required for AE Tier 2 testing
- Minimum test battery requirements were met and included:
- 1 earthworm, 1 soil arthropod reproduction tests
  - ***Eisenia andrei*** reproduction test (63-d)
  - *Folsomia candida* reproduction test (28-d)
- 2 definitive plant tests
  - Alfalfa (21-d), **northern wheatgrass** (21-d)
- Experimental Design
  - **QA/QC treatment** (Artificial soil)
  - **Background Soil** (Field-collected reference soil)
  - **Impacted Soil** (Bioremediated site soil)

Case Study 2 – Pass/Fail Results

A Tier 2 Pass for natural areas must meet the following criteria:

- When statistically significant differences are identified between reference and contaminated soils, the differences must be ≤ 25% for at least 75% of the endpoints
- Test organism mortality must be no greater in the contaminated soil than in the site soil
- Invertebrate reproduction in the contaminated soil must not be less than 50% of that in the reference soil
- No more than one endpoint per test species may exceed a 25% difference between site and reference soil
- the experimental design must have adequate power to detect a difference of 25% or more between treatments

Case Study 2 – Pass/Fail Results

Plant Test Endpoints	Percent Difference from Reference Control Soil		Invertebrate Test Endpoints	Percent Difference from Reference Control Soil	
	Alfalfa	Northern Wheatgrass		*Eisenia andrei*	*Folsomia candida*
Emergence	-5	0	Adult Survival	10	-25
Shoot Length	-10	-4	# Progeny Produced	58	-66
Root Length	16	24	Progeny Wet Mass	17	NA
Shoot Dry Mass	-8	2	Progeny Dry Mass	17	NA
Root Dry Mass	11	41			

Total Number of Endpoints	16
Number of Endpoints Adversely Affected	2
Percent of Endpoints Adversely Affected	13%

Case Study 2 – Discussion

All Tier 2 Pass criteria were met for all the criteria:

- ✓ When statistically significant differences are identified between reference and contaminated soils, the differences must be ≤ 25% for at least 75% of the endpoints
- ✓ Test organism mortality must be no greater in the contaminated soil than in the site soil
- ✓ No more than one endpoint per test species may exceed a 25% difference between site and reference soil
- ✓ the experimental design must have adequate power to detect a difference of 25% or more between treatments

Except…

Case Study 2 – Discussion/Conclusion

- **Invertebrate reproduction in the contaminated soil must not be less than 50% of that in the reference soil**
- Earthworm reproduction was 42% of control
- However, the organic matter content in the impacted soils was more than 2.5x less than that in the reference soil, and was below the optimal threshold of 3-4%
- Earthworm reproduction is sensitive to organic matter content

- The low organic matter content in the impacted soil relative to the reference soil likely adversely affected earthworm reproduction independently of, or in combination with, the effect of the PHCs.
- In consideration with other ancillary information, the toxicity data from this Tier 2 site-specific ecotoxicity testing contributed to the regulatory closure for this site.

Acknowledgements

The authors would like to thank Gemma Leighton-Boyce,
from WorleyParsons Komex, Calgary, Alberta,
for permission to present the Case Study 2 data