ECOTOXICOLOGICAL AND ECOLOGICAL HAZARD SCREENING OF Zn CONTAMINATED ACIDIC SOIL

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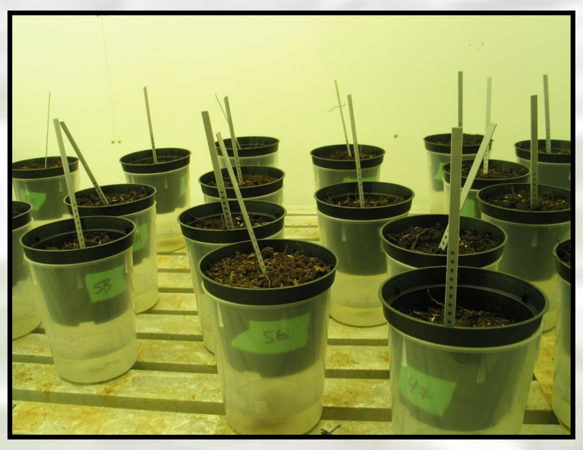
Introduction

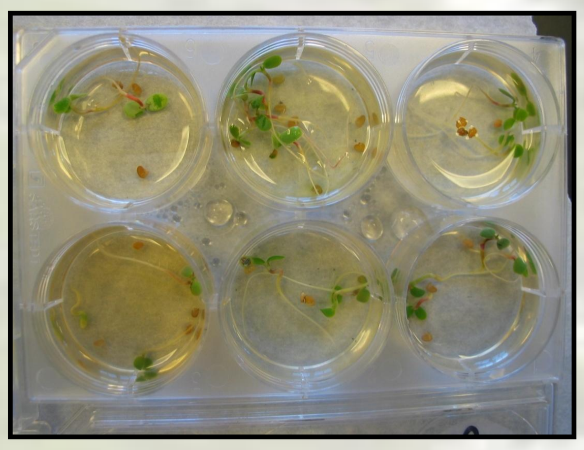
- Most standardized bioassays require extensive handling of the soil such as sieving, homogenization, pH alterations, and drying, which can lead to changes in the bioavailability of metals in the soil and thus the hazard.
- Ideally, the soil samples used for hazard screening assessment should be kept as intact soil cores collected from the field to minimize changes in metal speciation. Tests and organisms that are sensitive to metal contamination but can withstand, for example, naturally acidic soil needs to be evaluated. These organisms should preferably also be ecologically relevant.

Objectives

- To assess the sensitivity of a few selected bioassays and ecological screening tools in naturally acidic Zn spiked soil cores
- To assess which of these methods could be routinely used to aid in the hazard screening process of naturally acidic metal contaminated soil samples.

Materials and Methods





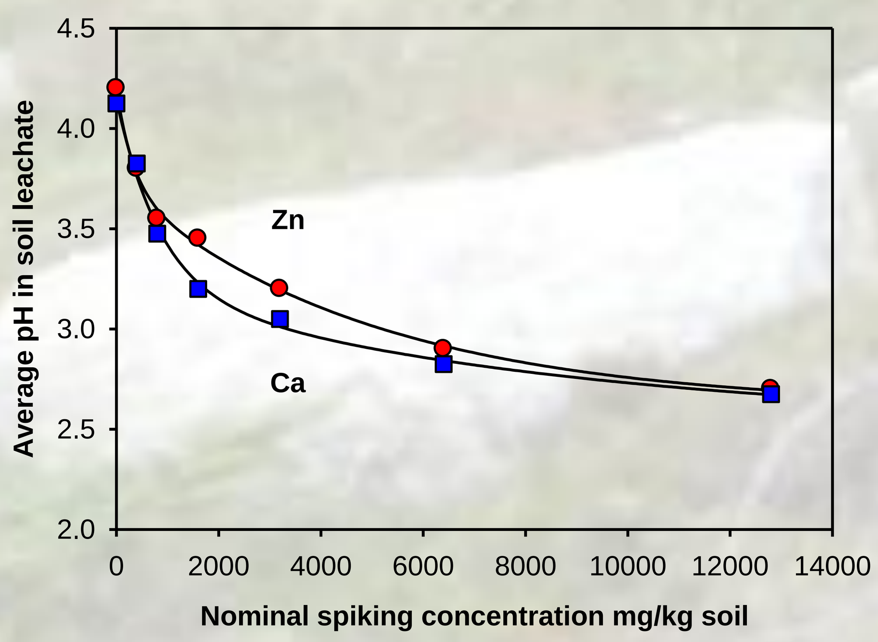
- Uncontaminated acidic (pH 4) soil samples were collected from the field. Soil samples were chosen so that they would be as similar as possible in terms of pH and soil type.
- The soil was transferred to pots and spiked to saturation aiming for 0, 100, 200, 400, 800, 1600, 3200, 6400, 12800 mg Zn/kg dw soil. Three replicates were used for each concentration. A similar molar series (with the exception of 100 and 200 mg/kg dw) was completed with Ca as a non-toxic reference.
- Transparent plastic beakers were placed underneath each pot to collect porewater draining from the pots
- Total metal analysis: aqua regia digestion of each soil sample followed by measurement by ICP-MS. Results were compared with CCME environmental soil quality guidelines.
- Total metal concentrations in soil leachate (ICP-MS) from pots. Results were compared with CCME freshwater quality guidelines for protection of aquatic life .

Table 1. Summary of tests completed in the undisturbed soil cores or pore water.

Test/organism	Duration of test
Test medium: Soil core	
Growth (root and shoot length) and emergence of red clover (*Trifolium pratense L.*), red fescue (*Festuca rubra L.*) and lettuce (*Lactuca sativa L.*)	4 weeks
MetSTICK, enzyme (beta-galactosidase) activity in *E. coli*.	4 hours
Bait lamina (soil microbial and soil invertebrate activity).	2 weeks
Test medium: Leachate from soil core	
Root growth of lettuce and clover	1 week (4 tests over 4 weeks)
Microtox, acute luminescent bacteria test (*Vibrio Fischeri*)	5 minutes, 15 minutes (pH adjusted and not-pH adjusted)
Mobility of *Hyallella azteca*	96 hours (pH adjusted)

Results

Figure 1. Average pH (week 1-4) in leachate from $ZnCl_2$ and $CaCl_2$ spiked soil samples. pH was significantly correlated (Spearman two-tailed R; 0.99, $p < 0.05$; N = 7) between the $ZnCl_2$ and $CaCl_2$ spiked soil samples every week. Nominal spiking concentrations with significantly different pH have been noted in the table below for each week.



Week	Sig. lower pH in Ca conc. in comparison with equivalent Zn conc. P < 0.05
1	1600
2	800, 1600
3	3200 - 12800
4	1600, 3200

Results (continued)

Table 2. Summary of results for bioassays and microorganism activity tests. W=week, ns=not sig., n/a=not analyzed due to lack of data.

Test/organism	Spiked Zn conc. sig. different from controls in test?	NOEC mg Zn /kg d.w. soil or µg/L leachate). Values in parenthesis (nominal column), represent Ca spiked samples.		Corr. with Zn conc. (R, N=27, P<0.05) in soil or leachate	Corr. with Ca conc. (R, N=21, P<0.05) in soil or leachate
		Measured	Nominal		
Plant growth test in soil, 28 days					
L. sativa shoot	N	9600	12800 (12800)	- 0.67	- 0.49
L. sativa root	N	9600	12800 (800)	- 0.72	- 0.54
T. pratense shoot	Y	490	400 (12800)	- 0.80	- 0.62
T. pratense root	Y	490	400 (1600)	- 0.86	- 0.77
F. rubra shoot	Y	5241	3200 (1600)	- 0.61	- 0.81
F. rubra root	Y	2309	1600 (3200)	- 0.63	- 0.75
Plant growth test in leachate, 7 days per test (4 weeks)					
L. sativa root	Y (W1-4)	W1: 700 W2: 13000 W3: 1000 W4: 70	W1: 400 (400) W2: 800 (400) W3: 800 (800) W4: 200 (800)	W1: - 0.95 W2: - 0.80 W3: - 0.91 W4: - 0.87	W1: - 0.91 W2: - 0.83 W3: - 0.91 W4: - 0.94
T. pratense root	Y (W1-4)	W1: 700 W2: 13000 W3: 6000 W4: 2400	W1: 400 (800) W2: 800 (800) W3: 1600 (6400) W4: 1600 (3200)	W1: - 0.92 W2: - 0.74 W3: - 0.63 W4: - 0.84	W1: - 0.76 W2: - 0.89 W3: - 0.58 W4: - 0.74
Invertebrate survival test in soil leachate					
H. azteca survival	Y (W1-4)	W1: 200 W2: 600 W3: 200 W4: 400	W1: 200 (12800) W2: 400 (12800) W3: 400 (12800) W4: 800 (12800)	W1: - 0.82 W2: - 0.85 W3: - 0.89 W4: - 0.88	W1-4: ns
Microorganism toxicity tests, Microtox in soil leachate and MetSTICK in soil					
Microtox (not pH adjusted W4, 5 min)	Y	400	800 (1600)	- 0.78	- 0.83
Microtox (not pH adjusted W4, 15 min)	Y	400	800 (1600)	- 0.85	- 0.82
Microtox (pH adjusted W2-4, 5 min)	Y (W2-4)	W2: 300 W3: 6000 W4: 400	W2: 200 (12800) W3: 1600 (12800) W4: 800 (12800)	W2: - 0.78 W3: - 0.61 W4: - 0.70	W2-4: ns
Microtox (pH adjusted W2-4, 15 min)	Y (W2-4)	W2: 80 W3: 1000 W4: 400	W2: 100 (12800) W3: 800 (12800) W4: 800 (12800)	W2: - 0.87 W3: - 0.77 W4: - 0.81	W2-4: ns
MetSTICK	Y	266	200 (12800)	- 0.40	ns
Soil invertebrate and microorganism activity test, 14 days					
Bait lamina	N	n/a	n/a	- 0.61	-0.61

Table 3. Potential factors influencing the tests other than Zn or pH. (W=week)

Test organism	W	Sig. higher conc. in Zn spike at LOEC, P < 0.05	Sig. higher conc. in Ca spike at LOEC, P < 0.05
V. fisheri (5 min not pH adjusted)	4	Cu*	Cd*
V. fisheri (15 min not pH adjusted	4	Cu*	Cd*
L. sativa (root length in leachate)	2	Cr*	Cd*
	3	---	Cd*
	4	---	Cd*
T. pratense (root length in leachate)	2	Cr*	Cd*
	3	---	Cd
	4	---	Cd

*Sig. correlation of contaminant concentration with test results, Spearman two-tailed R; $p < 0.05$; N = 27 (Zn) and N = 21 (Ca).

Conclusions

- Of the tests completed directly in the soil core, the MetSTICK test appears to be the most sensitive to Zn, with the lowest measured and nominal NOEC. The most sensitive plant growth test for Zn in the soil core was root and shoot growth of *T. pratense* (red clover). In addition, the MetSTICK test as well as the growth test with red clover appeared to be tolerant of low pH, which make these two tests ideal for assessing hazards of undisturbed metal contaminated acid soils.
- The growth tests with *L. sativa* (lettuce) and *F. rubra* (red fescue) in the soil cores appeared to be relatively insensitive to Zn in the soil and more sensitive to low pH. The high sensitivity to low pH makes these species a poor choice in evaluating hazards of undisturbed metal contaminated acid soils.
- The bait lamina test completed to assess the activity of invertebrates and microorganisms in the soil appeared to be more sensitive to the pH of the soil than to Zn concentrations. However, enough data could not be collected during this study to confirm NOEC and LOEC numbers for this test.
- The Microtox test and the test with *H. azteca* in soil leachate were sensitive to low pH and required pH adjustments before the tests. Bioavailability of the metals may have changed following pH adjustments. Hazard assessments based on results from these tests should take this potential effect into account.
- Of the plant growth tests (root) completed in soil leachate, *T. pratense* appeared more sensitive to Zn concentrations than to low pH. *L. sativa* appeared more sensitive to low pH and elevated Cd concentrations than to Zn concentrations in soil leachate.
- The results of this study suggests that MetSTICK in soil and plant growth tests with *T. pratense* in soil and soil leachate are well suited for hazard assessment of Zn contaminated undisturbed acid soils. Future projects should identify potential pH tolerant, metal sensitive invertebrate toxicity tests as well as further investigating the use of Bait Lamina and other ecological screening tools for the hazard assessment of metal contaminated acid soils.

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