

## Supplementary material

### **Ion-exchange technique (IET) for measuring Cu<sup>2+</sup>, Ni<sup>2+</sup> and Zn<sup>2+</sup> activities in soils contaminated with metal mixtures**

*D. M. Schwertfeger<sup>A,B</sup> and W. H. Hendershot<sup>A,C</sup>*

<sup>A</sup>Department of Natural Resources, McGill University, Macdonald Campus, 21111 Lakeshore Road, Sainte Anne de Bellevue, Quebec, H9X 3V9, Canada.

<sup>B</sup>Environment and Climate Change Canada, Biological Assessment and Standardisation, 335 River Road, Ottawa ON, K1A 0H3, Canada.

<sup>C</sup>Corresponding author. Email: [william.hendershot@mcgill.ca](mailto:william.hendershot@mcgill.ca)

## Test soils

Each of the three test soils (Farmington, St. Bernard, St. Zotique) were treated with four levels of single-metal additions of Cu, Ni or Zn (acetate forms), three binary metal mixture additions (Cu+Ni, Cu+Zn and Ni+Zn) and one tertiary mixture addition (Cu+Ni+Zn). Target metal concentrations for single metal treatments were meant to cover the range where we expected to see phytotoxicity based in range-finding tests conducted with *Hordeum vulgare*. EC50 estimates from the range-finding tests were used to set the target concentrations for the metal mixture treatments which were as follows:  $\frac{1}{2}$ EC50 for each metal in the binary mixtures;  $\frac{1}{3}$  EC50 for each metal in the tertiary mixture. Included were control samples, to which no metals were added. Metal acetate solutions were added to soil samples and thoroughly mixed by hand, equilibrated at room temperature for 24 h, then leached by slowly passing approximately 7.5x the soil pore volume of a weak electrolyte solution (200  $\mu$ M Ca, 200  $\mu$ M Cl, 100  $\mu$ M SO<sub>4</sub>) through a column of soil over 72 h, using a surface drip method. This amount of time was sufficient to leach the excess salts and acidity displaced from the dissolved metal additions, as indicated by stabilized leachate pH and electrical conductivity values by the end of the 3<sup>rd</sup> day. Details of the column spike/leach method are provided in Schwertfeger et al.<sup>[1]</sup> The spiked and leached soil samples were then used in a 14-d barley growth assay conducted in controlled environmental chambers following standard procedures,<sup>[2]</sup> which further aged the soils. After harvesting plants and removing roots from the soil, samples were air-dried and stored in sealed containers at room temperature for up to six months.

**Table S1. Soil Cu concentrations, soil Cu/Cu<sup>2+</sup> partition coefficients (Kd<sub>Cu</sub>) and extract pH for Cu treatments**

Test Soil	Treatment	Soil Cu (μmol/g)	Kd <sub>Cu</sub> (L/g)	Extract pH
Farmington	Cu	2.66	64	6.15
	Cu	4.82	70	6.22
	Cu	5.02	54	6.24
	Cu	8.01	46	6.07
	Cu + Ni	2.62	100	6.06
	Cu + Zn	2.34	50	5.92
	Cu + Ni + Zn	1.62	100	5.94
St. Bernard	Cu	2.23	>560	7.03
	Cu	9.96	685	6.90
	Cu	12.36	488	7.11
	Cu	23.23	293	7.05
	Cu + Ni	5.02	>1200	7.27
	Cu + Zn	5.11	985	6.90
	Cu + Ni + Zn	3.34	1347	6.77
St. Zotique	Cu	4.32	517	7.37
	Cu	9.94	402	6.94
	Cu	14.87	128	7.51
	Cu	30.07	45	7.21
	Cu + Ni	5.00	937	7.42
	Cu + Zn	4.84	340	7.24
	Cu + Ni + Zn	3.33	484	7.29

**Table S2. Soil Ni concentrations, soil Ni/Ni<sup>2+</sup> partition coefficients (K<sub>dNi</sub>) and extract pH for Ni treatments**

Test Soil	Treatment	Soil Ni ( $\mu\text{mol/g}$ )	K <sub>dNi</sub> (L/g)	Extract pH
Farmington	Ni	2.57	1.3	6.34
	Ni	3.85	0.9	6.28
	Ni	4.70	0.8	6.27
	Ni	8.65	0.8	6.41
	Cu + Ni	2.10	1.0	6.06
	Ni + Zn	1.90	1.0	6.19
	Cu + Ni + Zn	1.23	1.1	5.94
St. Bernard	Ni	10.45	3.1	7.26
	Ni	19.59	2.2	7.29
	Ni	26.88	2.2	7.16
	Ni	38.01	1.8	7.26
	Cu + Ni	11.16	2.9	7.27
	Ni + Zn	12.17	2.1	6.68
	Cu + Ni + Zn	7.80	2.6	6.77
St. Zotique	Ni	7.71	1.7	7.32
	Ni	13.43	1.5	7.23
	Ni	14.12	1.4	7.35
	Ni	24.38	1.2	7.32
	Cu + Ni	7.00	1.9	7.42
	Ni + Zn	6.55	1.5	7.29
	Cu + Ni + Zn	4.47	1.6	7.29

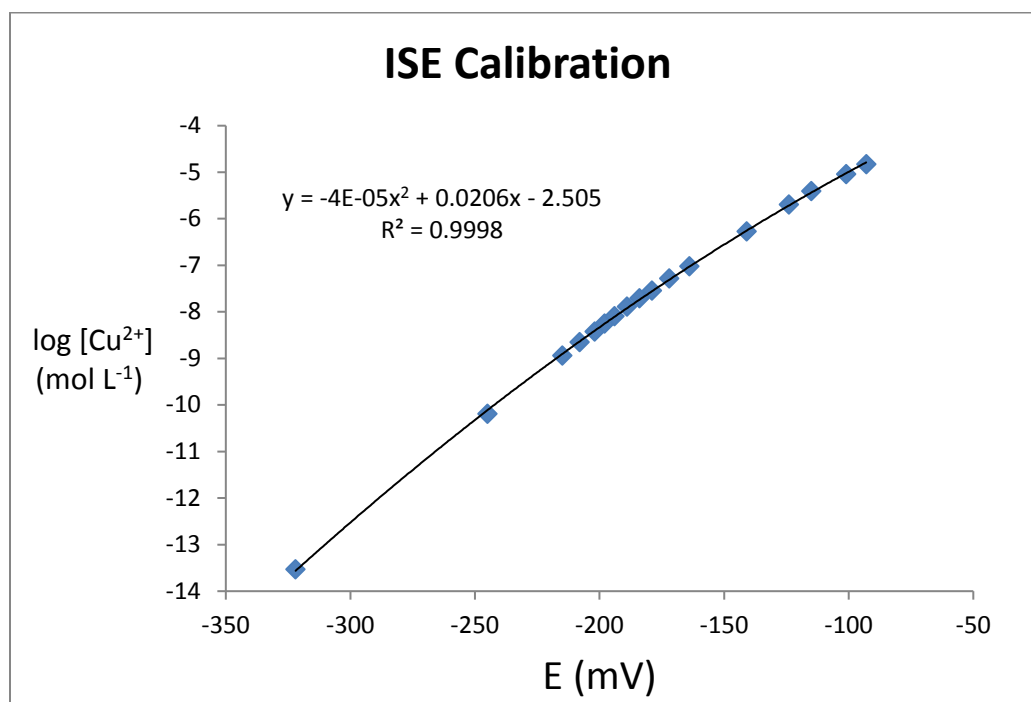
**Table S3. Soil Zn concentrations, soil Zn/Zn<sup>2+</sup> partition coefficients (Kd<sub>Zn</sub>) and extract pH for Zn treatments**

Test Soil	Treatment	Soil Zn ( $\mu\text{mol/g}$ )	Kd <sub>Zn</sub> (L/g)	Extract pH
Farmington	Zn	7.16	1.4	6.43
	Zn	13.92	0.9	5.88
	Zn	21.51	1.0	5.59
	Zn	28.67	0.7	6.04
	Cu + Zn	6.08	0.9	5.92
	Ni + Zn	6.11	1.1	6.19
	Cu + Ni + Zn	4.32	1.2	5.94
St. Bernard	Zn	32.09	3.3	6.84
	Zn	60.85	2.3	6.72
	Zn	89.06	1.9	6.61
	Zn	100.47	1.8	6.83
	Cu + Zn	41.80	2.8	6.90
	Ni + Zn	45.49	2.9	6.68
	Cu + Ni + Zn	26.67	3.5	6.77
St. Zotique	Zn	20.76	2.6	7.34
	Zn	34.69	1.2	7.19
	Zn	37.96	1.6	6.65
	Zn	54.45	0.8	7.15
	Cu + Zn	17.62	2.4	7.24
	Ni + Zn	17.47	3.0	7.29
	Cu + Ni + Zn	12.05	3.6	7.29

**Table S4. Regression relationships between concentration of dissolved organic carbon (DOC) in mg L<sup>-1</sup> (y) and absorbance at wavelength 254 nm (x) determined on subsets of extracts taken from the three different test soils, which included samples to cover the range of DOC found in each soil type**

Absorbance was measured on a spectrophotometer and DOC was measured on a Shimadzu® TOC analyser

Test soil	Regression	R <sup>2</sup>	n
Farmington	y = 42.99x + 0.3148	0.9993	6
St. Bernard	y = 51.60x + 0.056	0.9996	6
St. Zotique	y = 40.89x + 0.1599	0.9995	6



**Fig. S1.** Cu Ion Selective Electrode (ISE) calibration results relating electro-potential, E (mV), measured in calibration solutions to Cu<sup>2+</sup> (mol L<sup>-1</sup>) determined in each solution using chemical speciation software, *Visual MINTEQ*.

## References

- [1] D. M. Schwertfeger, W. H. Hendershot, Spike/leach procedure to prepare soil samples for trace metal ecotoxicity testing: method development using copper. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 1570.
- [2] Environment Canada, *Biological Test Methods: Tests for Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil* **2005** (Environment Canada: Ottawa, Ontario).