Effects of metal-contaminated forest soils from the Canadian shield to terrestrial organisms.

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Abstract

The effects of elevated metal concentrations in forest soils on terrestrial organisms were investigated by determining the toxicity of soils from six sites in northern Ontario and Quebec, using a battery of terrestrial toxicity tests. These biological tests used single species, field-collected soils, and acute and chronic exposures. Soils were collected from three sites on each of two transects, established downwind of smelter emissions in Rouyn-Noranda, Quebec, and in Sudbury, Ontario. Soils were collected in June 2001 from all sites, and in October 2001 from the Sudbury sites only. The concentrations of Cu, Ni, Pb and As measured in the site soils demonstrated a gradient among the three sites within each transect (high contamination, low contamination and background-level sites). Tests with one plant, earthworm, and arthropod (collembola) species were conducted with undiluted site soils and with site soils with high metal contamination diluted with the corresponding background site soil. Undiluted Rouyn-Noranda soils were not acutely toxic to earthworms or collembola. However, plant growth was reduced in sites with background and high concentrations of metals. Adverse effects to all three species occurred in response to chronic exposure to the background and high contamination soils relative to those in the low contamination soils. The Rouyn-Noranda transect sites did not provide an acceptable toxicity gradient, and the relationship between pH, metal contamination, and soil type was ambiguous; therefore, further toxicity testing proceeded with Sudbury soils only. The Sudbury soils were not acutely toxic to earthworm or collembola, with the exception of toxicity to collembola in the background soil collected in October. Adverse effects to earthworm and collembola occurred in response to chronic exposure to the Sudbury soils. The soils collected in October from the Sudbury background and high contamination sites adversely affected the survival and reproduction of collembola. Plant toxicity was observed following both acute and definitive exposure to the Sudbury site soils. The toxic effects observed corresponded to the metal contamination gradients.

Introduction

The Metals in the Environment Research Network (MITE-RN) is a network consisting of industry, government and academia formed to address research gaps in the understanding of the sources, pathways, and impacts of metals in the environment. The research presented in this paper had been designed to compliment existing MITE-RN studies, and to ultimately provide additional data required for assessing the potential risk to terrestrial organisms associated with elevated concentrations of metals in soil.

The primary objective of the research was to determine the toxicity of metal-contaminated forest soils from three sites on each of two established transects, with known contamination gradients, to a terrestrial test battery using single-species toxicity tests. The second objective was to assess the applicability of the terrestrial toxicity test methods, developed by Environment Canada for agricultural soils, to forest soils and to identify any methodological modifications that might be required. This paper will describe the results of the first research objective only. The result of this research can be found in more detail in ESG 2002.

Pre-existing transects had been established, by other MITE researchers, to reflect a metal concentration gradient in soils from high metal contamination to low, downwind of two Canadian smelting operations located near Sudbury, Ontario, and Rouyn-Noranda, Quebec. Soils were collected from each of three sites along both transects, and were evaluated in the laboratory using Environment Canada's biological draft test methods for earthworms, arthropods and plant species. The earthworm species was *Eisenia andrei*, a species commonly used to assess soil toxicity, and one that is found in Canada as a compost worm. The arthropod

species was *Onychiurus folsomi*, an indigenous springtail or collembolan species. The plant species tested was northern wheatgrass, *Elymus lanceolatus* (previously *Agropyron dasystachyum*), a native prairie grass species that is also found in northern Ontario.

Materials and Methods

Two transects were established downwind of smelting operations in Sudbury (SUD), Ontario, and Rouyn-Noranda (RN), Quebec. For both transects, Site 1 (i.e., SUD1, RN1) was located closest to the smelting operation, and soil from this site had the highest soil metal concentrations. Site 2 (i.e., SUD2, RN2) was located further downwind of the emission source and had lower metal soil concentrations than the Site 1 soils. Site 3 (i.e., SUD3, RN3) was farthest downwind of the emission source, and was far enough away to have metal concentrations that are considered background levels. Site 3 soils were used as the reference control site soils for testing with the corresponding contaminated SUD and RN soils.

Soils were collected from Sudbury in June and October, 2001. Rouyn-Noranda soils were collected in June, 2001 only. At each site along a transect, 4 to 6 sub-sites were chosen that were considered representative of the larger site. The leaf litter was gently raked away from these sub-sites, and using a hand trowel, soil was removed from a 1-m^2 plot to a depth ranging from 5 to 10 cm. Once the soil had been removed, the displaced leaf litter was replaced over each plot within the sub-site. Upon arrival at the laboratory, soils were air-dried for 48 hours to no less than approximately 10% moisture content, homogenized, and sieved through a 6 mm mesh. Once soils were sieved, they were homogenized again thoroughly and stored in 20-L plastic buckets until use. The soils were stored at room temperature (20°C ±2°C) until use.

Two experimental control soils were also used during the course of this research. They included an artificial sandy soil (AS) and a field-collected clay loam soil (RS). The artificial soil was formulated as described in the ASTM Standard Guide E 1676-97 (ASTM, 1995) and the clay loam soil, was collected from a site in Alberta. The clay loam soil was analyzed and found to be free of contaminants. Experimental control soils provided QA/QC data on test organism health and experimental conditions.

Laboratory testing was conducted in two phases. Phase 1 testing included acute and chronic tests with treatments that comprised 100% of each site soil collected (i.e., SUD (June and October) and RN (June only) soils from Sites 1, 2 and 3). Analysis of variance procedures followed by pairwise comparison tests were used to determine statistical differences among treatments. Phase 2 testing was conducted with Sudbury soils only, and included acute and chronic dilution tests in which treatments comprised a dilution series of the most contaminated site soil (i.e., SUD1) diluted with the background level soil (i.e., SUD3). Diluted soil treatments consisted of 0 (control), 10, 20, 40, 50, 60, 80 and 100% contaminated soil. Phase 2 testing was conducted with Sudbury soils collected in both June and October. The dilution series created by diluting SUD1 with SUD3 soils was used to generate acute IC (inhibition concentration) or LC (lethal concentration) for a specified percentage effect (e.g., 50 or 20%), as well as, chronic EC (effect concentration)/IC50s and 20s or NOAECs (no observable adverse effect concentrations) and LOAECs (lowest observable adverse effect concentrations). EC/IC50s and 20s were determined by applying to the data regression procedures that used linear or non-linear regression models that had re-parameterized to include the EC/ICp and the associated 95% confidence intervals (Stephenson et al. 2000). LOAECs and NOAECs were determined by applying analysis of variance procedures to the data followed by pairwise comparison tests.

The earthworm, plant and arthropod tests were conducted according to draft Environment Canada test methods (EC 2001, EC 1998a and b). Acute and definitive plant tests were 14 and 21 days in duration, respectively, and at the end of a test emergence, shoot and root length, wet and dry mass were recorded. Acute earthworm tests were 14 days in duration with an interim 7-

d check, with survival as the measured endpoint. Chronic earthworm tests were 63 days in duration and the reproduction metrics were number of juveniles produced, juvenile wet and dry mass, and production of hatched and unhatched cocoons. Adult 35-d survival was also evaluated. Acute lethality tests were 7 days in duration for springtails, whereas chronic tests were 35 days long and evaluated adult survival, number of juveniles produced and adult fecundity.

Results and Discussion

Total metal concentrations were measured for each of the site soils collected, and a concentration gradient along each transect was established for copper, nickel, lead and arsenic (Table 1). The physical and chemical characteristics of the site soils were also analysed. The soils collected were surficial soils and the organic matter content ranged from 47 to 52% for the Rouyn-Noranda (RN) soils, and from 14 to 38% for the Sudbury (SUD) soils. Soil pH was low in all soils, and ranged from 3.6 to 5.1, and 3.8 to 4.6 for the SUD and RN soils, respectively. The textures of the Rouyn-Noranda soils were a clay loam (RN 1 and RN3) and a loam (RN2) and, for Sudbury, the soil textures were a sandy loam (SUD3), a silt loam (SUD2) and a fine sandy loam (SUD3).

Phase 1 Testing: Undiluted Rouyn-Noranda Soils

Northern wheatgrass emergence was not affected following 14 or 21 days of exposure to the RN soils. Plant growth results were similar between the acute and definitive tests, where growth was significantly decreased in RN1 and RN3 soils, compared to growth in RN2 soils (Figure 1).

Earthworm survival was not affected following 7 and 14 days of exposure to the three RN soils. Earthworm reproduction was adversely affected in all three soils compared to the experimental control soils (Figure 2). The impact was similar between the RN1 and RN3 soils, but the impact on earthworm reproduction was significantly greater in the RN2 soils relative to the other site soils.

Adult springtail survival was not affected following 7 or 35 days of exposure to the three RN soils. However, production of juveniles and adult fecundity were significantly greater in the RN2 soil compared to the RN1 and RN3 soils (Figure 3).

The toxicity of the Rouyn-Noranda site soils did not correspond with metal concentrations in soil (Table 1). Although the soil pH was low in all three site soils, it was higher in RN2 than RN1 and RN3 (4.6 vs. 3.9 and 3.8, respectively). It is possible therefore, that pH influenced the growth and survival of the test organisms.

Phase 1 Testing: Undiluted Sudbury Soils

Northern wheatgrass emergence was not affected following 14 or 21 days of exposure to the SUD soils. The plant growth results of the 14-day tests were similar to those of the 21-day tests. Plant growth was significantly greater in the SUD3 soils compared to the SUD2 and SUD1 soils (Figure 4). The response of northern wheatgrass to exposure to the Sudbury soils collected in June was the same as that for the soils collected in October.

Adult earthworm survival was not affected following 7, 14 or 35 days of exposure to the SUD soils. Reproduction, however, was significantly decreased in the SUD1 and SUD2 soils, but not in the SUD3 soils, compared to the experimental control soils. Earthworm reproduction following exposure to the June- and October-collected soils were the same, except that reproduction was greater in SUD3 soils collected in October compared to that collected in June (Figure 5).

Adult springtail survival was not affected following 7 and 35 days of exposure to the June-collected SUD soils. Springtail reproduction in the June-collected soils was significantly decreased in the SUD1 soils compared to the SUD3 soils. Adult springtail survival was dramatically affected by exposure to Sudbury soil collected in October, 2001. Adult springtail survival in the SUD1 and SUD3 soils was significantly lower than that in the two

experimental control soils, and survival in SUD3 (the background-level soil) soil was significantly lower than survival in SUD1 (i.e., most contaminated soil) soil. Reproduction was also adversely affected by exposure to the SUD3 soils collected in October compared to exposure to the SUD1 soils. Adult survival and reproduction was greater following exposure to SUD1 soils compared to that in SUD3 soils (Figure 6).

Except for springtail toxicity in October-collected SUD3 soils, the toxicity of the undiluted Sudbury site soils did correspond to metal concentrations in soil (Table 1). Although the soil pH was low in all three site soils, it was higher in SUD3 than SUD2 and SUD1 (4.9 vs. 3.6 and 4.3, respectively). Although soil pH did not follow a gradient from SUD3 to SUD1, it is possible that pH also influenced the growth and survival of the test organisms. The adverse effects of the SUD3 soils collected in October on the survival and reproduction of springtails is believed to be unrelated to the metal concentration in the soil.

Phase 2 Testing: Diluted Sudbury Soils

Northern wheatgrass emergence was not affected following 14 or 21 days of exposure in either June- or October-collected soils. Plant growth decreased following exposure to increasing proportions of SUD1 collected in June soil, and the results were similar between acute and definitive tests (Table 2). SUD1 soils collected in October were less toxic than June-collected soils to northern wheatgrass following acute exposure (Table 3). Definitive plant growth in October-collected soils did not follow a concentration-response pattern, however, and only two endpoints (shoot and root length) were amenable to regression analysis (Table 3).

Adult earthworm survival was not affected following 14 days of exposure to the diluted SUD1 soil in either the June or October-collected soil. However, chronic adult earthworm survival and reproduction decreased following exposure to increasing proportions of SUD1 soil. The chronic toxicity of the June-collected soil was slightly greater than that of the October-collected soil (Table 4).

There was no effect upon springtail adult survival following exposure to diluted SUD1 soils collected in June. Springtail reproduction was adversely affected but only when the proportion of SUD1 soil exceeded 60% (Table 5).

The background Sudbury soil (SUD3) collected in October 2001 adversely affected acute and chronic adult springtail survival. No live adults were observed following 35 days of exposure to soil treatments with low concentrations of SUD1 (e.g., 0 to 50% of SUD1). Springtail reproduction was similarly affected, and juvenile reproduction was negligible at 10, 40, 50 and 60% of SUD1. No juveniles were produced in the 0 and 20% SUD1 soil treatments (Table 5). Adult fecundity could only be measured in treatments greater than 50% SUD1. Springtail reproduction was greater in the October-collected soil compared to the June-collected soil in the 100% SUD1 treatment.

The possible cause of the toxic effect of the October-collected SUD3 soil to springtails is unknown. The soil metal concentrations were similar between the June- and October- collected soils. However, a large quantity of fungus was observed in soil samples collected in October following storage that was not observed in June-collected soils. This fungus was only noticed in the SUD3 soils. Autumn is typically a period of peak biological activity in a forest ecosystem, and most of the biological activity occurs belowground. Storage of soil at room temperature is ideal for fungal growth, so perhaps the toxicity of the October-collected SUD3 soils is a result of the presence of this fungus and/or potential toxic metabolites.

Summary

The toxicity of exposure to elevated metal concentrations in forest soils to a plant, earthworm and springtail species were assessed. Earthworms were the most sensitive of the three species tested, and springtails the least. The response of the test organisms to the Rouyn-

Noranda soils did not correspond with metal concentrations in soil, and the relationship between metal concentrations, soil pH and soil characteristics was ambiguous. The response of the test organisms to the Sudbury soils did correspond with soil metal concentrations, except for the response of springtails to the background soil collected in October. The cause of the adverse effect of the SUD3 soil collected in October to springtails is unclear. In contrast, Sudbury soils collected in October were less toxic to earthworms and plants compared to soils collected in June.

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Table 1. Concentrations (mg/kg soil d.w.) of selected metals in each site soil.

Parameter (µg/g soil d.w.)	Soil								
	SUD 1 June	SUD 2 June	SUD 3 June	SUD 1 October	SUD 2 October	SUD 3 October	RN 1 June	RN 2 June	RN 3 June
As	110	<20	<20	101	9.9	3	27	<20	<20
Cd	<2	<2	<2	6	1	0.6	16	13	5.3
Cu	640	200	38	615	294	39	750	280	98
Ni	340	180	65	435	325	45	18	14	14
Pb	120	53	34	126	85	45	750	440	170
Zn	29	35	34	28	32	57	300	360	120

SUD indicates soil sampled from Sudbury
RN indicates soil sampled from Rouyn-Noranda
1 site closest to contaminant source (most contaminated site)
2 site farther from contaminant source (less contaminated site)
3 uncontaminated site (site far enough from contaminant source to have background contaminant concentrations)
June indicates that the soils were collected in June, 2001
October indicates that the soils were collected in October, 2001

Table 2. Summary of acute and definitive IC50s and IC20s (% of SUD 1) for two endpoints for northern wheatgrass grown in the most contaminated Sudbury site soil (SUD 1) diluted with the Sudbury background site soil (SUD 3) collected in June 2001. Numbers in brackets indicate upper and lower confidence limits.

Endpoint		EC50 (% of SUD1)	EC20 (% of SUD1)
Acute			
	Shoot Length	93.54 (89.12 – 97.96)	73.78 (67.37 – 80.19)
	Root Length	70.23 (65.80 – 74.66)	56.17 (49.49 – 62.85)
Definitive			
	Shoot Length	90.67 (82.41 – 98.94)	56.62 (47.01 – 66.23)
	Root Length	64.41 (60.26 – 68.56)	48.84 (43.19 – 54.49)

LCL Lower 95% Confidence Limit
UCL Upper 95% Confidence Limit

Table 3. Summary of acute and definitive IC50s and IC20s (% of SUD 1) for six endpoints for northern wheatgrass grown in the most contaminated Sudbury site soil (SUD 1) diluted with the Sudbury background site soil (SUD 3) collected in October 2001. Numbers in brackets indicate upper and lower confidence limits.

Endpoint		EC50 (% of SUD1)	EC20 (% of SUD1)
Acute			
	Shoot Length	222.80 (96.82 – 348.79)	35.36 (13.27 – 57.46)
	Root Length	91.36 (88.21 – 94.52)	80.73 (75.42 – 86.05)
Definitive			
	Shoot Length	96.52 (87.95 – 105.09)	66.86 (56.51 – 77.21)
	Root Length	93.41 (87.34 – 99.48)	85.44 (79.84 – 91.04)

LCL Lower 95% Confidence Limit
UCL Upper 95% Confidence Limit

Table 4. Summary of EC50s and EC20s (% of SUD 1) and NOAECs and LOAECs for *E. andrei* reproduction in the most contaminated Sudbury site soil (SUD 1) diluted with the Sudbury background site soil (SUD 3) collected in June and October 2001. Numbers in brackets indicate upper and lower confidence limits.

Endpoint	EC50 (% of SUD1)	EC20 (% of SUD1)	NOAEC (% of SUD1)	LOAEC (% of SUD1)
June Soils				
No. Juveniles	9.85 (2.78 – 16.92)	3.97 (0.00 – 9.32)	40	50
Juvenile Wet Mass	20.04 (6.67 – 33.40)	6.77 (0.00 – 15.38)	10	20
Juvenile Dry Mass	22.03 (6.88 – 37.18)	7.82 (0.00 – 17.75)	20	40
October Soils				
No. Juveniles	26.49 (20.71 – 32.28)	14.96 (8.83 – 21.09)	20	40
Juvenile Wet Mass	24.23 (15.88 – 32.59)	3.67 (0.00 – 15.10)	0	10
Juvenile Dry Mass	23.59 (16.10 – 31.09)	2.29 (0.00 – 13.94)	0	10

LCL Lower 95% Confidence Limit
UCL Upper 95% Confidence Limit

Table 5. *O. folsomi* reproduction following exposure to the most contaminated Sudbury soil (SUD1) diluted with the Sudbury soil at background metal concentrations (SUD3) collected in June and October, 2001. Values in brackets indicate one standard deviation of the mean.

Soil Treatment (% of SUD1)	Number of Juveniles	Adult Survival	Adult Fecundity
June Samples			
0	88.60 (77.68)	8.20 (3.29)	10.11(7.95)
10	137.70 (59.01)	8.80 (1.23)	15.94 (7.10)
20	108.60 (72.93)	9.70 (0.67)	27.83 (55.00)
40	87.00 (66.93)	9.00 (1.05)	9.70 (7.35)
50	92.10 (70.18)	8.90 (1.29)	10.52 (7.76)
60	124.33 (77.23)	8.00 (1.73)	15.04 (7.93)
80	72.30 (74.67)	8.70 (1.42)	7.41 7.35)
100	5.60 (9.00)	7.20 (2.53)	0.67 (0.95)
October Samples			
0	0	0	0
10	0.10 (0.32)	0	0
20	0	0	0
40	0.25 (0.71)	0	0
50	0.10 (0.32)	0	0
60	0.60 (1.35)	1.20 (2.39)	1.43 (2.23)
80	62.80 (65.38)	4.80 (3.79)	12.21 (8.35)
100	79.50 (60.86)	6.50 (2.37)	15.12 (14.93)

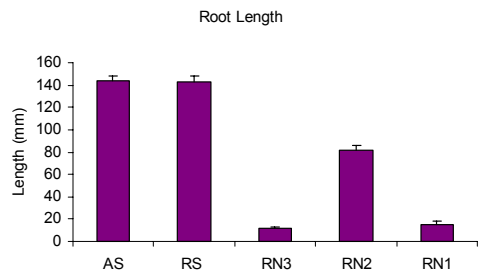

Figure 1. Northern wheatgrass root length following 21 days of exposure to Rouyn-Noranda site soils. AS and RS are experimental control soils. Bars indicate one standard error.

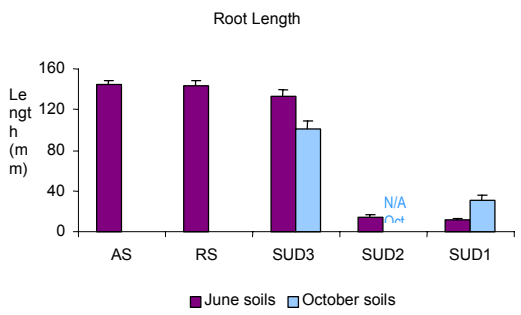

Figure 4. Northern wheatgrass root length following 21 days of exposure to Sudbury site soils. AS and RS are experimental control soils. Bars indicate one standard error.

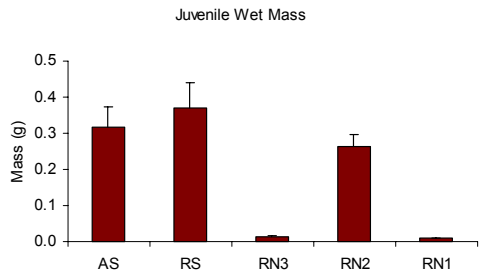

Figure 2. *Eisenia andrei* juvenile wet mass following 63 days of exposure to Rouyn-Noranda site soils. AS and RS are experimental control soils. Bars indicate one standard error.

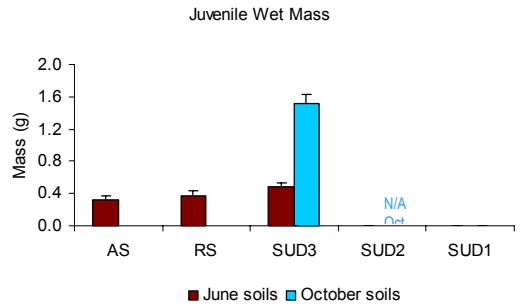

Figure 5. *Eisenia andrei* juvenile wet mass following 63 days of exposure to Sudbury site soils. AS and RS are experimental control soils. Bars indicate one standard error.

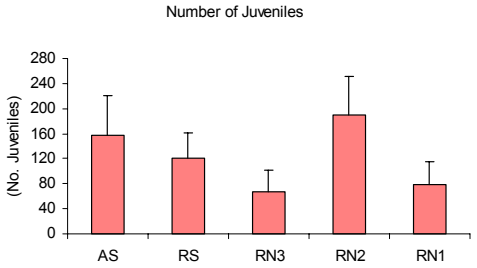

Figure 3. Production of juveniles by *Onychiurus folsomi* following 35 days of exposure to Rouyn-Noranda site soils. AS and RS are experimental control soils. Bars indicate one standard error.

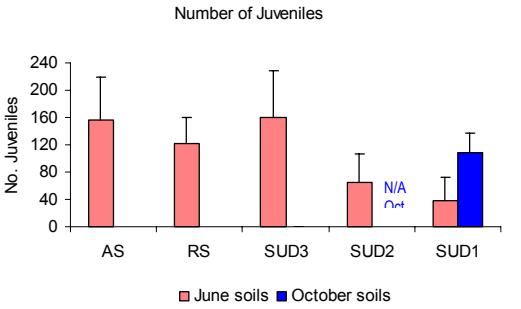

Figure 6. Production of juveniles by *Onychiurus folsomi* following 35 days of exposure to Sudbury site soils. AS and RS are experimental control soils. Bars indicate one standard error.