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Ecotoxicity of Aged Uranium in Soil Using Plant, Earthworm and Microarthropod Toxicity Tests

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Abstract Discrepancies about probable no effect concentrations (PNEC) for uranium in soils may be because toxicity tests used freshly contaminated soils. This study used 3 soils amended with a range of uranium concentrations 10 years previously. The toxicity tests with northern wheatgrass (*Elymus lanceolatus*); earthworm (*Eisenia andrei*) were not affected below ~1,000 mg U kg^{-1}, and the soil arthropod *Folsomia candida* was not affected below ~350 mg U kg^{-1}. Survival of *Orthonychiurus folsomi* was diminished 20% (EC_{20}) by ~85–130 mg U kg^{-1}, supporting a PNEC in the range of 100–250 mg U kg^{-1} as derived previously.

Keywords Arthropod · Collembola · PNEC · Uranium

Ecotoxicity of uranium (U) is important and challenging for a number of reasons. Most often the toxicity to humans and non-human biota is attributed to chemical effects, but with U these are potentially confounded by the always-concurrent radiological stress (Giovanetti et al. 2010). Additionally, the solubility of U in soil is highly dependent on soil pH in an atypical pattern (Echevarria et al. 2001). U is most soluble and hence probably most toxic in the mid-range (pH 5–7). Finally, there is concern in the literature regarding the reliability of the lowest effect concentrations, and some of the lowest reported effect concentrations are within the range of normal background (Sheppard et al. 2005).

Sheppard et al. (2005) in a review of ecotoxicity of U concluded that the majority of evidence supported a probable-no-effect-concentration (PNEC) in soil of 100 mg kg^{-1}. Earlier research reported lower effect concentrations but these were inadequately documented (Sheppard et al. 2005). A very recent paper (Giovanetti et al. 2010) indicated effect concentrations for biochemical endpoints (DNA damage and adverse effects on lysosomal membrane stability) in the range of 5–15 mg kg^{-1} soil, but the authors comment that these endpoints may not have ecological relevance. The effect level for endpoints that have clear ecological relevance (survival and weight reduction) was 600 mg kg^{-1} (Giovanetti et al. 2010).

One of the factors that contributed to the discordance of effect levels in the literature is that in preparing soils for toxicity tests, the length of time between the addition of the U to the soils and the start of the toxicity test may not have been sufficient for the U to reach sorption/bioavailability equilibrium. The purpose of this study was to assess the ecotoxicity of U in soils amended with a broad series of U concentrations that had been weathered and aged under natural conditions for 10 years, using toxicity test methods developed for and recently promulgated by (Environment Canada 2004, 2005, 2007; Stephenson et al. 2000).

Materials and Methods

Tens of litres of each of 3 soils were amended with a geometrically scaled sequence of U concentrations from control (0.9–3.7 mg kg^{-1}) to 1,000 mg kg^{-1} in ~1990 by Sheppard et al. (1992). These soils were used in outdoor lysimeters and remained outdoors in the lysimeters until

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2002, when they were excavated for the present study. One of the soils was a loam from Port Hope, Ontario (pH 7.5, 24% clay, 2.2% organic matter). The second soil was a limed Podzolic sand (pH 6.2, 2% clay, 1.0% organic matter). The third soil was a garden soil (pH 7.5 with carbonates present, 18% clay, 18% organic matter). The geometric mean soil solid/liquid partition coefficients (Kd) for the 3 soils across this range of U concentrations were 0.060, 0.29 and 0.066 L kg^{-1}, respectively, with a geometric standard deviation of 2. The Kd was measured on centrifugally extracted pore water using the methods of Thibault and Sheppard (1992). After excavation the soils were air dried only sufficiently to allow thorough mixing. All soil concentration data are expressed on a dry weight basis.

In addition to the 3 soils in the U-series, a negative control soil (ASTM 1995) was formulated from sand, clay and peat and included as a control treatment in each test for quality control and assurance purposes.

The previous toxicity tests conducted with these soils indicated toxicity below 1,000 mg kg^{-1} for some endpoints, but not for several others (Sheppard et al. 1992). In order to have a positive control (an assured effect of U) for most endpoints, aliquots of the soils at 1,000 mg kg^{-1} were further amended to achieve a 3,000 mg U kg^{-1} treatment. In all cases, the U concentrations in the actual soils used in the toxicity tests were measured by digestion with using Aqua Regia, perchloric and hydrofluoric acids, followed by Inductively Coupled Plasma–Mass Spectroscopy (ICP–MS, see Sheppard et al. 2008). These measurements were used to derive the effect levels. Note that for simplicity in describing the treatments, the treatment levels refer to the target concentrations. Recently amended soils were allowed to age moist at room temperature for at least 2 weeks before being used in toxicity tests.

Analysis of soils for ancillary physical and chemical attributes included pH in a 1:2 water:soil slurry (Hendershot et al. 1993), mineral particle size into 3 classes by the hydrometer method (Kalra and Maynard 1991), organic carbon and organic matter using the wet oxidation method (Tiessen and Moir 1993), and soluble carbonate and bicarbonate in saturated paste extract (Janzen 1993). The analyses by ICP–MS were done by Activation Laboratories Ltd., Ancaster, ON, Canada: accuracy relative to reference materials was within 10% and precision on blind duplicates was within 11%.

Toxicity tests followed protocols developed by Stephenson et al. (2000) on behalf of (Environment Canada 2004, 2005, 2007). The volume of soil available was less than optimal, so some modification of the test methods was required, including second use of the same soil aliquots in some cases. The earthworm and plant toxicity tests were conducted with the controls, 1,000 and 3,000-mg kg^{-1} treatments. The soil arthropod toxicity tests used less soil, and so a dilution series of the 3,000-mg kg^{-1} treatment was possible. This allowed comparison of a concentration series of aged soils with a series of recently amended soils. The target concentrations were 0, 100, 300, 1,000, 1,250, 1,750, and 3,000 mg U kg^{-1} for the garden soil and the Port Hope loam and 0, 100, 300, 500, and 1,000 mg U kg^{-1} for the aged and freshly-amended limed sand.

The earthworm toxicity test used the compost worm, *Eisenia andrei*. A 14-days acute lethality test was used for screening and a 56-days reproduction test was used as the more definitive test. There were 2 replicate units with 5 earthworms each per treatment for the screening test and 3 replicate units with 2 sexually mature adults each per treatment for the definitive test. Experimental conditions included 16/8 h (light/dark) photoperiod and temperature at 20 ± 2°C. The measurements for the screening test were survival on day 14, and for 56-days reproduction test the adult survival on day 35, when the adults were removed from the test units, and progeny count at day 56.

The plant toxicity test used northern wheatgrass (*Elymus lanceolatus*), and was conducted as a 22-days screening test and a 51-days definitive test, both with 3 replicate units per treatment and 2 or 5 seeds. Emergence, shoot length, root length and total seedling wet and dry weights were the endpoints measured. Experimental conditions included 16/8 h (light/dark) photoperiod and temperature at 20/16 ± 2°C.

The first arthropod test used the collembolan *Orthonychiurus folsomi* in two of the aged U-amended soils. Sexually mature adults of *O. folsomi* were collected from an asynchronous culture and added to the test soils on day 0 to start the 35-days survival and reproduction test. Ten organisms were placed into each test unit and there were 3 replicate units per treatment. The second arthropod test used even-aged neonates from synchronous cultures of the collembolan *Folsomia candida.* The toxicity test was a 28-days survival and reproduction test, with 10 organisms per test unit and 3 replicate units per treatment. For both toxicity tests, the experimental conditions included 16/8 h (light/dark) photoperiod and temperature at 20 ± 2°C.

Statistical analyses of the toxicity test results followed two procedures. For the screening toxicity tests that included the control, 1,000 and 3,000 mg U kg^{-1} treatments, analysis of variance followed by means comparison with Fisher's protected least significant difference were used. Assumptions of normality were evaluated using the Shapiro–Wilk normality test and Levene's test for homogeneity of variances. Log transformation was required for the collembolan reproduction data. Statistical analyses were conducted with SYSTAT 11 and Statistix 9.

With the exception of the collembolan toxicity tests, there were no effects at 1,000 mg U kg^{-1} and so no further

toxicity tests were considered necessary. For the collembolan toxicity tests used for more detailed assays, the statistical analysis followed those of Sheppard et al. (1992). A segmented linear equation was iteratively fitted to the data that defined a control-level response, a threshold U concentration, and a loglinear decreasing response at U concentrations above the threshold. In general, this equation was fit to untransformed data. However, in some cases the numbers of progeny for the collembolans was very large (over 400), and in order to deal with inhomogeneity of variances, log transformed data were used.

Results and Discussion

In general, the toxicity tests with collembola and the series of U concentrations produced consistent monotonic results (Fig. 1). In all cases, the endpoints measured in the negative control ASTM soil were within acceptable limits established by Environment Canada (2004, 2005, 2007), indicating that the procedures, conditions, and organism health were acceptable. It was hypothesized that differences in ecotoxicity of U from soil to soil might be related to the corresponding Kd values, but there was no consistent trend in Kd values across the soil U concentrations for the aged U in any of the three soils. The Kd values measured for freshly amended soils, either at about 170 mg U kg^{-1} or at 3,000 mg U kg^{-1}, were generally within the range observed for the aged soils, implying that U reacts quickly in soil and does not undergo substantial changes in sorption over time in the order of years.

The measurement endpoints for organisms exposed to the highest U concentration were significantly decreased relative to those for organisms in the control treatment (Table 1). This implies that for these endpoints the effect threshold was between ~840 and ~3,190 mg kg^{-1}, substantially higher than most proposed PNEC (Sheppard et al. 2005). The response of *O. folsomi* was an exception in that adverse effects on survival and reproduction were observed for organisms exposed in the 1,000 mg U kg^{-1} treatment with an inferred effects threshold below 1,000 mg U kg^{-1}.

The results from the segmented linear regressions for the more detailed toxicity tests with collembola gave the EC_{20} values shown in Table 2, and plots for the *O. folsomi* data, the most sensitive species, are shown as Fig. 1. The second collembolan species, *F. candida*, was not especially sensitive to U, with EC_{20} values above 350 mg U kg^{-1}: *F. candida* reproduces parthenogenically and is known to be relatively insensitive to metals whereas *O. folsomi* is a sexually reproducing species with a greater sensitivity to metals (Feisthauer et al. 2006). As noted in the previous toxicity tests (Table 1), *O. folsomi* was more sensitive than *F. candida* to U in soil, and in the limed sand soil in particular. For the limed sand, either with aged or fresh U, the EC_{20} for survival of adults and production of progeny was in the range of ~85–150 mg U kg^{-1} (Table 2). Aging neither influenced the Kd values for U, nor the results of the toxicity tests (Table 2). Note that the freshly amended U was aged 2 weeks prior to testing, perhaps this is sufficient to represent long-term contamination.

Although the lowest EC_{20} obtained was 85 mg U kg^{-1}, this is an interpolated value with the associated uncertainties, and the more certain conclusion is that the lowest EC_{20} effect level was closer to100 mg U kg^{-1}. The results with *O. folsomi* in the Port Hope soil are interesting (Table 2): adult survival had an EC_{20} of 210 mg U kg^{-1}, yet production of progeny had an EC_{20} of 1,000 mg kg^{-1}. Typically, progeny production is a more sensitive endpoint than adult survival. This may simply reflect variability, but

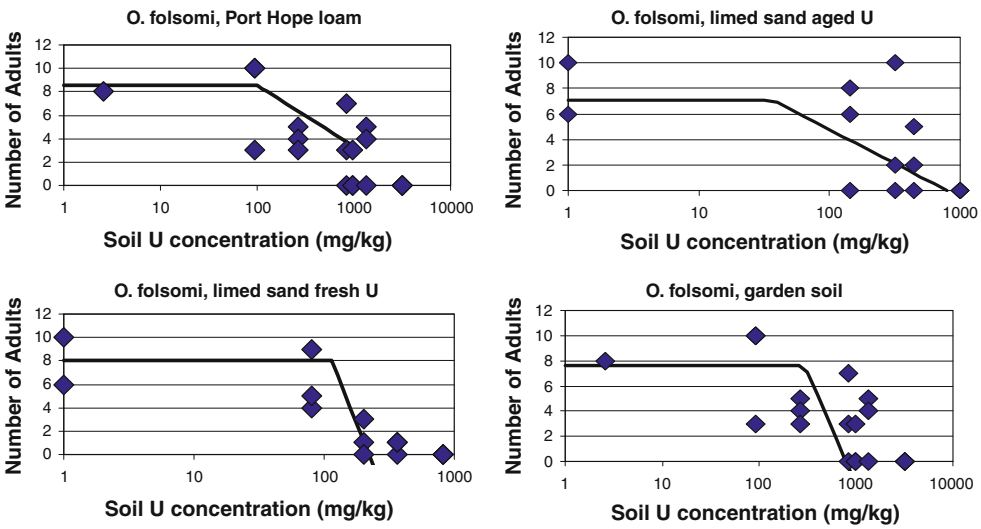

Fig. 1 Results for survival of adult *O. folsomi,* showing the individual observations and the iteratively fit segmented regression model. Note that the effect threshold concentration for the limed sand with aged U can only be reliably reported as <140 mg kg^{-1}

Table 1 Selected endpoints of soil toxicity tests in screening trials with control, 1,000 and 3,000 mg U kg^{-1} dry soil

Soil, species and endpoint	ASTM artificial soil	Control (2.3 mg U kg^{-1})	1,000 (mg U $kg^{-1)}$	3,000 (mg U kg^{-1})
Port hope soil				
O. folsomi 35-days adult survival (count)	9	9.7	9	1.3*[a]
35-days number of progeny (count)	47	49	41	0*
N. Wheatgrass shoot length (mm)	95.5	93.7	98.0	39.5*
Root length (mm)	164.0	155.0	206.0	7.8*
Earthworm survival (count)	5	5	5	–[b]
Number of progeny (count)	12	0.67	0.67	–
Limed sand soil				
O. folsomi 35-days adult survival (count)	9	9.7	1.3*	1.7*
35-days number of progeny (count)	47	19	5	1.3*
N. Wheatgrass shoot length (mm)	95.5	94.7	89.5	78
Root length (mm)	164	179	145	39*
Earthworm survival (count)	5	5	5	–
Number of progeny(count)	12	2.0	0	–
Garden soil				
Earthworm survival (count)	5	5	5	–
Number of progeny (count)	12	16	14	–

[a] Asterisk indicates significant difference compared to control by Fisher's protected least significant difference with $p < 0.05$), the 35-days progeny counts were log-transformed for statistical comparisons

[b] Dashes indicate the treatment was not included in the bioassay

Table 2 Results of collembolan toxicity tests with a series of 5 to 7 soil U concentrations and in the ASTM artificial soil (negative control)

Soil, species and endpoint	ASTM artificial soil	Control	Interpolated threshold (mg U kg^{-1})	EC_{20} (mg U kg^{-1})
Port hope soil				
O. folsomi 35-days adult survival (count)	9	8.0	100	210*[a]
35-days number of progeny (count)	2	10	930	1,000*
F. candida 28-days adult survival (count)	10	10	690	710*
28-days number of progeny (count)	291	115	760	840*
Limed sand soil, aged U				
O. folsomi 35-days adult survival (count)	9	7.5	45 (<140)[b]	85 (<300)*
35-days number of progeny (count)	23	0	nd[c]	nd ns[d]
F. candida 28-days adult survival (count)	10	8.8	>1,000	>1,000 ns
28-days number of progeny (count)	358	275	580	2,200 ns
Limed sand soil, aged plus fresh U				
O. folsomi 35-days adult survival (count)	9	8	114	130*
35-days number of progeny (count)	23	31	100	150*
F. candida 28-days adult survival (count)	9	10	200	350 ns
28-days number of progeny (count)	210	288	300	>3,000 ns
Garden soil				
O. folsomi 35-days adult survival (count)	9	8.2	300	360*
35-days number of progeny (count)	47	51	790	850*
F. candida 28-days adult survival (count)	10	10	1,000	1,030*
28-days number of progeny (count)	405	186	1,500	1,900*

[a] Asterisk indicates a significant overall effect on the endpoint by F-ratio in analysis of variance ($p < 0.05$)

[b] The threshold occurred between the control and 140 mg kg^{-1}, thus the interpolated value of 45 mg kg^{-1} is not as well founded as for the other soils where there were data between the control and the first apparent effect concentration

[c] *nd* not determined: progeny were not produced in the control and were only produced at 100 mg U kg^{-1}

[d] *ns* not significant ($p > 0.05$)

it is possible that progeny production was a compensatory response to toxic stress.

While the plant data indicated no adverse effects to emergence and growth, progeny production was compromised for *E. andrei* exposed to U in the Port Hope and limed sand soils. This effect on earthworm reproduction was attributed to the low organic matter content in these soils. Earthworms can survive in soils that are sub-optimal for reproduction. The test soils where minimal or no earthworm reproduction occurred had organic matter contents of 2.2% and 1%, respectively. Soils with organic matter content below 4% typically present challenges to earthworms depending upon other correlated potentially confounding physico-chemical factors (Chelinho et al. 2011; Van Gestel et al. 1992).

In conclusion, the plant and earthworm toxicity tests for U did not show effects at the 1,000 mg U kg^{-1} treatment level. One of two species of collembola was more sensitive. Survival and reproduction of *O. folsomi* were diminished when exposed to both freshly spiked U and aged U concentrations in soil with EC_{20} between 85 and 150 mg U kg^{-1} in the limed sand soil. This is not a typical agronomic soil, and is probably not optimal habitat for *O. folsomi*. Overall, the results support the conclusion from the literature (Sheppard et al. 2005) that most organisms in most soils would probably not be harmed by U concentrations below 100–300 mg U kg^{-1} soil. The toxicity of U aged in soil for 10 years was not markedly different from U added to soils within 2 weeks of the toxicity tests.

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