1 of 1

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Toxicity Test of the F-Area Seep Soils by Laboratory Lettuce Seed Germination and Seedling Growth^(U)

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Prepared for the U.S. Department of Energy under contract no. DE-AC09-89SR18035

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Abstract

This study is a follow-up of a similar study done by Loehle (1990). The objectives of the original study were to: (1) measure the toxicity of groundwater contaminated by the F-Area seepage basins where this water surfaces in a seepage along Fourmile Branch and (2) to evaluate the effectiveness of rainwater for washing contaminants from the soil. Results of seed germination tests show no significant difference between water extracted from one extraction of F-Area seepage soil, soil from a control area, the sixth consecutive extraction from F-Area soil, and a deionized water control. A root-growth assay on the same seeds shows a significant effect with the order of growth, first extraction of F-Area soil < control site < deionized water < sixth F-Area extraction. When compared to the results of the 1990 study, this suggests that there may be some improvement in the soil at the F-Area seepage, but there is still some evidence of phytotoxicity in this soil. As shown previously, the cause of the toxicity is removed by soil washing, suggesting that continued improvement should be expected.

Introduction

The F-Area Seepage is located along Fourmile Branch (Figure 1). The seepage is an area of springs where groundwater surfaces and produces swamps and small tributaries that empty into the adjacent creek. Seepages occur naturally along Fourmile Branch. However, it was realized quite early that some of the springs along Fourmile Branch were receiving water from the seepage basins used for disposal of low-level waste water from the F- and H-Area chemical separations facilities. Tree mortality has been observed along these seepages and the adjacent swamps (Killian et al. 1987, Mackey 1988).

Low-level liquid waste was released to the F- and H-Area seepage basins from the mid-1950s until 1988 when the basins were filled and covered with an impervious clay cap. The waste water entering the basins was typically acidic, high in dissolved ions, and contained low levels of radionuclides and dissolved metals (Killian et al. 1987). Seepage water contained elevated levels of sodium, nitrate, aluminum, and heavy metals. Elevated aluminum concentrations were also found, probably the product of leaching from the clay soil matrix by the acidic water.

Loehle (1990) conducted toxicity tests of soil collected earlier from the F-Area seepage for chemical and physical characterization (Richardson et al. 1990). The tests were

designed to assess toxicity before and after leaching of the soil by rainwater. The tests described in this paper were designed to evaluate changes in toxicity over the three-year period since Loehle's (1990) study. These tests were done on the same F-Area seepage soil used in the Loehle (1990) study, collected from (as closely as possible) the same location.

Methods

The methods used in this study duplicated, as closely as practical, those used by Loehle (1990). These methods were originally described by Carlson et al. (1989). Soils were gathered from two locations, adjacent to field marker in the F-Area seepage and at a control site in the flood plain of Fourmile Branch near its crossing with SRS Road E-1. The latter site is upstream of the seepages contaminated by the F- and H-Area seepage basin effluents.

Samples were taken by digging three trenches at each site to 18 inches and collecting a slice of soil that was of equal volume for each depth from 0 to 18 inches. The soil contained considerable organic material near the surface but graded to more mineral soil with depth.

The soil samples were removed to the laboratory where they were put in individual stainless steel pans and dried in an oven at 45° C until repeated weighing showed no fur-

ther weight loss. Water extractions were done by putting 100 gm of dry soil in a 250 mL polyethylene bottle and adding 150 mL of deionized water. The soil-water mixture was shaken five times initially and five times once each day for four days. After the mixture had been allowed to equilibrate for four days, it was placed in a centrifuge and the water and soil separated. The water was decanted from the bottle and filter to remove any remaining suspended soil. At the end of the first four days there were six water samples from the soils, three from the F-Area seepline and three from the control area. The water samples were stored in a refrigerator until used.

The F-Area seepline soil was extracted five more times. The same sequence of adding water, shaking, and equilibrating for four days was used for each extraction. Only 100 mL of water was added for these extraction rather than the 150 mL used in the first extraction. The soil samples were not dried between extractions. Only the water from the sixth set of extractions was saved. At the end of the extraction there were nine water samples, three from a single extraction from the control, three from a single extraction from the F-Area seepline samples, and three from the sixth extraction of the F-Area seepline samples.

Four water samples were prepared for toxicity testing. Water samples were prepared by mixing equal quantities of water from the three samples of each extraction type, control (C), first F-Area seepline extraction (F1), and sixth F-Area seepline extraction (F6). A deionized water sample was also prepared.

Filter papers (9 mm) were placed in 10 mm diameter petri dishes. Eight petri dishes were prepared for each type of water (total of 32 dishes). The petri dishes were on a table top near the window of the laboratory. The treatment (type of water added) used in each petri dish was randomly selected so that distance from the window would not be a factor. The filter paper in the petri dish was saturated with one of the types of water. Ten lettuce seeds were placed on the filter paper; the variety butter crunch bib was used. The tops were placed on the petri dishes and they were sealed with wax.

The seeds were allowed to germinate for three days. At the end of this period the germinated seeds were removed from the petri dishes and an image was recorded with a copy machine. Ungerminated seeds were not copied. The number of seeds germinating in each petri dish were counted. The length of the roots of the germinated seedlings was measured by using a line distance recorder run along the roots images.

Results

The results of the germination test are shown in Table 1 and Figure 2. An analysis of variance procedure was carried out on this data (Table 4). As is obvious from the data, there was no significant effect of the treatments. Even though the germination of the seeds exposed to water from the first extraction of the F-Area seepline soil is lower than the control or any of the other treatments, the difference is too small to be differentiated from random chance.

The results of the root-length measurements are shown in Table 2 and Figure 3. Unlike the seed germination, the root length does appear to be affected by the type of treatment. This is confirmed by an analysis of variance procedure, which shows significant treatment effects (Table 5). Comparison of the 95%, two-tailed confidence limits for each treatment indicates significant differences in treatments with the first extraction from F-Area seepline soil being significantly lower in length than any other treatment. However, it should be noted that the water extracted from the control soil also shows significant depression of root growth when compared to either the water from the sixth extraction from the F-Area seepline soil or deionized water. This suggests that there is toxicity to lettuce seedlings even in uncontaminated forest soils. This should not be too surprising since allelopathy (chemical competition) is common among forest tree species.

Discussion

When the results of this study are compared to the earlier study of Loehle, there does appear to be some decrease in toxicity (Table 3). This is shown most clearly in the seed germination studies. This is in agreement with the recovery of the die-back area from which the samples were collected at the F-Area seepline. While trees have not begun to grow in this area there are a number of herbaceous species which have begun to colonize this area.

The confirmation that the toxic agent(s) in the soil can be removed by successive washes, the decrease in toxicity to lettuce seed, and the revegetation of the area all suggest that the area will recover naturally given time for the toxic agent(s) to leach from the soil.

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Figure 2. Germination of Lettuce Seed. Vertical Line spans 95% Confidence Limits of Mean. Square is at the Mean Germination for Eight Petri Dishes.

Figure 3. Results of Lettuce Seedling Root Growth. Vertical line spans middle 50 percentile. Box spans 95% Confidence limits. Bars connected by line are at the mean length.

Table 1. Lettuce Seed Germination in Water from Different Sources.

Treatment	Petri Dish Number (Randomly Assigned)							
	D1	D2	D3	D4	D5	D6	D7	D8
F-Area, First Wash	8	8	7	10	9	10	10	10
Deionized Water	10	10	10	9	10	10	7	9
Control	10	10	10	8	9	9	9	10
F-Area, Sixth Wash	10	10	9	10	10	10	10	9

Table 2. Root Growth in Water from Different Sources (cm).

	Petri Dish Number (Randomly Assigned)							
	D1	D2	D3	D4	D5	D6	D7	D8
F-Area, First Wash	0.41	0.44	0.19	0.41	0.03	0.98	0.67	0.48
F-Area, First Wash	0.38	0.54	0.29	0.32	0.35	0.29	0.73	0.32
F-Area, First Wash	0.32	0.57	0.38	0.54	0.29	0.38	0.95	0.32
F-Area, First Wash	0.35	0.57	0.38	0.38	0.26	0.51	0.48	0.54
F-Area, First Wash	0.32	1.33	0.29	0.26	0.26	0.67	0.63	0.54
F-Area, First Wash	0.16	0.41	0.48	0.44	0.29	0.67	0.41	0.63
F-Area, First Wash	0.35	0.44	0.35	0.51	0.19	0.57	0.32	0.54
F-Area, First Wash	0.48	0.6	0	0.07	0.26	0.7	0.56	0.57
F-Area, First Wash	0	0	0	0.26	0.32	0.54	0.22	0.73
F-Area, First Wash	0	0	0	0.57	0	0.48	0.67	0.7
Deionized Water	1.77	0.82	0.98	1.33	1.3	1.08	1.08	1.08
Deionized Water	1.08	0.89	1.11	1.58	1.49	1.01	1.42	0.98
Deionized Water	1.33	0.95	1.68	1.65	1.14	1.52	1.68	1.14
Deionized Water	1.65	0.89	1.2	1.68	0.35	0.73	1.65	1.68
Deionized Water	1.61	1.05	1.84	1.39	0.38	1.61	1.71	0.98
Deionized Water	0.48	0.38	0.19	0.67	1.08	1.49	0.26	1.58
Deionized Water	1.61	0.89	1.11	1.74	1.3	1.71	0.76	1.27
Deionized Water	1.08	1.05	1.33	1.58	1.96	1.08	0	0.89
Deionized Water	1.27	0.73	1.61	1.33	1.65	1.14	0	1.23
Deionized Water	1.33	0.79	1.2	0	1.17	1.27	0	0
Control	0.6	0.76	0.48	0.6	0.76	0.35	1.3	1.46
Control	0.26	0.44	0.6	0.76	0.95	0.19	0.67	1.23
Control	0.76	0.73	0.57	0.63	0.35	0.38	1.01	1.17
Control	0.41	0.86	1.14	0.29	0.92	0.35	0.98	1.39
Control	0.41	0.89	1.14	0.29	0.41	1.17	1.42	1.48
Control	1.05	0.26	0.26	0.26	0.38	0.07	0.26	0.22
Control	0.67	0.38	0.7	0.73	0.38	0.38	0.35	0.76
Control	0.31	0.13	0.38	0.76	0.51	0.26	0.48	0.89
Control	0.41	0.38	0.6	0	0.38	0.38	1.23	0.6
Control	0.76	0.38	0.51	0	0	0	0	0.95
F-Area, Sixth Wash	0.82	1.01	0.82	0.76	0.98	1.71	0.92	1.36
F-Area, Sixth Wash	1.01	0.51	0.88	0.82	1.52	1.49	1.27	0.86
F-Area, Sixth Wash	1.07	0.82	0.88	1.23	0.82	1.2	1.01	0.89
F-Area, Sixth Wash	0.38	0.88	0.82	0.76	1.08	0.92	0.98	1.36
F-Area, Sixth Wash	0.32	0.95	0.38	1.2	0.76	1.01	1.23	1.33
F-Area, Sixth Wash	1.01	0.95	0.51	1.2	0.44	1.55	0.98	1.71
F-Area, Sixth Wash	0.63	0.95	0.57	1.08	0.54	1.14	0.73	1.23
F-Area, Sixth Wash	0.76	1.07	0.88	0.92	1.39	0.63	0.79	1.42
F-Area, Sixth Wash	0.82	1.14	0.7	1.05	1.11	1.3	1.2	0.51
F-Area, Sixth Wash	0.57	1.33	0	1.2	1.36	1.49	0.98	0

Table 3. Comparison of 1990 and 1993 Toxicity Tests.

Treatment	Germination		Length (cm)	
	1990	1993	1990	1993
F-Area, First Wash	4.4	9	0.76	0.41
Deionized Water	9.5	9.375	1.22	1.13
Control	7.5	9.375	1.18	0.6
F-Area, Sixth Wash	8.5	9.75	1.03	0.96

Table 4. Analysis of Variance Table for Seed Germination

Anova: Single-Factor

Summary

Groups	Count	Sum	Average	Variance
F-Area, First Wash	8	72	9	1.428571
Deionized Water	8	75	9.375	1.125
Control	8	75	9.375	0.553571
F-Area, Sixth Wash	8	78	9.75	0.214286

ANOVA

Source of Variation

	SS	df	MS	F	P-value	F crit
Between Groups	2.25	3	0.75	0.903226	0.452	2.947
Within Groups	23.25	28	0.83036			
Total	25.5	31				

Table 5. Analysis of Variance Table for Root Growth Toxicity Test

Anova: Two-Factor With Replication

Summary

	D1	D2	D3	D4	D5	D6	D7	D8	Total
F1									
Count	10	10	10	10	10	10	10	10	80
Sum	2.76	4.89	2.345	3.73	2.22	5.755	5.615	5.335	32.65
Average	0.276	0.489	0.2345	0.373	0.222	0.5755	0.5615	0.5335	3.265
Variance	0.0276	0.1351	0.0317	0.0238	0.0137	0.037	0.0457	0.0189	0.334
D1									
Count	10	10	10	10	10	10	10	10	80
Sum	13.175	8.41	12.225	12.925	11.79	12.6	8.535	10.805	90.47
Average	1.3175	0.841	1.2225	1.2925	1.179	1.26	0.8535	1.0805	9.047
Variance	0.1459	0.0367	0.2105	0.3013	0.254	0.0966	0.5499	0.2098	1.805
C									
Count	10	10	10	10	10	10	10	10	80
Sum	5.625	5.185	6.35	4.3	5.03	3.52	7.68	10.14	47.83
Average	0.5625	0.5185	0.635	0.43	0.503	0.352	0.768	1.014	4.783
Variance	0.0612	0.0707	0.0847	0.0907	0.0851	0.1015	0.2382	0.1679	0.9

Anova: Two-Factor With Replication

Summary

	D1	D2	D3	D4	D5	D6	D7	D8	Total
F6									
Count	10	10	10	10	10	10	10	10	80
Sum	7.39	9.61	6.44	10.205	9.98	12.41	10.075	10.645	76.76
Average	0.739	0.961	0.644	1.0205	0.998	1.241	1.0075	1.0645	7.676
Variance	0.0683	0.0459	0.0822	0.0368	0.1315	0.1087	0.0323	0.2588	0.765
Total									
Count	40	40	40	40	40	40	40	40	
Sum	28.95	28.095	27.36	31.16	29.02	34.285	31.905	36.925	
Average	2.895	2.8095	2.736	3.116	2.902	3.4285	3.1905	3.6925	
Variance	0.3031	0.2883	0.4091	0.4527	0.4844	0.3438	0.8661	0.6554	

ANOVA*Source of Variation*

	SS	df	MS	F	P-value	F crit
Sample	26.127	3	8.709	73.284	3E-35	2.636
Column	1.9135	7	0.2734	2.3003	0.027	2.0414
Interaction	7.7237	21	0.3678	3.0949	1E-05	1.5927
Within	34.225	288	0.1188			
Total	69.99	319				

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