Lettuce Seed Germination and Root Elongation Toxicity Evaluation of the F- Area Seepline Soils (U)

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ABSTRACT

This study is a continuation of similar studies conducted by Eaton and Murphy (1993) and Loehle (1990). The objectives of these studies are to: (1) assess the toxicity of the water-soluble constituents of soil in a seepline adjacent to the F-Area Seepage Basins and (2) evaluate the effectiveness of rainwater movements in reducing the toxicity of the soil. Soils from the F-Area seepline that were found to inhibit lettuce seed germination and radicle elongation in 1990 were not found to be significantly different from soils from an uncontaminated control site in this test. After six washings of the soil, the toxicity of the leachate was comparable to that of de-ionized water. This indicates that natural water movements may have rendered the F-Area seepline soils less toxic to lettuce seedlings than in previous tests.

INTRODUCTION

The F-Area seepline is located between the F-Area Seepage Basins and Fourmile Branch (Figure 1). Groundwater from the adjacent higher elevations, including the basin, comes to the surface in a series of naturally occurring seeps. Water from the seeplines forms wetlands and small tributaries that drain into Fourmile Branch. The seepage basins received waste water from F-Area chemical separations facilities that was acidic, high in dissolved ions, and contained low levels of radionuclides and

dissolved metals (Killian et al., 1987). Tree mortality was subsequently observed along these seeplines and in the adjacent wetlands (Mackey, 1988). Chemical analysis of surface water in several of the wetlands confirmed that the wetlands were being influenced by seepage from the basins (Looney et al., 1988). The F-Area Seepage Basins were filled and covered with an impervious clay cap in 1988. The H-Area Seepage Basins and seepline have very similar histories of waste disposal, basin closure, and tree-kill at the seepline.

A later study to determine the cause of the tree mortality in the seepline areas was unable to clearly define the causal agent (Greenwood et al., 1990). Among potential agents they listed were high levels of aluminum, manganese, cadmium, and zinc. Additionally, the areas had very low pH values and high sodium contents, and experienced a rainfall shortage during the period. The sodium was thought to have caused very extensive dispersion of the soil clay component, which led to greatly reduced soil structure and increased siltation.

Chemical analyses of the leachate from seepage area soil samples showed that the soils were low in pH and high in conductivity, sodium, nitrate, aluminum and other metals. Seed germination and root elongation tests demonstrated toxicity of the leachate and showed that repeated washing of the soil significantly reduced this toxicity (Loehle, 1990). Subsequent testing of soil samples from the same areas indicated that there might be some reduction in the phytotoxicity of the soil (Eaton and Murphy, 1993).

METHODS

This study was conducted using methods derived from Carlson et al. (1989), as used in the previous studies. Soil samples were collected from the seepline adjacent to the tree mortality area at the approximate location sampled by the two previous studies (groundwater sample location F19), and from a control site at a seepline in the same watershed that has not received contaminated groundwater (Fourmile Branch near the crossing by SRS Road E-1). Three samples, each representing the top 18 inches of soil, were collected at the impacted F-Area seepline and the control seepline, transported to the laboratory in plastic bags, and stored refrigerated until processing began. The samples were placed on a drying pan in an oven at 45° C until dry. When completely dry, as measured by no loss of weight with additional drying, they were passed through a number 16 sieve to mix the sample and remove large organic debris.

Water extracts were obtained from each sample by placing 100 gm of the dry soil sample in a 250 ml polyethylene bottle with 150 ml of de-ionized water. The soil-water mixtures were shaken initially and once each day of the experiment to form a slurry. During the extraction, all samples were stored in a refrigerator at 5° C .

After four days of equilibration, the samples were placed in a centrifuge for 15 minutes at 3000 rpm to separate the water from the soil. The leachate was decanted from the bottle and filtered through a 45 um filter in order to remove any suspended components. Three sample leachates from the two sites were obtained and labeled F1a-F1c from the contaminated seepline and C1a-C1c from the uncontaminated seepline. The F-Area soil samples were then washed 5 additional times by a process of adding 100 ml of de-ionized water, shaking daily, and allowing to equilibrate for a

period of 72 hours. They were then centrifuged to separate the water and soil, and the leachate decanted. The leachates from the sixth wash were filtered, collected and labeled F6a-F6c.

Experimental water samples were prepared by mixing equal parts of leachate for each sample type and by preparing an additional blank sample of de-ionized water, resulting in samples F1 (F-Area First Wash), C (control), F6 (F-Area Sixth Wash), and DI (De-ionized Water Blank). Eight petri dishes were prepared for each type of sample. Filter papers (48 mm) were placed in 50x10 mm disposable petri dishes and saturated with 2 ml of sample solution. Ten Bibb lettuce (*Lactuca sativa*) seeds were placed into each petri dish, the lids were replaced, and then sealed in Parafilm. The petri dishes were placed at room temperature in a randomized block pattern near a window where they received diffuse natural light.

After 72 hours, the seedlings were removed from the petri dishes, mounted on paper, and photocopied to produce a permanent record. The length of root elongation was measured from the cotyledons to the tip of the radicle, including the hypocotyl. Statistical analysis was conducted to determine: (1) if there was an experimental block effect due to placement of the petri dishes, (2) if there was a difference in the germination rates between samples, and (3) if there were differences in root elongation between sample types. Results of germination and average root elongation of the treatments were compared with results of the previous studies.

RESULTS

This study measured both the number of seeds that germinated and the elongation of the root and hypocotyl during the 72 hour test. Because the petri dishes were placed

on a lab bench-top, rather than an environmentally controlled germination cabinet, the affect of position on results was tested. An analysis of the variance due to the physical location of the petri dish in the replications indicated that there was no significant block effect (Table 1). This factor was therefore discarded from further consideration.

The seed germination experiment results are given in Table 2. Statistical differences in germination rates due to water samples in this study were not significant at the 95% confidence level (Table 3). Germination of seeds from the control, blank, and sixth wash of the F-Area samples were similar in 1994 to the results from 1990. However, the average germination of seeds from the first wash of the F-Area samples increased from 44% in 1990 to 84% in 1994. Germination order among the treatments have been similar over the three years tested. De-ionized water has generally produced the greatest germination values, with sixth wash values of the F-Area being nearly equal. First wash treatments have generally been lower, especially for the F-Area in previous studies. Much of the toxic affect on seed germination of this sample has been reduced over the years.

The analysis of the variation in root elongation found significant differences between treatments (Table 4). A Tukey multiple comparison analysis (Neter et al., 1990) found that the first wash treatments from the F-Area and the control area were different from the de-ionized water blank. No other differences were significant at the 95% confidence level, including between F-Area first wash and the control.

First washes of these wetland soils have generally reduced root elongation in each of the years tested (Table 5). It is possible that these soils possess a component that has natural suppression of seed germination, above and beyond the chemical toxicity of the impacted soils tested. The sixth wash of the impacted soil generally produced

higher germination and greater elongation than the first wash of the control soil and nearly as good as the de-ionized water sample.

In 1990, significant differences were found between the F-Area first wash and the control samples for both germination and root elongation. In this study, no significant differences were observed between F-Area and control samples. Average root elongation of F-Area first wash samples was 96% of the average elongation of the control sample in 1994 as opposed to 64% in 1990. After six washes root elongation in 1994 increased to a length comparable to that of the de-ionized water blank.

FUTURE DIRECTIONS

Evidence of long term toxicity on seed germination of seepline wetlands in the F-Area is diminishing. If subsequent work of this type occurs, a number of suggestions should be incorporated. During a recent routine screening of dried soil samples from a similar seepline area, radioactivity that was much higher than background was found. The tritium known to be present in these samples was removed by the drying process. Most of the material placed in the F- and H-Area Seepage Basins was not high level radioactive waste, but additional precautions during soil drying and handling would be advisable to insure no inhalation of the dust.

Alternate testing methods and species may be appropriate in subsequent tests. The general inhibition of germination of wetland extracts on lettuce seeds, while it is able to determine very large affects, now appears to be less dynamic at eliciting minor differences. The use of a wetland herbaceous species or the use of an *in-situ* testing method may be more powerful than the standard lettuce seed bioassay we have been using.

Testing of surface water samples from the impacted wetland areas has not been conducted due to low tritium levels that are present. The use of a more standardized and controlled set of germination conditions, e.g. dark, temperature controlled germination cabinets, could possibly reduce the variance component of the germination and elongation results. This could result in more uniform results that could differentiate minor difference that were not picked up by our procedure.

CONCLUSIONS

This study showed a greatly reduced toxic effect of the leachate from F-Area seepline soil samples on lettuce seed germination and growth as compared to control samples from an uncontaminated site. Leachate from both F-Area first wash and from the control sample had lower average elongation lengths than the de-ionized water blank. After six washings, the F-Area soil leachate sample average root elongation was the same as that of the de-ionized water blank. This study indicates that natural water transport of soluble contaminants has reduced the toxicity of the F-Area seepline soils.

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Table 1. Summary and analysis of variance for root elongation (mm) attributable to petri dish location.

Summary

Location	Count	Sum	Average	Variance
NE Quadrant	80	826	10.3	39.361
NW Quadrant	80	779	9.7	37.892
SE Quadrant	80	888	11.1	41.205
SW Quadrant	80	780	9.8	34.544

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	99.11	3	33.036	0.8637	0.4602	2.6332
Within Groups	12087	316	38.251			
Total	12186	319				

Table 2. Lettuce seed germination in water from different sources (Number of 10 seeds)

	Replication							
Treatment	1	2	3	4	5	6	7	8
F Area, First Wash	7	9	8	8	9	9	9	8
F Area, Sixth Wash	9	10	7	9	6	9	9	10
Control Area	6	9	7	9	7	9	7	6
Deionized Water	7	9	10	10	10	9	9	9

Table 3. Summary and analysis of variance for seed germination.

Summary

Treatment	Reps	Sum	Average	Variance
F Area, First Wash	8	67	8.4	0.554
F Area, Sixth Wash	8	69	8.6	1.982
Control Area	8	60	7.5	1.714
Deionized Water	8	73	9.1	0.982

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	11.094	3	3.698	2.827	0.0566	2.9467
Within Groups	36.625	28	1.308			
Total	47.719	31				

Table 4. Summary and analysis of variance for root elongation (mm).

Summary

Treatment	Count	Sum	Average	Variance
F Area, First Wash	80	706	8.8	21.412
F Area, Sixth Wash	80	894	11.2	45.159
Control Area	80	739	9.2	44.563
Deionized Water	80	934	11.7	37.108

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	475.21	3	158.40	4.2742	0.0056	2.6332
Within Groups	11711	316	37.06			
Total	12186	319				

Table 5. Comparison of results from previous toxicity testing.

Treatment	1990	1993	1994
		Germination (%)	
F Area, First Wash	44	90	84
F Area, Sixth Wash	85	98	86
Control Area	75	94	75
Deionized Water	95	94	91
		Root Elongation (mm)	
F Area, First Wash	7.6	4.1	8.8
F Area, Sixth Wash	10.3	9.6	11.2
Control Area	11.8	6.0	9.2
Deionized Water	12.2	11.3	11.7

Appendix 1. Root growth (mm) of germinant seeds in water from different sources.

TREATMENT	Replication 1	2	3	4	5	6	7	8
F Area, first wash	15	13	8	13	10	10	12	10
	11	10	6	15	13	12	11	10
	13	9	9	11	14	11	12	10
	11	11	9	15	15	12	9	9
	12	11	6	12	11	12	11	12
	12	11	10	10	11	10	4	10
	12	14	11	12	15	10	11	11
	0	11	5	10	10	9	9	3
	0	6	0	0	14	6	3	0
	0	0	0	0	0	0	0	0
F Area, sixth wash	13	12	17	25	16	13	17	13
	27	19	12	12	12	12	10	14
	13	20	15	12	11	10	13	12
	8	11	12	19	13	16	17	12
	7	27	12	12	11	9	16	12
	12	20	10	16	11	11	15	11
	11	11	17	12	0	3	10	10
	10	21	0	11	0	2	13	11
	3	13	0	22	0	1	25	3
	0	13	0	0	0	0	0	2
Control Area	15	14	17	12	16	10	9	6
	11	13	12	3	15	14	11	11
	14	11	5	13	3	13	17	13
	14	9	18	14	17	20	19	3
	12	15	10	14	17	15	18	12
	9	11	16	19	15	6	17	15
	0	16	16	14	13	17	3	0
	0	10	0	15	0	3	0	0
	0	11	0	6	0	2	0	0
	0	0	0	0	0	0	0	0
Deionized Water	17	9	11	15	14	10	23	13
	10	10	13	27	12	17	15	17
	9	19	14	21	17	17	21	16
	12	11	15	15	12	12	8	12
	17	9	20	3	13	14	12	7
	8	9	18	9	20	15	9	20
	5	6	10	10	11	16	11	15
	0	20	18	13	12	3	10	10
	0	17	19	6	9	2	9	4
	0	0	15	14	2	0	0	0

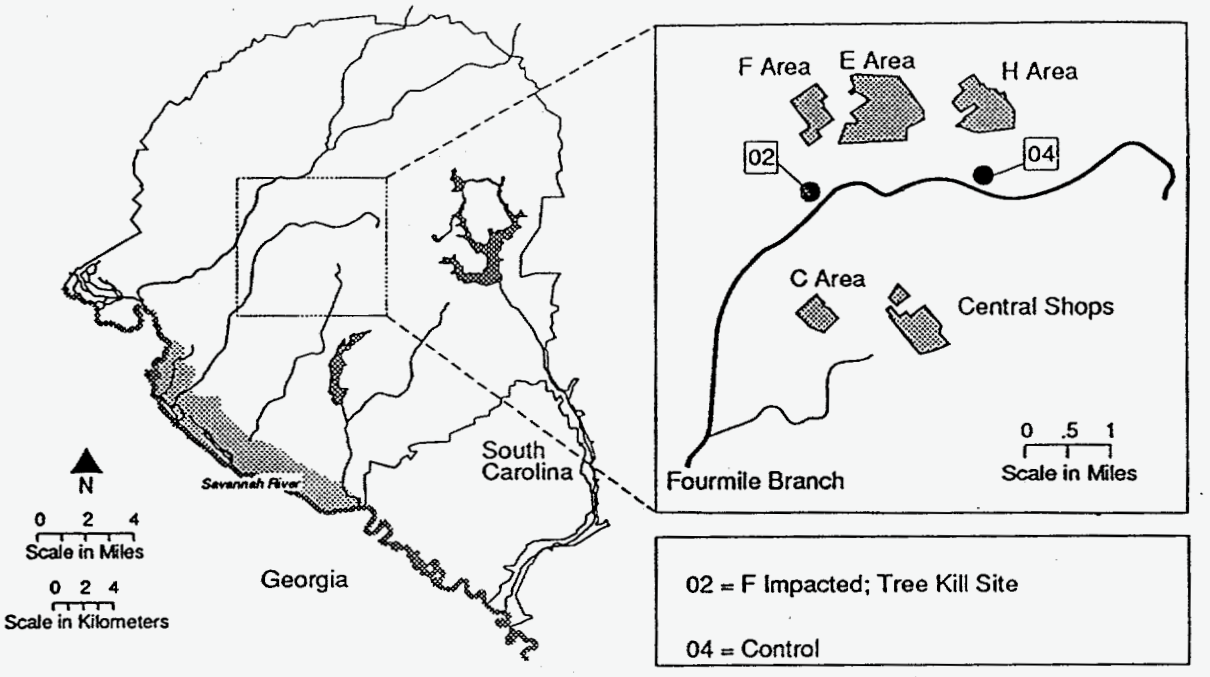

Figure 1. Map of study site locations at SRS