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Adjusting Hydration Water Volume to Decrease Preparation Time in Seed Germination Studies

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Soil toxicity tests are commonly used to assess the toxicity of contaminated soils, particularly from hazardous waste sites. As in the case of water-borne contaminants, chemical analysis of soils may not be indicative of the potential hazard of soils because 1) all contaminants may not be known and the list of analytes is, therefore, dependent upon limited data and subjective opinion and 2) conditions of the soil (e.g., the presence of organic material) may mitigate toxicity.

Toxicity test methods for water-column studies have been developed and refined over several years. There are now numerous documents that describe techniques for conducting both chronic and acute water column toxicity tests. Less developed methods are available for soil studies. Many researchers rely upon a single USEPA document (Greene et al. 1989) for test guidance, although other papers have been written (Wang 1987; Linder et al. 1990).

Soil toxicity testing commonly consists of earthworm and seed germination studies. In germination tests, soil from a site containing suspected toxicants is mixed in various concentrations with a laboratory control soil, consisting of washed silica sand. The mixed soil is then added to test chambers (e.g., petri dishes), the soil is hydrated with laboratory water, seeds are added to the soil, and a cover soil consisting of a larger-grain silica sand is placed over the seeds.

The final target concentrations (e.g., 6.25, 12.5, 25, 50, and 100 percent site soil) are based upon dry weight. The quantities of site and control soil mixed together, and the volume of hydration water, are dependent upon the existing moisture content of the soils (Moisture Fraction or MF) and the volume of water the soils can hold at saturation (Water Holding Capacity or WHC). After the soils have been mixed together and added to the test chamber hydration water is added to bring all test chambers up to the same MF. Current EPA guidance (Greene et al. 1989) recommends raising the MF to 85% of the WHC.

To achieve a MF of exactly 85% of the WHC, soil must be weighed for each replicate. This may take a considerable amount of time, particularly if multiple concentrations are being tested for several different soils. To reduce setup time, seed germination studies with contaminated soils previously conducted at ENSR Fort Collins Environmental Toxicology Laboratory were prepared by

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volumetrically measuring the amount of soil for each test chamber. When soils were hydrated to 85% of the WHC, inconsistent, highly variable results were observed. Excessive water was suspected to have adversely impacted seed germination, therefore, the primary objective of this study was to determine the effects of percent hydration on seed germination for four plant species.

MATERIALS AND METHODS

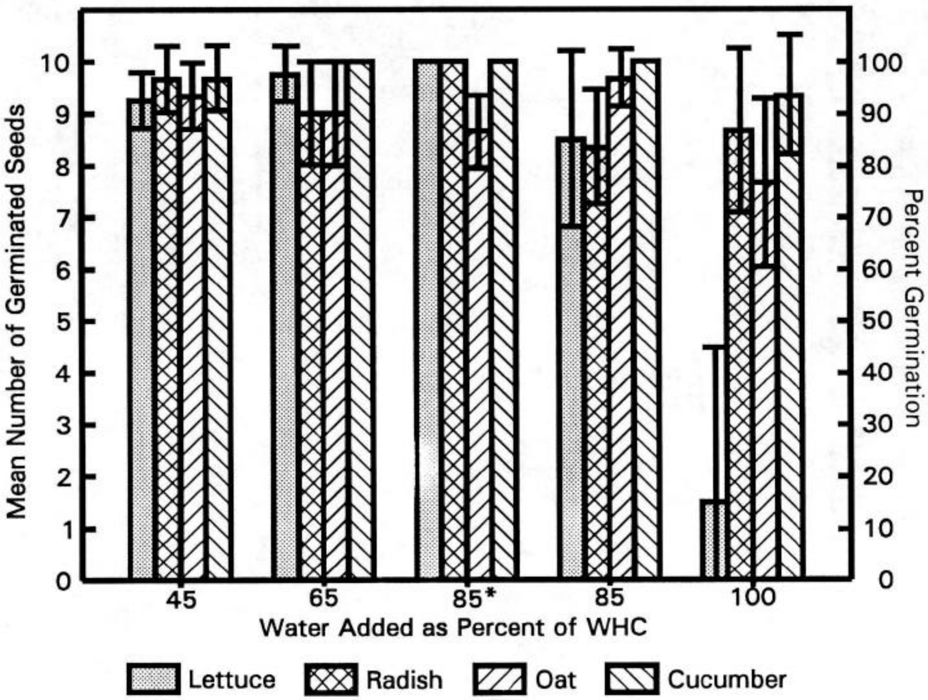
Four species were studied: lettuce (*Lactuca sativa* var. Buttercrunch), radish (*Raphanus sativus* var. Early Scarlet Globe), cucumber (*Cucumis sativus* var. Straight Eight), and oat (*Avena sativa* var. Nip). Washed silica sand (16-40 mesh) was used as artificial soil. Test chambers were plastic, 100x15 mm petri dishes. Five treatments were prepared for each species; four replicates per treatment. In four of the treatments approximately 50 grams of silica sand were added to each test chamber by filling a small beaker up to a predetermined line and then pouring the sand into the test chamber. In the fifth treatment 50 grams of silica sand, as measured on a top-loading balance, were added to the four test chambers. In the four treatments containing approximately 50 grams of sand, laboratory water (ASTM Type I, Milli-Q) was added by pipet, to equal approximately 45, 65, 85, or 100% of the WHC. In the fifth treatment, water equaling 85% of the WHC was added to the treatment containing exactly 50 grams of artificial soil. Ten seeds were added to each test chamber and the seeds were covered with 40 grams of 10-20 mesh silica sand.

Test chambers were placed in a plastic tray lined with wet paper towels. The trays were covered, taped shut, and placed into sealed black garbage bags. The covered trays were placed in an environmental chamber set at 25 ± 1°C for 48 h. After 48 h the trays were removed from the black plastic bags, the tops of the petri dishes were removed, and the uncovered dishes were returned to the trays. The trays were then put into clear, plastic bags for another 72 h. Lighting in the environmental chamber was 50 to 100 foot-candles with a 16 h:8 h light:dark cycle.

At the end of the study all test chambers were examined for germinated seeds, defined as any shoot that had visibly emerged through the cover sand. All treatments were compared to each other using analysis of variance with Tukey's multiple range test for studies meeting parametric assumptions or Kruskal-Wallis with Dunn's multiple range test for studies that did not meet parametric assumptions.

RESULTS AND DISCUSSION

Of the four species tested, lettuce was the most sensitive to excessive water. In the treatment containing water at 100% of the WHC, germination (15%) was significantly ($p \leq 0.05$) reduced, relative to the other treatments (Fig. 1); seeds germinated in only 1 of the 4 replicates. The best germination (100%) occurred in the 85% WHC treatment that contained exactly 50 grams of artificial soil; germination in the 85% WHC treatment that contained approximately 50 grams of soil was 85% (not significantly less than the other lettuce treatments). For the other three species, germination was not significantly ($p > 0.05$) reduced in



*** This treatment received exactly 50 grams of silica sand per chamber plus exactly 40 grams of cover sand. In all other treatments sand was added volumetrically and percent of WHC is, therefore, approximate.**

Figure 1. Mean seed germination (+/- 1 S.D.) of four species exposed to different volumes of hydration water.

the 100% WHC treatment, although oat germination was lowest in the high treatment. Variation among replicates was generally higher in the 100% WHC treatment and in the approximately 85% treatment.

Earlier studies with lettuce at ENSR using a hydrocarbon-contaminated soil had produced highly variable results (Fig. 2). This earlier study was rerun by hydrating the soil to 75% WHC (selected as an appropriate intermediate concentration between 65 and 85%). A different concentration series was used in the second round of tests. The results of that test were much more consistent, with a clear concentration-response curve, and reduced within-treatment variability (Fig. 3). All subsequent seed germination studies at ENSR have been performed using 75% WHC and have proven successful, with acceptable germination in the control and low variability between replicates.

Seed germination tests can be a valuable tool in the assessment of hazardous waste sites. Several researchers have used germination and root elongation studies to explore toxicity in contaminated soils at hazardous waste sites (Thomas et al. 1986; Nwosu et al. 1991; Hund and Traunspurger 1994). A major drawback of these studies is the time and effort needed to set the tests

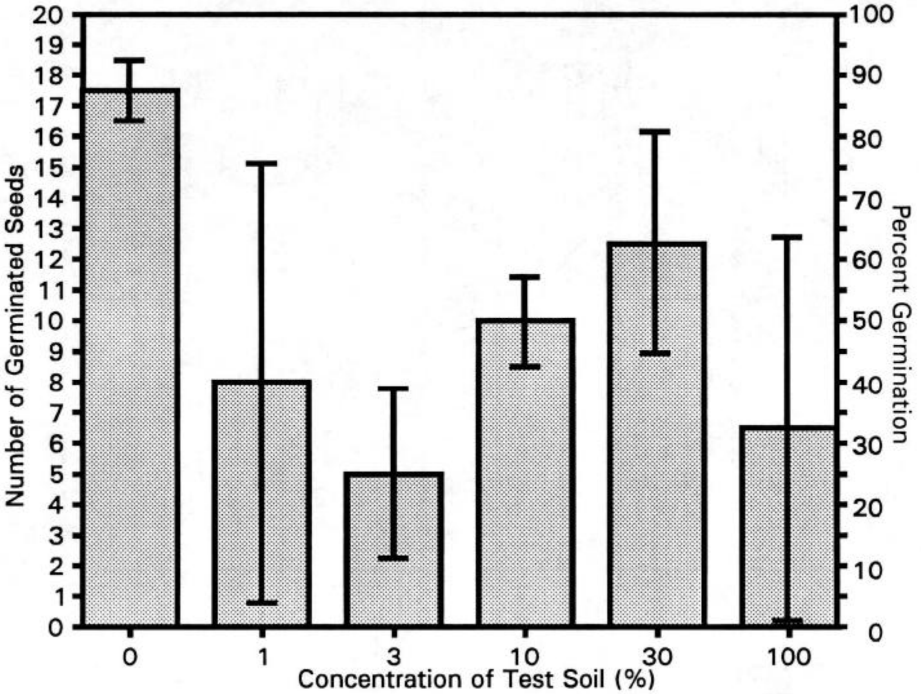


Figure 2. Germination of lettuce seeds (+/- 1 S.D.) in the first test exposed to hydrocarbon-contaminated soil. Soil was hydrated to 85% WHC.

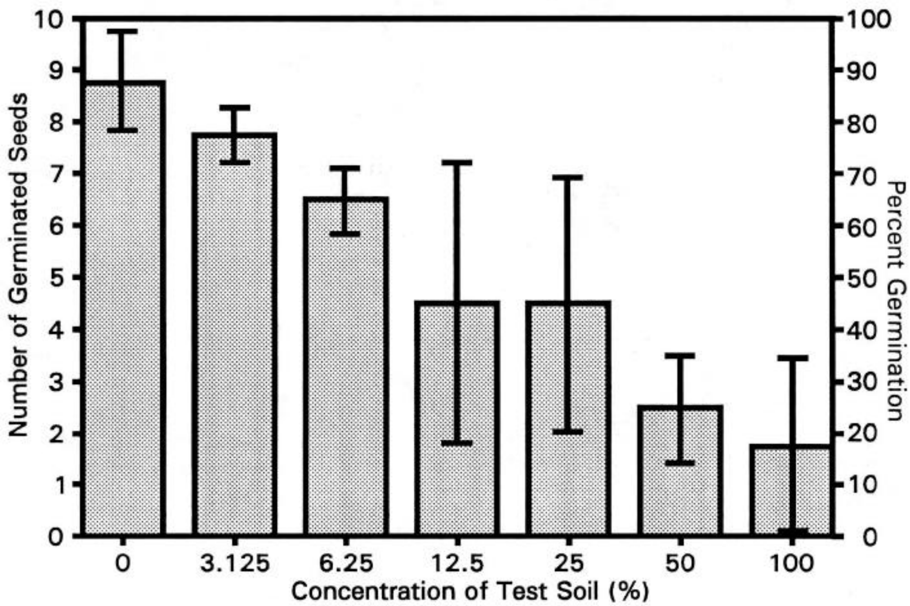


Figure 3. Germination of lettuce seeds (+/- 1 S.D.) in the second test exposed to hydrocarbon-contaminated soil. Soil was hydrated to 75% WHC.

up. If the amount of soil going into each test chamber is not measured out gravimetrically, then hydrating to 85% WHC may result in too much water in the test chamber, which can significantly reduce performance for lettuce. If the volume of water added to the test chambers is reduced from 85% to between 45 and 75%, seed germination rates for all four species are acceptable and the test soil may be measured out volumetrically rather then gravimetrically, thus reducing setup time. Most of the common species used for germination studies (Fletcher and Ratsch 1991) should perform well with lower moisture levels. Nwosu et al. (1991) got acceptable results using 45% WHC in field and laboratory studies with cucumber and wheat (*Triticum aestivum).*

Although “acceptable” levels of contaminants have been established for a variety of materials by different agencies and legislation (Beyer 1990), such levels are not universally applicable. For many contaminated soils, “clean” levels must be established on a site-specific basis. Toxicity tests are an important component in setting these levels. If the effort and associated cost required to run seed germination studies are reduced, these studies may become a more routine technique for assessing soil contamination.

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