

**RTDF TPH SUBGROUP COOPERATIVE
PHYTOREMEDIATION FIELD TRIALS
Plant Hydrocarbon Uptake Analysis Protocol**

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Objective:

Plants growing in petroleum hydrocarbon contaminated soil may increase ecological risk by creating potential pathways of exposure to petroleum hydrocarbons. This analysis seeks to document plant uptake of PAHs in the RTDF field trials.

Background:

PAHs in plant material can originate from a number of sources that are difficult to distinguish, including deposition of PAHs from ambient air, adsorption PAHs from contaminated soils, and plant uptake of PAHs. Based on a review of the literature, PAH uptake is expected to be small (Chaineau, 1997; EPRI, 1992, Wild et al. 1992). Carbon 14 studies show that most PAHs taken up by plants are confined to the roots (EPRI, 1993). It is difficult to distinguish between PAHs that are adsorbed to plant tissue surfaces and PAHs that are taken up by the plant. This is especially true for roots growing in contaminated soils. The potential for plant uptake is affected by the physical and chemical properties of the compounds such as the octanol:water partition coefficient and aqueous solubility. Two-ring and three-ring PAHs are more likely to be taken up than PAHs with four or more rings. It may be possible to compare PAH fingerprints to help distinguish between sources of PAHs such as plant uptake versus air deposition. Although plant uptake of PAHs during phytoremediation is not expected to be large, data is needed from phytoremediation plants plantings under varying contaminant and soil conditions to address the issue of potential contributions to ecological risk.

Considerations in developing the plant uptake sampling protocol:

1. The number of samples that can be analyzed from each RTDF site is limited due to the expense of analysis. RTDF participants agreed to take four plant tissue samples per site.
2. Plant tissue samples will be analyzed for PAHs and not for TPH.
3. A more detailed assessment of PAH uptake can be made at individual sites if resources are available to do additional analyses. Some suggestions for additional plant samples include:
 - a. Sample the same plant species from a nearby location where plants are growing in clean soil. This sample can serve as a control.
 - b. Sample individual plant species within a species mixture since PAH uptake may vary among species in a mixture.
 - c. Take plant samples from more than one replication of the RTDF trial.

4. Plant sampling should take place at the end of the third growing season to provide a common time to observe plant uptake at each experimental site and to sample at a time when plants are well established.
5. We assume volatile PAHs will not be recovered using this protocol. The weathered condition of the soils in the RTDF trials suggests volatile PAH concentrations will be low.

Plant Tissue Sampling Protocol:

1. Select the field trial replication with the highest concentrations of PAHs. The standard mix plot and the local option treatment plot from that replication will be sampled for PAH uptake.
2. At the Time 3 sampling at the end of the third growing season, flag the random sampling locations within the selected plots that will be used for soil sampling.
3. Prior to soil sampling, for plots with herbaceous vegetation clip a sample of biomass from each of the sampling locations. Clip sufficient biomass to obtain 100 grams of dry plant material. Take care to avoid sampling vegetation that has come into contact with the soil.
4. Store the samples at 4C until they can be rinsed and dried.
5. Rinse the aboveground plant material prior to drying to remove as much airborne dust as possible. Dry the samples in a drying oven at 40C.
6. Weigh the dried samples from each quadrat to obtain an estimate of biomass production.
7. Subsample and composite an equal quantity of plant material from each biomass to obtain 100 gm of dry sample. Store dry shoot material in labeled plastic bags and prepare for shipment to the analytical lab.
8. For tree plots, determine a sampling method to obtain 100 gm of dry sample. Most likely this should be leaf material. Composite samples and dry as in step 3.
9. At the same locations where aboveground tissue is sampled, sample roots from 0-15 cm to obtain sufficient root material to obtain 100 grams of dry plant material. Prior to washing store the samples at 4C.
10. Develop a system for washing the root material within 24 hours of sampling. Rinse the roots with water. Repeat root washing to remove as much soil as possible. Use a dilute detergent solution or a surfactant like sodium hexametaphosphate to remove soil adhering to the root surfaces.
11. Dry the root samples at 40C. Store dry root material in Ziploc bags. Prepare for shipment to the analytical lab.

Plant Tissue Analytical Protocol:

1. Dry plant tissue samples will be shipped to the contracting laboratory.
2. For the purposes of the RTDF trial, it is assumed that holding times will not be critical for the analysis. Therefore, dry plant tissue samples will be stored frozen at the laboratory prior to analysis.
3. Plant tissue samples will be analyzed for the extended list of PAHs.

4. Extraction and GC analysis techniques will be based on the laboratory's previous experience with plant tissue samples and consultation with protocols developed by EPA (Banks et al. 2000; Nadeau, personal communication).

References:

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