Appendix 1: SOIL SAMPLING PROTOCOLS FOR GEOCHEMICAL LANDSCAPES PILOT STUDIES – 2004

General comments regarding criteria for site selection:

1. Avoid sample sites nearer than 5 miles (8 km) downwind for known power plants, smelters, etc.
2. Avoid sample sites closer than about 200 meters from major highways.
3. Avoid sample sites closer than about 50 meters from rural roads.
4. Avoid sample sites closer than 100 meters from buildings.
5. Do not sample nearer than about 50 meters from end rows or other areas where large amounts of fertilizer could have been deposited.
6. Avoid any atypical or unusual landscapes, landforms, excessively disturbed ground, etc. (For example, mine dumps, landfills, construction sites.)

At each randomly selected site, the actual location where the sample is collected may be constrained by access. Common sense should prevail. Always request permission to sample on private land. Once an area is selected, determine a specific site that approximates the average landform of the area in view. For example, if you are in an area of mostly forested slopes and a few open fields, sample from the forested slopes. For sites within the United States, use the NRCS County Soil Survey maps and reports, if available, as a guide for site selection, identification of soil series sampled, and horizon depth and descriptions. Take several digital photographs of the area to be sampled, both of the surrounding area and the ground at the specific site. At each area, determine where each sample will be taken, and preserve the site until the sample is collected (i.e., do not walk all over the area to be sampled).

Field Form:

Filling out the field form is straightforward. Be neat and write legibly because all the information will be entered into a database.

1. Indicate what samples are collected at each site by checking the box.
2. The "Photographs From" field will be filled in if digital photographs are taken at the site. It is useful to include a piece of paper or a dry-erase board with the site number in the photos, if possible. Alternatively, take a "close up" of the piece of paper or dry-erase board showing the sample number immediately before a series of photographs of the site.
3. Latitude and longitude are collected as **decimal degrees**. Set up GPS accordingly.
4. Standardize coordinates on GPS to Map Datum WGS 84. If another map datum is preferred for an individual country, coordinates should be converted to WGS 84 for reporting purposes.
5. The "Landuse" field should include information related to cropland, range, etc. For example, Cropland, Rangeland, Wetland, etc. A complete list of landuse options is found in the following NRCS publication: *Field Book for Describing and Sampling Soils, September 2002.*
6. The "Landcover" field should include information related to the type of crop or range

site. For example: Sugarbeets, Wheat, Corn, Hayland, silty rangesite, Wetland rangesite.
7. Lots of room for comments – please comment liberally, as something of significance may be recorded inadvertently.

Samples to be collected:

At each site for the continental-scale N-S and E-W transect, the following samples will be collected:

1. O horizon (if present) for chemical analysis – 1 sample bag (approximately 1 kg)
2. A horizon for chemical analysis– 1 sample bag (approximately 1-2 kg)
3. C horizon for chemical analysis– 1 sample bag (approximately 1-2 kg)
4. Composite of the A horizon (using sterile collection methods) for microbial characterization – three sterile 50 ml centrifuge tubes
5. Composite of the A horizon for determination of soil moisture– 1 16 ounce (about 500 ml) plastic or metal container with screw top. The container must be watertight.
6. The top 5 cm of soil (regardless of horizon) for chemistry – 1 sample bag (approximately 1 kg)
7. The top 5 cm of soil (regardless of horizon) for analysis of organic compounds – 1 500 ml glass jar

The order in which the samples are collected is best determined by the individuals sampling, and conditions at the site. At the first site of the day, it may be useful to take the samples for organic compounds and microbial characterization first, so a stainless steel bowl, trowel, and sieve are then available for all additional sampling. Weigh each of the stainless steel bowls and write the weight on the bowl – this is useful if the bowl is used to weigh soil samples.

Soil sampling protocol for the O horizon, A horizon, and C horizon.

For the purposes of this project the following definitions are used:

O horizon – Surface accumulations of mainly organic material; may or may not be, or has been saturated with water.

A horizon – Accumulation of humified organic matter mixed with mineral fraction; the latter is dominant. Occurs at the surface or below an O horizon.

C horizon – A subsurface horizon, excluding consolidated bedrock, like or unlike material from which the soil formed, or is presumed to have formed. Lacks properties of A and B horizons, but includes materials in various stages of weathering.

(From Soil Survey Division Staff [1993])

Equipment needed:

Standard soil bucket auger, with one or more extensions
Stainless steel or plastic trowel (2-3)
Plastic tarpaulin
Hubco sample bags (approximately 7 inches [17.8 cm] wide x 12.5 inches [31.8 cm] deep)
Sharpie permanent markers
Portable scale (for example, MyWeigh briefcase scale)
Pinto beans (approximately 5 kilograms)
Plastic garbage bags
Tent stakes (4) and twine or string
Stainless steel bowl (big enough to hold 2-3 kilograms of pinto beans)
Soil pH meter (optional)
Shovel (sharpshooter or similar long-nose variety)
Rock hammer
Knife
2-mm stainless steel sieve
Zip lock bags (1 gallon)
Handheld GPS unit
Digital camera
Extra batteries for GPS, camera, and scale

For the O- and A-horizon samples, it may be useful for some purposes to convert from concentration data to loads (i.e., from ppm to grams/meter^2 or grams/meter^3). For this reason, we describe a volumetric sampling procedure for those horizons.

Volumetric sampling of O-horizon or organic litter: (Because the thickness of the O horizon is frequently very small (1-2 cm) and so compressible that the thickness is difficult to determine accurately, we get a weight of O per unit area rather than density. This still gives us the ability to calculate the amount of element per unit area.) To volumetrically sample the O horizon, at each site lay out a 60 cm by 60 cm area (about 2 ft^2), using tent pegs to mark the corners and outline the measured area with string or twine. Record the actual area on the field form. Use a knife to cut into the soil along the margins of the string to restrict sample material to measured area. Within this area, collect all the O-horizon or litter material lying on the mineral soil; **exclude live vegetation, woody debris, cones, droppings, charcoal, and roots.** Place all collected material on a plastic garbage bag and pick through by hand, cleaning and homogenizing, to remove any remaining wood or live plants. The distinction between the O-horizon and mineral soil can be somewhat arbitrary, but is often based on feel (grainy [mineral] vs. soft [organic]) and hand lens observation. If the O horizon is thick, measure the approximate depth at several points in the area to help calculate the volume of material removed. Record this information in the comments section of the field form.

All of the organic material collected from within the square is weighed, with the **total weight** recorded on the field form. A representative portion of the O-horizon material is then collected into a Hubco sample bag or similar container. If the amount of material from within the square does not fill the bag, additional comparable material may be collected from nearby. About 200-300 grams of material is required for chemical analysis plus additional material is needed for archival purposes. If possible, collect about 1 kg of O-horizon material. Label the sample bag with the site number and "**O-horizon/litter**". **Weigh the sample bag and record on both the field form and on the bag.** Do not subtract the weight of the sample bag – this can be checked later. Prior to submission for analysis, the air-dried sample bag will be reweighed and a final weight for the entire O-horizon sample within the measured area is recalculated based on the % weight change from wet to dry, and from the original bulk weight. **Do not store O-horizon/litter sample in a plastic bag as it can mold.**

Volumetric sampling of the A horizon: The A horizon is usually thick enough to permit an accurate measurement of depth to the A-B boundary. For this reason, volumetric sampling of the A horizon uses the weight and volume of soil collected to calculate a bulk soil density. This requires an accurate measurement of the depth of the A-horizon, preferable accurate to less than 2 cm. The A horizon can be collected from the area used for collection of the O horizon, or from anywhere considered to be typical. Remove any living plants. If the sample is collected from inside the O-horizon square, start in the middle of the square. Using a shovel and/or trowels, collect the A horizon down to what is determined to be the top of the B horizon, a distinction usually based on color change. In areas of thick A horizons, it is efficient to dig a small hole using the shovel or trowel, and then carefully auger to the base of the A, checking often for color change. This creates a fairly small volume suitable for the measuring by the cavity compensation method described below.

Place **all** soil from the sampled volume onto a plastic garbage bag or into a stainless steel bowl. This bulk soil sample is then weighed on a portable field balance—if weighed in a bowl, note the weight of the bowl. If rocks, roots, and woody debris represent a significant proportion of the volume, **sieve** the sample using a 2-mm stainless steel sieve. Sieve the entire quantity of A-horizon soil removed and weigh the sieved soil, entering the value onto the field sheet. If it is not possible to sieve (e.g., too wet or too much clay), or if there would be little difference between the sieved and unsieved weights, then estimate the approximate volume that was occupied by any rocks or roots that were removed—this will make the volume calculation more accurate. In other words, estimate what percent of the volume (not weight) was occupied by things other than soil. make a note on the field form of actions taken for the A-horizon sieving.

The volume of the A-horizon is calculated by a method known as *cavity compensation*. This involves filling the excavated hole with a material whose weight-to-volume ratio is known. This material is then removed from the hole, weighed, and the volume calculated. We have found that dried pinto beans work very well for this purpose. First, weigh the container with all the pinto beans to establish a baseline. Line the excavated

hole with a plastic bag (if the hole has been augered, make sure the bag goes all the way to the base of the hole). Pour the beans into the excavated hole until the beans are level with the original surface. Weigh the container with the remaining beans to determine the weight of the beans filling the hole by difference (do not weigh the beans in the hole). This process should be repeated two more times to obtain an average weight. It is not necessary to completely remove all the beans from the hole. Remove several handfuls of the beans from the hole and place them back in the container. Now refill the hole to the surface and weigh the container to determine the weight of the beans in the hole by difference. Record the measured weights of the beans remaining in the container on the field form—you can subtract this from the total weight of the beans to determine the weight of beans in the hole and then obtain the average later. When the arithmetic is completed, the calculated weight of beans in the hole will be entered on the field form. This weight can be extrapolated into a volume by a formula by a formula determined in the lab by multiple weighings of beans in fixed volumes. This can be accomplished using a large graduated cylinder or similar container with graduated volume markings.

Collect a representative subsample of the A-horizon material from the bowl or plastic bag and place into a sample bag—about 1-2 kilograms is required. Label the sample bag with the site number and **A horizon. Weigh the sample bag and record the weight both on the field form and on the bag label.** Do not subtract the weight of the sample bag – this can be checked later. **Measure the depth interval of the A horizon (assumed to start at 0 centimeters) and record the depth interval on the field form – accuracy is important!** Any left-over soil should be used to refill the hole when all sampling is complete.

Prior to submission for analysis, the air-dried sample bag will be reweighed and a final weight for the entire A horizon is recalculated on the % weight change from wet to dry, and from the original bulk weight.

C horizon: No volumetric sample is required. The C-horizon sample is collected from the A-horizon hole or from a separate hole using a bucket auger, shovel, or trowel. Auger or dig to the best depth estimate for the C horizon, or until bedrock is encountered. For sites in the United States, the NRCS soil survey data may be used as a guide. In especially deep soils, dig as deep as possible, at least 1 meter—more if possible. This may require some maneuvering around smaller rocks, or angle augering. Try to ensure that the shovel hole is free of A-horizon material, which may become smeared through the C-horizon sample by the auger. If necessary, each auger volume should be laid out on a plastic tarpaulin or plastic bag in order to observe color change to aid in identification of the different soil horizons. Collect a representative portion of the C horizon into a sample bag (1-2 kilograms) and label bag with the site location and **C horizon**. Remove as many of the rocks as practical—the more rocks, the smaller the amount of sample remaining after sieving. On the field form, record the depth interval from which the sample was collected.

Soil sampling of the A horizon for microbiological characterization

Equipment needed:

Sterile 50-ml centrifuge tubes with screw cap (3)
Rubbing alcohol
Nitrile gloves
Sterile stainless steel bowl (can be same bowl used in organic sampling, just be sure to clean and sterilize with alcohol)
Sterile stainless steel (or plastic) trowel
Cooler with blue ice or freezer for storing samples
Cooler with blue ice for shipping samples
Strapping tape for sealing cooler for shipping
Sharpies
Large zip lock (3 gallons or more) bags or plastic storage containers for holding sampling equipment for one site)
Small zip lock sandwich bags for storing centrifuge tubes after sampling
Kimwipes

The important consideration in sampling soil for microbiological characterization is to use only sterile tools and sample containers and to wear Nitrile gloves during sampling. If a hole from which the sample is to be taken was dug with a non-sterile shovel or auger, then the sides of the hole should be scraped "clean" with a sterile stainless steel (or plastic) trowel prior to sampling. This is to eliminate any cross-contamination from previous holes dug with the same shovel or auger. If the same trowel is to be used for collecting the sample, then it should be re-sterilized by wiping with rubbing alcohol.

A composite of the A-horizon soil is the sample medium for microbiological characterization. If the A horizon is shallow, then the soil may be sampled directly using a sterile stainless steel (or plastic) trowel. If the A horizon extends to a depth beyond which can be easily reached with the trowel, then a hole should be dug with a shovel or auger. If this is the case, then scrape the sides of the hole from which the sample will be collected with a sterile stainless steel (or plastic) trowel to avoid contamination from previous holes dug with the same shovel or auger. Using a sterile trowel, place the soil into a sterile glass or stainless steel bowl to homogenize. Then place the homogenized material into three (3) sterile 50-ml centrifuge tubes (or similar sterile containers). Fill each tube as full as possible. We normally place the three tubes from each site into a zip lock plastic bag for storage in a cooler or freezer. The samples must be kept cold from the time of sampling. If a portable freezer is available, place the samples there and freeze. If a freezer is not available, place the samples into a cooler and keep on ice. Unless a freezer is available, the samples should be sent on ice in a cooler by overnight mail so they arrive at the laboratory within three days of collection. If a freezer is available, then the samples can be sent to the labs when convenient.

Two of the centrifuge tubes should be sent to:

Dale Griffin
U.S. Geological Survey
600 4th St. South
St. Petersburg, FL 33701
727-803-8747, ext3113

One of the centrifuge tubes should be sent to Kate Scow's laboratory in care of her post-doctoral assistant, Rebecca E. Drenovsky:

Rebecca E. Drenovsky
Dept. of Land, Air, & Water Resources
University of California, Davis
PES Building
One Shields Ave
Davis, CA 95616
(530) 752-0146

Soil sampling of the A horizon for determination of moisture content

Equipment needed:

16 ounce (500 ml) polypropylene jars or similar watertight container (can be plastic or metal)
Trowel
Shovel
Plastic garbage bag
Portable scale

Collecting a sample of the A horizon for determination of soil moisture requires no special equipment other than a watertight container. A 500 ml (16 ounce) or similar size is adequate. Simply take a composite of the A horizon using a trowel or shovel and place a representative sample into the watertight container. It is probably advisable to weigh the container plus soil on site and to record this weight on the field form and on the jar. Assuming no moisture is lost from the container, this weighing can be performed in the laboratory as well. Once in the laboratory, the soil will be dried and weighed; the moisture content being the difference between the wet weight and the dry weight.

Soil sampling of the top 5 cm (0-5 cm)

Equipment needed:

Stainless steel or plastic trowel
Hubco sample bags (approximately 7 inches [17.8 cm] wide x 12.5 inches [31.8 cm] deep)
Plastic garbage bag or stainless steel bowl

Portable field scale

The topmost 5 cm of soil is the preferred sample medium of the public health community. This is the material that people most often come into contact with when walking, sitting, lying, or playing on the ground. Thus, this is the most important medium for health issues concerning chronic exposure to potentially toxic elements or compounds in soils. Volumetric sampling is required to convert concentration data to loads (ppm to grams/m^2 or grams/m^3). If the 0-5 cm interval is entirely within the A horizon, then the density calculated for the A horizon (using the cavity compensation method explained above) can be applied to this sample and no additional volumes need be determined. If this is the situation, simply excavate an area down to 5 cm and place the material in the sample bag, removing roots and rocks. About 1-2 kg is needed. Label the bag with the site name and **0-5 cm Environmental Health.**

However, if the 0-5 cm interval includes the O horizon, then the density of the mixed material must be determined by volumetric sampling. For the volumetric measurement by the cavity compensation method, follow the procedure described for the A-horizon sample. In short, excavate a volume down to 5 cm and put the soil onto a plastic bag or into a stainless steel bowl. Calculate the volume of the hole using the pinto bean method described for the A horizon. Weigh **all** of the soil removed from the hole and record the weight on the field form. Place the sample into a sample bag and weigh the bag, recording the weight both on the field form and on the bag. Label the bag with the site name and **0-5 cm Environmental Health.**

Soil sampling for organic compounds (0-5 cm)

Equipment needed:

500 ml baked amber glass jars
Stainless steel 10-mesh (2-mm) sieve
Stainless steel trowel
Stainless steel or glass bowl
Heavy-duty aluminum foil
Pesticide-grade or residue-grade methanol
Phosphate-free detergent
Large zip-lock plastic bags (3 gallon or more) or plastic containers (for storing and transporting cleaned equipment.
Labels
Cooler with blue ice or portable freezer
Cooler with blue ice for shipping samples
Strapping tape etc. for sealing cooler
Sharpies or similar permanent marker pens
Bubble wrap for cushioning jars during shipping (can use newspapers or foam worms)

The important point in taking soil samples to be analyzed for organic compounds is to only use equipment made of stainless steel, Teflon, or glass. All the equipment should be cleaned prior to sampling by washing in water and a phosphate-free detergent such as Liqui-Nox (available from Fisher Scientific) and then a final rinse with pesticide-grade or residue-grade methanol. The cleaned sampling equipment is then wrapped in heavy-duty aluminum foil and stored for transportation to the field in either a large (3 gallon more or less) zip lock plastic bag or in a plastic (e.g., Rubbermaid) storage container. It is certainly an acceptable alternative to clean all the equipment between each site, but it is much easier to do all of this in a motel room where you have a bathtub or sink and plenty of water. We have been preparing four separate sampling "kits" prior to each day in the field.

The sample is taken from 0-5 cm regardless of what horizon this might be. Using the properly cleaned stainless steel trowel, take soil from 0-5 cm and place into the cleaned stainless steel 2-mm sieve. Sieve the material into a cleaned glass or stainless steel bowl and then transfer into the 500 ml baked glass jars. Try to fill the jar reasonably full. Label both the jar and the screw cap, put the cap in place and then put the jar into either a portable freezer or cooler. It is important to keep the sample cold. If a portable freezer is not available, then the samples must be shipped by Federal Express or other overnight service so that the samples are in the lab where a freezer is available within three days of collection. If a portable freezer is available, then keep the samples frozen until returned to the lab or until it is convenient to ship to the labs by Federal Express. The samples can be sent to:

David B. Smith
U.S. Geological Survey
Denver Federal Center, MS 973
Denver, CO 80225
(303) 236-1849