Appendix 1. U.S. Geological Survey soil sampling manual for the North American Soil Geochemical Landscapes Project

The Soil Sampling Manual was provided to all field crews engaged in collecting soil samples during this project and provides the basis for standardization of the sampling protocols. See report text for details about the study. The manual is shown as it was provided to the sampling crews; some minor editorial changes have been made, but it has not been formatted to conform to U.S. Geological Survey publication standards.

Target Area Selection:

With the assistance of the U.S. Environmental Protection Agency, a spatially balanced array of 4,857 target sites has been established for the conterminous United States using a generalized random tessellation stratified (GRTS) design. Pre-selected GRTS coordinates are supplied to field crews before the initiation of sampling and provide the starting point for characterizing sample target areas and selecting the exact sample site based on considerations outlined in the following sections. The ***target area*** for each sample is defined as a 1-km^2 area centered on the GRTS coordinates.

Final Sample Site Selection:

Within the 1-km^2 target area, soil samples should be collected in the most representative geomorphic setting. For example, if the target area is characterized by rolling hills and valleys, the samples should be collected from near mid-slope and not from the hill top or the valley bottom. In addition, the set of broad criteria below should be considered in the final site selection:

1. Avoid sample sites within about 200 meters from major highways.
2. Avoid sample sites within about 50 meters from rural roads.
3. Avoid sample sites within about 100 meters from buildings.
4. In agricultural fields, do not sample within about 50 meters from end rows or other areas where abnormally large quantities of fertilizer could have been deposited.
5. Avoid sampling less than 5 km downwind from active major industrial activity, such as power plants or smelters.

However, in the practical world of field sampling, conditions arise in which access to the sampling site is difficult or impossible because of physical or legal considerations or the criteria cited above cannot reasonably be met. In such instances, an alternate site must be selected that represents the best compromise between the practicality of field operations and the preserved integrity of representative and unbiased sampling. In all cases, the landscape characteristics of the alternate site should, if possible, be similar to the original randomly selected target area.

In some cases, an inability to sample the primary target area is evident prior to field operations. Tracts of land such as military bases or tribal lands may be determined to be inaccessible during the planning phase of field operations, or air photos and maps could indicate that a target area is obviously accessible only with difficult and time-consuming overland travel. In such cases, available landscape maps can be used to compare the original area with the surrounding region to identify an alternate target area that matches the original one as closely as possible. The professional judgment of field crews is critical in this step, and the final sampling site requires a compromise between the practical aspects of timely progress of the survey and the integrity of the sample design. An alternate site may be as far as 20 km from the original GRTS site in some instances.

Digital Photos:

One or more wide-view digital photos should be taken at each sample site to capture the sense of the landscape and vegetation cover around the site. It is essential to include some indication of

the sample site number in the photo for archiving. One method that works well is to take a photo of a piece of paper or dry-erase board with the site number written on it and then follow with the series of photos at that site. This creates a visual tag for each site and makes cataloging the photos easier. When sampling is completed and the field form is fully filled out, take a close up photo of the field form to provide a digital back up for the paper form.

Field Form:

Sampling at each site requires completion of a paper field form (shown at the end of this manual). The paper field forms will be scanned for inclusion in the final database, so legibility is a requirement.

Each sample site is identified by the state *(line 1)* and the GRTS number *(line 2)*. The soil samples collected at each site will be designated by writing the field sample number (same as GRTS number) on the sample bag, followed by the soil sample name (PH [our abbreviation for soils collected from a depth of 0 to 5 cm], A, or C). It is also helpful, but not essential, to indicate the state on each sample bag. For example, the notation on a sample bag "10271A CA" would identify the A-horizon soil collected from sample site (GRTS number) 10271 in the state of California.

The latitude and longitude of each site is determined in the field at the actual place where the sample is collected (not the GRTS latitude/longitude) using GPS units set to WGS84 Datum, entered as decimal degrees to the full accuracy of the unit *(line 4)*. The month, day, and year the sample was collected and weather conditions are entered on *line 5*. The soil moisture content of the sample is indicated on *line 6* as either dry, moist, wet—nonsaturated (meaning that water films are present as seen by a glisten on soil particles but no free water is observed), or wet—saturated (meaning that free water is present). The last names of all the field crew members collecting the sample are entered on *line 7*.

The "Landscape/Landform" entry (*line 8*) refers to unique landscapes (broad assemblages or natural, spatially associated features) and landforms (discrete, natural, individual features mappable at common survey scales) observed in the target area. Examples of these include alluvial fan, beach, canyon, coastal plain, delta or delta plain, till plain, hill or hill slope, landslide, loess bluff, mesa, marine/river/stream terrace, lake plain, flood plain, lowland, mountain valley, valley slope, valley floor, mountain slope, river valley, or other broad terms that describe the geomorphic setting of the sample site.

Each sample site is either flat or sloped; for all sites that are not flat, circle the descriptor that best approximates the degree of slope at the sample site *(line 9)*. For "Slope Aspect" *(line 10)* (the principal compass direction that a slope faces, looking down slope) and "Slope Position" *(line 11)* (the parts of a line segment along a transect that run up and down the slope), circle or mark the appropriate entry that describes the sample site's setting.

"Land Use" *(line 12)* refers to the dominant land use, such as agriculture, grazing/pasture, urban, recreation, or fallow land.

"Land Cover" *(line 13)* is the dominant vegetation at the sample site. This description should include the larger site area as well as the specific site where the sample is taken. Possible options include the following:

Agricultural crops—Cultivated field crops. If known, note type of crop being grown as well as condition of crop (e.g., harvested, recently planted, corn 2 ft high, etc.)

Coniferous forest—Dominated by needle- and cone-bearing species. Note tree species (pine, fir, etc.) if known. Also note the base diameter (diameter 2 feet above ground) or range of diameters as an indication of stand age.

Deciduous forest—Dominated by broadleaf species. Note tree species (oak, maple, etc.) if known. Also note the base diameter (diameter 2 feet above ground) or range of diameters as an indication of stand age.

Grassland—Perennial native grassland or improved pasture.

Lichen—Dominated by lichens with significant amounts of mosses.

Mixed forest—Composed of both coniferous and deciduous tree species. Note species if known. Also note the base diameter (diameter 2 feet above ground) or range of diameters as an indication of stand age.

Parkland—A forest-grassland transition consisting of a mosaic of aspen stands interspersed with patches of cropland, grassland, and meadow.

Shrubland—Dominated by shrub species.

Tundra—Treeless or shrub-dominated terrain occurring at high altitudes, immediately above the forest zone.

Unvegetated surface—No vegetation observed.

Meadow—Dominated by sedges and cotton grass.

"Other Anthropogenic/Natural Features in area" *(line 14)* would include any details that could potentially influence soil chemistry, such as the presence of fixed irrigation, animals in an enclosed field, fencing, silos, wind turbines, prospect pits, transmission lines, or a tree farm.

The "Comments/Sketch" area *(line 15)* is for any information about the site that was not captured by the previous entries that may be relevant for soil chemistry. This can also include a sketch of the site area and (or) a sketch of the soil profile if desired.

Soil Samples Collected:

The following discussion provides descriptions of a standard set of samples that are collected at an ideal site and are applicable to a majority of sites in North America, generally consisting of

upland soils at mid-latitudes. Sampling is accomplished with a variety of hand instruments (trowels, shovels, augers) appropriate to the specific conditions of the site. Three sample types represent the basic set of samples that are to be collected at each site for inorganic geochemistry (with exceptions noted below). Those basic samples are soils from 0 to 5 cm in depth (abbreviated in this study as "PH"), the A horizon, and the C horizon. In addition, a separate sample of 0–5-cm soil is to be collected at each site for determination of *Bacillis anthracis* (anthrax) and other soil pathogens. Additional protocols are used for more specialized conditions such as wetlands, permafrost, and urban areas.

0–5 cm—collect about 2 kg of sample. Regardless of mixture of horizons or material, a composite of all soil (mineral and (or) organic) present from 0 to 5 cm, with 0 referring to the upper soil surface. The soil surface is considered to be the top boundary of the first soil layer that can support plant/root growth. This equates to:

1. for bare mineral soils: the air/earth interface

2. for vegetated mineral soils: the upper boundary of the first layer that supports root growth, excluding both freshly fallen plant litter (L horizon) and litter that has compacted and begun to decompose but remains somewhat recognizable (F horizon), but including decomposed organic material (H horizon).

At each site, collect a separate sample of the 0–5-cm material for determination of soil pathogens by filling a sterile 50-mL centrifuge tube.

A horizon—collect 2 kg of sample. Mineral soil formed at the soil surface or below an O horizon (if present), with little remnant rock structure, and both or either:

1. accumulation of humified organic matter but dominated by mineral matter, and not E or B horizons

2. cultivation properties (Ap).

The collected sample is a composite of the entire thickness of the horizon.

C horizon—collect 1 kg of sample. Mineral soil little affected by pedogenesis and lacking the properties of O, A, E, or B horizons. May or may not be parent material for overlying soil. Where soil profiles are relatively thin, the C horizon can be reached with hand instruments within the upper meter of the soil profile. In those cases, the collected sample consists of approximately the upper 15–20 cm of the C horizon. In thicker profiles, where the true C horizon cannot be reached within the upper meter, the sample consists of a composite of the first 15–20 cm of soil below about 1 m depth. In these cases, the sample is recorded on the field form as not being a true C-horizon but is included with other C-horizon samples for processing and analysis.

Equipment needed for sampling 0–5-cm, A-horizon, and C-horizon soil samples:

Handheld GPS unit set to decimal degrees and WGS84 Datum

Compass

Digital camera

Standard soil bucket auger with one extension—preferably 3-in bucket diameter

Steel heavy-weight, long-nose shovel (e.g., Montana sharpshooter)

Mason trowel

Large plastic sample bags for mineral soils—10 in x 18 in, 4 mils thick

50-mL centrifuge tube (for soil pathogen sample)

Tape for sealing soil sample bags

Small knife with sharp blade for sampling organic layers

Pruning shears for cutting roots and litter, if needed

Folding wood rule or tape measure, preferably in cm, for measuring soil depths

Plastic garbage bags

Cleaning cloths or tissues

Extra batteries for GPS unit and camera

Dry-erase board for sample labels for photos

Black permanent markers for writing on sample bags

Black markers for writing on dry-erase board

Suggested Onsite Procedures—Step-by-Step (Modify as needed)

1. All tools used during the sampling procedure must be cleaned before using to avoid any possible cross contamination. Any painted equipment must have the paint removed prior to sampling.

2. Proceed to the pre-selected potential site location, noting vegetation cover, exposed bedrock, possible sources of contamination, etc., in the vicinity of the selected location. Ideally, the collection of samples will take place at the designated site. If the site is not easily accessible (e.g., requires a 4-hr hike from a jeep trail) or unsuitable (e.g., someone's backyard,

landowner unavailable, etc.), select an alternate site preferably within 1 km of original site location, if possible, following guidelines stated previously (>200 m from highways, etc.). Recognize that in some (many?) cases, the alternate site may be several km from the target site because of access issues.

3. Take a digital photo of the field number on a dry-erase board or piece of paper to record the sample site number followed by photos (usually 2–4) of the field site that capture the typical landscape/land use/land cover.

4. At the site, use the auger to obtain several short soil profiles in the area by boring into the ground to the depth of the top of the auger bit (about 25 cm), removing the bit from the hole with the soil core in place and placing this core onto a plastic bag or tarpaulin. These short cores will help identify a preferred sampling site, avoiding large rocks or buried wood. This core also provides information on the upper soil profile, such as thickness and nature of forest floor organic material and underlying mineral soils. In forested sites, the surface soil is often composed of forest litter in various stages of decomposition, including relatively fresh litter (L horizon); partly decomposed, recognizable plant residues (F horizon); and well decomposed plant residues that are not recognizable (H horizon). Thin mineral A horizon is often underlain by a light-colored E horizon (mineral soil leached of Fe, Al, clay or organic matter). Recognizing the presence and thickness of each of these horizons prior to sampling will facilitate collection of the A-horizon sample.

5. The first sample to be collected is usually the A horizon. The mineral A horizon is the desired sample target, but at some sites, the thinness of the A horizon will make collecting sufficient sample material difficult. If, from the auger cores described above, it is apparent that the A horizon is thin and irregular, then only material from the L and F horizon (recognizable plant material) should be cleared and the humic material (organic residue) will be collected with the mineral A-horizon soil—**do not include AE material with the A-horizon sample**. Collect about 2 kg of soil—if the A horizon is thin, a large spatial area may need to be excavated. Estimate the relative percent of humic vs. mineral soil in the final sample and enter on field form. If the A horizon is sufficiently thick, collect only mineral A-horizon soil. Remove any large rocks or pebbles from the sample before putting it into a labeled plastic bag. If the soil has a plow zone (Ap horizon), composite the entire Ap zone. Note the depth interval from which the sample was collected on the field form.

6. The next sample to be collected is a C horizon. Dig or auger through the area where the A horizon was removed down to the C horizon or to a depth of about 1 m, if possible. Successive auger barrels or shovels of soil can be laid out on a plastic sheet or bag to help define soil profiles. The depth of the C horizon is dependent on the nature of the parent material and soil development—often an approximate depth can be inferred from soil series descriptions for the site, from color changes observed during augering, or from reaching bedrock beneath shallow soils. In the ideal case, the C horizon can be reached within the upper meter of the soil column, but there are many exceptions. Where the top of the C is reached at depths less than 1 m, the collected sample should be approximately the uppermost 15–20 cm of the C horizon. In deeper soil profiles, where the true C horizon cannot be reached within the upper meter, the sample consists of a composite of the first 15–20 cm of soil at about 1 m depth. If using the auger to collect the sample, after each bore (into the

same hole), discard the top few centimeters of material from the auger bit before putting into bags to remove any material that may fall back into the hole. The sample is hand-picked to remove larger stones and placed into a plastic bag. The depth interval of the sample is recorded on the field form.

7. For the PH, or 0–5-cm sample, work within an undisturbed area. This sample is often best collected from the side of the hole excavated for the A-horizon sample. If organic soils are present, clear away the L and F horizon material to expose H horizon material or upper mineral soil (usually A or AE horizon). Measure 5 cm below the exposed surface (i.e., the 0 centimeter level) and slide a flat trowel into the pit face to provide the base of the sample. Collect all soil above the trowel, moving the trowel as required to obtain sufficient quantity of sample. For the sample for pathogen determination, fill a 50-ml centrifuge tube with soil from 0 to 5 cm and label the tube with the sample site number. Check the box on the field form to indicate the sample was collected.

8. After collecting all samples, make sure the field form is complete, the photos were taken, and the bags and centrifuge tube are labeled. Take a close up photo of the completed field form making sure that all entries are legible on the photo. Fill in the holes to leave minimum disturbance. Move on to next site and repeat the procedure.

Samples for Microbial Characterization

This section describes the protocol for soil microbial sampling as part of the North American Soil Geochemical Landscapes project. Note, this is in addition to the sample of 0–5-cm material that is taken for determination of soil pathogens at each site. Soil microbial sampling refers to acquiring and handling a soil sample appropriate for analysis of phospholipid fatty acids (PLFAs) and DNA. The microbial sampling must accomplish the following:

1. Produce a scientifically rigorous microbial dataset that is interpretable in the context of microbial ecology and biogeochemistry on a continental scale,

2. Integrate with the protocols for geochemical sampling to allow for a paired microbial/geochemical sample.

We will collect and store the 0–5-cm interval, the A horizon, and the C horizon separately as microbial samples. We want to save all three ‘horizons’ because we want to be able to obtain as full a set of microbial data as possible.

Samples for soil microbiology will be collected at one site for every ten sampled for chemistry. For example, if you are sampling about 5 sites per day, take the soil microbial sample at the end of the second day.

The complete sampling method for soil microbiology is stated thusly:

a. The top 5 cm of soil (regardless of horizon) for soil microbiology (1 50-mL centrifuge tube—place in portable freezer until freezer is full)

b. A composite of the A horizon for soil microbiology (1 50-mL centrifuge tube—place in portable freezer until freezer is full)

c. A composite of the C horizon for soil microbiology (or a composite of material from a depth of about 60–100 cm, regardless of horizon, if the upper boundary of the C horizon is below 1 meter) (1 50-mL centrifuge tube—place in portable freezer until freezer is full)

Sampling for soil microorganisms

Equipment needed:

- Sterile 50-mL centrifuge tubes with screw caps and Styrofoam 'rack'
- Plug-in portable freezer or cooler with dry ice for temporarily storing samples for 'soil microbiology'
- Coolers with dry ice (preferable) or blue ice for shipping samples
- Strapping tape for sealing cooler for shipping
- Permanent marking pen
- Large (3 gallons or more) sealable plastic bags or plastic storage containers for holding sampling equipment for one site)
- There is no need to use sterile techniques for any of the samples, but be careful that tools are clean (i.e. rinsed with water and wiped dry) so that you do not cross contaminate with soil.

Using a clean trowel, place the soil into a plastic bowl to homogenize. As much as possible, remove large roots and gravel larger than 0.25 inch. Then place a subsample of the homogenized material into one sterile 50-mL centrifuge tube (or similar container). Fill each tube as full as possible and place in portable freezer. If a portable freezer is not available, place the samples in a cooler with freezer packs in the field. Freezer packs are colder than ice, so they

are preferable. Frozen samples should be maintained that way until you are prepared to send them. When the portable freezer is full, ship the samples to the laboratory in a small cooler.

Shipping:

Place sample tubes in a cooler with adequate dry ice (dry ice is preferable, blue ice is acceptable) to last for two or three days. Dry ice life can be extended by using a smaller cooler, triple-bagging blocks and filling headspace with packing material. Use overnight delivery for shipment to laboratory.

Contact the microbiology staff by phone or email to notify of shipment, and include tracking number in case the shipment does not show up. Please ship on a Monday or Tuesday so the samples do not arrive on the weekend when there is no USGS employee to receive them.

Specialized Protocols for Different Settings:

Urban settings: A few GRTS sites fall in urbanized areas where logistical (e.g., digging 1-m deep auger holes in private yards or public parks) and practical problems (e.g., digging into buried utility lines) make collection of a complete set of soil samples impossible. Pre-field site characterization should have identified urban sites where sampling is problematic because of urbanization. Rather than abandon these urbanized sites altogether, only the 0–5-cm sample should be collected from wherever possible, such as from nearby public land (e.g., a park or school yard). Further sampling in highly urbanized areas should not be attempted. Alternate sites (such as schools or parks) may have been determined prior to going into the field, or field crews may have to be creative. In any event, the final sample should be geographically as close as possible to the original GRTS site.

Wetlands settings: Protocols currently under development—if your site falls within a wetland, move to the nearest possible site with mineral soils. Do not skip the site.

Permafrost settings: Protocols currently under development.

Shipping Requirements:

Soils from certain locations in the United States are regulated in their movement about the country. A permit from the United States Department of Agriculture (USDA) is required to receive these soils unless they are sterilized. The USGS in Denver has such a permit and a copy will be provided to each sampling crew. More complete information about the regulation of soil can be found on the USDA Animal and Plant Health Inspection Service (APHIS) website at: http://www.aphis.usda.gov/plant_health/permits/organism/soil/index.shtml.

FIELD FORM

NORTH AMERICAN SOIL GEOCHEMICAL LANDSCAPES

Modified USGS Field Form 2010

1. State: _____

2. GRTS Site Number ID: _____

3. Datum: WGS84

4. Latitude (dd): _____ . _____ Longitude (dd): _____ . _____

5. Date: month _____; date _____; Weather Conditions:_____

6. Soil moisture (circle one)**: Dry Moist Wet - non-saturated (grains glisten) Wet - saturated (free water)**

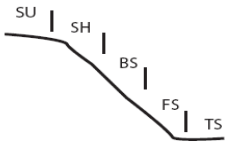
7. Crew:_____

8. Landscape/Landform:_____

9. Slope: Flat Gentle Slope Moderate Slope Steep Slope

10. Slope Aspect: (circle appropriate) **N NE E SE S SW W NW**

11. Slope Position: (mark location) **SU** = summit; **SH** = shoulder; **BS** = backslope; **FS** = footslope; **TS** = toeslope



12. Land Use:_____

13. Land Cover:_____

14. Other Anthropogenic/Natural Features in area: _____

15. Comments/Sketch:

SOIL SAMPLES COLLECTED

anthrax ○ **sample**

PH 0-5 cm:

Description:_____

A-horizon:

Description (include estimate of % humic vs. mineral): _____

Depth interval: _____cm

C-horizon:

Description: _____

Depth interval: _____cm