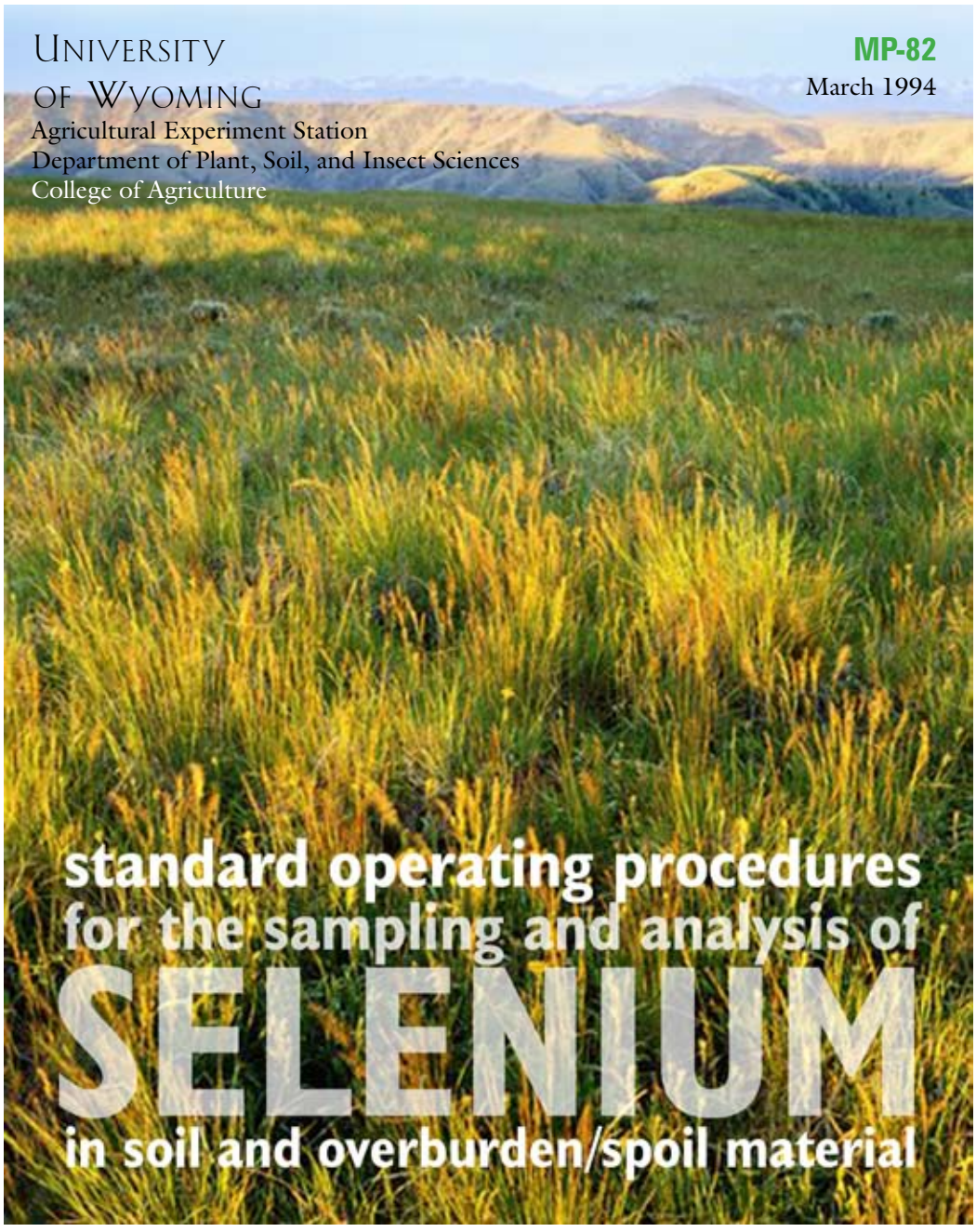

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Contents

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1. Introduction

This detailed protocol has been prepared to promote consistent and repeatable determinations of selenium in soil, spoil, and overburden samples. The procedures should be followed carefully to obtain useful and supportable results. Spoil, as used here, refers to overburden removed during the mining operation and does not include the marketable mineral, subsoil, or topsoil. The methods described are those thought to be the best at this time. The methods will be updated as more knowledge and data develop.

2. Sample Collection Procedures

A. Methods of Collection Wyoming Department of Environmental Quality – Land Quality Division (WDEQ-LQD Guideline No. 1, 1984):

1. Soil
2. Chips or cores for overburden and spoil

B. Tools for Collection

1. Shallow Phase Sampling – Collect samples using a hand auger or a mechanical soil sampler equipped with a core barrel or auger.
2. Deep Phase Sampling – Drill cores or chip samples should be collected. Cross-contamination between sub-samples should be minimized. Be careful to prevent contamination of samples from drilling fluids or pipe dope.

C. Sample Depths

Samples should represent the entire sample increment. For monitoring locations, permanently identify each sample location by marking and labeling. These monitoring locations will have soil and regraded spoil or overburden samples that must be drilled and collected within a 2.5 meter (8 foot)-circle of the permanent marker.

1. Soil
   a. Native – Sample by horizon, with sub-horizons where distinct sublayers exist.
   b. Reclaimed – If baseline data suggest extractable selenium may be above 0.1 parts per million and the soil is 30 centimeters (12 inches) thick or less, one sample should be collected. For soils greater than 30 centimeters (12 inches) thick, two samples at equal intervals should be collected.
2. Spoil
   a. Shallow Phase Sampling – Collect composite samples of regraded spoil material every 60 centimeters (2 feet) to a depth of 120 centimeters (4 feet). Sampling in reconstructed drainages should be to the depth at which suitable material was replaced.
   b. Deep Phase Sampling – Sample in accordance with the requirements of the permit, if applicable, and/or the most current Wyoming Department of Environmental Quality guidelines (WDEQ-LQD Guideline No. 1, 1984)
3. Overburden

   Sample in accordance with Guideline No. 1, (WDEQ-LQD, 1984) or as required by a specific project.

D. Sample Quantity

Collect approximately 1,500 to 2,000 grams (4 to 5 pounds) of sample for laboratory analysis

E. Treatment of Vegetation Before Sampling

Remove surface vegetation from the sample before processing or shipping. Do not include the vegetative portions of plants in the sample. Roots and other decaying plant material are considered part of the soil material and therefore are not removed from the soil or spoil material.

F. Sample Containers

Air dry the samples (see Section 4G) before storing samples in closed plastic containers with lids, paper bags lined with plastic, or in plastic bags. Seal and properly label all bags or containers. Label the containers, not just the lid.

Place appropriate information on sample container for ease in identification such as name, identification number, and date of sampling.

G. Record Keeping

1. Chain of custody – Document the sample location to maintain a complete record and expedite the analytical process. This record will add confidence to the results. This record also should accompany the sample from collection through data analysis.

2. Document all pertinent field information including, but not limited to, the following: sampler, date and time, climatic conditions, temperature, vegetation cover estimates, and conditions and duration of storage. Sample collection information will be critical in the overall quality control evaluation. Send uniform samples to the laboratories. A data form is provided as Attachment A for recording sample information.

H. Holding Times and Methods

Store the samples in a cool (15-20 degrees Celsius), dry environment. The samples should **not** be frozen nor exposed to heat or direct sunlight. Process or ship the samples to a laboratory within three days of collection.

I. Transportation and Delivery

Ship the samples directly to the laboratory. If direct shipment is not applicable, a reliable surface mail transportation system may be used. Place sample containers or bags in strong, adequately taped containers.

J. Storage Methods Before Laboratory Analysis

Complete the analysis of the samples within one month to minimize chemical changes that may occur with the samples. Store samples in a cool (15-20 degrees Celsius), dry area with limited exposure to sun, dust, or other contaminants to preserve the natural characteristics of the material.

3. Laboratory Practices

A. Laboratory Personnel

1. Dishwashing, sample preparation, and sample analysis must be handled by trained laboratory personnel. Personnel must be trained in safety procedures and laboratory hygiene, and able to properly use all equipment such as pipette, balances, microwaves, ovens, and volumetric flasks.

2. Identify the qualified laboratory personnel who perform sample preparation and analysis as part of the sample record.

B. Laboratory Safety and Housekeeping

1. Safe laboratory practices and operation of equipment must be followed for all analytical activities. Refer to each specific analytical procedure for more detailed discussion on safety items.

2. Good housekeeping procedures must be established and followed for all laboratory procedures. Examples of good housekeeping activities include keeping crushers and grinding equipment clean between uses with each sample. Keep sample preparation and storage areas clean to limit dust contamination.

C. Special Considerations

1. Deionized-distilled Water Specifications – Use deionized-distilled water for final rinsing of glass and plastic ware and for extracting solutions and standards. Deionized-distilled water must have an electrical conductivity (EC) no greater than 2µS cm^{-1} (µmho cm^{-1}). Water with EC higher than 2µS cm^{-1} indicates the ion-exchange columns are exhausted and must be replaced.

2. Chemicals – Use American Chemical Society (ACS) grade or better for all chemicals.

3. Hot Water Bath

a. Temperature – Use a constant temperature within 85-90 degrees Celsius (185-194 degrees Fahrenheit) for all procedures unless specified otherwise.

b. Time – Heating time is specific to each procedure.

c. Size – Bath size must be large enough to completely immerse the sample; however, care should be exercised to assure sample containers are not completely immersed. Immersion of containers may result in contamination of samples.

4. Microwave Oven

a. Temperature – It is difficult to measure temperature in a microwave; however, the objective is to obtain a constant temperature within 85-90 degrees Celsius (185-194 degrees Fahrenheit). A power calibration curve should be run to establish oven parameters if a temperature probe is not used.

b. Time – Microwave exposure is specific to each procedure.

c. Sample Orientation – Place samples within the microwave to ensure even heating of samples. Teflon digestion vessels are used as described in Section 10 below.

d. The times and power level of treating samples as outlined in these SOPs are based on a 600-watt microwave. IF a microwave other than 600 watts is used, it may be necessary to adjust the duration and power level of heating to adjust temperature. The time, power level of heating, and wattage must be reported with all samples extracted or digested with a microwave.

D. Laboratory Quality Assurance/Quality Control – Quality assurance and quality control are to be maintained throughout the analyses as follows:

Note: Standards should be prepared by quantitatively combining the same constituents used to prepare the samples.

1. Calibration – Establish an appropriate calibration protocol for each procedure and instrument. Analyze a standard reference sample every 20 samples to ensure the instrument remains properly calibrated. Recalibration of equipment will be necessary if rechecked standards are not within 5 percent of standard values.

2. Standards – Begin with 1000 ppm (mg L^{-1}) selenium stock. Prepare 0.01, 0.05, 0.10, 0.25, and 0.50 ppm (mg L^{-1}) selenium every 90 days using the same procedures used for the preparation of the sample extracts. Preserve to a pH of less than 2 using HNO_3 and keep refrigerated. Bring standards to room temperature before using.

3. Reagent Blanks – Analyze a reagent blank every 20 samples to evaluate possible sample contamination. Blanks are produced from the chemicals that contact the sample and are used to determine background concentrations.

4. Duplicate Analysis – Evaluate laboratory precision by duplicate analysis. Select 10 percent of the samples for duplicate analysis. Duplicates should be run during the same batch and run on different batches to evaluate the precision of the analysis.

5. Spikes – Spiking may be used for an additional quality control measure; however, this procedure should not be used for calibration and validation when other approaches are feasible.

6. Standard Reference Materials – A standard reference material, such as the NIST river sediment, should be run with every set of 20 samples for total Se analysis.

7. Audits – Audit samples should be analyzed regularly to ensure compliance with those Standard Operating Procedures (SOPs).

E. Dishwashing – Washing dishes is an important task in the lab. Follow proper procedure when washing dishes to limit contamination.

1. Remove sample identification numbers from tubes (a piece of cheesecloth moistened, not soaked, with 95 percent ethanol (EtOH) works well at removing "Sharpies" commonly used on glassware.)
2. Place dishes in a tub of warm, soapy water. Use Alconox or similar detergent that leaves no residue after rinsing to clean laboratory containers.
3. Non-Teflon Items
    a. Wash dishes well with the appropriate bottle brush.
    b. Rinse dishes with tap water two to three times.
    c. Carefully place dishes in acid bath (10 percent HCl, pH should be near 0.5), making certain they are fully submerged and let soak for a minimum of 15 minutes.
    d. Remove dishes from acid bath and rinse with deionized-distilled water as follows:

Apparatus Volume	Times to Rinse (minimum)
>100 ml	3
25-100 ml	4
<25 ml	5

    e. For every 10 to 15 items, fill one with deionized-distilled water and allow to set for at least 15 minutes. Check the conductivity of the water to determine if sufficiently rinsed. If conductivity readings are above 2µS cm^{-1}, re-rinse all glassware from that batch. This conductivity measurement is used to determine if Cl^- from the acid bath has been removed during rinsing.
    f. Place remaining items on clean racks and allow to dry completely.
4. Teflon items
    a. Wash dishes with a piece of cheese cloth wrapped around a brush handle.
    b. Rinse with tap water two to three times.
    c. Place in acid bath (10 percent HCl) and let soak for a minimum of 15 minutes.
    d. Rinse with deionized-distilled water a minimum of three times.
    e. See 3e above.
    f. Place on clean racks to dry.

4. Sample Preparation

A. Reference

Modified from USDA 1972, Soil Survey Investigations Report No. 1; Workman et.al., 1988, Soil testing methods used at Colorado State University for the evaluation of fertility, salinity, and trace element toxicity. Technical Bulletin LTB88-2.

B. Apparatus and Equipment
    1. Jaw crusher
    2. Pulverizer
    3. Riffle Splitter
    4. Drying pans
    5. Sample bags or containers with lids
    6. 10 mesh (2 mm screen) stainless steel sieve

C. Reagents

No reagents are required for sample preparation.

D. Samples must be inventoried and air-dried (Section 4G, below) within one week of receipt.

E. The dates and times of sample receipt, preparation, and analysis also must be incorporated as part of the sample record.

F. Percent moisture, cobble, gravel, and coarse fragment percentages can be estimated from uncrushed, air-dried samples.

G. Air Drying Procedures

1. If the overburden sample is cored, crush to 0.6 to 1.25 centimeters (¼ to ½ inch) before riffle splitting.
2. Run samples through a riffle splitter to ensure that a representative sample is obtained. The sample split must include enough material to fill a 28 x 18 x 4-centimeter (11 x 7 x 1.5-inch) drying pan.
3. Place sample into a pan. Transfer the sample identification number to the pan immediately.
4. Dry sample indoors at a temperature range of 24-27 degrees Celsius (75-80 degrees Fahrenheit). Document initial temperature, final temperature, and drying time. Also, identify the samples by name and sample identification number.
5. Air dry samples until visual inspection indicates no presence of moisture and the samples are dry to the touch. Drying time should take between 24 to 48 hours. Time will vary depending on percentage of moisture and texture of the sample and humidity in the sample drying room.

H. Crushing and Grinding

1. Soils – If clods are present, lightly crush samples with a wooden roller or ceramic mortar and pestle, or flail the sample. Do not try to grind rocks, pebbles, etc. Sieve samples through a 10-mesh (2.00 millimeter) sieve to remove rocks, pebbles, and larger pieces of plant materials.
2. Overburden and Spoil – Grind samples with a ceramic mortar and pestle, an automatic grinder equipped with ceramic plates, or a grinder equipped with a high density aluminum oxide auger.

   Clean the grinding apparatus between each sample. Clean all accessible parts of the equipment with a stream of pressurized air. Pass a portion of the next sampled through the grinder before processing the entire sample. This procedure will inoculate the inaccessible parts of the equipment with the sample to be analyzed. Contamination also will be minimized from the previous sample by following this procedure. Sieve the samples through a 10-mesh sieve until less than 5 percent of the sample remains.
3. Transfer sample to a plastic bag, plastic-lined paper bag, or plastic container with lid to limit contamination. Place name, identification number, and date of preparation on the sample container.
4. Samples are now ready for analysis and should be stored in a clean, dry area with temperatures at 20-23 degrees Celsius (65-70 degrees Fahrenheit).

I. General Comments

Consistent preparation of soil, spoil and overburden samples will produce accurate analytical data. Factors that have an influence upon final results include sampling and shipping techniques, temperature of drying, interval between sampling and preparation, homogeneity of sample, and degree of grinding. Selenium and other chemical parameters in soils may change if samples are kept moist at room temperature in plastic sample bags.

Increased grinding force and time may increase extractable metal levels. For example, minimal grinding time should be observed. Samples must be prepared carefully to obtain reproducible results.

5. AB-DTPA Extractable Selenium

A. Reference

Modification of a method by Soltanpour, P.N. and Workman (1980); Soltanpour, P.N., J.B. Jones, Jr., and S.M. Workman, 1982, Optical Emission Spectrometry, Chapter 3 In Methods of Soil Analysis, Part 2, A.L. Page (editor), Monograph No. 9, ASA, SSSA, Madison, WI.

B. Apparatus and Equipment:

1. balance (0.01 g readability)
2. 125 milliliter Erlenmeyer flasks with silicon stoppers or 50 milliliter plastic centrifuge tubes with caps. Rubber stoppers can be a source of trace metal contamination, particularly Zn and Fe. Silicon stoppers or rubber stoppers coated with parafilm should be used.
3. shaker (a wrist-action or reciprocal; capable of shaking frequency of 180 cpm)
4. Whatman #42 filter paper
5. filter rack
6. plastic funnels
7. 25 milliliter Erlenmeyer flasks, 25 milliliter solo cups (available from paper product supply companies), or 15 milliliter plastic (polyethylene or polypropylene) centrifuge tubes.
8. parafilm
9. pipettes (10 and 20 milliliter)
10. hot water bath (constant temperature control)
11. 50 milliliter test tubes with rack
12. ICP or AA spectrometer with hydride generator.

C. Reagents

1. Add chemical to deionized-distilled water in the order and amount listed below for the desired volume of AB-DTPA solution:

Chemical	---------- Desired Volume ----------		
	500 ml	1000 ml	2000ml
DTPA	0.99 g	1.98 g	3.97g
Ammonium Hydroxide (12.1 N)	0.4 ml	0.8 ml	1.6 ml
Ammonium Bicarbonate	39.53 g	79.06 g	158.13 g

2. Concentrated Hydrochloric Acid (HCl), 12.1 M
3. Hydrogen peroxide (H_2O_2), 30 percent

D. Extraction Procedure

1. Adjust the pH of the AB-DTPA extracting solution to 7.6 ± 0.05 using ammonium hydroxide to raise the pH or hydrochloric acid to lower the pH. The pH must be adjusted each day the extracting solution is used. The solution is unstable with regard to pH. Check the pH of the solution daily during use and adjust accordingly. Prepare a fresh solution at least every two weeks.
2. Weigh 10.0 grams of previously prepared sample into a 125 milliliter Erlenmeyer flask or plastic centrifuge tube.
3. Pipette 20.0 milliliters of extracting solution into each flask.
4. Shake stoppered flasks (or bottles with screw-on caps) for 15 minutes at approximately 180 cycles per minute. Samples must be shaken sufficiently to keep the sample in suspension for the duration of shaking.

   If samples are acidic, CO_2 evolution may cause problems with stoppered flasks. The CO_2 evolution will subside in about five minutes. Following the evolution of CO_2, the flasks can be stoppered for shaking.
5. Filter through Whatman #42 paper into 25 milliliter glass Erlenmeyer flasks, 25 milliliter solo cups, or 15 milliliter plastic centrifuge tubes.
6. Cover flasks with parafilm or cap the solo cups or tubes when done filtering.

E. General Comments

All QA/QC measures described in Section 3 above must be followed to ensure reproducible results. All laboratories performing analysis for mining operations in Wyoming are encouraged to strictly follow the directions from these SOPs. Any variations in these SOPs must be cleared through the WDEQ-LQD before analysis.

6. Hot Water Extractable Selenium

A. Reference

Modified from ASA Monograph No. 9, Part 2, 1st edition, Method 80-3: Hot Water Extraction, AAS-Hydride, pg 1122.

B. Apparatus and Equipment

1. balance (0.01 grams readability)
2. 50 milliliter (automatic) pipette, i.e. repipette dispenser
3. 125 milliliter bottles (inert and corrosion-resistant) or 50 milliliter plastic centrifuge tubes.
4. Whatman #42 filter paper
5. plastic funnels
6. filter rack
7. shaker (a wrist-action or reciprocal; capable of shaking frequency of 180 cpm)
8. 25 milliliter Erlenmeyer flasks, 25 milliliter solo cups with lids, or 15 milliliter plastic centrifuge tubes
9. pipettes (10, 15, or 20 milliliters)
10. 50 milliliter test tubes with rack
11. hot water bath with constant temperature control or microwave
12. ICP or AA spectrometer with hydride generator

C. Reagents

1. Extraction: 0.1 percent (weight/volume) $CaCl_2$ (1.00 g $CaCl_2$ diluted to one liter with deionized-distilled water)
2. Digestion: 30 percent hydrogen peroxide, concentrated HCl, deionized-distilled water

D. Extraction Procedure

1. Weigh 25 grams of 10-mesh material, and transfer into a 125 milliliter bottle. Alternatively, weigh 10 grams into a 50 milliliter centrifuge tube.
2. Add 50 milliliters of 0.1 percent (w/v) $CaCl_2$. Cap the bottles and shake by hand to ensure complete saturation. If centrifuge tubes are used, add 20 milliliters of 0.1 percent $CaCl_2$, cap, and shake. When making the extraction solution, the 0.1 percent $CaCl_2$ is added to deionized-distilled water to help flocculate the heavy clays in the sample and produce a clear filtrate.
3. Place the samples evenly in a microwave for even temperatures and heating. Loosen the caps to allow the samples to steam but not boil. Heat the samples on high (100 percent power, 600 watt) for 7.5 minutes. Alternatively, place tubes, loosely capped, in a hot-water bath set at 85-90 degrees Celsius (185-194 degrees Fahrenheit) for 20 minutes.

   The heating time is based on a sample load of 20 samples and must be adjusted according to sample number. A different wattage for the microwave of choice also will require adjustments to the heating time and intensity (see 3.C.4 above).
4. Remove samples from the microwave to cool for a few minutes. Place the bottles back in the microwave and keep together so they remain warm for another 30 minutes. For the tubes, remove from water bath and allow to cool at room temperature.

   Note: It is important to note that a hot-water bath may be substituted for a microwave. Prepare water bath as described in Section 3.C.3. Caution should be used with either method to ensure samples do not boil or go to dryness.
5. Set up a filter rack using #42 Whatman filters and appropriate-sized storage containers such as 25 milliliter solo cups with lids. After the 30-minute waiting period, filter the samples and collect the filtrate into the storage containers. Cap the samples.

E. General Comments

Results from this method have been used to measure available selenium since the 1930s. Several western states have guidelines for mining activities that regulate spoil placement by

their hot water soluble selenium levels; however, the precision of this method is often poor, providing results that are questionable.

This procedure attempts to standardize the methods to improve precision.

This extraction method, referenced from the ASA Monograph #9 part 2, has been modified to facilitate rapid extraction. Results between the condenser method and the microwave have shown comparable results (R. Pasch, IML; personal communication, 1991).

7. Saturated Past Extraction

A. Reference

Modified from USDA Agriculture Handbook 60 (1954); Paste pH Procedure

B. Apparatus and Equipment

1. balance (0.01 gram readability)
2. 500 to 1000 milliliter plastic containers
3. spatula
4. pipettes (10, 15, 20 milliliter)
5. test tubes (50 milliliter)
6. hot water bath (or a microwave)
7. drying cans with lids for determining moisture content
8. oven (convection or similar, stable to within ±2 degrees Celsius)
9. desiccator
10. ICP or AA spectrometer with hydride generator

C. Reagents

Deionized-distilled water

D. Extraction Procedure

1. Prepare pastes by adding deionized-distilled water to sample (200 to 400 grams) and mixing the two to obtain a glistening surface. Usually, just enough water to cover the sample 1 to 2 centimeters (about ½-inch) deep will percolate down and wet the entire sample. Close the container and allow sample to equilibrate for at least 24 hours.
2. Mix the sample with a spatula after one hour. The sample must flow slowly off a spoon or spatula as it is drawn from the mixture. Most saturated pastes will not stick to a clean spatula.

   Free water must not be standing on the surface when the container is gently tapped on the bench top; however, the surface will glisten. If water remains on the surface, more samples must be added because the paste is too wet.

If the sample has stiffened, more water is needed. Any additions to the sample will require the sample to re-equilibrate and be rechecked.

3. Upon obtaining a proper consistency, the sample must be covered with an air-tight lid. Allow the sample to set for 24 hours to establish equilibrium between the soil/spoil minerals and the water. The sample will have the consistency of a milkshake when ready for extraction.
4. The percentage of water of the saturation paste must be determined and reported with the sample results using the following procedure:

   a. Make a saturation paste and allow it to equilibrate for the required time.
   b. On the back of the lab sheet, set up the following table headings:

Can ID	wet wt. + can	dry wt. + can	can wt.

   c. Record the can identification number for each can with its empty weight.
   d. Immediately before any measurement (i.e., pH) of the sample, transfer about 20 grams of the saturated soil to the proper can.
   e. Record the wet weight of the sample and can.
   f. Place cans in an oven at 105 degrees Celsius (222 degrees Fahrenheit) for 24 hours.

g. After 24 hours, place the cans in a desiccator until cool and weigh them on the analytical balance. Record the dry weight and calculate percent water as follows:

$$\frac{\text{wet weight} - \text{dry weight} \times 100}{\text{wet weight}}$$

5. Following equilibration, filter the sample (using a filter press or Buchner filter funnel) to obtain the extract. The samples must be refiltered or discarded if the extractant solution is turbid before analysis. Camp the containers and store samples at 4 degrees Celsius (39 degrees Fahrenheit).

E. General Comments

Currently this method is used for pH, EC (emulsifiable) concentrate, soluble ions, and metals. This method is proposed as an alternative for selenium extractions. The procedure requires a larger sample size (10 to 200 times that of other extraction methods), which may promote consistent results. Experience is necessary to obtain the proper saturated paste extract.

8. Phosphate Extractable Selenium

A. References

Modified from Cappo, K.A., L.J. Blume, G.A. Raab, J.K. Bartz, and J.L. Engels. 1987. Extractable sulfate and nitrate. In *Analytical Methods Manual for the Direct/Delayed Response Project Soil Survey.* USEPA Rep. 600/8-87/020. Environ. Monitoring Systems Lab., USEPA, Las Vegas, NV. Section 12, 11 p.

B. Apparatus and Equipment

1. balance (0.0l gram readability)
2. 50-milliliter screw top centrifuge tubes
3. centrifuge (general purpose, capable of 2,000 rpm)
4. reciprocating shaker (a wrist-action or reciprocal; capable of shaking frequency of 180 cpm)
5. volumetric pipettes and flasks as needed
6. ICP or AA spectrometer with hydride generator

C. Reagents

1. Extraction: Phosphate extract solution (KH_2PO_4), 1.l M PO_4^{-3} – dissolve 68.045 grams KH_2PO_4 in deionized-distilled water. Dilute to 500 milliliters with deionized-distilled water.
2. Digestion: Concentrated (12.1 M) HCl, 30 percent hydrogen peroxide (H_2O_2)

D. Extraction Procedure

1. Place 5.0 grams of air-dried sample into a 50-milliliter centrifuge tube.
2. Add 25 milliliters of phosphate extracting solution.
3. Shake tube and contents horizontally on a reciprocating shaker for two hours. Make sure the soil is not accumulating at the base of the centrifuge tube by keeping the shaker at a rate that will keep the soil suspended or approximately 180 cpm.
4. Centrifuge for 15 minutes at 2,000 rpm. If supernatant is not clear, repeat centrifugation. Decant supernatant into a clean beaker or plastic centrifuge tube.
5. Camp and store the solution at 4 degrees Celsius (39 degrees Fahrenheit) until filtration and analysis. Analyze within 24 hours because biological activity in this nutrient-rich extract may occur.

E. General Comments

Selenium can be adsorbed to soil/overburden/spoil materials in several ways. These different processes in turn affect the extent to which selenium can be displaced or exchanged from the material surface. Phosphate (PO_4^{-3}) extraction provides a quantitative measure of the water soluble and adsorbed selenium. The ligand strength of the phosphate ion will provide nearly quantitative displacement of noncrystalline, inorganic selenium for solid material.

This method has been demonstrated to be an effective extractant of selenium species from certain minerals. The phosphate ion selectively replaces selenium adsorbed on kaolinite and montmorillonite (Bar-Yosef and D. Meeks. 1987. Selenium sorption by kaolinite and montmorillonite. Soil Science 144:11-19). The United States Environmental Protection Agency has also used this extractant to assess extractable sulfate from soils throughout the United State.

9. Total Metal Acid Digestion (Including Selenium)

Warning!! This method uses hydrofluoric and perchloric acids, which are very hazardous. Extreme caution is mandatory. Use appropriate safety procedures. A special perchloric hood is essential when using perchloric acid.

A. Reference

Modified from ASA Monograph No. 9, Part 2, 2nd edition, pg 1-1, 1982.

B. Apparatus and Equipment

1. perchloric hood
2. 40 milliliter graduated Teflon centrifuge tubes
3. balance (capable of 0.001 gram readability)
4. spatula
5. weighing paper
6. aluminum block
7. 30 milliliter polyethylene tubes
8. pipette (10 milliliter)

C. Reagents

1. concentrated perchloric acid (11.7N)
2. concentrated hydrofluoric acid
3. concentrated nitric acid (15.8 N)
4. 1 + 1 hydrochloric acid (6 M)
5. deionized-distilled water

D. Digestion Procedures

1. Weigh 0.500 grams of sample into 40 milliliter Teflon centrifuge tube.
2. Add 10 milliliters of concentrated nitric acid.
3. For soil/spoil, add 10 milliliters of concentrated perchloric acid to the samples in a perchloric hood.
4. Add 5 milliliters of concentrated hydrofluoric acid to soil/spoil samples.
5. Digest the samples for 16 hours at 100 degrees Celsius (212 degrees Fahrenheit); dense white fumes will appear at the end of this period. **DO NOT** let the samples go to dryness.
6. Remove the samples and allow to cool.
7. Slowly add 10 milliliters of 1+1 hydrochloric acid to the soil/spoil samples.
8. Bring samples to a final volume of 25 milliliters with deionized-distilled water, using the rinse water from the Teflon tube. **Caution:** be careful not to use too much deionized-distilled water while rinsing out the Teflon tube.

E. General Comments

Add acids in the concentrations and manners dictated in this procedure. Failure to follow these procedures could result in dangerous explosions.

Samples to be read for metals must be additionally diluted 1:10 with deionized-distilled water to help minimize matrix and inter-element interferences; however, this is **NOT** necessary for samples that will be pre-treated for arsenic and selenium analysis

The high level of iron and aluminum in soil/spoil samples also can cause inter-element interferences. Perchlorate will combine with potassium to form potassium perchlorate crystals. Special precautions must be taken to ensure the crystals are brought back into solution before analyzing for potassium.

10. Total Soil Selenium by Microwave Digestion

Note: This method is an alternative to the Total Metal Acid Digestion method described in section 9; however, the use of this method requires further testing. The use of the microwave requires a comparison with the Total Acid Digestion Method for all samples. This comparison is necessary because recent results have shown the microwave method is not digesting all the selenium present in a sample. The microwave method may be exclusively used if comparisons between the two methods are statistically equivalent.

WARNING!! This method uses hydrofluoric acid, which is very hazardous. Use appropriate safety procedures.

A. Reference

Modified from Introduction to Microwave Sample Preparation: Theory and Practice, Editors H.M. Kingston and L. Jassie, 1988, American Chemical Society, Washington, D.C.

B. Apparatus and Equipment

1. balance (top loading, 0.001 gram readability)
2. Teflon digestion vessel
3. microwave (600 watt)
4. pipettes
5. water bath
6. test tubes

C. Reagents

1. Trace metal grade, concentrated (12.1 M) hydrochloric acid (HCl)
2. Trace metal grade, concentrated (15.8 M) nitric acid (HNO_3)
3. 30 percent hydrogen peroxide (H_2O_2)
4. Metal grade, concentrated hydrofluoric acid
5. Deionized-distilled water

D. Digestion Procedures

1. Weigh out 0.500 g of 10-mesh sample into Teflon digestion vessels.
2. Add 5 milliliters of trace metal grade concentrated nitric acid.
3. Add 2 milliliters 30 percent H_2O_2
4. Cap the Teflon digestion vessel.
5. Digest for 30 minutes at 60 percent power (microwave, 600 watt).
6. Cool the samples and then open the digestion vessels.
7. Add 5 milliliters of trace metal grade concentrated hydrofluoric acid.
8. Cap the Teflon digestion vessel.
9. Digest for five minutes at 100 percent power, and then digest for 120 minutes at 80 percent power.
10. Allow samples to cool and then open digestion vessels.
11. Add 25 milliliters of deionized-distilled water.
12. Cap the digestion vessels.
13. Digest five minutes at 80 percent power.
14. Cool samples and dilute to 50 milliliters.
15. If using ICP rather than hydride generation, use saturated boric acid to dilute the acid in the sample before analysis.

11. Selenium Analysis

A. Sample Preparation

1. Pipette a 3 milliliter aliquot of extract into a 50 milliliter test tube. Add 12 milliliters of deionized-distilled water using a pipette.

   Note: the aliquot size will depend upon the sensitivity of the hydride generation equipment.

2. Add 1 milliliter of 30 percent H_2O_2.
3. Place the rack of tubes into a hot water bath that has been prepared previously at 85-90 degrees Celsius (185-194 degrees Fahrenheit). Cook for 20 minutes and then remove.

4. Using a 10 milliliter pipette, slowly add 10 milliliters of concentrated HCl to each tube. Place rack back in water bath for 20 minutes. Remove and let stand for 12 hours.

B. Analysis Procedure

1. Samples are then ready to be analyzed using ICP and AA Hydride generation.
2. The operation and maintenance of the analytical instruments are beyond the scope of these SOPs. Use manufacturers' specifications and guidelines in the operation of appropriate equipment.
3. The analytical method used must be incorporated as part of the sample record. The record should include type of instrument and manufacturer. The use of a simultaneous ICP instrumentation is recommended because of more accurate optics compared to sequential ICP instruments.

C. General Comments

The microwave digestion technique is a modified version of total digestion using hydrogen fluoride and perchloric acid solution. Special safety precautions must be followed during the microwave digestion as specified by the microwave manufacturer.

12. Acknowledgements

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Attachment A

Supplemental Data Sheet	
Date of Sampling	
Time of Sampling	
Name of Mine	
Name of Sampler	
Date of Backfill Regrading (if applicable)	
Date of Topsoil Placement (if applicable)	
Topsoil Source (stockpile or direct haul)	
Date of Permanent Revegation (if applicable)	
Slope Position (1-summit, 2 shoulder, 3-backslope, 4-footslope, 5-toeslope, 6-drainage, 7-closed depression)	
Slope Steepness (1-flat, 2-gentle, 3-moderate, 4-steep)	
Orientation (in degrees)	
Animal Signs (1-isolated, 2-scattered, 3-abundant)	
Animals Observed (Note: only the relative abundance of animals and not their type will be included here as a variable 1- isolated, 2 – few, 3- common, 4-many)	
Average Annual Precipitation (at closest station)	
Depth of soil	
Depth of A+B Horizons	
Reason for Site Selection (append relevant data)	
Date and Time of Sample Delivery to Laboratory	