

UNIVERSITY
OF WYOMING

Agricultural Experiment Station
Department of Plant, Soil, and Insect Sciences
College of Agriculture

MP-77

March 1994

**standard operating
procedures for sampling**

SELENIUM in vegetation



D.G. Steward, AMAX West, Inc. • J.G. Luther, Wyoming Department of Environmental Quality • P.K. Carroll, formerly with Black Thunder Coal Company • L.E. Vicklund, Cordero Mining Company • G.F. Vance, Department of Plant, Soil, and Insect Science, University of Wyoming • L.K. Spackman, Department of Environmental Quality, Land Quality Division

Contents

Introduction	1
Relationship Between Vegetation and Soil Samples	1
Sample Quadrat	1
Sample Quadrat Location	1
Management of Sample Locations	1
Sample Time	1
Growing Season Sample	1
Post-Growing Season Sample	1
Sampling Procedure	2
Cover Estimation	2
Selenium Analysis	2
Laboratory Preparation and Analytical Procedures	2
Procedure for Plant Analysis of Selenium	2
Reagents	2
Instrumentation	2
Quality Control	2
Blanks	2
Duplicates	2
Spikes	2
Control	2
Control Limits	2
Procedures	2
Method #1	2
Method #2	2
Calculations	3
Detection Limits	3
Comments	3
Parameters	3
Skills Required	3
Interferences	3
Sample Collection	3
Holding Time	3
Safety	3
Physical Safety	3
Apparatus and Materials	3
References	3
Attachment A: Field Data Sheet	4
Attachment B: Computer Code Data Sheet Format	5
Attachment C: Key to Sample Sheet	6

UNIVERSITY OF WYOMING

Persons seeking admission, employment, or access to programs of the University of Wyoming shall be considered without regard to race, color, religion, sex, national origin, disability, age, political belief, veteran status, sexual orientation, and marital or familial status. Persons with disabilities who require alternative means for communication or program information (Braille, large print, audiotape, etc.) should contact their local UW CES office. To file a complaint, write to the UW Employment Practices/Affirmative Action Office, University of Wyoming, 1000 E. University Ave., Department 3434, Laramie, WY 82071-3434.

Standard Operating Procedures for Sampling Selenium in Vegetation

Introduction

The protocol described in this document has been prepared to promote consistent and repeatable determinations of selenium in vegetation. The procedures should be followed carefully to obtain useful and supportable results. Vegetation cover is estimated as part of the protocol to gain an understanding of the relationship between plant community structure and selenium levels in vegetation.

Relationship Between Vegetation and Soil Samples

Locate vegetation samples in conjunction with soil samples if soil samples are required. When required, take soil samples from the center of the vegetation sample quadrat immediately after the vegetation is sampled. When soil samples are to be taken, follow the Standard Operating Procedures for the Sampling and Analysis of Selenium in Soil and Overburden/Spoil Material (Spackman et al., 1994).

Sample Quadrat

Sample quadrats are needed to estimate vegetation cover and to clip vegetation for selenium analysis. Cover estimates are best made in grasslands with 1-square meter quadrats; however, a 2-meter by 2-meter quadrat is needed to ensure sufficient material can be collected for an adequate sample. Therefore, vegetation cover should be estimated within a 1-meter by 1-meter quadrat centered within the 2-meter by 2-meter quadrat.

Under certain circumstances, another sampling approach may be considered that is not as time consuming as the method described above. The less time-consuming, but statistically valid, approach is to establish equidistant parallel compass lines over the area to be sampled and use random numbers to determine the number of paces along each line between sample quadrats.

Sample Quadrat Location

Locate sample quadrats randomilliterly, or based on previously obtained soils or vegetation information. When sample sites are to be located randomilliterly, use a 2-meter by 2-meter grid pattern and random number

generation to locate the sites. When preliminary soil samples are used to select vegetation sampling locations, place the vegetation sample quadrat 2-meters from the initial soil sample in a randomilliterly selected (random number generation between 0 and 360) direction. Orient the axis of the sample plot along true cardinal direction (0-180 and 90-270 degrees).

Relocate sample quadrats when random selection results in placement of the quadrat in areas with less than 10 percent absolute vegetation cover. Note in the sample records whenever relocation is necessary. If soil sampling is to be conducted simultaneously with vegetation sampling, collect a soil sample at sites with less than 10 percent absolute cover to verify that low vegetation cover is not due to high soil selenium levels.

Photograph (slide or print) the sample site before sampling. The photograph should show a plan view of the sample quadrat prior to disturbance. Survey or permanently mark the sample location with a device not attractive to animals after sampling is completed.

Management of Sample Locations

Record the type and amount of grazing by livestock and wildlife observed at the sample location. Manage sample locations consistent with surrounding lands so use patterns are similar. For example, do not fence the sample location or mark it with a device attractive to animals.

Sample Time

Growing Season Sample

Collect vegetation samples and associated soil samples when western wheatgrass, at or near the sample location, is between bolting and flowering. This timing will help ensure all samples are taken at a similar stage of plant development. If western wheatgrass meets the requirements described in the next section under selenium analysis number 1, then it should be sampled along with the other plant species that meet the criteria.

Post-Growing Season Sample

Collect vegetation and associated soil samples between October 15 and November 15.

Sampling Procedures

Cover Estimation

Following placement of the sample quadrat, estimate and record absolute vegetation cover and associated information (Attachment A) using the methods specified in Appendix A of the Wyoming Department of Environmental Quality – Land Quality Division (WDEQ-LQD) Rules and Regulations (1989).

Selenium Analysis

1. Clip samples of all species present at greater than or equal to 2 percent absolute cover, starting with the species of highest cover. If more than six species meet the 2 percent criterion, no more than six species need to be sampled.
2. Include only current year's growth in shrub clippings. Do not clip annual species.
3. Starting at the center of the plot, select and clip sufficient individuals from each species to obtain a 5-10 gram sample. It is not necessary to wear gloves or use specially cleaned sampling equipment; however, use reasonable care to prevent sample contamination.
4. Immediately place samples in airtight plastic bags. Evacuate air from the bags to the maximum extent possible before closing.
5. Immediately chill plant samples in the field and freeze within eight hours of collection. Transport samples to the laboratory within 60 hours of collection. Keep samples frozen until analysis. Analysis must begin within two weeks of receipt of sample.
6. Record all supplemental information on the data sheet shown in Attachment A.

Laboratory Preparation and Analytical Procedures

Digest plant samples with perchloric, hydrofluoric, nitric, hydrochloric acid (Modified from ASA Monograph No. 9, Part 2, 1982). Analyze for selenium using inductively coupled argon plasma atomic emission spectrometry (ICAP-AES) or atomic absorption spectroscopy-hydride generation (AAS-HG).

Procedure for Plant Analysis of Selenium

Reagents: Concentrated perchloric acid, concentrated hydrofluoric acid, concentrated nitric acid, 1+1 hydrochloric acid, distilled-deionized water.

Instrumentation: Comply with operating manual for instrumentation.

Quality Control: Run an appropriate NIST standard with every set of digestions, i.e., for plant selenium determination, use citrus leaves or wheat flour standards (include NIST number).

Blanks: Run a blank on each set of 10 samples.

Duplicates: Duplicate 10 percent of sample set.

Spikes: Include when appropriate. Spiking may introduce technical uncertainties due to the uncertainty of recovering the artificially added analyte with the same efficiency as a natural one. Spiking should not be used for calibration and validation when other approaches are feasible.

Control: Run a control sample with each set.

Control Limits: 95 percent confidence on checks and control sample.

Procedure:

Method #1

1. Weigh 0.500g plant tissue into a 40 milliliter Teflon centrifuge tube.
2. Add the following acids:
 - a. 10 milliliters of concentrated nitric acid.
 - b. 2 milliliters of concentrated perchloric acid (USE ONLY IN A PERCHLORIC HOOD).
 - c. 1 milliliter of concentrated hydrofluoric acid.
3. Digest the samples for 16 hours at 100 degrees Celsius. Dense white fumes will appear at the end of this time. Do **NOT** let the samples go to dryness.
4. Remove the samples and allow to cool.
5. Bring samples to a final volume of 25 milliliters with distilled-deionized water while rinsing out Teflon tube.

Method #2

1. Weigh 0.500g plant tissue into a 50 milliliter Pyrex Folin-Wu Digestion (N.P.N.) glass tube with 35 and 50 milliliter graduations.

2. Add the following acids:
 - a. 10 milliliters of concentrated nitric acid
 - b. 2 milliliters of concentrated perchloric acid (USE ONLY IN A PERCHLORIC HOOD).
3. Digest the samples for two hours at 100 degrees Celsius on an aluminum block digester. **Do NOT** let the samples go to dryness.
4. Remove the samples and allow to cool.
5. Dilute samples to a final volume of 35 milliliters using distilled-deionized water.

Drying of Plants: Plants should be dried at a maximum temperature of 50 degrees Celsius to prevent loss of selenium. If plants are to be “wet digested,” a subsample must be dried to determine selenium on a dry weight basis.

Calculations: Apply appropriate dilution factors to analytical results.

Detection Limits: Comply with operating manual for instrumentation.

Comments: It is important to add acids in the concentrations and manners dictated in this procedure. Failure to do so could cause dangerous chemical reactions. Samples to be read for metals should be additionally diluted 1:10 with distilled-deionized water to help minimize matrix and inter-element interferences. It is not necessary to do this to samples that will be pretreated for selenium.

Parameters: This procedure can be used in the analysis of the following elements: Cu, Zn, Ni, Mo, Cd, Co, Cr, Sr, B, Ba, Pb, V, As, Se, Al, Fe, Ti, Mn, P, Ca, Mg, Na, and K.

Skills Required: Knowledge of safety practices and practical chemistry knowledge.

Interferences: Perchloric acid will combine with potassium to form potassium perchlorate crystals. Special precautions must be taken to ensure the crys-

tals are brought back into solution before analyzing for potassium. This may be done by heating the digestion mixture of water and acid to 90 degrees Celsius for approximately one hour, mixing, and bringing to final volume.

Sample Collection: Use the polyethylene tubes for final storage because of possible decomposition of plastic containers such as Solo cups.

Holding Time: Samples are good for up to one month after digestion; however, the potassium perchlorate crystals may reform on prolonged storage.

Safety: The acids used in this procedure are very dangerous. Use extreme caution. Refer to Material Safety Data Sheets for proper handling of these materials.

Physical Safety: Wear lab coat, rubber gloves, and eye protection.

Apparatus and Materials: Perchloric acid hood, 40 milliliter FEP-type Teflon centrifuge tubes, balance accurate to 0.000 or 0.0000, spatula, weighing paper, aluminum block, 30 milliliter polyethylene tubes, repeater pipette and tips.

References

WDEQ-LQD, 1989, Rules and Regulations, Wyoming Department of Environmental Quality – Land Quality Division, Cheyenne, WY.

American Society of Agronomy, 1982, Chapter 1, Dissolution for total elemental analysis (C.H. Lim and M.L. Jackson) In Monograph No. 9, *Methods of Soil Analysis*, Part 2, 2nd edition, A.L. Page (ed.), SSSA, Inc., Madison, WI. Pp. 1-12.

Spackman, L.K., G.F. Vance, L.E. Vicklund, P.K. Carroll, D.G. Steward, and J.G. Luther, 1994, *Standard Operating Procedures for the Sampling and Analysis of Selenium in Soil and Overburden/Spoil Material*, University of Wyoming, College of Agriculture, MP-82.

Attachment A – Field Data Sheet

Variable	Value
Date	
Mine	
Sample ID	
Total Vegetation Cover (%)	
Litter and Rock Cover (%)	
Bare Soil (%)	
Total Cover (%)	
Cool Season Perennial Grass Cover (%)	
Warm Season Perennial Grass Cover (%)	
Shrub Cover (%)	
Forb Cover (%)	
Method* (random or non-random)	
Sample Type* (native or reclaimed)	
State Plane Coordinates (northing and easting)	
Date of Backfill Grading (if applicable)*	
Date of Topsoil Replacement (if applicable)*	
Topsoil Source (stockpile or direct haul)	
Date of Permanent Revegetation(if applicable)*	
Depth of Replaced Soil (Reclaimed) or Depth of A through B horizons (Native)	
Series (Mapping Unit if Native)	
Slope Position*	
Slope Steepness*	
Orientation (in degrees)	
Animal Sign*	
Animals Observed (type and amount)	
Average Annual Precipitation at Closest Station	
Type of Utilization*	
Estimated Utilization (%) or descriptive phase	
Plant Community Type*	
Date and Time of Sample to Laboratory	

*For listing of acceptable values, refer to “Key to Sample Sheet,” Attachment C.

Attachment B – Computer Code Data Sheet Format

OBS						
MINE						
SAMPLE						
TVEGC						
LITROCK						
BARESOIL						
TCOV						
CSPGC						
WSPGC						
SHRBC						
FORBC						
METHOD						
SAMTYPE						
EASTING						
NORTHING						
DBKFLGRD						
DTPSLREP						
DPERMVEG						
TSDEPTH						
ABDEPTH						
SERIES						
SLOPEPOS						
SLOPESTP						
ORIENT						
ANIMALSG						
ANIMALOBS						
PPTN						
UTILTYPE						
UTIL						
COMMTYPE						
SP1						
SESP1						
SP2						
SESP2						
SP3						
SESP3						
SP4						
SESP4						
SP5						
SESP5						
SP6						
SESP6						

Attachment C – Key to Sample Sheet

OBS – Site location number

MINE – Use a three digit code for the mine (example: Belle Ayr is BEL)

SAMPLE – Sample identification number

TVEGC – Percent total absolute vegetation cover – sample in accordance with Appendix A, WDEQ-LQD, 1989, Rules and Regulations

LITROCK – Percent absolute litter and rock cover – sample in accordance with Appendix A, WDEQ-LQD, 1989, Rules and Regulations

BARESOIL – Percent bare soil cover – sample in accordance with Appendix A, WDEQ-LQD, 1989, Rules and Regulations

TCOV – Total cover of vegetation, litter and rock

CSPGC – Cool Season Perennial Grass Cover (%)

WSPGC – Warm Season Perennial Grass Cover (%)

SHRBC – Shrub Cover (%)

FORBC – Forb Cover (%)

METHOD – Method of Site Selection

Random = 1

NonRandom = 2

SAMPTYPE – Native site (1) or reclaimed site (2)

EASTING – Easting coordinate of the sample location

NORTHING – Northing coordinate of the sample location

DBKFLGRD – Date of backfill grading, if site is reclaimed. Give date as last two digits of the year, followed by month using OX format, followed by day using OX format. Example: June 4, 1991 = 910604

DTPSLREP – Date of topsoil replacement, if site is reclaimed. Give date as last two digits of the year, followed by month using OX format, followed by day using OX format. Example: June 4, 1991 = 910604

DPERMVEG – Date of permanent revegetation seeding, if site is reclaimed. Give date as last two digits of the year, followed by month using OX format, followed by day using OX format. Example: June 4, 1991 = 910604

TSDEPT – Depth of replaced topsoil, if site is reclaimed

ABDEPTH – Depth of A + B horizon, if site is native

SERIES – Mapping unit number of the soil series, if site is native

SLOPEPOS – Slope position of sample

Summit	=	1
Shoulder	=	2
Backslope	=	3
Footslope	=	4
Toeslope	=	5
Drainageway	=	6
Closed Drainage	=	7

SLOPOESTP – Slope Steepness

Flat	=	1
Gentle	=	2
Moderate	=	3
Steep	=	4

ORIENT – Orientation in degrees

ANIMALSG – Animal Sign

Isolated	=	1
Scattered	=	2
Abundant	=	3

ANIMALOBS – Animals Observed (Note: only the relative abundance of animals and not their type will be included here as a variable.)

PPTN – Average Annual Precipitation

UTILTYPE – Type of livestock or wildlife utilization

Primarily Livestock	=	1
Primarily Wildlife	=	2

UTIL – Estimated percent utilization of the vegetation

COMMTYPE – Plant Community Type

Rough Breaks	=	1
Grassland	=	2
Shrubland	=	3
Grass/Shrub	=	4
Draw Bottom	=	5
Stream Bottom	=	6
Cropland	=	7
Pastureland	=	8

SP1, SP2, SP3, SP4, SP5, and SP6 – Sampled plant species in order of highest (SP1) to lowest (SP6) absolute plant cover. All plant species collected must be greater than or equal to 2 percent absolute cover.

SESP1, SESP2, SESP3, SESP4, SESP5, and SESP6 – Selenium concentration in corresponding species from laboratory analysis (e.g., SESP1 selenium concentration is for SP1 sampled plant species.)