SELENIUM AND MINING IN THE POWDER RIVER BASIN: PHASE II - THE ROOTING ZONE-PLANT RELATIONSHIP[1]

Lowell K. Spackman, D.G. "Mickey" Steward, Laurel E. Vicklund, George F. Vance, and Jim G. Luther[2]

Abstract. Members of the Land Quality Division of the Wyoming Department of Environmental Quality, the Wyoming Mining Association, and the University of Wyoming have formed an interactive research committee to address how mining affects selenium. To meet one of the objectives of this committee, a three-phase research project for soil, overburden, vegetation, and wildlife was initiated. Phase I and Phase III are discussed in separate papers in these proceedings. The purpose of Phase II was to meet, in part, an objective to develop predictive relationships between Se concentration in the environment and target organisms, and to identify appropriate suitability levels for Se in postmining backfill. This phase involved the analyses of Se in overburden, soils, and backfill, and the relationship of Se in this material to Se uptake by vegetation. Selection of research sites for Phase II were based on the range of Se values encountered in Phase I vegetation sampling. A Phase II pilot study was initiated to identify sites that represent a range in rooting zone and plant Se concentration. The sites selected for the full scale Phase II were characterized for several soil parameters. Both unmined and reclaimed areas were sampled according to the Standard Operating Procedures developed by this committee. Vegetation was resampled together with the soil, overburden, and backfill for each set of samples. The results of this study show that Se decreases in vegetation from summer to fall. There was also a consistent increase in concentration with depth of rooting zone material for both unmined and reclaimed sample areas. Se in vegetation appeared to be predicted by the "rate of change" of Se in the soil with depth. The absolute concentration of Se in vegetation appears to be influenced by several soil factors including: percent clay, total organic carbon, extractable soil Se, electrical conductivity, sulfates, and the age of the reclaimed site. Because of the complex and dynamic nature of the vegetation-soil system, it was not possible to find a direct relationship between vegetation Se and soil extractable Se with the number of samples collected. Characterization of Se in vegetation and soil of unmined and reclaimed sites in the Powder River Basin suggests reduced concern for Se toxicity in topographic high areas.

Additional Key Words: Vegetation Life-Forms, Statistical Analysis

Introduction

Surface mining activities in areas containing seleniferous geological materials have become a concern due to the potential for increasing selenium availability in the reclaimed environments. During surface mining activities, materials previously in reducing environments are exposed to atmospheric oxidizing conditions. Through the process of oxidation, the mobility of Se in the reclamation environment may increase. The consequences of this mobility may be increased Se availability to plants that results in greater plant uptake of Se, and in turn an increased concern for toxicity to animals.

Current soil and overburden guidelines of several western states, including Wyoming, were developed to aid in the sampling and analysis of Se or other parameters of concern. These guidelines are used by many federal agencies, state agencies, mining operators, and consultants. The guidelines were designed to identify

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[2] Submitted by the Wyoming State Subcommittee on Selenium in Soils, Vegetation, Overburden, and Wildlife: Lowell K. Spackman and Jim G. Luther, Wyoming Department of Environmental Quality, Land Quality Division, Cheyenne, WY; D.G. "Mickey" Steward, AMAX Coal West, Inc., Gillette, WY; Laurel E. Vicklund, Cordero Mining Company, Gillette, WY; George F. Vance, Plant, Soil, and Insect Sciences, University of Wyoming, Laramie, WY.

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unsuitable levels of soluble constituents which may potentially produce toxic or deleterious conditions in reclamation environments.

Guideline No. 1 of the Wyoming Department of Environmental Quality, Land Quality Division (WDEQ-LQD, 1984) defines marginal Se levels in soil, spoil, and overburden as being <0.1 μg Se/g determined by ammonium bicarbonate-diethylenetriaminepentacetic acid (AB-DTPA) or hot water soluble (HWS) extraction. The choice of this concentration of extractable Se was based on a correlation between 0.1 μg/g AB-DTPA extractable Se and 5 μg Se/g in vegetation from greenhouse studies (Soltanpour and Workman, 1980). The 5 μg Se/g concentration in forage is believed to be toxic to animals consuming this vegetation for several weeks (Thatcher, 1961). It is not known with any reasonable certainty whether 0.1 μg/g extractable Se in overburden translates into vegetation concentrations of greater than 5 μg Se/g, or what effects this level of Se would have on wildlife and livestock. Therefore, there is a need to define the correlation between plant Se concentrations to those in regraded spoil and surface zones to be revegetated (Ihnat, 1989). Since the primary land uses for most mine lands are livestock grazing and wildlife, there is just cause to focus attention and research efforts on the relationship between the rooting zone, vegetation, and target organisms within Powder River Basin. Additional testing is required to accurately correlate the true relationship between plant Se concentrations and Se contents of soils from mining sites to validate the use of the above criteria.

This three phase study of Se is a joint effort between the WDEQ-LQD, the Wyoming Mining Association (WMA) and the University of Wyoming as part of the Se Task Force Steering Committee. This paper was Phase II of a three-phase study conducted by the Joint Subcommittee on Soil, Vegetation, Overburden, and Wildlife (SVOW). Phase I was a characterization of vegetation on both unmined and reclaimed lands (Vicklund et al., 1995). Phase II evaluates the relationship of plant Se concentrations with Se in overburden, soil, and spoil material. The results of Phase II are presented in this portion of the study on "Selenium and Mining in the Powder River Basin, Wyoming." Phase III will attempt to identify the effect of Se on target organisms or those animals that may be affected by Se from vegetation and water consumption. A preliminary inventory of Se in deer mice, as part of Phase III is presented by Raisbeck et al., 1995. Most surface coal mines within the Powder River Basin participated in these studies by providing access and funds to analyze both vegetation and the earthen material.

The objectives of this research were to:

1. characterize both pre and post mining Se concentrations;
2. correlate seleniferous soil/spoil and uptake of Se in vegetation.

Materials and Methods

BKS Environmental Associates, Inc., of Gillette, WY, conducted sampling in the early summer and fall of 1993. The sample points were selected by the SVOW subcommittee based on characterization of Se in vegetation from the Phase I analysis and a pilot Phase II project. Sites were selected with relative Se values from medium to high as found in the Powder River Basin (Vicklund et. al., 1995). The Phase I sample sites were either randomly selected (random sites) or selected based on existing overburden/spoil or plant data that indicated the rooting material contained Se above 0.1 mg/kg (select sites). Select sites were collected in addition to random sites for two reasons: 1) one concern was that the random samples would not have elevated concentrations of Se, and 2) to test the assumption that study or monitoring sites could be selected by historical or baseline data. Both unmined and reclaimed sample points had been previously marked with metal fence posts or lathe during the Phase I sampling of vegetation. Each sample site was photographed before sampling.

Sample Time

Vegetation and soil/spoil/overburden samples were collected at two different times in 1993; between June 15 and July 15, and again between October 15 and November 15. Samples were collected from the same plots for both summer and fall. The plants were chosen to represent the dominant species and lifeforms; no attempt was made to sample the same plants. The time frame for sampling generally occurred soon after bolting and flowering of western wheatgrass *(Agropyron smithii)* in the early summer, and senescence for most species, specifically forbs, in the fall sampling. However, cool season grass species showed some green growth during fall sampling.

Vegetation Sampling and Analysis

All cover sampling was conducted in 1.0 m^2 plots. Absolute vegetation cover by species, as well as litter, rock, or bare ground, if applicable, were estimated to the nearest whole percent. Absolute vegetation cover values were determined before clipping. These estimates were made to determine the importance of various species or lifeforms to be sampled.

Four vegetative lifeforms were sampled, including: warm season grass, cool season grass, shrub, and perennial forb. Shrub clippings included only recent growth. If all four lifeforms were not present in the sample plot, dominant individual plant species, as determined by cover estimates, were sampled. Samples were collected from at least five individual plants for each dominant species, when possible. Plant samples were immediately chilled in the field and frozen within eight hours of collection. Samples were transported to the laboratory within 24-48 hours of collection. Samples were kept frozen until analysis, and analyzed within two weeks. Details on the sampling and analysis procedures for Se in the vegetation are provided in Steward et al. (1994). These procedures were initiated to limit field and laboratory errors during analysis.

Soil/Overburden/Spoil Sampling and Analysis

Soils were sampled at two locations, the southeast and northeast corners for each plot. An auger was used to collect the samples at each depth. Samples were collected in three depth increments at each reclaimed area location; one for the replaced topsoil and one for each two-foot increment of spoil below the replaced topsoil. Composites of the two locations within each plot were collected at each depth increment regardless of horizonation at the unmined locations, i.e., 0-24, 24-48, and 48-72 inches. The three depths for both unmined and reclaimed are referred to as shallow, moderate, and deep sample intervals.

Field information was collected on texture, color, and salinity. In addition, the presence of coarse fragments and carbonaceous material was documented. Samples were shipped to the laboratory at the end of each sample period. Parameters analyzed at the laboratory included: pH, electrical conductivity (EC), saturation percent, texture, total organic carbon (TOC), phosphates, and sulfates. Three methods were used to determine extractable Se concentration, i.e., HWS, AB-DTPA, and potassium dihydrogen phosphate (KH_2PO_4) Se. Standard Operating Procedures (SOPs) for the sampling and analysis of soil/overburden/spoil material in this study were provided in Spackman et al. (1994). These SOPs were developed and used to improve precision and reduced the variability in Se analytical results.

Results

Descriptive Statistics

Samples from vegetation and soils were stratified by location (uumined or reclaimed), season (summer or fall), and method of site selection (random or select). Descriptive statistics were determined separately for both vegetation and soil/overburden/spoil samples.

Vegetation

The highest vegetation Se concentration measured in this study was 320 mg/kg in the forb two-grooved milkvetch (*Astragalus bisulcatus*). On the average, forb Se concentrations were higher than shrub, and shrub concentrations were higher than grasses. Forbs and shrubs are more deeply rooted than grasses, therefore they may have access to greater amounts of Se. In addition, certain forbs such as two-grooved milkvetch have been identified as Se accumulators. These forbs can greatly skew or bias average values. However, even disregarding the accumulator species, forbs showed higher concentrations of Se accumulation on the average than either did shrubs or grasses.

In general, Se concentrations in grass were similar between species, and were not very different between unmined and reclaimed sites. Selenium concentrations in most grass species averaged around 0.5 mg/kg. Selenium concentrations in needle-and-thread grass (*Stipa comata*) were only about half of that found in most other grasses. The highest overall mean Se value in grasses was for green needle grass (*Stipa viridula*) at 0.71 mg/kg.

Results show seasonality plays a large part in vegetation Se concentration, and therefore it is logical that the generally high growth rates experienced by forbs early in the summer may contribute to relatively high rates of Se accumulation. Forbs demonstrated a dramatic decline in Se concentration from summer to fall (from an average of 22.84 mg/kg to an average of 4.08 mg/kg).

Inspection of the data show a summer-fall reduction trend for every individual species sampled except needle-and-thread grass; the reduction is especially dramatic for two-grooved milkvetch, a primary Se accumulator species. Such a decline was not evident in the majority of shrub species sampled (from an average of 1.40 mg/kg in the early summer to an average of 1.36 mg/kg in the fall). Shrub growth occurs more slowly than does forb growth and extends over a greater time period. This difference in growth may influence the rate of Se uptake.

Junegrass (*Koeleria macrantha*) showed the highest concentration of Se (10.1 mg/kg) for any one grass sample collected on a randomly chosen, unmined site in the summer. The maximum Se concentration encountered from any individual grass species in the fall was 0.9 mg/kg. A similar seasonal drop was seen in mean values for all of the grass species (Table 1).

The summer of 1993 was cool, with higher than average precipitation compared to other recent summers, and as such may have stimulated growth and Se accumulation beyond normal concentrations. As in the case of the four lifeforms, the difference between summer and fall concentrations was statistically significant for several individual species sampled. On Table 1, species labelled with a different letter between seasons were significantly different (alpha = 0.05); otherwise, samples were not significantly different.

Some trends, although not statistically significant, were observed between unmined and reclaimed sites. Selenium was slightly higher in thickspike wheatgrass (*Agropyron dasystachyum*) on reclaimed sites than unmined sites (0.49 vs. 0.63 mg/kg); and slightly higher in western wheatgrass (*Agropyron smithii*) on unmined sites (0.59 vs. 0.54 mg/kg). Selenium concentrations were higher on unmined sites than reclaimed sites for yellow sweet clover (*Melilotus officinalis*, 1.25 vs. 0.64 mg/kg), needle-and thread grass (0.27 vs. 0.17 mg/kg), green needlegrass (*Stipa viridula*, 0.71 vs. 0.70 mg/kg), and dandelion (*Taraxacum officinale*, 6.37 vs 2.69 mg/kg).

Average Se concentrations in two-grooved milkvetch were different between reclaimed and unmined sites, even if not statistically significant. The reclaimed sites far exceeded the unmined sites for two-grooved milkvetch. However, the general trend shows slightly higher concentrations of Se in vegetation on unmined sites in relationship to reclaimed sites. This relationship represents a change from the trend seen in Phase

Table 1.
Mean Values for Vegetation Selenium Concentrations

Species	Overall	Unmined	Reclaimed	Summer	Fall	Random	Select
Thickspike wheatgrass	0.56	0.49	0.63	0.83	0.30	0.53	0.60
n	30	14	16	15	15	16	14
sd	0.55	0.45	0.63	0.63 a	0.28 b	0.55	0.57
Western wheatgrass	0.56	0.59	0.54	0.72	0.39	0.52	0.74
n	48	23	25	25	23	39	9
sd	0.49	0.51	0.49	0.61 a	0.23 b	0.43	0.73
Big sagebrush (shrub)	1.20	1.20	-	1.43	0.91	1.26	0.68
n	18	18	-	10	8	16	2
sd	1.83	1.83	-	2.36	0.89	1.93	0.60
Two-grooved milkvetch (forb)	101.55	86.07	140.26	192.54	10.56	85.76	141.03
n	14	10	4	7	7	10	4
sd	130.64	125.23	155.26	132.58 a	8.89 b	127.19	150.04
Blue grama (grass)	0.55	0.55	-	0.74	0.35	0.58	0.35
n	19	19	-	10	9	17	2
sd	0.74	0.74	-	0.98	0.28	0.78	0.07
Broom snakeweed (halfshrub)	0.75	0.77	0.65	1.19	0.39	0.75	-
n	11	10	1	5	6	11	-
sd	1.14	1.20	-	1.66	0.21	1.14	-
Junegrass	0.64	0.64	-	0.96	0.29	0.65	0.52
n	31	31	-	16	15	28	3
sd	1.77	1.77	-	2.45	0.25	1.87	0.30
Yellow sweet clover (forb)	0.77	1.25	0.64	0.89	0.64	0.58	1.10
n	14	3	11	7	7	9	5
sd	0.54	0.70	0.45	0.40	0.67	0.39	0.67
Needle-and-thread grass	0.25	0.27	0.17	0.22	0.30	0.26	0.20
n	21	18	3	11	10	19	2
sd	0.24	0.25	0.18	0.33	0.33	0.25	0
Green needle grass	0.71	0.71	0.70	1.08	0.30	0.70	0.73
n	27	11	16	14	13	21	6
sd	1.05	1.16	1.01	0.12	0.19	1.56	0.65
Dandelion (forb)	4.26	6.37	2.69	4.56	-	4.26	-
n	7	3	4	6	-	7	-
sd	5.97	9.65	1.20	6.48	-	5.97	-

Mean and standard deviation expressed in units of mg/kg. Values followed by the letter a in the first column and b in the second column are significantly different at the 95% level of confidence.

I research (Vicklund et al., 1995). The sample size was small for this study, thus this trend toward higher concentrations in unmined areas could not be confirmed.

Soils

In addition to those descriptive statistics examined for vegetation, data were combined to provide an average by location in the plot (northeast or southeast) and depth (shallow, moderate, or deep) for each soils variable from each sample plot. Inspection of summary information for soil variables reveal very little difference between summer and fall observations (Table 2A). However, there was a slight trend for Se and certain other variables to be higher in unmined soils compared to reclaimed soils (Table 2B). Further, as might be expected, a trend toward elevated Se was observed for method of site selection (Table 2C), with select sites showing higher concentrations of soil Se than the randomly chosen sites.

In contrast to the subtle trends described above, there is a distinct increase with depth for all extraction methods of Se, electrical conductivity, and sulfates (Table 2B). This trend is consistent throughout the data set for these variables, although the difference between the moderate and deep material for the reclaimed sites was not significant. Recall that the shallow depths on the reclaimed sites were the topsoil, while the moderate and deep samples were the 0-2' and 2-4' depths, respectively for the regraded spoil surface. None of the other variables sampled, including texture, pH, total organic carbon, or phosphorus, showed a depth trend.

Evaluation of the extraction methods showed the KH_2PO_4 (phosphate) method to extract the most Se from the soils, overburden, and spoils. Phosphate extraction is followed by AB-DTPA and then HWS in terms of the amount of Se extracted.

Summary of Descriptive Statistics

In summary, there are two major trends evident from the descriptive statistics. The first is the difference in vegetation Se between seasons, and the second is a consistent increase with depth for extractable Se, electrical conductivity, and sulfates. Also of note is the striking, even if not statistically significant, difference between unmined and reclaimed sites for two-grooved milkvetch, with far higher plant Se concentrations on reclaimed areas.

Analytic Statistics and Graphical Analysis

Correlations and Regressions

Correlations were computed between dependent and independent variables with the goal of relating soil and vegetation results. For example, *Astragalus bisulcatus* was correlated with organic carbon at the three soil depths. Descriptors of a regression relationship (Table 3) were estimated for significant ($p<0.01$) correlation pairs and combinations of correlation pairs. The relationship between the four different growth forms and any of the extraction methods is absent from this table because of the poor correlation. In addition, plots were developed for the data sets in order to visually identify suspect relationships. Significant regressions that also showed a strong visual relationship were Se in big sagebrush (*Artemisia tridentata*) versus percent clay in the shallow layer of the soil; yellow sweet clover and shallow clay; and Se in alfalfa and shallow clay (Figure 1A, B, and C, respectively). This relationship held over both seasons, but was more striking in the summer, especially for big sagebrush.

In general, there was no well defined relationship between the grasses and the textural variables. However, thickspike wheatgrass did show a slight graphical relationship to shallow depth pH (Figure 2) in the summer samples. This relationship was the only case in which pH appeared to be at all correlated to plant Se concentrations.

Table 2
Mean Values for Soil Variables

(A) Seasonal, Summer and Fall

	Hot Water Extract Selenium (mg/kg)			ABDTPA Extract Selenium (mg/kg)			Phosphate Extract Selenium (mg/kg)			Electrical Conductivity* (mmhos/cm)			Sulfate* (meq/l)		
Depth	Overall	Summer	Fall	Overall	Summer	Fall	Overall	Summer	Fall	Overall	Summer	Fall	Overall	Summer	Fall
1	0.02	0.02	0.02	0.03	0.03	0.03	0.06	0.06	0.06	1.77	1.82	1.72	17.39	18.44	16.34
2	0.10	0.11	0.09	0.12	0.12	0.11	0.14	0.15	0.13	3.77	3.97	3.56	47.04	50.17	43.83
3	0.18	0.17	0.20	0.21	0.19	0.23	0.24	0.23	0.14	4.92	4.79	5.09	65.69	63.40	68.75

(B) Unmined and Reclaimed

	Hot Water Extract Selenium (mg/kg)		ABDTPA Extract Selenium (mg/kg)		Phosphate Extract Selenium (mg/kg)		Electrical Conductivity* (mmhos/cm)		Sulfate* (meq/l)	
Depth	Unmined	Reclaimed	Unmined	Reclaimed	Unmined	Reclaimed	Unmined	Reclaimed	Unmined	Reclaimed
1	0.03	0.01	0.04	0.02	0.07	0.05	1.56	2.01	13.35	22.08
2	0.12	0.08	0.13	0.10	0.16	0.12	3.67	3.90	45.04	49.55
3	0.24	0.09	0.27	0.11	0.30	0.14	5.66	3.80	77.27	48.10

(C) Random and Select Locations

	Hot Water Extract Selenium (mg/kg)		ABDTPA Extract Selenium (mg/kg)		Phosphate Extract Selenium (mg/kg)		Electrical Conductivity* (mmhos/cm)		Sulfate* (meq/l)	
Depth	Random	Select	Random	Select	Random	Select	Random	Select	Random	Select
1	0.01	0.07	0.01	0.08	0.04	0.11	1.56	3.08	12.71	31.92
2	0.08	0.18	0.09	0.20	0.12	0.21	3.67	5.15	42.56	62.22
3	0.19	0.13	0.23	0.15	0.25	0.17	5.66	4.96	66.59	62.83

*saturated paste extract

Table 3
Seleninm Levels in Vegetation as a Function of Environmental Values

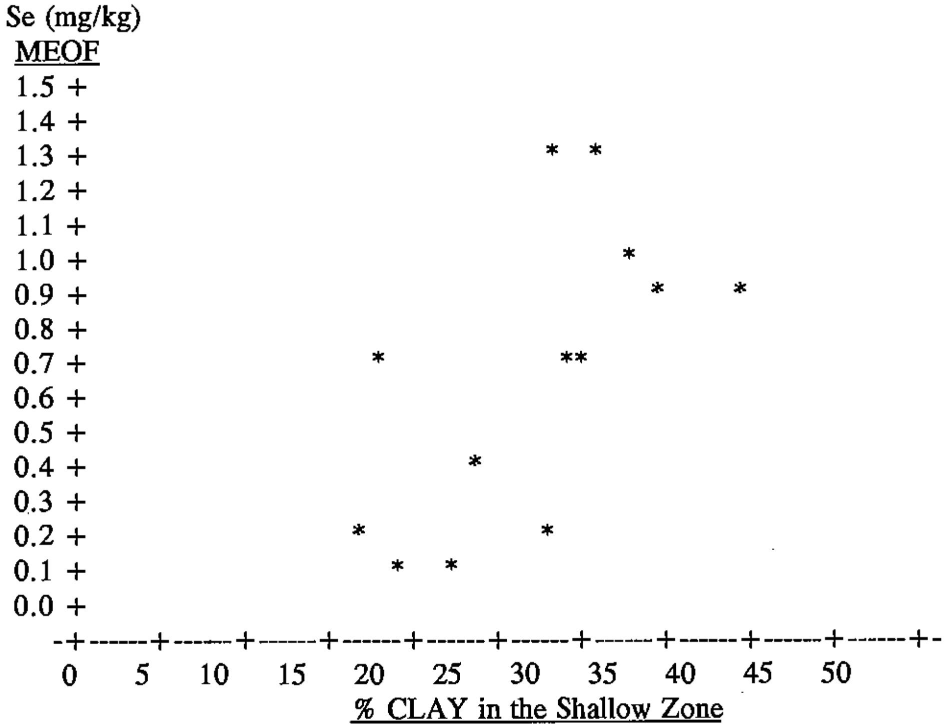
Dependent Variable	Independent Variable	F-Value	Probability	Cumulative R-square
Big sage	Deep Soil Sulfates	21.95	0.0004	0.63
Two-Groove milkvetch	Moderate Soil Total Organic Carbon	14.25	0.005	0.64
Broom snakeweed	Moderate Soil Phosphate Extract Selenium	25.53	0.007	0.86
	Deep Soil Phosphate Extract Selenium	49.07	0.006	0.99
	Hot Water Soluble Extract Selenium	8.55	0.10	0.999
Yellow sweet clover	Shallow Soil Sulfate	15.45	0.004	0.66
Alfalfa	Shallow Soil Total Organic Carbon	15.80	0.02	0.80
	Shallow Soil Saturation Percentage	4.47	0.12	0.92
Scarlet globemallow *Sphaeralcea coccinea*	Deep Soil Phosphorus	19.07	0.01	0.83

Figure 1 A
Selenium in Big Sage (*Artemisia tridentata*) versus Shallow Soil Clay

```
Se (mg/kg)
 ARTR
     8.0 +
     7.8 +
     7.6 +
     7.4 +                                       *
     7.2 +
     7.0 +
     6.8 +
     6.6 +
     6.4 +
     6.2 +
     6.0 +
     5.8 +
     5.6 +
     5.4 +
     5.2 +
     5.0 +
     4.8 +
     4.6 +
     4.4 +
     4.2 +
     4.0 +
     3.8 +
     3.6 +
     3.4 +                                        *
     3.2 +
     3.0 +
     2.8 +
     2.6 +                        *
     2.4 +
     2.2 +
     2.0 +
     1.8 +                                         *
     1.6 +
     1.4 +
     1.2 +
     1.0 +                            *
     0.8 +
     0.6 +                       * *
     0.4 +                *  *    *
     0.2 +                  *     ***
     0.0 +                    *      *
        -+----+----+----+----+----+----+----+----+----+----+-
         0    5    10   15   20   25   30   35   40   45   50
                      % CLAY in the Shallow Zone
```

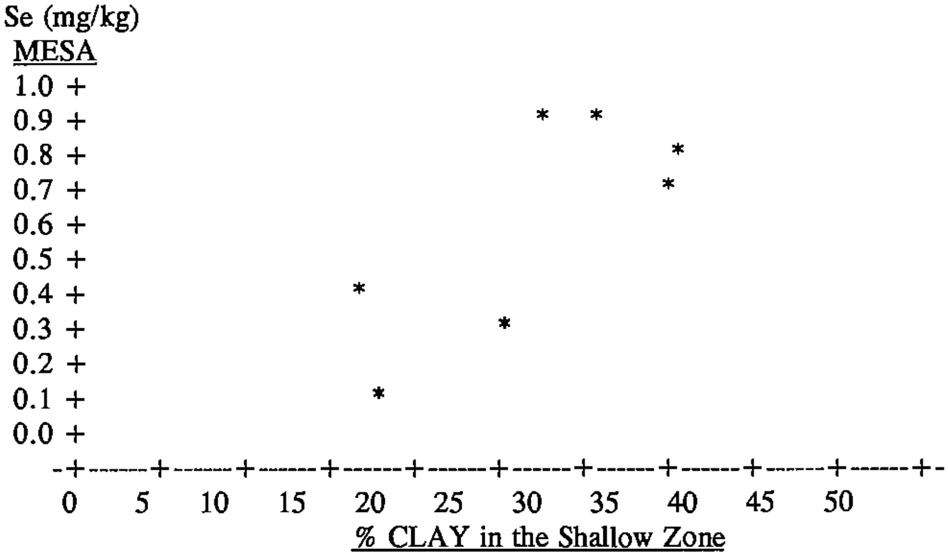
NOTE: 62 obs had missing values. 2 obs hidden. 2 obs were out of range.

FIGURE 1 B
Selenium in Yellow Sweet Clover *(Melilotus officinalis)* versus Shallow Soil Clay



NOTE: 67 obs had missing values. 2 obs were out of range.

FIGURE 1 C
Selenium in Alfalfa *(Medicago Sativa)* versus Shallow Soil Clay



NOTE: 73 obs had missing values. 2 obs were out of range.

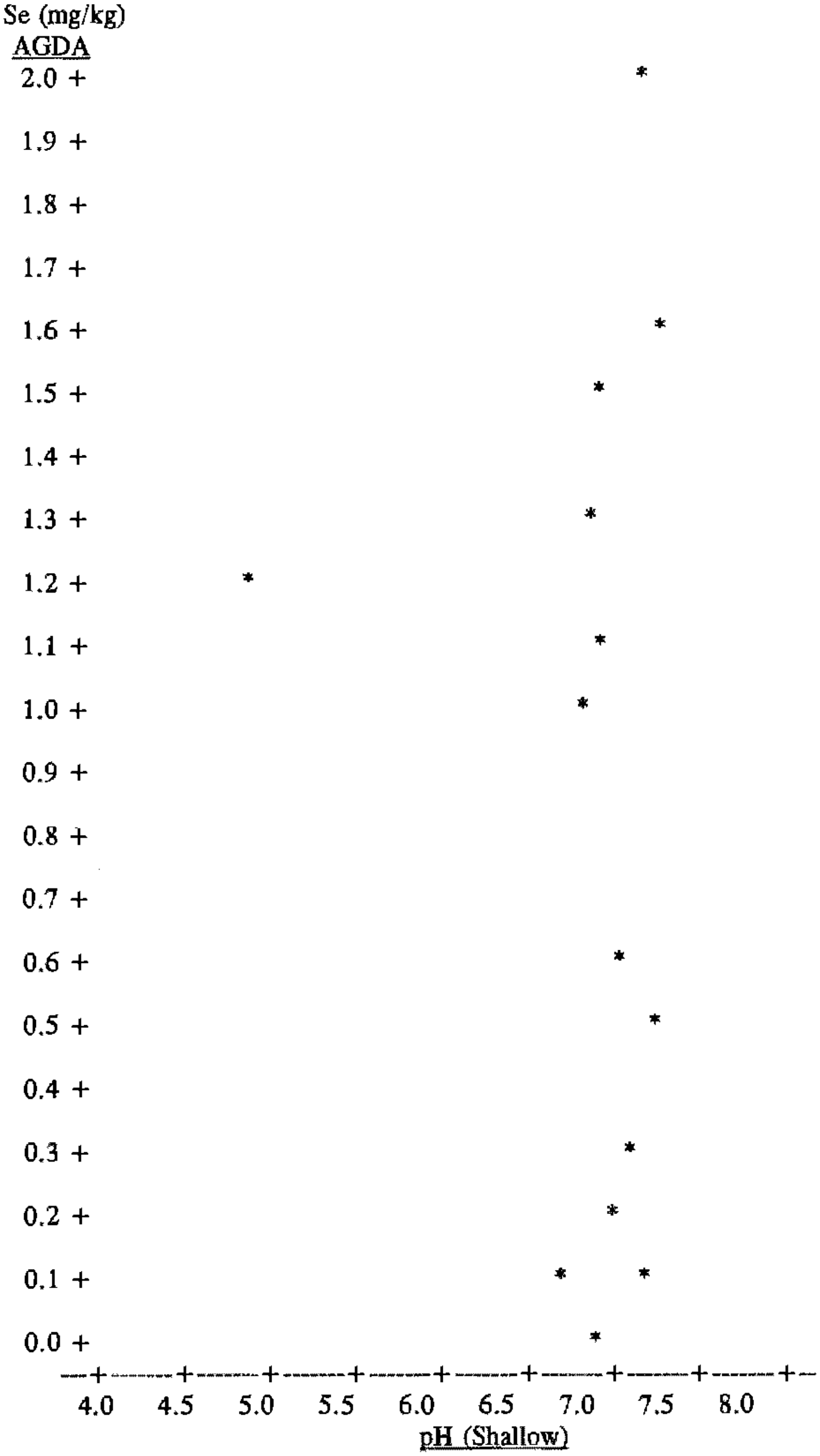
FIGURE 2

Selenium in

Thickspick wheatgrass *(Agropyron dasystachyum)*

versus Shallow Soil pH

Summer



NOTE: 26 obs had missing values. 1 obs were out of range.

Selenium in two-grooved milkvetch appeared to be related to total organic carbon, both statistically and graphically during both summer and fall (Figures 3A and B). A general conclusion to be drawn from these analyses is to expect plants with somewhat elevated Se in clayey soil, and possibly in soils with high organic carbon, although the selenium-organic carbon link may be limited plants to that are Se accumulators.

Rate of Change Analysis

The results, thus far, show consistent increase in Se with soil depth and a marked seasonal difference in Se in vegetation. These findings, and the difficulties in obtaining correlations between vegetation and extractable Se, suggest the vegetation-soils system is very dynamic. Some measure of rate of change, either with time or depth, may help in describing the dynamics of that system.

A measure of the "rate of change" was derived by calculating the change from one depth to another for those soil variables that increased with depth. Thus, the difference between the shallow and the moderate soil samples and between the moderate and the deep samples was computed for the phosphate extract soil Se, soil sulfates, and soil electrical conductivity. Phosphate extractable Se was chosen because this extract resulted in the widest range of Se values between depths.

These "rate of change" variables were then regressed stepwise against vegetation Se. In many cases the results were striking (Table 4). As was the case in some of the untransformed variables, some of the significant relationships were artifacts that could be identified visually by plotting. Nonetheless, although not in all cases, Se in vegetation appeared to be predicted by the "rate of change" of Se in the soil with depth. This relationship merits further exploration, especially as it appears to be governed by, or at least accompanied by, change in electrical conductivity and sulfates.

While seasonality also affected the change relationship, it is clear that, for some species, the "rate of change" relationship held regardless of season. Of particular note, is the relationship between Se in yellow sweet clover and change in phosphate extract Se from moderate to deep soils. Yellow sweet clover is a useful plant because it grows quickly and is easy to establish on both reclaimed and unmined areas.

In this case, 60% of the variation in sweet clover Se concentrations could be explained by the variation in phosphate extractable Se with depth. In addition, graphical analysis of the relationship showed it to be consistent and linear. According to the regression, the greater the difference in Se concentrations between the moderate and deep soils, the greater the Se concentrations in yellow sweet clover, regardless of the absolute concentrations of Se in the soil. Similar results were also seen for alfalfa; although, for alfalfa, the determinant variable was change in sulfate concentration.

Selenium concentrations in grasses appeared to be negatively related to change with depth in electrical conductivity for needle-and-thread grass in the summer, and related to change with depth in phosphate extractable Se (positive coefficient) and sulfates (negative coefficient) for green needle grass. The relationship between Se concentrations in vegetation and "rate of change" in soil Se with depth suggests that certain soil dynamics not clearly identified by antecedent soil Se concentrations result in increased Se uptake in plants. Avenues for further research and data analysis are recommended by these interesting findings. A study specifically designed to investigate Se change with depth allied with rooting depths might refine these preliminary results. It is also clear that texture, total organic carbon, sulfate, and electrical conductivity are important parameters that must be evaluated along with Se to understand the full range of Se interaction within the vegetation-soils system.

Extract Comparisons

Regressions were computed to describe the relationship between values for the three extracts used to analyze soil Se. The regressions were stratified by depth, but no consistent depth effect was apparent. Hot water extracted Se could be predicted from AB-DTPA extracted Se with a coefficient of determination (r^2)

FIGURE 3 A

Selenium in Two-grooved milkvetch *(Astragalus bisulcatus)* versus Moderate Soil Total Organic Carbon - Summer

```
Se (mg/kg)
 ASBI
250 +                                        *
240 +
230 +                                  *
220 +                                    *
210 +
200 +
190 +
180 +
170 +
160 +
150 +
140 +
130 +
120 +
110 +
100 +
 90 +
 80 +
 70 +
 60 +
 50 +
 40 +
 30 +
 20 +
 10 +                  *           *
  0 +
   -+------+------+------+------+------+------+------+------+------+------+------+-
    0      1      2      3      4      5      6      7      8      9      10
                                      % TOC2
NOTE: 33 obs had missing values.  3 obs were out of range.
```

FIGURE 3 B

Selenium in Two-grooved Milkvetch *(Astragalus bisulcatus)* versus Moderate Soil Total Organic Carbon

Fall

```
Se (mg/kg)
 ASBI
 30 +
 20 +                                     *  *
 10 +                             *
  0 +                    *   *    *
   -+------+------+------+------+------+------+------+------+------+------+------+-
    0      1      2      3      4      5      6      7      8      9      10
                                      % TOC2
NOTE: 33 obs had missing values.  1 obs hidden.  1 obs were out of range.
```

Table 4
Selenium Levels in Vegetation as a Function of Rate of Change of Phosphate-Extract Selenium (KH_2PO_4), Sulfates (SO4), and Electrical Conductivity (EC)

Dependent Variable	Independent Variable	F-Value	Probability	Cumulative R-square
Thickspike wheatgrass Summer	Shallow to Moderate KH_2PO_4	4.68	0.05	0.28
Big sage	Moderate to Deep EC Moderate to Deep SO4 Shallow to Moderate EC	10.26	0.002	0.74
Big sage Fall	Moderate to Deep SO4 Shallow to Moderate PO4 Shallow to Moderate EC Shallow to Moderate SO4	347.95	0.04	0.999
Blue grama Fall	Shallow to Moderate SO4	5.98	0.04	0.46
Broom snakeweed	Moderate to Deep KH_2PO_4	86.07	0.0002	0.95
Junegrass	Moderate to Deep EC Moderate to Deep SO4 Shallow to Moderate KH_2PO_4	6.01	0.03	0.75
Yellow sweet clover	Moderate to Deep KH_2PO_4	12.31	0.008	0.61
Alfalfa	Moderate to Deep SO4	4.77	0.09	0.54
Alfalfa Summer	Shallow to Moderate SO4	20.78	0.04	0.91
Scarlet globemallow *Sphaeralcea coccinea*	Shallow to Moderate KH_2PO_4 Shallow to Moderate SO4	8.99	0.05	0.86
Scarlet globemallow Summer	Shallow to Moderate KH_2PO_4 Shallow to Moderate SO4	8.99	0.05	0.86
Needle-and-thread grass Summer	Moderate to Deep KH_2PO_4	7.10	0.03	0.44
Green needle grass	Moderate to Deep KH_2PO_4 Moderate to Deep SO4	9.64	0.002	0.56
Green needle grass Summer	Moderate to Deep KH_2PO_4 Moderate to Deep SO4 Shallow to Moderate KH_2PO_4	8.90	0.006	0.77

of over 95% Slightly less dramatic, but still impressive, was the ability to predict phosphate extractable Se from AB-DTPA extractable Se (r^2=90% on the average) and phosphate extractable Se from HWS Se (r^2 =88% on the average). These relationships imply that the Se extraction methods are consistent and the precision of the different methods is acceptable.

Selenium Versus Site Age

Date of backfill grading was used as a rough measure of site age; the younger the site, the more recent the date of backfill grading. Regressions of Se, electrical conductivity, and sulfates against date of backfill grading were found to be significant at moderate soil depths (Table 5). Regressions were generally not significant in shallow or deep soil samples. Visual assessment of the simple regressions showed plausible fits for the significant regressions.

Analysis of Variance

The analysis of variance was designed to examine differences between unmined and reclaimed, random and select, and summer and fall sample locations. Sample sizes were not large, so only single factor analysis of variance was employed. The Duncan multiple range test at $p \leq 0.05$ was used to differentiate means that were significantly different. The results of these analyses could profitably be augmented by multiple factor analysis of variance and cluster analysis, if a larger database were available.

Vegetation

There was only one significant difference between unmined and reclaimed sites for any of the vegetation variables. Concentrations of Se in yellow sweet clover were significantly higher on unmined sites than reclaimed. At a similarly significant concentration, Se was higher in yellow sweet clover on select sites than randomly chosen sites. There were no other significant differences between random and select sites.

There were statistically significant differences between summer and fall only for thickspike wheatgrass, western wheatgrass, and green needlegrass. Despite the general lack of statistical significance, the concentrations of Se in vegetation were almost all lower in the fall than the summer. This condition led to a less traditional analysis of the data, which is further described below under "multiple variable t-test".

Soils

Phosphate extractable Se, sulfates, and electrical conductivity at three soil depths (shallow, moderate, and deep) were compared for unmined and reclaimed sites; random and select sites; and summer and fall samples. A statistically significant difference was detected between phosphate extractable Se in deep soils for unmined and reclaimed sites, with unmined sites showing approximately double the amount of Se found at that depth in reclaimed soils. The other two extracts of Se were assumed to respond in a fashion similar to phosphate extract. Sulfate and electrical conductivity were also approximately double in deep soil samples in unmined versus reclaimed soils, and these differences were also statistically significant. These relationships suggest that mineland reconstruction efforts can be effective in creating a suitable rooting zone for improved reclamation success. The analysis and comparison of reclaimed vs unmined soil characteristics should become more clear as the influence of the soil forming factors of this semi-arid environment, i.e., topography, climate, parent material, vegetation, and time, begins to affect the physical and chemical characteristics of the reclaimed soil/spoil profile.

Phosphate extractable selenium was significantly higher on select sites as compared to random sites for shallow and moderate soil depths, but not for deep soils. A similar pattern was evident for sulfates and electrical conductivity. This finding suggests that deliberate selection of sites containing relatively high concentrations of Se was successful. The ability to selected elevated Se sites based on previous knowledge had been questioned in the past. No seasonal difference was found for any of the soil variables analyzed.

Table 5
Date of Backfill Grading versus Soil Variables

VARIABLE	SHALLOW SOIL	MODERATE SOIL	DEEP SOIL
Probability that the regression coefficient is zero (Prob>F)			
Hot Water Extract Selenium	0.44	0.01	0.03
ABDTPA Extract Selenium	0.56	0.02	0.15
Phosphate Extract Selenium	0.06	0.02	0.35
Electrical Conductivity	0.19	0.01	0.56
Sulfates	0.20	0.02	0.76
Mean	**0.29**	**0.01**	**0.37**
Coefficient of Determination for the Regression			
Hot Water Extract Selenium	0.03	0.27	0.18
ABDTPA Extract Selenium	0.02	0.22	0.09
Phosphate Extract Selenium	0.14	0.22	0.04
Electrical Conductivity	0.07	0.26	0.01
Sulfates	0.07	0.23	0.004
Mean	0.07	0.24	0.07

Multiple Variable T-Test

Some of the trends observed in mean values for this Phase II data set were very prominent. For example, although many of the differences were not statistically significant, in every case (except the pioneer species needle-and-thread grass), the mean value for Se in the various plant species was higher in the summer than in the fall (Table 1). Similarly, in many of the cases, the Se concentration in both plants and soils was higher in selected sites than random sites. Selenium concentrations in vegetation from unmined sites were higher, to some extent than reclaimed sites, a trend also reflected in the soils variables.

A simple method was devised to compare the effect each of the variables (i.e. summer to fall, unmined to reclaimed, and random to select) had on the vegetation-soils system represented by this data set. The means for each variable for each treatment were divided by the overall mean for that variable. The resulting dimensionless values were then used to compute a mean for each treatment (mean of the mean/mean). In this way, all variables contributed to a single representation of each treatment. A t-test was then conducted between the treatment means to assess the differences (Table 6).

Using this method, the difference between summer and fall for the vegetation-soils system was markedly significant ($p<0.01$). In addition, the difference between summer and fall for plants only was even more significant ($p<0.001$). However, there was no significant difference between summer and fall when only soils variables were inspected. This result indicates that the vegetation component is far more responsive to seasonal changes than the soils component of the system.

The multiple variable t-test revealed significant differences between random and select sites for soil variables only. This result is very likely an artifact of the site selection procedure; the location of selected sites was based on prior information about the soils. Vegetation and soils data analyzed together, and vegetation data analyzed by itself, did not result in significant differences between random and select sites, which was expected. This lack of significance between random and select sites for vegetation and the combined soil-vegetation system may further support the inability to define a relationship between soil and vegetation Se, except perhaps on a site-by-site basis.

Results of this test were marginally significant for unmined versus reclaimed sites. In all three cases, i.e., vegetation-soils, soils only, and vegetation only, the mean of the mean/mean was greater for the unmined than the reclaimed treatment, indicating that, on the whole, values were lower for system variables on reclaimed land than on unmined land. Using the calculated value of the t-statistic as a measure, this result was more pronounced for the vegetation-soils system as a whole than for the soils alone or the vegetation alone.

Conclusions

On the average, forb Se concentrations were higher than shrub, and shrub concentrations were higher than grasses. Forbs and shrubs are more deeply rooted than grasses, and may thereby have access to greater amounts of Se. The generally high growth rates experienced by forbs early in the summer may contribute to relatively high rates of Se accumulation. Forbs demonstrated a dramatic decline in Se concentration from summer to fall. Such a decline was not evident in the majority of shrub species sampled, but a similar seasonal drop was seen in mean values for all of the grass species except needle-and-thread. In contrast, extractable Se concentrations were nonsignificantly different between summer and fall.

The slightly higher concentration of plant Se on unmined sites when compared to reclaimed sites represent a change from the trend seen in earlier research. However, this conclusion is based on a limited data set as compared to the Phase I data (Vicklund et al., 1995). In contrast to this general trend, two-grooved milkvetch was higher on reclaimed than unmined areas. Soil Se was also slightly lower in reclaimed than unmined samples. This may suggest that unsuitable material is being isolated from the rooting zone on reclaimed areas.

Table 6
Multiple Variable t-test

ALL VARIABLES, SOILS AND VEGETATION (only one extract of selenium to prevent overemphasis on the soil selenium variable)						
Variable	Summer	Fall	Native	Reclaimed	Random	Select
Count	40	40	40	40	40	40
variance	0.05	0.08	0.05	0.11	0.01	0.18
Mean	1.10	0.87	1.10	0.87	0.96	1.04
Pooled Variance	0.06		0.08		0.09	
t-test	3.983		2.303		-1.279	
t-statistic (0.01, 2-tailed)	2.640		2.640		2.640	
t-statistic (0.005, 2-tailed)	2.889		2.889		2.889	

SOILS VARIABLES ONLY (only one extract of selenium to prevent overemphasis on the soil selenium variable)						
Variable	Summer	Fall	Native	Reclaimed	Random	Select
Count	27	27	27	27	27	27
variance	0.01	0.01	0.01	0.02	0.0009	0.09
Mean	0.99	1.01	1.02	0.97	0.95	1.16
Pooled Variance	0.01		0.02		0.05	
t-test	-0.0448		1.393		-3.490	
t-statistic (0.01, 2-tailed)	2.779		2.779		2.779	
t-statistic (0.005, 2-tailed)	3.067		3.067		3.067	

VEGETATION VARIABLES ONLY						
Variable	Summer	Fall	Native	Reclaimed	Random	Select
Count	13	13	13	13	13	13
variance	0.08	0.10	0.14	0.24	0.01	0.29
Mean	1.30	0.59	10.0	0.65	0.97	0.80
Pooled Variance	0.09		0.19		0.15	
t-test	6.088		2.051		1.103	
t-statistic (0.01, 2-tailed)	3.012		3.012		3.012	
t-statistic (0.005, 2-tailed)	3.372		3.372		3.372	

All measures of extractable Se in soil, i.e., HWS extractable, AB-DTPA extractable, and phosphate extractable, increased with depth. A similar increase with depth occurred for electrical conductivity and sulfate. These trends were evident for both unmined and reclaimed sites. This depth trend was not apparent for the other soil variables.

Selenium concentrations in the soils did not seem to predict Se concentrations in plants. Instead, Se in vegetation appears to be predicted to some degree by the "rate of change" of Se in the soil with depth. The "rate-of-change" relationship suggests that certain soil dynamics not clearly identified by antecedent Se concentrations result in increased Se uptake in plants. This relationship merits further exploration, especially as it appears to be governed by, or at least accompanied by, changes in electrical conductivity and sulfates.

The relationship of the different extraction methods was generally good. The best correlation was AB-DTPA extractable Se to predict HWS extractable Se with a coefficient of determination (r^2) of over 95%.

A statistically significant difference was detected between phosphate extract Se in deep soils between unmined and reclaimed sites, with unmined sites showing approximately double the amount of Se found at that depth than in reclaimed soils. This difference suggests that special handling and mitigation of unsuitable material is effective for the upper four feet of the spoil surface. Perhaps pedogenic processes have not affected the accumulation of Se in the reclaimed soil/spoil profile because of the long-term nature of soil formation.

Phosphate extractable Se was significantly higher on select sites than random sites for shallow and moderate soil depths, but not for deep samples. This relationship suggests that relatively high Se sites can be selected based on previous soil analysis.

A synthetic test (multiple variable t-test) of differences between summer and fall for the vegetation-soils system was markedly significant. A similar test was marginally significant between unmined and reclaimed, and not significant between random and select sites. This latter result was expected, as these sites were selected based on previous soils data and not Se concentrations in vegetation. For the three cases of the synthetic test: vegetation-soils, soils only, and vegetation only, the unmined values were higher than the reclaimed values.

The relative concentration of Se in the various species seems to be controlled by the period of active growth for the plant. On the other hand, absolute concentrations of Se in vegetation appear to be correlated to a multitude of soil factors, including percent clay, total organic carbon (possibly in the form of coal), extractable Se, electrical conductivity, and sulfates, as well as time since reclamation.

Perhaps more importantly, the dynamics of an active relationship between plants and soils appear to control the concentration of Se in both plants and soil. This relationship is exemplified by the "rate of change" in Se between soil depths. Because Se is a micronutrient, and unless a source of new Se constantly replenishes Se faster than it can be utilized by the vegetation, the vegetation will eventually deplete plant available soil Se. In a steady state system that is not based on extremely high concentrations of Se in the parent material, Se availability (as well as other nutrients and micronutrients) in the upper part of the soil will be limited by the rate of erosion and the rate of soil formation. This hypothesis is reinforced by the consistent increase of Se with depth, whether in unmined, reclaimed, random, or deliberately selected sites. However, soil formation of these reclaimed soil/spoils may take decades to reach a steady state. In addition, this study did not account for the possible recirculation of Se from the decomposition of plant tissue.

Recommendations

The recommendations offered here should be tempered by the words of Mark Twain, who said, "There is something fascinating about science. One gets such a wholesale return of conjecture out of a trifling investment of fact." With that firmly in mind, there are some possible applications of the information available as a result of this study.

The dominant trends identified by this study in the vegetation-soils system were found to be similar on unmined and reclaimed lands. Therefore, system behavior is probably the same on both unmined and reclaimed lands. However, the system is clearly active and dynamic. Selenium concentrations in vegetation drop markedly once the growing season was over, as evidenced by seasonal changes. From these condensed conclusions, it would seem that:

- The highest <u>average</u> concentrations of plant Se observed in this study do not give reason for concern that Se accumulation in vegetation will affect the wildlife or domestic animals that use this area after reclamation. Although Se accumulator plants had significant concentrations, these plants are generally not foraged by wildlife nor domestic animals.
- The dynamic nature of the vegetation-soil system makes if difficult to predict vegetation Se concentrations from extractable soil Se concentrations. Plant physiology, in addition to chemical and physical characteristics of the soil, can greatly affect the mobility of Se. Site-by-site correlation of vegetation and soil Se may be possible when the vegetation-soil system is well defined.
- Selenium content of yellow sweet clover, a short-lived, fast-growing biennial, may be a good bio-indicator of the risk earthen materials pose for toxic accumulations of Se. It is worth noting that this study indicates that alfalfa may be a similarly good bio-indicator, and elevated Se in alfalfa is exactly what was observed in hay affected by the Kendrick Irrigation Project outside of Casper, Wyoming.
- Grasses resist Se accumulation; they are thus good species to use for reclamation of marginal lands containing Se.

In final summary, results of this research effort tend to diminish concern that mining and reclamation activities in the Powder River Basin will increase potential Se toxicity of animals grazing on upland areas of reclaimed lands. However, it is essential that a union of all recent Se research from mining areas of Wyoming be synthesized to fully comprehend the effects of mining on Se on these reclaimed lands. Surface mining reclamation is clearly different from the problems that are associated with irrigated cropland such as in Kesterson, CA, or Kendrick, WY, with respect to vegetation-soil interactions. Nevertheless, concerns with surface and subsurface Se concentrations and the impacts these concentrations have on aquatic environments has not been addressed by this study.

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SELENIUM AND MINING IN THE POWDER RIVER BASIN, WYOMING: PHASE III - A PRELIMINARY SURVEY OF SELENIUM CONCENTRATIONS IN DEER MICE (*Peromyscus maniculatus*) LIVERS[1]

M.L. Raisbeck, D.G. Steward, Laurel E. Vicklund, George F. Vance,
Lowell K. Spackman, and Jim G. Luther[2]

Abstract: Samples of liver tissue from deer mice trapped on not-yet-mined areas and reclaimed areas at five surface coal mines in the Powder River Basin of northeastern Wyoming were analyzed for selenium. The overall mean concentration of selenium in wet weight liver tissue was 1.685 ppm. The mean value from not-yet-mined areas was 1.437 ppm; the mean value from reclaimed areas was 1.910 ppm (significant at $p<0.1016$). When one not-yet-mined outlier was removed, significance rose to $p<0.0004$. Mine-to-mine comparison of samples stratified by type (that is, by not-yet-mined or reclaimed), showed average tissue concentrations from the reclaimed area of Mine 1 to be higher ($p<0.0143$) than reclaimed area samples from each of the other mines. Average tissue concentrations from the reclaimed area of Mine 1 were also higher ($p<0.0143$) then not-yet-mined area samples at Mine 1. No statistically significant differences were found between mines for samples from not-yet-mined areas, and no statistically significant differences were found between Mines 2, 3, 4, and 5 for samples from reclaimed areas. Multiple analysis of variance using the factors: site (mine) and type (not-yet-mined or reclaimed) was not statistically significant ($p<0.2115$). Simple linear regression showed that selenium concentrations in dry tissue could easily be predicted from wet tissue selenium (r2=0.9775), demonstrating that percent water in the samples was relatively constant. Animal body weight in general was not a predictor for either wet or dry tissue selenium concentrations, but was related to body weight at the higher tissue concentrations of selenium encountered in samples from the reclaimed area at Mine 1. Mouse body weights at Mine 1 were higher on the reclaimed area than mouse body weights from the not-yet-mined area.

Additional Key Words: surface coal mining, reclamation

Introduction

Surface coal mining in Wyoming is regulated by the Department of Environmental Quality. In 1991, that agency fostered the development of joint subcommittees, with members drawn from the regulatory agency, the surface coal mining industry, and the academic community, to investigate the relationship between selected environmental variables and the effects of selenium released or potentiated by surface coal mining. Interest in selenium was stimulated by reports of selenium toxicity in wildlife at various irrigation projects in the western United States. Concern was expressed by some that removal and replacement of overburden during mining might enhance the availability selenium in the postmining environment.

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[2] Submitted by the Wyoming State Subcommittee on Selenium in Soils, Vegetation, Overburden, and Wildlife: M.L. Raisbeck, Department of Veterinary Toxicology, University of Wyoming, Laramie, WY; D.G. Steward, AMAX Coal West, Inc., Gillette, WY 82717; Laurel E. Vicklund, Cordero Mining Company, Gillette, WY 82717; George F. Vance, Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071, Lowell K. Spackman and Jim G. Luther, WDEQ-LQD, Cheyenne, WY 82002