AB-DTPA extractable soil selenium and selenium content of plants
by Richard Allen Prodgers

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University

Abstract:
Twenty-nine soils were sampled near Lysite and Chalk Bluff, Wyoming, where livestock mortality has resulted from toxic (presumably seleniferous) forage. Ammonium bicarbonate - diethylenetriamine-pentaacetic acid (AB-DTPA) extractable selenium concentrations were determined for each soil horizon. Samples of foliage associated with these soils were collected, frozen, later digested in acid, and selenium concentrations determined. Plant species were assigned to one of three groups based on selenium concentrations. Relationships between plant tissue selenium levels and extractable soil selenium levels were statistically evaluated using linear regression and analysis of variance. Independent variables were extractable soil selenium concentrations for half-meter depth increments, average selenium concentration for the profile, and highest selenium concentration in each profile. Regressions were calculated for each group of species at each site. Near Lysite, where extractable soil selenium concentrations were rather low, significant soil/plant relationships were found for the more common, non-indicator species. An AB-DTPA extractable soil selenium concentration of approximately 0.07 Hg Se/g soil (weighted average for soil profile) is correlated with a tissue selenium concentration of 5 Hg/g tissue. As soil depth increases, higher concentrations of extractable soil selenium correlate with threshold toxic vegetation in non-accumulator species. At Chalk Bluff, where soil selenium concentrations were much higher, significant soil/plant relationships were found for selenium accumulator species, but not for non-accumulators. An average AB-DTPA extractable soil selenium concentration of 0.1 µg Se/g soil is correlated with 1900 µg Se/g plant tissue for selenium accumulator species. However, the r^2 for this relationship is only 0.40.

AB-DTPA EXTRACTABLE SOIL SELENIUM AND SELENIUM CONTENT OF PLANTS

by

Richard Allen Prodgers

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Land Rehabilitation

MONTANA STATE UNIVERSITY
Bozeman, Montana

March 1991

APPROVAL

of a thesis submitted by

Richard Allen Prodgers

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Mar 12, 1991
Date

Frank F. Munshower
Chairperson, Graduate Committee

Approved for the Major Department

3/15/91
Date

L. C. Gaynor
Head, Major Department

Approved for the College of Graduate Studies

4/4/91
Date

Henry J. Parsons
Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Dean of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Richard A. Rodgers

Date March 14, 1991

ACKNOWLEDGEMENTS

Thanks to Dr. Frank Munshower and Scott Fisher, Jr. for assistance in the collection of bulk samples. The Office of Surface Mining, U.S. Department of the Interior, funded portions of this study under Grant Number H5160072.

TABLE OF CONTENTS

TABLE OF CONTENTS--(Continued)

LIST OF TABLES

LIST OF TABLES--(Continued)

LIST OF FIGURES

ABSTRACT

Twenty-nine soils were sampled near Lysite and Chalk Bluff, Wyoming, where livestock mortality has resulted from toxic (presumably seleniferous) forage. Ammonium bicarbonate - diethylenetriamine-pentaacetic acid (AB-DTPA) extractable selenium concentrations were determined for each soil horizon. Samples of foliage associated with these soils were collected, frozen, later digested in acid, and selenium concentrations determined. Plant species were assigned to one of three groups based on selenium concentrations. Relationships between plant tissue selenium levels and extractable soil selenium levels were statistically evaluated using linear regression and analysis of variance. Independent variables were extractable soil selenium concentrations for half-meter depth increments, average selenium concentration for the profile, and highest selenium concentration in each profile. Regressions were calculated for each group of species at each site. Near Lysite, where extractable soil selenium concentrations were rather low, significant soil/plant relationships were found for the more common, non-indicator species. An AB-DTPA extractable soil selenium concentration of approximately 0.07 μg Se/g soil (weighted average for soil profile) is correlated with a tissue selenium concentration of 5 μg/g tissue. As soil depth increases, higher concentrations of extractable soil selenium correlate with threshold toxic vegetation in non-accumulator species. At Chalk Bluff, where soil selenium concentrations were much higher, significant soil/plant relationships were found for selenium accumulator species, but not for non-accumulators. An average AB-DTPA extractable soil selenium concentration of 0.1 μg Se/g soil is correlated with 1900 μg Se/g plant tissue for selenium accumulator species. However, the r^2 for this relationship is only 0.40.

INTRODUCTION

The objective of this thesis was to investigate relationships between concentrations of ammonium bicarbonate - diethylenetriamine-pentaacetic acid (AB-DTPA) extractable soil selenium and selenium concentrations in forage plants. Two study areas in Wyoming were selected because they contain known seleniferous soils and plants. Each study area encompasses a variety of plant communities and sites ranging from rock outcrops and uplands to drainage channels. Soils and plants were sampled at both seleniferous sites.

Selenium is one of the mineral elements present in healthy plant foliage that is both a necessary micronutrient and a potential poison for livestock. Although there are many qualifying factors, forage with over 5 μg Se/g dry plant tissue is generally harmful to livestock. Seleniferous forage plants grow in soils that formed as selenium-rich parent materials weathered under conditions that promoted the oxidation of selenium to selenate. This weathering must occur in conjunction with climatic conditions which provide little water to leach soluble selenium from the plant rooting zone. In arid alkaline environments, mining may create seleniferous soils if soil or overburden with high levels of plant-available selenium is placed in the plant root zone.

To prevent the creation of seleniferous soils in reclaimed areas, some mining regulations specify the maximum amount of extractable selenium permitted in the plant root zone. Montana coal reclamation regulations limit the amount of selenium in the upper eight feet to 0.1 μg extractable Se/g soil. The 0.1 μg/g value was tentatively suggested

by Soltanpour and Workman (1980), based on a greenhouse study in which alfalfa was grown in soils treated with sodium selenate and soil selenium was extracted using AB-DTPA, a chelating agent.

LITERATURE REVIEW

Chemistry

Selenium, a naturally occurring element found in soils, geologic formations, plants and animals, was first identified as an element in 1817 from a sulfuric acid production residue. Atomic weight is 78.96, atomic number is 34, atomic radius is 1.40 angstroms, and ionic radius is 1.91 angstroms (-2 charge). Selenium is chemically similar to sulfur in electron configuration of the outer valence shell, bond energies, ionization potentials and atom size. Consequently, selenium forms many organic compounds analogous to sulfur compounds. However, biological systems tend to oxidize the quadrivalent form of sulfur but to reduce selenite, the quadrivalent form of selenium. For example, plants take up selenium mostly as selenates and selenites, but within the plant these selenium forms may be reduced to selenides and incorporated into amino acids (NRC 1976). Ruminant fecal matter contains selenium mostly in the forms of selenides and elemental selenium, and little of this is absorbed by plants (Peterson and Spedding 1963).

Selenium occurs naturally in four oxidation states. Hydrogen selenide (H_2Se) is an example of selenium in the selenide (-2) form. In the atmosphere, H_2Se forms elemental selenium and water. Metal selenides are common in soils and are very insoluble. These compounds may act as selenium sinks (NRC 1976).

Elemental selenium (0) has been placed in the periodic table in Group VIA, the sulfur group. It has both metallic and nonmetallic

properties and is considered a metalloid. It is very insoluble in water.

Selenite (+4) may occur as selenious acid (H_2SeO_3, a weak acid) or various selenites, such as calcium selenite ($CaSeO_3$). Selenites are generally less soluble than selenates. Selenites are stable in mildly acid to alkaline environments and are most soluble in coarse texture soils of low iron content (NRC 1976). Selenium is strongly complexed in ferric selenite [$Fe_2(SeO_3)_3$], which is very insoluble. Selenites are readily reduced at low pH by mild reducing agents to elemental selenium.

Selenate (+6) occurs in selenic acid (H_2SeO_4, a strong acid) and various selenates, such as calcium selenate ($CaSeO_4$). This form is not strongly complexed in ferric selenate in alkaline environments. Selenates are the most soluble form of inorganic selenium. They are stable and soluble in high pH environments and are expected to occur in aerated alkaline soils and alkaline parent materials. Selenates are strongly implicated in plant uptake of selenium even if most of the selenium in the soil is in other forms (NRC 1976).

Organic compounds containing selenium include selenocysteine, selenocystine, selenohomocysteine, Se-methyl selenocysteine (prevalent in *Astragalus bisulcatus*), selenocystathionine (found in *A. pectinatus* and *Stanleya pinnata*), selenomethionine, Se-methyl selenomethionine, dimethyl selenide, dimethyl diselenide, trimethyl selenomium, selenotaurine, selenocoenzyme A and various unidentified selenoproteins and seleniferous waxes (Shamberger 1983). Water-soluble forms are often associated with selenium accumulating plant species, whereas less

soluble forms are usually found in non-accumulator species (Shrift 1973).

Natural Occurrence and Abundance

Most selenium in the biosphere originated during magma crystallization when metal sulfides and selenides formed. As crystallization continued, a residual fluid form of concentrated sulfur and selenium remained. This fluid flowed through fractures in the crystallized magma. Sulfide (and selenide) ore bodies formed where this fluid remained trapped in the earth's crust. Selenium most often occurs as a component of sulfide minerals or as selenides of silver, copper, mercury, or nickel but not as selenium ore *per se*. The Sudbury, Ontario, metal sulfide ore deposits have the greatest known selenium concentration in rock. Even in these materials, however, selenium concentrations are below the level at which selenium alone could be economically mined (Rosenfeld and Beath 1964).

Volatile sulfide and selenide gases sometimes escaped through volcanic discharges. Selenide in the atmosphere oxidized to the elemental form and fell to earth, for example in shallow Cretaceous seas where selenium accumulated in shale sediments. This is the source of most selenium-rich sedimentary geologic formations in western North America (Rosenfeld and Beath 1964).

Selenium is not abundant in the earth's crust, averaging less than 0.1 μg/g, or roughly 1/6,000 the abundance of sulfur (Lakin 1972). Most selenium occurs in the elemental form or as metal selenides (NRC 1976).

Adriano (1986) compiled a table of selenium contents in rocks, soils, and plants. His table is the basis for the following discussion.

Igneous rocks, sandstone and limestone usually contain less than 0.1 μg Se/g. The average concentration for shale is 0.6 μg/g, with levels up to 675 μg/g. Although the selenium content of limestone is usually low, the chalky shales and marls of the Niobrara formation (a Cretaceous shale that outcrops in South Dakota and Wyoming) have greatly elevated levels. Coal usually contains between 1 μg Se/g and 10 μg Se/g. Pillay et al. (1969) reported a mean concentration of 3.36 μg Se/g coal, based on 55 coal samples for the United States. The highest concentration (10.65 μg Se/g) came from a Pennsylvania coal. Selenium, often found near lignite seams, is positively correlated with total sulfur content (Clark et al. 1980, Pitt 1984). Phosphate rock and superphosphate are also high in selenium, often containing concentrations of several hundred micrograms per gram. Soils usually contain less than 0.2 μg total Se/g, with extreme concentrations as high as 5,000 μg/g.

Most of the selenium in parent materials is probably lost in the soil formation process (Moxon et al. 1939). Even in acid or neutral soils where selenium solubility is lowest, selenium is slowly lost from soils through time unless added to the system (NRC 1976, Geering et al. 1968). A mesic precipitation regime contributes to selenium leaching from soils and further selenium depletion (Oldfield 1972).

Many soils are deficient in selenium with respect to animal nutrition because of low initial selenium concentrations, insoluble forms of selenium and leaching. The parent material of these soils is

commonly igneous rocks. In the United States, selenium deficient forages are found in the Pacific northwest, Great Lakes to New England and Florida panhandle areas (Kubota et al. 1975).

Selenium cycling on a global scale involves volcanic discharge and selenium accumulation in seabeds over geologic time. National Research Council (1976) provides one such cycling model. Other models portray selenium cycling through soils, plants and animals (Lipman and Wakeman 1923, Shrift 1964, Allaway et al. 1967). Atmospheric emissions from industrial sources are causing selenium enrichment of many soils, though the contribution is usually minor (Mayland et al. 1989). Selenium cycling is in some ways similar to nitrogen and sulfur cycling. Each element exists in gaseous form at some stage and changes oxidation state at least once in the cycle.

Selenium in Soils

Trelease and Beath (1949) emphasized a direct relationship between plant selenium content and available selenium in the geologic formation from which the soil was derived. Byers and Lakin (1939) also observed a positive correlation between geological strata and selenium in soils. This relationship applies in arid to semiarid, neutral to alkali environments.

In the United States, most seleniferous soils have derived from Cretaceous sedimentary rocks. Lakin (1961) hypothesized that this may have resulted from the deposition of volcanic effluvia or redeposition of erosion products from formations of volcanic origin. Knight (1937) stressed that nearly all seleniferous soils are very thin mantles formed

by the mechanical disintegration of rocks *in situ*, as opposed to soils formed from transported parent materials.

In addition to geologic processes, "selenium converter plants" (Rosenfeld and Beath 1964) can contribute to plant-available selenium (Beath 1959). These species pump selenium from deeper soil horizons to above-ground plant parts. Upon decomposition, this organic selenium is deposited at the soil surface, where it may oxidize and become available to other plants, including non-accumulator species. Beath (1937) reported that grasses near an *Astragalus bisulcatus* plant contained 70 μg Se/g, while nearby grasses had 62 μg Se/g and grasses beyond the zone of influence of the *A. bisulcatus* plant contained 11 μg Se/g.

Selenium may be added to soils as an impurity in superphosphate and ammonium sulfate fertilizers (Rader and Hill 1935). Seleniferous soils can also result from mining activities (Rosenfeld and Beath 1964).

Seleniferous soils have traditionally been identified by the presence or abundance of selenium accumulator plant species, rather than a specified content of total or available selenium. Toxic seleniferous soils are those producing forage toxic to livestock. Potentially toxic seleniferous soils are those capable of producing toxic forage, but where the flora present is not toxic to livestock. Thus soils and plants interact to contribute to forage selenium content.

Swaine (1955) estimated that total selenium in soils is commonly 0.1 to 2 μg/g. Byers et al. (1938) measured soil selenium levels in the United States ranging from trace amounts to 82 μg/g. They concluded that seleniferous soils typically contain total concentrations of 1 to 6 μg Se/g. Most of the seleniferous soils Rosenfeld and Beath (1964)

analyzed contained less than 2 μg Se/g. Trelease and Beath (1949) found the selenium content of soils to vary greatly over rather small areas.

Total and Available Soil Selenium

Total soil selenium content is an unreliable index to available plant selenium (Johnson 1975, Lakin 1972, Nye and Peterson 1975). Although a general correlation sometimes exists between soil selenium content and plant selenium content (Miller and Byers 1937), other factors complicate this relationship. For example, selenium oxidation state and plant species properties affect plant uptake.

Low selenium content plants can grow on high selenium content soils (Byers et al. 1938, Byers et al. 1936). For example, Hawaiian soils with 6 to 15 μg total Se/g soil are not toxic, apparently because they are acidic (pH 4.5 to 6.5) and have abundant iron and aluminum compounds. Under humid conditions, these metals tightly bind selenium (Lakin 1972). In contrast, toxic seleniferous soils are usually alkaline in reaction and contain free $CaCO_3$ (Rosenfeld and Beath 1964).

The three principal factors that determine soil "selenium-supplying power" (Trelease and Beath 1949) are the forms and concentrations of selenium in the soil solution and concentrations of other substances (e.g. sulfates and protein derivatives). The forms of selenium most commonly used by plants are selenate, selenite and organic selenium (Johnson et al. 1967). Plants absorb selenate more readily than selenite, while the availability of organic selenium has been rated both higher than selenate and less than selenite (Trelease and DiSomma, 1944, Trelease and Beath, 1949, Hamilton and Beath 1963); the specific

form of organic selenium may preclude generalizations about availability.

In any case, water-soluble organic selenium can also be a significant selenium source for plants (Beath et al. 1937). Trelease and Beath (1949) reported a soil formed in a Niobrara outcrop in eastern Wyoming that supported an individual *Astragalus racemosus* plant that contained 14,920 μg Se/g. Organic selenium dominated the upper 50 cm, while selenate was most abundant in the 50 to 100 cm depth. Soil content of soluble selenites was not appreciable.

Where selenium is not deficient, selenates probably contribute the most inorganic selenium to plants (Byers 1936). Beath et al. (1946) concluded that selenate is the main water-soluble selenium form in soils associated with toxic vegetation. Selenates are stable in aerated alkali soils of semiarid areas (Lakin 1961).

Selenite compounds are the most stable oxidation state over most of the soil redox range (Bohn et al. 1979). This form of the element is generally less available to plants than selenate because selenite is more strongly adsorbed onto soil particles (Gissel-Nielsen et al. 1984). Selenite ions are less strongly adsorbed at pH 8 than below pH 8, and not adsorbed at all at pH 11 (Governor's Task Force on Selenium 1989). Alkaline environments favor oxidation of selenite to selenate (Geering et al. 1968).

Elemental selenium or selenides on soil colloids may contribute minutely to plant selenium, but their contribution is probably insignificant even in selenium-deficient areas (Gissel-Nielsen and Bisbjerg 1970). When 1 μg elemental Se/g soil was added to

selenium-deficient soils, alfalfa grown thereon had selenium concentrations well below the toxic level. A few months after application, concentrations were too low to protect mammals from selenium responsive disease (Cary et al. 1967).

Other factors such as pH, soil texture, sulfate abundance, and organic matter can influence plant selenium uptake. Soil reaction is important because of its relation to redox potential. Organic matter can be important in retaining selenium in soil surface horizons (Levesque 1974). Ferric selenides and soil colloids containing iron oxides bind selenium and reduce the amount of the element available to plants (Franke and Painter 1937, Gile and Lakin 1941, Gissel-Nielsen 1971). Adding sulfate to soils can decrease selenate uptake by plants (Gissel-Nielsen 1973).

Bisbjerg and Gissel-Nielsen (1969) and Cary and Allaway (1969) found soil clay content and selenium uptake by plants (red clover, barley, white mustard) to be inversely related. The soils they investigated were sandy with 12% average clay content, and pHs ranged from 4.8 to 7.4. Singh et al. (1981) found increasing soil clay content was positively correlated with selenite and selenate selenium sorption and that selenite and selenate were less strongly sorbed by saline and alkali soils than normal or calcareous soils. However, other variables such as CEC and soil type were colinear with clay content, and only five soils were used in this experiment. Pitt (1984) found no significant relationship between soluble selenium and pH in overburden from coal mines in Texas.

Water-soluble selenium has often been used as an index of the availability of selenium to plants (Lakin 1972, Rosenfeld and Beath 1964). Selenium absorption by plants has been correlated with water-soluble soil selenium in a greenhouse study (Olson and Moxon 1939). Soils with less than 0.5 μg water-extractable Se/g soil have been associated with toxic seleniferous vegetation (Lakin 1972).

More recently, the AB-DTPA selenium extraction has become an alternative to hot-water extraction. Coal mine spoil and overburden selenium determinations by hot-water and AB-DTPA were highly correlated, although AB-DTPA extracted about 60% more selenium than hot-water. Higher selenium concentrations in the AB-DTPA extracts were attributed to bicarbonate exchangeable selenium in the soil (Soltanpour and Workman 1980).

The relationship between extractable soil selenium and the concentration of selenium in plants can be quite complex. Jump and Sabey (1985) presented data for *Elymus smithii* grown in a greenhouse on naturally seleniferous soils. One soil with 0.08 μg AB-DTPA extractable Se/g produced plants with an average 2.0 μg Se/g tissue, while another soil with the same amount of extractable selenium produced plants with 8.5 μg Se/g tissue. To further complicate the pattern, a soil with 0.84 μg extractable Se/g produced grass foliage containing only 1.5 μg Se/g tissue.

Variability of Selenium Content of Soils

Distribution of selenium in the soil may be irregular and complex. Trelease and Beath (1949) found selenium concentrations in the soil to be so variable "it would be virtually impossible to obtain a soil sample

that adequately represented the soil mass from which the plant roots actually absorb their selenium." Many samples from the soil mass penetrated by roots would be needed to characterize the soil selenium content for a single plant. Moreover, these authors found selenium distribution in the soil profile to be rather uniform at some sites, yet highly variable at others. Byers et al. (1938) observed a lack of uniformity in selenium distribution in 20 soil profiles in eastern Colorado. Selenium distribution was not correlated with soil depth, location or origin. These authors stated that water-soluble selenium (determined by boiling water-extraction) did not adequately characterize selenium availability in the soil solution that bathes absorbing roots.

Natural processes can redistribute selenium in soils. Soluble selenium forms can be leached from the upper soil horizons and redeposited lower in the profile (Byers 1935, Beath et al. 1935, 1937, Olson et al. 1942b). For this reason, soils should be sampled for selenium by horizon or depth increments throughout the rooting depth.

Selenium in Forage

Different plant species rooted in the same seleniferous soil may have tissue selenium concentrations from trace amounts to several thousand micrograms per gram (Trelease and Beath 1949). A majority of plants from a variety of locations contained less than 1 μg Se/g tissue (Davis and Watkinson 1966, Ehlig et al. 1968). Most species do not accumulate more than 100 μg Se/g regardless of the soil selenium concentration. Grasses fall into this non-accumulator category. A sample of 135 western wheatgrass (*Elymus smithii*) specimens from western

South Dakota had an average concentration of 11.5 μg Se/g, with values ranging from 0 to 84 μg/g (Rosenfeld and Beath 1964). Olson et al. (1942a) suggested western wheatgrass is a good indicator of selenium content of the grasses in general.

Non-accumulator species including most crops exhibit symptoms of damage after accumulating no more than several hundred μg Se/g from inorganic sources (Hurd-Karrer 1937, 1934, 1933, Martin 1936, Shrift 1954 a,b, Trelease and Beath 1949, Hidiroglou et al. 1969, Higgs et al. 1972). However, soil selenium has not been documented to damage plants in a natural environment, but the absence of a species from a particular spot seldom gives unambiguous evidence as to why that species is not present. In a greenhouse study, Singh and Singh (1978) demonstrated that addition of 2.5 to 10 mg selenite/kg soil reduced wheat yields 28 to 89 percent, respectively. It is possible that soils high in available selenium provide beneficial environment for accumulator species and a disadvantageous site for at least some non-accumulator species.

Rosenfeld and Beath (1964) identified secondary selenium absorbers as plants not restricted to seleniferous soil, but which can be facultative selenium accumulators when grown in seleniferous soils. Plant-available soil selenium concentrations within ranges found in nature do not apparently harm these species. Some species or genera include *Aster ascendens*, *Atriplex gardneri*, *Castilleja*, *Grindelia squarrosa*, *Gutierrezia sarothrae*, and *Machaeranthera*.

Other plant species have a special affinity for selenium. These species have been referred to as selenium indicators, primary

accumulators, absorbers and converters (Rosenfeld and Beath 1964, Shrift 1973). The same species are usually indicated by any of these terms. Selenium accumulators have been defined as plants that can absorb 100 times more selenium from a soil than most plants (Lewis 1976). Beath et al. (1939) coined the phrase "selenium indicators" to refer to certain plant species, the mere presence of which indicated available soil selenium. Beath (1959) stated that of the 100 or so *Astragalus* species in the western states, 21 species are limited to soils containing selenium. These species include *Astragalus bisulcatus*, *A. grayi*, and *A. pectinatus*. Other indicator species include *Stanleya pinnata, Xylorhiza glabriuscula* and some species of the genus *Haplopappus*.

The quadrivalent form of selenium has enhanced the growth and vigor of some accumulator species (Trelease and Trelease 1939, Trelease and Beath 1949). Some investigators have suggested that selenium is necessary for normal growth of these species (Shrift 1969, Lewis 1976, Johnson 1975), but the status of selenium as a necessary micronutrient for most taxan is uncertain. Trelease and Beath (1949) and Rosenfeld and Beath (1964) did demonstrate that plant-available soil selenium could enhance plant vigor and production of certain species and suggested selenium may be a necessary micronutrient for some indicator species. Indicator plants containing several thousand micrograms selenium per gram of plant tissue exhibited no ill effects (Rosenfeld and Beath 1964). A possible detoxification mechanism that prevents the incorporation of Se-selenocysteine into proteins has been postulated for selenium accumulator species (Nigam and McConnell 1969, Peterson and Butler 1967, Shrift and Virupaksha 1965, Virupaksha and Shrift 1965).

In a book on botanical prospecting, Cannon (1952) stated that ore chemical constituents control the distribution of indicator plants and that selenium indicator species are useful in locating uranium and vanadium deposits. Among the indicator species identified were *Astragalus bisulcatus*, *Stanleya pinnata*, and secondary accumulators *Haplopappus armerioides* and *Grindelia squarrosa*.

McPhee (1986) reported that Wyoming geologist Dave Love, who early in his career had been assigned to look for seleniferous plants, observed that seleniferous vegetation did not cross non-seleniferous barriers naturally. Love reported that millions of acres in the Rocky Mountain region have since been converted to a seleniferous flora due to the transportation systems and activities of European man. This would be a classic case of potentially toxic seleniferous soils becoming toxic seleniferous soils as a result of change in flora.

Selenium accumulation in plants varies with growth stage (Trelease and DiSomma 1944, Moxon et al. 1939, Olson et al. 1942a). Rosenfeld and Beath (1964) present data suggesting, as a general trend, selenium content decreases with increasing maturity, but there are exceptions and the reason for this phenomenon is unknown. Beath et al. (1937) found older and larger plants of perennial species had higher selenium contents than smaller and younger plants. Presumably the older and larger plants had deeper and better developed root systems. The above-ground portions of accumulator plants usually contain more selenium than the roots, and fruits and seeds may be very high in selenium (Rosenfeld and Beath 1964).

Selenium in biological systems is associated with proteins and amino acids and tends to distribute with them in plant tissues. The amount of selenium in one type of plant tissue cannot be used reliably to predict how much will be found in another tissue (Bisbjerg and Gissel-Nielsen 1969, Gile and Lakin 1941, Gissel-Nielsen 1971, Rosenfeld and Beath 1964).

Livestock Tolerance

Selenium is both a necessary micronutrient and a possible toxic agent. Biological effects of selenium have been reviewed by the National Research Council (NRC 1976), the National Academy of Science (NAS 1983), and Shamberger (1983).

Schwartz and Folst (1957) first recognized selenium's nutritional essentiality. Muth et al. (1958) demonstrated the effectiveness of selenium in preventing white muscle disease in livestock (also known as nutritional muscular dystrophy). The amount of selenium necessary to prevent white muscle disease is 0.03 to 0.10 μg Se/g forage, depending on vitamin E abundance and dietary composition (Allaway et al. 1967). Trace amounts of selenium are necessary for vitamin E metabolism. Rosenfeld and Beath (1964) reviewed the early literature of selenium in nutrition.

Franke and Painter (1936) first demonstrated selenium toxicity to animals. Trelease and Beath (1949) and Rosenfeld and Beath (1964) described in detail both acute and chronic poisoning symptoms. Estimates for chronic poisoning are often 5 to 10 μg/g in forage, while acute poisoning usually involves plants with over 100 μg Se/g; obviously

there are many variables, including the form of selenium and dietary composition (Schwarz and Foltz 1957, NRC 1976, Underwood 1977).

In its 41 years of existence, the Wyoming Department of Veterinary Science laboratory has not confirmed a case of selenium toxicity in livestock. Analysis of 375 livestock tissue samples collected over a recent three-year period revealed many incidences of selenium deficiency, but none of toxicity (Governor's Task Force on Selenium 1989). Acute selenosis has not been reproduced in animals by administering pure selenium compounds, but blind staggers can be induced with accumulator plant extracts; therefore, an agent other than selenium may be involved (NAS 1983).

STUDY AREAS

Lysite

The Lysite study area is located in Fremont County, Wyoming, in the southern portion of section 27 and northern portion of section 34, T40N, R91W (Figure 1). Average elevation is 1740 meters. The nearest official weather station is at Lost Cabin, approximately 15 km southeast of the study area. At Lost Cabin, average annual precipitation for 1961-1980 was 21 cm. Highest monthly precipitation usually occurs in May and June (Martner 1986).

The surficial geologic strata is the Wagon Bed formation, a variable Eocene-age unit of volcaniclastic claystone and sandstone with minor conglomerate sometimes admixed with detrital material (Thaden 1981). The Wagon Bed formation near Lysite is listed among the most highly seleniferous formations in Wyoming (Case and Cannina 1988). The Wagon Bed was deposited as even-bedded airfall and mudflow or mass flow of drier materials, covering an extremely rough paleotopographic surface. The claystone is mostly pale green, and some material of this color was evident in the study area. However, the dominant appearance of the study area is one of a red-orange semi-desert. Thaden (1981) noted that "Detrital sand washed from highland areas is intertongued with the volcanic materials at many places; the color of the Wagon Bed in these places tends to be orange rather than green."

The three soil complexes mapped for this area by the Soil Conservation Service (SCS In Print) are described below.

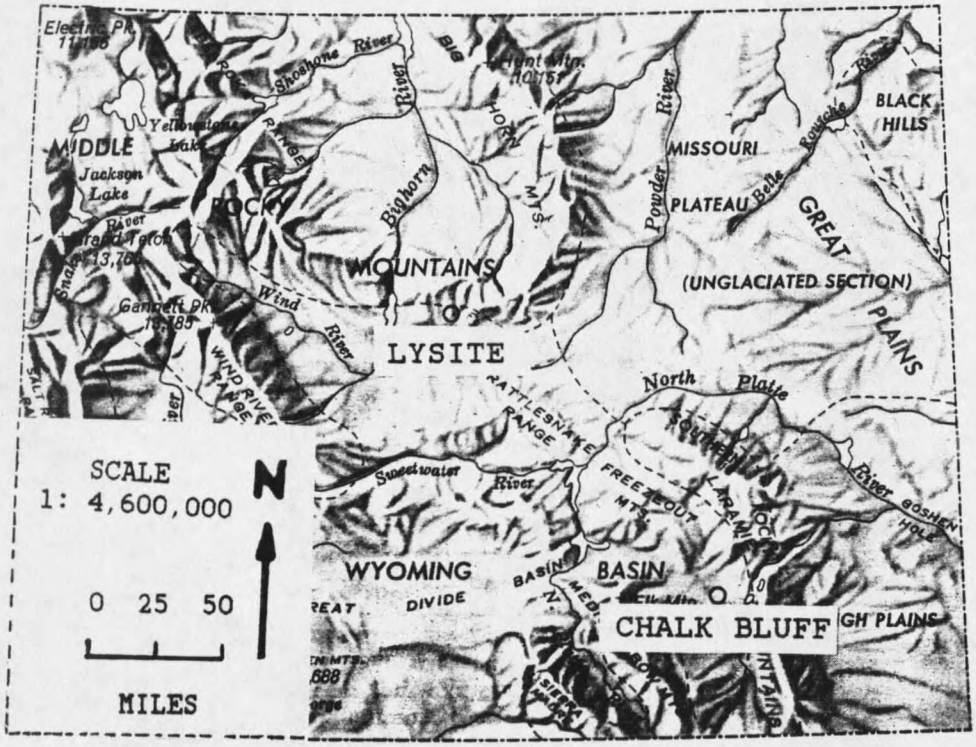

Figure 1. Location of the Lysite and Chalk Bluff study areas.

1. Frisite-Youngston complex, 1 to 8% slopes. This unit is comprised of 60% Frisite fine sandy loam on fans and terraces and 20% Youngston loam on floodplains, with some other inclusions. The Youngston series is a Typic Torriorthent, fine-loamy, mixed (calcareous), mesic. Soils are deep and well-drained, with effective rooting depths of 150 cm or more.

2. Persayo-Rock Outcrop complex, hilly. This mapping unit on 2 to 45% slopes comprises hills, ridges and escarpments. It consists of 65% Persayo clay loam and 15% rock outcrops with

other inclusions. The Persayo soil, formed from residual materials and slope alluvium derived from shale, is shallow and well-drained. The Persayo series is a Typic Torriorthent, loamy, mixed (calcareous), mesic, shallow. This soil is moderately permeable with an effective rooting depth of 25 to 50 cm.

3. Youngston-Lostwells complex, 1 to 3% slopes. This unit is typically 50% Youngston loam and 35% Lostwells loam and occurs on floodplains and fan aprons. Both soils are deep and well-drained, with effective rooting depths of 150 cm and more. The Lostwells series is a Typic Torrifluvent, fine-loamy, mixed (calcareous), mesic.

Based on 16 soil profiles sampled during this study, the most common textural class was sandy loam, followed by loam and sandy clay loam (Appendix B, Table 10). Average clay content for the 16 soils was 19% (Appendix B, Table 11). The average extractable selenium concentration of soils sampled at Lysite was 0.05 μg/g (Table 3).

Artemisia tridentata was a common dominant species at Lysite. Associated species included *Elymus smithii* and, on notably sandy soils, *Carex filifolia*, *Stipa comata* and *Elymus spicatus*. *Psoralea lanceolata* and *Elymus smithii* occurred in association with *Artemisia tridentata* in drainage channels.

In upland communities where *Artemisia tridentata* was not dominant, *Elymus smithii*, *E. spicatus*, and *Carex filifolia* predominated. In large, often flat-bottomed drainage channels, *Elymus smithii*, *Glycyrrhiza lepidota* and *Taraxacum officinale* were characteristic

species. In outwash channels from the Wagon Bed formation, *Elymus smithii*, *Astragalus grayi* and *Distichlis spicata* occurred.

The most striking type of plant community in this area is dominated by *Artemisia pedatifida*. This tiny sagebrush is usually only a few inches tall. Other species are not abundant in these communities, and boundaries to other plant communities are usually abrupt. Vascular plant species found at the Lysite study area are listed in Appendix A.

Chalk Bluff

The Chalk Bluff study area is located in Albany County, Wyoming, in the southern portion of Section 7, T21N, R74W (Figure 1). Average elevation is 2160 meters. The nearest official weather station is at Sybille Creek, about 27 km east of the study area where precipitation may be slightly higher than at Chalk Bluff. At Sybille Creek, average annual precipitation is 39 cm, with highest monthly precipitation usually occurring in April, May and June (Martner 1986).

Chalk Bluff is a coquina of Brachiopods deposited in the Niobrara Seaway about 75 to 85 million years ago. The Niobrara formation is a marine calcareous mudstone or chalky marl, often light gray to yellow. Most soils in this area are formed in residuum or alluvium from the Niobrara formation. In the southern portion of the study area, the Steele shale is at the surface. This soft gray shale contains beds of bentonite and lenticular sandstone (Love and Weitz 1953).

The SCS (In Press) has mapped three soils in the study area as described below.

1. Chaperton, moderately saline-Blazon complex, 8 to 20% slopes. This unit is associated with metastable remnant shale ridges and applies to Chalk Bluff and adjacent upland areas. This complex is usually comprised of 45% Chaperton loam, moderately saline, and 40% Blazon clay loam with other inclusions. The Chaperton series is a Borollic Camborthid, fine-loamy, mixed. The Blazon series is a Ustic Torriorthent, loamy, mixed (calcareous), frigid, shallow. Both soils have formed in colluvium and residuum of shale, are well-drained, and have effective rooting depths of 25 to 100 cm, below which weathered shale is usually encountered.
2. Poposhia - Chaperton association, 6 to 12% slopes. This complex is typically comprised of 45% Poposhia loam and 30% Chaperton clay loam, with other inclusions such as the Blazon loam. The Poposhia series is a Ustic Torriorthent, fine-loamy, mixed (calcareous), frigid. This complex is similar to the previously described upslope soil, but in the Poposhia - Chaperton association the soils are deeper and effective rooting depth is 50 to 150 cm or more. It occurs downslope from Chalk Bluff.
3. Poposhia - Forelle complex, 1 to 8% slopes. This complex occupies a low slope position in the southwest portion of the study area has deeper soils than the previously described complexes with an effective rooting depth of 150

cm or more. The Poposhia loam typically comprises 50% of this complex and the Forelle 25%. The Forelle series is a Borollic Haplargid, fine-loamy, mixed.

Most soil horizons from Chalk Bluff sampled for this study were clay loams, followed by loams and clays (Appendix C, Table 13). The average clay content for 13 soils was 33% (Appendix C, Table 14), and average extractable soil selenium was 0.8 $\mu g/g$ (Table 3).

The plant communities at Chalk Bluff were less discrete than at Lysite, reflecting a more continuous gradation of site factors. On the bluff outcrops, rock fragments predominate. Plant coverage is low, but common species include *Phlox muscoides*, *Elymus spicatus*, *Linum lewisii*, *Astragalus gilviflorus*, *Astragalus kentrophyta* and *Eriogonum brevicaule*. Between the outcrops, *Elymus spicatus* is well represented, sometimes with *Astragalus bisulcatus* or *Xylorhiza glabriuscula*. Immediately below the outcrops where plant coverage and soil development are limited, *Eriogonum brevicaule* and *Atriplex gardneri* are common species.

On better developed upland soils, plant communities are dominated by several combinations of species. *Chrysothamnus viscidiflorus* is often found in conjunction with *Krascheninnikovia lanata*, *Elymus* spp. and *Poa juncifolia*. Over much of the area, *Krascheninnikovia lanata* is often found in association with *Phlox hoodii* and *Elymus* spp. *Tetradymia canescens* is also associated with *Elymus* spp. in some areas. Common species of depositional channels include *Poa juncifolia*, *Elymus* spp.,

Astragalus bisulcatus, *Stanleya pinnata* and *Chrysothamnus viscidiflorus*. All species identified at Chalk Bluff are listed in Appendix A.

METHODS AND MATERIALS

Field Methods

Preliminary plant community locations were identified using aerial photographs. Tentative sample locations were chosen to represent all major plant communities visible on true color aerial photographs. Other sample locations were later chosen in the field to include communities not apparent from remote sensing.

Plant communities were traversed and inspected for species composition. Sample locations were selected in the following manner. Starting from some arbitrary point within a community, a random direction (azimuth) and distance were chosen, and the indicated point in the community located. If the vegetation at that location did not differ greatly from the community in general (e.g., a trail or a claypan in an area where claypans were rare), the plants and soil at that location were sampled.

Five by 15 m rectangular plots (hereafter referred to as "macroplots") were delineated parallel to topographic contours with a meter tape. Within each macroplot, all recognizable vascular plant species were listed. Unrecognized plant species were collected, identified by plot number and coverage, and identified later. Total plant canopy-coverage (Daubenmire 1959) was estimated, followed by canopy-coverage of each species. The sum of individual coverages was compared to the estimate of total canopy-coverage and adjusted if the estimates differed by 10 percent or more. The plot perimeter was read as a line intercept to provide another estimate of species coverage.

These data were used to resolve discrepancies between estimates of total plant cover and the sum of cover estimates for each speices. Thirty-one communities were sampled at Lysite and 39 communities at Chalk Bluff.

The term "macroplot" may have originated with Daubenmire and Daubenmire (1968). Daubenmire found that after sampling canopy-coverage in 50 0.1 m^2 plots in forests, an additional estimate from a single 125 m^2 macroplot was needed to provide data for species under-represented by the smaller plots. Mueggler and Stewart (1980) also used macroplots to augment data from 40 smaller plots in grassland and shrubland communities. Bray et al. (1959) used the largest plots feasible to decrease the ratio of perimeter to sampled area.

Canopy-coverage estimates for macroplots meet the criteria for vegetation sampling set forth by Junk (1973):

1. Sample area is large enough to represent effectively the composition of the plant community.
2. A homogeneous area can be sampled.
3. Samples provide the most important information efficiently.
4. The appropriate measurement (i.e. canopy-coverage) can be estimated for macroplots.

Soil pits were dug to the depth of lithic or paralithic material. Samples from soil pits were collected from each distinguishable horizon and retained for later analysis. Sixteen soil profiles were sampled at Lysite and 13 soil profiles were sampled at Chalk Bluff.

Plant tissue samples were collected within five meters of the soil pit. Species were chosen based on abundance or known tendency to accumulate selenium. Tissue samples consisted of current year's growth

(shrubs and sub-shrubs) or the terminal 23 cm of shoots (herbs). Samples were sealed in plastic containers, packed on ice in the field, and later frozen until analyzed for selenium content. Tissue samples collected in May, July and August were used in this study.

A limited analysis of spatial variation of selenium concentration in the soil of a single plant community was conducted at Lysite. First, an abrupt, clear and relatively straight boundary between two extensive plant communities was located. One plant community had four dominant plant species: *Artemisia tridentata*, *Carex filifolia*, *Stipa comata* and *Elymus spicatus*; the other had a single dominant species, *Artemisia pedatifida*. Parallel to the common boundary but 10 m into each community, parallel 15 m transects were delineated. Four soil pits were placed at 5 m intervals along each transect, and soils from the 0 to 5 cm and 50 to 55 cm depths were collected. This resulted in four samples for each depth increment from each plant community, from which AB-DTPA extractable soil selenium was determined.

Laboratory Methods

Particle-size analysis of soils was performed using the hydrometer method described by Day (1965). Selenium content of soils was evaluated using the extractant AB-DTPA (Soltanpour and Schwab 1977 as modified by Soltanpour and Workman 1979). Standards, blanks, and spikes were made up in 50 ml volumetric flasks with the equivalent sample matrix and heated in a hot water bath for 30 minutes. Extracts were analyzed by atomic adsorption spectroscopy via generation of selenium hydride (H_2Se). The lowest quantifiable concentration was 0.004 μg Se/g dry

soil based on a detection limit of 0.002 μg Se/ml and 15 g of soil in 30 ml of extracting solution. The value 0.003 μg Se/g was used in statistical analysis for concentrations below 0.004.

Each sample of plant tissue was split, two sub-samples of equal weight at field moisture content were selected. One sample was digested while the other was oven-dried at 70°C to constant weight. The resulting dry weight ratio was used to convert the selenium concentration in fresh tissue to a dry weight value. This method prevented volatile selenium loss from drying of plant tissues used for digestions. Plant tissue samples consisted of stems less than 1 mm diameter and leaves; flowers were excluded from digestion.

The digestion procedure was as follows (a modification of Jones et al. 1982): 25 ml of a solution of 3 parts nitric acid and 2 parts perchloric acid were added to the one gram of dry plant tissue in a 125 ml erlenmeyer flask. This mixture was heated overnight at 40° C. The hotplate temperature was then raised to 100° C and maintained at that temperature until the digestion solution turned light yellow and most plant material had dissolved. The hotplate temperature was then increased to 140° C until nitrous fumes were not evident. Three milliliters of sulfuric acid were added and the temperature was raised to 190° C until volume was reduced to about 5 ml. If the addition of a drop of hydrochloric acid produced yellow-orange fumes, nitric acid was still present and further heating was required. After cooling, 25 ml of concentrated hydrochloric acid were added and the solution was transferred to a 50 ml volumetric flask. The erlenmeyer flask was

rinsed twice with distilled water which was poured into the volumetric flask, and the solution brought to 50 ml with distilled water.

Samples of the digestion solution with over 1 mg Se/l were analyzed by flame atomic absorption spectroscopy. Samples with selenium concentration exceeding the standard curve were diluted. Samples of digestate with 1 mg/l or less selenium were analyzed by atomic absorption spectroscopy after generation of selenium hydride (H_2Se). The minimum quantifiable concentration was 0.1 μg Se/g dry tissue based on a detection limit of 0.002 μg Se/ml and one gram of tissue in 50 ml of solution. All tissue samples contained selenium above the detection limit.

Mathematical and Statistical Methods

Some plant species were sampled at several soil pits, some at only one or a few. Sufficient data were not available to derive separate soil/plant selenium analyses for each species. Because of the highly variable ability of different plant species to accumulate selenium from a given soil, plant species were assigned to one of three groups. These three groups correspond to selenium concentrations of 1 to 100 μg, 101 to 500 μg, and more than 500 μg/g soil.

The three groups were meant to reflect the selenium accumulating ability of each species. The actual amount of selenium in a given plant tissue sample reflects two major factors: selenium available to the plant and the accumulating proclivity of that plant. To emphasize the plant's contribution, or ability to accumulate selenium, only selenium concentrations above the median value for each species were averaged to

determine the selenium concentration values used to assign each species to one of the three groups. Tissue samples collected in May, July and August were used to provide a large data base.

Soil-plant relationships at Lysite (16 sites) and Chalk Bluff (13 sites) were statistically analyzed independently because foliar selenium data from each site showed different relationships to extractable soil selenium. For each group of species at each site, foliar selenium concentrations for plants collected in July were regressed on up to five soil selenium concentrations. These five selenium concentrations were weighted average extractable soil selenium concentrations in the upper 50 cm interval, 51 to 100 cm interval and 101 to 150 cm interval, weighted average extractable selenium for the entire soil profile and highest extractable selenium from any soil horizon. For a depth interval (e.g. 101 to 150 cm) to be included in analysis, more than half of that interval must have been sampled. For example, if a profile was sampled to 120 cm, the 101 to 150 cm interval was not evaluated for its relationship to plant selenium, since less than half of that depth interval was sampled. If a profile was sampled to 130 cm, then the 101 to 150 cm depth interval was considered to be sampled, and a regression was calculated. However, the extractable selenium content of all horizons was used to calculate the average concentration for the profile, and to determine the highest concentration for any horizon in that profile.

If a regression was significant at the .025 significance level, the role of that factor was further evaluated using analysis of variance. In the analysis of variance, sites were independent variables

and selenium concentrations for each plant in a species group considered individually were dependent variables. Because more than one plant from a species group were sampled in association with most sample sites, this analysis of variance identified the plant-to-plant residual. By subtracting the plant-to-plant residual from the regression residual, the site-to-site residual (not including the soil selenium component) was identified.

Extractable soil selenium was the numerator in the combined analysis of variance. The site-to-site component was used as the denominator to compute the F-ratio if the site-to-site residual was larger than the plant-to-plant residual. Some soil selenium/plant relationships that appeared to be significant from the regression alone were not significant ($p \geq .025$) when subjected to this more rigorous analysis.

If the plant-to-plant residual was larger than the site-to-site residual, a weighted average residual was computed from the plant-to-plant and site-to-site residuals. This is equivalent to the regression residual for extractable soil selenium, in which the residual represents the combined effects of all other factors. Regression alone is not as rigorous a test as the combined analysis of variance described above, but more appropriate when the plant factor outweighs the site factor. Only results significant at the .025 probability level have been reported.

RESULTS AND DISCUSSION

Plant Species Groups

Species groups and median concentrations are presented in Tables 1 and 2. Nomenclature follows Dorn (1988). Only those species sampled for selenium concentrations appear in these tables.

Group I species include many common range and revegetation species of the western Great Plains and intermountain region. Most species did not accumulate more than 60 μg Se/g tissue on any soils (Appendix A, Tables 7 and 9). However, few samples of some species were collected, and some species may not have been sampled on seleniferous soils. Therefore, some species placed in Group I may accumulate enough selenium to justify placement in Group II if sampled extensively on seleniferous soils.

Group II species usually contained several hundred micrograms selenium per gram tissue when grown on seleniferous soils, but one *Atriplex gardneri* sample contained over 1,000 μg Se/g tissue. Two species, *Stanleya pinnata* and *Xylorhiza glabriuscula*, may be indicative of available soil selenium, but the other three species in this group, *Atriplex gardneri, Gutierrezia sarothrae* and *Haplopappus nuttallii*, are more ubiquitous in the Great Plains.

Six species were placed in Group III, the group of species that usually accumulates more than 500 μg Se/g tissue when grown on seleniferous soils. However, individual plants may accumulate thousands of micrograms selenium per gram dry tissue. For example,