Phytoextraction of selenium and other metals from soil used for landfarming oil refinery waste
by Shane Allen Matolyak

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

Abstract:
Waste slurry emanating from an oil refinery wastewater treatment system was incorporated into soil at the Conoco Land Treatment Unit (LTU) since 1972. As a result, the soil contained a total selenium concentration (18.6 mg/kg) that approached the limit permitted by the state regulatory authority. Total concentrations of other elements included arsenic (34.4 mg/kg), chromium (159.6 mg/kg), lead (26.2 mg/kg), and zinc (185.8 mg/kg). This soil was saline (8.3 mmhos/cm), had a loam texture, and a pH of 7.2. The use of selenium accumulating plant species to decrease the soil selenium concentration was evaluated.

Selenium accumulating plant species (canola, desert prince's-plume, and Indian mustard) and selenium non-accumulating species (pubescent wheatgrass and tall fescue) were seeded at the LTU and harvested upon maturity. No significant change in soil metal concentration was measured. Based on scientific literature, it was expected that the selenium accumulating species would have tissue selenium concentrations in the range of 300 to 2000 mg/kg. Plant tissue selenium concentrations in canola (6.8 mg/kg), canola grown on phosphorous amended LTU soil (7.6 mg/kg), Indian mustard (10.4 mg/kg), and desert prince's-plume (111.6 mg/kg) were considerably lower than expected yet great enough to present a chronic toxicity hazard in grazing animals.

To determine whether lower than expected selenium accumulation was due to plant species selection, soil characteristics, or a characteristic of the waste slurry, selenium accumulating plant species were grown in replicated greenhouse trials on four different substrates; i) the LTU soil, ii) selenate-enriched LTU soil, iii) waste slurry-enriched sand, and iv) selenate-enriched sand. Mean plant tissue selenium concentrations in each substrate were 10.2 ± 6.5 mg/kg, 49.0 ± 27.8 mg/kg, 43.0 ± 37.5 mg/kg, and 683.9 ± 423.1 mg/kg, respectively. Plant selenium concentrations in selenate-enriched sand were significantly greater than in the other three substrates that received waste slurry as their principle supply of selenium.

It was concluded that waste slurry, when applied to soil, contained either i) a form of selenium that was in a reduced oxidation state and thus unavailable for plant uptake or ii) another chemical constituent was present that competed with selenium for plant uptake.

PHYTOEXTRACTION OF SELENIUM AND OTHER METALS FROM SOIL USED FOR LANDFARMING OIL REFINERY WASTE

by

Shane Allen Matolyak

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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.

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ABSTRACT

Waste slurry emanating from an oil refinery wastewater treatment system was incorporated into soil at the Conoco Land Treatment Unit (LTU) since 1972. As a result, the soil contained a total selenium concentration (18.6 mg/kg) that approached the limit permitted by the state regulatory authority. Total concentrations of other elements included arsenic (34.4 mg/kg), chromium (159.6 mg/kg), lead (26.2 mg/kg), and zinc (185.8 mg/kg). This soil was saline (8.3 mmhos/cm), had a loam texture, and a pH of 7.2. The use of selenium accumulating plant species to decrease the soil selenium concentration was evaluated.

Selenium accumulating plant species (canola, desert prince's-plume, and Indian mustard) and selenium non-accumulating species (pubescent wheatgrass and tall fescue) were seeded at the LTU and harvested upon maturity. No significant change in soil metal concentration was measured. Based on scientific literature, it was expected that the selenium accumulating species would have tissue selenium concentrations in the range of 300 to 2000 mg/kg. Plant tissue selenium concentrations in canola (6.8 mg/kg), canola grown on phosphorous amended LTU soil (7.6 mg/kg), Indian mustard (10.4 mg/kg), and desert prince's-plume (111.6 mg/kg) were considerably lower than expected yet great enough to present a chronic toxicity hazard in grazing animals.

To determine whether lower than expected selenium accumulation was due to plant species selection, soil characteristics, or a characteristic of the waste slurry, selenium accumulating plant species were grown in replicated greenhouse trials on four different substrates; i) the LTU soil, ii) selenate-enriched LTU soil, iii) waste slurry-enriched sand, and iv) selenate-enriched sand. Mean plant tissue selenium concentrations in each substrate were 10.2 ± 6.5 mg/kg, 49.0 ± 27.8 mg/kg, 43.0 ± 37.5 mg/kg, and 683.9 ± 423.1 mg/kg, respectively. Plant selenium concentrations in selenate-enriched sand were significantly greater than in the other three substrates that received waste slurry as their principle supply of selenium.

It was concluded that waste slurry, when applied to soil, contained either i) a form of selenium that was in a reduced oxidation state and thus unavailable for plant uptake or ii) another chemical constituent was present that competed with selenium for plant uptake.

CHAPTER 1

INTRODUCTION AND STUDY OBJECTIVES

Metals contaminate the soil resource in many areas throughout the world including 69 % of the sites on the United States National Priority List (Raskin and Ensley, 2000). This contamination can reduce soil productivity and pose a direct threat to the health of the biota. Primary methodologies to rehabilitate metal-contaminated soil include excavation and burial, acid-leaching, or in situ immobilization (Baker et al., 1994; Raskin and Ensley, 2000). In recent years, phytoextraction has been investigated as a means to rehabilitate metal contaminated soil.

Phytoextraction is the process of using plants to remove elements of concern from the soil. Certain plants, known as hyperaccumulators, can accumulate soil-borne metals in aboveground tissue to concentrations many times greater than that of the surrounding soil (Becker, 2000). By harvesting the metal-rich plant shoots, a net reduction of metals in the soil is possible.

Advantages of phytoextraction over other methods of remediating metal contaminated soils include less expense, less environmental disturbance, and higher acceptance by the public (Kumar et al., 1995; Morgante, 2000; Raskin and Ensley, 2000). Phytoextraction also provides an opportunity to recycle metals, possibly by using the metal-rich plants as nutritional supplements for livestock or as a source of high-grade metal ore in a process known as phytomining (Becker, 2000; Comis, 2000; Wood, 2000).

Study Objectives

The purpose of this study was to investigate phytoextraction methodologies to reduce the level of selenium (Se) in a soil receiving applications of sludge emanating from an oil refinery wastewater treatment system. The potential exists to remove notable amounts of Se from the soil at the Conoco Land Treatment Unit (LTU) if plant species can be identified and propagated that possess the ability to hyperaccumulate Se and produce large amounts of harvestable tissue. Specific objectives include the following:

- Identify Se accumulating plant species that will grow at the LTU.
- Identify a seed source for plant species to be tested.
- Determine which plant species accumulate the most Se at the LTU site.
- Determine the amount of Se and other metals removed from the soil by phytoextraction.

I conducted a field investigation over two growing seasons, beginning March 1999 and ending July 2001. Data collected during the field investigation were used to evaluate and compare the ability of selected Se-accumulating plant species to establish, develop, and uptake Se at the Conoco LTU site. A greenhouse investigation was conducted from May 2001 until September 2001 in order to assess substrate effects on Se uptake.

CHAPTER 2

LITERATURE REVIEW

Metal Hyperaccumulating Plant Species

The term "hyperaccumulator" was first used to describe plants that contained over 1000 mg Ni/kg in dry plant tissue when grown on serpentine soils (Brooks, 1977; Brooks, 1998; Raskin and Ensley, 2000). While somewhat arbitrary, this plant tissue nickel concentration was approximately 100 times greater than that of non-accumulating species found on serpentine soils (Brooks, 1998).

Tissue concentration levels used to determine hyperaccumulator status differ with the metal in question (Brooks, 1998; Raskin and Ensley, 2000). For zinc or manganese, plants with tissue concentrations equal to or greater than 10,000 mg/kg are considered hyperaccumulators while Se or nickel hyperaccumulators have tissue concentrations equal to or greater than 1000 mg/kg (Baker and Brooks, 1989; Brooks 1998). The threshold value defining nickel hyperaccumulating plants has been called into question as more data on these plants have been collected (Raskin and Ensley, 2000). Therefore it seems possible that the threshold values that define hyperaccumulators of nickel as well as other metals may change as more is learned about these plants.

The first hyperaccumulating plants were recorded in 1885, almost 100 years before the word was coined, when A. Baumann found that specimens of *Viola calaminaria* and *Thlaspi calaminare* growing over calamine deposits in Aachen, Germany contained over 10,000 mg/kg (dry weight) zinc (Baumann, 1885 as cited in

Raskin and Ensley, 2000; Brooks, 1998). Since that time approximately 400 hyperaccumulating plant species in 45 families have been identified (Raskin and Ensley, 2000). The discovery that certain plant species are able to accumulate large amounts of metals has since lead to the use of these plants in phytochemical studies, mineral exploration, phytoarchaeology, and phytoextraction of metals (Beath, 1939; Brooks, 1998; Brooks and Johannes, 1990; McGrath, 1993).

Phytoremediation and Phytoextraction

The words phytoremediation and phytoextraction have been used interchangeably in scientific literature, however these words have different meanings. Phytoremediation, the general use of plants to remove, degrade or stabilize environmental contaminants includes a number of sub-disciplines, including rhizofiltration, phytostabilization, phytodegradation, phytovolitilization, and phytoextraction (Morgante, 2000; Raskin and Ensley, 2000).

Phytoextraction involves the use of hyperaccumulating plants to transport metals from the soil into aboveground plant portions that are subsequently harvested and removed from the site, resulting in a decrease of the soil metal concentration (Morgante, 2000; Raskin and Ensley, 2000).

Phytoextraction offers several potential advantages compared to traditional methods used to rehabilitate metal contaminated soils. Phytoextraction is much less expensive than excavating and disposing of the contaminated soil. The current cost of excavation and disposal is approximately $150 to $350 per ton while the estimated cost

of phytoextraction, including off-site disposal of the biomass as a hazardous waste, is between $20 and $80 per ton of treated soil (Raskin and Ensley, 2000).

The implementation of phytoextraction does not require the removal of topsoil and does not necessarily require the incorporation of soil amendments. Therefore this method can be less environmentally disturbing and generally more acceptable to the public than other techniques (Kumar et al., 1995; Morgante, 2000; Raskin and Ensley, 2000).

An opportunity to recycle metals is provided by phytoextraction. It may be possible to use the metal enriched plants as nutritional supplements for livestock (Wood, 2000). The plants may also serve as a source of high-grade metal ore in a process known as phytomining (Becker, 2000; Comis, 2000).

Phytoextraction also has some disadvantages compared to other methods of rehabilitating metal contaminated soils. While traditional methods of rehabilitation are applicable at sites having multiple contaminants, hyperaccumulators are often specific with regard to the type of metal(s) they are able to accumulate (Brooks, 1998; Rosenfeld and Beath, 1964). Therefore it may be necessary to identify and establish multiple hyperaccumulating species in order to rehabilitate a site contaminated with multiple metals. Furthermore hyperaccumulators of some contaminants, such as arsenic, have yet to be identified (Brooks, 1998).

The efficiency of phytoextraction is dependent on the production of large amounts of metal rich aboveground plant tissue. Most hyperaccumulators that have been identified are small, slow growing, species with undefined growth requirements (Kumar

et al., 1995). Therefore successful clean-up using phytoextraction may be medium to long term while excavation or capping provides a relatively immediate remedy.

While high plant productivity is important, the amount of metal that a plant can concentrate in its tissue also has a great impact on the efficiency of phytoextraction (Brooks, 1998). The ability of any plant to concentrate metal is dependent on factors that influence soil metal availability such as soil pH, soil redox potential, chemical speciation of the metal in question, the presence of other elements that may compete for plant uptake, and clay content of the soil (Ahlrichs and Hossner, 1987; Banuelos and Meek, 1990; Bisbjerg and Nielsen, 1969; Singh et al., 1981; Williams and Thornton, 1972).

Phytoextraction of Selenium

Orville Beath and his associates were the first to identify plant species from genera such as *Astragalus* (milkvetch) and *Stanleya* (prince's-plume) that were able to hyperaccumulate Se to concentrations in excess of 1000 mg/kg (Beath et al., 1939; Rosenfeld and Beath, 1964). Ingestion of these plants, which Rosenfeld and Beath (1964) referred to as primary selenium accumulators, was the cause of chronic selenium toxicity in cattle. It has been suggested that Se is necessary for the normal growth of primary selenium accumulators (Johnson, 1975; Lewis, 1976; Shrift 1969). Canola and Indian mustard, both members of the genus *Brassica*, accumulate Se to concentrations ranging from 274 to 470 mg/kg and are classified with other plants that accumulate Se to the range of a few hundred mg/kg as secondary accumulators (Banuelos et al., 1997a; Banuelos et al., 1997b; Rosenfeld and Beath, 1964). Most cultivated crop plants, grains,

and native grasses usually accumulate Se to concentrations below 30 mg/kg regardless of the soil Se concentration (Rosenfeld and Beath, 1964).

Despite their ability to accumulate very high amounts of Se the species of *Astragalus* that have been evaluated for use in phytoextraction have proven to be difficult to establish and tend to produce small biomass (Bell et al., 1992; Duckart et al., 1992; Parker et al., 1991; Retana et al., 1993). *Stanleya* was only recently investigated as a Se phytoextractor, so there is little information regarding its biomass production (Feist and Parker, 2001). While canola and Indian mustard accumulate significantly less Se then primary accumulators, their relatively high biomass production and adaptability to a range of soil conditions make them attractive candidates for phytoextraction (Banuelos and Meek, 1990; Banuelos et al., 1996; Banuelos et al., 1997b; Banuelos et al., 1998).

Soils are defined as seleniferous when they support the growth of vegetation containing toxic concentrations of Se (Anderson and Scarf, 1983). While a dietary intake of 0.1 mg/kg Se in forage is required for livestock, it has been determined that five mg/kg presents a chronic Se toxicity hazard (NRC, 1976; Underwood, 1977). The soil Se concentration provides a poor index of potential toxicity because the availability of Se to plants is dependent on a number of factors (Fisher et al., 1987). Therefore, the success of Se phytoextraction on a specific soil is not guaranteed by the establishment of plants that act as hyperaccumulators on other soils.

Plant availability of Se is highly dependent on Se speciation, which is influenced by the soil redox potential. Se occurs in four oxidation states in soil: elemental Se (Se^0), selenide (Se^{-2}) (-2 oxidation state), selenite (SeO_3^{-2}) (+4 oxidation state), and selenate (SeO_4^{-2}) (+6 oxidation state) (Brooks, 1998). Elemental selenium and metal selenides are

very insoluble and therefore not available for plant uptake (NRC, 1976). Selenite and selenate are plant-available, although it has been shown that plants accumulate more selenium when presented with selenate than selenite (Banuelos and Meek, 1990; Bisbjerg and Nielsen, 1969; Brooks, 1998). High soil pH favors the oxidation of selenite to selenate (Geering et al., 1968). The plant-availability of Se in organic compounds varies greatly with plant species as well as the specific form of organic Se (Trelease and Disomma, 1944; Trelease and Beath, 1949; Hamilton and Beath, 1963).

Se speciation can be influenced by microbial activity, which can cause selenate or selenite to become reduced to insoluble forms (Levine, 1925; Lortie et al., 1992; Oremland et al., 1989; Tomei et al., 1992). In a study of microbial activity on Se transfer in a laboratory soil-plant system, selenate was the predominant species in the soil solution (Arbestain, 1988). Supplying the soil microbes with a carbon source (straw) caused a 92 – 97 % reduction in the Se concentration of the soil solution. Four – 5 % of the reduction was attributed to microbial volatilization, while the remainder was attributed to the formation of insoluble, reduced Se compounds. Sarathchandra and Watkinson (1981) reported what they believed to be the first observation of microbial oxidation of Se. In this report the soil bacterium *Bacillus megaterium* was found to oxidize up to 1.5 % of the elemental Se added to a soil to the selenite form.

Plant uptake of Se is also influenced by the presence of other chemical constituents in the soil. Sulfate reduces the amount of selenate accumulated by a plant. Sulfur and selenium share similar chemical characteristics such as electronegativity and atomic, covalent, and ionic radii (Rosenfeld and Beath, 1964). Sulfur and selenium can each exist in the + 6, + 4, and – 2 oxidation states, although selenium has less tendency

than sulfur to become oxidized to the + 6 state (Rosenfeld and Beath, 1964). Their chemical similarities cause selenate and sulfate to enter plant roots via the same carrier and compete strongly for uptake (Brooks, 1998; Legget and Epstein, 1956; Williams and Thornton, 1972). Primary selenium accumulators have the ability to preferentially accumulate selenate over sulfate while canola and Indian mustard display avid sulfate accumulation coupled with indiscriminant selenate uptake (Bell et al., 1992; Banuelos et al., 1997b).

Plant uptake of Se is also influenced by the clay and organic matter content of the soil with a decrease in plant Se concentration as clay and organic matter increase (Bisbjerg and Nielsen, 1969). Only 3 % of the Se added to a soil containing 12.8 % organic matter could be extracted by leaching with water while 20 to 30 % of the added Se could be extracted from soils with less than 3.5 % organic matter. The amount of Se accumulated by a plant was found to be highest when the plants were grown on sandy soils (Bisbjerg and Nielsen, 1969). It appears possible that otherwise available forms of Se can become adsorbed to the numerous cation exchange sites that are present on clays and organic matter, immobilizing the Se against leaching and making it unavailable to plants roots (Brady and Weil, 1999).

The sorbtion, and thus mobility and plant-availability, of selenate and selenite is dependent on soil solution pH (Goldberg and Glaubig, 1988). Selenite sorbtion on a calcareous, montmorillonitic soil was maximal near a pH 3 and sharply declined to pH 6. Selenite sorbtion on montmorillonite and kaolinite increased at low pH and peaked at pH 5 while selenite sorption on calcite peaked between pH 8 and 9 (Goldberg and Glaubig, 1988). Other researchers have also observed the trend of decreasing selenite sorption,

and increased mobility, with increasing pH with maximum sorbtion occurring at pH 3 to 4 (Alrichs and Hossner, 1987; Hingston et al., 1968; Neal et al., 1987).

Selenate sorption was not observed in the Goldberg and Glaubig (1988) experiment while Ahlrichs and Hossner (1987) observed that less than 1 % of the selenate added to a lignite overburden was adsorbed. However in another study it was found that selenate sorption was higher than that of selenite on five different soils (Singh et al., 1981). Singh summarized his findings by saying that sorbtion of both selenite and selenate is positively influenced by organic carbon, clay content, calcium carbonate, and cation exchange capacity while high salt content, alkalinity, and high pH negatively effect sorption. Singh et al. (1981) found that phosphate is effective at displacing selenite and selenate that had been adsorbed by the soil. In an earlier study a 336-fold increase in the concentration of Se in Indian mustard was measured when the plants were fertilized with 100 mg/kg phosphorous (Singh, 1979).

Ecotypic variation within a plant species could influence the efficiency of phytoextraction due to possible variation between populations with respect to Se accumulating abilities. Ecotypic variation with regard to metal tolerance and metal accumulation is a common phenomenon for plants adapted to high metal soils (Baker, 1987; Macnair, 1993). *Stanleya pinnata* (desert prince's-plume) seeds collected from sites having high soil Se concentrations matured into plants with greater Se accumulating ability than plants grown from seeds collected from areas with low soil Se concentrations (Parker and Feist, 2001).

Plant-Enhanced Selenium Volatilization

Selenium-accumulating plant species have been found to produce volatile methyl-selenide compounds that are subsequently released to the atmosphere from the plant leaves and/or root systems (Duckardt et al., 1992; Terry et al., 1992; Terry and Zayed, 1994; Zayed and Terry, 1992; Zayed and Terry 1994), with up to 6.1 % of the Se removed from a soil by two-grooved milkvetch attributable to plant-enhanced volatilization (Duckardt et al., 1992).

Volatilization is important to consider when air-drying samples of hyperaccumulator tissue prior to laboratory analysis. Up to 60 % of Se in species of *Astragalus* was lost through volatilization upon air-drying (Beath et al., 1935 and 1937; Evans et al., 1968). The most reliable results are obtained from wet tissue analysis of hyperaccumulating species, while air-drying plant tissue samples should be satisfactory for analysis of non-accumulators that contain little volatile Se (Shamberger, 1983).

CHAPTER 3

GROWTH AND SELENIUM ACCUMULATION OF PLANT SPECIES GROWN ON LAND TREATMENT UNIT SOIL

The ability of selected plant species to develop and accumulate Se as well as arsenic, chromium, lead, and zinc when grown in the Conoco Land Treatment Unit (LTU) soil was evaluated during a two year field investigation at the Conoco LTU.

Materials and Methods

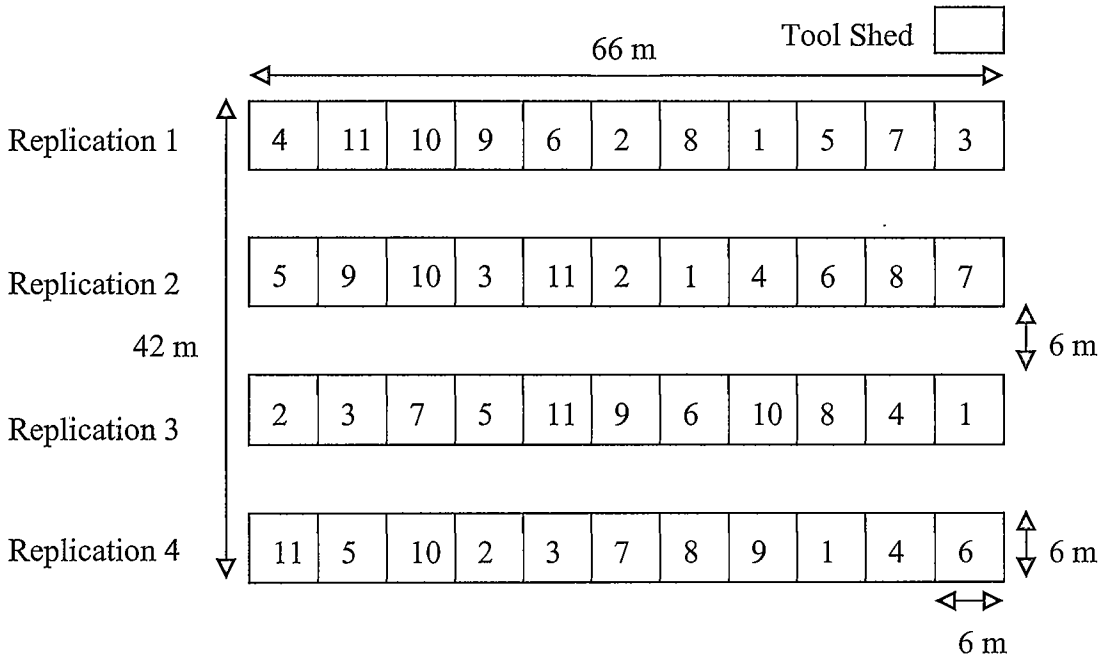
Field Site Description

The LTU is a nearly level, non-vegetated, 11-acre, fenced impoundment located 10 miles north of Billings, Montana. The LTU is divided into seven sub-areas referred to as cells. Since 1972, cells within the LTU received applications of waste emanating from an oil refinery wastewater treatment system. This waste was applied to the loam soil as a slurry then tilled to a depth of 15 cm. Conoco conducts periodic soil analysis for total metal concentrations in the 0 to 30 cm depth increment. Examples of such metals and their concentrations in cell number 7 include arsenic (45.1 mg/kg), chromium (227.0 mg/kg), lead (23.6 mg/kg), selenium (27.3 mg/kg), and zinc (216.0 mg/kg) (Conoco, 2000). The concentration of these and other metals are above average yet still within the range of natural soils with the exception of mercury which is present at a concentration (1.4 mg/kg) approximately 10 times greater than the national average for loam soils (0.13

mg/kg) (Kabata-Pendias, 2001; Williams and Schuman, 1987).

Experimental Design

A randomized complete block experimental design was implemented in cell number 7 at the LTU (Figure 1). Cell number 7 was chosen because its soil Se concentration was the highest in the LTU. This design consisted of four rows (i.e., replications or blocks) each containing 11 test plots. Each test plot within a replication was dedicated to a different treatment (i.e., plant species, species mixture). This



1 – Pubescent wheatgrass
2 – Two-grooved milkvetch
3 – Cicer milkvetch
4 – Tall fescue
5 – Indian mustard
6 – Canola
7 – Desert prince's-plume
8 – Fallow (control)
9 – Canola plus 100 mg/kg phosphorous
10 – Canola and tall fescue
11 – Extra plot

Figure 1. Randomized complete block experimental design at the Conoco LTU.

experimental design enabled the application of two-way analysis of variance and Fisher's Least Significant Difference method of mean separation analysis (SPSS Inc., 1992-1997) so that a determination could be made as to which treatment removed significantly more Se from the soil compared to other plant species. These tests of significance were completed at a 95 % probability level. The experimental design also had the capability to account for inherent field variability in the soil Se level. This means that if existing field variation resulted in the soil Se level being higher in test plots in replication 2 compared to replications 1, 3, and 4, this variation could be statistically removed so that a true and sensitive test was possible between treatments and not masked by field variation.

Plant Material Selection

An extensive review of the scientific literature lead to the identification of plant species that posses the ability to accumulate high levels of Se and could be found growing in Montana or bordering states. Seed for species that was available commercially or in limited amounts (i.e., less than 30 grams) through the United States Department of Agriculture's National Plant Germplasm System (NPGS), was selected for use in this investigation (Table 1). Non-accumulating species were also included to provide data regarding the minimum amount of Se that would be accumulated in plant tissue from the LTU soil.

Measurement of Seed Germination

The germination potential of each species of seed was tested by placing 10 seeds of an individual species between paper towels that had been moistened with tap water.

Table 1. Plant species used for the field investigation at the Conoco LTU.

Common Name	Scientific Name	Seed Source
Selenium accumulating species		
Canola	*Brassica napus*	Circle S Seeds. Three Forks, MT
Cream milkvetch	*Astragulus racemosus* [1]	Prairie Moon Nursery. Winona, MN
Desert prince's-plume	*Stanleya pinnata*	Western Native Seed. Salida, CO
Indian mustard	*Brassica juncea*	V & J Seed Farms. Woodstock, IL
Shadscale saltbush	*Atriplex confertifolia*	Western Native Seed. Salida, CO
Two-grooved milkvetch	*Astragalus bisulcatus* [1]	National Plant Germplasm System
Selenium non-accumulating species		
Cicer milkvetch	*Astragalus cicer*	Circle S Seeds. Three Forks, MT
Pubescent wheatgrass	*Agropyron trichophorum*	Circle S Seeds. Three Forks, MT
Tall fescue	*Festuca arundinacea*	Circle S Seeds. Three Forks, MT

[1] Less than 30 g of seed was available for these species.

The seeds were monitored over one month during which time the towels were kept moist and the number of germinated seeds recorded. This process was repeated for each species listed in Table 1.

Seeding

The following treatments were seeded at the LTU on April 25, 2000; cicer milkvetch, shadscale saltbush, Indian mustard, canola, desert prince's-plume, and a seed mixture consisting of equal amounts of shadscale saltbush and Indian mustard seeds. Each plot was scarified using hand rakes before hand-broadcasting the seeds at a rate of 700 seeds/m^2. The plots were lightly raked after seed application to cover the seed with soil.

A second seeding occurred on May 30, 2000, prompted by a lack of germination of shadscale saltbush and cicer milkvetch. Two Se non-accumulating species, pubescent wheatgrass and tall fescue, were seeded at this time. Pubescent wheatgrass was seeded into previously unused plots. Tall fescue was seeded into plots that had previously been seeded to shadscale saltbush including plots dedicated to the establishment of a mixture of Indian mustard and shadscale saltbush. Tall fescue failed to establish in plots dedicated to the seed mix so this treatment was not sampled during the study.

It was believed that successful germination of saltbush would be achieved during the second growing season if saltbush seeds were subjected to a period of vernalization. Plots previously seeded to cicer milkvetch received a seeding of shadscale saltbush (700 seeds/m^2) on December 10, 2000, however no germination was observed during the second field season.

Seeds of the species used in the field study were also planted immediately outside the LTU in order to provide a visual comparison of the ability of these seeds to mature in similar environmental conditions on uncontaminated soil. Likewise, seeds were planted inside the LTU cell number 7 at a location approximately 10 m east of the test plots. These seeds did not receive irrigation during the study in order to provide a visual comparison of the ability of the species to establish without supplemental irrigation.

Increased Phosphorous Treatment

To determine whether a 100 mg/kg increase in the amount of soil phosphorous would increase plant uptake of Se as had been observed during other research (Singh

1979), one plot in each replication received a 100 mg/kg increase of phosphorous and was seeded to canola. Four pounds of super triple phosphate fertilizer was incorporated into the top 15 cm depth increment of the plot using a hand operated rotary tiller immediately prior to seed application during the first seeding event.

Greenhouse Propagation of Milkvetch Plants

The limited amount of seed available for two-grooved milkvetch and cream milkvetch necessitated that these species be established in the greenhouse prior to transplanting them at the field site. Seeds were planted on March 30, 2000 in plastic containers (15 cm tall by 2.5 cm in diameter) filled with LTU soil that was disaggregated using a 2 mm sieve. One thousand seeds of each species were planted 1 per container. The containers were watered daily and two-grooved milkvetch seedlings were transplanted at the LTU on June 6, 2000, when the seedlings were approximately 4 cm tall. Poor germination of the cream milkvetch seeds resulted in too few of these seedlings to warrant transplanting at the LTU.

A total of 144 two-grooved milkvetch seedlings were planted at the LTU. Thirty-six seedlings were planted at a spacing of 1 plant/0.09 m^2 in 3.24 m^2 "mini-plots" located in the center of one test plot in each replication. The location of each seedling was marked with a plastic stake to facilitate monitoring of the seedlings.

Irrigation

During the first season of field study all test plots were irrigated once daily between seeding and June 24, 2000, and alternate days from June 26th until the plants

were harvested. Water was applied using a sprinkler system until water began to pond on the soil surface. Plants located outside of the LTU were irrigated in an identical fashion however irrigation was discontinued in late May after the plants had been eaten by antelope.

During the second season of field study, water was applied to all plots using soaker hoses. The change in irrigation technique was instituted because frequent strong winds at the site made operation of the sprinkler system too costly due to water blown off site during irrigation. Irrigation was performed from late May until July 9, 2001 during the second season of field study.

Vegetation Sampling

Plants comprising a given treatment were sampled from all replications when 50 % to 75 % of these plants reached the flowering stage. During the first growing season, canola and Indian mustard plants reached the flowering stage and were sampled on July 6, 2000 (10 weeks after seeding). Tall fescue and wheatgrass were sampled on October 17, 2000 (20 weeks after they were seeded). Tall fescue, wheatgrass, and prince's-plume were sampled on July 6, 2001 during the second field season.

For the purpose of vegetation sampling, steel stakes were driven into the corners of each test plot. During sampling events a transect was established in each plot by stretching a steel measuring tape between stakes located at diagonal corners of each test plot. Five 20 cm by 50 cm (0.1 m^2) Daubenmire frames were placed along each transect at 50 cm intervals starting 2 m from the endpoint of the transect (Daubenmire, 1965).

Plants encompassed by these frames were used for the measurement of plant density, canopy cover, and aboveground biomass production.

Transects were established between the northwest and southeast corners of each test plot during the first growing season and between northeast and southwest corners during the second growing season. This eliminated the possibility of measurements taken during the first growing season influencing those taken during the second growing season.

Plants remaining in test plots following sample collection were mowed to a height of approximately 10 cm within one week of the sample collection date in order to mimic harvesting practices that would take place if phytoextraction was implemented on an operational scale at the LTU.

Measurement of Plant Density

The density of each plant species seeded at the LTU was measured in order to determine the ability of that species to germinate and emerge in the LTU soil. This was performed 8 weeks after the plants were seeded. The mean number of plants in each of the five Daubenmire frames was calculated and multiplied by a factor of 10 in order to report mean plant density values for each respective test plot in units of plants/m^2.

Measurement of Percent Canopy Cover

The percent of canopy cover produced by each species was determined in order to indicate the ability of the plants to develop on the LTU. This measurement was performed immediately prior to harvesting the plants by visually estimating the

percentage of plant canopy cover present within the frames using the technique described by Daubenmire (1965). A mean percent canopy cover value was calculated for each test plot.

Measurement of Aboveground Plant Production

Aboveground biomass produced by each species was determined in order to assess overall growth as well as to facilitate the calculation of soil metal removed by phytoextraction. Plants encompassed by the previously described Daubenmire frames were clipped 2 cm above ground level. The plants were placed into a paper bag and placed into a drying oven at 70° C until reaching a constant weight. The dried plants were weighed and the average dry plant mass per square meter was calculated.

Measurement of the Survival and Development of Two-grooved Milkvetch

Due to the limited number of two-grooved milkvetch seedlings that were transplanted to the LTU, these plants were not subjected to the same measurements of plant density or percent canopy cover as those species that were planted as seed. Instead, the number of surviving milkvetch plants in each replication was counted five weeks after transplanting and a qualitative assessment of their development made.

Measurement of Plant Metal Concentrations

Plants from successfully established treatments were collected for laboratory analysis of arsenic, chromium, lead, selenium, and zinc concentrations. This was

performed during the plant harvest period by randomly selecting 10 plants in each test plot and clipping them 2 cm from ground level using stainless steel clippers. The clipped plants were immediately placed into plastic zip-lock bags and put into a cooler containing dry ice. The plants were frozen in order to inhibit metabolism that may otherwise have converted Se into a volatile form. After 4 days in a freezer at Montana State University, Bozeman campus, frozen plants were pulverized with a mortar and pestle, mixed, placed into glass jars, and shipped to Severn Trent Laboratories in Sacremento, CA. Total As, Cr, Pb, Se and Zn concentrations were determined using nitric acid digestion and inductively coupled plasma spectroscopy (EPA methods 3050 and 6010B) (U.S.E.P.A., 1986) (Table 2). Selenium analysis was performed using a trace instrument to achieve a lower detection limit for this element. A percent moisture correction was used so that the metal concentrations could be reported on a plant tissue dry weight basis even though the plants were not dried prior to metal analysis in order to prevent Se volatilization.

Measurement of Soil Metal Concentrations

In each test plot, soils were collected to determine the total concentrations of As, Cr, Pb, Se, and Zn both before seeding and after harvesting the test plots during the first season of field study. Soils were collected by taking five randomly located, 2-cm diameter soil cores from the 0 - 15 cm and 15 - 30 cm depth increments in each test plot. Soil cores were mixed to create two composite samples from each test plot; one from the 0 – 15 cm increment and another from the 15 – 30 cm increment. Composite soil samples were placed into glass jars and sent to Severn Trent Laboratories in Knoxville,