MANAGING HIGH SOIL SELENIUM WITH PHYTOREMEDIATION[1]

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Abstract: High concentrations of soil selenium (Se) are potentially toxic for the agricultural ecosystem in many regions of the western USA. As part of a strategy to manage the impact of this problem, a series of five experiments were conducted to evaluate the uptake, volatilization, and removal of Se by different plant species. In *Experiment I*, *Astragalus incanus L., Atriplex semibaccata, Brassica juncea, Festuca arundinacea,* accumulated more Se when grown in selenate–treated soil than selenite–treated soil. After harvest, Se levels were lowered in soils that supported growth of all tested plant species, especially *B. juncea*. In *Experiment II*, the variability of Se uptake was observed in different cultivars of tall fescue in which shoot Se concentrations ranged from 521 to 1191 mg kg^{-1} DM.

In *Experiment III*, the planting of *B. juncea, Brassica napus,* and *F. arundinacea* contributed to lowered levels of native soil Se concentrations measured after harvest. *B. juncea* was the most effective species for reducing soil Se concentrations. In *Experiment IV*, volatile Se was measured from the following *Brassica spp.* grown in water culture enriched with Se: *B. juncea, B. oleracea var. botrytis, B. oleracea var. capitata, B. oleracea var. cauliflora,* and *B. campesetris var. chinesis*. The rate of Se volatilization was found to be correlated with Se concentrations in shoot tissue. In *Experiment V*, field grown species of *B. juncea, F. arundinacea, Lotus corniculatus, Hibiscus cannabinus* L. accumulated native soil Se. After 3 years, levels of total soil Se supporting the four plant species were significantly lowered ($P<0.05$ level) at 0–75 cm, while soil supporting *B. juncea* was the most reduced at the $P<0.01$ level. Phytoremediation with the tested plant species in conjunction with improved water management may be a practical strategy to manage Se–laden soils and thus reduce Se–laden effluent produced in agricultural regions of the western United States.

Additional Key Words: Selenium, phytoremediation.

Introduction

High concentrations of selenium (Se), which may cause toxicity problems in livestock, wildlife, and humans, occur naturally in some soils [(e.g., those derived from Cretaceous shale parent material) (Gilliom, 1991)]. Selenium in well–aerated alkaline soils can be mobilized into solution through irrigation and reach hazardous concentrations in the irrigation drainage water. Elevated concentrations of Se in subsurface agricultural drainage water, which was collected from farmland on the westside of the San Joaquin Valley, and stored and concentrated in Kesterson Reservoir, have caused a high incidence of deformity and mortality in waterfowl using Kesterson National Wildlife Refuge in central California (Deverel et al., 1984; Presser and Barns, 1985). There is an increasing concern about similar environmental incidences related to high concentrations of Se in soils and drainage water occurring in other agricultural regions in California, Nevada, Utah, Wyoming, and Colorado, as well as other parts of the world.

After more than a decade of extensive research on Se cleanup in Kesterson Reservoir, management options for the remediation of this Se–laden soil are few, costly and only marginally effective. For example, it is expensive and only temporarily effective to excavate and remove Se–laden soil to another site or to cover (bury) it with layers of Se–free soil. In the latter approach, Se concentrations may again appear at unacceptable levels due to natural–weathering processes including rainfall, affecting physical and chemical characteristics over time at the soil surface (Tokunaga and Benson, 1992). Successful management of Se–laden soils requires limiting the movement of soluble Se and other trace elements into shallow groundwater and evaporation ponds. Phytoremediation is an alternative strategy for managing Se in Se–laden soils (Bañuelos and Meek, 1990; Terry et al., 1992). In this approach, selected plant species remove soluble forms of Se from the soil by plant uptake and volatilization.

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Plants are effective in absorbing Se from large volumes of soil because of their extensive root systems. Selenium–accumulating plants appear to be especially tolerant of high concentrations of soil Se since they can absorb Se, and may metabolize and store high concentrations of Se [(>1000 mg kg^{-1} DM) Mayland et al., 1989]. Several researchers have evaluated the ability of Se–accumulating plants (Rosenfeld and Beath, 1964; Bell et al., 1992) to accumulate and remove Se from Se–laden soil by plant uptake (Wu et al., 1988; Bañuelos and Meek, 1990; Wu and Huang, 1991; Parker et al., 1991; and Retana et al., 1993). From these studies, the results indicate that selected Se–accumulators may offer unique potential as a component of bioremediation strategies for saline, Se–contaminated soils, and sediments. Other researchers suggested the use of sulfur–affinity plants, i.e., *Brassica spp.* to remove soil Se (Bañuelos and Meek, 1989). These plants may absorb high concentrations of Se (Hurd–Karrer, 1937; Bañuelos and Meek, 1989; Bell et al., 1992) because of the reported similar mechanisms of absorption and translocation of selenate to those of sulfate (Brown and Shrift, 1982; Leggett and Epstein, 1956; Gissel–Nielsen et al., 1984). The availability of Se for plant uptake is, however, dependent upon factors which influence the solubility and mobility of Se, e.g., soil sulfate concentrations (Bell et al., 1992; Wu and Huang, 1991; Wu et al., 1988; Ferreri and Renosto, 1972), salinity (Mikkelsen et al., 1988) oxidation/reduction transformations in the soil (Blaylock and James, 1994; Zayed and Terry, 1994), and plant species.

An additional and potentially very important pathway of Se removal by plants is through biological volatilization (Lewis et al., 1966; Terry et al., 1992). In this pathway, once Se has entered the plant in the form of selenate, it is likely reduced to selenite and then selenide, followed by the incorporation of selenide into selenocysteine (Ng and Anderson, 1978). Selenocysteine is then converted to selenomethionine (Ng and Anderson, 1978), the most likely precursor eventually leading to the formation of volatile Se, dimethylselenide (Zayed and Terry, 1992). Generally, most of the Se in Se–non–accumulating plant species is found as protein–bound selenomethionine, whereas Se in accumulator plants is mostly water–soluble and found in non–protein forms like Se methylselenocysteine (Mayland, 1994). Plant volatilization of Se may not only keep the levels of Se to safe levels within the plant but the process may be an additional advantage to further reduce levels of Se in Se–laden soils with minimum damage to the environment (Zayed and Terry, 1994; Duckart et al., 1992; Wu and Huang, 1991). Thus, successful bioremediation should utilize plant species which both accumulate and volatilize Se at high rates to lower Se levels in the soil.

The objective of this present work was to determine if different plant species, lower levels of soil Se by accumulation and/or volatilization of Se. The following series of five experiments were conducted to evaluate the uptake, volatilization and removal of Se by different plant species:

1) Selenium uptake by potential Se–accumulating plant species grown in soils enriched with selenite or selenate under greenhouse conditions.

2) Selenium uptake by cultivars of tall fescue grown in water culture enriched with selenate.

3) Selenium uptake and removal by tall fescue and *Brassica spp.* grown in soils high in native soil Se under greenhouse contitions.

4) Selenium volatilization by *Brassica spp.* grown in water culture enriched with selenate.

5) Selenium uptake and removal by the above plant species grown in field soils high in native Se under field conditions.

Methods and Materials

Experiment I–Selenate vs. selenite

Selenium uptake by potential Se–accumulator plants was investigated under greenhouse conditions. Treatments consisted of adding Se as either aqueous Na_2SeO_4 or Na_2SeO_3, respectively, to the soil before transplanting to achieve a total soil Se concentration of 3.5 mg Se kg^{-1} soil in each pot. The following species were transplanted in groups of six or clumps (for tall fescue) into 4–L plastic pots: *Astragalus incanus* L. (no common name), creeping saltbush (*Atriplex semibaccata* R. Br. L.), Old Man saltbush (*A. nummularia* Lindl. L.), Indian mustard (*Brassica juncea* Czern. L.), and tall fescue (*Festuca arundinacea* Schreb. L.). 'Control soils' consisted of Se^{+4}– and Se^{+6}–treated soils without plants. Pots were each filled with 1 kg air–dried soil mix (30% organic matter, by dry wt.). Soil pH was initially adjusted between 6.5 and 7.0 by adding powdered hydrated lime to the soil and mixing well. The design

structure was completely randomized with 10 replications per Se treatment for each plant species, respectively. Indian mustard and tall fescue were clipped three times (includes final harvest); Creeping saltbush and Saltbush two times (includes final harvest); and *A. incanus* one time. Sixty–five to seventy days after plants were transplanted into Se–treated soil, all plants were harvested, separated into shoots and roots, and soil samples were collected from each pot. Soils and plant materials were analyzed for Se.

Experiment II–Genotypical variation of Se uptake

Selenium uptake by tall fescue was investigated in water culture to determine the genotypical variation of Se accumulation in different cultivars of tall fescue under greenhouse conditions. Treatments consisted of the following cultivars and germplasm lines: Alta, Fawn, Kentucky 31, Mustang, Olympic, Rebel, Australia (No. 150156), Chile (No. 427127), Italy (No. 237559), Israel (No. 200339), Japan, South Africa (No. 7749750, and Soviet Union (No. 283314). Twenty seeds of each respective cultivar of tall fescue were sown on an 8 cm diameter fiberglass sheet and a stretched nylon mesh supported by a styrofoam frame. The whole apparatus was then floated in a 2 liter plastic container of 0.25 strength modified Hoagland nutrient solution (Epstein, 1972) with 2.0 mg L^{-1} Se added as selenate. The solution pH was initially adjusted between 6.5 – 6.7. Each treatment was repeated three times and completely randomized on a greenhouse bench. After 3 weeks of growth, the plants were thinned to 10 plants per container. The plants were harvested at the end of the fifth week and separated into root and shoot tissues and analyzed for Se.

Experiment III–Selenium uptake in native soil

The uptake of soil–borne Se was investigated under greenhouse conditions for Indian mustard, tall fescue, and canola (*Brassica napus* L.). Each species was grown in 15 kg air–dried soil (Panoche fine–loamy mixed [calcareous], Thermic Typic Torriorthents) containing a concentration of 1.2 mg total Se kg^{-1} soil. The soil had a pH of 7.8 and an electrical conductivity (EC) of 3.2 dS/m in the soil saturation extract.

The experimental design was a randomized complete block with four treatments (three plant species and one 'control soil', containing no plants), three blocks, and six pots per treatment in each block (totalling 18 pots per treatment). Indian mustard was harvested 55 days after transplanting by clipping off the shoots at the soil level. Two–week old Indian mustard seedlings were then replanted in the same soil. The second planting of Indian mustard was harvested 45 days after transplanting. Canola was harvested 85–90 days after transplanting (one harvest only). Tall fescue was harvested 100 days after transplanting. Canola and tall fescue were not replanted in the soil, since an attempt was made to have each respective species growing for 90 to 100 days in their respective soil. After harvest, plants were separated into shoots and roots, and soil samples were collected from each pot. Soil and plant samples were analyzed for Se.

For *Experiment I, II,* and *III*, plant species were grown in temperature–controlled greenhouses at 21°±3C day and 18°±3C night temperatures with an average natural sunlight flux of 850 µmol $m^{-2}s^{-1}$. Soil moisture in potted soil experiments was maintained at 50–60% of field capacity by monitoring weight losses of individual planted pots. Applying only the volume of Se–free water lost by transpiration and evaporation helped maintain soil concentration of Se and minimize the production of drainage water.

Experiment IV–Volatilization of Se

Selenium volatilization was investigated using greenhouse water culture techniques for evaluating the following *Brassica spp*: broccoli (*Brassica oleracea var. botrytis*); cv. Green Valiant, cabbage (*B. oleracea var. capitata*); cauliflower (*B. oleracea var. cauliflora*); chinese mustard (*B. campesetris var. chinesis*); and Indian mustard (*B. juncea* Czern L.).

Plants were grown in growth chambers under the constant conditions of 500 µmol $m^{-2}S^{-1}$ photon flux density, 25±2°C, and 16 h photoperiod. Two–week old seedlings were grown in 0.25 strength Hoagland's solution for 2 to 3 weeks (Epstein, 1972). Selenium was added as sodium selenate at a concentration of 20 µM and the plants allowed to grow another 14 d before Se volatilization was measured. Selenium volatilization was measured three separate times for each plant species as described by Terry et al. (1992). The rate of Se volatilization was determined by measuring the amount of volatile Se given off from a plant mounted in a collection chamber (a 76 cm long Plexiglas cylinder 28 cm in diameter; volume=46.7 l) housed within a plant growth chamber for 24 h. Air from the collection chamber was passed through an alkaline peroxide trap solution (160 ml of 0.05 M NaOH and 40 ml of 30% H_2O_2) that oxidized the volatile Se into a form suitable for later measurement. Air movement within the chamber was promoted using a small fan. Air entered the chamber with the aeration of the culture. Sufficient vacuum was applied by a water aspirator to induce a slightly negative pressure in the collection chamber, generating two air changes per hour. To collect the

volatilized Se compounds from roots separate from shoots, two volatilization chambers were used. The plant root system was transferred to a two–liter container that was inserted inside a small chamber. The small chamber was placed inside a larger one with the shoot being outside the small chamber and inside the larger one. The air circulating inside each chamber was collected separately and passed through two separate trapping systems each consisting of two traps. After determining Se volatilization rates, the respective plant species was removed from the collection chamber, separated into roots and shoot tissue, and Se content was prepared as described elsewhere (Zayed and Terry, 1992) and analyzed for Se (Bañuelos and Meek, 1990).

Experiment V–Selenium uptake from native soil under field conditions

A three year field study was conducted to study uptake of native soil Se in different plant species grown in a soil typical of the westside of central California (Los Banos clay loam, fine mixed soil thermic Typic Haploxeralfs). The total soil Se concentration ranged from 0.75 to 1.25 mg Se kg^{-1} soil (depending on field location). Treatments consisted of four plant species grown under field conditions: Indian mustard, tall fescue, birdsfoot trefoil (*Lotus corniculatus* L.), kenaf (*Hibiscus cannabinus* L.) and bare plots (no plants). Each species was grown in a 10 by 10 m size plot. Plant densities were as follows: Indian mustard and kenaf (40 plants/m^2) and tall fescue and birdsfoot trefoil (125 to 150 plants/m^2). Treatment design was a completely randomized block with each treatment replicated three times. Irrigation scheduling for the sprinkler system was based in part on information (i.e., precipitation, Et_o) collected from the California Irrigation Management Information System (CIMIS) weather station (Howell et al., 1983). Water used for irrigation contained negligible concentrations of Se (<10 µg Se L^{-1}). Prior to planting (May 1) and at the end of each growing season (October 15) for each year of the study, four soil core samples were collected from the depth intervals of 0–30 and 30–75 cm in each plot, respectively. Each year of the study, tall fescue and birdsfoot trefoil were hand clipped from four 1 m^{-2} sampling sites in each plot approximately at 60, 85, and 115 days after May 1. Indian mustard was harvested at day 75 and kenaf at day 115 each year and both species were replanted approximately May 1 in the subsequent years. Soil and plant samples were analyzed for Se.

Plant and soil analyses for all experiments

Washed plant material was oven–dried at 50°C for 7 days and ground in a stainless steel Wiley mill equipped with a 1 mm mesh screen. Soil samples were oven dried at 45°C for 5 d, pulverized, and then passed through a nonmetallic 1 mm cheesecloth. Plant and soil Se samples were prepared and digested by either microwave or block digestion with $HNO_3/H_2O_2/HCl$ and analyzed for total Se by atomic absorption with continuous hydride generation. Temperatures and conditions used in sample preparation are described in full detail by Bañuelos and Akohoue (1994). All measurements were made at the most sensitive resonance line (196.0 mm). The NIST Standard Reference materials wheat flour (SRM 1567; Se content was 1.1±0.2 mg kg^{-1}, with a recovery of 94%) and coal fly ash (SRM 1633a; Se content was 10.3±0.6 mg kg^{-1}, with a recovery of 93%) were both used as external quality control for Se analyses for plant and soil samples, respectively.

Results

Experiment I

For all plant studies under study, the chemical form of Se present in the soil did not have any significant effect on above–ground dry matter weight yields (Table 1). Plant dry matter weight yields increased with subsequent clippings for all species. Selenium concentrations were higher in the above–ground plant material of each plant species grown in the selenate enriched soil than the same species grown in the selenite treated soil (Table 1). For both Se treatments, the tested plant species absorb Se in the following order: *A. incanus* <tall fescue <creeping saltbush, <Old Man saltbush <Indian mustard. Selenium concentrations in roots never exceeded 0.1 mg Se kg^{-1} DM (data not shown) of tested plant species grown in either selenate or selenite–treated soils. After harvest, selenite treated soils contained more of the initial soil Se than the selenate treated soils for all plant species (Table 1). Losses of soil Se were greatest in soils supporting Indian mustard of multiple clippings and least for *A. incanus* (Table 1). For all plant species, the amount of Se recovered in plant material including all clippings did not completely equate to the amount of Se lost in the soil. This fraction of Se was expressed as 'unaccounted for Se' (Table 1).

Table 1. The concentrations of selenium recovered in different plant species grown in Se-enriched soil, in postharvest soil and percentage of unaccounted for Se for *Experiment I*.†

Plant species	Se added to soil	Mean shoot dry matter (g pot^{-1})‡	Se in: Plant‡ (mg kg^{-1} DM)	Se in: Postharvest Soil§ (mg pot^{-1})	Unaccounted for Se (%)¶
Indian mustard	selenate	6.06(0.17)	274(14)	0.81(0.05)	25
	selenite	4.94(0.18)	27(3.6)	2.45(0.14)	26
Tall fescue	selenate	3.72(0.16)	52(5.9)	2.02(0.15)	36
	selenite	3.35(0.18)	10(1.8)	2.79(0.13)	19
Creeping saltbush	selenate	8.04(0.34)	60(4.9)	1.96(0.13)	23
	selenite	9.30(0.64)	95(8.7)	2.75(0.17)	19
Saltbush	selenate	9.15(0.37)	78(7.4)	2.14(0.13)	19
	selenite	8.33(0.32)	9(0.8)	2.69(0.12)	27
A. incanus	selenate	1.55(0.18)	64(6.1)	2.73(0.12)	19
	selenite	1.52(0.13)	7(0.9)	3.08(0.07)	12

†Values represent the mean from a minimum of 10 replications followed by the standard error in parenthesis.

‡Values represent the mean from three clippings (includes final harvest) for Indian mustard and tall fescue; the mean from two clippings for Creeping saltbush and Saltbush; and the mean from one clipping for *A. incanus*.

§Initial soil preplant concentration was 3.5 mg Se pot^{-1}. Values represent the final soil Se concentration irrespective of the number of clippings for the respective plant species.

¶Approximated percentage of initial soil Se not accounted for in the postharvest soil concentration and in plant material harvested for each pot.

Experiment II

Shoot tissue Se concentrations of the tested cultivars of tall fescue ranged from 521 to 1191 mg kg^{-1} dry weight (Table 2). The total shoot accumulation of Se in tall fescue is also shown for all species in Table 2. Root tissue Se concentrations ranged between 150 to 550 mg kg^{-1} dry weight (data not shown); root tissue Se concentrations were correlated positively (R=0.860; P<0.001) with the shoot tissue Se concentrations (data not shown). Analysis of variance indicates that among the 13 tall fescue cultivars the shoot and root dry weight production and tissue Se concentrations were significantly different. F values are as follows for the different parameters: shoot dry weight (7.15) root dry weight (4.37), shoot Se (7.75), and root Se (9.98); all significant at 0.001 level of probability. In general, Alta and Fawn cultivars accumulated Se in their tissues to the greatest degree (>2000 µg); cultivars from Australia, South Africa, and Soviet Union accumulated the least amount (<1000 µg); while the remaining cultivars accumulated intermediate levels of Se in their tissues [(between 1000 and 2000 µg) (Table 2)].

Table 2. Shoot dry matter (DM) production and Se accumulation of 13 tall fescue lines after 5 weeks growth in solution culture enriched with 2 mg Se L^{-1} for *Experiment II*.

Variety	Dry weight of 10 plants (mg DM)	Shoot tissue Se concentration (mg kg^{-1} DM)	Total Se accumulation (µg)
Alta	2900*a*†	706*de*	2138*a*
Fawn	3212*a*	649*ef*	2092*a*
Ky–31	1450*b*	767*cde*	1114*cde*
Mustang	2007*b*	755*de*	1518*b*
Olympic	2188*b*	733*de*	1607*b*
Rebel	1943*b*	714*de*	1388*bd*
Australia	1042*e*	929*b*	970*def*
Chile	1421*cde*	927*b*	1306*bcd*
Israel	936*e*	1191*a*	1106*cde*
Italy	1106*e*	925*a*	1018*de*
Japan	1482*cde*	843*bcd*	1258*bcde*
South Africa	932*e*	906*bc*	845*ef*
Soviet Union	1071*e*	521*f*	560*f*

†Means separated in columns by Duncan's new multiple range test, P<0.01. The same letters represent no significant difference between varieties.

Experiment III

Selenium concentrations were slightly higher in the above–ground plant material for both Indian mustard and canola than in root portions, but not in tall fescue grown in the native Se soil (Table 3). Conversely, Se concentrations in roots were higher in tall fescue than in canola or Indian mustard. For all three plant species, extractable and total Se levels were at least 30% lower after harvest than at pre–planting (Table 3). Losses of soil Se were greatest in soils supporting Indian mustard. 'Control soils', which were irrigated and contained no plants, had 23% less total Se at the end of the experiment.

Table 3. Changes of soil selenium and the accumulation of selenium in different plant species grown in soils high with soil borne Se for *Experiment III*.

Plant species	Preplant total soil Se (mg Se kg^{-1} soil)	Postharvest total soil Se (mg Se kg^{-1} soil)	Loss of soil Se (%)	Plant Se in: Shoot (mg Se kg^{-1} DM)	Plant Se in: Root (mg Se kg^{-1} DM)
Control (no plant)	1.17(0.10)	0.90(0.05)	23	NA	NA
Indian† mustard	1.17(0.10)	0.69(0.08)	41	1.11(0.10)‡	0.57(0.07)‡
Canola§	1.17(0.10)	0.81(0.09)	31	1.31(0.11)	0.37(0.09)
Tall fescue§	1.17(0.10)	0.72(0.10)	38	0.97(0.09)	0.94(0.05)

†Species was replanted in the same soil postharvest soil concentrations represents the residual Se after two plantings followed by the standard error in parenthesis.

‡Mean tissue concentrations of Se from plant material from two plantings.

§Species was planted only once.

Experiment IV

The data in Table 4 show the rate of Se volatilization expressed as per unit plant dry matter. From these data it appears that plant root system is the main site for Se volatilization. If the data for root and shoot volatilization are combined for all the tested plant species, Indian mustard has the highest rate of Se volatilization compared to the other species (Table 4). The highest volatilizers of Se tend to have higher concentrations of Se in their plant tissues than the others as described elsewhere (Terry et al., 1992). In this respect, when the data from all species were combined, the rate of Se volatilization was found to be correlated (r^2=0.664) with Se concentrations in shoot tissue.

Table 4. Rates of Se volatilization of shoot, root, and detopped root of four species grown in half strength Hoagland's solution enriched with 20μM Se L^{-1} for *Experiment IV*.

Plant Species	Rate of Se volatilization in:†	
	Intact Shoot	Intact Root
	(μg kg^{-1} d^{-1}; dry mass)	
Broccoli	125±59‡	1782±825
Cabbage	200±92	2930±14
Cauliflower	149±13	2775±1966
Chinese mustard	199±62	2010±1756
Indian mustard	885±92	2785±375

†Volatile Se was extracted from alkaline peroxide trap described in Zayed and Terry (1994).

‡Values represent the mean followed by the standard deviation.

Experiment V

In each year of the three years of the field study, Indian mustard accumulated higher concentrations of Se than the other plant species (Table 5). Tissue concentrations of Se did not increase with subsequent clippings of tall fescue or with birdsfoot trefoil (only the mean concentrations of Se from all clippings for each species are presented in Table 5). Shoot Se concentrations from all species were correlated with preplant soil Se levels (r=0.60 and τ=0.44; P<0.01 level). Levels of total soil Se in each of the tested plots were significantly lowered at 0–75 cm for all plant species in comparison to bare plots (P<0.05 level). Plots supporting Indian mustard were the most reduced (P<0.01 level) compared to other plant species.

Table 5. Mean DM yield, tissue concentrations of Se in different crops, and changes in soil Se concentrations from 0–75 cm in 1992–1994 for *Experiment V*.†

Plant Species	Dry matter yield	Se concentrations in: Shoots	Root	Preplant soil	Postharvest soil
	($g\ m^{-2}$)	($\mu g\ kg^{-1}$ DM)		(mg Se kg^{-1})	
1992 Experiment					
Control (bare plot)	NA‡	NA	NA	0.99(0.06)	0.94(0.04)
Indian mustard	1420(138)	1650(63)	890(65)	1.12(0.04)	0.81(0.02)
Tall fescue§	280(46)	280(14)	140(13)	1.25(0.06)	1.14(0.03)
Birdsfoot trefoil§	340(39)	340(22)	110(10)	0.98(0.08)	0.78(0.04)
Kenaf	2855(175)	620(33)	480(29)	0.96(0.06)	0.83(0.02)
1993 Experiment					
Control (bare plot)	NA	NA	NA	0.90(0.10)	0.87(0.03)
Indian mustard	1512(121)	1421(109)	875(78)	0.78(0.07)	0.67(0.02)
Tall fescue	454(42)	245(34)	110(9)	1.07(0.04)	0.97(0.04)
Birdsfoot trefoil	562(26)	322(29)	124(12)	0.70(0.09)	0.61(0.06)
Kenaf	2975(161)	575(36)	440(80)	0.84(0.10)	0.70(0.07)
1994 Experiment					
Control (bare plot)	NA	NA	NA	0.85(0.08)	0.82(0.05)
Indian mustard	1381(133)	1050(112)	652(34)	0.68(0.06)	0.60(0.05)
Tall fescue	560(29)	193(17)	108(10)	0.91(0.04)	0.87(0.02)
Birdsfoot trefoil	785(31)	224(23)	116(12)	0.58(0.08)	0.56(0.04)
Kenaf	3214(196)	438(25)	278(18)	0.71(0.06)	0.66(0.03)

†Values presented represent means followed by standard error in parenthesis for a minimum of 12 samplings for plant material and 24 samplings for soil (data presented represents combined data from 0–30 and 30–75 cm).
‡NA – not applicable.
§Values presented represent mean DM and plant concentrations of Se for the three clippings.

Discussion

The results of all the above studies show that Indian mustard, as a member of the sulfur affinity *Cruciferae* genus, absorbed the greatest amount of Se without any decrease and lowered soil Se levels to the greatest extent. The ability of Indian mustard to absorb the highest concentrations of Se among all tested species and volatilize Se at high rates (Table 4) may account for the losses of soil Se and for the 'unaccounted for Se' observed in *Experiment I*, since soil Se losses due to leaching were minimized with irrigation management. Selenite complexes were not as readily taken up as selenate by Indian mustard or the other tested plant species. The plants preference for selenate implies that the planting of primary or secondary Se accumulators may be more efficient in lowering levels of soil Se by uptake where the selenate predominates. Since uptake of water soluble Se (primarily selenate) did not account for the total losses of soil Se, presumably a transformation of Se occurred within the soil and/or plant.

Biological volatilization of Se may be achieved by plants, and/or by bacteria in association with the plant (Doran, 1982; Karlson and Frankenberger, 1989). Bacterial contribution could be indirect; bacteria might facilitate the production of Se intermediates that could be used in the plant biochemical pathway for Se volatilization (Terry and Zayed, 1994). Biological volatilization of soil Se is attractive because Se is bimethylated by plants or plant/microbe associations to produce relatively non–toxic volatile forms of Se. Whether plants rely on soil microflora to mineralize complexed insoluble organic Se into a soluble form before Se can be absorbed need additional investigation. The results

of all the present studies do not specify that reduction of soil Se was solely attributed to plant uptake, since Se was also lost from 'control soils' (without plants) as well. Microbial volatilization of Se may have been involved (Frankenberger and Karlson, 1990) and may be encouraged with the planting of certain plant species.

Although the oxidation state of Se is the predominate factor responsible for determining its availability for plant uptake, it may also play a role in the volatilization capability of plants. Zayed and Terry (unpublished results; 1994) have observed that the availability of Se for plant volatilization is governed among other factors by the chemical form of Se in the soil. Plants volatilize Se at a faster rate when supplied with selenite and much faster when supplied with organic forms of Se, e.g., selenomethionine. Apparently volatilization of Se by the plant from different Se chemical species occurs at a rate irrespective of the rates of Se uptake by the plant. Once Se is methylated, it is released into the atmosphere, diluted and dispersed by air currents away from the contaminated source. Dimethylselenide is reported to be 500 to 700 times less toxic than similar concentrations of aqueous selenite or selenate (Frankenberger and Karlson, 1988).

Additional investigations are necessary to determine the safest and most practical way to dispose of Se–containing plants used for bioremediation. Some options for harvested plant material include: 1) blending with animal feed rations; 2) incorporating in Se–deficient soils and growing forage crop; and 3) incorporating into soil to enhance volatilization of by microbes and plants. Extreme caution must be taken, however, when blending into soils for forage production or feeding Se–laden plant material directly to animals, since there is a narrow range between toxic and deficiency level of Se in animals. Not all Se absorbed by the animals is physiologically important. In this regard, some Se is metabolized to a methylated form that is re–excreted readily. The bioavailability of Se in feces, urine, and respiratory products is described elsewhere (Mayland 1984). Although a number of factors influence Se requirements, a dietary level of 0.3 ppm Se is near the minimum required to support health and optimal performance of food producing animals (Oldfield et al., 1994). Thus the perennial plant species used for phytoremediation in these reported studies, i.e., tall fescue, birdsfoot trefoil, may be more suited for use as animal forage, because of their lower tissue Se concentrations.

In conclusion, it is clear that some plants have the ability to remove measureable amounts of soil Se by uptake, accumulation and volatilization over a short period of time. Indian mustard seems to be an appropriate plant species for use in the vegetation management of Se. Under greenhouse conditions, Indian mustard contributed to lower soil Se concentrations by at least 30% irrespective of the species and concentration of Se in the soil. Under field conditions, Indian mustard contributed to lower soil Se concentrations by almost 50% between 0–75 cm after 3 years. Removing soil Se by phytoremediation is cost effective and does not require sophisticated chemical or mechanical processes. Plant volatilization of Se may comprise a significant portion of the Se lost from soils after planting and it should, therefore, be considered as a potential phytoremediation process in the vegetation management of Se. Future research should focus on incorporating the tested plant species in a crop rotation in Se–laden soils and then developing ways of significantly enhancing Se volatilization by plant in the field, i.e., genetic engineering of transgenic plant or applying chemical modifiers or microbial inoculation. It is important to realize that plants alone are not the long–term solution for managing high levels of soil Se. Phytoremediation in conjunction with improved irrigation practices and drainage water management can ameliorate the potential negative effects that high levels of Se exert on the agriculture ecosystem. Using vegetation and irrigation management in Se–laden soils is necessary to sustain irrigated agriculture in the western United States.

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