2003-2004
Technical Progress Reports

University of California

Salinity/Drainage Research Program

Division of Agriculture and Natural Resources
University of California

October 2004

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FOREWORD

The UC Salinity/Drainage Program was initiated in 1985 to develop, interpret, and disseminate research knowledge addressing critical agricultural and environmental problems of salinity, drainage, and toxic trace elements in the West Side of the San Joaquin Valley in California. The Water Resources Center and the Salinity/Drainage Program were administratively combined with the UC Center for Water Resources in 1993.

A major function of the UC Salinity/Drainage Program is to support research and extension activities that will contribute to developing optimal management strategies to cope with salinity/drainage/toxics problems in the western San Joaquin Valley. Funded research projects must be both relevant and scientifically sound. An external advisory committee consisting of UC scientists and individuals, listed on page iv, evaluates the merits of proposals. Appreciation is expressed to all the individuals that devoted time and made valuable contributions to the selection of the research to be supported.

This publication reports the research findings of projects funded by the Salinity/Drainage Program in 2003-2004. The number of projects supported during this year is significantly lower than in previous years because of the 47% reduction in budget as compared to previous years.

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Salinity/Drainage Annual Meeting

March 25, 2004
Red Lion Inn, Sacramento

7:45 am **Registration**

8:25 **Welcome, John Letey**, Director, UC Center for Water Resources, UC Riverside

8:30 **UC Salinity/Drainage Program Research Highlights**

9:45 **Update of the USBR San Luis Drainage Service**

Jason Phillips, USBR

10:15 ***BREAK – Posters***

10:40 ***Is Land Retirement Drainage Service?***

Thad Bettner, Westland Water District

11:10 ***Results from the CVPIA Land Retirement Demonstration Project***

Bea Olsen, USFWS,
Stephen Lee, USBR,
Curt Uptain, CSUS-ESRP,
Ken Lair, USBR

11:40 ***Selenium and Nitrate Removal from Agriculture Drain Water Using ABmet Process***

Tim Pickett, Applied Biosciences Corp.

12:10 pm ***LUNCH – Posters***

1:10 ***Advances in Reverse Osmosis Technology***

Jose Faria, DWR
Scott Irvine, USBR

1:10 ***Development and Application of New Solar Evaporator Regulations***

Wayne Verrill, SWRCB
Lonnie Wass, Central Valley Regional WQCB

2:50 ***BREAK – Posters***

3:15 ***USFWS Approaches to Drainwater Risk Management***

Steven Detwiler, DFWS
Thomas Maurer, DFWS

3:45 ***Landscape-Level Approaches to Mitigate Impacts of Selenium at Agricultural Drainwater Evaporation Basins.***

Scott Terrill, H.T. Harvey & Associates

4:15 ***Sensibility or Senility: Personal Reflections***

John Letey, UC Center for Water Resources, UC Riverside

Interaction of Se Biogeochemistry with Foodchain Disruption in Full-Scale Evaporation Basins and Pilot-Scale Drain Water Systems

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ABSTRACT:

The purpose of this project is to evaluate the Se bioremediation potential (via reduction of ecotoxic risk) of combined foodchain disruption and Se volatilization in full-scale TLDD evaporation basins and pilot-scale drain water systems. Our approach has been to assess the influence of ongoing brine shrimp harvest and fertilizer input on water chemistry, water and biota Se status, Se volatilization activities, and algal community. Analyses have been conducted for monthly water and brine shrimp samples from selected saline basin cells as well as for the annual collections of water and macroinvertebrate samples from both saline and less saline cells. Fertilizer input appeared to enhance algal populations which in turn regulated brime shrimp harvest in hypsersaline cells. Brine shrimp harvest did not appear to have a direct impact on water chemistry or the Se content of water-column biota. However, it exhibited a disruptive influence on Se incorporation into brine shrimp proteins and into benthic invertebrates. In addition, harvest activity and/or fertilization was relatable to a somewhat elevated Mn and Ni status (relative to P) in algal biomass with no significant change in other elements. Salinity continued to be the predominant factor in modulating Se food chain transfer and volatilization, namely, the most saline cell (SEB 8, instead of SEB 9, became most saline in 2003) posted the highest waterborne volatile Se content and the lowest biota Se status, regardless of the basin cell configuration or other factors. Moreover, DNA-based algal community analysis of harvested saline cells revealed two green algae that may support brine shrimp growth while the Se-volatilizing Synechococcus sp. could evade grazing and contribute to the high volatilization activity observed persistently in hypersaline cells.

PURPOSE:

The purpose of this project is to evaluate the Se bioremediation potential (via reduction of ecotoxic risk) of combined foodchain disruption and Se volatilization in full-scale TLDD evaporation basins and pilot-scale drain water systems.

Preliminary investigation in hypersaline ponds of TLDD indicates that Se volatilization may be combined with brine shrimp harvest to reduce Se load in waters and biota. In addition, it appears that both processes could be enhanced by manipulating the water chemistry via fertilizer input, which would increase microphyte population that functions to dissipate Se by volatilization and/or as food for brine shrimp. If mechanistically understood, this coupled process should prove to be a highly economical and flexible option for remediating Se ecotoxic risk in agricultural drainage systems. These advantages are in part due to a market demand for brine shrimp and the practicality of implementing the option together with other drainage mitigation plans such as IFDM and reverse osmosis.

INTRODUCTION:

OBJECTIVES

Our objectives are to investigate and understand the effect of fertilization and brine shrimp harvest on Se biogeochemistry and to uncover conditions that simultaneously favor Se volatilization and brine shrimp production while minimizing the accumulation of Se ecotoxic indicators. We will approach these objectives by both full-scale monitoring and pilot-scale studies as follows:

1. Change in Se status in TLDD hypersaline ponds (Hacienda A4 in particular since we have data on its Se status before harvest began) elicited by brine shrimp harvest;
2. Changes in water chemistry at TLDD associated with fertilizer input;
3. Effects of fertilizer input on nutrient status of microalgae and brine shirmp, microalgal community, as well as Se status in TLDD hypersaline ponds so that these effects may be related to changes in water chemistry, thereby guiding additional nutrient supplementation.
4. Establish pilot-scale drain water system at the Red Rock Ranch to better control the water chemistry (which in turn regulates microalgal populations and community) to optimize Se volatilization and brine shrimp harvest.

APPROACH

Rates of brine shrimp harvest and fertilization in each of the TLDD evaporation basins have been compiled from daily records provided by Novlek. On a monthly basis, water, microalgae, and brine shrimp samples have been collected from TLDD evaporation basins, processed, and analyzed for total Se and/or Se speciation into proteins. The microalgal community has also been profiled using 16S cDNA in combination with Denaturing Gradient Gel Electrophoresis (DGGE). In August, 2003, extensive field sampling was conducted at TLDD basins to collect water column and benthic macroinvertebrates, with the assistance of Julie Vance from Dept. of Water Resources, Fresno. Selenium status of these samples should indicate the distribution of Se in the ecological niches of the basins. In situ Se volatilization measurements were also made at selected TLDD basin cells during this field trip. Elemental analyses of microalgae collected from the evaporation basins monthly between February 2002 and January 2003 have been undertaken to investigate the nutritional status of the primary producers in the basins.

RESULTS:

CHANGES IN TLDD WATER MANAGEMENT

Diminished water delivery to the TLDD evaporation basins in 2003 relative to previous years changed some aspects of the basins, truncating harvest from Cell A4 of the Hacienda basins (HAC A4) in early summer and eliminating the un-harvested C4 comparison basin from monthly monitoring. Also, the sequential flow of water through the South Evaporation Basin was disrupted; linking basin cells 8 and 10 so that

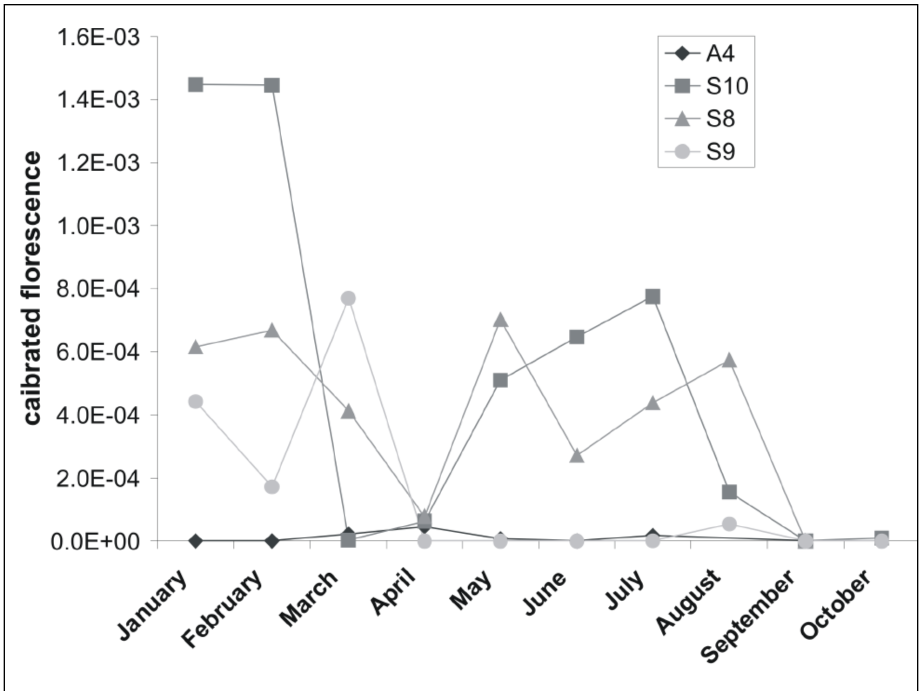

Figure 1. Monthly trend of Chl a fluorescence in water samples.

both SEB 9 and SEB 10 were directly supplied by SEB 8 and the salinity of those three basins fluctuated substantially. In terms of the annual average salinity, SEB 8 became the highest (125 ppth), SEB 9 was next (111 ppth) and SEB 10 was the least saline (86 ppth). Thus, while the most saline basin (SEB 8) continues to be the most Se volatilizing (cf. Fig. 17), it was not harvested much for brine shrimp. The basin with the highest brine shrimp yields actually had the lowest average salinity but this was probably due to the regular influx of relatively fresh water needed to maintain the water level for brine shrimp harvest operation.

WATER CHEMISTRY AND SE STATUS IN TLDD MICROALGAE AND MACROINVERTEBRATES

Water, algae, and invertebrate samples were collected monthly from TLDD basin cells and analyzed for chlorophyll a (chl a) fluorescence, total Se of water, algae, and brine shrimp, as well as protein Se content of algae and brine shrimp. Figure 1 shows the monthly trend of in vivo chl a fluorescence of the 4 monitored basin cells, where SEB 9, 10 and HEB A4 have been harvested while SEB 8 has not produced significant amounts of shrimp. The chl a fluorescence, which reflected total algal population, were the highest in SEB 8, followed by SEB10 and SEB9 while the chl a fluorescence in HEB A4 remained low year-round. This may be attributed to the low harvest activity in SEB 8. In addition, chl a fluorescence fluctuated widely from month to month in SEB 8 with peaks occurring in February, May and August, 2003.

Variations in fluorescence or algal population in the harvested ponds can be compared with the corresponding cumulative brine shrimp harvest, as shown in Figure 2. In HEB 10, the decline in algal population in March and April preceded the high shrimp production from late June to mid-July. Then, high fluorescence readings in July preceded the harvest in August and September. Similarly, in SEB 9 high algal populations in March were followed by high shrimp yields in April. The lowest fluorescence and algal populations were measured in HAC A4 with a small peak occurring in April which preceded the shrimp harvest in early May. The harvest activity was terminated after May due to the precipitous lowering of water levels in that basin in June, which led to the loss of algae and shrimp for the remainder of the year. The declining algae population at the end of the year (September and October) was met with tailing off of shrimp harvest in both active ponds (SEB 9 and 10). This account is consistent with the notion that brine shrimp grazing regulates microalgal population in these hypersaline basin cells.

In addition to chl a fluorescence, salinity and water Se concentration ([Se]) also exhibited seasonal changes

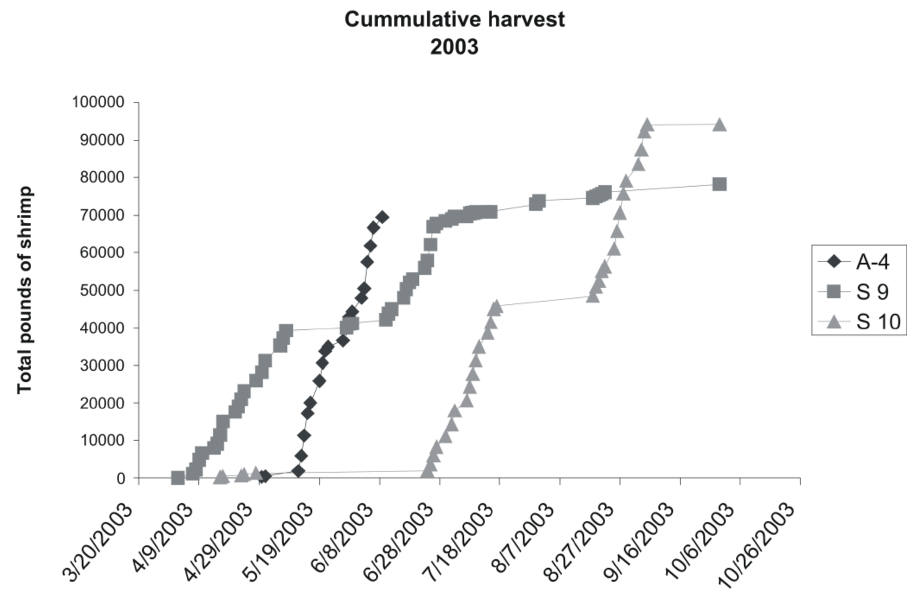

Figure 2. Cumulative brine shrimp harvest.

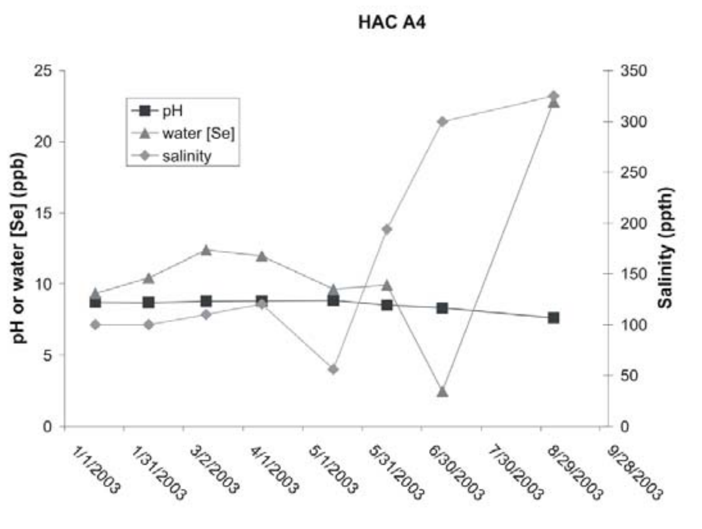

Figure 3. Salinity, pH and water selenium concentrations in monthly water samples from HAC A4.

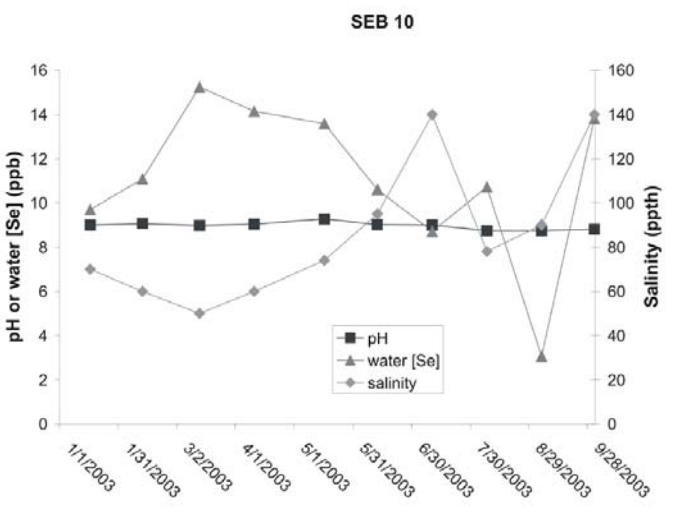

Figure 4. Salinity, pH and water selenium concentrations in monthly water samples from SEB 10.

and differences among the basin cells (as observed in previous years), which is shown in Figures 3-6. As with previous years, the salinity (in parts per thousand or ppth) of SEB 10 (Fig. 4) were largely lower than that of SEB 9 (Fig. 5) and HEB A4 (Fig. 3). However, the salinity of SEB 8 (Fig. 6) was higher than that observed previously. As stated above, this was due to the change in TLDD water management. The relative stability in salinity seen in SEB 10 may underlie the prolonged harvest and greater yield of brine shrimp from that basin compared to SEB 9 and HEB A4 (Fig. 2). The water levels in SEB 10 was better maintained when water availability limited input to other basins, which contributed to a more stable salinity (Fig. 3-5). As observed previously, salinity and water Se concentration did not correlate, as would be expected from simple evaporite chemistry. The pH was similar and stable over time in all cells (Fig. 3-6). The water Se concentration of all cells was comparable all year round, with the exception of the sharp increase in HAC A4 as it dried up towards the end of the year (Fig. 3). Water Se concentration, pH, and salinity were chronologically stable in the unharvested basin SEB 8 which supplied water to SEB 10 (Fig. 6).

The effect of brine shrimp harvest on algal Se status is shown in Figure 7 (algae Se concentrations), Figure 8, (Se bioconcentration factor or BCF based on dry mass), and Figure 9 (protein Se concentrations) in the TLDD basins. The algal Se body burden follow the same patterns over time in all four monitored cells, peaking towards the end of the year's harvest when salinity and water Se concentrations also fluctuated wildly (Fig. 7). Also noted is that the algal Se burden of the harvested cells (SEB10, SEB 9 and HEB A4) was lower at the start of the harvest season (April and May, Fig. 2) than that of the unharvested cell (SEB 8), during which time brine shrimp harvest rose rapidly. In July, however, that trend was reversed while brine shrimp harvest activity was low. The

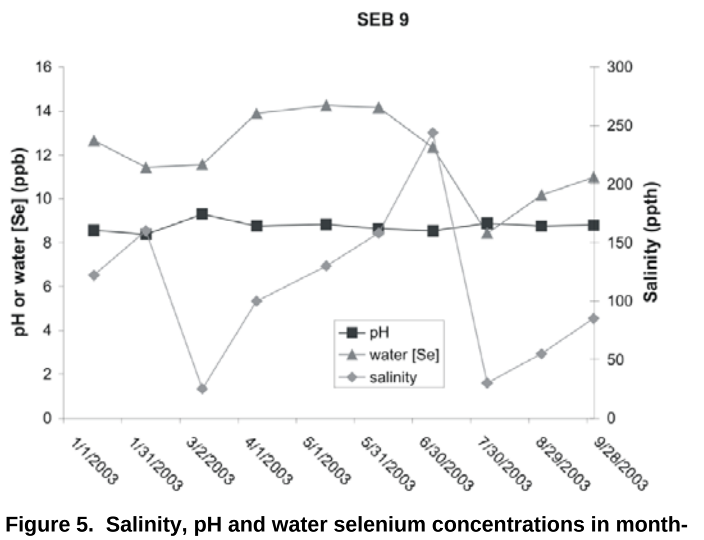

Figure 5. Salinity, pH and water selenium concentrations in monthly water samples from SEB 9.

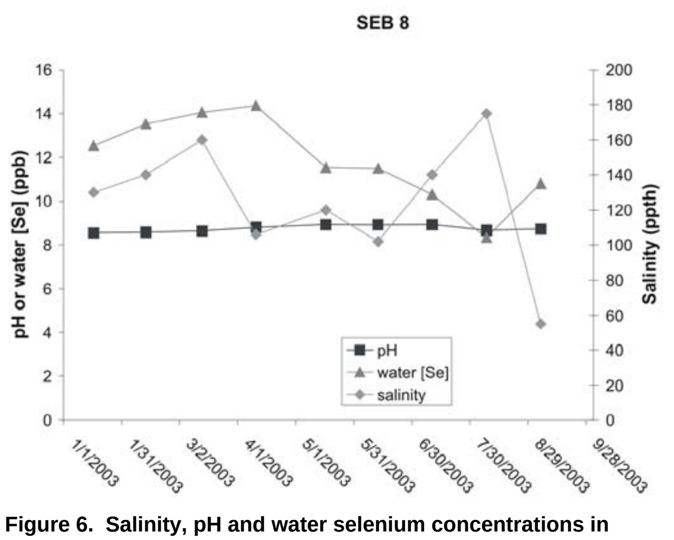

Figure 6. Salinity, pH and water selenium concentrations in monthly water samples from SEB 8.

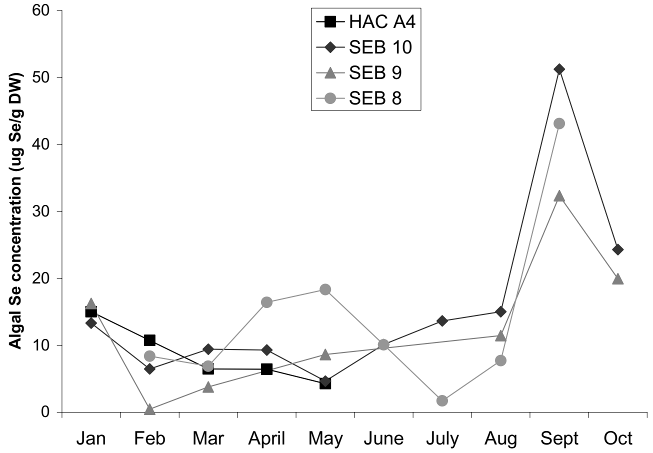

Figure 7. Algae Se concentrations.

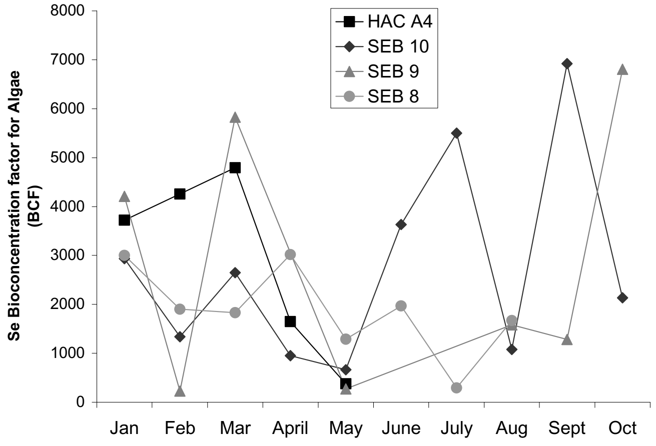

Figure 8. Se bioconcentration factor or BCF of algae, based on dry mass.

concentrations of Se in the protein of algae were similar in all ponds and generally followed the trend of the total Se body burden but unrelated to harvest activity. The Se BCF in algae varied widely in the harvested cells (HAC A4, SEB 9, and SEB 10) while it was relatively stable throughout the year in the unharvested basin, SEB 8 (Fig. 9).

As for the Se status in brine shrimp, the one effect of harvesting appears to be a disruption of Se transfer from microalgae to the macroinvertebrates, as evidenced by the following two observations. In unharvested basins (SEB 1, SEB 8, and HAC A3) the percent of the Se body burden present in the protein of the algae was correlated with that of the brine shrimp (Fig. 10). In harvested basins (SEB 9, SEB 10, and HAC A4) these two variables were much less correlated. Additionally, the negative correlation between water Se concentration and brine shrimp BCF is more scattered in the harvested basins than in those that were not harvested (Fig. 11). No data for the unharvested HEB C4 was available due to lack of adequate samples.

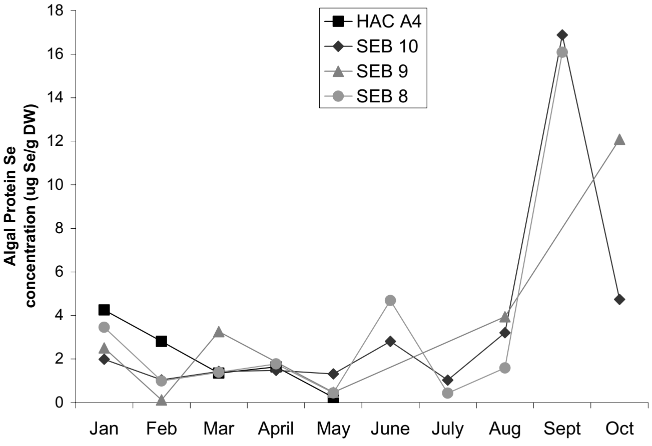

Figure 9. Algal protein Se concentrations.

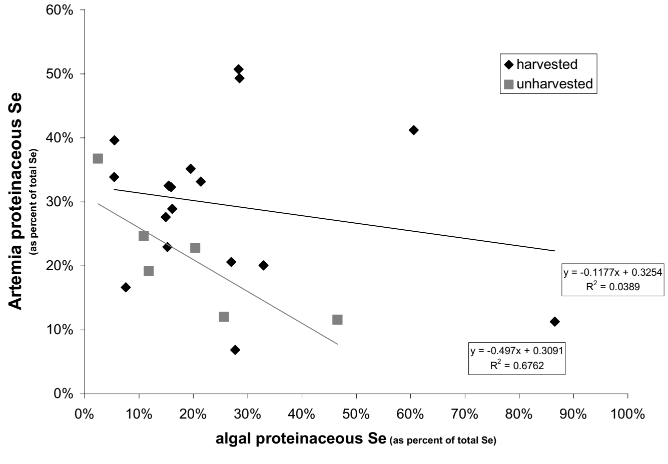

Figure 10. Percent of the Se body burden present in the protein of the algae and brine shrimp in harvested and unharvested basins.

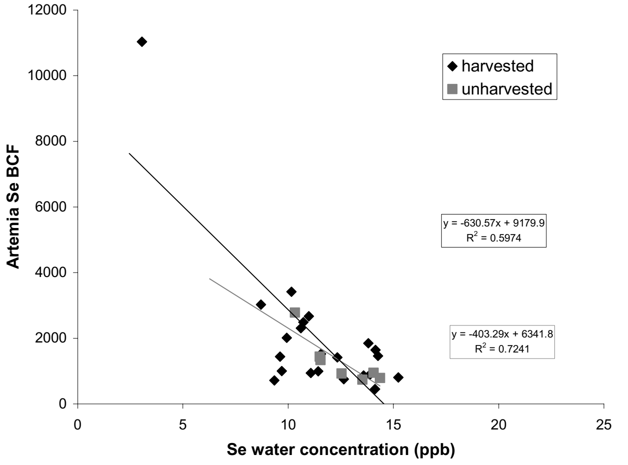

Figure 11. Water Se concentration and brine shrimp BCF.

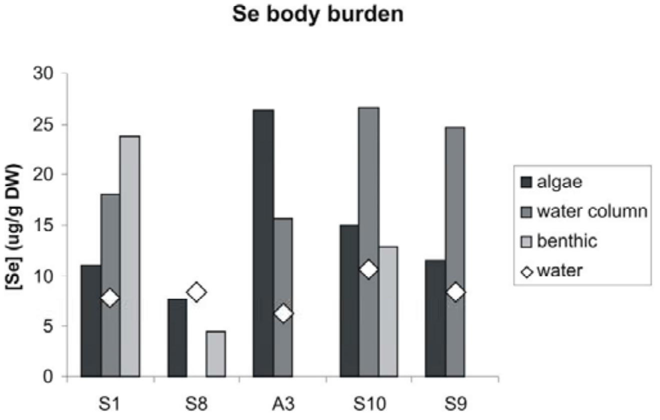

Figure 12. Total Se concentrations in algae, water column invertebrates, benthic invertebrates, and water samples collected on August 5, 2003.

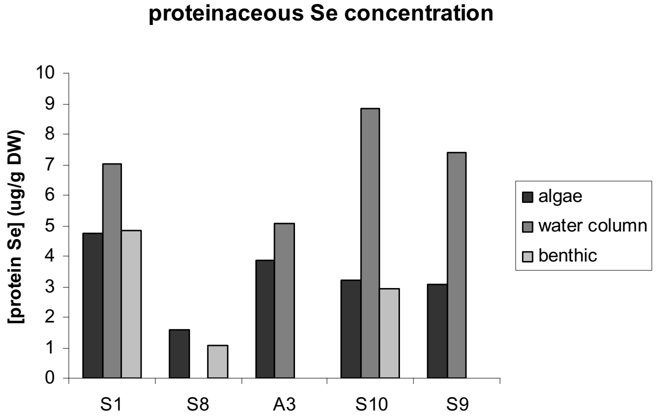

Figure 13. Proteinaceous Se concentrations in algae, water column invertebrates, benthic invertebrates, and water samples collected on August 5, 2003.

In addition to monthly sampling of algae and brine shrimp in hypersaline cells, a composite each of water column and benthic organisms was collected from TLDD basin cells of low to high salinity on August 5, 2003. The Se status of these samples along with the water [Se] is shown in Figure 12 (total Se) and 13 (protein Se). The total Se concentration measured in biota from the unharvested basins (SEB 1, SEB 8, and HAC A3) exhibit no trend related to taxa. In the harvested basins (SEB 9 and SEB 10), however, the Se body burden of the water column macroinvertebrates is consistently higher than that in the algae or in the benthic organisms. Basin fertilization and exhaustive harvest of the brine shrimp may help funnel Se to the water column organisms via enhancing algal growth, while reducing Se incorporation into benthic organisms by limiting detrital deposition and, thus, growth and consumption of Se by benthic organisms . A similar trend was observed for the proteinaceous Se burden of algae, water column and benthic macroinvertebrates composites (Fig. 13). In fact, total and proteinaceous Se concentrations were well correlated in the annual survey samples (data not shown). As observed in previous years, no clear correlation was discerned from the water [Se] to the Se burden of algae, water column or benthic invertebrates and from salinity (cf. Fig. 16) to water [Se]; the former indicates that water [Se] is not a good predictor of Se accumulation in aquatic biota. This is presumably due to the influence of complex Se biogeochemistry on Se bioaccumulation. The lack of water Se buildup with increasing salinity is consistent with Se removal via volatilization and/or brine shrimp harvest (see Se volatilization by Microalgae).

Monthly water samples collected from February 2002 to January 2003 were also filtered to trap the microalgae which were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the follow-

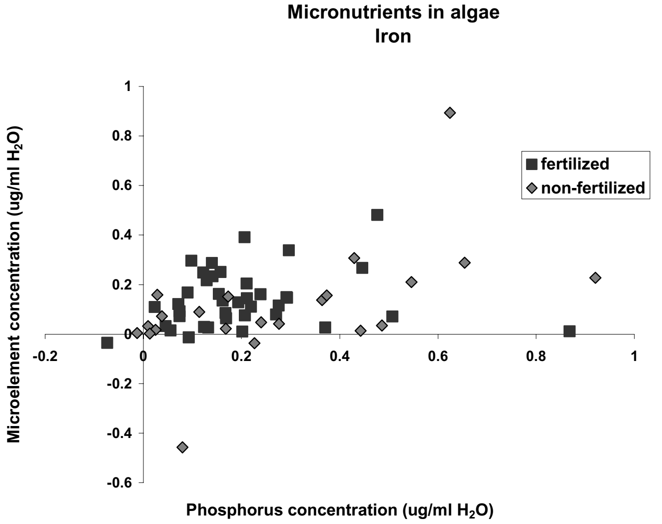

Figure 14. Concentrations of Iron relative to Phosphorus in algae collected from fertilized and non-fertilized evaporation basins.

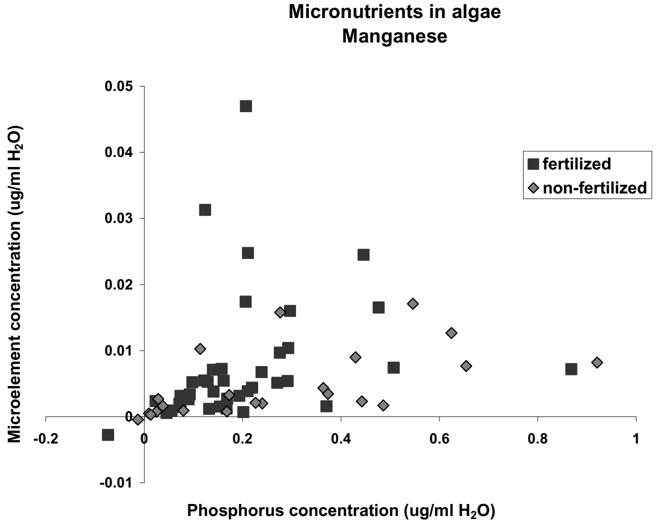

Figure 15. Concentrations of Manganese relative to Phosphorus in algae collected from fertilized and non-fertilized evaporation basins.

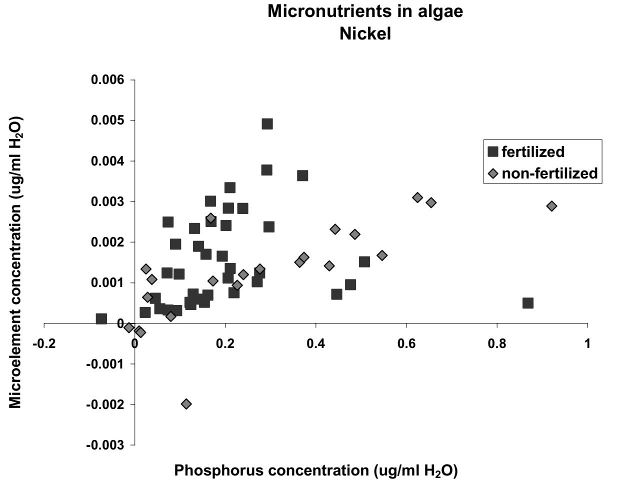

Figure 16. Concentrations of Nickel relative to Phosphorus in algae collected from fertilized and non-fertilized evaporation basins.

ing elements: phosphorus, calcium, magnesium, sulfur, silicon, boron, zinc, nickel, iron, potassium, sodium, copper, molybdenum, cobalt, strontium, lead, cadmium, beryllium, and cesium. The macronutrient phosphorus was used as the basis for comparing the variation in the content of other nutrient elements and toxic metals in algal biomass. Generally, iron, manganese, and nickel concentrations co-varied with phosphorus, as shown in Figures 14-16 respectively, and metals concentrations in algae were not affected by fertilization and brine shrimp harvest in the basins. For example, iron concentrations appear to be comparable in algae from harvested and un-harvested basins (Figure 14). However, manganese (Figure 15) and nickel (Figure 16) relative to phosphorus were elevated in algae of fertilized basins compared to un-fertilized ones . This could be due to the abundance of these metals in the poultry manure used as fertilizer. It could also result from a change in phosphorus utilization by algae in fertilized ponds . In any case, the elemental data collected in this study indicates that basin fertilization and brine shrimp harvesting do not create micronutrient deficiencies or toxic metal accumulation in algae.

SE VOLATILIZATION BY MICROALGAE

As in previous years, measurements of volatile Se of water collected at the upwind and downwind corners of the basins in both morning and afternoon were made on site during the annual field sampling trip (August 5, 2003). Figure 17 shows the volatile Se content of TLDD basin waters in two locations in the morning and at the downwind location in the afternoon in comparison with basin salinity. Volatile Se concentrations were generally greater at downwind locations than at upwind, with the exception of HAC A3. The diurnal pattern was less pronounced this year than previously, perhaps due to lower ambient temperatures. As seen previously, the highest volatile Se production took place in the most saline pond (SEB 8 or S8).

Figure 18 shows the average downwind volatile Se content of TLDD basin waters in comparison with the bioconcentration factor (BCF, on a dry wt basis) of Se by microalgae for the 2003 field measurement. The algal community in the basin with the highest Se volatilization (SEB 8) tended to accumulate the least amount of Se. This basin is only occasionally harvested and has not been fertilized since early 2000. It also had the highest average salinity for 2003. Basins SEB 9 and SEB 10, which were most heavily harvested, show mid-level Se volatilization and algal BCF, as does the un-harvested basin, SEB 1. Un-harvested basin HAC A3 shows low volatile Se but highest algal BCF, which has been repeatedly observed in less saline un-harvested basins in previous years . Moreover, SEB 9 has been consistently the most saline basin cell with highest volatile Se content in previous years but for 2003, it became less saline, which was accompanied by a lower volatile Se content. These results indicate that high salinity is a predominant factor in modulating Se volatilization.

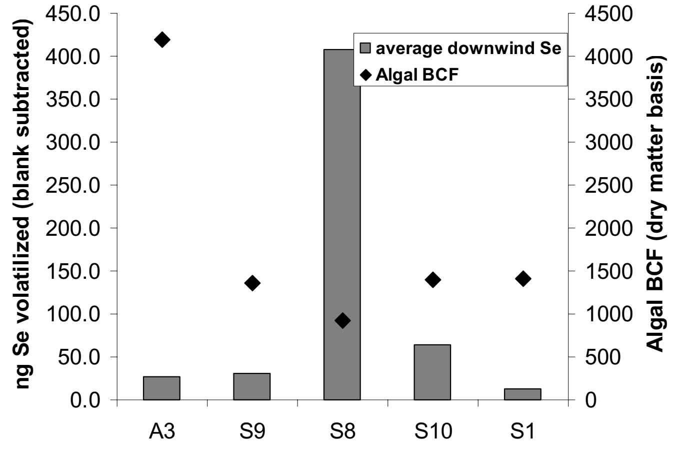

Figure 17. Volatile Se content of TLDD basin waters in two locations in the morning and at the downwind location in the afternoon in comparison with basin salinity.

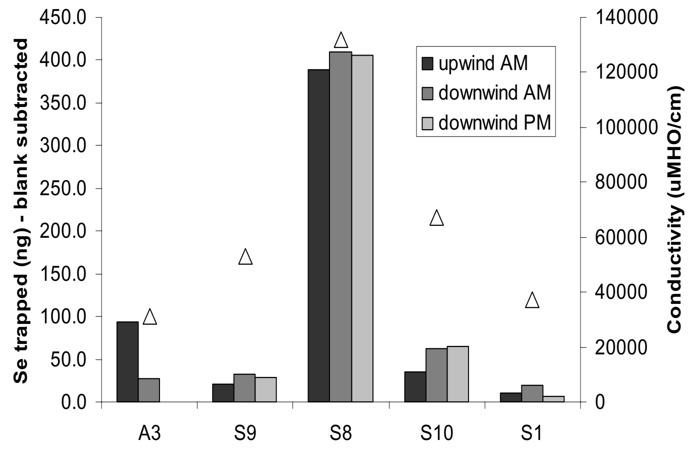

Figure 18. Average downwind volatile Se content of TLDD basin waters in comparison with the algal Se bioconcentration factor (BCF, on a dry wt basis).

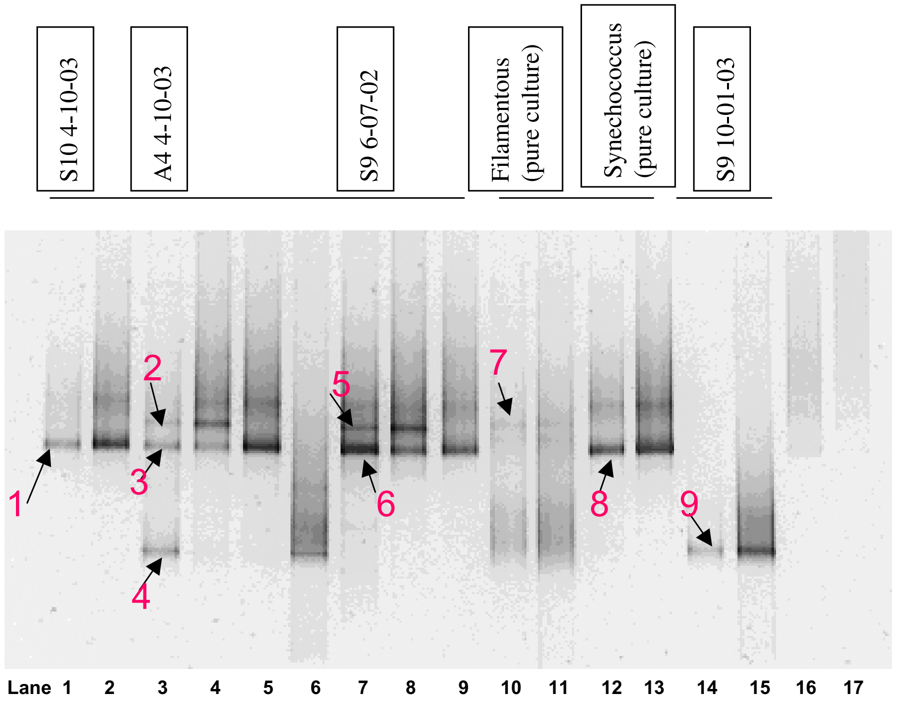

Figure 19. DGGE gel used for identification of algal strains through DNA sequencing. Various field and laboratory isolate samples are identified in lanes 1, 3, 7, 10, 12, and 14. Lanes immediately following each of these are further amplified samples of the numbered bands (e.g. band #1 in lane one was further amplified to produce bands in lane 2).

MICROALGAL COMMUNITY ANALYSIS

Monthly microalgal composite samples were extracted for total DNA, amplified with cyanobacterial 16S rDNA primers, and the resulting Polymerase Chain Reaction (PCR) products analyzed by Denaturing Gradient Gel Electrophoresis (DGGE). Figure 19 shows the DGGE gel patterns for environmental algae samples collected during 2003 from TLDD basins SEB 10, SEB 9, SEB 8, and HAC A4, along with two isolated monocultures. Our effort was to identify the dominant DGGE bands by DNA sequencing and thus reveal the microalgal community composition. Bands indicated with arrows (#1-9, Fig. 19) were cut, re-amplified, run on agarose gel, and purified with Quiagen kit before sending for sequencing. Based on sequencing comparison with the available 16S rRNA data base (Gene Bank and RDP) we identified the following algal species: Chlorella mirabilis (band # 1, 3, 6, and #8 –pure culture), Koliella spiculiformis (band # 2), Synechococcus sp. (band # 4), and Oscillatoria neglecta (# 7, pure culture filamentous alga). Band # 5, 9, and 10 are still under the process of sequence identification. Our previous observations of the gel patterns of algae collected during 2002-2003 were that a number of the samples had the same 2 gel bands (#2 and 3), now identified as the green algae Chlorella and Koliella sp. For example, the appearance of these 2 species for the HEB A4 samples seemed to relate to the strength of the harvest activity. The 2 green algae were present starting 4/4/02 and persisted until 7/19/02, after which they were not detected (cf. Fan's report, 2003). The opposite is true for the Synechococcus sp. (band # 4), which was not present during this intensive harvest period (4/4/02 to 7/19/02), but was very persistent after 7/19/02 and during the 2003 sampling. It is likely that the green algae Chlorella and Koliella sp. promote brine shrimp growth, while Synechococcus sp. was left behind by the brine shrimp. It should also be noted that Synechococcus sp. Is very active in Se volatilization (cf. Fan's report, 2003).

CONCLUSION:

The data acquired in 2003 indicate that waterborne Se, algal Se, and invertebrate Se did not increase as a result of increasing salinity, as repeatedly reported for previous years. In fact, the most saline cell (SEB 8) posted the lowest Se status . Also there is evidence that brine shrimp harvest reduced Se deposition to the benthic community while disrupting the food chain transfer of Se in harvested basins. There is some evidence for a change in the basin nutrient status based on the elemental composition of the microalgae, which could result from the combination of fertilization and brine shrimp harvest. Whether this leads to a change in microalgal community and/or micronutrient depletion accompanying the harvest operations awaits further investigations. Correlation of algal species composition with in situ Se volatilization activity revealed Synechococcus sp. as a candidate that underlies high Se volatilzation in hypersaline basin cells.

Removal of Selenium from Water by Zero-Valent Iron

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ABSTRACT

Zero-valent iron (ZVI) has been widely used in the removal of environmental contaminants from water. In this study, ZVI was used to remove selenate [Se(VI)] at a level of 1000 µg/L in the presence of varying concentrations of Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-, and PO_4^{3-}. Results showed that Se(VI) was rapidly removed during the corrosion of ZVI to Fe oxyhydroxides (Fe_{OH}). During 16 h of the experiments, 100 and 56% of the added Se(VI) was removed in 10 mM Cl^- and SO_4^{2-} solutions under a closed contained system, respectively. Under an open condition, 100 and 93% of the added Se(VI) were removed in the Cl^- and SO_4^{2-} solutions, respectively. Analysis of Se species in ZVI-Fe_{OH} revealed that selenite [Se(IV)] and nonextractable Se increased during the first 2-4 h of reaction, with a decrease of Se(VI) in the Cl^- experiment and no detection of Se(VI) in the SO_4^{2-} experiment. Two mechanisms can be attributed to the rapid removal of Se(VI) from the solutions. One is the reduction of Se(VI) to Se(IV), followed by rapid adsorption of Se(IV) to Fe_{OH}. The other is the adsorption of Se(VI) directly to Fe_{OH}, followed by its reduction to Se(IV). The results also show that there was little effect on Se(VI) removal in the presence of Cl^- (5, 50, and 100 mM), NO_3^- (1, 5 and 10 mM), SO_4^{2-} (5 mM), HCO_3^- (1 and 5 mM), and PO_4^{3-} (1 mM) and only a slight effect in the presence of SO_4^{2-} (50 and 100 mM), HCO_3^- (10 mM), and PO_4^{3-} (5 mM) during a 2 day experiment, whereas, 10 mM PO_4^{3-} significantly inhibited Se(VI) removal. This work suggests that ZVI may be an effective agent to remove Se from Se-contaminated water.

INTRODUCTION

Elevated selenium (Se) concentrations in agricultural drainage water have been found in many sites of California. In the San Joaquin Valley (SJV), California, Se in the drainage water is frequently at a concentration level of 140-1400 µg/L (Amweg et al., 2003; Cantafio et al., 1996; Sylvester, 1990). In the Salton Sea region, elevated Se is in the range of 3 to 300 µg/L in the subsurface drainwater (Setmire and Schroeder, 1998). In an effort to minimize environmental impacts of Se, the State of California Water Resources Control Board (SWRCB, 1989) has recommended an interim maximum mean monthly Se concentration of 2 to 5 µg/L in rivers and wetlands receiving agricultural drainage water. So far no treatment technology has proven economically feasible for meeting this criterion.

Several biotreatment systems have been used to treat Se-contaminated agricultural drainage water in the SJV. In a pilot-scale Se bioremediation system using a Se(VI) reducer, Thauera selenatis and acetate in liquid phase as an electron donor, Cantafio et al. (1996) reported that bacterial reduction of Se(VI) to elemental Se [Se(0)] proceeded rapidly in a series of four columns filled with Jaeger Tri-packs and /or silica sand. About 98% of Se(VI) and selenite [Se(IV)] in agricultural drainage water were reduced. However, high costs make it less feasible to use acetate as an electron donor and carbon source for bacteria to reduce Se(VI) to Se(0) during full-scale operation in field conditions.

By using economical organic carbon sources, an Algal-Bacterial Selenium Removal System (ABSRS) has been used as an economic way to remove large amounts of Se from drainage water (Lundquist et al., 1995). However, increase in concentration of the most bioavailable organic Se in the treated water creates greater bioavailability of Se to biota than that in the influent (drainage water) (Amweg et al., 2003), which could propose greater toxicological risk to biota if the treated water flows into nearby wetlands. Therefore, there is a need for alternative technique to remove Se from drainage water.

Zero-valent iron (ZVI) is an inexpensive and moderately strong reducing agent (Genin, et al, 1998). It is used as a catalyst for chemical synthesis in industrial applications (Campbell, 1988) and is capable of removing many common environmental contaminants, such as Cr(VI), U(VI), and NO_3^- (Alowitz and Scherer, 2002; Farrell et al., 1999; Huang et al., 1998; Powell et al., 1995; Qiu et al., 2000). The corrosion of ZVI is an electrochemical process during which iron is oxidized to soluble Fe^{2+}. Reaction of Fe^{2+} with OH^- forms $Fe(OH)_2$ that can be further oxidized to green rust I $[Fe_3(II)Fe(III)(OH)_8Cl]$, $([Fe_4(III)Fe_2(II)(OH)_{12}][CO_3 \cdot 2H_2O])$, green rust II $([Fe_4(III)Fe_3(II)(OH)_{12}][SO_4 \cdot 2H_2O])$, magnetite ($\alpha$-$Fe_3O_4$), lepidocrocite ($\gamma$-FeOOH), ferrihydrite $Fe(OH)_3$ and goethite (α-FeOOH) (Furukawa et al., 2002; Genin et al., 1998; Phillips et al., 2003).The green rust can also serve as a reducing agent to abiotically reduce Se(VI) to Se(IV) and Se(0) (Myneni et al., 1997; Refait et al., 2000). Ferrihydrite and goethite are also strong adsorbents that can be used to effectively remove Se(IV) from water (Balistrieri and Chao, 1987; 1990). Therefore, ZVI may be an inexpensive potential agent to remove Se(VI) from Se-contaminated water.

This study was conducted to determine the removal of Se(VI) by ZVI in varying concentrations of Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-, and PO_4^{3-}. The removal process was characterized in a series of batch experiments.

MATERIALS AND METHODS

Selenium standards used in this study included Se(IV) (selenite reference standard solution) purchased from Fisher Scientific (Pittsburgh, PA), Se(VI) (Na_2SeO_4) from Sigma (St. Louis, MO). Se(0) was obtained by the chemical reaction of Se(IV) with ascorbic acid (Sigma) (Combs. et al. 1996). After the reaction, a red Se(0) solution was passed through a 0.1 µm filter, followed by washing 6 times with deionized water to remove ascorbic acid and un-reacted Se(IV). Se(0) accumulated on the filter membrane was transferred to a 20 mL glass vial with deionized water and was then sonicated for 15 min prior to use. Other chemicals, such as sodium sulfate (Na_2SO_4), sodium chloride (NaCl), sodium hydroxide (NaOH), and sodium phosphate (NaH_2PO_4

and Na_2HPO_4) were purchased from Fisher Scientific. ZVI (40-60 mesh) was obtained from Peerless (Peerless Metal Powers and Abrasive, Detroit, MI) and was used as received. The surface area was 1.63 m^2/g.

Stability Test of Se Species in a PO_4^{3-} Extractant

Stability of Se species in extractants needed to be tested in order to correctly extract Se accumulated in ZVI-Fe oxyhydroxides (ZVI-Fe_{OH}) described below. In this study, the term ”Fe oxyhydroxides” is defined to represent all corrosion products of ZVI iron in NaCl and Na_2SO_4 solutions. Phosphate (PO_4^{3-}) and NaOH, the most commonly used extractants in Se speciation studies (Jackson and Miller, 2000), were selected for extracting Se species in ZVI-Fe_{OH}. The extractant solution containing 0.35 M of PO_4^{3-} with a pH range of 7-12.6 was used. In the test, 1.25 g of ZVI was added to each 40-mL glass vial, followed by 1 ml of 1 mM Cl^- and SO_4^{2-}. The vials were opened to the atmosphere and stood overnight allowing the formation of Fe_{OH} (yellowish-brown color) on the surface of some ZVI particles prior to the addition of the extractants. Afterwards, 24 mL of the extractant was added to each vial, followed by spiking with standard Se(VI), Se(IV), and Se(0) in different vials to a final concentration of 1000, 1000, 2600 µg/L, respectively. The vials were tightly capped and horizontally shaken in a gyrotory shaker for 2 and 5h at 180 rpm. Then, solution samples were collected for analysis of Se species after centrifugation for 14 min at 12000 rpm to precipitate ZVI-Fe_{OH}.

Characterization of Se(VI) removal

A series of time course experiments were conducted in the laboratory to determine the removal of Se(VI) by ZVI in a 10 mM Cl^- and a 10 mM SO_4^{2-} solution. These experiments were designed to examine whether Se(VI) can be removed from solution under natural conditions. Therefore, pH and Eh were not controlled during the experiments and dissolved O_2 in solution was not removed before the experiments. In a closed contained experiment, 25 ml of 10 mM Cl^- or SO_4^{2-} solution containing 1000 µg/L of Se(VI) was placed to 40-mL EPA glass vials, followed by 1.25 g of ZVI. The vials were tightly capped and placed horizontally in a gyrotory shaker for shaking at a speed of 180 rpm. The vials were removed from the shaker at the time of 0.125, 0.25, 0.5, 1, 2, 4, 7, and 16 h. Each sample was passed through a 0.2-µm membrane filter (Fisher Scientific) into another 40-ml glass vial for Se species, pH, and Eh analysis. ZVI-Fe_{OH} residue in the vials was washed two times with 5 mL of DI water and re-filtrated. The ZVI-Fe_{OH} on the filter membrane was replaced into the original vials for extraction of accumulated Se to ZVI-Fe_{OH} particles. During the extraction, 25 ml of 0.35 M PO_4^{3-}solution with a pH of 12.6 was added to each vial. The vials were tightly capped, placed horizontally in a gyrotory shaker, and shaken for 2 h at a speed of 180 rpm. Then, solution samples were collected for Se species analysis by centrifugation for 14 min at 12000 rpm to precipitate ZVI-Fe_{OH}. All of the experiments were run in triplicates at room temperature (21±1 °C).

In an open system experiment, 25 ml of 10 mM Cl^- or SO_4^{2-} solution containing 1000 µg/L of Se(VI) was placed in 50-mL Pyrex flasks, followed by 1.25 g of ZVI. The flasks were not capped and shaken in a gyrotory shaker at a speed of 180 rpm. The sample collection and extraction of accumulated Se to ZVI-Fe_{OH} were the same as the closed system experiment described above.

Effects of different anions on Se(VI) removal

Effects of different anions (Cl^-, SO_4^{2-}, HCO_3^-, PO_4^{3-}, and NO_3^-) on Se(VI) removal by ZVI were examined in a series of batch experiments under aerobic conditions. In these experiments, 150 ml of 1000 µg/L of Se(VI) containing one of the anions was placed in 150-mL Pyrex flasks, followed by 5 g of ZVI. These anions included a Cl^- and SO_4^{2-} level of 5, 50, and 100 mM, and a HCO_3^-, PO_4^{3-}, and NO_3^- range of 1, 5, and 10 mM. The flasks were not capped and shaken in a gyrotory shaker at a speed of 180 rpm. 1.5 mL of the samples was collected at the time of 0.5, 1, 2, 4, 7, 24, 31, and 48 h for Se species analysis by centrifugation for 14 min at 12000 rpm to precipitate ZVI-Fe_{OH}. pH and Eh in the samples were not determined. All of the experiments were run in triplicates at room temperature (21±1 °C). The rate constant of Se(VI) removal by ZVI in these experiments was calculated using a simple first order kinetics equation: $dSe(VI)/dt = -kSe(VI)$, where k is the rate constant of Se(VI) removal. Se data that were close to zero were not used in the calculation because Se(VI) in these samples was almost completely removed during the experiments.

Analysis

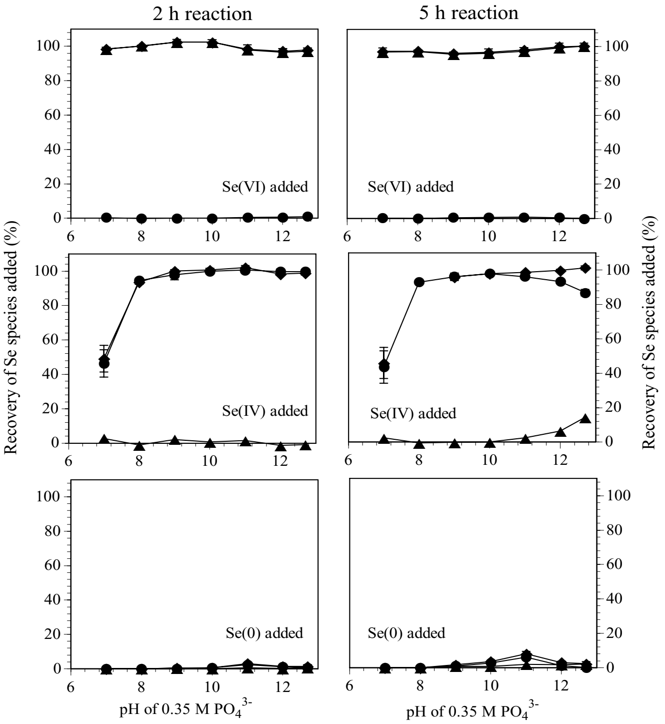
Redox potential and pH in the filtered samples were immediately measured after collection with a 720A pH/ISE meter (Thermo Orion, Beverly, MA). pH was measured using an Accumet pH combination electrode. The redox potential was measured with an Accumet combination platinum electrode (Ag/AgCl). The measured potential ($Eh_{measured}$) was converted to potential in the solution (Eh_{actual}) relative to a standard H electrode as $Eh_{actural} = Eh_{measured} + 224.4$ mV (Jayaweera and Biggar, 1996).

Only total Se and Se(IV) were monitored in the filtered samples and PO_4^{3-} extract because there were no organic materials and organic Se in the solution-ZVI-Fe oxyhydroxides system. Se(IV) in the samples was determined in a 6 N HCl solution. Total soluble Se in the samples was determined after a reduction of Se(VI) to Se(IV) in 6 N HCl (8.9 mL) added with 0.1 mL of 5% $K_2S_2O_8$ at 90 ºC for 15 min. Se concentrations in all prepared solutions were analyzed by hydride generation atomic absorption spectrometry (HGAAS) (Zhang et al., 1999a). Se(VI) concentration was calculated as the difference between total soluble Se concentration and Se(IV). Estimation of nonextractable Se was performed as the differences between the added Se and sum of the total soluble Se and total extractable Se in each sample.

RESULTS

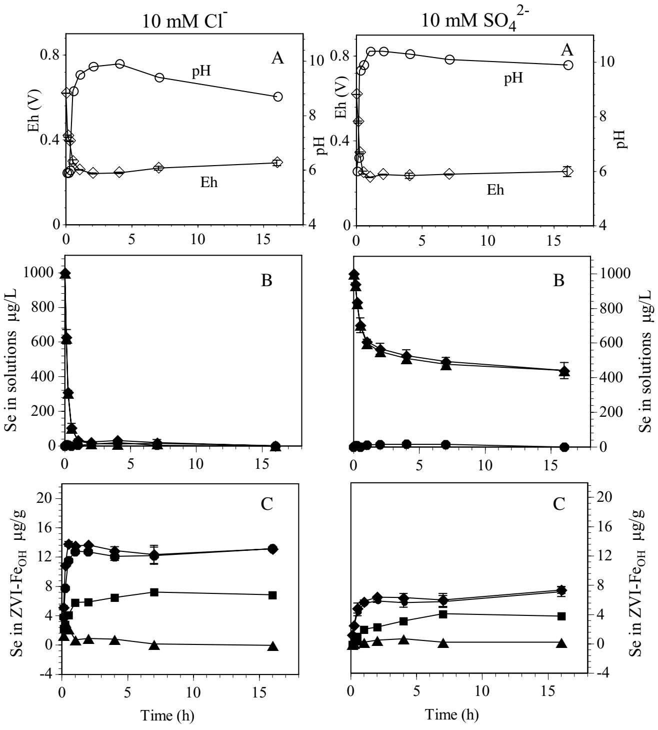
Stability of Se Species in the PO_4^{3-} Solutions with Different pH

Extraction effectiveness of adsorbed Se from soil constituents is commonly related to the composition and concentrations of extractants used (Jackson and Miller, 2000). Se(IV) adsorption results through its replacement of the surface hydroxyl groups and its adsorption decreases with increasing OH^- concentrations (as the pH increases). Phosphate is considered to have a similar affinity with Se(IV) binding on soil constituents (Balistrieri and Chao, 1987; Goldberg, 1985) and it can replace Se(IV) on adsorption sites. In this study, stability of Se species [Se(0), Se(VI) and Se(IV)] in a 0.35 M PO_4^{3-} solution with a pH range of 7 to 12.6 was tested in a ZVI-Fe_{OH} system (Fig.1). Se(VI) was stable in the PO_4^{3-} solutions during a 2 and 5 h reaction, indicating that Se(VI) was not removed by ZVI-Fe_{OH}. Se(IV) was also stable in the PO_4^{3-} solutions with a pH range of 9-12.6 during a 2h reaction. About 49 and 94% of the added Se(IV) were recovered in the pH 7 and pH 8 solutions, respectively. A similar reaction of Se(IV) was observed in the PO_4^{3-} solutions with a pH range of 7-11 during a 5h reaction. In the pH 12 and 12.6 solutions, 6 and 14% of the added Se(IV) were oxidized to Se(VI). Se(0) was relatively stable in the PO_4^{3-} solutions during a 2 h reaction with less than 3% of the added Se(0) being oxidized to Se(IV). Oxidation of the added Se(0) increased to 1.5-8% during a 5 h reaction. Considering stability of the Se species in the PO_4^{3-} solutions and high efficiency of the extraction of adsorbed Se species in a high pH solution (Jackson and Miller, 2000; Zhang et al., 1999b), the PO_4^{3-} solution with the highest pH (12.6) tested and a period of 2 h extraction were selected for extracting Se accumulated to ZVI-Fe_{OH} upon the removal of Se(VI) in the Cl^- and SO_4^{2-} solutions.



F : Total soluble Se; J : Se(IV); H: Se(VI)

Figure 1. Stability of Se species in a 0.35 M PO_4^{3-} solution containing ZVI-Fe_{OH}. Left figures: 2 h reaction and right figures: 5 h reaction. The pH range of PO_4^{3-} solution was 7 to 12.6. Error bars show one standard deviation (n=3).



F : Total Se; J : Se(IV); H: Se(VI); B : Nonextractable Se

Figure 2. Removal of Se(VI) in 10 mM Cl^- (left figures) and SO_4^{2-} (right figures) solutions by ZVI under a closed condition. A: Changes in pH and Eh during the removal of Se(VI). B: Removal of Se(VI) in the solutions. C: Se species in the ZVI-Fe_{OH} extracted by a 0.35 M PO_4^{3-} solution (pH 12.6). Error bars show one standard deviation (n=3).

Se(VI) Removal from 10 mM Cl^- and SO_4^{2-} Solutions

Removal of Se(VI) from a 10 mM Cl^- and SO_4^{2-} solution under a closed contained system by ZVI is present in the Fig. 2. Total soluble Se dropped rapidly from 1000 to 32 µg/L in the 10 mM Cl^- solution during the first h of the experiment. Se(VI) was the only dominant form of Se in the solution. pH increased rapidly from 5.9 to 9.5, with a rapid decrease of Eh from 0.62 to 0.26 V. Total soluble Se decreased to zero during rest of the experiment, with a slight change in pH and Eh. Total PO_4^{3-} extractable Se from ZVI-Fe_{OH} increased rapidly to 13.8 µg/g in the first half h reaction, and then stabilized at a range of 12.4-13.8 µg/g. Se(IV) was the major Se form, increasing from 3.83 to 12.8 µg/g at 0.125 to 1 h, respectively. Se(VI) was relatively low and had a peak of 3.16 µg/g at 0.25 h and then decreased to zero at 16 h.

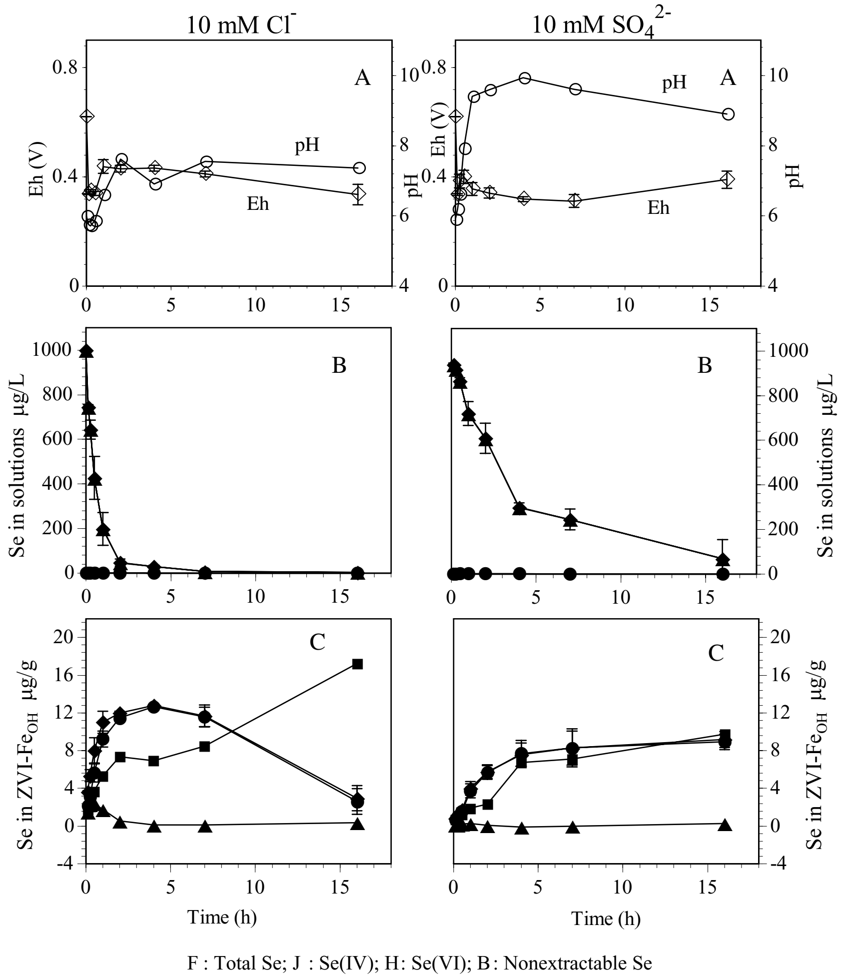


Figure 3. Removal of Se(VI) in 10 mM Cl$^-$ (left figures) and SO$_4^{2-}$ (right figures) solutions by ZVI under an open condition. A: Changes in pH and Eh during the removal of Se(VI). B: Removal of Se(VI) in the solutions. C: Se species in the ZVI-Fe$_{OH}$ extracted by a 0.35 M PO$_4^{3-}$ solution (pH 12.6). Error bars show one standard deviation (n=3).

Total soluble Se in a 10 mM SO$_4^{2-}$ solution decreased from 1000 to 610 µg/L, with a rapid increase in pH from 6 to 10.4 and a rapid decrease in Eh from 0.62 to 0.23 V during the first h of the experiment (Fig. 2). Afterwards, total soluble Se slightly decreased to 442 µg/L after 16, with a slight change in pH and Eh. Se(VI) was the major form of Se during 16 h of the experiment, accounting for 97 to 100% of total Se. Total PO$_4^{3-}$ extractable Se increased rapidly to 5.8 µg/g in the first h of the reaction, and then slightly increased to 7.37 µg/g at 16 h. Se(IV) also was the dominant form of Se in the extract, accounting 95-100%.

Under the open condition (Fig. 3), total soluble Se in the 10 mM Cl$^-$ solution decreased rapidly from 1000 to 46.9 µg/L, with an increase in pH from 5.7 to 7.6 and a drop of Eh from 0.62 to a range of 0.34-0.43 V during the first 2 h reaction. Total soluble Se dropped to 4 µg/L during rest of the experiment. pH and Eh stabilized at the levels of 6.9-7.6 and 0.34-0.43 mV, respectively. Se(VI) was the only Se species in the solution during 16 h of the experiment. Total PO$_4^{3-}$ extractable Se increased rapidly to 12.8 µg/g during the first 4 h of the experiment, and then decreased to 2.95 µg/g at 16 h. Se(IV) was the major form of Se in the extract, accounting for 59-99%. Se(VI) was relatively low, having a peak of 2.38 µg/g at 0.5 h.

Total soluble Se also dropped from 1000 to 68 µg/L in the 10 mM SO$_4^{2-}$ solution during 16 h of reaction (Fig. 3). Se(VI) was the only Se species in the solution. pH increased rapidly from 5.9 to 9.4 during the first h of the experiment and then remained at a level of 8.9-9.6. Eh decreased from 0.62 to a range of 0.31-0.4 V. Total PO$_4^{3-}$ extractable Se increased from 0.81 to 9.18 µg/g at 0.125 h to the end of the experiment. Se(IV) was the only Se species in the extract.

EFFECTS OF ANIONS ON SE(VI) REMOVAL

Effect of Cl$^-$, SO$_4^{2-}$, HCO$_3^-$, PO$_4^{3-}$, and NO$_3^-$ on Se(VI) removal by ZVI under aerobic conditions is illustrated in Figs. 4 and 5. Only Se(VI) (total Se) is presented in Figs. 4 and 5 because no measurable Se(IV) was detected in the samples. Se(VI) in the all of the solutions decreased during the experiments. More than 99% of the added Se(VI) was removed in the Cl$^-$ solutions (5, 50, and 100 mM) (Fig. 4 and Table 1). In contrast, 99, 87.4, and 79% of the added Se(VI) was removed, respectively in the 5, 50, and 100 mM SO$_4^{2-}$ solutions.

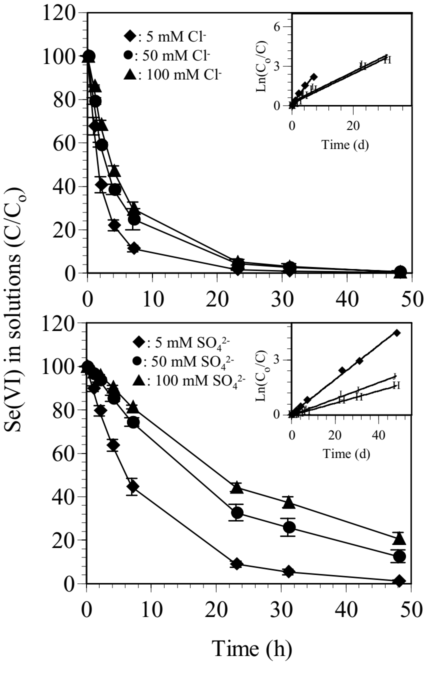


Figure 4. Effect of Cl$^-$ and SO$_4^{2-}$ concentrations on the removal of Se(VI) by ZVI. Error bars show one standard deviation (n=3). Inserted small figures show the first order kinetics of Se(VI) removal.

Table 1. Rate constant (k, h^{-1}) of Se(VI) removal by ZVI in the presence of Cl^- or SO_4^{2-} (See figure 4).

Anions	5 mM	50 mM	100 mM
Cl^-	0.309	0.115	0.113
SO_4^{2-}	0.093	0.043	0.033

Table 2. Rate constant (k, h^{-1}) of Se(VI) removal by ZVI in the presence of HCO_3^-, PO_4^{3-}, and NO_3^- (See figure 5).

Anions	1 mM	5 mM	10 mM
HCO_3^-	0.183	0.100	0.043
PO_4^{3-}	0.204	0.037	0.011
NO_3^-	0.463	0.296	0.255

In a range of 1, 5, and 10 mM of NO_3^-, removal of Se(VI) was much faster than that of HCO_3^- solutions, which was faster than that in the PO_4^{3-} solutions (Fig. 5 and Table 2). Se(VI) was almost completely removed in the 1, 5, and 10 mM NO_3^-, 1 mM HCO_3^- and 1 mM PO_4^{3-} solutions during the first 24 h. Approximately 99, 82., 88, and 43% removal of Se(VI) occurred in the 5 and 10 mM HCO_3^- and PO_4^{3-} solutions at the end of the experiment, respectively.

DISCUSSION

Zero-valent iron has been used to treat Cr(VI), NO_3^-, and U(VI)-contaminated waters as a reducing agent (Alowitz and Scherer, 2002; Farrell et al., 1999; Huang et al., 1998; Powell et al., 1995; Qiu et al., 2000). This study revealed that ZVI is also capable of removing Se(VI) from 10 mM Cl^- and SO_4^{2-} solutions. After a short period of time (16 h), the added Se(VI) was completely removed from the Cl^- solution, and 56 and 93% of the added Se(VI) was removed in the SO_4^{2-} solution under a closed contained and open condition, respectively.

The major mechanism for the rapid removal of Se(VI) by ZVI and $\mathrm{Fe}_{\mathrm{OH}}$ can be attributed to the reduction of Se(VI) to Se(IV) by Fe(II) oxidized from ZVI (Murphy, 1988), followed by rapid adsorption of Se(IV) to $\mathrm{Fe}_{\mathrm{OH}}$ due to lower redox potentials of Fe(III)/Fe(II) than that of Se(VI)/Se(IV) (Genin, et al, 1998) and stronger adsorption of Se(IV) to $\mathrm{Fe}_{\mathrm{OH}}$ than Se(VI) (Balistrieri and Chao, 1987; 1990). Zingaro et al. (1997) reported that 99% of added Se(VI) was rapidly reduced after 7 h reaction with Fe(II) at an initial Fe(II)/Se(VI) molar ratio of 9. Manning and Burau (1995) extracted both Se(VI) and Se(IV) from Fe precipitates after a reaction of Fe(II) with Se(VI) in 0.1 M NaClO_4 solution. In this study, little Se(IV) was found in both 10 mM Cl^- and SO_4^{2-} solutions during the removal of Se(VI), revealing that Se(IV) formed from the reduction Se(VI) to Se(IV) by ZVI and Fe(II) was rapidly adsorbed to $\mathrm{Fe}_{\mathrm{OH}}$. Analysis of Se species in the PO_4^{3-} extract shown that Se(IV) increased rapidly in the $\mathrm{Fe}_{\mathrm{OH}}$, with a rapid decrease of Se(VI) in the Cl^- and SO_4^{2-} solutions.

Another mechanism that can not be ruled out for the removal of Se(VI) might be the direct adsorption of Se(VI) to $\mathrm{Fe}_{\mathrm{OH}}$, followed by the reduction of adsorbed Se(VI) to Se(IV). Several studies have reported that Se(VI) can be adsorbed to $\mathrm{Fe}_{\mathrm{OH}}$ via surface complexes of outer-sphere layer or inner-sphere layer, and or both layers (Hayes et al., 1987; Manceau and Charlet, 1994; Su and Suarez, 2000). Refait et al. (2000) reported that SO_4^{2-} in green rust II [GR(SO_4^{2-})], one of the Fe corrosion products, can be replaced by SeO_4^{2-} to form GR(SeO_4^{2-}) and Se(VI) can be reduced to Se(IV) after the replacement. In a study on abiotic reduction of Se(VI) by green rust using X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopy, Myneni et al. (1997) reported that Se(VI) can be incorporated into the interlayers of green rust to form bidentate binuclear and edge-sharing complex with structural Fe(II) and reduced immediately to Se(IV). In this study, we visually observed that greenish $\mathrm{Fe}_{\mathrm{OH}}$ on the filter membranes after filtration at initial period of the experiments, showing the existence of green rust I in the Cl^- solution and green rust II in the SO_4^{2-} solution. Analysis of Se species in the ZVI and $\mathrm{Fe}_{\mathrm{OH}}$ revealed that extracted Se(VI) from ZVI-$\mathrm{Fe}_{\mathrm{OH}}$ accounted for as high as 30-40% of total extractable Se during first 0.5 h of the Cl^- experiment and then decreased rapidly to zero, providing evidence that Se(VI) was adsorbed to $\mathrm{Fe}_{\mathrm{OH}}$. These studies indicate that Se(VI) can be reduced rapidly to Se(IV) when it is adsorbed to $\mathrm{Fe}_{\mathrm{OH}}$ (e.g. green rust I and II).

Further reduction of Se(IV) to Se(0) might occur in the $\mathrm{Fe}_{\mathrm{OH}}$. Calculation of Se mass showed that nonextractable Se increased with time. At the end of the experiment, nonextractable Se was 17.3 and 9.75 µg/g in the Cl^- and SO_4^{2-} experiments under an open condition, respectively. Under a closed contained system, it was 6.86 and 3.78 µg/g in the Cl^- and SO_4^{2-} experiments, respectively. By using X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopy, Myneni et al. (1997) detected that Se(0) existed in the green rust after a reduction of Se(VI). Using X-ray absorption near edge structure (XANES), Roberson (1999) also reported that Se(IV) and Se(0) was observed in $\mathrm{Fe}_{\mathrm{OH}}$ during a study on the removal of Se(VI) by ZVI. After a reaction of Se(VI) with Fe(II), Zingaro et al. (1997) identified Se(0) in the Fe precipitates using XPS and TEM-EDS analysis. These results may suggest that nonextractable Se in the present study might partly be a form of Se(0), which cannot be extracted by a basic PO_4^{3-} extractant in a short period of the time (2 h). The increase of nonextractable Se may reveal a further reduction of Se(IV) to Se(0). Part of nonextractable Se might be attributed to the formation Fe(II)-

Se(IV) precipitates [$Fe_2(SeO_3)_3$ and $Fe_2(OH)_4SeO_3$] in the both Cl^- and SO_4^{2-} solutions (Elrashid et al., 1987).

Experimental conditions affect the removal of Se(VI) by ZVI. Under a closed contained system, ZVI in the vials can be reciprocally moved during shaking due to horizontal placement of the vials in a shaker. Rapid reaction of ZVI-Fe_{OH} with Se(VI) resulted in a removal of 97 and 40% of the added Se(VI) during the first h of the experiment in 10 mM Cl^- and SO_4^{2-} solutions, respectively. Removal of Se(VI) was slow in the SO_4^{2-} solution during rest of the experiment. One possible reason is that ZVI was aerobically oxidized rapidly to Fe(II) ($2Fe^o + 2H_2O + O_2 = 2Fe^{2+} + 4OH^-$) at an initial period of time when dissolved O_2 and air existed in the closed contained vials. After consuming O_2, anaerobic reactions of ZVI took place ($Fe^o + 2H_2O = Fe^{2+} + 2OH^- + H_2$), which produced H_2. Accumulation of H_2 limited continuous corrosion of ZVI under a closed contained system, thus slowing the production of Fe_{OH} used for the removal of Se(VI). Under an open condition, ZVI was not mobilized at the bottom of the flasks. Relatively slow corrosion of ZVI resulted in relatively slower removal of Se(VI) at an initial period of the experiment than that under the closed contained condition. However, continuous corrosion of ZVI by dissolved O_2 removed all added Se(VI) in the Cl^- solution at 7 h, and removed greater amounts of Se(VI) in the SO_4^{2-} solution at end of the experiment than that in the closed contained system. Improvement of Se removal from aqueous solution in the presence of dissolved O_2 by ZVI can also be found in Murphy's (1988) study, in which he reported that Se(VI) removal was much greater under open conditions than that under a condition of purging with argon. Recently, Meng et al (2002) reported that >97% of $SeCN^-$ was removed from water by ZVI in the presence of dissolved O_2, whereas only 8% of $SeCN^-$ was removed in water purged with N_2 after 7h.

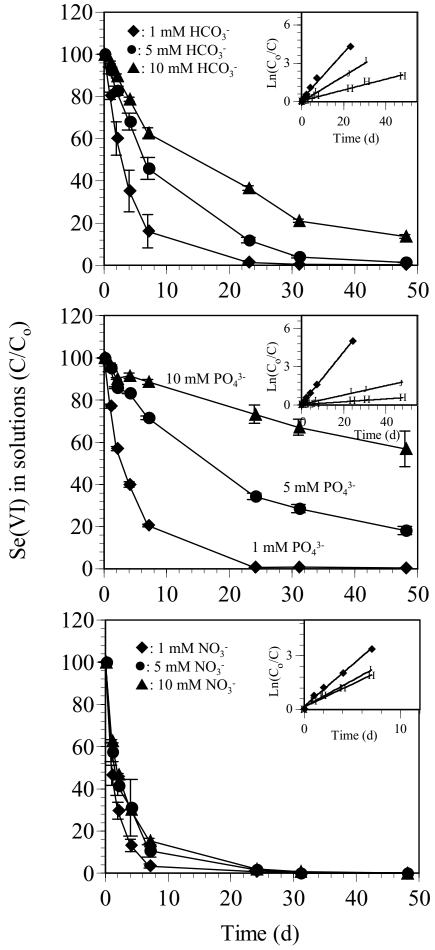


Figure 5. Effect of HCO_3^-, PO_4^{3-}, and NO_3^- concentrations on the removal of Se(VI) by ZVI. Error bars show one standard deviation (n=3). Inserted small figures show the first order kinetics of Se(VI) removal.

Relatively slower removal of Se(VI) in the SO_4^{2-} solutions than in the Cl^- solutions can be partially attributed to the similarities in the chemical properties of SO_4^{2-} and Se(VI). In this study, SO_4^{2-} was much greater than Se(VI). High SO_4^{2-} concentrations can compete for adsorption sites on Fe_{OH}, thus delaying and limiting the removal of Se(VI). Relatively slow removal of Se(VI) in the SO_4^{2-} solutions might also be partially attributed to rapid corrosion of ZVI in the SO_4^{2-} solutions (Hunt, 2000), resulting in a higher pH. This higher pH in the SO_4^{2-} solution can decrease Se adsorption, causing a relatively slow removal of Se(VI).

Higher PO_4^{3-} levels significantly affected the removal of Se(VI) by ZVI-Fe_{OH}. In comparison to Se(VI) removal in the 5 mM Cl^- solution, 1 mM PO_4^{3-} only had a slight effect on the removal of Se(VI). Increases in the concentrations of PO_4^{3-} to 5 and 10 mM significantly reduced Se(VI) removal, with low k values of 0.037 and 0.011 h^{-1}, respectively. This decrease of Se(VI) removal might be attributed to the reaction of Fe(II) with PO_4^{3-}. The formation of Fe-PO_4^{3-} minerals [e.g. $Fe_3(PO_4)_2(H_2O)_8$, vivianite] consumes Fe(II) used for reducing Se(VI), resulting in a lower removal of Se(VI). The presence of PO_4^{3-} can also compete for adsorption sites of Fe_{OH}, thus reducing adsorption of Se (Balistrieri and Chao,1987; Goldberg, 1985).

Higher levels of HCO_3^- can also reduce the removal of Se(VI) in HCO_3^-/CO_3^{2-}-ZVI-Fe_{OH}. Reaction of Fe(II) with CO_3^{2-} can form $FeCO_3$ (siderite) that is often found in ZVI barriers used for removing environmental contaminants from ground water (Phillips, et al, 2003), and reduce the amounts of Fe(II) available for reducing Se(VI). In this study, increasing the concentrations of HCO_3^- from 1 to 10 mM led to a 18% decrease of Se(VI) removal in ZVI-Fe_{OH} system.

Nitrate is commonly considered as a competitive electron acceptor in bacterial reduction of Se(VI) to Se(0) because the redox potential of NO_3^-/N_2 in an aquatic system is very similar to that of Se(VI)/Se(IV) and much higher than Se(IV)/Se(0) (Masscheleyn and Patrick, 1993). However, this study reveals that NO_3^- in a range of 1 to 10 mM had little effect on the removal of Se(VI) by ZVI-Fe_{OH} in the Se(VI)-NO_3^--ZVI system. Like the 5 mM Cl^- solution, about 100% of the added Se(VI) was removed from solution with a NO_3^- range of 1, 5, and 10 mM during the first 24 h of reaction. The most likely reason is that the major reduction of Se(VI) to Se(IV) was caused by Fe(II) so that the removal of Se(VI) is controlled by oxidation of ZVI to Fe(II) by dissolved O_2 under an open condition. The presence of NO_3^- did not significantly affect oxidation of ZVI to Fe(II) by dissolved O_2, thus resulting in little effect of NO_3^- on Se(VI) removal by ZVI.

CONCLUSIONS

Results from this study reveal that Se(VI) can be removed from aqueous solutions with varying concentrations of Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-, and PO_4^{3-}. During 16 h of the experiment, 100 and 56% of the added Se(VI) was removed in 10 mM Cl^- and SO_4^{2-} solutions under a closed contained system, respectively. Under an open condition, 100 and 93% of the added Se(VI) were removed in the Cl^- and SO_4^{2-} solutions, respectively. The experiments also showed that there was little effect of Cl^- (5, 50, and 100 mM), SO_4^{2-} (5 and 50 mM), NO_3^- (1, 5 and 10 mM), HCO_3^- (1 and 5 mM), and PO_4^{3-} (1 mM) and a slight effect of SO_4^{2-} (100 mM), HCO_3^- (10 mM), and PO_4^{3-} (5 mM) on Se(VI) removal. Only higher PO_4^{3-} (10 mM) levels significantly inhibited Se(VI) removal by ZVI. In California, Se-contaminated drainage water contains an average concentration of 987 (Cl^-), 2282 (SO_4^{2-}), 97 (NO_3^-), 214 (HCO_3^-), and 0.12 mg/L (PO_4^{3-}) (Oswald et al., 1989), which are much lower levels than those tested in our study. Our work suggests that ZVI may be an inexpensive agent that can be used to treat Se-contaminated drainage water.

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Does Saline Drainage Water Affect Crop Tolerance to Boron?

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ABSTRACT

A potential limitation in implementing a drainage water reuse system is determining the extent by which boron, a naturally occurring element in the drainage water, affects the selection, growth and yield of crops in the reuse system.

Boron is a concern for several reasons. First, boron is an element that is essential for crops but has a small concentration window between that what is considered deficient and that which is potentially toxic. Second, it has a higher affinity to the soil than common salts requiring much more water to reclaim soil B to pre-existing levels than it does to reduce the salinity to pre-salinization levels. Furthermore, the B concentration in San Joaquin Valley drainage water varies widely but in nearly all cases, it far exceeds levels that would result in toxic conditions based on B-tolerance guidelines.

Unlike guidelines for salt tolerance, the guidelines for boron tolerance are more limited. With the exception of a few sand tank studies that actually provide B coefficients (i.e. threshold and slope) for a few crops (see Maas and Grattan, 1999), most of the B classification has come from work conducted over a half a century ago by Eaton et al. (1944). More importantly, these older studies defined the B-tolerance limit based on the development of incipient injury on the crop (i.e. foliar burn), not yield response under a range of B concentrations.

The question is often raised, are the effects of salinity and boron on crops additive, synergistic or antagonistic? Despite the common occurrence of high boron and high salinity in many parts of the world, very little research has been done to study the interaction of the two. For those that have been done, contradictory results have been obtained. Greenhouse studies are underway to evaluate B tolerance particularly in relation to salinity. Our goal is to answer this question. Should the answer be that the effects of the two are antagonistic, B may not be as limiting a factor in reuse systems than previously thought.

A comprehensive greenhouse experiment was conducted in 2003 at the US Salinity Laboratory in Riverside to evaluate the interactions between B and saline drainage water on the performance of broccoli and to get a better indication how limiting B really is to plants grown in drainage reuse systems. Particular interest in this study was directed towards the composition of the salinizing solution to determine what role various salts have on the salinity-boron interaction. Results from this study indicate that both Cl-based salts and those characteristic of shallow saline drainage water (i.e. a mixture of salts dominated by sodium sulfate) showed a significant salinity-boron interaction. That is at high salinity, increased B concentration was less detrimental, both visually and quantitatively (i.e. biomass), than it was at low salinity. That is, plants could tolerate a higher solution B-concentration at higher salinity. However there was no significant difference between salt types. The effects on head weights were more exaggerated than those on shoot biomass. Therefore these data indicate that salinity and B are antagonistic.

Extensive ion analyses have been conducted over the past 9 months to evaluate the influence of salinity on ion uptake and distribution within broccoli shoots. To date, ion concentration data are only complete from plant samples taken on April 24th. This mid-season vegetative-harvest included whole shoot samples as well as leaf blade, leaf margins and petioles. Shoot B concentration was influenced by salinity, but interestingly the direction of influence was dependent upon the B concentration in the solution. Regardless of the composition of the salinizing solution, increased salinity increased shoot B concentration when B concentrations in the solution were relatively low (i.e. 0.5 mg/L). At the highest solution B concentration (28 mg/L), increased salinity reduced shoot B concentration. Solution B in itself had very little influence on shoot ion accumulation but both salinity (i.e. EC) and salinity composition had very strong influences on shoot tissue ion composition. A detailed summary is provided in the results along with a discussion on the distribution of ions within the shoot.

Cumulative water use of broccoli was evaluated in relation to the various treatments. Cumulative ET was, for the most part, directly related to cumulative biomass; the higher the cumulative biomass the higher the cumulative ET. Stable isotopic ratios of oxygen in the solution were used to separate evaporation and transpiration. With these estimates, we were able to provide insight into whether B uptake is truly passive with the transpiration stream as many have suggested in the literature or whether the plant is able to regulate the amount of B absorbs and transports to the shoot. In no treatment did shoot B accumulate to a level predicted based on transpiration volume times solution B-concentration. Plants treated with low B contained the largest percent of B uptake (10-60%), expressed relative to predicted passive uptake. Salinity treatments, regardless of composition, represented the higher percentage range. On the other hand, plants treated with high B (14 or 28 mg/L) only accumulated 1-2% of that predicted if uptake and accumulation were truly passive. Therefore based on our data, it appears that broccoli can reduce that amount of B it absorbs and/or transports to the shoot at high solution B concentrations.

Further experiments are needed using crops (e.g. cucumber and wheat) that have been known to show enhanced sensitivity to B in the presence of Cl salinity. Only after these experiments are conducted and the data analyzed and interpreted can one determine whether SJV salts reduces boron’s toxic effect to crops.

INTRODUCTION

Reuse of saline drainage water is a management option on the west side of the San Joaquin Valley (SJV) that is necessary for reducing the volume of drainage water (San Joaquin Valley Drainage Implementation Program, 2000). Several methods of utilizing saline water (i.e. sequential, cyclic and blending) have been tested experimentally or demonstrated under field conditions. In addition to these methods of reuse, saline water table control has also been tested as a means of allowing certain agronomic crops (such as cotton and safflower) to extract water directly from this saturated zone. Regardless of how crops utilize this saline drainage water, crop roots are exposed to water containing both high concentrations of sulfate and chloride salts as well as high concentrations of boron.

There is considerable controversy over the extent by which boron limits the reuse of SJV drainage water. On the one hand, there is concern that boron is one of the most limiting factors in the long-term success of drainage water reuse systems. The concern stems from several relationships. First, there is a small concentration window between the level in the soil that is required for optimal crop growth and that considered toxic (Gupta et al., 1985). Second, the boron concentrations in drainage water exceed the published boron tolerance coefficients for most crops grown in the San Joaquin Valley, despite the fact that many are classified as moderately B-tolerant to B-tolerant (Maas and Grattan, 1999). Third, boron is adsorbed tightly to the soil and therefore is not as readily leached from the crop rootzone as the other salts are. This phenomenon provides the opportunity for boron to accumulate in the root zone more rapidly than salinity, eventually affecting crop selection and ultimately having a negative effect on crop growth and yield. Because of these concerns, it has long been thought that B is a much more limiting factor in drainage water reuse than is the salinity of the drainage water.

On the other hand, some argue that the boron coefficients might be too conservative (e.g. Letey et al., 2001). Most of the coefficients are based on the concentration of B in the soil water that produces incipient injury and are not based on yield reduction criteria (i.e. yield reduction as a function of increased B in the soil solution). Moreover, these coefficients were developed in non-saline environments suggesting that they may not be appropriate for crops grown under saline conditions.

Toxicity occurs in crops when boron concentrations increase in either stem and leaf tissues to lethal levels, but soil and plant-tissue analyses can only be used as general guidelines for assessing the risk of B-toxicity (Nable et al., 1997). Although experimental evidence indicates that plants absorb B passively as H_3BO_3, contradictions between experimental results and observations in the field suggest that other factors, yet unknown, may affect B uptake (Hu and Brown, 1997). Once B has accumulated in a particular organ within the shoot, it has restricted mobility in most plant species but not all (Brown and Shelp, 1997). In some plant species, particularly those that produce substantial amounts of polyols, B is readily translocated as B-polyol complexes.

The question has recently been raised, are the effects of salinity and boron on crops additive, synergistic, or antagonistic? Despite the common occurrence of high boron and high salinity in many parts of the world, very little research has been done to study the interaction of the two (Grattan and Grieve, 1999). From a review of the limited number of studies that addressed the combined effects of salinity and boron on the plant, it appears that the results are contradictory.

In sand-culture experiments conducted in a greenhouse, researchers found that wheat responded to boron in the soil solution independently of salinity, made up of sodium chloride (NaCl) and calcium chloride ($CaCl_2$) salts (Bingham et al., 1987). That is, there was no salinity - B interaction with respect to leaf B concentration. Similarly, others have found that boron and salinity effects were independent of each other for corn, barley and alfalfa (Shani and Hanks, 1993 and Mikkelsen et al., 1988).

However in more recent studies, investigators found that Cl-based salinity enhanced B sensitivity in wheat (Grieve and Poss, 2000; Lauchli et al. 2001; Wimmer et al., 2003). Wheat is one of those crops that are tolerant to salinity but sensitive to B. Grieve and Poss (2000) found that Cl-salinity increased B accumulation in leaves and was associated with more injury. Wimmer et al., (2003) attribute its effect on B compartmentalization within the plant. They found that under saline conditions, total B concentration was reduced in the root, was unaffected in the basal portion of the leaf, and increased in the leaf tip. Therefore salinity enhanced B mobility within the plant.

In a recent greenhouse study using soil in pots, investigators found that NaCl salinity increased B sensitivity in tomato and cucumber (Alpaslan and Gunes, 2001). However they found that salinity reduced B concentration in tomato but increased it in cucumber. These results question the hypothesis that B is taken up passively by plants via the transpiration stream. Furthermore, these investigators found that NaCl increased membrane permeability but increasing B in the soil to toxic levels did not, except in the presence of salinity.

On the other hand, investigators who used a mixture of salts (i.e. Na^+, Ca^{2+}, Cl^- and SO_4^{2-}) found the opposite effect. In one field study conducted in Northern Chile, a number of vegetable crop species and prickly pear cactus were irrigated with saline water (8.2 dS/m) containing a mixture of ions including 17 mg/L of boron (Ferreyra et al., 1997). Plant growth and crop yields of artichoke, asparagus, broad bean, red and sugar beets, Swiss chard, carrot, celery, a local variety of sweet corn, potato, prickly pear cactus, onion, shallot, spinach, were all greater than expected based on published salt and boron tolerance coefficients. These investigators found that salinity reduced leaf boron levels. If separate effects of salinity and boron are additive, little or no growth would be expected for any of these crops. Interactions likely occur which increase the crop's tolerance for boron in the presence of saline conditions. The investigators suggested that a reduction in plant water uptake, due to higher salinity levels, would reduce the rate boron accumulation in the plant tissue thereby extending the time during which boron levels are not affecting plant growth.

Others also found that salinity, using a mixture of salts, reduced leaf B concentration of chickpea (Yadav et al., 1989), wheat (Holloway and Alston, 1992) as well as reduced B uptake and accumulation in the stem of several Prunus rootstocks (El-Motaium et al., 1994), thereby decreasing B-toxicity symptoms. In the latter study, the investigators found a negative relationship between B and SO_4^{2-} concentrations in tissue suggesting that SO_4^{2-} could be responsible for the salinity-induced reduction in tissue B. Others have also found that a mixture of chloride and sulfate salinity reduces leaf injury in Eucalyptus camaldulensis (Grattan et al., 1996) by reducing tissue B concentrations and in pistachio (Ferguson et al. 2002) by some unknown mechanism. Studies that include a mixture of salts (i.e Na^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}) are much more appropriate for conditions of the San Joaquin Valley as well as a number of coastal valleys than those using chloride salts alone.

In no study, however, were investigators able to suggest the actual mechanism that supports this phenomenon such as direct ion interactions, reduced transpiration in salt-stressed conditions or both. Consequently, many questions regarding the interactions between salinity and boron remain unresolved. Questions related to (1) the relationship between visual leaf symptoms and yield; (2) the dynamic relationships between boron concentration in irrigation water, adsorption of boron, boron uptake and distribution within the plant; (3) the influence of salinity, both concentration and composition, on boron tolerance of the crop; and (4) whether boron damage will ever exceed salinity damage when using saline drainage water.

The most important information that is needed is to have boron tolerance coefficients related to yield rather than visual symptoms, and that the boron tolerance coefficients be evaluated as a function of salinity (i.e. saline drainage water).

We are currently conducting experiments in sand cultures to address these questions. Sand-culture facilities are useful because salt concentrations and compositions can readily be controlled creating uniform profiles such that true comparisons can be made among the treatments. An interdisciplinary research project involving scientists from the University of California and the USDA-ARS with expertise in soils and irrigation management, plant physiology, salinity and plant nutrition are currently conducting experiments to evaluate salinity – B interactions and to develop crop response functions to B in both the presence and absence of salinity.

MATERIALS AND METHODS

A greenhouse experiment was conducted at the USDA-ARS, George E. Brown, Jr. Salinity Laboratory located at the UC Riverside campus. The experiment was designed to determine the interactive effects of salinity and boron on broccoli performance including growth, yield, injury, and ion relations. Broccoli (Brassica oleracea L., botrytis group, cv Seminis PX511018) was selected because it is a crop common to the Westside of the SJV and is known to be moderately sensitive to salinity and moderately sensitive to B in non-saline systems.

The greenhouse experiment was conducted using an elaborate sand tank system. This system creates a uniform and controlled environment and consists of 60 large tanks (1.2 m x 0.6 m x 0.5 m deep) filled with washed sand having an average bulk density of 1.2 Mg m^{-3}. At saturation, the sand has an average volumetric water content of 0.34 $m^3\,m^{-3}$. Each tank is irrigated with a solution prepared in individual reservoirs having a volume of approximately 890 L. Salinity-B treatments were complemented with modified half-strength Hoagland's nutrient solution. Solutions were pumped from reservoirs, located below the sand-tank facility, to the tanks and then returned to the reservoirs through a subsurface drainage system at the bottom of each sand tank. Each reservoir irrigated three replicate tanks. Irrigation frequencies (i.e. several times per day) were sufficient to allow the sand-water concentration to approach that in the irrigation water, thereby creating a uniform distribution of salt in the crop rootzone. Calculations indicate that the salinity of the irrigation water was more or less equivalent to that of the sand water and previous studies (Wang, 2002) have indicated that the EC of the sand water is approximately 2.2 times the EC of the saturated soil extract (ECe), the salinity parameter used to characterize salt-tolerance. Total evapotranspiration from each tank was measured by solution-volume changes in the storage reservoirs and water lost was replenished to maintain constant osmotic potentials in the treatment irrigation waters.

Several sand tanks were not planted but irrigated to provide maximum evaporation estimates. These tanks were also used as a reference for stable isotope analyses in order to separate evaporation from transpiration in the planted tanks using an approach described in a previous report (Grattan et al. 1998). The overall principle is that enrichment in the delta ^{18}O in the reservoirs occurs by evaporation only and that crop water uptake (i.e. transpiration) does not discriminate in isotopic differences of water. Delta ^{18}O (δ_{sample}) is defined below where $^{18}O/^{16}O$ is the isotopic ratio of $H_2^{18}O$ to $H_2^{16}O$ in the water sample or standard mean ocean water (SMOW).

$$\delta_{\text{sample}} = \frac{{}^{18}O/{}^{16}O_{\text{sample}} - {}^{18}O/{}^{16}O_{\text{SMOW}}}{{}^{18}O/{}^{16}O_{\text{SMOW}}}$$

Therefore if a system lost water by transpiration only, the reservoirs isotopic signature would not change as the reservoirs lost water. On the other hand, if evaporation occurs, the solution will become more enriched in $H_2^{18}O$. These measurements were done so that crop growth and ion accumulation can be expressed relative to consumptive water use (T) and that comparisons can be made to the total water consumed by the crop and the total boron absorbed by the crop (mg/plant) relative to its potential if truly a passive process (i.e. cumulative transpiration time solution boron concentration). This is an important component of the study since B uptake is thought to occur passively by plants and its distribution follows the transpiration stream (Marschner, 1995).

The irrigation treatments consisted of three salinity levels representing non-saline (1.5 dS/m), moderately saline (11dS/m) and saline (18 dS/m) conditions. Based on sand-water (ECsw)- ECe relations described above, these saline treatments translate into average rootzone salinities (ECe) of 0.7, 5.0 and 8.2 dS/m. Each salinity level was comprised of either 1) chloride dominated salts or 2) synthetic saline drainage water with an ion composition typical to that found in shallow, saline water tables in the western SJV. The compositions of each saline treatment are presented in Table 1. The SJV salt solutions were prepared from predictions based on appropriate simulations (Suarez and Simunek, 1997). Each of these treatments had three boron concentrations ranging from very low, such as that found in solution cultures (i.e. 0.23-0.5 mg/L), high (14 mg/L) and very high B concentrations (28 mg/L). The pH of the solutions was maintained between (5.7 and 6.7) using additions of sulfuric acid.

Broccoli was planted on 4 February, 2003 and salinization began 16 days later when plants had approximately two leaves. Several plants were periodically harvested from each tank for biomass and ion accumulation. Broccoli was first harvested on March 28th and again on April 24th. On April 24th, total shoot biomass was determined and plants were divided into leaf blades, leaf margins, and petioles. The remainder of the plants were harvested at maturity on May 21st (90 days after saliniization). Broccoli shoots were divided into heads, stems, young leaves (most fully expanded leaf and younger), and old leaves (all remaining leaves). Immature heads, from salt-stressed treatments, were given the opportunity to mature and these heads were harvested on May 29th. Fresh and dry weight measurements were made on all harvested biomass. Tissue ion concentrations (e.g. B, Na, Ca, Mg, Cl, K and S) were determined on total shoots and various organs from the April 24th harvest and will be determined on the various organs of the crop at final harvest. ANOVA and surface regression model analysis were performed on the data.

Remote sensing was used to characterize treatment effects on broccoli visual injury and to quantify specific reflectance data. The leaf normalized difference vegetative index (NDVI) has been used in many studies to quantify remotely sensed reflectance data. Primarily it has been related to chlorophyll concentration and photosynthetically active leaf area (Broge and Leblanc, 2000, Gamon and Surfus, 1999). For example, elephant grass exposed to salinity had reduced total chlorophyll content on a per plant basis and was proportional to a remotely determined single ratio vegetation index (SRVI). The salinity stress reduced the SRVI primarily by reducing reflectance in the near-infrared spectrum region (Wang, et al., 2002).

Leaf reflectance of the lower leaves in the canopy was measured near the initial development of the broccoli heads on March 15th, 2003. Percent reflectance after an optimization and calibration procedure every other measurement was obtained with an Analytical Spectral Devices™, FieldSpec Pro JR fitted with a High Intensity Contact Probe Model A122300 using a 4.25 VDC 4W reflectorized bulb. The portable instrument is a fast scanning spectroradiometer covering the 350nm to 2500nm region. The spectral resolution is 3nm at 700nm and 10nm at 1400 nm and 2100 nm. The 1.5-meter fiber optic input was adapted with the contact probe and light source.

Leaf reflectance NDVI was calculated as:

$$\text{NDVI} = \frac{(R_{745:755} - R_{700:710})}{(R_{745:755} + R_{700:710})}$$

where R is the reflectance at the specified spectral region (nm) of interest.

Two other indices were also calculated from the spot probe leaf spectra. The Derivative chlorophyll Index (Zarco-Tejada et al., 2002), is defined as the ratio of the derivative of the reflectance at 705 nm to the derivative of the reflectance at 722nm and is reported to be indicative of the chlorophyll content in plant leaves. The third index applied to this experiment was the pseudo-absorbance red-edge position index ,REPabs (Rinehart et al, 2002) which is reported to be a general stress indicator were also calculated.

RESULTS AND DISCUSSION

Biomass

Shoot biomass data indicate that both increased salinity and boron reduced shoot growth. There were no significant effects regarding the composition of the salt. Therefore Cl based salts behaved similarly to SJV-type salts. However, there was a significant salinity-B interaction. At low B concentration, increased salinity reduced biomass relatively more than it did at high B. Similarly, at low salinity, increased B was far more damaging than it was at high salinity. The predicted three-dimensional surface plot is shown in Figure 1. Therefore these data indicate that broccoli is more tolerant to boron in the presence of salinity sug-

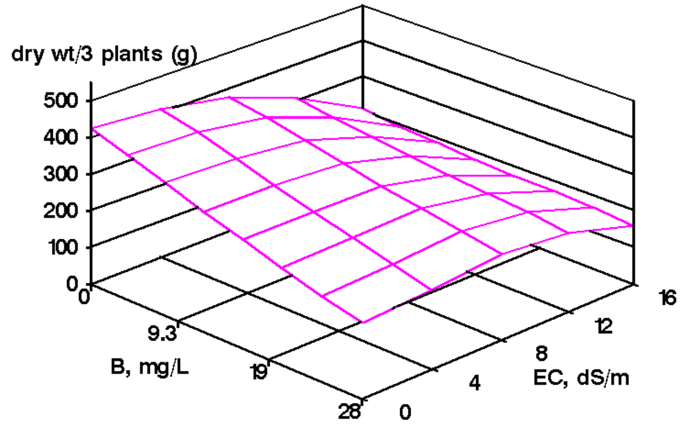

Figure 1. **Predicted surface plot based on dry-weights of three harvested broccoli shoots/tank at the final harvest in relation to both the salinity and boron in the irrigation water.**

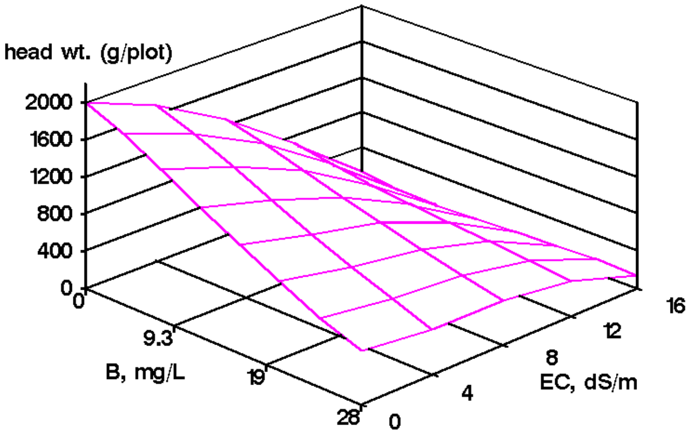

Figure 2. **Predicted surface relationship of broccoli head fresh weights at final harvest as a function of salinity and boron in the irrigation water.**

gesting that the salinity-B interaction is antagonistic.

Similar results were found with fresh broccoli heads (Figure 2). The only difference is that the relative effects are more exaggerated. These data indicate that head yields are more sensitive to both salinity and B than are broccoli shoots.

ION RELATIONS

To date, ion concentration data are complete from all plant samples from the April 24th harvest. Ion concentrations were determined in whole shoots as well as leaf blades, leaf margins and petioles. At this vegetative developmental stage, these shoot components comprised the entire shoot.

Total shoot

Shoot B concentration was influenced by salinity, but interestingly the direction of influence was dependent upon the B concentration in the solution (Table 2). Regardless of the composition of the salinizing solution, increased salinity increased shoot B concentration when B concentrations in the solution were relatively low (i.e. 0.3 mg/L). At the highest solution B concentration (28 mg/L), increased salinity reduced shoot B concentration. At 14 mg/L B, our intermediate treatment, increased salinity either had no effect (i.e. Cl-based salinity) or mixed results (i.e. SJV salts) depending on the salinity. Therefore these data indicate that the concentration of B in the solution determines whether salinity increases or decreases B concentration in the tissue.

Shoot Ca concentrations were higher in plants treated with Cl-based salts than SJV salts (Table 2). This is not surprising based on differences in salt compositions (Table 1). As salinity increased, Ca decreased in SJV salt-treated shoots whereas there was no significant difference in Cl salt-treated plants. Solution B had no effect on tissue Ca concentrations.

Just the opposite of shoot Ca, shoot Mg concentrations were higher in plants treated with SJV salts than those treated with Cl salinity (Table 2). As salinity increased, shoot Mg decreased in Cl-treated plants but increased in SJV treated plants. Again this is attributed to differences in ion compositions between Cl-based and SJV based salinities (Table 1). Solution B did not affect tissue Mg concentration.

Regardless of salt type, as salinity increased, shoot Na increased, however shoot Na concentration was generally higher in plants treated with SJV salts than those treated with Cl salts (Table 2). As solution B concentration increased at any given salinity level, shoot Na was not significantly affected.

Shoot K concentrations were higher in plants treated with Cl salinity than those treated with SJV salinity (Table 2). As salinity increased, shoot K decreased regardless of salt type. This type of phenomenon has been observed in numerous studies (Grattan and Grieve, 1999) where increasing solution Na decreased tissue K concentrations. Shoot K concentration was unaffected by changes in solution B concentration.

Shoot P concentrations tended to be higher in plants treated with SJV salts than those treated with Cl salts (Table 2). Although there was no significant effect, there was a trend for shoot P concentrations to decrease with increasing salinity. This was observed under both salt compositions. No significant effect was found with shoot P

Table 1. Targeted ionic compositions of the various salinity and boron treatments used in the experiment.

Salt Type	EC (dS/m)	Ca (mmol$_c$/L)	Mg (mmol$_c$/L)	Na (mmol$_c$/L)	K (mmol$_c$/L)	Cl (mmol$_c$/L)	SO_4 (mmol$_c$/L)
SJV	2	5.3	3.0	4.3	3.0	2.3	6.0
SJV	11	25.6	19.4	76.8	3.0	37.4	79.6
SJV	18	29.6	32.6	134.3	3.0	65.5	127.0
Cl-based	2	5.2	3.0	6.0	3.0	11.0	1.0
Cl-based	11	47.7	6.0	48.5	3.0	102.2	2.0
Cl-based	18	81.2	6.0	85.4	3.0	170.0	2.0

Table 2. Ion concentrations in broccoli shoots sampled 24 April, 2003. Comparison among salinity (EC, dS/m) levels at each of the three solution boron concentrations (mg B/L).

Ca (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	738.67a	785.00a	699.67a	705.00a	664.67a	630.33a
11.0	830.00a	874.00a	749.33a	635.33ab	615.67a	588.67a
18.0	747.67a	689.33a	747.67a	444.33 b	435.67 b	528.00a

Mg (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	143.33a	172.67a	192.33a	181.00 c	168.67 b	189.67 c
11.0	104.00 b	107.17 b	107.67 b	212.00 b	219.67a	226.00 b
18.0	114.67 b	102.67 b	110.67 b	258.67a	245.00a	266.33a

Na (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	255.00 b	274.33 b	311.33 b	263.00 c	226.67 b	283.00 b
11.0	484.67a	522.00a	506.67a	841.33 b	988.67a	920.00a
18.0	583.67a	563.67a	501.67a	978.00a	1083.33a	998.67a

K (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	1484.67a	1458.00a	1541.33a	1412.00a	1663.67a	1510.67a
11.0	1270.00a	1193.67 b	1273.67 b	929.67 b	995.00 b	1058.33 b
18.0	1276.67a	1253.67 b	1179.33 b	910.00 b	829.33 c	842.33 c

P (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	147.67a	149.00a	147.67a	155.00a	164.33a	155.00a
11.0	129.33a	130.67a	137.67a	148.67a	150.33a	150.67a
18.0	126.00a	118.33a	127.00a	137.67a	153.00a	152.67a

S (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	427.33a	486.67a	458.00a	398.00a	451.00a	465.33a
11.0	389.00a	419.67a	429.00ab	421.33a	478.33a	495.00a
18.0	386.67a	395.00a	364.33 b	483.00a	504.33a	472.00a

Cl (mmol/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	581.33 c	450.67 c	360.33 c	345.67 c	360.67 b	319.00 c
11.0	926.00 b	889.00 b	878.00 b	500.33 b	542.67a	456.33 b
18.0	1164.33a	1045.00a	1153.33a	581.00a	564.67a	619.00a

B (mg/kg dry wt.)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	43.73 b	232.00a	469.00a	51.40 b	192.67a	489.00a
11.0	79.93ab	118.90 b	292.33 b	48.60 b	127.67 b	245.67 b
18.0	117.67a	234.33a	269.00 b	125.33a	212.00a	224.00 b

Table 3. Ion concentrations in broccoli leaf blades sampled 24 April, 2003. Comparison among salinity (EC, dS/m) levels at each of the three solution boron concentrations (mg B/L).

Ca (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	928.00a	843.33a	850.67a	897.33a	783.67a	784.33a
10.0	974.00a	1002.00a	924.67a	824.67ab	754.67a	792.67a
16.0	957.33a	893.33a	846.00a	708.33 b	605.67 b	576.67 b

Mg (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	125.67a	143.33a	175.33a	159.00 c	141.33 c	172.33 b
10.0	86.23 b	90.47 b	92.63 b	216.67 b	216.33 b	234.67a
16.0	99.53 b	95.60 b	91.83 b	282.33a	277.00a	260.33a

Na (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	272.00 b	260.67 b	318.67 c	270.33 b	217.33 b	316.00 b
10.0	455.33a	522.67a	533.67 b	972.33a	1009.33a	1113.00a
16.0	549.67a	583.00a	627.67a	1180.33a	1169.33a	1238.33a

K (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	1273.00a	1224.67a	1396.00a	1314.67a	1482.33a	1379.67a
10.0	1037.67 b	1054.67 b	1184.67 b	810.00 b	846.00 b	910.00 b
16.0	1084.00ab	1066.00 b	1120.00 b	635.00 b	824.00 b	794.00 b

P (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	145.00a	149.33a	142.00a	154.33a	160.00a	163.33a
10.0	113.67a	113.67ab	115.00a	154.00a	142.00a	143.00a
16.0	105.97a	111.20 b	105.23a	147.67a	136.00a	156.00a

S (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	472.67a	589.33a	566.67a	491.33a	530.33 c	563.00a
10.0	437.00a	465.33 b	522.67ab	547.67a	580.67 b	620.33a
16.0	403.67a	449.00 b	458.67 b	630.00a	627.33a	610.33a

Cl (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	700.67 b	399.33 c	381.00 c	393.00 b	388.00 b	349.67 b
10.0	1274.00ab	1049.67 b	993.00 b	660.33a	612.00a	565.00a
16.0	1445.67a	1312.33a	1281.00a	739.67a	726.33a	704.67a

B (mg/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	46.07a	306.67a	421.33a	45.77a	159.67a	406.33a
10.0	75.93a	97.67a	229.00 b	44.40a	93.20 b	198.00 b
16.0	45.93a	102.70a	189.67 b	54.10a	89.60 b	142.33 b

Table 4. Ion concentrations in margins of broccoli leaves sampled 24 April, 2003. Comparison among salinity (EC) levels.

Ca (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	912.33a	840.33a	849.33ab	871.00a	766.33ab	810.67a
10.0	971.67a	986.33a	914.33a	847.67a	808.67a	803.00a
16.0	959.00a	862.67a	801.33 b	708.00 b	658.67 b	603.00 b

Mg (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	123.67a	147.33a	186.00a	152.00 c	136.67 c	174.33 b
10.0	84.53 b	91.10 b	93.87 b	224.00 b	225.67 b	239.00a
16.0	103.83ab	95.70 b	94.97 b	283.67a	289.33a	263.00a

Na (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	289.67 b	279.00 b	331.67 c	285.00 b	223.67 b	321.00 b
10.0	481.67a	531.33a	539.67 b	982.33a	1043.00a	1089.67a
16.0	561.67a	572.00a	603.33a	1163.67a	1153.67a	1209.00a

K (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	1057.00a	1247.33a	1322.33a	1128.67a	1356.33a	1245.67a
10.0	775.33 b	776.67 b	969.33 b	517.33 b	632.00 b	714.33 b
16.0	742.00 b	819.00 b	847.33 b	525.33 b	572.00 b	574.33 b

P (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	145.67a	149.00a	154.33a	143.00a	160.00a	173.00a
10.0	120.33ab	117.33a	104.17ab	161.00a	132.00 b	122.33 b
16.0	104.67 b	106.43a	93.07 b	140.00a	126.33 b	114.67 b

S (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	596.00a	608.67a	710.33a	560.33a	601.00 b	682.00a
10.0	541.67ab	550.00a	592.00 b	664.00a	700.00ab	716.00a
16.0	457.33 b	490.67a	474.00 c	650.00a	711.00a	710.00a

Cl (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	505.00 c	362.33 c	307.67 b	295.33 c	290.67 b	288.00 b
10.0	791.00 b	807.67 b	831.33a	481.33 b	477.00a	418.00ab
16.0	1195.67a	1008.00a	959.33a	670.67a	549.00a	477.67a

B (mg/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	84.23a	450.67a	1214.00a	143.00a	537.00a	1232.00a
10.0	184.83a	270.33a	758.67 b	88.40a	338.00 b	801.67 b
16.0	96.43a	291.00a	636.33 b	69.43a	284.67 b	515.00 c

Table 5. Ion concentrations in broccoli petioles sampled 24 April, 2003. Comparison among salinity (EC, dS/m) levels at each of the three solution boron concentrations (mg B/L).

Ca (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	790.67a	687.67a	637.00 b	669.33a	626.67a	652.00a
10.0	809.00a	759.33a	759.67a	624.33ab	530.00a	563.33 b
16.0	691.67a	660.67a	646.00 b	448.00 b	371.67 b	378.67 c

Mg (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	192.00a	205.33a	224.33a	208.33 b	201.33 c	232.33 b
10.0	124.00 b	123.67 b	127.00 b	258.33ab	247.00 b	271.00ab
16.0	126.00 b	124.00 b	123.33 b	294.67a	295.67a	291.67a

Na (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	276.67 b	242.67 b	299.67 c	239.67 b	207.00 b	323.00 b
10.0	447.67a	481.67a	522.00 b	861.33a	930.00a	981.33a
16.0	484.00a	536.67a	599.67a	996.00a	1005.00a	1089.00a

K (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	1974.00a	1881.67a	1858.33a	1870.67a	2067.67a	1986.67a
10.0	1730.33 b	1665.33a	1853.00a	1416.33 b	1446.33 b	1472.67 b
16.0	1693.33 b	1788.33a	1760.33a	1317.00 b	1465.00 b	1378.00 b

P (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	111.67a	127.33a	105.33a	116.67a	126.33a	113.67a
10.0	83.40 b	74.97 b	68.50 b	105.40a	104.20ab	87.20a
16.0	69.27 b	65.03 b	59.23 b	109.00a	92.00 b	105.00a

S (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	214.67a	251.33a	234.33a	221.00a	236.00a	237.00a
10.0	202.33a	196.67 b	215.33ab	235.67a	231.33a	238.00a
16.0	207.00a	206.00 b	211.00 b	223.33a	233.00a	248.00a

Cl (mmol/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	900.00 b	582.00 c	445.67 c	503.00 b	504.00 b	421.33 b
10.0	1056.00 b	1010.33 b	1040.33 b	686.33ab	650.00a	620.33a
16.0	1310.33a	1286.67a	1276.00a	798.67a	731.00a	719.33a

B (mg/kg dry wt.)

EC Level	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	26.50a	81.97a	94.93a	30.73a	61.87a	98.80a
10.0	31.70a	37.80 b	59.10 b	29.33a	43.23 b	50.87 b
16.0	26.90a	38.67 b	51.97 b	26.97a	40.50 b	52.63 b

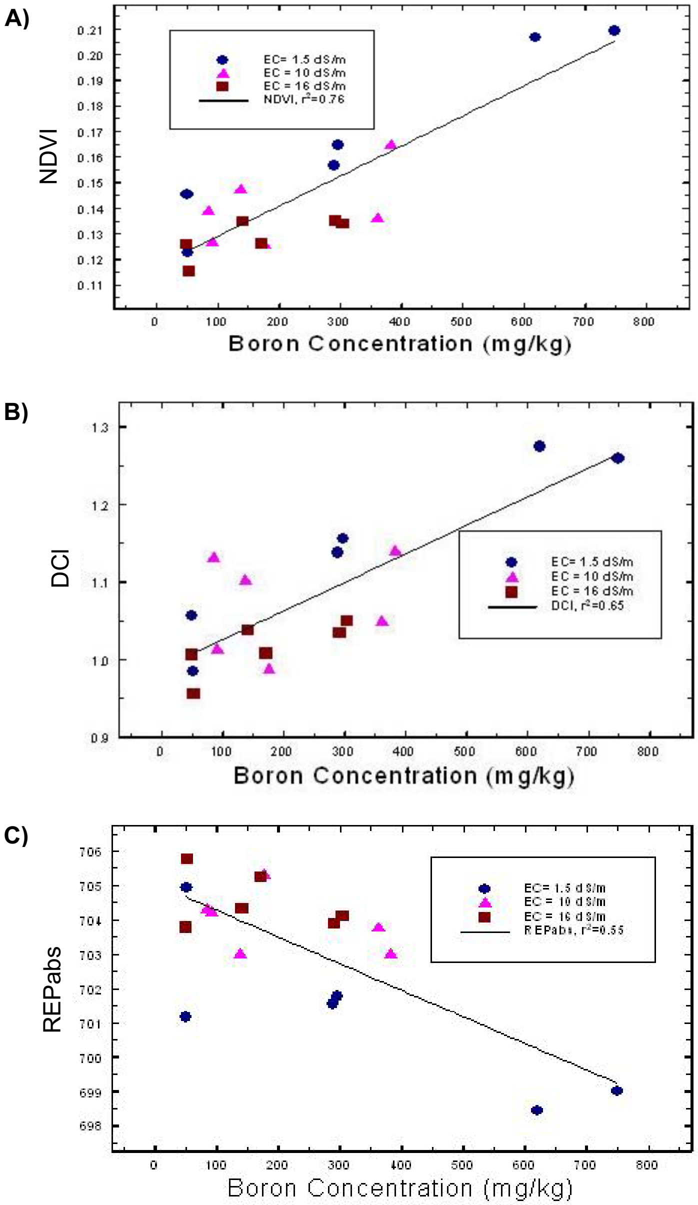

Figure 3. Linear regressions of (A) Normalized vegetation difference index, (B) Derivative chlorophyll index (DCI), and (C) Red-Edge position (nm), as a function of the boron in broccoli leaves for each of the three conductivity treatments. The ionic compositions were not significantly different so were pooled. Each point represents the average of three replications.

concentration as influenced by changes in solution B concentration.

Shoot S concentrations in SJV salt-treated plants were only slightly higher than those treated with Cl salts (Table 2). Shoot concentrations were reduced by Cl salinity but increased only slightly, and not always significantly, with increased SJV salinity. The lack of a profound response with increased SJV salts could be related to the high SO_4 solution concentration in the SJV control treatment. Shoot S was not influenced by solution B concentrations.

Not surprisingly, shoot Cl concentration was higher in Cl-treated plant than those treated with SO_4 dominated salts (Table 2). Regardless of salt type, however, increased salinity increased shoot Cl concentration. Interestingly, however, at high Cl salinity, increased solution B concentration decreased shoot Cl concentration. This effect was not observed with the SJV salts.

Ion distribution within the leaf

Ion distribution within the leaf depended upon the ion in question. For the divalent cations (Ca and Mg) as well as Na and P, ion concentrations in the margins were no different than those in the blades (Tables 3 and 4). K and Cl, on the other hand, were both higher in the blade portions while S and B were higher in the leaf margins. Regardless of salt type, an increase in substrate B concentration increased blade B concentration by a factor of about 9 at low salinity while it only increased blade B concentration by a factor of 3 at high salinity.

There were also differences among ions in the distribution between those in the leaves and those in the petioles. Petioles concentrated more Mg and K and less Ca, P, S and B in its tissue than did leaf blades (Table 5). At low salinity, Cl was higher in petioles than blades but these differences were not apparent as salinity increased.

REMOTE SENSING

The composition of salinity had no significant effect on leaf reflectance indices. When the compositions were pooled together, the effect of salinity and boron treatments had a significant effect on reflectance as did the interaction of salinity and boron for three separate vegetation indexes. The complete experimental model was significant but the NDVI index only accounts for about 57% of the variation within the experiment and 49% and 46% of the variation in the experiment was explained by the other two indices (DCI, and REPabs, respectively). Relationships between leaf boron concentrations and the interaction of boron and salinity treatments were highly significant as the reflectance was significantly reduced by the high B concentration - low EC combination (Figure 3a). These same plants that were under high boron stress at low salinity also showed visual symptoms atypical of the rest of the experimental population with extensive leaf curl and mottled chlorosis. This reduced index appears to be related to a reduction in chlorophyll content in the leaf, however, chlorophyll analysis was not performed to verify this observation.

Table 6. Percentage of total B (mg/shoot) found in the plant relative to its potential content if completely passive (cumulative transpiration times solution B concentration)

EC (dS/m)	Boron 1 Chloride	Boron 14 Chloride	Boron 28 Chloride	Boron 1 SJV	Boron 14 SJV	Boron 28 SJV
1.5	24.6	1.4	1.2	10.4	1.8	2.1
10.0	38.6	1.1	1.1	10.9	1.0	0.9
16.0	60.2	1.9	1.2	30.6	1.6	0.8

Two other remote indices were also calculated. Instead of normalized difference vegetation indices, these indices are based on the derivatives of certain portions of the spectra. The Derivative Chlorophyll Index (Zarco-Tejada et al., 2002), defined as the ratio of the derivative of the reflectance at 705 nm to the derivative of the reflectance at 722nm, was found to also be significantly related to boron concentrations in the leaves (Figure 3b). Another derivative index based on pseudo-absorbance, REPabs (Rinehart et al, 2002), is reported to be correlated with general stress and is also significantly correlated with boron in leaf tissue (Figure 3c). In each case the extremely chlorotic leaves associated with high boron are those that are associated with the greatest differences in the index. These relations with boron concentrations, it appears, are indirect effects of pigmentation changes induced by the treatments and not a direct indication of the B concentration in the leaves. The fact that these indices can segregate boron and salinity treatment effects is very encouraging and justifies further study in this area.

The data indicate little change in the NDVI, DCI, or REPabs indicies except for the case of low salinity and high B concentrations where the effect was profound. The NDVI index is comprised of wavelengths sensitive to leaf chlorophyll. Therefore B toxicity may be indirectly detected due to the effect on chlorophyll distribution, concentration, or development.

CUMULATIVE WATER USE

Cumulative water use of broccoli was evaluated in relation to the various treatments. Cumulative ET was, for the most part, directly related to cumulative biomass; the higher the cumulative biomass the higher the cumulative ET (data not presented).

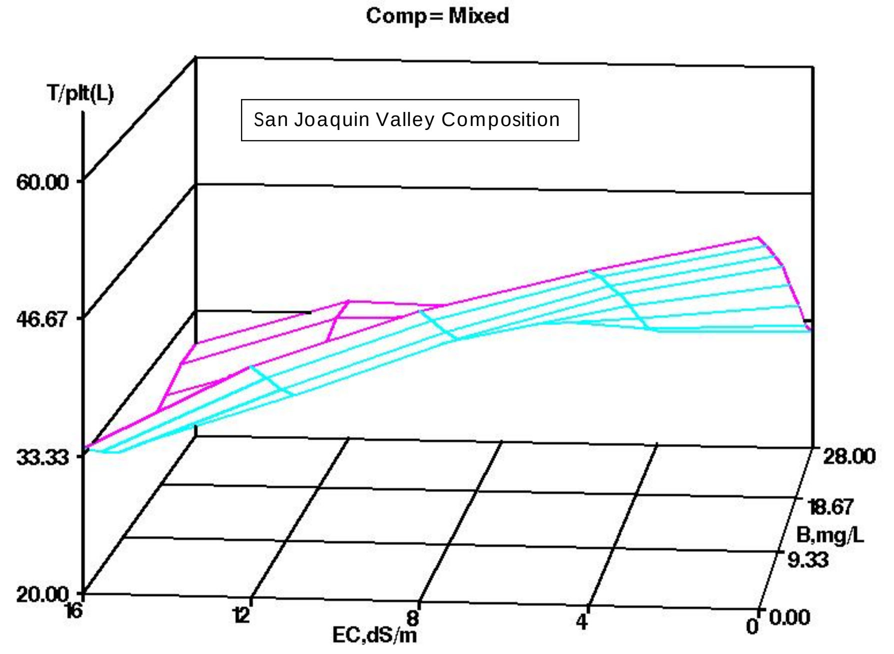

Figure 4. Cumulative transpiration (T) volumes (L/plant) in relation to various salinity (SJV) and boron treatments. Values are estimates for the April 24th harvest based on stable isotope analysis.

EVAPORATION VS TRANSPIRATION

Stable isotopic ratios of oxygen in the solution were used to separate evaporation and transpiration. Similar to cumulative ET, cumulative transpiration (Figure 4) was directly related to shoot biomass. Although both 3-D surface regressions (Figures 1 and 4) are presented from different angles, one can see that each follow the same basic shape. With these estimates of cumulative transpiration, we were able to provide insight into whether B uptake is truly passive with the transpirational stream as many have suggested in the literature or whether the plant is able to regulate the amount of B it absorbs and transports to the shoot. In no treatment did shoot B accumulate to a level predicted based on cumulative transpirational volume times solution B-concentration (Table 6). Plants treated with low B contained the largest percent of B uptake (10-60%), expressed relative to predicted passive uptake. Salinity treatments, regardless of composition, represented the higher percentage range. What is also interesting but not yet tested significantly is that the percentages were generally lower in SJV treatments as compared to corresponding Cl-based treatments. On the other hand, plants treated with high B (14 or 28 mg/L) only accumulated 1-2% of that predicted if uptake and accumulation were truly passive. Therefore under conditions of high external B, the amount of B that is absorbed and/or transported to shoots of broccoli (and perhaps other species) is controlled by some unknown mechanism, a finding that deserves further investigation.

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Integrated Drainwater Management In the Central Valley: A Regional Analysis of Irrigated Agriculture

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ABSTRACT

This research evaluates the efficiency of alternative management strategies towards maintaining agricultural productivity under various drainage constraints, economic considerations, and biophysical characteristics. A variety of strategies can be implemented in response to the closed basin restriction, including source control (e.g., changing crop mix or changing irrigation technology or intensity), reuse of drainage water on salt-tolerant crops, and drainage into an evaporation pond. Empirical analysis using data and parameters representative of the WWD demonstrates that efficiency generally requires the use of multiple management strategies. Reuse seems to be the most promising strategy, achieving drainage requirements at minimal losses (approximately 5% relative to the unconstrained case). Under the reuse option, drainage control is largely in the form of a transfer of freshwater land area to reuse production, with minimal source control on a per-acre basis (e.g. shift to higher uniformity irrigation systems). Evaporation ponds, alternatively, led to losses of between nearly 10 to 34% depending on whether compensating habitat acreage was required. Hence, evaporation ponds did not prove to be an efficient mechanism for hydrologic balance under the base conditions.

Issues that might impact the attractiveness of reuse as a means of disposal include decreasing cotton prices and increasing salinity levels. The primary reuse crop is cotton whose prices have recently declined. Empirical analysis considering a range of cotton prices did find significant reductions in agricultural income (approximately 36% differential across the range of cotton prices that represent recent highs and lows) and a partial shift to wheat as the reuse crop. However, agricultural returns were still higher under reuse than any other strategy. An investigation of the sustainability of agricultural production with and without the drainage constraint showed that a large part of price change affected income regardless of whether the hydrologic constraint was binding or not. Another concern with reuse as a single disposal strategy is that eventually salt concentrations will rise within the aquifer. However, our results suggest that agricultural production can still be profitably maintained even with a doubling of salt concentration. Furthermore, a dynamic analysis suggests that aquifer salt concentrations are likely to rise only very slowly over time.

The empirical analysis finds modest use, if any, of evaporation ponds. Pond areas range from zero to less than 7% of the land area depending on the range of management options considered. However, several additional factors need to be considered. The analysis here is only for average conditions and pond area comes out of productive land. In general there can be spatial variability such that some land may not be suitable for reuse; conversely there may be low-quality land available for ponds with minimal opportunity cost. Furthermore, if birds and wildlife can be kept off the evaporation ponds, then provision of the compensating habitat may be an environmental benefit, especially in areas such as the SJV where there has already been excessive wetland loss. Well-run evaporation ponds in conjunction with mitigation and compensation measures may well have a larger social role than that found (and analyzed) here.

INTRODUCTION

A variety of strategies for reducing external drainage and meeting closed basin restrictions have been investigated, promoted, and in some cases, implemented. One strategy is source control, which includes measures such as changing crop mix, installing more uniform irrigation systems, and varying irrigation timing to more closely meet plants needs. This category of methods has seen considerable economic research, as well as adoption of some of the lower cost options. A second category of drainage control is reuse of drainage water on salt-tolerant crops. This category, which provides both drainage disposal and water conservation services, has received considerable scientific research, yet relatively little economic research, and very limited adoption. Two additional strategies that have been and continue to be considered, especially in light of any disposal restrictions, include evaporation ponds and land retirement. Evaporation ponds serve as an in-region disposal option, while land retirement reduces the total amount of drainage by taking land, preferably with poor quality soils, out of production.

This research investigates integrated drainwater management for irrigated agriculture at the regional level. Schwabe and Knapp (2003) develop a regional mathematical programming model to evaluate integrated drainwater management in the San Joaquin Valley (SJV) in California. Research questions are efficient combinations and levels of various management strategies, response and sensitivity to alternate conditions, policy instruments for closed basin management, benefits to producers from external drainage facilities, and agricultural sustainability. Research this year developed theoretical insight into regional management with novel findings that general equilibrium effects could reverse what might be expected from partial equilibrium analysis. The empirical model was also expanded and applied to additional topics. A complete technical description of the analysis and results can be found in Schwabe, Knapp, and Kan (2004).

The regional programming model assumes a constant water table level and aquifer salt concentration; however, in principle these can evolve over time which can alter efficient management. Knapp (1997, 1998) develops a lumped parameter dynamic model for saline aquifers and reports preliminary results. Research this year developed the theory of common property usage and a revised empirical specification utilizing the structure and data of the static regional programming model and additional data on surface water costs. Overview of the general results and explicit new findings are described below. A complete technical description of the model as well as summary of the general results discussed below is contained in Knapp (2004).

STUDY AREA AND DATA

The empirical analysis focuses on the San Joaquin Valley (SJV), with particular attention towards the 570,000 acres of irrigable farmland comprising the Westlands Water District (WWD). The agricultural mathematical programming model includes six crops and five irrigation systems (table 1). The crops include cotton, processing tomatoes, wheat, lettuce, alfalfa, and bermuda grass. Non-water production costs and market prices for each cropping system are derived from both the UC Cooperative Extension Service (crop budgets) and Fresno County Crop Report (2000). The irrigation systems (and their respective Christensen Uniformity Coefficient) include Furrow 0.5 mile (CUC=70), Furrow 0.25 mile (CUC=75), Linear move sprinklers (CUC=85), Low-energy precise application system (CUC=90), and Subsurface drip (CUC=90). The costs of irrigating include amortized capital costs along with maintenance and operating costs. One option for disposal of drainage water is through the use of evaporation ponds. Our evaporation pond infrastructure construction and pumping costs come from Posnikoff and Knapp (1997). To calculate surface and groundwater costs, we assume a weighted-average of surface water prices in WWD for cost of surface water to growers. Groundwater pumping costs from the confined aquifer are calculated based on energy requirements (www. westland water .org). Finally, to account for heterogeneous land quality characteristics, we calibrate the model with attention to historic crop acreage allocations in WWD.

INTEGRATED MANAGEMENT AND EVOLVING BASIN CONDITIONS

Formed in 1952, WWD relied mostly on groundwater for irrigation until 1968, at which time it started receiving appreciable amounts of Central Valley Project surface water supplies following development of the San Luis Unit. The San Luis Unit, which consists of over a 1000 miles of conveyance and distribution pipes and canals, was built as part of a 1963 agreement between WWD and the Bureau of Reclamation. This agreement relegated the Bureau to supply 900,000 ac-ft of water to WWD at stipulated prices for a 40 year period, and provide drainage services for drainage originating in WWD.

Prior to 1985, WWD growers operated with essentially no constraints on drainage discharge. For those growers in drainage service areas, drainage was discharged into a system terminating at the Kesterson reservoir. The efficient solution under this pre-1985 scenario can be simulated with our model by relaxing the hydrologic balance constraint. The first column in table 2 reports the efficient solution when net flows to the watertable are not constrained. As shown, traditional irrigation systems are selected, no reuse is used, and deep percolation flows average slightly over 1 ft/yr, which is consistent with the historical average for deep percolation flows in this region (Tanji and Karajeh 1993).

To address the threat of a rising watertable, and fulfill its legal obligations associated with providing drainage services, in 1976 the Bureau began construction of a system of drain lines and a regional collector system, the San Luis Drain. This drainage system was intended to provide drainage services for nearly 42,000 acres of irrigated agricultural lands, with the outlet of the San Luis drain terminating in Kesterson Reservoir. In 1985, though, Kesterson Reservoir was closed and drainage services stopped following the discovery of high concentrations of selenium and bird deformities at the reservoir. The selenium, along with other toxic trace elements, was linked to drainage flows from upstream agriculture. Since then, the district has existed largely as a closed drainage basin. Consequently, the amount of acreage having a shallow groundwater depth of between 0 and 5 feet has increased from nearly 70,000 in 1985 to approximately 190,000 in 1998 (Westlands Water District 1998).

One response by growers to the cessation of drainage services was to implement various source control strategies for water conservation, primarily in the form of a shift in irrigation systems. In particular, there is evidence of a shift from traditional furrow irrigation systems (½ mile runs) to higher uniformity furrow systems (1/4 mile runs). Another response observed in the SJV region was disposal of collected drainage waters in evaporation ponds. This strategy required setting aside a fraction of land that would be used for disposal alone. Compensating habitat was not required nor was reuse practiced.

The hydrologic balance constraint is imposed in the empirical model to satisfy the in-region disposal requirement. The results are presented in column 2 of table 2. Without a reuse option nor compensating habitat requirement, efficient management entails both a substantial level of source control and evaporation pond construction. Total crop area declines to accommodate ponds, and irrigation systems switch from traditional to more uniform systems. Average deep percolation flows decline by over 50% due to improvements in irrigation efficiency and reductions in applied water. Evaporation pond acreage amounts to 8% of the regional area. Net benefits decline by 9% relative to the unconstrained case, an outcome that still seems economically viable and is consistent with the observed continued production in the region.

Similar to the events at the Kesterson reservoir, selenium bioaccumulation in the evaporation ponds eventually threatened wildlife. Consequently, evaporation pond operators were subject to more stringent environmental regulations, including a one-to-one compensating habitat to evaporation pond acreage requirement. From a partial equilibrium perspective, addi-

Table 1. Price, Cost, and Production Data

Crop prices & harvest costs[a]	***Cotton***	***Tom***	***Lettuce***	***Wheat***	***Alfalfa***	***B-grass***
Output Prices ($/ton)	*1489.7*	*55.2*	*596.5*	*133.3*	*109.3*	*75.2*
General harvest costs ($/acre)	*61.0*	*54.9*	*0.0*	*10.0*	*95.0*	*131.2*
Yield related costs ($/ton)	*12*	*11.95*	*232.6*	*29*	*13.3*	*15.0*
Revenue related costs	0.005	0.0	0.0	0.0	0.0	0.0
Irrigation System[b]	***F2***	***F4***	***Sprinkler***	***LEPA***	***Linear***	***Drip***
CUC	70	75	80	85	90	90
Pressure head (ft)	10	10	150	50	80	50
Capital Recovery Cost ($/ac/yr)	21.9	28.8	42.7	81.7	81.8	178.1
O & M Costs ($/acre/yr)	2.9	3.8	20.0	38.3	38.4	60.0
Fixed energy costs ($/acre/yr)	1.0	1.0	1.5	1.0	1.2	1.0
Pressurization cost ($/af)	1.1	1.1	16.5	5.5	8.8	5.5
Production costs[c]	***F2***	***F4***	***Sprinkler***	***LEPA***	***Linear***	***Drip***
Cotton ($/acre/yr)	607.8	628.1	678.5	683.1	680.3	744.9
Tomatoes ($/acre/yr)	636.8	661.2	718.8	704.9	701.5	751.2
Lettuce ($/acre/yr)	1652.1	1683.4	1729.6	1717.8	1700.5	1661.3
Wheat ($/acre/yr)	194.8	204.5	235.5	288.6	286.9	390.9
Alfalfa ($/acre/yr)	396.8	409.9	434.6	492.3	483.2	538.6
Bermuda grass ($/acre/yr)	507.1	541.6	609.8	557.3	548.8	627.2

[a] Price and cost data are 1999 dollars. Capital recovery costs assume a 5% interest rate. Output prices are average price per ton in WWD, California 1997-1999. Cost data from UC Cooperative Extension crop budgets with adjustment for inflation and irrigation system type. Tom=tomatoes, B-grass=Bermuda grass. F2 and F4 are furrow with ½ and ¼ mile runs, respectively.

[b] Irrigation system data are generally from Posnikoff and Knapp (1997) with adjustment for inflation. CUC = the Christensen Uniformity Coefficient; this is a measure of the uniformity of water application for an irrigation system, with larger numbers implying greater uniformity.

[c] Production costs include all production costs (seed, land preparation, machinery, fertilizer, etc.) except water, irrigation system, land and cash overhead.

Table 2. WWD drainwater management under evolving regulatory conditions

	No Reuse			Reuse	
	External Drains	No Compensating Habitat	Compensating Habitat	Compensating Habitat	Compensating Habitat
Crop Production					(*salt balance*)
Area (acres)	0.83	0.75	0.75	0.51	.50
Cotton(%)	58	53	53	37	36
Tomatoes(%)	24	27	27	39	40
Wheat(%)	4	4	4	-	-
Lettuce(%)	6	7	7	10	10
Alfalfa(%)	8	9	9	14	14
Water (ft/yr)					
Water Use	3.28	2.52	2.05	3.18	3.17
Deep Percolation	1.23	0.57	0.19	1.21	1.21
Irrigation (%)					
FUR2	94	-	-	90	90
FUR4	-	76	13	-	-
LINEAR	-	17	80	-	-
DRIP	6	7	7	10	10
Reuse Production					
Area (acres)	-	-	-	0.32	.25
Cotton(%)	-	-	-	91	88
Wheat(%)	-	-	-	9	12
Water(ft/yr)					
Water Use	-	-	-	3.85	3.8
Deep Percolation	-	-	-	1.89	1.86
Irrigation (%)					
FUR4	-	-	-	100	100
Land Disposal(acres)					
Evaporation Pond	-	0.08	0.03	-	0.02
Compensating Habitat	-	-	0.03	-	0.02
Welfare Measures					
Net Benefits ($)	311	282	206	295	262
Drainage Shadow Value ($/ac-ft)	-	74	305	19	305

Land areas and social net benefits are per regional acre. Irrigation system and crop specific results are percent of the respective crop or reuse areas. Water variables are average depths over the cropped areas in the crop production and reuse sectors. When used, compensating habitat is required at a 1:1 ratio with evaporation ponds. External Drains ~ no drainage constraint. F2 and F4 are furrow with ½ mile and ¼ mile runs respectively. Irrigation systems differ with respect to irrigation uniformity and costs. Salt balance ~ constraint requiring salt flows to water table equal salt flows from water table. Finally, 17% of the land is devoted to trees and fallowing.

tional compensating habitat requirements would likely be accommodated by (i) devoting fewer acres to crop production and (ii) increasing source control measures per unit area. However, contrasting general equilibrium effects might arise. For example, reducing applied water depths decreases crop profits and, consequently, decreases both the opportunity cost of land and the shadow value on drainage. Theoretical analysis suggests that, a priori, the impact of a compensating habitat requirement on acreage devoted to crop production and drainage is unknown.

Empirically, the quantitative effect of requiring compensating habitat (column 3 in table 2) is dramatic. The shift to more uniform irrigation systems is increasingly pronounced, and deep percolation flows are reduced by 85% relative to the unconstrained solution. Corresponding to the comparative static analysis, there is a reduction in surface water use, no change in the area devoted to crop production, and a decrease in pond size. Pond acreage is minimal (3%), although an equal amount goes into compensating habitat for a total of 6% of the regional land area. Most significantly, net benefits to agricultural production – while still positive – are reduced by 34% relative to the unconstrained level. Justifying compensating habitat on economic efficiency grounds would require substantial ecological benefits.

Despite a large amount of scientific research and funding, drainwater reuse has not been adopted in the District. Should one use evaporation ponds, reuse, or both in closed drainage basin management? Analytic derivations illustrate that reuse is the less costly alternative when land values are low, while evaporation ponds are preferable when land values are high. The trade-off arises because reuse, while less costly per-acre than ponds (and quite possibly having positive returns), generally disposes of less water per unit area than ponds. Thus the choice of disposal method depends, in part, on the profitability of freshwater crop production, a nominally independent entity.

Column 4 in table 2 presents results under the condition that efforts to meet the in-region drainage constraint can be achieved by both reuse and evaporation ponds (with the requisite compensating habitat). The results suggest that drainwater reuse offers great promise in maintaining agricultural production and hydrologic balance in the region. Compared to the unconstrained case, net benefits decline by only 5% under reuse. The area devoted to freshwater crop production is reduced quite substantially to allow for reuse production. Compared to the external drainage solution, reuse opportunities require little source control from growers. There is a 5% reduction in the use of less uniform irrigation systems, deep percolation flows increase slightly, and most of the drainwater reuse is applied to cotton. Cotton as a reuse crop is practical since it is moderately salt tolerant (Mass and Hoffman 1977) and, given the current groundwater salinity concentration, somewhat profitable. Note that with the reuse option, no acreage is allocated to evaporation ponds.

COTTON PRICES AND AQUIFER SALT CONCENTRATIONS

Over the past 8 years there has been a downward trend in upland cotton prices by nearly 50%. While cotton acreage is slowly being allocated towards prima cotton, whose price is substantially higher and hasn't experienced as great a downward trend, upland cotton still comprises over 70% of cropped cotton acreage. Given that the favorable drainage management strategy noted above relies on cotton as the reuse crop, it is prudent to evaluate the robustness of this conclusion to changing cotton prices. From an analytical perspective, results show that in an unconstrained setting, lower cotton prices discourage water use as one moves back along the production function. Lower cotton prices also decrease profits in the reuse sector, in effect increasing net drainage costs and again, negatively influencing water application rates. However, lower cotton prices also imply lower land opportunity costs and, hence, lower drainage prices. These latter results tend to have a contrary effect on water depths and source control.

Empirical results were presented in last year's report. Our results suggested that regardless of whether a drainage restriction is imposed, a reduction in cotton prices from the high level to the low level had dramatic implications for agricultural production as it resulted in a near 36% decrease in grower profits. This was identified as an upper bound on losses given the minimum bounds placed on crop acreage. However, the effects on drainage management from this price change are limited. When there are no drainage restrictions the qualitative impacts of lowering cotton prices is essentially borne by a reduction in crop production and a slight decrease in applied water rates. Alternatively, when the drainage constraint is imposed, the price change results in a similar reduction in freshwater crop acreage (mostly in cotton), a slight increase in applied water rates, and a substantial shift to less efficient irrigation in the reuse sector. More importantly, under either price scenario the efficient management response to the drainage constraint is reuse, not evaporation ponds. The reduction in net benefits from the drainage constraint relative to the unconstrained solution is only 5%. Thus while agriculture is substantially impacted by lower cotton prices, this is a general effect occurring regardless of the drainage regulation. The conclusions noted above about the desirability of reuse for drainage management and viability of agriculture in a closed drainage basin continue to hold.

A concern with reuse is the possible increase in aquifer salt concentration. Since ET is almost completely pure water and soil salinity has to be maintained at an appropriate level for crop production, reuse ultimately just recirculates salts in the basin while surface water inflows import salts. Given no significant drainage outflows from the basin, the efficient reuse strategy noted above will in principle cause increasing groundwater salt concentrations over time. General equilibrium effects complicate the analysis. A theoretical analysis shows that an increase in groundwater salt concentration decreases reuse profits and also decreases reuse ET given reuse water depths. As reuse ET is the drainage disposal mechanism, both of these effects work to increase the explicit cost of drainage disposal which tends to increase source control for crop production. However, this reduces crop profits and hence land opportunity costs, which tends to reduce implicit disposal costs and hence has a contrary effect on source control.

Empirical results were presented last year for groundwater salt concentrations ranging from the current level (10 ds/m) to a high level of 20 dS/m while maintaining hydrologic balance. As salt concentration increased, reuse acreage declined on average and there was a shift in acreage away from cotton towards wheat production. This shift is efficient given that cotton becomes a less profitable "reuse" alternative as salt concentrations increase, essentially serving as a means for disposal with very little, if any, profit potential; cotton, thus, loses its relative advantage and thus wheat becomes a less costly option for reuse. Concomitantly, there is an increase in both freshwater crop production, primarily arising from a shift towards cotton from tomatoes, and source control, in the form of higher uniformity irrigation systems. Compared to the existing salt concentration, there is a loss of $66 acre-1 yr-1 when aquifer salt concentration doubles.

Another approach is to force a steady-state by balancing salt flows to the water table with salt flows from the water table. This balance will maintain the groundwater salt concentrations at the current level, but does require the use of evaporation ponds and some salt disposal mechanism from the pond. The fifth column of table 2 reports results for the currently observed 10 dS/m salt concentration, yet maintaining salt balance. Crop production is little changed; however, reuse area is slightly reduced with the difference going to the evaporation pond and compensating habitat. The pond/CH area is quite small (4% of regional area) with net returns decreasing by $33 acre-1 yr-1 compared to column 1, and by $49 acre-1 yr-1 relative to column 1 in table 2 - the external drainage case.

Saline Groundwater Model

The second major line of research activity this year was continued development of a saline groundwater econonics model for analysis of dynamic reuse and other topics. Irrigated agriculture with surface water supplies and overlying a groundwater aquifer is considered (figure 1). Irrigation water may come from either or both of ground and surface water. Some irrigation water is not used by the crop and percolates back to the aquifer. This arises partially from salt leaching requirements but mostly from nonuniform irrigation. Drainage flows are extractions from the water table which are disposed of in some fashion. These typically occur only under high water table conditions to maintain the water table at an acceptable level for crop production.

There are 3 sectors in the agricultural production model: crop production with fresh-water, reuse production with groundwater, and an evaporation pond. Two crops are considered (cotton and tomatoes) with a traditional irrigation system. Crop areas and water application levels in the production and reuse sectors are variable, as is evaporation pond area. Crop-water production functions as previously reported by the authors [Kan, Schwabe, and Knapp (2002)] describe crop yield and quantity and quality of deep percolation flows as functions of applied water depth and salt concentration. Land area is constrained and surface water is obtained at increasing marginal cost and also subject to an upper

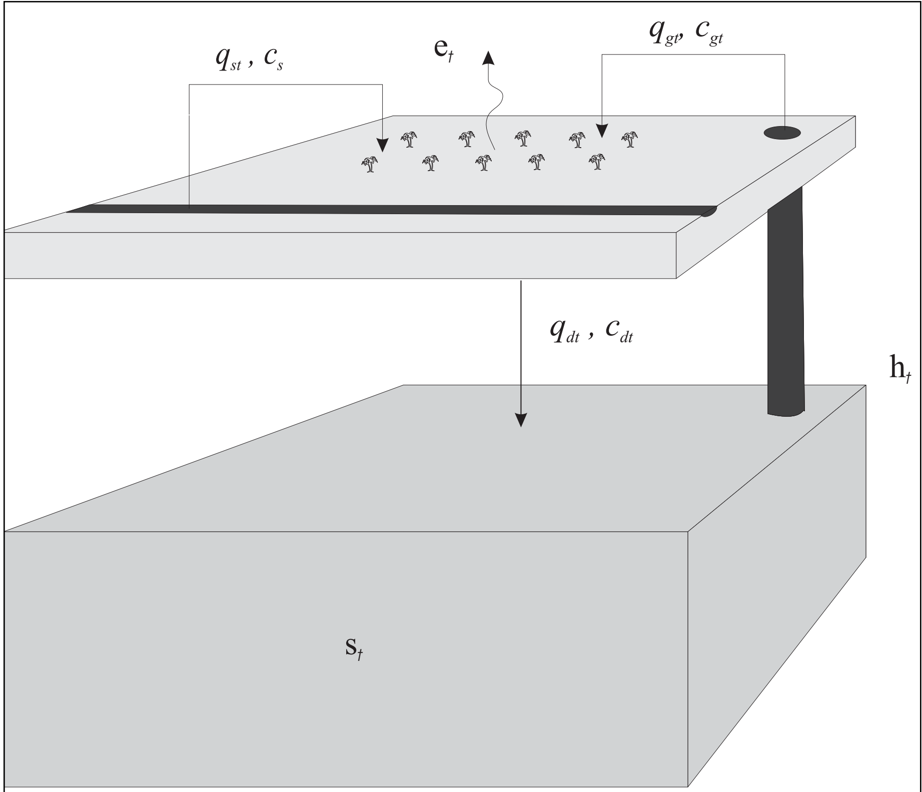

Figure 1. Regional agricultural production with surface water supply and overlying an aquifer system with water quality (salinity). Variables are q=water quantity, c = salt concentration, e = crop ET, h=hydraulic head, s = aquifer salt mass.

bound. Equations of motion describe hydraulic head and aquifer salt concentration over time in response to the various flows to and from the aquifer. The agricultural production model is empirically specified using the data described for the regional programming model, while Kern county data is used for the aquifer dynamics.

COMMON PROPERTY AND EFFICIENT MANAGEMENT: HISTORICAL ANALYSIS

Under common property usage there is no regulation of the resource system; individual growers make decisions strictly in their own interest and ignore effects on others. With many users, each of whom is small compared to the resource, the effect of individual current decisions on future water table levels and salt mass is negligible. Therefore it is reasonable to suppose that, with many users, decisions in each period maximize profits in that period without regard to future values of the state variables. A theoretical analysis of common property usage was developed for a single crop with fixed land area and surface water usage and assumed conditions for the crop-water production function. The results are reported in the phase-plane analysis (figure 2). Empirical results were also reported in the earlier reports which are qualitatively similar to the new analytical structure.

Initial conditions of a completely full aquifer and an initial salt concentration of 1 dS/m imply three distinct regimes for groundwater use. The first might be termed the Resource Extraction era. Here groundwater is relatively abundant and high-quality, so extractions exceed deep percolation back to the aquifer and the water table falls. As the water table falls pumping costs increase, and the salt concentration is rising due to increasing salt mass from water imports and the concentrating effect of reduced volume, and these tend to reduce groundwater extractions over time. At some point deep percolation flows exceed groundwater extractions and the water table begins to rise. This could be labeled the Waste Disposal era: although beneficial extractions occur, they are outweighed by deep percolation. Salt concentration continues to rise during this era as well. The final era is Drainage where the water table is at the maximum height. For irrigation to continue, hydrologic balance is maintained by balancing deep percolation flows with groundwater extractions. Initially this is accomplished via source control and reuse; however, aquifer salt concentration is still increasing making reuse increasingly less attractive as a solution. Consequently, a point is reached where evaporation ponds are the desired disposal option, the system is stabilized, and the economic value of the aquifer to the production system is exhausted.

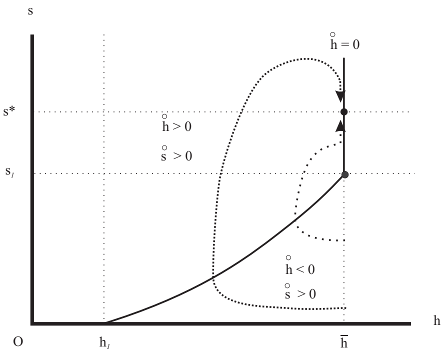

Figure 2. Phase plane diagram for the saline groundwater system. Aquifer salinity is on the vertical axis and water table height is on the horizontal axis.

Annual net benefits are initially declining over time as the water table falls, pumping costs increase, and quality declines. This occurs until the water table turning point. Somewhat surprisingly, annual net benefits then slightly increase over time. This appears to be a result of the rising water table. Although groundwater quality is substantially degraded at this point, the reduced pumping costs apparently outweigh the quality effects and annual net benefits increase. Once the Drainage era is reached, annual net benefits fall as aquifer salt concentration increases until a steady-state is reached with the onset of evaporation ponds. The system is therefore not sustainable in the sense of maintaining or improving incomes over time. A 50% drop in net returns is experienced over the horizon.

Economically efficient allocations are determined by maximizing the present value of net benefits. This differs from common property (CP) usage where users only maximize annual net benefits, consequently ignoring future impacts on hydraulic head and aquifer salt concentration. As a result, common property usage is inefficient. Efficient time paths are qualitatively similar to common property, but differ substantially quantitatively. Hydraulic head under economic efficiency follows the U-shaped path to a steady-state as previously observed; however, it remains at significantly higher levels than CP and, interestingly enough, reaches the steady-state maximum level earlier. Although salt concentration of the aquifer increases over time as in CP, the rate of increase is somewhat less and the steady-state level is approximately 20% below that under CP. Efficiency annual net benefits are generally declining until the steady-state as in CP. While they are initially lower than under CP for a decade or so, they are then generally higher throughout the remainder of the horizon. The difference can be quite substantial, amounting to some $ 67 acre-1 yr-1 in the steady-state for example. Basin-wide annualized net benefits are $530 million/yr and $552 million/yr under CP and efficiency respectively.

Efficient management implies restricting groundwater withdrawals in the early periods with the effect of maintaining a higher water table level than would otherwise occur. This reduces pumping costs and also has a dilution effect on aquifer salt concentration. Later on, evaporation ponds are used to stabilize aquifer salt mass (and concentration) at lower levels than would otherwise occur. These actions contribute to maintaining a higher level of annual net benefits in the basin over the majority of the horizon. Ultimately the differences occur because, under economic efficiency, withdrawals are chosen to reflect both current net benefits as well as impacts on the future, while common property only considers the former. Thus management can be viewed as an investment where growers give up net benefits early on in return for increased net benefits later, the investment being managed to achieve a market rate of return. While common property usage is theoretically and empirically inefficient, whether or not the resource should actually be managed depends on management benefits defined as the difference in present values between common property and economic efficiency. Under the base conditions as defined above and after annualizing, management benefits are $ 25.62 acre-1 yr-1. This estimate exceeds that found previously for this basin considering only water quantity.

DYNAMIC REUSE AND POLICY INSTRUMENTS

The dynamic model was specifically applied to the drainage problem area starting with a high water table condition and initial salt concentration of 10 dS/m. Results are illustrated in figure 3. As can be seen, hydraulic head actually declines very slightly indicating that reuse actually has some beneficial aspects even beyond maintaining salt balance. Aquifer salt concentration increases as expected; however, the increase over a 20-year period is close to negligible and would not likely even be measurable. Decision variables are not reported in order to save space; however, there is very little change over the horizon as would be expected from the small change in the state variables. For common property, there is no change in surface water use while there is a very slight decrease in reuse reflecting the slightly higher pumping costs. For efficiency there is a very slight decrease in surface water usage and a slight increase in reuse. Presumably the latter is to account for the increased salinity of water.

There are 2 conclusions that flow from these observations. First, the efficient management mix is – in principle – dynamic. That is, it will evolve over time in response to the underlying evolution of the aquifer system. Second, these results reinforce the findings of the static regional programming model. Although the system is dynamic, the time rate of salt buildup in the aquifer from reuse activities is very slow. This is due to the relatively small rate of salt mass imported into the basin relative to the existing size of the aquifer and the associated salt mass. Thus the management strategy identified earlier relying heavily on reuse should be viable for several decades, thus allowing development of a long-run sustainable solution to the salinity/drainage problem.

Both groundwater extractions and salt emissions generate externalities. Groundwater flows from an individual user affect hydraulic head and hence pumping and/or drainage disposal costs for all, while salt affects water suitability for production. Clearly salt emissions to the aquifer generate a negative externality. Groundwater withdrawals generate negative externalities in the classic case analyzed in the literature, but can also be a positive externality here, either by helping to reduce a high water table and thereby reducing drainage problems, or by reducing aquifer salt mass via pond disposal. In general there are a variety of policy instruments for alleviating nonpoint pollution. Quantity regulation and/or market establishment policies are reasonably straightforward from the proceeding results. The analysis concentrates on pricing policy.

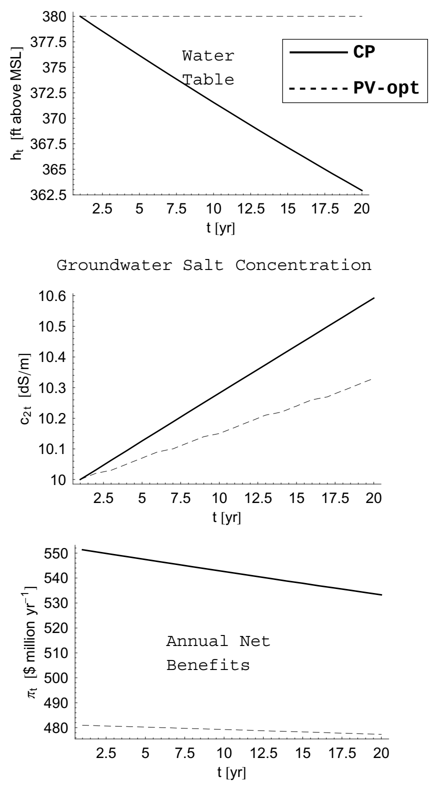

Figure 3. Aquifer dynamics: common property vs economic efficiency.

Price rules for extractions, deep percolation quantity and salt concentration are derived via comparison of optimal decisions under common property and efficiency. The price formulas make explicit the water table and aquifer salt concentration effects. Under classic conditions growers are charged for extractions and compensated for deep percolation, with the reverse under drainage conditions. Charges are always positive for salt emissions while compensation is paid for pond salt disposal.

Figure 4 plots state-dependent regulatory groundwater and salt emission prices. The extraction price is generally increasing in water table elevation, and increases with aquifer salt concentration up to a point, after which it is decreasing. The explanation for this pattern is largely driven by the associated level of efficient groundwater extractions. In particular, as extractions increase then either a unit reduction in pumping lift or a unit decrease in aquifer salt concentration translates into larger cost savings so the regulatory price is also higher. A final consideration is that these results apply for unconstrained drainage. As the aquifer approaches a saline, high water table condition, then extraction prices decrease in elevation, eventually turning negative. This reflects the fact that the high water table is now a liability and hence it is necessary to charge deep percolation flows and subsidize extractions.

Salt emission prices decline with water table elevation at low salinity levels but increase at high salinity levels, and increase with salt concentration up to an elevation-dependent point, after which they decline. As before a driving variable in explaining the pattern is the associated level of groundwater extractions. An additional consideration is the dilution effect apparently overriding the quantity effect resulting in the observed price decline with elevation at lower salinity levels. Figure 4 also reports time-series values evaluated at the efficient intertemporal allocation. The extraction price initially falls during the Resource Extraction era, rises during the Waste emission era, and then declines to an eventual negative level as the aquifer is increasingly salinized and elevated. Salt prices are generally increasing to a steady-state level following an initial transition period. The explanation for the time-series pattern follows from the considerations just noted for state-dependent prices.

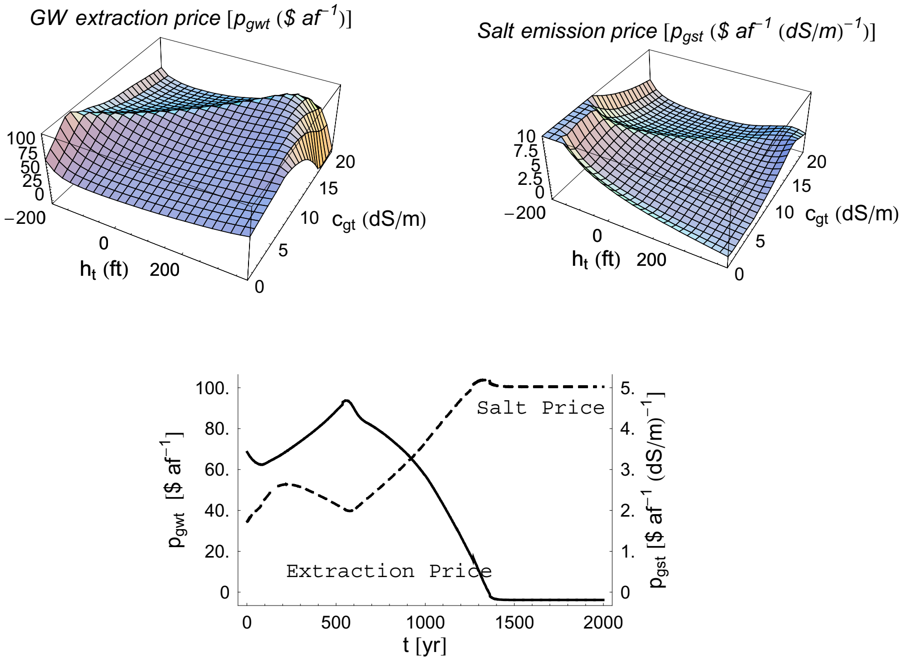

Figure 4. Regulatory prices for the agricultural production/saline aquifer system. (a) State-dependent groundwater extraction prices. (b) State-dependent groundwater salt emission prices. (c) Time-series for extraction and salt emission prices.

CONCLUSIONS

A regional model of agricultural production in a closed drainage basin is developed to provide an integrated analysis of a range of management options for salinity and drainage problems. The empirical analysis for WWD demonstrates that efficiency generally requires use of multiple management strategies. In the base conditions analyzed here, though, reuse was the primary strategy. Drainage control is largely in the form of a transfer of freshwater land area to reuse production, with minimal source control on a per-acre basis (e.g. shift to higher uniformity irrigation systems). Evaporation ponds did not prove to be an efficient mechanism for hydrologic balance under the base conditions.

While reuse appears to be an extremely promising strategy, there are concerns. For one, the primary reuse crop is cotton whose prices have recently been declining. Empirical analysis considering a range of cotton prices did find significant reductions in agricultural income and a partial shift to wheat as the reuse crop. However, reuse remained the primary efficient management strategy. The bulk of the income response from the price change occurred regardless of whether the hydrologic constraint was binding or not. Another concern with reuse as a single disposal strategy is that eventually salt concentrations will rise within the aquifer. However, our results suggest that agricultural production can still be profitably maintained even with a doubling of salt concentration.

The empirical analysis finds modest use, if any, of evaporation ponds. Pond areas range from zero to less than 7% of the land area depending on the range of management options considered. However, several additional factors need to be considered. The analysis here is only for average conditions and pond area comes out of productive land. In general there can be spatial variability such that some land may not be suitable for reuse; conversely there may be low-quality land available for ponds with minimal opportunity cost. Furthermore, if birds and wildlife can be kept off the evaporation ponds, then provision of the compensating habitat may be an environmental benefit, especially in areas such as the SJV where there has already been excessive wetland loss. Well-run evaporation ponds in conjunction with mitigation and compensation measures may well have a larger social role than that found (and analyzed) here.

In general, agricultural production in the basin appears to be economically and environmentally sustainable over intermediate time periods with appropriate management and without significant land retirement.

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Phytoremediation of Selenium-Contaminated Drainage Sediments with Different Transgenic Lines of Indian Mustard (Brassica juncea)

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ABSTRACT

Different genotypes of Indian mustard (Brassica juncea) plants were tested for their efficiency in removing Se from agricultural drainage sediment taken from the San Luis Drain. Analysis of the sediment showed that it had an alkaline pH (8.42), a high EC value (100 dS m-1), and high extractable concentrations of sodium (Na), sulfur (S), magnesium (Mg), selenium (Se), boron (B), and molybdenum (Mo). Indian mustard plants grown in pots in a greenhouse were able to survive and grow only if the soil contained no more than 15% (v/v) sediment; at 0 to 10% sediment, the survival rates were 100% compared to 67% when the soil contained 15% sediment. On 10% sediment, Indian mustard lines maintained between 38 and 70% of their growth on clean soil. The concentration of Se in the shoots of Indian mustard plants increased when the proportion of sediment was increased from 0 to 15%. Transgenic Indian mustard plants overexpressing ATP sulfurylase (APS) and γ-glutamylcysteine synthetase (γ-ECS) accumulated 2 and 3 times more Se in the shoots than wildtype, respectively. Shoot Se concentrations were strongly correlated with shoot S concentrations in Indian mustard transgenic lines overexpressing ATP sulfurylase (APS) and glutathione synthetase (GS).

After Indian mustard plants had been grown for a period of 22 days in pots containing 10% sediment/soil mixture, the total soil Se concentration decreased by 27 and 34% for WT and APS8 lines, respectively, while water-soluble Se decreased by 40% for both WT and APS8 lines. In the sediment/soil without plants (blanks), total Se decreased by 16%; this was due to a 33% loss of water-soluble Se by biological Se volatilization. The insoluble fraction of Se, which did not change in the blank treatment, decreased by 15 and 26% in WT and APS8 lines. Chemical speciation of the water-extractable Se showed that selenate accounted for 92-94% of the Se loss in blank-, WT-, and APS8-treated sediments, while selenite and reduced Se remained constant between untreated sediment and blank-, WT-, and APS8-treated sediments. A mass balance analysis of Se showed that treatment with APS8 plants reduced total Se in the sediment by 37%, while treatment with WT decreased total sediment Se by 23%; total Se in the blank sediment decreased 19%. These results suggest that the APS8 line can be used for more efficient phytoextraction of Se from the drainage sediment.

Salt tolerance of Indian mustard was improved by transforming plants with the AtNHX1 gene encoding the tonoplast sodium/proton antiporter from Arabidopsis thaliana (transformation was achieved using Agrobacterium tumefaciens and hypocotyls). Successful transformants (selected by resistance to hygromycin) showed higher tolerance than WT when grown on 100 mM NaCl. While WT plants had signs of necrosis, the AtNHX1 plants displayed no symptoms of toxicity.

INTRODUCTION

Large amounts of Se-contaminated sediments have been deposited in drainage canals in the Central Valley of California. For example, the drainage sediments in the Broadview Water District of the San Luis Drain contain ~50 to 60 mg Se kg-1. Such high levels of Se pose a substantial ecotoxic risk to local wildlife. In this regard, the EPA has expressed serious concerns about future treatment of the drainage sediment in the San Luis Drain (Federal Register: March 23, 2001, V. 66, No. 57).

Another potential source of Se-contaminated sediments could arise from the use of constructed wetlands as a treatment system for removing Se from agricultural drainage water. Constructed wetlands have been shown to be highly efficient at removing Se from oil refinery wastewater (Hansen et al., 1998), and there is considerable promise in using them as a treatment system for agricultural drainage water, i.e., to decrease the ecotoxicity of solar evaporation ponds (Terry, 1998; Tanji, 1999). Our research at the Tulare Lake Drainage District (TLDD) flow-through constructed wetland at Corcoran showed that vegetated constructed wetland cells removed, on average, 69% of the monthly mass Se input to each wetland cell (Lin and Terry, 2000). In the wetland study carried out at the Chevron oil refinery, almost 90% of the Se was removed from the inflow (Hansen et al., 1998). However, constructed wetlands suffer from the disadvantage that substantial quantities of Se are immobilized in the sediments. Most of the Se removed from drainage water at the TLDD wetlands was retained in the top 10-cm layer of sediment; for example, this Se sink accounted for up to ~56% of the total Se mass input in the wetland cell planted with cordgrass (Lin and Terry, 2000). Over three years, the Se concentration in the top 5-cm layer of sediment in the TLDD wetlands increased from their original background level of ~0.25 to ~10 mg Se kg-1. This buildup of Se in wetland cells over time poses a potential ecotoxic risk to wildlife, which must be considered and dealt with if constructed wetlands are to be used as a cost-effective water treatment system for agricultural drainage water.

There is an urgent need to remediate the large amount of drainage sediments that already exist from drainage canals and will exist if constructed wetlands are to be used to treat agricultural drainage water. One way of achieving this cleanup is by phytoremediation. This is a relatively inexpensive and environment-friendly approach for Se removal (Terry and Banuelos, 2000). There are two ways in which plant- (or plant-microbe-) based systems can be used to remove Se from soils or sediments: phytoextraction and phytovolatilization. The effectiveness of phytoextraction has already been demonstrated in experiments carried out by Banuelos et al. (1997a). These researchers tested the ability of several different crop plants to remediate Se-contaminated soils, showing that Indian mustard vegetation reduced

~47% of the Se from the contaminated soil under field conditions (Banuelos et al., 1997b).

Phytovolatilization is the use of plant-microbe systems to absorb selenate or selenite from soil and metabolize it to volatile Se forms. Measurements of volatilization at the TLDD wetland suggest that volatilization (by plants and microbes) can represent a significant pathway of Se removal: for example, up to 49% of the Se mass input was removed via volatilization in June of 1998 in the rabbitfoot grass cell. This observation is in line with our other Se volatilization studies in an upland ecosystem in which significant amounts of Se were volatilized from contaminated soil in a Salicornia field (Lin et al. 2000; Lin et al. 2002). The removal of Se from sediments by volatilization has distinct advantanges as a remediation technology. This is because volatilization removes Se from the local sediment-plant ecosystem into the atmosphere, thereby minimizing its entry into the food chain. The predominant form of volatile Se produced by plants and microbes, dimethyl selenide (DMSe), is ~600 times less toxic than selenate (Wilber, 1980). Even if volatile Se is deposited at sites distant from the point of origin, this is a relatively small problem in California because most of the state is deficient in Se, and farmers currently provide Se supplements to their sheep and cattle to overcome the deficiency. Thus, the development of Se volatilization as a strategy for the remediation of Se-contaminated sediments is an innovative and environment-friendly way to clean up Se.

Research in our laboratory over the past seven or eight years has centered on developing plant-based systems for enhancing phytoextraction and phytovolatilization of Se. To this end, we have genetically engineered Indian mustard plants that have different potentials for Se phytoremediation. One such transgenic line (referred to as APS) overexpresses the gene encoding ATP-sulfurylase, the rate-limiting enzyme responsible for the reduction of selenate to selenite (Pilon-Smits et al. 1999). Transgenic Indian mustard lines overexpressing γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthase (GS) have increased production of γ-glutamylcysteine (γ-EC) and glutathione (GSH), compounds that are important in protecting against oxidative and metal/metalloid stresses (Zhu et al. 1999a and 1999b). GSH is a major component of the active oxygen scavenging system of the cell and protects plant cells from metal-related oxidative stress damage (Noctor and Foyer, 1998). The APS lines have superior abilities to 1) tolerate high concentrations of Se, and 2) take up 3-fold more Se per plant than the wild type. As the mechanism of the toxicity of selenite is reportedly similar to oxidative stress in yeast (Pinson et al., 2000), overexpressing (γ-ECS and GS could increase the tolerance of transgenic plants to high Se.

Our major goal in this work is to test the efficacy of using different phytoremediating plant systems for the remediation of drainage sediments. We are especially interested in comparing genetically engineered Indian mustard for their efficiencies in Se phytoextraction and phytovolatilization. Thus, we are currently investigating three different phytoremediation treatments, i.e., wildtype Indian mustard, genetically engineered Indian mustard, and unplanted control. The experiments are carried out by growing plants in pots containing drainage sediments in a greenhouse. The pots are used because 1) they avoid the need for special regulatory permission to do field tests with genetically modified organisms, 2) they are far less expensive than field studies, and 3) they allow for more accurate control over experimental conditions. Indian mustard was chosen because of its known ability for Se phytoextraction (Banuelos et al., 1997b); it is tolerant to many toxic conditions, accumulates high concentrations of Se, and rapidly produces a large biomass. The use of the genetically engineered Indian mustard enables us to determine whether these transgenic plants, which have performed well under hydroponic conditions, are also capable of superior Se phytoremediation when planted in soil.

Salinity is a major limiting factor often associated with high Se level in soils typical of the west side of the California Central Valley. Researchers have suggested that plants to be used for phytoremediation of Se must also be salt-tolerant (Banuelos et al. 1996). Tolerance to salinity may involve the exclusion of sodium ions (Na+) from the cytosol and the accumulation of osmoprotectants (such as betaines). Overexpression of a vacuolar Na+/H+ antiporter, AtNhx1, has been reported to successfully confer salt tolerance to Arabidopsis (Apse et al. 1999). In order to improve the salt tolerance of WT and other transgenic lines of Indian mustard, we introduced the AtNHX1 gene into these plant materials via hypocotyl transformation. Successful transformants should have increased tolerance to the saline environment of the sediment, thus leading to more efficient removal of Se from the sediment.

Below we report the results of pot experiments designed to determine 1) the proportion of Se-contaminated sediments to clean soil for establishing the Indian mustard plants, 2) the growth and Se accumulation of different genotypes of Indian mustard on the sediment, 3) changes in the chemical speciation of Se in the sediment during phytoremediation, and 4) the mass balance of Se before and after phytoremediation. Preliminary tests for WT and transgenic Indian mustard (referred as APS8) to volatilize Se and the salt tolerance of WT and AtNHX1 transformants are also reported.

MATERIALS AND METHODS

PLANT AND SEDIMENT MATERIALS

Wildtype Indian mustard (Brassica juncea) seeds were acquired from the North Central Regional Plant Introduction Station, Ames, IA (accession no. 173847). Different transgenic lines of Indian mustard were chosen to compare their efficiency for drainage sediment phytoremediation. The transgenic plants were developed by earlier researchers in our laboratory

(Pilon-Smits et al., 1999; Zhu et al., 1999a and 1999b). APS lines (APS8 and APS9, overexpressing ATP sulfurylase) were chosen because of their higher tolerance and accumulation of selenate. ECS lines (ECS3 and ECS8, overexpressing γ-glutamylcysteine synthetase) and GS lines (GS2 and GS7, overexpressing glutathione synthase) were selected for their increased contents of glutathione, beneficial in scavenging any oxidative stress caused by selenite (Pinson et al., 2000).

The AtNHX1 gene was kindly provided by Dr. Roberto Gaxiola (University of Connecticut, Gaxiola et al. 1999). The NcoI fragment from pRG433 flanking AtNHX1 was cloned by PCR. An XbaI site was introduced to facilitate cloning into the binary vector pCAMBIA1302, which carries the hph gene for hygromycin resistance. AtNHX1 was introduced into WT, APS8, and ECS3 lines of Indian mustard by hypocotyl transformation via Agrobacteria tumefaciens carrying pCAMBIA 1302 with AtNHX1. Calli that successfully developed shoots and roots were transferred to greenhouse for the collection of seeds. Progeny selection was performed by sowing seeds on 1/2 MS agar plates containing 30 mg/L of hygromycin.

Drainage sediment was collected from the San Luis Drain, east of Mendota, at depths ranging from 0-25 cm. After being air-dried at room temperature, the sediment was ground to fine powder to pass a 2-mm sieve before being mixed with clean potting soil.

Growth Condition of the Plant Materials

Indian mustard seeds were sown on a synthetic potting medium moistened with half-Hoagland's solution and kept at 4°C for 2 days to ensure high germination rates. After two weeks, the plants were transferred to 6-inch pots containing compost (Genetic Mix) in the Oxford Greenhouse Facility at UC Berkeley and watered with half-Hoagland's solution every other day. Three weeks after germination, the plants were transferred to soils containing drainage sediment while carefully maintaining the root systems in the original potting soil. The growth conditions of the greenhouse were 22±2 °C and 16 h light/day.

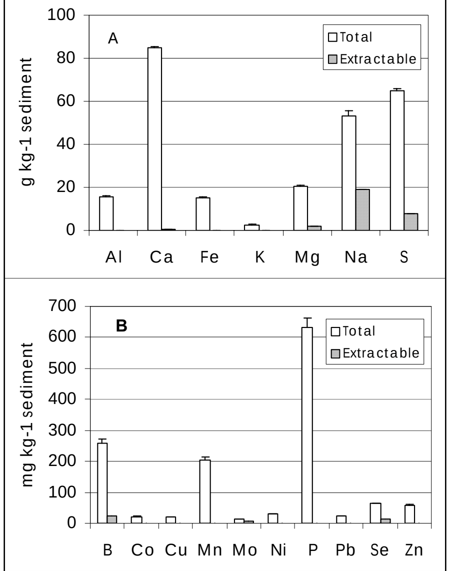

Figure 1. Concentrations of 17 elements in the sediment from San Luis Drain. Total and water-soluble concentrations of A) aluminum (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), and sulfur (S) are expressed in g kg-1 sediment, and B) boron (B), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), phosphorus (P), lead (Pb), selenium (Se), and zinc (Zn) are shown in mg kg-1 sediment. Bars represent the mean and s.e. of 3 replicates.

Pot Experiment

Three week-old Indian mustard plants were transferred into 12-inch pots containing either clean potting soil or potting soil containing different ratios of sediment mixed with clean soil. Plants grown in clean soil were irrigated with half-Hoagland's solution regularly, while those grown in soils containing sediments were watered with deionized water to avoid further buildup of salinity. A plastic tray was placed under each pot to collect the leachate, which was returned to the same pot in each case to avoid the leaching of Se and other salts. On the 10th and 22nd days, the plants were harvested, and their shoots washed with clean deionized water; they were then dried in an oven at 55°C for two days. Plants were harvested according to their developmental stage; the first batch was harvested at the start of bolting and the second at the end of production for greater biomass. After drying, the samples were weighed and ground into a fine powder for the measurement of Se and S contents. The sediment-soil was left to dry in the greenhouse. After removing any plant debris and gravel, the sediment-soil was well mixed and passed through a 2-mm sieve. Soil samples were then collected for the determination of total and extractable Se as described below.

Collection of Volatile Selenium

Se volatilization from Indian mustard plants was determined according to Lin et al. (1999). The plants were left in a volatilization chamber in the greenhouse and the volatile Se collected into a trap solution (5 mM NaOH, 6% H_2O_2) with a pump (25 cm3 min-1) for 24 hours. The air in the volatilization chamber was circulated by a CPU fan to avoid high temperature buildup and to ensure that the concentration of volatile Se inside the chamber was homogenous. The recovery of volatile Se was 99% according to a preliminary test using pure DMSe (containing 5 μg Se) for 24 hours.

Analyses of Plant and Sediment Samples

Total Se concentrations in plant and sediment/soil samples were determined following the procedures described in USEPA Methods 3050B (USEPA 1996) using HGAAS (hydride generation atomic absorption spectrometer). The electrical conductivity (EC) values of the sediment were determined by 1:1 soil-to-water method, using an Orion Model 150 conductivity meter. The water-extractable concentrations of different kinds of metals were determined in the extract of 1:1 soil-to-water solution using inductively-coupled plasma atomic emission spectrometry (ICP-AES). The chemical speciation of soluble Se in the sediment was carried out according to Zhang

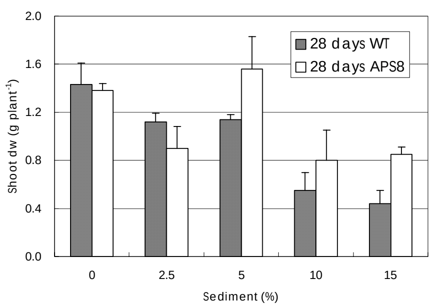

Figure 2. Growth of wildtype and transgenic (line APS8) Indian mustard plants on 0 to 15% of sediment mixed with clean potting soil for 28 days. Bars represent the means and s.e. of three samples.

et al. (1999). The statistical analyses (one-way ANOVA, Pearsons product's moment correlation) were performed using SAS Enterprise Guide software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

PROPERTIES OF DRAINAGE SEDIMENT

The concentrations of 17 elements present in the drainage sediments are shown in Fig. 1. Sodium (Na), sulfur (S), calcium (Ca), iron (Fe), potassium (K), and magnesium (Mg) were present at high concentrations when compared with soils from the same area (e.g., San Joaquin standard reference soil from NIST, no. 2709). The water-extractable concentrations of these elements showed that the predominant solutes were Na (19.04±0.11 mg g-1 sediment), S as sulfate anion (7.98±0.12 mg g-1 sediment), Mg (1.88±0.02 mg g-1 sediment), and Ca (0.28±0.04 mg g-1 sediment). The drainage sediment had an alkaline pH (pH 8.42) and a high EC value (100.5 dS m-1). Total Se concentration in the sediment was ~60-70 mg kg-1 (Fig. 1B). This is high compared to the average Se concentration in most soils, which ranges from 0.1 to 4 mg kg-1 (Kabata-Pendias and Pendias, 1992). Up to 23% of the total Se was extractable. Since B toxicity has been reported in soils containing 20-50 mg kg-1 extractable B and Mo toxicity at extractable Mo levels in soil of 0.2 mg kg-1 (Peverill et al. 1999), the soluble concentrations of boron (B) and molybdenum (Mo) observed here are considered to be high and are probably toxic for plant growth (Fig. 1B).

GROWTH OF DIFFERENT LINES OF INDIAN MUSTARD PLANTS ON SEDIMENT/SOIL

Because the Se-contaminated sediment contained high levels of different salts, plant growth was severely inhibited. In order to determine the optimal ratio of sediment to clean soil for the establishment of Indian mustard plants, pot experiments were carried out in the greenhouse. In a preliminary experiment, three-week-old WT and APS8 Indian mustard plants were transferred to soils with different ratios of sediment from 0 to 40%; both genotypes of Indian mustard were unable to survive in soils with a sediment/soil proportion higher than 20% (data not shown). In a follow-up experiment, the growth of WT and APS8 was determined for sediment/soil mixtures ranging from 0 to 15%. Over the 28-day growth period, shoot dry weights of wild type plants decreased as the proportion of sediment increased; the shoot dry weight of the APS8 line, on the other hand, remained high at 5% sediment/soil and did not decrease until 10 and 15% sediment/soil proportions were reached (Fig. 2). At 15% sediment/soil, the shoot biomass of the APS8 line was 2-fold greater than that of WT. These results show that the APS8 line was better able to tolerate the high levels of Se and other toxic aspects of the sediment/soil than wild type.

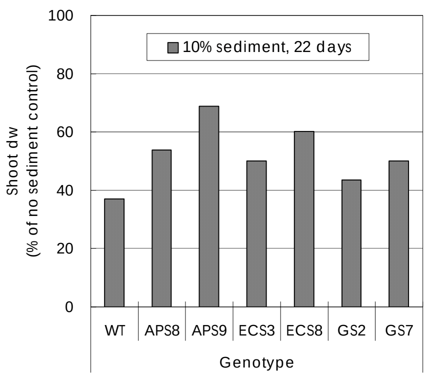

Figure 3. Growth of different genotypes of Indian mustard on 10% sediment/soil mixture compared to that on no-sediment soil for 22 days. Bars represent means and s.e. of 3 to 6 replicates.

Since the survival rates of both genotypes decreased to 67% in 15% sediment (data not shown), the 10% sediment/soil mixture was determined to be the optimum percentage of sediment/soil on which plants should be grown. The growth of different genotypes of Indian mustard was therefore determined on a sediment/soil mixture of 10%. The results showed that transgenic lines of APS (APS8 and APS9), ECS (ECS3 and ECS8), and GS (GS2 and GS7) maintained 40 to 70% of the growth on clean soil, while WT had 38% of that of control (Fig. 3). The highest shoot biomass was attained by the APS9 line.

ACCUMULATION OF SELENIUM BY DIFFERENT LINES OF INDIAN MUSTARD PLANTS

Shoot Se concentration and the total amount of Se accumulated per shoot were determined for wild type and for different transgenic lines of Indian mustard plants grown on 10% sediment (Fig. 4). The ECS lines attained the highest Se concentrations (Fig. 4 A) and accumulated more Se per shoot (Fig. 4B) than other genotypes: after 22 days on 10% sediment, the ECS3 and

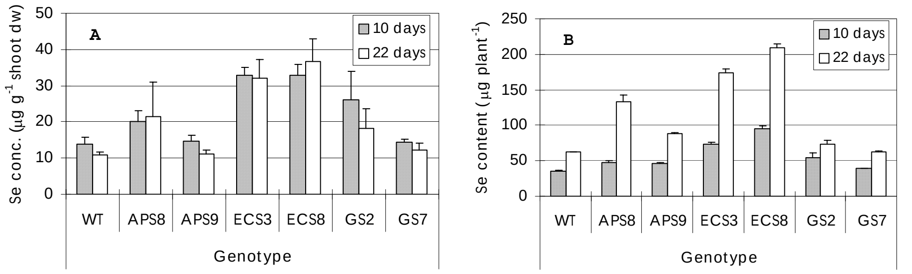

Figure 4. Concentration (A) and total accumulation (B) of Se in the shoots of different genotypes of Indian mustard plants after growing on 10% sediment for 10 and 22 days. Bars represent mean and s.e. of 3 to 6 samples.

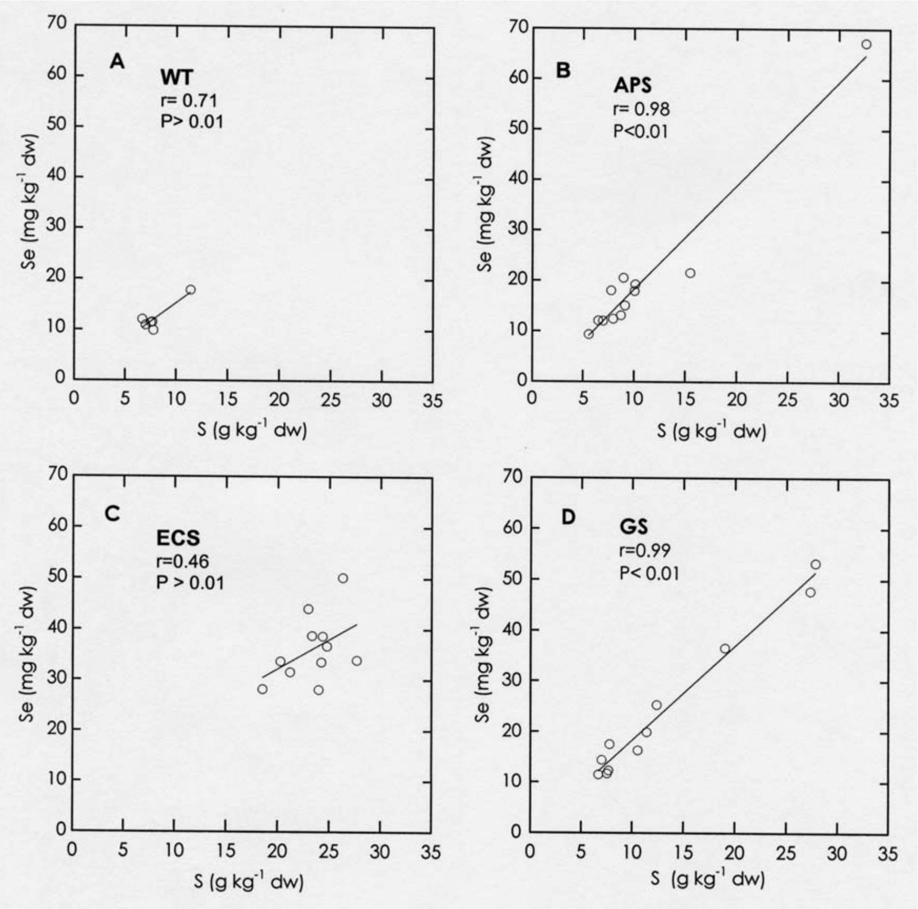

Figure 5. Correlations between S and Se concentrations in the shoots of WT (A), APS (B), ECS (C) and GS (D) lines of Indian mustard plants. The data points are from 10-day and 22-day plants growing on 10% sediment.

ECS8 lines had 2.4- to 3.3-fold higher Se concentrations and 2.8- to 3.4-fold higher Se accumulation in their shoots than WT. The APS8 and APS9 lines accumulated 2.2-times and 45% more Se, respectively, than WT after 22 days on 10% sediment.

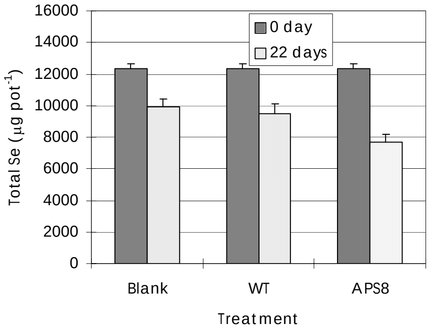

Figure 6. Total Se in 10% sediment before (0 day) and after 22 days of phytoremediation with WT and APS8 lines of Indian mustard. Bars indicate mean and s.e. from at least 6 replicates.

The GS2 line had 89% and 65% higher Se concentrations in the shoot than WT after 10 and 22 days, respectively. However, the total accumulation of Se in both GS2 and GS7 lines was not significantly higher than WT, due to the smaller size of the plants.

APS, ECS, and GS lines are known to accumulate higher Se and S concentrations in their shoots than wild type (Pilon-Smits, 1999; Zhu et al., 1999a and 1999b). This was also observed in the present work. ECS plants accumulated significantly higher concentrations of Se and S (37.16±2.10 mg Se kg-1 dry wt., 23.40±0.79 g S kg-1 dry wt.) than APS (19.34±4.45 mg Se kg-1 dry wt., 10.82±2.11 g S kg-1 dry wt.) or GS (24.29±4.51 mg Se kg-1 dry wt., 13.19±2.39 g S kg-1 dry wt.) plants, which in turn had higher Se and S concentrations than wild type (12.30±1.15 mg Se kg-1 dry wt., 8.01±0.70 g S kg-1 dry wt.).

Because selenate is taken up and assimilated via the sulfur (S) assimilation pathway, there is often a strong correlation between Se and S concentrations in plant tissues (Terry et al., 2000). This was indeed the case in two instances: positive correlations of Se with S were obtained for the shoot tissues of APS (Fig. 5B, r = 0.98, P < 0.01) and GS (Figure 5D, r = 0.99, P < 0.01) lines. However, no such correlations were obtained of Se with S in the ECS or wild type plants (Fig. 5A and C). Interestingly, there was some indication that APS and GS plants were able to take up selenate selectively over sulfate. This is based on the fact that these lines had higher [Se]/[S] ratios in their shoot tissues than ECS or wild type, i.e., 1.842x10-3 for APS and 1.841x10-3 for GS compared to 1.588x10-3 for ECS and 1.535x10-3 for wild type.

CHANGES IN TOTAL AND SOLUBLE FRACTIONS OF SOIL SELENIUM AFTER PHYTOREMEDIATION

A major concern of phytoremediation is its impact on the chemical transformation of Se in the growth substrate. The concentration and amount of total Se in the 10% sediment/soil was determined after 22 days of phytoremediation. The total amount of Se in the pots planted with the APS8 line decreased by 37%, compared to 23% for wild type, and 19% for the blank (unplanted sediment/soil) (Fig. 6). In the sediments treated with WT and APS8 lines of Indian mustard the total Se concentrations decreased by

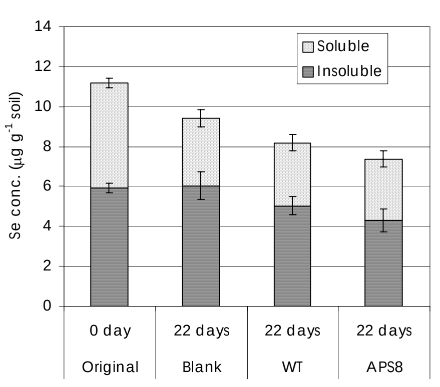

Figure 7. Changes in Se concentration after 22 days of phytoremediation with WT and APS8 lines of Indian mustard plants. Bars indicate mean and s.e. from at least 6 replicates.

27 and 34%, respectively, and by 16% in the sediment without any plant treatment (Fig. 6). The decrease in the insoluble fraction of Se was 0, 15, and 26% in blank, WT, and APS8 treatments, respectively, while the soluble Se concentration decreased 33% in blank and 40% in both WT and APS8 lines (Fig. 7). Thus, the decrease in total Se concentration in the blank was due to a loss of soluble Se (most likely due to loss of Se by microbial volatilization, see below) and not to a change in insoluble Se. The presence of plants growing in the sediment/soil caused a decrease in insoluble Se, the decrease being particularly great when the transgenic line, APS8, was planted.

Speciation of the water-extractable fraction of Se in the sediment/soil after 22 days of treatment (WT, APS8, or blank) revealed that the amounts of Se(II) and Se(IV) did not change significantly from those in the original 10% sediment; 92 to 94% of the loss of Se in the soluble fraction was due to a decrease in Se(VI) (Fig. 8).

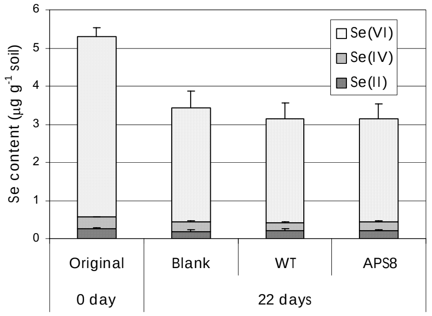

Figure 8. Changes in different chemical species in the soluble fraction of Se before and after 22 days of phytoremediation. Bars indicate mean and s.e. from at least 6 replicates.

SELENIUM VOLATILIZATION OF DIFFERENT INDIAN MUSTARD LINES

Selenium can be removed from the sediment by biological volatilization (plants and microbes). Rates of Se volatilization of WT and APS8 lines were measured during a 22-day period of phytoremediation. There was no significant difference between the two lines of Indian mustard (Fig. 9). Moreover, more volatile Se was recovered from the blank (no plants) than from the pots of WT and APS8. The most likely explanation for this result is that biological volatilization in the soil without plants was greater than in the soil with plants due to a higher moisture content; plants transpired water at such a large rate that significant drying of the soil took place in the planted pots.

IMPROVEMENT OF SALT TOLERANCE OF INDIAN MUSTARD PLANTS

The high saline background of the drainage sediment significantly limits the growth of different lines of Indian mustard plants and, therefore, their capacities to remove Se from the sediment by phytoextraction and phytovolatilization. The introduction of a salt tolerance gene, AtNHX1, into the different lines of Indian mustard improved their tolerance to salts in that WT plants growing on 1/2 MS (Murashige and Skoog) medium with 100 mM NaCl exhibited greater necrosis on their young leaves, while the AtNHX1 transformants showed no symptoms of salt toxicity (Fig. 10).

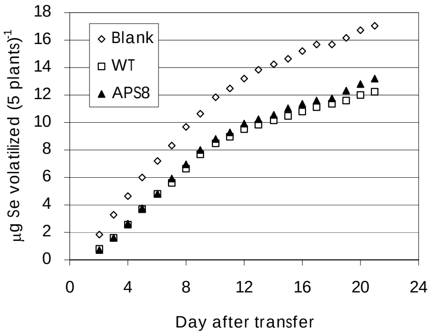

Figure 9. Volatilization of Se during a 22-day period of phytoremediation without plants (blank) and with WT and APS8 Indian mustard lines. Each point is the total volatilized Se collected from 5 independent plants for 24 h.

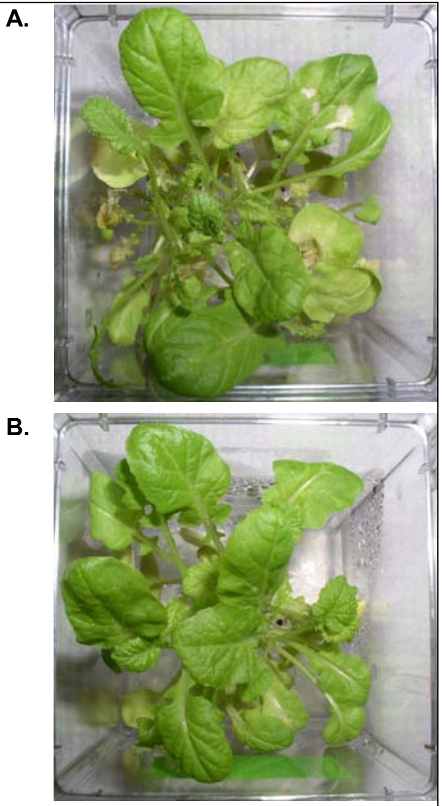

Figure 10. Salt tolerance of WT and WT/AtNHX1 transformant of Indian mustard growing on 100 mM NaCl for 21 days. Each magenta box contained five plants of each genotype.

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