

Salinity/Drainage Program Annual Report 2000-2001



UC Center for Water Resources

2000-2001 Technical Progress Reports

The logo of the University of California Center for Water Resources is a circular seal. It features a shield in the center with a stylized water drop or flame shape. The text "University of California" is arched over the top, and "Center for Water Resources" is arched along the bottom. A vertical line is positioned to the right of the seal.

University of California
**Salinity/Drainage Research Program
and Prosser Trust**

Division of Agriculture and Natural
Resources, University of California

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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. John Letey serves as Director of the Center for Water Resources and is the coordinator of the Salinity/Drainage Program. He also has administrative responsibility for the Prosser Trust Fund. This report documents the 2000-2001 accomplishments of the Salinity/Drainage Program.

A major function of the UC Salinity/Drainage Program is to support research and extension activities which 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. The relevancy of proposals is evaluated by an external advisory committee consisting of individuals listed on page iv. The scientific validity of each proposal is evaluated by peer reviewers. The evaluations of the Advisory Committee and peer reviewers are provided to the Technical Committee which makes recommendations to the Program Coordinator on funding priorities. Members of the Technical Committee are listed on page v. Appreciation is expressed to all the individuals that devoted time and made valuable contributions to the selection of the research to be supported.

The annual Salinity/Drainage Program meeting was held March 28, 2001, in Sacramento. Part of the day was devoted to oral 5-minute synopses of research projects and a half-day poster session displaying the research findings in detail. The full research reports on each project are contained in this report. Reports on other topics of interest were also presented. See page vi for the complete program.

SALINITY/DRAINAGE PROGRAM 2001 ADVISORY COUNCIL

Manucher Alemi

Department of Water Resources
1020 Ninth Street
Sacramento, CA 95814

Ronald Amundson

UC Berkeley
ESPM-Division of Environmental Science
Berkeley, CA 94720

Andrew Chang

UC Riverside
Dept. of Environmental Science
Riverside, CA 92521-0436

Doug Davis

Tulare Lake Drainage District
P.O. Box 985
Corcoran, CA 93212

Mike Delamore

U.S. Bureau of Reclamation
2666 North Grove Industrial Drive
#3106
Fresno, CA 93727-1551

Jose Faria

Department of Water Resources
San Joaquin District
3374 East Shields Avenue
Fresno, CA 93726

Sarge Green

Tranquility Irrigation District
P.O. Box 487
Tranquility, CA 93668

Dan Johnson

U.S. Department of Agriculture - NRCS
2121-C Second Street
Davis, CA 95616

Steve Shaffer

California Department of
Food & Agriculture
1220 N Street
Sacramento, CA 95814

Wayne Verrill

State Water Resources Control Board
Division of Water Quality
1001 I Street, 15th Floor
Sacramento, CA 95814

Robert Zasoski

UC Davis
Department of LAWR
One Shields Ave.
Davis, CA 95616

SALINITY/DRAINAGE PROGRAM 2001 TECHNICAL COMMITTEE

Masoud Ghodrati

UC Berkeley
Dept. of Ecosystems Sciences
151 Hilgard Hall
Berkeley, CA 94720-3110

Roland Meyer

LAWR/Hoagland
One Shields Avenue
129 Hoagland Hall
Davis, CA 95616

Albert Page

UC Riverside
Dept. of Environmental Sciences
2217-A Geology Building
Riverside, CA 92521-0424

Mike Singer

LAWR/Hoagland
One Shields Avenue
171 Hoagland Hall
Davis, CA 95616

UC Annual Salinity/Drainage Conference

March 28, 2001

AGENDA

8:00am **Registration**

8:30 **Welcome**

8:35 **Proposition 204 (Drainage Reuse Subaccount) Projects Update**
Jose Faria, California Department of Water Resources, Fresno

9:05 **Demonstration Projects on Drainage Water Reuse for Irrigation:
What Have We Learned?**
David Birkle & John Letey, University of California, Riverside

9:45 **BREAK**

10:15-Noon

Blaine Hanson, *Response of Crop Yield and Water Table to Subsurface Drip Irrigation of Processing Tomato Under Saline, Shallow Groundwater Conditions (Prosser Trust)*

Norman Terry, *Fate of Selenium in Constructed Wetlands Treating Agricultural Drainage Water: Role of Sediment Se Deposition and Se Volatilization*

Kenneth Tanji, *Mass Balance and Modeling for Se Remediation in TLDD Flow-Through Wetland Cells*

Richard Higashi, *Mitigating Selenium Ecotoxic Risk by Combining Foodchain Breakage with Natural Remediation*

Teresa Fan (Richard Higashi), *Se Ecotoxicology and Application to In Situ Se Bioremediation*

William Frankenberger, *Transformations of Selenium in Tulare Lake Evaporation Ponds*

Michael Fry, *An Investigation into the Ecotoxicology of Selenium Bioaccumulation in Birds*

John Trumble, *Evaluation of Atriplex as a Potential New Crop for Integrated Production Systems in the San Joaquin Valley*

Steve Grattan, *Evaluation of Salt-tolerant Forages for Sequential Reuse Systems*

Steve Kaffka, *Using Forages and Livestock to Manage Drainage Water in the San Joaquin Valley*

Kurt Schwabe, *Economics of Integrated Drainwater Management in the Western San Joaquin Valley*

David Goldhamer (Mario Salinas), *Evaluation of Orchard Irrigation Scheduling Using Trunk Diameter Fluctuations and Shaded Leaf Water Potential (Prosser Trust)*

Noon **LUNCH & POSTER SESSION**

2:00pm *Selenium Bioremediation of Drainage Water in Broadview Irrigation District*
Carla Scheidlinger, Agrarian Research & Management Co.

2:30 *Using Invertebrates as a Biological Treatment for the Reduction of Selenium in Agricultural Drainage Systems. Can We Make Biomagnification Work for Us?*
Panel: John Shelton, California Department of Water Resources, Fresno
Robert Rofen, Novalek Inc.
Teresa Fan, University of California, Davis

4:00 *Video: The San Joaquin Valley Drainage: In Pursuit of Solutions*
Manucher Alemi, California Department of Water Resources, Sacramento



Mitigating Selenium Ecotoxic Risk by Combining Foodchain Breakage with Natural Remediation

Project Investigators:

Teresa W-M. Fan, Dept. Land, Air & Water Resources, UC Davis

Richard M. Higashi, Crocker Nuclear Laboratory, UC Davis

Collaborators:

Dr. Jarka Komarkova, Hydrobiological Institute AS CR, Czech Republic

Dr. Robert Rofen, Novalek, Inc.

Julie Vance, California Department of Water Resources

Research Staff:

Jian Huang, LA

Mia Park, PGRI

Teresa James, SRAIII

ABSTRACT

Se undergoes complex biogeochemistry in aquatic environments. In particular, Se biotransformations and foodchain accumulation by aquatic biota represent a key to ecotoxic risk and site-specific variations in Se impact as well as mitigation of Se risk. Se volatilization by aquatic algae could be an important force in removing total and bioavailable Se from the food source, although algae also bioconcentrate Se to pose risk. To resolve this dilemma, we are working with Dr. Robert Rofen to develop and test the concept of combining algal volatilization with macroinvertebrate harvest to remove Se while disrupting foodchain transfer to top predators. Since the key to the success of this scheme is the understanding of algal community for sustaining Se volatilization and macroinvertebrate harvest, we also elicit the expertise of Dr. Jarka Komarkova to characterize algal community and dynamics at TLDD evaporation basins where brine shrimp harvest is occurring. Three objectives will be fulfilled with this project: (1) assessing effects of fertilizer inputs on algal community for optimizing the harvest of brine shrimp (*Artemia franciscana*) and other macroinvertebrates as well as Se volatilization so that total and bioavailable Se are reduced in TLDD basins; (2) evaluating Se status in algae and invertebrate populations under intensive commercial harvest of brine shrimp and other macroinvertebrates in TLDD basins; (3) uncovering reliable Se ecotoxic indicators in agricultural drainage systems to assist with Objectives (1) and (2). Initial characterization of Se volatilization potential in different cells of TLDD evaporation basins indicates that this potential was much higher in fertilized hypersaline cells (where intensive *Artemia* harvest are occurring) than less saline cells. The Se forms (dimethylselenide and dimethyldiselenide) volatilized also differed among cells, suggesting contribution from different algal activities and/or biogeochemical conditions. Corresponding algal community analysis indicates that the species variation was more diverse in less saline than hypersaline cells. The latter was predominated by *Chlorella* or *Oocystis*, suggesting that these species may be conducive for both *Artemia* growth and Se volatilization. The effect of *Artemia* harvest on

Se status in waters, algae, and macroinvertebrates was also examined. Relative to the less saline cells, the harvested hypersaline cells showed a lower algal Se burden, in addition to a stronger negative correlation of algal Se burden with water salinity or waterborne Se concentration. This suggests that the combination of Se volatilization and *Artemia* harvest could lower the algal Se burden and underlie the less-than-expected buildup of waterborne Se concentration during high evaporation months. These initial results are encouraging but need to be substantiated with further research including validation on the trend in ecotoxic indicators. For uncovering Se ecotoxic indicator(s), we found that proteinaceous Se, and the selenomethionine (Se-Met) form in particular, was biomagnified up the foodchain, which shows promise to be a good indicator for evaluating the ecotoxic status in *Artemia*-harvested systems.

KEYWORDS: Se volatilization, brine shrimp (*Artemia franciscana*), foodchain disruption, bioremediation, ecotoxic risk, algae, macroinvertebrates, proteinaceous selenomethionine, evaporation basin

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BACKGROUND & OBJECTIVES

The use of evaporation ponds for agricultural drainage disposal in the San Joaquin Valley has resulted in the buildup of salts and contaminants (Se in particular) in these ponds, which threatens agricultural production in California. Selenium undergoes a complex biogeochemical cycling in the environment, including biotransformations of inorganic oxyanions (the main contaminant forms in the aquatic environment) into organo-selenium compounds by primary and secondary producers, which are subsequently transferred and accumulated through the aquatic foodweb. It is clear that Se biotransformations and foodchain transfer/accumulation are the key to its adverse effects expressed in top predator bird and fish species (EPA Peer Consultation Workshop, 1998; Adams et al., 1997; Riedel et al., 1996; Maier and Knight 1994; Saiki et al. 1993; Skorupa and Ohlendorf, 1991). A mechanistic understanding of these

processes not only is important to developing in situ means for remediating Se contamination problems but also is needed for evaluating the efficacy of the approach. Unfortunately, our current knowledge in this area is very limited.

Our research aim has been and continues to be generating understanding in Se biotransformations and foodchain transfer such that sustainable measures for mitigating Se risk in agricultural drainage disposal systems can be developed. Our previous work has shown that Se volatilization by algal activity may be an important route for removing Se from agricultural drainage evaporation basins of the Tulare Lake Drainage District (TLDD) (Fan et al., 1998b; Fan and Higashi, 1998; Salinity/Drainage reports, 1999&2000). On the other hand, while Se is volatilized by algae, it is also bioconcentrated into algal biomass, which could pose ecotoxic risk. To resolve this dilemma, we initiated a collaboration with Dr. Robert Rofen at Novalek (who has decades of experience in brine shrimp biology and commercial harvest) to investigate the efficacy of combining algal volatilization of Se and macroinvertebrate harvest to remediate Se risk in TLDD evaporation basins. The conceptual framework of this approach is illustrated in Figure 1. One key factor to the success of this scheme is the ability to manipulate appropriate algal populations to sustain both Se volatilization and brine shrimp harvest. Thus, detailed information on algal community and dynamics is essential. To gain insight into these aspects in evaporation basins, we initiated another collaboration with Dr. Jarka Komarkova of the Czech Republic Academy of Sciences, who is an international expert on aquatic algal ecology. Moreover, we continue to collaborate with Julie Vance of the Department of Water Resources on macroinvertebrate ecology.

Our research efforts at the TLDD evaporation basins have the following three objectives:

- (1) assessing effects of fertilizer inputs on algal community for optimizing the harvest of brine shrimp (*Artemia franciscana*) and other macroinvertebrates as well as Se volatilization so that total and bioavailable Se are reduced in TLDD basins;
- (2) evaluating Se status in algae and invertebrate populations under intensive

commercial harvest of brine shrimp and other macroinvertebrates in TLDD basins;

- (3) uncovering reliable Se ecotoxic indicators in agricultural drainage systems to assist with Objectives (1) and (2).

SUMMARY OF PROGRESS

SE VOLATILIZATION AND ALGAL COMMUNITY IN TLDD EVAPORATION BASINS (OBJECTIVE 1)

From our previous characterization of Se volatilization from TLDD evaporation basins, it is clear that algae are active players in these euryhaline waters (Fan et al., 1997; Fan et al., 1998b; Fan and Higashi, 1998). These results were derived mainly from laboratory studies. In the past two years, we have been characterizing Se volatilization property in situ in several TLDD systems including the Hacienda and South Evaporation basins (HEB and SEB) as well as the demonstration flow-thru wetland (FTW). The Se volatiles from waters were purged on-site with He and trapped in liquid argon for Se forms or in alkaline peroxide for total Se. Total Se in the alkaline peroxide trap was determined using the microfluorescence method (Fan et al., 1998b) while the volatile Se forms in the liquid argon trap were analyzed by GC-MS. This approach measures the Se volatilization potential from site waters, which was shown by us to be positively related to the in situ rate of Se volatilization (Salinity/Drainage report, 2000). Figure 2 show the comparison of volatile Se form profiles from waters of FTW, HEB, and SEB. The dominant forms dimethylselenide (DMSe) and dimethyldiselenides (DMDSe) varied over an order of magnitude among site waters, presumably reflecting different producer communities (see Table 1) and/or biogeochemical conditions. The volatile S form dimethylsulfide (DMS) also differed among site waters and did not follow the same pattern as DMSe, except for the SEB waters. This deviation of DMS and DMSe release suggests a different biological mechanism underlying their production and that DMS cannot be used as a surrogate for predicting DMSe production. It is also clear that Se volatilization potential was related to the salinity of the water, being highest from the most saline waters of SEB and lowest in the least saline waters of FTW.

Initial microscopic examination of HEB, SEB, and FTW waters revealed a number of algal species, some of which are illustrated in Figure 3. Major algal groups including cyanobacteria, diatoms, and green phytoplankton are all present in these waters. A closer survey of the evaporation basin waters yielded different profiles of algal community, as shown in Tables 1 and 2. The % algal biomass (or abundance) was dominated by *Oocystis* and/or *Chlorella* in the hypersaline waters of HEB and SEB cells (i.e. HEB A4 and C4; SEB 9 and 10) while *Prymnesium*, pennate diatoms, *Synechocystis*, *Cyclotella*, and *Chaetoceros* were abundant in less saline cell waters. As expected, the algal species diversity was substantially reduced in the hypersaline waters relative to the less saline waters (Tables 1&2). It is interesting to note that the algal community in HEB A cell series differed significantly from that in HEB C cell series, even though these two cell series received the same subsurface drain water. This difference was also noted in our previous investigation and was related to a different Se volatilization characteristics (Salinity/Drainage report, 1998). Moreover, the changes in algal abundance was rather dynamic, varying significantly over a 2-week period, as shown in Figure 4. In particular the dominance algal species in HEB A1 cell changed from *Synechocystis* to *Chaetoceros*.

Despite the variability and dynamics described above, the hypersaline cells that demonstrated the highest Se volatilization potential (Figure 2) (SEB 9, in particular) were predominated by a green alga, *Chlorella*. This observation brings up the possibility that *Chlorella* may be a dominant player in Se volatilization in these saline waters. In addition, SEB 9 had received turkey manure and phosphoric acid as fertilizers, which supported the highest *Artemia* harvest activity in the past year (R. Rofen and M. Almeida, personal communication). This suggests the possibility that *Chlorella* may be a good source of food for *Artemia*.

SE STATUS IN ARTEMIA-HARVESTED TLDD EVAPORATION BASINS (OBJECTIVES 2)

To investigate the influence of fertilization samples from various cells of SEB and HEB for Se analysis. Figure 5 shows the plot of water salinity (A) or Se concentration (B) against Se

burden in algae collected from cells with and without *Artemia* harvest activity as well as Se burden in harvested *Artemia*. In addition, Figure 6 compares the monthly amount of *Artemia* harvest with the corresponding waterborne Se concentration and algal Se burden. The algae Se burden in hypersaline waters with *Artemia* harvest was generally lower than that in less saline waters without harvest. Overall, a negative correlation was observed for algal Se burden and water salinity but the correlation was much weaker for without harvest than with harvest (R^2 shown, Fig. 5A). Similar trends applied for waterborne Se concentration versus algal Se burden, where algal Se burden tended to be lower and less scattered in harvested cells than cells without harvest, even though the waterborne Se concentration was sometimes higher in the harvested cells (Fig. 5B). This means that the Se bioconcentration factor for algae from harvested waters was generally lower than that from waters without harvest. Moreover, the Se burden in harvested *Artemia* and their algal food source appeared to be comparable, which indicates that *Artemia* did not bioconcentrate Se from algae (Fig. 5). It should also be pointed out that since hypersalinity is a crucial condition for sustainable *Artemia* harvest, it is at present difficult to distinguish the effect of hypersalinity from *Artemia* harvest. In any rate, the two factors combined seemed to reduce the Se load in algae and attenuate the buildup of waterborne Se concentration due to evaporation (Fig. 5B & 6). These effects are likely to be a result of Se removal via both volatilization activity (see Fig. 2) and *Artemia* harvest. As for the Se status in the corresponding sediments, a reducing trend in Se load was observed for *Artemia*-harvested cells, when compared with those without the harvest (see report by Richard Higashi).

Therefore, these preliminary results suggest that sustained algal activity and *Artemia* harvest in the hypersaline TLDD evaporation ponds could lead to a reduction of both Se load and ecotoxic risk in these ponds. A longer-term characterization of the Se status and ecotoxic indicators in the TLDD ponds should help substantiate these initial findings and contribute towards developing efficacious means for reducing Se risk in evaporation basins.

SE ECOTOXIC INDICATOR(S) IN AGRICULTURAL DRAINAGE SYSTEMS (OBJECTIVE 3)

As indicated above, reliable Se ecotoxic indicator(s) are crucial to evaluating the success of utilizing macroinvertebrate harvest and algal volatilization for reducing Se risk in agricultural drainage systems. A reliable Se ecotoxic indicator should be one that is successively enriched up the foodchain and related to Se toxicity in top predators. To uncover such indicator(s), we have developed the methodology to speciate organic Se forms in the foodchain organisms of TLDD basins. Our focus has been on determining the proteinaceous Se forms, since these forms are highly bioavailable, and therefore likely to be bio-magnified up the foodchain. Figure 7 shows the bioconcentration factors (BCF) of total Se in biomass, total Se in protein fraction, and proteinaceous selenomethionine (Se-Met) from water for algae and macro-invertebrates collected from HEB and SEB. Due to the large

range of BCF, this axis was displayed in log scale. It is interesting to note the trend that the BCF of total Se burden for macroinvertebrates was comparable to that for algae (see also Fig. 5), while the BCF's of proteinaceous Se and Se-Met for macroinvertebrates were progressively higher than the respective algal BCF's. This result indicates that protein Se (and the Se-Met component, in particular) is biomagnified from algae to macroinvertebrates.

Thus, proteinaceous Se-Met or Se should be a more reliable indicator of Se foodchain transfer processes than total Se burden or waterborne Se concentration. It is, however, still unclear whether protein-bound Se is linked to Se toxicity such as reproductive failure and embryonic deformity in sensitive bird or fish species. Such information would require parallel field and laboratory feeding studies.

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PUBLICATIONS & REPORTS

MANUSCRIPT SUBMITTED

Fan, T.W.-M., Teh, S., Hinton, D.E., and Higashi, R.M., Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Submitted to *Aquatic Toxicology*, a special Se issue.

MANUSCRIPT IN PREPARATION

Fan, T.W.-M, Pruszkowski, E., and Shuttleworth, S. Speciation of selenoproteins in Se-contaminated wildlife by gel electrophoresis and laser ablation-ICP-MS.

REPORTS

"Se Biogeochemistry, Ecotoxicology, and Bioremediation", Invited talk at the Annual meeting of the American Chemical Society (Apr., 2000).

"Se biogeochemistry and In situ bioremediation in Se-laden aquatic ecosystems", PacificChem meeting of the American Chemical Society (Aug., 2000)

"Bioremediating Selenium Ecotoxic Risk: A Biogeochemical Approach", Invited talk at the Dept. of Soil and Environmental Sciences, Univ. of California, Riverside (Feb., 2001)

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Table 1. Summary of algal community in HEB¹, June-July 00

Sites	A4	Sites	A3	Sites	A2	Sites	A1
Salinity	156 ppt	Salinity	28 ppt	Salinity	15 ppt	Salinity	6 ppt
Total Biomass²	192.2 mg/L	Total Biomass	15.5 mg/L	Total Biomass	59.3 mg/L	Total Biomass	138.7 mg/L
Diversity index	0.47	Diversity index	0.44	Diversity index	0.91	Diversity index	0.60
Algae Species	% Biomass	Algae Species	% Biomass	Algae Species	% Biomass	Algae Species	% Biomass
Oocystis cf.	64.0	Prymnesium sp.round	72.2	Prymnesium sp.round	20.4	Chaetoceros sp.	47.5
Prymnesium sp.round	17.2	Oocystis parva	16.9	Oocystis cf.	19.3	Chaetoceros 8	28.4
Chlorella sal.	10.2	Oocystis cf.	2.6	Oocystis cf.	13.5	Oocystis cf.	10.2
Chlorella sal.	7.1	Cryptomonas salin.smallg	1.9	Pennátní rozsivky	12.5	Oocystis cf.	9.5
Dunaliella narrow	1.1	Gymnodinium spec.	1.6	Oocystis parva	11.7	Pennátní rozsivky	2.1
Dunaliella narrow	0.1	Stephanodiscus hantzschii	1.5	Cryptomonas marssonii	9.4	Chlamydomonas spec.	0.5
Indeterminatae genera	0.1	Pyramimonas salin.	1.3	Limnithrix amphigranulata	5.6	Pseudanabaena limnetica	0.4
Synechococcus sal.	0.1	Limnithrix amphigranulata	0.5	Indeterminatae genera	3.9	Cryptomonas marssonii	0.4
Synechococcus sal.	0.0	Rhodomonas minuta	0.4	Gymnodinium spec.	1.9	Chlamydomonas spec.	0.3
		Chlorella sal.	0.3	Chlorella sal.	1.1	Chlorella sal.	0.3
		Stephanodiscus parvus	0.3	Chlorella sal.	0.4	Rhodomonas minuta	0.1
		Chlamydomonas spec.	0.2	Chlamydomonas spec.	0.4	Synechocystis salin.	0.1
		Chlorella sal.	0.2	Aphanocapsa delicatissima	0.0	Anabaenopsis salin.	0.1
				Cylindrospermopsis catemaco	0.0	Scenedesmus spec.	0.1
						Aphanothece minutissima	0.0
Sites	C4	Sites	C3	Sites	C2	Sites	C1
Salinity	136 ppt	Salinity	66	Salinity	28 ppt	Salinity	6 ppt
Total Biomass	4.3 mg/L	Total Biomass	59.8	Total Biomass	101.5 mg/L	Total Biomass	5.6 mg/L
Diversity index	0.61	Diversity index	0.73	Diversity index	1.07	Diversity index	0.95
Algae Species	% Biomass	Algae Species	% Biomass	Algae Species	% Biomass	Algae Species	% Biomass
Chlorella sal.	44.8	Pennátní rozsivky	41.4	Aphanocapsa +	23.4	Cyclotella saline	31.3
Stichococcus spec.	34.2	Aphanocapsa +	21.8	Synechocystis salin.	13.4	Chaetoceros sp.	14.9
Chlorella sal.	7.9	Limnithrix amphigranulata	16.0	Synechocystis salin.2b.	12.7	Monoraphidium capric.	14.6
Synechocystis salin.	4.7	Chlorella sal.	9.7	Oocystis parva	10.3	Stephanodiscus salin.	8.8
Spermzop salin.	2.5	Indeterminatae genera	2.7	Pyramimonas salin.	7.5	Oocystis parva	5.9
Synechococcus sal.	2.1	Chlorella sal.	1.8	Indeterminatae genera	5.1	Pennátní rozsivky	5.3
Leibleinia sal.	1.6	Leibleinia sal.	1.7	Synechocystis sal.	4.2	Chaetoceros 8	4.0
Synechococcus sal.S	1.3	Chlorella sal.	1.7	Chaetoceros 8	4.1	Cyanodictyon salin.	3.5
Synechococcus sal.S	0.9	Synechocystis salin.	1.6	Chlamydomonas spec.	3.0	Indeterminatae genera	3.3
		Stichococcus spec.	0.5	Synechocystis salin.	2.8	Synechocystis salin.	2.3
		Indeterminatae genera	0.4	Limnithrix amphigranulata	2.8	Cyclotella pseudostelligera	1.5
		Synechococcus sal.S	0.4	Leibleinia sal.	2.7	Pennátní rozsivky	1.5
		Cylindrospermopsis catemaco	0.2	Pyramimonas salin.	1.7	Oocystis cf.	1.5
		Synechococcus sal.S	0.1	Synechococcus sal.S	1.6	Chlorella sal.	0.6
				Anabaenopsis salin.	1.4	Leibleinia sal.	0.6
				Cylindrospermopsis catemaco	1.1	Rhodomonas sal.sp	0.2
				Indeterminatae genera	1.1	Prymnesium sp.round	0.2
				Dunaliella narrow	0.5		
				Chlorella sal.	0.5		

¹HEB, Hacienda evaporation basin

²Total biomass was estimated from algal cell volumes, assuming cell density is approximately 1 g/ml.

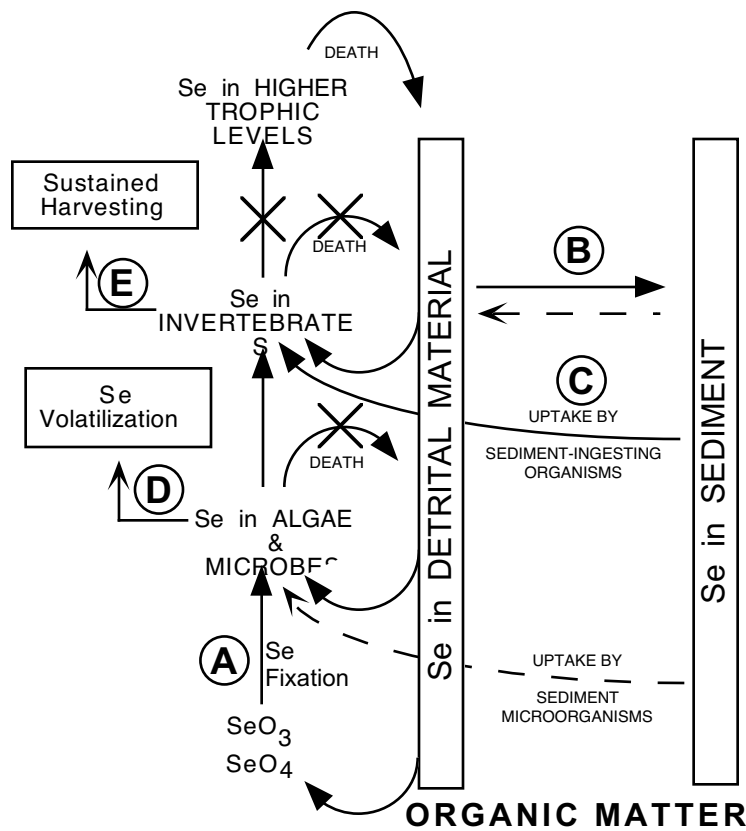


Figure 1. Reducing Se ecotoxic risk in drainage basins by invertebrate harvest and Se volatilization

In this "biogeochemical reflux" scheme, the drainage inorganic Se forms are initially biologically fixed by aquatic algae & microbes (A). The fixed Se does not directly head up the foodchain in the water column, as is often portrayed. Instead, a major fraction enters into organic matter, taking a detour through detritus (recently dead organic matter) and sediment, then re-entering the foodchain at several trophic levels. Over longer periods, part of the detrital material is converted to recalcitrant humic material (B), locking up the Se until sediment-ingesting organisms reintroduce them to the foodchain (C).

Through sustained harvesting (D) of water-column invertebrates that consume algae and microbes, the bioavailable Se is removed from water, plus detrital formation resulting from the death of water-column organisms is also blocked. Both types of blockages are shown by the three "X"s. In turn, this would help minimize the sediment-detritus foodchain pathway for Se. In the meanwhile, additional Se can be removed by manipulating the algae/microbe community for optimal Se volatilization (E). This scheme would greatly improve the ecological "safety" margin of operating drainage basins. The economic efficacy of the approach is clear: (a) much of the scheme is water management; (b) costs of encouraging and managing brine shrimp growth is offset by marketing harvested materials.

Fig. 2

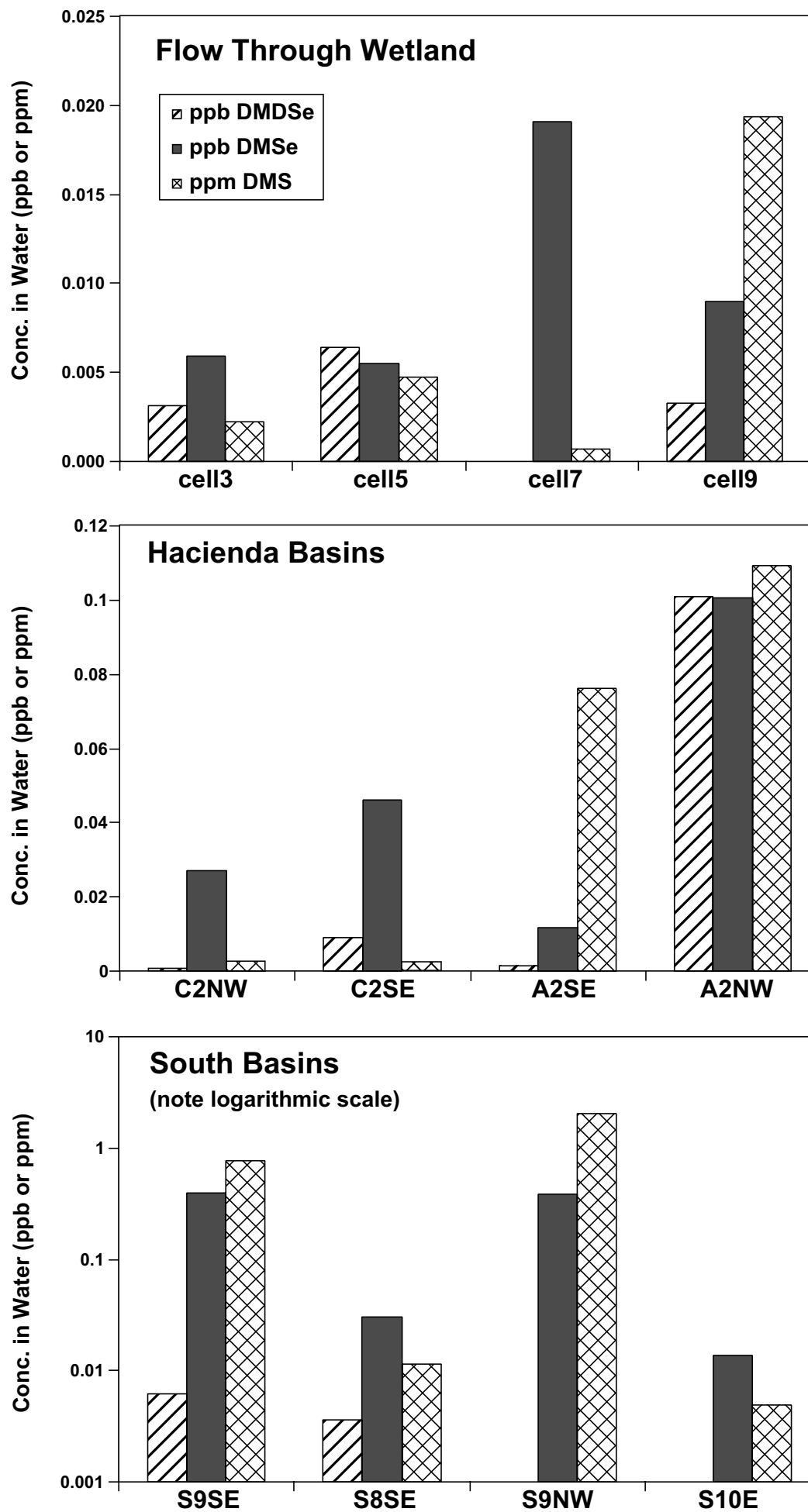


Fig. 3



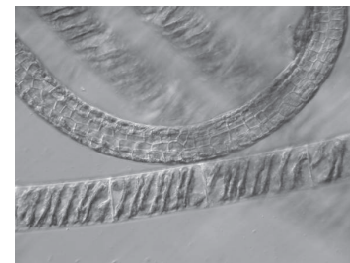
Lyngbya



Diatom mix



Oscillatoria



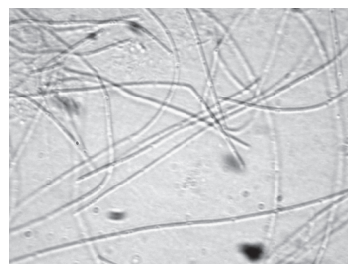
Spirogyra&Filam



Spirulina



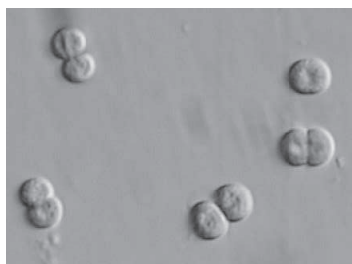
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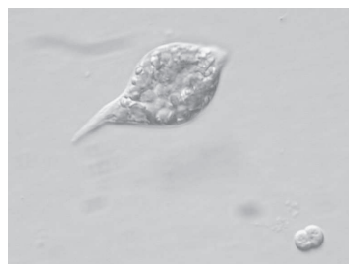
Limnothr.



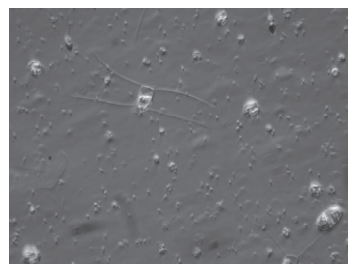
Oscillatoria&Phormidium



Synechocystis



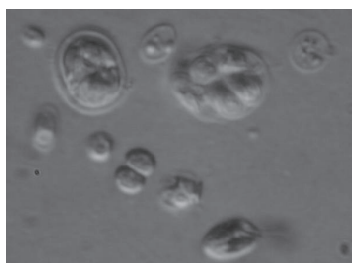
Euglena



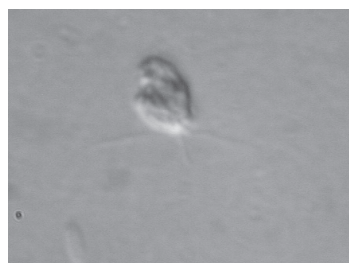
Chaetoceros



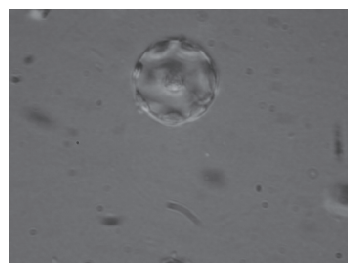
Anabaenopsis



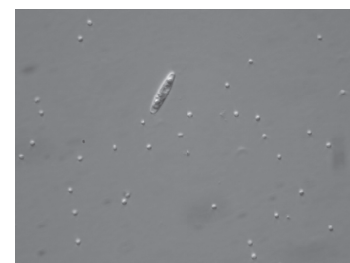
Oocystis



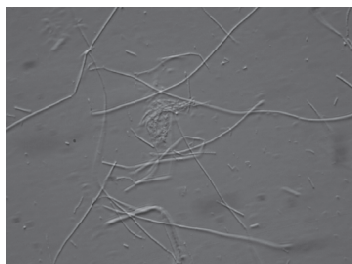
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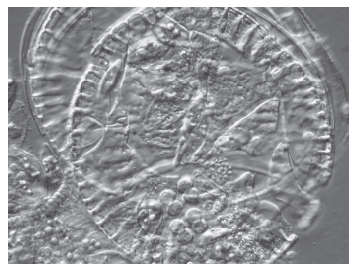
Cyclotella



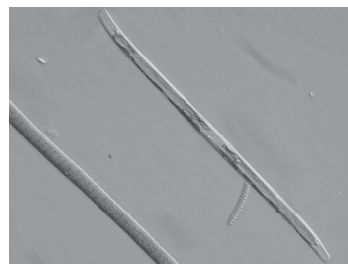
Navicula&Chlorella



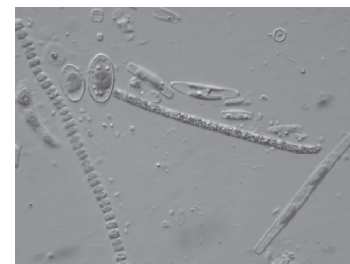
Leibleinia



Campylo



Nitzschia



Beggiatoa&Ozvocit

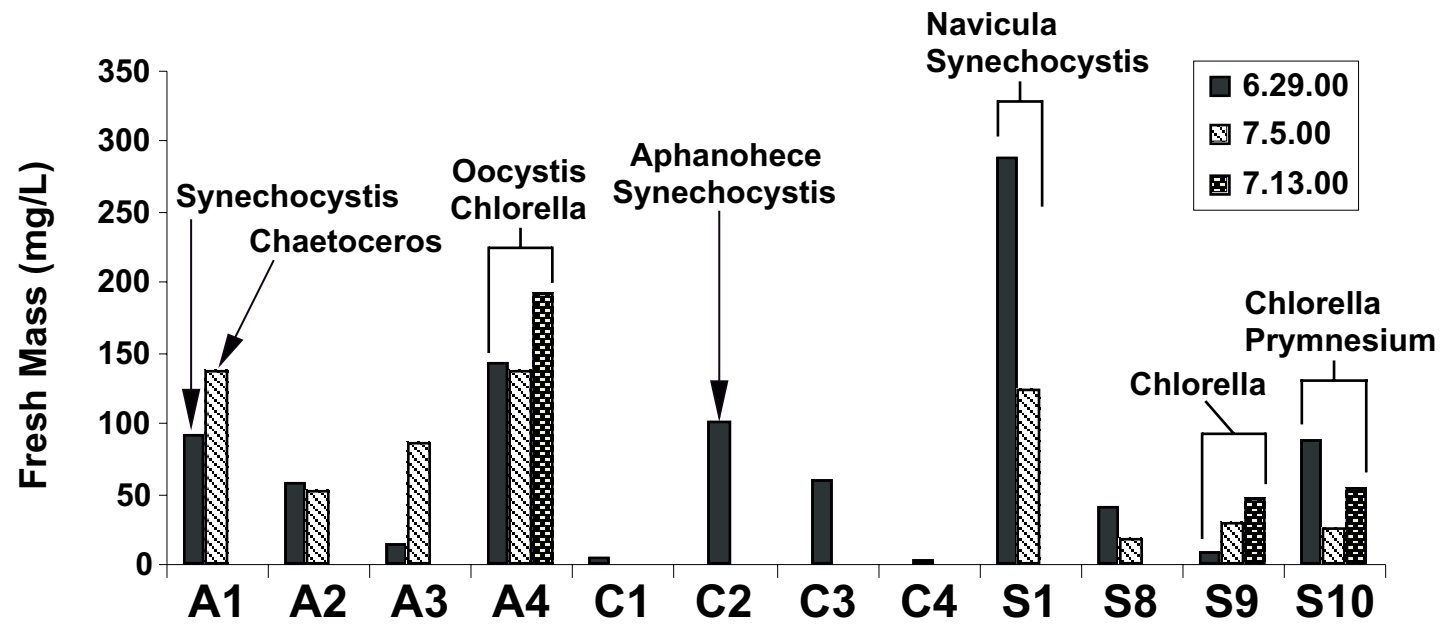
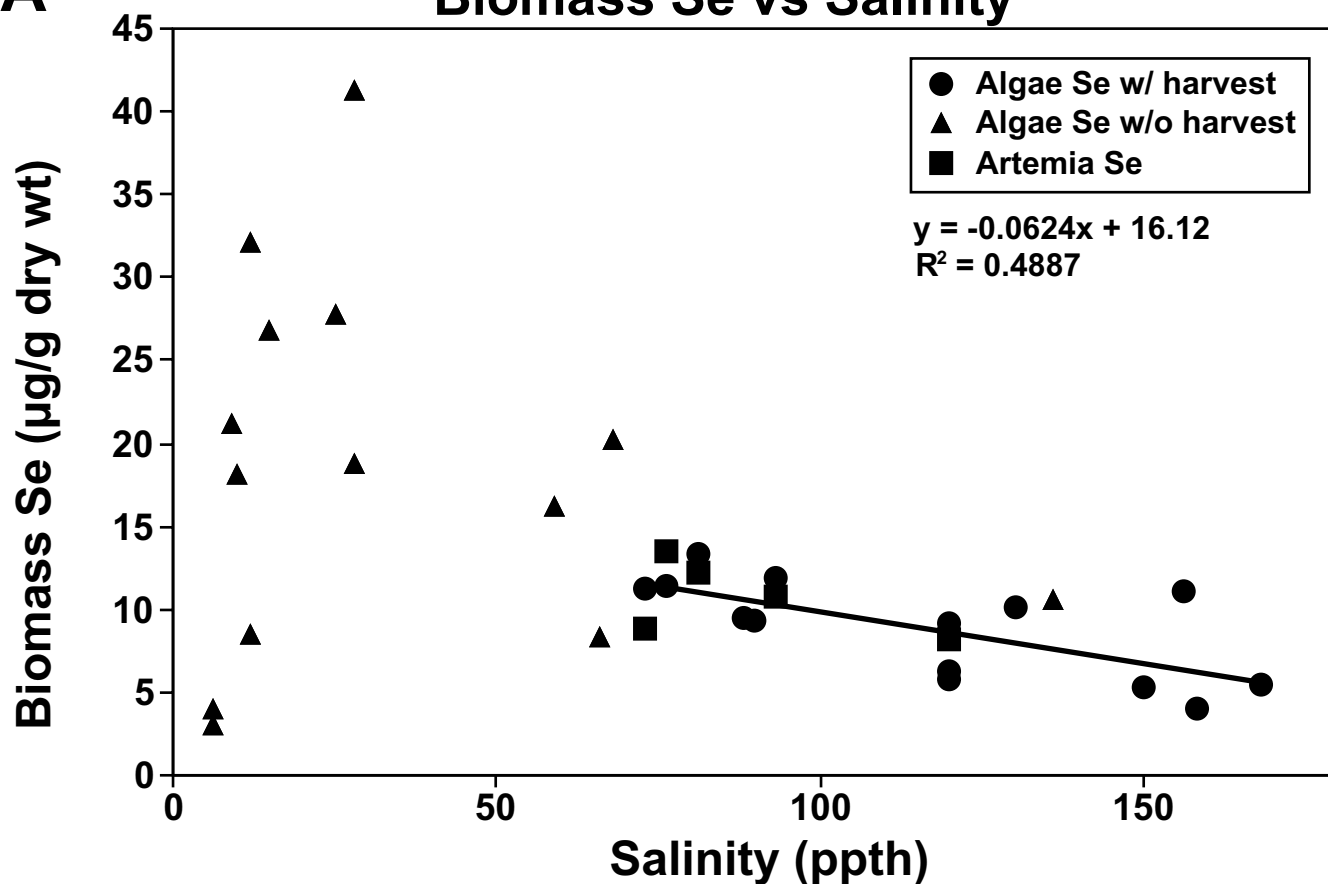


Fig. 5

A

Biomass Se vs Salinity



B

Biomass Se vs Waterborne Se

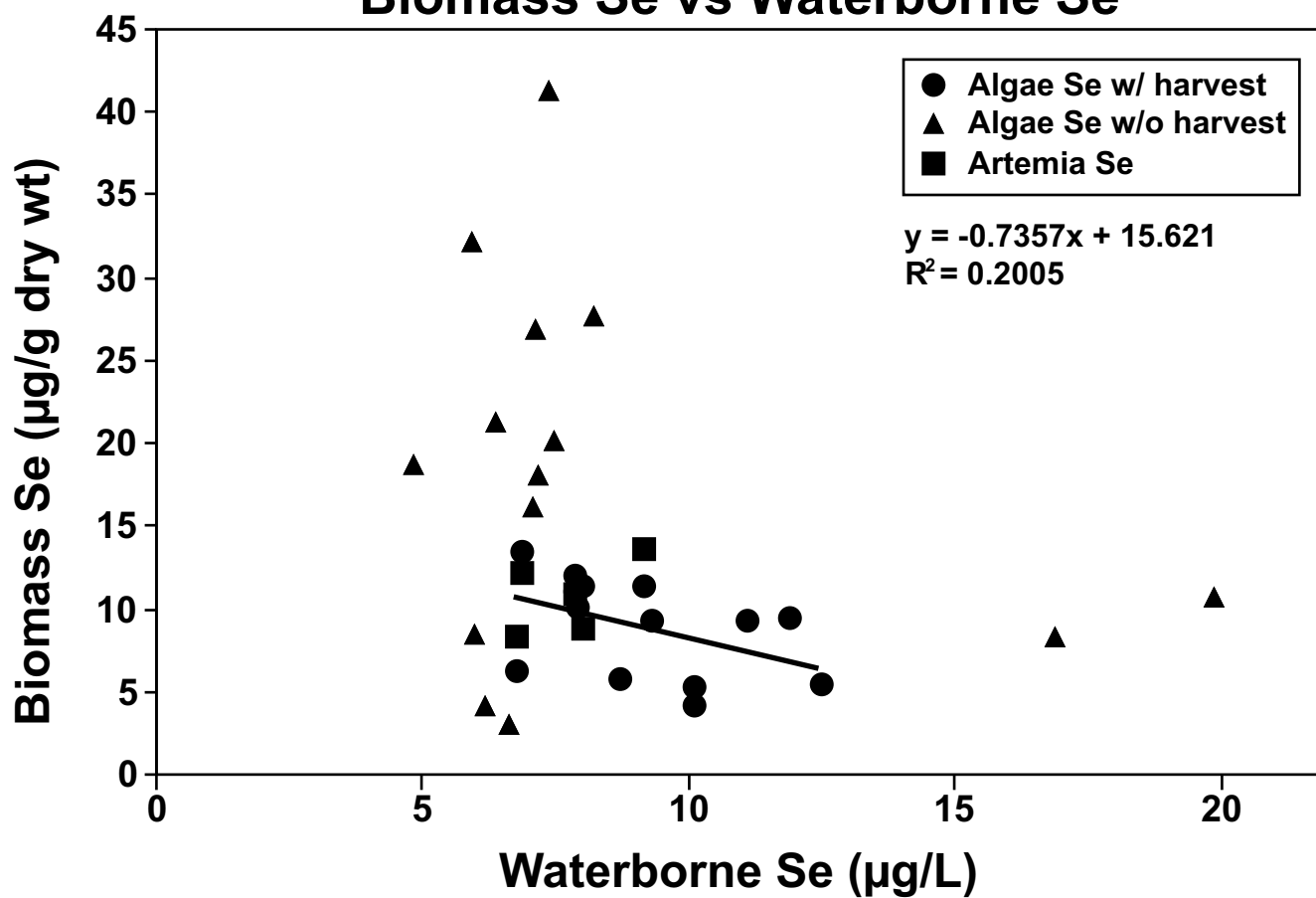


Fig. 6

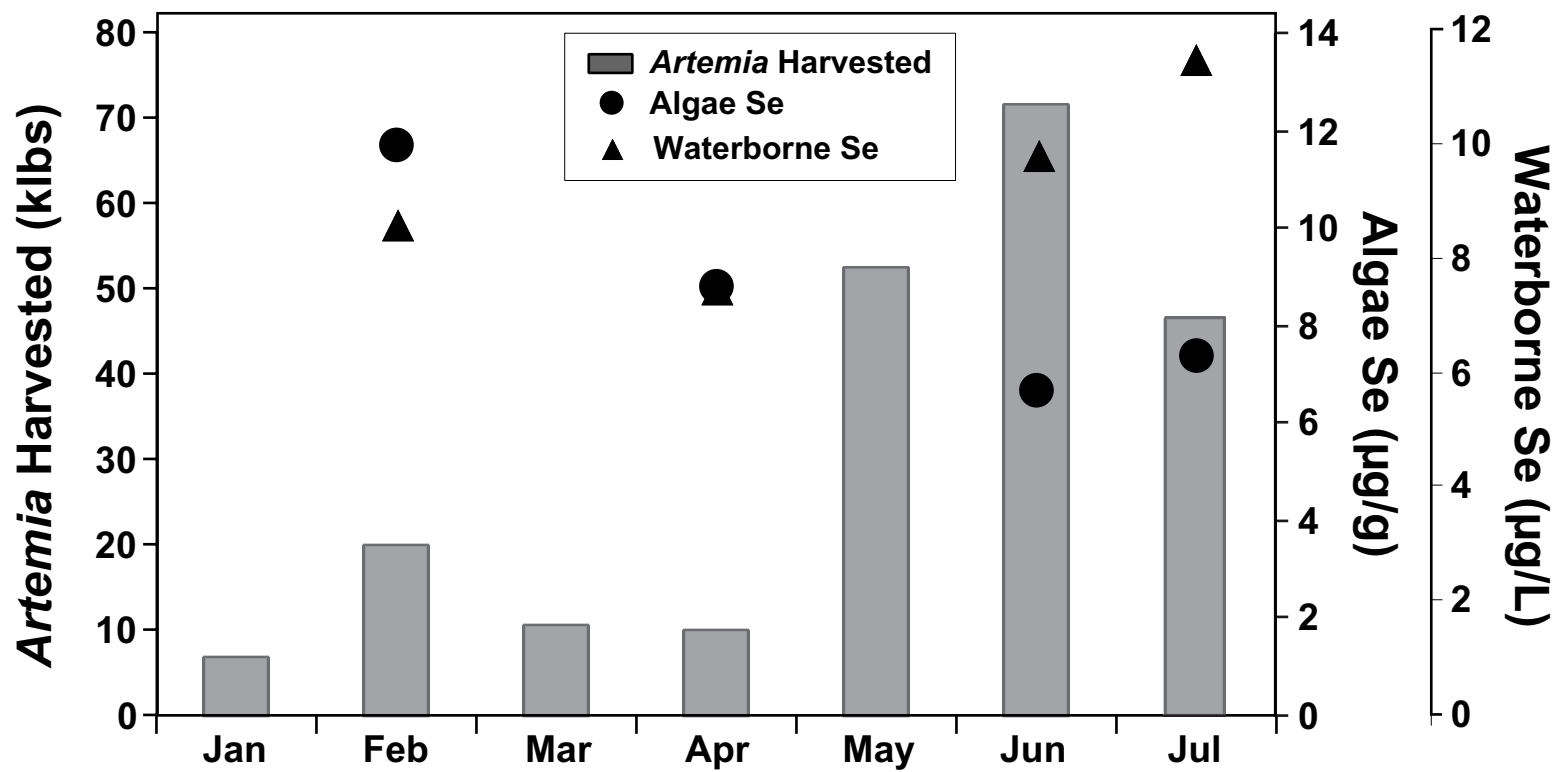


Fig. 7

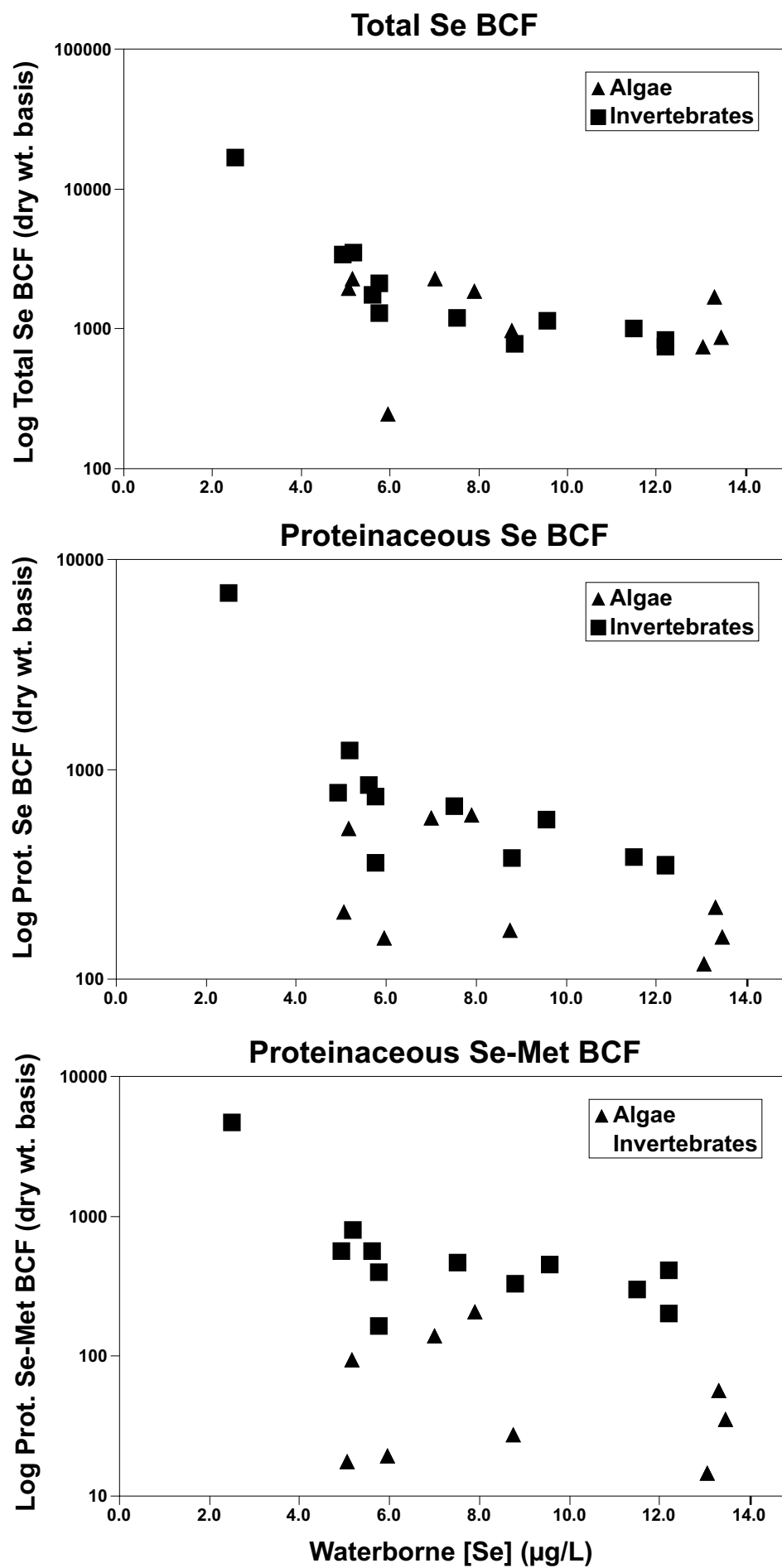
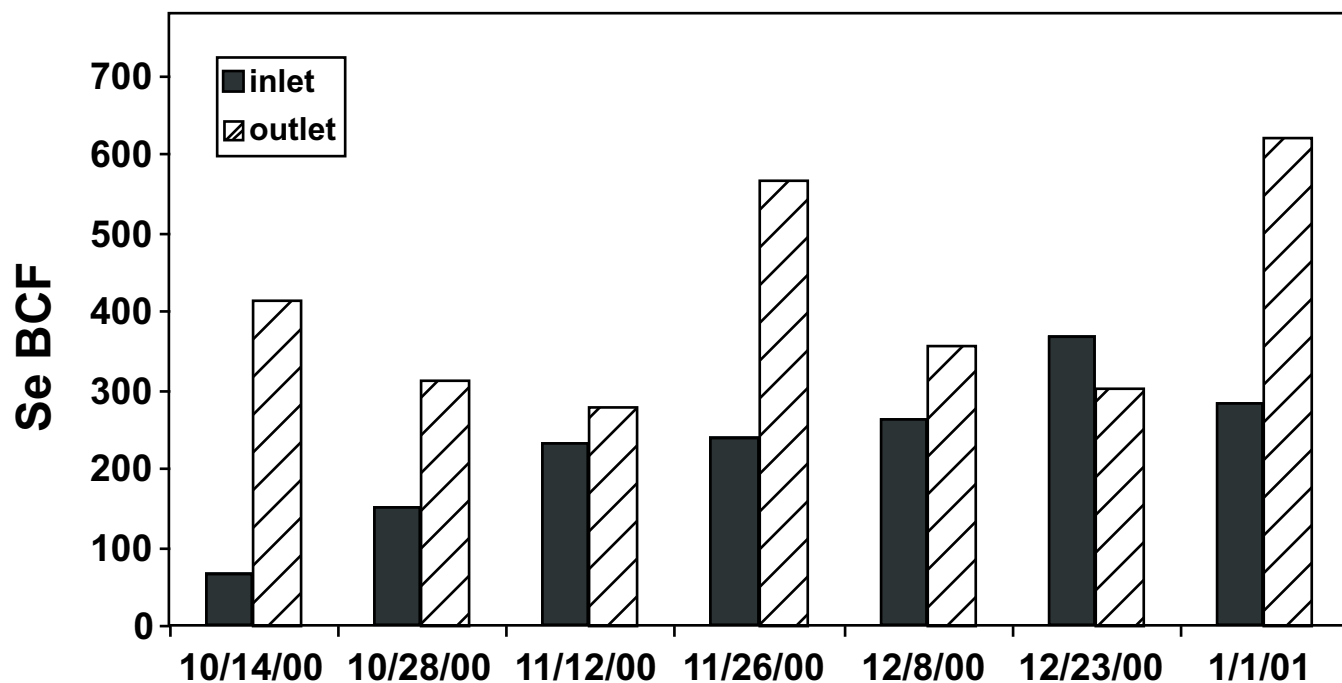


Figure 8

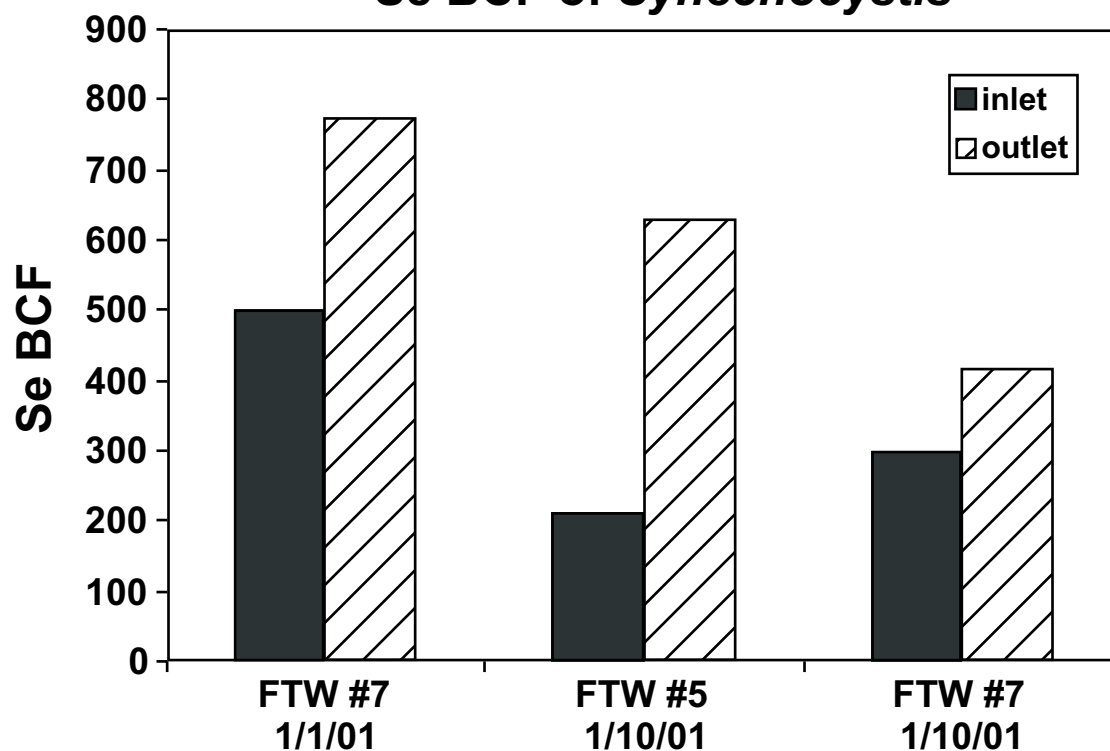
A

Se BCF of Native Algae of FTW #7



B

Se BCF of *Synechocystis*





Transformation of Selenium in Tulare Lake Evaporation Ponds (Measurement of Selenium Speciation in the TLDD Sediments)

Principal Investigator:

W. T. Frankenberger, Jr.

Department of Environmental Sciences, University of California, Riverside

Research Staff:

Y. Q. Zhang

ABSTRACT

Determination of selenium (Se) speciation in wetland sediments is very important in understanding the bioavailability and bioaccumulation of Se in wetland systems. Sequential extraction of Se in sediments is often used in speciation studies. Because of several drawbacks of the sequential extraction (e. g. large dilution factors, loss of light material, and less extraction efficiency with clay samples), an alternative method was developed by parallel extraction of Se in sediments with deionized (DI) water, NaOH, Na₂SO₃ and H₂O₂-HCl, and determination of Se species by differences. Results showed that accumulated Se from spiked Se in sediments during DI water extraction can be quantitatively recovered with 0.1 M NaOH and 1 M Na₂SO₃. Mass recovery of Se from sediments by parallel extraction and a tedious sequential extraction was very close, indicating that sequential extraction can be replaced by parallel extraction in Se speciation studies. Selenite (Se [IV]), elemental Se (Se [0]), or organic material-related Se (OM-Se) and organic Se were major forms of Se, respectively accounting for an average of 26.7, 17.7, 32.1 and 19.8% of the total Se in Tulare Lake Drainage District wetland sediments. Reduction of selenate (Se [VI]) to reduced Se (Se [IV]+ Se [0]) and Se uptake by wetland organisms with incorporation of these organisms into the sediment are two major immobility processes that accumulate Se in wetland sediments.

■ ■ ■ ■ ■

INTRODUCTION

Irrigation in certain parts of the western United States has produced selenium (Se)-impacted drainage water, which can contaminate nearby wetlands. Bioaccumulation of Se in these wetlands has caused deformities in waterfowl (Ohlendorf, 1989). The biogeochemistry of Se in wetland systems is very complex because Se can exist in four different oxidation states (-II, 0, IV and VI) and

as a variety of organic compounds. The bioavailability of Se as a toxicant in wetlands is largely dependent on the speciation of Se present (Besser et al., 1993). Studies by Besser et al. (1993), Davis et al. (1988), and Maier et al. (1988) showed that organic forms of Se generally are more toxic than selenite (Se [IV]) and selenate (Se [VI]), and bioaccumulate more rapidly (Besser et al., 1993). Therefore, information about Se speciation in wetland systems is much more important than total Se to scientists and wetland managers.

A wetland system is a complex aggregate of water, sediment and various types of organic materials. Se [VI] is often the dominant Se species in agricultural drainage water. Se [VI] reacts with the wetlands through a series of adsorption, reduction, and transformation processes, causing Se accumulation in wetland systems as different forms of Se (Maier et al., 1988; Tokunaga et al., 1996; Zawislanski and Zavarin, 1996; Zhang and Moore, 1996; 1997a). Wetland sediment is the largest reservoir of Se, accounting for more than 90% of total Se (George et al., 1996; Weres et al., 1989). Therefore, speciation of Se in wetland sediments is a major reflection of the various biogeochemical processes in wetland systems.

Sequential extraction has been widely used in speciation and fractionation studies of Se in sediments and soils (Martens and Suarez, 1997; Zawislanski and Zavarin, 1996; Zhang and Moore, 1996). Soluble Se species, exchangeable Se, organically bound Se and elemental Se (Se [0]) are the major Se forms which are currently determined by a sequential extraction procedure. However, we have found several drawbacks with the sequential extraction procedure. The first is that sequential extraction of Se in soil and sediment often uses 4-5 extractions that are performed in a set sequence. After each extraction, the residue is washed 1-2 times in order not to affect the next extraction. Therefore, it may not be possible to measure Se species in samples containing low Se due to the large dilution factor. The second is that a sequential extraction requires a series of shaking, centrifuge, and filtration processes. Upon separation, light materials can be removed from the sediment samples, causing

a loss of Se in the next series of extractions. The third is that the extraction efficiency is low for samples containing high clay content due to the difficulty of re-suspending the sample residue after each extraction and centrifugation. All of these drawbacks indicate that sequential extraction for determination of Se speciation may only be successfully used in sediment samples containing relatively high levels of Se, less light material and less clay texture, which may not be the case in most of wetland sediments. Therefore, we have developed an alternative method which can not only overcome these drawbacks, but also accurately determines Se speciation in wetland sediments.

The main objective of this study was to develop an alternative method to determine Se speciation in wetland sediments. We used deionized (DI) water, sodium hydroxide (NaOH), sodium sulfite (Na₂SO₃) and hydrogen peroxide (H₂O₂)-hydrochloric acid (HCl) to extract different Se species in sediment samples, and used concentration differences to determine Se species in the sediments.

METHOD DEVELOPMENT

An efficient extraction procedure for different Se species in sediments needs to meet two criteria: (i) Extractants will not interfere with Se measurements as determined by hydride generation atomic absorption spectrometry (HGAAS), and (ii) Target Se can be extracted in each extraction step and speciated. DI water, NaOH, Na₂SO₃, H₂O₂ and HCl used in this study should work well for extracting different Se species from sediments because these solutions meet these two criteria. For examples, HCl and NaOH are two reagents used in hydride generation acid and sodium borohydride (NaBH₄) channels in a hydride generation system. They, therefore, will not interfere with Se measurements by HGAAS. Na₂SO₃ and H₂O₂ will not interfere with Se measurements by HGAAS because Na₂SO₃ is oxidized to sulfate during oxidization of all Se to Se [VI] by H₂O₂. Surplus H₂O₂ can be easily destroyed to water by heating the H₂O₂ solution under a basic condition (Zhang et al., 1999a).

DI water, NaOH, Na₂SO₃, H₂O₂ and HCl will extract different Se species based on the physical-chemical characteristics of Se species in sediment samples. Se [VI] is the most oxidized form of Se, and is highly soluble in water (Mikkelsen et al., 1989). DI water, therefore, can be used to dissolve most of Se [VI] in the sediment. DI water can also dissolve soluble Se [IV] and organic Se.

Selenite has a strong affinity to sorption sites of sediment and soil constituents (Balistrieri and Chao, 1990; Bar-Yosef and Meek, 1987; Frize and Hall, 1988; Neal et al., 1987a; 1987b). Se [IV] adsorption results through the replacement of the surface hydroxyl groups with Se [IV] (Balistrieri and Chao, 1987; Hayes et al., 1988; Sposito et al., 1988). At pH > 10-12, Se [IV] adsorption in different soils is close to zero (Balistrieri and Chao, 1987; Hayes et al., 1988; Neal et al., 1987a; 1987b; Sposito et al., 1988). Therefore, Se [IV] can be efficiently extracted by 0.1 N NaOH (pH 13). 0.1 M NaOH can also extract some of the organically-bound Se (humic substances-related Se), and Se [VI]. Because this direct NaOH (0.1 M) extraction can dissolve all soluble Se species extracted by the DI water, the concentration difference of Se species between the direct NaOH (0.1 M) extraction and the DI water extraction should be equal to the Se species extracted by 0.1 M NaOH after DI water soluble Se is removed from samples.

Elemental Se is a thermodynamically stable form under some anoxic conditions, and it is relatively insoluble (Elrashidi et al., 1987). Se [0] can be extracted with a high concentration of Na₂SO₃ (Velinsky and Cutter, 1990). When all Se [0] is extracted by 1 M Na₂SO₃ (pH 7) extractant, the 1 M Na₂SO₃ extractant is adjusted to pH 13 with 5 M NaOH for continuing extraction. This combined extraction should extract Se [0] and all 0.1 M NaOH extractable Se. Therefore, the concentration difference of Se species between this direct 1 M Na₂SO₃ extraction and the direct 0.1 M NaOH extraction should be equal to Se [0] in the samples.

Hydrogen peroxide is a strong oxidant, which can oxidize organic Se, Se [0], organic materials-related Se (OM-Se) and Se [IV] to Se

[VI]. OM-Se is defined as the Se in sediment organic materials after soluble and 0.1 M NaOH extractable organic Se are removed from the samples. Therefore, the concentration difference of Se between the direct H₂O₂ extraction and the direct Na₂SO₃ extraction should be equal to OM-Se after Se [VI], Se [IV], Se [0], water soluble and 0.1 M NaOH extractable organic Se are removed from the samples.

Total Se can be determined in the sediments by using a two-step (H₂O₂-HCl) digestion procedure (Zhang and Moore, 1997b). The concentration difference of Se between this extraction and the direct H₂O₂ extraction should be equal to the residue Se.

Based on these characteristics of Se species described above, our new procedure to extract different Se species is proposed in Figure 1.

MATERIAL AND METHODS

Sample treatment and extraction

Wetland sediment samples used in this study were collected from Tulare Lake Drainage District wetlands (TLDD), CA. Two sediment samples used for the method development were collected from Kesterson Reservoir pond 4 (Pond 4), CA and Stewart Lake (SL), UT. All sediment samples were air-dried at room temperature (21°C) and ground to 100 mesh prior to extraction.

The speciation of Se in the sediment samples was extracted in triplicates using a procedure described in Figure 1, in which four parallel extractions were used to extract different Se species.

1) DI water extraction: DI water extracted soluble Se [VI], Se [IV] and organic Se. In this extraction, 1 g sediment samples were placed in 40-ml Teflon centrifuge tubes, followed by 30 ml of deionized water. The centrifuge tubes were tightly capped and placed horizontally in a gyrotory shaker and shaken for 20 hours. Then, the tubes were centrifuged at 17300 x g (R.C.F) for 20 min. The supernatant from each tube was passed through a 0.45-µm Supor membrane filter (Gelman Sciences) into a 40-ml glass vial.

2) 0.1 M NaOH extraction: This extraction extracted 0.1 NaOH extractable Se [VI], Se [IV] and organic Se. In this extraction, 1 g sediment samples were placed in 40-ml Teflon centrifuge tubes, followed by 30 ml of 0.1 M NaOH. The centrifuge tubes were tightly capped and placed horizontally in a gyrotory shaker and shaken for 20 hours. The centrifuge and filtration procedures were the same as the DI water extraction described above.

3) 1 M Na₂SO₃ extraction: 1 M Na₂SO₃ extracted elemental Se, and 0.1 NaOH extractable Se [VI], Se [IV] and organic Se. In this extraction, 1 g sediment samples were placed in 40-ml Teflon centrifuge tubes, followed by 30 ml of 1 M Na₂SO₃. In a previous recovery test of red elemental Se in a 1 M Na₂SO₃, we found that a relatively long-time shaking was needed to recover 95% of elemental Se. Therefore, the centrifuge tubes were tightly capped and placed horizontally in a gyrotory shaker and shaken for 40 hours. After that, the 1 M Na₂SO₃ extractant was adjusted to pH 13 with 5 M NaOH, and the tubes were shaken again for 20 hours. The centrifuge and filtration procedures were the same as the DI water extraction described above.

4) H₂O₂ (30%) extraction: 30% H₂O₂ oxidized organic Se, Se [0], OM-Se and Se [IV] to Se [VI]. In this extraction, 0.3-1 g sediment samples were placed in 40-ml Teflon centrifuge tubes. Then, 0.5 ml of 30% H₂O₂ were added. After effervescence stopped, an additional 0.5-1 ml of H₂O₂ was added. This procedure was repeated several times. Then, 5 to 10 ml of H₂O₂ were added to the tube. The sample was heated in a hot water bath at 60°C until the sediment residue in the tube had settled to the bottom of the tube. Then, several drops of 1 M NaOH were added to the tube to facilitate the decomposition of the surplus H₂O₂. After the slurry was cooled to the room temperature, the tubes were centrifuged at 17300 x g (R.C.F) for 20 min. The supernatant from each tube was transferred to a 60-ml polyethylene bottle. The sample was then rinsed and re-suspended once with deionized water and centrifuged. The final combined supernatant of the sediment sample was adjusted to 40-50 ml with

deionized water, and then passed through a 0.45- μm Supor membrane filter into another polyethylene bottle. After the H_2O_2 extraction, 5 ml of 6 N HCl were added to the tube, mixed with the sediment residue, and heated in a hot water bath at 95 °C for 3-4 hours. After cooling to room temperature, the separation of the supernatant and sample residue was performed using a procedure described in the H_2O_2 extraction.

Se recovery tests

In order to accept the procedure proposed in Figure 1, two important tests were performed. The first was used to test the recovery of spiked Se during extractions. To initiate this test (see Figure 2), we spiked 0.2 ml of 15 $\mu\text{g}/\text{ml}$ Se [IV] in three batches of sediment samples to examine (i) the accumulation of spiked Se during the DI water extraction (Figure 2, P1); (ii) the recovery of accumulated Se during the 0.1 M NaOH extraction (Figure 2, P2); (iii) the recovery of accumulated Se during 1 M Na_2SO_3 extraction (Figure 2, P3).

The second test was used to determine whether the amount of Se detected from the procedure described in Figure 1 was similar to the mass recovery of Se from sequential extraction. In conducting this test, we carried out four different extraction procedures (Figure 3, P1-P4) to test whether Se species directly extracted from the sediment samples was equal to the sum of Se species extracted with sequential extraction. Duration, the amount of sediment samples and extractants used in the sequential extraction were the same as the parallel extraction described in Figure 1.

Two sediment samples (Pond 4 and SL) were selected to test the mass recovery of Se in different extraction procedures and the recovery of spiked Se in sediment samples because these two sediment samples contained relatively high Se concentrations (14.314-17.861 $\mu\text{g}/\text{g}$) and the residue of these two samples can be easily resuspended after each extraction. All extractions in these tests were run in triplicates.

Se species analysis

Selenium species in the DI water and 0.1 M NaOH extracts were determined using a

speciation method developed by Zhang et al. (1999c) after a slight modification. This modification includes the adjustment of solution pH to 10.3 instead of 10 and the addition of 0.2-0.4 ml 0.2 M sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$) to each sample when the sum of Se [IV] and organic Se was determined. In brief, this speciation was carried out as follows: Se [IV] in the extracts was determined in a pH 7 buffer solution. The sum of Se [IV] and organic Se was determined when the organic Se in the extracts was oxidized to Se [IV] by $\text{S}_2\text{O}_8^{2-}$, which was indicated by precipitation of Mn oxides. The organic Se concentration was calculated as the difference between Se in this solution and Se (IV) concentration determined in another subsample. Total Se in the DI water and 0.1 M NaOH extracts was determined by oxidizing all Se to Se [VI] by $\text{S}_2\text{O}_8^{2-}$, followed by reduction to Se [IV] in 6 N HCl. Se [VI] concentration was calculated as the difference between total Se concentration, and Se [IV] and organic Se concentration determined in another subsample.

For determination of total Se in the Na_2SO_3 and H_2O_2 extracts, Na_2SO_3 was oxidized to sulfate by H_2O_2 , and then surplus H_2O_2 was decomposed to water under a basic condition in a hot water bath (Zhang et al., 1999a). Total Se in the sediment was determined in a solution mixed with the H_2O_2 and HCl extracts in the parallel extraction. Se concentrations in all prepared solutions were determined by HGAAS (Zhang et al., 1999b; 1999c). Total Se [VI], Se[IV] and organic Se in the TLDD sediment samples was determined in the 0.1 M NaOH extract. Elemental Se was calculated as the difference between total Se in the 1 M Na_2SO_3 extract and 0.1 M NaOH extract. OM Se was determined by the difference between total Se in the H_2O_2 extract and 1 M Na_2SO_3 extract. Residue Se was determined by the difference between total Se in the H_2O_2 extract and total Se in sediment.

RESULTS AND DISCUSSION

Se recovery tests

Selenate, Se [IV] and organic Se (e.g. selenomethionine) are commonly used as spiked Se species in speciation studies. In this

work, only Se [IV] was selected because Se [IV] has a strong affinity to sorption sites of sediments. After the DI water extraction, a significant amount of Se [IV] can remain in the sediment. Recovery of the accumulated Se [IV] in the sediment can be obtained by the 0.1 M NaOH and 1 M Na₂SO₃ extractions. After 20 h of shaking with DI water (Figure 2, P1), about 72% and 28% of the spiked Se [IV] were, recovered in the Pond 4 and SL sediments, respectively (Table 1), indicating that part of added Se [IV] was accumulated in sediment samples. When the DI water was adjusted to pH 13 (0.1 M NaOH), and then shaken for another 20 h (Figure 2, P2), the accumulated Se [IV] was completely recovered as Se [IV] in the Pond 4 sediment and about 84% was recovered in the SL sediment, revealing that hydroxyl (OH) groups can quantitatively replace the accumulated Se [IV] on the surface of sediments (Balistreri and Chao, 1987; Hayes et al., 1988; Sposito et al., 1988). In another test with these two sediment samples, there was no accumulation of Se [IV] in the sediment when spiked Se [IV] was directly added in a 0.1 M NaOH extraction solution (data not shown). When the DI water was adjusted to 1 M Na₂SO₃ at pH 7 (Figure 2, P3), and then shaken for another 40 h, followed by 20 h of shaking at pH 13, the accumulated Se in both sediments was completely recovered as total Se. These results revealed that spiked Se can be quantitatively recovered with the 0.1 M NaOH and 1 M Na₂SO₃ extractions.

Selenium concentrations determined with different extraction procedures are present in Table 2. The sum of Se fractions from the four extraction procedures were very similar, ranging from 13.84 to 14.29 µg/g in the Pond 4 sediment and 17.37 to 17.58 µg/g in the SL sediment. The sum of Se fractions were also very close in the first two fractions (DI water and 0.1 M NaOH) and in the first three fractions

(DI water, 0.1 M NaOH and 1 M Na₂SO₃) (Table 2). Considering Se speciation in the DI water and the 0.1 M NaOH extracts (Table 3), the sum of Se species in the Pond 4 and SL sediments was also close. All these tests show that a complicated and tedious sequential extraction can be replaced by direct extraction with different extractants and concentrations of Se species can be determined by the difference.

The advantages of this direct extraction method not only overcome the drawbacks (described in the Introduction section) of sequential extraction, such as a large dilution factor, loss of light material during filtration and less extraction efficiency for some samples containing high clay content due to the difficulty in resuspending the sample residue after each extraction and centrifugation, but also this direct extraction method can be easily operated. For example, a sequential extraction procedure needs to continuously extract Se for several days, and each extraction requires separation (centrifuged and/or filtration) and resuspension of the residue 2-3 times. Whereas, in this direct extraction method, each extraction can be performed at different times, each extraction is only centrifuged one time, and the residue samples do not need to be resuspended. Also, Se species in the DI water and 0.1 M NaOH extracts can be performed on the same day after centrifugation and filtration. This eliminates the problems caused by changes in Se species during storage of extract solutions.

■ ■ ■ ■ ■

ACKNOWLEDGMENTS

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Table 1.Recovery of spiked Se ($\mu\text{g/g}$) in different extracts as described in Figure 2.

Samples	Se in extracts	Spike Se	Se measured	Recovery (%)
Procedure 1 (P1)				
Pond 4	1.018 $\pm$ 0.018	2.998 $\pm$ 0.012	3.189 $\pm$ 0.037	72
SL	1.781 $\pm$ 0.020	2.996 $\pm$ 0.011	2.608 $\pm$ 0.068	28
Procedure 2 (P2)				
Pond 4	3.213 $\pm$ 0.029	2.995 $\pm$ 0.015	6.319 $\pm$ 0.028	104
SL	7.179 $\pm$ 0.080	2.985 $\pm$ 0.012	9.686 $\pm$ 0.128	84
Procedure 3 (P3)				
Pond 4	8.401 $\pm$ 0.174	2.985 $\pm$ 0.009	11.371 $\pm$ 0.218	99
SL	14.229 $\pm$ 0.315	2.993 $\pm$ 0.001	17.281 $\pm$ 0.123	102

Table 2.

Mass balance of Se ($\mu\text{g/g}$) released from the different extraction procedures as described in Figure 3.

Samples	DI water	0.1 M NaOH	1 M Na ₂ SO ₃	H ₂ O ₂	Sum ^a
Procedure 1 (P1)					
Pond 4	1.018 $\pm$ 0.018	2.566 $\pm$ 0.031	4.987 $\pm$ 0.338	5.717 $\pm$ 0.073	14.288
SL	1.781 $\pm$ 0.020	5.953 $\pm$ 0.039	7.287 $\pm$ 0.184	2.409 $\pm$ 0.041	17.430
Procedure 2 (P2)					
Pond 4		3.362 $\pm$ 0.025	4.684 $\pm$ 0.096	5.793 $\pm$ 0.080	13.839
SL		7.231 $\pm$ 0.014	7.569 $\pm$ 0.186	2.642 $\pm$ 0.281	17.442
Procedure 3 (P3)					
Pond 4			8.332 $\pm$ 0.174	5.734 $\pm$ 0.071	14.066
SL			14.672 $\pm$ 0.577	2.699 $\pm$ 0.049	17.371
Procedure 4 (P4)					
Pond 4				14.006 $\pm$ 0.211	14.006
SL				17.583 $\pm$ 0.182	17.583

Table 3.

Comparison of Se species ($\mu\text{g/g}$) released from two different extraction procedures (Figure 3, P1 and P2).

Samples	Se [IV]	Se[IV]+ Organic Se	Total Se	Se [VI] Calculated	Organic Se Calculated
Procedure 1 (P1)-DI water extract					
Pond 4	0.348 $\pm$ 0.012	0.459 $\pm$ 0.021	1.018 $\pm$ 0.018	0.559	0.111
SL	0.457 $\pm$ 0.003	0.529 $\pm$ 0.011	1.781 $\pm$ 0.020	1.252	0.072
Procedure 1 (P1)-0.1 M NaOH extract					
Pond 4	1.168 $\pm$ 0.071	2.257 $\pm$ 0.080	2.566 $\pm$ 0.031	0.309	1.089
SL	4.546 $\pm$ 0.137	5.699 $\pm$ 0.091	5.953 $\pm$ 0.039	0.254	1.153
Procedure 2- (P2) 0.1 M NaOH extract					
Pond 4	1.518 $\pm$ 0.034	2.362 $\pm$ 0.032	3.362 $\pm$ 0.025	1.000	0.844
SL	4.769 $\pm$ 0.060	5.596 $\pm$ 0.073	7.231 $\pm$ 0.014	1.635	0.827

Table 4.Speciation of Se ($\mu\text{g/g}$) in the TLDD sediments.

Samples	Se [IV]	Se [VI]	Organic Se	Se [0]	OM-Se	Residue Se	Total Se
Cell 1	1.017	0.120	0.879	0.449	1.421	0.028	3.914
Cell 3	0.886	0.055	0.215	0.818	0.620	0.037	2.631
Cell 6	0.381	0.043	0.127	0.189	0.360	0.042	1.142
Cell 7	0.476	0.047	0.748	0.200	0.790	0.012	2.273
Cell 9	0.600	0.014	0.744	0.624	1.046	0.031	3.059

Table 5.Speciation of Se ($\mu\text{g/g}$) in the DI water extract, and the 0.1 M NaOH extract after DI water soluble Se species was removed from samples.

Samples	Se [IV]	Se [VI]	Organic Se	Total Se	Se [IV]	Se [VI]	Organic Se	Total Se
Cell 1	0.358 $\pm$ 0.005	0.023	0.118	0.498 $\pm$ 0.005	0.659	0.097	0.761	1.519
Cell 3	0.136 $\pm$ 0.003	0.014	0.079	0.229 $\pm$ 0.004	0.750	0.041	0.136	0.927
Cell 6	0.153 $\pm$ 0.003	0.018	0.052	0.224 $\pm$ 0.003	0.228	0.025	0.075	0.327
Cell 7	0.216 $\pm$ 0.002	0.04	0.122	0.378 $\pm$ 0.004	0.260	0.007	0.626	0.892
Cell 9	0.126 $\pm$ 0.003	0.012	0.089	0.228 $\pm$ 0.002	0.474	0.002	0.655	1.130

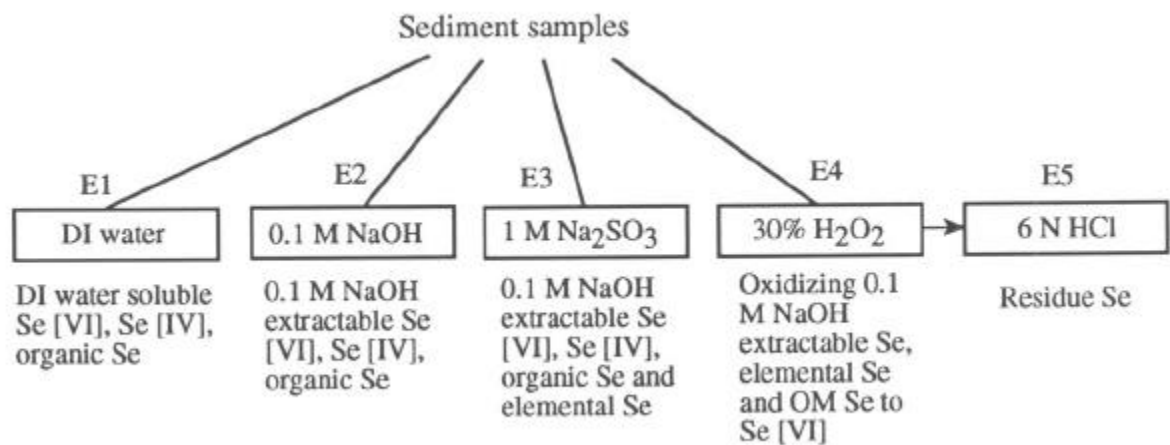


Figure 1. A parallel extraction procedure to determine Se species in wetland sediments.

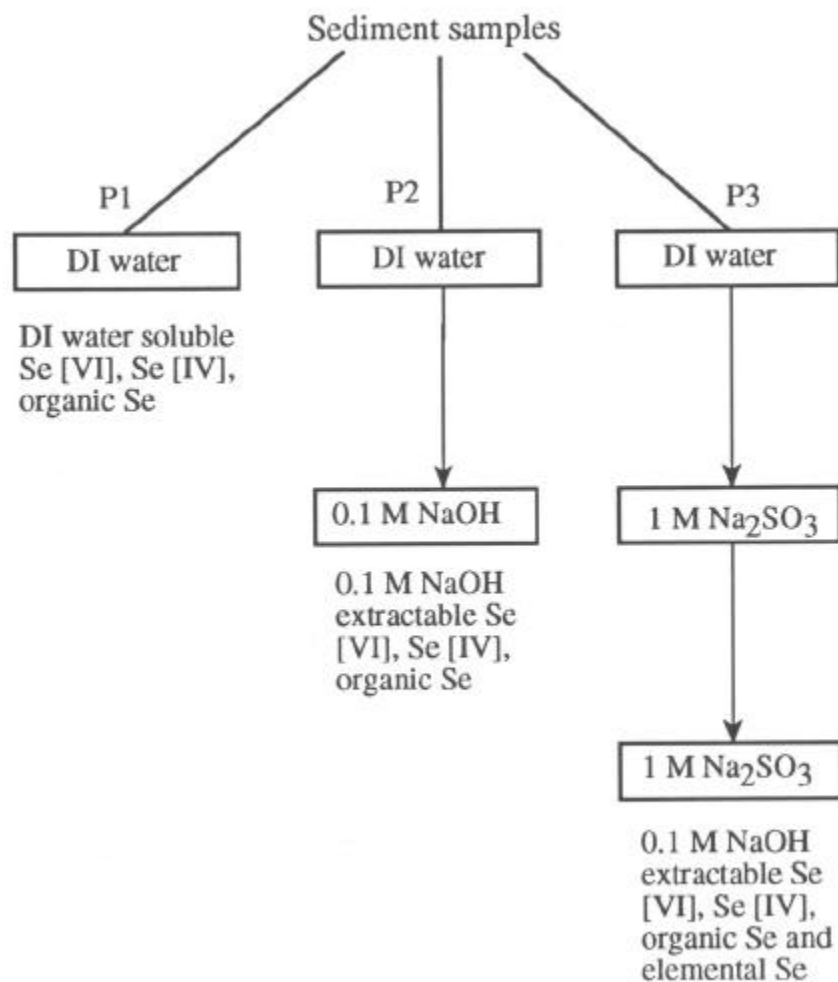


Figure 2. A procedure to determine the recovery of spiked Se in wetland sediments.

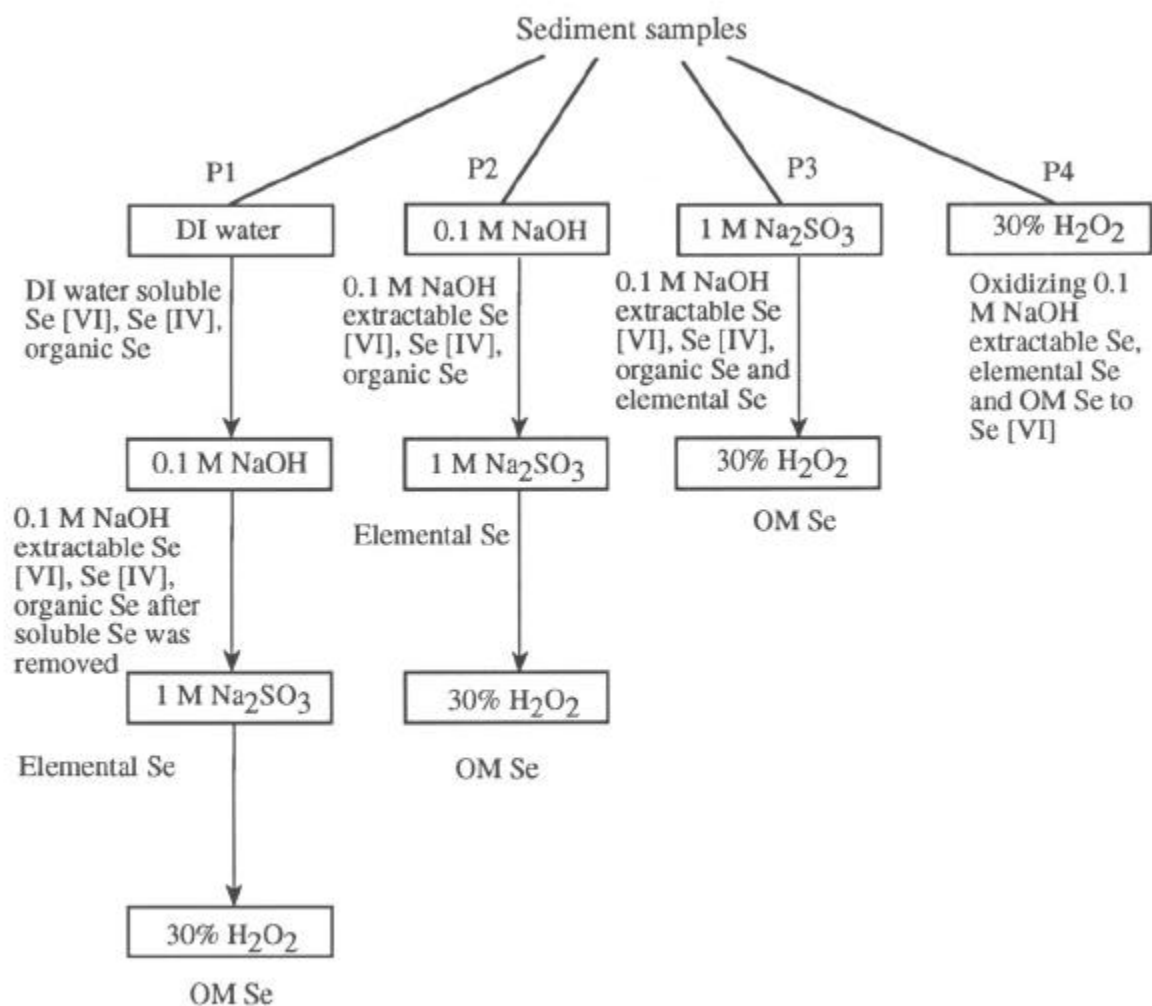


Figure 3. Four different extraction procedures (P1-P4) to determine the mass recovery of Se in wetland sediments.



An Investigation into the Ecotoxicology of Selenium Bioaccumulation in Birds

PROJECT INVESTIGATOR:

D. Michael Fry, Department of Animal Science, UC Davis, CA 95616-8521

RESEARCH STAFF:

1 RA: Steven Detwiler (50%)

2 Lab Assistants: Uland Wong (10%), Janice Rahman (10%)

ABSTRACT

Marked variation between wild bird species exists in sensitivity to the teratogenic effects of selenium. Our working hypothesis is that more tolerant species have the ability to distinguish between methionine and selenomethionine during protein synthesis, and as a result, the embryos of resistant species incorporate less Se into proteins, and are thus protected from the teratogenic effects of Se. As one measure of evaluation, both wild bird eggs and chicken eggs have been separated into yolk, albumin, and embryo fractions and analyzed for Se to evaluate the ability of different species to prevent Se incorporation into protein.

Work to this point has included the examination of 231 eggs from 9 species of wild birds nesting in selenium contaminated environments. The data suggest that more tolerant avian species (American avocet) effectively exclude selenium from embryonic tissues relative to more sensitive species with similar dietary ecology.

A laboratory feeding trial was completed in 2001 exposing chickens to SelenoMax[®] selenized yeast, containing about 70% of selenium as selenomethionine. The dietary transfer of selenium into eggs, the chemical forms of selenium sequestered or utilized, and the net toxic effects to embryos were measured in chicken eggs to compare with wild birds.

Hen weights during the feeding trial were monitored before and after each dose administration. Dose-dependent cachexia (wasting syndrome) was observed in the study hens. Measurements of chicken embryonic masses at defined incubation period were also collected to detect dose-dependent retardation of embryogenesis. A decrease in embryo weight was detected in eggs from the 4 ppm selenium diet, without detected teratogenesis. At dietary levels of 8 ppm. selenium, embryo weights were severely reduced and 17% of embryos were abnormal. At 12 ppm. selenium in the diet, 97% of the embryos were deformed, and live embryos averaged only 37% of control weights.

A feeding trial with Mallards duplicating the chicken feeding is being conducted

during 2001, with 2 elevated SelenoMax[™] selenized yeast dose groups. Ducks were housed in floor pens with 5 females and 2 males per treatment group. Eggs were collected and incubated to ¾ term, and broken out exactly as in the chicken experiment. 390 eggs were collected for analysis, with at least 80 eggs per treatment group. A comparison will be made for the dietary transfer of Se into eggs, the Se incorporation in protein, and the sensitivity of the two species to Se induced malformations during embryogenesis. The comparative data for these two species will provide the links for interpreting the dose response, protein bound Se, and terata observed in more resistant wild species.

KEYWORDS: Selenium, Birds, Ecotoxicology, Bioaccumulation

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INTRODUCTION

In 1983, scientists discovered significant reproductive impairment and avian mortality at Kesterson Reservoir—a waterfowl enhancement area operated by the U.S. Bureau of Reclamation as a unit of the Federal National Wildlife Refuge System in the San Joaquin Valley of California (Ohlendorf et al., 1986a). Embryo toxicosis (embryo deaths and deformities) and/or reduced hatchling survival were observed in American coots (*Fulica americana*), eared grebes (*Podiceps nigricollis*), black-necked stilts (*Himantopus mexicanus*), and several dabbling duck species (mallard [*Anas platyrhynchos*], gadwall [*Anas strepera*], cinnamon teal [*Anas cyanoptera*], and northern pintail [*Anas acuta*]) nesting at the reservoir (Ohlendorf et al., 1986b; Ohlendorf, 1989). In subsequent years, similar observations were reported in embryos of killdeer (*Charadrius vociferus*) and American avocets (*Recurvirostra americana*) (Ohlendorf et al., 1986b; Ohlendorf et al., 1989). Researchers attributed the observed reproductive impairment and adult mortality to the effects of elevated concentrations of selenium in the subsurface agricultural drain-water which supplied the reservoir (Hoffman et al., 1988; Ohlendorf, 1989; Ohlendorf and

Skorupa, 1989; Ohlendorf et al., 1988; Ohlendorf et al., 1986a; Ohlendorf et al., 1986b).

Beginning in 1987, similar ecotoxicological results to those documented at Kesterson Reservoir have been reported at several sub-surface agricultural evaporation ponds further south in California's Tulare Lake Basin (Moore et al., 1990; Skorupa and Ohlendorf, 1991).

BIOLOGICAL EFFECTS IN BIRDS

In birds, reproductive impairment is manifested as reduced hatchability due to increased embryo mortality, induced deformities, and/or embryo malpositioning. All of these are fatal and result in failure to hatch. Typical deformities include exencephaly, beak malformations, and reduced or absent eyes and limbs. Classic selenium deformities are generally overt, and include some or all of the aforementioned conditions concurrently. A relatively good degree of information has been generated regarding feed supplementation, tissue distributions, and toxicity due, in large part, to the observations of selenium's toxicological and teratogenic properties in poultry fed seleniferous grain.

INTERSPECIFIC VARIABILITY IN SELENIUM TOLERANCE

The US Fish and Wildlife Service has monitored breeding populations of birds throughout the San Joaquin Valley during the past 15 years. An extensive database has been generated, including observations from nest monitoring and quantitative analyses of wild bird eggs.

The results, presented in our previous annual reports, demonstrate that selenium dose-response curves for avian populations in contaminated environments vary by species. Ducks are the most sensitive in this dataset, with an EC50 (effective concentration to cause 50 % deformities) of about 230 ppm Se egg concentration (dry weight basis), compared to an EC50 for stilts of 55ppm, and for avocets of about 100ppm. (Skorupa, 1998). In addition, monitoring of western snowy plover (*Charadrius alexandrinus nivosus*), revealed no deformities despite exposures as high as 100ppm. Laboratory studies have demonstrated that chickens (EC50 of 10ppm) are

more sensitive to selenium exposure than mallards (EC50 of 20ppm), screech owls and black-crowned night herons (Heinz, 1989; Smith et al., 1988).

CHEMICAL FORM DETERMINES TOXICITY

The chemical form of selenium determines its metabolism and toxicity. In aquatic systems, it has been shown that selenomethionine is preferentially taken up as compared to inorganic selenite and selenate (Besser et al, 1989). Smith et al (1938) observed this same pattern in lab animals. Heinz et al (1987) confirmed that dietary selenium as selenomethionine was much more readily taken up by mallard hens and passed into the egg. Selenomethionine is also the most teratogenic chemical form (Heinz et al, 1989; Heinz et al, 1987; Hoffman and Heinz, 1988; Palmer et al, 1973; Martin, 1988), and appears to produce qualitatively different deformities than other forms. In mallards, dietary selenite generally caused edema and stunted growth, whereas selenomethionine resulted in overt, multiple malformations including microphthalmia, hydrocephaly, bill, foot, and leg deformities (Hoffman and Heinz, 1988). The mechanism for differences in teratogenesis of different chemical forms is as yet not understood. The detailed study of Se biochemistry in bird egg tissues, along with the understanding of the bioaccumulation of different chemical forms is a critical part of defining this relationship.

STUDY OBJECTIVES

Our working hypothesis is that species resistant to the teratogenic effects of Se have a better ability to distinguish between methionine and selenomethionine during protein synthesis, and as a result, the embryos of resistant species incorporate less Se into proteins, and are thus protected from the teratogenic effects of Se.

The four-fold difference between wild species' tolerance to selenium toxicosis (that observed between duck species versus avocets) should be detectable in wild collected eggs as differences in protein bound Se in embryos. Chickens are even more sensitive, and the measurement of Se transfer from diet into eggs and incorporation of Se

into chicken embryos should provide a comparison for other species.

This year we began a dietary study with mallard ducks that should provide a bridge between laboratory studies with chickens and the studies of field collected eggs of wild species. We believe that the correlation of protein bound Se with toxicosis should provide a better estimate of toxicological risk, and the speciation of Se in other organisms within the food web will give a better understanding of the bioaccumulation and toxicity of Se to both plants and animals.

Specific experimental objectives of this study:

1. To determine the protein-bound fraction (and specific forms) of selenium in bird eggs.
2. To determine if eggs and tissues of species exhibiting greater and lesser tolerance to selenium poisoning contain variable concentrations of specific selenium metabolites.
3. To determine whether the incorporation of Se into protein correlates with teratogenicity, and whether Se tolerant species have the ability to reduce the incorporation of Se into protein.
4. To investigate the metabolism and toxicokinetics of selenium through inferred metabolic and ecological pathways using tissues and proteins from field-collected eggs.
5. To provide laboratory confirmation of 3) and 4) by conducting similar analyses on eggs from domestic chickens fed diets comprised with natural-source Se in known doses under controlled conditions.
6. To bridge the laboratory data set and the field data by conducting a feeding trial with mallard ducks using a similar protocol as for chickens.
7. To determine if a particular form of selenium (e.g., selenomethionine) is a better measure of ecotoxic risk across species and between systems (i.e., a more universal regulatory standard than total recoverable selenium).

During the past two years we have addressed Objectives 1-3, and have

completed Objective 5. We have completed the feeding trial portion of Objective 6, and are in the process of processing and analyzing the mallard duck eggs and embryos.

PROGRESS

CHICKEN LABORATORY FEEDING TRIALS

A feeding trial has been conducted with domestic (white leghorn) chickens for a focused study under controlled conditions. Since no firmly established dose response data were available for selenized yeast fed to chickens, the experiment was conducted in a paired block design—where all six treatment birds were placed on control, followed by treatment diets. This particular experimental design allowed for the use of fewer eggs and hens through increased statistical power by removing inter-individual variability. When dietary Se was changed, hens were fed the new diet for a minimum of two weeks prior to insemination and egg collections to allow egg [Se] to normalize. Previous investigators (Arnold et. al, 1971; Latshaw and Osman, 1975) observed that egg [Se] plateaus within a period of 2 weeks or less on an amended diet. The initial dietary [Se] was 4 ppm, followed by 12 ppm. The very high toxicity of the nominal 12 ppm diet prompted us to test an intermediate diet of 8 ppm. Se. Hens were placed on control diet for approximately 2.5 weeks before beginning the final 8 ppm dietary interval.

The nominal Se levels in the diets were 0.5ppm in the reference diet, and 4, 8 and 12 ppm in the treatment diets. The actual measured concentrations of Se in the diets were 0.53, 4.87, 9.43, and 13.2 mg Se/kg diet, respectively.

Eggs were collected during each of the treatment periods, and were incubated artificially to 16 days of incubation, at which time eggs were broken out to examine embryo development, and to collect embryo, yolk sac, and albumin contents, as in the field collection experiments. All fractions were saved for Se analysis, to determine partitioning and Se uptake by embryos. Chemical analyses for total Se were contracted to the California Animal Health and Food Safety

Laboratory (CAHFS) (formerly the California State Veterinary Diagnostic Laboratory) at UC Davis. Duplicate samples were analyzed in the laboratory of Teresa Fan at UCD using the microdigestion and fluorescence-based microanalysis (Fan et. al, 1997) previously used for field collected eggs to compare the variation between the two laboratories. Results of the Se analysis have not yet been compiled and reviewed.

Hen weights during the feeding trial were monitored before and after each dose administration. Dose-dependent cachexia (wasting syndrome) was observed in the study hens. Measurements of chicken embryonic masses at defined incubation period were also collected to detect dose-dependent retardation of embryogenesis (as suggested in the literature).

The feeding trial successfully produced eggs from the range of dietary exposures. Figure 1 demonstrates the decrease in embryo weight observed with the nominal 4, 8, and 12 ppm selenium diets. At 4 ppm (4.87ppm wet weight in the diet), embryos demonstrated decreased weight relative to eggs from the reference diet, and at higher selenium levels embryo weights were very significantly reduced. Figure 2 shows that no malformations or terata were observed in embryos from the control or 4 ppm diets, but terata and viability of embryos was markedly reduced for the higher selenium levels (17% at 9.43ppm, and 97% in the 13.2ppm diet). The slight decrease in embryo weight shown in Figure 1 at the 4 ppm dietary level appears to be the most sensitive parameter for detecting an effect of selenium. Statistical analyses are incomplete to date, but it is expected that this pattern will be quantitatively confirmed for visually "normal" chicken embryos.

The chicken embryos, yolk sacs, and albumin fractions have been collected using the same methods as for wild bird eggs, and Se levels have been measured in each fraction. The complete analysis of selenium from the

separated egg fractions has been completed, but the data analysis and comparison of chicken data with the data on wild birds have not been completed. The selenium partitioning data will be presented in 2002.

MALLARD DUCK FEEDING TRIAL

In early 2001 a selenium feeding trial was initiated with Mallard ducks to essentially duplicate the chicken feeding trial in an effort to determine the species differences in Se incorporation into eggs and into embryonic tissues. Because individual housing of ducks is not practical, groups of 5-6 female and two male ducks were housed together in floor pens using the same procedures as for toxicity testing conducted by the US EPA. Pens of ducks were randomly assigned to three groups: reference controls, 4 ppm. Se, and 8 ppm. Se as nominal treatments. Eggs were collected from each group prior to introduction of treatment feed as pre-treatment controls, and the groups were fed control or treatment diets for 2 months, with eggs collected on a daily basis. Eggs were collected and stored at 12°C for up to one week, and artificially incubated to 21 days of the normal 28 day incubation period, to produce embryos $\frac{3}{4}$ through the normal incubation period. This embryo age duplicates our studies with chickens and wild bird species.

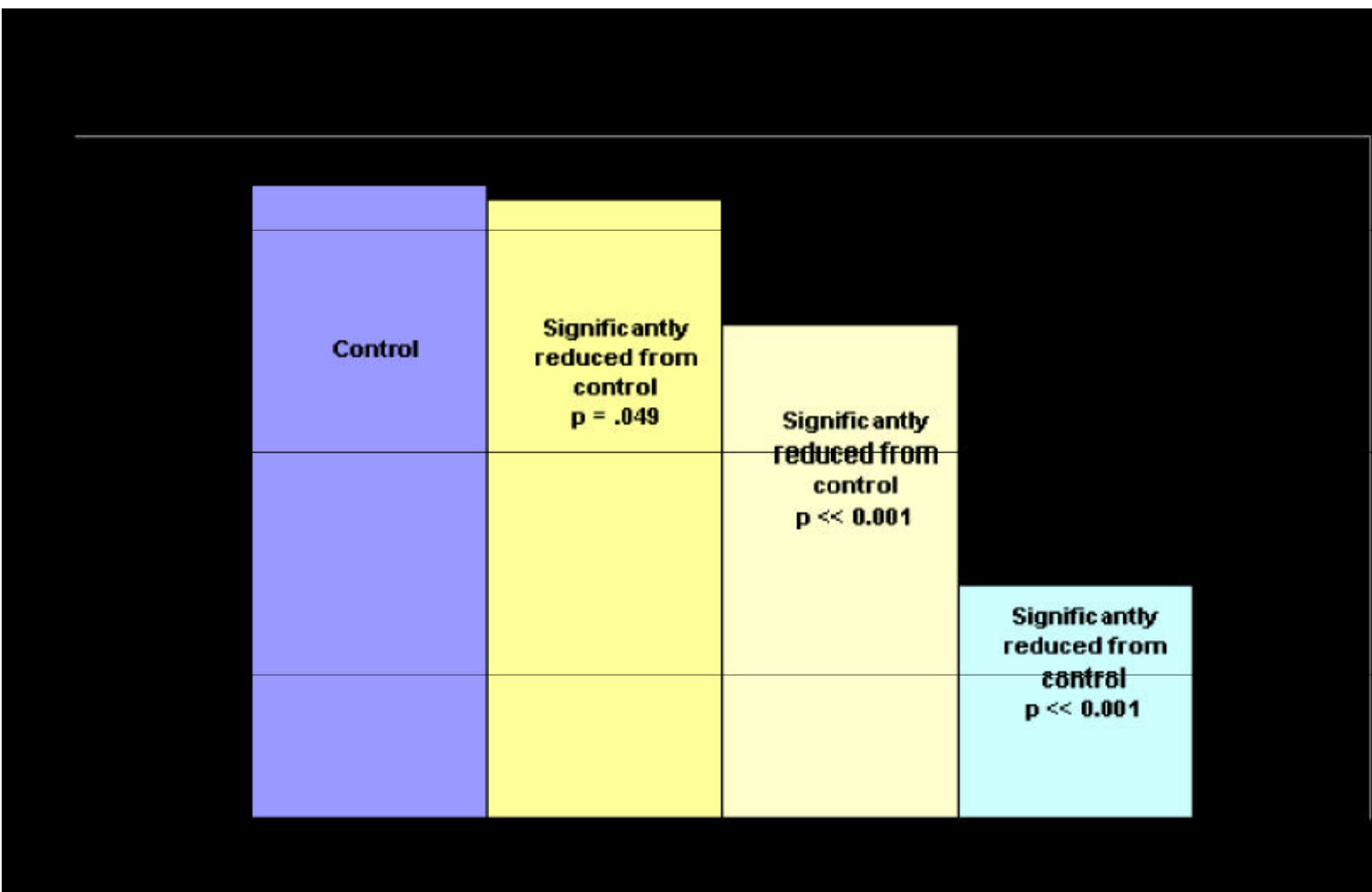
148 eggs were collected from ducks in the 4 ppm diet treatment group, 95 from the 8 ppm treatment, and 103 from the pretreatment and reference diet. Eggs were weighed, broken out, and contents separated into embryo, yolk, and albumin fractions, exactly as in the chicken study. We are currently in the process of preparing the samples for Se analysis at CAHFS. Selenium partitioning results will be compared to the chicken and wild bird studies, to evaluate the ability of each species to prevent incorporation of selenium into proteins which result in terata.

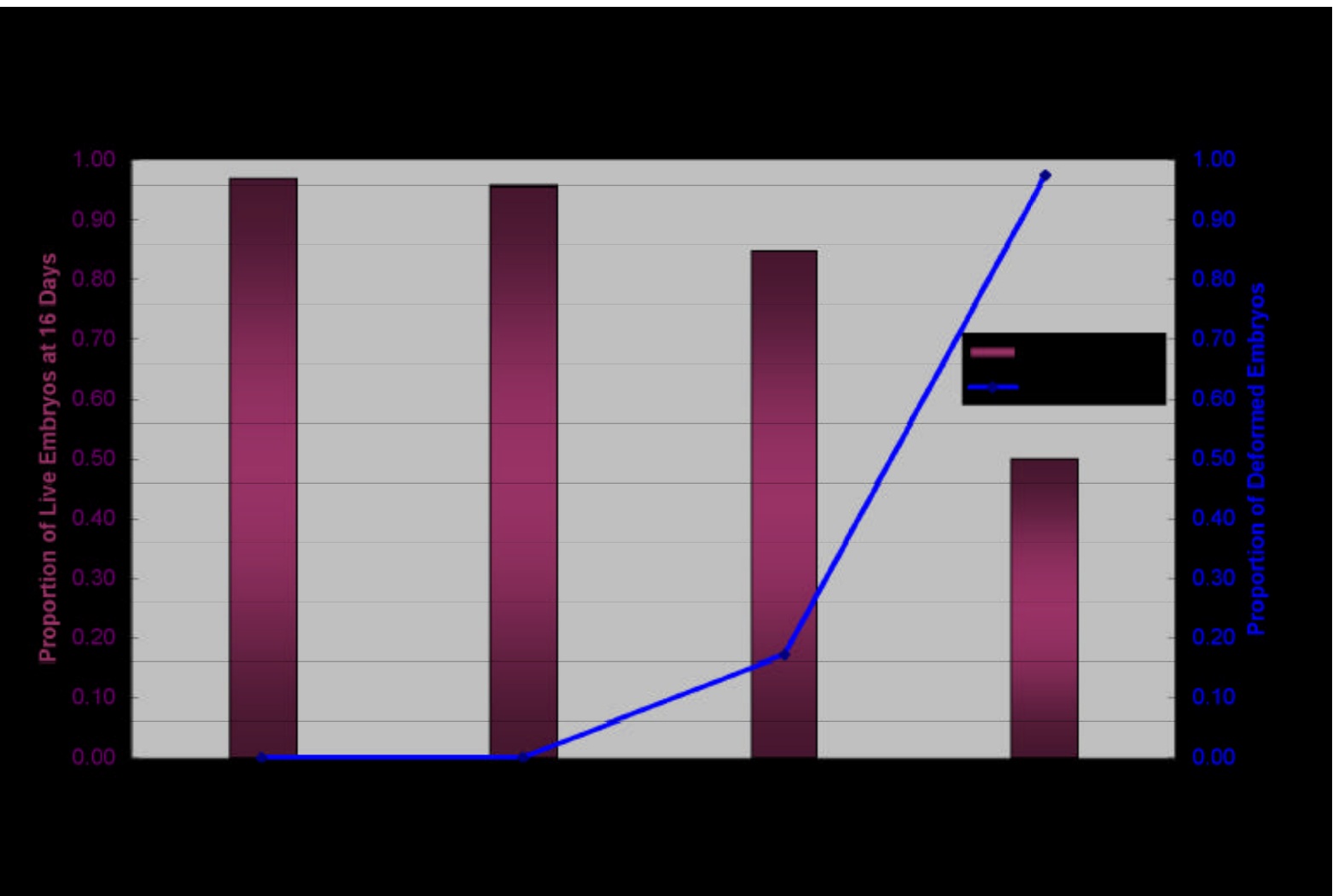
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Validation of Protocols for Using Trunk Diameter and Tree Water Potential Measurements in Orchard Irrigation Scheduling

Project Investigators:

David A. Goldhamer, Kearney Ag. Center/Dept. of LAWR, UC Davis

R. Scott Johnson, Kearney Ag. Center/Dept. of Pomology, UC Davis

Ken Shackel, Dept. of Pomology, UC Davis

Research Staff:

Mario Salinas, RA

Jesus Salinas, RA

Miguel Marquez, RA

ABSTRACT

Irrigation scheduling protocols involving two different parameters associated with tree water status were tested in a mature, commercial almond orchard. Maximum daily trunk shrinkage (MDS) gleaned from trunk diameter fluctuations measured with linear variable displacement transducers (LVDTs) and shaded leaf water potential (LWP) taken with a pressure chamber were the operative parameters involved. Signals for each parameter were calculated as the actual measurement divided by reference values taken on trees that received 150% of the ranch practice and were thus, fully irrigated. The scheduling protocols called for a 10% decrease in the rate of applied water when the signal exceeded a threshold value of 1.25 for 3 consecutive days. Conversely, there was a 10% increase in the rate of applied water when the signal was less than 1.25 for 3 consecutive days. These protocols resulted in applied water for both the MDS and shaded LWP approaches greatly exceeding estimated evapotranspiration (ETc). We believe this was due to the Control trees having much lower MDS and higher shaded LWP throughout the season than those in the scheduling plots. This occurred despite the fact that all trees received water in excess of ETc and would, thus, have been expected to have the same MDS and shaded LWP. An independent study confirmed the fact that tree water status may change as irrigation rates in excess of ETc change. This raises questions concerning the use of in-field Control, reference, or baseline trees in scheduling protocols for plant-based indicators.

Keywords: Irrigation scheduling, plant sensors, linear variable displacement transducer, LVDT, stem water potential

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BACKGROUND AND JUSTIFICATION

The most popular methods of irrigation scheduling in orchard crops are based on soil and atmospheric parameters even though these measured parameters are only indirectly related to the well-being of the tree. It has long been recognized that the plant is

the best indicator of its water status but until recently, plant-based monitoring involved discrete measurements in both space and time. Assessing plant water status requires that a technician travel to the field with a rather cumbersome pressure chamber. The timely detection of critical plant water stress depends on the monitoring frequency and how much labor is allocated for this approach.

The current state of the art in plant water status monitoring is measuring stem water potential (SWP). This technique requires that a small, foil covered polyethylene bag be placed on the leaf for a given amount of time prior to the leaf being excised and placed in the pressure chamber. In our previous work, we found that interior, shaded leaf water potential (LWP) was highly related to stem water potential (SWP) in almond trees. Thus, using shaded LWP saves time compared with SWP.

Another problem in using plant water status measurements as indicators for water management is that they are very dynamic, reflecting the balance between soil water supply and atmospheric demand. Thus, a single measurement may not be very informative as the value is influenced by both soil water level and weather conditions. What's needed are instruments that continuously record plant water status or a plant feature that is related to water status. The development of instruments of this type and associated protocols for their use can result in the automation of irrigation scheduling (coupling of plant-based indicator to computer/ electronic irrigation controller), as suggested by Huck and Klepper (1976) and Huguet et al., (1992).

Our work during the past two years shows that changes in trunk diameter correlate with tree water status (Goldhamer et al., 1999; Goldhamer et al., 2000). We use linear variable displacement transducers (LVDTs) to continuously record trunk diameter at the micron level. Trunk diameters have diurnal oscillations; maximum and minimum sizes occur in the early morning and late afternoon, respectively. While there are various parameters that can be gleaned from the trunk diameter oscillations, we have found that maximum daily trunk shrinkage (MDS), defined as the difference between daily maximum and

minimum values, is related to plant water status in mature trees.

Last season, we developed and tested MDS and shaded LWP-based scheduling protocols for the first time in a daily irrigated, mature, commercial almond orchard. The performance of these parameters was assessed based on applied water, plant water status during the season, and yield component results.

OBJECTIVE

To evaluate MDS and shaded LWP-based irrigation scheduling protocols in a mature, commercial almond orchard.

METHODS

This work was conducted in cooperation with Paramount Farming Co., West Valley Ranch, located near Lost Hills. The experimental orchard is irrigated with a buried drip system that contains multiple irrigation sets (blocks), applies water 2-3 times/day, and can be irrigated independently. Three sites were established in two adjacent blocks. The three sites will be referred to as: (1) LVDT Indicator, (2) SLWP Indicator, and (3) Control. The Control plot provided “baseline” data for fully irrigated trees, which was insured by installing a micro-sprinkler on each tree in addition to the existing buried drip emitters. Thus, the Control trees received 150% of the water added to as the “ranch practice”. We assumed this would always exceed estimated evapotranspiration (ET_c) and that the well drained, sandy loam soil would allow for deep percolation of the excess water and the trees in the Control plot would not be subject to anoxia.

Each site contained 4 trees instrumented with LVDT's (Model 2.5 DF Solartron Metrology, Bagnor Regis, U.K.). The LVDTs were installed on primary scaffolds on the north side of each tree. They were mounted on holders built of aluminum and INVAR—an alloy comprised of 64% Fe and 35% Ni that has minimal thermal expansion (Li et al, 1989)—and covered with silver foil to provide constant shade. Measurements were taken every 30 secs and the datalogger (Model CR 10, Campbell Scientific, Logan, Utah, US) was programmed to report 20 min means. Data were downloaded to a

laptop computer every 3-4 days and taken to the Kearney Ag. Center for analysis.

Interior, shaded water potential was measured using a pressure chamber (Model 3005 Soil Moisture Equipment Co., Santa Barbara, CA) each weekday on each of the 12 LVDT-instrumented trees. The SLWP procedure involves covering leaves with a damp cloth prior to excision. The cloth is removed just prior to placing the leaf in the pressure chamber. These measurements were made between 12:30 and 1:30 pm. Precautions recommended by Hsiao (1990) were taken to prevent leaf water loss during the measurement.

Our development of protocols for using MDS and shaded LWP for irrigation scheduling is predicated on the assumption these measurements can be used to indicate the presence of very mild water stress. Moreover, that almond trees tolerate this mild stress without negative impacts on production. We used the following protocol (Goldhamer and Fereres, In Press) for the LVDT and SLWP Indicator blocks in this study:

- 1) The irrigation starting date is selected and a fixed amount (irrigation duration) is applied.
- 2) Reference MDS and shaded LWP are determined in the Control trees.
- 3) The LVDT and SLWP indicator signals are calculated as the MDS of the indicator trees divided by the MDS and shaded LWP reference values.
- 4) When the Indicator signals exceeds 1.25 for 3 consecutive days, the daily irrigation rate is raised by 10%. Similarly, if the Indicator signals do not reach 1.25 for 3 consecutive days, the irrigation rate is decreased by 10%.

Water meters were used to measure applied water. At the end of the season, the 4 monitored trees at each site were harvested and a 2 kg fruit samples per tree were collected. The sample was returned to the lab where each nut was analyzed to determine hull split, and hull, shell, and kernel weights.

RESULTS AND DISCUSSION

The MDS and shaded LWP values varied between irrigation treatments over the season

(Figs. 1a and 1b, respectively). The Control, which received the most water, had consistently lower MDS and higher shaded LWP than the LVDT and SLWP Indicator regimes.

Both the LVDT and SLWP regimes were well managed, as evidenced by the fact that signal values for each technique oscillated around the 1.25 threshold over the season (Fig. 2). However, the greater sensitivity of the MDS measurements to tree water status as compared with the shaded LWP measurements is evident by the higher "peaks" and lower "valleys." While the irrigation regimes were well managed and followed the scheduling protocol as well as can be expected, all irrigation treatments applied water far in excess of estimated orchard ETC, both on a seasonal and rate basis (Figs. 3a and 3b, respectively). This resulted in few differences in yield and yield component values (Table 1).

Since all the treatments received water in excess of ETC and one would expect that the extra water would drain out of the root zone, conventional wisdom dictates that none of the trees would be water stressed and thus, the MDS and shaded LWP values would be the same for all treatments. This did not occur. Control values, from trees that received the most water, had consistently lower MDS and higher shaded LWP values throughout the season. These low MDS and high shaded LWP values can be interpreted as indicating that these trees were under less water stress than those in the other irrigation regimes.

In view of these results, we conducted an independent test of the impact of irrigation in excess of ETC during a daily irrigation cycle in late July. Normal irrigation (approximately 250% of ETC) to the Control trees occurred from 7:00-9:30 am. At 11:45 am, an additional 35 gals. of water were applied to two of the four instrumented Control trees. Diurnal measurements of stem water potential (SWP) and stomatal conductance were taken every hour from 4:30 am to 2:30 pm. While the two tree sets had the same SWP from predawn through 11:00 am, the trees that received the extra water at 11:45 am had significantly higher SWP in the subsequent six measurement periods (Fig. 4a). The patterns of stomatal conductance were not similarly affected (Fig. 4b).

Our current guidelines for interpreting trunk diameter measurements and the Shackel et al. (1997) approach using SWP assume that the Control, "reference," or "baseline" indicator values of "stress" will not vary with applied water in excess of ETC; that excess water will simply travel below the root zone. The fact that high soil water levels, far greater than field capacity, in a relatively large amount of the root zone changed plant water status when compared to other irrigation regimes that less received water but also in excess of ETC raises questions concerning the use of in-field Control, reference, or baseline trees in scheduling protocols for plant-based indicators. This affects the validity of the current scheduling guidelines suggested by our group and Shackel. It's possible that since soil hydraulic conductivity increases exponentially with soil water content, maintaining high soil water content at the root/soil interface may decrease flow resistance and thus, increase (make less negative) the plant water potential required to sustain a given transpiration rate.

While establishing the validity of using Control, reference, or baseline values in interpreting plant-based measurements for irrigation scheduling is an important issue, one approach to adapting protocols where these values are used is to adjust signal "threshold" values. Last year, our threshold value of 1.25 was clearly too low. We believe that these values can be set such that the high stress sensitivity of MDS becomes an asset to this procedure and results in applied water being less than orchard ETC. Performance using the adjusted signal threshold values will be evaluated in 2001.

To help expand our knowledge of how excess irrigation affects plant water status, we propose to irrigate peach trees surrounding the large, weighing lysimeter at the Kearney Ag. Center at four rates: 100, 125, 150, and 175% of lysimeter ETC. We believe that the transport properties of the soil are sufficient to allow for drainage even with irrigation at 175% ETC. Tree water status, trunk diameter fluctuations, and fruit growth will be evaluated.

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Table 1. Almond yield and yield component values for the 2000 season.

			Dry	Dry		Fresh			
Irrigation	FulHull	Kemel	Kemel	Kemel	Fruit	Kemel	Kemel	Shell	Hull
Regimes	Split	Weight	Kemel+Shell	Nut	Load	Yield	Hydration	Hydration	Hydration
	(%)	(gm /kemel)	(%)	(%)	(#/tree)	(bs/acre)	(%)	(%)	(%)
SLW P	96.6	1.06 ab	64.0 a	28.6	5460	1282	4.17 a	8.6 a	11.0 a
LVDT	96.2	1.00 a	64.4 ab	28.5	5803	1241	5.68 ab	10.6 ab	14.8 ab
Control	94.3	1.11 b	66.8 b	29.3	4852	1348	8.92 c	14.8 c	25.0 c
	NSD			NSD	NSD	NSD			

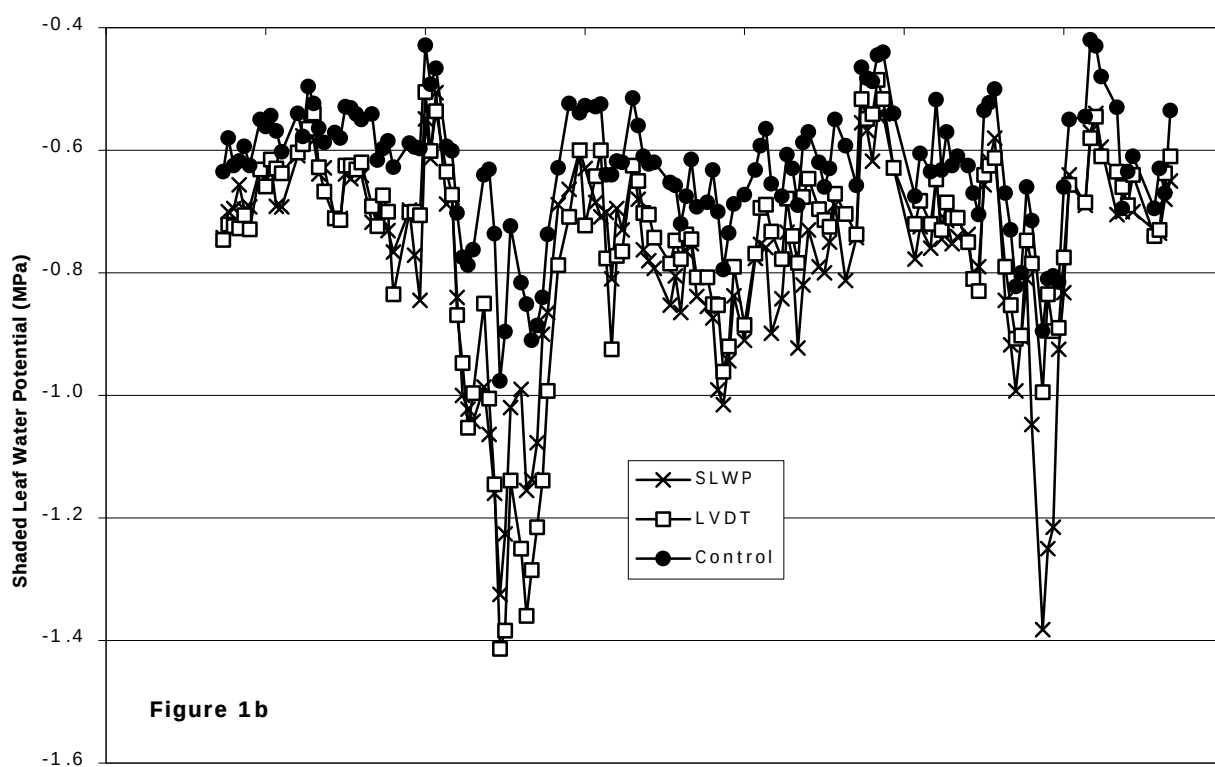
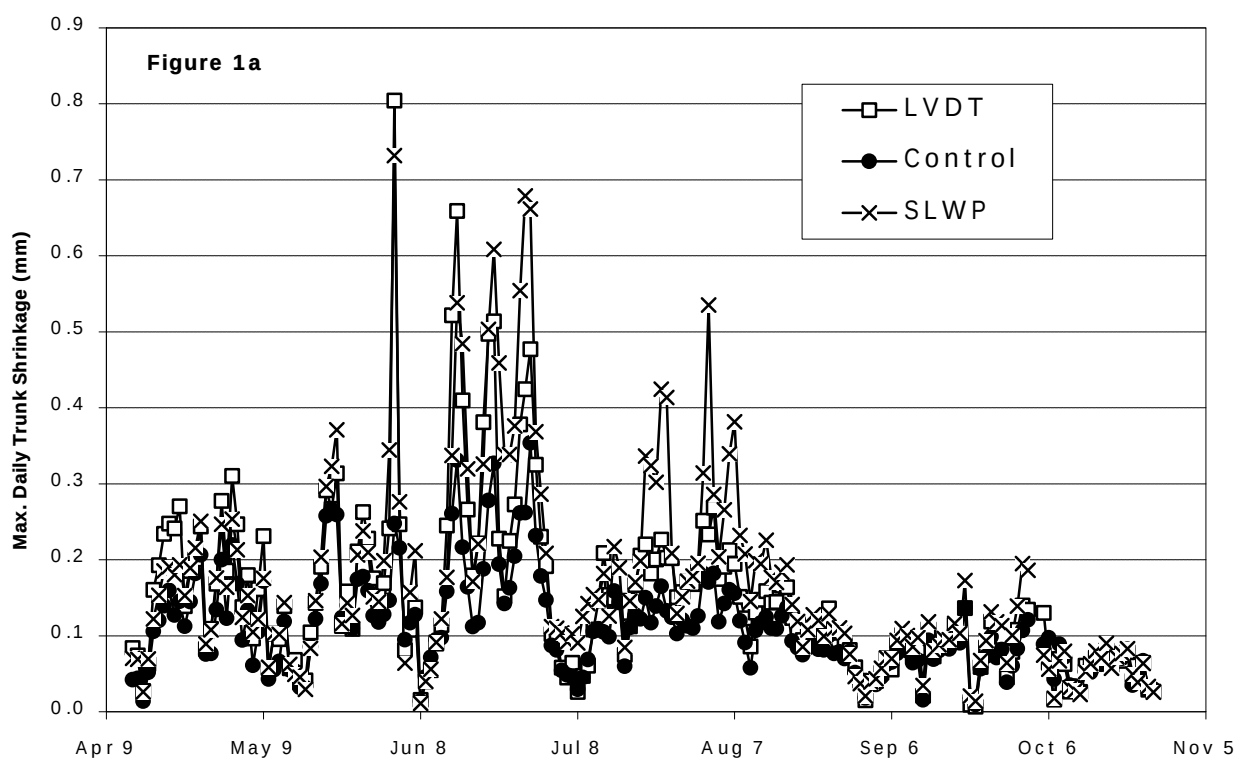


Figure 1. MDS (a) and shaded LWP (b) with time over the 2000 season for the LVDT and SLWP Indicator regimes.

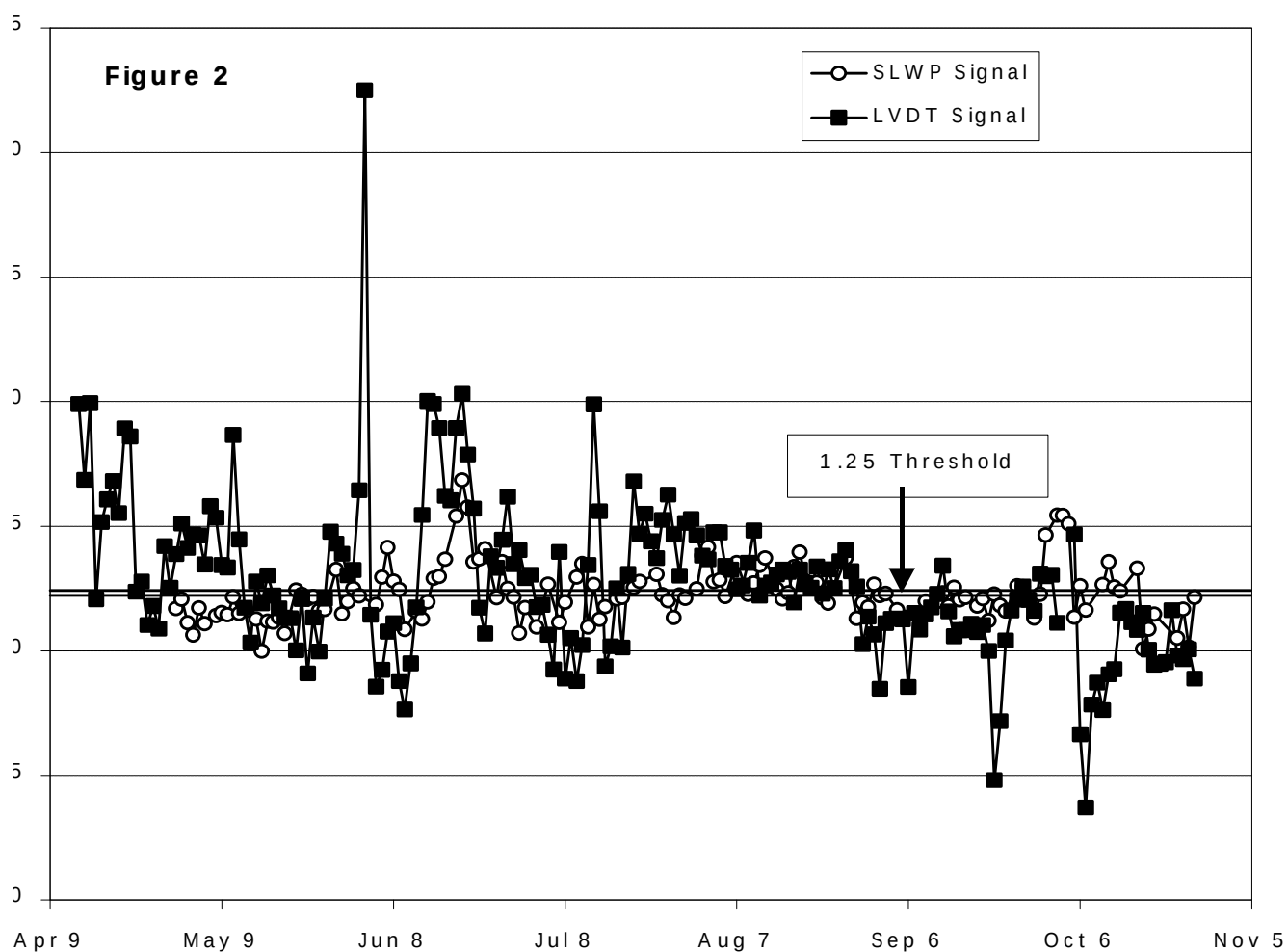


Figure 2. Signals (Indicator plot values/reference values) for LVDT and SLWP measurements

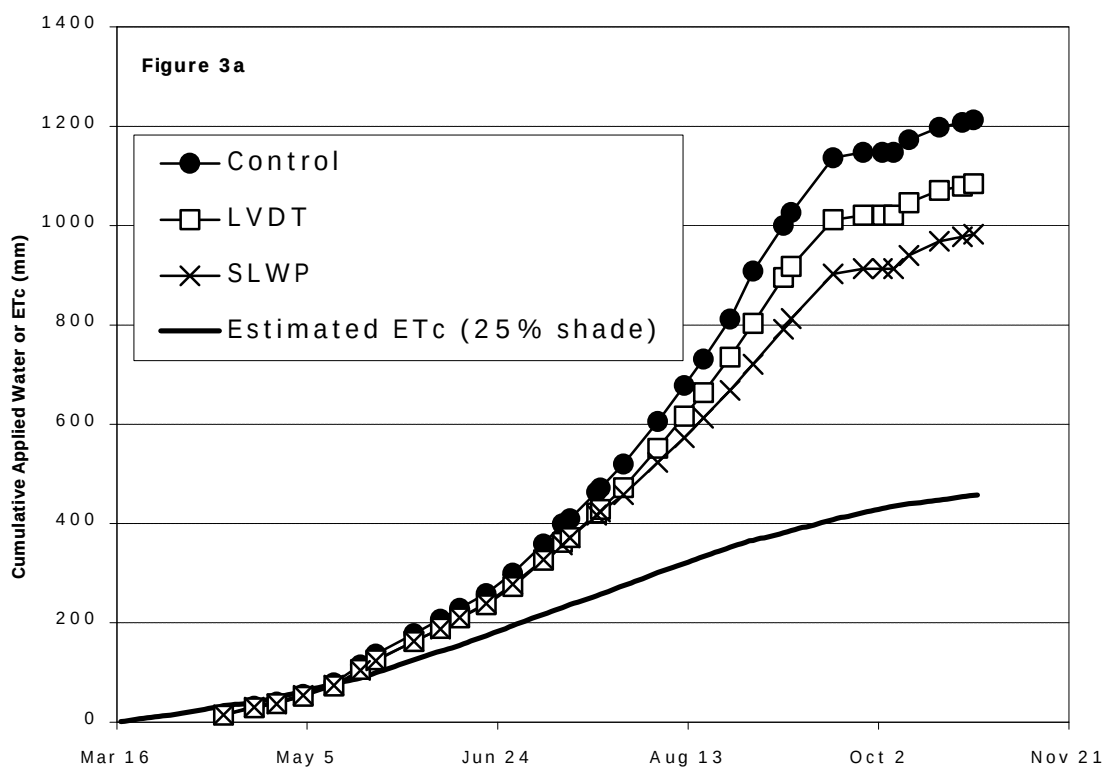
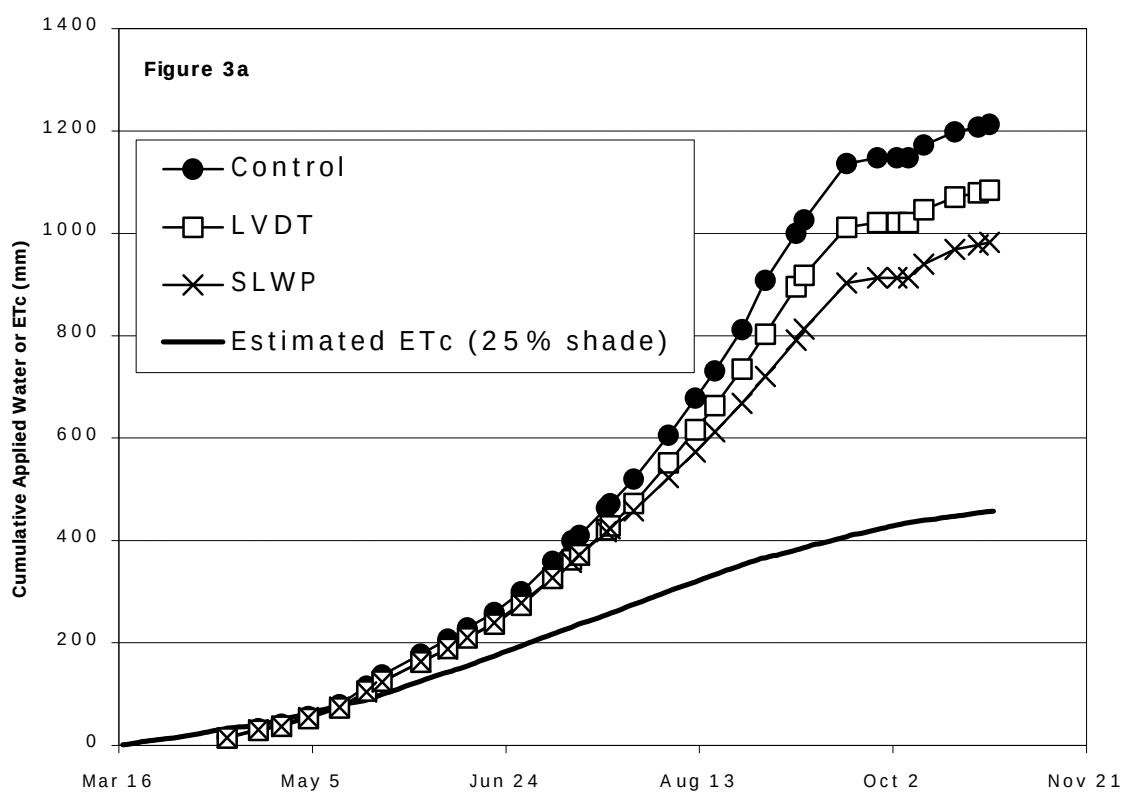


Figure 3. Cumulative (a) and rates (b) of applied water and estimated orchard ETc during the 2000 season.

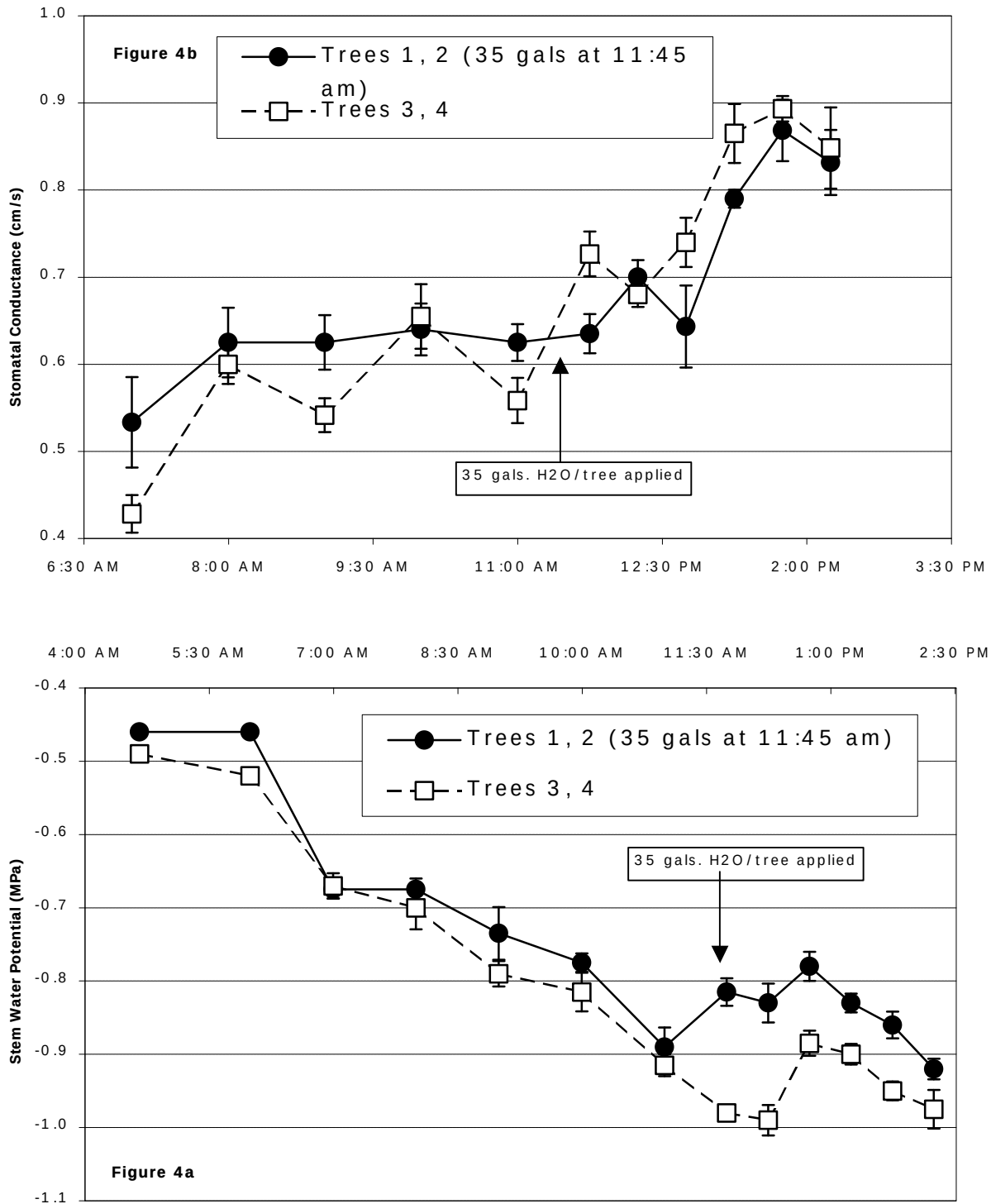


Figure 4. Stem water potential (a) and stomatal conductance (b) on Control trees during July 26 diurnal study.



Evaluation of Salt-tolerant Forages for Sequential Reuse Systems

Project Investigators:

Steve Grattan*, Dept. LAWR, UC Davis, Davis, CA

Catherine Grieve, USDA/ARS, US Salinity Lab, Riverside, CA

Jim Poss, USDA/ARS, US Salinity Lab, Riverside, CA

Sharon Benes, Dept. of Plant Science, CSUF, Fresno, CA

Dong Wang, USDA/ARS, US Salinity Lab, Riverside, CA

Don Suarez, USDA/ARS, US Salinity Lab, Riverside, CA

Peter Robinson, Dept. of Animal Science, UC Davis, Davis, CA

***Team Leader**

Research Staff:

Four UCR students, casual labor (1.5 FTE)

ABSTRACT

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). There are a number of salt-tolerant forages that may play an important role in reducing drainage water volumes in the San Joaquin Valley while at the same time producing a food source for sheep and dairy cattle. Their actual suitability for sequential reuse systems, however, will depend upon their production potential under saline-sodic conditions and their resulting forage quality.

A controlled study using an elaborate sand-tank system is underway at the US Salinity Laboratory to evaluate a number of promising forage crops including Bermuda grass, 'Salado' and 'SW9720' alfalfa, 'Duncan' and 'Polo' paspalum, 'Big' and 'Narrow leaf' trefoil, Kikuyu grass, Jose tall wheatgrass, and Alkali sacaton. Forages are irrigated frequently with synthetic drainage waters with an EC of either 15 or 25 dS/m. Forages are cut periodically and biomass is presented as a function of cumulative thermal time. Plant material is currently being analyzed for mineral content, trace elements, and the standard forage quality parameters.

The forage species tested performed differently in terms of absolute biomass accumulation, biomass accumulation relative to salinity level, and various forage quality parameters, which also varied from cutting to cutting.

At 25 dS/m, Kikuyu grass produced the largest amount of biomass at 5000 °C-d, followed closely by alfalfa "SW 9720", Jose tall wheat grass, bermuda grass, alfalfa 'salado' and Narrow leaf trefoil. While Kikuyu produced well in these conditions, its forage quality was among the lowest. Those forages that were of good to high quality were the two alfalfa varieties, Duncan paspalum, narrow leaf trefoil, bermuda grass, Jose tall wheat grass and polo paspalum.

The difficulty in comparing species is complex particularly when trying to rank cultivars by their combined forage production

and quality potentials. Therefore the ranking is based on both quantitative and qualitative factors. Overall the alfalfa cultivars have performed the best under these controlled conditions. Their performance will likely decline at higher salinity because these cultivars were found to be the most salt-sensitive. Narrow leaf trefoil and bermuda grass fall into the next class followed closely by Jose tall wheatgrass. Under field conditions, other soil, climate and biological factors will interact affecting forages differently and therefore affecting the ranking based on this controlled study.

KEYWORDS: biomass, canopy reflectance, drainage, forage-quality, molybdenum, salinity, selenium.

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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 are being demonstrated under field conditions. Under 'sequential reuse', part of the farm or sub-region is designated as the reuse area. It consists of a sequence of fields, within the boundaries of a farm or an irrigation district, that are irrigated with saline drainage water of increasingly higher concentrations. The main purpose is to manage the salt and drainage water on the farm, reduce the area affected by shallow water tables and reduce the volume of drainage water requiring disposal without sacrificing the potential productivity of these lands.

High quality forages for dairy cattle, beef cattle, and sheep are in short supply in the Central Valley. Dairy production alone has risen 50% in the last decade. Identifying salt-tolerant forage crops that could grow well under irrigation with saline drainage water could not only increase forage supplies, but

could play a key role in drainage water management. Their actual suitability for reuse systems, however, will depend upon their production potential under saline-sodic conditions and their resulting forage quality.

Although some studies have been conducted that address forage quality in salt-stressed land (e.g. Atiz-ur-Rehman et al. 1999), a considerable amount of additional research in this area is needed (San Joaquin Valley Drainage Implementation Program, 2000). Currently, field studies and field demonstrations are underway to test the feasibility of a few salt-tolerant forages and forage cropping strategies (S. Benes and S. Kaffka, personal communication; Oster et al., 1999) for irrigation with saline-sodic water. Some promising species include bermudagrass, saltgrass, siltgrass, alkali sacaton, Jose Tall wheatgrass, creeping wild rye, "salado" alfalfa, salicornia, purslane and atriplex (S. Benes, V. Cervinka, S. Kaffka and F. Menezes, personal communication; Grieve and Suarez, 1997, Marcum, 1999, Shannon et al. 1998).

Naturally occurring trace elements in soils in the SJV and in the underlying shallow-groundwater adds a new dimension to the management of saline drainage waters (van Schilfgaarde, 1990). Selenium (Se) and molybdenum (Mo) are trace elements of particular interest. They occur in relatively high concentrations (<1.0 to 5,000 µg/L for Mo and <1.0 to 3,800 µg/L for Se) at many locations in the geochemically mobile and biologically available forms i.e. selenate and molybdate (Deverel et al., 1984).

Due to the presence of these trace elements, there are potential toxicological concerns regarding livestock whose diet may rely almost entirely on forage grown with drainage water that contains appreciable levels of these constituents. Selenium and molybdenum are essential nutrient elements for animals, however, the concentration range in forage that would result in deficiency or toxicity to livestock is rather narrow. Selenium toxicity can occur in livestock that graze on forage containing high levels of Se (Rosenfeld and Beath, 1964). Alkali disease is one form of selenosis that can result from ingestion of forage containing as low as 35

mg/kg Se (Klasing and Schenker, 1988). Molybdenosis is a nutritional disorder that ruminant animals, particularly sheep and dairy cows, may develop if the animals feed on forage containing high levels of molybdenum (Barshad, 1948; Ward, 1978). Molybdenosis results from a molybdenum-induced Cu deficiency and is often called a molybdenum-induced hypocuprosis (Mason, 1990). However susceptibility to this disorder is more complex than a simple molybdenum toxicity and involves interactions with copper, zinc and sulfur nutrition as well (Ishizaki et al., 1991).

A number of investigations funded by the UC Salinity/Drainage Task Force showed that sulfate had a profound influence on reducing selenate and molybdate accumulation in alfalfa and other plants. Therefore, the presence of sulfate in the drainage water may in fact maintain forage quality by preventing excessive accumulation of these potentially toxic elements. Furthermore, saline drainage water applications could increase total digestible nutrients and protein within the forage (Rhoades et al., 1988). Although forage productivity may be reduced when irrigated with drainage water, we hypothesize that if managed appropriately, the quality and production of forage in well-grazed pastures can be sufficiently maintained to support acceptable rates of livestock growth.

Therefore an interdisciplinary research project was developed involving scientists from the University of California, USDA-ARS, and Cal State University Fresno. The team members have expertise in soils and irrigation management, plant physiology, salinity and plant nutrition, and ruminant nutrition. We are currently conducting a controlled study to evaluate a number of promising forage crops as suitable candidates for drainage water reuse systems.

RESEARCH OBJECTIVES

The overall objective of this study is to evaluate a number of promising forage crops in terms of their production potential and forage quality when irrigated with saline-sodic drainage water, containing selenate and molybdate.

SPECIFIC OBJECTIVES

1. To investigate the interactive effects of saline-sodic drainage water containing SeO_4^{2-} and MoO_4^{2-} on (a) forage production at different salinities and (b) the accumulation, distribution and selected ratios of Se, S, Mo, Cu and other inorganic elements in plant shoots.
2. To assess the effects of saline-sodic drainage water on forage quality (i.e. in-vitro gas evolution, Acid Detergent Fiber (ADF), Total Digestible Nutrients (TDN), Neutral Detergent Fiber (NDF), crude protein, and trace element concentration) in reference to ruminant nutrition. The influence of sequential harvests on forage quality will also be examined.

RESEARCH METHODS

The experiment is currently being conducted in greenhouse sand-tanks at the USDA-ARS, George E. Brown, Jr. Salinity Laboratory located on the U. C. Riverside campus. The sand tank system creates a uniform and controlled rootzone such that actual production potentials among forages can be compared. There are 30 large tanks (1.2 m x 0.6 m x 0.5 m deep) filled with washed sand and that has an average bulk density of 1.4 Mg m^{-3} . At saturation, the sand has an average volumetric water content of $0.34 \text{ m}^3 \text{ m}^{-3}$. Each tank is irrigated with a complete nutrient solution salinized to either 15 or 25 dS/m. The salt solutions were prepared to simulate the composition of typical drainage water in the San Joaquin Valley and from predictions based on appropriate simulations (Suarez and Simunek, 1997) (Table 1). Tanks were irrigated three times daily for a 15-min duration. These irrigations allowed the sand to become completely saturated, after which the solutions drained to 765 L reservoirs below the sand tanks for reuse in the next irrigation. Therefore the salinity of the irrigation water is more or less equivalent to that of the sand water. The irrigation waters were analyzed by inductively coupled plasma optical emission spectrometry (ICPOES) to confirm that target ion concentrations were maintained. Chloride in the solutions was determined by coulometric-amprometric titration.

Water lost by evapotranspiration is replenished automatically to maintain constant volumes and osmotic potentials in the irrigation waters using a pulse output water meter coupled to an electronic water level detector installed in each reservoir. A Class I agrometeorological station is situated immediately adjacent to the experimental site. Micrometeorological data, including sand and air temperature, pan evaporation, photosynthetically active radiation (PAR), relative humidity, and wind velocity are recorded.

Ten forages are grown in sand tanks at two salinity levels (15 or 25 dS/m) and each treatment is replicated three times. The forage species chosen for this study were: alfalfa (*Medicago sativa*) cvs. 'Salado' and 'SW 9720', narrow leaf trefoil (*Lotus glaber*), big trefoil (*L. ulginosus*), kikuyu grass (*Pennisetum clandestinum*) cv. Whittet, alkali sacaton (*Sporobolus airoides*), paspalum (*Paspalum vaginatum*) cvs. 'Polo' and 'Duncan', tall wheatgrass (*Agropyron elongatum*) cv. 'Jose', and bermuda grass (*Cynodon dactylon*) cv. 'Tifton'. The date of planting, the source of the material and the date salinization began are provided in Table 2. In each tank, two different forages were planted in a $0.6 \times 0.6 \text{ m}$ area, separated by a plastic partition extending ~8" below and 4" above the surface of the sand. With the exception of bermuda grass, all species were well-established in the tanks by irrigation with complete nutrient solution (EC_w) prior to application of salinity.

Harvest scheduling depended on growth pattern of the forage in question. For example, alfalfa cultivars were sampled at first flowering; alkali sacaton, kikuyu, tall wheatgrass, on plant height; the trefoils, paspalums, and bermudagrass on biomass production (Table 2). At each harvest, herbage was cut 2 to 4" above the surface of the sand. Shoot material was weighed, washed in deionized water, dried in a forced-air oven at 70°C for 72 hr. Statistical analyses were performed by analysis of variance with mean comparisons at the 95% level based on Tukey's studentized range test.

Dry-ground forage material is currently being analyzed for a number of inorganic constituents including Ca, Mg, Na, K, P, N, Cl,

S, B, Se and Mo. Total-S, total P, Ca^{2+} , Mg^{2+} , Na^+ , and K^+ are being determined on nitric-perchloric acid digests of the tissues by ICPOES. Chloride is being determined on nitric-acetic acid extracts by coulometric-amperometric titration. For tissue Se analysis, the method described by Briggs and Crock (1986) will be followed. Data are not yet available so no discussion of these elements are included in this annual report.

In vitro gas production was carried out using 30 ml of buffered rumen fluid according to the *in vitro* gas method of Menke et al. (1979). Approximately 200 mg sample was incubated in water bath at 39°C and gas production at 24 h was recorded and corrected for blank incubation (buffered rumen fluid with no sample). Organic matter (OM) was determined according to Association of Official Analytical Chemists (AOAC, 1990). *In vitro* true degradability (IVTD), neutral detergent fiber (NDF), and *in vitro* neutral detergent fiber degradability (dNDF) were determined by incubating the samples in multi-layer polyethylene polyester cloth bags (F57 filter bag; ANKOM, NY) as described by Robinson et al. (1999).

Forage quality determinations were statistically analyzed as a factorial experiment with salinity, forage, cut and the forage by harvest interaction as factors in the model. In a number of forage nutritive value descriptors, the cut by forage interaction was statistically significant (i.e., $P < 0.05$). Therefore forages were statistically analyzed within cuts and data presented represent all forages that were cut once, thrice and five times.

Salinity effects on biophysical parameters related to canopy development is determined with remote sensing techniques (Weigand et al., 1992; Penuelas et al., 1997; Wang et al., 2001). In this study, canopy reflectance of alfalfa cultivar 'SW 9720' was measured with a hand-held crops can multi-spectral radiometer (MSR) consisting of wavebands centered at 460, 560, 660, 661, 710, 810, 830, 900, 950 nm. Reflectance was computed from the simultaneous radiance measurements of paired sensors; one downward-facing and one upward-facing. The reflectance measurements were made on September 11,

2000. Scans were in triplicate on plants in two unshaded tanks in the greenhouse. These measurements are preliminary due to effects of greenhouse architecture on radiation uniformity. Outdoor measurements in greater detail are planned for the upcoming season. During each measurement the MSR was held 25 cm above the canopy at an angle perpendicular to the horizontal plane. One vegetation index, the SRVI (sample ratio vegetation index) developed by Wang et al., 2001, was calculated as the ratio of reflectance measurements between 830 and 660 nm.

RESULTS

BIOMASS ACCUMULATION

Shoot biomass accumulation is shown in relation to cumulative thermal time (i.e. degree-days) (Figures 1-10). There are a couple important features that can be derived from these graphs.

First, the biomass accumulation represents potential accumulation under field conditions if the average rootzone salinity of the soil water is either 15 or 25 dS/m. However under field conditions, there may be additional stresses that could affect plant growth such as anoxia, increased soil strength or pathogenic pressures such as phytophthora.

Given the obvious limitations of comparing species that have different optimal preferences for temperature and different planting dates, we have ranked the forage production potential based on cumulative production (kg/ha dw) at 5000 growing degree-days after planting. At 15 dS/m, alfalfa SW 9720 > salado alfalfa = kikuyu > JTWG = Bermuda > Duncan paspalum = AS = NL trefoil > Polo paspalum > big trefoil. At 25 dS/m, the ranking differs slightly: Kikuyu > SW 9720 > JTWG = Bermuda = salado alfalfa = NL trefoil > AS > Duncan paspalum > Polo paspalum > big trefoil.

The second important feature in the figures is the relative difference in the slopes (cumulative biomass as a function of thermal time) between the 15 and 25 dS/m treatments. The larger the difference in slopes or the smaller the ratio of slope at 25 / slope at 15 dS/m, the

more sensitive the crop is to salinity. In this study, with the exception of Big trefoil that died at 25 dS/m shortly after salinization, both alfalfa cultivars are among the most salt-sensitive along with perhaps Duncan paspalum. The other forages show considerably more tolerance to this synthetic drainage water suggesting that the performance rankings would differ from those given above should the salinity increase further. Therefore the alfalfa cultivars grow vigorously despite showing less overall tolerance to salinity.

FORAGE QUALITY

Forage quality was evaluated based on five parameters; organic matter (OM), neutral detergent fiber (NDF), digestible NDF (dNDF), in vitro true digestibility (IVTD) and gas evolution. The NDF is an estimate of the cell wall minus pectin and the dNDF is a measure of the NDF that is digestible in vitro at 30 h. The gas evolution at 24 h in vitro estimates digestion when fed to cows at a high level of production and is used later to estimate energy value. In general the quality or energy value of the forage increases as: OM increases, NDF decreases, dNDF increases, IVTD increases and as Gas production increases

First Cut

The OM content of Bermuda grass was higher at 25 dS/m than it was at 15 dS/m, but the reverse was true for Big trefoil ($P<0.05$) (Table 3). The NDF levels of 'Salado' Alfalfa, Big Trefoil and Jose Tall Wheatgrass were lower ($P<0.05$) for 25 dS/m vs. 15 dS/m material, but the opposite was true for Bermudagrass ($P<0.05$). The digestibility of NDF (i.e. dNDF) and the in vitro digestibility of DM (IVTD) were not influenced by salinity. However gas production, an indicator of the energy value, was higher ($P<0.05$) in Jose tall Wheat grass and Kikuyu grass at 25 dS/m as compared to material from the 15 dS/m treatment.

Third Cut

The NDF levels of forage material decreased as salinity increased from 15 dS/m to 25 dS/m ($P<0.001$) (Table 4). The IVTD in all forage material, on the other hand, increased ($P<0.05$). Other nutritive descriptors were not influenced by the salinity of the applied water.

Fifth Cut

The OM content of all forages was higher ($P<0.001$) for 25 dS/m vs. 15 dS/m material (Table 5). The NDF content of Alfalfa SW 9720 was lower ($P<0.05$), and the gas production was higher ($P<0.05$), for 25 dS/m vs. 15 dS/m material. NDF digestibility and IVTD were not influenced by the salinity of the applied water.

Salinity influenced various forage quality parameters including NDF, OM, Gas production and IVTD but their significance varied among species and cuttings. Whenever salinity influenced these quality parameters, it did so in a positive manner. The only exceptions were NDF for Bermuda and OM in Big trefoil during the first cutting.

The ability to compare forages per se is very limited statistically and we did not test for significant differences among forages between quality descriptors. Furthermore, we still need to integrate the gas with the CP and fat values to get a real energy value. Nevertheless, based on our forage quality results for the first, third and fifth cuttings, the quality rankings from highest to lowest as a first approximation are:

First Cut

Duncan paspalum > alfalfa 'SW 9720' = alfalfa 'salado' = bermuda = polo paspalum = narrow trefoil > alkali sacaton = Jose tall wheatgrass > big trefoil > kikuyu grass

Third Cut

Narrow leaf trefoil > Jose tall wheat grass = alfalfa 'SW 9720' = alfalfa 'salado' > alkali sacaton > kikuyu grass

Fifth Cut

Jose tall wheat grass = alfalfa 'SW 9720' = alfalfa 'salado' > kikuyu grass

REMOTE SENSING

Canopy reflectance of alfalfa cultivar SW 9720 as well as several other species was measured with a hand-held cropscan multi-spectral radiometer (MSR) consisting of various wavebands ranging between 460 and 950 nm. Our intent was to determine whether such

information could serve as an indicator of salinity stress.

One vegetation index, the SRVI (sample ratio vegetation index) developed by Wang et al., 2001, was calculated as the ratio of reflectance measurements between 830 and 660 nm. The reflectance data at 15 dS/m were significantly different than reflectance data obtained from plants grown at 25 dS/m at all wavebands except 559 and 950 nm. The SRVI was significantly reduced from 15.7 (se 0.2) to 13.0 (se 0.1) as salinity increased ($P < 0.05$). Data on the other species are limited at this time.

CONCLUSIONS TO DATE

The forage species tested performed differently in terms of absolute biomass accumulation, biomass accumulation relative to salinity level, and various forage quality parameters, which also varied from cutting to cutting.

At 25 dS/m, Kikuyu grass produced that largest amount of biomass at 5000 °C-d, followed closely by alfalfa 'SW 9720', Jose tall wheat grass, bermuda grass, alfalfa 'salado' and Narrow leaf trefoil. While Kikuyu produced well under these conditions, its forage quality was among the lowest. Those forages that were of good to high quality were the two alfalfa varieties, Duncan paspalum, narrow leaf trefoil, bermuda grass, Jose tall wheat grass and polo paspalum. The overall quality and various quality descriptors varied depending on the cutting.

The difficulty in comparing species has already been emphasized. It becomes even more complex when trying to rank cultivars by their combined forage production and quality

potentials. The investigators understand the importance of attempting such a ranking in the interest of mission of the Salinity/Drainage Program. Therefore the ranking is based on both quantitative and qualitative factors. We hesitate to provide such a ranking for fear that it could be misused or misleading. Under field conditions, other soil, climate and biological factors will interact affecting forages differently and therefore affecting the ranking based on this controlled study. Nevertheless this provides a fair starting point and can be useful provided the limitations are understood.

Overall the alfalfa cultivars have performed the best under these controlled conditions. Their performance will likely decline at higher salinity because these cultivars were found to be the most salt-sensitive. That is the relative decline in forage production as salinity increased from 15 to 25 dS/m was the highest. Narrow leaf trefoil and bermuda grass would fall into the second class followed closely by Jose tall wheatgrass.

The reader must also realize that the EC of the sand water (i.e. the EC's provided here in this study) do not equal the average rootzone salinity expressed as a saturated soil extract (ECe). As a general rule of thumb, the EC of the soil water is roughly twice that of the ECe. Therefore, as a first approximation to estimate the production potential of these forages under field conditions (i.e. average rootzone salinity), ECe is calculated as the treatment EC divided by two. Since all other stresses are eliminated from this sand-tank study, one could not expect them to perform better in the field.

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PUBLICATIONS AND REPORTS: **None to date.**

Table 1. Ionic composition of the simulated drainage water treatments. Both means and standard errors (se) are provided.

Salinity	Ca mmolc/ L	Mg mmolc/ L	Na mmolc/ L	K mmolc/ L	S mmolc/ L	Cl mmolc/ L	B mg/L	Se mg/L	Mo mg/L
15 dS/m	24.2	28.8	126	3.7	112.0	57.8	0.25	0.50	0.50
se	0.3	0.2	1	0.3	0.6	0.6			
30 dS/m	23.8	55.3	246	4.7	195.0	106.0	0.25	0.50	0.50
se	0.3	0.3	4	0.2	2.0	3.0			

Table 2. Forage type and source, planting date, and date salinization began.

Forage	Planting Date	Salinization Date	Source
Alfalfa, 'Salado'	7-17-00*	8-14-00	D. Davis via ABI Alfalfa, Nampa, ID
Alfalfa 'SW 9720'	7-17-00*	8-14-00	S&W Seed Co., Five Points, CA
Narrowleaf Trefoil	7-17-00*	8-25-00	J. Steiner, USDA-ARS, Corvallis, OR
Big Trefoil	7-17-00*	8-25-00	J. Steiner, USDA-ARS, Corvallis, OR
Tall Wheatgrass 'Jose'	7-28-00*	8-25-00	S&W Seed Co., Five Points, CA
Alkali Sacaton	7-28-00*	8-25-00	USDA/NRCS Plant Materials Center
Kikuyu grass	8-03-00*	9-19-00	Agrotera, McMinnville, OR
Paspalum 'Polo'	8-31-00†	1-18-01	R. Duncan, University of Georgia
Paspalum 'Duncan'	8-31-00†	1-18-01	R. Duncan, University of Georgia
Bermuda grass 'Tifton'	1-22-01†‡	1-22-01	J. Guerrero, UC Davis

*Seeded

†Sprigs

‡Planted in pre-salinized sand tanks

Table 3. Effect of salinity level on nutritive value of different species of forages at first cut.

Forage species	Salinity dS/m	OM (% DM)	NDF (%DM)	dNDF (% NDF)	IVTD (% DM)	Gas (ml/g DM)
Alfalfa 'SW 9720'	15	88.5	34.1	47.5	81.7	220
	25	88.5	25.1	49.6	87.2	233
Alfalfa 'salado'	15	88.8	31.6 ^a	50.4	84.3	239
	25	88.7	26.5 ^b	49.8	86.7	229
Alkali sacaton	15	86.6	55.8	55.6	75.3	209
	25	87.4	53.9	61.6	79.3	201
Bermuda grass	15	87.5 ^b	55.9 ^b	67.3	81.8	216
	25	89.3 ^a	60.9 ^a	64.4	78.3	221
Big trefoil	15	82.4 ^a	19.1 ^a	59.8	92.3	188
	25	77.4 ^b	16.1 ^s	61.0	93.7	175
Duncan paspalum	15	88.0	59.9	81.7	89.0	277
	25	86.0	59.7	79.9	87.9	267
Jose tall wheat grass	15	86.2	46.2 ^a	77.4	89.6	187 ^b
	25	87.1	43.5 ^b	76.9	89.9	205 ^a
Kikuyu grass	15	82.6	49.5	56.1	78.3	99 ^b
	25	84.0	43.9	55.2	80.2	133 ^a
Narrow leaf trefoil	15	87.5	25.1	55.8	88.9	221
	25	88.0	22.9	54.8	89.7	219
Polo paspalum	15	87.3	53.8	75.0	86.5	230
	25	86.5	51.8	72.8	85.9	214
SEM		0.27	0.53	1.20	0.59	2.6
Salinity		NS	***	NS	NS	NS
Species		***	***	***	***	***
Species x salinity		*	*	NS	NS	*

Different letters between salinity levels within species indicate significant differences (P<0.05).
*, P < 0.05; **, P < 0.01; ***, P < 0.001.

Table 4. Effect of salinity level on nutritive value of different species of forages at third cut.

Forage species	Salinity DS/m	OM (% DM)	NDF (%DM)	dNDF (% NDF)	IVTD (% DM)	Gas (ml/g DM)
Alfalfa 'SW 9720'	15	88.2	31.1	56.6	85.9	215
	25	88.2	24.3	56.3	89.3	232
Alfalfa (solado)	15	87.9	25.5	55.4	88.7	212
	25	88.3	22.4	55.9	90.1	234
Alkali sacaton	15	87.9	59.8	46.0	67.8	203
	25	88.4	59.4	52.7	71.9	212
Jose tall wheat grass	15	84.4	46.1	75.7	88.8	228
	25	86.5	40.2	77.2	90.9	208
Kikuyu grass	15	83.3	48.0	68.4	84.8	168
	25	84.4	40.6	70.4	88.0	148
Narrow leaf trefoil	15	85.3	22.6	65.1	92.1	245
	25	85.9	21.8	60.5	91.4	250
SEM		0.02	0.77	2.00	0.97	5.5
Salinity		NS	***	NS	*	NS
Species		***	***	***	***	***
Salinity x Species		NS	NS	N.S	NS	NS

Different letters between salinity levels within species indicate significant differences (P<0.05).

*, P < 0.05; **, P < 0.01; ***, P < 0.001

Table 5. Effect of salinity level on nutritive value of different species of forages at the fifth cut.

Forage species	Salinity DS/m	OM (% DM)	NDF (%DM)	dNDF (% NDF)	IVTD (% DM)	Gas (ml/g DM)
Alfalfa 'SW 9720'	15	87.8	27.6 ^a	59.8	88.9	220 ^b
	25	88.4	24.3 ^b	56.6	89.4	245 ^a
Alfalfa 'salado'	15	87.5	25.2	57.2	89.4	230
	25	88.6	22.2	58.2	90.7	241
Jose tall wheat grass	15	86.3	42.2	80.7	91.9	229
	25	87.3	44.2	78.8	90.6	240
Kikuyu grass	15	82.6	44.6	59.7	82.0	140
	25	83.8	43.3	47.9	77.3	111
SEM		0.18	0.58	2.59	1.18	6.2
Salinity		**	*	NS	NS	NS
Species		***	***	***	***	***
Salinity x Harvest		NS	*	NS	NS	*

Different letters between salinity levels within species indicate significant differences ($P < 0.05$).
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

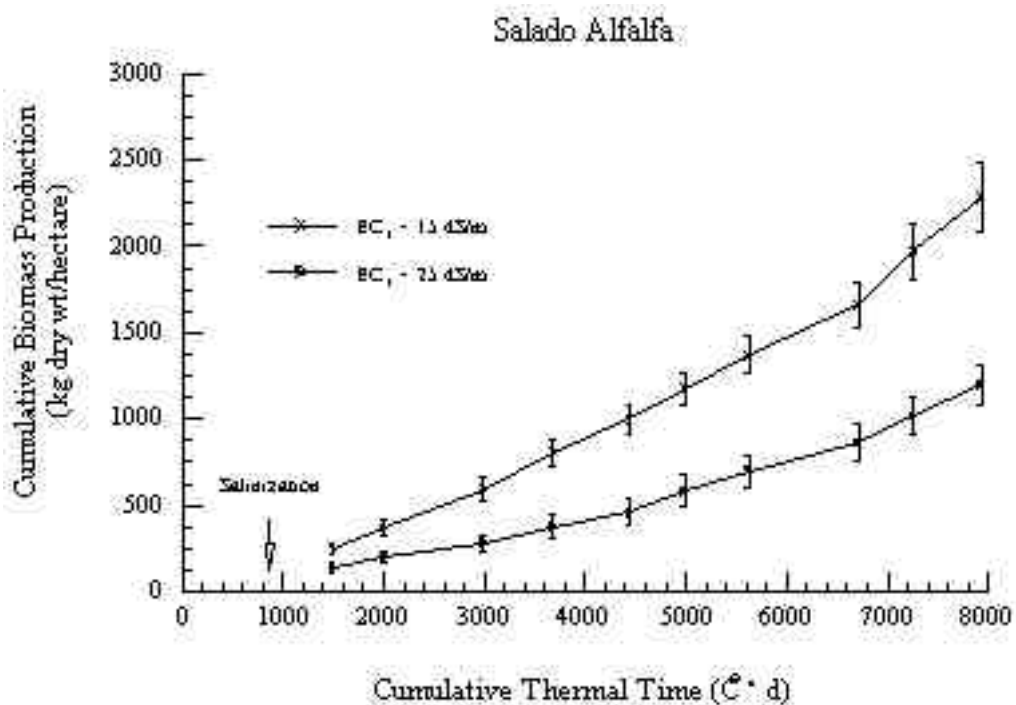


Figure 1. Cumulative biomass production of salado alfalfa (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

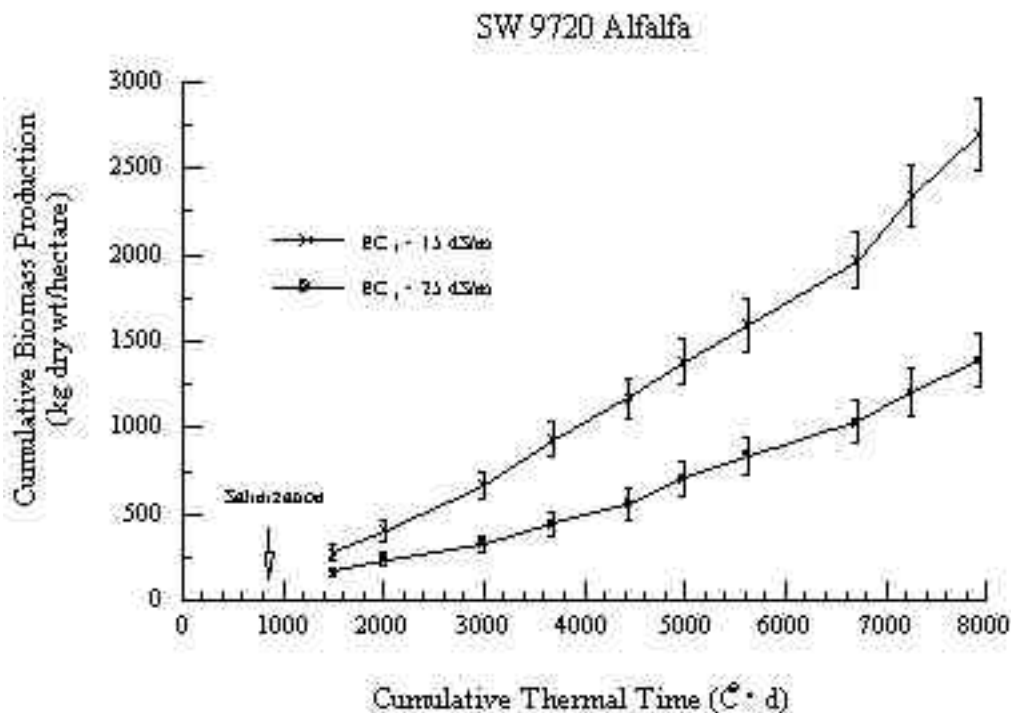


Figure 2. Cumulative biomass production of SW 9720 alfalfa (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

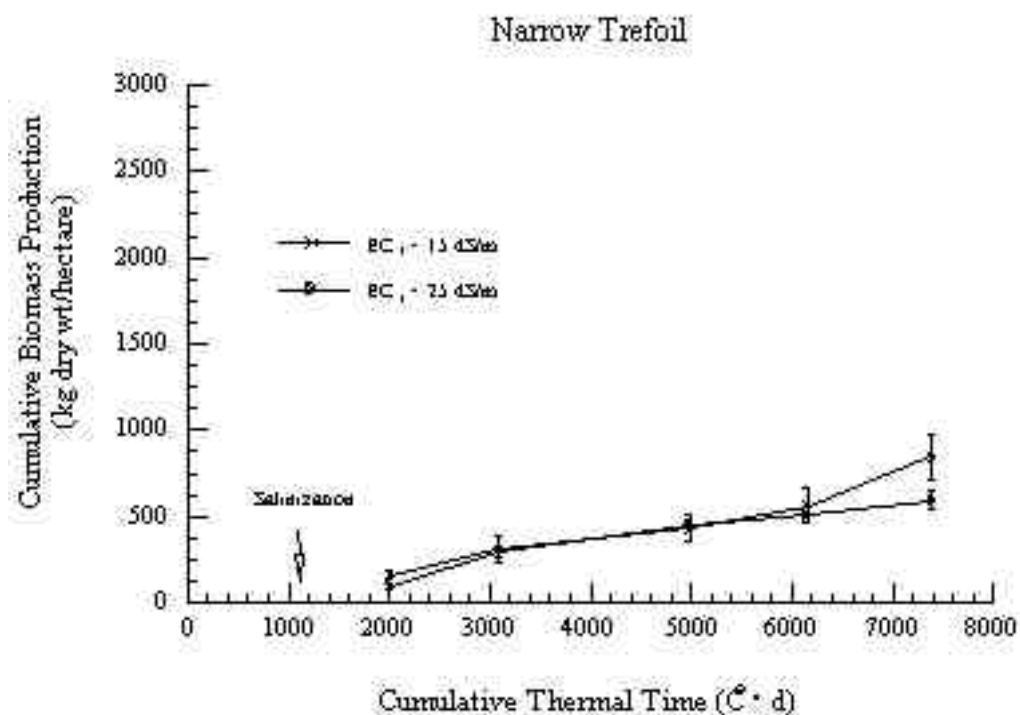


Figure 3. Cumulative biomass production of Narrow leaf trefoil (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

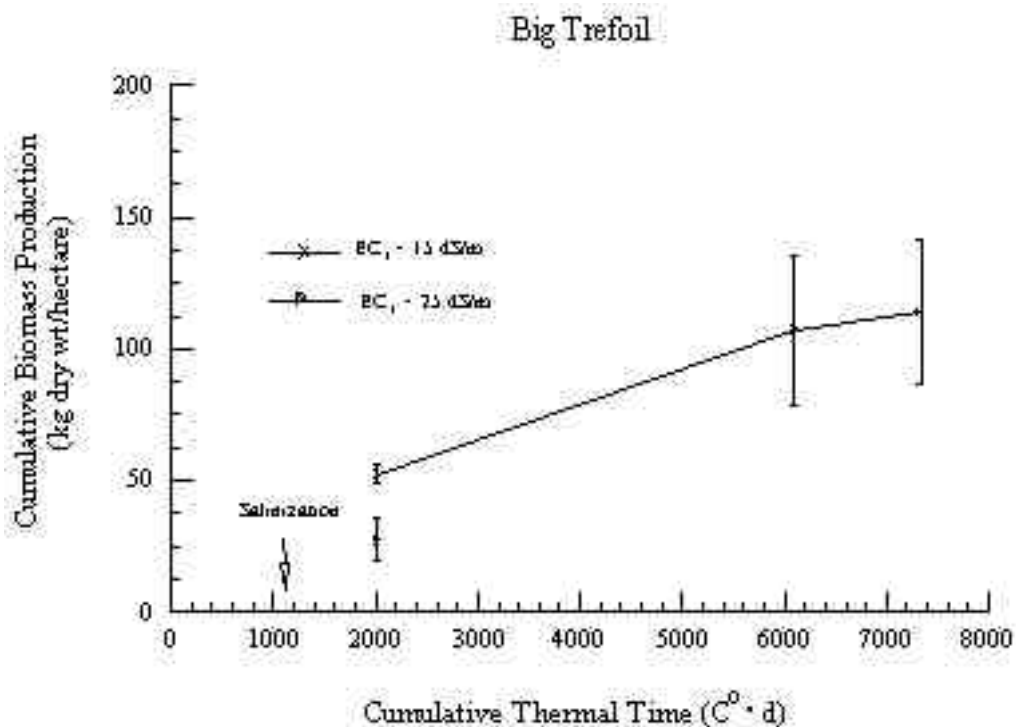


Figure 4. Cumulative biomass production of Big Trefoil (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

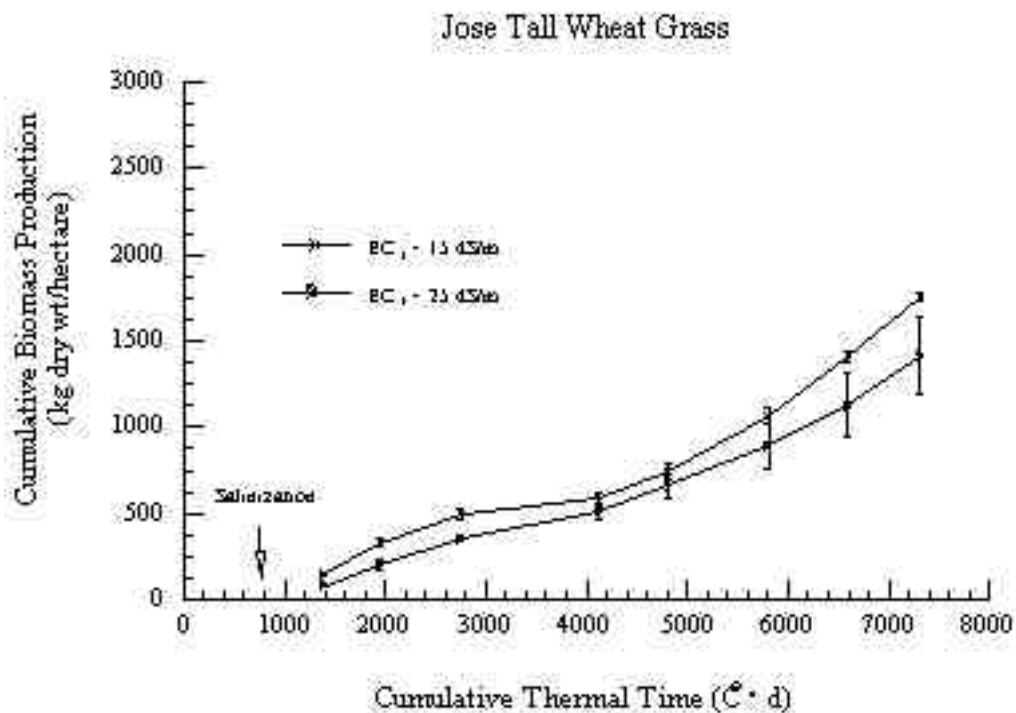


Figure 5. Cumulative biomass production of Jose Tall Wheat Grass (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

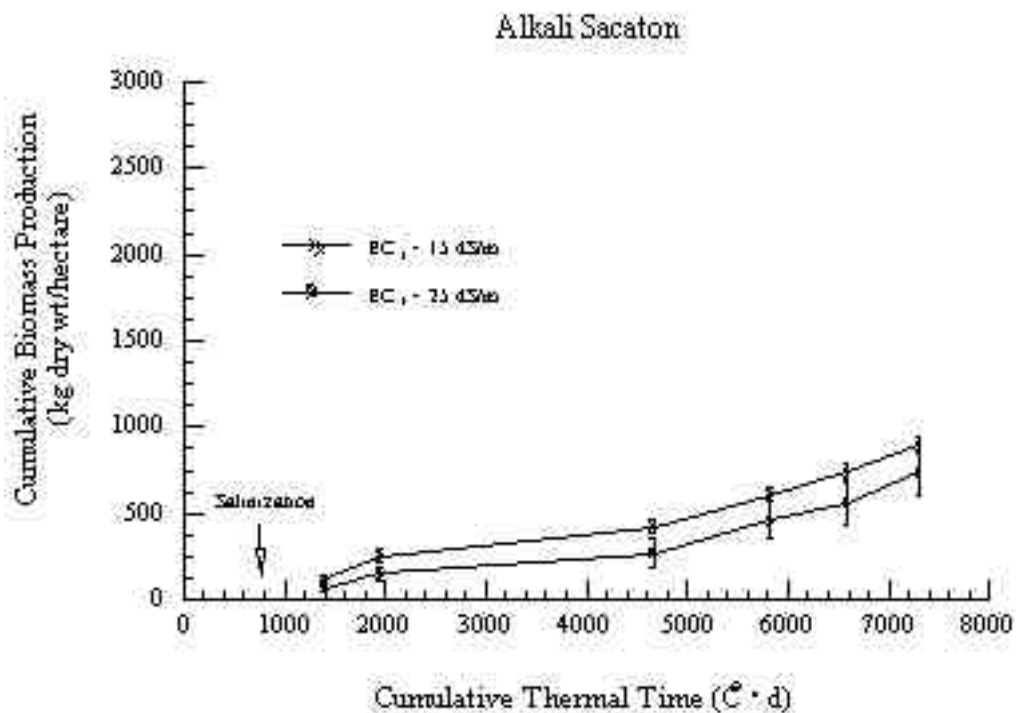


Figure 6. Cumulative biomass production of Alkali Sacaton (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

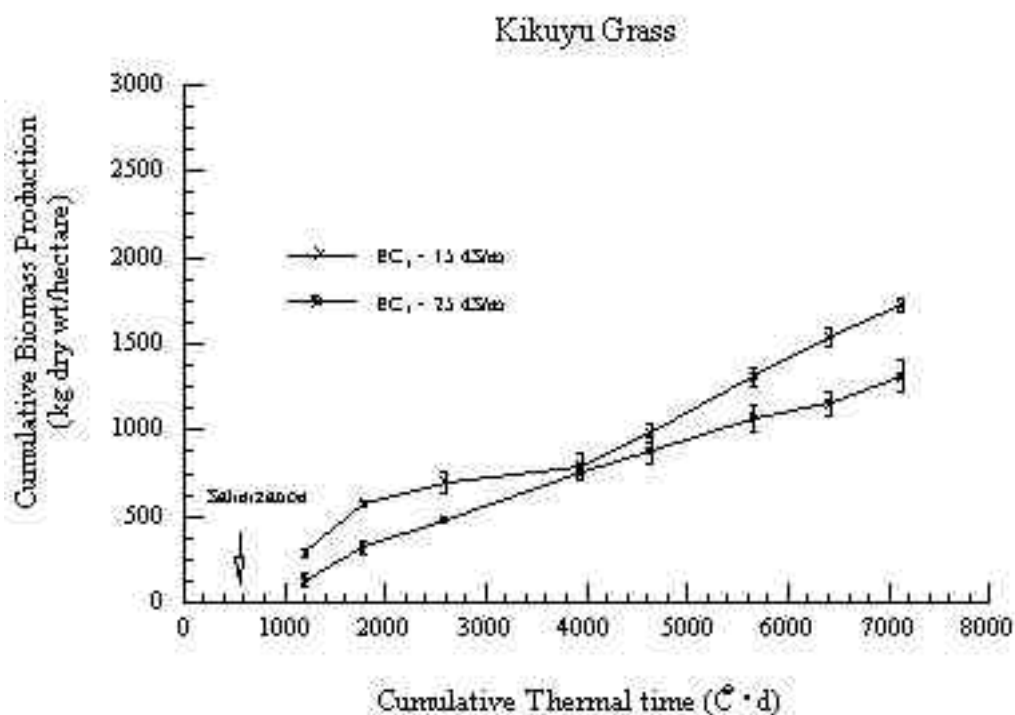


Figure 7. Cumulative biomass production of Kikuyu Grass (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

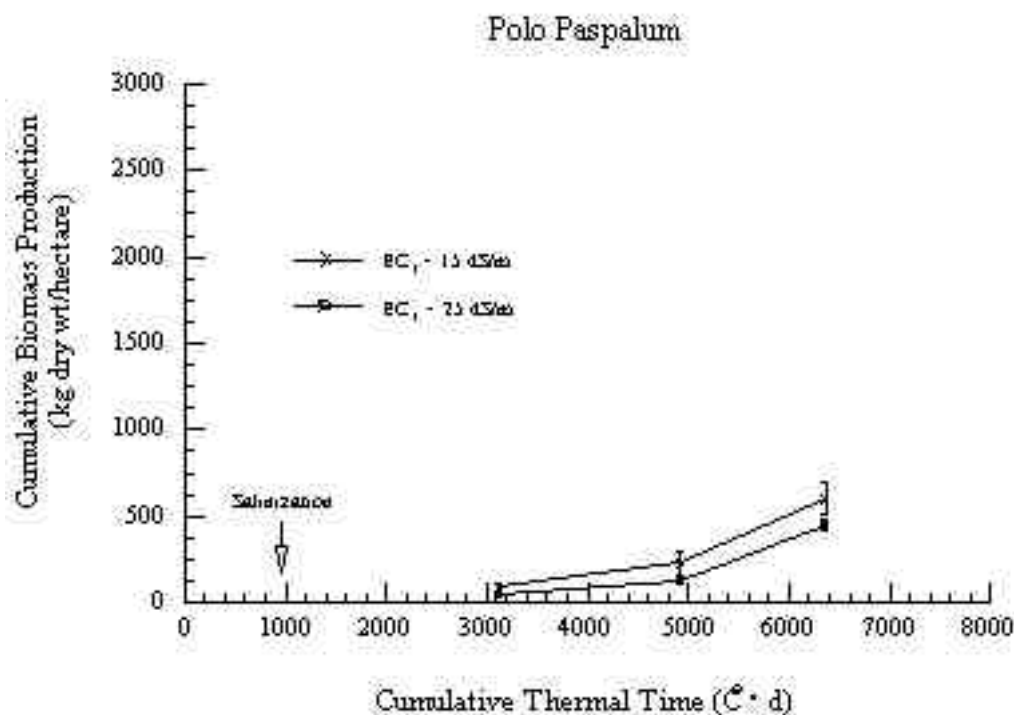


Figure 8. Cumulative biomass production of Polo Paspalum (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

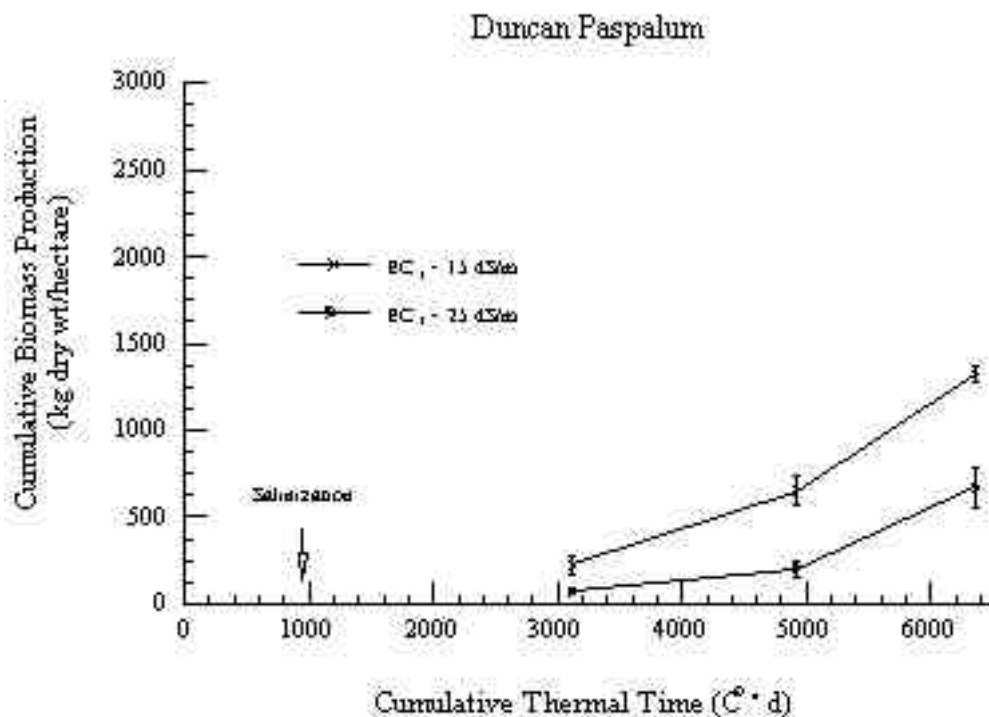


Figure 9. Cumulative biomass production of Duncan Paspalum (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.

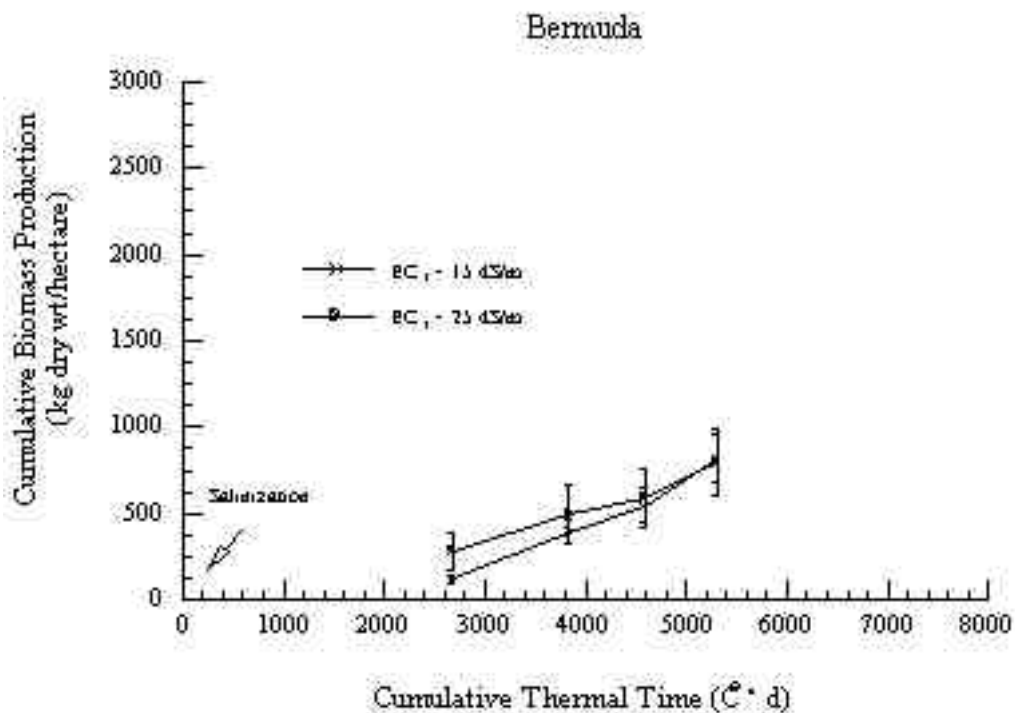


Figure 10. Cumulative biomass production of Bermuda Grass (kg/ha dry wt.) at either 15 or 25 dS/m in relation to thermal time.



Response of Crop Yield and Water Table to Subsurface Drip Irrigation of Processing Tomato Under Saline, Shallow Groundwater Conditions

Project Investigators:

Blaine Hanson, Department of Land, Air and Water Resources, UC, Davis

Don May, Farm Advisor Emeritus, UC Cooperative Extension, Fresno County

Research Staff:

1 SRA

2 Field Assistants

ABSTRACT

Subsurface drip irrigation systems were installed in three fields in the Westlands Water District to evaluate the effect of drip irrigation on processing tomato in areas with saline, shallow groundwater. At each site, treatments of different irrigation amounts were also conducted to determine the minimum amount of irrigation water that can be applied without severe yield reductions. Yield and applied water of the field-wide drip systems were compared with those of adjacent sprinkler irrigated fields. Results showed field-wide tomato yield to be 13 to 22 Mg/ha more compared with the sprinkler-irrigated field for about the same amount of applied water. Solids content of the tomato increased with increasing soil salinity. Yields were independent of soil salinity levels, but solids content increased with increasing soil salinity. Results of the irrigation treatments revealed relatively large yield decreases for small reductions in irrigation water for those sites with relatively high levels of soil salinity. For the field with soil salinity levels less than the threshold value for tomato, large reductions in applied water resulted in relatively small reductions in crop yield.

KEYWORDS: irrigation, processing tomato, salinity, shallow groundwater

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INTRODUCTION

Subsurface drainage problems continue to afflict many areas along the west side of the San Joaquin Valley of California. Concurrent with the drainage problems are excessive levels of soil salinity, the result of upward flow of the shallow, saline ground-

water and insufficient leaching. Subsurface drainage systems, normally installed to drain fields affected by shallow groundwater, cannot be used because no economically, technically, and environmentally feasible drain water disposal method exists for the San Joaquin Valley. Thus, the drainage problem must be addressed through options other than drainage systems such as better management of irrigation water to reduce percolation below the root zone and to induce maximum crop water use of the shallow groundwater without any yield reductions and drainage water reuse for irrigation.

One option for improving irrigation water management is to convert from furrow or sprinkler irrigation to drip irrigation. Drip irrigation can apply water both precisely and uniformly compared with furrow and sprinkler irrigation. Drip irrigations can occur more frequently compared with furrow and sprinkler irrigation, which can control soil salinity in the upper part of the root zone. Drip irrigation systems can be managed to reduce subsurface drainage by decreasing percolation below the root zone and encouraging shallow groundwater use, while at the same time, controlling soil salinity.

The main disadvantage of drip irrigation is its cost, about \$2,500/ha. For drip irrigation to be at least as profitable as the other irrigation methods, higher yields and reduced irrigation and cultural costs must occur. Yet, several large-scale comparisons of furrow and drip irrigation of cotton revealed much uncertainty in the economic benefits of drip irrigation (Hanson and Trout, 2000). Thus, growers converting to drip irrigation assume an economic risk.

During the past three years, a project has been conducted in the Westlands Water District to evaluate subsurface drip irrigation of processing tomato in fine-textured soil underlain by saline, shallow groundwater. The objective of this project is to determine the effect of drip irrigation on yield and yield quality, soil salinity, and depth to the water table. Because processing tomato is a high cash value crop, a better potential for increased profitability with drip irrigation exists compared to cotton. On the other hand,

tomato is much more sensitive to soil salinity, which could result in reduced crop yields in the areas with the saline groundwater.

PROCEDURE

In 1999, drip irrigation systems were installed at two locations in the Westlands Water District. Results from these systems were compared with adjacent sprinkler irrigated fields. Westlands irrigation water was used for these fields. In 2000, drip irrigation was installed in a third site irrigated with well water containing about 3 times more salt compared with the district water. Measurements made at all sites were the field-wide yield (machine harvested), yield quality, depth to the water table, irrigation water salinity, groundwater salinity, and applied water.

At each site, low-flow 22-mm diameter drip tape was buried about 200 mm deep. Emitter spacing ranged from 0.3 m to 0.45 m, depending on the type of tape used in a particular field. Dripline lengths were about 400 m at all sites.

At each site, an experiment consisting of different irrigation amounts was conducted to determine the minimum amount of applied water needed to prevent yield reductions under these shallow groundwater conditions. Target amounts of applied water were 90, 75, 60, 45, and 30 percent of the potential crop evapotranspiration. The potential evapotranspiration was estimated as the product of a crop coefficient and a reference crop evapotranspiration obtained from a CIMIS station at the Westside Research and Extension Center, located about 3 to 5 kilometers away from the sites. At Site DP, three varieties were grown for the differential irrigation treatments. Measurements made during these experiments were yield, fruit quality, soil salinity, soil moisture content, applied water, and canopy growth.

The experimental design used for the irrigation treatments consisted of dividing the field length into five blocks, each block 15 beds wide. Each treatment plot was three beds wide. Block lengths were about 80 m. For a given treatment, the inflow rate of a plot in a given block was the outflow rate of that

treatment from the previous block. The treatments were arranged in a Latin square design to minimize any bed effects on the treatment results (Ott and Longnecker, 2001). Cumulative applied water of a given treatment was measured at the inlet of the first block. Linear regression was used by regressing total yield along the field length with cumulative applied water for each treatment.

All plots were machine harvested along the length of the plots. Harvest data were total yield, solids content, percent red, green, and nonmarketable fruit, and color. Soil moisture contents were measured throughout the irrigation crop season with a neutron moisture meter. Soil salinity was determined at the beginning and end of the crop season for three of the irrigation treatments. Canopy growth was measured with a digital, infrared camera mounted on a pole about 2.4 m above the crop. The canopy coverage (fraction of the bed area shaded by the canopy) was calculated from the camera images using software supplied by the manufacturer.

RESULTS

YIELD CHARACTERISTICS

Table 1 shows applied water, yield, solids, and color for both 1999 and 2000. 1999 yields of the drip-irrigated fields were 13 to 22 Mg/ha (6 to 10 tons/acre) more compared with the sprinkler-irrigated fields. Applied water of the sprinkler systems was available for only Site BR, similar for both irrigation methods. After the 1999 crop, the growers converted the adjacent sprinkler-irrigated fields at Sites BR and DI to drip irrigation.

In 2000, yield of the drip irrigated field at Site DP was about 22 Mg/ha (10 tons/acre) more compared with the sprinkler field. About 152 mm more water was applied to the drip field, which was irrigated about two weeks longer compared with the sprinkler field. The yield at Site BR was relatively low, the result of a late replanting due to hail damage in late April. However, this yield was considered to be excellent by the grower for a late-season crop. Normally, late-season yields are 56 to 67

Mg/ha. At Site DP, the grower converted the sprinkler-irrigated field to drip irrigation after the 2000 crop.

For both years, the solids content was acceptable for all sites. However, the lowest solids occurred at Site DI in 2000, the site with the lowest soil salinity. The higher solids contents occurred on those fields with higher levels of soil salinity.

The 1999 irrigation treatments showed a slight reduction in yield with applied water for variety H9557 at Site DI, where the soil salinity generally was less than about 2 dS/m. Applied water ranged from 378 mm to 587 mm while corresponding yields ranged from 91.3 Mg/ha to 101.6 Mg/ha. The relationship between yield and applied water was linear with a coefficient of determination of 0.99. A 36 % reduction in applied water caused a 10 % reduction in yield.

At Site BR, where soil salinity greatly exceeded the threshold salinity for tomato (discussed later), yields range from 61.4 Mg/ha to 84.39 Mg/ha as applied water ranged from 319 mm to 481 mm. The coefficient of determination at this site was 0.5. These data show that a 33 % reduction in applied water caused a 26 % reduction in yield.

In 2000, we were unable to attain the desired irrigation amounts for the differential irrigation treatments because of coordination problems with the irrigators. Yields of the differential treatments of Site DI (not reported) were considerably less compared with the field-wide yield caused by a very late harvest of these treatments due to excessively wet soil from a leak in the plots.

The results of the differential treatments at Site DP for 2000 are shown in Table 2 for the three varieties. A linear relationship was found between crop yield and applied water with coefficients of determination of 0.74 (H8897), 0.82 (H9965), and 0.86 (Hally). Interestingly, although the applied water amounts differed only by about 102 mm among the treatments, a significant decrease in yield with decreasing applied water was found. A 12 % reduction in applied water resulted in a 22 to 24 % yield reduction among the three varieties. Yields of H8892 were significantly less than those of the

other varieties. Little differences were found in both solids content and color for the irrigation treatments at this site.

At Site BR, applied water of the irrigation treatments ranged from 330 mm to 422 mm, while crop yield ranged from 42 to 62.3 Mg/ha. A linear relationship was found between yield and applied water with a coefficient of determination of 0.99. Thus, at this site, decreasing the applied water by about 22 percent decreased the yield by about 33 percent.

These results suggest that for relatively high levels of soil salinity such as at Sites BR and DP, a slight decrease in applied water can substantially reduce yield. For soils with relatively low salt levels such as at Site DI, reductions in applied water did not significantly reduce yield.

WATER QUALITY

The electrical conductivity of irrigation and groundwater water, listed in Table 3 for both 1999 and 2000, shows the low-salinity irrigation water at Sites BR and DI to be about 0.34 dS/m. However, at Site DP, the electrical conductivity of the irrigation water (well water) was about 1.06 dS/m, about three times higher compared with the other sites. The electrical conductivity of the shallow groundwater was the least at Site BR (4.7 dS/m) and the highest at Site DP (16.4 dS/m).

SOIL SALINITY

Soil salinity at Site DI, shown in Figure 1 for 2000, was found to be less than the threshold value of tomato (2.5 dS/m) for both years. The threshold value is the maximum soil salinity at which yield reductions should not occur according to Maas, 1990. A slight increase in soil salinity occurred with time for depths less than about 2 feet. At the other sites, however, salinity levels in 2000 were much higher than the threshold salinity for the largest irrigation amounts, with values ranging from about 2.5 to 7 at Site BR and about 3.5 to 7 at Site DP (see Figure 1). Similar levels were found in 1999 at Site BR. At both of these sites, soil salinity increased with time for depths less than about 0.3 m to 0.46 m and decreased with time for the deeper depths. Soil salinity profiles of the

treatment receiving the least amount of irrigation water (not shown) showed an increase in soil salinity with time at nearly all depths for all sites compared with the salinity profiles of the largest irrigation amounts.

In spite of the higher levels of soil salinity at Sites BR and DP, field-wide crop yields were similar to those of Site DI (except for the 2000 yield of Site BR which was affected by a late planting) even though soil salinity at Sites BR and DP was much higher than that at Site DI. This behavior indicates for the maximum amounts of applied water, crop yields were independent of soil salinity levels for the range of soil salinity found at these sites.

Patterns of soil salinity about the dripline are shown in Figures 2, 3, and 4 for the three locations in 2000. At Site DI (Figure 2), low values of soil salinity occurred throughout the soil profile. A slight increase in salinity occurred near the surface. At Site BR (Figure 3), salinity was the least near the dripline with values less than about 1 dS/m. Salinity increased with distance and depth from the dripline to values of about 7 dS/m. Salinity also increased near the soil surface. The area of relatively low soil salinity (less than the threshold value of 2.5 dS/m) extended to about 400 mm horizontally from the dripline and to about 560 mm deep below the drip line. This pattern shows that most of the root zone probably consisted of low salinity soil.

A much different pattern of soil salinity was found at Site DP (Figure 4). Soil salinity was the highest near the dripline and decreased with horizontal distance. Values near the dripline were about 3 to 4 dS/m. At distances beyond about 200 to 400 mm, soil salinity was less than 2.5 dS/m. The high salinity near the dripline reflects the salinity of the irrigation water. The low levels of salinity near the edge of the pattern, however, suggest that for depths less than about 400 mm, considerable leaching of salt had occurred prior to irrigation, reasons for which are not clear because the preplant irrigation was applied with a sprinkler system using the well water (EC = 1.06 dS/m). The only possible explanation for this behavior is that during the spring, the storm that destroyed the crop at Site BR caused much ponding of

water at Site DP, which probably leached much of the salt near the ground surface.

SOIL MOISTURE CONTENT

Little trend in soil moisture content with time was found for Sites DI and DP, shown in Figure 5. Soil moisture content increased with depth. However, at Site BR (Figure 5), relatively high soil moisture contents were found in July compared with August and September. This behavior was evident for depths down to about 1 m. In August and September, soil moisture contents were much smaller, particularly for the shallower depths. This behavior was caused by early water applications with the drip system greatly exceeding the crop evapotranspiration in an attempt to control soil salinity. These water applications had a considerable impact on the water table (discussed later). When this impact became evident, irrigation amounts were reduced to about 80 percent of the potential crop evapotranspiration about mid-July.

WATER TABLE DEPTH

Figures 6 and 7 show the depth to the water table at Sites BR and DI in 2000. Similar data were not collected at Site DP because of the difficulty in installing observations wells in that soil. At Site BR, the spikes from about June 28 to about July 15 are the water table response to drip irrigation. For example, between July 10 and July 11, the water table raised by about 0.5 m, to a depth of 0.4 m below the ground surface, the result of the July 10 irrigation. In the later part of July, irrigation amounts were decreased to less than the potential crop evapotranspiration, and as a result, the depth to the water table increased with time. A slight response of the water table to irrigations still could be found between mid July and into August. At Site DI, the depth to the water table was relatively constant with time throughout the irrigation season. However, a slight response of the water table to drip irrigation could be found with changes in the water table depth of a few inches during irrigation. At Site DP, periodic measurements showed the depth to the water table to be about 1.5m.

CROP EVAPOTRANSPIRATION

Crop evapotranspiration was determined using measurements of canopy coverage with time and a computer simulation model (Hsaio and Henderson, 1985). Table 4 shows the amounts of cumulative crop ET and applied water for 1999 and 2000. In 1999, applied water was less than ET for both sites, whereas in 2000, applied water exceeded ET at Sites DI and DP. Figure 8 shows the total crop evapotranspiration with time after planting for 2000. Note that crop evapotranspiration changed little with time until about 50 days after planting. Thereafter, cumulative crop ET increased rapidly with time. The rate of increase was similar for all sites. Also shown in Figure 8 is the total amount of applied water at each site in 2000. Applied water and cumulative crop ET was about the same for Site BR, but for the other sites, the applied water exceeded the cumulative crop ET.

CONCLUSIONS

These results indicate that processing tomato can be successfully grown with drip irrigation on salt affected soils overlying saline, shallow groundwater. Yields under drip irrigation were 13 to 22 Mg/ha more compared with sprinkler irrigation. At the same time, amounts of applied water generally were similar for the two irrigation methods.

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Crop yield appeared to be independent of soil salinity for the three sites for the maximum amounts of applied water. In two of the fields, soil salinity greatly exceeded the threshold soil salinity for tomato. However, yields were similar to the field with soil salinity levels less than the threshold salinity for tomato.

The solids content generally was acceptable for all three locations. However, solids content increased with increasing levels of soil salinity.

Under saline conditions, applied water should be equal or slightly exceed crop evapotranspiration. Slight reductions of applied water under saline soil appear to cause substantial reductions in crop yield. Under conditions of low salinity where salinity levels are less than the threshold value, large reductions in applied water had a slight effect on crop yield.

Water table depths can be controlled with properly managed drip systems. However, if drip system applications are excessive, substantial decreases in the depth to the groundwater can occur as were found at Site BR in 2000.

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Table 1. Yields and quality characteristics for 1999 and 2000

Irrigation System	Variety	Applied Water (mm)	Yield (Mg/ha)	Soluble Solids (°Brix)	Color
<u>Site BR (1999)</u>					
Sprinkler	H8892	427	81.69	5.3	24.2
Drip	H8892	406	103.62	6.0	21.1
<u>Site DI (1999)</u>					
Sprinkler	H9557	**	78.98	5.2	24.8
Drip	H9557	564	90.94	5.0	22.8
<u>Site BR (2000)</u>					
Drip	Halley 3551	427	78.33	5.4	23.4
<u>Site DI (2000)</u>					
Drip	H9492	737	103.89	4.8	21.0
<u>Site DP (2000)</u>					
Sprinkler	H9557	579	63.91	5.5	23.9
Drip	H9557	711	86.49	5.6	23.7

Table 2. Yield results of the differential irrigation treatments at Site DP. Average values with the same letters were statistically similar at a level of significances of 5 %.

	Applied Water (mm)	Yield (Mg/ha)	Brix (%)	Color	Red Fruit (%)	Green Fruit (%)	Non- marketable (%)
<u>Halley</u>							
90	610	96.23	6.3	22.6	87.6	2.4	10.0
75	612	95.16	6.1	22.8	88.8	2.6	8.6
60	579	81.00	6.4	22.3	86.4	4.4	9.2
45	556	84.11	6.3	23.2	86.0	2.0	12.0
30	531	75.42	6.5	23.4	90.4	2.0	7.6
Average		86.35a	6.3a	22.8a	87.8a	2.7a	9.5ab
<u>H9665</u>							
90	610	106.22	5.4	23.4	9.02	2.4	7.4
75	612	98.36	5.6	23.4	92.4	3.6	4.0
60	579	87.09	5.9	23.2	90.2	3.6	6.2
45	556	88.23	5.6	24.2	91.2	2.6	6.2
30	531	80.30	6.3	22.8	85.2	2.8	12.0
Average		92.04a	5.8a	23.4a	89.7a	3.0a	7.2b
<u>H8892</u>							
90	610	89.76	5.45	23.0	85.6	3.6	10.8
75	612	82.97	6.1	20.8	83.6	4.8	12.8
60	579	69.93	5.5	21.2	88.4	2.8	8.8
45	556	88.23	5.8	21.4	82.8	2.4	14.8
30	531	68.48	6.3	21.0	83.8	1.8	14.5
Average		75.91b	5.8a	21.5b	85.1b	3.1a	11.9a

Table 3. Electrical conductivity (dS/m) of the irrigation water and groundwater for all sites.

	Irrigation Water	Ground Water
Site BR (1999)	0.34-0.46	6.9
Site DI (1999)	0.34-0.46	9.8-11.1
Site BR (2000)	0.34	4.7-7.4
Site DI (2000)	0.34	7.9-10.8
Site DP (2000)	1.06	13.6-16.4

Table 4. Total amount of applied water and cumulative crop ET for 2000.

Site	Applied Water (mm)	Cumulative Crop ET (mm)
<u>1999</u>		
BR	388	462
DI	564	584
<u>2000</u>		
BR	427	424
DP	709	615
DI	737	605



Chemical Nature of Selenium in Agricultural Drainage Sediments and its Implications for Bioavailability

Project Investigators:

Richard M. Higashi, Crocker Nuclear Laboratory, UC Davis

Robert G. Flocchini, Crocker Nuclear Laboratory, UC Davis

Research Staff

Jian Huang, LA

Teresa A. James

Project Collaborator:

ABSTRACT

A crucial element in the design and implementation of remediation on Se ecotoxic impact is the biogeochemistry of Se. Part of the biogeochemistry is direct trophic transfer of Se which is being examined by Salinity/Drainage investigators and other agencies. However, the chemical basis of bioavailability- that is, the molecular mechanisms of lower trophic level entry of Se into the foodchain - remains largely unknown and unstudied. There is only a general consensus that organic forms are much more bioavailable than inorganic forms of Se, yet such impressions have already worked their way into the regulatory arena for water (EPA, 1996; EPA, 1997). Moreover, the sediment is the major Se sink, yet it is essentially uninvestigated in these regards. Relatively new fields such as "organic matter environmental chemistry" and "bio-inorganic chemistry" have sprung up in other research areas to address similar gaps, but so far have not been applied to environmental Se concerns.

We have embarked on research that aims to harness experimental strategies and techniques from those scientific fields to the drainage water Se problems in the Central Valley. The main research emphasis is on physico-chemical basis of bioavailability in sediment. The principal benefits of the research to sustainable agriculture will be to provide key information for evaluation of short- and long-term prospects regarding management/treatment options. The information will consist of: (a) vital chemical principles (currently unknown) to help interpret the observed biological accumulation of Se and Se forms from sediments in complex foodweb/detrital systems, and (b) thereby help identify key theoretical vs. practical control points for remediation of Se ecotoxic impacts.

In particular, key missing information is how the environmental chemistry of Se relates to its initial fixation into the foodchain by microphytes ("algae"). In the previous year of research, we focused on the Tulare Lake Drainage District (TLDD) "Hacienda" (HEB) and "South" (SEB) evaporation basins, which are currently among the best-studied sites in the

world with regards to algal environmental biochemistry of Se. We surveyed the carbon and nitrogen relationships with total Se, including natural abundance isotope ratios, of the detritus/sediment at HEB and SEB. Both total C and total N independently showed some surprisingly tight relationships with total Se; not surprisingly, this was dependent on the basin (differences in salinity, operations, etc.) and location within basins (downwind where detritus is blown to, or upwind). The natural abundance isotope ratios lacked a clear trend, which is expected given the multiple cycling and pathways of organic material in these systems.

This year, we compared the extant sediment with newly-deposited sediments, both of which shared the same linear relationship between organic carbon vs. total Se. However, the newly-deposited material were generally several-fold higher in both organic carbon and total Se. Therefore, we fractionated the organic matter (OM) of the newly-deposited sediment to examine the chemistry. In particular, we focused on the assessment of both organic carbon and proteinaceous material in various OM fractions, in relation to the Se content of these fractions. We found evidence of proteinaceous OM in most fractions, and the load of total Se roughly tracked the load of proteinaceous OM. This opens the possibility that much of the Se in OM is in proteinaceous form, and the future focus will be on direct chemical evidence of proteinaceous Se in OM.

One of the by-products of Se metabolism by algae are volatile Se compounds. Since these products are short-lived in the water due to their high propensity to evaporate from the water (high Henry's parameter), the profile of the volatile Se compounds could reflect algal activity and the resulting eco-biogeochemical pathways of Se fixation. We repeated the previous year's survey of HEB and SEB with regards to concentrations of volatile Se forms. Again, we found large differences between sites and basins in the profiles and total amounts. Unlike the previous survey, SEB cells were not higher in dimethylselenide than HEB cells, and HEB-A4 in particular now rivaled SEB-S9 in concentrations of volatile Se. This could

be due to natural variation, but could also be due to the fact that A4 had been recently fertilized in an attempt to increase microphytes as food for brine shrimp. This year, we added total volatile Se (tvSe) measurement in tandem with the chemical forms analyses, which revealed that DMSe plus dimethyldiselenide (DMDSe) accounted for only about half of the volatile Se; part of this difference could be due to analytical limitations. The nature of the other forms is under investigation.

KEYWORDS: Se volatilization, ecotoxic risk, microphytes, detritus, sediment, organic matter, evaporation basin, flow-through wetland

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BACKGROUND & OBJECTIVES

It has been known that the major risk of Se toxicosis to aquatic top predators such as shorebirds occurs thru their diet, and therefore thru the foodchain (e.g. Skorupa, 1998 and references cited therein). Other routes of exposure, e.g. direct exposure to Se-contaminated water, is not considered to be significant. The chemical form(s) of Se that moves through the foodchain is not known, but a recent consensus (e.g. EPA Office of Water, 1998) proposes that protein-bound Se in food organisms may be the most available form to the next trophic level. Currently, UC Salinity/Drainage Program projects (those of Fan and Fry), as well as other agencies, are investigating these aspects.

On the other hand, little attention has been paid to the non-living forms of Se which enter the foodchain in the first place: that is, the bioavailability of Se to lower trophic levels. Organic forms appear to be important, as laboratory studies have shown that, directly from water, organic Se as selenomethionine (Se-Met) is much more available to algae and invertebrates than the typical inorganic forms, selenite (SeO_3) and selenate (SeO_4) (Rosetta and Knight, 1995; Maier and Knight, 1994 and references cited therein). However, commercially-available organic forms such as Se-Met occur at vanishingly low concentrations

in the water (Fan and Higashi, unpublished data), so they may not be relevant model compounds. Luoma et al. (1992) have shown that more complex, but unknown organic form(s) such as Se-labeled diatoms and sediments have high bioavailability to clams. The sediment is often the largest pool of Se and considered to be an important source of foodchain Se (e.g., EPA Office of Water, 1998). In the sediment, Se is likely to be resident in all particle sizes, ranging from algal mats and detrital floc to colloidal and small-molecule sizes; but only the latter has been investigated. Thus, what is needed are studies of bioavailability of the various organic forms and sizes of Se—in water, food items, and particularly the sediment—to gain an understanding of how Se enters the foodchain.

Unfortunately, such studies are not feasible at the present stage of knowledge, because the relevant forms of Se in the sediment are simply not known. This is not a trivial list to compile, if we consider briefly the biogeochemical cycles of Se. Figure 1 illustrates a sort of biogeochemical “refluxing” of Se depicting the relatively simple system of an evaporation basin, which is devoid of vascular plants and infaunal vertebrates. Waterborne and sediment Se as SeO_3 and SeO_4 are initially “fixed” into organic forms mostly by algae and microbes, some of which can head up the foodchain, or turn into organic material that is relatively unaltered—termed detritus—upon death. The detrital material can re-enter the foodchain immediately via microbes or invertebrates that are exposed (physically or thru ingestion) to the detritus. Other paths are for Se to re-enter the foodchain thru microbes or invertebrates after considerable chemical transformation has occurred to the detrital material—for the purposes of brevity, this aged material is lumped into the term humic material.

In any of the steps in Fig. 1, multiple chemical forms of Se are involved, and for the most part these forms are unknown. In most cases, even the physical state (gaseous, soluble, insoluble) is unknown, which is also important to bioavailability. Furthermore, the size distribution of organic Se as it converts from detritus to humic material is likewise unknown.

Size is of gross importance as it determines the target organism, exposure route, and chemistry of Se uptake, ranging from direct sorption or membrane transport for molecular-sizes, to ingestion and digestion for macroscopic particles and debris.

Clearly, it is vital to survey the physical state and chemistry of Se forms that comprise their physico-chemical basis for bioavailability. Also, since Se undergoes extensive chemical and biochemical transformations, it is important to gain experimental insight into how the forms might interconvert over longer periods; of course, such changes are expected to affect bioavailability. This means that the overall organic matter chemistry (the non-Se part) may be a key factor. As the largest pool of Se, the sediment is a key compartment to study in these regards (e.g. Canton and Van Derveer, 1997; EPA Office of Water, 1998), especially since waterborne Se has only an indirect connection to ecotoxic effects, as summarized specifically for Central Valley evaporation ponds by Maier and Knight (1992).

These considerations formed the impetus for the present project. Evaporation basin systems (alkali ponds) in the southern San Joaquin Valley is a good place to conduct these studies, since the biogeochemistry and ecology are simpler relative to most other systems, and is already under study by several other projects relevant to this proposal. In particular, key missing information is how the environmental chemistry of Se relates to its initial fixation into the foodchain by microphytes ("algae").

In this first year of research, we focused our efforts at the Tulare Lake Drainage District (TLDD) "Hacienda" (HEB) and "South" (SEB) evaporation basins, which are currently among the best-studied sites in the world with regards to algal environmental biochemistry of Se. The lead researcher in this area, Dr. Teresa Fan, is a collaborator on this project. We have started by surveying the carbon and nitrogen relationships with total Se, including natural abundance isotope ratios, of the detritus/sediment at HEB and SEB. Both total C and total N independently showed some surprisingly tight relationships with total Se; not surprisingly, this was dependent on the basin

(differences in salinity, operations, etc.) and location within basins (downwind where detritus is blown to, or upwind). The natural abundance isotope ratios lacked a clear trend, which is expected given the multiple cycling and pathways of organic material in these systems.

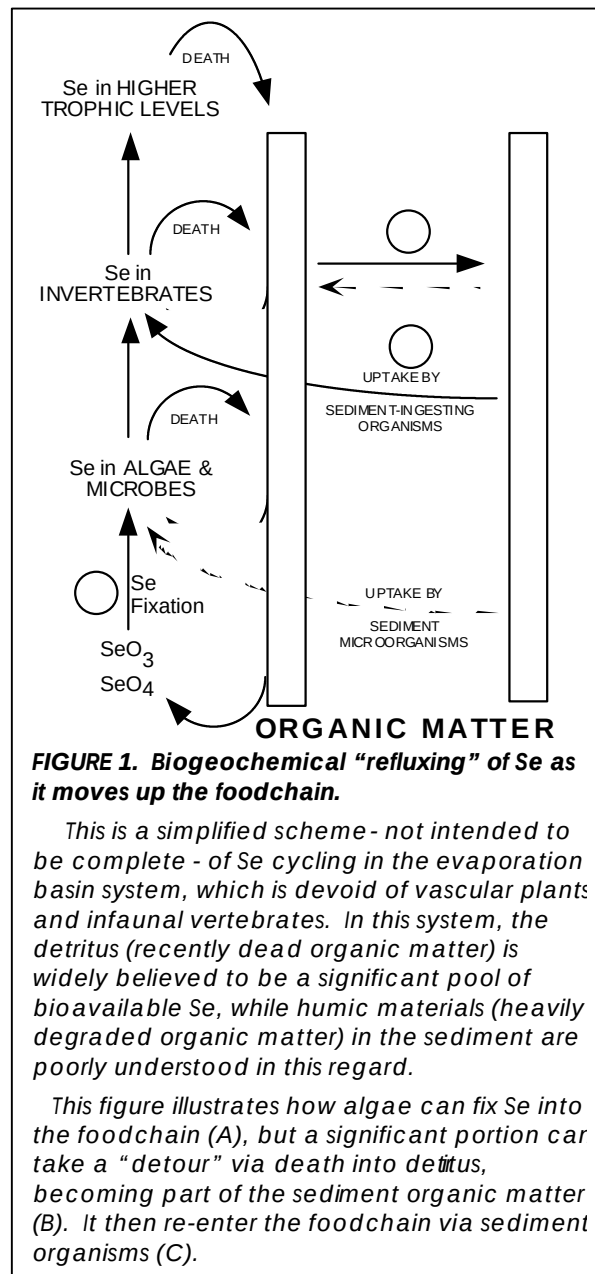


FIGURE 1. Biogeochemical "refluxing" of Se as it moves up the foodchain.

This is a simplified scheme - not intended to be complete - of Se cycling in the evaporation basin system, which is devoid of vascular plants and infaunal vertebrates. In this system, the detritus (recently dead organic matter) is widely believed to be a significant pool of bioavailable Se, while humic materials (heavily degraded organic matter) in the sediment are poorly understood in this regard.

This figure illustrates how algae can fix Se into the foodchain (A), but a significant portion can take a "detour" via death into detritus, becoming part of the sediment organic matter (B). It then re-enter the foodchain via sediment organisms (C).

A parallel direction is to survey the volatile by-products of Se metabolism by algae. Since these volatile Se products are short-lived in the water due to their high propensity to evaporate from the water (high Henry's

parameter), the profile of the volatile Se compounds could reflect algal activity and the resulting eco-biogeochemical pathways of Se fixation. Using a modified version of our on-site water-purge-cryotrap method, we surveyed HEB, SEB, and the flow-thru wetlands (FTW) with regards to volatile Se forms. We found large differences between sites and basins in the profiles and total amounts. For example, SEB cells were typically two orders of magnitude higher (high parts-per-trillion, or ppt) in dimethylselenide than FTW cells (low ppt to high parts-per-quadrillion). In addition, it is frequently theorized that Se shares the same biochemical pathways as sulfur (S). However, from a biogeochemical standpoint, we saw no volatile forms relationship between Se and S in the field.

SUMMARY OF PROGRESS

SITE RATIONALE

As stated above, we have continued to focus on TLDD HEB and SEB evaporation basins because of the extensive algal environmental Se biochemistry work (e.g. Fan and Higashi, 1997; Fan et al., 1997; Fan and Higashi, 1998; Fan et al., 1998a; Fan et al., 1998b) underway in collaboration with Dr. Teresa Fan. However, the flow-thru wetlands (FTW) Se trial on the TLDD premises has been terminated, and was not sampled this year.

SEDIMENT/DETRITUS STUDY

As depicted in Fig. 1, the sediment/detritus can harbor large pools of Se. The figure illustrates how algae can fix Se into the food chain (A), but a significant portion can take a “detour” via death into detritus, becoming part of the sediment organic matter (B). It then re-enters the food chain via sediment organisms (C). Thus the Se biogeochemical cycle is driven by the organisms, and is therefore intimately tied to the carbon cycle.

There was no attempt to define or separate “detritus” from inorganic “sediment” at the time of sampling, since this will be addressed in chemical fractionation. For sediment cores, only the top 5 cm or less was sampled, since that is where sediment organisms are generally

found (Cooper et al. 1997), and therefore of greatest interest in terms of bioavailability of Se. We used our previous method for evaporation basins (Fan and Higashi, 1997), which employed a large, all-Teflon syringe to obtain a composite of 10-15 sediment cores in a 2 m² area. The sediments were frozen and transported back to UC-Davis, whereupon they were lyophilized and ground. Total carbon and nitrogen analyses, along with their natural abundance isotope ratios, were performed at the UC Davis Stable Isotope Facility using a PDZ-Europa ANCA elemental analyzer interfaced to a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer. Total Se was analyzed as described previously (Fan et al., 1998).

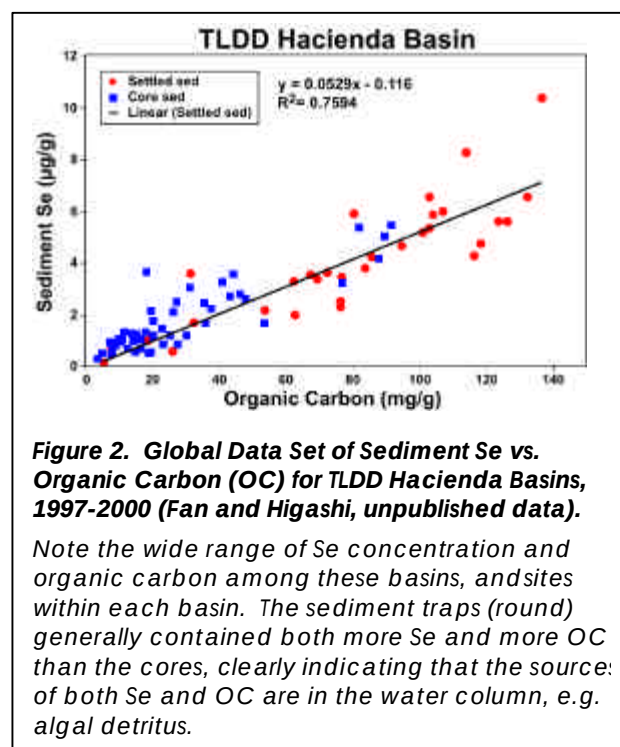


Figure 2 shows the entire data set of the sediment and trap contents for 1997-2001. Note that the traps (dots) are generally higher in both OC and Se concentrations than the cores (squares). This is consistent and expected from trap contents, which contain primarily “new” (<3 months old) detrital material. In contrast, cores would be “diluted” with non-detrital as well as older Se-laden material. Thus, comparison of Se forms in cores

vs. traps would be important to interpretations of bioavailability.

Figure 2, which depicts a positive OC-Se relation, is evidence for the probable strong coupling between the organic carbon (OC) and Se biogeochemical cycles, as alluded to back in Fig. 1. At first, this appears to support contentions by some (most notably, Canton and Van Derveer, 1997) that sediment OC can be utilized for ecotoxic risk assessment of Se.

However, Figure 3 shows that caution should be exercised, and that a more mechanistic approach is needed. These data from the TLDD South basins, which have a truncated biogeochemistry due to brine shrimp harvest, show very little sediment relationship between OC and Se concentrations. Thus, when

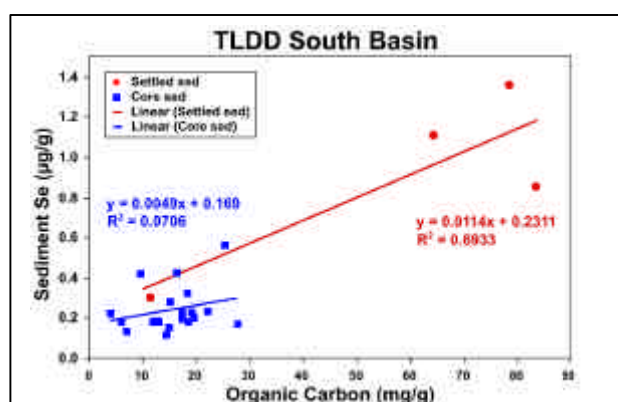


Figure 3. Global Data Set of Sediment Se vs. Organic Carbon (OC) for TLDD South Basins, 1999-2000 (Fan and Higashi, unpublished data).

Some of these basins are being harvested for brine shrimp, thereby truncating the food chain. There is no clear relationship present, in contrast with the non-harvested Hacienda basins. Sediment trap data, as in Fig.2, are in a higher concentration range for Se, but the number of samples are currently insufficient to draw conclusions.

the biogeochemistry is disturbed - as WILL be the case for ecotoxic remediation schemes such as shrimp harvesting - potential indicators, such as the OC-Se relationship in Fig. 2, are no longer reliable. It follows that simple indicators such as OC is not valid for assessing the progress of biogeochemical-based remediation technologies. And, since ecotoxic risk is a function of the biogeochemistry, the

latter cannot be readily dismissed in remediation efforts.

To improve the mechanistic under-standing of bioavailability, we performed a more detailed examination of the sediments. We have applied a general scheme fractionation scheme shown in Fig. 4 to survey sediment trap contents. Figure 5 reveals clearly that "sediments" are not just a single compartment;

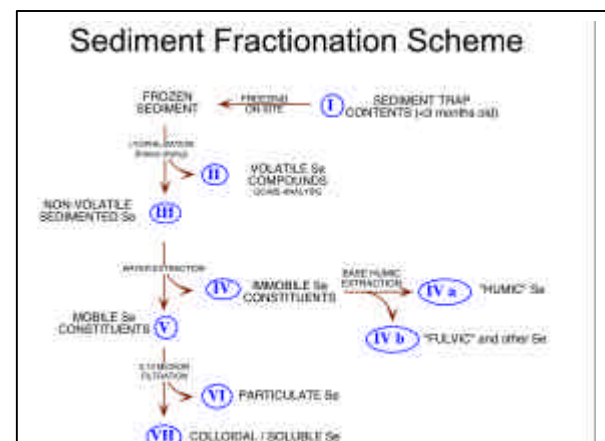


Figure 4. Chemical Fractionation Scheme Designed for Bioavailability Studies.

The general design follows the toxicity identification chemical fractionation approach pioneered by the PI (Higashi et al., 1992). In the present case, the fractions are based on bio-availability considerations, not chemical classification. For example, both the volatile and humic forms may be "organic selenium", but are very unrelated in terms of exposure and bioavailability. An important consideration is that water-column organisms will be exposed to mobile, water-soluble Se, but generally not to the immobile Se. The secondary consideration would then be the chemical form in each of these fractions.

the legend explains the importance of distinguishing between the "mobile" and "immobile" fractions of the sediment. Thus, the traditional pore water approach of assigning "mobile" and "bioavailable" is probably not useful in highly saline systems such as these basins. Therefore, we implemented the more bioavailability-oriented fractionation of Fig. 4, which directly accounts for the effect of fresher water flux events that occur in these basins.

As stated previously, protein-bound Se is hypothesized to be very bioavailable, so this

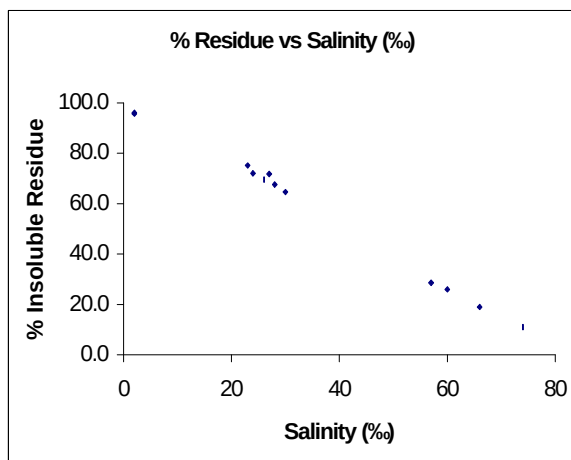


Figure 5. Non-soluble residue of sediment samples as a function of basin salinity.

These data from sediment traps deployed in TLDD basins (Apr–July, 2000) illustrates that “sediment” samples may consist of salts that can dissolve. These are very transient and are not a feature of the fixed sedimented material. Since evaporation basins routinely experience fluxes of fresher water, it is important to distinguish between such soluble (“mobile”) and insoluble (“immobile”) materials. Specifically, sediment and water column algae and other organisms will be exposed differently to the mobile and immobile fractions of sediment and any associated Se.

was a major focus of the fractionation approach. Since detrital material is - by definition - only slightly degraded, considerable proteinaceous material should still be present. Even in many humics, which are highly degraded biogenic substances, there is thought to be substantial proteinaceous material still intact (Hayes, 1991 and references cited therein), as we have recently shown (Fan et al., 2000).

It should be noted that conventional protein isolation and analysis procedures (e.g. Bradford method) for intact protein is not likely to yield representative protein data from sediments, since it is chemically altered. Instead, we employed peptide backbone structure detection by pyrolysis-GC/MS (Fig. 6), which enables analysis of degraded or bound protein fragments directly from sediments. The rationale for this technique is explained in Fig. 6.

Using pyrolysis-GCMS (Fig. 6) in conjunction with total Se analysis throughout the scheme in Fig.

4, we determined protein and Se in various newly deposited sediment OM fractions. The fractionation is outlined in Fig. 4. Briefly described, the freeze-drying operation (Fig. 4) chemically stabilized the non-volatile sediment

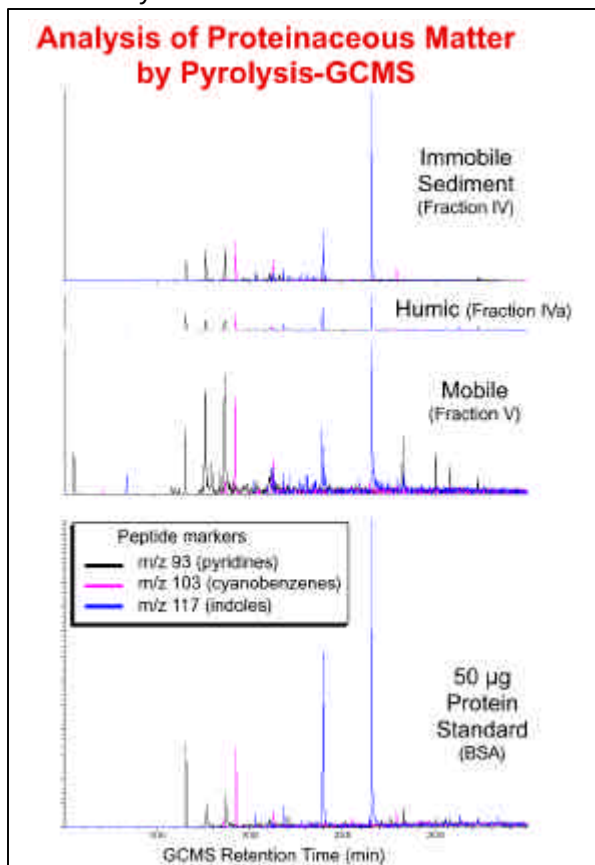


Figure 6. Analysis of Total Peptidic Structures by Pyrolysis-GC/MS (Fan et al., 2000)

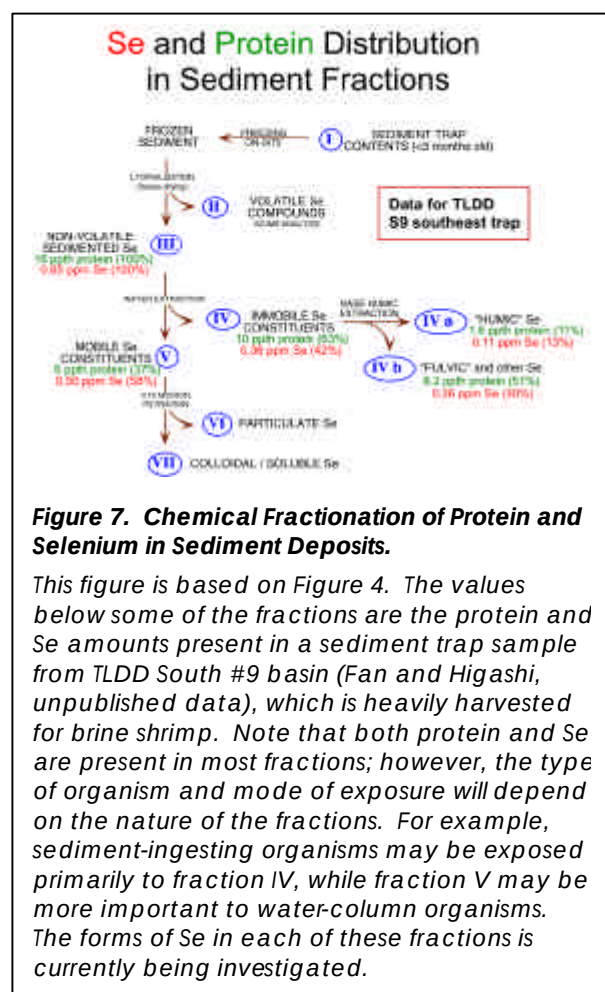
This figure illustrates the analysis for degraded or bound peptidic structures in actual sediment fractions that would otherwise escape conventional protein analyses. At the bottom is shown analysis of a protein standard. Further comparison with total Se and selenoamino acid analysis will enable, for example, estimation of selenoprotein that is/is not bioavailable via known invertebrate digestive processes. In turn, this information can be compared with Se burdens in the invertebrates.

for further processing. The immobile, or solid state is operationally defined as negative buoyancy, particulate-bound Se that is transported only with significant water current (generally not present at evaporation basins), and generally must be ingested to become

bioavailable. This was the residual Se (Fig. 4, IV) following removal of the gaseous and mobile Se. This fraction was further extracted by base to obtain humic- or fulvic-bound Se (Fig. 4, IVa & b), using chemically mild methods developed by us (Olk et al., 1995; Higashi, Fan, and Lane, 1998).

Figure 7 illustrates one result of fractionation via the scheme in Fig. 4. The values below some of the fractions show the concentrations (in terms of the whole sample dry weight) of Se and protein residues. Interestingly, both Se and protein (which potentially harbor the highly available proteinaceous Se) appear in most fractions. Thus, it appears important to pursue the chemical forms of Se in most of the fractions.

SE VOLATILE FORMS



For the survey of volatile forms from various basins, we used a modified version of our on-

site purging/cryotrapping/SPME-GCMS method described in our pilot study (Fan and Higashi, 1999). This was done in tandem with the alkaline peroxide trapping for total volatile Se (tvSe) (Fan et al., 1998). Figure 8 shows the relationship of tvSe to the sum of the two methyl-selenides, DMSe and DMDSe. The slope is approximately 0.5, indicating that about half of the tvSe was accounted for by the cryotrapping/SPME-GCMS method. Part of this difference may be somewhat variable recoveries, particularly at the SPME sampling step; although this is a convenient technique to obtain volatile Se speciation, the SPME device suffers from lack of precision.

Figures 9 and 10 show the concentrations of tvSe, DMDSe, and DMSe measured in the basin waters of the Hacienda (Fig. 9) and South Basin (Fig. 10) facilities. Generally, highest concentrations of volatile Se compounds were measured in water from basins of highest

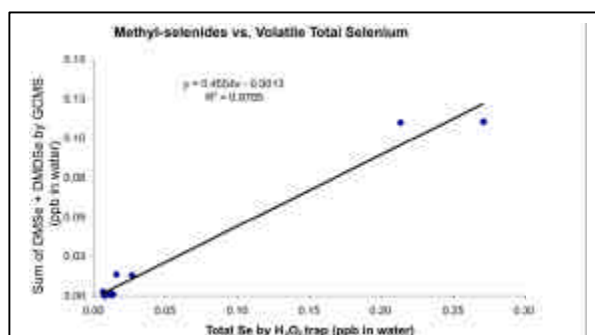


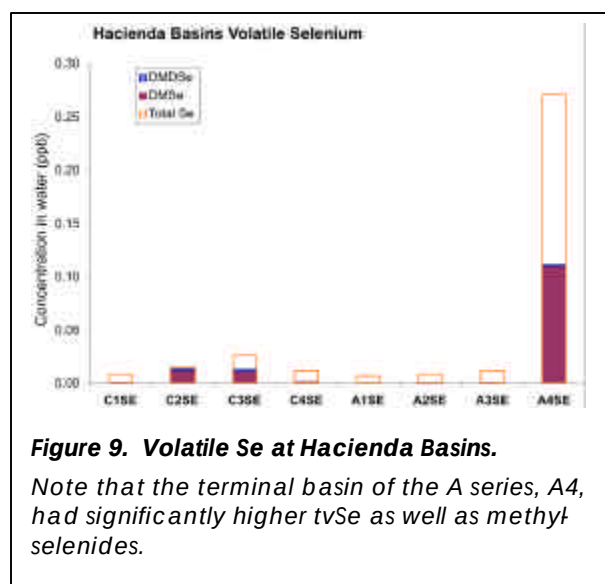
Figure 8. Relationship of methyl-selenides to total volatile Se.

Tandem measurements yielded a slope of approximately 0.5, indicating that the methyl selenides accounted for approximately half of the total volatile Se.

salinity, e.g. the terminal basin of the Hacienda A facility (A4, Fig. 9) as well as South Basin 9 (Fig. 10) where brine shrimp harvests have been intensively conducted.

As we observed in previous years at the Hacienda facility, the A basins have a distinctly different volatile Se profile from the C basins. The C cells with the largest portion of volatile Se present as DMDSe + DMSe were numbers C2 and C3, while A4 had by far the largest amount of DMDSe and DMSe measured

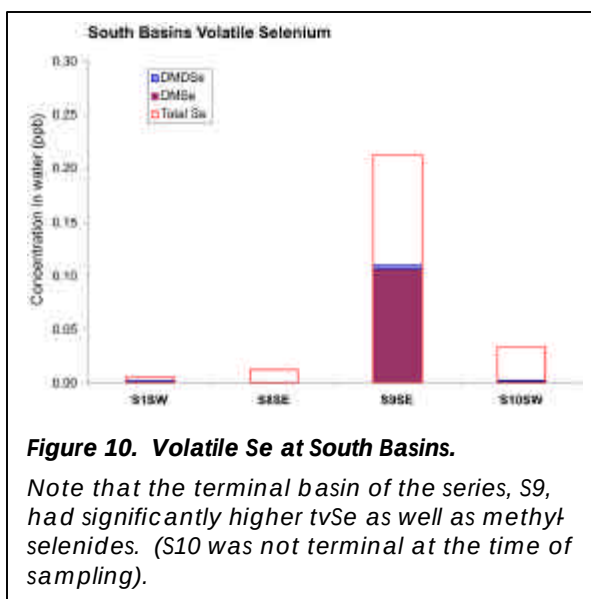
among the A basins (Fig. 9). This could be due to natural variation, but could also be due to



the fact that A4 had been recently fertilized in an attempt to increase microphytes as food for brine shrimp.

Total amounts of volatile Se in all forms measured in 2000 were about the same as those measured in 1999-2000, for Hacienda basins that were monitored in both years (C2SE

and A2SE). In the South basins (Fig. 10), cell 9 had very high concentrations of tvSe and DMSe. The high concentrations measured in this most heavily brine shrimp-harvested pond are consistent with last year's findings and indicate that it has a different biogeochemistry from other basins (also see Fig. 3).



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PUBLICATIONS & REPORTS

MANUSCRIPT ACCEPTED

Fan, T.W.-M., Teh, S., Hinton, D.E., and Higashi, R.M., Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Accepted in Aquatic Toxicology, a special Se issue.

REPORTS

"Se biogeochemistry and In situ bioremediation in Se-laden aquatic ecosystems", PacificChem meeting of the American Chemical Society (Aug., 2000)

"Bioremediating Selenium Ecotoxic Risk: A Biogeochemical Approach", Invited talk at the Dept. of Soil and Environmental Sciences, Univ. of California, Riverside (Feb., 2001)

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Using Forage and Livestock to Manage Drainage Water in the San Joaquin Valley

Project Investigators:

Stephen Kaffka*, Agronomy and Range Science, UC Davis

Jim Oster*, Environmental Sciences, UC Riverside

Carol Collar, UCCE-Kings Co.

Dennis Corwin, USSL, Riverside

Steve Grattan, LAWR, UC Davis

Jan Hopmans, LAWR, Davis

Ceil Howe II, Ceil Howe III, Westlake Farms, Stratford

Keith Knapp, Environmental Sciences, UC Riverside

John Maas, Veterinary Medicine, UC Davis

Peter Robinson, Animal Science, UC Davis

Bruce Roberts, UCCE-Kings Co.

Chris van Kessel, Agronomy and Range Science, UC Davis

*report authors

ABSTRACT

A long-term research, multidisciplinary project has been established in the Tulare Lake Basin to investigate the sustainability of reusing saline drainage water for forage and livestock production. Initial project activities have focused on site preparation and characterization. The site has been leveled, tile drained, and fenced. Drainage and irrigation volume and quality monitoring equipment has been installed. Site characterization using electrical induction methods linked to GPS and soil sampling has been completed. Forages have been established, cattle grazing begun, and a regular program of forage, soil, and water sample collection and analysis begun. ECe from soil samples combined over a depth of 1.2 m varied from 12.8 to 36.6 dS m⁻¹, SAR from 28.8 to 88.8. Boron and Molybdenum concentrations varied from 11.5 to 32.2 ppm and 480 to 1960 ppm respectively. Bermuda grass established and grew well except in the most saline areas of the site (ECe > 22 dS m⁻¹).

KEYWORDS: electrical conductivity, ECe, drainage water reuse, salinity, Mo, B, San Joaquin Valley

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OVERVIEW

Without a means to dispose of drainage water in the western SJV, increasing amounts of farmland will become salt impaired. The use of underlying groundwater and limited numbers of evaporation basins for disposal of the large volume of drainage water produced is not sustainable. A multi-disciplinary team has assembled to test the hypothesis that saline-sodic drainage water can be used in an environmentally sound manner for forage and livestock production. Our goal is to use salt tolerant forages to provide a year-round supply of high quality feeds that can support economic weight gain by cattle or sheep, or be sold to dairies. If livestock production can be based on the reuse of drainage water, it may transform saline drainage water from an environmental burden into an economic asset, reduce dramatically the amount of water that must be disposed to groundwater or in evaporation ponds, provide the means and incentives

to install needed artificial drainage systems to improve soil quality, and facilitate the ultimate disposal of salts in ponds or through technical means.

OBJECTIVES

1. Measure forage biomass accumulation, nutrient and trace element uptake, and the quality of salt tolerant forages produced using saline-sodic drainage water (Kaffka, Robinson).
2. Monitor the mineral status, and general health, measure the growth rate of cattle fed or grazing forages produced with drainage water (Mass, Robinson, Kaffka).
3. Quantify the effects of saline-sodic drainage water on overall water use, drainage water quantity and quality, salt and trace metal balances, soil organic matter, and soil chemical and physical properties over time (Corwin, Grattan, Oster, Hopmans, Van Kessel).
4. Model changes in important soil chemical, physical and biological properties at the local and field scale (Corwin, Hopmans, Oster).
5. Estimate the costs and returns associated with forage and livestock production based in the use of saline-sodic drainage water (Knapp, Kaffka, Robinson).
6. Develop an economic management model for drainage water reuse in irrigated forage-livestock operation (Knapp, Kaffka).

PROGRESS TO DATE

1. The site has been leveled for irrigation and drainage tiles and an irrigation system for saline water installed. Each plot can be irrigated separately. Irrigation is being carried out on a two week schedule.
2. A detailed baseline soil assessment for soil physical and chemical properties has been carried out. Soil samples have been analyzed and archived. Analyses include cations and anions, pH, ECe, SAR, texture, bulk density, and trace metals. A second detailed survey is scheduled for October 2001. (fig. 1, 2, 3, 4)
3. The assessment of soil hydraulic conductivity and correlated properties in the laboratory and field has been initiated.

4. Irrigation with saline water and irrigation and drainage volume monitoring are taking place. (Table 1; fig. 5)
5. The site has been fenced for livestock and divided into eight paddocks to facilitate grazing and the control of livestock.
6. Bermuda grass has been established Forage samples are being collected twice a month at 36 to 72 locations. Two 0.25 m² samples are collected at each location. Forages are being analyzed for crude protein, quality, ash, and mineral composition. Remote sensing flights are carried out periodically in coordination with ground sampling and combined analyses are being carried out.
7. Grazing has begun with an initial small group of cattle. Weights, body condition scores, blood and liver samples are collected regularly from the test cattle to evaluate performance and test for the accumulation of trace elements and overall health and performance.

Irrigation and drainage events were monitored during 2000, although the data record is not complete for every event. Available data are summarized in Table 1.

Results are initial estimates. Drainage depth in the table does not take into account water that passes through the root zone, raising the water table. This occurred in 2000. The water table was below the drains, located at a depth of 4 ft, and rose to an elevation above the drain with the start of irrigation. Assuming the water table rose 1 ft and the volumetric water content increased 10 %, another 1.2 inches could be added to the drainage depths (Col. 4) in the table. Leaching fractions would then average 0.063 +/- 0.002 for the four plots, and the salt balance ratio (Col. 8) for plots 2, 6 and 7 would be 1.0, 1.2, and 1.1 respectively. The salt balance ratios in the Table were calculated as: The product of irrigation depth times EC_{iw} was divided by the product of drainage depth times EC_{dw}.

ACKNOWLEDGEMENTS

The UC Salinity/Drainage Program, the UC Proesser Fund, the Kearney Foundation for Soil Science, and the California Department of Water Resources have helped fund this research. Nahum Gat of Optiscan, Inc, has provided remote sensing images.

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Table 1. Irrigation and Drainage Summary (2000)

Location	Date	Irrigation depth (ac in)	Drainage depth (ac in)	LF	EC _{dw} flow weighted average dS/m	EC _{iw} dS/m	Salt balance ratio (C3*C7/ C4*C6)
Drain 4, plot 2	7/14 -18	8.8	NM	--		NM	---
	8/4	6.5	0.07	0.01	40.1	3.0	7
	9/8-16	6.4	0.13	0.02	30.9	1.9	3
Drain 6 Plot 3	7/14 -18	8.8	NM	--		NM	---
	8/4	6.5	NM	--	51 ^a	3.0	--
	9/15	6.4	NM	--	40 ^a	1.9	--
Drain 12 Plot 6	7/25	10.7	0.15	0.01	NM	3.3	NM
	8/5	6.5	0.20	0.03	37.5	3.0	2.6
	8/22-9/16	17	0.54	0.03	40.5	4.1	3.3
Drain 14 Plot 7	7/25	10.7	NM	NM	25 - 50	3.3	--
	8/5	6.5	0.09	0.01	49.4	3.0	4.6
	8/25-8/30	17	0.40	0.02	46.5	3.6	3.3

^aSalinity level recorded near the time irrigation occurred. NM: not measured. LF: leaching fraction, EC: Electrical conductivity, dw : drainage water; iw: irrigation water

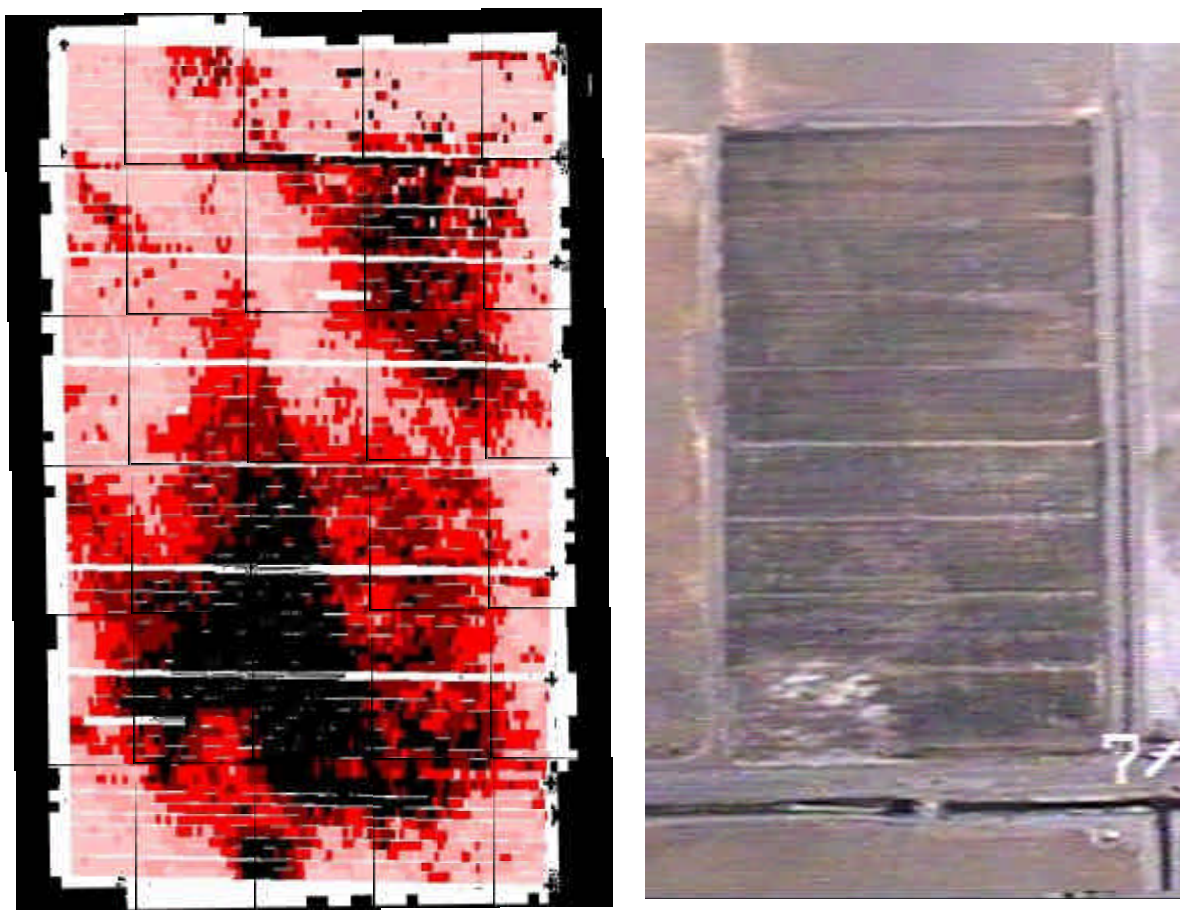


Figure 1. A. Initial soil assessment bulk ECa map, July 1999. Soil quality varies across the site. Darker colors are larger values. (Corwin data) B. Aerial image of site showing Bermuda grass establishment, July 2001. Soil variation provides a basis for comparison of forage response to saline soil and water. All soil and forage samples are collected using a GPS system. (OKSI Optisystems).

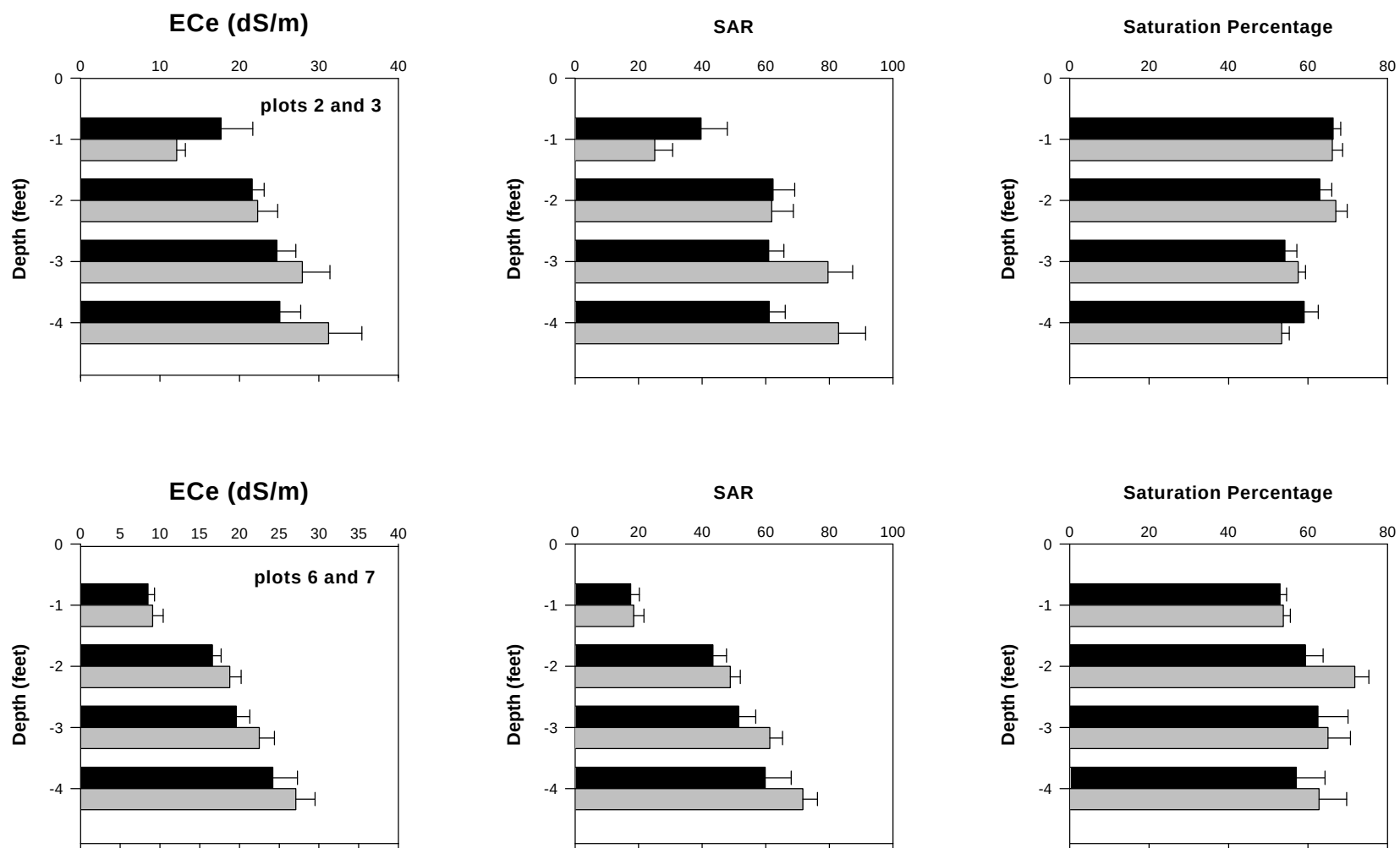


Figure 2. Correlations between soil depth and selected soil physical and chemical properties in plots monitored for drainage water discharge volume and composition. Values are plot means and standard errors (Corwin data). The dark, upper bar is for the first of the two plots

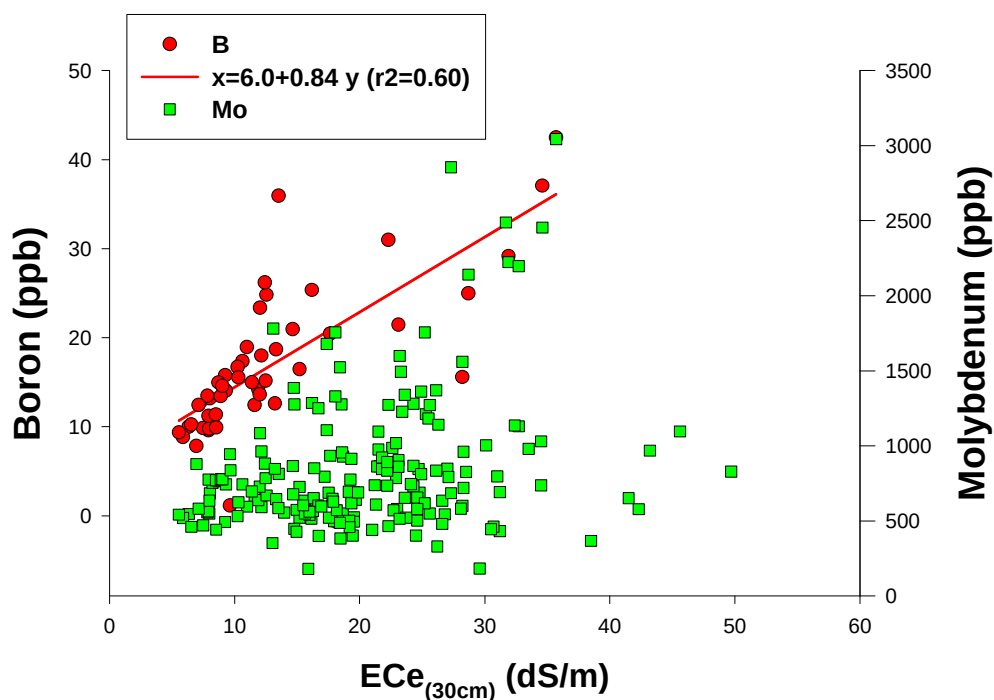


Figure 3. Boron and molybdenum: correlation with salinity, from initial assessment soil samples, July 1999 (Corwin data). Mo is present in soils at the site in large amounts, but its abundance is not apparently correlated to salinity in a simple manner.

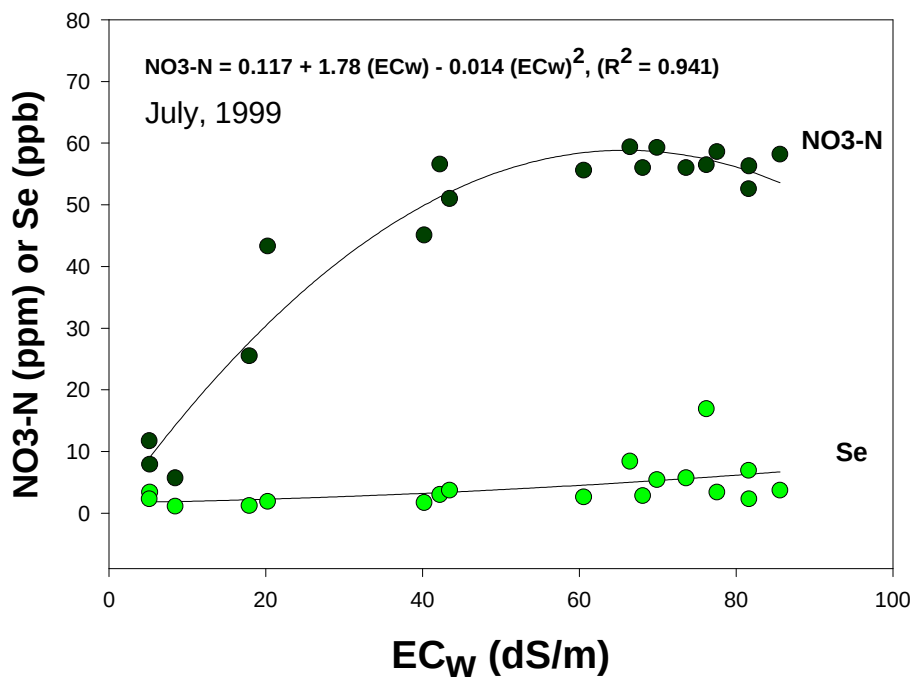


Figure 4. Water quality characteristics for samples collected from tile drains during the baseline irrigation event (August 1999). The site is low in Se but high in nitrates.

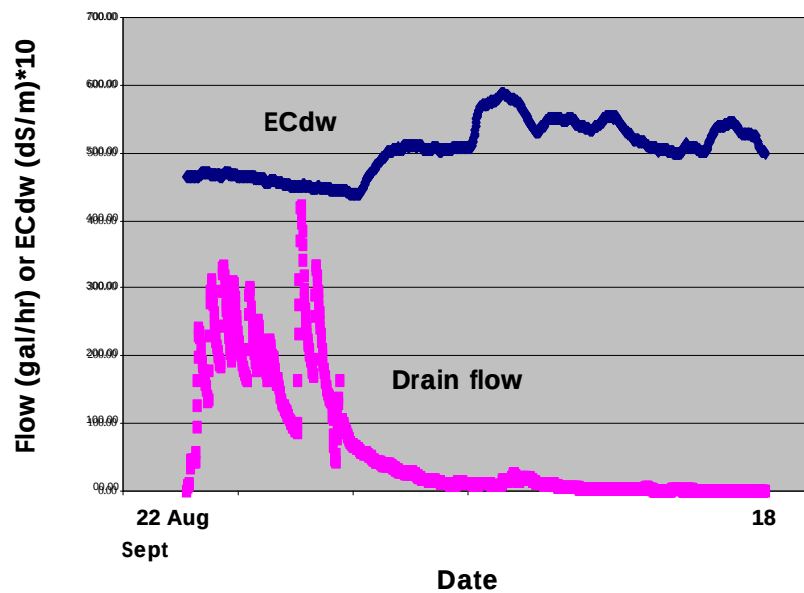


Figure 5. Tile drainage salinity (ECdw , $\text{dS m}^{-1} \cdot 10$) and raw drainage volumes (gal/hr) for an irrigation event (August-September 2000) in plot 6. These patterns and values are representative of the other irrigations monitored in 2000.

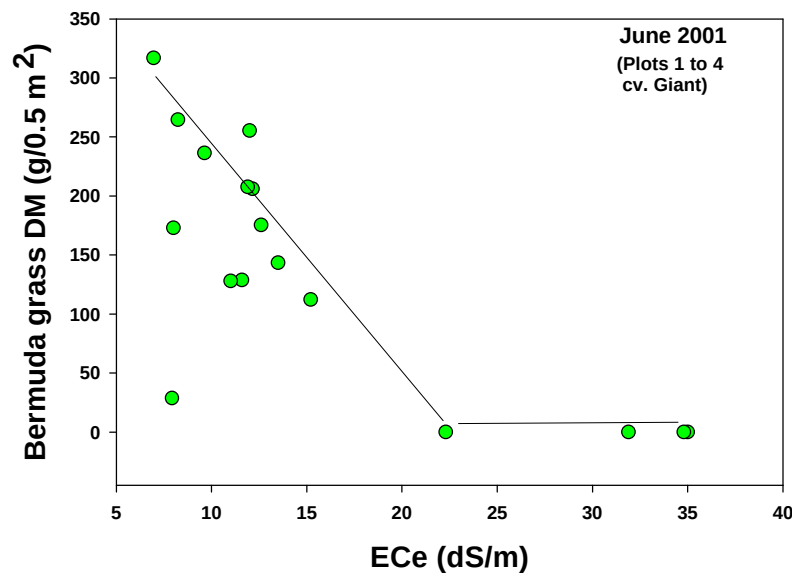


Figure 6. Dry matter yield of Bermuda grass (cv. Giant) samples collected in June 2001 (plots 1 to 4). The outlier is from an area that did not receive adequate irrigation water to establish the crop. No Bermuda grass established or grew in areas with ECe levels greater than approximately 20 dS m^{-1} . These areas correspond to the southwest corner of the site, and are visible in figure 1.



The Economics of Integrated Drain Water Management in the Central Valley

This research is a combination of the following two projects:

An Economic Analysis of Alternative Methods to Control Salinity In the Western San Joaquin Valley

Principle Investigator:

Kurt A. Schwabe, Department of Environmental Sciences, UC, Riverside

and

The Economics of Integrated Drain Water Management

Principle Investigator:

Keith Knapp, Department of Environmental Sciences, UC, Riverside

Co-Investigator on both projects:

Iddo Kan, Department of Environmental Sciences, UC, Riverside

ABSTRACT

In response to restrictions on out-of-region discharges of drainage effluent off irrigated fields, a number of alternative management strategies to the drainage problem have been suggested. Such options include source control, drainage water reuse on salt-tolerant crops, drainage reclamation, and in-region disposal options. The objective of this research is to develop a regional economic model that can evaluate and compare alternative management strategies confronting growers in the San Joaquin Valley with recognition of the current regional environmental restrictions associated with drainage water. For analysis, a mathematical programming model is developed that includes response functions that describe both the yield and deep percolation for different levels and salinity of applied-water under five irrigation systems. Focusing on the Westlands Water District (WWD), preliminary results from the first stage of the model's development suggest that reuse can be an economically attractive option, increasing per acre profits by roughly 14% when compared to a scenario prohibiting reuse. Furthermore, while cotton is currently the only reuse crop in our model, initial estimates show that between 18% and 23% of the land is devoted to growing this crop with reuse water depending on how we specify restriction on regional drainage.

KEYWORDS: Economics, irrigation, mathematical programming, regional management, reuse, salinity, salt-tolerant crops, San Joaquin Valley, source control, Westlands Water District

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INTRODUCTION

Traditionally, the installation of drainage systems that discharged effluent to streams solved many of the problems associated with high water tables that arose due to deep percolation flows from irrigated fields. Environmental damages linked to this effluent, however, led to restrictions on out-of-

region discharges. Consequently, solutions to the drainage problem have shifted to alternate management strategies, including source control, drainage water reuse on salt-tolerant crops, drainage reclamation, and in-region disposal options. Given the array of on-farm management decisions confronting growers and recognition of the physical links between these decisions and their potential environmental impacts, developing a management strategy that is both economically attractive and environmentally sustainable can pose a significant task to any grower or policy maker in the region.

The objective of this research is to develop a regional economic model that can evaluate and compare alternative management strategies confronting growers in the WWD with recognition of the current regional environmental restrictions associated with drainage water. Management strategies are subject to constraints on land availability, out-of-region drainage water discharge, and available surface water supply. Model inputs include: (i) response functions that describe both the yield and deep percolation associated with different levels and salinity of applied-water under five different irrigation systems, and (ii) economical data, including crop prices, non-water production costs, surface water application costs, groundwater and reused water options, and water disposal costs. By varying input parameters and/or constraints, this mathematical programming model can be to analyze the efficiency of both on-farm and regional management strategies. Furthermore, assumptions regarding inputs, constraints, and/or the applied physical relationships can be investigated via sensitivity analyses. Preliminary results from the first stage of the model's development are presented below. The current application is the West-lands Water District (WWD).

THE STUDY AREA AND DATA

For the current application, our focus is on the WWD, which consists of approximately 600,000 acres and is located in the agriculturally rich San Joaquin Valley. A high water table due to irrigation that takes place

over the impermeable Corcoran clay layer affects the WWD. Initially, a system of drainage tiles were installed as a response to the high water table easing both the potential for water-logging of the crops and the leaching of toxic contaminants from the soil surface. For a period of time, the drainage waters from these tile systems were exported out of the region along the federal San Luis Drain with an interim terminus at Kesterson Reservoir. Following a series of environmental incidences in the early 1980s associated with these discharges, such out-of-region disposal relief was eliminated.

Our preliminary model includes four crops and five irrigation systems. The crops include cotton, processing tomatoes, wheat, and lettuce. These four crops represent 64% (40%, 15%, 5%, and 4%, respectively) of the total cropped farmland in the WWD. Non-water production costs and market prices for each cropping system 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 (<http://www.westlandswater.org/>). Finally, to account for heterogeneous land quality characteristics, we calibrate the preliminary model with attention to historic crop acreage allocations in WWD.

MODELING FRAMEWORK

The modeling framework for this analysis uses a mathematical programming model that will evaluate the economic efficiency and profitability of various strategies confronting growers to reduce saline drainage water. In effect, the problem can be presented as one for which we have an objective of maximizing regional agricultural profits with specific constraints on land availability, surface water availability, groundwater availability, watertable depth, and out-of-region discharges. Profits consist of the revenues generated from producing some combination of four crops less the costs of both producing the crops and disposing of the drainage water.

RESPONSE FUNCTIONS

In addition to economic data associated with the crop production, irrigation, and disposal, our model includes response functions for yield, deep percolation, and salinity. Yield-Water-Salinity and Deep-percolation-Water-Salinity response functions are derived for each combination of crop and irrigation system via a two-stage process. First, we produce data sets consisting of yields, Y (ton/acre), and deep-percolation flows, D (acre-feet), as an outcome from different combinations of applied-water, AW (acre-feet), salinity-of-applied-water, EC

$$Y = [a_{11} + a_{12}EC + a_{13}EC^2 + a_{14}EC^3] + [a_{21} + a_{22}EC + a_{23}EC^2 + a_{24}EC^3]AW^{(a_{31} + a_{32}EC + a_{33}EC^2 + a_{34}EC^3)} [a_{41} + a_{42}EC + a_{43}EC^2 + a_{44}EC^3]$$

$$D = (b_{11} + b_{12}EC + b_{13}EC^2 + b_{14}EC^3) + (b_{21} + b_{22}EC + b_{23}EC^2 + b_{24}EC^3)AW + (b_{31} + b_{32}EC + b_{33}EC^2 + b_{34}EC^3)AW^2 + (b_{41} + b_{42}EC + b_{43}EC^2 + b_{44}EC^3)AW^3$$

- (1) **Figure 1** provides an illustration of these fitted functions for cotton under two irrigation systems assuming 1.3 AW 4.8 and 0 EC 10
- (2)

(ds/m) and irrigation system using an evapotranspiration model. Second, we fit designated response functions to the data sets via regression analysis. The specific functional forms for yield and deep percolation flows are presented in equations (1) and (2):

ALTERNATIVE MANAGEMENT STRATEGIES:

A number of potential management options have been suggested to address the salinity and drainage problems arising from irrigated agriculture. Our model will evaluate the economic efficiency and profitability of such options, which include:

- Source Control
 - changing cropping patterns
 - improving irrigation systems
 - reducing water application rates
 - Reuse
- viable cropping alternatives for reused water
- allowing higher salt concentrations in reused water
- reducing reused water application rates
- increase acreage allocated to crops receiving reused water
- Disposal
 - traditional evaporation ponds and compensating habitat
 - solar evaporation ponds
 - desalination measures
 - markets for both compensating habitat and salt

POLICY INSTRUMENTS:

Given our model identifies management solutions that meet the prescribed environmental conditions associated with the salinity and drainage problem, we can then evaluate the ability of various policy instruments in achieving those solutions. Instruments that address this problem directly may include:

- Emission Charges on drainage – By placing charges on drainage effluent, will likely see a reduction in this pollutant up to the point where the costs of reducing the pollutant are equal to the charge. Can be cost-effective, yet nonpoint source nature of pollutant may limit this strategy's effectiveness.
- Standards on drainage – By placing limits on allowable drainage, can reduce pollutant to desired level. Nonpoint source nature of pollutant may limit this strategy's effectiveness.
- Charges / Subsidies on inputs – The use of charges or subsidies on input usage or technology adoption may provide correct incentive that will ultimately lead to a reduction in drainage.
- Standards on inputs – By placing limits on input usage or allowable technologies may be able to reduce drainage to desired level.

Alternatively, potential instruments that may achieve the management solutions indirectly include:

- Water Pricing / Transfers – may create incentives to conserve water or consider value of water in alternative uses which transfers would open up. Pricing will induce conservation, reward efficiency and penalize inefficiency
- Land Retirement – retire problem land, thereby reducing salt burden, with adequate compensation to growers.

SUSTAINABILITY:

Finally, this framework can be used to evaluate concerns associated what may be considered sustainability issues. Three issues are identified below along with a brief description of each.

- (I) Profitability in closed basins: Deep percolation emissions are an inevitable consequence of irrigated agriculture.

Many basins currently do not have access to external drainage facilities. Can irrigated agriculture continue to maintain an adequate level of profitability in closed basins? Is agriculture sustainable over the intermediate- and long-run when all emissions are disposed of within the region?

- (II) Cost of drainage reduction: Drainage emissions to the San Joaquin River and elsewhere cause environmental damages. What is the cost to agriculture of reducing these emissions? These costs need to be balanced against the benefits of reducing emissions.
- (III) External drainage facilities: External drainage facilities allow increased agricultural production and reduce the costs to irrigated agriculture of within-region disposal. This model can be used to estimate the benefits to agriculture of constructing external disposal facilities. These benefits then need to be weighed against the construction cost of the facilities as well as the consequent environmental damages.

PRELIMINARY RESULTS

To illustrate some of the relationships we can investigate and evaluate with this model, we present the solutions to three scenarios we evaluated using the first stage of our preliminary model. For simplification purposes, the scale of our analysis is a single acre of land in the WWD. Our objective function maximizes net returns to land and management. The grower is cropping cotton, wheat, tomatoes, and lettuce, choosing among five alternative irrigation systems. Currently, the only reuse sector crop is cotton. The solutions are intended to illustrate the potential impacts on grower profit from changes in three potential components involved in the water management issue. These components include:

- 1) in-region (IRD) vs. out-of-region (ORD) discharge constraints
- 2) reusing drainage water on salt-tolerant crops (R)

- 3) allowing for evaporation ponds as a means of disposal (EP).

Specifically, we compare the following three scenarios:

- Scenario A: The model requires out-of-region disposal (ORD), reuse (R), and evaporation ponds (EP)
- Scenario B: The model requires in-region disposal (IRD), but allows for reuse (R) and evaporation ponds (EP).
- Scenario C: The model requires in-region disposal (IRD), allows for evaporation ponds (EP), but does not allow for reuse (i.e., no R).

In all three scenarios, we assume that approximately 9% of the land is left fallow. The results are presented in Table 1.

Under Scenario A, and given the objective of allocating land and water so as to maximize net returns to land and management, reuse (R) is shown to be an optimal strategy and is allocated nearly 18% of the land. Furthermore, all crops except tomatoes use furrow irrigation with half-mile runs. Under Scenario B and given that no out-of-region discharges are allowed, maximizing net returns to land and management leads to a choice of reuse again as the optimal strategy, even though evaporation ponds are an option. As shown, the limitation on out-of-region discharges actually increases the amount of land devoted to reuse crops compared to Scenario A. The main influence driving the observed differences in net regional profits under Scenarios' A and B is the cost associated with the in-region drainage disposal requirement. Finally, it should be noted that the optimal choice of irrigation system under Scenario B is furrow with quarter-mile runs.

Scenario C represents a situation where both reuse and out-of-region disposal are prohibited; hence, evaporation ponds are used. The difference between the net profits under Scenarios' B and C can be attributed

to the costs of the reuse prohibition. Conversely, one can consider the increase in profit under Scenario B when compared to Scenario C as a measure of the potential gain to growers if they engage in the reuse option.

SUMMARY

This progress report describes the development of a regional economic model that can evaluate and compare alternative management strategies confronting growers in the WWD with recognition of the current regional environmental restrictions associated with drainage water. Significant progress this year includes estimating the yield and deep percolation response functions that differ with respect to both applied water and EC, estimating and updating the economic price data, and developing a mathematical programming model that includes both salt tolerant and salt sensitive crops. Our preliminary results are promising in that the model is responding in a manner consistent with our a priori expectations. In particular, our solutions suggest that as the cost of disposing of drainage water increases, not only is there an increase in the amount of

land that uses more efficient, albeit costly, irrigation systems, there is also an increase in land devoted to crops grown with reused water. While currently our model includes cotton alone as the single reuse crop, the results suggest that even under a scenario where costless out of region discharge is allowed, 18% of the cultivated land is devoted to reuse. In effect, this outcome suggests that the additional savings in from using less fresh water outweigh the decrease in cotton production from reusing the water.

Two developments are currently underway. First, we are analyzing individual crops (cotton, tomatoes, and sugarbeets) to understand the specific characteristics of their yield and deep percolation functions, both theoretically and empirically, in terms of how they respond to changes in the prices of fresh-, reused-, and drainage water as well as to changes in EC and AW. Second, this data that is used in these individual specific crop analyses is being incorporated into the regional model.

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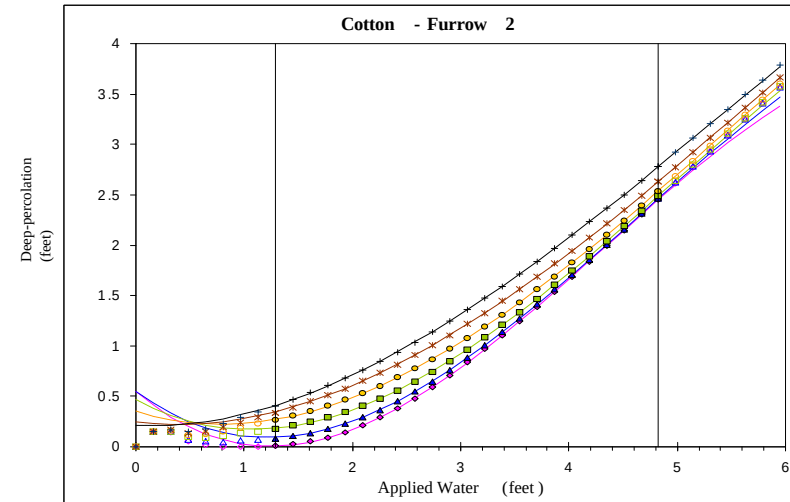
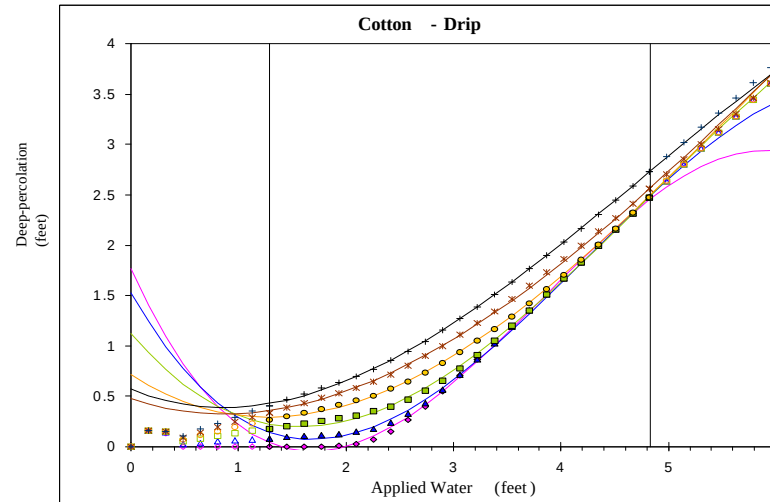
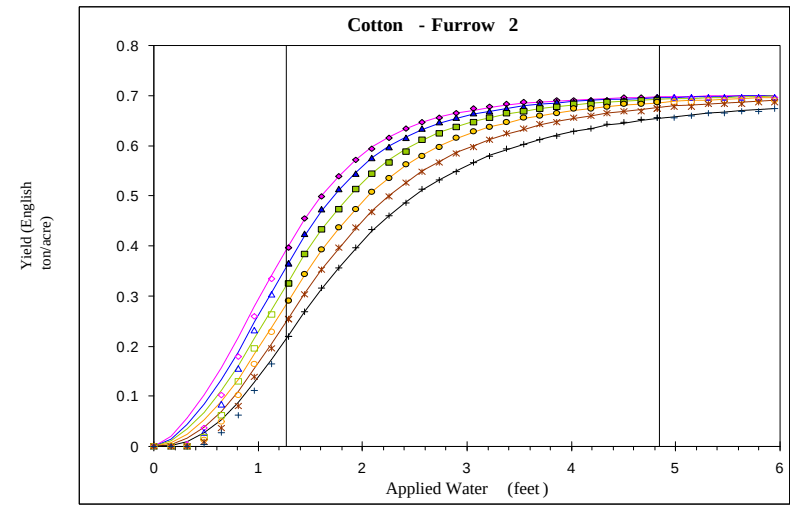
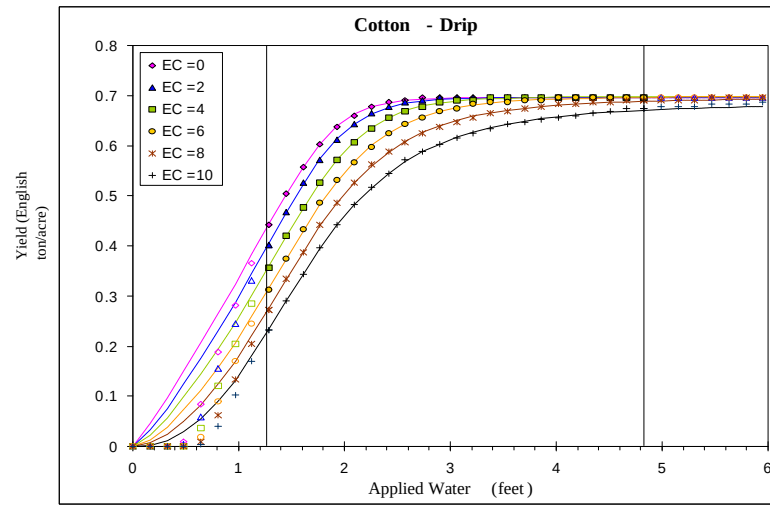


Figure 1. Examples of yield (Y) and deep percolation (D) functions vs. applied water (AW) under different irrigation systems (Drip and Furrow) and various salinity (EC) levels

Table 1. Preliminary Results under Three General Scenarios.

Scenario	Scenario A (ORD, R, EP)	Scenario B (IRD, R, EP)	Scenario C (IRD, EP, no R)
Net regional profit (\$/acre/year, 1992)	463.1	460.8	406.9
<u>Fresh water sector</u>			
Cotton	40.6	37.6	46.1
Land (%)	Furrow 2	Furrow 4	Furrow 4
Irrigation system			
Tomatoes	10.0	9.9	10.2
Land (%)	Furrow 4	Furrow 4	Linear
Irrigation system			
Wheat	13.6	10.3	16.8
Land (%)	Furrow 2	Furrow 4	Furrow 4
Irrigation system			
Lettuce	10.4	10.3	10.7
Land (%)	Furrow 2	Linear	Linear
Irrigation system			
Total fresh water (acre-feet)	1.90	1.55	1.74
Total deep-percolation (acre-feet)	0.71	0.45	0.42
<u>Reuse sector (cotton)</u>			
Land (%)	17.9	23.3	na
Irrigation system	Furrow 2	Furrow 4	na
Fresh water (acre-feet)	0.25	0.02	na
Reused water (acre-feet)	0.61	1.09	na
Deep-percolation (acre-feet)	0.46	0.64	na
Salinity of applied water (ds/m)	7.3	9.8	na
<u>Evaporation Ponds</u>			
Land (%)	0.0	0.0	7.9
Evaporated volume (acre-feet)	0.00	0.00	0.42



Selenium Removal and Mass Balance in a Constructed Flow-through Wetland System

Project Investigators:

Kenneth Tanji, Department of Land, Air and Water Resources, UC Davis

Suduan Gao, Department of Land, Air and Water Resources, UC Davis

Collaborators:

Norman Terry, Department of Plant and Microbial Biology, UC Berkeley

Zhi-Qing Lin, Department of Plant and Microbial Biology, UC Berkeley

ABSTRACT

A field plot study on the removal of Se from subsurface irrigation drainage waters was conducted from June 1996 to February 2001 in the Tulare Lake Drainage District (TLDD) of San Joaquin Valley, California. The flow-through wetland system consisted of ten 15.2x76.2 m cells with various substrates (Salt-marsh bulrush, Baltic rush, Smooth cord grass, Rabbitsfoot grass, Saltgrass, Cattail, Tule and Widgeon grass). Cell 3 was left non-vegetated. The objectives of this research were to evaluate Se removal efficiency of each wetland cell and perform a mass balance on Se. The Se removal efficiency was evaluated on the data collected from May 1997 to December 2000. Mass balance was performed for the period May 1997 to September 2000 when a comprehensive sampling was carried out on standing water, sediment pore water, fallen litter, organic detritus layer, and mineral sediment.

The inflow drainwater to the cells had an average concentration of 19 L-1 Se dominated by selenate (SeVI, 95%). Residence times for the ponded water varied from about 7 to 20 days. Se removal efficiencies are reported by [outflow]/[inflow] ratios based on concentration of Se as well as mass of Se (concentration x water volume). Vegetated cells showed higher Se removal than that of the non-vegetated cell. Se concentration ratios ranged from an average of 0.45 to 0.79 and mass ratio from 0.22 to 0.52 for year 2000 indicating 21 to 55% reduction in Se concentration in outflow waters and 48 to 78% removal in mass of Se.

Mass balance showed that on the average about 41.1% of the total inflow Se left the wetland cells via outflow (35.3%), seepage losses (3.6%), and volatilization losses (2.2%). In the internal compartments, about 53.1% of the total Se from inflow was retained within the wetland cells in surface (0-20 cm) sediment (32.5%), organic detritus layer above the mineral sediment (17.9%), fallen litter (1.9%), standing plants (0.6%), and surface water (0.4%). About 5.6% of the total Se from inflow was unaccounted for. Thus, the surface mineral sediment was the largest sink. The outlet waters contained higher percentages of SeIV and Org-Se than the inlet water indicating

reduced conditions in the cells. The goal of reducing Se in outflow water to about 2.4 $\mu\text{g L}^{-1}$ was not achieved but several wetland cells averaged about 4 $\mu\text{g L}^{-1}$ in their outflows.

The potential hazards to wildlife, particularly water birds, of accumulated Se in various internal compartments of the wetland cells and organic forms of Se in outflow waters are of concern and are under investigation by another research team.

KEYWORDS: Se removal efficiency, residence time, mass balance, seepage, volatilization, evapotranspiration

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INTRODUCTION

The UC Salinity/Drainage Program, Tulare Lake Drainage District (TLDD), and the California Department of Water Resources established an experimental flow-through wetland system near Corcoran, California in 1996 (Fig. 1 and Table 1). The objectives of this research were to evaluate Se removal efficiency of each cell in the TLDD constructed flow-through wetland cells and perform mass balance on Se. The Se removal efficiency was evaluated based on the data collected from May 1997 to December 2000. Se mass balance was performed for the period of May 1997 to September 2000 when a final comprehensive sampling was carried out on standing water, sediment pore water, fallen litter, organic detritus layer, and mineral sediment. Such a comprehensive finding would be valuable in appraising flow-through wetland systems for Se removal from agricultural drainage water or other wastewaters contaminated with Se.

EXPERIMENTAL SECTION

TLDD FLOW-THROUGH WETLAND SYSTEM AND SAMPLING

Water inflow was monitored with a totalizing flow meter fitted with terminal plastic orifice pressure regulators and outflow measured with a v-notch weir on weekly to twice a week schedule. Inflow and outflow water sampling began in April 1997 after the flow measuring devices were installed by the end of March 1997. Total Se concentration in

the inflow and outflow waters was determined weekly and water Se speciation was determined several times during each year. Se volatilization was measured monthly from June 1997 to September 1999. A final comprehensive sampling was conducted in September 2000. The wetland system was shut down in February 2001. Water flows were monitored to December 2000.

WATER FLOW AND RESIDENCE TIME

The weekly inflow rate was calculated based on the total volume of water discharged into the cell divided by the time interval between meter readings. The outflow rate was calculated from $Q_{out} = 448.8 \times 2.49 \times (h/305)^{2.48}$ where Q_{out} is the outflow rate in gallons per minute (gpm) and h is the reading of water height in mm above the V-notch of the weir.

Taking the wetland cell as the control volume and treating any fluxes cutting across the boundary of the control volume as inputs and outputs, the global mass balance of water (Fig. 2) is defined by:

$$\frac{\Delta V}{\Delta t} = Q_{in} + Q_{precip} - Q_{out} - Q_{et} - Q_{seep} \quad (1)$$

where $\frac{\Delta V}{\Delta t}$ is the change in water volume per unit time (taken as duration of monitored period herein) within the cell and since a constant water depth was maintained throughout, $\frac{\Delta V}{\Delta t}$ was set to zero. Q_{in} and Q_{out}

are respectively the measured inflow and outflow, Q_{precip} is the measured precipitation from a nearby weather station, Q_{et} is the estimated evapotranspiration rate obtained from $ET = ETo \times Kc \times \text{surface area}$ where ETo, the reference evapotranspiration rate, was obtained from a nearby weather station (Stratford) and Kc, the crop coefficient, was assumed to be 1.2. Seepage losses from the cell were initially measured by drop in head in a plastic column that was inserted into the bottom mud and filled with water. Since this method of measuring seepage losses was laborious, seepage was obtained as a closure term in eq. (1) with small difference as compared to measured values (Fig. 3). Thus,

seepage losses throughout the study was estimated from

$$Q_{seep} = Q_{in} + Q_{precip} - Q_{out} - Q_{et} \quad (2)$$

The residence time of water in the cell was calculated (Kadlec, 1989) from

$$t = \frac{L \times W \times d \times e}{Q} \quad (3)$$

where t is the residence time in days, L and W are the length and width in m, d is the depth of water in m, e is the substrate porosity (correcting for fraction of water column occupied by vegetation), and Q is the volumetric flow rate in $m^3 \text{day}^{-1}$. Thus, the calculated residence time reduces to:

$$T = \frac{Vol \times e}{Q} \quad (4)$$

where T is the residence time in days and Vol is the water volume in the cell ($L \times W \times d$) in m^3 .

The residence time in the cells is adjusted by changing the standing water depth and/or the inflow rate. The water depth was initially set at about 8 cm in Cells 5 and 6 (grasses), about 15 cm for most cells, and variable depths for Cells 8 and 9, i.e., 15 cm. for shallow areas and 60 cm for deeper areas. The design for the bottom surface for Cells 8 and 9 followed the pattern of shallow-deep-shallow-deep-shallow by dividing the cell into 5 sections corresponding to various vegetation regime as shown in Table 1. The substrate porosity was taken as 1.0 for Cells 3 and 8, 0.95 for Cells 1, 2, 4 and 9, and 0.9 for Cells 5, 6, 7 and 10.

The actual residence time was adjusted for reasons to be stated but was stabilized at about 7 and 20 days in 1997. The initial residence time was set at 2-4 days. Due to comparatively small reductions in Se concentration in the outflow relative to the inflow, the residence time in many cells was increased in August 1997 by decreasing the inflow rate. The goal was a target of residence time about 7 days for Cells 1 to 7, 14 days for Cell 10, and 20 days for Cells 8 and 9.

PROBLEMS ENCOUNTERED IN TLDD WETLAND MONITORING AND MAINTENANCE

The management and operation of the 10-cell TLDD flow-through system encountered various problems, especially before early 1999.

The main problems included: (i) occasional electricity cutoffs that shuts down inlet water pump and the need to be reset manually; (ii) the malfunctioning of the filter assembly in regard to plugging from sediments; (iii) burrowing muskrats between Cells 1 and 2, and between Cells 6 and 7 that affects pumping of inlet water from the tile sump and repairs done to leaky drainpipe and ditch. By December 1999, item (i) was solved by an automated reset switch, item (ii) by an electronic triggering to produce back-flush at a preset pressure differential, item (iii) compaction of levees between cells with heavy caterpillar tracks (except for leak from Cell 1 to 2), and item (iv) was partially corrected by adjusting the height of the pump in the sump. The cattail in Cell 7 was accidentally burnt in the fall of 1998 while controlling weeds on the banks, but recovered well in 1999. Cattail in Cell 10, however, died in late 1999 due to the deep standing water depth, preventing regeneration of new cattail plants. In June 2000, Cell 10 was drained in order to promote re-growth of cattail and seeding but was not successful due to the saline surface soil. The occurrence of these events must be taken into consideration while evaluating and interpreting residence times, water balance and Se mass balance. It should also be pointed out that by January 2001, the monoculture cells were beginning to be invaded by cattail through seed dispersal from the cells and surrounding drain ditches.

SE MASS BALANCE

Taking the wetland cell as the control volume and treating any fluxes cutting across the boundary of the control volume as inputs and outputs, the global mass balance on Se (Fig. 4) is defined by

$$\frac{\Delta M}{\Delta t} = \frac{M_{t+\Delta t} - M_t}{\Delta t} = \dot{M}_{in} - \dot{M}_{out} - \dot{M}_{gas} - \dot{M}_{seep} \quad (5)$$

where $\frac{\Delta M}{\Delta t}$ is the change in mass of Se within the cell accounting for M_t , the initial Se in the soil, $\dot{M}_{in}$ and $\dot{M}_{out}$ are respectively the mass (concentration x volume) of Se in the inflow and outflow waters, $\dot{M}_{gas}$ is the mass of Se

volatilized, and $\dot{M}_{seep}$ is the mass of Se in seepage losses.

Within the control volume (cell) there are several internal Se compartments that were measured in this study. Thus, an independent

calculation of $\frac{\Delta M}{\Delta t}$ in equation (5) can be

obtained from

$$\frac{\Delta M_{int}}{\Delta t} = \dot{M}_{sed} + \dot{M}_{org} + \dot{M}_{lit} + \dot{M}_{pl} + \dot{M}_{sw} \quad (6)$$

where $\frac{\Delta M_{int}}{\Delta t}$ is the change in storage of Se in

the internal compartments to differentiate from the global mass balance, and

$\dot{M}_{sed}$, $\dot{M}_{org}$, $\dot{M}_{lit}$, $\dot{M}_{pl}$, and $\dot{M}_{sw}$ denote mass of Se in the sediment, organic detritus layer, fallen litter, plants, and standing water, respectively.

COMPREHENSIVE SAMPLING FOR SE MASS BALANCE

A comprehensive sampling of each TLDD wetland cells was conducted in September 2000 for standing water, sediment pore water, fallen litter, organic detritus layer, the underlying mineral sediments to 20- and 100-cm depths, and plants. Speciation and total Se in the standing water and Se concentrations in the various samples were determined. Then, estimates on mass of Se in each compartment were performed considering bulk density and biomass.

Figure 5 presents the detailed sampling plan. Twenty surface water samples were taken from each cell in 4 rows with 5 samples in each row. Surface water samples were taken prior to any other samples and all the remaining samplings in each cell were taken simultaneously as noted in the legend. Water samples were stored in ice-coolers and kept refrigerated in the laboratory until analysis. Total Se concentration and speciation for Se(VI), Se(IV), and org-Se were performed on the waters.

To estimate Se in seepage losses, three ceramic suction cup lysimeters were installed in early May 2000 in three locations in each cell, ~1/4 distance from inlet, center, and ~1/4

distance from outlet. The cups were installed at depths below rooting zone, at ~35 cm below the mineral sediment surface level. Vacuum was applied to the lysimeters to collect pore water and the water samples were collected into previously acid-washed glass vials. Total Se in the pore water samples was analyzed. Sampling of sediment pore water was conducted in May, July, and September of the year. The mass of Se in rootzone drainage was obtained from the product of average annual Se concentration in the pore water and annual seepage volume estimated from the water balance equation.

To estimate the mass of Se in fallen litter in each cell, six 3,716-cm² areas were drawn using a square top- and bottom-open aluminum box at designated locations in each cell (Fig. 5) and the fallen litter was picked up by hand. The fallen litter samples were dried at ~55°C, weighed, ground and analyzed for total Se. The total mass of fallen litter was calculated by multiplying the total area of the cell and the unit area sampling weight. The mass of Se in fallen litter was then obtained from the product of biomass of fallen litter and Se concentration.

To estimate the mass of Se in fine organic detritus matter in the cell bottom, a layer right above the mineral sediment was collected using 30 acrylic tubes with an inside diameter of 10 cm pressed to depth of 10 cm. These tubes were inserted into the undisturbed sediment surface after large pieces of litter were carefully removed. Surface water was decanted and the brown-colored fine detritus layer was scraped off with spatulas. Three sub-samples were collected from each cell by compositing each of the 10 tubes into one sub-sample. A second set of samples was collected by directly scraping off the detritus layer from the six 20-cm deep sediment core samples (described below). The mass of Se in fine and thin organic detritus layer was obtained from the average of the two sampling methods described above. The processing, preparation and analysis of detrital layer samples were analogous to that of fallen litter.

A previous study showed that Se was mainly accumulated in the top surface sedi-

ment in this study (Gao et al., 2000). Se in the surface sediment was collected by six cores using 5-cm inside diameter acrylic tubes with an approximate length of 25 cm. The tubes were pushed down into the sediment and pulled up manually after inserting to about 20 cm deep. The acrylic tubes were then capped immediately on both ends and sealed with duct tapes. The core samples were sectioned into 0-5, 5-10, 10-15 and 15-20 cm segments. After air-drying, the samples were ground and analyzed for total Se.

To investigate Se concentration in deeper sediment, auger samples (~2.5 cm diameter) up to 1-m depth were taken at 3 locations in each cell (Fig. 5). Sediment samples were sectioned in 10-cm increments, air-dried, ground and analyzed for total Se.

The analytical methods for Se speciation in water samples and total Se analysis in water, fallen litter, detritus layer, and sediment were described in detail in Gao et al., (2000). Se speciation in water samples was performed using the method by Zhang et al. (1999). Total Se in water samples was determined using the procedure by Cutter (1982) and Yoshimoto (1992). Total Se analysis in the fallen litter, detritus layer and sediments followed a modified digestion procedure by Zasoski and Burau (1977). Hydride generation atomic absorption spectroscopy (AAS) technique was used for quantifying Se concentrations.

For sampling standing plant materials, three whole green plants were removed using a spade from each of five randomly selected sampling quadrates (Fig. 5). Dead, above ground, standing litter was also collected along with green plant samples. After the roots were rinsed on-site to remove sediments, all plant samples were placed in *Ziploc* bags and kept at 4 °C during their transport to the laboratory for total Se analysis. The plant coverage (%) in each cell was recorded, and the standing above ground plant biomass was determined by this harvesting method. The root biomass was estimated by the ratio of the shoot to the root that was previously determined for each whole green plant (Zayed et al., 1998).

The wetland plant samples were dried at 60°C, and ground in a Wiley Mill to pass a 0.43-mm mesh screen. The ground samples were acid-digested with HNO₃ and H₂O₂ (USEPA, Method 3050B). Total Se was measured with AAS with hydride generation (VGA-77) following the US EPA Method 7742. Standard reference materials of SRM-1567a (wheat flour) was measured as internal quality controls for analyses of Se in plant samples.

Se volatilization was measured monthly in Cells 1 to 7 starting in June 1997 to September in year 2000 in order to minimize disturbance inside the cells for final comprehensive sampling for Se mass balance.

Volatile Se was collected using an open-flow sampling chamber system (Lin et al., 1999) and pulling air under suction from the chamber through charcoal filters with a vacuum pump, which allowed an air outflow rate of 0.85 m³ h⁻¹ from the chamber. The charcoal filters were composed of activated carbon impregnated onto a polyester fiber mesh and was inserted into the air inlet and outlet for each chamber. The dimensions of a single module *Plexiglas* chamber were 71-cm long, 71-cm wide and 76-cm high. The double module chambers with a total height of 1.5 m were used to accommodate taller plants (such as cattails in Cell 7) that did not fit in the single module chamber. The sampling chamber enclosed a ground surface area of 0.5 m², and an internal space of 0.38 m³ for the single module chamber and 0.75 m³ for the double module chamber. The open-bottom chambers were inserted 3-5 cm into the sediment, thereby sealing off the sampling area from the surrounding wetland. The chambers were then covered by shade canopies (e.g., *EZ Up DOME*) and allowed to collect volatile Se over 24 hrs. At the end of the collection period, the charcoal filters were collected from the chambers, placed into *Ziploc* bags, labeled, and stored in a cooler at 4 °C.

Se volatilization measurement was conducted in triplicates in Cells 1-7 on a monthly basis. To analyze total Se from the charcoal trap material, the Se-containing charcoal filters were weighed and cut into sub-samples. The sub-samples were then placed into a 500-ml Nalgene bottles, and immersed in 200-ml of

extraction solution (160 ml 0.05 M NaOH + 40 ml 30% H₂O₂, v/v). After extraction for two days, the extraction solution was filtered with 1 mm filters and H₂O₂ was added to the aliquots, then boiled off at 91°C for 30 min. Selenium in the sample was then reduced by adding an equal volume of concentrated HCl, and heated to 91°C for 30 min. Total Se was determined by AAS with hydride generation following the Varian's procedure.

RESULTS AND SUMMARY

OVERALL PERFORMANCE OF WETLAND CELLS AND SE REMOVAL EFFICIENCIES

Se removal efficiency by flow-through wetlands can be appraised by either a decrease in concentration of Se from the inlet to the outlet (concentration affects biota) or a decrease in the mass of Se from the inlet to outlet (evaluates treatment efficacy). Water Se concentrations are currently used to evaluate its toxic risk to fish and wildlife such as levels of concerns for impounded waters (Skorupa, 1998) and the national chronic criterion for surface waters (U.S. Environmental Protection Agency, 1987). However, it has been recently realized that it is more important to know the dietary exposure to Se (both water and sediment Se) in determining the risk potential for chronic effects in aquatic organisms. (USEPA, 1998). Thus, the changes in both Se concentration and mass in the outflow need to be evaluated.

Figure 6 shows the average annual hydraulic residence time for the wetland cells for years 1998, 1999, and 2000. In 1998, the residence times were higher and fluctuated more than the latter two years due to plumbing problems mentioned earlier. In 1999 and 2000, the residence times were relatively more stable except for a couple of cells. Horizontal crossover flow from Cells 1 to 2 and from Cells 6 to 7 from muskrat burrows occurred in 1999. The latter leak was fixed in late September 1999 and the former, in early 2000. These crossovers are reflected in the longer residence time for Cell 1 and 6 as compared to Cells 2 and 7 in 1999. Rabbit-foot grass in Cell 5 is not a wetland plant and suffered from inappropriate high water depth and the water level was adjusted during the course of this research as

shown by the decrease of residence time in 2000. Cattail in Cell 10 whose residence time was about twice that in Cell 7 died in late 1999 from high water depth. The cell was completely drained in June 2000 in an effort to reestablish the cattail but was unsuccessful. Thus, the monitored data for Cell 10 is only up to the drain date. Considering all of these factors, the more reliable data for Se removal efficiency are those collected in 1999 and 2000.

Figure 7 presents the annual average concentrations of Se in inflow and outflow waters. There were some variations in Se concentrations in inflow and outflows. The water Se concentrations in outflows were all lower than that of inflow water. Cell 3 that was not vegetated had some Se removal because of volunteer colonies of algae and microbes. In year 2000, outflow Se in Cells 2, 4, and 6 were the lowest, followed by Cells 8, 9, and 10, and highest in Cells 1 (high inflows due to crossover flow), 3 (non-vegetated), 5 (non-wetland plant with shallow water depth) and 7 (accidental burning). None of the vegetated cells, however, had reduced the Se concentration down to or below the target level of $2 \mu\text{g L}^{-1}$ although some of the cells in some seasons had reduced Se concentration close to or below the national chronic criterion of $5 \mu\text{g L}^{-1}$ (USEPA, 1987).

Figure 8 plots the average annual ratios of [outflow]/[inflow] in year 2000 when there were the least problems in maintaining the wetland system as compared to previous years. In terms of Se concentration, the cells showing better performance in [outflow]/[inflow] ratio were Cells 2, 4, 6, and 10 which had values < 0.5 , i.e., over 50% Se concentration reduction while all other cells did not reduce Se concentration substantially. However, in terms of mass, Cells 4 and 6 had the lowest ratio of 0.24 (highest Se removal) and other vegetated cells ranging from 0.31 to 0.44 with Cell 3, the non-vegetated control cell, having the highest ratio of 0.52 (the lowest Se removal). Overall, Se concentration ratio ranged from 0.45 to 0.79 and mass ratio from 0.22 to 0.52 indicating respectively 21-55% in Se concentration reduction and 48-78% Se mass removal. Cells 4 (Smooth cord-grass) and 6 (Saltgrass) had highest Se removal efficiency in either Se

concentration or Se mass reduction. It was clear that Cell 3 (non-vegetated) had the lowest Se removal efficiencies, confirming the important role of vegetation in Se removal in wetland systems.

SE REMOVAL EFFICIENCIES AND RESIDENCE TIME

The relationship between Se removal efficiencies and water residence time was evaluated. Figure 9 shows Se ratios vs. residence time by combining all the data for the 10 cells for year 2000. Although a trend seems exist the correlation was not significant due to many variables involved in the wet-land performance such as plant type, losses from volatilization and seepage, microbial community, etc. However, if the data is evaluated for each cell as shown in Figure 10 where Se mass ratio is plotted vs. residence time, a clearer relationship between the Se removal and residence time is observed. It can be concluded that for a wetland system that has a fixed physical setting a longer residence time results in higher Se removal up to a certain extent. Longer residence time allows water Se to interact with the organic-rich sediment that promotes Se reduction and immobilization.

SELENIUM TRANSFORMATION IN WETLAND CELLS

Figure 11 presents Se speciation in inflow and outflow waters for year 2000. The inflow water was dominated by oxidized form of Se, SeVI (95%), with a smaller percentage of SeIV (5%), and negligible Org-Se. In contrast, the proportion of reduced Se forms, SeIV and Org-Se, were significantly higher in the out-flow compared to the inflow water indicating that Se reduction processes occurred in the wetland. The percentages of reduced Se forms increased gradually in the three consecutive study years when compared to the results monitored in 1998 and 1999 (Gao et al., 2000). This indicates that as the wetland accumulates organic matter, increased reduction of Se occurs and more Se is retained in the wetland.

Figure 12 shows the longitudinal distribution of reduced Se forms as SeIV and Org-Se in standing water for Cells 1-9 in September 2000. The proportion of reduced Se forms increases in most cells along the direction from inlet to outlet except for Cell 3. Cell 8 had more open

water body and conditions were close to Cell 3 where no vegetation was present. Almost identical patterns in the distribution of reduced Se species was observed in Cells 3 and 8. The much lower proportion of reduced Se species in Cell 1 might be due to the shut down of inflow for a few months when an effort was made to find the location of muskrat burrow that caused crossover flow from Cell 1 to 2. Cell 5 had a much shallower water depth than other cells and thus a lower portion of reduced Se species was expected.

SE ACCUMULATION IN SURFACE SEDIMENT

Figure 13 shows a distinct pattern for Se accumulation in the surface sediment, obtained from the extensive sampling in September 2000. The highest Se concentration was found in the surface and dramatically dropped with increasing sediment depth. This pattern was observed in previous years of studies (Gao, et al., 2000). It was concluded that the accumulation and immobilization of Se in surface sediment was due to the reduction of Se to elemental Se and association with organic matter (Gao et al., 2000). The accumulation of Se was confined to the top 20 cm of the mineral sediment.

Figure 14 shows that Se accumulation in the mineral surface sediment (0-20 cm) of Cells 1, 3, and 5 progressively increased annually since the wetland was established. The initial Se concentration in the sediments was about 0.11 mg kg^{-1} . The large variation of Se accumulation in the surface was mainly due to the difficulty and consistency in defining the surface of mineral sediment from the organic detrital layer. But, the trend and pattern of Se accumulation in the wetland cells are clearly shown.

SE DISTRIBUTION IN WETLAND CELLS

Figure 15 gives the results of Se concentrations in fallen litter, fine organic detritus layer, and surface mineral sediment (0-5 cm). Fallen litter contains the highest Se concentration followed by the detritus-rich layer, which was only slightly higher than in the surface 0-5 cm of the mineral sediment.

Figure 16 shows that the mass distribution of Se is in an opposite direction compared to the

concentration pattern in Figure 15. The surface sediment accumulated (background value was corrected) the most of the Se followed by the detritus layer. Although the concentration of Se in the fallen litter was the highest, the mass of Se in the fallen litter was very small. Although organic materials do not contain massive amounts of Se, it was the driving force for Se reduction and accumulation in the surface mineral sediment. The highest Se concentration was associated with organic matter-rich compartment.

Figure 17 gives the longitudinal distribution and depth of Se accumulation in the sediments in the cells. Similar patterns of Se accumulation were observed for each cell with no apparent differences along the direction of inflow to outflow (A to C). Figure 18 further validates that immobilization of Se is confined to the surface sediment when sampled to the 1 m depth. Low Se concentrations in deeper sediments were observed in the cells except in those cells with shallow Se-rich ground water present in a patchy pattern beneath the cells.

WATER BALANCE

Table 2 shows the water balance for each cell as well as the total for 10 cells. Total water applied to the TLDD wetland system during the period of May 1997 to September 2000 was $386,954 \text{ m}^3$ of which about 30% was discharged as outflow, 18% as ET and 51% as seepage loss, and 0.6% present as standing water. There were some variations due to the crossover flow (horizontal seepage) from Cell 1 to 2, and Cell 6 to 7 as indicated by the excessive seepage in Cells 1 and 6, and much lower percentages as outflow as compared to those values of Cells 2 and 7. In Se mass balance to be given later, we will combine the water flows of Cells 1 and 2 as well as Cells 6 and 7. When the flows are combined the seepage and outflows are closer to the average values.

SE MASS BALANCE

Table 3 shows the results of global Se mass balance (equation 5) for each cell based on data collected during the period of May 1997 to September 2000. The mass of Se in the inflow, outflow and seepage was calculated

from the average Se concentration and the volume of water. Se loss by volatilization was measured monthly for over two years and estimates were made for those months with no measurements based on previously measured data for the cell in question when the field site was inaccessible during wet winter months and for year 2000 when volatilization was not monitored.

The total Se introduced into the 10 cells was 8.108 kg of which about 41.1% (3.337 kg) left the wetland system via outflow (35.3%), seepage (3.6%), and volatilization (2.2%). Thus, about 58.9% of the applied Se was retained in the cells or the $\frac{\Delta M}{\Delta t} = 4.771 \text{ kg}$. When the combined outputs for cells 1 and 2 as well as cells 6 and 7, Se outflow ranged from 24.6 to 51.7% of the inputs. Volatilization and seepage losses were about an order of magnitude smaller.

Table 4 contains data on mass of Se in the internal compartments of the 10 cells taken together. About 53.1% of the total Se from inflow was retained in the wetland cells in surface sediment (0-20 cm) (32.5%), organic detritus layer above the mineral sediment (17.9%), fallen litter (1.9%), standing plants (0.6%), and surface water (0.4%). The $\frac{\Delta M_{\text{int}}}{\Delta t}$ is 4.292 kg Se as compared to the global $\frac{\Delta M}{\Delta t}$ of 4.771 kg Se (58.8% of the total inputs). When global outputs and Se in the internal compartments are considered, about 94.4% of the inputs are accounted for. The measured recovery of 94.3% of the Se or 5.6% of the Se unaccounted for is considered as remarkable for a field study of this magnitude and duration.

When Cells 1 and 2 and Cells 6 and 7 are combined, the range of Se in the various

compartments of the cells are considerably narrowed. The largest sinks of Se in the cells are mineral sediment (24 to 41% of the inputs) and organic detrital layer (12 to 34% of the inputs). On an individual cell basis, from 86 to 110% of the Se inputs were accounted for when cells 1 and 2 and cells 6 and 7 are combined.

The results obtained from the TLDD flow-through wetland cells are for site-specific conditions such as dimensional configuration (15 m x 76 m), residence times (7 to 20 days), medium levels of Se concentration in the inflow water (mean concentration of 19-22 $\mu\text{g L}^{-1}$ Se dominated by SeVI, and climate. Although much has been learned from a science point of view, additional studies are needed to extend the current findings to engineering design and performance evaluation for a scaled-up operational flow-through system. We plan to continue our research with conceptual modeling utilizing and integrating the information and database obtained.

Other concerns should be addressed on using wetland in Se removal from agricultural drainage water or contaminated wastewaters, especially the potential ecotoxicity aspects. Although wetland can remove more than 50% of the Se mass, most of the Se was accumulated inside the wetland in the organically rich surface sediment. Reduction of Se in concentration and mass is accompanied by an increase in the proportions of reduced species, as SeIV and Org-Se, in the standing water and outflow that are more toxic than SeVI, the dominant form in the inflow water. It might be difficult to prevent ecotoxicological impacts. However, research is underway to reduce or break the transfer of Se in the foodchain of water birds (Fan and Higashi, 2000).

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Table 1. TLDD wetland cells with various vegetation, water depth, and target residence time.

Cell #	Vegetation	Water depth in cm	Target residence time in days
1	Saltmarsh bulrush	15	7
2	Baltic rush	15	7
3	Non-vegetated (control)	15	7
4	Smooth cordgrass	8	7
5	Rabbitfoot grass	8	7
6	Saltgrass	8	7
7	Cattail	15	7
8	Bulrush-Widgeon grass-Wigeon- Wigeon-Bulrush	15-60-15-60-15	20
9	Cattail-Tule-Wigeon grass-Tule- Cattail	15-60-15-60-15	20
10	Cattail	30	14

Table 2. Water balance in TLDD flow-through wetland cells for the period of May 1997 to September 2000.

	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Sum
Total applied											
cubic meters	68313	27005	33678	33843	37624	49011	36298	33716	33700	33765	386954
combined volume		95318					85309				
Total outflow											
cubic meters	7077	16727	14979	11083	10660	8451	15404	8717	12029	10143	115271
% of applied	10	62	44	33	28	17	42	26	36	30	30
combined %		25					28				
ET											
cubic meters	6830	6986	7188	7004	6726	7031	7226	7019	7519	7322	70851
% of applied	10	26	21	21	18	14	20	21	22	22	18
combined %		14					17				
Standing water:											
cubic meters	295	151	172	160	97	202	156	419	427	316	2395
% of applied	0.4	0.6	0.5	0.5	0.3	0.4	0.4	1.2	1.3	0.9	0.6
combined %		0.5					0.4				
Seepage loss											
cubic meters	54111	3141	11340	15596	20140	33328	13512	17560	13725	15984	198438
% of applied	79	12	34	46	54	68	37	52	41	47	51
combined %		60					55				

Table 3. Global Se mass balance in TLDD flow-through wetland cells for the period of May 1997 to September 2000

	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Sum	% of Se In
Total Se (g) in (5/97-9/00)	1405.7	563.9	711.9	741.5	773.4	1013.7	780.1	711.4	710.4	695.7	8107.7	
combined	1,969.6					1,793.8						
Total Se (g) out (5/97-9/00)	285.4	284.2	368.2	264.4	289.3	249.6	381.7	243.7	241.8	254.5	2862.9	
(% of total Se in)	20.3	50.4	51.7	35.7	37.4	24.6	48.9	34.3	34.0	36.6		35.3
combined %	28.9					35.2						
Total Se (g) volatilized*	5.8	12.0	32.9	16.0	39.2	11.8	12.7	18.6	18.6	12.4	179.8	
(% of total Se in)	0.4	2.1	4.6	2.2	5.1	1.2	1.6	2.6	2.6	1.8		2.2
combined %	0.9					1.4						
Total Se (g) in seepage (5/97-9/00)	83.7	4.5	19.2	18.9	24.7	35.4	18.6	18.3	30.0	41.1	294.4	
(% of total Se in)	6.0	0.8	2.7	2.5	3.2	3.5	2.4	2.6	4.2	5.9		3.6
combined %	4.5					3.0						

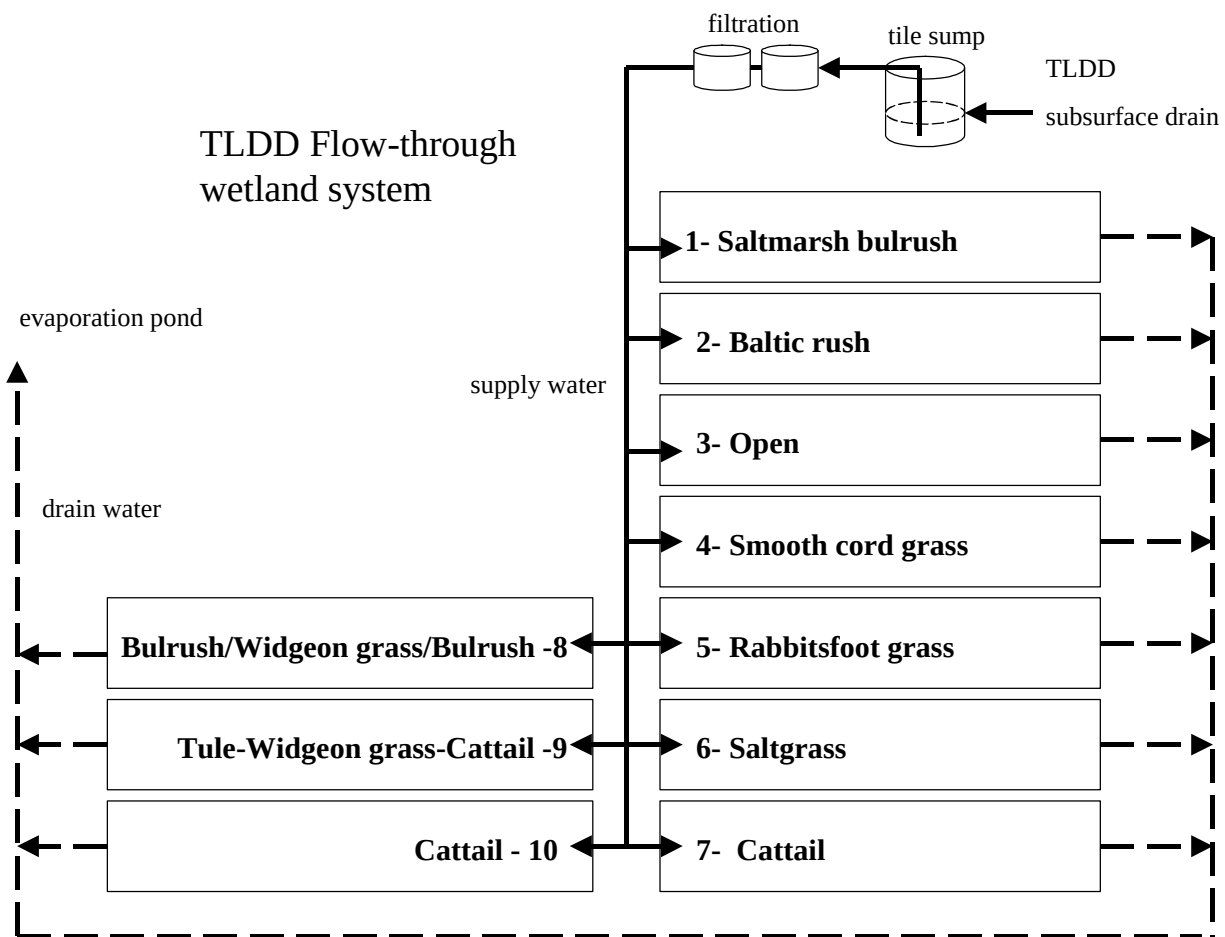


Figure 1. Schematic diagram of the layout of the TLDD flow-through wetland system

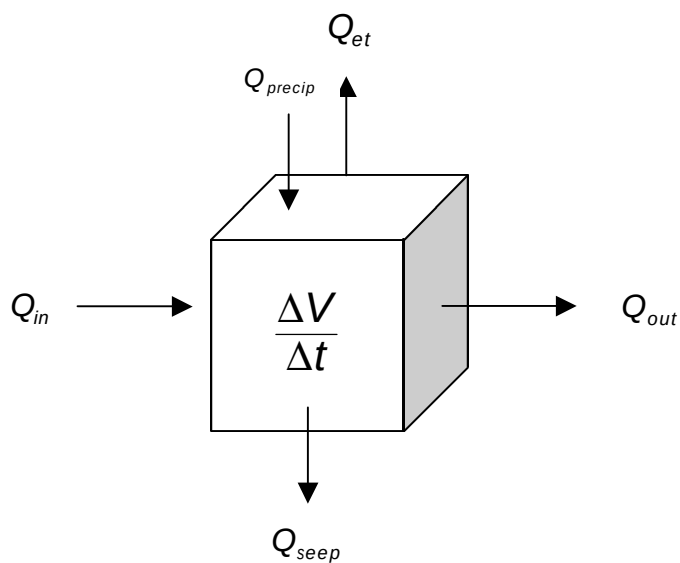


Figure 2. Global mass balance on water

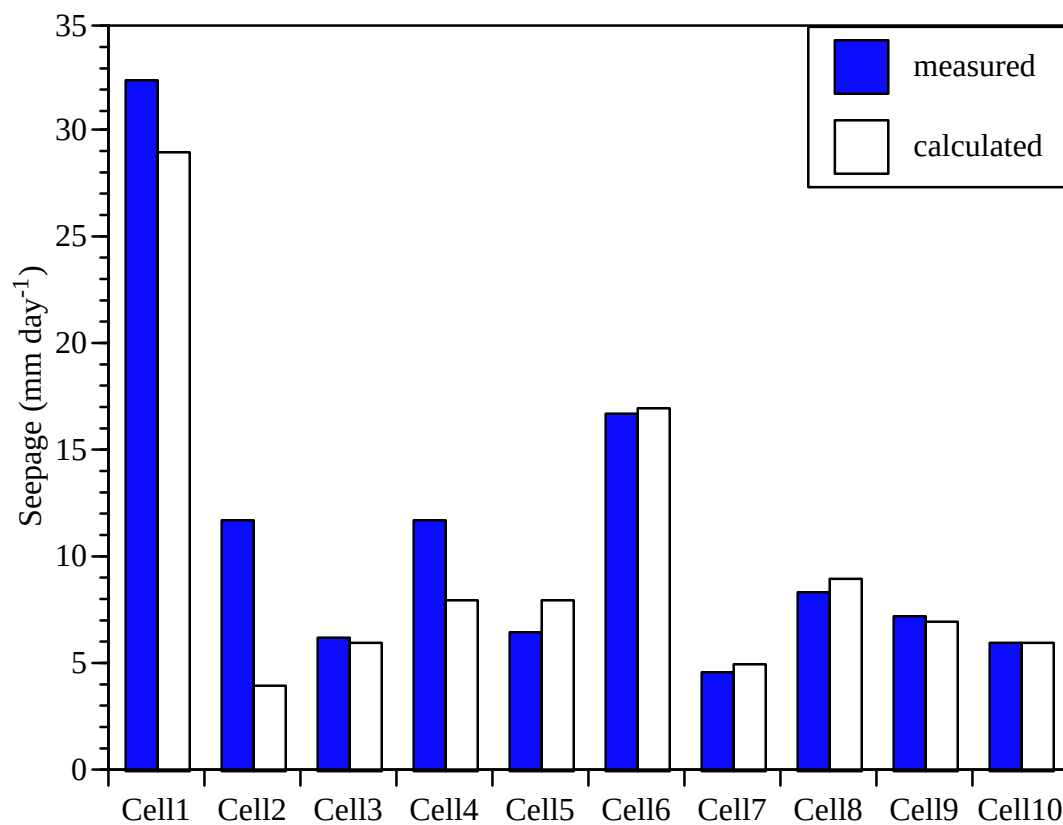


Figure 3. Comparison of measured seepage and seepage obtained by difference in the water balance

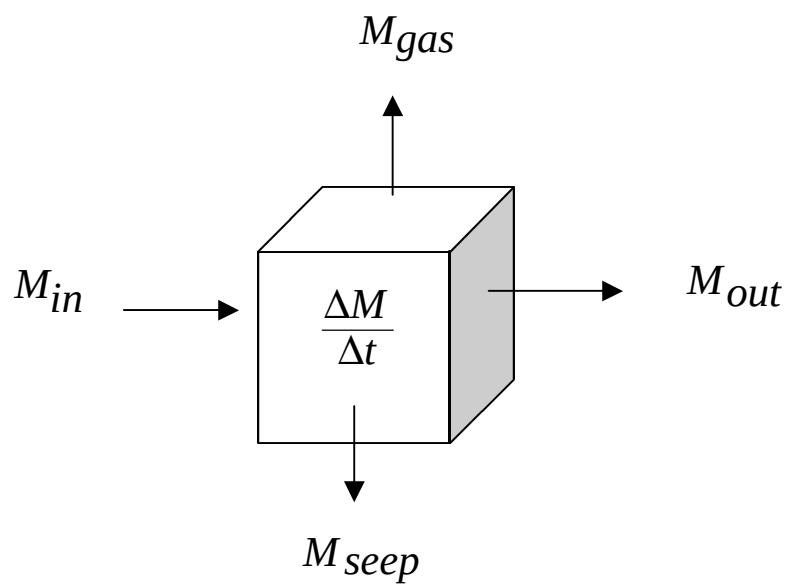


Figure 4. Global mass balance on Se

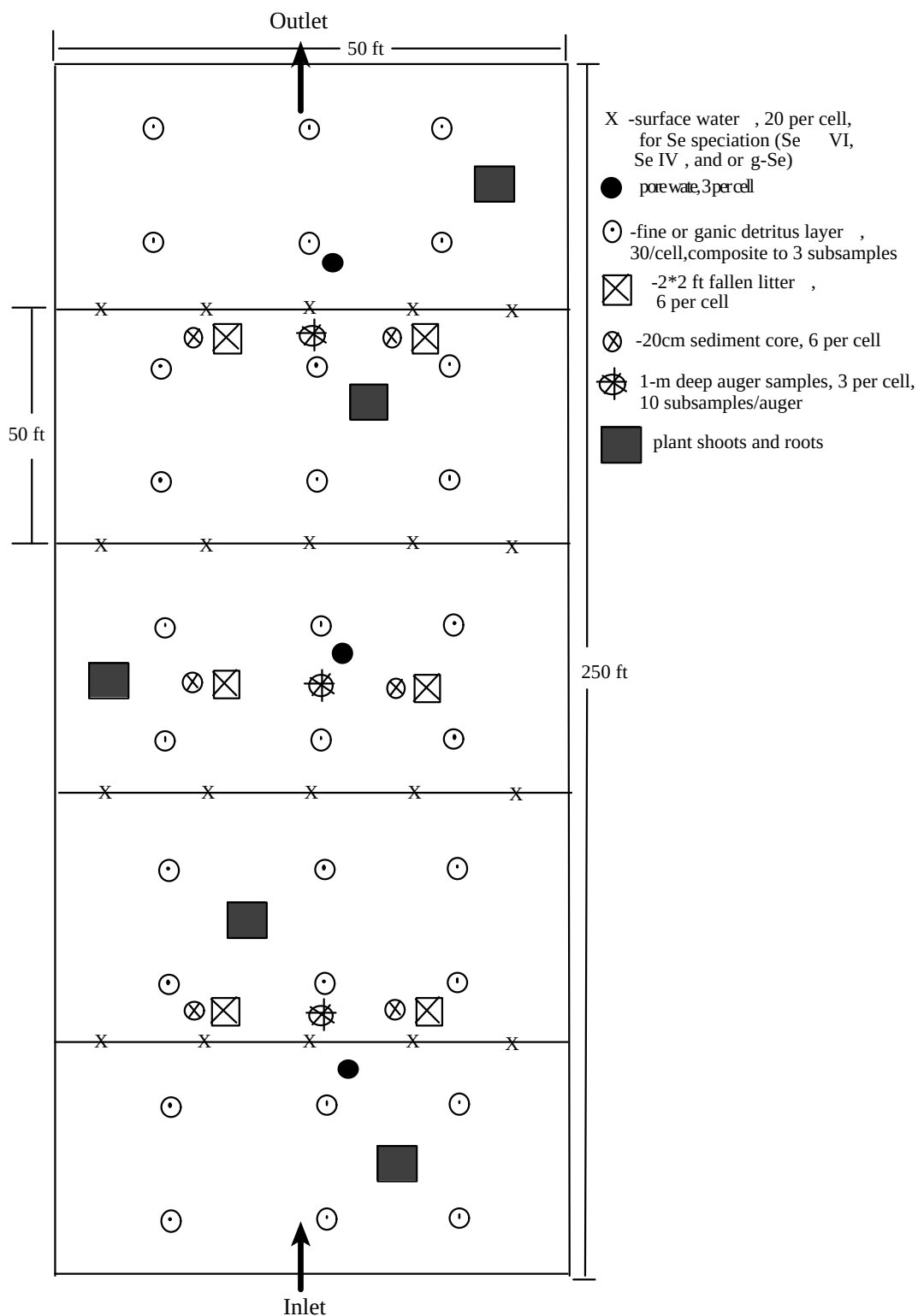


Figure 5. Sketch of the comprehensive sampling for mass balance in September 20-22, 2000

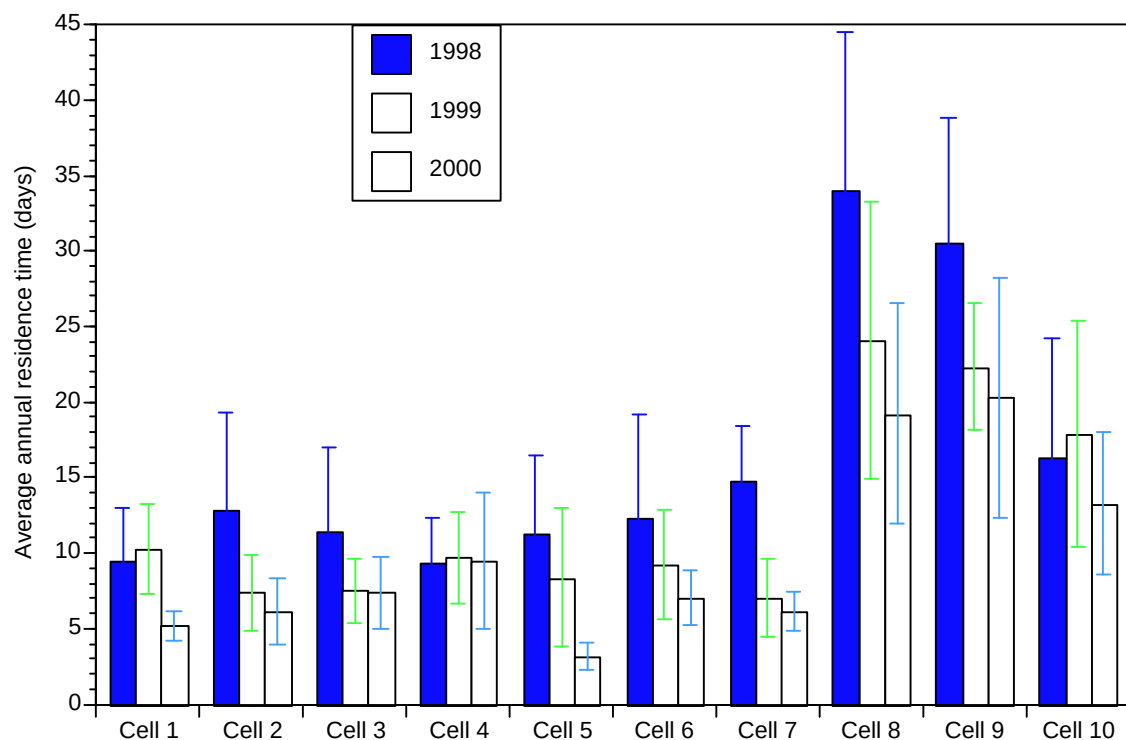


Figure 6. The average annual residence time in 1998, 1999 and 2000

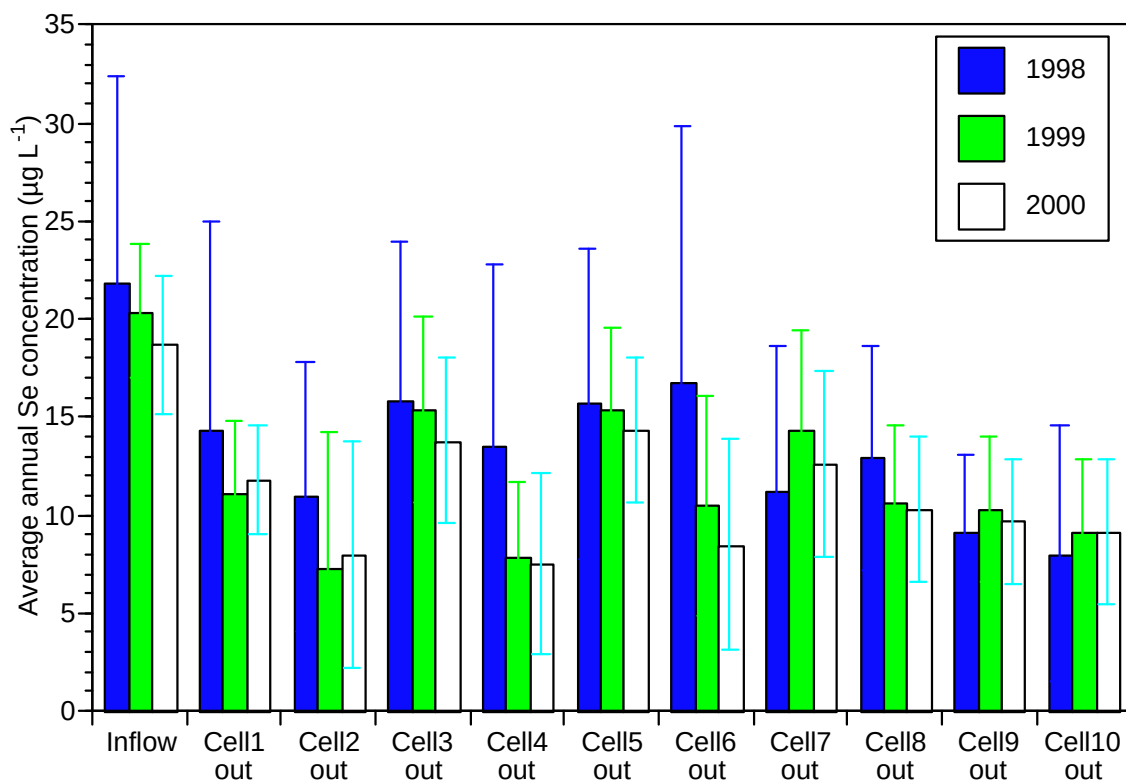


Figure 7. Average annual Se concentration in inflow and outflow waters in 1998, 1999 and 2000

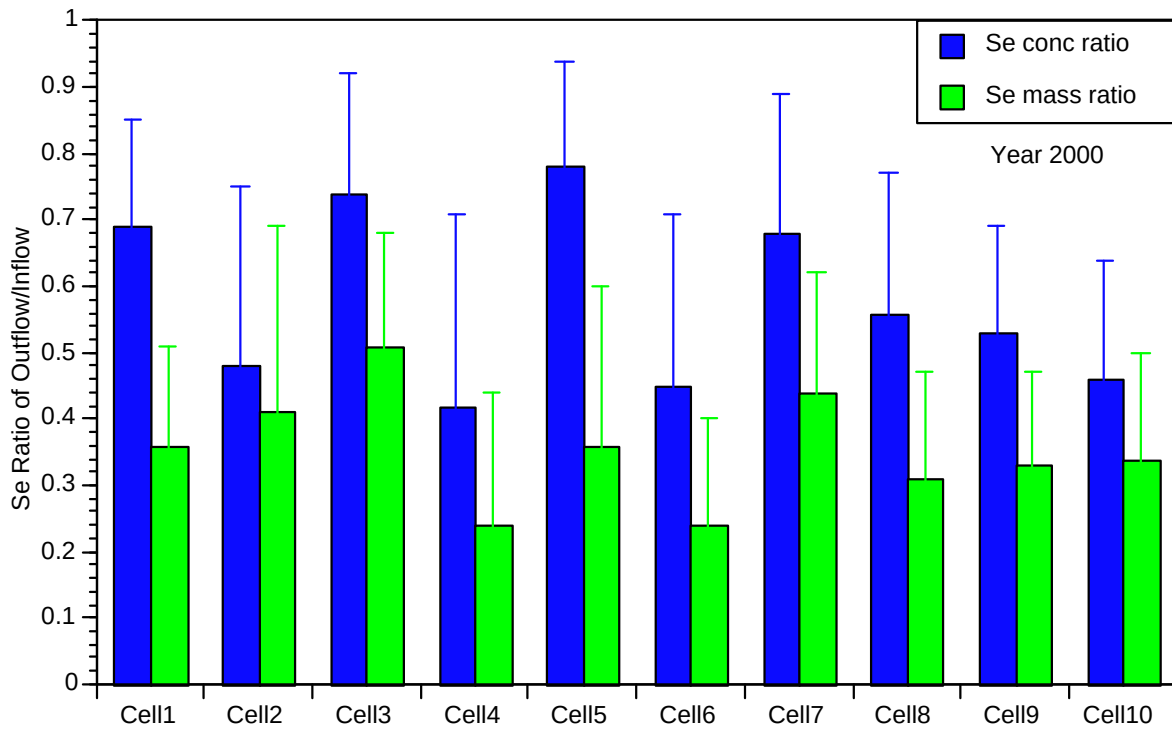


Figure 8. Se concentration ratio and mass ratio of [outflow]/[inflow] in 2000

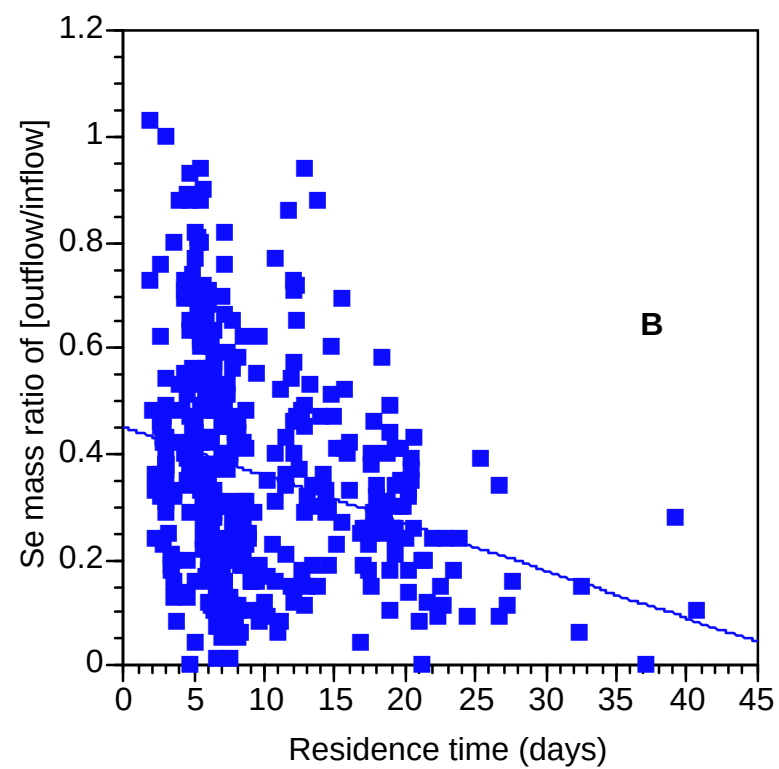
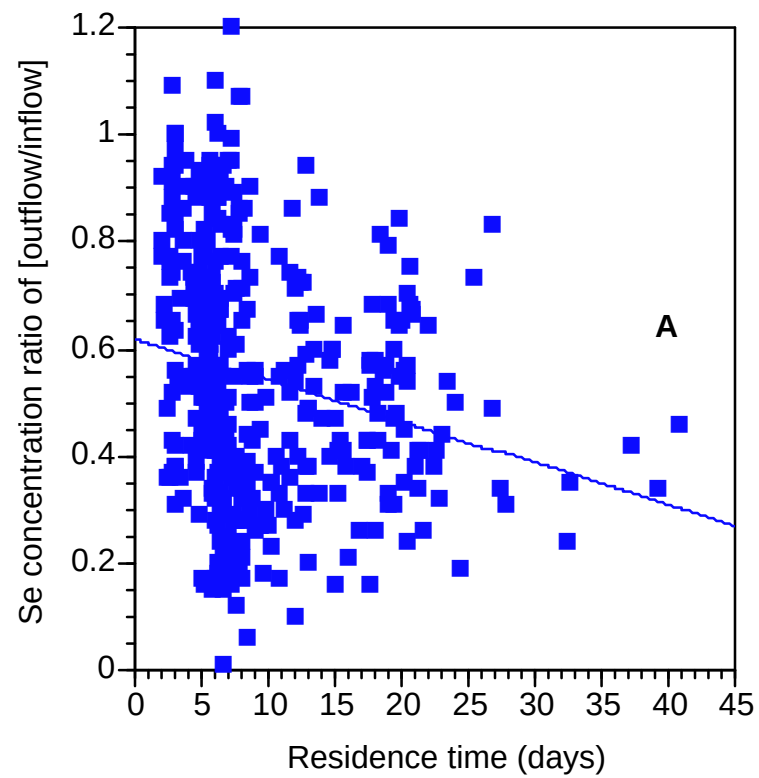


Figure 9. Se concentration ratio (A) and mass ratio (B) of [outflow]/[inflow] vs. residence time

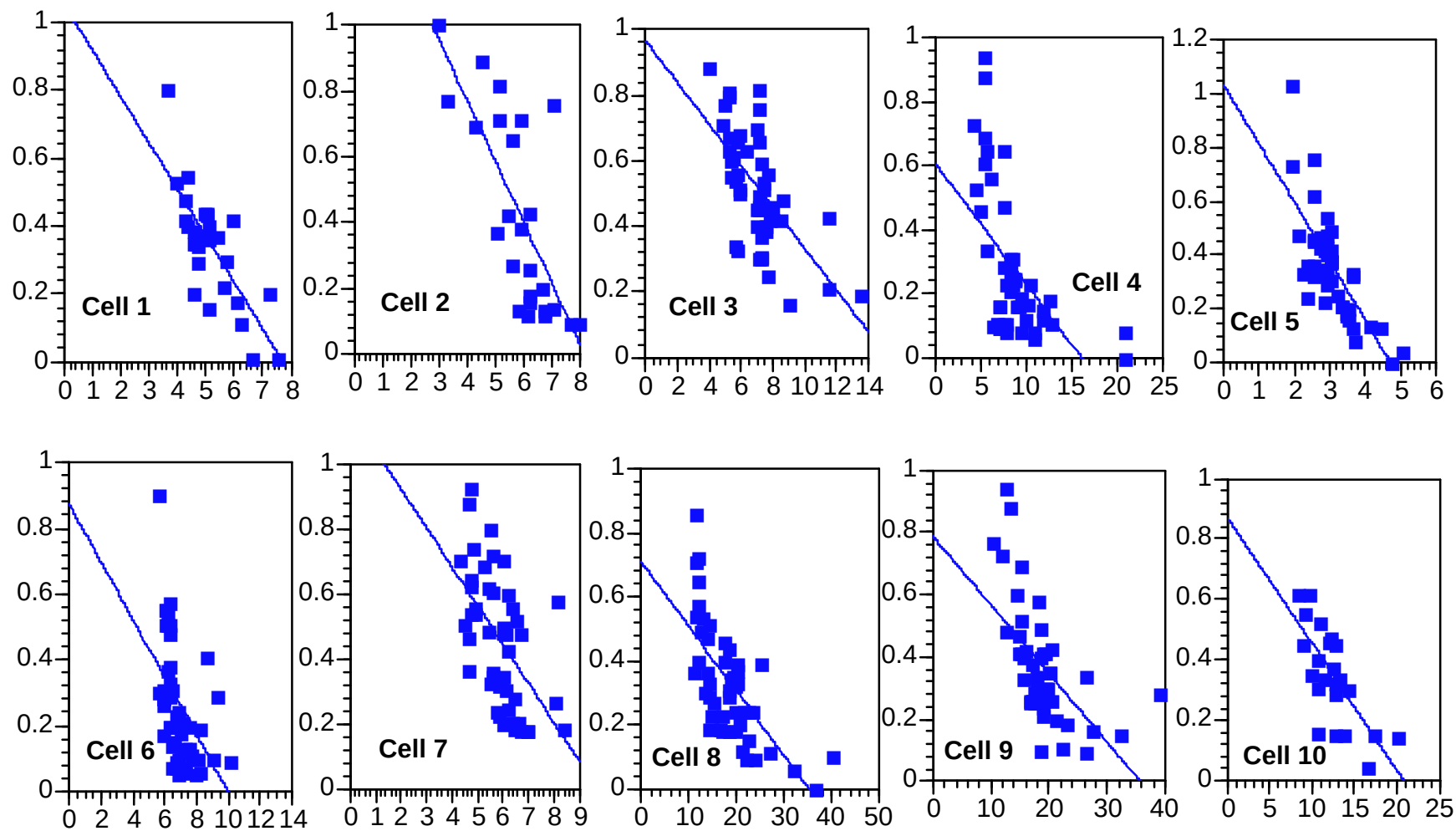


Figure 10. Se mass ratio (y-axis) vs. residence time (x-axis) in year 2000

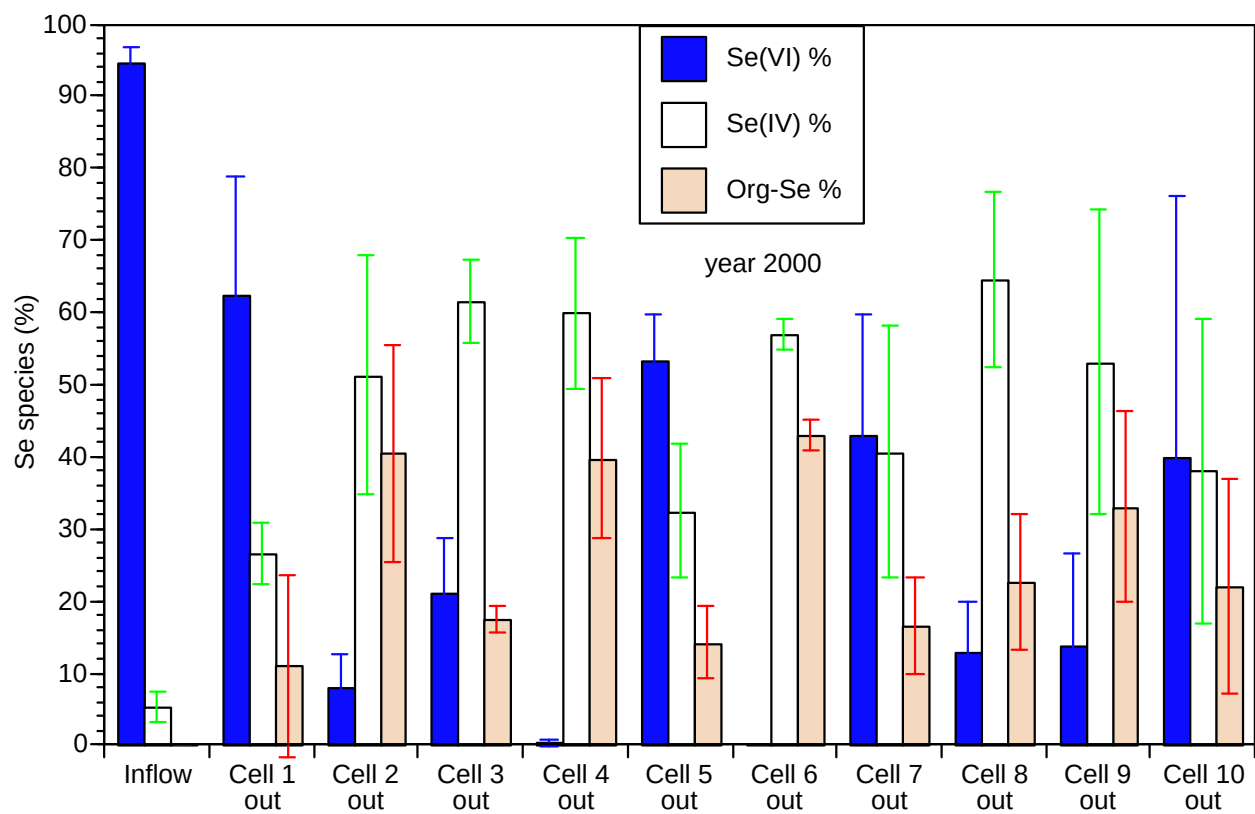


Figure 11. Se speciation in inflow and outflow waters in year 2000

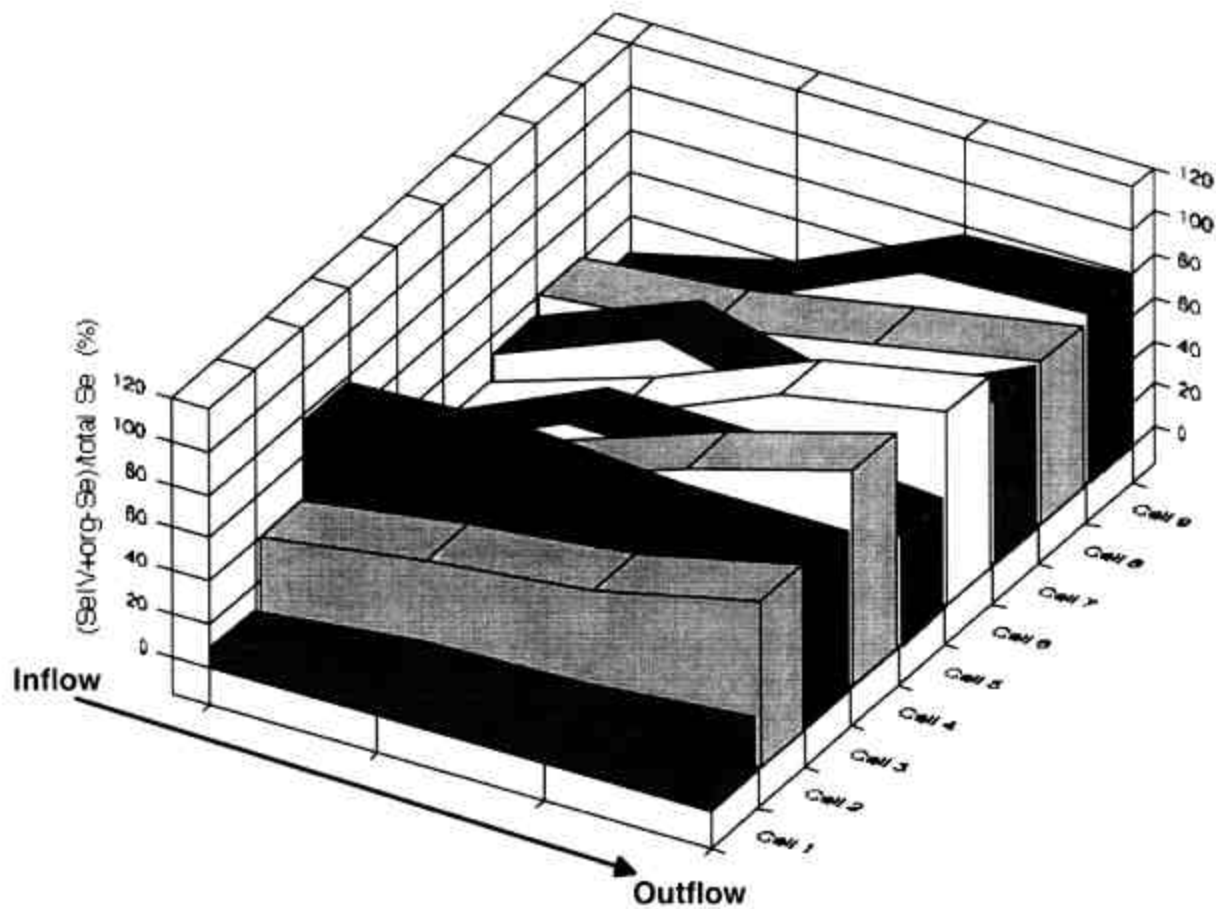


Figure 12. Distribution of reduced Se species $[(\text{Se IV}) + \text{org-Se}]$ in standing water in September 2000

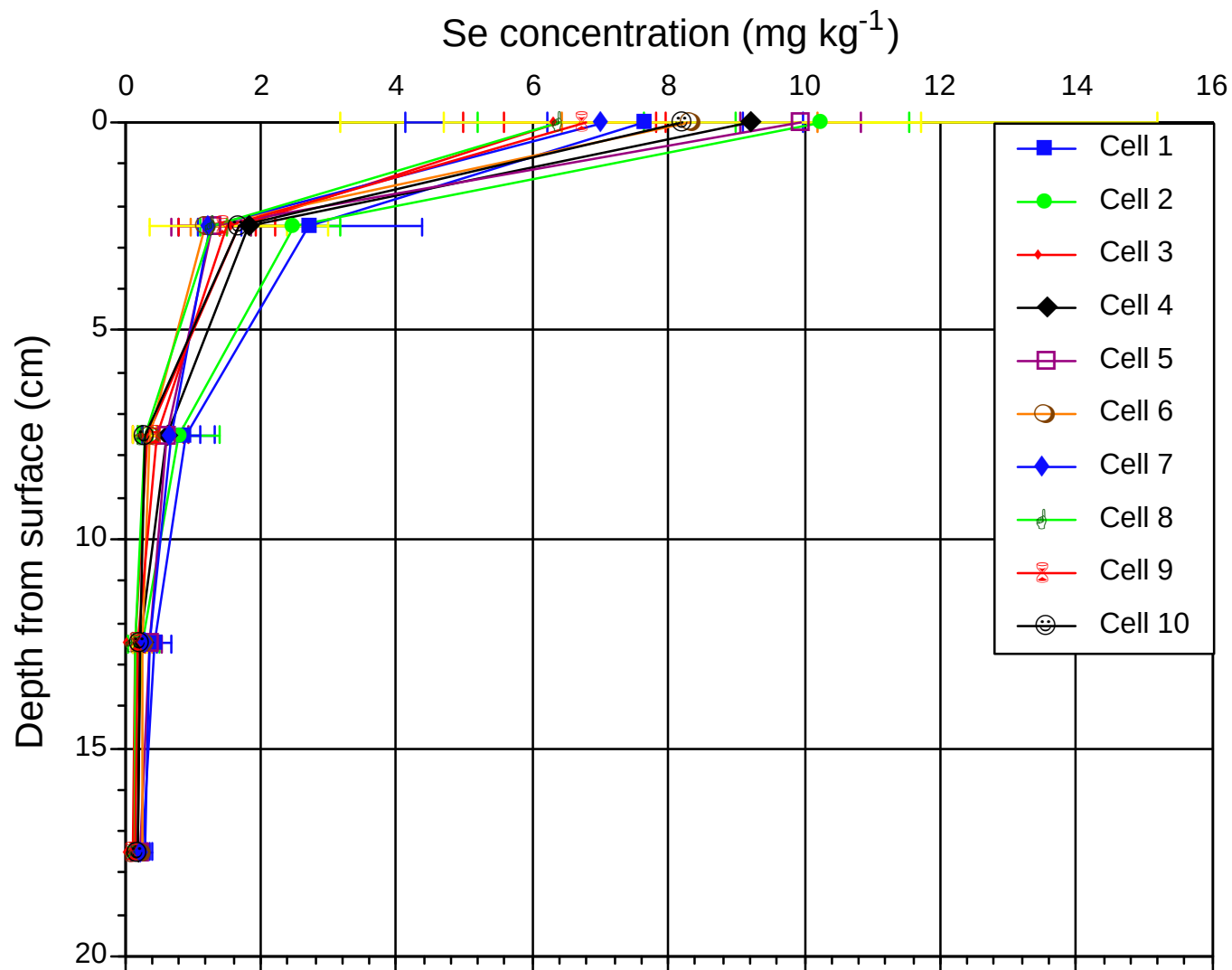


Figure 13. Se concentration in sediments in 2000. Values at depth 0 cm represents Se concentration in the fine organic detritus layer

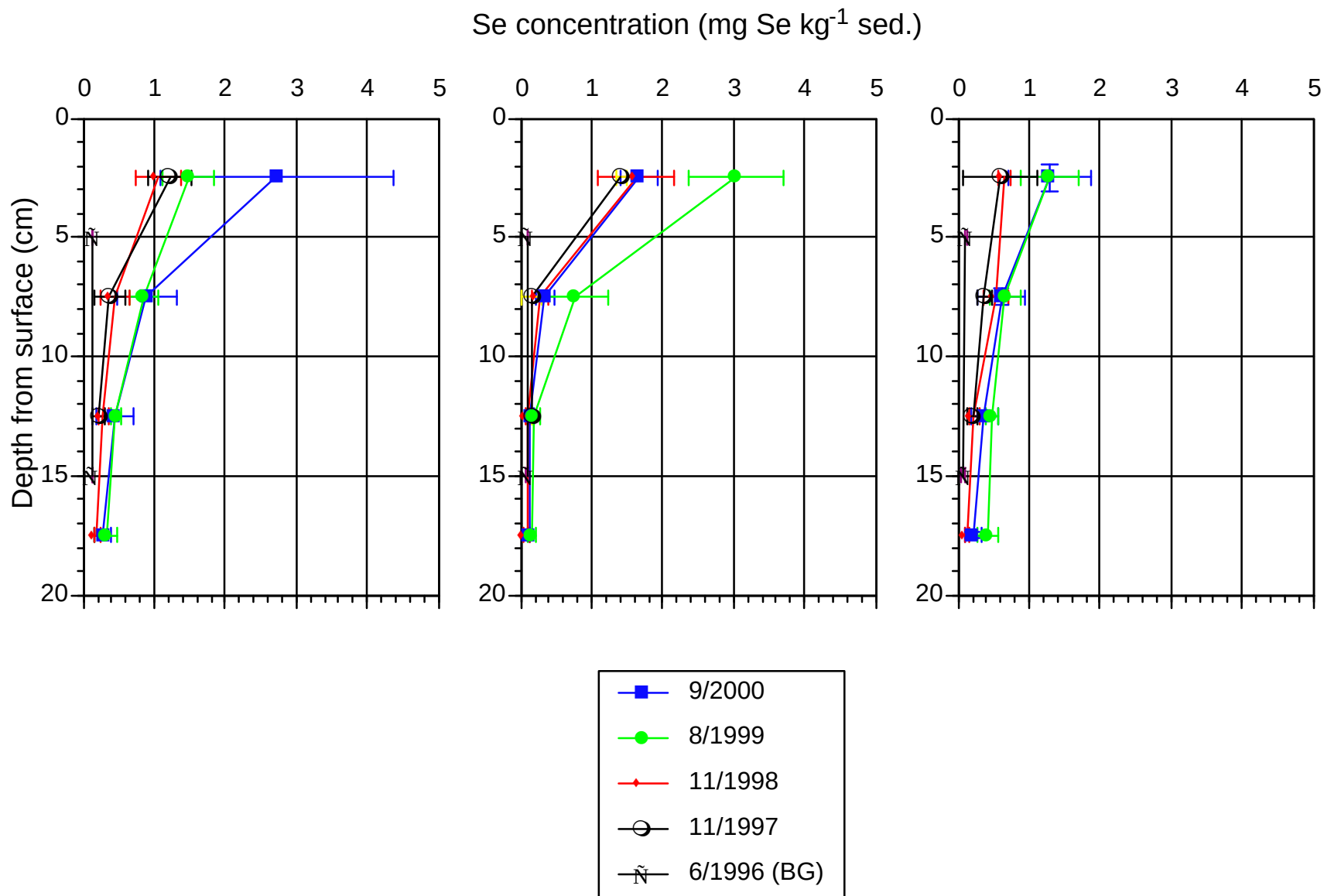


Figure 14. Sediment Se concentration in Cells 1, 3, and 5 from 1996 to 2000

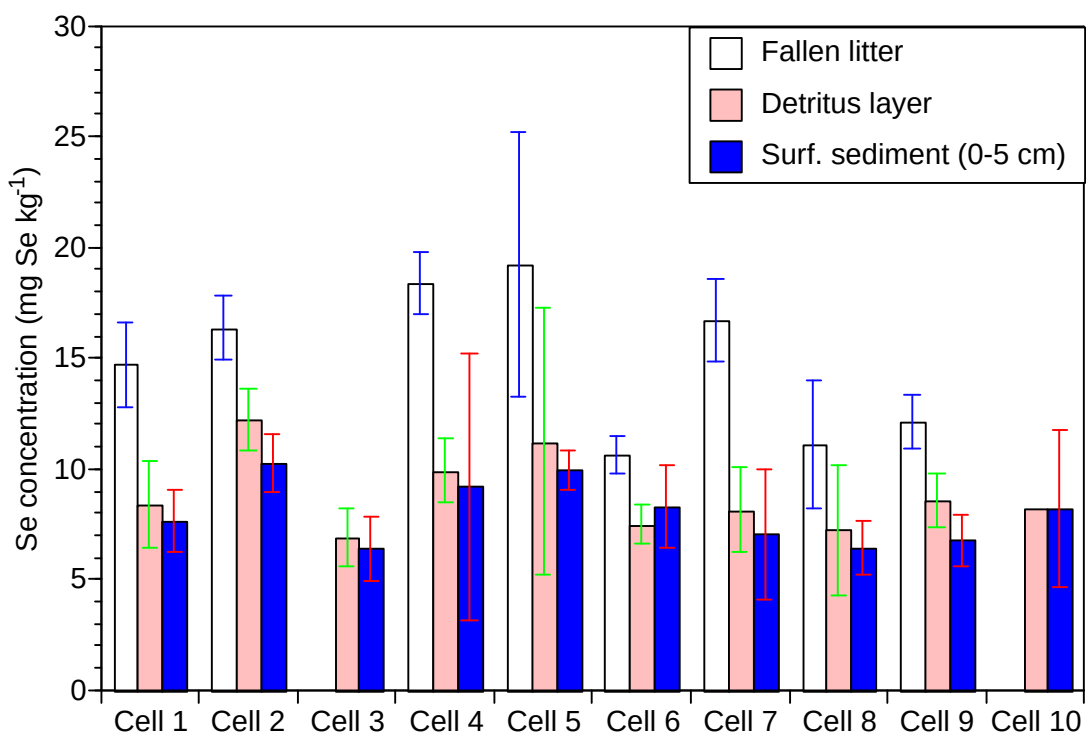


Figure 15. Se concentration in fallen litter, organic detritus layer and surface sediment (0-5 cm) in September 2000

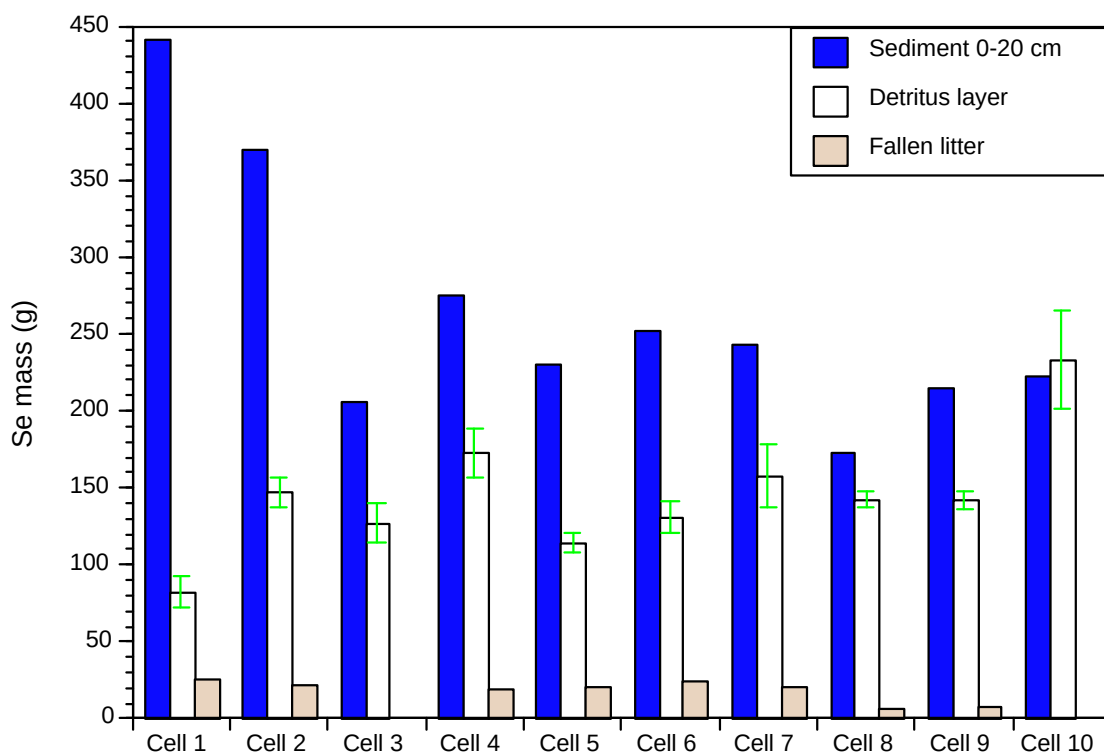


Figure 16. Mass of Se accumulated in the 0-20 cm sediment, detritus layer and fallen litter in September 2000

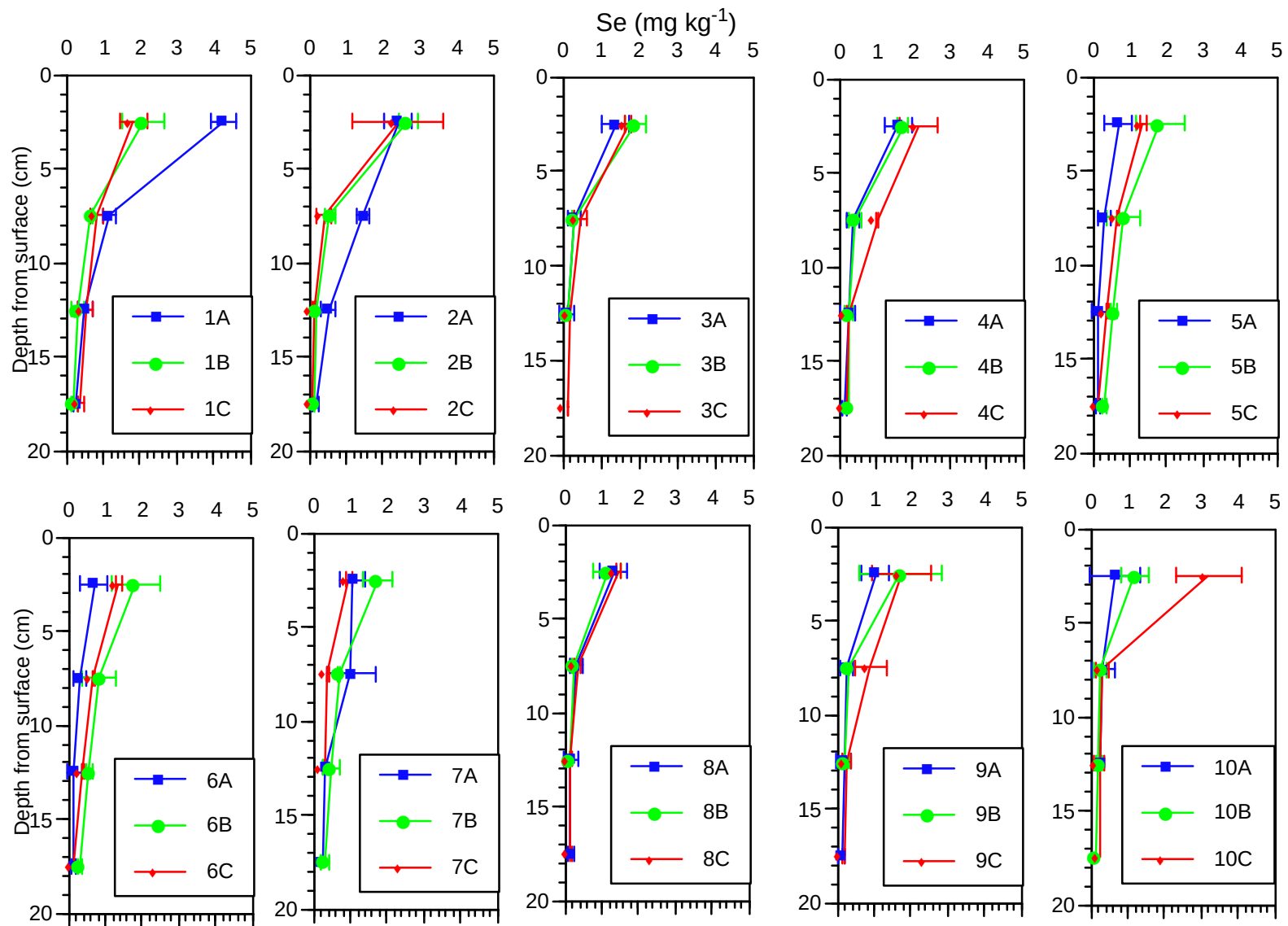


Figure 17. Se concentration in surface sediment in location A (1/4 distance from inlet) , B (center) and C (1/4 distance from outlet) in September 2000. Numerals present the Cell number

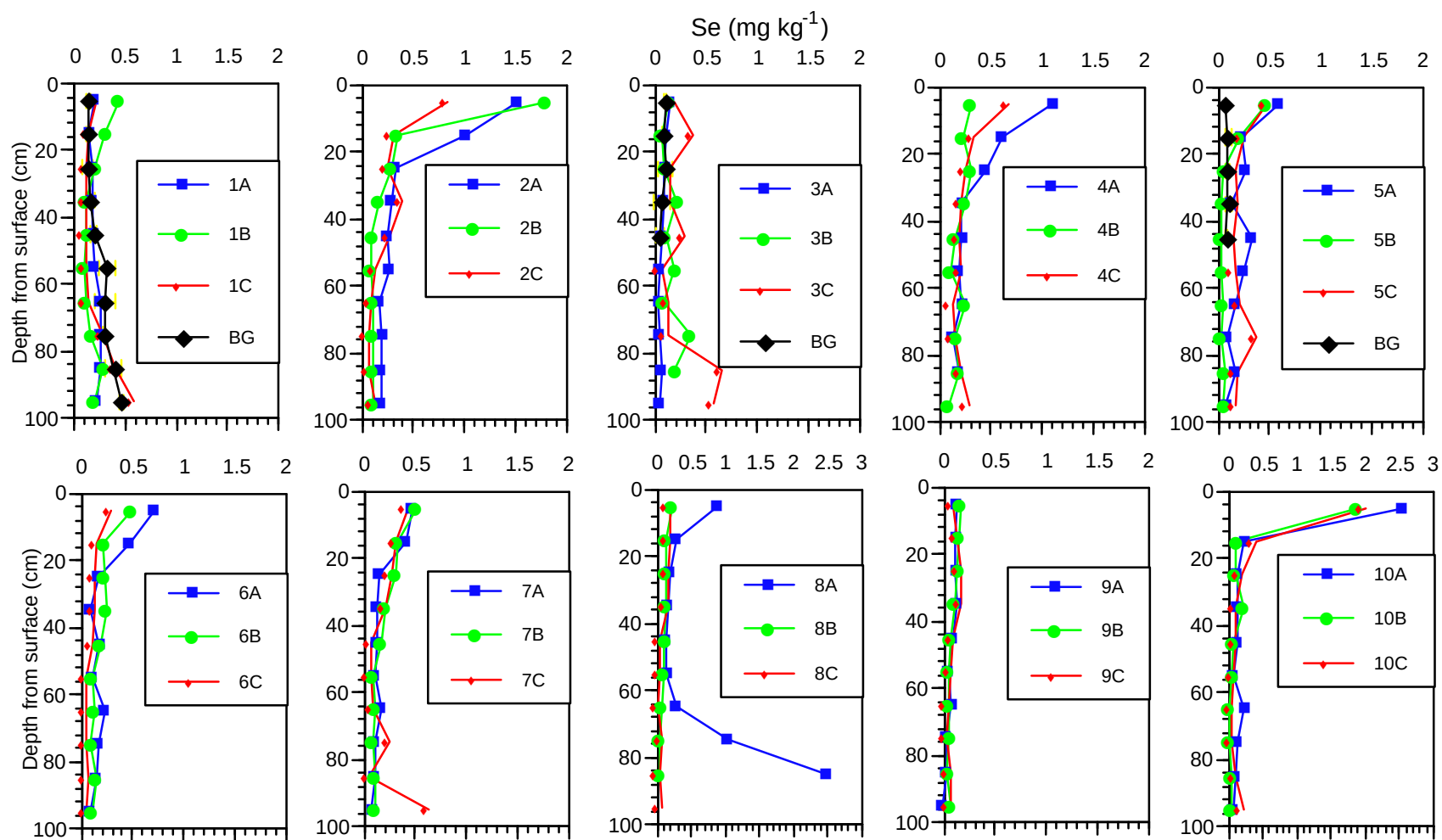


Figure 18. Se concentration sediment profiles in location A (1/4 distance from inlet) , B (center) and C (1/4 distance from outlet) in September 2000. Numerals present the Cell number. Background values are available for cells 1, 3, and 5 only



FATE OF SELENIUM IN FLOW-THROUGH CONSTRUCTED WETLANDS TREATING AGRICULTURAL TILE-DRAINAGE WATER

Project Investigator:

Norman Terry, Department of Plant and Microbial Biology, UC Berkeley

Report Prepared by:

Zhi-Qing Lin, Patricia Fox, Hussein Saad-Hussein, and Norman Terry

ABSTRACT

Earlier data showed that the Corcoran flow-through wetland cells removed 70% of the total Se mass from the agricultural drain-age water over a 3-year period. The present study confirms that the sediment was the major sink for Se removal by the wetland; about 60% of the total Se mass input was retained in the sediment. Sediment Se concentrations were highest in the top 3-cm layer, and decreased with increasing sediment depth. Greater amounts of Se were retained in sediments that were from vegetated regions of wetland cells compared to sediments that were from unvegetated areas. The accumulation of Se in plant materials, including living shoot and root, and standing and fallen litter, accounted for less than 2% of the total Se mass input, while Se volatilization removed 2-5% of the total Se mass from the wetland cells. Selenium speciation by X-ray absorption spectroscopy showed that a substantial proportion of selenate in the drainage water entering the wetland cells was converted to reduced chemical forms. Most of the Se in the surface bulk sediment was in elemental and organic forms, but in the sediment immediately surrounding the roots Se was mainly in organic forms. The Se in the tissues of roots, sediment invertebrates and fish was mainly in the organoselenium form. The vegetated wetland cells were generally more effective in Se removal than the unvegetated wetland cell; cattail and saltmarsh bulrush were among the most competitive plant species in the constructed wetland cells, compared to rabbit-foot grass and saltgrass species.

KEY WORDS: selenium (Se), fate, constructed wetland, sediment, phytoextraction, speciation, volatilization

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INTRODUCTION

The management of large volumes of selenium (Se)-contaminated agricultural tile-drainage water is one of the major environmental issues confronting California agriculture. Currently, the contaminated drainage water is disposed into evaporation ponds/

basins, where it is contained and evaporated. However, previous studies indicated that such disposal resulted in elevated concentrations of Se, As, B, and Mo in the evaporation ponds, which approached current water quality criteria for hazardous waste (Ong et al., 1997). The goal of this research program is to test the idea that flow-through constructed wetlands can effectively and safely remove Se from agricultural drainage water before it is disposed into evaporation ponds.

The idea for this research came from a study carried out at the Chevron Water Enhancement Wetland at Richmond, California; in this study, the flow-through constructed wetland was shown to remove 89% of the Se from selenite-contaminated oil refinery effluent (Hansen et al., 1998). The success of the Chevron wetland study led to a proposal made to the UC Salinity/Drainage Task Force to evaluate the use of constructed wetlands to remove Se from agricultural drainage water. As a result, a pilot flow-through wetland project was developed to determine the efficiency of various wetland plant systems (e.g., mono- and mixed-cultures of different wetland plant species) for Se remediation. Ten wetland cells were built in May 1996 at Corcoran, California, and nine wetland cells were vegetated; the unvegetated cell was used as control. The monthly-monitoring program began in May 1997, and continued through December 1999. Measurements were made of Se concentrations in the influent and effluent surface waters, sediment pore water, sediments and plant tissues, plant population densities and biomass, Se volatilization, the total carbon in sediment, and microbial biomass in sediment. In addition, measurements were made of some important physicochemical properties of surface waters, including water pH, electrical conductivity (EC), dissolved oxygen (DO), and temperature in the wetland cells.

Although our previous research at Corcoran has shown that constructed wetlands removed significant amounts of Se from the agricultural tile-drainage water (Terry, 1998, Terry, 1999, Lin and Terry, 2000), there are some uncertainties or questions that need to be elucidated and answered. For example, to

better evaluate the performance of the flow-through wetland treatment system, an accurate Se mass balance model needs to be developed for the wetland treatment system. It is important that we obtain an accurate determination of the accumulation of Se in the “bulk” sediments and deposition of Se in localized regions of the sediments, such as rhizosphere region or the surface of sediment in association with fallen litter or organic detritus layers, in which Se might be accumulated in amounts sufficient to provide a significant component of the mass balance for Se removal. In order to answer these questions, and in collaboration with scientists from University of California at Davis (Tanji Lab), a study was carried out in the September 2000 to achieve a complete assessment of the partitioning of Se in the wetland. In addition, Se was speciated using high energy X-ray absorption spectroscopy to provide insights into the mechanism of Se immobilization and deposition in the sediments, as well as the potential ecotoxic risks to wildlife associated with Se accumulation in the wetland ecosystem. Thus, the objectives of this study were 1) to develop a mass balance for Se in selected wetland cells, with special emphasis on the compartmentalization of Se in the sediments and accumulation in plant materials of standing and fallen litter and detritus organic layer; 2) to explore the mechanism of Se retention in sediments of vegetated wetland cells; and 3) to identify major chemical forms of Se in the wet-land ecosystem, including sediment, plant materials, and invertebrates.

MATERIALS AND METHODS

CONSTRUCTED WETLAND

Ten unlined wetland cells were constructed in 1996, each measuring 15 m wide and 77 m long (approximately 0.29 acres per wetland cell). Eight cells were constructed to have a constant water depth of 30 cm, and the remaining wetland cells (Cells 8 and 9) had variable water depths of 30 to 60 cm. The wetland inlets consisted of a 5-cm PVC irrigation pipe with a PVC butterfly control valve and a 5-cm impeller flow meter installed inside the inlet pipe to regulate and monitor inlet water-flow. Each wetland outlet

consisted of a 25-cm galvanized metal pipe with a measured v-notch weir. Tile-drainage water was pumped from a central collection basin under relatively constant pressure via a submerged pump to all wetland cell inlets. Initially the residence time was low and variable (i.e., ranging from 1.5 to 10.0 days) due to low water depths in some cells and fast water flow rates in other cells. The residence time was adjusted for all wetland cells on August 1997, by increasing water depth, to achieve a residence time of 5 to 7 days on all cells except for Cells 8 and 9, which had an average residence time of 21 days, and for Cell 10 with a residence time of 14 days.

The 10-wetland cells were tilled and fertilized with 16-16-16 type granular fertilizer at a rate of approximately 100 lb. per acre. The wetland cells were then flooded, and transplants of cattail (*Typha latifolia* L.) (Cells 7 and 10), baltic rush (*Juncus balticus* Willd.) (Cell 2), cordgrass (*Spartina alternifolia* Loisel.) (Cell 4) and saltgrass (*Distichlis spicata* (L.) Greene) (Cell 6) were planted by hand into the cells at a plant spacing of 50 to 100 cm depending on the plant type. Saltmarsh bulrush (*Scirpus robustus* Pursh) (Cell 1) and rabbitfoot grass (*Polypogon monspeliensis* (L.) Desf.) (Cell 5) were planted from seeds. Monotypic stands of individual wetland plant species were planted in all vegetated wetland cells, except in Cells 8 and 9. Cell 8 had a combination of saltmarsh bulrush and widgeon grass (*Ruppia maritima* L.), and Cell 9 contained cattail, widgeon grass, and tule (*Scirpus lacustris* L.). Cell 3 remained unplanted as a control. The soil was classified as a Westcamp loam (a fine-silty, mixed, calcareous, thermic Typic Endoaquepts).

SAMPLING STRATEGY

The sampling and *in situ* measurements began in May 1997, about 10 months after the wetland was initially vegetated in June 1996 and continued until December 1999. Further details of the sampling strategy are given previously (Terry, 1998). Briefly, monthly measurements included in-field physical-chemical measurements of water pH, EC, DO, and temperature. Sampling also included whole plant (root and shoot) grab samples, sediment profiles (0-15 cm depth), inlet and

outlet water samples, and sediment pore water (0-10 cm) samples. All samples were transported and stored at 4 °C until analyzed for Se. The aboveground plant biomass was determined seasonally over three randomly selected 0.25 m² areas in each cell by the harvesting method (Cheremisinoff et al., 1980), while the under-ground root biomass was estimated by the ratio of shoot biomass to root biomass of the plant species.

In September 2000, 4 years after the wetland's inception, a special effort was made to perform a complete characterization of the wetland. The results from this sampling will allow a complete mass partitioning of the Se in the wetland system to be determined. For the biomass measurements, five sampling areas (0.5 m²) were randomly assigned for each vegetated wetland cell. All aboveground shoots, standing litter and fallen litter were collected in each of these sampling areas, and the fresh weight and dry weight were recorded. The plant coverage was determined for each plant species (planted or invaded) in each wetland cell using a 2 x 2-m grid-map. At least 5 samples of each sample type (e.g. living shoots and roots, and standing and fallen litter) from each cell were brought back to the laboratory for total Se analysis. Additionally, five sediment cores were collected from Cells 2 (baltic rush cell), 3 (unvegetated control cell), and 5 (rabbitfoot grass and invasive cattail cell), respectively. The cores included plant material and any overlying layers of detritus and organic material. Rhizosphere sediment attached to plant roots was also collected. These sediment core samples were collected in order to determine the accumulation of Se in localized areas of the wetland sediments, such as rhizosphere sediments and detritus layers on the surface of sediments.

COLLECTION OF SEDIMENT AND PLANT SAMPLES AND TOTAL SE ANALYSIS

Sediments (0-25 cm depth) were collected with a sediment core sampler equipped with a replaceable butyrate liner (30 cm L x 5 cm D). The thin-walled probe was inserted into the sediment beyond the desired depth. The probe was then removed from the sediment, and the liner was removed from the probe and capped. The cores were then brought back

to the lab with dry ice, and frozen at -20 °C. After defrosting, the cores were sectioned into the following layers: detrital or sediment organic layer (0-3 cm), mixed/transition layer (3-5 cm) for the top 5-cm, and then every 5-cm up to a depth of 25 cm. Sub-samples (1-g) of each layer were kept frozen at -80°C for XAS analysis (discussed below). Samples were air-dried and ground to fine powder. Sediment samples were acid-digested with HNO₃/H₂O₂/HCl according to EPA method 3050B (USEPA, 1996), followed by Se measurements with AAS-hydride generation in adherence to Varian's procedure for the VGA-77 vapor generator.

Whole plants were removed using a spade. During the growing season, plant samples consisted of aboveground green shoot (leaf and stem) and root tissues. During the fall and winter months, dead plant litter (standing and fallen litter) was collected along with root samples. Dry weights of shoots and roots were recorded for seasonal biomass measurements. After roots were rinsed on-site to remove sediments, all plant samples were placed in Ziploc bags. Wetland plant samples were dried at 60°C, and ground in a Wiley Mill to pass a 0.43-mm mesh screen. Ground samples were acid-digested with HNO₃/H₂O₂/HCl according to EPA method 3050B (USEPA, 1996), followed by Se measurements with AAS-hydride generation following Varian's procedure for the VGA-77 vapor generator.

MEASUREMENT OF SELENIUM VOLATILIZATION

To measure Se volatilization in the wetland cells, Plexiglas chamber systems were set up in the wetland. The sampling chamber enclosed a ground surface area of 0.5 m² (0.71x 0.71-m), and a total internal volume of 0.38 m³ or 0.75 m³ (for cattail plants). A complete description of chamber design and calibration is given elsewhere by Lin et al. (1999). Volatile Se was collected by pulling air under suction from the Plexiglas chamber through the charcoal filters with a vacuum pump, which allowed an air outflow rate of 0.85 m³ h⁻¹ from the chamber. Sun canopies (EZ Up DOME) were used to shade chambers from direct sunlight in the field. The sun canopy shading helped reduce the temperature buildup inside the chamber

due to high air temperature in the San Joaquin Valley in the summer.

On a monthly basis, the Se volatilization measurement was conducted in each cell over 24 hours. The open-bottom chambers were inserted 3-5 cm into the sediment, thereby sealing off the sampling area from the surrounding wetland. At the end of the collection period, charcoal filters were collected from the chambers, placed into Ziploc bags, labeled, and stored in a cooler at 4 °C. To analyze volatile Se from the charcoal trap material, the Se-containing charcoal filters were weighed and cut into sub-samples. The sub-samples were then placed into labeled 500 ml Nalgene bottles. The charcoal filter sub-samples were immersed in 200 ml of extraction solution (160 ml 0.05 M NaOH + 40 ml 30% H₂O₂). After extraction for two days, the extraction solution was filtered with 1 mm filters. H₂O₂ was added to the aliquots, and then boiled off at 91°C for 30 min. Selenium in the sample was then reduced by adding equal volume of concentrated HCl and heating at 91°C for 30 min. Total Se was determined by AAS with hydride generation following the Varian's procedure. A detailed description on analysis of the volatile Se samples was reported previously by Lin et al. (1999).

TOTAL CARBON IN SEDIMENTS

Measurements of total carbon in sediments were primarily focused on the top 5-cm depth. Sub-samples of air-dried sediments were ground to a very fine powder, and 50 mg of sediment was weighed on a balance with 1-mg accuracy. The sediment samples were wrapped in a tin capsule and flash-combusted using a Carlo-Erba NC 2100 analyzer equipped with a mass spectrometer to determine total percent carbon content.

SELENIUM SPECIATION BY HIGH ENERGY X-RAY ABSORPTION SPECTROSCOPY (XAS)

The XAS analysis was conducted at beam line 4-3 at the Stanford Synchrotron Radiation Laboratory (SSRL). Sediment and plant samples were frozen with dry ice immediately after collection in wetlands. Plant shoots and roots were rinsed in deionized distilled water, frozen in liquid nitrogen, and then ground to a fine

texture and stored at -80°C. Samples were care-fully packed into Lucite sample holders with a 2-mm path length and sealed with Mylar tape.

Samples were placed in a sample chamber at a 45° angle to the X-ray beam and were maintained at 15K in a flowing liquid helium cryostat. The source electron energy was about 3.0 GeV with a current ranging from 70 to 100 mA. A Si (220) double crystal monochromator was used, with 50% detuning for harmonic rejection, and positioned with a 1-mm entrance slit that produced a beam of about 1 eV band width. A Canberra 13 element Germanium X-ray fluorescence detector was used to increase X-ray resolution. Fluorescent X-ray spectra were obtained by a series of replicate scans for samples and for appropriate standard reference materials (Pickering et al., 1995). The energy was calibrated with the spectrum of hexagonal Se(0). Data were collected and analyzed using the program suites XAS-Collect (George, 2000) and EXAFSPAK (<http://www-ssrl.slac.stanford.edu/exafspak.html>), respectively.

Quantitative analysis was carried out using an edge-fitting method (Pickering et al. 1995), in which the normalized edge spectrum of a sample containing unknown Se species was fit to a linear combination of the spectra of standard Se compounds by using a least-squares minimization procedure. The fractional contribution of a standard spectrum to the fit was equivalent to the fractional abundance of Se in that chemical species in a sample. In the present work, spectra were fit to 10 mM solutions of selenate, selenite, selenomethionine (SeMet), and selenocysteine (SeCys), chosen to be representative of potential Se species present (Pickering et al. 1999). It is important to note that the use of selenocysteine in a fit does not necessarily quantify this particular compound, but rather aliphatic diselenides in general. The precision of the determined fractional abundances of Se chemical species are three times the estimated standard deviations, calculated from the diagonal elements of the variance-covariance matrix, and are equivalent to the 95% confidence limits. The accuracy of the

values obtained depend upon the degree of similarity between the standard spectra chosen and their counterparts in the plant spectra, and are generally larger than the precisions.

MASS BALANCE OF SE

A conceptual model of Se mass balance for a flow-through constructed wetland is illustrated in Figure 1. Expressing the total Se mass partitioned in different components as a percentage of the total Se mass loaded into the wetland during a certain period of time ($DT = T_{\text{start}} - T_{\text{end}}$) will formulate a Se mass balance model for the flow-through constructed wetlands. In this study, the time period (DT) for the Se mass balance was defined from May 1997 (T_{start}) to August 1999 (T_{end}).

Due to a lack of measurements of invertebrate biomass and of the total Se concentration in invertebrates, the mass balance of Se in this study will not include the partitioning of Se in invertebrates. However, we hypothesized that even though the Se concentrations in invertebrates were high, the invertebrate biomass would account for only a relatively small percentage of the Se entering the wetland cell. The mass balance of Se presented in this report incorporates the dominant pathways of the Se in the wetland cells.

STATISTICAL ANALYSIS

This field study involved the collection of multiple samples from each experimental unit (i.e., wetland cell) sequentially every month during the 3-year study period. Since it was difficult to establish true replicates for each treatment due to the large scale of the wetland system and the great cost of establishing and constructing the wetlands, this field study was conducted with temporal pseudoreplication (Hurlbert, 1984). Repeated sampling on different sampling dates was taken to represent replicated treatments, and statistical significance tests were applied since different sampling and measurements were considered to be independent. Statistical analysis was performed using the Statistical Analysis System (SAS).

RESULTS

PLANT COMPOSITION AND BIOMASS IN THE WETLAND CELLS

The composition of plant species in some wetland cells has changed significantly four years after construction of the wetland. The new plant species composition in each wetland cell is shown in Table 1. By the sampling time in September 2000, Cells 5 and 6 were dominated by cattail, while the rabbitfoot

	Saltmarsh		Baltic	Rabbitfoot			Hardstem		Open	
Cell	Bulrush	rush	Cordgrass	Grass	Saltgrass	Cattail	Tule	rush	Cedar	Water
1	97.2									2.8
2	2.4	83.2				0.2		8.5		5.7
3										100
4			78.9		6.4	1.2				13.5
5	14.4			6.8		78.4				0.5
6	7.3			1.2	6.8	80.3			0.2	4.3
7						99.5				0.5
8	15.6									84.4
9						11.4	10.9			77.7
10						2.4				97.6

Table 1. The plant coverage of each species in wetland cells observed in Fall 2000. The surface area of each wetland cell was 1155 m². Values in bold present the species planted originally (or open water area, Cell 3) for each wetland cell. Cell 3 was unvegetated as a control, and the total originally assigned vegetated-area for Cell 8 and Cell 9 was 40% and 60%, respectively. Widgeon grass grows in the open water areas of Cells 8 and 9 during the summer months.

grass and salt-grass (originally planted) each covered about 6% of Cell 5 and 6, respectively. We conclude that saltmarsh bulrush and cattail species were the most competitive species in the wetland system, and that other species would eventually disappear after a long-term wetland operation.

The biomass (g DW m^{-2}) of each species in the wetland is shown in Fig. 2. Saltmarsh bulrush had the highest biomass production ($\text{g m}^{-2} \text{ y}^{-1}$) of living shoots in year 2000. Cattail plants had the greatest biomass of standing litter. The biomass of plant fallen litter was less than 300 g m^{-2} , and only accounted for small proportion of the total plant materials in Fall 2000. Overall, saltmarsh bulrush and tule produced the greatest amount of plant materials (living shoot and root tissues and dead standing and fallen litter) ($\sim 4 \text{ kg m}^{-2}$), followed by cattail and saltgrass ($\sim 3 \text{ kg m}^{-2}$); then, in descending order, baltic rush, rabbit foot grass, and cord-grass. A relatively low biomass of cattail or cordgrass was associated with the lower density of each species in the wetland cell at the sampling time.

CHANGES IN SEDIMENT SE CONCENTRATION WITH DEPTH, TIME AND IN VEGETATED VERSUS UNVEGETATED REGIONS OF THE WETLAND CELL

The concentration of Se in the sediment decreased significantly with increasing sediment depth (Fig. 3). In the top 3-cm layer of sediment, Se concentration was ~ 3 to 4 times higher than the concentration in the second layer (3-5 cm). Sediment Se concentrations below 10-cm depth remained near the background level ($< 0.5 \text{ mg kg}^{-1}$). Thus, Se was retained mainly in the surface layer of sediment. The results showed a similar distribution pattern of Se in the sediment profile in both the vegetated wetland cell (Cell 2) and the unvegetated wetland cell (Cell 3).

The Se concentration in the sediment increased over time. The levels of Se in the sediment of the wetland cells have been monitored since the wetland was first established in May 1996. Our results show that a significant increase in Se accumulation in the sediment over time was observed in the surface layers (0-5 cm) of the wetland cells (Fig 4, illustrated by Cells 2, 3, and 5). After four

years of operation of the wetland treatment system, the Se concentration in the top sediment layer increased 2- to 19-fold over the initial back-ground levels (i.e., $\sim 0.5 \text{ mg kg}^{-1}$). Some accumulation of Se over time occurred at 5-10 cm depth, with no significant increase at 10-15 cm (data not shown).

In order to examine the potential effects of vegetation on the retention of Se in sediments, sediment samples were collected from the baltic rush areas versus nearby unvegetated areas in Cell 2. Our results show that the surface layers of sediment and the sediments from the root zone in vegetated areas appear to have higher levels of Se concentration compared to the sediments from unvegetated areas, although the differences are not statistically significant (Fig. 5). For the sediments that immediately surrounded the roots (i.e., rhizosphere region) in the vegetated wetland cells, the Se concentrations were 2 to 4 times greater than the bulk sediment at a similar depth (Fig. 6). Thus, sediments from the vegetated area, especially in the root zone, appeared to accumulate more Se than from the unvegetated area, showing that plants may substantially increase Se retention in sediment.

SELENIUM CONCENTRATIONS IN PLANT TISSUES

The concentrations of Se in different plant materials of each species are shown in Fig. 7. The Se concentration in living shoot or standing litter was generally $< 5 \text{ mg kg}^{-1}$, except in the standing litter of rabbitfoot grass in Cell 5. Selenium concentrations in roots varied from 1 to 8 mg kg^{-1} , and the highest Se concentration was observed in rabbitfoot grass roots. The fallen litter of each plant species in different wetland cells accumulated similar levels of Se ($\sim 10 \text{ mg kg}^{-1}$). The fallen litter acted as an important sink for Se adsorption or deposition in the wetland, regardless of vegetation type. In comparing these Se concentration values with ones reported in our previous reports (Terry, 1998, 1999; Lin and Terry, 2000), there was no significant increase of Se concentration in plant shoots or roots in the wetland cells with time from year to year, except in Cells 4 and 5 where Se concentrations in living plant shoot tissues were highest in Fall 2000. For example, the Se concentration in cordgrass roots

changed from $0.9 \pm 0.2 \text{ mg kg}^{-1}$ in 1997 to $2.9 \pm 0.3 \text{ mg kg}^{-1}$ in 2000.

PARTITIONING OF SE AMONG THE SEDIMENTS AND PLANT MATERIALS

The total Se mass distributed in the different vegetation components of the wetland cell, i.e., living shoot and root, and standing litter and fallen litter, was determined for each vegetated wetland cell in Fall 2000 (Fig. 8). Our results show that the living root tissue accumulated more Se than shoot tissue, and that salt-marsh bulrush roots contained the greatest amount of Se mass, followed by saltgrass and cattail. The Se accumulation in standing litter ranged from 0.6 g per cell for saltmarsh bulrush to 1.5 g per cell for cattail. Cattail and salt-marsh bulrush had the greatest amount of Se mass accumulated in fallen litter (2.7 g and 2.5 g Se per cell, respectively) at the sampling time. Overall, Cell 1 retained the highest amount of Se in living and non-living plant tissues, followed by Cell 4 and Cell 6 (Fig. 9). The low levels of Se mass accumulated in plant materials in Cells 8, 9, and 10 were due to small plant coverage areas in Cells 8 and 9 and plant dieback in Cell 10 (i.e., almost no plant coverage at the sampling time, see Table 1).

mass was retained in the top 15-cm layer of sediment, which includes sediments at the root-zone depth in vegetated wetland cells. Within the sediment profile top 3-cm sediments are the major pool of Se retained in the wetland cell.

MASS BALANCE OF SE IN WETLANDS

The operation of the 10 wetland cells had encountered various difficulties during the course of this study. A detailed description regarding the problems (e.g., muskrat burrows or malfunctioning of the sand filtration assembly) and the correcting measures was given by Tanji (1999). Based on their water balance monitoring results in each wetland cell, our mass balance model was developed only for two wetland cells, Cell 2 (baltic rush) and 3 (unvegetated), during the study period from May 1997 to August 1999.

To develop a mass balance model of Se in the wetland cell, the total amounts of Se that flowed into each wetland cell over the period (i.e., May 1997 to August 1999) were calculated as $\sum V_i C_i$, where V_i = the volume of the inflow water during the sampling month, C_i = Se concentration in the inlet water, and i =

	Baltic Rush Cell	Unvegetated Cell
Total Se mass input (g per cell)	382.2	419.6
Discharged in outflow water (%)	33	39
Se remaining in standing water* (%)	0.4	0.4
Volatilized into the atmosphere (%)	2	4.7
Accumulated in top 10-cm sediment (%)	68	57
Accumulated in plant materials* (%)	1.2	N/A
<i>*These values refer to the amounts present in these compartments at the time of sampling, September 2000.</i>		

Table 2. The total Se mass input and the partitioning of the Se among different major pathways in the baltic rush vegetated cell (Cell 2) and the unvegetated wetland cell (Cell 3) during a two-year study period from May 1997 to August 1999. The partitioning of Se in each pathway is presented as a percentage of the total Se mass input during the study period. N/A: not applicable.

In general, the total Se mass accumulated in plant materials was relatively small. Our recent results show that plant materials, including living shoot and root, plant standing litter and fallen litter, accounted for less than 2% of the total Se mass that was retained in the sediment and plant system in the wetlands at the sampling time of September 2000 (Fig. 9). In comparison, more than 95% of the total Se

the number of sampling month (i from 1 to 28). Similarly, the total Se mass discharged from each wetland cell was calculated as the product of the Se concentration in the outflow water and the volume of the outflow water during the sampling month. The Se removal by volatilization was calculated as the product of the rate of Se volatilization determined for 24 hours and the number of days of the specific

study month during which the measurement was made. The amounts lost through vertical seepage are negligible according to our measurements of sediment Se concentrations (down to 25-cm deep) in Cells 2 and 3 (Fig. 3).

Most of the Se retained in the wetland was held by the surface layer of sediments; much less Se was retained by living and dead plant tissues, and by the standing water. The mass balance of Se (i.e., the recovery rate for the total mass of Se loaded into the wetland from May 1997 to August 1999) among the different compartments of the wetland cell ecosystem is shown in Table 2 for the vegetated wetland cell, Cell 2, and for the unvegetated wetland cell, Cell 3. Since there was no significant ($P < 0.05$) increase in Se concentration at depths lower than 10 cm, the total Se mass accumulated in sediment was determined only for the top 10-cm of sediment. The data show that the 0-10 cm sediment layer accounted for 57-68% of the total Se input, 33-39% by the outflow water, 2-4.7% by volatilization, 1.2% by accumulation into plant materials, and 0.4% by the standing water remaining in the cell at the end of August 1999. The total Se mass recovery rate was 105% for Cell 2 and 101% for Cell 3.

SPECIATION OF SE IN THE WETLAND ECOSYSTEM

Selenium can exist in variety of chemical forms. Selenium in the agricultural drainage water entering the wetland was predominantly selenate, which is soluble, bioavailable and potentially toxic. The reduced, elemental form

of Se on the other hand, is insoluble, not bioavailable, and essentially non-toxic as long as it is not reoxidized. Organic forms of Se such as free selenomethionine are soluble, bioavailable and are toxic. Therefore, it is very important to determine how the chemical forms of Se changes after selenate enters the wetland cells. This was determined by speciating Se in various compartments of the wetland cell by X-ray absorption spectrometry (XAS) analysis. In comparing the XAS spectra of our samples with the spectra of Se standards, the data show that selenate was reduced in the surface sediments to elemental Se and organic Se in a comparable proportion (Fig. 10, Table 3). In the sediment immediately surrounding roots, however, most of the Se retained was in organic forms (Table 3). There was no detectable difference in the spectra collected from surface sediments in different wetland cells.

CHANGES IN TOTAL CARBON IN SEDIMENT

The total carbon in the wetland sediments was highest in the top sediment and decreased with depth (Fig. 11). The top 3-cm layer of the sediment contained $4.8 \pm 1.7\%$, with $2.3 \pm 1.7\%$ in the 3-5 cm layer, and about $0.5 \pm 0.1\%$ in the deeper layers in Fall 2000. Overall, levels of the total carbon varied from 1.0 to 1.5% in the top 5-cm layer of sediments; the highest levels were observed in Cells 2 (baltic rush) and 3 (unvegetated) and the lowest levels in Cells 5 (rabbitfoot grass) and 6 (saltgrass). During a three-year study period,

	SeO ₄ ²⁻	SeO ₃ ²⁻	SeMet	Se ⁰	Fit-error
Roots	<5	14±1	75±27	18±15	1.61E-2
Shoot	31±2	16±5	23±12	27±7	0.31E-2
Surface sediment (Cell 3)	<5	15±3	36±7	38±4	0.11E-2
Surface sediment (Cell 5)	<5	11±5	47±9	45±6	0.20E-2
Sediment surrounding the roots	<5	23±6	62±15	11±9	0.48E-2
Fish	<5	<5	83±7	12±5	0.14E-2

Table 3. Percentage contributions to the fit for Se K-edge spectra for rabbitfoot grass shoot and root, surface (0-2-cm) sediment in Cells 3 (unvegetated) and 5 (rabbitfoot grass) and fish samples. Data are presented with 3 times the estimated standard deviation and indicates the 95% confidence limit of the parameter in the fit.

there was no significant seasonal variation in the total carbon level; however, a general increase of the total carbon in the top 5-cm sediments occurred during 2000 in the wetlands (Fig. 12, illustrated by Cells 2, 3, 6, and 7). The results show that, compared to other vegetated wetland cells, the unvegetated wetland cell (Cell 3) had a big temporal variation in the total carbon content in the top sediment layer.

DISCUSSION

Our previous studies show that flow-through constructed wetlands are capable of significantly reducing the concentration of Se in drainage water (Terry, 1998; Terry, 1999; Lin and Terry, 2000). The efficiency of the wetland cells in removing Se improved significantly with each successive year as the wetland matured (Lin and Terry, 2000). Plants play a major role in Se removal by wetlands; the unvegetated wetland cell, Cell 3, was the least efficient at removing Se from drainage water, compared to the vegetated cells. Selenium removal through volatilization was greatest in the rabbitfoot grass cell, where about 5.2% of the total Se inflow was volatilized to the atmosphere over the 2-year study period. The earlier studies showed that most of the Se removed by the wetland cells was retained in the surface sediments and this finding was confirmed in the more detailed study that we, and the Tanji group at UC Davis, conducted in Fall 2000. Our (the Terry Lab) research showed that most of the Se retained by the Corcoran wetland was accumulated in the top 15-cm layer of the sediment; this layer retained more than 95% of the total Se mass removed by the wetland cells. Plant materials, on the other hand, accumulated less than 2% of the Se flowing into each vegetated wetland cell. Similar results with respect to the extent of accumulation of Se in sediments and plant tissues have been obtained with other wetlands (Zhang and Moore, 1996; Hansen et al., 1998).

ROLE OF VEGETATION IN SE RETENTION

Among the plant species originally planted in the Corcoran wetland, only cattail (Cell 7), saltmarsh bulrush (Cell 1), baltic rush (Cell 2), and cordgrass (Cell 4) successfully maintained

high levels of coverage over the four years, i.e., almost 100, 97, 83, and 79%, for each of the four species, respectively. Rabbitfoot grass (Cell 5) and saltgrass (Cell 6) did not maintain their initial coverage; by the fourth year, these cells were heavily invaded by cattail and saltmarsh bulrush. Although it is clear that the vegetated cells removed more Se than the unvegetated Cell 3 (Terry, 1999; Lin and Terry, 2000), there was no evidence that planting any particular species or combination of species resulted in a greater removal of Se from drainage water. The accumulation of Se in plants accounted for only a minor proportion of the total Se mass removal by the wetland cells. Overall, less than 2% of the total Se mass input was located in living (shoot and root) and dead plant materials (standing and fallen litter). Because of their high Se concentrations and large biomass, living roots accounted for the highest proportion of the Se mass accumulated by plant materials (Fig. 8). Selenium concentrations were higher in the fallen litter and living roots ($\sim 10 \text{ mg kg}^{-1}$) compared to the Se concentrations in living shoots and standing litter ($< 5 \text{ mg kg}^{-1}$). Unlike the retention of Se in sediments, the accumulation of Se in most plant species did not increase significantly with time from year to year, except for rabbitfoot grass and cordgrass species in which higher concentrations of Se were found in Fall 2000.

Wetland plants play many roles in Se (or other trace element) removal by constructed wetlands. These include stabilizing the wetland ecosystem to provide an effective structural filter for the sedimentation of trace element-bearing particles and ensuring a circuitous path for the water flowing through the wetland. However, the present research with Se, as well as research with other trace elements, indicates that the role of plants is not due to the accumulation of trace elements in plant tissues (Hansen et al., 1998; Horne, 2000; Ye et al., 2001). Probably the major role of plant life, including algae, is to provide fixed carbon (from photosynthesis), through their death and decay, for the growth of microbes. Sediment microbes carry out important chemical conversions of trace elements to non-mobile forms, which in the case of Se, involves the reduction of selenate to elemental

and organic Se (Lin and Terry, 2000). In addition, plants provide fixed carbon to microbes in root zone sediments through the secretion of root exudates and from the death and decay of fine roots. The increased population of microbes in the root zone, which is supported by this fixed carbon, facilitates the buildup of Se (mainly organoselenium) in the sediments surrounding the roots.

MECHANISMS OF SE RETENTION IN THE SEDIMENTS

The last year's research confirmed three important facts about retention of Se in the sediments. Firstly, most of the Se was accumulated in the surface layers of the sediments, the accumulation of Se in the sediment profile decreasing with depth. Secondly, high concentrations of Se were found in the sediments surrounding the roots of plants (i.e., rhizosphere region) compared to the bulk sediment at the same depth of unvegetated regions of the wetland cell. Thirdly, Se concentration in the sediments increased with time as the wetland cells matured. As the wetland matured from 1997 to 2000, sediment Se concentrations increased from 0.5 (background level) to 5 mg kg⁻¹, with most of increase occurring during the last two years (Fig. 4). Of the Se accumulated in the sediment and plant systems, most was retained in the top 3-cm layer of the sediment (i.e., about 59% of the Se flowing into the wetland cells), followed by about 15% in the 3-5 cm and the 5-10 cm layer, respectively. This finding agrees with other observations, showing that the Se concentration decreased with increasing depth of sediment in this wetland (Gao et al., 2000), as well as in other wetlands (Zhang and Moore, 1996). Additionally, our study showed that root zone sediment accumulated from 2- to 5-fold more Se than the bulk sediment at the same depth in an unvegetated area (Fig. 5, Fig. 6).

Several mechanisms may account for the retention of Se from the water column to the surface sediments. These include various physical-chemical and biological processes (Bowie et al., 1996): 1) the precipitation of Se-associated particulates from water to the sediment, 2) the reduction of soluble selenate to less soluble chemical forms, and 3) the

decay of Se-laden biological materials and their transfer to the surface sediment. Selenium can be sorbed onto suspended particulate matter (both mineral and organic) that flows into the wetland. As the water filters through the wetland, the particles will settle on the sediment surface. This has been shown to be the major input of Se to sediments in two wetlands located in the San Francisco Bay in California (Zawislanski et al., 2001). Selenium solubility is lowest under reducing conditions and highest under oxidizing conditions (Masscheleyn et al., 1991). Selenate can be reduced to selenite (SeO₃²⁻) biologically or chemically under moderately reducing conditions (Tokunaga et al., 1996). Selenite is strongly adsorbed onto surfaces of common soil minerals and organic matter (Christensen et al., 1989). Our results showed that about 10 to 20% of the Se retained in the surface sediments was present as selenite. Selenate can also be reduced to organic Se (e.g. selenomethionine) or to elemental Se through both chemical and biological mechanisms (Frankenberger and Karlson, 1994; Myneni et al., 1997). Insoluble elemental Se will be precipitated into the surface sediments. It has been proposed that the dissimilatory reduction of selenate to elemental Se carried out by microbes is the major process for the selenate removal from waters and retention in the surface sediments (Zhang and Moore, 1997; Steinberg and Oremland, 1990; Weres et al., 1989; Tokunaga et al., 1998). Our results were consistent with those early observations, showing that nearly half of the total Se was present largely in the elemental form in the top 2-cm layer of sediment.

The decomposition of algae, bacteria, or plant materials containing high levels of Se on the surface of sediment will also contribute to the large proportion of Se mass retained in the surface sediment. Gustafsson and Johnsson (1992) reported that higher Se retention in the top sediment layer was associated with high organic matter in the sediment. A correlation between the Se distribution and the sediment organic matter content was also observed at Kesterson Reservoir by Tokunaga et al. (1991). The organic matter in the surface sediments results from the decay of many different types of organism including plants, algae, bacteria

and invertebrates. Our analysis of total carbon in the sediments showed that the total carbon content was highest in the top 3-cm sediment and increased with time as the wetland became mature (Figs. 11 and 12). A similar distribution pattern or temporal increase in total carbon content as the one in Se accumulation in sediments supports those previous findings that organic matter is a significant factor affecting Se accumulation and distribution in the wetland sediment.

POTENTIAL ECOTOXICITY OF SE SPECIES IN THE WETLAND ECOSYSTEM

The ecotoxicity of Se is determined by the bioaccumulation of Se and its biotransformation as it passes through the food chain (Skorupa and Ohlendorf, 1991). The protein-bound forms of Se are generally considered to be more toxic, particularly concerning chronic toxicity, than free selenoamino acids (USEPA, 1998; Fan et al., 1998). At prevailing environmental concentrations, dissolved selenate and selenite are only moderately toxic to aquatic organisms (Lemly, 1985). Organoselenium forms on the other hand, particularly selenomethionine (SeMet), are much more toxic within the food chain, despite their lower concentrations in water and soil (Hoffman and Heinz, 1988; USEPA, 1998). While plants and microorganisms may take up Se directly from the soil or water, most organisms at higher trophic levels, such as birds, obtain most of their Se from organisms containing protein-

bound Se. Fairly high concentrations of Se were found in the Corcoran wetland study. Selenium concentrations in living plant tissues were generally less than 5 mg kg⁻¹, except in rabbitfoot grass roots where the maximum concentration reached up to 25 mg kg⁻¹ (one sample only, Fall 2000). In addition, high levels of Se were found in plant fallen litter (10-17 mg kg⁻¹) and in the top 3-cm of sediment (8-15 mg kg⁻¹). Our XAS study showed that most of the accumulated Se in rabbitfoot grass roots (about 75%) and fallen litter was present as organic forms of Se, which, because of its greater ecotoxicity and bioavailability, present a potential threat to wildlife. However, this threat will be attenuated to some extent because organic Se forms, particularly selenomethionine, may be volatilized at greater rates by both plants and microbes (Terry et al., 2000), and volatilization is beneficial because it removes Se from the local ecosystem (Lin et al., 2000). Although volatilization was generally low (2-5% of the total Se mass input to the wetland cells), in some months it reached very high levels (e.g., 48% June 1998). In the surface sediments, some Se was present in organic forms (36-47%), but a significant proportion (45-48%) of the Se was present as insoluble elemental Se. Elemental Se represents a low ecotoxic risk since it is unavailable for biological uptake as long as the wetland remains wet and anaerobic.

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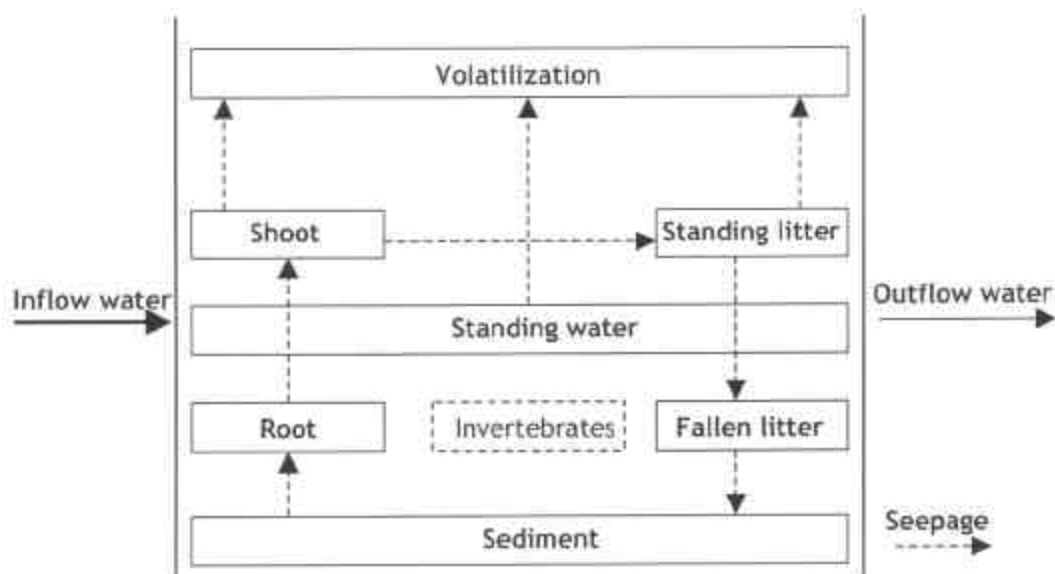


Figure 1. A conceptual model for the mass balance of Se in TLDD constructed wetlands

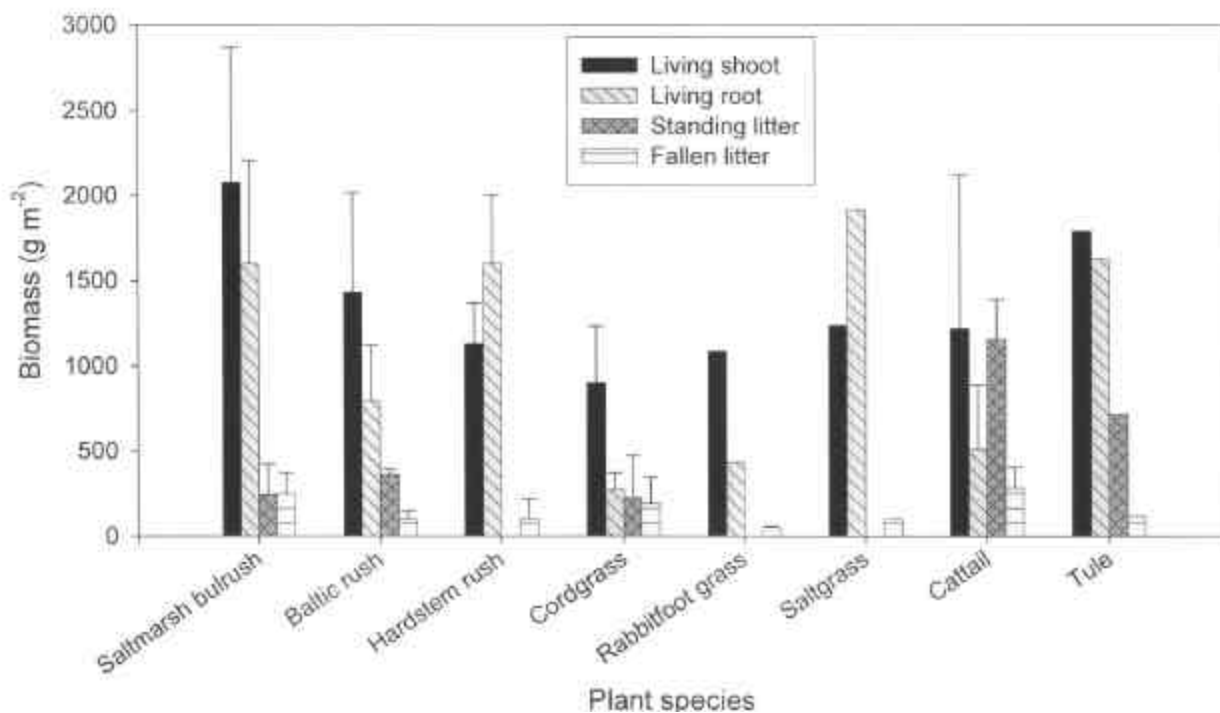


Figure 2. Biomass (DW) of plant shoot, root, standing litter, and fallen litter of each species in vegetated wetland cells in September 2000. Data shown are means and standard deviation (n=5), except for rabbitfoot grass, saltgrass, or tule, which, because of the limited coverage, yielded a single pooled sample.

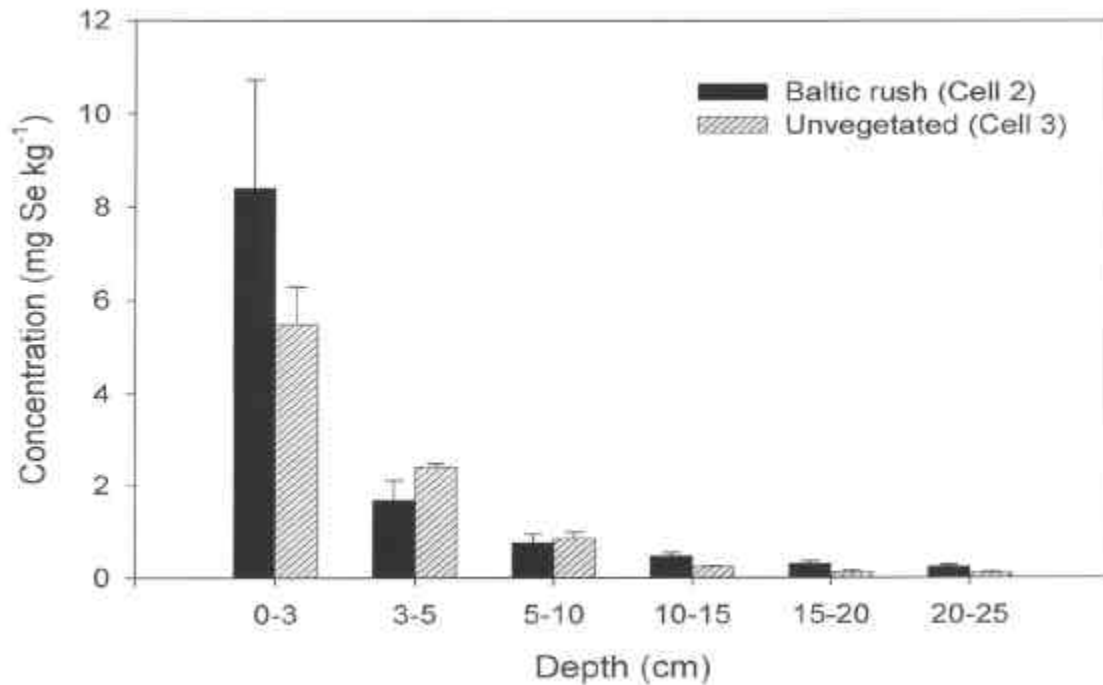


Figure 3. Selenium concentration at different depths of the sediment in two wetland cells in September 2000. Data shown are means and one standard error (n=6 for Cell 2, n=3 for Cell 3).

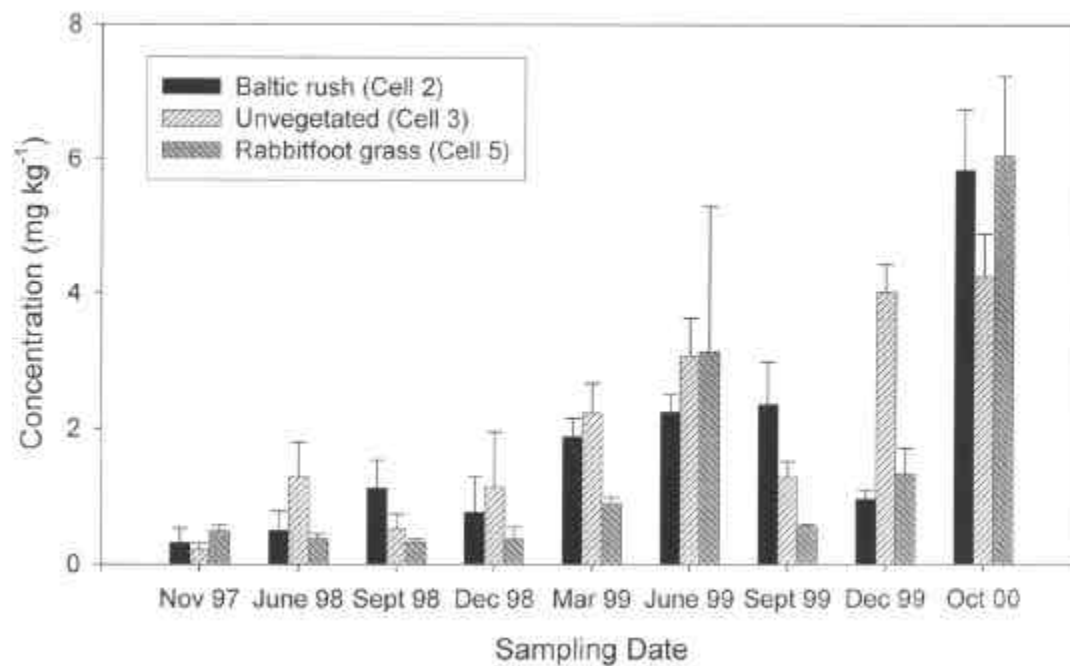


Figure 4. Increasing Se concentration in the top 5-cm depth of sediment in Cells 2 (Baltic rush), 3 (unvegetated), and 5 (rabbitfoot grass) from 1997 to 2000.

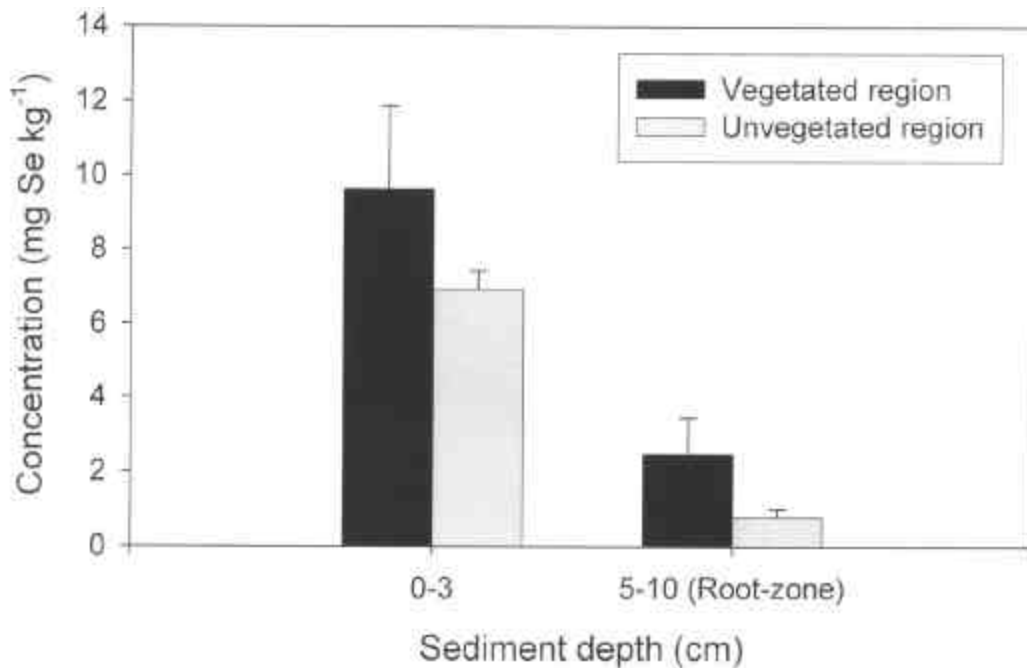


Figure 5. Selenium concentrations in the top 3-cm sediments and sediments at the root zone in vegetated or unvegetated areas of the wetland cells in Fall 2000. Data shown are means and standard deviation (n=5).

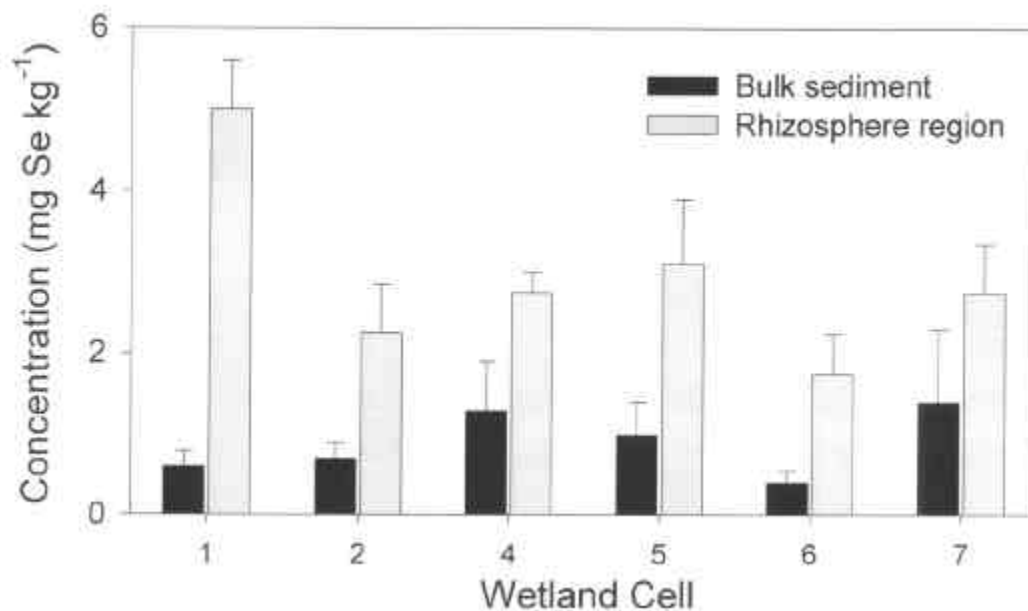


Figure 6. Selenium concentrations in the sediments immediately surrounding the roots and the bulk sediments (5-10 cm layer) from unvegetated areas in September 1998. Data shown are means and standard deviation.

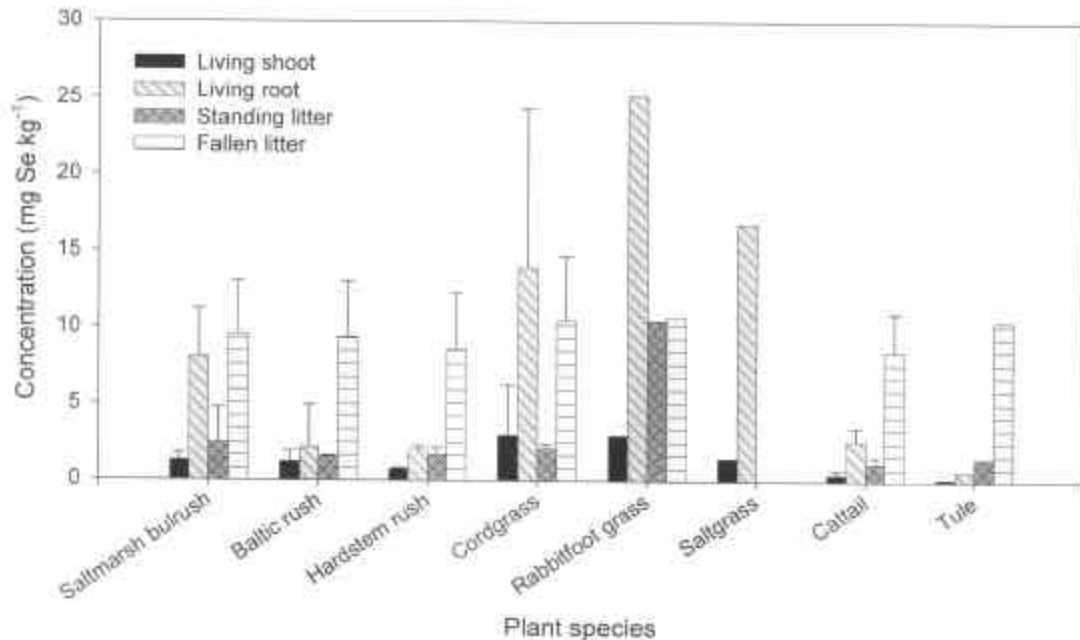


Figure 7. Selenium concentrations in different plant materials of each species in September 2000. Data shown are means and standard deviation (n=5), except a single pooled-sample for rabbitfoot grass, saltgrass, and tule species.

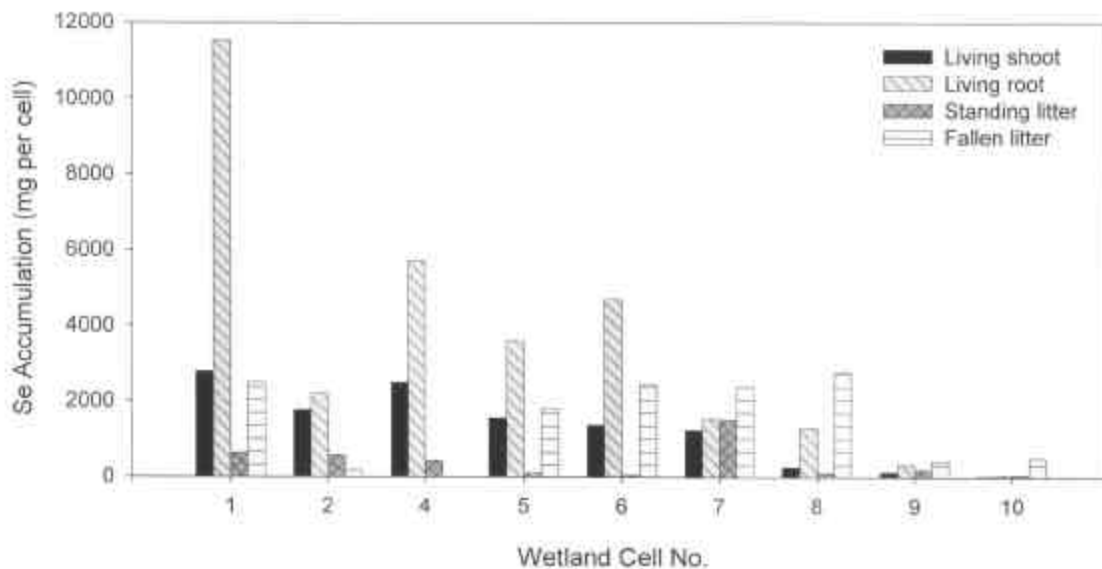


Figure 8. The total Se mass accumulated in plant materials, including living shoot and root, stand-ing litter, and fallen litter, in each vegetated wetland cell in September 2000. The total Se mass included the accumulation of Se in the original planted species and all other invasive species.

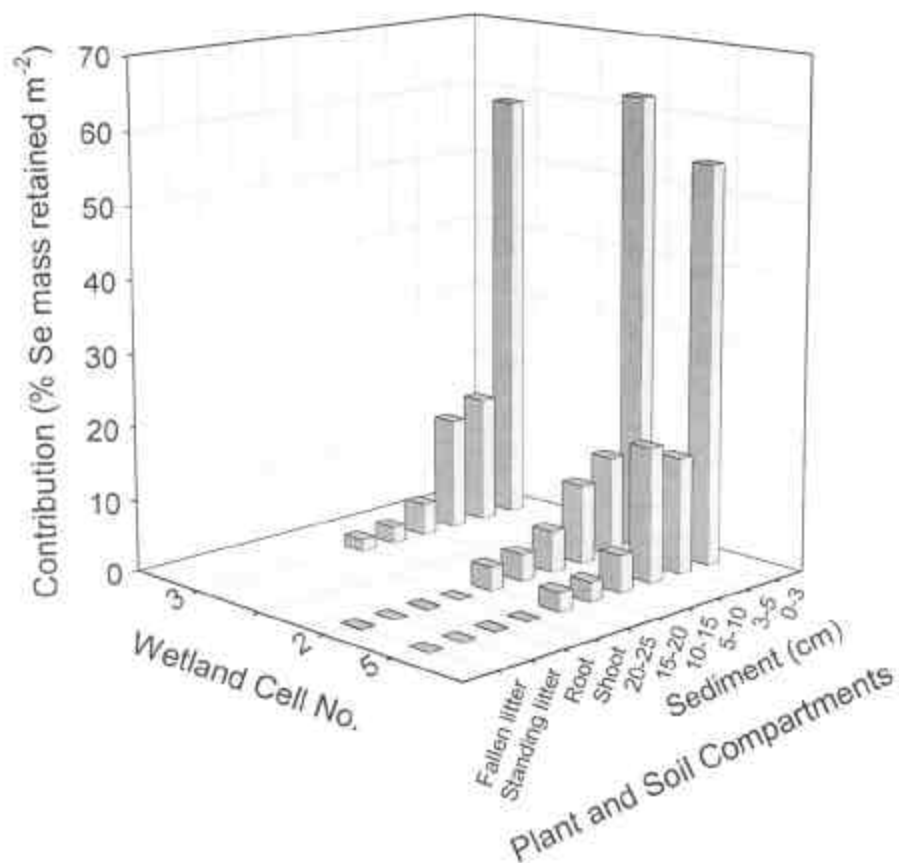


Figure 9. Partitioning of Se mass in the sediment and plant system in September 2000. Data shown are percent contribution of Se mass accumulated in each compartment of the wetland cell to the total Se mass retained in the sediment and plant system.

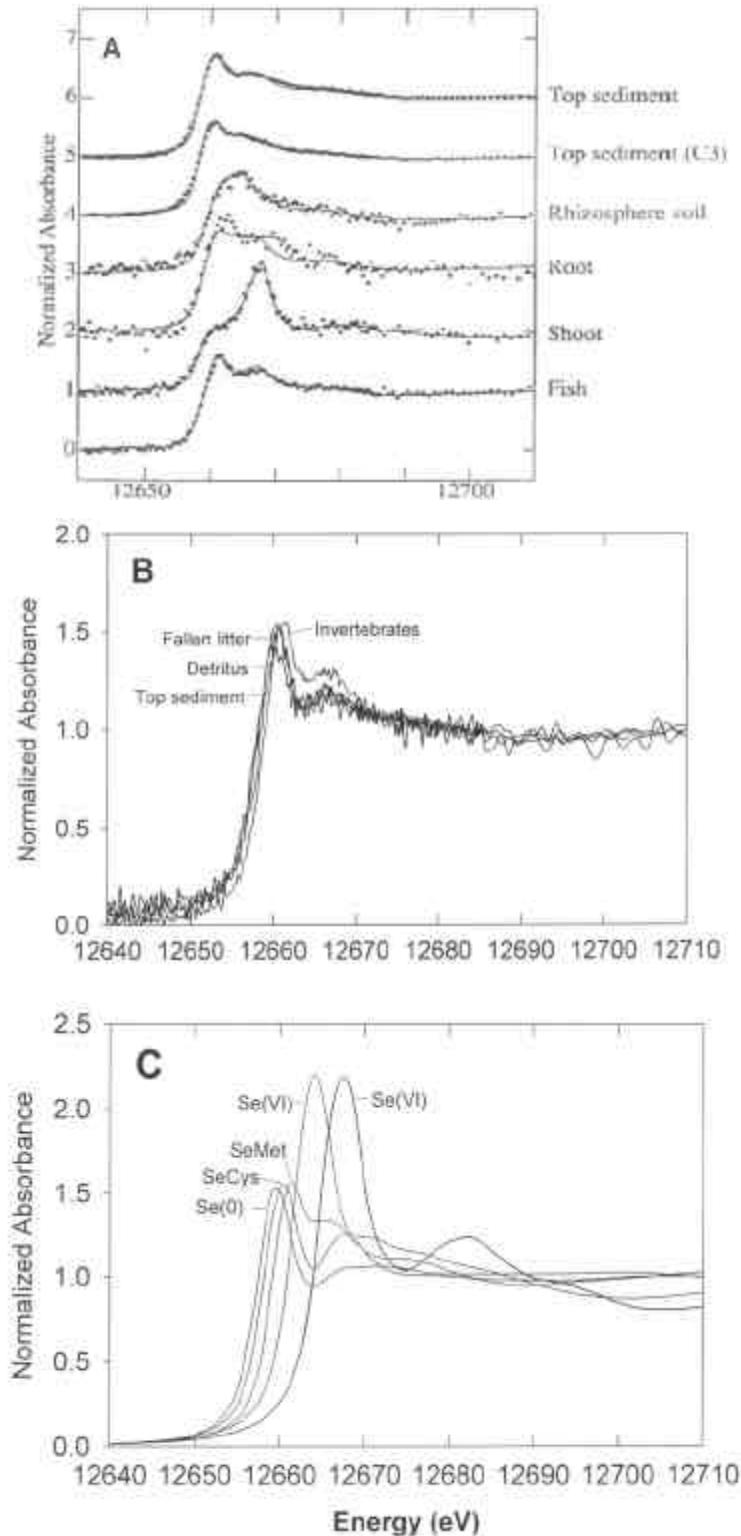


Figure 10. A) Selenium K-edge spectra of the samples collected in Cell 5 (rabbitfoot grass), and Cell 3 (unvegetated, C3). The circles are the data and solid lines are the fit. **B)** Se K-edge spectra of the samples taken from Cell 4 (cordgrass). **C)** Se K-edge spectra of five standards. Se(VI), selenate; Se(IV), selenite; Se(0), elemental Se; SeCys, selenocystine; SeMet, selenomethionine.

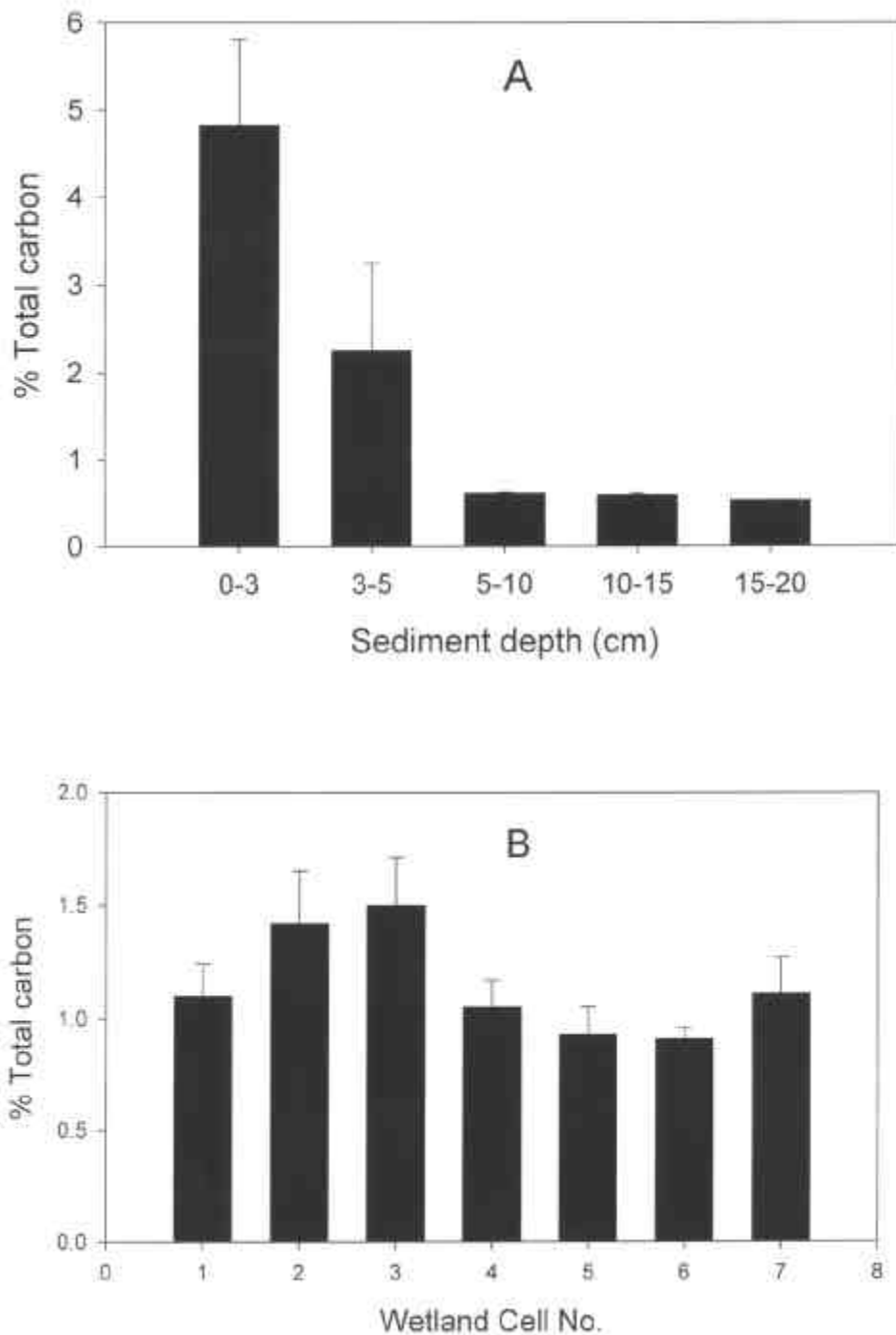


Figure 11. A) Change in total carbon in sediments of Cell 2 with depth. The data shown are the mean and standard deviation (n=5). Samples were collected in Fall 2000. B) Comparison of total organic carbon for different wetland cells. shown are means, averaged from June 1997 to November 1999, and one standard error (n=28).

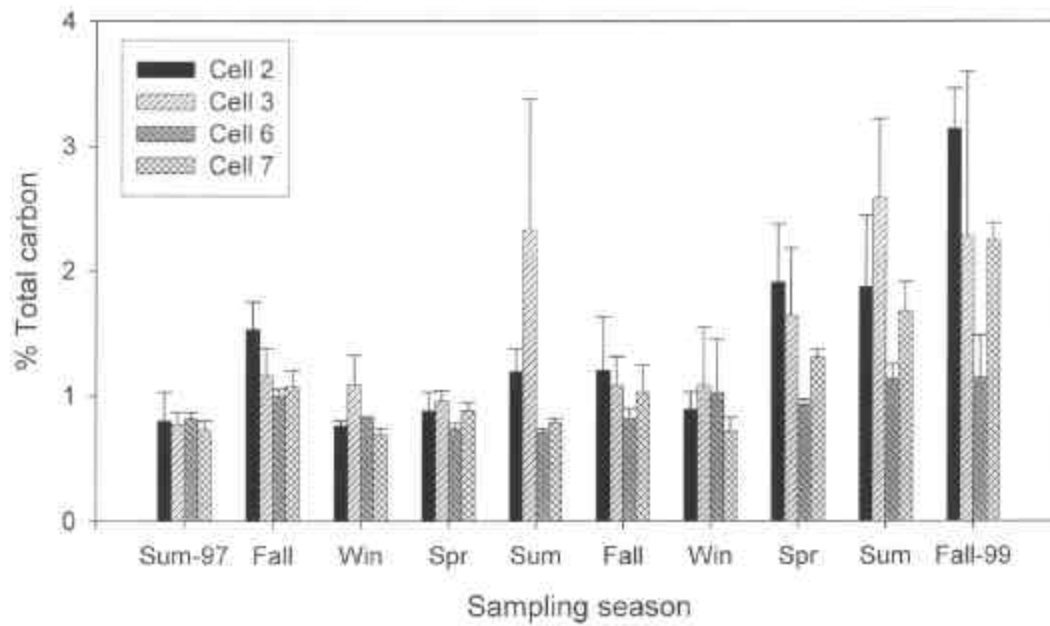


Figure 12. Changes in total carbon in surface sediments (0-5 cm) over time shown for various wetland cells. Data shown are means and one standard error (n=9).



Evaluation of *Atriplex* as a Potential New Crop for Integrated Production Systems in the San Joaquin Valley

Project Investigators:

J.T. Trumble, Dept. of Entomology, UC Riverside

M.C. Shannon and C.M. Grieve, USDA ARS Salinity Laboratory, UC Riverside

G.S. Bañuelos, USDA ARS Water Management Research Lab, Fresno, California

Research Staff:

D.B. Vickerman, RA

G. Kund, SRA

ABSTRACT

Remediation strategies proposed for the San Joaquin Valley (SJV) include removal of soil selenium (Se) through microbial and plant volatilization, and by plant harvest and removal. We conducted plant screening studies to determine the ability of 62 *Atriplex* lines to selectively accumulate Se in the presence of chloride and sulfate salts characteristic of SJV soils. Insect bioassays were conducted on 30 of these plant entries to determine if any of the best lines could be used in a phytoremediation program without creating an insect pest problem. Plants were tested using three irrigation treatments: 1) control, no Se; 2) NaCl and CaCl₂ salts plus 1 mg L⁻¹ sodium selenate; and 3) iso-osmotic to treatment 2 containing high amounts of sulfate plus 1 mg L⁻¹ sodium selenate. Insect bioassays included measures of survival, growth, and development. *Atriplex patula* *patula*, *A. spongiosa* 415862, *A. hortensis* *hortensis*, *A. hortensis* 379088 and *A. hortensis* 379092 were among the top biomass producers and Se accumulators, yet significantly reduced insect growth, developmental rates, and survival of the key pest, *Spodoptera exigua* (Hübner). A phytoremediation program based on these plant lines is attractive, as their use in such a program will reduce populations of *S. exigua* and minimize selenium biomagnification.

KEYWORDS: *Atriplex*, herbivory, integrated production program, phytoremediation, selenium, *Spodoptera exigua*, saline drainage

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INTRODUCTION

Selenium (Se) is an essential trace element important to humans and most other animals. Unfortunately, the boundaries between deficiency and toxicity are very narrow (Frankenberger and Benson 1994). Because this element naturally enters the food chain through water uptake, it can be acquired and readily biomagnified in the food chain. Intoxication of wildlife, domestic animals, and humans is surprisingly common (Heinz et al.

1990, Daniels 1996). The primary effect appears to be the replacement of sulfur with selenium in amino acids resulting in incorrect folding of the protein in the tertiary conformation and consequently malformed, nonfunctional proteins and enzymes (Lemly 1998). Although plants accumulate Se as inorganic salts and non-protein amino acids (Brown and Shrift 1982, Lauchli 1993), in agricultural drainage water sodium selenate is the most common soluble form (Cutter 1982, Terry and Zayed 1994).

Remediation strategies include removal of soil Se through microbial and plant volatilization, and by plant harvest and removal; both agricultural and uncultivated plant species have been proposed for use in reclamation programs (phytoremediation) for Se-contaminated soils (Khattak et al. 1991, Nyberg 1991, Bañuelos et al. 1996, Wu, Vanmantgem, and Guo 1996, Bañuelos et al. 1997, Losi and Frankenberger 1997). Use of plants in Se-remediation programs will result in the availability of Se to insect herbivores. Insect response to plants proposed for phytoremediation must be further investigated to ensure that such plants not only accumulate/volatilize adequate amounts of selenium, but do not serve a source of pest insects in surrounding cropping systems.

The influence of low levels of Se on insect herbivores can be dramatic. Increased developmental times and substantially increased mortality have been documented with *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) using artificial diet and vary with the form and concentration of selenium (Trumble, White, and Kund 1998). Although all forms of Se tested are toxic to these insects, choice tests demonstrated a preference for control diet over sodium selenate treatments and sodium selenite treatments, but no preference between selenocystine or selenomethionine and untreated controls (Vickerman and Trumble 1999). Additionally, recent studies with insects have shown that canola plants (*Brassica spp.*) such as Indian mustard grown on Se-treated soils became resistant as hosts to cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) whereas control plants were defoliated by the pest (Bañuelos et al. 1999).

They hypothesized that phytophagous insects may avoid feeding on Se-rich plants and thus reduce the likelihood of biotransfer from plant to insect.

Another aspect of this proposal is that Na_2SO_4 -dominant salts can reduce the uptake of selenium in some plant species. In a recent study in greenhouse sand cultures in Riverside, CA, wheat (cv. Yecora Rojo) grown with 1 mg l⁻¹ Se in the irrigation water accumulated over 400 mg kg⁻¹ Se in the leaf tissues under non-saline conditions. Under salt stress, leaf tissues accumulated as much selenium as the controls when NaCl was used as the predominant salt; whereas Se in wheat leaf tissues was less than 10 mg kg⁻¹ when Na_2SO_4 was the dominant salt (Grieve, Suarez, and Shannon 1999). Thus it is important, not only that plants accumulate and volatilize biologically active concentrations of Se, but also that they can do so in the presence of high sulfate salinities characteristic of SJV soils. The proposed screening studies will measure the ability of the accessions to selectively accumulate and volatilize Se in the presence of chloride and sulfate salts.

Atriplex spp. (Chenopodiaceae) have been proposed as potential crop plants for integrated production systems using drainage water in the San Joaquin Valley (SJV) due to their high growth potential with saline drainage water. These drainage waters are typically high in Na_2SO_4 -dominant salts and are Se-laden, prohibiting their disposal into drainage channels and solar evaporation ponds. Preliminary studies have shown that species of *Atriplex*, *A. patula* and *A. nummularia* can be grown in the SJV using drainage water as the only source for irrigation (Hoffman and Shannon 1986, Watson and O'Leary 1993). Additionally, recent studies conducted at the USDA Salinity Laboratory have shown that *A. hortensis*, which is used as a salad green, also has a high salt tolerance as compared to other vegetables (Shannon et al. 1998). Na_2SO_4 -dominant salts can reduce the uptake of Se in some plant species (Grieve, Suarez, and Shannon 1999), thus it is important that plants chosen for Se phytoremediation studies are able to accumulate and volatilize Se in the presence of high sulfate salinities characteristic of SJV soils.

There are at least 30 different species of *Atriplex* native to California and a wider range of genetic diversity world-wide. However, *Atriplex* grows through the winter months and is a known host for the generalist herbivore and major crop pest *S. exigua*, making it a potential overwintering host for the economically important insect. Possible biotransfer of Se from plant to insect may not only have consequences for the pest insect (Trumble, White, and Kund 1998), but also for the invertebrates and vertebrates that feed on them (Wu, Enberg, and Biggar 1994).

OBJECTIVES

- 1) Screen *Atriplex* entries to test their ability to produce high biomass under saline conditions.
- 2) Screen *Atriplex* entries to test their abilities to selectively accumulate Se against a high chloride and Na_2SO_4 background.
- 3) Bioassays with *S. exigua* to screen the most favorable candidate(s) for potential endogenous insecticidal properties.

METHODS

PLANTS.

Atriplex germplasm was collected through both commercial sources and from Western Regional Plant Introduction Station, USDA, ARS, National Plant Germplasm System, Pullman, WA. All plants were grown in sand cultures at the USDA Salinity Laboratory outdoor facilities in Riverside CA, using large cement containers which were irrigated daily from reservoirs containing treatment solutions. Sixty-two *Atriplex* entries were examined (M.C.S. et al. unpublished) and the most promising 30 lines were tested in insect bioassays. Assays were conducted in sets of ten plant lines and each set was labeled consecutively as test 1, test 2, and test 3. Treatment solutions included: 1) a no Se control, containing tap water plus nutrients; 2) Se + Cl, containing nutrients and added NaCl and CaCl_2 salts (EC ~ 30 dS/m) plus 1 mg L⁻¹ sodium selenate; and 3) Se + Cl + SO_4 , using simulated SJV drainage water (iso-osmotic to Se + Cl treatment) containing high amounts of sulfate plus 1 mg L⁻¹ sodium selenate. Plant

harvest was conducted in three phases labeled harvest 1, 2 and 3. Whole plants were dried and plant weight was recorded and used as a measure of biomass. Leaf samples collected were analyzed for Se by first using acid digestion with the $\text{HNO}_3/\text{H}_2\text{O}_2/\text{HCl}$ procedure described by Bañuelos and Akohoue (1994), and then identified with atomic absorption using an automatic vapor accessory.

An additional plant, a tã tã (Medicago sativa L.) was grown in sand culture in the U. C. Riverside Entomology Department greenhouses and was irrigated daily with half strength modified Hoagland's nutrient solution after Khattak et al. (1991). Since alfalfa is known to be an acceptable host plant for *S. exigua*, it was used as an absolute control for all tests to document insect survival and development on a plant known to be an acceptable host. This also indicated that the insect colony did not vary in terms of survival potential or development rate over the course of the experiments. However, the alfalfa data were excluded from statistical analyses because this plant was not grown in the outdoor sand culture tanks.

INSECTS.

Spodoptera exigua was chosen as a model insect for this study because it is a generalist feeder and a crop pest of economic importance throughout the U.S.A., including areas where selenium is a problem. In California, its host range includes native and introduced plants in the families Lilaceae, Fabaceae, Solanaceae, Malvaceae, Chenopodiaceae, Apiaceae, Asteraceae, and Amaranthaceae that can be found in both cultivated and uncultivated areas (Metcalf and Flint 1962, Peterson 1962, Pearson et al. 1989). In addition, this species has highly mobile larvae, which select feeding sites by moving between plants (Berdegué, Reitz, and Trumble 1998).

All experiments were initiated with first instar larvae (standardized within 12h of eclosion) obtained from a laboratory colony maintained at $28 \pm 2^\circ\text{C}$ and 16:8 (L:D) photoperiod. Insects were contained in individually labeled containers and fed plant tissue ad libitum. Ten replicates of one insect each were tested for each of the three

treatments per plant line (in addition to the alfalfa control). Insect mortality, and the developmental stage of newly dead and all surviving larvae were recorded every other day. On day 30, the developmental stage of insects which had not yet become adults was recorded and the experiment was terminated. In our experience, few insects will achieve the adult stage if development requires over 30 d. Developmental stages were numbered as follows: larval instars 1 to 5 were stages 1 - 5, stage 6 was the pre-pupal stage, stage 7 was the pupa, and stage 8 the adult moth. Developmental stage at death is important because this information can; 1) serve as an indicator of how much plant damage has occurred (consumption by this herbivore greatly increases after molting into stage four, so ideally plant lines would be chosen for their ability to suppress insect development at or before this stage) and, 2) determine if insect live long enough for beneficial parasitoids to potentially have the resources to complete development.

Percent survival to pupation and adult, mean days to insect pupation, and mean days to adult were calculated for insects in each plant line and all treatments. Survival to the adult stage, by comparison to the data on survival to the pupal stage, can be used to determine if additional mortality occurred during the pupal stage. Pupation is a rigorous process requiring increased production of enzymes and reorganization of proteins in insects. Since Se can replace sulfur in amino acids resulting in incorrect folding of the protein in the tertiary conformation and consequently malformed, nonfunctional proteins and enzymes (Daniels 1996) it is reasonable to expect that presence of Se may slow or impede the development process.

A measure of growth, the Relative Growth Index (RGI; Zhang, Chaduri, and Kubo 1993), was calculated for day eight, the day before any of the absolute control group developed into the pupal stage. This value measures herbivore growth rate by determining relative values of what developmental stage could have been achieved under excellent growth conditions versus what was achieved in the treatments.

STATISTICAL ANALYSIS

Analyses included two-way analysis of variance (GLM) and protected Least Significant Difference (LSD) post hoc tests (SAS 1996). For the percent survival analyses a theoretical interaction term was calculated as described in Steel and Torrie (1980) in order to test for a significant interaction. Following a two-way analysis of variance (GLM, SAS 1996), the protected LSD post hoc procedure was used where the 'no interaction' model was chosen. When interactions were documented, no additional statistical analyses could be conducted (Steel and Torrie 1980). Growth index (GI) and RGI (relative growth index) values were calculated as described by Zhang et al. (1993), where GI is calculated as:

$$GI = \frac{\sum_{i=1}^{i_{\max}} [n_{(i)} \times i] + \sum_{i=1}^{i_{\max}} [n_{(i)} \times (i - 1)]}{N \times i_{\max}}$$

$i_{\max}$ = 5, the highest attainable instar of the insect at 8 days and n = the number of insects. RGI was determined as:

$$RGI = \frac{GI \text{ of the test group}}{GI \text{ of the control group}}$$

RESULTS

PLANTS.

Plant biomass serves as an indicator of salt and Se tolerance of the plant entry. In the Se + Cl + SO₄ treatment most characteristic of irrigation waters from the SJV, the plant entries *A. hortensis* 379088, *A. hortensis* 379092, *A. hortensis hortensis*, *A. spongiosa* 415862, *A. patula patula*, *A. muelleri* 224963, *A. leptocarpa* 342565, *A. pseudocampanulata* 342567, *A. lindleyi* 415865, and *A. leucolada* 355940 were in the top 30% of biomass accumulators (Table 1).

Selenium concentrations in leaf samples are reported for each plant entry in each treatment (Table 2). In the Se + Cl + SO₄ treatment most characteristic of irrigation waters from the SJV, plant entries *A. hortensis* 379088, *A. hortensis* 379092, *A. inflata* 330660, *A. halimus* 415863, *A. leucolada* 339807, *A. semibaccata* 299489, *A. patula patula*, *A.*

lindleyi 415865, and *A. leucolada* 355940 were the top 30% of Se accumulators. Many lines had only minimal biomass and low Se accumulation, and were therefore less suitable for use in phytoremediation.

INSECTS.

Insect survival to pupation was reduced in all ten of the *Atriplex* lines as compared to alfalfa in test 1 (Figure 1a-c). In addition, the Se + Cl treatment and the Se + Cl + SO₄ treatment had significantly reduced survival as compared to the control ($F_{2,18} = 4.26$, $p = 0.031$). In plants assigned to test 1 there also was a significant difference between plant lines ($F_{9,18} = 4.50$, $p = 0.003$). In two plant lines, the *A. hortensis* and 330658, the Se containing treatments allowed no survival. A similar pattern was observed in tests 2 and 3, but significant interaction terms ($p < 0.05$) precluded statistical analysis. However, entry *A. hortensis* 379092 in test 2 was noteworthy for not allowing any insect to reach the pupal stage, regardless of the irrigation treatment.

Survival to the adult stage was significantly different between treatments in test 1, with the Se + Cl + SO₄ treatment causing the most mortality ($F_{2,18} = 3.82$, $p = 0.042$) (Figure 2a). Insect survival was significantly decreased in six of the plant lines bioassayed in test 1 ($F_{9,18} = 3.87$, $p = 0.007$). The significant interaction terms in tests 2 and 3 ($p < 0.05$) precluded statistical comparisons. However, because the patterns of survival to the adult stage (Figure 2 a-c) were nearly identical to the patterns of survival to the pupal stage (Figure 1a-c), we can conclude that most mortality occurred during the larval stage rather than during the pupation process.

Development stage at death varied for insects depending on plant line and irrigation treatment (Figure 3a-c). In plants examined in test 1, these differences were significant between irrigation treatments ($F_{2,270} = 4.31$, $p = 0.014$), with the Se treatments causing insects to die earlier in their life cycles. Differences in developmental stage at death were also significant between plant lines ($F_{9,270} = 7.84$, $p < 0.001$). The mean developmental stage at death was before stage four in the Se + Cl + SO₄ treatment for plant entries *A. semibaccata* 299488, *A. glauca glauca*, *A. patula patula*,

A. hortensis hortensis, and *A. canescens* 330658 from test 1. There were no significant plant line*treatment interactions in test 1.

Similarly, in test 2, the between plant differences were significant ($F_{9,270} = 10.27$, $p < 0.001$) Fig. 3b). Feeding on *A. halimus* 415853, *A. hortensis* 379088, and *A. hortensis* 379092 resulted in death before stage four. However, the most rapid mortality was seen on plant line *A. hortensis* 379092. Differences between irrigation treatments were not significant. Plant*treatment interactions also were not significant.

In test 3 plant line*treatment interactions were significant at the $p = 0.05$ level, so additional statistical analyses were not possible. This test included few plant lines that appeared to offer substantial reductions in developmental stage (Fig. 3c). Only *A. lentiformis* 330661 consistently caused larval death before stage four.

The Relative Growth Index (RGI) indicated that several *Atriplex* lines which supported some insect growth did so at a greatly reduced development rate as compared to the absolute control (Figure 4a-c). Differences in the RGI between plant lines were significant for plant entries in test 1 ($F_{9,270} = 5.78$, $p < 0.001$) and test 2 ($F_{9,270} = 7.44$, $p < 0.001$), and between irrigation treatments in test 2 (the no Se control plants grew significantly faster than plants in Se irrigation treatments; $F_{2,270} = 5.14$, $p = 0.006$). Plant entries *A. canescens* 330658 from test 1, and *A. canescens canescens* and *A. hortensis* 379092 from test 2 significantly reduced insect development in the Se + Cl + SO_4 treatment as measured by RGI. Test 3 plant line*treatment interactions were significant at the $p = 0.05$ level.

Day of death (Table 3) differed significantly between irrigation treatments and between plant lines in test 1 ($F_{2,270} = 5.13$, $p < 0.007$; $F_{9,270} = 6.90$, $p < 0.001$), and between plants in test 2 ($F_{9,270} = 7.99$, $p < 0.001$) and test 3 ($F_{9,270} = 2.09$, $p = 0.031$). There were no significant plant line*treatment interactions in any of the tests. Day of death on control plants in test 1 was significantly later than in the two Se irrigation treatments. For plants *A. glauca glauca*, *A. hortensis hortensis*, and *A. canescens* 330658 from test 1, *A. halimus*

415853, and *A. hortensis* 379092 from test 2, and *A. lentiformis* 330661 from test 3, the average day of death was before the mean day to pupation for insects in the absolute control group fed alfalfa. For those insects which die, a relatively early day of death reduces the amount of time the insect is available to the next trophic level, particularly predators, and so reduces the potential for Se biotransfer.

Mean days to pupation were included specifically to establish if any plants were toxic primarily to larval stages. Toxicity could result in either death or an increase in developmental time. Insects which completed development to the pupal stage varied in their mean days to pupation between plant lines and between irrigation treatments (Table 4). In plants examined in test 1, these differences were significant between irrigation treatments ($F_{2,270} = 5.26$, $p = 0.007$) and between plant lines ($F_{9,270} = 4.08$, $p < 0.001$). Mean days to pupation on control plants in test 1 was significantly longer than that on the Se + Cl + SO_4 treatments, but there were no differences between the two Se treatments. In plants examined in tests 2 and 3, plant line*treatment interactions were significant at the $p = 0.05$ level.

Mean days to adult (for those insects which made it to the adult stage) varied between plant lines and between irrigation treatments (Table 5). Insects fed alfalfa, the absolute control, developed to adult in 20 days or less while insects fed *Atriplex* entries had mean developmental times of as long as 28 days. In plants examined in test 1, differences were significant between plant lines ($F_{9,270} = 4.20$, $p < 0.001$) but not between treatments. In plants examined in tests 2 and 3, plant line*treatment interactions were significant at the $p = 0.05$ level, and no additional analyses were possible.

CONCLUSIONS

Plant lines chosen for phytoremediation programs should have high biomass, high Se accumulation or volatilization, and should not support growth of agriculturally important insects into the adult stage. As expected in this study, selenium accumulation was

reduced in the presence of high sulfate levels. Additional information on how much Se was volatilized by these plants would be desirable, and allow a comprehensive model of the phytoremediation potential of the *Atriplex* species examined. The presence of Se in the irrigation water did increase plant resistance to insects, which is in agreement with the data generated for canola (Bañuelos et al. 1997). This suggests that Se may enhance plant resistance, but the current sample size in the literature is too limited for generalizations.

Several *Atriplex* plant lines appear to have potential for use in phytoremediation, particularly in areas where other plants are not suitable. Of the five plant entries in the top 30% of both biomass producers and Se accumulators, *A. hortensis* 379088 and *A. hortensis* 379092 had no survival to pupation and only 10% of the insects fed *A. patula patula* survived. Thus, these plant lines would be unlikely to host significant *S. exigua* populations or allow substantial selenium bioaccumulation in the food chain.

Other plant lines that justify further investigation include *A. hortensis hortensis*, which had no insect survival and reduced insect development (also in the top 60% of Se accumulators and in the top 30% of plant biomass producers), and *A. spongiosa* 415862,

which reduced insect survival to 30% (top 30% of plant biomass producers and top 33% of Se accumulators).

Unfortunately, other lines with high biomass and Se accumulation in the Se + Cl + SO₄ treatment such as *A. lindleyi* 415865 and *A. leucolada* 355940 allowed 90% survival to adult eclosion making them unsuitable candidates for a phytoremediation program. Although plant entries *A. glauca glauca*, *A. nummularia* 419463, *A. canescens* 330658, *A. halimus* 415853, *A. canescens canescens*, and *A. lentiformis* 330661 suppress insect survival to 30% or less and reduce insect development in the Se + Cl + SO₄ treatment, they were excluded because of low biomass production and low Se accumulation.

Our results suggest that these plant lines could improve grower participation in phytoremediation programs by potentially reducing local populations of a key insect pest. However, the most promising 4 - 6 plant lines should be subjected to additional studies designed to determine if these plants are attractive for oviposition, and for effects on other key insect pests such as *Circulifer tenellus*, a vector of curly top virus.

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Species	Line	Dry Wt/Plant (g)			Origin
		Control	Se+Cl	Se+Cl+SO ₄	
Harvest 1					
<i>A. hortensis</i>	379088	42.56	11.76	16.46	WRPIS ^a
<i>A. hortensis</i>	379092	15.27	13.38	12.43	WRPIS
<i>A. hortensis</i>	hortensis	13.87	5.50	10.89	J. L. Hudson
<i>A. spongiosa</i>	330668	7.32	4.05	6.59	WRPIS
<i>A. hortensis</i>	372512	11.72	5.83	4.62	WRPIS
<i>A. glauca</i>	<i>glauca</i>	5.23	3.32	4.07	Carter Seeds
<i>A. inflata</i>	330660	1.71	1.66	4.00	WRPIS
<i>A. halimus</i>	415853	3.12	1.22	3.43	WRPIS
<i>A. suberecta</i>	368854	7.49	1.41	2.91	WRPIS
<i>A. canescens</i>	<i>canescens</i>	0.86	0.42	0.79	Carter Seeds
Harvest 2					
<i>A. spongiosa</i>	415862	20.25	0.57	14.30	WRPIS
<i>A. patula</i>	<i>patula</i>	13.36	7.53	9.80	S & S Seeds
<i>A. semibaccata</i>	415860	4.46	1.22	7.95	WRPIS
<i>A. semibaccata</i>	368853	5.55	1.39	6.27	WRPIS
<i>A. muelleri</i>	380751	8.56	2.66	5.39	WRPIS
<i>A. nummularia</i>	419462	5.11	1.41	4.07	WRPIS
<i>A. leucolada</i>	339807	3.56	0.61	2.73	WRPIS
<i>A. nummularia</i>	419463	4.38	2.73	2.66	WRPIS
<i>A. canescens</i>	<i>canescens</i>	2.00	0.66	1.41	Carter Seeds
<i>A. semibaccata</i>	299489	5.10	1.72	1.31	WRPIS
<i>A. semibaccata</i>	299488	4.21	1.26	0.60	WRPIS
Harvest 3					
<i>A. muelleri</i>	224963	25.85	25.39	42.17	WRPIS
<i>A. leptocarpa</i>	342565	20.62	2.66	32.49	WRPIS
<i>A. pseudocampanulata</i>	342567	10.22	13.51	28.29	WRPIS
<i>A. lindleyi</i>	415865	22.58	22.36	21.05	WRPIS
<i>A. leucolada</i>	355940	29.94	5.58	12.30	WRPIS
<i>A. lentiformis</i>	409121	7.51	1.46	5.92	WRPIS
<i>A. canescens</i>	<i>canescens</i>	4.09	1.29	3.48	Carter Seeds
<i>unknown sp.</i>	330670	5.07	2.41	3.01	WRPIS
<i>A. lentiformis</i>	330661	2.38	1.54	2.93	WRPIS
<i>A. hortensis</i>	NSSL 9810501	1.61	0.71	0.72	NSSL
<i>A. canescens</i>	330658	8.57	0.04	0.58	WRPIS

^aWRPIS = Western Regional Plant Introduction System, Pullman, WA; part of the USDA, ARS, National Plant Germplasm System

Table 1. Dry plant weight of *Atriplex* plant entries tested in bioassays from three irrigation treatments.

Species	Line	Total Se mg/kg		
		Control	Se+Cl	Se+Cl+SO ₄
Harvest 1				
<i>A. hortensis</i>	379088	0.08	40.80	3.04
<i>A. hortensis</i>	379092	0.14	40.00	2.70
<i>A. inflata</i>	330660	0.50	66.00	2.40
<i>A. halimus</i>	415853	0.16	82.80	2.27
<i>A. spongiosa</i>	330668	0.14	38.20	1.91
<i>A. hortensis</i>	372512	0.17	27.00	1.73
<i>A. glauca</i>	<i>glauca</i>	0.16	61.50	1.58
<i>A. hortensis</i>	<i>hortensis</i>	0.19	39.70	1.54
<i>A. canescens</i>	<i>canescens</i>	0.14	42.40	1.54
<i>A. suberecta</i>	368854	0.10	33.40	1.40
Harvest 2				
<i>A. leucolada</i>	339807	0.40	39.10	3.20
<i>A. semibaccata</i>	299489	0.30	33.80	2.30
<i>A. patula</i>	<i>patula</i>	0.10	37.60	2.00
<i>A. spongiosa</i>	415862	0.09	39.70	1.96
<i>A. canescens</i>	<i>canescens</i>	0.15	48.20	1.95
<i>A. semibaccata</i>	368853	0.10	34.70	1.90
<i>A. nummularia</i>	419462	0.14	49.80	1.82
<i>A. semibaccata</i>	299488	0.30	32.20	1.80
<i>A. semibaccata</i>	415860	0.15	35.10	1.77
<i>A. muelleri</i>	380751	0.40	40.10	1.70
<i>A. nummularia</i>	419463	0.10	33.20	1.20
Harvest 3				
<i>A. lindleyi</i>	415865	0.24	41.16	2.61
<i>A. leucolada</i>	355940	0.12	65.25	2.11
<i>A. leptocarpa</i>	342565	0.19	47.76	1.45
<i>unknown sp.</i>	330670	0.15	45.00	1.42
<i>A. pseudocampanulata</i>	342567	0.21	35.16	1.42
<i>A. canescens</i>	<i>canescens</i>	0.17	48.70	1.41
<i>A. lentiformis</i>	409121	0.22	47.88	1.40
<i>A. muelleri</i>	224963	0.20	33.24	1.38
<i>A. hortensis</i>	NSSL 9810501	0.19	42.24	1.25
<i>A. lentiformis</i>	330661	0.16	45.00	1.15
<i>A. canescens</i>	330658	.	.	.

Table 2. Selenium concentrations of *Atriplex* plant entries tested in bioassays from three irrigation treatments.

Species	Line	Mean day of death			
		Control	Se+Cl	Se+Cl+SO ₄	
Test 1		A	B	B	
<i>M. sativa</i>	(alfalfa)	30.0			
<i>A. semibaccata</i>	299489	22.6	27.4	22.8	A
<i>A. hortensis</i>	372512	27.7	18.8	23.4	AB
<i>A. suberecta</i>	368854	23.8	23.6	16.8	ABC
<i>A. semibaccata</i>	299488	21.0	19.4	16.0	BCDE
<i>A. glauca</i>	<i>glauca</i>	23.0	16.6	9.2	DEF
<i>A. spongiosa</i>	415862	21.4	19.8	19.6	ABCD
<i>A. nummularia</i>	419463	16.4	9.2	17.6	EF
<i>A. patula</i>	<i>patula</i>	14.4	20.2	13.6	CDEF
<i>A. hortensis</i>	<i>hortensis</i>	18.8	3.4	11.8	FG
<i>A. canescens</i>	330658	12.2	9.4	4.4	G
Test 2		A	A	A	
<i>M. sativa</i>	(alfalfa)	30.0			
<i>A. leucolada</i>	355940	24.4	29.4	27.2	A
<i>A. semibaccata</i>	415860	24.8	26.8	22.2	AB
<i>A. nummularia</i>	419462	15.4	24.4	27.6	ABC
<i>A. spongiosa</i>	330668	23.0	23.4	24.8	AB
<i>A. leptocarpa</i>	342565	16.8	26.0	19.0	BCD
<i>A. hortensis</i>	NSSL 9810501	20.8	18.4	21.6	BCD
<i>A. halimus</i>	415853	21.6	17.2	13.6	AB
<i>A. canescens</i>	<i>canescens</i>	30.0	15.2	12.4	D
<i>A. hortensis</i>	379088	20.4	17.4	15.6	CD
<i>A. hortensis</i>	379092	8.6	4.0	11.2	E
Test 3		A	A	A	
<i>M. sativa</i>	(alfalfa)	28.8			
<i>A. semibaccata</i>	368853	30.0	30.0	25.0	A
<i>A. muelleri</i>	380751	27.4	24.6	30.0	AB
<i>A. lentiformis</i>	330661	28.4	26.8	12.2	BC
<i>A. inflata</i>	330660	27.4	24.6	28.6	AB
<i>A. leucolada</i>	339807	25.8	23.0	28.2	AB
<i>A. pseudocampanulata</i>	342567	25.0	21.8	24.8	ABC
<i>unknown sp.</i>	330670	24.8	24.2	20.8	BC
<i>A. muelleri</i>	224963	19.4	24.4	27.2	ABC
<i>A. lindleyi</i>	415865	24.6	26.4	27.2	AB
<i>A. lentiformis</i>	409121	20.0	18.0	22.2	C

Table 3. Comparison of day at death for *S. exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines and between treatments are indicated by capital letters, values with the same letters are not significantly different (LSD, SAS 1996).

Species	Line	Mean days to pupation			
		Control	Se+Cl	Se+Cl+SO ₄	
Test 1		A	B	AB	
<i>M. sativa</i>	(alfalfa)	12.2			
<i>A. semibaccata</i>	299489	13.7	14.7	12.3	A
<i>A. hortensis</i>	372512	15.3	21.0	16.9	C
<i>A. suberecta</i>	368854	14.8	12.3	16.0	AB
<i>A. semibaccata</i>	299488	12.5	15.0	14.5	A
<i>A. glauca</i>	<i>glauca</i>	13.7	14.0	13.0	A
<i>A. spongiosa</i>	415862	16.0	18.0	15.3	BC
<i>A. nummularia</i>	419463	16.5	18.0	14.0	ABC
<i>A. patula</i>	<i>patula</i>	15.0	18.5	18.0	C
<i>A. hortensis</i>	<i>hortensis</i>	18.0	..	..	C
<i>A. canescens</i>	330658	17.0	20.0	..	C
Test 2					
<i>M. sativa</i>	(alfalfa)	12.6			
<i>A. leucolada</i>	355940	13.0	12.4	15.6	
<i>A. semibaccata</i>	415860	12.0	14.3	12.6	
<i>A. nummularia</i>	419462	13.8	17.0	15.4	
<i>A. spongiosa</i>	330668	12.9	16.3	14.3	
<i>A. leptocarpa</i>	342565	12.8	14.3	13.3	
<i>A. hortensis</i>	NSSL 9810501	15.0	15.3	17.3	
<i>A. halimus</i>	415853	18.0	15.3	..	
<i>A. canescens</i>	<i>canescens</i>	14.3	16.0	18.0	
<i>A. hortensis</i>	379088	17.0	21.3	..	
<i>A. hortensis</i>	379092	..	..	..	
Test 3					
<i>M. sativa</i>	(alfalfa)	12.7			
<i>A. semibaccata</i>	368853	12.6	13.4	12.3	
<i>A. muelleri</i>	380751	12.7	13.5	14.2	
<i>A. lentiformis</i>	330661	14.7	15.8	15.3	
<i>A. inflata</i>	330660	13.8	14.6	14.0	
<i>A. leucolada</i>	339807	12.7	13.0	12.2	
<i>A. pseudocampanulata</i>	342567	13.0	15.0	14.0	
<i>unknown sp.</i>	330670	14.0	15.7	14.4	
<i>A. muelleri</i>	224963	14.3	13.5	12.4	
<i>A. lindleyi</i>	415865	13.8	15.3	12.5	
<i>A. lentiformis</i>	409121	14.0	14.4	13.3	

Table 4. Comparison of mean days to pupation for *S. exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines and between treatments are indicated by capital letters, values with the same letters are not significantly different (LSD, SAS 1996). Plant entries which did not support survival to the adult stage are indicated with the symbol ‘..’.

Species	Line	Mean days to adult			
		Control	Se+Cl	Se+Cl+SO ₄	
Test 1		A	A	A	
<i>M. sativa</i>	(alfalfa)	19.0			
<i>A. semibaccata</i>	299489	21.1	21.0	19.7	AB
<i>A. hortensis</i>	372512	22.6	26.0	23.4	C
<i>A. suberecta</i>	368854	19.5	19.7	22.7	A
<i>A. semibaccata</i>	299488	19.5	22.0	19.3	A
<i>A. glauca</i>	<i>glauca</i>	21.0	21.0	22.0	AB
<i>A. spongiosa</i>	415862	22.5	24.0	22.0	BC
<i>A. nummularia</i>	419463	23.0	26.0	22.0	C
<i>A. patula</i>	<i>patula</i>	22.0	24.7	24.0	CD
<i>A. hortensis</i>	<i>hortensis</i>	24.0	''	''	CD
<i>A. canescens</i>	330658	26.0	28.0	''	D
Test 2					
<i>M. sativa</i>	(alfalfa)	19.2			
<i>A. leucolada</i>	355940	19.7	19.3	21.0	
<i>A. semibaccata</i>	415860	17.8	20.8	19.4	
<i>A. nummularia</i>	419462	20.0	23.5	22.0	
<i>A. spongiosa</i>	330668	20.7	23.0	21.0	
<i>A. leptocarpa</i>	342565	19.0	21.5	19.0	
<i>A. hortensis</i>	NSSL 9810501	22.0	22.7	24.0	
<i>A. halimus</i>	415853	26.7	22.0	''	
<i>A. canescens</i>	<i>canescens</i>	21.0	24.0	25.0	
<i>A. hortensis</i>	379088	23.0	28.0	''	
<i>A. hortensis</i>	379092	''	''	''	
Test 3					
<i>M. sativa</i>	(alfalfa)	20.2			
<i>A. semibaccata</i>	368853	19.2	19.6	19.7	
<i>A. muelleri</i>	380751	19.8	20.3	21.0	
<i>A. lentiformis</i>	330661	21.3	23.0	22.0	
<i>A. inflata</i>	330660	20.9	21.1	20.8	
<i>A. leucolada</i>	339807	18.8	19.0	18.2	
<i>A. pseudocampanulata</i>	342567	19.0	22.0	19.0	
<i>unknown sp.</i>	330670	20.3	22.0	21.2	
<i>A. muelleri</i>	224963	21.0	20.3	19.0	
<i>A. lindleyi</i>	415865	20.0	23.0	19.5	
<i>A. lentiformis</i>	409121	19.3	21.6	20.3	

Table 5. Comparison of mean days to adult for *S. exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines and between treatments are indicated by capital letters, values with the same letters are not significantly different (LSD, SAS 1996). Plant entries which did not support survival to the adult stage are indicated with the symbol ''.

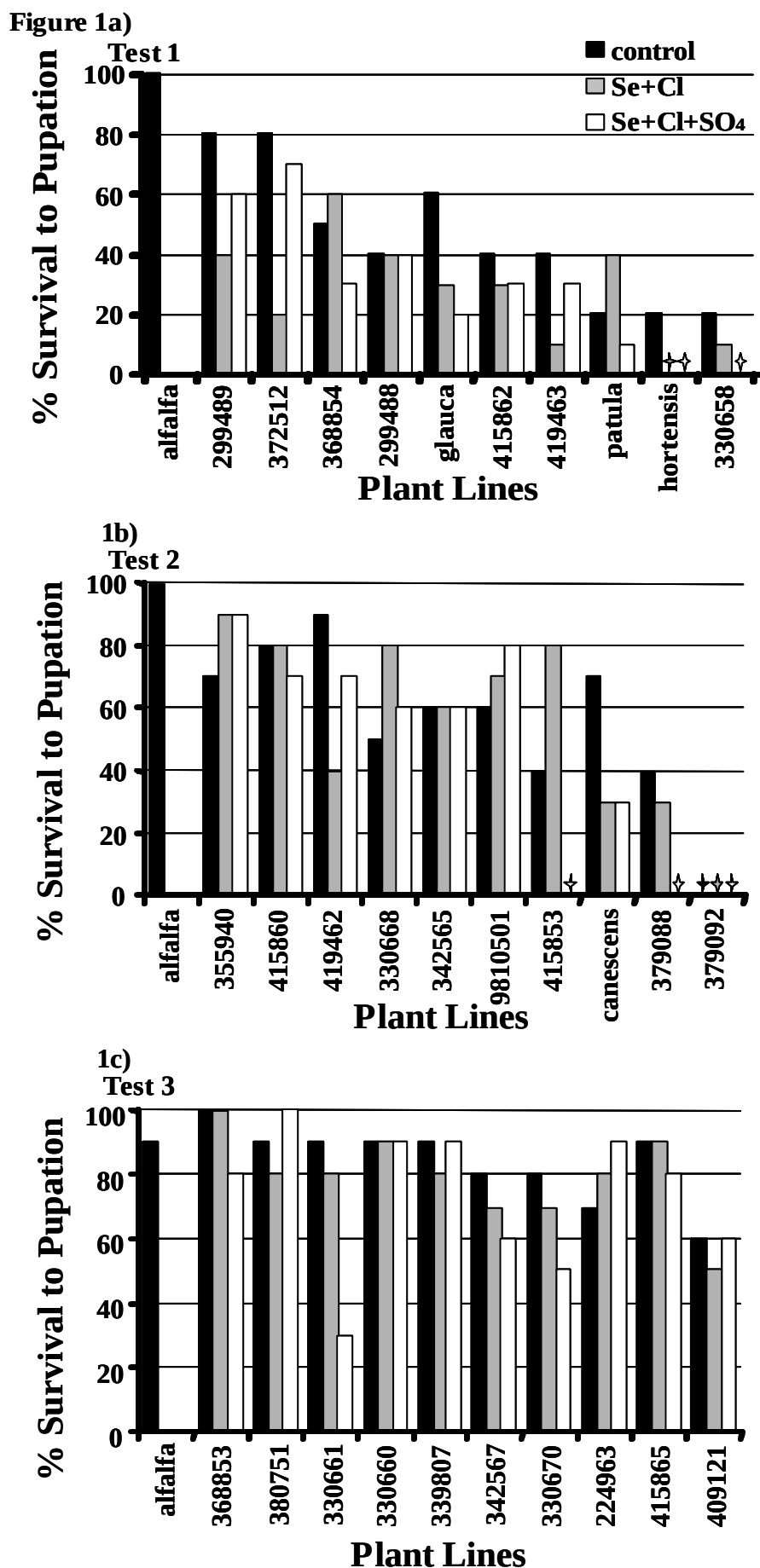
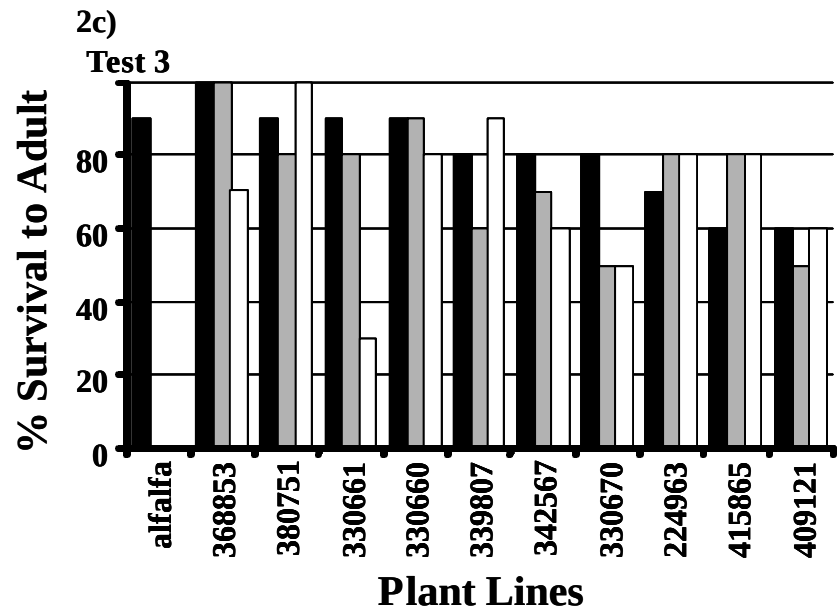
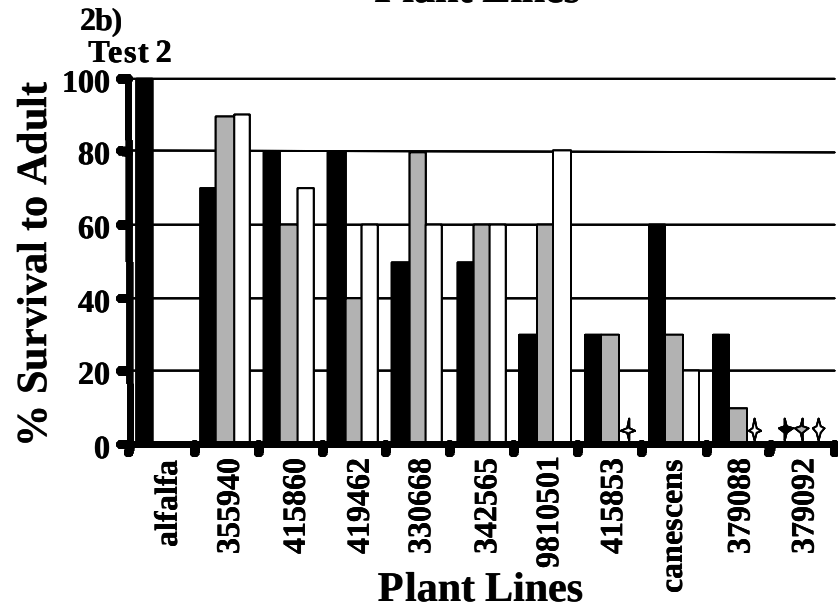
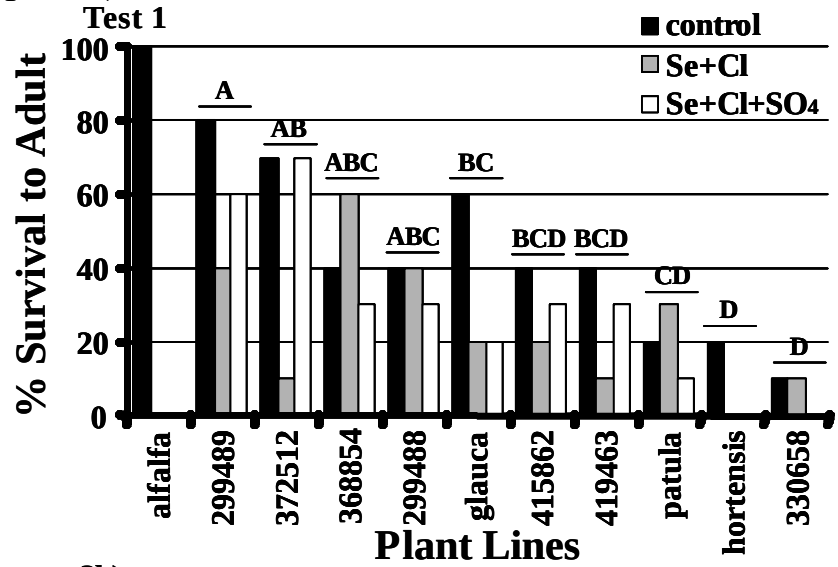


Figure 1. Percent survival to pupation for *Spodoptera exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In test 1 the control treatment was significantly different from both the Se + Cl and the Se + Cl + SO₄ treatments (see text; LSD, SAS 1996). Tests 2 and 3 had a significant plant line*treatment interactions, so additional statistical comparisons were not possible. Plant entries which did not support survival to the pupal stage are indicated with the symbol ‘*’.

Figure 2. Percent survival to adult for *Spodoptera exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In test 1 the control treatment was significantly different from the Se + Cl + SO₄ treatment (see text; LSD, SAS 1996). Tests 2 and 3 had a significant plant line*treatment interactions, so additional statistical comparisons were not possible. Plant entries which did not support survival to the adult stage are indicated with the symbol ‘*’.

Figure 2a)



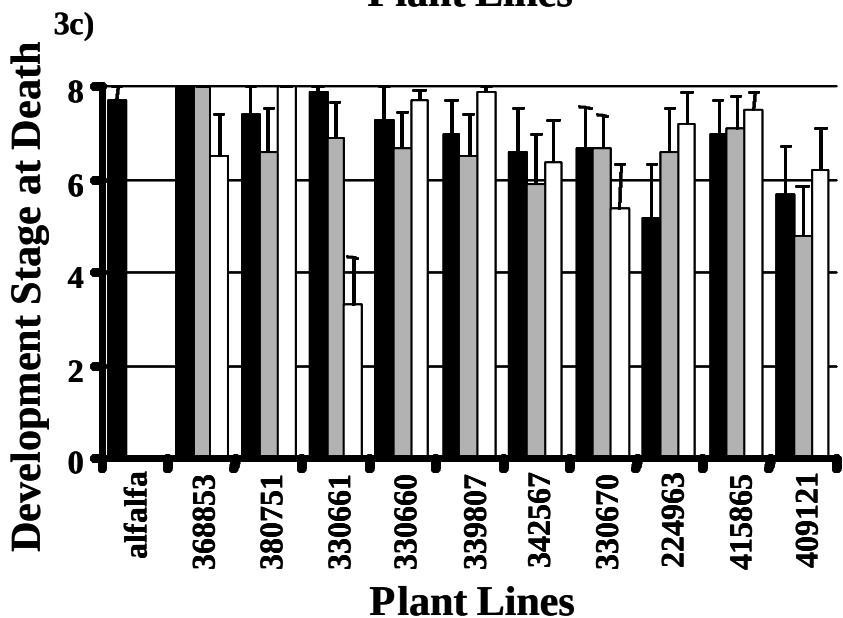
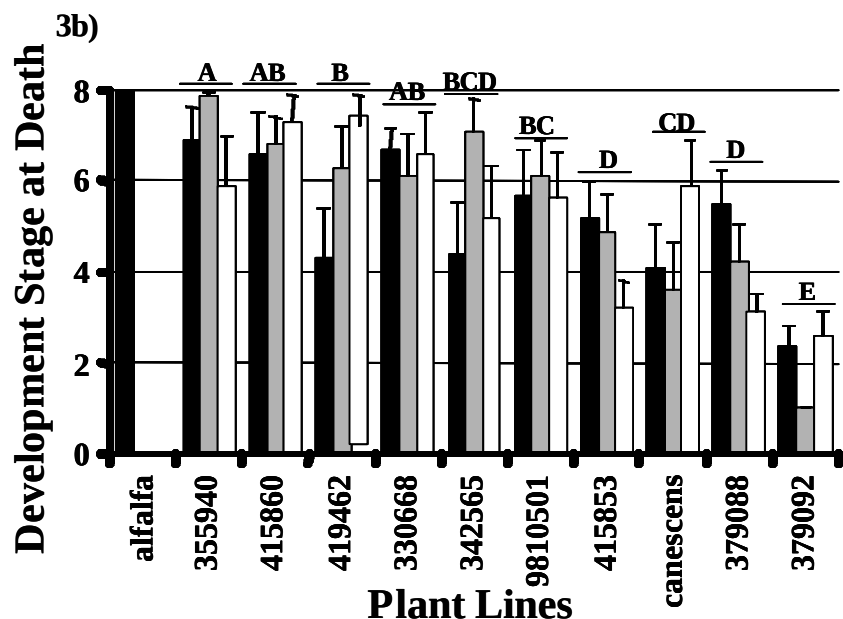
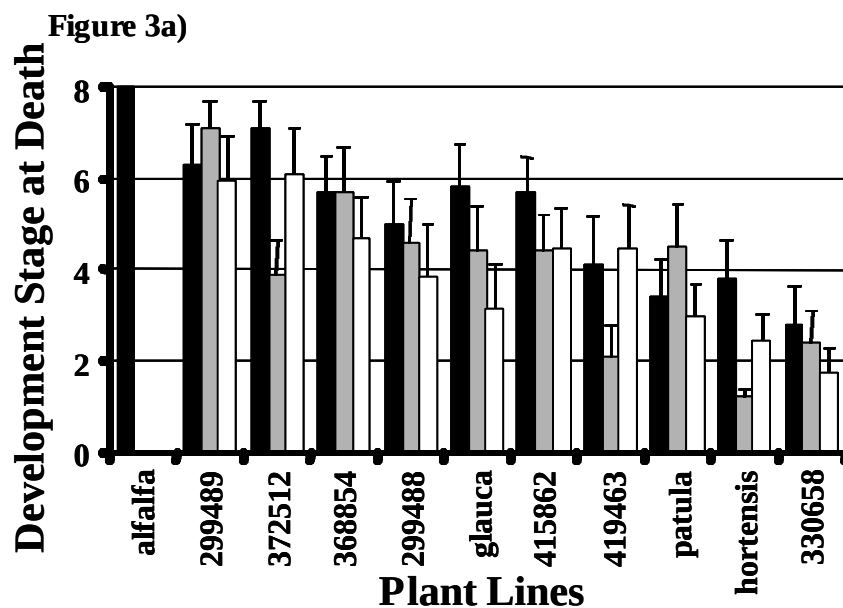


Figure 3. Development stage at death for *Spodoptera exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In tests 1 and 2 there were no significant treatment differences (LSD, SAS 1996). Test 3 had a significant plant line*treatment interaction, so additional statistical comparisons were not possible.

Figure 4. Relative growth index for *Spodoptera exigua* larvae fed *Atriplex* plant entries from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In test 1 there were no significant treatment differences, in test 2 the control treatment was significantly different from the Se + Cl + SO₄ treatment (see text; LSD, SAS 1996). Test 3 had a significant plant line*treatment interaction, so additional statistical comparisons were not possible.

