Selenium Incorporation and Partitioning in "Jose" Tall Wheatgrass (*Thinopyrum ponticum*) Irrigated with Saline Drainage Water

Jaya Ram K.C.[1]
Sharon Benes, Ph.D.[1]
Peter Robinson, Ph.D.[2]
Steve Grattan, Ph.D.[3]
Suduan Gao[4]
John Bushoven[1]

[1]Department of Plant Science, California State University, Fresno, 2415 E San Ramon Ave., M/S AS72, Fresno, CA 93740

[2]Department of Animal Science and [3]Department of Land, Air and Water Resources, University of California, Davis, 1 Shields Ave., Davis, CA 95616.

[4]Water Management Research Lab, USDA- ARS, 9611 South Riverbend Avenue, Parlier, CA 93648-9757

Corresponding author: sbenes@csufresno.edu

Abstract: In the western San Joaquin Valley (SJV) of California, saline drainage water (DW) has been utilized for irrigation primarily to extend irrigation water supplies and to dispose of these saline waters. High levels of selenium (Se) in this DW, however, requires that measures be taken to minimize the exposure of wildlife to this selenium. 'Jose' tall wheatgrass (TWG) (*Thinopyrum ponticum* var. 'Jose'), a highly salt tolerant forage which can be grown in soils of even 20 dS/m ECe and with high Se has accumulated up to 10 mg Se/kg in the dry matter. Conversely, in the eastern SJV, soils are low in Se and dairy cattle producers often supplement their animals with inorganic sodium selenate. A greenhouse study was initiated in 2009 at California State University, Fresno to assess the selenium accumulation in TWG with irrigation waters of two salinities (EC 3 and 12 dSm^{-1}) and two selenium concentrations (350 and 1000 ppb), along with three cutting heights (20, 40, 60 cm) arranged in a split-plot design. Initial results showed significant effects of irrigation water combination and cutting heights on forage Se accumulation which was as high as 15 mg/kg for the 60 cm cuts.

Keywords: Reuse, drainage water, selenium, "Jose" tall wheatgrass

Introduction

The San Joaquin Valley (SJV) which is the southern half of the Central Valley of California extends approximately 402 km from the San Joaquin-Sacramento River Delta on the north to the Tehachapi Mountains to the south. The valley often called the food basket of the world, contributes about 12.8% of United States agricultural production (2009) and includes the top five counties of United States in terms of agricultural production (Fresno, Tulare, Kern, Merced, Monterey). This enormous production which is brought about by irrigated agriculture has also brought the problem of salinity in the western part of the valley.

The western side of the SJV is mostly made of sedimentary deposits enriched in trace elements such as selenium (Se), molybdenum (Mo), and boron (B). Fine-textured soils along with shallow water tables in wet years contribute to these salinity problems. Conversely, soils on the east side of the valley which are granitic in origin and mostly coarse-textured, contain few native salts and much lower concentrations of selenium and boron. Interestingly, besides being an environmental hazard, Se is an essential element required for livestock, humans and cattle. It is the essential component of glutathione peroxidase and thyroid oxidase in mammals which are enzymes responsible for regulating reproductive health and immunity. Consequently, in the eastern SJV where selenium is often deficient in the soils, dairy farmers commonly supplement their animals with sodium selenate for proper animal nutrition. This area has some of the nation's largest dairy industries thus the importation of Se in the form of these dietary supplements represents a significant import of Se into the valley.

Drainage water (DW) management to cope with salinity has been approached in the past by the use of sub-surface drains. Reuse of saline DW to produce salt tolerant forages has emerged as an attractive option to reduce drainage volumes and produce high quality forage for the large beef and dairy cattle industry in the Central Valley of California. In areas of the western SJV where soils contain high levels of Se, forages enriched in Se due to DW irrigation have potential to be processed into organic Se supplements to replace the sodium selenate currently used by producers in Se-deficient areas (Suyama et al, 2007b, Grattan and Diaz, 2009, Robinson et al, 2004). As the problem of salinity in the WSJV is inseparably associated with the presence of Se, any salinity management approaches must address the issue of Se. The objectives of this study were to evaluate the effects of salinity and Se levels in the irrigation water and cutting height on Se accumulation in 'Jose' tall wheatgrass (TWG) (*Thinopyrum ponticum* var. 'Jose') in order to assess its potential as a local, organic source of dietary Se for dairy cattle.

Methodology

Experimental set-up and design

The study was conducted in a greenhouse at California State University, Fresno. Soils were collected from Red Rock Ranch in Five Points in western Fresno County, CA and passed through a screen. The soil thus screened was mixed with sand in a 60:40 (soil:sand) ratio to ensure better drainage in the pots while maintaining the cracking clay characteristics common to soils in the western SJV. Four irrigation water combinations consisting of two levels of salinity (3 and 12 dS/m) representing low (LS) and high (HS) salinity levels and two levels of Se (350 - 400 and 1000 ppb) as low (LSe) and high (HSe) selenium levels were utilized as the main plot factor. The LSe treatment level could not be set more precisely because the lowest level was determined by the amount of source water that had to be added to reach the HS (high salinity) level. Cutting heights of 20cm, 40cm, and 60cm were used as sub-plot factor which resulted in a split-plot design (Fig. 1).

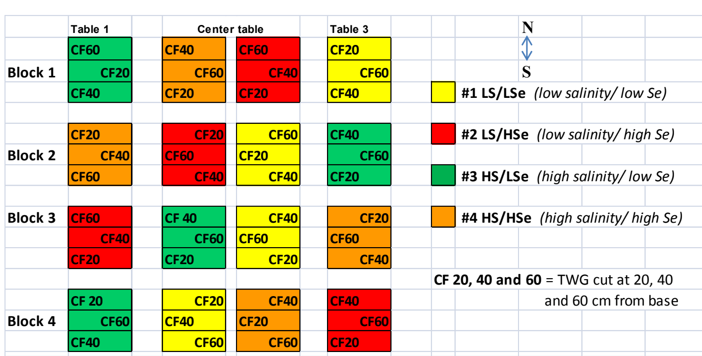

Fig. 1: split-plot design for arrangement of pots in the greenhouse.

Plant establishment, salinization, and irrigation

The pots used in the study (25.5 cm diameter x 30 cm deep= 15.3 L volume) were seeded directly and thinned to 12 plants per pot once the seedlings were several inches tall. The tall wheatgrass variety used was “Westside Wheatgrass” from S&W Seed, Five Points, CA. For the first several weeks all the plants were irrigated with non-saline tap water supplemented with basic nutrients (3 mmol/L of KNO_3, 0.5 mmol/L of KH_2PO_4 and 20μmol/L of Fe-DTPA). Concentrated DW from Panoche Water District, CA was collected from a drainage sump and used as the saline water source. To reach the target salinities, this saline water was introduced weekly in step-wise increments (¼, ½, full strength). From laboratory analysis, the Se input from the DW was determined for each irrigation water treatment and then supplementary Se in the form of sodium selenate was added to reach target Se levels. Once desired salinity, selenium and nutrient levels (as listed above) were reached, irrigation water samples were taken for complete chemical analysis.

Large plastic irrigation tanks (378.5 L) were used in a re-circulating system in which all the drainage water from the pots returned to the source tank. Tap water was used to replenish the water lost to evapotranspiration when the water level in the tank fell below 90%. Irrigation water salinity and nitrate concentrations were measured weekly and the targets levels were maintained. Irrigation tank waters were changed bi-monthly and fresh nutrients were added. The pots were irrigated 3-4 times a week initially and then daily or twice daily during the peak of summer to maintain a sufficient leaching fraction to maintain soil salinities in the pots close to the irrigation water salinities. To calculate the leaching fraction (LF), drainage was collected from selected pots and the LF calculated as the ratio of the drainage volume to the volume of irrigation water. An LF of approximately 20-30 % was maintained to keep salinity in the high salinity treatment at or below 15 dS/m.

Water and soil sampling

Irrigation tank waters were changed bi-monthly with samples collected one day after mixing and at the end of the tank mix. These water samples were analyzed for EC, pH, B, Se, Cl-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, and NO_3-N. Beginning with the second tank mix, it was observed that Se levels in the irrigation water were depleting substantially over the two month period between mixes. Thus beginning with tank mix 2, Se spikes (50 ppb for LSe and 150 ppb for HSe treatments) in the form of Na selenate were added to the irrigation tanks every two weeks. At the end of the experiment soil samples representing the entire depth of the pot were taken from each pot and saturated soil pastes were prepared. Salinity (EC_e), pH, B, Cl^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, and Na^+ were measured on the saturated paste extracts and total Se and NO_3^- were measured on dry soil samples using established procedures.

Forage Sampling

Forage samples were harvested when the plants grew to 20cm, 40cm, and 60cm height. A complete harvest was considered to be completed when the 60 cm plants reached their full height. Samples thus obtained were rinsed three times in deionized water to remove surface salts and dust. Samples were then air-dried in forced air oven at 60ºC for 48 hours and weighed. The dried tissue was then ground to pass a 1 mm sieve using a Wiley mill. The samples were analyzed individually for total Se, but for the analysis of other mineral nutrients (Cu, Zn, B, S, Ca, Mg, total N and crude protein) samples from multiple cuts within a harvest period, as occurred for the 20 cm plants, were composited.

Data and statistical analysis

Total plant Se concentrations (mg/kg) and mineral nutrient concentrations were statistically analyzed using a general linear model with irrigation treatment (salinity/Se level), cutting height, and the interaction (irrigation treatment x cutting height) as fixed factors and block as a random factor using SPSS 17 (SPSS, Inc., Chicago, Illinois). The data sets were tested to see if they meet the assumptions of the analysis of variance (ANOVA), but no transformation was required. Since the 2-way ANOVA indicated significant differences at the 0.05 significance level amongst irrigation water treatments and cutting heights, Tukey's HSD test was used for mean separation.

Results and Discussion

Irrigation water composition

The average salinity (EC_w), pH and ionic composition (other than Se) of the water used to irrigate the pots are shown in Table 1. Low salinity (LS) treatments had salinities of 3.3 to 3.4 dS/m EC_w, boron concentrations of 4.2 mg/L, and SAR of 10.3. In contrast, the high salinity (HS) treatments had an EC_w of 10.7 dS/m, boron concentrations of 17-18 mg/L and SAR of 27. Nitrate (NO_3-N) levels were 36-40 mg/L for the LS and 41-47 mg/L for the HS irrigation waters.

Table 1: Irrigation water composition (averages for six tank mixes and for samples taken at the beginning and end of each mix). Selenium data are shown in Table 2.

Irrigation treatment	EC_w (dS/m)	pH	Se (ug/L)	B (mg/L)	NO_3-N (mg/L)	Cl^-	SO_4^{2-}	Na^+	Ca^{2+}	Mg^{2+}	SAR
						............................(meq/l)				....	
LS/LSe	3.3	7.9	313	4.2	39.9	12.4	15.6	23.0	5.1	5.9	10.2
LS/HSe	3.4	8.0	761	4.2	36.1	13.0	16.1	23.5	5.4	5.9	10.4
HS/LSe	11.5	8.0	369	17.8	47.5	59.2	70.7	103.2	14.1	13.5	27.8
HS/HSe	11.1	8.2	787	18.3	41.3	56.5	67.7	98.2	13.2	12.8	27.3

Selenium levels in the irrigation waters (initial and final concentrations for each of the six tank water mixes) are shown in Table 2. It can be observed that for tank mix 1, Se levels in the irrigation water depleted during the two month period prior to re-mixing. For tank mixes 2 to 7, there was less depletion of Se because beginning with tank mix 2, Se spikes were added to the irrigation tanks, initially every month and then every two weeks from tank mix four onward.

Table 2: Selenium levels (ug/L) in the irrigation water with initial and final values shown for each tank mix

Irrigation	Mix 1		Mix 2		Mix 3		Mix 4		Mix 5		Mix 6		Mix 7	
treatment	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
LS/LSe	271	108	310	154	420	321	371	320	337	392	385	462	428	255
LS/HSe	.	627	800	305	971	455	990	916	835	1420	943	1150	783	172
HS/LSe	362	218	360	177	315	184	347	244	317	453	963	795	368	690
HS/HSe	891	323	877	177	883	655	995	856	914	1140	1090	1100	946	174

**Spiking of Se was done monthly from Mix 2 onward with 50 and 200 mg/L sodium selenate for LSe and HSe treatments, respectively. From Mix 4 onward spiking was done every 2 weeks with 50 and 150 mg/L sodium selenate for LSe and HSe.*

Soil chemical composition

Soil salinities at the end of season for the LS treatments were 4.5 to 4.7 dS/m EC_e. For the high salinity treatments the values were 12.8 to 13.3 dS/m. The ratio of soil salinity to irrigation water salinity (EC_e/EC_w) was 1.26 for the LS treatments and 1.22 for the HS treatments which indicates a leaching fraction of about 20% (Ayers and Wescot, 1986). Measured leaching fraction (volume of drainage from pots/volume of water applied) was 20-30%. This range in measured LF values was likely due to differences in water use between the 20, 40, and 60 cm plants. LF values were higher for the 20 cm plants, lower for 40 cm and lowest for 60 cm plants.

Table 2: Soil chemical composition (samples taken at end of experiment)

Irrigation Treatment	EC_e (dS/m)	pH	Soluble Se (ug/L)	B	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}
				.... mg/L				
LS/LSe	4.7 ± 0.4	7.7 ± 0.03	100 ± 10	6.2 ± 0.2	561 ± 23	1362 ± 223	140 ± 25	53 ± 8
LS/HSe	4.5 ± 0.2	7.9 ± 0.1	110 ± 3	6.4 ± 0.7	611 ± 97	1125 ± 186	134 ± 9	53 ± 5
HS/LSe	13.3 ± 1.8	7.6 ± 0.1	100 ± 5	19.8 ± 1.9	1980 ± 359	4160 ± 620	342 ± 42	174 ± 25
HS/HSe	12.8 ± 0.1	7.7 ± 0.1	100 ± 10	18.1 ± 1.3	1847 ± 98	3699 ± 311	305 ± 23	153 ± 1

Boron concentrations were high (17-18 mg/L) in the irrigation water for the HS treatments and accordingly, soluble B was high in the soil at the end of the experiment (Table 3) being nearly 20 mg/L. In both the irrigation water and the soil, SO_4^{2-} was more predominant than was Cl^-. High levels of sulfur in soil and irrigation water are of particular importance because sulfate has been shown to inhibit Se uptake by plants (Grieve et al., 2001; Bañuelos et al., 2003) and high levels of sulfate in forage tissue can be detrimental to ruminant health (Grattan et al., 2004). Soluble Se concentrations were very low in the soil (100 to 110 ug/L soil paste extract) and they were similar amongst LSe and HSe treatments. Total Se concentrations are a better indicator of Se potentially available for plant uptake, but these data are not yet available.

Forage Selenium accumulation

Irrigation water composition (salinity/selenium level) had a significant effect on Se accumulation in TWG forage ($P \leq 0.0001$). For plants cut at 40 and 60 cm, Se accumulation in the herbage was greatest under HSe irrigation (10.1 to 12.1 mg/kg = ppm, average for six harvests), but not significantly different for low and high salinity conditions. For the 20 cm plants, the effect of salinity on Se accumulation was more significant as the LS/HSe plants had higher Se in the herbage (10.8 ± 0.51 mg/kg, average of six harvests) as compared to the HS/LSe (8.3 ± 0.51 mg/kg). Overall, it appeared high sulfate levels in the irrigation did not substantially inhibit Se accumulation in the HS/HSe plants, with the possible exception of the plants cut at 20 cm at T2, T3, and T4 (Fig. 1d). However, it cannot be determined if reduced Se accumulation under high salinity at these harvests was due to sulfate inhibition of Se uptake or an effect of salinity on evapotranspiration (ET) which in turn could have reduced Se uptake. Irrigation with low Se irrigation water resulted in lower Se accumulation in the forage, averaging from 3.8 to 5.8 mg/kg Se, averages for six harvests) under both low and high salinity conditions.

Cutting height also had a statistically significant effect on Se accumulation in the TWG herbage ($P \leq 0.003$). Differences in Se accumulation in response to cutting height were greatest for the HS/HSe treatment (Fig. 1d) and for this treatment, Se accumulation was lowest for plants cut at 20 cm suggesting that the osmotic effect of salinity may have impacted water and Se uptake to a greater extent in these frequently cut plants with younger tissue. For the T3 and T4 cuts, plants irrigated with low salinity and high selenium (LS/HSe) irrigation water and cut at 40 cm and 60 cm accumulated the most Se (12.1 ± 1.4 and 15.5 ± 1.0 mg/kg, respectively) which was significantly higher than for the 20cm cuts. At the T5 cut, Se accumulation was highest for 60 cm plants under both HS/HSe and LS/HSe irrigation which were not significantly different from one another. The final harvest (T6) produced highest Se accumulation for LS/HSe treatments which was not statistically different within cutting heights, nor from the 60cm plants in the HS/HSe treatment (Fig. 1c,d).

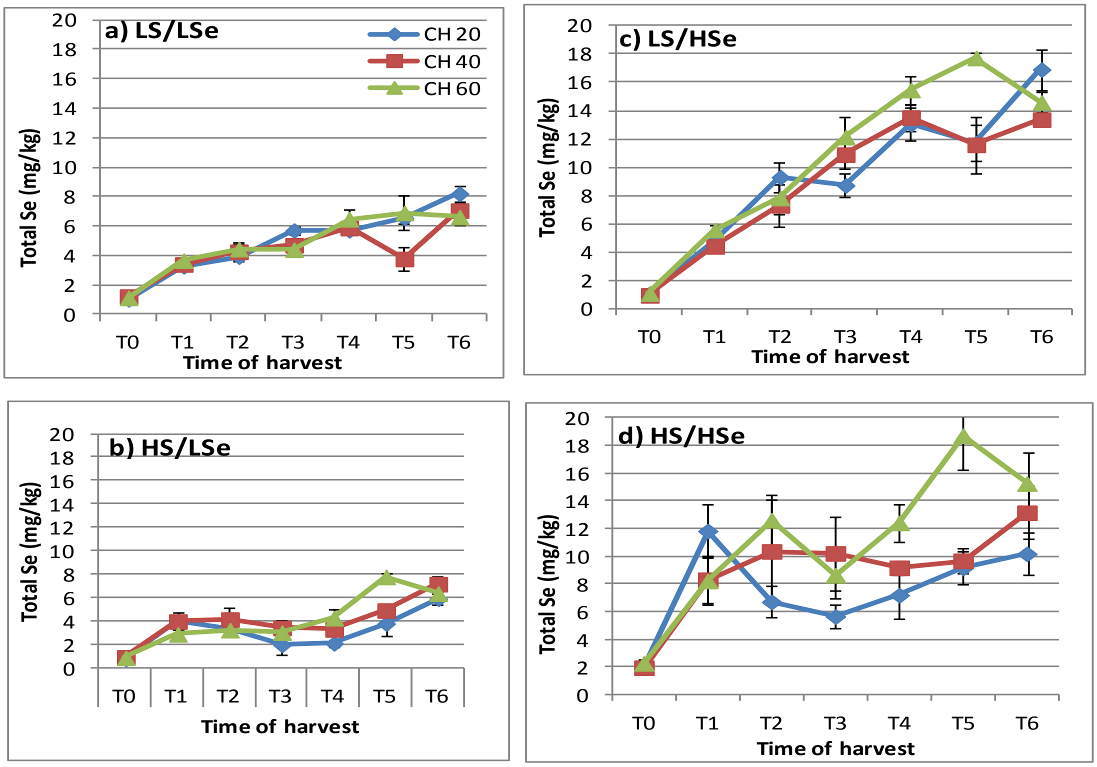

Fig.1. Total Se accumulation (mg/kg) in "Jose" tall wheat grass irrigated under irrigation with a) low salinity, low selenium (LS/LSe), b) low salinity, high selenium (LS/HSe) c) high salinity, low selenium (HS/LSe) and d) high salinity, high selenium (HS/HSe) water for plants cut at 20, 40 or 60 cm heights. Six cuts of the forage were made over the one year period.

For the plants cut at 40 and 60 cm, Se concentrations in the herbage generally increased over time likely due to the increased exposure to the selenium enriched irrigation waters. Interestingly, there was little difference in final concentrations of Se in the herbage of plants cut at 40 vs. 60 cm which could mean that as the tissue started to age the uptake and accumulation of Se slowed down. It is important to note that with 20 cm plants, frequent cuttings resulted in low biomass production per pot and the death of a number of plants initially. With frequent cutting these plants became fewer and finer. With the overall objective of harvesting tall wheatgrass as an organic Se supplement for dairy cows, the greater biomass production obtained from the plants cut at 40 and 60 cm would be desirable.

Conclusion

The highest Se accumulation was obtained for tall wheatgrass plants cut at 60 cm under LS/HSe (17.7 ± 0.4 mg/kg) and HS/HSe (18.7 ± 2.4 mg/kg) irrigation and these plants also had the greatest biomass production (data not shown). These data suggest that there is potential to use high Se drainage waters of high or low salinity to produce Se-enriched 'Jose' tall wheat grass in salt- and drainage-affected areas of the western SJV. The Se enrichment achieved in the TWG forage under DW irrigation (up to 18 mg/kg = ppm dry matter) would be sufficient to provide adequate Se to meet the nutritional requirement of dairy cattle (0.05 mg Se/ kg of ration) (Minson, 1990) when added to the diet at low percentages (< 5%). Utilization of Se-enriched tall wheatgrass forage in place of sodium selenate supplements currently used by the industry would have the environmental benefit of reducing Se imports into the SJV in the form of dietary supplements for cattle.

Acknowledgements

Funding for this project was provided by the California State University Agricultural Research Initiative Program (CSU-ARI). The authors acknowledge the help of the many individuals, including P. Vasquez, M. Triano, B. Pultrini-Aquilante, and Drs. Anil Shrestha and Denis Bacon.

References

Ayers R.S., and D.W. Westcot. 1985. Water quality for agriculture. FAO Irrigation and Drainage paper No 20 (rev. 1). FAO United Nations, Rome, Italy. 174 p.

Bañuelos, G.S., Sharmarsakar, S., Cone, D. and G. Stuhr. 2003. Vegetative approach for improving the quality of water produced from soils in the Westside of central California. Plant Soil 249, 229–236.

Diaz, F.J. and S.R. Grattan. 2009. Performance of tall wheatgrass (*Thinopyrum ponticum*, cv. 'Jose') irrigated with saline-high boron drainage water: Implications on ruminant mineral nutrition. Ag Ecosyst Envi. 131: 128–136

Grattan, S.R., Grieve, C.M., Poss, J.A., Robinson, P.H., Suarez, D.L. and S.E. Benes. 2004. Evaluation of salt-tolerant forages for sequential reuse systems: III. Implications for ruminant mineral nutrition. Agric. Water Manage. 70, 137–150.

Grieve, C.M., Poss, J.A., Suarez, D.L. and D.A. Dierig. 2001. Lesquerella growth and selenium uptake affected by saline irrigation water composition. Ind. Crops Prod. 13, 57–65.

Minson, D.J., 1990. Forage in Ruminant Nutrition. Academic Press, New York, NY, USA.

Robinson, P.H., Grattan, S. R., Getachew, G., Grieve, C.M., Poss, J.A., Suarez, D.L., Benes S.E. 2004. Biomass accumulation and potential nutritive value of some forages irrigated with saline-sodic drainage water. Animal Feed Sci Technol 111, 175-189.

Suyama, H., Benes, S.E., Robinson, P.H., Getachew, G., Grattan, S.R., and C.M. Grieve. 2007a. Productivity and quality of forages under long-term irrigation with saline-sodic drainage water
on the Westside San Joaquin Valley. Animal Feed Sci Technol 135, 329–345.

Suyama H., Benes, S. E., Robinson, P. H., Grattan, S. R., Grieve, C. M. and G. Getachew. 2007b. Forage yield and quality under irrigation with saline-sodic drainage water: Greenhouse evaluation. Agricultural Water Management 88, 159-172.