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# Irrigation Method Affects Selenium Accumulation in Forage *Brassica* Species

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## ABSTRACT

A greenhouse study was conducted in sand cultures to compare the effects of saline irrigation waters applied by two different methods, flooding and above-canopy sprinkling, on selenium (Se) accumulation by the forage brassicas, kale (*Brassica oleracea* L., cv. "Premier") and turnip (*B. rapa* L., cv. "Forage Star"). The composition of the irrigation water was designed to simulate saline (7 dS $m^{-1}$) drainage effluent commonly encountered in the San Joaquin Valley of California, and being evaluated for reuse by irrigation of salt tolerant crops. The experimental design was a randomized complete block with two irrigation methods, two plant species (kale and turnip), four Se concentrations (0.25, 0.50, 1.0, and 2.0 mg $L^{-1}$ Se–$SeO_4{}^{2-}$), and three replications. Kale was generally a more efficient Se accumulator than turnip. Shoot Se concentrations in kale and turnip increased with increasing

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Se in the irrigation waters regardless of irrigation method. Selenium was readily taken up by the leaves of the sprinkled plants to give shoot-Se concentrations that were two- to three-fold higher than in plants of the same cultivar grown under flood irrigation. Both kale and turnip can accumulate Se to concentrations that would be toxic to animals if exclusively fed this material. These Se-enriched forages may be useful as an additive to Se-deficient fodders in order to meet the nutritional requirements of livestock. The potential for phytoremediation of Se contaminated soils or waters is greatly enhanced by sprinkler irrigation via the mechanism of foliar absorption of Se. This enhanced uptake is especially important in the presence of elevated sulfate concentrations, which normally reduce Se uptake by plants.

*Key Words:* Flood irrigation; Foliar uptake; Kale; Salinity; Sprinkler irigation; Turnip.

## INTRODUCTION

Forage *Brassica* species have increased in popularity as supplemental herbage crops in North America due to their palatability, high protein content and high dry matter digestibility.[1] Several cultivars show promise for incorporation in drainage water reuse systems such as the one proposed for the San Joaquin Valley (SJV) of California.[2] The reuse of saline agricultural effluents would conserve significant amounts of good quality water, and substantially reduce the volumes of drainage water that require disposal. The composition of saline drainage effluents in this region is typically a mixture of salts with $Na^+$, $SO_4{}^{2-}$, $Cl^-$, $Mg^{2+}$, and $Ca^{2+}$ predominating, in that order, on an equivalent basis.

A limitation to the use of saline drainage waters is that they are often contaminated with potentially toxic trace elements, such as selenium (Se). In parts of the SJV, drainage waters may contain as much as $2\,\mathrm{mg\,Se\,L^{-1}}$,[3] a concentration that poses a potential health threat to humans, livestock, and wildlife. Concerns about Se toxicity to wildlife have resulted in restrictions on the discharge of irrigation drainage waters to surface ecosystems. Limits have been placed on Se concentrations allowed in drainage waters as well as development of maximum allowable daily, monthly and yearly loads of Se that can be discharged in the drainage water of various districts. Enhanced uptake of Se by crops grown with recycled drainage water would serve to reduce not only the volume of water discharged but also the Se loading to the receiving rivers and other bodies of water.

Selenium-enriched forages, if they exceed Se-feed guidelines, are still highly desirable. The forages can be used as nutritional supplements by blending with Se-deficient fodders that exist in adjacent areas (e.g., the east side of the SJV) to provide a product that would meet the nutritional requirement of livestock.

Plant Se concentrations are influenced by the plant species and by numerous soil factors, including the form and concentration of Se in the soil, soil pH, clay content, soil salinity, and the concentration of competitive ions in the soil solution.[4] Cruciferous plants have an exceptionally high requirement for sulfur.[5] This characteristic is generally associated with efficient selenium absorption,[6] although Se is not known to be essential for higher plants. Members of the *Brassica*, e.g., *B. oleracea*, *B. juncea*, *B. napus*, have been identified as strong Se-accumulators.[7,8] However, the uptake of both $SO_4^{2-}$ and $SeO_4^{2-}$ in plants is mediated by a high affinity permease and the anions compete for binding sites on this cell membrane carrier.[9]

The presence of elevated $SO_4^{2-}$ concentrations has been shown to dramatically reduce the uptake of Se for many plants[10–12] including forages grasses [clover and fescue,[14] tall fescue.[15]] For example, for tall fescue at a Se solution concentration of 3 mg $L^{-1}$ (37 μM), the Se in the shoots was 1060, 26.2 and 13.8 μg $g^{-1}$ in the presence of 0.25, 10 and 30 mM $SO_4$.[15] Several other factors, in addition to anion competition, may contribute to reductions in plant-Se, e.g., reduced $SeO_4^{2-}$ activity in saline solutions and feedback inhibition from internal tissue S levels.[16]

Selenate readily penetrates the leaf cuticle. Foliar applications of Se are generally effective in raising crop-Se levels in regions where Se concentrations are inadequate to provide proper Se nutrition and assure optimum growth of livestock.[17] However, this is not true for all species.[18] Plant-Se concentration is linearly correlated with the concentration of foliarly-applied Se under conditions of Se deficiency.[19] Likewise, $SO_4^{2-}$ is rapidly absorbed by leaves and translocated in the phloem to other plant parts.[20] Under sprinkler irrigation, an ion present in the irrigation water can accumulate in plant leaves by two pathways: (a) by root uptake and transport to the leaves; and (b) by penetration of the leaf cuticle and translocation to other areas of the leaves and to other plant organs. Inasmuch as the ion enters the plant by two discrete pathways under sprinkler irrigation, its concentration in the leaves would be expected to be higher than in leaves of flood-irrigated plants. The composition of the irrigation water may influence Se uptake by either or both processes of root uptake and foliar absorption. For example, the competitive processes involved in Se uptake and transport in the roots may not be present when irrigation waters containing both $SO_4^{2-}$ and $SeO_4^{2-}$ are applied as a foliar spray, as is the case with sprinkler irrigation. To our knowledge, no data have been reported on the Se accumulation by plants sprinkle-irrigated with sulfate-dominated waters that are also contaminated with Se.

This study was conducted to: (a) determine the uptake of Se in forage kale and forage turnip as related to Se concentration in sulfate-dominated saline waters, and (b) to evaluate the use of sprinkler irrigation instead of surface irrigation as a management practice to enhance Se uptake (via foliar adsorption) in the presence of sulfate-dominated saline waters.

## MATERIALS AND METHODS

Forage kale (cv. Premier) and forage turnip (cv. Forage Star) were grown in 24 sand tanks in a greenhouse in Riverside, CA. The tanks (1.2 by 0.6 by 0.5 m deep) contained washed sand having an average bulk density of $1.4\,\mathrm{Mg\,m^{-3}}$. At saturation, the sand had an average volumetric water content of $0.34\,\mathrm{m^3\,m^{-3}}$. On 7 June 1999 seeds of each species were planted in four rows per tank. The rows were spaced about 12 cm apart with 25–30 seeds per row. The plants were irrigated three times daily with a saline solution consisting of (in mM): $7.3\,\mathrm{Ca^{2+}}$, $5.7\,\mathrm{Mg^{2+}}$, $50.9\,\mathrm{Na^{+}}$, $6.0\,\mathrm{K^{+}}$, $25.9\,\mathrm{SO_4{}^{2-}}$, $24.7\,\mathrm{Cl^{-}}$, $6.0\,\mathrm{NO_3{}^{-}}$, $0.17\,\mathrm{KH_2PO_4}$, 0.050 Fe (as sodium ferricdiethylenetriamine pentaacetate, $0.023\,\mathrm{H_3BO_3}$, $0.005\,\mathrm{MnSO_4}$, $0.0004\,\mathrm{ZnSO_4}$, $0.0002\,\mathrm{CuSO_4}$, and $0.0001\,\mathrm{H_2MoO_4}$ made up with city of Riverside municipal water. Electrical conductivity ($EC_1$) of the solution was $7\,\mathrm{dS\,m^{-1}}$. Twelve tanks were flood-irrigated; 12 were sprinkler-irrigated. All tanks received the same amount of water throughout the experiment. Each irrigation cycle was of 15 min duration, sufficient to completely saturate the sand, after which the solutions drained back into the 765 L reservoirs for reuse in the next irrigation. Water lost by evaporation was replenished automatically each day to maintain constant electrical conductivities in the solutions. One week after planting four Se treatments were initiated by adding $Na_2SeO_4$ to the saline irrigation waters. Selenium concentrations were: 0.25, 0.50, 1.0, and $2.0\,\mathrm{mg\,L^{-1}}$ (3.18, 6.35, 12.7, and $25.4\,\mu\mathrm{M}$, respectively). Each Se treatment was imposed on plants in three tanks by flood irrigation and in three tanks by sprinkler irrigation. The pH of the irrigation waters was essentially constant ($\sim$7.8) due to buffering by the alkalinity present in the water.

Four weeks after planting, shoots were harvested for Se analysis. Samples were washed in deionized water to remove the residual Se on leaf surfaces, dried in a forced-air oven at 70–75° for 48 h, then ground in a Wiley mill to pass a 60-mesh screen. Selenium was analyzed by the method described by Briggs and Crock.[21] Total-S, Total-P, Ca, Mg, Na, and K were determined on nitric–perchloric acid digests of shoot tissues by inductively coupled plasma optical emission spectrometry.

A split plot design was used to evaluate differences between species and Se concentrations. Statistical analyses of the Se data were performed by analysis of variance with mean comparisons at the 95% level based on Tukey's studentized range test. SAS release version 6.12 was used.[22]

## RESULTS AND DISCUSSION

As shown in Fig. 1, turnip shoot Se concentrations increased with increasing Se in the irrigation water. The increase in shoot Se was approximately linear with the increase in Se solution both for sprinkler irrigation and flood irrigation. The presence of $SO_4^{2-}$ served to greatly suppress Se uptake. At a Se concentration of 2 mg $L^{-1}$ in the presence of nutrient concentrations (~1.5 mM) $SO_4^{2-}$ other brassicas contain about 1000 mg Se per kg dry weight.[23] In contrast, turnip Se concentrations were only 60 mg $kg^{-1}$ dry weight in the presence of 2 mg $L^{-1}$ Se and 25.9 mM $SO_4^{2-}$ in the irrigation water (Fig. 1). Selenium uptake by turnip was greatly enhanced by foliar absorption, as the Se concentrations in the shoots of the sprinkler irrigated plants were approximately twice that of the flood irrigated plants (Fig. 1).

Selenium uptake by kale also increased with increasing Se in the irrigation water (Fig. 2). Selenium uptake was suppressed by the presence

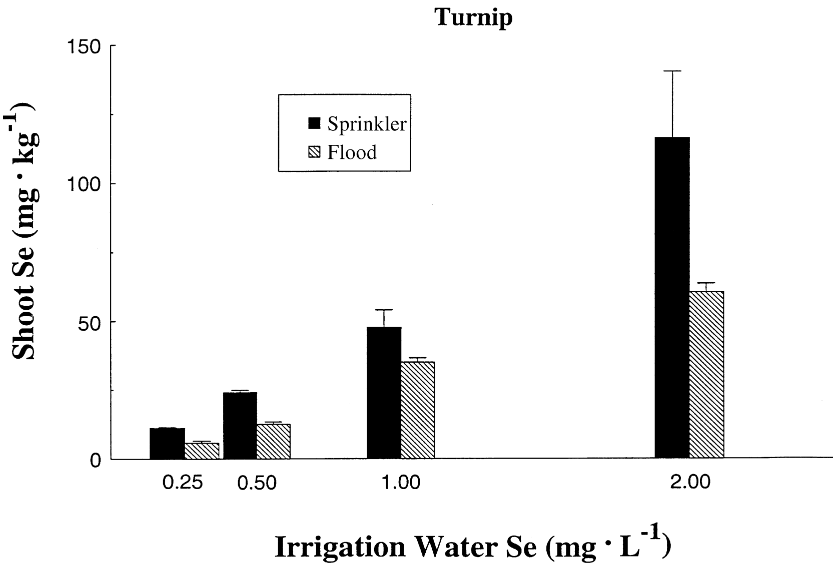

*Figure 1.* Turnip shoot Se concentration as a function of Se concentration in the irrigation water for sprinkler irrigated and flood irrigated treatments.

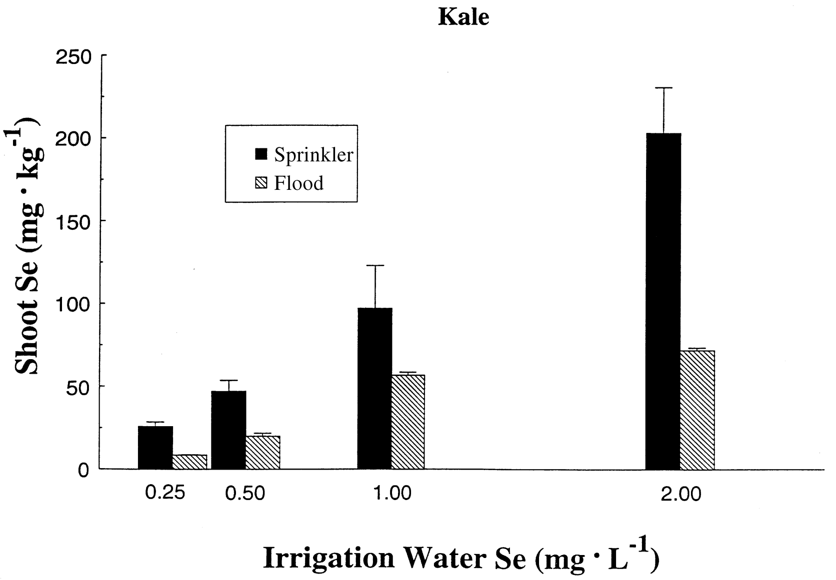

***Figure 2.*** Kale shoot Se concentration as a function of Se concentration in the irrigation water for sprinkler irrigated and flood irrigated treatments.

of $SO_4^{2-}$ as Se concentration was only 75 mg kg$^{-1}$ dry weight in the shoots. As with turnip, the increase in shoot Se was approximately linear with Se in the irrigation water for the sprinkler treatment. At the highest Se concentration under flood irrigation there was an indication that we may be approaching a plateau in the shoot Se concentration. Shoot Se in sprinkler treatments was approximately double that in the flood irrigation treatments for all but the highest Se concentration. In contrast to flood irrigation results, shoot Se in the sprinkler treatment continued to increase approximately linearly with increase in Se in the irrigation water.

The data in Figs. 1 and 2 indicate that kale is a slightly better Se accumulator than turnip under flood irrigation (root uptake). This is consistent with the increased S uptake of kale as compared to turnip (Table 1). However, kale had more than twice the foliar uptake of Se as compared to turnip (Fig. 3). Foliar uptake in Fig. 3 was calculated as the difference in Se uptake in the flood and sprinkler treatments. The increased foliar uptake of Se by kale as compared to turnip is likely the result of morphological differences, as the rough leaf surface of kale may hold more of the sprinkled water. Consistent with this idea, the S (Table 1) and Cl (data not shown) concentrations also

***Table 1.*** Chemical analyses of kale and turnip grown under flood and sprinkler irrigation at different irrigation Se (selenate) concentrations.

| Species | Irrigation Method | Irrigation Water Se (mg/L) | Plant composition (mmoles $Kg^{-1}$ dry weight) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ca | Mg | Na | K | P | S |
| Kale | Flood | 0.25 | 694 | 296 | 1,110 | 1,150 | 114 | 634 |
| | | 0.50 | 651 | 327 | 1,130 | 1,140 | 161 | 740 |
| | | 1.00 | 693 | 373 | 1,500 | 843 | 153 | 978 |
| | Sprinkler | 0.25 | 497 | 408 | 3,460 | 563 | 88 | 1,150 |
| | | 0.50 | 510 | 393 | 3,380 | 428 | 94 | 1,150 |
| Turnip | Flood | 0.25 | 752 | 248 | 411 | 1,870 | 101 | 415 |
| | | 0.50 | 770 | 263 | 478 | 1,830 | 134 | 481 |
| | | 1.00 | 752 | 306 | 767 | 1,340 | 150 | 596 |
| | Sprinkler | 0.25 | 725 | 310 | 1,410 | 1430 | 104 | 597 |
| | | 0.50 | 717 | 308 | 1,700 | 1200 | 123 | 651 |
| | | 1.00 | 649 | 434 | 2,180 | 636 | 142 | 933 |

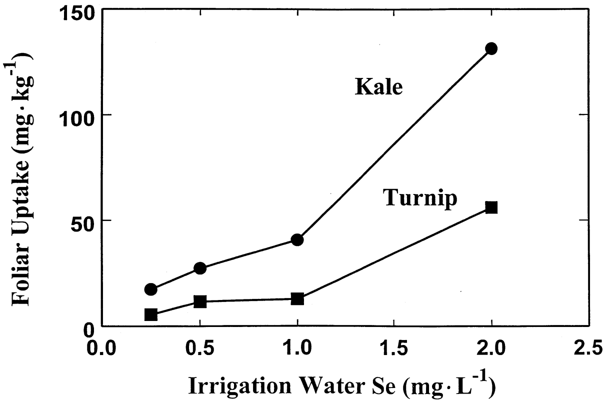

***Figure 3.*** Foliar Se uptake for turnip and kale as a function of Se concentration in the irrigation water.

increased more for kale as compared to turnip as a result of sprinkler irrigation.

Selectivity of Se over S in the plant was made by comparison of the Se/S ratios in solution and in the shoot. As shown in Fig. 4, both kale and turnip have moderate preference for Se as compared to S, regardless of irrigation

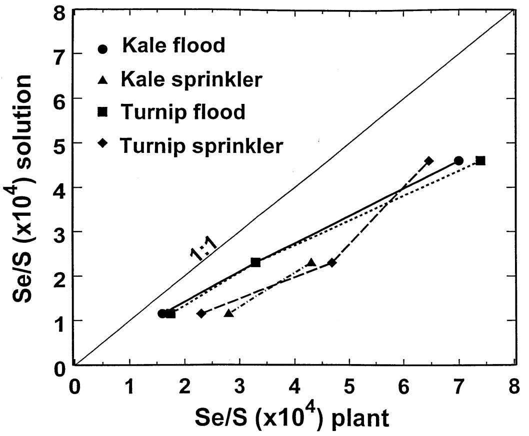

***Figure 4.*** Molar Se/S ratio in solution as compared to Se/S ratio in the plant. Multiply the reported values by $10^4$ to obtain the actual values.

method. Foliar uptake showed slightly greater Se preference than root uptake. The Se/S selectivity was the same for both plants, suggesting a similar mechanism and that Se uptake can be related to the Se/S ratio in solution and the S uptake of the plant.

The Se uptake of kale and turnip can be contrasted to that of other *Brassica* species. Banuelos et al.[24] measured Se uptake by wild mustard (*B. juncea*) of 1.9 mg kg$^{-1}$ in roots to 2.8 mg kg$^{-1}$ in young leaves in the presence of 0.2 mg L$^{-1}$ Se and 60 mM $SO_4$. If we consider that the Se uptake is proportional to the Se/S ratio in solution as found in our study then the uptake of 2.0–2.8 μg g$^{-1}$ for wild mustard leaves at a Se/S ratio of $41.4 \times 10^{-6}$ in the irrigation water is comparable to our measured values for flood irrigation. Under flood irrigation we determined 5.7 and 8.5 μg g$^{-1}$ Se for turnip and kale, respectively at a Se/S solution ratio of $120 \times 10^{-6}$. On this basis root Se uptake was relatively similar for all three species, and the Se/S ratio in solution served as a good predictor of Se/S uptake. Similarly, Kopsell and Randle[8] reported on Se accumulation of *B. oleracea* at three concentrations of added Se in the presence of 1.5 mM S. From their data we calculate Se/S ratios of each of the Se additions as follows: at 1.25 mM Se the Se/S ratio was 0.026 in solution and 0.026 in the leaves, at 2.5 mM Se the Se/S ratio was 0.052 in solution and 0.053 in the leaves; at 7.5 mM Se the Se/S ratio was 0.078 in solution and 0.072 in the leaves. Similar results are obtained from their analysis of the roots and shoots. These results indicate that for *B. oleracea* there is no discrimination of Se over S. Based on the results in Fig. 4 and the calculations based on previously published reports we conclude that for *Brassica* species studied, there is slight to no discrimination for Se over S in regards to uptake and transport.

For livestock fodder, the critical Se level between nontoxic and toxic concentrations has been established as 5 mg kg$^{-1}$ dry matter.[25] Animals that consume feed containing Se in excess of this concentration over a period of several weeks may exhibit disorders associated with chronic selenosis. These critical concentrations were exceeded by all treatments in this experiment and are consistent with the ability of these forages to accumulate Se. This fodder cannot be used without dilution, but should be an especially valuable resource for Se supplementation of forage in adjacent, Se deficient regions. In order to be economically viable the supplement would need to provide high Se concentrations that would necessitate the export of only small volumes of supplemental forage. Both species will satisfy this criterion.

The Se uptake results indicate that sprinkler irrigation of high Se drainage water is a potentially useful management option for doubling plant Se uptake and decreasing Se leaching to ground or drainage waters. Sprinkling may thus enhance the phytoremediation process for high Se waters, although Se uptake is highly suppressed in the presence of S. For kale and turnip the Se/S ratio in the plant was within a factor of 1.5 to 2 times greater than that in solution.

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