



## The Potential of Phytoremediation Techniques for Selenium Removal

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### Introduction

The San Joaquin Valley in California was historically a landscape with extensive wetlands. Due to the fact that the soil was very rich, 90% of the wetlands were converted to farmland. Because of the poor drainage in these areas, the Bureau of Reclamation proposed construction of the San Luis Drain. The San Luis Drain was to be a 188-mile concrete channel that would carry drain water from 300,000 acres of farmland. In addition to the channel, regulatory reservoirs were planned along the drain to hold and evaporate excess water during certain times of the year to keep the flow out of the drain even. Twelve 100-acre, 3-ft.-deep drainage cells were constructed on a 5900-acre parcel of land on the Kesterson site in 1971. Construction on the drain was later halted, so flow ended at Kesterson. The site was later managed by the U.S. Fish and Wildlife Service as a replacement of a small portion of lost habitat and was consequently named Kesterson National Wildlife Refuge. Until 1978, the water flowing into the refuge was fresh surface water. After this time, an increase in irrigation in the surrounding fields caused the water entering the Kesterson reservoir to be entirely sub-surface agricultural drain water (Engberg et al. 1998). By 1982, the U.S. Fish and Wildlife Service had begun to see high rates of reproductive failures and birth defects in waterfowl nesting at Kesterson. From 1982 to 1985, the area was investigated for sources of contamination, and the cause determined to be high levels of selenium (Se) from irrigation runoff (U.S. Fish and Wildlife Service 1996). Water Se concentrations greater than  $5 \text{ ug L}^{-1}$  are cause for aquatic life protection by EPA standards. The levels at Kesterson were about  $300 \text{ ug L}^{-1}$  (Engberg et al. 1998). In 1986, the U.S. Bureau of Reclamation halted the routing of agricultural wastewater to Kesterson. In 1988, they dewatered the site and filled in all low-lying areas to 15 cm above the expected average seasonal rise of groundwater, so the site would not have standing water again. Selenium contamination was thought to be less harmful to terrestrial animals than to the waterfowl (Ohlendorf and Santolo 1994).

Selenium poses a unique environmental problem. In small quantities, it is an essential nutrient in the diets of most animals. In slightly greater concentrations, it becomes toxic to wildlife, as was made tragically evident in the San Joaquin Valley. In addition to endangering waterfowl, high selenium concentrations in terrestrial forage plants have caused "alkali disease" symptoms in grazing livestock. Such symptoms include hoof disorders, horn lesions and loss of mane and tail hair. Selenosis can also cause liver and heart degeneration (O'Toole and Raisbeck 1998). Excessive amounts of selenium in the diets of fish results in the accumulation of selenium in eggs and teratogenic birth defects in the offspring. The difference between a beneficial and a detrimental dose is quite small; as little as 7 to 30 times the dietary requirement in fish will cause teratogenic defects (Lemly 1998). In 1999, fifteen years after the discovery of the original waterfowl deformities, researchers discovered that one-third of the mice they examined inhabiting the former Kesterson Wildlife Refuge had dual sex organs. The cause has not yet been determined (Vogel 1999).

Simply filling contaminated wetlands with soil, as was done at the Kesterson reservoir, is not a solution for high selenium levels, because the excess selenium is still present and potentially mobile. A way must be found to not only clean up the areas with particularly high Se contamination, but also a method to prevent future accumulation in high risk areas. An inexpensive, environmentally-friendly method of selenium remediation is in great need for the Kesterson area as well as other areas with high selenium concentrations. In this paper, phytoremediation, a promising technique for selenium clean-up will be described and the results of recent research discussed. The direction selenium phytoremediation may take in the future will also be addressed.

## **Selenium in the Environment**

Selenium cycles through the environment. It volatilizes from marine waters and terrestrial deposits and is suspended in the atmosphere. Atmospheric Se is deposited back on the Earth's surface either through rain or through particles dropping out of suspension. Selenium can also move from the marine system to the terrestrial as continents thrust upward from the sea. When left undisturbed, this cycle balances itself. Anthropogenic activities, such as the refining and combustion of fossil fuels, metal production, mining and farming nonarable land have drastically increased the load of selenium being cycled through the environment. These human endeavors release Se into the environment that would have remained immobile for millions of years.

Soils with naturally high concentrations of Selenium occur in regions with arid climates where the soil comes from sedimentary rock of marine origin. In such areas, like California, irrigation is essential for any agricultural production. In these soils, selenium is most often present in the form of selenate ( $\text{SeO}_4^{2-}$ ) and selenite ( $\text{SeO}_3^{2-}$ ), both of which are water-soluble and very mobile (Haygarth 1994). When agricultural fields with seleniferous soils are excessively irrigated to flush out the saline build-up from irrigation, trace elements, including selenium, are carried away with the salts. The mobile selenium can then accumulate wherever water accumulates, such as the drainage reservoir at the Kesterson Wildlife Refuge (Haygarth 1994).

## **Background on Phytoremediation**

Phytoremediation is a form of bioremediation, which is the use of biological processes to detoxify a site. Phytoremediation specifically is the use of plants to remove pollutants from the environment or render them harmless (Raskin 1996). Several species of plants have been shown to have the ability to grow in contaminated soils and actually extract the pollutant from the growth medium. These plants function in several different ways. Some plants can hyperaccumulate toxic trace elements in their tissues. Hyperaccumulators can accumulate hundreds and thousands of milligrams of Se per kilogram dry matter (DM), while nonaccumulator plants do not accumulate more than 50 mg Se  $\text{kg}^{-1}$  DM (Bañuelos et al. 1997). Others can convert the pollutants to less toxic compounds and volatilize them (Terry and Zayed 1994, Brooks 1998). Some aquatic plants' roots can filter contaminants from water (Brooks and Robinson 1998). Phytoremediators have been studied for use in cleaning up heavy metals like aluminum (Al), cadmium (Cd), chromium ( $\text{Cr}^{3+}$  and  $\text{Cr}^{6+}$ ), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). Phytoremediation has also been tested for clean-up of explosives like 2,4,6-trinitrotoluene (TNT), trichloroethylene (TCE) and other volatile organic chemicals,

and organic compounds such as petroleum compounds (Cunningham and Ow 1996, Thompson et al. 1998). If effective, phytoremediation can be an attractive alternative to current remediation methods because the treatment can be done *in situ*, the cost of plants is lower than most other current technologies and it is relatively environmentally safe. Using this technology lowers the total cost of the clean-up project and minimizes the disturbance the remediation will cause in the environment. There are limitations, however. One of the problems associated with phytoremediation is that the technology is still very new and is not completely understood. The use of chelators to mobilize the metal ions is necessary in some instances for uptake by plant roots, and the results can be unpredictable. If the plants do not take up the metals rapidly enough, the pollutants could move off site (Cunningham and Ow 1996). The plants that are the best hyperaccumulators are very small plants and do not produce high biomass (Bañuelos et al. 1997). A phytoremediation project may take several years to show results (Cunningham and Ow 1996).

### **Phytoremediation and Selenium**

After the discoveries at Kesterson were made, research into clean up procedures became a priority. The U.S. Department of Agriculture has had selenium phytoremediation studies going on for several years (Bañuelos et al. 1997), and the University of California Berkeley Phytoremediation Lab has done extensive work on selenium as well (Phytoremediation Research Lab). Several different methods are emerging. Plants that hyperaccumulate and plants that volatilize selenium have been identified in both terrestrial systems and aquatic systems. Extensive lab work and small field studies have been done and published for both terrestrial and aquatic systems.

Elemental selenium is not mobile, and thus, relatively benign. The total soil selenium concentration, therefore, is not the issue of concern in remediation. It is the concentration of mobile selenium, usually selenate, that determines the need for treatment. The change in mobile Se concentration is the measure of success. Plants that accumulate or volatilize selenium take up what is extractable. Since mobile, extractable selenium is presumed to be what is harmful, chelators have not been necessary. Studies are still needed to determine whether elemental selenium is mobilized when extractable Se is removed, and the rate at which this process occurs (McGrath 1998).

### **Phytoaccumulation**

Early investigations into phytoaccumulation sought to identify high biomass-producing species with the ability to hyperaccumulate selenium. Members of the Brassicaceae family have shown the ability to take up selenium at high rates, especially Indian mustard (*Brassica juncea* Czern. L.) and canola (*Brassica napus* cv. Westar). Study plots at Kesterson Reservoir were directly seeded with canola in rows at a density of 25 to 30 plants per square meter. The plants were irrigated to replace water lost due to evaporation only. Total soil Se concentrations prior to planting were 25.5 mg Se kg<sup>-1</sup> soil to 30 cm soil depth, 6.8 mg kg<sup>-1</sup> from 30-60 cm deep and 1.8 mg kg<sup>-1</sup> from 60-90cm in depth. The extractable Se concentrations for these depths were .45 mg Se L<sup>-1</sup> water, .32 mg Se L<sup>-1</sup> and .60 mg Se L<sup>-1</sup>, respectively. Nearly all extractable selenium was removed from the canola plots after 90 growing days. The post-plant concentrations at all 3 soil depths were less than .01 mg Se L<sup>-1</sup> (Bañuelos et al. 1998). A three year field study was done

using the same procedures comparing Indian mustard, tall fescue (*Festuca arundinacea* Schreb. L.), birdsfoot trefoil (*Lotus corniculatus* L.) and kenaf (*Hibiscus cannabinus* L.). Each year a reduction in the soil levels was seen for all four species. The Indian mustard accumulated higher concentrations of Se than the other plants at the end of each season. Indian mustard accumulated an average of 1373 mg Se kg<sup>-1</sup> DM in its shoots and 805 mg kg<sup>-1</sup> DM in its roots each season. The other species averaged between 239 and 544 mg kg<sup>-1</sup> DM in their shoots and between 117 and 399 mg kg<sup>-1</sup> DM in their roots. Indian mustard accumulated the highest total quantity of Se and reduced the total soil Se content by almost 50% at a depth of up to 75 cm after 3 years (Bañuelos et al. 1995).

The disposal of accumulator crops after use in the field is currently being researched. Bañuelos and Mayland (1999) performed a study done on sheep who were fed canola from Se-laden sites, and results show that accumulators can be carefully blended with non-Se accumulating forage to provide the dietary requirement of selenium (Agricultural Research Service 1999). Clippings from perennial accumulators such as birdsfoot trefoil and tall fescue may be more suited for use as animal feed due to lower tissue accumulation (Bañuelos et al. 1995).

### **Phytovolatilization**

Superior terrestrial Se volatilizers also tend to be members of the Brassicaceae family, such as cabbage (*Brassica oleracea* var. *capitata*), broccoli (*Brassica oleracea* var. *botrytis* cv. Green Valiant), cauliflower (*Brassica oleracea* var. *cauliflora*) and Indian (called wild brown mustard in this paper) and Chinese mustards (*Brassica juncea* Czern. L. and *B. campestris* var. *chinesis*, respectively). Other plants, such as rice (*Oryza sativa* L.) (Zayed and Terry 1994) and hybrid poplars (*Populus tremula* H *alba* L.) (Pilon-Smits et al. 1998) have been shown to have high rates of selenium volatilization. Under optimum lab conditions, these plants can generally volatilize Se at rates of 1.5-2.5 mg<sup>-1</sup> kg dry mass day<sup>-1</sup> when supplied nutrients solutions containing 1.6 mg Se L<sup>-1</sup> (Bañuelos et al. 1997). In growth chamber studies by Zayed and Terry (1994), the roots of these plants volatilized 7-20 times faster than the shoots, despite the fact that the roots make up less than 20% of the plant dry mass. Once the roots were determined to be the primary site of volatilization, the rate of volatilization from the detopped roots was measured. In broccoli, rice, cabbage, cauliflower and the mustards, the rate of Se volatilization was 1.5-5 times faster than that of the intact root. The rate continued to increase for the following 48 hours getting up to 20-30 times the intact root rate (Zayed and Terry 1994, Terry and Zayed 1994). Planting and harvesting annual crops, such as rapidly growing *Brassica* species, in rotation may be a possible method to remove extractable soil selenium (Terry and Zayed 1994). These plants would be seeded as they normally would in cultivation for production, because high biomass is desired. They would be disposed of in the same manner as the hyperaccumulators.

### **Rhizofiltration**

Few studies have been done on rhizofiltration of Se by wetland species. In the lab, duckweed (*Lemna minor* L.), a floating aquatic plant, was shown to accumulate comparable concentrations to other known Se accumulating plants (Zayed et al. 1998). In a similar study, water hyacinth (*Eichhornia crassipes*) was shown to be a moderate accumulator of Se. Both of these plants are commonly used in constructed wetlands for wastewater treatment (Zhu et al. 1999).

Laboratory investigations have documented several aquatic species which also volatilize selenium. In an experiment comparing twenty wetland species, mare's tail (*Hippuris vulgaris* L.) and azolla (*Azolla caroliniana* Willd.) were responsible for the two highest rates of both selenate and selenite volatilisation. These plants showed similar rates of volatilization to Indian mustard (Pilon-Smits et al. 1999). Saltmarsh bulrush (*Scirpus robustus*) and cattail (*Typha latifolia* L.) also showed respectable rates in this experiment and have been shown to perform well in field experiments (Hansen et al. 1998). According to the results of the investigation by Pilon-Smits et al. (1999), a constructed wetland will be able to extract five times more Se from selenate-contaminated wastewater than from selenite-wastewater, therefore a constructed wetland will be more efficient in treating agricultural runoff than oil refinery wastewater. Selenate is more prevalent in agricultural drainage and selenite occurs in higher concentrations in refinery wastewater (Pilon-Smits et al 1999).

### **Future of Selenium Phytoremediation**

Investigations into the use of phytoremediation for selenium removal are still needed. Much more field work needs to be done to determine the extent to which plants can remediate selenium-laden soils. Multi-year field studies are in progress in California. Bañuelos and Chaney are currently including phytoremediators as a part of normal agronomic crop rotations on 40 acres of contaminated farmland (G. Bañuelos, personal communication). Practical field studies employing multiple methods of remediation are also needed; for example, phytoremediation in coordination with use of Se-volatilizing microbes, improved irrigation practices, and treatment of irrigation effluent (Bañuelos et al. 1995).

Lab-based investigations will likely focus on improving the ability of certain plants to hyperaccumulate and volatilize selenium. Investigations into the mechanisms for volatilization and accumulation will hopefully lead to a better understanding of these processes, and ways of enhancing the processes could be delineated (Terry and Zayed 1994). A breeding program could produce better and stronger phytoremediators from the species already identified. The target characteristics for this breeding program would include high rates of Se absorption and volatilization, fast growth rate, high biomass production, salinity tolerance and low water requirements. Identifying the genes responsible for volatilization and hyperaccumulation and ultimately transferring them into plants producing high biomass could increase the efficiency and potentially decrease the time frame for Se control (Bañuelos et al. 1997).

### **Conclusion**

Recognizing the limitations of phytoremediation for Se-contaminated sites will be the key to putting this technique to use. Soil phytoremediation is a slow process, due in part to the small size of the plants that best hyperaccumulate selenium and in part to the poor conditions of the sites for plant growth. Sites predisposed to Se contamination are usually arid, have high salinity levels due to the necessary irrigation and are generally poor environments for plant production. However, phytoremediation shows great potential to be a tool for managing contaminated sites, as long as it is not looked at as the complete solution. It will probably be most successful when combined with other methods of management, such as reduced irrigation levels and drainage water treatment.

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