

# Removal of Aqueous Selenium by Four Aquatic Plants

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## ABSTRACT

Several aquatic species were examined as potential phyto-removal agents for selenium in aqueous solutions. Selenium was initially present in concentrations of 0-100 ppm Se (as sodium selenite) in 10% Hoagland's medium, and aquatic plants were grown in the medium for one week. Four aquatic plants were studied: Cattail (*Typha domingensis*), duckweed (*Lemna obscura*), hydrilla (*Hydrilla verticillata* Royle), and swamp lily (*Crinum americanum*). Analyses were done by atomic absorption spectrometry using hydride reduction. Four replicates were done for each analysis. Each system was examined for change in fresh weight, percent removal of selenium from solution, and accumulation of selenium in the plant. At selenium concentrations of 100 ppm or less, fairly good to excellent removal was achieved (65 to 100%), depending on the plant. Exposure to concentrations greater than 100 ppm had an inhibitory effect on plant growth, so concentrations

less than 100 were studied in more detail. During a one-week period, hydrilla quantitatively removed the selenium, and the fresh weight and dry weights of the plant increased. Other plants were less effective in removal of selenium or were more adversely affected by added selenium.

*Key words:* phytoremediation, hydrilla, duckweed, cattail, and swamp lily.

## INTRODUCTION

The process by which aquatic plants improve water quality by transferring and accumulating metals and excessive nutrients into their biomass is known as phytoremediation. Phytoremediation is an innovative technology, which involves the use of naturally occurring plants and microorganisms to remediate contaminated sites.

The use of plants and microorganisms that can naturally volatilize the selenium and remove it from the soil or water has been studied with promising results (Pilon-Smits et al. 1999). Selenium can be removed from soils by plant uptake and accumulation (phytoaccumulation), plant volatilization (phytovolatilization),

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tilization), and removal in the rhizosphere (rhizodegradation) (Terry and Zayed 1998). Selenium removal by phytoaccumulation depends upon which chemical species of selenium is present in the polluted soils and water (Mikkelsen et al. 1989, Blaylock and James 1994). The oxidized forms of selenium, selenate and selenite, are more readily available to plants because of their high solubility. The reduced forms, elemental selenium and most selenides (excluding the alkali metal selenides), are insoluble and, therefore, are unavailable. Furthermore, the uptake of selenate and organic selenium is metabolically driven, unlike the uptake of selenite, which may be passive (Ulrich and Shrift 1968, Abrams et al. 1990). Organic forms of selenium, such as dimethyl selenide, may be more readily available for uptake by plants rather than inorganic forms, such as elemental selenium (Williams and Maryland 1992).

Once the plants have successfully accumulated and stored selenium in their plant tissues, the plant tissue must be harvested, removed from the contaminated site, and disposed safely. An advantage with selenium remediation is that selenium is an essential trace element for adequate nutrition and health in mammals. One safe disposal method would involve using the selenium enriched plant material as forage for animals with low selenium levels. Another option would be to use the selenium-enriched plant material as organic selenium fertilizer and add it to forage crops. Finally, if the selenium-enriched plant material also absorbed undesired toxic elements such as mercury and arsenic at levels that exceeded the safe limits for animal consumption, the plant tissue could be used as fuel to generate electricity.

Selenium can also be removed by phytovolatilization. The concept of biological volatilization of selenium from selenium-contaminated soils is based upon interactions between the soil and microbes (Frankenberger and Karlson 1988) and plants (Duckart et al. 1992, Terry et al. 1992, Biggar and Jayaweera 1993). The idea of using plants, which are able to perform phytovolatilization of selenium, is a very attractive method of phytoremediation because the selenium is completely removed from the local ecosystem by being released to the atmosphere in relatively nontoxic volatile forms (Terry et al. 1992, Terry and Zayed 1994). Studies have shown that the addition of plants to the soil increased the rate of volatilization of selenium (Zieve and Peterson 1984, Biggar and Jayaweera 1993, Duckart et al. 1992). It has also been demonstrated that the volatile forms of selenium, released by the plant, are released directly through the plant tissues (Beath et al. 1935, Lewis et al. 1966). The volatile forms of selenium released vary between selenium accumulators and non-accumulators. Typically, plants referred to as selenium accumulators predominately release dimethyldiselenide (Evans et al. 1968), while plants referred to as non-accumulators mainly release dimethylselenide.

The last mechanism of phytoremediation that has been observed in selenium-contaminated areas is rhizodegradation. It has been observed that when the shoots of plants have been removed, increased rates of selenium volatilization result. This has been explained by the fact that when the shoots of a plant are removed, reduced carbon compounds, including free amino acids, leak into the rhizosphere thus accelerating the production of volatile selenium rhizosphere microorganisms (Terry and Zayed 1998). Studies have shown

that although volatilization of selenium can occur without rhizosphere microbes, the rate of volatilization is increased in their presence (Terry and Zayed 1994, Azaizeh et al. 1997).

In our studies, *Typha domingensis*, *Lemna obscura*, *Hydrilla verticillata*, and *Crinum americanum* were individually evaluated for phytoremediation of selenium. The percent removal of selenium, amount of selenium accumulated in the plant tissue, and changes in the weight of the plants after exposure to selenium were examined. The maximum contaminate level of selenium in drinking water allowed by the EPA is a value of 0.05 ppm (United States Environmental Protection Agency, Office of Ground Water and Drinking Water). In this study, concentrations significantly higher than the maximum amount allowed were selected, in order to examine the phytoremediation potential of these selected wetland species when exposed to extremely elevated levels of selenium in the water.

## MATERIALS AND METHODS

*Plants and plant sources.* The plants selected were aquatic species common to Florida wetlands. Cattails (*Typha domingensis*) and hydrilla (*Hydrilla verticillata* Royle) were obtained from the pond behind University Square Mall, which is adjacent to the west side of the USF Tampa campus. Duckweed (*Lemna obscura* Aust.) and swamp lily plants (*Crinum americanum* L.) were obtained from Delany Creek at the point that it crosses South 36th street in south Tampa. The plants were collected in the morning and brought back to the laboratory where they were rinsed in deionized water, and any unwanted debris was removed prior to being placed in half-strength Hoagland's medium (Steward and Elliston 1973) and kept in the Phytotron room. The medium was changed biweekly and no studies were done before a month had elapsed.

*Phytoremediation studies.* Several studies were conducted in order to determine if selected wetland species were capable of reducing toxic soluble forms of selenium into either an insoluble or volatile and less toxic form. All phytoremediation studies were conducted in the Phytotron room (Environmental Growth Chambers, Chagrin Falls, OH), located in room 119 of the Science Center with a photo period of 12 hours light/12 hours dark, a relative humidity of 80%, constant temperature of 26C, and light intensity of 190  $\mu\text{mol photon m}^{-2} \text{sec}^{-1}$  (as measured with a LI-COR model LI-185A photometer). Sodium selenite was used in all phytoremediation experiments. A stock solution was diluted to the desired concentrations with Hoagland's medium. The wetland plants chosen were aquatic species common to Florida wetlands. All plants chosen were obtained from local sites in the Tampa Bay area. The plants used in the phytoremediation studies were cattails (*Typha domingensis*), duckweed (*Lemna obscura*), hydrilla (*Hydrilla verticillata* Royle), and swamp lily (*Crinum americanum*).

Once the wetland species were adapted to their new environment in the Phytotron room, several selenium remediation experiments were conducted using each of the specified wetland plants. In the experiments using hydrilla and duckweed, two-gram samples were placed in sterile foam-stoppered 500-mL erlenmeyer flasks with selenium solutions containing 0, 1, 2, 5, 10, 20, 50 ppm in Hoagland's medium. In the experiments using cattails and swamp lilies, higher concentrations, 100-200 ppm were used because of the larger

biomass of the plants. Samples were in triplicate and were exposed to the selenium-enriched solutions for one week. After one week of exposure, a liquid aliquot was taken from each sample and refrigerated until analysis by atomic absorption.

The entire plant was removed from the solutions and prepared for digestion in the following manner. Each individual plant was weighed, cut, and blended. The initial fresh weight was compared with the fresh weight measured after exposure, and any physical differences observed were recorded. The plant was allowed to dry in the phytotron for one week. A dry weight was taken and each sample was placed in a 150-mL Teflon beaker. Next, 5 mL of nitric acid (16 M) and 5 mL of deionized water were added. Each sample was covered with a watch glass and warmed for 10 to 15 minutes without boiling (90 to 95°C). The sample was then allowed to cool, another 5 mL of nitric acid (16 M) was added and the sample was warmed for 30 minutes. This step was repeated, then 2 mL of deionized water and 3 mL of 30% hydrogen peroxide were added slowly, and the mixture was heated until effervescence ceased. Finally, 5 mL of hydrochloric acid (12 M) was added and the mixture was refluxed for 10 to 15 min. The sample was cooled to room temperature, then diluted to 100 mL with 6% (v/v) HCl. Next, the sample was vacuum filtered in an all glass filtration apparatus using a 0.45 µm Millipore membrane filter. Finally, the aliquot was diluted to 100 mL using a volumetric flask and then analyzed by hydride generation atomic absorption using a custom-built apparatus (Carvalho et al. 2000).

## RESULTS AND DISCUSSION

The results from all of the experiments showed that all plants examined were able to remove selenium from the hydroponic environment. All of the plants examined accumulated selenium in their tissues, but cattails and swamp lilies seemed to tolerate higher concentrations of selenium (100, 200 ppm) better. Hydrilla and duckweed were studied at lower concentrations (<50 ppm). Cattails, swamp lilies, and duckweed decreased by 75-85% in fresh weight during the studies (whereas controls showed a 4% increase). Hydrilla

showed an 56-130% increase in fresh weight over the concentration range studied during the study period.

Cattails and swamp lily plants retained the highest amounts of selenium in their tissues. However, duckweed accumulated the most selenium on a fresh-weight basis, accumulating as much as 4.95% selenium per mg of biomass. In contrast, cattails and swamp lilies accumulated only between 0.002 to 0.003% selenium per mg of dry biomass. More attention was focused on the removal effectiveness of duckweed and hydrilla over the range of 1 to 50 ppm Se (Table 1). The percent removal was essentially quantitative for hydrilla and only slightly less for duckweed (95.0 to 99.8%). The pattern was different, however, for the other two plants. Duckweed became more effective in removing selenium with increasing concentration (1-50 ppm), and at 5 to 50 ppm Se, the effectiveness was nearly quantitative (99 to 99.8%). Hydrilla, in contrast, exhibited quantitative removal at lower concentrations (1 to 10 ppm Se), but showed reduced removal effectiveness at 20 ppm (94.3% removal) and 50 ppm (92.0%). It would be tempting to ascribe the reduction to a toxic effect. A good increase in fresh weight was obtained at 0 to 20 ppm Se, then the value at 50 ppm was about the same. It is a remarkable thought that selenium would stimulate the growth of this nuisance species.

Through additional studies under confined environments (so that products of volatilization could be collected), it was verified that the main mechanism for selenium removal was phytovolatilization (Carvalho et al. 2000). All of the plants studied increased the amount of selenium volatilized, and, using gas chromatography coupled with mass spectrometry (GC-MS) analyses, it was found that the major form of selenium products produced by volatilization was some organic selenium species (Carvalho et al. 2000). This is a promising result, because organic forms of selenium are much less toxic than inorganic forms.

From these studies, we can conclude that hydrilla, duckweed, swamp lily, and cattail plants could be effective at removing selenium from the environment, and could also convert the inorganic selenium into a less toxic form. Of the

TABLE 1. PERCENTAGE OF SELENIUM REMOVAL BY AQUATIC PLANTS.

Plant	Initial solution Se conc (ppm)	Final solution Se conc (ppm)	Conc retained in Plant (ppm)	Biomass (mg)	% Se removal	% Se removal/weight
Cattails	100.3 ± 5.8	45.9 ± 2.7	50.1 ± 3.7	16300 ± 2400	54.2	0.003
Cattails	200.8 ± 4.1	62.9 ± 5.2	80.0 ± 2.8	51800 ± 7200	68.7	0.001
Swamp Lily	200.4 ± 5.2	78.0 ± 3.5	30.6 ± 4.6	27600 ± 3900	61.1	0.002
Duckweed	1.00 ± 0.05	0.05 ± 0.03	0.20 ± 0.02	50 ± 20	95.0	1.9
Duckweed	2.00 ± 0.02	0.05 ± 0.01	0.10 ± 0.04	40 ± 10	97.5	2.4
Duckweed	5.00 ± 0.78	0.05 ± 0.02	0.15 ± 0.07	40 ± 10	99.0	2.5
Duckweed	10.00 ± 1.18	0.10 ± 0.04	0.25 ± 0.06	30 ± 10	99.0	3.3
Duckweed	20.00 ± 1.82	0.10 ± 0.12	0.30 ± 0.03	20 ± 20	99.5	4.95
Duckweed	50.00 ± 2.13	0.10 ± 0.08	0.45 ± 0.12	30 ± 10	99.8	3.3
Hydrilla	1.00 ± 0.05	0.00 ± 0.08	1.10 ± 0.08	110 ± 10	100.0	0.91
Hydrilla	2.00 ± 0.02	0.00 ± 0.03	1.30 ± 0.14	130 ± 50	100.0	0.77
Hydrilla	5.00 ± 0.78	0.00 ± 0.05	0.90 ± 0.17	120 ± 10	100.0	0.83
Hydrilla	10.00 ± 1.18	0.00 ± 0.21	1.15 ± 0.06	80 ± 10	100.0	1.25
Hydrilla	20.00 ± 1.82	1.15 ± 0.22	0.85 ± 0.03	100 ± 20	94.3	0.94
Hydrilla	50.00 ± 2.13	4.00 ± 0.38	1.00 ± 0.23	150 ± 80	92.0	0.61

four plants studied, the most effective plant for removing selenium was hydrilla, but it is doubtful that anyone would advocate its use for this purpose. On the other hand, duckweed was also effective, and is an environmentally acceptable plant for phytoaccumulation of heavy metals.

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