Bioaccumulation Study of the Algal-Bacterial Selenium Removal System

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Abstract Selenium levels in agricultural drainage water of the San Joaquin Valley, CA have been found to be toxic to aquatic ecosystems. Researchers have been looking for cost-effective ways to reduce selenium concentrations in drainage water. This study looks at the effectiveness of the Algal-Bacterial Selenium Reduction System to reduce the selenium available to aquatic communities. Microcosms containing four species of aquatic invertebrates were established in each part of the system and used to study bioaccumulation. Water and soil samples from the microcosms were also taken to analyze selenium speciation throughout the system. It was found that the system is successfully reducing total selenium levels in the drainage water. However, it is also releasing a significant amount of the remaining selenium in the forms of selenite and organo-selenium, which are more bioavailable to wildlife. Invertebrate bioaccumulation showed that body burdens increased with higher levels of organo-selenium in water. Due to increasing selenite and organo-Se through the system, the selenium in the effluent of the system may be more available to wildlife than the influent. The water and soil samples confirmed that selenium is being transformed into more bioavailable forms, especially by the High Rate Pond. Recommendations were made to modify the Algal Bacterial Selenium Removal System in order to reduce the formation of these more bioavailable forms. Removal of the High Rate Pond from the system may reduce the amounts of available selenium and will be investigated in further studies. When modified, the Algal Bacterial Selenium Removal System may prove to be an effective low-cost method of selenium removal.

Introduction

Selenium (Se) is a semi-metallic element, often called a metalloid (Wilber, 1983). Although it is an essential element for animal and human nutrition, in high concentrations it has been shown to create toxicity problems in livestock and wildlife (Bainbridge et al. 1988). These high concentrations of selenium can occur due to natural enrichment of Se in soil originating from Cretaceous marine sedimentary rock (Presser et al. 1994). Selenium exists in different oxidation states. Selenate (Se^{6+}) and Selenite(Se^{4+}) are both water soluble inorganic species commonly found in aerobic water sources and elemental Se is more readily found in anaerobic sediments (Rosetta and Knight, 1995). Se also forms into selenoproteins, such as selenomethionine, analogous to the essential amino acid methionine, in which sulfur is replaced by selenium (Alaimo et al. 1994). Toxic effects of inorganic and organic Se are caused by the alteration of protein three dimensional structure and the impairment of enzymatic function of an organism (Demayo et al. 1979).

The San Joaquin Valley in California has been found to contain soils with naturally high concentrations of Se (Losi and Frankenberger, 1997). Agricultural use of the lands in the San Joaquin Valley have resulted in leaching of selenium into drainage water. Drainage water can contain selenium concentrations, mainly in the form of selenate, of 230 to 640 μg/liter (Cantafio et al. 1996). The USEPA safe criterion level for chronic exposure of aquatic life to Se is 5 μg/liter (Dobbs, 1997). In 1986, high concentrations of Se in the drainage water at Kesterson Reservoir were shown to be causing embryonic mortality and abnormalities in aquatic birds (Ohlendorf et al. 1986). These negative effects on aquatic birds from Se were due to biomagnification, the increase in concentration of a substance in living tissue as it moves through the foodweb. Through feeding and direct uptake Se can bioaccumulate, be absorbed and stored, in organisms such as small aquatic invertebrates. Birds then feed on these organisms and with each feeding more Se is being ingested than excreted and Se concentrations within the birds increase. Kesterson was declared a toxic site in 1987 and has since been filled (Presser et al. 1994). However, farmland irrigation water continues to be drained into the San Joaquin River or into on-farm evaporation ponds and levels of selenium are well above the suggested limit to protect aquatic wildlife (Presser, 1994).

Many research groups have been looking for cost-effective methods of selenium removal. It has been found that certain bacteria have the ability to reduce Se into less toxic forms or into forms that can be more easily removed (Losi and Frankenberger, 1997). An Environmental Engineering group at UC Berkeley led by Professor W.J. Oswald has developed a selenium removal system called the Algal-Bacterial Selenium Removal (ABSR) System. Agricultural drainage water enters the system where selenium levels are theoretically reduced by the bacterial and algal processes and the resulting discharge is less contaminated. This system uses a combination of ponds containing algae and bacteria in which selenate, the major form of selenium in the drainage water, is reduced to selenite and elemental selenium which are more easily removed from the system (Lundquist et al. 1994). Influent (IN) water undergoes four steps in the system. The steps of the process are summarized as follows:

1) In an anaerobic Reduction Pond (RP), bacteria reduce selenate to selenite and elemental selenium which precipitate out into a sludge on the bottom of the pond.
2) In a High Rate Pond (HP), water is re-oxygenated and cleaned for surface discharge by algae cultures and high rate movement.
3) In a Dissolved Air Flotation (DAF) device, ferric sulfate polymerizes algal cells into flocs which are carried to the surface by air bubbles and then skimmed off and removed.
4) In a slow sand filter (SSF), any remaining particles are filtered out of the water.

A pilot study using this pond system has been set up in the Panoche Water District near Los Banos, approximately 285 miles Southeast from Berkeley. Preliminary results have shown that the method may be able to reduce the total Se in the drainage water up to 80%, causing discharged water to be at concentrations much closer to suggested levels for wildlife safety (Quinn et al. 2000).

Although much of the total selenium may be removed using the ABSR system, it is essential that the remaining selenium is in a form that is not readily bioavailable to aquatic organisms. The toxicity of Se is directly related to its bioavailability (Losi and Frankenberger, 1997). Different forms of Se are more bioavailable than others. Elemental Se is the least bioavailable followed by Selenate, and then Selenite; the most bioavailable form is organic selenium, such as selenomethionine (Maier et al. 1993). In the ABSR system, if the Se that remains has been transformed into a more bioavailable form, then the system could possibly be increasing the concentrations of Se absorbed by aquatic life. In order to assure that the system is reducing the bioavailability of Se, it is necessary to study how the system effects bioaccumulation. The purpose of this study is to analyze the effectiveness of the ABSR in terms of reducing the bioaccumulation of Selenium.

In this study, microcosms were established in each step of the ABSR system to investigate Se invertebrate bioaccumulation. Other studies have shown microcoms to be an effective method for testing the bioaccumulation of toxins in invertebrates (Maloney, 1996). Se speciation in water and soil was also analyzed throughout the different steps of the ABSR system. This study hopes to show whether or not the ABSR system is successfully reducing the bioavailability and therefore the bioaccumulation of Se to aquatic organisms.

Methods

The site of the ABSR system is near Firebaugh in the San Joaquin Valley, California. Microcosms were created to test bioaccumulation in water from each of the five parts of the north ABSR system: Influent (IN), Reduction Pond (RP), High Rate Pond (HP), Dissolved Air Flotation (DAF), and Slow Sand Filter (SSF). There were a total of fifteen microcosms, with three microcosms for each part of the ABSR system. For this experiment, a sand filter was set up between the RP and the microcosm receiving the RP water in order to filter out particulates. This sand filter was only used for the water passing to the three RP microcosms and not for the water continuing on to the HP and the other microcosms.

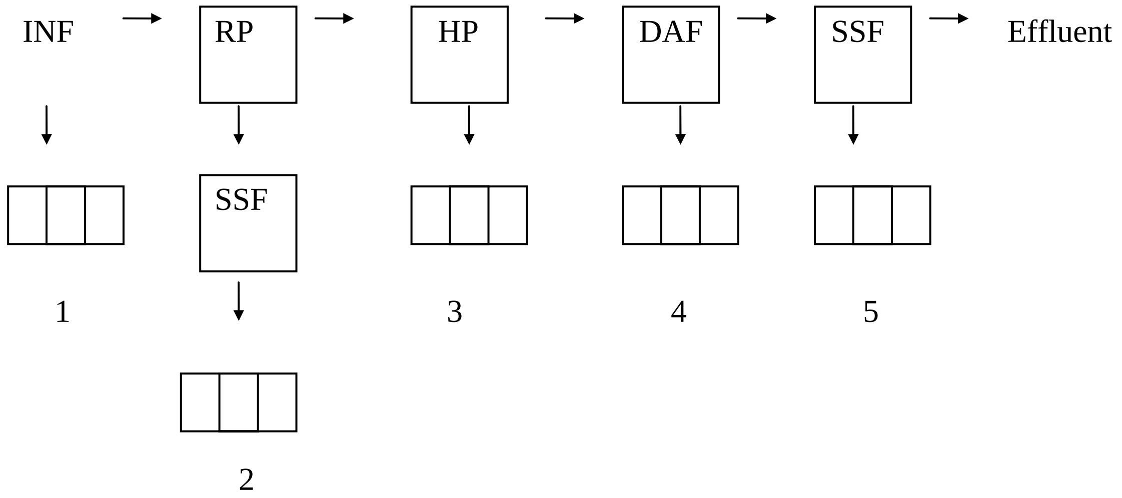

Figure 1: The ABSR System with Microcosms. In each of the five parts of the ABSR System, water is diverted to boxes containing three microcosms each.

To create the microcosms, 20 cm wide x 20 cm high x 30 cm deep polyethylene boxes were used with a 2 cm lip around the top. Rectangles 9 cm wide and 13.5 cm high were removed from two opposite sides of the box and a 200 micrometer screen was placed over it using nylon screws to attach it. Lids were created for the boxes using acrylic with a dozen small holes drilled in the top for air circulation. A cheesecloth mesh was then attached to the lid to prevent organisms from getting in or out of the holes.

Soil was collected to go into each microcosm. Soil needed to be collected from a site that would have minimal Se contamination. The collection site was located in the Sacramento Valley, an area known to have lower levels of Se. Soil was collected from ponds located behind S&W Fish Farm, located off Highway 99 in Herald, CA. About 3/4 inch to 1 inch of soil from the ponds was placed into each of the fifteen microcosms.

Five different invertebrate species were collected to put into the microcosms. The four species used were: *Chironomus tentans*, *Lumbriculus variegatus*, *Hyallella azteca*, and two different types of snails: *Helisoma* and *Physa*. The chironomids, lumbriculus, and hyallella were all obtained from a lab which raises invertebrates for other studies (M. Lydy, Wichita, KS). This insured that the organisms would have no initial Se contamination. Because the lab did not have snails, the snails had to be collected along with the soil from the ponds near the fish farm.

To accommodate the microcosms, water from each of the five steps of the ABSR system was diverted into 48 cm wide x 48 cm high x 60 cm deep boxes (holding boxes) that contained three microcosms each (Figure 1). The microcosms were placed so that water entered and ran through the screens. Water entered the holding boxes at a rate less than 1 liter per minute. A drain was installed and water in the holding boxes was kept level, about 20 cm high, just above the screens.

The soil was put into the microcosms first and then after a week the invertebrates were added. In each microcosm 30 2nd-4th instar chironomids, ~150 lumbriculus, 60-80 hyallella, 1 Physa snail, and 6-7 Helisoma snails were added. Additional samples from each species were taken for initial Se analysis. The invertebrates were put into the microcosms with the system running on September 9, 2000 and were taken down on October 10, 2000. Water samples from within each holding box were taken each week during that time. When the microcosms were taken down, all remaining invertebrates and soil samples were collected and analyzed.

To analyze the invertebrates for Se the method from He and Tulisalo (1996) was used. In this method, the invertebrates are digested in acid and the resulting liquid is then read by Atomic Absorption Spectrometry (AAS) giving Se concentrations. From the data given by the AAS and knowing the mass of the samples, the Se body burdens of the invertebrates can be found.

For the water samples, a method developed by Zhang, Moore, and Frankenberger (1999) was used. The method uses different procedures to analyze for selenite, organo-selenium, and selenate concentrations in the water. To analyze the soil, after a series of centrifuging, the final supernatant was treated using the same methods as for the water. The water and soil samples were also run in the AAS for Se concentrations. Because the water samples analyzed contained higher levels of Se than the methods were designed for, several changes were made in the protocol for Organic-Se. Different amounts of Mn^{2+} and S_2O_8 were tested for the best recoveries of Organic Se. For best results, Mn^{2+} used was increased to 105 μl and S_2O_8 increased to 600 μl. For each analytical method used, invertebrate and water procedures, standards of known Se concentrations were used to test the accuracy of the methods.

Results

Many of the invertebrate samples did not survive through the weeks of the test. There were insufficient hyallella and chironimids to make body burden comparisons between the different steps of the system. However, enough samples for at least two replicates of lumbriculus and helisoma snails (except for the HP) were found in every step. With the lumbriculus data, due to large concentration variations between samples, no significant differences were found between

Se body burdens in different steps of the system. Helisoma snail data showed a significance level of p=.0136 using Kruskall-Wallis. The Conover test for multiple comparisons showed an overall significant increase between the influent (IN) and the effluent (SSF) of the system, a significant increase between the IN and the RP, and a significant decrease between the RP and the DAF.

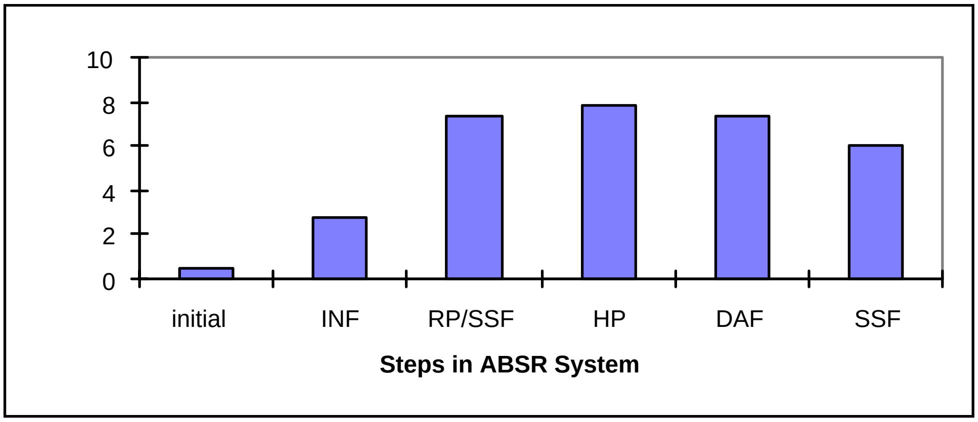

Figure 2: Mean selenium body burdens in lumbriculus samples from each step of the ABSR System.

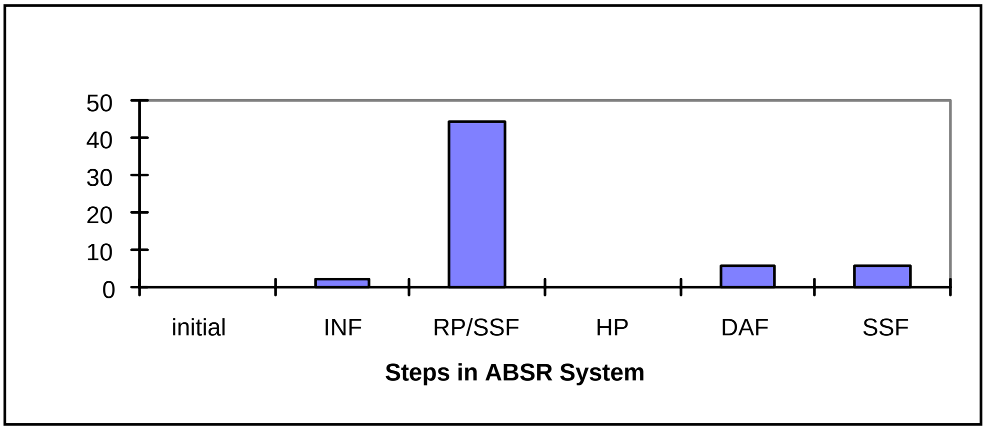

Figure 3: Mean selenium body burdens in helisoma snails from each step of the ABSR System. Missing data point at HP is due to lack of samples.

Water sample analyses resulted in concentrations of Selenate, Selenite, and Organo-Se for each step of the ABSR system. The Kruskall-Wallis test showed significant results for all three: Organo-Se (p=.0009), Selenite (p=.0002), and Selenate (p=.0039). The Conover test for multiple comparisons was used to find significance for each species of Se between the different steps of the system (Figure 4). How these three forms of Se added up to the total Se in each step of the system was also studied (Figure 5). Differences between IN and SSF show that Selenate and total Se significantly decrease through the system and Organo-Se and Selenite significantly increase.

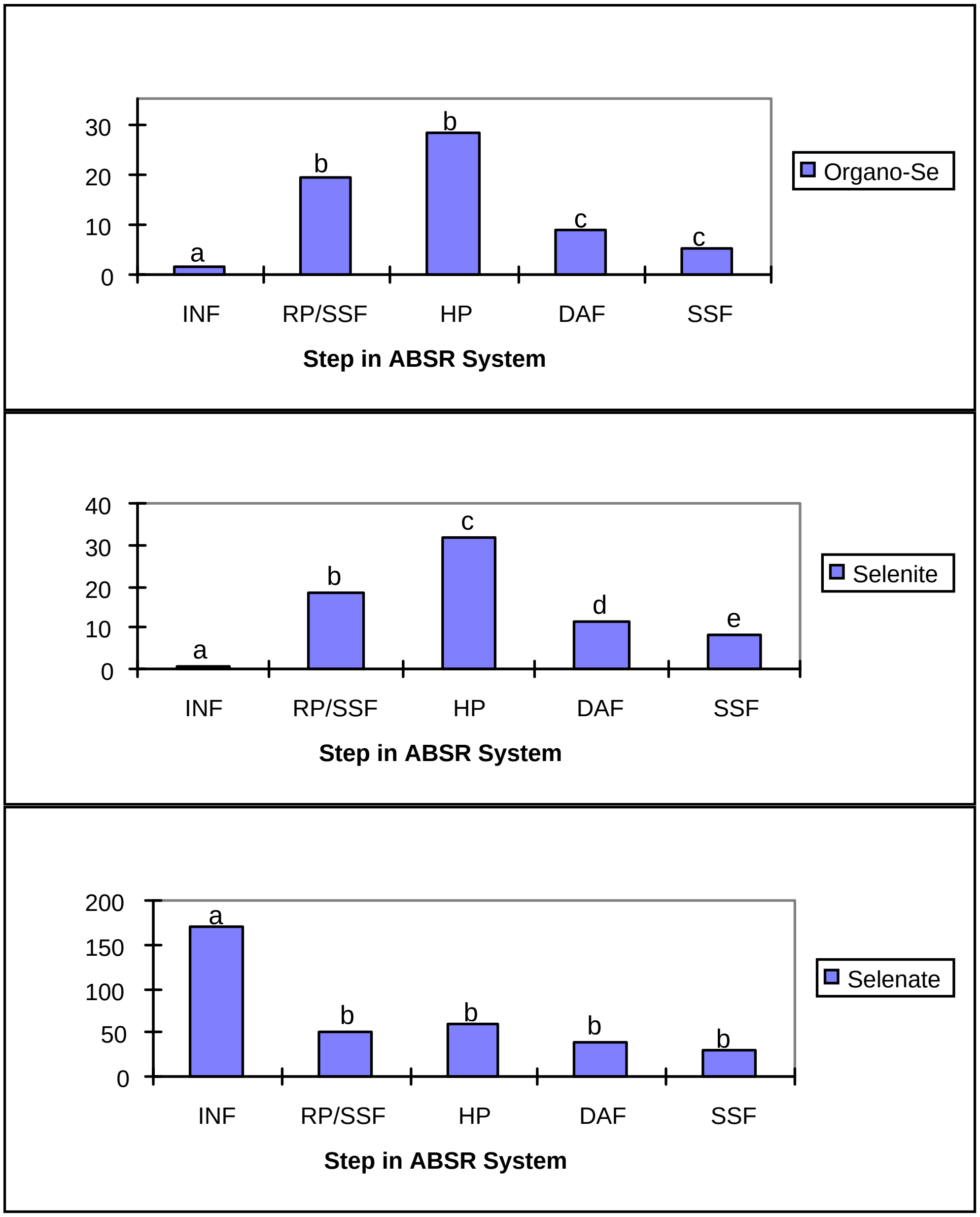

Figure 4: Mean concentrations of Organo-Se, Selenite, and Selenate found in water from microcosms in each step of the ABSR System. Letters of the alphabet indicate significant differences in concentrations between positions in the ABSR System.

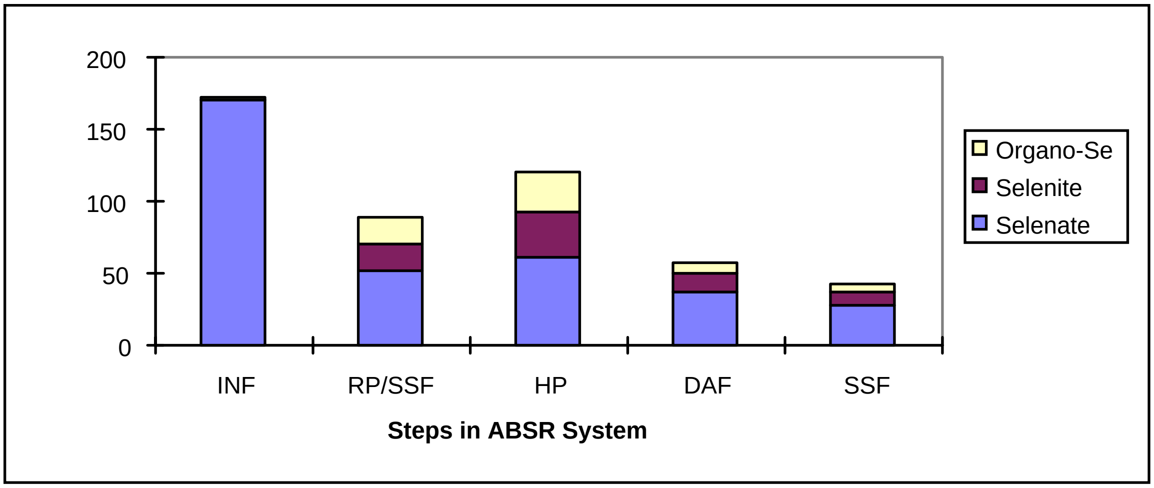

Figure 5: Mean concentrations (from all 5 weeks) of Organo-Se, Selenite, and Selenate found in water samples shown together representing total Se found in each step of the system.

Microcosm soil samples were also analyzed looking at the three dominant species of Se (Figure 6). Again the Kruskall-Wallis test showed significant results for all three: Organo-Se (p=.0438), Selenite (p=.0096), and Selenate (p=.0463). The Conover test for multiple comparisons showed significant differences between steps of the process. All Selenium levels, except Organo-Se, significantly increase between when the samples were initially gathered (initial) and exposure to the INF water. Total Se, Organo-Se, and Selenite all significantly increase between IN and RP/SSF.

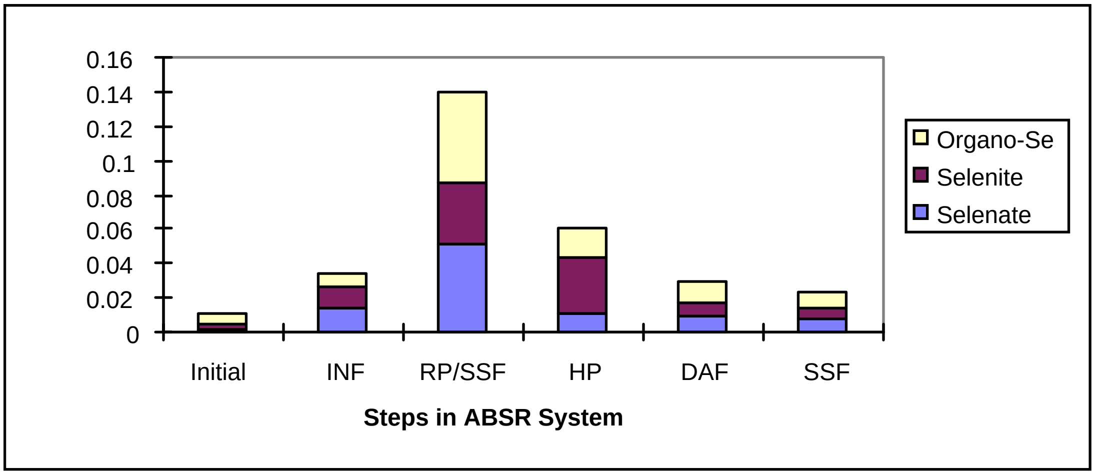

Figure 6: Mean concentrations of Organo-Se, Selenite, and Selenate found in soil from microcosms in each step of the ABSR System. Three species of Se add up to Total Se.

Total Se body burdens in lumbriculus and helisoma snails showed a significant relationship with Organo-Se concentrations. For lumbriculus $R^2 = 0.5654$, y = 0.14 (+/-0.03) x + 4.49 (+/-

0.56) (Figure 7). For helisoma snails $R^2 = 0.9041$, y = 2.45 (+/-0.69) x - 6.74 (+/- 8.7) (Figure 8). This regression only has four points and is not significant without the one outstanding point.

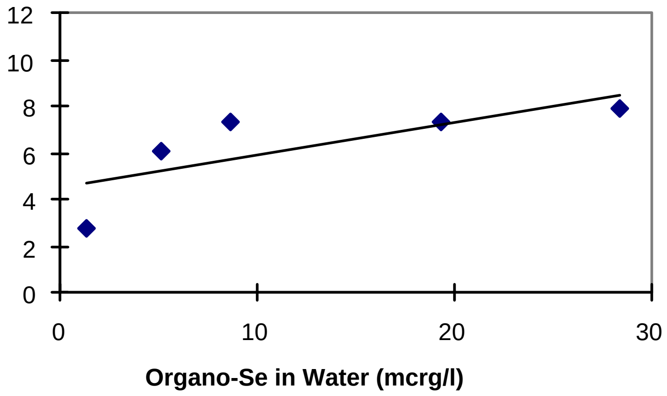

Figure 7: Regression of total selenium lumbriculus body burdens on Organo-Se water concentrations.

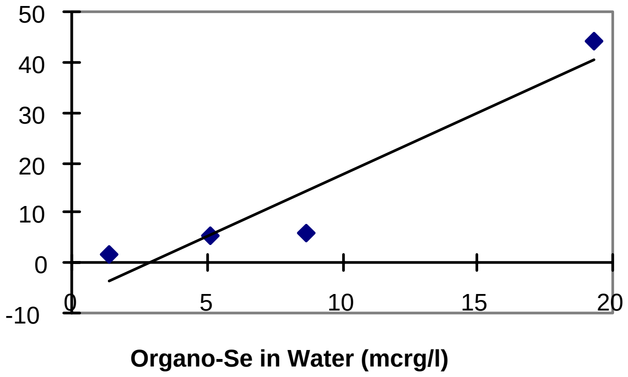

Figure 8: Regression of total selenium Helisoma snails body burdens on Organo-Se water concentrations.

Bioconcentration Factors were calculated using invertebrate body burdens and total Se water concentrations. Lumbriculus and helisoma snail BCFs show a general trend to increase in the ABSR system (Figure 9).

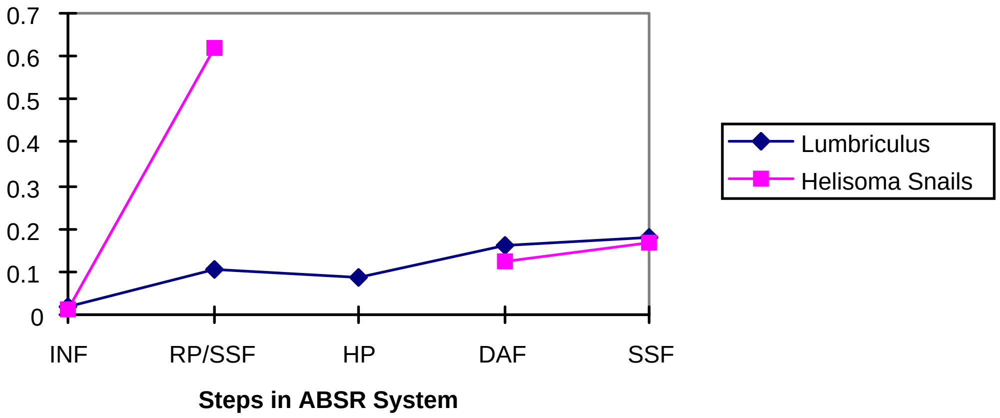

Figure 9: Bioconcentration Factors from lumbriculus and helisoma snail samples. Missing data point for snails from the HP is due to lack of samples.

Standards of known Se concentrations were tested along with the invertebrate and water samples and resulted in percent recoveries. For invertebrate samples bovine liver, with known Se concentrations, was used as a standard and percent recoveries were roughly 100%. Percent recoveries were found for the water and soil sampling method for each form of Se (Table 1).

FORM OF SELENIUM	% RECOVERY	STANDARD DEVIATION
Selenite	112.5	7.3
Organo-Se	83.5	19.5
Selenate	137.6	32.9
Total Selenium	74.3	7.1

Table 1: Selenium standards recovery and standard deviation.

Discussion

The results of this study suggest that the ABSR system may not be successfully reducing the bioavailability of Se to aquatic organisms. Although microcosm data was limited, results lead to the conclusion that certain steps of the system may be increasing bioavailability. It was shown that the system is successfully reducing the total Se levels in agricultural drainage water, but small amounts of more bioavailable forms of Se are being created in the system which may be causing an increase in the bioavailability of the Se to aquatic organisms.

The mortality found in this experiment is most likely due to factors other than selenium soil and water concentrations. Loss of samples, especially in hyallella, chironomids, and physa snails, were found in water from many parts of the ABSR system, regardless of water and soil selenium levels. It is also known that several of the test species are found in abundance living in the RP and HP. Therefore mortality in this study is probably not due to selenium levels. Laboratory tests with invertebrates and selenium (Thomas et al. 1999) have also shown that low percent survival of test species can be due to factors other than selenium. In this experiment mortality was most likely due to changes in water quality, temperature, and sunlight.

The only species found in every part of the ABSR system was lumbriculus; however, these samples varied widely in body burdens so that no significant difference was found in Se concentrations between steps in the system. There is a general trend in the lumbriculus data which is probably similar to the actual impact of the system on Se bioaccumulation. The results for helisoma snails did show significant differences in Se body burdens between different steps of the ABSR System. Most noteworthy is the significant increase in body burdens between the influent and the effluent of the system, suggesting that organisms will be able to absorb slightly more Se in the outflow of the system than in untreated drainage water. It is important to acknowledge that species differ in their absorption of Se and that certain Se levels will lead to high body burdens in some species and not in others. Due to the small invertebrate sample sizes caused by high mortality, this experiment does not give as much evidence of the effects of the ABSR system on bioaccumulation as it had intended, but still provides a rough estimate.

The water samples from the weeks of the experiment show more definite evidence as to what is happening in the ABSR system and its potential effects on bioaccumulation. The water samples showed over a 70% reduction in total Se between the influent and the effluent of the system with total concentrations being much closer to the 5 μg/liter safe criterion level (Dobbs, 1996). However, almost all of the influent into the system is Selenate and over 30% of the effluent consists of Selenite and Organo-Se. These two forms, especially Organo-Se, are much more bioavailable and therefore more harmful to wildlife.

Processes in the system are converting Se into Selenite and Organo-Se so that there is more in the outflow than existed in the untreated water. The RP is causing a significant decrease in Selenate, but an increase in both Selenite and Organo-Se. This RP was also connected to a sand filter which takes out particulate Se. This means the Selenite and Organo-Se results from the microcosm water may in fact be less than is actually being transferred to the HP and on through the system. The RP sand filter affects the RP microcosm water but not the water in the HP and the rest of the microcosm. This explains the apparent increase in total Se between the RP/SSF and the HP. The HP shows an even greater increase in Selenite and Organo-Se, and may be the major cause for increased Selenite and Organo-Se in the system as a whole. The DAF and the last SSF reduce levels of all three forms of Se and although the final effluent has less total Se and Selenate, Selenite and Organo-Se levels are both significantly greater than in the influent.

Attempting to tie the water sample and invertebrate results together, regression analysis was done. Because Organo-Se is the most bioavailable form it was tested to see if it had a direct correlation with Se body burdens. Although some relationship was found, if additional studies could show better correlations, in the future water Organo-Se concentrations could be studied rather than invertebrate body burdens.

Soil results showed a significant increase in Selenite, Organo-Se, and total Se in the RP/SSF microcosm soil. Both lumbriculus and helisoma snails also showed a great increase in body burdens in the RP/SSF microcosms. This increase, however, was not seen in the water samples. It has been found that particulate (sediment) Se is a more accurate predictor of adverse biological effects than waterborne Se (Van Derveer et al. 1997). Although no significance was found between the influent and effluent microcosm sediments, considering the importance of sediment Se levels as an indicator, future research may wish to further study how the effluent of the system may effect drainage sediment Se concentrations.

For future water and soil sampling, it may be best to modify the methods used. The percent recoveries from the standards indicate that the results found may be over or under the actual concentrations present. Levels of Mn^{2+} and S_2O_8 were altered in an attempt to find the best recoveries, but the levels could be further manipulated to find more accurate results. Looking at the percent recoveries, Organo-Se levels may be underrepresented and Selenate over represented in the results of this experiment. This means that the Organo-Se water and soil concentrations could actually be higher than this study found.

The most important finding in this study is that a significant amount of Se is being converted to Organo-Se in the ABSR system. Organic forms of Se have been found to be much more harmful to wildlife than all other forms. Selenomethionine concentrations less than 1 μg/liter have been shown to bioconcentrate by a factor of 50,000 in algae and 350,000 in daphnids, much higher than biomagnification levels reached by other forms of Se (Presser et al. 1994). Tests with Daphnia magna also showed much higher toxicity for seleno-DL-methionine than Selenite and Selenate (Maier et al. 1993). Although the ABSR system is successfully reducing total Se, it is important that Organo-Se is not being released in the effluent.

The ABSR system could be modified so that less Organo-Se is being created. The HP is where much of the Se is being converted into Organo-Se and could possibly be removed from the system. Although the RP may also be contributing to Organo-Se levels, the bacteria are essential in the removal of total Se. The HP contains large amounts of algae that primarily serve the purpose of reducing the high BOD from the molasses used to feed the bacteria in the RP. It has been found that algae can readily transform inorganic Se into various forms of organic Se (Thomas et al. 1999). It was recently discovered that the RP can function efficiently without the addition of molasses, and therefore the HP is no longer needed and could be removed from the system (Lundquist, pers.com, 2001). The removal of the HP from the ABSR system should show a reduction in Organo-Se formation. After the removal of the HP, additional tests should be done to assure that significant levels of Organo-Se are no longer being released in the effluent.

The high selenium levels in the San Joaquin Valley drainage water has been shown to be a serious ecological hazard that needs to be addressed. This problem is not isolated to the San Joaquin valley, but affects many western states. There are 200 wildlife refuges and management areas in the western US that receive water from more than 400 water projects, most of which consist of agricultural drainage facilities (Lemly et al. 1993). A cost-effective method to reduce the threat of Se in drainage water is needed throughout the west in order to protect wildlife from the same effects seen at Kesterson Wildlife Refuge. The ABSR system is one of the most economical and therefore easily adopted selenium removal systems. With modifications, it may prove to be a viable option for widespread selenium removal.

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