Bull. Environ. Contam. Toxicol. (1996) 57:779-786

Bulletin of Environmental Contamination and Toxicology

Parameters for Removal of Toxic Heavy Metals by Water Milfoil (*Myriophyllum spicatum*)

T. C. Wang,[1] J. C. Weissman,[2] G. Ramesh,[1] R. Varadarajan,[2]
J. R. Benemann[3]

[1]Harbor Branch Oceanographic Institution Inc., Ft. Pierce, Florida 34946, USA
[2]Microbial Products, Inc., Vero Beach, Florida 32968, USA
[3]Consultant, Walnut Creek, California 94508, USA

Received: 20 October 1995/Accepted: 5 May 1996

Due to the increasing awareness of toxic heavy metals contamination to the environment, studies of metal accumulation from the view point of metal removal from contaminated water have been performed (Kuyucak and Volesky, 1988; Darnall et al., 1986). Conventional methods including precipitation, oxidation, reduction, ion exchange, filtration, electrochemical treatment, membrane technologies and evaporation recovery are expensive or ineffective, especially when the metal concentrations are very low in the order of 1 to 100 mg/L (Volesky, 1990). The use of biological systems for removing metals from low metal solution has the potential to achieve greater performance at lower cost.

Aquatic plants and/or algae are known to accumulate metals and other toxic elements from contaminated water (Wolverton and McDonald, 1975; Muramoto and Ohi, 1983; Green and Bedell, 1990; Maeda and Sakaguchi, 1990; Wilde and Benemann, 1993). The bioremoval process using aquatic plants often exhibits a two-stage uptake process: an initial fast, reversible, metal-binding process (biosorption), followed by a slow, irreversible, ion-sequestration step (bioaccumulation). The initial metal biosorption by different parts of cells can occur via complexation, coordination, chelation of metals, ion exchange, adsorption and microprecipitation. The bioaccumulation process is an active mode of metal accumulation by living cells. This process is dependent on the metabolic activities of the cell, which in turn can be affected by the presence of the metallic ions (Wilde and Benemann, 1993). The bioremoval application merits consideration when comparing with other methods (Kuyucak, 1990). The advantages of bioremoval of metals are:

1. Metals at low concentration can be selectively removed to meet regulatory level. This could serve as a polishing step after the treatment of effluents from conventional process, or for treatment of contaminated surface or groundwater with low levels of contamination.

Correspondence to: T. C. Wang

This process can result in the recovery and reuse of the heavy metals bound by the biomass. This can be achieved by eluting with a metal chelator, a low or high pH solution to reduce metal ion bindings. The metal-laden biomass can be reduced in volume by drying or incineration; this reduces the volume of hazardous waste.

2. The biosorption process can either be performed on-site with contaminated water flowing over the biomass for metal removal or the biomass is transported to a specific site or reactor for metal removal.

3. A biosorbent has a very low affinity for calcium and magnesium ions. This could make them very competitive with ion-exchange resins in hard water.

4. The system offers low capital investment and low operating cost. It can be operated over the broad range of pH values (3-9) and temperatures (4° to 90°C).

Plants that are potentially useful in such a process were identified through our initial screening program. Water milfoil, a rooted aquatic higher plant, has been identified as one of the potential plants for bioremoval process (Wang et al.,). The goal of this study was to determine the range of metal adsorptive capabilities by water milfoil under different pH conditions. Five metals, Cd, Zn, Ni, Pb, and Cu were used for the study. Both the maximum specific adsorption capacity and the lowest residual metal concentration attainable were measured.

MATERIALS AND METHODS

Fifty mL of known metal solution ranging from 1 to 16 mg/L was added to a 125-mL Erlenmeyer flask with a screw cap. About one gram of water milfoil biomass (wet weight) was added to the flasks and placed on a shaker for 60 min for metal contacting experiments. All experiments were conducted in triplicate. The pH and temperature of the contact solution both before and after experiments were measured. At the end of the experiment, the contents of the flask were filtered to separate biomass from the solution. Both biomass and filtrate were analyzed to determine metal contents in both samples.

The biomass sample (0.5 - 1.0 g wet weight) was digested with 10 mL of concentrated nitric acid using a microwave digester before metal analysis. Dry weight analysis was performed as per AWWA standard methods. The pH solution was measured using a Cole-Palmer portable pH meter, and temperature was recorded with a thermometer. All samples were analyzed using an atomic absorption spectrophotometer (AAS) or an inductively coupled argon plasma (ICAP) trace analyzer. The latter was used for determining low metal concentrations (<0.1 mg/L). The results of metal analysis was used to calculate:

1. percent of metal remaining in solution;
2. percent metal recovered by biomass;
3. specific adsorption (mg metal adsorbed/kg of biomass, dry weight).

The specific adsorption obtained was plotted against the residual metal concentration to define the sorption characteristics for the plant biomass. The maximum adsorption was calculated using the Langmuir adsorption equation (Cris et al., 1992); $C/Y = C/Y_m + 1/kY_m$, where Y_m is the maximum adsorption; k, the equilibrium constant related to the affinity of the binding site; and Y, the specific adsorption at residual metal concentration C. From a plot of C/Y vs. C, the slope ($s=1Y_m$) gave Y_m and the intercept ($I=1/k\ Y_m$), gave k constant. The specific adsorption capability and residual metal concentration were plotted in the sorption isotherms. A steep isotherm from the origin at low residual concentration indicated high affinity of the biomass for the given metal species.

RESULTS AND DISCUSSION

Metal contact time and pH of metal solution affect metal adsorption. Our preliminary work showed that pH above neutral resulted in precipitation of copious amounts of Pb and Cu when no water milfoil was present. Use of phosphate buffer was also found to co-precipitate the same two metals. The preliminary results also showed that all of the metal removal occurred within 60 min. The experiments indicated that water milfoil added to the metal solution quickly raised the pH from 3 to 8. A minimum of 20 mM acetate solution was normally required to maintain a constant pH solution for the contacting experiment. Due to all of these potential experimental procedures with pHs and different types of buffers, protocols were established to measure adsorption as a function of metal concentrations at acidic conditions with no buffer added. A pH3 solution was obtained directly from metal standard solution and pH5 solution was obtained by titrating the initial metal solution to pH5 with NaOH. Figures 1 and 2 show the specific adsorption and linearized plots of the Langmuir model at pH5. Table 1 summarizes the maximum specific adsorption Y_{max} for each metal. By weight, the maximum adsorptions were about 0.5% for Ni, 0.8% Cd, 1.3% Cu and 5.5% Pb. Lead has the highest and Ni has the least metal adsorption. The minimum residual metal concentrations attained are also shown in Table 1. The results indicated that the minimum residual concentration was about 0.01 mg/L for Cd, Ni, and Cu, 0.1 mg/L for Zn and below 0.004 mg/L for Pb. All of these residual concentrations were within EPA surface water-quality criteria (EPA, 1986).

A comparison was made to demonstrate the effect of acetate buffer concentration on the metal adsorption. It was observed that the specific adsorption of Cd at 4 ppm was reduced almost by 50% from 2,400 mg/kg to 1,100±50 mg/kg when the buffer concentration was increased from 10 to 40 mM at a biomass density of 0.02 mg/L. However, there was little difference in the adsorption of Cd when the

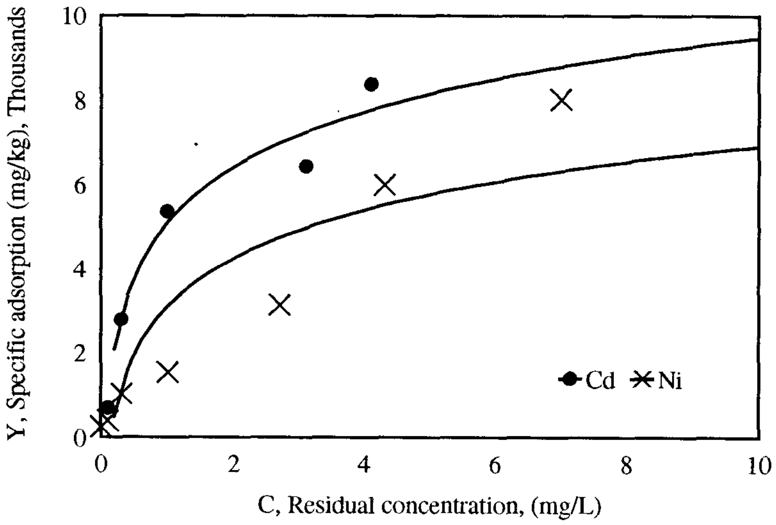

Figure 1 Langmuir adsorption isotherm of metals onto water milfoil (pH=5-8.0)

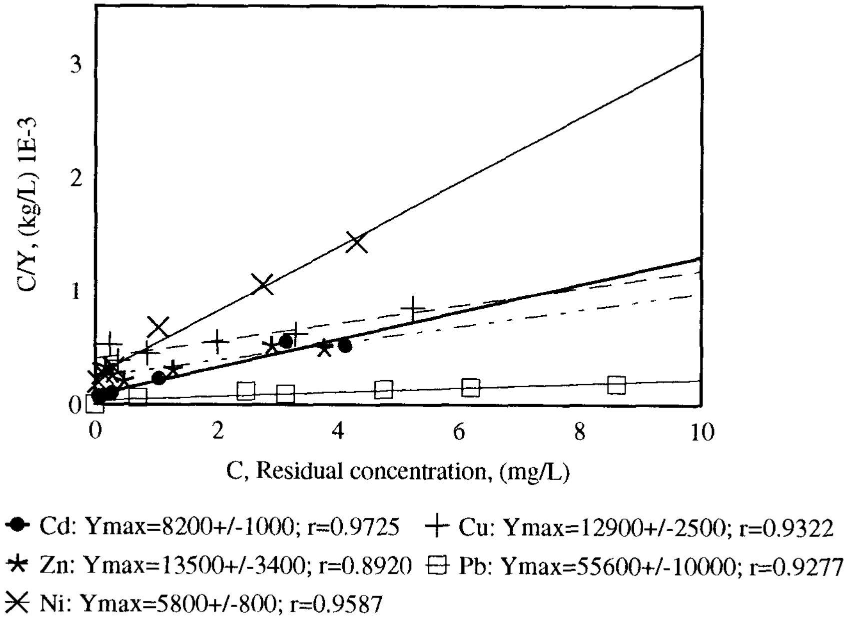

Figure 2 Linear Langmuir adsorption isotherm of metals onto water milfoil. Biomass density was 0.02 kg/L for all metals except Pb 0.005 kg/L (pH=5-8.0)

Table 1. Maximum specific adsorption and minimum residual concentration attained by water milfoil at ph 5 without buffer presence.

Metal	Y_{max} (mg/kg)	Minimum Residual Metal Concentration. (mg/L)	EPA Fresh Water Quality Criteria (mg/L)
Cd	8,200±1,000	0.007±0.001	0.0011
Zn	13,500±3,400	0.10±0.004	0.047
Pb	55,600±10,000	<0.004	0.0032
Ni	5,800±800	0.029±0.002	0.96
Cu	12,900±2,500	0.008±0.007	0.012

Table 2. Maximum specific adsorption by water milfoil at different pH.

Metal	Y_{max}	pH	Buffer
Cd	2,800±200	3-8	No buffer
	6,200±400	5	Acetate (20 mM-NaOH)
	8,200±1,000	5-7.8	NaOH - No buffer
Zn	3,600±300	3-8	No buffer
	8,300±900	7	Phosphate (10-40 mM)
	13,500±3,400	5 -7.8	NaOH - No buffer
Pb	1,300±100	1.9-3.5	No buffer
	36,500±2,300	3-7	No buffer
	55,600±10,000	5-7.4	NaOH - No buffer
Ni	2,400±60	3-8	No buffer
	5,800±800	5-7.4	NaOH - No buffer
Cu	8,200±500	3-8	No buffer
	7,100±600	3	Phos. acid (10-40 mM)
	12,900±2,500	5-7.8	NaOH - No buffer

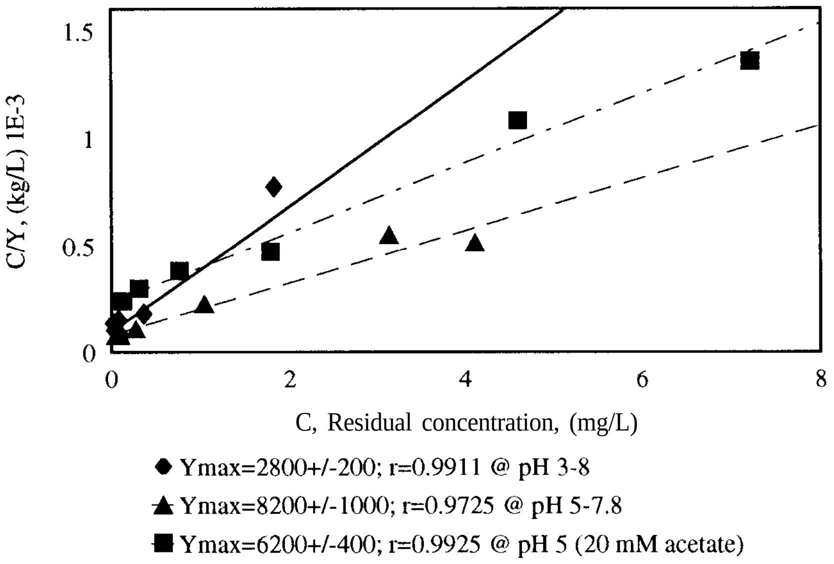

Figure 3 Effect of pH on adsorption of cadmium onto water milfoil. Biomass density was 0.02 kg/L

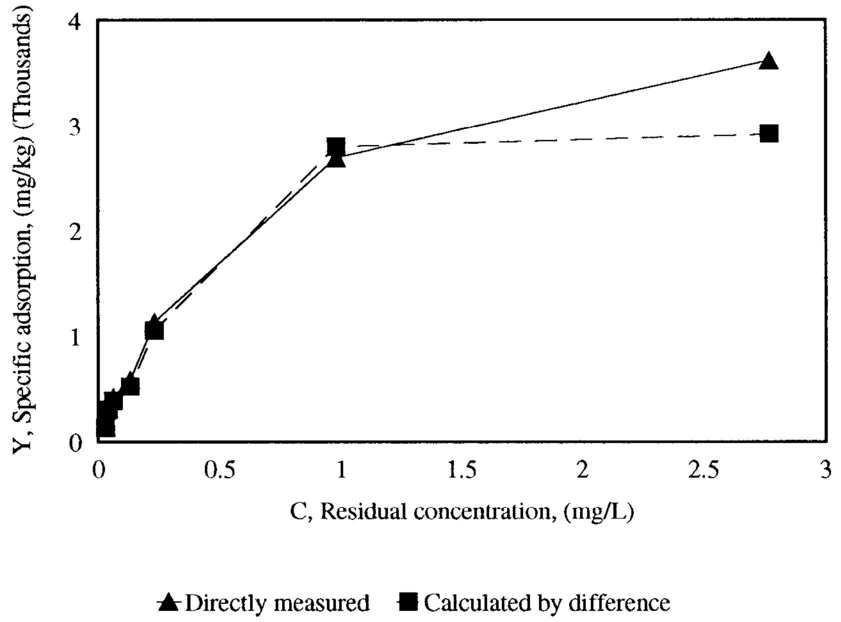

Figure 4 Comparison between measured and calculated specific adsorption for zinc. Biomass density was 0.02 kg/L (pH 3-8)

acetate buffer was changed from 5 to 10 mM. Due to the effect of buffer on metal adsorption, experiments were performed with metals in different pH and buffer solutions. Figure 3 shows a typical adsorption curve with Cd at pH5 with and without buffer in the solution. The results indicated that pH5 solution without buffer had the highest metal adsorption capability for all metals shown in Table 2. Specific adsorption determined by direct measurement of metal in the biomass pellet was compared with specific adsorption calculated from the difference between the initial and final metal concentration. Figure 4 shows a comparison of specific adsorption for Zn at different residual concentrations. Comparisons were generally agreeable. Some discrepancies were observed when residual metal concentrations were greater than 2.5 mg/L. These discrepancies were primarily due to errors generated in calculating specific adsorption from initial and final metal concentrations.

This study demonstrated that water milfoil has a high metal adsorption capability in a relatively short contact time. This plant is naturally immobilized and is a very common component of many natural communities. Those characteristics indicate water milfoil can be used for metal removal in a biosorption process.

Acknowledgment. This research was performed under subcontract No 94-C189-CR from MSE, Inc., Butte, Montana, under U.S. Department of Energy Contract number DE-AC22-881D 12735, In Situ Remediation Integrated Program, Office of Technology Development, Office of Environmental Management.

REFERENCES

Crist RH, Oberhoisk K, McGarthy J, Crist DR, Johnson JK, Brittsan JM (1992) Interaction of metals and protons with algae 3. Marine algae with emphasis on lead and aluminium. Environ Sci Technol 26: 496-502

Darnall D, Green WB, Henzl MT, Hosea JM, McPherson RH, Sneddon J, Alexander MD (1986) Recovery of heavy metals by immobilized algae. In: Thompson R (ed.) Trace Metal Removal from Aqueous Solution, Whitstable Litho Ltd, 1-24

Green B, Bedell GW (1990) Immobilize algae for metal recovery. In: Akatsuka I (ed.) Introduction to Applied Phycology, Academic Publishing, The Hague, Netherlands 109-136

Kuyack N, Voleksy B (1988) Biosorbents for removal and recovery of metals from industrial solutions. Biotech Lett 10: 137-142

Kuyack N, Voleksy B (1990) Biosorption of algal biomass. In: Volesky B (ed.), Biosorption of heavy metals, CRC Press, Boca Raton 174-198

Maeda S, Sakaguchi T (1990) Accumulation and detoxification of toxic metal elements by alga. In: Akatsuka I (ed.) Introduction to applied phycology, Academic Publishing, The Hague, Netherlands 109-136

Muramoto S, Ohi Y (1983) Removal of some heavy metals from polluted water by water hyacinth. Bulletin Environ Contam Toxicol 30: 170-177

Wang TC, Weissman JC, Ramesh G, Varadarajan R, Benemann JR (1995) Bioremoval of toxic elements with aquatic plants and algae. In: Hinchee R, Means J, Burris D (eds) Bioremediation of inorganics, Battelle Press, Columbus, OH, 65-69

Wilde EW, Benemann JR (1993) Bioremoval of heavy metals by the use of microalgae. Biotech Adv 11:781-812