Determination of cadmium accumulation capabilities of aquatic macrophytes *Ceratophyllum demersum*, *Bacopa monnieri* and *Rotala rotundifolia*

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Abstract

In the present study, cadmium (Cd) accumulation capabilities of aquatic macrophytes *Ceratophyllum demersum*, *Bacopa monnieri* and *Rotala rotundifolia* were determined after treating both individual and triple macrophytes. The macrophytes were treated with Cd at 0, 0.1, 1 and 10 mg/L concentrations in a controlled chamber for 12-days. Cadmium accumulations increased with increasing Cd concentrations in all macrophytes. Bioconcentration factor (BCF), which indicates the efficiency of the macrophyte to accumulate Cd, decreased with increased external Cd concentrations. When compared to single macrophyte applications, reductions were observed in Cd accumulation of the combined macrophyte treatments. The maximum Cd accumulation was recorded in *R. rotundifolia* followed by *C. demersum* and *B. monnieri* in both single and combined macrophyte applications. Consequently, the macrophytes in both applications proved highly effective in the accumulation of Cd. Thus, they may be used especially in the abatement and monitoring of Cd pollution.

Keywords: Cadmium accumulation, *Ceratophyllum demersum*, *Bacopa monnieri*, *Rotala rotundifolia*

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Introduction

Among heavy metals, Cd is a heavy metal with no known essential biological functions in higher plants. Although it is naturally released into the environment, substantial sources of Cd in the environment are human activities. Such activities include power stations, heating systems, metal working industries, urban traffic or phosphate-containing fertilizers. Cadmium is widely used in electroplatinig, pigments, plastic stabilizers, and Ni-Cd batteries (Sanita di Toppi and Gabrielli, 1999). Because of its high solubility in water and high toxicity, it is recognized as a highly significant environmental pollutant (Pinto *et al*., 2004).

Macrophytes grow in many different types of aquatic ecosystems, such as lakes, reservoirs, wetlands, streams, rivers, marine environments and even rapids and falls. This variety of colonized environments results from a set of adaptive strategies achieved over evolutionary time (Wetzel, 2001; Kalff, 2002). They are known to have great importance, forming a substantial component of the primary production in many aquatic habitats (Pip, 1990). Vascular aquatic macrophytes may accumulate considerable amounts of heavy metals in their tissues (Kovacks *et al*., 1984). Several of the submerged, emergent and free-floating aquatic macrophytes are reported to accumulate heavy metals in natural waters as well as after exposure to wastewaters (Greger, 1999; Shirvani Mahdavi *et al*., 2012).

C. demersum L., a submersed rootless species, is a free-floating aquatic macrophyte. It is frequently found growing in ponds, lakes and streams. Heavy metal accumulation capabilities of the macrophyte have been studied by many authors (Ornes and Sajwan, 1993; Dogan and Demirors Saygideger, 2009). *B. monnieri* L. grows very fast with its creeping stem in wetlands. Heavy metal accumulation capabilities of *B. monnieri* have been reported in many studies (Ali *et al*., 2001; Singh *et al*., 2006). Although the origin of *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne, an aquarium macrophyte, is in southeast Asia, it is stored in many local fish stores. There is no study about the Cd accumulation capacity of the macrophyte.

In literature, there are many studies about the heavy metal accumulation capabilities of aquatic macrophyte both macrophytes collected from natural environments and those grown under laboratory conditions. In laboratory studies, either heavy metal accumulation(s) in a single macrophyte or combined macrophytes have been determined. The aim of the present study was to evaluate (i) Cd accumulation levels of aquatic macrophytes *C. demersum*, *B. monnieri* and *R. rotundifolia* treated individually and (ii) Cd accumulation capabilities of the aquatic macrophytes treated together.

Materials and Methods

Test materials, *C.demersum* (Ceratophyllaceae), *B. monnieri* (Scrophulariaceae) and *R. rotundifolia* (Lythraceae) were obtained from aquarium retailers. For 2 weeks, the macrophytes were acclimatized in 10% Arnon and Hoagland nutrient solution (Arnon and Hoagland 1940), under 22–24°C, 16 h light (6000 μE $m^{-2}.s^{-2}$) and 8 h dark periods in a climate chamber (Snijders Scientific, Netherlands). After acclimatization, the macrophytes (6-7 g fresh weight) were cultured in glass vessels containing 1000 mL solution for Cd treatment for 12-days. Two different applications were carried out. In the first application, macrophytes were individially treated with different Cd concentrations. In the second application, macrophytes were treated with different Cd concentrations combined together. Three replicates of macrophytes were exposed to Cd as $CdCl_2$ at 0.1, 1 and 10 mg/L concentrations which were added to the 10% nutrient solution. The test media were changed every third day, and Cd concentrations were replenished. Macrophytes without added Cd served as controls. The pH of solutions was adjusted to 6.5-6.7. All chemicals used in the study were of analytical grade.

After harvesting, macrophytes were carefully washed three times with distilled water. Three replicates of each treatment were dried to a constant weight at 80°C in an electric furnace. After that, the samples were pulverized using a mortar and pestle. To determine Cd contents the samples were dissolved in 14 M HNO_3 and residues were dissolved in 1 M HCl. After mineralization, Cd contents were determined using an atomic absorption spectrometer (Perkin Elmer AAnalyst 400). Bioconcentration factor (BCF) was estimated from the ratio of Cd concentrations in macrophyte tissues (mg/kg dry weight) to Cd concentrations in the test medium (mg/L).

Statistical analyses were carried out with three replicates. Data were analyzed byanalysis of variance (ANOVA) using SPSS 11.0 for Windows. The significance of differences was determined with the least significant difference (LSD) test.

Results

Treatment of *C. demersum*, *B. monnieri* and *R. rotundifolia* with Cd concentrations ranging from 0.1 to 10 mg/L for 12-days caused enhanced Cd accumulation in individual macrophyte applications (Table 1). Cadmium was not detected in control macrophytes. Considering individual macrophyte applications, the highest Cd accumulation was observed in *R. rotundifolia* tissues. In contrast, the lowest Cd accumulations were determined in *B. monnieri* tissues. At 0.1 mg/L, Cd accumulation of *R. rotundifolia* was 2.8 and 5.3 times higher than *C. demersum* and *B. monnieri*, respectively. Cd accumulation of *R. rotundifolia* tissues

compared to *C. demersum* and *B. monnieri* were higher as well.

Unlike Cd accumulation in macrophytes, bioaccumulation factor decreased with increased external Cd concentrations (Table 2). Whereas the highest BCF was established in *R. rotundifolia* at 0.1 mg/L Cd concentration, the lowest value was determined in *B. monnieri* at 10 mg/L Cd concentration. Also findings indicate that *R. rotundifolia* has higher Cd accumulation capability compared to *C. demersum* and *B. monnieri.*

Cadmium accumulations of combined macrophyte applications are given in Table 3.

Unlike single macrophyte applications, Cd was not determined in *B. monnieri* tissues at 0.1 mg/L concentration, however, Cd accumulations increased with increasing Cd concentrations in all macrophytes. As with single macrophyte applications, the highest Cd accumulation was determined in *R. rotundifolia* tissues. The lowest Cd accumulation was found in *B. monnieri* at 0.1 mg/L. Cd accumulation of *R. rotundifolia* tissues was 2.5 times higher than *C. demersum*. Similarly, the increase in Cd accumulation of *R. rotundifolia* tissues was determined as1.02 and 1.2-folds at 1 mg/L Cd concentration and 2.2 and 2.3-folds at 10 mg/L Cd concentration, when compared to *C. demersum* and *B. monnieri*, respectively.

BCFs in combined macrophyte applications decreased with increasing Cd concentrations (Table 4). The highest BCF was determined in *R. rotundifolia* at 0.1 mg/L Cd concentration and the lowest value of this factor was observed in *B. monnieri* at 10 mg/L Cd concentration in the single macrophyte application.

Table 1: Cadmium accumulations in *Ceratophyllum demersum*, *Bacopa monnieri* and *Rotala rotundifolia* after 12-days individual applications.

Cd concentrations (mg/L)	Cd accumulations in single macrophyte applications (mg/g DW)		
	C. demersum	***B. monnieri***	***R. rotundifolia***
0	ND	ND	ND
0.1	41.9 c,x	22.3 c,x	117.0 c,y
1	230.5 b,x	196.3 b,x	317.2 b,y
10	824.7 a,x	757.4 a,y	1458.7 a,z

Values expressed as mean of the three replicates. Letters a, b and c show the differences among concentrations in a macrophyte; letters x, y and z show the differences among macrophytes in same concentration (p<0.05). ND: not detected.

Table 2: Bioconcentration factor (BCF) of *Ceratophyllum demersum*, *Bacopa monnieri* and *Rotala rotundifolia* after 12-days individual applications.

Cd concentrations (mg/L)	BCFs in single macrophyte applications		
	C. demersum	*B. monnieri*	*R. rotundifolia*
0.1	418.7 a,x	223.3 a,y	1170.0 a,z
1	230.5 b,x	196.3 b,x	317.2 b,y
10	82.5 c,x	75.7 c,x	145.9 c,y

Values expressed as mean of the three replicates. Letters a, b and c show the differences among concentrations in a macrophyte; letters x, y and z show the differences among macrophytes in same concentration (p<0.05). ND: not detected.

Table 3: Cadmium accumulations in *Ceratophyllum demersum*, *Bacopa monnieri* and *Rotala rotundifolia* after 12-days combined macrophyte applications.

Cd concentrations (mg/L)	Cd accumulations in combined macrophyte applications (mg/g DW)		
	C. demersum	*B. monnieri*	*R. rotundifolia*
0	ND	ND	ND
0.1	35.3 c,x	ND	88.1 c,y
1	201.3 b,x	173.6 b,y	205.4 b,x
10	537.1 a,x	512.7 a,x	1172.2 a,y

Values expressed as mean of the three replicates. Letters a, b and c show the differences among concentrations in a macrophyte; letters x and y show the differences among macrophytes in same concentration (p<0.05). ND: not detected.

Table 4: Bioconcentration factor (BCF) of *Ceratophyllum demersum*, *Bacopa monnieri* and *Rotala rotundifolia* after 12-days combined macrophyte applications.

Cd concentrations (mg/L)	BCFs in combined macrophyte applications		
	C. demersum	*B. monnieri*	*R. rotundifolia*
0.1	352.7 a,x	ND	881.0 a,y
1	201.3 b,x	173.6 a,y	205.4 b,z
10	53.7 c,x	51.3 b,x	117.2 c,y

Values expressed as mean of the three replicates. Letters a, b and c show the differences among concentrations in a macrophyte; letters x, y and z show the differences among macrophytes in same concentration (p<0.05). ND: not detected.

Discussion

Metal concentrations in plant tissues are generally a function of the metal concentration in the growth solution (Kabata-Pendias and Pendias 1984). Cadmium accumulation by aquatic macrophytes has been reported by many researchers (Gupta and Chandra, 1996; Saygideger and Dogan, 2004; Aravind and Prasad 2005). In a study, Cd accumulation capacities of macrophytes (*Eichhornia crassipes*, *Pistia stratiotes* and *Lemna minor*) was determined. *L. minor* accumulated the highest concentration of metals in the dry biomass (20.11 mg/g dry wt) followed by *P. stratiotes* (9.045 mg/g dry wt) and *E. crassipes* (6.29 mg/g dry wt) Goswami *et al*. (2009). According to our findings, The maximum Cd accumulation was observed in *R. rotundifolia* followed by *C. demersum*

and *B. monnieri* at both single and combined macrophyte applications. Submerged macrophytes like *C. demersum* and *R. rodundifolia* up take metals directly from the water column. Thus, these macrophytes have higher metal accumulation capabilities than *B. monnieri,* an emersed macrophyte.

When compared to single macrophyte applications, reductions were determined in Cd accumulations in the combined macrophyte treatments. The reduction rates at 0.1, 1 and 10 mg/L Cd concentrations were estimated to be 15.8%, 12.7% and 34.9% for *C. demersum* and 24.7%, 35.3% and 19.6% for *R. rotundifolia*, respectively. Cd accumulations of *B. monnieri* tissues decreased to 11.6% and 32.3% at 1 and 10 mg/L, respectively, with respect to single macrophyte applications. Metal accumulation depends on plant species, and numerous abiotic factors like temperature, pH and dissolved ions in water (Lewis, 1995; Lewander *et al*., 1996). In addition to this, according to our findings metal accumulations may depend on the competitive strength among macrophytes. Comparing Cd contents of macrophytes in single and combined applications, this suggestion can be supported.

BCF, defined as the concentration ratio of Cd in a macrophyte to that in the tested solution, is used to measure the effectiveness of a macrophyte in concentrating Cd in its biomass. BCFs decreased with increased Cd concentrations in the external solution for all the three species. Compared to data in both applications, high BCFs can be seen in single macrophyte applications. When compared to macrophytes, the highest BCFs can be seen in *R. rodundifolia* followed by *C. demersum* and *B. monnieri* in both single and combined macrophyte applications. Consequently, the macrophytes in both applications proved highly effective in the accumulation of Cd.

The toxic heavy metal Cd is especially released to the environment as a result of human activities. This metal has detrimental effects in plants, animals and humans. Remediation of toxic metals such as Cd from contaminated water is very important for organisms. Aquatic macrophytes have great potential to accumulate heavy metals inside their body. Thus, they are often used for phytoremediation of water polluted with heavy metals. When both single and combined plant applications are considered together, it can be seen that *R. rotundifolia* has a higher Cd accumulation capability than *C. demersum* and *B. monnieri*. According to these results, *R. rotundifolia* can be used as a new macrophyte for the remediation of Cd contaminated waters. Accumulation capabilities of the macrophyte for other heavy metals should be determined. Results obtained in the present study can be a source for further investigation dealing with similar subjects.

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