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VERONICA BECABONGA L. AS A HYPERACCUMULATOR PLANT FOR CADMIUM

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ABSTRACT

Heavy metal pollution of water is a major environmental problem facing the modern world. The major objective of this research was to evaluate the potential of water-speedwell plant, *Veronica becabonga* L. to uptake and accumulate heavy metal cadmium under greenhouse conditions. *Veronica becabonga* L., were cultured in 3% Hoagland's nutrient medium, which was supplemented with 0, 25, 50,100, 200, 300 mg/l of $Cd(NO_3)_{2.4}H_2O$ over one week treatment period. Plants were harvested at the end of this period and heavy metals from the entire shoot tissue was extracted using the closed Teflon vessel method and metal content in the extract was estimated using a Flame atomic absorption spectrophotometry. The results showed that the uptake and accumulation of Cd in *V. becabonga* L. showed significant increase when metal concentration was increased. The highest amount of Cd accumulation was detected at 100mg/l $Cd(NO_3)_{2.4}H_2O$ in the culture solution that was 20660.3 mg/kg DW of shoots. The linear pattern of uptake suggest the involvement of both active and passive transport mechanisms for Cd uptake. Finally, since the high concentrations cadmium accumulation in shoots of plants has far exceeded 0.01% DW *V. becabonga* L. is a hyperaccumulator plant for this metal and has potential for phytoremediation of water contaminated with cadmium.

KEYWORDS

Cadmium; Hyperaccumulator; Metal accumulator; Metal uptake; *Veronica becabonga L.*; Phytoremediation

1 INTRODUCTION

Heavy metal pollution of aqueous streams is a major environmental problem facing the modern world. Several methods of removing heavy metals from water based on ion exchange or chemical and microbiological precipitation have been developed and used with some success [7, 12]. These technologies have different efficiencies for different metals and may be very costly if large volumes, low metal concentrations, and high cleanup standards are involved.

Recently, there has been some research into the use of living and nonliving bacteria and algae for the bioremediation and recovery of heavy metals from aqueous streams. In addition, live

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or dead cultured cells of Datura innoxia, a higher plant, can be used to remove Ba2+ from solution (6, 12, 18). Commercial applications of this research are still hampered by the high cost of growing pure cultures of cells and microorganisms and by the need for their immobilization or separation from the aqueous stream [17].

Metal-accumulating fungi and *Azolla filiculoides*, an aquatic fern, [14] were also proposed as metal biosorbers capable of remediating industrial effluents. Aquatic higher plants have also been utilized for water purification. Water hyacinth (*Eichhornia crassipes*) [11, 13], pennywort (*Hydrocotyle umbellata*), duckweed (*Lemna minor*) [10], and water velvet (*Azolla pinnata*) can remove various heavy metals from solution. However, the efficiency of metal removal by these plants is low because of their small size and slow-growing roots.

Phytoremediation technology is originated from the identification of hyperaccumulator plants, which can take up large amounts of heavy metals and accumulate high concentrations in their above ground tissues with no adverse effects on growth [1, 2, 3]. Currently, the defining plant tissue concentrations for hyperaccumulation are Cd>100mg.kg^{-1}; Co, Cu, Ni, Pb>1000 mg kg^{-1}; Mn, Zn>10000 mg kg^{-1}. However, plants with such a large accumulation of heavy metals typically produce relatively small amounts of biomass and have a slow growth habit (4).

In contrast, identification of new plant species with fast growth and robust growth habit, coupled with ability to tolerate and accumulate metals has become an important aspect of phytoremediation.

The aquatic plant *Veronica becabonga* L. is a submerged plant growing in cold water channels and ponds profoundly.

The overall objective of the current investigation was to evaluate the potential of this species to uptake and accumulate major metal pollutant, cadmium from solution.

2 MATERIAL AND METHODS

PREPARATION OF TEST MATERIAL

V. beccabunga were collected from natural ponds and grown in 3% Hoaglands nutrient medium [5] in the laboratory under controlled conditions (18±2°C, 3000 lux, 12h/day light period, relative humidity 70%). The final pH of the solution was 6.5. Two-week old *V. becabonga* from the stock culture were used in the experiments

METAL EXPOSURE

The 3% Hoagland's nutrient medium was supplemented with five concentrations (25, 50, 100, 200, 300 mg/l) of $Cd(NO_3)_{2.4}H_2O$. The final pH of the solutions was 6.5. *V. beccabunga* plants (18 g fresh weight) were inoculated into each flask containing various concentrations of Cd. Plants cultured in the nutrient medium without heavy metals were treated as control. All experiments were performed in triplicates.

METAL ANALYSIS

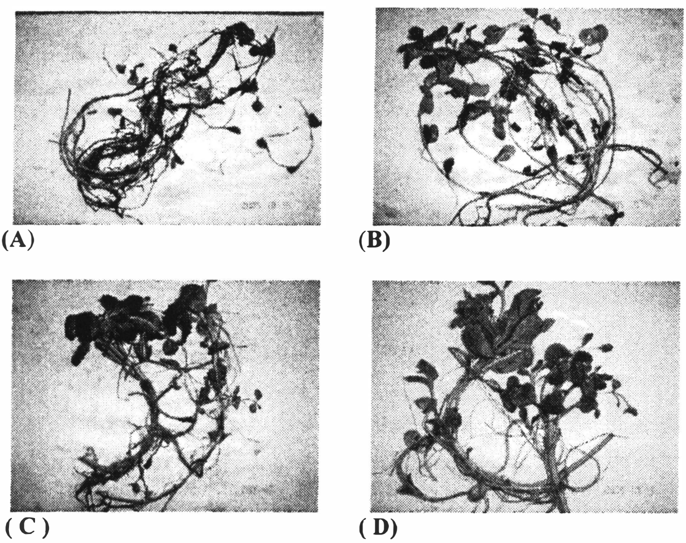
Plants from all the treatments were harvested at the same time, washed thoroughly with distilled deionized water, and divided into root and shoot biomass. Metal from the entire biomass was extracted using the closed teflon vessel method as described by Topper and Kotuby-Amacher (16). Briefly, the tissue was cut into small pieces and oven dried at 80 °C for 2 d. Oven-dried material was weighted and a sample of 1g each was placed in teflon vessels. The plant material was digested by adding 30mL of trace metal grade nitric acid (70%, Merck) and the teflon vessels were placed in an oven overnight (110 °C). The metal content in the extract was estimated using a flame atomic absorption spectrophotometry (AAS), Model Perkin-Elmer. Standard solutions were used to assess the concentrations of samples.

3 STATISTICAL ANALYSIS

Analysis of Variance (One -way ANOVA) was carried out using the Statistical software, SPSS,11 to determinate if there were significant differences in metal accumulation as a result of metal treatments. Significant differences between the means assessed by Duncan test at $P<0.05$.

4 RESULTS

Exposure of water-speedwell to various concentrations of cadmium induced morphotoxicity symptoms on the plants exposed to the higher levels (200,300 mg/l) (Fig. 1a). These plants died at first day but in 100 mg/l plants showed toxicity symptoms. The symptoms were mostly observed on the mature leaves (at 7^{th} day), which indicated signs of chlorosis and early senescence (Fig 1b).
The results showed that the uptake and accumulation of Cd in *V. becabonga* L. showed significant increase when metal concentration was increased (Fig 2.) The highest amount of Cd accumulation was detected at 100mg/l $Cd(NO_3)_{2.4}H_2O$ in the culture solution that was 20660.3 mg/kg DW of shoots. The linear pattern of uptake suggest the involvement of both active and passive transport mechanisms for Cd uptake.



(A) (B) (C) (D)

Figure1. Morpho-phytotoxicity symptoms in water-speedwell plants (Veronica becabonga), following exposure to 0(d), 25, 50(c), 100(b) or 200,300(a) mg.L^{-1} $Cd(NO_3)_{2.4}H_2O$ for 7 days.

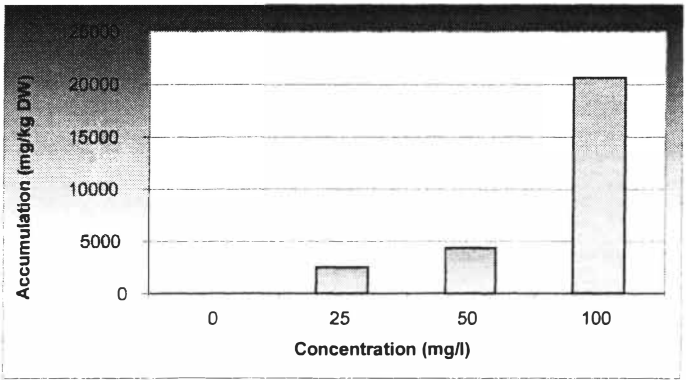


Figure 2. Cadmium content (mg. kg^{-1} DW) in shoots of water-speedwell plants (Veronica becabonga), exposed to various levels of cadmium for 7 days.

5 DISCUSSION

The high concentrations of cadmium found in shoots of water-speedwell plants has far exceeded 0.01% DW which is considered as a standard for defining cadmium hyperaccumulator plants in a natural environment (3) and it is likely be a new hyperaccumulator plant for this metal.

Metal transport to the shoot primarily takes place through the xylem. Cadmium loading into the xylem sap of *Brassica juncea* displays biphasic saturation kinetics (13), suggesting that xylem loading of metal ions is facilitated by specialized membrane transport processes. Movement of metal ions, particulary Cd, in xylem vessels appears to be mainly dependent on transpiration-driven mass flow (13).

Because xylem cell walls have a high cation exchange capacity, they are expected to retard severely the upward movement of metal cations. Therefore, noncationic metal-chelate complexes, such as Cd-citrate, should be transported more efficiently in transpiration stream (15). Theoretical studies have predicted that the majority of the Fe(II) and Zn(II) in xylem sap should be chelated by citrate, whereas Cu(II) should be chelated by various amino acids including histidine and asparagines (White et al.,1981). Isolation of a citratonickelate(II) complex from the latex of the Ni hyperaccumulator *Serbertia acuminata* supports the role of organic acids in metal transport (8).

X-ray absorbance fine structure (EXAFS) analysis showed that Cd in xylem sap of *B. juncea* was chelated by oxygen or nitrogen atoms, suggesting the involvement of organic acids in Cd translocation (13). EXAFS analysis produced no evidence for sulfur coordination of Cd, confirming that phytochelatins and other thiol-containing ligands play no direct role in Cd transport in the xylem.

In the present investigation we have demonstrated the cadmium accumulation potential of water-speedwell. This research indicates the efficacy of Veronica becabonga for decontamination of cadmium polluted water bodies.

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