Uptake of Cadmium by Alfalfa *(Medicago sativa* L.*)* grown in Liquid Medium

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ABSTRACT

Cadmium is a toxic heavy metal for humans, animals and plants which enters the environment mainly from anthropogenic sources including smelters and by applications of fertilizers and sewage sludge. Effective and economical technologies are needed to remediate cadmium from contaminated terrestrial and aquatic environment. Phytoremediation is a novel, cost-effective, eco-friendly 'green' remediation technology for remediation of heavy metals from contaminated environment. Researchers have observed that some plant species endemic to metalliferous soils can tolerate greater than usual amounts of heavy metals and accumulate them in their aerial biomass. In the present study, the uptake of cadmium by alfalfa (*Medicago sativa* L.) was studied under *in vitro* culture conditions. Plants initially grown in liquid media containing Murashige & Skoog's medium were transferred to Hoagland's medium spiked with cadmium as Cd $(NO_3)_2.4H_2O$. The concentrations of cadmium used in this study were 0, 5, 10, 20 and 50 ppm and the experiments carried out upto a period of 21 days. After 21 days of treatment, the cadmium content in plant tissues was quantified using Atomic Absorption Spectroscopy. The results showed that 80-85% of cadmium from solution (50 ppm) was remediated by alfalfa plants within 21 days of culture. Most of the cadmium i.e.12360 ìg gm^{-1} was accumulated in roots, while 1920 ìg gm^{-1} was translocated to shoots. The present study that alfalfa plants could remediate cadmium will be useful to remediate cadmium from contaminated solutions.

INTRODUCTION

Increased industrialization, mining, smelting, electroplating, agriculture and anthropogenic activities have contributed to elevated levels of various heavy metals and their compounds in the soil and water. Removal of these pollutants is necessary for the survival of life system and maintenance of ecosystem. Unlike organic compounds, heavy metals cannot be degraded and tend to bioaccumulate in the living organisms. Heavy metal causes toxicity and environmental impact although toxicity is entirely dependent

on the particular element, speciation, concentration and environmental parameters (Fulekar, 2005).

Cadmium is a heavy metal naturally present in soil at concentrations of slightly more than 1mg Kg^{-1} (Peterson and Alloway, 1979). Cadmium is highly toxic to most organisms, having toxicity 2-20 times higher than many other metals (Vassilev et al, 1998). Cadmium poses a significant health risk to living organisms. The cadmium concentrations above the threshold limit values have been found to be carcinogenic, mutagenic and terratogenic for a large number of animal species (Degraeve, 1981). It is considered as the most serious pollutant of the modern age.

The environmental cleanup of toxic metals such as cadmium is of paramount importance. There are various methods for the remediation of heavy metals contaminated soil and they are roughly classified into physical, chemical and bioremediation (Zhou and Song, 2004). The limitations of the first two methods are high cost, destruction of soil structures and adverse effects on biological life. The development of phytoremediation technique is being driven primarily by the high cost of many other remediation methods, as well as the desire to use "green", sustainable process (Pulford and Watson, 2003). Phytoremediation is an environmental friendly method, which utilizes the ability of plants for to take up heavy metals from the soil-water environment. Researchers have observed that some plant species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds (Peralta et. al., 2000). The plants which accumulate high amounts of heavy metals in their aerial biomass under natural conditions are known as hyperaccumulators. Hydroponic media contain all the major and minor nutrients, required for the growth and development of the plants. The advantage of this method is the short period required, and it is easy to observe changes in rhizosphere of plants (Zhi-xin et al., 2007). Miller et al. (1995) reported that alfalfa plants had the ability of accumulating cadmium from soils receiving high rates of sewage sludge.

Alfalfa (*Medicago sativa* L.) is a flowering plant in the family Fabaceae. It is a cool season perennial legume from three to twelve years, depending upon climate and variety. The plant grows to a height of upto 1 metre, and has a deep root system sometimes stretching to 4.5 meters, which makes it very resilient, specially to droughts. It has a tetraploid genome.

In the present study, experiment was conducted to evaluate the uptake of cadmium by alfalfa plant grown in liquid media under *in vitro* conditions.

MATERIALS AND METHODS

Alfalfa (*Medicago sativa* L. var. Lucerne Col) seeds, obtained from seed suppliers Ratanshi Agro-Hortitech (Byculla, Mumbai) were sterilized with 70% ethanol for 30 seconds followed by sterilization with 0.1% mercuric chloride for 5 min. The seeds were thoroughly washed 5 times with sterile distilled water. These sterilized seeds were inoculated in test tubes containing MS (Murashige and Skoog, 1962) basal medium

supplemented with 3% sucrose and kept in a gyratory shaker at 30-50 rev/ min. Seedlings were allowed to grow for one month under *in vitro* condition.

Seedlings (one month old) of similar size were transferred to Steinberg solution (Steinberg, 1953), containing various plant nutrients spiked with different concentrations of cadmium as cadmium nitrate [Cd $(NO_3)_2$. $4H_2O$]. The different treatments of cadmium used in this study were 0, 5, 10, 20, and 50 µg ml^{-1}. The experiment was carried out in triplicates and mean values are reported. Sample of 0.5 ml aliquots were withdrawn from each concentration on 0^{th}, 1^{st}, 7^{th}, 14^{th} and 21^{st} day of treatment. These samples were used for the analysis of cadmium. The treatment was carried out for 21 days. The plants after treatment were harvested, roots and shoots separated, dried at 60°C to constant weight and dry weight noted. The dried plant material was digested with HNO_3: $HClO_4$ (5:1 v/v) acid mixture on a hot plate, till a clear solution was obtained The volume was made up with double glass distilled water and the cadmium content analyzed for cadmium using GBC 932 B+ Atomic Absorption Spectrophotometer. Experimental data were analyzed for uptake of cadmium. Further transport index and bioaccumulation coefficient were computed using the following formulae:

(i) Transport index (TI) = $\frac{\text{Shoot content}}{\text{Total plant content}} \times 100$

(ii) Bioaccumulation Coefficient (BC) = $\frac{\text{Cadmium content } g^{-1} \text{ dry plant tissue}}{\text{Cadmium content } ml^{-1} \text{ nutrient solution}}$

Least significance difference [LSD (p = 0.05)] test was used for the comparison between the treatment means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The uptake of cadmium metal by *M. sativa* at various concentrations was investigated in hydroponic medium for a period of 21 days. Results on dry matter yield (g) and uptake of cadmium by *M. sativa* are shown in Table 1. No significant difference was noticed in the dry matter yield of plants grown in nutrient solution spiked with different concentrations of cadmium from 0 to 50 µg ml^{-1}. However, the uptake of cadmium by both root and shoot increased gradually with respect to increase in cadmium concentrations. Significantly high amount of cadmium was taken up at 50 µg ml^{-1} treatment by both root and shoot as compared to other treatments. Root accumulation of cadmium was found to be higher than that of shoot accumulation; it was 3.6 times and 6.4 times more at the lowest and highest treatment levels of 5 and 50 µg ml^{-1}, respectively. Jiang et. al. (2004) in their plant uptake studies from soil has reported similar findings where the accumulation of cadmium by roots was more than that of shoots in Indian mustard.

The bioaccumulation coefficient and transport index data of cadmium in alfalfa plant, in general (Table 2), indicated that these parameters decreased with increased cadmium concentrations of external nutrient solution. The lowest treatment of 5 µg ml^{-1}

resulted in highest amount of translocation of cadmium in the shoot of alfalfa plant showing thereby the potential of this plant to absorb cadmium readily.

The potential of plants for phytoremediation is based on the depletion of metal level from the nutrient solution at various concentrations. Figure 1 demonstrated the

Table 1: Uptake of Cadmium by *M. sativa* (alfalfa) plant grown at varying \concentrations of cadmium in nutrient solution

Cadmium concentrations (µg/ml)	Plant dry weight (g) Root	Shoot	Cadmium uptake (µg g^{-1} dry wt) Root	Shoot
Control	0.036	0.116	ND	ND
5	0.028	0.099	1546	431
10	0.028	0.099	3876	778
20	0.028	0.107	6270	1292
50	0.026	0.104	12360	1920
LSD (p=0.05)	NS	NS	4914	659

(Duration of cadmium treatment: 21 days). ND = Not detected; NS= Not significant

Table 2: Bioaccumulation coefficient and transport index of *M. sativa*

Cadmium concentration (µg/ml)	Bioaccumulation Coefficient	Transport index (%)
5	395.50	21.80
10	465.43	16.71
20	378.12	17.08
50	285.60	13.44
LSD (p=0.05)	171.12	8.34

depletion of cadmium metal from hydroponic solution at various concentration of cadmium metal studied. The reduction of cadmium concentration in the nutrient solution was attributed to its uptake by the plants. It showed that *M. sativa* had taken up 85% of cadmium from the nutrient solution at the end of 21 days of treatment. The result further confirmed that the quantity of cadmium accumulated by plants increased with the increase of cadmium concentration and the accumulation of cadmium was found high in the root tissues as compared to the shoot tissues. Cataldo et al. (1983) in their studies on soybean has reported that cadmium ions are mainly retained in the roots and only small amounts are transported to the shoots. As reported by Ernst et al., (1992) potential tolerance

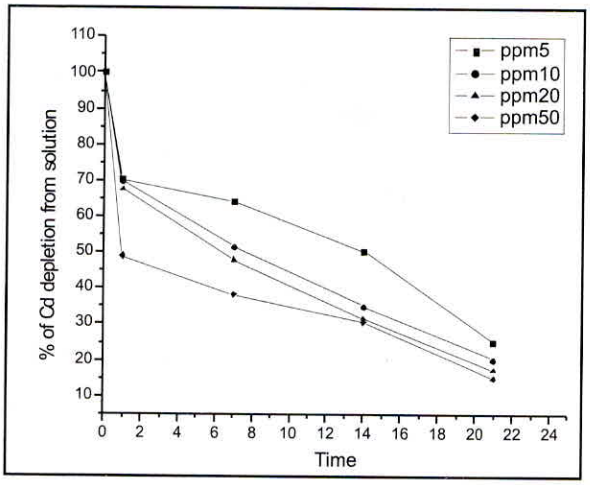

Fig. 1: Depletion of cadmium metal from the solution

(various concentration of Cd) which shows the uptake of metal by alfalfa plants (M. sativa)

mechanism operating in the roots may be responsible for reduced translocation of cadmium to shoots of alfalfa.

The accumulation of metal in plant shoot indicate that the soluble metals can enter into the root symplast by crossing the plasma membrane of the root endouermal cells or they can enter the root apoplast through the space between the cells. For xylem transport, the metals have to cross the endodermal cells. The solutes travel up through the plants by apoplastic flow by xylem. To enter the xylem, metal must cross a membrane, probably through the action of a membrane pump or channel. Once loaded into the xylem, the flow of the xylem sap will transport the metal to the aerial parts, where it must be loaded into the cells of the leaf, again crossing a membrane. Cadmium uptake is likely mediated through transporters or channels for other divalent ions (Cosio et al., 2004). Several of zinc and iron transporting ZIP genes in plants have been shown to transport cadmium, although with a wide range of affinities (Grotz et al., 1998; Ramesh et al, 2003).

The present research study shows that, during 21 days of treatment, the heavy metals were depleted from the nutritive solution, suggesting absorption of cadmium metal by the plants. It is noted from our studies that *M. sativa* is found to be a suitable plant for phytoremediation operation, which can accumulate high concentration of cadmium from the hydroponic solutions.

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