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Potential of *Medicago sativa* for uptake of cadmium from contaminated environment

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Abstract

Cadmium is associated with industrial processes such as metal plating and the production of nickel-cadmium batteries, pigments, plastics and other synthetics. Cadmium is a potential environmental hazard. A cost effective technologies are needed to remove cadmium from the contaminated soil-water environment. Phytoremediation is a novel, cost effective and eco-friendly 'green' remediation technology for environmental cleanup. In the present research study the uptake of cadmium by Medicago sativa (alfalfa, var. CoI) has been studied under in vitro culture (aseptic) conditions. Plants initially grown in liquid media containing Murashige & Skoog medium were transferred to Steinberg solution spiked with cadmium as Cd $(NO_3)_2.4H_2O$ at concentration 0, 5, 10, 20 and 50 µg ml^{-1}. The experiment was continued for a period of 21 days till complete metal uptake takes place. It has been observed that the growth of alfalfa plants was affected at higher concentration i.e. at 20 and 50 µg ml^{-1}; whereas the lower concentration of cadmium was uptaken without any effects on growth of plant. The cadmium content in plant tissues was quantified using Atomic Absorption Spectroscopy. The result shows that most of the cadmium upto 12360 µg gm^{-1} was located in roots, while 1920 µg gm^{-1} was translocated to shoots when exposed to 50 µg ml^{-1} concentration of cadmium. The phytoremediation of cadmium using alfalfa plant in hydroponic solution shows that, during the period of the experiment (i.e. 21 days), the plant was found to have potential to uptake 80-85% of cadmium.

Keywords: *Medicago sativa*, cadmium, uptake, hydroponic, potential

Introduction:

The increased industrialization, mining, smelting, electroplating, agriculture and anthropogenic activities have concentrated various heavy metals and their compounds into the soil- water environment. The 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 [6].

Cadmium is a heavy metal naturally present in soil at concentrations of slightly more than 1mg Kg^{-1}[11]. Cadmium is highly toxic to most organisms, having toxicity 2-20 times higher than many other metals [16]. 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 teratogenic for a large number of animal species [4]. It is considered as the most serious pollutant of the modern age.

The environmental cleanup of toxic metals such as cadmium is paramount important. There are various methods for the remediation of heavy metals contaminated soil and roughly classified into physical, chemical and phytoremediation [18]. The limitations of first two methods are high cost, destruction of soil structures and had an adverse effect on biological life. The development of phytoremediation technique is being driven primarily by the high cost of many other remediation methods, as well as desire to use “green”, sustainable process [13]. Phytoremediation is environmental friendly method which utilizes the uptake ability of plants for the removal of heavy metals from the soils-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 [12]. The plants which accumulate high amount of heavy metals are known as hyperaccumulators. Hyperaccumulators can be selected by growing the plants in hydroponics media supplemented with metals. Hydroponic media contain all the major and minor nutrients, required for the growth and development of the plants. The advantages of this method are short period and maneuverable though there are some distinct characteristics between soil and liquid and it is easy to observe changes in rhizosphere of plants [17]. Miller et al. (1995) reported that alfalfa had the ability of accumulating cadmium in soils receiving high rates of sewage sludge (equivalent to 4.6 Kg Cd/hm^2) [9].

Medicago sativa (alfalfa) is a flowering plant in the pea family Fabaceae. It is a cool season perennial legume from three to twelve years, depending upon climate and variety. The plants grows to a height of upto 1 metre (3 ft), and has a deep root system sometimes stretching to 4.5 meters (15 ft), which makes it very resilient, specially to droughts. It has a tetraploid genome.

In the present study an experiment was conducted to evaluate the uptake of cadmium by alfalfa plant grown in hydroponics media under *in vitro* condition.

Materials and Methods

Medicago sativa (Alfalfa, var. Lucerne CoI) seeds, obtained from seed suppliers Ratanshi Agro-Hortitech (Bycalla, 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 [10] basal medium supplemented with 3% sucrose. Seedlings were allowed to grow for one month under *in vitro* condition.

Seedlings (one month old) of similar size were transferred to Steinberg solution [15] containing various plant nutrients spiked with different concentrations of Cadmium metal as Cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$). 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 average values are reported. Sample of 0.5 ml aliquots were withdrawn from each concentration on 0th, 1st, 7th, 14th and 21st 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 into roots and shoots, dried at 60^{o}C to constant weight and dry weight noted. The dried plant material was digested HNO_3: $HClO_4$ (5:1 v/v) with acid mixture on a hot plate till to get a clear solution. The volume was made up with double glass distilled water and the cadmium content analyzed for cadmium using GBC 932 B+ Atomic Absorption Spectroscopy.

Statistical analysis:

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

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

Least significance difference [LSD ($p = 0.05$)] was used for the comparison between the treatment means [8].

Results and Discussion

The uptake of Cadmium metal by *M. sativa* at various concentrations has been conducted in hydroponic medium under *in vitro* condition for a period of 21 days. The uptake of cadmium metal by alfalfa plant listed in Table 1 was based on the dry weight basis of harvested plant material. The significant increase in level of metal in roots and shoots has been found with increase in metal concentrations. The cadmium content was found more in roots than shoots of alfalfa plant. The cadmium concentration was found 1546 µg g^{-1} in roots and 431 µg g^{-1} in shoots when alfalfa plant was exposed to 5 µg ml^{-1} metal; whereas the cadmium concentration was 3876 µg g^{-1} (roots) and 778 µg g^{-1} (shoots) when the plants was exposed to 10 µg ml^{-1} initial concentration. The alfalfa plant at initial concentration of 20 µg ml^{-1} in hydroponic solution showed the uptake of 6270 µg g^{-1} in roots and 1292 µg g^{-1} in shoots. Cadmium concentration in roots and shoots reached an average of 12360 µg g^{-1} and 1920 µg g^{-1} respectively when exposed to 50 µg ml^{-1} of cadmium metal.

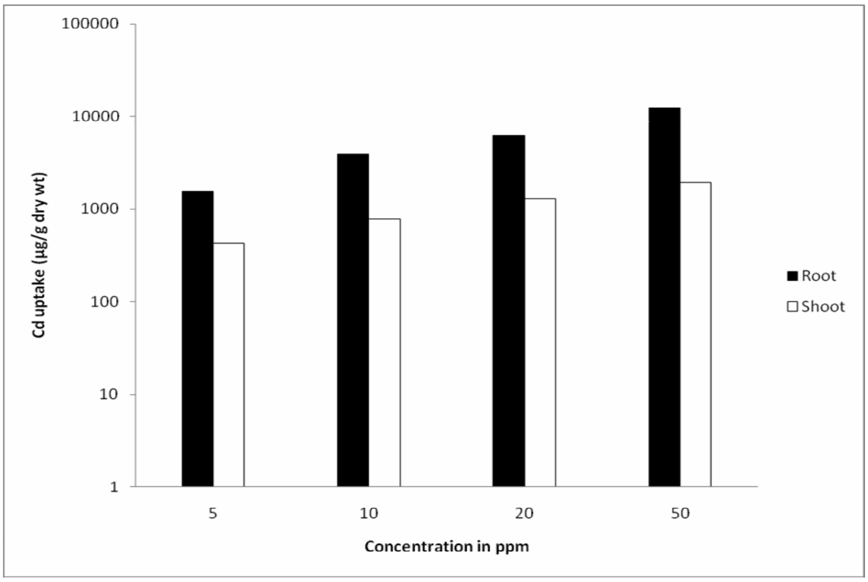

Figure 2. Cd accumulations in the dry biomass of roots and shoots of alfalfa plants (*M. sativa*) cultivated in hydroponics media containing different concentrations of Cd.

Table 1. Uptake of Cadmium (µg g^{-1} dry wt) by *M. sativa* (alfalfa) plant grown at varying concentrations of cadmium in nutrient solution (Duration of cadmium treatment: 21 days).

Cadmium concentrations (µg/ml)	**Cadmium uptake (µg/g dry wt)**		**Plant dry weight (g)**	
	Root	**Shoot**	**Root**	**Shoot**
Control	ND	ND	0.036	0.116
5	1546	*431*	0.028	0.099

10	3876	*778*	0.028	0.099
20	6270	*1292*	0.028	0.107
50	12360	*1920*	0.026	0.104
L.s.d. (p=0.05)	4914	*659*	NS	NS

ND = Not detected; NS= Not significant

Table 2 demonstrated the bioaccumulation coefficient and transport index of cadmium metal by *M. sativa* at 5, 10, 20 and 50 µg ml^{-1}. The bioaccumulation coefficient is found high at 10 µg/ml (465.4) and low at 50 µg ml^{-1} (28.1). The highest transport index is found 21.80% at 5 µg ml^{-1} and the lowest is 13.44% at 50 µg ml^{-1}. The potential of plants for phytoremediation is also based on the depletion of metal level from the nutrient solution at various concentrations. The reduction of cadmium concentration in the nutrient solution was attributed to its uptake by the plants.

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

Cadmium concentration (µg/ml)	Bioaccumulation Coefficient	Transport index (%)
5	395.4	21.80
10	465.4	16.71
20	378.1	17.08
50	285.6	13.44

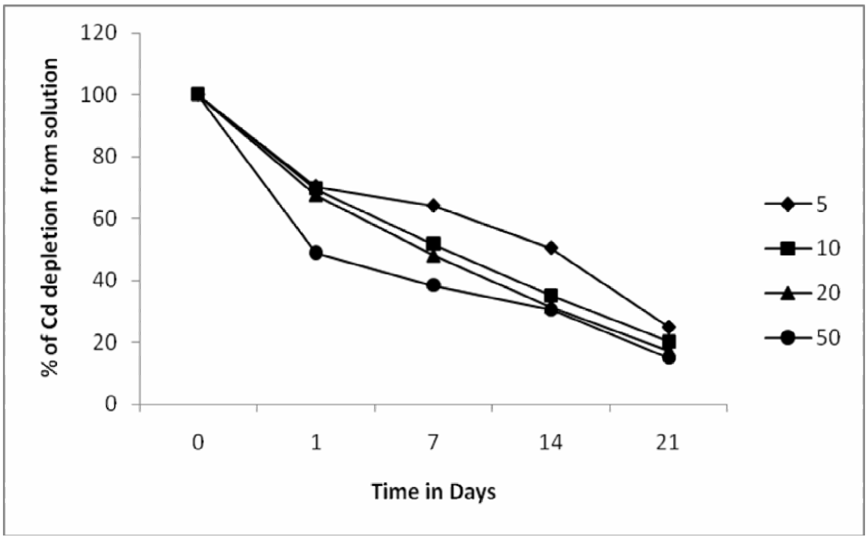

Figure 1. Depletion of cadmium metal from the solution (various concentration of Cd) shows the uptake of metal by alfalfa plants (*M. sativa*)

Figure 1 demonstrated the depletion of cadmium metal from hydroponic solution at various concentration of cadmium metal studied. It showed that uptake was highest at the first 4 days and then decreased continuously until 21 days. *M. sativa* has uptaken 85% of cadmium metal from the nutrient solution after 21 days of the exposure treatment. The result has shown that the quantity of cadmium accumulated by plants increased with the increase of cadmium concentration. The uptake of Cadmium metal was found high in the root tissues as compare to the shoot tissues. Cataldo et al. (1983) has reported that normally cadmium ions are mainly retained in the roots and only small amounts are transported to the shoots [2]. Roots of the plant acts as a barrier against heavy metal translocation and this may be a potential tolerance mechanism operating in the roots [5].

The uptake of metal in plant (root and shoot) parts indicate that the soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between the 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 [3]. Several of zinc and iron transporting ZIP genes in plants have been shown to transport cadmium, although with a wide range of affinities [7, 14].

The present research study shows that, during 21 days of exposure treatment, the heavy metals were depleted from the nutritive solution, suggesting absorption of cadmium metal by the plants. It is noted that *M. sativa* is the suitable candidate plant for phytoremediation operation, which has a lower biomass but accumulate a higher concentration of cadmium metal from the hydroponic solution.

Conclusion

The present research study indicates that the alfalfa plant has higher potential to uptake the toxic metal like cadmium from the concentration ranging from 5 to 50 $\mu g\ ml^{-1}$. the exposure of cadmium metal in hydroponic solution upto 50 $\mu g\ ml^{-1}$ concentration during the period of 21 days shows that the uptake of cadmium was 12360 $\mu g\ g^{-1}$ in shoots and 1920 $\mu g\ g^{-1}$ in roots; which has 285.6 bioaccumulation coefficient and transport index is 13.44%. The research finding shows that alfalfa plant has uptaken 80-85% of cadmium from hydroponic solution which will have direct application to remediate toxic metals from the contaminated soil-water environment.

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