A HYDROPONIC VETIVER SYSTEM TO REMOVE CADMIUM AND CHROMIUM FROM CONTAMINATED WATER

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Abstract

Phytoremediation is one of the biological methods used to purify industrial effluents and preserve the natural environment. The aim of the present study is to determine the possibility of using vetiver to purify cadmium and chromium contaminants in the Orathupalayam reservoir of Noyyal River in Tamil Nadu, India. One month old, hydroponically grown vetiver plants were used to treat contaminated reservoir water and synthetic wastewater with varying concentrations of cadmium and chromium. The data have shown that vetiver accumulates a maximum of about 625 $mgkg^{-1}$ of cadmium and 2847 mg kg^{-1} chromium. Vetiver could completely remove the cadmium (0.00052 ppm) and chromium (0.0577 ppm) present in Orathupalayam reservoir water. The antioxidant activities of SOD, calalase and peroxidase enzymes were found to be higher in roots than shoots of treated vetiver plants. The results demonstrated that vetiver plant has the capacity to remove Cd and Cr simultaneously and could be used to remediate sites affected with heavy metals. Results also showed that heavy metal toxicity reduced the plant's biomass, thereby decreasing the amount of metal accumulation.

Key words: Phytoremediation, Cadmium, Chromium, Hydroponics

1. Introduction

River water is often contaminated with industrial effluents, sewage water, municipal wastes, fertilizers and pesticides. Orathupalayam reservoir built in 1992 to store flood water from Noyyal river in South India, lies on the border between Kangayam and Perundrai taluks in Erode district of Tamil Nadu and has an ayacut of 500 acres. This dam is polluted by 75-100 million litres/day of effluent water from more than 700 dyeing and bleaching industries situated in the town of Tiruppur, 20 km upstream of the river (Furn, 2004). Effluents from the industries contaminate surface water, as well as soil and groundwater due to the presence of soluble solids, suspended solids, organic matter, heavy metals and toxic constituents. Toxic metal contamination of the river water poses a major environmental and human health problem, which is still in need of an effective and affordable technological solution.

Most conventional remediation approaches do not provide acceptable solutions to toxic metal pollution. The value of metal accumulating plants for environmental remediation is now fully recognized (Baker *et al.,* 1994; Raskin *et al.,* 1994). Phytoremediation is an emerging technology using specially selected and/or engineered metal accumulating plants for environmental clean-up with many advantages such as lower costs, generation of a recyclable metal-rich plant residue, applicability to a range of toxic metals and radionuclides, minimal environmental disturbance, elimination of secondary air or water borne wastes and public acceptance (Salt *et al*., 1995). Vetiver (*Chrysopogan zizanoides*), a perennial grass of the Poaceae is widely cultivated in the tropics. Many earlier studies have reported that vetiver grass grows well in soil contaminated with heavy metals (Troung and Baker, 1998; Zheng *et al.,* 1998; Roongtanakiat and Chairoj, 2001). Heavy metals accumulate both in the roots and shoots indicating the high tolerance of vetiver to heavy metals. All these early studies have proved vetiver as a phytoremediating species for contaminated soil, however, reports on its ability to remediate aquatic systems is lacking. This is a pioneering effort to use vetiver for remediating contaminated water. The research presented here aimed at using hydroponic vetiver system for absorption of heavy metals (cadmium and chromium) from contaminated water samples of the Orathupalayam reservoir and synthetic water samples with varying concentrations of cadmium and chromium.

2. Materials and methods

2.1 Characterization of contaminated water

The contaminated water sample was collected from the Orathupalayam reservoir located on the Noyyal River in South India. The water was collected from different points in the reservoir, pooled together to get a composite sample and used for the experiments. Cadmium and chromium concentration in the Orathupalayam water were determined using Atomic Absorption Spectrophotometer (Varian AA240, Australia).

2.2 Cultivar source and growth conditions

The vetiver slips were obtained from Agricultural Research Station, Tamil Nadu Agricultural University, Aruppukottai, India. The vetiver slips were grown in hydroponic condition for a month in a greenhouse and then used for the treatment. Clay pots lined by polythene bags were used for the growth of plants to avoid the adherence of the chemicals to the walls of the clay pots. The vetiver slips were fixed on the thermocol in each pot containing 4.0 l of 0.5X Hoagland's nutrient solution (Hoagland and Arnon, 1950). The vetiver slips obtained were allowed to grow in distilled water for overnight. Then they were gradually subjected to grow in 0.25X concentration of Hoagland's solution for one day and in 0.5X concentration of Hoagland's solution for a month. The nutrient solution was changed once in three days in order to avoid the depletion of macro and micro nutrients. The tillering of shoot and development of shoots were observed. The plants were grown for a month to get sufficient biomass and then subjected to various treatments for 14 days.

2.3 Treatment of plants with heavy metals

After growing vetiver for a month in hydroponics, the plants were weighed and the plants with uniform biomass were subjected to various treatments using cadmium nitrate and potassium dichromate (Table 1). Each treatment pot contained one plant. The treatment was done in four replications and was carried out for fourteen days. During the treatment period 100 ml of 0.1X of Hoagland's solution was added once in three days in order to compensate the water loss due to evaporation and nutritional loss.

Table 1: Heavy metal treatment conditions for vetiver. O_s is the Orathupalayam reservoir water sample.

Treatments	Treatment condition	
	Cd as $Cd(NO_3)$ (ppm)	Cr as $K_2Cr_2O_7$ (ppm)
T1	0	0
T2	5	0
T3	10	0
T4	20	0
T5	0	10
T6	5	10
T7	10	10
T8	20	10
T9	0	50
T10	5	50
T11	10	50
T12	20	50
T13	0	75
T14	5	75
T15	10	75
T16	20	75
T17	O_s	O_s
T18	O_s + 5	O_s
T19	O_s	O_s + 10
T20	O_s + 5	O_{s+} 10

2.4 Plant harvest and sample preparation

After the treatment, plants were harvested and the fresh and dry weights were measured. The fresh samples of leaves and roots were used for antioxidant assays. The dried samples were powdered by using Wiley mill and used for further analysis.

2.5 Analysis for cadmium and chromium

A known weight of powdered sample was digested with tri-acid mixture (9:2:1 of HNO_3: H_2SO_4: $HClO_4$) and the samples were analyzed for cadmium and chromium in Atomic Absorption Spectrophotometer (Varian AA240, Australia). Similarly, the water samples were analysed at the end of the treatment to determine the depletion of heavy metals in the treatment samples using AAS.

2.5 Antioxidant enzyme assays

The antioxidant assays were performed in shoot and root samples. Enzyme extraction was carried by grinding 0.5 g of sample with 7 ml of 0.1 M phosphate buffer (pH 7) at 4°C followed by centrifugation at 12,000 rpm for 15 min. Peroxidase activity was measured by the method of Lobarzewski and Ginalska (1995). Catalase activity was measured by the procedure of Aebi (1984). The assay of SOD was performed according to Das *et al.,* (2000).

2.6 Statistical analysis

The experimental data were analyzed using LSD and their statistical significance was determined. Statistical analysis was performed using SPSS Software.

3. Results and discussion

3.1 Characterization of Orathupalayam water

Water samples from Orathupalayam reservoir had 0.00052 ppm of cadmium which was less than the permissible limit of 0.01 ppm and 0.0577 ppm of chromium which was slightly higher than the permissible limit of 0.05 ppm (Table 2) (BIS, 1991). The heavy metal concentration of the water in the reservoir may vary due to various factors like heavy rainfall, quality and quantity of effluents let out by the nearby industries, etc. Since the levels of heavy metals were not much higher in the collected sample, Orathupalayam water spiked with synthetic salts of cadmium and chromium were also used in the study to simulate conditions of high metal concentrations.

Table 2: Characterization of Orathupalayam reservoir water

Parameters	Results	Permissible limit (BIS Guideline value)
pH	8.5	6.5-8.5
TSS (mg/L)	371	100
TDS (mg/L)	2910	2000
Cadmium (ppm)	0.00052	0.01
Chromium (ppm)	0.0577	0.05

3.2 Plant growth

Cadmium and chromium both affect vetiver as seen by the toxicity symptoms such as stunted growth, a poorly developed root system, curled and discolored leaves, and even complete wilting and death of exposed plants, similar to the observations made by Das et al, (1997) for cadmium toxicity. Chromium at 50 and 75 ppm was found to be highly toxic to the plants as they did not survive until the end of the treatment period. Chromium is known to cause oxidative damage to the cells which affect the uptake of mineral nutrients and water, leading to mineral nutrition deficiency particularly chlorosis and eventually death. Plants under all the treatments showed reduction in biomass in the 14-day period which could be the result of toxicity of the metals affecting various metabolic functions of the plant. Similar results were observed in the various other plants including *Alyssum* due to cadmium and nickel stress reported by Hedva and Hadar (1999). The biomass accumulation was inversely proportional to the heavy metal concentration (Fig. 1). The maximum effect was observed in plants subjected to 20 ppm $CdNO_3$ and 75 ppm $K_2Cr_2O_7$ (T16). The plants grown in the un-spiked reservoir water, also showed lesser accumulation compared to control plants (T1).

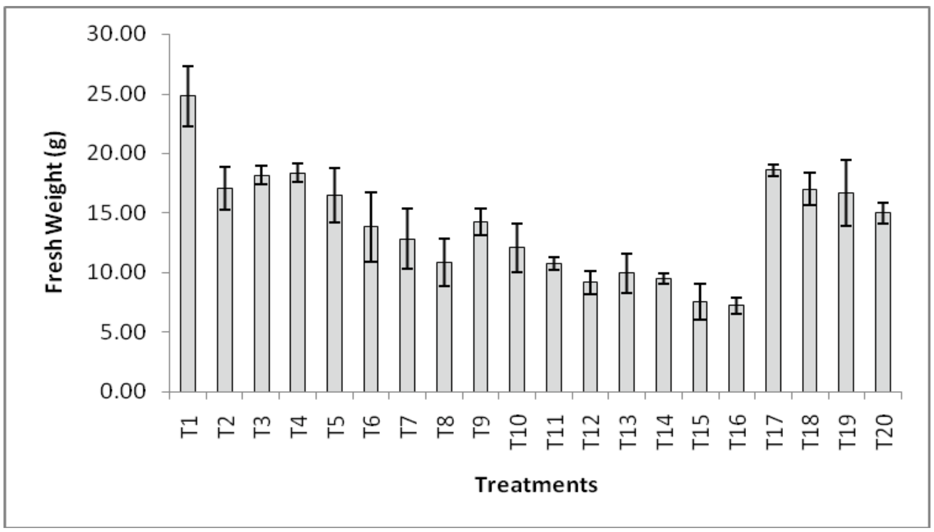

Fig. 1: Biomass accumulation represented as fresh weight (g), by vetiver grown under various treatment conditions. The values are mean of triplicates. Treatments T7 to T16 did not survive until the end of the experimental period, the biomass indicated are the maximum achieved by vetiver in these treatments.

2.7.3 Heavy metal accumulation in plants

Vetiver could effectively remove heavy metals from the various treatments to a maximum of 89.6% cadmium and 38.82% chromium in treatments T4 and T13 respectively. Cadmium and chromium were completely removed from the un-spiked Orathupalayam reservoir sample at the end of the treatment (Table 3). Similar to many other plants, in vetiver also the accumulation of metals were higher in the roots compared to shoots (Table 4). Maximum cadmium accumulation of 625.63 $mgkg^{-1}$ was observed in the treatment of 20 ppm $CdNO_3$ and 10 ppm $K_2Cr_2O_7$ (T8), while maximum translocation of cadmium to the shoot was about 9.95% noticed in the treatment of 20 ppm $CdNO_3$ alone (T4). Cadmium uptake by roots was 5-10 times higher than that translocated to the shoots. Maximum chromium accumulation by vetiver of 2847.28 mg kg^{-1} was seen at 75 ppm $K_2Cr_2O_7$ treatment (T13) (Table 4).

Table 3: Depletion of Cd and Cr in the water sample after 14 days of vetiver growth.

Depletion of heavy metal in water sample						
Treatment	Initial conc. ppm		Depletion ppm		Depletion %	
	Cd	Cr	Cd	Cr	Cd	Cr
T1	0	0	-	-	-	-
T2	5	0	4.09	0.00	81.72	-
T3	10	0	8.24	0.00	82.35	-
T4	20	0	17.92	0.00	89.60	-
T5	0	10	0.00	2.95	-	29.52
T6	5	10	3.15	2.78	63.07	27.77
T7	10	10	8.10	2.69	80.98	26.86
T8	20	10	17.48	2.35	87.40	23.54
T9	0	50	0.00	18.11	-	36.22
T10	5	50	2.54	17.64	50.83	35.29
T11	10	50	6.94	17.04	69.36	34.08
T12	20	50	16.58	16.52	82.89	33.04
T13	0	75	0.00	29.11	-	38.82
T14	5	75	2.85	28.55	57.08	38.07
T15	10	75	7.37	28.25	73.71	37.67
T16	20	75	17.02	27.48	85.09	36.64
T17	0.00052	0.0577	0.00	0.06	100.00	100.00
T18	5.00052	0.0577	3.25	0.06	65.05	100.00
T19	0.00052	10.0577	0.00	2.58	100.00	25.64
T20	5.00052	10.0577	3.96	2.11	79.16	20.98

Note: "-" denotes values for those treatments where there was no addition of that particular metal(s). Values are mean of four replicates

Similar to cadmium the accumulation in roots were more than that of shoots and the maximum Cr translocation to shoot was 10.15% observed for treatment T11 with 10 ppm $CdNO_3$ and 50 ppm $K_2Cr_2O_7$. Similar results were obtained by Chandra *et al*. (1997) on studies on the ability of *Scirpuslacustris*, *Phragmites karka* and *Bacopa monnieri* to absorb, translocate and concentrate Cr in their tissues. Their results showed that most of the Cr removed by these species was accumulated in their roots with very low Cr being concentrated in their shoots. These differences in root and shoot uptake might be explained by the fact that one of the normal functions of roots is to selectively acquire ions from the soil solution, whereas shoot tissue does not normally play this role (Salt *et al*, 1997). Many earlier studies have reported higher accumulation of metals in the roots compared to shoots (Chandra *et al.,* 1997; Andra *et al.,* 2009). The roots are the first plant structures coming in contact with the metal containing media and the metals have to be translocated to the shoots. Accumulation of Cd by roots is found to be dependent on the number of root tips (Berkelaar and Hale, 2000). The accumulation could also be dependent on the tonoplast based cation trasnporters that were seen to affect cadmium accumulation in roots (Koren'kov *et al.,* 2007). Vetiver with its fibrous roots is able to accumulate relatively higher amount of the metals in its roots.

Table 4: Metal accumulation by vetiver grown for 14 days under various treatment conditions

Treatment	Initial Conc in solution ppm		Metal accumulation in plant parts			
			Conc of Cd mg/kg		Conc of Cr mg/kg	
	Cd	Cr	Shoot	Root	Shoot	Root
			Mean±STD	Mean±STD	Mean±STD	Mean±STD
T1	0	0	0.000	0.000	0.000	0.000
T2	5	0	35.650±2.275	455.700±35.705	0.000	0.000
T3	10	0	42.800±3.251	468.950±26.940	0.000	0.000
T4	20	0	60.825±6.803	550.300±21.648	0.000	0.000
T5	0	10	0.000	0.000	155.830±6.284	1481.850±11.895
T6	5	10	33.775±4.152	425.350±46.573	146.840±5.836	1405.700±15.757
T7	10	10	38.175±5.698	512.200±24.297	140.485±6.083	1302.850±21.747
T8	20	10	56.725±5.786	568.900±16.325	126.448±10.953	1219.500±52.342
T9	0	50	0.000	0.000	209.918±14.851	2088.050±39.886
T10	5	50	28.150±3.943	367.150±27.169	186.675±8.494	1910.475±17.904
T11	10	50	39.025±6.664	415.700±21.966	175.008±10.066	1724.250±28.234
T12	20	50	54.775±5.890	495.900±13.841	154.575±10.736	1642.325±27.963
T13	0	75	0.000	0.000	270.063±18.197	2577.225±28.103
T14	5	75	25.225±5.496	323.850±57.323	249.188±13.496	2451.800±40.636
T15	10	75	40.100±5.735	449.380±52.060	225.583±10.619	2290.875±20.775
T16	20	75	55.350±7.378	558.970±22.150	213.633±13.190	2150.475±23.470
T17	0.00052	0.0577	1.600±0.271	39.150±15.470	31.215±5.643	431.100±31.579
T18	5.00052	0.0577	40.800±5.350	492.130±34.855	29.358±6.777	408.975±21.219
T19	0.00052	10.0577	0.700±0.245	12.050±7.576	118.155±12.854	1207.975±53.141
T20	5.00052	10.0577	38.275±6.830	464.305±26.420	97.620±9.715	1057.280±25.065

Note: Values are mean of four replicates

2.7.4 Antioxidant enzyme activity

Peroxidase, catalase and superoxide dismutase activities in roots and shoots were analysed as indicators of plant tolerance. Vetiver subjected to 50 and 75 ppm $K_2Cr_2O_7$ did not survive till the end of the treatment so only those grown under 0. 5, 10 and 20 ppm $CdNO_3$ and 0 and 10 ppm $K_2Cr_2O_7$ were used for antioxidant analysis. All the plants subjected to heavy metal stress showed higher antioxidant enzyme activity and the activity was higher in the roots than the shoots for all the treatments (Fig 2 and 3). Maximum antioxidant activity was seen in vetiver grown in 20 ppm $CdNO_3$ (T4) and that in Orathupalayam reservoir sample spiked with 5 ppm $CdNO_3$ and 10 ppm $K_2Cr_2O_7$ (T20). The peroxidase and SOD activities were higher in roots of treatment T20, being 5.279 µM min^{-1} mg^{-1} protein and 1.4321 U min^{-1} mg^{-1} protein respectively, while the maximum catalase activity of 3.7286 µM min^{-1} mg^{-1} protein in roots of T4.

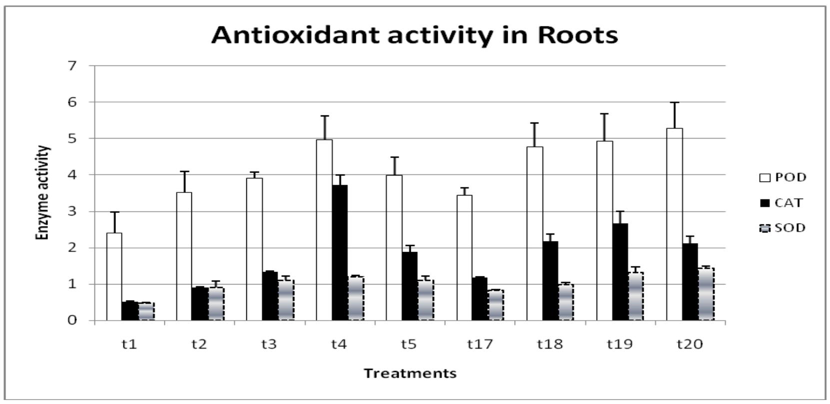

Fig. 2: Antioxidant enzyme activities in the roots of vetiver grown under various treatment conditions. The values are mean of triplicates. POD - peroxidase, µM min^{-1} mg^{-1} protein, SOD - superoxide dismutase, U min^{-1} mg^{-1} protein, CAT – catalase, µM min^{-1} mg^{-1} protein

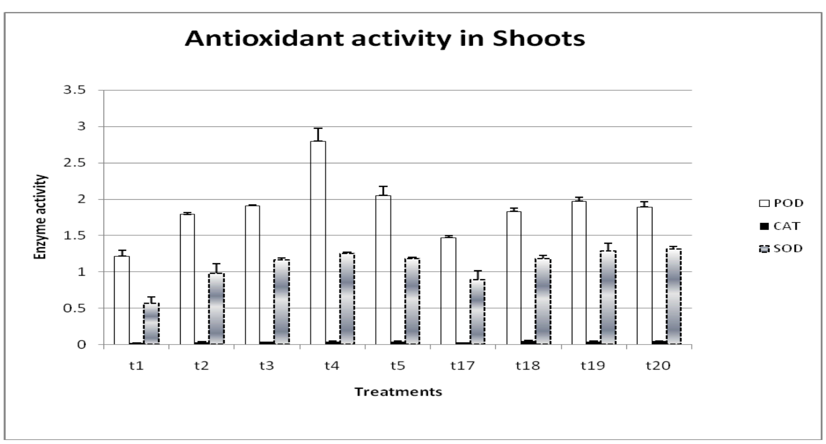

Fig. 3: Antioxidant enzyme activities in the shoots of vetiver grown under various treatment conditions. The values are mean of triplicates. POD - peroxidase, µM min^{-1} mg^{-1} protein, SOD - superoxide dismutase, U min^{-1} mg^{-1} protein, CAT – catalase, µM min^{-1} mg^{-1} protein

From the results, it is evident that the oxidative stress increased with the increase in heavy metal concentration. Similar results with increase in POD, CAT and SOD activity was noticed in garlic treated with high concentrations of cadmium (Zhang *et al.*, 2005). However, there are varied reports on antioxidant responses among different plant species, tissues, metal responsible and also the intensity of the stress. In *Phaseolus aureus* seedlings, Cd induced elevated guaiacol peroxidase, but decreased catalase activities (Shaw, 1995) whereas in *Populus canescens* roots, severe diminution of ascorbate peroxidase, catalase, and glutathione reductase activities were observed (Schutzendubel *et al*, 2002). An increase in CAT activity under Cd stress has been found in radish (Vitoria *et al*, 2001). Dixit *et al.,* (2001) had shown that pea plants stressed with Cd showed an induction in antioxidant activities in roots over a period of 7 days and transient induction in leaves. In our study, the accumulation of both Cd and Cr was higher in the roots than the shoots this could be the reason for the relatively higher antioxidant activities seen in the roots. Cd is known to cause lipid peroxidation suggesting that the tissues suffered from oxidative stress. The involvement of antioxidant enzymes in plant responses against cadmium toxicity is unclear because Cd does not belong to the group of transition metals like copper, iron, zinc etc. which may induce oxidative stress via Fenton-type reactions (Schutzendubel *et al*, 2002).

In the present study, the metal content in the samples from the reservoir were relatively low and its effect on biomass reduction was also the least (Fig 1). Upon spiking with heavy metals to simulate conditions wherein the metal concentration in the reservoir water would be high, the trend in the response of vetiver was similar to that seen in the synthetic samples, although on comparison the biomass reduction of synthetic sample containing 5 ppm $CdNO_3$ and 10 ppm $K_2Cr_2O_7$ (T6) was more than that of the Orathupalayam reservoir sample spiked with 5 ppm $CdNO_3$ and 10 ppm $K_2Cr_2O_7$. There are earlier reports that nutrient limitations could lead to higher metal accumulation (Lombi *et al.,* 2002). It has also been found that presence of ammonium nitrogen will facilitate the mobilization of sparingly soluble Cd (Zaccheo *et al.,* 2006) increasing its accumulation in plants (Padmavathiamma and Loretta, 2008). The reservoir receives effluents from various types of industries, including textile dyeing and bleaching industries and tanneries apart from waste from residential settlements (Furn, 2004), there is also variation in the water quality due to the variation in effluents that mingle with it or the rain water. Metal accumulation could be affected by the water's characteristics. In the present study it was found that in the Orathupalayam reservoir samples spiked with heavy metals (T18 and T20), the cadmium accumulation was marginally higher than that of the synthetic samples with similar amount of heavy metals (T2 and T6) although the chromium accumulation was relatively reduced (Table 3)

The heavy metals inhibit leaf growth, dry matter accumulation, and photosynthesis of leaves, and enhance the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and the tolerance is dependent on different mechanisms that nullify the toxic effects of heavy metals. Our results suggest that the accumulation of Cd and Cr in roots and shoots causes active oxygen species generation, which are ameliorated by the increases in the antioxidant enzyme activities. These observations imply that heavy metal tolerance is a characteristic of vetiver, which could be exploited for phytoremediation.

2.8 Conclusions and Recommendations

Hyperaccumulators are plants have the ability to accumulate toxic metal ions in their tissues. Our results show that vetiver could accumulate >100mg/kg cadmium and >1000mg/kg chromium. Hydroponic vetiver system can be used as one of the wastewater treatment methods for industrial effluents with high content of heavy metals. In our study, we have investigated the potential of vetiver to uptake and translocate cadmium and chromium hydroponically and found it to be a possible candidate for use in remediating cadmium and chromium contaminated water bodies. Further studies into the mechanism of heavy metal removal by vetiver needs to be carried out to fully exploit its potential.

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