CHROMIUM ACCUMULATION BY PHYTOREMEDIATION WITH MONOCOT WEED PLANT SPECIES AND A HYDROPONIC SAND CULTURE SYSTEM

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ABSTRACT

The accumulation of chromium by three monocot species Cynodon dactylon, Echinochloa colonum and Vetiveria nemoralis cultivated in a hydroponic sand culture system was investigated in this study. The capacity for chromium removal and the accumulation of chromium in different parts of the three monocot species were studied at concentrations of 0, 5, 10 and 15 mg CrVI/kg of sand for growing periods of 30, 60, 90 and 120 days. The results obtained from this study show that the highest accumulation of chromium in Cynodon dactylon occurred at a concentration of 5 mg CrVI/kg of sand after 60 days and equalled 94.59 mg/kg dry weight of plant. For the concentrations of 10 and 15 mg CrVI/kg of sand, chromium accumulations were 47.72 and 459.52 mg/kg dry weight of plant at 30 days, respectively. Cynodon dactylon showed the highest accumulation at the period of 30-60 days but had a tendency toward decreased accumulation levels as harvesting time increased. Echinochloa colonum showed the most accumulation of chromium at 120 days and was equal to 58.22, 90.80, and 246.35 mg/kg dry weight of plant at concentrations of 5, 10, and 15 mg CrVI/kg of sand, respectively. The highest accumulation of chromium in all parts of Vetiveria nemoralis for the concentration of 5 mg CrVI/kg of sand occurred at 90 days and was equal to 49.88 mg/kg dry weight of plant and also equal to 63.02 and 77.83 mg/kg dry weight of plant at 120 days for the concentrations of 10 and 15 mg CrVI/kg of sand, respectively. The results of this study clearly indicate that the highest chromium accumulation in all three monocot species occurred in the root, and that the chromium subsequently translocated to stem and leaf. This process is called phytoextraction or phytoaccumulation and the results show that Cynodon dactylon performed this function better than Echinochloa colonum and Vetiveria nemoralis.

Key Words : Chromium, Phytoremediation, Hydroponic, Monocot species, Cynodon dactylon, Echinochloa colonum, Vetiveria nemoralis

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INTRODUCTION

Presently, environmental pollution resulting from the use of chemicals in agriculture and the production of hazardous waste in industrial processes is one of the world’s most important problems. Chemicals, both organic and inorganic, are released into the environment and this results in soil, water and air pollution. The contamination by heavy metals such as arsenic, copper, mercury, cadmium, lead, nickel, chromium, manganese and zinc in water and soil sludge is especially worrying. Ninety percent of hazardous waste is a product of industrial processing. In the past, the heavy metal chromium (Cr), was often used in the tanning industry and spread widely into the environment, contaminating the soil, underground water and surface water resources, causing harm to humans, animals and plants. Trivalent Chromium - Cr(III) and Hexavalent Chromium - Cr(VI) are the two species of chromium which are found naturally in the environment. Cr(VI) appears to be more toxic than Cr(III)\(^1\). Many technologies, physicochemical and biological techniques, precipitation, oxidation reduction, microbial absorption and phytoremediation have been used for chromium removal. Phytoremediation is a biological methodology in which selected plants are used for treating heavy metals that have contaminated soil and/or water. The processes in Phytoremediation are, 1) Phytoextraction or Phytoaccumulation: the uptake of contaminants by plant roots and translocation within the plants, 2) Phytostabilization: the use of plants and plant roots to immobilize contaminant migration and transportation through soil and groundwater, 3) Rhizofiltration: the adsorption or precipitation of contaminants solution surrounding the root zone onto plant roots, or absorption into the roots by biotic or antibiotic processes, 4) Phytovolatilization: the uptake and transpiration of a contaminant by a plant, 5) Phytodegradation: the breakdown of contaminants taken up by plants through metabolic processes within the plant and, 6) the breakdown of an organic contaminant in soil through microbial activity that is enhanced by the presence of the root zone and referred to as Rhizodegradation or Enhanced Rhizosphere Biodegradation or Phytostimulation or Plan-tered Assisted Bioremediation/Degradation\(^2,3\). Phytoremediation technology has been receiving attention lately as an innovative and cost-effective alternative to the more established treatment methods used at hazardous waste sites\(^4\). Therefore, the removal of chromium contaminate from soil and water by using expendable monocot species such as Cynodon dactylon, Echinochloa colonum and Vetiveria nemoralis is of interest as an alternative technology.

Objectives

1. To study the removal capacity of three monocot species: Cynodon dactylon, Echinochloa colonum and Vetiveria nemoralis cultivated in sand media contaminated with chromium synthetic wastewater.

2. To study the accumulation and translocation of chromium in the plant root, stem and leaf of all three monocot species at various lengths of time and concentrations of Cr(VI).

3. To compare the accumulation and transformation of chromium in each part of three different monocot species which were grown in sand media.
MATERIAL AND METHODS

Material preparation

River sand was collected and air dried for 1-2 days, then soaked in 10% nitric acid for 2-3 days for cleaning, and subsequently air-dried. Plastic pots with 30-cm diameter used in this study were soaked with 10% nitric acid for 1-2 days then washed with tap and deionized water. All pots were air-dried for 2-3 hrs. Next, 5 kilograms of sand were placed in each pot.

Plant preparation

Monocot species: *Cynodon dactylon*, *Echinochloa colonum* and *Vetiveria nemoralis* as illustrated in Fig. 1 were used in this study.

1) *Cynodon dactylon*, *Echinochloa colonum* and *Vetiveria nemoralis* seedlings were selected and pruned to have similar height of stem and length of roots.

2) Selected monocot species were grown in tap water for 3-5 days in order to stimulate the growth of plant roots.

3) The young plants were transplanted into pots that contained 5 kilograms of sand. They were kept properly watered in a greenhouse for 2-3 weeks in order to observe their survival potential before Cr(VI) solution was added to the sand.

![Fig. 1: Monocot species used in the study](image)

(1) *Cynodon dactylon*, (2) *Echinochloa colonum* and (3) *Vetiveria nemoralis*

Synthetic wastewater preparation (chromium solution)

Synthetic wastewater (chromium solution) was prepared by dissolving Potassium Dichromate (K$_2$Cr$_2$O$_7$) in deionized water until reaching concentrations of 5, 10 or 15 mg Cr(VI)/kg of sand. A control environment was also prepared containing 0 mg Cr(VI)/kg of sand.

Experimental design and procedure

The 12 young samples of each plant species: *Cynodon dactylon*, *Echinochloa colonum* and *Vetiveria nemoralis* were planted into plastic pots containing 5 kilograms of sand, and maintained in a nursery for 2-3 weeks. Next, synthetic wastewater (chromium solution) at concentrations of 5, 10 or 15 mg Cr(VI)/kg of sand was added to the pots. In addition, the control group (0 mg Cr(VI)/kg of sand) was also prepared. No studied group received any fertilizer. Sand and plant samples were collected every 30 days throughout the 120-day trial period.

Sample collection

After 30, 60, 90 and 120 days, 5 grams of sand were collected randomly. The samples were air-dried at room temperature

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for 2 – 3 hr in order to determine fresh weight. After that, sand samples were allowed to dry at 105°C to measure moisture content. The TCr, Cr(VI) and Cr(III) in the sand samples were determined.

Plant samples were harvested after 30, 60, 90 and 120 planting days. Samples were washed and cleaned with tap water twice, rinsed with deionized water, and then air-dried at room temperature for 2 – 3 hrs in order to determine fresh weight. After that, they were separated into three parts: roots, stems and leaves. Subsequently moisture content of each part was determined. The samples were dried at 105°C for 24 hrs and dry-matter yields were also determined. Next, the samples were ground with an electric mill and thoroughly mixed to homogenize. They were then analyzed for TCr, Cr(VI) and Cr(III) levels.

Sample analysis
1) Synthetic wastewater samples were measured for pH, oxidation-reduction potential (ORP) and conductivity.
2) Total chromium in sand and plant samples were analyzed by US EPA method 3052 (microwave-assisted acid digestion of siliceous and organically-based matrices). Then the digested solution was analyzed by Atomic Absorption Spectroscopy; AAS.
3) The analysis of Cr(VI) in sand and plant samples was conducted by the colorimetric method: US EPA method 3060 and subsequently measured by using a UV spectrophotometer.
4) The amount of Cr(III) in samples was obtained by calculating the difference between TCr and Cr(VI).

RESULTS AND DISCUSSION
The accumulation of chromium in three monocot species: Cynodon dactylon, Echinochloa colonum and Vetiveria nemoralis was examined in the present study. All species were planted in sand media contaminated with Cr(VI) synthetic wastewater for 120 days. All three monocot species were grown normally without the addition of fertilizer. The results are summarized as follows.

The characteristics of chromium synthetic wastewater

Oxidation – Reduction Potential (ORP)
The analysis of chromium synthetic wastewater at concentrations of 5, 10 and 15 mg Cr(VI)/kg of sand shows that the oxidation reduction potential tended to decrease with increasing concentration. Moreover, the concentrations of synthetic wastewater (Cr solution) planted with the three monocot species: Cynodon dactylon, Echinochloa colonum and Vetiveria nemoralis tended to decrease with increasing length of the harvesting period. Cr(VI) is a strong oxidizing agent with a reduction potential of +1.331 volts. Therefore, the transformation of Cr(VI) to Cr(III) can occur and also leads to a decreased oxidation-reduction potential.

pH
For pH values of synthetic wastewater (Cr solution) measured at the different harvesting times (30, 60, 90 and 120 days) the results show that pH values of the synthetic wastewater at a concentration of 5 mg Cr(VI)/kg of sand tended to increase with increasing time for all three monocot species. This result may be due to the concentration of CO2 and CO3^2- species in synthetic wastewater. However, changes of the carbonate balance, resulting from biochemical reaction and nutrient absorption, could be a factor in increasing pH values of the Cr solution. Moreover, the decreasing mole and amount of Cr(VI) could also contribute to the increase in pH values. This phenomenon may result from the acidity state that occurred when Cr(VI) is dissolved.
Conductivity

The conductivity of synthetic wastewater at 30, 60, 90 and 120 days was also measured. The results from the study indicate that the conductivities trended to increase with increasing Cr concentration. This finding may be explained by the dissolved ions in water, changing pH value, and other factors which could be causing the deionization of organic compounds\(^8\). Therefore, increased pH and changes in other control factors can influence conductivity.

Chromium accumulation in sand media

For the analysis of total Cr accumulation in *Cynodon dactylon*, *Echinochloa colonum* and *Vetiveria nemoralis* cultivated sand, the results from statistical analysis show a significantly different total Cr accumulation in sand media at the concentration of 5 mg Cr\(_{\text{VI}}\)/kg of sand for values of 4.59, 6.17, 7.13 and 7.81 mg/kg at 30, 60, 90 and 120 days, respectively. For a concentration of 10 mg Cr\(_{\text{VI}}\)/kg of sand, the study found Cr accumulations of 19.07, 15.32, 9.45 and 5.55 mg/kg for a concentration of 10 mg Cr\(_{\text{VI}}\)/kg of sand at 30, 60, 90 and 120 days, respectively. The study found Cr accumulations of 19.07, 15.32, 9.45 and 5.55 mg/kg for a concentration of 10 mg Cr\(_{\text{VI}}\)/kg of sand at 30, 60, 90 and 120 days, respectively (Fig. 2.2). In addition, Cr accumulation in the *Echinochloa colonum*-planted sand media mostly appeared in the form of Cr\(_{\text{III}}\). Moreover, the accumulation of TCr in the *Echinochloa colonum*-planted sand media tended to decrease with increasing time.

The data for total chromium accumulation in the *Vetiveria nemoralis*-planted sand media are presented in Fig. 2.3. For the concentration of 5 mg Cr\(_{\text{VI}}\)/kg of sand, the data indicate a significant difference (p<0.05) of chromium accumulation in the sand media ranging from 11.59, 14.42, 10.51 and 19.37 mg/kg for the harvesting times of 30, 60, 90 and 120 days, respectively. For the concentration of 10 mg Cr\(_{\text{VI}}\)/kg of sand, the results are 13.17, 15.52, 10.52 and 11.62 mg/kg, respectively, however, these differences are not statistically significant. There was a significant difference (letters a and b) for chromium accumulation in the sand media at the concentration of 15 mg Cr\(_{\text{VI}}\)/kg of sand at 30, 60, 90 and 120 days, respectively. In addition, Cr\(_{\text{III}}\) was the preferable species for chromium accumulation in the sand media compared to Cr\(_{\text{VI}}\) as presented in Fig. 2.3. Moreover, the results also indicate that, at a concentration of 5 mg Cr\(_{\text{VI}}\)/kg of sand, total Cr accumulation in the sand media tended to
increase slightly. However, at the concentrations of 10 and 15 mg Cr(VI)/kg of sand, the accumulation of Cr in *Vetiveria nemoralis* cultivated sand media had a tendency to decrease with increasing time as presented in Fig. 2.3. This finding may result from the accumulation of Cr in various parts of *Vetiveria nemoralis*.

**Fig. 2**: Chromium accumulations in sand media

1) *Cynodon dactylon* 2) *Echinochloa colonum* and 3) *Vetiveria nemoralis*

Note: The same letter shows no significant difference at p<0.05 by Duncan’s multiple range tests.
Chromium accumulation in plants

As illustrated in Fig. 3.1, Cr accumulated in various parts of *Cynodon dactylon* at the concentrations of 5 mg Cr(VI)/kg of sand at 30, 60, 90 and 120 days. Cr was found mostly in the roots followed by leaves and stems, and equaled 94.59, 93.77 and 86.59 mg/kg dry weight after 60 days respectively, but these differences were not significantly different. However, the results did indicate a significant difference at p<0.05 (letters a, b and c) for samples at 30, 90 and 120 days. The results of this study show that the lowest accumulation of chromium in *Cynodon dactylon* occurred at 90 days. For the concentration of 10 mg Cr(VI)/kg of sand, the highest accumulations of Cr in various parts of *Cynodon dactylon* occurred at 30 days and were equal to 47.72, 38.04 and 31.23 mg/kg dry weight of plant for root, stem and leaf, respectively (Fig. 3.2). In addition, these differences were significant at p<0.05 (letters a, b and c) for the accumulation of Cr in the different plant parts for the periods of 30, 60 and 90 days, but not significantly different at 120 days. For a concentration of 15 mg Cr(VI)/kg of sand, Cr accumulation in the root, stem and leaf of *Cynodon dactylon* at 30 days was equal to 459.52, 210.24 and 183.96 mg/kg dry weight of plant, respectively. Significant differences (p<0.05) of Cr accumulation in different parts of *Cynodon dactylon* were found at 30 and 60 days (letters a, b and c). The lowest accumulation of Cr was found at the end of the study (120 days). (See data in Fig. 3.3.) The results of this study indicate that the most efficient part of *Cynodon dactylon* is the root, which accumulated approximately 10-100 times more Cr than the stem and leaf. This finding may be the result of the translocation of Cr into the xylem which is mostly found in the roots of plants. The accumulations of Cr in various parts of *Cynodon dactylon* had a tendency to decrease with increasing time.

![Graph showing chromium accumulation in different parts of Cynodon dactylon](chart.png)

1)
Fig. 3: Chromium accumulations in root, stem and leaf of *Cynodon dactylon* at different chromium concentrations: 1) 5 mg Cr/kg of sand 2) 10 mg Cr/kg of sand and 3) 15 mg Cr/kg of sand.

Fig. 4 demonstrates the accumulation of chromium in various parts of *Echinochloa colunum* at the different concentrations of chromium (5, 10 and 15 mg Cr\(_{VI}\)/kg of sand). The data show significant differences (p<0.05; letters a, b and c) for 30, 60, 90 and 120 days at all concentrations of Cr. The results of this study show that the greatest accumulation occurred at 120 days at a concentration of 5 mg Cr\(_{VI}\)/kg of sand. The highest accumulation was in the root which accumulated an amount of Cr equivalent to 58.22 mg/kg dry weight of plant. The accumulation of Cr in *Echinochloa colunum* also tended to increase with increasing time. Moreover, Cr accumulations in the other plant parts (stem and leaf) were found (Fig. 4.1). Fig. 4.2 displays the accumulations of Cr at the concentration of 10 mg Cr\(_{VI}\)/kg of sand, and shows that Cr accumulated mostly in the root and was equal to 90.80 mg/kg dry weight of plant at 120 days. The lowest accumulation in the root of *Echinochloa colunum* occurred at 60 days and was equal to 23.11 mg/kg dry weight. For the concentration of 15 mg Cr\(_{VI}\)/kg of sand, the
results show that the highest accumulation of Cr in the root was equal to 246.35 mg/kg dry weight and occurred at the period of 120 days. The lowest accumulation was equal to 109.48 mg/kg dry weight at 30 days (Fig. 4.3). Cr accumulations were also found in both stem and leaf of *Echinochloa colonum*. The highest accumulation of Cr occurring in the root may be due to its more direct contact to the Cr solution. Also, the root has a high capacity for cation exchange because of its high level of inorganic and amino acids. These acids could join with heavy metals and translocate heavy metal ions to the root of the plant. The accumulation of Cr in the stem and leaf of plants results from the translocation process. The results of this study also conform to those of who studied the accumulation of chromium and lead in *Hydrocotyle umbellate* L. The results of that study showed increasing chromium and lead accumulation in *Hydrocotyle umbellate* L. with increasing concentrations of heavy metals and time, and also found that the most accumulation of chromium and lead occurred in the root compared to other parts (i.e., the stem and leaf).
Fig. 4: Chromium accumulations in root, stem and leaf of *Echinochloa colonum* at different chromium concentrations: 1) 5 mg Cr/kg of sand 2) 10 mg Cr/kg of sand and 3) 15 mg Cr/kg of sand.

The results of Cr accumulation in the root, stem and leaf are illustrated in Fig. 5.1 to Fig. 5.3. The highest accumulation by *Vetiveria nemoraris* was in the root. For a concentration of 5 mg Cr(VI)/kg of sand, the differences were statistically significant. We also found that the greatest accumulation of Cr occurred in the root and was equal to 49.88 mg/kg dry weight at 90 days. The accumulation of Cr tended to increase through 90 days; after that, it tended to decrease until 120 days at which point it was equal to 18.42 mg/kg dry weight of plant.

For concentrations of 10 and 15 mgCr(VI)/kg of sand, the results were significantly different at 30, 90 and 120 days. However, statistical analysis showed no significant difference of Cr accumulation in various parts of *Vetiveria nemoraris* at the period of 60 days. The figure shows the highest and lowest accumulations of Cr in the root which are 63.02 and 8.67 mg/kg dry weight of plant for a concentration of 10 mg Cr(VI)/kg of sand at 120 and 60 days, respectively. In addition, this study found that the accumulation of Cr at the concentration of 15 mg Cr(VI)/kg of sand had a tendency to increase during the period of 30-120 days. The lowest Cr accumulation in *Vetiveria nemoraris* was at 30 days and was equal to 22.54 mg/kg dry weight of plant; the highest accumulation was found at 120 days and was equal to 77.83 mg/kg dry weight of plant. The results of this study are in accordance with the study of H in the removal of Cr in wastewater from the plating industry by using *Nymphaea spontanea* (*lotus* plant). That study reported the most accumulation by *Nymphaea spontanea* in the root at a concentration of 10 mg Cr/kg. Less accumulation was found in the leaf and stem.
Moreover, the researchers also found that Cr(III) accumulated more readily than Cr(VI).

Fig. 5: Chromium accumulations in root, stem and leaf of *Vetiveria nemoraris* at 1) 5 mg Cr/kg of sand 2) 10 mg Cr/kg of sand and 3) 15 mg Cr/kg of sand
CONCLUSION

The accumulation of Cr in three monocot species: *Cydon dactylon*, *Echinochloa colonum* and *Vetiveria nemoralis* cultivated in sand media contaminated with synthetic wastewater (Cr solution) was investigated in this study. The capacity of Cr removal and the accumulation of Cr in different parts of three monocot species were studied at different concentrations: 0, 5, 10 and 15 mg Cr(VI)/kg of sand; and for different harvesting times: 30, 60, 90 and 120 days. The results of this study can be summarized as follows:

1. The *Cydon dactylon*, *Echinochloa colonum* and *Vetiveria nemoralis* in the control environment grew normally. For the plants exposed to the experimental conditions, the highest accumulation of Cr occurred in the root followed by the stem and leaf (root>stem>leaf), respectively. The highest accumulation of Cr in all three monocot species occurred at a concentration of 5 mg Cr(VI)/kg of sand, followed by 10 and 15 mg Cr(VI)/kg of sand, respectively. Moreover, it was shown that the highest accumulation of chromium in *Cydon dactylon* root occurred at a concentration of 5 mg Cr(VI)/kg of sand: 80.85, 94.59, 61.03 and 69.00 mg/kg dry weight of plant for the harvesting times 30, 60, 90 and 120 days, respectively. For *Echinochloa colonum*, the results showed that at a concentration of 5 mg Cr(VI)/kg of sand Cr mostly accumulated in the root, with values of 49.72, 25.60, 48.05, and 58.22 mg Cr/kg dry weight of plant at 30, 60, 90, and 120 days, respectively. At a concentration of 5 mg Cr(VI)/kg of sand, the highest accumulating part of *Vetiveria nemoralis* was the root.

This system absorbed 24.87, 33.60, 49.88 and 18.42 mg/kg dry weight of plant for 30, 60, 90 and 120 days, respectively. The accumulation rate of Cr concentration in all three monocot species at 10 and 15 mg Cr(VI)/kg of sand appeared to increase slightly when compared to the extent of accumulation at a concentration of 5 mg Cr(VI)/kg of sand.

2. The plant part with the highest Cr(VI) accumulation in all three monocot species was the root, followed by stem and leaf (root>stem>leaf), respectively. Also, the results clearly indicate that the Cr accumulation in each part of all three studied monocot species was the non-toxic Cr(III) form rather than the toxic Cr(VI).

The results of this study suggest that uptake by the root of Cr(VI) and the subsequent translocation through the stem and leaf of all three monocot species is one potential process in the phytoremediation of contaminated land by phytoextraction or phytoaccumulation. These three monocot species were chosen for their wide distribution, fast growth, survivability and, more importantly, by the fact that they are non-edible and have a short life span and produce a large biomass, thus making them highly suitable for phytoremediation.

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REFERENCES


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