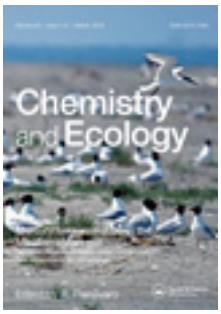

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Impact of organic manure on the phytoremediation potential of *Vetiveria zizanioides* in chromium-contaminated soil

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Chromium is a pollutant present in electroplating waste water and its removal is necessary for the protection of the environment. *Vetiveria zizanioides* (VZ) was grown in chromium effluent concentrations of 50, 100 and 200 mg kg^{-1} soil amended with organic manure and the potential for phytoremediation was determined. The amounts of Cr in plant tissues (root and shoot), soil and percentage electrolyte leakage of VZ roots were analysed. From the results, VZ amended with organic manure showed the greatest potential for Cr removal because of its faster growth and larger biomass achieved over the whole length of the experiment. In this study, 92.25% Cr removal efficiency was obtained with a Cr concentration of 50 mg kg^{-1} soil and removal efficiencies of 90.5% and 85% were obtained with 100 and 200 mg kg^{-1}, respectively after a period of two months of VZ growth.

Keywords: chromium; electroplating effluent; phytoremeditation; *Vetiveria zizanioides*

1. Introduction

Industrial effluents are usually loaded with heavy metals which are harmful to humans and other forms of life. Their toxic nature has a severe environmental impact on ecosystems [1]. Chromium is one of the top sixteen toxic pollutants. It is a serious health risk because of its carcinogenic and teratogenic effects on humans [2]. Chromium exists in the environment in either its hexavalent form, Cr(VI), or its trivalent form, Cr(III). The metal species Cr(VI) is considered to be highly toxic in that it can act as a carcinogen, mutagen and teratogen in biological systems [3]. Cr(VI) causes skin irritation, gastric distress, liver damage and yellow colouring of the tounge and skin [4]. Ion-exchange resins [5], reverse osmosis [6] and an electrolysis system [7] all have been investigated as methods of removing of Cr contamination. However, these methods are expensive and are often not considered to be cost-effective. These processes have several disadvantages, such as incomplete metal removal, high reagent and energy requirements, and the generation of toxic waste products that require disposal and further treatment.

Phytoremediation is a green and sustainable technology that has continued to generate considerable research and commercial interest worldwide [8–12]. The ability to stay healthy, and therefore continue to grow, is an important factor in the choice of plants for phytoremediation [13]. There

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are four different plant-based phytoremediation technologies, each having a different mechanism of action for remediating metal-polluted soil, sediment or water: (1) phytostabilisation, where plants stabilise, rather than remove contaminants by plant roots metal retention; (2) phytofiltration, using plants to clean various aquatic environments; (3) phytovolatilisation, utilising plants to extract certain metals from soil and then release them into the atmosphere by volatilisation; and (4) phytoextraction, in which plants absorb metals from the soil and translocate them to harvestable shoots where they accumulate [14]. Recently, research is going on the removal of Cr by phytoremadiation [15–19]. The uptake, translocation and form of Cr in the plant are dependent on the form and concentration of the supplied Cr. Chromium was found predominately in the +3 oxidation state, regardless of the Cr source supplied to the plant. Although at high Cr(VI) treatment concentrations, Cr(VI) and Cr(V) were also observed. At low Cr(VI) concentrations, the plant effectively reduced toxic Cr(VI) to less toxic Cr(III) [20,21], which was observed both as a Cr(III) hydroxide phase in the roots and a Cr(III–organic complex in the roots and shoots. At low Cr(VI) treatment concentrations, Cr in the leaves was observed predominately around the leaf margins, whereas at higher concentrations Cr accumulated in the leaf veins [20]. Cr toxicity is thought to be caused by reaction of the Cr with reducing agents, such as NADPH, which in turn react with hydrogen peroxide (H_2O_2) to generate damaging hydroxide (OH) radicals, in addition to Cr reacting with the carboxyl and sulfhydryl groups of enzymes, thereby inhibiting their activity [22]. The ability of a plant to minimise these effects and thereby withstand greater concentrations indicates a plants tolerance to the metal.

Recent studies have shown that vetiver grass is a promising plant to use in phytoremediation of heavy metals [23,24]. Vetiver is a fast-growing perennial grass with a complex root system that can penetrate into the deeper layers of the soil [25,26]. The current study investigated the potential for the phytoremediation of Cr directly from effluent of the electroplating industry using *Vetiveria zizanioides* (VZ) amended with and without organic manure. Generally, in chrome-plating, Cr is present as chromates, dichromates and chromic acid; total Cr content was analysed. From previous reports, organic amendments (cow manure and compost) produce active phytoremediation followed by natural attenuation, and are effective for the remediation of pyrite-polluted soil [27,28].

The objectives of this study were: (1) to investigate the total Cr uptake ability of roots and shoots of VZ in different Cr-contaminated soil amended with and without organic manure (cow dung); and (b) to study percentage electrolyte leakage of VZ root.

2. Materials and methods

2.1. *Experimental*

Cr effluent was collected from the electroplating industry at Ernakulam District in Kerala and was diluted to various concentrations (50, 100 and 200 mg L^{-1}). Air-dried and sieved soil was collected and used to determine pH, soil moisture and organic matter content [29]. The cation-exchange capacity (CEC) was determined using the ammonium acetate saturation method. The physico-chemical propertoes of the soil and manure are given in Tables 1 and 2.

The clumps VZ of root length 8 cm and shoot length 10 cm were collected from the nursery and were allowed to grow for 60 days in plastic containers containing Cr effluent of different concentrations (50,100 and 200 mg Cr kg^{-1} soil). The effect of organic matter on Cr uptake was analysed by adding ~ 1 g kg^{-1} of organic manure (powdered cow dung) in plastic containers along with soil. Plants were harvested at 15-, 30-, 45- and 60-day intervals. They were dried in a hot air oven and the dried plant materials (root and shoot) were powdered and digested.

Table 1. Physico-chemical analysis of soils used in the study.

pH	6.1
Moisture content (%)	7.33
Organic carbon (%)	0.80
Organic matter (%)	1.38
Total nitrogen (%)	0.08
Exchangeable Na (cmol kg^{-1})	0.52
Exchangeable K (cmol kg^{-1})	0.25
Exchangeable Ca (cmol kg^{-1})	2.42
Exchangeable Mg (cmol kg^{-1})	0.8
CEC (cmol kg^{-1})	3.85
Sand (%)	75
Silt (%)	15
Clay (%)	10

Table 2. Chemical analysis of manure used in the study

Organic manure	Organic carbon (%)	Organic matter (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Cow dung	13.2	22.75	1.4	0.6	0.8	0.95	0.48

2.2. *Analysis of Cr in plant tissues*

For 1 g of plant materials, a mixture of conc. HNO_3 (2 mL) at 65% and H_2O_2 (1 mL) at 30% was used in the digestion reaction. After cooling to room temperature, the residue was diluted with deionised water to 10 mL and analysed for total Cr using inductively coupled atomic emission spectroscopy (ICP–AES) [30].

2.3. *Analysis of Cr in soil*

Approximately 1 g of sludge was weighed and digested with 2 mL HNO_3, 1 mL $HClO_4$ and 5 mL HF at a temperature of 90 ± 190°C for 16 h. The residue was then dissolved in 2 mL of 4 mol L^{-1} HCl and analysed for total Cr using ICP-AES [30].

2.4. *Determination of percentage electrolyte leakage of VZ root*

Approximately 200 mg of root was incubated in distilled water at 25°C for 3 h in a glass tube and the initial conductivity of the solution was measured (E1). Tubes containing root material were then boiled for 30 min to release all of the electrolytes, cooled to 25°C, and the conductivity (E2) was measured again. Electrolyte leakage was calculated as, $EL = E1/E2 \times 100$ [31].

2.5. *Statistical analysis*

Statistical analysis was performed as described by Bennet and Franklin [32]. The results were analysed using statistical program SPSS/PC+, version 11.5. A one-way analysis of variance (ANOVA) was employed for the comparison among groups. Duncan's post-hoc multiple comparison test of significant differences among groups were determined. All values are expressed as means $\pm$ SD ($n = 6$).

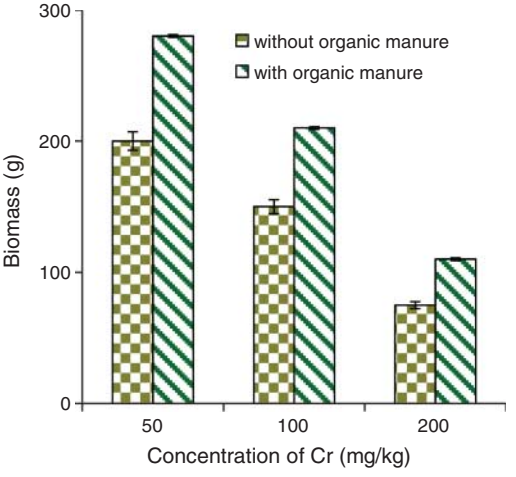

Figure 1. Vetiver biomass production after 2 months of growth in Cr-contaminated soil. Values represent the mean ± SD ($n = 6$).

3. Results and discussion

3.1. *Chromium concentration and vetiver biomass*

The effect of Cr contamination on vetiver grass biomass yield is shown in Figure 1. Vetiver biomass decreased with the increase in soil Cr concentration. But high biomass production was observed in soil with organic manure. At 200 mg Cr kg^{-1}, soil without organic manure showed minor yellowing of leaves and a decrease in biomass.

3.2. *Effect of Cr on plant growth with soil pH and organic manure*

Soil pH is an important factor controlling the solubility of metals in soils. In acidic effluent, Cr(VI) was present as $Cr_2O_7^{2-}$. The additions of effluent directly into the soil made the pH value 4. Previous studies have shown that lowering the pH of a soil will decrease the adsorption of heavy metals and thus increase their concentration in the soil solution [33]. Organic matter content is one of the most important soil properties and it has the ability to retain heavy metals in an exchangeable form. It has been reported that dissolved organic matter in soil could increase the mobility and uptake of heavy metals to plant roots [34,35] and manure has been shown to allow significant increases in C, N and CEC of soil contents [36]. In our study, it was observed that the addition of organic manure increased Cr mobility in soil and its uptake by VZ.

3.3. *Accumulation of Cr in vetiver tissues*

The accumulations of Cr in shoots and root tissues of VZ with Cr concentration of 50, 100 and 200 mg kg^{-1} soil with and without organic manure after 2 months are shown in Figures 2–7. The growth of plants at different Cr concentrations was explained by the phenomenon of chemical hormesis, which is the capacity of a living organism to adapt to a low level exposure to chemical agents [37]. In the current study, Cr accumulation was higher in root tissue and increased with the increase in Cr concentration. Cr accumulation in VZ roots was higher than in leaves because of the high CEC of roots that can absorb Cr when in direct contact with wastewater and then transmit Cr to the leaves [38].

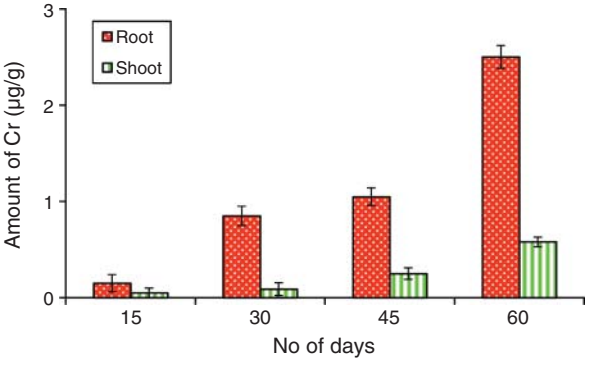

Figure 2. The amount of Cr ($\mu g\,g^{-1}$) in root and shoot of VZ after planting in a Cr concentration of 50 mg kg^{-1} soil without organic manure. Values represent means ± SD ($n = 6$).

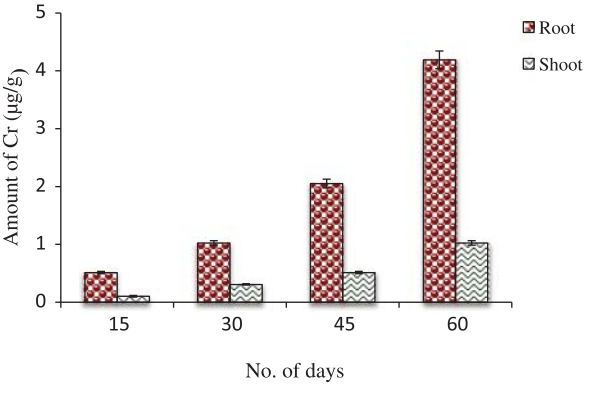

Figure 3. The amount of Cr ($\mu g\,g^{-1}$) in root and shoot of VZ after planting in a Cr concentration of 50 mg kg^{-1} soil amended with organic manure. Values represent the means ± SD ($n = 6$).

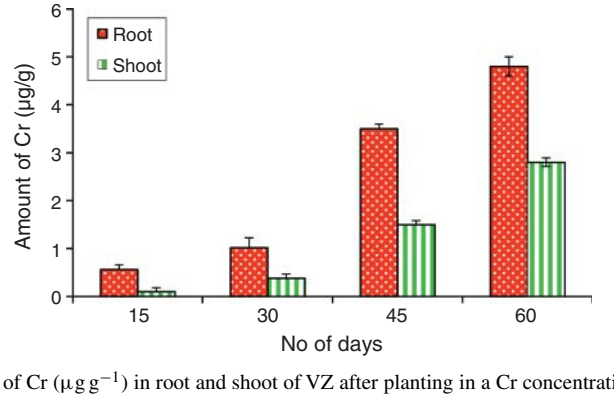

Figure 4. The amount of Cr ($\mu g\,g^{-1}$) in root and shoot of VZ after planting in a Cr concentration of 100 mg kg^{-1} soil without organic manure. Values represent means ± SD ($n = 6$).

The low mobility of Cr from root to shoot was because there are barriers or lack of a transport mechanism suitable for Cr transport from roots to shoots [39]. No significant toxicity symptoms were detected in VZ grown in soil contaminated with Cr. The Cr concentration in the plants and whether they appear healthy or not may indicate the tolerance of that plant to the metal concerned, and therefore their potential for phytoremediation [13].

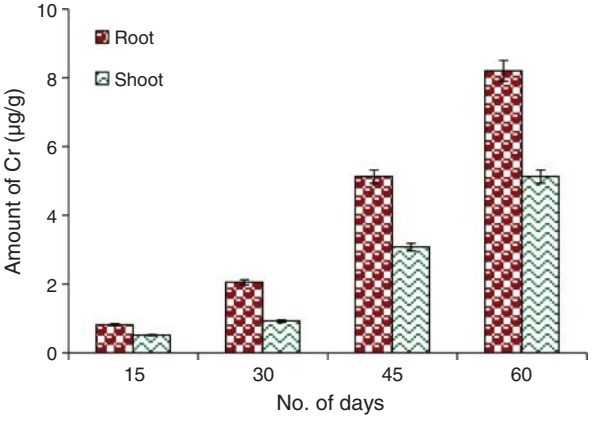

Figure 5. The amount of Cr ($\mu g\, g^{-1}$) in root and shoot of VZ after planting in a Cr concentration of 100 mg kg^{-1} soil amended with organic manure. Values represent means ± SD ($n = 6$).

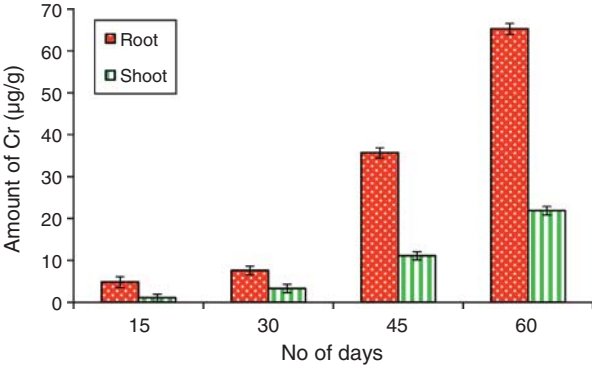

Figure 6. The amount of Cr ($\mu g\, g^{-1}$) in root and shoot of VZ after planting in a Cr concentration of 200 mg kg^{-1} soil without organic manure. Values represent means ± SD ($n = 6$).

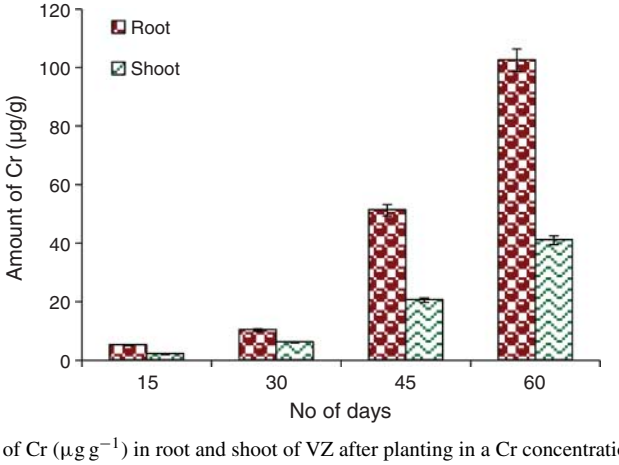

Figure 7. The amount of Cr ($\mu g\, g^{-1}$) in root and shoot of VZ after planting in a Cr concentration of 200 mg kg^{-1} soil amended with organic manure. Values represent means ± SD ($n = 6$).

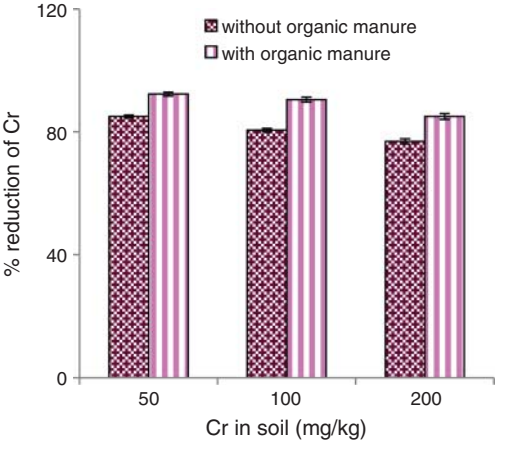

Figure 8. Percentage reduction of Cr in soil after 2 months of VZ growth. Values represent means ± SD ($n = 6$).

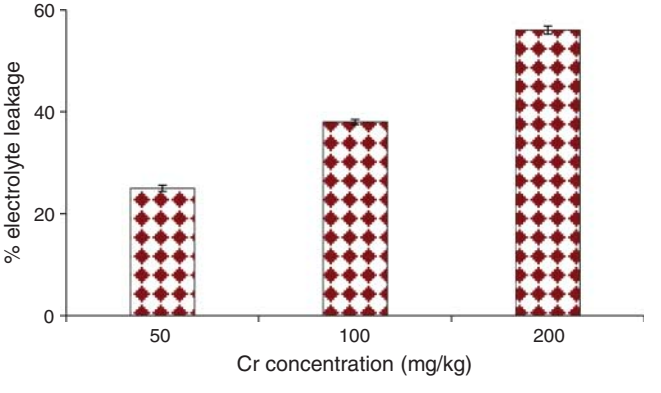

Figure 9. Variation in percentage electrolyte leakage of VZ root with Cr concentration.

3.4. *Reduction of Cr in soil*

The percentage reduction in Cr in soil amended with organic manure after 2 months of VZ growth is shown in Figure 8. It was observed that 92.25% removal efficiency was obtained with 50 mg Cr kg^{-1} and 90.5 and 85% removal efficiencies were obatined with with 100 and 200 mg Cr kg^{-1}, respectively, in soil amended with organic manure.

3.5. *Determination of percentage leakage of electrolyte with Cr concentration*

The variation in percentage electrolyte leakage in VZ root with Cr is shown in Figure 9. It was concluded that electrolyte leakage from VZ root increases with the increase in Cr concentration.

3.6. *Determination of translocation factor, accumulation factor and bioaccumulation factor of VZ with Cr concentration*

Plant species with a translocation factor (TF) <1 as 'shoot metal excluders', are suitable for the phytostabilisation of metal-contaminated soils [40]. TF is the ratio of the metal concentration in the above ground part of the plant to that in the roots [30]. As shown in Table 3, TF values

Table 3. TF, AF and BF values for VZ grown in soil with varying Cr concentrations and amended with organic manure.

Cr concentration (mg kg^{-1} soil)	TF	AF	BF
50	0.9 ± 0.05	0.8 ± 0.06	0.6 ± 0.05
100	0.8 ± 0.03	0.7 ± 0.05	0.5 ± 0.06
200	0.5 ± 0.05	0.6 ± 0.05	0.4 ± 0.08

were <1, indicating that Cr accumulated by VZ was largely retained in roots. As per a previous study [23], VZ is not a phytoextractor because the TF is always <1. The accumulation factor (AF) was obtained by dividing shoot concentration by the total soil metal concentration [41]. The bioaccumulation factor (BF) is the metal concentration in shoots versus the initial metal concentration in the substrate [42]. TF, AF and BF values for VZ grown in soil with varying Cr concentrations and amended with organic manure are given in Table 3.

4. Conclusion

The results obtained from this study demonstrated the ability of vetiver grass to tolerate and accumulate Cr. In this study, 95.25, 90.5 and 85% total Cr removal efficiency was obtained with Cr concentrations of 50, 100 and 200 mg kg^{-1} soil, amended with organic manure after 2 months of VZ growth. Whereas only 85, 80.5 and 76.87% Cr removal was observed in soil without organic manure. Percentage electrolyte leakage of VZ root also increased with the increase in Cr concentration, and confirmed the high uptake of Cr in plant tissues. Determination of TF, AF and BF proved that VZ is not a hyperaccumulator. Hence, phytoremediation using *Vetiveria zizanioides* in soil amended with organic manure is proposed as a good strategy for soil contaminated with chromium.

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