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Original Research Article

Phytoextraction of chromium from electroplating effluent by *Tagetes erecta* (L.)

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ABSTRACT

Industrialization has made developing countries 'hot-spots' of metal pollution. Being non-biodegradable, they persist in the environment and result in bioaugmentation. To remediate this persistent pollutant, the green technology, phytoremediation has been attempted in the present study. Analysis of variance showed the probability (P) of significant chromium (Cr) uptake by shoot ($P \leq 0.03$) and highly significant Cr accumulation in root ($P \leq 0.0001$). Cr-induced physiological changes were observed in the form of significant decrease in chlorophyll content ($P \leq 0.004$) and significant increase in biomass ($P \leq 0.002$), from day 7 to day 35 when exposed to 2, 4 and 6 mg kg^{-1} of chrome effluent. *Tagetes erecta* in association with rhizobacteria (*Bacillus cereus*-CK 505 and *Enterobacter cloacae*-CK 555) was found to accumulate high levels (94%) of Cr within a short period of 35 days.

1. Introduction

Heavy metal pollution is a significant problem in the industrialized world. The major sources of heavy metal discharges include chrome plating, mining, pharmaceutical, electroplating, agriculture and automotive industries [1]. Non-biodegradability and toxicity of heavy metals pose serious ecological consequences [2] which results in poor crop yield and ultimately causes biomagnification [3]. Of all heavy metals, hexavalent chromium (Cr(VI)) is a highly toxic metal which is used in most of the industries. India discharges about 2.0–3.2 kt of Cr(VI) annually from tannery industries [4]. Next to tannery industries, electroplating industries are major sources of discharge of chrome-effluent. In India, most of the electroplating industries are operating as small scale sectors with over 3,00,000 units [5]. As per discharge standards of the Central Pollution Control Board (CPCB) of India it is 2 mg L^{-1} for chromium [6]. Cr causes skin irritation and is carcinogenic to humans [7] with toxicity levels being assessed by its oxidative state. Besides its redox potential, Cr undergoes transformation like precipitation and dissolution. A considerable amount of Cr(III) is essential for carbohydrate and lipid metabolism in humans [7]. In the plant system, it is non-essential and has no beneficial effect; however, plants take up the metals via roots and store them in harvestable parts in a process known as phytoremediation [8]. Among the phytoremediation strategies, phytoextraction holds the pollutants in the plant parts. Thus an ideal plant, possessing multiple traits, such as fast growth with high biomass production, a deep rooting system, and ability to tolerate and accumulate a wide range of heavy metals in its aerial and harvestable parts, is needed to achieve a considerable metal uptake [9]. *Tagetes erecta* belongs to the family of Asteraceae. It possesses all the above mentioned criteria of an ideal phytoextractor. Moreover; it is a plant producing flowers of commercial importance and can be grown on contaminated soil for phytoremediation as well as floriculture. Hence, the aim of this study has been focused on determining the Cr uptake capacity of *T. erecta* (African marigold) in soil containing electroplating effluent (i.e., Cr). Besides the plant system, microbial communities associated with its rhizosphere are also involved in promoting metal uptake. Metal mobility at the rhizosphere can be controlled and be made readily available to the plants by plant release of root exudates contributing to chelating agents, acidification, phosphate solubilisation and redox changes [10]. Notably, plant growth-promoting bacterial species associated with rhizosphere exert some beneficial effects on plant growth by providing nutrients through a

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number of mechanisms such as nitrogen fixation, production of phytohormones and siderophores [10]. Reports indicate that *Enterobacter cloacae* and *Bacillus cereus* species are well-documented for their role in contributing to redox potential and chromate resistance, wherein their participation is remarkable by making metals available to plants, besides functioning as potential plant growth promoters [11,12]. In bacterial species, antibiotic resistance is directly proportional to their ability to concentrate heavy metals, wherein, the genes for both characteristics are probably in a cluster on the same bacterial plasmid [2]. As the standards of CPCB for Cr is 2 mg L^{-1} [6], the current study made an attempt to investigate the response of the chosen plant towards Cr with initial concentration of 2 mg kg^{-1}. Hence, the objectives of the pot study are focused on (i) Cr extraction potential of *T. erecta*, (ii) investigating the role of rhizobacteria in phytoextraction of Cr, which is proportional to their antibiotic resistance.

2. Materials and methods

2.1. Analysis of Physico-chemical characteristics

Pot experiments were conducted to study Cr uptake by *T. erecta* and its effect on plant growth. As the combination of red soil and sandy-clay loam soil permits slower movement of water and retain moisture [13], they were mixed in the ratio of 1:1 (w/w). Soil and electroplating effluent were characterized for physico-chemical parameters. Cr concentration in effluent collected from an electroplating industry located in Coimbatore, India, was 247 mg L^{-1} which was used to prepare desirable concentrations in soil (2, 4 and 6 mg kg^{-1}).

T. erecta seeds were purchased from Tamil Nadu Agriculture University, Coimbatore and sown in trays containing red sandy-clay-loam soil. The seeds were allowed to grow for 7 d. At the end of day 7, the plantlets were transplanted into both experimental and control pots. Here, the pot study was performed for a period of 35 days after the day of transplant.

Experimental plants were exposed to the above concentrations while control plants were grown in soil without effluent. Plants were harvested on day 7, 14, 21, 28 and 35 for estimation of metal accumulation. Biomass, chlorophyll content and metal accumulation in the roots and shoots were analysed separately.

2.2. Soil sample preparation for metal analysis

5 g of soil samples were collected in triplicates from a depth of 0–20 cm below surface and then dried at 50 °C for 48 h. Well-mixed samples were digested with aqua regia (3 HCl:1 HNO_3) (0.05 M HCl and 0.5 M HNO_3) at 80 °C. The digested samples were made up to 250 mL with 0.5 M nitric acid. The samples were analysed for metals in Atomic Absorption Spectrophotometer (Elico: SL173).

2.3. Analysis of plant material for Cr accumulation

The plants were harvested and separated into root and shoot (inclusive of leaves). The plants were washed with distilled water and dried in hot air oven at 80 °C overnight. The dried material was manually crushed using mortar and pestle and acid-digested in Kjeldahl digestion apparatus with aqua regia. Digestion was carried out until a clear solution was obtained. The solution was filtered through Whatman no. 1 (11 μm) filter paper to remove the debris and then with Whatman no. 42 (2.5 μm) filter paper which was further analysed for Cr in an Atomic Absorption Spectrophotometer.

2.3.1. Aqua regia digestion mixture

Total recoverable heavy metal in plant material was determined by aqua regia digestion [14]. Aqua regia was freshly prepared.

2.4. Total chlorophyll content

Total chlorophyll content was estimated according to Jayaraman [15]. Wet weight of fresh leaves (1 g) was minced with scissors and ground with a mortar and pestle. 5 mL of water was added and homogenized in a blender. The final volume was made up to 10 mL. An aliquot (0.5 mL) was taken and mixed with 4.5 mL of 80% acetone (v/v), mixed thoroughly and placed in dark for 10 min. The supernatant was collected by centrifugation at 4000 rpm for 10 min and its optical density was measured at two wavelengths, 645 and 663 nm. The concentration of pigments was directly calculated using the following formula,

$$\text{Chlorophyll}\left(\text{mg g}^{-1}\right) = [8.02 \times OD_{663} + 20.20 \times OD_{645}] \times \text{V}/1000 \times \text{W}$$

where V = volume of the extract (mL); W = Weight of fresh leaves (g).

2.5. Plant biomass

Harvested plants were washed and filtered. The root and shoot were separately dried in hot air oven at 80 °C overnight, weighed and recorded as dry biomass (g).

2.6. Microbial characterization

Soil sample collected at the rhizosphere was diluted with distilled water from 10^{-1} to 10^{-10} and used as an inoculum. An aliquot of 0.1 mL was spread-plated onto nutrient agar (Himedia-M001) (composition (g L^{-1}): peptic digest of animal tissue, 5; sodium chloride, 5 beef extract, 1.5; yeast extract, 1.5; agar, 15) plates from appropriate dilution tubes and incubated at room temperature. Colony morphology was studied after 24–48 h and individual colonies were isolated.

2.7. Biochemical characterization of rhizobacteria

Biochemical characterization such as Indole test, citrate utilization test, gelatin hydrolysis, casein hydrolysis, hydrogen sulphide test, urease test, nitrate reduction and Voges-Proskauer were performed according to Bergey's Manual [16]. Results were interpreted by observing colour change and growth of the colonies after 24–48 h.

2.8. Molecular characterization of rhizobacteria

16S rRNA gene sequencing was performed for identifying the rhizobacteria present in rhizosphere of the proposed plant. 16S rDNA universal primer was used according to Weisburg et al. [17], Forward primer: AGAGTTTGATCCTGGCTCAG, Reverse primer: ACGGCTACCTTGTTACGACTT. The evolutionary relationship of isolates was predicted by maximum likelihood method using Mega 6.0. The constructed tree was evaluated using Bootstrap method with 1000 replications.

2.9. Cr resistance assay

Different concentrations of chromium were prepared by dissolving required amounts from stock potassium dichromate (100 mg L^{-1}); concentrations of 20, 40, 60, 80 and 100 mg L^{-1} were

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