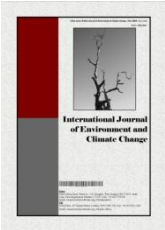

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Effect of Chromium Species and Plant Growth Promoting Microorganisms on Growth Parameters of *Amaranthus gangeticus*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In agricultural regions close to industrial areas, especially at the vicinity of tannery industry, chromium contamination poses a serious environmental risk. A pot experiment was carried out in the current study to assess the impact of hexavalent and trivalent chromium under the influence of microorganisms on *Amaranthus gangeticus* growth characteristics. Nine different concentrations of chromium (Cr) were used which consists of 0, 50, 100, 150, and 200 mg/kg Cr^{+6} as $K_2Cr_2O_7$ and 50, 100, 150, and 200 mg/kg Cr^{+3} as $KCr(SO_4)_2$, in conjunction with four different levels of microorganisms, consisting of control, *Azospirillum*, Phosphate solublizing Bacteria (PSB), and Potassium Releasing Bacteria (KRB). The study revealed that when Cr concentrations increased, steady decline was observed in shoot, root length, leaf length, leaf breadth, number of leaves, and fresh weight. But when supplemented with KRB, the treatments had an ameliorative impact on chromium and, in comparison, enhanced the growth characteristics.

Keywords: Hexavalent chromium; trivalent chromium; microorganisms; Amaranthus.

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1. INTRODUCTION

Chromium is a non-essential metal that is harmful to plants and microbes. Chromium (Cr) has developed into a significant contaminant in a variety of environmental contexts as a result of its extensive industrial use. The tanning industry may be a major source of Cr introduced into the environment by humans. Cr may be found in a number of oxidation states, from Cr (-2) to Cr (+6). Often, Cr is found in the +3 and +6 oxidation states [1]. Oxidation of Cr^{+3} often yields hexavalent chromium, a compound that is extremely dangerous and cancer-causing. On the other hand, trivalent chromium is considered to be a crucial trace element in human nutrition and is the most stable form of chromium that can be found naturally [2]. In most of cases, soil Cr (VI) quickly transforms into far less dangerous Cr (III). Research has unequivocally shown that Cr^{+3} is essential for insulin's proper operation, making it useful in the management of type 2 diabetes (Hua et al*.,* 2011). Low concentrations of Cr (III) can even be beneficial for plants [3]. The considerable difference in toxicity between Cr (VI) and Cr (III) is linked to the much lower values of the standard electrode potential and prooxidant activity of Cr (III) [4]. When Cr is present in the environment, the plant growth and development pattern is severely affected. Due to inefficient production, nutrient uptake, and nutrient partitioning to the productive parts of the plant, the influence of Cr on plant activities during early growth and development results in loss in yield and total dry matter. In brief, Cr has a negative indirect effect on plant production and dry matter [5]. Many bacteria and macrofungi can endure high levels of heavy metals, which frequently cause severe toxic symptoms in higher plants. Hexavalent chromium-reducing microorganisms can lower hazardous levels of the metal and improve plant growth in contaminated regions when applied to soil as bio-fertilizers.

Amaranthus is one of the earliest varieties of food plants. The *Amaranthus* species is a superior source of calcium, potassium, iron, zinc, magnesium, and a sizeable amount of carotenes and vitamin C [6]. Pharmacological analysis of the seeds and leaf extracts of *Amaranthus* revealed the bioactive chemicals' potency in preventing serious ailments including diabetes, hyperlipidemia, and diarrhoea as well as their antioxidant, antihelminthic, antimalarial, anti-inflammatory, and antifungal capabilities [7]. *Amaranthus* employ the C4 photosynthetic pathway to provide for sustenance, making the plant easily tolerant of severe environmental circumstances. *Amaranthus* has been included in the list of plants with heavy metal resistance and the capacity to clean the environment of dangerous heavy metals [8]. The current study focuses on the effect of chromium and plant growth promoting microorganisms on the growth of *Amaranthus*.

2. MATERIALS METHODS

2.1 Pot culture Experiment

To investigate the impact of interaction of chromium and microorganisms, the present pot culture study was conducted at the Department of Soils and Environment, Agricultural College and Research Institute, Madurai in the year 2022. *Amaranthus* was used as a test crop. In each pot, 15 kg of soil was filled and the moisture level was maintained to the field's capacity. *Amaranthus* seeds were sown in pots after imposing the treatments. To maintain the desired plant population of 10 plants per pot, the plants were gradually thinned at a height of 5 cm. All package of practices for *Amaranthus* crop were carried out in accordance with TNAU Crop production manual.

2.2 Experimental Design

The pot culture study was laid out in Factorial Completely Randomized Block Design (FCRD) with two factors (9 levels of chromium treatments and 4 levels of microorganism treatments) and replicated thrice.

2.3 Treatments

Pots were treated with Cr through potassium dichromate ($K_2Cr_2O_7$) i.e. Cr (VI) with five different levels T_1 – Control, T_2-50, T_3-100, T_4-150, T_5-200 mg/kg soil, and Potassium chromium sulphate [$KCr(SO_4)_2$] i.e. Cr (III) with four different levels T_6-50, T_7-100, T_8-150, T_9-200 mg/kg soil and maintained for 15 days to create chromium stress. After 15 days of application of chromium, 4 levels of Plant growth promoting microorganisms (M) i.e. *Azospirillum lipoferum*, *Bacillus megaterium* (PSB) and *Paenibacillus mucilaginosus* (KRB) were applied to the soil in the form of liquid formulations in recommended dosage and mixed thoroughly. A control (without microorganism) was also maintained. All the pots received uniform dose of NPK, and irrigation was done to maintain field capacity.

2.4 Growth Parameters

Plant samples were collected at 25 days after sowing. These samples were observed for the growth parameters like shoot height, root length, number of leaves, leaf area index, fresh weight and dry weight. Shoot length (cm) was measured from the stem base up to the tip of main stem using a meter rod and mean values were calculated.

2.5 Statistical Analysis

From the pot trial, the data associated to growth and yield parameters were statistically analyzed to assess the significance of the factors (Chromium treatments (T) and microorganism treatments (M)) and their interaction.

3. RESULTS AND DISCUSSION

The growth of *Amaranthus* was significantly affected by chromium. The results showed that the growth parameters showed a gradual decline with increasing chromium concentrations. It can be observed that hexavalent chromium have a significant effect on growth parameters than trivalent chromium. However, the effect was more obvious with Cr concentrations more than 100 mg L^{-1}, which limit growth more than control.

3.1 Effect of Chromium on Shoot Height

Inhibitory effect of Cr on plant growth was evaluated and it was reported that Cr significantly ($p< 0.05$) reduced the plant growth. Average plant height was significantly decreased by 23.26% and 18.38% when plants are exposed to highest 200 mg L^{-1} hexavalent Cr (T_5) and trivalent Cr (T_9) as compared to control (T_1). Therefore the effect of hexavalent chromium on plant growth was more prominent than trivalent chromium. The maximum shoot length of 56.6 cm is observed in plants grown in control (T_1) and minimum shoot length was observed in plants grown in 200 mg L^{-1} of hexavalent Cr (T_5) (Table 1). It was observed that shoot height was on par with control at low concentrations of 50 and 100 mg L^{-1} of Cr. The application of KRB and PSB to chromium spiked pots relatively increased plant growth as compared to control (M_1). This relative increase in shoot height in microorganisms treated pots maybe due to the reduction of toxic hexavalent chromium thereby mitigating the toxic effects of chromium on plant growth [9]. The reduction of shoot length in chromium spiked pots may be due to the inhibition of uptake of nutrients by Cr. This diminished nutrient absorption may be caused by a loss in root development and impairment of root penetration under Cr toxicity, or it may be caused by a reduction in essential element translocation due to the removal of nutrients from the physiologically significant binding sites [10]. Cr toxicity is therefore said to have an impact on plant development and hinder several of their crucial metabolic processes [5]. Similar findings were made in the sunflower (*Helianthus annuus L.*) by Fozia et al*.,* [11], where there was a remarkable reduction in various growth parameters.

Table 1. Effect of Cr and microorganisms on shoot length (cm) of *Amaranthus*

Cr treatments	Microorganisms			
	Control (M_1)	***Azospirillum* (M_2)**	**PSB (M_3)**	**KRB (M_4)**
T_1 - Control	50.2	51.8	55.8	57.3
T_2- 50 mg/L Cr^{+6}	48.5	49.4	51.6	53.2
T_3- 100 mg/L Cr^{+6}	45.6	48.1	48.4	50.6
T_4- 150 mg/L Cr^{+6}	40.2	42.8	44.6	45.9
T_5- 200 mg/L Cr^{+6}	38.9	40.1	42.3	43.2
T_6- 50 mg/L Cr^{+3}	49.4	50.4	53.8	55.4
T_7- 100 mg/L Cr^{+3}	48.8	52.0	52.5	53.4
T_8- 150 mg/L Cr^{+3}	44.2	47.7	48.6	48.9
T_9- 200 mg/L Cr^{+3}	41.8	43.4	44.2	45.6
	T	M	TxM	
SEd	0.485	0.323	0.971	
C.D. (at 5%)	0.967	0.856	2.568	

SEd - Standard deviation, C.D – Critical Difference

3.2 Effect of Chromium on Root Length

Chromium had a significant effect ($p<0.05$) on root length. The maximum and minimum value of 15.3 cm and 8.6 were observed in control (T_1) and 200 mg/l of Cr^{+6} (Table 2). Hexavalent Cr had more visible effect on root length when compared to trivalent chromium treatments. Shanker et al*.,* [5] observed that root growth of green gram (*Vigna radiate*) had been inhibited comparatively more in hexavalent Cr treatments than in trivalent Cr treatments. As roots directly contact with Cr in medium, root cell division/root elongation or the extension of cell cycle in the roots is inhibited by Cr toxicity thereby root growth is declined consequently the ability of roots to absorb water is also affected [12]. As the concentration of Cr increases there is a decline in root growth. But a substantial rise in root growth was noticed in plants grown in Cr spiked pots with the microorganism factor. This effect may be due to reduction of Cr^{+6} by microorganisms and also increased supply of nutrients by these plant growth promoting microorganisms which support the plant growth during heavy metal stress [13]. The highest increase in root length is observed in KRB treatments among the microorganisms.

3.3 Effect of Chromium on Number of Leaves, Leaf Length and Width

It was observed that as the concentration of Chromium in the media is increasing there is a considerable decrease in the leaf length and width. The maximum leaf length (Table 3) of 15.2 cm and leaf width (Table 4) of 7.2 cm was noted in control. The minimum leaf length of 12.2 cm and leaf width of 4.8 cm was observed in plants grown in 200 mg L^{-1} Cr^{+6}. This reduction of leaf length and width is more significant in hexavalent chromium treatments than trivalent chromium treatments. Davies et al*.,* (2002) found that Cr toxicity significantly decreased the parameters of transpiration rate, net photosynthetic rate, intercellular CO_2 concentration, and stomatal conductance in the leaves by 71%, 36%, 25%, and 57%, respectively. Chandra and Kulshreshtha [14] reported that plants under Cr stress displayed reduced leaf area and chlorophyll contents, which may be caused by the disruption of chlorophyll production. Cr also had a significant effect ($p< 0.05$) on number of leaves (Table 5). Highest number of leaves was observed in control and lowest number was observed in plants grown in 200 mg L^{-1} Cr^{+6}. It was observed that the effect of chromium was ameliorated in treatments combined with microorganisms. This effect may be due to tolerance to hexavalent Cr by KRB when compared to PSB and *Azospirillum*.

3.4 Effect of Chromium on fresh Weight

The average fresh weight of the samples followed a decreasing trend as the concentration of chromium in the soil is increased. The highest value of fresh weight of 28.8 grams was observed in control (T_1) combined with KRB treatment (Table 6). Least fresh weight of 20.3 grams was found in 200 mg/L of hexavalent Cr (T_5). Similar results were reported by Amin et al*.,* [15,16] on gradual decrease of fresh weight in *Hibiscus esculentus* with increase in chromium levels. This reduction of fresh weight is the indirect effect of Cr on all the growth parameters.

Table 2. Effect of Cr and microorganisms on root length (cm) of *Amaranthus*

Cr treatments	Microorganisms			
	Control (M_1)	***Azospirillum* (M_2)**	**PSB (M_3)**	**KRB (M_4)**
T_1	13.5	12.4	14.7	15.3
T_2	12.3	11.9	13.1	13.6
T_3	10.8	12.4	12.2	13.4
T_4	8.2	10.1	10.5	11.2
T_5	7.6	9.2	10.1	11.3
T_6	12.9	13.2	14.3	14.9
T_7	13.1	12.9	14.1	14.1
T_8	12.2	12.1	13.5	13.8
T_9	10.6	11.7	11.2	12.7
	T	M	TxM	
SEd	0.117	0.078	0.234	
C.D. (at 5%)	0.234	0.156	0.620	

SEd - Standard deviation, C.D – Critical Difference

Table 3. Effect of Cr and microorganisms on leaf length (cm) of *Amaranthus*

Cr treatments	Microorganisms			
	M_1	M_2	M_3	M_4
T_1	14.3	14.5	14.6	15.2
T_2	13.7	14.6	14.0	14.5
T_3	13.5	13.9	13.8	14.9
T_4	12.9	13.5	13.8	14.3
T_5	12.2	13.8	13.6	14.2
T_6	14.5	14.3	14.8	15.3
T_7	13.8	13.5	14.1	14.6
T_8	13.1	13.6	13.5	14.9
T_9	13.5	14.6	14.3	14.7
	T	M	TxM	
SEd	0.136	0.091	0.273	
C.D. (at 5%)	0.272	0.181	0.544	

SEd - Standard deviation, C.D – Critical Difference

Table 4. Effect of Cr and microorganisms on leaf width (cm) of *Amaranthus*

Cr treatments	Microorganisms			
	M_1	M_2	M_3	M_4
T_1	6.8	7.0	6.9	7.2
T_2	5.8	6.1	6.5	6.5
T_3	5.5	5.9	6.2	6.1
T_4	5.1	5.8	6.3	6.2
T_5	4.8	5.5	5.7	5.9
T_6	6.2	6.4	6.4	6.8
T_7	5.9	6.1	6.4	6.6
T_8	6.2	5.9	6.1	6.4
T_9	5.8	5.9	6.0	6.2
	T	M	TxM	
SEd	0.060	0.040	0.121	
C.D. (at 5%)	0.120	0.080	0.241	

SEd - Standard deviation, C.D – Critical Difference

Table 5. Effect of Cr and microorganisms on number of leaves of *Amaranthus*

Cr treatments	Microorganisms			
	M_1	M_2	M_3	M_4
T_1	10	10	11	12
T_2	9	9	11	11
T_3	9	8	9	10
T_4	9	10	10	11
T_5	7	8	9	9
T_6	10	11	11	9
T_7	9	11	10	12
T_8	9	10	10	11
T_9	8	10	9	9
	T	M	TxM	
SEd	0.109	0.073	0.219	
C.D. (at 5%)	0.219	0.146	0.437	

SEd - Standard deviation, C.D – Critical Difference

Table 6. Effect of Cr and microorganisms on fresh weight (gm) of *Amaranthus*

Cr treatments	Microorganisms			
	M_1	M_2	M_3	M_4
T_1	25.2	27.3	26.9	28.8
T_2	22.6	25.9	25.5	26.7
T_3	23.1	25.3	24.9	27.4
T_4	21.8	22.9	22.7	23.4
T_5	20.3	22.1	21.6	22.5
T_6	26.2	25.2	25.7	27.8
T_7	24.9	24.8	26.3	26.1
T_8	23.7	24.2	23.8	24.6
T_9	22.9	22.8	24.1	23.9
	T	M	TxM	
SEd	0.275	0.183	0.549	
C.D. (at 5%)	0.548	0.365	1.095	

SEd - Standard deviation, C.D – Critical Difference

As Cr had an inhibitory effect on the growth parameters which contribute to fresh weight of plant there was a decline in fresh weight of plants grown in increasing Cr concentrations. The fresh weight was relatively higher in all the treatments which are combined with Microorganisms.

4. CONCLUSION

In summary, Cr^{+6} has a significant influence on growth of *Amaranthus* than Cr^{+3} as it is the most soluble form and readily translocated in plants. Maximum values for growth parameters like shoot height, root length, number of leaves, leaf length, leaf width and fresh weight were recorded in control (T_1) nevertheless root length and shoot height at 50 and 100 mg L^{-1} of Cr were similar to control. At higher concentrations of chromium there is a decline in growth parameters due to Cr toxicity. However, application of plant growth promoting microorganisms reduced the toxic effects of Cr possibly by reduction of hexavalent Cr and improving the nutrient status in soil. This ameliorative effect is most pronounced in KRB applied pots proving its efficiency in detoxification and tolerance to Cr. Hence, the application of plant growth promoting organisms like KRB, PSB and *Azospirillum* can mitigate the chromium toxicity in polluted soils. This chromium detoxifying property of plant growth promoting microorganisms needs extensive research so as to improve plant growth and concurrently, phytoremediation of chromium stressed soils.

FUTURE SCOPE

Due to the contradictory effects produced by the two species of chromium, it is essential to study the detailed chemistry of chromium in soil-plant systems. The ability of microorganisms to tolerate and reduce high concentrations of toxic chromium concentrations can be exploited for remediation purposes in heavy metal contaminated soils. More research should be undertaken to evaluate Cr (VI) detoxification mechanisms used by bacteria, their limitations and assess their applications for large-scale remediation of Cr (VI).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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