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Impacts of Cd and Cr toxicity on morphological and physiological properties of *Tagetes erecta* L.

Akanksha Verma, Samuel Gorden Singh, Talad Khan, Harihar
Department of Botany, St. John's College, Dr. Bhimrao Ambedkar University, Agra, Uttar Pradesh, India

***Abstract- Tagetes erecta* L. was used to explore the use of techniques for phytoremediation. The treatment of heavy metals such as Cd and Cr was provided to soil for phytoremediation experiment using by plants of African marigold. In the present study the plants were grow in given treatments of heavy metals' for a period of 120 days. This study was carried out to determine impact of Cd and Cr toxicity on morphological and physiological parameters of *Tagetes erecta* L. Plants were grown in Cd 10mg/Kg, Cr 10mg/Kg and Cd + Cr (each 10mg/Kg), respectively with garden soil including control. The results was showed that plant length (in cm.) was reduced in T2 and T3 of 28 ± 0.5 and 27.84 ± 0.29, respectively than control of 35.34 ± 0.29 and in T1, it was increased (37.34 ± 0.29) than control. Total fresh weight of T1 was observed 33.232 ± 1.32 (in gm.) which is higher than control (29.473 ± 1.93) while T2 (17.63 ± 1.89) and T3 (17.433 ± 1.26) was lower than control. Same as dry biomass was found in T1 8.59 ± 0.88 (in gm.) i.e. higher than control (6.39 ±0.6) and lower in T2 (5.23 ± 0.2) and T3 (4.89 ± 0.5). Total chlorophyll for T2 and T3 were less (4.379 ± 0.229 and 4.522 ± 0.291) than control and T1 (6.229 ± 0.432 and 6.117 ± 0.610). Control and T1 value for chlorophyll a, chlorophyll b and total chlorophyll were approximately same. The results were concluded that Cr inhibited normal growth for *Tagetes erecta* but plants were grow in Cr stress condition with slow growth than normal while adequate growth in *T. erecta* with Cd treated plant (T1) than control. So *Tagetes erecta* can be valuable tool for the phytoremediation of Cr and Cd contaminated soil.**

***Index Terms- Tagetes erecta* L., Cadmium, Chromium, Morphological Characters, Soil Pollution, Phytoremediation.**

I. INTRODUCTION

Heavy metals (HMs) pollution is increasingly becoming a problem and is a great concern due to its adverse effects around the world. Heavy metal pollutants are being discarded in water, soil and air due to the rapidly growing metal industries, agriculture, fertilizers, pesticides and improper waste disposal. Some hazardous metals' affect biological growth and function where other metals accumulate in different body organs in humans causing many serious problems including cancer (Briffa, 2020). Mining activities are the main source of inorganic contamination of the environment. Most heavy metals like As, Cr, Cu, Ni and Zn showed a low potential ecological risk while slightly higher potential of ecological risk by Cd, Pb and Hg is reported (Liu *et al.,* 2019).

The world health organization (WHO) recommends safe limits of 0.003 mg/L or mg/Kg (ppm) in both waste water and agricultural soil, respectively. Elevated cadmium doses are carcinogenic to humans. Exposure can lead to health problems like kidney damage and bone issues. Uncontaminated soil in India, the permissible limit for cadmium is generally considered to be around 1-3 mg/Kg. It's important to note that naturally occurring cadmium in soil can range from <0.01 to 0.8 mg/Kg. The AIP (American Institute of Physics) Organization indicates that the critical limit of cadmium in soil is 3-8 ppm (Sukarjo *et al.,* 2019). But when Cd levels in soil exceed 8 mg/Kg or bioavailable Cd becomes greater than permissible rang plant may exhibit toxicity symptoms.

Exposure of hexavalent chromium through contaminated soil as well as water can lead to various health issues including respiratory problems, skin damage and increased cancer risk for human according to the Indian Journal of Pharmaceutical Sciences (IJPS), the National Institutes of Health (NIH) and Adimalla *et al.,* 2019. In normal soil, a non-toxic level of hexavalent chromium is generally considered to be 0.05 mg/L for waste water and 0.1 mg/Kg for soil. The NIH suggests that hexavalent chromium can be severely harmful to plants at concentrations as low as 5 mg/Kg in soil and 0.5 mg/L in solution. But in present, the concentration of chromium in soil generally ranges from 10 to 50 mg/Kg.

Presently, the phytoremediation through plants is a better option to solve this problem. Physical and chemical methods used to reduce the heavy metals from polluted soil have serious limitation like high cost, harmful irreversible changes in soil properties, destruction of soil micro-flora and generation of secondary pollutants whereas, phytoremediation is cost-effective and environmentally favorable techniques. It helps to reduce the risk of heavy metals to accumulation in humans and ecosystem. Different plants remediate different heavy metal at different rates by one or more mechanism of phytoremediation (Etim, 2012; Ali *et al.,* 2013 and Kafle *et al.,* 2022). For phytoremediation purpose, the exploitation of ornamental plants can be additional option due to their good impacts on landscape (Capuana, 2020) such as *Tagetes erecta* L. commonly known as African marigold, member of Asteraceae family and it is classified as a non-edible plant species. In a study, the *T. erecta* was able to tolerate Cr concentrations up to 6 mg/Kg without affecting the plant growth (Chitraprabha and Sathyavathi, 2018). This research work focused on morphological and physiological impact of Cd and Cr toxicity on *T. erecta*.

II. MATERIALS AND METHODS

In this study, soil was collected from the Botanical garden of St. John's College, Agra, Uttar Pradesh, India. The soil was sandy loam with an average pH value and electrical conductivity (EC) of 7.29 ±0.05 and 0.63 ±0.21 dS/m, respectively. Organic matter content of the soil (1.82%) was moderate.

Pots experiment were prepared in triplicate for each heavy metal (HM) treatment including control. Each pot could hold 4 kg soil and added 10 mg/Kg cadmium (Cd) and chromium (Cr) in soil as $CdCl_2$ salt and $K_2Cr_2O_7$, respectively. Control pots contained without metal treatment. The seeds of *Tagetes erecta* were sown in each pot. Three replicates of each treatment of heavy metals were taken, so as to get the precise and accurate result.

Experimental setup

1. Control- Garden Soil
2. T1- Garden Soil + Cd (10mg/Kg)
3. T2- Garden Soil + Cr (10mg/Kg)
4. T3- Garden Soil + Cd(10mg/Kg) + Cr (10mg/Kg)

Fig 1: (A) Prepared pot experiment, (B) experimental setup of *Tagetes erecta* L., (C) Flowering in control and (D) Flowering in T1(Cd)

These pot experiments were conducted for 4 months. During experiment, every 40 day interval, plants were observed for morphological character such as their plant length and number of leaves per plant. After 120 days, plants were harvested and root, stem and leaf sample along with soil (in triplicate) and determined physiological impact of HM toxicity on plants.

Physicochemical parameters of soil samples

The physicochemical parameters of collected soil sample such as pH and electrical conductivity (EC) were determined by pH meter and EC meter and organic carbon (OC) was determined by Walkley-Black method (Nelson and Sommers, 1982)

Plant growth parameters with Cd and Cr toxicity

Plants were washed with distilled water to remove soil and dust particles after harvesting. Length of plants were measured using measuring scale and plants were weighed separately for their fresh weight. The biomass was estimated by air drying the plants parts. Chlorophyll content was also estimated according to Arnon (1949).

Statistical Analysis

Mean and standard deviation (SD) were used for collected data from each replicates of experiments.

III. RESULTS AND DISCUSSION

The physiological parameters of soil samples are presented in Table 1. The soil pH was above 7 in control as well as all treatments, indicating slightly alkaline nature of soil. The organic matter of soil was 1.83% in control and T1. This finding is mainly because of the presence of many organic waste residues and heavy metals. That contributes more organic matter after their decay. EC is a good indicator of plant growth. The EC levels observed in control and all treatments are slightly different. In all the treatments including control, EC was with in normal range.

Table 1: Physicochemical parameters of different HM treated soil after experiment including initial and control. Results were presented as mean ± SD (n=3).

	Control	T1	T2	T3	Normal range
pH	7.3 ± 0.21	7.46 ± 0.22	7.57±0.11	7.4 ± 0.2	6-8.2
EC (dS/m)	0.43 ± 0.12	0.52 ± 0.15	0.62±0.3	0.63 ± 0.11	0.2-0.8
OC (%)	1.83	1.8	1.7	1.72	0.5-6.0

Result of experiment revealed that the accumulation of heavy metals in *Tagetes erecta* had significant impacts on height and biomass of the plants as shown in Table 2. The height of Cd treated plants (T1) was seems to be increased as 37.34 cm on 120 days than control. On the other hand, Cr treated plants (T2) was 23.5, 27.33 and 28 cm on 40, 80 and 120 days, respectively. While comparing the height of combination of Cd and Cr treated plants (T3) were decreased (27.84 cm) followed by control which was 35.34 cm. Plant height was significantly reduced in T2 and T3 treated plants while T1 plants with increased height than control.

Total fresh weight of T1 was increased (33.232gm) while T2 (17.63gm) and T3 (17.433gm) were decreased than control (29.473gm). Same as dry biomass was increased in T1 (8.59gm) than control (6.39gm) while reduced in T2 (5.23gm) and T3 (4.89gm). T2 and T3 values were approximately same (Table 2). Biomass of *Tagetes erecta* using Cd treatment was increased as a compared to control and other treatments.

Table 2: Effect of Cd and Cr concentration on plant height (cm) and fresh and dry weight in *Tagetes erecta* L. (Each value is the mean of triplicates; n=3)

	Plant height (cm)			Total fresh wt. of plant (gm)	Total dry biomass of plant (gm)
Treatment	40 Days	80 Days	120 Days		
Control	25 ± 0.5	32.84 ± 0.29	35.34 ± 0.29	29.473 ± 1.93	6.39 ± 0.6
T1	27.84 ± 0.29	34.5 ± 0.5	37.34 ± 0.77	33.232 ± 1.34	8.59 ± 0.88
T2	23.5 ± 0.5	27.33 ± 0.77	28 ± 0.5	17.6303 ± 1.89	5.23 ± 0.2
T3	23.66 ± 0.77	26.66 ± 0.77	27.84 ± 0.29	17.433 ± 1.26	4.89 ± 0.5

Number of leaves in T1 were more (45.33) than control (42.67) on 120 days while in T2 and T3 less (30.67 and 31, respectively) than control and result showed same variation for number of flowers (Table3).

In the present study, the Cd treated plants (T1) were shows that increased plant height and biomass while the Cr treated plants (T2) and the combination of Cd and Cr treated plants (T3) were inhibits the plant height and total biomass. Coelho *et al.,* 2017, reported similar effect on linear growth with increasing concentration of trivalent Cr.

Research data revealed that chlorophyll content of *Tagetes erecta* among different heavy metals as shown in Table 4. On T1 condition, total chlorophyll was 6.17 mg/g. On T2, it was 4.37 mg/g; on T3 i.e. the combination of both Cd and Cr, total chlorophyll was observed 4.52 mg/g. The clearly stated that chlorophyll contents with Cd treated plants were approximately same as control which was 6.22 mg/g while chlorophyll contents with Cr treated plants and the combination of Cd and Cr treated plants were less than control.

Table 3. Number of leaves in different HM treatments and number of flowers per plants of *Tagetes erecta* L. (Mean±SD)

	No.of leaves			No. of flowers per plant (approx)
Treatment	40 Days	80 Days	120 Days	
Control	18 ± 2	32.34 ± 0.57	42.67 ± 1.15	8
T1	20.67 ± 1.15	35.34 ± 1.15	45.33 ± 1.15	8
T2	12.67 ± 1.15	23.67 ± 1.52	30.67 ± 1.15	3
T3	12.34 ± 1.52	22.67 ± 0.57	31 ± 1.73	4

Table 4: Effect of Cd and Cr toxicity on Chlorophyll content in *Tagetes erecta* L. (Values in Mean±SD)

	Chl. 'a'	Chl. 'b'	Total Chl.
Control	4.196 ± 0.567	2.034 ± 0.269	6.229 ± 0.432
T1	4.293 ± 0.616	1.825 ± 0.642	6.117 ± 0.610
T2	3.367 ± 0.160	1.014 ± 0.130	4.379 ± 0.229
T3	3.779 ± 0.116	0.749 ± 0.189	4.522 ± 0.291

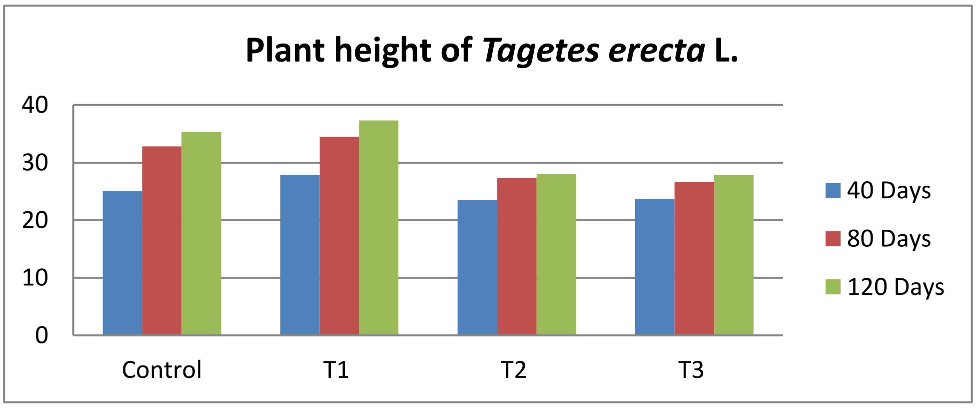

Fig 2: Effect of Cd and Cr toxicity on plant height

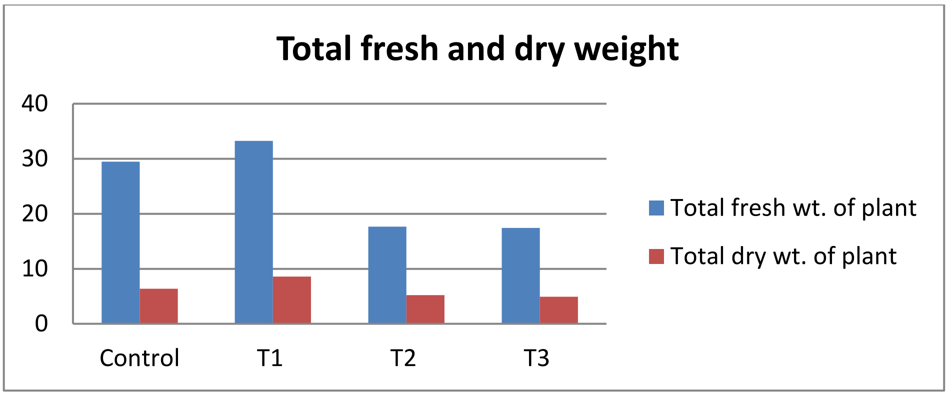

Fig 3 Effect of Cd and Cr toxicity on Fresh and dry weight of *Tagetes erecta* L.

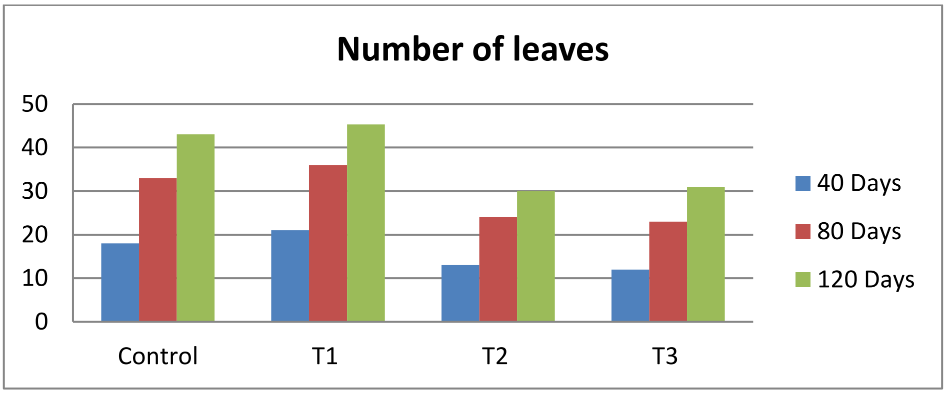

Fig 4: Variance in numbers of leaves

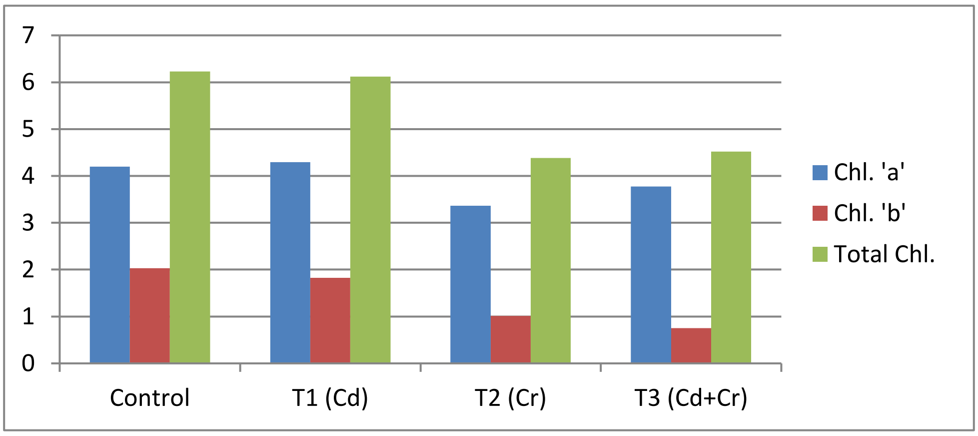

Fig 5: Effect of Cd and Cr toxicity on Chlorophyll content in *Tagetes erecta* L.

IV. CONCLUSION

The results were conducted to determine the effects of different treatments of Cd and Cr on different morphological and physiological parameters such as plant height, biomass, number of leaves and chlorophyll content in *Tagetes erecta* L. The results were concluded that Cr (10 mg/Kg) inhibited normal growth with Cd (T3) as well as alone (T2) for *Tagetes erecta* but plants were grow in Cr stress condition with slow growth than normal. While adequate growth in *Tagetes erecta* with Cd (10 mg/Kg) treated plant (T1) than control. So *Tagetes erecta* can be valuable tool for the phytoremediation of Cr and Cd contaminated soil because *Tagetes erecta* is non-edible ornamental plant species with have low risk of food contamination.

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