GMR

The Original

CHELATE-ENHANCED REDUCTION OF HEXAVALENT CHROMIUM (CR^{6+}) TOXICITY IN WHEAT SEEDLINGS: EFFECTS ON GROWTH, PHYSIOLOGICAL, BIOCHEMICAL, AND METAL UPTAKE RESPONSES

Ansul Sarangi[1], Manoja Das[2], Nibedita Samal[1], Kambaska Kumar Behera*[3]

[1] Ph.D Scholar, Department of Life Sciences, Gandhi Institute of Engineering and Technology University, Odisha, Gunupur-765022, India.
[2] Assistant Professor, Department of Biotechnology, Gandhi Institute of Engineering and Technology University, Odisha, Gunupur-765022, India.
[3] Professor, Department of Pharmacognosy, AIPER, Cuttack-754022, Odisha, India

Abstract

Hexavalent chromium (Cr^{6+}) toxicity adversely affects plant growth and metabolism, limiting crop productivity. This study evaluated the effects of Cr^{6+} (20–80 mg L^{-1}) and the mitigating role of chelating agents (DTPA and EDTA, 20 mg L^{-1}) on growth, physiological, biochemical, and metal accumulation responses of wheat (Triticum aestivum L. cv. HD-2428) under pot culture conditions. Chromium stress caused a dose-dependent reduction in shoot and root growth, biomass, photosynthetic pigments, proteins, and soluble sugars, while proline accumulation increased in response to stress. Chelate application significantly alleviated Cr-induced toxicity by improving growth, pigment content, and biochemical attributes. It also enhanced chromium uptake and accumulation, as evidenced by increased bioconcentration factor (BCF) and total accumulation rate (TAR), while maintaining a high translocation index (Ti). The tolerance index (TI) increased to >1.0 under chelate treatment, indicating improved plant tolerance. These findings demonstrate that DTPA and EDTA effectively reduce Cr^{6+} toxicity while enhancing chromium accumulation, supporting their potential application in wheat-assisted phytoremediation of chromium-contaminated soils.

KEYWORDS: Cr^{6+}; Triticum aestivum; Chelating agents; Bioconcentration factor; Tolerance index

1. INTRODUCTION

Heavy metal contamination of agricultural soils has become a serious environmental issue due to rapid industrialization, urbanization, mining, electroplating, leather tanning, textile manufacturing, and the use of untreated industrial effluents for irrigation. Among these metals, chromium (Cr) is particularly hazardous due to its persistence, toxicity, and environmental mobility, affecting human and animal health through the food chain (Srivastava et al., 2021; Rai et al., 2019; Zayed & Terry, 2003). Chromium exists mainly in two stable oxidation states: trivalent (Cr^{3+}) and hexavalent (Cr^{6+}). Cr^{6+} is far more toxic, highly soluble, and bioavailable, enabling it to enter plants through sulfate and phosphate transporters. Inside cells, Cr^{6+} triggers excessive production of reactive oxygen species (ROS), causing oxidative stress, lipid peroxidation, enzyme damage, impaired photosynthesis, nutrient imbalances, and hindering plant growth and development (Shanker et al., 2005).

Wheat (Triticum aestivum L.) is a crucial cereal crop globally, serving as a key source of calories and protein. Chromium contamination in wheat-producing areas jeopardizes crop yields, grain quality, and food safety by accumulating toxic metals in plant tissues. Therefore, it is vital to develop sustainable and effective methods to reduce Cr^{6+} toxicity, supporting agricultural productivity and minimizing health risks from heavy metal pollution. In India, chromium (Cr) contamination mainly occurs near tannery complexes and industrial discharge zones. Hotspots include Jajmau in Kanpur (Uttar Pradesh), the tannery region of Vellore (Tamil Nadu), and various industrial areas of Gujarat, where untreated or partially treated industrial wastewater is often used for irrigation. Persistent use of chromium-contaminated water has led to the buildup of Cr in soils, negatively impacting wheat growth and increasing the likelihood of chromium entering the food chain, thereby raising environmental and health concerns (Parmar & Patel, 2015).

Chelating agents like EDTA and DTPA are noteworthy for their ability to bind metal ions, influence metal availability, affect uptake and movement within plants, and reduce oxidative damage caused by heavy metals. In addition to controlling chromium mobility, these chelators can enhance nutrient uptake, preserve membrane integrity, and boost antioxidant defenses, thereby improving plant tolerance under heavy metal stress. The effectiveness of various chelating agents depends on their chemical stability, metal-binding strength, and interactions with plant physiological processes (Mussio et al., 2025). However, studies comparing EDTA and DTPA for reducing Cr^{6+} toxicity in wheat seedlings are limited. Furthermore, comprehensive analyses covering growth, physiological, biochemical, antioxidant, and chromium accumulation responses to understand chelate-based detoxification mechanisms are scarce.

This study assesses how EDTA and DTPA can reduce Cr^{6+} toxicity in wheat seedlings by examining growth, physiological, biochemical, and chromium accumulation responses. The results will deepen insights into chelate-based chromium detoxification processes and help pinpoint effective methods to lower chromium toxicity. This research aims to promote sustainable management of chromium-contaminated soils, boost wheat yields, and decrease chromium entry into the food supply.

2.MATERIALS AND METHODS

2.1. Planting material: The experimental material used in this study was wheat (Triticum aestivum L. cv. HD-2428), procured from the Odisha State Seeds Corporation Ltd. Uniform, healthy seeds were selected and surface-sterilized using 0.1% mercuric chloride ($HgCl_2$) for 2–3 minutes. The seeds were then thoroughly rinsed with tap water, followed by distilled water to remove any residual sterilant. Subsequently, the seeds were sown in pots filled with pre-prepared sand, maintaining uniform spacing and consistent planting depth to ensure even germination and optimal growth conditions.

2.2.Pot-culture experiment: River sand was collected and thoroughly cleaned by removing debris and foreign materials. It was washed under running tap water to remove adhering soil and mud, then soaked in 0.1% sulfuric acid (H_2SO_4) for 3 days to remove residual nutrients. The sand was then repeatedly rinsed with distilled water to ensure complete removal of the acid, as verified with litmus paper. Cleaned sand (3.0 kg per pot) was filled into pots measuring 11.5 cm in height and 12.5 cm in diameter. The pots were arranged in a completely randomized design (CRD) comprising treatments T_0 to T_{12}, each with three replications.Ten surface-sterilized seeds were sown in each pot at uniform depth and spacing. The pots were irrigated alternately with water and full-strength Hoagland's nutrient solution (Hoagland & Amon, 1934)for the first five days. Germination occurred within 2–3 days. After establishment, five uniform, healthy seedlings were retained per pot, and excess seedlings were removed to ensure uniform growth and stress application. Five days after seedling emergence, twelve treatments involving chromium (Cr) alone and in combination with chelating agents (DTPA and EDTA) were imposed: T_1–T_4 (Cr at 20, 40, 60, and 80 mg L^{-1}), T_5–T_8 (Cr + DTPA at 20 mg L^{-1}), and T_9–T_{12} (Cr + EDTA at 20 mg L^{-1}). Hexavalent chromium (Cr^{6+}) was applied using potassium dichromate ($K_2Cr_2O_7$) as the source. A control (T_0) was maintained with only water and nutrient solution. Chelating agents (DTPA and EDTA) were applied at a uniform concentration of 20 mg L^{-1}.In addition to metal treatments, half-strength Hoagland's nutrient solution (50 mL per pot) was supplied at three-day intervals throughout the experimental period, which continued up to 15 days after treatment application. The experiment was conducted in a net house under controlled environmental conditions, maintaining temperatures between 25±2°C and 30±2°C. Seedlings were harvested at 20 days for physio-biochemical analyses and metal accumulation. All treatments were performed in triplicate to ensure the reliability of the results.

2.3. Assessment of Growth Parameters: To evaluate seedling growth under hexavalent chromium (Cr^{6+}) stress, plants were harvested on the 15th day of exposure to metal and chelate treatments. Collected seedlings were thoroughly washed with distilled water,then with Milli-Q water, to remove surface contaminants. Roots and shoots were carefully separated, and their lengths were measured. Fresh weights of roots and shoots were recorded using five randomly selected samples per treatment, each in triplicate. Dry weights were determined after oven-drying the samples at 70–80°C for three days until a constant weight was achieved (Liu et al., 2008).

2.4. Physiological Parameters

2.4.1. Photosynthetic Pigment Analysis: Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were estimated from fresh primary leaf tissues using 80% chilled acetone as the extraction solvent. The absorbance of the extracts was recorded at 663, 645, and 475 nm using a spectrophotometer. Pigment concentrations were calculated according to Arnon (1949), using the following standard equations.

chlorophyll a mg/g tissue $= 12.7(A663) - 2.69(A645) \times V/1000 \times W$

chlorophyll b mg/g tissue $= 22.9(A645) - 4.68(A663) \times V/1000 \times W$

total chlorophyll mg/g tissue $= 20.2(A645) + 8.02(A663) \times V/1000 \times W$

Carotenoid mg/g tissue $= (A480) + (0.114+(A663) - (0.638x(A645)$

Where, A = Absorbance, V = final volume of the extract, W = fresh weight of leaf sample taken for extraction

2.4.2.Estimation of Soluble Sugar Content: Soluble sugars were extracted by homogenizing 0.5 g of leaf tissue in 80% ethanol. The homogenate was incubated at 60°C for 15 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and the extraction process was repeated 2–3 times to ensure complete recovery of sugars. The pooled extracts were used to estimate sugar content following the anthrone method described by Yoshida et al. (1972). The reaction mixture, consisting of the extract and freshly prepared anthrone reagent, was heated in a boiling water bath for 8 minutes. Absorbance was recorded at 630 nm using a spectrophotometer, and total sugar content was quantified using D-glucose as the standard.

2.4.3. Proline (Amino Acid) Estimation: Proline content was determined following the method of Szabados and Savouré 2010. Fresh leaf tissue (0.5 g) was homogenized in 3% sulfosalicylic acid and centrifuged at 5000 rpm for 20 minutes. The supernatant was treated with acid ninhydrin and glacial acetic acid, then incubated at 100°C for 1 hour. After cooling, toluene was added, and the mixture was vortexed. The absorbance of the chromophore was measured at 520 nm, and proline concentration was expressed as mg per gram of fresh weight.

2.4.4. Protein Content Analysis: Protein content was estimated using the method of Lowry et al. (1951). Leaf samples (0.5 g) were homogenized in 20% trichloroacetic acid (TCA) and centrifuged. The residue was washed with ethanol, then treated with 0.3 N NaOH and incubated below 40°C for 24 hours. The assay involved mixing the sample with alkaline copper reagent, followed by the addition of Folin–Ciocalteu reagent. After incubation, absorbance was recorded at 750 nm. Protein concentration was determined using a standard curve prepared with bovine serum albumin (BSA).

2.4.5. Heavy metal analysis in the plant part: The bioavailability of hexavalent chromium ($Cr^{6+)}$ in the seedlings' roots and shoots was assessed based on the methodology described by Shahid et al. (2017). Before analyzing the Cr content in the plant parts, plant samples were gently washed with distilled water, then with 0.01 M EDTA to remove surface-bound metals, and finally rinsed with deionized water. Subsequently, roots and shoots were separated, oven-dried at 80 °C for 3 days until a stable biomass was achieved, and ground individually into fine powders using a stainless-steel grinder to prepare the samples. For digestion, a 10:1 mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added to the weighed samples, and the mixture was left to stand overnight, following Baruah et al (2019). Digestion was carried out using an MDS-8 Microwave Digestion System until a clear, soluble solution was obtained. The resulting digested solutions were filtered through Whatman No. 1 filter paper and diluted to a final volume of 100 mL with deionized water. Cadmium and lead concentrations in different plant parts were quantified using an Atomic Absorption Spectrophotometer (PerkinElmer Analyst 200, USA).

2.4.6.Metal accumulation and tolerance indices: There are multiple methods available to quantify and represent metal accumulation in plants. The plant's biomass was used to compute the bioconcentration factor (BCF), total accumulation rate (TAR), transportation index (Ti), and tolerance index (TI) as per the formulae adopted by Ghosh (2005).

$$\mathrm{BCF} = \frac{\text{Average concentration of Cr(VI) in the plant tissue(mg kg}^{-1})}{\text{Average concentration of Cr(VI) added in to the soil (mg kg}^{-1})}$$

$$\mathrm{TAR} = \frac{[(\text{Cr(VI) concentration in the shoot x Shoot biomass})+(\text{Cr(VI)concentration in root x Root biomass})]}{[(\text{Shoot biomass+ Root Biomass}) \text{ x Days of plant growth}]} \text{ mg kg}^{-1}$$

$$\mathrm{Ti} = \frac{\text{Concentration of Cr(VI) in the shoot(mg kg}^{-1})}{\text{Concentration of Cr(VI) in the root (mg kg}^{-1})} \text{x100}$$

$$\mathrm{TI} = \frac{\text{Dry weight of treated Plants}}{\text{Dry weight of control plant}}$$

2.4.7. Statistical Analysis: Data from the seedlings were collected, and a statistical analysis was conducted. The treatments were administered in triplicate, and the mean ± SE (standard deviation of the mean of three repetitions) values are presented in the figures and tables. Analysis of variance (ANOVA) for all measured variables was performed using R version 4.4.3 (Copyright (C) 2025). The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64. The treatment means were separated using Duncan's multiple range test (DMRT), taking $P<0.05$ as significant.

3. RESULTS AND DISCUSSION

3.1. Effect of Cr^{6+} on Shoot, Root Growth, and Biomass Accumulation:

Hexavalent chromium (Cr^{6+}) adversely affected the growth of 20-day-old wheat seedlings (Triticum aestivum L. cv. HD-2428) in a concentration-dependent manner (Table 1). Compared with the control (T_0), shoot length decreased by 9.3–37.2%, while root length declined by 10.0–60.0% under increasing Cr concentrations (T_1-T_4). Similarly, shoot biomass decreased by 5.8-52.9% and root biomass by 12.5–43.8%, indicating that roots were more sensitive to chromium toxicity than shoots. The reduction in growth and biomass is attributed to chromium-induced oxidative stress, disruption of nutrient and water uptake, inhibition of cell division, and impairment of photosynthesis, ultimately restricting plant development (Zhang et al., 2007; Gill & Tuteja, 2010).

Table 1. Shoot length, Root length(cm), Shoot biomass, and Root biomass(g) of 20-day-old Wheat Pot culture seedlings

Treatments	Wheat			
	Shoot length (cm)	**Root length (cm)**	**Shoot biomass (g)**	**Root biomass (g)**
T_0	14.33^{a}	10.00^{ab}	1.38^{abc}	0.16^{a}
T_1	13.00^{a}	9.00^{ab}	1.3^{abcd}	0.14^{a}
T_2	11.67^{a}	7.67^{ab}	1.04^{bcd}	0.14^{a}
T_3	10.00^{a}	5.67^{ab}	0.79^{cd}	0.12^{a}
T_4	9.00^{a}	4.00^{b}	0.65^{d}	0.09^{a}
T_5	20.67^{a}	11.67^{a}	1.82^{a}	0.22^{a}
T_6	19.67^{a}	10.67^{a}	1.74^{ab}	0.21^{a}
T_7	19.00^{a}	9.33^{ab}	1.65^{ab}	0.21^{a}
T_8	17.00^{a}	8.33^{ab}	1.58^{ab}	0.19^{a}
T_9	20.00^{a}	11.00^{a}	1.75^{ab}	0.22^{a}
T_{10}	18.67^{a}	9.67^{ab}	1.65^{ab}	0.21^{a}
T_{11}	17.67^{a}	8.33^{ab}	1.57^{ab}	0.2^{a}
T_{12}	16.00^{a}	7.00^{ab}	1.47^{abc}	0.17^{a}

T0-Control, T1- Cr 20 mg L^{-1}, T2- Cr 40 mg L^{-1}, T3- Cr 60 mg L^{-1} , T4- Cr 80 mg L^{-1},T5-T1+ DTPA-20 mg L^{-1},T6-T2+DTPA-20 mg L^{-1}, T7-T3+ DTPA-20 mg L^{-1},T8-T4+ DTPA-20 mg L^{-1}, T9-T1+EDTA-20 mg L^{-1} , T10-T2+ EDTA-20 mg L^{-1},T11-T3+ EDTA-20 mg L^{-1},T12-T4+ EDTA-20 mg L^{-1}. The same letter in a column shows no significant effect at 5% level of significance(Duncan test, $p < 0.05$).

Application of DTPA and EDTA markedly improved seedling growth under chromium stress. DTPA-treated plants (T_5-T_8) showed greater shoot length (17.00-20.67 cm), root length (8.33–11.67 cm), shoot biomass (1.58-1.82 g), and root biomass (0.19-0.22 g) than Cr-treated plants, while EDTA (T_9–T_{12}) also enhanced these parameters, although to a lesser extent. The improved growth suggests that both chelators reduced Cr^{6+} phytotoxicity by complexing chromium, improving nutrient availability, and alleviating oxidative damage. DTPA was more effective than EDTA in promoting growth recovery, indicating its greater potential to mitigate chromium toxicity in wheat seedlings, consistent with previous reports on chelator-assisted heavy metal detoxification (Singh et al, 2020;Sarangi et al.,2025).

3.2. Effect of Cr^{6+} on Photosynthetic Pigments: Hexavalent chromium (Cr^{6+}) caused a concentration-dependent reduction in photosynthetic pigments of wheat seedlings (Table 2). Compared with the control (T_0), chlorophyll-a decreased from 2.37 to 1.63 mg g^{-1} FW (31.2%), chlorophyll-b from 1.63 to 0.97 mg g^{-1} FW (40.5%), total chlorophyll from 4.00 to 2.60 mg g^{-1} FW (35.0%), and carotenoids from 0.21 to 0.08 mg g^{-1} FW (61.9%) under the highest Cr concentration (T_4). The decline in chlorophyll and carotenoid contents indicates severe impairment of the photosynthetic apparatus under chromium stress.The reduction in photosynthetic pigments may be attributed to chromium-induced oxidative stress, inhibition of chlorophyll biosynthesis, degradation of chloroplast ultrastructure, and disruption of nutrient uptake, particularly magnesium and iron, which are essential for chlorophyll synthesis. Singh et al.(2020) investigated the effects of hexavalent chromium (Cr^{6+}) toxicity on two chickpea (Cicer arietinum L.) varieties under hydroponic and pot culture conditions. They reported that increasing Cr concentrations significantly inhibited chlorophyll content.Similar decreases in chlorophyll content under chromium stress have been reported in wheat and other crop plants (Sarwar, 2025).

Table 2. Photosynthetic Pigment Composition (mg g^{-1}fresh weight) of Wheat 15-day-old Pot-culture seedlings

Treatments	**Wheat**			
	Chlorophylla	**Chlorophyllb**	**Total Chlorophyll(a+b)**	**Carotenoid**
T_0	2.37^{abc}	1.63^{abc}	4.0^{abc}	0.21^{abc}
T_1	1.92^{bc}	1.43^{abc}	3.35^{bc}	0.17^{abc}
T_2	1.83^{c}	1.23^{bc}	3.06^{c}	0.12^{bc}
T_3	1.7^{c}	1.1^{c}	2.8^{b}	0.11^{bc}
T4	1.63^{e}	0.97^{a}	2.60^{a}	0.08^{c}
T_5	3.0^{a}	1.96^{a}	4.96^{a}	0.28^{a}
T_6	2.91^{a}	1.83^{ab}	4.74^{ab}	0.26^{ab}
T_7	2.8^{ab}	1.77^{abc}	4.57^{abc}	0.24^{abc}
T_8	2.65^{abc}	1.67^{abc}	4.32^{abc}	0.22^{abc}
T_9	2.93^{a}	1.82^{ab}	4.75^{ab}	0.26^{ab}
T_{10}	2.83^{ab}	1.72^{abc}	4.55^{abc}	0.24^{ab}
T_{11}	2.70^{abc}	1.64^{abc}	4.34^{abc}	0.23^{abc}
T_{12}	2.6^{ab}	1.55^{abc}	4.15^{ab}	0.21^{bc}

T0-Control, T1- Cr 20 mg L^{-1}, T2- Cr 40 mg L^{-1}, T3- Cr 60 mg L^{-1} , T4- Cr 80 mg L^{-1},T5-T1+ DTPA-20 mg L^{-1},T6-T2+DTPA-20 mg L^{-1}, T7-T3+ DTPA-20 mg L^{-1},T8-T4+ DTPA-20 mg L^{-1}, T9-T1+EDTA-20 mg L^{-1} , T10-T2+ EDTA-20 mg L^{-1},T11-T3+ EDTA-20 mg L^{-1},T12-T4+ EDTA-20 mg L^{-1}. The same letter in a column shows no significant effect at 5% level of significance(Duncan test, $p < 0.05$).

Chelator application markedly alleviated the adverse effects of chromium on photosynthetic pigments. DTPA-treated seedlings (T_5–T_8) recorded higher chlorophyll-a (2.65–3.00 mg g^{-1} FW), chlorophyll-b (1.67–1.96 mg g^{-1} FW), total chlorophyll (4.32–4.96 mg g^{-1} FW), and carotenoids (0.22–0.28 mg g^{-1} FW) than chromium-only treatments. Likewise, EDTA-treated plants (T_9–T_{12}) significantly improved pigment contents, although the recovery was slightly lower than that observed with DTPA. The highest pigment contents were recorded in T_5 (Cr 20 mg L^{-1} + DTPA), exceeding even the control values.The enhanced pigment content following DTPA and EDTA application suggests that chelators mitigated chromium toxicity by reducing chromium bioavailability, protecting chloroplast integrity, improving nutrient uptake, and enhancing antioxidant defense against oxidative damage. The relatively superior performance of DTPA indicates its greater efficiency in preserving photosynthetic pigments under chromium stress. These findings agree with previous reports demonstrating that EDTA- and DTPA-assisted treatments improve chlorophyll stability and photosynthetic efficiency in heavy metal-stressed plants (Sangwan et al., 2013).

3.3. Effect of Cr^{6+} on Biochemical Parameters: Hexavalent chromium (Cr^{6+}) induced marked biochemical changes in wheat seedlings (Table 3). Protein content declined progressively from 0.66 mg g^{-1} FW in the control to 0.56 mg g^{-1} FW under the highest Cr concentration (T_4), while soluble sugar decreased from 0.79 to 0.58 mg g^{-1} FW. In contrast, proline content increased more than eleven-fold, from 0.05 mg g^{-1} FW in the control to 0.57 mg g^{-1} FW under T_4, indicating enhanced osmotic adjustment in response to chromium-induced stress. Although protein content showed no significant differences among treatments according to Duncan's test, a consistent decline was observed as chromium concentration increased.

The reduction in protein and soluble sugar contents may be attributed to chromium-induced inhibition of protein synthesis, degradation of cellular proteins, impaired photosynthetic carbon assimilation, and disruption of carbohydrate metabolism. Conversely, the substantial accumulation of proline represents an adaptive response that helps maintain osmotic balance, stabilize proteins and membranes, and scavenge reactive oxygen species under heavy metal stress. Similar biochemical responses have been reported in wheat and other crop plants exposed to chromium toxicity (AbdElgawad et al., 2023).

Table 3. Biochemical Parameters: Protein, Proline, and Sugar content (mg g^{-1} fresh weight) of Wheat 15-day-old Pot-culture seedlings

Treatments	Wheat		
	Protein	Soluble sugar	Proline
T_0	0.66^{a}	0.79^{ab}	0.05^{a}
T_1	0.64^{a}	0.71^{ab}	0.15^{b}
T_2	0.59^{a}	0.68^{ab}	0.28^{c}
T_3	0.57^{a}	0.64^{ab}	0.43^{ab}
T4	0.56^{a}	0.58^{b}	0.57^{abc}
T_5	0.72^{a}	0.90^{a}	0.3^{bc}
T_6	0.68^{a}	0.85^{a}	0.41^{ab}
T_7	0.65^{a}	0.79^{ab}	0.52^{abc}
T_8	0.61^{a}	0.74^{ab}	0.63^{a}
T_9	0.69^{a}	0.85^{a}	0.28^{c}
T_{10}	0.66^{a}	0.78^{ab}	0.38^{abc}
T_{11}	0.62^{a}	0.74^{ab}	0.49^{abc}
T_{12}	0.57^{a}	0.69^{ab}	0.60^{ab}

T0-Control, T1- Cr 20 mg L^{-1}, T2- Cr 40 mg L^{-1}, T3- Cr 60 mg L^{-1} , T4- Cr 80 mg L^{-1},T5-T1+ DTPA-20 mg L^{-1},T6-T2+DTPA-20 mg L^{-1}, T7-T3+ DTPA-20 mg L^{-1},T8-T4+ DTPA-20 mg L^{-1}, T9-T1+EDTA-20 mg L^{-1} , T10-T2+ EDTA-20 mg L^{-1},T11-T3+ EDTA-20 mg L^{-1},T12-T4+ EDTA-20 mg L^{-1}. The same letter in a column shows no significant effect at 5% level of significance(Duncan test, $p < 0.05$).

The application of DTPA and EDTA alleviated the adverse effects of chromium stress by improving protein and soluble sugar contents and modulating proline accumulation. DTPA-treated seedlings (T_5–T_8) recorded higher protein (0.61–0.72 mg g^{-1} FW) and soluble sugar (0.74–0.90 mg g^{-1} FW) contents than chromium-only treatments, with the highest values observed in T_5. Similarly, EDTA treatments (T_9–T_{12}) enhanced protein (0.57–0.69 mg g^{-1} FW) and soluble sugar (0.69–0.85 mg g^{-1} FW). Proline content remained elevated in chelator-treated plants, particularly at higher chromium concentrations, suggesting that osmotic protection continued while growth recovery was enhanced (Aloud et al., 2024). Overall, DTPA showed slightly greater effectiveness than EDTA in maintaining biochemical attributes under chromium stress.

The beneficial effects of DTPA and EDTA are likely due to their ability to complex chromium ions, reduce metal-induced oxidative damage, improve nutrient availability, and preserve cellular metabolism. These findings are consistent with previous reports demonstrating that chelating agents mitigate heavy metal toxicity by maintaining protein synthesis, improving carbohydrate metabolism, and enhancing stress tolerance through osmolyte accumulation (Ashraf Foolad, 2007; Gupta et al., 2010; Rai et al., 2024).

3.4.Effect of Cr^{6+} on BCF, TAR, TI, and Ti: Chromium stress and chelator application significantly influenced chromium uptake, accumulation, translocation, and tolerance in wheat seedlings (Figs. 1–4). Under chromium-only treatments (T_1–T_4), the bioconcentration factor (BCF) decreased progressively from 0.24 to 0.12 with increasing Cr concentration, whereas the total accumulation rate (TAR) increased from 0.12 to 0.25. Similarly, the translocation index (TI) increased from 58.94% (T_1) to 89.71% (T_4), indicating greater movement of chromium from roots to shoots at higher Cr levels. In contrast, the tolerance index (TolI) declined markedly from 0.94 to 0.48, reflecting severe growth inhibition under increasing chromium stress.

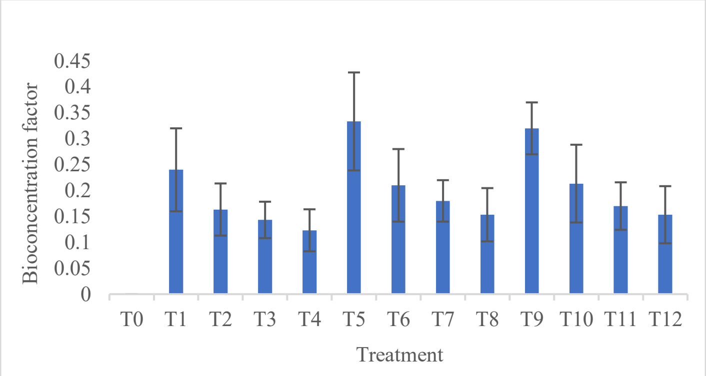

Fig. 1. Bioconcentration factor of 20-day-old wheat seedlings. T0 - Control, T1- Cr 20 mg L^{-1}, T2- Cr 40 mg L^{-1}, T3 - Cr 60 mg L^{-1}, T4 - Cr 80 mg L^{-1}, T5 - T1 + DTPA-20 mg L^{-1}, T6 - T2 + DTPA - 20 mg L^{-1}, T7 - T3 + DTPA-20 mg L^{-1}, T8 - T4 + DTPA 20 mg L^{-1}, T9 - T1 + EDTA-20 mg L^{-1}, T10 - T2 + EDTA-20 mg L^{-1}, T11 - T3 + EDTA-20 mg L^{-1}, T12 - T4 + EDTA-20 mg L^{-1}

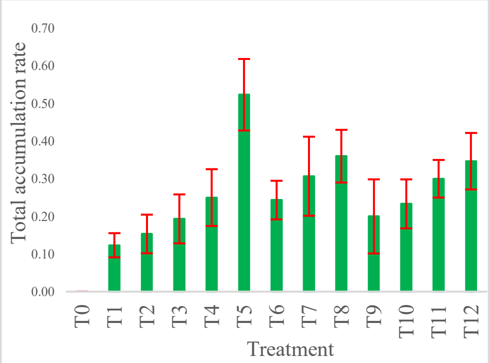

Fig. 2. Total accumulation rate of 20-day-old wheat seedlings. T0 - Control, T1- Cr 20 mg L-1, T2- Cr 40 mg L-1, T3 - Cr 60 mg L-1, T4 - Cr 80 mg L-1, T5 - T1 + DTPA-20 mg L-1, T6 - T2 + DTPA -20 mg L-1, T7 - T3 + DTPA-20 mg L-1, T8 - T4 + DTPA 20 mg L-1, T9 - T1 + EDTA-20 mg L-1, T10 - T2 + EDTA-20 mg L-1, T11 - T3 + EDTA-20 mg L-1, T12 - T4 + EDTA-20 mg L-1

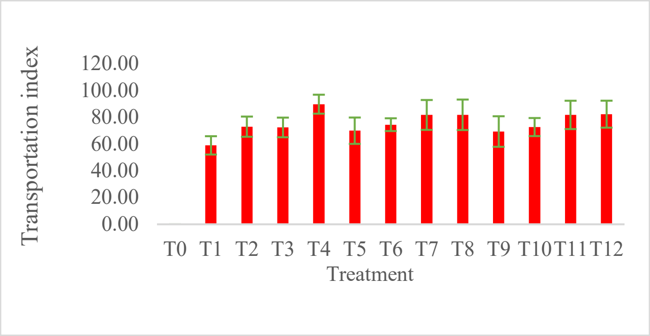

Fig. 3. Transportation index of 20-day-old wheat seedling. T0 - Control, T1- Cr 20 mg L^{-1}, T2- Cr 40 mg L^{-1}, T3 - Cr 60 mg L^{-1}, T4 - Cr 80 mg L^{-1}, T5 - T1 + DTPA-20 mg L^{-1}, T6 - T2 + DTPA -20 mg L^{-1}, T7 - T3 + DTPA-20 mg L^{-1}, T8 - T4 + DTPA 20 mg L^{-1}, T9 - T1 + EDTA-20 mg L^{-1}, T10 - T2 + EDTA-20 mg L^{-1}, T11 - T3 + EDTA-20 mg L^{-1}, T12 - T4 + EDTA-20 mg L^{-1}

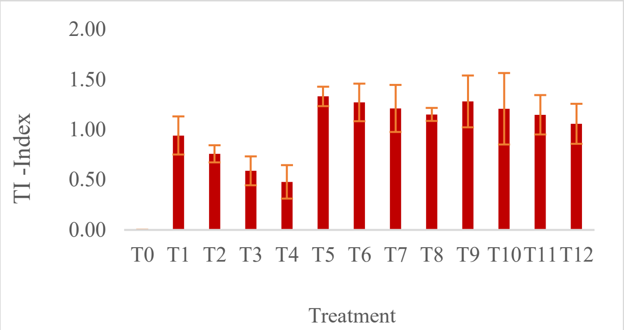

Fig. 4. Tolerance index of 20-day-old wheat seedling. T0 - Control, T1- Cr 20 mg L^{-1}, T2- Cr 40 mg L^{-1}, T3 - Cr 60 mg L^{-1}, T4 - Cr 80 mg L^{-1}, T5 - T1 + DTPA-20 mg L^{-1}, T6 - T2 + DTPA -20 mg L^{-1}, T7 - T3 + DTPA-20 mg L^{-1}, T8 - T4 + DTPA 20 mg L^{-1}, T9 - T1 + EDTA-20 mg L^{-1}, T10 - T2 + EDTA-20 mg L^{-1}, T11 - T3 + EDTA-20 mg L^{-1}, T12 - T4 + EDTA-20 mg L^{-1}

Applying DTPA and EDTA significantly enhanced chromium uptake and plant tolerance. Seedlings treated with DTPA (T_5–T_8) showed higher BCF (0.15–0.33), TAR (0.24–0.52), and tolerance index (1.15–1.33) compared to chromium-only treatments. EDTA-treated plants (T_9–T_{12}) also displayed increased BCF (0.15–0.32), TAR (0.20–0.35), and tolerance index (1.06–1.28). Both chelator treatments maintained high translocation indices (69.27–82.25%), indicating efficient movement of chromium from roots to shoots. DTPA was slightly more effective than EDTA, especially at lower chromium levels, shown by the highest BCF (0.33), TAR (0.52), and tolerance index (1.33) in T_5. As chromium concentration increased, BCF decreased, likely due to saturation of root uptake sites and activation of defense mechanisms, while TAR and TI increased, reflecting continued chromium accumulation and translocation. Chelators improved chromium mobility by forming soluble Cr–chelate complexes, thereby facilitating uptake and xylem transport and reducing the toxicity of free Cr ions. Higher tolerance indices in DTPA- and EDTA-treated seedlings suggest that chelators reduce oxidative stress, support nutrient uptake, and sustain physiological functions for growth. DTPA's greater effectiveness indicates a stronger ability to regulate chromium bioavailability and boost plant tolerance than EDTA in this study. These results support recent research indicating that synthetic chelating agents enhance heavy metal absorption, transport within plants, and the effectiveness of phytoremediation (Prakash et al., 2022). They also enhance plant resilience by reducing oxidative stress and fortifying antioxidant defenses. The increased tolerance observed here suggests that chelate-assisted remediation could be an effective approach to treating chromium-contaminated agricultural lands while maintaining wheat growth and yield(Nazmul et al., 2021).

3.5. Comparative Performance of DTPA and EDTA in Mitigating Cr^{6+} Toxicity in Wheat Seedlings: A comparison of the two chelating agents revealed that both DTPA and EDTA effectively reduced Cr^{6+} toxicity in wheat seedlings, with DTPA showing slightly better performance across most measures (Table 5). In terms of growth traits, DTPA consistently led to greater increases in shoot length, root length, shoot biomass, and root biomass compared to EDTA at similar chromium levels. For instance, at 20 mg L^{-1} Cr, DTPA resulted in a shoot length of 20.67 cm versus 20.00 cm with EDTA, and shoot biomass reached 1.82 g compared to 1.75 g. Similar patterns appeared at higher chromium concentrations, indicating improved growth recovery with DTPA. Regarding photosynthetic pigments, seedlings treated with DTPA maintained higher levels of chlorophyll a (3.00 vs. 2.93 mg g^{-1} FW), chlorophyll b (1.96 vs. 1.82 mg g^{-1} FW), total chlorophyll (4.96 vs. 4.75 mg g^{-1} FW), and carotenoids (0.28 vs. 0.26 mg g^{-1} FW) than those treated with EDTA, implying better protection of the photosynthetic system against chromium-induced oxidative stress. Regarding biochemical parameters, DTPA proved more effective in preserving protein (0.72 vs. 0.69 mg g^{-1} FW) and soluble sugar (0.90 vs. 0.85 mg g^{-1} FW) levels. Both chelators similarly induced proline accumulation under chromium stress. The increased protein and carbohydrate levels suggest better maintenance of cellular metabolism in DTPA-treated plants. Additionally, chromium uptake metrics showed DTPA's advantage, with the highest bioconcentration factor (0.33), total accumulation rate (0.52), and tolerance index (1.33) compared to EDTA's 0.32, 0.20, and 1.28, respectively. Although both chelators enhanced chromium translocation, DTPA led to greater chromium accumulation and higher plant tolerance, indicating a more effective balance between metal uptake and detoxification.

Table 5. Comparative Performance of DTPA and EDTA in Mitigating Cr^{6+} Toxicity in WheatSeedlings

Sl.No.	Parameter	DTPA	EDTA	Comparative Observation
1	Shoot length	Greater increase (17.00–20.67 cm)	Moderate increase (16.00–20.00 cm)	DTPA > EDTA
2	Root Length	8.33–11.67 cm	7.00–11.00 cm	DTPA slightly superior
3	Shoot biomass	1.58–1.82 g	1.47–1.75 g	DTPA > EDTA
4	Root Biomass	0.19–0.22 g	0.17–0.22 g	DTPA marginally better

5	Chlorophylla	2.65–3.00 mg g^{-1} FW	2.60–2.93 mg g^{-1} FW	DTPA higher
6	Chlorophyllb	1.67–1.96 mg g^{-1} FW	1.55–1.82 mg g^{-1} FW	DTPA higher
7	Total chlorophyll-(a+b)	4.32–4.96 mg g^{-1} FW	4.15–4.75 mg g^{-1} FW	DTPA higher
8	Carotenoid	0.22–0.28 mg g^{-1} FW	0.21–0.26 mg g^{-1} FW	DTPA higher
9	Protein	0.61–0.72 mg g^{-1} FW	0.57–0.69 mg g^{-1} FW	DTPA is a better-maintained protein
10	Proline	0.74–0.90 mg g^{-1} FW	0.69–0.85 mg g^{-1} FW	DTPA higher
11	Soluble sugar	0.30–0.63 mg g^{-1} FW	0.28–0.60 mg g^{-1} FW	Comparable; DTPA slightly higher under severe stress
12	BCF	0.15–0.33	0.15–0.32	DTPA slightly higher
13	TAR	0.24–0.52	0.20–0.35	DTPA markedly higher
14	Ti	69.90–81.78%	69.27–82.25%	Nearly similar; EDTA marginally higher at the highest Cr level
15	TI	1.15–1.33	1.06–1.28	DTPA higher tolerance
16	Overall effectiveness	Stronger mitigation of Cr^{6+} toxicity, better growth recovery, higher pigment retention, improved biochemical status, and greater phytoremediation efficiency	Effective but generally less efficient than DTPA	DTPA performed by

The results show that both DTPA and EDTA effectively reduce chromium toxicity. However, DTPA consistently offers better protection by promoting growth, maintaining photosynthetic pigments, supporting biochemical balance, and increasing chromium uptake and tolerance. These results indicate that DTPA is a more effective chelating agent than EDTA for alleviating Cr^{6+} toxicity in wheat seedlings and may be more suitable for chelate-assisted phytoremediation of chromium-contaminated soils. Similar findings have been reported in recent research, which shows that DTPA generally provides stronger metal complexation and enhances growth recovery (Panda & Choudhury, 2005;Zulfiqar et al., 2023). Nevertheless, the effectiveness of DTPA versus EDTA varies with metal type, plant genotype, and soil conditions (Shahid et al., 2014;Khan et al., 2015).

CONCLUSION

The current study found that hexavalent chromium (Cr^{6+}) severely harms wheat seedlings, significantly reducing shoot and root growth, biomass, photosynthetic pigments, proteins, and soluble sugars. In response to oxidative and osmotic stress, proline levels increased markedly. Higher chromium levels also affected uptake dynamics by decreasing the bioconcentration factor and tolerance index, while raising total accumulation and translocation rates. Using DTPA and EDTA chelators effectively mitigated chromium toxicity by enhancing plant growth, preserving chlorophyll and carotenoids, maintaining biochemical balance, and boosting chromium tolerance through better metal complexation and nutrient availability. DTPA was more effective than EDTA in restoring physiological and biochemical functions and improving chromium uptake and tolerance, making it a superior chelator for reducing Cr^{6+} toxicity in wheat. These results suggest that chelator-assisted strategies, especially with DTPA, can reduce oxidative damage, strengthen plant resilience, and improve phytoremediation in chromium-contaminated soils. Overall, DTPA shows promise as a sustainable amendment for growing wheat in polluted environments and provides a scientific foundation for future field applications and integrated heavy-metal remediation efforts.

REFERENCE

1. AbdElgawad H, Abuelsoud W, Elkelish A, Hasanuzzaman M, Ibrahim MF, Mohamed HI, &Hozzein WN (2023). Selenium and salicylic acid mitigate chromium toxicity in faba bean by enhancing the antioxidative defense system and photosynthesis. Journal of Hazardous Materials 446:130437 https://doi.org/10.1016/j.jhazmat.2022.130437
2. Aloud BM, Alyemeni MN, Ahanger MA, Alsahli AA, Alamri S, Ahmad P, & Siddique KHM (2024). Chelation-assisted mitigation of chromium toxicity in faba bean: Modulation of antioxidant system and photosynthetic machinery. Plant Physiology and Biochemistry 196: 42–53 https://doi.org/10.1016/j.plaphy.2023.11.018
3. Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology 24: 1–15 https://doi.org/10.1104/pp.24.1.1

4. Ashraf M &Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59(2): 206–216 https://doi.org/10.1016/j.envexpbot.2005.12.006
5. Baruah N, Mondal SC, Farooq M, Gogoi, N (2019). Influence of heavy metals on seed germination and seedling growth of wheat, pea, and tomato. Water Air & Soil Pollution, 230(12). https://doi.org/10.1007/s11270-019-4329-0
6. Ghosh, M. (2005). A Review on Phytoremediation of heavy metals and utilization of Its Byproducts. Applied Ecology and Environmental Research, 3(1), 1–18. https://doi.org/10.15666/aeer/0301_001018
7. Gill SS and Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48(12): 909-930 https://doi.org/10.1016/j.plaphy.2010.08.016
8. Gupta DK, Huang HG, & Corpas FJ (2010). Lead tolerance in plants: Strategies for phytoremediation. Environmental Science and Pollution Research 17(1): 1–3 https://doi.org/10.1007/s11356-009-0252-8
9. Hoagland DR. & Amon DI (1934). The water-culture method for growing plants without soil. University of California, College of Agriculture, Berkeley, Calif. Circ 347:1–39https://doi.org/10.1016/S0140-6736(00)73482-9
10. Khan MI, Fatma M, Per TS, Anjum NA, & Khan NA (2015). Salicylic acid alleviates the adverse effects of heat stress on photosynthesis by altering proline production and ethylene formation. Plant Signaling&Behavior 10(3): e1057360 https://doi.org/10.1080/15592324.2015.1057360
11. Liu D, Jiang W & Gao X (2008). Effects of cadmium on root growth, cell division, and nucleoli in root tip cells of garlic. Biologia Plantarum 52(2): 215–219 https://doi.org/10.1007/s10535-008-0042-0
12. Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193(1): 265–275 https://doi.org/10.1016/S0021-9258(19)52451-6
13. Mussio C, Garcia-Perez P, Moret E, Catena S, Lucini L (2025). The role, mechanisms, and evaluation of natural chelating agents in food stability: a review. Food Chemistry, 496(Pt 1), 146682. https://doi.org/10.1016/j.foodchem.2025.146682
14. Nazmul Huda A, Noman M, Javed MT, Sohail MI, Khan SU, Arif MS, & Ahmad I (2021). Chelator-assisted phytoremediation of chromium: Recent advances and prospects. Environmental Technology & Innovation 22: 101504 https://doi.org/10.1016/j.eti.2021.101504
15. Parmar P, Kumari N & Sharma V (2013). Structural and functional alterations in the photosynthetic apparatus of plants under cadmium stress. Botanical Studies 54:45 https://doi.org/10.1186/1999-3110-54-45
16. Prakash CA, Nair S, Sebastian D, Mohanan S, Sindhu E, George S, &Puthur J (2022). Chelate-assisted phytoextraction for effective rehabilitation of heavy metal (loid)s contaminated lands. International journal of phytoremediation 25:1-16 10.1080/15226514.2022.2124233
17. Rai A, Sharma NK, Singh VK, Rai A, Kumar V, Kumar A, Singh JS, Kudesia S, Rai PK (2024). Use of biowaste to ameliorate chromium-contaminated soils to improve crop productivity. Waste Management Bulletin 2(1): 276-288 https://doi.org/10.1016/j.wmb.2024.02.004
18. Rai UN, Singh S, Gupta A, & Singh H (2019). Chromium-induced physiological responses in terrestrial plants and their uptake in the food chain. Plant Biology 21:315–325. https://doi.org/10.1111/plb.12881
19. Sangwan P, Kumar V, Khatri R.S, and Joshi U.N.(2013). Chromium (VI) Induced Biochemical Changes and Gum Content in Cluster Bean (Cyamopsis tetragonoloba L.) at Different Developmental Stages. Hindawi Publishing Corporation Journal of Botany Volume 2013, Article ID 578627, 8 pages http://dx.doi.org/10.1155/2013/578627
20. Sarangi, A., Das, M., Behera, K. K., & Samal, N. (2025). Comparative study of chromium stress in pot-cultured wheat and rice seedlings: role of DTPA and EDTA chelation in bioaccumulation and antioxidant responses: Chelator-mediated chromium stress response. ORYZA-An International Journal of Rice, 62(03), 289-300. https://epubs.icar.org.in/index.php/OIJR/article/view/172219
21. Sarwar G, Fatima M, Danish S et al. (2025). Enhancing wheat growth under chromium toxicity using gibberellic acid and microbial inoculants as modulating agents. Sci Rep 15, 8356. https://doi.org/10.1038/s41598-025-92828-6
22. Shahid M, Dumat C, Pourrut B, Nadeem M, Aslam M, and Pinelli E (2014). Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Environmental Science and Pollution Research 21(6): 15132-15150 https://doi.org/10.1007/s11356-014-2731-5
23. Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N. K., Dumat, C., & Rashid, M. I. (2017). Chromium speciation, bioavailability, uptake, toxicity, and detoxification in soil-plant system: A review. Chemosphere, 178, 513–533. https://doi.org/10.1016/j.chemosphere.2017.03.074
24. Shanker AK, Cervantes C, Loza-Tavera H, &Avudainayagam S (2005). Chromium toxicity in plants. Environment International 31(5): 739–753 https://doi.org/10.1016/j.envint.2005.02.003
25. Singh D, Sharma NL, Singh CK, Sarkar SK, Singh I, Dotaniya ML (2020). Effect of chromium (VI) toxicity on morpho-physiological characteristics, yield, and yield components of two chickpea (Cicer arietinum L.) varieties. PLoS ONE 15(12): e0243032. https://doi.org/10.1371/journal. pone.0243032
26. Srivastava D, Tiwari M, Dutta P, Singh P, Puja S, Chawda K, Kumari MK, & Chakrabarty D (2021). Chromium stress in plants: toxicity, tolerance and phytoremediation. Sustainability 13:4629. https://doi.org/10.3390/su13094629
27. Szabados L, &Savouré A (2010). Proline: A multifunctional amino acid. Trends in Plant Science 15(2): 89–97 https://doi.org/10.1016/j.tplants.2009.11.009
28. Yoshida S, Forno DA, Cock JH & Gomez KA (1972). Laboratory manual for physiological studies of rice. International Rice Research Institute, Los Baños, Philippines https://doi.org/10.22004/ag.econ.92011
29. Zayed AM & Terry N (2003). Chromium in the environment: factors affecting biological remediation. Plant and Soil 249:139–156. https://doi.org/10.1023/A:1022504826342

30. Zhang FQ, Wang YS, Lou ZP & Dong JD (2007). Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (Kandeliacandel and Bruguiera gymnorrhiza**).** Chemosphere 67:44–50. https://doi.org/10.1016/j.chemosphere.2006.10.007

31. Zulfiqar U, Hussain A, Anwar S, Al-Sadi AM, Khan MA, Zahir ZA, & Saqib M (2023). Role of chelating agents in enhancing chromium accumulation and tolerance in wheat: A step toward sustainable phytoremediation. Environmental Pollution 317: 120754 https://doi.org/10.1016/j.envpol.2022.120754

****