Effect of chromium (VI) on wheat seedlings and the role of chelating agents

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Toxic effect of chromium concentration with and without chelating agents was studied on wheat seedlings grown hydroponically. Graded dry wheat seeds (*Triticum aestivum* L.C.V., UP 262) obtained from Odisha University of Agriculture and Technology, Bhubaneswar were sterilized and germinated under controlled condition at 25°C in darkness for two days. The seedlings were grown in growth chamber with 7 h/16 h light and dark period with a photon flux density of 52 μeM^{-2} S^{-1}. The growth parameters, i.e. root and shoot length, and fresh and dry matter of 5–7-day-old seedlings were determined. Different chromium concentrations (5–100 μM) with chelating agents (EDTA, citric acid, $ZnSO_4$; 50 μM) were used during growth. Biochemical analysis of chlorophyll content was made from 7-day-old primary leaves of seedlings grown in different Cr (VI) concentrations with or without chelating agents using spade meter. Cr (VI) contents in root and shoot were analysed on hydroponically grown 7-day-old seedlings with the help of AAS. The study showed overall growth retardation of wheat seedlings with increase in Cr (VI) concentration. However, lower concentration of Cr (VI) was found to be stimulating chlorophyll biosynthesis in wheat plants.

Keywords: Chelating agents, chlorophyll, chromium, hydroponics, wheat seedlings.

CHROMIUM (Cr) is the seventh most abundant element on Earth and 21st among crustal rocks. Cr constitutes 0.1–0.3 mg kg^{-1} of the Earth's crust. The world production of Cr is in the order of 10^7 tonnes per year and India is the third largest producer of chromites. About 60–70% of the world's Cr production is used in alloys, including stainless steel because of its wear resistance and attractive surface preventing corrosion and 15% is used in chemical industrial processes, mainly leather tanning, pigments, electroplating, wood preservation, textile and aircraft industries. Arsenic (As) and chromium metals are potential pollutants due to their toxic and carcinogenic effects[1]. Their compounds are widely used as pesticides, herbicides and wood preservatives[2] and in tanning of skin and hide, chrome plating, dye, pigments, etc.[3]. Environmental contagion and exposure to As and Cr are a matter of grave concern. Many places in the world are contaminated by these metals. Extensive industrial use has resulted in their accumulation in soils, and further contamination of aquifers has become a serious environmental issue in some parts of the world, including India[4,5].

The anthropogenic sources contribute Cr (VI) to the environment which is referred to as industrial chromium and sources of chromium in soils include direct discharges from industry and indirect atmospheric deposition. Cr (VI) considered as the most toxic form, known to cause cancer and birth defects, usually occurs in the form of oxy anions, chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). In contrast, Cr (III) is in the form of oxides, hydroxides, sulphates or organically bound in soil and aquatic environments. Cr (VI) in the presence of organic matter is reduced to Cr (III); this transformation is faster in acid environments such as acidic soils. However, high levels of Cr (VI) may exceed the reducing capacity of the environment and thus persist as a toxic pollutant. In addition, Cr (III) is also oxidized to Cr (VI) by MnO_2 and molecular O_2 and transformed again to the more toxic form.

High concentrations of chromium exhibit severe chlorosis, necrosis and a host of other growth abnormalities and anatomical disorders in plants[6]. The effects of heavy metal on barley germination[7], Cr (VI) on *Lemna minor* and *Pistia startiotes*[8], peas[9,10], radish[11], *Euglena grasifis*[12], *Myriophyllum spicatum*[13], maize[14], potato[15], wheat[16,17] have been studied. Chromium (III)–iron interaction in Fe-deficient and Fe-sufficient conditions[18], the distribution of Cr (VI) in lowland rice[19], the effect of increasing levels of Cd, Cr or Ni on tomatoes in nutrient solution[20], and reduction of Cr (VI) to Cr (III) by wetland plants[21] have also been studied.

Chromium interferes with several metabolic processes causing toxicity to plants. It leads to a decrease of root growth and biomass, chlorosis, photosynthetic impairment and finally plant death. Thus remediation of As and Cr contaminated soil has become an important environmental issue. Traditional methods like soil washing,

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encapsulation, vitrification, precipitation, ion exchange, flocculation, carbon adsorption, etc. for heavy metal remediation are expensive, laborious and often degrade the environment. As the traditional physio-chemical methods to clean up are often expensive, difficult and inefficient, current research in biotechnology includes investigation that has less detrimental effects on soil structure and fertility with great efficiency. One such technique that utilizes plants to facilitate reclamation is called phytoremediation. It is an emerging clean-up technology that uses the ability of a plant to accumulate and remove a variety of metals and chemical elements and transport them from the substrate to above-ground parts[22–28]. It is an eco-friendly and sustainable alternative, cheap and viable on small as well as commercial scale. This approach includes overall biological, chemical and physical process that enables uptake, sequestration, degradation and metabolization of contaminants by plants[29,30]. It offers an attractive, non-interactive, effective, aesthetically pleasing, socially accepted and economically viable method. Phytoextraction[29,31] is one of the different approaches of phytoremediation which could be used to absorb heavy metals from the environment and accumulate in plant biomass[29,32]. As the plants absorb, concentrate and precipitate toxic metals from the contaminated soils and accumulate in the biomass, phytoextraction is best suited for remediation of diffusely polluted areas, where pollutants occur only at relatively low concentration and not in greater depth of the soil[33]. Discovery of hyper accumulator plant species which have the unusual ability of accumulating metals such as As, Cr, Zn, Ni and Cu to very high concentrations[34,35] has further given a boost to this technology. This approach has arisen because plants have a remarkable ability to extract, concentrate and metabolize materials from air, water and soil. Baker[36] proposed that plants respond to soil contaminants in three ways; they can act as contaminant accumulators, indicators, or excluders, based on the way they take up and translocate constituents above the ground biomass. Accumulators are those plants that survive despite contaminants concentrating in their aerial tissues. Indicator plants have a mechanism that controls the translocation of contaminants from the root to the shoots. Excluders restrict contaminant uptake to the biomass. Among the known arsenic hyper accumulators, *Pteris vittata* (brake fern) is one of the most efficient and extensively studied plants[37–39]. Arsenic and chromium hyper accumulation by an ecotype of *P. vittata* was reported for phytoextraction prospective from contaminated water and soil[40]. The phytotoxic effect of chromium was reported several decades ago[41]. Uptake of chromium by plants results in reduced rate of growth, damage to cell walls and membranes and changes in metabolic status of plants[16].

Most applications to date have focused on the remediation of nutrients, trace metals and organics. Phytoremediation is emerging as an attractive method due to its simplicity, relatively low cost and *in situ* 'green clean approach'. Remediation of inorganic contaminants involves either physical removal or conversion into biologically inert forms[42]. To optimize phytoremediation, chelating agents are often used[24,43].

As there is notable dearth of information in the literature pertaining to this accumulation of chromium in plants, it requires substantial investigation. The current study was designed to compare the influences of several chelating agents like EDTA, citric acid and zinc on chromium accumulation and their effects on certain physiological and biochemical parameters in wheat seedlings.

Materials and methods

Plant material

Graded dry seeds of wheat (*Triticum aestivum* L. CV. UP 262) were obtained from Odisha University of Agriculture and Technology, Bhubaneswar and stored in a dark and cool place for experimental use. Uniform seeds were selected and surface-sterilized by soaking in 0.1% $HgCl_2$ for 5 min and then washed several times with tap water followed by distilled water. The surface of petri plates over-saturated with cotton pads was sterilized and used for germination. Thirty millilitres of either distilled water (control) or solutions of potassium dichromate ($K_2Cr_2O_7$) containing specific concentrations of chromium (VI) were poured into each petri plate. The seeds were germinated under controlled condition at 25°C in darkness for two days and emergence of 2 mm primary root was used as the operational definition at germination.

After two days of germination, the seeds were transferred to well-aerated Hoogland's nutrient solutions (full strength) placed in glass culture vessels. The seedlings were grown in growth chamber with 7 h/16 h light and dark period. White light was provided by filtered cool white fluorescence tubes (36W Philips TLD) with a photon flux density of 52 μ eM^{-2} S^{-1} (PAR).

The growth parameters study

The growth parameters like root length, shoot length, fresh and dry matter content of 5–7-day-old seedlings were used for study, following the standard procedure. Different Cr (VI) concentrations (5–100 μM) with chelating agents (EDTA, citric acid (CA), $ZnSO_4$) or without chelating agent were used during the growth parameter study. Fresh and dry matter content of both control and treated samples was measured using electronic balance (Shimadzu Corporation). The dry matter of the seedlings was measured keeping the seedlings in an oven at 80°C for a period of three days or more till constant dry weights were determined.

Nutrient culture experiment

The seedlings were grown in different Cr (VI) concentrations (10 and 100 μM) with or without chelating agents. A control pot was also run side by side with the respective treatments. The pH of nutrient solution was adjusted to 6.8 with the help of a pH meter (Hanna, digital pH meter). For chromium bioavailability study, the seedlings were grown in different concentrations of Cr (VI) (10 and 100 μM) separately and with chelating agents (Cr^{+6}–EDTA, Cr^{+6}–CA, Cr^{+6}–Zn^{2+}) 50 μM. After five days of treatment, chromium bioavailability on seedlings was measured using atomic absorption spectrometer. The growth parameters and chlorophyll content of seedlings were determined.

Analysis of chlorophyll content

Biochemical analysis of chlorophyll-*a* and chlorophyll-*b* was done for both control and treated seedlings grown in different toxic concentrations of Cr (VI) (10–100 μM) and with chelate-assisted Cr(VI) for 7-day-old primary leaves of wheat. Chlorophyll content was calculated with the help of spade-meter and using acetone as solvent[44].

Analysis of total chromium content

Hydroponically grown 5-day-old wheat seedlings (control and treated) were oven-dried at 80°C for five days and ground to fine powder. Nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio 10 : 1 were added and volume was made up to 30 ml by adding distilled water to the weighed and ground plant powder samples (roots and shoots separately) and kept for 24 h. Then the acid-mixed plant samples were digested until a clear solution was obtained followed by their filtration and the final volume was made up to 25 ml. The total chromium content in roots and shoots was analysed with the help of a Perkin–Elmer atomic absorption spectrophotometer using the 7000A EPA-method[45]. To ensure precision and accuracy of Cr analyses, at the end a known concentration standard was included.

Statistical analysis

All the experiments were done in triplicate and the data presented in the figures are the means of three independent experiments. The data were analysed statistically and standard errors of mean were calculated.

Results

Treatment with different chromium (VI) concentrations (5–50 μM) along with presence or absence of chelating agents (EDTA, CA, Zn^{2+}) showed marked changes in the different growth parameters of 7-day-old wheat seedlings (Table 1). The root length of wheat seedling grown under different conditions of chromium treatment revealed increase with increase in growth period of the seedlings and the same decreased markedly with increase in Cr (VI) concentration, i.e. seedlings treated with Cr^{+6} (100 μM) had smaller roots than those treated with Cr^{+6} (10 μM). The root length of control was maximum in comparison to all other Cr (VI)-treated seedlings. Among different conditions of treatment, the seedlings treated with Cr^{+6}–CA (50 μM) had maximum root length. A marked decrease in root length was also observed in the seedlings treated with Cr^{+6}+Zn^{2+} (50 μM) compared to those treated with other chelated agents. The order of increase in root length is as follows

$$Cr^{+6}\ (100\ \mu M) < Cr^{+6}\text{–}Zn^{2+}\ (50\ \mu M) < Cr^{+6}\text{–EDTA}\ (50\ \mu M) < Cr^{+6}\text{–CA}\ (50\ \mu M) < \text{control} < Cr^{+6}\ (10\ \mu M) < Cr^{+6}\ (5\ \mu M).$$

The shoot length of wheat seedlings grown under different conditions showed an increase with extended growth period. The shoot length of control seedlings was higher compared to other conditions and decreased with increase in Cr^{+6} concentration, i.e. Cr^{+6} (100 μM) treated seedlings showed minimum growth (Table 1). But seedlings treated with Cr^{+6}–EDTA (50 μM) had maximum shoot length and Cr^{+6}–CA (50 μM) had minimum shoot length. The order of increase in shoot length under different treatments is as follows

$$Cr^{+6}\text{–CA}\ (50\ \mu M) < Cr^{+6}\text{–}Zn^{2+}\ (50\ \mu M) < Cr^{+6}\text{–EDTA}\ (50\ \mu M) < Cr^{+6}\ (100\ \mu M) < Cr^{+6}\ (10\ \mu M) < Cr^{+6}\ (5\ \mu M) < Cr^{+6}\ (\text{control}).$$

Wheat seedlings grown under nutrient conditions revealed increase in root fresh matter with increase in the growth period. The root fresh weight gradually decreased with increase in Cr(VI) concentration (Table 1). A marked increase in root fresh weight was observed in the seedlings treated with Cr^{+6}–EDTA (50 μM) compared to other treatments. The order of increase in root fresh weight is as follows

$$Cr^{+6}\text{–}Zn^{2+} < Cr^{+6}\text{–CA}\ (50\ \mu M) < Cr^{+6}\text{–EDTA}\ (50\ \mu M) < Cr^{+6}\ (100\ \mu M) < Cr^{+6}\ (10\ \mu M) < Cr^{+6}\ (5\ \mu M) < Cr^{+6}\ (\text{control}).$$

Wheat seedlings grown under hydroponic condition showed increase in shoot fresh weight with increase in the growth period. The seedlings under control had the maximum value and the shoot fresh weight gradually decreased with increase in Cr^{+6} concentration. Among the seedlings treated with chelating agents, Cr^{+6}–EDTA (50 μM) had maximum shoot fresh weight and Cr^{+6}–CA

Table 1. Effect of Cr (VI) treatments on shoot length, root length, fresh weight and dry weight of 7-day-old wheat seedlings grown in nutrient solution

Growth parameter	Control	Cr (VI) treatment			Cr (VI): chelated agents (μm)		
		5 μM	10 μM	100 μM	Cr^6 : EDTA (50 μM)	Cr^6 : CA (50 μM)	Cr^6 : Zn^{2+} (50 μM)
Root length (cm)	10.79 ± 0.017	13.46 ± 0.011	13.24 ± 0.011	3.53 ± 0.017	4.44 ± 0.011	5.18 ± 0.034	4.16 ± 0.020
Shoot length (cm)	12.07 ± 1.193	11.85 ± 0.012	10.53 ± 0.021	6.7 ± 0.020	4.45 ± 1.154	2.21 ± 0.241	3.52 ± 0.017
Root fresh wt (mg)	0.693 ± 0.577	0.605 ± 1.452	0.575 ± 1.154	0.183 ± 0.831	0.046 ± 0.377	0.027 ± 0.681	0.022 ± 1.156
Shoot fresh wt (mg)	1.235 ± 1.452	1.204 ± 1.732	0.876 ± 2.08	0.439 ± 0.881	0.336 ± 1.154	0.117 ± 2.157	0.280 ± 0.426
Root dry wt (mg)	0.530 ± 1.452	0.054 ± 1.154	0.053 ± 0.881	0.028 ± 0.881	0.392 ± 1.166	0.313 ± 1.732	0.266 ± 0.881
Shoot dry wt (mg)	0.161 ± 0.577	0.160 ± 2.031	0.130 ± 1.150	0.082 ± 1.201	0.301 ± 0.868	0.303 ± 1.666	0.360 ± 0.440

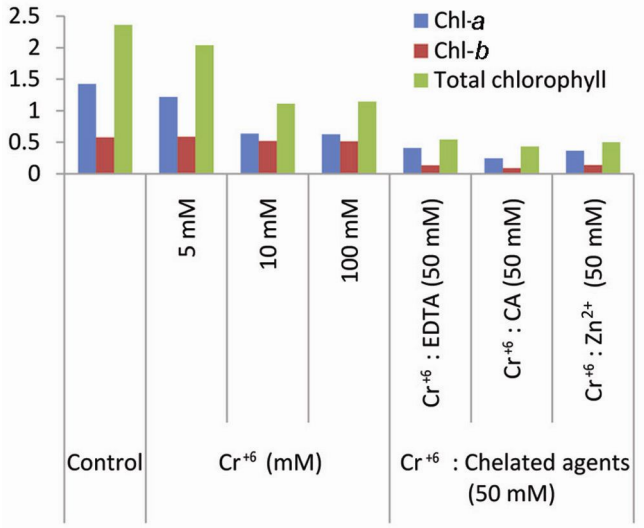

Figure 1. Effect of Cr^{+6} treatments on chlorophyll content (mg g^{-1} dry wt) of 7-day-old wheat seedlings grown in hydroponic condition.

(μM) had minimum value. The order of increase in fresh shoot weight is as follows

Cr^{+6}–CA (50 μM) < Cr^{+6}–Zn^{2+} (50 μM) < Cr^{+6}–EDTA (50 μM) < Cr^{+6} (100 μM) < Cr^{+6} (10 μM) < Cr^{+6} (5 μM) < Cr^{+6} (control).

The changes in root dry weight of wheat seedlings in growth under hydroponic condition were found to be highest when grown under control condition. The order of increase in root dry weight is as follows

Cr^{+6} (100 μM) < Cr^{+6} (50 μM) < Cr^{+6} (5 μM) < Cr^{+6}–Zn^{2+} (5 μM) < Cr^{+6}–CA (50 μM) < Cr^{+6}–EDTA (50 μM) < Cr^{+6} (control).

Wheat seedlings grown under nutrient conditions showed a decrease in shoot dry weight with increase in Cr^{+6} concentration. The shoot dry weight of the seedling was highest under supplemented Cr^{+6}–Zn^{2+} (50 μM). The order of increase in shoot dry weight is as follows

Cr^{+6} (100 μM) < Cr^{+6} (10 μM) < Cr^{+6} (5 μM) < Cr^{+6} control < Cr^{+6}–EDTA (50 μM) < Cr^{+6}–CA (50 μM) < Cr^{+6}–Zn^{2+} (50 μM).

Treatment with different chromium (VI) concentrations (10 μM, 100 μM) in the presence or absence of chelating agents (EDTA, CA, Zn^{2+}) showed marked changes in the chlorophyll content of 7-day-old wheat seedlings grown in hydroponic conditions (Figure 1).

A marked increase was found in chlorophyll *a* content of the seedlings grown in controlled condition with increase in growth period. But with increase in Cr (VI) concentration, Chl *a* content decreased. But among the seedlings treated with chelating agents (EDTA/CA/Zn^{2+}), the Chl *a* content was maximum in those treated with Cr^{+6}–EDTA (50 μM). Also there was a marked decrease in the Chl *b* content of the seedlings treated with Cr^{+6} (50 μM). The order of Chl *b* content in the seedlings treated with chelating agents is as follows

Cr^{+6}–Zn^{2+} (50 μM) < Cr^{+6}–EDTA (50 μM) < Cr^{+6}–CA (50 μM).

Appreciable increase in chlorophyll content was found for the seedlings treated with EDTA solution and the total chlorophyll content decreased with increase in Cr (VI) concentration. The order of total chlorophyll content among the seedlings treated with chelating agents is as follows

Cr^{+6}–EDTA (50 μM) < Cr^{+6}–Zn^{2+} (50 μM) < Cr^{+6}–CA (50 μM).

The pH value of normal solution varied with different concentrations with and without chelating agents (Figure 2). The order of decreasing pH value of different solutions is as follows

Cr^{+6}–Zn^{2+} (50 μM) > Cr^{+6} (50 μM) > Cr^{+6} (5 μM) > Cr^{+6} (control) > Cr^{+6}–EDTA (50 μM) > Cr^{+6}–CA (50 μM).

After 7 days of seedling growth, the pH varied with different concentrations of the solution. The order of decrease in pH value is as follows

Cr^{+6} (5 µM) > Cr^{+6}–EDTA (50 µM) > Cr^{+6} (control) > Cr^{+6} (50 µM) > Cr^{+6}–Zn^{2+} (50 µM) > Cr^{+6}–CA (50 µM).

Distribution of chromium in the plant cells and cellular levels varied with the chemical form added to the nutrient medium. Irrigated wheat plants were cultivated in Hoagland nutrient solution. The stable chromium levels used were determined in a preliminary experiment in order to avoid any toxicity symptoms in wheat plants. The chromium content in the nutrient solution after 7 days of seedling growth varied remarkably. In the Cr^{+6}–CA solution, the bioavailability of Cr was found to be maximum and in Cr^{+6} (5 µM) solution, Cr– was found to be minimum (Figure 3). The order of Cr bioavailability is as follows

Cr^{+6}–CA (50 µM) > Cr^{+6}–EDTA (50 µM) > Cr^{+6}–Zn^{2+} (50 µM) > Cr^{+6} (50 µM) > Cr^{+6} control > Cr^{+6} (5 µM).

The chromium content in roots of the seedlings increased with increase in Cr^{+6} concentration. Its availability in the roots treated with Cr^{+6} and chelating agents was found to be in the following order

Cr^{+6} (50 µM) > Cr^{+6}–EDTA (50 µM) > Cr^{+6}–Zn^{2+} (50 µM) > Cr^{+6}–CA (50 µM).

The chromium content increased with increasing Cr^{+6} concentration and its availability in shoots treated with Cr^{+6} and chelating agents was found to be in the following order (Figure 4)

Cr^{+6}–EDTA (50 µM) > Cr^{+6} (50 µM) > Cr^{+6}–Zn^{2+} (50 µM) > Cr^{+6}–CA (50 µM).

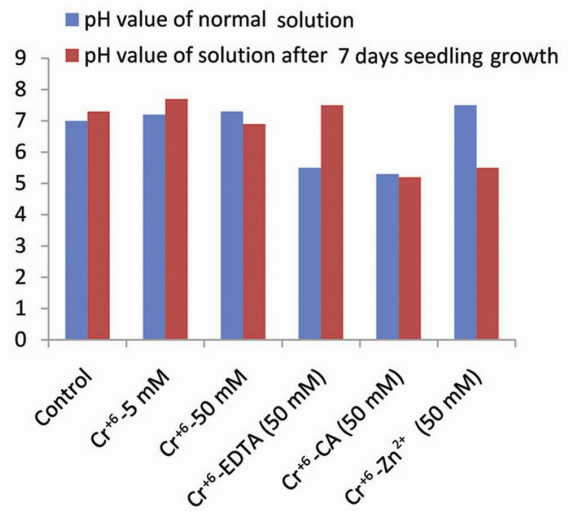

Figure 2. Effect of pH of solution before and after 7 days of seedling growth.

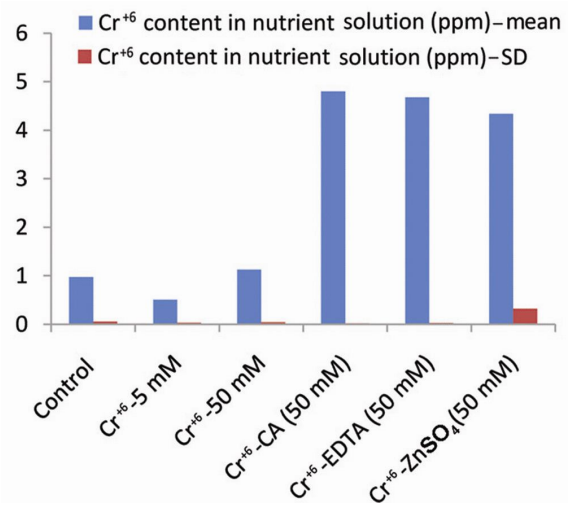

Figure 3. Effect of Cr^{+6} treatment on chromium content (ppm) in nutrient solution after 7 days of growth of wheat seedlings.

Discussion

The effects of the interaction of Cr (VI) with chelating agents on the changes of growth parameters (root length, shoot length, fresh and dry weight) and biochemical lesions revealed that wheat seedlings exhibited growth retardation at increasing chromium concentrations, i.e. at 100 µM (Table 1), but Cr at 5 and 10 µM concentration showed favourable growth compared to controlled treatments. Chromium in association with EDTA at 1 : 1 ratio

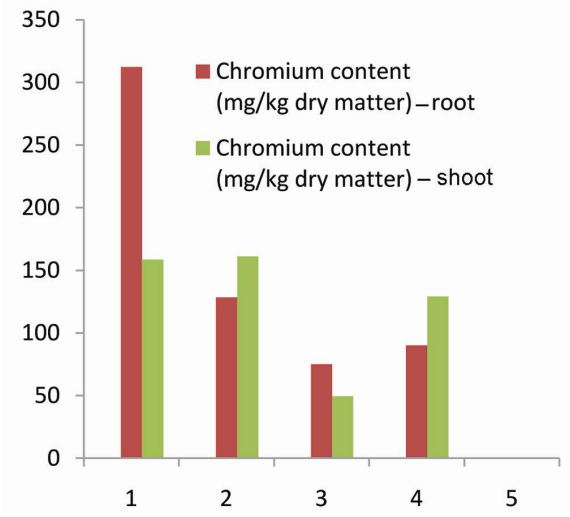

Figure 4. Effect of Cr^{+6} and chelating agents on chromium bioavailability (mg/kg dry matter) in roots and shoots of wheat seedlings grown in nutrient culture.

and 50 μM concentration showed high growth rate than all the treatments (Table 1). The present study shows the toxic effect of hexavalent chromium and its uptake in the presence of different chelating agents like EDTA, CA and Zn^{2+}. Chelators are substances that render insoluble cations soluble and thus become available to plants[46,47]. Low toxicity, multidentate chelating agents such as EDTA are used to enhance the bioavailability of heavy metals for plant uptake[48]. The enhancement of Cr uptake by different chelators differs from each other. It has been observed that chelating agents increase the toxic effect of Cr(VI) to a greater extent, which is marked by the stunted growth of the seedlings and subsequent chlorophyll content estimation. Among the three types of chelated chromium compounds, Cr(VI)–CA (50 μM) showed lowest growth rate and determination of Cr content in root and shoots revealed that maximum uptake of Cr(VI) was due to CA chelation. Further Cr bioavailability in roots and shoots using chelated Cr^{+6} compounds may be useful in the detoxification, phytoextraction and phytoremediation processes[24,40–43].

Among six types of treatments studied, lower Cr concentrations were found to be stimulating chlorophyll biosynthesis in plants. It has been reported that over 90% of the supplied chromium is incorporated in roots in barley and wheat plants with poor translocation to aerial parts[49]. This is reflected in the present study as well. The study also reveals a higher level of chromium bioaccumulation in plants treated with high Cr (VI) concentration and presence of chelators. But this translocation of chromium to the aerial parts (shoots) decreased compared to the roots.

Overall the study showed that chelated chromium compounds have effective role in growth hindrance and ameliorating the toxicity effect of chromium. It has also been demonstrated that among the six types of treatment, control showed high chlorophyll content and at higher concentration of Cr (i.e. at 100 μM), chlorophyll concentration was low. This is due to the fact that the seedlings exhibited stunted growth for which unusual metabolism was noticed. The amelioration of chromium toxicity has been proved from the Cr content estimation in roots and shoots and measurement of total chlorophyll. Therefore, the role of heavy metal chelators, viz. EDTA, CA, Zn^{2+} in enhancing chromium accumulation proves useful in the phytoremediation and phytoextraction processes in the region where Cr content in the soil is high.

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