

Research Article

Comparative study of phytoremediation of chromium contaminated soil by *Amaranthus viridis* in the presence of different chelating agents

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Abstract

Chromium is a harmful heavy metal to the environment due to the toxicity induced by it to plants and other living organisms. High concentration of Cr in soil poses severe toxicological problems ecosystem. Phytoremediation using different plants is an economical and environment-friendly method for removing Cr from soil. The addition of chelating agents augments the phytoex-traction using plants. The present study aimed to augment the Cr phytoremediation capacity of *Amaranthus virdis*, a predominant plant species in the Cr-contaminated open dumpsites of Bangalore. Phytoextraction of Cr by *Amaranthus virdis* was studied in the presence of different chelating agents viz. ethylenediaminetetraacetic acid (EDTA), citric acid (CA), growth promoting hormone- indoleacetic acid (IAA) and NPK fertiliser. *A. viridis* grown under different concentrations (5, 10 and 20 mg/Kg) of Cr were treated with 0.5g EDTA/Kg of soil, 0.5g CA/Kg of soil, 1mg IAA/Kg of soil and NPK (125 mg of nitrogen, 45 mg of phosphorous and 156 mg of potassium per Kg of soil). Results indicated that CA, at 10 mg/kg Cr supply, induced the highest uptake (up to 29.25 µg/plant). Furthermore, the study revealed that CA amendment induced maximum Cr uptake in *A. viridis* at all levels of Cr supply as compared to other amendments. This was due to the increased solubility of Cr in the presence of citric acid and the amelioration of oxidative stress due to Cr to plants by citric acid. This study inferred that the non-hyperaccumulating plant, *A. viridis* could be used as a phytoremediator for Cr in the presence of citric acid in the places where it is grown abundantly.

Keywords: Amaranthus viridis, Chelating agents, Chromium, Phytoremediation

INTRODUCTION

Soil contamination by heavy metals is a concern around the world. Heavy metals in soil gain attention because of their toxicological importance in the environment, agriculture and human health (Alloway, 1990). Metals like Mn, Fe, Cu, Ni, Zn and Co are required for the normal metabolic processes of living organisms, but when these are present beyond the permissible limit, it becomes harmful. Changes in physiological and biochemical processes due to heavy metal pollution in the soil cause a reduction in growth, which has been recorded and expressed in various studies. Heavy metal contamination has dire ecological consequences and it affects agricultural yield, soil fertility and soil microorganisms. Chromium (Cr) occurs in earth's crust in a trivalent state (Cr^{3+}), but hexavalent (Cr^{6+}) found in dumpsites are pollutants from man-made products

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(Feng et al., 2019). Chrome tanning used in leather making is considered the major source of Cr contamination in dumpsites (Abebaw and Abate, 2018). Apart from tanning, Cr contamination occurs in the paint industry, wood preservation, metal and household waste (Dotania et al., , 2014). Chromium compounds are highly toxic to most higher plants at 0.1 µg/g. Chromium toxicity in plants inhibits enzyme activity, seed germination, root growth, photosynthesis and photophosphorylation (Sayantan and Shardendu, 2013; Sharma et al., 2020). Phytoremediation is a safe eco-technology that is also an economical way of removal of contaminants from the environment. Phytoremediation is one of the best-emerging technologies for metal remediation from contaminated soils as it is cost-effective and ecofriendly (Wiszniewska et al., 2016). Moreover, such treatment causes minimal disturbance to the remediated soil, which can be used for agricultural practices, as the source of nutrients (Farraji et al., 2016). Phytoremediation of heavy metals is majorly of two types - natural and induced phytoremediation. In the natural mode of phytoremediation, plants remove heavy metals under natural conditions without any amendment to the soil, whereas in induced phytoremediation the bioavailability of heavy metals is increased by using chelating agents (Menhas et al., 2022). When chelates are applied to the soil, it helps in the dissolution of the insoluble metal compounds and forms water-soluble metal-chelate complexes, which the plants subsequently take up. Some of the chelating agents widely used include synthetic chelators like EDTA (ethylene diamine tetra acetic acid), ammonium sulphate, EDDS (SS-ethylene diamine disuccinic acid), elemental sulphur, indole acetic acid (IAA) and organic chelators like citric acid, malic acid and tartaric acid (Yang et al., 2019). So many reports exist on the phytoremediation of Cr-contaminated soil using different plants (Riaz et al., 2019; Samarana et al., 2020; Basit et al., 2022; Manar et al., 2022;). Different species of Amaranthaceae family were used for phytoremediation of chromium (Bashri et al., 2016; Wang et al., 2019). Variuos authors have studied the phytoremediation capacity of Amaranthus virdis for chromium. Ramanlal et al., (2020) assessed the potential of Cr uptake capacity of Amaranthus viridis grown in soil irrigated with paint industry effluent. Comparison study of Cr phytoextraction of A. Virdis with Alternanthera philoxeroides, Euphorbia hirta, Solanum nigrum and Portulaca oleracea revealed that A. virdis was more tolerant towards Cr toxicity (Pal 2020). Fast growing, deep rooted, easily propagated and hyperaccumulating plants are used for phytoremediation. A.virdis exhibits first three characteristics making it a good candidate for Cr accumulation (Zhou et al., 2007; Liu et al., 2008). Since it is not a hyperacuumulator for Cr, a study was conducted to select a chelating agent that increases the Cr uptake capacity of A. virdis. Further a previous study by authors revealed the prevalence of A. virdis in the dumpsites of Bangalore city, Karnataka, India. The present study is a comparative analysis of the Cr phytoremediation enhancement potential of synthetic and non-synthetic chelators like NPK, indole acetic acid (IAA), ethylene di-amine tetra acetic acid (EDTA) and citric acid (CA). There was no study reported on the use of Amaranthus viridis for the phytoremediation of Cr-contaminated soil in conjugation with chelating agents. This plant, belonging to the family of Amaranthaceae, was chosen for the present study due to its high biomass, extensive root system, short lifecycle and tolerance to heavy metals (Zhou et al., 2007; Liu et al., 2008). The present work aimed to determine the effect of a synthetic chelating agent (EDTA), natural chelating agent (citric acid), NPK fertiliser and growth-promoting hormone (IAA) on the phytoremediation of soils contaminated with Cr using the plant A. viridis.

MATERIALS AND METHODS

Plant description

Amaranthus viridis, commonly known as slender amaranth or green amaranth, is an annual herb belonging to the botanical family Amaranthaceae. It has a green stem that grows about 60-80 cm in height and produces many branches from the base. The ovate-shaped leaves 19 alternate, 3-6 cm long and 2-4 cm wide, with a long petiole of about 5 cm. The inflorescence is a terminal panicle with a few branches and small green flowers. Flowers are unisexual with lanceolate-ovate bracts, which are membranous with a short awn. Perianth consists of 3 segments about 1.5mm long. Male flower consists of 3 stamens. Female flowers consist of bicarpellary or tricarpellary gynoecium, unilocular with three stigmas and one erect ovule. Fruits are dry, indehiscent, endospermic and one-seeded.

Preparation of contaminated soil

Red soil received from Lalbaugh botanical garden, Bangalore, was sieved to remove the bulky lumps and stones, which would have prevented the uniform mixing of the metal solution. Each pot used for the study was of size 7cm diameter and 15cm depth. 4 kg of the soil was taken in each pot. 0.5 mL of 20 mg/mL Cr solution was added to the Hoagland solution to make the total volume 500 mL. This solution was added to a pot containing 4 kg of soil to get 1 mg/Kg Cr contaminated soil. Likewise, 1 mL, 2 mL and 4 mL of 20 mg/mL of cr solution was mixed with Hoagland solution and was used to make 5, 10 and 20 mg/Kg cr contaminated soil.

The composition of Hoagland solution included 2mmol/ L calcium nitrate $[Ca(NO_3)_2]$, 0.75mmol/L potassium sulphate (K₂SO₄), 0.1mmol/L potassium chloride (KCl), 0.25mmol/L potassium dihydrogen phosphate (KH₂PO₄), 0.65mmol/L Magnesium sulphate (MgSO₄), 0.1mmol/L ferric EDTA solution (EDTA-Fe), 0.01mmol/ L boric acid (H₃BO₃), 0.001mmol/L manganese sulphate (MnSO₄), 0.001mmol/L zinc sulphate (ZnSO₄), 0.0001mmol/L copper sulphate (CuSO₄) and 0.000005 mmol/L ammonium molybdate [(NH₄)Mo₇O₂₄)].

Preparation of mother culture

The soil was filled in the coco peat trays; five to six seeds of *A. viridis* were sown in each coco peat. The trays were kept at a suitable place in the greenhouse, exposed to 70-80% of sunlight. The trays were watered daily with Hoagland solution. After 3-4 days, the seeds germinated into small plantlets. Photograph of the Mother culture of *A. viridis* seedlings is given in Fig. 1.

Experimental set-up

The experimental set-up for metal uptake study in the presence of amendments by *A. viridis* is given in Figs-2. Controls for this experiment set-up were plants grown in only Cr amendment soil without chelators and plants grown in soils amended with chelators without Cr. The metal solution was allowed to settle in each pot for a day. The next day, 11-day-old plant seedlings were taken from the mother culture and planted in the pots. Four plants were grown in each pot. After about 15 days, the chelators, such as NPK, EDTA, CA and IAA solution, were added to each pot. The plants were watered with Hoagland solution every 3-4 days. Morphological characteristics of plants were observed in regular intervals.

The amendment solutions were prepared as follows: EDTA solution, 10% (w/v): 10g sodium salt of EDTA was dissolved in 100 mL of distilled water. 20 mL of this

solution was diluted to 100 mL with Hoagland solution and added into each pot to get an amendment concentration of 0.5g EDTA/Kg of soil.

Citric acid solution, 10% (w/v): 10g citric acid was dissolved in 100 mL of distilled water. 20 mL of this solution was diluted to 100 mL with Hoagland solution and added into each pot to get an amendment concentration of 0.5g citric acid/Kg of soil.

NPK solution: NPK stock solution was prepared by dissolving 14.3 g of ammonium nitrate and 7.89 g of potassium dihydrogen phosphate and 7.65g of potassium chloride into 100 mL solution. 10 mL of this solution was diluted to 100 mL with Hoagland solution used to water each pot to get an amendment which is equivalent to 125 mg of nitrogen, 45 mg of phosphorous and 156 mg of potassium per Kg of soil. Indole acetic acid solution (IAA), 0.1% (w/v): 0.1g of indole acetic acid was dissolved in 100 mL of 1:1 mixture of ethanol and distilled water. 4 mL of this solution was diluted to 100 mL with Hoagland solution and used to water each pot equivalent to 1mg/ Kg of the amendment.

Harvesting of plants

Plants of *A. viridis* were harvested after growing for two months. Roots were initially washed gently under running tap water to remove loosely adhered sand particles, followed by rinsing with 3% HCl for leaching out of minerals adsorbed on the surface of roots. The shoots and acid-rinsed roots were washed at least three times with distilled water.

The plant growth parameters such as stem length (cm), root length (cm) and number of leaves per plant were recorded. After harvesting, fresh weight of leaves, stem and roots were measured, then samples were kept in



Fig. 1. Mother culture of Amaranthus viridis seedlings



Fig. 2. Experimental set-up for metal uptake study in the presence of amendments by Amaranthus viridis (The first row of the above set-up represents the control concerning the chelator ammendment, while the first column represents the control concerning the Cr supply)

aluminum foil, labelled and oven dried at 100°C for three to four days and dry weights were measured until constant weight was obtained. Dry weight (DW) biomass (in grams) per plant was determined using an electronic balance.

Digestion of plant material

For analysis of Cr, dried stem, leaves and root samples were ground using mortar and pestle. The entire amount of ground sample was weighed and transferred into a conical flask. Samples were digested with 5 mL of Conc.HNO₃ and 1mL of 40 % (v/v) H_2O_2 . The contents of the flasks were evaporated to incipient dryness. The process was repeated three times. The residue was dissolved in 10 ml of 3% HNO₃.

Estimation of Cr plant material by ICP-AES

The concentration of Cr in the solution prepared from plant residues was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; JY Horiba 2000 France) after calibrating the instrument with respective standards. Wavelength selected for analysis is 205.552 nm.

Statistical analysis

All the values presented in the bar graphs are means of three replicates \pm standard deviation. Student's t-test was performed to determine the significance of the difference between the control and the test values. The statistical analysis was performed by using Microsoft

Office Excel - 2016.

RESULTS AND DISCUSSION

Effect of Cr uptake in *A. viridis* in the presence of different chelators

The combined effect of Cr supply and the chelators viz., NPK, EDTA, CA and IAA on Cr uptake by A. viridis is represented in Fig. 3. In the soil without any external supply of Cr (control), IAA amendment showed maximum uptake (6.1µg/plant) followed by CA (4µg/plant), NPK (3.2µg/plant) and EDTA (1.5 µg/plant). It is mainly due to residual Cr in the control soil. All chelators, at 1 and 5 mg/kg Cr supply showed an increase in Cr uptake in a dose-dependent manner and further increase in Cr supply resulted in a decrease in the Cr uptake (Fig. 6), except in the case of CA. Compared to control, at 5 mg/kg Cr supply, chelator efficiency in enhancing the Cr uptake was found to be highest for EDTA > NPK > CA > IAA with 13.17 fold (19.7 µg/plant vs 1.5µg/ plant), 6.09 fold (19.5 µg/plant vs 3.2 µg/plant), 5.7 fold (22.5 µg/plant vs 4.0 µg/plant) and 3.11 fold (19.0 µg/ plant vs 6.1 µg/plant) increase, respectively. Even though EDTA (19.8 µg/plant) showed a maximum increase at 5 mg/kg supply, when the amount of Cr uptake was taken into account, it was found that CA, at 10 mg/kg Cr supply, induced the highest uptake (up to 29.25 µg/plant). At 10 mg/kg Cr supply uptake was in the order CA (29.25 µg/plant)> NPK (13.9 µg/plant)> EDTA(13.4 > >IAA (11.1 µg/plant). Furthermore, it was clear from the findings that CA amendment induced maximum Cr uptake in A. viridis at all levels of Cr supply as compared to other amendments. Even at 20 mg/ kg Cr supply CA amendment (10 µg/plant) showed increased uptake compared to EDTA (3.2 µg/plant), NPK (5.2 µg/plant) and IAA (5.1 µg/plant).

Cr uptake by control plants is from the Cr present in the natural soil, which mainly exists in a non-bio-available form (Qureshi et al., 2020). In soil, Cr exists as Cr (III), but uptake of Cr from soil usually takes place as Cr (VI). Cr (III) present in the soil is converted to Cr (VI) by some oxidizing agents present in the soil. Due to their structural resemblance, Cr (VI) is transported through the root via sulphate ion carriers (Srivastava et al., 2021). Cr (III) absorption occurs through cation exchange process. (Sharma et al., 2020). The present study showed that the uptake of Cr from control soil in the presence of IAA and CA was higher than other amendments. This can be attributed to the increasing capacity of these two reagents to form soluble complexes with Cr in the soil. CA, a strong metal chelating agent, can convert insoluble Cr (III) in the soil to soluble Cr citrate complex, which plants can easily absorb. Increased uptake and accumulation of Cr in the presence of citric acid is also due to the formation of soluble and highly mobile Cr citrate complex, which can be easily absorbed by the root (Ehsan *et al.*, 2014). The growth-promoting hormone IAA stimulates the root exudates to produce organic acids, which form complexes with Cr which in turn is absorbed by the plant (Saleh *et al.*, 2020). Low uptake of control in the presence of EDTA may be attributed to its inability to complex Cr (III) present in the soil. This result is in tune with the findings of other studies. (Jean-Soro *et al.*, 2012; Song *et al.*, 2016;). Better uptake shown by the NPK amendment compared to EDTA can be due to increased biomass produced due to the increased nutrition available to the plants.

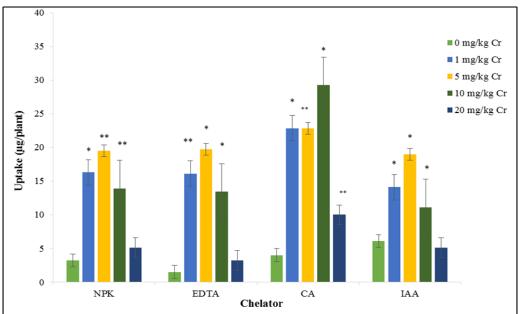
Cr uptake showed an increasing trend in all amendments from 1 mg/Kg to 5 mg/Kg and after that showed a decrease except in CA. In the case of CA amendment, maximum Cr accumulation was observed at 10 mg/Kg. This can be attributed to the capacity of CA to help the plant withstand the toxic stress induced by Cr. Farid et al. (2017), in their studies, pointed at an increase in the production of antioxidant enzymes in the presence of CA. These antioxidant enzymes have an ameliorative effect on Cr stress. Chromium toxicity induces reduced plant growth, morphological changes, and reduction in photosynthesis. These effects can be attributed to the excess production of reactive oxygen species (ROS), which disrupts the redox balance in plants (Anjum et al., 2017). Hence it is inevitable for plants to develop some sort of defensive mechanism to counter this oxidative stress. As a defence mechanism to alleviate the ROS stress, plants trigger the production of antioxidant enzymes like, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase

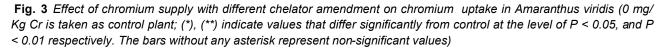
(CAT) (Malik et al., 2022).

Mild Cr stress increases antioxidant activities whereas increased Cr stress decreased the production of antioxidants (Qureshi et al., 2020) Excessive Cr stress reduces the production of these antioxidants leading to the damage of growth and physiological parameter in plants. However, the exogeneous application of CA increased the production of antioxidant enzyme there by reducing the negative impacts of Cr stress on plants (Maqbool et al., 2018). Mallhi et al., 2020 in their study on citric acid assisted Cr phytoremediation using sunflower plants, have inferred that folial application of citric acid improved the plant's morphological characteristics, which might be due to the increased uptake of vital nutrients by plants. In the present study, exogeneous application of citric acid increased the Cr uptake. This might be due to the increased production of antioxidant enzymes which ameliorated the toxic effect of Cr. The findings of the present study are well supported by the previous authors. Even though NPK, EDTA and IAA could enhance Cr uptake, it is seen from the results that only citric acid showed an enhanced uptake at a higher Cr supply (10mg/Kg).

Effect of Cr uptake on total biomass of A. viridis

All amendments except IAA showed a drastic reduction in biomass with an increase in Cr uptake (Fig. 4). Biomass reduction of plants at all concentrations of Cr supply was in the order IAA<NPK<EDTA<CA. Biomass of CA-amended plants was drastically (83%) reduced. In the presence of CA, uptake per unit biomass increased drastically compared to other amendments. This biomass reduction can be attributed to increased





Cr uptake, which in turn caused an increase in toxicity to the plant. This can be correlated to the decrease in Cr uptake by IAA amended plant. However, with almost similar Cr uptake, NPK and EDTA amendment showed an increased reduction in biomass compared to IAA amendment. This can be ascribed to the enhanced plant growth in the presence of IAA in metalcontaminated soils by absorption of nutrients and metals by proliferating plant roots. Effect of Cr uptake on plant height of A. viridis The height of A. viridis grown under Cr stress in soil amended with various chelators is given in Fig. 5. Plant height showed an inverse relationship with Cr supply in soil amended with different chelators. The highest inhibition in plant height was observed in 20 mg/kg Cr supply with all chelators, in the range of 80.4% to 90.1% (p<0.05) reduction with respect to control. In the case of 5 and 10 mg/kg supply of Cr, plant

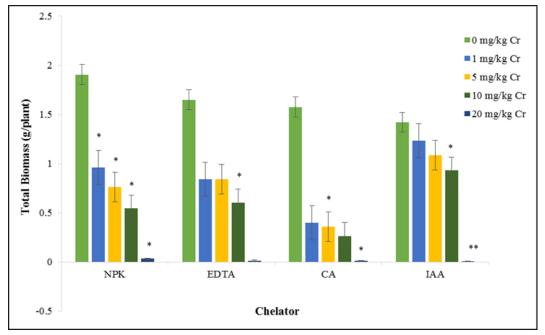


Fig. 4. Effect of chromium supply with different chelator amendment on biomass in Amaranthus viridis (0 mg/Kg Cr is taken as control plant; (*), (**) indicate values that differ significantly from control at the level of P < 0.05, and P < 0.01 respectively. The bars without any asterisk represent non-significant values)

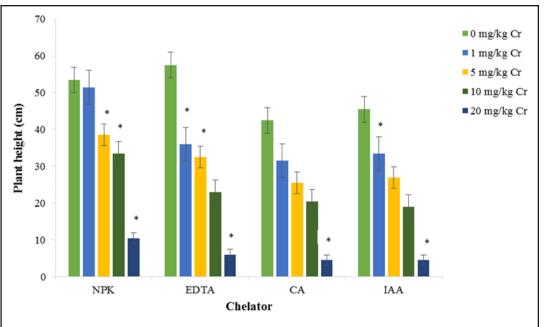


Fig. 5. Effect of chromium supply with different chelator amendments on plant height of Amaranthus viridis (0 mg/Kg Cr is taken as control plant; (*), (**) indicate values that differ significantly from control at the level of P < 0.05, and P < 0.01 respectively. The bars without any asterisk represent non-significant values)

height was reduced from 28% to 60 %, respectively, with respect to the control plant. At 5 mg/kg of Cr supply, reduction in the plant height with respect to chelator amendment was in the order of CA > IAA > EDTA > NPK, proving CA to be the most efficient chelator, helping in maximising the uptake of Cr, in turn, conferring maximum toxicity in the plant.

Effect of Cr uptake on root length of *A. viridis* The combined effect of Cr stress and chelators on the root length of *Amaranthus viridis* is presented in Fig. 6. Root length showed reduction with an increase in Cr supply in a dose-dependent manner in soil amended with different chelators. Maximum detrimental effect of Cr supply and chelator amendment on root length was

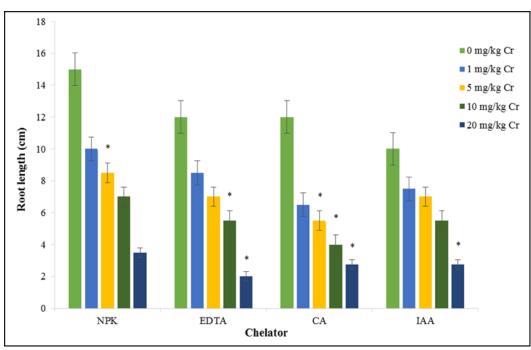


Fig. 6. Effect of chromium supply with different chelator amendment on root length in Amaranthus viridis (0 mg/Kg Cr is taken as control plant; (*), (**) indicate values that differ significantly from control at the level of P < 0.05, and P < 0.01 respectively. The bars without any asterisk represent non-significant values)

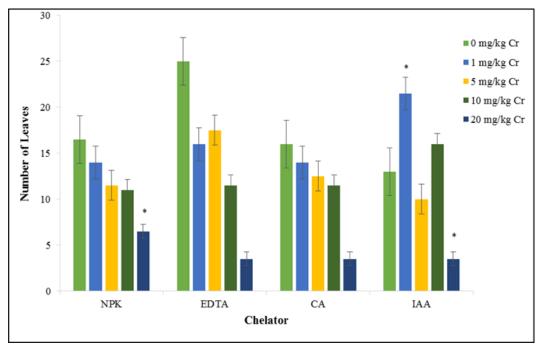


Fig. 7. Effect of chromium supply with different chelator amendment on root length in Amaranthus viridis (0 mg/Kg Cr is taken as control plant; (*), (**) indicate values that differ significantly from control at the level of P < 0.05, and P < 0.01 respectively. The bars without any asterisk represent non-significant values)

at 20 mg/kg supply. At 5 and 10 mg/kg supply, the reduction in root length was in the range of 30% to 66.7%, with CA showing maximum reduction followed by EDTA, NPK and IAA. The reduction in root length in comparison with control was found to be statistically significant at 5% level in 5 mg/Kg amended with NPK, 10 and 20 mg/kg amended with EDTA, 5, 10, 20 mg/Kg amended with CA and 20 mg/Kg amended with IAA.

Effect of Cr uptake on number of leaves of A. viridis Amaranthus viridis showed dose dependent decrease in number of leaves at 1-20 mg/kg Cr in all the chelator amendments (Fig. 7). At 20 mg/kg supply, the highest reduction in number of leaves was observed in EDTA amended soil with 86% reduction and the lowest was reported in NPK amendment with 60.6% reduction. At 5 and 10 mg/kg supply, A. viridis showed inhibition in number of leaves in the range of 21.9% to 54% in the order CA>NPK>EDTA > IAA , in comparison to respective control plants. In comparison with the control, the reduction in number of leaves was found to be statistically significant only at 5% in 20 mg/Kg amended with NPK and 1 and 20 mg/Kg amended with IAA.

All amendments showed a decrease in, biomass, plant height, root length and number of leaves with increase in Cr uptake. Biomass reduction can be ascribed to increased Cr stress, which hampers biomass production, cellular membrane damage and imbalances nutrient uptake due to oxidative stress (Malik *et al.*, 2022). Decreased biomass is also due to the reduced intake of water and other nutrients, which is due to decreased metabolic activities and photosynthesisunder Cr stress (Asgher *et al.*, 2018; Al Mahmud *et al.*, 2019). A decrease in root length was more compared to decrease in shoot length and number of leaves in all amendments. This may be because even in the presence of amendments, Cr accumulates more in the root compared to the above ground parts.

Amin et al. (2019), in their study of Cr accumulation in biofuel plants, have shown that Cr accumulated more in roots compared to aerial parts of the plants. According to them, the high accumulation of Cr in roots could be due to the immobilisation of Cr (VI) in the vacuoles of root cells to render it non-toxic, a natural response of the plant to toxicity. Another important reason for the lack of transport of Cr from roots to shoots could be the lack of a specific mechanism for the transport of Cr, as it is a toxic and non-essential element to plant growth. According to the studies conducted by Cervantes et al. (2001), it was reported that there was a greater degree of Cr accumulation in the roots than shoot in Triticum vulgare when grown in Cr-enriched soil amended with various organic compounds such as oxalic acid, malate and glycerine. The present study is in agreement with the above reports, which signify that Cr gets accumulated mostly in roots, followed by the shoot, in the presence of complexing amendments.

Cr uptake in the presence of CA showed a pronounced increase compared to other amendments. Due to this, toxicity introduced into the plant is also more in the case of CA amendment. But compared to other amendments, CA had a greater capacity for enhancing Cr uptake. Even at a decreased biomass due to the Cr stress, it could help the plant withstand increased Cr uptake for A. viridis due to the ameliorating effects of CA. A. virdis, is a well adapted and highly prevailing plant species in Bangalore dumpsite. It is not a hyperaccumulator of Cr. However, it can be converted into Cr hyperaccumulating species in presence of citric acid. It is always advantageous to select a plant well adopted to environment and enhance its phytoremediating capacity by the addtiotn of chelators than to use a hyperaccumulating plant not adopted to experimental conditions. A. virdis satisfies this condition and citric acid being a natural chemical, its addition to soil will not affect the soil.

Conclusion

The present study demonstrated the Cr accumulation potential of A. virdis with a comparative assessment of the efficiency of different chelators in heavy metal uptake and their toxicity on the plant. This was done to identify the optimum conditions for effective phytoremediation of Cr using A. viridis as a model plant. In Cr uptake studies, citric acid amendment showed a maximum Cr accumulation of 29.25µg/Plant, a 7.31fold increase in uptake. The efficacy of different chelators in enhancing Cr uptake was found to be in the order of IAA<NPK<EDTA<CA. The increased uptake of Cr in the presence of CA by plants can be attributed to the increased solubility of Cr citric acid complex in the soil. The capacity of citric acid to help the plant withstand the toxic stress induced by Cr by increasing the production of antioxidant enzymes which can ameliorate the oxidative stress in A. viridis. Further extension of the study requires the evaluation of the selected plant in phytoremediation of heavy metals in field conditions, with the complex interaction of biotic and abiotic factors affecting plant growth and uptake.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

 Abebaw, G. & Abate, B. (2018). Chrome Tanned Leather Waste Dechroming Optimization for Potential Poultry Feed Additive Source: A Waste to Resources Approach of Feed for Future. *J Environ Pollut Manag.*, 1(1), 16–18.

- Alloway, B.J. (1990). Heavy Metals in Soils, Blackie and Son Ltd., Glasgow, 100-124.
- Al Mahmud, J., Bhuyan, M.H.M.B., Anee, T.I., Nahar, K., Fujita, M., Hasanuzzaman, M. (2019). Reactive Oxygen Species Metabolism and Antioxidant Defense in Plants Under Metal/Metalloid Stress. In: Hasanuzzaman, M., Hakeem, K., Nahar, K., Alharby, H. (eds) Plant Abiotic Stress Tolerance. Springer, Cham. https:// doi.org/10.1007/978-3-030-06118-0 10
- Amin, H., Arain, B. A., Abbasi, M. S., Amin. F, Jahangir. T. J. & Soomro. N. A. (2019). Evaluation of chromium phytotoxicity, phyto-tolerance, and phyto-accumulation using biofuel plants for effective phytoremediation, *Int. J Phytoremediation.*, 21(4), 352-363, https:// doi.org/10.1080/15226514.2018.1524837
- Anjum, S.A., Ashraf, U., Khan, I., Tanveer, M., Shahid, M., Shakoor. A. & Wang, L. (2017). Phyto-toxicity of chromium in maize: Oxidative damage, osmolyte accumulation, anti-oxidative defense and chromium uptake. *Pedosphere.*, 27, 262–273. https://doi.org/10.1016/S1002-0160(17)60315-1
- Asgher, M., Per, T.S., Verma, S. *et al.* (2018). Ethylene Supplementation Increases PSII Efficiency and Alleviates Chromium-Inhibited Photosynthesis Through Increased Nitrogen and Sulfur Assimilation in Mustard. *J Plant Growth Regul.*, **37**, 1300–1317. https://doi.org/10.1007/ s00344-018-9858-z
- Bashri. G., Parihar, P., Singh. R., Singh. S., Singh. V. P. & Prasad. S. M. (2016). Physiological and biochemical characterization of two *Amaranthus* species under Cr(VI) stress differing in Cr(VI) tolerance. *Plant Physiol. Biochem.*, 108, 12-23. https://doi.org/10.1016/ j.plaphy.2016.06.030.
- Basit, F., Bhat, J. K., Dong, Z., Mou, Q., Zhu, X., Wang, Y., et al., (2022). Chromium toxicity induced oxidative damage in two rice cultivars and its mitigation through external supplementation of brassinosteroids and spermine. Chemosphere., 320, https://doi.org/10.1016/ j.chemosphere.2022.134423
- Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., TorresGuzmán, J. C & Moreno-Sánchez, R. (2001). Interactions of chromium with microorganisms and plants. *FEMS Microbiol. Rev.*, 25(3), 335–347. https://doi.org/10.1111/j.1574-6976.2001.tb00581.x
- Dotania, M. L., Thakur, J. K., Meena, V.D., Jajoria, D.K & Gopal.R. (2014). Chromium Pollution -A threat to environment -A review. *Agric. Rev.*, 35(2), 153-157. http:// dx.doi.org/10.5958/0976-0741.2014.00094.4
- Ehsan, S., Ali, S., Noureen, S., Mahmood, K., Farid, M., Ishaque, W. & Rizwan, M. (2014). Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. *Ecotoxicol. Environ.* Saf., 106, 164–172. https:// doi.org/10.1016/j.ecoenv.2014.03.007
- Farid, M., Ali, S., Ali, R.Q., Abbas, F., Bukhari, S. A. H., Saeeda, R. & Wuc, L. (2017). Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. *Ecotoxicol. Environ. Saf.*, 145, 90-102. https://doi.org/10.1016/ j.ecoenv.2017.07.016
- Farraji, H., Zaman, N. Q., Tajuddin, R. M. & Faraji. H. (2016). Advantages and disadvantages of phytoremedia-

tion: A concise review. Int. J. Environ. Tech. Sci., 2, 69-75.

- Feng, Y., Yu, X., Mo, C. & Lu. C. (2019). Regulation Network of Sucrose Metabolism in Response to Trivalent and Hexavalent Chromium in Oryza sativa. J. Agric. Food Chem., 67(35), 9738-9748. https://doi.org/10.1021/ acs.jafc.9b01720
- Jean-Soro, L., Bordas, F. & Bollinger, J. (2012). Column leaching of chromium and nickel from a contaminated soil using EDTA and citric acid. *Environ. Pollut.*, 164, 175-181. https://doi.org/10.1016/j.envpol.2012.01.022.
- Liu, L., Li, W., Song, W & Guo, M. (2018). Remediation techniques for heavy metalcontaminated soils: Principles and applicability. *Sci. Total Environ.*, 633, 206–219. https://doi.org/10.1016/j.scitotenv.2018.03.161
- Mallhi, A.I., Chatha, S. A. S., Hussain, A. I., Rizwan, M., Bukhar, S. A. H., Hussain. A., *et al.* (2020). Citric Acid Assisted Phytoremediation of Chromium through Sunflower Plants Irrigated with Tannery Wastewater. *Plants.*, 9, 380. https://doi.org/10.3390/plants9030380
- Malik, Z., Afzal, S., Dawood, M., Abbasi, G. H., Khan, M. I., Kamran, M., *et al.* (2022). Exogenous melatonin mitigates chromium toxicity in maize seedlings by modulating antioxidant system and suppresses chromium uptake and oxidative stress. *Environ. Geochem. Health*, 44, 1451– 1469. https://doi.org/10.1007/s10653-021-00908-z
- Manar, S. M., Mfarrej, F. B., Rizwan, M., Hussain, A., Shahid, M.J.,*et al.* (2022). Microbe-citric acid assisted phytoremediation of chromium by castor bean (*Ricinus communis* L.). *Chemosphere.*, 296. https:// doi.org/10.1016/j.chemosphere.2022.134065
- Menhas, S., Yang, Y., Hayat, K., Aftab, T., Bundscuh, J., et al. (2022). Exogenous Melatonin Enhances Cd Tolerance and Phytoremediation Efciency by Ameliorating Cd Induced Stress in Oilseed Crops: A Review. J. Plant Growth Regul., 41, 922-935. https://doi.org/10.1007/ s00344-021-10349-8
- Maqbool, A., Ali, S., Rizwan, M., Ishaque, W., Rasool, N., et al. (2018). Management of tannery wastewater for improving growth attributes and reducing chromium uptake in spinach through citric acid application. *Environ. Sci. Pollut. Res.*, 25, 10848–10856. https://doi.org/10.1007/ s11356-018-1352-4
- Pal, S. (2020). Screening of chromium tolerance potential of few weeds of Kolkata and assessment of phytoextraction efficiency. *Pollut. Res.*, 39(3), 753-762.
- 23. Qureshi, F. F., Ashraf, M. A., Rasheed, R., Ali, S., Hussain, I., *et al.* (2020). Organic chelates decrease phytotoxic effects and enhance chromium uptake by regulating chromium-speciation in castor bean (*Ricinus communis* L.). *Sci. Total Environ.*, 716, 137061. https:// doi.org/10.1016/j.scitotenv.2020.137061
- 24. Ramanlal, D. B., Kumar, R. N., Kumar, N & Thakkar, R. (2020). Assessing potential of weeds (*Acalypha indica* and *Amaranthus viridis*) in phytoremediating soil contaminated with heavy metals□rich effluent. *SN Appl. Sci.*, 2, 1063. https://doi.org/10.1007/s42452-020-2859-0.
- Riaz, M., Yasmeen, T., Arif, M. S., Ashraf, M.A., Hussain, Q., *et al.* (2019). Variations in morphological and physiological traits of wheat regulated by chromium species in long-term tannery effluent irrigated soils. *Chemosphere*, 222, 891-903. https://doi.org/10.1016/j.chemosphere.20

19.01.170

- Saleh, D., Sharma, M., Seguin, P. & Jabaji, S. (2020). Organic acids and root exudates of Brachypodium distachyon: effects on chemotaxis and biofilm formation of endophytic bacteria. *Canadian J. Microbiol.*, 13, 418. https://doi.org/10.1139/cjm-2020-0041
- Samarana, S., Ali, A., Muhammad, U., Azizullah, A., Ali, H., *et al.* (2020). *Environ. Pollut.*, 266(1). https:// doi.org/10.1016/j.envpol.2020.115394
- Sayantan, D & Shardendu. (2013). Amendment in phosphorus levels moderate the chromium toxicity in *Raphanus sativus* L. as assayed by antioxidant enzymes activities. *Ecotoxicol. Enivron. Saf.,*, 95, 161-170. https:// doi.org/10.1016/j.ecoenv.2013.05.037
- Sharma, A., Kapoor, D., wang, J., Shahzad, B., Kumar, V., *et al.* (2020). Chromium bioaccumulation and its impacts on plants: An Ooverview. *Plants.*, 9(1), 100 https:// doi.org/10.3390/plants9010100
- Song, Y., Ammami, M.T., Benamar, A., Mezazigh, S. & Wang. H. (2016). Effect of EDTA, EDDS, NTA and citric acid on electrokinetic remediation of As, Cd, Cr, Cu, Ni, Pb and Zn contaminated dredged marine sediment. *Environ. Sci. Pollut. Res.*, 23, 10577–10586. https://

doi.org/10.1007/s11356-015-5966-5

- Srivastava, D., Tiwari, M., Dutta, P., Singh, P., Chawda, K., *et al.* (2021). Chromium Stress in Plants: Toxicity, Tolerance and Phytoremediation. *Sustainability*, 13, 4629. https://doi.org/10.3390/su13094629
- Wang, K., Liu, Y., Song, Z., Wang, D. & Qiu. W. (2019). Chelator complexes enhanced *Amaranthus hypochondriacus* L. phytoremediation efficiency in Cd-contaminated soils. *Chemosphere*, 237, 1-8. https://doi.org/10.1016/ j.chemosphere.2019.124480
- Wiszniewska, A., Hanus-Fajerska, E., MuszyŃska, E. & Ciarkowska, K. (2016). Natural organic amendments for improved phytoremediation of polluted soils: a review of recent progress. *Pedosphere*, 26(1), 1–12. https:// doi.org/10.1016/S1002-0160 (15)60017-0
- Yang, J., You, S. & Zheng. J. (2019). Review in strengthening technology for phytoremediation of soil contaminated by heavy metal. *Conf. Series: Earth and Environ. Sci.* 242, https://doi.org/10.1088/1755-1315/242/5/052003
- Zhou, J. M., Dang, Z., Chen, N., Xu, S. & Xie, Z. (2007). Enhanced phytoextraction of heavy metal contaminated soil by chelating agents and auxin indole-3-acetic acid. *Huan Jing Ke Xue.*, 28(9), 2085-2088.