



Chelator enhanced phytoextraction of Chromium using Guinea grass

¹P.A. Shahidha, ¹A. Bharani*, ²G. K. Dinesh, ³T. Kalaiselvi, ¹M. Maheswari, and ⁴E.Kokiladevi and ¹Raveena Ravi

1 - Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore

-

641 003, Tamil Nadu, India

2 - Division of Environment Science, ICAR – Indian Agricultural Research Institute, New Delhi – 110012, India

3 - Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore

- 641 003, Tamil Nadu, India

4 - Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641

003, Tamil Nadu, India

*Corresponding author – alagirisamybharani@gmail.com

Abstract

Using biodegradable chelators to assist phytoextraction may be an effective approach to enhance the heavy-metal remediation efficiencies of plants. A pot experiment was conducted to investigate the effects of various synthetic chelators and low molecular weight organic acids (LMWOAs) on the growth of hyperaccumulator *guinea grass* and its Chromium uptake and accumulation in tannery-contaminated soil. The addition of 5-mmol kg⁻¹ of Citric acid, (dose selected as per literatures) has a significant uptake of total and Hexavalent (VI) Chromium among the chelators at the end of the pot experiment (90th day) as 17.98 ppm and 2.94 ppm, respectively. Among the synthetic chelators, EDTA @ 5mmol/kg had resulted in the maximum uptake of Chromium as 2.42 mg/kg (hexavalent Cr) and 15.76 mg/kg (Total Cr), which indicates the best chelator next to citric acid. The phytoremediation technology can be best studied using chelators, which makes Chromium more bioavailable in Guinea grass, a hyperaccumulator plant.

Keywords: Chelators, Bioavailable Chromium, Guinea grass, phytoextraction

Introduction

Chromium (Cr) is a hazardous contaminant that harms all living things, including plants and humans as it moves up the food chain. A large amount of Cr is released into the environment by many industries especially tanneries. The rising accumulation of Cr on agricultural soil reduces the yield and quality of commercially essential crops significantly.

Plant growth and development are inhibited by Cr-induced biochemical, molecular, cytotoxic, genotoxic, and hormonal deficits (Wakeel & Xu, 2020). To effectively reduce health hazards, obtaining Cr toxicity data before creating soil quality criteria/standards is critical. Chromium has received significant attention due to their toxicity, persistence, and biological accumulation, which can pose a substantial hazard to ecosystems and human health via the food chain (Liu et al., 2021). Over the last few years, biologists have become increasingly concerned about chromium waste generated by numerous human activities (Rahman et al., 2023). Chromium is a heavy metal that plants do not require essentially for the growth of plants. It was discovered in two chemical states in the soil: Cr (III) and Cr (VI), with Cr (VI) being more hazardous than Cr (III) (Montes et al., 2013; Peralta et al., 2009). Although it promotes growth and increases crop production at low concentrations, at higher concentrations it may cause growth inhibition, reduced carbon dioxide uptake, decreased chlorophyll synthesis, and chlorosis (Nichols et al., 2000).

Cr (VI) is also known to impair the absorption of critical heavy metal (HM) like Fe, Mg, and Ca at high amounts. Complexation enhances chromium solubility and, thus, mobility across the root xylem. Cr (VI) enters the root cells via the symplastic pathway, whereas Cr (III) enters via the apoplastic pathway. After then, Cr (VI) is decreased and sequestered in the cortex. Despite limited translocation to aerial regions, Chromium accumulates inside tissues depending on its chemical forms (Peralta-Videa et al., 2009; Saha et al., 2011). Cr (VI) compounds have a higher solubility and oxidizing potential than Cr (III) compounds. They are hazardous carcinogens that can kill animals and people (Zayed et al., 1998). One of the most prevalent heavy metal pollutants in soil, sediments, and groundwater is Chromium (Cr). The ability of a plant to ingest and translocate heavy metals, which is determined by phenotype and genotype, is critical to phytoremediation success (Chen et al., 2003). Several studies have shown that chelating agents such as ethylene diamine triacetic acid (EDTA), N-(2 hydroxyethyl)-EDTA, and citric acid (CA) can be used to increase metal mobility, thereby improving phyto extraction (Elless & Blaylock, 2000; Chen et al., 2003).

Extensive work has been carried out to remove the Cr from contaminated soils but there is not much research on the phytoextraction of the metal and subsequent recovery of the metal in the elemental form for reuse in the tannery industry. In view of the seriousness of Cr pollution, considerable efforts have been made to develop suitable methods for the remediation of chromium-contaminated soil. There is no previous studies on the phytoextraction of Chromium using Guinea grass and there is a need of suitable cost effective technologies for Chromium recovery processes. If the hyperaccumulating plants after

phytoextraction are again disposed to another site as bulk means it will create serious environmental issues further. So in order to assess the bioavailability of Chromium and quantification of Chromium recovery employing high biomass producing guinea grass through pot experiments this study has been conducted.

Materials and Methods

pH and Electrical Conductivity of the Guinea grass grown soil

The pH was determined using a pH metre (1:2.5 ratio) Standard methodology by Jackson (1973) was used to measure electrical conductivity (1:2.5 ratio). The mixed content was allowed to settle for 30 minutes before measuring the EC of the supernatant. The mixture was well mixed again, and the pH was determined.

Organic Carbon of the Guinea grass grown soil

The organic carbon was assessed using the chromic acid wet digestion method (Walkley & Black, 1934). In a 500 ml conical flask, 0.5 g of soil sample was taken. It was combined gently with 10ml of 1N potassium dichromate and 20ml of strong sulphuric acid. After then, the materials were allowed to stand for 30 minutes. After 30 minutes, 200 mL of distilled water, 10 mL of phosphoric acid, and 1 mL of diphenylamine indicator were mixed together and titrated against 0.5 N Ferrous Ammonium Sulphate until a vivid green colour developed.

Cation Exchange Capacity of the Guinea grass grown soil

Ten grams of the sample was taken in a 250 ml beaker along with 50 ml of neutral ammonium acetate solution and kept overnight. The sample was then transferred to the filter paper (Whatman No.3) and leached with 30-40 portions of $\text{CH}_3\text{COONH}_4$ for 6-8 times. After that, a pinch of ammonium chloride was added and leached with 60 per cent alcohol until the filtrate ran free of chloride. The sample and filter paper were transferred to the distillation flask, and 500 ml of distilled water and 10 ml of 40 per cent NaOH were added. The NH_3 evolved during distillation was collected in 25 ml of 0.1 N H_2SO_4 added with two drops of methyl red indicator. The excess acid was titrated against 0.1 N KOH, and CEC was calculated (Jackson, 1973).

$$\text{CEC} = (V - V_1) \times 0.1 \times 100/10 \text{ cmol kg}^{-1} \text{ soil}$$

Note: V- Volume of 0.1 N H_2SO_4 ; V_1 - Volume of 0.1 N KOH consumed

Total Chromium content of the Guinea grass-grown soil

From November 2022 to January 2023, the Guinea grass plants were grown in 3kg pots for 90 days in a controlled environment in the greenhouse (Supplementary Fig.1). The

stem above the soil surface was cut to harvest as the plant biomass (shoot), and the below portion is considered as root biomass. The plant roots were removed after immersing the pots and their contents in a water bath and gently cleaning the soil from the roots. Before drying at 70°C, the roots and shoots were washed with deionized water and weighed to determine the dry matter yield of roots and shoots. Following digestion with concentrated nitric acid and 30% H₂O₂, the chromium concentration in the shoots and roots was measured using Atomic Absorption Spectrophotometer with air-acetylene flame of Perkin Elmer, AA200 (USEPA, 1979a).

Hexavalent Chromium (ppm) content of the Guinea grass-grown soil

The hexavalent Cr concentration in soil is determined using Diphenyl carbazide (DPC) method (USEPA, 1979b). One gram of the soil sample was taken in a 50ml polypropylene centrifuge tube, and 25ml of distilled water was added. The content was shaken using an end-over-end shaker for 2 hours. The content was centrifuged at 8000 rpm for 10 minutes and filtered through Whatman No.1 filter paper. About 10 ml of this extract was taken in a 50 ml volumetric flask, to this 10 ml of 1 N H₂SO₄ and 4 ml of 1, 5 – diphenyl carbazide reagent were added, and the volume made up to 50 ml with distilled water. The content was allowed to stand for 20 minutes for purple-violet colour development. The absorbance of the colour was measured at 540nm using Spectrophotometer and compared with that of the standard Cr solution. The indicator 1, 5 – diphenyl carbazide was prepared by dissolving 0.25 g of 1, 5 – diphenyl carbazide and 4 g of phthalic anhydride in 100 ml of 95% ethyl alcohol.

Results and Discussion

pH and Electrical Conductivity (dSm⁻¹) of the Guinea grass grown soil

Increase in the pH of the control (T₁) was observed as the days advanced from the initial to the 90th day of the pot experiment (.031 units) and recorded the highest pH value (7.82) on the 90th day of the experiment (Fig.1). The application of chelators showed a reduction in pH as the days advanced (90 days). As the chelate treatments are concerned, contaminated soil with Citric acid (@5mmol/kg) is showing the lowest value (7.11), followed by oxalic acid (7.27) at 5mmol/kg. Among the synthetic chelators, EDTA has the lowest (7.36), and EDDHA has the maximum (7.47) value at the end of the 90th day. The pH value was found to be somewhat lower in chelate-assisted Cr-treated soil (Patra *et al.*, 2018). In pot research with lupine and yellow mustard, lower soil solution pH resulted in significantly more excellent Cd and Zn contents (Römken *et al.*, 2002). The increase of the pH in the

rhizosphere may be due to the accumulation of cations (Ca^{2+} and Mg^{2+}) across the rhizosphere (Youssef & Chino, 1988)

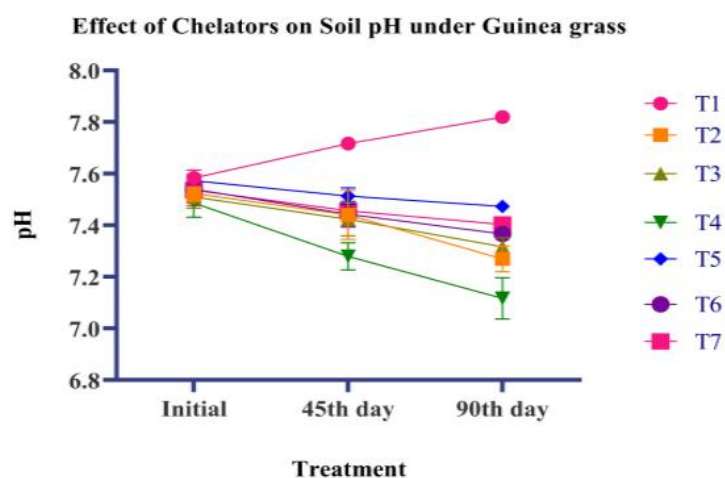


Fig.1 Effect of chelators on soil pH in Guinea Grass grown soils

The EC also showed a similar trend as pH, decreasing as the days advanced. The EC values showed a declining trend overall in the experiment (Fig. 2). The control (T1) without any chelators decreased by 0.18 dS/m throughout the experiment. Citric acid-treated soil showed a significant decline of EC that is 0.57 dS/m compared to the control. The oxalic acid showed a drop of 0.55 dS/m on the final day. Compared to the control, least decrease was observed in the DTPA-treated soil, which is 0.4 dS/m compared to the control. The EC value of soil solutions represent the soil's solute content. The EC values in control reduced dramatically with plant growth, possibly due to the decrease in solute concentration absorbed by the hyperaccumulator plants (Wang *et al.*, 2020).

Effect of Chelators on soil EC (dS/m) under Guinea grass

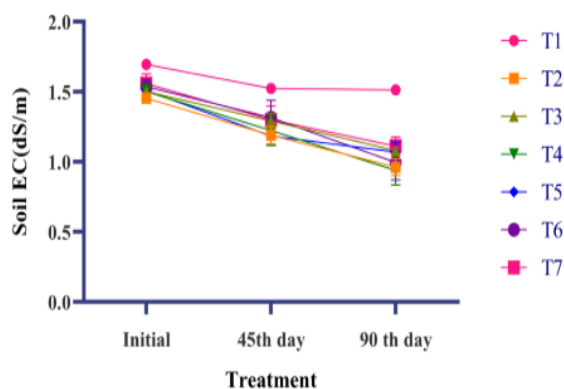
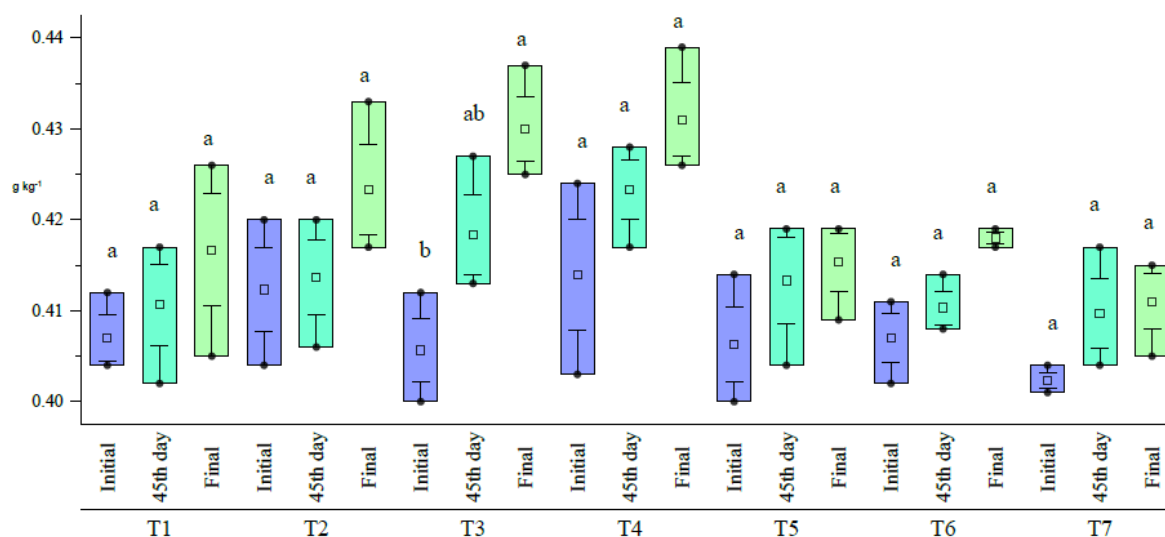


Fig. 2 Effect of chelators on soil EC (dS/m) in Guinea grass grown soils**Organic Carbon of the Guinea grass grown soil**

The soil organic carbon showed a decreasing trend due to the application of chelators (Fig. 3). There is no significant change in the treatments, even though the control showed a drop in organic carbon percentage. On the 90th day, all the treatments showed an increase in the soil organic carbon compared to the control, among which the citric acid-treated soils had the highest (0.431 g kg⁻¹), while the lowest value was observed in the DTPA-treated soil (0.402 g kg⁻¹) at the end of the pot experiment. Each treatment had a decreasing trend in soil organic carbon as time advanced, but the change was very low. The real availability of heavy metals was high due to the low soil pH combined with the organic matter and clay content's inadequate binding capacity (Römken *et al.*, 2002). Because soil organic matter tends to decrease mobile Cr (VI) to comparatively immobile Cr (III), its organic matter concentration has a considerable impact on Cr mobility in soil (Banks *et al.*, 2006).

**Fig.3 Effect of chelators on soil organic carbon (g kg⁻¹) in Guinea Grass grown soils****Cation Exchange Capacity (CEC) of the Guinea grass-grown soil**

The CEC showed an increasing trend along the treatments except for the control. The control showed a decline in the CEC value at the end of the experiment, while all other treatments with chelators showed an increase in the CEC (Fig.4). In the control alone, there was a drop of CEC about 3.9 cmol (+) kg⁻¹ soil at the end of 90th day of the experiment. In the case of organic chelators, citric acid-treated soil recorded the highest CEC (19.06 cmol (+)

kg⁻¹ soil) followed by iron chelator EDDHA (18.10 cmol (+) kg⁻¹ soil). The Cation Exchange Capacity (CEC) of a soil denotes its ability to hold nutritional cations in readily available forms. It refers to the amount of nutritional cations found in exchangeable forms. It provides plants with direct mineral nourishment. In general, chromium absorption and adsorption to the soil surface reduces chromium availability in the system. As a result, the bigger the system's cation exchange capacity, the greater the absorption, adsorption, and arrest of the Chromium (Patra *et al.*, 2018).

Effect of Chelators on CEC of soil under Guinea grass

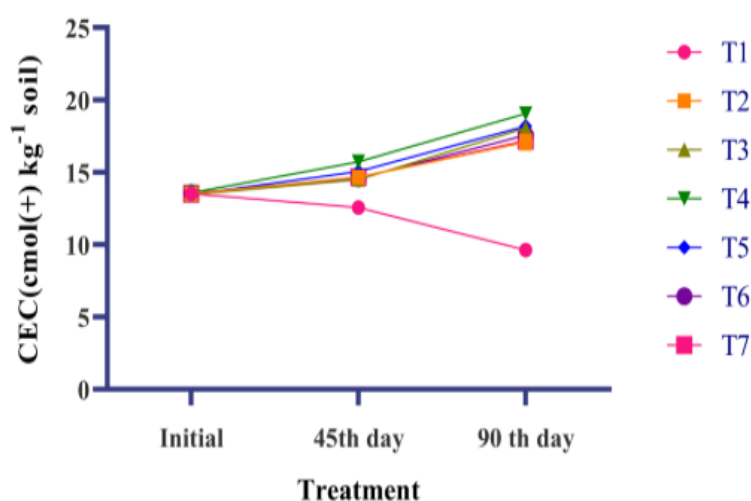


Fig.4 Effect of chelators on soil CEC in Guinea grass grown soils

Total Chromium and hexavalent Chromium concentration in Guinea grass grown soil

The application of chelators showed a significant increase in Cr (VI) and total Chromium concentration. At the end of the experiment, the highest concentration of Cr (VI) recorded in the Citric acid-treated soil (T4) at about 2.94 ppm. The lowest concentration of Cr (VI) was recorded in control at 0.30 ppm (Fig.5). Same trend was obvious in the case of total chromium concentration on the 90th day of the experiment such that 1.38 ppm in the control and 17.98 ppm in the Citric acid-treated soil respectively (Fig.6). Among the synthetic chelators, the EDTA @5mmol/kg treated soil was having the highest concentration of Cr (VI) and total Chromium which is of 2.42 and 15.76 ppm respectively.

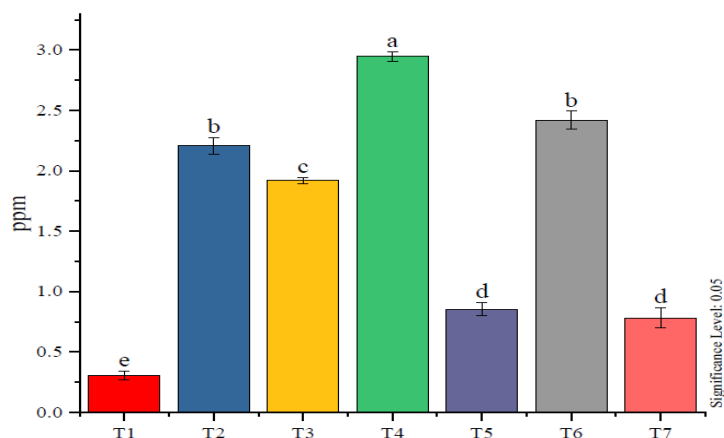


Fig.5 The effect of chelators on the hexavalent chromium in Guinea grass-grown soil

The addition of chelators such as EDTA, DTPA, Citric acid, salicylic acid, and metal ions enhanced the development of Lemongrass. The treated plants with lower transportation index values accumulated more Chromium in the roots than in the shoots (Patra *et al.*, 2018). Chelating compounds have been shown in studies to reduce the harmful effects of Cr (VI). Chelating compounds containing Cr (VI) increased Cr bioavailability in plants. (Mohanty & Patra, 2012).

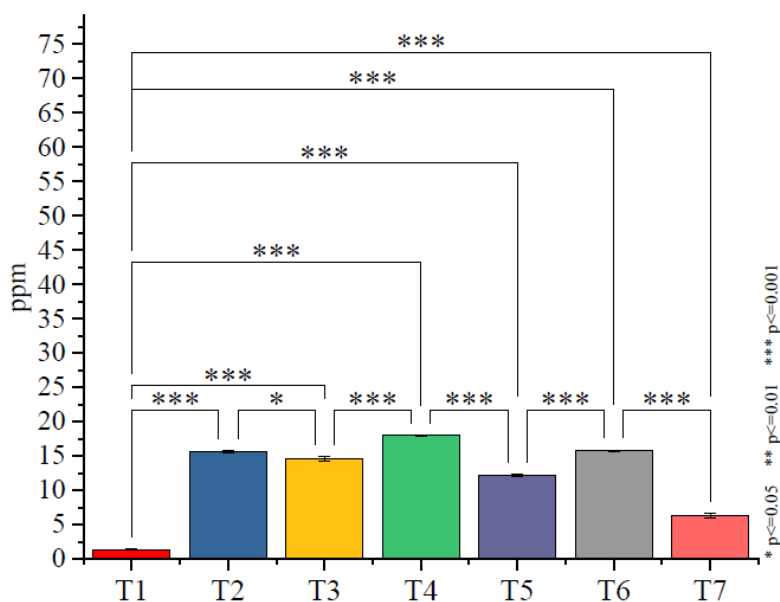


Fig.6 The effect of chelators on the total Chromium content of the Guinea grass-grown soil

Dey (2016) opined that heavy metals such as chromium, lead, copper, and zinc absorption efficiencies of Vetiver grass were significantly increased by chelating agent (EDTA or citric acid) treatment. The increase in citric acid dose showed a progressive rise in absorption efficiency in their experiments. Chelators considerably improved Cr solubilization in soil and raised Cr accumulation in plant tissues. Compared to the control, EDTA and citric acid were more effective at increasing Cr uptake by plant shoots, resulting in a fivefold increase of Chromium (Almaroai *et al.*, 2012).

Conclusion

The chelators like malic acid, citric acid, oxalic acid, EDTA, EDDHA and DTPA at 5mmol/kg assisted the phytoextraction process of chromium using Guinea grass. Among these low molecular weight organic chelators, citric acid was the best chelator which helps in the maximum chromium accumulation thereby Cr bioavailable in the plants. As the synthetic chelators were concerned, EDTA had the best chelation property, resulting in chromium's maximum phytoextraction. Developing a cost-effective technology for Cr recovery from Guinea grass grown in the contaminated sites are feasible. It will benefit the tannery industry itself, if at all the Chromium salts can be recovered in the elemental form.

Declaration of interest statement

The authors have no financial or proprietary interests in any material discussed in this article. The authors have no competing interests to declare.

References

- Banks, M. K., Schwab, A. P., & Henderson, C. (2006). Leaching and reduction of chromium in soil as affected by soil organic content and plants. *Chemosphere*, 62(2), 255–264.
- Jackson, M. L. (1973). Soil chemical analysis, pentice hall of India Pvt. Ltd., New Delhi, India, 498, 151–154.
- Mohanty, M., & Patra, H. K. (2012). Effect of chelate-assisted hexavalent chromium on physiological changes, biochemical alterations, and chromium bioavailability in crop plants—An in vitro phytoremediation approach. *Bioremediation Journal*, 16(3), 147–155.
- Montes, M. O., Peralta-Videa, J. R., Parsons, J. G., Diaz, B. C., & Gardea-Torresdey, J. L. (2013). Spectroscopic determination of the toxicity, absorption, reduction, and

- translocation of Cr (VI) in two Magnoliopsida species. *International Journal of Phytoremediation*, 15(2), 168–187.
- Patra, D. K., Pradhan, C., & Patra, H. K. (2018). Chelate based phytoremediation study for attenuation of chromium toxicity stress using lemongrass: *Cymbopogon flexuosus* (nees ex steud.) W. Watson. *International Journal of Phytoremediation*, 20(13), 1324–1329.
- Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. *The International Journal of Biochemistry & Cell Biology*, 41(8–9), 1665–1677.
- Saha, R., Nandi, R., & Saha, B. (2011). Sources and toxicity of hexavalent chromium. *Journal of Coordination Chemistry*, 64(10), 1782–1806.
- USEPA. (1979a). Method 218.1. Atomic absorption direct aspiration. In *Methods for chemical analysis of water and wastes*. Environmental Monitoring and Support Laboratory.
- USEPA. (1979b). Method 281.4. Chromium, hexavalent. In *Methods for chemical analysis of water and wastes*. Environmental Monitoring and Support Laboratory, USEPA.
- Wang, G., Pan, X., Zhang, S., Zhong, Q., Zhou, W., Zhang, X., Wu, J., Vijver, M. G., & Peijnenburg, W. J. G. M. (2020). Remediation of heavy metal contaminated soil by biodegradable chelator-induced washing: Efficiencies and mechanisms. *Environmental Research*, 186, 109554.
- Youssef, R. A., & Chino, M. (1988). Development of a new rhizobox system to study the nutrient status in the rhizosphere. *Soil Science and Plant Nutrition*, 34(3), 461–465.

Supplementary Fig.1

