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# PHYTOREMEDIATION OF URANIUM TAILINGS: ROLE OF CHELATORS IN TRIGGERING URANIUM ACCUMULATION IN WHEAT

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**ABSTRACT**

Chelator-assisted phytoextraction has been proposed as a potential tool for phytoremediation of uranium contaminated tailings. The purpose of the present investigation was to test the efficiency of the four various chelators namely, citric acid (CA), oxalic acid (OA), NTA and EDTA and to screen out the most effective chelator with promising concentration of it in increasing the U uptake and accumulation for phytoremedial programmes. Three kilograms of mixture (25:75; tailing: garden soil) was filled in the earthen pots. Treatment pots were prepared by applying- 0.1, 0.5, 2.5 and 12.5 mmol kg-1 concentrations of each of the chelator (CA, OA, NTA and EDTA). Optimum concentrations of the chelators were recorded considering biomass production, tolerance index and U uptake. Each chelator produced severe toxicity symptoms at 12.5 mmol kg-1 treatment level. Lowest depression in respect of growth was observed with NTA while OA and CA were proved less toxic than EDTA. Highest inhibition was recorded in EDTA treatments at respective levels. U uptake and accumulation was concentration dependent for each of the chelator amendment. Maximum U uptake (3.4-fold) in the roots occurred at 2.5 mmol kg-1 of CA while NTA proved to be the weakest for the same purpose. Not with standing, EDTA and NTA are stronger complexion agents than CA but in contrary, the use of CA proved beneficial in U tailing phytoremediation in the present investigation. The growth of the wheat plants was affected by each of the chelator, which in general follows the order: NTA < OA < CA < EDTA, whereas the order for U accumulation was recorded as- CA > EDTA > OA > NTA. On the basis of this study it can be suggested that the use CA over EDTA is better, as it is easily biodegradable, less toxic and has lower leaching risk..

***Keywords:*** Chelator, Phytoremediation, Radionuclides, U tailings

## INTRODUCTION

Uranium possess radiotoxic as well as chemotoxic effects (Stojanović *et al.,* 2010). Radionuclides existing in soil can be dissolved in solution, or ion exchanged in reaction, complexed with soil organics or precipitate as pure or mixed solids (Gavrilescu *et al.,* 2009). For moderately polluted soils, *in situ* phytoremediation is an environmentally attractive but time consuming solution (Evangelou *et al.,* 2007; Jensen *et al.,* 2009). For severely polluted soils, a possible operational solution is soil washing by *ex situ* extraction or *in situ* flushing with aqueous solutions containing a strong ligand [*e.g.* EDTA, nitrilo-triacetic acid (NTA) or similar harsh compounds] although other cleaning or stabilization methods exist (Sun *et al.,* 2001; Di Palma *et al.,* 2003; Kim *et al.,* 2003; Lim *et al.,* 2005; Ehsan *et al.,* 2006; Dermont *et al.,* 2008; Leštan *et al.,* 2008; Rao *et al.,* 2008).

Phytoremediation, especially phytoextraction has emerged as a cost-effective and eco-friendly technology for the restoration of metal contaminated soils. It is a green technology that uses plants to remove inorganic contaminants particularly heavy metals, from soils and waters (Salt *et al.,* 1998; Garbisu and Alkorta, 2001; Saifullah *et al.,* 2010; Moosavi and Seghatoleslami, 2013; Paz-Alberto and Sigua, 2013; Amanullah *et al.,* 2016; Sarwar *et al.,* 2017; Sheoran and Sheoran, 2017). Research toward increasing the efficiency of this technology has been evolving in two main directions: firstly, the use of hyperaccumulating plant species and secondly, the use of high-biomass producing plant species with induced accumulation of trace metals. The rationale behind the second approach is that hyperaccumulators are generally metal specific and yield a low annual biomass production, thus limiting the overall amount of heavy metals that can be extracted per harvest. Enhancing uptake and/or translocation of potentially toxic metals in high-biomass producing species has the prospect of achieving higher amounts of contaminants being extracted per harvest. The use of soil amendments to increase the phytoavailability and/or translocation of heavy metals has been suggested in numerous publications (Cooper *et al.,* 1999; Blaylock and Huang, 2000; Jiang *et al.,* 2003; Soleimani *et al.,* 2010; Gunawardana *et al.,* 2011; Bulak *et al.,* 2014; Jagetiya *et al.,* 2014).

In the chemically assisted phytoextraction approach, high biomass plant species are used when grown in soils that have been treated with various chelators (Saifullah *et al.,* 2009; Mihalík *et al.,* 2012; Shahida *et al.,* 2012; Jagetiya and Sharma, 2013; Shakoor *et al.,* 2013; Shahid *et al.,* 2014; Anning and Akoto, 2018). Various organic acids may be used to enhance U desorption in soil solution from soil particles and

resulted in an higher U uptake (Laroche *et al.,* 2005; Mihalík *et al.,* 2012; Tauqeer and Sagir, 2018). These agents influence the distribution of metals in soils by converting them from insoluble to soluble fractions (Liu *et al.,* 2011). Order of chelation of various heavy metals with chelators is follows: EDTA (EDTA related synthetic chelators) > NTA > citric acid (CA) > oxalic acid (OA) > acetic acid (AA) as demonstrated earlier in many comparative studies (Hong and Pintauro, 1996; Krishnamurti *et al.,* 1998; Wenger *et al.,* 1998; Evangelou *et al.,* 2007; Duquéne *et al.,* 2009). More than 100-fold increases of Pb concentrations in the biomass of crops were reported when EDTA was applied to contaminated soils (Ulmer-Scholle *et al.,* 2004; Meers *et al.,* 2005). Most metals present in soils exist in large quantities in unavailable forms and thus soil conditions need to be altered to elicit phytoextraction since this phenomenon depends on a relatively high concentration of soluble metal(s) in soils to enable significant uptake of a target metal (Saifullah *et al.,* 2010). U phytoavailability can be enhanced by adding various chelators or ligands (Huang *et al.,* 1998; Shahandeh and Hossner, 2002). The potential of a chelating agent for metal complexation is based on the number of available sites for metal fixation on the molecule. The strength of the metal-chelator complex is expressed by the thermodynamic stability constant (log k) and the concentration of competing cations in soils (cations and other metals). A large log k means a high ratio of chelated to un-chelated or free metal when equivalent amounts of metal and chelating agent are present (Duquène *et al.,* 2009). Complexes with multidentate ligands are usually much more stable than those with monodentate ligands. In the soil matrix, the selective complexation of one metal in the presence of others depends on the difference between thermodynamic stability constants for the two metals (Duquène *et al.,* 2008).

For more than 50 years, synthetic chelators, including EDTA, have been used to supply plants with micronutrients both in soil and hydroponics. EDTA was considered as a chelating agent for the assisting the process of phytoextraction during late 1980s and early 1990s. In earlier reports on the use of EDTA has suggested over 100-fold enhancement in uptake and accumulation of U (Grčman *et al.,* 2001).

For the last 50 years NTA was primarily used in detergents and is known as biodegradable chelating agent. Inspite of its expected positive properties, few studies have been performed with NTA as the ligand to assist phytoextraction of metals. Meier et al. (2010) believe that low molecular weight organic acids are involved in the metal transport and storage in plants.

Due to complexing properties natural low molecular weight organic acids (NLMWOAs), such as CA, OA or malic acid (MA), may be useful in heavy metal desorption in soil solution (Mench and Martin, 1991; Krishnamurti *et al.,* 1998; Nigam *et al.,* 2001; Bao *et al.,* 2011). Earlier study suggests that NLMWOAs have the capability to detoxify intracellular heavy metals via binding. CA enhances metal solubility and plant uptake via formation of soluble citrate-metal complexes (Qu *et al.,* 2011).

Main purpose of the present investigation was to test the efficiency of the four various chelators namely, EDTA, NTA, CA and OA and to screen out the most effective chelator with promising concentration of it in increasing the U uptake and accumulation for phytoremedial programmes. Selection of chelators was done due to their reported effective nature towards metal uptake as well as mobilisation.

## MATERIAL AND METHODS

### Soil Preparation

Tailings of U of Umra region, Rajasthan have been collected and a mixture was prepared with garden soil in the proportion of 25:75. Physical and chemical properties and characteristics of garden soil and the mixture are presented in Table 1. The mixture of tailing and garden soil was kept to equilibrate for duration of four weeks. Three kilograms of this mixture was filled in the earthen pots for each of the chelator amendment. For CA, OA, NTA and EDTA, treatment pots were prepared by applying- 0.1, 0.5, 2.5 and 12.5 mmol $kg^{-1}$ concentrations of each of the chelator. One set of pot was not treated with any of the chelator, and considered as control set. The experiment was conducted in completely randomized block design with three replicates for each of the treatment. Ten seeds of wheat were sown equidistantly at the depth of 2.5 cm in each of the pot. Standard and recommended agronomic practices have been used to irrigate these pots with deionised water (DIW). In order to retain leachate, collection trays have been placed beneath the treatment and control pots. The leachate collected so was re-applied immediately to the respective pots. During the vegetative stage of growth the observations for the biomass production, tolerance and uptake parameters were observed. Biomass production, tolerance and U accumulation potential were considered to find out the effective concentration of the chelators. These parameters have been used for the comparison of chelators for their phytoremediation efficiency. Pellet fluorometer was used to analyse the U concentrations in plant parts. The data of the experiment were subjected to statistical analysis for the computation of differences between control and amended pots. Mean values based on three replicates for biomass production and uranium accumulation were calculated while differences between treatments were considered significant and highly significant at $p = 0.05$ and 0.01, respectively.

## RESULTS AND DISCUSSION

The results presented in Figs. 1-3 indicate that CA was found most effective chelator for U uptake than EDTA, OA and NTA. Though 2.5 mmol $kg^{-1}$ concentrations were observed suitable for phytoremediation but 12.5 mmol $kg^{-1}$ of each chelator was resulted in death of the wheat plants. Application of 2.5 mmol $kg^{-1}$ of CA resulted in several fold increase in U accumulation in wheat roots.

### Effect of Chelators on Biomass Production

Biomass of wheat in terms of shoot-root fresh mass as well as dry mass when tailing-soil mixture was amended with chelators is presented in Fig. 1. The fresh as well as dry mass

production at 0.1 and 0.5 mmol $kg^{-1}$ of only NTA among the four chelators resulted in mild increase. 2.5 mmol $kg^{-1}$ treatment of NTA was deleterious and produced toxicity symptoms in wheat plants and resulted in 27-30% and 23-36% reduction in shoot-root fresh and dry mass, respectively. All the applications of OA, CA and EDTA produced growth reduction and toxicity symptoms. OA produced moderate (24-62%, 5-51%) while CA resulted a noticeable reduction (32-81%, 13-57%) in fresh and dry mass. EDTA was most toxic towards the biomass production and inhibited fresh and dry mass in the range of 49-91% and 20-73%. All these chelators resulted in death of the plants at their respective highest application concentration.

**Tolerance Index as Affected by Chelators Amendment**

The tolerance index of wheat varied depending on the chelators and their various application levels during the study (Fig. 2). Lowest TI values have been observed for EDTA, whereas the TI values for OA and CA were ranged between NTA and EDTA. The sequence for TI values observed under influence of chelators is as follows: NTA > OA > CA > EDTA.

**Effects of Chelators on U Uptake**

Fig.3 depicts the values for U uptake in shoot and root tissues of wheat plants. CA was proved most effective chelator for uptake of U followed by EDTA, OA and NTA. Higher accumulation occurred in the roots as compared with shoots. Compared to the control plants, 2.5 mmol $kg^{-1}$ CA treatment roots accumulated U by a factor of more than three.

**Biomass Production**

Amendments including synthetic aminopolycarboxylic acids (APCAs) such as EDTA and diethylene triamine pentaacetic acid (DTPA), natural APCAs like ethylene diamine disuccinate (EDDS) and NTA as well as NLMWOAs such as CA, OA and MA were investigated to enhance uptake of U and other heavy metals by many authors (Evangelou *et al.,* 2007; Bao *et al.,* 2011; Al-Saad, 2012; Mihalík *et al.,* 2012; Shahid *et al.,* 2012; Siva Ananthi *et al.,* 2012; Jagetiya and Sharma, 2013; Shakoor *et al.,* 2013). Though the EDTA was found more effective in various studies but due to lower rate of biodegradation and their affinity with heavy metals, EDTA-heavy metal complexes are toxic to plants and soil microorganisms (Chiu *et al.,* 2005; Quartacci *et al.,* 2005; Evangelou *et al.,* 2007). Chaney et al. (1997) and Grčman et al. (2001) acknowledged the efficiency of EDTA in induced phytoextraction, yet pointed out that necessary measures would be required to prevent offsite migration. Both plants and microorganisms of rhizosphere are known to release NLMWOAs to increase mineral nutrient solubility by acidification and formation of organic-mineral complexes. Among root exudates, a wide range of NLMWOAs can be found, these are AA, OA, tartric acid, MA, CA, propionic acid and lactic acid etc. (Meers *et al.,* 2004). They function as natural chelators, and considering their application as a soil amendment, NLMWOAs have a potential advantage over compounds like EDTA in that they are more readily degraded in the environment (Wasay *et al.,* 1998). Among the NLMWOAs tested CA was reported as much effective than OA in solubilising U and various heavy metals in soil (Peters, 1999; Duquéne *et al.,* 2009). NTA has been used commonly as a detergent in the last 50 years is a biodegradable chelating agent. There are reports that in 3 to 7 days under aerobic conditions NTA is decomposed (Bucheli-Witschel and Egli, 2001) and proved as relatively better substance in much effective chelator for heavy metal uptake and accumulation (Tandy *et al.,* 2004; Quartacci *et al.,* 2006).

During the present study chelators (EDTA, CA, OA and NTA) amendments resulted moderate to heavy growth reduction and toxicity with more depression in roots than shoots, except lower concentrations of NTA (0.1 and 0.5 mmol $kg^{-1}$). The highest concentration (12.5 mmol $kg^{-1}$) of these chelators proved highly toxic for wheat plants and resulted in death. NTA and CA at this concentration (12.5 mmol $kg^{-1}$) come up with few seeds to germinate while EDTA and OA completely inhibited seed germination at this treatment level. Roots suffered more reduction than shoots under all chelators' treatments. This happened as they were the most sensitive organs towards the substrate combinations as well as they were the organs of higher U storage. NTA enhanced growth at lower applications (0.1 and 0.5 mmol $kg^{-1}$) which might be due to two possible reasons, firstly, NTA increases the bioavailability and secondly, the uptake of essential nutrients.

Ryegrass biomass was found unaffected even after the treatment with EDDS, CA, NH4-CA/CA mixture, OA and NTA while biomass of Indian mustard shoots on EDDS and CA treated soil was significantly decreased. Another amendment (NH4-CA/CA mixture, OA and NTA) had no inhibitory symptoms on biomass (Duquéne *et al.,* 2009). In contrary to the findings of Vandenhove et al. (2001); Shahandeh and Hossner (2002); Lesage et al. (2005), Vandenhove et al. (2009) reported that CA-assisted U phytoextraction hampered the growth of ryegrass. Certain reports demonstrates inhibitory effects of NTA on plant growth (Meers *et al.,* 2004; Quartacci *et al.,* 2005) while Duquéne et al. (2009) found that NTA did not produce any toxicity and growth inhibition in plants.

Outcomes of the present investigation indicate that among all the four chelators, EDTA was found most phytotoxic. Severe biomass inhibition due to application of EDTA has also been observed by Chen et al. (2005); Lesage et al. (2005); Ruley et al. (2006); Hernández-Allica et al. (2007); Sinegani and Khalilikhah (2008); Jagetiya and Sharma (2013). Stability and permeability of cell walls through enhanced uptake of metals by the use of EDTA has been reported (Saifullah *et al.,* 2010). It exhibits an inhibitory effect, most probably due to removal of $Ca^{2+}$ and $Mg^{2+}$ from the outer cell membrane which affects membrane integrity (Chavez de Paz *et al.,* 2010; Krujatz *et al.,* 2012). When EDTA applied at too high concentrations it could enhance metal translocation from roots to shoots apart from its inhibition potential on plant biomass production (Chen and Cutright, 2001).

**U Uptake**

U uptake and accumulation in the roots of wheat plants was increased from the control value of 170 ppb to 585 ppb at

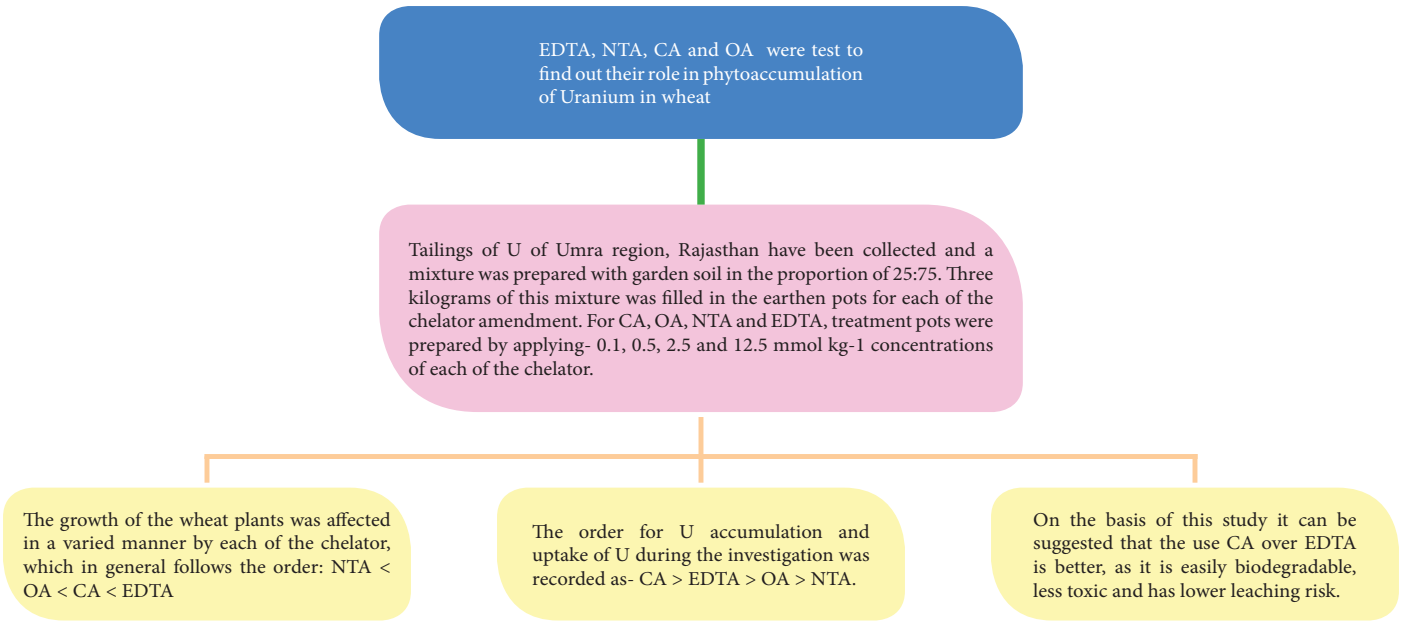


**Table1.** Physico-chemical properties of garden soil and mixture of U tailing and garden soil.

| Physico-chemical properties | Garden soil | Mixture |
|---|---|---|
| pH (H2O) | 7.31±0.07 | 7.78±0.09 |
| Conductivity (S m-1) | 0.79±0.09 | 0.76± 0.12 |
| Biological carbon (%) | 0.060±0.31 | 0.58±0.10 |
| Phosphate (kg ha-1) | 28.0±5.70 | 24± 6.65 |
| Potash (kg ha-1) | >348 | >348 |
| Uranium concentration (µg g-1) | ND | 36± 9.50 |

Results are the mean of three replicates.

2.5 mmol $kg^{-1}$ of CA treatment, whereas, shoots accumulated U content from the control value of 92 ppb to 300 ppb. More than 3-fold increase in U accumulation with 520 ppb and 247 ppb, respectively in roots and shoots was observed at 2.5 mmol $kg^{-1}$ EDTA treatment. An uptake of U at levels of 436 ppb in roots and 212 ppb in shoots was noticed during OA treatment at similar level. NTA treatment could not enhance any significant U accumulation at 0.1 and 0.5 mmol $kg^{-1}$ applications while at 2.5 mmol $kg^{-1}$ treatment the values for U in roots and shoots were 392 ppb and 180 ppb, respectively. Lower concentrations (0.1 and 0.5 mmol $kg^{-1}$) of each chelator lagged behind 2.5 mmol $kg^{-1}$ in terms of enhancing U uptake, hence it is proposed that 2.5 mmol $kg^{-1}$ of chelators concentration is the optimum for 25:75 (tailing:soil) mixture. These results for uranium uptake with relation to CA, EDTA, OA and NTA applications are in agreement with the findings of Ebbs et al. (1998); Huang et al. (1998); Sun et al. (2001); Shahandeh and Hossner (2002); Duquéne et al. (2009); Vandenhove et al. (2009); Vera Tomé et al. (2009); Jagetiya and Sharma (2013).

It is confirmed during the present investigation that CA was found most effective chelator than EDTA, OA and NTA in desorption of U in soil to soil solution as well as increasing U availability for plant uptake (Fig. 3). The efficiency of EDTA in complexation and accumulating U was found little less than CA but far better than OA while NTA did not prove useful at respective concentrations of these chelators. OA and NTA during the present investigation did not demonstrate significant potential for U uptake. These results are in agreement with the work of Duquéne et al. (2009); Vandenhove et al. (2009); Jagetiya and Sharma (2013). Elements such as Al, Fe, Mg and Ca might be competing U for chelation with NTA and OA, which as a consequence inhibited uptake of U. Further, entry of essential nutrients may have assisted with the treatment of NTA and OA in plants thereby decreasing the toxicity with these chelators (Duquéne *et al.,* 2008; Jagetiya and Sharma, 2013).

EDTA treatments resulted in higher growth inhibition at respective treatment levels when compared to the other chelators. Higher accumulation of U was noticed at each levels of CA than the EDTA, OA and NTA. This might happened due to the differential mechanism of CA, EDTA, OA and NTA for U desorption and complexation with the chelators, interaction between plant roots and metal chelator complexes as well as influence of physico-chemical properties of the substrate. EDTA, due to its toxicity caused more harm to the biomass production, thereby decreasing the further chances of further U uptake and accumulation. CA on the other hand produced less toxic role with higher uptake results. Reports of Huang et al. (1998); Sun et al. (2001); Umer-Scholle et al. (2004) favored EDTA assisted U-uptake whereas, our results on EDTA do not match with aforesaid workers who advocated the ability of EDTA for U uptake. Ebbs et al. (1998) reported that chelating agents like EDTA and DTPA did not increase U solubility

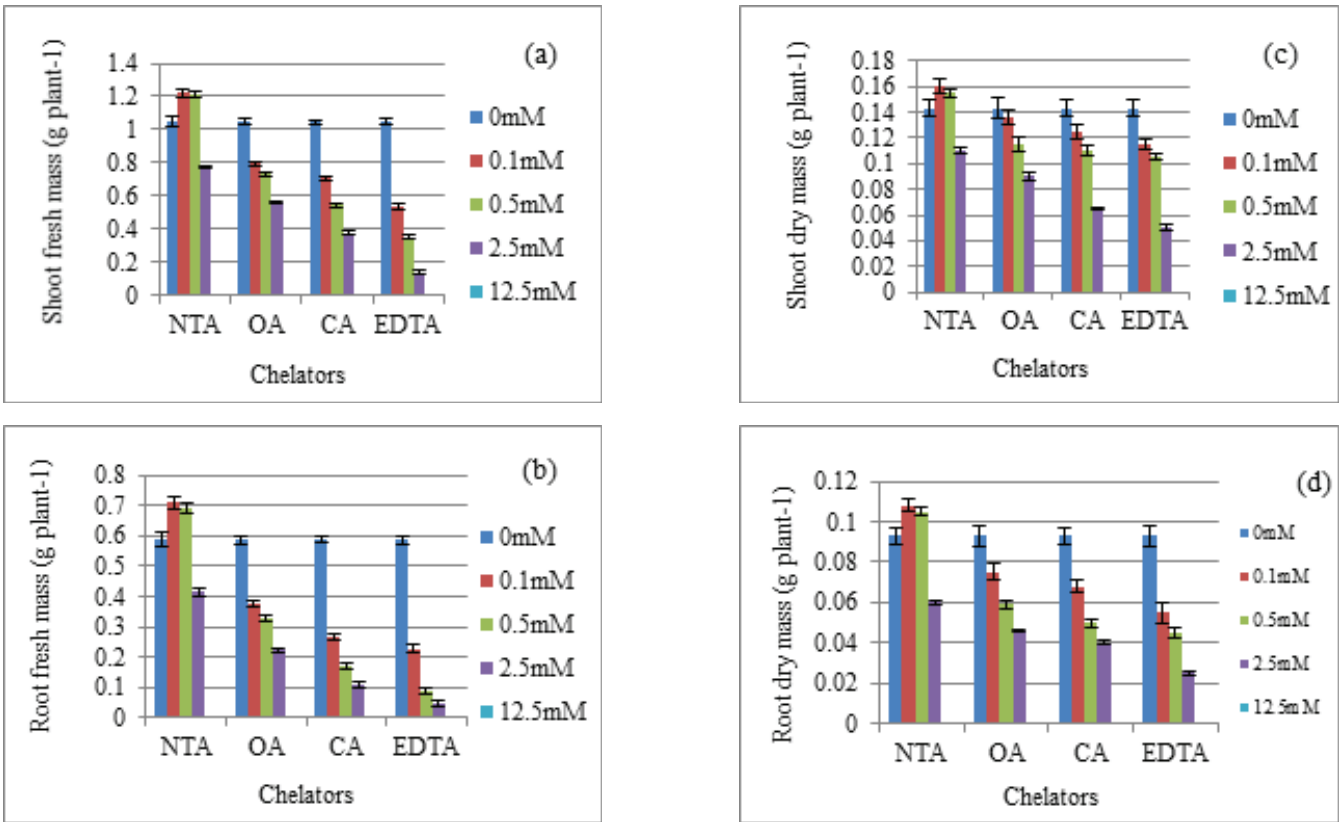


**Fig.1.** Effect of chelators on biomass production in terms of shoot- root fresh mass (a, b) and dry mass (c, d) in wheat

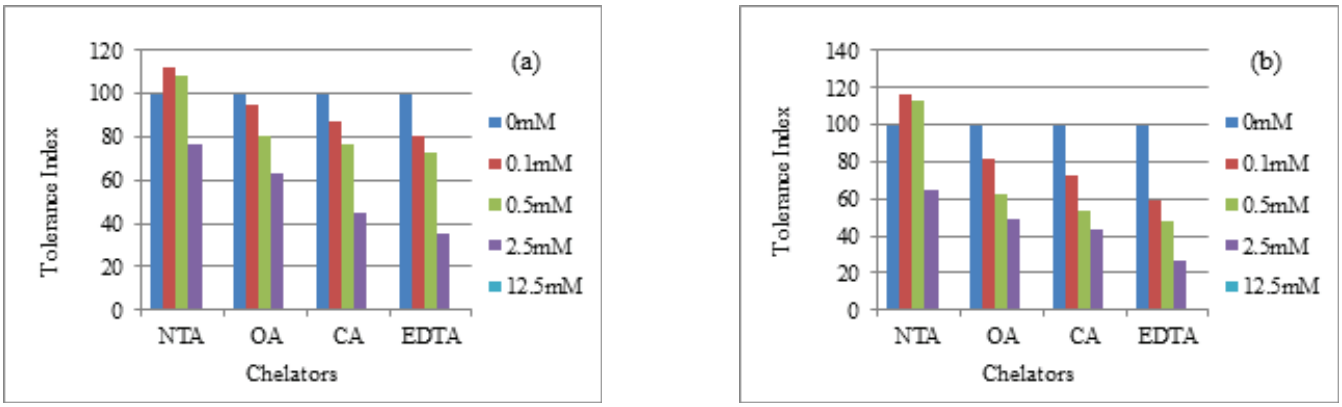


**Fig. 2.** Tolerance index values of wheat shoot (a) and root (b) treated with chelators.

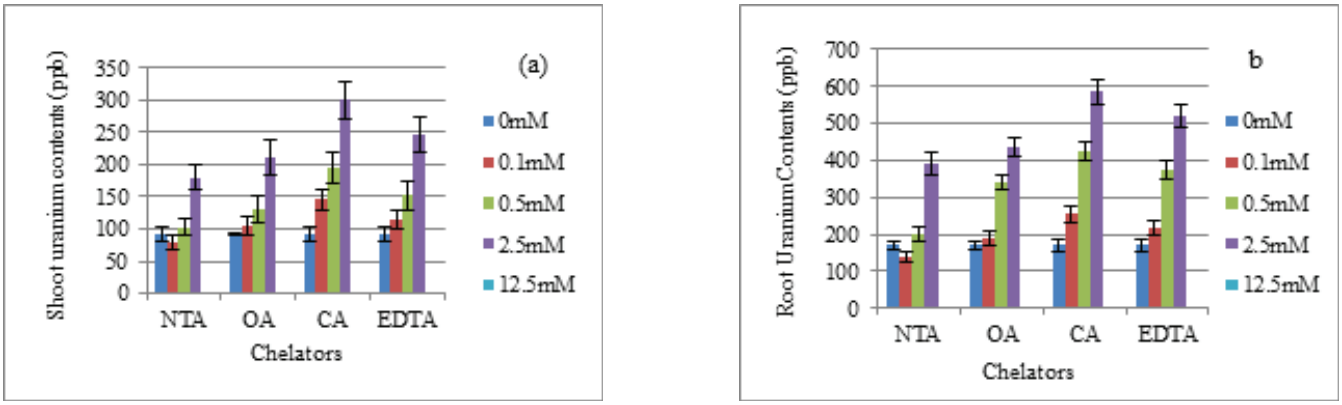


**Fig. 3.** Effect of chelators on accumulation of U by shoot (a) and root (b) in wheat.

significantly.

Outcomes of the present investigations suggest that CA may be the better candidate over EDTA as it is a less toxic, easily biodegradable and has lower leaching risk. The uptake data obtained at the vegetative stage shows more accumulation of U in the roots of plants, while only a little proportion of it was transferred to the shoots. Shahandeh and Hossner (2002) reported that the main portion of U was accumulated in the roots, where its concentration can reach up to quantities 100-fold higher than in the shoots.

## CONCLUSION

The upshots of the present investigations have highlighted the order of chelators for chemically assisted phytoremediation of U. This does not follow the typical order but similar order was reported by Duquéne et al. (2008), who hold the idea that thermodynamic stability constants of complexes did not predict the efficiency of selected amendments on U release in soil solution and they observed CA to be superior then others during the experiment. Notwithstanding, EDTA and NTA are stronger complexion agents than CA but in contrary, the use of CA proved beneficial in U tailing phytoremediation in the present investigation. The growth of the wheat plants was affected in a varied manner by each of the chelator, which in general follows the order: NTA < OA < CA < EDTA, whereas the order for U accumulation and uptake of U during the investigation was recorded as- CA > EDTA > OA > NTA. On the basis of this study it can be suggested that the use CA over EDTA is better, as it is easily biodegradable, less toxic and has lower leaching risk. This is also suggested that verification of the efficiency of these chelators in promoting U uptake by biomass crops for large scale field application programmes.

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