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Technical Note

Optimization of chelators to enhance uranium uptake from tailings for phytoremediation

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HIGHLIGHTS

- ▶ Four chelators (CA, EDTA, OA and NTA) were compared for U phytoremediation.
- ▶ Biomass production, tolerance and U accumulation were the criteria for comparison.
- ▶ Biomass production series during the investigation was as follows – NTA > OA > CA > EDTA.
- ▶ For the uptake and accumulation of U the order of chelators was – CA > EDTA > OA > NTA.
- ▶ CA was found to be the most effective chelator for uranium phytoremediation.

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ABSTRACT

A greenhouse experiment was set up to investigate the ability of citric acid (CA), oxalic acid (OA), nitrilotriacetic acid (NTA) and EDTA for phytoremediation of uranium tailings by Indian mustard [*Brassica juncea* (L.) Czern. *et* Coss]. Uranium tailings were collected from Umra mining region and mixed with 75% of garden soil which yielded a 25:75 mixture. Prepared pots were divided into four sets and treated with following different concentrations – 0.1, 0.5, 2.5 and 12.5 mmol kg^{-1} soil additions for each of the four chelators. Control pots which were not treated with chelators. Experiments were conducted in completely randomized block design with triplicates. The optimum concentrations of these chelators were found on the basis of biomass production, tolerance and accumulation potential. The data collected were expressed statistically. EDTA produced maximum growth depression whereas, minimum occurred in the case of NTA. Maximum U uptake (3.5-fold) in the roots occurred at 2.5 mmol of CA, while NTA proved to be the weakest for the same purpose. Severe toxicity in the form of reduced growth and plant death was recorded at 12.5 mmol of each chelator. Minimum growth inhibition produced by chelators occurred in NTA which was followed by OA, moderate in CA and maximum was traced in EDTA applications. Chelator strengthened U uptake in the present study follows the order: CA > EDTA > OA > NTA.

1. Introduction

In mining, uranium and its decay products buried deep in the Earth are brought to the surface and the rock containing them is crushed into fine sand. After the uranium is chemically removed, the sand is stored in huge reservoirs. These leftover piles of radioactive sand are called ‘uranium tailings’ (Prasad and Freitas, 2003). Uranium mill tailings are normally dumped as sludge in special ponds or piles, known as ‘Tailing Dams’ or ‘Tailing Impoundments’ or ‘Tailing Retention System’ (Mukhopadhyay and Maiti, 2010). Mine tailings are poorly textured with low pH, high concentrations of heavy metals, high electrical conductivity, lack of nutrients and low water retention capacity (Conesa et al., 2009).

U and its compounds are highly carcinogenic and highly toxic, causing acute kidney failure and death in high concentration as well as resulting in brain, liver and heart diseases. Because of this high toxicity, an oral reference dose of 3.0 μg kg^{-1} d^{-1} was recommended by the US Environmental Protection Agency. The minimal risk level for oral exposure was developed by the Agency for Toxic Substances and Disease Registry as 2.0 μg kg^{-1} d^{-1} for an intermediate duration (Craft et al., 2004).

Phytoremediation proves to be a cost effective, safe and superior method for the remediation of contaminated soils (Jagetiya and Sharma, 2007; Nwoko, 2010; Roongtanakiat et al., 2010; Saier and Trevors, 2010). The thriving plants display a particular potential for remediation, they act as natural vacuum cleaners sucking pollutants out of the soil and depositing them in various plant parts (Rajalakshmi et al., 2011).

Certain biomass crops such as *Brassica juncea* (L.) Czern. *et* Coss (Indian mustard) can be utilized for phytoremediation purpose in

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conjunction with chelators for an efficient translocation of metals from soil to plant tissues. Chemically enhanced phytoremediation makes use of chemicals that enhance the mobility of metals in soil. Some organic acids can be added to soil to increase uranium desorption from soil to soil solution and to trigger a rapid uranium accumulation in plants (Laroche et al., 2005). In soils, complexation of heavy metals with various complexing agents may follows the order, EDTA and related synthetic chelators > nitrilotriacetic acid (NTA) > citric acid (CA) > oxalic acid (OA) > acetic acid, as was shown by several comparative experiments (Krishnamurti et al., 1998; Evangelou et al., 2007).

In late 1980s and early 1990s EDTA was suggested as a chelating agent for the assistance of phytoextraction process. In many of the pot experiments described in the literature, the influence of EDTA has ranged from non-significant to over 100-fold enhanced accumulation (Grčman et al., 2001). NTA is a biodegradable chelating agent, which has been used in the last 50 years primarily in detergents. The effect of the addition of NTA on the mobilization and uptake of heavy metals was observed in various studies (Chiu et al., 2005; Quartacci et al., 2005). Natural low molecular weight organic acids (NLMWOAs), such as CA, OA or malic acid (MA), because of their complexing properties are of particular importance and play a significant role in heavy metal solubility and plant uptake (Nigam et al., 2001; Qu et al., 2011).

The aim of this study is to assess the differential phytoextraction influence of chelators on Indian mustard for the elimination of uranium under defined chemical conditioning of soil. The outcomes of the investigation will be useful to compare the effect of various concentrations of chelators as potential soil amendments for enhancing phytoremediation of uranium by *B. juncea* (L.) Czern. *et* Coss.

2. Materials and methods

2.1. Soil preparation and plant parameters

U tailings were collected from Umra mining region and mixed with garden soil in the ratio of 25:75. Physical and chemical properties of garden soil were as follows: pH (H_2O) 7.31 ± 0.07, conductivity, 0.79 ± 0.09 S m^{-1}, biological carbon 0.60 ± 0.31%, phosphate 28 ± 5.70 kg ha^{-1} and high potash (>348 kg ha^{-1}). The mixture was then allowed to equilibrate for a period of 4 weeks and 3 kg of the mixture were filled in the earthen pots (physical and chemical properties of this mixture are presented in Table 1). During the experiment, amended pots were prepared by adding – 0.1, 0.5, 2.5 and 12.5 mmol kg^{-1} soil, for each of the four chelators, whereas one set of pots was not amended with any chelator, and kept as control. The design of the experiment employed three replicates for each treatment in a completely randomized block design. Ten seeds of *B. juncea* (L.) Czern. *et* Coss were sown equidistantly at the depth of 2.5 cm. Pots were watered as recommended in agronomic practices. Collection trays were placed beneath each pot in order to retain any leachate, which was re-applied immediately to the same pot. The observations were recorded during the vegetative stage. Most effective concentration of the chelators was found on the basis of biomass production, tolerance and accumulation potential. Effects of different concentrations of chelators were noted on growth parameters, which includes, shoot–root length and shoot–root fresh as well as dry mass. Uranium accumulation in different plant parts was recorded through pellet fluorometric method.

Table 1
Showing various physical and chemical properties of uranium tailings (Umra region, Udaipur) amended with garden soil (25:75).

Parameter	Value
pH (H_2O)	7.78–7.91
Conductivity (S m^{-1})	0.76 ± 0.12
Biological carbon (%)	0.58 ± 0.10
Phosphate (kg ha^{-1})	24 ± 6.65
Potash (kg ha^{-1})	>348
Uranium concentration (μg g^{-1})	36 ± 9.50

Results are the mean of three replicates.

2.2. Statistical analysis

The data of growth performance and uranium uptake were subjected to statistical analysis for the computation of differences between control and amended pots. Mean values based on three replicates was calculated. Differences between treatments were considered significant and highly significant at $p = 0.05$ and 0.01, respectively.

3. Results

The results (Figs. 1–3) obtained from the study indicates that CA was superior than EDTA, OA and NTA for chelator assisted phytoremediation. 2.5 mmol of each chelator was found to be the suitable concentration, whereas 12.5 mmol showed a depression in the growth of Indian mustard. Application of 2.5 mmol of CA can produce several fold increase in uranium accumulation in roots as well as translocation to the shoots.

3.1. Effect of chelators on the growth performance

3.1.1. Growth parameters

Growth response of Indian mustard in terms of shoot–root length, shoot–root fresh mass as well as dry mass when amended with chelators is illustrated in Fig. 1. All the growth parameters at 0.1 and 0.5 mmol of NTA showed an enhancement in comparison to the control. Higher levels of NTA (2.5 and 12.5 mmol) caused mild to severe toxicity. OA, CA and EDTA at all their application rates proved toxic. OA produced 35–42%; 43–53%; 35–40% reduction in the above growth parameters at 2.5 mmol. CA stood third in the series, which reduced the growth at a value of 42–47%; 56–60%; 40–51%, respectively for shoot–root length, fresh as well as dry mass. EDTA was harshest towards the growth performance of test crop. Highest application rate resulted into plant death while 2.5 mmol produced decrease in all the observed parameters with a value of 55–69%; 75–83%; 70–79%.

3.1.2. Tolerance index

The tolerance index (TI) of Indian mustard differed according to the chelator treatments used during the investigation (Fig. 2). Minimum values for TI were reported in the case of EDTA. The TI values for OA as well as CA ranged between NTA and EDTA. The resultant order for TI values is as follows: NTA > OA > CA > EDTA.

3.2. Uranium uptake

Fig. 3 displays the data for uranium uptake in different plant parts of Indian mustard. The efficiency of chelators follows the order: CA > EDTA > OA > NTA. Higher accumulation occurred in the roots in comparison to the shoots.

4. Discussion

Several authors investigated amendments to enhance uptake of U and other heavy metals by plants. The complexing agents tested were synthetic aminopolycarboxylic acids (APCAs) including EDTA