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Effect of biodegradable chelant nitrilotriacetic acid on lead (pb) phytoextraction efficiency of *coronopus didymus* grown in pb-contaminated soil

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ABSTRACT

The current study investigated the use of nitrilotriacetic acid (NTA), a biodegradable chelating agent, on the phytoextraction of lead (Pb) from soil using Coronopus didymus. The plants were exposed to three different Pb concentrations: 3500, 4500, and 5500 mg kg^{-1} alone and in combination with 10 mM kg^{-1} of NTA. The morphological traits such as plant growth and biomass, the Bioconcentration Factor (BCF) values, and Pb accumulation in roots, stems, and leaves were measured. The plant growth and dry biomass were found to be maximum at 3500 mg kg^{-1} Pb and minimum at 5500 mg kg^{-1} Pb as compared to the control under Pb alone treatments and in combination with NTA. The supplementation of NTA significantly enhanced the Pb accumulation in different plant parts and was in the order: roots > stems > leaves. Compared to the BCF values of stems and leaves, the BCF_{roots} values, which ranged from 89.3 to 257, were highest for all the treatments. NTA application resulted in the highest BCF value at 3500 mg kg^{-1} Pb concentration in roots. The present study suggests that the use of NTA as a chelating agent significantly increased the ability of C. didymus plants to remove Pb from the environment. This approach can be widely used for efficient remediation of contaminants from the metal-contaminated soils.

Keywords: Biodegradable chelants, bioconcentration factor, metal accumulators, nitrilotriacetic acid, phytoremediation

INTRODUCTION

Heavy metals are the transition metals that have atomic mass and specific gravity above 20 and 5, respectively. These are the major contaminants of the soil throughout the world as they pose serious health issues to humans and spread largely over the globe (Rascio *et al.*, 2011). Lead (Pb) is the 2nd most toxic heavy metal after arsenic (Ji *et al.*, 2022). Due to its physical properties and low cost, Pb and its related compounds are present in different products such as batteries, pipes, ceramics, cosmetics, poly-vinyl chloride (PVC) toys, gasoline, and paints (Njati and Maguta, 2019). Removal of heavy metals from the environment requires high cost and an advanced equipment system (Yao *et al.*, 2012). Also, the use of chemical and physical methods for the remediation of soil degrades the quality of soil by negatively impacting the activity of useful microorganisms, thereby making it less suitable for the plant growth (Ghosh and Singh, 2005). Phytoremediation is one of the bioremediation methods that involve an engineering incorporation of green plants for the remediation of toxic heavy metals from the soil (Balkrishna *et al.*, 2022). Successful phytoremediation process involves a hyper-accumulator plant which uptakes high levels of heavy metal from the contaminated soil to the roots and translocate it to their shoots (Mahar *et al.*, 2020). Plants of the genus *Brassica* have an inherent potential to accumulate heavy metals in their tissues (Diarra *et al.*, 2021). *Coronopus didymus* (L.) Sm. Brassicaceae) is a wild, unpalatable herb of greater biomass, native to South America, but is broadly dispersed all over the globe. So, keeping in mind the capability of Brassicaceae plants in hyper-accumulation of heavy metals, *C. didymus* was selected for the present study. The phytoremediation efficiency of a plant is limited by factors such as low accessibility and transferability of metals from the soil. To overcome these difficulties several chelating agents which include biodegradable and non-biodegradable have been used (Yan *et al.*, 2017).

In the present study, it is proposed to evaluate nitrilotriacetic acid (NTA), another synthetic but biodegradable chelating agent, for the phytoextraction of Pb by *C. didymus*. NTA is

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highly biodegradable and is metabolized to CO_2, H_2O, and inorganic N by microorganisms (Thompson and Duthie, 1968). The key objectives of the study were to investigate (a) the effect of Pb alone or in combination with NTA on the morphological parameters like growth and biomass of the test plant, and b) the role of NTA in enhancing uptake and accumulation of Pb in leaves, stems, and roots by *C. didymus* from Pb-spiked soils. The results of the study will be helpful to understand the efficacy of NTA as a chelating agent in removal of Pb from polluted soils using *C. didymus*.

MATERIAL AND METHODS

Soil treatments and *Coronopus didymus* plant transplantation

Soil was collected from 0-15 cm depth from an unpolluted area in Panjab University, Chandigarh. It was air dried and sieved using 2 mm sieve. The sieved soil was mixed with sand and cow dung manure in the ratio 2:1:1, respectively. Pb-contaminated soil (3500 mg kg^{-1}, 4500 mg kg^{-1}, and 5500 mg kg^{-1}) was prepared by supplying $Pb(NO_3)_2$. For two-weeks, the contaminated soil was drenched with distilled water and air dried for another two weeks. Soil was filled in 1 kg capacity polypropylene pots. For seedling preparation of *C. didymus*, fifteen-days-old seedlings of similar sizes were collected from an unpolluted area. Seedlings were immediately washed with distilled water and transplanted to the plastic trays containing 10 kg of wet sand. After one week, seedlings were transferred into the pots containing 1 kg of artificially contaminated soil and allowed to grow under an average day and night temperature of 19–26ºC and 7–14ºC, respectively (Sharma *et al.*, 2022).

Plants were allowed to grow for six weeks in Pb-contaminated soil. NTA was supplied @ 10 mM kg^{-1} to the plants in the solution form, and these were further allowed to grow for seven days. There were three replicates of each treatment and in total there were eight treatments: T_1: C (control); T_2: C + NTA; T_3: $Pb_{3500;}$ T_4: Pb_{3500}+NTA; T_5: $Pb_{4500;}$ T_6: Pb_{4500}+NTA; T_7: Pb_{5500}; T_8: Pb_{5500}+NTA representing 0, 3500, 4500 and 5500 mg kg^{-1} Pb concentration with and without NTA, respectively. The whole experiment was carried out under experimental dome conditions. Plants were harvested after 42 days of treatment and washed gently with deionized water. The growth variables such as root length, shoot length, above-ground (shoot) and below-ground (root) dry biomass were measured (Sharma *et al.*, 2022). One hundred milligrams of dried leaf, stem, and root powder of plants grown in each treatment were digested separately using nitric acid (HNO_3) and perchloric acid ($HClO_4$) mixture (4:1, *v/v*) (10 ml) at 150ºC until the white-colored fumes were formed. The digested material was filtered through Whatman #42 and raised to 20 ml by adding distilled water (Sharma *et al.*, 2022). Pb concentration in leaves, stem, and roots was estimated using atomic absorption spectrophotometer (AAS) (LABINDIA, AA8000) at 244.8 nm wavelength and expressed in terms of mg kg^{-1} dry weight.

The mobility of heavy metals and their accumulation in plants can be determined by Bioconcentration factor (BCF) values (Parven *et al.*, 2022). BCF represents the quantitative measurement of heavy metals that are transferred to the plant from the soil (Soriano-Disla *et al.*, 2014).

$BCF_{roots} = C_{root}/C_{soil}$

$BCF_{stems} = C_{stems}/C_{soil}$

$BCF_{leaves} = C_{leaves}/C_{soil}$

Where, BCF_{roots}, BCF_{stems} and BCF_{leaves} represent Bioconcentration factor for roots, stems, and leaves, respectively. Further, C_{root}, C_{stems}, C_{leaves} and C_{soil} represent Pb concentration in root, stem, leaves and soil, respectively.

Data was analysed by one-way Analysis of Variance (ANOVA). Comparison of means for different treatments was done by applying post hoc Tukey's test at $P≤0.05$. All the statistical analysis was conducted using SPSS software package (ver. 16.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Plant Growth

Root and shoot length were significantly *(P≤0.05)* decreased with the increasing Pb treatments, over the control (Table 1). At 3500, 4500 and 5500 mg kg^{-1} Pb concentrations, the root length was reduced by 11.5%, 39%, and 66%, respectively, with reference to the control. Further, the addition of NTA to all the three treatments, i.e., 3500, 4500 and 5500 mg Pb kg^{-1}

significantly decreased the root length of *C. didymus* by 10%, 54% and 76%, respectively, over the control. Similarly, at 4500 and 5500 mg kg^{-1} Pb treatments, the decrease in shoot length was 26% and 55%, respectively, over the control. However, a significant *($P \leq 0.05$)* increase of 50% was noticed at 3500 mg kg^{-1} Pb concentration, as opposed to the control. Upon addition of NTA to 3500 mg kg^{-1} Pb concentration, a significant rise *($P \leq 0.05$)* in shoot length was noticed, whereas at 4500 and 5500 mg kg^{-1} Pb levels, the shoot length was decreased by 5.4% and 39.3%, respectively, as compared to the control. NTA application has positive effect on shoot length of *C. didymus* plants when set against the plants exposed to Pb only treatment. Compared to the 3500, 4500 and 5500 mg kg^{-1} Pb treatments alone, the supplementation of NTA increased the shoot length by 16%, 28% and 34%, respectively (Table 1). The results are in line with those of Zhang *et al.* (2005) who observed that Pb toxicity significantly reduced the shoot and root length of rice seedlings. The possible reason for the reduced growth might be due to the non-selective inhibition of both cell elongation and division in the tissues (Eun *et al.*, 2000).

Table 1: Variation in different plant growth parameters of *Coronopus didymus under treatments* of lead (Pb) alone and in combination with nitrilotriacetic acid (NTA)

Treatments (mg kg^{-1})	Root length (cm)	Shoot length (cm)	Dry root biomass (g)	Dry shoot biomass (g)
Control	35.0 ± 0.33a	20.3 ± 0.88b	0.44 ± 0.003a	1.0 ± 0.09c
Pb_{3500}	31.0 ± 0.60b	30.5 ± 0.60a	0.31 ± 0.013c	3.1 ± 0.06a
Pb_{4500}	21.3 ± 0.73c	15.0 ± 0.93bc	0.20 ± 0.007e	1.7 ± 0.32b
Pb_{5500}	12.0 ± 0.88e	9.2 ± 0.73d	0.04 ± 0.003f	0.3 ± 0.03d
Control + NTA	35.1 ± 0.01a	22.0 ± 0.67b	0.45 ± 0.003a	2.1 ± 0.07b
Pb_{3500} + NTA	31.5 ± 0.30b	35.4 ± 0.52a	0.38 ± 0.007b	3.3 ± 0.07a
Pb_{4500} + NTA	16.0 ± 0.30d	19.2 ± 0.40b	0.25 ± 0.003d	1.6 ± 0.09b
Pb_{5500} + NTA	8.50 ± 0.29f	12.3 ± 0.15cd	0.06 ± 0.006f	0.1 ± 0.01d

Data represented as Mean±SE; and means followed by a different small letter within a column indicate significant differences among treatments at $P \leq 0.05$.

Plant biomass

Dry root biomass of the plant under the 3500, 4500 and 5500 mg kg^{-1} Pb concentrations was decreased by 29.5%, 54.5% and 91%, respectively, over the control (Table 1). On the contrary, addition of NTA enhanced the dry root biomass, as compared to Pb alone treatments. However, with reference to the control, Pb+NTA treatment declined the dry root biomass by 13.6%, 43.2% and 86.4% at 3500, 4500 and 5500 mg kg^{-1} Pb concentration, respectively. The dry shoot biomass of the plant under 3500 mg kg^{-1} Pb concentration was increased significantly *($P \leq 0.05$)* by 3.1 folds, with respect to the control. However, at 4500 and 5500 mg kg^{-1} Pb treatments the dry shoot biomass was significantly decreased. Dry shoot biomass was decreased by 3.3 folds in 5500 mg kg^{-1} Pb treatment. Supplementation of NTA also showed a similar trend with the increased Pb concentrations, i.e., a significant rise at 3500 mg kg^{-1} Pb concentration whereas, a gradual decrease at higher concentrations, against the control. At 5500 mg kg^{-1} Pb concentration, the addition of NTA decreased the dry shoot biomass by 10 folds, over the control (Table 1). It is worthwhile to understand that the supplementation of NTA markedly increased the dry shoot and root biomass of the plants under Pb stress, when compared against the Pb only treatments (Table 1).

Similar to our study, Yang *et al.* (2022) reported a significant increase in the biomass of *Sasa argenteostriata* under Pb stress when supplemented with NTA as a chelating agent. The degradation product of NTA is NH_3 which is required for plant's growth and development (Wang *et al.*, 2019). Thus, this might be the reason for the increased dry root and shoot biomass of the plant.

Accumulation of Pb in plants

In general, Pb accumulation in *C. didymus* was in the order roots > shoots > RIYA leaves. The accumulation of Pb was found to be 6465.3, 8030.2, and 2758 mg kg^{-1} DW in the roots at 3500, 4500, and 5500 mg kg^{-1} Pb concentrations, respectively (Table 2). Similar trends, such as increased Pb content at Pb_{4500}+chelant treatment and reduced Pb content values at Pb_{5500}+chelant treatment, were observed in roots with the addition of NTA. The amounts of Pb detected at the Pb_{3500}+NTA, Pb_{4500}+NTA, and Pb_{5500}+NTA treatments were 7748.3, 7969.8, and 5248.3 mg kg^{-1} DW, respectively. In comparison to the plant's roots, the stems had a lower Pb concentration. The amount of Pb accumulated in the stems was 754.5, 1696.6, and 1135.5 mg kg^{-1} DW, respectively, at 3500, 4500, and 5500 mg kg^{-1} Pb concentrations. This quantity was further enhanced with the addition of NTA, and the observed values were 1577.9, 4642.5, and 2266.7 mg kg^{-1} DW, respectively (Table 2). Pb accumulation in leaves was 234, 1148.3, and 528.2 mg kg^{-1} DW at 3500, 4500, and 5500 mg kg^{-1} Pb concentrations, respectively. The presence of NTA enhanced the concentration of Pb in the leaves, which was 367.9, 790.3, and 794 mg kg^{-1} DW at doses of 3500, 4500, and 5500 mg kg^{-1} of Pb, respectively (Table 2). Because Pb has a positive charge, it can connect to negatively charged sites in the cell walls of roots, which promotes its absorption in the root portion (Yang *et al.*, 2005). Greater accumulation of heavy metal by the addition of NTA has already been observed in *Siegesbeckia orientalis* during the study of efficiency of some biodegradable chelants (Lan *et al.*, 2013).

Table 2: Metal accumulation potential of *Coronopus didymus under treatments* of lead (Pb) alone and in combination with nitrilotriacetic acid (NTA)

Treatments	Pb content in roots (mg kg^{-1} DW)	Pb content in stems (mg kg^{-1} DW)	Pb content in leaves (mg kg^{-1} DW)	BCF_{roots}	BCF_{stems}	BCF_{leaves}
Pb_{3500}	6465.3 ± 120.7b	754.5 ± 14.9e	234.0 ± 1.2de	194.1 ± 13.1b	22.7 ± 1.5d	7.0 ± 0.6e
Pb_{4500}	8030.2 ± 142.5a	1696.6 ± 78.2c	1148.3 ± 52.2a	239.4 ± 5.2a	50.8 ± 4.3bc	34.2 ± 0.2a
Pb_{5500}	2758.0 ± 115.5d	1135.5 ± 22.3de	528.2 ± 55.4c	92.5 ± 7.4c	38.0 ± 2.2c	17.5 ± 1.2c
Pb_{3500} + NTA	7748.3 ± 264.4a	1577.9 ± 5.8cd	367.9 ± 26.1cd	257.0 ± 12.0a	52.2 ± 0.5b	12.2 ± 1.0d
Pb_{4500} + NTA	7969.8 ± 17.5a	4642.5 ± 217a	790.3 ± 27.9b	256.0 ± 6.2a	148.9 ± 4.2a	25.4 ± 0.5b
Pb_{5500} + NTA	5248.3 ± 196.0c	2266.7 ± 134.2b	793.6 ± 42.8b	89.3 ± 6.6c	38.4 ± 2.6c	13.3 ± 1.2d

Data represented as Mean±SE. Means followed by a different small letter indicate significant differences among treatments at P≤0.05.

Bioconcentration Factor (BCF_{roots}, BCF_{stems}, BCF_{leaves})

Under a 4500 mg kg^{-1} Pb treatment, the highest BCF values for roots, stems, and leaves were observed and measured as 239.4, 50.8, and 34.2, respectively (Table 2). The BCF_{roots} values were highest at all Pb treatments when compared to the stem and leaf BCF values. This merely denotes that the plant's roots acquired larger levels of Pb. Additionally, greater Pb accumulation was observed at 4500 mg kg^{-1} Pb treatment (Table 2). While the BCF values for the roots, stems, and leaves significantly increased when NTA was supplemented at 3500 and 4500 mg kg^{-1} Pb, they did not enhance at 5500 mg kg^{-1} Pb compared to the Pb alone treatments (Table 2). The impact of NTA in raising the BCF values just highlighted its significance in strengthening the ability of *C. didymus* ability to remove metal from polluted soil. The outcomes were consistent with the findings of Arán *et al.* (2017) who highlighted that the plant *Limnobium laevigatum* had greater BCF values when exposed to various Pb concentrations. The results of the present study demonstrated that the plant *C. didymus* can be utilised in combination with NTA, a biodegradable chelating agent, for the remediation of Pb-contaminated soils, and that it can be regarded as a potential Pb accumulator.

It may be concluded that the potential of *C. didymus* plant in combination with organic chelating agent such as NTA in the phytoextraction of Pb from the soils was examined in this study. The plant accumulated higher amounts of Pb in its tissues such as roots, stems and leaves. The Bioconcentration factor values were also greater than one and were more pronounced in the roots as compared to the stems and leaves of *C. didymus* plants. The study highlights the application of NTA as a chelating agent with *C. didymus* plants for the remediation of Pb contaminated soil as a promising approach to reduce metal toxicity in humans, plants, and animals. However, while the plant reveals excellent Pb accumulation capability, further research is necessary to establish its suitability for phytoremediation under actual field conditions.

REFERENCES

Arán, D.S., Harguinteguy, C.A., Fernandez-Cirelli, A. and Pignata, M.L. (2017) Phytoextraction of Pb, Cr, Ni, and Zn using the aquatic plant *Limnobium laevigatum* and its potential use in the treatment of wastewater. *Environmental Science and Pollution Research* **24**: 18295-18308.

Balkrishna, A., Dabhade, N.R., Sharma, N., Sharma, J., Mishra, S. and Arya, V. (2022) Phytoremediation: Current Techniques and Futuristic Opportunities. *Indian Journal of Ecology* **49**: 1057-1067.

Diarra, I., Kotra, K. K. and Prasad, S. (2021) Assessment of biodegradable chelating agents in the phytoextraction of heavy metals from multi–metal contaminated soil. *Chemosphere* **273**: 128483.

Eun, S.O., ShikYoun, H. and Lee, Y. (2000) Lead disturbs microtubule organization in the root meristem of Zea mays. *Physiologia Plantarum* **110**: 357-365.

Ghosh, M. and Singh, S.P. (2005) A review on phytoremediation of heavy metals and utilization of it's by products. *Asian Journal on Energy Environment* **6**: 214-231.

Ji, X., Wan, J., Wang, X., Peng, C., Wang, G., Liang, W. and Zhang, W. (2022) Mixed bacteria-loaded biochar for the immobilization of arsenic, lead, and cadmium in a polluted soil system: effects and mechanisms. *Science of the Total Environment* **811**: 152112.

Lan, J., Zhang, S., Lin, H., Li, T., Xu, X., Li, Y., Jia, Y. and Gong, G. (2013) Efficiency of biodegradable EDDS, NTA and APAM on enhancing the phytoextraction of cadmium by *Siegesbeckia orientalis* L. grown in Cd-contaminated soils. *Chemosphere* **91**: 1362-1367.

Mahar, A., Ali, A., Lahori, A.H., Wahid, F., Li, R., Azeem, M., Fahad, S., Adnan, M., Rafiullah., Khan, I.A. and Zhang, Z. (2020) Promising Technologies for Cd-Contaminated Soils: Drawbacks and Possibilities. *In:* Fahad, S, Hasanuzzaman, M, Alam, M, Ullah, H, Saeed, M, Khan, I.A. and Adnan, M. *(eds.) Environment, Climate, Plant and Vegetation Growth*. Springer, Cham, 63-91. DOI: 10.1007/978-3-030-49732-3_3

Njati, S.Y. and Maguta, M.M. (2019) Lead-based paints and children's PVC toys are potential sources of domestic lead poisoning–A review. *Environmental Pollution* **249**: 1091-1105.

Parven, S., De, A. and Gupta, A. (2022) Cu and Pb accumulation and removal from aqueous medium by *Enydra fluctuans* Lour. (Asteraceae)—a medicinal plant with potential for phytoremediation. *Environmental Science and Pollution Research* **1**-11. https:// doi.org/10.1007/s 11356-022-20483-9

Rascio, N. and Navari-Izzo, F. (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Science* **180**:169-181.

Sharma, P., Rathee, S., Ahmad, M., Batish, D.R., Singh, H.P. and Kohli, R.K. (2022) Biodegradable chelant-metal complexes enhance cadmium phytoextraction efficiency of *Solanum americanum*. *Environmental Science and Pollution Research* **29**: 57102–57111.

Soriano-Disla, J.M., Gómez, I., Navarro-Pedreño, J. and Jordán, M.M. (2014). The transfer of heavy metals to barley plants from soils amended with sewage sludge with different heavy metal burdens. *Journal of Soils and Sediments* **14**: 687-696.

Thompson, J.E. and Duthie, J.R. (1968) The biodegradability and treatability of NTA. *Journal of Water Pollution Control Federation* **40**: 306-319.

Wang, K., Liu, Y., Song, Z., Wang, D. and Qiu, W. (2019) Chelator complexes enhanced *Amaranthus hypochondriacus* L. phytoremediation efficiency in Cd-contaminated soils. *Chemosphere* **237**: 124480.

Yan, L., Li, C., Zhang, J., Moodley, O., Liu, S., Lan, C., Gao, Q. and Zhang, W. (2017) Enhanced phytoextraction of lead from artificially contaminated soil by *Mirabilis jalapa* with chelating agents. *Bulletin of Environmental Contamination and Toxicology* **99**: 208-212.

Yang, X., Feng, Y., He, Z. and Stoffella, P.J. (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. *Journal of Trace Elements in Medicine and Biology* **18**: 339-353.

Yang, Y., Liao, J., Chen, Y., Tian, Y., Chen, Q., Gao, S., Luo, Z., Yu, X., Lei, T. and Jiang, M. (2022) Efficiency of heterogeneous chelating agents on the phytoremediation potential and growth of *Sasa argenteostriata* (Regel) EG Camus on Pb-contaminated soil. *Ecotoxicology and Environmental Safety* **238**: 113603.

Yao, S., Lai, H. and Shi, Z. (2012) Biosorption of methyl blue onto tartaric acid modified wheat bran from aqueous solution. *Iranian Journal of Environmental Health Science & Engineering* 9: 1-6.

Zhang, S., Hu, J., Chen, Z.H., Chen, J.F., Zheng, Y.Y. and Song, W.J. (2005) Effects of Pb pollution on seed vigor of three rice cultivars. *Rice Science* **12**: 197-202.