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Appraising the role of environment friendly chelants in alleviating lead by *Coronopus didymus* from Pb-contaminated soils

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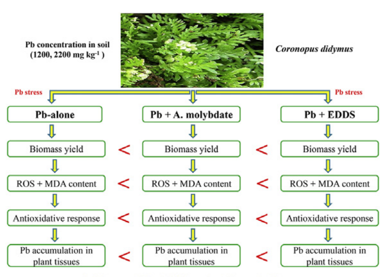
HIGHLIGHTS

- Two eco-friendly chelants, A. molybdate and EDDS were compared for Pb accumulation and extraction by *C. didymus.*
- Evaluation of Pb uptake and accumulation, biomass yield, photosynthetic activity, were the criteria for comparison.
- *C. didymus* accumulated Pb more in roots than shoots.
- EDDS was more effective in solubilising and mobilising Pb than A. molybdate.
- *C. didymus* showed good efficacy to remediate Pb-contaminated soils.

GRAPHICAL ABSTRACT



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ABSTRACT

In a screenhouse experiment, we investigated the role of two environment friendly chelants, Ammonium molybdate and EDDS for Pb mobilisation and its extraction by *Coronopus didymus* under completely randomized controlled conditions. Seedlings of *C. didymus* were grown in pots having Pb-contaminated soil (1200 and 2200 mg kg^{-1}) for 6 weeks. Plants were harvested, 1 week after the addition of A. molybdate and EDDS. Results revealed that A. molybdate and EDDS enhanced the uptake and accumulation of Pb in roots and shoots of *C. didymus*. At 2200 mg kg^{-1} Pb level, compared to Pb-alone treatment, the maximal concentration of Pb was increased upto ~10% and ~19%, in roots whereas ~8% and ~18%, respectively, in shoots on addition of 2 mmol kg^{-1} A. molybdate and EDDS. Additionally, Pb + EDDS treatments enhanced the plant biomass and triggered strong antioxidative response, more efficaciously than Pb + A. molybdate and Pb-alone treated plants. In this study, EDDS relative to A. molybdate was more efficient in mobilising and extracting Pb from soil. Although, EDDS followed by A. molybdate had good efficacy in mitigating Pb from contaminated soils but *C. didymus* itself has the

Abbreviations: A. molybdate, ammonium molybdate; BCF, bioconcentration factor; CAT, catalase; EDDS, S,S-ethylenediaminedisuccinic acid; H_2O_2, hydrogen peroxide; LPO, lipid peroxidation; MDA, malondialdehyde; PCs, phytochelatins; ROS, reactive oxygen species; SOD, superoxide dismutase; $O_2^{\bullet-}$, superoxide ion; TF, translocation factor.

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Lead Mobilisation

inherent affinity to tolerate and accumulate Pb from contaminated soils and hence in future, can be used either alone or with some other eco-friendly amendments for soil remediation purposes.

1. Introduction

Soil contamination due to heavy metal toxicity is a worldwide problem. During the last few decades, the onset of developing countries on the global market has accelerated the release of heavy metal contaminants in the environment (Baldantoni et al., 2014). Anthropogenic activities like electroplating, metallurgical processes, extensive use of fertilizers, pesticides, sewage sludge and faulty mining activities contribute to the discharge of lead (Pb) in the environment. Pb, a heavy metal has no biological relevance to plants and its toxicity can exhibit seed dormancy, stunted growth, lessened chlorophyll content (Shahid et al., 2012) and even death at elevated levels. Pb toxicity in plants induces oxidative stress (Sidhu et al., 2016) and thus imparts pessimistic effect on photosynthesis, antioxidative response and nutrient uptake (Shakoor et al., 2014). Therefore, there is a critical need to device and use environment friendly techniques for the remediation and detoxification of Pb-contaminated soils.

Conventional cleanup techniques like excavation, physical separation, stabilisation, electrochemical processes and disposal to a landfill site are often expensive and laborious (Gómez-Sagasti et al., 2012). Alternatively, phytoremediation is a reliable, *in situ*, cost-effective technology in which green plants are exploited to remediate and lessen the impact of hazardous contaminants in the environment. Due to the intrinsic potential, members of Brassicaceae family, having genus *Alyssum*, *Thlaspi* and *Brassica* have been employed for remediation of soils contaminated with heavy metal (Anjum et al., 2014). Therefore, it is pertinent to explore wild plant species of Brassicaceae family that are tolerant towards heavy metal stress. Thus *C. didymus* (35–45 cm), a wild, fast growing, annual herb with abundant biomass was selected for the present work. It is commonly known as lesser swine–cress having profusely branched root and shoot system. This annual herb has a wide distribution throughout the world and is a native of South America. It grows during the winters (October–February) along the roadsides, vacant areas, plots and gardens in northern parts of India.

The efficacy of high biomass yielding plant species for metal extraction can be incremented with the assistance of synthetic chelants (Meers et al., 2009). Among synthetic chelants, EDTA is the most effective chelant used to solubilise metals in soil and enhance metal uptake and accumulation in plants (Cutright et al., 2010). However, its persistent nature due to poor degradability, having half-life of 6 months in soil and leaching poses serious environmental concerns (Meers et al., 2009; Zhao et al., 2011). Besides, commercial field application of EDTA is indecorous due to inadmissible toxicity and tenacity in the ecosystem. Keeping in mind the pessimistic effects of synthetic chelants, two environment friendly chelants, Ammonium molybdate and EDDS have been used to enhance Pb uptake and accumulation from the Pb-contaminated soils. The half-life of both the chelants is short and the [S,S]-isomer of EDDS easily degrades in the soil (Koopmans et al., 2008). EDDS mineralises within 2–8 days in the soil (Yang et al., 2013). The dissociation of A. molybdate generates NH^{4+} that could chelate with heavy metals and form soluble fractions as chelating complexes. The chelant assisted solubilisation enhances the bioavailability of heavy metals in the soil and thus, promote the uptake and accumulation of heavy metal contaminants. According to Qu et al. (2011), A. molybdate facilitates the removal of Cd, Ni and Cu from multi-metal polluted soils by forming soluble fractions. In previous findings, EDDS was reported to initiate the solubilisation and mobilisation of Cu, Zn, Ni and Pb in the soils (Koopmans et al., 2008).

To the best of our knowledge, no previous study has illustrated the role of A. molybdate and EDDS in ameliorating the uptake and accumulation of Pb by *C. didymus*. Herein, the main objectives of the present work were to evaluate (i) the efficacy of eco-friendly chelants (A. molybdate and EDDS) to enhance Pb uptake and accumulation by *C. didymus* from Pb-contaminated soils (ii) the effect on plant biomass, photosynthetic machinery, oxidative status and antioxidative response towards Pb stress (iii) the role of chelants on *C. didymus* for alleviating Pb from contaminated soils. The findings of the current work will be useful to equate the potency of these chelants for enhancing the remediation and mitigation of Pb by *C. didymus* plants from contaminated soils.

2. Material and methods

2.1. Plant material and soil samples

The seeds of *C. didymus* and soil samples were collected locally from a non-contaminated site at Panjab University campus, Chandigarh, India. Surface sterilised seeds (sodium hypochlorite) were sown in plastic tray having 10 kg soil in a screenhouse. Soil specimens were collected from peripheral layer (0–20 cm), mixed with cow dung manure (soil and manure ratio 5:1), air dried and sieved through 2 mm mesh. The selected soil was sandy loam having pH 6.69 ± 0.07, electrical conductivity 139.9 ± 1.51 μS, organic carbon $0.98 \pm 0.04\%$ and organic matter content $1.68 \pm 0.07\%$. In polythene bags, 1 kg soil was filled and kept in plastic pots. The soils were spiked by two levels of Pb, 1200, 2200 mg kg^{-1} supplied as lead nitrate [$Pb(NO_3)_2$]. Spiked soils were incubated for 2 weeks.

2.2. Experimental setup

In a screenhouse, 15 day old seedlings having uniform size (4–6 cm long shoots and 3–6 cm long roots) were transplanted in Pb-spiked soils. After transplantation into individual pots, plants were watered as required until harvest. There were four replicates (pots) for each treatment having five plants per pot. Dissolved 2 mmol kg^{-1} A. molybdate and EDDS were smeared to soils, 5 weeks after sowing. The pots were arranged in a completely randomized manner as follows: C (control), C + A [A. molybdate (2 mmol kg^{-1})], C + E [EDDS (2 mmol kg^{-1})], Pb_{1200}, Pb_{1200}+A, Pb_{1200}+E, Pb_{2200}, Pb_{2200}+A, Pb_{2200}+E, where, (A and E) refers to A. molybdate and EDDS, respectively. Controls were set up without lead nitrate (C) and containing the same concentration of A. molybdate and EDDS (2 mmol kg^{-1}). Control soils were fertilised with 200 mg N kg^{-1} soil supplied as ammonium nitrate (NH_4NO_3) so as to normalise the effect of nitrates present in Pb salt. Plants were harvested, 1 week after A. molybdate and EDDS additions. Plant root-shoot tissues were rigorously rinsed thrice in 0.2 mM $CaSO_4$, washed for approximately 3 min with distilled water and then oven dried at 75 °C for 72 h. The dried plant tissues were weighed, recorded, grounded to powder and sieved through 2 mm stainless mesh.