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Ethylenediamine disuccinic acid enhanced phytoextraction of nickel from contaminated soils using *Coronopus didymus* (L.) Sm.

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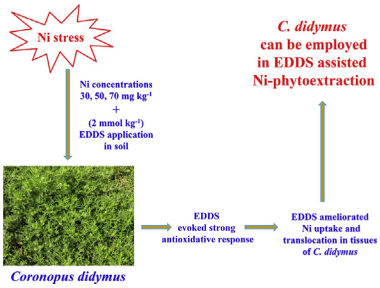
HIGHLIGHTS

- Ni treatment enhanced the growth and biomass of *C. didymus*.
- EDDS amendment to soil effectively mounted Ni translocation to aerial tissues.
- EDDS application elicited strong antioxidative response in *C. didymus*.
- *C. didymus* in combination with EDDS can be exploited for Ni-phytoextraction.

GRAPHICAL ABSTRACT



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ABSTRACT

In a screenhouse, the applicability of biodegradable chelant ethylenediamine disuccinic acid (EDDS) to enhance Ni-phytoextraction by *Coronopus didymus* was tested for the first time. This study assayed the hypothesis based upon the role of EDDS on physiological and biochemical alterations and ameliorating phytoextraction capacity of *C. didymus* under nickel (Ni) stress. Pot experiments were conducted for 6 weeks and *C. didymus* plants were cultivated in soil artificially contaminated with 30, 50, and 70 mg kg^{-1} Ni treatments. Soil was amended with EDDS (2 mmol kg^{-1}). Plants were harvested, 1 week after EDDS application. At 70 mg kg^{-1} Ni level, EDDS application dramatically enhanced the root and shoot Ni concentration from 665 and 644 to 1339 and 1338 mg kg^{-1}, respectively. Combination of Ni + EDDS induced alterations in biochemical parameters of plants. EDDS addition posed pessimistic effects on growth, biomass, photosynthetic activity and protein content of the plants. Besides, application of EDDS stimulated the generation of superoxide anion, H_2O_2 content and MDA level. However, EDDS assisted mount in antioxidant activities (superoxide dismutase, catalase and glutathione peroxidase) considerably neutralised the toxicity induced by reactive oxygen species in plant tissues. The results revealed EDDS efficacy to ameliorate the performance of antioxidant enzymes and improved Ni translocation in plant tissues, thus strongly marked its affinity to be used together with *C. didymus* for Ni-phytoextraction.

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; EDDS, S,S-ethylenediamine disuccinic acid; EDTA, ethylenediamine tetraacetic acid; EL, electrolyte leakage; GPX, glutathione peroxidase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MTs, metallothioneins; ROS, reactive oxygen species.

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1. Introduction

Soil contamination by inorganic contaminants is, nowadays, a serious environmental concern with calamitous future repercussion to our ecosystem. Various anthropogenic activities like metallurgical processes, smelting, burning of fossil fuels, vehicular emissions and untreated industrial effluents have led to the release of heavy metal contaminants in soil ecosystems (Sidhu, 2016; Sidhu et al., 2018). Among the inorganic contaminants, nickel (Ni), is an indispensable micro element for plants, but high Ni concentrations in soil could prove noxious to them (Chen et al., 2009). Ni is the twenty-second most abundant element found in the earth's crust and is found in igneous rocks either in free or in complex state with iron. Although, Ni is a redox inactive metal but its peaked level in plants induce oxidative stress by generating reactive oxygen species (ROS), alterations in nutrient and water uptake mechanisms, hindrance in uptake and transport of vital micro nutrients (Zn, Cu, Fe), promote chlorosis and impede photosynthesis and respiration processes (Chen et al., 2009). In humans, Ni can enter into the human bodies through food and is related to various skin allergies like dermatitis (Bocca et al., 2007); lung and nervous disorders (Haber et al., 2000).

High Ni concentration disturbs the cellular redox equilibrium by generating ROS in plants (Yusuf et al., 2011). ROS instigates membrane peroxidation, vandalise vital bio-molecules like lipids and proteins; ultimately cause cellular destruction. For survival, plants have evolved certain protective mechanisms to alleviate and repair cellular impairment induced by ROS. Various antioxidant enzymes like SOD, CAT, APX and GPX confer a consequential part to sequester ROS, generated and accumulated during oxidative stress in plants (Sidhu et al., 2016). The vigorous antioxidative response is the one mechanism evolved by the plants to counteract oxidative stress induced by ROS accumulation (Sidhu et al., 2016, 2017a, 2017b).

Keeping in mind about Ni toxicity, it is obligatory to remediate Ni-contaminated soils. Various mechanical or chemical techniques have been employed to mitigate the concentration of metal contaminants present in the soils (Sidhu et al., 2018). Among all, phytoextraction is the best suitable *in situ* strategy used for the remediation of contaminated soils. It is a cost-effective, sustainable, aesthetically pleasing, environment friendly technique. The success of phytoextraction is dependent upon certain factors: (i) high biomass production of aerial plant tissues (ii) plant tolerance towards metal stress and (iii) high metal concentration translocated from roots to the aerial plant tissues. The amount of metal accumulated and translocated in plant tissues is either natural or chemically assisted (Saifullah et al., 2010). The natural metal accumulation and translocation approach deploys hyperaccumulator plants having exceptionally high metal-accumulating ability. The aerial tissues of such plants contain $>$ 100 mg kg^{-1} Cd, $>$1000 mg kg^{-1} Ni, Pb, Cu or Cr and $>$ 10,000 mg kg^{-1} Zn or Mn (Pollard et al., 2002). Members of family Brassicaceae are well known for their potential to accumulate heavy metals in their tissues (Mourato et al., 2015). Some of the known metal hyperaccumulators are *Brassica juncea* (Hsiao et al., 2007); *Thlaspi caerulescens* (Munn et al., 2008); *Sedum alfredii* (Wang et al., 2009) and *Coronopus didymus* (Sidhu et al., 2017a). The chemically assisted strategy utilises high biomass yielding species whose phytoextraction efficiency can be incremented in the presence of chelants. Therefore, use of easily biodegradable chelant like S,S-ethylenediamine disuccinic acid (EDDS) has been recommended for enhancing the phytoextraction ability of the plants. The half-life of EDDS 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). EDDS is used as an alternative of EDTA for chelant-assisted phytoremediation, as unlike EDTA, it is biodegradable. Moreover, the concentration of both extracted metal and biodegradable EDDS decreased substantially with the increase in soil depth and time (Wang et al., 2012). It has also been asserted that EDDS addition does not impart perceptible residuary effects on the crop yield in terms of their biomass production (Yang et al., 2013). Additionally, EDDS instigates significant mobilisation of Ni, together with Cu and Zn in soil matrices (Koopmans et al., 2008). Recently, studies have revealed that EDDS significantly enhances the metal solubility and phytoextraction ability of *Macleaya cordata* (Li et al., 2015) and *Chrysopogon zizanioides* (Attinti et al., 2017). Sidhu et al. (2017b) demonstrated that EDDS application enhanced the biomass yield, oxidative stress and antioxidative response in *C. didymus* on exposure to incremented Pb treatments. Nevertheless, not much information has been available in the literature regarding the effect of EDDS on photosynthetic pigments and antioxidative response in plants undergoing phytoextraction of metal contaminants. Hence, we need to assess the effect of EDDS on physiological attributes, oxidative status and antioxidative response of Ni exposed *C. didymus* plants, so as to better understand the practical implications of EDDS-mediated Ni-phytoextraction.

Coronopus didymus commonly known as lesser swine cress is a wild, unpalatable plant that has been reported earlier to accumulate lead (Sidhu et al., 2016) and cadmium (Sidhu et al., 2017a). It is a fast growing, easily adaptable annual herb with greater biomass. It grows along the road sides, vacant plots and gardens during winter season (October to February) in the northern parts of India. It has a speedy growth with profusely branched root and shoot system, having short harvest period. The sampling period was selected for 6 weeks as the *C. didymus* plants showed luxuriant growth and high biomass yield during this period. To the best of our knowledge, no previous work has elucidated the effect of EDDS on the phytoextraction of Ni-contaminated soils using *C. didymus* plants. The principle objective of this work were to analyse (i) the effect of EDDS on phytoextraction of Ni from contaminated soils using *C. didymus* (ii) the accumulation and translocation of Ni from plant roots to aerial tissues and (iii) the effect of EDDS on physiological attributes, oxidative status and antioxidative response of *C. didymus* plants under varied Ni treatments.

2. Materials and methods

2.1. Preparation of pot plants and soil

The seeds of *C. didymus* were obtained locally from Botanical garden, Panjab University campus, Chandigarh, India. Before sowing, seeds were surface sterilised with 1.5% sodium hypochlorite for 10 min and then rinsed five times with distilled water. After sterilisation, in a screenhouse, seeds were germinated in plastic tray containing 10 kg soil (5 parts of soil and 1 part of cow dung manure). The soil used in the pots contains similar composition. The selected soil used for the experiment was sandy loam having pH 6.69 ± 0.07, electrical conductivity 139.9 ± 1.51 μScm^{-1}, organic carbon 0.98 ± 0.04% and organic matter content 1.68 ± 0.07%. The soil moisture of each pot was maintained at 60% of the water-retaining capacity by adding distilled water as needed until harvest.

2.2. Treatment of Ni and EDDS

In a screenhouse, *C. didymus* plants were grown in the presence of different Ni and EDDS treatments. The soil was spiked by three levels of Ni (30, 50, and 70 mg kg^{-1}), supplied as nickel sulphate ($NiSO_4 \cdot 6H_2O$). Before plantation, spiked soils were incubated for 2 weeks. 15 day old *C. didymus* plants having identical size (4–6 cm