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Synergistic Effect of Salt and Cadmium Stresses on Phytoremediation Potential of Lepidium Sativum

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Abstract

In this study *Lepidium sativum* ability to accumulate cadmium (1M Cd) under increasing doses of NaCl was investigated. Results revealed *L. sativum* reduced Cd accumulation by simultaneously application of high NaCl dose. Combined stress (Cd+NaCl) induced reduction of dry matter production and photosynthetic pigments contents. Nevertheless, foliar tissues contained important Cd amounts independently of presence or not of NaCl. Lipid peroxidation parameter as an indicator of internal damage induced was markedly accumulated in leaves of treated plants. Furthermore, Cd enhanced antioxidant enzymes such superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX) and glutathione reductase (GR) activities in order to minimize the harmful effects of reactive oxygen species (ROS) generated by Cd. In fact, addition of Cd in nutrient solution containing increasing salt doses of NaCl (100, 200, 300mM), showed an inhibition of antioxidant system efficiency. Our results suggest that *L. sativum* has the ability to maintain effectively phytoremidiation under salt and Cd stresses.

Keywords: Cd Bioaccumaulation, Interaction Cd/NaCl, antioxydant system, *Lepidium sativum*.

Introduction:

Water pollution by heavy metals has become acute in recent years because metal ions from natural, domestic and industrial sources tend to concentrate in the organic residue at the sewage treatment works. The problem return to the non-degradability of inorganic pollutants like heavy metals which are hazardous when discharged into a water body (Afzaal et al., 2022). However, water pollution constitutes a risk of agricultural soils contamination through irrigation. It is well suggested that excess concentrations of heavy metals in soils such as Cd have caused the disruption of natural aquatic and terrestrial ecosystems (Kosar et al., 2023). In the other hand,

salinity is one of the major abiotic stresses which menace crop production and human health. However, salinization is still expanding, posing a threat to sustainable agriculture development. One third of the world's irrigated soils and a large proportion of soils in dry-land agricultural regions are saline (Singh, 2021). Besides, soil salinity has been shown to increase Cd concentration in crops grown on soils fertilized with phosphorous fertilizers containing Cd (Rashid et al., 2023; Wang et al., 2023). Several studies have interested to different plant species playing a major role in phytoremiodiation. However, those plant species has abnormally high capacities of trace element removal from water (Mick et *al*., 2020; Moceck-Plociniak *et al.,* 2023). Soltan and Rashed (2003) have shown that there is a relation sheep between metal concentrations of hyperaccumulator plants and the water column. Nevertheless, interaction of Cd and salinity should be taken into consideration where both stresses are expected to impact crop growth and yield. It has been previously documented that NaCl and Cd stress in combination resulted in more severe growth inhibition than Cd or NaCl stress alone (Ondrasek et al., 2021), yet interaction of salinity and cadmium stresses on the ROS levels regenerated is little unknown. Thus, it is imperative to elucidate whether the interaction of salinity and Cd leads to a further influence on antioxidant enzymes for understanding the combinational stress of NaCl and Cd on plants growth and survivor. In the present research, we focused principally on the combined stress of salinity (NaCl) and Cd on the level of lipid peroxidation of membranes and several antioxidant enzymes activities include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR).

Interaction of Cd and salinity should be taken into consideration where both stresses are expected to impact crop growth and yield. In fact, Fertilizers extensively applied to soils to alleviate salt stress to crops resulted in a dramatic increase of Cd content in these soils. In our data, we demonstrated that the ability of *L. sativum* seeds to remove Cd still important regardless salt contamination of nutrient solution. The loss of tolerance capacity to cadmium by presence of salt was directly linked to depression in antioxidant enzymes activities as consequent of perturbation of mineral nutrient.

2. Material and Methods

2.1. Plant Material and Growth Conditions

Seeds of *L. sativum* were germinated in petri dishes in the dark. Seedlings were transferred and grown under continuous aeration in a nutrient solution containing KH_2PO_4, 0.5 mM ; $Ca(NO_3)_2$, 1.25 mM ; KNO_3, 2mM ; $MgSO_4$, 0.5 mM ; Fe-K-EDTA, 50 µM ; $MnSO_4 4H_2O$, 5 µM ; $ZnSO_4 7H_2O$, 1 µM ; $CuSO_4 5H_2O$, 1µM ; H_3BO_3, 30 µM ; $(NH_4)_6 Mo_7O_{24} 4H_2O$, 1 µM. Plants were grown in a growth chamber under controlled conditions: a 16h-light (150 µmol m^{-2} s^{-1} PAR)/8h dark cycle, 23°C (light) /18°C (night) and 65% relatively humidity. After six weeks, part of seedlings was maintained grown in control condition. The other seedlings were transferred on nutrient solution containing 1M $CdCl_2$ in absence or in presence of NaCl (100mM, 200mM, 300mM). Treatment period was limited to only five days.

2.2. Determination of Metal Contents

Cadmium contents was analyzed by digestion of dried samples with an acid mixture ($HNO_3/HClO_4$, 4/1 v/v). Metal concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer, AAnalyst 300).

2.3. Lipid Peroxide Determination

Lipid peroxide was determined by measuring the concentration of thiobarbituric acid-reacting substances (TBARS), as described by ALIA et *al*. (1995). The leaves were homogenized in 5 % (w/v) trichloroacetic acid (TCA). After centrifugation, a sample of the supernatant was added to 20 % TCA containing 0.5 % (w/v) thiobarbituric acid (TBA). The mixture was incubated at 95°C for 30 min. The concentration of thiobarbituric acid reacting substances was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

2.4. Photosynthetic Pigments Determination

Chla and carotenoides contents were determined by the method of Arnon (1949). The absorbance of a sample was read at 645 and 663nm.The

pigment concentrations were calculated by equations allowing a simultaneous determination of Chl a and carotenoids**.**

2.5. Enzyme Assay

Total SOD (EC 1.15.1.1) activity was measured spectrophotometrically at 560 nm according to Beyer and Fridovich (1987), based on the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT). One unit of enzyme activity was defined as the quantity of SOD required for 50 % inhibition of NBT reduction.

Total CAT (EC 1.11.1.6) activity was assayed in presence of H_2O_2, according to Chaparro-Giraldo et al. (2000), by monitoring the decline in absorbance at 240 nm, as H_2O_2 was consumed. Enzyme activity was calculated using the extinction coefficient of 40 mM^{-1} cm^{-1} for H_2O_2.

Total APX (EC 1.11.1.11) activity was assayed in the presence of ascorbate by following the decline in absorbance of the oxidized ascorbate at 290 nm, according to Chen and Asada (1989). Enzyme activity was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate.

Activity of GPX (EC 1.11.1.11) was measured by monitoring the H_2O_2 dependent oxidation of reduced guaiacol at 470 nm (Decleire, 1982). One unit was defined as the enzymic amount which oxidizes 1 µM guaiacol min.

Total GR (EC 1.6.4.2) activity was determined by following the rate of NADPH oxidation, as measured by the decrease in the absorbance at 340 nm (Rao et al., 1996).

2.6. Statistics

The data are presented in the figures and tables as the average of at least six replicates per treatment. Each experiment was conducted in duplicate. The mean values ± S.E. are reported in figures and tables. The significance of differences between control and treatment was determined at the 0.05 level of probability.

3. Results and Discussion

In this study, it was shown that uptake and bioaccumulation of Cd in leaves of *L. sativum* was often enhanced regardless presence of salt in nutrient solution (Table 1).

Table 1. DW, Cd, *Chl*a, carotenoids and MDA contents in leaves of *Lepidium sativum* treated with Cd alone or in combination with NaCl. Each point represents the mean ± SD of six determinations. Different letters above bars indicate significant differences between treatments (ANOVA).

	DW(mg)	Cd (µg/gDW)	Chla (mg/g FW)	Carotenoids (mg/g FW)
Control	330±10.02^{a}	0.012±0.001^{d}	1.02±0.01^{a}	0.893±0.057^{a}
Cd	308± 9.163^{b}	1.217±0.12^{a}	0.95±0.008^{a}	0.902±0.062^{a}
Cd+ 100mM NaCl	255.71±15.04^{bc}	1.12±0.153^{b}	0.861±0.035^{b}	0.818±0.01^{b}
Cd+ 200mM NaCl	125±6.58^{c}	1.03±0.184^{b}	0.417±0.012^{c}	0.543±0.038^{c}
Cd+ 300mM NaCl	93±8.71^{d}	0.925±0.0862^{bc}	0.321±0.11^{d}	0.12±0.01^{d}

Nevertheless presence of high dose of NaCl (300mM) reduced Cd content in foliar tissues. Previous studies examined the bioaccumulation of Cu, Zn, Ni and found that high amount of those heavy metals could be accumulated by *L. sativum* (Montvydiene and Marciulioniene, 2007; Szczodrowska et *al*., 2016; Bozim and Mizerna, 2021; Bozim, 2022). In comparison with Cd alone, the combination of NaCl and Cd treatments led to significant decline in dry matter production in leaves, but no significant difference was found between Cd treatment and control (Table 1). Growth inhibition caused by combination of NaCl and Cd application, seemed to be the result of mineral nutrition perturbation. However, rate of uptake and distribution of certain essential nutrients in plants treated with both Cd and salt stress, may be responsible for mineral deficiencies/imbalance and depression of the plant growth (Zhang et al., 2002; Dražić et al., 2004, Huang et al., 2007). In other hand, presence of Cd alone didn't affect chlorophylla and carotenoides tenors (Table 1). However, the combined stress (NaCl+Cd) stresses caused significant decline in these photosynthetic pigments. It seems that, the nutritional disorders and the lost of nutrient availability caused by salt (Wei et al., 2003),

resulted in reduction in biosynthesis of each photosynthetic pigments. On the whole, the inhibiting effect of NaCl on growth of Cd-treated plants increased with rise of salt concentration added in nutrient culture. It has been identified that salt was an important determinant of Cd concentration in crops (Weggler et al., 2000; Zulfiqar et al., 2022). It was reported that Cd uptake was enhanced when plants grew in soils with higher NaCl content (Ayachi et al., 2023). Although these is an inconsistency between experiments using soil or hydroponic solution. In hydroponics' culture, it might be possible that injured roots due to high salinity weaken the capacity of ion uptake, or Na ion competitively inhibits Cd uptake (Huang et al., 2007). In our work regardless that hydroponics cultures were conducted, we found that *L. sativum* typically accumulated relatively high heavy metal amounts independently of stress applied. However, this specie saved its ability to take up and accumulate Cd in both stress conditions (NaCl+Cd), but showed senescence symptoms earlier than in Cd-treated ones.

In another hand, it is well demonstrated that increase in ROS engender mitochondrial membrane lipid peroxidation, which can cause damage to these organelles. Even so, in our data we showed that membrane lipid peroxydation was not enhanced by presence of Cd alone (Table 1). But, when the combined stress (NaCl + Cd) was applied an increment in lipid peroxidation level was detected and level of this parameter increased with rise of NaCl concentration. We can suggest that Cd complexation or immobilization process were inhibited. Since mineral nutrient was unbalanced by presence of NaCl (Alharbi et al., 2022) synthesis of different constituent of defense system was blocked. For example GSH which play a crucial role and a central molecule in defense against Cd toxicity (Cui et al., 2020). Activation of antioxidative defense systems began by stimulation of the first group of enzymes such as SOD and CAT (Fig1A, B). Grow in SOD and CAT activities were observed in plants treated with Cd alone. But, these key enzymes of all the defense system were inhibited in plants simultaneously stressed by salt and Cd. Implication of these enzymes in plant tolerance was evident (Queiroz et al., 2023). Concurrently to SOD and CAT decline after both stress (NaCl+Cd) exposure, a more increase in MDA amount and the start of visual toxicity symptoms, was more pronounced in those plants. This confirms SOD role played in alleviation of oxidative stress by scavenging ROS from cell compartment. In second stage, ascorbate/glutathione cycle intervened with the other enzymes (APX, GPX, GR) to control and re-establish the homeostatic equilibrium of the redox status in cells. Since participation of those enzymes in ROS scavenging was suggested, our data shown that changes of APX, GPX and GR activities were similar to SOD (Fig 2A, B, C). Cd alone enhanced dynamics of those enzymes (APX, GPX and GR) but, application of both Cd and salt reduced them. Since both stress simultaneously applied, perturbed mineral nutrition, different important trace elements were not available. As consequence, deficiency of different prosthetic groups (Fe, Cu/Zn or Mn) essential for catalytic action of SOD. These results put out major key role of SOD affecting all the other different enzymes of defense system (Rajbout et al., 2021; Zengh et al., 2023).

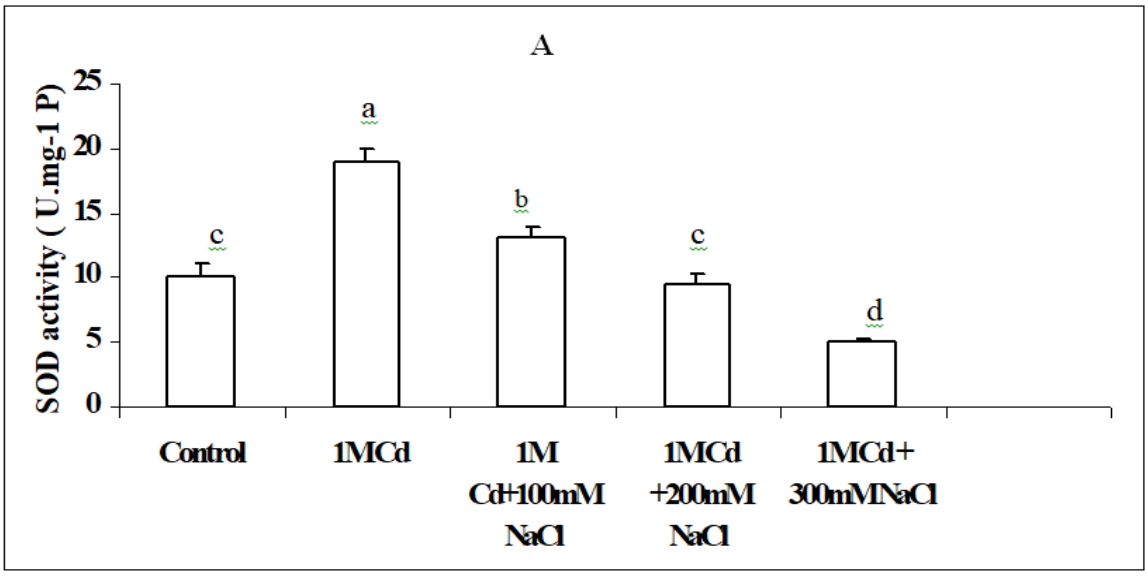
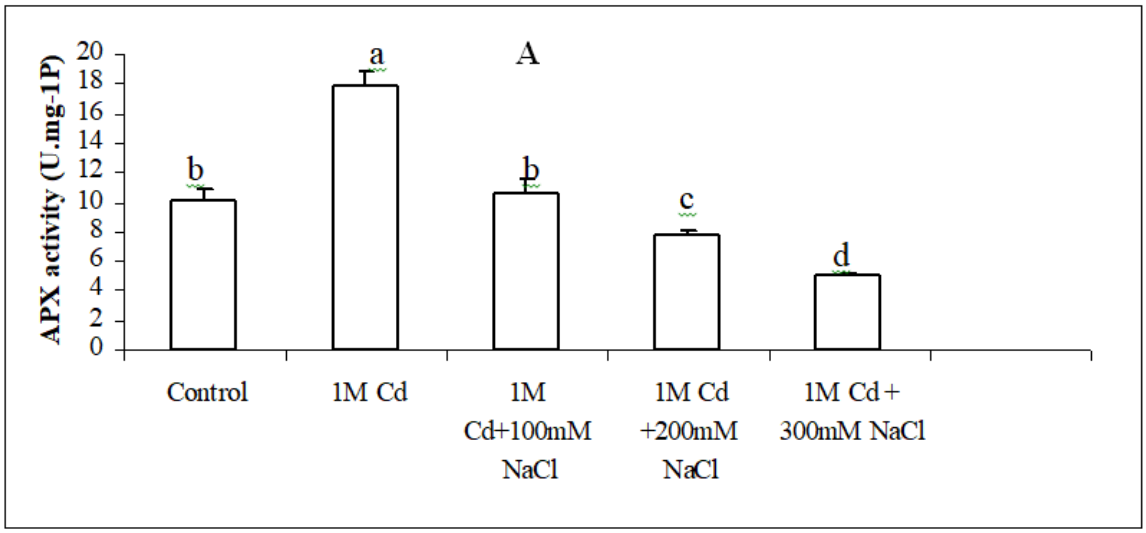
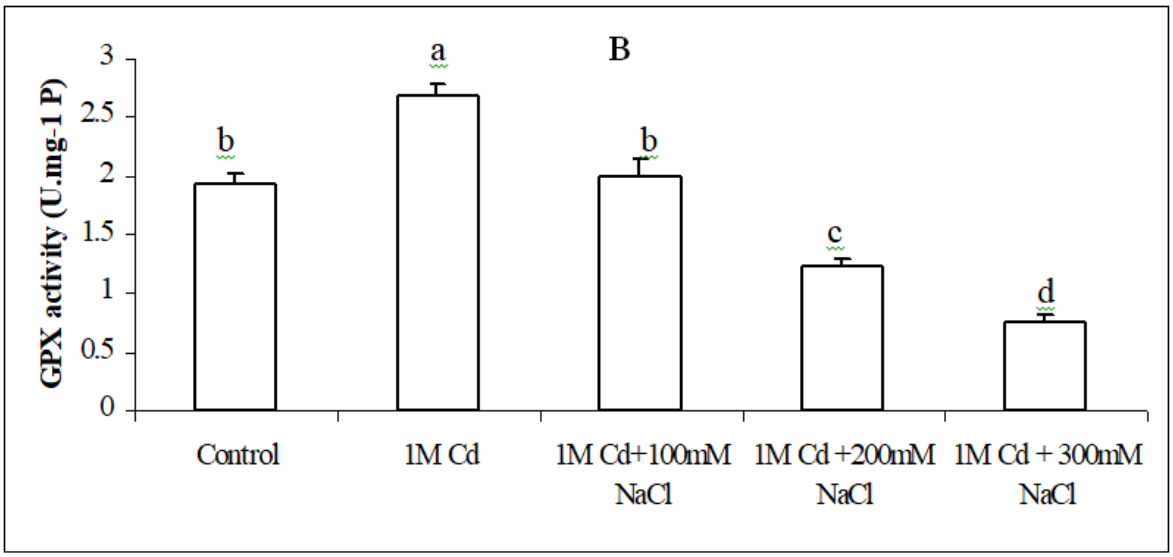


Figure 1. SOD (A), CAT (B), activities in leaves of *Lepidium sativum* treated with Cd alone or in combination with NaCl. Each point represents the mean ± SD of six determinations. Different letters above bars indicate significant differences between treatments (ANOVA).





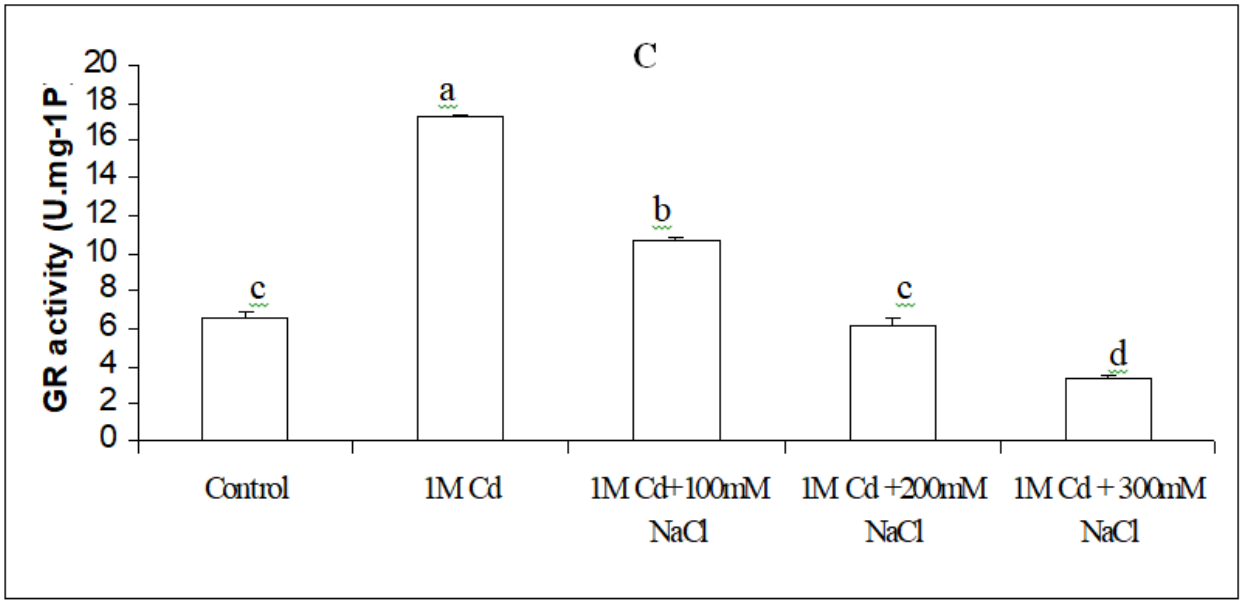


Figure 2. APX (A), GPX (B), and GR (C) activities in leaves of *Lepidium sativum* treated with Cd alone or in combination with NaCl. Each point represents the mean ± SD of six determinations. Different letters above bars indicate significant differences between treatments (ANOVA).

Conclusion

The study reported to date about the effect of salt stress on Cd-treated *L. sativum*. We demonstrated how Cd-hyperaccumulation ability of this specie was saved in salt condition. The ultimate strategy of *L. sativum* containing important amounts of Cd, is based on earlier senescence. This proximate strategy contributed to reduced seeds recruitment produced in such stress conditions. Growth inhibition under simultaneous stress condition (Cd+NaCl) reflected the lost of defense antioxidant system efficiency. Infact, SOD and CAT activities were reduced by salt and inhibition degree rise with increase of salt dose. More that, enzymes (APX, GPX, GR) involved in ascorbate-gluthatione cycle, showed a diminution in dynamics.

Acknowledgments

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