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EFFECTS OF LEAD AND CADMIUM ON SEED GERMINATION, SEEDLING GROWTH AND ANTIOXIDANT ENZYMES ACTIVITIES OF MUSTARD (*Sinapis arvensis* L.)

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ABSTRACT

The effect of different concentrations of cadmium nitrate (0 to 1000 µM) and lead nitrate (0 to 1500 µM) on seed germination, seedling growth and antioxidant enzymes activities of mustard (*Sinapis arvensis* L.) after 10 days of incubation were investigated. The results revealed that Cd and Pb adversely influenced the seed germination. The effective of Cd and Pb concentrations causing 5.6% and 10.23% inhibition of germination of seeds, respectively. The root and shoot of mustard plants were also decreased with increasing concentrations of Cd and Pb metals. When plants were treated with Pb and Cd, the activity of antioxidant enzymes such as APX, GPX and CAT were increased in shoot plants. In addition, it was observed that cadmium cased higher antioxidative activity in mustard plant than lead. Results in this study showed that at the highest concentration of heavy metals, the activity of CAT and APX activity were higher in Cd treatment than Pb treatment.

Keywords: seed germination, seedling growth, antioxidant enzymes, heavy metal.

INTRODUCTION

Different levels of heavy metals contained in soil, water and air cause pollution after reaching certain concentrations. There are different reasons for this kind of pollution. Metals are continuously released into the biosphere by volcanoes, natural weathering of rocks and by industrial activities such as mining and the combustion of fossil fuels and the release of sewage (De Abreu *et al*., 1998). Heavy metal contamination of agricultural soil has also been observed to be increased due to industrialization. Therefore, heavy metal contamination represents a risk for primary and secondary consumers and ultimately humans (Zeller and Feller, 1999).

Agricultural soils, as an essential part of the environment, are no exception of this phenomenon. Cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) are among the most abundant heavy metals in the agricultural soils (Förstner, 1995). These metals are mostly absorbed by plants easily and prove toxic to plants that can be observed as growth retardation as a result of alterations in biochemical process like inhibition of enzyme activity, protein penetration and impaired nutrition etc. (Arun rtal, 2005). In the recent era, lead (Pb) contamination has gained a considerable attention as a potent environmental pollutant. Significant increases in the Pb content of cultivated soils have been observed near urban and industrial areas where it tends to accumulate in the surface ground layer (Di Toppi and Gabrielli, 1999). Despite regulatory measures adopted in many countries to have a check on Pb input in the environment, it continues to be one of the most serious global environmental hazards in the developing world (Yang *et al*., 2000).

Cadmium (Cd), being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health (Di Toppi and Gabrielli, 1999). The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis (Padmaja, 1990). Excessive amount of Cd may cause decreased uptake of nutrient elements, inhibition of various enzyme activities, induction of oxidative stress including alterations in enzymes of the antioxidant defence system (Sandalio *et al*., 2001).

The aim of present study is to assess the effect of different concentrations of lead and cadmium on germination, seedling growth and some antioxidant enzymes activities of Mustard (*Sinapis arvensis* L.).

MATERIALS AND METHODS

Mustard seeds were obtained from the Zabol Agricultural Research Institute, Zabol, Iran, and were used as plant material and Cadmium nitrate [$CdSO_4.8H_2O$] and lead nitrate [Pb $(C_2H_3O_2)_2$] as heavy metals. The seeds were immersed in 3% v/v formaldehyde/deionized water for five minutes to avoid fungal contamination. After that, the seeds were washed with deionized water. 50 seeds were placed in glass culture dishes containing 20mL of the different concentrations of test solutions. Seeds were arranged in such away that each seed did not touch each other nor touch the side of the dish.

Three replicates of each test solution were prepared. Solutions were also changed every 24 hours. Mustard (*Sinapis arvensis* L.) was subjected to eight different concentrations of Cd (0, 150, 300, 450, 600, 750, 900 and 1000 µM) or Pb (0, 150, 300, 600, 750, 900, 1200 and 1500 µM). The seeds were set under a photoperiod of 12 hr, and 25/18 °C day/night temperature. The seedlings

were harvested after 10 days and the germination percentage, root and shoot length and some antioxidant enzymes activities were recorded.

Enzyme assays

Ascorbate peroxidase

The enzyme was extracted in 50mM phosphate buffer (pH=7). The activity of ascorbate peroxidase (APX EC 1.11.1.11) was measured the using method of Nakno and Asada (1981). Shoot (0.20g) powder was homogenized in a mortar and pestle with 4ml of ice-cold extraction buffer (100 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA). The homogenate was filtered through muslin cloth and centrifuged at 14000 g* for 15 min. the supernatant fraction was used as crude extract for enzyme activity.

The reaction mixture consisted of 50 mM sodium phosphate buffer (pH=7) containing 0.2 mM EDTA, 0.5 mM ascorbic acid (sigms), 50 mg of BSA (Sigma), and crude enzyme extract. The reaction was started by additin of H_2O_2 at final concentration of 0.1 mM. Oxidation of ascorbic acid as a decrease in absorbance at 290 nm was followed 2 min after starting the reaction. The difference in absorbance was divided by the ascorbate molar extinction coefficient (2.8 $mM^{-1}.cm^{-1}$) and the enzyme activity expressed as µmol of H_2O_2 min^{-1} mg^{-1} protein, taking into consideration that 1.0 mol of ascorbate is reqired for the reduction of 1.0 mol of H_2O_2 (McKersie and Leshem., 1994).

Catalase

Catalase (CAT, EC 1.11.1.6) activity was assayed spectrophotometrically by monitoring the decrease in absorbance of H_2O_2 at 240 nm. CAT was measured according to the method of Noctor and Foyer (1998). The enzyme was extracted in 50 mM phosphate buffer (pH=7). The assay solution contained 50 mM phosphate buffer and 10 mM H_2O_2. The reaction was started by addition of enzyme aliquot to the reaction mixture and the change in absorbance was followed 2 min after starting and the reaction. Unite activity was taken as the amount of enzyme, that decomposes 1 M of H_2O_2 in one min.

Guaiacol peroxidase

Total GPX (EC 1.11.1.7) activity was determined as described by Urbanek *et al* (1991) in a reaction mixture (0.2 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 5.0 mM guaiacol, 15 mM H_2O_2 and 50 µL enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}).

Statistical analysis

All data were analyzed with SAS Institute Inc., Version 6.12 software and first analyzed by ANOVA to determine significant ($P \forall \leq 0.05$) salinity treatment and genotype effects. Significant differences between individual means were determined using Fisher's protected least significant difference (LSD) test.

RESULTS AND DISCUSSIONS

Effects of Cd and Pb on seed germination and the growth of mustard seedlings

The percentage of germination may reflect the reaction rate of plant seeds to their living environment. From Table-1, it can be seen that Cd and Pb significantly affect on seed germination and seedling growth of mustard plants.

Table-1. Results of variance analysis (ANOVA) of Cd and Pb concentrations on seed germination and seedling growth and antioxidant enzymes activities

Treatments	Cadmium	Lead
Germination	17.12^{*}	28.35^{**}
Root length	3.08^{**}	16.56^{**}
Shoot length	3.05^{**}	1.48^{**}
Fresh weight	0.0065^{**}	0.0083^{**}
Dry weight	0.0021^{**}	0.000042^{*}
APX	0.00073^{**}	0.00058^{**}
GPX	0.0011^{**}	0.0044^{**}
CAT	0.00013^{**}	0.000078^{**}

Number represents *F*-values at 5% level
^{ns} Non-Significant, * and ** significant at $P<0.05$ and $P<0.01$

The results of the present study revealed that Cd and Pb adversely influenced the seed germination. The effective of Cd and Pb concentrations causing 5.6% and 10.23% inhibition of germination of seeds respectively (Table-2). When the concentration of metals exceeded certain levels, an abnormal germination was resulted. Neither Cd nor Pb at relatively low concentrations (up to 300 µM Cd and 150 µM Pb) had any significant effect on seed germination. The decrease in seed germination of Sinapis *arvensis* can be attributed to the accelerated breakdown of stored food materials in seed by the application of lead and cadmium. Chugh and Sawhney (1996) reported that seed germination of pea (*Pisum sativum* L.) was affected by up to 0.5mM of Cd doses. Barcelo and Kahle (1992) indicated that Cd affected water relations not only by decreasing water absorption and transport, but also by lowering water stress tolerance.

The root and shoot of mustard plants were also decreased with increasing concentrations of Cd and Pb metals. Table-2 summarize root and shoot growth of germinating seeds. All concentrations of cadmium and lead inhibited seed germination shoot and root growth, though to a different extent. The effects of the heavy metals over the shoot growth were different as compared to the effects on root growth. The biomass of shoots and

roots of mustard plants in the control treatment were significantly higher than that in the metal treatment ($P < 0.01$).

The inhibition of root growth can be attributed in part to the inhibition of mitosis, the reduced synthesis of cell-wall components, damage to the Golgi apparatus and changes in the polysaccharide metabolism, while browning is caused by suberin deposits (Punz and Sieghardt, 1993). An interaction of heavy metals with salinity factors in soils and plants is present under field conditions and stronger soil salinity might increase the contents of heavy metals and specific metabolites in plant products considerably (Bergmann, 1996).

The activity of antioxidant enzyme

Results of this study showed, the activity of antioxidant enzymes are elevated with increased Cd and Pb concentration (Tables 1 and 2). When plants were treated with Pb and Cd, the activity of antioxidant enzymes such as APX, GPX and CAT were increased significantly in shoot plants (Table-2). Table-2 shows that the highest concentration of Cd and Pb the CAT, GPX and APX activity increased 62.9%, 63.1% and 84.7% in Pb and 86.2%, 50.9% and 91.8% in Cd than control treatment, respectively.

In this study, the higher antioxidative ability was observed in mustard plants, indicating that the increased antioxidative activity might reflect a damage response to stress factors, which was in agreement with the report of Mittal and Dubey (1991), who presumed that high lipid peroxidation and anti-oxidative ability both were parts of a damage response to salinity in rice cultivars.

The results of the present study showed that the seed germination and root development in mustard plant was gradually reduced with the increase of Cd concentration. We also found that the root growth and seed germination were sensitive to lead than cadmium stress. In addition, it was observed that cadmium cased higher antioxidative activity in mustard plant than lead. Results in this study showed that the highest concentration CAT and APX activity was in Cd treatments.

Table-2. Seed germination and seedling growth and antioxidant enzymes activities of mustard at different Cd and Pb concentration

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Antioxidant enzymes		
						APX	GPX	CAT
						(µmol/ m/mg FW)		
Cd (µM)								
0	100a	2.98a	2.93a	0.163a	0.009ab	0.0093h	0.025g	0.0003e
150	100a	0.22b	1.28b	0.082b	0.07bc	0.022g	0.021h	0.00047ed
300	100a	0.12b	0.48c	0.038c	0.009ab	0.027f	0.032f	0.00062d
450	98.8ab	0.11b	0.34c	0.033c	0.008ab	0.028e	0.033e	0.0013b
600	97.8ab	0.106b	0.08c	0.036c	0.011a	0.037d	0.036d	0.00091c
750	96.3a	0.10b	0.09c	0.032c	0.011a	0.044c	0.047c	0.0031a
900	95.3ab	0.10b	0.066c	0.036c	0.0116a	0.052b	0.083a	0.0034a
1000	94.4b	0.046b	0.053c	0.024c	0.004c	0.055a	0.053b	0.0037a
Pb (µM)								
0	97.7a	6.41a	3.78a	0.27a	0.008ab	0.023g	0.055h	0.00061d
150	95.4ab	5.96a	3.86a	0.21ab	0.01a	0.019h	0.057g	0.00014e
300	95.5ab	2.15b	2.32b	0.18dc	0.007bc	0.033f	0.059g	0.00021e
600	94.4ab	1.84b	2.75b	0.18dc	0.0093ab	0.037e	0.063f	0.00051d
750	94.4ab	1.47b	2.7b	0.19dc	0.0083abc	0.040d	0.099e	0.00061d
900	94.3ab	0.75b	2.08b	0.14d	0.008bc	0.041c	0.125c	0.0023c
1200	91.67bc	0.7b	2.28b	0.16dc	0.006c	0.051b	0.129b	0.0037b
1500	87.7c	0.67b	2.2b	0.10d	0.0061c	0.062a	0.149a	0.004a

Different letters indicate significant difference between means at *P<0.05*.

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