Australian Journal of Crop Science AJCS
AJCS 5(4):375-383 (2011) ISSN:1835-2707

Biochemical responses of Gouan (*Aeluropus littoralis*) to heavy metals stress

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Abstract

Heavy metals affect growth, morphology and metabolism of plants in several ways. To reveal relationship between metal toxicity, oxidative stress and detoxification responses, the effects of four different heavy metals (Cd, Co, Pb and Ag) on *Aeluropus littoralis* was determined. So, one-month-old plantlets were treated with different concentrations (50 µM and 100 µM) of heavy metals for one week and the contents of photosynthetic pigments**,** phenolic compounds, proline, soluble proteins and the activities of enzymatic antioxidants (superoxide dismutase**,** guiacol peroxidase and catalase) as marker for oxidative stress were investigated. Survey results indicated that the amount of non-enzymatic antioxidant compounds (phenol and proline) significantly were increased in all treatments but a significant decrease was observed in photosynthetic pigments in the most treatments. Also, the accumulation of soluble proteins and increase in superoxide dismutase activity of which are respectively recognized as primary and secondary response to metal toxicity that can be pointed to the great capability of *A. littoralis* for accumulation and tolerance to excess amount of heavy metals. These results collectively show that different response in control and treated plants is due to induction oxidative stress and heavy metal toxicity in plants because of the accumulation of reactive oxygen species in treated plants.

Keywords: enzymatic antioxidants; phenoilc compounds; proline; protein; SDS PAGE.
Abbreviations: CAT-catalase; DHAR-dehydroascorbate reductase; GPX-Glutathione peroxidase; GR-glutathione reductase; MDHAR-monodehydroascorbate reductase; POX-peroxidase; ROS-reactive oxygen species; SOD-superoxide dismutase.

Introduction

Heavy metal stress is one of the major abiotic stresses that causes environmental pollution in recent decades. These metals unlike other organic pollutants are not degraded and converted into harmless compounds via biological processes (Gisbert et al., 2003). Hence, they can persist for a long time in the environment and entry into food chain. A common feature of environmental stress is their ability for production of toxic oxygen derivatives (Arora et al., 2002). Reactive oxygen species (ROS) are continuously produced at low level during normal metabolic processes (Arora et al., 2002). But in biological systems, increasing the synthesis of ROS is one of the initial responses to different stress factors (Singh and Sinha, 2005). ROS induce damage to the biomolecules through peroxidation of membrane lipids, alteration of protein functions, DNA mutation, damage to chlorophyll and disruption some of metabolic pathways (electron transport chain and ATP production) (Ruley et al., 2004; Semane et al., 2010). Therefore the tolerance of plants to stress conditions depends on their ability to make balance between the production of toxic oxygen derivatives and capacity of antioxidative defense systems. Therefore, plants have complex ROS scavenging mechanisms at the molecular and cellular levels. These mechanisms with inhibition or slow the oxidation of biomolecules and oxidative chain reactions (Michalak, 2006) decrease the cellular oxidative damage and increase resistance to heavy metals. The plant antioxidant defense systems include antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) and low-molecular weight quenchers (cycteine, ascorbic acid, thiols, proline (Singh and Sinha, 2005), α-tocopherol, glutation, carotenoids, phenolic and nitrogen compounds (Michalak, 2006). *Aeluropus littoralis* is a C4 halophyte monocotyledonous grass (Wang, 2004), that has recently been discovered as a genetic resource for biotechnological researches because of small diploid genome (324 Mb) and tolerance to salt, drought and heat stresses (Zouari et al., 2007), but to date, no study has performed about response of the antioxidative defence system to heavy metals effects in this plant. Therefore, the present study aimed to explore the effects of four heavy metals on some biomarkers of oxidative stress in order to study relationship between metal toxicity, oxidative stress and detoxification responses.

Material and methods

Cultivation of Plants and Experimental Design

Seeds of *A. littoralis* were prepared from Pakan Seed Research Centre, Isfahan, Iran. The seeds were surface sterilized for 20 min in 1% (v/v) sodium hypochlorite, and then were washed several times with distilled water. The sterilized seeds were planted in plastic pots in sand and grit

Table 1. Photosynthetic pigments (chlorophyll a, b and carotenoids) content in different treatments.

Treatment	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)	Total chlorophyll (a+b)	Chlorophyll a/b ratio	Carotenoids (µg/g FW)	Chl (a+b)/ carotenoids
Cd (µM)						
0	0.995^{a}	0.639^{a}	1.634	1.56	93.8^{abc}	0.017
50	0.847^{b}	0.561^{b}	1.408	1.51	84.2^{abc}	0.016
100	0.713^{f}	0.488^{cdf}	1.201	1.46	69.5^{c}	0.017
Co (µM)						
0	0.995^{a}	0.639^{a}	1.634	1.56	93.8^{abc}	0.017
50	0.822^{c}	0.497^{cde}	1.319	1.65	87.1^{abc}	0.015
100	0.712^{f}	0.422^{g}	1.134	1.69	72.8^{bc}	0.015
Pb (µM)						
0	0.995^{a}	0.639^{a}	1.634	1.56	93.8^{abc}	0.017
50	0.794^{d}	0.517^{c}	1.311	1.53	99.1^{ab}	0.013
100	0.673^{g}	0.469^{ef}	1.142	1.43	79.6^{abc}	0.014
Ag (µM)						
0	0.995^{a}	0.639^{a}	1.634	1.56	93.8^{abc}	0.017
50	0.860^{b}	0.509^{cd}	1.369	1.69	105.3^{a}	0.013
100	0.747^{e}	0.480^{de}	1.227	1.56	71.5^{bc}	0.017

Values represent mean ± S.E. (n = 5). Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

Table 2. Effect of different heavy metals on enzyme activity in *A. littoralis* leaves.

Treatment	SOD (U/mg protein)	GPOx (U/mg protein)	CAT (U/mg protein)
Cd (µM)			
0	0.611^{ab}	0.321^{cd}	0.438^{cd}
50	1.12^{e}	0.402^{a}	0.485^{d}
100	0.701^{b}	0.346^{bc}	0.539^{e}
Co (µM)			
0	0.611^{ab}	0.321^{cd}	0.438^{cd}
50	0.671^{b}	0.358^{b}	0.471^{cd}
100	0.962^{cd}	0.194^{e}	0.505^{d}
Pb (µM)			
0	0.611^{ab}	0.321^{cd}	0.438^{cd}
50	1.026^{de}	0.354^{b}	0.386^{b}
100	1.342^{f}	0.297^{d}	0.315^{a}
Ag (µM)			
0	0.611^{ab}	0.321^{cd}	0.438^{cd}
50	0.844^{c}	0.338^{bc}	0.504^{d}
100	0.494^{a}	0.303^{d}	0.348^{ab}

Values represent mean ± S.E. (n = 5). Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

(1:1, v/v) in a growth chamber (60%-80% relative humidity, 16 h of light and 25/16º C day/night temperature). The pots were irrigated daily with MS basal-medium. After one month, uniform plants were selected and treated with MS medium containing two different concentrations (50 µM and 100 µM) of $CoCl_2$, $CdCl_2$, $HgCl_2$ and $PbNo_3$ for 7 days, separately. The experiments were carried out in a completely randomized design with five replicates. On the 7th day, plants were harvested and washed thoroughly with distilled water. Harvested leaves were frozen in liquid nitrogen and stored at -80º C for future analysis.

Proline Measurment

Proline was measured spectrophotometrically at 520 nm according to Bates et al. (1973).

Pigment Content Measurement

0.2 g leaves were homogenized with 10 ml of 80% acetone. The extract was centrifuged at 3000 g for 5 min. The upper phase was transferred into a new tube and its absorbance was measured at 663, 646 and 470 nm, respectively, for chlorophyll a, b and carotenoid with acetone 80% as a blank. The chlorophyll a, b and carotenoid content measured according to Lichtenthaler and Wellburn Method (1983).

Phenolic Compounds Measurement

Total phenolic compound concentration was measured by Folin-Ciocalteu method (Campbell and Ellis, 1992) at 725nm. Total phenolic compounds were determined by standard curve using cafeic acid.

Protein Measurement

The concentration of protein was measured by the Bradford method (1976) using bovine serum albumin as standard.

Superoxide Dismutase Activity Measurement

Superoxide dismutase activity was determined by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Dhindsa et al., (1981). One unit of activity is expressed as the quantity of enzyme required to the reduction of NBT to formazan by 50%. The activity of SOD was expressed as unit per milligram protein.

Glutathione Peroxidase Activity Measurement

Glutathione peroxidase activity was assayed according to the method of Polle et al., (1994).

Catalase Activity Measurement

The activity of catalase was assayed spectrophotometrically by measuring the H_2O_2 decomposition in time at 240 nm according to Aebi procedure (1984). The enzyme activity was expressed as units (mmol of H_2O_2 destroyed per minute) per milligram of total protein (extinction coefficient= 43.6 mM-1 cm−1).

Gel Electrophoresis

The separation of GPX isozymes carried out by native polyacrylamide gel electrophoresis on 10% separating gel and 5% stacking gel as described by laemmli (1970). 80 µg of samples was loaded and electrophoresis carried out with a constant current of 30 mA for 5 h at 4 ºC. Gel staining was performed by the method of Graham (1965).

Statistical Analyses

All data were analyzed by analysis of variance (ANOVA) procedures using MSTATC software package. Treatment means were separated by Duncan's multiple range tests at (Duncan 0.05).

Results

The results showed a positive relationship between heavy metal concentration and proline accumulation in all treatments (Fig. 1). The same results have been previously reported for *Triticum aestivum* (Amani, 2008), *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005), and *Raphanus sativus* (Teklić et al., 2008). In addition, the comparison of different treatments revealed that maximum proline accumulation was observed when the plants were treated with Cd (Fig. 1). The results showed that phenolic compounds significantly increased between 4.6% and 23.2% in comparing to control. Also results indicated that the increase of phenolic compounds was dependent on the concentration of heavy metals. The highest phenolic compounds accumulation was observed in Cd, Pb, respectively (Fig. 2). In comparison to control a significant reduction of total soluble protein content was observed in Cd treatment while there was an increase significant in both levels of Ag treatment (Fig. 3). There was a negative relationship between metal concentration and protein content in Cd treatment (Fig. 3). Our results coincide with those of other researchers (Guo

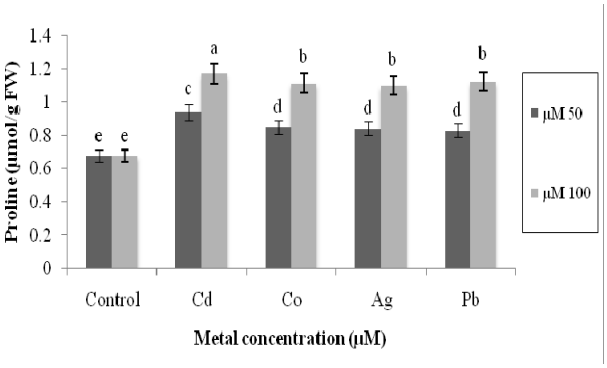

Fig 1. Effect of different heavy metals on proline content in *A. littoralis* leaves. Values represent mean ± S.E. (n = 3). Similar letters are not significantly different at p<0.05, according to Duncan's test.

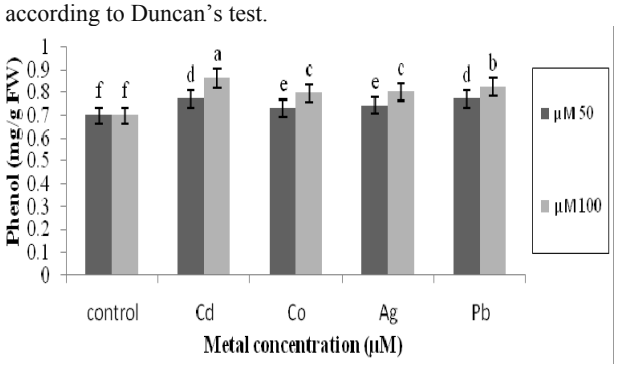

Fig 2. Effect of different heavy metals on phenolic compounds in *A. littoralis* leaves. Values represent mean ± S.E. (n = 3). Similar letters are not significantly different at p<0.05, according to Duncan's test.

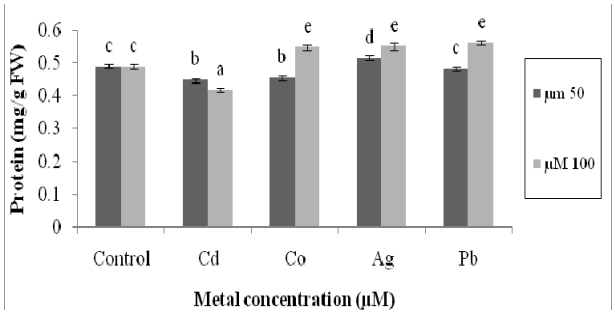

Fig 3. Effect of different heavy metals on protein content in *A. littoralis* leaves. Values represent mean ± S.E. (n = 3). Similar letters are not significantly different at p<0.05, according to Duncan's test.

et al., 2007; John et al., 2009; Szőllősi et al., 2009; liu et al., 2005; Mohan and Hosetti, 1997). In Co treatment, total protein content decreased at 50 µM but an increase was observed in 100 µM. Protein content didn't change considerably in Pb at 50 µM as compared to control, but with increase in metal concentration, protein content significantly increased (Fig. 3). Our results indicated that carotenoid content decreased with increasing metal concentrations in Co and Cd treatments at both levels (Table 1). When the plants were treated with Ag and Pb, the carotenoid content increased in 50 µM, but decreased with increasing heavy metal concentration (Table 1). In the case of all heavy metals, the carotenoid content decreased in 100 µM in comparison to control.Chlorophyll a and b are essential for photosynthesis and they are very sensitive to environmental stresses such as

heavy metals (Ekmekçi et al., 2008). Hence, chlorophyll content was measured in different treatments. As shown in Table 1, total chlorophyll content significantly decreased in all treatments. Similar results were obtained by other researchers (Wu et al., 2003; Wang et al., 2009; Zengin and Munzuroglu, 2005; John et al., 2009; Mobin and khan, 2007). Also with increase in metal concentration in all treatments, the content of both chlorophyll a and b had a decreasing trend (Table 1). The data showed that content chlorophyll a decreased in sequence of Pb>Co>Cd>Ag treatments. The maximum and minimum decrease in content chlorophyll b was observed in Co and Cd treatment, respectively (Table 1). The chlorophyll ratio (a/b) decreased progressively with increasing heavy metal concentration in Pb and Cd treatments but increased in Co treatment. Also Ag increased the chlorophyll ratio in 50 µM level but there is no remarkable change in 100 µM level in comparison with control (Table 1). The comparison of SOD activity in different treatments revealed that SOD activity increased with increasing heavy metal concentration in Co and Pb treatments. The increase in SOD activity was more marked in plants that were treated with Pb than with Co. The enzymatic activity markedly increased under both levels of Cd, but there was a negative relationship between metal concentration and enzyme activity. Although, the plants were treated with Ag showed an increase in SOD activity in 50 µM, but SOD activity decreased with increase in Ag concentration (Table 2).As shown in Table 2, GPX activity increased significantly in Cd, Co and Pb treatments in 50 µM level as compared to control. In 100 µM level, the enzyme activity significantly decreased just in Co treatment, but about other metals there is no significant difference with control (Table 2). The analysis of CAT activity showed considerable changes in its activity after treating with different heavy metals (Table 2). As expected, CAT activity in the leaves of plants were grown in medium supplemented with different heavy metals increased in comparison with control. In Pb treatment, enzyme activity inhibited with increase in concentration of metals, while the CAT activity initially increased in low concentration of Ag treatment and then dropped in high concentration. It is agree with previous studies (Schickler and Caspi, 1999; Stroinski, 1999; Fatima and Ahmad, 2005). Maximum and minimum enzyme activity was observed in Cd (100 µM) and Pb (100 µM) treatment, respectively.Figure 4 demonstrates that the isozyme profiles of peroxidases of *A.littoralis* variety in different heavy metal treatments. Although a clear band of GPX isoenzymes activity was observed in all treatments, but the band sharpness significantly changed in different heavy metals treatments. The sharpest band observed in Pb (50 µM) and Co (50 µM) treatments while band sharpness didn't change very significantly in other treatments.

Discussion

Proline

Previous studies indicated that significant decrease in the mitochondrial electron transport activity accompany increase in proline accumulation under environmental stresses (Saradhi et al., 1995). The induction of proline accumulation in response to abiotic stress may be due to increase its *de novo* synthesis or decrease degradation (Kasai et al., 1998) and the effect of proline on the permeability of membrane (Pesci and Reggiani, 1992). It was suggested that this amino acid acts as an osmolyte by antioxidative, osmoprotection properties and metal chelator (Farago and Mullen, 1979), takes part in reconstruction of chlorophyll (Carpena et al., 2003), regulation of cytosolic acidity (Gajewska and Skłodowska, 2008) tolerance to stress by osmoregulation and stabilization of protein synthesis (Kuznetsov and Shevyakova, 1997), stabilize the macromolecules and organelles (John et al., 2008) the protection of enzymes from denaturation (Gajewska and Skłodowska, 2008) and also serve as source of nitrogen and energy in recovery growth (Chandrashekhar and Sandhyarani, 1996). In our study, increase in proline levels as a proteinogenic amino acid, in all treatments reinforces the hypothesis that this amino acid by detoxification reactive oxygen species causes tolerance to heavy metals. Therefore, the accumulation of proline can be considered as an indicator of tolerance to heavy metals stress.

Chlorophyll

Decrease in chlorophyll content may be due to reduce synthesis chlorophyll due to inhibition of enzymes activity such as δ-aminolevulinic acid dehydratase (ALA-dehydratase) (padmaja et al., 1990) and protochlorophyllide reductase (Van Assche and Clijsters, 1990), replacement of Mg with heavy metals in chlorophyll structure (küpper et al., 1998), decrease in the source of essential metals that involved in chlorophyll synthesis such as Fe^{2+} and Zn^{2+} (Van Assche and Clijsters, 1990; Küpper et al., 1996), destruction of chloroplast membrane by lipid peroxidation due to increase in peroxidase activity and lack of antioxidants such as carotenoids (Prasad and Strzalka, 1999), decrease in density, size and the synthesis of chlorophyll and inhibition in the activity of some enzymes of Calvin cycle (Baryla et al., 2001; Benavides et al., 2005). Regarding the pigment content, heavy metal-treated plants showed a remarkable decrease of chlorophyll that causes photosynthesis rate enormously decrease in response to elevated heavy metal concentration. In another word, chloroplast contains many different parts that respond to heavy metal stress therefore any changes in chlorophyll synthesis and activity used as the index of direct toxic effects of heavy metals. Also increase in chlorophyll ratio (a/b) in Co and Ag (50 µM) treatments shows that chlorophyll 'b' is more sensitive to Co and Ag that disrupt the balance between energy trapping in photosystem II and cause a decrease in electron transport (Falkowski and Raven, 2007). While decrease in chlorophyll ratio (a/b) in response to Cd and Pb treatments suggest that chlorophyll 'a' is more sensitive to Cd and Pb.

Carotenoid

The carotenoid content decreased in response to all treatments except in low concentration (50 µM) of Ag and Pb. That may indicate a severe effect of Ag and Pb on cell and its component parts in compared to other heavy metals. Hence, it can be suggested that at first carotenoid content increased to protect the cell against these heavy metals, but in high concentration (100 µM) these heavy metals activate some mechanisms and degrade carotenoid pigments. Carotenoid is a non-enzymatic antioxidant pigment that protects chlorophyll, membrane and cell genetic composition against ROS under heavy metals stress (Hou et al., 2007). In plant cell protective role of this pigment might be due to quenching triplet chlorophyll, replacing peroxidation and destruction of chloroplast membrane (Kenneth et al., 2000). Previous studies evidenced that decrease in carotenoid content is a common response to metal toxicity (Rout et al., 2001), but increase is due to important role of this pigment in detoxifying ROS (Tewari et al., 2002; Chandra et al., 2009).

Phenolic Compounds

The increase in phenolic content may be due to protective function of these compounds against heavy metal stress by metal chelation and ROS scavenging (Brown et al., 1998; Lavid et al., 2001). Increase in phenol content suggests an antioxidative activity for these compounds under stress conditions. In fact, previous studies showed that phenolic compounds beside ascorbate can protect cell against oxidative stress by phenol-coupled APX reaction (Polle et al., 1997). It has been reported that the antioxidative properties of phenolic components is due to their ability to chelating transition metal ion, the inhibition of superoxide-driven Fenton reaction (Rice-Evans et al., 1997; Arora et al., 1998), and membranes stability by decreasing membrane fluidity (Blokhina et al., 2003).

Total Soluble Protein Content

Lipids and proteins are important constituents of the cell that easily damage in environmental stress condition (Prasad, 1996). Hence, any change in these compounds can be considered as an important indicator of oxidative stress in plants. The results of this study showed variable changes in soluble protein content in different metal treatments that reflect different levels of antioxidant defense. It is thought that decrease in total soluble protein content under heavy metals stress may be due to increase in protease activity (Palma et al., 2002), various structural and functional modifications by the denaturation and fragmentation of proteins (John et al., 2009), DNA-protein cross-links (Atesi et al., 2004), interaction with thiol residues of proteins and replacement them with heavy metals in metalloproteins (Pál et al., 2006). The most decrease was observed in cadmium treatment. It has been reported that cadmium is able to decrease protein content by inhibiting the uptake of Mg and K and promote posttranslational modification (Gardea-Torresdey et al., 2004; Romero-Puertas et al., 2007), decrease in synthesis or increase in protein degradation (Monteiro et al., 2009) and the prevention of Rubisco activity (Muthuchelian et al., 2001; Siedlecka et al., 1997). The increase in total soluble protein content under heavy metal stress may be related to induce the synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins (Verma and Dubey, 2003; Mishra et al., 2006).

SOD Activity

Superoxide dismutase (SOD) is an essential component of plant antioxidation system that can be used as biomarker of environmental stress (Dazy et al., 2009). Superoxide dismutase is the first enzyme in ROS detoxifying process that with converting O^{2-} to H^2O^2 in cytosol, chloroplast and mitochondria plays a axial role in cellular defense mechanisms against the risk of OH formation (Gratão et al., 2005; Salin, 1988). Increase in SOD activity in almost all treatments indicate on high accumulation of ROS under heavy metals stress in order to activate antioxidative defense enzymes to inhibit oxygen radical accumulation. Increase in SOD activity appears to be probably attributed to superoxide radical accumulation, de-novo synthesis of the enzymatic proteins (Verma and Dubey, 2003) and induction the expression of genes encoding SOD (Alvarez and Lamb, 1997). Possible explanations for the decrease in SOD activity under 100 µM Ag treatments may be linked to inactivation of enzyme by the production of excess ROS and unspecific enzyme degradation (Filek et al., 2008) or the binding of non-essential heavy metals to the active center of the enzyme (Stroinski and Kozlowska, 1997).

GPX Activity

Peroxidases (POX) with large number isoenzymatic forms participate in a variety of cellular functions such as growth, development, differentiation, senescence, auxin catabolism, and lignifications (Cui and Wang, 2006). SOD activity results in H_2O_2 production that should be detoxified by some other oxidative enzymes such as APX, GPX and CAT to H_2O and O_2 (Anuradha and Rao, 2007). Although any change in GPX activity can consider as a typical response to oxidative stress, but diversity in peroxidase activity under heavy metals stress depends on plant species (physiological status and genetic potential of plant), time of treatment and metal concentration (Schützendübe and Polle, 2002; Tamàs et al., 2008). Our results showed that POX activities increased in treatment with the low-level of treatments (50 µM) but exception Cd, enzyme activity decreased under severe stress (100 µM) (Table 2). The decrease in GPX activities may result in the cytotoxicity due to blocking of essential functional groups, replacement of essential metals with heavy metals, changes in structure or the integrity of proteins and the interruption of signal transduction pathways of antioxidant enzymes because of poisonous active oxygen species (AOS) derivatives (Alvarez and Lamb, 1997; Stroinski and Kozlowska, 1997; Schützendübel and Polle, 2002). In comparison with control, GPX activity increased in both levels of Cd treatments. Van Assche and Clijsters (1990) demonstrated that increase in GPX activity might be a result of increase in *de novo* protein synthesis or the activation of enzymes already present in plant cells to diminish ROS deleterious effects.

CAT Activity

Increase in CAT activity was expected because increase in SOD activity lead to H_2O_2 generation that will be detoxified in further steps by CAT or POX to maintain the cellular redox state. There are two pathways in ROS scavenging: SOD/CAT and ascorbate-glutathione cycle (Foyer et al., 1994). CAT is one of the most important component of plant protective mechanisms that exist in mitochondria and peroxisomes (Gupta et al. 2009) and has important role in scavenge free radicals specially H_2O_2 generated during photorespiration (Bowler et al., 1992) and stress condition (Mittler, 2002; Foyer and Noctor, 2005). This enzyme by catalyzing H_2O_2 to H_2O and O_2 via two-electron transfer (Wang et al., 2008) prevent the generation of $OH^{\bullet}$ and protect proteins, nucleic acids and lipids against ROS (Imlay and Linn 1988). In present work CAT activity increased in all treatments except Pb and Ag (100 µM) which can be considered as a circumstantial evidence for role of CAT in detoxification of H_2O_2 that induced under heavy metals stress. Under Pb stress a significant dose-dependent decrease in activity was observed and enzyme activity at higher concentration (100 µM) was less than 50 µM and this indicate that increase in metal concentration cause the inhibition of enzyme activity because in high concentration of metal CAT is not properly able to protect cell against ROS. These results are in agreement with results of Verma and Dubey (2003). It

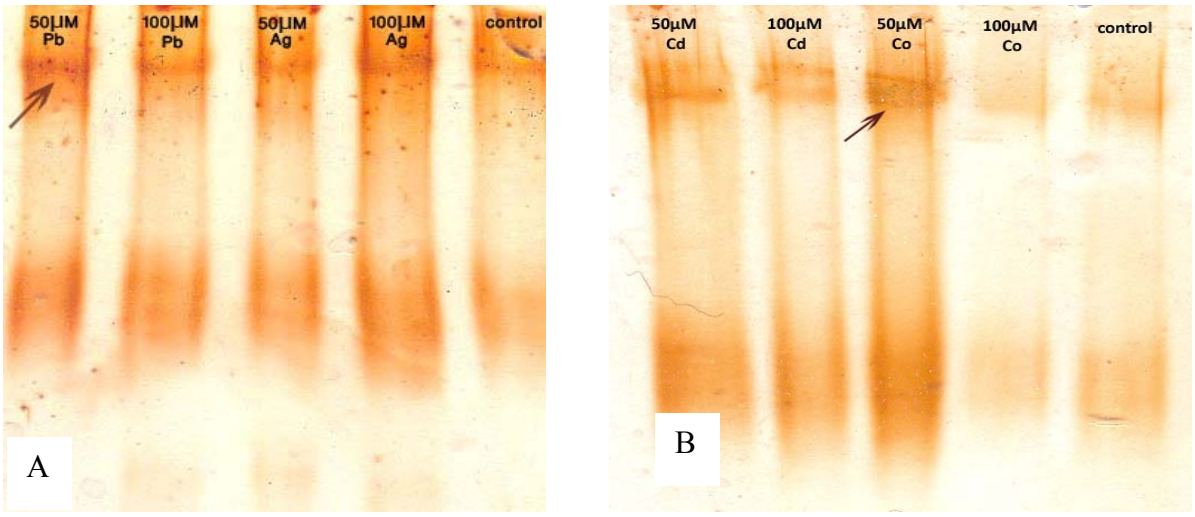

Fig 4. (A) Effect of Pb and Ag on GPX isozyme intensity in *A. littoralis* leaves. (B) Effect of Cd and Co on GPX isozyme intensity in *A. littoralis* leaves. The isozyme band was shown by arrow.

has been reported that decrease in CAT activity under Pb treatment resulted in increase in lipid peroxidation because of decrease in H_2O_2 detoxification (Halliwell and Gutteridge, 1985). It also reported that decrease in SOD and CAT activities cause increase in lipid peroxidation by convert Fe^{+3} to Fe^{2+} for generation OH°. Some of the reasons for decrease in CAT activity under stress conditions are changes in the assembling of CAT subunits and enzyme inactivation or proteolytic degradation by peroxisomal protease (MacRae and Fergusam, 1985; Cakmak, 2000), changes in enzyme structure due to binding non-essentional metals to them (Florence and Stauber, 1986). Also increase in GPX and decrease in CAT activity in Pb 50 µM support this hypothesis that most H_2O_2 produced by SOD, detoxified by peroxidase in oxidation processes.

Gel Electrophoresis

It has been previously reported that any quantitative and qualitative changes in isozyme profile of POX can be considered as an indicator of heavy metals stress (Karataglis et al., 1991; Baccouch et al., 1998). Our data showed variable changes in bands sharpness under different treatments. These results proved that changes in isozymes activity may be dependent on the kind of metal and its concentration. The increase in GPX activity might be due to the role of this enzyme in detoxifying of H_2O_2 by ROS scavenging and cell wall cross-linking.

Conclusion

The present study demonstrated that under heavy metals stress, antioxidative system in *A. littoralis* underwent biochemical changes to survive under high concentrations of metals. Increase in metal chelate components (free phenols and proline) in all treatment levels proves this fact. On the other hand, decrease in chlorophyll and protein (in some treatments) may be considered as circumstantial evidence for the toxicity of heavy metals. The main cause in the variation of different activity of the detoxification enzymes (SOD, GPX and CAT) may be that they exist in different parts of the cell and having different threshold tolerance to heavy metals (Hou et al., 2007). Increase in total soluble protein in most treatments suggests that there are some special proteins that have active roles in the tolerance of this plant to high concentrations of heavy metals. SOD showed the highest enzymatic activity among others, although there is no direct evidence for the role of this enzyme, but it can be pointed to its important role in the tolerance mechanism to heavy metals stress. Our results showed that this halophytic plant have a high tolerance to different heavy metals stress and can survive under high concentration of these metals. There are different mechanisms that different factors such as enzymes, phenolic compounds, proteins and amino acids, pigments are involved in.

Acknowledgements

This work was funded by Shiraz University. The authors express appreciation to Biotechnology Center staff for technical assistance.

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