Plant Archives Vol. 19, Supplement 1, 2019 pp. 284-292 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF LEAD METAL STRESS ON ANTIOXIDANT GENES EXPRESSION IN LEAVES OF SUNFLOWER (*HELIANTHUS ANNUUS* L. CV. AQMAR) PLANTS

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Abstract

To evaluate the expression of the *HaMt1* and *CAT* genes under lead stress, thirty days old sunflower seedlings, cv. Aqmar were treated with 0, 50, 100,150,200 and 250 mg Pb.kg^{-1} soil , and then gene-encoding metallothioneins (*HaMt1*) and antioxidant (*CAT*) gene were measured three times after 0, 24, 48, 72 hours and 10 days of exposure to Pb. The result of using all the concentrations 50, 100, 150, 200 or 250mgPb.kg^{-1} soil showed difference in the response of *CAT* gene after 0, 24, 48 and 72 hours or 10 days of treatment . Expression patterns of *HaMt1* gene were differing from antioxidant gene (*CAT*) patterns. The metallothioneins gene *HaMt1*was expressed in control and lead at 100 mg Pb after 72 h and 10 days, and at 150mgPb after 24, 48, 72 hr of 10 days of treatment comparing with control treatment. These results indicate that the sunflower cultivar Aqmar is a promising plant for phytoremediation of lead-polluted soils.

***Keywords*:** Sunflower, lead, gene expression, RT-PCR.

Introduction

Heavy metals are very persistent in the environment, they do not biodegrade or thermodegrade, and they accumulate to toxic levels (Stoikou *et al*., 2017), which poses a severe threat to organisms exposed to high levels of such pollutants. Metals are essential to the biological functions of plants and animals but at elevated levels, they interfere with metabolic reactions in systems of organisms (Ojuederie and Babalola, 2017). Soil and plants can be contaminated by lead from car exhaust, dust, and gases from various industrial sources which can be primarily attributed to its large industrial use (industry of extraction, petroleum, accumulators, inks and dyes etc.) (Kacálková *et al*., 2014) or applications of lead-contaminated media (sewage sludge and fertilizers) to land (Grover *et al*., 2010). It could accumulate in agricultural soils and get into the food chain, thereby becoming a major threat to food security (Ojuederie and Babalola, 2017).

Lead may enter the nucleus (Małecka *et al.,* 2008) and bind directly to the DNA or indirectly to protein. After binding to DNA, lead disrupts DNA repair and replication mechanisms. Lead does not induce direct genotoxic effects until it becomes attached to naked DNA (Valverde *et al*., 2001). To face with the increased production of ROS and to avoid oxidative damage, plants have a system of antioxidant enzymes that scavenge the ROS that are present in different cell compartments (Gupta *et al.*, 2010). Plants have many strategies to withstand oxidative stress (Gill and Tuteja, 2010). The first line of defence is the activation and synthesis of enzymatic antioxidants and non-enzymatic compound to handle with the excessive production of ROS and its scavenging (Hossain *et al*., 2012).

Catalase was the first antioxidant enzyme discovered and characterized (Mhandi *et al*., 2010). It is a haemoprotein that plays a significant role in the antioxidant defense system of all aerobic organisms by catalyzing the dismutation of H_2O_2 into water and dioxygen (Roychoudhury *et al*., 2012). Metallothioneins (MTs) are a group of cysteine-rich proteins with two structural domains (Cys-rich and metal-binding) participate in metal homeostasis and detoxification (Choppala *et al.,* 2014; Gu *et al*., 2014). MT genes appear to be differentially regulated in a tissue-specific manner and in relation to developmental stage and also in response to a number of stimuli, including HMs (Castiglione, 2007). The physiological function of MTs has been proposed include (i) participation in maintaining the homeostasis of essential transition HMs, (ii) sequestration of toxic HMs and (iii) protection against intracellular oxidative damage **(**Gasic and Korban, 2006).

In few decades ago, much interest has been taken on the use of sunflower for phytoremediation of organic pollutants and heavy metals due to its high production of biomass and strong ability to accumulate heavy metals (e.g., Cd, Cr, Cu, Hg, Ni, Pb, Zn) (Hao *et al*., 2011; Shaheen and Rinklebe, 2015). The studies on the antioxidative efficiency under lead metal stress are well

documented but reports on gene expression due to excess Pb induced oxidative stress under experimental conditions are very rare. The present study focuses on *CAT* and *HaMt1* gene expression in sunflower under stress of Pb in plants grown in soil-pot culture at an excess supply of this element.

Materials and Methods

Plant Materials

The experiment was carried out on sunflower plants (*Helianthus annuus* L. cv. Aqmar) obtained from Field Crop Department, College of Agriculture, University of Baghdad**.**

Experimental Layout

The study was carried out in the woody shade, Department of Horticulture and Gardens, College of Agriculture - University of Baghdad. Seeds were grown in plastic pots filled with 10 kg of soil. Surface soil were gathered at depths ranging from 0-30 cm, the soil was air-dried and then passed through 2 mm sieve, and large stones and plant root debris were removed. The experiment was laid out in Completely Randomized Block Design with five levels 50, 100, 150, 200 and 250 $mg.kg^{-1}$ soil of lead acetate [$Pb(CH_3COO)_2$], in addition to control treatment (tap water). Lead treatments were added to the soil at one month seedling age .Each treatment was dissolved in 0.250 liter of water for the purpose of distributing it evenly to pot soil; the pot bottom was sealed off to prevent lead leaching.

Preparation of Leaves Samples

The leaves samples were collected after 24, 48 and 72 hours and after 10 days of treatment with lead doses. Outer surface of the plants were washed with distilled water to remove residual contaminants. In addition, Scissors and forceps were washed with ethanol (70 v/v) and then with distilled water after each sample. After cutting of leaf samples, 1 ml of RNA secure solution (Bioland Scientific LLC, USA) was added to the ependrof tubes for each sample to protect RNA products from degradation until RNA extraction.

RNA Extraction and Reverse Transcriptase

RNA was extracted from 100 mg of each sample, TRIzol® protocol (Pattemore, 2014) was used for RNA extraction from sunflower leaf. The principle method of RNA extraction was related to using Traizol solution. Traizol is anacid guanidinium thiocyanate-phenol-chloroform mixture; It is a powerful method for DNA/RNA extration (Chomczynski and Sacchi, 1987). This procedure was done using commercially available RNA extraction kit (GENEzol Tri RNA Pure Kit - Geneaid - Bioner, Korea), the procedure was explained in details in user's manual. The solution was incubated at 60^{o} C for 10 minutes. In this step, the solution was ready for convert reverce transcriptase RNA to cDNA.RNA integrity was verified on a 1% agarose gel. RNA was stored at −80ºC until use. First strand cDNA synthesis was performed by the protocol of Wiz Script RT FDmix Kit (Wizbiosolution, Seongnam, South Korea).

Real Time Reverse Transcription PCR (RT-PCR)

SYBR Green I fluorescent dye was used in this study for measuring quantitative PCR. The thermal cycling programs were carried out in a real time PCR system (Rotor-Gene Q, Qiagen, Germany) supplememted with the WIZpURE ^{Tm} qPCR Master (SYPR) Kit (Go Taq G2 Green Master - Promega, USA). Two primers pair (*CAT*and *HaTm1*) genes with *Actin* as internal standard for normalization were used (Table 1).

Table 1 : Primers pair for RT- PCR .

Gene		Primer Sequence	Product Size	Reference
CAT	F	CTTCCCGCTTGAATGTGAAG	248	Pena *et al*., 2011
	R	CCGATTACATAAACCCATCATC		
HaMt1	F	ATGTCTTGCTGTGGTGGAAG	400	Wińska-Krysiak *et al*., 2015
	R	TTTGCAGGTACAAGGGTTGC		
Actin	F	AGGGCGGTCTTTCCAAGTAT	320	Fernandez *et al*., 2008
	R	ACATACATGGCGGGAACATT		

Components of RT-PCR reaction and mixing amounts are shown in Table 2. Optimization of PCR reaction was accomplished after several trials, thus the following programs were adopted, and the PCR reaction was carried out as shown in Table 3. Theaverage expression level of the testing *CAT*, *HaMt1* and *actin* genes from qRT-PCR analysis were utilized from the result of three replicates for examination using $2^{-\Delta\Delta CT}$ (Double logarithm Dalta Dalta Ct) method (Zhao *et al*., 2013). The cDNAs for qRT-PCR was used after dilution to 1×10^{-1} as templates.

Table 2 : RT-PCR reaction components.

Component	Concentration(µl)
GoTaq® qPCR Master (SYBR)	12.5
DNA	8
Primer F	0.5
Primer R	0.5
Nuclease-Free Water	3.5
Total	25

Table 3 : PCR program for SYBR Green I fluorescence.

No.	Step	Temp.(°C)	Time	Cycle
1.	Initial Denaturation	95	4 min.	1
2.	Denaturation	95	30 sec.	45 cycles
3.	Annealing	62	35 sec.	
	Extension	72	48sec.	
4.	Melting Curve analysis	60 to 95	5 sec.	1

The presence of a single band of the expected size on a 2% agarose gel which was stained with ethidium bromide at 70 volt/cm^{2}and 50 current for 1.5 hour in 1 X TBE buffer. After that, it was visualized under UV light using ultraviolet transilluminater. A 1kb -100 bp DNA ladder (Promega, USA) was used and the gel was photographed by a digital camera.

Results

Total RNA was successfully extracted from all samples using commercially available RNA extraction kit (GENEzol Tri RNA Pure Kit - Geneaid – Bioner, Korea). The concentration of total RNA ranged from 210 to 250 ng/ µl, and the purity of total RNA samples ranged from 1.7 to 1.9 ng/µl. The efficiency of cDNA concentration was assessed through gel electrophoresis to detected cDNA before real time application (Figure1).

The qRT-PCR method was used to examine the expression levels of the antioxidant *CAT* gene in sunflower plant under Pb stress. The results showed that the stress treatments increased the transcript levels of the tested gene in over expressing plants compared to the normal growing conditions. The expression levels of the genes were related with the degree of tolerance to heavy metal stress. The result of using all concentrations (50, 100, 150, 200 or 250 mg Pb.kg^{-1} soil) showed the difference in the response of *CAT* gene after 0, 24, 48 and 72 hours or 10 days of treatment (Table 4 and Figure 2). The real-time qPCR results showed that the expression was increased after 24 hours at all concentrations by increasing the $\Delta\Delta^{CT}$ by -1.77, -0.26, -5.15 -0.39 and -0.01 $\Delta\Delta^{Ct}$ or approximately 3.41, 1.20, 35.51, 1.31 and 1.07 fold-differences, respectively. Also the expression increased at 200 and 250 mg Pb.kg^{-1}soil after 10 days by -0.59 and -0.51 $\Delta\Delta^{Ct}$ or approximately 1.51 and 1.42 fold-differences, respectively, comparing with control treatment. Similar results obtained with the logarithmic scale (Figure 3). The results of *CAT* gene expression were different in Ct threshold. After 24 hours of treatment with Pb.kg^{-1} soil, the concentration 150 mg Pb showed high Ct threshold -5.15 $\Delta\Delta^{Ct}$ followed by 50mg Pb.kg^{-1} soil -1.77which were 35.51 and 3.41 fold .

Table 4 : CAT gene expression at Pb concentrations.

Treatment	Average ct	Ct ±StDv	$\Delta\Delta^{Ct}$ ±StDv	$\Delta\Delta^{Ct}$	Folding	incorporating StDv into fold difference (min & max)
Reference	27.15	27.15 ± 0.27				
control	30.55	30.55 ± 0.47	3.4 ± 0.54	0	1	1 (0.69 - 1.46)
50-24H	28.78	28.78 ± 0.61	1.63 ± 0.67	-1.77	3.41	3.41(2.15 - 5.42)
50-48H	31.04	31.04 ± 0.21	3.89 ± 0.34	0.49	0.71	0.71 (0.56 - 0.90)
50-72H	31.40	31.40 ± 0.32	4.25 ± 0.42	0.85	0.55	0.55 (0.42 - 0.74)
50-10D	0.00	0		0	0.00	0
100-24H	30.29	30.29 ± 0.63	3.14 ± 0.69	-0.26	1.20	1.20 (0.74 - 1.93)
100-48H	32.81	32.81 ± 0.19	5.66 ± 0.33	2.26	0.21	0.21 (0.17 - 0.26)
100-72H	0.00	0	0.0	0	0.00	0
100-10D	31.14	31.14 ± 0.53	3.99 ± 0.59	0.59	0.66	0.66 (0.44 - 1.00)
150-24H	25.40	25.4 ± 0.82	-1.75 ± 0.9	-5.15	35.51	35.51 (19.52 - 64.59)
150-48H	32.06	32.06 ± 0.72	4.91 ± 0.77	1.51	0.35	0.35 (0.21 - 0.60)
150-72H	31.08	31.08 ± 0.44	3.93 ± 0.5	0.53	0.69	0.69 (0.48 - 0.99)
150-10D	31.36	31.36 ± 0.52	4.21 ± 0.59	0.81	0.57	0.57 (0.38 - 0.86)
200-24H	30.16	30.16 ± 0.64	3.01 ± 0.7	-0.39	1.31	1.31 (0.81 - 2.12)
200-48H	44.48	44.48 ± 0.28	17.33 ± 0.39	13.93	0.00	0
200-72H	33.38	33.38 ± 0.56	6.23 ± 0.6	2.83	0.14	0.14 (0.09 - 0.22)
200-10D	29.96	29.96 ± 0.17	2.81 ± 0.32	-0.59	1.51	1.51 (1.21 - 1.88)
250-24H	30.45	30.45 ± 0.33	3.30 ± 0.4	-0.1	1.07	1.07 (0.80 - 1.44)
250-48H	33.15	33.15 ± 0.46	6.00 ± 0.53	2.6	0.16	0.16 (0.11 - 0.24)
250-72H	31.88	31.88 ±0.73	4.73 ± 0.8	1.33	0.40	0.40 (0.23 - 0.68)
250-10D	30.04	30.04 ± 0.34	2.89 ± 0.43	-0.51	1.42	1.42 (1.05 - 1.92)

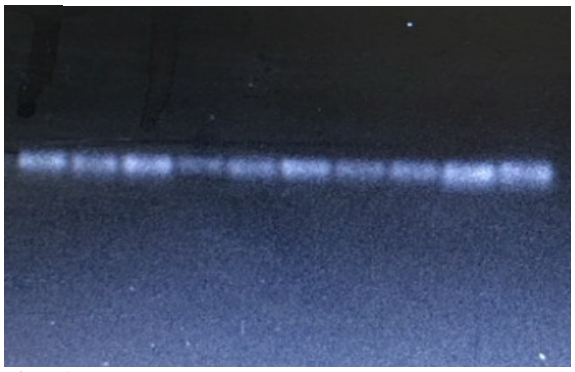

Fig. 1 : Gel electrophoresis detected cDNA bands (agarose 1%, TBE buffer (1X), 70/cm for 30 min stained with ethidium bromide).

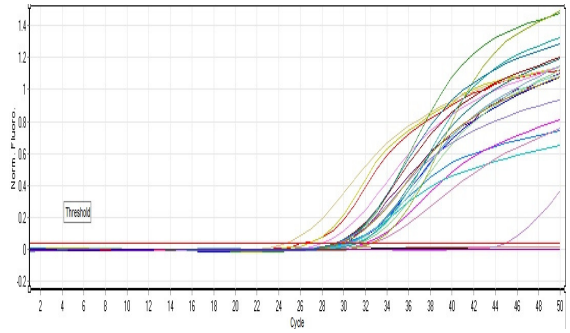

Fig. 2 : Real time -PCR results of CAT gene after 0, 24, 48, 72 hours and 10 days with action a reference gene at Pb concentrations.

CAT gene expressions were analysed with RT-PCR by using samples' cDNAs as a template. PCR products were then resolved on a 2% agarose gel. Expected band size for CAT (248 bp) was observed in all expressed samples, and did not observed r in samples that did not expressed (Figure 4).

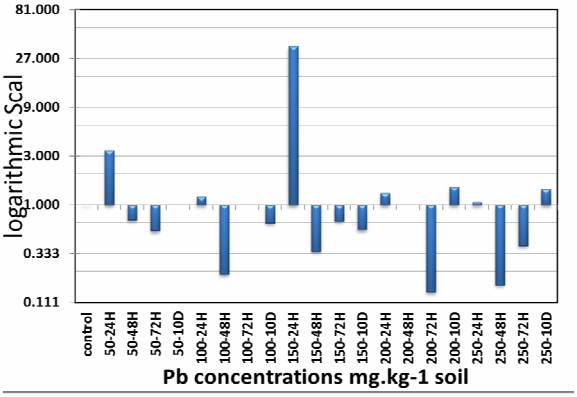

Fig. 3 : Logarithmic scale of CAT gene expression at Pb concentrations.

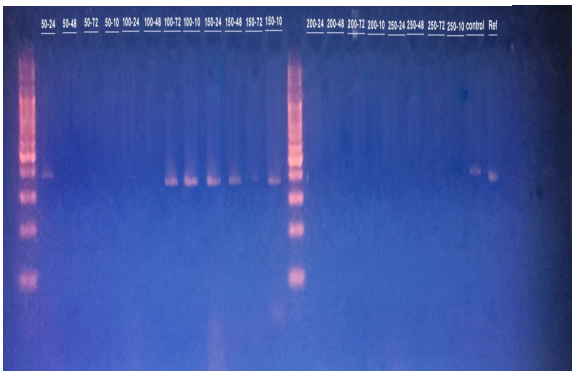

Fig. 4 : Effect of Pb treatment on the expression of *CAT* (248bp)(RT- PCR amplification) in leaves of *Helianthus annuus* L. plants. Effects were evaluated after 0, 24, 48, 72 hours and 10 days of exposure to lead (0, 50, 100, 150, 200 or 250 mg $Pb.kg^{-1}$ soil). Lane 1: 100 bp ladder, Lane 1, 7-12 : Positive for *CAT* gene; Lane 21,: control ; Lane 22 reference gene detected by 2 % agarose gel electrophoresis and 70 volts for 1.5 h, 1X TBE buffer).

The expression patterns of *HaMt1* gene were differing from the above-mentioned antioxidant gene (*CAT*) patterns. The metallothioneins gene *HaMt1* was expressed in control and lead at 100 mg Pb after 72 h and 10 days, and at 150 mg Pb after 24, 48, 72 hr of 10 days of treatment (Table 5 and figure 5).The expression of *HaMt1* was at high levels after 72 hr and 10 days at 100 mg of Pb by increasing the $\Delta\Delta^{CT}$ by -9.87 and -8.69 or approximately 935.76 and 431.00 fold-differences, respectively, and after 24 hr at 150 mg of Pb. Also the expression was at high level at 150 mg $Pb.kg^{-1}$ soils after 24 hr of treatment by increasing the $\Delta\Delta^{CT}$ by -7.29 or approximately 156.50 fold-differences comparing with control treatment. The expression levels were reduced after 10 days of treatment with 150 mg of Pb by increasing the $\Delta\Delta^{CT}$ by -1.01 or approximately 2.01fold-differences comparing with control treatment. Whereas showed more reduction after 48 and 72 hr of treatment with 150 mg of Pb to $\Delta\Delta^{CT}$ by 0.85 and 5.19 or approximately 0.55 and 0.03 fold-differences comparing with control treatment. Similar results obtained with the logarithmic scale (Figure 6). *HaMt1* gene expressions were analysed with RT-PCR by using samples' cDNAs as a template. PCR products were then resolved on a 2% agarose gel. Expected band size for *HaMt1* (400 bp) was observed in all expressed samples, and did not observed in samples that did not expressed (Figure 7).

Table 5 : *HaMt1* gene expression at Pb concentrations.

Treatment	Average ct	Ct ±StDv	$\Delta\Delta^{Ct}$ ±StDv	$\Delta\Delta^{Ct}$	Folding	incorporating StDv into fold difference
Reference	27.28	27.28 ±0.27				
control	35.21	35.21 ±0.41	7.93 ± 0.49	0	1	1 (0.71-1.41)
50- 24H	42.89	42.89 ±0.26	15.61 ± 0.37	7.68	0.00	0 (0.00-0.01)
100-72H	25.34	25.34 ±0.25	-1.94 ± 0.37	-9.87	935.76	935.76 (725.10 -1207.63)
100-10D	26.52	26.52 ±0.94	-0.76 ± 0.98	-8.69	413.00	413.00 (209.67- 813.51)
150-24H	27.92	27.92 ±0.88	0.64 ± 0.92	-7.29	156.50	156.50 (82.68 -296.21)
150-48H	36.06	36.06 ±0.25	8.78 ± 0.37	0.85	0.55	0.55 (0.43-0.72)
150-72H	40.4	40.40 ±0.47	13.12 ± 0.54	5.19	0.03	2.01 (1.49 - 2.72)
150-10D	34.2	34.20 ±0.34	6.92 ± 0.4	-1.01	2.01	2.01 (1.49 - 2.72)

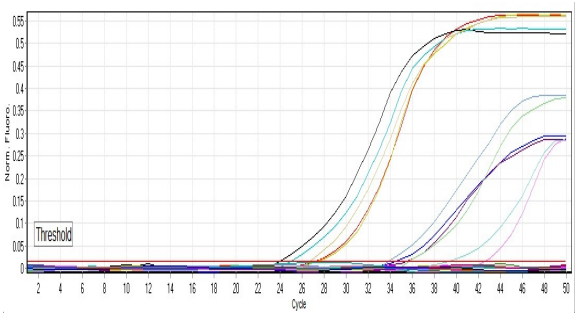

Fig. 5 : Real time -PCR results of *HaMt1* gene after 0, 24, 48, 72 hours and 10 days with actin a reference gene at Pb concentrations.

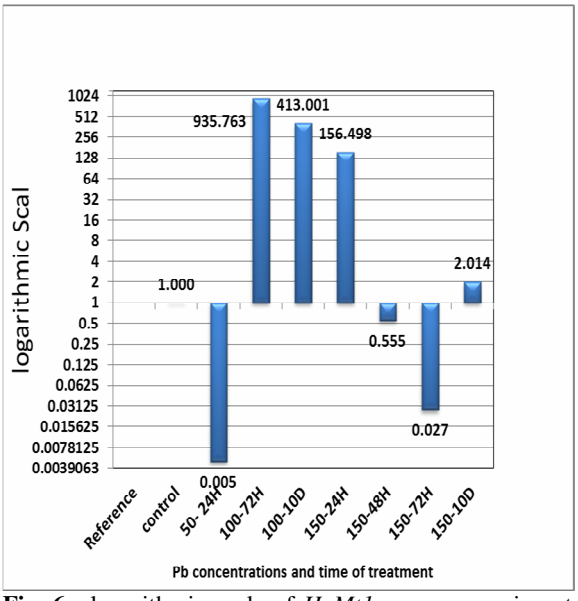

Fig. 6 : logarithmic scale of *HaMt1* gene expression at Pb concentrations

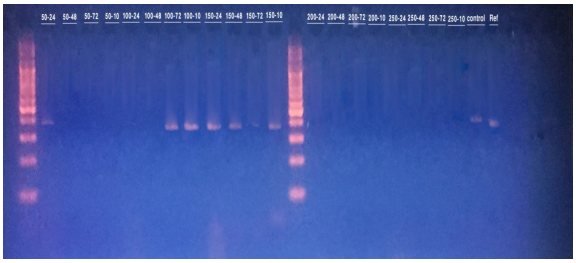

Fig. 7 : Effect of Pb treatment on the expression of *HaMt1* (400 bp) (RT- PCR amplification) in leaves of *Helianthus annuus* L. plants. Effects were evaluated after 0, 24, 48, 72 hours and 10 days of exposure to lead (0, 50, 100, 150, 200 or 250 mg Pb.kg^{-1} soil). Lane 1: 100 bp ladder, Lane 7-12 : Positive for *HaMt1* gene; Lane 21: control; Lane 22 reference gene detected by 2% agarose gel electrophoresis and 70 volts for 1.5 h, 1X TBE buffer).

Discussion

In this study, RNA was extracted from leaf seedlings tissues without the use of liquid nitrogen. Prior to this study, several tests were performed to extract DNA and RNA from freshly seedlings belonging to some species using liquid nitrogen and without use it and gave close results (Kang and Yang, 2004). The aim of using liquid nitrogen is to broken the cell wall. Since seedlings are soft and freshly, the cell wall can be broken by crushing and grinding the sample into a fine paste using a sterile pestle and mortar. It has given similar results to the use of nitrogen liquid in addition to being cheaper, faster and safer. Al-Jobori and AL-Tamemy (2018) extracted DNA without the use of liquid nitrogen. Magdum (2013) presented reliable protocol independent to use of liquid nitrogen, and yielding higher quantity of genomic DNA.

The enzyme catalase represents one of the essential enzymatic defenses against oxidative stress. It is substantial in all oil seeds and its activity has been demonstrated to be closely related to the germination rate in the sunflower (Bailly *et al*., 2004; Tseng *et al*., 2007) revealed that antioxidants are involved in the mechanism that scavenges the ROS generated by heavy metal stress. This was harmonic with the results of previous studies, which declare that heavy metals were toxic to plants and caused injuries through the generation of ROS (Scoccianti *et al*., 2006). Sunflower is a plant capable of accumulating high concentration of metals, and it has been declare that many plant species accumulate significantly greater amounts of metals in roots than in shoots (Groppa *et al*., 2007). Lead has considerable effect on the activities of antioxidant enzymes in leaves and roots of sunflower at stress levels of Pb. In the present study, the effect of Pb on the *CAT* gene expression 30 days of developing sunflower seedlings was investigated. The addition of Pb enhances the expression of *CAT* gene and was higher at all Pb concentrations after 24 hr of treatment decreased at 200 and 250 mg Pb after 10 days (Table 4)

In response to metal stress including Pb stress, plants cells have developed antioxidant defense mechanism to decrease oxidative damage. Plant cells are equipped with a protective system including antioxidant enzymes like catalase and peroxidase which can inhibit free radicals (Cho and Park, 2000). Decrease in enzyme synthesis or change in subunits arrangement may be a cause for decline in catalase activity (Hertwig *et al*., 1992).The antioxidant enzymes involves SOD, POD, CAT, APX, and GR which regulate the cellular superoxide (O_2^-) and hydrogen peroxide (H_2O_2) concentration, therefore inhibiting the production of -OH radicals (Rucinska-Sobkowiak and Pukacki, 2006). SOD and CAT play a key role in elimination of oxidative stress (Gomes-Junior *et al.,* 2006). In present study, expression of *CAT* gene increased under the Pb stress (Figures 2, 3). Several studies have revealed that treatment of heavy metal increases ROS formation, and therefore, major increase in the activities of SOD, CAT, and APX (Bharwana *et al*., 2013; Bashri and Prasad, 2015).

Plants normally face the oxidative stress when exposed to heavy metals. Oxidative injury observed to be implicated in Pb stress as indicated by decrease in some antioxidants and rise in ROS (O_2^-, H_2O_2) activities (Kanwal and Ali, 2014). In this study, exposure sunflower plants to Pb stress caused several fold increase in *CAT* expression in leaves (Table 4). The different response of *CAT* transcripts in roots and cotyledons of sunflower seedling could represent the beginning of the biogenesis of the peroxisomal core, in which both temporal and spatial changes in the pattern of catalase isozyme expression occur during the course of normal post-germinative development (Kleff *et al*., 1997).

Metallothioneins (MT) proteins are classified into mammalian Class I and plant Class II, and plant MTs can be further subdivided into four types based on the number and arrangement of cysteine residues and the length of spacer region (Cobbett and Goldsbrough, 2002). These four-type plant MTs exhibited certain tissue-preferential expression patterns. Type 1 MTs are expressed much higher in roots than in shoots (Hudspeth *et al*., 1996), whereas Type 2 MTs are found mainly in leaves (Hsieh *et al*., 1995). Type 3 MTs are expressed abundantly in the ripe fruits (Reid and Ross, 1997) and expression of Type 4 MTs, also known as the Ec type, was only found in developing seeds (Chyan *et al*., 2005). In this study, the expression of the gene of metallothionein (*HaMT1*) was observed in sunflower leaves at 100mgPb after 72 hr and 10 days, and at 150 mg Pb after 24, 48, 72 hr and 10 days (Table 5 and Figures 5,6). These results are not consistent with Wińska-Krysiak *et al*. (2015) who observed the expression of the gene of metallothionein (*HaMT1*) in stems only after 24 and 48 hours of treatment, they reported that the expression of *HaMt1* was more pronounced in plants treated with 15 mg Pb dm-3 than in the controls, and concluded that metallothioneins are only involved in Pb detoxification when the stress low or moderate. A family of genes encoding a protein similar to metallothionein (*htMT2*) was identified in *Helianthus tuberosus*. The transcripts of these genes were detected in stems and – at low levels – in leaves, but not in roots, and declined after the plants' exposure to zinc and copper ions (Chang *et al*., 2004).

Defense mechanisms used by plants include: production of the enzyme phytochelatin synthase that readily binds to heavy metals at lethal levels (Jan and Parray, 2016), production of metallothioneins and proline that acts as a compatible and metabolic osmolyte, a component of cell walls, free radical scavenger, antioxidant and macromolecule stabilizer (Ehsanpour *et al*., 2012). It has been reported that metallothioneins expression is organ-dependent (Zhou and Goldsbrough, 1995), and regulated at the transcription level, and may be induced by the presence of hormones, toxins and heavy ions (Mejare and Bulow, 2001). In *A.thaliana*, the synthesis of mRNA *MT2* was strongly induced by the presence of Cu, Cd, Zn ions (Zhou and Golsbrough, 1994).

Apart from detoxification of heavy metals, plant MTs also play a role in maintenance of the redox level, repair of plasma membrane (Emamverdian *et al*., 2015),

cell proliferation and its growth, repair of damaged DNA and scavenge ROS (Grennan, 2011). The mechanisms of Pb-detoxification include the sequestration of Pb in the vacuole, phytochelatin synthesis and binding to glutathione and amino acids. Pb tolerance is associated with the capacity of plants to restrict Pb to the cell walls, the synthesis of osmolytes and the activation of an antioxidant defense system. The role of metallothioneins is to bind metal ions by cysteine thiol groups (Gavanji *et al*., 2014). Metallothioneins appear not to be the major determinants of metal hyper accumulation. However, as they are expressed when sunflower plants exposure to high levels of Pb. Metallothioneins might be involved in metal tolerance as modifiers rather than as primary determinant. Alternatively they may be involved in the homeostasis of copper in a high-Zn or high-Cd cellular environment (Hassinen, 2009). In this study, expected band size (248 bp for *CAT*) approximately were observed in all samples(Figure 4).Whereas expected band size (400 bp for*HaMt1*) were observed in treatments of 100 mgPb after 72 hr and 10days, and in treatments of 150mgPb after 24, 48,72 hr and 10days (Figure 7). Results of *HaMt1* gene are similar with the results of Wińska-Krysiak *et al*. (2015) who found that after 24 and 48 hours of treatment, the expression of *HaMT1*was more pronounced in plants treated with 15 mg $Pbdm^{-3}$.

Conclusions

Metallothioneins appear not to be the major determinants of metal hyper accumulation. Since *HaMt1* gene not expressed at all Pb concentrations. The expression of *CAT* gene changed under all treatments of Pb metal. The highest level of *CAT* gene expression was measured at 150 mg $Pb.kg^{-1}$ soil compared to control plants, which might be preventing enzyme inactivation and H_2O_2 accumulation.

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