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Biochemical responses to cadmium and lead stresses in date palm (*Phoenix dactylifera* L.) plants

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Abstract. Investigations were conducted on young date palm (*Phoenix dactylifera* L.) plants derived from tissue culture to evaluate their biochemical responses to cadmium and lead treatments through irrigation water. Changes in date palm metabolisms were observed as a response to heavy metals accumulation, the reductions in photosynthetic pigments as chlorophyll and carotenoides were obvious after 180 days of treatments, compared with the increase in anthocyanin pigments which was twofold more than control plants. It is noteworthy, that Cd and Pb treatments induced a significant decrease in chlorophyll stability index (CSI) to 66.97 and 58.79%, in Cd (9 mg/kg) and P (276 mg/kg), respectively. Stressed *P. dactylifera* plants tend to accumulate more carbohydrates, free amino acid and proline in their leaves as consequences of heavy metal treatments. Interestingly, the level of total soluble proteins was found to be less, up to twofold, than those in non-stressed plants. An excessive production of reactive oxygen species (ROS) was generated by *P. dactylifera* plants exposed to Cd and Pb, an increase in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) accumulations was observed in stressed plants, with a significant reduction in membrane stability index (MSI), the MSI was 78.8% in non-stressed plants and decreased significantly to 66.17 and 60.90%, under Cd and Pb at high concentrations, respectively. The activity of antioxidant enzyme peroxidase and total phenolic compounds were enhanced under Cd and Pb treatments. The present study supports the potential toxic effect of Cd and Pb on date palm by hampering metabolism and interfering with biological functions.

Key Words: chlorophyll, *P. dactylifera*, heavy metals, lipid peroxidation, membrane stability.

Introduction. Heavy metals (HMs) can be defined as elements having densities higher than 5 g cm^3, 53 out of 90 naturally occurring elements are HMs, and more likely have toxic effects such as aluminum (Al), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), and zinc (Zn), and metalloids, including arsenic (As) and boron (B) with severe impacts on agriculture and health (Weast 1984; MacFarlane & Burchett 2001; Pinto et al 2003). The HMs levels in the environment has risen dramatically during the past decades; several sources of HMs account for this increase including anthropogenic activities like mining, metal production, wood production and phosphate fertilizers; additionally, natural activities lead to an increase in the level of heavy metals in the environment (Galloway et al 1982; Jackson & Alloway 1991; Angeleon & Bini 1992; Sanita di Toppi et al 1999). Another reason for the increase of HMs in the environment is the non-biodegradability of these metals which leads to their persistence (Mustafa & Komatsu 2016).

The bioavailability of HMs in soils depends mainly on soil physical and chemical characteristics, as well as, the species of growing plants (Mortvedt 1994; Viehweger & Geipel 2010). Different uptake routes have been reported in plant HMs uptake; the first route is passive uptake which depends entirely on the concentration gradient across the membrane and the active uptake that is driven by inducible substrate-specific and energy dependent (Nies 1999; Williams et al 2000).

Exposure of plants to heavy metals at elevated levels for specific time periods generally provoked profound responses, the type of plant responses, as well as, their intensity are mainly dependent on the type, available form and concentration of the heavy metal used, the species of examined plants, the analyzed plant tissue and the

route and time of exposure (Gupta et al 1999; Prasad et al 1999; Cho & Park 2000; Madhava Rao & Sresty 2000; Shainberg et al 2000; Aldoobie & Beltagi 2013; Chen et al 2015). The most common response pattern in heavy metals-stressed plants is associated with oxidative injury (Schutzendubel & Polle 2002; Mustafa & Komatsu 2016), HMs are a well-known disrupter of cellular redox homeostasis, which consequently lead to increase production and accumulation of reactive oxygen species (ROS) such as anion (O^{-}_{2}); hydroxyl radical (OH^{-}) and hydrogen peroxide (H_2O_2) (Saidi et al 2013; Howladar 2014), as a consequence of heavy metals overproductions in plant cells, different types of damages have been identified including lipid peroxidation which alters the structure and function of membrane, for many years the malondialdehyde (MDA) product of lipid peroxidation has been considered as a biomarker of oxidative injury (Unyayar et al 2006; Gonclaves et al 2009; Badmus et al 2011). Additionally, ROS cause damage at the DNA level either by breaks of DNA strands or inhibition of DNA replication by binding protein involved in replication, resulting in alteration of encoded proteins and lead to a malfunction of protein in plant cells (Lin et al 2007; Nanda & Agrawal 2016). Induction of electrolyte leakage is related with exposed plants to heavy metal stress which associated with cell membrane stability (Garty et al 2000).

Regarding biochemical responses of stressed plants, several responses have been characterized with elevated heavy metals, such as photosynthesis inhibition, a decrease of chlorophyll *a* and *b*, as well as, carotenoides was observed with heavy metals treatments in different plants (Jiang et al 2012; Pooja et al 2012; Chen et al 2015; Zouari et al 2016a). An increase in the amount of MDA, proline content; antioxidant enzymes (such as CAT; SOD and POD) and protein content was associated with heavy metals treatments in *Vigna* seedlings, *Medicago sativa*, *Olea europaea* and *Phoenix dactylifera* (Pooja et al 2012; Chen et al 2015; Zouari et al 2016b,c). A significant decrease in chlorophyll stability index and membrane stability index was observed in *Vigna* seedlings treated with cadmium (Pooja et al 2012).

Date palm (*Phoenix dactylifera* L.) is a dioecious monocotyledon of the family Arecaceae, cultivated mainly for their nutritive fruits, and as ornamental plants, with a pivotal importance, in agricultural, cultural, socioeconomic around the world, more specifically, in Middle East regions (Al-Khyari et al 2015). The remarkable survivability of *P. dactylifera* trees enable these plants to grow in different areas, as well as, a wide range of temperatures and grow almost in all type of soils. Most importantly, it can grow over large areas by roadsides in industrial, rural, residential and agricultural areas even when a high level of pollution exists (Al-Khashman et al 2011; Abass et al 2015).

In the present work, the biochemical responses of young date palm plants to Cd and Pb treatments were assessed to understand the role of these heavy metals in plant toxicity.

Material and Method

Plant materials and heavy metal treatments. For the present study tissue culture-derived *P. dactylifera* offshoots of Barhee cultivar at two years old were selected (their genetic uniformity were ascertained).Two different concentrations of Cd and Pb were selected according to the maximum and minimum levels recorded in Basra governorate soil (Al-Jabbary et al unpublished data), to evaluate their affects on *P. dactylifera* plant on biochemical level. *P. dactylifera* offshoots were cultured in pots, each pot filled with 5 kg of soil, soil chemical characteristics were: pH= 7.62; electrical conductivity = 5.23 ds m^{-1}; cation exchange capacity = 9 cmole and organic matter = 0.81 %. Soil texture was loam (silt = 45.65; sand = 32.44 and clay = 21.91).

Transplanted offshoots were subjected to laboratory conditions during the trail (10,000 Lux light density; 27±2°C temperature and relative humidity of 60–70%). Heavy metals treatments were as follow:

Control: non-stressed *P. dactylifera* plants; Cd_1: 3 mg kg^{-1}; Cd_2: 9 mg kg^{-1}; Pb_1: 100 mg kg^{-1}; Pb_2: 276 mg kg^{-1}.

Cadmium chloride and lead acetate (Sigma-Aldrich) were used as sources of heavy metals and applied to each treatment by irrigation using RO (reverse osmosis)

water, the chemical properties of used water were: pH = 7.6; EC = 1.3 ds m^{-1}; Ca^{+} = 5.9; Mn^{+} = 15.8; Na^{+} = 19; Cl^{-} = 15 and K^{+} = 1.9 ppm.

The trail was conducted with triplicates for each treatment at the Date Palm Research Centre, University of Basra. Plants were irrigated with heavy metals treatment for 180 days during the period between 1st/ January/ 2016 to 1st/ July/ 2016.

Cadmium and lead analysis in soil and P. dactylifera leaves. Total concentrations of Cd and Pb in soil and *P. dactylifera* leaves were analyzed using a Flame Atomic Spectrophotometer (Perkin Elmer AAS Analysis 300, USA) at zero time and 180 days after treatment. Acid digestion procedure was followed to measure the total heavy metals content in soil according to Davidson (2013). Briefly, 1 g of dry soil finely ground, moistened with distilled water heated in 100 mL Teflon beaker with 10 mL of HNO_3, and evaporated to a small volume, 5 mL of HNO_3, 5 mL of 70% $HClO_4$ and 10 mL of hydrofluoric acid (HF) were added, the mixture was heated to perchlorate fume, and then left for 30 min. 10 mL HCl (1/1, v/v) was added to the mixture and boiled for 10 min, cooled and diluted to 100 mL using distilled water.

Wet acid digestion of the *P. dactylifera* leaves was performed using HNO_3/ $HClO_4$ digestion (Jones 1984). Briefly, 5 mL of HNO_3 (70%) and 1.5 mL $HClO_4$ (60%) were added to 0.5 g of sample, and the solution was heated until the disappearance of the brown fume, then cooled, subsequently a 5 mL of diluted (1:1) HCl was added, finally the mixture was diluted with distilled water to 25 mL.

Biochemical responses of stressed P. dactylifera plants. The responses of *P. dactylifera* plants to heavy metal stresses were evaluated at zero time and 180 days of treatment.

Pigments content. Leaf pigment contents were determined in *P. dactylifera* leaves. The extraction procedure of Arnon (1949) was followed, 200 mg of fresh leaves were homogenized in 8 mL acetone (80%), followed by a centrifugation at 3,000 rpm for 30 min. Absorbance of supernatant were determined at 645, 663, 534 and 470 nm. The following formulas were used to estimate the pigments and the values were expressed in mg g^{-1} of fresh weight (Asare-Boamah et al 1986):

Chlorophyll-*a* = 12.7 (OD_{663}) -2.69 (OD_{645}) x Vol./Wt).

Chlorophyll-*b* = 22.9 (OD_{645}) - 4.68 (OD_{663}) x Vol./Wt)

Total chlorophyll = 20.2 (OD_{645}) + 8.02 (OD_{663}) x Vol./ Wt.)

Anthocyanins = 0.0821 x A534 – 0.0439 A643- 0.002423 x A661

Carotenoids (mg/g) = (A 740-17.1 x (Chl–*a* + Chl-*b*) - 9.479 x anthocyanins) /119.26

Chlorophyll stability index (CSI). Chlorophyll stability indices were calculated in stressed and control plants following the formula (Sairam et al 1997):

$$CSI\ (\%) = \frac{Total\ Chlorophyll\ in\ stressed\ \ plant}{Total\ Chlorophyll\ in\ control\ \ plant} \times 100$$

Total carbohydrates. The procedure of Watanabe et al (2000) was followed to determine the total carbohydrates in stressed and control *P. dactylifera* plants. Briefly, 0.5 g of fresh weight leaves were homogenized with 80% of ethanol, followed by a centrifugation at 5,000 rpm for 10 min, 1 mL of supernatants was transferred into a new tube and 3 mL of anthrone reagent (50 mL of 95% H_2SO_4 + 50 mg anthrone) was added, and left for 10 min in a water bath at 100°C, then cooled on ice. Total carbohydrates were measured at 620 nm, using glucose as a standard.

Proline content. Proline content was determined according to Bates et al (1973). 500 mg of leaf tissues was homogenized in 10 mL of 3% aqueous sulfosalicylic acid, 5 min of centrifugation at 6,000 rpm was followed, 2 mL was taken from the supernatant and transferred into a new test tube, and 2 mL of glacial acetic acid and 2 mL of acid ninhydrine were added to the supernatant, the mixture was heated at 100ºC for 1 hour and then the reaction was stopped by placing on ice. 4 mL of toluene was added to the

mixture and stirred well for 30 seconds. The absorbance at 520 nm was measured against a toluene blank. L-Proline was used as the standard.

Free amino acid content. Free amino acid contents were measured according to Lee & Takahashi (1966) by utilizing ninhydrine reagent. In brief, 0.5 g tissue was incubated in 70% ethanol overnight at room temperature, and then washed with double-distilled water. A 1.5 mL of 55% glycerol and 0.5 ml ninhydrin solution were added, boiled at 100°C for 20 min and cooled down. The final volume was made up to 6 mL using double-distilled water, followed by the measuring of the optical density at 570 nm.

Total soluble protein. Total soluble protein extraction was carried out according to Bavei et al (2011). Briefly, 300 mg of *P. dactylifera* leaves in each heavy metals treatments were ground in liquid nitrogen and homogenized in 3 mL Tris-HCl buffer (0.1 M, pH 7.5) containing 1 mM phenylmethanesulfonylfluoride (PMSF), at 4°C. The homogenate was centrifuged at 13,000 rpm for 30 min. Total soluble protein content was measured according to Bradford (1976) method using Bradford reagent (containing: 100 mg Coomassie Brilliant Blue R-250 in 50 mL 95% ethanol, 100 mL 85% (w/v) phosphoric acid), a crystalline bovine albumin (5 to 100 micrograms protein) was used to establish a standard curve at 595 nm.

Oxidative stress responses of stressed *P. dactylifera* plants

Hydrogen peroxide accumulation in stressed P. dactylifera plants. Hydrogen peroxide (H_2O_2) accumulation in *P. dactylifera* leaves was determined as described by Sergiev et al (1997). A 0.5 g sample of fresh leaves was homogenized with 5 mL of trichloroacetic acid (TCA; 0.1%: w/v). The homogenate was centrifuged at 13,000 rpm for 15 min, then, 1 mL of the supernatant was added to 0.5 L of potassium phosphate buffer (10 mM, pH = 7.0) and 1 mL of potassium iodide(1 M). The absorbance of the supernatant was measured at 390 nm. Hydrogen peroxide was used to establish a standard curve.

Peroxidase activity. Peroxidase activity was evaluated according to the method of Kim & Yoo (1996). Each single unit of peroxidase catalyzed the oxidation of guaiacol in 1 min U/min/g. The variation in the absorption as a result of tetraguaiacol formation was measured at 470 nm.

Malondialdehyde (MDA) content. MDA content was measured according to Heath & Packer (1968); in *P. dactylifera* leaves, 500 mg of leaf tissue was homogenized in 5 mL TCA (0.1%, w/v). Then the homogenate was centrifuged at 10,000 rpm for 5 min, 1 mL of supernatant and 4 mL of thiobaributric acid (TBA; 0.5% w/v) prepared in 20% TCA was boiled, after 30 min the reaction was terminated by placing the mixture on ice, another centrifugation at 10,000 rpm for 15 min was done. The absorbance of supernatant was measured at 532 and 600 nm, and MDA content was calculated using an extinction coefficient of 155.

Membrane stability index (MSI). Membrane stability index (MSI) was measured in *P. dactylifera* leaves. 0.25 g of fresh palm leaves in each heavy metal stressed and control treatments were cut into small parts, and homogenated using 10 mL of deionized water and incubated at room temperature on a rotary shaker for 24 h. Subsequently, the initial electrical conductivity of the medium (*C1*) was measured. The samples were placed in an oven at 90°C for 2 h to expel all electrolytes, and cooled at 25°C, and then the second electrical conductivity (*C2*) was measured. Leaf membrane stability index was calculated using the following formula (Lutts et al 1996):

$$\text{Membrane stability index (MSI \%)} = \left[1 - \left(\frac{C1}{C2}\right)\right] \times 100$$

Total phenolic content

The method of Singleton & Rossi (1965) was followed to determine the total phenolic content in stressed and control plants using Folin-Ciocalteu reagent; which was previously prepared (prediluted 10-fold with distilled water) and left at room temperature for 5 min, followed by addition of sodium bicarbonate (1.2 mL: 7.5%,w/v) to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent (mg GAE/ g). Gallic acid was used as a reference standard.

Statistical analysis. All experiments were done according to randomized design. The obtained data were analyzed statistically with SPSS-21 statistical software (SPSS In., Chicago, IL., USA). Data were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared by least significant difference (LSD) test at $P \leq 0.05$ levels with triplicates for each treatment. The bars on each diagram represent standard deviations. Each data of tables and figures represent the means of triplicates.

Results and Discussion

Cd and Pb content in soil and P. dactylifera leaves. Table 1 shows that at zero time no Cd was detected in either soil or *P. dactylifera* leaves, while an average of 1.04 and 0.04 mg kg^{-1} DW where recorded at Pb treatments in soil and leaves of *P. dactylifera*, respectively. After 180 days of *P. dactylifera* exposure to different concentrations of examined heavy metals, an increase was observed at all treatments, in soil, the level of Cd was increased from 0 to 15.36 mg kg^{-1} at high Cd concentration (9 mg kg^{-1}), and from 0.99 to 86.49 mg kg^{-1} at high Pb concentration (276 mg kg^{-1}). Regarding the heavy metals analysis in treated *P. dactylifera* leaves, results showed that the increase of heavy metals in soils led to accumulate high levels of heavy metals in leaves, thus, was evident with both Cd and Pb at high concentrations, the highest leaf contents of Cd and Pd were recorded (8.02 and 39.20 mg kg^{-1} DW) in Cd and Pd treatments of 9 and 276 mg kg^{-1}, respectively.

P. dactylifera plants exposed to heavy metal stresses (Cd and Pb) through irrigation showed higher accumulations of these heavy metals in leaves, the high accumulation levels of heavy metals in aerial parts of *P. dactylifera* is in a good accordance with many other results revealed the accumulation of several heavy metals in aerial parts of different plants such as cardoon (*Cynara cardunculus*); cole (*Brassica campestris*); common bean (*Phaseolus vulgaris*) and date palm (Yang et al 2011; Papazoglou 2011; Aldoobie & Beltagi 2013; Zouari et al 2016a). The significant increases of HMs in soils after 180 days of treatment were proportionally correlated with the increases of heavy metal contents in *P. dactylifera* leaves, thus, was revealed by several studies on different plants (Reber 1989; Kádár 1995; Brown et al 1998; Smolders 2001). Different uptake routes have been described for heavy metals uptake by plants; passive uptake which involves heavy metals diffusions in the soil solutions into root endodermis and does not require any energy, while, the other heavy metals uptake route is the active uptake which occurs against concentrations and requires metabolic energy (Williams et al 2000; Reichman 2002). Regarding heavy metals transportation from root to shoot system, there are different mechanisms directing this transportation, either by mass flow of water which takes place by transpiration stream (Kochian 1991), or by chelators such as organic acid and amino acid, which facilitate heavy metals translocations through xylem (Clemens et al 2002; Lesage et al 2005).

Table 1

Cd and Pb contents (mg kg^{-1}) in soil and *Phoenix dactylifera* leaves, before and 180 days after treatment

Treatment	Soil (mg kg^{-})		Leaves (mg kg^{-} DW)	
	Time of exposure (days)			
	0	180	0	180
Cd_1	0^{b}	$7.1\pm0.42^{d*}$	0^{c}	2.59 ± 0.13^{d}
Cd_2	0^{b}	15.36 ± 1.03^{c}	0^{c}	8.02 ± 0.21^{c}
Pb_1	1.04 ± 0.03^{a}	52.96 ± 1.13^{b}	0.04 ± 0.02^{b}	22.93 ± 0.94^{b}
Pb_2	0.99 ± 0.02^{a}	86.49 ± 3.25^{a}	0.09 ± 0.04^{a}	39.20 ± 0.68^{a}
$RLD_{0.05}$	0.07	0.16	0.02	0.03

* Values represent the averages of triplicates per treatment ± standard deviation. Different letters indicate significant differences between treatments using LSD (0.05). Cd_1: 3 mg kg^{-1}, Cd_2: 9 m kg^{-1}, Pb_1: 100 mg kg^{-1}, Pb_2: 276 mg kg^{-1}.

Photosynthetic pigment contents in P. dactylifera leaves. The photosynthetic pigment contents in *P. dactylifera* leaves under the treatments of Cd and Pb were measured after 180 days of treatment, exposure of young *P. dactylifera* plants to these HMs resulted in significant reductions ($p \leq 0.05$) in chlorophyll *a* contents, the reduction was up to twofold in Pb treatment at 276 mg kg^{-1}, compared to control treatment (Table 2), Cd treatments at 3 and 9 mg kg^{-1} led to a decrease in chlorophyll *a* from 4.16 mg g^{-1} FW in control plants to 2.86 and 2.51 mg g^{-1} FW, in Cd treatments, respectively. Significant decreases of chlorophyll *b* in each of Cd and Pb treatments at high concentrations 9 and 276 mg kg^{-1} were recorded as 1.22 and 1.13 mg g^{-1} FW, respectively, compared with 1.41 mg g^{-1} FW at control treatment. Similar reduction trends were observed in *P. dactylifera* leaves grown under Cd and Pb stresses for total chlorophyll, a reduction of total chlorophyll percentage reached 30 and 41%, respectively, in comparison to the control treatment.

Results describing the carotenoid contents for Cd and Pb treatments revealed that the content was 1.87 mg g^{-1} FW at control treatment and reduced significantly at Cd and Pb treatments (9 and 276 mg kg^{-1}) and reached 1.17 and 1mg g^{-1} FW, respectively. It is noteworthy, the opposite was observed with anthocyanin pigments (Table 2), an increase in anthocyanin content was recorded in *P. dactylifera* leaves under the treatment of Cd and Pb, the increase level reached 47 and 42% in Cd and Pb treatments at high concentrations compared to control ones.

Table 2

Photosynthetic pigments (mg g^{-} FW) contents of *Phoenix dactylifera* plants treated with different concentrations of Cd and Pb

HM treatment	*Chlorophyll a*	*Chlorophyll b*	*Total chlorophyll*	*Carotenoides*	*Anthocyanins*
Control	$4.16 \pm 0.08^{a*}$	1.41 ± 0.11^{ab}	5.58 ± 0.06^{ab}	1.87 ± 0.14^{a}	0.068 ± 0.003^{d}
Cd_1	2.86 ± 0.06^{b}	1.52 ± 0.03^{a}	4.38 ± 0.10^{a}	1.50 ± 0.07^{b}	0.082 ± 0.003^{c}
Cd_2	2.51 ± 0.15^{c}	1.22 ± 0.13^{bc}	3.74 ± 0.13^{bc}	1.17 ± 0.08^{c}	0.118 ± 0.003^{a}
Pb_1	2.36 ± 0.07^{cd}	1.56 ± 0.08^{a}	3.92 ± 0.02^{a}	1.64 ± 0.04^{ab}	0.099 ± 0.008^{b}
Pb_2	2.15 ± 0.13^{d}	1.13 ± 0.06^{c}	3.28 ± 0.14^{c}	1.00 ± 0.04^{c}	0.129 ± 0.003^{a}
LSD (0.05)	0.3	0.25	0.29	0.24	0.012

* Values represent the means of triplicates per treatment ± standard deviation. Cd_1: 3 mg kg^{-1}, Cd_2: 9 mg kg^{-1}, Pb_1: 100 mg kg^{-1}, Pb_2: 276 mg kg^{-1}. Different letters indicate significant differences between treatments using LSD (0.05).

Regarding chlorophyll stability index (CSI), statistical analysis proved that the exposure of young tissue culture-derived *P. dactylifera* plants to Cd and Pb heavy metals resulted in a profound reduction, the CSI average was 100% in control treatment, and reduced significantly to 69.97 and 58.79%, in Cd and Pb at high concentrations respectively, hence, the low concentrations of Cd and Pb induced the reduction in CSI parameters compared to control plants (Figure 1a).

Obtained results showed a significant decline in chlorophyll content in *P. dactylifera* leaves exposed to Cd and Pb stress; a twofold of decrease in chlorophyll *a* was noticeable consequent to Cd and Pb treatment at high concentrations (9 and 276 mg kg^{-1}, for Cd and Pb; respectively). Referring to many published research studies the collapse in chlorophyll content is a common response to heavy metals stresses (Jamers et al 2009; Liu et al 2013; Zhang et al 2014; Gomes et al 2015; Zouri et al 2016a). The collapse in chlorophyll content could be the result of the disruption of chlorophyll synthesis caused by enzymes inhibition including δ-aminolevulinic acid dehydratase and proto- chlorophyllide reductase which are essential for synthesis of chlorophyll, as well as, Mg and Zn deficiency which accompanied heavy metal stress and these elements are required for chlorophyll synthesis (Cenkci et al 2010; Parmar et al 2013; Elloumi et al 2014). Additionally, chlorophyll degradation was correlated with different heavy metals stresses in numerous plants (Cozzolino et al 2010; Gupta et al 2013), and a disruption of

protein synthesis involved in chlorophyll and photosynthesis was reported as a response to heavy metals treatment, such as Chl *a/b* binding proteins and subunits of photosystem *II* (Duquesony et al 2009; Walliwallagedara et al 2010; Zeng et al 2011).

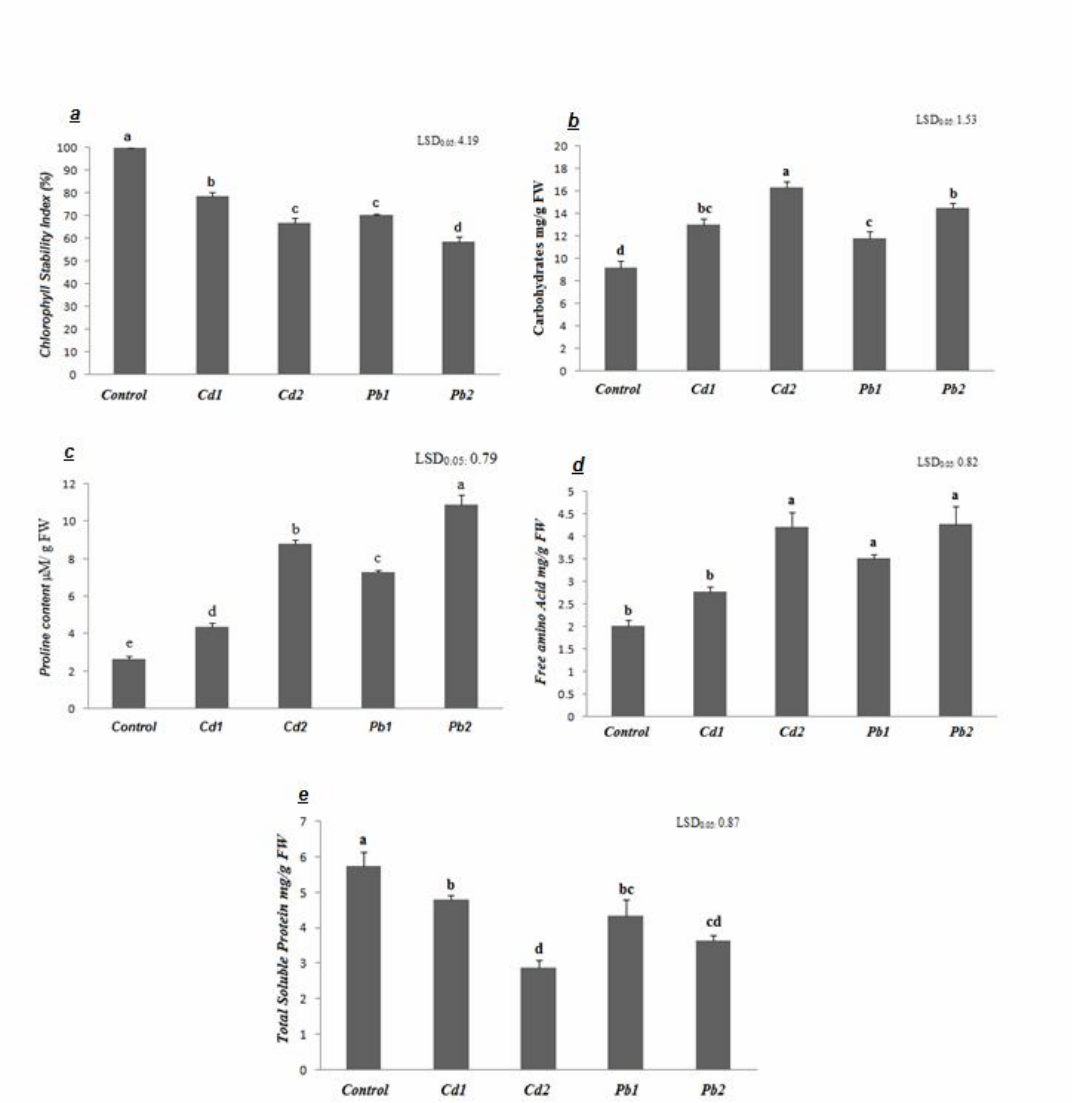

Figure 1. *Phoenix dactylifera* responses to different concentrations of Cd and Pb (mg/kg). (*a*): Chlorophyll stability index (%); (*b*): Carbohydrates content (mg g^{-1} FW); (*c*): Proline content µmole g^{-1} FW; (*d*): Free amino acid (mg g^{-1} FW); (*e*): Total soluble protein (mg g^{-1} FW). Values represent the average of triplicates for each treatment ± standard deviation value. Different letters indicate significant differences between treatments according to LSD test at 0.05 level. Cd_1:3 mg kg^{-1}, Cd_2: 9 mg kg^{-1}, Pb_1: 100 mg kg^{-1}, Pb_2: 276 mg kg^{-1}.

It is noteworthy, that chlorophyll *a* was more sensitive to both Cd and Pb treatments than chlorophyll *b* at high concentrations, this finding is in agreement with many other results which showed that chlorophyll *a* decreased much more than chlorophyll *b* as a response to heavy metal stress (Parmar et al 2013; Aldoobie & Beltagi 2013; Elloumi et al 2014; Zouari et al 2016b). Chlorophyll stability index was decreased significantly as a consequence to Cd and Pb treatments, the reduction was up to 50% at high concentrations, the decrease in chlorophyll stability is a common sensitive parameter to heavy metal exposures in wide range of organisms and associated with the inhibition of chlorophyll biosynthesis (Bajpai & Upreti 2012; Pooja et al 2012).

The pronounced reduction in carotenoid pigments in stressed *P. dactylifera* plants compared to control ones, especially at high concentrations of Cd (9 mg kg^{-1}) and Pb (276 mg kg^{-1}) is in agreement with several study results on different plants such as maize to copper stress (Prasad, 1995); *Spirodela polyrhiza* and *Lemna minor* to Ni stress (Appenortha et al 2010); *Triticum aestivum* to Ni and Pb (Dar et al 2010); *Paulownia fortunei* to copper treatments (Jiang et al 2012) *Medicago sativa* to different heavy metals (Chen et al 2015) and *P. dactylifera* to Cd stress (Zouari et al 2016b). Herein, the inhibition rate of chlorophyll pigments was much more than those recorded in carotenoids; it is generally known that the sensitivity of chlorophyll pigments is higher than carotenoids to heavy metals exposure (Fargasová 1998, 2001; Singh et al 2015). Controversial results were found in different plant responses to heavy metals by increasing the accumulation of carotenoids pigments in their leaf tissue (Sinha et al 2003; Strzalka et al 2003; Pinto et al 2011; Baek et al 2012); this could be attributed to the metal type, concentration, plant species, duration and exposure route of heavy metals (Sinha et al 2003; Baek et al 2012).

Carotenoids are linear polyenes pigments produced by different organisms and widely distributed as an accessory pigment (Voet & Voet 2011). In deciduous trees, the carotenoid pigments function as light-harvesting pigments, with an excellent absorption at the range of the wavelength 400–480 nm (Wong & Choong 2014).

An opposite trend of results was found with anthocyanins pigment, both Cd and Pb treatments at high concentrations induced accumulation of this pigment in stressed *P. dactylifera* leaves up to 40% more than those analyzed in non-stressed plants, this finding is in a good agreement with many results revealed that anthocyanins accumulated in plants as a response to heavy metal stresses (Hale et al 2001; Collin et al 2008; Baek et al 2012). Anthocyanin accumulation in stressed plants is believed to protect plant from various types of stress and is considered pivotal to mitigate the phytotoxic affects of heavy metals through shielding chloroplasts from excess high energy quanta and scavenging ROS, as nonenzymatic antioxidant mechanism (Krupa et al 1996; Neill & Gould 2003).

Carbohydrates and proline content in stressed date palm leaves. Cd and Pb treatments significantly ($p \leq 0.05$) increased the level of carbohydrates in leaves of *P. dactylifera* in comparison with non-stressed plants, the highest level was observed in Cd at a high concentration (9 mg kg^{-1}) which was 16.35 mg/g FW and was 43% more than the level of carbohydrates in control treatment (9.21 mg g^{-1} FW), followed by Pb treatment at high concentration (Figure 1b). High accumulation levels of carbohydrates, up to 40% more than those in control plants, were induced by Cd and Pb treatments; the increase in carbohydrates content is considered as an indicator for plant responses to stresses, and plants tend to accumulate more carbohydrates to provide energy to cope with these stresses (Rhodes & Wooltorton 1978; Abass et al 2016). These results are in accordance with Aldoobie & Beltagi (2013) findings that carbohydrates were increased significantly in common bean plants as a response to Cd; Pb and Ni treatments.

Similar increases were observed with proline content in examined *P. dactylifera* leaves after exposure to heavy metals (Figure 1c), the level of proline was 2.67 µg mole g^{-1} FW in control plants, this level was increased up to five and fourfold in Pd and Cd treatments at high concentrations (9 and 267 mg kg^{-1}), and recorded the averages of 10.88 and 8.80 µg mole g^{-1} FW, respectively. The lowest proline content was recorded in Cd treatment at low concentration and reached 4.36 µg mole g^{-1} FW, and was significantly higher than the control level. Enhanced production of proline (up to fivefold) in heavy metals stressed *P. dactylifera* plants is in accordance with many research studies that showed that plants exposed to different heavy metals led to a significant accumulation of proline in their leaves (Aldoobie & Beltagi 2013; Asgher et al 2014; Khan et al 2015; Chen et al 2015; Zouari et al 2016c); there are different explanations for proline role in plant responses to heavy metal stress including *(a)*: direct role of proline as an antioxidant agent to scavenge and control ROS in stressed plants *(b)*: indirect role through modulation redox balance by activation of antioxidant responses (Dawood et al 2014; Singh et al 2015), additionally, proline can act as a metal chelating agent (Sharma

et al 1998). Proline accumulation is considered one of the plant adaptive strategies to different stressful conditions such as temperature, water deficiency, high plant auxin concentrations, salinity and fungicides (Naidu et al 1991; Hare et al 1998; Abass & Morris; 2013; Al-Samir et al 2015; Abass 2016; Abass et al 2016).

Free amino acid and protein content in stressed P. dactylifera leaves. *P. dactylifera* plant responses to different concentrations of Cd and Pb were evaluated as free amino acid and protein content, heavy metals stresses resulted in a significant increase in *P. dactylifera* leaf content of free amino acid, twofold increase was observed in exposed *P. dactylifera* plants to high concentrations of Cd and Pb (Figure 1d), the level of free amino acid was 2.01 mg kg^{-1} FW in non-stressed plants and reached the values of 4.28 and 4.22 mg kg^{-1} FW in Pb and Cd at 9 and 276 mg kg^{-1} concentration, respectively. While, the Cd at low concentration did not induce any change in free amino acid content compared to control plants.

Interestingly, the opposite trends of results were revealed with protein analysis in stressed *P. dactylifera* plants, a profound reduction in total soluble protein was recorded as a response to heavy metal treatments, especially, at high concentrations, the highest reduction in protein content was observed in Cd treatment at high concentration. The protein level was 5.74 mg g^{-1} FW in control plants and reduced significantly to 2.89 mg g^{-1} FW under high Cd concentration (9 mg kg^{-1}; Figure 1e). Pb treatment led to a decrease in protein content at high and low concentration to 4.34 and 3.65 mg g^{-1} FW, respectively.

Changes in total soluble proteins were observed in *P. dactylifera* leaves exposed to heavy metals, with a twofold reduction at high concentrations of Cd and Pb, this reduction in protein content was reported in several studies as a response to heavy metal treatments which were attributed to inhibition of protein synthesis or to protein degradation induced by heavy metal accumulation in plant cells (Wang et al 2009a; Chen et al 2010; Chen et al 2015). Proteins are considered as one of important constituents of the cell, and known to be damaged easily under different environmental stress conditions (Prasad 1996; Wu et al 2010). Controversial findings were found in the results of Heiss et al (2003) and Sabatini et al (2009), which demonstrated the increase of total soluble protein under the treatments with Zn and Ni heavy metals, thus, could be explained by the type and concentration of the examined heavy metals, as well as, plant species under stress.

The opposite trend of results was observed with total free amino acid in treated *P. dactylifera* plants with heavy metals, an increase (up to twofold) of free amino acid was obtained after 180 days of Cd and Pb treatments; the increase of free amino acid in plant leaves is considered as a common response to several heavy metal treatments in many plants (Hsu & Kao 2003; Chaffei et al 2004; Bhardwaj et al 2009; Vassilev & Lidon 2012). Heavy metals are known to alter the amino acid metabolite in exposed plants (Megateli et al 2009), many research studies showed an increase in the synthesis of asparagine, cysteine and histidine amino acid as responses to different heavy metal stresses (Smirnoff & Stewart 1987; Harmens et al 1993; Kramer et al 1996). The increase of free amino acid in plant cells could be attributed to the degradation of proteins, substantial increases of proteolysis enzymes such as protease have been reported in many plants exposed to heavy metals which led to reduce total soluble proteins and increase free amino acid (Hsu & Kao 2003; Vassilev & Lidon 2012). Amino acid involvements in heavy metal responses in plants are well known as signaling molecules; osmolyte regulators, detoxification and chelating agents (Xu et al 2012).

Oxidative stress responses and phenolic content in stressed P. dactylifera leaves. Figure 2 illustrates the responses of *P. dactylifera* plants to Cd and Pb stresses as oxidative damages, statistical analysis showed that *P. dactylifera* plants responded to both heavy metals by accumulating more H_2O_2 in leaves, the level of H_2O_2 was 0.84 µM g^{-1} FW in non-stressed plants, this level increased significantly up to 2.5 fold in Cd treatment at a high concentration and reached 2.31 µM g^{-1} FW, followed by Pb treatment at a high concentration (1.42 µM g^{-1} FW; Figure 2a). Similar trends of results were

observed for peroxidase activity, this enzyme activity was 21.88 unit/g/min in control plants, while the treatment of Cd and Pb heavy metals induced more activity of peroxidase, reaching the values of 36.74 and 41.71 unit/g/min, in Pb and Cd treatments at high concentrations, respectively (Figure 2b).

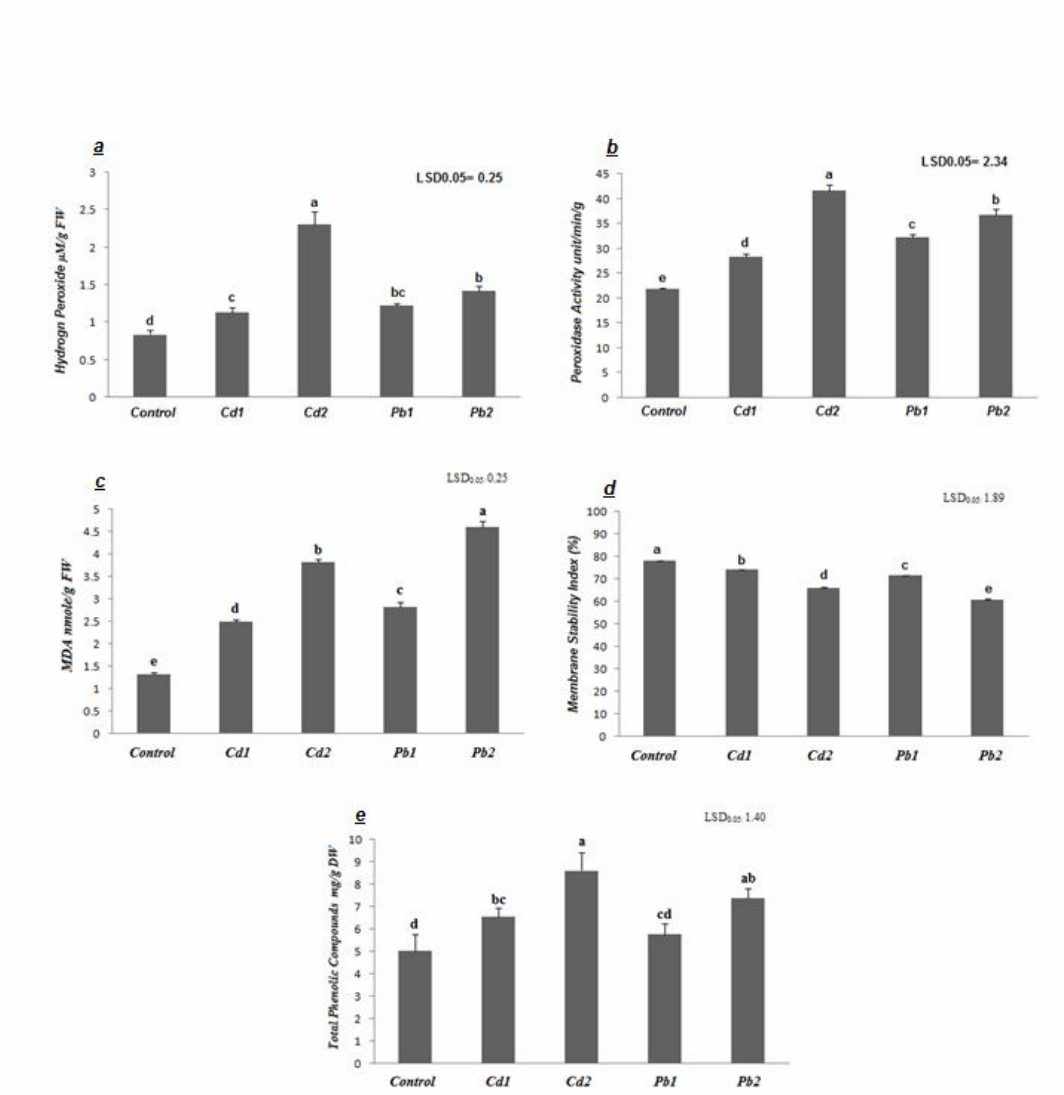

Figure 2. *Phoenix dactylifera* responses to different concentrations of Cd and Pb (mg/kg). (*a*): Hydrogen peroxide (µM/g FW); (*b*): Peroxidase activity (unit/g/min); (*c*): MDA (nmole g^{-1}); (*d*): Membrane stability index (%); (*e*): Total phenolic compounds (mg g^{-1} DW). Values represent the average of triplicates for each treatment ± standard deviation value. Different letters indicate significant differences between treatments according to LSD test at 0.05 level. Cd_1:3 mg kg^{-1}, Cd_2: 9 mg kg^{-1}, Pb_1: 100 mg kg^{-1}, Pb_2: 276 mg kg^{-1}.

Oxidative responses of *P. dactylifera* plants to heavy metals have been studied and the present results showed that H_2O_2 productions increased up to 2.5 fold by increasing the concentrations of examined heavy metals compared to those in control plants. H_2O_2 is one kind of reactive oxygen species (ROS) in plants with different roles in many physiological functions, as well as, plant responses to biotic and abiotic stresses (Abass & Morris 2013; Abass 2016). HMs are widely known to generate ROS in stressed plants, either via Fento and Harber-Weiss reactions or indirectly by inhibiting plant antioxidant mechanisms (Romero-Puertas et al 2007). Several studies showed that the level of hydrogen peroxide increased in response to heavy metal stresses (Cho & Park 2000; Maksymiec & Krupa 2006; Zouari et al 2016c). The increase of hydrogen peroxide levels in heavy metal stressed plants was accompanied with an increase in peroxidase activity

up to twofold; the changes in peroxidase activity in plant cells are a good biomarker of biotic and abiotic stresses (Gill & Tuteja 2010; Gulsen et al 2010; Doganlar & Atmaca 2011); including heavy metal stress (Singh et al 2006; Wang et al 2009b; Doganlar 2013). The peroxidase enzymes have important roles in plant life, and are involved in different physiological events including growth, respiration, transpiration and gas exchange (MacFarlane & Burchett 2001; Yurekli & Porgali 2006); another vital role of peroxidase enzymes in plant cells is their effective scavenging of H_2O_2.

Lipid peroxidation level in *P. dactylifera* leaves was measured as a malondialdehyde content, the obtained results revealed that the MDA level was increased in *P. dactylifera* leaves as a response to Cd and Pb stresses, thus, was evident by the increment of MDA level up to threefold in Pb treatment at high concentration and reached 4.60 nmole g^{-1} FW, compared to those in control treatment (1.32 nmole g^{-1}), followed by Cd at high concentration (9 mg kg^{-1}) which induced lipid peroxidation up to twofold (3.82 nmole g^{-1}) compared to non-stressed plants (Figure 2c). The high lipid peroxidation activity in heavy metals stressed *P. dactylifera* plants was accompanied with a significant reduction in membrane stability, the results showed that Pb treatment at high concentration decreased the MSI to 60.90%, compared to the stability of 78.30% observed in non-stressed plants (Figure 2d), followed by Cd treatment at high concentration which recorded the MSI of 66.17%.

A threefold increase of malondialdehyde was detected in *P. dactylifera* leaves after 180 days of treatment with Cd and Pb; MDA is considered as a cytotoxicend product of lipid peroxidation especially polyunsaturated fatty acid (PUFA) (Agadjanyan et al 2006), this increase in MDA is in consonance with many results showed MDA accumulation during heavy metal stress (Unyayar et al 2006; Pooja et al 2012; Doganlar 2013; Chen et al 2015; Zouari et al 2016a). The increased level of hydrogen peroxide is well correlated with lipid peroxidation, this observation is in accordance with the results of Sharma et al (2012) and Howladar (2014).

The increases of MDA content in stressed *P. dactylifera* leaves were accompanied with a significant reduction in membrane stability index (MSI), which was obvious in high concentrations of Cd and Pb treatments. RO is well known to attack on the phospholipid molecules of PUFA present in cell membrane, and the toxic end product MDA is responsible of cell membrane damage (Halliwell & Gutteridge 1989; Sharma et al 2012). This finding is in a good agreement with many other researchers showed that heavy metals stresses caused a significant reduction in MSI (Howladar 2014; Abu-Muriefah 2015; Zouari et al 2016b).

Analysis of total phenolic compounds of stressed *P. dactylifera* plants showed similar patterns as observed in oxidative response, the highest content of phenolic compounds was recorded in the treatment of Cd at high concentration which reached 8.63 mg/g DW, without any significant difference than those in Pb treatment at high concentration (276 mg kg^{-1}) which recorded the content of 7.39 mg g^{-1} DW, compared to the lowest level of phenolic content obtained in control plants (5.03 mg g^{-1} DW; Figure 2e). The content of stressed *P. dactylifera* leaves of phenolic compounds found to be increased with the increases of both Cd and Pb. Phenolic compounds are diverse secondary metabolites such as flavonoids, tannins, hydroxycinnamate esters, and lignin which serve as potent non-enzyme antioxidants, with several mechanisms including scavenging molecular species of active oxygen, inhibition of lipid peroxidation by trapping the lipid alkoxyl radical and modification of lipid packing order and decrease fluidity of the membranes (Arora et al 2000; Sharma et al 2012).

Several studies revealed that the level of phenolic compounds was increased significantly with the increase of heavy metal concentrations (Michalak 2006; Janas et al 2009; Guo et al 2016; Zouari et al 2016b).

Conclusions. Current experiments concluded that higher given concentrations of Cd and Pb affected the metabolism of young *P. dactylifera* plants (Barhee cv.). Stressed *P. dactylifera* plants tend to accumulate more Cd and Pb in their leaves; this accumulation was accompanied with a decrease in their chlorophyll (*a*; *b* and total) and carotenoids pigments; however, the increases in anthocyanin pigments; proline, carbohydrates and

free amino acid contents were observed with increasing proportions of examined heavy metal. Oxidative injury was induced in stressed *P. dactylifera* plants by high concentrations of Cd and Pb, thus was obvious by ROS generated in their leaves, the ROS biomarker H_2O_2 increased more than twofold than those in non-stressed plants, with a pronounced accumulation of lipid peroxidation as MDA. Peroxidase enzyme activity and total phenolic compounds observed to be higher in stressed plants compared to control date palm plants.

References

Abass M. H., 2016 Responses of date palm (*Phoenix dactylifera* L.) callus to biotic and abiotic stresses. Emir J Food Agric 28(1):66-74.

Abass M. H., Morris P. C. M., 2013 The *Hordeum vulgare* signaling protein MAP kinase 4 is a regulator of biotic and abiotic stress responses. J Plant Physiol 170:1353-1359.

Abass M. H., Hassan Z. K., Al-Jabary K. M. A., 2015 Assessment of heavy metals pollution in soil and date palm (*Phoenix dactylifera* L.) leaves sampled from Basra/Iraq governorate. AES Bioflux 7(1):52-59.

Abass M. H., Al-Utbi S. D., Al-Samir E. A. H., 2016 Morphological and biochemical impact of different decontamination agents on date palm (*Phoenix dactylifera* L.) procallus. Australian Journal of Crop Sciences 10:1022-1029.

Abu-Muriefah S. S., 2015 Effects of silicon on membrane characteristics, photosynthetic pigments, antioxidative ability, and mineral element contents of Faba bean (*Vicia faba* L.) plants grown under Cd and Pb stress. Int J Adv Res Biol Sci 2(6):1–17.

Agadjanyan Z. S., Dugin S. F., Dmitriev L. F., 2006 Cumene peroxide and $Fe^{(2+)}$-ascorbate-induced lipid peroxidation and effect of phosphoglucose isomerase. Mol Cell Biochem 289:49-53.

Aldoobie N. F., Beltagi M. S., 2013 Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress. Afr J Biotechnol 12(29):4614-4622.

Al-Khashman O. A., Al-Muhtaseb A. H., Ibrahim K. A., 2011 Date palm (*Phoenix dactylifera* L.) leaves as biomonitors of atmospheric metal pollution in arid and semi-arid environments. Environ Pollut 159(6):1635-1640.

Al-Khayri J. M., Jain S. M., Johnson D. V. (eds), 2015 Date palm genetic resources and utilization, Africa and the Americas. Vol. 1, Springer, Dordrecht.

Al-Samir E. A. H., Al-Utbi S. D., Abass M. H., 2015 Phytotoxic effect of 2, 4-D and dicamba on date palm (*Phoenix dactylifera* L.) tissue cultures at initiation stage. AAB Bioflux 7(2):96-108.

Angelone M., Bini C., 1992 Trace elements concentrations in soils and plants of western Europe. In: Biochemistry of tree metals. Adriano D. C. (ed), pp. 17-60, Boca Raton, FL: Lewis Publishers.

Appenroth K. J., Krech K., Keresztes A., Fischer W., Koloczek H., 2010 Effects of nickel on the chloroplasts of the duckweeds *Spirodela polyrhiza* and *Lemna minor* and their possible use in biomonitoring and phytoremediation. Chemosphere 87:216-23.

Arnon D. I., 1949 Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiol 24:1-15.

Arora A., Byrem T. M., Nair M. G., Strasburg G. M., 2000 Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys 373:102–109.

Asare-Boamah N. K., Hofstra G., Fletcher R. A., Dumbroff E. B., 1986 Triadimefon protects bean plants from water stress through its effects on abscisic acid. Plant Cell Physiol 27:383-390.

Asgher M., Khan N. A., Khan M. I. R., Fatma M., Masood A., 2014 Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. Ecotoxicol Environ Saf 106:54–61.

Badmus J. A., Adedosu T. O., Fatoki J. O., Adegbite V. A., Adaramoye O. A., Odunola O. A., 2011 Lipid peroxidation inhibition and antiradical activities of some leaf fractions of *Mangifera indica*. Acta Pol Pharm 68(1):23-29.

Baek S., Han T., Ahn S., Kang H., Cho M. R., Lee S., Im K., 2012 Effects of heavy metals on plant growths and pigment contents in *Arabidopsis thaliana*. Plant Pathol J 28:446-452.
Bajpai R., Upreti D. K., 2012 Accumulation and toxic effect of arsenic and other heavy metals in a contaminated area of West Bengal, India, in the lichen *Pyxine cocoes* (Sw.) Nyl. Ecotoxicol Environ Saf 83:63-70.
Bates L. S., Waldren R. P., Teare I. D., 1973 Rapid determination of free proline for water stress studies. Plant Soil 39:205-207.
Bavei V., Shiran B., Khodambashi M., Ranjbar A., 2011 Protein electrophoretic profiles and physiochemical indicators of salinity tolerance in sorghum (*Sorghum bicolor* L.) Afr J Biotechnol 10(14):2683-2697.
Bhardwaj L. P., Chaturvedi A. K., Prasad P., 2009 Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris*. Nature and Science 7:63-75.
Bradford M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 38:248-252.
Brown S. L., Chaney R. L., Angle J. S., Ryan J. A., 1998 The phytoavailability of cadmium to lettuce in long-term biosolids-amended soils. J Environ Qual 27:1–8.
Cenkci S., Cigerci I. H., Yildiz M., Ozay C., Bozdag A., Terzi H., 2010 Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environ Exp Bot 67(3):467-473.
Chaffei C., Pageau K., Suzuki A., Gouia H., Ghorbel M. H., Masclaux-Daubresse C., 2004 Cadmium toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. Plant Cell Physiol 45:1681–1693.
Chen F., Wang F., Wu F., Mao W., Zhang G., Zhou M., 2010 Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. Plant Physiol Biochem 48:663–672.
Chen F., Wang S., Mou S., Azimuddin I., Zhang D., Pan X., Al-Misned F. A., Mortuza M. G., 2015 Physiological responses and accumulation of heavy metals and arsenic of *Medicago sativa* L. growing on acidic copper mine tailings in arid lands. J Geochem Explor 157:27–35.
Cho U. H., Park J. O., 2000 Mercury- induced oxidative stress in tomato seedlings. Plant Sci 156(1):1-9.
Clemens S., Palmgrn M. G., Kramer U., 2002 A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309-15.
Collin V. C., Eymery F., Genty B., Rey P., Havaux M., 2008 Vitamin E is essential for the tolerance of *Arabidopsis thaliana* to metal-induced oxidative stress. Plant Cell Environ 31:244–257.
Cozzolino V., Pigna M., Di Meo V., Caporale A. G., Violante A., 2010 Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth of *Lactuca sativa* L. and arsenic and phosphorus availability in an arsenic polluted soil under non-sterile conditions. Appl Soil Ecol 45:262–268.
Dar S. H., Agnihotri R. K., Sharma R., Ahmad S., 2010 Nickel and lead induced variations in pigment composition of *Triticum aestivum* L. Research Journal of Agricultural Sciences 1:128-131.
Davidson C. M., 2013 Methods for the determination of heavy metals and metalloids in soil. In: Heavy metals in soil. Alloway B. J. (ed), pp. 97-140, Springer, Netherlands.
Dawood M. G., Taie H. A. A., Nassar R. M. A., Abdelhamid M. T., Schmidhalter U., 2014 The changes induced in the physiological, biochemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under sea water stress. S Afr J Bot 93:54–63.
Doganlar Z. B., 2013 Metal accumulation and physiological responses induced by copper and cadmium in *Lemna gibba*, L. minor and *Spirodela polyrhiza*. Chemical Speciation & Bioavailability 25(2):79-88.

Doganlar Z. B., Atmaca M., 2011 Influence of airborne pollution on Cd, Zn, Pb, Cu, and Al accumulation and physiological parameters of plant leaves in Antakya (Turkey). Water Air Soil Pollut 214:509-523.
Duquesnoy I., Goupil P., Nadaud I., Branlard G., Piquet-Pissaloux A., Ledoigt G., 2009 Identification of *Agrostis tenuis* leaf proteins in response to As(V) and As(III) induced stress using a proteomics approach. Plant Sci 176:206–213.
Elloumi N., Zouari M., Chaari C., Jomni C., Ben Rouina B., Ben Abdallah F., 2014 Ecophysiological responses of almond (*Prunus dulcis*) seedlings to cadmium stress. Biologia 69(5):604–609.
Fargasová A., 1998 Root growth inhibition, photosynthetic pigments production, and metal accumulation in *Sinapis alba* as the parameters for trace metals effects determination. Bull Environ Contam Toxicol 61:762–769.
Fargasová A., 2001 Phytotoxic effects of Cd, Zn, Pd, Cu, and Fe on *Sinapis alba* L. seedlings and their accumulation in roots and shoots. Biol Plant 44:471–473.
Galloway J. N., Thornton J. D., Norton S. A., Volcho H. L., McLean R. A., 1982 Trace metals in atmospheric deposition: a review and assessment. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/J-82/451 (NTIS PB86133055).
Garty J., Weissman L., Tamir O., Beer S., Cohen Y., Karnieli A., 2000 Comparison of five physiological parameters to assess the vitality of the lichen *Ramalina lacera* exposed to air pollution. Physiol Plant 109(4):410-418.
Gill S. S., Tuteja N., 2010 Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909-930.
Gomes M. P., Le Manac'h S. G., Maccario S., Labrecque M., Lucotte M., Juneau P., 2015 Differential effects of glyphosate and aminomethylphosphonic acid (AMPA) on photosynthesis and chlorophyll metabolism in willow plant. Pestic Biochem Physiol 130:65-70.
Goncalves J. F., Antes F. G., Maldaner J., Pereira L. B., Tabaldi L. A., Rauber R., Rossato L. V., Bisognin D. A., Flores V. L. M., Nicoloso F. T., 2009 Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions. Plant Physiol Biochem 47:814–821.
Gulsen O., Eickhoff T., Heng-Moss T., Shearman R., Baxendale F., Sarath G., Lee D., 2010 Characterization of peroxidase changes in resistant and susceptible warm-season turf grasses challenged by *Blissus occiduus*. Arthropod Plant Interact 4:45-55.
Guo B., Liu C., Ding N., Fu Q., Lin Y., Li h., Li N., 2016 Silicon alleviates cadmium toxicity in two cypress varieties by strengthening the exodermis tissues and stimulating phenolic exudation of roots. J Plant Growth Regul 35:420-429.
Gupta M., Cuypers A., Vangronsveld J., Clijsters H., 1999 Copper affects the enzymes of ascorbate-glutathion cycle and its related metabolites in the root of *Phaseolus vulgaris*. Physiol Plant 106:262-267.
Gupta D. K., Huang H. G., Nicoloso F. T., Schetinger M. R., Farias J. G., Li T. Q., Razafindrabe B. H., Aryal N., Inouhe M., 2013 Effect of Hg, As and Pb on biomass production, photosynthetic rate, nutrients uptake and phytochelatin induction in *Pfaffia glomerata*. Ecotoxicology 22(9):1403–1412.
Hale K. L., McGrath S. P., Lombi S. E., Stack S. M., Terry N., Pickering I. J., George G. N., Pilon-Smits E. A. H., 2001 Molybdenum sequestration in *Brassica* species. A role for anthocyanins? Plant Physiol 126:1391–1402.
Halliwell B., Gutteridge J. M. C., 1989 Free radicals in biology and medicine. Oxford University Press, Oxford, UK, 2rd edition.
Hare P., Cress W. A., Staden V. J., 1998 Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21:535-553.
Harmens H., Den Hartog P. R., Ten Bookum W. M., Verkleij J. A. C., 1993 Increased zinc tolerance in *Silene vulgaris* (Moench.) Garcke is not due to increased production of phytochelatins. Plant Physiol 103:1305–1309.
Heath R. L., Packer L., 1968 Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198.

Heiss S., Wachter A., Bogs J., Cobbett C., Rausch T., 2003 Phytochelatin synthase (PCS) protein is induced in *Brassica juncea* leaves after prolonged Cd exposure. J Exp Bot 54:1833-1839.
Howladar S. M., 2014 A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. Ecotoxicol Environ Saf 100(1):69–75.
Hsu Y. T., Kao C. H., 2003 Changes in protein and amino acid contents in two cultivars of rice seedlings with different apparent tolerance to cadmium. Plant Growth Regul 40:147–155.
Jackson A. P., Alloway B. J., 1991 The transfer of cadmium from sewage sludge amended soil into the edible food crops. Water Air Soil Pollut 57:873-881.
Jamers A., Lenjou M., Deraedt P., Bockstaele D. V., Blust R., Coen W., 2009 Flow cytometric analysis of the cadmium-exposed green alga *Chlamydomonasre inhardtii* (Chlorophyceae). Eur J Phycol 44:541–550.
Janas K. M., Amarowicz R., Zielinska-Tomaszewska J., Kosinska A., Posmyk M. M., 2009 Induction of phenolic compounds in two dark-grown lentil cultivars with different tolerance to copper ions. Acta Physiol Plant 31:587–595.
Jiang Z. F., Huang S. Z., Han Y. L., Zhao J. Z., Fu J. J., 2012 Physiological response of Cu and Cu mine tailing remediation of *Paulownia fortunei* (Seem) Hemsl. Ecotoxicology 21:759–767.
Jones J. J., 1984 Plants. In: Official methods of analysis. Williams S. (ed), pp. 38–64, Association of Official Analytical Chemists, Arlington, Virginia, USA.
Kádár I., 1995 Effect of heavy metal load on soil and crop. Acta Agricult Hung 43:3–9.
Khan M. I. R., Nazir F., Asgher M., Per T. S., Khan N. A., 2015 Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. J Plant Physiol 173:9–18.
Kim Y. H., Yoo Y. Z., 1996 Peroxidase production from carrot hairy root cell culture. Enzyme Microb Technol 18:531-536.
Kochian L. V., 1991 Mechanisms of micronutrient uptake and translocation in plants. In: Micronutrients in agriculture. Mortvedt J. J. (ed), pp. 251–270, Soil Science Society of America, Madison, WI,.
Kramer U., Cotter-Howells J. D., Charnock J. M., Baker A. J. M., Smith J. A. C., 1996 Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638.
Krupa Z., Baranowska M., Orzot D., 1996 Can anthocyanins be considered as heavy metal indicator in higher plants? Acta Physiol Plant 18:147-151.
Lee Y. P., Takahashani T., 1966 An improved colorimetric determination of amino acids with the use of ninhydrine. Anal Biochem 14:71-77.
Lesage E., Meers E., Vervaeke P., Lamsal S., Hopgood M., Tack F. M., Verloo M. G., 2005 Enhanced phytoextraction: II. Effect of EDTA and citric acid on heavy metal uptake by *Helianthus annuus* from a calcareous soil. Int J Phytoremediation 7:143-152.
Lin A. J., Zhang X. H., Chen M. M., Cao Q., 2007 Oxidative stress and DNA damages induced by cadmium accumulation. J Environ Sci (China) 19:596–602.
Liu Y., Li M., Han C., Wu F., Tu B., Yang P., 2013 Comparative proteomic analysis of rice shoots exposed to high arsenate. J Integr Plant Biol 55:965-978.
Lutts S., Kinet J. M., Bouharmont J., 1996 NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann Bot 78:389–398.
MacFarlane G. R., Burchett M. D., 2001 Photosynthetic pigments and peroxidase activity as indicators of heavy metals stress in grey mangrove, *Avicennia marina (*Forsk.) Vierh. Mar Pollut Bull 42(3):233-240.
Madhava Rao K. V. M., Sresty T. V. S., 2000 Antioxidative parameters in the seedlings of pigeonpea (*Cajanus caja* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci 157(1):113-128.
Maksymiec W., Krupa Z., 2006 The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*. Environ Exp Bot 57:187–194.

Megateli S., Semsari S., Couderchet M., 2009 Toxicity and removal of heavy metals cadmium, copper and zinc by *Lema gibba*. Ecotoxicol Environ Saf 72(6):1774-1780.
Michalak A., 2006 Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol J Environ Stud 15(4):523–530.
Mortvedt J. J., 1994 Plant and soil relationships of uranium and thorium decay series radionuclides- a review. J Environ Qual 23:643–650.
Mustafa G., Komastu S., 2016 Toxicity of heavy metals and metal-containing nanoparticles on plants. Biochim Biophys Acta 1864:932-944.
Naidu B. P., Paleg L. G., Aspinall D., Jennings A. C., Jones G. P., 1991 Amino acid and glycine beatin accumulation in cold-stressed wheat seedlings. Phytochemestry 30:407-409.
Nanda R., Agrawal V., 2016 Elucidation of zinc and copper induced oxidative stress, DNA damage and activation of defence system during seed germination in *Cassia angustifolia* Vahl. Environ Exp Bot 125:31–41.
Neill S. O., Gould K. S., 2003 Anthocyanins in leaves: light attenuators or antioxidants? Funct Plant Biol 30:865-873.
Nies D. H., 1999 Microbial heavy metals resistance. Appl Microbiol Biotechnol 51(6):730-750.
Papazoglou E. G., 2011 Responses of *Cynara cardunculus* L. to single and combined cadmium and nickel treatment conditions. Ecotoxicol Environ Saf 74:195–202.
Parmar P., Kumari N., Sharma V., 2013 Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. Bot Stud 54:45.
Pinto E., Sigaud-Kutner T. C. S., Leitao M. A. S., Okamoto O. K., Colepicolo P., 2003 Heavy metals induced oxidative stress in algae. J Phycol 39(6):1008-1018.
Pinto E., Carvalho A. P., Cardozo K. H. M., Malcata F. X., Maria, dos Anjos F. M., Colepicolo P., 2011 Effects of heavy metals and light levels on the biosynthesis of carotenoids and fatty acids in the *Macroalgae gracilaria tenuistipitata* (var. liui Zhang and Xia). Braz J Pharmacogn 21(2):349-354.
Pooja V., Ram A., Gadi B. R., 2012 Effect of salicylic acid on photosynthetic pigments and some biochemical content in *Vigna* seedlings under cadmium stress. J Chem Bio Phy Sci B 2(4):1801-1809.
Prasad M. N. V., 1995 Inhibition of maize leaf chlorophylls, carotenoids and gasexchange functions by cadmium. Photosynthetica 31:635–640.
Prasad T. K., 1996 Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. Plant J 10:1017-1026.
Prasad K. V. S. K., Pardha S. P., Sharmila P., 1999 Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environ Exp Bot 42:1-10.
Reber H. H., 1989 Threshold levels of cadmium for soil respiration and growth of spring wheat (*Triticum aestivum* L.), and difficulties with their determination. Biol Fertil Soils 7:152–157.
Reichman S. M., 2002 The responses of plant to metal toxicity. A review focusing on copper, manganese and zinc. Melbourne: Australian Mineral and Energy Environmental Foundation.
Rhodes J. M., Wooltorton L. S. C., 1978 The biosynthesis of phenolic compounds in wounded plant storage tissues. In: Biochemistry of wounded plant tissues. Kahl G. (ed), pp. 243-276, Walter de Gruyter and Co., Berlin, Germany.
Romero-Puertas M. C., Corpas F. J., Rodriguez-Serrano M., Gomez M., del Río L. A., Sandalio L. M., 2007 Differential expression and regulation of antioxidative enzymes by Cd in pea plants. J Plant Physiol 164:1346–1357
Sabatini S. E., Chaufan G., Juárez A. B., Coalova L., Bianchi L., Eppis M. R., de Molina M. C. R., 2009 Dietary copper effects in the estuarine crab, Neohelice (*Chasmagnathus granulate*), maintained at two different salinities. Comp Biochem Physiol C Toxicol Pharmacol 150(4):521-527.

Saidi I., Ayouni M., Dhieb A., Chtourou Y., Chaïbi W., Djebali W., 2013 Oxidative damages induced by short-term exposure to cadmium in bean plants: protective role of salicylic acid. S Afr J Bot 85:32–38.
Sairam R. K., Shukla D. S., Saxena D. C., 1997 Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. Biol Plant 40:357-364.
Sanita di Toppi L. S., Lambardi M., Pazzagli L., Cappugi G., Durante M., Gabbrielli R., 1999 Response to cadmium in carrot *in vitro* plants and cell suspension cultures. Plant Sci 137:119-129.
Schutzendubel A., Polle A., 2002 Plant responses to abiotic stresses: heavy-metal induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351-1365.
Sergiev I., Alexieva V., Karanov E., 1997 Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Proceedings of the Bulgarian Academy of Sciences 51:121–124.
Shainberg O., Rubin B., Rabinowitch H. D., Libal Y., Tel O. E., 2000 Acclimation of beans to oxidative stress by treatment with sub lethal iron level. J Plant Physiol 157:93-99.
Sharma S. S., Schat H., Vooijs R., 1998 *In vitro* alleviation of heavy metal-induced enzyme inhibition by proline. Phytochemestry 46:1531–1535.
Sharma P., Jha A. B., Dubey R. S., Pessarakli M., 2012 Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot Volume 2012, Article ID 217037, 26 pages doi: 10.1155/2012/217037.
Singh S., Eapen S., D'Souza S. F., 2006 Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. Chemosphere 62:233-246.
Singh M., Singh V. P., Dubey G., Prasad S. M., 2015 Exogenous proline application ameliorates toxic effects of arsenate in *Solanum melongena* L. seedlings. Ecotoxicol Environ Saf 117:164–173.
Singleton V. L., Rossi J. A., 1965 Colorimetry of total phenolics with phosphomolybdic-phosphotungsic acid reagents. Am J Enol Vitic 16:144-158.
Sinha S., Bhatt K., Pandey K., Singh S., Saxena R., 2003 Interactive metal accumulation and its toxic effects under repeated exposure in submerged plant *Najas indica* Cham. Bull Environ Contam Toxicol 704):696−704.
Smirnoff N., Stewart G. R., 1987 Nitrogen assimilation and zinc toxicity to zinc-tolerant and non-tolerant clones of *Deschampsia cespitosa* (L.) Beauv. New Phytol 107:671–680.
Smolders E., 2001 Cadmium uptake by plants. Int J Occup Med Environ Health 14(2):177-183.
Strzalka K., Kostecka-Guga A., Latowski D., 2003 Carotenoids and environmental stress in plants: significance of carotenoid-mediated modulation of membrane physical properties. Russian Journal of Plant Physiology 50(2):168–173.
Unyayar S., Çelik A., Cekic F. C., Gozel A., 2006 Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis 21:77-81.
Vassilev A., Lidon F., 2012 Cd-induced membrane damages and changes in soluble protein and free amino acid contents in young barley plants. Emir J Food Agric 23(2):130-136.
Viehweger K., Geipel G., 2010 Uranium accumulation and tolerance in *Arabidopsis halleri* under native versus hydroponic conditions. Environ Exp Bot 69:39–46.
Voet J. G., Voet C. W., 2011 Biochemistry. 4th edn., United States of America: John Wiley and Sons Inc. Ch. 25:905-908.
Walliwalagedara C., Atkinson I., van Keulen H., Cutright T., Wei R., 2010 Differential expression of proteins induced by lead in the Dwarf Sunflower *Helianthus annuus*. Photochemistry 71:1460–1465.
Wang H., Wang P. F., Zhang H., 2009a Use of phosphorus to alleviate stress induced by cadmium and zinc in two submerged macrophytes. Afr J Biotechnol 8:2176-2183.

Wang C., Zhang S. H., Wang P. F., Qian J., Hou J., Zhang W. J., Lu J., 2009b The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. Chemosphere 76:938-945.
Watanabe S., Kojima K., Ide Y., Sasakii S., 2000 Effect of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica in vitro*. Plant Cell Tissue Organ Cult 63:199-206.
Weast R. C., 1984. CRC handbook of chemistry and physics. 64th edn., Baco Raton, CRC Press.
Williams L. E., Pittman J. K., Hall J. L., 2000 Emerging mechanisms of heavy metals transport in plant. Biochim Biophys Acta 1465:104-126.
Wong S., Choong C., 2014 Rapid detection of heavy metals with the response of carotenoids in *Daucus carota*. International Journal of Environmental Science and Development 5:270-273.
Wu G., Kang H., Zhang X., Shao H., Chu L., Ruan C., 2010 A critical review on the bioremoval of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174:1-8.
Xu J., Zhu Y., Ge Q., Li Y., Sun J., Zhang Y., Liu X., 2012 Comparative physiological responses of *Solanum nigrum* and *Solanum torvum* to cadmium stress. New Phytol 196:125–138.
Yang Y., Nan Z., Zhao Z., Wang S., Wang Z., Wang X., 2011 Chemical fractionations and bioavailability of cadmium and zinc to cole (*Brassica campestris* L.) grown in the multi-metals contaminated oasis soil, northwest of China. J Environ Sci (China) 23:275–281.
Yurekli F., Porgal Z. B., 2006 The effects of excessive exposure to copper in bean plants. Acta Biol Crac Ser Bot 48:7-13.
Zeng X. W., Qiu R. L., Ying R. R., Tang Y. T., Tang L., Fang X. H., 2011 The differentially expressed proteome in Zn/Cd hyperaccumulator *Arabis paniculata* Franch. in response to Zn and Cd. Chemosphere 82:321–328.
Zhang X., Zhang X., Gao B., Li Z., Xia H., Li H., Li J., 2014 Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of an energy crop, king grass (*Pennisetum americanum* x *P. purpureum*). Biomass Bioenergy 67:179–187.
Zouari M., Ben Ahmed C., Zorrig W., Elloumia N., Rabhi M., Delmaild D., Ben Rouinab B., Labroussec P., Ben Abdallaha F., 2016a Exogenous proline mediates alleviation of cadmium stress by promoting photosynthetic activity, water status and antioxidative enzymes activities of young date palm (*Phoenix dactylifera* L.). Ecotoxicol Environ Saf 128:100–108.
Zouari M., Elloumia N., Ben Ahmeda C., Delmaild D., Ben Rouinab B., Ben Abdallaha F., Labroussec V., 2016b Exogenous proline enhances growth, mineral uptake, antioxidant defense, and reduces cadmium-induced oxidative damage in young date palm (*Phoenix dactylifera* L.). Ecological Engineering 86:202–209.
Zouari M., Ben Ahmed C., Elloumi N., Bellassoued K., Delmail D., Labrousse P., Ben Abdallah F., Ben Rouina B., 2016c Impact of proline application on cadmium accumulation, mineral nutrition and enzymatic antioxidant defense system of *Olea europaea* L. cv Chemlali exposed to cadmium stress. Ecotoxicol Environ Saf 128:195–205.

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