COMPARATIVE STUDIES OF THE BIOCHEMICAL AND MOLECULAR RESPONSES OF *HALOXYLON AMMODENDRON* GROWN ON HEAVY METAL POLLUTED SOIL IN AL-JOUF REGION OF SAUDIA ARABIA

ALHAITHLOUL, H. A. S.

Biology Department, College of Science, Jouf University, Sakaka 2014, Kingdom of Saudi Arabia (e-mail: haifasakit@ju.edu.sa)

(Received 30th May 2023; accepted 1st Aug 2023)

Abstract. Several factors, including biochemical patterns and gene expression patterns, were examined in *Haloxylon ammodendron* plants growing in two separate locations in the Al-Jouf region of Saudia Arabia. The soil in the two distinct locations has different properties, and the soil in one region has substantial quantities of heavy metals and agrochemicals, therefore it is deemed to be polluted. Samples were collected from the *H. ammodendron* growing on polluted and unpolluted soils and analyzed for different parameters. Plants growing on polluted soils showed significantly higher concentration of heavy metals and agrochemicals as compared to ones growing on unpolluted soil while as exhibited a significant decline in the content of essential mineral ions including potassium, calcium, magnesium, manganese and iron. Lipid peroxidation and hydrogen peroxide increased in both leaves and stem of *H.* ammodendron growing on polluted soils with significant decline in nitrate and oxalate content. Plants that were grown in contaminated soils have enhanced activity of antioxidant enzymes like catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD). In addition, the content of secondary compounds including total phenols, tannins, saponins and alkaloids increased significantly in plants growing on polluted soils as compared to the plants growing on normal soil. The expression patterns of genes including heavy metal ATPase (HMA4), phytochelatin synthase (PCS1), heat shock protein 70 (HSP70) and zinc transporter (ZIP4) also showed considerable increase in the plants growing on polluted soils as compared to the plants gown in unpolluted soil. The instant study reveals that heavy metal and agrochemical pollution has significant influence on the growth and metabolism of H. *ammodendron* vis-à-vis has the potential to up-regulate tolerance mechanisms to alleviate the damaging effects of heavy metals and agrochemicals.

Keywords: *agrochemicals, gene expression, H ammodendron, heavy metal, lipid peroxidation, secondary metabolites*

Introduction

Soil pollution through heavy metals and agrochemicals like pesticides has become a serious problem for scientists all over the globe. It has been estimated that millions of hectares of agricultural lands is contaminated by heavy metals and pesticides and can be rendered as unproductive thereby reflecting in significant decline in annual global food production (Xie et al., 2016). Key metal pollutants include cadmium, lead, mercury, chromium, arsenic, nickel, copper etc. in addition of the agrochemical including pesticides, weedicides, fungicides, and insecticides which significantly accumulate in soils (Alengebawy et al., 2021). These toxic metals and chemicals are continuously added to soil through natural and anthropogenic means. Bioaccumulation, translocation, and mechanism of action of these toxic metals and agrochemical have been studied (Pasricha et al., 2021). The bioavailability and uptake of these toxic metals and agrochemicals into the plant system has been reported to impart adverse effects on key growth and metabolic attributes (Ahmad et al., 2021). These contaminants are rapidly transported to higher trophic levels in the food chain due to their high mobility and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

bioaccumulation (Lin and Aarts, 2012). These heavy metals and agrochemicals, if translocated into biological systems, cause serious health complications in both human and animals (Jaishankar et al., 2014; Singh et al., 2017).

Moreover, the influence of these toxic metals and agrochemicals on the plants growing on the polluted soils significantly affects their growth and development hence influencing their productivity (Srivastava et al., 2017; Alengebawy et al., 2021). Among the key adverse effects of metals and agrochemicals on the plants are included: restricted root and shot growth, photoinhibition, enzyme inhibition and impaired nutrient uptake and assimilation (Petit et al., 2012; Ahanger et al., 2020; Ahmad et al., 2021; Qin et al., 2022). Metal and agrochemical pollution significantly affects the microbial diversity of soil thereby affecting the soil health as well as the plant growth (Xie et al., 2016; Ahemad and Khan, 2012). Reactive oxygen species (ROS) are produced in excess because of heavy metals and pesticides used in farming. These toxic ROS damage proteins, lipids and nucleic acids resulting in irreversible changes in metabolism and under extreme conditions can lead to death of plant (Ahanger et al., 2017). Stress induced ROS accumulation triggers oxidative damage to key macromolecules and affects the functioning of cellular organelles including chloroplast and mitochondria (Das and Roychoudhury, 2014; Li and Kim, 2022). Plants have indigenous mechanisms to mitigate the damaging effects of stresses including heavy metal or agrochemicals (Skuza et al., 2022; Ahmad et al., 2020; Chen et al., 2022). In addition of the antioxidant system, osmolyte accumulation and secondary metabolite accumulation, the efficient sequestration and compartmentalization of toxic metals and other chemicals significantly contribute to lessen the damaging effects on normal plant growth (Sharma et al., 2016; Hasan et al., 2017). Toxic metal ions in cytosol are eliminated either by phytochelatins or metallothioneins followed by the compartmentalization into the vacuole's concomitant with the repair or removal or degradation of damaged proteins (Hasan et al., 2017).

Growing plants on the polluted soils is really a challenging task, however nature has endowed some potential mechanisms to plants which help them to endure the harsh conditions to considerable extent. *Haloxylon ammodendron* is a xerophytic shrub having potential to thrive in extreme conditions of dry sand deserts, sand dunes etc. It has been reported that *H. ammodendron* has the potential to thrive under such conditions through the unique and distinct tolerance mechanisms thereby making it among the ideal plants to restore and assist in reclamation of polluted soils (Fan et al., 2016). In addition of this, it has been observed that camels when fed with the *H. ammodendron* growing in polluted areas develop health complication and more often leads to their death. This toxic effect of *H. ammodendron* has not been observed from the unpolluted regions. In this backdrop, in present study *Haloxylon ammodendron* growing in two distinct regions/areas of Al-Jouf, Saudia Arabia was analyzed for (a) physiological, biochemical and gene expression parameters to find the adaptability mechanisms and (b) identify the potential constituents in *H. ammodendron* growing in polluted soils leading to health complications or death in camels.

Materials and methods

Plant materials and sampling

Leaves and stem samples of *Haloxylon ammodendron* were collected from two different regions of Al-Jouf region, Saudi Arabia. Region I was containing heavy metal

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

(HMs) polluted soil while as region II was having normal soil. Leaf and stem samples were brought to laboratory for further analysis. Soil samples (30-60 cm) were collected under the roots of *H. ammodendron* plants from both different regions. It was confirmed from the soil elemental and heavy metal analysis that region was heavy metal polluted (*Tables 1* and *2*). Leaf and stem samples were oven dried at 70°C for 48 h. Thereafter samples were finely powdered and used for different estimations discussed below. However, for enzyme activity, lipid peroxidation and hydrogen peroxide estimation fresh leaf samples were used which were frozen in liquid nitrogen immediately after their collection.

Table 1. *Content of essential mineral ions and heavy metals in normal and polluted soils collected from Al-Jouf region of Saudia Arabia. Data presented is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

	Normal	**Polluted soil**
Co (mg g^{-1} DW)	ND	1.75 ± 0.02
Cu (mg g^{-1} DW)	0.0212 ± 0.0015b	9.937 ± 0.51a
Fe (mg g^{-1} DW)	0.283 ± 0.0245b	14.29 ± 1.40a
Cd (mg g^{-1} DW)	0.0128 ± 0.0030b	5.186 ± 0.25a
C (mg g^{-1} DW)	0.9066 ± 0.092b	3.66 ± 0.041a
K (mg g^{-1} DW)	23.88 ± 2.12a	16.91 ± 1.17b
Mn (mg g^{-1} DW)	25.23 ± 2.08a	13.52 ± 0.321b
Al (mg g^{-1} DW)	0.119 ± 0.017b	4.33 ± 0.12a
Ca (mg g^{-1} DW)	11.52 ± 1.05a	1.76 ± 0.085b
Na (mg g^{-1} DW)	4.161 ± 0.25b	1.16 ± 0.051a
Mg (mg g^{-1} DW)	8.976 ± 0.52a	4.63 ± 0.027b
Pb (mg g^{-1} DW)	0.1261 ± 0.015b	1.02 ± 0.025a
Ni (mg g^{-1} DW)	0.5819 ± 0.020b	2.41 ± 0.035a

Table 2. *Agrochemical (mg g^{-1} DW) concentration in normal and polluted soils collected from Al-Jouf region of Saudia Arabia. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

	Normal	**Heavy metal polluted**
Toxaphen	ND	ND
Lambda-Cyhalothrin	0.0081 ± 0.00012b	4.88 ± 0.022a
Malathion	0.0062 ± 0.00013b	2.98 ± 0.011a
Chlorpyrifos	0.0011 ± 0.00014b	2.87 ± 0.013a
Diazinon	0.0021 ± 0.00013b	2.02 ± 0.009a
Lindane	ND	3.05 ± 0.019
Atrazine	ND	ND
Methomyl	0.0010 ± 0.00013b	1.24 ± 0.006a
Carbendazm	0.0010 ± 0.00011b	4.04 ± 0.021a
PP-DDT	ND	ND
PP-DDE	ND	2.04 ± 0.003
Dimethoate	ND	ND
Cypermethrin	0.0021 ± 0.00011b	11.92 ± 0.66a

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

Determination of total phenolics

Dry powdered leaf and stem samples were extracted in ethanol and homogenate was centrifuged at 10,000 g. Supernatant was added and Folin Ciocalteau's reagent followed by addition of one mL 10% Na_2CO_3 solution and mixed thoroughly. After 1 h at room temperature, at 700 nm, absorbance was determined (Singleton et al., 1999). The standard curve for gallic acid was used in computations.

Determination of total tannins

Tannin content in leaf and stem of *H. ammodendron* was determined according to Amadi et al. (2004) and Ejikeme et al. (2014). Briefly, one-gram powdered plant sample was extracted distilled in water by boiling the samples on electric hot plate for 1 h and extracts was filtered through Whatman No.1 filter paper. After centrifuging the extract, an aliquot was combined with Na_2CO_3 and the Folin-Denis reagent. For 30 min, the mixture was placed in a water bath to develop its colour. After that, the optical density at 700 nm was measured, and the tannic acid standard curve was used to calculate the results.

Determination of total saponins

Following Obadoni and Ochuko's (2001), the saponin content was assessed. By heating the tubes on a water bath for 4 h while continuously stirring at 55°C, dry powdered 5 g samples were extracted in 10% ethanol and computed at 700 nm. Extracts were mixed and evaporated to 40 ml over a water bath at 90°C after being filtered, with the residue being re-extracted after the first extraction. The extract was then mixed thoroughly with 10 ml of diethyl ether. A 30 ml addition of n-butanol was added after recovering the aqueous layer and discarding the ether layer. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride, and the leftover solution was then heated in a water bath. The samples were evaporated, followed by oven drying to a consistent weight, and the saponin concentration was determined.

Determination of alkaloid content

Alkaloid content was determined according to Misra and Gupta (2006). Half a gram powdered leaves and stem samples were extracted in 90% ethanol and extract was filtered and dried. The dried residue was redissolved in ethanol, diluted with water, and then 3% HCl was added. By moving the mixture to a separating funnel and adding hexane to it, the mixture was extracted three times and shaken for 15-20 min. The lower water layer was poured into a beaker, brought to a pH of 8.5 with 3% ammonium hydroxide, and then chilled to 10°C. The mixture was then once again poured into a separate funnel while being added three times more chloroform. The content was then shaken for 15-20 min. The upper chloroform layer was decanted, while the lower layer was discarded. Decanted layer was put to a dry porcelain plate that had been previously weighed, and it was then evaporated until dry. The dish weight was then measured once again. Total alkaloids were expressed as percent alkaloid content in the dry plant powder.

Estimation of total terpenoides

Terpenoids content in dried powdered samples of *H. ammodendron* was estimated according to Ferguson (1956). In a nutshell, 5 g of plant powder that was extracted in alcohol for 24 h, filtered, and then extracted with petroleum ether was treated as total terpenoids.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

Determination of glycosides

Glycosides were determined in dried powdered samples according to Amadi et al. (2004) and Ejikeme et al. (2014). Briefly, 1 g dried powder was extracted in distilled water for 2 h. Thereafter, distillation was carried out in conical flask containing 2.5% NaOH and antifoaming agent (tannic acid) was also added. Subsequently, 6 M NH_4OH and 5% KI were added to the distillate and mixed followed by titration with 0.02 M $AgNO_3$ using a micro burette against a black background.

Measurement of lipid peroxidation and hydrogen peroxide

Lipid peroxidation was measured as the formation of malonaldehyde (MDA) content in accordance with the method of Heath and Packer (1968). Fresh 0.5 leaf samples were extracted in 10 mL trichloroacetic acid and followed by centrifugation at 10,000 × *g* for 10 min. Thereafter 1 mL supernatant was added to 2 mL of thiobarbituric acid (TBA, 0.65% prepared in 20% TCA) and mixture was boiled for 30 min. After cooling the mixture centrifugation was done again for 5 min and absorbance was read at 600 and 532 nm. For measurement of hydrogen peroxide method of Velikova et al. (2000) was followed and standard curve of hydrogen peroxide was used for calculation.

Assay of antioxidant enzymes

Fresh 500 mg of tissue was homogenized in cold sodium phosphate buffer (50 mM; pH 7.0) containing polyvinyl pyrrolidine (1%), 0.1 mM PMSF, and 1 mM EDTA for the extraction of antioxidant enzymes using a pre-chilled pestle and mortar. The supernatant was collected and used for the enzyme assay after centrifuging the extract at 15,000 g for 15 min at 4°C. According to Bayer and Fridovich (1987), superoxide dismutase (SOD, EC 1.15.1.1) activity was measured, and optical density at 560 nm was recorded. Activity of catalase (CAT, EC 1.11.1.6) was measured according to Luck (1974) and change in absorbance was measured at 240 nm for 2 min. Using Nakano and Asada's (1981) approach, the change in absorbance at 290 nm was recorded for 3 min to measure the activity of APX.

Estimation of nitrate and oxalate

Oxalate determination in dry powder was carried using titratimetric reaction method described by Ejikeme et al. (2014) and Munro and Bassir (1969). Nitrate content was determined according to Bremner (1965) and Carlson and Keeney (1971).

Analysis of toxic elements and agrochemicals

The dried stem and leaves of *H. ammodendron* and the soil samples of two regions were analyzed for the mineral contents using Perkin Elmer 100 Atomic Absorption Spectrometer. For estimation of pesticides 5 g of powdered leaves and stem, 10 g soil sample were extracted according to the QuEChERS (quick easy cheap effective rugged and safe) method of Anastassiades et al. (2003). To an aliquot of homogenized sample was added 10 mL of acetonitrile followed by vortexing for 1 min. Thereafter, magnesium sulphate and sodium chloride were added and samples were centrifuged. The clean-up was carried out by transferring the supernatant into another tube containing 20 mg of primary and secondary amine sorbent (PSA), 10 mg of graphite carbon black (GCB), and 50 mg of $MgSO_4$. After this clean-up procedure, the final

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

dried extract was dissolved in 0.5 ml acetonitrile and subjected to gas chromatography-mass spectrometry (GC-MS) analysis., by employing GC-MS/MS (GC-MS-QP2010 Ultra analytical equipment Shimadzu, Japan), pesticide residues in the samples were found and verified. Sigma Aldrich provided purities > 95% of the pesticide reference standards that were acquired. A DB-5 column (60 m 0.25 mm, 0.25 m thick) was used to inject the sample at a flow rate of 1.5 mL for 1 min. The temperature of the GC oven was programmed to start at 70°C for 2 min, rise to 150°C at a rate of 25°C/min (held for 0 min), rise to 200°C at a rate of 3°C/min (held for 0 min), rise from 200 to 280°C at a rate of 8°C/min (held for 10 min). It took 16 min to complete the experiment with samples injected in non-split mode. To identify the components of the sample based on comparison of their relative indices and mass spectra, the GC-MS system computer matched with WILEY and National Institute of Standards and Technology (NIST08) library data (http://webbook.nist.gov).

RNA extraction and gene expression analysis

Total mRNA was isolated from 250 mg of *H. ammodendron* tissue using total RNA extraction kit (Sigma-Aldrich) according to the manufacturer's protocol. The extracted RNA was analyzed and spectrophotometrically quantified using a 1% agarose gel. A reverse transcription of RNA was performed. 2.5 l of 5X buffer, 2.5 l of $MgCl_2$, 2.5 l of 2.5 mM dNTPs, 4 l of oligo (dT), 0.2 l of reverse transcriptase (5 unit/l; Promega, Germany), and 2.5 l of RNA were used in the reaction. A thermal cycler PCR with RT-PCR amplification was set at 42°C for 1 h and 72°C for 20 min was employed. The primer sequences used in quantitative real-time PCR (qRT-PCR) were listed in *Table 3*. Quantitative real-time PCR for gene expression analysis used a SYBR® Green-based technique. Real-time analysis was conducted using Rotor-Gene 6000 (Germany) employing primers from four metal responsive genes and a housekeeping gene (reference gene). An amount of 20 L was used throughout the entire reaction. For the reactions, a total volume of 20 L was used, which included 2 L of template, 10 L of SYBR Green Master Mix, 2 L of reverse primer, 2 L of forward primer, and sterile dist. water. The stipulated sequences were used for the PCR assays that include: 40 cycles of 30 s at 95°C and 30 s at 60°C were performed after 15 min at 95°C. The CT of each sample was used to calculate ΔCT values (target gene CT subtracted from β-Actin gene CT). The relative gene expression was determined using the 2-ΔΔCt method (Togawa and Dunn, 2008).

Table 3. *Oligonucleaotide primers used in qRT-PCR analysis*

Primer Name		Sequence (5′–3′)
HMA4 – heavy metal ATPase	F	5′ACTGAAGCCACTTGAAGGAGT-3′
	R	5′-CGCGAGCAATAACCCTGATA-3′
PCS1 – phytochelatin synthase	F	5′-AGCCAAGCATGCAACTTTCC-3′
	R	5′-CCCCGCCATTGAATTTGCTT-3′
HSP70	F	5′-CCCATCTTGGTGGTGAAGAT-3’
	R	5’-GTCCTCAGCAGACACGTTCA-3’
ZIP4	F	5′-ACGAGGATTGAAACGAGTGC-3′
	R	5′-AAACCCCACCTTCGCTTTTC-3′
GAPDH	F	5′-TTGGTTTCCACTGACTTCGTT-3′
	R	5′-CTGTAGCCCCACTCGTTGT-3′
β-Actin	F	5′-GTGCCCATTTACGAAGGATA-3′
	R	5′-GAAGACTCCATGCCGATCAT-3′

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

Statistical analysis

Data presented is mean of three replicates. Data was statistically analyzed, and significant difference was calculated at $P < 0.05$.

Results

Results showing the mineral content, toxic metal ions and pesticides in soil samples of both sites are given in *Tables 1* and *2*. Significant variation was observed in the content of essential mineral ions with considerable decline in soil samples collected from polluted site. Relatively to control (unpolluted soil), content of Cd, Pb, Al, Cr, Ni, Cu, and Co showed significant increase (*Table 1*). The content of different agrochemical including Toxaphen, lambda-cyhalothrin, malathion, chlorpyrifos, diazinon, lindane, atrazine, methomyl, carbendazm, PP-DDT, PP-DDE, dimethoate and cypermethrin was more in infected soil over the unpolluted site (*Table 2*).

Relative to control (unpolluted), plants grown on polluted soils exhibited a significant decline in the content of key mineral elements like K, Ca, Fe, Mg and Mn while as an increase was observed in toxic metal ions including Cd, Pb, Ni, Cr, Al, Cu, and Co (*Table 4*). Decline in K, Ca, Fe, Mg and Mn in leaves was 11.12, 3.37, 8.31, 2 and 1.93-fold respectively. Moreover, plants grown on polluted soils exhibited considerable increase in the contents of pesticides including taxophen, lamdha-cyhalothrin, malathion, chlorpyrifos, diazinon, lindane, atrazine, methomyl, carbendazm, PP-DDT, PP-DDE, dimethoate and cypermethrin (*Table 5*).

Content of total phenols, tannins, saponins, alkaloids and glycosides exhibited a significant increase in plants grown on the polluted soil over the control. Relative to control (unpolluted), percent increase in total phenols was 109.61% and 63.53%, tannins were 81.78% and 49.19%, saponins was 24.14% and 34.64%, alkaloids were 43.17% and 14.24% and glycosides was 41.51% and 26.09% in leaves and stem of plants grown on polluted soil (*Fig. 1A-E*). However, terpenoids did not show so much difference between the plants grown on normal and polluted soils (*Fig. 1F*).

Table 4. *Mineral contents concentration in Leaf and Stem as affected by control and stressed condition. Data is mean (±SE) of three replicates and different letters denote significant difference at p < 0.05*

Mineral element	Leaf		Stem	
	Control (unpolluted soil)	Stressed (polluted soil)	Control (unpolluted soil)	Stressed (polluted soil)
K (mg g^{-1} DW)	47.26 ± 4.05a	4.25 ± 0.35b	8.489 ± 0.66a	6.64 ± 0.05b
Ca (mg g^{-1} DW)	12.96 ± 1.42a	3.84 ± 0.44b	9.085 ± 1.15a	3.29 ± 0.64b
Fe (mg g^{-1} DW)	12.30 ± 1.01a	1.481 ± 0.17b	7.626 ± 0.190a	7.44 ± 0.88a
Na (mg g^{-1} DW)	1.22 ± 0.42a	1.457 ± 0.15a	2.775 ± 0.23b	6.050 ± 0.54a
Mg (mg g^{-1} DW)	7.56 ± 0.34a	3.785 ± 0.53b	1.264 ± 0.163b	5.892 ± 0.48a
Mn (mg g^{-1} DW)	3.18 ± 0.55a	1.642 ± 0.21b	2.383 ± 0.55a	1.8.65 ± 0.16a
Co (mg g^{-1} DW)	Nd	1.096 ± 0.18	0.071 ± 0.0059b	0.9666 ± 0.015a
Al (mg g^{-1} DW)	1.11 ± 0.03b	3.203 ± 0.24a	9.476 ± 0.84a	2.62 ± 0.12b
Cu (mg g^{-1} DW)	0.7433 ± 0.59b	6.259 ± 0.49a	0.9233 ± 0.76b	8.08 ± 0.51a
Cd (mg g^{-1} DW)	0.0761 ± 0.0021b	4.126 ± 0.38a	0.090 ± 0.020b	3.94 ± 0.50a
Cr (mg g^{-1} DW)	0.08133 ± 0.012b	2.64 ± 0.102a	0.04366 ± 0.011b	2.346 ± 0.032a
Pb (mg g^{-1} DW)	1.47 ± 0.020b	11.49 ± 0.46a	0.9136 ± 0.015b	2.645 ± 0.026a
Ni (mg g^{-1} DW)	3.575 ± 0.28b	10.94 ± 0.63a	3.618 ± 0.35b	11.12 ± 0.721a

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

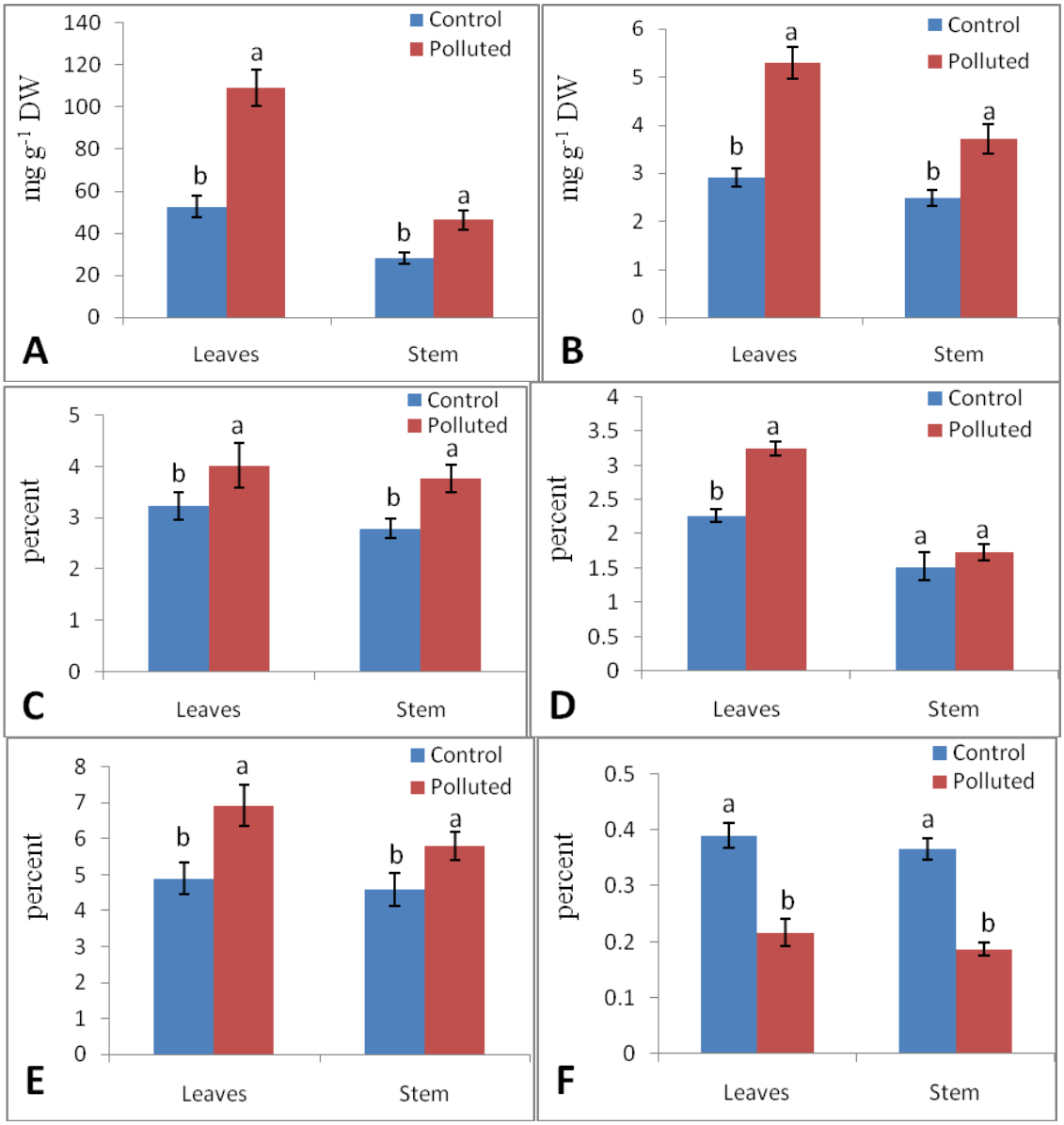

Figure 1. *Content of (A) total phenols, (B) tannins, (C) saponins, (D) alkaloids, (E) terpenoids and (F) glycosides in H. ammodendron plants grown on control (unpolluted) and polluted soils. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

Plants grown on polluted soils exhibited significant increase in the lipid peroxidation in both leaves and stem. Relative to control, lipid peroxidation increased by 91.02% and 120.06% in leaves and stem respectively (*Fig. 2A*). In addition to this the concentration of hydrogen peroxide in plants grown on polluted soils was significantly higher than the ones grown on normal soil. Relative to plants grown on the normal soil, hydrogen peroxide increased by 4.93 and 3.76-fold in leaves and stem of plants grown on polluted soils (*Fig. 2B*).

Plants grown on polluted soils exhibited a significant increase in the activities of the antioxidant enzymes assayed. Percent increase in the activities of SOD, CAT and APX was 119.80%, 196.60% and 119.81% respectively in leaves over the normal grown

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

plants. Similar trend was observed in stem exhibiting significant increase in antioxidant enzyme activities over the plants grown on normal soils (*Fig. 3A-C*).

Table 5. *Mineral contents concentration in Leaf and Stem as affected by Control and stressed condition. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

Mineral element	Leaf		Stem	
	Control (unpolluted soil)	Stressed (polluted soil)	Control (unpolluted soil)	Stressed (polluted soil)
Toxaphen	ND	ND	ND	3.03 ± 0.021
Lambda-Cyhalothrin (mg g^{-1} DW)	0.012 ± 0.003b	2.12 ± 0.011a	0.011 ± 0.0011b	7.27 ± 0.045a
Malathion (mg g^{-1} DW)	0.012 ± 0.002b	6.06 ± 0.031a	ND	3.45 ± 0.23
Chlorpyrifos (mg g^{-1} DW)	0.011 ± 0.0021b	4.14 ± 0.017a	0.010 ± 0.0002b	0.101 ± 0.044a
Diazinon (mg g^{-1} DW)	ND	1.81 ± 0.091	ND	0.141 ± 0.011
Lindane (mg g^{-1} DW)	ND	0.901 ± 0.056	ND	ND
Atrazine (mg g^{-1} DW)	ND	ND	ND	11.11 ± 0.88
Methomyl (mg g^{-1} DW)	ND	0.404 ± 0.014	ND	0.222 ± 0.010
Carbendazm (mg g^{-1} DW)	ND	1.098 ± 0.028	ND	ND
PP-DDT (mg g^{-1} DW)	ND	ND	ND	1.01 ± 0.002
PP-DDE (mg g^{-1} DW)	ND	2.02 ± 0.019	ND	ND
Dimethoate (mg g^{-1} DW)	ND	ND	ND	2.07 ± 0.011
Cypermethrin (mg g^{-1} DW)	0.010 ± 0.003b	11.15 ± 0.67a	0.010 ± 0.001b	11.12 ± 0.721a

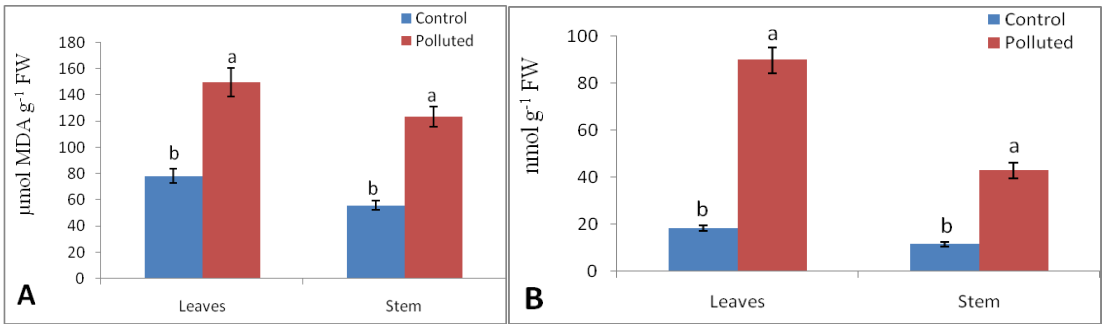

Figure 2. *Lipid peroxidation (A) and hydrogen peroxide (B) in H. ammodendron plants grown on control (unpolluted) and polluted soils. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

Nitrate and oxalate content declined significantly in plants grown on polluted soils as compared to the plants grown on normal soil. Percent decline in nitrate and oxalate in plants grown on polluted soils was 26.12% and 24.53% in leaves and 11.18% and 30.43% in stem respectively over the control plants (*Fig. 4A, B*).

Gene expression results of four genes namely HMA4, PCS1, HSP70 and ZIP4 are shown in *Figure 4*. The expression of HMA4, PCS1, HSP70 and ZIP4 was increased significantly in plants grown on polluted soils with percent increase of 12.67, 16.57, 14.74 and 8.39-fold in leaves and 10.38, 13.5, 9.17 and 7.79-fold in stem (*Fig. 5A-D*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

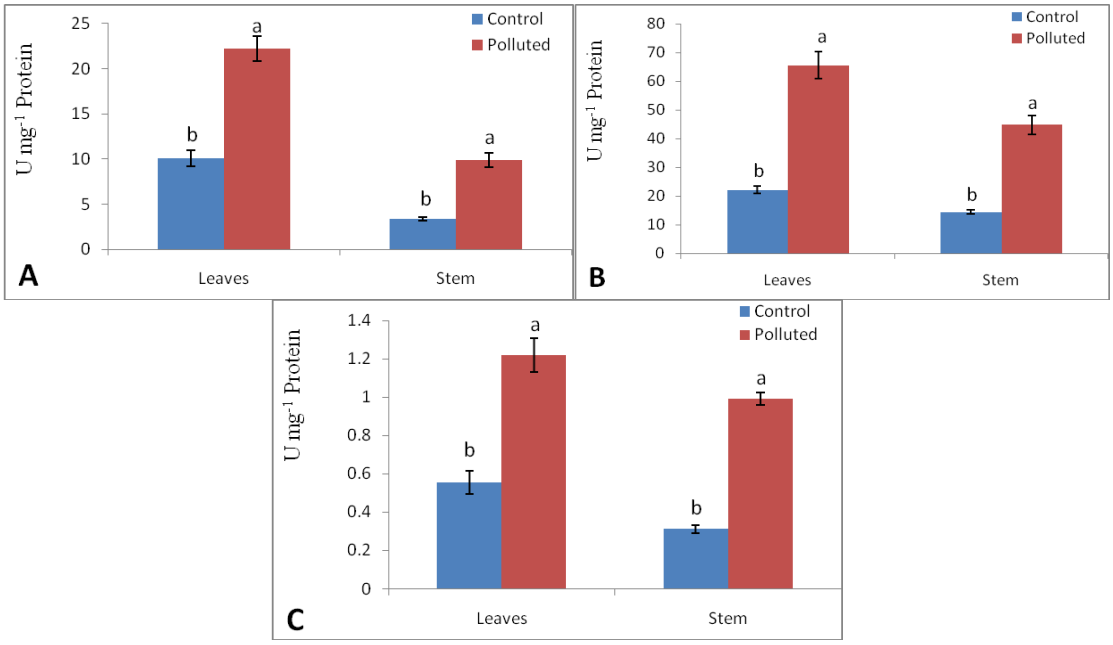

Figure 3. *Activity of (A) superoxide dismutase, (B) catalase and (C) ascorbate peroxidase in H. ammodendron plants grown on control (unpolluted) and polluted soils. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

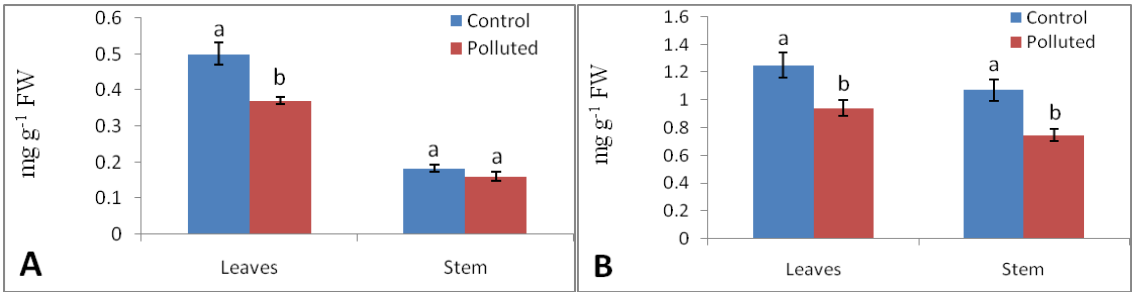

Figure 4. *Content of (A) nitrates and (B) oxalate in H. ammodendron plants grown on control (unpolluted) and polluted soils. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

Discussion

With rapid industrialization majority of agricultural lands are getting adversely affected by the industrial effluents which are rich in toxic metals, pesticides, herbicides, and other toxic agrochemicals. The accumulation of these toxic metal ions and agrochemicals in the soils directly affects the plants growing. In present investigation the influence of pollution from industrial effluents on soil properties and the growth of *H. ammodendron* was analyzed. Soil analysis of the polluted region revealed the presence of significant quantity of toxic metal ions concomitant with decline in beneficial elements. Presence of toxic metal ions in polluted soils significantly affected the growth of *H. ammodendron* by triggering decline in content of essential mineral elements including K, Ca, Mg, Na and Mn in leaf and stem. Earlier the availability of toxic metal ions has been reported to decline the content of essential mineral ions by others as well (Khan et al., 2015; Ahanger et al., 2020;

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

Ahmad et al., 2018; Alyemeni et al., 2018). Similar studies carried by Karahan et al. (2020) have reported considerable accumulation of toxic metal ions in different parts of medicinal plants grown in eastern mediterranean region of Turkey, thereby affecting their medicinal efficacy. On the contrary *H. ammodendron* plants grown on normal soil exhibited significantly increased content of the essential elements. Optimal availability and uptake of key mineral ions regulates the plant growth and metabolisms at physiological, biochemical and molecular levels often reflected as better growth and yield performance (Nazar et al., 2012; Ahanger et al., 2017). Availability and uptake of mineral elements significantly alleviates the damage to key physiological and biochemical functions of plants by strengthening the tolerance mechanisms (Sarwar et al., 2010; Ahmad et al., 2015; Khan et al., 2015; Sanches-Virosta et al., 2019; Qin et al., 2022). Despite involving specific transporters, heavy metals affect the functioning of transporters involved in uptake and translocation of key mineral ions to aboveground plant parts (Haney et al., 2005; Peiter et al., 2007; Gustin et al., 2011; Ricachenevsky et al., 2013; Jain et al., 2018). Accumulation of heavy metals including Cd, Pb, Cr, Ni etc. triggers damaging effects on the key attributes including root growth, photosynthesis, enzyme functioning, electron transport and tolerance mechanisms thereby altering the normal growth and development in plants (Nazar et al., 2012; Chandra and Kang, 2015; Soliman et al., 2019; Ahanger et al., 2020).

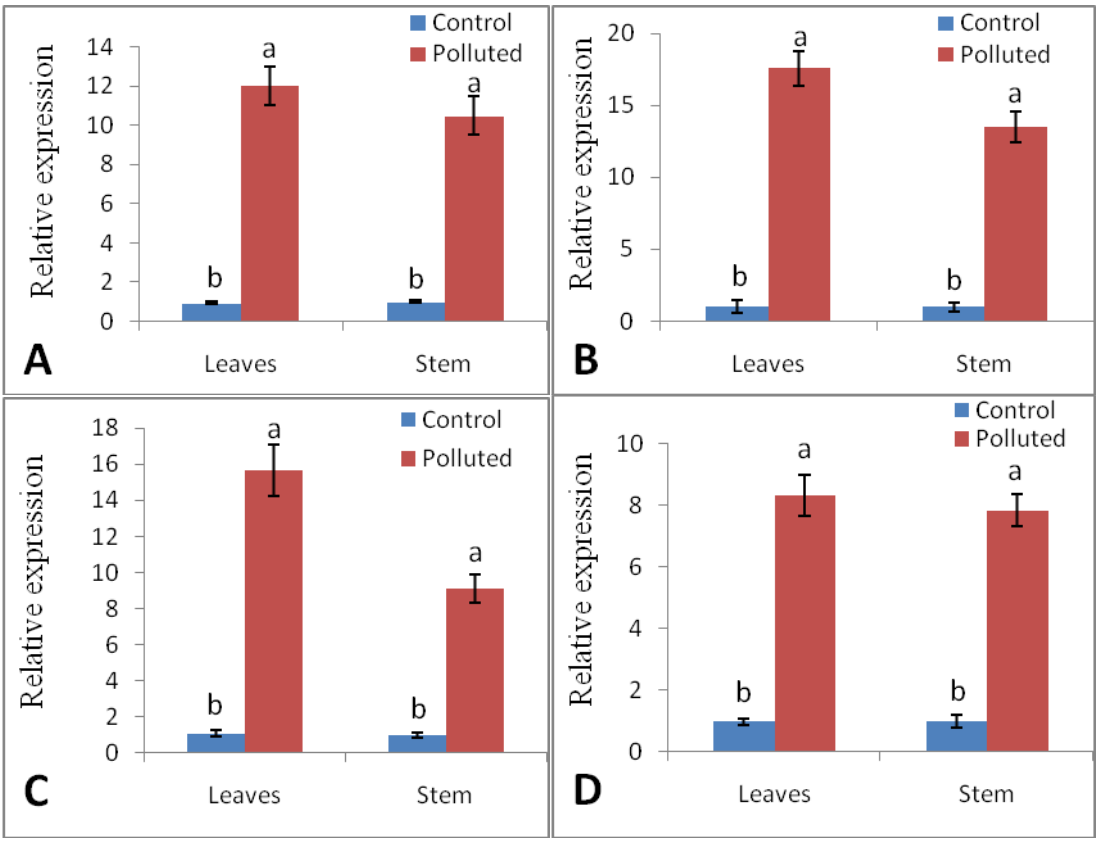

Figure 5. *Relative expression of (A) heavy metal ATPase4, (B) phytochelatin synthase1, (C) heat shock protein 70 and (D) ZIP4 transporter in H. ammodendron plants grown on control (unpolluted) and polluted soils. Data is mean (±SE) of three replicates and different letters denote significant difference at $p < 0.05$*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

Moreover, the growth decline in plants grown on polluted soil may be reflected to the presence of considerable amounts of agrochemicals including fungicides, pesticides etc. Accumulation of fungicides, pesticides, insecticides, and other agrochemicals in soils lead to soil degradation, reduced fertility and soil microbiome thereby influencing the vegetation considerably (Kalia and Gosal, 2011; Narayanan et al., 2022). Fungicides, including systematic as well as contact fungicides, induce alterations in the photosynthesis thereby reducing the generation of photoassimilates through the deleterious impact on the chloroplast structure, PSII activity and chlorophyll biosynthesis (Petit et al., 2012). In present study the polluted soils contained significant concentration of agrochemicals and their subsequent accumulation in *H ammodendron* may have adversely affected the growth and metabolism. Liu et al. (2021) have demonstrated that exposure of wheat to fungicide (difenoconazole) triggers oxidative stress and hampers chlorophyll biosynthesis reflecting in reduced growth and development. Agrochemicals can prove toxic to environment and plants and can affect the humans as well after entering the food chain. The usage of agrochemicals can benefit when applied in threshold concentrations while as can prove damaging in higher concentrations. For example, below threshold levels of fungicide and pesticide improve the yield of soybean (Henry et al., 2011). Treatment of kitazin, hexaconazole and carbendazim reduced growth and yield of pea by inducing oxidative damage and reduction in chlorophyll content (Shahid et al., 2018).

Oxidative damage resulting from the pollutants in the soil significantly contributes to growth decline. In present study it was observed that *H ammodendron* grown on soils collected from polluted sites exhibited a significant enhancement in the lipid peroxidation. Lipid peroxidation determines the extent of damage to membrane structural and functional integrity which directly results from excessive generation of toxic reactive oxygen species (Soliman et al., 2019). Stress induced excessive generation of toxic radicals including hydrogen peroxide, hydroxyl, and superoxide etc. trigger peroxidation of membrane lipids thereby hampering their structural and functional integrity (Ahanger et al., 2020; Qin et al., 2022). Besides the presence of considerable amounts of heavy metals, the occurrence of several agrochemicals like fungicides, pesticides and insecticides may also trigger the lipid peroxidation through excessive generation of reactive oxygen species. Antioxidant system consisting of enzymatic and non-enzymatic components alleviates the damaging effects of toxic radicals to considerable extent and protects the functioning of cell and tissues. In present study also, the activity of antioxidant enzymes was significantly upregulated in plants grown on polluted soils reflecting in the potential of *H. ammondendron* to alleviate the damaging effects of excess free radicals. Earlier, Shakir et al. (2018) has demonstrated that treatment of higher concentration of pesticides induce reactive oxygen species production reflecting in increased lipid peroxidation and cell injury concomitant with decline in cell viability in *Solanum lycopersicum*. Similarly, treatment of different fungicides elevated the accumulation of reactive oxygen species and hence inducing lipid peroxidation (Mohamed and Akladious, 2017).

The presence of heavy metal and agrochemicals in the soils resulted in significant decline in the nitrate and oxalate content in *H ammodendron*. Nitrate reduction in plants is accompanied by the formation of organic acid like oxalic acid and these acids contribute to maintenance of pH by neutralizing it after being alkalized during nitrate reduction (Tanaka et al., 2001). Nitrate concentration as well as assimilation influences the oxalate generation and the quality of edible plant products (Tian et al., 2008).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

Organic acids including citric acid or oxalate have great affinity for heavy metals and sequester them into vacuole (Rauser, 1999) thereby preventing the damage to growth and cellular functioning. Oxalates potentially function in improving tolerance to pests and grazing animals (Prasad and Shivay, 2017). In present study *H ammodendron* grown on soils polluted with heavy metals and agrochemicals resulted in significant decline in oxalates and nitrate which affect significantly to heavy metal sequestration and the nitrogen metabolism respectively. Nitrate is taken through specific transport system and its metabolism is specifically regulated (Forde, 2000), and heavy metal as well as agrochemicals may have significantly influenced its uptake as well as metabolism.

The *H. ammodendron* grown on the polluted soils exhibited a significant enhancement in the content of secondary metabolites including total phenols, tannins, alkaloids, saponins and glycosides. Accumulation of secondary compounds has a key role in mitigating the adverse effects of stress factors on the growth and development of plants (Ahanger et al., 2020; Soliman et al., 2019). Heavy metals trigger the accumulation of phenolic compounds by improving the functioning of the biosynthesizing enzymes (Chen et al., 2019). Secondary compounds neutralize the toxic reactive oxygen species thereby preventing the damage to key cellular and metabolic structures and there functioning as well (Chen et al., 2022). Under stressful conditions, like the polluted soil in present case, plants tend to mitigate toxicity of heavy metals and other compounds by modulating secondary metabolite metabolism which act as metal precipitators, scavengers of reactive oxygen species and chelating agents (Anjitha et al., 2021). The generation or synthesis of specific type of metabolite in plants depends on the species, genotype, environmental condition, physiology (Isah, 2019) and in present study plants grown on metal and agrochemical polluted soils exhibited significant increase in accumulation of secondary metabolites.

In addition of improving the physiological and biochemical mechanisms to alleviate the damaging the effect of heavy metals and agrochemicals on the growth the *H. ammodendron* plants exhibited a significant modulation of the expression pattern of some key genes having prime role in metal and agrochemical tolerance. Relative to plants grown on normal soil, the H ammodendron plants grown on polluted soils exhibited a significant up-regulation of the expression levels of genes including HMA4, PCS1, HSP70 and ZIP4. Heavy metal ATPase4, is one of the P18-type ATPase heavy metal transporter that pump toxic metals across membrane in an ATP dependent manner (Moller et al., 1996). Increased expression of HMA4 results in increased translocation of Zn to shoot (Mollar et al., 1996; Hanikenne et al., 2008) and contribute to increased accumulation of Zn and Cd (Lochlainn et al., 2012). HMA genes sequester metal ions into vacuole thereby restrict the toxic effects to considerable levels as has been reported by Liu et al. (2017). Phytochelatin synthase (PCS) mediates the synthesis of phytochelatins from glutathione thereby provide protection to plants against the toxic metal ions (Shine et al., 2015; Filiz et al., 2018). Phytochelatins are the metal binding peptides in plants that carry the detoxification of toxic metal ions thereby protect plants. Plants over-expressing PCS gene exhibit significant improvement in metal tolerance through greater production of phytochelatins resulting in improved growth and lower oxidative stress in terms of lipid peroxidation and radical generation (Zhang et al., 2018; Zhu et al., 2021). On the other hand, ZIP also contributes to prevent the damaging effects of toxic metal and improve the uptake and transport of beneficial ions (Guerinot, 2000). There have very rare reports available discussing the influence of metal and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

agrochemical pollution on the gene expression of plants. HSPs have key role in stress adaption and mitigation in plants (Al-Whaibi, 2011). HSPs regulate stress responses either or indirectly by controlling the expression of other key genes (Li et al., 2021). Increased expression of the mentioned genes in *H. ammodendron* grown on polluted soils reflects its potential to endure the harsh growth condition to some extent. Increased expression of HSP70 genes in *Sogatella furcifera* has been reported in response to pesticide treatment (Zhou et al., 2020).

Conclusion

Conclusively, it can be said that *H ammodendron* plants grown on polluted soils exhibit significant decline in growth, biochemistry and gene expression. Plants grown on normal soil exhibited relatively low oxidative damage in terms of lipid peroxidation which was correlated with the low accumulation of heavy metals and agrochemicals in them as compared to the plants grown on polluted soils. Polluted soils have significantly higher concentrations of heavy metals and agrochemicals which probably imparted oxidative damage, and the accumulation of secondary compounds in plants grown on such soils reflects in the induction of tolerance mechanisms in them. Gene expression studies reflected that *H. ammodendron* plants exhibited significant up-regulation of marker genes including HMA4, ZIP, HSP70 and PCS1. Presence of increased concentrations of agrochemicals and the accumulation of metabolites in plants can be potential reasons for developing the health complication in camels.

Data availability statement. The datasets used and/or analyzed during the current study are available from the author on reasonable request.

REFERENCES

[1] Ahanger, M. A., Tomar, N. S., Tittal, M., Argal, S., Agarwal, R. M. (2017): Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. – Physiology and Molecular Biology of Plants 23(4): 731-744.

[2] Ahanger, M. A., Aziz, U., Alsahli, A. A., Alyemeni, M. N., Ahmad, P. (2020): Combined kinetin and spermidine treatments ameliorate growth and photosynthetic inhibition in *Vigna angularis* by up-regulating antioxidant and nitrogen metabolism under cadmium stress. – Biomolecules 10: 147. DOI: 10.3390/biom10010147.

[3] Ahemad, M., Khan, M. S. (2012): Effect of fungicides on plant growth promoting activities of phosphate solubilizing Pseudomonas putida isolated from mustard (Brassica compestris) rhizosphere. – Chemosphere 86(9): 945-950.

[4] Ahmad, P., Ahanger, M. A., Alam, P., Alyemeni, M. N. (2018): Modification of osmolytes and antioxidant enzymes by 24-epibrassinolide in chickpea seedlings under mercury (Hg) toxicity. – J Plant Growth regulation 37(1): 309-322.

[5] Ahmad, P., Alyemeni, M. N., Wijaya, L., Ahanger, M. A., Ashraf, M., Alam, P., Paray, B. A., Rinklebe, J. (2021): Nitric oxide donor, sodium nitroprusside, mitigates mercury toxicity in different cultivars of soybean. – Journal of Hazardous Materials 408 124852 https://doi.org/10.1016/j.jhazmat.2020.124852.

[6] Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., Wang, M. Q. (2021): Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications. – Toxics. Mar. 9(3): 42. DOI: 10.3390/toxics9030042.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

[7] Al-Whaibi, M. H. (2011): Plant heat-shock proteins: a mini review. – Journal of King Saud University - Science 23(2): 139-150.
[8] Alyemeni, M. N., Ahanger, M. A., Wijaya, L., Alam, P., Bhardwaj, R., Ahmad, P. (2018): Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum lycopersicum* L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. – Protoplasma 255(2): 459-469.
[9] Amadi, B. A., Agomuo, E. N., Ibegbulem, C. O. (2004): Research Methods in Biochemistry. – Supreme Publishers, Owerri.
[10] Anastassiades, M., Lehotay, S. J., Stajnbahar, D., Schenck, F. J. (2003): Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. – J AOAC Int 86(2): 412-31.
[11] Anjitha, K. S., Sameena, P. P., Puthur, J. T. (2021): Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. – Plant Stress 2: 100038 https://doi.org/10.1016/j.stress.2021.100038.
[12] Bayer, W. F., Fridovich, J. L., (1987): Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. – Anal. Biochem. 161: 559-566.
[13] Bremner, J. M. (1965): Total Nitrogen. – In: Black, C. A. (ed.) Methods of Soil Analysis. Part 2. Am Soc Agron, Madison, WI, pp 1216-1219.
[14] Carlson, R. M., Keeney, D. R. (1971): Specific Ion Electrodes: Techniques and Uses in Soil, Plant, and Water Analysis. – In: Walsh, L. M. (ed.) Instrumental Methods for Analysis of Soils and Plant Tissue. Soil Sci Soc Am, Madison WI, pp. 39-65.
[15] Chandra, R., Kang, H. (2015): Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. – Forest Science and Technology 12(2): 55-61 https://doi.org/10.1080/21580103.2015.1044024.
[16] Chen, D., Mubeen, B., Hasnain, A., Rizwan, M., Adrees, M., Naqvi, S. A. H., Iqbal, S., Kamran, M., El-Sabrout, A. M., Elansary, H. O., Mahmoud, E. A., Alaklabi, A., Sathish, M. and Din, G. M. U. (2022): Role of promising secondary metabolites to confer resistance against environmental stresses in crop plants: current scenario and future perspectives. – Front. Plant Sci. 13: 881032. DOI: 10.3389/fpls.2022.881032.
[17] Chen, M., Zhang, X., Jiang, P., Liu, J., You, S., Lv, Y. (2022): Advances in heavy metals detoxification, tolerance, accumulation mechanisms, and properties enhancement of Leersia hexandra Swartz. – Journal of Plant Interactions 17(1): 766-778. DOI: 10.1080/17429145.2022.2096266.
[18] Chen, S., Wang, Q., Lu, H., Li, J., Yang, D., Liu, J., Yan, C. (2019): Phenolic metabolism and related heavy metal tolerance mechanism in Kandelia Obovata under Cd and Zn stress. – Ecotoxicol Environ Saf. 169: 134-143. DOI: 10.1016/j.ecoenv.2018.11.004.
[19] Das, K., Roychoudhury, A. (2014): Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. – Front. Environ. Sci. https://doi.org/10.3389/fenvs.2014.00053.
[20] Ejikeme, C. M., Ezeonu, C. S., Eboatu, A. N. (2014): Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. – European Scientific Journal 10(18): 247-270.
[21] Fan, B., Zhang, A., Yang, Y., Ma, Q., Li, X., Zhao, C. (2016): Long-term effects of xerophytic shrub haloxylon ammodendron plantations on soil properties and vegetation dynamics in Northwest China. – PLoS One 11(12): e0168000. DOI: 10.1371/journal.pone.0168000.
[22] Ferguson, N. M. (1956): A Text Book of Pharmacognosy. – MacMilan Company, New Delhi.
[23] Filiz, E., Saracoglu, I. A., Ozyigit, I. I., Yalcin, B. (2018): Comparative analyses of phytochelatin synthase (PCS) genes in higher plants. – Biotechnology and Biotechnological Equipment 33(1): 178-194 https://doi.org/10.1080/13102818.2018.1559096.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

[24] Forde, B. G. (2000): Nitrate transporters in plants: structure, function and regulation. – Biochimica et Biophysica Acta (BBA) - Biomembranes 1465(1-2) 219-235.
[25] Guerinot, M. L. (2000): The ZIP family of metal transporters. – Biochimica et Biophysica Acta (BBA) - Biomembranes 1465(1-2): 190-198.
[26] Gustin, J. L., Zanis, M. J., Salt, D. E. (2011): Structure and evolution of the plant cation diffusion facilitator family of ion transporters. – BMC Evol Biol. 11(1): 76. DOI: 10.1186/1471-2148-11-76.
[27] Haney, C. J., Grass, G., Franke, S., Rensing, C. (2005): New developments in the understanding of the cation diffusion facilitator family. – J Ind Microbiol Biotechnol. 32(6): 215-226. DOI: 10.1007/s10295-005-0224-3.
[28] Hanikenne, M., Talke, I. N., Haydon, M. J., Lanz, C., Nolte, A., Motte, P., et al. (2008): Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*. – Nature 453: 391-5. DOI: 10.1038/nature06877.
[29] Hasan, M. K., Cheng, Y., Kanwar, M. K., Chu, X. Y., Ahammed, G. J., Qi, Z. Y. (2017): Responses of plant proteins to heavy metal stress - a review. – Front Plant Sci. 8: 1492. DOI: 10.3389/fpls.2017.01492.
[30] Heath, R. L., Packer, L. (1968): Photoperoxidation in isolated chloroplasts. – Arch. Biochem. Biophys. 125: 189-198.
[31] Henry, R. S., Johnson, W. G., Wise, K. A. (2011): The impact of a fungicide and an insecticide on soybean growth, yield, and profitability. – Crop Protection 30(12): 1629-1634.
[32] Isah, T. (2019): Stress and defense responses in plant secondary metabolites production. – Biol Res. 52: 39. DOI: 10.1186/s40659-019-0246-3.
[33] Jain, S., Muneer, S., Guerriero, G., Liu, S., Vishwakarma, K., Chauhan, D. K., Dubey, N. K., Tripathi, D. K., Sharma, S. (2018): Tracing the role of plant proteins in the response to metal toxicity: a comprehensive review. – Plant Signal Behav. 13(9): e1507401. DOI: 10.1080/15592324.2018.1507401.
[34] Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., Beeregowda, K. N. (2014): Toxicity, mechanism and health effects of some heavy metals. – Interdiscip Toxicol. 7(2): 60-72. DOI: 10.2478/intox-2014-0009.
[35] Kalia, A., Gosal, S. K. (2011): Effect of pesticide application on soil microorganisms. – Archives of Agronomy and Soil Science 57(6): 569-596. https://doi.org/10.1080/03650341003787582.
[36] Karahan, F., Ozyigit, I. I., Saracoglu, I. A., Yalcin, I. E., Ozyigit, A. H., Ilcim, A. (2020): Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern mediterranean region of Turkey. – Biol Trace Elem Res. 197(1): 316-329. DOI: 10.1007/s12011-019-01974-2.
[37] 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.
[38] Li, C., Cao, S., Wang, K., Lei, C., Ji, N., Xu, F., Jiang, Y., Qiu, L., Zheng, Y. (2021): Heat shock protein HSP24 is involved in the BABA-induced resistance to fungal pathogen in postharvest grapes underlying an npr1-dependent manner. – Front. Plant Sci. https://doi.org/10.3389/fpls.2021.646147.
[39] Li, M., Kim, C. (2022): Chloroplast ROS and stress signaling. – Plant Communications 3(1): 100264. https://doi.org/10.1016/j.xplc.2021.100264.
[40] Lin, Y. F., Aarts, M. G. (2012): The molecular mechanism of zinc and cadmium stress response in plants. – Cell. Mol. Life Sci. 69: 3187-3206. DOI: 10.1007/s00018-012-1089-z.
[41] Liu, H., Zhao, H., Wu, L., Liu, A., Zhao, F. J., Xu, W. (2017): Heavy metal ATPase 3 (HMA3) confers cadmium hypertolerance on the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. – New Phytologist 215(2): 687-698 https://doi.org/10.1111/nph.14622.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

[42] Liu, R., Li, J., Zhang, L., Feng, T., Zhang, Z., Zhang, B. (2021): Fungicide difenoconazole induced biochemical and developmental toxicity in wheat (*Triticum aestivum* L.). – Plants 10(11): 2304. https://doi.org/10.3390/plants10112304.
[43] Lochlainn, S., Bowen, H. C., Fray, R. G., Hammond, J. P., King, G. J., White, P. J., Graham, N. S., Broadley, M. R. (2012): Tandem quadruplication of *HMA4* in the zinc (Zn) and cadmium (Cd) hyperaccumulator *Noccaea caerulescens.* – PLoS ONE 6:e17814.
[44] Luck, H. (1974): Catalases. – In: Bregmeyer, H. U. (ed.) Methods of Enzymatic Analysis. Vol. 2. Academic Press, New York.
[45] Misra, N., Gupta, A. K., (2006): Effect of salinity and different nitrogen sources on 12the activity of antioxidant enzymes and indole alkaloid content in Catharanthus roseus seedlings. – Journal of Plant Physiology 163: 11-18.
[46] Mohamed, H. I., Akladious, S. A. (2017): Changes in antioxidants potential, secondary metabolites and plant hormones induced by different fungicides treatment in cotton plants. – Pesticide Biochemistry and Physiology 142: 117-122.
[47] Moller, J. V., Juul, B., leMaire, M. (1996): Structural organization, ion transport, and energy transduction of P-type ATPases. Biochimica et Biophysica Acta – Reviews on Biomembranes 1286: 1-51.
[48] Munro, A., Bassir, O. (1969): Oxalate in Nigerian vegetables. – W. A. Journal of Biological and Applied Chemistry 12(1): 4-8.
[49] Nakano, Y., Asada, K., (1981): Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. – Plant Cell Physiol. 22: 867-880.
[50] Narayanan, M., Kandasamy, S., He, Z., Kumarasamy, S. (2022): Ecological Impacts of Pesticides on Soil and Water Ecosystems and Its Natural Degradation Process. – In: Singh, P. et al. (eds.) Pesticides in the Natural Environment. Elsevier, Amsterdam, pp. 23-49 https://doi.org/10.1016/B978-0-323-90489-6.00002-1.
[51] Nazar, R., Iqbal, N., Masood, A., Khan, M. I. R., Syeed, S., Khan, N. A. (2012): Cadmium toxicity in plants and role of mineral nutrients in its alleviation. – American J Plant Sci. 3(10) DOI: 10.4236/ajps.2012.310178.
[52] Pasricha, S., Mathur, V., Garg, A., Lenka, S., Verma, K., Agarwal, S. (2021): Molecular mechanisms underlying heavy metal uptake, translocation and tolerance in hyperaccumulators-an analysis: heavy metal tolerance in hyperaccumulators. – Environmental Challenges 4: 100197. https://doi.org/10.1016/j.envc.2021.100197.
[53] Peiter, E., Montanini, B., Gobert, A., Pedas, P., Husted, S., Maathuis, F. J. M., Blaudez, D., Chalot, M., Sanders, D. A. (2007): Secretory pathwaylocalized cation diffusion facilitator confers plant manganese tolerance. – PNAS-USA 104: 8532-8537. DOI: 10.1073/pnas.0609507104.
[54] Petit, A. N., Fontaine, F., Vatsa, P., Clement, C., Vaillant-Gaveau, N. (2012): Fungicide impacts on photosynthesis in crop plants. – Photosynth Res. 111(3): 315-26. DOI: 10.1007/s11120-012-9719-8.
[55] Prasad, R., Shivay, Y. S. (2017): Oxalic acid/oxalates in plants: from self-defence to phytoremediation. – Current Science 112(8): 1665-1667.
[56] Qin, C., Shen, J. and Ahanger, M. A. (2022): Supplementation of nitric oxide and spermidine alleviates the nickel stress induced damage to growth, chlorophyll metabolism, and photosynthesis by up-regulating ascorbate–glutathione and glyoxalase cycle functioning in tomato. – Front. Plant Sci. 13: 1039480. DOI: 10.3389/fpls.2022.1039480.
[57] Rauser, W. E. (1999): Structure and function of metal chelators produced by plants. The case for organic acids, amino acids, phytin and metallothioneins. – Cell Biochem. Biophys. 31: 19-48. 10.1007/BF02738153.
[58] Ricachenevsky, F. K., Menguer, P. K., Sperotto, R. A., Williams, L. E., Fett, J. P. (2013): Roles of plant metal tolerance proteins (MTP) in metal storage and potential use in biofortification strategies. – Front Plant Sci. 4: 144. DOI: 10.3389/fpls.2013.00144.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

[59] Sanches-Virosta, P., Espin, S., Ruiz, S., Stauffer, J., Kanerva, M., Garcia-Fernandez, A. J., Eeva, T. (2019): Effects of calcium supplementation on oxidative status and oxidative damage in great tit nestlings inhabiting a metal-polluted area. – Environmental Research 171: 484-492.

[60] Sarwar, N., Saifullah, Malhi, S. S., Zia, M. H., Naeem, A., Bibi, S., Farid, G. (2020): Role of mineral nutrition in minimizing cadmium accumulation by plants. – J Sci Food Agric 90(6): 925-37. DOI: 10.1002/jsfa.3916.

[61] Shahid, M., Ahmed, B., Zaidi, A., Khan, M. S. (2018): Toxicity of fungicides to Pisum sativum: a study of oxidative damage, growth suppression, cellular death and morpho-anatomical changes. – RSC Adv. 8: 38483-38498 DOI: 10.1039/C8RA03923B.

[62] Shakir, S. K., Irfan, S., Akthar, B., Rehman, S. U., Daud, M. K., Taimur, N., Azizullah, A. (2018): Pesticide-induced oxidative stress and antioxidant responses in tomato (Solanum lycopersicum) seedlings. – Ecotoxicology 27(7): 919-935. DOI: 10.1007/s10646-018-1916-6.

[63] Sharma, S. S., Dietz, K. J., Mimura, T. (2016): Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. – Plant Cell Environ. 39(5): 1112-26. DOI: 10.1111/pce.12706.

[64] Shine, A. M., Shakya, V. P., Idnurm, A. (2015): Phytochelatin synthase is required for tolerating metal toxicity in a basidiomycete yeast and is a conserved factor involved in metal homeostasis in fungi. – Fungal Biol Biotechnol 2: 3. https://doi.org/10.1186/s40694-015-0013-3.

[65] Singh, N., Gupta, V. K., Kumar, A., Sharma, B. (2017): Synergistic effects of heavy metals and pesticides in living systems. – Front. Chem. https://doi.org/10.3389/fchem.2017.00070.

[66] Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. – Methods Enzymol. 299: 152-178.

[67] Skuza, L., Szucko-Kociuba, I., Filip, E., Bozek, I. (2022): Natural molecular mechanisms of plant hyperaccumulation and hypertolerance towards heavy metals. – Int. J. Mol. Sci. 23: 9335. https://doi.org/10.3390/ijms23169335.

[68] Soliman, M., Alhaithloul, H. A., Hakeem, K. R., Alharbi, B. M., El-Esawi, M., Elkelish, A. (2019): Exogenous nitric oxide mitigates nickel-induced oxidative damage in eggplant by upregulating antioxidants, osmolyte metabolism, and glyoxalase systems. – Plants 8: 562. DOI: 10.3390/plants8120562.

[69] Srivastava, V., Sarkar, A., Singh, S., Singh, P., de Araujo, A. S. F., Singh, R. P. (2017): Agroecological responses of heavy metal pollution with special emphasis on soil health and plant performances. – Front. Environ. Sci. https://doi.org/10.3389/fenvs.2017.00064.

[70] Tanaka, F., Kim, T. H., Yoneyama, T. (2001): Relationship between Oxalate Synthesis and Nitrate Reduction in Spinach (*Spinacia oleracea* L.) Plants Tracing by 13C and 15N. – In: Horst, W J. et al. (eds.) Plant Nutrition. Developments in Plant and Soil Sciences. Vol. 92. Springer, Dordrecht. https://doi.org/10.1007/0-306-47624-X_145.

[71] Tian, H., Jiang, L., Liu, E., Zhang, J., Liu, F., Peng, X. (2008): Dependence of nitrate-induced oxalate accumulation on nitrate reduction in rice leaves. – Physiol Plant. 133(2): 180-9. DOI: 10.1111/j.1399-3054.2008.01052.x.

[72] Togawa, T., Dunn, W. A., Emmons, A. C., Nagao, J., Willis, J. H. (2008): Developmental expression patterns of cuticular protein genes with the R&R Consensus from *Anopheles gambiae*. – Insect Biochem Mol Biol. 38: 508-519.

[73] Velikova, V., Yordanov, I., Edreva, A. (2000): Oxidative stress and some antioxidant systems in acid rain-treated bean plants. – Plant Sci 151: 59-66.

[74] Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., Fu, J. (2016): Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. – Front. Plant Sci. https://doi.org/10.3389/fpls.2016.00755.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387

[75] Zhang, X., Rui, H., Zhang, F., Hu, Z., Xia, Y., Shen, Z. (2018): Overexpression of a functional Vicia sativa PCS1 homolog increases cadmium tolerance and phytochelatins synthesis in *Arabidopsis*. – Front. Plant Sci. https://doi.org/10.3389/fpls.2018.00107.

[76] Zhou, C., Yang, X. B., Yang, H., Long, G. Y., Wang, Z., Jin, D. C. (2020): Effects of abiotic stress on the expression of Hsp70 genes in *Sogatella furcifera* (Horváth). – Cell Stress Chaperones 25(1): 119-131. DOI: 10.1007/s12192-019-01053-4.

[77] Zhu, S., Shi, W., Jie, Y. (2021): Overexpression of BnPCS1, a novel phytochelatin synthase gene from ramie (*Boehmeria nivea*), enhanced Cd tolerance, accumulation, and translocation in *Arabidopsis thaliana*. – Front. Plant Sci. 15 https://doi.org/10.3389/fpls.2021.639189.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_53695387