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EFFECTS OF INHIBITION THRESHOLD IN HEAVY METALS ON THE GROWTH AND DEVELOPMENT OF *ARUNDINARIA FORTUNEI*

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Abstract

Extreme amount of heavy metals and the contamination in metropolitan areas caused by industrial sediments, factory pollution, and car exhaust lead to a negative effect on ornamental plants' growth. However, small amounts of some heavy metals as nutrients may help the plants to achieve a better growth. Our research was carried out to detect the effects of copper, lead, and zinc on enzymatic activity, lipid production, soluble protein, photosynthesis properties, and plant growth indexes on *Arundinaria fortunei* in Nanjing City, east of China. For this purpose, three heavy metals (Cd, Pb, and Zn) at four concentrations (0.500, 1000, and 2000mg/kg) were chosen based on their dispersion in the district. The results showed an increase in antioxidant enzyme activity in low concentration that ultimately reveals a downward trend with excess of heavy metals; this trend continued in protein structure and led to an increase in MDA content and soluble protein. Moreover, the results obtained by photosynthesis indexes showed that the effects of heavy metals have a negative impact on photosynthesis properties. High concentrations of Cu, Pb, and Zn revealed toxicity symptoms, such as chlorosis, and reduced the plant growth indexes in *Arundinaria fortunei*, while low concentrations of heavy metals improved the plant growth. In this study, *Arundinaria fortunei* is introduced as one species that is sensitive to high doses of heavy metals, particularly to a high concentration of Pb, used to monitor the contamination of urban areas.

Key words: Contamination, Enzyme's activity, HMs, Ornamental bamboo plant, Plant growth, Photosynthesis.

Introduction

The problems caused by heavy metals for the plants are obvious (Mathur *et al.*, 2016; Ivanov *et al.*, 2016).Heavy metals known in nature can be divided into two main groups: 1-the essential heavy metals, such as Cu, Zn, Mn, and Fe that their trace is essential for plants' growth, and the plants use them as a nutrient. 2-non-essential heavy metals, such as Cd, Ag, Pb that are usually detrimental to plants' growth and cause high levels of toxicity to plants (Tangahu *et al.*, 2011; Mera *et al.*, 2016).The extreme dose of heavy metals in plants impairs the structures of plants, including substituting essential ions with a generation of free radicals and structures of proteins and enzyme activities by binding to their constituents (Das *et al.*, 2015; Singh *et al.*, 2015). The high dose of heavy metals in plants impairs the physiological processes, such as respiration-photosynthesis, and also harms the absorption of essential nutrients by the root organs, and finally leads to the production of ROS by auto-oxidation (Das *et al.*, 2015). The ROS is composed of several chemical structures, such as (O2•−), (OH•−), and hydrogen peroxide (H_2O_2) (Zouari *et al.*, 2016b; Farnese *et al.*, 2016). Having ROS released with the production of oxidative stress, a negative effect imposes on the lipids and proteins of cell membranes, permeability, electrolyte leakage, DNA, and photosynthesis properties, and finally affects the growth and development of plants (Hossain *et al.*, 2012; Blasco *et al.*, 2015 ; Zouari *et al.*, 2016a). On the other hand, plants faced with ROS activate the antioxidant defense systems, including enzyme activity superoxide dismutase (SOD), pro oxidase (POD), and catalase (CAT) (Blasco *et al.*, 2015; Nanda & Agrawal, 2016).Evaluation of these activities is one of our purposes in the present study. Ornamental plants are known as resistant plants against heavy metal stress in urban areas. Having phytoremediation properties in contaminated areas, these plants are used as one remediation method (González-Chávez Mdel & Carrillo-González, 2013). Besides beautifying the city, they play an important role in the urban environment as an indicator and inhibitor of atmospheric pollution and sewage contamination (Liu *et al.*, 2008). In this study, we chose *Arundinaria fortunei*, which is counted as a bamboo species and cultivated for the ornamental purpose in Jiangsu, Zhejiang, and other provinces in China (but, originated from Japan) (Flora of china *et al.*,____). It has several white stripes appearing on the leaves (Okamura & Tanaka, 1986). In this study, the *Arundinaria fortunei* is exposed to three well-known heavy metals (Cu, Pb, and Zn). Because of wide use and high production, these heavy metals have great increase and spread in the environment (Mera *et al.*, 2016). The purpose of this research is to survey the effects of heavy metals stress (Cu, Pb, and Zn) on enzyme activities, growth, and physiology indexes, and to evaluate the role of antioxidant defense mechanisms in alleviation of heavy metals, toxicity, and ameliorate plant growth indexes on the two-year-old ornamental bamboo species.

Material and Method

In this study, we examined the effectiveness of HMs (Cu, Pb, and Zn) in four different concentrations (0 (for control), 500mg/kg, 1000mg/kg, and 200mg/kg), by five replications for each treatment in one bamboo species ("*Arundinaria fortunei*"*).* Analysis of variance of factorial experiment based on CRD and means comparison using Turkey's test at the $p>0.05$ probability level was performed by the statistical software package R. For pre

experiments the pots (2years old bamboo species) were exposed under heavy metals stress conditions. The experiment period was 60 days. The concentrations of elements (500mg/Kg, 1000mg/Kg and 2000 mg/Kg) were applied in the form of aqueous solution including ($Cu\ SO_4\ .5H_2O$) _ ($Pb(NO_3)_2$) _ ($Zn(\ SO_4$). 7 H_2O).Weight of dry soil for pots was 0/710kg = 710 g**.** After measuring the photosynthesis and morphological indexes, the samples were moved out to the physiology laboratory of the Nanjing Forestry University for measuring the protein content and enzyme activity (free from pollution and sterilized).After removing all interior organ treatments by liquid nitrogen, preparation process was carried out using 2mg of 7.8 buffer at 7000rpm centrifuged during 10 minutes.

SOD (EC1.15.1.1) was measured using Zhang's method, (Zhang,1992) by 470nm spectrophotometer to address the photo diminution of Nitro Blue Tetrazolium (NBT).In this experiment, 0.1g/1000ml of NBT was used in reaction with 0.01g /100ml of riboflavin (Rib) and 1g/50ml of methionine MET and 2.1g/100ml of EDTA. POD (POD, E.C. 1.11.1.7) was conducted by Zhang's method (Zhang, 1992) using 4ml of 2 metroxy phenol and 0.2ml of H_20_2 in 20 micro lit.sample, and 0.8ml of ph7. This experiment was conducted based on the variations in absorbance of 470 nm wavelength exposed to the heavy metal stress spectrophotometer. The catalase CAT (EC 1.11.1.6) activity was measured according to Aebi's model, (Aebi, 1984) to evaluate catalase based on H_2O_2 catalysis at 240 nm by 1ml of Tris-HCL, 1.6ml of water and 0.1ml of sample plus 0.2 ml of H_2O_2.

In our study, experiment of MDA was conducted by Duan's method (Duan *et al.*, 2005). For this purpose, 10mL trichloroacetic acid (TCA), and 2ml of thiobarbituric acid (TBA) centrifuged at 7,000×g during 10 min. Then, the soluble was keeping in boiled water during 30min, and then centrifuged at 7,000×g during 10 min, the Soluble at 450, 532, and 600nm was measurement with a spectrometer. The final results obtained by the following formula:

$$C\ (\mu g\ g{-}1){=}6.45(A532{-}A600) - 0.56\ A450$$

The soluble protein was used to measure the changes in protein concentration in bamboo species as affected by heavy metal treatments. This protein estimation was done using Coomassie Brapt Blue G25, which is according to the procedure described by (Bradford, 1976). Photosynthesis properties were determined by photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) Powered with light sources consisting of blue-red light-emitting diodes (LI-6400-02B). The measurements were conducted at photosynthetic photon flux density (PPFD) of 1000μMm−2s−1, leaf temperature of 25°C, and constant CO2 of 380±5 μM (CO2) mol−1 in the sample chamber provided with buffer volume.

Morphological indexes include the length of shoot and the number of emerged plants. To determine the percentage of shoot length, prior to the experiment, the heights of three to four of the tallest shoots in each pot were measured, and their means were computed. After the application of HMs treatments, the heights' means of the three to four tallest shoots in each pot along with the control ones were measured. To determine the percentage of emerged plant, prior to the experiment, the number of shoots in each pot were measured. After the application of HMs treatments, the number of shoots in each pot along with the control ones was measured.

Results

Effects of HMs on SOD activity: As shown in (Table 1), under heavy metal excess, no statistical difference was found among heavy metals; however, concentrations were significantly affected ($p<0.001$). The effects of heavy metals on SOD activity in *Arundinaria fortunei* showed an upward trend in SOD levels when it was treated with 500mg/kg Cu, Pb, and Zn and then started to decline with the further increase in high concentrations of heavy metal (1000, 2000mg/kg). However, SOD activity showed an increasing trend where SOD levelsin all concentrations increased to approximately 30%, 23%, and 30% by Cu, Pb, and Zn compared with control treatment, respectively (Table 1).

Table 1. The effect of HMs (Cu, Pb, and Zn) on the SOD, POD, CAT, MDA, and soluble protein of *Arundinaria fortunei*. Each data point is the mean value ± SE of five replicates. The capital letters are the demonstration of statistical significance between different concentrations of HMs, and the small letters are the demonstration of statistical significance between different HMs in each concentration.

Heavy metal	Treatment	SOD	POD	CAT	MDA	SP
	Mg/kg	u.g.Fw	u.g.Fw	u.g.Fw	μmol.g.Fw	mg/g.Fw
Cu	0	0.18 ± 0.07 ^{Aa}	0.32 ± 0.23 ^{Aa}	0.025 ± 0.008 ^{Ba}	0.028 ± 0.009 ^{Aab}	0.039 ± 0.016 ^{Aa}
	500	0.26 ± 0.05 ^{Aa}	0.47 ± 0.12 ^{Aa}	0.046 ± 0.007 ^{Ab}	0.040 ± 0.16 ^{Aa}	0.050 ± 0.012 ^{Aa}
	1000	0.23 ± 0.04 ^{Aa}	0.35 ± 0.07 ^{Aa}	0.040 ± 0.012 ^{ABa}	0.046 ± 0.19 ^{Aa}	0.058 ± 0.007 ^{Aa}
	2000	0.20 ± 0.04 ^{Aa}	0.37 ± 0.11 ^{Aa}	0.033 ± 0.012 ^{ABa}	0.048 ± 0.16 ^{Ab}	0.061 ± 0.011 ^{Aa}
Pb	0	0.18 ± 0.03 ^{Aa}	0.30 ± 0.09 ^{Aa}	0.032 ± 0.005 ^{Ba}	0.024 ± 0.006 ^{Bb}	0.038 ± 0.006 ^{Ba}
	500	0.25 ± 0.07 ^{Aa}	1.35 ± 0.07 ^{Aa}	0.049 ± 0.001 ^{Aab}	0.028 ± 0.018 ^{ABa}	0.048 ± 0.010 ^{ABa}
	1000	0.24 ± 0.03 ^{Aa}	0.35 ± 0.17 ^{Aa}	0.050 ± 0.012 ^{Aa}	0.047 ± 0.005 ^{ABa}	0.059 ± 0.014 ^{ABa}
	2000	0.18 ± 0.05 ^{Aa}	0.34 ± 0.14 ^{Aa}	0.043 ± 0.008 ^{ABa}	0.049 ± 0.016 ^{Ab}	0.070 ± 0.015 ^{Aa}
Zn	0	0.18 ± 0.05 ^{Aa}	0.27 ± 0.16 ^{Aa}	0.025 ± 0.007 ^{Ca}	0.048 ± 0.017 ^{Ba}	0.037 ± 0.006 ^{Aa}
	500	0.25 ± 0.03 ^{Aa}	0.47 ± 0.21 ^{Aa}	0.058 ± 0.006 ^{Aa}	0.045 ± 0.017 ^{Ba}	0.042 ± 0.007 ^{Aa}
	1000	0.23 ± 0.02 ^{Aa}	0.37 ± 0.16 ^{Aa}	0.038 ± 0.001 ^{Ba}	0.049 ± 0.018 ^{Ba}	0.052 ± 0.043 ^{Aa}
	2000	0.22 ± 0.05 ^{Aa}	0.29 ± 0.11 ^{Aa}	0.031 ± 0.006 ^{BCa}	0.091 ± 0.011 ^{Aa}	0.056 ± 0.007 ^{Aa}

Effects of HMs on POD activity: According to POD results, no statistical significance was found in POD level of heavy metals; however, there was a significant difference among concentrations ($p<0.05$). Similar to the SOD response, under heavy metal stress, a conspicuous increase occurred in their POD content in low concentrations, and then it slightly decreased in high concentrations of three heavy metals. The results also showed that Cu about 1.23 fold, Pb about 1.16 fold, and Znabout 1.38 fold increase the POD levels in all three concentrations compared to the control treatment (Table 1).

Effects of HMs on CAT activity: The comparison of mean values for the effect of heavy metals (Cu, Pb, and Zn) on CAT activity showed there were significant differences among heavy metals ($p<0.05$) and also within each concentration ($p<0.001$) (Table 1), which indicate a pronounced increase in CAT activity by raising the level of Cu, Pb, and Zn. However, the amount of increase was the lowest for the Pb and the greatest for Zn compared with their control treatments (Table 1). Moreover, CAT level in all concentrations of heavy metals increased to approximately 57%, 45%, and 68% by Cu, Pb, and Zn compared to the control treatment, respectively.

Effects of HMs on MDA contents: The mean values obtained from the comparison of MDA activities exposed to different concentrations of heavy metals (Cu, Pb, and Zn) demonstrated that all heavy metals were significantly affected in each and across the concentrations ($p<0.001$). The amount of MDA for all species was increased by increasing the amounts of heavy metals (Table 1). Although the Pb had the highest level of MDA under control treatment, it exhibited the greatest increase in MDA content when heavy metal concentrations were mounted to 1000 and 2000 mg/kg. Overall, MDA amount under the Cu,Pb, and Zn, showed approximately 1.61-, 1.74-, and 1.27-fold increases under different levels of heavy metals as compared to the control treatment.

Effects of HMs on Soluble protein contents: Statistically, significant differences were found in soluble protein contents (SP) of our bamboo species when they were compared in each heavy metals ($p<0.05$) as well as across their concentrations ($p<0.001$) (Table 1). Under heavy metal stress, the greatest soluble protein contents (SP) level (0.0702 U.g.FW) was observed in the Pb at the high concentration of 2000 mg/kg, whereas the lowest soluble protein contents (SP) (0.037 U.g.FW) was detected in the Zn at the low concentration of 500 mg/kg (Table 1). Overall, a soluble protein (SP) amount under the Cu,Pb, and Zn showed approximately 1.43-, 1.55-, and 1.35-fold increases under different levels of heavy metals as compared to the control treatment.

Gas exchange parameters (photosynthesis indices): As shown in (Table 2), the results obtained from the impact of heavy metals on the gas exchange properties revealed that by increasing the concentration of Cu, Pb, and Zn, photosynthesis properties, including net photosynthetic rate (PN), conductance to H_2O (Cond), intercellular CO2 concentration (Ci), and transpiration rate (Tr) significantly reduced. The results show that a low concentration of heavy metals (500 mg/kg) had a light impact on reducing the photosynthesis properties, while high concentration of heavy metals (1000 mg/kg, 2000 mg/kg) caused highest reduction in photosynthetic properties (Table 2). Also, their amount under the Cu, Pb, and Zn showed approximately 1.27-, 1.41-, and 1.14-fold increases under different levels of heavy metals as compared to the control treatment (Table 3).

Table 2. The effect of HMs (Cu, Pb, and Zn) on gas exchange parameters (photosynthesis indices) of *Arundinaria fortunei*. Each data points shows the mean value ± SE of five replicates. The capital letters are the demonstration of statistical significance between the different concentrations of HMs, and the small letters are the demonstration of statistical significance between the different HMs in each concentration.

Heavy metal	Treatment	Net photosynthetic rate (Pn)	Conductance to H2O (Cond)	Intercellular CO_2 concentration (CI)	Transpiration rate (Trimmol)
	Mg/kg	µmol CO_2 m^{-2} s^{-1}	mol H2O m^{-2} s^{-1}	µmol CO_2 mol^{-1}	mmol H_2Om^{-2} s^{-1}
Cu	0	75.99 ±5.51 ^{Aa}	0.09 ± 0.05 ^{Aa}	41.52 ± 16.8 ^{Aa}	1.9 ± 0.41 ^{Ab}
	500	70.82 ± 1.10 ^{Aa}	0.07 ± 0.01 ^{Aa}	34.97 ± 6.67 ^{Aa}	1.8 ± 0.20 ^{Aa}
	1000	61.00 ± 1.92 ^{Bb}	0.06 ± 0.03 ^{Aa}	26.69 ± 5.28 ^{ABb}	1.5 ± 0.33 ^{Aa}
	2000	53.33 ± 2.27 ^{Cb}	0.06 ± 0.01 ^{Aa}	12.16 ± 3.89 ^{Bb}	1.4 ± 0.46 ^{Aa}
Pb	0	80.48 ± 2.95 ^{Aa}	0.14 ± 0.05 ^{Aa}	39.96 ± 7.89 ^{Aa}	2.8 ± 0.32 ^{Aa}
	500	73.34 ± 1.54 ^{Ba}	0.08 ± 0.006 ^{Ba}	33.19 ± 7.00 ^{ABa}	1.9 ± 0.37 ^{Ba}
	1000	65.93 ± 2.47 ^{Ca}	0.06 ± 0.03 ^{Ba}	22.01 ± 6.54 ^{BCb}	1.6 ± 0.46 ^{Ba}
	2000	55.71 ± 2.32 ^{Db}	0.04 ± 0.01 ^{Ba}	18.42 ± 9.63 ^{Cab}	1.6 ± 0.31 ^{Ba}
Zn	0	75.45 ± 3.03 ^{Aa}	0.12 ± 0.07 ^{Aa}	48.74 ± 11.02 ^{Aa}	2.3 ± 0.48 ^{Aab}
	500	73.35 ± 1.94 ^{Aa}	0.10 ± 0.05 ^{Aa}	48.23 ± 13.67 ^{Aa}	2.1 ± 0.33 ^{Aa}
	1000	67.58 ± 1.65 ^{Ba}	0.09 ± 0.02 ^{Aa}	36.16 ± 4. 66 ^{ABa}	2.05 ± 0.25 ^{Aa}
	2000	61.33 ± 2.30 ^{Ca}	0.07 ± 0.03 ^{Aa}	28.41 ± 4.66 ^{Ba}	2.03 ± 0.32 ^{Aa}

Table 3.The percentages of photosynthesis properties reduction in three heavy metals at all concentrations compared to their control treatments.

Heavy metal	Pn (%)	Cond (%)	Ci (%)	Trimmol (%)	Total (%)
Cu	18.8	29.89	46.18	14.92	27.44
Pb	19.24	55.78	54.4	37.98	41.85
Zn	10.64	24.79	13.11	9.21	14.43

Table 4. The effect of HMs (Cu, Pb, and Zn) on the percentage of shoot length and emerge plants of *Arundinaria fortunei*. Each data points shows the mean value ± SE of five replicates. The capital letters are the demonstration of statistical significance between the different concentrations of HMs and the small letters are the demonstration of statistical significance between the different HMs in each concentration.

Heavy metal	Treatment	Percentage of shoot plants	Percentage of emerge plants
	Mg/kg	(%)	(%)
Cu	0	15.83 ± 9.44 ^{ABa}	177.83 ± 32.35 ^{ABa}
	500	29.69 ± 9.81 ^{Aa}	196.84 ± 49.56 ^{Aa}
	1000	20.11 ± 9.62 ^{ABa}	154.91 ± 22.60 ^{ABa}
	2000	10.75 ± 3.45 ^{Ba}	130.06 ± 34.66 ^{Ba}
Pb	0	16.47 ± 8.69 ^{Aa}	178.35 ± 50.55 ^{Aa}
	500	16.37 ± 7.95 ^{Aa}	197.92 ± 36.50 ^{Aa}
	1000	10.24 ± 6.57 ^{Aa}	134.45 ± 26.72 ^{Aa}
	2000	7.10 ± 2.94 ^{Aa}	129.31 ± 55.55 ^{Aa}
Zn	0	15.96 ± 4.66 ^{Aa}	118.95 ± 22.80 ^{ABa}
	500	21.97 ± 7.10 ^{Aa}	154.32 ± 42.88 ^{Aa}
	1000	17.32 ± 4.29 ^{Aa}	115.88 ± 42.05 ^{ABa}
	2000	12.98 ± 4.42 ^{Aa}	76.55 ± 24.99 ^{Ba}

Effects of HMs on the plant growth (percentage of shoot length and percentage of emerge plants): Considering the trend depicted in (Table 4), shoot length and emerge plants in the bamboo species exposed to heavy metal excess increased when they received 500kg/mg of this HM; however, when the heavy metal excess mounted to the extreme concentrations of 1000 and 2000 kg/mg, a conspicuous decrease was observed in the growth indexes. Consequently, there was a slight change in the growth of shoot length and emerge plants when plants were treated with 500 kg/mg of Pb concentrations, but ultimately, it underwent a pronounced decline as the concentration of the HMs was increased to 2000 kg/mg, under which the lowest values were recorded for this trait in all heavy metals. The mean values obtained from the comparison for the effect of heavy metal excess on shoot length of three bamboo species showed that the heavy metals were significantly affected in each ($p<0.001$) and across ($p<0.05$) their concentrations (Table 4). As shown in (Table 4), there are statistical significant differences in the percentage of emerged plants in our bamboo species in each and across Cu concentrations ($p<0.001$) (Table 4). The results obtained by effects of HMs (Cu, Pb, Zn) on plant growth indexes (percentage of shoot length and percentage of emerge plants) in *Arundinaria fortunei* revealed that, compared with control treatments, the percentage of the plants' growth mainly increased with low concentration (500mg/kg) of HMs (Cu, Pb, and Zn), and then significantly decreased with the excess of HMs (1000 and 2000mg/kg) (Table 4). This reduction was shown in copper, lead, and Zn by 32%, 47%, and 18% of shoot length, and 19%, 26%, and 19% of emerge plant (Table 4). Moreover, an increase in low concentration was shown in copper, lead, and Zn by 57%, 0%, and 23% of shoot length and 10%, 10%, and 29% of emerge plant.

Discussion

In order to make the plants to cope with ROS caused by the extreme dose of heavy metals, the antioxidant mechanisms, such as superoxide dismutase SOD (EC1.15.1.1) per oxidases (POD, EC 1.11.1.7) or catalase (CAT, EC 1.11.1.6), were used (Zouari *et al.*, 2016b). Where the SOD is the frontline of plant mechanisms in dealing with the increase of ROS in a wide range of intracellular organs (mitochondria to cytosol), which could be the decomposition superoxide radical to oxygen molecular and H_2O_2 (Nanda & Agrawal, 2016). H_2O_2 scavenging was conducted by per-oxidases (POD) and catalase (CAT), (Sun *et al.*, 2010; Cvjetko *et al.*, 2010) and the H_2O_2 decomposition to water and oxygen was conducted by catalase (CAT, EC 1.11.1.6) (Srivastava *et al.*, 2006).This trend was also found in our results and indicated an upward trend in the antioxidant activities of SOD, POD, and CAT encountering with different concentrations of heavy metal stress (Cu, Pb, and Zn) compared with their control. However, the increase in low concentrations (500mg/kg) was higher than the high concentrations (1000-2000mg/kg).It showed that the degrade of antioxidant enzyme effectiveness encountered an excess of heavy metal concentrations which can be the plant threshold point in coping with oxidative stress. However, depending on different genetic environments, it is different in various plants (Hall, 2002). The results observed in numerous research works acknowledge our finding (Jabeen *et al.*, 2016; He *et al.*, 2016; Malar *et al.*, 2014a,b). On the other hand, the ROS caused by the excess of heavy metals leads to bio-membrane deterioration by lipid production (Das *et al.*, 2015), and consequently leads to an increase in malondialdehyde (MDA), which can be counted as one of the cell membrane lipid productions – (Zouari *et al.*, 2016b; Liu *et al.*, 2014). The results obtained by our finding showed that MDA content increased with the excess of heavy metals. That cause of this decline can be a reduction of antioxidant efficiency. This issue was confirmed by several other studies (Hassan & Mansoor, 2014, 2017; Liu *et al.*, 2008; Belkhadi *et al.*, 2010). Moreover, ROS with a dysfunctional amino acid chain and a generation of carbonyl groups (Chen *et al.*, 2013) and also a cross-link between MDA and protein (intra molecular and intermolecular) (Liu *et al.*, 2014) causes a disorder in soluble protein structures, which led to an increase in soluble protein contents in our study. Much research has pointed to heavy metal cases to inhibit the net photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency (Brown & Wells, 1990; Zaheer *et al.*, 2015; Kanwal *et al.*, 2014; Dong *et al.*, 2005; Zahoor *et al.*, 2018). The results of the current study indicated a downward trend with excess of heavy metals on gas exchange properties, including net photosynthetic rate (PN), conductance to H2O (Cond), intercellular CO2 concentration (Ci), and transpiration rate (Tr) that could be more intense in high concentrations and nonessential heavy metal (Pb) and get slighter in low concentrations. Excess of heavy metals disturbs the photosynthesis properties; for instance, the chlorophyll synthesis impairs the synthesis of enzyme

activities and also the reduction ratio of chlorophyll (a/b) and their balance (Mera *et al.,* 2016), and after affecting the electron transport system, leads to a reduction in the efficiency of reaction center or light harvesting complex (LHC) PS II (Babu *et al.,* 2010; Linger *et al.,* 2005). It is observed in our experiment that the reduction of antioxidant effectiveness in excess of heavy metals causes most photosynthesis properties to decrease so that it decreases the growth indexes. The results obtained by our data analyses indicate that the enhancement of heavy metals concentrations (1000mg/kg-2000mg/kg) significantly reduced the growth indexes, such as percentage of shoot length and percentage of emerge plants which could be directly attributed to a reduction in photosynthesis caused by antioxidant deficiency. Numerous studies reported that high concentration of heavy metals (by Cu (Chai *et al.,* 2014; Jain *et al.,* 2010;Vidaković-Cifrek *et al.,* 2015), pb (Xu *et al.,* 2009; Malar *et al.,* 2014b), and Zn (Jain *et al.,* 2010; Michael & Krishnaswamy, 2011) leads to a reduction in the growth indexes that approve our finding. On the other hand, we noticed that low concentrations of heavy metals could further improve the plant growth in our bamboo species (*Arundinaria fortunei*), which revealed the role of the essential ions in plant nutrition although this amount in Pb was negligible. The previous studies have confirmed the growth acceleration at low concentrations of heavy metals (Aydinalp & Marinova, 2009; Fatnassi *et al.,* 2015).

Conclusion

Ornamental plants in urban areas could be used as a biomarker of heavy metals toxicity where higher concentrations of heavy metals may be alarming for the human environment and food chain. On the other hand, ornamental plants with phytoremediation properties could act as biodefense against the excess of heavy metals and prevent the entry of heavy metals into the human life cycle. The results of our study for *Arundinaria fortunei* showed that the concentration of 500mg/kg at three heavy metals of Cu, Pb, and Zn could be the inhibition threshold of heavy metals on plant growth. The development and excess of heavy metal concentration (1000, 2000) can be a toxicity warning for environment and impair the biology process in *Arundinaria fortunei* as one of the contamination biomarkers in the area. The results indicated that Pb has considerable toxic effects on plant growth as compared to other two heavy metals (Cu and Zn). It is also showed that Cu and Zn in low concentration could act as nutrients in plant growth.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

Aebi, H. 1984. Catalase *In vitro*. *Methods Enzymol*., 105: 121.

Aydinalp, C. and S. Marinova. 2009. The effects of heavy metals on seed germination and plant growth on alfaalfa plant (*Medicago Sativa*). *BJAS*., 15: 347-350.

Babu, N.G., P.A. Sarma, I.H. Attitalla and S.D.S. Murthy. 2010. Effect of selected heavy metal ions on the photosynthetic electron transport and energy transfer in the thylakoid membrane of the cyanobacterium, spirulina platensis. *AJPS*., 3: 46-49.

Belkhadi, A., H. Hediji, Z. Abbes, I. Nouairi, Z. Barhoumi, M. Zarrouk, W. Chaïbi and W. Djebali. 2010. Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum* L. *Ecotoxicol Environ Saf*., 73: 1004-1011.

Blasco, B., N.S. Graham and M.R. Broadley. 2015. Antioxidant response and carboxylate metabolism in Brassica rapa exposed to different external Zn, Ca, and Mg supply. *J Plant Physiol*., 176: 16-24.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*., 72: 248.

Brown, D.H and J.M. Wells. 1990. Physiological effects of heavy metals on the moss *Rhytidiadelphus squarrosus*. *Ann Bot*., 66: 641-647.

Chai, M., F. Shi, R. Li, G. Qiu, F. Liu and L. Liu. 2014. Growth and physiological responses to copper stress in a halophyte spartina alterniflora (Poaceae).*Acta Physiol. Plant*., 36: 745-754.

Chen, Y.P., Q. Liu, X.Z. Yue, Z.W. Meng and J. Liang. 2013. Ultrasonic vibration seeds showed improved resistance to cadmium and lead in wheat seedling. *Environ Sci. Pollut Res Int*., 20: 4807-4816.

Cvjetko, P., S. Tolić, S. Sikić, B. Balen, M. Tkalec, Z. Vidaković-Cifrekand and M. Pavlica. 2010. Effect of copper on the toxicity and genotoxicity of cadmium in duckweed (*Lemna minor* L.). *Arh. Hig. Rada. Toksikol*., 61: 287-96.

Das, S.K., J.K. Patra and H. Thatoi. 2015. Antioxidative response to abiotic and biotic stresses in mangrove plants: A review. *Int. Rev. hydrobiol*., 101: 3-19.

Dong, J., F.B. Wu and G.P. Zhang. 2005. Effect of cadmium on growth and photosynthesis of tomato seedlings. *J. Zhejiang Univ. Sci. B*., 6: 974-980.

Duan, B., Y. Lu, C. Yin, O. Junttila and C. Li. 2005. Physiological response to drought and shade in two contrasting *Picea asperata* population. *Physiol. Plant*., 124: 476.

Farnese, F.S., P.E. Menezes-Silva, G.S. Gusman and J.A. Oliveira. 2016. When bad guys become good ones: the key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. *Front Plant Sci*., 7: 471.

Fatnassi, I.C., M. Chiboub, O. Saadani, M. Jebara and S.H. Jebara. 2015. Impact of dual inoculation with *Rhizobium* and PGPR on growth and antioxidant status of *Vicia faba* L. under copper stress. *C.R. Biol*., 338: 241-254.

Flora of china (FOC) Vol. 22. Pp. 121-126. http://www.efloras.org/

González-Chávez Mdel, C and R. Carrillo-González. 2013. Tolerance of Chrysantemum maximum to heavy metals: The potential for its use in the revegetation of tailings heaps. *J. Environ. Sci*., 25: 367-75.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot*., 53: 1-11.

Hassan, M. and S. Mansoor. 2014.Oxidative stress and antioxidant defense mechanism in mung bean seedlings after lead and cadmium treatments. *Turk. J. Agri. For*., 38: 55-61.

Hassan, M. and S. Mansoor. 2017. Priming seeds with phytohormones alleviates cadmium toxicity in mung bean (*Vigna radiata* L. Wilczek) seedlings. *Pak. J. Bot*., 49(6): 2071-2078.

He, J., Z. XJi, Q.Z. Wang, C.F, Liu and Y.B. Zhou. 2016. Effect of Cu and Pb pollution on the growth and antionxidant enzyme activity of *Suaeda heteroptera*. *Ecol. Eng*., 87: 102-109.

Hossain, M.A., P. Piyatida, J.A. Teixeira da Silva and M. Fujita. 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and Methylglyoxal and in heavy metal chelation. *J. Bot*., 872875: 37.

Ivanov, Y.V., A.V. Kartashov, A.I. Ivanova, Y.V. Savochkin and W. Kuznetsov. 2016. Effects of zinc on Scots pine (*Pinus sylvestris* L.) seedlings grown in hydroculture. *Plant Physiol. Biochem*., 102: 1-9.

Jabeen, N., Z. Abbas, M. Iqbal, M. Rizwan, A. Jabbar, M. Farid, S. Ali, M. Ibrahim and F. Abbas. 2016. Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. *Arch. Acker PFL Boden*., 62: 648-662.

Jain, R., S. Srivastava, S. Solomon, A.K. Shrivastava and A. Chandra. 2010. Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (*Saccharum* spp.). *Acta Physiol. Plant*., 32: 979-986.

Kanwal, U., S. Ali, M.B. Shakoor, M. Farid, S. Hussain, T. Yasmeen, M. Adrees, S.A. Bharwana and F. Abbas. 2014. EDTA ameliorates phytoextraction of lead and plant growth by reducing morphological and biochemical injuries in *Brassica napus* L. under lead stress. *Environ Sci. Pollut Res. Int*., 21: 9899-9910.

Linger, P., A. Ostwald and J. Haensler. 2005. Cannabissativa, growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. *Bio Plantarum.*, 49: 567-576.

Liu, D., T.Q. Li, X.E. Yang, E. Islam, X.F. Jin and Q. Mahmood. 2008. Effect of Pb on leaf antioxidant enzyme activities and ultrastructure of the two ecotypes of *Sedum alfredii* Hance. *Russ. J. Plant Physiol*., 55: 68-76.

Liu, J.J., Z. Wei and J.H. Li. 2014. Effects of copper on leaf membrane structure and root activity of maize seedling. *Bot. Stud*., 55: 47.

Liu, J.N., Q.X. Zhou, T. Sun, L.Q. Ma and S. Wang. 2008. Growth responses of three ornamental plants to Cd and Cd-Pb stress and their metal accumulation characteristics. *J. Hazard. Mater*., 151: 261-267.

Malar, S., R. Manikandan, P.J. Favas, S.S. Vikram and P. Venkatachalam. 2014b. Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in *Sesbania grandiflora*: a potential plant for phytoremediation. *Ecotoxicol. Environ. Saf*., 108: 249-257.

Malar, S., S.S. Vikram, P.J. C. Favas and V. Perumal. 2014a. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Bot. Stud*., 55: 54.

Mathur, S., H.M. Kalaji and A. Jajoo. 2016.Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. *Photosynthetica*., 54: 185-192.

Mera, R., E. Torres and J. Abalde. 2016. Influence of sulphate on the reduction of cadmium toxicity in the microalga *Chlamydomonas moewusii*. *Ecotoxicol. Environ. Saf*., 128: 236-245.

Michael, P.I. and M. Krishnaswamy. 2011. The effect of zinc stress combined with high irradiance stress on membrane damage and antioxidative response in bean seedlings. *Environ. Exp. Bot*., 74: 171-177.

Nanda, R. and V. Agrawal. 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.

Okamura, H. and Y. Tanaka. 1986. The horticultural bamboo species in Japan. The description of horticultural bamboo species. Hata Okamura. Japan.

Singh, V., B.N. Tripathi and V. Sharma. 2015. Interaction of Mg with heavy metals (Cu, Cd) in *T. aestivum* with special reference to oxidative and proline metabolism. *J. Plant Res*., 129: 487-497.

Srivastava, S., S. Mishra, R.D. Tripathi, S. Dwivedi and D.K.Gupta. 2006. Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. *Aquat. Toxicol*., 80: 405-415.

Sun, B.Y., S.H. Kan, Y.Z. Zhang, S.H. Deng, J. Wu, H. Yuan, H. Qi, G. Yang, L. Li, X.H. Zhang, H. Xiao, Y.J. Wang, H. Peng and Y.W. Li. 2010. Certain antioxidant enzymes and lipid peroxidation of radish (*Raphanus sativus* L.) as early warning biomarkers of soil copper exposure. *J. Hazard. Mater*., 183: 833-8 .

Tangahu, B.V., S.R. Sheikh Abdullah, H. Basri, M. Idris, N. Anuar and M. Mukhlisin. 2011. A review on heavy metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. *Int. J. Chem. Eng*., 939163: 31.

Vidaković-Cifrek, Ž., M. Tkalec, S. Šikić, S. Tolić, H. Lepeduš and B. Pevalek-Kozlina. 2015. Growth and photosynthetic responses of *Lemna minor* L. exposed to cadmium in combination with zinc or copper. *Arh. Hig. Rada. Toksikol*., 66: 141-52 .

Xu, Z., Q. Zhou and W. Liu. 2009. Joint effects of cadmium and lead on seedlings of four Chinese cabbage cultivars in northeastern China. *J. Environ. Sci.*, 21: 1598-1606.

Zaheer, I.E., S. Ali, M. Rizwan, M. Farid, M.B. Shakoor, R.A.Gill, U. Najeeb, N. Iqbal and R. Ahmad. 2015. Citric acid assisted phytoremediation of copper by *Brassica napus* L, *Ecotoxicol. Environ. Saf.*, 120: 310-317.

Zahoor, A., F. Ahmad, M. Hameed and S.M.A. Basra. 2018. Structural and functional aspects of photosynthetic response in *Eichhornia crassipes* (Mart.) Solms under cadmium stress. *Pak. J. Bot*., 50(2): 489-493.

Zhang, X.Z. 1992. The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In: Zhang XZ (ed) research methodology of crop physiology. *Beijing Agriculture Press*. 208.

Zouari, M., C. Ben Ahmed, N. Elloumi, K. Bellassoued, D. Delmail, P. Labrousse, F. Ben Abdallah and B. Ben Rouina. 2016a. 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.

Zouari, M., C.H. Ben Ahmed, W. Zorrig, N. Elloumi, M. Rabhi, D. Delmail, B. Ben Rouina, P. Labrousse and F. Ben Abdallah. 2016b. 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.

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