African Journal of Biotechnology Vol. 11(47), pp. 10717-10725, 12 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB12.973
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Heavy metal induced ecophysiological function alterations in the euhalophyte *Suaeda salsa*

Zhaoxiang HAN[1,2,3]*, Jiaying LI[4], Dandan WU[2] and Chunxia LÜ[5]

[1]Chongqing Key Laboratory of Micro/Nano Materials Engineering and Technology, Chongqing City, 402160, China.
[2]School of Chemical Engineering, Huaihai Institute of Technology, Lianyungang City, Jiangsu,222005, China.
[3]Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection, Yancheng City, Jiangsu, 224002, China.
[4]Department of Chemistry and applied Chemistry, Changji University, Changji City, Xinjiang, 831100, China.
[5]Jiaonan Agricultural Bureau, Qingdao, Shandong, 266400, China.

Accepted 11 May, 2012

Heavy metal accumulation affects the physiological status of plants. *Suaeda salsa* L. is used to investigate the toxic effects of cadmium (Cd) and lead (Pb) either alone or mixtures under the static test conditions. Cd-Pb mixture exposure can decrease lignin content and weaken the increase. Mitochondrial calcium content significantly reduced at 30 µM Cd and Pb exposure. Cd-Pb mixture exposure can increase calcium content under the same concentration exposure. Soluble sugar levels noted a significant decrease in Cd, Pb and Cd-Pb mixture exposure. The accumulations of Cd, Pb in *S. salsa* were significantly increased with exposure time. Soluble protein (SP) in *S. salsa* at 30 µM concentration treatments decreased with exposure time. Heat shock protein 70 (HSP70) was enhanced lightly along with the increase of added Cd-Pb from 30 to 70 µM and then decreased below the controls which present a synergistic effect. Heat shock protein 60 (HSP60) increased slightly with the increase of Cd-Pb from 30 to 110 µM, and then decreased hereafter and significantly inhibited at 150 µM ($p<0.05$). Moreover, Cd-Pb mixture exposure significantly increased the Rubisco activity under lower concentration and presented antagonistic effect. At the same time, the viability percent decreased as increase Cd-Pb concentration exposure ($p<0.05$), it presents a dose-dependent manner. Mitochondrial cells treated with Cd-Pb exposure obviously reduced the reactive oxygen species (ROS) levels in mitochondrial cells.

Key words: *Suaeda salsa*, heavy metal, ecophysiological function.

INTRODUCTION

Heavy metals are environmental pollutants released from both industrial and agricultural sources affecting the biosphere in many places worldwide. Among them, cadmium (Cd), a non essential element present in the atmosphere, soil and water, is one of the most aggressive and persistent elements in natural environment. Accumulation of Cd in plant tissues elicits symptoms ranging from growth reduction, wilting, and chlorosis to cell death (Benavides and Gallego, 2005; Finger-Teixeira et al., 2010). Lead (Pb) is one of the most useful and toxic metals present in the environment on a global scale (Arshad et al., 2008; Uzu et al., 2009). When exposed to this metal, even at low concentrations, plants usually experience harmful effects such as micronucleus induction (National Toxicology Program, 2003), DNA damage (Gichner et al., 2008), alterations in membrane permeability (Sharma and Dubey, 2005) and inhibition or activation of enzymatic activities (Reddy et al., 2005) along with various physiological impacts. Pb has an enhancing effect on the accumulation of Cd by plants (Carlson and Rolfe, 1979; Lai and Chen, 2006). In this

*Corresponding author. E-mail: hjyyzx@sina.com. Tel: +86 518 85895409.

study, they were applied to artificially Cd, Pb and Cd–Pb-spiked soils that were planted with *S. salsa* L. to study the effect of Cd, Pb and Cd–Pb on ecophysiological function.

MATERIALS AND METHODS

Seed, soil sample collection, experiment design and plant growth

S. salsa L. is a succulent euhalophytic herb, which occurs both on saline soil and in the intertidal zone. The experiment was design according to our previous study.

Measurement of lignin and calcium content

After the incubation period, dry roots (0.5 g) were homogenized in 50 mM potassium phosphate buffer (7 ml, pH 7.0) with a mortar and pestle and transferred to a centrifuge tube. The pellet was centrifuged (1400 g, 4 min) and washed by successive stirring and centrifugation as follows: twice with phosphate buffer pH 7.0 (7 ml); 3 × with 1% (v/v) Tritons X-100 in pH 7.0 buffer (7 ml); 2 × with 1 M NaCl in pH 7.0 buffer (7 ml); 2 × with distilled water (7 ml); and 2 × with acetone (5 ml).The pellet was dried in an oven (60°C, 24 h) and cooled in a vacuum desiccator. The pellet was dried at 60°C, dissolved in 0.5 M NaOH, and diluted to yield an appropriate absorbance for spectrophotometric determination at 280 nm. Lignin was expressed as mg $LTGAg^{-1}$ DW. The mitochondria were isolated by the method of Cain and Skilleter (1987).

Assay of sugars and soluble proteins content

Sugars were estimated following modified protocol of Xu and Zhou (2007). The soluble protein concentrations of tissues were determined by the pierce method using bicinchoninic acid according to Smith et al. (1985) with an Uptima kit.

Determination of heat shock protein 70 (HSP70) and heat shock protein 60 (HSP60)

Total protein was extracted in 0.1 M Tris-HCl, pH 8.0, 10% (v/v) glycerol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% (v/v) TritonX-100, 2 mM DTT, 1mM phenylmethylsulphonyl fluoride (PMSF) at 4°C according to the method of Wang et al. (2011).

Measurement of rubisco activity, cell viability and ROS activity

The activity of rubisco was measured following the method of Sato et al. (1980). The reaction mixture for the assay volume of 3 ml consisted of 50 mM Tris-HCl buffer (pH7.8), 10 mM $NaHCO_3$, 5 mM $MgCl_2$,2 Mm NADH,1 mM ATP,1 mM DTT, 0.5 mM RuBP,5 U glyceralde-hyde-3-phosphodehydrogenase and 5 U phosphoglycerate kinase. The change in absorbance was measured at 340 nm (Bhupinder et al., 2011). The effect of Cd, Pb on cell viability was determined by using the MTT assay. Cells were treated with Cd, Pb at 30, 70, 110 and 150 µM, respectively. Twenty-four hours later, 50 µl of MTT stock solution (2 mg/ml) was added and after 4 h incubation, the absorbance at 540 nm was then measured on a scanning multi-well spectrophotometer (Carmichael et al., 1987). Mitochondrial ROS measurement: Cells were seeded in a 96 well plate at 2×10^4 cells/well. Sixteen hours (16 h) later, the cells were treated with Cd, Pb at 30, 70, 110 and 150 µM. Cells were incubated for an additional 30 min at 37°C. After addition of 20 µM of DHR 123 solution for 10 min, the fluorescence was detected using flow cytometer (Kyung et al., 2011).

RESULTS AND DISCUSSION

Effects of Cd and Pb on lignin and calcium content

As a consequence of Cd, Pb exposure, lignin content increased from 17.8, 20.6, 22.8 and 24.8% to 150.2, 166.5, 200.3 and 251.5% after 30 to 150 µM treatment with respect to control, respectively. However, lignin content increased from 19.2 to 155.5% after 30 to 150 µM Cd-Pb treatment with respect to the control, respectively (Figure 1). Cd-Pb mixture exposure can decrease lignin content and Cd-Pb mixture exposure weakens the increase and present antagonistic effect. It is ascertained that lignin content has been already affected by Cd or Pb. Moreover, the reduction in root growth has been considered one of the first effects of the Cd, Pb associated with lignin production and related parameters (Yang et al., 2007; Finger-Teixeira et al., 2010). As described earlier, the biosynthesis of lignin involves the polymerization of monolignols primarily derived from the phenylpropanoid pathway, which commences with the deamination of phenylalanine by PAL to form cinnamate, followed by the other derivatives (Ferrarese et al., 2002; Ferrer-Teixeira et al., 2008).

Based on the preliminary study results, 30 µM of Cd, Pb exposure concentration and 120 h of exposure time were chosen as standard procedure for the measurements on mitochondrial calcium contents. The effect of Cd, Pb on *S. salsa* was determined by analysis of mitochondrial calcium contents because heavy metal as, Cd and Pb can cause significant alteration of mitochondrial Ca^{2+}-ATPase activity (Aline Finger-Teixeira et al., 2010; Sangita and Subhra, 2011). As expected, *S. salsa* mitochondrial calcium content was found significantly reduced (0.50 ± 0.02, 0.70 ± 0.03, $p<0.05$) at 30 µM Cd and Pb exposure, compared to the control (1.50 ± 0.06) respectively. Cd-Pb mixture exposure can increase calcium content under the same concentration exposure and was found to restore this level significantly (1.13 ± 0.04, $p<0.05$) (Figure 2). Ca^{2+}, Mg^{2+}-ATPase is chiefly associated in the uptake and exchange of different ions. It was also reported that inhibition of heavy metal as, Cd and Pb exposure could lead to the impairment of uptake and transport of vital ions, which in turn may be responsible for heavy metal toxicity (Mukherjee et al., 1992; Bansal and Murthy , 1985).

Effects of Cd and Pb on sugars and soluble proteins content

Production of total soluble sugars was severely affected in Cd, Pb exposed *S. salsa* compared to the control. That is to say, soluble sugar levels noted a significant decrease in Cd and Pb exposed *S. salsa* (Figure 3). The reduction in

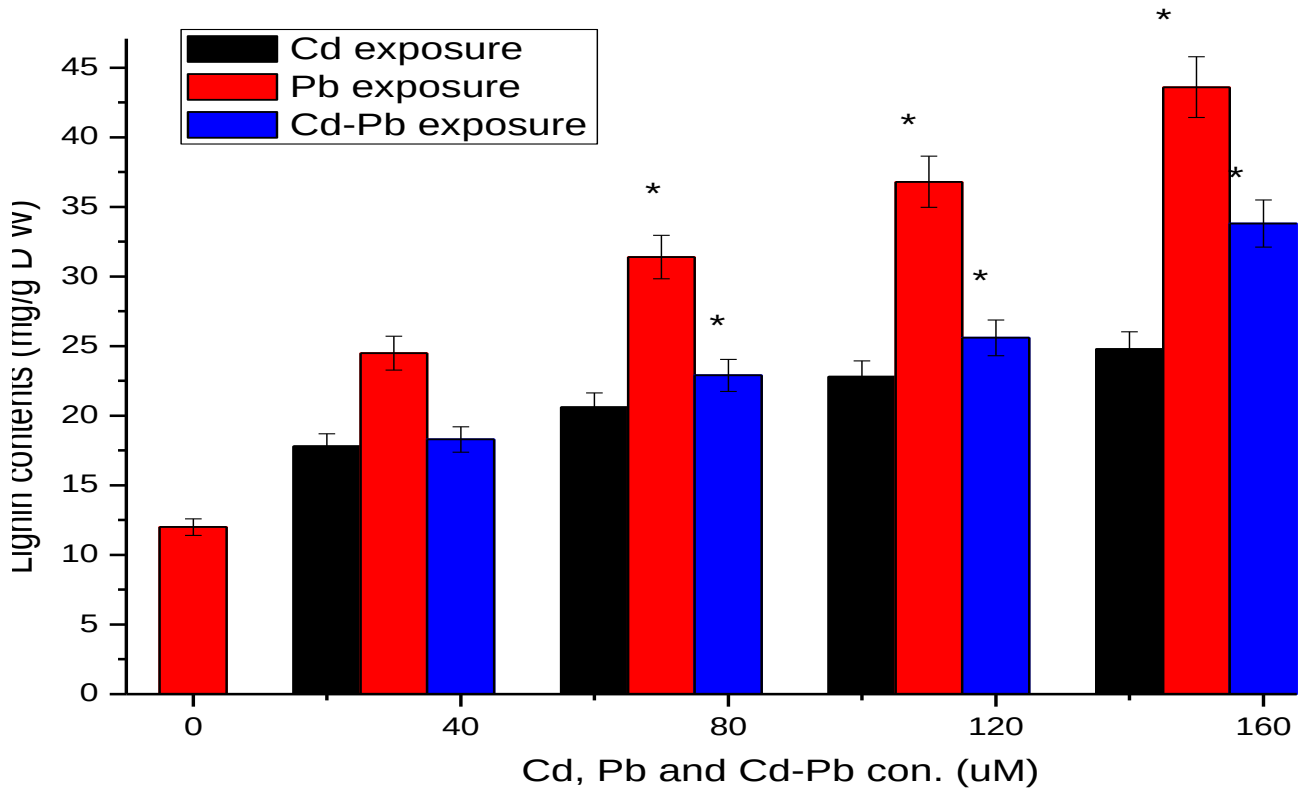

Figure 1. Effects of Cd, Pb, Cd-Pb on lignin contents. * $p<0.05$.

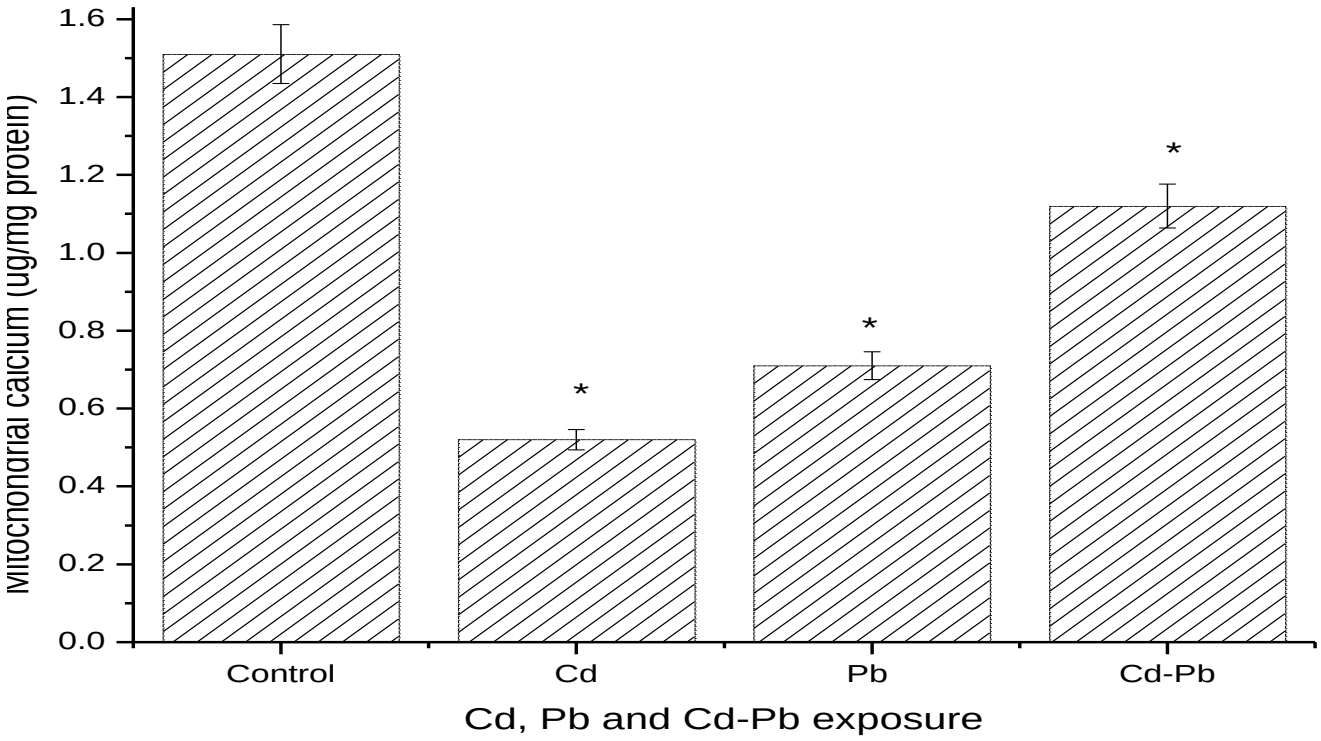

Figure 2. Cd, Pb, Cd-Pb-induced changes in mitochondrial calcium content. * $p<0.05$.

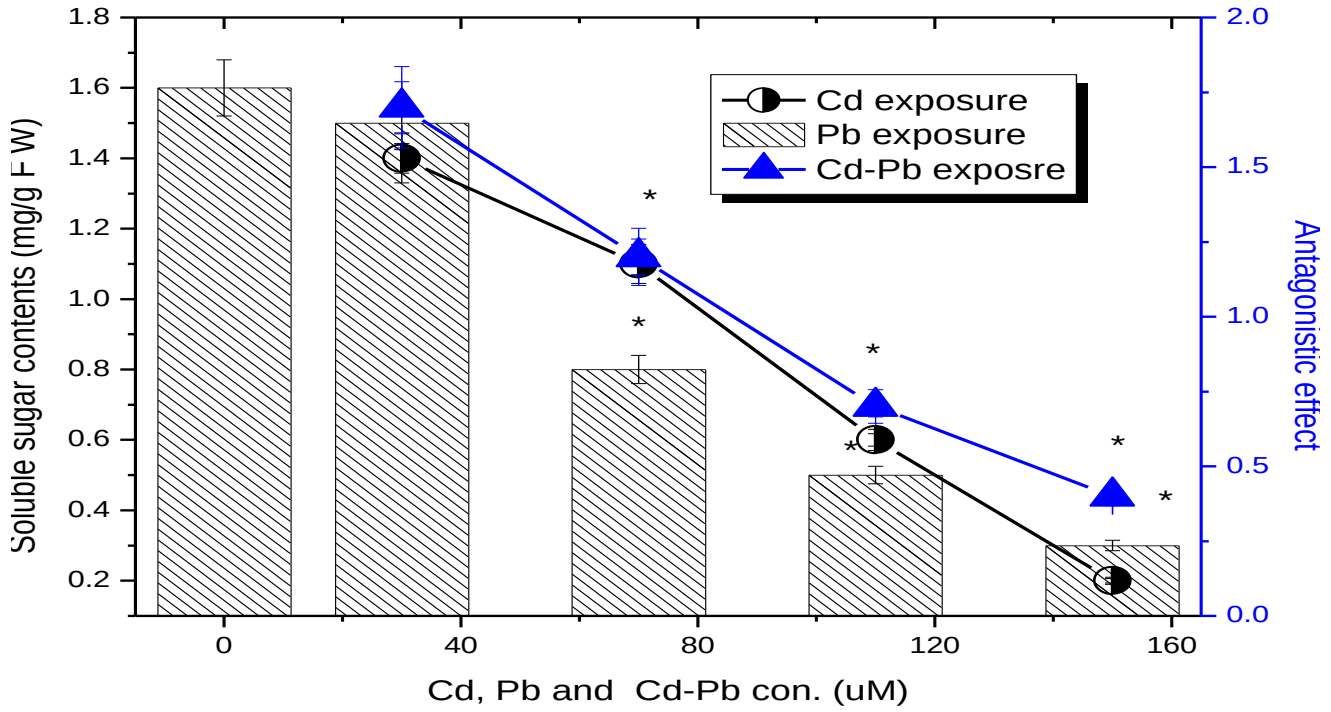

Figure 3. Effects of Cd, Pb, Cd-Pb on sugars* $p<0.05$.

carbohydrate production might be the result of decline in photosynthetic capacity (Hou et al., 2007) or reduced photochemical activities (John et al., 2008; Mishra and Tripathi, 2008; Xing et al., 2010). Moreover, heavy metal Cd-Pb mixture exposure significantly decreased production of total soluble sugars as increased concentration and presented antagonistic effect (Figure 3). In order to operate the experiment easily, we selected 30 µM (Cd, Pb) as the standard exposure concentration for this experiment, respectively. The accumulation of Cd, Pb in *S. salsa* significantly increased with exposure time.

Soluble protein (SP) in *S. salsa* at 30 µM concentration treatments decreased with exposure time (Figure 4). Pb has a synergistic effect on enhancing the accumulation of Cd by *Brassica rapa* grown in artificially Cd-contaminated soils (Chen et al., 2010). The coexistence of Pb in Cd-contaminated soil promoted the accumulation of Cd of the shoots of *B.rapa*. The Cd concentration in the shoot of *B. rapa* grown in Cd-Pb was higher (11.9±2.5 mg/kg) compared with that in Cd. The relative increase was more significant (2.8 fold) in the Cd-Pb treatment soil because of higher plant uptake of Cd from soil (Chen et al., 2010). The existence of Pb in Cd contaminated soil raises the Cd concentration in the soil solution, increases the availability of Cd, and thus promotes its uptake by plants. The synergistic effect of soil Pb on the accumulation of Cd by plants in this experiment was similar to those observed in previous studies (Carlson et al., 1979; Wong et al. 1996; Lai and Chen, 2006; Chen et al., 2010).

Effect of Cd and Pb on HSP70 and HSP60 content

HSP70 and HSP60 productions were investigated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. Figure 5 displays HSP70 and HSP60 levels obtained after simultaneous exposure of Cd, Pb. Cd and Pb caused induction of HSP70 and HSP60 in all mitochondrial cells which is indicated by the calculated regressions. HSP70 increased with the increase of the added Cd from 30 to 110 µM and declined thereafter owing to HSP70 inactivation by Cd toxicity at high concentration exposure, HSP70 increased with the increase of the added Pb from 30 to 150 µM after 120 min exposure. However, HSP70 enhanced lightly along with the increase of added Cd-Pb from 30 to 70 µM, and then decreased below the controls and present synergistic effect (Figure 5A). Significant increase in HSP60 was found in all treatments compared to the controls after 120 h Cd, Pb exposure ($p<0.05$). HSP60 increased slightly with the increase of Cd-Pb from 30 to 110 µM, and then decreased hereafter and was significantly inhibited at 150 µM ($p<0.05$) (Figure 5B). HSPs are present in small amounts in non-stressed cells on a constitutive basis with important functions in normal cellular processes and protein housekeeping, for instance as chaperones. Adaptation of HSPs reflecting past exposure histories can result in both changes in constitutive levels, and in the inducible response, as found for *S. salsa* tested in our study which showed an

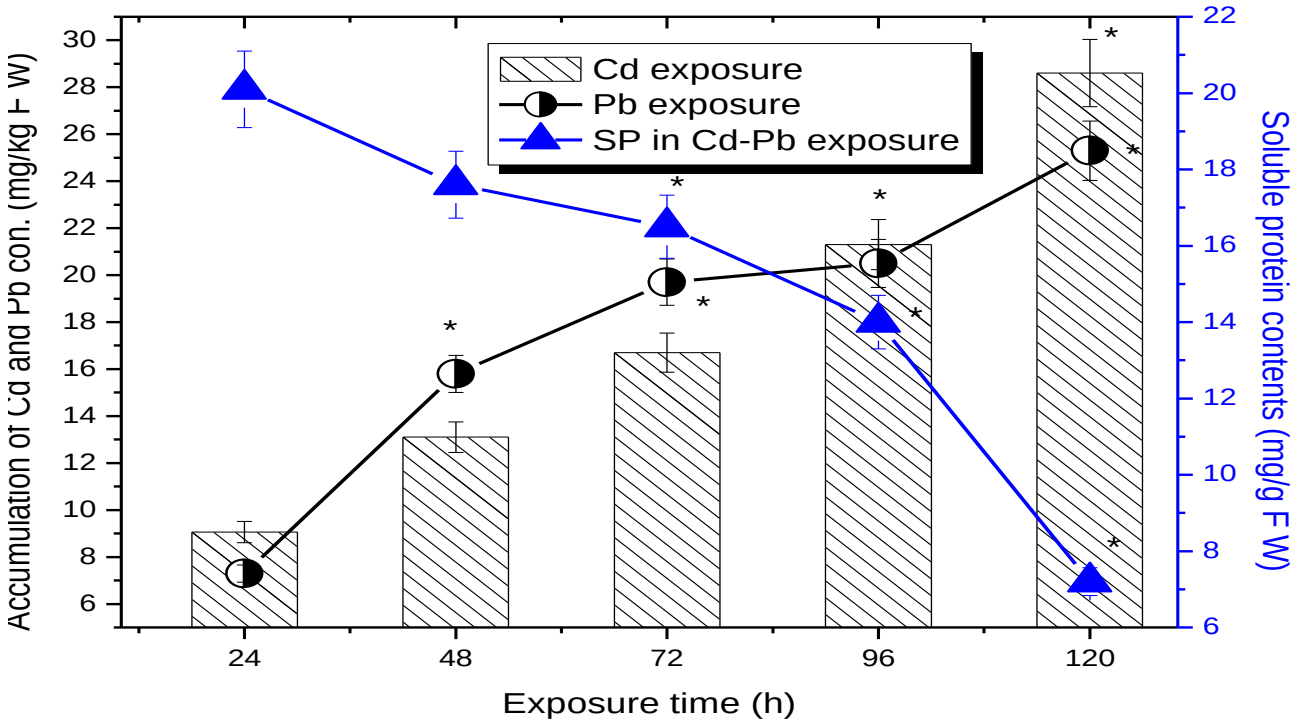

Figure 4. Accumulation of Cd, Pb and soluble protein content. * $p<0.05$.

inverse relationship between Cd, Pb sensitivity HSP70 and HSP60 levels.

Chankova et al. (2009) and Stefan et al. (2010), showed that heat pretreatment resulted in overproduction of HSP70 and HSP90. There are reports that a short heat stress pretreatment induced thermotolerance in Chlorella or tolerance to cadmium in cell-suspension cultures of *Lycopersicon peruvianum* (Neumann et al., 1994; Tukaj and Tukaj, 2010). Heat shock protein 70 (HSP70) was induced sensitively by soil Pb, which was to alleviate oxidative damage (Wang et al., 2008).Other recent proteomic studies include induction of heat-shock (HSP60) in response to combined exposures of arsenic and heavy metals in *Plationus pattulus* (Rios-Arana and Alberti, 2005). Proteins were involved in sulfur metabolism in Arabidopsis thaliana roots by Cd (Sun and Zhou, 2008) and induction of cysteine synthase in response to aluminum stress in rice (Yang et al., 2007; Walliwalagedara and Atkinson, 2010). We know that the HSP70 proteins are induced by different toxins, including metals and the chaperoning capacity of two main classes of stress proteins, HSP70 and HSP60, which both are involved in protein folding, transmembrane passage and restoration ((Heinz-R. KÖhler et al. 2005).

Effects of Cd and Pb on rubisco activity, cell viability and ROS activity

S. salsa exposed to heavy metals Cd, Pb depicted an inhibition in rubisco activity. A significant decline of about 42 and 49% was noted in Cd, Pb (30 µM) and the inhibition rate decreased about 56 and 61% in Cd, Pb (150 µM) compared with the control, respectively and that heavy metal Cd-Pb mixture exposure significantly increased the rubisco activity under lower concentration and presented antagonistic effect. But, the rubisco activity declined after exposure at 70 µM Cd-Pb coexistence shown synergistic effects until 150 µM Cd-Pb (Figure 6). The carbon fixation potential was severely affected in metal exposed plants (Chen et al., 2010). The loss of rubisco activity results from deactivation of the enzyme. The substitution of Mg, Co, Zn and Cu by metal ions in the rubisco complex causes loss of carbon fixation potential (Van Assche and Clijsters, 1990; Chen et al., 2010). Similar responses have been reported earlier in few aquatic species (Pietrini et al., 2003; Al-Hamdani and Blair, 2004; Hou et al., 2007; Chen et al., 2010).

The cell viability at different concentrations (30, 70, 110 and 150 µM) of Cd, Pb were assessed by using the MTT test. The results (Figure 7) found that the effects of Cd, Pb on viability of mitochondrial cells have significant differences under different concentration ($P<0.05$), the cell viability was 49.5, 43.8% at 30 µM, 42.3, 37.5% at 70 µM, 40.7, 33.1% at 110 µM, 35.4, 31.8% at 150 µM of Cd, Pb compared to control, respectively. However, the viability percent decreased by 47.1, 40.8, 32.5 and 28.6% compared to the control levels when treated with 30, 70, 110 and 150 µM for Cd-Pb, respectively ($P<0.05$). That is

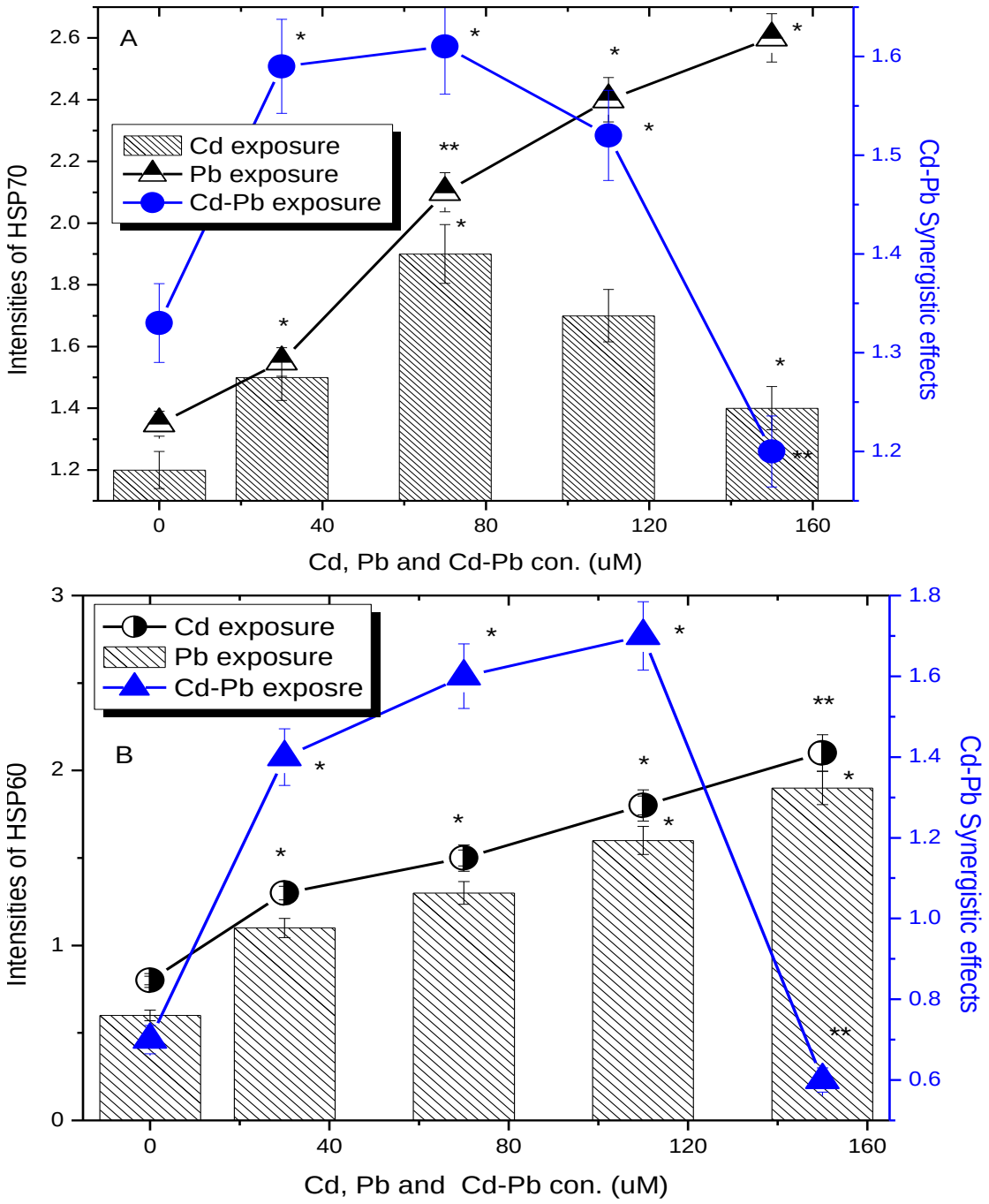

Figure 5. Effects of Cd, Pb, Cd-Pb on HSP70 (A) and HSP60 (B),* $p<0.05$, ** $p<0.001$.

to say, the viability percent decreased with the increase of Cd-Pb concentration exposure ($p<0.05$), it presents a dose-dependent manner. Pollen viability was tested as a potential indicator of pollution and strictly related to Pb and Cd levels in leaves. Presented data indicate that soybean cells grown in culture are very sensitive to cadmium ions, The Cd^{2+} concentrations tested actually caused the decrease of cell viability, The Cd and Pb induced stimulation of growth is correlated with the increased cell viability (Calzoni and Antognoni, 2007; Sobkowiak and Deckert, 2003) and lead has a synergistic effect on enhancing the accumulation of Cd by *B. rapa* grown in artificially Cd-contaminated soils (Chen et al., 2010).

The intensity of fluorescence intensity is directly proportional to the level of ROS in the cells, to determine

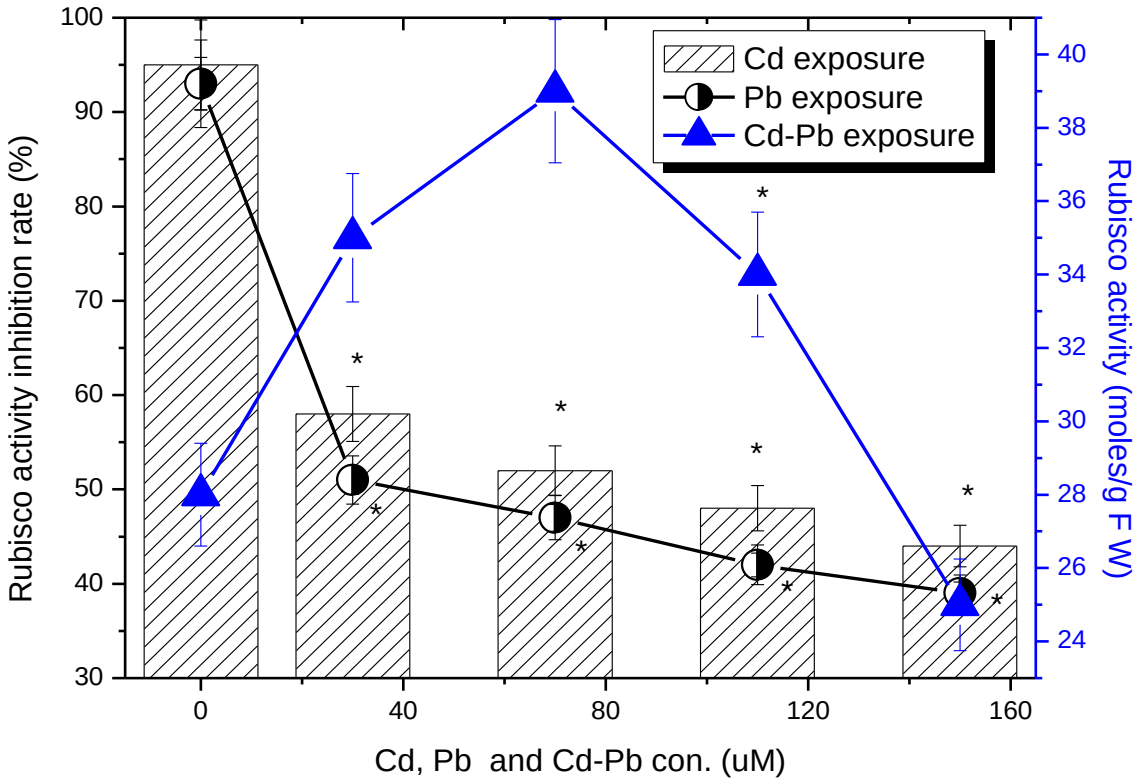

Figure 6. Effects of Cd, Pb, Cd-Pb on Rubisco activity.* $p<0.05$.

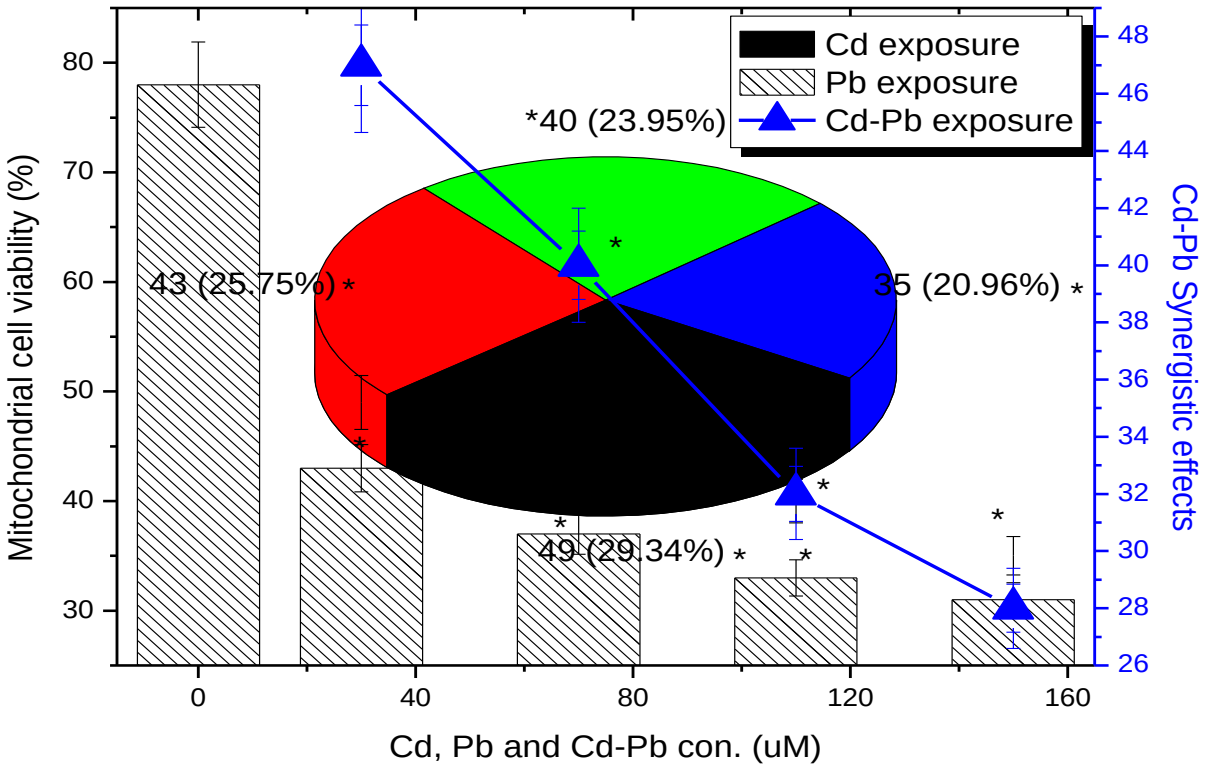

Figure 7. Effects of Cd, Pb, Cd-Pb on cells viability.* $p<0.05$.

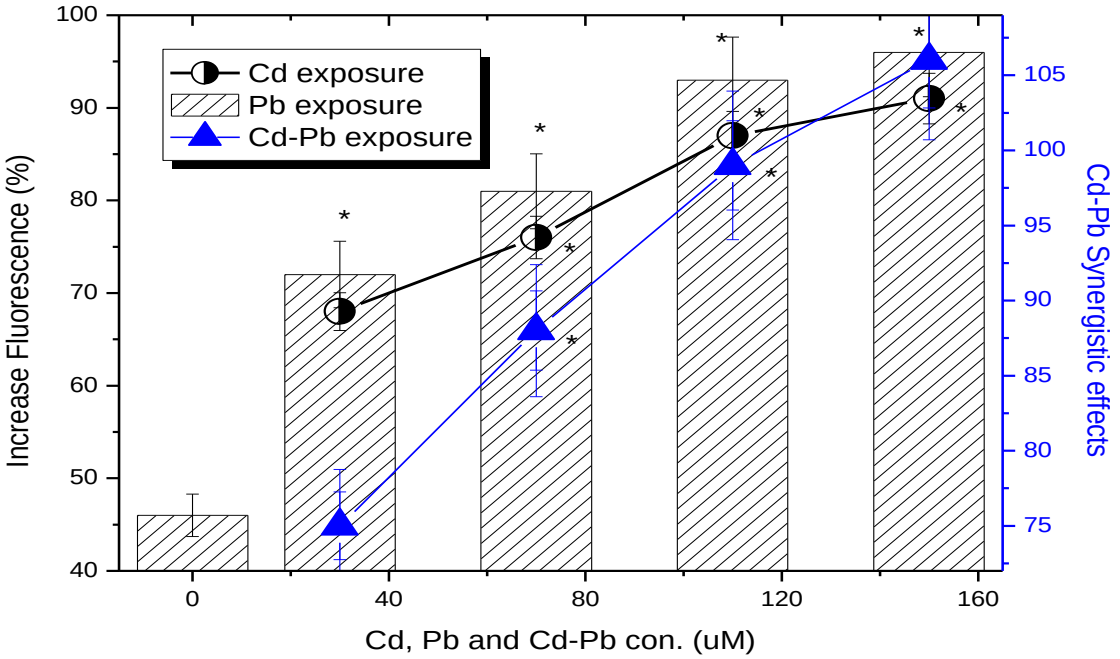

Figure 8. Effects of Cd, Pb, Cd-Pb on ROS generation.* $p<0.05$.

whether or not Cd, Pb could induce ROS generation. The fluorescence dye DHR 123 was used to detect mitochondrial ROS in cells after Cd, Pb treatment at 30, 70, 110 μM, and 150 μM (Figure 8). Mitochondrial cells exposed to Cd, Pb for 120 min displayed a significant increase in the mitochondrial level of ROS relative to the levels seen in control cells. But, mitochondrial cells pretreated with 30, 70, 110 μM, and 150 μM Cd-Pb exposure obviously reduced the ROS levels in mitochondrial cells and 70 to 150 μM of measurements statistically significantly increased when compared to the controls in the dose-response curves for Cd-Pb exposure. Linear regression analysis showed highly significant dose-response patterns for Cd-Pb exposure with p-values ($p<0.05$). Heavy metal (Cd, Cu, and Pb) can enhance toxicity which will lead to increase in metal biosorption and ROS generation (Piotrowska-Niczyporuk and Bajguz, 2011; Wang et al., 2011).

Conclusion

The present study reveals that the application of Cd, Pb, and Cd-Pb to *S. salsa* causes the following effects: Cd-Pb mixture exposure decrease lignin content and Cd-Pb mixture exposure weakens the increase and presents antagonistic effect. Mitochondrial calcium content significantly reduced at 30 μM Cd and Pb exposure compared to the control. Cd-Pb mixture exposure can increase calcium content under the same concentration exposure. Soluble sugar levels noted a significant decrease in Cd, Pb and Cd-Pb mixture exposure. The accumulations of Cd, Pb in S. salsa were significantly increased with exposure time. Soluble protein (SP) in *S. salsa* at 30 μM concentration treatments decreased with exposure time.

HSP70 enhanced lightly along with the increase of added Cd-Pb from 30 to 70 μM and then decreased below the controls and present synergistic effect. Significant increases in HSP60 in all treatments compared to the controls after 120 h Cd, Pb exposure ($p<0.05$). HSP60 increased slightly with the increase of Cd-Pb from 30 to 110 μM and then decreased hereafter and was significantly inhibited at 150 μM ($p<0.05$). Cd-Pb mixture exposure significantly enhanced the rubisco activity under lower concentration and presented antagonistic effect. But, the rubisco activity declined after exposure at 70 μM Cd-Pb coexistence shown synergistic effects until 150 μM Cd-Pb. The viability percent decreased as increase Cd-Pb concentration exposure ($p<0.05$), it presents a dose-dependent manner. Mitochondrial cells pretreated with Cd-Pb exposure obviously reduced the ROS levels in mitochondrial cells.

ACKNOWLEDGEMENTS

This research was supported by Chongqing Key Laboratory of Micro/Nano Materials Engineering and Technology (No.KFJJ1106), Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection (No.JLCBE11004), and Jiangsu Marine Resources Development Research Institute (No.JSIMR10E04).

REFERENCES

Aline Finger-Teixeira, Maria de Lourdes LF, Anderson RS (2010). Cadmiu-induced lignification restricts soybean root growth. Ecotoxicol. Environ. Saf., 73: 1959-1964.

Arshad M, Silvestre J, Pinelli E, Kallerhoff J, Kaemmerer M (2008). A field study of lead phytoextraction by various scented Pelargonium cultivars. Chemosphere, 71: 2187-2192.

Al-Hamdani SH, Blair SL (2004). Influence of copper on selected physiological responses in Salvinia minima and its potential use in copper removal. Am. Fern. J., 94: 47-56.

Piotrowska-Niczyporuk A, Bajguz A (2011). Phytohormones as regulators of heavy metal biosorption and toxicity in green alga Chlorella vulgaris (*Chlorophyceae*). Plant Physiol. Biochem., 11: 23-34.

Benavides MP, Gallego SM (2005). Cadmium toxicity in plants. Braz. J. Plant Physiol., 17: 21-34.

Bhupinder D, Sharmila P, Pardha PS (2011). Heavy metal induced physiological alterations in *Salvinia natans*. Ecotoxicol. Environ. Saf., 74: 1678-1684.

Cain K, Skilleter DN (1987). Preparation and use of mitochondria in toxicological research Biochemical Toxicology. Oxford: IRL Press: 217-254.

Carlson RW, Bazzaz FA (1979). Growth reduction in American sycamore (*Plantanus occidentalis* L.) caused by Pb–Cd interaction. Environ. Pollut., 12: 243-253.

Wang ChG, Gu XY, Wang XR (2011). Stress response and potential biomarkers in spinach (*Spinacia oleracea* L.) seedlings exposed to soil lead. Ecotoxicol. Environ. Saf., 74: 41-47.

Carmichael J, DeGraff WG, Gazdar AF (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. Cancer Res., 47: 936-941.

Chankova SG, Yurinab NP, Dimova EG, Ermohina OV, Oleskina (2009). Pretreatment with heat does not affect double-strand breaks DNA rejoin in gin *Chlamydomonas reinhardtii*. J. Therm. Biol., 34: 332-336.

Wang ChG, Wang XR (2008). Oxidative stress and potential biomarkers in tomato seedlings subjected to soil lead contamination. Ecotoxicol. Environ. Saf., 71: 685-691.

Walliwalagedara C, Atkinson I (2010). Differential expression of proteins induced by lead in the Dwarf Sunflower *Helianthus annuus*. Phytochemistry, 71: 1460-1465.

Ferrarese MLL, Zottis A, Ferrarese-Filho O (2002). Protein-free lignin quantification in soybean (*Glycine max*) roots. Biologia, 57: 541-543.

Ferrer JL, Austin MB, Stewart Jr C, Noel JP (2008). Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiol. Biochem., 46: 356-370.

Gichner T, Znidar I, Szakova (2008). Evaluation of DNA damage and mutagenicity induced by lead in tobacco plants. Mutat. Res., 652: 86-190.

Calzoni GL, Antognoni F (2007). Active biomonitoring of heavy metal pollution using Rosa rugosa plants. Environ. Pollut., 149: 239-245.

Heinz-R. Köhler, Gerd Alberti, Stanisaaw Seniczak, Anna Seniczak (2005). Lead-induced hsp70 and hsp60 pattern transformation and leg malformation during postembryonic development in the oribatid mite, Archegozetes longisetosus Aoki. Comp. Biochem. Physiol. Part C, 141: 398-405.

Hou W, Chen X, Song G, Wang Q (2007).Excessofcopperand cadmium on heavy metal polluted water body restoration by duckweed (*Lemna minor*). PlantPhysiol. Biochem., 45: 62-69.

Chen HL, Lai HY, Wang SM (2010). Effect of Biosolids and Cd/Pb Interaction on the Growth and Cd Accumulation of *Brassica rapa* Grown in Cd-Contaminated Soils. Water Air Soil Pollut., 206: 385-394.

In Kyung L, Kyoung AK, Zhang R (2011). Mitochondria protection of baicalein against oxidative damage via induction of manganese superoxide dismutase. Environ. Toxicol. Pharmacol., 31: 233-241.

John R, Ahmad P, Gadgil K, Sharma S (2008). Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ., 54: 262-270.

Lai HY, Chen ZS (2006). The influence of EDTA application on the interactions of cadmium, zinc, and lead and their uptake of rainbow pink. J. Hazard. Mater., 137: 1710-1718.

Mukherjee S, Upreti RK, Tekwani BL (1992). Biochemical analysis of jejunal brush border membrane of golden hamster: pathogenic modulations due to ancylostomiasis. Indian J. Biochem. Biophys., 29: 82-86.

Mishra VK, Tripathi BD (2008). Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresour. Technol., 99: 7091-7097.

National Toxicology Program (2003). Report on carcinogens: background document for lead and lead compounds. Department of Health and Human Services, Research Triangle Park, NC.

Neumann D, Lichtenberger OG¨ unther D, Tschiersch K, Nover L (1994). Heat-shock proteins induce heavy-metal tolerance in higher plants. Planta, 94: 360-367.

Pietrini F, Iannelli MA, Pasqualini S, Massacci A (2003). Interactionofcadmium with glutathione and photosynthesis in developing leaves and chloroplasts of Phragmites australis *Trin.ExSteudel*. Plant Physiol., 133: 829-837.

Reddy AM, Kumar SG, Jyothsnakumari G, Thimmanaik S (2005). Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflo*rum and bengalgram. Chemosphere, 60: 97-104.

Rios A, Alberti G (2005). Lead-induced hsp70 and hsp60 pattern transformation and leg malformation during postembryonic development in the oribatid mite, Archegozetes longisetosus Aoki. Comp. Biochem. Physiol. Part C., 141: 398-405.

Sobkowiak R, Deckert J (2003). Cadmium-induced changes in growth and cell cycle gene expression in suspension-culture cells of soybean. Plant Physiol. Biochem., 41: 767-772.

Sharma P, Dubey RS (2005). Lead toxicity in plants. Braz. J. Plant Physiol., 17: 35-52.

Sato FK, Nishida K, Yamada Y (1980). Activities of carboxylation enzymes and products of $^{14}CO_2$ fixation in photoautotrophically cultured cells. Plant Sci. Lett., 20: 91-97.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem., 150: 76-85.

Sangita M, Subhra K (2011). Arsenic-induced hepatic mitochondrial toxicity in rats and its amelioration by dietary phosphate. Environ. Toxicol. Pharmacol., 31: 107-118.

Bansal SK, Murthy RC (1985). The effects of some divalent metals on cardiac and branchial Ca^{2+}-ATPase in a freshwater fish *Saccobranchus fossilis*. Ecotoxicol. Environ. Saf., 9(3): 373-377.

Shen H, Lee YK (1997). Thermo tolerance induced by heatshock in Chlorella. J. Appl. Phycol., 9: 471-475.

Stefan T, Zbigniew T (2010). Distinct chemical contaminants induce the synthesis of Hsp70 proteins in green microalgae Desmodesmus subspicatus: Heat pretreatment increases cadmium resistance. J. Therm. Biol., 35: 239-244.

Sun FH, Zhou QX (2008). Oxidative stress biomarkers of the polychaete Nereis diversi color exposed to Cd and petroleum hydrocarbons. Ecotoxicol. Environ. Saf., 70: 106-114.

Uzu G, Sobanska S, Aliouane Y (2009). Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation. Environ. Poll., 157: 1178-1185.

Van Assche F, Clijsters H (1990). Effects of metals on enzyme activity in plants. Plant Cell Environ., 13: 195-206.

Wong JWC, Li GX, Wong MH (1996). The growth of *Brassica chinensis* in heavy metal contaminated sewage sludge compost from Hong Kong. Bioresour. Technol., 58: 309-313.

Xu ZZ, Zhou GS (2007). Photosynthetic recovery of a perennial grass Leymus chinensis after different periods of soild rought. Plant Prod. Sci., 10: 277-285.

Xing W, Huang W, Liu G (2010). Effect of excess iron and copper on physiology of aquatic plant *Spirodela polyrrhiza* (L.). Schleid. Environ. Toxicol., 25: 103-112.

Yang YJ, Cheng LM, Liu ZH (2007). Rapid effect of cadmium on lignin biosynthesis in soybean roots. Plant Sci., 172: 632-639.

Yang Q, Wang Y, Zhang J, Shi W (2007). Identification of aluminu responsive proteins in rice roots by a proteomic approach: cysteine synthase as a key player in Al response. Proteomics, 7: 737-749.