Physiological mechanisms of some aquatic plants to tolerate lead element pollution in water.

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ABSTRACT:

Lead (Pb) heavy metal pollution in waterways has become one of the major issues across the world. A hydroponics experiment was aimed at investigating of the physiological and the biochemical responses and phytoremediation ability of three aquatic floating macrophytes, *Eichhornia crassipes*, *Pistia stratiotes* and *Ludwigia stolonifera,* plants grown under different treatments of Pb (CH_3COO). With an increase in Pb concentration in growth medium, the element's accumulation in plant parts increased and the accumulation of the element increased in the roots more the shoots for all tested plants. For studying the response of these plants to Pb stress, we observed inhibited plant growth% and decreased photosynthetic pigments, membrane stability index (MSI) and total proteins. In addition, heavy metal induced oxidative damage as observed by increased lipid peroxidation (MDA) and electrolyte leakage (EL) levels in all species. Antioxidative enzymes activity such as catalase (CAT) and peroxidase (POX) and proline accumulation were positively correlated with Pb treatment. The plants were ideal for phytoremediation because of their rapid rate of growth, extensive root system, high biomass output, and capacity to accumulate and tolerate Pb.

Keywords*:* Aquatic plants; Pb accumulation; tolerant mechanisms; antioxidant enzymes; proline.

INTRODUCTION

Growing industrialization besides accelerating urbanisation and economic development in recent decades are regarded to be the primary causes of the worsening the environment quality and heavy metal contamination (Khalid *et al.*, 2020). In contrast to soil contamination, the water cycle allows water pollution to spread quickly and globally. Artificial pesticides and plastics were two particularly dangerous anthropogenic water contaminants (Bell *et al.*, 2019). Due to rapid population expansion and the completion of the Grand Ethiopian Renaissance Dam on the Nile River Egypt's biggest challenge going forward will be water scarcity. Therefore, it is crucial to manage the available water resources particularly wastewater cleanup and reuse in order to combat the challenges associated with the expected drought (Abdelhafez *et al.*, 2020). Heavy metals are metallic elements with comparatively high atomic weight and density ranging 4.0 - 5.0 g/cm3 and are non-degradable. Therefore, they are poisonous to a greater extent to plants, animals, and humans (Farid *et al.*, 2019). Among heavy metals, lead (Pb) is a significant environmental contaminant and a highly poisonous metal whose extensive use has adversely affected public health (Jaishankar *et al.*, 2014). According to (Kamran *et al.*, 2015) the amount of Pb in aquatic ecosystems at 4 million tonnes. Lead levels in water, soil, and plants are typically < 0.2 µg/l, 20 mg/kg, and 1 µg/g, respectively (Afaj *et al.*, 2017). However, primarily as a result of human activity, its levels occasionally exceed these limits.

Phytoremediation is the use of plants and their associated soil and water conditions, agronomic techniques and rhizospheric microorganisms to remove organic and inorganic contaminants in soil and water without endangering the environment (Mojiri *et al.*, 2021). Researchers' interest in the potential for employing plants in environmental remediation has grown over the last few years and the technique has started to take off as an alternative to restoring contaminated sites (Oh *et al.*, 2014). Utilizing native plants is essential for phytoremediation because they considerably outperform introduced plants from different environments in terms of endurance, development, and reproduction under environmental stress (Galal *et al.*, 2018). Hyperaccumulating plant has the innate ability to flourish in environments where other plants would typically be harmed by high metal concentrations in the soil or water. They are able to tolerate high metal concentrations in their tissues without showing any harmful effects (Rascio and Navari-Izzo, 2011). The majority of aquatic plants exhibit varying capacities for survive in relatively high-contaminated water and function as an efficient natural filter for various metals and dangerous contaminants (Sharma *et al.*, 2015). Aquatic plants always have enormous root systems making them prime candidates for the accumulation of contaminants in their roots and shoots (Stoltz and Greger, 2002). Particularly, floating plants have a significant capacity to ingest, translocate, and stabilize a variety of hazardous metals in their harvestable parts (Rahman and Hasegawa, 2011). A variety of floating plants, including *E. crassipes*, *P. stratiotes* and *L. stolonifera*, have demonstrated their ability to remove metals from different kinds of wastewaters (Galal and Farahat, 2015; Eid and Shaltout, 2016; Galal *et al.*, 2020). *E. crassipes* in the irrigation canals of the Nile Delta have the capacity in just 16 days to double their biomass (Eid and Shaltout, 2017). According to Liao and Chang (2004) *E. crassipes* is capable of absorb and translocate many of heavy metals like the cadmium, lead, copper and zinc in the plant's tissue as a root or shoot. Furthermore, it is 3 to 15 times better to locate the elements into the roots rather than the shoots. According to many research, *P. stratiotes* has the ability to remove heavy metals as Zn, Mn, Fe, Cd, Pb, Cu, As, Cr, and Ni (Kumar *et al.*, 2019; Kumar *et al.*, 2019). Although the lead had accumulated at higher concentrations primarily in the root system in this plant it had shown various patterns of lead elimination (Singh *et al.*, 2011). *L. stolonifera* is an invasive macrophyte with a rapid rate of growth and reproduction and is thought to be an effective living species for phytoextraction of a range of metals and is playing an important role in removing various pollutants from the aquatic environment (Saleh *et al.*, 2017). Furthermore, it has been demonstrated that 2 g of *L. stolonifera* can absorb and translocate more than 95 percent of radiocobalt and 65 percent of radio-cesium from radioactive waste solution, as well as eliminate up to 65 percent, 97 percent, and 99 percent of Cd, Cr, and Pb respectively (Saleh *et al.*, 2019).

Pb exposure even at low concentrations prevents the growth of plant parts (Kopittke *et al.*, 2007). However, roots exhibit a larger Pb-induced growth inhibition than other plant components, which may be connected to the increased lead content of the roots (Liu *et al.*, 2008). Nevertheless, Pb-induced suppression of plant development may be related to a decline in photosynthesis, plant water relations, and nutrient metabolism (Rady *et al.*, 2021). Pb is a non-redox active chemical alters this redox equilibrium via a number of indirect ways including the replacement of crucial cations in cellular macromolecules and modulation the activities of some enzymes and finally led to enhanced the generation of ROS (Chen *et al.*, 2016). By scavenging ROS antioxidant defense mechanisms work in concert to protect plant cells from oxidative damage and prevent cascades of uncontrolled oxidation (Gill and Tuteja, 2010). Higher levels of resistance to abiotic stressors are positively associated with the expression of several antioxidant enzymes (Caverzan *et al.*, 2016). According to the research, plant pigments adversely affected from heavy metals. The levels of carotenoids and chlorophyll of plants changes when plants are exposed to heavy metals (Houri *et al.*, 2020). Pb inhibit chlorophyll synthesis may be due to increased chlorophyllase activity (Drazkiewicz, 1994). Although large quantities of lead may reduce the protein pool, its impact on the total protein is unknown (Piotrowska *et al.*, 2009). However, under lead stress some amino acids such proline increase (Qureshi *et al.*, 2007).

The goals of the current investigation were to: (1) compare and assess tolerance and accumulation of Pb in these three macrophyte species in hydroponics after 21 days of treatment (2) evaluate the physiological response and mechanisms of tolerance of these species to Pb stress.

MATERIALS AND METHODS

Experimental set-up and Pb exposure

Eichhornia crassipes, Pistia stratiotes and Ludwigia stolonifera plants were used for this study. The plants were collected and acclimatized in a tank for 10 days containing tap water. All species were grown in plastic bowl containing 10% Hoagland nutrient solution (20 L) (Hoagland and Arnon, 1950) with continuous aeration. The pH of the nutrient solution was 5.8. Plants were treated with different concentrations of Pb $(CH_3COO)_2$ (5, 10, 15 mg/L) for 21 days, while plants without Pb treatment were used as a control and every 7 days solutions were refreshed. The plants were grown in hydroponics with three uniform plants of each species in each container. For each treatment, triplicates were maintained. Root and shoot samples were used for Pb content analysis.

Pb estimation in plant samples

After 21 days of treatment, the plants were thoroughly washed with 20 mM Na_2-EDTA for 15 min to desorb putatively surface adsorbed Pb then washed three times with distilled water, oven-dried at 70 ℃ till constant weight, milled and sieved to < 1 mm. To determine the content of Pb^{+2} , we used the modified digestion method of (Chapman and Pratt, 1978). Determination of heavy metal (Pb) contents in plant samples were carried out by graphite furnace Atomic Absorption Spectrometer. Amount of heavy metal in different samples of plant was calculated using dilution factor.

Metal (mg/ g DW) in plant = metal reading of digested sample (mg L^{-1}) × dilution factor.

The bio-concentration factor (BCF) was calculated as follows (Rezania *et al.*, 2016):

BCF = metal concentration in plant (mg/Kg)/ metal concentration in medium (mg/ L)

Translocation factor (TF) is defined as the ratio of metals concentration in the shoots (mg kg^{-1}) to that in the roots (mg kg^{-1}). It shows the ability of a plant to translocate metals from its roots to the shoots (Yoon *et al.*, 2006).

Growth (%)

Biomass on the fresh weight (g) basis was estimated and the growth (%) of plants was measured as follows (Das *et al.*, 2021):

Growth % = {(Final weight after the exposure duration – Initial weight at 0 time)/ Initial weight at 0 time)} × 100

Photosynthetic pigments determination

Photosynthetic pigments were extracted from leaves using 90% aqueous methanol solution and chlorophyll *a*, *b*. Carotenoids content was determined spectrophotometrically at 666, 653 and 470 nm according to (Lichtenthaler and Wellburn, 1985) and expressed in mg/g FW.

Leaf relative water content (RWC)

The estimation of leaf RWC was conducted according to the method described by (Weatherley, 1950).

Membrane stability index (MSI)

Leaf membrane stability index (MSI) was determined according to the method of (Premachandra *et al.*, 1990).

Malondialdehyde MDA contents determination

The degree of MDA was estimated following the method described by (Heath and Packer, 1968). The MDA content was estimated using the following formula (Davenport *et al.*, 2003).

MDA (μmol g-1 FW) = (6.45 x (A532 − A600) − (0.56 xA450)) × Vt/W.

Where, Vt = 0.0021; W = 0.2 g

Estimation of total Protein Content

Total proteins were estimated through the method developed by (Bradford, 1976). Absorption and concentration were measured at the 595 nm wavelength using a spectrophotometer. The total protein content of the samples was recorded as milligram per gram of fresh weight using bovine serum albumin as a standard.

Antioxidant enzymes determination

Antioxidant enzymes including peroxidase (POX) and catalase (CAT) were evaluated spectrophotometrically. Fresh leaves samples (0.2 g) were ground in liquid N_2 and homogenized in an ice-bath in 4 mL homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v polyvinylpyrrolidone (pH 7.8). The homogenate was centrifuged at 14000 rpm at 4°C for 10 min and the resulting supernatant was utilized for enzyme assays. Assay of Catalase (CAT): Catalase action was precise according to (Aebi, 1984).

Assay of peroxidase (POX): The determination of POX activity at 420 nm using the method of (Chance and Maehly, 1955).

Anthocyanin estimation

For anthocyanin determination, plant samples (200 mg of FW) were incubated with 6 mL of methanol: HCl (99:1) and kept in the dark for 24 h. The extract was then centrifuged at 13,000 x g for 10 min. The absorbance of the supernatant was measured at 550 nm using spectrophotometer. Anthocyanin concentration (nmol g−1 FW) was calculated using an extinction coefficient of 33,000 mol−1 cm−1 (Wagner, 1979).

Absorbance (A) = Ab530- (1/3×Ab657)
Anthocyanin content = (A×Mol.wt×DF×1000) / Ɛ

Estimation of proline content

Proline was extracted from 0.2 g leaf tissues homogenized in 4 ml 3% aqueous sulfosalicylic acid using the method described by (Bates *et al.*, 1973). Final proline concentration was calculated by the standard curve.

Statistical Analysis

Statistics software CoStat (version 6.3) was used to analyze the data statistically. A one-way ANOVA was calculated by using Danken's multiple range test to determine the significant value (p < 0.05) between the means. Pearson's correlation of all the data were performed by using Microsoft excel 2016.

RESULTS AND DISCUSSION

Lead accumulation in plants

The metal concentration in the shoots and roots of all the three macrophyte species after their exposure to various concentration of Pb depicted in (Fig. 1 A). The acquired data demonstrated that Pb concentrations in the plant tissues varying among species reflecting their various metal uptake capacities. Pb accumulation in shoot and root of *E. crassipes*, *P. stratiotes* and *L. stolonifera* plants increased significantly ($P<0.05$) with increasing concentration of Pb in the growth medium. Generally, the root had acquired more metal than the shoot, according to accumulation patterns. *E. crassipes* showed the highest accumulation of Pb (801.9 mg/Kg DW) at 5 mg/l exposure level and (245.9 mg/Kg DW) at 15 mg/l Pb in root and shoot respectively. *P. stratiotes* exhibited a maximum accumulation (608.1 mg/Kg DW) in root at 15 mg/l and (209.5 mg/Kg DW) at 15 mg/l in the shoot. The maximum Pb uptake in the root and shoot of *L. stolonifera* was (851.5 mg/Kg DW and 249.3 mg/Kg DW at 5 mg/l and 15 mg/l respectively. According to the results, uptake of metal in shoot tissues of these three macrophytes reveals tow trends in terms of Pb exposure concentrations. One, as metal levels in the growth medium rise Pb uptake gradually increases as seen in *E. crassipes* and *L. stolonifera*. Second, in *P. stratiotes* there is a gradual decrease in Pb uptake as the amount of metal in the nutritional medium increases. These aquatic macrophytes showed three patterns in metal uptake in root tissues in regard to Pb exposure levels. One, a progressive increase in Pb absorption as the amount of metal in the growth medium increases as seen in *P. stratiotes*. Second, as metal levels increased Pb uptake gradually decreased as seen in *L. stolonifera* roots. The third, showed an initial rise in metal uptake followed by a decrease with increasing metal concentrations in the growth medium as in *E. crassipes*. Our findings are consistent with the reports of many similar studies. For example, known plant species like *Glycine max* L accumulate higher Pb concentrations in their roots compared to other tissue parts (Khalofah and Farooq, 2023). Also, our result is in consistent with (Langley-Turnbaugh and Belanger, 2010; Kumar *et al.*, 2017). The decrease in metal removal at 15 mg/L in the roots of *E. crassipes* and *L. stolonifera* could be caused by the saturation of Pb selective sites and also the tolerance limit of the plants towards Pb when the concentration was further increased (Yuanqing *et al.*, 2013). Our findings is consistent with (Das *et al.*, 2021). As plant roots prevent the transport of heavy metals this may be a potential tolerance mechanism employed in the roots (Ernst *et al.*, 1992). Because of differences in physiology, metal concentrations, environmental factors, duration of exposure, species, and developmental stages various plant species have varying Pb absorption efficiencies (Pourrut *et al.*, 2011). For the majority of plants Pb concentrations in plant tissues between 30 and 300 μg/g are crucial and negatively affect metabolism (Ramachandra *et al.*, 2018). Therefore, it is possible to assume that the highest Pb concentration resulted in phytotoxicity, which hindered its future uptake.

The translocation factor value was found to be below than 1 (Fig. 1B). Despite that most Pb was concentrated in plant roots during all Pb treatments little Pb were transferred to the shoot. Because of its great affinity for attaching to chemicals in cell walls, Pb has little mobility from root to shoot generating precipitates and crystals. Because plants lack transport channels it appears that Pb is bound to carboxylic groups of mucilage uronic acids on root surfaces (Kabata-Pendias and Pendias, 2011). The three macrophytes in this study had an advantage in terms of covered surface area for metal uptake due to their substantial root biomass, extensive water surface covering through rapid growth and proliferation, or both. Therefore, regardless soil cleaning these macrophytes can be utilized to clean up industrial wastewater and contaminated water. The roots of free-floating macrophytes accumulate the most heavy metal (Kumar and Prasad, 2018). This limitation on metals in the roots acted as a safety net to keep damage to the shoots' photosynthetic system to a minimum (Rezania *et al.*, 2016). In accordance with our data, *Typha angustifolia* and *E. crassipes* exposed to Pb-containing wastewater absorbed substantially more Pb in the roots than in the shoots (Sricoth *et al.*, 2018).

Our results indicate that, the bioconcentration factor (BCF) significantly decreased with an increase of Pb concentration in hydroponic nutrient medium after 21 days in three species (Fig 1C). The highest value of BCF was at 5 mg/L, where was the BCF value (141.9, 144.2 and 195.9 mg/ Kg) for *E. crassipes*, *P. stratiotes* and *L. stolonifera* respectively. While the lowest value was at 15 mg/L in all three plant species. Our result is in agreement with (Velichkova *et al.*, 2019). Similarly, (Yuanqing *et al.*, 2013) revealed that water lettuce subjected to 20 ppm Pb had lower BCF values, while plants treated to 15 ppm Pb had the highest BCF values. The BCF offers details on the intake of metal, its mobilization into plant tissues and its storage in aerial parts of plant (Newman and Unger 2nd, 2003). A high BCF for metal elements at low external concentrations is important for phytoremediation because it makes the process more cost-efficient than the conventional method for treating large amounts of wastewater with concentrations of contaminants (Kamal *et al.*, 2004).

Chlorophyll content

According to this study, a dose-dependent decrease was found in chl *a*, chl *b* and carotenoids content of *E. crassipes* and *L. stolonifera* leaves, While *P. stratiotes* showed a higher stability of chl *b* and carotenoids content in different concentrations of lead as shown in (Fig. 2 A, B and C). Significant differences were observed for chlorophyll content among all the treatments for most of the species except for *P. stratiotes*. The lowest content of chl *a*, chl *b* and carotenoids were determined in the leaves of plants exposed to 15 mg/L Pb in all plants. According to estimates, the decrease in chl *a* relative to the relevant controls was 33.5%, 28.5 and 17.7% at 15 mg/L in *E. crassipes, P. stratiotes* and *L. stolonifera* respectively. as well as the decrease in chl *b* compared to their respective controls were estimated to be 52%, 3.3% and 43.1 % at same concentration in *E. crassipes, P. stratiotes* and *L. stolonifera* respectively. carotenoid content reveled a gradual decrease with increase in concentration with a maximum of 59.1%, 4.2% and 22% decline at 15 mg/L in *E. crassipes, P. stratiotes* and *L. stolonifera* respectively. Our results corroborate the previous studies (Ibrahim *et al.*, 2022; Khalofah and Farooq, 2023) , but disagree with the findings of (Gajewska *et al.*, 2006). This might be because different plants have different mechanisms for defending themselves against heavy metal stress. The result of chloroplast membrane peroxidation due to an increase in ROS formation during Pb treatment may be a decrease in the rate of photosynthetic pigment accumulation (Malar *et al.*, 2014). Additionally, under Pb stress increased MDA levels and electrolyte leakage may harm chloroplast membranes and reduce the formation of photosynthetic pigments (Khalofah and Farooq, 2023). Reduction of the chlorophyll contents was attributed to Pb stress by reducing chlorophyll synthesis and prohibiting plants absorbing vital nutrients such as Mg and Fe (Rucińska-Sobkowiak, 2016). As a result, it destroyed chlorophyll in response to increasing chlorophyllase activity and therefore harming the photosynthetic system (Sharma and Dubey, 2005).

Plant growth

The obtained results revealed that growth % gradually decreased with an increase in the lead level in the three plant species (Fig. 3 A). The highest grade of growth inhibition was in concentration 15 mg/L in all plants. *E. crassipes* showed the minimal percentage while *P. stratiotes* showed the maximal percentage of growth inhibition in all concentrations compared to their respective control. The growth inhibition compared to their corresponding controls were estimated to be 16.7%, 20.3% and 34.8% at 15 mg/L for *E. crassipes, L. stolonifera* and *P. stratiotes* respectively. It is concluded that high concentration of heavy metals in water can have a negative impact on plant growth, as these metals interfere with various physiological and biochemical processes, inhibition of photosynthesis and respiration and degeneration of major cell organelles which can even result in plant death (Schmidt, 2003; Afzal *et al.*, 2006).

Relative water content (RWC)

The level of relative water content**.** (RWC) in *E. crassipes, P. stratiotes* and *L. stolonifera* was estimated (Fig. 3 B). According to the results Pb exposure levels show two trends in the RWC in the leaves of these aquatic macrophytes. One, shows a reduction in RWC when metal levels in the growing medium increasing in comparison to the control as seen in *E. crassipes.* Second, where a slight initial increase up to 5 mg/L followed by a decrease with rising metal concentrations in the growth medium as in *P. stratiotes* and *L. stolonifera*. Data indicated that the highest rate of decrease in RWC was at a concentration of (15 mg/L) where the value of decreasing was (1, 3.5 and 4.4%) in *E. crassipes, P. stratiotes* and *L. stolonifera* respectively Our results is in agreement with previous studies (Malar *et al.*, 2014; Mishra *et al.*, 2014). Plants treated with Pb had a little more relative water in their leaves than untreated plants. The Pb treatment probably led to stomatal closure, which was activated throughout the experiment as a result of the reduced atmospheric carbon-fixing activities (Brunet *et al.*, 2008). The increase in proline accumulation could be led to the rise in water content.

Membrane stability index (MSI) and electrolyte leakage (EL)

The level of degradation of cell membrane in *E. crassipes*, *P. stratiotes* and *L. stolonifera* plants was estimated (Fig. 4 A). The obtained results showed that the membrane stability decreased gradually in all plants with increasing the level of lead concentrations in the nutrient solution, and the highest rate of decrease in the membrane stability was at a concentration of (15 mg/L). *L. stolonifera* showed the highest rate of membrane stability in all concentrations and this may be due to the increased activity of antioxidant enzymes (CAT and POX) compared to other plants. The reduction in membrane stability in treated plants in comparison to the corresponding controls were estimated to be 15.5%, 18.7 % and 7.9 % at (15 mg/L) in *E. crassipes*, *P. stratiotes* and *L. stolonifera* respectively. In contrast, EL were increased with increasing the level of lead in all plants (Fig. 4 B). The maximum EL value was 35.7%, 40.2% and 35% at (15 mg/L) in *E. crassipes*, *P. stratiotes* and *L. stolonifera* respectively. In intact plant cells, the leaking of electrolytes is a sign of stress response and "a measure" of plant stress tolerance (Levitt, 1980). The obtained results are consistent with previous studies (Janmohammadi *et al.*, 2013). When a plant is under Pb stress the ROS generation is the primary production (Israr *et al.*, 2011). The peroxidation of membrane lipids which results in the generation of aldehydes as mlondialdehyde is one of the reactions that accelerates in the presence of reactive oxygen species (Jiang and Huang, 2001). High levels of malondialdehyde accelerate the oxidation of cell membrane fatty acids and lipid peroxidation which ultimately lowers the cell membrane stability index (DaCosta and Huang, 2007).

The effects of Pb on MDA concentration

Lipid peroxidation levels in *E. crassipes*, *P. stratiotes* and *L. stolonifera* plants was estimated by MDA content (Fig. 5 A). After 21 days of Pb exposure, MDA content increased along with the rising lead levels in growth medium. According to the data, *L. stolonifera* showed the lowest value of lipid peroxidation in all concentrations. The results showed that the maximum MDA content was at a concentration of 15 mg/l in three aquatic macrophytes. The increasing rate of MDA in plants exposed to a concentration of 15 mg/L compared to control plants was 63.6, 63.5 and 38% in *E. crassipes*, *P. stratiotes* and *L. stolonifera* respectively. Earlier studies indicated that MDA content increased with increase Pb levels (Singh *et al.*, 2010; Malar *et al.*, 2014). When plants are stressed lipid peroxidation produces MDA which is frequently used as a marker of the severity of oxidative stress (Hu *et al.*, 2012). Inducing oxidative stress in plants as a result of increased generation of (ROS) is one of Pb's toxic effects. Lipid peroxidation, a sign of the generation of ROS, indicated the beginning of oxidative damage (Hattab *et al.*, 2016) which caused by harmful impact s of Pb on plants (Mihailovic *et al.*, 2015).

Protein contents

According to obtained data, all macrophytes showed reduction in protein content with a progressive increase in Pb concentration (Fig. 5 B). The minimum content of protein was observed in all plants exposed to 15 mg/L Pb. The reduction in protein in treated plants to their controls were estimated to be 10.6%, 9.3 and 3.6% at 15 mg/L in *E. crassipes*, *P. stratiotes* and *L. stolonifera* respectively. It was noticed that *E. crassipes* showed the highest value of decrease in protein content in all concentrations of Pb compared to other plants, while *L. stolonifera* showed the lowest reduction in protein content compared to other plants. Numerous studies revealed that Pb accumulation reduced the protein content of aquatic macrophytes (Gupta, 2014; Dogan *et al.*, 2018). One of the most important nutrients, nitrogen is a component of biomolecules including proteins and nucleic acids. An earlier study found that Pb prevented aquatic macrophytes from absorbing nitrogen (Saygideger and Dogan, 2005). During Heavy metals transport into plants can act at various places to block numerous enzymes with functional sulphydryl groups by impeding on protein synthesis processes which has an adverse effect on the normal protein shape (Dua and Sawhney, 1991). A decrease in protein content in the presence of heavy metal ions may be caused by the breakdown of soluble protein or by an increase in the activity of protease or other catabolic enzymes which were activated and destroyed the protein molecules (Mishra *et al.*, 2009).

Antioxidant enzymes

CAT and POX activities in plant leaves under the effects of Pb are shown in (Fig. 6 A, B). According to of Pb treatment, CAT and POX activities significantly increased in *L. stolonifera,* where was the highest rate of CAT and POX activity in treated plants compared to its control was at 15 mg/L by 47% and 103%

respectively. *P. stratiotes* showed significantly increase in the activity of POX in the treated plants in comparison to the control with the highest activity rate at 15 mg/L by 395%, while it showed a slight increase in the activity of CAT with an increase in the concentration of lead. *E. crassipes* showed a non-significant increase in CAT and POX activity at low levels of lead and then, the activity of both enzymes decreased with increasing lead concentration. Our result is in agreement with previous studies (Wang *et al.*, 2012; Wang and Song, 2019). The ROS is the primary product when a plant is under Pb stress (Israr *et al.*, 2011), which may quickly result in the production of lipid peroxides and damage to membranes (Weckx and Clijsters, 1996). Consequently, increasing the activity of two important antioxidant defense system enzymes (SOD and POX) in the three aquatic species. to prevent oxidative damage for plants to adapt and ultimately survive in stressful environments antioxidant enzymes and certain plant metabolites are crucial for minimizing oxidative damage (Zhang *et al.*, 2007)

Anthocyanin content

The obtained results showed an increase in the leaves content of anthocyanins in low concentrations of Pb, then the content of leaves of anthocyanins decreased in high concentrations compared to control plants as in *E. crassipes* and *P. stratiotes*. Whereas, in *L. stolonifera* the leaf content of anthocyanins increased in the treated plants in comparison to the control plants (Fig. 7 A). Our results corroborate the previous studies. These data revealed that *L. stolonifera* could reduce the loss of anthocyanins even at higher Pb concentrations (Dube *et al.*, 1993; Kiran and Prasad, 2017). Here, anthocyanins appear to play a unique role in Pb tolerance as they are known to combine with heavy metals and sequester them in the vacuole (Hale *et al.*, 2001). Anthocyanin shows effective defense against ROS generation (Kumar and Prasad, 2015). Anthocyanin which is biosynthesized through the phenylpropanoid pathways has the potential to scavenge free radicals and also has the capacity to bind heavy metal ions. It is hypothesised that heavy metal-induced stress targets phenylalanine ammonia lyase (PAL) a crucial enzyme in the synthesis of flavanoids which inhibits the manufacture of anthocyanins (Dube *et al.*, 1993).

Proline accumulation

Under Pb stress, results of this study showed increases in free proline concentrations as osmoprotectants (Fig. 7 B). Results showed that proline concentration was significantly higher in treated plants in compare with their control. The results reveled that maximum accumulation of proline at 15 mg/L in *E. crassipes* and *P. stratiotes* (3.11 and 3.31 µmoles/g FW) respectively. while *L. stolonifera* showed the highest proline accumulation (2.84 µmoles/g FW) at a concentration of 10 mg/l Our results are in agreement with data reported by Khalofah and Farooq (2023) who stated a significant accumulation of proline in *Glycine max* as an effective defense mechanism to Pb treatments. Also, with those of (Mahdavian *et al.*, 2016; Ramana *et al.*, 2021). As one of the amino acids and part of a general adaptation syndrome to unfavorable environmental conditions, free proline is probably one of the most prevalent metabolites produced in response to stress (Al-Ghzawi *et al.*, 2019). The increased proline in Pb-exposed plants might be linked with protein breakdown (Khalofah and Farooq, 2023). Proline alleviates metal-induced oxidative stress due to its ability to scavenge ROS (Ben Rejeb *et al.*, 2014).

Heat Map Analysis

Data heat map analysis (Fig. 8 A, B, C) showed that treatments with Pb levels significantly decreased in most parameters as growth %, protein, Chl *a*, Chl *b*, total Chl, Car, anthocyanin, MSI and RWC in all species. While, Pb accumulation, MDA, EL, POX, CAT and Proline content showed highest values in all three species at 15 mg/L compared with control**.**

Pearson correlation coefficients

For calculating the correlation between measurements, Person correlation coefficients were determined in three species as presented in (Fig. 9). The obtained data obviously showed very strong positive Pearson correlations were observed between MDA and proline (r = 0.999) then followed by total Chl and MSI (r = 0.991) and a strong negative correlation occurs between total MSI and EL (-1.00) then Pb accumulation and growth % (r = -0.991) in *E. crassipes* (Fig. 9 A). *P. stratiotes* showed very strong positive Pearson correlations between EL and proline (r = 0.995) then followed by MDA and proline (r = 0.983) and a strong negative correlation between total MSI and EL (-1.00) then MSI and proline (r = -0.996) (Fig. 9 B). *L. stolonifera* (Fig. 9 C) appeared very strong positive Pearson correlations between Chl a and anthocyanin (r = 0.991) followed by MDA and CAT (r = 0.988).

Conversely, a strong negative correlation between total MSI and EL (-1.00) then MDA and Chl b (r = -0.996).

CONCLUSION

We conducted the current study to examine the feasibility of the potential of phytoremediation for lead of three types of aquatic plants in terms of significant metal accumulation in plant tissues and the physiological ability of those plants to resist lead stress. Based on our findings, all three macrophytes *E. crassipes*, *P. stratiotes* and *L. stolonifera* showed high efficiency for the Pb removal from different metal concentrations. The maximum absorption rate of metal was observed in the roots of all aquatic macrophytes. Whereas, *L. stolonifera* showed the highest accumulation rate for whole plant in all concentrations. Results strongly suggest that these macrophytes are less affected by oxidative stress, in spite of the presence of higher dose of Pb in the hydroponic medium, as would be expected for a species that has efficiently survived in a highly polluted environment. Increased CAT and POX activity appear to play key roles in the antioxidant defense response of these aquatic plants when exposed to Pb heavy metal toxicity particularly *L. stolonifera*, where showed the highest (MSI) value and protein stability. In addition, gave the lowest (EL) value and MDA content in compare with other species. While *P. stratiotes* showed the highest rate of stability of chlorophyll in stressed plants.

REFERENCES

Abdelhafez, A.A., Metwalley, S.M., Abbas, H.H. 2020: Irrigation: Water Resources, Types and Common Problems in Egypt. In: Technological and Modern Irrigation Environment in Egypt: Best Management Practices & Evaluation, E.-S. E. Omran and A. M. Negm, (Eds.). Springer International Publishing, Cham: pp: 15-34.

Aebi, H. 1984: Catalase in vitro. In: Methods in Enzymology. Academic Press: pp: 121-126.

Afaj, A.H., Jassim, A.J., Noori, M.M., Schüth, C. 2017: Effects of lead toxicity on the total chlorophyll content and growth changes of the aquatic plant *Ceratophyllum demersum* L. International Journal of Environmental Studies, 74(1): 119-128.

Afzal, I., Basra, S.M.A., Hameed, A., Farooq, M. 2006: Physiological enhancements for alleviation of salt stress in wheat. Pakistan Journal of Botany, 38(5): 1649-1659.

Al-Ghzawi, A.L.A., Al Khateeb, W., Rjoub, A., Al-Tawaha, A.R.M., Musallam, I., Al Sane, K.O. 2019: Lead toxicity affects growth and biochemical content in various genotypes of barley (*Hordeum vulgare* L.). Bulgarian Journal of Agricultural Science, 25: 55-61.

Bates, L.S., Waldren, R.P., Teare, I.D. 1973: Rapid determination of free proline for water-stress studies. Plant and Soil, 39(1): 205-207.

Bell, C.H., Gentile, M., Kalve, E., Ross, I., Horst, J., Suthersan, S. 2019: Emerging contaminants handbook. CRC Press.

Ben Rejeb, K., Abdelly, C., Savouré, A. 2014: How reactive oxygen species and proline face stress together. Plant Physiology and Biochemistry, 80: 278-284.

Bradford, M.M. 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1): 248-254.

Brunet, J., Repellin, A., Varrault, G., Terryn, N., Zuily-Fodil, Y. 2008: Lead accumulation in the roots of grass pea (*Lathyrus sativus* L.): a novel plant for phytoremediation systems? Comptes Rendus Biologies, 331(11): 859-864.

Caverzan, A., Casassola, A., Brammer, S.P. 2016: Antioxidant responses of wheat plants under stress. Genetics and Molecular Biology, 39: 1–6.

Chance, B., Maehly, A.C. 1955: Assay of catalases and peroxidases. In: Methods in Enzymology. Academic Press: pp: 764-775.

Chapman, H., Pratt, P. 1978: Methods of Analysis for soils, plants and waters. Division of Agric. Sci. Univ. California, Berkeley, USA: 3043 pp.

Chen, Q., Zhang, X., Liu, Y., Wei, J., Shen, W., Shen, Z., Cui, J. 2016: Hemin-mediated alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity; suppressed metal uptake and oxidative stress in rice seedlings. Plant Growth Regulation, 81(2): 253-264.

DaCosta, M., Huang, B. 2007: Changes in Antioxidant Enzyme Activities and Lipid Peroxidation for Bentgrass Species in Response to Drought Stress Journal of the American Society for Horticultural Science, 132(3): 319-326.

Das, S., Das, A., Mazumder, P.E.T., Paul, R., Das, S. 2021: Lead phytoremediation potentials of four aquatic macrophytes under hydroponic cultivation. International Journal of Phytoremediation, 23(12): 1279-1288.

Davenport, S.B., Davenport, S.B., Gallego, S.M., Benavides, M.P., Tomaro, M.L. 2003: Behaviour of antioxidant defense system in the adaptive response to salt stress in *Helianthus annuus* L. cells. Plant growth regulation, 40(1): 81-88.

Dogan, M., Karatas, M., Aasim, M. 2018: Cadmium and lead bioaccumulation potentials of an aquatic macrophyte Ceratophyllum demersum L.: A laboratory study. Ecotoxicology and Environmental Safety, 148: 431-440.

Drazkiewicz, M. 1994: Chlorophyllase: occurrence, functions, mechanism of action, effects of external and internal factors. Photosynthetica, 30: 321–330.

Dua, A., Sawhney, S.K. 1991: Effect of chromium on activities of hydrolytic enzymes in germinating pea seeds. Environmental and Experimental Botany, 31(2): 133-139.

Dube, A., Bharti, S., Laloraya, M.M. 1993: Inhibition of anthocyanin synthesis and phenylalanine ammonialyase activity by Co2+ in leaf disks of *Terminalia catappa*. Physiol Plant, 88(2): 237-242.

Eid, E.M., Shaltout, K.H. 2016: Bioaccumulation and translocation of heavy metals by nine native plant species grown at a sewage sludge dump site. International Journal of Phytoremediation, 18(11): 1075-1085.

Eid, E.M., Shaltout, K.H. 2017: Population dynamics of *Eichhornia crassipes* (C. Mart.) Solms in the Nile Delta, Egypt. Plant Species Biology, 32(4): 279-291.

Ernst, W., Verkleij, J., Schat, H. 1992: Metal tolerance in plants. Acta botanica neerlandica, 41(3): 229-248.

Farid, M., Ali, S., Saeed, R., Rizwan, M., Bukhari, S.A.H., Abbasi, G.H., Hussain, A., Ali, B., Zamir, M.S.I., Ahmad, I. 2019: Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. International Journal of Phytoremediation, 21(8): 760-767.

Gajewska, E., Skłodowska, M., Słaba, M., Mazur, J. 2006: Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biologia Plantarum, 50(4): 653-659.

Galal, T.M., Al-Sodany, Y.M., Al-Yasi, H.M. 2020: Phytostabilization as a phytoremediation strategy for mitigating water pollutants by the floating macrophyte *Ludwigia stolonifera* (Guill. & Perr.) P.H. Raven. International Journal of Phytoremediation, 22(4): 373-382.

Galal, T.M., Eid, E.M., Dakhil, M.A., Hassan, L.M. 2018: Bioaccumulation and rhizofiltration potential of *Pistia stratiotes* L. for mitigating water pollution in the Egyptian wetlands. International Journal of Phytoremediation, 20(5): 440-447.

Galal, T.M., Farahat, E.A. 2015: The invasive macrophyte *Pistia stratiotes* L. as a bioindicator for water pollution in Lake Mariut, Egypt. Environmental Monitoring and Assessment, 187(11): 701.

Gill, S.S., Tuteja, N. 2010: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48(12): 909-930.

Gupta, D.K., Huang, H.G., Yang, X.E., Razafindrabe, B.H.N., Inouhe, M. 2010: The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione. Journal of Hazardous Materials, 177(1): 437-444.

Gupta, K. 2014: Biomonitoring of lead (Pb) toxicity through aquatic macrophyte *Eichhornia crassipes*. International Journal of Environment, 3(2): 12-19.

Hale, K.L., McGrath, S.P., Lombi, E., Stack, S.M., Terry, N., Pickering, I.J., George, G.N., Pilon-Smits, E.A.H. 2001: Molybdenum Sequestration in BrassicaSpecies. A Role for Anthocyanins? Plant Physiology, 126(4): 1391-1402.

Hattab, S., Hattab, S., Flores-Casseres, M.L., Boussetta, H., Doumas, P., Hernandez, L.E., Banni, M. 2016: Characterisation of lead-induced stress molecular biomarkers in *Medicago sativa* plants. Environmental and Experimental Botany, 123: 1-12.

Heath, R.L., Packer, L. 1968: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics, 125(1): 189-198.

Hoagland, D.R., Arnon, D.I. 1950: The water-culture method for growing plants without soil. California Agricultural Experimental Stat, 347(2nd edit): 1–32.

Houri, T., Khairallah, Y., Zahab, A.A., Osta, B., Romanos, D., Haddad, G. 2020: Heavy Metals Accumulation Effects on The Photosynthetic Performance of Geophytes in Mediterranean Reserve. Journal of King Saud University - Science, 32(1): 874-880.

Hu, R., Sun, K., Su, X., Pan, Y.X., Zhang, Y.F., Wang, X.P. 2012: Physiological responses and tolerance mechanisms to Pb in two xerophils: *Salsola passerina* Bunge and *Chenopodium album* L. Journal of Hazardous Materials, 205-206: 131-138.

Ibrahim, M.A., Waziri, M.S., Ibrahim, M., Kyari, B.A., Apagu, B., Adams, K.J. 2022: Uptake and growth response of water lettuce (*Pistia stratiotes* L.) in lead (Pb)-spiked water. Arid Zone Journal of Basic and Applied Researc, 1: 183-191.

Israr, M., Jewell, A., Kumar, D., Sahi, S.V. 2011: Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of *Sesbania* *drummondii*. Journal of Hazardous Materials, 186(2): 1520-1526.

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.

Janmohammadi, M., Bihamta, M., Ghasemzadeh, F. 2013: Influence of rhizobacteria inoculation and lead stress on the physiological and biochemical attributes of wheat genotypes. Cercetări Agronomice în Moldova, 1 49-67.

Jiang, Y., Huang, B. 2001: Drought and Heat Stress Injury to Two Cool-Season Turfgrasses in Relation to Antioxidant Metabolism and Lipid Peroxidation. 41(2): 436-442.

Kabata-Pendias, A., Pendias, H. 2011: Trace elements in soils and plants. 4 th ed, Boca Raton (FL): CRC Press. Taylor & Francis Group.

Kamal, M., Ghaly, A.E., Mahmoud, N., Côté, R. 2004: Phytoaccumulation of heavy metals by aquatic plants. Environment International, 29(8): 1029-1039.

Kamran, M.A., Syed, J.H., Eqani, S.A.M.A.S., Munis, M.F.H., Chaudhary, H.J. 2015: Effect of plant growth-promoting rhizobacteria inoculation on cadmium (Cd) uptake by *Eruca sativa*. Environmental Science and Pollution Research, 22(12): 9275-9283.

Khalid, A., Farid, M., Zubair, M., Rizwan, M., Iftikhar, U., Ishaq, H.K., Farid, S., Latif, U., Hina, K., Ali, S. 2020: Efficacy of *Alternanthera bettzickiana* to Remediate Copper and Cobalt Contaminated Soil Physiological and Biochemical Alterations. International Journal of Environmental Research, 14(3): 243-255.

Khalofah, A.M., Farooq, S. 2023: Physiological, Morphological, and Biochemical Responses of Soybean [*Glycine max* (L.) Merr.] to Loquat (*Eriobotrya japonica* Lindl.) Leaf Extract Application on Pb-Contaminated Soil. Sustainability, 15(5): 4352.

Kiran, B.R., Prasad, M.N.V. 2017: Responses of *Ricinus communis* L.(castor bean, phytoremediation crop) seedlings to lead (Pb) toxicity in hydroponics. Selcuk Journal of Agriculture and Food Sciences, 31(1): 73-80.

Kopittke, P.M., Asher, C.J., Kopittke, R.A., Menzies, N.W. 2007: Toxic effects of Pb2+ on growth of cowpea (*Vigna unguiculata*). Environmental Pollution, 150(2): 280-287.

Kumar, A., Prasad, M.N.V. 2015: Lead-induced toxicity and interference in chlorophyll fluorescence in *Talinum triangulare* grown hydroponically. Photosynthetica, 53(1): 66-71.

Kumar, A., Prasad, M.N.V. 2018: Plant-lead interactions: Transport, toxicity, tolerance, and detoxification mechanisms. Ecotoxicology and Environmental Safety, 166: 401-418.

Kumar, B., Smita, K., Cumbal Flores, L. 2017: Plant mediated detoxification of mercury and lead. Arabian Journal of Chemistry, 10: S2335-S2342.

Kumar, V., Singh, J., Kumar, P. 2019: Heavy metal uptake by water lettuce (*Pistia stratiotes* L.) from paper mill effluent (PME): experimental and prediction modeling studies. Environmental Science and Pollution Research, 26(14): 14400-14413.

Kumar, V., Singh, J., Saini, A., Kumar, P. 2019: Phytoremediation of copper, iron and mercury from aqueous solution by water lettuce (*Pistia stratiotes* L.). Environmental Sustainability, 2(1): 55-65.

Lamhamdi, M., Bakrim, A., Aarab, A., Lafont, R., Sayah, F. 2011: Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. Comptes Rendus Biologies, 334(2): 118-126.

Langley-Turnbaugh, S.J., Belanger, L.G. 2010: Phytoremediation of Lead in Urban Residential Soils of Portland, Maine. Soil Horizons, 51(4): 95-101.

Levitt, J. 1980: Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses. Academic Press.

Liao, S., Chang, W. 2004: Heavy Metal Phytoremediation by Water Hyacinth at Constructed Wetlands in Taiwan. Journal of Aquatic Plant Management, 42: 60-68.

Lichtenthaler, H., Wellburn, A. 1985: Determination of total cartionoids and chlorophyll A and B of leaf in different solvents. Biochemical Society Transactions, 11: 59-592.

Liu, D., Li, T.Q., Jin, X.F., Yang, X.E., Islam, E., Mahmood, Q. 2008: Lead induced changes in the growth and antioxidant metabolism of the lead accumulating and non-accumulating ecotypes of *Sedum alfredii*. Journal of Integrative Plant Biology, 50(2): 129-140.

Mahdavian, K., Ghaderian, S.M., Schat, H. 2016: Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous *Peganum harmala* L. under Pb exposure. Environmental and Experimental Botany, 126: 21-31.

Malar, S., Shivendra Vikram, S., Jc Favas, P., Perumal, V. 2014: Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Botanical Studies, 55(1): 55, 54.

Mihailovic, N., Andrejić, G., Dželetović, Ž. 2015: Tolerance of *Portulaca grandiflora* to Individual and Combined Application of Ni, Pb and Zn.

Bulletin of Environmental Contamination and Toxicology, 94(1): 103-107.

Mishra, K., Gupta, K., Rai, U.N. 2009: Bioconcentration and phytotoxicity of chromium in *Eichhornia crassipes*. J Environ Biol, 30(4): 521-526.

Mishra, M., Pradhan, C., Satapathy, K.B. 2014: Decontamination of Lead from aquatic environment by exploitation of floating macrophyte *Azolla microphylla* Kauf. Journal of Environmental Science, Toxicology and Food Technol, 8: 17-23.

Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S., Gupta, D.K. 2006: Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere, 65(6): 1027-1039.

Mojiri, A., Zhou, J.L., Ratnaweera, H., Ohashi, A., Ozaki, N., Kindaichi, T., Asakura, H. 2021: Treatment of landfill leachate with different techniques: an overview. Journal of Water Reuse Desalination, 11(1): 66-96.

Newman, M., Unger 2nd, M., 2003: Fundamentals of Ecotoxicology, 2nd Edn Lewis Publishers, Boca Raton. Florida.

Oh, K., Cao, T., Li, T., Cheng, H. 2014: Study on application of phytoremediation technology in management and remediation of contaminated soils. Journal of Clean Energy Technologies, 2(3): 216-220.

Overall, R.A., Parry, D.L. 2004: The uptake of uranium by Eleocharis dulcis (Chinese water chestnut) in the Ranger Uranium Mine constructed wetland filter. Environmental Pollution, 132(2): 307-320.

Piotrowska, A., Bajguz, A., Godlewska-Żyłkiewicz, B., Czerpak, R., Kamińska, M. 2009: Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). Environmental and Experimental Botany, 66(3): 507-513.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E. 2011: Lead Uptake, Toxicity, and Detoxification in Plants. In: Reviews of Environmental Contamination and Toxicology Volume 213, D. M. Whitacre, (Ed.). Springer New York, New York, NY: pp: 113-136.

Premachandra, G.S., Saneoka, H., Fujita, K., Ogata, S. 1990: Cell membrane stability and leaf water relations as affected by phosphorus nutrition under water stress in maize. Soil Science and Plant Nutrition, 36(4): 661-666.

Qureshi, M.I., Abdin, M.Z., Qadir, S., Iqbal, M. 2007: Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Biologia Plantarum, 51(1): 121-128.

Rady, M.M., El-Yazal, M.A.S., Taie, H.A., Ahmed, S.M. 2021: Physiological and biochemical responses of wheat (*Triticum aestivum* L.) plants to polyamines under lead stress. Innovare Journal of Agricultural Sciences 9(1): 1-10.

Rahman, M.A., Hasegawa, H. 2011: Aquatic arsenic: phytoremediation using floating macrophytes. Chemosphere, 83(5): 633-646.

Ramachandra, T.V., Sudarshan, P.B., Mahesh, M.K., Vinay, S. 2018: Spatial patterns of heavy metal accumulation in sediments and macrophytes of Bellandur wetland, Bangalore. Journal of Environmental Management, 206: 1204-1210.

Ramana, S., Tripathi, A.K., Bharati, K., Singh, A.B., Kumar, A., Sahu, A., Rajput, P.S., Dey, P., Saha, J.K., Patra, A.K. 2021: Tolerance of cotton to elevated levels of Pb and its potential for phytoremediation. Environmental Science and Pollution Research, 28(25): 32299-32309.

Rascio, N., Navari-Izzo, F. 2011: Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant science, 180(2): 169-181.

Rezania, S., Taib, S.M., Md Din, M.F., Dahalan, F.A., Kamyab, H. 2016: Comprehensive review on phytotechnology: Heavy metals removal by diverse aquatic plants species from wastewater. Journal of Hazardous Materials, 318: 587-599.

Rotkittikhun, P., Kruatrachue, M., Chaiyarat, R., Ngernsansaruay, C., Pokethitiyook, P., Paijitprapaporn, A., Baker, A.J.M. 2006: Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand. Environmental Pollution, 144(2): 681-688.

Rucińska-Sobkowiak, R. 2016: Water relations in plants subjected to heavy metal stresses. Acta Physiologiae Plantarum, 38(11): 257.

Saleh, H.M., Aglan, R.F., Mahmoud, H.H. 2019: *Ludwigia stolonifera* for remediation of toxic metals from simulated wastewater. Chemistry and Ecology, 35(2): 164-178.

Saleh, H.M., Bayoumi, T.A., Mahmoud, H.H., Aglan, R.F. 2017: Uptake of cesium and cobalt radionuclides from simulated radioactive wastewater by *Ludwigia stolonifera* aquatic plant. Nuclear Engineering and Design, 315: 194-199.

Saygideger, S., Dogan, M. 2005: Influence of pH on lead uptake, chlorophyll and nitrogen content of *Nasturtium officinale* R. Br. and *Mentha aquatica* L. J Environ Biol, 26(4): 753-759.

Schmidt, U. 2003: Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. Journal of Environmental Quality, 32(6): 1939-1954.

Sharma, P., Dubey, R.S. 2005: Lead toxicity in plants. Journal of Plant Physiology, 17: 35-52.

Sharma, S., Singh, B., Manchanda, V. 2015: Phytoremediation: role of terrestrial plants and aquatic macrophytes in the remediation of radionuclides and heavy metal contaminated soil and water. Environmental Science Pollution Research 22(2): 946-962.

Shi, W.G., Liu, W., Yu, W., Zhang, Y., Ding, S., Li, H., Mrak, T., Kraigher, H., Luo, Z.B. 2019: Abscisic acid enhances lead translocation from the roots to the leaves and alleviates its toxicity in *Populus × canescens*. Journal of Hazardous Materials, 362: 275-285.

Singh, D., Gupta, R., Tiwari, A. 2011: Phytoremediation of lead from wastewater using aquatic plants. International Journal of Biomedical Research, 7: 411-421.

Singh, R., Tripathi, R.D., Dwivedi, S., Kumar, A., Trivedi, P.K., Chakrabarty, D. 2010: Lead bioaccumulation potential of an aquatic macrophyte Najas indica are related to antioxidant system. Bioresource Technology, 101(9): 3025-3032.

Sricoth, T., Meeinkuirt, W., Pichtel, J., Taeprayoon, P., Saengwilai, P. 2018: Synergistic phytoremediation of wastewater by two aquatic plants (*Typha angustifolia* and *Eichhornia crassipes*) and potential as biomass fuel. Environmental Science and Pollution Research, 25(6): 5344-5358.

Stoltz, E., Greger, M. 2002: Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environmental and Experimental Botany, 47(3): 271-280.

Velichkova, K., Sirakov, I., Slavcheva-Sirakova, D. 2019: Bioaccumulation, growth and photosynthetic response of a new found in bulgaria invasive species *Lemna minuta* and *L. valdiviana* to heavy metal pollution. Planta Daninha, 37.

Wagner, G.J. 1979: Content and Vacuole/Extravacuole Distribution of Neutral Sugars, Free Amino Acids, and Anthocyanin in Protoplasts Plant Physiology, 64(1): 88-93.

Wang, F., Song, N. 2019: Salinity-induced alterations in plant growth, antioxidant enzyme activities, and lead transportation and accumulation in *Suaeda salsa*: implications for phytoremediation. Ecotoxicology, 28(5): 520-527.

Wang, P., Zhang, S., Wang, C., Lu, J. 2012: Effects of Pb on the oxidative stress and antioxidant response in a Pb bioaccumulator plant *Vallisneria natans*. Ecotoxicology and Environmental Safety, 78: 28-34.

Weatherley, P.E. 1950: Studies in the water relations of the cotton plant. New Phytologist, 49(1): 81-97.

Weckx, J.E.J., Clijsters, H.M.M. 1996: Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiologia Plantarum, 96(3): 506-512.

Yoon, J., Cao, X., Zhou, Q., Ma, L.Q. 2006: Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of The Total Environment, 368(2): 456-464.

Yuanqing, Z., Shu Ying, L., Yundong, S., Wei, L., Taibo, S., Qilin, H., Yinke, L., Zhaolu, W., 2013: Phytoremediation of Chromium and Lead Using Water Lettuce *Pistia stratiotes* L. In: Applied Mechanics and Materials. Trans Tech Publ: pp: 2071-2075.

Zhang, F.Q., Wang, Y.S., Lou, Z.P., Dong, J.D. 2007: Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere, 67(1): 44-50.

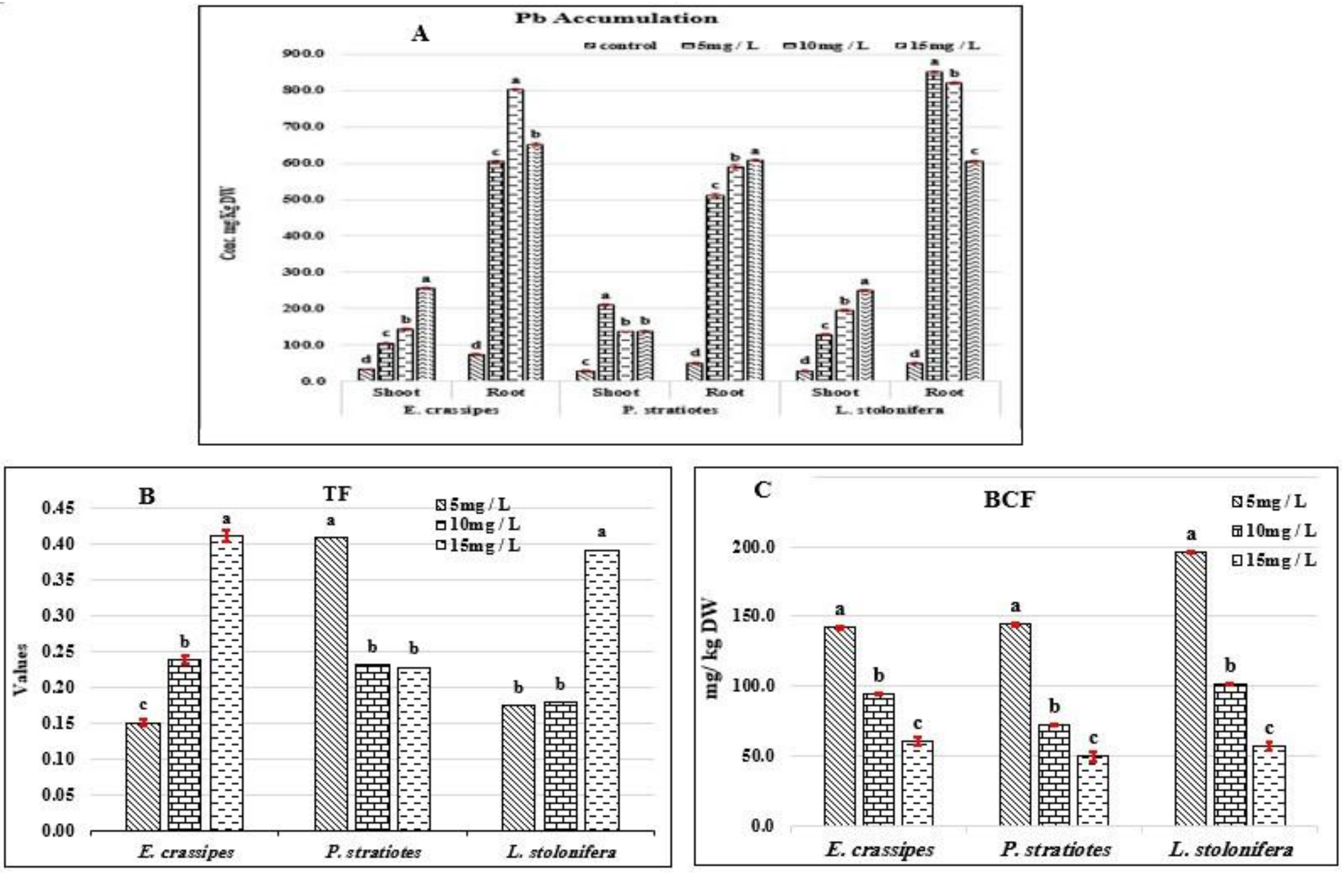


Figure 1: Pb accumulation (mg/Kg DW) in shoot and roots (A), Translocation factor (TF) (B) and bioconcentration factor (BCF) (C) of three aquatic plants grown for 21 days in Hoagland nutrient solution with addition of different Pb concentration.

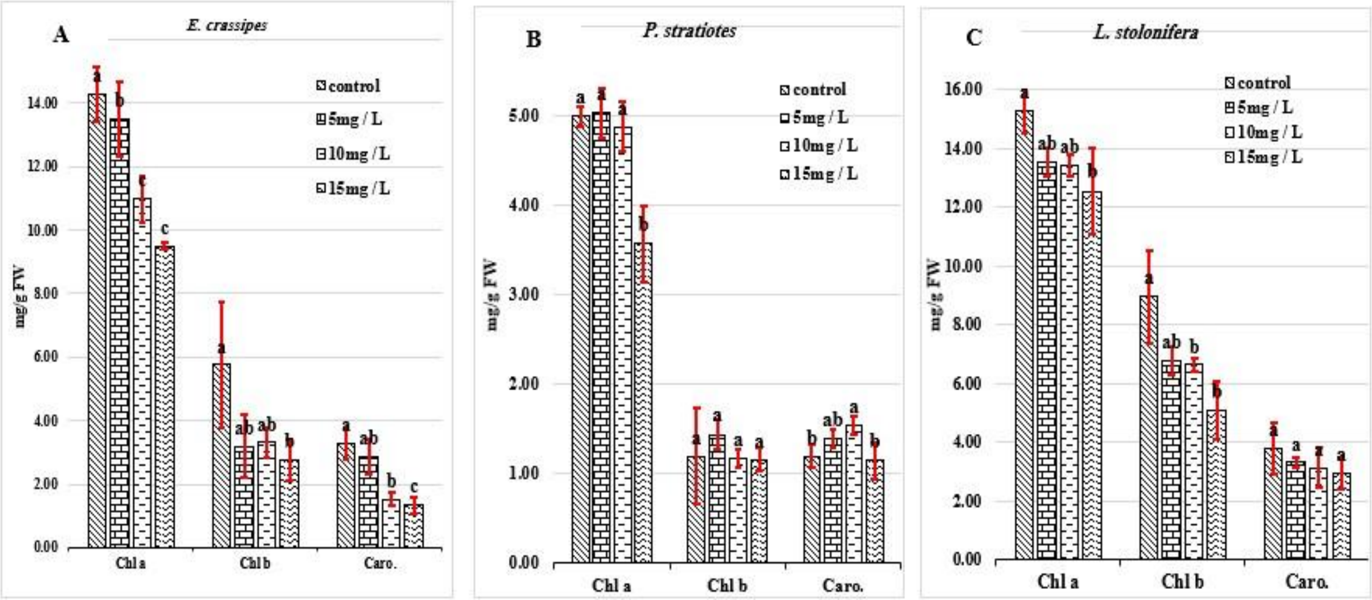


Figure 2: Chlorophyll (Chl. *a,* Chl *b* and carotenoids) of three aquatic plants *E. crassipes* (A), *P. stratiotes* (B) and *L. stolonifera* (C) grown for 21 days in Hoagland nutrient solution with addition of different Pb concentrations.

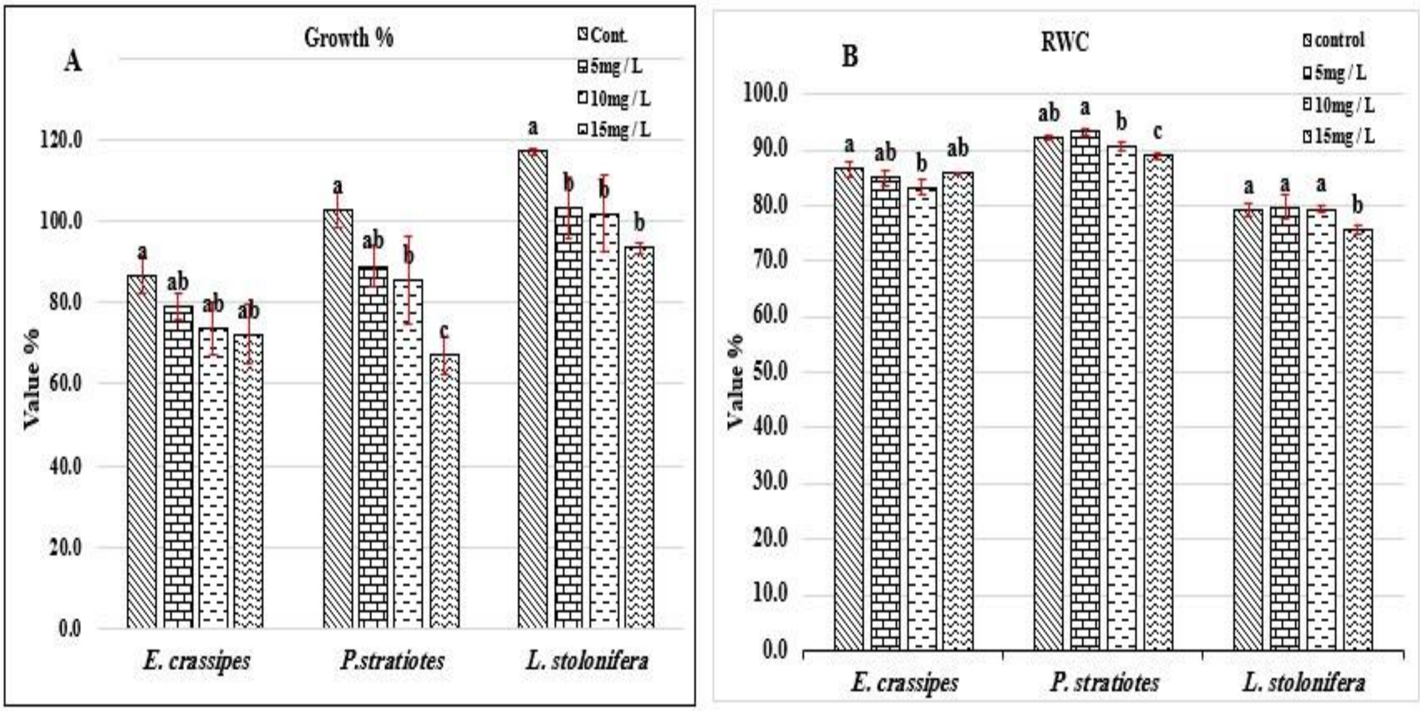


Figure 3: Growth % (A) and relative water content RWC (B) of three aquatic plants grown for 21 days in Hoagland nutrient solution with addition of different Pb concentrations.

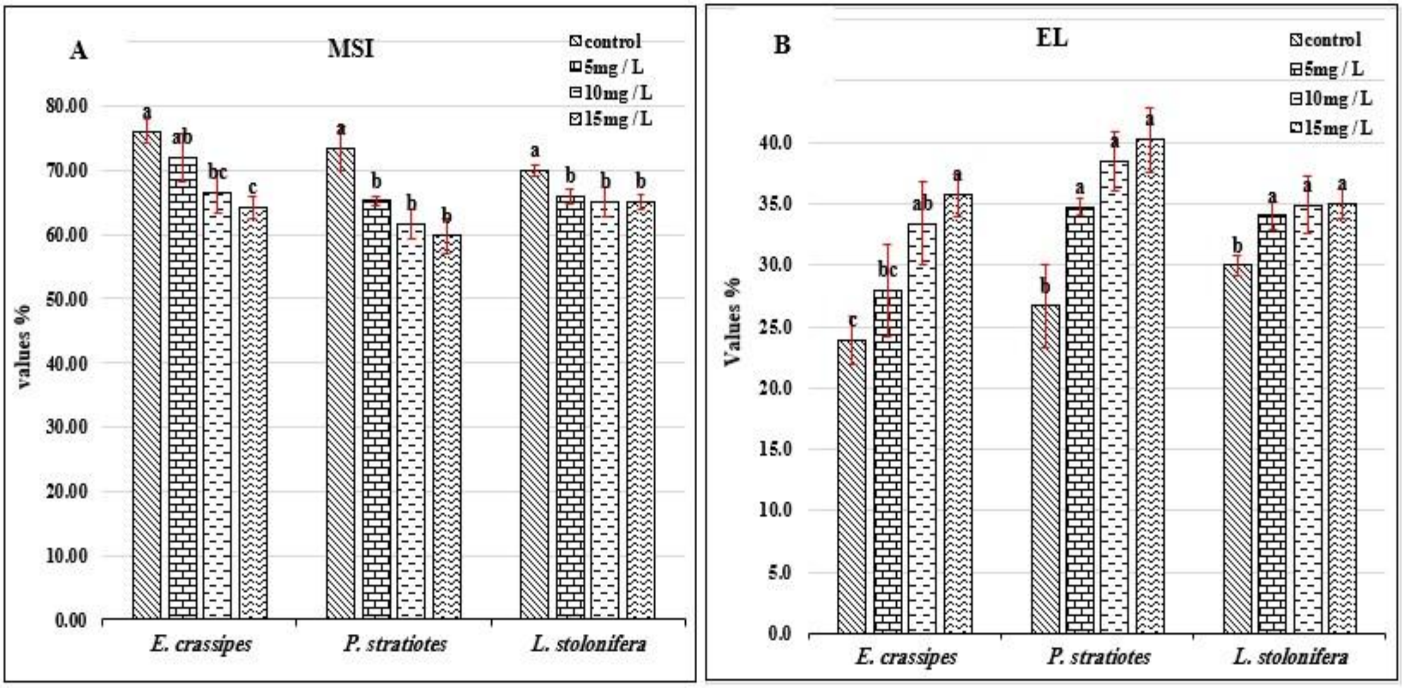


Figure 4: Membrane stability index MSI (A) and electrolyte leakage EL (B) of three aquatic plants grown for 21 days in Hoagland nutrient solution with addition of different Pb concentrations.

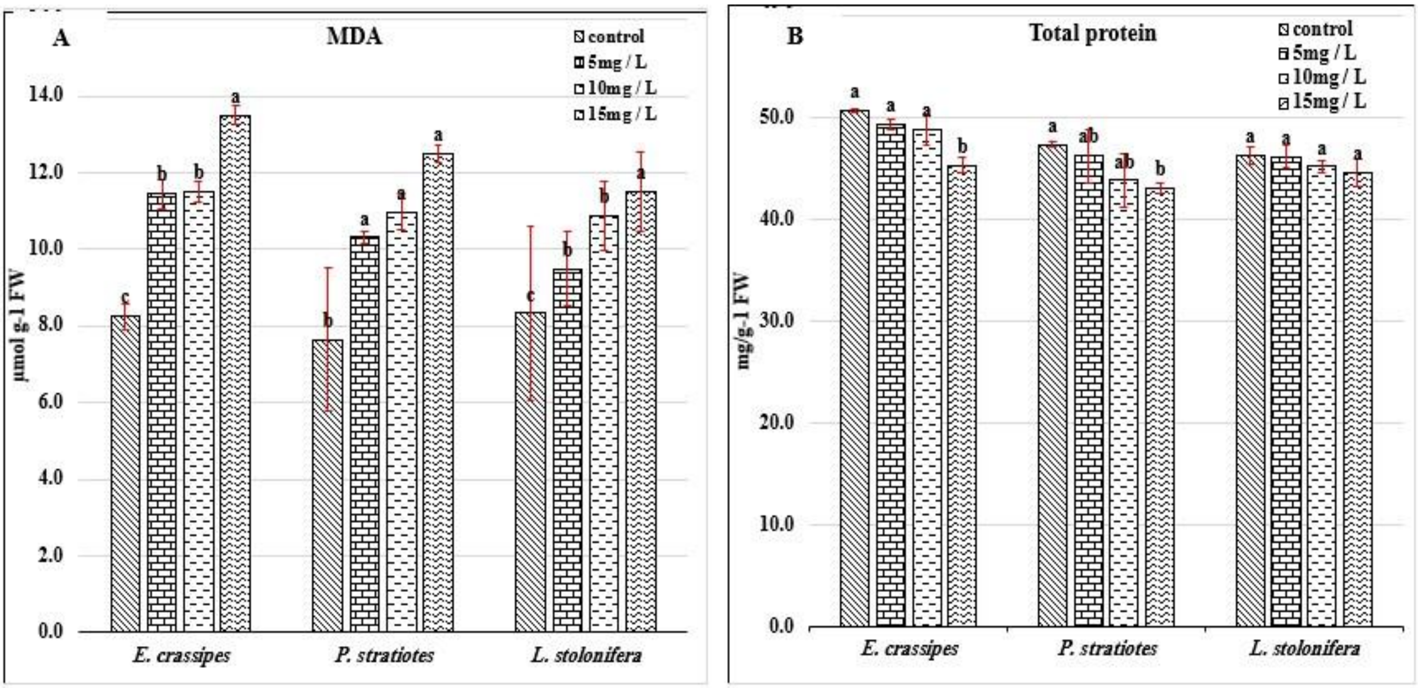


Figure 5: Malondialdehyde MDA (A) and total Protien content (B) for three aquatic plants grown for 21 days in Hoagland nutrient solution with addition of different Pb concentrations.

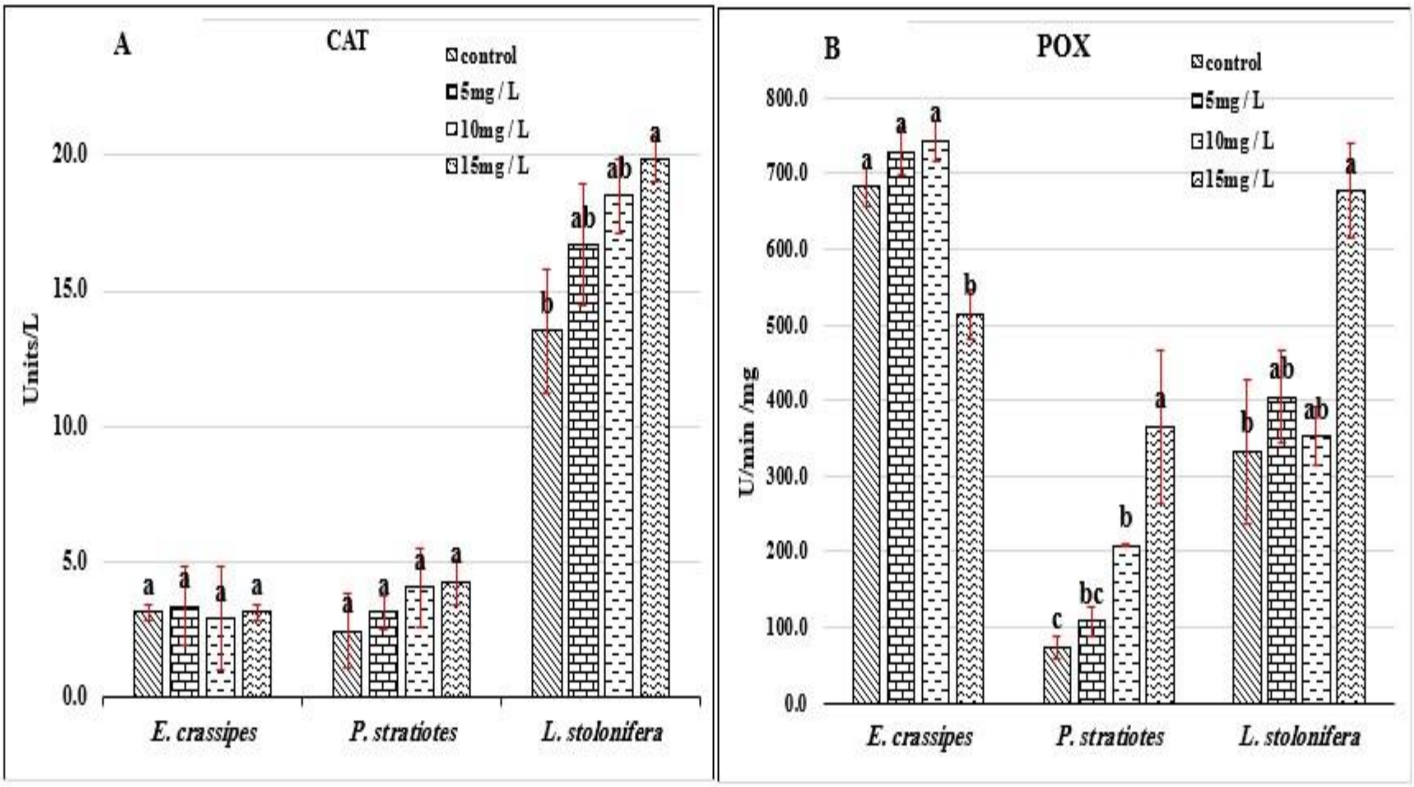


Figure 6: Antioxidant enzymes Catalase "CAT" activity (A) and peroxidase "POX" activity (B) of three aquatic plants grown for 21 days in Hoagland nutrient solution with addition of different Pb concentrations.

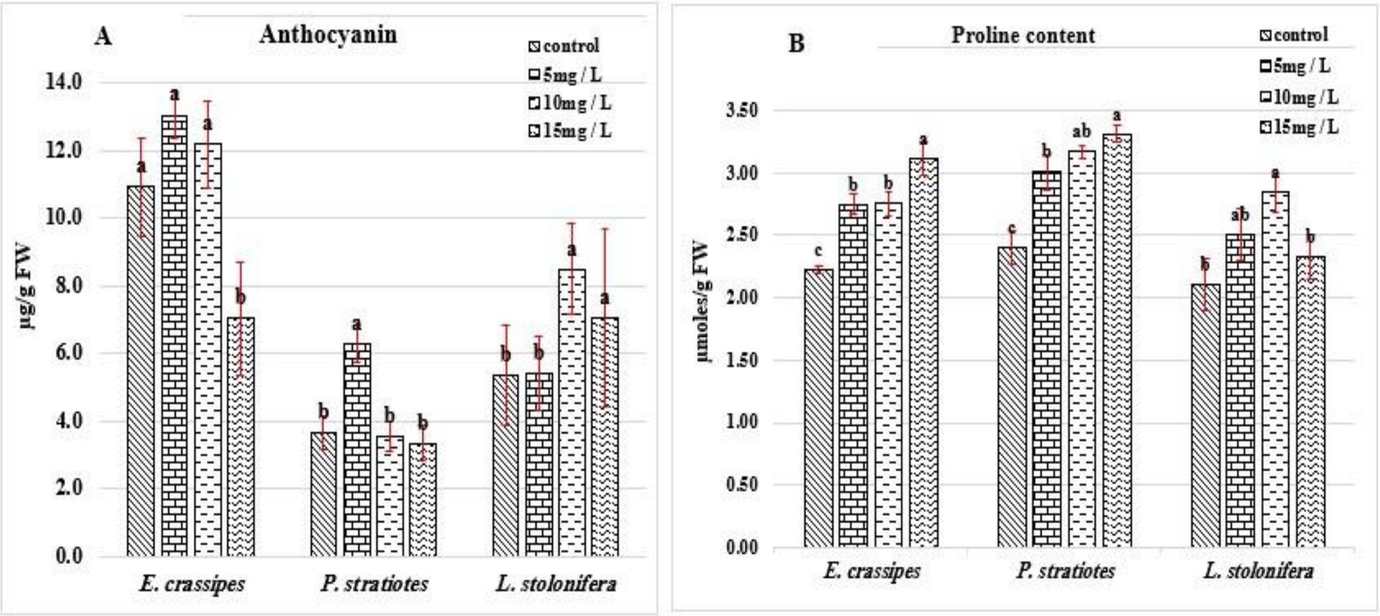


Figure 7: Anthocyanin content (A) and proline content (B) of three aquatic plants grown for 21 days in Hoagland nutrient solution with addition of different Pb concentrations.

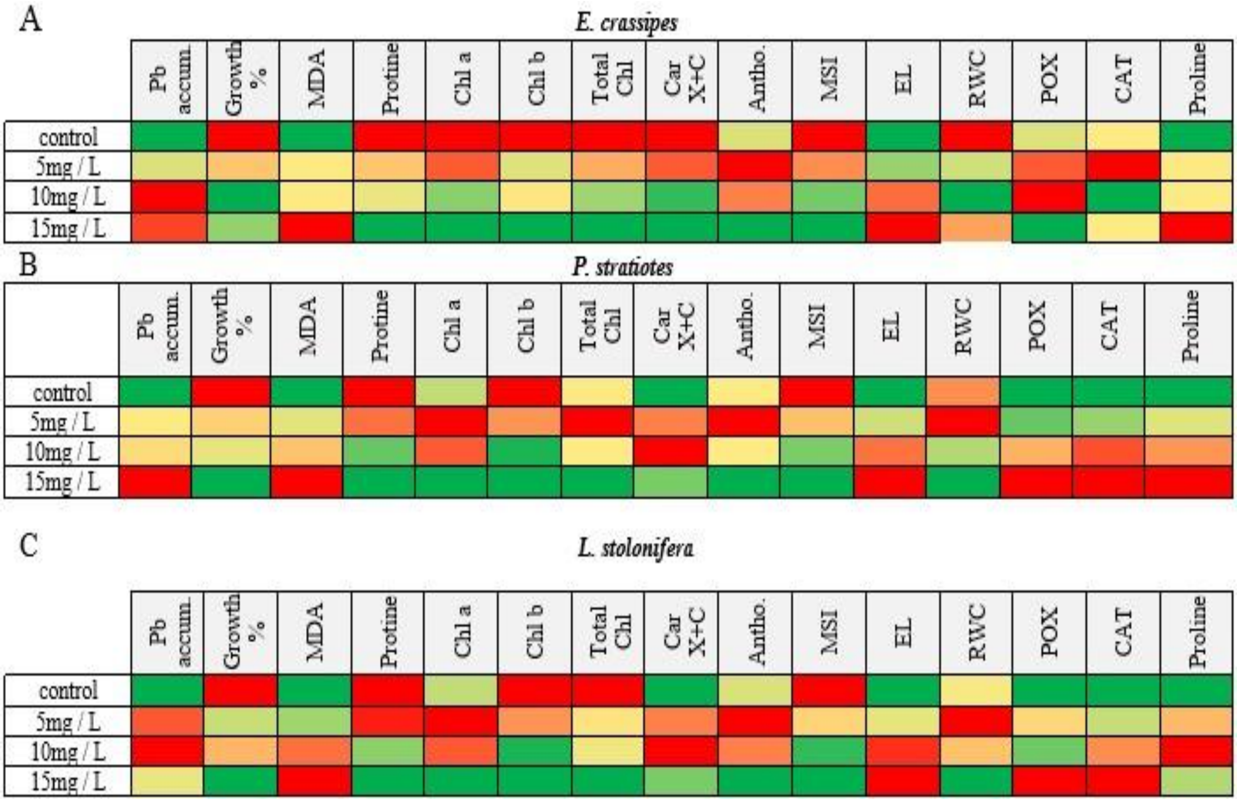
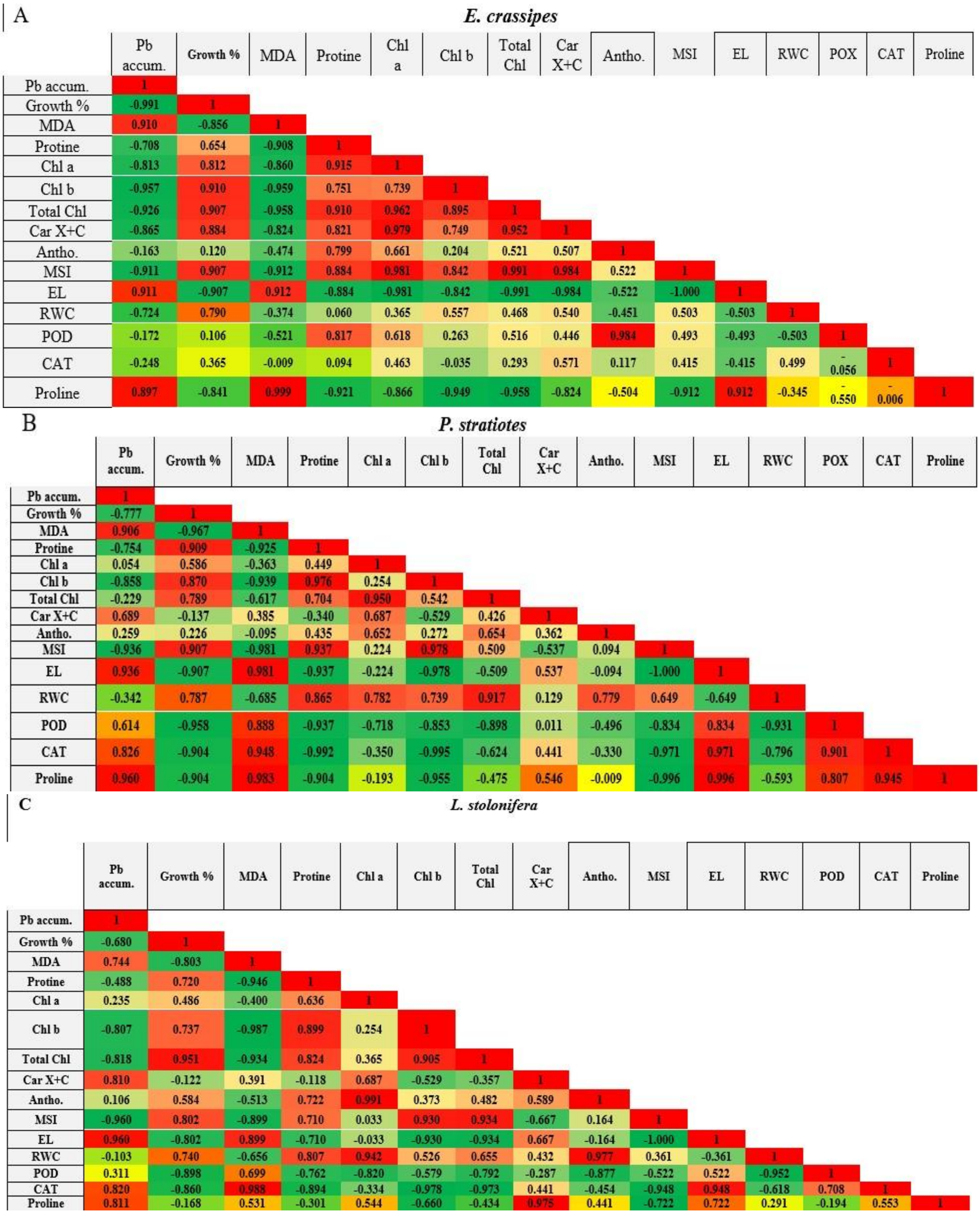


Figure 8: Cluster heat map analysis summarizing Pb concentrations on physiological and phytochemical characteristics of *E. crassipes* (A), *P. stratiotes (B)* and *L. stolonifera* (C).



A

E. crassipes

	Pb accum.	Growth %	MDA	Protine	Chl a	Chl b	Total Chl	Car X+C	Antho.	MSI	EL	RWC	POX	CAT	Proline
Pb accum.	1														
Growth %	-0.991	1													
MDA	0.910	-0.856	1												
Protine	-0.708	0.654	-0.908	1											
Chl a	-0.813	0.812	-0.860	0.915	1										
Chl b	-0.957	0.910	-0.959	0.751	0.739	1									
Total Chl	-0.926	0.907	-0.958	0.910	0.962	0.895	1								
Car X+C	-0.865	0.884	-0.824	0.821	0.979	0.749	0.952	1							
Antho.	-0.163	0.120	-0.474	0.799	0.661	0.204	0.521	0.507	1						
MSI	-0.911	0.907	-0.912	0.884	0.981	0.842	0.991	0.984	0.522	1					
EL	0.911	-0.907	0.912	-0.884	-0.981	-0.842	-0.991	-0.984	-0.522	-1.000	1				
RWC	-0.724	0.790	-0.374	0.060	0.365	0.557	0.468	0.540	-0.451	0.503	-0.503	1			
POD	-0.172	0.106	-0.521	0.817	0.618	0.263	0.516	0.446	0.984	0.493	-0.493	-0.503	1		
CAT	-0.248	0.365	-0.009	0.094	0.463	-0.035	0.293	0.571	0.117	0.415	-0.415	0.499	-0.056	1	
Proline	0.897	-0.841	0.999	-0.921	-0.866	-0.949	-0.958	-0.824	-0.504	-0.912	0.912	-0.345	-0.550	-0.006	1

B

P. stratiotes

	Pb accum.	Growth %	MDA	Protine	Chl a	Chl b	Total Chl	Car X+C	Antho.	MSI	EL	RWC	POX	CAT	Proline
Pb accum.	1														
Growth %	-0.777	1													
MDA	0.906	-0.967	1												
Protine	-0.754	0.909	-0.925	1											
Chl a	0.054	0.586	-0.363	0.449	1										
Chl b	-0.858	0.870	-0.939	0.976	0.254	1									
Total Chl	-0.229	0.789	-0.617	0.704	0.950	0.542	1								
Car X+C	0.689	-0.137	0.385	-0.340	0.687	-0.529	0.426	1							
Antho.	0.259	0.226	-0.095	0.435	0.652	0.272	0.654	0.362	1						
MSI	-0.936	0.907	-0.981	0.937	0.224	0.978	0.509	-0.537	0.094	1					
EL	0.936	-0.907	0.981	-0.937	-0.224	-0.978	-0.509	0.537	-0.094	-1.000	1				
RWC	-0.342	0.787	-0.685	0.865	0.782	0.739	0.917	0.129	0.779	0.649	-0.649	1			
POD	0.614	-0.958	0.888	-0.937	-0.718	-0.853	-0.898	0.011	-0.496	-0.834	0.834	-0.931	1		
CAT	0.826	-0.904	0.948	-0.992	-0.350	-0.995	-0.624	0.441	-0.330	-0.971	0.971	-0.796	0.901	1	
Proline	0.960	-0.904	0.983	-0.904	-0.193	-0.955	-0.475	0.546	-0.009	-0.996	0.996	-0.593	0.807	0.945	1

C

L. stolonifera

	Pb accum.	Growth %	MDA	Protine	Chl a	Chl b	Total Chl	Car X+C	Antho.	MSI	EL	RWC	POD	CAT	Proline
Pb accum.	1														
Growth %	-0.680	1													
MDA	0.744	-0.803	1												
Protine	-0.488	0.720	-0.946	1											
Chl a	0.235	0.486	-0.400	0.636	1										
Chl b	-0.807	0.737	-0.987	0.899	0.254	1									
Total Chl	-0.818	0.951	-0.934	0.824	0.365	0.905	1								
Car X+C	0.810	-0.122	0.391	-0.118	0.687	-0.529	-0.357	1							
Antho.	0.106	0.584	-0.513	0.722	0.991	0.373	0.482	0.589	1						
MSI	-0.960	0.802	-0.899	0.710	0.033	0.930	0.934	-0.667	0.164	1					
EL	0.960	-0.802	0.899	-0.710	-0.033	-0.930	-0.934	0.667	-0.164	-1.000	1				
RWC	-0.103	0.740	-0.656	0.807	0.942	0.526	0.655	0.432	0.977	0.361	-0.361	1			
POD	0.311	-0.898	0.699	-0.762	-0.820	-0.579	-0.792	-0.287	-0.877	-0.522	0.522	-0.952	1		
CAT	0.820	-0.860	0.988	-0.894	-0.334	-0.978	-0.973	0.441	-0.454	-0.948	0.948	-0.618	0.708	1	
Proline	0.811	-0.168	0.531	-0.301	0.544	-0.660	-0.434	0.975	0.441	-0.722	0.722	0.291	-0.194	0.553	1

Figure 9: Pearson correlation coefficients and their significance for all parameters after treatments.

الآليات الفسيولوجية لبعض النباتات المائية لتحمل عنصرالرصاص الملوث للمياه

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الملخص العربي

يعتبر الرصاص ملوثًا معدنى شديد السمية حيث يتواجد فى كثير من مناطق المياه المصرية بتركيزات تتعدى التركيزات المسموح بها من المنظمات الدولية. أجرينا الدراسة الحالية لفحص جدوى إمكانية المعالجة النباتية للرصاص بواسطة ثلاثة أنواع من النباتات المائية من حيث التراكم المعدني في أنسجة النبات وفهم بعض الآليات الفسيولوجية لتلك النباتات لتحمل إجهاد الرصاص. بناءً على النتائج التي توصلنا إليها ، أظهرت جميع النباتات الثلاثة (ورد النيل ، خس الماء ، اللدوجيا) كفاءة عالية في إزالة الرصاص من تركيزات المعدن المختلفة . لوحظ أقصى معدل تراكم للرصاص في جذور كل النباتات المائية المختبرة بالمقارنه لتراكمه فى المجموع الخضرى. بينما أظهرت نبات اللدوجيا أعلى معدل تراكم لكامل النبات في جميع التراكيز .تشير النتائج بقوة إلى أن تلك النباتات أقل تأثراً بالإجهاد التأكسدي على الرغم من وجود جرعة أعلى من الرصاص في وسط الزراعة المائية ، كما هو متوقع بالنسبة للأنواع التي نجت بكفاءة في بيئة شديدة التلوث. يبدو أن زيادة نشاط مضادات الأكسدة الإنزيمية (الكاتليز و البروكسيديز) والغير إنزيمية (الأنثوسيانين والبرولين) يلعبان أدوارًا رئيسية كآلية تحمل لتلك النباتات عند تعرضها لإجهاد الرصاص وخاصة نبات اللدوجيا، حيث أظهر أعلى قيمة لثبات الغشاء وإستقرار البروتين . بالإضافة إلى ذلك أعطى أقل قيمة للتسرب الكهربى ومحتوى المالونديالدهيد مقارنة بالأنواع الأخرى. بينما أظهر نبات خس الماء أعلى معدل ثبات للكلوروفيل في النباتات المختبرة تحت ظروف الإجهاد.

الكلمات الاسترشادية: النباتات المائية، تراكم الرصاص، آليات التحمل، الإنزيمات المضادة للأكسدة البرولين.