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Biochemical Studies on the Effects of Zinc and Lead on Oxidative Stress, Antioxidant Enzymes and Lipid Peroxidation in Okra (*Hibiscus esculentus* cv. *Hassawi*)

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ABSTRACT

Hibiscus esculentus plants were grown in 5, 10, 20 and 40 mM of Zn^{2+} or/and Pb^{2+} for 4 weeks. The treated *Hibiscus esculentus* plants were analyzed with focus on the effects of Zn^{2+} or/and Pb^{2+} on antioxidant enzymes and antioxidants in shoots. An enhanced level of lipid peroxidation and an increased of hydrogen peroxide (H_2O_2) in shoots indicated that Zn^{2+} or/and Pb^{2+} caused oxidative stress in *Hibiscus esculentus* plants. Zn^{2+} or/and Pb^{2+} induced a diminish in superoxide dismutase (SOD) activity whereas catalase (CAT) was significantly increased. Glutathione peroxidase (GPOD) activity decreased, enhancement in glutathione reductase (GR) activity was recorded more at 20 and 40 mM than at 5 and 10 mM Zn^{2+} or/and Pb^{2+}. While, ascorbate peroxidase (APOD) exhibit maximum stimulation at high concentrations of Zn^{2+} or/and Pb^{2+} in shoots. Augmented activities of some antioxidant enzymes in Zn^{2+} or/and Pb^{2+} treated plants propose that they have some additive function in the mechanism of metal tolerance in *Hibiscus esculentus* plants. The levels of reduced glutathione (GSH) in shoots of *Hibiscus esculentus* plants treated with Zn^{2+} or/and Pb^{2+} were declined. Zn^{2+} or/and Pb^{2+} decreased the amount of glutathione disulphide (GSSG) as a whole and this decrease was concentration-dependent. Ascorbate (AS) level was augmented slightly at lower levels but decreased at higher levels of Zn^{2+} or/and Pb^{2+}, while increments in dehyroascorbic acid (DAS) content was observed at either 20 or 40 mM of Zn^{2+} or/and Pb^{2+}. Therefore, the AS/DAS relation declined with Zn^{2+} or/and Pb^{2+} treatment.

Key words: Heavy metals, plants, oxidative stress, antioxidant enzymes, antioxidants

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INTRODUCTION

Pollution of environment by toxic metals arises as a result of various industrial activities and has turned these metal ions into major health issue[1]. Soil pollution with Heavy Metals (HM) is a universal problem, leading to agricultural losses and hazardous health effects as metals enter the food chain. HM can infect soils in town areas, between many of them, Zn^{2+} and Pb deriving from galvanized metals, rubber production batteries, petrol, steel mill residues and paints. The phytotoxicity of the heavy metals because of industrial pollution has serious implications in soil degradation[2]. This may diminish both the quality and productivity of plants. Heavy metals show a discrepancy according to their role in metabolic functions. Microelements, such as zinc, are necessary and are involved in numerous physiological processes[3]; however, at high concentrations, they are strongly toxic and prejudice plant growth[4]. Heavy metals as cadmium are very toxic even at low concentrations[5]. Zinc is a main industrial pollutant of the global and aquatic environment[6]. Zinc toxicity leads to chlorosis in little leaves and inhibits photosynthesis at different steps and through different mechanisms. Zinc is evidence for a specific effect on the Calvin cycle[7] and photosystem activities[8]. Plants act in response to heavy metal ion stress in different ways including exclusion and chelation, expression of stress protein genes.

Zinc is an indispensable trace element for the normal healthy growth and reproduction of some plants; it required in relatively small concentrations in the plant tissues[9]. It is extremely toxic at elevated concentrations and can slow down plant growth and disrupt various essential physiological processes[10,11]. Zn^{2+} was diminished respiratory rate and increased of membrane damage in sunflower plants[12]. An excess of Zn^{2+} is indicated by diminishing in growth and development, metabolic activity and an induction of oxidative damage in various plant species[13]. Zn^{2+} nutrition affected the accumulation

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of other nutrients in different plants[12]. However, because of the similarities in ion radius of divalent cations, excess zinc can shift certain physiological equilibrium by local competition at various sites[14].

Lead (Pb^{2+}) is a heavy metal, with specific toxic action and central sources of environmental pollution. Because of its use as antiknock in internal combustion engines, it has been widely scattered, so may be present in many areas[14]. Lead inhibits metabolic procedures such as nitrogen assimilation, photosynthesis, respiration, water uptake and transcription[15-20]. Binding nucleic acids to lead causes aggregation and condensation of chromatin, as well as stabilization of the DNA double helix inhibiting the processes of replication and transcription[17] Lead causes unfavorable processes in biological systems such as, inactivates several enzymes by binding with their SH-groups[21]. Moreover, lead ions, as those of other heavy metals, can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress[22,23]. These processes, which destructively affect cell structure and metabolism, are mutually connected and stimulate each other, which may result in a decreased efficiency of oxidation-reduction enzymes or the electron transport system leading to fast production of ROS in the cell[24]. The oxidative stress results in production of toxic free radicals in plants[25]. Heavy metals accelerate production of Reactive Oxygen Species (ROS) that have the ability to initiate lipid peroxidation and degrade proteins, lipids and nucleic acids[26]. Plants act in response to heavy metals by changes in the levels of antioxidants and antioxidative enzymes[27,28].

The okra plant (*Hibiscus esculentus* L., member of Malvaceae) is established in many parts of Africa, the Mediterranean region. Plant parts, as flowers and green tissues, may be consumed as specialty foods, while the seed oil may be used for industrial or food purposes. The knowledge of the toxic effects of Zn^{2+} and Pb^{2+} on biochemical and physiological processes in Hassawi okra is potentially useful to establish criteria for selection of plants to be used as bio-indicators. The rationale of this investigation is to evaluate the influence of the external Zn^{2+} and Pb^{2+} concentrations on some physiological parameters and oxidative enzymes in Hassawi okra in an attempt to find differential characteristics to be used as bioindicators of toxic levels of Zn^{2+} or/and Pb^{2+}. In addition, this study will afford better understanding to the tolerance mechanisms of Hassawi okra to the toxicty of Zn^{2+} or/and Pb^{2+}. Hassawi okra is local cultivar ad was chosen because it cultivated widely in Al-Hassa region (eastern region) in Saudi Arabia.

It could be concluded that Zn^{2+}or/and Pb^{2+} induced increase in the levels of some antioxidative enzymes may represent a secondary defensive mechanism against oxidative stress that are not as direct as the primary defensive responses such as phytochelatins and vacuolar compartmentalization. Also, acute concentrations of Zn^{2+}or/and Pb^{2+} may adversely affect the activity of certain defense enzymes either by inhibiting their synthesis or by their inactivation and down regulation.

MATERIALS AND METHODS

Plant materials and treatments: Homogenous okra (*Hibiscus esculentus* cv. Hassawi) Plants were grown in plastic pots (12 cm in diameter and 30 cm in height) lined with polyethylene bags and filled with soil composed of air dried clay and sand (1:1 by volume) in growth chamber under constant conditions. Seeds were surface sterilized with 0.1% sodium hypochlorite for 5 min and washed three times with sterile water. Sterile seeds were germinated in dark on plastic trays covered with wet filter papers and were kept wet by spraying with sterile water. After complete germination, five uniformly germinated seeds were sown at a depth of 1 cm in each pot.

Zinc and lead treatment: The *Hibiscus esculentus* cv. Hassawi plants were divided into 4 groups. The first supplied with sterile water (control). The second group were supplied with 5, 10, 20 and 40 mM Zn^{2+} as $Zn(NO_3)_2$. The third group were supplied with 5, 10, 20 and 40 mM Pb^{2+} as $Pb(NO_3)_2$. The forth group were supplied with 5, 10, 20 and 40 mM of both $Zn^{2+}$and Pb^{2+}. The soil was then thoroughly mixed with the applied concentrations of Zn^{2+} and Pb^{2+}or both. Three replicates for each treatment were prepared. The plants were watered with distilled water to field capacity by weighing the pots daily and left to grow until the end of experiment [thirty days after sowing (DAS)].

Harvesting: At the end experiment, okra plants were harvested and washed with distilled water three times.

Enzyme extract: Shoots of *Hibiscus esculentus* plants (5 g) were ground with a mortar and pestle in homogenization buffer containing 50 mM potassium phosphate (pH 7.5), 1 mM EDTA, 0.1% Triton x-100 and 1.0% polyvinyl pyrophosphate. The homogenate was filtered throughout four layers of muslin fabric and centrifuged at 5000 rpm for 15 min at 4°C. Supernatant equivalent of 50 µg protein was used for the estimation of enzyme activity.

Enzyme assays: Superoxide dismutase (SOD) activity was determined by the method described by Spitz and Oberley[51]. One unit (U) of SOD is the amount of enzyme necessary to cause 50% inhibition of the colour reaction at 25°C and pH 7.8.

Catalase assay: (CAT) activity was measured as described by Claiborne[52]. One unit of catalase was defined as the amount that decomposes 1 µmol H_2O_2 per min at 25°C and pH 7.8.

Glutathione peroxidase assay: (GPOD) was assayed according to Gunzler and Flohe[53]. One unit of GPOD activity was defined as the amount oxidizing 1 µmol NADPH per min at 25°C and pH 7.0.

Ascorbate peroxidase assay: (APOD) was measured as described by Asada[54]. One unit of APOD activity was defined as the amount oxidizing 1 µmol ascorbate per min at 25°C and pH 7.0.

Glutathione reductase assay: (GR; EC 1.6.4.2.) was determined using the method of Racker[55]. One unit of GR activity was defined as the oxidation of 1 µmol NADPH per min at 25°C and pH 7.6.

Dehydroascorbic acid reductase assay: (DHAR) was measured by the method adopted by Asada[54]. One unit of DHAR activity was defined as the formation of 1 µmol ascorbate at 25°C and pH 6.5.

Estimation of antioxidants compounds: Ascorbate (AS), dehydroascorbate (DAS), reduced glutathione (GSH) and glutathione disulphide (GSSG) were extracted and measured according to the method of Doulis[56] and Kingston[57].

Lipid peroxidation: Lipid peroxidation was estimated in root tissues as described by Du and Bramlage[58].

Hydrogen peroxide determination: The hydrogen peroxide level was colorimetrically determined according to the method of Jena and Choudhuri[59].

Determination of proteins: Proteins were determined according to Bradford[60].

RESULTS AND DISCUSSION

Lipid peroxidation: The malondialdehyde (MDA) level is one of the most significant 2-thiobarbituric acid (TBA) reactive metabolites is decreased only in 5 mM Zn^2 but increased in Zn^{2+} or/and Pb^{2+}treated *Hibiscus esculentus* plants (Fig. 1). Such increased of lipid peroxides indicates that Zn^{2+} or/and Pb^{2+} caused oxidative damage to plants. Gallego[29] reported that in sunflower and pea, heavy metals induced oxidative stress was mediated by generation of reactive oxygen species (ROS). In many plant species heavy metals have been reported to origin oxidative damage due to production of ROS. To resist oxidative damage, the antioxidant

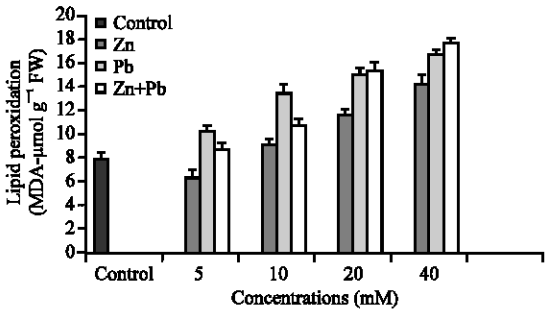

Fig. 1: Effect of Zn^{2+} or/and Pb^{2+}-treatment on lipid peroxidation in *Hibiscus esculentus* shoots. Values are means of three measurements ± SE

enzymes and definite metabolites existent in plants play a vital role leading to adaptation and ultimate survival of plants during periods of stress[30]. The present study suggests that Zn^{2+} and Pb^{2+} toxicity leads to production of lipid peroxides and induces some of the key enzymes of antioxidant defense system in *Hibiscus esculentus* plants. Induction in the activities of antioxidant enzymes is a common approach adopted by plants to overwhelmed oxidative stress due to the imposition of environmental stresses[31]. Lipid peroxidation is a biochemical indicator for the free radical mediated damage. Our results display an increase in the level of lipid peroxides with increasing concentrations of Zn^{2+} or/and Pb^{2+}, indicating that Zn^{2+} or/and Pb^{2+} induce oxidative stress in *Hibiscus esculentus* plants. The results in the present work are in conformity with the explanations of Panda *et al.*[13] who stated heavy meatl induced oxidative stress in wheat leaves cells. Similar to our comments, higher lipid peroxidations have been stated under Pb^{2+} [19], Cd^{2+} and Cu^{2+} toxicity[25] in different plant species.

Hydrogen peroxide level: As a signal in plants, the H_2O_2 could be induced by many environmental stresses and an important reactor in the resistance of plants to environmental stresses[31]. The action of H_2O_2 in stress resistance of plants was involved in many regulation processes[32,33]. The accumulation of H_2O_2 could reflect the oxidative stress and the changes of antioxidants in plant. In this study, H_2O_2 content in shoots of *Hibiscus esculentus* was decreased only at 5 mM Zn^{2+} but increased depending on Zn^{2+} or/and Pb^{2+} concentrations (Fig. 2). The content of H_2O_2 with Pb^{2+} was much higher than that with Zn^{2+} treatment. In support, Chao *et al.*[34] reported rapid generation of H_2O_2 by Zn^{2+} treated plant cells.

Superoxide dismutase activity: SOD activity at 10, 20 and 40 Zn^{2+} or/and Pb^{2+}exposed plants mostly remained lower than the control in shoots whereas, only at 5 mM Zn^{2+} the SOD activity is increased (Fig. 3). SOD is an

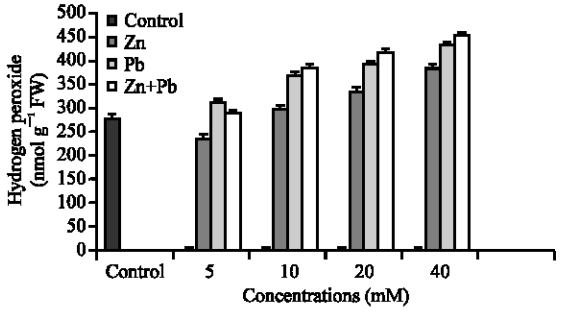

Fig. 2: Effect of Zn^{2+} or/and Pb^{2+}-treatment on the induced-generation of H_2O_2 in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

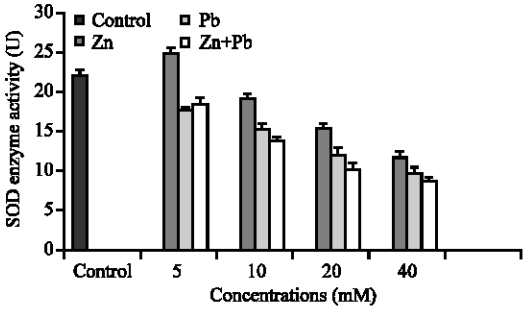

Fig. 3: Effect of Zn^{2+} or/and Pb^{2+}-treatment on SOD in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

essential component of antioxidant defense system in plants and it dismutates two superoxide radicals ($O_2^{\bullet-}$) to water and O_2. Our results show increased activity of SOD in *Hibiscus esculentus* plants growing under toxic levels of Zn^{2+} or/and Pb^{2+}. Previous reports revealed a variable response of an increase or decrease in SOD activity in plants exposed to different metals including Zn^{2+} and Cu^{2+} [30,35]. A reduction in SOD activity in the metal treated plants has been attributed to an inactivation of the enzyme by H_2O_2 that is formed in different cellular compartments where SOD catalyses the scavenging of superoxide radicals. A number of non-enzymatic and enzymatic processes in cells[36,37] can also produce H_2O_2. SOD activity has been reported to increase under salinity[32], Zn, Cd, Cu, Ni and Pb toxicity[4,15,17,25,35]. Increase in SOD activity in response to stress appears to be probably due to *de-novo* synthesis of the enzymes protein[38]. Transgenic plants over-expressing super oxide dismutase, show increased tolerance towards oxidative damage caused due to harsh environmental conditions and among antioxidant enzymes, the activity levels of SOD are of more relevance in maintenance of the overall defense system of plants subjected to oxidative stress[46].

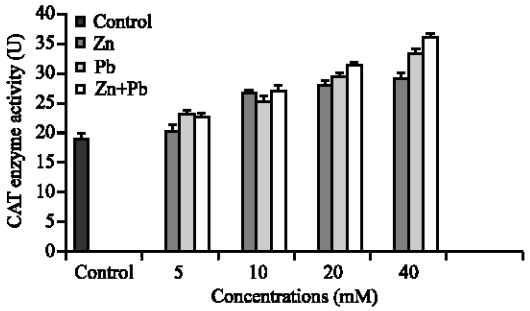

Fig. 4: Effect of Zn^{2+} or/and Pb^{2+}-treatment on CAT in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

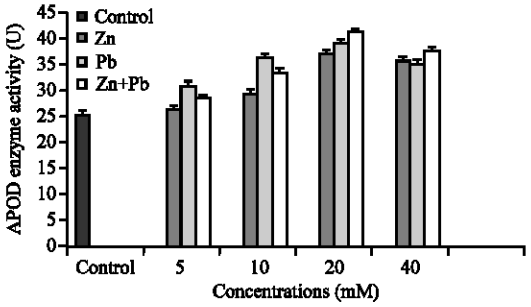

Fig. 5: Effect of Zn^{2+} or/and Pb^{2+}-treatment on APOD in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

Azooz and Abou-Elhamd[32] reported that, in maize cultivars, SOD activity increased sharply in the salt tolerance cultivars, whereas there was no visible increase in the salt sensitive cultivars.

Catalase and ascrobate peroxidase activities: Catalase is universally present oxidoreductase that decomposes H_2O_2 to water and molecular oxygen and it is one of the key enzymes involved in removal of toxic peroxides. Zn^{2+} or/ and Pb^{2+} treatment induced the activities of CAT and APOD in shoots of *Hibiscus esculentus* (Fig. 4, 5). These results are in harmony with those of Erdeil[38] and indicate that the two enzymes were functioning in shoots to remove H_2O_2. A decline in catalase activity under Zn^{2+} or/and Pb^{2+} toxicity was observed in our study that suggests a possible delay in removal of H_2O_2 and toxic peroxides mediated by catalase and in turn, an enhancement in the free radical mediated lipid peroxidation under Zn^{2+} and Pb^{2+} toxicity. However, similar to our studies in subcellular compartments of pea root cells increased catalase activity was observed when plants were grown in nutrient medium containing 0.5 or 1 mM Pb^{+2} [18]. In contrary, decline in catalase activity was reported in sensitive

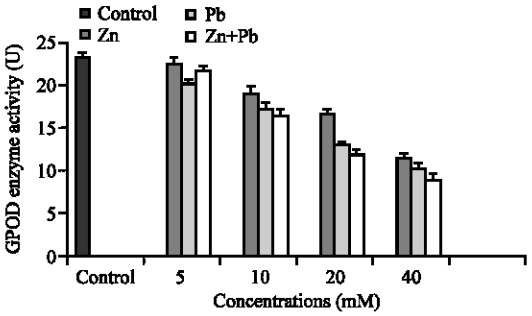

Fig. 6: Effect of Zn^{2+} or/and Pb^{2+}-treatment on GPOD in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

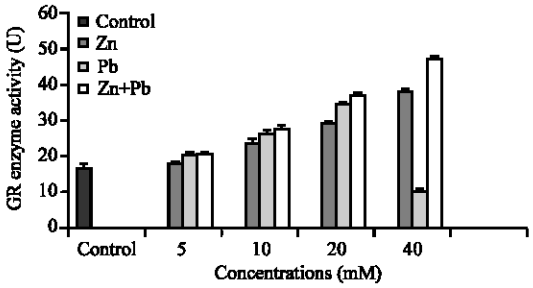

Fig. 7: Effect of Zn^{2+} or/and Pb^{2+}-treatment on GR in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

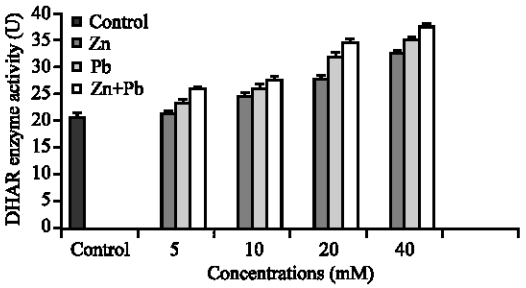

Fig. 8: Effect of Zn^{2+} or/and Pb^{2+}-treatment on DHAR in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

maize cultivars grown under salinity stress[32]. A reduction in catalase activity under stressful conditions has been attributed to the inactivation of enzyme protein due to ROS decrease in enzyme synthesis or change in assembly of enzyme subunits[26]. The inadequate response of CAT activity to Zn^{2+} or Pb^{2+} in shoots was compensated by the increased activity of APOD in this part of plant. Moreover, the induction of APOD provides an additional defense against metal toxicity and keeps the metabolic activities in *Hibiscus esculentus* shoots. The enzymes components associated with defense against ROS include SOD, catalase, ascrobate peroxidase and enzymes of ascorbate/glutathione cycle. SOD and catalase have been identified as enzymatic protectors against peroxidation reactions[34].

Glutathione peroxidase and reductase activities: GPOD catalyzes the reduction of H_2O_2, organic hydroperoxides and lipid hydroperoxides by GSH. GPOD activity is diminished in *Hibiscus esculentus* shoots of the Zn^{2+} or/and Pb^{2+}- treated *Hibiscus esculentus* (Fig. 6). Activation of the ascorbate glutathione cycle has been found to be indispensable in stressed plants to combat oxidative damage[39]. GR is another enzyme of the ascorbate glutathione cycle, which reduces GSSG to GSH that maintains the cellular redox status and also serves as substrate for phytochelatin synthesis. GR was activated in shoots of the Zn^{2+}or /and Pb2^{+}-treated in *Hibiscus esculentus* plants (Fig. 7). GR from other plant sources has been shown to be induced by heavy metals as Cd^{2+}, Zn^{2+} [30,35,40]. Struzyñska and Bokara[41,42] reported that Pb^{2+}stimulate GR enzyme. However, Cd^{2+} decreases the activity of this enzyme in *Helianthus annuus*[29]. The increase in APOD and GR activities in Zn^{2+} and Pb^{2+}-exposed *Hibiscus esculentus* plants may maintain ascorbate and glutathione turnover and activation of the H_2O_2 scavenging ascorbate glutathione cycle.

Ascorbate peroxidase and dehydroascorbic acid reductase activity: DHAR showed a similar behavior to GR (Fig. 8) at both Zn^{2+}or/and Pb^{2+} concentrations. Gallego[29] reports similar results. Usually, metal ions may enhance either the generation of ROS, by expressing the electron transfer in single electron reactions involving metal cations, or as a consequence to metal inactivated metabolic reactions[43]. However, the fluctuation of ROS is most probably high in shoots because it is photosynthetic tissue.

Ascorbate peroxidase (APOD) and glutathione reductase (GR) are indispensable components of the ascorbate glutathione pathway, required to scavenge H_2O_2 produced mainly in chloroplasts and other cell organelles and to maintain the redox state of the cell[36]. APOD utilizes the reducing influence of ascorbic acid to abolish potentially harmful H_2O_2. Our results indicate an enhancement in the activity of APOD in response to Zn^{2+} and Pb^{2+} stress. Similar induction was reported in response to mild salt stress[32], Cu toxicity[17,25]. Catalase along with APOD and SODs are considered as vital enzymes within the antioxidant defense mechanism, which directly determine the cellular concentration of $O_2^{\bullet-}$ and H_2O_2[25]. Glutathione reductase (GSH) catalyzes the NADPH dependent reduction of oxidized glutathione (GSSG) to reduced glutathione. Owing to its redox active thiol group, GSH is involved in the redox

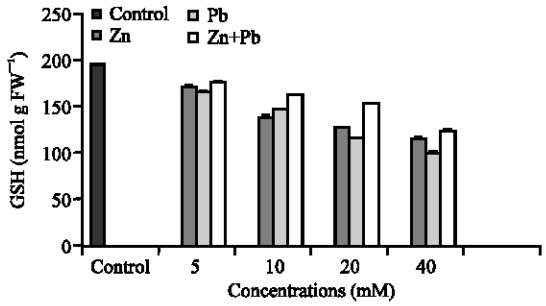

Fig. 9: Effect of Zn^{2+} or/and Pb^{2+}-treatment on GSH contents in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

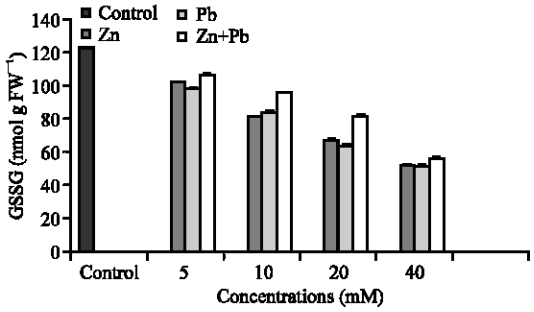

Fig. 10: Effect of Zn^{2+} or/and Pb^{2+}-treatment on GSSG contents in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

regulation of the cell cycle[37,41,45] and has often been considered to play an important role in defense of plants and other organisms against oxidative stress[47]. Being a main water soluble antioxidant in plant cells, GSH directly reduces most active oxygen species, while GR uses NADPH to reduce GSSG to GSH[37]. Various free radicals and oxidants are able to oxidize GSH to GSSG[25]. Higher cellular GSH levels are associated with heavy metal tolerance in tomato cells[9,32] and heavy metal exposure leads to accelerated GSH synthesis in roots and cultured cells[47].

Levels of glutathione reduced and oxidized forms: In the present study GSH showed a concentration dependent decrease in its level in *Hibiscus esculentus* shoots of Zn^{2+}or/and Pb^{2+} exposed plants (Fig. 9). These results suggested a close relation between the GSH content and the H_2O_2 content in the shoots of the *Hibiscus esculentus.* The results also imply that H_2O_2 may have toxic effect and acts as a negative signal for the accumulation of Zn^{2+}or/and Pb^{2+} in *Hibiscus esculentus* shoots. The turn down level in GSH may be attributed to low down GR activity[44,45]. Our results show increased GR activity in Zn^{2+}or/and Pb^{2+} treated *Hibiscus esculentus* shoots which

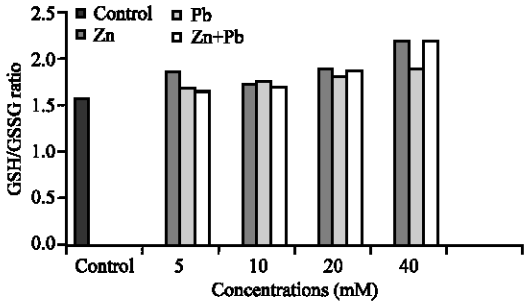

Fig. 11: Effect of Zn^{2+} or/and Pb^{2+}-treatment on GSH/GSSG ratio in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

suggests possible involvement of GR in regenerating GSH from GSSG (Fig. 10) under Zn^{2+}or/and Pb^{2+} toxicity conditions to increase GSH/GSSG ratio (Fig. 11) and the total glutathione pool[41]. Similar to our findings, induction in GR activity has been reported in the leaves of Cd and Cu stressed marrow roots[25]. Increase in the activity of GR has been attributed to the *de novo* synthesis of the enzyme protein[22]. The results suggest that Pb^{2+} toxicity causes oxidative stress in *Hibiscus esculentus* shoots and the enzymes peroxidases, SOD and GR appear to play a pivotal role in combating oxidative stress in plants. As, unlike iron, Pb is not an oxido-reducing metal, the oxidative stress induced by Zn^{2+} or/and Pb^{2+} in *Hibiscus esculentus* shoots appears to be an indirect effect of Zn^{2+}or/and Pb^{2+} toxicity leading to production of ROS with a simultaneous increase in tissue levels of SOD, peroxides and GR. Another reason for an overall decrease in the endogenous level of GSH might be due to its utilization as a reducing substrate in the synthesis of ascorbate. In addition, GSH is probably consumed and degraded with the purpose of protecting cellular membranes from lipid peroxidation[46]. The interaction of GSH with peroxyl radicals during peroxidation of liposomes initiated in the aqueous phase may also be a cause of GSH depletion[47]. The total of GSSG as Zn^{2+} or/and Pb^{2+} decreased a whole and this decrease was concentration dependent (Fig. 6, 7). The ratio of GSH/GSSG remained more or less constant. These results are in harmony with those of Rauser[21]. In addition, DeVos.[48] reported more than a 50% decrease in GSH level in roots of heavy metal treated *Silene cucubalis.*

Levels of Ascorbate and dehyroascorbic acid: Ascorbate (AS) level (Fig. 12) increased slightly at 5 mM of Zn^{2+} but it was decreased at the higher levels of Zn^{2+}or/and Pb^{2+}. On the other hand, increments in dehyroascorbic acid (DAS) content were observed at 10, 20 and 40 mM of Zn^{2+}or/and Pb^{2+} (Fig. 13). Therefore, the AS/DAS ratio (Fig. 14) declined with Zn^{2+}or/and Pb^{2+}treatments.

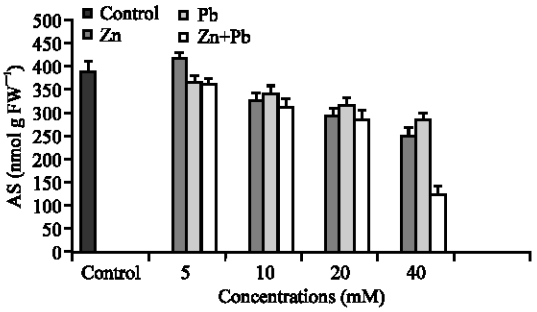

Fig. 12: Effect of Zn^{2+} or/and Pb^{2+}-treatment on AS contents in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

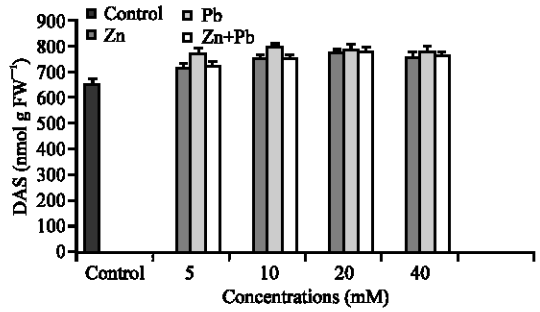

Fig. 13: Effect of Zn^{2+} or/and Pb^{2+}-treatment on DAS contents in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

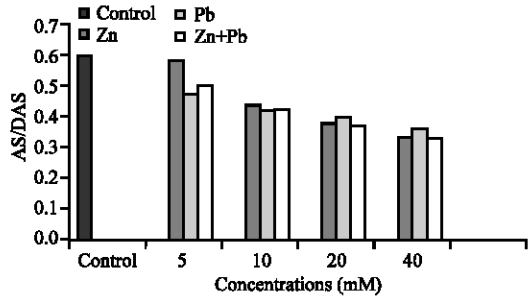

Fig. 14: Effect of Zn^{2+} or/and Pb^{2+}-treatment on AS/DAS ratio in *Hibiscus esculentus* shoots. Values are means of three measurements ±SE

Zn^{2+} and Pb^{2+} are the greatest abundant heavy metals polluting the soil environment. These heavy metals are readily absorbed by plants mostly through the root system and thereafter exert their toxicity symptoms. Metal phytotoxicity happens when metal transfer from soil to plant roots and are additional transported to different sites in the shoots. The effects of Zn^{2+} and Pb^{2+} phytotoxicity include inhibited growth, blackening of the root systems[49]. Our results indicated a decrease in growth of *Hibiscus esculentus* seedlings under increasing levels of Zn^{2+} or/and Pb^{2+} in culture experiments. We conducted experiments with Zn^{2+} or/and Pb^{2+} of increasing concentrations up to 40 mM and based on our observations (data not reported here) we concluded a Zn^{2+} or/and Pb^{2+} level of 40 mM as moderately toxic and 80 mM as highly toxic (Lethal level)[49]. Decreased growth in *Hibiscus esculentus* due to Zn^{2+} and Pb^{2+} could possibly be attributed to the interference of Zn^{2+} and Pb^{2+} with the metabolic and biochemical processes associated with normal growth and development of the plant. The present results show that Pb^{2+} is more phytotoxic than Zn^{2+}. This is because Zn^{2+} is an essential element for plants and may be absorbed and become involved in metabolic pathways more readily than Pb^{2+}. DeVos and Azooz[48,49] suggested that Zn^{2+} induced damage to integral proteins, through the formation of disulfide links, resulted in increased cell membrane permeability and ion efflux.

The present results suggest that Zn^{2+}or/and Pb^{2+} induced increase in the levels of some antioxidative enzymes may represent a secondary defensive mechanism against oxidative stress that are not as direct as the primary defensive responses such as phytochelatins and vacuolar compartmentalization[50]. Also, acute concentrations of Zn^{2+}or/and Pb^{2+} may adversely affect the activity of certain defense enzymes either by inhibiting their synthesis or by their inactivation and down regulation.

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