ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Changes in Growth and Antioxidant Enzymes in Bean (*Phaseolus vulgaris* L.) Under Heavy Metal Stress.

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ABSTRACT

The impact of different concentrations of copper sulphate, lead nitrate and mercuric chloride [12, 25, 37.4 mg/kg for ($CuSO_4.5H_2O$) , 30, 60, 80 mg/kg for [Pb ($NO_3)_2$] and 8, 16, 24 mg/kg for (HgC_{l2})] on morphological parameters (root and shoot length, fresh and dry weight and water content) and antioxidant enzymes (catalase, poly-phenol oxidase and peroxidise) were investigated in bean seedlings {Phaseolus vulgaris cv. Giza 6) grown in peat moss. Our findings show that all morphological parameters decreased as concentration of metals was increased as compared to control plants. The present results showed that the increment of copper sulphate and lead nitrate concentration in the soil was associated with the enhancement in catalase activity. CAT activity increased in coincidence with low concentrations of Hgcl2 while decreased under higher level. Activity of both Peroxidise (POX) and polyphenol oxidase (PPO) stimulated with increasing doses of the metals studied. Based on these results it is concluded that the strongest effect on growth and antioxidant enzymes was found in bean plants exposed to mercury, followed by the sequence Cu++> Pb++.

Keywords: Catalase, peroxidase, polyphenol oxidase, mercury, copper, lead.

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ISSN: 0975-8585

INTRODUCTION

The term "heavy metals" is indicative of any metallic element that has a relatively high density and is toxicant even at low concentration. Furthermore, it specifies to the group of metals and metalloids with atomic density greater than 4 g/ cm3, or 5 times or more, bigger than water. Heavy metals have greatest presence in soil and aquatic ecosystems and to a relatively littler rate in the atmosphere as participate or vapours **(Nagajyoti et al., 2010).**

In plants, heavy metal toxicity changes with plant species, specific metal dosage, chemical form, soil constitution and pH. Plants are immobile; thereby roots of a plant are the primary contiguity location for heavy metal ions. Metal contamination questions are becoming mostly widespread in Egypt and elsewhere, with several documented cases of metal toxicity in the mining industry, metallurgic industry, smelters, coal-fired power plants, mud dumps and agriculture. Heavy metal accumulation in soils is of worry especially in agricultural production due to the inimical impacts on food safety and marketability, crop growth due to phytotoxicity, and environmental health of soil organisms **(Nagajyoti et al., 2010; Shah et al., 2010).** It has been found that plants growing in metal- polluted locations display changed metabolism, growth reduction, lower biomass production and metal accumulation. However, few metals such as copper, manganese, cobalt, zinc and chromium are fundamental to plant metabolism in trace amounts. Therefore, it is only when metals are existent in bio-obtainable forms and at excess levels, they have the possibility to become poisonous **(Nagajyoti et al., 2010).** Metals such as Cu, Zn, Fe, Mn, Mo, Ni and Co are essential micronutrients, whose absorption in overabundance to the plant needs leads to toxic effects. They are also designated as trace elements since they are present in trace (10 mg kg"1, or mg L'1) or in ultra trace (1 ug kg"1,or ug L"1) amounts in the environmental matrices **(Lebrun, 2001; Monni et al., 2000; Nagajyoti et al., 2010).**

Two principal functions of intrinsic heavy metals are the following: (1) Participating in redox reactions, and (2) Being complementary part of numerous enzymes. Several other metals have no biological role (e.g. Cd, Al, Ag, Pb, and Hg), and are dispensable and probably toxicant to microorganisms **(Kumara, 2011; IMagajyoti et al., 2010).**

Copper is an essential heavy metal for higher plants and algae, especially for photosynthesis. Cu is a constituent of primary electron donor in pho-tosystem 1 of plants. Cu can easily acquire and lose an electron, for that reason it is a cofactor of oxidase, mono- and di-oxygenase (e.g., amine oxidases, ammoniamonoxidase, ceruloplasmin, lysyl oxidase) and of enzymes implicated in the removal of superoxide radicals (e.g., superoxide dismutase and ascorbate oxidase) **(Nagajyoti et al., 2010).**

At high subjection lead is toxic to the majority of living organisms and there is no apparent biological requirement. Interference of lead with growth, maintenance, and/or photosynthesis processes can be anticipated in view of the fact that most of the physiologically active tissues of plants are implicated in the abovementioned processes. Indeed, excepting near smelters or mines, such reactions have been noticed in laboratory experiments at lead doses greater than those usually found in the nature **(Fargasova, 2004).**

Mercury (Hg) is a toxic metal. When liberated from its natural and anthropogenetic activeness, it gets into the atmosphere originally in vapor or elemental state, as inorganic, mono- or divalent salts; or as an organo- mercurial such as methyl mercury. Plants are able to cumulate Hg under higher dosages, which lessens their photosynthetic pigments and has inimical impacts on seedling growth, development, and metabolism **(Ali et al., 2002).**

The purpose of current study is to dissect whether Cu-, Pb- and Hg-stimulated phytotoxicity evinced as growth prevention in bean seedlings is intermediated by oxidative stress.

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MATERIALS AND METHODS

Plant material and heavy metal

Treatment

Seeds of bean (*Phaseolus vulgaris* cv. Giza 6) purchased from agriculture directorate, Zagazig, Egypt. Prior to germination, to prevent any fungal/bacterial contamination seeds were surface-sterilized with 5% (v/v) sodium hypochlorite (NaOCI) for 10 minutes and rinsed several times with distilled water, then soaked for 5 h in distilled water. The sterilized seeds were planted in plastic pots containing peat moss (obtained from Aamer company of agricultural production, New Salheya) under natural conditions of light and temperature in a random block design in the green house of Botany department, Faculty of Science, Zagazig University. 500 g peat moss was taken in plastic pots of 16cm diameter and 14cm height. In each pot 5 seeds were sown. All the pots were watered to field capacity daily. Plants were thinned to a single plant per pot 1 week after germination.

The different concentration of heavy metals chosen were 12, 25, 37.4 mg/kg for copper sulphate (CuSO4.5H2O) (M.W-249.7), 30, 60, 80 mg/kg for lead nitrate Pb (NO3)2 (M.W-331.2) and 8, 16, 24 mg/l for mercuric chloride [Hg Cl2] (M.W-272). After heavy metal exposure, plant samples were harvested, washed, frozen in liquid nitrogen and stored in a -20 °C deep freezer for subsequent steps of measurement of various biochemical constituents. Both controls and heavy metal-treated experiments were analyzed in triplicate.

In case of Cu- and Pb-treated plants morphological parameters and antioxidant enzymes were studied at 4 days interval upto twelve days after initiation of heavy metal stress. While in case of Hg-treated plants they were studied at one day interval upto four days after initiation of heavy metal stress.

Morphological observations

Morphological observations like root length, shoot length, fresh and dry weight were recorded after each harvest. Fresh and dry weight was taken with the help of digital balance (Citizen CY 204). Dry masses (dried at 70°C for 72 hr). Fresh and dry weight used to determine the plant water content [100- (dry massxlOO/fresh mass)] **(Kovacik et al., 2010).**

Antioxidant enzymes

Enzymes extraction

A known fresh weight of plant material (0.5 g) was homogenized in 50 mM cold phosphate buffer (pH 6.5) contained 1 mM EDTA, Na2 and centri-fuged at 10.000 rpm for 10 min. The supernatant was completed to a total known volume and used as enzyme source **(Ma et al., 2012).**

Assay of catalase activity

Five mL of assay mixture comprising 300 uM of phosphate buffer (pH 6.8), 100 uM of H2O2 and 1 mL of the crude extract were prepared. After incubation at 25°C for 5 min, the reaction was stopped by the addition of 10 mL 2% (v/v) H2SO4. The residual H2O2 was titrated against 0.01 N KMnO4 until a faint purple color persisted for at least 15 seconds. A blank activity was run at the same time, in which the enzyme activity was stopped at zero time. One unit of catalase activity is defined as the amount of enzyme required to decompose 1 umol of H2O2 in 1 minute under the assay conditions described. The activity was expressed as U/ g fresh weight **(Turkan et al., 2005).**

Assay of peroxidase activity

Five mL of the assay mixture comprising 300 uM of phosphate buffer (pH 6.8), 50 uM catechol, 50 uM H2O2 and lmL of crude enzyme extract were prepared. After incubation at 25 °C for 5 min, the reaction was stopped by the addition of lmL 10% (v/v) H2SO4. The optical density of the produced color was measured at 430 nm using spectrophotometer (WP 0803006). One unit of POX activity was defined as the amount of

enzyme that produces 1 absorbance change at 430 nm per minute under the assay conditions described. The activity was expressed as U/ g fresh weight **(Racusen and Foote, 1965).**

Assay of polyphenol oxidase activity

Five mL of assay mixture comprising 125 uM of phosphate buffer (pH 6.8), 100 uM of pyrogallol and lmL of crude extract were prepared. After incubation at 25°C for 5 min, the reaction was stopped by the addition of lmL 10% (v/v) H2SO4. The optical density of the produced color was measured at 430 nm using spectrophotometer (WP 0803006). One unit of PPO activity was defined as the amount of enzyme that produces 1 absorbance change at 430 nm per minute under the assay conditions described. The activity was expressed as U/ g fresh weight **(Beyer and Frido-vich, 1987).**

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) using SPSS 16.0.

Least significant differences (LSD) were estimated at P <0.05 (level of probability). Data are presented as means ± standard error (SE) of three replicates (three independent replicates of every treatment).

RESULTS

Morphological parameters

Seedling biomass (Fresh wt. and Dry wt.) declined slightly with increasing concentrations of all the heavy metals and increased with an increase in age of the plants. On 12th day, fresh weight decreased by 37.1%, 45%, 54.7% for different concentrations of copper sulphate i.e. 12, 25, 37.4 mg/kg (CuSO4.5H2O) consecutively (Fig. 2A). Fresh weight of seedling under different levels of lead nitrate i.e. 30, 60, 80 mg/kg [Pb (NO3)2] showed a decrease by 33.4%, 42.6%, and 48.9% on 12th day respectively (Fig. 2B). For diverse doses of mercuric chloride i.e. 8, 16, 24 mg/kg HgCI2, fresh weight declined by 19.2%, 29.7%, and 38.7% on 4th day successively with reference to the control (Fig. 2C).

On 12th day, dry weight reduced by 36.8%, 42.5%, and 47.4% for different concentrations of copper sulphate respectively (Fig. 3A). For varied doses of lead nitrate, dry weight dropped by 35%, 39.5%, and 44.4% after 12 days of interval respectively (Fig. 3B). In addition, dry weight fell by 7%, 15.2%, and 24.8% under various supplies of mercuric chloride on 4th day successively in respect of the control (Fig. 3C).

Likewise, water content dropped proportionately with increasing the doses of (CuSO4.5H2O), [Pb (NO3)2] and HgCI2. In different concentrations of copper sulphate water content decreased on 12th day by 1.62%, 3%, and 4.1% respectively (Fig. 4A). Under varying doses of lead nitrate water content also went down by 1.2%, 2.2%, and 3.7% on 12th day successively (Fig. 4B). As for variegated mercuric chloride treatments, water content declined by 3.24%, 4.8%, and 6% on 4th day consecutively as compared to control (Fig. 4C).

There was a negative relationship between all metal concentrations and plant height. At different concentrations of copper sulphate, shoot length decreased noticeably at 12 days after metal supply by 33.8%, 43.5%, and 51.7% respectively (Fig. 1A, 5A). For varied doses of lead nitrate, shoot length lessened by 17.9%, 32.4%, and 42% after 12 days of interval respectively (Fig. IB, 5B). In respect of various levels of mercuric chloride, shoot length was lowered by 8.7%, 17.3%, and 24.2% on 4th day respectively with respect to the control (Figs. 1C, 5C). Under different concentrations of copper sulphate root length went down by 51.8%, 64.3%, and 71.4% after 12 days of interval respectively (Fig. 1A, 6A). For varied doses of lead nitrate it fell by 44.6%, 57.1%, and 63.5% at 12 days after metal supply consecutively (Fig. IB, 6B). Moreover, root length was diminished at different concentrations of mercuric chloride by 12%, 32.1%, and 48% at 4 days after metal treatment successively in comparison with control (Fig. 1C, 6C).

Antioxidant enzymes activities

The data pertaining to the effect of different concentrations of the selected heavy metals on CAT activity was depicted in Fig. 7. Compared with the control, CAT activity increases linearly with increasing levels

ISSN: 0975-8585

of both copper sulphate and lead nitrate. CAT activity was elevated under different concentrations of copper sulphate by 25%, 35.3%, and 49.1% at 12 days after metal supplement respectively (Fig. 7A). At varied doses of lead nitrate CAT activity rose 17.8%, 24.5%, and 35.6% at 12 days after metal supplementation consecutively (Fig. 7B). Finally, on 4th day CAT activity increased initially 54.8%, 67.8% at 8 and 16 mg/kg HgCI2 successively, then decreased smoothly at 24 mg/kg HgCI2 but remained higher than for the control by 15.4% (Fig. 7C).

The peroxidase enzyme was elevated in a dose-dependent manner under different concentrations of the studied heavy metals. There was a consistent increase in peroxidase of 104.8%, 170.5%, and 254.2% at varied doses of copper sulphate at 12 days after metal supplement respectively (Fig. 8A). The activity of POX was induced by 86.7%, 113.3%, and 162.7% at various levels of lead nitrate after 12 days of interval successively (Fig. 8B). Concerning varied levels of mercuric chloride POX was stimulated dramatically by of 43.6%, 144.6%, and 289.1% on 4th day consecutively (Fig. 8C).

Trend of increase in the polypheno! oxidase enzyme was observed in all concentrations of the selected heavy metals. At varying copper sulphate supply PPO rose 45.1%, 57.1%, and 151.4% on 12th day consecutively (Fig. 9A). At 12 days exposure to diverse lead nitrate concentrations PPO activity was enhanced by 28.6%, 48.6%, and 93.1% respectively (Fig. 9B). In terms of variant doses of mercuric chloride there was a significant rise in PPO activity by 151%, 234%, and 488.6% on 4th day successively (Fig. 9C).

Figure 1: Correlative morphology of P. Vulgaris seedlings grown under different metals (A, Cu; B, Pb; C, Hg) (after 12 days growth in A and B and 4 days in C)

ISSN: 0975-8585

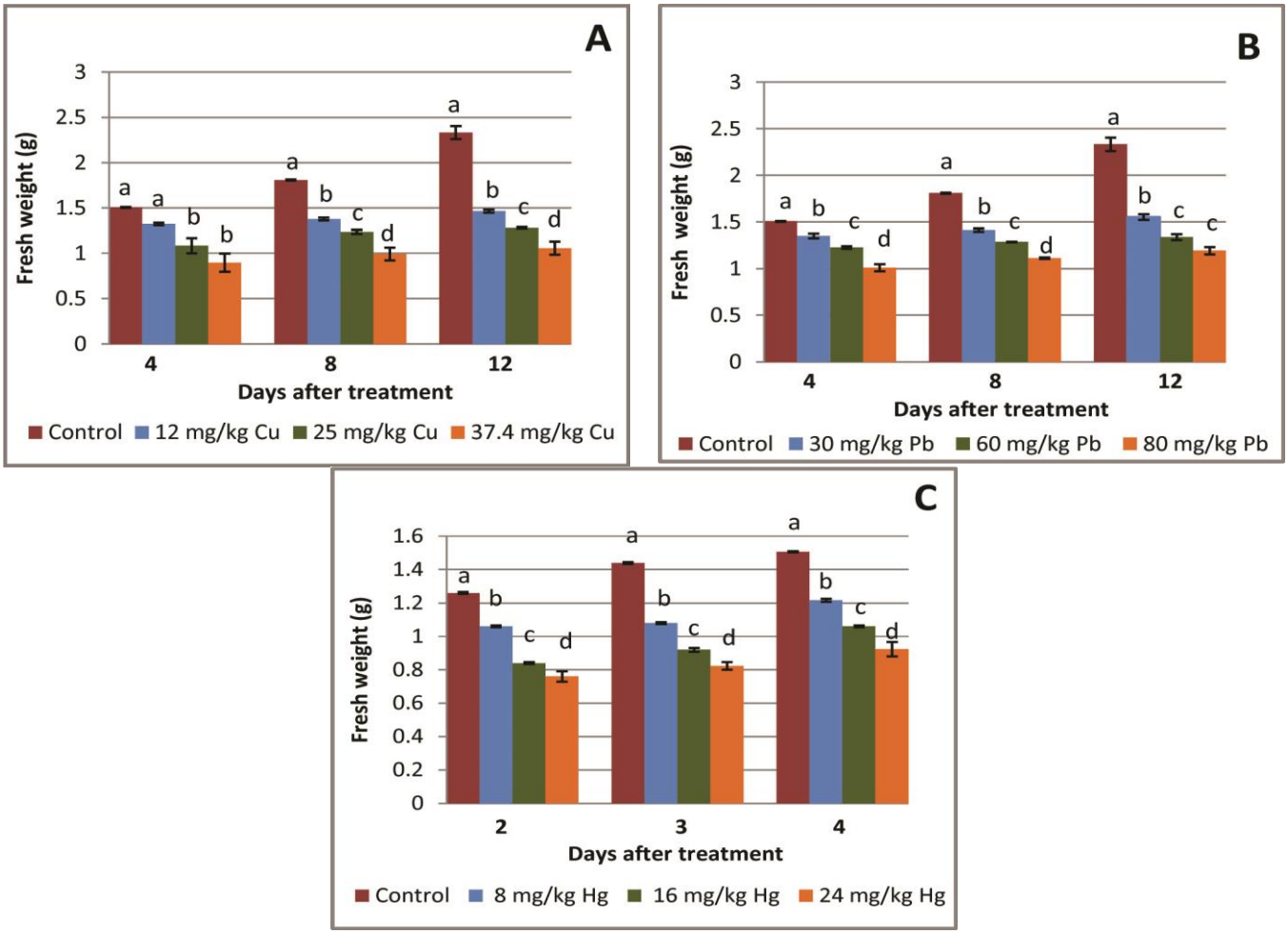
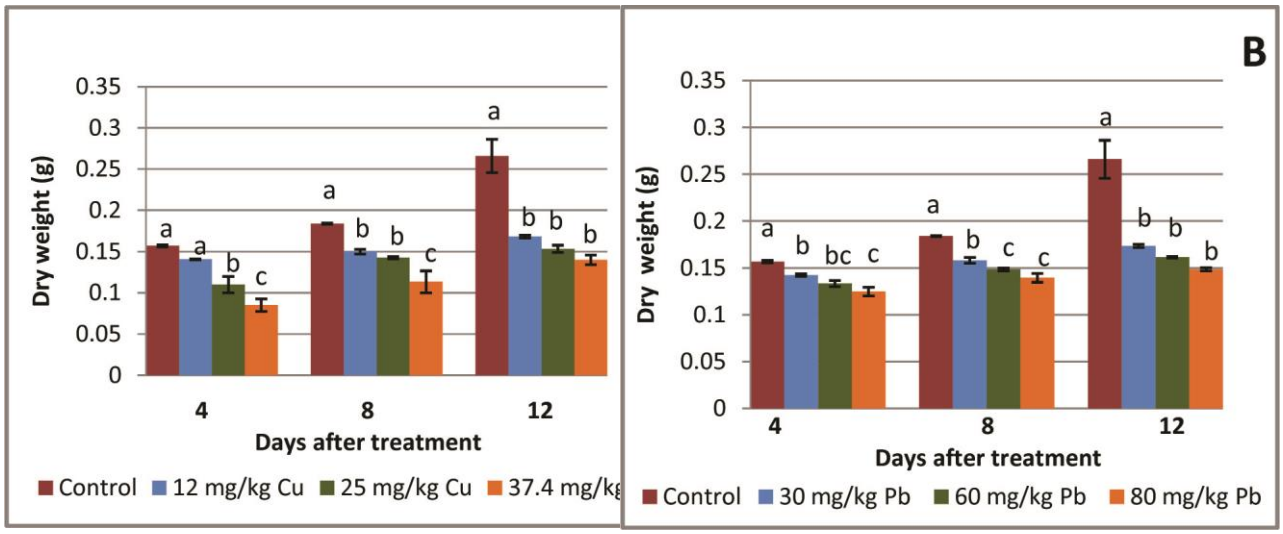


Figure 2: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on fresh weight (g) in Phaseolus vulgaris. Values are means of three replicates ± SE. Values marked with the same letters are not significantly ($p<0.05$) different according to LSD test.



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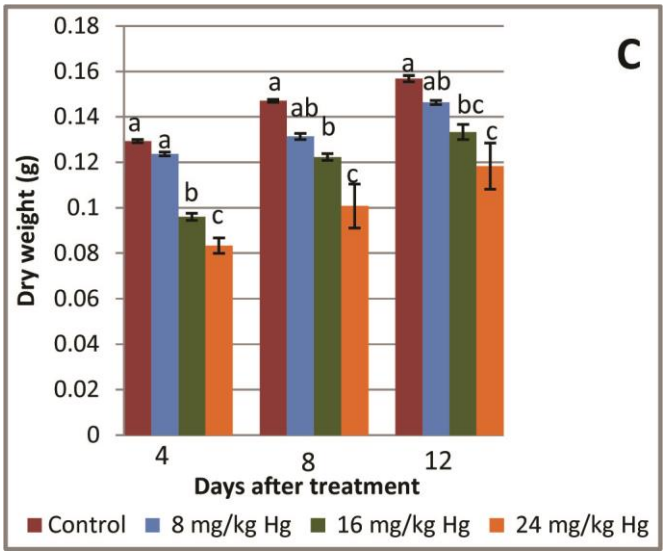

Figure 3: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on dry weight (g) in Phaseolus vulgaris. Values are means of three replicates ± SE. Values marked with the same letters are not significantly (p<0.05) different according to LSD test.

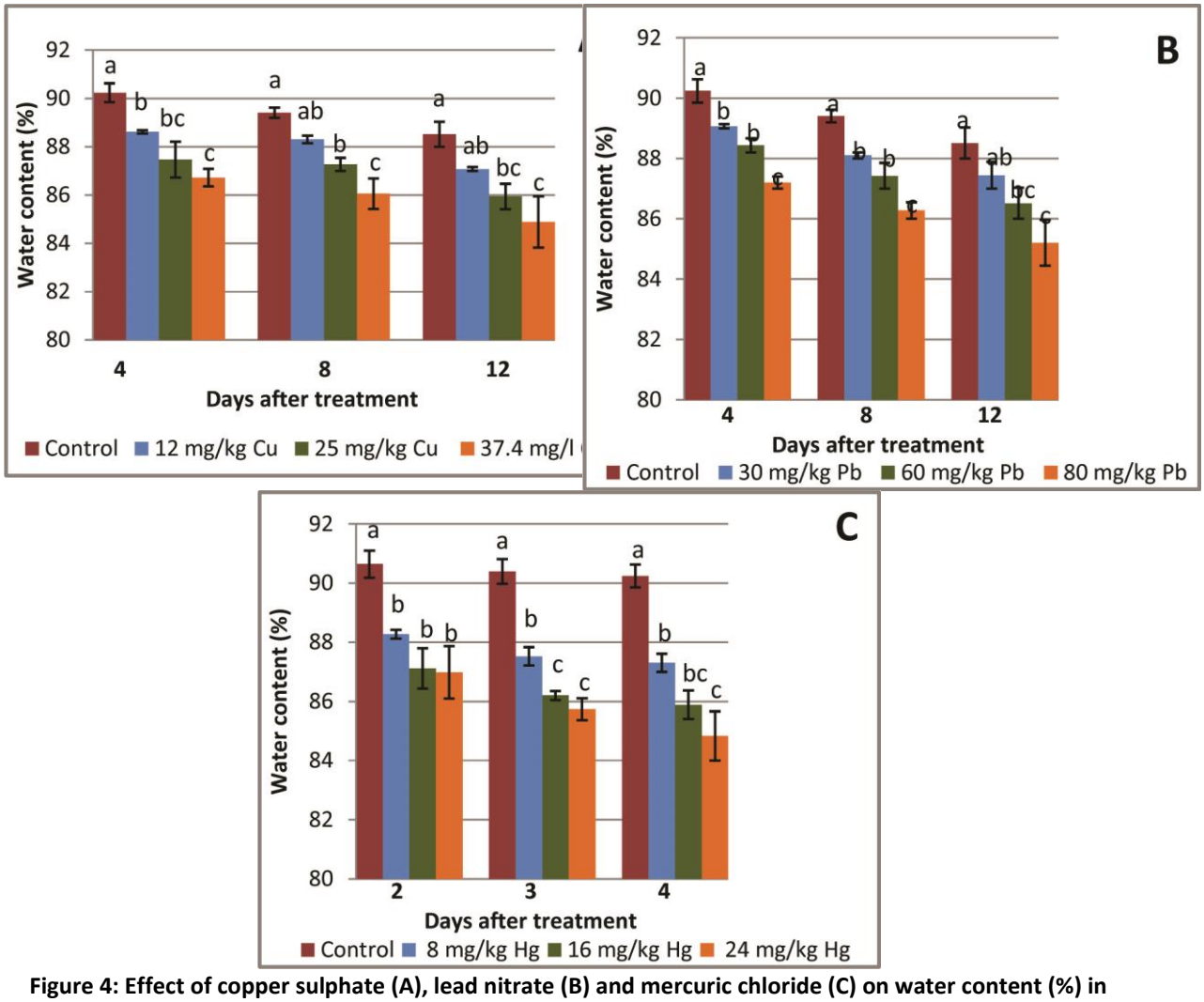

Figure 4: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on water content (%) in Pha-seolus vulgaris. Values are means of three replicates ± SE. Values marked with the same letters are not significantly (p<0.05) different according to LSD test.

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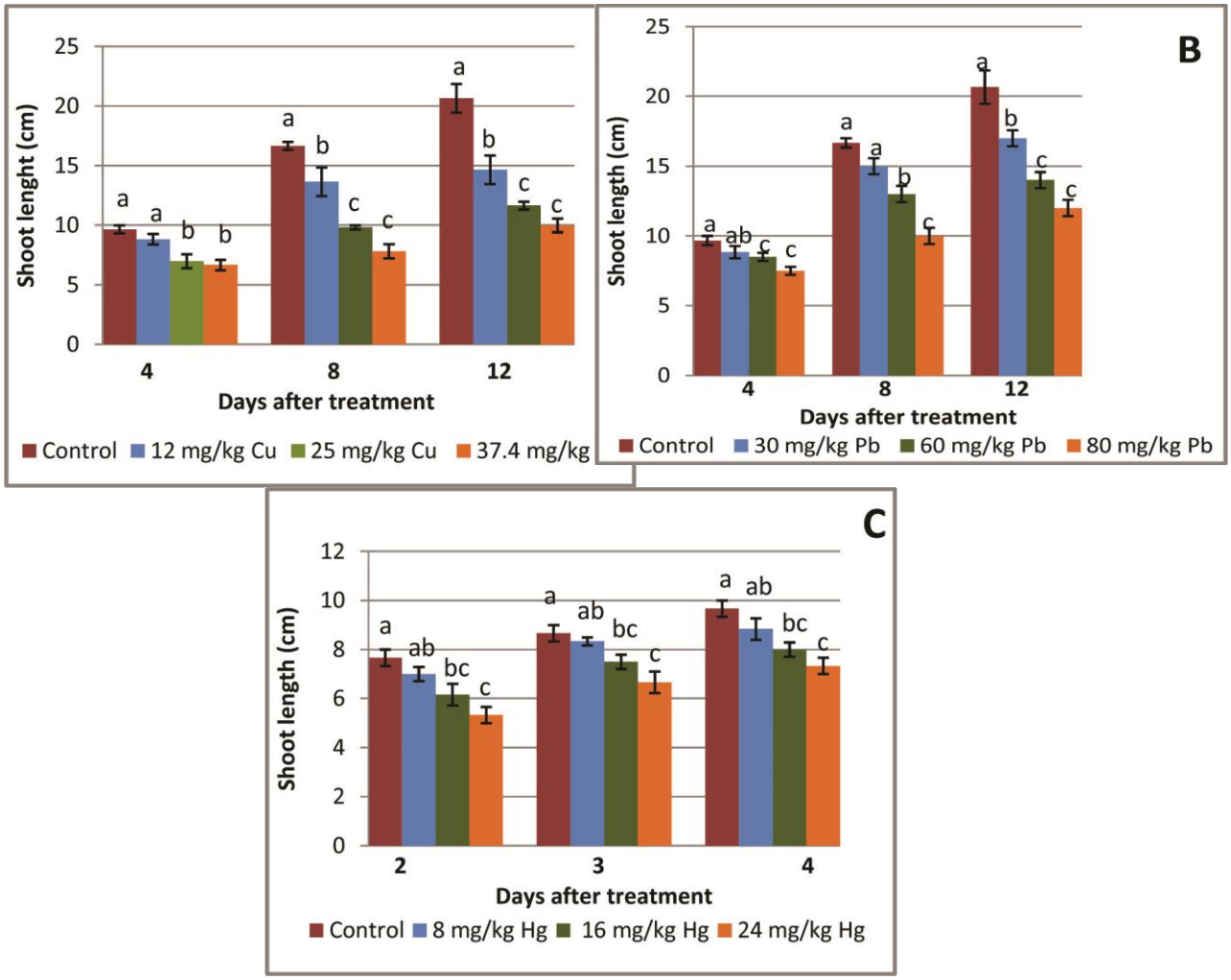
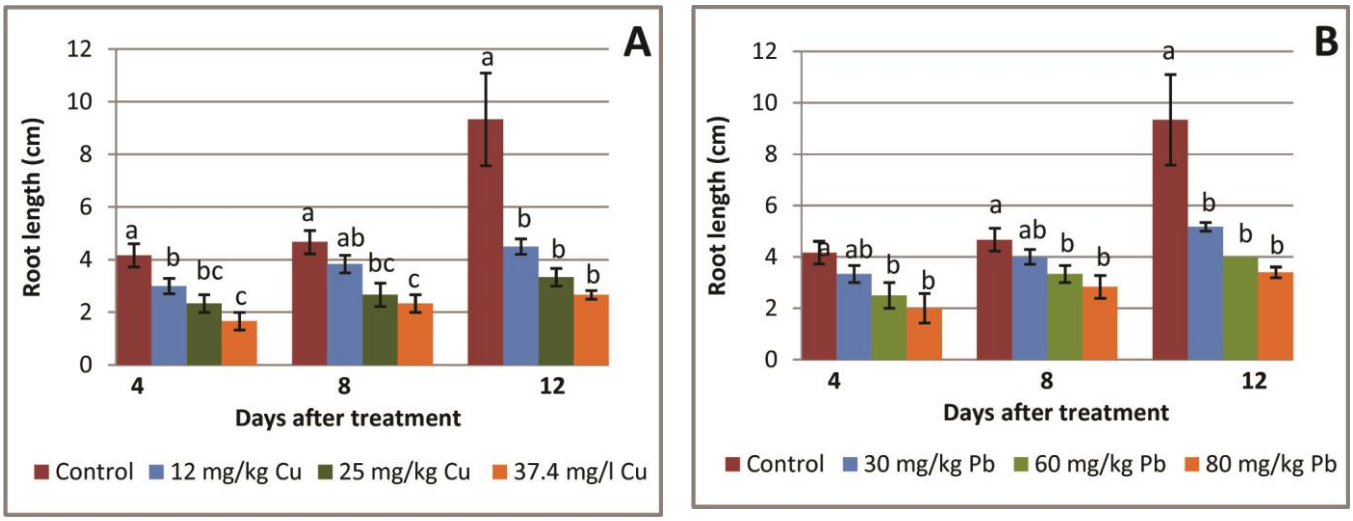


Figure 5: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on shoot length (cm) of Phaseolus vulgaris. Values are means of three replicates ± SE. Values marked with the same letters are not significantly (p<0.05) different according to LSD test.



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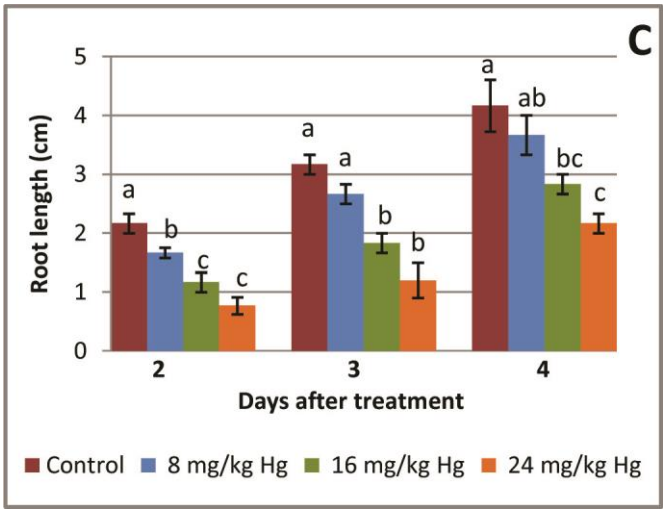


Figure 6: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on root growth in Phaseolus vulgaris. Values are the mean of three replicates ± SE. Values marked with the same letters are not significantly (p<0.05) different according to LSD test.

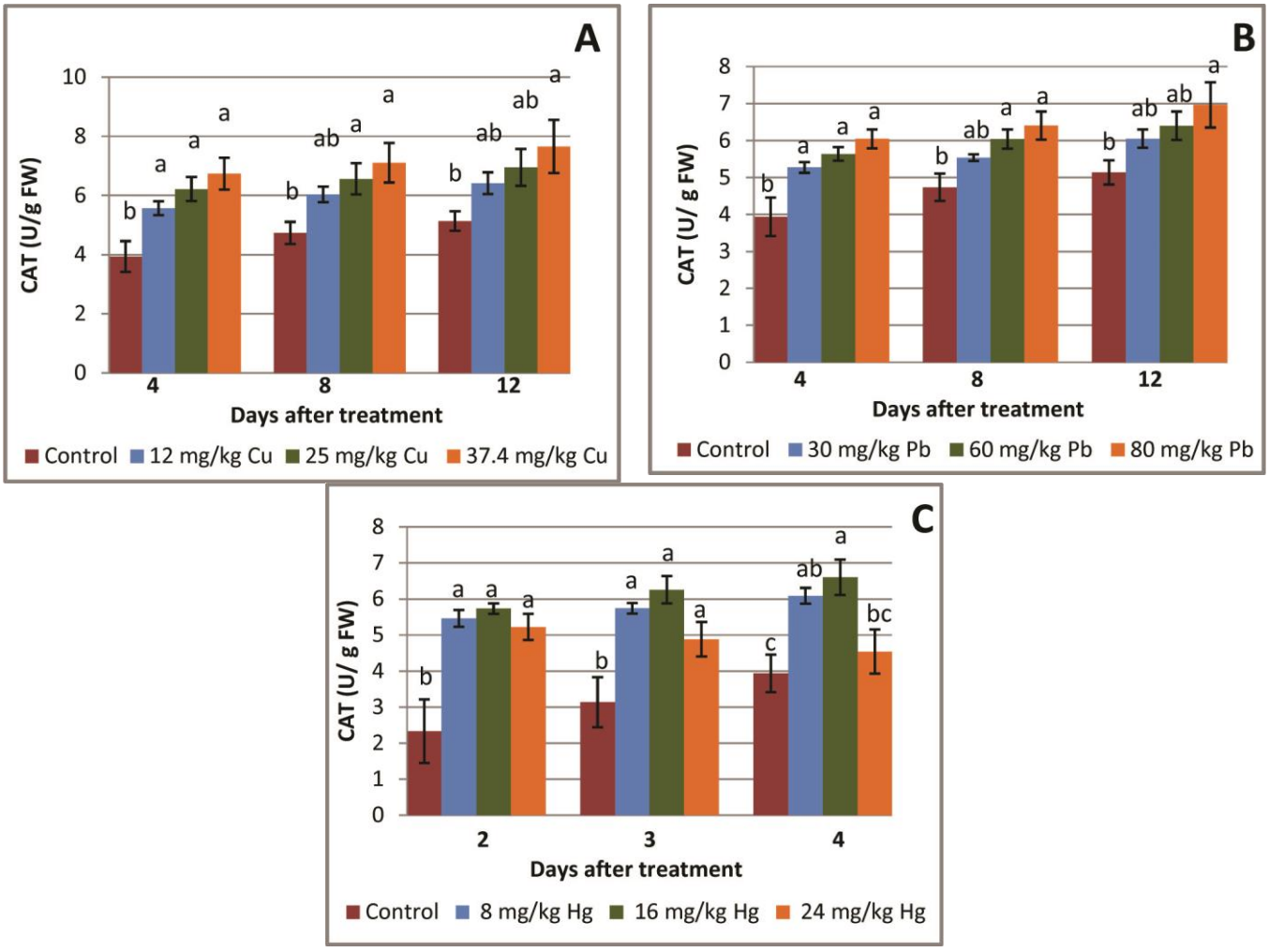


Figure 7: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on CAT activity in Phaseolus vulgaris. Values are the mean of three replicates ± SE. Values marked with the same letters are not significantly (p<0.05) different according to LSD test.

ISSN: 0975-8585

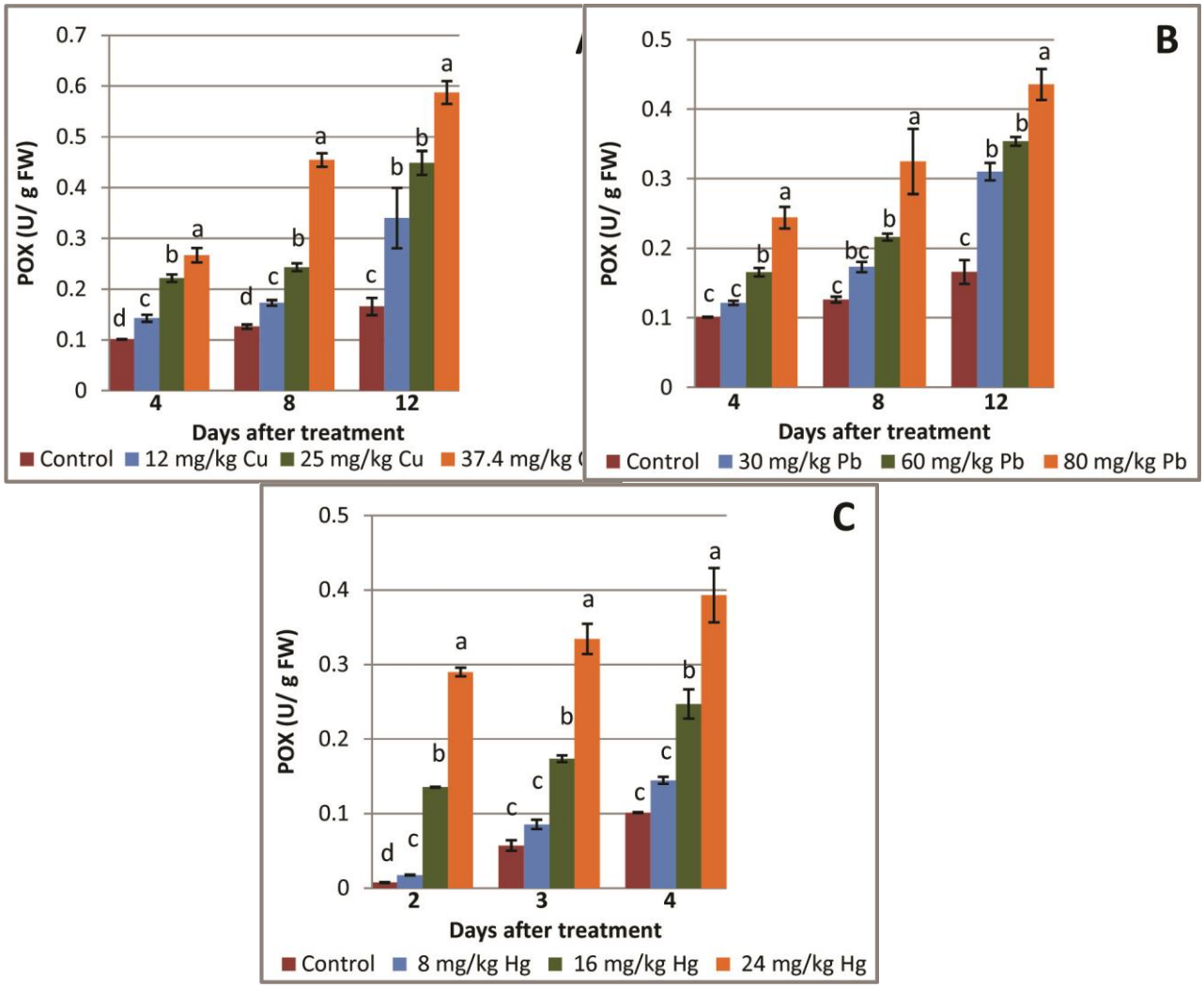
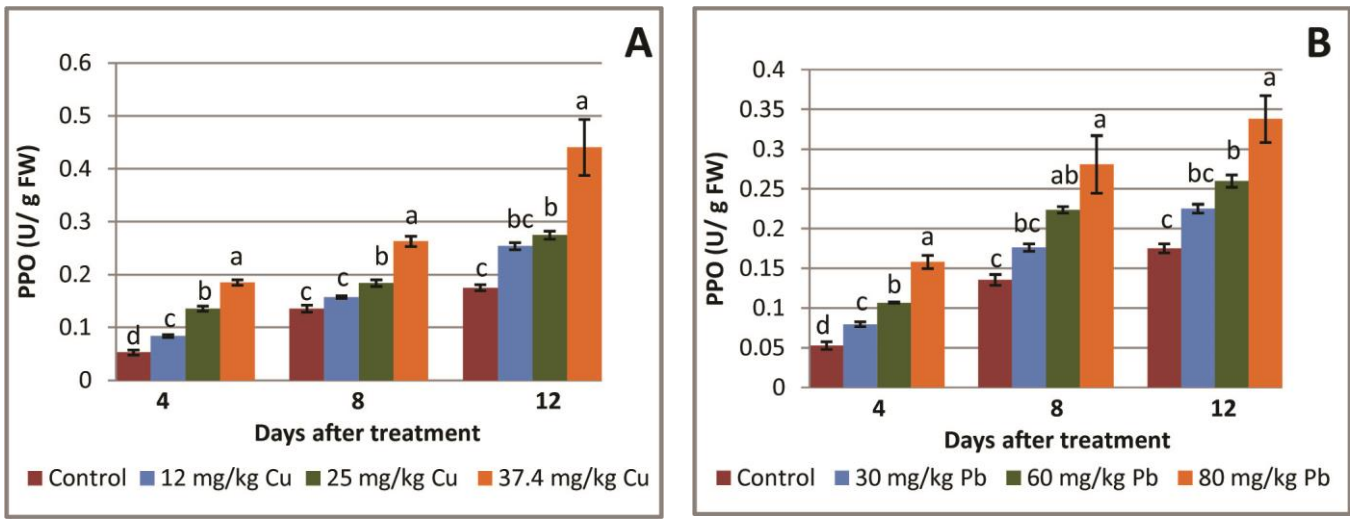


Figure 8: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on POX activity (U/ g FW) in Phaseolus vulgaris. Values are the mean of three replicates ± SE. Values marked with the same letters are not significantly ($p<0.05$) different according to LSD test.



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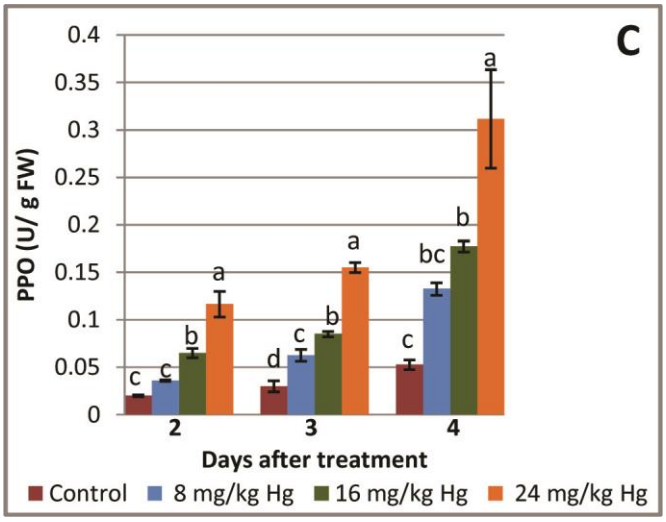

Figure 9: Effect of copper sulphate (A), lead nitrate (B) and mercuric chloride (C) on PPO activity (U/ g FW) in Phaseolus vulgaris. Values are the mean of three replicates ± SE. Values marked with the same letters are not significantly ($p<0.05$) different according to LSD test.

DISCUSSION

Of grain legumes, beans are of great import for direct human consuming in the world. Bean seeds comprise between 20 and 25% proteins, great deal of that is composed of the storage protein phaseolin. Legumes are much preferable to cereals as sources of micronutrients first inasmuch as legumes have a paramount initial content of minerals, and second since polishing should be done to several cereals before eating. On the other hand, most legumes, comprising common beans, are consumed whole. Beans are momentous source of iron, phosphorus, magnesium, manganese, and in lower degree, zinc, calcium and copper **(Broughton et al., 2003).**

Heavy metals in soils are an increasing solicitude of environmental pollution. For that reason we exposed Phaseolus vulgaris plants to different levels of copper sulphate, lead nitrate and mercuric chloride. As a result they exhibited morphological and biochemical alterations. Our results of decline in fresh and dry weight corroborated with the findings of other researchers. **Bhardwaj et al. (2009)** in their investigation on the bean plant observed a diminution in fresh weight of seedling and also similar reports have been found by other researchers in T. Sativum **(Ouzounidou et al., 1997a)** and Lens esculanta **(Mesmar and Jaber, 1991).** Reduction in fresh weight may be referred to toxicity of (CuSO4.5H2O), [Pb (NO3)2] and HgCl2, thereby these toxic materials can retard normal physiological mechanisms and finally have unfavorable impacts on biomass.

Depression in dry matter yield of plants under heavy metal stress is congruous with results reported in earlier studies by **Vijayarengan (2004) (nickel), Vijayarengan (2012a) (cobalt) and Xiong (1997)** (lead). However, in opposition to our study, there are some reports that the heavy metals augmented the dry matter yield of multifarious plants at lower doses **(Shrikrishna and Singh, 1992; Vijayarengan, 2012b).** The limited biomass of bean plants in the presence of (CuSO4.5H2O), [Pb (NO3)2] and HgCl2 probably the outcome of exiguous protein formation in such situations. Laceration of nitrogen metabolism in bean by polluters has been perceived **(Zeid and el-Ghate, 2007).** Parallel conditions have been remarked with overplus Cr in wheat **(Sharma et al., 1995)** as well as other plant species **(Hunter and Vergnano, 1953).** Considering nitrogen is one of the prime requisite nutrients involved as a component of biomolecules such as nucleic acids, nitrogen bases, coenzymes and proteins, any deviance in these constituents is sufficient to deter the growth and yield of plants extremely **(Chatterjee and Chatterjee, 2000).**

Copper sulphate, lead nitrate, and mercuric chloride at the tested levels also induced a change in plant water status. The water content was negatively related to metal concentration. It has been proved that metal toxicity can influence the plasma membrane permeability, bringing about a dwindling in water content, even if the seedlings are grown hydroponically **(Barcelo and Poschenrieder, 1990; Doncheva, 1998; Pandolfini etal., 1996).**

Root and shoot length of bean plants decreased with an increase in the level of the selected heavy metals in the soil. Reduction in shoot growth could be ascribed to the decline in chlorophyll contents and activity of photosystem I engendered by heavy metal stresses **(F.R et al., 2008).** Equally, metal elements transmitted to above ground plant part minified height by deactivating the cellular metabolism of the shoots **(Panda and Choudhury, 2005). Cuypers et al. (2001)** have proposed that shoot growth may be diminished by mercury-stimulated degradation of the photosynthetic pigments. On the grounds that mercury has a potent propensity to react with sulfhydryl groups in plant membranes, this metal also may impede the entrance of nutrients such as Ca, Mg, and K, thereby taking part in lessening of plant growth **(AM et al., 2002).**

Root development is the sequel of cell division at the root tip and cell elongation in the extension zone **(Woolhouse, 1983).** Roots are more liable to heavy metal toxicity compared to shoots **(F.R et al., 2008; Oncel et al., 2000),** subsequently the sterner dropping in the length of roots may be owing to their immediate contact with copper, lead and mercury polluted soil. Corresponding perceptions have been noted by **Mesmar and Jaber (1991)** (lead) and **Vijayarengan (2012a)**(cobalt). The selected heavy metals ((CuSO4.5H2O), [Pb (NO3)2] and HgCI2) may hinder the root growth directly by retardation of cell division or cell elongation or union of both, generating the delimited scouting of the soil volume for absorption and locomotion of nutrients and water and induce mineral lack **(Foy et al., 1978).** The results of the present study also supported these views. In addition, numerous workers have promulgated the inhibition of root growth and of cell divisions in root tips, along with mitotic aberrances, impairments to microtubules and instability of the cellular membranes **(Seregin and Kozhevnikova, 2008). Sengar et al. (2008)** suppose also that the key cause of cell growth repress originates from a lead-induced stimulation of indole-3-acetic acid (IAA) oxidation. Besides, lead may handcuff auxin-regulated cell elongation, which can be showed in an Avena coleoptile assay. Unlike our results, a marked increase in plant organs of corn seedlings was proclaimed owing to an augmentation in the synthesis of cell wall polysaccharides ensuing from lead liability **(Obroucheva et al., 1998).**

The activities of CAT, POX, and PPO were investigated to determine whether copper, lead and mercury exposure influenced these antioxi-dant enzymes. Emergence of oxidative stress in plants subjected to heavy metals is considerably attributed to heavy metal induced imbalance between the production of toxicant oxygen radicals viz., superoxide radical (O2-~), hydrogen peroxide (H2O2), hydroxyl radical (-OH), and singlet oxygen (XO2), etc and their elimination via the anti-oxidative defense mechanism. The latter is regarded as an effectual system for detoxification and getting rid of the toxic oxygen species by means of upregulation of anti-oxidative enzymes such as SOD, CAT, POD, PPO, and APX and enhancement of cumulation of cellular antioxidants such as AsA-GSH cycle (also known as the Halliwell-Asada cycle) which scavenge hydrogen peroxide by reducing it to water and repressing the conversion of the super oxide ions to the highly reactive and genotoxic hydroxyl (OH) ions **(Bhardwaj et al., 2009; Jayakumar et al., 2007; Latowski et al., 2010).**

Among the detoxifying enzymes that we assayed, CAT is one of the most substantial constituent of plant defensive mechanisms that existent in mitochondria and peroxisomes and plays a crucial role in scavenging free radicals particularly H2O2 arised during photorespiration and stress conditions. This enzyme catalyzes H2O2 to H2O and O2 through two-electron transfer and ultimately blocks the production of hydroxy radical OH« and maintain proteins, nucleic acids and lipids against ROS **(Bowler et al., 1992; Foyer and Noctor, 2005; Gupta et al., 2009; Imlay and Linn, 1988; Mittler, 2002; Rastgoo and Alemzadeh, 2011; Wang et al., 2008).** The present results show that the increment of copper sulphate and lead nitrate concentration in the soil was related with the rise in catalase activity. These results are in agreement with the findings of **(Lamhamdi et al., 2011; Singh et al., 2007),** indicating that H2O2 diffuse from the chloroplasts to the peroxisomes, entailing an induction in CAT activity. Another possibility is that the augmentation in catalase could be demonstrated by an increase in photorespiration in consequence of the toxicant action of the studied heavy metals on the primary reactions of photosynthesis and inhibitory influence on photosynthetic electron transport principally at PSII. Thus, a decrease in the carboxylation/oxygenation ratio of the Rubisco reaction may occur, thereby enhancing photorespiration in the peroxisomes **(Ouzounidou et al., 1997b; Shainberg et al., 2001; Shioi et al., 1978).**

Albeit mercury does not engender ROS directly as perform other heavy metals, it does generate oxidative stress by altering the plant's antioxidative defense systems **(Ali et al., 2002).** Increased CAT levels in synchronization with low dosages of Hg is in consonance with results of other researchers **(Ali et al., 2002; Cargnelutti et al., 2006). Ali et al. (2002)** found that CAT activity in Oryza sativa leaves increased initially up to 10 uM Hg, then progressively decreased but remained higher than for the control. **Cargnelutti et al. (2006)**

observed that at 10 days, catalase activity increased in cucumber seedlings at a moderately toxic level of Hg, whereas at the higher concentration (500 uM), there was a manifest inhibition. **Rastgoo and Alemzadeh (2011)** observed that CAT activity in Aeluropus littoralis at higher concentration (100 uM Ag) was less than 50 uM. The inhibition of protein synthesis and other oxidase protein may cause decreases in CAT activity at high Hg concentrations **(Ali et al., 2002).** In addition, **Patra and Sharma (2000)** reported that elevated concentrations of Hg may result in protein precipitation thus reducing the functions of some enzymes. Rastgoo and Alemzadeh (2011) as-sume that under stress conditions assembling of CAT subunits modifies causing enzyme deactiva-tion or peroxisomal protease may give rise to proteolytic degradation of CAT. A part from that, binding of CAT to non-essentional metals can alters its structure.

POD is regarded the most important marker for stress and metal toxicity **(Doganlar and Atmaca, 2011).** POD lies in cytosol, cell walls, vacuoles and extracellular spaces. It has a wide specialty for phenolic substrates and more binding affinity for H2O2 than CAT. POD uses up H2O2 to produce phenoxy compounds that are polymerized to generate cell wall components such as lignans **(Lamhamdi et al., 2011; Reddy et al., 2005).** Indeed, POX has been presumed to solidify the cell wall via POX-mediated lignification that reduces the cell wall plasticity, and hence lessens cell elongation **(Cho and Park, 2000).** Increase in POD is correlated with copper, lead and mercury stress suggesting it to be a substantial defense tool **(Verma and Dubey, 2003).** Increased POD activity shown in our results can be attributed to the release of peroxidase centralized in the cell walls **(Gaspar, 1982).** This finding is consistent with previous studies such as **Teisseire and Guy (2000)** who have found that POD has enhanced activitiy under Cu stress in Lemna minor. Moreover, **Ali et al. (2002)** have reported that POD activity increased in Hg-treated versus untreated leaves of rice. **Lamhamdi et al. (2011)** have also recorded that POD activity were significantly stimulated in wheat in the presence of lead in a dose-dependent manner.

Polyphenol oxidases (PPO) are designated also as tyrosinases. They are enzymes with a dinu-clear copper centre, that are capable of inserting oxygen in a position ortho- to an existent hy-droxyl group in an aromatic ring, succeeded by the oxidation of the diphenol to the corresponding quinine which create brown pigments in wounded tissues. Because the phenomenon decreases fruit quality, PPO has been considered to be a significant enzyme in food technology **(Mayer, 2006; Tang and Newton, 2004).** The copper centre in the active site of PPO that is highly conserved is bound to six or seven his-tidine residues and a single cysteine residue. Despite its approximately universal distribution in animals, plants, fungi and bacteria much is still unknown about the biological function of POD, particularly in plants and fungi **(Mayer, 2006).** The trend of Polyphenol oxidase activity with all dosages of the (CuSO4.5H2O), [Pb (NO3)2] and HgCI2 was upward. Our results are in good agreement with previous reports by **(Vijayarengan, 2012a)** who found that cobalt treatment increased PPO (except 50 mg kg- 1) in cowpea with an increase in cobalt level in the soil. Further, **Jayakumar et al. (2007)** found that PPO activity increased in radish with an increase in the Co level of soil (except at 50 mg Co kg-1 soil).

Finally, It has been shown that mercury-treated plants had varied levels of antioxidant enzymes and this can be explained as redundancy in reactive oxygen intermediates (ROI) scavenging mechanisms **(Mittler, 2002; Sinam et al., 2011).** Hence, Hg-treated plants with low CAT production (at 24 mg/kg HgCI2) induce POX and PPO to compensate for the reduced activity of CAT.

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