ACTA BIOLOGICA CRACOVIENSIA Series Botanica 47/2: 157–164, 2005

EFFECTS OF SOME HEAVY METALS ON CONTENT OF CHLOROPHYLL, PROLINE AND SOME ANTIOXIDANT CHEMICALS IN BEAN (*PHASEOLUS VULGARIS* L.) SEEDLINGS

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Received January 6, 2005; revision accepted September 30, 2005

The effects of lead, copper, cadmium and mercury on the content of chlorophyll, proline, retinol, α-tocopherol and ascorbic acid were investigated in 17-day-old bean seedlings (*Phaseolus vulgaris* L.) grown in Hoagland solution spiked with various concentrations of Pb, Cu, Cd and Hg. Control and heavy metal-treated plants were grown for 10 days in Hoagland solution. Content of total chlorophyll (*a* and *b*), proline, retinol, α-tocopherol and ascorbic acid was measured in ten-day-old primary leaves by High Performance Liquid Chromatography (HPLC). Total chlorophyll content declined progressively with increasing concentrations of heavy metals. A significant increase of proline, retinol, α-tocopherol and ascorbic acid content was detected in primary leaves after ten-day exposure to heavy metals. The strongest effect on total chlorophyll, proline, retinol, α-tocopherol and ascorbic acid content was found in plants exposed to mercury, followed by the sequence $Cd^{++} > Cu^{++} > Pb^{++}$.

Key words: Chlorophyll, heavy metal, proline, retinol, α-tocopherol, ascorbic acid.

INTRODUCTION

Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedelkoska and Doran, 2000). They present a risk for primary and secondary consumers and ultimately humans (Zeller and Feller, 1999).

Heavy metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. However, elevated concentrations of both essential and nonessential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall, 2002). Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects (van Assche and Clíjsters, 1990). In addition, a heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Dietz et al., 1999). The lifetime of active oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage. The antioxidative system comprises numerous enzymes and compounds of low molecular weight (Noctor and Foyer, 1998). The antioxidant properties of plants exposed to various stress factors have been studied (Havaux and Kloppstech, 2001), but studies related to the effect of heavy metal-induced stress on vitamin levels in plants are limited. Lead and mercury was reported to cause an increase in ascorbic acid and α-tocopherol levels in two *Oryza sativa* cultivars (Mishra and Choudhuri, 1999), and mercury exposure was found to increase the ascorbic acid levels in *Bacopa monnieri* (Sarita et al., 1996).

Detailed studies indicate that heavy metals have effects on chlorophyll content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clíjsters, 1990). The amount of chlorophyll was reduced in *Triticum aestivum* cv. Vergina grown on Cu-enriched soil (Lanaras et al., 1993), and in *Brassica oleracea* var. Botrytis cv. Maghi exposed to Cu^{2+}, Co^{2+} and Cr^{2+} (Chatterjee and Chatterjee, 2000).

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PL ISSN 0001-5296

Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles. Heavy metal stress leads to proline accumulation (Alía and Saradhi, 1991). Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins (Charest and Phan, 1990).

This study examined the effects of four heavy metal cations, namely lead, copper, cadmium and mercury, on the content of total chlorophyll, proline, retinol, α-tocopherol and ascorbic acid in bean seedlings. We studied the effect of various concentrations of metals on some physiological parameters in *Phaseolus vulgaris* L.

MATERIALS AND METHODS

Seven-day-old bean seedlings (*Phaseolus vulgaris* L.) were used. The heavy metal salts used in this study included $PbCl_2$, $CuCl_2$, $CdCl_2 \cdot H_2O$ and $HgCl_2$. The heavy metal concentrations in the stock solutions were 1.5, 2.0 and 2.5 mM for lead; 0.1, 0.2 and 0.3 mM for copper; 0.05, 0.06, and 0.08 mM for cadmium; and 0.02, 0.04 and 0.06 mM for mercury. The recovery rates of standards were determined as 96.2% for retinol, 96.5% for α-tocopherol and 95.7% for ascorbic acid. Separation times, at a flow rate of 1 ml/min, were 3.2 min for retinol, 3.6 min for ascorbic acid, and 5.6 min for α-tocopherol.

The bean seeds were surface-sterilized in 10^{-3} M $HgCl_2$ for 2 min (Sresty and Madhova Rao, 1999), washed in distilled water, and germinated between wet paper towels at 25˚C in the dark for 3 days. Then the plants were cultivated hydroponically in a growth chamber at light intensity of 4500 µmol·quoute·m^{-2}·s^{-1} under a 96 h photoperiod and constant 25˚C. After an initial growth period of 7 days, the seedlings were taken from their plastic pots and their roots were washed with distilled water. Then a sponge was used to take 20 seedlings for each concentration and place them in 500 ml jars (two seedlings per jar) containing 200 ml of the given heavy metal solution (Hoagland solution for control). The jars were covered with aluminum foil. After 10 days of heavy metal treatment, the primary leaves were harvested and used for pigment, proline, retinol, α-tocopherol and ascorbic acid analyses. Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf tissue was homogenized in sulfosalicylic acid, and the homogenate was centrifuged at 3000 × g for 20 min. The supernatant was treated with acetic acid and acid-ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Chlorophyll was determined according to Monni et al. (2001). Extraction of retinol and

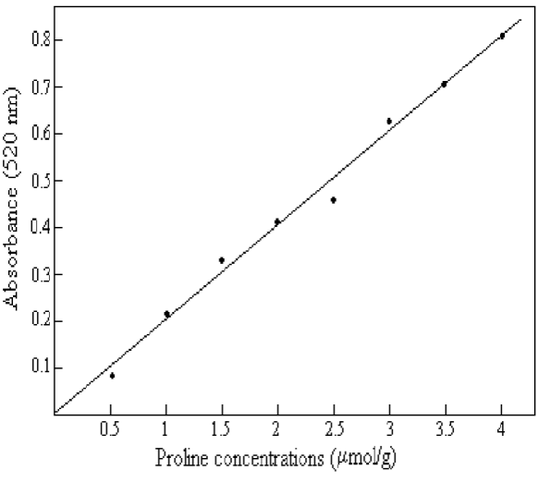

Fig. 1. Standard curve of proline.

α-tocopherol was done according to Catignani (1983) and Miller et al. (1984). Leaf tissues were homogenized in ethanol and the homogenate was centrifuged at 4500 × g for 5 min. The supernatant was treated with n-hexane. Retinol and α-tocopherol were extracted twice in hexane phase and the collected extract was dried in liquid nitrogen. The dried extract was solubilized in 0.5 ml methanol for HPLC. Injections were made in duplicate for each sample. Quantification was according to Catignani (1983) and Miller et al. (1984) at absorption maxima of 326 nm for retinol and 296 nm for α-tocopherol. HPLC separations were done at room temperature with a Perkin-Elmer liquid chromatography system (Series 1100) consisting of a sample injection valve (Cotati 7125) with a 20 l sample loop, UV spectrophotometric detector (Cecil 68174), integrator (HP 3395) and Techsphere ODS–2 packed (5 µm particle and 80 Å pore size) column (250 × 4.6 ID) with a methanol:acetonitrile:chloroform (47:42:11, v/v) mobile phase at 1 ml min^{-1} flow rate. Extraction of ascorbic acid was done according to Cerhata et al. (1994). Leaf tissues were homogenized in perchloric acid and the volume was adjusted to 1 ml by adding ddH_2O. The mixture was centrifuged at 4500 × g for 5 min at 4˚C. The supernatant was filtered as above and the ascorbic acid level was determined with the method of Tavazzi et al. (1992) by HPLC utilizing a column (250 × 4.6 ID) packed with Li–60 reversed-phase material (10 µm particle size) with mobile phase (3.7 mM phosphate buffer, pH 4.0) at 1 ml min^{-1} flow rate.

Statistical analysis employed SPSS ver. 10.0. The significance of differences between variables at $p < 0.01$ or $p < 0.05$ was checked with a multiple comparison (LSD) test. All values are means ± SE. Increases or

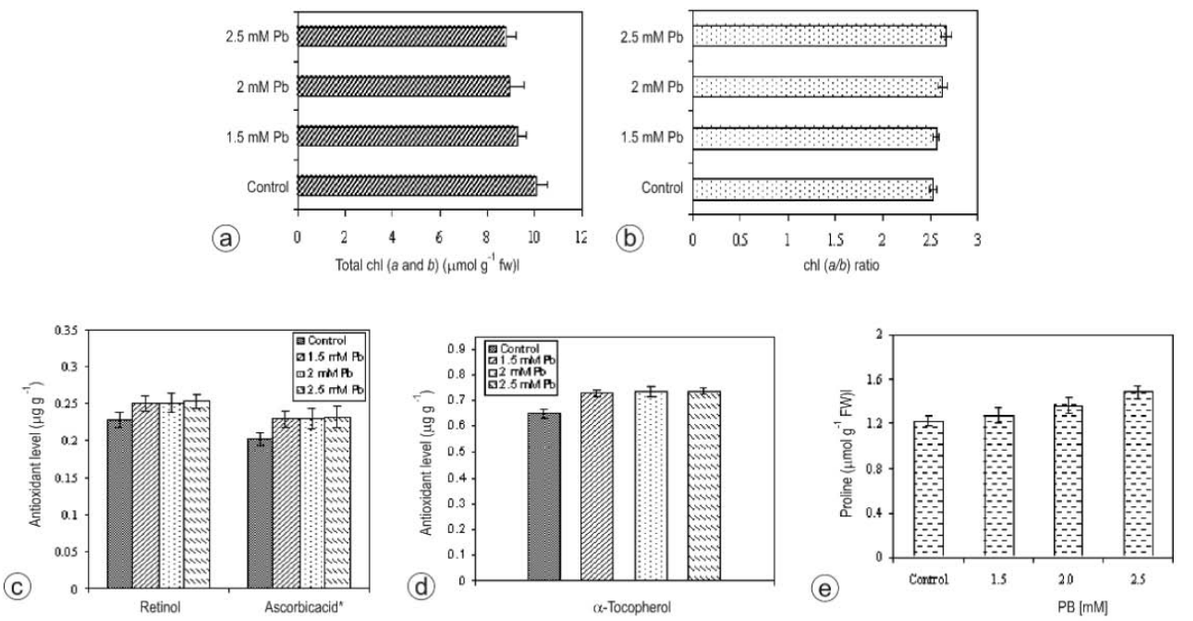

Fig. 2. (**a**) Total chlorophyll (*a* and *b*) content [µmol g^{-1} (FW)], (**b**) Chlorophyll *a*/*b* ratio, (**c**) Retinol and ascorbic acid [µg.g^{-1} (FW)], (**d**) α-tocopherol [µg.g^{-1} (FW)], and **(e)** Proline content [µmol g^{-1}(FW)] in primary leaf of bean seedlings dosed with different concentrations of lead ($PbCl_2$). Error bars indicate ± SE. *Ascorbic acid × 10^{+2}.

decreases are expressed as percentages versus the control.

RESULTS

Figure 1 shows the standard curve of proline. Figures 2–5 summarize the results for the effects of selected heavy metals (Pb, Cu, Cd, Hg) on total chlorophyll, retinol, α-tocopherol, ascorbic acid, proline content and chlorophyll ratio (*a*/*b)* in primary leaves of the bean seedlings. The chlorophyll ratio increased slightly with increasing heavy metal. Total chlorophyll content declined progressively with increasing concentrations of the heavy metal. Significant increases of primary leaf proline, retinol, α-tocopherol and ascorbic acid content were detected after ten-day exposure to heavy metal.

LEAD

Lead concentrations above 1.5 mM caused a significant reduction of total chlorophyll content (8.04%–12.51%) (Fig. 2a), and a slight increase of the chlorophyll ratio (1.5%–5.5%) (Fig. 2b). Exposure of seedlings to lead resulted in an increase of retinol, α-tocopherol and ascorbic acid content in primary leaves (Fig. 2c,d). In primary leaf treated with 1.5, 2.0 and 2.5 mM lead, retinol content increased by 9.6%, 10.0% and 10.1%, respectively. α-tocopherol content in the seedlings increased 12.1%–13.7% ($p < 0.01$). With 1.5, 2.0 and 2.5 mM lead concentrations, ascorbic acid content increased in a dose-dependant manner (12.7%, 13.2%, 14.2%). The content of the stress-indicating amino acid proline increased with the concentration of lead, between 4.6% and 21.2% ($p < 0.05$) (Fig. 2e).

COPPER

At 0.1, 0.2 and 0.3 mM $CuCl_2$ concentrations, total chlorophyll content in primary leaves decreased significantly by 14.4%, 15.9% and 17.0%, respectively ($p < 0.05$) (Fig. 3a). Copper spiking increased the chlorophyll ratio 3.1%–6.7% ($p < 0.05$) (Fig. 3b). Content of retinol, α-tocopherol and ascorbic acid in primary leaves increased with the copper concentration (Fig. 3c,d). Retinol content in the seedlings increased 16.2%–25%. In seedlings treated with 0.1, 0.2 and 0.3 mM copper, α-tocopherol content increased by 19.8%, 20.3% and 24%, respectively ($p < 0.05$). Ascorbic acid content increased 13%–15%. Proline content increased by 12.2% (0.1 mM Cu), 21.3% (0.2 mM Cu) and 30.9% (0.3 mM Cu) ($p < 0.05$) (Fig. 3e).

CADMIUM

Figure 4 summarizes the results for the effects of cadmium on total chlorophyll content of retinol, α-tocopherol, ascorbic acid and proline, and the chlorophyll ratio in primary

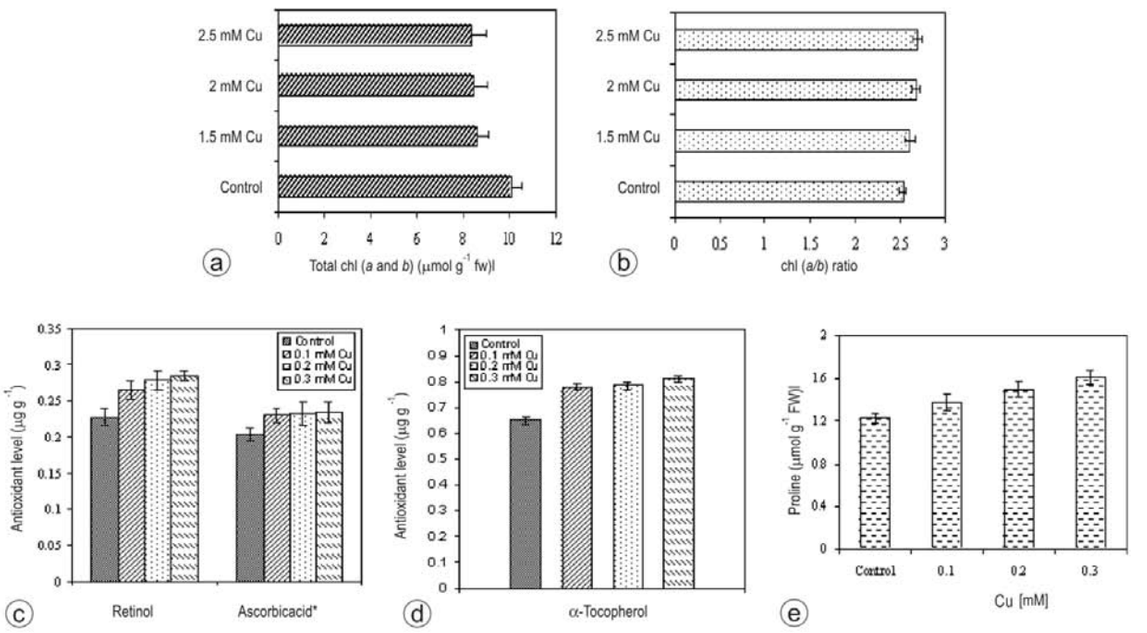

Fig. 3. (**a**) Total chlorophyll (*a* and *b*) content [µmol g^{-1} (FW)], (**b**) Chlorophyll *a*/*b* ratio, (**c**) Retinol and ascorbic acid [µg.g^{-1} (FW)], (**d**) α-tocopherol [µg.g^{-1} (FW)], and (**e**) Proline content [µmol g^{-1}(FW)] in primary leaf of bean seedlings dosed with different concentrations of copper ($CuCl_2$). Error bars indicate ± SE. *Ascorbic acid × 10^{+2}.

leaves of bean seedlings. Total chlorophyll content in primary leaves decreased 18.3%–22.5% at 0.05, 0.06 and 0.08 mM concentrations of cadmium ($p < 0.05$) (Fig. 4a). The chlorophyll ratio increased 4.7%–9.8% ($p < 0.05$) (Fig. 4b). In Cd-treated seedlings, retinol, α-tocopherol and ascorbic acid content increased significantly ($p < 0.05$) (Fig. 4c,d). Cadmium treatment (0.05 and 0.08 mM) increased the retinol content 14%–18% ($p < 0.05$). In seedlings treated with 0.05, 0.06 and 0.08 mM cadmium, α-tocopherol increased by 14.4%, 14.8% and 15.1%, and ascorbic acid content increased by 14.2%, 15.7% and 17.2%, respectively ($p < 0.05$). Under the same treatments, proline content rose 34.1%, 49.5% and 54.4% ($p < 0.05$) (Fig. 4e).

MERCURY

Total chlorophyll content of primary leaves decreased with increasing mercury concentration. It decreased 24.9–29.2% under treatments with 0.02, 0.04 and 0.06 mM $HgCl_2$ ($p < 0.01$) (Fig. 5a). In mercury-treated seedlings the chlorophyll ratio increased 5.1%–10.3% ($p < 0.01$) (Fig. 5b). Retinol, α-tocopherol and ascorbic acid content increased with the mercury concentration (Fig. 5c,d). In primary leaves of seedlings treated with 0.02, 0.04 and 0.06 mM mercury, retinol content increased by 17.5%, 18.4%, and 19.3%, respectively. α-tocopherol content increased 16.6%–18.3% ($p < 0.05$), and ascorbic acid content increased in a dose-dependent manner (18.1%, 18.6%, 19.2%) ($p < 0.05$). Proline content increased by 45.5%, 56% and 60.1% ($p < 0.01$) (Fig. 5e).

DISCUSSION

In the present study, exposure to heavy metals affected different parameters of bean: proline content, total chlorophyll content, chlorophyll ratio, retinol content, α-tocopherol content and ascorbic acid content. Exposure of ten-day-old bean seedlings to lead, copper, cadmium and mercury decreased the total chlorophyll content, and increased the chlorophyll ratio slightly. Metal-treated seedlings also showed significantly higher retinol, α-tocopherol and ascorbic acid. Increased lead, copper, cadmium and mercury concentrations significantly enhanced proline accumulation in bean seedlings.

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh, 1990). Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals. In two wheat varieties to which Cd and Pb were applied, total chlorophyll decreased

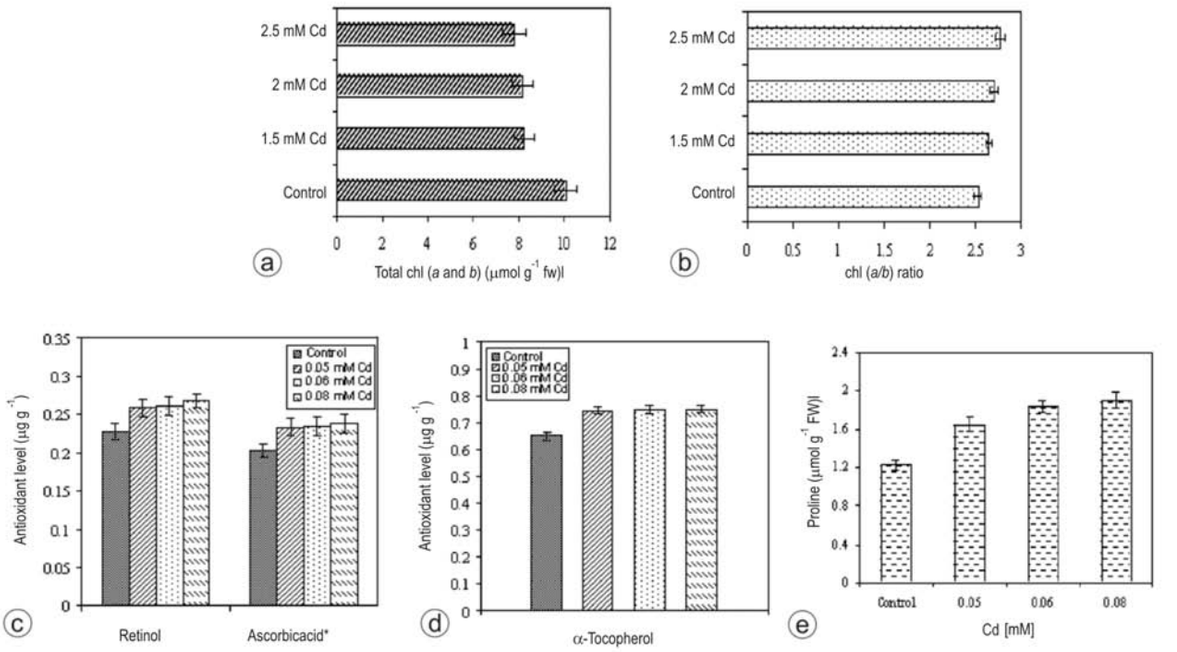

Fig. 4. (**a**) Total chlorophyll (*a* and *b*) content [µmol g^{-1}(FW)], (**b**) Chlorophyll *a*/*b* ratio, (**c**) Retinol and ascorbic acid [µg.g^{-1} (FW)], (**d**) α-tocopherol [µg.g^{-1} (FW)], and (**e**) Proline content [µmol g^{-1}(FW)] in primary leaf of bean seedlings dosed with different concentrations of cadmium ($CdCl_2.H_2O$). Error bars indicate ± SE. *Ascorbic acid × 10^{+2}.

50% (*Triticum aestivum* cv. Gerek 79) and 70% (Bolal 2973) (Oncel et al., 2000). Heavy metals inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium was reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid (ALA) synthesis (Stobart et al., 1985).

The chlorophyll ratio, which is used as a stress indicator, increased slightly with increasing metal treatments. This was also seen in *Empetrum nigrum* leaves near a copper and nickel smelter in the field (Monni et al., 2001). Increased chlorophyll ratios due to environmental stress have been reported in spinach leaves (Delfine et al., 1999). A high chlorophyll ratio also indicates a change in the PSII/PSI ratio in stressed leaves (Anderson, 1986).

Free radical generation is one the initial responses of plants to stress. Generation of free radicals and reactive oxygen species is stimulated in the presence of metals (Halliwell and Gutteridge, 1993), and this can seriously disrupt normal metabolism through oxidative damage to cellular components. To mitigate and repair the damage initiated by active oxygen, plants have developed a complex antioxidant system. These antioxidants play an important role in the cellular defense strategy against oxidative stress, inducing resistance to metals by protecting labile macromolecules (Zhang and Kirkham, 1994; Galli et al., 1996). It is widely accepted that detoxification of metal ions within plant tissues usually depends on chelation by appropriate ligands. Antioxidants like systeine, proline, ascorbic acid and nonprotein thiol (sulfhydryl) play an important role in detoxification of toxic metal ions (Singh and Sinha, 2005). Ascorbate is a ubiquitous soluble antioxidant in photosynthetic organisms, and the most important reducing substrate for H_2O_2 detoxification (Singh et al., 2005). It has been suggested that pollutants produce oxyradicals in plants (Sakaki et al., 1983). These radicals cause widespread damage to membranes and associated molecules, including chlorophyll pigments (Sakaki et al., 1983). Several authors have reported that ascorbic acid can serve as a free radical scavenger against O_3 (van Hove et al., 2001; El-Khatib, 2003). Ascorbate occurs in the cell wall, where it is a first-line defense against ozone. Cell wall ascorbate and cell wall-localized ascorbate oxidase (AO) have been implicated in control of growth. High AO activity is associated with rapidly expanding cells, and a model linking wall ascorbate and ascorbate oxidase to cell wall extensibility has been presented (Smirnoff, 1996). Ascorbate has also been implicated in

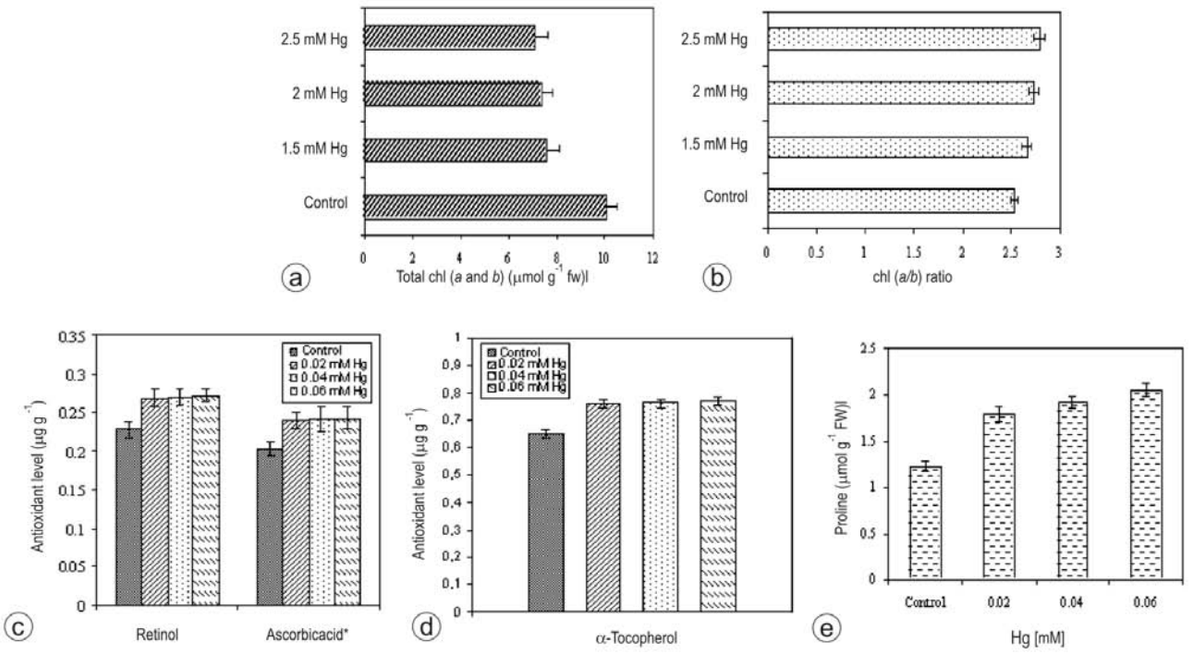

Fig. 5. (**a**) Total chlorophyll (*a* and *b*) content [µmol g^{-1} (FW)], (**b**) Chlorophyll *a*/*b* ratio, (**c**) Retinol and ascorbic acid [µg.g^{-1} (FW)], (**d**) α-tocopherol [µg.g^{-1} (FW)], and (**e**) Proline content [µmol g^{-1}(FW)] in primary leaf of bean seedlings dosed with different concentrations of mercury ($HgCl_2$). Error bars indicate ± SE. *Ascorbic acid × 10^{+2}.

regulation of cell division, through its influence on progression from the G1 to S phases of the cell cycle. There is a need to increase our understanding of this enigmatic molecule, since it could be involved in a wide range of important functions from antioxidant defense and photosynthesis to growth regulation (Smirnoff, 1996). It was found to act as a chain-breaking scavenger for peroxy radicals and also as a synergist with vitamin E (tocopherol), since vitamin C can donate a hydrogen atom to the vitamin E-derived phenolate radical, thus regenerating its activity. Vitamin E (tocopherol) is one of the best quenchers for singlet oxygen, and can act as a chain-breaking antioxidant (Chanwítheesuk et al., 2005). α-tocopherol is the major form found in green parts of plants. Tocopherols protect lipids and other membrane components by physically quenching and reacting chemically with singlet oxygen. Scavenging of singlet oxygen by α-tocopherol in chloroplasts results in the formation of, among other products, α-tocopherol quinone, a known contributor to cyclic electron transport in thylakoid membranes, thereby providing photoprotection for chloroplasts. Given that α-tocopherol increases membrane rigidity, its concentration, together with that of other membrane components, might be regulated to afford adequate fluidity for membrane function (Munné-Bosch and Alegre, 2002.). Furthermore, α-tocopherol may effect intracellular signaling in plant cells. The effects of this compound in intracellular signaling may be either direct, through interaction with key components of the signaling cascade, or indirect, through prevention of lipid peroxidation or the scavenging of singlet oxygen. In the latter case, α-tocopherol may regulate the intracellular concentrations of reactive oxygen species and plant hormones, such as jasmonic acid, which control both the growth and development of plants, and also the plant response to stress (Munné-Bosch and Alegre, 2002). More recently it has been shown that the extent of α-tocopherol quinine accumulation in Mediterranean plants exposed to drought stress depends on the severity of the stress, the stress sensitivity of the species, and the presence of alternative mechanisms of antioxidant protection (e.g., diterpenes, flavonoids) (Munné-Bosch and Alegre, 2003; Hernàndez et al., 2004). It has also been shown that α-tocopherol quinone correlates with dynamic photoinhibition of photosynthesis, supporting the contention that this compound may be involved in cyclic electron transport around photosystem II (Kruk et al., 2000; Kruk and Strzalka, 2001). In our study it was determined that all four metals (Pb^{++}, Cu^{++}, Cd^{++}, Hg^{++}) caused a significant increase in retinol, α-tocopherol

and ascorbic acid levels. Exposure of *Bacopa monnieri* to various concentrations of mercury for 14 days caused an increase in ascorbic acid levels (Sarita, 1996).

For over 40 years, plant physiologists have studied the accumulation of free proline in a number of species subjected to hyperosmotic stress. Terrestrial plants experience dehydration not only under conditions of water deficit and elevated soil salinity, but also following exposure to low temperature (Hare et al., 1999). Accumulation of free proline in response to heavy metal exposure seems widespread among plants (Costa and Morel, 1994). Exposure to heavy metals, especially Cd (Barceló et al., 1986), is known to disturb the plant water balance. Proline accumulation in plants under Cd stress is induced by a Cd-imposed decrease of the plant water potential, and the functional significance of this accumulation would lie in its contribution to water balance maintenance; proline-mediated alleviation of water deficit stress could substantially contribute to Cd tolerance (Costa and Morel, 1994). Proline increases the stress tolerance of plants through such mechanisms as osmoregulation, protection of enzymes against denaturation, and stabilization of protein synthesis (Kuznetsov and Shevyakova, 1997). Accumulation of free proline in response to Cu, Cd and Zn was determined in nontolerant and metal-tolerant *Silene vulgaris* (Moench) Garcke; the constitutive proline concentration in leaves was 5 to 6 times higher in the metal-tolerant ecotype than in the nontolerant ecotype (Schat et al., 1997).

The present results showed that lead, copper, cadmium and mercury toxicity decreased the total chlorophyll content of the leaves of bean seedlings. In response to heavy metal stress, the plants increased their proline, retinol, α-tocopherol and ascorbic acid content. The highest increases in proline, retinol, α-tocopherol and ascorbic acid content and greatest reduction in total chlorophyll were found in plants exposed to mercury, followed by the sequence cadmium > copper > lead.

ACKNOWLEDGEMENTS

We are grateful to dr. Suleyman Aydin (Firat University, School of Medicine) for his critical reading of the manuscript, editing and suggestions.

REFERENCES

ALÍA P, and SARADHI PP. 1991. Proline accumulation under heavy metal stress. *Journal of Plant Physiology* 138: 554–558.

ANDERSON JM. 1986. Photoregulation of the composition, function and structure of the thylakoid membranes. *Annual Review of Plant Physiology* 37: 3–136.

BARCELÓ J, CABOT C, and POSCHENRIEDER C. 1986. Cadmium induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv Contender), II effects of Cd on endogenous abscisic acid levels. *Journal of Plant Physiology* 125: 27–34.

BATES LS, WALDREN SP, and TEARE ID. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205–207.

CATIGNANI GL. 1983. Simultaneous determination of retinol and tocopherol in serum or plasma by liquid chromatography. *Clinical Chemistry* 2914: 708–712.

CERHATA D, BAUEROVA A, and GINTER E. 1994. Determination of ascorbic acid in blood serum using high-performance liquid chromatography and its correlation with spectrophotometric. *Ceska-Slov-Farm* 43: 166–168.

CHANWÍTHEESUK A, TEERAWUTGULRAG A, and RAKARÍYATHAM N. 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry* 92: 491–497.

CHAREST C, and PHAN CT. 1990. Cold acclimation of wheat (*Triticum aestivum*) properties of enzymes involved in proline metabolism. *Physiologia Plantarum* 80: 159–168.

CHATTERJEE J, and CHATTERJEE C. 2000. Phototoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution* 109: 69–74.

COSTA G, and MOREL J-L. 1994. Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiology and Biochemistry* 32: 561–570.

DELFINE S, ALVINO A, VILLIANI MC, and LORETA F. 1999. Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiology* 119: 1101–1106.

DIETZ K-J, BAIER M, and KRÄMER U. 1999. *Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants: from molecules to ecosystems*, 73–97. Springer-Verlag, Berlin.

EL-KHATIB AA. 2003. The response of some common Egyptian plants ozone and their use a biomonitors. *Environmental Pollution* 124: 419–428.

GALLI U, SCHIIEPP H, and BRUNOLD C. 1996. Thiols in cadmium and copper-treated maize (*Zea mays* L.). *Planta* 198: 139–143.

HALL JL. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* 53: 1–11.

HALLIWELL B, and GUTTERIDGE JMC. 1993. *Free radicals in biology and medicine.* Clarendon, Oxford.

HARE PD, CRESS WA, and VAN STADEN J. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *Journal of Experimental Botany* 50: 413–434.

HAVAUX M, and KLOPPSTECH K. 2001. The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. *Planta* 213: 953–966.

HERNÀNDEZ I, ALEGRE L, and MUNNÉ-BOSCH S. 2004. Drought-induced changes in flavonoids and other low-molecular-weight antioxidants in *Cistus clusii* plants grown under Mediterranean field conditions. *Tree Physiology* 24: 1303–1311.

KRUK J, SCHMID GH, and STRZALKA K. 2000. Interaction of α-tocopherol quinine, α-tocopherol and other prenyllipids with photosystem II. *Plant Physiology and Biochemistry* 38: 271–277.

KRUK J, and STRZALKA K. 2001. Redox changes of cytochrome b559 in the presence of plastoquinones. *Journal of Biological Chemistry* 276: 86–91.

KUZNETSOV VV, and SHEVYAKOVA N I. 1997. Stress responses of tobacco cells to high temperature and salinity. Proline accumulation and phosphorylation of polypeptides. *Physiologia Plantarum* 100: 320–326.

LANARAS T, MOUSTAKAS M, SYMEONÍDÍS L, DIOMANTOGLOU S, and KARATAGLÍS S. 1993. Plant metal content, growth responses and some photosynthetic measurements of field-cultivated wheat growing on ore bodies enriched in Cu. *Physiologia Plantarum* 88: 307–314.

MILLER KW, LORR NA, and YANG CS. 1984. Simultaneous determination of plasma retinol, α-tocopherol, lycopene, α-carotene, and β-carotene by high performance liquid chromatography. *Analytical Biochemistry* 138: 340–345.

MISHRA A, and CHOUDHURI MA. 1999. Monitoring or phytotoxicity of lead and mercury from germination and early seedling growth induces two rice cultivars. *Water, Air, and Soil Pollution* 114: 339–346.

MONNI S, UHLIG C, JUNTTILA O, HANSEN E, and HYNYNEN J. 2001. Chemical composition and ecophysiological responses of *Empetrum nigrum* to above ground element application. *Environmental Pollution* 112: 417–426.

MUNNÉ-BOSCH S, and ALEGRE L. 2002. The function of tocopherol and tocotrienols in plants. *Critical Review in Plant Sciences* 21: 31–57.

MUNNÉ-BOSCH S, and ALEGRE L. 2003. Drought-induced changes in the redox state of alpha-tocopherol, ascorbate, and the diterpene carnosic acid in chloroplasts of Labiatae species differing in carnosic acid contents. *Plant Physiology* 131: 1816–1825.

NEDELKOSKA TV, and DORAN PM. 2000. Characteristics of heavy metal uptake by plants species with potential for phytoremediation and phytomining. *Minerals Engineering* 13: 549–561.

NOCTOR G, and FOYER CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual Review in Plant Physiology and Molecular Biology* 49: 249–279.

ONCEL I, KELES Y, and USTUN AS. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environmental Pollution* 107: 315–320.

PAREKH D, PURANÍK RM, and SRÍVASTAVA HS. 1990. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochemie Physiologie der Pflanzen* 186: 239–242.

SARITA S, MANISHA G, and PRAKASH C. 1996. Bioaccumulation and biochemical effects of mercury in the plant *Bacopa monnieri* L. *Environmental Toxicology and Water Quality* 11: 105–112.

SCHAT H, SHARMA SS, and VOOIJS R. 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and non-tolerant ecotype of *Silene vulgaris*. *Physiologia Plantarum* 101: 477–482.

SINGH A, AGRAWALSB, and RATHORE D. 2005. Amelioration of Indian urban air pollution phytotoxicity in *Beta vulgaris* L. by modifying NPK nutrients. *Environmental Pollution* 134: 385–395.

SINGH S, and SINHA S. 2005. Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. *Ecotoxicology and Environment Safety* 62: 118–127.

SMIRNOFF N. 1996. Botanical briefing: the function and metabolism of ascorbic acid in plants. *Annals of Botany* 73: 661–669.

SRESTY TVS, and MADHOVA RAO KV. 1999. Ultrastructural alterations in response to zinc and nickel stress in the root cell of pigeonpea. *Environmental Experimental Botany* 41: 3–13.

STOBART AK, GRIFFITHS WT, AMEEN-BUKHARI I, and SHERWOOD RP. 1985. The effect of Cd^{+2} on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum* 63: 293–298.

TAVAZZI B, LAZZARINO G, DI-PIERRO D, and GIARDINA B. 1992. Malondialdyde production and ascorbate decrease are associated with reperfusion of the isolated postischemic rat heart. *Free Radical Biology and Medicine* 13: 75–78.

VAN ASSCHE, and CLIJSTERS H. 1990. Effect of metals on enzyme activity in plants. *Plant Cell Environment* 13: 195–206.

VAN HOVE LWA, BOSSEN ME, SON GABINO BG, and SGREVA C. 2001. The ability of apoplastic ascorbate to protect poplar leaves against ambient ozone content rations: a quantitative approach. *Environmental Pollution* 114: 371–382.

ZHANG J, and KIRKHAM MB. 1994. Drought stress induced changes in activities of superoxide dismutase, catalase and peroxide in wheat species. *Plant Cell Physiology* 35: 785–791.

ZELLER S, and FELLER U. 1999. Long-distance transport of cobalt and nickel in maturing wheat. *European Journal of Agronomy* 10: 91–98.