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The Palliative Effect of Lead Pollution in *Vicia faba L.* by using Stigmasterol

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Abstract. Environmental pollution by toxic heavy metals is one of the most serious issues that world populations have to cope with. Lead is one of the most toxic for humans and plants with great damage for the environment.

Faba bean (*Vicia faba* L.) is an annual legume crop which is consumed as plant foods for human and animal nutrition. Thus, this study was undertaken to evaluate the effect of stigmasterol at recommended dose (500 µM), a promising plant development regulatory substance in alleviating the deteriorative effect of Pb at 0.0 and 400 mg/l on faba bean plants. The photosynthetic pigments, photosynthetic activity ($^{14}CO_2$-assimilation), transpiration rate, antioxidant enzymes activities, glutathione contents, free proline, total protein, total phenol, lipid peroxidation (malondialdehyde content) and inorganic nutrient contents (nitrogen, phosphorus and potassium) were investigated.

The results revealed that lead-stressed bean plants treated with stigmasterol had increased levels of photosynthetic pigments (chlorophyll a+b and carotenoids) as compared with lead-stressed plants. The level of antioxidant system components (superoxide dismutase, peroxidase, catalase, and glutathione peroxidase) increased in response to stigmasterol treatment as compared to lead-stressed plants. Enhanced antioxidant activities helped to decrease oxidative damage and develop tolerance against lead stress in stigmasterol-treated faba bean plants. An increase in the degree of lead tolerance induced by stigmasterol was indicated by the improvement of photosynthetic pigments and consequently the photosynthetic activity and weight of 100 grain. Lead treatments decreased the transpiration rate as compared to the control plant. Meanwhile, transpiration rate increased in response to stigmasterol treatment as compared to lead-stressed plants.

Lead treatments decreased the macronutrient level (N, P and K) as compared to the control plants. Meanwhile lead-stressed bean plants pretreated with stigmasterol had an increased level of these element contents.

The data provided evidence that stigmasterol treatment reduced the adverse effects of lead stress on faba bean plants, and might play a key role in providing stress tolerance by stimulation of the antioxidant system as a stress protection mechanism. Eventually, the results of the present investigation clearly manifested that the addition of the stigmasterol (500 µM), to faba bean plants grown in soil contaminated by lead up to 400 mg/l, boosted plants to overcome or even reduce lead toxicity and thus obtained relatively better growth, better quality and yield, as well as better chemical composition.

Key Words: *Vicia faba*, stigmasterol, lead, antioxidant enzymes, free proline.

1. Introduction

The global problem concerning the contamination of the environment as a consequence of anthropogenic activities is increasing. Most of the environmental contaminants are chemical by-products and heavy metals such as lead (Pb). The sources of lead included smelting, combustion of leaded gasoline, or applications of lead-contaminated media (sewage sludge and fertilizers) to land [1,2,3,4], intensive agriculture, municipal wastes, pesticides, power transmission and military operations [5,6]. Despite a long history of its beneficial use by humankind, lead has no known biological function in living organisms [7].

Lead released into the environment makes its way into the air, soil and water [8]. Lead is a cumulative poison which when present at higher doses causes serious irreversible damage to the human brain, kidneys, nervous system and decline in mental, cognitive and physical health [9]. Diverse biochemical changes in green plants in response to Pb have been reported by several authors, which includes reduction in the rates of photosynthetic activity and transpiration rate, net CO_2 uptake, photosystem II efficiency, stomatal conductance [10], DNA synthesis and mitotic activity [11,12]. It also reduces plant growth, namely the length of roots and shoots, leaf area, the fresh and dry mass of both roots and shoots [11,13,14], plant survival rates [15], the number and size of leaves, tillers and inflorescence and finally plant yield [16,17]. Moreover, lead accumulation negatively affected the contents of each of chlorophyll a,b, a+b and carotene as a result of photosynthetic pigment degradation [17,18,19,20,21,22], the levels of total sugars, nitrogen, protein, nitrate and free amino acids in various tested plants [14,16,17] and the absorption of calcium, phosphorus, potassium and magnesium by polluted plants as it dramatically affects the permeability of plasma membranes to these elements resulting in nutrient imbalance [23-25]. In stress conditions (biotic or abiotic stresses), different protective processes are enhanced such as accumulation of compatible solutes (proline and soluble sugars) which play an important role in osmoregulation, enzyme protection and scavenging of free radicals [26-28], increase in the activities of detoxifying enzymes and enhanced tissue lipid peroxidation. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage [29].

Lead-exposed plants may have high concentrations of phenols produced as an enzymatic strategy to cope with metal-caused oxidative stress [30].

Faba bean, *Vicia faba* L., is one of the world's most important legume crops as a source of protein. In Egypt it is used in the human diet, as animal feed, and for industrial purposes [31,32]. The nutritional value of faba bean has been attributed to its high protein content, which ranges from 25 to 35%. The seeds are also a good source of sugars, minerals and vitamins; being particularly rich in calcium and iron, and the contents of thiamin, tocopherols, niacin and folic acid are high as compared with other grains [33]. In Egypt, faba bean (*Vicia faba* L.) is produced in an average area of 69720 ha with an average yield of 1896 kg/ha [34]. Although Egypt is the third highest producer of faba beans, the country imports a considerable quantity from China to satisfy the local requirement.

Stigmasterol is a structural component of the lipid core of cell membranes and is the precursor of numerous secondary metabolites, including plant steroid hormones, or as carriers in acyl, sugar and protein transport [35]. Sterols play an important role in plant development [36,37]. Stigmasterol is considered to be one of the mostly free or conjugated sterols that play essential functions in plant growth [35]. A number of studies have provided evidence that stigmasterol plays a role in response to biotic and abiotic stresses [38,39].

The objective of the current study was to investigate the effect of stigmasterol at recommended dose (500 µM), a promising plant development regulatory substance in alleviating the deteriorative effect of Pb at 0.0 and 400 mg/l on faba bean plants. The photosynthetic pigment, photosynthetic activity ($^{14}CO_2$-assimilation), transpiration rate, antioxidant enzymes activities (superoxide dismutase, peroxidase and catalase), glutathione contents, free proline, total protein, total phenol, lipid peroxidation (malondialdehyde content) and inorganic nutrient contents (nitrogen, phosphorus and potassium) were investigated.

2. Materials and Methods

Plant Material and Experimental Conditions.

Uniform-sized faba bean seeds (*Vicia faba* L.*)* cv. Giza 2 was purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt. Grains were surface sterilized in 0.1% (w/v) sodium dodecyl sulphate solution and then thoroughly rinsed with sterile deionized water. The seeds were then soaked overnight (12 h) in either distilled water or 500 μM freshly prepared stigmasterol solution (solution was prepared by dissolving stigmasterol in a minimum amount of chloroform then complete to the total volume by distilled water) the recommended dose (RD, 500 μM) was selected according to El-Greedly and Mekki [40].

Ten seeds per treatment were sown in each pot at 3 cm depth. The seeds were germinated in pots (40 cm high × 35 cm diameter), each filled with 15 kg sandy loam soil with 2.5% organic matter and available N, P and K concentrations of 170, 80 and 200 mg kg^{-1}, respectively. To simulate soil pollution, the soil was amended with lead nitrate $Pb(NO_3)_2$ at a concentration of 400 mg/l. The treatments are, T_1 (water, control), T_2 (lead at 400mg/l),T_3 (stigmasterol at 500 μM), and T_4 (lead at 400 mg/l + stigmasterol at 500 μM).

After emergence, the seedlings were thinned to four healthy seedlings per pot. Plants were grown in a controlled environment growth chamber with 15-h photoperiod; 65%–75% relative humidity; and day and night temperatures of 22 and 20°C. Photosynthetic photon flux density at maximum plant height was about 440 $\mu Mm^{-2}s^{-1}$. Cultural practices, such as weed control and irrigation, were performed as needed. The experimental design was randomized complete block design with three replicates.

All determinations were carried out at the flowering stage (50-day-old). Weights of 100 seed were determined at the harvest stage.

Chemical Analysis.

Photosynthetic activity ($^{14}CO_2$-assimilation) was measured in the Radioisotope Department, Atomic Energy Authority, Cairo, Egypt, with the method of Moussa [31]. Transpiration rate was determined as described by Ludlow and Muchow [41]. Chlorophyll *a,b* and total carotenoids were determined according to Dere *et al.* [42]. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the thiobarbituric acid reaction as described by Madhava Rao and Sresty [43]. The catalase (CAT, EC 1.11.1.6) activity was assayed from the rate of H_2O_2 decomposition following the method of Aebi [44]. The glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined as the decrease in absorbance at 340 nm due to the oxidation of NADPH by the method of Navrot *et al.* [45]. Superoxide dismutase activity (SOD, EC 1.15.1.1) was assayed by the nitrobluetetrazolium modified method from that described by Dhindsa *et al.* [46]. Peroxidase (POD, EC 1.11.1.7) following the method of Macheix and Quessada [47]. Free proline content was estimated photometrically in acidic ninhydrin assay according to the method adopted by Bates *et al.* [48]. Total phenols were determined in the ethanolic extract following the method described by Simons and Ross [49] using folin reagent.

Elemental Analysis.

Determination of potassium in shoot of faba bean was done by flam photometer (Jenway, PFP-7). The method of Prokopy [50] was applied for phosphorus estimation. Total nitrogen was determined using the Kjeldahal method according to AOAC [51].

Statistical Analysis.

Data were analyzed using a stat view ANOVA program. Statistically different groups were determined by Duncan's test ($P < 0.01$) as described by Moussa [31].

3. Results and Discussion

Photosynthetic pigments, weights of 100 grain, photosynthetic activity ($^{14}CO_2$-assimilation) and transpiration rate.

Data presented in Table (1) revealed that Pb treated plants showed significant decrease in photosynthetic pigments (chlorophyll a+b and carotenoids), weights of 100 grain, photosynthetic activity ($^{14}CO_2$-assimilation) and transpiration rate by 25, 44, 25.6, 28.3 and 51.2 %, respectively as compared to the control plants. These results were in correspondence with those of Larbi *et al.* [23], Zengin and Munzuroglu [52] and John *et al.* [53] concerning the deteriorative effect of lead on chlorophyll a,b & total chlorophyll. Our results also corroborated with those of Romerio *et al.* [11] and Ali and Al-Homaidan [18] concerning lead retardation of photosynthetic and transpiration rates and net CO_2 assimilation.

Pb negatively affects many processes vital to the photosynthetic pathway as chlorophyll biosynthesis, as it inhibits the activity of delta aminolevulinic acid dehydratase enzyme responsible for the biosynthesis of heme pigments [54], alters the action of ribulose 1,5-bisphosphate carboxylase-oxygenase, interferes with the dynamics of the thylakoid membrane, inhibits electron transport system in both photosystem I and II and finally reduces the metabolites of the carbon reduction cycle [13,18]. Any or all of these effects can explain the significant damage and death, or to even less extent, the reduction in growth, metabolism and yield of faba bean plants.

Table 1. Effect of lead (400 mg/l) in *Vicia faba* plants pre-treated with stigmasterol (500 μM) on chlorophyll *a+b* (mg g^{-1} FW), carotenoids (mg g^{-1} FW), weight of 100 grain (g), photosynthetic activity (10^3 Becquerel mg^{-1}FW) and transpiration rate (mMH_2O $m^{-2}s^{-1}$).

Treatment	Chlorophyll (*a+ b*)	Carotenoids	Weights of 100 Grain	Photosynthetic Activity	Transpiration Rate
T_1	**3.59^b**	**1.67^a**	**55.8^b**	**13.720^b**	**4.3^a**
T_2	**2.71^d**	**0.94^c**	**41.5^d**	**9.830^d**	**2.1^b**
T_3	**3.69^a**	**1.69^a**	**56.7^a**	**14.625^a**	**4.2^a**
T_4	**3.38^c**	**1.43^b**	**52.9^c**	**12.546^c**	**4.0^c**

Data are means of three replicates. Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01).The treatments are, T_1 (control),T_2 (lead at 400mg/l),T_3 (stigmasterol at 500 μM), and T_4 (lead at 400 mg/l + stigmasterol at 500 μM).

The decrease in chlorophyll content in lead-stressed faba bean plants concomitant with the increase in proline content (Table 2) is consistent with the suggestion that nitrogen might be redirected to the synthesis of proline instead of chlorophyll [55].

Meanwhile, plant treatment with stigmasterol (500 μM) + lead (400 mg/l) showed a significant decrease in the photosynthetic pigments (chlorophyll a+b and carotenoids), weights of 100 grain, photosynthetic activity ($^{14}CO_2$-assimilation) and transpiration rate by 5.8, 14.4, 5.2, 8.5 and 6.8 %, respectively as compared to the control plants. Plants pretreated with stigmasterol (500 μM) alone or in combination with lead (400 mg/l) stimulated the photosynthetic pigments, weights of 100 grain, photosynthetic activity ($^{14}CO_2$-assimilation) and transpiration rate which were consistent with those of [39,56,57].

In agreement with these results, Kalinich *et al*. [58] stated that spray application of stigmasterol enhanced the photosynthetic apparatus and enzyme activity in beans. In addition, Abd El-Wahed [37] found that the contents of the photosynthetic pigments chl *a*, chl *b* and carotene were increased in maize as sitosterol concentration increased.

A strong relationship exists between Pb application and a decrease in photosynthesis of the whole plant and is believed to result from stomatal closure rather than a direct effect of Pb on the process of photosynthesis [39].

Antioxidant enzyme activities (catalase, peroxidase and superoxide dismutase), glutathione peroxidase, lipid peroxidation levels (malondialdehyde), free proline, total phenols and total protein.

The results presented in Table (2) revealed that Pb treated plants showed a significant decrease in antioxidant enzyme activities (superoxide dismutase, peroxidase and catalase), glutathione peroxidase and total protein by 36, 27, 40, 37.2 and 34.2 %, respectively as compared to the control plants. Meanwhile, Pb treated plants showed a significant increase in lipid peroxidation levels (malondialdehyde), free proline and total phenols by 60.7, 50, and 26 %, respectively as compared to the control plants. These results are in agreement with that of [10,39].

Plants treated with stigmasterol (500 μM) + lead (400 mg/l) showed a significant decrease in the antioxidant enzyme activities (superoxide dismutase, peroxidase and catalase), glutathione peroxidase and total protein by 5.1, 8.3, 14.3, 11.5 and 8 %, respectively as compared to the control plants. Also, decreased the lipid peroxidation levels (malondialdehyde), free proline and total phenols by 21.4, 14.8, and 5.6 %, respectively as compared to the control plants. These results were consistent with those of [10,39].

In addition, proline accumulation was reported to serve as a nitrogen storage compound and protect cellular structure [59]. It is also evident from the present study that the level of proline increased in *Vicia faba* plants treated with lead and decreased with stigmasterol treatment. This finding might be explained by the fact that stigmasterol enhances the biosynthesis of other amino acids and their incorporation into protein [10].

Table 2. Effect of lead (400 mg/l) in *Vicia faba* plants pre-treated with stigmasterol (500 μM) on SOD (units mg^{-1} protein), POD (units mg^{-1} protein), CAT (μMH_2O_2/min.gFW), GPX (μMNADPH/min.gFW), MDA (μM g^{-1} FW), free proline (μmol g^{-1} FW), total phenol (mg g^{-1} DW), and total protein (mg g^{-1} FW).

Treatment	SOD	POD	CAT	GPX	MDA	Free Proline	Total Phenol	Total Protein
T_1	**7.8^b**	**10.8^b**	**3.5^a**	**7.8^b**	**2.2^c**	**213^c**	**17^c**	**149^b**
T_2	**5.0^d**	**7.9^d**	**2.1^d**	**4.9^d**	**5.6^a**	**427^a**	**23^a**	**98^d**
T_3	**8.1^a**	**11.7^a**	**3.2^b**	**8.4^a**	**2.0^d**	**205^d**	**19^b**	**162^a**
T_4	**7.4^c**	**9.9^c**	**3.0^c**	**6.9^c**	**2.8^b**	**250^b**	**18^c**	**137^c**

Data are means of three replicates. Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01).The treatments are, T_1 (control), T_2 (lead at 400mg/l), T_3 (stigmasterol at 500 μM), and T_4 (lead at 400 mg/l + stigmasterol at 500 μM).

Inorganic Macronutrient Contents (nitrogen, potassium and phosphorus).

It is evident from Tables (3) that the content of most major nutrient elements estimated in this study (N, P and K) decreased in Pb (400 mg/l) treated plants by 23.5, 33.8, 43.7 %, respectively as compared to the control plants. Several comparable studies confirmed the current data [25]. Accordingly, it is ascertained that Pb toxicity causes disorder in plant mineral nutrition, an alteration in membrane permeability and its physiological activity and lowering pH of soil thus rendering most nutritive elements in soil unavailable to plant roots [10].

Table 3. Effect of lead (400 mg/l) in *Vicia faba* plants pre-treated with stigmasterol (500 μM) on the macronutrient contents of N, P and K (mg g^{-1}DW).

Treatment	N	P	K
T_1	**98^a**	**77^a**	**65^a**
T_2	**75^d**	**51^d**	**37^d**
T_3	**93^b**	**73^b**	**61^b**
T_4	**89^c**	**68^c**	**56^c**

Data are means of three replicates. Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01).The treatments are, T_1 (control), T_2 (lead at 400mg/l), T_3 (stigmasterol at 500 μM), and T_4 (lead at 400 mg/l + stigmasterol at 500 μM).

However, when these elements were uptaken by plants, Pb induced an imbalance in their distribution and their concentrations in different plant tissues; thus, they became less beneficial to Pb-treated plants. This opinion was strengthening by the remarks of both [57,60].

Meanwhile, plants treated with stigmasterol (500 μM) + lead (400 mg/l) showed a significant increase in the above parameters by 5.1, 11.6 and 13.8 %, respectively as compared to the control plants. These results were confirmed by the results of [35,56].

4. Conclusion.

Subsequently, it appears that the stigmasterol (500 μM), can drastically inhibit the hazardous effect of Pb by exclusion, chelation, compartmentalization of lead and other toxic heavy metals in soil away from faba bean roots or in the apoplastic tissues of root and hampered their translocation to shoots, and/ or by synthesizing metal detoxifying substances (phenols) or enzymes (antioxidant enzymes) or osmoprotectants (proline), and/or by inducing nutrient balance which resulted from progressive uptake of beneficial elements. All these effects render faba bean plants capable of enduring lead toxicity and restitute normal growth and productivity which were indicated in the present results. The method of soaking seeds in stigmasterol is a simple and economic method for improvement of lead tolerance of plants and is environmentally safe.

The present study revealed that stigmasterol treatment (seeds pre-treated with 500 μM stigmasterol solution) induced augment of enzymatic antioxidant system (SOD, POD and CAT) and non-enzymatic antioxidant system (GPX), reducing oxidative damage (MDA) in lead stressful conditions.

The increase in the degree of lead tolerance induced by stigmasterol was also reflected in the improvement in the photosynthetic pigments, photosynthetic activity, weights of 100 grain and total protein.

Eventually, the results of the present investigation clearly manifested that the addition of the stigmasterol (500 μM), to faba bean plants grown in soil contaminated by lead (400 mg/l), boosted plants to overcome or even reduce lead toxicity and thus obtained relatively better growth, better quality and yield, as well as better chemical composition.

References

[1] Piotrowska A, Bajguz A, Godlewska-Zylkiewicz B, Czerpak R, Kaminska M (2009). Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). Environmental and Experimental Botany, 66, 507-513.

[2] Gupta D, Nicoloso F, Schetinger M, Rossato L, Pereira L, Castro G, Srivastava S, Tripathi R (2009). Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. Journal of Hazardous Materials**,** 172, 479-484.

[3] Sammut M, Noack Y, Rose J, Hazemann J, Proux O, Depoux Ziebel M, Fiani E (2010). Speciation of Cd and Pb in dust emitted from sinter plant. Chemosphere, 78, 445-450.

[4] Grover P, Rekhadevi P, Danadevi K, Vuyyuri S, Mahboob M, Rahman M (2010). Genotoxicity evaluation in workers occupationally exposed to lead. International Journal of Hygiene and Environmental Health, 213, 99-106.

[5] Nedelkoska TV and Doran PM (2000). Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Min. Eng., 13: 549-61.

[6] Mercier G, Duchesne J, Carles-Gibergues A (2002). A simple and fast screening test to detect soils polluted by lead. Environ. Pollut., 118: 285-96.

[7] Maestri E, Marmiroli M, Visioli G, Marmiroli N (2010). Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environmental and Experimental Botany, 68, 1-13.

[8] Paz-Alberto AM, Sigua GC, Bellrose GB, Prudente JA (2007). Phytoextraction of lead contaminated soil using vetivergrass (*Vetiveria zizanioides* L.), Cogongrass (*Imperta cylindrica* L.) and Carboagrass (*Paspalum conjugatum* L.). Environ. Sci. Pollut. Res., 14(Pt7): 505-509.

[9] Skipton SO, Dvorak BI, Woldt W, Drda S (2008). Drinking Water; Lead. Neb. Guide, 3: 1-4.

[10] Mona M Abdalla and Nada El-Khoshiban (2012). The Palliative Effect of Bio-Organic Fertilizer on Lead Pollution in *Lycopersicum esculentum* Plants. Journal of Basic and Applied Sciences, 8, 399-410.

[11] Romerio S, Lagoa AMMA, Furlani PR, deAbreu CA, deAbreu MF, Erismann NM (2006). Lead uptake and tolerance of *Ricinus communis* L. para chumbo. Braz. J. Plant Physiol., 18(Pt4): 1-10.

[12] Wierzbicka M (1999). The effect of lead on the cell cycle in the root meristem of *Allium cepa* L. Protoplasma, 207: 186-94.

[13] Nicholis AM and Mal TK (2003). Effects of lead and copper exposure on growth of an invasive weed, *Lythrum salicaria* L. (Purple Loosestrife*).* Ohio J. Sc., 103(Pt 5): 129-33.

[14] Xiong ZT, Zhao F, Li MJ (2006). Lead toxicity in *Brassica pekinensis* Rupr, Effect on nitrate assimilation and growth. Environ. Toxic., 21(Pt2): 147-53.

[15] Rotkittikhun P, Chaiyarat R, Kruatrechue M, Pokethitiyook P, Baker A (2007). Effects of soil amendment on growth and lead accumulation in grass *Vetiveria zizanioides* and *Thysanolaena maxima* grown in lead-contaminated soil. Environ. Pollut., 12: 1-10.

[16] Moftah AE (2000). Physiological responses of lead-polluted tomato and eggplant to the antioxidant ethylene diurea. Minufiya J. Agric. Res., 25(Pt4): 933-954.

[17] Chatterjee C, Dube BK, Sinha P, Srivastava P (2004). Detrimental effects of lead phytotoxicity on growth yield and metabolism of rice. Comm. Soil Sci. Plant Anal., 35(Pt 1and2): 255-65.

[18] Ali AA and AL-Homaidan AA (2007). Removal of lead ions from polluted water using *Plantago vulgaris* L. Aus. J. Basic and App. Sci., 1(Pt4): 467-72.

[19] Ewais EA (1997). Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. Biol. Plant., 39:403-410.

[20] Xiong ZT (1997). Bioaccumulation and physiological effects of excess lead in a roadside pioneer species *Sonchus oleraceus* L. Environ. Pollut., 97:275-279.

[21] Kastori R, Plesnicar M, Sakac Z, Pancovic D, Arsenijevic Maksimovic I (1998). Effect of excess lead on sunflower growth and photosynthesis. J. Plant Nutr., 21:75-85.

[22] Fargasova (2001). Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapis alba* L. seedlings and their accumulation in roots and shoots. Biol. Plant., 44:471-473.

[23] Larbi A, Morales F, Abadia A, Gogorcena Y, Lucena JJ, Abadla J (2002). Effects of Cd and Pb on sugar beet plants grown in nutrient solution: induced Fe deficiency and growth inhibition. Funct Plant Biol., 29(Pt12): 1453-4.

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

[25] Yassen AA, Badran NM, Zaghloul SM (2007). Role of some organic residues as tools for reducing metals hazard in plant. World J. Agric. Sci., 3(Pt2): 204-209.

[26] Choudhary M, Jetley UK, Klan MA, Zutshi S, Fatma T (2006). Effect of heavy metal Stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacerium *Spirulina plantensis-S5.* Ecotoxic. Environ. Safety, 6: 143-49.

[27] Talanova VV, Titov AF, Boeva NP (2004). Effect of increasing concentrations of lead and cadmium on cucumber seedlings. Biol. Plant., 43(Pt3): 441-44.

[28] Zare S, Afagi A, Heidari R, Asadpoor Y, Shiri S (2007). Effects of lead nitrate $Pb(NO_3)_2$ on the glucose and cortisol hormone levels in common, *Cyprinus carpio*. Pak. J. Biol. Sci., 10: 2587-2590.

[29] Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem., 95: 351.

[30] WANG C, GU X, WANG X, GUO H, GENG J, YU H, SUN J (2011). Stress response and potential biomarkers in spinach (*Spinacia oleracea* L.) seedlings exposed to soil lead. Ecotoxicology and environmental safety, 74(1): 41-47.

[31] Moussa HR (2008). Gamma irradiation effects on antioxidant enzymes and G_6PDH activities in *Vicia faba* plants. J. New Seeds, 1:89-99.

[32] Crepon K, Marget P, Peyronnet C, Carrouée B, Arese P, Duc G (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crop Res., 115: 329-339.

[33] Larralde J and Martinez JA (1991). Nutritional value of faba bean: effects on nutrient utilization, protein turnover and immunity. Option Mediterraneennes-Serie Siminaires, 10:111-117.

[34] AOAD (2007). Arab Agriculture Statistics Year Book, 27. Arab Organization for Agricultural Development Khartoum, Sudan.

[35] Genus JMC (1978). Steroid hormones and growth and development. Phytochem., 17:1-44.

[36] Clouse SD and Sasse JML (1998). Brassinosteroid: Essential regulatore of plant growth and development. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49:427-451.

[37] Abd El-Wahed MS (2001). Sitosterol stimulation of root growth, yield and some biochemical constituents of maize. J Agr Sci Mansoura Univ., 26: 2563-2577.

[38] Arnqvist L, Persson M, Jonsson L, Dutta PC, Sitbon F (2008). overexpression of CYP710A1 and CYP710A4 in transgenic Arabidopsis plants increases the level of stigmasterol at the expense of sitosterol. Planta, 227: 309-317.

[39] Hassanein RA, Hashemand HA, Khalil RR (2012). Stigmasterol treatment increases salt stress tolerance of faba bean plants by enhancing antioxidant systems. Plant Omics journal, 5(5):476-485.

[40] El-Greedly NH and Mekki BB (2005). Growth, yield and endogenous hormones of two sesame (*Sesamum indicum L.*) cultivars as influenced by stigmasterol. J. Appl. Sci. Res., 1: 63-66.

[41] Ludlow MM and RC Muchow (1990). A critical evaluation of trials for improving yield crops in water-limited environments. Adv. Agro., 43:107-153.

[42] Dere S, Gines T, Sivaci R (1998). Spectrophotometric determination of chlorophyll-a,b and total carotenoid contents of some algae species using different solvents. Tr. J. Botany, 22: 13-17.

[43] Madhava Rao KV and Sresty TVS (2000). Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. Plant Sci., 157: 113-28.

[44] Aebi H (1983). Catalase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. 3. Weinheim-Verlag, Chemie, Germany. pp. 273-277.

[45] Navrot N, Rouhier N, Gelhaye E, Jacquot JP (2007). Reactive oxygen species generation and antioxidant systems in plant mitochondria. Physiol. Plant., 129(1): 185-195.

[46] Dhindsa RA, Dhindsa PP, Thorpe TA (1981). Leaf senescence correlated with increased permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 126: 93-101.

[47] Macheix JJ and Quessada MP (1984). Caractérisation d'une peroxydase impliquée spécifiquement dans la lignification, en relation avec l'incompatibilité au greffage chez l'abricotier. Physiologie Végétale, 22:533-540.

[48] Bates LS, Waldron RP, Teare ID (1973). Rapid determination of free proline in water stress studies. Plant Soil, 29: 205-207.

[49] Simons TS and Ross AF (1971). Changes in phenol metabolism associated with system resistance to tobacco mosaic virus in Samsun NN tobacco. Phytopath., 61, 1261-1268.

[50] Prokopy WR (1995). Phosphorus in acetic acid extracts. *Quickchem Method.,* 12: 115-117.

[51] AOAC, Official Methods of Analysis (1995). Arlington, Virginia, USA, 16th Ed.

[52] Zengin FK and Munzuraglu O (2005). Effects of some heavy metals on concept of chlorophyll, proline and some antioxidant chemicals in bean *(Phaseolus vulgaris* L.) seedlings. Acta Biol. Cracov. Seri. Bot., 47(Pt2): 157-64.

[53] John R, Ahmed P, Gadgil K, Sharma S (2008). Effect of cadmium and lead on growth biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ., 54(Pt6): 262-70.

[54] Sinha SK, Srivastava HS, Tripathi RD (2001). Influence of some growth regulators and cations on inhibition of chlorophyll biosynthesis in maize. Bull Environ Contamin Toxic., 1(Pt2): 241-46.

[55] Da La Rosa-Ibarra MM and Maiti RIK (1995). Biochemical mechanism in glossy sorghum lines for resistance to salinity stress. J. Plant Physiol., 146: 515-519.

[56] Abd El-Wahed MS and Gamal El-Din KM (2004). Stimulation on growth, flowering, biochemical constituents and essential oil of chamomile plant (*chamomilla recutita* L.) with spermidine and stigmasterol application. Bulg. J. Plant Physiol., 30: 89-102.

[57] Rolf GL and Bazzaz FA (2001). Effect of lead contamination on transpiration and photosynthesis of loblolly pine and autumn Olive. Forest Sci., 21: 33-35.

[58] Kalinich JF, Mandava NB, Todhunter JH (1985). Relationship of nucleic acid metabolism to brassinolide induced response in beans. J. Plant Physiol., 120: 207-214.

[59] Hare PD and Cress WA (1997). Metabolic implications of stressinduced proline accumulation in plants. Plant Growth Regul., 21: 79-102.

[60] Olivares E (2003). The effect of lead on the phytochemistry of *Tithonia diversifolia* exposed to roadside automotive pollution or grown in pots of Pb-supplemented soil. Braz. J. Plant Physiol., 15(Pt3): 1-13.

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