

Lead Accumulation and its Effect on Photosynthesis and Free Amino Acids in *Vicia faba* Grown Hydroponically

Hedaya Ahmed Kamel

Radioisotopes Department, Atomic Energy Authority, 12311, Cairo, Egypt.

Abstract: The effects of the heavy metal lead (Pb) on the chlorophyll content, ¹⁴C- fixation capacity and free amino acids contents of *Vicia faba* plants were examined to understand the impact of lead accumulation on the growth of *Vicia faba*. Plants were treated with different concentrations of Pb(NO₃)₂ ranging from 0-48 mM in hydroponic solution. Pb contents in both root and shoot tissues gradually increased with increasing Pb concentration in the nutrient solution. More lead was accumulated in root tissues than that translocated to shoot tissues. The higher concentration of lead (48 mM) caused a significant decrease in the fresh weight although it caused a significant increase in dry weight. Low doses of Pb (0.48 mM) increased chlorophyll content during the first 24 hrs of treatments. High concentration of Pb (48 mM) caused a significant decrease in chl-a after 24 h followed by a significant increase throughout the treatment period. The Chl-a / b ratio was increased when Pb concentration increased up to 4.8 mM relative to the control, while 48 mM Pb decreased the ratio. ¹⁴C –fixation was decreased by all applied Pb concentrations. There was no change in the total amino acids content except at 4.8 mM where, total amino acids content reached 228.67 % of the control.

Key words: *Vicia faba*; Pb accumulation; growth; ¹⁴C-fixation; free amino acids

INTRODUCTION

Lead (Pb), a heavy metal, is a potent environmental pollutant (Sharma and Dubey, 2005). Pb contamination has resulted from mining, smelting activities, Pb-containing paints, gasoline, explosives, as well as from the disposal of municipal sewage sludge enriched in Pb (Jackson and Watson, 1977; Levine *et al.*, 1989). Although it is not essential for plants; it is absorbed and accumulated in different plant tissues (Kabata-Pendias and Pendias 1999) with the highest amount in the root tissues (Kumar *et al.*, 1995; Piechalak *et al.*, 2002; Malkowski *et al.*, 2002; Verma and Dubey, 2003). Pb accumulation in root takes place by binding with polysaccharides (Seregin and Ivanove, 1998), complexing with organic acids (Harmens *et al.*, 1994) or binding to the cell walls in the roots (Sieghardt, 1981 and 1984) and xylem vessels (Fathi, 1983) and thus might become immobile. The low allocation ratio of Pb between the roots and shoots may be because of the Pb movement in the root tissues which is usually prevented by the endodermis (Sieghardt, 1981 and 1984; Wierzbicka, 1987). Plants exposed to Pb ions showed a considerable decrease in dry weight of different plant parts (Kosobrukhov, 2004), and a decline in the total chlorophyll and thus photosynthetic efficiency (Heckathorn *et al.*, 2004; Kambhampati *et al.*, 2005). High concentrations of Pb inhibits chlorophyll synthesis by impaired uptake of other essential ions by plants like Mg and Fe (Bruzynski, 1987), or due to increased chlorophyllase activity (Drazkiewicz, 1994). It has been shown that plants exposed to Pb ions showed a decline in the photosynthetic rate as a result of distorted chloroplast, restrained synthesis of chlorophyll, obstructed of electron transport, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO₂ as a result of stomatal closing (Sharma and Dubey, 2005).

Nitrogen metabolism is central to the plants response to heavy metals; upon exposure to metals, plants often synthesize a set of diverse metabolites that accumulate to concentrations in the millimolar range, particularly specific amino acids (Sharma and Dietz, 2006). Glycine and glutamic acid are involved in the synthesis of glutathione and phytochelatin, which play a role in metal binding. Arginine is involved in the synthesis of polyamines, which act as signaling molecules and antioxidants (Sharma and Dietz, 2006). The proteinogenic amino acid proline functions as a radical scavenger, electron sink, stabilizer of macromolecules, cell wall component (Matysik *et al.*, 2002), osmoregulation and in metal chelation (Sharma and Dietz, 2006).

Corresponding Author: Hedaya Kamel, Radioisotopes Department, Atomic Energy Authority, 12311, Cairo, Egypt.
E-mail: hedaya_kamel@yahoo.com

Vicia faba plants have a higher resistance to lead and a faster initiation of the detoxication system than other legumes when cultivated hydroponically in Hoagland nutrient solution supplemented with 1 mM Pb(NO₃)₂ (Piechalak *et al.*, 2002). Because the contamination with Pb is not likely to decrease in the near future, and due to the many Pb pollution sources which are indispensable for modern human life, (Yang *et al.*, 2000), we investigated the effect of different concentrations of Pb (0- 48 mM) on growth, chlorophyll content, ¹⁴C-fixation capacity, lead accumulation and free amino acids of *Vicia faba* plants grown in nutrient solution supplemented with different concentrations of Pb.

MATERIALS AND METHODS

Plant:

Seeds of *Vicia faba* Misr 1 were purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt.

Chemical:

Pb(NO₃)₂ (Sigma, USA) and NaH¹⁴CO₃ (Radiochemical laboratory, Ameresham, England) with an original specific activity 23.2 MBq / mg were used in this study.

Growth Condition:

Uniform *Vicia faba* seeds were soaked in distilled water for six hours, and then germinated in the dark on filter paper moistened with distilled water at 24°C. Two-day old seedlings were transferred to a 16 cm diameter polyethylene pot (5 seedlings /pot) containing 2 kg of sandy soil (pH 7.6, Ec 0.33 ds/m, organic matter 0.44 %, SAR 0.85) Pots were kept in the growth chamber under controlled conditions (light intensity of 100 μmol m⁻² s⁻¹, 10-h light/14-h dark cycle at 28 °C), and irrigating with full strength Hoagland nutrient solution (pH 5.8).

Lead Treatment:

Fifteen days after sowing, plants were collected and the roots were washed in a nutrient solution to remove attached sand particles. Plants of average fresh weight 6.12 ± 0.09 g/ plant were divided into 5 independent groups (126 plants/ group divided into three replicates). Plant roots of each group were exposed to 4 litres of Hoagland nutrient solution to which KH₂PO₄ was replaced with an equivalent molar concentration of KCl to avoid Pb²⁺ precipitation by PO₄³⁻ ions (Antosiewicz, 2005). Such solution was supplemented with Pb(NO₃)₂ to give 0.00 (control), 0.048, 0.48, 4.8 and 48 mM. Plants were grown in the growth room and the roots were shielded from light. At 24, 48, 72 and 96h post treatment, plants were sampled randomly and measured for growth, chlorophyll content and ¹⁴C fixation. Pb accumulation and free amino acids were determined after 96h of treatment.

Chlorophyll Content:

Total chlorophyll was extracted from fresh leaves using dimethylsulfoxide (DMSO). The absorbance (A) of the extract was measured at 648.2 nm and 664.9 nm using a (Uv-Vis recording spectrometer, UV-240 PC Shimadzu). Chlorophyll contents in the leaves were calculated according to (Barnes *et al.*, 1992) using the following equations.

- Chl- a = 14.85 A_{664.9} - 5.14 A_{648.2}
- Chl- b = 25.48 A_{648.2} - 7.36 A_{664.9}

Photosynthetic Activity (¹⁴C- Application and Fixation Measurement):

Plant samples collected at different time periods were transferred to paper cups containing Hoagland nutrient solution supplemented with the same concentration of Pb and exposed to ¹⁴CO₂ generated in an illuminated chamber from a reaction between 10 % HCl and NaH¹⁴CO₃ diluted with cold NaHCO₃. After 15 min, plants were collected and frozen at -20°C for 10 min and then oven dried at 80°C. The oven-dried plants were subjected to combustion using a Harvey Biological Oxidiser (OX-600). The ¹⁴CO₂ evolved was trapped in carbosorb scintillation cocktail and counted in a TRI-CARB 2300 liquid Scintillation Analyzer, (PACKARD). The amount of ¹⁴C-fixed was determined as disintegration per sec (Bq)/g dry wt.

Determination of Lead Content in Plant Tissues:

Plants grown for 96 hrs were harvested and separated into shoots and roots. Roots were rinsed with 1 mM citrate at 4°C to remove Pb adsorbed on the surface. Tissues were oven dried at 80°C overnight (Yang *et al.*, 2000). A known weight of the dried samples (shoot or root) were digested in glass tubes (sugar tubes) containing 5 ml of 65% HNO₃ until the solution became clear. The sample volumes were raised to 50 ml with deionised water. The concentration of total Pb in the tissues was measured by an atomic absorption spectrometer (Spectr AA, Varian). The conditions for Pb determination were as follows:

- Lamp current 10 mA,
- Slit width 1nm,
- Wave length 217 nm,
- Fuel support (Acetylene: Air, 4.5: 1.5).
- Standard curve was in the concentration range of 2.5 to 20 ppm.

Determination of Free Amino Acids:

Frozen plant samples were homogenised in a blender with 80% ethanol. Plant tissues were removed by centrifugation at 4000 rpm for 15min and the clear extract was mixed with chloroform (1:3 v/v). The upper phase was vacuum dried and stored at -25°C (Raggi, 1994). Residues were dissolved in 1 ml of buffer solution containing sodium acetate (8.2 g/L), methanol (7.5 %), formic acid (0.3 %), acetic acid (1.5 %) and octanoic acid (0.001%), membrane filtered and analyzed by LC3000 Amino Acid Analyser (Eppendorf, Biotronic, Maintal, Germany) equipped with a 75 x 6.0 mm BTC guard column and a 145 x 3.2 mm BTC 2140 main column. The running conditions used a flow rate of 0.2 ml/min., pressure of buffer from 0.0 to 50 bar, pressure of reagent from 0.0 to 150 bar, and reaction temperature of 123 °C. Amino acids derived from the column with ninhydrine were quantified at 440 nm for primary amino acids and at 570 nm for proline.

Statistical Analysis:

The data was subjected to One-way ANOVA and the differences between means at the 5% probability level were determined using Duncan's new multiple range test. The software SPSS, version 10 (SPSS, Richmond, USA) was used as described by Dytham (1999).

RESULTS AND DISCUSSION**Results:**

After 48 h of treatment a black colour appeared along the midrib and veins of the leaves treated with 48 mM as compared with untreated plants or plants grown in lower concentrations of Pb (Fig. 1). The intensity of the black colour increased with time and a complete wilting occurred after 96 h.

Table 1: Free amino acids ($\mu\text{g/g}$ fr. wt) and % of control of *Vicia faba* plants grown for 96h in Hoagland nutrient solution (KH₂PO₄ replaced with equivalent molar of KCl) containing 0.0 to 48 mM Pb.

Amino acids	Pb mM							
	0.0		0.048		0.48		4.8	
	μg	%	μg	%	μg	%	μg	%
Glutamic acid	4.13	100	3.36	81.36	3.36	81.36	9.81	237.53
Arginine	0.45	100	1.90	422.22	0.03	6.67	4.88	1084.44
Proline	1.85	100	1.35	72.97	0.75	54.05	4.34	234.59
Histidine	2.9	100	2.36	81.38	3.69	127.24	8.11	279.66
Aspartic acid	6.98	100	3.91	56.02	5.84	83.67	6.75	96.70
Threonine	29.62	100	27.96	94.35	30.71	103.68	66.76	225.39
Isoleucine	5.45	100	3.66	67.16	6.89	126.42	3.87	71.01
Lysine	1.99	100	0.07	3.52	1.51	75.89	7.61	382.41
Leucine	1.71	100	1.68	98.24	1.48	86.55	6.09	356.14
Valine	5.37	100	5.11	95.16	4.82	89.76	18.48	344.13
Tyrosine	2.90	100	3.03	104.48	3.80	131.03	11.39	392.76
Phenylalanine	0.76	100	0.06	7.89	1.27	167.11	3.21	422.37
Serine	0.96	100	0.93	96.88	1.21	126.04	0.11	11.45
Glycine	2.24	100	1.63	72.77	2.12	94.64	3.51	156.70
Total	67.31	100	57.01	84.7	67.34	100.4	153.92	228.67

Table 2: Pb accumulation (mg/g dry wt.) in shoots and roots of *Vicia faba* plants grown for 96 h in Hoagland nutrient solution (KH₂PO₄ replaced with equivalent molar of KCl) containing 0.0 to 48 mM Pb.

Plant part	Pb concentrations				
	0.0	0.048	0.48	4.8	48
shoot	0.115 ± 0.00	0.283 ± 0.01	0.543 ± 0.01	2.548 ± 0.06	40.590 ± 1.42
Root	0.173 ± 0.01	0.710 ± 0.02	3.064 ± 0.12	17.175 ± 0.40	190.000 ± 3.04
Root: shoot	1.5	2.52	5.65	6.74	4.68

Data are mean of three replicates ± standard error

**Fig. 1:** Leaves of *Vicia faba* plants grown for 48 h on Hoagland nutrient solution containing 0.0 (left) and 48 mM Pb (right) showing a black colour in the midrib and veins.

In addition to the morphological distortion observed in leaves, the fresh and dry weights were also significantly affected. There was a significant decrease in the fresh weight and a significant increase in the dry weight of *Vicia faba* plants throughout 96 h of growth in nutrient solution supplemented with 48 mM Pb compared with either control or other Pb concentrations (0.048 to 4.8 mM) (Fig. 2).

Chl-a content of *Vicia faba* plants collected after 24 h significantly increased with increasing Pb concentrations reaching the maximum at 4.8 mM relative to the control (Fig. 3, upper panel). Later in the experiment (48-96 h) there was no significant effect on chl-a content due to Pb concentration up to 4.8 mM. The highest concentration of Pb (48 mM) caused significant decrease in chl-a after 24 h followed by significant increase throughout the experimental period (Fig. 3, upper panel). On the other hand Chl-b was less affected. It was reduced in plants treated with 48 mM Pb (Fig. 3, middle panel). Chl-a/b ratio was increased when Pb concentration increased up to 4.8 mM relative to control, where the highest increase was due to 0.48 mM Pb. The 48 mM Pb concentration decreased this ratio (Fig. 3, lower panel).

In order to test if the reduction in chl-a content affected photosynthetic efficiency, we measured the rate of ¹⁴CO₂ fixation (Fig. 4). The results indicated that all Pb concentrations decreased ¹⁴C-fixation. The decrease in ¹⁴C-fixation due to 4.8 and 48 mM Pb was significant throughout the experimental period when compared with the untreated sample.

Amino acid content was found to be affected in response to heavy metal stress. Since plants treated with 48 mM Pb were wilted at 96 h, amino acids analysis was performed in plants treated with 4.8 mM. Basically there was no change in the total amino acids content except at 4.8 mM, where a significant increase occurred in all amino acids except iso-leucine and serine (Table 1). Although, the highest amount was in threonine (29.62 µg/g fresh weight in control compared to 66.67 µg/g fresh weight in 4.8 mM Pb) the highest increase was in arginine (1048.44 compared to control).

There was significant increase in Pb accumulation in both root and shoot tissues with increasing Pb in the nutrient solution (Table 2). More Pb was accumulated in root than in shoot as shown from the ratio of Pb in roots and shoots. Although, the highest amount of Pb was in plants treated with 48 mM, the highest root: shoot ratio was in plants treated with 4.8 mM.

Discussion:

Pb is one of the most toxic metals in the environment and causes drastic morphological and physiological deformities in *Ipomoea lacunose* plant (Kambhampati *et al.*, 2005). In this study, some morphological changes occurred in the leaves of *Vicia faba* plants treated with 48 mM Pb. Blackening appeared in the midrib and veins of the leaves after 24h and increased with time.

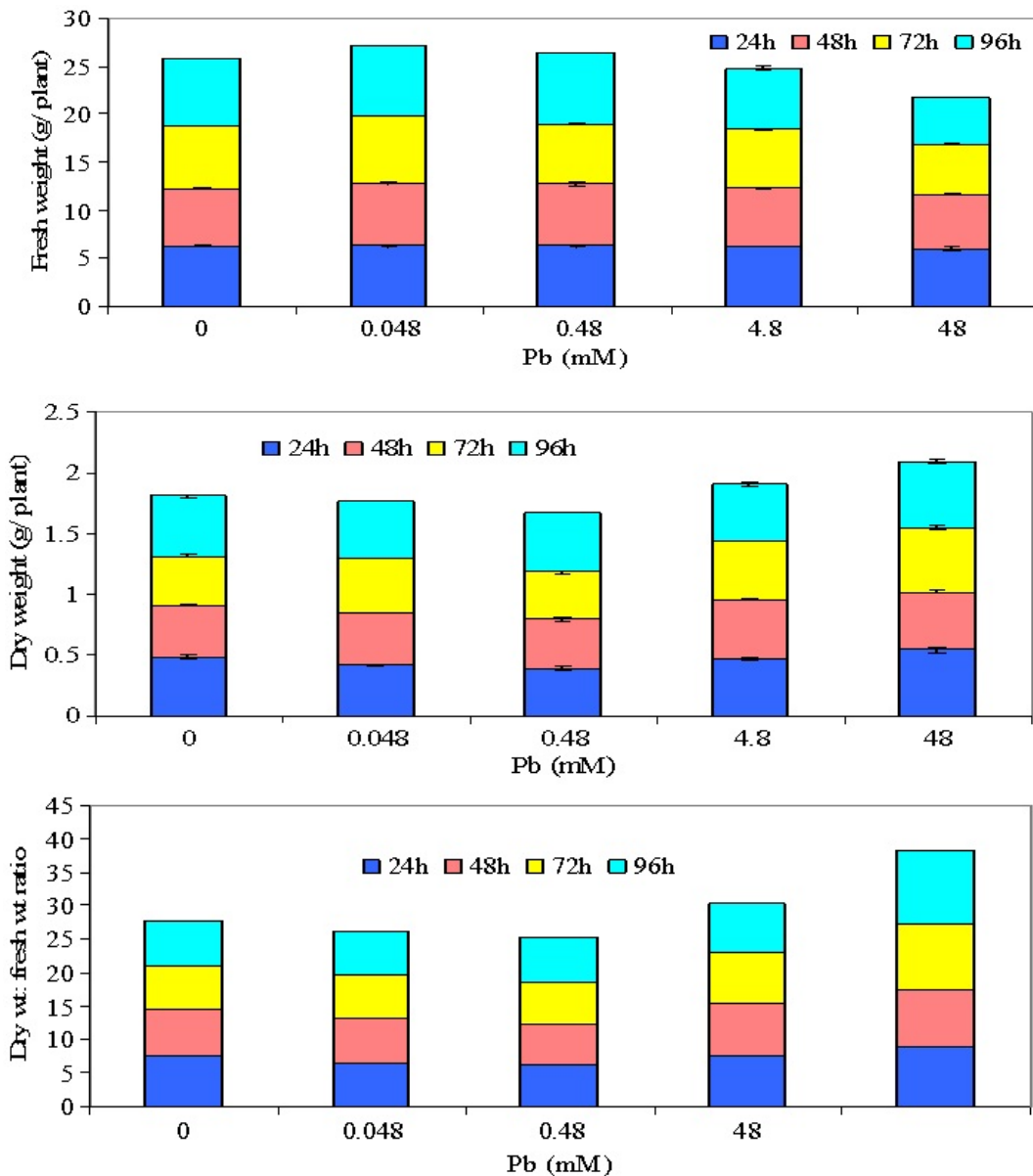


Fig. 2: Changes in fresh and dry weight (g /plant) and % of dry to fresh weight of *Vicia faba* plants grown for 96 h in Hoagland nutrient solution (KH_2PO_4 was replaced with equivalent molar of KCl) containing 0.0 to 48 mM Pb.

Although the fresh weight was reduced by Pb treatment, the dry weight was increased significantly. The increase in dry weight with Pb did not agree with Kosobrukhov (2004), who observed a considerable decrease in the dry weights of plant parts under Pb treatment. On the other hand, Wierzbicka (1998) explained that the increase in dry weight of corn seedlings resulted from Pb exposure, was due to the increase in the synthesis of cell wall polysaccharides. The highest level of Pb (48 mM) was most effective to increase the percent of dry to fresh weight (Fig. 2). This was due to the decrease in fresh weight which might be attributed to the

loss of water. Wierzbicka (1995) observed that Pb ions caused water deficit by disturbing water balance. Higher concentrations of Pb significantly affected plant water status causing water deficit (Patra *et al.*, 2004).

Results showed that Pb up to 4.8 mM increased chl-a after 24h while, 48 mM decreased its content. Chl-a content decreased by extended exposure to various Pb concentrations. These findings agreed with those of Nyitrai *et al.* (2003) who found that low-doses stressors (Cd, Pb, Ni and DCMU) either applied in the nutrient solution or sprayed onto the leaves, facilitated chlorophyll synthesis. Sarvari *et al.*, (2002) also observed an increase in the chlorophyll content either in Photosystem II core or light-harvesting chlorophyll a/b-protein

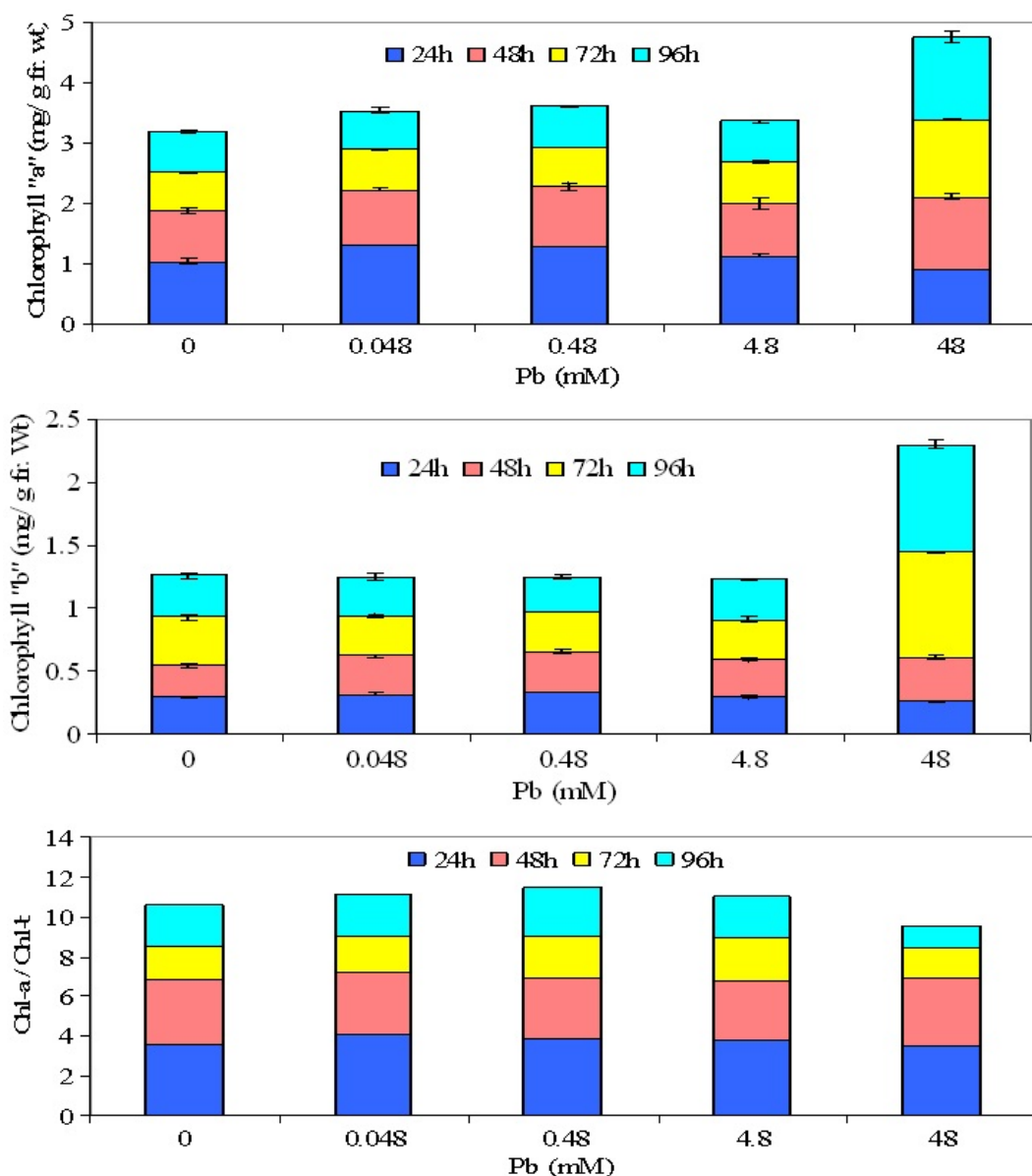


Fig. 3: Chlorophyll "a", "b" (mg/ g fr. wt) and % of Chl-a /chl-b of *Vicia faba* plants grown for 96 h in Hoagland nutrient solution (KH_2PO_4 was replaced with equivalent molar of KCl) containing 0.0 to 48 mM Pb.

complex of photosystem II at low concentrations of Pb treatment. Sengar and Pandey, (1996) claimed that, at 50 mM Pb the concentration of Pb inside the leaf was high enough to directly inhibit chlorophyll synthesis. Pb may inhibit chlorophyll synthesis by impaired uptake of Mg and Fe by plants (Bruzynski, 1987), or due

to increased chlorophyllase activity (Drazkiewicz, 1994). The decrease in the chl-a / b ratio may indicate a proportionately greater effect on photosystem reaction centers compared to light-harvesting complexes (LHC), since the reaction centers are relatively rich in chl-a, while the LHCs are rich in chl-b (Taiz and Zeiger, 1998).

The photosynthesis process is adversely affected by Pb. The results presented in Fig. (4) show that all Pb concentrations decreased ^{14}C -fixation. Pb at 500 to 2000 mg/ l decreased net photosynthetic rate of *Ipomoea lacunose* (Kambhampati *et al.*, 2005). In *Zea mays* exposed to varying soil concentration of Cu, Ni, Pb and Zn net photosynthesis was decreased by all metals and more at higher levels and by longer exposure (Heckathorn *et al.*, 2004). Pb may reduce photosynthetic activity of *Vicia faba* plants according to the explanation of Kupper *et al.* (1996). The substitution of the central atom of chlorophyll (magnesium) by Pb *in vivo*; prevents photosynthetic light-harvesting in the affected chlorophyll molecules.

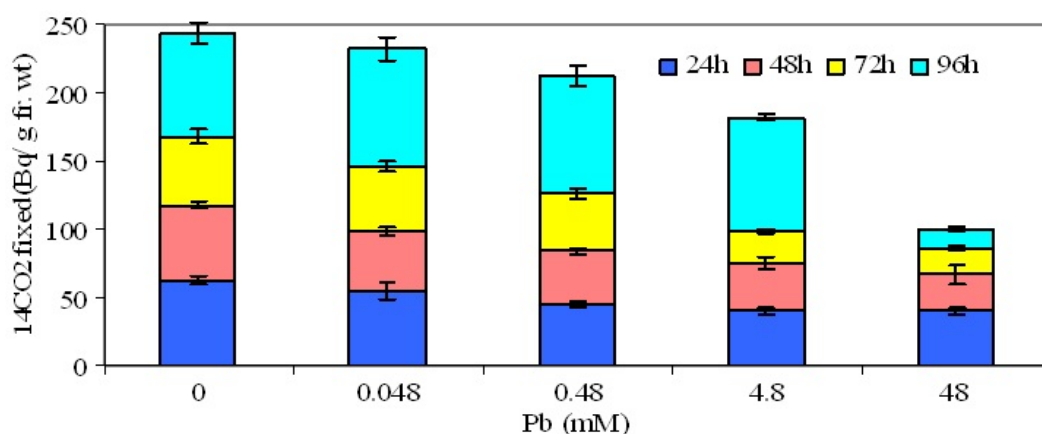


Fig. 4: $^{14}\text{CO}_2$ Fixed (Bq /g fr. wt) by *Vicia faba* plants grown for 96 h in Hoagland nutrient solution (KH_2PO_4 was replaced with equivalent molar of KCl) containing 0.0 to 48 mM Pb.

Sharma and Dubey (2005) reported that plants exposed to Pb ions showed a decline in the photosynthetic rate as a result of distorted chloroplast, restrained synthesis of chlorophyll, obstructed electron transport, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closing.

The level of Pb accumulated in *Vicia faba* roots and shoots at the end of 96h period increased with Pb concentrations (Table 2). Results also showed that more Pb was localized in roots than in the above ground parts. This result agrees with that of Malkowski *et al.* (2002) also showed that the Pb concentration in the roots of corn seedlings increased with increasing Pb concentration in the solution. About 90% of Pb accumulated in a number of species of the Brassicace family and other plants were in the roots (Kumar *et al.*, 1995). Roots can accumulate up to 3-50 times more Pb than leaves (Wozny *et al.*, 1995). Lead translocation from roots to shoots took place by loading in the xylem sap and translocating to the aboveground parts through the transpiration stream (Briat and Lebrun, 1999). Pb accumulation in the root takes place by binding with polysaccharides (Seregin and Ivanove, 1998), complexing with organic acids (Harmens *et al.*, 1994) or binding to the cell walls in the roots (Sieghardt, 1981 and 1984) and xylem vessels (Fathi, 1983) and thus might become immobile. The low allocation ratio of Pb between the roots and shoots may also be because of Pb movement in the root tissues which is usually prevented by the endodermis (Sieghardt, 1981 and 1984; Wierzbicka, 1987).

Of the 14 amino acids detected, only isoleucine and serine were decreased with the increase in Pb concentration. The other 12 amino acids were increased by Pb at 4.8 mM. According to Jones *et al.* (1980), Rabe (1990) and Mansour (2000), 6 of the 14 amino acids were known to play a vital role in the osmotic adjustment of plants. These amino acids are arginine, proline, leucine, valine, serine and glycine. Glycine and glutamic acid are involved in the synthesis of glutathione and phytochelatin, which plays a role in metal binding while arginine is involved in the synthesis of polyamines, which act as signaling molecules and antioxidants (Sharma and Dietz, 2006). The proteinogenic amino acid proline functions as a radical scavenger, electron sink, stabilizer of macromolecules, cell wall component (Matysik *et al.*, 2002), osmoregulation and metal chelation (Sharma and Dietz, 2006). Proline was found to accumulate in *Helianthus*

annuus due to Pb, Cd, Cu and Zn (Kastori *et al.*, 1992). The exposure of Ni-hypoaccumulating *Alyssum lesbiacum* to Ni resulted in a large and proportionate increase in histidine concentration in xylem sap (Krämer *et al.*, 1996). Salt *et al.* (1999) identified Zn-histidine complex in the roots of Zn-hyperaccumulator *Thlaspi caerulescens*. Ni-hyperaccumulation relies on histidine-dependent root-to-shoot translocation (Kerkeb and Krämer, 2003).

Conclusion:

For all aspects that were measured, Pb at 4.8 mM or 48 mM had a different effect than Pb at lower concentrations. Results of growth, chlorophyll, photosynthesis and free amino acids indicated that *Vicia faba* plants survived and continued their growth. According to the hypothesis of (Baker and Brooks, 1989) plant species in which lead concentration exceeds 0.1% of their dry weight are considered as hyperaccumulators; *Vicia faba* considered a Pb hyperaccumulator.

ACKNOWLEDGMENT

The author acknowledges the efforts and quick responding of Dr. Salah E. Abdel-Ghany and Naja M. Hessein for reviewing the article language and grammar.

REFERENCES

- Antosiewicz, D.M., 2005. Study of calcium-dependent lead-tolerance on plants differing in their level of Ca-deficiency tolerance. *Environ. Poll.*, 134: 23-34.
- Baker, A.G.M. and R.R. Brooks, 1989. Terrestrial higher plants which hyper-accumulate metallic elements a review of their distribution. *Ecology and phytochemistry. Biorecovery*, 1: 81-126.
- Barnes, J.D., L. Balaguer, E. Manrique, S. Elvira and A.W. Davison, 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environ. Exp. Bot.*, 32: 85-100.
- Briat, J.F. and M. Lebrun, 1999. Plant responses to metal toxicity. *Comptes Rendus Acad. Sci. Ser. III-Sci.*, 322: 43-54.
- Bruzynski, M., 1987. The influence of lead and cadmium on the absorption and distribution of potassium, calcium, magnesium and iron in cucumber seedlings. *Acta Physiol. Plant*, 9: 229-238.
- Drazkiewicz, M., 1994. Chlorophyll-occurrence, functions, mechanism of action, effects of internal and external factors. *Photosynthetica*, 30: 321-331.
- Dytham, C., 1999. *Choosing and Using Statistics: A Biologist's guide*. Blackwell Science Ltd., London UK.
- Fathi, M., 1983. Binding of mercury, cadmium and lead in plant and animal tissues with respect to human nutrition. *Z. Anal. Chem.*, 316: 589-613.
- Harmens, H., P.L.M. Koevoets, J.A.C. Verklrij and W.H.O. Ernst, 1994. The role of low molecular weight organic acids in the mechanism of increased zinc tolerance in *Silene vulgaris* (Moench) Garcke. *New Phytol.*, 126: 615-621.
- Heckathorn, S.A., J.K. Mueller, S. LaGuidice, B. Zhu, T. Barrett, B. Blair and A. Dong, 2004. Chloroplast small heat-shock proteins protect photosynthesis during heavy metal stress. *American J. Bot.*, 91: 1312-1318.
- Jackson, D.R. and A.P. Watson, 1977. Disruption of nutrient pools and transport of heavy metals in a forested water-shed near a lead smelter, *J. environ. Qual.*, 6: 331-338.
- Jones, M.M., C.B. Osmond and N.C. Turner, 1980. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficit. *Aust. J. Plant Physiol.*, 7: 193-205.
- Kabata-Pendias, A. and H. Pendias, 1999. *Biogeochemistry of Trace Elements* (in Polish). PWN, Warszawa, Poland.
- Kambhampati, M.S., G.B. Begonia, M.F.T. Begonia and Y. Bufford, 2005. Morphological and physiological responses of Morning glory (*Ipomoea lacunose* L.) grown in a lead- and chelate-amended soil. *Int. J. Environ. Res. Public Health*, 2: 299-303.
- Kastori, R., M. Petrovic and N. Petrovic, 1992. Effect of excess lead, cadmium, copper and zinc on water relations in sunflower. *J. of Plant Nutr.*, 15: 2427-2439.
- Kerkeb, L. and U. Krämer, 2003. The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. *Plant Physiol.*, 131: 716-724.
- Kosobrukhov, A., I. Knyazeva and V. Mudrik, 2004. *Plantago major* plants responses to increase content of lead in soil: growth and photosynthesis. *Plant Grow. Regul.*, 42: 145-151.

Krämer, U., J.D. Cotter-Howells, J.M. Charnock, A.J.M. Baker and J.A.C. Smith, 1996 Free histidine as a metal chelator in plants that accumulate nickel. *Nature*, 379: 635-638.

Kumar, P.B.A.N., V. Dushenkov, H. Motto and I. Raskin, 1995. phytoextraction: the use of plants to remove heavy metals from soils. *Environ. Sci. Technol.*, 29: 1232-1238.

Kupper, H., F. Kupper and M. Spiller, 1996. Environmental relevance of heavy metal substituted chlorophylls using the example of water plant. *J. Exp. Bot.*, 47: 259-266.

Levine, M.B., A.T. Stall, G.W. Barrett and D.H. Taylor, 1989. Heavy metal concentration during ten years of sludge treatment to an old-field community. *J. Environ. Qual.*, 18: 411-418.

Malkowski, E., A. Kita, W. Galas, W. Karcz and M. Kuperberg, 2002. Lead distribution in corn seedling (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. *Plant and Soil.*, 37: 69-76.

Mansour, M.M., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant*, 43: 491-500.

Matysik, J., B. Alia and P. Mohanty, 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Current Sci.*, 82: 525-532.

Nyitrai, P., K. Bóka, L. Gáspár, E. Sárvári, K. Lenti and A. Keresztes, 2003. Characterization of the stimulating effect of low-dose stressors in maize and bean seedlings. *J. Plant Physiol.*, 160: 1175-1183.

Patra, M., N. Bhowmik, B. Bandopadhyay and A. Sharma, 2004. Comparison of mercury, Lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Exp. Botany*, 52: 199-223.

Piechalak, A., B. Tomaszewska, D. Baralkiewicz and A. Malecka, 2002 Accumulation and detoxification of lead ions in legumes. *Phytochemistry*, 60: 153-162.

Rabe, B., 1990. Stress Physiology: the functional significance of the accumulation of nitrogen containing compounds. *J. Hort. Sci.*, 65: 231-243.

Raggi, V., 1994. Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. *Physiol. Plant.*, 91: 427-434.

Salt, D.E., R.C. Prince, A.J.M. Baker, I. Raskin and I.J. Pickering, 1999. Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy. *Environ. Sci. Tech.*, 33: 713-717.

Sarvari, E., L. Gaspar, L. Fodor, E. Cseh, K. Kropfl, A. Varga and M. Baron, 2002. Comparison of the effects of Pb treatment on thylakoide development in poplar and cucumber plants. *Acta Biol. Szeged.*, 46: 163-165.

Sengar, R.S. and M. Pandey, 1996. Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments. *Biol. Plant.*, 38: 459-462.

Seregin, I.V. and V.B. Ivanove, 1998. The transport of cadmium and lead ions through root tissues. *Russian J. Plant Physiol.*, 45: 780-785.

Sharma, S.S. and K.J. Dietz, 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.*, 57: 711-726.

Sharma, P. and R.S. Dubey, 2005. Lead toxicity in plants. *Braz. J. Plant Physiol.*, 17: 35-52.

Sieghardt, V.H., 1984. Eine anatomisch-histocemische Studie zur Bleiverteilung in Primarwurzeln von *Pisum sativum* L. *Mikroskopie (Wien)*, 41: 125-133.

Sieghardt, V.H., 1981. Ein histochemischer Nachweis zur Bleiverteilung in juvenilen Wurzeln von *Zea mays* L. Eine lichtmikroskopische Studie, *Mikroskopie (Wien)*, 38: 193-199.

Taiz, L. and E. Zeiger, 1998. *Plant Physiology*. Sinauer, Sunderland, Massachusetts, USA.

Verma, S. and R.S. Dubey, 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.*, 164: 645-655.

Wierzbicka, M., 1998. Lead in the apoplast of *Allium cepa* L. root tips-ultra structure studies. *Plant Sci.*, 133: 105-119.

Wierzbicka, M., 1995. How lead loses its toxicity to plant. *Act. Soc. Bot. Pol.*, 64: 81-90.

Wierzbicka, M., 1987. Lead accumulation and its translocation barriers in roots of *Allium cepa* L. Autoradiographic and ultrastructural studies. *Plant Cell Environ.*, 10: 17-26.

Wozny, A., J. Schneider and E.A. Gwozdz, 1995. The effects of lead and kinetin on greening barley leaves. *Biol. Plant.*, 37: 541-552.

Yang, Y.Y., J.I.Y. Jung, W.Y. Song, H.S. Suh and Y. Lee, 2000. Identification of Rice varieties with high tolerance or sensitivity to lead and characterisation of the mechanism of tolerance. *Plant Physiol.*, 124: 1019-1026.