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Full Length Research Paper

Response of tomato (*Solanum lycopersicum* L.) to lead toxicity: Growth, element uptake, chlorophyll and water content

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The effect of lead on accumulation of Pb; mineral composition in roots, shoots and leafs; growth-development; chlorophyll and water content of tomato (*Solanum lycopersicum* L. cv. Falcon) seedlings was tested under doses of 0-control, 75, 150 and 300 mg L^{-1} Pb. Lead accumulation was defined by elevated concentrations as 75 to 300 mg L^{-1} Pb in root, shoot and leaf tissues. Element uptake by roots, shoots and leafs was negatively affected by in raised lead concentrations, especially in 300 mg L^{-1} Pb. Root elongation and plant height; root, shoot and leaf fresh and dry biomass; leaf area were negatively affected by increasing lead concentrations. Tissue water content (TWC), growth tolerance index (GTI), chlorophyll a, chlorophyll b and total chlorophyll content limited by lead toxicity.

Key Words: Lead (Pb), toxicity, growth, nutrient, chlorophyll, water status.

INTRODUCTION

Lead is major pollutant in both terrestrial and aquatic ecosystems. Besides natural weathering process the main sources of Pb pollution are exhaust fumes of automobiles, chimneys of factories using Pb, effluents from the storage battery, industry, mining and smelting of Pb ores, metal plating and finishing operations, fertilizers, pesticides and additives in pigments and gasoline (Sharma and Dubey, 2005). Heavy metals can accumulate in plants through both foliage and root systems. Many studies have been conducted to determine the toxic levels of heavy metals for certain plants, especially those metals considered as public health threats (Reeves and Baker, 2000; Abou-Arab and Abou Donia, 2000). The toxicity of heavy metals to plant metabolism has received extensive research interest for several decades (Markert, 1993). This is critical in soils used for agricultural production, because pollutants can be accumulated in crops. This can cause threats to human health (Spona and Baum, 1993), and exceed legal limits.

Lead naturally occurs in soils but is in relatively low concentrations. Lead concentrations in uncontaminated soils are generally in the range of 20 to 50 mg kg^{-1} (Nriagu, 1978), with the median concentration in the United Kingdom of 40 mg kg^{-1} and arithmetic mean of 74 mg kg^{-1} (MAFF, 1993). Non-polluted soils usually contain less than 100 mg kg^{-1} Pb, with soils in unpolluted polar soils buried before the industrial revolution contain less than 5 mg kg^{-1} (Meggeson and Hall, 1999). Soils that contain 400 - 800 mg kg^{-1} lead, soil are regarded as significantly affected with a possibility that food grown on it will exceed the legal limit used in the United Kingdom (MAFF, 1993) if the pH is below 6.0. In industrialized areas up to 1000 mg kg^{-1} Pb and above has been recorded (Angelone and Bini, 1992). Although lead is not essential element for plants, it gets easily absorbed and accumulated in different plant parts. Uptake of Pb in plants is regulated by pH, particle size and cation exchange capacity of the soils as well as by root exudation and other physico-chemical parameters.

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Abbreviations: GTI, Growth tolerance index; **Pb,** lead; **TWC,** tissue water content; **Cu,** copper; **Ca,** calcium; **g,** magnesium; **k,** potassium, **Fe,** iron; **Zn,** zinc, **Mn,** manganese; **Na,** sodium.

Excess Pb causes a number of toxicity symptoms in plants as stunted growth, chlorosis, and blackening of root system. Pb inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability (Sharma and Dubey, 2005). However, results of these investigations are rather contradictory, as the nature of heavy metal effect depends on the species, variety and age of plants and the concentrations, duration of effect, physical and chemical properties of contaminants. Therefore plant cultivation in contaminated soils is problematic due to hyper accumulation of HM in fruits and vegetables. (Bashmakov et al., 2005). Effects of heavy metals must be investigated in all aspects of every cultivated species.

The aim of this study is to define lead effects on (1) the accumulation and distribution of lead and other elements in roots, shoots and leaves, and (2) growth and development of plant organs (3) water contents of tissues and chlorophyll amounts of leafs in tomato.

MATERIALS AND METHOD

Plant material, culture and treatments

The experiment was carried out in a greenhouse. Seeds of tomato (*Solanum lycopersicum* L. cv. Falcon) were germinated and cultivated in pots (600 cm^3) containing a topsoil - manure mixture (3:1) in natural light, with a mean air temperature of 25/15±3°C (day/night), a relative air humidity of 75±10%. Each pot or seedling was watered every two days with tap water (100 cm^3) and thirty days after germination. Then, Pb was added every four days (5 times in 20 days) to the pots 0 (control), 75 (low), 150 (moderate) and 300 (high) mg L^{-1} of Pb with 100 cm^3 water as experimental treatment. The experiment was arranged in a randomized blocks design. Each treatment was replicated three times and each replicate included twenty-five plants. When phytotoxicity symptoms (necrotic lesions, and tips) begun to be visible, plants collected from each treatment after 30 days of Pb treatments.

Measurement of lead and mineral elements

Five plants were taken from each treatment and separated into roots, shoots and leaves. The leaves, shoots and roots were washed in tap and deionized water three times and dried at 65°C for 48 h. The dried tissues were weighed and ground into a fine powder before wet digestion in $HClO_4$:HNO_3 (4:1, v:v) solution. Calcium, magnesium, potassium, iron, zinc, copper, manganese, and lead were determined by atomic absorption spectrophotometer (Perkin Elmer 3100, USA) of plant part (Jones and Case, 1990). The phosphors content of plant tissues were determined by vanadomolibdophosphoric method (Kuo, 1996).

Plant growth, biomass and growth tolerance index

Seedlings were harvested, washed with deionised water and separated into roots, shoots, and leaves at the end of the study. Washed seedlings were evaluated for elongation, fresh and dry biomass, number and area of roots, shoots, and leaves. Dry biomass was determined after oven drying the samples at 65°C for 2 – 3 days until constant weight gained. Pb tolerance was calculated as the growth tolerance index (GTI) which gives the integrated percentage of Pb - treated to Pb - untreated control seedling parts, and it gives opinion for effect of applied stress factor on plant grown and development. Index of growth tolerance (GTI) was calculated as well using the established values of all morphological parameters using the formula obtained from equations of Rout et al. (2000) and Miteva et al. (2005):

$$GTI = \frac{1}{n}\sum_{i=1}^{n}\frac{PLi}{Pci} \times 100$$

where *PLi* is the established value of the respective *i*-th parameter of the lead applied plants, *Pci* is the established value of the respective *i*-th parameter of the control plants, and n is the number of the used morphological parameters (root length-cm, root fresh and dry weight-g, stem length-cm, shoot fresh and dry weight-g, leaf area-cm^2, leaf fresh and dry weight-g).

Chlorophyll and water content

Third and fourth leaves were used for chlorophyll determination. Chlorophyll a and b were extracted in 99% acetone using a blender, and vacuum filtered used 1 g chopped fresh leaf sample. The filtrate was determined for chlorophyll a and b at 645 and 663 nm measured spectrophotometrically and calculated according to the formula of Witham et al. (1971). Tissue water content (TWC) was obtained from the Fresh Weight-Dry Weight/ Dry Weight ratio (Gill et al., 2003).

Statistical analysis

The results are the means of three replicates. The analysis of variance for all the parameters was computed on statistically significant differences, determined based on the appropriate F-tests. Differences between the mean values were compared utilizing Fischer's least significant test.

RESULTS

Lead uptake and accumulation

All lead applications caused an increase in Pb concentrations root, shoot and leaf tissues (Figure 1). Low lead concentration increased lead contents of the tissues of tomato seedlings. Exposure to excess lead, particularly moderate, and high Pb, led to more increased accumulation of Pb in roots, shoots, and leaves. Lead levels of plant were found 510 in roots, 130 in shoots, and 312 mg kg^{-1} in leaves at low Pb concentration. In addition, the lead levels of plant were found 1438 and 2520 in roots, 750 and 1022 in shoots, 917 and 1750 mg kg^{-1} in leaves at the medium and high Pb applications, respectively.

Elements uptake

Analysis of variance indicated that macro elements accumulations in roots, shoots and leaves of tomato

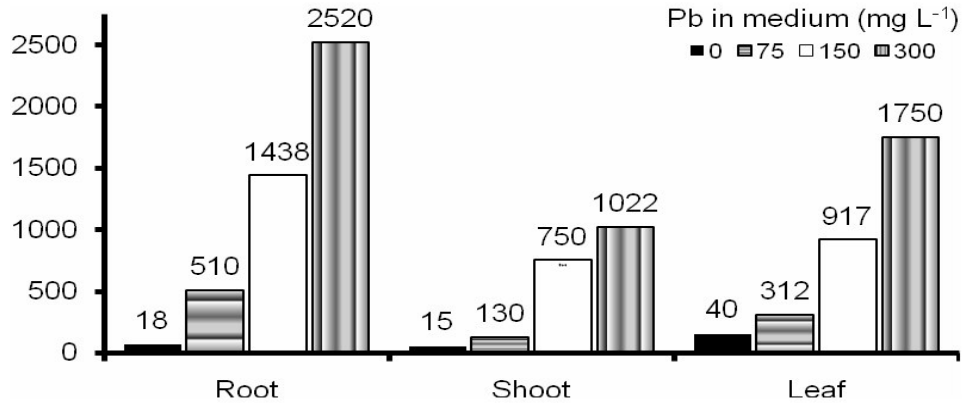

Figure 1. Lead concentrations in root ($p<0.001$, $LSD_{0.05}$: 591.3), leaf ($p<0.01$, $LSD_{0.05}$: 16.93) and shoot ($p<0.01$, $LSD_{0.05}$: 817.2).

Table 1. Macroelement, microelement and Na content in roots, shoots and leafs of tomato seedlings as affected by different lead concentrations. (Different letters indicate a statistically significant difference)

Lead (mg L^{-1})	Ca mg g^{-1}	Mg mg g^{-1}	K mg g^{-1}	P mg g^{-1}	Na mg g^{-1}	Fe mg kg^{-1}	Zn mg kg^{-1}	Cu mg kg^{-1}	Mn mg kg^{-1}
					Roots				
Control	15.8	9.4	10.9b	2.6a	4.5a	158.3a	38.3	11.7ab	48.3a
75	16.3	9.9	11.5b	2.5a	4.2b	156.7b	35.0	18.3a	55.0a
150	14.4	9.9	19.4a	2.4a	3.9c	143.3b	31.7	13.3ab	43.3a
300	13.2	8.8	9.5b	1.4b	2.6c	141.7b	21.7	10.0b	21.7b
P	*ns*	*ns*	*<0.05*	*<0.01*	*ns*	*<0.05*	*ns*	*<0.05*	*<0.01*
$LSD_{0.05}$	*3.59*	*2.71*	*6.60*	*0.46*	*1.99*	*28.49*	*42.70*	*6.87*	*14.02*
					Shoots				
Control	23.3c	8.7a	22.9ab	1.5b	2.7a	16.7	58.3a	5.0a	56.7a
75	28.4b	8.3ab	27.0a	2.1a	2.0b	13.3	48.3b	8.3a	46.7b
150	34.4a	7.9b	24.9ab	2.1a	1.9b	10.0	43.3b	6.7a	43.3b
300	28.7b	7.5c	20.2b	1.6b	1.7b	8.3	41.7b	5.0b	20.0c
P	*<0.01*	*<0.05*	*<0.05*	*ns*	*<0.05*	*ns*	*<0.05*	*<0.05*	*<0.001*
$LSD_{0.05}$	*4.44*	*2.21*	*5.47*	*0.69*	*0.54*	*9.42*	*8.65*	*3.72*	*3.72*
					Leafs				
Control	33.6a	7.7ab	18.7bc	4.6a	1.3b	30.0bc	103.3a	13.3c	63.3
75	28.6ab	9.4a	28.5a	3.7ab	1.9a	56.7a	91.7a	40.0a	56.7
150	30.1ab	8.9ab	23.2ab	3.4ab	1.2b	41.7b	80.0ab	35.0ab	51.7
300	17.5b	7.0b	15.1c	2.3b	1.0b	25.0c	63.3b	28.3b	43.3
P	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.01*	*<0.05*	*<0.01*	*ns*
$LSD_{0.05}$	*13.44*	*1.81*	*7.46*	*1.62*	*0.55*	*14.80*	*27.00*	*8.81*	*21.84*

seedlings were affected by excess Pb applications (Table 1). The highest level of lead generally inhibited the uptake of all mineral elements compared with the low-level lead treatment. Calcium and magnesium content of roots were not significantly different at all lead levels.

Calcium and magnesium concentration of shoot and leafs was significantly decreased with increasing Pb concentrations. Potassium content in roots shoots and leafs decreased with only 300 mg L^{-1} Pb level compared with control. A difference was not obtained between control

Table 2. Growth parameters in root, shoot and leaf of tomato seedlings as affected by different lead concentrations, (Different letters indicate a statistically significant difference).

Lead (mg L^{-1})	Root			Shoot				Leaf	
	Length (cm)	Fresh weight (g)	Dry weight (g)	Height (cm)	Fresh weight (g)	Dry weight (g)	Area (cm^2)	Fresh weight (g)	Dry weight (g)
0	14.30a	3.43a	0.89a	30.7a	11.7a	2.25a	174.9a	18.4a	1.86a
75	12.9b	2.92a	0.78ab	27.5b	9.2b	2.16ab	124.0b	15.4b	1.53ab
150	10.33c	1.95b	0.66bc	20.7c	6.1c	1.87b	51.6c	12.1c	1.51b
300	8.37d	1.49b	0.58c	13.5d	4.1d	1.48c	39.2c	9.3d	1.26b
P	<0.001	<0.001	<0.01	<0.001	<0.001	<0.01	<0.001	<0.001	<0.05
$LSD_{0.05}$	0.51	0.51	0.14	3.01	1.87	0.3	18.62	2.28	0.33

with low and moderate Pb levels. The phosphorus content of roots were increased with low and moderate Pb levels; but high Pb level decreased the content of P below that of the control. The phosphorus content of shoot and leaf were significantly decreased with increasing Pb levels. Na content in roots, shoots and leafs was significantly affected and decreased by increasing 75 to 300 mg L^{-1} Pb levels.

Also Table 1 showed that lead toxicity significantly affected iron, zinc, copper and manganese concentrations in the root, shoot, and leaf of tomato seedling, except iron content of shoot, zinc content of root, manganese content of leaf. On increasing lead concentrations, iron contents were significantly declined in roots, all Pb and in leaf only high Pb levels. Lead concentrations were gradually limited zinc contents of shoot and leaf While Pb at low level of Pb increased the amount of copper uptake into the root, the effect increased with moderate and high levels of Pb. With Pb concentration increased, the distribution of Cu was leaf>root>shoot. The Mn content of root increased with low Pb, but high Pb level reduced the content of Mn below that of the control. With Pb concentration increased, the Mn reduced in shoot and leaf.

Plant growth, biomass and growth tolerance index

Excess Pb concentrations restrained growth parameters (Table 2). Length and fresh and dry weight of root and shoot were decreased compared to the controls. Area, fresh and dry weight of leafs were decreased compared to the controls. These results showed that lead contamination negatively affected root, shoot and leaf tissue.

Growth tolerance index (GTI) is an integrated calculation of particular parameters and makes for a summary assessment of effect of stress factor on plant growth and development (Figure 3). GTI was affected by lead that is the applied stress factor in our study, $P<0.001$. The GTI of plants was calculated based on morphological parameters and showed inhibition of growth and biomass synthesis in all lead concentrations. The value of GTI was 100 in controls while 85, 64 and 50 in 75, 150 and 300 mg L^{-1} Pb, respectively.

Chlorophyll and water content

Chlorophyll a, b, a+b, were affected negatively by increasing Pb levels. Chlorophyll a ($P<0.001$), chlorophyll b ($P<0.05$) and chlorophyll a+b ($P<0.001$) was significantly affected by increasing lead concentrations, but chlorophyll a/b was insignificant (Figure 2). Chlorophyll a 1.35, 1.17 and 1.29; chlorophyll b 1.91, 1.45 and 1.74; chlorophyll a+b 3.24, 1.70 and 2.52 were fold greater than control in 75, 150 and 300 mg L^{-1} Pb, respectively.

Root, shoot and leaf water contents were decreased in all Pb levels compared with control, except in leaf at 75 mg L^{-1} Pb (Figure 3). Loses of the water content in root were by 4.53, 30.66 and 42.51% at 75, 150 and 300 mg L^{-1} Pb according to control, respectively. Loses in shoots were much more than in roots as 22.09, 46.79 and 57.01% at low, moderate and high Pb levels. Leaf water contents were increased in 75 mg L^{-1} Pb, but were decreased in 150 and 300 mg L^{-1} Pb as 9.28, 7.07 and 6.37, respectively.

DISCUSSION

Lead uptake and accumulation

Excess lead caused accumulation of Pb in tissues of tomato seedlings. While the Pb concentration increased, the distribution of Pb within the plant followed the trend root>leaf>shoot. At the high lead concentrations, roots accumulated 2.27 fold

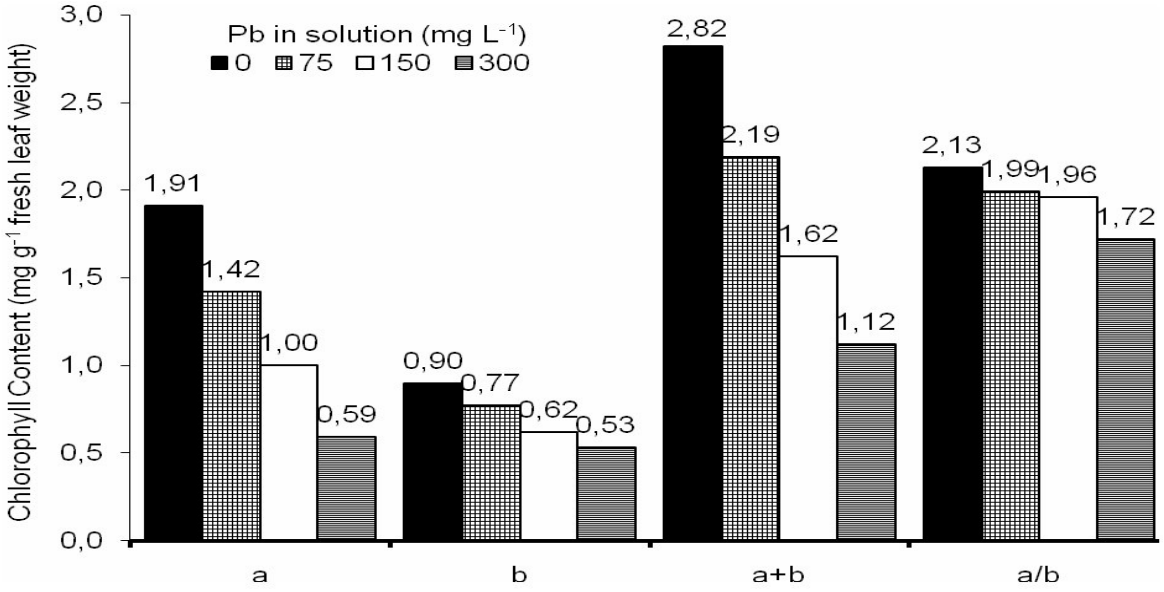

Figure 2. Chlorophyll a ($p<0.001$, $LSD_{0.05}$: 0.38), chlorophyll b ($p<0.05$, $LSD_{0.05}$: 0.25), chlorophyll a+b ($p<0.001$, $LSD_{0.05}$: 0.22) and chlorophyll a/b (ns) content in leafs of tomato seedling as a function of lead concentration in solution.

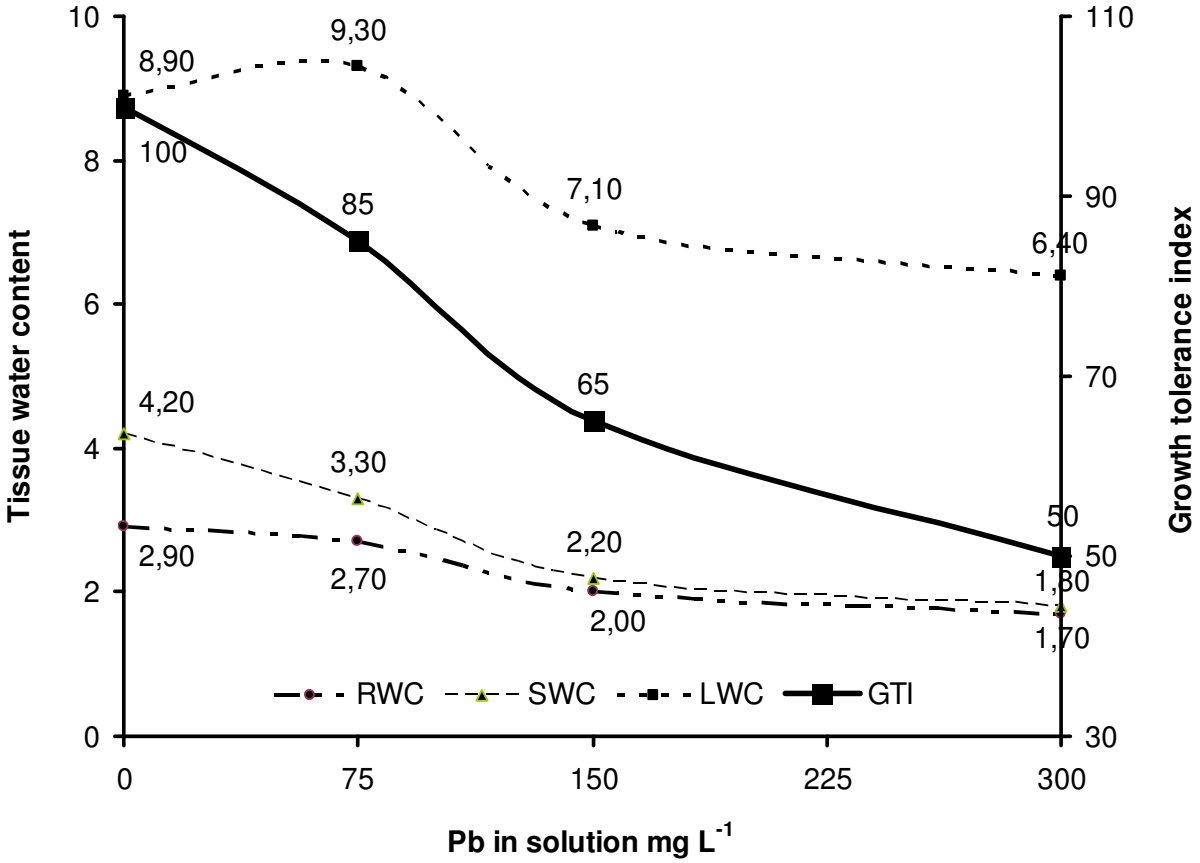

Figure 3. Tissue water contents of roots (RWC; $p<0.05$, $LSD_{0.05}$: 0,97), shoots (SWC; $p<0.01$, $LSD_{0.05}$: 2.16) and leafs (LWC; $p<0.01$, $LSD_{0.05}$: 1.39); growth tolerance index (GTI; $p<0.001$, $LSD_{0.05}$: 4.65) in tomato seedlings as affected by different lead concentrations.

more lead than shoots and 1.44 fold more than leaves. The roots Pb concentration were 1.92 times higher than in shoots and 1.57 times higher than in leaves at moderate Pb application, respectively. The roots Pb concentration were 3.92 times higher than in shoots and 1.65 times higher than in leaves at low Pb application, respectively. There must be different mechanisms for uptake and accumulation for lead in general and for the different plant parts themselves. Dahmani et al. (2000) found similar results in three plant (*Armeria maritima. Agrostistenuis* and *Cardaminopsis.halleri*). They reported that Pb, the large differences between root and leaf concentrations indicate an important restriction of the internal transport of metals from the roots towards shoots and green leaves. In addition, Ye et al. (1998) in *Typha latifolia* and Gothberg et al. (2004) in water spinach found that the highest Pb accumulated in roots, followed by shoots. So, root tissue acts as a barrier to Pb translocation to the shoot. Our results were partly compatible with these findings that Pb was stored in tomato roots. This event can be useful for environment. Actually high lead deposition in corn root tips was observed than in upper organs by Malkowski et al. (2002). So, they pointed that rhizofiltration is the use of plant roots to remove contaminants as heavy metals from contaminated water can have implications for this emerging environmental cleanup technology.

Elements uptake

There is little information about Pb stressed tomato on mineral accumulation in plants. The metal concentrations in roots, shoots, and leaves of the tomato under study may be influenced by an exclusion mechanism triggered by high external Pb concentrations, resulting in a reduction in root, shoot, and leaf metal concentrations. The levels of Ca showed significant decreases with increasing Pb applications, especially 300 mg L^{-1} in leaf. The levels of Mg showed significant decreases in shoots and leafs of tomato. The levels of potassium in roots, shoots, and leaves showed significant decreases with increasing Pb applications.

There must be different mechanisms for uptake for macro elements for the different plant parts under Pb press. Kim et al. (2002) showed that Ca and Mg inhibit the transport of Pb into rice roots. He indicated negatively effects between Pb with Ca and Mg transport into rice roots. These results are similar to our results with Ca and Mg contents decreasing in root at moderate and high Pb applications. Paivoke (2002) found similar results that the potassium content decreased, and in the shoot, a significant negative correlation with the soil lead concentration was established. Malkowski et al. (2002) found that the positive correlation between lead concentration (10^{-5}, 10^{-4}, and 10^{-3} M) and calcium concentrations found in seedling roots might be connected with high constitutional tolerance of corn to lead. But they defined negative correlation was observed between lead and potassium concentration in corn roots. It is postulated that inhibition of corn root growth is connected with potassium leakage from root cells. The levels of Pb in roots and leaves showed significant decreases with increasing Pb applications. Paivoke, (2002) also indicated that the root and shoot phosphorus contents showed a significant negative correlation with the soil lead concentration controls.

Similarly micro element contents were affected from increasing lead concentrations. The level of iron in roots, and leaves showed decreases with increasing Pb applications. This result was explained that decreases in leaf chlorophyll concentration caused by heavy metals are generally attributed to an induced Fe deficiency (Wallace et al., 1992). The level of Zn in shoots and in leaves showed significant decreases with increasing Pb applications. The levels of Cu in roots, shoots showed significant decreases with increasing Pb applications, while an opposite trend was noticed for Cu content in leaves of tomato. The levels of Mn decreased roots and shoots at the all Pb levels, except in roots at low Pb level. The similar results that manganese content in the roots of adult plants increased were indicated by Paivoke (2002).

Plant growth, biomass, and growth tolerance index

The growth - development, fresh-dry biomass and growth tolerance index of root, shoot and leafs were negatively affected by Increasing lead concentrations in tomato seedlings. Similar results were obtained by some other studies at the evaluated Pb concentration: root, shoot and leaf growth; fresh and dry biomass is significantly reduced in *Pisum sativum* (Kevresan et al., 2001), in *Zea mays* (Małkowski et al., 2002; Çimrin et al., 2007), in *Paspalum distichum* and *Cynodon dactylon* (Shua et al., 2002), in *Lycopersicon esculentum* (Jaja and Odoemena, 2004), in *Ipomoea aquatica* (Gothberg et al., 2004)., in *Phaseolus vulgaris* and *Lens culinaris* (Haider et al., 2006).

Tomato seedlings treated with Pb resulted in a decrease in growth tolerance index (GTI). Tolerance index can be accepted strength ability to stress factor that generally indicates growth in plants metal treated as a percentage of the control in most stress investigations. Findings of Shua et al. (2002) confirmed our results that the value of tolerance index decreases 5 - 40 mg L^{-l} lead in *Paspalum distichum* and *Cynodon dactylon* roots. Also, a decrease of tobacco root growth tolerance index was recorded during exposition to 4 and 5 mM Pb^{2+} in plants comparison with controls (Wojas et al., 2007). Tolerance index is also reduced with pressure of increasing lead in water spinach (Gothberg et al., 2004). Pb disrupts photosynthesis in different ways. The decline of the photosynthetic rates results from the distorted chloroplast

ultra structure; the restrained synthesis of chlorophyll, plastoquinone, and carotenoids; the obstructed electron transport; the inhibited enzyme activities of the Calvin cycle; and CO_2 deficiency due to stomatal closure. All the metabolic changes produced by Pb listed above dramatically modify plant growth and development. Morphogenetic distortions are a nonspecific symptom of the effects exerted by diverse stress factors convenient for assessing the phytotoxicity of these factors (Seregin and Ivanov, 2001).

Chlorophyll and water content

The results of this experiment show that phytotoxicity of increasing Pb 75 to 300 mg L^{-1}, which is apparent from the reduction of chlorophyll concentration in eggplant. There is very strong inhibitory effect on chlorophyll a and b content in mustard (Fargasova, 2001). The studies of photosynthesis can illustrate the direct and indirect effects of Pb. One of the direct effects includes the inhibition of chlorophyll. The inhibition of chlorophyll synthesis by heavy metals is often manifesting as chlorosis. All these changes produced by Pb dramatically modify plant growth and development. Morphogenetic distortions are a nonspecific symptom of the effects exerted by diverse stress factors convenient for assessing the phytotoxicity of these factors (Seregin and Ivanov, 2001). Similarly, chlorophyll content of *Phaseolus vulgaris* and *Lens culinaris* were highly decreased and inhibited at 250 mg L^{-1} Pb concentration. The spectral change due to enhancement of Pb dose, led to significant reduction in chlorophyll biosynthesis. This may be related with accumulation of Pb in tissues. The change in structure of chlorophyll, which indicated that absorption of Pb as compare to essential mineral ion was higher. Pb replaces the other minerals as magnesium. It may be attributed with less concentration of Mg in chlorophyll (Haider et al., 2006).

Negative effect was determined the Pb concentration on tissues water contents in this study. This finding was supported by Jaja and Odoemena (2004) that they was defined a great decrease of leaf water content in tomato 0.001, 0.01, 0.1 and 1% of lead acetate comparison with control. Many researchers reported a decline in transpiration rate and water content in plants treated with Pb^{2+}. Various mechanisms underlie these effects. First, growth retardation results in the reduced area of leaves, the major transpiring organ. Second, the guard cells are sometimes smaller in the plants treated with Pb^{2+}, whereas, in other cases, the guard cells are relatively more numerous, because these heavy metals generally affect leaf growth to a greater extent than the particular differentiation of stomata. Third, Pb^{2+} lower the contents of the compounds maintaining cell turgor and cell wall plasticity and thus lower the water potential; the latter effect becomes an important factor of growth inhibition.

Fourth, these metals increase the content of ABA, thus inducing stomata closure. Fifth, disordered respiration and oxidative phosphorylation can also cause disarray in the plant water regime (Seregin and Ivanov, 2001). Consequently this study showed that plant growth, biomass, macro and microelements uptake and distribution, chlorophyll and water content of *Solanum lycopersicum* L. were negatively affected elevated doses of Pb. Therefore, tomato can be used as an indicator plant in the metal contaminated areas as lead and production risk level can be defined before agricultural activities.

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