INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
07–277/MFA/2008/10–2–167–172
http://www.fspublishers.org

Full Length Article

Photosynthetic Performance of Two Mung Bean (*Vigna radiata*) Cultivars Under Lead and Copper Stress

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ABSTRACT

Thirty-days old plants of two mung bean [*Vigna radiata* (L.) Wilczek] cultivars (Mung-1 & Mung-6) were exposed to either 25 or 50 mg L^{-1} of both lead and copper separately along with a control variant. Application of copper and lead in both Mung bean cultivars caused significant reduction in the CO_2 exchange and photosynthetic pigments. Higher concentration of lead (50 mg L^{-1}) particularly caused significant inhibition of photosynthetic and transpiration rates and stomatal conductance compared to the same doses of copper. Among the plant pigments chlorophyll *b* was more strongly affected by both metals in both cultivars. The pronounced stomatal limitation seemed to be the main factor for photosynthetic inhibition. Nevertheless this photosynthetic inhibition itself may be attributed to the considerable reduction in the plant pigments caused by copper and lead treatments. In general, Mung-1 was ranked as lead and copper tolerant and Mung-1 as a sensitive one.

Key Words: Copper; Lead; Photosynthetic and transpiration rates; Stomatal conductance; Water use efficiency; photosynthetic pigments

INTRODUCTION

Worldwide biosphere has been severely contaminated by metals through natural weathering processes and anthropogenic activities. Among the metals, lead particularly has become a cosmopolitan environmental pollutant (Sharma & Dubey, 2005). It is commonly used in gasoline to improve the efficiency of fuel, but when released through vehicle exhaust pipes it substantially pollutes the urban environment particularly. Other sources of lead pollution include mining and smelting activities, use of Pb containing paints and drainage of untreated municipal sewage (Chaney & Ryan, 1994). It is a non essential element for plants but shows high tendency for its uptake and accumulation in different plant organs (Sharma & Dubey, 2005). Toxic effects of lead on plants grown on lead contaminated soils include inhibition of photosynthesis, deficient mineral nutrition uptake and the problem of water imbalance, which considerably reduce both the vegetative and reproductive growth of plants (Johnson & Eaton, 1980; Sharma & Dubey, 2005).

Copper, another metal, originates from metal smelting and electric power plants, pesticides, sewage sludge, etc. It being a micronutrient, improves plant growth at natural concentrations. However, at higher concentrations it also proves very toxic for plants. The phytotoxic effects related to higher concentrations of copper include inhibition of photosynthetic efficiency and as a result reduced crop productivity (Moustakas *et al.*, 1994).

Vigna radiata (L.) Wilczek locally known as "Mung bean" has great value as food, fodder and green manure. Like other legumes it can considerably improve soil fertility through biological nitrogen fixation. The climate in Pakistan is very suitable for Mung bean cultivation and it is commonly grown during two growing seasons spring and autumn at optimum temperature (27-35^{o}C) for its growth. In this study we aimed to determine the effects of lead (Pb) and copper (Cu) toxicity on some photosynthetic parameters and plant pigments in two Mung bean cultivars.

MATERIALS AND METHODS

This study was conducted in earthen pots lined with polyethylene bags, during spring 2004 to examine the effect of lead and copper on some photosynthetic features of two mung bean [*Vigna radiata* (L.) Wilczek] cultivars (Mung-1 & Mung-6). Each cultivar was treated with solutions containing 25 and 50 mg L^{-1} lead and copper. All experimental pots were filled in with 5 kg of well-washed sand and placed in a completely randomized design with six replicates under natural conditions. Seeds of the two mungbean cultivars were obtained from the Pulses Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan and were surface sterilized with 10% v/v hydrogen peroxide before sowing. Eight seeds moisturized with distilled water were sown in each pot and after complete germination only five seedlings of uniform size were maintained in each pot by thinning. Plants were watered with distilled water whenever felt it necessary and fertilized at ten days interval with 1/8th-strength nutrient solution

To cite this paper: Ahmad, M.S.A., M. Hussain, S. Ijaz and A.K Alvi, 2008. Photosynthetic performance of two mung bean (*Vigna radiata* (L.) wilczek) cultivars under lead and copper stress. *Int. J. Agri. Biol.,* 10: 167–72

(Hoagland & Arnon, 1950). Thirty days after germination plants were treated with 25 or 50 mg L^{-1} soluble chloride salts of both copper and lead dissolved in distilled water, while control plants were treated with distilled water only. The applied concentrations based on the concentration of both metals in the sewage water being drained into irrigation water (UNIDO, 2000). They are comparable to concomitant studies (Xiong, 1998), as well.

The data for different attributed were recorded 14 days after treatment. Chlorophyll content was estimated following Withan *et al*. (1971). Fresh leaves (0.5 g) were triturated in a porcelain mortar with 80% acetone and then the extract was filtered. Thereafter the filtrate was filled up to 50 mL and thoroughly mixed. For spectrophotometric determination of chlorophyll *a*, *b* and total chlorophyll contents, the absorbance of the extracts were measured at 645 and 663 nm, respectively. The absorbance at 480 nm was measured for carotenoid determinations (Hitachi Model U 2001, Japan).

For measuring the net assimilation rate (*A*), stomatal conductance (g_s) and transpiration rate (*E*) of the youngest fully developed intact leaves, an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company Ltd, Hoddeson, England) equipped with a PLCB-4 chamber was used. Measurements were made from 9.30 to 11.30 a.m. with following chamber specification: leaf area 6.25 cm^2, ambient CO_2 concentration (C_{ref}) 290.1 µmol mol^{-1}, leaf chamber temperature (T_{ch}) varied from 31 to 33.8ºC, leaf chamber gas flow rate (V) 394 mL min^{-1}, leaf chamber gas flow rate (U) 256.6 µmol s^{-1}, ambient pressure (P) 98.9 k Pa, water vapor pressure into chamber ranged from 4.4 to 6.6 mbar, molar flow of air per unit leaf area (U_s) 410.6 mol m^{-2} s^{-1} and PAR (Q_{leaf}) at leaf surface was 1948 µmol m^{-2} s^{-1}. The values of assimilation rate (*A*) and transpiration rate (*E*) were used for calculation of water use efficiency (*A*/*E*).

To determine uptake and accumulation of Cu and Pb in roots and leaves, dried plant material was ground and digested with H_2SO_4 and H_2O_2 following Wolf's (1982) method. The concentrations of both metals were determined using an atomic absorption spectrophotometer (Hitachi AAS-Z-8200 with Polarized Zeeman Effect). The collected data were analyzed using COSTAT statistical package (CoHort Software, Minneapolis, MN, USA) by two ways analysis of variance and Duncan's New Multiple Range Test (DMRT) at 5% level of probability was applied to compare means.

RESULTS

The visual observation of 30-days old Mung bean plants exposed to both Cu treatments (i.e., 25 & 50 mg L^{-1}) did not show any toxicity symptoms even after fourteen days of metal application. Similarly, the lower dose of Pb (25 mg L^{-1}) did not indicate any lethal effects. However, well expressed leaf chlorosis and necrosis were observed in the lower mature leaves in response to higher Pb treatment (50 mg L^{-1}).

All metal treatments caused significant reduction in the chlorophyll *a* content of treated plants compared with control (Table I). The difference between chlorophyll *a* content after both Cu treatments plants was not significant but was significantly different in both lead treatments. As regards the interaction between cultivars and treatments, in Mung-1, chlorophyll *a* content of all metal treated plants differed significantly from control but the differences between the lower and higher concentrations for both metals were non significant. In contrast in Mung-6, Chl a contents were significantly higher in plants treated with 25 ppm Pb than that of 50 ppm Pb (Table I) Chlorophyll *a* content in Mung-6 were reduced up to 8.00% at lower concentration of Cu and differed non-significantly from control plants. However, the higher concentration of Cu caused 13.60% reduction in chlorophyll *a* content that differed significantly from control plants but non-significantly from the lower concentrations of both metals. Nevertheless, the higher concentration of Pb caused maximum (30.40%) reduction in the chlorophyll *a* content (Table I).

The chlorophyll *b* content of metal treated plants gradually decreased with the increase of external metal levels and significantly ($P\leq0.05$) differed from control plants. In Mung-1, both Cu concentrations (25 & 50 mg L^{-1}) caused 4.90 and 17.65%, respectively reduction in chlorophyll *b* content differing significantly ($P\leq0.05$) from control plants and with each other, as well. Similarly, 25.49 and 31.37%, reduction in chlorophyll *b* content was found when exposed to 25 and 50 mg Pb L^{-1}, respectively. In Mung-6, chlorophyll *b* content for all metal treatments differed significantly ($P\leq0.05$) from control plants but the differences among the higher Cu concentration and lower Pb level were non significant (Table I).

Likewise, total chlorophyll contents showed a gradual significant decrease in response to increasing concentrations of both metals. In Mung-1, all metal treated plants differed significantly from control plants but the differences between the lower and higher concentrations of both metals were non significant. Nevertheless, in Mung-6, total chlorophyll content pertaining to all metal treatments with the exception of lower copper concentration, differed significantly from control plants.

The carotenoid content of Mung bean plants was significantly influenced by all metal treatments. Moreover, higher concentrations of both metals caused more reduction in the carotenoids compared with respective lower concentrations. In cv. Mung-1, similar carotenoid contents were recorded from control lower level of Cu. However, it differed significantly from rest of the treatments. In both the cultivars, both levels of Cu and Pb differed significantly from each other but there was no difference between higher level of Cu and the lower level of Pb.

Photosynthesis of the plants growing under metal stress was severely inhibited by both metal treatments. A

Table I. Effect of Cu and Pb application on the chlorophyll and carotenoid contents of two Mung bean [*Vigna radiata* (L.) Wilczek] cultivars (Mung-1 & Mung-6)

Parameter	Cultivars	Treatments: Control	Cu (mg L^{-1}) 25	Cu (mg L^{-1}) 50	Pb (mg L^{-1}) 25	Pb (mg L^{-1}) 50	Cultivar Means
	Mung-1	1.38±0.038 a x	1.17±0.023 b x	1.13±0.025 bc x	1.03±0.021 cd x	0.92±0.023 d x	1.12 a
	% inhibition	-	-15.22	-18.12	-25.36	-33.33	
Chlorophyll *a*	Mung-6	1.25±0.021 a y	1.15±0.024 ab x	1.08±0.025 b y	1.07±0.041 b x	0.87±0.032 c y	1.08 b
(mg/ g FW)	% inhibition	-	-8.00	-13.60	-14.40	-30.40	
	Tr. Means	1.31 a	1.16 b	1.11 b	1.05 c	0.90 d	
LSD (5%) [Cultivar means = 0.056, Treatment means = 0.036, Cultivar x Treatment means = 0.051]							
	Mung-1	1.02±0.029 a x	0.97±0.010 b x	0.84±0.010 c x	0.76±0.06 d x	0.70±0.017 e x	0.84 a
	% inhibition	-	-4.90	-17.65	-25.49	-31.37	
Chlorophyll *b*	Mung-6	1.02±0.034 a x	0.87±0.007 b y	0.74±0.009 c y	0.74±0.004 c x	0.69±0.013 d x	0.81 b
(mg/ g FW)	% inhibition	-	-14.71	-27.45	-27.45	-32.35	
	Tr. Means	1.02 a	0.88 b	0.79 c	0.75 d	0.69 e	
LSD (5%) [Cultivar means =0.033 , Treatment means = 0.021, Cultivar x Treatment means = 0.048]							
	Mung-1	2.08±0.060 a x	1.73±0.024 b x	1.66±0.065 b x	1.73±0.073 b x	1.61±0.065 b x	1.77 a
Total	% inhibition	-	-16.83	-20.19	-16.83	-22.60	
chlorophyll	Mung-6	1.88±0.033 a y	1.84±0.067 a x	1.62±0.044 b x	1.64±0.049 b x	1.45±0.017 c y	1.70 b
(mg/ g FW)	% inhibition	-	-2.13	-13.83	-12.77	-22.87	
	Tr. Means	1.89 a	1.79 b	1.69 bc	1.64 c	1.53 d	
LSD (5%) [Cultivar means 0.067 = , Treatment means = 0.106, Cultivar x Treatment means = 0.150]							
	Mung-1	1.61±0.036 a x	1.51±0.015 a x	1.37±0.049 b x	1.42±0.083 b x	1.27±0.022 c x	1.43 a
	% inhibition	-	-6.79	-16.05	-13.58	-21.60	
Carotenoids	Mung-6	1.58±0.028 a x	1.48±0.033 b x	1.36±0.025 c x	1.34±0.012 c y	1.06±0.036 d y	1.37 b
(mg/ g FW)	% inhibition	-	-3.18	-13.38	-12.74	-32.48	
	Tr. Means	*1.60 a*	*1.50 b*	*1.38 c*	*1.36 c*	*1.17 d*	
LSD (5%) [Cultivar means = 0.049, Treatment means = 0.078, Cultivar x Treatment means = 0.079]							

Mean values sharing the same letter within rows or columns differ non-significantly ($P \leq 0.05$)
Treatment means and cultivar x treatment interaction means have been grouped horizontally while cultivar means have vertical grouping.

Table II. Effect of Cu and Pb application on photosynthetic attributes of two Mung bean [*Vigna radiata* (L.) Wilczek] cultivars (Mung-1 & Mung-6)

Parameter	Cultivars	Treatments: Control	Cu (mg L^{-1}) 25	Cu (mg L^{-1}) 50	Pb (mg L^{-1}) 25	Pb (mg L^{-1}) 50	Cultivar Means
	Mung-1	5.94 ±0.251 a x	5.62±0.173 a x	5.14±0.217 b x	5.20±0.095 ab x	4.82±0.132 b x	5.34 a
	% inhibition	-	-5.39	-13.47	-12.46	-18.86	
A	Mung-6	5.87±0.143 a x	5.50±0.137 a x	5.02±0.072 b x	4.48±0.234 c y	4.05±0.124 c y	4.98 b
(μ mol m^{-2}) S^{-1})	% inhibition	-	-6.30	-14.48	-23.68	-31.01	
	Tr. Means	5.90 a	5.56 b	5.08 c	4.84 c	4.43 d	
LSD (5%) [Cultivar means = 0.213, Treatment means = 0.336, Cultivar x Treatment means = 0.473]							
	Mung-1	3.89±0.042 a x	3.68±0.048 a x	3.43±0.096 ab x	2.96±0.140 c x	2.30±0.059 d x	3. 25 a
	% inhibition	-	-5.40	-11.83	-23.91	-40.87	
E	Mung-6	3.78±0.027 a x	3.44±0.072 b y	3.20±0.163 b x	2.55±0.042 c y	2.18±0.114 d x	3.03 b
(m mol m-^{2} S^{-1})	% inhibition	-	-8.99	-15.34	-32.54	-42.33	
	Tr. Means	3.83 a	3.56 b	3.32 c	2.76 d	2.24 e	
LSD (5%) [Cultivar means = 0.116, Treatment means = 0.183, Cultivar x Treatment means = 0.258]							
	Mung-1	0.184±0.005 a x	0.149±0.004 b x	0.109±0.006 c x	M	0.086±0.003 d x	0.119 a
	% inhibition	-	-19.02	-40.76	-54.89	-53.26	
g_s	Mung-6	0.179±0.002 a x	0.150±0.002 b x	0.094±0.002 c x	0.076±0.003 d y	0.060±0.001 d y	0.114 b
(m mol m^{-2} s^{-1})	% inhibition	-	-16.20	-47.49	-57.54	-66.48	
	Tr. Means	0.181 a	0.149 b	0. 101 c	0.079 d	0.064 e	
LSD (5%) [Cultivar means =0.0041 , Treatment means = 0.0066, Cultivar x Treatment means = 0.0093]							
	Mung-1	1.52±0.021	1.59±0.023	1.61±0.040	1.77±0.023	2.09±0.015	1.72
A/*E*	% promotion	-	+ 4.61	+ 5.92	+ 16.45	+ 37.50	
(μmol CO_2/ m	Mung-6	1.54±0.012	1.55±0.011	1.58±0.035	1.75±0.026	1.87±0.036	1.66
mol H_2O)	% promotion	-	+ 0.65	+ 2.60	+ 13.64	+ 21.43	
	Tr. Means	1.53 c	1.57 c	1.60 c	1.76 b	1.98 a	
LSD (5%) Cultivar means = ns , Treatment means = 0.152, Cultivar x Treatment means = ns							

Mean values sharing the same letter within rows or columns differ non-significantly ($P \leq 0.05$)
Treatment means and cultivar x treatment interaction means have been grouped horizontally while cultivar means have vertical grouping.

strong relationship in reduction in *A* and *E* and g_s was observed in both cultivars. All the treatments reduced the assimilation rate significantly except lower dose of Cu, which behaved similar to that of control. As regards the interaction between cultivars and treatments, with the exception of lower dose of Cu, all other metal treatments caused significant reduction in assimilation rate in Mung-1 as compared with control variant.

Both metals significantly ($P \leq 0.05$) affected transpiration rate (*E*) of treated Mung bean plants. Mung-1

Table III. Effect of Cu and Pb application on the Cu and Pb uptake in two Mung bean [*Vigna radiata* (L.) Wilczek] cultivars (Mung-1 & Mung-6)

Plant organs	Cultivars	Treatments										Cultivar Mean
		Control		Cu ($mg\ L^{-1}$)				Pb ($mg\ L^{-1}$)				
				25		50		25		50		
Cu^{2+} uptake in root ($mg\ kg^{-1}$)	Mung-1	1.02±0.16 d	x	3.46±0.22 bc	x	4.91±0.14 a	x	0.98±0.09 d	x	1.00±0.16 d	x	2.27 a
	% change	-		+ 239.22		+ 381.37		-3.92		-1.96		
	Mung-6	0.95±0.15 d	x	2.21±0.18 c	y	3.75±0.15 b	y	0.98±0.10 d	x	0.98±0.09 d	x	1.77 b
	% Change	-		+ 132.63		+ 294.74		+ 3.16		+ 3.16		
	Tr. Means	0.99 c		2.84 b		4.33 a		0.98 c		0.99 c		
LSD (5%) [Cultivar means = 0.43, Treatment means = 1.19, Cultivar x Treatment means = 1.13]												
Cu^{2+} accumulation in leaf ($mg\ kg^{-1}$)	Mung-1	1.48±0.14 d	x	9.42±0.76 bc	x	11.10±0.51 a	x	1.11±0.10 d	x	0.86±0.08 d	x	4.79 a
	% increase	-		+ 536.49		+ 650.00		-25.00		-41.89		
	Mung-6	1.24±0.16 d	x	8.28±0.53 c	y	9.20±0.43 b	y	0.84±0.05 d	x	0.71± 0.11 d	x	4.05 b
	% increase	-		+ 567.74		+ 641.94		-32.26		-42.74		
	Tr. Means	1.36 c		8.85 b		10.15 a		0.97 c		0.78 c		
LSD (5%) [Cultivar means = 0.69, Treatment means = 1.14, Cultivar x Treatment means = 1.19]												
Pb^{2+} uptake in root ($mg\ kg^{-1}$)	Mung-1	0.001±0.00 c	x	0.002±0.00 c	x	0.003±0.00 c	x	2.630±0.12 c	x	3.570±0.29 a	x	1.20 a
	% increase	-		+ 100.00		+ 200.00		+ 262900.00		+ 178400.00		
	Mung-6	0.002±0.00 c	x	0.003±0.00 c	x	0.003±0.00 c	x	2.206±0.11 d	y	3.348±0.27 b	y	1.16 b
	% increase	-		+ 50.00		+ 50.00		+ 110200.00		+ 334700.00		
	Tr. Means	0.002 c		0.002 c		0.003 c		2.418 b		3.459 a		
LSD (5%) [Cultivar means = 0.13, Treatment means = 0.21, Cultivar x Treatment means = 0.29]												
Pb^{2+} accumulation in leaf ($mg\ kg^{-1}$)	Mung-1	0.002±0.00 c	x	0.003±0.00 c	x	0.003±0.00 c	x	1.000±0.15 b	x	1.686±0.19 a	x	0.54 a
	% increase	-		+ 50.00		+ 50.00		+ 49900.00		+ 84200.00		
	Mung-6	0.002±0.00 c	x	0.004±0.00 c	x	0.005±0.00 c	x	0.814±0.08 b	x	1.050±0.09 b	y	0.38 b
	% increase	-		+ 100.00		+ 150.00		+ 40600.00		+ 52400.00		
		0.002		*0.003*		*0.004*		*0.907*		*1.368*		
LSD (5%) [Cultivar means = 0.011, Treatment means = 0.092, Cultivar x Treatment means = 0.014]												

Mean values sharing the same letter within rows or columns differ non-significantly ($P \leq 0.05$)
Treatment means and cultivar x treatment interaction means have been grouped horizontally while cultivar means have vertical grouping.

plants treated with 25 and 50 mg Pb L^{-1} was found 23.91 and 40.87% reduction of transpiration rate, respectively; while in Mung-6 the corresponding reductions were recorded 32.54 and 42.33%, respectively. In Mung-1, there was no significant effect of both Cu treatments on transpiration rate.

Data pertaining to stomatal conductance (g_s) of metal treated Mung bean plants showed highly significant ($P > 0.01$) differences among treatments, between cultivars and for interaction. As regard the main effects of metal treatments, all the metal treatments caused significant ($P \leq 0.05$) reduction in the stomatal conductance, which gradually reduced with the increasing external metal concentrations. Higher concentration of both metals caused significantly more reduction than respective lower concentrations (25 mg L^{-1}). Maximum reduction in stomatal conductance was noted in Mung bean-6 treated with higher dose of Pb that was similar to that both the Pb doses in Mung bean-1 and lower Pb dose in Mung bean-6.

The analysis of data relating to water use efficiency (*A*/*E*) of metal treated plants showed non significant ($P > 0.05$) differences among studied cultivars and interaction terms and highly significant ($P \leq 0.01$) differences among metal treatments. Despite of the fact that overall treatment means were significantly different, the difference in higher and lower concentration of Cu was non significant.

Application of metal significantly increased Cu uptake by roots in both Cu stress treatments. On the other hand, it was slightly reduced by in plants under lead stress. The highest Cu uptake in both cultivars was observed in higher Cu treatment followed by its lower dose. Although, Cu content were increased under Cu as well as Pb stress, it differed significantly in Cu treatments and were non-significantly different in Pb treatments. Mung-1 absorbed relatively higher levels of Cu in roots and exhibited accelerated accumulation in leaves. Overall, Cu translocation from root to leaves was observed in Cu stressed plants (Table III). Similar results were obtained for Pb uptake in the metal stress plants. There was no difference in both Cu treatments Pb content in roots and leaves. On the other hand, Pb content was significantly different in both Pb treatments. It was observed that in contrast to Cu, which was translocated to leaves, Pb showed a restricted translocation to aerial parts and mainly stored in roots of Mung bean plants (Table III).

DISCUSSION

The translocation of metals from the roots into the shoot is a controversial issue. As roots remain completely immersed and fully exposed to higher metal concentrations of growth medium, majority of the metals become sequestered in the roots. The metals (Cu & Pb) used for this study showed a different response for their translocation from the roots into the shoot. Copper being an essential microelement showed more translocation towards shoots as compared to Pb, which became highly sequestered in the roots (Burzynski & Grabowski, 1984; Yruela, 2005). The uptake of Cu and Pb by Mung bean roots and their translocation into the shoot when exposed to both these metals at the concentrations of 25 and 50 mg L^{-1} seemed to be strongly correlated with the external metal concentrations (Table III) (Bibi *et al*., 2005). In Mung bean-1, there was 100 and 200% higher Cu accumulation roots treated with lower and higher Cu levels, while there was only 50% more Cu accumulation in roots after both Cu treatments in soils (Baker, 1981; Antosiewicz, 1992).

The increasing concentrations of copper and lead i.e., 25 and 50 mg L^{-1} caused a gradual reduction in the observed plant pigments (chlorophyll a, chlorophyll b, total chlorophyll & carotenoids) in both studied cultivars (Tu Shu & Brouillette, 1987). Ouzounidou (1993; 1995) related destruction of photosynthetic pigment in catchfly (*Silene compacta*) to the negative effects of excessive copper application on photosynthetic electron transport. Similarly in our study, the gradual decline in chlorophyll contents may be attributed to the gradual degradation of photosynthetic pigments. Moreover, high Pb and Cu concentrations inside the leaf might have been high enough to directly inhibit chlorophyll synthesis (Sengar & Pandey, 1996). Lead accumulation in different organs is considered a general protoplasmic poison (Johnson & Eaton, 1980). Moreover, the key enzyme of chlorophyll biosynthesis, δ-amino laevulinate dehydrogenase has been reported to be strongly inhibited by Pb (Prassad & Prassad, 1987).

The process of photosynthesis (*A*) was adversely affected by Pb and Cu toxicity. Plants exposed to both metals showed a decline in photosynthetic rate, which might have resulted from distorted chloroplast structure, restrained photosynthesis of chlorophyll and carotenoids, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closure (Vojtechova & Leblova 1991; Moustakas *et al*., 1994). A strong relationship exists between Pb application and a decrease in photosynthesis and it is believed to result from stomatal closure rather than a direct effect of Pb on the process of photosynthesis (Bazzaz *et al*., 1975). According to Kosobrukhov *et al*. (2004), the photosynthetic activity is affected by many factors including stomatal conductance. The inactivation of Rubisco (ribulose-bisphosphate carboxylase/oxygenase) a key-enzyme of Calvin cycle and its two accompanying enzymes i.e., Rubisco activase (RCA) and carbonic anhydrase (CA) under the stress conditions caused by copper and lead (not examined) may be regarded another possible factor (Vojtechova & Leblova, 1991; Moustakas *et al*., 1994).

It is a common observation that Pb toxicity causes disorder in the plant water regime including transpiration rate (*E*). In our studies, both metals significantly affected *E* of treated Mung bean plants. It was observed that despite low foliar concentrations, lead proved more toxic and decreased *E* to a greater extent as compared to copper treated plants (because of it is essential microelement) (Hernandez-Allica *et al*., 2003). Transpiration exhibited similar trend to photosynthesis suggesting that an appreciable part of the inhibition of the two processes is related to increased stomatal resistance as a result of stomatal closure. In addition, Pb and Cu treatments caused growth retardation, which results in reduced leaf area, the major transpiring organ (Iqbal & Mushtaq, 1987) and hence reduced *E*.

Stomatal conductance (g_s) of metal treated Mung bean plants showed significant reduction with the increasing concentration of both the metals in the substrate. The reduced stomatal conductance seemed to be a consequence of reduced K^+ and Ca^{2+} uptake under Pb and Cu stresses (Yruela, 2005), both ions being the main controlling factors of the stomatal opening. Similarly, reduced photosynthesis under both the metal stresses might also explain this reduction in g_s (Moustakas *et al*., 1994). On the other hand, a beneficial effect of decreased stomatal conductance can be reduction in Cu or Pb transport with the transpiration flow to the aerial parts.

CONCLUSION

The higher concentration of Pb (50 mg L^{-1}) particularly caused more inhibition of assimilation rate, transpiration rate, stomatal conductance and sub-stomatal conductance and considerably promoted water use efficiency compared to the same doses of Cu. Among the plant pigments, chlorophyll *b* was more strongly affected by both metals in both Mung bean cultivars. The pronounced stomatal limitation seemed to be the main factor for photosynthetic inhibition. Nevertheless this photosynthetic inhibition itself may be attributed to the considerable reduction in the plant pigments caused by copper and lead treatments. Despite Mung-1 accumulated higher levels of both metals ion roots and leaves as compared to Mung-6, the later cultivar was more sensitive to both metals (Cu & Pb) in general and leads (Pb) in particular.

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(Received 18 August 2007; Accepted 17 September 2007)