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Effect of Copper and Lead on Photosynthesis and Plant Pigments in Black Gram [*Vigna mungo* (L.) Hepper]

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The discharge of untreated chemicals by different industries and their incorporation in to aquatic ecosystems is creating great environmental problem in Pakistan not only for the aquatic biota but also, for the terrestrial biota such as cultivated crops and natural plant communities. The heavy metal component of these effluents is greater than international standards (EPTI 1997). Entering the plant body heavy metals inhibit the metabolic process and substantially reduce crop production (Xian, 1989).

Among the heavy metals, lead (Pb^{2+}) toxicity has particularly become important due to its cosmopolitan presence in the environment. After emission from industries, motor vehicles, and stationary fuel its accumulation in plant organs up to an undesirable level poses inhibitory effects on physiological processes i.e. photosynthesis (Stribrova *et al.*, 1986) and chlorophyll synthesis (Fargasova 2001), which subsequently reduce crop yield (Moftah, 2000). Copper (Cu^{2+}) originates from electric power plants, metal smelting plants, agrochemicals (pesticides) and sewage sludge. At lower concentrations it improves plant growth but its higher concentrations also prove toxic for plants. Its phytotoxic effects include deterioration of photosynthetic efficiency that ultimately reduces crop productivity (Moustakas *et al.* (1997).

Black gram [*Vigna mungo* (L.) Hepper] locally known as "Mash" belongs to family Papilionaceae. It has great value as food, fodder and green manure. Besides having the potential to improve soil fertility through biological nitrogen fixation by nitrifying bacteria located in root nodules, it proves a cheap source of protein (22-24%), and vitamin A and B (James 1981). The optimum temperature for its growth being 27-35°C, it can be grown in both winter and summer seasons. The climate of Pakistan along-with other South Asian countries falls under arid and semi-arid zones is very suitable for black gram [*Vigna mungo* (L.) Hepper] cultivation. However, the areas under black gram cultivation in Pakistan are prone to higher concentrations of toxic metals due to rampant discharge of untreated industrial effluents in to rivers, the major source of irrigation water in Pakistan. Hence, this project was designed to determine the toxic effects of lead (Pb^{2+}) and copper (Cu^{2+}) on photosynthetic parameters and plant pigments in two black gram cultivars.

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MATERIALS AND METHODS

This experiment was conducted in earthen pots lined with polyethylene bags, during spring 2003 to evaluate the effect of 25 & 50 mg L^{-1} concentrations of copper and lead on photosynthetic attributes and plant pigments in black gram [*Vigna mungo* (L.) Hepper] cultivars i-e Mash-95018 (V_1) and Mash-97 (V_2). Pots were filled in with 5 kg sand and placed in a completely randomized design with six replicates under natural conditions in the Botanical Garden, University of Agriculture, Faisalabad, Pakistan. Before sowing seeds were surface sterilized with 10% v/v hydrogen peroxide following Vassilev et al (2002). Initially eight seeds were sown in each pot, moisturized with distilled water and after complete germination only five seedlings uniform in 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 modified Hoagland solution (Hoagland and Arnon 1950). Thirty days after germination plants were treated with soluble chloride salts of both copper @ 25 (T_1) and 50 (T_2) mg kg^{-1} and lead @ 25 (T_3) and 50 (T_4) mg kg^{-1} in a liquid form by dissolving their finely ground powder in distilled water, while control (T_0) plants were treated with distilled water only. The applied concentrations based on our previous studies (unpublished), the concentration of both metals in the sewage water being drained into irrigation water (UNIDO 2000). They are comparable to cited studies (Xiong 1998) as well.

For chemical analysis we harvested plants ten days after treatment. During this period soil moisture was kept constant. Plant material was dried, ground, and digested with H_2SO_4 and H_2O_2 following Wolf's (1982) method. Atomic absorption spectrophotometer (Hitachi AAS-Z-8200 with polarized Zeeman effect) was used for determining $Cu2^+$ and Pb^{2+} uptake by black gram plants. Despite both metals were sequestered in the roots, Cu being a micro nutrient showed slightly higher translocation to leaves for its both concentrations in both black gram cultivars than lead treatments (Table 1).

Table 1. Copper and lead uptake (mean effective concentrations and standard deviation) by two black gram cultivars viz. Mash-95018 (V_1) and Mash-97 (V_2).

Plant organs	Varieties	Treatments means for metal uptake (mg kg^{-1})			
		Copper		Lead	
		25 mg kg^{-1}	50 mg kg^{-1}	25 mg kg^{1}	50 mg kg^{-1}
Leaf	V_1	1.54 ± .32	2.75 ± 0.52	0.31 ± 0.13	0.52 ± 0.11
	V_2	1.75 ± 0.37	2.83 ± 0.58	0.46 ± 0.14	0.91 ± 0.16
Root	V_1	5.91 ± 0.64	8.27 ± 1.5	2.17 ± 0.64	3.10 ± 0.52
	V_2	8.08 ± 1.52	10.02 ± 1.79	2.22 ± 0.38	3.31 ± 0.40

An open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company Ltd, Hoddeson, England) equipped with a PLCB-4 chamber was used for measuring the assimilation rate, stomatal conductance, sub-stomatal conductance and transpiration rate of the youngest fully developed intact leaves. Measurements were made from 10.00 to 11.30 a. m. with chamber specification i. e. leaf area $6.25cm^2$, ambient CO_2 concentration (Cref) 290.1μmole $mole^{-1}$, leaf chamber temperature (Tch) varied from 41 to 43.8 °C, leaf chamber gas flow rate (V) 394 mL min^{-1}, leaf chamber gas flow rate (U) 256.6μ mol S^{-1}, ambient pressure (P) 98.9k Pa, water vapor pressure (e ref) into chamber ranged from 4.4 to 6.6 m bar, molar flow of air per unit leaf area (Us) 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 to work out the intercellular water use efficiency.

For the estimation of plant pigments, leaves were chopped in to small pieces and extracted in 100% acetone solution. The absorbance of the extract was measured at 645 and 663 nm for chlorophyll a, b and total chlorophyll respectively while the absorbance at 480 nm was measured for carotenoids contents using a spectrophotometer (Hitachi Model U 2001, Japan). The concentration of different pigments was worked out using the formula given by Davis (1972). Data were analyzed using the ANOVA procedure for a Completely Randomized Design with a two factor factorial arrangement (2 varieties and 5 treatments) (Steel and Torrie 1986). We used COSTAT statistical package (CoHort Software, Minneapolis, MN, USA) to analyze the data by two ways analysis of variance and Duncan's New Multiple Range Test (DMRT) at 5% level of probability to compare means.

RESULTS AND DISCUSSION

The assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (*gs*), sub-stomatal conductance and water use efficiency (WUE) of two black gram [*Vigna mungo* (L.) Hepper] cultivars were significantly ($p \leq 0.05$) influenced by copper (Cu^{2+}) and lead (Pb^{2+}) treatments (Table 2). As compared to control, assimilation rate was 23.70% and 36.51% less in V_1 and 21.68 % and 28.51% less in V_2 in response to 25 and 50 mg Pb kg^{-1} respectively. In contrast, the higher dose (50 mg kg-1) of copper caused maximum (18.57%) reduction in assimilation rate in V_1 as compared to control.

Although both heavy metals significantly affected transpiration rate of treated black gram plants, but lead despite its low foliar concentrations proved more toxic and decreased it to a greater extent compared to copper treated plants. In V_1 plants treated with 25 and 50 mg Pb kg^{-1} showed 29.59% and 42.21% reduction in their transpiration rate while in V_2 the corresponding reductions were recorded 19.82% and 44.08% respectively. These findings are in accordance with the results of Elenany and Hamada (1995) who treated sunflower (*Helianthus annuus*), black-eyed pea (*Vigna sinensis*) and wheat (*Triticum vulgare*) with copper and observed that transpiration rate gradually decreased as the metal concentration in the medium increased. Similarly Hernandez-Allica et al (2003) noted considerable reduction in the transpiration rate of Cardoon seedlings (*Cynara cardunculus*) with the application of lead.

Application of both heavy metals i. e. copper and lead significantly reduced stomatal conductance and sub-stomatal conductance of treated black gram plants.

The differences between the lower (25 mg kg^{-1}) and higher (50 mg kg^{-1}) doses of both metals were also significant. Nevertheless, water use efficiency of both cultivars was significantly influenced by the higher dose of lead (50 mg Pb kg-1) that enhanced it up to 28.08% and 27.78% in V_1 and V_2 respectively as compared to control.

The changes in *A, E, gs* and sub-stomatal conductance in the copper and lead treated plants agree with the findings of Ali et al (1999) who observed the physiological response of white mustard (*Bacopa monnieri*) to copper stress that severely inhibited stomatal conductance. The stomatal limitation caused by copper and lead treatments in both black gram cultivars in this study as well, may be regarded the main cause of considerable reduction in the photosynthetic parameters including the drastic change in water use efficiency. In a similar study Landberg et al (1994) treated pea (*Pisum sativum*) plants with copper and cadmium in the presence of selenium and examined no difference in the photosynthetic attributes. The contradiction in the results may be due to the variable response of crop cultivars under study and the difference in the environmental conditions under which the different studies were undertaken. However, our results agree with findings of Moustakes *et al.* (1994) showing that photosynthetic characters of oats (*Avena sativa*) under copper and lead stress limited overall photosynthetic functioning as observed during the present study.

The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of copper and lead treated plants show significant differences from control (Table 3). For chlorophyll a content, despite a slight variation both black gram cultivars showed similar response to 25 and 50 mg Cu kg^{-1} as well as 25 mg Pb kg^{-1}. However, V_2 was more sensitive towards 50 mg Pb kg^{-1} that caused 11.67% reduction in its chlorophyll a content as compared to that in V_1 (7.94%). The chlorophyll b content of both black gram cultivars was substantially influenced by both heavy metals. The increasing concentration of copper, as well as, lead caused gradual reduction in chlorophyll b content. In V_1 it went down to 30.56% and 47.32% with the application of 25 and 50 mg Pb kg-1 while in V_2 the same concentrations of Pb reduced it by 33.24% and 50.21% respectively.

Total chlorophyll and carotenoid contents of both black gram cultivars showed similar pattern in response to copper and lead application. For both parameters, lead concentrations, as 25 and 50 mg kg^{-1} were more toxic. A comparison among the black gram cultivars indicates that for both parameters under consideration, V_2 proved more sensitive to copper and lead treatments than its counterpart (V_1).

The increasing applied or effective concentrations of copper and lead i. e. 25 and 50 mg kg^{-1} caused a gradual reduction in the observed plant pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) in both black gram cultivars. The sensitivity of these pigments may be ranked as: Chlorophyll b > total chlorophyll > carotenoids > chlorophyll a, that seems in accordance with the findings of Shu et al. (1987). Fargasova (2001) also observed considerable reduction in chlorophyll a, b, total chlorophyll and carotenoid contents in white mustard (*Sinapis alba*). Our studies corroborate the findings of Lidon et al. (1992)

Table 2. Effect of copper and lead on photosynthetic characters of black gram (*Vigna mungo mungo* (L.) Hepper] cultivars, ($p \leq 0.05$).

Parameters	Varieties	Control (T_0)	Copper (Cu^{2+}) mg kg $^{-1}$		Lead (Pb^{2+}) mg kg $^{-1}$		Variety Means
			25 (T_1)	50 (T_2)	25 (T_3)	50 (T_4)	
Assimilation rate (μmol m^{-2} S^{-1})	Mash-95018 (V_1)	5.93	5.64	4.83	4.52	3.76	4.95 a
	Decrease (%)		-4.79	-18.57	-23.70	-36.51	
	Mash-97 (V_2)	5.38	5.12	4.91	4.21	3.85	4.69 b
	Decrease (%)		-4.89	-8.80	-21.68	-28.51	
	Tr. Means	5.65 a	5.38 b	4.87 c	4.37 d	3.80 e	
Transpiration rate (mmol m^{-2} S^{-1})	Mash-95018 (V_1)	3.01	2.28	2.21	2.120	1.74	2.27 a
	Decrease (%)		-24.31	-26.54	-29.59	-42.21	
	Mash-97 (V_2)	2.43	2.24	2.11	1.95	1.36	2.02 b
	Decrease (%)		-8.10	-13.16	-19.82	-44.08	
	Tr. Means	2.72 a	2.26 b	2.16 bc	2.04 c	1.55 d	
Stomatal conductance (mmol m^{-2} S^{-1})	Mash-95018 (V_1)	0.17	0.12	0.07	0.06	0.03	0.09 a
	Decrease (%)		-30.23	-53.49	-65.12	-82.56	
	Mash-97 (V_2)	0.11	0.06	0.06	0.03	0.02	0.05 b
	Decrease (%)		-46.43	-55.36	-73.21	-82.14	
	Tr. Means	0.14 a	0.09 b	0.06 c	0.05 cd	0.03 d	
Sub-stomatal conductance	Mash-95018 (V_1)	209	179	171	162	146	173 a
	Decrease (%)		-14.52	-18.13	-22.60	-30.14	
	Mash-97 (V_2)	187	168	153	136	118	152 b
	Decrease (%)		-10.34	-17.98	-27.24	-37.04	
	Tr. Means	198 a	173 b	162 c	149 d	132 e	
Water use efficiency (μmol CO_2/ m mol H_2O)	Mash-95018 (V_1)	1.96	2.18	2.48	2.12	2.52	2.26 a
	Increase (%)		+11.18	+26.18	+8.17	+28.08	
	Mash-97 (V_2)	2.21	2.28	2.32	2.155	2.84	2.36 b
	Increase (%)		+3.17	+5.02	+2.53	+27.78	
	Tr. Means	2.08 a	2.23 b	2.40 bc	2.14 a	2.67 c	

Mean values sharing the same letter differ non-significantly ($p \leq 0.05$)

Table 3. Effect of copper (Cu) and lead on the chlorophyll and carotenoid contents of two black bean [*Vigna mungo* (L.) Hepper] cultivars, ($p \leq 0.05$).

Parameters	Varieties	Treatments					Varity means
		Control	Copper (Cu^{2+}) mg kg $^{-1}$		Lead (Pb^{2+}) mg kg $^{-1}$		
		(T_0)	25 (T_1)	50 (T_2)	25 (T_3)	50 (T_4)	
Chlorophyll "a" (mg/ g FW)	Mash-95018 (V_1)	1.26	1.29	1.18	1.17	1.16	1.21 a
	Decrease (%)		-2.38	-6.35	-7.14	-7.94	
	Mash-97 (V_2)	1.20	1.14	1.15	1.11	1.06	1.13 b
	Decrease (%)		-5.00	-4.17	-7.50	-11.67	
	Tr. Means	1.23 a	1.22 b	1.17 b	1.14 c	1.11 d	
Chlorophyll "b" (mg/ g FW)	Mash-95018 (V_1)	0.88	0.83	0.65	0.61	0.46	0.69 a
	Decrease (%)		-5.25	-26.45	-30.56	-47.32	
	Mash-97 (V_2)	0.71	0.62	0.54	0.47	0.35	0.54 b
	Decrease (%)		-12.16	-24.05	-33.24	-50.21	
	Tr. Means	0.79 a	0.73 a	0.59 b	0.54 b	0.41 c	
Total chlorophyll (mg/ g FW)	Mash-95018 (V_1)	2.14	1.93	2.07	1.78	1.62	1.91 a
	Decrease (%)		-9.79	-3.45	-16.93	-24.35	
	Mash-97 (V_2)	1.91	1.77	1.69	1.58	1.42	1.67 b
	Decrease (%)		-7.28	-11.47	-17.08	-25.62	
	Tr. Means	2.03 a	1.85 b	1.88 c	1.68 c	1.52 d	
Carotenoids (mg/ g FW)	Mash-95018 (V_1)	1.68	1.60	1.56	1.52	1.51	1.58 a
	Decrease (%)		-4.52	-7.38	-9.35	-9.88	
	Mash-97 (V_2)	1.59	1.54	1.45	1.39	1.35	1.47 b
	Decrease (%)		-3.45	-8.91	-12.55	-15.38	
	Tr. Means	1.64 a	1.57 b	1.50 c	1.46 d	1.43 d	

Mean values sharing the same letter differ non-significantly ($p \leq 0.05$)

as well. They reported the negative effect of excess copper application on the photosynthetic pigments of rice due to the degradation of photosynthetic attributes. Somaskekaraiah et al (1992) examined the phytotoxic effects of Cd ions on the growing seedlings of mung bean (*Phaseolus vulgaris*) and reached the same conclusion. Nevertheless, Ozounidou (1993) related destruction of photosynthetic pigment in catchfly (*Silene compacta*) to the negative effects of excessive copper application on photosynthetic electron transport. In this study as well, the gradual decline in chlorophyll contents may be attributed to the gradual degradation of photosynthetic parameters.

In conclusion application of both copper and lead, in both black gram cultivars caused significant reduction in the photosynthetic gas exchange and pigment contents as compared with control. The higher concentration of lead (50 mg kg-1) particularly caused severe inhibition of assimilation rate, transpiration rate, stomatal conductance and sub-stomatal conductance and considerably promoted water use efficiency compared to the same doses of copper. Among the plant pigments chlorophyll b was more strongly influenced by both heavy metals in both black gram varieties. The pronounced stomatal limitation 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. 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. Among the black gram cultivars V_2 (Mash-97) had non-significantly higher effective concentrations of both metals in its leaves. However, it proved comparatively more sensitive to both heavy metals ($Cu2^{+}$ and Pb^{2+}) in general and lead (Pb^{2+}) in particular. It may be regarded a consequence of variation in the genetic make up of black gram cultivars. This study has great implication for the selection of suitable black gram cultivars for cultivation in the areas being irrigated with water excessively contaminated with heavy metals particularly copper and lead.

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