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Accumulation of Nutrients and Metal Ions by Two Mung Bean [*Vigna radiata* (L.) Wilczek] Cultivars Treated with Copper and Lead

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The discharge of untreated industrial effluents into fresh water bodies has become a critical problem in Pakistan. The amount of metals particularly copper (Cu), cadmium (Cd), chromium (Cr), zinc (Zn), lead (Pb), manganese (Mn) and nickel (Ni) incorporated in these industrial effluents is considerably high (EPTI, 1997). When taken up by crop plants in higher concentrations, they create a dual problem. Entering the plant body, they not only inhibit metabolic process and reduce crop production in plants (Foy et al., 1978) but also, become incorporated in the food chain subsequently (Somers, 1974) and can cause liver and brain disorders. Therefore, the problem posed by increasing amounts of metals released into the environment demands a complete understanding of their phytotoxic effects on soil-plant-animal system.

Copper is a well-known essential micronutrient for higher plants among metals, but its higher concentration causes deficiency of other essential nutrients by competitive exclusion at uptake sites (Lin and Wun, 1994). Besides, it reduces enzyme production (Angelov et al, 1993) and suppresses membrane functioning (Nag et al., 1980).

Lead, another metal has become a worldwide dangerous environmental pollutant. After emission from industries, motor vehicles, and stationary fuel, it becomes incorporated in the food chain and poses detrimental influences on a number of physiological processes in plants (Mesmar and Jaber, 1991) and in human beings it badly damages brain and nervous system (Body et al., 1991).

Mung bean [*Vigna radiata* (L.) Wilczek] is a leguminous crop. It has great value as food, fodder and green manure. In addition to improving the soil fertility, it proves a cheap source of protein for direct human consumption. Chemical analysis of mung bean seed indicates 24.9% protein, 1.3% fats, 60.0% carbohydrates on dry weight basis and a fair amount of minerals such as calcium, phosphorus, iron, sodium, and potassium, in addition to some vitamins like thiamine, riboflavin and vitamin A (John, 1991). It is an important summer pulse crop of many South Asian countries including Pakistan, India, Bangladesh, Thailand and Korea. The climate of Pakistan that falls under the arid and semi-arid zones is very suitable for mung bean cultivation. However, the areas under its

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cultivation in different parts of Pakistan are prone to extensive metal contamination through irrigation water receiving industrial effluents highly contaminated with metals. So the aim of this study was to investigate the effect of copper and lead on ion uptake (K^+, Ca^{2+}, Cu^{2+} and Pb^{2+}) by two mung bean [*Vigna radiata* (L.) Wilczek] cultivars. To get an insight regarding the feasibility of cultivation of mung bean in areas irrigated with contaminated water was also an objective of this study.

MATERIALS AND METHODS

This experiment was conducted to evaluate the effect of copper (Cu) and lead (Pb) on K^+, Ca^{2+}, Cu^{2+} and Pb^{2+} uptake by the root, leaves, and seeds of two mung bean [*Vigna radiata* (L.) Wiczek] cultivars i-e Mung-1 (V_1) and Mung-6 (V_2). The experiment was carried out in earthen pots lined with polyethylene bags, during spring season 2003. Pots were filled in with 5 kg sand and placed in a completely randomized design with five replicates under natural conditions in a net house in the Botanical Garden, University of Agriculture, Faisalabad, Pakistan.

Seeds were surface sterilized with 10% v/v hydrogen peroxide before sowing and sand was moisturized with distilled water. Initially eight seeds were sown in each pot and after complete germination only five seedlings uniform in size were maintained in each pot by thinning. Modified Hoagland nutrient solution ($1/8^{th}$ strength) (Hoagland and Arnon 1950) was applied at ten days interval and plants were watered with distilled water whenever felt it necessary.

Thirty days after germination plants were treated with soluble chloride salts of both copper @ 25 (T_1) and 50 (T_2) mg L^{-1} and lead @ 25 (T_3) and 50 (T_4) mg L^{-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 of both metals based on their content detected in sewage water being drained into irrigation water (UNIDO 2000). They are comparable to cited studies (Xiong 1998) as well.

For the determination of nutrient (K^+ and Ca^{2+}) and metal (Cu^{2+} and Pb^{2+}) content in the roots and leaves, keeping in view the period of maximum metal uptake we harvested black gram plants ten days after treatment application, a period of maximum metal uptake following Qu et al. (2003) and Bibi and Hussain (2005). During this period, the sand moisture measured gravimetrically after oven drying at the start of experiment, was kept constant by reweighing the pots daily and watering them up to field capacity. Nevertheless, the nutrient and metal contents of seeds were determined at the maturity of the crop.

Plant material was ground in a grinder, and was digested with H_2SO_4 and H_2O_2 following the method of (Wolf, 1982). Ca^{2+} and K^+ cat-ions were determined using a flame photometer (Jenway, PFP-7) whilst Cu^{2+} and Pb^{2+} were determined with atomic absorption spectrophotometer (Hitachi AAS-Z-8200 with polarized

Zemaan effect) as described by Bibi and Hussain (2005). To ensure the quality of the analysis, a certified reference material, Sea Lettuce (*Ulva lactuca*) BCR/CRM 279 (EU Community Bureau of Reference) along with procedural blanks was analyzed after every five samples. The percentage recovery of certified reference material remained 91% and 97% for Cu^{2+} and Pb^{2+} respectively. The data collected were analyzed statistically using analysis of variance (Steel and Torrie, 1986) while for pairwise comparisons Tukey's test was applied using SYSTAT statistical package (Walkinson, 1990).

RESULTS AND DISCUSSION

Plant nutrient uptake and their translocation to different plant organs are detrimentally influenced by abiotic stresses i. e. salinity, drought, water logging, hypoxia, and metal toxicity. During this study the uptake of potassium (K^+) by mung bean plants treated with lead (Pb^{2+}) and copper (Cu^{2+}) showed highly significant ($P<0.01$) differences among treatments means, between mung bean cultivars and for variety x treatment interaction (Table 1). All the three plant organs i. e. leaves, seeds and roots in both mung bean cultivars showed a similar trend for K^+ uptake. As compared to control, application of copper @ 25 and 50 mg kg^{-1} caused 10% and 13 % reduction in K^+ uptake by leaves in Mung-1 and 16% and 22% reduction in that relating to Mung-6 respectively. The same doses (25 and 50 mg kg^{-1}) of lead caused 19% and 39% reduction in K^+ uptake in Mung-1 leaves and 28% and 38% respectively in Mung-1 leaves.

The K^+ content in the seeds of Mung-1 was significantly reduced by both lead as well as copper treatments. However, they did not differ significantly among themselves. In Mung-6 as well, both lead treatments and higher dose of copper (T_2) significantly reduced K^+ uptake as compared to control and varied non significantly among themselves. Nevertheless, lower dose of copper (T_1) also significantly reduced K^+ content but it war significantly ($P<0.05$) higher than both lead treatments.

Both mung bean cultivars showed similar trend for the uptake of K^+ by their roots. However, higher concentration of lead (50 mg L^{-1}) only caused significant ($P<0.05$) reduction in K^+ uptake as compared to control while both copper treatments and lower concentration of lead neither varied significantly ($P<0.05$) with control nor among themselves.

The Ca^{2+} uptake by roots, leaves and seeds of both mung bean cultivars was significantly ($P<0.05$) influenced by metal treatments (Table 2). The Ca^{2+} content in the leaves of Mung-1 plants treated with both concentrations of lead and higher concentration of copper (50 mg kg^{-1}) was significantly less than control plants but lower concentration of copper (25 mg kg^{-1}) did not reduce it to a significant level. In contrast all the four metal treatments significantly reduced Ca^{2+} content in the leaves of Mung-6. However, the reduction in Ca^{2+} content caused by higher concentrations of both metals (T_2 & T_4) did not vary significantly from those resulting from their lower concentrations (T_1 & T_3).

The Ca^{2+} content in the seeds of both black gram cultivars was also significantly ($P<0.05$) influenced by both metals as compared to control. Both lead treatments (T_3 & T_4) showed comparatively more drastic effects on Ca^{2+} content in the seeds of both mung bean cultivars, as compared to resembling copper treatments. However, they varied non- significantly ($P>0.05$) with one another and with the higher concentration of copper (T_2). The Ca^{2+} content in the seeds of both mung bean plants was significantly ($P<0.05$) reduced by the lower concentration of copper as well, but it remained significantly ($P<0.05$) higher than remaining metal treatments.

The Ca^{2+} content in the roots of both mung bean plants showed almost the same trend as that recorded in case of Ca^{2+} content pertaining to leaves and roots. Despite both metals caused significant ($P<0.05$) reduction in Ca^{2+} uptake by roots, their higher concentrations reduced it more drastically than their lower concentrations and lead treatments proved more toxic than resembling copper treatments. These results are supported by some previous studies (Larbi, et al., 2002). Cook et al. (1997) also observed reduction in K^+ and Ca^{2+}, uptake in *Phaseolus* plants treated with copper and attributed it to the toxic effects of copper. Our results are consistent with the findings of Ouzounidou (1994) as well. They investigated photosynthetic functioning of *Alyssum montanum* L. plant and noted that high copper content in plant tissue negatively affected the uptake and translocation of K^+ and Ca^{2+} ion in plant.

Plant species greatly vary in their nutrient uptake potential under different abiotic stresses including that resulting from metal toxicity. Those well adapted to stress conditions usually show slight reduction in their nutrient ions uptake particularly K^+ and Ca^{2+} as compared to sensitive species under stress conditions. During this study copper and lead treated mung bean plants showed a considerable reduction in the K^+ and Ca^{2+}, uptake by their roots, leaves and seeds and Mung-1 proved more sensitive than Mung-6. A comparison among the mung bean cultivars indicates that Ca^{2+} uptake by all the three plant organs (leaves, seeds and roots) in Mung-6 was comparatively more strongly influenced than that recorded for Mung-1. Overall Ca^{2+} uptake by both mung bean cultivars followed almost the same pattern (leaves>roots>seeds) as that recorded in the case of K^+.

The uptake of Cu^{2+} is a controversial issue for plant scientists Hall (2002). In spite of being a plant micronutrient, does not show high translocation from the roots to different plant organs. However, some studies indicate its considerable accumulation and translocation to different plant organs (Liao, et al., 1999).

Data regarding the copper (Cu^{2+}) contents in the leaves, seeds and roots of two mung bean cultivars treated with copper and lead treatments is given in Table 3. Its statistical analysis indicated highly significant ($P<0.01$) differences among treatment means, variety means as well as for variety x treatment interaction. In both mung bean cultivars, plants receiving copper treatments showed very high Cu^{2+} contents in their stems, seeds and roots. Nevertheless, in lead treated plants,

Table 1. Effect of copper and lead application on K^+ uptake (mg kg^{-1}) by the leaves, roots and seeds of two mung bean cultivars viz. Mung-1 and Mung-6 ($P<0.05$).

Plant organs	Varieties	Treatments					Variety Means
		Control	Copper (mg kg^{-1})		Lead (mg kg^{-1})		
		(T_0)	25 (T_1)	50 (T_2)	25 (T_3)	50 (T_4)	
Leaf	Mung-1	19.00±1.12 a	17.20±1.18 ab	16.50±0.94 bc	15.40±0.96 cd	11.58±0.68 e	15.94±1.71 b
	Mung-6	24.20±1.15 a	20.14±0.86 b	18.90±0.55 c	17.50±1.12 cd	15.08±0.71 e	19.16±1.21 a
	Tr. Means	21.60±1.96 a	18.67±1.93 b	17.70±1.46 b	16.45±1.55 b	13.33±1.91 c	
Seed	Mung-1	12.54±1.03 a	9.73±0.80 a	9.12±0.65 a	7.51±0.84 a	7.55±1.48 a	9.29±1.18 b
	Mung-6	16.83±1.26 a	13.06±1.27 b	10.44±1.03 bc	9.10±1.09 c	8.57±0.98 c	11.60±1.40 a
	Tr. Means	14.68±1.98 a	11.40±1.18 b	9.78±1.07 bc	8.30±1.24 c	8.06±1.30 c	
Root	Mung-1	12.30±1.14 a	12.81±1.08 a	9.80±0.57 bc	10.40±1.15 ab	7.80±1.14 c	10.62±1.33 b
	Mung-6	15.70±1.11 a	12.95±1.23 a	11.75±0.35 ab	9.47±1.03 bc	8.70± 0.54 bc	11.71±1.97 a
	Tr. Means	14.00±1.95 a	12.88±1.52 a	10.77±1.12 b	9.94±1.55 bc	8.25±1.25 c	

Mean values sharing the same letter differ non-significantly. Treatment means and variety x treatment interaction means have been grouped horizontally while variety means have vertical grouping.

Table 2. Effect of copper and lead application on Ca^{2+} uptake (mg kg^{-1}) by the leaves, roots and seeds of two black gram cultivars viz. Mung-1 and Mung-6 ($P<0.05$).

Plant organs	Varieties	Treatments					Variety Means
		Control	Copper (mg kg^{-1})		Lead (mg kg^{-1})		
		(T_0)	25 (T_1)	50 (T_2)	25 (T_3)	50 (T_4)	
Leaf	Mung-1	17.00±1.00 a	13.40±1.14 ab	8.80±1.28 de	10.06±0.90 bc	9.13±1.19 cd	11.68±3.78 b
	Mung-6	19.75±1.14 a	13.60±1.22 bc	12.26±1.24 cd	10.20±1.10 cde	8.10±1.02 e	12.78±4.18 a
	Tr. Means	18.38±1.11 a	13.50±1.46 b	10.53±1.51 bc	10.13±0.95 c	6.62±1.52 c	
Seed	Mung-1	3.97±0.24 a	3.24±0.40 b	2.50±0.30 c	1.72±0.19 cd	1.16±0.07 d	2.52±1.06 b
	Mung-6	4.69±0.53 a	3.28±0.53 b	2.27±0.25 c	2.11±0.31 cd	1.46±0.34 d	2.76±1.21 a
	Tr. Means	4.33±0.54 a	3.26±0.44 b	2.39±0.29 c	1.92±0.32 d	1.31±0.28 e	
Root	Mung-1	7.04±0.30 a	6.31±0.53 ab	4.01±0.21 bc	2.78±0.25 c	1.95±0.28 d	4.62±2.03 b
	Mung-6	7.91±1.21 a	5.93±0.35 b	5.07±0.72 b	2.91±0.20 c	2.05±0.19 c	4.77±2.23 a
	Tr. Means	4.47±0.95 a	6.12±0.47 b	4.54±0.75 c	2.84±0.23 d	2.00±0.23 e	

Mean values sharing the same letter differ non-significantly. Treatment means and variety x treatment interaction means have been grouped horizontally while variety means have vertical grouping.

Table 3. Effect of copper and lead application on Cu^{2+} uptake (mg kg^{-1}) by the leaves, roots and seeds of two mung bean cultivars viz. Mung-1 and Mung-6 (P<0.05).

Plant organs	Varieties	Treatments					Variety Mean
		Control (T_0)	25 mg Cu kg^{-1} (T_1)	50 mg Cu kg^{-1} (T_2)	25 mg Pb kg^{-1} (T_3)	50 mg Pb kg^{-1} (T_4)	
Leaf	Mung-1	1.48±0.14 c	9.42±0.76 b	11.10±0.51 a	1.11±0.10 c	0.86±0.08 c	4.79± a
	Mung-6	1.24±0.16 c	8.28±0.53 a	9.20±0.43 a	0.84±0.05c	0.71± 0.11c	4.05± b
	Tr. Means	1.36±0.11 c	8.85±0.85 b	10.15±0.71 a	0.97±0.11 c	0.78±0.15c	
Seed	Mung-1	0.02±0.01 c	0.38±0.07 b	1.01±0.12 a	0.01±0.00 c	0.01±0.00 c	0.29± a
	Mung-6	0.01±0.00 c	0.30±0.02 b	0.62±0.07 a	0.01±0.00 c	0.01±0.00 c	0.19± b
	Tr. Means	0.02±0.01 c	0.35±0.09 b	0.82±0.11 a	0.01±0.00 d	0.01±0.00 d	
Root	Mung-1	1.02±0.16 c	3.46±0.22 b	4.91±0.14 a	0.98±0.09 c	1.00±0.16 c	2.27± a
	Mung-6	0.95±0.15 c	2.21±0.18 b	3.75±0.15 a	0.98±0.10 c	0.98±0.09 c	1.77± b
	Tr. Means	0.99±0.19 c	2.84±0.25 b	4.33±0.17 a	0.98±0.11 c	0.99±0.15 c	

Mean values sharing the same letter differ non-significantly. Treatment means and variety x treatment interaction means have been grouped horizontally while variety means have vertical grouping.

Table 4. Effect of copper and lead application on Pb^{2+} uptake (mg kg^{-1}) by the leaves, roots and seeds of two mung bean cultivars viz. Mung-1 and Mung-6 (P<0.05).

Plant organs	Varieties	Treatments					Variety Means
		Control (T_0)	25 mg Cu kg^{-1} (T_1)	50 mg Cu kg^{-1} (T_2)	25 mg Pb kg^{-1} (T_3)	50 mg Pb kg^{-1} (T_4)	
Leaf	Mung-1	0.001±0.00 d	0.002±0.00 d	0.003±0.00 d	2.630±0.12 b	3.348±0.27 a	1.20± a
	Mung-6	0.002±0.00 d	0.003±0.00 d	0.003±0.00 d	2.206±0.11 c	3.570±0.29 a	1.16± b
	Tr. Means	0.002±0.00 c	0.002±0.00 c	0.003±0.00 c	2.418±0.14 b	3.459±0.30 a	
Seed	Mung-1	0.002±0.00	0.003±0.00	0.003±0.00	0.576±0.08	0.998±0.16	0.32± a
	Mung-6	0.001±0.00	0.002±0.00	0.002±0.00	0.463±0.05	0.960±0.03	0.26± a
	Tr. Means	0.002±0.00 b	0.002±0.00 b	0.003±0.00 b	0.463± 0.07 b	0.979±0.09 a	
Root	Mung-1	0.002±0.00 c	0.003±0.00 c	0.003±0.00 c	1.000±0.15 b	1.686±0.19 a	0.54± a
	Mung-6	0.002±0.00 c	0.004±0.00 c	0.005±0.00 c	0.814±0.08 b	1.050±0.09 b	0.38± b
	Tr. Means	0.002±0.00 c	0.003±0.00 c	0.004±0.00 c	0.907±0.21 b	1.368± 0.15a	

Mean values sharing the same letter differ non-significantly. Treatment means and variety x treatment interaction means have been grouped horizontally while variety means have vertical grouping.

Cu^{2+} contents of all the three plant parts was almost at par with or varied very slightly from control. Cook et al. (1997) treated *Phaseolus* plants with copper and observed a positive correlation between its external concentrations and those accumulated by roots, leaves and stems. In this study as well, we observed sequestering of a high amount of Cu^{2+} in the roots rather than in leaves and seeds that simultaneously increased with the increase in its external concentration.

The uptake and translocation of Pb^{2+} also, still remains an unsolved issue (Yang et al., 2000). To promote Pb^{2+} uptake from contaminated soil, some chelators are being investigated (Vassil et al., 1998) keeping in view the risk of getting it leached to ground water. Nevertheless, Huang et al. (1996) examined lead transport in corn, *Brassica*, ragweed, wheat and in some *Thalaspi* species. They observed significant differences among different plant species in their Pb^{2+} uptake and translocation, as examined during this study. We examined that the uptake of Pb^{2+} by the leaves, and roots of mung bean plants showed significant ($P<0.05$) differences among treatments, varieties and for variety x treatment interaction (Table 4). A comparison among the treatment means indicated significantly higher Pb^{2+} content in the leaves and roots of both mung bean cultivars receiving both lead treatments (25 & 50 mg Pb^{2+} kg^{-1}) as compared with control (T_0), and both copper treatments. Both copper treatments (25 & 50 mg Cu^{2+} kg^{-1}) very slightly influenced the Pb^{2+} content in both mung bean cultivars. However, lead treated plants showed maximum Pb^{2+} content in their roots as compared to that in their leaves and seeds, in both mung bean cultivars, and it increased simultaneously with the increase in its external concentration. Our results have support from Sing et al. (1997) as well. They also examined the response of higher plants to lead contamination and noted that plant absorbed lead and accumulated it in stem, roots and seeds, which gradually increased with the increase in its exogenous level.

In conclusion, the uptake of nutrient ions, as well as metal ions by the leaves, roots and seeds of two mung bean cultivars seems concentration dependent. The presence of high K^+ and Ca^{2+} in the leaves, roots and seeds of Mung-1 as compared to those in Mung-6 may be regarded an important adaptive character. We found relatively more Cu and Pb^{2+} in roots followed by leaves but a low concentration of both metals in the seeds of both mung bean cultivars that may be regarded a time dependent phenomenon. It seems that when the crop had reached the maturity stage, metals might have been sequestered in the roots and leaves.

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