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GROWTH, TISSUE CONCENTRATION AND BIOACCUMULATION OF CADMIUM BY DIFFERENT MUNGBEAN CULTIVARS IN A HYDROPONIC STUDY

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The necessity of artificial irrigation due to arid climate of Pakistan and the shortage of canal water force the use of raw industrial effluents to grow crops, especially around big cities. The sewage contains heavy metal ions, including cadmium. The cultivars of the same crop do differ in their ability to absorb and translocate Cd. Four varieties of mungbean were investigated for their responses to Cd concentration, translocation from roots to shoots and bioaccumulation. The varieties NM-28, NM-54, NM-92 and NM-98 were tested in hydroponics with Cd application rates of 0.04, 0.08, 0.13, 0.17, 0.22 mM. For root dry weight varieties ranked in the order as NM-98 > NM-54 > NM-92 > NM-28, for shoot dry weight the order was NM-54 > NM-98 > NM-28 ~ NM-92. The NM-28 had the highest root Cd concentration followed by NM-92, NM-54 and NM-98. For shoot Cd concentration the varieties ranked as NM-28 > NM-54 > NM-92 > NM-98. All varieties retained most of the absorbed Cd in their roots and also these all have the ability to accumulate Cd in their roots and shoots. For root and shoot Cd bioaccumulation factor, the varieties were found to be in the decreasing order as NM-28 > NM-92 > NM-54 > NM-98. From the results, NM-98 was found to be a good source for future mungbean breeding programs with the aim to produce low Cd accumulating varieties. It may also be recommended when mandatory to cultivate mungbean on slightly Cd contaminated areas while NM-28 seemed better to avoid under such conditions.
Keywords: Cadmium, mungbean, tissue concentration, translocation factor, municipal sewage, peri-urban agriculture.

INTRODUCTION

The shortage of fresh water persuades the use of raw municipal waste effluent, consisting of domestic refuse and industrial effluents, to irrigate crops in urban and peri-urban areas around big towns in Pakistan and other water scarce developing countries (Ghafoor *et al*., 2004). Agriculture in urban and peri-urban areas of big cities like Karachi, Lahore, Faisalabad, Gujranwala, Multan, Rawalpindi, Peshawar and Hyderabad is dependent upon raw sewage irrigation. Easy access, regular supply and nutrient value of raw sewage attract its use, despite it contains heavy metals (Cd, Ni, Zn etc.), soluble salts, pathogens, suspended particles (Ghafoor, 2002) and other organic toxins. Because of continuous irrigation with these effluents, threat of heavy metals toxicity to agriculture is quite prominent (Ghafoor *et al*., 2004). Contamination of soils with toxic metals could become important not only regarding yield and quality of crops, but also due to adverse effects on quality of atmospheric and aquatic environment, and health of human beings upon their entry into food chain (Chen *et al*., 2000).

Among metals present in raw effluents, cadmium (Cd) is considered as the most important pollutant, being able to be easily absorbed by and translocated into different plant parts (Gratao *et al*., 2005) where it can accumulate to high levels (Yu *et al*., 2006) probably due to bioconcentration and genetic architecture of plants. It is found that Cd may pose risk to human and animal health at plant tissue concentration that is not phytotoxic (Jackson and Alloway, 1992). Therefore, Cd is one of the most important metals to consider in terms of food chain contamination.

Mungbean (*Vigna radiata* L.) is an important pulse crop of summer season in Pakistan owing to be source of human diet, feed and fodder for animals. It contains 22.2% proteins, 60% carbohydrates and a fair amount of vitamins A and B as well as minerals like Ca, P and K (Saleemi, 1998). Identified as a high yielding crop in many Asian countries, mungbean sensitivity to potential toxicants like Cd remains to be comprehensively assessed (Bindhu and Bera, 2001).

Genetic differences among plant types for Cd absorption and accumulation have been observed (Liu *et al*., 2007). Since cultivars of the same crop species differ widely in their response to heavy metal stress (Yu *et al*. 2006), the most appropriate one may be selected for such conditions (Liu *et al*., 2003, 2007; Yu *et al*., 2006). Thus, using cultivars that have low metal accumulation potential could provide an option for farmers to cope with the risk, and to reduce the influx of pollutants into human food chain. In this regard, a solution culture study was conducted with the basic objective to investigate simultaneously regarding mungbean growth response, varietal differences for Cd concentration, bioaccumulation and translocation into above ground harvestable parts.

MATERIALS AND METHODS

The present study was conducted in the wire house, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Four varieties of mungbean including NM-28, NM-54, NM-92 and NM-98 were used. The seeds were grown in polythene lined trays containing acid pre-washed sand using distilled water. The experiment was laid out in a completely randomized design each with four replications. Two-week old seedlings of uniform size were transplanted 2 per hole into tubs containing foam-plugged holes in thermo pore sheets floating on continuously aerated half strength Johnson's solution (Johnson *et al*., 1957). Aeration was also supplied through aquarium pumps. The Cd stress was imposed 3 weeks (21 days) after transplanting mungbean nursery. Cadmium concentrations (i.e., 0, 0.04, 0.08, 0.13, 0.17, 0.22 mM) using $CdCl_2$. 2.5 H_2O salt were established in growth medium. The pH of solution in all the tubs was maintained daily at 6.0 ± 0.5 with HCl or NaOH. The mungbean plants were harvested 21 days after Cd treatments application. Plant samples were washed with tap water followed by washing with distilled water. To compare the response of mungbean cultivars against Cd stress, root and shoot dry weights, root and shoot-Cd concentrations, translocation factor and bioaccumulation of Cd in roots and shoot were determined. Samples were oven-dried to constant weight at 65°C in an oven. Then, root and shoot dry matter were recorded. Plant samples (roots and shoots) were analysed for Cd concentration following standard procedures (AOAC, 1990). First, samples (roots and shoots) were ground in a Wiley mill fitted with stainless steel blades (MF 10 IKA, Werke, Germany) and stored for further analysis. Then, the di-acid (2:1 mixture of nitric acid to perchloric acid) at 150°C (Miller, 1998) was used for extraction and Cd concentration in plant roots and shoots (1-g portion) with the help of flame atomic absorption spectrophotometer (FAAS; Model Thermo S-Series, Thermo Electron Corporation, Cambridge, UK) having Cd lamp in place.

The consistency of the digestion and analytical technique was also confirmed by including blanks with every lot of sample digest. Reagent blanks and at least three replicates of all samples were used to guarantee perfection and correctness in the analysis. The five calibration standards were primed using the standard solution, which was accredited by the supplier. All the instrumental conditions for FAAS were adjusted for the maximum sensitivity as specified by manufacture's manual.

Translocation factor for Cd in plants was determined by using the ratio of shoot-Cd concentration to root-Cd concentration (Majid *et al*., 2012). The bioaccumulation factor for Cd in roots and shoots was determined by the ratio of Cd concentration in part of plant (roots or shoot) to Cd concentration in the solution (Hadi and Bano, 2010).

The data regarding metal accumulation patterns of cultivars were analyzed following Analysis of Variance technique and Least Significant Difference (LSD) test was applied (Steel *et al*., 1997) to differentiate the varieties according to their relative concentration and translocation of Cd, using M-STATC version 1.10 computer software package.

RESULTS

***Root dry weight*:** In present study, among all tested mungbean varieties, NM-54 had statistically the highest root dry weight under control conditions. The root dry weight of all varieties decreased gradually with increasing application rates of Cd. At the highest rate of applied Cd (i.e., 0.22 mM), the root growth of NM-24 was highly affected and had the lowest root dry weight. Whereas, cultivar NM-98 had the highest root dry weight which was statistically at par with NM-54. Thus, NM-98 consistently showed better performance at all Cd application rates as compared to the other varieties (Table 1).

***Shoot dry weight*:** Under control conditions, the highest shoot dry weight was observed for NM-54, while all other varieties produced statistically similar shoot dry weight. With increasing application rate of Cd, the shoot dry weight of all varieties decreased gradually. At the highest application rate of Cd (i.e., 0.22 mM), NM-98 produced the

Table 1. Dry weights (g) of mungbean roots as affected by applied Cd.

Cd (mM)	Varieties				Mean	Decrease over control (%)
	NM-28	NM-54	NM-92	NM-98		
0	0.606 cd	1.059 a	0.753 b	0.662 c	0.770 a	-----
0.04	0.575 d	0.407 fg	0.652 c	0.575 d	0.552 b	28.31
0.08	0.314 hij	0.402 fg	0.420 fg	0.485 e	0.405 c	47.40
0.13	0.286 ij	0.323 hi	0.329 hi	0.442 ef	0.345 d	55.19
0.17	0.231 k	0.315 hij	0.260 jk	0.366 gh	0.293 e	61.95
0.22	0.156 l	0.304 ij	0.229 k	0.329 hi	0.254 f	67.01
Mean	0.361 c	0.468 a	0.440 b	0.476 a		

LSD: Treatment (T) = 0.026, Variety (V) = 0.021, T × V = 0.052

Table 2. Dry weights (g) of mungbean shoots as affected by applied Cd.

Cd (mM)	Varieties				Mean	Decrease over control (%)
	NM-28	NM-54	NM-92	NM-98		
0	3.30 bc	6.66 a	3.26 bc	3.53 b	4.19 a	-----
0.04	2.76 d	2.63 de	2.86 cd	3.30 bc	2.89 b	31.01
0.08	2.33 ef	2.30 efg	1.83 hij	2.20 efgh	2.16 c	48.31
0.13	2.17 fgh	2.00 fghi	1.76 hij	1.86 ghij	1.95 d	53.46
0.17	1.60 ij	1.83 hij	1.67 ij	1.76 hij	1.71 e	59.02
0.22	1.100 k	1.40 jk	1.50 jk	1.70 ij	1.42 f	66.01
Mean	2.21 c	2.80 a	2.15 c	2.39 b	-----	-----

LSD: Treatment (T) = 0.30, Variety (V) = 0.16, LSD: T × V = 0.40

Table 3. Root Cd (mg kg-1) in mungbean as affected by applied Cd.

Cd (mM)	Varieties				Mean	Percent increase over control (000)
	NM-28	NM-54	NM-92	NM-98		
0	5.8 l	5.5 l	4.6 l	4.3 l	5.1 f	-----
0.04	992.4 j	901.0 jk	851.4 jk	799.0 k	886.0 e	17.27
0.08	1493.0 gh	1401.0 hi	1381.0 hi	1302.0 i	1394.0 d	27.23
0.13	1660.0 fg	1793.0 f	2123.0 e	1711.0 f	1821.0 c	35.60
0.17	3051.0 b	2420.0 d	2658.0 c	2463.0 d	2648.0 a	51.81
0.22	4213.0 a	2151.0 e	2181.0 e	1659.0fg	2551.0 b	49.91
Mean	1903.0 a	1445.0 c	1533.0 b	1323.0 d	-----	-----

LSD: Treatment (T) = 83.9, Variety (V) = 68.5, T × V = 168.0

Table 4. Shoot Cd (mg kg-1) in mungbean as affected by applied Cd.

Cd (mM)	Varieties				Mean	Percent increase over control (000)
	NM-28	NM-54	NM-92	NM-98		
0	2.0 i	1.9 i	1.7 i	1.9 i	1.9 f	-----
0.04	24.0 h	20.0 h	21.8 h	20.0 h	21.4 e	1.02
0.08	33.3 g	44.5 f	56.1 e	31.7 g	41.4 d	2.06
0.13	44.9 f	52.1 e	69.3 d	56.7 e	55.7 c	2.81
0.17	160.7 a	109.0 b	92.3 c	56.7 e	104.7 a	5.36
0.22	114.3 b	70.3 d	31.2 g	21.0 h	59.2 b	2.99
Mean	63.2 a	49.7 b	45.4 c	31.3 d		

LSD: treatment (T) = 3.0, Variety (V) = 2.4, T × V = 6.0

highest shoot dry weight which was statistically at par with NM 92 and NM- 54 (Table 2). Similar trends in results were also observed at Cd applied levels of 0.08, 0.13 or 0.17 mM.

***Root-Cd*:** The concentration of Cd in mungbean roots was very low in all varieties under control conditions. However, a significant increase was found in root Cd of all varieties with increasing Cd application rates. At the highest Cd application rate (i.e., 0.22 mM) root Cd was the highest in NM-28, while NM 98 had significantly the lowest root Cd (Table 3).

***Shoot-Cd*:** The shoot Cd concentration in tested mungbean varieties was very low in control treatment and shoot Cd increased gradually with increasing Cd application rates. The shoot Cd was the lowest in NM-98 at highest Cd application rate (i.e., 0.22 mM). However, NM-28 had the highest shoot Cd at this treatment followed by NM-54 and NM-92 (Table 4).

***Translocation factor for Cd*:** In mungbean, Cd application rates had significant effects on Cd translocation factor in mungbean, whereas mungbean varieties and the interaction between Cd levels and varieties (T × V) remained non-significant. The maximum translocation factor was recorded with the control treatment, the translocation factor decreased gradually with increasing Cd application rate and minimum translocation factor was found at the highest Cd application rate. For all the mungbean varieties, the translocation ratio was <1 (Table 5).

***Root bioaccumulation of Cd*:** No Cd accumulation was recorded for control plants. At Cd application rate of 5 mg L^{-1}, the maximum Cd accumulation was found in NM-28 and it was significantly higher than other varieties. With increasing Cd application rates, the root Cd accumulation decreased in all varieties except NM-28 in which it was increased. At the highest Cd application rate (i.e., 0.22 mM), NM-98 had significantly the lowest root Cd accumulation while NM-28 had the highest root Cd accumulation. All varieties had root Cd bioaccumulation ratio >1 (Table 6).

Table 5. Translocation factor of Cd in mungbean cultivars as affected by applied Cd.

Cd (mM)	Varieties				Mean
	NM-28	NM-54	NM-92	NM-98	
0	0.355	0.351	0.371	0.446	0.381 a
0.04	0.024	0.022	0.026	0.025	0.024 b
0.08	0.022	0.032	0.041	0.024	0.029 b
0.13	0.027	0.029	0.033	0.033	0.030 b
0.17	0.053	0.045	0.035	0.023	0.038 b
0.22	0.027	0.033	0.014	0.013	0.021 b
Mean	0.085	0.085	0.087	0.094	

LSD: Treatment (T) = 0.037, Variety (V) = ns, T × V = ns

Table 6. Bioaccumulation factor of Cd in mungbean roots as affected by applied Cd.

Cd (mM)	Varieties				Mean
	NM-28	NM-54	NM-92	NM-98	
0	0.0m	0.0m	0.0m	0.0m	0.00f
0.04	198.6a	180.3b	170.3bc	159.8cd	177.2a
0.08	149.3de	140.1ef	138.1f	130.2fgh	139.4b
0.13	110.7j	119.5hij	141.4ef	113.0ij	121.1d
0.17	152.6d	121.0hij	132.9fg	123.2ghi	132.4c
0.22	168.5c	86.0k	87.2k	66.3l	102.0e
Means	129.9a	107.8b	111.7b	98.7c	

LSD: Treatment = 5.0, Variety = 4.1, T×V= 10.1

Table 7. Bioaccumulation factor of Cd in mungbean shoots as affected by applied Cd.

Cd (mM)	Varieties				Mean
	NM-28	NM-54	NM-92	NM-98	
0	0.00l	0.00l	0.00l	0.00l	0.00e
0.04	4.80c	4.01e	4.37d	4.00e	4.29b
0.08	3.33gh	4.46cd	5.62b	3.17ghi	4.14b
0.13	2.99hi	3.47fg	4.64cd	3.78ef	3.72c
0.17	8.03a	5.46b	4.62cd	2.83i	5.23a
0.22	4.57cd	2.81i	1.24j	0.84k	2.36d
Mean	3.95a	3.37b	3.41b	2.44c	

LSD: Treatment = 0.18, Variety (V) = 0.14, T × V = 0.36

***Shoot bioaccumulation of Cd*:** There was no accumulation in the control plants but a gradual increase was observed. The highest shoot Cd accumulation was recorded for NM-28 at 0.17 mM Cd in the growth medium. The shoot Cd accumulation initially increased in all varieties with increasing Cd application rate but then decreased at the highest Cd application rate. This pattern of Cd accumulation corresponds to dry matter yield. Varieties had significant differences ($P \leq 0.05$) for their shoot Cd accumulation at all the Cd application rates but the shoot Cd accumulation was >1 (Table 7).

DISCUSSION

Today, there is an increasing concern regarding how to produce safe agricultural products in heavy metal contaminated soils. For this purpose, effective evaluation and identification of low Cd accumulating crop species and/or genotypes in edible parts are a pre-requisite for successful development of safer food production (Zeng *et al.*, 2008). Thus, the assessment of the crop genotypes within the same genus for heavy metal tolerance is an important tool in defining whether growth, physiological traits, bioaccumulation and translocation responses are related to heavy metal tolerance (Niaz *et al.*, 2010; Zhivotovsky *et al.*, 2011). In present solution culture study, growth response, tissue concentration, bioaccumulation and translocation of Cd by different mungbean cultivars were investigated.

Growth reduction of roots has been one of the most sensitive parameters to be measured while using mungbean seedlings as bio-indicators against Cd stress (Wahid and Ghani, 2008). The Cd could reduce pigment synthesis above an endogenous concentration of 5 mg kg^{-1} dry weight, increase the stigma sterol to sitosterol ratio in roots and could induce a redistribution of sugars among plant parts. Putrescine increased in mungbean roots above an endogenous Cd concentration of 27 mg kg^{-1} dry weight and this accumulation of Putrescine in the roots occurred before any growth reduction could be seen (Geuns *et al*., 1997).

Root sensitivity to Cd stress in mungbean has also been reported by Athar and Ahmad (2002), Azmat *et al*. (2005) and Simonova *et al*. (2007). Gul *et al*. (2007) studied the variability among 16 mungbean genotypes for yield and yield components. It was reported that NM-54 acquired more root weight under uncontaminated conditions than that of NM-28, NM-92 and NM-98. The results are also in line to those of Wahid *et al*. (2008) where NM-54 recorded maximum root dry weight for control while NM-98 exhibited minimum root dry weight due to Cd accumulation.

A reaction of Cd with constituent biosynthetic enzymes is reported to induce premature senescence and lower pigment content (Geuns *et al*., 1997) and hence resultantly lower shoot weight in mungbean. Gul *et al*. (2007) reported that NM-54 exhibited more shoot weight under uncontaminated conditions than that of NM-28, NM-92 and NM-98 among the 16 tested cultivars. Similarly, Wahid *et al*. (2008) also reported that NM-98 and NM-92 produced higher shoot dry weight compared to those of some common mungbean cultivars.

The results indicate that mungbean cultivars with better root weight absorbed comparatively less Cd from the solution. In other words, good root growth in mungbean was due to less Cd absorption from the growth medium. This is opposite to what the other authors noticed in case of rice roots (Niaz *et al*., 2010) when Cd tolerant rice varieties owing to higher root activity originally absorbed more Cd into roots. Later the tolerance character resulted in translocation and accumulation of less Cd in the above ground (edible) parts. Results are consistent to those of Athar and Ahmad (2002), Azmat *et al*. (2005), Kumar and Dingra (2005) and Simonova *et al*. (2007) who reported that Cd sensitive mungbean cultivars absorbed more Cd in their roots than the tolerant varieties.

By comparing root- and shoot-Cd in mungbean, it is evident that very low Cd is recorded in shoots compared to that in roots. Simonova *et al*. (2007) suggested that a barrier for Cd transport in mungbean might exist between the roots and the hypocotyls; therefore, a very small amount of Cd is usually transported into shoots leading ultimately more concentration in roots. Geuns *et al*. (1997) also reported that most of the Cd uptake by mungbean plants was retained in the roots.

Translocation factor of Cd was low for all the Cd application rates which seems associated with comparatively low shoot Cd concentration than that in roots and also for all the mungbean varieties, the translocation ratio was <1 (Table 5) which, according to Liu *et al*. (2007), indicates that most of the absorbed Cd was retained in roots and less was translocated to shoots. Higher translocation factor for the control conditions could be explained in that the plants were very good with full leaf area expansion, efficient photosynthetic apparatus, heavy Cd load in both xylem and phloem conducting tissues caused Cd to move actively from roots to shoots (Geuns *et al*., 1997). This is consistent with the findings of Kabata-Pendias and Pendias (2001) who concluded that the transport of Cd in plants was directly and positively related with their metabolic activity.

Likewise, in the lights of recommendations from Liu *et al*. (2007), root bioaccumulation ratio being >1 indicates that all the test varieties not only absorbed but also accumulated Cd in their roots. Similarly, >1 Cd bioaccumulation factor in shoots of all the varieties indicates that they have the ability to accumulate Cd in their shoots preferably. The present results are in consonance with those of Geuns *et al*. (1997), Rout *et al*. (2000), Kumar and Dhingra (2005), Simonova *et al*. (2007) and Wahid and Ghani (2008).

***Conclusion*:** On the basis of present study results, it can be concluded that mungbean cultivars were bit sensitive to Cd but varietal difference did persist. All varieties retained most of the absorbed Cd in their roots rather than had the ability to accumulate Cd in their shoots. The cultivars NM-98, NM-54 and NM-92 showed comparatively less reduction in root and shoot growth than NM-28. From the present results, NM-98 can be found as a precious resource for future mung bean breeding programs when aimed at higher Cd tolerance. It may also be recommended when mandatory to grow on slightly Cd contaminated areas because of its comparatively less shoot Cd concentrations while NM-28 found as the worst choice in this regard. Thus, selection of suitable cultivars may be the pre-requisite when cultivating mungbean in moderately Cd contaminated soils. It also persuades the plant breeders to search for genes of metal tolerance in mungbean.

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