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Physiological, Biochemical and Growth Responses of Lady's Finger (*Abelmoschus esculentus* L.) Plants as Affected by Cd Contaminated Soil

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Abstract Cadmium contamination of the soil is a common cause of environmental concern in the suburban areas of developing cities in India The present research paper describes the changes in physiological, biochemical, growth and yield characteristics, and bioaccumulation potential of lady's finger (*Abelmoschus esculentus* L.), an important vegetable crop at different levels of Cd in the soil. Cadmium accumulation was maximum in roots followed by stems, leaves and fruits at 100 mg Cd kg^{-1} in the soil. Cd accumulation in lady's finger negatively affected the physiological and biochemical characteristics, growth and yield. The magnitude of negative effect enhanced with increasing Cd concentration. The study suggests that due to higher potential of bioaccumulation of Cd in lady's finger and consequent reductions in growth and yield, this plant may not be a suitable option for cultivation in Cd contaminated soil.

Keywords Cd · Bioaccumulation · Yield · Growth · Lady's finger

Toxic heavy metals are major health risk concerns due to their high bioaccumulation potential, persistent nature and harmful biological effects (Singh and Tewari 2003; Sharma and Agrawal 2005). The ubiquity of toxic heavy metals as global pollutants has been well documented in the literature (Singh and Agrawal 2007; Sharma et al. 2007, 2008; Singh et al. 2008). Cadmium belongs to the toxic heavy metals, which can cause adverse effects on the organisms even at a very low concentration. Cadmium has high mobility in soil–plants system and gets accumulated easily into the food chain. Cadmium is especially very dangerous to plants as it accumulates at very large quantities without any visible symptoms (Lehoczky et al. 1996, 1998). High levels of heavy metals particularly Cd are reported in soil, irrigation water (wastewater) as well as vegetables grown in suburban areas (Singh et al. 2004; Sharma et al. 2007). Heavy metal accumulation in soil and consequently in food items can pose health risks to the human being. Many studies conducted at national and international levels reported heavy metal contamination in the soil and food items (Tripathi et al. 1997; Radwan and Salama 2006; Singh and Kumar 2006; Sharma et al. 2008, 2009a).

Previous studies showed that Cd concentration in edible portions of commonly consumed vegetables often exceeded the safe limits of both national and international standards (Sharma et al. 2006, 2007, 2009a; Singh et al. 2010). In the present study, Cd was selected as a heavy metal to study its effects and accumulation in lady's finger (*Abelmoschus esculentus* L.) whose edible fruits are commonly consumed by the poor urban population in India. The present study aimed to study (i) bioaccumulation potential of Cd in lady's finger, and (ii) its effects on physiological, biochemical and growth characteristics, and yield of tested plant.

Materials and Methods

A pot experiment was conducted using lady's finger (*Abelmoschus esculentus* L.) plant at an Agriculture Farm of

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Table 1 Physico-chemical properties of experimental soil

Parameters	Unit	Values
Soil type		Alluvial
pH		7.65
Electrical conductivity	d Sm^{-1}	0.20
Organic carbon	%	0.68
Available P	kg ha^{-1}	30.56
Available N	kg ha^{-1}	197.45
Available K	kg ha^{-1}	250.04
Total Cd	mg kg^{-1}	1.98
Available Cd	mg kg^{-1}	0.07

Values are mean of three replicates

Institute of Agricultural Science, Banaras Hindu University, Varanasi from March to May 2006. The physico-chemical properties of the experimental soil are given in Table 1. Forty pots were filled with 10 kg of uniformly air dried soil mixed with recommended dose of nitrogen, phosphorus and potassium (40:50:30 kg ha^{-1}) as urea, ammonium phosphate and potassium muriate, respectively and farmyard manure (20 t ha^{-1}). The total pots were separated into four groups of 10 each. Five healthy seeds (soaked in water for 24 h) of lady's finger were hand sown at 2 cm depth at identical distance. To maintain the uniform moisture content, same volume of water was given to each pot. After 10 days of seed germination, plants were thinned to three plants per pot. Fifteen days after seed germination, first, second, third and forth group of pots (10 each) were supplied, respectively with 0, 10, 50 and 100 mg Cd L^{-1} in form of $CdCl_2$ H_2O @ 100 ml pot^{-1}. The supplementation of respective pots with Cd was repeated at an interval of 10 days up to 70 days after seed germination.

The measurements of photosynthesis and transpiration rates, stomatal conductance, and chlorophyll induction kinetics were conducted on second leaf from the top by using a Portable Photosynthetic System (LI-6200, LI-COR, INC, Lincon, NE, USA) and a Plant Efficiency Analyzer (PEA, MK2 9414, Hansatech Instruments, Ltd., UK), respectively under ambient climatic conditions, after 30 days of first supplementation of Cd. During the measurement of above characteristics, photosynthetically active radiation and ambient CO_2 concentration varied between 1,000–1,200 μmol m^{-2} s^{-1} and 320–342 g m^{-3}, respectively. The same leaves were used for quantifying biochemical characteristics.

Chlorophyll and carotenoid contents were extracted in 0.1 g of leaf tissue in 80% acetone and quantified as mg g^{-1}dry weight according to the formulae given by Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Peroxidase activity (μM purpurogallin. formed $min^{-1}g^{-1}$ FW) was assessed in 100 mg of fresh leaf homogenized in 10 ml of 0.1 M phosphate buffer containing 5 mM of cystein (pH 6.8) at 4°C by the methodology of Britton and Mehley (1955). Extract of 0.1 ml was added to 4 ml of assay mixture (containing 125 μM phoshpate buffer, 50 μM pyrogallol, 50 μM H_2O_2) and incubated at 25°C for 5 min. The reaction was terminated by adding 0.5 ml of 5% H_2SO_4 (v/v) and the coloured product was extracted in 10 ml ether to take the absorbance at 420 nm using UV–VIS spectrophotometer (Model 119, Systronics, India). Lipid peroxidation (LPO) was determined in term of malondialdehyde (nM MDA mL^{-1} fresh weight) content by thiobarbituric acid (TBA) reaction (Heath and Packer 1968). Leaf tissue of 0.5 g was homogenized in 5 ml of 5% trichloroacetic acid (TCA) and centrifuged. To 1 ml of the supernatant, 4 ml of 0.5% thiobarbituric acid was added and the mixture was heated at 80°C for 30 min and then centrifuged at 0°C after cooling. The absorbances were taken at 532 and 600 nm using the UV–VIS Spectrophotometer (Model 119, Systronics, India). Thiol (μg g^{-1} fresh weight) in fresh leaf sample was determined by the method of Fahey et al. (1978). Fresh leaf tissue (0.1 g) was boiled in 10 ml of 80% ethanol for 15 min and 1 ml of cooled extract was added to 5 ml of 6 mM of 5 5′ dithiobis 2-nitrobenzoic acid (in 0.1 M phosphate buffer, pH 7.5). After centrifugation, the absorbance was measured at 412 nm using UV–VIS Spectrophotometer (Model 119, Systronics, India).

Proline content (mg g^{-1} fresh weight) in 0.5 g of leaf tissue was quantified after extraction in 10 ml of 3% aqueous sulpho-salisilic acid (Bates et al. 1973). To 1 ml of the filtrate, 2 ml of ninhydrin was added and kept in water bath for 60 min at 80°C. After cooling, 4 ml of toluene was added and mixed vigorously to separate the chromophore containing toluene. The absorbance was measured at 520 nm using UV–VIS Spectrophotometer (Model 119, Systronics, India). Total phenolics (mg g^{-1} fresh weight) in 0.1 g of fresh leaf tissue were extracted in 10 ml of 80% acetone (Bray and Thorpe 1954). To 1 ml of supernatant, 1 ml of Folin–Ciocalteau regent (1 N) and 2 ml of 20% Na_2CO_3 were added and the final volume was maintained to 10 ml with double distilled water and heated for 1 min. The absorbance of cooled solution was measured at 650 nm using UV–VIS Spectrophotometer (Model 119, Systronics, India). The ascorbic acid content (mg g^{-1} fresh weight) in 1 ml of leaf extract (0.5 g of fresh leaf tissue homogenized in 20 ml of 0.5% oxalic acid containing 0.75 mg EDTA) was determined by adding 5 ml of 20 μg $g^{-1}$of 2, 6 dichloro phenol indophenols against the standard prepared for ascorbic acid (Keller and Schwager 1977). The total soluble protein content in the leaf tissue was quantified by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Growth characteristics such as number of leaves, leaf area, root and shoot lengths and biomass of tested plants were measured at 80 days after first treatment. Leaf area was measured using a portable leaf area meter (Model LI-COR-3000, LI-COR, Inc., Lincon, NE, USA). For component wise biomass determination, roots, stems, leaves and fruits were separated and oven dried at 80°C till constant weight. Dry weights of each plant parts were measured separately and were added for the total biomass.

For analysis of Cd, 1 g of air dried soil/oven dried plant parts was digested in 15 ml of tri-acid mixture (Allen et al. (1986), and concentration in the filtrate was determined using atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, INC., Norwalk, CT, USA). Precision and accuracy of Cd analyses were ensured through replicate analyses of samples against standard reference material (SRM-1570) of National Institute of Standard and Technology. The results were found within the certified values (±2%).

Statistical analyses to test the significance of differences between the treatment means were determined using Duncan's multiple range tests at a 0.05 probability level with the help of SPSS software version 12. Results were presented as means ± standard error (SE) of three replicates.

Result and Discussions

The results of the present study showed that at increasing Cd concentrations in the soil, significant increments in Cd in all plant parts were observed (Fig. 1). Earlier studies have also shown variations in Cd accumulation in different plant parts with Cd concentrations and types of vegetables (Sharma and Agrawal 2006; Islam et al. 2007). Cd accumulation was highest in roots and lowest in fruits at 100 mg Cd kg^{-1} in soil as fruits had 13.5 times lower accumulation than roots. The results showed that roots and fruits have maximum and minimum accumulation efficiency for Cd, as the bioconcentration factor (BCF) for roots and fruits were above and below than an unit, respectively (Table 2). Bioconcentration factor of a plant part is defined as the ratio of Cd concentration in plant part to that of soil. With increasing concentrations of Cd, BCF for fruits decreased significantly indicating that accumulation of Cd in fruits was very low as compared to Cd present in soil. The decreasing trend of BCF may also be ascribed to increased levels of phytoavailable Cd in soil. The concentration of Cd in edible portion of lady's finger was below the safe limit of Indian standard (1.5 mg kg^{-1}), whereas the same in roots and stems was above the safe limits (Fig. 1). Cadmium accumulation tendency in the fruits increased at increasing application rates of Cd. Thus

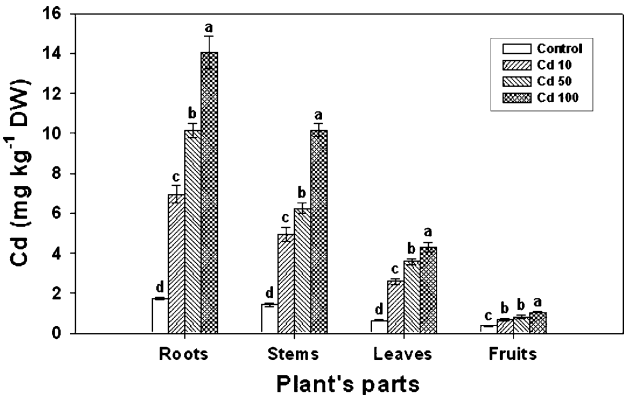

Fig. 1 Cd accumulation in different parts of *A. esculentus* L. plants. *Bars* with *different letters* in each group are significantly different from each other at $p \leq 0.05$ (Duncan's multiple range test)

the long term consumption of edible portion of tested vegetable may pose health risks to consumers.

The results showing the effects of different concentrations of Cd on growth, biomass and yield of tested vegetable are shown in Table 3. The number of leaves reduced significantly with increasing Cd concentration as compared to the control. Significant reductions in leaf area, biomass and yield were also observed at increasing Cd concentrations. The reduced biomass in the present study is due to the lowering of carbon accumulation resulted from decreased photosynthesis rate (Tables 3 and 4). The reductions in number of leaves as well as leaf area negatively affected photosynthetic carbon fixation and consequently reduced dry matter accumulation in different components of tested plant. The reduction in leaf area is correlated with inhibition of cell division and cell elongation (Arduini et al. 1994). Growth inhibition due to the metal toxicity is found to reduce biomass accumulation (Chaoui et al. 1997; Quariti et al. 1997). Excessive Cd accumulation in soil resulted into reduction in root growth causing reduced mineral absorption, altered carbohydrate metabolism leading to reduced dry matter production in palak (Singh and Agrawal 2007) and rice (Moya et al. 1993).

The reduction in biomass at increasing Cd concentrations may also be attributed to inhibition of chlorophyll biosynthesis as well as photosynthetic carbon fixtation (Baszynski et al. 1980; Padmaja et al. 1990). The percent reductions in economic yield of the tested plant were 22, 39 and 46 at 10, 50 and 100 mg Cd kg^{-1}in soil, respectively (Table 3). The yield reductions in *Beta vulgaris* due to heavy metals present in fly ash and sewage sludge amended soil have been reported (Singh et al. 2008; Singh and Agrawal (2007).

The ratio of variable fluorescence and maximum fluorescence (Fv/Fm) representing the efficiency of photosystem

Table 2 Bio-concentration factor (BCF) of Cd accumulated in roots and fruits of *A. esculentus* L. plants

Cd (mg L^{-1})	Root	Fruit
0	$2.11^{b} \pm 0.09$	$0.44^{a} \pm 0.02$
10	$3.42^{a} \pm 0.28$	$0.34^{b} \pm 0.01$
50	$1.99^{b} \pm 0.07$	$0.16^{c} \pm 0.03$
100	$0.92^{c} \pm 0.07$	$0.07^{d} \pm 0.01$

Values are mean ± 1SE of three replicates. Values followed by different letters in each *column* are significantly different at $p \leq 0.05$ (Duncan's multiple range test)

II did not show significant difference at 10 mg Cd L^{-1}, but decreased significantly at 50 and 100 mg Cd L^{-1} as compared to the control (Table 4). Photosynthesis rate and stomatal conductance decreased significantly at increasing Cd concentrations with maximum reduction at 100 mg Cd L^{-1} (Table 4). Dong et al. (2005) have reported that addition of 1.124 mg Cd L^{-1} to the growth medium reduced the photosynthesis rate by 62% in tomato seedlings as compared to the control. The higher magnitude of reduction in photosynthesis rate at increasing concentration of Cd is caused by inhibition of PS II activities as shown by reduction in Fv/Fm ratio as well as reduction in photosynthetic pigments (Table 5). The highest dose of Cd caused 38, 65, 43 and 32% reductions in chlorophyll a, b, total and carotenoid contents, respectively as compared to the control. The result suggests that chl b is more sensitive than Chl a to Cd stress. Greater reduction in chl b has also been reported in *Avicennia marina* and other mangrove species under heavy metal stress (Macfarlane and Burchett 2001; Chen et al. 2008).

Malondialdehye (MDA) content representing the peroxidation of membrane lipids increased at increasing Cd concentrations (Table 5). At 100 mg Cd kg^{-1}, MDA content increased by 255% as compared to the control (Table 5). Higher degree of lipid peroxidation is correlated with more production of free radicals under heavy metal stress (Luna et al. 1994; Chaoui et al. 1997). During the present study, significant increments in thiol, proline and total phenolic contents and peroxidase activity were also observed at increasing Cd concentrations, but ascorbic acid content did not show significant variations between control and Cd treated plants (Table 5). As compared to the control, Cd applied @ 100 mg L^{-1} increased thiol, proline and

Table 3 Effect of different Cd concentrations on growth, biomass accumulation and economic yield of *A. esculentus* L. plants

Parameters	Cd (mg L^{-1})			
	0	10	50	100
No of leaves (plant^{-1})	$13.33^{a} \pm 0.24$	$12.67^{b,c} \pm 0.24$	$12.33^{c} \pm 0.24$	$12.00^{c} \pm 0.41$
Leaf area (cm^2 plant^{-1})	$2029^{a} \pm 26.84$	$1380^{b} \pm 10.88$	$1170^{c} \pm 10.73$	$1024^{d} \pm 24.62$
Root length (cm plant^{-1})	$34.00^{a} \pm 0.67$	$29.40^{b} \pm 0.59$	$24.40^{c} \pm 1.04$	$23.83^{c} \pm 0.85$
Shoot length (cm plant^{-1})	$58.57^{a} \pm 0.94$	$55.03^{a} \pm 2.48$	$48.87^{b} \pm 1.17$	$48.07^{b} \pm 1.64$
Total plant length (cm plant^{-1})	$92.57^{a} \pm 1.04$	$84.43^{b} \pm 1.93$	$73.27^{c} \pm 0.33$	$71.90^{d} \pm 1.07$
Root biomass (g plant^{-1})	$0.168^{a} \pm 0.06$	$0.12^{b} \pm 0.003$	$0.10^{c} \pm 0.006$	$0.08^{d} \pm 0.003$
Stem biomass (g plant^{-1})	$1.40^{a} \pm 0.01$	$1.20^{b} \pm 0.01$	$1.09^{c} \pm 0.004$	$0.72^{d} \pm 0.01$
Leaf biomass (g plant^{-1})	$0.63^{a} \pm 0.004$	$0.52^{b} \pm 0.006$	$0.44^{c} \pm 0.006$	$0.38^{d} \pm 0.004$
Fruit biomass (g plant^{-1})	$1.45^{a} \pm 0.07$	$1.14^{a,b} \pm 0.16$	$0.88^{b,c} \pm 0.04$	$0.79^{c} \pm 0.11$
Total plant biomass (g plant^{-1})	$3.64^{a} \pm 0.08$	$2.98^{b} \pm 0.17$	$2.51^{c} \pm 0.04$	$1.98^{d} \pm 0.11$
Economic yield (g plant^{-1} FW)	$26.33^{a} \pm 1.31$	$20.67^{a,b} \pm 2.87$	$16.00^{b,c} \pm 0.82$	$14.33^{d} \pm 2.09$

Values are mean ± 1SE of three replicates. Values followed by different letters in each *row* are significantly different at $p \leq 0.05$ (Duncan's multiple range test)

Table 4 Effect of different Cd concentrations on physiological characteristics of *A. esculentus* L. plants

Parameters	Cd (mg L^{-1})			
	0	10	50	100
Photosynthesis rate (μM CO_2 m^{-2} s^{-1})	$7.22^{a} \pm 0.08$	$5.44^{b} \pm 0.04$	$4.33^{c} \pm 0.02$	$3.91^{d} \pm 0.02$
Transpiration rate (M H_2O m^{-2} s^{-1})	$0.71^{a} \pm 0.01$	$0.64^{b} \pm 0.01$	$0.54^{c} \pm 0.01$	$0.39^{d} \pm 0.01$
Stomatal conductance (cm s^{-1})	$1.09^{a} \pm 0.04$	$0.80^{b} \pm 0.008$	$0.62^{c} \pm 0.006$	$0.51^{d} \pm 0.009$
Fv/Fm	$0.76^{a} \pm 0.004$	$0.74^{a} \pm 0.01$	$0.70^{b} \pm 0.003$	$0.70^{b} \pm 0.01$

Values are mean ± 1SE of three replicates. Values followed by different letters in each *row* are significantly different at $p \leq 0.05$ (Duncan's multiple range test)

Table 5 Effect of different Cd concentrations on biochemical parameters of *A. esculentus* L. plants

Parameters	Cd (mg L^{-1})			
	0	10	50	100
Chlorophyll a (mg g^{-1} DW)	$10.05^{a} \pm 0.16$	$7.59^{b} \pm 0.52$	$6.65^{c,d} \pm 0.41$	$6.19^{d} \pm 0.22$
Chlorophyll b (mg g^{-1} DW)	$3.97^{a} \pm 0.07$	$2.52^{b} \pm 0.08$	$1.78^{c} \pm 0.03$	$1.53^{d} \pm 0.04$
Total chlorophyll (mg g^{-1} DW)	$14.02^{a} \pm 0.16$	$10.11^{b} \pm 0.50$	$8.42^{c} \pm 0.38$	$7.73^{c} \pm 0.18$
Carotenoid content (mg g^{-1} DW)	$3.96^{a} \pm 0.04$	$3.11^{b} \pm 0.20$	$2.89^{b,c} \pm 0.07$	$2.68^{c} \pm 0.01$
Lipid peroxidation (nM MDA mL^{-1})	$0.56^{d} \pm 0.01$	$0.67^{c} \pm 0.01$	$0.94^{b} \pm 0.01$	$1.99^{a} \pm 0.05$
Protein content (mg g^{-1} DW)	$10.53^{a} \pm 0.32$	$8.82^{b} \pm 0.36$	$8.81^{b} \pm 0.13$	$6.79^{c} \pm 0.18$
Thiol content (μg g^{-1} FW)	$1.28^{d} \pm 0.01$	$1.56^{c} \pm 0.02$	$1.81^{b} \pm 0.03$	$2.05^{a} \pm 0.02$
Proline content (mg g^{-1} FW)	$0.15^{d} \pm 0.002$	$0.22^{c} \pm 0.01$	$0.36^{b} \pm 0.01$	$0.50^{a} \pm 0.01$
Total phenol content (mg g^{-1} FW)	$10.26^{c} \pm 0.22$	$11.80^{b,c} \pm 0.40$	$11.15^{b} \pm 0.09$	$16.29^{a} \pm 0.54$
Ascorbic acid content (mg g^{-1} FW)	$1.18^{a} \pm 0.01$	$1.18^{a} \pm 0.01$	$1.19^{a} \pm 0.005$	$1.19^{a} \pm 0.004$
Peroxidase activity (μM pur. formed $min^{-1}g^{-1}$ FW)	$4.04^{d} \pm 0.08$	$5.62^{c} \pm 0.17$	$6.06^{b} \pm 0.05$	$8.67^{a} \pm 0.03$

Values are mean ± 1SE of three replicates. Values followed by different letters in each *row* are significantly different at $p \leq 0.05$ (Duncan's multiple range test)

total phenolic contents and peroxidase activity by 60, 230, 50 and 112%, respectively (Table 5). Increase in peroxidase has been suggested to be an early indicator of heavy metal stress in the plants (Van Assche and Clijsters 1990; Dietze et al. 1999). Increased activity of peroxidase in the tested plant denotes an enhancement in oxidative stress due to increase in hydrogen peroxide leading to disruption of the plasmalemma through lipid peroxidation under elevated accumulation of Cd (Singh et al. 2006). Total soluble protein content in leaves decreased significantly at increasing Cd concentrations (Table 5). Decline in soluble protein content has been reported under heavy metal stress (Vyas and Puranic 1993; Bhattacharya and Choudhuri 1994; Sharma et al. 2009b). The simultaneous decline in soluble protein content and increase in peroxidase activity strongly suggest an increase in catalytic activities under Cd stress. Heavy metal stress induces senescence through enhancement of catabolism of the key metabolites including chlorophylls and proteins. The reduction in soluble protein content of the leaves may be correlated with decline in the growth of the plants (Khudsar et al. 2004).

This study indicated that Cd accumulation in edible portion of tested plant was 13.5 times lower than the roots. Accumulated Cd negatively affected the biochemical and physiological characteristics in tested plant leading to reductions in growth, biomass accumulation and economic yield. Though Cd accumulation in edible portion is below the safe limit, but its long term dietary intake may lead to health hazards to the consumers. The present study further concludes that lady's finger grown in Cd contaminated soil not only showed loss in the economic yield but was also found not safe for long term consumption due to related risk to human health.

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