

EFFECT OF INCREASING LEVELS OF APPLIED CADMIUM ON GROWTH, BIOCHEMICAL ATTRIBUTES AND MICRONUTRIENT UPTAKE BY WHEAT AND RICE

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Transfer of cadmium (Cd) through food crops have become a sturdy plight globally, due to increasing Cd pollution in agricultural soils. The Cd accumulation in food crops is not only quandary for food safety but also a prominent threat to agricultural productivity due to its severe biological toxicity which hinders plant growth and physiology by affecting nutrient homeostasis. Thus, a hydroponic experiment was executed to investigate the various levels of Cd on growth, physiology, mineral nutrients and Cd accumulation in different tissues of rice and wheat plants. The plant's seedlings grown from acid washed seed in sand culture were shifted to foam plugged holes in a polystyrene sheet floating on plastic tubs (8 L) containing Hoagland's solution. Four Cd levels (0, 5, 10 and 15 μM) with four replicates were applied after seven days of transplantation and harvested after twenty days of Cd application. The results explained that increasing levels of Cd resulted in severe toxicity and declined the physiological performance and growth of plants. Similarly, Cd uptake by roots of wheat and rice was increased up to 42 and 304 $\mu\text{g pot}^{-1}$ respectively in Cd containing rooting solution. Compare to control, the 15 μM Cd treatment decreased the copper (Cu), manganese (Mn) and zinc (Zn) contents by 6-61%, 6-41% and 6-17% in wheat and 18-39%, 6-29% and 4-32% in rice, respectively. Interestingly, root iron (Fe) contents in wheat were increased up to 28% at 10 μM Cd level while rice (both root and shoot) Fe contents were reduced at 10 and 15 μM Cd stress.

Keywords: Cadmium, growth, hydroponic, micronutrient, physiology, rice, wheat .

INTRODUCTION

The incessant accrual of heavy metals (HMs) in agricultural soils due to robust anthropogenic activities has clutched the attentiveness of researchers (Adrees *et al.*, 2015; Rehman *et al.*, 2017). Among HMs, Cd is biologically non-essential, divalent cation, readily accessible and absorbed by roots growing even at moderately contaminated medium (Uraguchi *et al.*, 2011; Akhtar *et al.*, 2016). Food chain contamination with Cd has become a prime potential threat to human health (Khan *et al.*, 2016; Qayyum *et al.*, 2017; Yang *et al.*, 2017). Due to higher mobility, solubility and toxicity of Cd at low concentrations, it negatively alters the growth and physiology of plants. The Cd is ranked at number 7 among toxic substances listed by USA Environment Protection Agency (ATSDR, 2013). Although Cd is non-essential element but its uptake and accumulation in plants may exceed many essential mineral nutrients (Pendias and Pendias, 2001). Rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) are staple foods for large parts of the world. Amongst cereals, more than half of the population utilizes rice (Meharg *et al.*, 2013; Kosolsaksakul *et al.*, 2014) and wheat (FAO, 2014) to fulfill their dietary requirements. In Pakistan, wheat-rice rotation is the major cropping system, especially in the Punjab province

(Hussain *et al.*, 2012). Both, wheat and rice can accumulate higher Cd in the edible plant parts (Song *et al.*, 2015; Rizwan *et al.*, 2016b).

In plants, Cd toxicity can aggravate morphological, physiological and biochemical alterations leading to reduced productivity. The most prominent phyto-toxic effects of Cd includes stunted growth and root damage (Das *et al.*, 1997; Tkalec *et al.*, 2008) by producing reactive oxygen species (Rizwan *et al.*, 2016b; 2016c), reduction in leaf water content and mineral nutrient uptake (Sanita di Toppi and Gabrielli, 1999), lipid peroxidation (Balen *et al.*, 2011), disturb photosynthetic and gas-exchange activities (Akhtar *et al.*, 2016), inactivation of enzymes, membrane dysfunctions, and hormonal imbalance (Chien and Kao, 2000; Sarwar *et al.*, 2010). Reduction in photosynthetic pigment and gas exchange characteristics are the major outcome of Cd toxicity reported for number of plant species (Lysenko *et al.*, 2015; Tauqeer *et al.*, 2016).

Nutrient imbalance through reduction in uptake and replacement of minerals ions with Cd in cellular compartments is believed to be prime toxicity mechanism in plants (Clemens *et al.*, 2002; Akhtar *et al.*, 2016). The uptake and distribution of micronutrients such as Cu, Fe, Mn and Zn has been reported to be negatively affected by Cd stress in

soybean, wheat, rice, barley and maize (Wu and Zhang, 2002 a,b; Zhang *et al.*, 2002 a,b; Yujing *et al.*, 2008; Akhtar *et al.*, 2016). It has been reported that Cd has an antagonistic effect on essential nutrients (Wu and Zhang, 2002a; Liu *et al.*, 2003). For example, the Cd in growth medium decreased the Mn translocation to the shoots of wheat (Zhang *et al.*, 2002a) and Zn, Cu in barley (Wu *et al.*, 2003). Similarly, in rice distribution of Cu, Fe and Zn was significantly minimized under the presence of Cd (Liu *et al.*, 2003). The disturbance in nutrient uptake under Cd stress is due to disturbed plasma membrane permeability (Sarwar *et al.*, 2010). Nan *et al.* (2002) documented a synergistic relation among Cd and Zn uptake in field cultivated corn and wheat, whereas Yujing *et al.* (2008) reported Cd-induced reduction in shoot Fe and Zn concentration and their uptake in rice genotypes under hydroponic conditions.

Thus, the existing literature urged to investigate the physiological response of wheat and rice plants under varying levels of Cd to explore the mechanism, how increasing level of Cd alters the nutrient homeostasis and growth of globally top produced cereals. Present experiment was accomplished with objective to study the effect of Cd on availability, uptake, accumulation and translocation of selected micronutrients from roots to aerial parts of hydroponically grown wheat and rice.

MATERIALS AND METHODS

Experiment location and growth conditions: Hydroponic experiment was conducted in glass house of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan (31° 25'59.55" N, 73° 04' 20" E, 183 m above sea level). The inside temperature varied from a minimum of 10°C to a maximum of 35°C with a mean value of 25±5°C, relative humidity ranged from 45% to 85% at day and night respectively, light intensity varied between 300 and 1400 µMol photon m² S⁻¹ depending upon day length and cloud conditions during the growth period.

Experimental design and treatment application: Seeds of wheat (Galaxy-2014) and rice (Basmati-515) were collected from Ayub Agricultural Research Institute Faisalabad, Pakistan. Seedling of both crops was grown in polyethylene lined plastic trays containing sand sequentially washed with (10% HCl solution) and then with deionized water. At “two leaf” stage, seedlings were plugged with foam and shifted into holes (two seedlings per hole) of polystyrene sheets floating on plastic tubs (8 L capacity) containing Hoagland’s nutrient solution (Hoagland and Arnon, 1950). Tap water (chemical analysis given in Table 1) was used in solution medium. To ensure oxygen supply to roots, nutrient solution was kept aerated continuously using aeration pumps connected to each tub via plastic tubes. The nutrient solution was monitored daily to adjust pH of solution to 6.0-6.50 using 1 N hydrochloric acid (HCl) and/or 1 N sodium hydroxide

(NaOH). While replacing nutrient solution on completion of one week of growth, Cd concentrations of 5, 10 and 15 µM as cadmium sulphate (3CdSO₄.7H₂O) were introduced to plant roots after seven days. These Cd levels were selected to understand micronutrient uptake and translocation at lower Cd concentrations. A control without Cd treatment was also maintained to compare the treatments response.

Table 1. Analysis of water used in hydroponic medium.

Parameter	Unit	Value	*Permissible limit
EC	dS m ⁻¹	1.12	<1.5
SAR	(mmol L ⁻¹) ^{1/2}	3.30	<7.5
RSC	mmolc L ⁻¹	1.25	<2.0
Cd	mg L ⁻¹	Nil	0.01
Ni	"	"	0.20
Pb	"	"	5.00
Cr	"	"	0.10
Co	"	"	0.05
Zn	"	"	2.0
Remarks	-	Suitable	-

*Ayers and Westcot (1985)

Measurements of gas exchange attributes and total chlorophyll contents: Eighteen days after Cd exposure, photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (*C_i*) and sub-stomatal CO₂ (*g_s*) concentration were measured on second upper fully expanded leaves, using a portable infrared gas analyzer (LCA-4, Analytical Development Company, Hoddesdon, England). Readings were taken by clamping the central part of the leaf in the chamber of the instrument on the same time of the day (from 10:00 am to 12:00 pm) to ensure unchanged photon flux density and temperature. Chlorophyll measurement, in terms of SPAD value, was performed on second upper leaves with the help of chlorophyll meter (SPAD-502 Konika Minolta Sensing Inc., Japan).

Membrane stability index (MSI): Fully expanded younger leaves were harvested after 18 days of Cd imposition. The harvested leaves after single washing with deionized water were placed in test tube containing 10 mL deionized water and then test tubes were placed in water bath and allowed to heat at 40 °C for 30 minutes. After prescribed time test tubes were taken out and electrical conductivity (EC) of suspension was measured that was named as EC₁. Then again same test tubes were heated at 100 °C for 10 minutes and EC was measured i.e. EC₂. The MSI was calculated according to formula described by Premchandra (1990) and Sairum (1994)

$$MSI = \left(1 - \frac{EC_1}{EC_2}\right) \times 100$$

Growth and biomass yield: Plants were harvested after twenty days of exposure to Cd stress, washed with tap water followed by washings with deionized water. Plants were blotted dry and separated into roots and shoots. Prior to plant drying, root and shoot lengths were measured using stainless

steel scale by placing on the uncontaminated board. Wiped samples were stored in labeled paper bags and air dried. Air dried samples were placed at 65±5 °C in a forced air-driven oven till constant weight was achieved and dry biomass of root and shoot was recorded by using digital weighing balance (Uni Bloc model AUW 120 D, Shimadzu Corporation, Japan).

Chemical analyses of plants

Wet digestion of plant tissues: Wet digestion of dry plant samples was performed as described by AOAC (1990). One gram of dried ground plant sample was mixed with 10 mL of concentrated HNO₃ and HClO₄ (3:1) mixture in a conical flask and kept for overnight. On next day, digestion was done using hot plat until material remained clear. After cooling, samples were diluted to 50 ml with deionized water, filtered using Whatman filter paper 42 and stored in plastic bottles at room temperature (25±2 °C).

Measurement and calculations of Cd and micronutrients: Cadmium and micronutrients viz. Cu, Fe, Mn and Zn were measured by using calibrated atomic absorption spectrometer (Model Thermo Electron S-Series) with a series of Cd and other required micronutrient standard solutions. The regression relation between concentration and absorbance of the standard solutions were used to calculate the concentration of unknown samples. The corrected concentration of Cd and micronutrients were calculated as follows;

$$\text{Metal concentration (mg kg}^{-1}\text{)} = \frac{\text{Concentration from regression equation} \times \text{DF}}{\text{Dilution factor}}$$

Here, DF stands for dilution factor. Cadmium and micronutrient uptake (µg pot⁻¹) was calculated by using following equation;

$$\text{Cd, micronutrient uptake} = \frac{\text{Cd or micronutrient conc. in root or shoot} \times \text{root or shoot dry biomass}}{\text{Pot}} \times 100$$

The Cd translocation from roots to shoots of both crops was described by translocation index (TI) calculated by following formulae

$$\text{TI(\%)} = \frac{\text{Shoot Cd Conc.}}{\text{Root Cd Conc.} + \text{Shoot Cd Conc.}} \times 100$$

(Baker and Whiting, 2002)

The Cd and micronutrient concentration was taken as mg kg⁻¹ and dry biomass as g pot⁻¹.

Statistical analysis: The data regarding plant growth response and Cd and micronutrients accumulation was statistically analyzed following ANOVA technique (Steel *et al.*, 1996). Treatments were differentiated by using least significant difference (LSD) test using Statistix 8.1 software (Version 8.1 Software package).

RESULTS

Effect of Cd on growth and biomass of wheat and rice:

Growth and biomass yields of both wheat and rice plants were significantly (p ≤ 0.05) reduced growing under the presence of Cd either 10 or 15 µM concentrations (Table 2). The values for these parameters were noted minimum in 15 µM Cd stressed plants and maximum in control (0 µM Cd) plants of both crops. The Cd stress was more prominent on shoot dry weight of wheat and rice compared to rest of growth parameters. Compared to control, shoot and root dry biomass were reduced by 44 and 28% in wheat and 59 and 56% in rice respectively at 15 µM Cd treatment. Shoot length of wheat and rice was decreased by 21 and 36% whereas, root length was reduced by 36 and 46% respectively in 15 µM Cd stressed plants relative to control.

Effect of Cd on membrane stability index, total chlorophyll and gas exchange characteristics:

Application of Cd significantly reduced MSI, highest reduction relative to control was observed in plants of wheat (60%) and rice (47%) grown in 15 µM Cd followed by 10 and 5 µM Cd applied solution (Fig. 1). Significant (p ≤ 0.05) reduction in total chlorophyll contents (SPAD-value) and gas-exchange attributes of both crops was noted as a result of applied Cd (Fig. 1). Minimum SPAD value of wheat and rice was

Table 2. Effect of different Cd levels on growth and biomass of wheat and rice.

Cadmium (µM)	Wheat			
	Shoot Length (cm)	Root Length (cm)	Shoot dry weight (g pot ⁻¹)	Root dry weight (g pot ⁻¹)
0	*42 a ± 0.48	22 a ± 0.71	1.42 a ± 0.06	0.50 a ± 0.009
5	38 b ± 0.65 (10)	19 b ± 0.91 (14)	1.23 b ± 0.04 (13)	0.49 ab ± 0.012 (2)
10	36 c ± 0.41 (14)	17 b ± 0.63 (22)	1.18 b ± 0.01 (17)	0.46 b ± 0.007 (8)
15	33 d ± 0.28 (21)	14 c ± 0.41 (36)	0.80 c ± 0.05 (44)	0.36 c ± 0.011 (28)
LSD	1.46**	2.12**	0.14**	0.031**
	Rice			
0	75 a ± 0.96	28 a ± 0.95	7.89 a ± 0.33	2.44 a ± 0.08
5	63 b ± 1.04 (16)	24 b ± 0.75 (14)	6.88 b ± 0.21 (13)	2.23 b ± 0.02 (9)
10	52 c ± 0.91 (31)	18 c ± 0.63 (36)	3.42 c ± 0.11 (56)	1.28 c ± 0.07 (47)
15	48 d ± 0.65 (36)	15 d ± 0.48 (46)	3.23 c ± 0.22 (59)	1.08 d ± 0.06 (56)
LSD	2.78**	2.22**	0.71**	0.195**

*Mean value (n=4), ± Standard error, () percent decrease compared to 0 µM Cd, **Significant difference among treatments on the basis of p ≤ 0.05

recorded in 15 μM Cd treated plants and was decreased by 30% and 23% respectively relative to control.

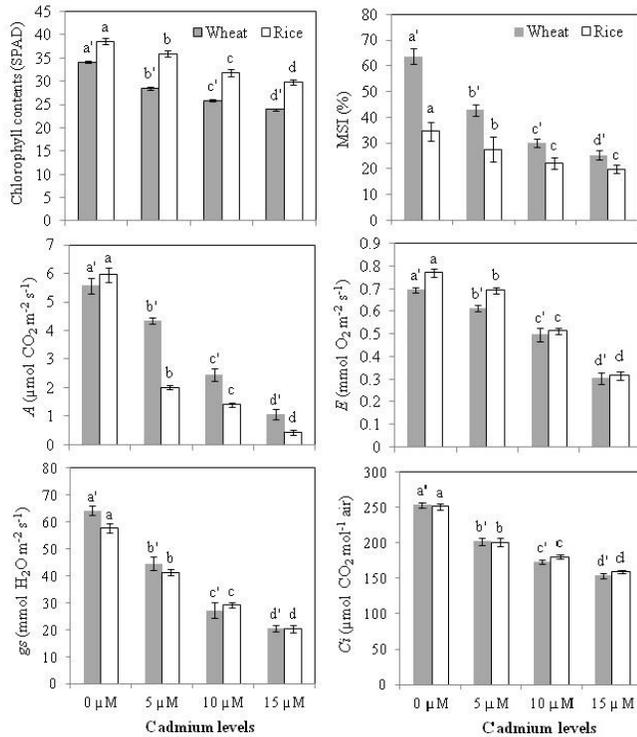


Figure 1. Effect of Cd on Chlorophyll, MSI and gas exchange attributes. MSI = membrane stability index; A = photosynthetic rate; E = transpiration rate; gs = stomatal conductance; Ci = substomatal CO_2 concentration.

Gas exchange characteristics were recorded lowest in plants of both crops grown in 15 μM Cd applied solution (Fig. 1). These attributes viz. A, E, Ci and gs of wheat were reduced by 81, 56, 68 and 39% respectively whereas in rice, reductions were 93, 47, 64 and 37% respectively in 15 μM Cd received plants as compared to 0 μM Cd. Among described gas exchange characteristics, Cd stress highly affected the “A” of both crops (decreased up to a range of 22-93%) as compared to other gas exchange attributes while among crops, rice showed higher reduction in “A” compared to wheat.

Effect of applied Cd on wheat and rice Cd concentration, uptake and translocation: Exogenous application of Cd in rooting solution had significantly ($p \leq 0.05$) increased both root and shoot Cd at all three levels (Fig. 2). Wheat and rice plants grown in 15 μM Cd applied growth medium showed highest root (119.1 and 210.5 mg kg^{-1}) and shoot (29.1 and 61.6 mg kg^{-1}) Cd concentration respectively whereas, no Cd was analyzed in plants grown in Cd untreated solution.

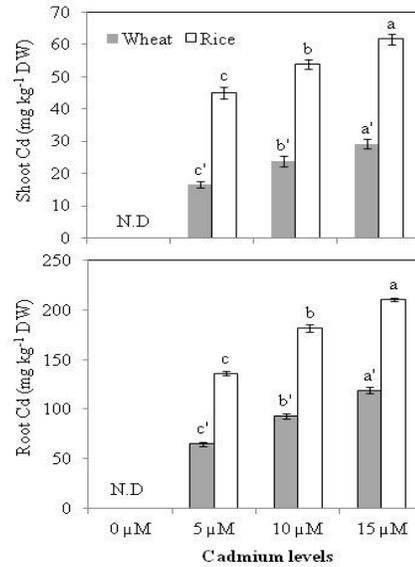


Figure 2. Effect of applied Cd on root and shoot Cd concentration (N.D = not detected).

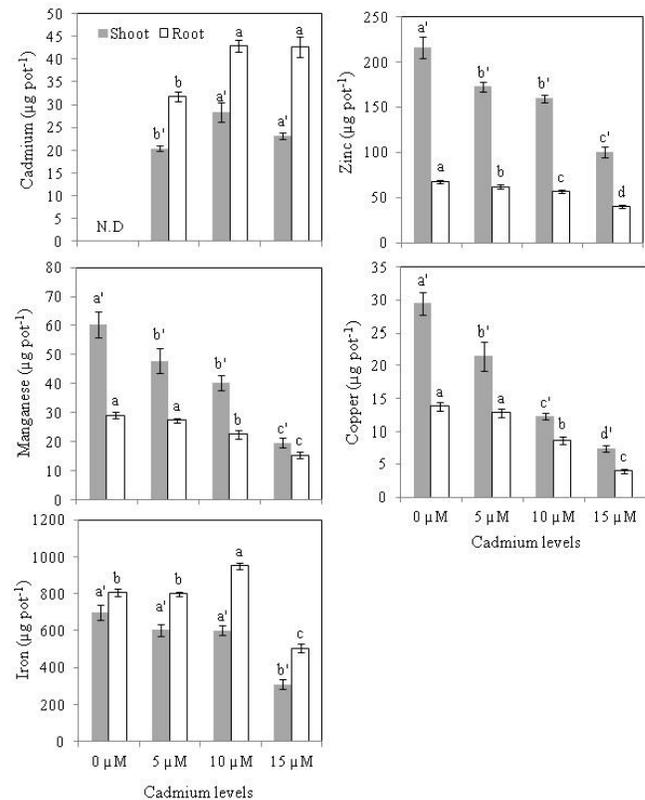


Figure 3. Effect of Cd imposed stress on metal uptake ($\mu\text{g pot}^{-1}$) by wheat (N.D = not detected).

The Cd uptake by wheat was enhanced with increasing Cd levels (Fig. 3). Compared to shoot, root Cd uptake in both wheat and rice was noted higher. Wheat Cd uptake was lowest

in 5 μM Cd received plants and highest in 15 μM Cd stressed plants. As explained by statistical treatment of data, no variation occurred in Cd uptake by wheat grown in 10 and 15 μM Cd treated solution among each other (Fig. 3).

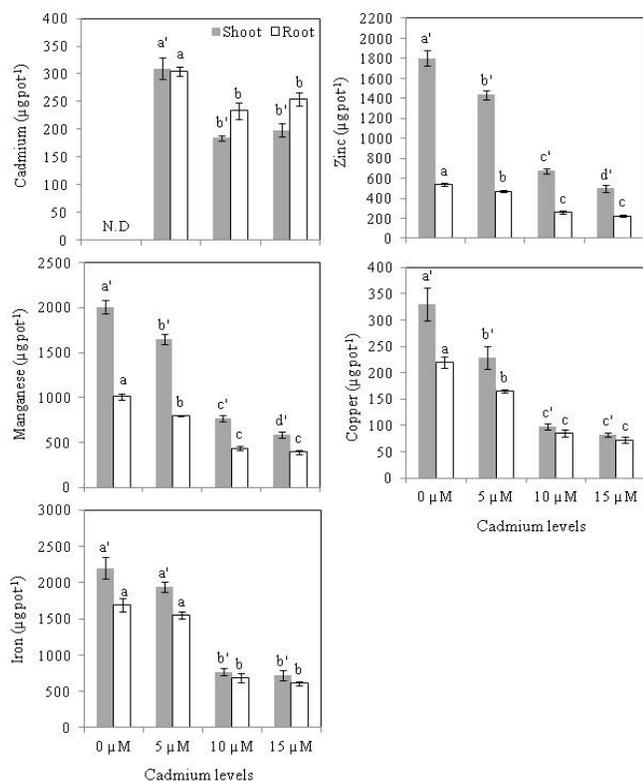


Figure 4. Effect of Cd imposed stress on metal uptake ($\mu\text{g pot}^{-1}$) by rice (N.D = not detected).

In rice, root ($305 \mu\text{g pot}^{-1}$) and shoot ($310 \mu\text{g pot}^{-1}$) Cd uptake was highest in plants grown in 5 μM Cd applied solution as

compared to Cd levels while plants grown under 10 μM and 15 μM Cd treatment showed similar Cd uptake among each other (Fig. 4). Translocation of Cd from root to shoot was calculated as similar among all levels of applied Cd in both rice and wheat (Fig. 5).

Effect of Cd on micronutrient/ ionic composition and uptake by wheat and rice: Root and shoot micronutrient (Cu, Mn and Zn) contents were negatively affected by applied Cd levels. Effect of Cd stress on micronutrients including Fe was most significant ($p < 0.05$) in 15 μM Cd stressed plants (Table 3). Gradual reduction in root and shoot Zn contents of both crops were noted with increasing Cd levels. Root and shoot Zn contents were minimized by 17 and 16% respectively in wheat exposed to 15 μM Cd (Table 3) whereas, reduction was 16 and 32% in root and shoot of rice grown in 15 μM Cd applied solution over control (Table 4).

Highest wheat and rice shoot Mn was recorded in 0 μM Cd whereas lowest in 15 μM Cd treated plants. Shoot Mn contents were lowered by 41 and 29% in 15 μM Cd treated wheat and rice plants respectively. Root Mn concentration was decreased by 41 and 21% in wheat and rice respectively exposed to Cd (15 μM) treated solution over control (Table 4). Root and shoot Cu contents of both crops were reduced especially at higher level of applied Cd. Shoot Cu was decreased by 57 and 39% in wheat and rice respectively exposed to higher level of applied Cd. Whereas, root Cu was decreased by 61 and 34% in 15 μM Cd applied wheat and rice plants respectively over control.

Interestingly, root Fe contents were increased in wheat plants grown in 10 μM Cd applied rooting solution. Shoot Fe was statistically similar in wheat exposed to 5 and 10 μM Cd and lowered by 19% in plants treated with Cd @ 15 μM whereas in rice shoot Fe was reduced by 21% in plants grown in higher level of Cd treated growth medium. Root Fe was improved by 28% in wheat grown in Cd (10 μM) applied solution and lowered by 12% grown in higher level of Cd added solution

Table 3. Effect of applied cadmium on ionic composition (mg kg^{-1} dry weight) of wheat and rice.

Cd μM	Wheat							
	Root				Shoot			
	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn
	(mg kg^{-1} DW)							
0	*28a \pm 1.4	1614b \pm 25	58a \pm 1.5	136a \pm 3	20a \pm 0.97	481a \pm 10	41.4a \pm 1.27	149a \pm 2.6
5	26a \pm 1.4	1631b \pm 24	56a \pm 1.6	126b \pm 2	17b \pm 1.22	491a \pm 15	38.6ab \pm 2.23	140b \pm 1.3
10	19b \pm 1.0	2064a \pm 36	49b \pm 2.3	123b \pm 3	10c \pm 0.42	508a \pm 21	34.0b \pm 1.97	135b \pm 2.8
15	11c \pm 0.8	1415c \pm 44	43c \pm 1.5	112c \pm 2	09c \pm 0.45	388b \pm 28	24.4c \pm 1.34	125c \pm 2.2
LSD	3.7**	102**	5.5**	7.54**	2.59**	60**	5.40**	7.1**
	Rice							
0	90a \pm 2.8	692a \pm 20	413a \pm 2.7	221a \pm 2.4	41a \pm 2.3	278.9a \pm 16	254.4a \pm 2.10	228a \pm 3.8
5	74b \pm 1.3	694a \pm 24	357b \pm 2.1	211b \pm 2.8	33b \pm 2.4	282.3a \pm 11	239.4b \pm 2.42	209b \pm 2.5
10	66c \pm 1.4	530b \pm 22	341c \pm 2.2	202c \pm 2.8	28bc \pm 1.6	223.6b \pm 08	225.5c \pm 3.73	197c \pm 2.5
15	59c \pm 2.9	506b \pm 17	325d \pm 1.9	184d \pm 2.6	25c \pm 1.2	221.7b \pm 11	180.6d \pm 2.51	154d \pm 3.0
LSD	6.9**	64.7**	7.8**	8.2**	6.06**	36**	8.50**	9.2**

*Mean value (n=4), \pm Standard error; **Significant difference among treatments on the basis of $p \leq 0.05$

whereas, root Fe was minimized by 26% in rice grown in higher level of Cd treated rooting solution relative to control. Uptake of micronutrients *viz.* Cu, Fe, Mn and Zn by wheat shoot as influenced by Cd levels was observed higher except Fe (Fig. 3). Increasing Cd levels significantly reduced Zn uptake by wheat root and shoot. Wheat Zn uptake was highest with control followed by 5 μ M, 10 μ M and 15 μ M Cd respectively. Uptake of Cu and Mn was reduced by exogenous Cd and was found lowest in plants of both crops grown in 15 μ M Cd treated rooting medium. Wheat roots grown in 10 μ M Cd applied solution showed higher Fe uptake followed by control and 5 μ M Cd while, wheat (both root and shoot) exposed to higher level of applied Cd showed minimum uptake of Fe (Fig. 3).

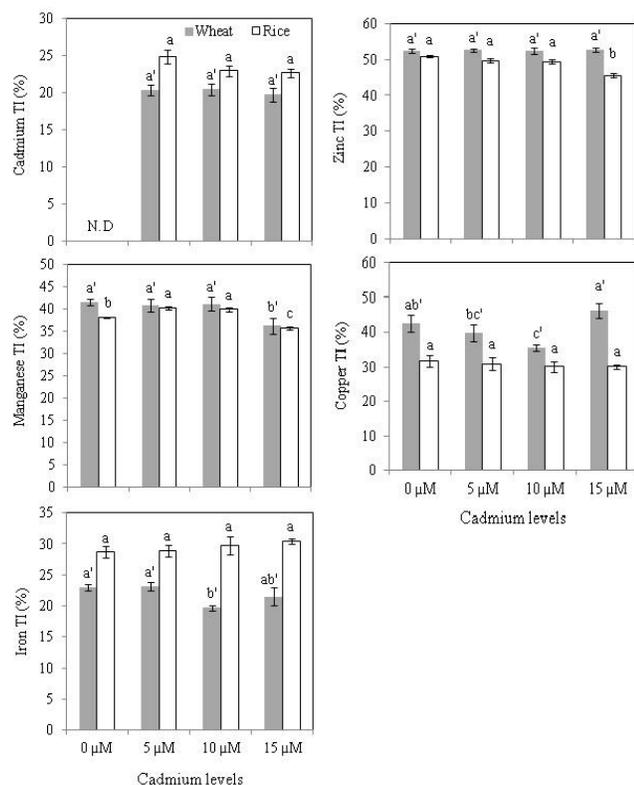


Figure 5. Effect of Cd exposure on root-shoot translocation of different metals in wheat and rice.

In rice, shoot uptake of micronutrient ions was found higher than root uptake (Fig. 4). Uptake of Zn by shoot and root grown in uncontaminated solution was higher ($p < 0.05$) than Cd stressed plants. Highest reduction in root and shoot Zn uptake was noted in 15 μ M Cd applied plants. As a result of Cd addition, ionic uptake (Cu, Fe and Mn) decreased especially in plants exposed to 15 μ M Cd (Fig. 4).

Root-shoot Zn translocation was similar at all Cd levels in wheat plants while it was lowest in 15 μ M Cd stressed rice

plants. The Mn translocation was minimum in both crops exposed to higher level of applied Cd (Fig. 5).

DISCUSSION

In present experiment, increasing Cd levels resulted in decreased shoot and root length and dry biomass with varying extent (Table 2), same phenomenon was reported in hydroponically grown wheat (Ci *et al.*, 2009, 2010) and rice (Liu *et al.*, 2009) that might be resulted due to Cd inactivation of enzymes responsible for cell division at root growing regions (Zhang *et al.*, 2008) and due to direct exposure of cellular membranes to toxic environment which might had caused injury in cortex cells (Sridhar *et al.*, 2007; Cosio *et al.*, 2005). Stunted shoot growth of both wheat and rice was clearly visible and symptom appeared in current experiment (Table 2) at increasing Cd toxicity which probably resulted due to disruption in H-S bonding leading to protein inactivation (Lin *et al.*, 2007). Reduction in growth as a result of Cd stress can also be reasoned by inactivation of photosynthesis due to disturbances in leaf photosystems (Rodriguez-Serrano *et al.*, 2009). Plants of both crops exposed to Cd showed limited chlorophyll and photosynthetic activity due to reduced gas exchange in present experiment (Krzeslowska, 2011; Ali *et al.*, 2013a; Akhtar *et al.*, 2016). Lowest photosynthetic activity at higher Cd level (15 μ M Cd) in both crops can be due to disturbed metabolic activities and stomatal closure (Wu *et al.*, 2004; Wu *et al.*, 2006), inactivation of rubisco and limited pigments (chlorophyll and carotenoids) (Imonova *et al.*, 2007) and lower sub-stomatal CO_2 (Cui *et al.*, 2006). Ali *et al.* (2013a) explained Cd based reductions in photosynthetic activity are due to negative effects of Cd on chlorophyll, stomatal conductance and gas exchange. Addition of Cd in growth medium resulted in abrupt reduction in chlorophyll contents possibly due to disruption in chlorophyll lamellar formation, restricted formation of chlorophyll precursor and variations in thylakoid membrane (Imonova *et al.*, 2007; Vaculik *et al.* 2015). Among other factors responsible for restricted chlorophyll formation under Cd stress includes inactivation of amino levulinic acid and protochlorophyll reductase (used in chlorophyll formation) that disturbs pigment and protein bonding of photosystems (Costa and Spitz, 1997; Ali *et al.*, 2013a). Differential response of wheat and rice under Cd treatment was possibly due to variation in tolerance ability of both crops against Cd application (Parrotta *et al.*, 2015).

The Cd Solution applied at rate of 15 μ M Cd suppressed the micronutrients *viz.* Cu, Mn and Zn, accumulation in both crops (Table 3). These results of this study are in line with Safarzadeh *et al.* (2013) and Farooq *et al.* (2016). In present study, antagonistic relationship was noted between Cd and Zn as these results might be due to their chemical similarity. This antagonism has been reported by various researchers (Zhang *et al.*, 2002b; Aravind and Prasad, 2003; Wu *et al.*, 2003;

Balen *et al.*, 2011; Akhtar *et al.*, 2016). Highest uptake of micronutrient was recorded in plants grown in uncontaminated growth medium while uptake was gradually decreased by increasing Cd levels. This inverse relationship between Cd and micronutrients might be due to plant preference and solution concentration of micronutrients. Synergistic and antagonistic relationship of Cd with other nutrient ions can be reasoned for imbalanced uptake of nutrients under Cd stress (Nan *et al.*, 2002; Yujing *et al.*, 2008). Mineral nutrients uptake by roots is function of selective properties of plasma membrane. The presence of Cd affects nutrients uptake by altering permeability and elemental transport process of plasma membrane (Dong *et al.*, 2006; Sarwar *et al.*, 2010). Due to strong affinity as ligand to S-H and C-OO groups, Cd²⁺ denatures proteins like enzymes, transporters and regulator proteins (Assche and Clijsters, 1990). Cadmium stress consequently resulted in nutrients deficiency/imbalance and reduction in plant growth as Cd²⁺ disturbs uptake and translocation of certain nutrients particularly micronutrients with similar valences such as Zn, Cu, Fe and Mn (Dong *et al.*, 2006). Wu *et al.* (2004) observed lower shoot Zn, Cu and Fe at 1 and 10 µM applied Cd whereas these nutrients were higher in root, depicting that Cd prevent translocation of micronutrients from root to shoot whereas Liu *et al.* (2003) observed positive correlation between Cd and Zn, Cu and Fe depicting synergistic interaction of Cd in absorption and translocation of these nutrients. Wu *et al.* (2006) tested different rice genotypes for Zn and Fe content grown under Cd stress and resulted that shoot Zn content was improved up to 0.1 mM Cd while root Zn up to 0.5 mM Cd, whereas Fe in shoot and root was increased up to 0.1 mM Cd and reduced abruptly at higher Cd levels.

In our experiment, root Cd was found in higher concentrations as compared to shoot Cd, which is might be due to higher adsorption of Cd on negative charges found on cell wall of plant roots (Polle and Schutzendubel, 2004). Roots can accumulate three times more Cd to its shoot Cd (Stingu *et al.*, 2010). Root Cd uptake is dependent on its structure and activities (Stritsis *et al.* 2014). In present experiment, Cd uptake and translocation was linearly increased with increasing Cd levels that was already reported by number of researchers in hydroponic medium (Wang *et al.* 2013; Nath *et al.*, 2013).

Conclusion:

In current study, increased Cd concentrations declined the growth and physiological activities for both wheat and rice crops. Rice accumulated higher concentrations of Cd compared to that with wheat leading to higher decrease in rice biomass than that of wheat. Cadmium partitioning reflected higher accumulation of metal in roots than shoot for both the crops due to less mobility of Cd in plant tissues. Different mineral nutrients like Cu, Fe, Mn and Zn showed antagonistic effect with elevated concentration of Cd for both rice and wheat crops. The future research must be focused on the

screening and genetic modifications of low Cd accumulating different genotypes of wheat.

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