

Essential Nutrients' Uptake Potential and Biochemical Characterization of Four Genotypes of Wheat (*Triticum Aestivum* L.) Under Various CdCl₂ Stress Conditions

Munazza Gull, Abida Kausar

Received: 20 October 2017 / Received in revised form: 15 January 2017, Accepted: 16 January 2018, Published online: 17 January 2018
© Biochemical Technology Society 2014-2018
© Sevas Educational Society 2008

Abstract

The major objective of the study was to determine the effect of different concentrations of cadmium chloride on nutrients that are available and biochemical properties of four varieties of wheat PUNJAB-2011, MILLAT-2011, LASANI and AARI. It has been estimated that chlorophyll a, chlorophyll b and total chlorophyll contents were decreased under CdCl₂ stress conditions. However, the uptake of cadmium was increased in all levels of cadmium stress (0, 50, 100 and 150 mM) in both shoots and roots (mg/g dry weight) of all cultivars. Cadmium adversely effected all the tested biochemical parameters like, chlorophyll a, b and total chlorophyll contents. Total carotenoids of wheat and the availability of necessary ions such as calcium, phosphorus, zinc and iron also decreased in the cadmium stress conditions. The extent of cadmium stress has increased and distinguished with increasing concentrations of cadmium chloride that led to having adverse effects on the yield and production of tested wheat genotypes.

Keywords: Cadmium Chloride, Nutrients, Biochemical Studies, Wheat.

Introduction

Industrialization of society, population explosion and the use of pesticides are main contributing factors causing heavy metal toxicity in the soil. The source of heavy metals in the environment is mainly sewage water from different industries. Natural aquatic and terrestrial ecosystems are destroyed by high concentration of heavy metals in soil (Azevedo and Azevedo 2006). Cadmium is one of the most toxic pollutants in air, water and soil (Yildiz 2005). The accumulation of cadmium in soil is increased by city and industrial wastes and fertilizers (Amirjani 2012). Cadmium is used in many industries for the preparation of dyes and paints. At low

concentration, some heavy metals are useful for plants. By increasing the concentration of heavy metals, growth pattern of many plants is inhibited (Rajkumar et al. 2013; Dikkaya and Ergun 2014).

Agricultural crops can be injured severely when exposed to high levels of heavy metals stored in soil. The increasing amount of cadmium in the environment affects numerous physiological and biochemical methods in plants (Tran and Popova 2013). The heavy metals causing reduction in germination growth, disturb the morphological characters of various plant species (Amirjani 2012; Januškaitienė, 2012; Liu et al. 2013; Dikkaya and Ergun 2014). The cadmium in the soil is taken up by the roots, and then transported across the plant tissues and finally accumulate in roots (Xiao 2014), shoots, fruits, and grains (Dikkaya and Ergun 2014). The increased cadmium concentration decreased the photosynthesis, chlorophyll contents (Amirjani 2012), mineral nutrients (Ashraf et al. 2013), enzyme activities (Sergent et al. 2014), activities of Rubisco, photosystem II, and Calvin cycle (Dikkaya and Ergun 2014) and modification of gene expressions (Sergent et al. 2014). It destroyed the chloroplast membranes in plants that fail to prepare their food properly (Rehman 2012). Due to the inhibition of photosynthesis, cadmium has a direct effect on the nucleus and other parts of the plant by reducing shoot and root length (Hussain et al. 2012). And ultimately, the reduction in plant growth and yield reduced the yield of plants (Ashraf et al. 2013). It inactivated different enzymatic processes and modified the cell redox balance (Sergent et al. 2014).

Oxidative damage has also been an important problem which induces in plants under the accumulation of cadmium (Sergent et al. 2014; Bavi et al. 2011; Dikkaya and Ergun 2014). The presence of toxic metal such as cadmium in the cell leads to the formation of reactive oxygen species, which causes severe oxidative damage to different cell organelles and biomolecules (Tkalec et al. 2014). The activity of various enzymes like catalases, superoxide dismutase (Zhang et al. 2004) peroxidase, α -amylase and β -amylase was suppressed by increasing the concentration of cadmium (Hussain et al. 2012). The activity of glutathione

Munazza Gull*

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

*Email: munagull @ hotmail.com

Abida Kausar

Department of Botany, GC University, Faisalabad, Pakistan

Email: abida.bot.gcw @ gmail.com

reductase and ascorbate peroxidase was also reduced under cadmium stress. Under cadmium exposure, the leaves of wheat accumulate lipid peroxidation products about 63% (Irfan et al. 2013; Sergent et al. 2014).

Wheat (*Triticum aestivum* L.) is the leading and most important grain crop among the cereals. World wheat production has been estimated as 708.9 million tons (FAO, 2013-14). Pakistan is an agricultural land, and most of its economy depends upon agricultural products. In Pakistan, more than 80% of people use wheat as major food. Wheat grows on 8.66 million hector area per year in Pakistan (FAO, 2013-14). Wheat is a staple food, and its production in Pakistan is 24.231 million tons per year (FAO, 2013-14). In temperate countries, wheat is a dominant crop being used for humans and livestock feed because it provides essential amino acids, minerals, vitamins, beneficial phytochemicals and dietary fiber to the human diet (Yang et al. 2011). Wheat lowers the risks of heart diseases, because it contains low fat contents. It also regulates blood glucose levels in diabetic patients. Problems such as obesity, mineral deficits, tuberculosis, chronic infection, gallstones, asthenia and breast cancer get improved by consuming wheat. Sterility is also treated with wheat oil. The seeds of wheat are consumed for the treatment of gastrointestinal ailments, skin problems, respiratory diseases, and cardiovascular illnesses. Keeping in view of the importance of wheat, the research work has been planned and the toxic influence of cadmium on wheat has been examined. The aim of this research work was to determine the reduction in growth biochemical attributes of four varieties of wheat under cadmium stress.

Materials and Methods

Biochemical attributes

Various levels of cadmium were used in sand culture to determine the reduction in biochemical attributes and nutrients' uptake in four varieties of wheat. The seeds of four cultivars i.e., PUNJAB-2011, MILLAT-2011, LASANI and AARI were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Healthy seeds of almost the same size of all genotypes were selected for the purpose of the experimentation. Different concentrations of cadmium i.e. 0, 50, 100 and 150 mM were used. The seeds were sown in Hoagland's nutrient solution in the sand culture. The experiment was carried out in pots filled with washed, river sand, and put out in a completely randomized design with four replications. Five plants from each treatment were removed carefully, and the data were recorded to find out the behavior of four wheat cultivars.

Chlorophyll contents were observed by the method given by Arnon (1949) and Davies (1976) from 60 days old fresh leaves. Chopped leaves (0.5g) were extracted overnight in 5 ml of 80% acetone, and after the extraction, the spectrophotometer readings were measured at 480nm, 645nm and 663nm. The chlorophyll contents were calculated by the formula (Davies 1976).

The sample of dried ground material (0.5 g) was taken, then put in digestion tubes, and added 5ml from sulphuric acid (95%) to each tube and incubated overnight at room temperature. After that, 0.5 ml from H₂O₂ (35%) was poured in the tubes and heated at 350°C. After 30 minutes, the tubes were removed, and H₂O₂ (0.5 ml) was slowly added. The above step was repeated until the digested material was colorless. The volume of the extracts was up to 50ml in volumetric flasks. The extract was filtered, and used for the determination of zinc, cadmium and iron using atomic absorption spectrophotometer.

Pipette aliquot till 25 ml not more than 0.1 meq of calcium into a 4-inch diameter was used. Diluted volume approximately 25 ml, and 5 drops of reagent blue naphthol, and approximately 50 ml solution were prepared. It was titrated against reagent using a 10 ml micro burette. The color change was observed from orange red to purple color as the end point, the solution F was added as a drop every 5 to 10 seconds, and the color change was observed. A blank sample has contained solutions, and the drop of reagent aided in distinguish to the endpoint. Phosphorous (P) was determined by a spectrophotometer (Jackson, 1962). Barton's reagent (5ml) was added to the extracted material (5ml), and the total volume was made to 50ml. These samples were kept for 30 min, then analyzed for phosphorous contents. The phosphorous was calculated using the standard curve.

Statistical Analysis

Data collected in different experiments were analyzed statistically using different techniques, and the STATISCA computer program was used. The least significant differences test at 5% eventuality level was used to assess the differences between significant means.

Results

In order to accomplish the objectives of the present study, the effect of cadmium on four cultivars of wheat was determined. Different levels of cadmium (0, 50, 100 and 150 mM) were used in sand land to determine the reduction in uptake of essential nutrients and biochemical attributes. The wheat seeds of four cultivars i.e., PUNJAB, MILLAT, LASANI and AARI were used.

Chlorophyll a

Chlorophyll a in four varieties of wheat (PUNJAB, MILLAT, LASANI and AARI) was non significantly affected by the application of cadmium. Chlorophyll a was higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 1, Fig 1).

Maximum Chlorophyll a was observed in MILLAT at 0mM (0.454 mg/g f. wt) cadmium stress followed by 50 (0.398 mg/g f. wt), 100 (0.312 mg/g f. wt) and 150 mM (0.282 mg/g f. wt) cadmium stress. The minimum Chlorophyll a was observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium as compared to the stressed plants. The minimum Chlorophyll a was recorded in LASANI at 150 (0.2 mg/g f. wt), 100 (0.23 mg/g f. wt)

and 50mM (0.25g) cadmium stress conditions. The interaction between treatment and variety was not significant.

Table 1: Hoagland's nutrient solution composition

Compound	Stock	mg/g	Concentration
KNO ₃	1 M	5	0.005 M
Ca(NO ₃) ₂ ·4H ₂ O	1 M	5	0.005 M
MgSO ₄ ·7H ₂ O	1 M	2	0.002 M
KH ₂ PO ₄	1 M	1	0.001 M
H ₃ BO ₃	3.54 g L ⁻¹	1	0.5 mg L ⁻¹
MnCl ₂ ·4H ₂ O	1.80 g L ⁻¹	1	0.5 mg L ⁻¹
ZnSO ₄ ·7H ₂ O	0.22 g L ⁻¹	1	0.05 mg L ⁻¹
CuSO ₄ ·7H ₂ O	0.078 g L ⁻¹	1	0.02 mg L ⁻¹
(NH ₄) ₂ MO ₄ O ₂₄	0.18 g L ⁻¹	1	0.01 mg L ⁻¹
Ferric Citrate	27.7 g L ⁻¹	1	5.00 mg L ⁻¹

Chlorophyll b

Chlorophyll b in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of cadmium. Chlorophyll b was higher in plants growing under 0 mM cadmium stress as compared to the treated plants with cadmium (Table 1, Fig 1). The behavior of four wheat cultivars non significantly differed with the application of cadmium. Maximum Chlorophyll b was observed in MILLAT at 0 mM (0.273 mg/g f. wt) cadmium stress followed by 50 (0.213 mg/g f. wt), 100 (0.19 mg/g f. wt) and 150 mM (0.16 mg/g f. wt) cadmium stress. The minimum Chlorophyll b was observed in LASANI followed PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum chlorophyll a was recorded in LASANI at 150 (0.1 mg/g f. wt), 100 (0.107 mg/g f. wt) and 50mM (0.145 g) cadmium stress conditions. The interaction between treatment and variety was not significant.

Total Chlorophyll

Total chlorophyll in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) was significantly affected by the application of cadmium stress (Table 1). Total chlorophyll was higher in plants growing under 0 mM cadmium stress as compared to the treated plants with cadmium.

Maximum total chlorophyll was observed in MILLAT at 0mM (0.697 mg/g f. wt) cadmium stress followed by 50 (0.546 mg/g f. wt), 100 (0.516 mg/g f. wt) and 150 mM (0.488 mg/g f. wt) cadmium stress. The minimum total chlorophyll was observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum total chlorophyll was recorded in LASANI at 150 (0.12 mg/g f. wt) followed by 100 (0.29 mg/g f. wt) and 50mM (0.31 g) cadmium stress conditions. The interaction between treatment and variety was not significant (Table 1).

Carotenoids

Carotenoids in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of

cadmium (Table 1). Carotenoids were higher in plants growing under 0 mM cadmium stress as compared to the treated plants with cadmium (Table 1).

The behavior of four wheat cultivars significantly differed with the application of cadmium (Table1). Maximum carotenoids was observed in MILLAT at 0mM (2.47 mg/g f. wt) cadmium stress followed by 50 (2.31 mg/g f. wt), 100 (2.007 mg/g f. wt) and 150 mM (1.59 mg/g f. wt) cadmium stress. The minimum carotenoids were observed in LASANI followed PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum carotenoids were recorded in LASANI at 150 (1.01 mg/g f. wt), 100 (1.23 mg/g f. wt) and 50mM (1.44 g) cadmium stress conditions. The interaction between treatment and variety was not significant.

Iron contents in shoots (mg/g d.wt.)

Shoot iron contents in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were non significantly affected by the application of cadmium (Table 2). Shoot iron contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 2, Fig 1).

The behavior of four wheat cultivars significantly differed with the application of cadmium (Table 2). Maximum shoot iron contents was observed in MILLAT at 0mM (12.1 mg/g d. wt) cadmium stress, 50 (9.1 mg/g d. wt), 100 (7.1 mg/g d. wt) and 150 mM (6.1 mg/g d. wt) cadmium stress. The minimum shoot iron contents were observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium condition as compared to the stressed plants. The minimum shoot iron content was recorded in LASANI (2.71 mg/g d. wt) at 150, 100 (4.17 mg/g d. wt) and 50mM (4.19 mg/g d. wt) cadmium stress conditions. The interaction between treatment and variety was not significant (Table 2).

Iron contents in roots (mg/g d.wt.)

Root iron contents in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of cadmium (Table 2). Root iron contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 2, Fig 1).

The behavior of four wheat cultivars non significantly differed with the application of cadmium (Table 2). Maximum root iron contents was observed in MILLAT at 0mM (12.1 mg/g d. wt) cadmium stress followed by 50 (9.1 mg/g d. wt), 100 (7.1 mg/g d. wt) and 150 mM (6.1 mg/g d. wt) cadmium stress. The minimum root iron contents were observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum root iron content was recorded in LASANI (2.87 mg/g d. wt) at 150, 100 (4.76 mg/g d. wt) and 50mM (5.9 mg/g d. wt) cadmium stress conditions. The interaction between treatment and variety was not significant (Table 2).

Phosphorous contents in shoots (mg/g d.wt.)

Shoot phosphorous contents in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were non significantly affected by the application of cadmium (Table 3, Fig 3). Shoot phosphorous contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 3). Maximum shoot phosphorous contents were observed in MILLAT at 0mM (27.01 mg/g d. wt) cadmium stress, 50 (23.98 mg/g d. wt),

100 (20.48 mg/g d. wt) and 150 mM (17.84 mg/g d. wt) cadmium stress. The minimum shoot phosphorous contents were observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum shoot phosphorous content was recorded in LASANI (8.26 mg/g d. wt) at 150, 100 (10.43 mg/g d. wt) and 50mM (12.87 mg/g d. wt) cadmium stress conditions. The interaction between treatment and variety was not significant (Table 3).

Table 2- Mean squares values from the analysis of variance (ANOVA) of data for the pigment analysis iron and P contents of four cultivars of wheat at 0, 50, 100 and 150 mM cadmium stress.

SOV	df	Chl _a	Chl _b	Chl _t	carotenoids	ShootFe contents	RootFe contents
Treatment(T)	3	2537.15 ns	0.095*	0.033**	5.7772***	3.4363 ns	0.0954*
Variety(V)	3	2534.092ns	0.022ns	0.018ns	0.7804*	53.685***	0.0220 ns
T x V	9	2533.148ns	0.042ns	0.010ns	0.1377 ns	3.1494 ns	0.042 ns
Error	32	2532.668	0.023	0.006	0.2016	6.2858	0.0422

*, **, ***= Significant at 0.05, 0.01, 0.001 levels, ns= non-significant

Phosphorus contents in roots (mg/g d.wt.)

Root phosphorous contents in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were non significantly affected by the application of cadmium (Table 3, Fig 3). Root phosphorous contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 3).

The behavior of four wheat cultivars significantly differed with the application of cadmium (Table 3). Maximum root phosphorous contents were observed in MILLAT at 0mM (20.3 mg/g d. wt) cadmium stress, 50 (17.63 mg/g d. wt), 100 (14.59 mg/g d. wt) and 150 mM (13.88 mg/g d. wt) cadmium stress. The minimum root phosphorous contents were observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum root phosphorous content was recorded in LASANI (6.26 mg/g d. wt) at 150, 100 (8.23 mg/g d. wt) and 50mM (10.09 mg/g d. wt) cadmium stress conditions. The interaction between the treatment and variety was not significant.

Calcium contents in shoots (mg/g d.wt.)

Calcium contents in four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were non significantly affected by the application of cadmium (Table 3). Calcium contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 3). The behavior of four wheat cultivars did non significantly differ with the application of calcium (Table 3, Fig 2). At 0 mM concentration of cadmium, the maximum calcium content was observed in MILLAT (6.67 mg/g d. wt), AARI (6.2 mg/g d. wt), PUNJAB (4.9 mg/g d. wt), and LASANI (3.5 mg/g d. wt). At 50 mM cadmium concentration, the maximum calcium content was observed in MILLAT (5.4 mg/g d. wt), AARI (5.09 mg/g d. wt), PUNJAB (4.01 mg/g d. wt) and LASANI (2.58 mg/g d. wt). At 100 mM concentration of cadmium, the maximum calcium content in shoot was observed in MILLAT (4.56 mg/g d. wt, AARI (4.02 mg/g d. wt), PUNJAB (2.93 mg/g d. wt), and LASANI (2.47 mg/g d. wt). At 150mM concentration, the maximum shoot calcium content was observed in MILLAT (3.59 mg/g d. wt) followed by AARI (3.28 mg/g d. wt), PUNJAB (2.3 mg/g d. wt) and LASANI (2.1 mg/g d. wt). The interaction between the treatment and variety was not significant (Table 3).

Table 3: Mean squares values from the analysis of variance (ANOVA) of data for P, Ca, Zn and Cd contents of four cultivars of wheat at 0, 50, 100 and 150 mM cadmium stress.

SOV	df	ShootP contents	RootP contents	ShootCa contents	RootCa contents	ShootZn contents	RootZn contents	ShootCd contents	RootCd contents
Treatment(T)	3	147.244 ns	147.244 ns	273.1695 ns	4277.74***	1044.44***	2908.491***	29.930***	250.033***
Variety(V)	3	157.66 *	157.66*	992.6058 ns	279.675**	348.712***	1858.861***	11.075**	7.6202***
T x V	9	9.17134 ns	9.1713 ns	588.0169 ns	45.828 ns	153.295**	124.443**	0.8721 ns	4.7424 ns
Error	32	53.832	53.832	366.3786	62.146	38.8875	39.265	1.933	3.4696

*, **, ***= Significant at 0.05, 0.01, 0.001 levels, ns= non-significant.

Calcium contents in roots (mg/g d.wt.)

Calcium contents in roots of four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of cadmium (Table 3, Fig 2). Calcium contents were higher in plants growing under 0mM cadmium stress as

compared to the treated plants with cadmium (Table 3). The behavior of four wheat cultivars significantly differed with the application of calcium (Table 3). At 0 mM concentration of cadmium, the maximum calcium content was observed in MILLAT (7.24 mg/g d. wt) followed by AARI (5.62 mg/g d. wt), PUNJAB (4.6 mg/g d. wt), and LASANI (3.93 mg/g d. wt). At 50

mM cadmium concentration, the maximum calcium content was observed in MILLAT (68.3 mg/g d. wt), AARI (4.93 mg/g d. wt), PUNJAB (4.93 mg/g d. wt) and LASANI (3.21 mg/g d. wt). At 100 mM concentration of cadmium, the maximum calcium content in roots was observed in MILLAT (6.15 mg/g d. wt), AARI (4.13 mg/g d. wt), PUNJAB (3.14 mg/g d. wt), and LASANI (2.01 mg/g d. wt).

At 150mM concentration, the maximum root calcium content was observed in MILLAT (5.08 mg/g d. wt), AARI (3.39 mg/g d. wt), PUNJAB (2.74 mg/g d. wt) and LASANI (1.79 mg/g d. wt) (Fig 2). The interaction between treatment and variety was not significant (Table 3).

Zinc content in shoots (mg/g d.wt.)

Shoot zinc contents of four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of cadmium (Table 3). Shoot zinc contents were higher in plants growing under 0 mM cadmium stress as compared to the treated plants with cadmium (Table 3).

The behavior of four wheat cultivars significantly differed with the application of cadmium (Table 3). Maximum shoot zinc contents were observed in MILLAT at 0 mM (5.37 mg/g d. wt) cadmium stress, 50 (4.27 mg/g d. wt), 100 (3.89 mg/g d. wt) and 150 mM (2.92 mg/g d. wt) cadmium stress. The minimum shoot zinc contents were observed in LASANI followed by Punjab, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum shoot zinc content was recorded in LASANI (1.84 mg/g d. wt) at 150, 100 (2.19 mg/g d. wt) and 50mM (2.41 mg/g d. wt) cadmium stress conditions. The interaction between the treatment and variety was significant (Table 3, Fig 2).

Zinc content in roots (mg/g d.wt.)

Root zinc contents of four varieties of wheat (AARI, PUNJAB, MILLAT AND LASANI) were significantly affected by the application of cadmium (Table 3). Root zinc contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 3). The behavior of four wheat cultivars significantly differed with the application of cadmium (Table 3, Fig 2). Maximum root zinc contents were observed in MILLAT at 0mM (8.32 mg/g d. wt) cadmium stress, 50 (6.26 mg/g d. wt), 100 (4.94 mg/g d. wt) and 150 mM (3.27 mg/g d. wt) cadmium stress. The minimum root zinc contents were observed in LASANI followed by PUNJAB, AARI and MILLAT at non stressed cadmium conditions as compared to the stressed plants. The minimum root zinc content was recorded in LASANI (1.7 mg/g d. wt) at 150, 100 (1.98 mg/g d. wt) and 50mM (2.79 mg/g d. wt) cadmium stress conditions. The interaction between treatment and variety was significant (Table 3, Fig 2).

Cadmium contents in shoots (mg/g d.wt.)

Shoot cadmium contents of four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of cadmium (Table 3, Fig 3). Shoot cadmium contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 3).

The behavior of four wheat cultivars significantly differed with the application of cadmium (Table 3). At 0mM concentrations, the minimum cadmium contents were observed in MILLAT (2.06 mg/g d. wt) cadmium stress followed by AARI (2.48 mg/g d. wt), PUNJAB (3.01 mg/g d. wt) and LASANI (4.56 mg/g d. wt). At 50mM concentrations, the minimum cadmium contents were observed in MILLAT (3.01 mg/g d. wt) cadmium stress followed by AARI (3.1 mg/g d. wt), PUNJAB (4.01 mg/g d. wt) and LASANI (5.32 mg/g d. wt). At 100mM concentrations, the minimum cadmium contents were observed in MILLAT (3.28 mg/g d. wt) cadmium stress, AARI (3.56 mg/g d. wt), PUNJAB (4.59 mg/g d. wt) and LASANI (6.17 mg/g d. wt).

The maximum concentration of cadmium at 150 mM was observed in LASANI (7.1 mg/g d. wt), AARI (5 mg.92 mg/g d. wt), PUNJAB (4.71 mg/g d. wt) and LASANI (3.61 mg/g d. wt). The cadmium was increased in all varieties of wheat. The interaction between treatment and variety was not significant. (Table 3).

Cadmium content in roots (mg/g d.wt.)

Root cadmium contents of four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI) were significantly affected by the application of cadmium (Table 3). Root cadmium contents were higher in plants growing under 0mM cadmium stress as compared to the treated plants with cadmium (Table 3).

The behavior of four wheat cultivars significantly differed with the application of cadmium (Table 3, Fig 3). At 0mM concentrations, the minimum cadmium contents were observed in MILLAT (6.67 mg/g d. wt) cadmium stress followed by AARI (7.56 mg/g d. wt), PUNJAB (9.2 mg/g d. wt) and LASANI (10.45 mg/g d. wt). At 50mM concentrations, the minimum cadmium contents were observed in MILLAT (8.94 mg/g d. wt) cadmium stress, AARI (10.67 mg/g d. wt), PUNJAB (12.09 mg/g d. wt) and LASANI (13.01 mg/g d. wt). At 100mM concentrations, the minimum cadmium contents were observed in MILLAT (9.65 mg/g d. wt) cadmium stress, AARI (12.65 mg/g d. wt), PUNJAB (14.5 mg/g d. wt) and LASANI (15.98 mg/g d. wt).

The maximum concentration of cadmium at 150 mM was observed in LASANI (19.24 mg/g d. wt), AARI (18.01 mg/g d. wt), PUNJAB (16.98 mg/g d. wt) and LASANI (14.71 mg/g d. wt). The cadmium was increased in all varieties of wheat. The interaction between treatment and variety was not significant. (Table 3).

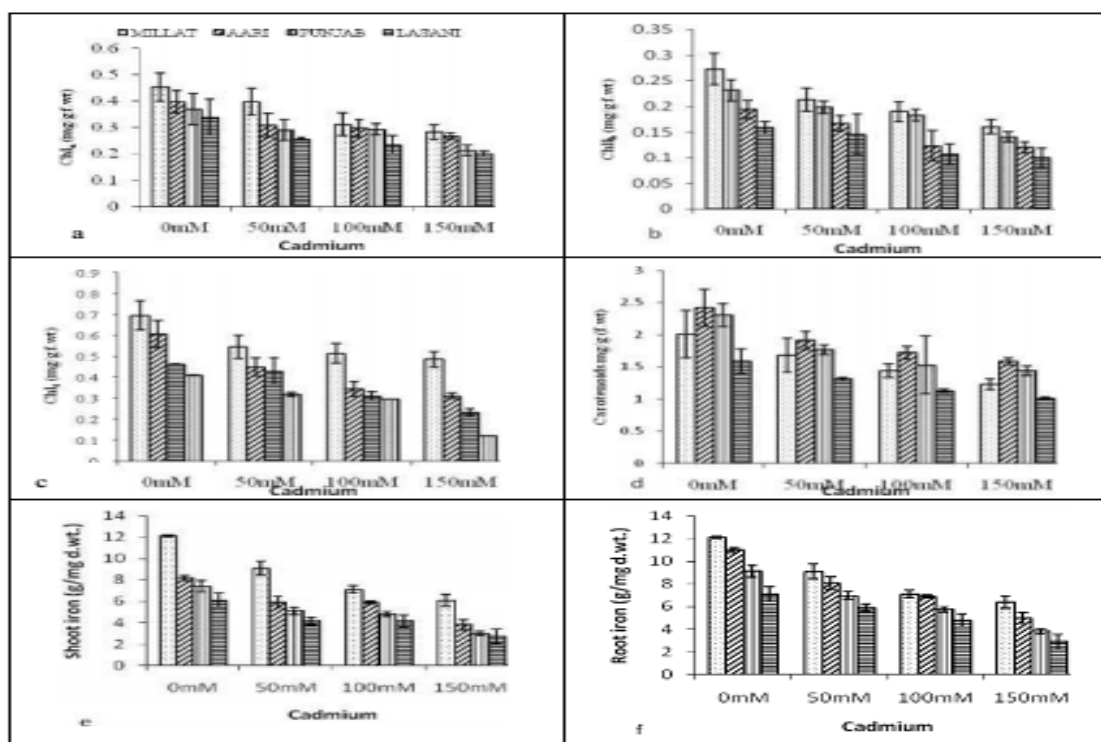


Figure 1: Effect of Cadmium stress on chlorophyll a, b and iron contents in both shoot & root of four wheat cultivars

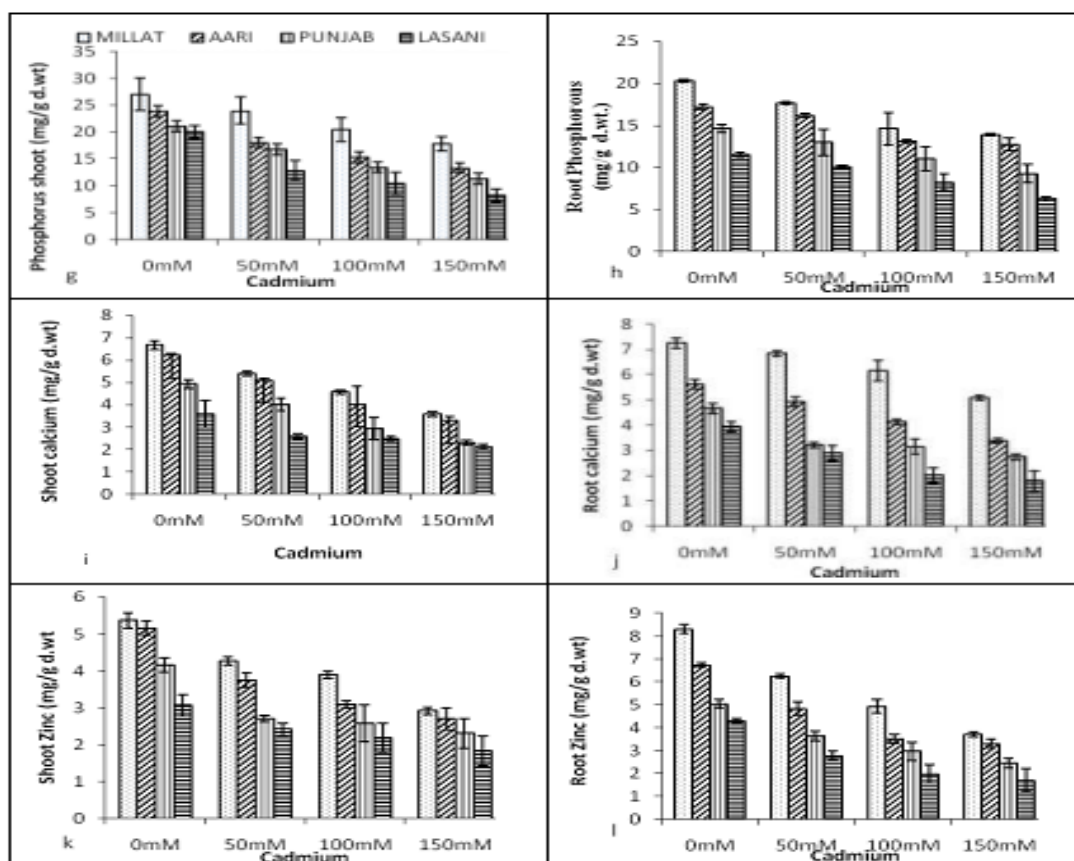


Figure 2. Effect of cadmium stress on Zn, calcium & phosphorus contents in shoot and roots of four wheat cultivars.

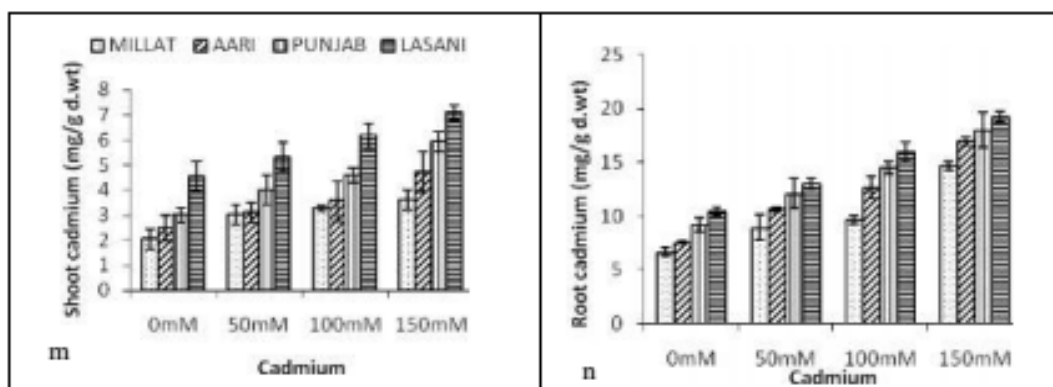


Figure 3. Accumulation of cadmium in shoot and roots of four wheat cultivars under cadmium stress conditions

Discussion

The aim of the present research work was to determine the effect of cadmium on essential ions available, and some biochemical parameters of four varieties of wheat (AARI, PUNJAB, MILLAT and LASANI). It has been observed that cadmium adversely affected the growth of plants (Ashraf et al. 2013). It has been concluded that cadmium caused premature death of roots and shoot cells especially the epidermal cells, loosened cell walls, reduced the uptake of water and stomatal closure, and changed the structure of chloroplast (Dikkaya and Ergun 2014; Gupta and Abdullah 2012; Haneef et al. 2013) which ultimately decreased the overall growth of the plants (Ozden and Kutbay 2011; Hussain et al. 2012). Present investigations showed that the biochemical attributes like chl_a, chl_b, total chl. and total carotenoids were also reduced with the application of cadmium stress. These results were in agreement with Anjana et al. (2006) and Khan et al. (2013) that chlorophyll contents and carotenoids were reduced in cadmium stress environments in mustard plants. It might cause lowering in photosynthesis of plants (Januskaitienė, 2012; Xu et al. 2013)). Under Cadmium exposure, the leaves of wheat accumulated lipid peroxidation products about 63% (Zhang et al. 2004). The oxygen release during photosynthesis decreased in Cd stressed plants (Pagliano et al. 2006) which ultimately reduced the overall growth of plants (Haneef et al. 2013). It has been established that cadmium reduced the activity of photosystem-II, and destroyed the chloroplast, as a consequence, the growth of plants and net photosynthesis also lowered under cadmium stress (Vassilev et al. 1995). It was also recorded that the activity of different enzymes related to photosynthesis might decrease by cadmium stress. The antioxidant system might damage under cadmium application, and more uptake of cadmium reduced the uptake of other essential nutrients which ultimately reduced the rate of photosynthesis (Hegeduset al. 1995; Zhang et al. 2004; Hayat et al. 2012; Irfan et al. 2012; Liu et al. 2013).

The cadmium contents were increased in the cadmium applied plants. The increase was more observed at the higher concentrations as compared to the low concentrations of cadmium. It might be due to more availability of cadmium in the environments which ultimately increased cadmium contents in the

plants under cadmium stressed conditions (Irfan et al. 2013; Liu et al. 2013). The essential ions uptake like phosphorus, iron, zinc and calcium were decreased in the environments enriched with cadmium in four cultivars of wheat. The decrease in zinc contents was also reported by Mondal et al. (2013). The decrease in uptake of many essential nutrients i.e. phosphorus, potassium, zinc, sulphur and calcium were also observed by Metwally et al. (2004) and Khan et al. (2013) in cadmium applied plants. The cadmium toxicity decreased the nitrogen, phosphorus, potassium and calcium contents in wheat plants under cadmium applied environments (Yang et al., 2011).

Conclusion

The study was conducted to find the effect of cadmium stress as a biochemical marker, and study the essential ions uptake potential of four cultivars of wheat. The present investigation concluded that cadmium adversely effected the biochemical parameters i.e., chl. a, chl. b, total chl. contents and total carotenoids of wheat, and the availability of absolutely necessary ions such as calcium, phosphorus, zinc and iron decreased in the cadmium stressed environments which led to having adverse effects on yield and production of wheat genotypes.

References

- Amirjani RM (2012) Effects of cadmium on wheat growth and some physiological factors. *Int J Forest, Soil and Ero 2* (1): 50-58
- Anjana, Umer S, Iqbal M (2006) Functional and structural changes associated with cadmium in mustard plant: effect of applied sulphur. *J Soil Sci Plant anal* 47: 1205-1217
- Arnon DI (1949) Copper enzyme in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*, *Plant Physiol* 24:1-15
- Ashraf M Y, Rafique N, Ashraf M, Azhar N, Marchand M (2013). Effect of supplemental potassium (K⁺) on growth, physiological and biochemical attributes of wheat grown under saline conditions. *J Plant Nutri*, 36: 443-458
- Azevedo JA, Azevedo RA (2006) Heavy metals and oxidative stress: where do we go from here. *Int J Agri Biol* 1(2): 135-138

- Bavi K, Kholdebarin B, Moradshahi A (2011) Effect of cadmium on growth, protein content, and peroxidase activity in pea plants. *Pak J Bot* 43(3):1467-1470.
- Davies B (1976) Carotenoids, In: Chemistry and biochemistry of plant pigments (Ed. Goodwin, T.W.). Academic Press, London, 2nd ed. 38-165.
- Dikkaya T, Ergun N (2014) Effects of cadmium and zinc interactions on growth parameters and activities of ascorbate peroxidase on maize (*Zea mays* L.). *Euro J Exp Biol* 4(1): 288-295.
- FAO (2013-14) Food and Agriculture Organization. Pakistan review of wheat sector and grain storage issues.
- Gupta D, Abdullah (2011) Effect of cadmium and copper on germination and seedling growth of maize (*Zea mays* L.). *Ind J Res Sci* 2(3): 67-70
- Haneef I, Faizan S, Parveen R, Kausar S (2013) Impact of bio-fertilizers and different levels of cadmium on the growth, biochemical contents and lipid peroxidation of *Plantago ovata* Forsk. *Saudi J Biol Sci* 21:305-310
- Hayat S, Alyemeni NM, Hasan AS (2012) Foliar spray of brassinosteroids enhance yield and quality of *Solanum lycopersicum* under cadmium stress. *Saudi J Biol Sci* 19(3): 325-335.
- Hegeduset A, Iordanov I, Chakalova E, Kervin V (1995) Effect of cadmium stress on growth and photosynthesis of young barley plants (*Hordeum vulgare* L.) plants. Structural and functional changes in the photosynthetic apparatus. *Bulg J Plant Physiol* 21(4): 12-21.
- Hussain I, Iqbal M, Qurat-ul-ain S, Rasheed R, Mahmood S, Parveen A, Wahid A (2012) Cadmium dose and exposure-time dependent alterations in growth and physiology of maize (*Zea mays*). *Int J Agri Biol* 14:959-964.
- Irfan M, Ahmed A, Hayat S (2013) Effects of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. *Saudi J Biol Sci* 21(2):125-131.
- Irfan M, Hayat S, Ahmed A, Alyemeni NM (2012) Soil cadmium enrichment: allocation and plant manifestation. *Saudi J Biol Sci* 20(1):1-10.
- Jackson, M.L., 1962. Soil Chemical Analysis. 1st Edn., Prentice Hall, New Jersey, USA.
- Januškaitienė (2012) The effect of cadmium on several photosynthetic parameters of pea (*Pisum sativum* L.) at two growth stages. *J Agri* 99:71-76.
- Khan MD, Mei L, Ali B, Chen Y, Cheng X, Zhu S J (2013) Cadmium-induced upregulation of lipid peroxidation and reactive oxygen species caused physiological, biochemical, and ultrastructural changes in upland cotton seedlings. *BioMed Res Int Article ID* 374063: 1-10.
- Liu HC, Chao YY, Kao HC (2013) Effect of potassium deficiency on antioxidant status and cadmium toxicity in rice seedlings. *J Bot Stud* 54(2): 1-10.
- Metwally A, Safronova V, Belimov A, Dietz K (2004) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. *J Exp Bot* 56:167-178.
- Mondal K, Das C, Roy S, Data J, Banerjee A (2013) Effect of varying cadmium stress on chickpea plant seedlings: An ultra structural study. *J Env Sci* 7: 59-70
- Ozdeney Y, Kutbay G H (2011) Physiological and biological responses of the leaves of *Verbascum wiedemannianum* F. on cadmium. *Pak J Bot* 43(3):1521-1525
- Pagliano C, Raviolo M, Vecchia F D, Gabbriellini R, Gonnelli C, Rascio N, Barbato R, Rocca NL (2006) Evidence for PSII donor-side damage and photoinhibition induced by cadmium treatment on rice (*Oryza sativa* L.). *J Photochem Photobiol* 84:70-78
- Rajkumar M, Prasad VNM, Swaminathan S, Freitas H (2013) Climate change driven plant-metal-microbe interactions. *Env Int J* 53:74-86
- Rehman Z, Ahmed G, Ghdan A, Manzer H, Siddiqui MH, Whaibi M, Hayssam M, Ali M, Ahmed M, Sakran M (2012) Effect of sulfur on cadmium stress tolerance in *Triticum aestivum* L. *Afri J Biotech* 11(43):10108-10114
- Sergent, K, Kieffer P, Dommes J, Hausman FJ (2014) Proteomic changes in leaves of poplar exposed to both cadmium and low temperature stress. *J Env Exp Bot* 4(3): 2771-2779.
- Tkalec I M, Peharec P, Tefanic S, Cvjetko P, Sandra S, Pavlica M, Balen B (2014) The effects of cadmium-zinc interactions on biochemical responses in tobacco seedlings and adult plants. *PLOS ONE* 9(1): e87582
- Tran T A, Popova L P (2013) Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turkish J Bot* 37:1-13
- Vassilev, A., I. Yordanov, E. Chakalova, V. Kerin, 1995. Effect of cadmium stress on growth and photosynthesis of young barley (*H. vulgare* L.) plants. 2. Structural and functional changes in photosynthetic apparatus. *Bulg. J. Plant Physiol.*, 21(4), 12-21
- Xiao L, Chao XX, Fang Y X, Wenli S, Fang CK (2014) Absorption, transfer and distribution of cadmium in indica and japonica rice under cadmium stress. *Chinese J Rice Sci* 28(2):177-184
- Xu D, Chen Z, Sun K, Yan D, Kang M, Zhao Y (2013) Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (*Solanum tuberosum* L.). *J Ecotoxicol Env Safety* 47:147-153
- Yang C, Huang G, Chai Q, Luo Z (2011) Water use and yield of wheat/maize intercropping under alternate irrigation in the oasis field of northwest China. *Field Crops Res* 124: 426-432
- Yildiz N (2005) Response of tomato and corn plants to increasing the cadmium in nutrient culture. *Pak J Bot* 37(3):593-599
- Zhang H, Jiang Y, He Z, Ma M (2005) Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). *J Plant Physiol* 162:977-984