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Photosynthetic efficiency, ion analysis and carbohydrate metabolism in *Brassica juncea* Plants under cadmium stress

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Abstract

Measurements of photosynthetic efficiency of *Brassica juncea* plants were made after subjecting them to cadmium (Cd) stress. Seeds were treated with 0.2, 0.4 and 0.6mM concentration of Cd. Observations were made on elemental analysis (Na⁺ & K⁺ ions and carbon, hydrogen, nitrogen and sulphur content), Cd accumulation, level of photosynthetic pigments (chlorophylls, carotenoids, anthocyanins, xanthophylls and flavonoid), gaseous exchange parameters (photosynthetic rate, stomatal conductance, transpiration rate, vapour pressure deficit, intrinsic mesophyll rate, water use efficiency and intercellular CO₂ concentration) and quantitative/qualitative estimation of sugars in 90 days old plants. Studies revealed that increased Cd toxicity altered the level of elements. Metal stress adversely affected the photosynthetic system. However, results showed the essential role of carbohydrates in the cell metabolism and plant defence by their quantitative prevalence in response to Cd stress.

Keywords: Cd stress, *Brassica juncea*, Photosynthesis, Carbohydrates, Elements

Introduction

Various agronomically important crops at different developmental stages are affected by abiotic stresses [1]. Among various abiotic stresses, heavy metals (HMs) are major environmental pollutants and their increasing toxicity lead to menace for ecological and environmental means. The major cause of long-lasting existence of heavy metals in the environment is their non-biodegradable nature [2]. Primary responses in the plants due to heavy metal toxicity are alteration in the process of photosynthesis, stomatal conductance, osmotic potential and combination of all such dynamics. These effects may further disrupt the morphological, cellular, biochemical and physiological mechanisms of plants [3].

Cadmium (Cd) is considered as environmentally important toxic metal, which is present in soil, air, water and sediment. It causes inhibition in photosynthesis, water and nutrient uptake in plants. Most commonly visible toxicity symptoms of Cd are chlorosis, necrosis and other physiological disorders [4]. Besides, Cd accumulates in soil as a result of rigorous usage of fertilizers in agriculture or industrial processes. Heavy metal ion homeostasis and redox homeostasis are strongly entwined and high doses of HM generate stress responses like oxidative stress in plants. Production of reactive oxygen species (ROS) triggers the oxidative stress that causes cellular damage, mutation or even death [5].

In plants, accumulation of metabolites is the most reliable strategy to avoid the toxic effects of heavy metals. Increased synthesis of carbohydrates provides defence against stressful conditions. Taking into account that *Brassica juncea* is habitual in facing the heavy metal stress; therefore, present investigation was undertaken to study the effects of Cd metal on level of ions, photosynthesis and sugar metabolism.

Materials and Methods

To study the effects of Cd metal on *Brassica juncea* plants, a field experiment was conducted in Botanical Garden of Guru Nanak Dev University, Amritsar. 20 X 20 feet area was taken for the experimentation and soil: manure in a ratio of 3:1 was added into it. Certified and disease free seeds of *Brassica juncea* L. var. RLC-1 were procured from Punjab Agricultural University, Ludhiana, (Punjab) and surface sterilized with 0.01% mercuric chloride solution, followed by the repeated washings of sterile double distilled water (DDW). Seeds were sown in different blocks. Different treatments of Cd metal were given (0, 0.2, 0.4 and 0.6 mM Cd) Plants were then harvested after 90-days of germination to study following parameters:

Cd uptake

Dried plant samples were first digested by the method given by Allen *et al.* [6]. 0.5 g of dried plant samples were taken in digestion tubes. Nitric acid and perchloric acid in the ratio of 2:1 were taken for digestion. Digested samples were followed by filtration and diluted upto the volume 50 ml. Standard solutions of CdCl₂ were prepared (1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, 20 ppm) before the estimation of the copper uptake in the plant samples. Readings of the standard solutions and test samples were taken on AAS (Atomic Absorption Spectrophotometer).

Na⁺ and K⁺ ion content

Potassium and sodium ion content was measured by flame emission photometer (systronics 128). Ions content was analyzed by method given by Allen *et al.* [7, 8]. Dried plant material (0.5 g) was digested by adding nitric acid (HNO₃) and perchloric acid (HClO₄) in the ratio of 2:1. The digested samples were made up to 50 ml by adding distilled water to it and the extract was filtered and used for analysis. Standardization of the flame photometer was done by running different concentrations of the standards of KCl and NaCl and plotted a calibration curve.

CHNS analysis

The percentage of carbon, hydrogen, nitrogen and sulphur in 90- days old plants were determined with the help of CHNS analyzer (Elementar Vario ELIII). Samples were dried completely in oven at 80 °C temperature. They were crushed to make fine powder. 10 mg of powdered samples was used to analyze the carbon, hydrogen, nitrogen and sulphur content by vario micro cube instrument run at CHNS mode. CHNS content was displayed in percentage (%).

Photosynthetic Pigments**Chlorophyll content**

Chlorophyll content was estimated by method given by Arnon [9]. 1g fresh plant tissue was homogenized in the chilled pestle and mortar, using 4ml of 80% acetone. The crushed material was subjected to centrifugation for 20 minutes at 13,000 rpm at 4 °C. The supernatant was collected for the analysis of chlorophyll a, b and total chlorophyll content and the absorbance was taken at 645 and 663nm.

Calculations

Total Chlorophyll Content (mg/g FW):

$$= (\text{Absorbance}_{645} \times 20.2) + (\text{Absorbance}_{663} \times 8.3) \times (V/1000 \times W)$$

Chlorophyll A content (mg/g FW):

$$= \text{Absorbance}_{663} \times (0.058) - (\text{Absorbance}_{645}) \times 0.032$$

Chlorophyll B content (mg/g FW):

$$= \text{Absorbance}_{645} \times (0.096) - (\text{Absorbance}_{663}) \times 0.01872$$

Where,

V = Volume of plant extract

W = fresh weight

Carotenoid content

Carotenoid content was estimated by method of Maclachlan and Zalik [10]. 1g fresh shoot tissue was homogenized in chilled pestle and mortar using 80% acetone. Then centrifugation was carried out for 20 minutes at 13,000 rpm. The supernatant was collected and absorbance was taken at 480 and 510nm.

Calculations

Total carotenoid content (mg/g FW)

$$= 7.6 (\text{O.D}_{480}) - 1.49 (\text{O.D}_{510}) \times (V/d \times W \times 1000)$$

Where,

V = Volume of plant extract

W = fresh weight

d = path length of cuvette (1cm)

Anthocyanin content

Anthocyanin content was determined by following the method given by Macinelli [11]. 1gm fresh plant tissue was homogenized with 3ml of extraction mixture (methanol: water: HCl, 79:20:1). Homogenized material was centrifuged for 20 minutes at 13,000 rpm at 4 °C and supernatant was collected for the analysis of anthocyanin content. The absorbance of the supernatant was taken at 530 and 657nm.

Calculations

Absorbance₅₃₀ – 0.25 Absorbance₆₅₇

Xanthophyll content

Xanthophyll content was estimated by the method proposed by Lawrence [12]. 0.05g of dried plant material was homogenized with 30 ml of extraction mixture in 100 ml of flask. Then flask was refluxed on water bath at 56 °C, followed by cooling. After cooling, flask containing samples were kept in dark for 1 hour then pipette 30ml of hexane into the flask and shake for 1 minute. After that volume was made up with 10% sodium sulphate solution kept for 1 hour. The upper phase was collected in 50 ml volumetric flask and the volume was made up by hexane and measured spectrophotometrically at 474 nm.

Calculations

Total xanthophylls (g/kg sample) = Absorbance₄₇₄ × D/ w × 236

Where,

w = weight of samples in grams

D = final dilution (50 × 100/ 3)

236 = translation specific absorbivity for 1g/Litre xanthophylls yield (Kg/ha)

Flavonoid content

Total flavonoid content was estimated by the method proposed by Kim *et al.* [13].

Preparation of extract

1g of fresh plant tissue was homogenized in chilled pestle and mortar using 3ml of absolute methanol. The crushed material was then subjected to centrifugation using Eltek cooling centrifuge for 20 minutes at 13,000 rpm at a temperature of 4 °C. The supernatant from the plant extract was collected for the further analysis of total flavonoid content.

1ml of the plant extract was added in 4ml of double distilled water. 0.3ml of sodium nitrite (NaNO₂) and 0.3ml of aluminum chloride (AlCl₃) were added to it. Then incubation was given for 5 minutes. Followed by addition of 2ml sodium hydroxide (NaOH) and pink color was developed. Then 2.4 ml of distilled water was added in it and absorbance was taken at 510nm. 1mg/ml of Rutin was used as standard for flavonoid content determination.

Calculation

A graph of absorbance v/s concentration for standard solutions of flavonoid was plotted and the amount of flavonoid in sample was calculated from graph. The amount of flavonoid is expressed as mg/g tissue.

Gaseous Exchange Parameters

Gaseous exchange parameters of plants like Photosynthetic rate and water use efficiency were measured with the help of (IRGA) infra-red gas analyzer (Li-COR 6400). The measurement was performed within the time period 9.00–11.00 h maintaining the air temperature, air relative humidity, CO₂ concentration and photosynthetic photon flux density (PPFD) at 25 °C, 80–90%, 400 μmol mol⁻¹ and 1000 μmol m⁻²s⁻¹ respectively.

Quantitative analysis of Sugars

Total sugars were quantitatively detected by the method given by Scott and Melvin [14]. In 25 mg of plant sample, 1.25 ml of 2.5 N HCl was added and cooled it to room temperature. Na₂CO₃ was added to neutralize it and made final volume to 25 ml. Then 4 ml of anthrone reagent was added to 1 ml of supernatant. Heated for 8 minutes in boiling water bath. Cooled it and read the optical density when the dark green colour appeared at 630 nm on UV-Vis PC Based Double Beam Spectrophotometer (Systronics 2202).

Calculations

A graph of absorbance v/s concentration for standard solutions of glucose was plotted and the amount of total sugars in sample was calculated from graph.

Qualitative analysis of Sugars

Sugar content of 90 days old plants were measured by Metrohm Ion Chromatograph (Orion-960). Plant samples were first oven dried and crushed to form powder. 2 g of powdered extract was mixed with 10 ml of DDW and filtered by 0.22 micron pore sized filter paper. Supernatant was used for analysis of sugar content. Flow rate was 1 ml /min at 32 °C of column temperature. Column used was metrosap carb 1-250. Dimensions of column were 4.6 X 250 mm. Sorbitol, mannitol, arabinose, xylose, fructose and cellobiose standards were run and chromatograms of samples were correlated with the standards.

Statistical analysis

Each experiment was conducted in three replicates. Data was expressed in Mean±SE. To check the significant differences between the treatments, one way-ANOVA was carried out by using Assisat version 7.7 beta.

Table 2: Effect of Cd on Carbon, Hydrogen, Nitrogen and Sulphur Content in 90 days old *B. juncea* Plants

Treatments	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)
0.0 mM	34.20±3.33 ^b	8.162±1.49 ^a	3.56±0.56 ^a	0.026±0.002 ^c
0.2mM	41.01±3.84 ^{ab}	6.107±0.82 ^b	3.45±0.12 ^a	1.151±0.067 ^a
0.4mM	45.97±5.98 ^{ab}	6.799±0.77 ^b	2.98±0.03 ^{ab}	0.530±0.01 ^b
0.6mM	52.47±2.79 ^a	2.53±0.53 ^c	0.96±0.15 ^b	1.162±0.08 ^a

Data presented in mean ± SE. Different letters (a, b, c & d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6mM) are significantly different (Fisher LSD *post hoc test*, $p \leq 0.05$) and signify the effect of Cd metal on Elemental analysis.

Photosynthetic Pigments

Chlorophyll content

Total chlorophyll, chl a and chl b content was found to decrease with increasing Cd dose (Table 3). Maximum level of total chl (22.63mg g⁻¹ FW), chl a (14.11mg g⁻¹ FW) and chl b (8.2mg g⁻¹ FW) was observed in control plants. With metal doses, total chl (21.14mg g⁻¹ FW) and chl b (6.96mg g⁻¹ FW)

Results

Cd uptake

Maximum metal uptake was recorded in the plants exposed to 0.6mM (101.22μg g⁻¹ DW). It was found less in case of plants treated with 0.4mM Cd (85.79μg g⁻¹ DW) and 0.2mM Cd (69.92μg g⁻¹ DW). In untreated control no metal uptake was noticed (Table 1).

Elemental analysis

Na and K ions were significantly decreased with the Cd stress in 90 days old plants (Table 1). In control plants, highest content of Na and K ions were observed (9.18 and 9.01ppm respectively). With increasing the concentration of Cd, contents of Na and K ions were found to decrease. Lowest Na and K contents were recorded in 0.6mM Cd stressed plants (4.73 and 7.04ppm). Decrease in the level of Na ion observed due to Cd stress was from 6.13 (0.2mM Cd) to 4.73ppm (0.6mM Cd) and that of K ion level decreased from 8.91 (0.2mM Cd) to 7.04ppm (0.6mM Cd).

Alteration in N and H content due to Cd stress assessed the same trend. As the Cd concentration increased, N and H contents were found to decrease except in 0.4mM Cd treated plants in case of H. This concentration showed slight increase in the level of H (6.79%), but it was less than the value of control (8.16%). Minimum value of N and H content was recorded in the plants subjected to 0.6mM Cd stress (0.96 and 2.53% respectively) with respect to control plants (3.56 and 8.16 respectively). A continuous increase in C and S content was noticed with metal treatment in plants as compared to control (34.20 and 0.026% respectively). Highest value of C and S was observed as 52.47% and 1.162% respectively, which was found in 0.6mM Cd treated plants (Table 2).

Table 1: Effect of Cd on Sodium, Potassium and Cd Metal Content in 90 days old *B. juncea* Plants

Treatments	Sodium ion (ppm)	Potassium ion (ppm)	Cd metal (μg g ⁻¹ DW)
0.0 mM	9.18±0.79 ^a	9.01±0.44 ^a	0.0±0.0 ^d
0.2mM	6.13±0.21 ^b	8.91±0.14 ^{ab}	69.92±4.07 ^c
0.4mM	5.64±0.39 ^b	7.94±0.45 ^{ab}	85.79±3.22 ^b
0.6mM	4.73±0.3 ^b	7.04±0.51 ^b	101.22±2.82 ^a

was highest in 0.4mM Cd treated plants as compared to 0.2mM (19.32 and 6.64mg g⁻¹ FW respectively) and 0.6mM Cd (16.09 and 4.66mg g⁻¹ FW respectively). Whereas, a continuous decrease in chl a content was noticed from 0.2mM (11.17mg g⁻¹ FW) to 0.6mM Cd (8.07mg g⁻¹ FW) treated plants with respect to control.

Carotenoid content

It was noticed that carotenoid content declined with Cd treatment (Table 3). Highest carotenoid was found in the control plants (11.02mg g⁻¹ FW). Decrease in the level of carotenoid was recorded from 9.46 to 8.53mg g⁻¹ FW i.e.,

from 0.2mM to 0.4mM Cd stressed plants. 0.6mM Cd caused slightly rise in the carotenoid content ($8.85\text{mg g}^{-1}\text{ FW}$) as compared to 0.4mM Cd.

Anthocyanin content

Cd metal altered the anthocyanin content in 90 days old plants of *B. juncea* by rising it as compared to control ($6.74\text{mg g}^{-1}\text{ FW}$) plants. A sharp increase was observed in 0.6mM Cd stressed plants ($9.89\text{mg g}^{-1}\text{ FW}$), which was found as highest value. 0.2mM and 0.4mM Cd treated plants showed less anthocyanin content (8.79 and $9.4\text{mg g}^{-1}\text{ FW}$ respectively) and these values are higher than the control plants (Table 4).

Xanthophyll content

2.03 folds rise in xanthophyll content was noted in 0.4mM Cd stressed plants (Table 4). Xanthophyll content was enhanced from control ($4.15\text{mg g}^{-1}\text{ DW}$) to 0.4mM Cd ($8.43\text{mg g}^{-1}\text{ DW}$). Increased level of xanthophylls was recorded in 0.2mM ($5.59\text{mg g}^{-1}\text{ DW}$) and 0.6mM Cd ($6.55\text{mg g}^{-1}\text{ DW}$) treated plants in comparison to control ($4.15\text{mg g}^{-1}\text{ DW}$).

Flavonoid content

Flavonoid content decreased with Cd metal treatment from 0.2mM to 0.4mM Cd (3.88 to $2.97\text{mg g}^{-1}\text{ FW}$). In 0.6mM Cd stressed plants, flavonoid content was further increased ($2.97\text{mg g}^{-1}\text{ FW}$), which was lower than control ($4.79\text{mg g}^{-1}\text{ FW}$) (Table 4).

Table 3: Effect of Cd on Total Chlorophyll, Chl A, Chl B and Carotenoid Content in 90 days old *B. juncea* Plants

Treatments	Total Chl ($\text{mg g}^{-1}\text{ FW}$)	Chl A ($\text{mg g}^{-1}\text{ FW}$)	Chl B ($\text{mg g}^{-1}\text{ FW}$)	Carotenoid ($\text{mg g}^{-1}\text{ FW}$)
0.0 mM	22.63 ± 1.58^a	14.11 ± 1.86^a	8.2 ± 0.99^a	11.02 ± 0.74^a
0.2mM	19.32 ± 1.35^{ab}	11.17 ± 0.80^{ab}	6.64 ± 1.19^{ab}	9.46 ± 0.54^b
0.4mM	21.14 ± 1.14^{ab}	9.5 ± 0.43^{ab}	6.96 ± 0.78^{ab}	8.53 ± 0.67^c
0.6mM	16.09 ± 0.82^b	8.07 ± 0.93^b	4.66 ± 0.66^b	8.85 ± 0.48^c

Table 4: Effect of Cd on Anthocyanin, Xanthophyll and Flavonoid Content in 90 days old *B. juncea* Plants

Treatments	Anthocyanin ($\text{mg g}^{-1}\text{ FW}$)	Xanthophyll ($\text{mg g}^{-1}\text{ DW}$)	Flavonoid ($\text{mg g}^{-1}\text{ FW}$)
0.0 mM	6.74 ± 0.87^b	4.15 ± 0.46^c	4.79 ± 0.55^a
0.2mM	8.79 ± 0.79^{ab}	5.59 ± 0.39^{bc}	3.88 ± 0.57^{ab}
0.4mM	9.4 ± 0.35^{ab}	8.43 ± 0.26^a	2.87 ± 0.22^b
0.6mM	9.89 ± 0.51^a	6.55 ± 0.38^b	2.97 ± 0.54^b

Data presented in mean \pm SE. Different letters (a, b, c & d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6mM) are significantly different (Fisher LSD *post hoc test*, $p\leq 0.05$) and signify the effect of Cd metal on Photosynthetic system.

Gaseous Exchange Parameters

A sharp decline in photosynthetic rate was noted in 90 days old plants (Table 5). Highest photosynthetic rate was observed in control plants ($31.76\text{m mol CO}_2\text{ m}^{-2}\text{s}^{-1}$). Cd toxicity caused reduction in the values as compared to control. But very small alterations were found in Cd treated plants i.e., from 0.2mM ($26.91\text{m mol CO}_2\text{ m}^{-2}\text{s}^{-1}$) to 0.6mM Cd ($24.79\text{m mol CO}_2\text{ m}^{-2}\text{s}^{-1}$). Transpiration rate was decreased from control ($1.209\text{m mol H}_2\text{O m}^{-2}\text{s}^{-1}$) to 0.6mM Cd stressed plants ($1.107\text{m mol H}_2\text{O m}^{-2}\text{s}^{-1}$). In case of 0.4mM Cd toxicity, reduction in transpiration rate was observed from (0.2mM Cd) 1.313 to 1.134 $\text{m mol H}_2\text{O m}^{-2}\text{s}^{-1}$. Transpiration rate was found to be highest in the plants exposed to 0.2mM Cd ($1.313\text{m mol H}_2\text{O m}^{-2}\text{s}^{-1}$). A significant reduction in stomatal conductance was observed due to the toxicity of Cd metal in comparison to control plants. Its highest value was found in

the control plants ($0.476\text{mol m}^{-2}\text{s}^{-1}$). 0.2mM and 0.4mM Cd revealed less stomatal conductance (0.25 and $0.24\text{mol m}^{-2}\text{s}^{-1}$) as compared to control ($0.48\text{mol m}^{-2}\text{s}^{-1}$), but more than 0.6mM Cd stressed plants ($0.19\text{mol m}^{-2}\text{s}^{-1}$). Increase in Cd concentration, caused decrease in intercellular CO_2 concentration (Ci). In 90 days plants, maximum value of Ci was showed by 0.4mM Cd dose (375.99ppm), which was slight higher than control. Control plants was found to contain 374.91ppm Ci , which was further very slightly decreased by 0.2mM Cd treatment (372.78ppm). Minimum Ci level was noted in 0.6mM Cd treated plants (367.46ppm). Control plants showed highest vapour pressure deficit (0.35kPa), which was reduced due to Cd toxicity (Table 5). A minimum value of vapour pressure deficit was found in 0.6mM Cd treated plants (0.23kPa). Decrease in value was also observed from 0.2mM (0.27kPa) to 0.4mM (0.29kPa). A decrease in WUE was recorded due to Cd toxicity. Highest WUE value was contained by control plants ($23.82\text{mgCO}_2\text{g}^{-1}\text{H}_2\text{O}$). Metal treated plants did not show any significant variations. 0.4mM Cd treated plants showed slightly higher value ($22.83\text{mgCO}_2\text{g}^{-1}\text{H}_2\text{O}$) than 0.2mM Cd stressed plants ($22.33\text{mgCO}_2\text{g}^{-1}\text{H}_2\text{O}$). Application of 0.6mM Cd revealed minimum WUE ($22.39\text{mgCO}_2\text{g}^{-1}\text{H}_2\text{O}$). It was noticed that 90 days old plants showed reduction in intrinsic mesophyll rate due to toxic effects of Cd metal. But highest value was possessed by 0.4mM Cd treated plants ($0.084\text{mgCO}_2\text{m}^{-3}$). Overall decrease in the mesophyll rate was observed from control ($0.072\text{mgCO}_2\text{m}^{-3}$) to 0.6mM Cd ($0.067\text{mgCO}_2\text{m}^{-3}$) treatment (Table 6).

Table 5: Effect of Cd on Photosynthetic rate, Transpiration rate, Stomatal Conductance and Intercellular CO_2 concentration in 90 days old *B. juncea* Plants

Treatments	Photosynthetic rate ($\text{m mol CO}_2\text{ m}^{-2}\text{s}^{-1}$)	Transpiration rate ($\text{m mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	Stomatal Conductance ($\text{mol m}^{-2}\text{s}^{-1}$)	Intercellular CO_2 Concentration (ppm)
0.0 mM	31.76 ± 0.27^a	1.209 ± 0.044^{ab}	0.476 ± 0.056^a	374.91 ± 0.58^{ab}
0.2mM	26.91 ± 0.23^b	1.313 ± 0.041^a	0.247 ± 0.019^b	372.78 ± 0.29^b
0.4mM	25.85 ± 0.12^{bc}	1.134 ± 0.035^b	0.238 ± 0.024^b	375.99 ± 0.29^a
0.6mM	24.79 ± 0.36^c	1.107 ± 0.002^b	0.192 ± 0.005^b	367.46 ± 3.92^c

Table 6: Effect of Cd on Vapour Pressure Deficit, H₂O Use Efficiency and Intrinsic Mesophyll Rate in 90 days old *B. juncea* Plants

Treatments	Vapour Pressure Deficit (kPa)	H ₂ O Use Efficiency (m mol H ₂ O m ⁻² s ⁻¹)	Intrinsic Mesophyll Rate (mol m ⁻² s ⁻¹)
0.0 mM	0.346±0.011 ^a	23.82±0.99 ^a	0.072±0.001 ^b
0.2mM	0.274±0.006 ^b	22.33±1.01 ^b	0.069±0.00 ^{bc}
0.4mM	0.296±0.002 ^b	22.83±0.61 ^b	0.084±0.001 ^a
0.6mM	0.235±0.005 ^c	22.39±0.33 ^b	0.067±0.001 ^c

Data presented in mean ± SE. Different letters (a, b, c & d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6mM) are significantly different (Fisher LSD *post hoc test*, $p \leq 0.05$) and signify the effect of Cd metal on Photosynthetic system.

Quantitative analysis of Sugars

B. juncea plants exposed to Cd stress showed elevation in sugar content (Fig 1). Sugar content was found to enhance

from 13.15 to 16.84 μ mol g⁻¹ FW. Minimum sugar content was showed by control plants (13.15 μ mol g⁻¹ FW), which further noticed to increase with 0.2mM Cd (16.15 μ mol g⁻¹ FW) and 0.4mM Cd (13.40 μ mol g⁻¹ FW) treatment. Highest value of sugars was observed in 0.6mM Cd treated plants (16.84 μ mol g⁻¹ FW).

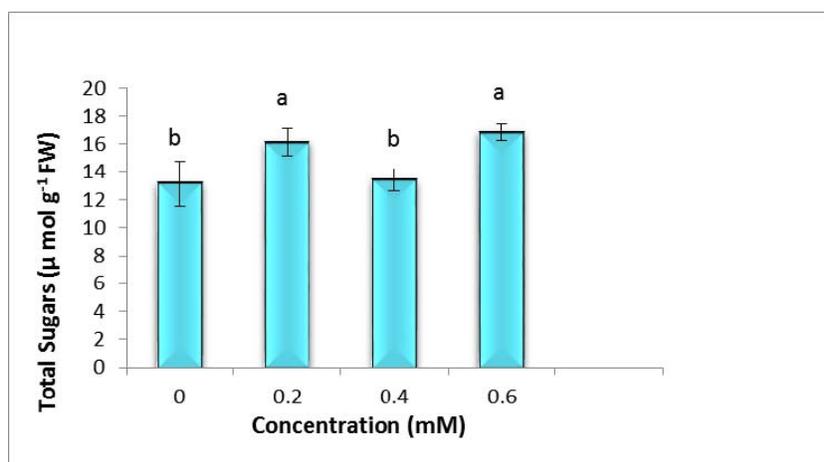


Fig 1: Effect of Cd on Total sugar content (μ mol g⁻¹ FW) in 90-days old Plants of *Brassica juncea*.

Qualitative analysis of Sugars by Ion Chromatography

In 90 days old plants of *B. juncea*, mannitol, fructose and cellobiose sugars were observed (Fig 2-5). Additional distinctive peak of glucose sugar was expressed in 0.2mM Cd stressed plants and increase in the amount of sugars mannitol (from 3.959 to 6.234ppm), fructose (9.542 to 15.976ppm) and

cellobiose (from 0.050 to 0.729ppm) were found. In 0.4mM Cd stress, glucose was also expressed, but decrease in concentration of mannitol and fructose sugars was noticed. Quantity of cellobiose was increased from 0.050 (control) to 0.264ppm (0.4mM Cd). Two additional sugars namely xylose and arabinose were expressed in 0.6mM Cd treatment (Table 7).

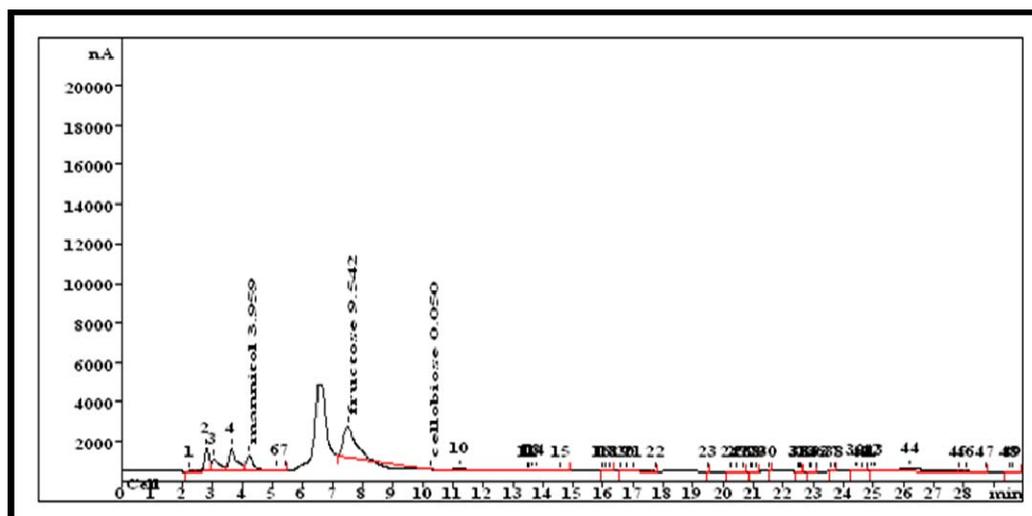


Fig 2: Qualitative analysis of Sugars in untreated control plants of *Brassica juncea*

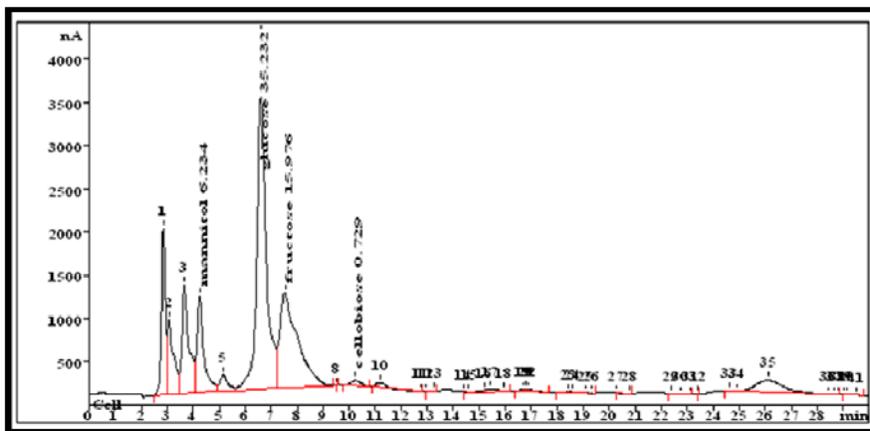


Fig 3: Qualitative analysis of Sugars in 90 days old *Brassica juncea* plants treated with 0.2mM Cd

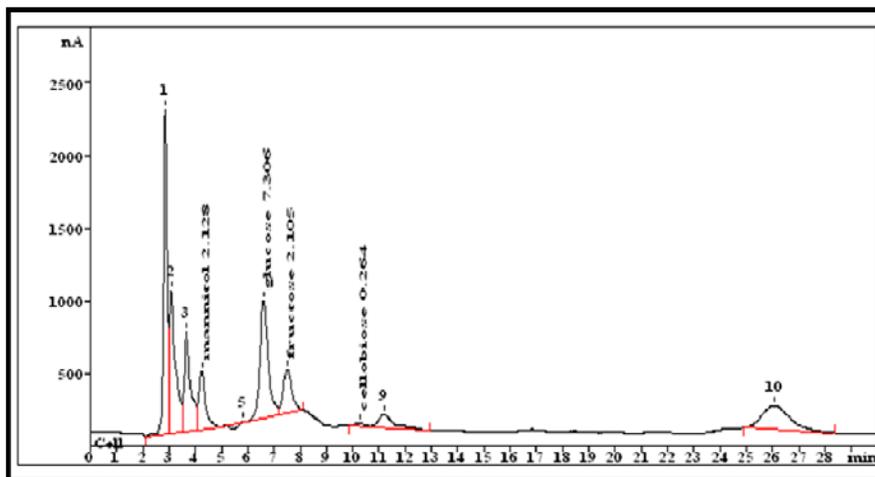


Fig 4: Qualitative analysis of Sugars in 90-days old plants of *Brassica juncea* treated with 0.4mM Cd

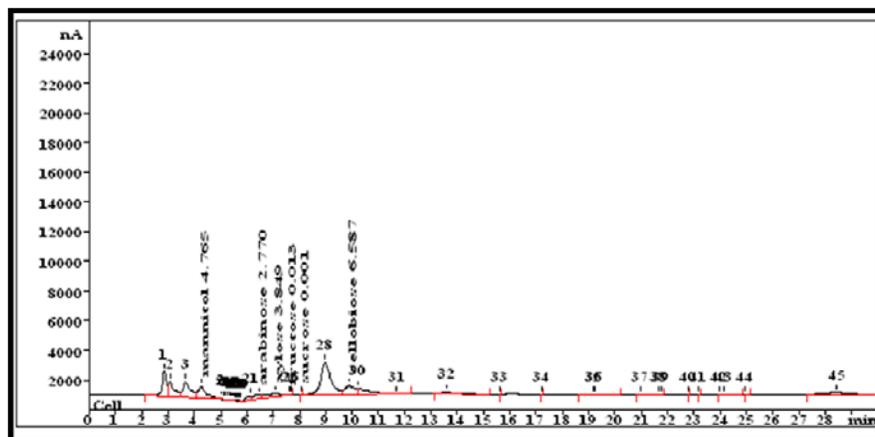


Fig 5: Qualitative analysis of Sugars in 90-days old plants of *Brassica juncea* treated with 0.6mM Cd

Table 7: Concentrations of Sugars in 90 days old *Brassica juncea* plants treated with Cd stress

S. No.	Sugars	Concentrations (ppm)			
		Control	0.2mM Cd	0.4mM Cd	0.6mM Cd
1.	Mannitol	3.950	6.234	2.128	4.765
2.	Glucose		35.232	7.306	
3.	Fructose	9.542	15.976	2.105	0.013
4.	Cellobiose	0.050	0.729	0.264	6.587
5.	Sucrose	-	-	-	0.001
6.	Arabinose	-	-	-	2.770
7.	Xylose	-	-	-	3.849

Discussion

Plant metabolism and yield is adversely affected by heavy metal stress^[15]. Under stressed conditions, ROS production is drastically increased in the organelles like chloroplast, peroxisomes and mitochondria due to the imbalance between the production and scavenging of ROS. ROS like hydrogen peroxide, singlet oxygen etc. are the toxic molecules cause oxidative damage to lipids, proteins and DNA. Several evidences have revealed that Cd phytotoxicity leads to oxidative stress^[16]. The findings of the present study revealed the better uptake efficiency in 90 days old *B. juncea* plants and metal uptake increased with increasing concentration of metal. Maximum uptake of metal was noticed in highest concentration. Metal hyperaccumulators like *Brassica juncea* accumulate metal ions in their shoots or leaves in extremely higher doses as compared to other plants^[17]. A report suggested that Cd uptake was found to enhance with enhancing the exposure period of Cd metal in radish seedlings^[18]. K^+ & Na^+ content and percentage of nitrogen & hydrogen was found to decrease in 90 days old plants in response to increase in the concentration of the Cd in comparison to untreated plants. Whereas, carbon and sulfur contents were found to enhance with Cd toxicity. Maximum reduction was recorded at highest metal dose i.e. 0.6mM Cd. Metal toxicity negatively affects the uptake and transport of mineral nutrients in the plants^[19]. The results of present work was also supported by the report of Souza *et al.*^[20], where Cu toxicity caused sharp decline in mineral nutrients like K^+ ion concentration in *Theobroma cacao* seedlings. A decrease in level of these ions often indicates their efflux across a plasma membrane and excess dose of metal damage them by inducing the production of malondialdehyde. Further this damage causes loss of membrane selectivity and enhances permeability^[21]. Sulfur is a major nutrient for most of the organisms which plays a key role in metal accumulator/hyperaccumulator plants to resist the heavy metal stress through synthesis of phytochelatins (PCs), a class of metal-binding peptides with the general structure (r-Glu-Cys)_n-Gly^[22]. In *B. juncea* plants, sulfur assimilation was stimulated to some extent by the presence of high metal concentrations in the soil. Fabio *et al.*^[23] reported that Cd could induce S uptake in maize due to the influence of Cd on high-affinity sulphate transport in maize roots. The level of elements raised by the enhanced metal concentrations in plant shoots, suggesting that *B. juncea* might have a specific physiological need for these elements if exposed to potential metal toxicity. In present study, decrease in Chl a, Chl b, total Chl, carotenoids and flavonoid contents in 90 days old plants has been observed under Cd stress. It may be due to the fact that Cd causes reduction of Fe and leads to chlorosis of leaves, thus negatively affects chlorophyll metabolism. Cd toxicity inactivates δ -aminolevulinic acid dehydrogenase that induces the biosynthesis of chl in the leaves^[24]. Inactivation of enzyme activities and replacement of essential micronutrients by the HM, which are required for biosynthesis of pigments, are the major reasons which lead to photosynthesis impairment. Heavy metal ion also leads to oxidative burst that give rise to the formation of ROS and thus causes oxidative damage to the membrane. Destruction of membrane directly or indirectly influences the photosynthetic pigments. The observations are also in coherence with the findings of Aldoobie and Beltagi^[25], where chl and carotenoid content was recorded to decline with Pb, Ni, Zn and Cr stress. On the other hand, anthocyanin and xanthophylls content was

found to increase with Cd metal stress in 90 days old plants of *B. juncea*. This might be due to the activation of antioxidative enzyme glutathione-S-transferase, which stimulates the synthesis of anthocyanin pigment. Biochemical evidence suggested that violaxanthin (xanthophyll), is a precursor for abscisic acid (ABA) synthesis that contribute in the protection of photosynthesis against oxidative stress^[26] and therefore also acts as antioxidant. The results of present work are in coherence with the observations of Amiri *et al.*^[27]. Gaseous exchange parameters like photosynthetic rate, transpiration rate, stomatal conductance, vapour pressure deficit, intercellular CO₂ concentration, water use efficiency and intrinsic mesophyll rate were inhibited with increasing Cd doses in *Brassica juncea* plants. Regarding the toxicity of Cd, it was investigated that Cd binds in the sites of both the acceptor and the donor sides of PSII^[28]. Cd²⁺ replaces the Ca²⁺ cofactor in the Ca/Mn cluster on the donor side, which constitutes the oxygen-evolving centre with high affinity that leads to inhibition of photosynthetic oxygen evolution. Cd also reduces the transfer of electron from redox-active tyrosine residues D1-161. Results of present study was in coherence with the findings of Fariduddin *et al.*^[29], where photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and water use efficiency was declined with enhancing NaCl toxicity. In present investigation, level of sugars was enhanced under Cd stress. Carbohydrates like glucose, fructose, sucrose etc. are important compatible solutes^[30]. Sugars contribute in removal of free radicals generated during stressed conditions and thus their enhanced synthesis provides protection against stress. These maintain the osmotic potential as well as involve in redox reactions and contribute in maintaining the structures of macromolecules and membranes^[31]. Findings of present study are in coherence with the observations of Gengmao *et al.*^[32], where carbohydrates were reported to increase in *Salvia miltiorrhiza* plants under NaCl toxicity.

Conclusion

It is concluded from the present investigations that Cd stress adversely affected the photosynthesis of *Brassica juncea* plants. But carbohydrate metabolism is positively influenced by metal toxicity. Enhanced accumulation of these osmotically active compounds protects the plants from metal stress by removal of reactive oxygen species.

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